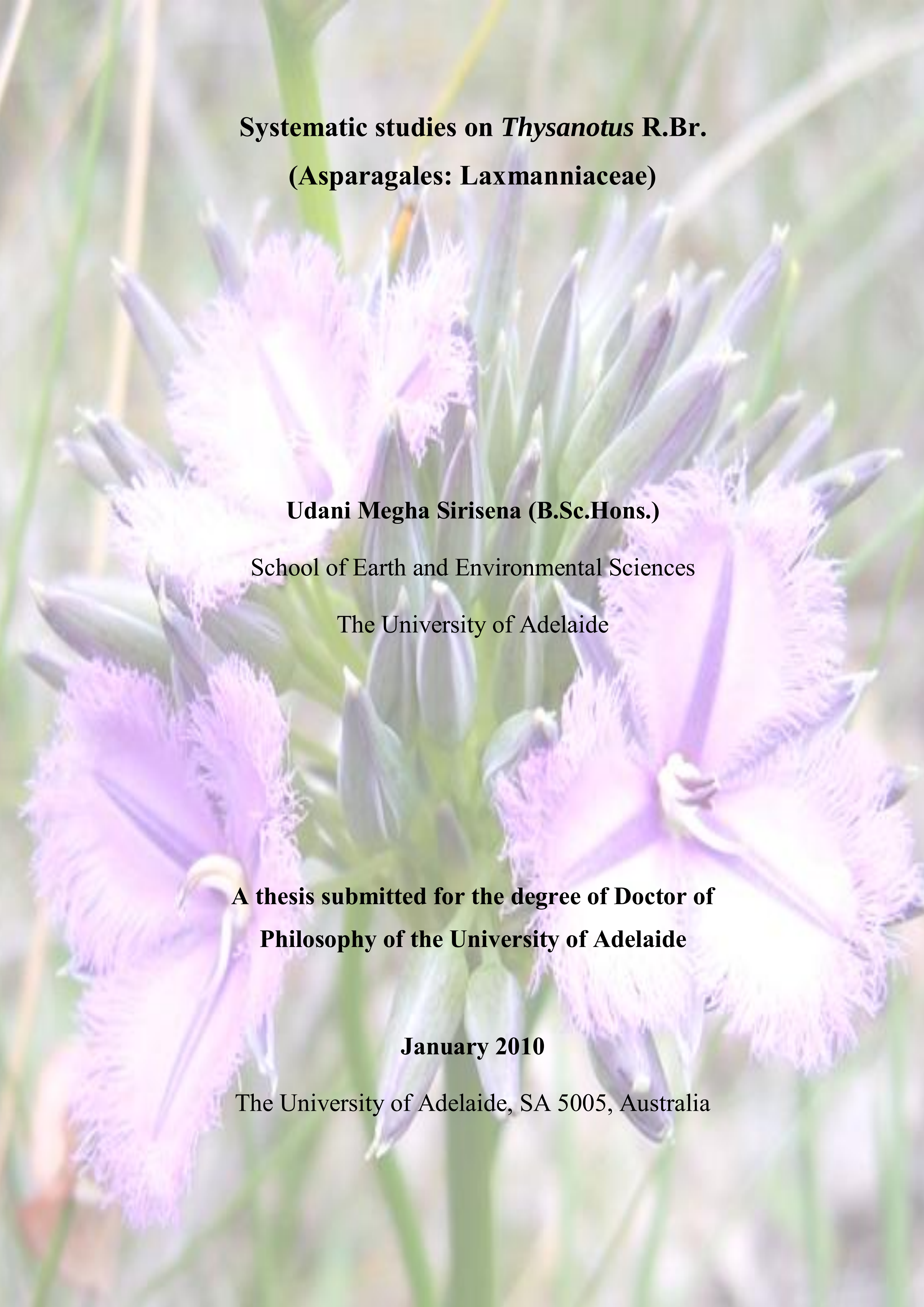


Systematic studies on *Thysanotus* R.Br. (Asparagales: Laxmanniaceae)

Udani Megha Sirisena (B.Sc.Hons.)

School of Earth and Environmental Sciences

The University of Adelaide

A thesis submitted for the degree of Doctor of Philosophy of the University of Adelaide

January 2010

The University of Adelaide, SA 5005, Australia

The systematic significance of seed coat morphology in *Thysanotus* R.Br. and related Laxmanniaceae

Udani M. Sirisena[A], *John G. Conran*[A] *and Terry D. Macfarlane*[B]

[A]Australian Centre for Evolutionary Biology and Biodiversity, School of Earth and Environmental Sciences, DX 650 312, The University of Adelaide, SA 5005, Australia

[B] Western Australian Herbarium, Manjimup Research Centre, Department of Environment and Conservation, Locked Bag 2, Manjimup, Western Australia 6258, Australia

Statement of contribution

Ms. Udani M. Sirisena carried out the field collections, experimental work, data analysis and preparation of the manuscript. Dr. John Conran collected specimens and helped in data analysis and manuscript preparation. Dr. Terry Macfarlane collected specimens and helped in manuscript preparation.

Udani M. Sirisena (January 2010)

John G. Conran (January 2010)

Terry D. Macfarlane (January 2010)

The systematic significance of seed coat morphology in *Thysanotus* R.Br. and related Laxmanniaceae

---

Abstract. The limited literature on seed morphology in Laxmanniaceae indicates that although taxonomically useful, seed characters are largely unknown for *Thysanotus*. Accordingly, seed morphology in *Thysanotus* and other Laxmannioideae was investigated in order to determine which characteristics have systematic and/or phylogenetic value. Seed patterns observed in *Thysanotus* and Laxmannioideae generally were phylogenetically important. Variation was observed in seeds to distinguish different species and different groups of species, for example, angular seeds were unique to most of the annual climbing *Thysanotus* species (except *T. manglesianus*), while elongated seeds with long stalked arils were restricted to all 3–staminate *Thysanotus* species

Introduction

Thysanotus R.Br. is a widespread native in Australia with about 49 species (Brittan 1981, 1987) described to date. The genus is morphologically very diverse, but generally characterised by its fimbriate inner tepals and flowers usually borne on umbels. The genus thrives in very different ecological conditions even extending to desert regions. However with very limited morphological and molecular systematic studies carried out on the genus, it is relatively unexplored and requires further work.

Molecular studies and characteristic nucellar data placed *Thysanotus* in a new family Laxmanniaceae along with *Arthropodium* R.Br., *Eustrephus* R.Br., *Sowerbaea* Sm., *Lomandra* Labill., *Cordyline* Comm.ex R.Br. and *Chamaescilla* F.Muell., *Acanthocarpus* Lehm., *Chamaexeros* Benth., *Romnalda* P.F.Stevens, *Xerolirion* A.S.George, *Laxmannia* R.Br., *Murchisonia* Brittan and *Trichopetalum* Lindl. (Chase *et al.* 1995; Chase *et al.* 1996; Fay *et al.* 2000). The current members of Laxmanniaceae have been placed previously within several polyphyletic families, including Anthericaceae, Philesiaceae, Xanthorrhoeaceae and Agavaceae.

The two subfamilies within the family: Lomandroideae and Laxmannioideae although only recently formalised (Thorne and Reveal 2007), these groups have been recognised for a while (Rudall and Chase 1996); however, according to Conran (1998) the Lomandroideae are the only morphologically readily recognisable group within the family, with generally distichous phyllotaxy, xeromorphic anatomy, and the absence of phytomelan in the seed coat, mucilage or storage roots. The literature on the Laxmannioideae implies a generally low reliability of traditional vegetative and floral features for describing taxonomic relationships (Chase *et al.* 1995; Chase *et al.* 1996; Rudall and Chase 1996; Conran 1998); therefore seed morphology could be possible source of information in recognition of the group and/or generic relationships. Although Thorne and Reveal (2007) formally described the subfamilies, Mabberley (2008) and APG III (2009) assigned all Laxmanniaceae genera into an expanded Asparagaceae, subfam. Lomandroideae, with no formally named lower taxa listed.

Barthlott (1984) reported the importance of seed morphology in classification of plants following Heywood (1969) and it had proved taxonomically useful in a range of families including Aizoaceae (Hassan *et al.* 2005), Byblidaceae (Conran *et al.* 2002), Droseraceae (Dwyer 1983; Boesewinkel 1989), Hemerocallidaceae: *Dianella* (Henderson 1987), Lentibulariaceae (Taylor 1989; Degtjareva *et al.* 2004) and Liliaceae: *Calochortus* (Ness 1989).

The limited literature on seed morphology in Laxmanniaceae indicates that seed characters are largely unknown for *Thysanotus*. Campylotropous ovules were considered as an indicator of a close relationship between *Thysanotus*, *Arthropodium* and *Dichopogon* (Huber 1969; Dahlgren and Clifford 1982; Conran 1998). Stringer and Conran (1991) examined seed cuticle morphology in *Arthropodium s. str.* and *Dichopogon*, supporting their merger, and species-level seed differences were also noted between two New Zealand *Arthropodium* species (Heenan *et al.* 2004). Based on these studies, we anticipate that seed morphology could offer a basis for distinguishing between different *Thysanotus* species and/or species groups, as well as relationships within and between them and other Laxmanniaceae. Accordingly, we present here information on seed morphology in *Thysanotus* and other

Laxmannioideae taxa to determine those which characteristics have systematic and/or phylogenetic value.

Materials and methods

Representative taxa (Table 1) were selected based on the revisions of *Thysanotus* (Brittan 1981, 1987). Seed material of as many taxa as possible was included in the study; however, some were excluded due to lack or poor quality of suitable material.

To understand the seed variation of other Laxmannioideae genera *Arthropodium*, *Chamaescilla*, *Cordyline*, *Eustrephus, Laxmannia, Murchisonia, Sowerbaea, Thysanotus* and *Trichopetalum* seeds were included for comparison, based largely on the gene tree of Chase *et al*., (1995), the family circumscription of Conran (1998) and the molecular studies in Chapter 4 (Sirisena 2010). *Murchisonia* was particularly included, since Black (1943) originally considered *M. volubilis* Brittan to be a variety of *T. patersonii* R.Br. (var. *exfimbriatum* J.M.Black) and despite segregating *Murchisonia* as a separate genus, Brittan (1971; 1987) still regarded it to be a close relative of *Thysanotus* with entire-petals.

Seeds were obtained from field collections and herbarium specimens, with seeds of at least two samples examined for each species, depending on availability. The seeds were air dried on aluminium stubs with carbon tops, sputter coated with carbon and gold, viewed with a Phillips XL 20 SEM at an acceleration voltage of 10Kv and photographed. Seed morphological characters were derived initially from those of Barthlott (1981), Ness (1989) and Hassan *et al.* (2005), with additional characters added as necessary.

After removing invariant characters, 16 characters and their respective states were used (Table 2). These seed characters were mapped on to a most parsimonious tree derived from molecular analyses presented in Chapter 3 using WinClada ver.1.00.08 (Nixon 2002) in order to understand the patterns of seed character evolution in *Thysanotus*.

Table 1. Sources of seeds used in the study

Specimen	Collector/source	Herbarium	Accession no
T. arbuscula Baker	N.H. Brittan	PERTH	3001547
T. arenarius Brittan	T.A. Halliday	PERTH	3016099
T. asper Lindl.	F. Lullfitz	PERTH	1978365
T. banksii R.Br.	D.E. Murfet	AD	186839
T. banksii R.Br.	John G. Conran	ADU	815
T. baueri R.Br.	Hj. Eichler	AD	96651021
T. brevifolius Brittan	Udani M. Sirisena & T. D. Macfarlane	PERTH	38
T. britanii H.R.White & T.D.Macfarl.	A. Chant	PERTH	6417728
T. chinensis Benth.	W.R. Barker	AD	9792103
T. cymosus Brittan	E.T. Bailey	PERTH	1041878
T. dichotomus (Labill.) R.Br.	N.H. Brittan	PERTH	2938367
T. exiliflorus (NT form) F.Muell.	H.P. Vonow	AD	199674
T. exiliflorus (SA form) F.Muell.	T.S. Te	AD	233
T. fastigiatus Brittan	N.H. Brittan	PERTH	6289037
T. formosus Brittan	Udani M. Sirisena & T. D. Macfarlane	PERTH	7
T. fractiflexus Brittan	Hj. Eichler	AD	96650163
T. gageoides Diels	A.S. George	PERTH	1041908
T. glaucifolius Brittan	N.H. Brittan	PERTH	6265405
T. glaucus Endl.	B.J. Keighery & N. Gibson	PERTH	4463501
T. gracilis R.Br.	A.S. George	PERTH	1743953
T. isantherus R.Br.	N.H. Brittan	PERTH	6238858
T. juncifolius (NSW form) (Salisb.) J.H.Willis & Court	Dalby et. al.	PERTH	94/09
T. juncifolius (SA form) (Salisb.) J.H.Willis & Court	Hj. Eichler	AD	96645033
T. manglesianus Kunth	D.J.E. Whibley, P.E. Trezise	AD	96019053
T. manglesianus Kunth.	John G. Conran	ADU	2210
T. multiflorus R.Br.	F.H. Mollemans, M.P. Mollemans	AD	97021056
T. multiflorus R.Br.	Udani M. Sirisena & T. D. Macfarlane	PERTH	8
T. nudicaulis Brittan	C.R. Alcock	AD	96718643
T. patersonii (SA form) R.Br.	John G. Conran	ADU	2119
T. patersonii (SA form) R.Br.	Udani M. Sirisena	ADU	1
T. patersonii (SA form) R.Br.	Udani M. Sirisena	ADU	3
T. patersonii (SA form) R.Br.	Udani M. Sirisena	ADU	4
T. patersonii (WA form) R.Br.	Hj. Eichler	AD	96310272
T. patersonii (WA form) R.Br.	John G. Conran	ADU	2255
T. pauciflorus R.Br.	C. Andrews	PERTH	2638193
T. pseudojunceus Brittan	Udani M. Sirisena & T. D. Macfarlane	PERTH	5
T. rectantherus Brittan	C.A. Gardner	PERTH	1978454
T. sp. aff. *baueri*	F.J. Badman	AD	99324113
T. sparteus R.Br.	N.H. Brittan	PERTH	4339452
T. scaber Endl.	C. Andrews	PERTH	1979302
T. speckii Brittan	A.S. George	PERTH	2638096
T. tenellus (SA form) Endl.	B. Copley	AD	96702146
T. tenellus Endl.	Udani M. Sirisena & T. D. Macfarlane	PERTH	12
T. tenuifolius sp.nov.	Udani M. Sirisena & T. D. Macfarlane	PERTH	11
T. tenuis Lindl.	Udani M. Sirisena & T. D. Macfarlane	PERTH	26
*T. thyrsoideus*Baker	D. Fell	PERTH	3020975
T. triandrus (Labill.) R.Br.	E. Pritzel	AD	96021079
T. triandrus (Labill.) R.Br.	Udani M. Sirisena & T. D. Macfarlane	PERTH	6
T. tuberosus ssp. *parviflorus* (Benth.) Brittan	John G. Conran	ADU	324
T. tuberosus R.Br. ssp. *tuberosus*	P. Trezise, R. Hill	AD	97705408
T. unicupensis Sirisena, T.Macfarlane & Conran	Udani M. Sirisena & T. D. Macfarlane	PERTH	13
T. virgatus Brittan	Dalby et. al.	PERTH	94/08

T. volubilis	T.D. Macfarlane	PERTH	8001146
T. wangariensis Brittan	C.R. Alcock	AD	96807209
T. wangariensis Brittan	A.S. George	PERTH	WAC02980568
Arthropodium milleflorum R.Br.	D.N. Kraehenbuehl	AD	99113194
Eustrephus latifolius R.Br. ex. Ker.Gawl.	John G. Conran	ADU	ADBG880587
Murchisonia fragrans Brittan	T.D. Macfarlane	PERTH	4044
Murchisonia volubilis Brittan	Lindley D. Williams	AD	98425782
Cordyline sp.	John G. Conran	ADU	1490
Chamaescilla corymbosa (R.Br.) Benth.	C.Ray Alcock	AD	97927233
Laxmannia gracilis R.Br.	John G. Conran	ADU	334
Sowerbaea alliacea F.Muell.	P. Stewart	AD	98113250
Trichopetalum plumosum J.F.Macbr.	Orto Botanico Università degli Studi di Napoli Federico II, Facoltà di Scienze MM. FF. NN.	ADU	Index Seminum 2008: 699

Table 2. Seed characters and their respective character states

1. Seed shape: orbicular/globose=0, elliptic/ovoid=1, elongate=2, angular=3
2. Median ridge: absent=0, present=1
3. Aril/ arillate appendage: absent=0, present=1
4. Aril stalk with aril positioning: sessile with proximal aril =0, long stalk with distal aril positioned distant=1, aril absent=2
5. Aril shape: elongated=0, flat=1, aril absent=2
6. Shiny seed coat: absent=0, present=1
7. Seed surface appearance/texture: smooth=0, rough=1
8. Seed length: ≤ 2mm=0, > 2mm=1
9. Outer epidermal cell shape: polygonal=0, rounded= 1, irregular=2
10. Outer periclinal wall shape: strongly convex=0, slightly convex=1, flat=2
11. Convex periclinal wall surface shape: flat=0, rounded=1, uneven=2
12. Cell surface microsculpturing: absent=0, present=1
13. Verrucose microsculpturing: absent=0, present=1
14. Striated microsculpturing: absent=0, present=1
15. Anticlinal cell wall boundary: raised=0, channelled=1
16. Shallowly channelled anticlinal boundaries: absent=0, present=1

Results

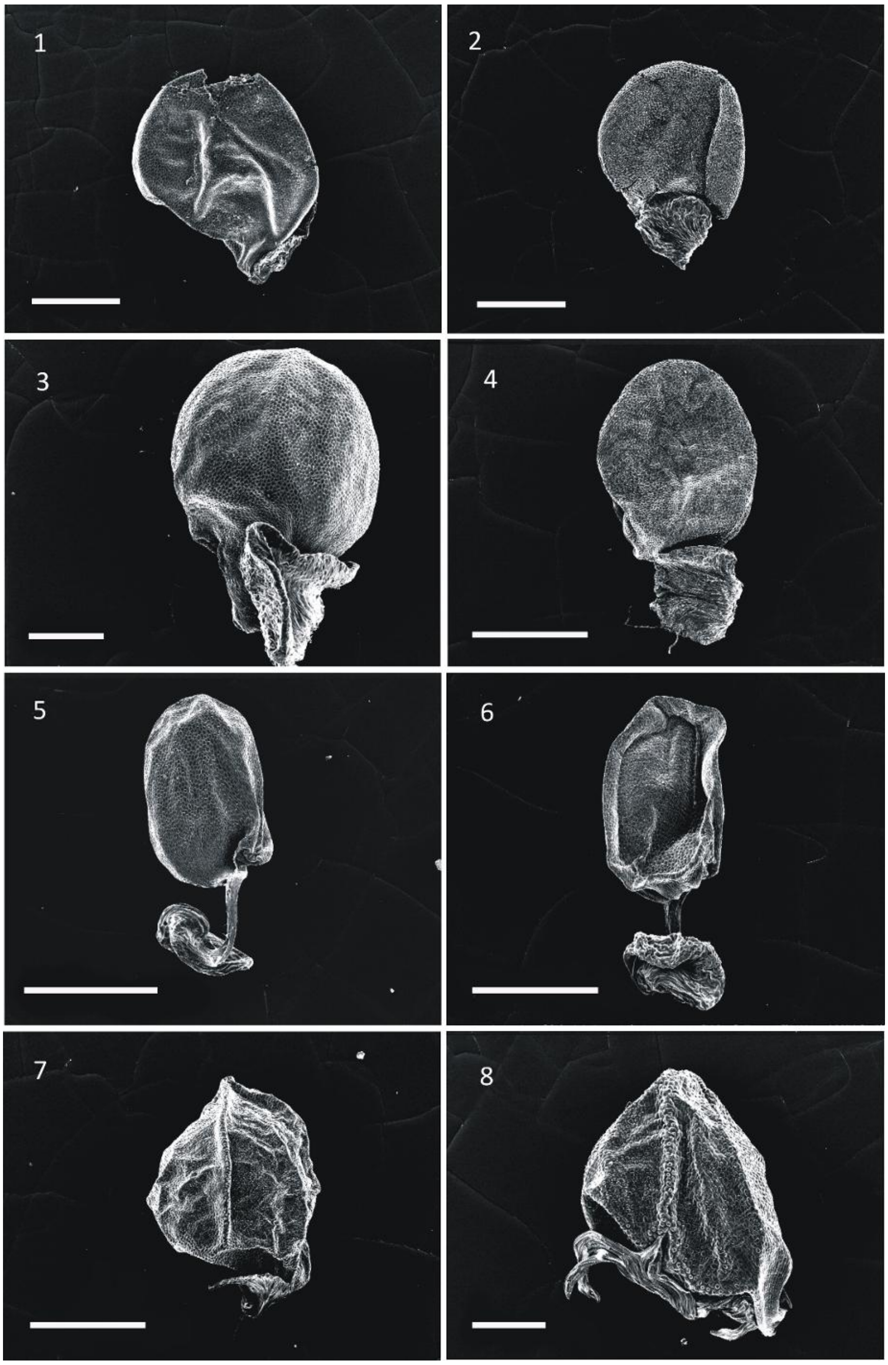
Examination of seed coat morphology of *Thysanotus* and the Laxmannioideae taxa indicated that there was sufficient variation, to serve as a possible source for recognising different taxa, species groups and evolutionary trends.

Seed epidermal cells in *Thysanotus* were fairly uniform, with polygonal to elongate polygonal cells, straight anticlinal walls and convex outer periclinal walls. Nevertheless, there was variation in appendage morphology, epidermal cell elongation, anticlinal boundaries, outer periclinal wall features and surface sculpturing.

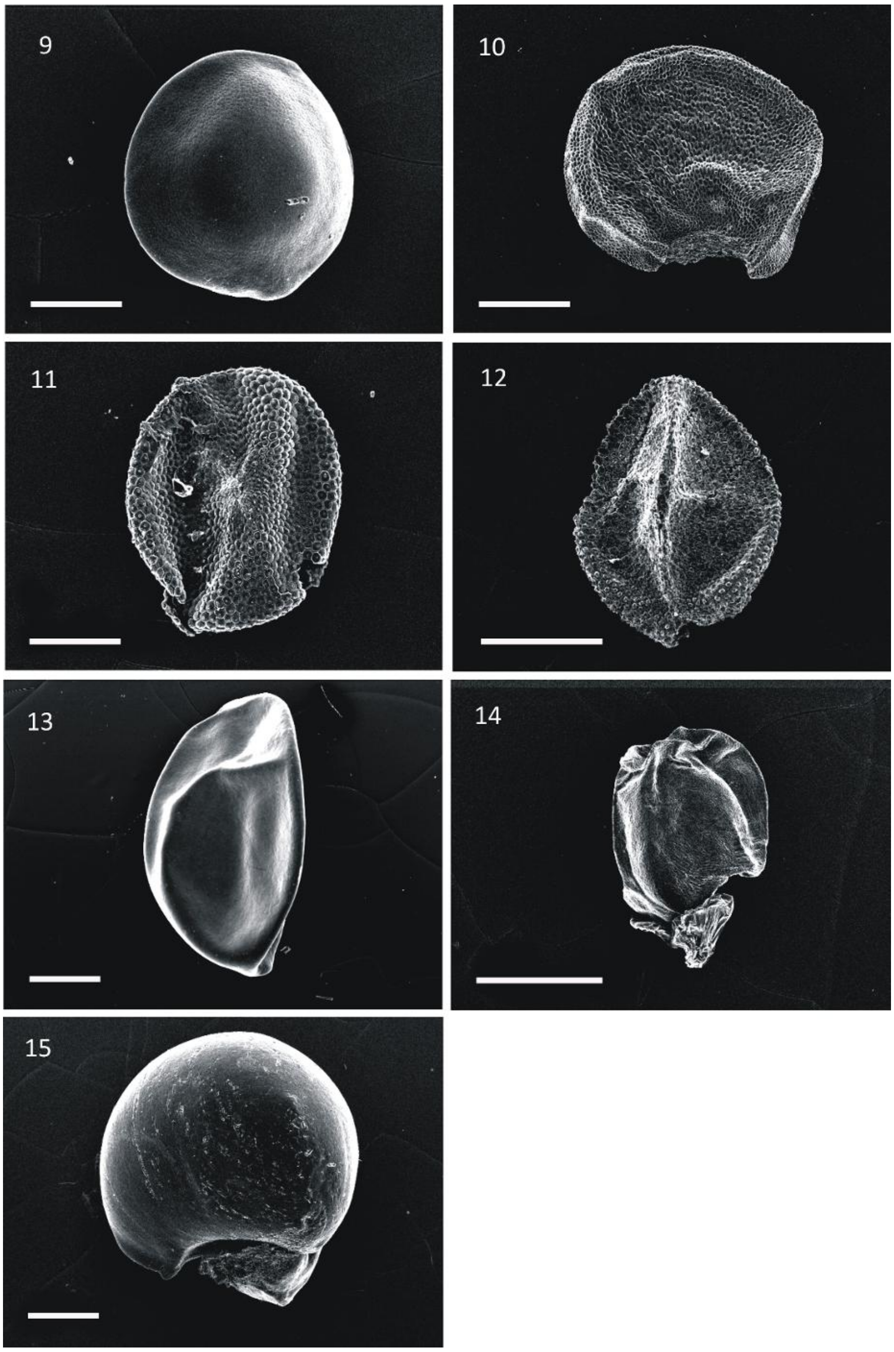
Seed shape

There were four basic seed shapes in *Thysanotus*; orbicular-globose (Figs. 1–2); elliptic-ovoid (Figs. 3–4); elongated (Fig. 5–6); and angular with a median ridge (Fig. 7–8). Elliptic and globose seeds were generally common in *Thysanotus*, while elongated and angular seeds were more restricted. Angular seeds were observed in *T. patersonii* populations from both South Australia and Western Australia; however the South Australian *T. patersonii* specimens all possessed an acute tip (Fig. 7), while the western Australian ones had a blunt tip (Fig. 8). *Thysanotus nudicaulis* and *T. tenuis* also possessed angular seeds. In contrast, elongated seeds were restricted to the 3–staminate species: *T. multiflorus*, *T. triandrus*, *T. pauciflorus* and *T. glaucifolius*.

No typical seed shape defined the Laxmannioideae, but three shapes were noted (Figs. 9–15): *Chamaescilla* and *Trichopetalum* with lenticular/rounded seeds (Figs. 9–10), sometimes flattened due to packing within the capsule (Fig. 10); *Laxmannia, Arthropodium, Sowerbaea, Eustrephus* and *Murchisonia* with angular seeds (Figs. 11–14); and *Cordyline* with globose seeds (Fig. 15).



Figs. 1–8. Scanning electron micrographs showing *Thysanotus* seed shapes. Fig. 1. *T. baueri* (AD96651021), orbicular/globbose. Fig. 2. *T. gracilis* (P1743953), orbicular. Figs. 3–4. *T. juncifolius* (AD96645033, SA form) and *T. wangariensis* (AD96807209) elliptic. Figs 5–6. *T. glaucifolius* (P6265405) and *T. triandrus (*AD96021079*)* elongated with long-stalked appendage. Fig. 7. *T. patersonii* from SA (JGC2119), triangular, apex acute. Fig. 8. *T. patersonii* from WA (JGC2255), triangular, apex blunt. (Scale bars: 1, 2, 4, 5, 6, 7 = 1 mm; 3, 8 = 500 µm).



Figs. 9–15. Scanning electron micrographs of Laxmanniaceae seeds. *Chamaescilla corymbosa* (AD97927233), *Trichopetalum plumosum* (2008: 699), *Laxmannia gracilis* (JGC334)*, Sowerbaea alliacea* (AD98113250), *Eustrephus latifolius* (ADBG880587), *Murchisonia volubilis* (JGC1186)*,* and *Cordyline sp.* (JGC1490). General shapes and arils are shown. (Scale bars: 9, 10, 11 = 500 µm; 12, 13, 14, 15 = 1 mm).

Appendages

Arils were common to all *Thysanotus* species, although there was variation in shape and attachment. Most species possessed sessile arils, but *T. multiflorus*, *T. triandrus*, *T. pauciflorus, T. glaucus* and *T. glaucifolius* possessed arils attached by a relatively long stalk and arils were positioned distantly from the seed (Figs. 5–6). *Thysanotus patersonii, T. manglesianus* and *T. tenellus* possessed relatively flattened arils compared to those of other species. Outside *Thysanotus*, arils were seen only in *Murchisonia, Eustrephus* and *Cordyline*.

Epidermal cell shape

Epidermal cell margin shape varied from polygonal/elongated polygonal shape (Fig. 16) to more or less rounded (Fig**.** 17). Polygonal/elongated-polygonal epidermal cells were the dominant type present in most *Thysanotus* species. In contrast, *T. nudicaulis*, *T. speckii* and *T. tenellus* were the only species observed where the epidermal cells were rounded. Among the other examined Laxmannioideae, rounded epidermal cells were seen only in *Arthropodium*.

Anticlinal boundaries

Anticlinal wall boundaries in *Thysanotus* were raised (Fig. 18) or channelled (Fig. 19). Channelled cell boundaries varied further from shallow to deep channel. Deeply channelled cell boundaries were seen in *T. baueri*, *T. fractiflexus, T. gracilis* and *T. juncifolius* (NSW and SA). Shallow channelled cell boundaries occur in *T. banksii*, *T. chinensis*, *T. glaucifolius*, *T. pauciflorus*, *T. speckii* and *T. wangariensis*. All other examined species displayed raised cell boundaries.

Raised anticlinal wall boundaries (both slightly and strongly) were present in most Laxmannioideae (Figs. 20–21); however, *Laxmannia* and *Sowerbaea* also possessed channelled cell boundaries.

Outer periclinal wall shape

Outer periclinal wall shapes also proved useful for distinguishing different *Thysanotus* taxa. The characteristic cell wall shape in *Thysanotus* was convex, but slightly convex walls were present in *T. baueri* and *T. tenellus* making it a useful character to distinguish them from other *Thysanotus* taxa.

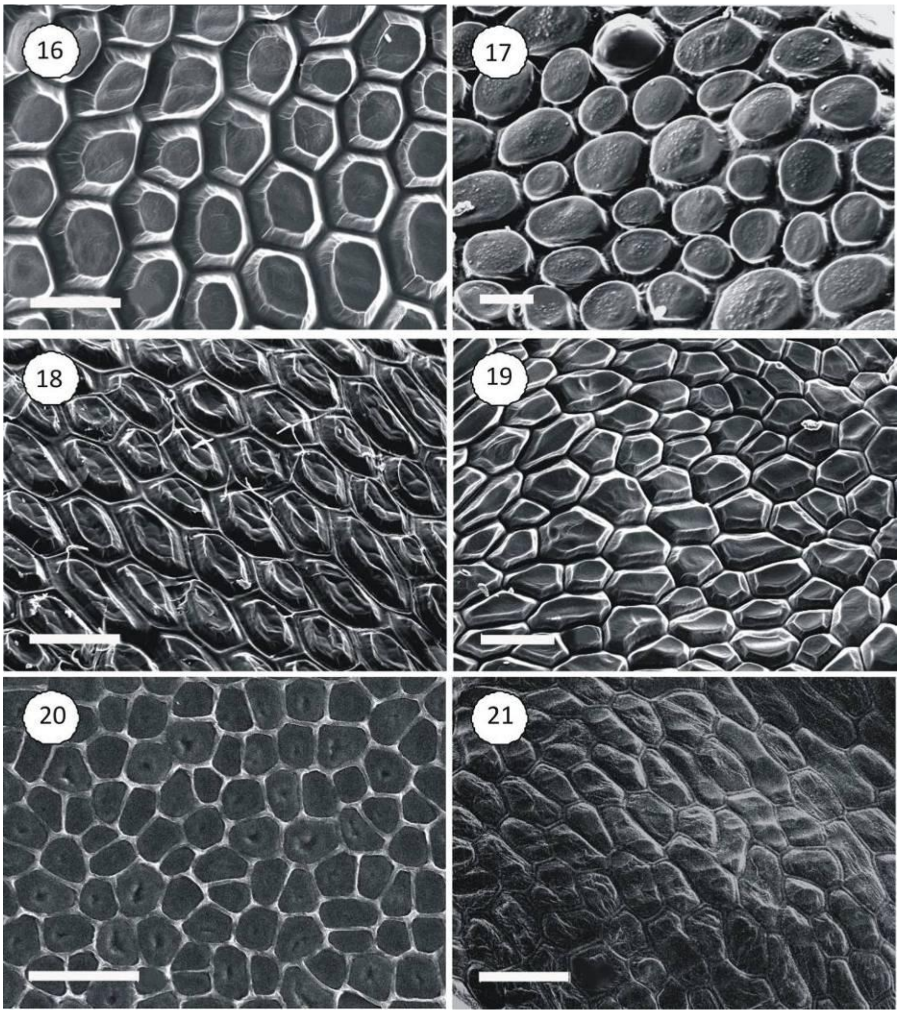
Periclinal wall surface shape was also useful for distinguishing between different taxa and species groups. Many, such as *T. arenarius, T. asper, T. juncifolius*, *T. pauciflorus,* and *T. triandrus* possessed flat periclinal wall surfaces (Figs. 22–23), whereas the group of leafless, annual inflorescence climbers (including *T. patersonii* and *T. manglesianus*) possessed strongly convex, but uneven surfaces (Fig. 24). Rounded periclinal wall surfaces were observed only in *T. fractiflexus* (Fig. 25) distinguishing it from rest of the genus.

Strongly convex periclinal walls were also seen in *Laxmannia, Murchisonia, Sowerbaea* and *Trichopetalum*, while *Eustrephus* and *Chamaescilla* had slightly convex periclinal walls (Figs. 26–27). The periclinal wall surface of *M. volubilis* seeds resembled those of the twining *Thysanotus* species *T. patersonii* and *T. manglesianus* (see Figure 21). The only Laxmannioideae genus with flattened periclinal walls was *Cordyline*, whereas papillate periclinal walls were present only in *Sowerbaea* and *Laxmannia* (Figs. 28–29) and the rest of the Laxmannioideae displayed uneven periclinal wall surfaces.

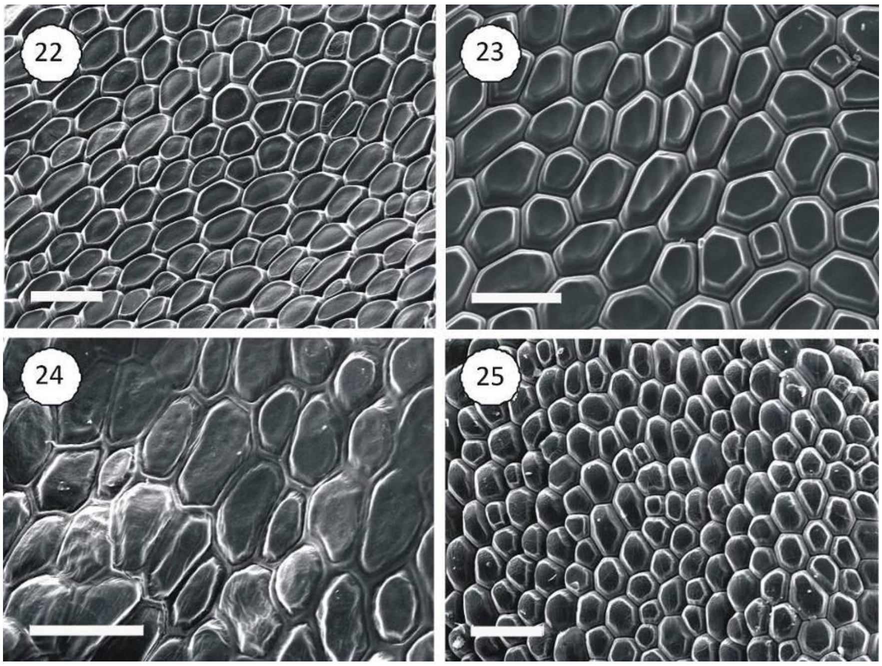
Surface microsculpture

Verrucose and striated surface microsculpturing occurred in some *Thysanotus* species. Verrucose microsculpturing was restricted to *T. baueri* (Fig. 30) and *T. nudicaulis* while striated microsculpturing (Fig. 31) was observed in *T. fractiflexus*, *T. gageoides, T. patersonii, T. rectantherus, T. scaber, T. speckii, T. tenuis* and *T. unicupensis.* All other species sampled possessed smooth periclinal wall surfaces.

Among the other Laxmannioideae, verrucose microsculpturing was observed in *Laxmannia*, striated microsculpturing seen in *Murchisonia*, *Sowerbaea* and *Trichopetalum* with the remainder displaying smooth surfaces.



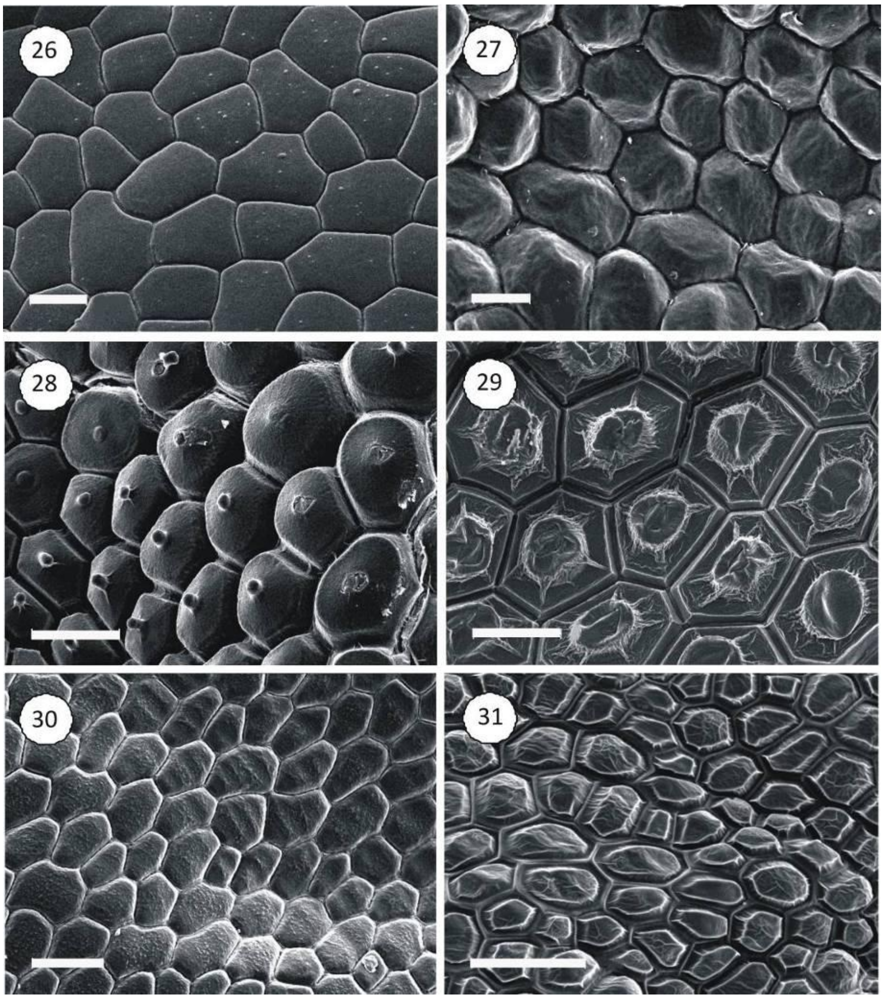
Figs. 16–21. Scanning electron micrographs of *Thysanotus* and Laxmannioideae seed coat morphology. Fig. 16. *T. asper* (P1978365) with polygonal shaped epidermal cells, strongly convex periclinal walls and smooth periclinal wall surfaces. Fig. 17. *T. nudicaulis* (AD96718643) with rounded epidermal cells, strongly convex periclinal walls and verrucose periclinal wall surfaces. Fig. 18. *T. arenarius* (P3016099) with raised anticlinal wall boundaries and smooth periclinal walls. Fig. 19. *T. wangariensis* (AD96807209) with shallow channelled anticlinal wall boundaries. Fig. 20. *Eustrephus latifolius* (ADBG880587) with polygonal shaped epidermal cells, convex periclinal walls and raised anticlinal wall boundaries. Fig. 21. *Murchisonia volubilis* (JGC1186) with polygonal shaped epidermal cells, strongly convex periclinal walls and raised anticlinal wall boundaries. (Scale bars: 16, 18, 19, 21 = 50 µm; 17, 20 = 20 µm).



Figs. 22–25. Scanning electron micrographs of *Thysanotus* seed coat morphology. Figs. 22–23. *T. juncifolius* (AD96645033) and *T. pauciflorus* (P2638193) with flat periclinal wall surfaces. Fig. 24. *T. manglesianus* (AD96019053) with uneven periclinal wall surfaces. Fig. 25. *T. fractiflexus* (AD96650163) with rounded periclinal wall surfaces. (Scale bars: 50 µm)

Seed character evolution in Thysanotus

When seed micromorphological characters were plotted on to the molecular tree from Chapter 3 (Fig. 32), *Thysanotus* could not be defined by any seed morphological synapomorphies. However, general seed and aril shapes common for each clade could be recognised, for example, species in Clade 1 possesses elongated seeds with long stalked arils; Clades 2, basal Grade 3 and Clade 3a possess elliptic/globose seeds with sessile arils. Clade 3b possesses elliptic/globose seeds with sessile arils and angular seeds with median ridges and flattened arils (Fig. 33).



Figs. 26–31. Scanning electron micrographs of Laxmanniaceae seed coats. Fig. 26. *Chamaescilla* (AD97927233) seed coat with slightly convex periclinal walls. Fig. 27. *Trichopetalum* seed coat with strongly convex periclinal walls. Figs. 28–29. *Laxmannia* (JGC334) and *Sowerbaea* (AD98113250) seed coats with papillate periclinal walls. Fig. 30. *T. baueri* (AD96651021) with periclinal wall surfaces with verrucose microsculpturing. Fig. 31. *T. rectantherus* (P1978454) with periclinal wall surfaces with striated microsculpturing. (Scale bars: 26, 27 = 20 µm; 28–31 = 50 µm).

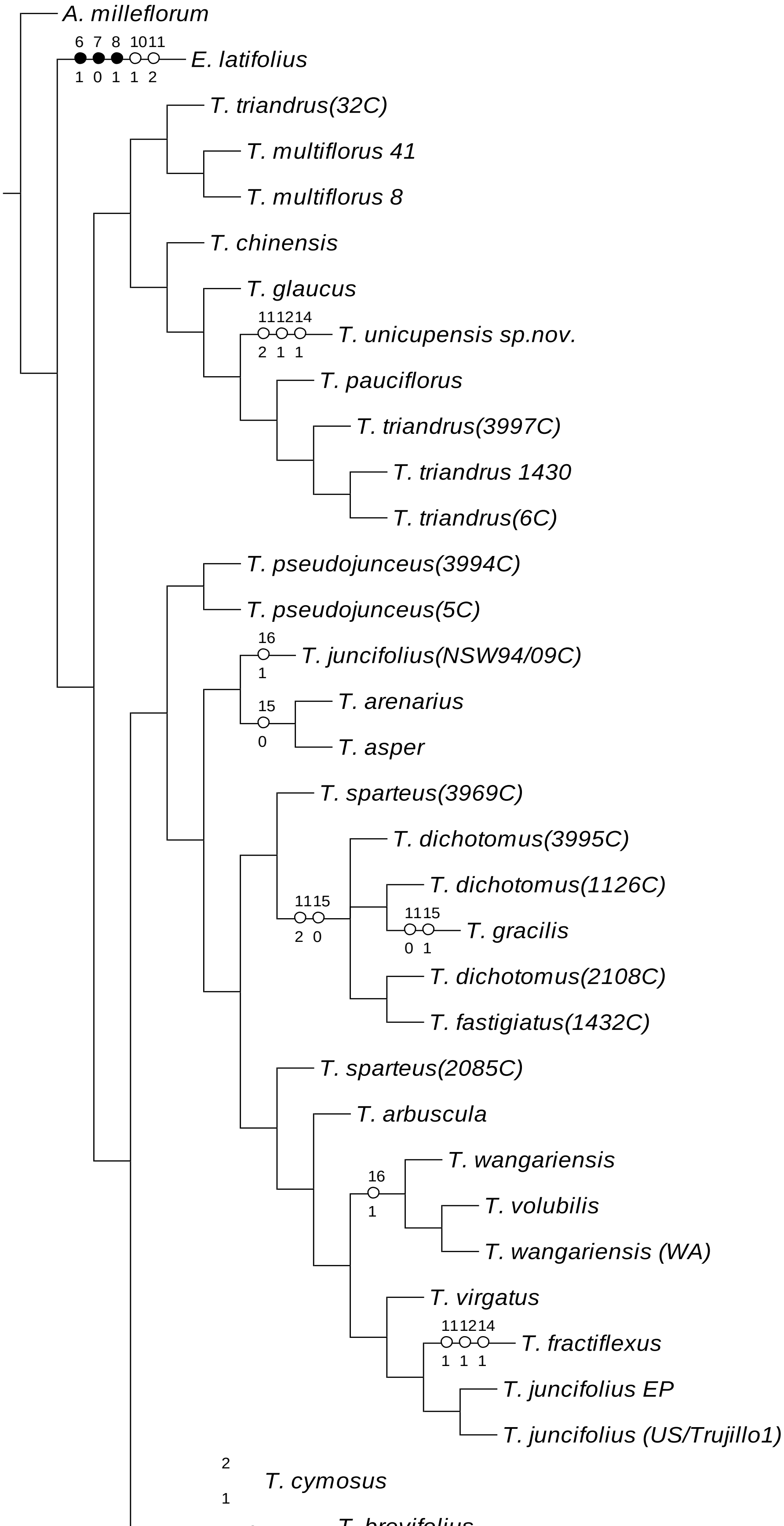


Fig. 32. Seed characters mapped onto the most parsimonious tree obtained from molecular phylogenetic analysis. Open circles indicate homoplasious characters and closed circles indicate synapomorphies.

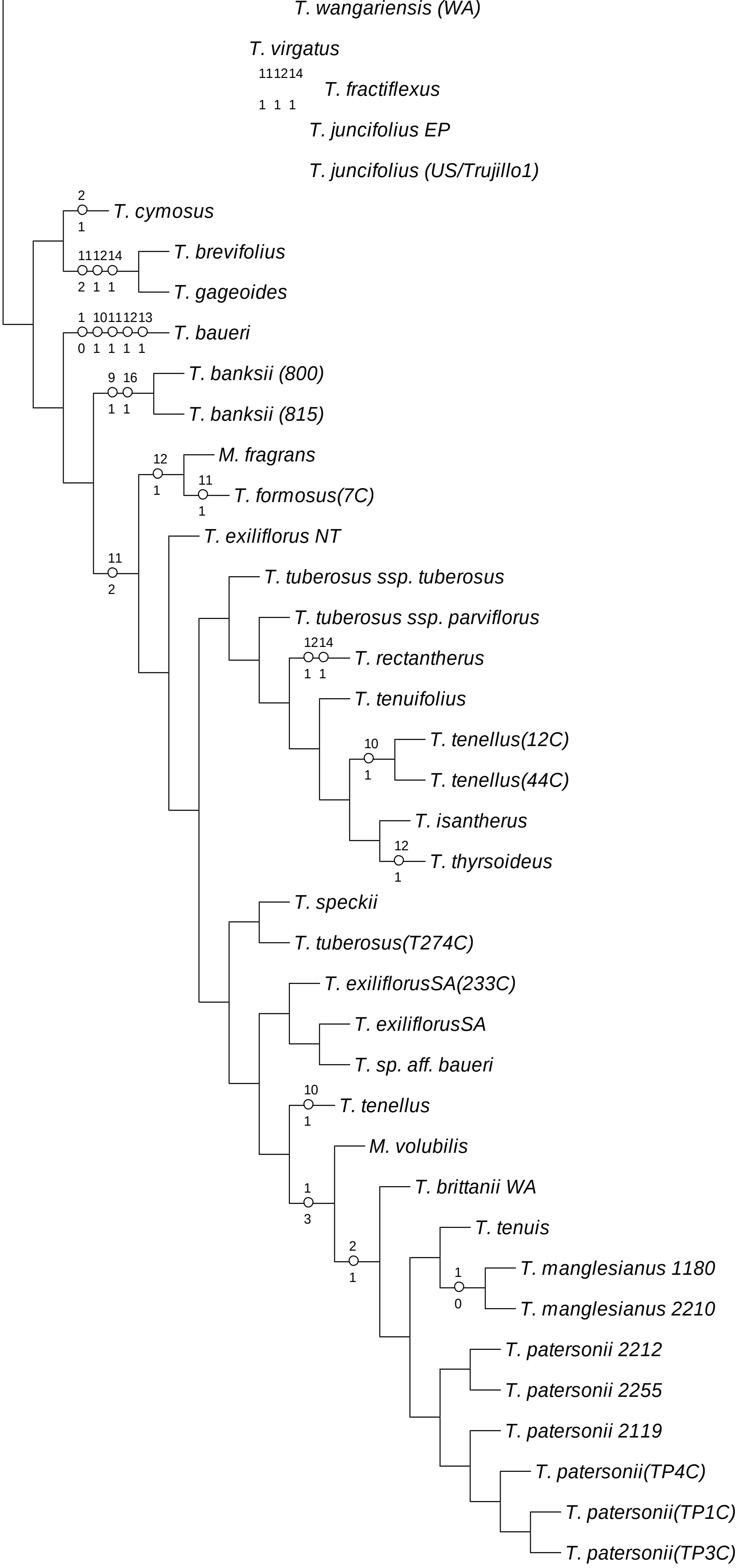


Fig. 32. Seed characters mapped onto the most parsimonious tree obtained from molecular phylogenetic analysis continued.

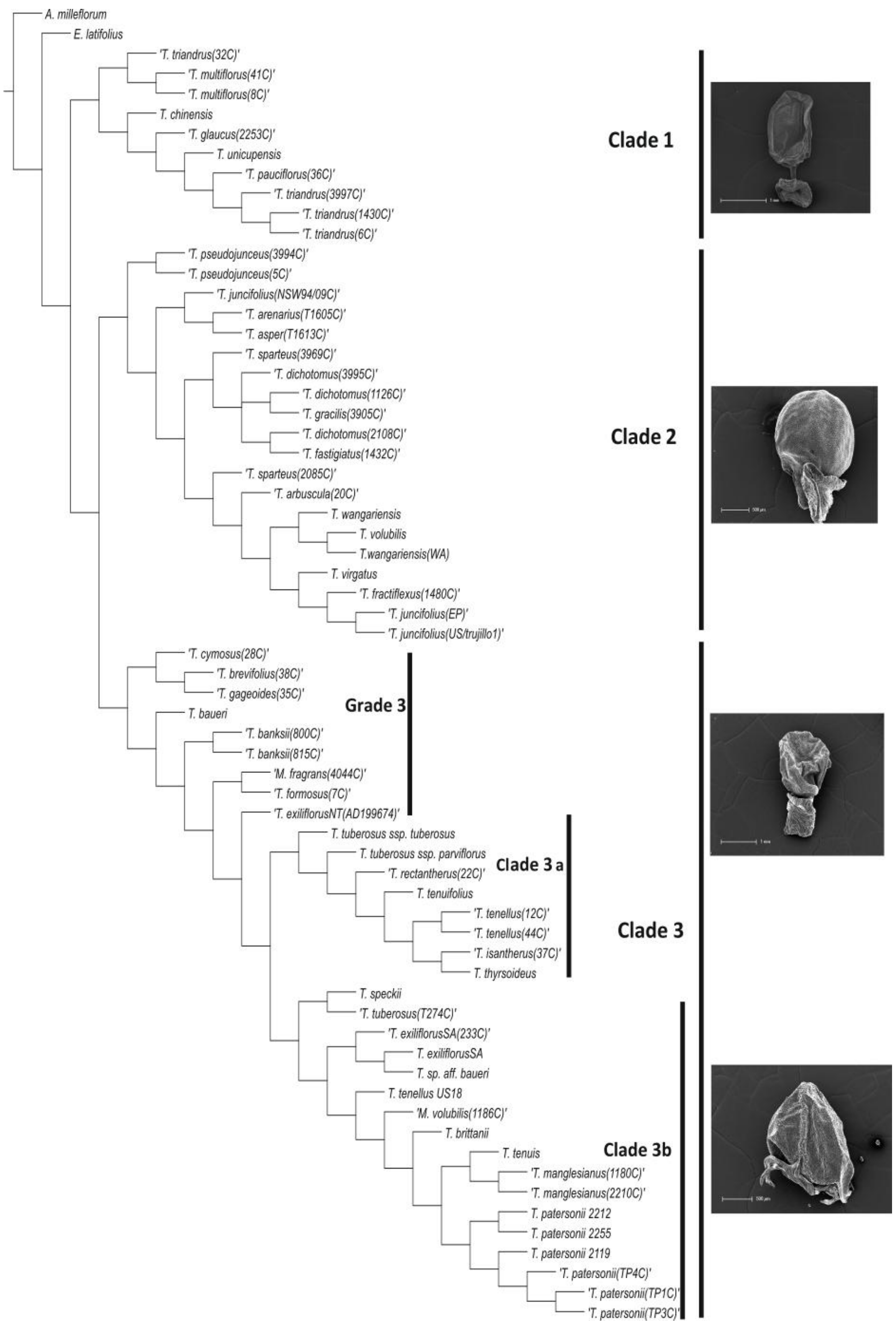


Fig. 33. Major seed shapes in major clades of the most parsimonious tree obtained from molecular phylogenetic analysis are illustrated on to the right of the tree. Species in Clade 1 possesses elongated seeds with long stalked arils; Clades 2, basal Grade 3 and the Clade 3a possess elliptic/globose seeds with sessile arils. Clade 3b possesses elliptic/globose seeds sessile arils and angular seeds with a median ridge and flattened arils.

Discussion

According to Barthlott (1981), differences in epidermal characters of comparable organs reflect genetic differences between the plants concerned. He also mentioned that while many surface characters are of minor taxonomic importance, some characters (e.g. secondary wall thickenings and many characters of the cell boundaries in seed coat) can still be used to characterise groups of related species, genera or other taxonomic categories, up to the family level.

Arils were observed in all *Thysanotus* and *Murchisonia* taxa, as well as *Eustrephus,* but were absent from the other outgroup taxon *Arthropodium*. However, *Eustrephus* was distinctive, with synapomorphic characters such as the presence of a shiny seed coat (6/1), smooth surface appearance/texture (7/0) and relatively large (>2 mm long) seeds (8/1).

Seed shape was highly variable in *Thysanotus*. Although shape can be affected by the number of seeds and how seeds are packed within the capsule (Ness 1989; Hassan *et al.* 2005), angular seeds (1/3) were unique to most of the annual climbing *Thysanotus* species (except *T. manglesianus*) while elongated seeds with long stalked arils were restricted to all 3–staminate *Thysanotus* species. In contrast, orbicular/globose seeds (1/0) were homoplasious, occurring in *T. baueri* and *T. manglesianus*.

Thysanotus and the outgroup Laxmannioideae all share convex periclinal wall surfaces but these varied from slightly to strongly convex (10). Slightly convex periclinal walls (10/1) were homoplasious in *Thysanotus* seen in *T. baueri* and *T. tenellus*. Although strongly convex periclinal walls were noted in the outgroup *Arthropodium milleflorum* (10/0) during this study, Stringer and Conran (1991) reported that the periclinal walls of *Arthropodium* species usually range from slightly convex through to raised papillae. Therefore, it is difficult to comment on the phylogenetic significance of this character solely based on this study.

The character, convex periclinal wall surface shape (11) has changed several times in the tree and was homoplasious. The outgroups *A. milleflorum* and *E. latifolius* possess flat or uneven periclinal wall surfaces, respectively. Rounded periclinal wall surfaces (11/1) also seem to have originated independently in species such as *T. fractiflexus, T. baueri* and *T. formosus* and therefore can be considered a parallelism. Uneven periclinal wall surfaces (11/2) occurred in *Eustrephus* and at several places within *Thysanotus* is therefore also homoplasious. All the annual climbing species including *Murchisonia* and several of the more proximal non-climbing species in Clade 3 also all shared this feature.

Variable seed characters such as cell surface microsculpturing (12), verrucose microsculpturing (13), striated microsculpturing (14), anticlinal cell wall boundary (15) and shallowly channelled anticlinal wall boundaries (16) were homoplasious when mapped onto the tree. Many characters of cell wall boundaries in seed coats are known to be important in characterising groups of related species, genera or taxonomic categories up to family level (Barthlott 1981, 1984).

Thysanotus multiflorus, T. triandrus, T. glaucus and *T. pauciflorus* are all 3-staminate with simple scapes and formed a distinct clade in the molecular tree in Chapter 4 (Sirisena 2010) with two 6-staminate species with simple scapes (*T. chinensis* and *T. unicupensis*). Elongated seed shape (1/2) and the aril attached with a long stalk positioning it distant from the seed (4/1) were restricted to all the 3–staminate species and could perhaps be synapomorphic seed conditions for 3–staminate species. Although *T. chinensis* and *T. unicupensis* were embedded within the 3–staminate *Thysanotus* species, the seeds of these species were globose or elliptic with sessile arils indicating that they represent parallelisms or reversals for conditions seen elsewhere in the genus.

SEM characters of *T. multiflorus* and *T. triandrus* seeds revealed that no seed character distinguish them separately in the cladogram supporting the close affinity between the two species indicated by the molecular tree. Since both species are closely related and polymorphic (Brittan 1981), it is difficult to comment on the taxonomic value of the seed characters for this species complex without further, more detailed, multi-character studies.

Individual seed morphological differences were prominent in *Thysanotus* taxa, and some species or species groups possessed distinct seed features which could be utilised in morphological keys. For example, *T. baueri* had verrucose seed surfaces; angular seeds with median ridge occurred in annual climbing species; and long stalked arils were present in the 3-staminate species.

Thysanotus and Laxmannioideae

All studied Laxmannioideae including *Thysanotus* shared many common seed morphological features and some groups (e.g. *Murchisonia* vs *Thysanotus*) possessed relatively large number of common seed characteristics. *Thysanotus* possessed arils and shared this feature with *Murchisonia* and *Cordyline* however, polygonal (sometimes rounded) epidermal cells shape, and convex periclinal walls were common to *Thysanotus* and all other Laxmannioideae, except *Cordyline*. *Thysanotus* mostly possessed raised anticlinal wall boundaries and rarely channelled boundaries. The seed size was conspicuously larger in *Eustrephus* and *Cordyline* (>2 mm long) while all other Laxmannioideae had smaller seeds (<2 mm long).

The close resemblance of *Murchisonia* and *Thysanotus* seeds and molecular results (Sirisena 2010) may lead to the reconsideration of *M. volubilis* as a *Thysanotus*, though not as *T. patersonii* var. *exfimbriatum* where it had been placed initially by Black (1943), as well as the transfer of *M. fragrans* to *Thysanotus* though separate from *M. volubilis*. The leafless, climbing annual-stemmed *Thysanotus* and *Murchisonia* both possess arils, elliptic–angular seeds, polygonal epidermal cells with convex periclinal walls and raised anticlinal wall boundaries indicating their closer relationship.

Although *Thysanotus* and *Eustrephus* share fimbriate inner tepals, the two genera did not share many common seed features to support a close relationship. When seed characters were mapped on to the molecular tree, *Eustrephus* possessed unique synapomorphies (6/1, 7/0, 8/1) which distinguished it from *Thysanotus* as well as the other outgroup taxon *Arthropodium*. The seeds of *Eustrephus* possess a shiny seed surface in naked eye (6/1), a smooth surface appearance/texture (7/0) and obviously a larger seed size which is >2 mm long which were absent from *Thysanotus*, *Arthropodium* and *Murchisonia* in the tree. Angular seed shape however was common to some species of *Thysanotus*, *Murchisonia* and *Arthropodium*.

A close relationship between *Sowerbaea* and *Laxmannia* has been recognised previously by Kheighery (1984), Thongpukdee (1989) and Conran

(1998) due to the possession of an unusual ligule like structure above the leaf sheath, and similar inflorescence and chromosome characteristics. Seed features in these two genera were also similar supporting a close relationship. The seeds were elliptic–angular with polygonal epidermal cells, convex and papillate periclinal walls and channelled anticlinal wall boundaries and they were the only Laxmannioideae that possessed papillate periclinal walls.

Seed morphology study of *Thysanotus* and other Laxmannioideae also give some indication of seed character evolution in Laxmannioideae and *Thysanotus* i.e. which seed feature are plesiomorphic or apomorphic; however, further studies including more seed characters and wider sampling species of non-*Thysanotus* Laxmannioideae are recommended.

Acknowledgments

The research was supported by funding from a Conservation Biology Grant from the Nature Conservation Society of South Australia, as well as student support from the The School of Earth and Environmental Sciences, The University of Adelaide. The School of Earth and Environmental Sciences and the Adelaide and Western Australian Herbaria are thanked for the provision of facilities to undertake part of the research.

References

APG III (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* **161**, 105–121.

Barthlott, W (1981) Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nordic Journal of Botany* **1**, 345–355.

Barthlott, W (1984) Microstructural features of seed surface. In 'Current concepts in plant taxonomy.' (Eds VH Heywood, DM Moore.) pp. 95–105. (Academic Press: London)

Black, JM (1943) Cyatheaceae-Orchidaceae. In 'Flora of South Australia.' (South Australian Government Printer: Adelaide)

Boesewinkel, FD (1989) Ovule and seed development in Droseraceae. *Acta Botanica Neerlandica* **38**, 295–311.

Brittan, NH (1971) *Murchisonia* Brittan, a new monotypic genus of Liliaceae from Western Australia. *Journal of the Royal Society of Western Australia* **54**, 95–98.

Brittan, NH (1981) The revision of the genus *Thysanotus* R.Br. (Liliaceae). *Brunonia* **4**, 67–181.

Brittan, NH (1987) *Thysanotus* R.Br. In 'Flora of Australia.' (Ed. AS George.) Vol. 45 pp. 345–348. (Australian Government Publishing Service: Canberra)

Chase, MW, Duvall, MR, Hillis, HG, Conran, JG, Cox, AV, Eguiarte, LE, Hartwell, J, Fay, MF, Chaddick, LR, Cameron, KM, Hoot, S (1995) Molecular phylogenetics of Lilianae. In 'Monocotyledons: systematics and evolution.' (Eds PJ Rudall, P Cribb, DF Cutler, CJ Humphries.) pp. 109–137. (Royal Botanic Gardens Kew)

Chase, MW, Rudall, PJ, Conran, JG (1996) New circumscriptions and a new family of asparagoid lilies: genera formerly included in Anthericaceae. *Kew Bulletin* **51**, 667–680.

Conran, JG (1998) Lomandraceae. In 'The families and genera of vascular plants. Vol. 3. Flowering plants. Monocotyledons: Lilianae (except Orchidaceae).' (Ed. K Kubitzki.) pp. 354–365. (Springer Verlag: Berlin)

Conran, JG, Lowrie, A, Moyle-Croft, J (2002) A revision of *Byblis* (Byblidaceae) in south-western Australia. *Nuytsia* **15**, 11–20.

Dahlgren, RMT, Clifford, HT (1982) 'The monocotyledons: a comparative study.' (Springer Verlag: Berlin)

Degtjareva, G, Casper, J, Hellwig, F, Sokoloff, D (2004) Seed morphology in the genus *Pinguicula* (Lentibulariaceae) and its relation to taxonomy and phylogeny. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **125**, 431–452.

Dwyer, TP (1983) Seed structure in carnivorous plants. *Carnivorous Plant Newsletter* **12**, 8–22; 33–38; 45–50.

Fay, MF, Rudall, PJ, Sullivan, S, Stobart, KL, de Bruijn, AY, Reeves, G, Qamaruz-Zaman, F, Hong, W-P, Joseph, J, Hahn, WJ, Conran, JG, Chase, MW (2000) Phylogenetic studies of Asparagales based on four plastid DNA regions. In 'Monocots: systematics and evolution.' (Eds KL Wilson, DA Morrison.) pp. 360–370. (CSIRO: Melbourne)

Hassan, NMS, Meve, U, Leide-Schumann, S (2005) Seed coat morphology of Aizoaceae–Sesuvioideae, Gisekiaceae and Molluginaceae and its systematic significance. *Botanical Journal of the Linnean Society* **148**, 189–206.

Heenan, PB, Mitchell, AD, de Lange, PJ (2004) *Arthropodium bifurcatum* (Lomandraceae), a new species from northern New Zealand. *New Zealand Journal of Botany* **42**, 233–246.

Henderson, RJF (1987) Liliaceae, 13. *Dianella*. In 'Flora of Australia.' (Ed. AS George.) Vol. 45 pp. 194–225, 434–437, 476–485. (Australian Government Publishing Service: Canberra)

Heywood, VH (1969) Scanning electron microscopy in the study of plant material. *Micron* **1**, 1–14.

Huber, H (1969) Die Samenmerkmale und Verwandschaftsverhältnisse der Liliiflorae. *Mitteilungen botanisches der Staatsammel München* **8**, 219–538.

Kheighery, GJ (1984) Chromosome numbers of Australian Liliaceae. *Feddes Report* **95**, 523–532.

Mabberley, DJ (2008) 'Mabberley's plant-book. A portable dictionary of plants, their classifications, and uses. 3rd edition.' (Cambridge University Press: Cambridge)

Ness, BD (1989) Seed morphology and taxonomic relationships in *Calochortus* (Liliaceae). *Systematic Botany* **14**, 495–505.

Nixon, KC (2002) 'WinClada version 1.00.08.' (Nixon, K.C: Ithaca)

Rudall, P, Chase, MW (1996) Systematics of Xanthorrhoeaceae *sensu lato*: evidence for polyphyly. *Telopea* **6**, 629–647.

Sirisena, UM (2010) Systematic studies on *Thysanotus* R.Br. (Asparagales:Laxmanniaceae). Ph.D thesis, University of Adelaide, Adelaide.

Stringer, S, Conran, JG (1991) Stamen and seed cuticle morphology in some *Arthropodium* and *Dichopogon* species (Anthericaceae). *Australian Journal of Botany* **39**, 129–135.

Taylor, P (1989) 'The genus *Utricularia* – a taxonomic monograph: Kew Bulletin additional series XIV.' (HMSO: London)

Thongpukdee, A (1989) The taxonomic position of the genus *Tricoryne* R.Br. Ph.D. thesis, University of Queensland, St. Lucia.

Thorne, R, Reveal, J (2007) An updated classification of the class Magnoliopsida ("Angiospermae"). *The Botanical Review* **73**, 67–181.

The systematic significance of aerial stem anatomy in *Thysanotus* R.Br. (Asparagales: Laxmanniaceae)

Udani M. Sirisena[A], *John G. Conran*[A] *and Terry D. Macfarlane*[B]

[A]Australian Centre for Evolutionary Biology and Biodiversity, Discipline of Ecology and Evolutionary Biology, School of Earth and Environmental Sciences, DX 650 312, The University of Adelaide, SA 5005, Australia
[B]Western Australian Herbarium, Manjimup Research Centre, Department of Environment and Conservation, Locked Bag 2, Manjimup, Western Australia 6258, Australia

Statement of contribution

Ms. Udani M. Sirisena carried out the field collections, experimental work, data analysis and preparation of the manuscript. Dr. John Conran collected specimens and helped in data analysis and manuscript preparation. Dr. Terry Macfarlane collected specimens and helped in manuscript preparation.

Udani M. Sirisena (January 2010)

John G. Conran (January 2010)

Terry D. Macfarlane (January 2010)

The systematic significance of aerial stem anatomy in *Thysanotus* R.Br. (Asparagales: Laxmanniaceae)

Abstract. The systematic aerial stem anatomy of *Thysanotus* R.Br. was investigated to determine the phylogenetic significance of anatomical features and if the floral and vegetative variation seen in some species was also reflected in their anatomy. Forty four *Thysanotus* species were examined, including multiple specimens of variable taxa, as well as *Murchisonia volubilis* Brittan and *M. fragrans* Brittan due to controversy over the generic status of *Murchisonia* separate from *Thysanotus*. When anatomical characters were mapped on to a most parsimonious molecular tree, synapomorphies and homoplasies could be recognised and anatomical character evolution could be understood. Although most multi-sampled species were coherent, some showed differences in stem anatomy consistent with previously noted morphological differences. For example, *Thysanotus juncifolius* (Salisb.) J.H.Willis & Court from NSW was distant from SA collections based on anatomical characteristics, suggesting that the latter represents a different species. Stem anatomy of *Murchisonia* was similar to *Thysanotus*, supporting placing it into synonymy.

Introduction

The genus *Thysanotus* R.Br. (Laxmanniaceae) is an Australian native with a high diversity of species concentrated in Western Australia. It includes 49 validly published species (Brittan 1987), one of which, *T. chinensis* Benth., extending the range to the mainland of China, Hong Kong, Thailand, the Philippines, Lesser Sunda Islands, Celebes, Aru islands and New Guinea and a second species (*T. banksii* R.Br.) extending to New Guinea and the islands of the Torres Strait (Brittan 1981). The genus is relatively unstudied, with no detailed systematic analyses (molecular or morphological) to determine species boundaries or phylogenetic relationships (Conran 1998).

One of the major problems associated with *Thysanotus* is that making definitive identifications of some species is problematic using vegetative and floral morphology, due to greater similarity of these features across the genus.

However, characteristics relating to the internal structure of plants have been employed for over 150 years and are useful in both practical identification and determining phylogenetic relationships (Judd *et al.* 2002). There have been numerous previous systematic studies on the general vegetative anatomy of monocotyledons. Cheadle (1942, 1943a, 1943b, 1944, 1953) carried out studies on different vessel types, origins and specialization in monocotyledons. More general investigations also include those of Rudall (1995), Prychid and Rudall *et al.* (1999), Prychid, Rudall and Gregory (2003), Linder and Rudall (2005) and de Menezes *et al.* (2005). There have also been several anatomical studies at the genus and family level, such as Conover (1983) on the vegetative anatomy of the reticulate-veined Liliiflorae, Rudall (1994) on Iridaceae, Rudall *et al.* (1998) on Asteliaceae and Hypoxidaceae, Rudall *et al.* (1999) on structure and systematics of *Hanguana* (Hanguanceae), Sajo and Rudall (1999) on *Xyris* (Xyridaceae), Conran (1999) on *Behnia* (Behniaceae) and Schneider and Carlquist (1998, 2005) and Carlquist and Schneider (1998) on the origins and nature of vessels of several monocot families.

Anatomical evidence can also be useful in separating intraspecific categories. *Thysanotus* contains putative intraspecific variants because it covers a wide geographical range, and therefore there is greater likelihood of the development of intraspecific variation (Brittan 1962). This is mainly because it is likely to include several different ecological conditions together with the possibility of isolation into small populations. For example, a preliminary anatomical study of *T. patersonii* R.Br. (Brittan 1970) reported that there are differences in the stem anatomy between south-eastern and Western Australian morphotypes. Accordingly, the major objectives of this project were:

1. Primarily, to understand the above-ground stem internal tissue arrangement of *Thysanotus* and the phylogenetic significance of aerial stem anatomical characters as there is only a single preliminary study by Brittan (1970).

2. To determine whether the aerial stem anatomy of *Thysanotus* is useful in recognising species group patterns and intraspecific variation (i.e. to find out whether there are differences in the internal tissue arrangement that could merit any taxonomic separation of intraspecific categories).

3. Whether the external morphological variation seen is also reflected in the aerial stem anatomy of *Thysanotus*.

Materials and methods

Fresh materials of the specimens were collected, fixed in 70% FAA (formalin: acetic acid: alcohol) and stored in 70% ethanol. Herbarium material was resolved in boiling water with a drop of detergent. Then the material was hand sectioned simply using a razor blade and stained with 0.05% aqueous Toluidine Blue. The stained sections were mounted in glycerine and observed under the light microscope. The slides were photographed using a digital camera.

The study included *Thysanotus* species as well as *M. volubilis* and *M. fragrans*. The list of examined specimens and their details is given in Table 1.

Character mapping

We used a randomly selected most parsimonious molecular tree for character mapping to understand the evolution of stem anatomical characters in *Thysanotus* (Fig. 27).

Table 1. *Thysanotus* species and specimens used for the present study.

Specimen	Collector/source	Herb.	Accession no
A. milleflorum R.Br.	D.N. Kraehenbuehl	AD	99113194
Eustrephus latifolius R.Br. ex. Ker.Gawl.	John G. Conran	ADU	ADBG880587
M. fragrans Brittan	T. D. Macfarlane	PERTH	4044
M. volubilis Brittan	John G. Conran	ADU	1186
T. anceps Lindl.	F. Lullfitz	PERTH	1041401
T. arbuscula Baker	A. Morrison	PERTH	1089889
T. arenarius Brittan	T. E. H. Aplin	PERTH	1978411
T. arenarius Brittan	T. D. Macfarlane	PERTH	1605
T. asper Lindl.	C. A. Gardner	PERTH	1978381
T. banksii R.Br.	John G. Conran	ADU	800
T. banksii R.Br.	John G. Conran	ADU	815
T. baueri R.Br.	F. J. Badman	AD	99106157
T. brachiatus Brittan	N.H. Brittan	PERTH	3001172
T. brevifolius Brittan	Udani M. Sirisena & T. D. Macfarlane	PERTH	38
T. britanii H.R.White & T.D. Macfarlane	A. Chant	PERTH	6417728
T. chinensis Benth.	W.R. Barker	AD	9792103
T. cymosus Brittan	E.T. Bailey	PERTH	1041878
T. dichotomus (Labill.) R.Br.	John G. Conran	AD	1126
T. dichotomus (Labill.) R.Br	John G. Conran	AD	2108
T. dichotomus (Labill.) R.Br	T. D. Macfarlane	PERTH	3995
T. exiliflorus F.Muell. NT	H. P. Vonow	AD	199674
T. exiliflorus F.Muell. SA	John G. Conran	ADU	2178
T. exiliflorus F.Muell. SA	T. S. Te	AD	233
T. exiliflorus F.Muell. WA	K.F. Kenneally & D.J. Edinger	PERTH	6705286

T. fastigiatus Brittan	John G. Conran	ADU	1432
T. formosus Brittan	Udani M. Sirisena & T. D. Macfarlane	PERTH	7
T. fractiflexus Brittan	G. Jackson	AD	96449063
T. gageoides Diels	Udani M. Sirisena & T. D. Macfarlane	PERTH	35
T. glaucus Endl.	B.J. Keighery & N. Gibson	PERTH	4463501
T. gracilis R.Br.	T. D. Macfarlane	PERTH	3905
T. isantherus R.Br.	N.H. Brittan	PERTH	6238858
T. juncifolius (Salisb.) Willis & Court (NSW)	Dalby et. al.	PERTH	94/09
T. juncifolius (Salisb.) Willis & Court (SA)	Udani M. Sirisena & Cecilia trujillo	ADU	1
T. juncifolius (Salisb.) Willis & Court (EP)	B. Saunders	ADU	*s.n.*
T. manglesianus Kunth.	John G. Conran	ADU	1180
T. manglesianus Kunth.	John G. Conran	ADU	2210
T. multiflorus R.Br.	Udani M. Sirisena	PERTH	41
T. multiflorus R.Br.	Udani M. Sirisena & T. D. Macfarlane	PERTH	8
T. patersonii R.Br.	John G. Conran	ADU	2119
T. patersonii R.Br. WA	John G. Conran	ADU	2212
T. patersonii R.Br. WA	John G. Conran	ADU	2255
T. patersonii R.Br. SA	Udani M. Sirisena	ADU	1
T. patersonii R.Br. SA	Udani M. Sirisena	ADU	3
T. patersonii R.Br. SA	Udani M. Sirisena	ADU	4
T. pauciflorus R.Br.	N. Hoyle	PERTH	1743937
T. pseudojunceus Brittan	T. D. Macfarlane	PERTH	3994
T. pseudojunceus Brittan	Udani M. Sirisena & T. D. Macfarlane	PERTH	5
T. ramulosus Brittan	Peter G. Wilson & R. Rowe	PERTH	6033318
T. rectantherus Brittan	C.A. Gardner	PERTH	1978454
T. sabulosus Brittan	J.S. Beard	PERTH	1053507
T. sp. aff. *baueri*	F.J. Badman	AD	99324113
T. sparteus R.Br.	John G. Conran	ADU	2085
T. sparteus R.Br.	T. D. Macfarlane	PERTH	3969
T. speckii Brittan	A.S. George	PERTH	2638096
T. tenellus Endl. SA form	Udani M. Sirisena	ADU	18
T. tenellus Endl. WA form	Udani M. Sirisena & T. D. Macfarlane	PERTH	12
T. tenellus Endl. WA form	Udani M. Sirisena & T. D. Macfarlane	PERTH	44
T. tenuifolius sp.nov.	Udani M. Sirisena & T. D. Macfarlane	PERTH	11
T. tenuis Lindl.	Udani M. Sirisena & T. D. Macfarlane	PERTH	26
T. teretifolius Brittan	C.A. Gardner	PERTH	3020789
T. thyrsoideus Baker	John G. Conran	ADU	2271A
T. triandrus (Labill.) R.Br.	John G. Conran	ADU	1430
T. triandrus(Labill.) R.Br.	Udani M. Sirisena	ADU	32
T. triandrus (Labill.) R.Br.	T. D. Macfarlane	PERTH	3997
T. triandrus (Labill.) R.Br.	Udani M. Sirisena & T. D. Macfarlane	PERTH	6
T. tuberosus ssp. *parviflorus* (Benth.) Brittan	John G. Conran	ADU	324
T. tuberosus R.Br. ssp. *tuberosus*	John G. Conran	ADU	JGC home garden
T. tuberosus R.Br.	T. S. Te	AD	274
T. unicupensis Sirisena, T.Macfarlane & Conran	Udani M. Sirisena & T. D. Macfarlane	PERTH	13
T. virgatus Brittan	Dalby et. al.	PERTH	94/08
T. volubilis sp.nov.	T. D. Macfarlane	PERTH	
T. wangariensis Brittan	T. S. Te	PERTH	238
T. wangariensis Brittan WA	A. S. George	PERTH	02980568

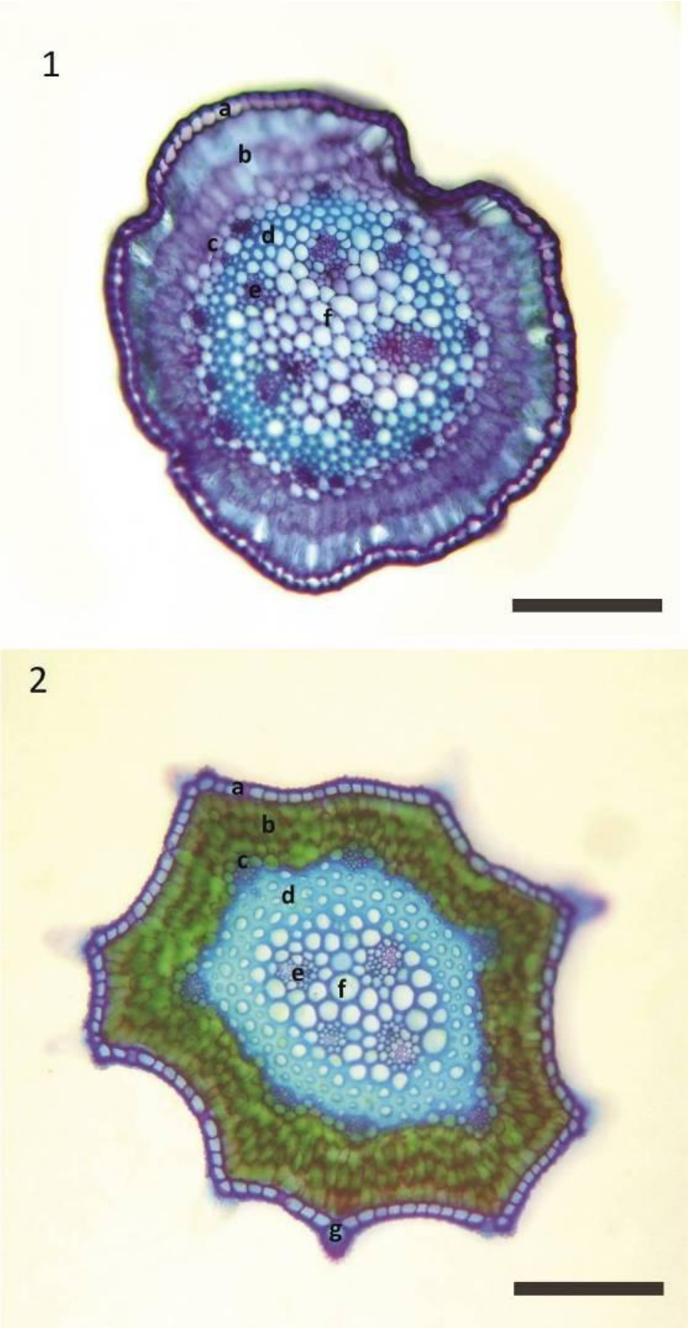
Table 2. List of aerial stem anatomical characters with their respective states

1. Shape of stem in transverse section: terete = 0, elliptic = 1, polygonal = 2, other = 3
2. Cuticle thickness: 0–10 µm = 0, 10.1–20 µm = 1, 20.1–30 µm = 2, >30 µm = 3
3. Projections on surface: absent = 0, present = 1
4. Length of projections: NA = 0, ≤ = 200 µm = 1, >200 µm = 2
5. Epidermal cells h/w ratio: <1 = 0, 1 = 1, >1 = 2
6. Rectangular epidermal cells: absent = 0, present = 1
7. Square epidermal cells: absent = 0, present = 1
8. Columnar epidermal cells: absent = 0, present = 1
9. Irregular shaped epidermal cells: absent = 0, present = 1
10. Chloroplasts in epidermal cells: absent = 0, present = 1
11. Elliptic chlorenchyma: absent = 0, present = 1
12. Globose chlorenchyma: absent = 0, present = 1
13. Elongated chlorenchyma: absent = 0, present = 1
14. Irregular shaped chlorenchyma: absent = 0, present = 1
15. Raphide canals in chlorenchyma: absent = 0, present = 1
16. Raphide canals around sclerenchyma: absent = 0, present = 1
17. Raphide canals in pith: absent = 0, present = 1
18. Shape of xylem: irregular = 0, v-shaped = 1,semicircular = 2, half square = 3, straight = 4
19. Lignified pith: absent = 0, present = 1

Results

The preliminary survey of the stem anatomy for 27 *Thysanotus* species by Brittan (1970) revealed that stem structure was in general uniform; with an outer ring of smaller vascular bundles closely associated with a ring of collenchyma or sclerenchyma and enclosed within a ring of endodermal cells. There was another series of small bundles in an irregular circle occurring towards the centre of the stem and further, larger bundles were located near the centre. Rudall (1995) and Rudall and Cutler (1995) reported the presence of secondary thickening meristems in the underground stem of *T. spiniger* Brittan.

Nevertheless, despite this general tissue arrangement, we noticed differences between various species and some populations in the epidermal cells, chlorenchyma, vascular tissues and pith, and presence/absence of raphides. In general, the above-ground stem anatomy was consistent with Brittan's (1970) description, but with considerable variation among different species.



Figs. 1–2. Transverse stem sections of *T. chinensis* and *T. dichotomus* showing the general stem anatomy and tissue arrangement. Fig. 1. Transverse stem section of *T. chinensis*, with a general tissue arrangement consisted of a single layered epidermis without epidermal hairs (a), and then few layered chlorenchymatous region (b), a ring of parenchyma cells below chlorenchyma (c), adjacently possessing sclerenchyma (d). Vascular bundles (e) occur towards the centre more or less in whorls. Pith is made up of parenchymatous tissue (f). Fig. 2. Transverse stem section of *T. dichotomus*, with a general tissue arrangement possessing lignified pith (f) and epidermal hairs (g) (Scale bars: 300 µm)

Important stem anatomical features in *Thysanotus*

General stem anatomy (Figs. 1 and 2)

The stems examined consisted of a single-layered epidermis (Figs. 1a and 2a), with many species possessing epidermal hairs (Fig. 2g), then a few-layered chlorenchymatous region (Figs. 1b and 2b), a ring of parenchyma cells below chlorenchyma (Figs. 1c and 2c), adjacent to sclerenchyma (Figs. 1d and 2d) or collenchyma layers. Vascular bundles occur towards the centre more or less in whorls (Figs. 1e and 2e). Pith is made up of parenchymatous (Figs. 1f) or lignified tissues (Figs. 2f).

Shape of transverse section

Stem shape in transverse section was variable in *Thysanotus* however, the common most shapes were, terete, elliptic and polygonal (Figs. 3, 4 and 5). An unusual shape was observed in *T. anceps* (Fig. 6). The shape was more or less elliptic with three conspicuous ridges.

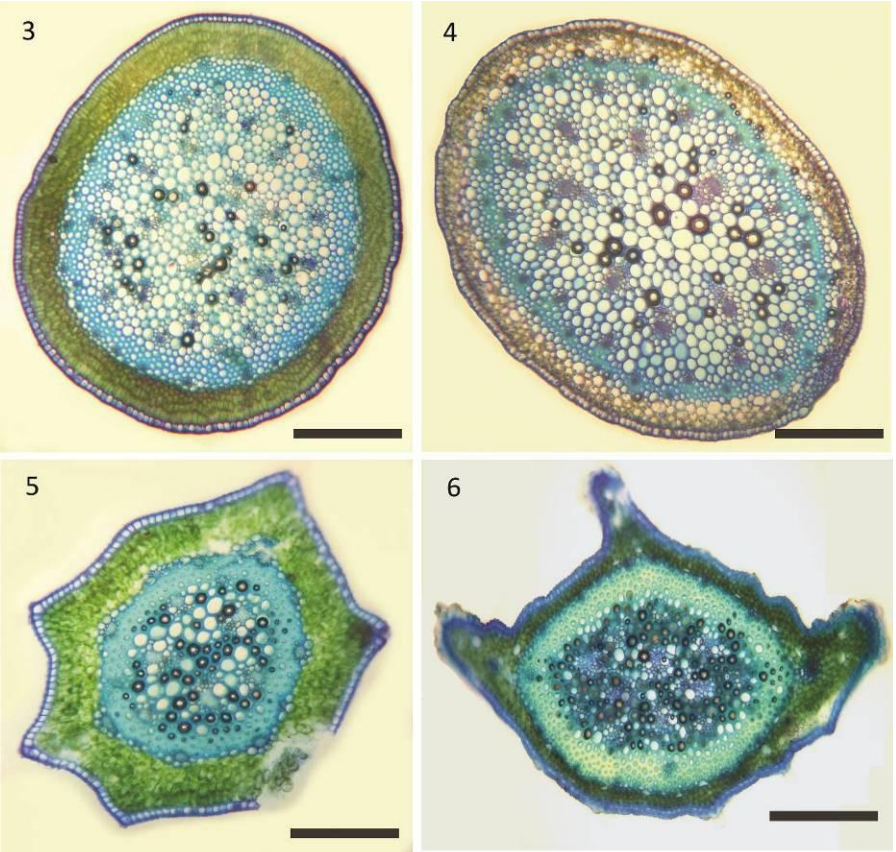
Cuticles and cuticle properties

Relatively thick cuticles were present in almost all *Thysanotus* species, varying from <10 µm to >30 µm. Many species possessed cuticles <10 µm thick, whereas *T. juncifolius* (SA form) ranged from 10–20 µm and *T. sparteus* cuticles were >20 µm thick (Figs. 7 and 8). Even and ferrulate cuticle surfaces were also distributed among the species (Figs. 9 and 10).

Epidermis

The epidermal cells were of variable shapes: rectangular, square, columnar or irregular. Many species possessed rectangular to square epidermal cells (Figs. 11 and 12) while few species possessed columnar (Fig. 13) and irregular shaped (Fig. 14) epidermal cells. Rectangular and square shaped epidermal cells were seen in species such as *T. arenarius, T. baueri, T. dichotomus, T. manglesianus, T. patersonii, T. wangariensis, T. virgatus, T. unicupensis*. Columnar shape was restricted to species such as *T. arbuscula, T. cymosus, T. multiflorus*, *T. sparteus* while irregular shape was observed in relatively few species such as *T. glaucus, T. juncifolius* (NSW)*, T. pauciflorus* and *T. tenellus*.

Chlorophyllous epidermal cells were seen only in NSW form of *T. juncifolius* (Fig. 15) and *T. tenellus*.



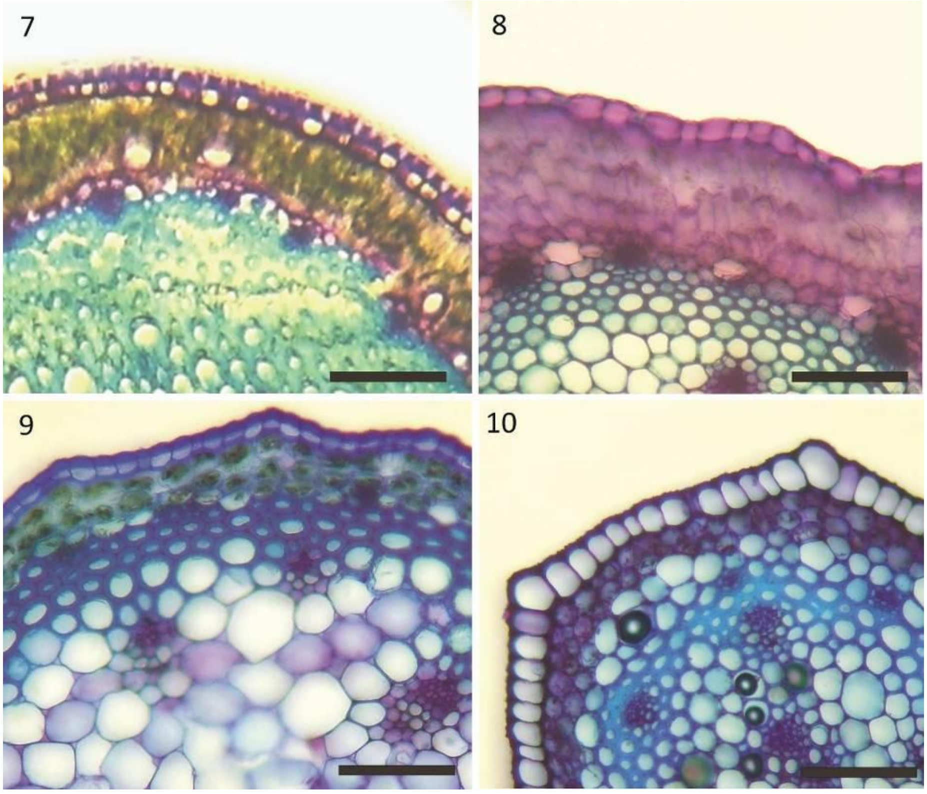
Figs. 3–6. Different shaped transverse stem sections observed in *Thysanotus*. Fig. 3. Terete transverse section of *T. pseudojunceus*. Fig. 4. Elliptic transverse section of *T. triandrus*. Fig. 5. Polygonal transverse section of *T. gracilis*. Fig. 6: Unusual shaped transverse section of *T. anceps*. (Scale bars: 500 µm)

Chlorenchyma

Chlorenchyma was variable in shape, number of layers and presence/ absence of raphide canals. Observed chlorenchyma shapes were elliptical, globose, elongate and irregular; however, the most common shape was elongate.

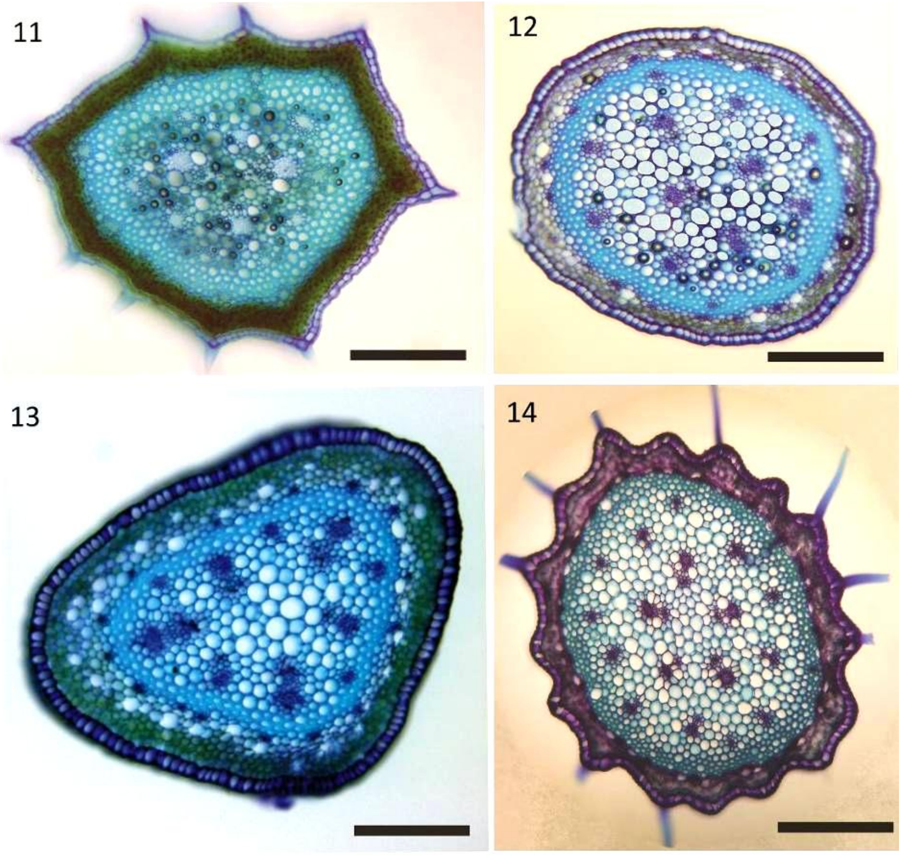
Elongated chlorenchyma (Fig. 16) occurs in *T.* sp. aff. *baueri*, *T. arbuscula*, *T. asper*, *T. brachiatus*, *T. chinensis*, *T. dichotomus*, *T. exiliflorus* (WA and NT forms), *T. fastigiatus, T. fractiflexus, T. juncifolius* (SA form)*, T. manglesianus, T. patersonii, T. pseudojunceus, T. ramulosus, T. sparteus, T. spiniger, T. virgatus* and *T. wangariensis*. Elliptical chlorenchyma (Fig. 17) was present in *T. baueri, T. banksii, T. cymosus, T. exiliflorus* (SA form), *T. formosus, T. isantherus, T. lavanduliflorus, T. parviflorus, T. pauciflorus, T. rectantherus, T. speckii, T. teretifolius, T. triandrus* (6, 1430), *T. tuberosus ssp.*

parviflorus (324) and *T. unicupensis,* while globose chlorenchyma (Fig. 18) were seen in *T. anceps, T. glaucus, T. gracilis, T. tenellus* (WA and SA), *T. tenuifolius* and *T. tuberosus* (274). Irregular chlorenchyma was only observed in *T. arenarius, T. sabulosus* and *T. juncifolius* (NSW form, see Fig. 15). Many species possessed ≤3 chlorenchyma cell layers, while only a few (*T. pseudojunceus*, *T. gracilis* and *T. virgatus*) had thick chlorenchyma with more than four tiers.



Figs.7–10. Epidermal features of *Thysanotus* species with different cuticle properties. Fig. 7. Epidermis of *T. sparteus* with a relatively thick cuticle (> 20.1 µm). Fig. 8. Epidermis of *T. ramulosus* with a thin cuticle (< 10 µm). Fig. 9. Epidermis of *T. parviflorus* with a smooth and even cuticle surfaced. Fig. 10. Epidermis of *T. multiflorus* with a ferrulate cuticle surface. (Scale bars: 200 µm)

Raphide canals were distributed in chlorenchyma in species such as *T. anceps, T. brachiatus, T. exiliflorus* (SA, WA), *T. formosus, T. juncifolius, T. lavanduliflorus, T. manglesianus, T. multiflorus, T. tenellus* (SA)*, T. tenuifolius, T. tuberosus* (274) while others possessed chlorenchyma free of raphides and canals.



Figs.11–14. Epidermises of *Thysanotus* species with different shaped epidermal cells and projections. Fig. 11. *T. fastigiatus* with rectangular epidermal cells. Fig. 12. *T. unicupensis* with square epidermal cells. Fig. 13. *T. tuberosus* with columnar epidermal cells. Fig. 14. *T. asper* with irregular shaped epidermal cells and hairy projections. (Scale bars: 20 µm)

Parenchyma below chlorenchyma

A parenchymatous layer below the chlorenchyma was observed in all *Thysanotus* species except in *T. tenellus* and *T. glaucus*.

Sclerenchyma

Sclerenchyma tissue was common below the chlorenchymatous region in all the species, and was present in a relatively consistent thickness.

Vascular tissues

Vascular bundles are of monocotyledonous type, collateral and surrounded by a parenchymatous bundle sheath. A ring of smaller bundles were distributed adjacent to and below the chlorenchyma, and then another ring occurs in the sclerenchyma layer. One or more rings of relatively larger bundles also occur towards the centre of the pith. The number of central vascular bundles and bundle rings were variable, with central vascular bundle number varying from

<10 to >21 while the number of central vascular bundle rings varied from 1 to >2.

Xylem shape was variable, with irregular, V-shaped, semicircular, half square and straight shapes being noted (Figs. 19, 20, 21, 22). The average number of xylem vessels in a bundle ranged from <4 to >10 among different species.

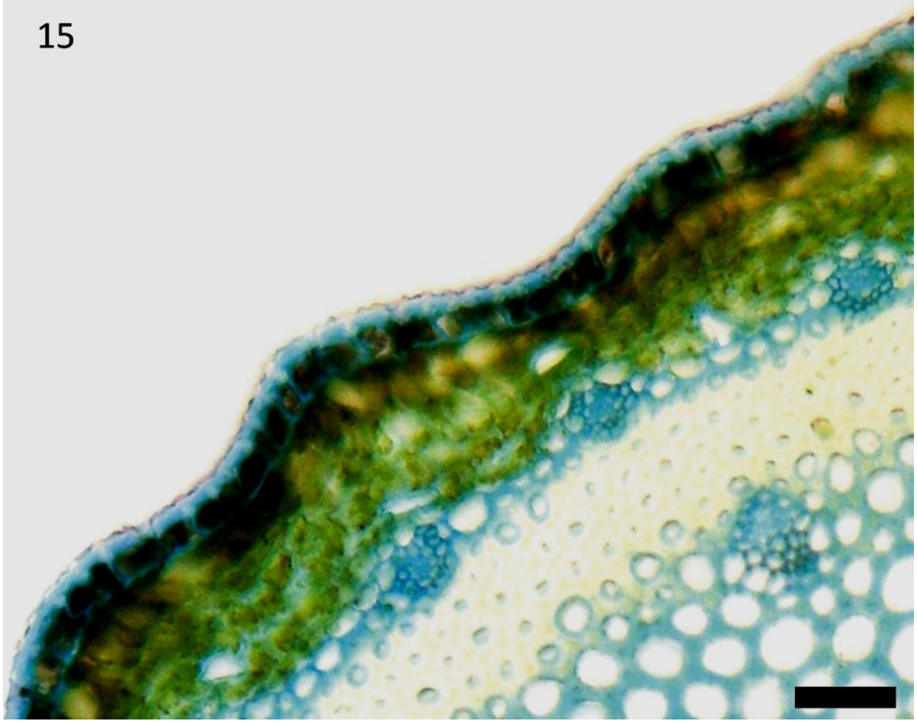


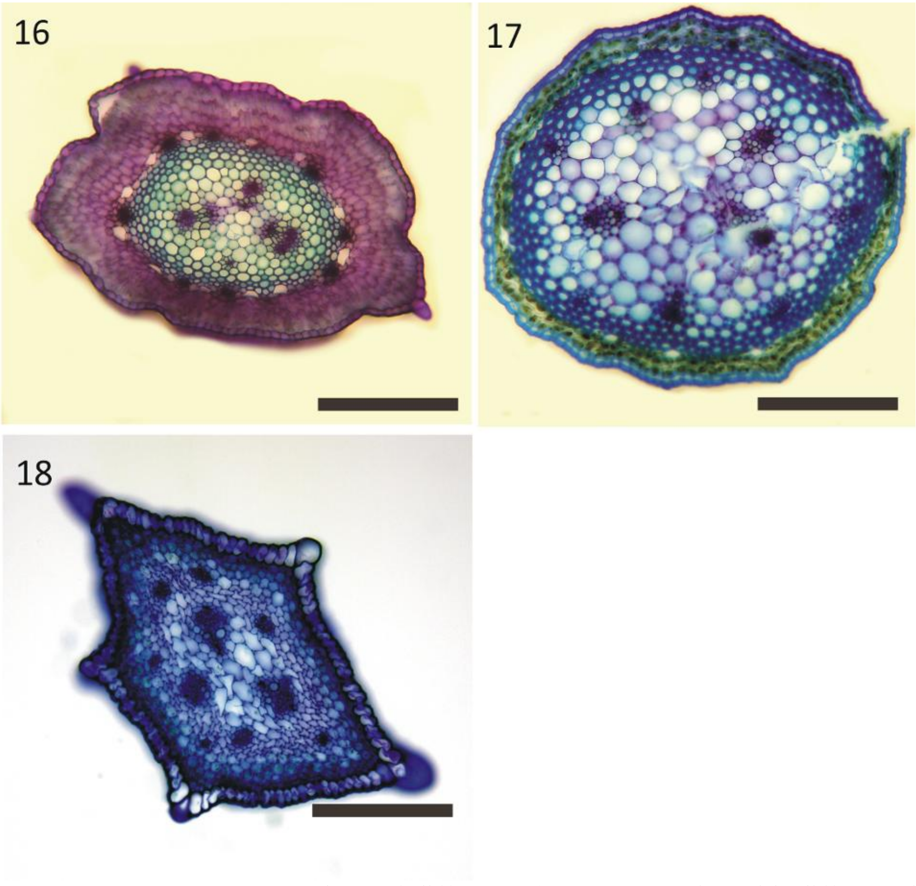
Fig. 15. *T. juncifolius* from NSW with chlorophyllous epidermis and irregular shaped chlorenchyma layers. (Scale bar: 20 µm)

Pith

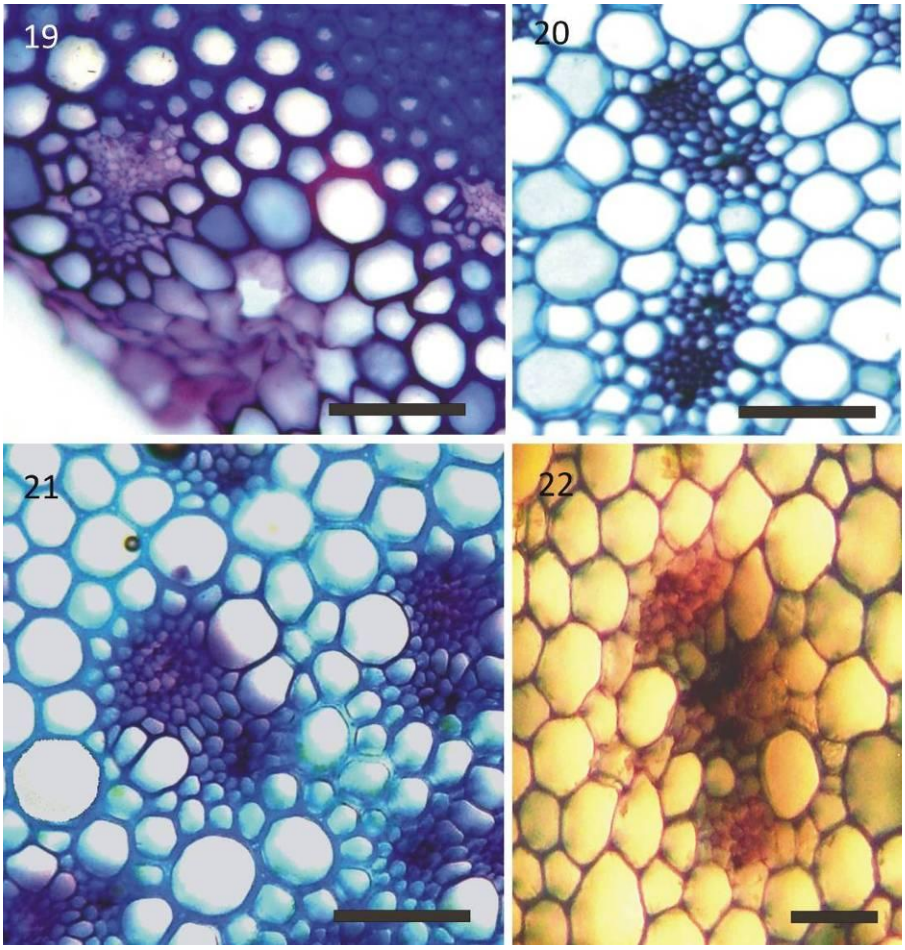
Pith cells were parenchymatous or rarely lignified. Parenchymatous pith was common in most species, while lignified pith was noted only in *T. anceps, T. brachiatus, T. dichotomus, T. fastigiatus, T. fractiflexus, T. juncifolius, T. sabulosus, T. sparteus, T. spiniger* and *T. virgatus* (Fig. 23).

Crystals/raphides

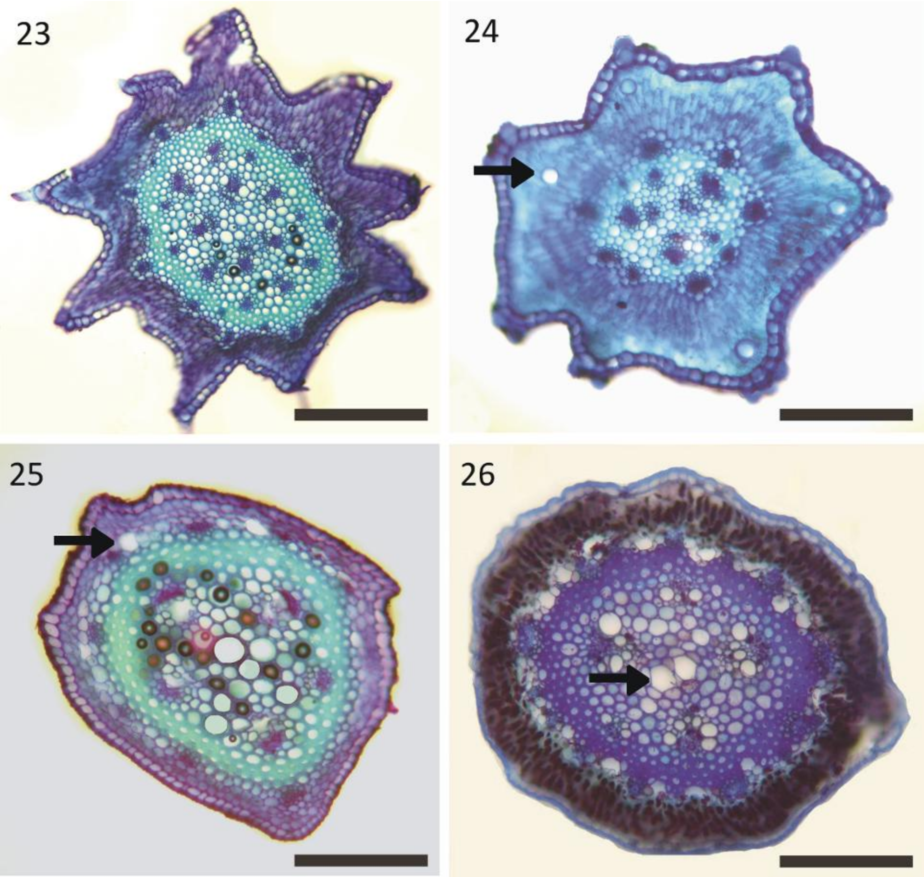
The results indicated that the presence/absence of raphides or raphide canals (Fig. 24) is particularly useful for differentiating between *Thysanotus* taxa. The distribution and systematic importance of calcium oxalate crystals in Dicotyledons and Monocotyledons has been emphasised by many systematists e.g. Prychid and Rudall (1999), Kauff, Rudall and Conran (2000). Brittan (1970) reported inter- and intra-plant raphid size variation in *Thysanotus*, noting that they were present as canals, mostly in the chlorenchyma of the stems and leaves. Similarly, we observed raphides and raphide canals mostly in the chlorenchyma (Fig. 24); below the chlorenchyma and above sclerenchyma (Fig. 25); or, in few species such as *T. exiliflorus,* in the pith (Fig. 26).



Figs.16–18. Transverse stem sections of different *Thysanotus* species with different shaped chlorenchyma. Fig. 16. Transverse stem section of *T. ramulosus* with elongated chlorenchyma. Fig. 17. Transverse stem section of *T. parviflorus* with elliptical chlorenchyma. Fig. 18. Transverse stem section of *T. tenellus* (SA) with globose chlorenchyma. (Scale bars: 300 µm)



Figs.19–22. Different xylem shapes in *Thysanotus*. Fig. 19. V-shaped xylem in *T. banksii*. Fig. 20. Semicircular xylem in *T. wangariensis*. Fig. 21. Half square xylem in *T. sparteus*. Fig. 22. Straight xylem in *T. patersonii* from WA, two adjacent vascular bundles are shown (Scale bars: 50 µm)



Figs.23–26. Lignified pith and raphides in *Thysanotus*. Fig. 23. Lignified pith *T. virgatus*. Fig. 24. Raphide canals in chlorenchyma of *T. brachiatus.* Fig. 25. Raphide canals above sclerenchyma (below chlorenchyma) in *T. isantherus*. Fig. 26. Raphide canals in pith of *T. exiliflorus* from SA. (Scale bars: Figs. 23–26 = 500 µm)

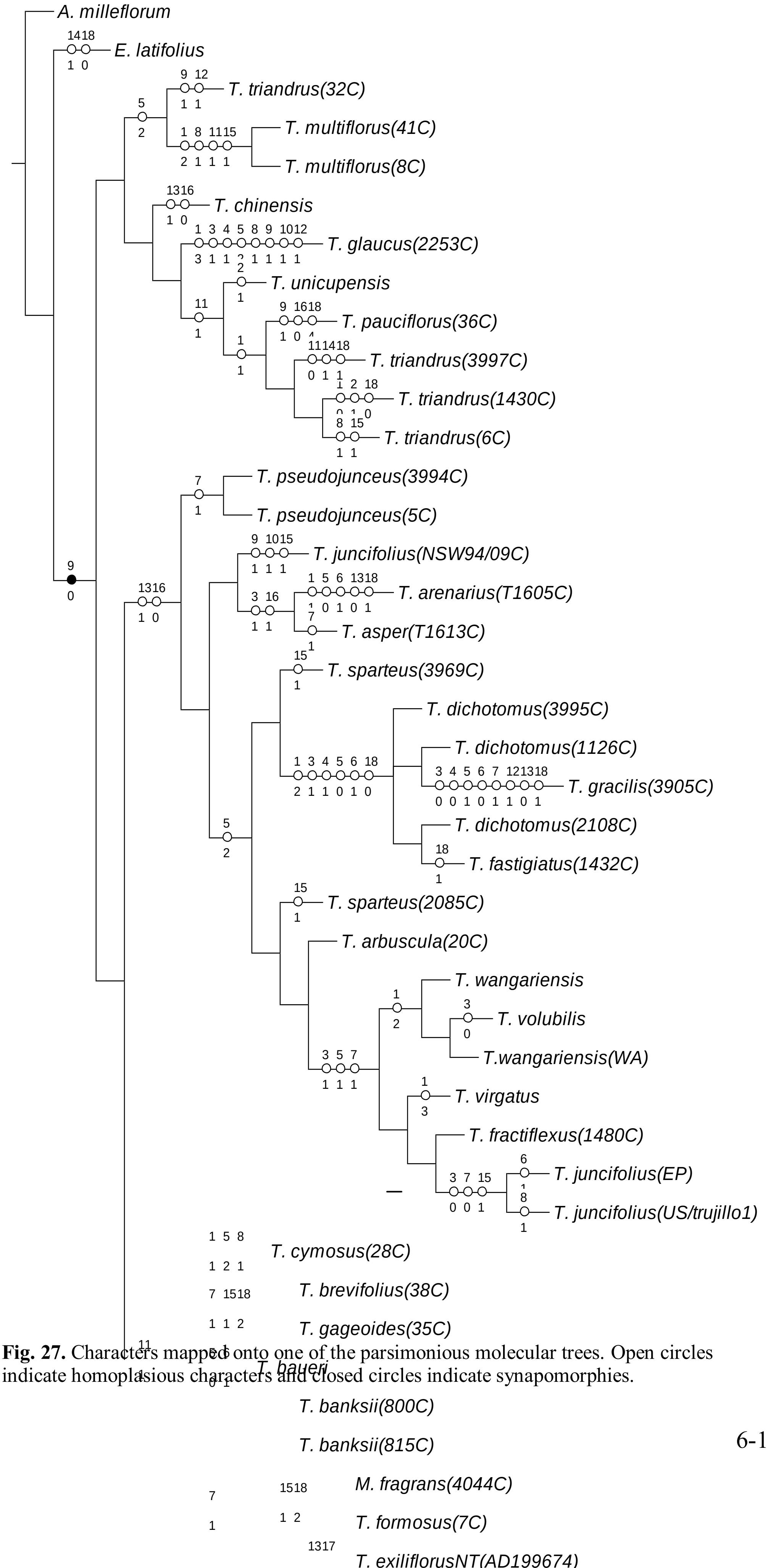


Fig. 27. Characters mapped onto one of the parsimonious molecular trees. Open circles indicate homoplasious characters and closed circles indicate synapomorphies.

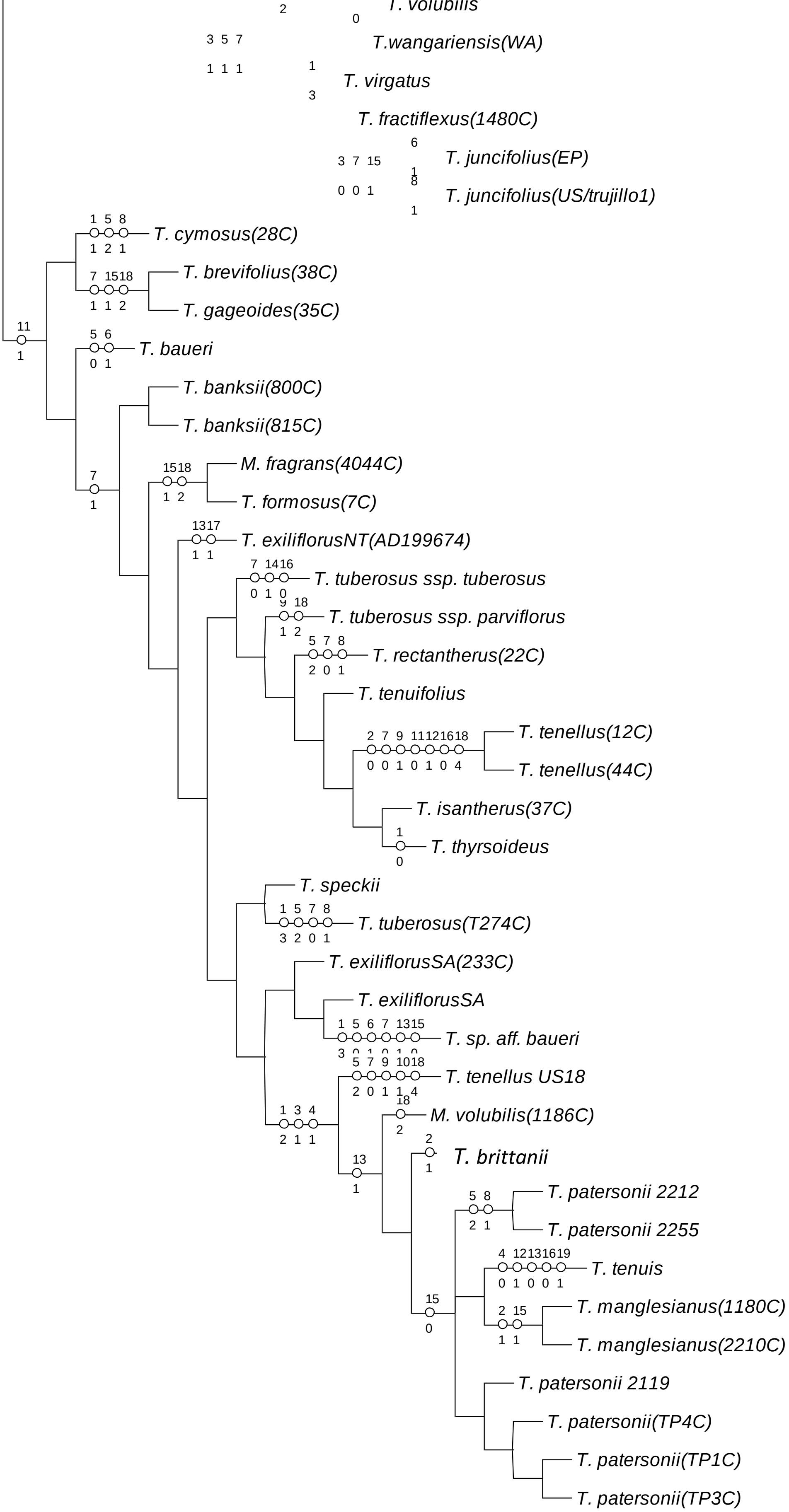


Fig. 27. Characters mapped onto one of the parsimonious molecular trees continued.

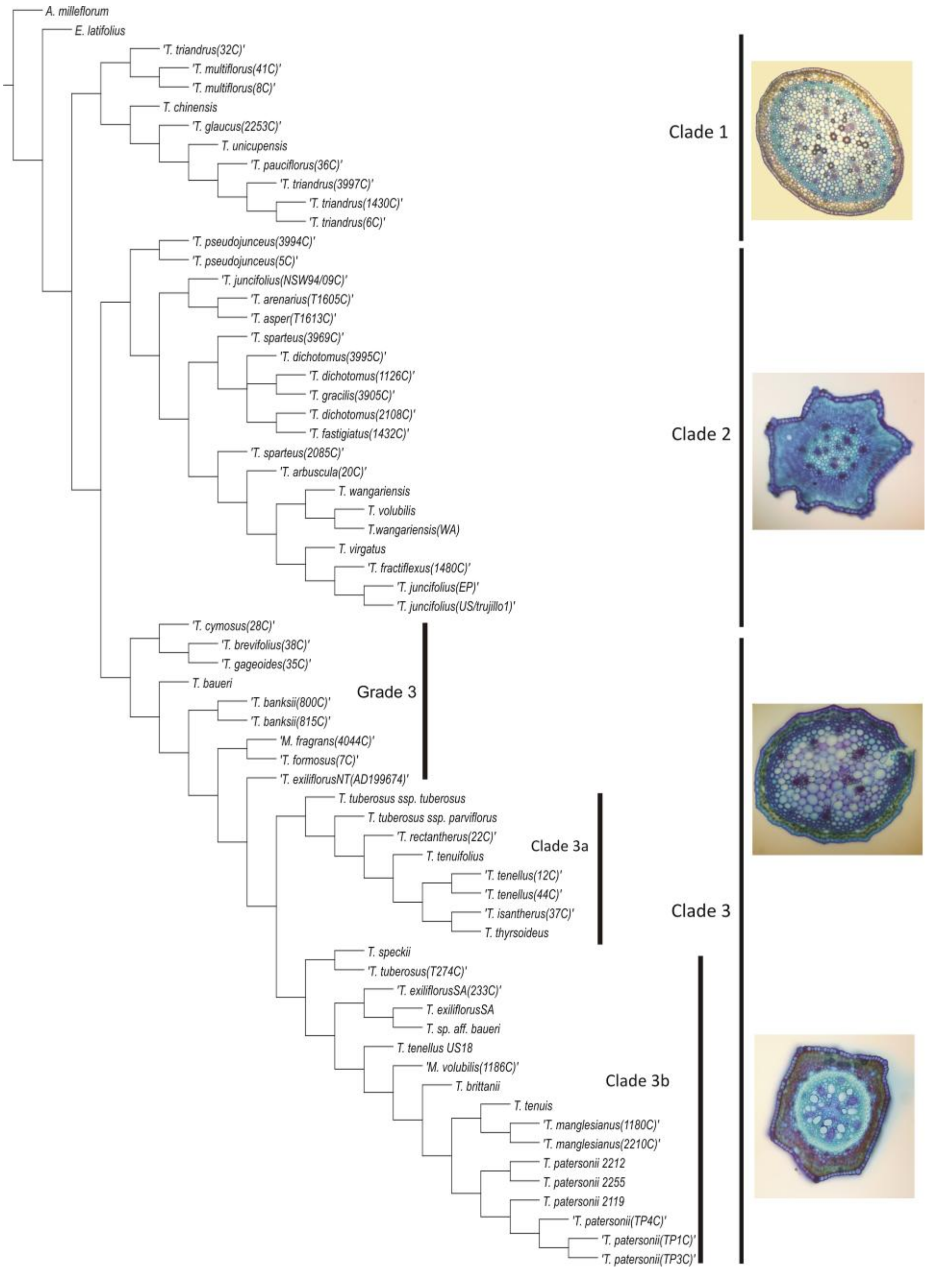


Fig. 28. General anatomical features in major clades of the most parsimonious molecular tree. In Clade 1:elliptic shaped stem transverse sections, elliptic/globose chlorenchyma, absence of raphide canals in chlorenchyma, presence of raphide canals around sclerenchyma (below chlorenchyma) and parenchymatous pith cells; Polygonal transverse stem sections were present in Clades 2 and 3 (in terminal crown lineage). Anatomical features of Clade 1 reappeared in Clade 3 (in Grade 3, Clade 3a and basal taxa of Clade 3b). Terminal crown lineage that includes basal *M. volubilis* defined by homoplasies: elongated chlorenchyma, absence of raphide canals in chlorenchyma, presence of raphide canals around sclerenchyma (below chlorenchyma) and parenchymatous pith.

Discussion

Phylogenetic significance of anatomical characters

When the stem anatomy characters were mapped onto the molecular tree (Fig. 27), only a single anatomical synapomorphy defined *Thysanotus*: the absence of irregular shaped epidermal cells (9/0); and none of the major clades within the genus possessed unique anatomical synapomorphies. Nevertheless, number of homoplasies could be identified to recognise major Clades 2 and 3. For example, Clade 2 could be defined by the presence of elongated chlorenchyma (13/1) and absence of raphide canals around the sclerenchyma (16/0) and Clade 3 by the presence of elliptical chlorenchyma (11/1). The two subclades (Clade 3a and 3b) were not defined by any stem anatomical character.

Major stem Shapes of stem TS were highly variable and homoplasious. Polygonal stem transverse sections (1/2) were common to Clade 3b with all the annual climbing species, including *M. volubilis* and *T. tenuis* in the terminal crown lineage. Terete (1/0) and polygonal (1/2) stem TS shapes in *Thysanotus* are apparently reversals to the plesiomorphic conditions also seen in *Eustrephus* and *Arthropodium* respectively, while elliptic (1/1) and other stem shapes (1/3) appear to have been the result of parallel evolution. Major stem TS shapes associated with major clades in the molecular analysis are given in Figure 28.

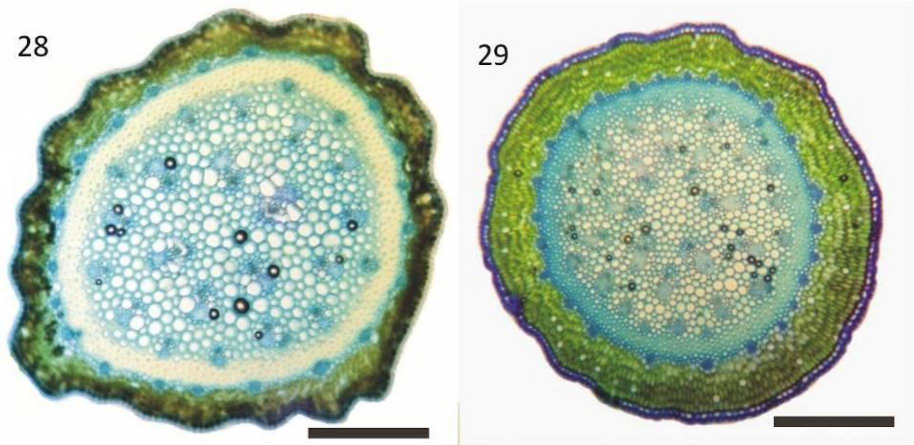
Cuticle thickness was also highly variable and homoplasious in *Thysanotus*. This feature can be highly governed in plants by environmental factors. Presence/absence of projections (3/0, 3/1) of stem surface has occurred several times in the tree thus homoplasious. Small, non-spiny epidermal projections generally occur in plant stems as an adaptation to reduce water loss and this feature has evolved in parallel across all major clades; for example, in *T. glaucus* (Clade 1), *T. arenarius* and *T. wangariensis* (Clade 2), *T. tenellus* and in the annual climbing species (Clade 3). A similar pattern was also observed for epidermal projection length.

Calcium oxalate crystals are widespread in flowering plants both in Dicotyledons and Monocotyledons and the distribution of these crystals in plants is known to be of systematic importance (Prychid and Rudall 1999). The main type of crystals observed in *Thysanotus* was raphides and Brittan (1970) also noted that in *Thysanotus* raphides occur mostly in the chlorenchyma of the stems and leaves. The patterns of occurrence and distribution for raphides and raphide canals in *Thysanotus* are homoplasious according to our analyses, with raphide canals in the chlorenchyma (15/1) were seen in most species. This feature was present in *Arthropodium*, lost in *Eustrephus* and reappeared in *Thysanotus* suggesting a reversal. Raphide canals were also distributed as a layer above the sclerenchyma (below chlorenchyma) in *Thysanotus* (16/1); a derived feature shared with *Eustrephus,* but absent from *Arthropodium*. Absence of this condition in *Thysanotus* (16/0) indicates a reversal/loss. Raphides in the pith (17/1) were only seen in *T. exiliflorus*. However, *Thysanotus exiliflorus* (NT/WA) was distant from the SA specimens of this species in the molecular analysis and has been recognised informally as *T. ineaqualis* (Macfarlane pers.comm) indicating that in this case the presence of raphide canals and raphides in the pith is convergent.

Shape of xylem tissue (18) and presence/absence of lignified pith (19) are also homoplasious in *Thysanotus*. Lignified stem pith is absent in *Arthropodium* and *Eustrephus,* but originated separately in several different species lineages of *Thysanotus*.

Although character mapping did not identify any unique synapomorphies, the species of Clade 1 nearly all had terete/elliptical stems in transverse section, except for *T. multiflorus* which had slightly polygonal transverse sections. The chlorenchyma was also elliptic/globose in all species except *T. chinensis*. Raphide canals were absent from the chlorenchyma, but present around the sclerenchyma (below the chlorenchyma). The pith cells were parenchymatous in all species in this clade.

Clade 2 could be defined by the presence of elongated chlorenchyma (13/1) and absence of raphide canals around the sclerenchyma (16/0). Morphologically members of this clade also shared features such as the absence of leaves during flower and fruit, presence of rhizomes and absence of tubers reflecting correlation between similar morphology vs. similar anatomy. Within the clade, *T. juncifolius* from NSW could be defined by presence of irregular shaped epidermal cells (9/1), presence of chloroplasts in epidermal cells (10/1) and presence of raphide canals in chlorenchyma (15/1) whereas South Australian specimens could be defined by absence of square epidermal cells (7/0) and presence of raphide canals in chlorenchyma (15/1) (Figs. 28 and 29). Anatomical features distinguish the NSW *T. juncifolius* specimens from the SA populations, supporting the decision to separate them and create a new species for the SA and western Victorian taxon (see Chapter 8).



Figs. 28-29. Transverse sections of *T. juncifolius* from NSW and SA. Fig. 28. *T. juncifolius* from NSW with a chlorophyllous epidermis and irregular shaped chlorenchyma. Fig. 29. *T. juncifolius* from SA with a non-chlorophyllous epidermis and elongated chlorenchyma cells. (Scale bars: Figs. 48–49 = 250 µm)

Presence of elliptic chlorenchyma (11/1) anatomically helps to define Clade 3 within which *M. fragrans* is also embedded. All of the species in Grade 3 were geophytes with annual inflorescences and leaves. Anatomically, these species generally possessed stems with terete/elliptic transverse sections, elliptic/globose chlorenchyma, raphide canals around the sclerenchyma (below chlorenchyma) and parenchymatous pith. Within Clade 3, the annual climbing species (as a terminal crown lineage including *M. volubilis*) were further united by the presence of elongated chlorenchyma (13/1). The climbers also possessed polygonal stems in TS, elongated chlorenchyma, raphide canals around the sclerenchyma below the chlorenchyma and parenchymatous pith.

Although the stem anatomical features in *Thysanotus* seem to be mostly homoplasious, and with numerous reversals for a range of features, there are nevertheless patterns within each clade that indicate anatomical adaptations by each lineage. There are also further anatomical changes associated with lifestyle shifts, such as those seen in the terminal crown lineage of annual climbing species. This indicates that anatomy as well as morphology has been driven at the lineage level, with ancestral lifestyle/habit patterns largely determining the patterns seen in the descendents.

Acknowledgments

The research was supported by funding from a Conservation Biology Grant from the Nature Conservation Society of South Australia and by the University of Adelaide. The School of Earth and Environmental Sciences, The University of Adelaide and the Adelaide and Western Australian Herbaria are thanked for the provision of facilities to undertake part of the research.

References

Brittan, NH (1962) Variation, classification and evolution in flowering plants – with particular reference to *Thysanotus* R. Br. *Journal of the Royal Society of Western Australia* **45**, 1–11.

Brittan, NH (1970) A preliminary survey of stem and leaf anatomy of *Thysanotus* R.Br. (Liliaceae). *Botanical Journal of the Linnean Society* **63**, 57–70.

Brittan, NH (1981) The revision of the genus *Thysanotus* R.Br. (Liliaceae). *Brunonia* **4**, 67–181.

Brittan, NH (1987) *Thysanotus* R.Br. In 'Flora of Australia.' (Ed. AS George.) Vol. 45 pp. 345–348. (Australian Government Publishing Service: Canberra)

Carlquist, S, Schneider, EL (1998) Origins and nature of vessels in monocotyledons. 3. Lowiaceae, with comments on rhizome anatomy. *Blumea* **43**, 219–224.

Cheadle, VI (1942) The occurence and types of vessels in the various organs of the plants in the Monocotyledonae. *American Journal of Botany* **29**, 441–450.

Cheadle, VI (1943a) The origins and certain trends of specialization of the vessels in Monocotyledonae. *American Journal of Botany* **30**, 11–17.

Cheadle, VI (1943b) Vessel specialization in the late metaxylem of the various organs in the Monocotyledonae. *American Journal of Botany* **30**, 484–490.

Cheadle, VI (1944) Specialization of vessels within the xylem of each organ in Monocotyledonae. *American Journal of Botany* **31**, 81–92.

Cheadle, VI (1953) Independent origin of vessels in the monocotyledons and dicotyledons. *Phytomorphology* **3**, 23–44.

Conover, MH (1983) The vegetative anatomy of the reticulate-veined Liliiflorae. *Telopea* **2**, 401–412.

Conran, JG (1998) Lomandraceae. In 'The families and genera of vascular plants. Vol. 3. Flowering plants. Monocotyledons: Lilianae (except Orchidaceae).' (Ed. K Kubitzki.) pp. 354–365. (Springer Verlag: Berlin)

Conran, JG (1999) Anatomy and morphology of *Behnia* (Behniaceae) and its relationships within Lilianae: Asparagales. *Botanical Journal of the Linnean Society* **131**, 115–129.

de Menezes, NL, Silva, DC, Arruda, RCO, Melo-De-Pinna, GF, Cardoso, VA, Castro, NM, Scatena, VL, Scremin-Dias, E (2005) Meristematic activity of the endodermis and the pericycle in the primary thickening in monocotyledons. Considerations on the "PTM". *Anais Da Academia Brasileira De Ciencias* **77**, 259–274.

Judd, WS, Campbell, CS, Kellogg, EA, Stevens, PF, Donoghue, MJ (2002) 'Plant systematics: a phylogenetic approach.' (Sinauer Associates, Inc: Sunderland)

Kauff, F, Rudall, PJ, Conran, JG (2000) Systematic root anatomy of Asparagales and other monocotyledons. *Plant Systematics and Evolution* **223**, 139–154.

Linder, HP, Rudall, PJ (2005) Evolutionary history of Poales. *Annual Review of Ecology Evolution and Systematics* **36**, 107–124.

Prychid, CJ, Rudall, PJ (1999) Calcium oxalate crystals in monocotyledons: a review of their structure and systematics. *Annals of Botany* **84**, 725–739.

Prychid, CJ, Rudall, PJ, Gregory, M (2003) Systematics and biology of silica bodies in monocotyledons. *Botanical Review* **69**, 377–440.

Rudall, P (1994) Anatomy and systematics of Iridaceae. *Botanical Journal of the Linnean Society* **114**, 1–21.

Rudall, P (1995) New records of secondary thickening in monocotyledons. *IAWA Journal* **16**, 261–268.

Rudall, PJ, Chase, MW, Cutler, DF, Rusby, J, De Bruijn, AY (1998) Anatomical and molecular systematics of Asteliaceae and Hypoxidaceae. *Botanical Journal of the Linnean Society* **127**, 1–42.

Rudall, PJ, Cutler, DF (1995) Asparagales: a reappraisal. In 'Monocotyledons: sytematics and evolution.' (Eds PJ Rudall, PJ Cribb, DF Cutler, CJ Humphries.) pp. 164. (Royal Botanic Gardens: Kew)

Rudall, PJ, Stevenson, DW, Linder, HP (1999) Structure and systematics of *Hanguana*, a monocotyledon of uncertain affinity. *Australian Systematic Botany* **12**, 311–330.

Sajo, MG, Rudall, PJ (1999) Systematic vegetative anatomy and ensiform leaf development in *Xyris* (Xyridaceae). *Botanical Journal of the Linnean Society* **130**, 171–182.

Schneider, EL, Carlquist, S (1998) Origin and nature of vessels in monocotyledons. 4. Araceae subfamily Philodendroideae. *Journal of the Torrey Botanical Society* **125**, 253–260.

Schneider, EL, Carlquist, S (2005) Origin and nature of vessels in monocotyledons. 7. Philydraceae and Haemodoraceae. *Journal of the Torrey Botanical Society* **132**, 377–383.

Thysanotus unicupensis (Laxmanniaceae), a new species discovered in Unicup Nature Reserve, south-west Western Australia

Udani M. Sirisena[A], Terry D. Macfarlane[B] and John G. Conran[A]

[A]Centre for Evolutionary Biology and Biodiversity, Discipline of Ecology and Evolutionary Biology, School of Earth and Environmental Sciences, DX 650 312, The University of Adelaide, SA 5005, Australia

[B]Western Australian Herbarium, Manjimup Research Centre, Department of Environment and Conservation, Locked Bag 2, Manjimup, Western Australia 6258, Australia

For original published paper see:

Sirisena, U.M., Macfarlane, T.D. and Conran, J.G. (2009) Thysanotus unicupensis (Laxmanniaceae), a new species discovered in Unicup Nature Reserve, south-west Western Australia.
Nuytsia, v.19 (2), pp. 259-263, 2009

Statement of contribution

Ms. Udani M. Sirisena carried out the field collections, experimental work and preparation of the manuscript. Dr. Terry Macfarlane collected specimens and helped in manuscript preparation. Dr. John Conran helped in manuscript preparation.

Udani M. Sirisena **(**January 2010)

Terry D. Macfarlane **(**January 2010)

John G. Conran **(**January 2010)

Thysanotus unicupensis (Laxmanniaceae), a new species discovered in Unicup Nature Reserve, south-west Western Australia

---

Abstract. Sirisena, U.M., Macfarlane, T.D. and Conran, J.G., *Thysanotus unicupensis* (Laxmanniaceae), a new species discovered in Unicup Nature Reserve, south-west Western Australia. *Nuytsia* **19** (2): 259–263 (2009). *Thysanotus unicupensis* is described as a new species from south-west Western Australia. This species shows some affinities to *T. chinensis, T. formosus* and *T. parviflorus* and is apparently localised on lateritic soils in moderately sunny areas within Jarrah/Marri (*Eucalyptus marginata*/*Corymbia calophylla*) woodlands. The key published in *Flora of Australia* (1987) is amended to include the new species.

Introduction

The genus *Thysanotus* R.Br. *nom. cons.* (Asparagales: Laxmanniaceae *sensu* APG (2003)) consists of over 50 species, all endemic or native to Australia, with *T. banksii* R.Br. and *T. chinensis* Benth. also in Papua New Guinea and the latter species extending to Malesia, Thailand, China and the Philippines (Jessop 1979; Conran 1998; Chen and Tamura 2000). Before being moved to Laxmanniaceae, the genus was variously placed in Liliaceae (Cronquist 1981), Anthericaceae (Marchant *et al.* 1987) or Lomandraceae (Chase *et al.* 1996; Conran 1998). The majority of the species occur in south-west Western Australia and following the treatments of Baker (1876) and Bentham (1878), Brittan described many new species from Western Australia (Brittan 1960, 1972), South Australia (Brittan 1971a, 1978) and New South Wales (Brittan 1971b) . In his subsequent revision of the genus, Brittan (1981) recognised 47 species and in the later treatment for the *Flora of Australia* (Brittan 1987) this was increased to 49 species, of which 42 are Western Australian. Since then several new *Thysanotus* species have been recognised (Paczkowska and Chapman 2000) although not yet described formally, and most require further collecting and study to define them more precisely.

While collecting material in Western Australia for a larger phylogenetic study of *Thysanotus* in late spring 2007, we observed and collected specimens of a species in the Unicup Nature Reserve *c.* 50 km east of Manjimup, south-west Western Australia which was not recognisable immediately and which was found subsequently to be morphologically distinct from all other known *Thysanotus* species. Further examination of material both from our collections and from accessions in the Western Australian Herbarium (PERTH) revealed that these specimens represented an unnamed species of *Thysanotus*. Accordingly, we here describe this new species following the descriptive terminology and criteria for species within the genus used by Brittan (1981, 1987).

Description

Thysanotus unicupensis Sirisena, T. Macfarlane & Conran, *sp. nov.*

Herba erectus. Radices fibrosae, non tuberosae. Folia recta, *c.* 30 cm longa, *c.* 2mm lata, glabra, basin versus vaginis membranaceis. Inflorescentia sessile umbellarum (1–5) terminalium. Scapus simplex, 15 cm altus, teres, glaber. Umbellae floribus 2–3, bracteis lanceolateis, 5–6 mm longis, membrenaceis, acuminatis. Pedicelli 7–9 mm longi, prope basin articulati, florescentes erecti, fructiferi erectes. Segmenta perianthii 8–9.5 mm longa: tepala exteriora lanceolata, 1.2–1.5 mm lata, mucronata; tepala interiora latus elliptica, *c.* 3.2–3.5 mm lata, fimbriis *c.* 1.3 mm longis. Stamina 6, antherae strictae, non tortae vel haud tortae, 4 mm longae. Ovarium utroque loculis, ovulis 2. Stylus rectus vel haud curvatus. Capsula perianthio persistenti inclusa, 5 x 3 mm longa. Semina nigra, arillata.

Typus: 1.5 km along a track from Lake Unicup Road at the south western corner of Unicup Nature Reserve, Western Australia, 34°21’24’’S 116°41’53”E, 28 Nov 2007, *U.M. Sirisena & T.D. Macfarlane* 13 (*holo*: PERTH).

Perennial *herb c.* 30 cm tall, with small rhizomes and fibrous-fleshy non-tuberous roots 10–12 cm long. *Leaves* green at flowering time, usually up to 5 per plant, basal, terete, glabrous, *c.* 30 cm long, *c.* 2 mm wide, expanding into membranous wings at base, margins entire; leaf bases enclosed by flat, white, oblong membranous bracts. *Scape* half to three quarters of length of leaves, usually unbranched. *Inflorescence* made of umbels aggregated in a terminal cluster which superficially resembles a single umbel, usually 2–4 flowers per umbel, the whole cluster of 7–17 flowers but sometimes with 1 or 2 bracts either empty or subtending sessile or shortly pedunculate secondary umbel cluster; umbel bracts usually 3, ovate to lanceolate, 5–7 (–10.5) mm long, 2–3.5 mm wide, margin broad, membranous, apex acuminate. *Pedicel* 7–9 mm long, articulated towards the base, erect in flower and fruit. *Tepals* 8–9.5 mm long, outer 3 lanceolate, 1.2–1.5 mm wide, mucronate, inner three broadly elliptic-circular, 3.2–3.5 mm wide; fimbriae *c.* 1.3 mm long. *Stamens* 6, filaments *c.* 1.5 mm long; anthers subequal, basifixed, purple, paler towards the tip, straight, slightly twisted or not twisted, dehiscence by a terminal pore; pores *c.* 0.5 mm long, the back lip exceeding the front lip, outer anthers 3.6 mm long, inner anthers 3.8–4 mm long. *Ovary* trilocular with 2 ovules per loculus, style straight to slightly curved. *Capsule* ellipsoid, *c.* 5 by 3 mm, enclosed within persistent perianth segments forming only a short tail. *Seed* black, narrowly ellipsoid, *c.* 1.5 mm long, *c.* 1 mm wide with strongly convex periclinal walls and striated microsculpturing; aril straw coloured and sessile. (Figure 1)

Specimens examined. WESTERN AUSTRALIA: 1.5 km along a track from Lake Unicup Road at the south-west corner of Unicup Nature Reserve, 34°21'24''S 116°41'53"E, 28 Nov 2007, *U.M. Sirisena & T.D. Macfarlane* 16 (PERTH); same location, 7 Dec 2007, *T.D. Macfarlane* 4183 *& R.W. Hearn* (PERTH); Tinkers Flat Road, Kingston Forest Block, 34° 04' 39.7" S, 116° 13' 30.5" E, 20 Oct. 1997, *A.D. Robinson* K 69 (PERTH); Kingston Forest Block, 34° 04' 51.7" S, 116° 12' 8.5" E, 25 Nov. 1997, *A.D. Robinson & B.G. Ward* K 89 (PERTH); Mullalyup, For. Ref. 5141, 33° 46' 50.0" S, 115° 49' 20.0" E, 5 Dec. 1991, *G.S. McCutcheon* 2435 (PERTH); Cell 13, Site 181, Parsons Swamp Road, ca 350 m W of T junction with Whistlers Road, bearing NE, [E of Boyup Brook], 33° 54' 29.7" S, 116° 33' 19.5" E, 29 Oct. 1998, *R. Davis* 8564 (PERTH).

Distribution and habitat. Known from Unicup Nature Reserve to the Mulallyup and Boyup Brook areas, south-west Western Australia. *Thysanotus unicupensis* grows on dry lateritic and grey sandy soils in moderately sunny places within Jarrah/Marri forests.

At the Unicup Nature Reserve *Thysanotus patersonii* R.Br., *T. multiflorus* R.Br. and *T. thyrsoideus* Baker co-occur with *T. unicupensis*, but show spatial and/or temporal separation. *Thysanotus patersonii* and *T. unicupensis* both grow in moderately sunny places within the Jarrah/Marri woodland, but *T. patersonii* was already fruiting when the latter was flowering. In contrast, *T. multiflorus* and *T. thyrsoideus* flowered simultaneously with *T. unicupensis*, but the former two grew on the more exposed, sunny edges of the forest rather than the more sheltered interior.

Phenology. Flowering late October to early December.

Conservation status. Currently listed as priority Two under DEC Conservation Codes for Western Australia. The species has only been collected a few times although over a range of approximately 100 km in an area with extensive forest including conservation reserves. A systematic survey has not yet been carried out although some localised searches have been conducted, and the various collections were only recognised as belonging together as a single taxon after we saw live plants and understood its distinctness.

Etymology. Named after the Unicup Nature Reserve where we first recognised the species.

Notes. Possession of fleshy roots in *Thysanotus unicupensis* may indicate a relationship to *T. chinensis*, *T. formosus* Brittan and *T. parviflorus* Brittan which also possess fleshy, non-tuberous roots. Although *T. chinensis* was described by Payens (1957), Jessop (1979) and Brittan (1987) as having fibrous roots, examination of live specimens from Queensland and the Northern Territory revealed that it possesses fleshy, non-tuberous roots when alive. These three species share several other morphological features such as an erect habit, glabrous scape and leaves, terete leaves, six stamens and anther dehiscence by a terminal pore. *Thysanotus unicupensis* is most easily distinguished from these other species by having straight to slightly twisted anthers of more or less equal length, sessile umbels clustered towards the scape apex and leaves more or less twice as long as the scape. Out of these three putative relatives, the new species seems closer to *T. formosus* than to either *T. parviflorus* or *T. chinensis,* as it shares with the former features such as a simple scape with sessile umbels and erect pedicels in flower and fruit. In contrast, the scape of *T. parviflorus* is unbranched to 4-branched, bearing pairs of closely appressed umbels at the apex, whereas the buds and fruits of *T. chinensis* are pendant (Brittan 1981).

The inflorescence structure of *T. unicupensis* and *T. formosus* is unusual in the genus where the umbels are usually discrete and easily delimited, despite varying from 1–2–flowered to many –flowered. In those two species the umbels are less easily delimited. The whole inflorescence of *T. unicupensis*, when best developed, consists of a terminal aggregation of flowers which looks superficially like a many-flowered umbel, and one or more smaller umbels below, either sessile or on short, obscure branches. Whilst these smaller umbels may be discrete and clearly separated from the terminal flowers, the terminal aggregation on closer examination looks more like a loose collection of several small umbels bearing as few as two flowers. *Thysanotus formosus* likewise has small umbels, usually 2-flowered, that aggregate to resemble superficially a larger umbel.

Amendments to published key to *Thysanotus* species

The key published in *Flora of Australia* (Brittan 1987) is amended to accommodate *Thysanotus unicupensis* by inserting a new couplet 10a within couplet 10. Couplet 10 is reached with plants that have six stamens having equal or subequal anthers, an annual scapose inflorescence that is not paniculate, and flowers usually two or more per umbel.

10 Inflorescence not paniculate, a single terminal umbel or cluster, sometimes with 1 or 2 subterminal umbels sessile or on a short branch

10a Roots tuberous; anthers dehiscing by slits; pedicels of fruit nutant **T. isantherus**

10a: Roots not tuberous; anthers dehiscing by terminal pores; pedicels of fruit erect **T. unicupensis**

10: Inflorescence paniculate

Acknowledgements

The Western Australian Department of Environment and Conservation is thanked for permission to collect plants from lands under their control. The Director of PERTH is thanked for access to collections, and the Manjimup Research Centre (DEC) and School of Earth and Environmental Sciences at the University of Adelaide are thanked for the provision of facilities to undertake the research, which was conducted as part of a PhD degree by UMS.

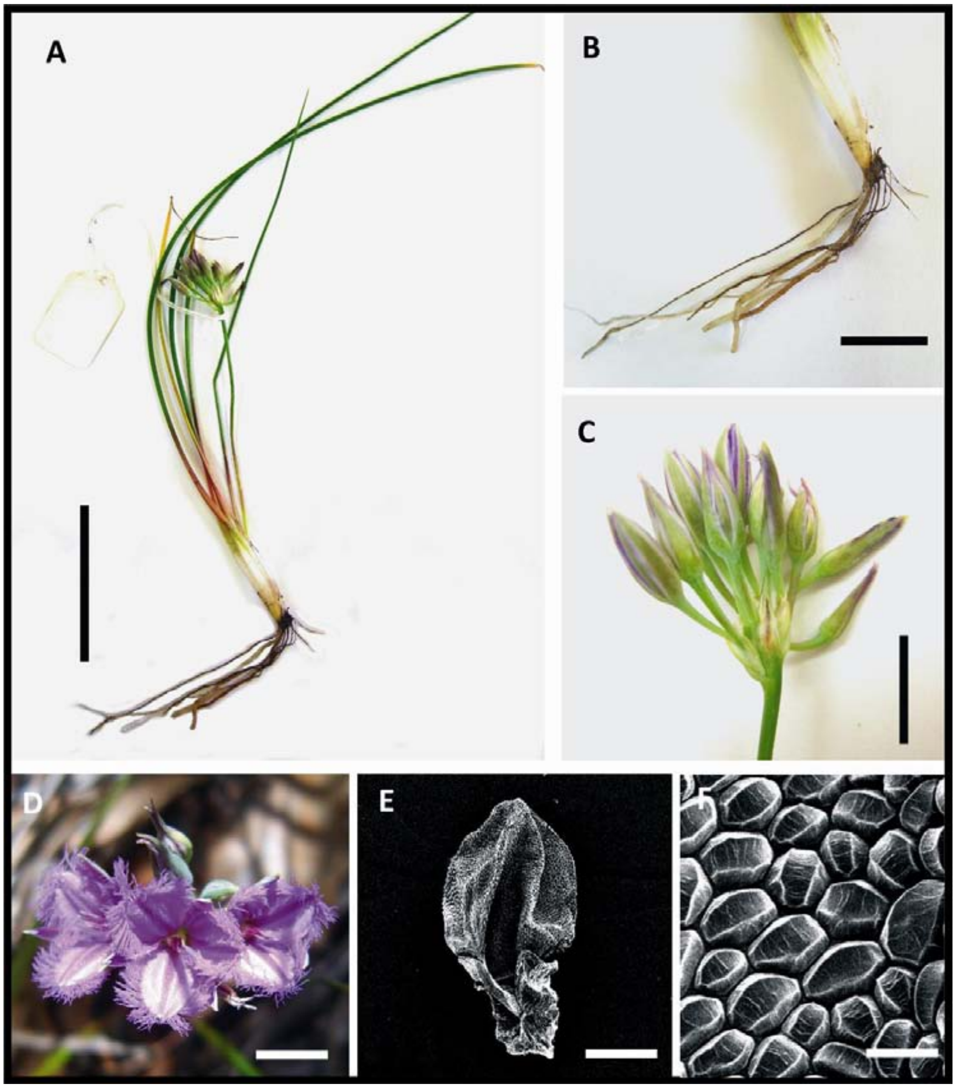


Figure 1. *Thysanotus unicupensis*. A – habit; B – fleshy roots; C – inflorescence; D – flowers and fruits; E – seed; F – periclinal walls on seed surface. Scale bars: A = 10 cm; B = 5 cm; C–D = 10 mm; E = 500 µm; F = 50 µm. Voucher: *Sirisena & Macfarlane* 13 (PERTH). Photographs by Udani M. Sirisena.

References

APG II (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Botanical Journal of the Linnean Society* **141**, 399–436.

Baker, JG (1876) Revision of the genera and species of Anthericae and Eriospermae. *Journal of the Linnean Society, Botany* **XV**, 335–341.

Bentham, G (1878) 'Flora Australiensis.' (Reeve: London)

Brittan, NH (1960) New Western Australian species of *Thysnotus* R.Br. (Liliaceae). *Journal of the Royal Society of Western Australia* **43**, 10–29.

Brittan, NH (1971a) *Thysanotus fractiflexus sp.nov.* (Liliaceae) endemic to Kangaroo Island, South Australia. *Transactions of the Royal Society of South Australia* **95**, 109–111.

Brittan, NH (1971b) *Thysanotus virgatus sp.nov.* (Liliaceae) from Royal National Park, New South Wales *Contributions from the New South Wales National Herbarium* **4** 265–266.

Brittan, NH (1972) New Western Australian species of *Thysanotus* R.Br. (Liliaceae). *Journal of the Royal Society of Western Australia* **54**, 76–93.

Brittan, NH (1978) A new species of *Thysanotus* R.Br. (Liliaceae) from Eyre Peninsula, South Australia. *Transactions of the Royal Society of South Australia* **102**, 55–58.

Brittan, NH (1981) The revision of the genus *Thysanotus* R.Br. (Liliaceae). *Brunonia* **4**, 67–181.

Brittan, NH (1987) *Thysanotus* R.Br. In 'Flora of Australia.' (Ed. AS George.) Vol. 45 pp. 345–348. (Australian Government Publishing Service: Canberra)

Chase, MW, Rudall, PJ, Conran, JG (1996) New circumscriptions and a new family of asparagoid lilies: genera formerly included in Anthericaceae. *Kew Bulletin* **51**, 667–680.

Chen, X-Q, Tamura, MN (2000) *Thysanotus* R.Brown. In 'Flora of China.' (Eds ZY Wu, PH Raven.) Vol. 24 pp. 132. (Science Press and Missouri Botanical Garden Press: Beijing and St Louis)

Conran, JG (1998) Lomandraceae. In 'The families and genera of vascular plants. Vol. 3. Flowering plants. Monocotyledons: Lilianae (except Orchidaceae).' (Ed. K Kubitzki.) pp. 354–365. (Springer Verlag: Berlin)

Cronquist, A (1981) 'An integrated system of classification of flowering plants.' (Columbia University Press: New York)

Jessop, JP (1979) Liliaceae I. In 'Flora Malesiana ' Vol. 9 pp. 189–235.

Marchant, NG, Wheeler, JR, Rye, BL, Bennett, EM, Lander, NS, Macfarlane, TD (1987) 'Flora of the Perth region.' (Department of Agriculture WA: South Perth)

Paczkowska, G, Chapman, AR (2000) 'The Western Australian flora: A descriptive catalogue.' (Wildflower Society of Western Australia: Nedlands, WA)

Payens, JPDW (1957) Revision of some Papuan Liliaceae. *Nova Guinea n. ser.* **8**, 389-390.

Thysanotus racemoides (Asparagales: Laxmanniaceae), a new species from South Australia and western Victoria

Udani M. Sirisena[A], *Terry D. Macfarlane*[B] *and John G. Conran*[A]

[A]Australian Centre for Evolutionary Biology and Biodiversity, Discipline of Ecology and Evolutionary Biology, School of Earth and Environmental Sciences, DX 650 312, The University of Adelaide, SA 5005, Australia

[B]Western Australian Herbarium, Manjimup Research Centre, Department of Environment and Conservation, Locked Bag 2, Manjimup, Western Australia 6258, Australia

Statement of contribution

Ms. Udani M. Sirisena carried out the field collections, experimental work and preparation of the manuscript. Dr. Terry Macfarlane collected specimens and helped in manuscript preparation. Dr. John Conran collected specimens and helped in manuscript preparation.

Udani M. Sirisena (January 2010)

Dr. Terry Macfarlane (January 2010)

Dr. John Conran (January 2010)

Thysanotus racemoides (Asparagales: Laxmanniaceae), a new species from South Australia and western Victoria

Abstract. Sirisena, Udani. M, Macfarlane, Terry. D. and Conran, John. G. 2009. *Thysanotus racemoides*, a new species from South Australia and western Victoria. *Telopea* X(X): XX–XX. *Thysanotus racemoides* T.Macfarlane, Sirisena & Conran, a new species from South Australia and western Victoria is described and distinguished from the previously conspecific *T. juncifolius* (Salisb.) J.H.Willis & Court. The key in the *Flora of Australia* is amended to accommodate the new species.

Introduction

Detailed taxonomic research is scarce on *Thysanotus* R.Br. since the pioneering work carried out by Brittan (Brittan 1960, 1962, 1970, 1971, 1972, 1978, 1981, 1987). Recent molecular analyses recognised *Thysanotus* in a new family Laxmanniaceae (also known as Lomandraceae) (Chase *et al.* 1996; APG II 2003) which was considered as an odd aggregation of genera due to the lack of obvious morphological synapomorphies to define the predominantly Australian family (Chase *et al.* 1995; Rudall and Chase 1996; Conran 1998). Most recently, the family was placed into a greatly expanded Asparagaceae as subfam. Lomandroideae (Mabberley 2008; APG III 2009), but for the purposes of this study, the previous, smaller family unit is retained.

Considering the lack of taxonomic research on the genus, a phylogenetic study was began in mid-2006 by the first author as part of a PhD project. After morphological and molecular analyses, it was apparent that the geographically disjunct South Australian and western Victorian form of *T. juncifolius* is phylogenetically distant from populations in NSW and East Gippsland in Victoria. Further herbarium investigations with multiple sampling revealed that specimens from South Australia and western Victoria represent a new species which is clearly and consistently morphologically distinguishable from the NSW and eastern Victoria form. Because the type of *T. juncifolius* is from Port

Jackson, New South Wales, the South Australian and western Victorian form is described here as a new species. We follow the descriptive terminology of Brittan (1981, 1987) to describe this new species, as well as to redefine *T. juncifolius* s. str.

Methods

Live and herbarium specimens were examined using SEM (Scanning Electron Microscopy), light microscopy and dissecting microscopy. Morphological descriptions were based on these observations.

Stem anatomical studies were carried out using dried material, rehydrated in warm water with a drop of detergent and hand sectioned. The specimens were then stained in 0.05% aqueous Toluidine blue and mounted in Glycerine.

Results

Taxonomy

1. Thysanotus racemoides. T.Macfarlane, Sirisena & Conran sp. nov.

Herba perenis. Rhizoma cylindricum, ca 1–5 cm longum, radices fibrosae haud tuberosae. Planta aphylla, stirpis c. 60 cm altus, tuberculatus vel glaber. Umbellae floribus 1–2, bracteis ovata, c. 2 mm longis, membrenaceis. Pedicelli 5–6 mm longi, prope basin articulati, florescentes erecti, fructiferi erectes. Segmenta perianthii 8–9 mm longa: tepala exteriora lanceolata, 1.2–1.5 mm lata, mucronata; tepala interiora latus elliptica, c. 3.2–3.5 mm lata. Stamina 6; poris terminalibus dehiscentes; 3 exteriores strictae, tortae, 3–6 mm longae. 3 interores curvatae, tortae, c. 6–12 mm longae. Capsula 4–7 mm x 3-5 mm, ab perianthio persistenti inclusa. Semina nigra, arillata, c. 1.5 mm x 1.5 mm.

Typus: Lucindale Road, [Hundred] of Coles, south east South Australia, 37°17’01’’S, 140°27’51”E, 16 Dec 1963, *A.C. Beauglehole 5923*; 96446095 (AD).

Perennial herb with a rhizomatous rootstock, horizontal and more or less cylindrical 1–5 cm long, pale brown or straw coloured. *Roots* fibrous, not tuberous. *Plant* leafless when flowering, aerial stems (scapes) to c. 60 cm tall with along the scape, 2.5–5 mm long. *Scapes* ascending, branches often 2–3 per node, sometimes further subdivided; node bracts lanceolate; branches ridged, tuberculate basally, tuberculate or glabrous distally. *Umbels* terminal above several sessile, subterminal umbels, commonly 2-flowered sometimes 3–4; bracts ovate, membranous, c. 2.5 mm long. *Pedicels* 5–6 mm long, erect in flower and fruit, articulating basally. *Perianth* segments 12–15 mm long, outer three linear-oblong, c. 2 mm wide, inner three elliptical, c. 6 mm wide, fimbriate, fimbriae c. 4 mm long. *Stamens* 6: dehiscing by posteriorly extended terminal pores, outer 3 anthers 3–6 mm long, purple (sometimes yellow), straight, slightly twisted, inner three anthers, purple, curved, twisted, 6–12 mm long; filaments c. 3 mm long. *Ovary* sessile, globular, ovules 2 per locule; style curved, 5–10 mm long. *Capsule* globose, 4–7 × 3–5 mm, enclosed in persistent perianth remains, forming a short tail. *Seeds* black, globose, c. 1.5 × 1.5 mm; testa periclinal walls strongly convex, without microsculpturing; aril straw-coloured (Fig.1).

Derivation of epithet: Derived from the fact the sessile subterminal umbels make the flowering branches resemble a raceme.

Flowering period: Late October to mid to late November.

Habitat: The new species is restricted to the deep sands of inland western Victoria eastern South Australia and the sand plains and lateritic gravels of Kangaroo Island (Brittan 1981). The species grows in a wide range of habitats from dry open forest to mallee woodland and low heaths.

Distribution: The species occurs in south eastern Australia from the Eyre Peninsula and Kangaroo Island in South Australia to western Victoria near Geelong (Fig. 2).

Proposed Conservation status: Currently listed as ‘not threatened’ under Conservation Codes for South Australia and Victoria.

Specimens examined: **South Australia**: *South eastern region*: Mount Burr Forest Reserve, c. 12 km E of Millicent, *P. Wilson 1093*, 11 Nov 1959 (AD96020128); Beachport, *R. Tate s.n.*, 13 Nov 1982 (AD96021026); Hundred of Robertson NW, *D. Hunt 258*, 21 Oct 1961 (AD96151151); Big Heath National Park, Hundred of Spence; 1 mile [1.6 km] S of and parallel to the fire break, mid-narrow neck, *C.R. Alcock 2903*, 4 Nov 1969 (AD96949199); Hundred of Caroline, Section 389, Caroline forest, *D.N. Kraehenbuehl 2562*, 30 Dec 1975 (AD97625135); Big Heath National Park, middle of park (firebreak), *J.Z. Weber 1836*, 6 Nov 1969 (AD97015200).

Kangaroo Island: 6.4 km SW of Cape Willoughby Lighthouse, *B. Overton, P. Canty, S. Kinnear NPKI 40876* (AD99003160); Muston, Pelican Lagoon, *H.M. Cooper s. n.* 26 Nov 1962 (AD97915042); Some 32 km S of Kingscote and 6 km N of Destrees Bay, *C.R. Alcock 10706* 22 Oct 1986 (AD99020004);

Eyre Peninsula: c. 50 m E of Long Beach Rd c. 100 m south of Jubilee Dr, Coffin Bay, Eyre Peninsula SA, *B. Saunders s. n.,* 22 Nov 2002; AD 97936175 (not entered in the herbarium data base).

Murray region: Chauncey's Line - near Monarto South, *J.B. Cleland s.n.*, 30 Nov 1963 (AD96405327).

Southern Lofty region: S of Mount Compass, just before tree area of Square Waterhole, *D.E. Symon 309*, 25 Jan 1960 (AD98666202); Spring Mount, c. 8 km SE of Adelaide, *D.J.E. Whibley 1704*, 23 Nov 1966 (AD96849142); Scrub near Myponga, *D. Hunt 2904*, 26 Dec 1968 (AD97011206); Cox Scrub Conservation Park near turn to Kyeema Conservation Park, *C. Trujillo US/Trujillo 1*, 17 Nov 2007 (ADU).

Western Victoria: Big Desert, c. 98.6 km from Murrayville, *E. Gardiner, G. Gardiner s.n.*, 22 Nov 1981 (AD98151033); 1 mile [1.6 km from] L. Wartook, *N.H. Brittan 59/89*, 15 Dec 1959 (2971976 PERTH); S of Anglesea, *N.H. Brittan 59/91*, 17 Dec 1959 (2971798 PERTH); Slopes between great Ocean road and Harvey street, Anglesea, *R.V. Smith* 59/357, 3 Nov 1959 (1527769MEL); 21.5 km WNW of Rainbow township, on unnamed track, *D.M. Parkes*, 11 Nov 1985, (1545142MEL); Heath Road, Kentbruck, *C & D Woolcock* 1598, 27th Dec 1983 (1524045MEL); Portland South west Portland, Emu hill area, between gorge west of mount Richmond, *A.C. Beauglehole* ACB19537, 1946 (535881MEL); Portland far south west, Kentbruck heath, Heath Road, *A.C. Beauglehole* ACB39458, 1964 (535883MEL); Tea tree creek, Red rock creek area, S of Glenisla station, *A.C. Beauglehole* ACB39458, 1968 (535882MEL); Grampians, black range, track up east side of double headed mount, west side of black range road, *A.C. Beauglehole* ACB30040, 11 Dec 1968 (536387MEL); Wyperfeld National Park *A.C. Beauglehole* ACB29546, 13 Nov 1968 (535880MEL); Little desert, 1 km SE of Broughton's Water Hole, *A.C. Beauglehole* ACB66628 and *A.J. Hicks*, 27 Nov 1979 (1595480MEL); Grampians, *A.C. Beauglehole* ACB 67116, 8 Nov 1979, (1597877MEL).

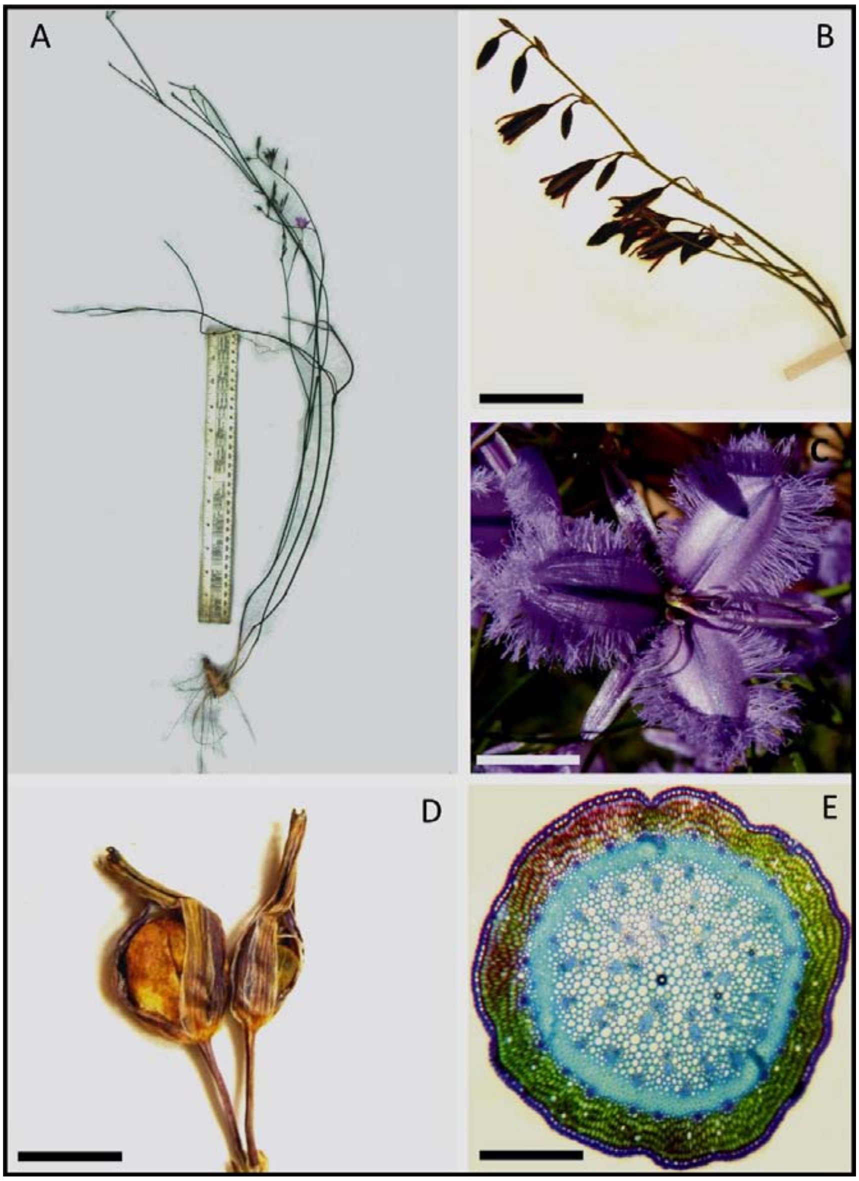


Fig. 1. *T. racemoides*. A. Habit. B. Sessile subterminal umbels below a terminal umbel. C. Flower. D. Fruit. E. Transverse stem section (Specimens – A. *B. Saunders s.n.*; B. AD96020128; C,E. US & Trujillo 1; D. AD96446095. Scale bars: B = 3 cm; C = 12 mm; D = 5 mm; E = 500 µm. Photos–A. JGC; B, D, E. UMS; C. Cecilia Trujillo)

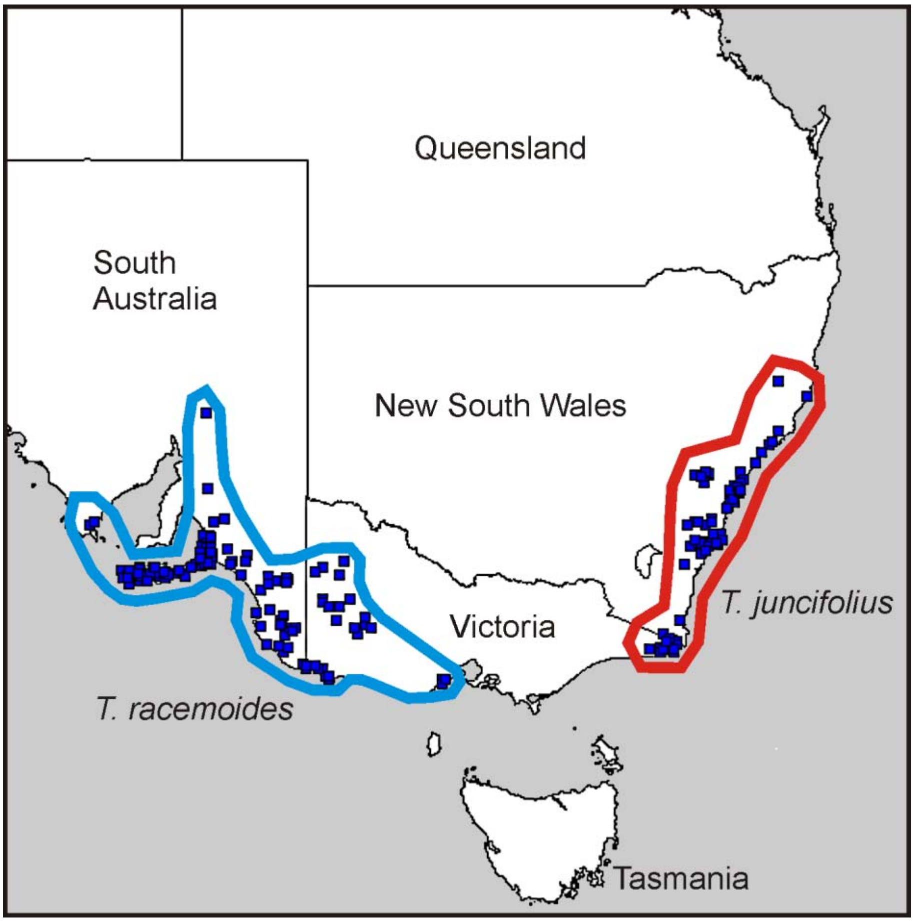


Fig. 2. Distribution of the *T. juncifolius* complex in south eastern Australia.

2. T. juncifolius (Salisb.) Willis & Court

Typus: Port Jackson, New South Wales, *R. Brown* (*Bennett 5684*, *holo*:NSW, *Lecto:*BM, *Iso*:E, K, MEL)

Perennial herb with rhizomatous rootstock. *Roots* fibrous, not tuberous. *Plant* leafless when flowering, aerial stems (scapes) to c. 60 cm tall. *Scapes* ascending, branching monopodially, rarely with 2–3 branches per node, ridged, smooth; node bracts lanceolate, 2–5 mm long. *Umbels* terminal, 1–5 flowered; bracts ovate, membranous, outer ones c. 1.4 mm long. *Pedicels* erect in flower and fruit, articulated near the base. *Perianth* segments to c. 12 mm long, outer three linear-oblong, c. 1.3 mm wide, inner three narrowly elliptical, 3.5–4 mm wide, fimbriate, fimbriae c. 1.5 mm long. *Stamens* 6, dehiscing by posteriorly extended terminal pores, outer 3 anthers 2.2–2.8 mm long, purple (sometimes yellow), straight, slightly twisted, inner three anthers, purple, curved, twisted, 6–8 mm long. *Ovary* sessile, globular, ovules 2 per locule. Capsule globose, 3–5 × 2–3 mm, enclosed in persistent perianth segments which form a short tail. Seeds globose, black, c. 1 mm diam.; aril straw-coloured (Fig.3).

Flowering period: October–early November

Habitat: *Thysanotus juncifolius* grows in very shallow, loamy soils in the Blue Mountains and sandy gravels over Hawkesbury sandstone in NSW and on coastal sands in eastern Victoria (Brittan 1981).

Distribution: The distribution occurs in East Gippsland, Victoria and southern and central coastal New South Wales (Fig. 2).

Proposed Conservation status: Currently listed as 'not threatened' under Conservation Codes for NSW and Victoria.

Specimens examined: **New South Wales:** Port Jackson district, Robert Brown 1802, (244288MEL-Isolectotype); New South Wales North coast, West of Tilligery creek, H. van Rees, 6 Nov 1979 (620710MEL); Yambulla creek, N.A. Wakefield, 21 Dec 1948 (541193MEL); Bullahdelah, *Anonymous Collector*, Nov 1923 (2214370MEL); Lane Cove, *Anonymous Collector*, Oct 1910, (2214365MEL); Port Jackson district, *Anonymous collector*, Oct 1897 (AD96021075 & AD96028166); Sydney, *J.B. Cleland s.n.* Oct 1897 (AD96021076); Near Bowens Creek, Mount Wilson-Bilpin, *N.H. Brittan 59/108-1*, 31 Dec 1959 (3000834 PERTH); Belrose, *R. Coveny & P. Hind RC 11059*, (1979000 PERTH); Princes Highway, ca 1 mile S of Sutherland, *N.H. Brittan 59/105-4*, 29 Dec 1959 (3000664 PERTH); Princes Highway, ca 1 mile S of Sutherland, *N.H. Brittan 59/105-2*, 29 Dec 1959 (3000516 PERTH).

Eastern Victoria: Princes Highway between Mount Drummer and Genoa, *N.H. Brittan 59/ 94,* 20 Dec 1959 (2934728 PERTH); *Beauglehole* and *E.W. Finck* ACB 31977, 22 Nov 1969 (535886MEL); Victoria Gippsland region, Tobins Creek, *D.L. Jones* (18118) and *B.E. Jones*, 15 Nov 2001 (2283147MEL). Princes

Highway, between Mount Drummer and Genoa, between 318 and 319 mile pegs [c. 22 km E of Genoa], *N.H. Brittan 59/94-2*, 20 Dec 1959 (2934744 PERTH); Maramingo Creek, Princes Highway, 4 miles [6.4 km] E Genoa (329 mile peg), *N.H. Brittan 59/96*, 20 Dec 1959 (2934701 PERTH); Maramingo Creek, Princes Highway, 4 miles [6.4 km] E Genoa (329 mile peg), *N.H. Brittan 59/96-2*, 20 Dec 1959 (2934736 PERTH); Mallacoota Inlet National Park, Spotted dog mine area, *A.C. Beauglehole* and *E.W. Finck* ACB 32469, 16 Dec 1968 (535885MEL); Mallacoota Inlet National Park, ½ km south of marshmead property, *A.C. Beauglehole* and *J.H. Willis* AC 31614 (535884MEL); Glue Pot Creek, near Genoa, *R. Melville* (2705) and *N. Wakefield*, 7 Jan 1953 (537755MEL); East Gippsland, Marramingo Creek, *N.A. Wakefield*, 19 Dec 1947 (2214345MEL); East Gippsland, Lower Reedy Creek, *N.A. Wakefield* 2868, 11 Dec 1948 (541195MEL); Cicada Trail, midway between Mueller and Wingan rivers, *A.C.Beuglehole* and *E.W. Finck* ACB 31977, 22 Nov 1969 (535886MEL); Victoria Gippsland region, Tobins Creek, *D.L. Jones* (18118) and *B.E. Jones,* 15 Nov 2001(2283147MEL).

Notes: Morphologically, anatomically and genetically there is a clear distinction between *T. racemoides* and *T. juncifolius* (Sirisena 2010 Chs 3-4). The presence of subterminal, sessile umbels towards the stem apex has sometimes lead South Australian collectors to misidentify *T. racemoides* as *T. baueri*, as the latter may also possesses sessile umbels towards the apex. However, the branched perennial habit, rhizomes and lack of tuberous roots clearly distinguish *T. racemoides* from *T. baueri*.

Thysanotus juncifolius lacks sessile subterminal umbels, which seems to be the most obvious distinguishing character separating it from the new species. Furthermore, the stems of *T. juncifolius* are always ridged and the anthers and perianth segments are shorter than of the new species (Table 1). Anatomically there are differences in the epidermis and chlorenchyma of the scape in transverse section (See table below). Molecular and combined data analyses indicate a close relationship between *T. racemoides* and the Western Australian *T. sparteus* R.Br., while *T. juncifolius* is distant and instead more closely related to *T. asper* Lindl. and *T. arenarius* Brittan, both also from Western Australia (Sirisena 2010 Chs 3-4).

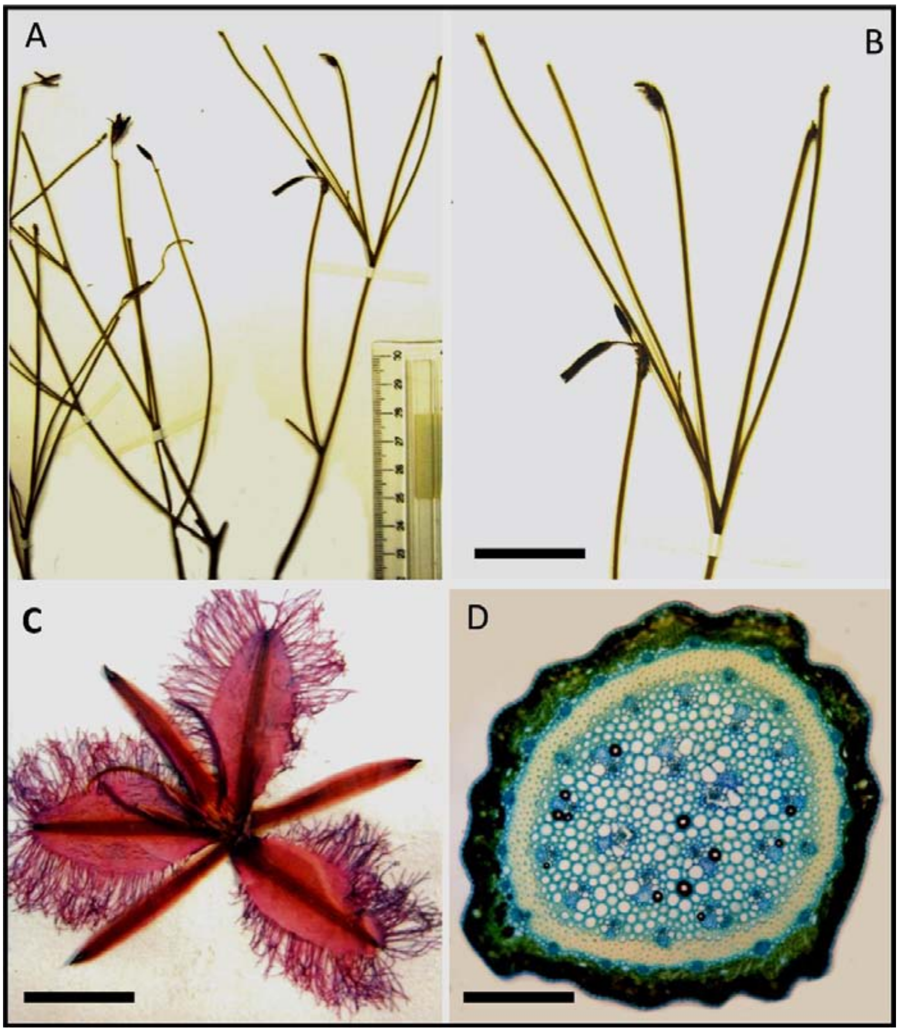


Fig. 3. *T. juncifolius*. A. Habit. B. Absence of sessile umbels below the terminal umbel. C. Flower. D. Transverse stem section. (Specimens – A&B. AD96028166; C. PERTH03000516; D. 94/09. Scale bars: B = 2 cm; C = 7 mm; D = 500 µm. Photos–Udani M. Sirisena)

Table 1. Character comparison of *T. racemoides* and *T. juncifolius*

Character	*T. racemoides*	*T. juncifolius*
Nodes with 2–3 branches	Common	Rare
Subterminal sessile umbels	Present	Absent
Number of flowers per umbel	Mostly 2 occasionally 3–	1–5
Length of perianth segments (mm)	12–15	Mostly <12
Outer anther length (mm)	2–6	<4.0
Inner anther length (mm)	7–12	6–8
Chloroplasts in epidermis scape	Absent (Fig. 1)	Present (Fig. 3)
Chlorenchyma shape	Always elongate (Fig. 1)	Mostly irregular (Fig. 3)

Amended key to *Thysanotus* species

The key published in *Flora of Australia* (Brittan 1987) is amended to accommodate *T. racemoides* by inserting a new couplet after couplet 51. All amendments are indicated in asterisks.

48: Stems terete, smooth to ridged

49 Stems strongly ridged, with short, dense simple or tuberculate hairs

50 Stems with tuberculate hairs; umbels to 8-flowered; perianth segments 7–8 mm long .... **T. brachiatus**

50: Stems with short, dense, simple hairs, at least basally; umbels 1–3 flowered; perianth segments 10–12 mm long .... **T. virgatus**

49: *Stems rounded or slightly ridged, glabrous at least distally

51 *Subterminal umbels absent, all umbels terminal ....**T. juncifolius**

51: *Subterminal umbels always present below terminal umbels

52 * Subterminal umbels always sessile ..... **T. racemoides**

52: * Subterminal umbels pedunculate, rarely sessile
.... **T. sparteus**

Acknowledgements

The South Australian Department of Environment and Heritage is thanked for permission to collect plants from lands under their control. The Directors of AD, PERTH and MEL are thanked for access to collections. AD, PERTH and School of Earth and Environmental Sciences at the University of Adelaide are thanked for the provision of facilities to undertake the research, which was conducted as part of a PhD degree by UMS.

References

APG II (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Botanical Journal of the Linnean Society* **141**, 399–436.

APG III (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**, 105-121.

Brittan, NH (1960) New Western Australian species of *Thysnotus* R.Br. (Liliaceae). *Journal of the Royal Society of Western Australia* **43**, 10–29.

Brittan, NH (1962) Variation, classification and evolution in flowering plants – with particular reference to *Thysanotus* R. Br. *Journal of the Royal Society of Western Australia* **45**, 1–11.

Brittan, NH (1970) A preliminary survey of stem and leaf anatomy of *Thysanotus* R.Br. (Liliaceae). *Botanical Journal of the Linnean Society* **63**, 57–70.

Brittan, NH (1971) *Thysanotus fractiflexus sp.nov.* (Liliaceae) endemic to Kangaroo Island, South Australia. *Transactions of the Royal Society of South Australia* **95**, 109–111.

Brittan, NH (1972) New Western Australian species of *Thysanotus* R.Br. (Liliaceae). *Journal of the Royal Society of Western Australia* **54**, 76–93.

Brittan, NH (1978) A new species of *Thysanotus* R.Br. (Liliaceae) from Eyre Peninsula, South Australia. *Transactions of the Royal Society of South Australia* **102**, 55–58.

Brittan, NH (1981) The revision of the genus *Thysanotus* R.Br. (Liliaceae). *Brunonia* **4**, 67–181.

Brittan, NH (1987) *Thysanotus* R.Br. In 'Flora of Australia.' (Ed. AS George.) Vol. 45 pp. 345–348. (Australian Government Publishing Service: Canberra)

Chase, MW, Duvall, MR, Hillis, HG, Conran, JG, Cox, AV, Eguiarte, LE, Hartwell, J, Fay, MF, Chaddick, LR, Cameron, KM, Hoot, S (1995) Molecular phylogenetics of Lilianae. In 'Monocotyledons: systematics and evolution.' (Eds PJ Rudall, P Cribb, DF Cutler, CJ Humphries.) pp. 109–137. (Royal Botanic Gardens Kew)

Chase, MW, Rudall, PJ, Conran, JG (1996) New circumscriptions and a new family of asparagoid lilies: genera formerly included in Anthericaceae. *Kew Bulletin* **51**, 667–680.

Conran, JG (1998) Lomandraceae. In 'The families and genera of vascular plants. Vol. 3. Flowering plants. Monocotyledons: Lilianae (except Orchidaceae).' (Ed. K Kubitzki.) pp. 354–365. (Springer Verlag: Berlin)

Mabberley, DJ (2008) 'Mabberley's plant-book. A portable dictionary of plants, their classifications, and uses. 3rd edition.' (Cambridge University Press: Cambridge)

Rudall, P, Chase, MW (1996) Systematics of Xanthorrhoeaceae *sensu lato*: evidence for polyphyly. *Telopea* **6**, 629–647.

Sirisena, UM (2010) Systematic studies on *Thysanotus* R.Br. (Asparagales:Laxmanniaceae). Ph.D thesis, University of Adelaide, Adelaide.

General discussion

Systematic relationships of the native genus *Thysanotus* R.Br. (Asparagales Laxmanniaceae) are poorly understood and affinities with other related genera remain still unresolved (Chase *et al.* 1996; Conran 1998). To date, no detailed systematic study based on molecular and/or non-molecular data, has been undertaken to determine the phylogenetic relationships of *Thysanotus* taxa. The present study therefore aimed to do a detailed systematic study based on non-molecular and molecular data.

Despite the various re-circumscriptions, the relationships of most genera within Laxmanniaceae–Lomandraceae are still uncertain (Chase *et al.* 1995; Rudall and Chase 1996; Conran 1998; Fay *et al.* 2000) and therefore require further work. APG II (2003), Mabberley (2008) and APG III (2009) merged Laxmanniaceae into a broader Asparagaceae. However, within Asparagales, many families are considered still problematic, including Asparagaceae, and still in a state of flux (APG III 2009) and subfamily Lomandroideae (Chase *et al.* 2009) is only a recently validated group. In order to explore relationships within the former family more thoroughly as well as the status of its two former subfamilies, Laxmanniaceae *sens. lat.* is therefore retained for the purpose of this study.

Our results based on *trn*L intron and *trn*L–F intergenic spacer sequence data and combined data were congruent with the circumscription for Lomandraceae–Laxmanniaceae in Chase (1995) and Fay *et al.* (2000). Tentatively placed genera in Laxmanniaceae, such as *Murchisonia* and *Trichopetalum* (Chase *et al.* 1996) were confirmed to be allied to the rest of the genera based on our data. In the present study, the *Lomandra* group (Lomandroideae *sens. lat.*) was well supported (100%) and clearly defined by characters such as the absence of tubers and phytomelan (black seeds), which were apomorphic to the group. An arthropodioid clade was defined by characters such as absence of tufted herbs, presence of tubers, absence of perennial leaves, and phytomelanic seeds and numerous ovules per locule.

In contrast, *Laxmannia* was sister to *Sowerbaea* and more closely related to the Lomandroideae than to the arthropodioids, indicating that of the two subfamilies as defined by Thorne and Reveal (2007), Laxmannioideae are artificial, representing the black-seeded "not-Lomandroideae". The *Laxmannia–Sowerbaea* clade is defined by the presence of an unusual ligule-like structure above the leaf sheath (7/1) and bracteate umbel-like flowering heads (10/1). Thus the present study recognises three potential subfamilies within Laxmanniaceae: Lomandroideae, Laxmannioideae and "Arthropodioideae". These would then become tribes within Asparagaceae: Lomandroideae once the composition and phylogeny within Asparagaceae *sens. lat.* is properly resolved.

Amplification of the ITS2 region for *Acanthocarpus*, *Chamaexeros* and *Romnalda* could not be improved during this study; however, the study by Donnon (2009) accommodating more species of each of these genera, along with the amplification of other gene regions including ITS2 region should provide better resolution between the relationships of the Lomandroideae genera. Similarly, *Murchisonia* and *Trichopetalum* presently lack embryological data and this is the focus of ongoing studies.

The present study also included a comprehensive morphological phylogenetic construction of *Thysanotus* based on morphological data in order to understand species relationships and recognise any major lineages, subgenera or sections within the genus, as well as to provide insights to any intra-specific variants or new species of the genus for several of the more morphologically diverse and/or widespread taxa. Phylogenetic construction is useful for an improved understanding of species relationships and facilitating the recognition of infrageneric lineages and character evolution within the genus. Also, any clades recognised during the process could be utilised in future classifications.

Two distinct species clades were recovered within *Thysanotus*, each with a characteristic combination of morphological features, as illustrated in Chapter 2. The clades were mostly based on features such as habit, presence/absence of tubers and rhizomes, presence/absence of leaves at anthesis, branching of the flowering stem, number of stamens, and the type of anther dehiscence. The single synapomorphy for *Thysanotus* and *Murchisonia* relative to the related outgroup taxa was the absence of pendent flowers (43/0).

We were also able to provide insights to intraspecific categories and new species of *Thysanotus,* using combined morphological and anatomical data for species such as *T. patersonii* and *T. juncifolius*, but for some of these, larger phenetic studies with wider sampling of specimens are recommended for the separation of intraspecific categories. Furthermore, the present study only included South Australian and Western Australian specimens of *T. patersonii*, but the species is widely distributed in extra-tropical Australia and any future study should be carried out including specimens from all *T. patersonii* populations across Australia, which was beyond the scope of this present PhD.

Molecular and combined (molecular and morphology) analyses were carried out to obtain a more resolved phylogeny for the first time in the taxonomic history of the genus, evaluate resulting inter-species and intra-species relationships and to recognise any sections within the genus. We used non-coding cp DNA (*trn*L intron and *trn*L–F intergenic spacer) and ITS2 sequence data for the molecular analysis. Based on both our molecular and combined morphological data sets it is confirmed that *Thysanotus* (including *Murchisonia*) is monophyletic. Monophyly is supported by 100% BS value in both analyses. After mapping the morphological characters onto the molecular tree, absence of pendent flowers (43/0) was the most reliable morphological synapomorphy.

The molecular data and the combined data yielded highly resolved consensus trees and enabled us to recognise three lineages (representing three sections) within the genus. The most important characters in recognising these lineages are the possession of tuberous roots and rhizomes, possession of annual/perennial leaves, presence/absence of branched inflorescence axes and habit of inflorescences. Furthermore, intra- and inter-species variations and relationships were also discernable from both the molecular and the combined trees.

Murchisonia was clearly polyphyletic and nested within *Thysanotus* in both analyses showing a need for reinstatement of both species as *Thysanotus*. Its separation from *Thysanotus* by Brittan (1987) has always been controversial without a proper systematic study. *Murchisonia fragrans* was sister to *T. formosus* while *M. volubilis* was basal to the leafless, annual climbing inflorescence *Thysanotus* species. *Thysanotus* and *Murchisonia* were known to be closely related, possessing umbellate inflorescences and our results support merging *Murchisonia* back into *Thysanotus*.

The molecular phylogeny could be improved by incorporating more gene regions which are fast evolving as well as easily amplified. Further, broader sampling is also recommended to obtain a more accurate phylogeny. Incorporating RFLP and AFLP studies will be helpful in recognising genetic variation in species complexes and/or wide spread *Thysanotus* species. This work should alone complement a diverse and larger project for the future.

Seed coat morphology of *Thysanotus* and the Laxmannioideae and arthropodioids indicated that there was sufficient variation, to serve as a possible source for recognising different taxa, species groups and evolutionary trends. Seed epidermal cells in *Thysanotus* were fairly uniform, with polygonal to elongate polygonal cells, straight anticlinal walls and convex outer periclinal walls. Nevertheless, there was variation in appendage morphology, epidermal cell elongation, anticlinal boundaries, outer periclinal wall features and surface sculpturing.

Thysanotus and *Murchisonia* could not be separated on seed morphological synapomorphies. Elongated seed shape (1/2) and aril attached with a long stalk and positioned distant from the seed (4/1) were restricted to all 3–staminate species; however, not synapomorphic seed conditions for the 3–staminate species. The seed SEM results therefore, are not in accordance with Thongpukdee‟s (1989) recognition of a distinct 3–staminate species clade, based on a morphological phylogenetic analysis. Angular seed is the plesiomorphic condition in arthropodioids, observed in the outgroup taxa *Arthropodium* and *Eustrephus,* and in *Thysanotus* mainly occurs within the annual climbing *Thysanotus* species, where it would be a reversal. Derived seed features in *Thysanotus* seem to be the elongated seeds with long stalked arils which were seen in all 3–staminate species (Clade 1) and globose/elliptical seeds with sessile arils observed in Clade 2 and the proximal members of Clade 3.

Seed micro-morphological studies could be extended to include Lomandroideae to obtain a clearer understanding of the evolution of seeds and generic relationships in Laxmanniaceae and adding more species from each genus is also recommended for future studies. Furthermore, seed characters indicated that they might be useful in recognising intraspecific categories (e.g. *T. patersonii*) and species boundaries of *Thysanotus* which will require much broader sampling across the geographic range.

The systematic examination of stem anatomy of *Thysanotus* showed that the stems consisted of a single-layered epidermis, with many species possessing epidermal hairs, then a few-layered chlorenchymatous region, a ring of cortical parenchyma cells below the chlorenchyma, adjacently possessing sclerenchyma or collenchyma layers below the cortical parenchyma. Vascular bundles occur towards the centre of the stem, more or less in whorls. The pith is generally parenchymatous, or rarely lignified. Despite the generally uniform tissue arrangement, there were still differences in the epidermal cells, chlorenchyma, vascular tissues and pith, and presence/absence of raphides. Only a single anatomical synapomorphy to define *Thysanotus* could be recognised after mapping anatomical characters onto the molecular tree i.e. the absence of irregular shaped epidermal cells (9/0). Major clades could not be defined uniquely by stem anatomical synapomorphies, nevertheless, a number of homoplasies could be identified to recognise the major Clades 2 and 3. For example, Clade 2 possesses elongated chlorenchyma (13/1) and absence of raphide canals around sclerenchyma (16/0) while Clade 3 shows the presence of elliptic chlorenchyma (11/1).

The anatomical results indicated that floral and vegetative variation seen in some species was also reflected in their anatomy. For example, Clade 2 could be defined by two homoplasies: presence of elongated chlorenchyma (13/1) and absence of raphide canals around sclerenchyma (16/0). Morphologically they also shared similar features such as the absence of leaves at anthesis, presence of rhizomes and absence of tubers. Similarly, all the annual climbing species shared similar anatomical characteristics such as polygonal stem TS, elongated chlorenchyma, raphide canals around sclerenchyma (below chlorenchyma) and parenchymatous pith.

Stem anatomy was also useful in delimiting species boundaries and intraspecific categories. For example, *T. juncifolius* from NSW could be defined by presence of irregular shaped epidermal cells (9/1) and presence of chloroplasts in epidermal cells (10/1) where as the South Australian specimens could be defined by absence of square epidermal cells (7/0). Presence of raphide canals in chlorenchyma (15/1) was common to both. Therefore, anatomical features distinguish NSW *T. juncifolius* specimens from and SA populations. Separation of other intra-specific categories of *Thysanotus* (in Chs 3 and 4) should be complemented with future sampling using multiple specimens per each taxa.

Another future direction of anatomical investigations might be the incorporation of leaf anatomy. However, a drawback of leaf anatomical investigations is that many of the „leafless‟ annual- or perennial-stemmed *Thysanotus* species only produce leaves when the plants are first sprouting, if at all, so that obtaining suitable leaf material for anatomical studies can be difficult.

Although the results of the morphological analysis was in accordance with previously recognised 3–staminate and 6–staminate lineages (Bentham 1878; Thongpukdee 1989), the molecular analyses contradict this. In the molecular analysis, 3–staminate species formed a distinct clade with two of the 6–staminate species. Instead, from both the morphological and molecular analyses, three major lineages could be recognised: non-rhizomatous, perennial-leaved and fibrous-rooted with annual inflorescences; leafless, fibrous-rooted and rhizomatous with perennial inflorescences; and leaves annual, tuberous-rooted geophytes with annual inflorescences. In both analyses, within the leafy tuberous-rooted geophytes with annual inflorescences, there was a specialised crown lineage of leafless, tuberous-rooted geophytes with annual, twining inflorescences. The three main lineages recognised represent the new sections for *Thysanotus*.

References

APG II (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Botanical Journal of the Linnean Society* **141**, 399–436.

APG III (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* **161**, 105–121.

Bentham, G (1878) 'Flora Australiensis.' (Reeve: London)

Brittan, NH (1987) *Murchisonia* Brittan. In 'Flora of Australia.' (Ed. AS George.) Vol. 45 pp. 340–341. (Australian Government Publishing Service: Canberra)

Chase, MW, Duvall, MR, Hillis, HG, Conran, JG, Cox, AV, Eguiarte, LE, Hartwell, J, Fay, MF, Chaddick, LR, Cameron, KM, Hoot, S (1995) Molecular phylogenetics of Lilianae. In 'Monocotyledons: systematics and evolution.' (Eds PJ Rudall, P Cribb, DF Cutler, CJ Humphries.) pp. 109–137. (Royal Botanic Gardens Kew)

Chase, MW, Reveal, JL, Fay, MF (2009) A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Botanical Journal of the Linnean Society* **161**, 132–136.

Chase, MW, Rudall, PJ, Conran, JG (1996) New circumscriptions and a new family of asparagoid lilies: genera formerly included in Anthericaceae. *Kew Bulletin* **51**, 667–680.

Conran, JG (1998) Lomandraceae. In 'The families and genera of vascular plants. Vol. 3. Flowering plants. Monocotyledons: Lilianae (except Orchidaceae).' (Ed. K Kubitzki.) pp. 354–365. (Springer Verlag: Berlin)

Donnon, MJ (2009) Molecular systematics of the *Lomandra* Labill. complex (Asparagales: Laxmanniaceae). Ph.D. thesis, The University of Adelaide, Adelaide.

Fay, MF, Rudall, PJ, Sullivan, S, Stobart, KL, de Bruijn, AY, Reeves, G, Qamaruz-Zaman, F, Hong, W-P, Joseph, J, Hahn, WJ, Conran, JG, Chase, MW (2000) Phylogenetic studies of Asparagales based on four plastid DNA regions. In 'Monocots: systematics and evolution.' (Eds KL Wilson, DA Morrison.) pp. 360–370. (CSIRO: Melbourne)

Mabberley, DJ (2008) 'Mabberley‟s plant-book. A portable dictionary of plants, their classifications, and uses. 3rd edition.' (Cambridge University Press: Cambridge)

Rudall, P, Chase, MW (1996) Systematics of Xanthorrhoeaceae *sensu lato*: evidence for polyphyly. *Telopea* **6**, 629–647.

Thongpukdee, A (1989) The taxonomic position of the genus *Tricoryne* R.Br. Ph.D. thesis, University of Queensland, St. Lucia.

Thorne, R, Reveal, J (2007) An updated classification of the class Magnoliopsida (“Angiospermae”). *The Botanical Review* **73**, 67–181.