Chapter 1: General introduction and aims

Bulbine Wolf

Bulbine (Asphodelaceae or Xanthorrhoeaceae s. lat.) is currently recognised as one of only a small number of herbaceous genera with an exclusively Africa – Australia occurrence (Watson 1986a). The genus occurs predominantly in South Africa, where there are c. 72 species (South African National Biodiversity Institute 2013). In Australia seven species are currently recognised: *B.bulbosa* (R.Br.) Haw., *B. semibarbata* (R.Br.) Haw, *B. alata* Baijnaith, *B. glauca* (Raf.) E.M.Watson, *B. vagans* E.M.Watson, *B. pendula* Keighery and *B. crassa* Morris & Durretto (Figure 1.1).

The most recent taxonomic treatments of Australian *Bulbine* (Baijnath 1977; Watson 1987) have implicitly employed a morphological species concept; i.e. species defined by suites of covarying attributes (Stuessy 1990). The biological species concept based on reproductively isolated populations (Mayr 1970) was tested by Baijnaith (1977) and Watson (1986a; 1986b). Their breeding experiments, however, did not prove useful in delimiting species.

Following the success of the morphological approach in delimiting Australian *Bulbine* (Baijnath 1977; Watson 1987), I have used this approach as a principle line of evidence. In line with de Queiroz (2007) I have sought to incorporate additional lines of evidence to assess species' limits and status within a phylogenetic context.

Australian *Bulbine*

Bulbine glauca, Rock Lily, was first described as *Anthericum semibarbatum* R.Br. from material Robert Brown collected in 1802 from Memory Cove and Kangaroo Island, South Australia (Brown 1810). In his treatment of the Australian Liliaceae, Brown (1810) followed closely the classification of de Jussieu and recorded his collection of two herbaceous species as *Anthericum bulbosum* R.Br and *A. semibarbatum* R.Br. The former was characterised by a "bulbous" base and also displayed filaments that were all hairy. *Anthericum semibarbatum* was distinguished by its fibrous radix and outer stamens with glabrous filaments (Baijnath 1977).

Haworth (1821) assigned *Anthericum semibarbatum* and *A. bulbosum* to *Bulbine*, providing the new combinations *B. semibarbata* (R.Br.) Haw. and *B. bulbosa* (R.Br.) Haw. respectively. Schultes (1830) proposed that the Tasmanian specimens attributed to *A. semibarbatum*, but which did not fit the descriptions of *B. bulbosa* or *B. semibarbata*, were a new species. However, Schultes did not formalise a name (Watson 1987). Hooker (1832, t. 3129) maintained plants that he recognised as corresponding to Schultes' description of specimens from Tasmania under '*Anthericum semibarbatum*. Half bearded Anthericum'. Rafinesque (1836) later described a new species, *Nemopogon glaucum* Raf., based on a specimen cultivated in Glasgow of Tasmanian provenance, which agreed with Schultes' and Hooker's descriptions, in having glaucous leaves and six bearded stamens.

In 1897, Borzi incorporated *Bulbine semibarbata* and *B. bulbosa* into a new genus *Bulbinopsis* Borzi. Although a detailed description was presented, it is not easy to isolate the salient features used to create the new genus. As described by Baijnath (1977), the characters recorded agree with those of the genus *Bulbine* except for anthers basifixed and two ovules in each locule. The anthers are now known to be dorsifixed and ovule numbers range from two per locule in *B. semibarbata* and 4–8 per locule in *B. bulbosa* (Watson 1987). *Bulbine* has been conserved and all species of *Bulbinopsis* appear under *Bulbine* (Govaerts 1996; Pope 2001).

Watson (1987) transferred *Nemopogon glaucum* to *Bulbine* as *B. glauca* (Raf.) E.M.Watson. As Rafinesque's type could not be located, Watson (1987) lectotypified *N. glaucum* by specimens in Royal Botanic Gardens Kew (K) that corresponded to the description of 'Half bearded Anthericum' by Hooker (1832) and annotated as cultivated from seed from Tasmania. The complete sequence of taxon descriptions in Australian *Bulbine* is outlined in Table 1.1.

Table 1.1 Nomenclatural history of names relating to Australian *Bulbine.* All previously published names are listed in alphabetical order following the year of publication and accepted names are in bold type. Names in roman type are synonyms or misapplied names; if a name is accepted, that which is used in this account is italicized and given in parenthesis. The type citation is listed for each published name although where relevant, the basionym rather than the type is recorded.

Year	Publication and type citation
1810	*Anthericum semibarbata* R.Br. (=*B. semibarbata* (R.Br.) Haw.): J.D.M. (Brown 1810) [Kangaroo Island and Memory Cove (Stearn 1960; Watson 1987)].
	Anthericum bulbosum R.Br. (=*B. bulbosa* (R.Br.) Haw.): J (Brown 1810) [between Parramatta and Hawkesbury in Western Sydney (Stearn 1960; Watson 1987)].
1821	***B. semibarbatum* (R.Br.) Haw**.: *Anthericum semibarbatum* R.Br. (Haworth 1821).
	***B. bulbosa* (R.Br.) Haw**.: *Anthericum bulbosum* R.Br. (Haworth 1821).
1825	*B. australis* Spreng. (=*B. bulbosa* (R.Br.) Haw.): *Anthericum semibarbatum* R.Br.
1832	*Anthericum bulbosum auct. non* R.Br.: W.J.Hooker (=*B. bulbosa* (R.Br.) Haw.)
	Anthericum semibarbatum auct. non R.Br. W.J.Hooker (=*B. glauca* (Raf.) E.M.Watson)
1836	*Nemopogon glaucum* Raf. (=*B. glauca* (Raf.) E.M.Watson): Tasmanian Anthericum semibarbatum R.Br. Lod.

Year	Publication and type citation
	Bot. C-b. 330, Hooker bot. Mag. 3129 (Rafinesque 1836).
	Blephanthera bulbosum Raf. (=*B. bulbosa* (R.Br.) Haw): *Anthericum bulbosum* R.Br
	Blephanthera hookeri Raf. (=*B. bulbosa* (R.Br.) Haw): Australia (Rafinesque 1836) [cultivated at Glasgow, Scotland, from a tuber collected probably in Qld in 1828 by C. Fraser (Watson 1987)].
	Blephanthera depressa Raf. (=*B. bulbosa* (R.Br.) Haw): *Anthericum bulbosum* R.Br.
1838	*Bulbine suavis* Lind. (=*B.glauca* (Raf.) E.M.Watson): was found by Major Mitchell in his last journey into the interior of NSW, in 1836. It flowered in the garden of the Horticultural Society in May 1838 (Lindley 1838).
1843	*Bulbine hookeri* Kunth (=*B. glauca* (Raf.) E.M.Watson): insula van Dieman (Kunth 1843)
	Bulbine fraseri Kunth (=*B. bulbosa* (R.Br.) Haw.): Nova Hollandia (New South Wales) Fraser *legit* (Kunth 1843)
1878	*Bulbine floribunda* Benth. (=*B. semibarbata* (R.Br.). Haw.):
1891	*Phalagium semibarbatum* (R.Br.) Kuntze (=*B. semibarbatum* (R.Br.). Haw.): *Anthericum semibarbatum* R.Br.
	Phalagium bulbosum (R.Br.) Kuntze (=*B. bulbosa* (R.Br.). Haw.): *Anthericum bulbosum* R.Br.
1897	*Bulbinopsis semibarbatum* (R.Br.) Borzi (=*Bulbine semibarbata* (R.Br.) Haw.): *Anthericum semibarbatum* R.Br.
	Bulbinopsis bulbosum (R.Br.) Borzi (=*Bulbine bulbosa* (R.Br.) Haw.): *Anthericum bulbosum* R.Br.
1912	*Bulbine semibarbatum f. gracilescens* Domin (=*Bulbine semibarbata* (R.Br.) Haw.): Forma haec respondent speciminibus quae a cl. R. Brown sub no. 5675 collecta vidimus (Domin 1912)
1932	*B. semibarbata var depilata* J.M. Black (=*B. alata* Baijnath): Near Marree, July, 1931; coll. D.W. George (Black 1932)
1981	*B. semibarbata* var A Jessop (=*B. semibarbata* (R.Br.) Haw.
1986	***B. alata* Baijnath** (=*B. semibarbata var. depilata* J.M. Black; *B. semibarbata var. depilata* (J.M. Black) H. Eichler): South Australia, Maralinga, clay pan c. 900 ft 3 Sept. 1956. Hill 714 (BM holotype) (Baijnath 1978).
	***B. glauca* (Raf.) E.M.Watson**: (=*B. suavis* Lindl., *Bulbinopsis terrae-victoriae* Poelln.): *Nemopogon glaucum* Raf. (Watson 1987).
1987	***B. vagans* E.M.Watson**: Cultivated Australian National Botanic Gardens Canberra, ACT, 13 Jan 1984, E.M.Watson 152 (from transplant CBG 7702723, from Amys Peak, Kroombit Tableland Qld, 1977, M.D. Crisp 2832); holo: CBG; ISO: AD, BRI, K. (Watson 1987).
2004	***B. pendula* Keighery**: 5 km E of Juna Downs Homestead, Hamersley Range, 22.51’S, 118 32’E West. Aust. 9 Aug 1973, M.E. Trudgen 370) holo: PERTH 06088074)(Keighery 2004).
2006	***B. crassa* D.I. Morris & Duretto**: Tasmania: Furneaux: Neds Reef, about 1 km off Neds Point on the north coast of Cape Barren Is, 40 20’S 148 04’E, 1 Dec. 1986, S. Harris (holotype: HO 312703) (Morris & Duretto 2005).

The number of records provided on Australia’s Virtual Herbarium (CHAH 2014) gives some measure of the relative abundance of each currently recognised species (Fig. 1.1). *Bulbine bulbosa* and *B. semibarbata* with 1029 and 1120 records respectively are the commonest species. *Bulbine glauca* is less common, being represented by 164 records, with the least abundant *B. vagans* (67 records) *B. pendula* (21 records) and *B. crassa* (28 records).

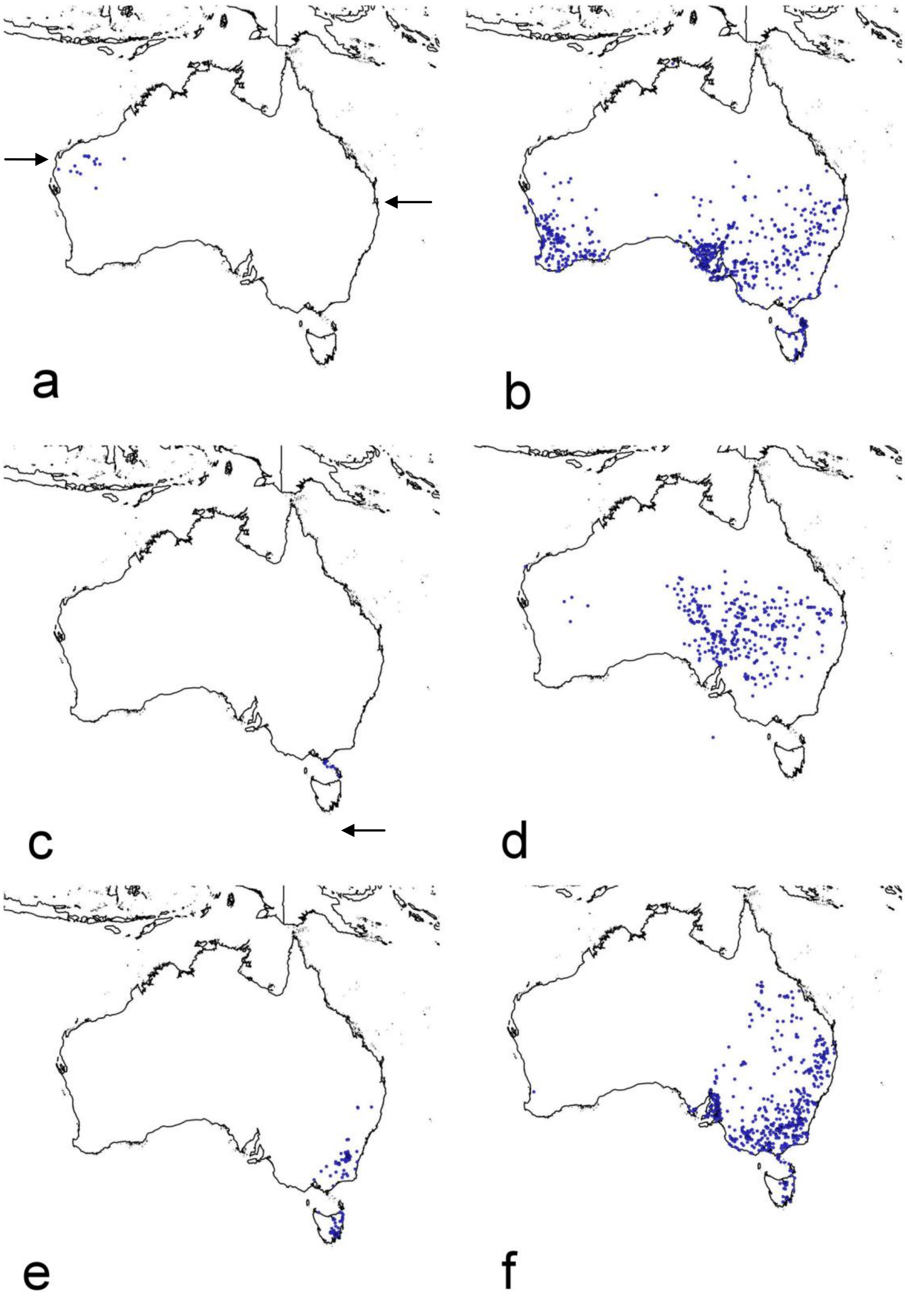

Fig 1.1. Distribution of Australian *Bulbine*: **a.** *B. pendula* (west coast) and *B. vagans* (east coast); **b**. *B. semibarbata*; **c**. *B. crassa*; **d**. *B. alata*; **e**. *B. glauca*; **f**. *B. bulbosa.* Arrows highlight areas of localised distribution

Bulbine glauca complex

Within the Australian *Bulbine,* the perennial species *B. glauca s.lat.* shows considerable variability in morphology and habit (Figure 1.2), which is captured in taxonomic descriptions of *Bulbine glauca* (cf. Godden 1983; Watson 1987; Conran & Walsh 1994); e.g. variation in rootstock morphology, leaf colour and seed surface patterning. This infraspecific variation begs the question, is it a complex and what are the species' limits in *Bulbine glauca s.lat.*?

More recently, McDougall and Walsh (2002) reported a population of *Bulbine glauca* at Nungar Plain in the Snowy Mountains of New South Wales having leaf dimensions exceeding those normally given for the species (cf. Watson 1987, Godden 1993). Similarly, we found populations at Mt Kaputar, in the Northern Tablelands of New South Wales to have leaf and scape dimensions exceeding those normally given for *B. glauca*.

Our observations indicate there is variation in the leaf colour, depth of leaf channelling, shape and size of floral bracts, seed colour and seed surface patterning between geographically disjunct populations of *Bulbine glauca s.lat.* from Mount Kaputar, Mount Canobolas, Bungonia and Bega in NSW, Mount Coree on the ACT/NSW border, Organ Pipes National Park in Victoria, and central and eastern Tasmania.

Morphological variation between populations of *Bulbine glauca s.lat.* corresponds with their extremely localised distribution (Figure 1.1). *Bulbine glauca s.lat.* occurs only on the eastern highlands of Australia and, to the south, on the coast adjacent to the highlands (Watson, 1987). The species is generally confined to higher rainfall areas, often at higher altitudes, in rock crevices or depressions, and sometimes in exposed positions on mountain tops. As described by Watson (1986a), the country rock is often granite but not invariably so; e.g. in southern Tasmania, *B. glauca* is found on sandstone (Pilchers Hill) and dolerite (Lindisfarne). The range of habitats in which *B. glauca s.lat.* occurs include: grassy cliff ledges (Mt Kaputar and Mt Canobolas NSW, Mt Coree, ACT-NSW border), shrubby rocky slopes above stream beds (Uriarra Crossing, ACT, Queanbeyan, NSW, Organ Pipes National Park, Vic.), open forest scree slopes in gorges (Shoalhaven River, NSW), closed forest on coastal sand dunes (Lake Tyers, Vic., Scamander, Tas.) and alpine plain herbfields (Nungar Plain, NSW). Based on our observation of morphology, there are clear differences between populations of *Bulbine glauca s.lat.* from Mount Kaputar, Mount Canobolas, Mount Coree and Shoalhaven River, Bungonia, Moruya and Gretna.

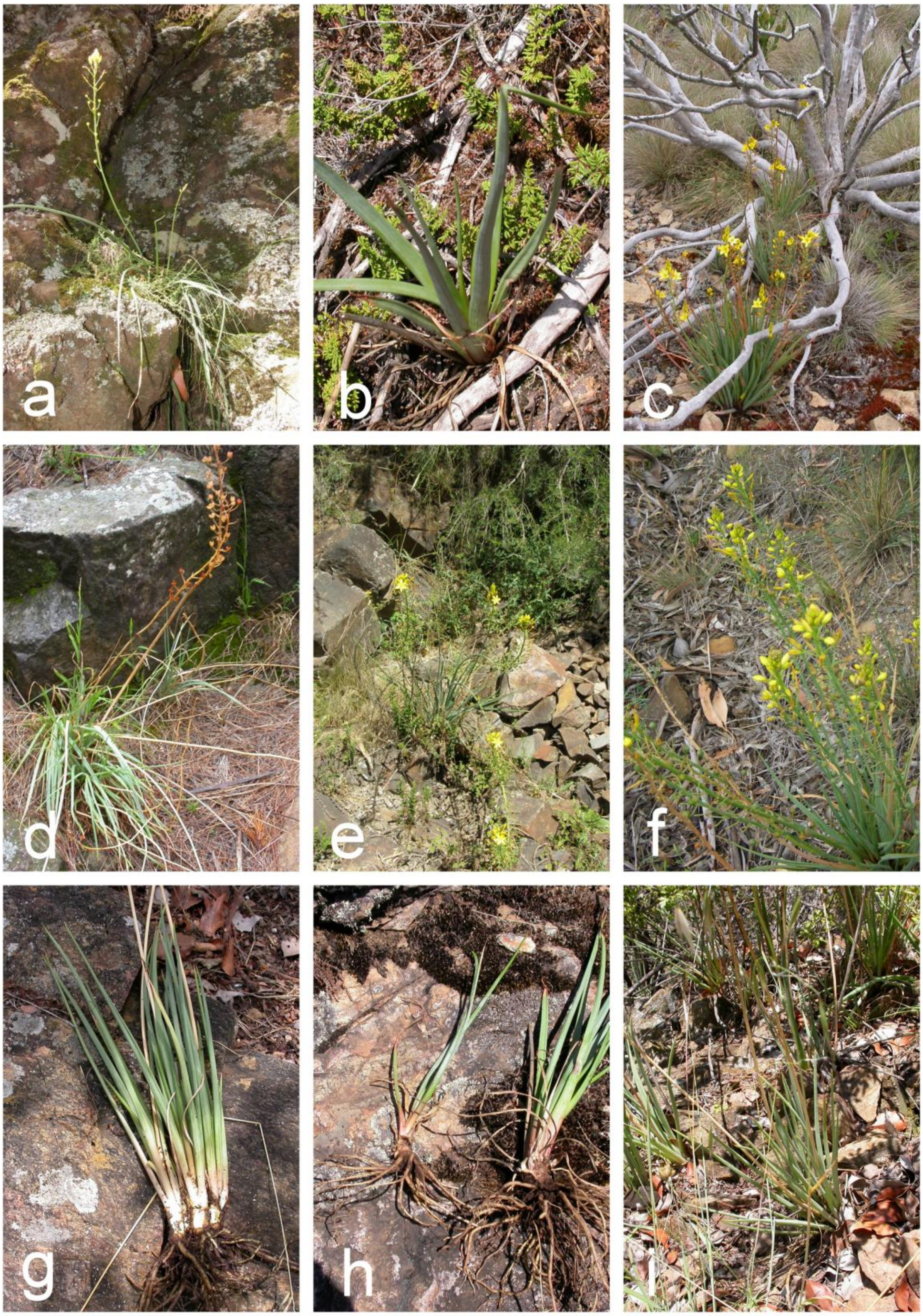

Fig 1.2. Variability in the morphology and habit of specimens of *Bulbine glauca s.lat.*, growing at: **a**. Mount Kaputar, NSW (*I.S.F.Moore 64*); **b.** Mount Canobolas, NSW (*I.S.F.Moore 83*); **c.** Mount Coree, ACT (*I.S.F.Moore 74*); **d**. Uriarra, ACT (*I.S.F.Moore 77*); **e**. Shoalhaven River, Bungonia, NSW (*I.S.F.Moore 108*); **f.** Bothwell, TAS (*I.S.F.Moore 105*); **g.** Queanbeyan, NSW (*I.S.F.Moore 80*); **h**. Mount Canobolas (*I.S.F.Moore 83*); **i.** Queanbeyan (*I.S.F.Moore 80*).

The circumscription of *Bulbine glauca* was based principally on the cytological findings of Watson (1986a), rather than a comprehensive morphological analysis of *B. bulbosa s.lat.* Watson (1986a) first clearly distinguished Watson's "Rock Lily group" (sensu *B. glauca*) from the wider "bulbosa group" following an extensive breeding and crossing program, which formed part of an earlier study by Watson (1977). Whilst largely sympatric with the bulbosa group, the Rock Lily group differs in chromosome number (2n = 46; cf. 2n = 24, 48, 72 in the bulbosa group). Chromosome counts were predominantly 2n = 46 (71%), but aneuploid numbers of 45 (15%), 44 (6%), 48 (3%), 47 (2%), 40 (2%) and 41 (1%) were also encountered (Table 1.3). The rock lilies also had a completely different karyotype pattern and a number of morphological and ecological differences, as described by Watson (1986a). The extent of karyotypic variation further suggests a need to test the species limits in the *Bulbine glauca* complex using other sources of data (cf. de Queiroz 2007).

Table 1.3. Recorded chromosome numbers in Australian *Bulbine*

Species	Chromosome number (2n)	Reference
B. bulbosa	24, 48, 72	Watson (1986a)
B. semibarbata	26, 50, 51, 52, 54, 74, 76, 77, 78	Watson (1986b)
B. alata	28	Watson (1986b)
B. vagans	46	Watson (1986a)
B. glauca	44, 45, 46, 47, 48, 40, 41	Watson (1986a)

Aims and relevance of the project

The morphological variation present within *Bulbine glauca s.lat.* indicates that this species is a complex with unresolved species limits. This project aimed to investigate, test and set species limits within the *B. glauca* complex.

No Australian *Bulbine* are currently listed as rare or endangered; however, this situation may be an artefact of the apparently broad species concept. Resolving species limits within the *B. glauca* complex will present a better estimate of Australian biodiversity and its conservation value with relevance to land and species management.

The potential of *Bulbine natalensis* Baker extract as a prosexual pharmaceutical (Yakubu and Afolayan 2009) raises the possibility of similar therapeutic value of Australian *Bulbine*. Any utilisation of the Rock Lily group for horticulture or chemical mining for compounds of pharmaceutical value will likely be more useful if accurate species limits inform sampling. Fundamental knowledge on cytology of the group should also stimulate further work in the genus to understand processes of evolution of breeding systems and speciation in plants.

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Chapter 6: Conclusion

Introduction

This chapter presents a summary of the general conclusions that address the aims of the thesis. All of the data sources investigated are reviewed and their taxonomic utility discussed along with the limitations of this study. Comments on the conservation of species of *Bulbine* of the study group are provided. Future directions for systematic studies in Australian *Bulbine* are suggested.

Overall contribution of this project to taxonomic knowledge

This project has contributed broadly to taxonomic knowledge of Australian *Bulbine* through investigation of the aims (Chapter 1; see below). The findings present a better estimate of Australian biodiversity and its conservation value with relevance to land and species management. This understanding provides the potential for more appropriate sampling for ecological studies and for compounds of pharmaceutical value. Fundamental knowledge on cytology should also stimulate further work in the genus to understand processes of evolution of breeding systems and speciation in plants.

My study demonstrates the taxonomic adage that it is best to utilise as many data sources as possible (Stuessy 1990); certainly, this is the case here in testing species limits and estimating evolutionary relationships.

Summary of findings against the aim of the project

Investigate and test species limits within the Bulbine glauca *complex*

Morphological phenetic analysis of OTUs of the *Bulbine glauca* species complex resolved the group into six species: the narrowly endemic *B. kaputarensis, B. watsoniae*, *B. petraea*, *B. ardua*, and the widespread and morphologically variable *B. terrae-victoriae* and *B. glauca.* The phenetic results incorporate cytomorphological variation among the species.

Phylogenetic reconstruction of plastid DNA and nuclear DNA sequences using *mat*K, *rbc*La, *trn*H-*psb*A and ITS sequence data indicate that the *Bulbine glauca* species complex contains monophyletic groups that indicate up to six species in the complex, although support is low. Combining the molecular and morphological analyses provides strong support for six species in the complex and indicates that populations within the *B. glauca* complex have diverged recently with incomplete lineage sorting. Therefore, I base my conclusions on the morphological analyses, recognising six species in the *B. glauca* complex, each with fixed diagnostic characters which are coherent with their current distributions.

Allopatric groups of populations of *Bulbine glauca s. lat.* are separated by large distances and I think that niche divergence has been responsible for speciation in the *B. glauca* complex. Such recent divergence makes the combined morphology and molecular data set more useful for studying speciation than the molecular data set alone. Thus, it was advantageous to gather morphological and molecular data simultaneously to detect recently diverged taxa, because the molecular data alone was unable to provide resolution.

Taxonomic utility of data sources used

Non-molecular data were extremely effective in testing species limits of the *Bulbine glauca* complex of species. Living plant material was more useful than herbarium specimens, especially in measuring variation in leaf colour and morphology for succulent leaves.

Cytomorphological data also proved useful in testing species limits. Additional analysis across populations of *Bulbine crassa* is merited.

Individual *mat*K, *rbc*La, *trn*H-*psb*A sequence data only partially resolved phylogeny within the *Bulbine glauca* complex. More resolution and support were provided by the combined plastid data. The ITS sequence data provided the greatest resolution of relationships between populations than other individual sequence data. However, the combined plastid and ITS sequence data provided the greatest information on phylogenetic relationships, and when combined with the non-molecular data resolved clear well supported monophyletic groups.

Approaches to future collection and studies in *Bulbine*

High quality herbarium specimens are essential for taxonomic studies. Phenetic analysis using only herbarium specimens has the advantage of being able to include type specimens which is therefore useful for the correct application of names. However, it has the disadvantage that not all characters are available for study – they were either not developed on the plants at the time of collection, or they were present, but only available through destructive sampling.

Care is needed in collecting plant samples of members of the *Bulbine glauca* species complex – flowers are open for only a few hours, and for future studies ensure samples are pressed with open flowers. Ideally plants should be collected when they have both open flowers and ripe seeds available, which happens only over at most a few weeks and not for all plants. It is acknowledged that plants may not be at this ideal stage of growth when encountered in the field.

The pressing process distorts inflorescences and leaves. It is difficult to count all buds in herbarium specimens. It is also impossible to establish the colour, extent of channelling in and orientation of leaves. Measuring size and shape of floral bracts is also difficult. Establishing the orientation of stamina hairs is impossible. To provide access to undistorted leaves it would ideal for some specimens in any collection to be preserved in liquid, such as FAA (by volume 40% formaldehyde 1: glacial acetic acid 1: 95% alcohol 8: water 10) or even 70% ethanol. In addition, some clean and fresh, green or white plant material could be preserved in silica gel or 95% ethanol, for the possibility of conducting molecular analysis on such tissue in the future. Given mucilaginous nature of leaves, it is important to have living collections. Living collections are invaluable in undertaking phenetic analysis, particularly in measuring floral parts, orientation of stamina hairs, orientation of leaves, colour of leaves, measuring floral bracts.

Undertaking cytology assessment requires healthy living specimens, with harvesting of root tips required during a relatively short growing period of Spring – early Summer. Collecting root tips in the field is a possibility, storing in 2 mM 8-hydroxyquinoline solution, but care is needed to ensure solution can be maintained at 4ºC until returned to laboratory. This requires an appropriate cooler or ice pack.

Conducting the research over an extended period has the advantage of allowing sampling of a large number of populations over successive growing seasons and development of extensive datasets for analysis.

Limitations of the current study

Limitations of the current study were as follows:

- Due to time constraints, not all recorded populations of the *Bulbine glauca* complex of species could be sampled. For completeness it would have been useful to have included more populations, particularly from north-eastern Victoria.

- Part-time research made it difficult to repeat laboratory work, and develop and refine skills in photography and image preparation.

- Molecular sampling was limited to only a few populations due to budgetary and time constraints. The need for fresh root tips meant harvesting was difficult for plants collected late in the study.

Recommendations for further work

The species status and veracity of the classification of the remaining members of the Australian *Bulbine* remain untested and controversial, in particular the *B. bulbosa* group (Watson 1986a) in relation to polyploidy (Soltis *et al.* 2007). The results of this study show that phenetic analysis can help resolve the status of a complex of species, by highlighting discrete groups and assigning names that denote discrete entities (in this case *B. kaputarensis*, *B. watsoniae*, *B. petraea*, *B. ardua*, *B. terrae-victoriae* and *B. glauca*). For best results, measurements would be preferentially taken from living samples with open flowers, ripe seeds and undistorted leaves.

Not all members of the *Bulbine glauca* complex were included in the molecular analysis. It is recommended that the range of entities be increased, to include populations from montane and subalpine New South Wales and Victoria. This would enable the molecular differences to be compared with the morphological differences discovered from the phenetic analysis allowing a better definition of the distributional boundaries between *B. watsoniae* and *B. terrae-victoriae*.

Use of next generation sequencing (NGS) to provide greater resolution of species limits in the *Bulbine glauca* complex of species is also recommended.

Variations in cytomorphology between populations suggests a need for further cytology investigations to explore the full extent of variation within the complex. It is recommended that the range of entities also be increased for cytology, to include populations from montane and subalpine New South Wales and Victoria.

Conservation of Australian species of *Bulbine* of the study group.

The current study has identified three putative species of *Bulbine* with limited distributions: *B. kaputarensis*, *B. petraea* and *B. ardua*. Fortunately, these species occur in protected reserves. However, known populations have fewer than 50 plants. Until assessed, a threat category of ‘Vulnerable’ is suggested under the *Environmental Protection and Biodiversity Conservation Act* 1999 (Commonwealth) and the *Threatened Species Conservation Act* 1995 (New South Wales). Conserved in Mount Kaputar National Park. As estimated population numbers of these three putative species are fewer than 250 mature individuals, a threat category of ‘Endangered’ (EN) is suggested under the International Union for Conservation of Nature (IUCN) Red List.

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