

Chapter 1: General introduction and aims

“The history of science, like the history of all human ideas, is a history of irresponsible dreams, of obstinacy, and of error. But science is one of the very few human activities perhaps the only one in which errors are systematically criticized and fairly often, in time, corrected. This is why we can say that, in science, we often learn from our mistakes, and why we can speak clearly and sensibly about making progress there.” (Popper 1963 p. 216)

Proteaceae and *Banksia*

The flowering plant family Proteaceae is predominantly distributed in the Southern Hemisphere and represents a classic Gondwanan clade, with fossils dating to c. 94 Mya, i.e., shortly after the separation of Africa from the rest of Gondwana (Guerin and Hill 2006). The family comprises about 80 genera with c. 1700 species, c.1450 of which are distributed in Australia and South Africa, which have the greatest concentrations of diversity (APG III 2009). There are also about 83 species in 8 genera in South and Central America (Prance and Plana 1998).

Well known genera in the Proteaceae clade include *Telopea*, *Protea*, *Banksia*, *Grevillea*, *Hakea*, and *Macadamia*. The New South Wales floral emblem is the Waratah (*Telopea speciosissima*); *Banksia*, *Grevillea*, and *Leucadendron* are popular cut flowers, while the nuts of *Macadamia integrifolia* are widely grown commercially. The genus *Banksia* L.f. (Proteaceae subfam. Grevilleoideae) was first described on the basis of four species collected by Banks and Solander during the Cook voyage in 1770 (Thiele and Ladiges 1996; Collins *et al.* 2009).

Banksia consists of c. 212 species (Thiele and Ladiges 1996). Only one species, *Banksia dentata*, occurs naturally outside of Australia, and extends to New Guinea and the Aru Islands (Thiele and Ladiges 1996; Mast *et al.* 2002). The growth habit of *Banksia* ranges from small prostrate woody mats to trees reaching 40 m tall (Weston 2007). The majority of species of *Banksia* are found in Western Australia with c. 15 species presently recognised in eastern Australia (Collins *et al.* 2009).

The focus of this study is the *Banksia spinulosa* complex, which is widespread in the mesic, fire-prone sclerophyllous woodland, heath and wallum communities of eastern Australia,

generally on acidic, nutrient-poor soils. Taxa in the *B. spinulosa* complex mostly occur on and east/south of the Great Divide. They have spectacular and recognisable spike-like cylindrical confluences (George 1984; Mast *et al.* 2005) (Fig. 1). The *Banksia spinulosa* complex has a complicated taxonomic history (Chapter 2, Table 1). Smith (1793) first described and named *B. spinulosa* Sm., and subsequent botanists named two close relatives, *B. collina* R.Br. and *B. cunninghamii* Sieber ex Rchb. (Brown 1810; Reichenbach 1827).

Classifications

It was not until George (1981) included *B. collina* R.Br. and *B. cunninghamii* Sieber ex Rchb. as varieties of *B. spinulosa* and published his infrageneric classification that formal recognition of these taxa as a complex occurred (Thiele and Ladiges 1996). The addition of *B. spinulosa* var. *neoanglica* (George 1988), brought the number of generally recognised taxa in the complex to four: *B. spinulosa* var. *spinulosa*, *B. spinulosa* var. *collina*, *B. spinulosa* var. *cunninghamii* and *B. spinulosa* var. *neoanglica* (George 1981, 1988).

The known sympatry of *B. spinulosa* and *B. cunninghamii* prompted Peter H. Weston (Herbarium NSW; pers. comm. 2007) to advise Harden (1991) to recognize two species in the *B. spinulosa* complex: *B. spinulosa*, with two varieties, *B. spinulosa* var. *spinulosa* and *B. spinulosa* var. *collina*, and *B. cunninghamii*, with two subspecies, *B. cunninghamii* subsp. *cunninghamii* and *B. cunninghamii* subsp. A (= *B. spinulosa* var. *neoanglica*). Weston's classification has been used by the National Herbarium of New South Wales since 1991. Most herbaria across Australia use George's (1981, 1988, 1999) classification. Stimpson *et al.* (2012) raised *Banksia cunninghamii* subsp. A (= *B. spinulosa* var. *neoanglica*) to species rank i.e. *B. neoanglica* and Stimpson *et al.* (2014; Chapter 2) described *B. vincentia*, bringing the number of known taxa in the *B. spinulosa* complex to five.



Fig. 1. Morphological variation in the *Banksia spinulosa* complex. A. *B. spinulosa*; B. *B. vincentia*; C. *B. cunninghamii*; D. *B. neoanglica*.

Geographical range of the *Banksia spinulosa* complex

The current classifications of the *B. spinulosa* complex *sensu* (George 1981 1988, 1999; Harden *et al* 2000, 2002) imply that the *B. spinulosa* complex has the broadest latitudinal and ecological amplitude of any species in the genus (Thiele and Ladiges 1996). George's (1981, p. 393) circumscription of the *Banksia spinulosa* complex explicitly states "*Banksia spinulosa* is a complex species, widely distributed near the east coast of Australia from Atherton in north Queensland to Wilsons promontory in Victoria. Three varieties are recognised here but each is variable and some populations do not fit readily into any of them". The geographic boundaries for *B. spinulosa* var. *spinulosa* according to AVH (2013–2015) range from Mossman in north Queensland, to Wilsons Promontory in Victoria, with four isolated populations in central Queensland (George 1981; Harden *et al.* 2000, 2002) at Blackdown Tableland, Isla Gorge, Expedition and Carnarvon Gorge National Parks (Fig. 2).

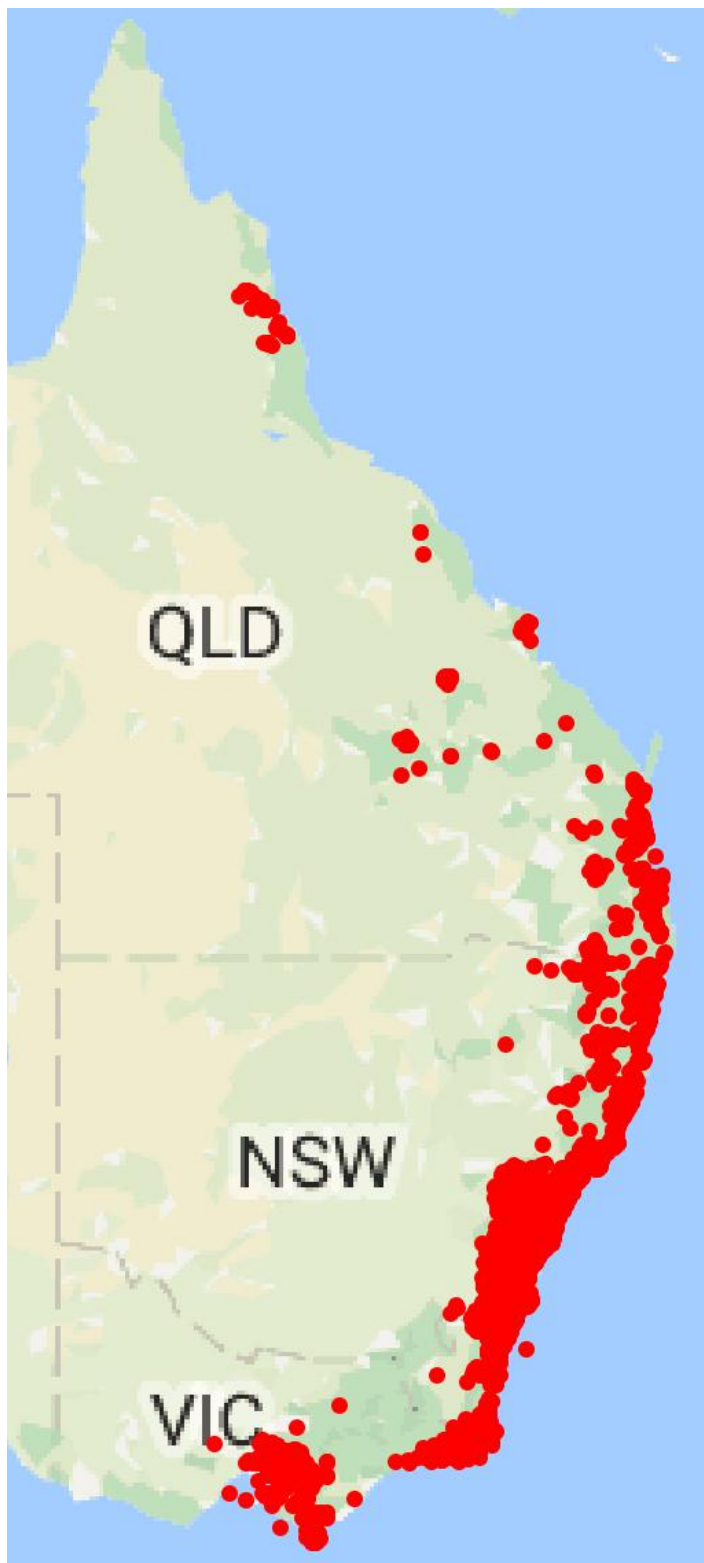


Fig. 2. Distribution of *Banksia spinulosa* complex. Occurrences in South Australia (not shown) are misidentifications of *B. marginata* (Stimpson pers. obs.). Outliers in Victoria are suspect. (Source: AVH, Accessed 6 Sep 2016)

Species concepts in the *Banksia spinulosa* complex

In George's (1981, 1988, 1999) classifications species are delimited using "significant and consistent differences in the morphology of the flowers and/or fruit. Generally, such differences are accompanied by others in habit, leaves, seeds, flowering time, etc. In two cases, where taxa are distinct in habit and to some extent in leaf morphology, I have retained them as varieties because they cannot be separated on the basis of their flowers and fruit" (George 1981, p. 243). These classifications were based on intuitive assessments of morphology. Harden's *et al.* (2000, 2002) classification is fundamentally based on the biological species concept, which implies reproductive barriers between one species and another, as in the case of *B. cunninghamii* and *B. spinulosa*, which grow sympatrically over a wide geographic area with no known hybridisation. Having two different classifications for the same complex does not aid nomenclatural stability nor provide clarity for science in general (Knapp *et al.* 2004).

Taxonomy should be the building and application of biological classifications based on hypotheses of phylogeny, and character homology, and is an important scientific discipline underpinning conservation and management of biodiversity. Nomenclatural stability and recognition of evolutionary units should be goals of taxonomy (Baum and Smith 2013). Neither of the current classifications for the *B. spinulosa* complex is based on a corroborated hypothesis of evolutionary relatedness or indeed, a comprehensive morphological analysis of specimens from the full range of the distribution. Instead, the classifications have been based on intuitive taxonomic assessments of visible morphological traits, coupled with information on the sympatric occurrence of two of the constituent taxa (*B. spinulosa* and *B. cunninghamii*) in the case of the classification of Harden *et al.* (2000, 2002). As demonstrated above, different species concepts result in different circumscriptions, which is an ongoing problem for science and other users of taxonomy.

"One of the complications in defining what constitutes a species is that speciation involves a sequence of events that span an extensive time frame; therefore, it is rarely possible for a scientist to observe those changes directly or to reproduce them in the laboratory" (Niklas 1997 p. 63). Species concepts are many and varied. No species concept that has been published so far is applicable to every organism (Crisp and Chandler 1993). That individuals can be grouped into populations and that populations can be grouped into discrete biological units called species,

underpins all biology (Niklas 1997). “Species concepts matter because they affect: 1) the taxonomic rank of diagnosable populations; 2) estimates of species diversity; 3) our understanding of patterns of gene flow within and among populations; 4) delineation of areas of endemism; 5) decisions on regeneration; and 6) which species will be given protection under local, national and international legal instruments” (Cracraft 2000 p. 7).

Taxonomic decisions about species and uncertainty in identifying species are not just issues for scientists. Species concepts affect everyone. Humanity needs to be able to think about and talk about kinds of organisms. A fisherman, a hunter, a birdwatcher, a gardener, or a person in the grocery store who shops for fruit and vegetables, depends on being able to distinguish among kinds of organisms. Doctors and other medical professionals need to be able to identify different kinds of parasites, including bacteria and viruses; farmers must be able to tell the difference between crop plants and weeds (Hey 2009).

There are currently 22 species concepts in modern literature all with defining properties (Wilkins 2006). Under the Biological Species Concept, species have to be reproductively isolated, that is, reproductive isolation differentiates one species from another (Dobzhansky 1937; Mayr 1942). Under the Ecological Species Concept; species are differentiated by the occupation of different ecological niches. Under the phenetic species concept one taxon has to be distinguishable by phenetic analysis from another. Under the phylogenetic species concept (monophyletic version), all members of a clade have to have a single common ancestor and extinction of the ancestor is assumed (Horandl 2006; De Queiroz 2007). The discussion over the nature of species has beset biological systematics from early in its history (Ereshefsky 2010; Mishler 2010).

Criticisms of particular species concepts are often stated in pejorative terms and usually reflect systematists/biologists preference for another concept: “this species concept is...typological,...merely morphological,...non-biological,...non-evolutionary,...non-genetic, ...arbitrary or...subjective” (Cracraft 2000 p. 8). Regardless of the species concept adopted, all species limits are based on characters and data derived from the organisms being studied to delimit species. However, a different answer may be arrived at by the use of different methods

and different species concepts. Each systematist/biologist brings their own prejudices, experiences, data and the theoretical framework of the species concept in which they operate when delimiting species. Herein lays the challenge: all systematists see the history of life as diversifying lineages and all species concepts are trying to capture that diversification.

The quest for a universally applicable species concept that will fit every living organism remains something of a holy grail (Niklas 1997). Almost all species concepts have merit but the main difficulty is that scientists try to reduce the infinite diversity of life into a single formula (Niklas 1997). Currently there is no consensus across the scientific community on the proper definition of “species” or in fact if a species category exists in nature (Mishler 1999, 2003). Mishler (2010) advocates that species properly defined are real entities but not uniquely real, and also asserts that life is organized in a hierarchy of nested monophyletic groups some of them very fine scale well below the level we currently call species.

Hennigan principles (cladistics) and classifications are routinely used, by systematists and monophyly is widely accepted as the only criterion for grouping taxa (Horandl 2006; Cellinese *et al.* 2012; Thiele & Ladiges 1994). The differences between proponents of monophyly and paraphyly reflect conceptual differences of evolutionary (Darwinian) and cladistic (Hennigan) classifications. Darwinian classifications request the consideration of two criteria **similarity** and **common descent** which allows grouping and ranking of taxa according to the Linnaean Hierarchies (George 2014). Hennigan classification accepts a single criterion i.e. **common descent** of monophyly for definition of taxa. Phylogenetic systematists regard and depict relationships as single evolutionary lines and with species as basic indivisible units. Hennigan principles are based solely on dichotomous branching patterns, where the ancestral taxon splits up and no terminal taxon can at the same time be ancestral, as the ancestor is by definition extinct or at least unknowable (Horandl 2006).

Proponents of Darwinian classifications argue that evolution is a process below the level of species. Hennigan proponents start at species level and paraphyletic taxa are an anathema to many systematists (Crisp and Chandler 1996). Darwinian classification considers that most evolutionary processes result in descendants without extinction of the parental group which

results automatically in paraphyly of the parental group (Horandl 2006). Whenever a monophyletic species is circumscribed it is possible that a paraphyletic or metaphyletic residual species is simultaneously recognised (Crisp and Chandler 1996). Attempts to place all organisms in a monophyletic taxon at every rank, regress to the population level where monophyly is no longer applicable leaving paraphyletic residuals. Both the phylogenetic species concept and the composite species concept predict non-monophyly of many species (Crisp and Chandler 1996).

The differences between proponents of strict monophyly and paraphyly are conceptual and are more than philosophical and will be the cause of much heated debate in the future. We are no closer to finding a single universally accepted definition of species and a single universal operational species concept than we were in Darwin's time (Cellinese *et al.* 2012). All of the species concepts discussed above in one way or another are unable to cover every variation for every living thing. All scientists faced with describing an organism must have a view on what constitutes a species in order to describe the particular organism or organisms they are looking at. Whatever species concept is used to delimit species, it has to be pragmatic, operationally useable and based in science. Without an operational species concept, no organisms can be described or named.

“In short we shall have to treat species in the same manner as those naturalists treat genera, who admit that genera are merely artificial combinations made for convenience. This may not be a cheering prospect: but at least we shall be freed from the vain search for the undiscovered and undiscoverable essence of the term species” (Darwin 1859 p. 485).

Operationally we have used a morphometric species concept with characters that identify the smallest groups of individuals that are consistently distinguishable using graphical and statistical analysis to test species limits (Phenetic analysis) (Sneath and Sokal 1973) (Chapters 2, 3 & 4). In conjunction with the use of diagnostic morphological characters, we have examined seedling morphology (Chapter 4), explored the taxonomic implications of floral colour in named and putative entities, (Chapter 5) and examined the development of lignotubers (Chapter 6). In addition we have scrutinized limited molecular markers (unpub. data) all of which are additive in delimiting species. Philosophically and operationally I seek to apply the phylogenetic, integrated species concept discussed and advocated by De Queiroz (2007).

Taxonomic issues

Extensive disjunctions

The extensive geographic disjunctions in the *B. spinulosa* complex appear to have been ignored in previous classifications, as have the implications of the resultant lack of gene flow. For example, the type specimen of *B. cunninghamii* was collected from Mount York in the Blue Mountains in New South Wales, but closely resembles plants distributed as far south as the New South Wales and Victorian border. It is reasonable to ask whether the population identified as *B. cunninghamii* (also identified in some herbaria as *B. spinulosa*) found at Wilsons Promontory is the same taxon as *B. cunninghamii* found 270 km to the north east in Lind National Park. Gene flow between the two populations is now most unlikely, given the disjunct nature of the populations at Wilsons Promontory; since there are also distinctive morphological differences between these populations (Chapter 3) it seems reasonable to treat these as two different taxa (*cf.* Evans *et al.* 2001). Similarly, we should ask whether populations of *Banksia collina sens. lat.* found at Expedition National Park or Isla Gorge National Park in central eastern Queensland are the same taxon as the geographically isolated and morphologically distinct populations of *Banksia collina sens. str.* found on the north coast of New South Wales (*cf.* Milner *et al.* 2012). A final example is provided by the populations of *Banksia spinulosa* found in New South Wales, south of the Hawkesbury River, which seem unlikely to belong to the same taxon as the morphologically distinct populations found at Kuranda National Park in north Queensland (also called *Banksia collina* in some herbaria). In addition to the natural disjunctions many populations have been fragmented by anthropogenic activity such as construction of highways, towns, and farms.

It is likely that climatic changes over time have contributed to the formation of these disjunctions. During the Quaternary period species ranges repeatedly shifted northward and southward and re-occurring glacial events contracted species into disjunct refugial populations (Hewitt 1996, 2000, 2004; Rossetto *et al.* 2012). Climate change over time and space has shifted the intensity of natural selection towards local optima. Although a species may shift to spatially track the same climatic range of temperature and moisture, species would still have to adapt to both biotic and abiotic interactions such as species competition, differing photoperiods, and

differing fire interaction (Mimura and Aitkin 2007). Geographic or habitat isolation are amongst the most important factors responsible for establishing the levels of reproductive segregation that lead to measurable genetic differentiation (Rossetto *et al.* 2011). Throughout much of Australia's east coast, the existence of terrestrial 'islands' (inselbergs) such as mountain tops, isolated headlands, and areas cut off by disturbances caused by anthropogenic activities or frequent fires, can function to isolate populations and reduce or halt gene flow (Narina *et al.* 2011).

Flower colour in the Banksia spinulosa complex (Fig. 3)

A further complication in the delimitation of specific and infraspecific taxa in the *Banksia spinulosa* complex is the occurrence of monomorphic, yellow-flowered populations in some locations and a mixture of red- and yellow-styled individuals in varying proportions in other locations. Populations that are polymorphic for flower colour have been variously identified as *B. collina*, *B. spinulosa*, or intermediate between *B. collina* and *B. spinulosa* (George 1988, 1999; Harden *et al.* 2000).

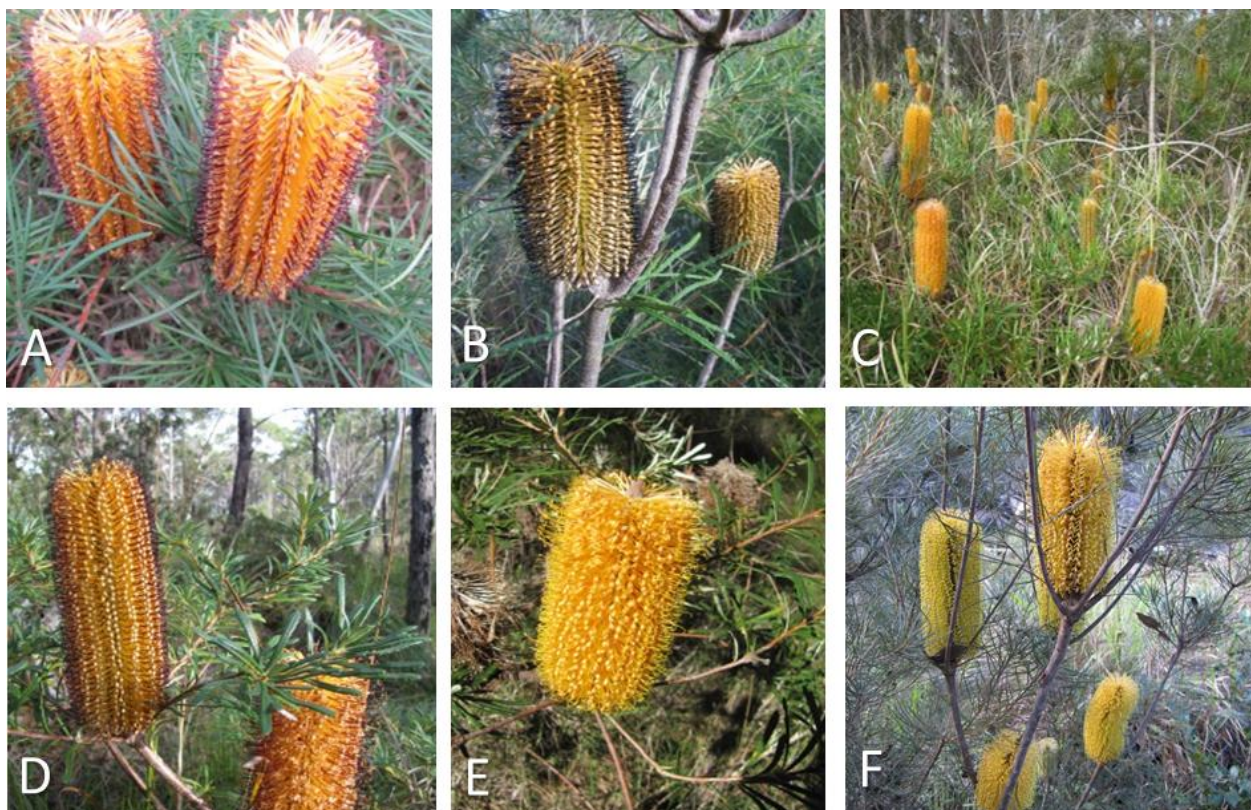


Fig. 3. Variation in style colour in the *Banksia spinulosa* complex. A. Wallum Trig, Queensland; B. Wilsons Promontory National Park, Victoria; C. Bongil Bongil National Park, New South

Wales; D–E. *B. collina sens. str.* Morisset, New South Wales; F. Blackdown Tableland National Park, Queensland.

Retention of juvenile leaf tothing into adulthood

The complexity in the pattern of the retention of juvenile tothing in leaves into adulthood in some populations and not in others has been the cause of much taxonomic confusion in the *Banksia spinulosa* complex. Juvenile tothing is almost universal in *Banksia*, and retention of numerous marginal teeth into adulthood has been used as a diagnostic character state for *B. collina s. str.* (George 1981, 1988, 1999; Harden *et al.* 2000; Stimpson *et al.* 2012.). However, using tothing along the leaf margins for identification without knowing when the population was last burnt or slashed is problematic (pers. obs. 2008–2015).

Relevance of this project

Biodiversity

The two current circumscriptions of the *Banksia spinulosa* complex are both limited by the lack of delimitation of species boundaries along the full range of the complex. The morphological variation, extensive disjunctions and a very broad distribution of the *Banksia spinulosa* complex are indicators that there is likely to be more diversity within the complex than any of the current circumscriptions have recognized. Resolution of the species limits within the *B. spinulosa* complex is likely to present an informed estimate of the diversity in the complex.

Conservation

Banksia vincentia has now received critically endangered species status 7 years after its discovery. In 2009 there were only 14 plants of *B. vincentia* in Australia now there are only eight living plants in the field. A conservation and regeneration strategy is urgently needed for this plant species to continue to survive. Many of the disjunct populations within the *B. spinulosa* complex are obvious candidates to test their taxonomic status to accurately assess their conservation requirements. No other taxon in the *B. spinulosa* complex has endangered species status possibly due to the artefacts of a broad species concept and the lack of extensive investigation into the *B. spinulosa* complex. An investigation into the full range of taxa in the *B. spinulosa* complex will inform any conservation strategies that may or may not be required to manage this diverse complex.

Horticultural value

Banksia species are one of the top ten taxa that account for 95% of the cut flower trade for export in Australia. Europe is a very competitive and quality-conscious market; Australia holds a niche market for high-quality *Banksia*. Within the next 30 years, Asia will represent the largest consumer market in the world. There is potential to expand into new Asian markets with perhaps the most significant being China where a very small rise in per capita consumption will translate into large sales (NSW DPI). Four of the five named species in the *B. spinulosa* complex are resprouters which allows for clonal propagation from adventitious shoots from a lignotuber.

Pharmaceutical value

It has been suggested that anthocyanins protect humans against chronic diseases because they are powerful anti-oxidants. However, the bioavailability of anthocyanins is low (<1%), making it highly unlikely that they promote health themselves as antioxidants. (Glover and Martin 2012). All known species and many of the putative entities in the in the *Banksia spinulosa* complex have some level of anthocyanin in the conflorescences and other tissues (Chapter 5). An investigation into the chemical composition of anthocyanins in the *B. spinulosa* complex may provide useful knowledge broadly relevant to the pharmaceutical use of anthocyanins.

Aims of the study

- 1) To explore limits of species in the *Banksia spinulosa* complex using phenetic analysis of morphological characters. Specifically, the general taxonomic goal of this project is to provide a stable, evidence-based classification that reflects both patterns of morphological variation and the limits of reproductive compatibility demonstrated by sympatric, phenetically distinct populations. The *B. spinulosa* complex was analysed morphometrically across its geographic range, to characterise and quantify patterns of morphological and geographic variation within the complex. In doing so, I aim to test whether any of the existing classifications accurately reflect morphological variation within the complex. The taxonomic implications of the morphometric patterns were considered in light of competing species concepts.

- 2) To describe seedling and juvenile morphology of species and putative species in the *Banksia spinulosa* complex. Specifically, the aims were to:
 - a) Test whether germination times differ significantly among recognised species and putative entities in the *B. spinulosa* complex;
 - b) Test whether the results of phenetic analyses of seedling characters are congruent with those of adult characters, and if so, determine the age at which such patterns emerge; and
 - c) Test seedling morphology to assess its taxonomic value.

- 3) To test the taxonomic value and understand the significance of flower colour in the *Banksia spinulosa* complex. Specifically, the aims were to:
 - a) Test whether style colour is of any taxonomic value in delimiting species in the *B. spinulosa* complex;
 - b) Chemically characterize anthocyanins for known and putative entities in the *B. spinulosa* complex;
 - c) Test the importance of edaphic factors (soil pH and N) in relation to species specific locations; and
 - d) Test the potential effect of pH on the ionic state of the anthocyanins in the *Banksia spinulosa* complex.

- 4) To explore the structure and function of the cotyledonary node in the *Banksia spinulosa* complex. Specifically, the aims were to:
 - a) Investigate the structure and function of the cotyledons and cotyledonary node;
 - b) Test the effects of mimicked herbivory on seedlings;
 - c) Test the effects of environmental stress on seedlings; and
 - d) Investigate the bud structure and development of lignotubers in seedlings and juvenile plants.

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Chapter 2, Paper 1

Could this be Australia's rarest *Banksia*? *Banksia vincentia* (Proteaceae), a new species known from fourteen plants from south-eastern New South Wales, Australia

Margaret L. Stimpson, Jeremy J. Bruhl and Peter H. Weston.

(2014)

Phytotaxa **163**, 269–286.

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This paper has been removed as it was published as the following journal article:

Stimpson, M., Bruhl, J. and Weston, P. (2014). Could this be Australia's rarest Banksia? *Banksia vincentia* (Proteaceae), a new species known from fourteen plants from south-eastern New South Wales, Australia. *Phytotaxa*, 163(5), pp.269-286.

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Chapter 3, Paper 2

A morphometric analysis of the *Banksia spinulosa* complex (Proteaceae) and its complex taxonomic implications

Margaret L. Stimpson, Peter H. Weston, R. D. B (Wal) Whalley and Jeremy J. Bruhl.

(2016)

Australian Systematic Botany **1**, 55–86.

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Stimpson, M., Weston, P., Whalley, R. and Bruhl, J. (2016). A morphometric analysis of *the Banksia spinulosa* complex (Proteaceae) and its complex taxonomic implications. *Australian Systematic Botany*, 29(1), pp.55-86.

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Chapter 4, Paper 3

**Seedling morphology within the *Banksia spinulosa* complex
(Proteaceae) is surprisingly diverse**

Margaret L. Stimpson, Boyd R. Wright, Peter H. Weston, R. D. B. (Wal) Whalley and
Jeremy J. Bruhl.

[Paper follows the manuscript format of *Australian Systematic Botany*]



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Chapter 5, Paper 4

**Floral colour in the *Banksia spinulosa* complex (Proteaceae)
is not mediated by pH**

Margaret L. Stimpson, R. D. B. (Wal) Whalley, Lynette McLean, Nicholas J Sadgrove,
Ben-Erik Van-Wyk, Jonathon Clay and Jeremy J. Bruhl.

[Paper follows the manuscript format of *Australian Systematic Botany*]



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Chapter 6, Paper 5

Cotyledonary node in the *Banksia spinulosa* complex (Proteaceae) is
linked to functional traits related to seedling survival

Margaret L. Stimpson, Peter H. Weston R. D. B. (Wal) Whalley, Christina J. Prychid, and
Jeremy J. Bruhl.

[Paper follows the format of *Australian Systematic Botany*]



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Chapter 7: General conclusions

Introduction

Not surprisingly, entities in the *B. spinulosa* complex have been taxonomically difficult for both specialist and non-specialists to delimit, given that: 1) all have juvenile toothed leaf margins; 2) some of the entities retain juvenile tothing into adulthood; 3) some populations are monomorphic for flower colour and some populations are polymorphic for flower colour; and 4) many of the named and putative entities exhibit extensive geographic disjunctions. The focus of this study was to investigate the taxonomy, ecology and biology of the *Banksia spinulosa* complex.

A morphometric analysis of specimens from the full range of the *Banksia spinulosa* complex resulted in 17 distinct groups (Chapter 3; see also Chapter 2). Five of these are named and there are also 12 putative new entities. Subsequent analysis of seedling morphology was taxonomically informative and corroborated the results of the adult morphometric analysis (Chapter 4). Phytochemical analysis provided information regarding the structures of some of the anthocyanins found in the conflorescences of entities in the *B. spinulosa* complex (Chapter 5). Observations of lignotuber development in lignotuberous and non-lignotuberous entities in the *B. spinulosa* complex have broadened our knowledge and understanding of resprouting entities within the *B. spinulosa* complex (Chapter 6), which may prove useful for regeneration for those entities that may receive endangered species status or propagation for the horticultural market.

Summary of findings against aims of the project

Species limits

In the preceding chapters multiple data sources were utilised to delimit species in the *Banksia spinulosa* complex. Phenetic analysis of morphological characters was used to delimit *Banksia vincentia* from the four known species in the *B. spinulosa* complex; viz., *B. spinulosa*, *B. neoanglica*, *B. cunninghamii* and *B. collina sens. lat.* (Chapter 2; Stimpson *et al.* 2014). That narrowly focused study was expanded to encompass collections from the full range of the *B. spinulosa* complex to assess the extent of the morphological variation within the complex (Chapter 3; Stimpson *et al.* 2016). The

results of these two analyses provided support for the five named species and for an additional 12 putative new entities.

Seedling Morphology

These studies of adults and seedlings have contributed substantially to our knowledge of the biodiversity found in the *B. spinulosa* complex, and will provide a solid framework for a complete revision of the complex. Seedling morphology provided additional morphological information that was previously unknown (Chapter 4); for example, some of entities in the *B. spinulosa* complex are heteroblastic and some are homoblastic. Patterns of tothing along the leaf margins across seedling and adults has provided novel information that corroborates the recognition of several of the putative entities based only on adult morphology.

Phytochemistry

This thesis presents novel information regarding the chemical structures of the anthocyanins that are found in the conflorescences of entities in the *B. spinulosa* complex and information from HPLC analysis has proved to be a taxonomically useful tool. Analysis using HPLC demonstrated that style colour differences were related to chemical differences in the anthocyanins and are not pH mediated ionic states, as are the petal colours of some garden ornamentals. Regression analyses completed on pH and N showed that each entity was restricted to soils with a specific pH and N range and that the ranges for many entities were statistically significant ($p < 0.05$).

Lignotubers

This is the first study of lignotubers applied to entities in the *Banksia spinulosa* complex. In fire-prone ecosystems 50–100% of the flora is composed of species that are capable of resprouting after fire (Williams *et al.* 2002; Enright *et al.* 2007). The majority of the entities in the *Banksia spinulosa* complex are resprouters with subterranean lignotubers. One of the species, *B. neoanglica* has two forms of habit, one that is a short multi-stemmed shrub and the other a single stemmed tree to 6 m (Stimpson *et al.* 2012, 2016). When seedlings of both forms were grown under glasshouse conditions a lignotuber did not develop on the tree form of *B. neoanglica* but the multi-stemmed entity developed a lignotuber. The ability to form a lignotuber is genetically controlled but the expression of that ability may depend on the influence of

edaphic and physiological factors on its development in seedlings (Beadle 1968; Weir 1969; Whittock *et al.* 2003). This work allows us to have a greater understanding of seedling survival and exactly how and where lignotubers start to develop in entities in the *B. spinulosa* complex.

Relevance of the project

Biodiversity and conservation

This thesis provides substantial scientific evidence to underpin a complete taxonomic revision of the *Banksia spinulosa* complex, i.e. an updated classification of its species, which needs to incorporate the biodiversity within the complex exposed in this thesis. Of the 17 groups identified by the phenetic analysis, *Banksia vincentia* is the only entity with critically endangered species status under New South Wales legislation. There are several other entities identified in this thesis that may also require endangered species such as *B. Carnarvon Gorge*, and *B. Croajingolong* and possibly the *B. McPherson Range* group. It is important that the previously hidden biodiversity in the *B. spinulosa* complex is given appropriate recognition, which will draw attention to those species and facilitate appropriate conservation initiatives.

Future Directions

Taxonomy

The circumscriptions of (George 1981, 1988, 1999; Harden *et al.* 2000) do not reflect the current biological reality within the *Banksia spinulosa* complex. Neither of the current classifications for the *B. spinulosa* complex is based on a corroborated hypothesis of evolutionary relatedness or indeed, a comprehensive morphological analysis of specimens. Meanwhile, in the absence of compelling evidence, users of taxonomy have been left in a state of confusion due to these two competing classifications. Under the unified species concept (De Queiroz 2007) and given the clear taxonomic evidence presented in this thesis, there is strong support for the recognition of at least 9 putative entities as species and further investigation on another 3. My first priority is to publish descriptions of those putative new entities shown to be valid as a result from this study. It is critically important to have an accurate record of the existing biodiversity.

Seedling morphology

Seedling morphology was particularly informative, despite the limited dataset due to unfortunate operational watering issues. Based on the taxonomic value that we were able to obtain from our results, a complete data set similar to the adult dataset, with seedlings from the full range of entities in the *Banksia spinulosa* complex would likely provide further insights and taxonomic clarity for the *B. spinulosa* complex.

Genetic analysis

The determination of chromosome numbers and ploidy would assist in providing more certainty with respect to the status of the *B. McPherson Range* populations. It is possible that polyploidy and or hybridization may reflect the residual taxonomic confusion concerning these populations. While our phenetic analysis (Chapter 3) placed the Lamington National Park and Mount Barney National Park entities (*B. McPherson Range*) in one group, our seedling analysis (Chapter 4), supported this to the extent of the material that was available for examination. Preliminary unrepresented data for ITS and microsatellite markers provided some confusing results for this particular group. Next Generation Sequencing approaches that skim more regions may be more productive.

Phytochemistry

Analysis of anthocyanin structures on all of the 17 groups identified in the phenetic analysis is incomplete. Identification of all of the flavonoid and anthocyanin structures may well provide us with a diagnostic character that is chemically and taxonomically useful.

Lignotubers

We now know the where, and how, of lignotuber development in the *B. spinulosa* complex, but do not know what the trigger for the expression of a lignotuber is. Given the presence of lignotubers in most members of the *Banksia spinulosa* complex and the variability of this trait in one species, the group provides a useful study group to understand and identify the trigger for lignotuber development in all species of *Banksia*.

Final remark

“Science does not rest upon solid bedrock. The bold structure of its theories rises, as it were, above a swamp. It is like a building erected on piles. The piles are driven down from above into the swamp, but not down to any natural or ‘given’ base; and when we cease our attempts to drive our piles into a deeper layer, it is not because we have reached firm ground. We simply stop when we are satisfied that they are firm enough to carry the structure, at least for the time being.” (Karl Popper 1959, p. 278)

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