THE WINSTON CHURCHILL MEMORIAL TRUST OF AUSTRALIA

Dr Kelly A. Shepherd – 2012 Churchill Fellow

The Australian Biological Resources Study Churchill Fellowship to investigate species diversity among fan flowers and other unique Australian plants

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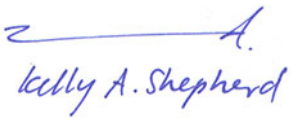
Signed  Dated 11 November 2013

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INTRODUCTION

The aim of my Winston Churchill Memorial Trust Fellowship was to investigate plant diversity and resolve potential new genera and species among two of my focus research groups – the fan flower family Goodeniaceae, and the genera *Lasiopetalum* and *Thomasia* in the family Malvaceae. My Fellowship had two broad aims: i) to examine important historical Australian plant collections including 'type' specimens housed in various European herbaria, and ii) to work with collaborators based in the USA to learn up-to-date molecular and micromorphological techniques to study floral development and symmetry in the family Goodeniaceae.

Scaevola aemula (Goodeniaceae)

Modern day taxonomists rely on a broad range of techniques to investigate relationships among related species. We examine the form and structure of plants in microscopic detail, utilising herbarium specimens and material we collect in the field to distinguish unique diagnostic features that characterise new species. We can also utilise molecular methods to understand the evolutionary relationships among related groups of plants, and compare the DNA of these species to confirm the closest relatives and determine if a plant is genetically unique. Molecular methods and statistical analyses of sequence data are being developed and refined all the time and scientists are continually gaining new insights into the drivers of species diversification.

This Churchill Fellowship provided me with a unique opportunity to travel to herbaria in the United Kingdom, Sweden and Austria to examine key historical collections to clarify my understanding of species concepts of a wide range of Australian plants. I then travelled to New York and Memphis in the United States to work closely with two research groups learning new molecular and micromorphological techniques to study floral development within the large (> 420 species), predominantly Australian plant family Goodeniaceae. We aim to determine if floral symmetry coincides with novel genes, highlighting possible drivers of biodiversity in this stunning plant family. We also worked towards completing a large, comprehensive molecular phylogeny of the family, which will clarify evolutionary relationships and inform my work on resolving the status of potentially new species and genera. This part of my Fellowship provided me with an excellent opportunity to foster a productive collaboration with world-class scientists that are at the forefront of new research. In the final week of my Churchill Fellowship I travelled to New Orleans with my American colleagues to the Botanical Society of America (BSA) 2013 Conference 'Celebrating Diversity', where we presented a poster we coauthored together. This broad plant science conference was a joint meeting between the BSA, International Association for Plant Taxonomy, American Fern Society, American Society of Plant Taxonomists and the American Bryological and Lichenological Society. There were more than 1000 attendees and a broad range of topics were covered including plant genomics, taxonomy, evolution, molecular systematics, ecology and conservation, biodiversity and climate change.

I would like to gratefully acknowledge the Winston Churchill Memorial Trust and the Australian Biological Resources Study (Department of Environment) who funded this Fellowship. Thanks to the following staff at the Western Australian Department of Parks and Wildlife: Drs Kevin Thiele and Stephen van Leeuwen for supporting my application, Dr Juliet Wege for providing advice on which European herbaria to visit and Bruce Maslin for letting me photocopy his handwriting samples of early European taxonomists. I would also like to thank the many people who assisted me during my travels and made my institutional visits so pleasant and productive. In the UK I

thank: Dr Martin Cheek and Tivvy Harvey, Royal Botanic Gardens, Kew; Jacek Wajer, British Natural History Museum; Christine Bartram, Cambridge University Herbarium and Mark Bickmore and his partner Janine Beeney who looked after us one weekend and took us to visit Winston Churchill's home 'Chartwell' near Westerham, Kent. In Europe I thank: Drs Ulf Arup and Torbjörn Tyler, Lund University Herbarium and Drs Vitek Ernst, Bruno Wallnöfer, Lia Pignotti, Naturhistorisches Museum in Vienna, Austria. In the USA I would especially like to thank the fantastic members of our informal *Goodeniaceae Working Group* including Drs Dianella Howarth and Brent Berger, St John's University (as well as their delightful students who supplied an endless flow of coffee and Dianella's husband Matt who cooked us breakfast every morning!), Drs Rachel Jabaily and Andy Gardner and their hard working undergrads Eden Johnson, Alex DeGenova and John Menz at Rhodes College in Memphis. Thanks also to Professor Scott Newstok and family who let us house-sit their lovely home in Memphis. We loved being so close to Rhodes College, Memphis Zoo and listening to live music at the Levitt Shell, and grew very fond of their three chooks. Finally, I would like to thank my husband Spencer Willis who accompanied me on this journey and was volunteered and trained by the *Goodeniaceae Working Group* to take detailed field photographs for our floral symmetry studies and has subsequently been working hard amongst the flies during the 2013 spring field season in the south-west of Western Australia.

Statue of Sir Winston Churchill in the town centre of Westerham in Kent, UK.

EXECUTIVE SUMMARY

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Project Description

The aim of my Winston Churchill Memorial Trust Fellowship was to examine historical Australian collections including 'type' specimens from the families Goodeniaceae and Malvaceae housed at the Royal Botanic Gardens Kew, Natural History Museum London and Cambridge University Herbarium in the United Kingdom, Lund University Herbarium in Sweden and the Naturhistorisches Museum in Austria. I also travelled to the United States of America to work with my collaborators in the *Goodeniaceae Working Group* (GWG) to learn new molecular and micromorphological techniques and discuss our ongoing research on the diverse and charismatic fan-flower family Goodeniaceae.

Highlights

- Examining important historical Australian plant specimens including collections made by Sir Joseph Banks and Dr Daniel Solander at Botany Bay in 1770.
- Visiting the Cambridge University Herbarium to examine the John Lindley collection, which will help me to resolve the status of many new Malvaceae species.
- Discovering so many unrecognised Goodeniaceae type specimens at the Naturhistorisches Museum in Austria.
- Learning flower bud dissection techniques in Dr Dianella Howarth's lab at St John's University, Queens and updating my molecular sequencing skills with Dr Andy Gardner in Dr Rachel Jabaily's lab in Memphis.
- Helping their undergrad student Eden Johnson prepare a poster for a conference.
- Working collaboratively with the GWG on joint publications and planning future work including both labs visiting Australia in 2014 with students to undertake fieldwork.
- Attending the Conference in New Orleans and doing the Honey Island Swamp tour.

Lessons

- While there is a global movement to scan type specimens, it is clear that Australian taxonomists still have a significant role to play in assessing collections in overseas institutions as many type specimens remain unrecognised.
- Without adequate knowledge of these earliest collections it is difficult to clarify the taxonomic status of potentially new species that are being discovered today.
- Notifying European herbaria of any research outcomes will ensure that their collections can be kept up-to-date and the new data can be made available online.
- Molecular DNA sequencing not only highlights evolutionary relationships but can prove useful in focusing taxonomic research in very large plant families.
- New techniques I have learned that preserve RNA from floral buds to assess floral symmetry can be communicated to local scientists to investigate other Australian plant families.
- International collaborations can be extremely fruitful and mutually beneficial. Overseas researches may have funding opportunities but limited access to Australian material. Aspects of this research will be jointly published in peer-review journals.
- Taxonomic resolution of putatively new taxa will provide description and distribution information and updated conservation assessments, resulting in more effective conservation management.

PROGRAMME

Royal Botanic Gardens, Kew
Richmond, London, Surrey, United Kingdom
Host: Dr Martin Cheek
3–5 June 2013

Natural History Museum, London
Cromwell Rd, London, United Kingdom
Host: Jacek Wajer
6–7 June 2013

Cambridge University Herbarium
Sainsbury Laboratory, Cambridge University, Cambridge, United Kingdom
Host: Christine Bartram
12–14 June 2013

Botanical Museum Lund University
Biological Museums, Porfyrvägen, Lund, Sweden
Hosts: Dr Ulf Arup and Dr Torbjörn Tyler
17–18 June 2013

Naturhistorisches Museum, Wien
Burgring, Vienna, Austria
Host: Dr Vitek Ernst and Dr Bruno Wallnöfer
20–21, 24 June 2013

St John's University, Queens USA
Utopia Parkway, Queens, New York, USA
Host: Dr Dianella Howarth
26–28 June 2013

Rhodes College
Memphis, Tennessee, USA
Hosts: Dr Rachel Jabaily and Dr Andy Gardner
1–24 July 2013

Botany Society of America 2013 Conference 'Celebrating Diversity'
Riverside Hilton, New Orleans, Louisiana, USA
26–31 July 2013

MAIN REPORT

i) Historical Australian plant collections

Background

Photographing the potentially new species *Anthotium* sp. Darling Range (F. Hort & B. Hort 2431), which is currently listed as threatened as it is only known from a small number of populations just east of Perth.

As a plant taxonomist my day-to-day work involves discovering, naming and describing new species and elucidating evolutionary relationships among related plant groups. I am based in Western Australia, which is a region of remarkable biodiversity, particularly in the floristically rich south-west. Currently there are over 12,000 native plant taxa (species, subspecies or varieties) described for the state. What many may find surprising is that we still have a large taxonomic backlog, as we have around 1500 plant taxa that are thought to be new but have not yet been named and described. Worryingly, more than 40% of these unnamed plants are poorly understood and are currently only known from a few populations and as such, are recognised as being rare or potentially under the threat of extinction. Perhaps of greatest concern is that many of these unnamed plants also lack accurate descriptive or distribution information and they are not included in general identification guides and keys. This means they cannot be readily identified by the general public, botanical consultants, industry or conservation practitioners. Moreover, new plant taxa are being discovered all the time through ongoing biological surveys or continued taxonomic study of known groups. In the face of escalating threats to our unique biodiversity, it is essential that taxonomic effort is focused on these vulnerable, unnamed plants to ensure accurate and up-to-date information is made widely available, which can in turn inform appropriate conservation and management decisions.

During the early exploration of Australia, naturalists made extensive plant collections which were distributed to herbaria all over Europe. Resident taxonomists began to utilise these specimens to name many of our currently known species. Unfortunately, only a few lines of Latin text often described these species and they were rarely illustrated. The scientific process that governs the formal naming of species (botanical nomenclature) has been refined over the last two centuries. There are now many rules that govern this process, outlined in an International Code of Nomenclature. To determine if a taxon should be recognised as new, a taxonomist must first decide what plants constitute that taxon (be it a subspecies, species or genus and so on) and what characters distinguish it as unique. Once circumscribed, a taxon must be published with a globally unique, Latin scientific name, comprising the genus and specific epithet. A key rule to naming a new species is allocating a ‘type’ specimen, which is a representative specimen(s) or illustration to which the name is permanently attached. In the 18th and 19th centuries taxonomic authors usually didn’t allocate a single type specimen (‘holotype’) but listed a number of collections in which they developed their taxon concept (‘syntypes’). Given the large species diversity in some groups, many of which show subtle variations that can only be described in detail, these early Latin descriptions were inadequate to distinguish closely related taxa. Moreover, because these authors had access to only limited material sent from Australia they may not have had an adequate understanding of the variation across all populations of a particular taxon. In some instances what we now understand as a widespread and variable species, may have been described as more than one species.

In this case, the earliest published name is the valid one and the subsequent name is then recognised as a synonym. When revising a plant group one of the most important tasks for a taxonomist is to clarify the type collections of known species and confirm if there are any synonyms present. In the case of syntypes, one specimen needs to be selected as the name-bearing type ('lectotype'). This helps to minimise confusion, particularly as it can become evident that more than one species may have been included in the original list of syntype specimens cited by the taxon author. Understanding the history behind the taxonomist who named a species and the specimens they examined is often critical in helping to resolving typification. The process then provides a sound basis to clarify the status of potentially new species within the group.

Accessing important historical specimens has always posed a challenge for Australian plant taxonomists. Specimens can be loaned to home institutions but overseas herbaria often send only a few samples of particular interest groups and not the whole collection. Sending material also constitutes a potential threat to these invaluable collections, as they could be lost or damaged in the post. In recent decades scanning technology has improved significantly and in 2003 a bold global project was initiated with the aim to digitise the world's type specimens. This involves taking high resolution images of around two million specimens and making them available online. This Global Plants Initiative (GPI), funded by the Andrew W. Mellon Foundation, now includes 166 herbaria from 57 countries around the world. This project is a particularly invaluable resource for Australian taxonomists, as this increases our access to collections that are housed in herbaria overseas. While the GPI project has already made significant headway, many type specimens are still to be scanned. More significantly, there are a large number of specimens currently stored in general collections that have not yet been recognised as type material. Taxonomic specialists therefore still have an important role to play in examining herbarium collections, as they are able to recognise these highly significant specimens and ensure they can be then be scanned and made available to all. The Australian Biological Resources Study sponsored Churchill Fellowship specifically provides funding for taxonomic research on Australian flora and fauna overseas and is an invaluable resource for this unique kind of research.

European Herbaria

The first aim of my Churchill Fellowship was to visit a number of European herbaria to examine collections focusing on two of my research interest groups, namely the family Goodeniaceae and the genera *Lasiopetalum* and *Thomasia* in the family Mavaceae.

There is a significant amount of taxonomic work required in both of these groups. The Goodeniaceae is ninth largest plant family in Australia with more than 420 species currently recognised (a few of which occur overseas, particularly in the Hawaiian Islands). Within Australia, 20% of the known species in this family are listed as threatened. The family Goodeniaceae was revised for the *Flora of Australia* series 21 years ago but since that time 37 taxa from five different genera have been named and there are at least 48 potentially new but as yet unnamed taxa, with one third of these listed as endangered. From the ongoing molecular DNA sequencing work undertaken by the *Goodeniaceae Working Group* it is clear that the taxonomic boundaries of some of the genera in the family are not clear and new genera may need to be recognised. The genera *Lasiopetalum* and *Thomasia* in the family Mavaceae have not been revised since the first volume of Bentham's *Flora Australiensis*, which was published in 1863! Of the 58 currently recognised species, 46% are threatened and of the 21 potentially new species more than half are also under threat of extinction.

The reason for visiting European herbaria was to identify type specimens from my resaerch interest groups, including multiple syntypes of a taxon so that I can begin the process of

selecting suitable lectotypes (the single name-bearing specimen of a taxon). Early collectors in Australia frequently made multiple collections of a plant to which they may have allocated a single collection number before shipping them to herbaria across Europe. In the situation where a taxon is found to be represented by multiple specimens with the same collection number, we aim to select a single lectotype based on a specimen that we believe the botanist who named and described the species actually examined. We can determine this if the specimens are housed in the institution where the author worked, or visited, or if the specimens have a label or notation with the author's handwriting. This obviously poses a challenge, as it is difficult to accurately determine the handwriting of particular authors who were working in the 18th and 19th centuries! There are some publications that provide examples of handwriting and prior to leaving on my Fellowship I was able to examine a wonderful lever arch folder of photocopied herbarium labels that Bruce Maslin, the resident *Acacia* expert at the Western Australian Herbarium, had made during visits to various European herbaria in the 1970s and 1980s. This proved to be helpful for the detective work I was about to undertake.

Royal Botanic Gardens, Kew

After arriving in London and settling into a B&B in Chiswick run by local artist Bob Osborne, my husband Spencer and I spent the weekend getting over our jet lag and exploring the local area (and acquainting ourselves with the local brews). A particularly lovely Sunday morning was spent wandering through the gardens at Hyde Park and being amused by the rhetoric spouted at Speaker's Corner – a proud tradition that has been upheld since 1872.

On Monday I headed to the new front reception of Kew Herbarium at the Royal Botanic Gardens. Established in 1853, Kew now houses over seven million plant specimens. It includes numerous historically significant collections such as those of Sir William Hooker, the first Director of Kew, and the prodigious taxonomist George Bentham, who completed the seminal *Flora Australiensis* (1863–1878). Many Australian taxonomists long to make the pilgrimage to Kew and even though I had visited 10 years ago I still felt a thrill of excitement as I walked through the front door. You never quite know what you are going to find once you start looking through their historical collections. Once notified of my arrival I was greeted by Dr Martin Cheek, the Head of the Wet Tropics Africa team, who has an interest in the family Malvaceae. Martin walked me through Kew's safety procedures and set me up at a desk in one of the old wings. Over the next three days I happily worked my way through the collections making detailed notes and photographing sheets and labels.

A wing of Kew Herbarium where specimens are housed over three floors, connected by red wrought iron stairs.

While at Kew I examined a total of 500 herbarium sheets and took 1950 photographs. These herbarium specimens included 416 sheets of 63 different taxa housed in the general collection and a further 75 type specimens representing 51 taxa, mostly in the genera *Lasiopetalum* and *Thomasia*. I also found 'new' type material including nine specimens of six different taxa that had previously not been recognised as significant. I annotated these sheets with a *Determinavit* slip and the specimens have already been scanned as part of the Global Plants Initiative and can be viewed online. Due to time constraints I did not annotate many of the specimens other than providing correct names for a number of sheets that were misidentified. I will be able to examine my images in detail over time and provide further feedback to Kew for other taxa that may be incorrectly identified.

Natural History Museum, London

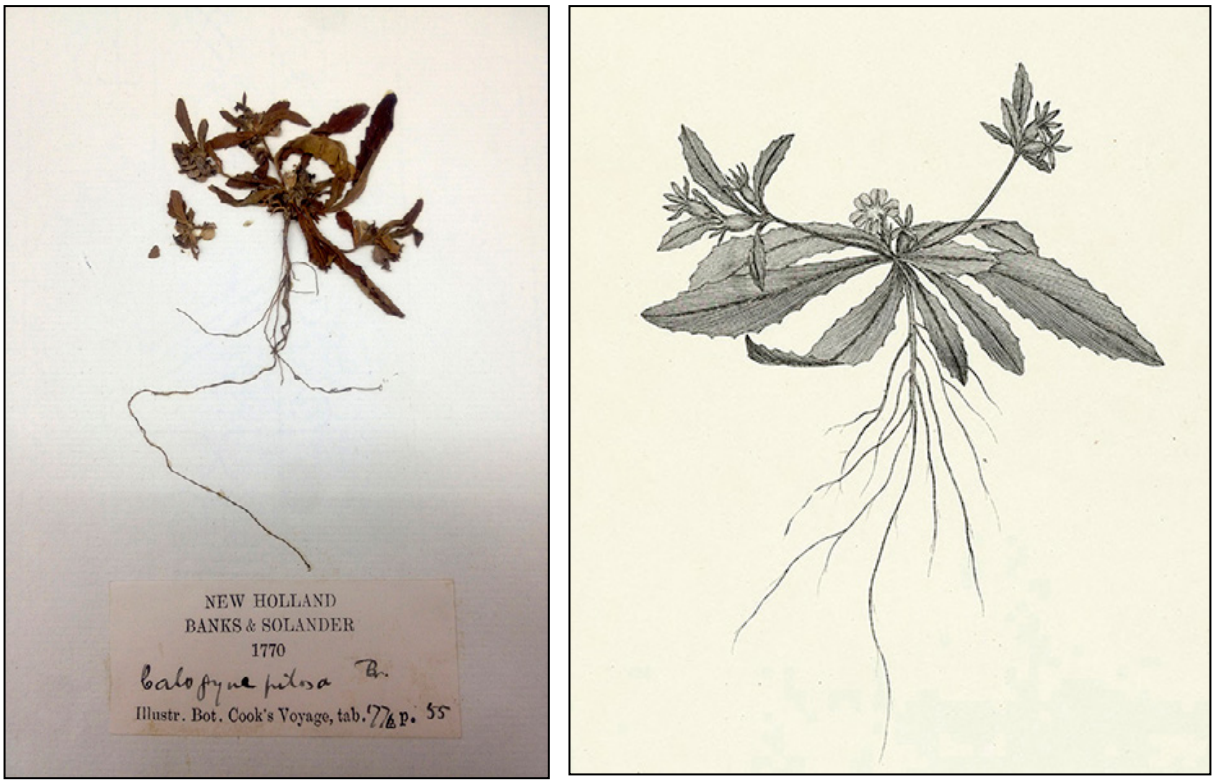
Following my visit to Kew I made my way to the Natural History Museum, London (NHM). It is a little overwhelming knowing there are an astonishing 70 million items housed in the collection of the NHM. This feeling is exacerbated by the wall of noise that hits you in the main hall due to the large numbers of school children excitedly looking at exhibits, and the looming presence of a massive 32m long replica of a dinosaur skeleton (*Diplodocus carnegii*). I smiled when I spotted the dignified statue of Charles Darwin himself, as I recalled that Dr Paul Doughty, another Western Australian ABRS Churchill Fellow, had been photographed standing in this exact spot two years earlier when he visited the collection to examine important Australian specimens of his beloved frogs and reptiles.

In contrast to Kew, the Herbarium of the Natural History Museum is now housed in the very modern climate-controlled Darwin Centre. Once I notified reception of my arrival I was greeted by Jacek Wajer who led me through a maze of rooms and back hallways (I got lost trying to retrace this path after lunch) and eventually through to the herbarium collection, stored in rows and rows of metal compactor cupboards. This herbarium, the first to be established in the United Kingdom, includes early botanical collections dating back to the 1600s and has expanded to now include more than six million specimens. There are many collections of special significance to Australians. Probably the most well-known are the specimens collected by Sir Joseph Banks when he voyaged to Australia in 1770 under Captain James Cook on the *Endeavour*, accompanied by the botanist Dr Daniel Solander and the talented botanical artist Sydney Parkinson. The *Endeavour* landed on the east coast of Australia on the 29 April 1770 and Banks and Solander made so many collections over the following days (more than 130), that Cook later named the area Botany Bay. Also of great significance to Western Australians in particular, are the collections made along the south coast in 1801/02 by the botanist Robert Brown who accompanied Matthew Flinders on the *Investigator* when he circumnavigated Australia.

During my time at NHM I examined more collections of *Lasiopetalum* and *Thomasia* as well as some interesting specimens of Goodeniaceae including a specimen of *Goodenia pilosa* (previously known as *Calogyne pilosa*) that was collected by Banks and Solander in 1770. Sydney Parkinson (who sadly died from Malaria during the voyage home) illustrated the plant and later an engraving was made, which wasn't published until 1901! In all, I examined 314 sheets of 51 different taxa and took 580 photographs. Of particular interest were type collections I examined of the small Western Australian genus *Anthotium* (Goodeniaceae), as I am working with my *Goodeniaceae Working Group* colleagues at Rhodes College to revise this genus using both morphological and DNA data. We included some of our findings in a poster we presented at the BSA Conference that we attended in New Orleans.

I also examined Australian collections of *Viola* (family Violaceae) on behalf of Dr Kevin Thiele, the Curator of Western Australian Herbarium, as he is trying to determine if there is a type specimen of *Viola hederacea* var. *elatines* as this taxon appears to have been described in 1862 from material cultivated in a garden "belonging to the Apothecaries Company, at Chelsea." Unfortunately, I found no corresponding specimen at NHM or later when I visited the herbarium at Cambridge University.

Unfortunately, I came down with a serious cold at this point and had to miss my last day at NHM. While I spent the next weekend sleeping, my husband Spencer continued his avid exploration of the museums of London. The following weekend Spencer's cousin Mark Bickmore and his partner Janine Beeney took us to Kent to visit Chartwell, Sir Winston Churchill's principle home near Westerham. I especially enjoyed the surrounding gardens as many plants were in full bloom and it is easy to see why Churchill loved this place so much.



A collection of *Goodenia pilosa* (formerly *Calogyne pilosa*) made by Sir Joseph Banks and Daniel Solander in 1770. Sydney Parkinson illustrated the plant, and later an engraving was made and included in the book published in 1901 “Illustrations of Australian plants collected in 1770 during Captain Cook's voyage round the world in H.M.S. Endeavour /by the Right Hon. Sir Joseph Banks and Daniel Solander, with determinations by James Britten.” http://plantillustrations.org/illustration.php?id_illustration=148097

Syntype collection of *Anthotium rubiflorum* by James Drummond (bottom row) at the Natural History Museum and a photo of the same species in the field.

Cambridge University Herbarium

The following week we travelled to Cambridge where I spent a very busy three days at the Cambridge University Herbarium. The collection was recently relocated to the basement of the impressive new Sainsbury Laboratory, a plant science research centre located in the Cambridge University Botanic Garden. While not open to the public, researchers are welcome to use the collection. Christine Bartram is currently the only full time staff member in the Herbarium and she was incredibly helpful. She directed me to the specimens I was most interested in, namely the John Lindley collection (which includes 58,000 sheets). Lindley was a botanist who worked for Banks and in 1840 published *Sketch of the Vegetation of the Swan River Colony* in which he described nearly 300 plant species from collections sent to him by James Drummond and Georgiana Molloy, among others. The importance of Drummond's collections have long been recognised as from 1835–1851 he collected nearly 50,000 plant and fungal specimens in Western Australia. However, Molloy's efforts have long been overlooked. Arriving in the colony as a young woman in 1830, Georgiana Molloy had a very difficult life as an early settler and tragically died giving birth to her seventh child only 13 years later at the age of 37. However, during her time in the fledgling colony she developed an avid love of Australian plants. She collected specimens and seeds for Drummond, the German botanist Ludwig Preiss, and Captain James Mangles who had a horticultural interest in south-western Australian plants. Mangles grew many of these species in his garden in London. It is apparent that some of these specimens made their way into George Bentham's and Lindley's herbaria where it seems that Mangles was credited for their collection rather than Molloy. A particular frustration for taxonomists is that Lindley's publication gave only very brief Latin descriptions and supplied no information on the specimens on which he based his taxon concepts. Consequently, his specimens at Cambridge are critical as we can see what sheets he personally examined.

During my time at the Cambridge University Herbarium I examined 90 specimens of 80 different taxa, made extensive notes and took 394 photographs. I also found 17 specimens that had previously not been recognised as type specimens. I also provided updated identification labels for specimens when I could. One morning while I was working through the collection Christine Bartram had some important visitors to the Herbarium and she gave a great talk on some of their most interesting collections, including specimens collected by Alfred Russel Wallace, who came to the theory of natural selection in parallel with Darwin. We also enjoyed walking through the lovely botanic gardens and glasshouses nearby.

Examining the type collections of the Cambridge University herbarium and making notes on the specimens of *Brunonia australis* (Goodeniaceae).

Botanical Museum Lund University

Folks sitting in front of the Malmö Rådhus (Old City Hall)

Leaving England we flew to Copenhagen in Denmark and then took a short train ride across the Baltic Sea to Malmö in Sweden. We spent the weekend exploring Malmö Castle, the 14th Century St Peter's Church, and admiring Old City Hall and various parks and gardens. On Monday I travelled to the town of Lund to visit the Botanical Museum of Lund University, which holds over 2.5 million specimens. My reason for visiting was to examine collections made by the German botanist J.A. Ludwig Preiss, who visited the Swan River Colony in late 1838. For three years Preiss travelled around the south-west and in that time he collected 3000–4000 specimens. These collections were subsequently divided and sent to 25 or more institutions across Europe and together with Drummond's collections, formed the foundation for early taxonomic studies on the Western Australian flora. A key reference set later used by J.G.C. Lehmann to describe numerous species in the multi-volume *Plantae Preissianae* (1844–48), is currently held at Lund University.

The Biological collections held by Lund University have been amalgamated into new facilities in recent years. On my trip there I began to worry that I had caught the wrong bus from the town centre as we progressed toward what appeared to be a light industrial area. When I stepped off the bus, I followed a guy who looked like he might be a science-type (there is something universal about my fellow geeks) and he unknowingly led me straight towards the facility. After notifying front reception of my arrival I was met by Dr Torbjörn Tyler, who took me through numerous doors and stairways past wonderful biological specimens (including large stuffed animals) to the herbarium collection. Over the next two days I had the opportunity to work through 102 specimens of 93 taxa and took a total of 542 photographs. I also found 24 hitherto unrecognised type specimens and made notes on barcode numbers of a further 39 types to be used in future publications. The information I was able to provide to the herbarium staff on specimen identifications has also already been added to their online database.

Naturhistorisches Museum, Wien

View from the Herbarium where you can see the line of statues on the roof of the Naturhistorisches Museum,

After visiting Lund we travelled back to Copenhagen and made our way to our last European stop in Vienna. I wanted to visit the Herbarium in the Naturhistorisches Museum, which houses more than 30 million objects and has a very active science program. On the morning I was due at the Museum I made my way to the back entrance (after stopping at a Viennese coffee house on the way). Once I had made it clear to security who I was (my high school German not helping me much at all) I was allowed into the inner courtyard and found my way to the lift that would take me up to the Herbarium. It is always fun to be allowed behind the scenes and, like many historical buildings, the oldest part of the Herbarium felt

like it was stuffed to the gills with specimens that were taking up every spare bit of space. I was met by Dr Bruno Wallnöfer, who showed me around and then set me up in a wing on the top floor, where I had a lot more space and could work at a long bench near a window that flooded the area with natural light. There was a fabulous view out over the city and I could see the back of a series of statues that line the edge of the roof of the Museum. It turned out that Dr Lia Pignotti, a Herbarium staff member working on the GPI scanning project, had been working through scanning Goodeniaceae type material very recently so I hoped my time at the Museum would be relatively straight forward. Over the next three days I was rather harried, taking 1750 photographs of the 361 sheets of 188 taxa that I examined. Among these I found an astonishing 63 specimens that were unrecognised type sheets! This is surprising as most of the types were of species in the family Goodeniaceae, which has already been scanned for the GPI project. Moreover, the family Goodeniaceae was revised more than 20 years ago for Volume 35 of *Flora of Australia* and I had assumed most of the type material would have been recognised at that time. This final Herbarium visit really highlighted how important these historical collections are, and the continuing vital role Australian botanists have to play in assessing this material and using it to resolve current taxonomic issues. Another benefit of looking at these specimens is that they may include important ancillary information such as notes and illustrations that may have been produced from living material at the time, for example the simple line drawings of flowers found attached to a *Brunonia australis* specimen.

Herbarium specimen of *Brunonia australis* collected from Dandenong, Victoria in the 1870s (left); line drawings of dissected flowers attached to the specimen (top right); field image of *Brunonia australis* showing an inflorescence that forms a compact head of multiple individual flowers.

ii) Molecular and micromorphological techniques to study floral development and symmetry in the family Goodeniaceae

Background

With 12 genera and more than 420 species, the Goodeniaceae comprise a diverse and often eye-catching element of the Australian and Pacific Island floras. The family includes herbs, scramblers, shrubs and small trees, and display considerable variation in floral morphology, including petal structure, colour and symmetry. Many of the more colourful species are considered to be of significant horticultural value. The Goodeniaceae are also characterised by an interesting and unique pollination system, of a cup-like stylar indusium that collects pollen as it develops in bud, which is presented to potential pollinators as the flower opens. Many species in the family are narrow-range endemics that are under significant pressure from a wide range of natural and anthropogenic threats.

Scaevola globulifera has a 5-petaled flower, which exhibits the fan-like orientation typical for the genus. The cup-like indusium, unique to the family Goodeniaceae, can also be seen at the apex of the curved style.

The Goodeniaceae were revised more than twenty years ago and significant taxonomic changes have occurred since then, such as the inclusion of the family Brunoniaceae and the description of 37 new taxa from five genera. Work is ongoing, as there are 48 potentially new taxa that remain undescribed, many of which are considered to be of conservation concern and potentially under threat of extinction. An international collaboration colloquially named the *Goodeniaceae Working Group* has been using molecular DNA sequences to elucidate the evolutionary relationships within the family. Two major groups were resolved from these analyses with the genera *Lechenaultia*, *Anthotium* and *Dampiera* forming a related group and a larger 'Core Goodeniaceae clade' comprising *Brunonia australis* next to *Scaevola* (including the monotypic genus *Diaspasis*) and a group of the remaining genera *Coopernookia*, *Goodenia*, *Selliera*, *Velleia*, *Verreauxia* and *Pentaptilon*. These data also suggest that the largest genus, *Goodenia,* resolves into three different groups and that the genus *Selliera* and species *Scaevola collaris* should be included within it. Current infra-generic classifications are also problematic. It is evident that a lot more detailed morphological investigations are required to resolve the status of genera and infra-generic groups, as well as the undescribed, potentially new species in this interesting family.

Goodeniaceae Working Group

The *Goodeniaceae Working Group* (GWG) represents an informal but dedicated International collaboration between myself and two labs in the US including Drs Dianella Howarth and Brent Berger at St John's University, Queens and Drs Rachel Jabaily and Andrew 'Andy' Gardner at Rhodes College in Memphis. The group has been collaborating for a number of years after discovering we were working in parallel on a molecular phylogeny of the family. Instead of competing against each other and rushing to publish independently, we decided to join forces to produce a coauthored paper. Together we have a broad range of research skills in molecular systematics, developmental genetics and taxonomy and this relationship has proved so fruitful we've planned ongoing projects. Currently we are working together on a National Science Foundation funded grant *Phylogenetics and Floral Symmetry Evolution of the Core Goodeniaceae*.

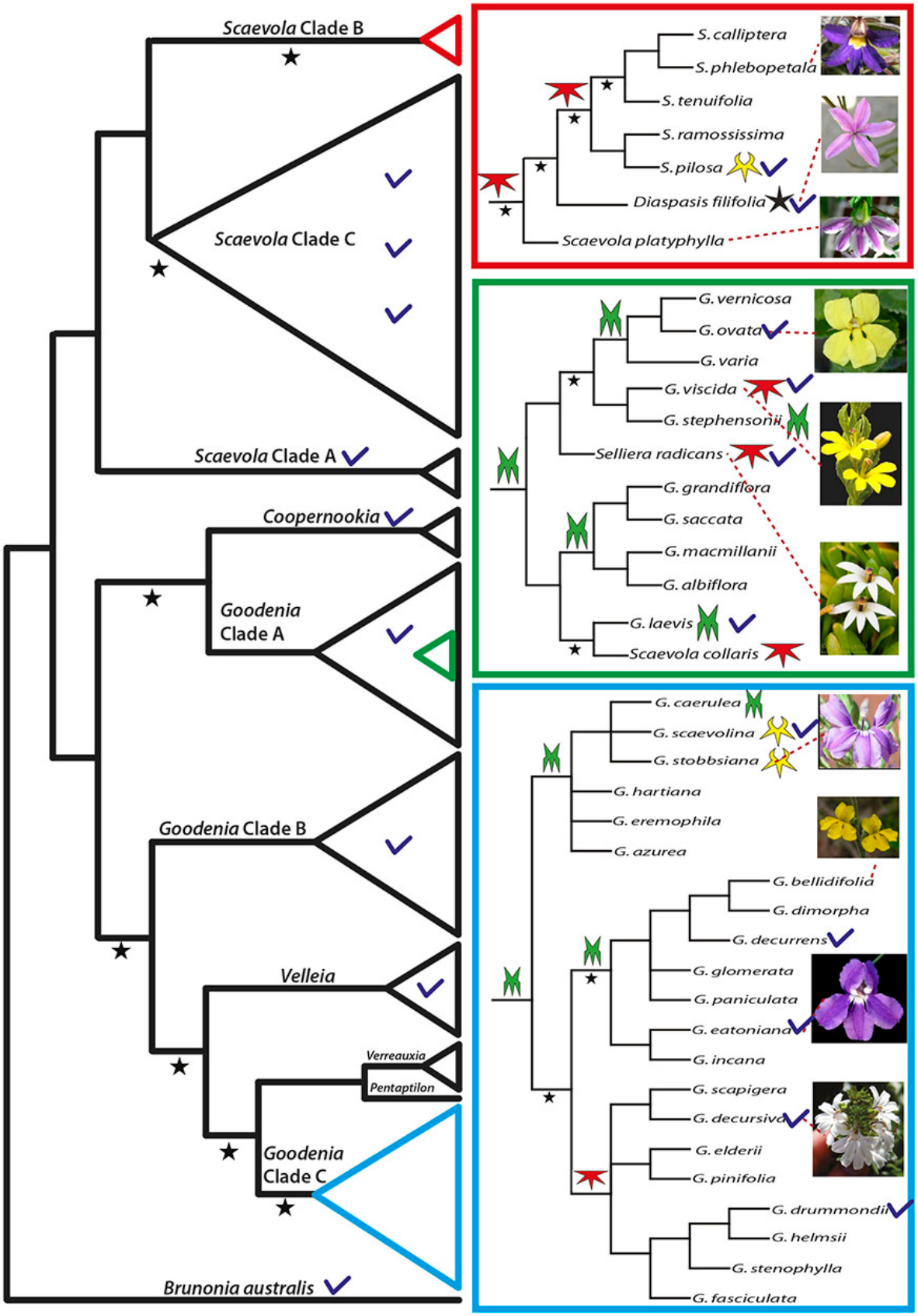
It is evident to us that the genus *Goodenia* is much more variable in floral morphology than *Scaevola*. Typically flowers of *Scaevola* have a fan-like orientation. However, *Diaspasis filifolia*,

The genus *Diaspasis* currently includes only a single species, *D. filifolia* shown here with its distinctive almost radially symmetrical flowers. Surprisingly, recent DNA evidence suggests that this species belongs to the genus *Scaevola*, which typically has fan-like flowers.

which has been shown to be so genetically close to *Scaevola* that it should be included within the genus, has flowers that are pseudo-actinomorphic (radially symmetrical). In contrast, the majority of flowers in *Goodenia*, *Velleia* and *Verreauxia* are bilabiate and generally yellow. However, there are exceptions and fan-like flowers have evolved multiple times within *Goodenia* (see image below). Intermediate symmetry forms with dorsal petals angled between the bilabiate and fan-flowered positions also occur. The actual number and direction of transitions in floral symmetry, and the evolutionary significance of these shifts is yet to be determined but we hypothesise that these shifts have been a key driver of lineage diversification.

In order to interpret the evolutionary history of transitions in floral symmetry, we need to understand the genetic developmental pathways by which those morphologies are produced. To test this we must produce a robustly sampled and resolved phylogeny (evolutionary framework of the family), objectively categorise floral symmetry types and determine developmental patterns in bud, as well as identify and trace the evolutionary history of the genes involved in controlling floral symmetry. In our current NSF funded project, the GWG aim to continue using molecular sequencing, including new Next-generation methods, to establish a robust phylogeny with near complete sampling of the Core Goodeniaceae. For a select group of species we will determine differences in floral symmetry development using Scanning Electron Microscopy (SEM) analysis of multiple developing flower bud series, and quantify floral symmetry types using geometric morphometric analysis of flower photographs. We will also use this target group of taxa to examine RNA expression of key floral symmetry candidate genes in floral tissue, so we can begin to understand the genetic pathways that influence floral symmetry shifts, which will also help us to better interpret the homology of floral morphology interaction with pollinators.

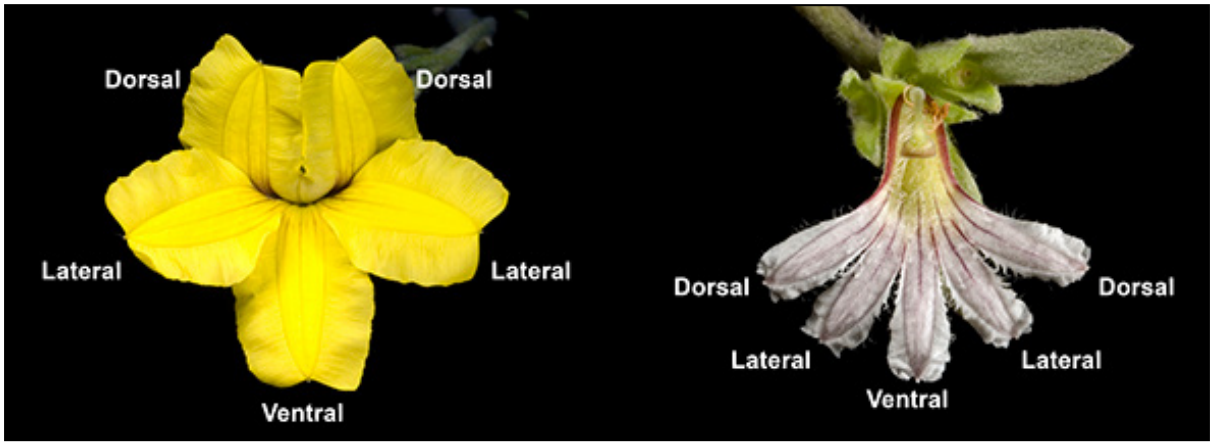
While labs in the US may have access to funds, it is difficult or them to undertake research on overseas plant groups. From the Australian perspective, working with international collaborators provides access to teams that are undertaking cutting-edge research. Training opportunities are limited, as to my knowledge no labs in Australia are currently doing this kind of floral evolutionary development (‘evo-devo’) research. This Churchill Fellowship allowed me to spend time in both US labs learning new techniques. I am already implementing these new methods by collecting samples during the 2013 spring season for our NSF study, but I will also be able to impart these skills to other interested researchers. This Fellowship also provided me with a chance to finally meet all of the team in person! This time together has allowed us to plan our ongoing research and set up a virtual lab management system and plan online lab meetings, which will all result in a series of publications that will significantly increase our current knowledge on one of Australia’s most beautiful plant families.



Phylogeny of Core Goodeniaceae from Jabaily et al., (2012) with mapped floral symmetry transitions. Ancestral state reconstruction of basic floral symmetry type is depicted at nodes with fan-shaped (),pseudo-bilabiate (fan-shaped with curved dorsal petals) (), true bilabiate (), or pseudo-actinomorphic (). The 20 target species for sampling in Australia in 2013/14 are denoted with blue checks.

St John's University, Queens

We arrived in New York and met Rachel Jabaily at the designated spot in front of a coffee shop in JFK airport. Even though we have only spent a short time together in person at a conference in Australia and when she visited the Western Australian Herbarium with her mentor the late Dr Tim Motley, I felt I was meeting an old friend after years of online correspondence and skype chats. We were soon bundled into Dianella's minivan after a hug hello, finally meeting her for the first time in 'real life.' Dianella had kindly insisted on us all staying with her family in Queens and so started a week of non-stop talking. While my husband Spencer spent his days wandering New York City, the GWG would gather at Dianella's lab at St John's University to discuss the scope of our project, everyone's respective roles and the planned publications that would result from our research, sustained by an endless flow of coffee. Dianella and her postdoctoral fellow Brent Berger spent time teaching us how to sample young floral buds for RNA extraction for our evo-devo work using Dianella's beloved 'Purple Fan Fare' *Scaevola aemula* pot plant, the only Australian Goodeniaceae available in horticulture in the US. Good eyesight and a steady hand were required as I had to learn to gently unfold the petals from each bud and separately sample the petal groups into separate tubes. We discussed the age of the buds required for the RNA extractions as well as what would be needed for the Scanning Electron Microscopy (SEM) development study. The team also spent considerable time selecting the species we would target for intensive sampling across all groups as this would dictate the field work I would undertake on returning to Australia.



Goodeniaceae flowers comprise 5 petals: two dorsal, two lateral and a single ventral petal. Typical *Goodenia* 'bilabiate flowers' (left) *Scaevola* 'fan-flower' (right). Petals need to be sampled from young, unopened flower buds and placed in separate tubes that contain RNA fixative. Whole, very young buds are also sampled as a control along with two tubes of young leaf material.

We developed protocols for handling large data sets between the different labs, and created shared documents in Dropbox to ensure everyone had access to the same information and could keep up-to-date.

Dr Dianella Howarth at St John's University, Queens showing Drs Andy Gardner (left) and Brent Berger (right) how to sample buds of *Scaevola aemula* for our RNA expression work.

Towards the end of our stay, Dianella had a family BBQ and invited all of her students to dinner. It was a lovely way to end our time with such a dynamic group. We spent our last evening in the city amongst the chaos of Times Square and after having dinner in a

typical NY Diner we explored the High Line. This is an amazing 1.6 km narrow park established on an elevated old railway line on the West Side, full of local prairie plants and astonishing views of the Manhattan streets below. On our last morning we again braved the Big Apple. Mesmerised by the beautiful works in the Metropolitan Museum of Art I wished for a moment that I was an artist…. but considering I have zero artistic ability I reconciled myself to sticking with science!

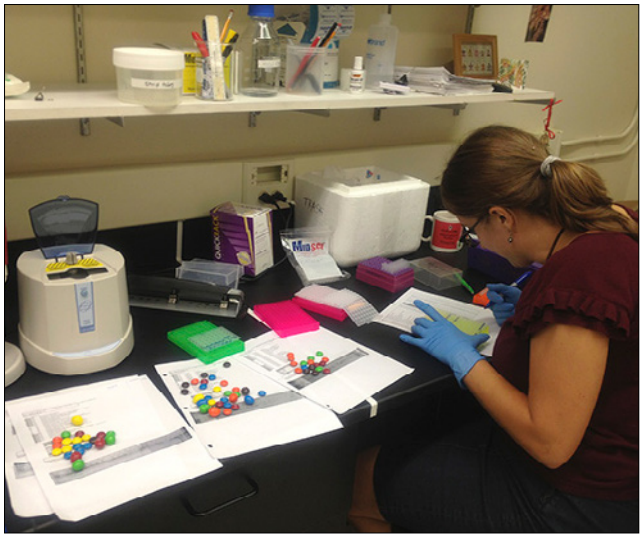
Goodeniaceae Working Group post-lab meeting in Times Square with Drs Andy Gardner, Rachel Jabaily and Brent Berger.

Rhodes College, Memphis

The beautiful library and grounds at Rhodes College

At last we made it to Memphis. After more than five weeks on the go it was nice to settle into one place for a while. As we aimed to be in the city for nearly a month, Rachel organised for us to stay at Dr Scott Newstok's house, a Shakespearean Professor at Rhodes who was away on holiday with his young family. We enjoyed looking after their three chooks and had lovely chats with their elderly neighbours who were very curious about the Australian couple staying next door. The next few weeks were intensive as I spent long hours working in the lab with Rachel and Andy Gardner and their hard-working undergrads Eden Johnson and Alex DeGenova. We aimed to get as much molecular sequencing of the samples I had previously sent from Australia done as possible, as well as continue to analyse the data. It was great to update my lab skills and we spent considerable time assessing and organising the mountain of data we were generating. We also spent time helping Eden prepare a poster for the BSA Conference on the south-west Australian genus *Anthotium* (Goodeniaceae).

A consideration of our molecular phylogeny work is that there are still some groups within the Core Goodeniaceae that are not fully resolved and this is necessary before we can make



M&Ms to help Rachel and the team get through the long hours of lab work at Rhodes College

decisions to taxonomically recognise new genera within the family. We decided that in order to clarify these relationships, we would try whole plastome sequencing via Next Generation Sequencing technology. We refined the list of exemplar species for this sequencing and subsequent evo-devo work, so that it adequately represent all of the major, currently well-supported clades. This provided me with a final hit list for spring sampling once we were back in Australia.

Along with the floral evo-devo work we want to statistically assess the symmetry of mature Goodeniaceae flowers using geometric morphometric software analysis. To achieve this aim, a series of comparable photos of the mature flowers of each of the target species needs to be taken. The flowers also need to be orientated properly, so that points could be landmarked on the images for subsequent analysis. My husband Spencer, who would spend some afternoons in the lab with us before heading to the Rhodes College pool, was 'volunteered' by the team to take these images for our planned field work while I would do the rest of the sampling for RNA, DNA and SEM work as well as taking herbarium specimens and field photos. Spencer took this with good grace and had a few practice sessions with Andy to work out the best methods for taking the images.

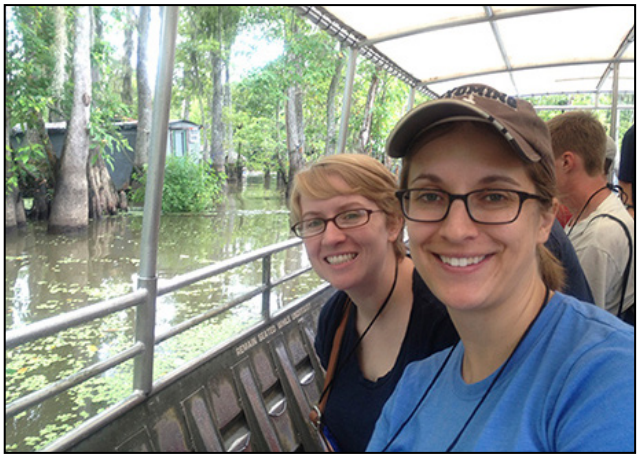
The lab took great care of us during our time in Memphis with meetings at the local Café Eclectic, family dinners and traditional BBQs. We were taken to a Baseball game, BB Kings Restaurant on the famous Beale Street and we watched the 4th July fireworks from the roof of an apartment building overlooking the mighty Mississippi and the Bridge to Arkansas. We had a lab outing to Overton Park to look for plants for the College herbarium, a trip to the Memphis Zoo and of course a memorable Saturday afternoon at Graceland – speechless!

BSA Conference, New Orleans

We found it hard to say goodbye to Memphis as we thoroughly enjoyed our time there and felt we had experienced a small but memorable taste of life in the South. However, I was soon distracted as we headed to New Orleans to attend a workshop and Botanical Society of America Conference 'Celebrating Diversity'. Field excursions were offered as part of the conference and we opted to do the Honey Island Swamp boat tour, which was a fantastic opportunity to see some unique Louisiana plants and animals.

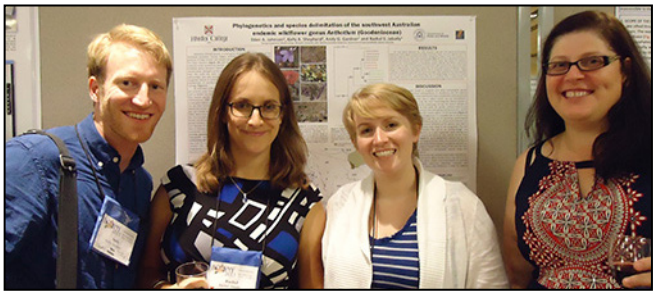
The (mad?) Honey Island Swamp tour operator leaning out of our boat to grab the tail of an alligator.

At the conference I attended an iPlant workshop that introduced some of the tools and data management cyberinfrastructure that the iPlant Collaborative is developing to handle large datasets. I also attended the annual meeting of the US Virtual Herbarium



Eden Johnson and Dr Rachel Jabaily on the Honey Island Swamp Tour.

and it was interesting to contrast their approach and progress with Australia's Virtual Herbarium project, which has been providing online information on Australia's plant specimen data for some time. Over the next three days I almost suffered from information overload. I was amazed at the number of attendees at the Conference and just how big the plant science community is in the US compared to Australia. The range of talks were interesting and varied and with so many joint sessions it was sometimes difficult to decide on which talks to attend. I learned a great deal and thought many talks had relevance to our own research interests. It was also enjoyable to see Eden attend her first conference and present our poster (abstract below). She is planning on attending graduate school next year and is currently considering pursuing a thesis project on Australian Goodeniaceae!



Poster coauthers (R to L) myself, Eden Johnson and Drs Rachel Jabaily and Andy Gardner.

Abstract Number: PSY008 **Abstract ID:** 568

Phylogenetic analysis and species delimitation of *Anthotium* (Goodeniaceae), a charismatic clade of Australian wildflowers.

Johnson, Eden [1], Shepherd, Kelly [2], Jabaily, Rachel [3]

1 - Rhodes College, 2000 N. Parkway, Memphis, TN, 38112, USA
2 - Western Australian Herbarium, Department of Environment And Conservation, Locked Bag 104, Bentley Delivery Centre, Bentley, 6983, Australia

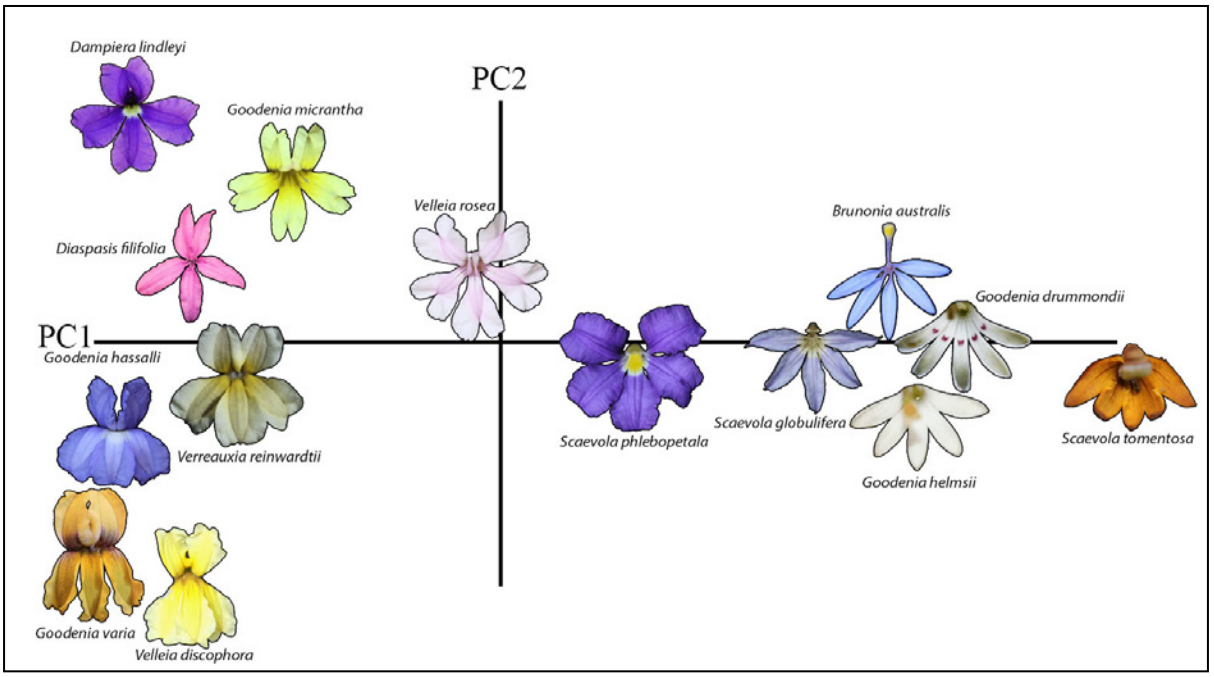
3 - Rhodes College, Botany, 2000 N. Parkway, Memphis, TN, 38112, USA

A molecular phylogenetic analysis of intra and inter-specific relationships within *Anthotium* (Goodeniaceae), a monophyletic genus of wildflowers endemic to the global biodiversity hotspot of the Southwest Australian Floristic Region (SWAFR), is presented. Species of this small genus are distinguished from representatives of the closely allied and much larger genus *Dampiera* in their flower color and form, the presence of numerous ovules and a distinct capsular fruit. Unlike related genera in the Goodeniaceae, *Anthotium* is confined to the SWAFR, a unique area that supports an extraordinarily diverse flora and fauna. This ancient and complex landscape has undergone significant fragmentation in the last 120 years due to extensive clearing for agriculture, and current vegetation remnants are under further threat from mining, urbanization, Phytophthora dieback, increased fire frequency and weed invasion. Two potentially new taxa have been identified in the genus from the Darling Range, a low escarpment on the Swan Coastal Plain east of Perth, and from the coastal region some 300 km to the south. Taxonomic delineation of closely related taxa can be difficult in regions such as the SWAFR where the flora often exhibit complex patterns of subtle morphological variation and genetic structuring. This molecular research was conducted, in part, to provide an independent data set that will complement morphological assessment of these taxa. The current study produced DNA extractions of multiple individuals from each of the four currently recognized species and two potentially new taxa from throughout their geographical range. PCR amplification of ca. 2500 basepairs of chloroplast spacer regions *matK, psbA-trnH*,*trnL-trnF* and nuclear ribosomal region ITS was sequenced, aligned with Geneious 6.0 and analyzed with maximum parsimony, maximum likelihood and Bayesian inference. Accessions of*Anthotium* divide into two major well-supported clades, and currently recognized species including the morphologically diverse *A. humile* may not be monophyletic. This research, in conjunction with ongoing morphological assessment of the genus, will result in a more robust taxonomy, which is essential in a region where conservation actions may be dependent on understanding the taxonomic status of potentially new but as yet undescribed taxa.

It was truly hard to say goodbye to the GWG after the Conference ended but I take comfort in the fact that we will see them next year when they plan to travel to Western Australia to do further field work. As I had organised to take annual leave at the end of my Churchill Fellowship, Spencer and I then headed off on a memorable month-long trip through the US.

Returning to Australia

We returned to Australia just as spring started, so after dealing with my most urgent emails I began to plan a number of field trips so we could begin sampling the target species needed for the Goodeniaceae evo-devo and floral symmetry work. In the nine weeks since we have been back, we have travelled more than 3000km and managed to sample almost all of our target species. Spencer has been busily photographing flowers using a small stand with alligator clips and a light box while fighting off the flies. Two batches of bud petals have also been shipped to the US for RNA extraction and we are anxiously waiting to see how they have worked. We have shared the flower photographs with the GWG through Dropbox and Andy Gardner and an undergrad student John Menz have already begun preliminary analyses of the images. They have tested the number of landmark points most appropriate to accurately capture floral symmetry and have run principal components analyses on the images with very promising results. It is incredibly satisfying to see our efforts already being put to such good use.



A preliminary Principal Components analysis of floral images shows that the x axis (PC1) explains 90% of the variation in symmetry represented by the angle between the two dorsal petals. For example the petals of *Goodenia hassallii* (far left) are close together, while the same petals in *Scaevola tomentosa* (far right) are almost 270 degrees apart. The second most correlated measure in the y axis (PC2) representing 9% of variation, is explained by the distance between the two lateral petals either side of the ventral petal at the bottom. For example lateral petals of *Damperia lindleyi* (top left) are spread far apart while the same petals in *Velleia discophora* (bottom left) are much closer together.

CONCLUSIONS

My Churchill Fellowship was an interesting contrast between examining the oldest of Australian plant collections stored in herbaria in Europe and learning the most recent and sophisticated methods of RNA and DNA extraction and analysis in the United States. Both of these divergent methods can be utilised to help us understand species diversity among important Australian plant groups.

My examination of historical collections, all of which have been captured in a digital library for future reference, has provided me with a solid foundation for my ongoing taxonomic research. The analysis of these specimens and more recent collections held in Australian herbaria, in combination with targeted field work, will help clarify the circumscription of numerous putatively new taxa, many of which are under conservation threat. The naming and description of these new taxa will provide essential information that in turn will result in more effective management of these vulnerable plants. Taxonomic work will also improve the curation of herbarium collections both in Australia and overseas, clarify distribution data, and provide updated conservation assessments of threatened taxa.

It is also important to realise that while ambitious projects like the Global Plants Initiative are scanning historical type specimens and making them available online, significant numbers of unrecognised type specimens may be present in the general collections. This can result in potential issues in selecting suitable lectotype ‘name-bearing’ specimens and could result in future taxonomic confusion. It is clear that it is still worthwhile for Australian taxonomists to visit overseas herbaria to examine historical specimens.

Molecular data and novel information from floral evolution studies obtained through continued collaborative research on the family Goodeniaceae, will provide further baseline information to underpin conservation management of this diverse group, and will clarify evolutionary relationships, aallowing the status of genera and species within the family to be resolved. By studying floral symmetry evolution within Goodeniaceae and the genes that underlie it, we can also identify how different methods of diversification, such as pollination syndrome, habitat specialisation, and dispersal ability affect speciation.

We still know so little about much of our local flora and this research will help us to learn more about our uniquely Australian natural heritage.

RECOMMENDATIONS

- Historical plant collections still have relevance in clarifying current taxonomic issues, highlighting the critical role Herbaria play in protecting these invaluable specimens and facilitating present day research.
- Australian taxonomists still have a significant role to play in assessing overseas collections and locating unrecognised type specimens and should be supported to visit key institutions.
- Providing feedback to overseas institutions enables new taxonomic findings to be immediately updated on online databases, ensuring they are available to all.
- Molecular DNA sequencing can prove useful in focusing taxonomic research in very large plant families.
- Fostering successful international collaborations provides a means to access resources

and share expertise, enabling new skills to be brought to Australian shores.

- International collaborations can be mutually beneficial and successfully managed through prudent use of online data sharing tools and video conferencing.
- The Goodeniaceae family includes numerous beautiful species that show great horticultural potential. Information on this popular family can be made widely available not only to the scientific community but also the general public through publications, online image data, and presentations to community groups such as local wildflower societies and garden groups.