Taxonomic Study of Rhododendron calophytum and its Immediate Allies

A Systematic Consideration of their Relationships to the rest of Subsection Fortunea within Subgenus Hymenanthes

Thesis submitted in partial fulfilment for the MSc in Biodiversity and Taxonomy of Plants

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Cover photo:

Rhododendron calophytum

var. *calophytum* in the

woodland garden at

Inverleith, RBGE. Wilson

#4279

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document were taken by

the author.

Abstract

The classification of *Rhododendron* subgenus *Hymenanthes* divides the species in this subgenus between 24 subsections is based on traditional morphological characters but its evolutionary history is poorly understood. Hymenanthes has recently been shown (using cpDNA) to contain two clades; the Tertiary Relict clade containing eight species from subsection *Pontica*, along with three anomalous species: *R. calophytum*, *R. praevernum* and *R. insigne*, and the Southeast Asian clade, containing all remaining species sampled from *Hymenanthes.* This study explored the relationships between the rogue species from subsection *Fortunea; R. calophytum,* and *R. praevernum,* and their closest allies: *R. asterochnoum* and *R. sutchuenense*, henceforth the 'Calophyta group'. Relationships of the *Calophyta* group to the rest of *Fortunea,* and *Hymenanthes* were then also unsertaken. A taxonomic revision of the study group species based upon a classic morphometric analysis was carried out in conjunction with phylogenetic analyses exploring the two distinct evolutionary lineages of *Hymenanthes.*

The morphological, molecular and biogeographical evidence provided is consistent with the hypothesis that a common ancestor of the *Calophyta* group hybridised with a *Pontica* species after a geographical split from other "proto-*Fortuneas*" resulting in chloroplast capture by introgression, followed by rapid speciation.

If it is confirmed that both *Fortunea* (s.s.) (i.e. excluding the *Calophyta* group) and the *Calophyta* group are monophyletic, using additional nuclear loci, then it is recommended that a new subsection be recognised within subgenus *Hymenanthes:* subsection *Calophyta*, to include only the species described in the taxonomic account

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1 Introduction

The magnificent morphological diversity of *Rhododendron* L. (Ericaceae) has elicited great passion and enthusiasm for the genus from amateur gardeners, horticulturists and scientists alike. The legacy of introductions from the Himalayas and South East Asia to British horticulture in the 19th and 20th centuries can be seen today in numerous spectacular public and private collections, from Trewithen and Caerhays in Cornall, to Glenarn and Benmore in Argyll and Bute, and Corrour in the Scottish Highlands, to name but a few.

Rhododendron is a large genus of more than 1,025 species (Chamberlain et al., 1996) in the 77 million year old (Liu et al., 2014) *Ericoideae* tribe of *Ericaceae* (The Angiosperm Phylogeny Group, 2009). The genus is mainly distributed in the northern hemisphere and has two main centres of diversity: Southwest China, and tropical Southeast Asia. Distinguished from other *Ericaceae* primarily by their zygomorphic flowers and anthers with dehiscent pores (Byng, 2014), they range from creeping alpine shrubs barely 10 cm tall, to canopy trees in the broadleaf temperate forests of Southwest China, with almost every variation in between, including epiphytes. Equally diverse in floral and vegetative characters, it is hardly surprising they have been so consistently popular. However, the very diversity which makes the genus so attractive to us, and has ensured both ecological success in the wild, and commercial success in cultivation, has proved problematic to the study of the genus.

A modern review of the genus coordinated by the Royal Botanic Garden Edinburgh (RBGE) from 1978 to 1996 resulted in an alpha-taxonomy of *Rhododendron* which remains largely unchallenged. However, large areas of the taxonomy still remain unclear, species boundaries are often blurred, and infra-generic relationships are still poorly understood. This study is intended to expand the evolutionary understanding of the genus by undertaking a taxonomic review of a group of closely related species, and then exploring the relationships between this group and its subsection and subgenus.

The *Calophyta* group contains *R. calophytum* Franchet, along with its "immediate allies": *R. asterochnoum* Diels, *R. praevernum* Hutchinson, and *R. sutchuenense* Franchet. These species are all classified as being in subsection *Fortunea* (Tagg) Sleumer, a large and variable subsection which could merit subdivision if clear-cut biosystematics evidence were presented (Chamberlain, 1982). *Fortunea* was shown to be non-monophyletic based on cpDNA (Milne et al., 2010), with *Fortunea*(s.s.)[1] in a poorly resolved clade with all sampled species from subgenus *Hymenanthes* (Blume) K. Koch excluding eight species from subsection *Pontica* Sleumer, both species sampled from the *Calophyta* group, and *R. insigne* Hemsley & Wilson which formed a second clade within the subgenus. The surprising placement of the two species sampled from the *Calophyta* group: *R. calophytum, R. praevernum,* justifies further study using more samples, to test whether all species in the *Calophyta* group share the same cpDNA, making *Fortunea* polyphyletic for cpDNA.

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[1]i.e. excluding species in the *Calophyta* group

1.1 A Brief Taxonomic History of *Rhododendron*

In *Species Plantarum* in 1753 Carolus Linneaus recognised just 5 species in the genus *Rhododendron* L.*,* and a further four in *Azalea* L.. This division still causes confusion today, with one of the most frequently asked questions at Rhododendron focused events being; "So what is the difference between Azaleas and Rhododendrons?" (Pers. Ex., 2011-2016), (Cox and Cox, 1997), (Leach, 1961).

With the limited number of species known to Linneaus, (all except *R. indicum* L. from marginal areas of the genus's range), the two were easily separated as *Azaleas* in "Pentandria Monogyna" have 5 stamens, and *Rhododendrons* in "Decandria Monogyna" have 10 stamens. However, with this major difference in mind, we must conclude that either Linneaus sometimes miscounted, or he felt other characters were sometimes more important, as he placed *R. lapponicum* L. in *Azalea* when this species generally has 10 stamens rather than 5. As more species were discovered, the characters which had been used to distinguish these two genera, such as stamen number, were found to be of little or no significance as intermediate species were described.

The genus had grown to 57 species by 1834 when George Don published his '*General history of the Dichlamydeous Plants*' (1834), in which he split the genus into 8 sections: Ponticum, Booram, Pogonanthum, Lepipherum, Chamaecistus, Tsutsuzi, Pentanthera and Rhodora. The next major revision of the genus was undertaken by C. J. Maximovicz, curator of St. Petersburg Botanic Gardens in 1870. By this point many more species had been introduced, most notably by J. D. Hooker, enabling Maximovicz to refine the work of G Don by incorporating a new suite of diagnostic characters. In classifications used today the influence of Don and Maximovicz is still evident.

The fruitful expeditions of Ernest Henry Wilson, George Forrest, and Frank Kingdon-Ward in the early 20th century lead to another great influx in the number of known species, and in the living collection at the Royal Botanic Garden Edinburgh. To catalogue this growing collection, the regius keeper Bayley Balfour created a temporary artificial system of classification by grouping seemingly related species together into series and subseries. Balfour intended to perform a comprehensive revision of this series system when he found the time, unfortunately he died before accomplishing his intended revision of the series system. Tagg, Rehder and Hutchinson followed Balfour's series system and expanded upon it with their own work, publishing *The species of Rhododendron*, in 1930 in which the genus was divided into 39 series. Cowan and Davidian also supported Balfour's series system, making only minor adjustments in their revisions in *The Rhododendron Yearbook* of the Royal Horticultural Society.

However, as is often the case in taxonomy, another group held opposing views; Herman Sleumer and James Cullen felt that the series system was inadequate in several areas:

- Firstly, the characters used were horticulturally important, such as flower colour, whilst ignoring more fundamental morphological characters and geographical distribution.
- Secondly, some species were named based on material from a single cultivated plant of unknown origin, others from plants of wild origin which stood out as being unusual in

collections; these could merely be extreme morphotypes of a variable species, or of hybrid origin.

- Thirdly, the series system lacked a hierarchy so all series held equal rank, implying that all series were equally related to one another.
- Finally, they felt that the species concept in the Balfourian classification was too narrow and did not allow for great variation within a species, even one very widely distributed.

Focusing on taxonomically important characters, Sleumer developed a much more comprehensive, hierarchical classification system by including the ranks of subgenus and section (then replacing series with subsection) based upon the earlier work of Don and Maximovicz. His treatment was largely ignored by horticulturists, but formed the foundation of the modern Edinburgh revision of *Rhododendron*, begun in 1972 and culminating in the publication of *The Genus Rhododendron: Its classification and synonymy* (Chamberlain et al., 1996).

1.2 Rhododendron Classification

Whilst new classifications based upon molecular phylogenetic studies are being explored and proposed (Kurashige et al., 2001),(Goetsch, Eckert, and Hall, 2005) ,the most widely accepted and followed classification of the genus is still that by Chamberlain et al.(1996), and will be used here. This system splits the genus in to three large subgenera, *Rhododendron, Hymenanthes* (Blume) K. Koch, *Tsutsusi* (Sweet) Pojarkova*, and five smaller subgenera as outlined in Table 1.

Table 1: Classification of *Rhododendron*
Subgenera and sections listed alphabetically. Approximate number of species in each section listed in right hand column (Chamberlain et al., 1996)

Rhododendron L.	1,025
Subgenus Azaleastrum Planch. (1854)	
Section Azaleastrum (Planch.) Maxim (1870)	11
Section Chionastrum Franch. (1886)	19
Subgenus Candidastsrum Franch. (1886)	1
Subgenus Hymenanthes (Blume) K. Koch (1872)	302
Section Ponticum G. Don (1834)	
Subgenus Mumeazalea Sleumer (1949)	1
Subgenus Pentanthera (G.Don) Pojarkova (1952)	
Section Pentanthera G.Don (1834)	23
Section Rhodora (L.) G.Don	2
Section Sciadorhodion Rehder & Wilson (1921)	4
Section Viscidula Matsum. & Nakai (1916)	1
Subgenus Rhododendron	
Section Pogonanthum Aitch. & Hemsl. (1880)	21
Section Rhododendron	211
Section Vireya (Blume) Copel.f (1929)	310
Subgenus Therorhodion (Maxim.) A.Gray (1878)	2
Subgenus Tsutsusi (Sweet) Pojarkova (1952)	
Section Brachycalyx Sweet (1831)	23
Section Tsutsusi Sweet (1833)	94

1.3 Subgenus Hymenanthes

All 302 species of subgenus *Hymenanthes* are contained within a single section: *Ponticum*, which is then further divided into 24 subsections. The subgenus has a clear identity and is repeatedly recovered as monophyletic in phylogenies (Kron and Judd, 1990), (Goetsch, Eckert, and Hall, 2005),(Milne et al., 2010). Synapomorphies for the subgenus include complex, dendritic hair types (Seithe, 1980), a complex nodal anatomy (Philipson & Philipson, 1968), and the presence of caryatin in leaves (Harborne and Williams, 1971). In contrast, the subsections within *Hymenanthes* appear at first to represent natural groups, but are based on Balfour's artificial groupings and although horticulturally useful, may not have any evolutionary significance (Hyam, 1997). Chamberlain (1982) expressed concern over a lack of sufficient data to support these informal groupings as formal taxonomic ranks:

> *"The 24 subsections recognised in this account are related to one another in a complex manner. The distinctions between them may well be obscured by hybridisation. In cultivation species from different subsections will cross freely and hybrids clearly also occur in the wild. Furthermore, the taxonomic significance of the morphological differences on which the classification is based is not always obvious."* Chamberlain (1982, page 459).

Chamberlain goes on to state that some of the subsections maintained in his revision are highly variable and could be further subdivided if clear-cut, biosystematics evidence can be found to support such divisions.

Hyam (1997) concluded that *Hymenanthes* is a large and complex group of species with little or no hierarchical structure, with two hypotheses suggested as to why this is the case; firstly, that the subgenus has undergone such explosively rapid evolution from a single ancestral stock that all resultant taxa are equally distantly related to one another and their ancestral taxon. Secondly, that breeding barriers between the species are so permeable that gene exchange between morpholocially distinct populations is relatively common resulting in the entire subgenus acting as a single evolutionary unit. A combination of these two hypotheses is the most likely explanation for the lack of structure within *Hymenanthes* (Hyam, 1997).

1.4 Consequences of Hybridisation

Hybridisation is known to occur frequently, both in cultivation and in the wild, within the larger subgenera of *Rhododendron* (*Rhododendron, Hymenanthes, Tsutsusi*), with the resultant offspring being fertile in most cases (Cox & Cox, 1997), (Chamberlain & Hyam, 1998). The subgenus *Hymenanthes* is remarkable for being incredibly diverse despite extraordinarily weak species barriers (Milne 2010), supported by numerous recordings of natural hybridisation in the wild (Chamberlain, 1982), (Zha, Milne, and Sun, 2008). Interfertility on this scale is relatively common among clades where rapid adaptive radiation has occurred (Smissen, Breitwieser, and Ward, 2004), (Wang, Yang, and Liu, 2005), but in *Hymenanthes,* even older species such as those in subsection *Pontica* are highly interfertile (Milne et al., 1999,2003). With such high levels of interfertility it is highly likely that hybridisation was a driving factor of diversification and rapid radiation within the

genus, as Masueli et al. suggested for *Solanum* (2009), and has similarly been shown in some animal groups (Seehausen, 2004), (Schliewen and Klee, 2004).

In the majority of cases hybridisation events do not have any evolutionary consequence. However, as hybridisation is so common in *Hymenanthes* it is reasonable to assume that evolutionarily significant hybridisation events have occurred within the group and so it is important to consider subsequent outcomes: introgression and hybrid speciation.

Introgression

The 2nd most likely outcome following hybridisation is introgression, whereby genetic information is transferred from one species to another by repeated back-crossing of the initial hybrid and its subsequent generations with the more abundant species in a habitat (Anderson & Hubricht, 1938). Introgression has been observed numerous times in *Hymenanthes* (Milne, et al., 1999), (Milne & Abbott, 2000), (Chung, et al., 2007), and can lead to chloroplast capture (or transfer) (Rieseberg & Wendel, 1993), resulting in phylogenetic incongruence across nDNA and cpDNA.

Hybrid speciation

There are two forms of hybrid speciation; allopolyploid and homoploid. Homoploid speciation occurs between parent species with low genetic divergence when the F1 is partially or fully fertile (Rieseberg, 1997) . No change in chromosome number takes place. Allopolyploid speciation is more likely to occur when parent species have a high genetic divergence, and the F1 generation is sterile, or almost so (100% sterility of F1s though common in animals is extremely rare in plants). It results in chromosome doubling and is rare in *Rhododendron,* rarer still in *Hymenanthes* (Ammal, Enoch, and Bridgewater, 1950) with most species being diploid (i.e. 2n=26) and highly interfertile. For the purpose of this report, the term hybrid speciation may henceforth be assumed to mean homoploid hybrid speciation.

1.5   Area of Study and Taxonomic Group

As detailed above, *Rhododendron* subgenus *Hymenanthes* is a large and complex natural grouping lacking in internal structure. Milne's study of the subgenus (2010) found strong support for two distinct clades (see Figure 1), but lacked resolution among the SE Asian Species, perhaps reflecting multiple reticulation events. The two clades present a biogeographic pattern in *Hymenanthes* of slow diversification outside of SE Asia followed by the rapid diversification of one lineage within SE Asia, most likely linked to hybridisation. Surprisingly, *R. praevernum, R. calophytum* (both in subsection *Fortunea*) and *R. insigne* (subsection *Argyrophylla*) came out nested within the more basal clade in which the other 8 species were from subsection *Pontica* and from outside of SE Asia. It is worth noting that the phylogeny in Figure 1 is incongruent with the rDNA phylogeny of Goetsch et al. (2005).
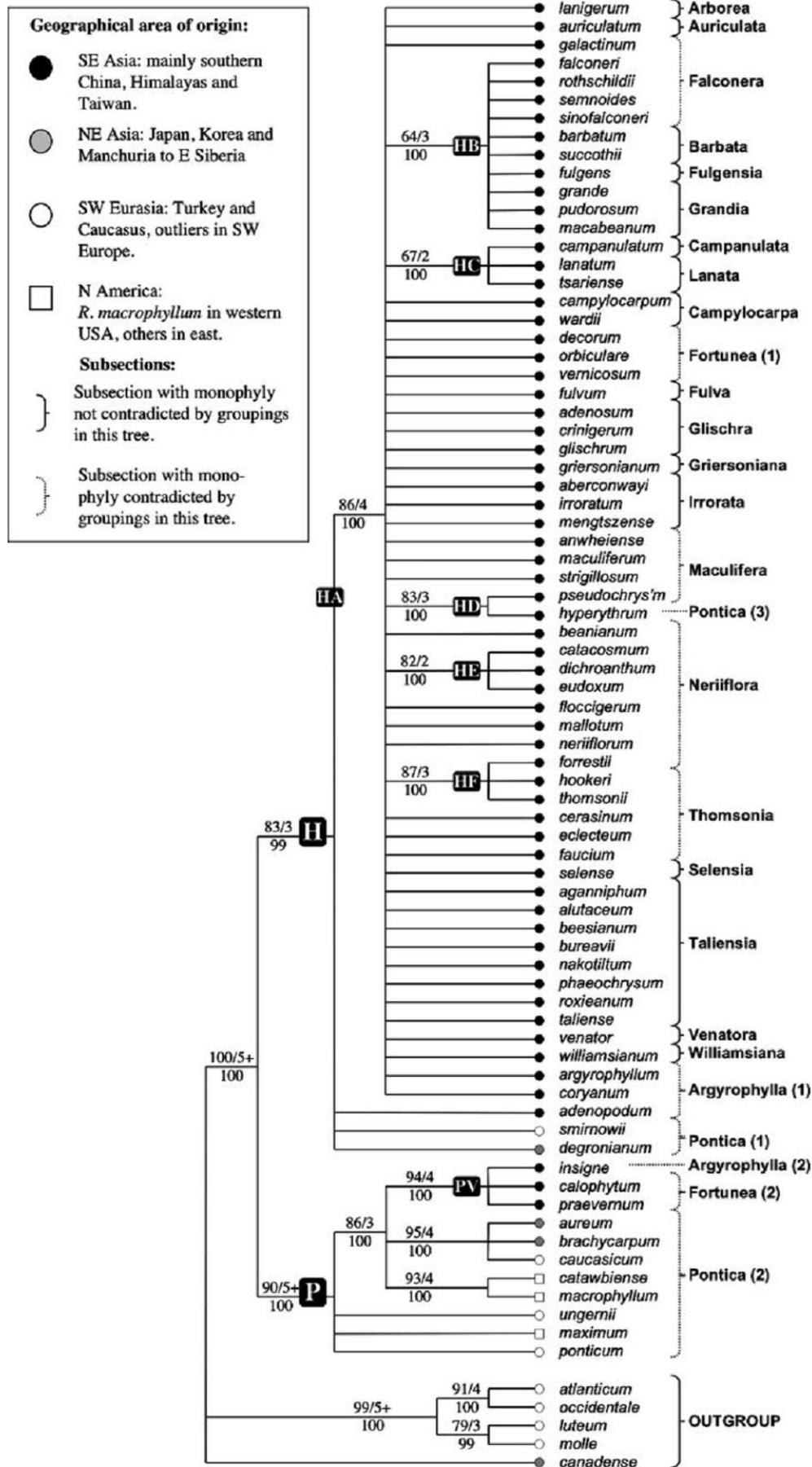
Figure 1: Phylogeny from Milne (2010) based upon a combined dataset for cpDNA (matK and trnL-trnF)

Few individual subsections have been examined using molecular data, however a phylogeny for *Hymenanthes* subsection *Fortunea* was generated for rDNA, from intron regions of RPB1, RPB2d, RPB2i, RPC1 and E19 genes using sequences of 100bp tracts extending in both directions from all of the Pst1 sites of 56 accessions, comprising 24 taxa (Hall et al., 2015) .

Within this subsection, four species consistently cluster together forming a basal clade within the subsection, inferring a shared ancestry quite distinct to that of the rest of the subsection based on nuclear markers (Hall et al., 2015). These species are: *R. sutchuenense, R. praevernum, R. calophytum* and *R. asterochnoum.* These species also appear morphologically distinct enough to merit further investigation into whether there is sufficient evidence to support either further subdivision of subsection *Fortunea,* or elevation to a separate subsection (Chamberlain, 1982). Unfortunately, the detailed study of the subsection (Hall et al., 2015) only included outgroup species from monotypic subsections putatively closely related to *Fortunea*: *Auriculata, Williamsiana*. These samples nested within different clades of the phylogeny rather than coming out separately. Combined with the morphological similarities with certain species in *Fortunea,* the genetic evidence suggests they may indeed be better placed in *Fortunea* (Hall et al., 2015)*.* However, much more extensive sampling across the subgenus *Hymenanthes* would be needed to thoroughly test this hypothesis, and to test the monophyly of *Fortunea* within the subgenus. This should be carried out for multiple gene regions, including both cpDNA, and nDNA*.*

1.6 Aims and Objectives

This project has been undertaken to bridge the gap between Milne's broad investigation of *Rhododendron* subgenus *Hymenanthes* and Hall's detailed study of just subsection *Fortunea* to contribute to our understanding of the evolution within *Hymenanthes*.

This will be achieved by testing a series of hypotheses with the first five examining relationships between the study group species, and the next four examining the relationship of this group of species to the rest of *Hymenanthes.*

$H_{1.0}$: Study group species are all well-defined, clearly separated species, supported by morphological and genetic characters.

$H_{1.1}$: Study group species ill-circumscribed with confusion over identity of specimens commonplace. Species boundaries unclear, morphology contradicts genetic characters.

$H_{2.0}$: *R. asterochnoum* is a one off natural hybrid of, or variation of *R. calophytum*.

$H_{2.1}$: *R. asterochnoum* is a stable, definable species

$H_{3.0}$: *R. sutchuenense* and *R. praevernum* are best described as distinct species.

$H_{3.1}$: *R. sutchuenense* and *R. praevernum* are best described as extreme morphotypes of one variable species.

$H_{4.0}$: *R. calophytum* var. *pauciflorum* is a well-supported variety.

H_{4.1}:   *R. calophytum* var. *pauciflorum* is not well supported. Plants grown under this name are often actually recent hybrids.

H_{5.0}:   *R. calophytum* var. *openshawianum* is a well-supported variety.
H_{5.1}:   *R. calophytum* var. *openshawianum* is not well supported.

H_{6.0}:   Subsection *Fortunea* is monophyletic for cpDNA.
H_{6.1}:   Subsection *Fortunea* is non-monophyletic for cpDNA.

H_{7.0}:   Study group species monophyletic for cpDNA.
H_{7.1}:   Study group species non-monophyletic for cpDNA.

H_{8.0}:   Study group species evolved from a single ancestral *Pontica* subsection species, with morphological links to subsection *Fortunea* merely convergent evolution.
H_{8.1}:   A now extinct ancestor of the study group species hybridised with a *Pontica* species after geographical split from other *"proto-Fortuneas"* resulting in chloroplast capture, followed by rapid speciation
H_{8.2}:   Homoploid hybrid speciation: both cpDNA and nDNA obtained by study group species from *Pontica* lineage along with morphological traits.

H_{9.0}:   *R. insigne* represents a second chloroplast capture event from *Pontica*.
H_{9.1}:   *R. insigne* gained its *Pontica* cpDNA type from one of the introgressed *Fortuneas*
H_{9.2}:   *R. insigne* is an anomalous member of the *calophyta* clade, unrelated to A*rgyrophylla*.

2 Morphological Materials and Methods

2.1 Species Concept

Species were delimited using a morphological species concept as no data on population genetics was available. Groupings in the phylogenetic study were consistent with the species delimitations established from direct observation of both macro-morphological and micro-morphological characters, and statistical analysis of morphometric characters, but based only upon plastid DNA so a phylogenetic species concept was not used.

2.2 Definitions

Terms are used as defined in J. G. Harris & M. W. Harris, "*Plant Identification Terminology, an illustrated glossary*", 2nd edition, (2001) except for hair type which follows J.M. Cowan, "*The Rhododendron Leaf*", (1950) (see Figure 4, p15), and flower shape which follows J.F.J. McQuire and M.L.A. Robinson, "*Pocket Guide to Rhododendron species*", (2009).

2.3 Material Studied

Herbarium Specimens

Morphological characters were studied from a total of 45 existing specimens (syn. exsiccatae) from the following herbaria: Royal Botanic Garden Edinburgh (E), Paris (P), Harvard University (A, GH), Royal Botanic Garden Kew (K), Swedish Museum of Natural History (S), and Universitat Wien (WU). All specimens were used for a preliminary study to inform on important taxonomic characters (Figure 2). Of the 36 specimens studied directly at the Royal Botanic Garden Edinburgh, two contained only a few fragments. The remaining 34 had sufficient material to be measured and analysed in detail. A table of exsiccatae studied is included in Appendix 1.

Figure 2: Preliminary study sorting specimens into taxon groups

Exsiccatae were generally in good condition but some were very old and degraded, or only a few small fragments. Out of those specimens measured in detail: almost all specimens had complete leaves, although some were curled or damaged so the leaf apex shape was difficult to determine. Only 12 had enough floral material to make reasonable measurements. 17 specimens were in fruit, with capsules in varying stages of dehiscence. No specimens had both fruit and flowers present. 6 specimens comprised only vegetative material.

Collection of Material

In order to supplement the available exsiccatae, fresh material was collected from 46 plants in cultivation at Edinburgh, Dawyck, Benmore, Corrour, Glenarn and Glendoick. Details of material collected are in table 2 in Appendix 1. The study group are early flowering species so efforts were made to press specimens in April when the plants were in flower, however, due to time constraints and a conflict of flowering time with course examinations, it was not possible to collect floral material for every specimen. A field press was used to collect material, with samples transferred to the drying room at RBGE either the same or the following day. The red herbarium press bag shown in Figure 3 made a good working surface for laying out specimens. Samples were placed between sleeves of newspaper labelled with an ID and location. They were then stacked between two sheets of cardboard with a slatted wooden frame at the bottom and top of this stack. Straps held this bundle together and began pressing the samples. Transferred to the drying room, sheets of blotting paper were placed between newspaper sleeves and the stack weighted down to speed up drying/pressing process as seen in Figure 3.

Figure 3: Left - Field equipment for sample collection; Right - Samples in the drying room

Observations were recorded in the field, and photographs taken to preserve details such as indumentum texture and colours which may be lost during the drying process. A selection of the photographs used to inform the taxonomic study are included in Appendix 2. Silica gel collections for molecular work were made at the same time as voucher specimens. For each plant sampled, multiple sheets were pressed. Mature foliage was always included, along with developing buds or young foliage if present. For specimens still in flower at time of collection, one to three trusses were pressed intact, along with numerous opened out corollas. When material was collected after plants had finished flowering, several trusses of developing capsules were collected and pressed.

2.4 Characters Used

Morphological characters such as leaf texture, indumentum quantity, location, and colour, were obtained by observing both living material and herbarium specimens wherever possible. Measurements of plant height and width were made directly from living specimens or inferred from collector's descriptions on exsiccatae when available. The length of one season's growth was measured from living specimens and from herbarium material, the difference between fresh and dried having been observed early on as minimal compared to natural standard deviation from one branch to another. All other measurements were taken from herbarium specimens.

Characters recorded in the data gathering phase were chosen after first performing a preliminary sort of the specimens into potential taxa groups and noting characters that stood out as important immediately. Closer examination of specimens revealed subtler characters which hinted at being informative so these were also recorded. Previous literature was then consulted(Chamberlain, 1982), (The American Rhododendron Society et al., 1980),(Davidian, 1989), with any commonly used characters not yet included but felt to be relevant to the study group species added to the morphological character matrix. Specimens were studied using a dissecting microscope. Detailed measurements were then recorded for 57 specimens: all 36 exsiccatae listed in Table 1, Appendix 1, plus the 21 fresh specimens indicated as measured in Table 2, Appendix 1.

Taxonomic characters informative for *Hymenanthes* (Chamberlain, 1982) and hence studied here are introduced below.

Habit

Species are trees or shrubs. Trees are defined as having one dominant trunk, which is unbranched for at least one third the height of the plant, or being unbranched for at least half its height, with no dominant trunk thereafter. Shrubs are multi-stemmed or deliquescent (with no dominant leading branch) from within the lower third of the trunk. Many specimens in living collections were crowded by other plants and their habit was affected by this. This was noted when severe. A distinction was made between rounded and flat-topped shrubs/trees as this seems an important character for the study group species.

Bark

Both texture and colour of bark vary greatly through the subgenus, ranging from the dull, rough grey-brown of *R. arboreum,* to the shining, smooth pink-grey of *R. thomsonii* and the marvellous, deep red, peeling bark of *R. barbatum*. The diversity of bark in the genus has not been used much as a taxonomic character and seems to be noted only when especially showy and thus of horticultural interest. With further study this may prove to be a helpful character in better understanding the relationships between subsections.

Buds and Bud Scales

The shape and size of terminal buds varies hugely across *Hymenanthes*, influenced of course by the shape and quantity of bud scales per bud. Bud scales vary greatly in colour size, shape and indumentum, even within one bud. They also are usually abscissed shortly after bud break, so it is hardly surprising that the character has been overlooked by many authors. However, they may yet prove to be helpful in delimiting species.

Leaves

In subgenus *Hymenanthes,* leaves are persistent for at least one season (premature leaf abscission can occur if a plant is very stressed, e.g. suffering a very severe case of powdery mildew, usually resulting in death). Leaves are highly variable within the subgenus in colour, texture, indumentum, petiole characteristics, shape and size. Leaf shape has been used to delimit subsections in the past, sometimes leading to unnatural classifications (Chamberlain, 1982) as in Spethmann (1987).

Mature leaves may be completely glabrous, densely indumented on both surfaces, or anywhere between these two extremes. The emerging foliage of most species is densely hairy; even in species with glabrous leaves at maturity one may expect a few hairs to be present of the unfurling leaves which are subsequently lost as the lamina expands. Note, "leaf" is henceforth used to refer to the entire organ (lamina and petiole).

Indumentum

Within the subgenus, indumentum is highly variable and provides numerous taxonomic characters which are useful in delimitation at both subsection and species level. Vesicular hairs for example only occur on veins on the abaxial surface of *R. vesiculiferum* (*Glischra*) leaves, whereas stiff, setose hairs are a key diagnostic character for *Barbata*.
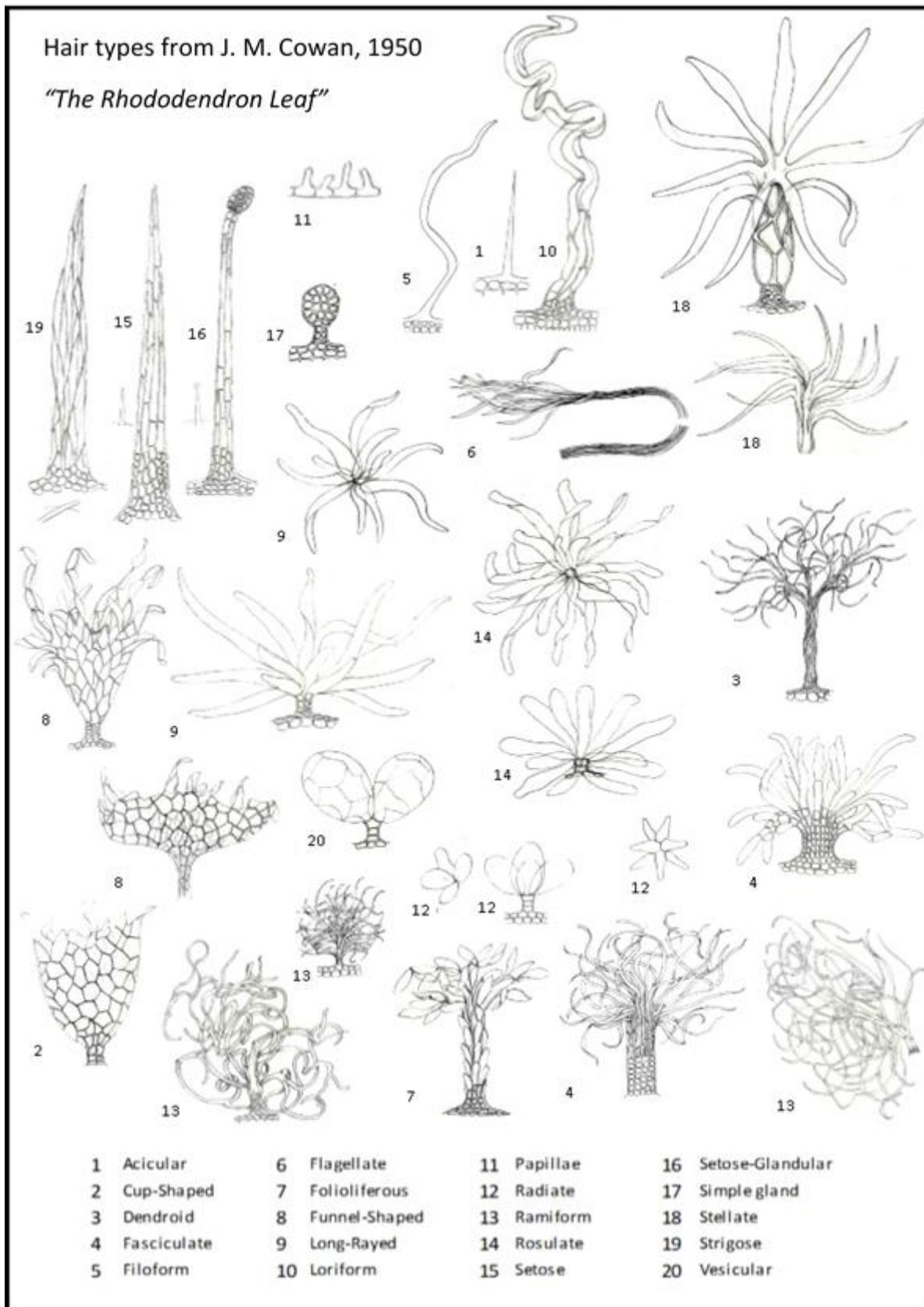
Figure 4: Trichome morphology

Leaf indumentum (or a lack there-of) is the most valuable taxonomic character and has been well studied. Trichome (hair) morphology is incredibly diverse as illustrated in by the drawings in Figure 4, amalgamated from "*The Rhododendron Leaf: A study of the epidermal appendages*" (Cowan,

1950). More comprehensive classifications of hair types have been produced but the additional subdivisions presented e.g. Seithe (1980), do not provide additional insights into classification of species within the genus. Terms used to describe complex hair types used in this study follow Cowan (1950) and are defined in Table 2.

Table 2: Trichome definitions and characteristics

Trichome Term	Description	Common location	Commonly found in
Setose hairs	Stiff, somewhat bristle-like hairs that may or may not have a glandular tip	Young shoots Petioles Leaf lamina	*Barbata Auriculata (Fortunea s.l.) Maculifera*
Glands	Clusters of cells producing a secretion at apex of setose hairs, on a short stalk, or sessile to lamina surface	Any part of the plant Rare on corolla Common on young shoots	*Campylocarpa Fortunea Glischra Thomsonia*
Radiate hairs	Sessile or shortly stalked rosettes of short cells	Leaf lamina, often in conjunction with dendroid cells Petioles	*Taliensia*
Rosulate and long-rayed hairs	Sessile or shortly stalked rosettes of long cells. Gives compressed, matted appearance if only trichome type present	Leaf lamina Petioles	*Fulva Grandia Taliensia*
Stellate hairs	With long, rigid arms spreading from a well-developed stalk	Young shoots Leaves	*Parishia*
Dendroid hairs	Characterised by a well-defined stalk several cells thick. Arms are unbranched, flexuous, and may be several cells long	Leaf lamina Petioles Along secondary veins on abaxial leaf surface	*Fulvum Maculifera Neriiflora Thomsonia*

The term indumentum is used in the broad sense to refer to any covering of hairs and also in the strict sense when discussing leaves to refer to any trichomes on the abaxial leaf surface at maturity. Tomentum is used to discuss indumentum which may not persist, such as on new growth, the upper surface of leaves and branchlets.

Inflorescence

In subgenus *Hymanenthes* the inflorescence (or truss) is always a terminal raceme. It may be one to many-flowered, forming a dense, compact truss as in *R.barbatum* and *R. niveum*, or lax as in *R.*

dichroanthum. The rhachis can be very short created an umbel-like truss, or elongated as in *R. griffithianum*. The rhachis and pedicels may be glabrous, indumented, or glandular.

The floral bud scales of *R.griersonianum* and *R. auriculatum* are noted as being cuspidate in contrast to the usual oblong or ovate bud scales of most other species(Chamberlain, 1982). They may be glabrous, densely indumented or glandular and can be strikingly coloured. Bud scales have not generally been used as a taxonomic character which may be down to them being overlooked. They are studied here to test if there may be potential for greater use of bud scales as a taxonomic tool in delineating taxa.

Calyx

In some species the calyx is well developed and ornamental, matching the colour of the corolla, or else contrasting strikingly with it. It can be cupular in shape, or lobed. In many species though, the calyx is reduced, sometimes extremely so, to be just a slightly fleshy rim the same colour as the pedicel. It can be glabrous, indumented or glandular and lobes often have a ciliate margin. Being so diverse it provides several useful diagnostic characters.

Corolla

In all species the corolla is weakly zygomorphic. Most species have retained the pleisiomorphic character of a 5-lobed corolla, but some subsections show a tendency towards increased merosity with species in *Auriculata, Falconera, Fortunea* and *Grandia* often being 6-9-lobed. Within the subgenus corolla length ranges from 2.5cm to 11cm (Chamberlain, 1982) and is highly diverse in shape, Figure 5 illustrates some of the common corolla shapes in *Rhododendron.*
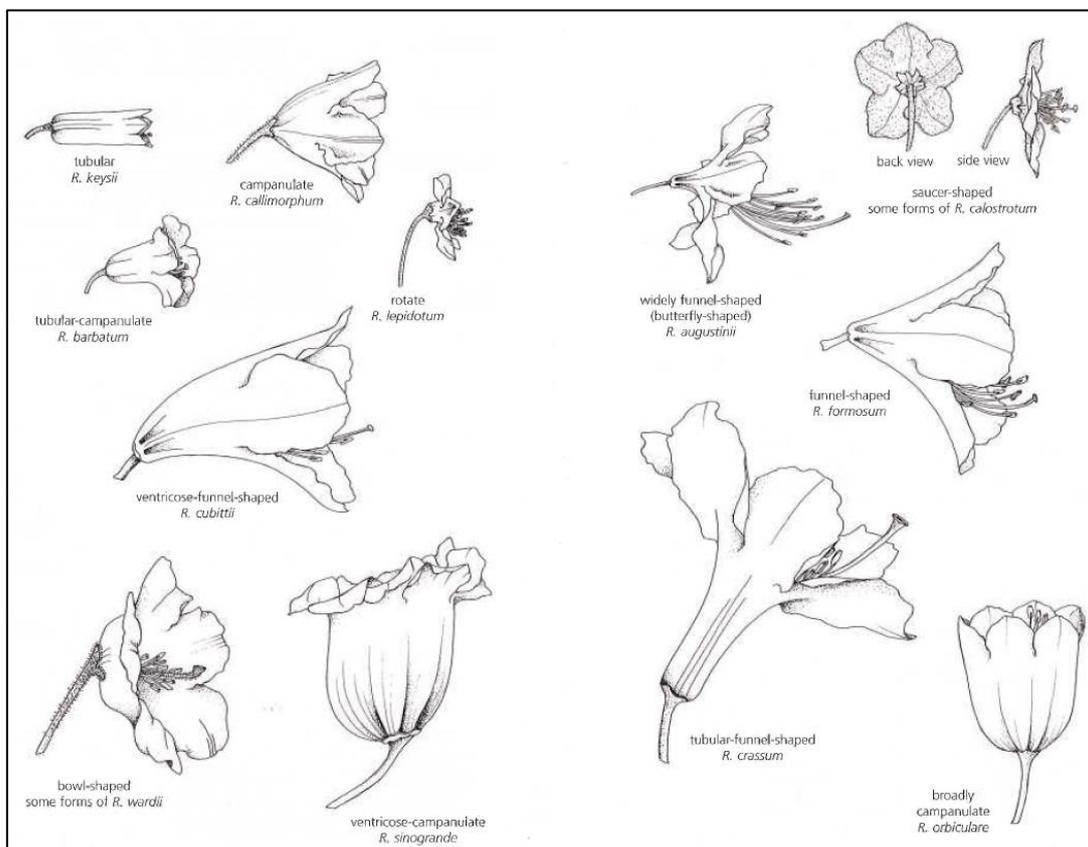
Figure 5: Inflorescence types in *Rhododendron*

Although corolla colour shows definite trends by subsection, e.g. reds in *Nerriflora* and *Thomsonii*, pinks in *Fortunea,* mauve in *Pontica,* it is a weak character taxonomically beyond the distinction between strong, dark colours, and paler, subtler hues. Corolla colour can vary greatly within a population and never ought to be a defining character of a species as there are always exceptions, and it is commonly noted that colour can vary from year to year, perhaps affected by climatic conditions or pH. Much more taxonomically significant than colour is corolla markings; prescence/absence of a blotch and or speckling in the throat of the corolla. The presence/absence of nectar pouches or marked depressions that may be coloured differently from the rest of the corolla can also be a useful taxonomic character. Pubescence on either the inner or outer surface of the corolla can also be informative to classification within the subgenus.

Androecium

The number of stamens is normally about twice the number of corolla lobes, obdiplostemonous (Byng, 2014), but can be more numerous, e.g. in *R. calophytum* (15-20). Stamens are usually declinate, contributing to the overall zygomorphy of the flower and may be glabrous or puberulous for a portion of their length.

Gynoecium

Within *Hymenanthes,* the number of locules per ovary varies from 5 to 18, and does not necessarily correlate with number of corolla lobes. The ovary may be glabrous, indumented or glandular, (fully or partially) and is often coloured. It contracts abruptly at the apex to form the style in all subsections except *R. neriiflorum* in which it tapers. The style is declinate like the stamens, often ascendent apically. It may be glabrous or glandular (sometimes indumented). In the majority of species, the stigma is capitate. However, in subsections *Falconera, Grandia* and species *R. calophytum, R, asterochnoum* it is large and prominently discoid.

Capsule

Any ovary indumentum generally does not persist until capsule maturity, but is reduced instead to protuberant hair bases. One exception is the Vietnamese species *R. suoilenhense* which is still densely indumented with floccose hairs at capsule maturity. The capsule is cylindrical, oblong to linear and can be straight or curved (rarely circinnate). Capsule characters are taxonomically important for some species, but are not widely used in classification.

Seeds

Variation in seed morphology is of little taxonomic value within *Hymenanthes,* although it has been well documented (Hedegaard, 1980). Seeds are fusiform and almost always winged. Seed size is variable.

2.5   Scanning Electron Microscopy

Light microscopy was sufficiently powerful to allow informative observations of most characters used but the intricacies of hair type were not discernible to the untrained eye. Scanning Electron Microscopy (SEM) was carried out on 12 samples in order to try and determine the hair types present on the samples, and to become familiar with the textures of indumentum produced by the

different hair types in the hope that this might inform future observation using the light microscope.

The specimens selected for SEM are given in Table 3. As all samples of *R. asterochnoum* are from the same introduction (since no other collections were found) and morphology seems consistent across specimens, only one sample was used for this species for SEM. In addition to this, species sampled were: *R. calophytum* (x4), *R. praevernum* (x2), *R. sutchuenense* (x4, including material from the type: Sutch-Far), and the putative hybrid Strig-X-sutch. High sampling of *R. praevernum, R. sutchuenense* was done to try and establish whether hair type could be one of the characters used to distinguish between the two species, or if there was no significant different in hair type between them. The putative hybrid Strig-X-sutch was sampled to image the glands (visible under the light microscope, but unclear) as *R. strigillosum* is glandular-setose whereas *R. sutchuenense* is eglandular.

Table 3: Samples used for SEM

Taxon name	Accession/Barcode	Name for discussion
R. asterochnoum	Glendoick1	Ast-GD
R. calophytum	19724038A	Cal-Wil
R. calophytum	19952865C	Cal-CEE
R. calophytum	19960429H	Cal-SICH
R. calophytum	E00757363	Cal-Yu
R. praevernum	357	Prae-1
R. praevernum	404	Prae-2
R. sutchuenense	595	Sutch-C
R. sutchuenense	E00010418	Sutch-Far
R. sutchuenense	Glenarn	Sutch-GA
R. sutchuenense	Glendoick	Sutch-GD
R. X strigillosum	599	Strig-X-sutch

Preparation of Material

After studying pressed specimens under the dissecting microscope to assess indumentum quantity and locate suitably clean leaf portions, 5x3cm fragments were removed and taken down to the microscopy laboratory. Samples were mounted onto stubs by cutting out 10x10mm squares of midrib-bisected lamina tissue and carefully placing the sample onto stub. Care had to be taken not to damage the hairs whilst placing the samples on the sticky carbon disc atop the stub by squashing them with the tweezers. Working on the ventilated bench Acheson ElectroDAG 1515m (Agar Scientific, UK) was painted onto the stubs to connect the edges of the foliage sample to the metal of the stub. Specimens were left on the ventilated bench for >30mins to allow the ElectroDAG to dry before sputter coating using the Emitech K575x Sputter coater. Organic materials act as an insulator and earth the electron beam, reducing the quality of any image recorded. Stubs were coated with platinum which acts as a conductor and improves the resultant image quality by increasing the

number of secondary electrons that can be detected from the surface. It also minimises charging of the specimen.

Examination under SEM and Image Capture

Samples were processed in two batches, six at a time. Stubs were loaded into the specimen holder and secured by tightening the screws. Specimens were then placed inside the LEOsupra 55VP SEM (Carl Zeiss Microscopy GmbH, Germany). Samples were examined using the SmartSEM Image Navigation interface system (v2.1.1, Carl Zeiss Microscopy GmbH, Germany). Scanning the specimens was carried out under the following criterion; operating mode: normal, scan speed: 4, store resolution: 1024x768. Once a suitable subject had been located, it was magnified far beyond the magnification required for the image to allow refined focusing. The magnification was then set to frame the feature nicely, and brightness and contrast altered so as to give the most detail. Parameters were then altered to; store resolution: 2048x1536, scan speed: 9 and the image frozen. The captured image was saved in TIF format in the author's folder and in the RBGE microscopy database.

2.6 Morphometric Analysis

Data collection – Morphological Character Matrix

55 specimens were examined for morphometric analysis, 34 existing specimens along with 21 of the new specimens created as part of this project. Data entry and manipulation was carried out in Microsoft Excel. Morphological characters were divided into vegetative characters (41), floral characters (49), and fruit characters (4) giving a maximum of 90 morphological characters recorded for each specimen. Missing data was recorded as "~", to note that it had been absent, not missed. Quantitative data was gathered for 40 morphological characters (measurements e.g. lamina length and quantities e.g. number of stamens). Qualitative data on 50 characters was gathered using discrete character states (e.g. glabrous/ indumented/glandular). Wherever enough material was available, five measurements were taken for each quantitative data character. These five values were then averaged to find the mean measurements for each sample. For discrete morphological characters, data was entered by selecting a possible character state from a drop down list to ensure consistency (see Tables 1 and 2, Appendix 3).

Principal Component Analysis

Morphometric analysis was conducted in Excel through the add-in XLSTAT (Addinsoft, 2016) using Principal Component Analysis (Legendre, Sneath, and Sokal, 1974). PCA requires normal distribution of continuous data, as input data to produce a valid result. Observations missing data for some variables cannot be included in PCA. As specimens did not have a uniform composition of leaves, flowers and fruits, the initial data matrix was reduced by removing columns and taxa until there was no missing data for the characters being considered. In this way four reduced datasets were produced; PCA1 with only eight vegetative characters, containing 54 specimens. PCA2 containing 42 specimens which was a combination of 8 vegetative and 4 floral characters, PCA3 contained 24 specimens and a higher proportion of floral characters (10 floral to 8 vegetative), and PCA4 which

contained only *R. praevernum R. sutchuenense* and *R. X geraldii* with the data gathered from their corolla markings, complemented by other floral characters (dataset matrices in Appendix 4).

Each continuous character was tested for normality using Anderson-Darling (again through XLSTAT). Numerous characters justified transformation to ensure normal distribution before the PCA was carried out. All such characters were transformed (e.g. using the LOG10 function in excel) and retested for normality before continuing.

3 Morphological Results

The morphological analyses grouped specimens into three clear species units, comprising 5 units in total: *R. asterochnoum-* 1 unit*, R. calophytum,* 2 units: - *R. calophytum* var. *calophytum* and *R. calophytum* var. *openshawianum* and *R. sutchuenense,* 2 units which for the purpose of presenting morphological results are termed *R.praevernum* and *R. sutchuenense* reflecting the current classification, (Chamberlain, 1982), which will be discussed in detail in chapters 7.3 and 8.

3.1   Qualitative Morphological Character Results

Many characters studied were found to be uninformative. Some such as habit provided general trends, but no definitive distinction between closely related taxa. Morphological characters recorded as discrete character states used in writing the taxonomic account are explored for the study group species below. Morphological characters recorded numerically are analysed in section 3.2 Quantitative Morphological Character Results.

Habit

None of study group species were only trees, or only shrubs. *R. calophytum* and *R. asterochnoum* usually form a flat-topped shrub. However, these species often do not have sufficient space in collections as their rate of growth is often much faster than anticipated. Specimens grown in good light, with plenty of space made neatly rounded shrubs, clothed to the ground with leaves, (as in Figure 6). Specimens of *R. calophytum* var. *openshawianum* were observed as being generally less dense than the autonym, but were only examined growing in high levels of shade so cannot be directly compared.

Figure 6: Habits. Left; a magnificent rounded shrub specimen of R. calophytum var. calophytum at Glendoick. Right; R. asterochnoum at Benmore, showing the typical flat-topped shrub habit

Of the specimens studied, *R. praevernum* is usually a rounded shrub whereas *R. sutchuenense* forms a rounded shrub when young, developing into an upright shrub or tree in time. Little difference in habit was found between specimens of *R.praevernum* at Dawyck, and *R. sutchuenense* at Glendoick and Glenarn. Habit was therefore found to be useful in delimiting *R. calophytum* and *R. asterochnoum from R.praevernum* and *R. sutchuenense*, but not in separating individual species.

Bark

Bark was found to be a difficult character to describe, with differences between the study group species being minimal. *R. asterochnoum* was found to have browner bark than the other species, with *R.praevernum* and *R. sutchuenense* being pinkish-grey and *R. calophytum* grey to grey-brown. Texture varied from flaky to rough in all taxa. Due to great potential for errors due to subjectivity, the bark characters were not given any significance, though it perhaps deserves more thought it future.

Buds and Bud Scales

Buds and bud scales proved inconclusive as taxonomic characters due to the detail of study required to utilise these characters effectively being well beyond the scope of this project. Figure 7 illustrates some of the diversity in shape, colour and form of the outer/lower bud scales on vegetative buds. In *R. calophytum* and *R. asterochnoum* the outer-most bud scales are always acuminate, often with a prominent, curved apiculus. Buds on *R. calophytum* were found to have more tomentum than those on *R. asterochnoum. R. praevernum* and *R. sutchuenense* outer bud scales are cuspidate, or tipped with a small mucron and always green. They have far less tomentum than those of *R. calophytum* and *R. asterochnoum.*

Figure 7: Terminal vegetative buds showing diversity of bud scale shape and colour
Left: *R. calophytum* var. *calophytum*: original Wilson collection 4279 (19724038A). Centre: *R. calophytum* var. *calophytum*, young plant just reaching maturity from expedition to Tibet and Sichuan, SICH1656 (19960429I). Right: *R. praevernum* (19698798A) growing at RBGE

The upper bud scales which enlarge as the bud breaks for new growth to emerge are also highly diverse. They range in shape from obovate through spathulate to almost linear. The apex may be acuminate, cuspidate, rounded or bifid. Colours vary from green through yellow tinged with pink to vivid reds. *R. praevernum* and *R. sutchuenense* have green outer bud scales. The inner bud scales of *R. praevernum* may be red or yellow-green. *R. sutchuenense* was only observed as having yellow green bud scales on new growth, but only three samples had new growth present. *R. calophytum* and *R. asterochnoum* mostly had red or yellow tinged with red bud scales, but both species also have examples of plants with yellow green inner bud scales, illustrated in Figure 8.

Figure 8: New growth showing striking bud scale diversity
From left to right: *R.* calophytum var. *openshawianum* at Glendoick. *R. calophytum* var*. calophytum* at Glendoick. *R. asterochnoum* at The Hutts. *R. sutchuenense* at RBGE

Foliage bud scales may be glabrous or indumented; hairs, if present, are usually on the inner surface, not the outer surface. Inflorescence bud scales are often densely lanulose on both surfaces. Although diverse, bud scale characters were not helpful in delimiting the study group species as few samples were collected whilst plants were in growth, so most samples lacked this character. However, they may prove to be taxonomically useful in future with further study.

Leaves

Leaf shape was found to be a taxonomically important character for distinguishing between species. All of the study group species had a variation on oblanceolate leaves, with *R. praevernum* and *R. sutchuenense* having shorter, squatter, leaves than *R. calophytum* and *R. asterochnoum.* A key character used to distinguish varieties of *R. calophytum* was the leaf apex shape; *R. calophytum* var. *calophytum* has an acute to cuspidate apex, whereas *R. calophytum* var. *openshawianum* has a distinctive narrowly acuminate apex (see Figure 9). *R. calophytum* var. *openshawianum* sometimes also has a thinner leaf with a glossier adaxial surface, but this was not a constant character. The leaves of *R. calophytum* var. *calophytum* are usually thick and sturdy, and may be keeled, whereas the leaves of *R. calophytum* var. *openshawianum* are flat. *R. asterochnoum* has acute cuspidate to acuminate leaf apices, but is easily distinguished from *R. calophytum* by its indumentum.

**Figure 9: Distinctive leaf apices of *R. calophytum* var. *calophytum* (left) and *R. calophytum* var. openshawianum *(right)*

R. praevernum and *R. sutchuenense* are much more difficult to separate by vegetative characters. They both have acute to cuspidate leaf apices, and slightly recurved leaf margins. Superficially, *R. praevernum* has smaller leaves with mean length 13.7cm, and *R. sutchuenense* has larger leaves, with mean length 16.7cm. However, 3cm cannot be considered a significance difference as the standard deviation is 2.58cm for *R. praevernum* and 3.26cm for *R. sutchuenense*.

Indumentum

R. asterochnoum is easily separated from all other species in the study group by its indumentum. It is unusual among the group in having rusty-brown indumentum instead of white (to fawn) indumentum. In addition to this, it has distinctive stellate trichomes as found in subsection *Parishia* (Chamberlain, 1982), which are visible with a x10 hand lens. Indumentum is very dense along the sides of the midrib, it is common along secondary veins and sparsely scattered across the lamina, though it does not persist well on the lamina. Young growth is densely indumented with a mixture of stellate, radiate and long simple hairs. Branchlets are tomentose at emergence with yellowish-white tomentum usually persisting for 1-2 years (see Figure 10).

Figure 10: R. asterochnoum at Benmore. Left: expanding leaves of new growth covered in dense tomentum. Centre: characteristic rusty brown indumentum of the species. Right: tomentum on new stem
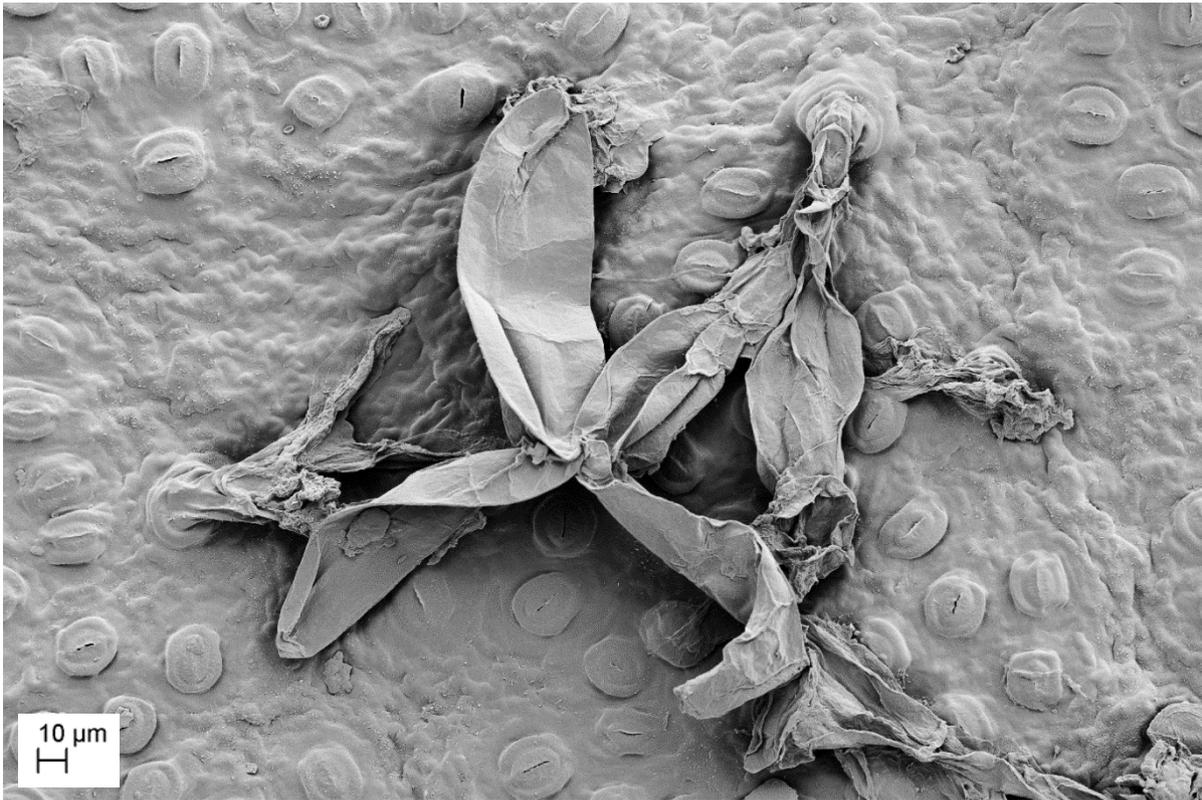
Figure 11: SEM of stellate hair on *R. asterochnoum* lamina. Some hairs damaged, but base of stalk for remaining hair in top right of image. Image taken at 6000x magnification with a working distance of 12.4mm

Figure 12: New growth on R. calophytum var. calophytum. Left: sparse fawn-coloured juvenile tomentum. Right: very young leaves densely tomented

Indumentum was not a very useful character for separating *R. calophytum* var. *calophytum* from *R. calophytum* var. *openshawianum* as neither variety consistently had persistent indumentum. Some specimens appeared glabrous, others had a sparse scattering of radiate hairs or persisting juvenile tomentum across the lamina, and a few patches of matted, squat, simple hairs plastered along the midrib. Tomentum was common on new growth but did not differ noticeably from the other species except that it was sometimes fawn coloured.
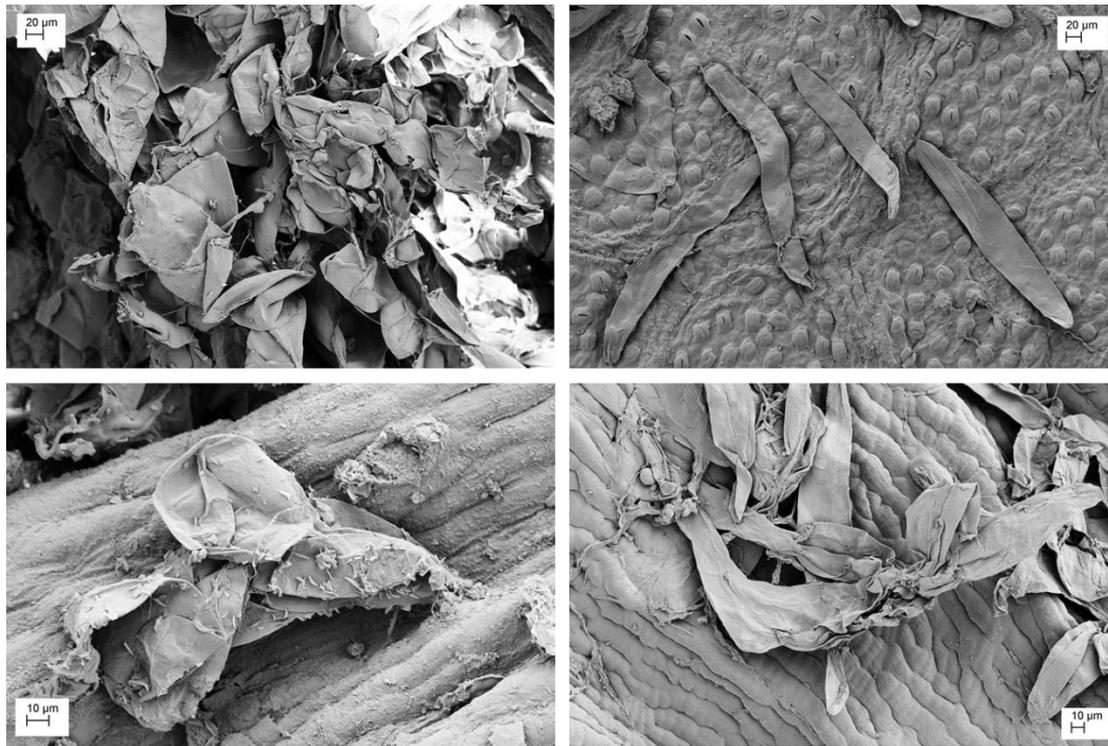
Figure 13: SEM images for R. calophytum. Top left: chaotic indumentum along midrib, mostly squat hairs (Mag = x400, WD = 11.6mm). Top right: simple, persisting juvenile tomentum on the underside of lamina (Mag = x350, WD = 10.2mm). Bottom left: radiate hair on midrib. Bottom right: simple dendroid hair on midrib

A range of hair types were found on samples of *R. calophytum* under the SEM. The simple, sporadically persisting juvenile tomentum type shown in Figure 13was most common. Radiate hairs with short, squat arms were found, along with some simple dendroid hairs. Indumentum along the midrib was too chaotic to interpret.

R. praevernum and *R. sutchuenense* could not be sufficiently separated into different species by trichome characters despite indumentum being fundamental to the initial description of *R. praevernum.* Juvenile and mature leaves from accessions of both species were studied. The new growth from all accessions emerges more or less identical. Hair characters differ only in mature leaves, in the quantity of persistent indumentum. This difference did not divide the accessions as expected into two distinct groups, but rather described the whole spectrum between entirely glabrous and with a persistent, dense indumentum along the sides of the midrib. Older plants especially were found to be less indumented than expected, e.g. *R. sutchuenense* at Glendoick, a Wilson original plant which matches the floral description of *R. sutcheunense* perfectly, but had surprisingly glabrous leaves.

Simple, juvenile, sporadically persistent tomentum was found on both specimens under SEM, as with the other study group species. Uniquely to these two species, alongside the juvenile tomentum, a second tomentum type that does not persist was found, illustrated in Figure 14. This tomentum consisted of a cob-web like network of thin trichomes that presumably snap off as the leaf expands increasing the tension between stalks. Indumentum along the midrib was usually sinuous in texture and looked to be composed of a mix of long, simple hairs, and medium stalked dendroid hairs.

Figure 14: Juvenile foliage of *R. praevernum* (outer two images) and *R. sutchuenense* (central two images)
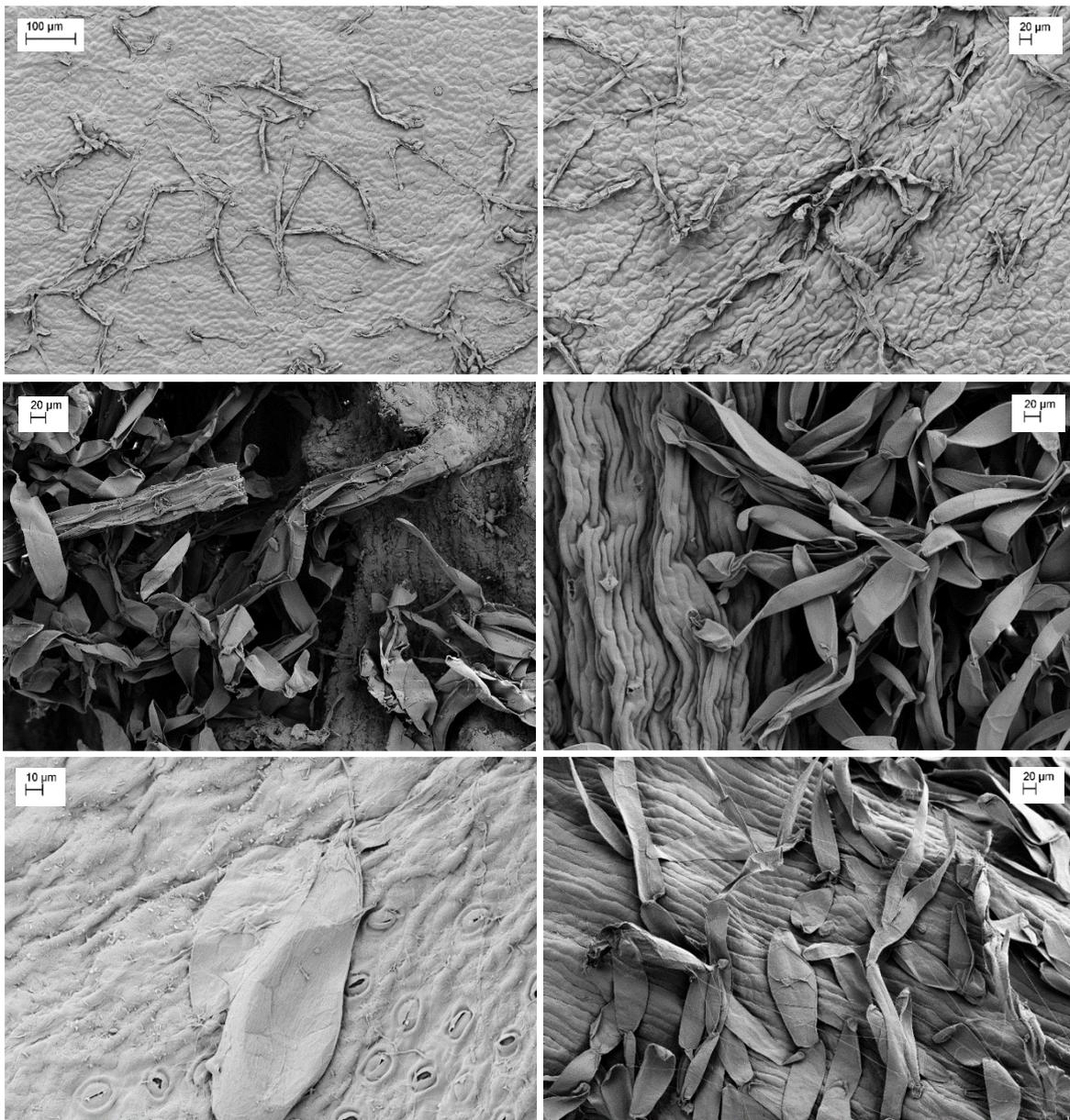
Figure 15: SEM images for R. praevernum and R. sutchuenense. Clockwise from top left: 1) R. praevernum cob-webby tomentum on abaxial side of juvenile leaf. 2) R. sutchuenense cob-webby tomentum on abaxial side of juvenile leaf. 3) R. sutchuenense, dendroid hairs persisting on midrib of mature leaf. 4) R. sutchuenense, mixture of dendroid hairs and simple, sinuous hairs persisting on midrib. 5) R. praevernum, simple hairs sporadically persisting on lamina. 6) R. sutchuenense, sparse indumentum of long, simple hairs, with some persistent juvenile tomentum on midrib

Inflorescence

Taxonomically informative inflorescence characters include overall habit, pedicel length and colour, rhachis length and number of flowers.

R. praevernum and *R. sutchuenense* have few-flowered trusses, with approx. 10 flowers per inflorescence. The rhachis is short, 8-15mm, and pedicels are short too creating a compact, flat-topped truss. Pedicels are brightly coloured red or red-purple, contrasting strongly with the corollas.

No taxonomically significant details were found between the inflorescences of *R. calophytum* and *R. asterochnoum*. *R. calophytum* var. *calophytum* may be distinguished from *R. calophytum* var. *openshawianum* by a combination of the following characters; having more flowers per truss (average of 18 vs 12), a longer rhachis (avg. 21 mm vs 15mm), and longer pedicels (avg. 58mm vs 45mm)

Calyx

The calyx in the study group species is highly reduced which limits its ability to be taxonomically informative. *R. asterochnoum* was found to have a larger calyx than all other species, being between 1.5mm and 2mm long, whereas most samples had a calyx just 0.5mm long.

Corolla

The study group species could be easily divided into two by corolla shape with *R. calophytum* and *R. asterochnoum* widely-campanulate to funnel campanulate, and *R. praevernum* and *R. sutchuenense* widely-funnelled to funnelled. All taxa had pinkish buds opening to a variation on pale-pink to white. *R. calophytum* and *R. asterochnoum* were more intensely pigmented than the other species, had usually glabrous corollas, and were always blotched. *R. praevernum* was always marked, usually with a dark crimson, prominent blotch in the upper half of the corolla. This blotch always disintegrated into dark speckling, sometimes abruptly, sometimes gradually. This solid blotched area was c. 20mmx18mm in most specimes studied, however in three specimens, the solid area of the blotch was c. 10x8mm, and in one specimen there was hardly a blotch at all, just a denser cluster of speckling. Two samples received as *R.* sutchuenense also had a blotch. The remaining samples of *R.* sutchuenense only had speckling, no blotch, and again the quantity of speckling varied between samples. Both *R. praevernum* and *R. sutchuenense* were puberulous on the inner surface of the corolla from the base for between one third and a half its length.

Androecium

R. calophytum var. *calophytum* consistently had 18-22 stamens whereas *R. calophytum* var. *openshawianum* had 20-25. *R. asterochnoum* was observed as having 15 to 20 stamens, but only one inflorescence was available for study so this figure cannot be used with any certainty. A slight distinction among these taxa was found in the puberulousness of their filaments, with *R. calophytum* var. *openshawianum* sometimes appearing glabrous, and other times being puberulous for just 1-2 mm (instead of 6-11mm). *R. praevernum* and *R. sutchuenense* both had c. 15 stamens, and were puberulous for roughly one third.

Gynoecium

Stigma size is an important character within this group. The number of locules was found to be variable for each species along with colour. *R. praevernum* and *R. sutchuenense* had 10-15 locules and attractively patterned ovaries; a light green with deep purple speckling condensing to a solid deep purple at the base. Their stigmas were capitate, 2-3mm in diameter and often pink tinged. *R. asterochnoum* and *R. calophytum* had 12-16 locules and usually a bright green ovary with no purple markings. Their stigmas were prominent and discoid (a trait in common with subsection *Grandia*), 6-8mm across and yellow.

Capsule

Taxonomically important capsule characters include shape and size. Texture, i.e. markedly ridged vs smooth does not appear to be informative for the study group but this could be due to poor representation in specimens. Capsule shape splits the taxa, into two groups; *R. praevernum* and *R. sutchuenense* have broadly cylindrical, slightly curved capsules with a rounded-truncate apex. *R. calophytum* var. *calophytum* is cylindrical to broadly cylindrical with an abruptly truncate apex. *R. calophytum* var. *openshawianum* is broadly cylindrical to very broadly cylindrical, again with an abrubtly truncate apex. No capsule sample was available for *R. asterochnoum.*

Seeds

No significant difference in seed morphology was found between the species.

3.2   Quantitative Morphological Character Results

Principal Component Analysis (PCA) found significant morphological differences between species, but only weakly supported varieties. The variation (%) and eigenvalues for the top five components of each PCA are given in Table 4. A summary of reduced datasets and PCA results are given in Table 5. Samples used for PCA were analysed under the taxon name assigned by the author during preliminary morphological study when samples were sorted into taxon groups (see sample details in Tables 1 and 2, Appendix 1)

Table 4: Variation and Eigenvalues of the top 5 components of each PCA

Component	PCA 1		PCA 2		PCA 3		PCA 4	
	Eigenvalue	Variability (%)	Eigenvalue	Variability (%)	Eigenvalue	Variability (%)	Eigenvalue	Variability (%)
1	3.525	44.059	4.436	36.971	7.313	40.627	9.742	32.475
2	1.751	21.888	2.141	17.842	3.533	19.626	6.521	21.736
3	1.038	12.970	1.207	10.056	1.408	7.825	3.900	13.000
4	0.678	8.470	0.965	8.043	1.244	6.909	2.341	7.803
5	0.598	7.475	0.819	6.825	0.966	5.367	2.092	6.972

Table 5: A summary of reduced datasets and PCA results

PCA	Reduced dataset description	Number of specimens	Number of characters	Taxa differentiated	Component	Eigenvalue	Variability (%)	Important Characters
1	Vegatative only	54	8	R. asterochnoum R. calophytum (s.l.) R. sutchuenense (s.l.)	1	3.525	44.059	Lamina length, 1 seasons growth, Lamina width, Petiole length
					2	1.751	21.888	Petiole length/Lamina length, Lamina width/Lamina length
2	Vegetative and floral	42	12	R. asterochnoum R. calophytum (s.l) R. sutchuenense (s.l.)	1	4.436	36.971	Lamina length, Pedicel length
					2	2.141	17.842	Petiole length
3	Mostly floral	24	18	R. asterochnoum R. calophytum var. calophytum R. calophytum var. openshawianum R. sutchuenense (s.l.)	1	7.313	40.627	Lamina length, Pedicel length, Stigma diameter
					2	3.533	19.626	Calyx size, style diameter, petiole length
4	Floral: R. praevernum and R. sutchuenense only	13	12	R. sutchuenense (s.l.)	1	9.742	32.475	Puberulous length of shortest stamen, puberulous length of longest stamen, number of flowers per truss
					2	6.521	21.736	Solid blotch area, solid blotch height, solid blotch width, Width/Height of corolla markings

PCA 1 (Figure 16) separates the samples into three clear species groups with 3 anomalous observations using the first two axes. The first axis with variance 44.06% has important characters: lamina length (0.932), length of one season's growth (0.792), lamina width (0.751) and petiole length (0.747). Axis 2 has variance 21.89% and is composed of the ratio between lamina length and petiole width (0.817), and the ratio between lamina length and width (0.674). The two axes have cumulative variation 65.95%.

R. asterochnoum (Group 1) is separated from group 2 by axis 2, and from group 3 by (mostly) axis 1. Group 2 is separated from groups 1 and 3 by axis 2. This group contains all but three of the samples determined as *R. calophytum.* One of these anomalous samples was a poor quality herbarium specimen with just one and a half leaves so it may be regarded as low quality data and ignored. The other two samples have a shared origin, one an herbarium specimen from the Chengdu-Edinburgh Expedition, the other from its progeny grown at Dawyck. Group 2 does not provide any clear further subdivisions correlating to the two varieties of *R. calophytum* recognised by this study. Group 3 (excluding the anomalies mentioned earlier) comprises *R. praevernum, R. sutchuenense* and *R. X geraldii,* again with no further subdivision of these taxa possible. For the rest of this discussion, the term *R. sutchuenense* (s.l.) is used to refer taxa here united in group 3. The species *R. decorum* is included to test the hypothesis that the sample is distinct from the study group species and the previous determination of *R. calophytum* var. *openshawianum* was incorrect. Its position in Figure 16 is insufficient evidence to either accept or reject this hypothesis as group 2 could easily be extended to include the data point with minimal effect. The vegetative continuous data characters

of lamina length, length of one season's growth, lamina width and petiole length may be used in conjunction with the ratios between lamina length and petiole width, and lamina length and width to separate the three groups: *R. asterochnoum, R. calophytum* and *R. sutchuenense* (s.l.).
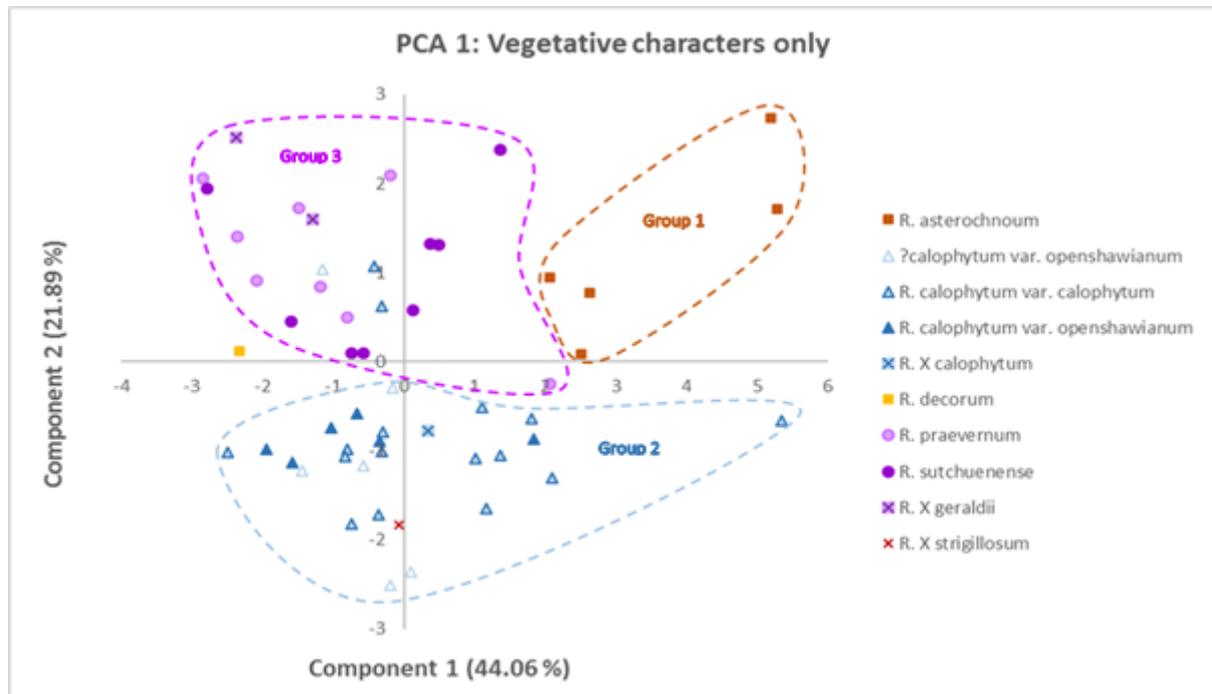
Figure 16: PCA 1 scatter plot of the first two axes, Component 1 and Component 2 with cumulative variability of 65.95%

PCA 2 separates the taxa into the same three groups as in PCA 1. This is not surprising as some characters are included in both datasets. The first axis with variance 36.97% has important characters: lamina length (0.934), pedicel length (0.773), the ratio between lamina length and the number of flowers per truss (0.695). Axis 2 has variance 17.84% with the most important character petiole length (0.774). The two axes have cumulative variation 54.81%. Groups are separated by the combination of axes 1 and 2, rather than by either alone. Only one sample of *R. asterochnoum* had flowers so the scatter plot in Figure 17 is not informative about expected variation within the species, however, it does cluster outside groups 2 and 3 inferring that were more data available, a distinct cluster as in Figure 16 is likely.

Group 2 contains all accessions of *R. calophytum* but again lacks any subdivision corresponding with varieties. Incorporating a few floral characters into the dataset has resulted in neater clustering of species overall confirming (the seemingly obvious) that the species are more easily distinguished from one another both vegetative and floral characters are considered together. Group 3 again comprises *R. sutchuenense* (s.l), with the two species and their described natural hybrid intermingled, leading to the question of whether there are in fact two distinct species here. Instead, is *R. sutchuenense* just a highly variable species which ought to include, within its circumscription, *R. praevernum*, thus explaining *R. X geraldii* as natural variation within the species?
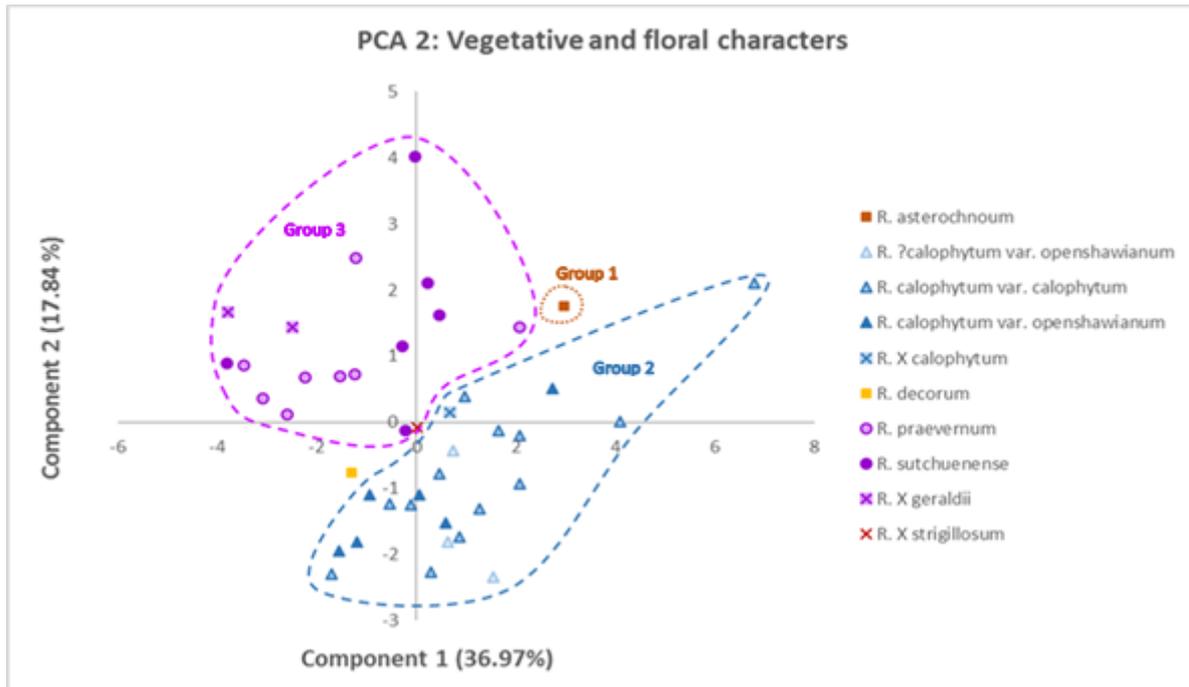
Figure 17: PCA 2 scatter plot of the first two axes, Component 1 and Component 2 with cumulative variability of 54.81%
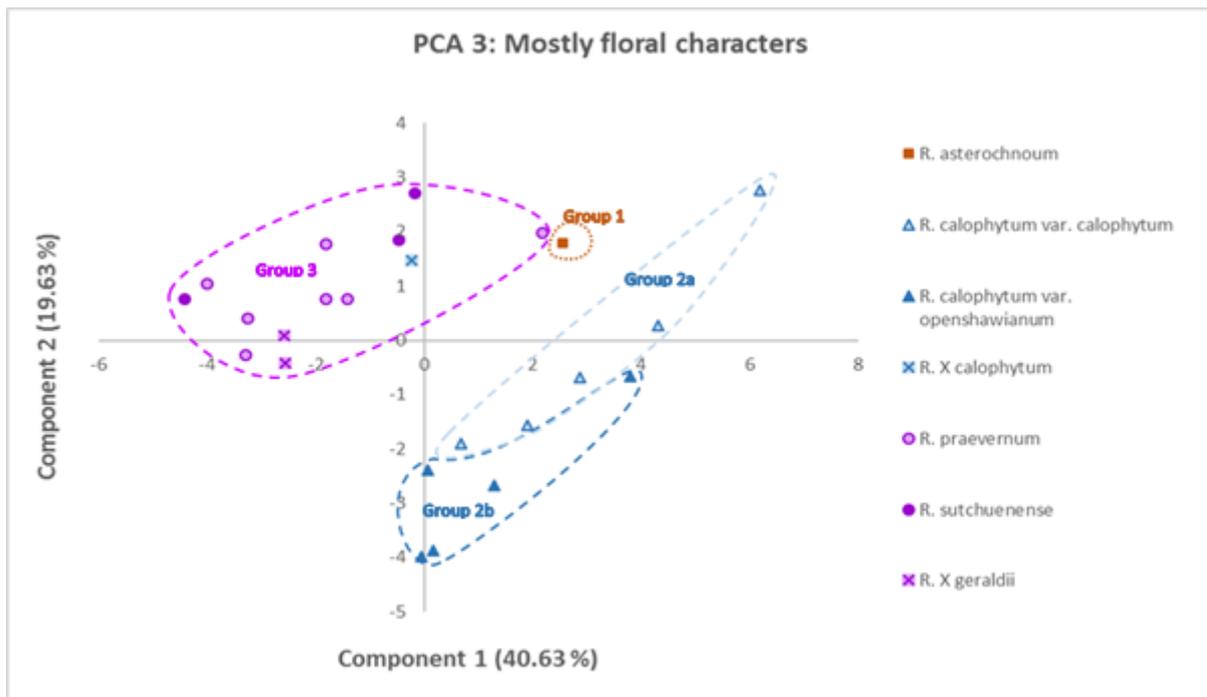
Figure 18: PCA 3 scatter plot of the first two axes, Component 1 and Component 2 with cumulative variability of 60.26%

PCA 3 separates the taxa into the same three groups as in PCA 1 and PCA 2. However, this time group 2 is subdivided. The first axis with variance 40.63% has important characters: lamina length (0.921), pedicel length (0.894), and stigma diameter (0.794). Axis 2 has variance 19.63% with the most important characters: calyx size (0.705), petiole length (0.638) and style length (0.507). The two axes have a high cumulative variation of 60.26%. Groups are separated by the combination of axes 1 and 2. Again, *R. asterochnoum* is not informative about expected variation within the

species, but does sit outside groups 2 and 3 inferring that were more data available, a distinct cluster as in Figure 16 is possible.

Group 2 contains all accessions of *R. calophytum* but this time internal structure is visible, with the subdivisions of group 2a and group 2b corresponding with *R. calophytum* var. *calophytum* and *R. calophytum* var. *openshawianum* respectively. Of all the numerical characters recorded, the combination of lamina length, pedicel length, and stigma diameter with calyx size, petiole length and style length may be used to separate the species *R. calophytum* into its two varieties.

Group 3 is separated from groups 1 and 2 by a combination of axes 1 and 2. Again, there is no further division of this cluster available which reflects the currently recognized classification.
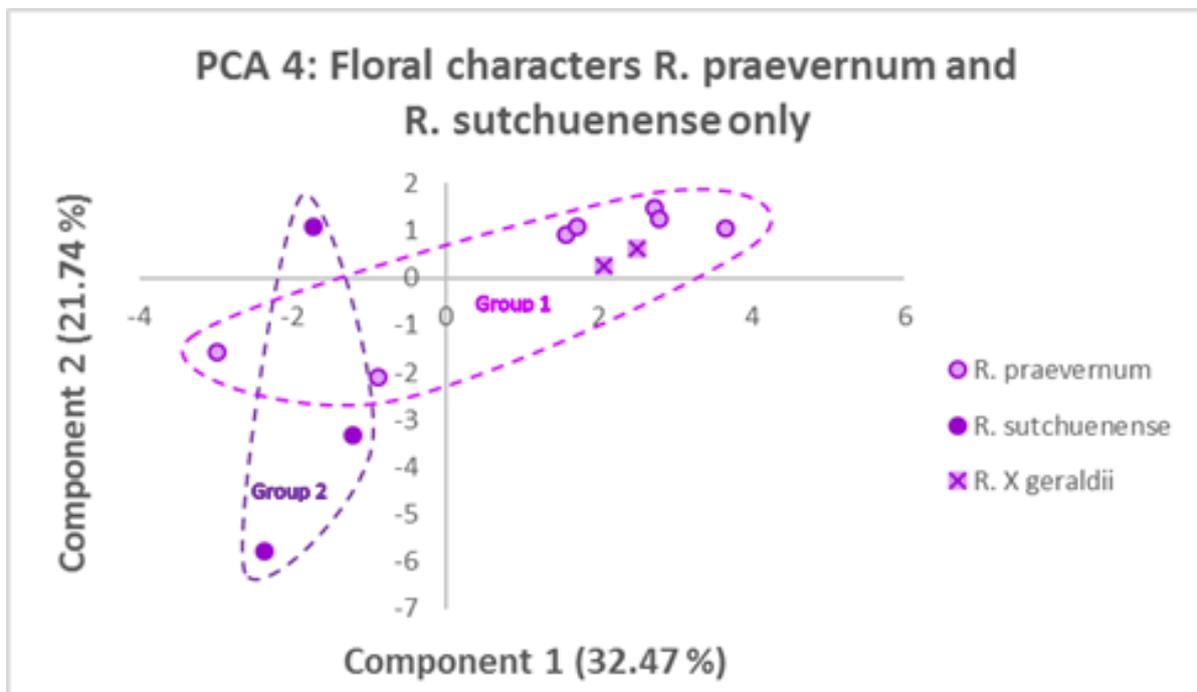
Figure 19: PCA 4 scatter plot of the first two axes, Component 1 and Component 2 with cumulative variability of 54.21%

PCA 4 was based upon measurements from just 12 samples as this was all the available floral material for *R. sutchuenense* (s.l.). The first axis with variance 32.47% has important characters: puberulous length of shortest stamen (0.928), puberulous length of longest stamen (0.864), total area of markings in corolla throat (0.677). Axis 2 has variance 21.74% with the most important characters: area of solid blotch (0.854), ratio of blotch height to width (0.742), length of fused corolla (0.648). The two axes have a cumulative variation of 54.21%.

Here the artificial groups 1 and 2 reflect species as described, with group 2 *R. sutchuenense*, in which corollas lack a blotch, and group 1 *R. praevernum* composed of samples with an obvious blotch. This traditional character does not separate the two species in this analysis. However, with so few samples, it is difficult to justify any conclusions besides a need for better sampling. Perhaps with more observations, this character would encourage the species to cluster.

4 Molecular Materials and Methods

4.1 Taxon Sampling and Material Collection

Material was collected for molecular analysis from *R. calophytum* (var. *calophytum* 3 samples, var. *openshawianum* 3 samples, var. *pauciflorum* 3 samples)*, R. asterochnoum* (4 samples)*, R, praevernum* (5 samples)*, R. sutchuenense* (7 samples), and *R.* X *geraldii* (2 samples). Specimens used are given in Table 6. Sampling was limited by the amount of available material, and the limited number of introductions to cultivation. In addition to the 27 samples spread across these study species and their suspected natural hybrids, *R. insigne, R. planetum* and *R. facetum* were sequenced. *R. insigne* appeared anomalous in its placement in Figure 1; as the voucher specimen could not be located, a placement due to misidentification or mix-up of samples could not be excluded so it has been resampled here that we may confirm its identity. *R. planetum* is another early flowering fortunea of unclear taxonomic status, a putative natural hybrid between *R. sutchuenense* and *R. oreodoxa. R. facetum* (section *Parishia*) was included to ensure each section of *Hymenanthes* was represented once new sequences were added to those from Milne (2010) .

Leaf material for molecular analysis was collected from RBGE, Benmore, Dawyck, Glendoick, Corrour and Glenarn (Table 6). Voucher specimens were made for each sample at the time of material collection; these will be placed in the herbarium at RBGE. Since this year's growth was not fully expanded at the time of observation, terminal leaves were selected from the previous year's growth. Only healthy, clean leaves were used. Approximately 4cm^2 laminar tissue (free from midrib and secondary veins) was torn into small pieces and deposited in a pre-labelled ziplock bag with 20g of silica gel with indicator crystals. Samples were left to dry for a minimum of 3 days (usually 7) before DNA extraction.

Table 6: Samples used for molecular work.

Taxon Name	Accession Number	Accession Qualifier	Location	Date of Silica Collection	Provenance	EDNA ID	Sample ID	Collector(s) / Expedition	Collection number
R. asterochnoum	20040714	A	V31	12th May 2016	wild origin	EDNA16-0045031	HANWIL2	Peter Cox & Peter Hutchinson	C&H7051
R. asterochnoum	20040714	B	V42	12th May 2016	wild origin	EDNA16-0045033	HANWIL4	Peter Cox & Peter Hutchinson	C&H7051
R. asterochnoum	20040714	C	YJ5	18th May 2016	wild origin	EDNA16-0045425	HANWIL9	Peter Cox & Peter Hutchinson	C&H7051
R. asterochnoum	-	-	Glendoick	24th May 2016	wild origin	EDNA16-0045426	HANWIL10	Peter Cox & Peter Hutchinson	C&H7051
R. calophytum var. calophytum	19952865	C	V32	12th May 2016	wild origin	EDNA16-0045037	HANWIL8	Chengdu Edinburgh Expedition	CEE172
R. calophytum var. calophytum	19960422	A	Benmore	7th June 2016	wild origin	EDNA16-0045441	HANWIL25	Tibet & Sichuan Expedition	SICH1630
R. calophytum var. calophytum	19960429	I	C14	10th May 2016	wild origin	EDNA16-0045036	HANWIL7	Tibet & Sichuan Expedition	SICH1656
R. calophytum var. openshawianum	19960770	A	V09	12th May 2016	unknown	EDNA16-0045030	HANWIL1		
R. calophytum var. openshawianum	19795452	-	V09	12th May 2016	unknown	EDNA16-0045034	HANWIL5		
R. calophytum var. openshawianum	-	-	Glendoick	24th May 2016	wild origin	EDNA16-0045427	HANWIL11	Peter Cox & Peter Hutchinson	C&H7055
R. calophytum var. pauciflorum	19960655	A	YK9	18th May 2016	wild origin	EDNA16-0045428	HANWIL12	Kunming Yunnan expedition	AC1054
R. calophytum var. pauciflorum	19960655	C	YK7	18th May 2016	wild origin	EDNA16-0045429	HANWIL13	Kunming Yunnan expedition	AC1054
R. calophytum var. pauciflorum	19960655	D	YK7	18th May 2016	wild origin	EDNA16-0045430	HANWIL14	Kunming Yunnan expedition	AC1054
R. facetum	19962558	A	Benmore	7th June 2016	wild origin	EDNA16-0045442	HANWIL26	Keith Rushforth	KR3986
R. insigne	19698662	I	F15/S	8th July 2016	unknown	EDNA16-0045568	HANWIL31		
R. planetum	19270460	C	Benmore	7th June 2016	from plant at caerhays	EDNA16-0045443	HANWIL27		
R. praevernum	19698798	A	C07/02	10th May 2016	cultivated	EDNA16-0045035	HANWIL6		
R. praevernum	19795174	B	V05	12th May 2016	unknown	EDNA16-0045431	HANWIL15		
R. praevernum	387	-	Corrour	9th June 2016	unconfirmed	EDNA16-0045436	HANWIL20		
R. praevernum	389	-	Corrour	9th June 2016	unconfirmed	EDNA16-0045437	HANWIL21		
R. praevernum	404	-	Corrour	9th June 2016	unconfirmed	EDNA16-0045438	HANWIL22		
R. sutchuenense	19865006	B	V05	12th May 2016	unknown	EDNA16-0045032	HANWIL3		
R. sutchuenense	-	-	Glendoick	24th May 2016	wild origin	EDNA16-0045432	HANWIL16	E H Wilson	
R. sutchuenense	181	-	Corrour	9th June 2016	unconfirmed	EDNA16-0045435	HANWIL19		
R. sutchuenense	559	-	Corrour	9th June 2016	unconfirmed	EDNA16-0045439	HANWIL23		
R. sutchuenense	595	-	Corrour	9th June 2016	unconfirmed	EDNA16-0045440	HANWIL24		
R. sutchuenense	-	-	Glenarn	8th June 2016	wild origin	EDNA16-0045444	HANWIL28	J D Hooker	
R. sutchuenense	-	-	Private garden	16th June 2016	unknown	EDNA16-0045445	HANWIL30		
R. X geraldii	19913262	B	V32	12th May 2016	wild origin	EDNA16-0045433	HANWIL17	Chengdu Edinburgh Expedition	
R. X geraldii	19913262	A	V32	12th May 2016	wild origin	EDNA16-0045434	HANWIL18	Chengdu Edinburgh Expedition	

4.2   DNA Region Selection

Different genomes evolve at different rates (Wolfe, Li, and Sharp, 1987). In order to build a useful phylogeny, it was vital to choose appropriate gene regions for the group under study. Phylogenies will be poorly resolved if the genome regions used evolve too quickly (causing long-branch attraction), or too slowly (causing polytomies) as a result of increased homoplasy. Higher plant DNA sequences evolve at different rates according to their location (Wolfe, Li, and Sharp, 1987), with nuclear DNA the fastest evolving, chloroplast DNA evolving at half the rate of nDNA, and mitochondrial DNA evolving at least 5 times slower than nDNA (Wolfe, Li, and Sharp, 1987). Chloroplast DNA is often used to investigate the genus and species level relationships of higher plants as it generally results in well resolved phylogenies, and it removes any risk of zoological contamination. For this study, the chloroplast gene regions trnL-trnF and matK were chosen. These regions have been extensively used within *Rhododendron* so choosing them meant the new sequences could be incorporated into existing phylogenies. This was the primary reason for selecting matK and trnL-trnF as resources limited the possible number of new sequences to 30, an insufficient dataset for exploring relationships within *Hymenanthes*. The first region examined, trnL-trnF, is a fragment of approximately 1kb, comprising the trnL intron, and trnL-trnF intergenic spacer. This region is noncoding and has been used in phylogenetic studies at many different taxonomic levels across the angiosperms to great effect (Gielly and Taberlet, 1996) (Kusumi et al., 2000) (Wallander and Albert, 2000), and within the genus *Rhododendron* (Kurashige et al., 2001), (Milne, 2004) (Brown et al., 2005) (Milne et al., 2010).

The second region used was the matK (extending to part of the trnK intron). The gene matK is a coding region approximately 1550 base pairs long, nested within a 2600 base pair intron between two trnK exons (Johnson and Soltis, 1995) , as shown in Figure 20. The gene matK encodes a maturase for splicing the trnK group IIA introns from RNA transcripts and is instrumental in staging early chloroplast development (Zoschke et al., 2010). MatK was chosen for this study as it has been used in conjunction with trnL-trnF in previous studies in *Rhododendron* (Kurashige et al., 2001), (Milne, 2004), (Milne et al., 2010) for effective analysis of sectional and species relationships. Working with frequently sequenced regions enabled expanded taxon sampling to include species from across the genus and thus construct a phylogeny to show how the study group species relate to the rest of subsection *Hymenanthes*, within the genus.
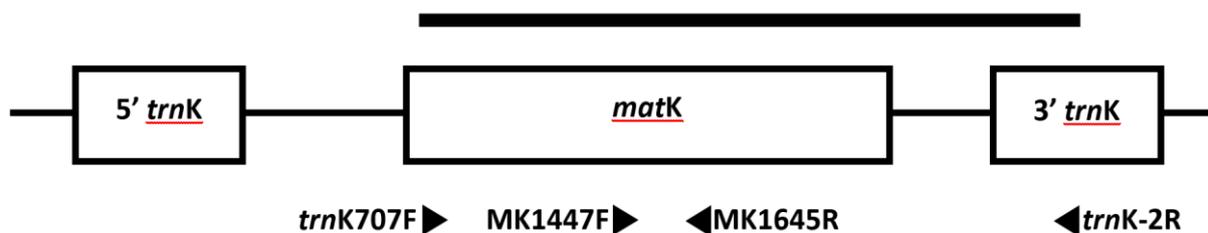
Figure 20: Structure of matK cpDNA region. Thick bar above diagram indicates region sequenced. Arrows indicate location and direction of primers.

4.3   DNA Extraction

DNA was extracted using the DNeasy Plant Mini Kit following the Qiagen DNeasy Plant Mini Kit protocol (Swofford, 2002).

Complete and quick disruption of starting material is essential to ensure high DNA yields and to avoid DNA degradation. To extract total genomic DNA 20g dry weight of silica gel dried leaf material was removed from the silica gel bags with tweezers and placed in a 2.0ml Eppendorf tube with one tungsten carbide 3mm Retsch cone ball. Silica gel is an irritant so gloves were worn and a fume bench used when handling it. Between samples, tweezers were cleaned using 70% ethanol to prevent cross contamination. Leaf material was then disrupted by processing the samples in TissueLyser II (Qiagen) set to 30seconds at 20Hz. This was repeated until a fine powder was obtained, with the adapters rotated between grinding cycles. Most material achieved a fine powder after three or four cycles. One sample (HPW19) was insufficiently dry for this method to be effective and had to be hand ground in a 1.5ml Eppendorf tube using a mini pestle and a pinch of sand. This could have affected DNA extraction but the sample was checked throughout the process and all subsequent steps were successful.

Samples were chemically lysed by adding 400µl Buffer AP1 and vortexing vigorously before incubation for 1 hour at 65 °C in Thermomixer C (Eppendorf) set at 800rpm. RNase is unnecessary for extractions from dried material so it was omitted. It is important to vortex before incubation to remove any clumps of tissue as these will not lyse properly. Following lysis, 130 µl Buffer P3 was added to the lysate and incubated on ice for 5min to precipitate detergent, proteins and polysaccharides.

The presence of large amounts of precipitates can result in shearing of the DNA in subsequent steps so lysates were then centrifuged in a Heraeus Pico 17 centrifuge (Thermo Electron Coorperation) for 5 min at 13,000rpm to condense the precipitates at the base of the Eppendorf tube. The clear lysate was then pipetted (taking care not to disturb the precipitates) into the QIAshredder Mini spin column, placed in a 2ml collection tube. Samples were centrifuged for 2min at 13,000rpm. The QIAshredder Mini spin column allows the lysate to pass through, whilst removing most precipitates and cell debris. Any remaining precipitates and cell debris formed a pellet in the collection tube.

Taking care not to disturb this pellet, lysate flow-though was pipetted into a new collection tube and mixed with 650 µl Buffer AW1 by pipetting. Buffer AW1 (with added ethanol) promotes binding of the DNA with the DNeasy membrane in a DNeasy mini spin column. 650 µl of this solution was transferred to the DNeasy mini spin column placed in a 2ml collection tube and centrifuged at 8,000rpm for 1min.

Flow-through was discarded and this step repeated with the remaining lysate mixture before transferring the DNeasy Mini spin column to a new 2ml collection tube. The DNeasy Mini spin column was then centrifuged twice with Buffer AW2 (once for 1min at 8,000rpm, then for 2min at 13,000rpm) and the flow-through discarded each time to remove any residual ethanol, proteins and polysaccharides which could interfere with subsequent reactions, and to dry the membrane. Elution of the pure DNA was carried out by applying AE Buffer directly onto the DNeasy Mini spin column

membrane, transferred into a clean 1.5ml microcentrifuge tube. AE buffer contains EDTA which stops Mg degrading DNA. Elution was carried out twice with 75 µl of AE buffer each time for 1 min at 8,000rpm to wash the DNA from the membrane into the microcentrifuge tube. Extractions were stored at -20 °C when not in use to prevent denaturation.

4.4 Polymerase Chain Reaction

Two cpDNA regions were amplified for all accessions; trnL-trnF and matK. The trnL-trnF region was amplified using the primers designed by Taberlet et al. (1991). The MatK region is easily amplified as it is flanked by highly conserved coding regions (Johnson and Soltis, 1995).

Table 7: Primers used for gene amplification

Region	Primer	Sequence 5'-3'
trnL-trnF	trnLF-c	CGA AAT CGG TAG ACG CTA CG
		(Taberlet et al., 1991)
trnL-trnF	trnLF-f	ATT TGA ACT GGT GAC ACG AG
		(Taberlet et al., 1991)
matK	trnK707F	ACT GTA TCG CAC TAT GTA TC
		(Milne, 2004) modified from trnK710F (Johnson and Soltis, 1995)
matK	trnK-2R	AAC TAG TCG GAT GGA GTA G
		(Johnson and Soltis, 1995)

The PCR recipe given in Table 8 was used for both matK and trnL-F, the only difference being the primers used. Each time a PCR was carried out, a master mix was made by multiplying the volumes in Table 8 by 1+(the number of samples to be processed) for all reagents except the template DNA. These were added to a 1.5ml Eppendorf tube, vortexed and centrifuged to create a master mix. 24 µl of the master mix was then aliquotted into each of the 0.2ml reaction tubes. The excess master mix was used as a negative control and was always aliquoted last. 1 µl of template DNA was then added to each reaction tube (except the negative control), giving a reaction volume of 25 µl. CES is as an effective, low-cost PCR enhancer shown to improve qualitative and/or qualitative output of PCRs (Ralser et al., 2006).

Table 8: Reagent volumes for one sample for PCR

Reagent	Volume (µl) per sample
dH$_2$O	12.05
10xNH$_4$ Buffer	2.5
MgCl$_2$	1.25
dNTPs	2.5
Primer A	0.75
Primer B	0.75
CES	4
Biotaq polymerase	0.2
Template	1
Total Volume(µl)	25

"Hot-start" PCR reactions (Ashraft and Paul, 2009) were performed under the program outlined in Table 9 (Brown, 2002), (Milne et al., 2010) using a Tetrad II DNA Engine peltier thermal cycler (Biorad). The cycle lasted 1hour54mins30secs, after which the PCR product was visualised using gel electrophoresis.

Table 9: Region amplification PCR programme

Step	Temp. (°C)	Duration (seconds)	Process	Repeats
1	94	150	Initial Denaturation: Heating breaks hydrogen bonds holding complimentary DNA strands together, resulting in double stranded DNA being separated into two single strands.	none
2	94	30	Denaturation (from second cycle onwards): Heating breaks hydrogen bonds resulting in newly synthesised target strand being separated from single stranded DNA.	Repeat 34 times
3	55	60	Annealing: Reduced temperature results in hydrogen bonds forming either between single DNA strands, or, as deisired, between primers and their target sequence.	
4	72	90	Extension: Taq polymerase synthesises DNA in 5' to 3' direction by facilitating the binding and joining of dNTPs.	
5	72	420	Extension: Taq polymerase synthesises DNA in 5' to 3' direction by facilitating the binding and joining of dNTPs.	none

4.5   Gel Electrophoresis

Molecules can be separated according to size using gel electrophoresis; a process in which an electric field is used to move macromolecules through a matrix. DNA molecules are negatively charged and so migrate towards the anode. The rate of migration of a DNA fragment is determined by its length; the shorter the molecule, the faster it travels through the gel matrix. Gel electrophoresis was used to visualise the PCR products to check that amplification of only one region had occurred, and that the master mix had not been contaminated. A single, uniform, clear band for each sample would indicate that all fragments are the same length and that amplification was successful.

A 1% agarose gel was made by adding 1g of agarose powder (Bioline, UK) to a conical flask with 100ml of 1xTBE buffer (Affymetrix UK Ltd, UK) and heating the solution in a microwave, mixing periodically until the agarose has all dissolved. The solution was allowed to cool until the bottom of the conical flask was comfortable to touch. To allow visualisation, 5μl SYBR safe DNA gel stain (Invitrogen, UK) was added and mixed in by gentle swirling of the conical flask. The solution was then poured into a medium gel tray with two gel combs which create loading wells. SYBR safe is photosensitive so the gel tray was covered with black card to prevent degradation whilst the gel was left for 30mins to set.

Samples were prepared for loading in a 96well plate by mixing 3μl PCR product with 2μl gel loading solution (Sigma-Aldrich Company Ltd, UK), containing glycerol to make the sample sink into the bottom of the well, and a blue dye so that migration of the samples can be monitored. Once the agarose gel had solidified the combs were removed and it was placed in an electrophoresis tank (Wide Mini-sub cell GT, Biorad) which was then topped up with TBE buffer to just above the surface of the gel. Samples were loaded left to right, top to bottom by pipetting, with a 1Kb plus DNA ladder (Invitrogen, UK) in the central well. The Power Pac 300 (Biorad) was then run for 30min at 80V, again with black card protecting the photosensitive SYBR safe.

When the electrophoresis was completed, the agarose gel was placed in the Syngene G:BOX F3 Flourescence Imaging System, on the UltraBright LED blue light transilluminator, beneath an orange filter panel. Images were captured using Genesys Image Acquisition Software for every gel run. Figure 21 is an example of a captured image (images for other runs included in appendices). One clear band is present in for each sample, inferring amplification was successful. This run shows 22 samples for matK, one sample for trnLF and a negative control for each. The negative controls are blank, showing no contamination. You can also see that trnLF is shorter than matK as it has travelled further down the gel. The second sample in on the bottom line is unusually faint and could suggest that PCR had not worked and would need to be repeated for this sample, or that insufficient template DNA was added so the PCR product is weak and the amount of template added to the sequencing PCR reaction should be increased for this sample. However, in this instance, the error is known: an ill-timed shaking hand resulted in only half the mix being loaded into the well, the other half lost, mixed in with the TBE buffer.
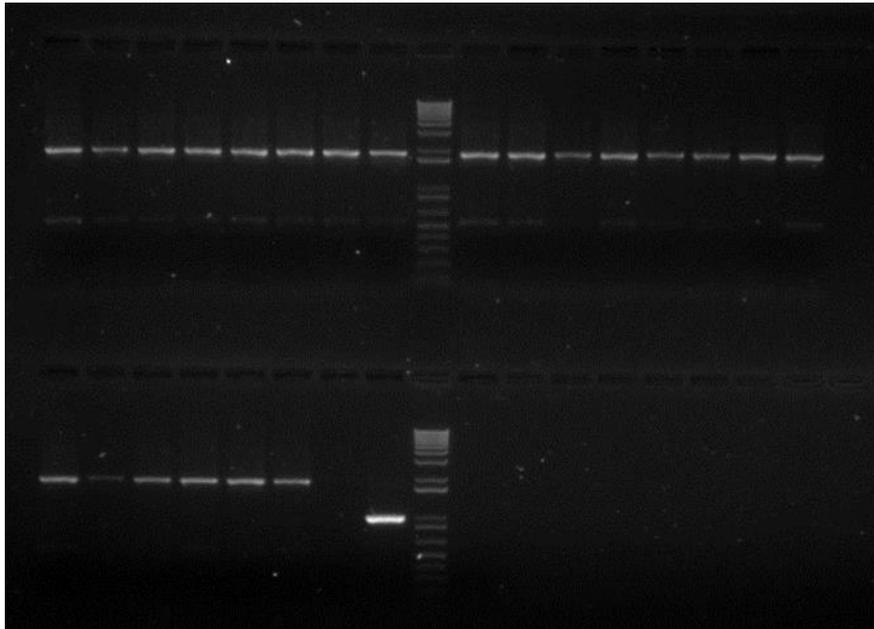
Figure 21: Image captured from gel electrophoresis of PCR products on 13th July 2016. Central well on both rows 1kb plus ladder. Samples left to right, top to bottom are: HPW9-HPW28, HPW30, HPW31, PCR product for matK amplification, negative control for matK master mix, HPW31; PCR product for trnL-F, negative control for trnL-F PCR to right of lower ladder

4.6   PCR Product Purification

PCR products were purified using ExoSAP IT (GE Healthcare) to remove unincorporated dTNPs and primers which can interfere with subsequent reactions, resulting in an unreadable sequence. ExoSAP IT is an enzyme-based purification treatment; Exonuclease I and Shrimp Alkaline Phosphatase degrade primers and dephosphorylate excess dNTPs (Bell, 2008). 2µl ExoSAP IT was added to 5µl PCR product before incubation in a Tetrad II DNA Engine peltier thermal cycler (Biorad) for 15mins at 37°C followed by a further 15mins at 80°C to inactivate the enzymes (Bell, 2008).

4.7   Sequencing PCR

TrnL-trnF was sequenced in two parts using the same two primers as for amplifying PCR. However, as matK is a long region, performing a sequencing PCR using just two primers results in low quality sequence. This is because long sequence fragments in the product occur in only miniscule proportions since the probability of a ddNTP being added (thus terminating extension) instead of a dNTP increases as fragment length increases. It is therefore best to sequence matK in four parts (two portions), using four primers. The same two external primers flanking the region used for amplification, with an additional two internal primers for sequencing designed by Milne et al, (2010), using existing matK sequences for subsection *Pontica* (Milne, 2004). Primers used for sequencing PCR are listed in Table 10, their relative position indicated in Figure 20, p39.

Table 10: Primers used in sequencing PCR

Region	Primer	Sequence 5'-3'
trnL-trnF	trnLF-c	CGA AAT CGG TAG ACG CTA CG (Taberlet et al., 1991)
trnL-trnF	trnLF-f	ATT TGA ACT GGT GAC ACG AG (Taberlet et al., 1991)
matK	trnK707F	ACT GTA TCG CAC TAT GTA TC (Milne, 2004) modified from trnK710F (Johnson and Soltis, 1995)
matK	trnK-2R	AAC TAG TCG GAT GGA GTA G (Johnson and Soltis, 1995)
matK	MK1447F	CGC TCA ATA TCT TCT GAA ACC TT (Sang, Crawford, and Stuessy, 1997)
matK	MK1645R	AGC CAA AAT GGC TTT TCC TC (Sang, Crawford, and Stuessy, 1997)

A sequencing reaction mix was made to the PCR recipe given in Table 11 was used for both matK and trnL-F. A master mix was made for each primer so that only one fragment was amplified per reaction tube during PCR. Master mixes were made by multiplying the volumes in Table 11 by 1+(the number of samples to be processed) to allow for measurement/pipetting error. These volumes of reagents were then added to a 1.5ml Eppendorf tube, vortexed and centrifuged. 9.5µl of the master mix was then aliquotted into each of the 0.2ml reaction tubes and 0.5µl of purified PCR product added giving a reaction volume of 10µl.

Table 11: Reagent volumes for one reaction for sequencing PCR

Reagent	Volume (µl) per sample
dH$_2$O	6.68
5x BigDye® Terminator v1.1 & v3.1 5X Sequencing Buffer (Applied Biosystems,UK)	2
10uM Primer	0.32
BigDye® Terminator v3.1 (Applied Biosystems, UK)	0.5
Volume of master mix	9.5
Purified PCR Product	0.5
Reaction volume	10

Samples were vortexed and placed in the Tetrad II DNA Engine peltier thermal cycler (Biorad) programmed to the cycle sequencing profile in Table 12, lasting 2hours50secs.

Table 12: Sequencing PCR programme

Step	Temp. (°C)	Duration (seconds)	Process	Repeats
1	95	30	Denaturation: Heating breaks hydrogen separating doubled stranded PCR product into single stranded fragments.	Steps 1-3 repeated 24 times.
2	50	20	Annealing: Reduced temperature results in hydrogen bonds reforming. As only one primer has been added, only one strand is amplified.	
3	60	240	Extension: Taq polymerase synthesises DNA in 5' to 3' direction by facilitating the binding and joining of dNTPs. When a labelled dNTP(ddNTP) is incorporated, extension stops resulting in a product containing fragments of different lengths.	
4	4	forever	Storage: at the end of the programme products are kept at 4°C to preserve products until they are moved to the freezer.	

Samples were sent to the Genepool facility (Edinburgh Genomics, University of Edinburgh) for sequencing, with resultant sequences returned by email. All accessions were successfully sequenced.

4.8 Sequence Editing

Sequences were edited using Sequencher v5.1 – Build 10627 (Gene Codes Coorporation, 2012). All sequences were trimmed to remove primers and poor quality data. Forward and reverse (and internal for matK) sequence data were aligned by 'assembly by name' to form contigs. These contigs were then manually checked to ensure sequences ran in the correct direction (i.e. 5' to 3'). Every contig was manually checked for ambiguities between sequence fragments which were resolved by eye using the chromatograms. If still unclear then sequences were checked against a matrix of aligned sequences which were to be used in addition to the new sequences, with more confidence placed in highly conserved regions in the matrix, and ambiguities retained if basepair positions in the aligned matrix were heterozygous. The consensus sequence was generated for each contig and saved as a FASTA file.

4.9 Phylogenetic Methods

Outgroup Selection
In order to test the monophyly of my study species within *Hymenanthes* it was important to sample across the whole subgenus. As *Hymenanthes* is known to lack internal structure from previous

studies (Hyam, 1997), (Milne et al., 2010), the dataset was expanded so as to include representatives from all other subgenera, and at least one taxon from each section to allow comparison of the overall topology more easily with other works. Wherever possible, the same accession was used for both gene regions. Rather than omit some subgenera and sections from the analyses, where only one region was available for an accession, a different accession had to be used to ensure sequences were obtained for both gene regions. Details of species included in analyses, their section and accessions used can be found in Table 1, Appendix 2.

Data from matK and trnL-F regions were initially considered separately. An alignment file for each region was created in Bioedit (Hall, 1999) combining the consensus sequences obtained from molecular work with the 108 sequences from genbank. Sequences were aligned manually by eye. Gaps were coded as missing data, informative indel characters of two or more base pairs were coded for by adding a single character to the end of the data matrix, coded as DNA for parsimony analysis and numbers (datatype = standard) in Bayesian analysis. Indels were assumed to have no greater or lesser phylogenetic significance than substitutions and so were given the same weight as substitutions in parsimony analysis and partitioned as a separate dataset in Bayesian analysis.

Parsimony Analysis

Maximum parsimony (MP) analyses were conducted in PAUP*version 4.0a149 (Swofford, 2002). First a heuristic search with 10,000 replicates was run to generate starting trees under the following conditions: optimality criterion = parsimony, all characters unordered and of equal weight, gaps treated as missing, multistate taxa interpreted as uncertainty, starting trees obtained via step-wise addition, addition sequence random, branch-swapping algorithm: none, branches collapsed if minimum branch length is zero ("amb-"). The trees generated were filtered to include only the best score trees, then saved to file.

These trees were then used to run a second, more thorough heuristic search under the following criterion: optimality criterion = parsimony, all characters unordered and of equal weight, gaps treated as missing, multistate taxa interpreted as uncertainty, starting trees: use trees stored in memory, branch-swapping algorithm: tree-bisection-reconnection (TBR) with reconnection limit = 8, steepest descent option in effect, MulTrees option in effect, branches collapsed if minimum branch length is zero ("amb-"). Tree length, consistency index (CI) and retention index (RI) were calculated in PAUP*. Strict and 50% majority rule consensus trees were generated, and a heuristic bootstrap analysis was conducted to assess support for consensus trees under the following conditions: 10,000 replicates, starting trees obtained via random step-wise addition, TBR and MulTrees in effect.

Data from matK and trnL-F were analysed separately first and then as one single data matrix after a partition homogeneity test conducted in PAUP* showed that the data were not incongruent. A hypervariable region of AT repeats was identified in trnLF. The combined maximum parsimony analysis was rerun with this region excluded and the results compared. With the region included, the 28,495 most parsimonious trees had length 604, consistency index (CI) = 0.7781, or 0.5890 excluding uninformative characters, retention index (RI) = 0.9152 and homoplasy index (HI) = 0.2219, or 0.4110 excluding uninformative characters. When the hypervariable region was

excluded, the 8,411 most parsimonious trees had length 587, consistency index (CI) = 0.7871, or 0.6019 excluding uninformative characters, retention index (RI) = 0.9196 and homoplasy index (HI) = 0.2129, or 0.3981 excluding uninformative characters. This shows that the hypervariable region increases homoplasy in the data, so the final parsimony analysis was run on the combined dataset with the hypervariable region excluded.

Bayesian Analysis

Following the partition homogeneity test, Bayesian analysis was conducted on the combined dataset using MrBayes version 3.2.5 x64 (Ronquist et al., 2012). Coding frames of matK were identified using the ExPASy Translate Tool (Artimo et al., 2012). The dataset was partitioned into 8, see Table 13.

Table 13: Partitions of combined dataset for Bayesian analysis

Partition label	Partition description	Base pairs
matK1st	Base pairs in 1st frame position within coding region of matK	1-1551\3
matK2nd	Base pairs in 2nd frame position within coding region of matK	2-1551\3
matK3rd	Base pairs in 3rd frame position within coding region of matK	3-1551\3
matK intron	Non-coding region	1552-1761
matK gap matrix	indels coded as datatype=standard	1762-1765
trnL-trnFnohyp	trnL-trnF excluding hypervariable region	1766-2095, 2122-2770
trnL-trnFhyp	trnL-trnF hypervariable region only	2096-2121
trnL-trnF gap matrix	indels coded as datatype=standard	2771

All substitution types were given the same rate for the coded indels and for the hypervariable region of trnL-trnF. Substitution models for the remaining 5 partitions were found using MrModelTest2 (Nylander, 2004), executed through PAUP*. For partitions matK1st and trnL-trnF, GTR+I+G was the best model (nst=6, rates=invgamma). For partitions matK2nd and matKintron, HKY+G was the best fit model (nst=2, rates =gamma), and for partition matK3rd, the best fit model was GTR+G (nst=6, rates=gamma). The analysis was run for 5million generations with trees sampled every 1000 generations, with 4 chains and 2 simultaneous runs. The first 1000 trees were discarded as burnin (20%).

5 Molecular Results

The combined data matrix for matK (including park of trnK) and trnL-trnF contained 2,766 bases, plus 5 additional characters coding for presence or absence of indels. After excluding beginning and end sections where a high proportion of sequences were missing data, the data matrix was 2,587 characters long and contained 138 accessions from 115 different taxa.

Maximum Parsimony Summary

Excluding the hypervariable region, the combined dataset used for parsimony analysis, was 2,561 characters in total, of which; 2150 were constant and 250 were variable but parsimony-uninformative, leaving 161 parsimony informative characters. The strict consensus tree of 8,411 most parsimonious trees had a very similar topology to that of the bootstrap consensus trees, differing only where bootstrap support values were low. Relationships were slightly more resolved in the strict consensus tree. Bootstrap support values were generally strong, but phylogenies lacked detailed resolution, as expected.

Bayesian Analysis Summary

The combined dataset used for the Bayesian analysis contained the hypervariable region and so was 2,587 characters long. The consensus tree from the Bayesian analysis had a similar topology to the strict consensus parsimony tree but with higher resolution. Support values were generally strong.

Figure 22: Consensus tree of Bayesian consensus and Parsimony strict consensus
Parsimony bootstrap support values have been transposed onto the Bayesian consensus tree with nodes lacking a bootstrap value collapsed. Subgenera are indicated in black. Clades of particular interest are coloured. All taxa represented by sequences produced for this project are coloured by species. Putative hybrids red

Molecular Analysis

Only clades with Bayesian support values >70% were retained. The phylogeny shows relatively good resolution for the genus, with all subgenera strongly supported as monophyletic except *Pentanthera* and *Azaleastrum* in which the sections are individually monophyletic, whilst subgenera are polyphyletic. Subgenus *Hymenanthes* is strongly supported as a monophyletic with bootstrap support of 99% and 100% Bayesian support. Within the *Hymenanthes* clade, taxa are divided between two clades which correspond to those found by Milne et al., (2010). This distinct division of the subgenus was observed across all consensus trees from all parsimony analyses as well as those from Bayesian analysis.

The first clade (H1) is very strongly supported with 96% bootstrap and 100% Bayesian support, it comprises 8 species of subsection *Pontica* (as in Milne 2004, 2010), alongside all accessions of the study group species (whose given identity is supported by morphology) and *R. insigne* (subsection A*rgyrophylla*). Within this clade, the study group species and *R. insigne* form a monophyletic subclade (henceforth "the calophyta clade") with bootstrap 81% and 100% Bayesian support. The placement of this subclade within the larger clade containing *R. catawbiense, R. macrophyllum, R. aureum, R. caucasicum* and *R. brachycarpum,* all from outside of SE Asia, is also strongly supported with bootstrap 86% and 100% Bayesian support. In the bootstrap analysis, four further subclades received meaningful support, the first contained both accessions of *R. insigne*(bootstrap 88%, Bayesian 100%), the second contained all accessions of *R. asterochnoum* along with *R. calophytum* var. *openshawianum* (EDNA00160045427) and *R. calophytum* var. *pauciflorum* (EDNA00160045430) (bootstrap 82%, Bayesian 100%), the third was not as strongly supported, with bootstrap 55% and Bayesian support 98%, it contained a mixture of species (*R. calophytum* var. *openshawianum*, *R. calophytum* var. *calophytum, R. praevernum, R.* X *geraldii*). The Bayesian analysis resolved a fourth subclade containing all remaining *R. praevernum* and all *R. sutchuenense* in the calophyta clade with 99% Bayesian support.

The second clade in subgenus *Hymenanthes* (H2) is also well supported with 88% bootstrap and 100% Bayesian support. This clade contains species from the remaining 23 subsections of *Hymenanthes* as well as three more species from subsection *Argyrophylla* (*R. adenopdum, R. coryanum, R. argyrophyylum),* three from *Pontica* (*R. degronianum, R. smirnowii, R. hyperythrum*) and the rest of those species sampled from *Fortunea.* Within clade H2, relationships between species were poorly resolved. *R. degronianum* was strongly supported as sister to the rest of the H2 clade, but the relatively basal positions of *R. smirnowii* and *R. adenopodum* were only moderately supported, with bootstrap 60%, 64% and 93%, 97% Bayesian support respectively. Most species fell within a strongly supported subclade of H2: HA, with 88% bootstrap and 100% Bayesian support. Relationships within clade HA were poorly resolved. Of the 67 accessions in clade HA, 19 fall within 5 subclades (HA1, HA2, HA3, HA4, HA5) with low/moderate to good support (52-86% bootstrap support); the remaining 48 unresolved in a large polytomy.

Clade HA1 had strong Bayesian support (100%), but low bootstrap support (52%). It comprised 9 species, all species examined from subsection *Barbata* (*R. barbatum, R. succothii*)*, Fulgensia* (*R. fulgens*) along with some of the species examined from *Grandia* (*R. grande, R. pudorosum*)*,* and

Falconera (*R. falconeri, R. rothschildii, R. semnoides and R. sinofalconeri*). Clade HA2 (86% bootstrap, 100%Bayesian support) comprised *R. forrestii* (*Nerriflora*), *R. thomsonii* and *R. hookeri* (both *Thomsonia*). Clade HA3 (80% bootstrap, 100%Bayesian support) contained *R. dichroanthum* (*Nerriflora*) and a subclade (79%bootstrap, 100% Bayesian support) comprising *R. catacosmum* and *R. eudoxum* (both *Nerriflora*)*.* Clade HA4 (63%bootstrap, 99% Bayesian support) contained two accessions; *R. strigillosum* (*Maculiferum*) and *R.* X *sutchuenense* (a suspected hybrid between *R. sutchuenense* and *R. strigillosum*). Clade HA5 (60% bootstrap, 100% Bayesian support) contained *R. pseudochrysanthum* (*Maculifera*) and *R. hyperythrum*, a species in *Pontica* sensu Chamberlain et al. 1996, which Goetsch et al. (2005) suggested moving to *Maculifera* based upon strong cpDNA and nDNA evidence.

The Bayesian analysis offered much more detailed resolution with a clade of four species (*R. lanatum, R. tsariense, R. campanulatum, R. delavayi*) strongly supported as sister to the rest of clade HA, (100% Bayesian support). Of the remaining 63 accessions, 28 were spread through 8 subclades with Bayesian support >70% (Bayesian consensus tree in Appendix 5).

6 Biogeography

Combining the phylogeny in Figure 22, and the geographic distribution of *Rhododendron* subgenus *Hymenanthes* provides some insight into its evolutionary history. *Hymenanthes* may be considered in two parts, as biogeographic entities with discrete, non-overlapping distributions. The first group, here termed the Tertiary Relict group, has a typical tertiary relict distribution, occurring disjunctly in SE and NE America, SW Eurasia and NE Asia (including Japan). This group comprises all the species in subsection *Pontica* included in the study, except for *R. hyperythrum* which may actually be better placed in *Maculifera* or in a subsection of its own based upon cpDNA (Milne et al., 2010), nrDNA (Goetsch et al., 2005) and weak morphological links to *Pontica*. The second group, referred to as the SE Asian group contains *R. hyperythrum* and all species in the remaining 23 subsections. This group has a narrower geographical range and exhibits highest diversity in the Eastern Himalayas. Figure 23 illustrates these two biogeographic entities.
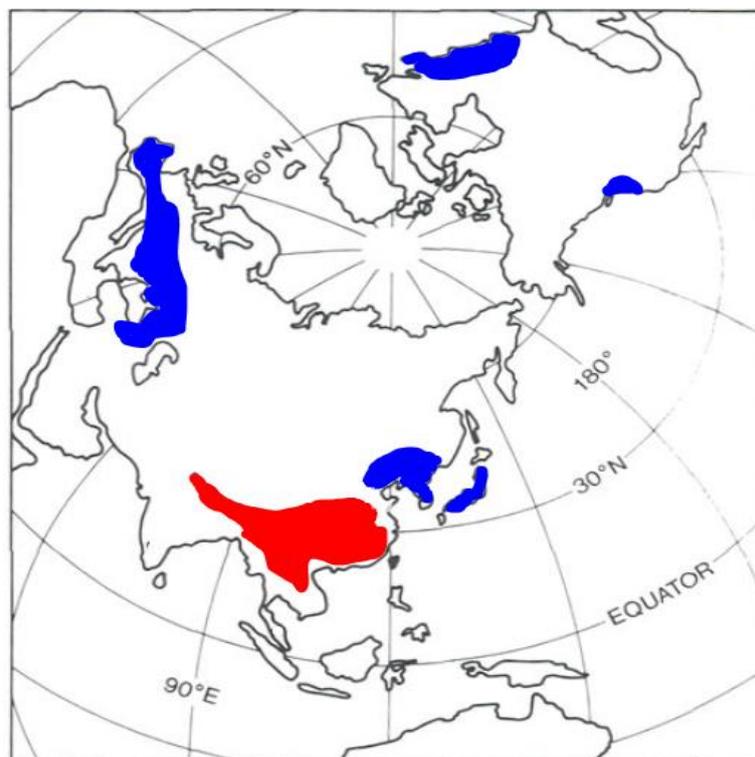
Figure 23: Distribution of Hymenanthes. Blue = tertiary relict distribution of Pontica (excluding R. hyperythrum). Red = distribution of the SE Asian group

Hymenanthes has the overall biogeographic pattern of slow diversification outside SE Asia followed by more rapid diversification of one lineage within SE Asia c. 5-3 million years ago, possibly as a result of hybridisation, followed by immigration of at least one additional lineage to the region (Milne, 2010), diversifying to form the *calophyta* clade c. 5-3 million years ago.

Ecological factors and the availability of environmental niches (Danley and Kocher, 2001), (Sakai et al. 2006) were almost certainly important driving factors of the rapid speciation within *Hymenanthes* given the narrow distributions of many species and their different ecological requirements (Chamberlain 1982).

The two suggested migratory routes within *Fortunea* from an origin in NW Sichuan (Hall, et al., 2015) correspond to species grouped by similar elevation ranges, see Table 14. These groups are purported to represent rapid diversification during migration. The first group (*R. orbiculare* Decaisne*, R. platypodum* Diels*, R. vernicosum* Franchet*, R. griffithianum* Wight) with altitudinal range 1,690-3,200m is proposed to have migrated southwards, following the higher elevations. The second group (*R. fortunei* Lindley*, R. faithiae* Chun*, R. maoerense* W.P.Fang & G.Z.Li*, R. magniflorum* W.K.Hu), better adapted to lower elevations of 1,320-2,100m and now distributed in Hunan, Guizhou and Guangxi is purported to have followed the chain of lower elevation ridges Eastwards, and then Southwards from NW Sichuan (Hall et al., 2015).

Table 14: *Fortunea* species altitudinal range, grouped by suggested migratory route

Group	Species	Altitudinal Range (m)
Study	R. asterochnoum	3,500 - 4,000
	R. calophytum	1,400 - 4,000
	R. praevernum	1,500 - 1,800
	R. sutchuenense	1,600 - 2,300
1	R. griffithianum	2,100 - 2,800
	R. orbiculare	1,400 - 3,500
	R. platypodum	1,800 - 2,200
	R. vernicosum	2,600 - 4,300
2	R. faithiae	1,000 - 1,400
	R. fortunei	600 - 2,000
	R. magniflorum	1,700 - 1,800
	R. maoerense	1,800 - 1,900

6.1 Methods

Distribution maps were created for each of the study group species from two datasets, firstly from occurrence records downloaded from GBIF (GBIF, 2016), and secondly from the exsiccatae studied. The distributions of samples used for this study were then compared to distribution maps compiled from the larger GBIF dataset for each species to check if sampling represented the species well or was biased towards one particular location.

When collecting additional fresh material for study, specimens of known wild origin were prioritised. However, the range of material available in cultivation from our study group species is limited, so collections were made from plants of unknown origin where a species would otherwise have been poorly represented in the study.

None of the exsiccatae at Edinburgh had latitude and longitude coordinates, but most had names of the mountain they were collected from, or a nearby town. Google earth was used to search for these locations, checking herbarium label descriptions of nearby features and altitude (if recorded) against the results. Wilson collection localities were determined using "*Mapping the collecting localities of E. H. Wilson in China*" (Clausen, 1980) to find more recent place names, and then checking the coordinates given in the article against Google Earth to see if the topography of the

area matched the description given on the herbarium label. A further two localities were identified by searching through the country file for China provided by the GEONet Names Web Team (NGA) (Table 3, Appendix 1). Distributions were then mapped in DIVA-GIS (Hijmans, 2001).

6.2 Results

All species in the *calophyta* clade are native to Central-Southern China, with an overall distribution encompassing the following Chinese Provinces: Guangxi, Guizhou, Henan, Hubei, Hunnan, Shaanxi, Sichuan and Yunnan, illustrated in Figure 24. The distribution of studied specimens of each taxa, and the group as a whole must be compared to the overall distributions to see if sampling is sufficiently broad to allow the assumption that the cpDNA types indicated by the samples studied for this project are the normal cpDNA types for the species.
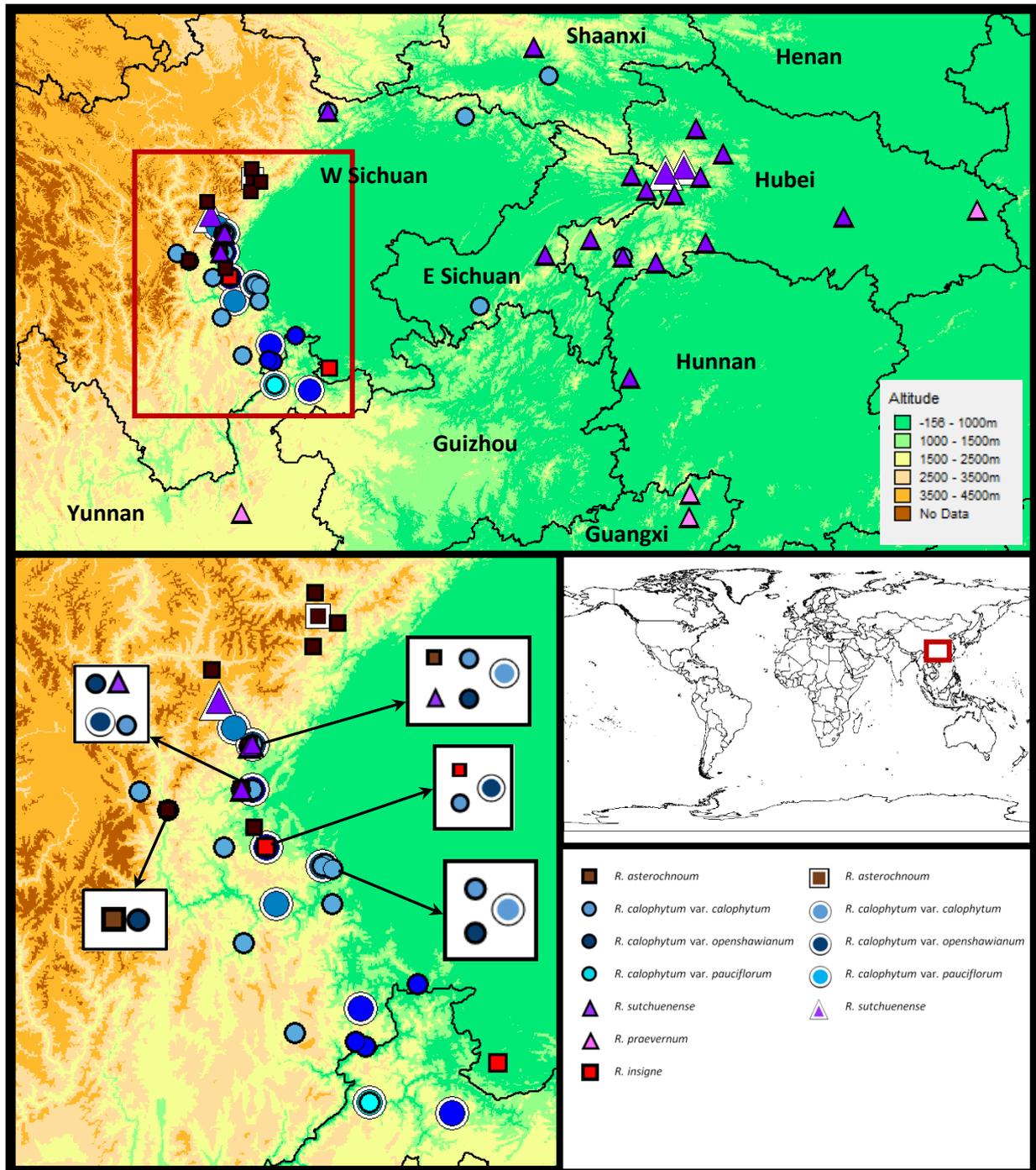
Figure 24: Distribution map for taxa in the *calophyta* clade
Left hand column of legend represents occurrences plotted using data from GBIF. Right hand column of legend represents occurrences plotted from location details of specimens studied (larger icons). A zoomed in map of the red boxed area in Central-Western Sichuan is included as an inset (lower left) to allow easier interpretation of the dense distribution of data points in this region. Some points are overlapping, especially in this area, due to a lack of accuracy regarding localities, for example, all specimens identified as being from Emei-shan (/Omei-shan) are mapped to the same point when realistically, samples were collected from multiple locations on Emei-shan

Species Distributions

The altitudinal data on the map shows that the area these species occupy is at the eastern edge of the Himalayas and is predominantly low-lying, with c. 80% of the area framed having an altitude of 2500m or less. The lowest recorded altitude for the specimens studied is 1,360m (K L Chu #2310, *R. calophytum* var. *openshawianum*). Clearly the lowlands of Central Sichuan do not currently provide the environmental niches required by these species.

All study group species except for *R. asterochnoum* have a disjunct distribution, spanning the lowlands of Sichuan. This implies that either these species were distributed across the entire region before the ancestors of Group 1 and Group 2 migrated, i.e. before speciation within Groups 1 and 2 occurred, or that the study group species migrated with Groups 1 and 2, but unlike the groups, did not undergo rapid diversification during migration. Rapid speciation is common throughout *Hymenanthes* with species examined by previous studies diversifying with the last 5 – 3 million years (Milne, 2004). It is therefore likely that the species in the *calophyta* clade and the clade HA which contains species from both groups (Figure 22) evolved contemporaneously. Furthermore, it is unlikely that the factors causing rapid speciation of groups 1 and 2 in *Fortunea* would have at the same time had no effect upon the study group species. It is therefore more likely that the ancestors of the study group species had a wider distribution than the species do today and the cpDNA type found in extant *Calophyta* Clade species.

Sampling

R. asterochnoum has a relatively narrow distribution in Western Sichuan, as shown in Figure 24 (main map and inset), with a known range of c. 210x130km and altitudinal range of 3,500-4,000m. The northern half of its range appears disjunct from that of any other *calophyta* clade taxa, whereas the southern half of its range overlaps the ranges of *R. sutchuenense, R. calophytum* var*. calophytum, R. calophytum* var*. openshawianum* and *R. insigne.* Despite sampling multiple accessions from this taxon, it is believed that all plants of this species currently in cultivation are from the same collection, represented by one cross on the map, central in the cluster of points at the northern end of its range. A single point cannot be assumed to represent a whole species, but may contribute to our understanding of the evolutionary history of the whole group.

The centre of diversity of *R. calophytum* is C W Sichuan, and Yunnan, with a range extending across into E Sichuan and Shaanxi. Only *R. calophytum* var. *calophytum* is found in E Sichuan and Shaanxi but all of the accessions studied for this taxon are from W Sichuan. *R. calophytum* var. *openshawianum* is only known from W Sichuan, where its distribution overlaps with those of *R. asterochnoum* and *R. sutchuenense* in the Northern third of its range, and with *R. calophytum* var. *calophytum,* and *R. insigne* for the entirety of its range of c. 320x200km. The studied specimens of *R. calophytum* var. *openshawianum* give a good representation of its known range, including specimens from both North and South extremes of its limit, and a reasonably spread of samples between the two. It can therefore be assumed that the cpDNA type found in *R. calophytum* var. *openshawianum* is the normal cpDNA type for this variety. *R. calophytum* var. *pauciflorum* lies within the range of the other two varieties, but is of unclear taxonomic status and the specimens analysed are thought to be of hybrid origin (see discussions in 7.1,9) based upon morphology and its different cpDNA types. The species as a whole then is poorly sampled as no accessions from E Sichuan or Shaanxi were included.

R. praevernum has a wide distribution to the South and East of the ranges of *R. asterochnoum* and *R. calophytum*, in Yunnan, Guangxi and Hubei.The distribution of *R. praevernum* overlaps that of *R. sutchuenense* in one place, but is distinct from all other *Calophyta* Clade taxa. All accessions of *R. praevernum* were either cultivated, or of unknown or unconfirmed origin. Specimens from Corrour

may be of wild origin (see further discussion in Chapter 9: Horticultural Implications) but as this could neither be confirmed nor denied within the scope of this project, it cannot be used here. Hence no conclusions can be drawn about whether the cpDNA types found for *R. praevernum* are a reflection of cpDNA types for the taxon.

R. sutchuenense, like *R. praevernum*, has a wide distribution, stretching 1,000 miles from W Sichuan to C Hubei (W-E), and 650 miles from Shaanxi to Hunan (N-S). *R. praevernum* lies outside of the range of *R. sutchuenense* except for one record; all other taxa in the *Calophyta* Clade are distributed within and hence overlapping with the range of *R. sutchuenense.* The samples for this taxon represent only a narrow band of its North-South range, but come from both W Sichuan and W Hubei, and hence from disjunct populations, separated by the lowlands of central Sichuan. Sampling may therefore be considered a fair, but not excellent representation of the species.

It may be concluded that due to the limitations on sampling imposed by available material in cultivation, at the species level, taxa are poorly sampled to represent their geographical distribution. However, they do provide a fair representation of the distribution of the group as a whole, inferring that it is likely the clade evolved from a single ancestor, which had the same cpDNA type as the extant taxa in the *Calophyta* clade.

7 Discussion

7.1 Anomalous or Doubtfully Identified Accessions

Five of the samples used for this study were of dubious identity at time of collection: Calo-op-1, Strig-X-sutch, Calo-pau-1, Calo-pau-2 and Calo-pau-3 (describe in Chapter 9). Both morphological and molecular characteristics have been found to support their status as putative hybrids. The anomalous accessions have been excluded from this discussion, and were not included in the specimens used to write the taxonomic account or explore species distributions. Individual cases are discussed in Chapter 9: Horticultural implications.

7.2 Classification of Study Species

Hypotheses Testing

$H_{1.0}$: Study group species are all well-defined, clearly separated species, supported by morphological and genetic characters.

$H_{1.1}$: Study group species are ill-circumscribed with confusion over identity of specimens commonplace. Species boundaries unclear, morphology contradicts genetic characters.

Consulting the literature, the four species and their subspecific ranks appeared to be well defined as distinct entities by unique character combinations. However, an initial sort of specimens into taxon groups found only three clearly distinct units, two of which were highly variable, containing seemingly very different specimens which don't naturally group together, but also the full range of intermediate morphologies. The three clearly distinct units corresponded to *R. asterochnoum, R. calophytum,* and *R. sutchuenense* (s.l.) as described in chapter 3.2: Quantitative Morphological Character Results, and illustrated there by the groupings in the figures of PCA scatter plots (Figure 16, Figure 17, Figure 18, Figure 19).

Numerous specimens were misidentified, most notably CEE172, represented by two vegetative specimens (V1, V2) from the expedition and one fresh specimen (F1) pressed from a plant raised from this seed collection at Dawyck, with floral material. V1 had been determined as *R. asterochnoum,* V2 as *R. sutchuenense* and F1 as *R. calophytum*. All three specimens lacked the stellate hairs characteristic of *R. asterochnoum* and although the leaf shape is very similar to that of *R. sutchuenense*, and the specimens are well indumented, the leaves are much larger than is usual for that species, and the floral material placed it quite comfortably in *R. calophytum.*

Some specimens determined as *R. sutchuenense* had a prominent blotch on the corolla, despite hand-written descriptions stating otherwise (Wilson 2537, SABE 1231). In addition to the mislabelled herbarium specimens, it is evident that there has been much confusion about the distinction between *R. praevernum* and *R. sutchuenense* since *R. praevernum* was published. In the collection of plates in the archives at RBGE, images labelled *R. sutchuenense* from J. C. Williams are clearly prominently blotched.

Correspondence between Sir John Stirling Maxwell who created the *Rhododendron* plantings at Corrour, H. F. Tagg, Erskine Jackson, Professor Wright-Smith and Dr Cowan regarding the identity of plants raised at Pollock, Glasgow and sold on or planted at Corrour by Sir John Stirling Maxwell discusses the differences between the two species, concluding that the plants in question are most likely *R. praevernum,* but that a number of intermediate forms are known, and the two should really be regarded as one very variable species. The genetic characters do not offer enough resolution to contradict the morphological characters but it may be concluded that the species are not all currently effectively circumscribed, and a taxonomic revision of the group would be useful.

7.3 Relationships between Study Species

The hypotheses found to be true are shown in bold font in the section below.

Hypotheses Testing

$H_{2.0}$: *R. asterochnoum* is a one off natural hybrid of, or variation of *R. calophytum*.

$H_{2.1}$: *R. asterochnoum* is a stable, definable species

In the molecular analysis, *R. asterochnoum* clusters as monophyletic for cpDNA. This was expected since as far as can be ascertained, all samples are from the same collection, C&H7051.

If it were a first generation natural hybrid, then these results support inheritance of cpDNA being maternal within the subgenus. However, these specimens by contrast reflect broad-sampling by being remarkably consistent in morphological characters and as such are highly unlikely to be the result of a recent hybridisation event. *R. asterochnoum* is easily distinguished from *R. calophytum* by the unique morphological character within the study group of a presence of rusty-brown, stellate hairs. In the phylogeny in Figure 22, *R. asterochnoum* is sister to a clade containing two accessions: the first, Calo-pau-2 is a putative hybrid of *R. calophytum* discussed in chapter 9: Horticultural Implications, the second is a specimen of *R. calophytum* var. *openshawianum* (C&H7055) from the same locality as the *R. asterochnoum* collection.

Morphologically, C&H7055 is striking by its difference from *R. asterochnoum*, with glabrous, narrower leaves tapering to a narrowly acuminate apex, thinner branches and a much laxer habit. Gene flow between the two species could be ongoing with morphological distinctness retained due to strong selection on parts of the genome (Wu, 2001), (Via, 2009), or this placement could just represent a single, local chloroplast capture event. Further study of plants from these collections would need to be undertaken in conjunction with additional field work to understand fully what is going on here. For the purpose of this project, it may be concluded that based upon the material studied and evidence found, there is no reason to assume that *R. asterochnoum* is not worthy of specific rank, noting that additional information may change our understanding of this taxa in the future.

H$_{3.0}$: *R. sutchuenense* and *R. praevernum* are best described as distinct species.

H$_{3.1}$: *R. sutchuenense* and *R. praevernum* are best described as extreme morphotypes of one variable species.

Within the study group species, *R. sutchuenense* and *R. praevernum* group together easily with many common characters that are taxonomically important for classification within *Fortunea* such as indumentum hair types, stigma diameter, leaf shape and inflorescence structure. Historically two key characters were used to divide the taxa: the quantity of persistent indumentum, and the presence or absence of a marked blotch in the corolla throat.

R. praevernum is described as having leaves with a glabrous midrib at maturity in contrast to *R. sutchuenense* which has persistent indumentum along its midrib (Hutchinson, 1920), (Chamberlain, 1982). Both these extremes are observed, but no clear cut line can be drawn between them as many plants exhibit intermediate levels of indumentum (Leach, 1961), (Cullen, 2005), (McQuire and Robinson, 2009) so although *R. praevernum* may be characterised as generally having less indumentum than *R. sutchuenense*, this distinction cannot be quantified. Furthermore, as discussed in chapter 3.1: Qualitative Morphological Character Results; they have the same indumentum hair types, and tomentum on their new growth.

The second important character used for distinguishing the species was that *R. praevernum* has a conspicuous dark purple blotch, and speckling, whereas *R. sutchuenense* has no blotch and may have dark or light speckling (Cullen, 2005), (Chamberlain, 1982). Again, flowers fitting both descriptions are found, commonly in conjunction with the appropriate indumentum characters, but intermediate forms with small blotches, or faint blotches, smears rather than a conspicuous mark, or a patch of more condensed speckling are all found. These forms are not commonly grown as gardeners have selected and propagated only the extreme forms which are of much higher horticultural merit, distorting our understanding of diversity within the species. "Hybrids" between *R. praevernum* and *R. sutchuenense* have been observed on numerous occasions both in cultivation and in the wild (Hutchinson, 1920), (Leach, 1961), and have been given the name R. × geraldii.

As the two species only have overlapping ranges for a small portion of their range in W Hubei, it seems likely that the variable morphology of R. × geraldii combined with the extreme morphotypes of *R. praevernum* and *R. sutchuenense* represents the natural levels of variability often found within single wide ranging species such as in *R. wardii* (Chamberlain, 1982), and in other genera, e.g. *Nassauvia* (*Asteraceae*), (Nicola, Johnson, and Pozner, 2014). Noting that evolution is not static, it is also worth considering that these species may still be in the process of diverging and that in another few million years, morphological distinctions between the extant *R. praevernum* and *R. sutchuenense* will be both numerous and consistent.

The molecular evidence presented in Figure 22 does not resolve relationships between the two morphotypes, it merely places all accessions for both species as equally closely related to each other as they are to the other taxa in the *calophyta* clade. However, both the strict consensus tree for the maximum parsimony analysis, and the consensus tree from the Bayesian analysis resolved a

further clade within the *calophyta* clade, which contained all accessions for both species as a monophyletic group for cpDNA (consensus trees in Appendix 5).

R. praevernum and *R. sutchuenense* have at least a small portion of their range overlapping in Western Hubei, as shown in Figure 24, with *R. praevernum* mostly occurring at low elevations, up to 1,800m, and *R. sutchuenense* occurring at higher elevations (inferred from mapping as no altitudinal data was recorded on herbarium labels of either taxa). One hypothesis is that *R. praevernum* and *R. sutchuenense* are simply high and low altitude versions of the same species (Cox, 2016). The distribution described above is consistent with this, as the two taxa are geographically isolated by elevation.

Considering the weak genetic signal, the overlapping morphological characters and the biogeography of the two taxa, it may be concluded that there is insufficient evidence to maintain both taxa at the specific level.

H$_{4.0}$: *R. calophytum* var. *pauciflorum* is a well-supported variety.

H$_{4.1}$: *R. calophytum* var. *pauciflorum* is not well supported. Plants grown under this name are often actually recent hybrids.

The taxon *R. calophytum* var. *pauciflorum* was not included in the main study group species as preliminary research failed to find enough material to study the taxa in any detail. No exsiccatae were found under this name. The only material available for study under this epithet was in the RBGE living collection at Benmore; three plants from a single collection. All three are morphologically distinct and discussed in detail in Chapter 9: Horticultural Implications. The conclusions of this study is that the characters given in accounts for this variety are insufficiently distinct to maintain the variety without further field work and evidence of greater differences than having fewer flowers per truss and a short rhachis. However, there is insufficient evidence to justify sinking the taxa at this point.

H$_{5.0}$: *R. calophytum* var. *openshawianum* is a well-supported variety.

H$_{5.1}$: *R. calophytum* var. *openshawianum* is not well supported.

Due to misidentifications in collections and limited sampling, no conclusions on the validity of this variety can be drawn from the molecular part of this study. However, the variety is well represented by exsiccatae with 10 specimens confidently determined as this variety, and a further 6 specimens believed to belong in this taxon group, but lacking enough material to confidently be placed in either variety of *R. calophytum*, some had no seed capsules or flowers, and three specimens were purely vegetative, but with every single leaf apex on the herbarium sheet damaged so that the shape of this key distinguishing morphological factor between could not confidently be determined. *R. calophytum* var. *openshawianum* is easily distinguished from *R. calophytum* var. *calophytum* by its smaller leaves with narrowly acuminate, long leaf apices, shorter, squatter capsules and fewer

inflorescences per truss, combined with shorter, slightly thicker pedicels than the autonym. *R. calophytum* var. *openshawianum* and *R. calophytum* var. *calophytum* are not geographically isolated and so are best recognised as different at the varietal level.

H_{6.0}: Study group species monophyletic for cpDNA.

H_{6.1}: Study group species non-monophyletic for cpDNA.

Excluding the putative hybrids discussed in Chapter 9, the study group species formed a monophyletic clade with *R. insigne* (subsection *Argyrophylla*), nested within 8 species from subsection *Pontica*, making the study group species paraphyletic for cpDNA. Implications of this are further discussed under Hypothesis 9.

7.4 Relationship of Study Species to the Rest of Fortunea and Hymenanthes

Hypotheses Testing

H_{7.0}: Subsection *Fortunea* is monophyletic for cpDNA.

H_{7.1}: Subsection *Fortunea* is non-monophyletic for cpDNA.

Little detail of the relationships between species within *Hymenanthes* was found, but the subgenus was shown to be monophyletic for cpDNA, as noted in previous studies (Kurashige et al., 2001), (Milne et al., 2004),(Goetsch, Eckert, and Hall, 2005), (Milne et al., 2010). The cpDNA phylogeny in FIGURE clearly shows *Hymenanthes* divided into two distinct clades, as shown by Milne (2004,2010). Species from subsection *Fortunea* were found in both clades, making the subsection polyphyletic for cpDNA.

H_{8.0}: The *calophyta* clade species evolved from a single ancestral *Pontica* subsection species, with morphological links to subsection *Fortunea* and *Argyrophylla* merely convergent evolution.

H_{8.1}: A now extinct ancestor of the study group species hybridised with a *Pontica* species after geographical split from other "proto-*Fortuneas*" resulting in chloroplast capture, followed by rapid speciation.

H_{8.2}: Homoploid hybrid speciation: both cpDNA and nDNA obtained from *Pontica* lineage along with morphological traits.

Before discussing the origin of the *calophyta* clade, recent chloroplast transfer either in cultivation or in the wild must be considered as this could explain a seemingly anomalous phylogenetic placement. However, plastid transfer as a result of introgression in cultivation can be eliminated for

15 out of the 27 accessions examined as they are of known wild origin (see Table 6: Samples used for molecular work.). Whilst introgression following hybridisation has been observed numerous times in *Hymenanthes* (Milne, et al., 1999), (Milne & Abbott, 2000), (Chung, et al., 2007), it may be regarded as unlikely that this has occurred independently 15 times across 3 different species in the wild, and 9 times in cultivation, and extremely unlikely given that the cpDNA type is consistent for ALL 27 sampled members of the group.

Within clade H1, *R. aureum* is geographically the nearest extant species in subsection *Pontica* to the study group species; occurring 2,000km to the north of the distribution of any species in the *calophyta* clade (Milne et al., 2010), (Chamberlain, 1982, p.314). Given this great distance between the species of the *calophyta* clade and the rest of clade H1, we can rule out chloroplast capture from a known extant species of subsection *Pontica*. Although it is possible that plastid transfer could have been facilitated by an undescribed clade H1 species, such a species would have to be abundant, with a wide distribution to come into contact with all of the *calophyta* clade species. It is therefore highly unlikely that such a species exists and has been overlooked since *Rhododendron* has been well studied in China, especially Yunnan and Sichuan provinces where our species predominantly occur. Furthermore, the study group species are geographically separated rather than all sharing exactly the same distribution (as illustrated in Figure 24) and this study shows that all of them have this H1 cpDNA type, suggesting a common origin. Hence, it is far more likely that the cpDNA type of these closely related species was inherited from an ancestor common to them which had this cpDNA type as a result of a historical chloroplast transfer event from either an extant or extinct species. We may therefore conclude that the cpDNA types of the *calophyta* clade are not the result of recent introgression either in cultivation or in the wild. There remain three possible explanations for the *calophyta* clade's position, nested within eight *Pontica* species; either the species in the *calophyta* clade are pure members of *Pontica*, an ancient hybridisation event resulted in their ancestors (common or not) acquiring the *Pontica* type cpDNA, or homoploid hybrid speciation occurred.

The taxa in the *calophyta* clade appear, from cpDNA, to belong to subsection *Pontica*, but are actually assigned to two different subsections: *Fortunea* (*R. asterochnoum, R. calophytum, R. sutchuenense*) and *Argyrophylla* (*R. insigne*), (Chamberlain, 1996). The key characteristics of these subsections are summarised in Table 15.

Table 15: Key differences in taxonomically informative morphological characters between *Pontica*, *Fotunea* (s.s.), the *Calophyta* clade and *Argyrophylla*

	Pontica	Fortunea (s.s.)	Calophyta Group	Argyrophylla
Colour of corolla markings	Green to yellow or orange	Pink to deep crimson-purple	Pink to deep crimson-purple	White or pale pink to violet
Corolla lobes	5(-7)	6-7(-8)	5(-7)	5
Corolla Markings	With or without speckling, no blotch	Speckling No Blotch	Speckling, often blotched	With or without speckling, no blotch
Corolla shape	Deeply lobed, widely funnel-shaped	funnel-campanulate to open-campanulate	funnel-campanulate to open-campanulate	funnel-campanulate to open-campanulate
Indumentum	Glabrous or with dendroid unistrate hairs	Often glabrous, or else sparse, clustered along midrib, matt	Sometimes glabrous, usually some hairs persistent along midrib	Copious, thin and plastered or thick, woolly, shiny or matt
Inflorescense	Candelabroid	Lax	Lax or dense	Lax or dense
Leaf shape	Linear to broadly elliptic or obovate	Highly variable	Oblanceolate, oblong, elliptic	Narrowly oblanceolate to elliptic
Leaves	Smooth	Smooth	Coriaceous	Rugulose
Pedicels	Long	Long or short	Long or short	Medium
Rhachis	Long	Long or short	Long or short	Short
Stamens	10	10 to 16	10 to 25	usually 10
Stellate hairs	Absent	Absent	Sometimes present	Absent
Style	Glabrous	Glandular	Glabrous	Glabrous or glandular

The four *calophyta* clade taxa have no morphological links to *Pontica* beyond that which is common to all *Hymenanthes* and have much stronger links to the subsections they're placed in. With hybridisation excluded from the equation, convergent evolution is the only plausible explanation for their morphological links to *Fortunea* and to *Argyrophylla.* Outside of SE Asia, *Pontica* has been shown to have a slow rate of diversification (Milne, 2004).

Hybridisation is thought to be a key factor driving rapid speciation and diversification within *Hymenanthes* (Milne, 2010). With hybridisation removed from the equation, diversification within the *calophyta* clade must have been influenced instead by factors such as topography and environmental niches. Convergent evolution to two different subsections within a short time-frame is also unlikely but there is currently no strong evidence with which to reject the hypothesis that the *calophyta* clade members have evolved from *Pontica* without hybridisation and then undergone convergent evolution creating superficial associations with *Fortunea* and *Argyrophylla.*

Homoploid hybrid speciation between *Pontica* and ancestors of the *calophyta* clade could explain the position of the *calophyta* clade in the phylogeny. However, this would result in both cpDNA and nuclear DNA being obtained from *Pontica,* along with morphological traits. As shown in TABLE, the *calophyta* clade species have no strong morphological links to *Pontica* beyond those common to all *Hymenanthes.* In order to test this hypothesis thoroughly, a complementary study of *Hymenanthes* would need to be completed using nuclear markers. If the phylogeny produced from this analysis was topographically compatible with the phylogeny in Figure 22, this would support a possible origin of the *calophyta* clade through homoploid hybridisation. However, the lack of strong morpholoigical congruence allows us to reject the hypothesis as being unlikely based upon the evidence available.

The third possible explanation for the position of the *calophyta* clade species (excluding *R.insigne*) within H1 is that a now extinct ancestor of the study group species hybridised with a *Pontica* species after geographical isolation from the other "proto-Fortuneas" resulting in chloroplast capture,

followed by rapid speciation. Introgression is known to have occurred numerous times during the evolution of *Hymenanthes* (Milne, et al., 1999), (Milne & Abbott, 2000), (Chung, et al., 2007), and so is highly likely to have occurred at some point in the evolutionary history of the study group species. Given the strong morphological links to *Fortunea* and the placing of the group within a clade dominated by *Pontica* it is reasonable to accept this hypothesis as the most likely explanation based upon the available evidence.

$H_{9.0}$: *R. insigne* represents a second chloroplast capture event from *Pontica*.

$H_{9.1}$: *R. insigne* gained its *Pontica* cpDNA type from one of the introgressed *Fortuneas*

$H_{9.2}$: *R. insigne* is an anomalous member of the *calophyta* clade, unrelated to A*rgyrophylla*.

R. insigne is morphologically distinct from the study group species due to an adpressed, shiny, compacted indumentum embedded in a surface film, unlike any indumentum types found in *Pontica* or *Fortunea* species, but present in other *Argyrophylla* species. However, *R. insigne* For *R. insigne* to have evolved directly from *Pontica* or from *Fortunea*, without hybridisation, it must have undergone convergent evolution. Whilst this cannot be ruled out, it is more likely *R. insigne* acquired its cpDNA type by introgression for the same reasons laid out above for the study group species. There are then three possibilities for the origin of *R. insigne's cpDNA:* it could have obtained the cpDNA directly from *Pontica*, at about the same time as the *calophyta* clade did, or later, from one of the introgressed F*ortuneas*, or else, *R. insigne* could be an morphologically anomalous member of the *calophyta* clade whose resemblance to *Argyrophylla* is superficial and does not reflect its evolutionary history. Figure 24shows the distribution of *R. insigne* relative to the study group species. The distribution of *R. insigne* overlaps that of *R. calophytum,* sitting comfortably within the overall range of the study group species. Noting that the nearest extant *Pontica* species occurs in excess of 2,000km away, it may be assumed that if *R. insigne* did obtain its cpDNA from *Pontica,* then it probably did so at the same time as the ancestor of the other species in the *calophyta* clade; when a member of *Pontica* was still present in the region. This would be supported if *R. insigne* was sister to the others, rejected if it was nested among them. If rejected by future analysis, then it may be concluded that either *R. insigne* as a member of *Argyrophylla* acquired its cpDNA from one of the introgressed *Fortuneas,* or, *R. insigne* is an anomalous member of the *calophyta* clade, evolving from the same ancestor as the study group species, but resulting in a species so morphologically distinct that it warrants being placed in a new subsection of its own. The phylogeny presented in this study is not sufficiently resolved to support either scenario; more in depth research into *R. insigne* and the rest of *Argyrophylla* is required before this hypothesis can be fully addressed.

Taxonomic Implications

The current classification of the study group species as explored in Chapter7.3 is no longer supportable. Evidence presented in this report has highlighted the need for a taxonomic revision of the three species and their sub-specific taxa supported by this evidence, which is given in Chapter 8. *Fortunea* as currently circumscribed is polyphyletic.

The study group species consistently group together morphologically and have a shared evolutionary history different to that of the rest of the subsection. It is proposed that if the findings presented here can be supported by additional fieldwork to confirm the biogeographical findings, and a study based upon nuclear markers showing *Fortunea*(s.s.) and the *Calophyta* group to be monophyletic, then these species be split from subsection *Fortunea,* and a new subsection *Calophyta* described*.*

As no such data is currently available, for the purposes of the taxonomic account, the study group species will be grouped together as a taxonomic entity the *Calophyta* clade within *Fortunea*, distinct from *Fortunea*(s.s.), containing all other species in the subsection.

8 Taxonomic Account

RHODODENDRON Linnaeus, Sp. Pl. 1: 392 (1753).

Subgenus **Hymenanthes** (Blume) K. Koch, Dendrologie 2: 170 (1872).

> Bas.: Genus *Hymenanthes* Blume, Bijdr. Fl. Ned. Ind. 15: 862 (1826).
>
> Syn.: Subgenus *Eurhododendron* auct. Plur. Incl. Sleumer (1949), non K. Koch (1872).

Description as for section *Ponticum*, the only section in the subgenus.

> Type species: *Rhododendron japonicum* (Blume) Sneider (*Hymenanthes japonica* (Blume)).

Section **Ponticum** G. Don, Gen. Hist. 3: 843 (1834).

> Syn.: Section *Leiorrhodium* Rehder, J. Arnold Arbor. 15: 269 (1934).

Large trees to dwarf creeping shrubs. Foliage evergreen, rarely aromatic (*R. taliense*). All parts may be glabrous or indumented to some degree, tomented, setose, glandular to hairy, or glabrous. Scales always absent. Inflorescence terminal, umbellate to racemose, many to few flowered, rarely one flowered. Calyx small, obsolete to well developed. Corolla 5-8-lobed, ventricose campanulate to tubular campanulate, with or without nectar pouches at base. Stamens 10–20, declinate. Cvary 5–20-locular. Style slender, long. Stigma capitate or discoid. Capsule roughly cylindrical, straight to curved, hard, dehiscing by woody valves. Seeds often with a thin membranous wing.

> Type species: *R. ponticum* L., Sp. Pl., ed. 2. 1: 562, (1762).

Subsection **Fortunea** (Tagg) Sleumer, Bot. Jahrb. Syst. 74: 546, (1949).

> Syn.: *Rhododendron* series *Fortunei* sensu Tagg in Stevenson (ed.), The Species of Rhododendron, 257 (1930).

Shrubs or trees to 15m. Bark rough, sometimes flaky. Young shoots with dense to sparse white to grayish floccose indumentum at emergence, soon glabrescent. Leaves oblanceolate, oblong, elliptic, rounded, ovate or orbicular, adaxial surface glabrous when mature, abaxial surface floccose or not along midrib, rarely with a rusty brown stellate indumentum (*R. asterochnoum*), lamina sometimes with sparse indumentum. Inflorescence lax to dense, 5–30-flowered. Rhachis 3–70 mm. Calyx minute to well-developed, 1–20 mm. Corolla 5–7(–8)-lobed, funnel-campanulate to open-campanulate, white or pale pink to purple, nectar pouches usually absent (apparently present in *R. praeteritum*). Stamens 10–16(–25). Ovary stipitate-glandular or glabrous. Style stipitate glandular to tip or glabrous. Stigma capitate or discoid.

> Type species: *R. fortunei* Lindley, Gard. Chron., 868 (1859); Hook. f. Bot. Mag. t. 5596 (*Fortunei*).

Distribution: Bhutan, China, India, Myanmar, Nepal, Sikkim, Vietnam. Thirty-one species.

A heterogeneous group of species, some of which exhibit affinities to other subsections, e.g. *R. oreodoxa* shares some characters with subsection *Campylocarpa*, *R. asterochnoum* and *R. calophytum* may have a distant affinity with subsection *Grandia*. Nevertheless, they are more closely related to one another than to any other subsections.

Calophyta Clade

Shrubs or trees to 12m. Bark rough, sometimes flaky. Young shoots with dense white to yellowish floccose indumentum at emergence, glabrescent usually within one year. Leaves oblanceolate, oblong, elliptic, 10–40 × 3–9 cm, adaxial surface glabrous when mature, abaxial surface floccose or not along midrib, rarely with a rusty brown stellate indumentum (*R. asterochnoum*), lamina sometimes with sparse indumentum. Leaf base cuneate. Inflorescence lax to dense, 9–25(–40)-flowered. Rhachis 8–25 mm. Pedicel eglandular. Calyx minute, 0.5–2.5 mm. Corolla 5–(7)-lobed, tubular-funnel-campanulate to open-campanulate, white, pale pink or rose pink, with or without blotch and or speckling in throat, nectar pouches absent. Stamens 12–25. Ovary glabrous. Style glabrous. Stigma capitate or discoid.

> Type species: *R. calophytum* Franchet, Null. Soc. Bot. France 33: 230 (1886).

Distribution: China: Guangxi, Guizhou, Henan, Hubei, Hunnan, Shaanxi, Sichuan, Yunnan. Three species.

1. *R. asterochnoum* Diels., Feddes Repert. Spec. Nov. Regni Veg. 17: 196 (1921).

> Type: China, Sichuan, Wen tchuan hsien, in valle Scha pa, 3,500-4,000 m, 27/04/1914, *Limprict* 1347 (iso **K**).

Small tree or flat-topped shrub, 3–7 m. Bark grey-brown. Last season's branches thick, to 2 cm diam. Young shoots densely tomentose, yellowish tomentum matures grey, persists for >1 year. Petiole winged, 20–50 mm, tomentose when young. Lamina sturdy, coriaceous, broadly oblanceolate, rarely oblong-elliptic, 20–35 × 4–9 cm, base cuneate, apex acute-cuspidate or acuminate, adaxial surface dark green, glabrous, abaxial surface pale green, indumentum rusty-brown, stellate hairs, discontinuous, dense along midrib, sparse along secondary veins, sparse on lamina, gradually glabrescent. Inflorescence 15–20-flowered. Rhachis 18–25 mm. Pedicels 3–6 cm, glabrescent. Calyx minutely 5-lobed, 1.5–2 mm. Corolla 5-lobed, oblique, funnel-campanulate, usually glabrous, white flushed pale pink, 4–5 cm, with small red blotch. Stamens, 18–20, 1.5–3 cm, filaments puberulous for basal third. Ovary oblique, ca. 13-locular, ca. 6 × 4mm, glabrous, pale green. Style ca. 3 cm, glabrous; stigma yellow, discoid, 5–6 mm in diam. Capsule not seen.

Closely allied to *R. calophytum* from which it is distinguished by its rusty-brown indumentum of stellate hairs and larger calyx lobes.

Forests, valleys, roadsides: 2,200–4,000 m. W Sichuan.

Flowering time: late April.

Figure 25: Distribution map of *R. asterochnoum* based on data from GBIF

2. *R. calophytum* Franchet, Null. Soc. Bot. France 33: 230 (1886).

Tree or flat-topped shrub, 2–12 m. Bark grey-brown. Last season's branches 1-2 cm diam. Young shoots densely tomentose, tomentum white, persists for >1 year. Petiole winged, 14–25 mm, tomentose when young. Lamina sturdy, coriaceous, oblong-oblanceolate, rarely oblong-elliptic, 15–40 × 3–7 cm, base cuneate, apex acute-cuspidate to narrowly-acuminate, adaxial surface pale to dark green, glabrous, abaxial surface pale green, glabrous at maturity or with radiate and/or simple dendroid hairs along midrib, somewhat glabrescent, lamina sometimes with persisting juvenile tomentum. Inflorescence 8–30(–40)-flowered. Rhachis 9–25 mm. Pedicels 3–9 cm, glabrous. Calyx minute, ca. 1 mm. Corolla 5(–6)-lobed, oblique, open-campanulate, usually glabrous, white, or white flushed pale pink, or pink, 4–5.5 cm, with dark red blotch and some speckling. Stamens, 16–25, 1–3 cm, filaments puberulous for basal third. Ovary, 12–16-locular, ca. 4–9 × 4–5 mm, glabrous, pale green. Style 25–30 mm, glabrous; stigma yellow, discoid, 7–8 mm in diam. Capsule 20–40 × 8–20 mm.

Forests, valleys, roadsides: 1,400–4,000 m. Shaanxi, Sichuan and Yunnan.

Flowering time: March – April.

2a. *var. **calophytum***

> Type: China, Sichuan, Moupin (=Baoxing), 4000m, 1870, *Père David* (iso **E**).

> Illust.: Bot. Mag. 153:t. 9173 (1927); Fang, Pl. Omeiens. T. 26 (1942).

2b. var. ***openshawianum*** (Rehder & Wilson) Chamberlain, Notes R.B.G. Edinb. 37: 330 (1979).

> Syn.: *R. openshawianum* Rehder & Wilson in Pl. Wilsonianae 1: 543 (1915).

> Type: China, W Sichuan, Yung Ching Hsien, Wa Wu Shan, 2,300-2,800 m, 18/09/1908, *Wilson* 3414 (holo. A; iso. K).

> Illust.: Fang, Pl. Omeiens. T. 27 (1942).

Leaf apex shape is key to distinguishing the varieties: *R. calophytum* var. *calophytum* has an acute to cuspidate apex, whereas *R. calophytum* var. *openshawianum* has a distinctive narrowly acuminate apex. *R. calophytum* var. *openshawianum* sometimes also has a thinner, flatter leaf with a glossier adaxial surface in contrast to the leaves of *R. calophytum* var. *calophytum* which are usually thick and sturdy, and may be keeled. In flower they are similar, but var. *openshawianum* generally has fewer flowers per truss, and shorter pedicels. The capsules of *R. calophytum* var. *openshawianum* are squat and barrel-shaped, whereas *R. calophytum* var. *calophytum* has cylindrical, narrower capsules.
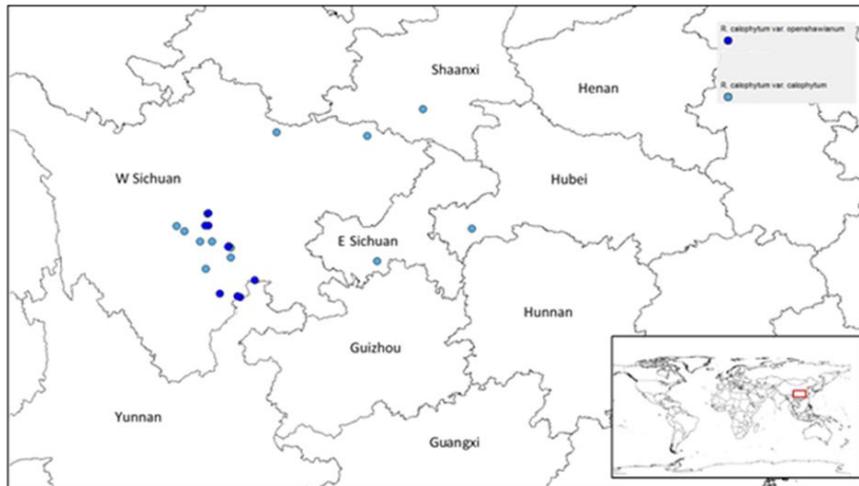
Figure 26: Distribution map of *R. calophytum* based on GBIF data. Light blue circles: *R. calophytum* var. *calophytum*. Dark blue circles: *R. calophytum* var. *openshawianum*

Figure 27: *R. calophytum* var. *calophytum* line drawing showing prominent discoid stigmas, small basal corolla blotch and characteristic leaf shape
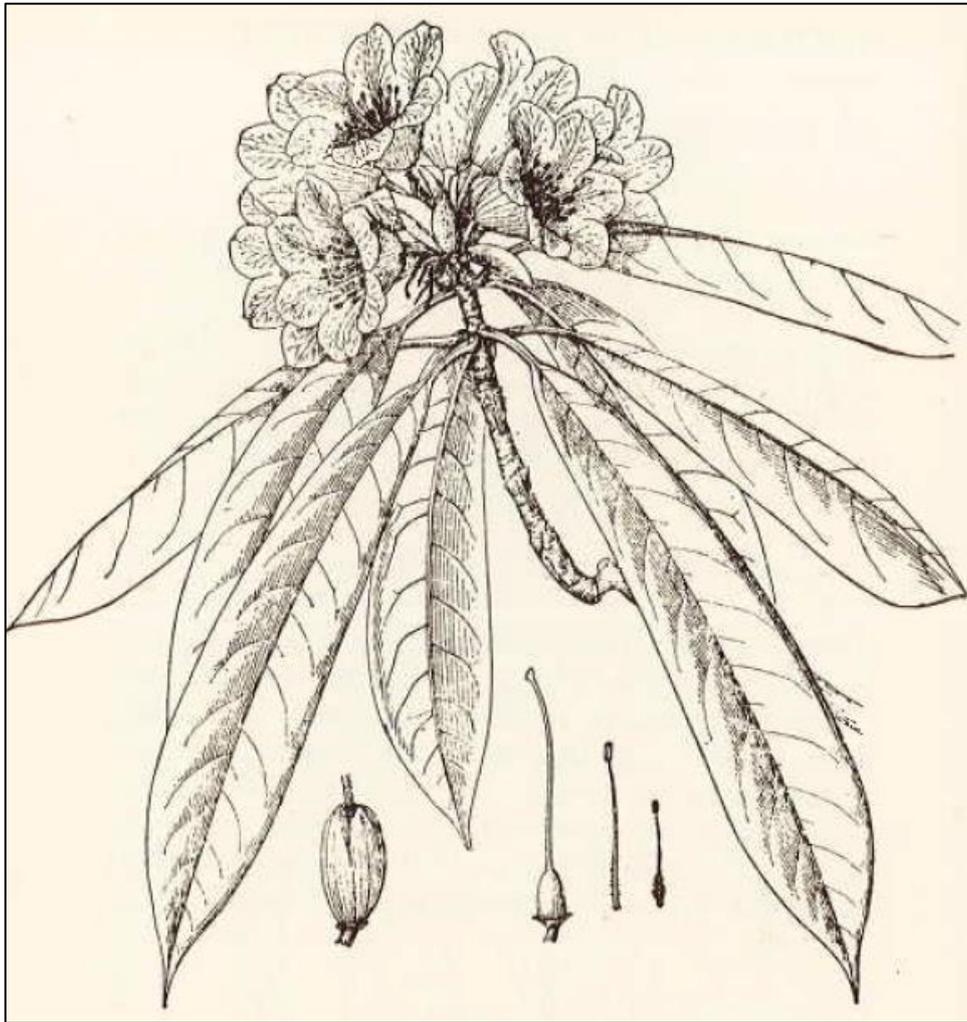
Figure 28: *R. calophytum* var. *openshawianum*. Illustration shows the smaller truss, wider capsule, and distinctive leaf apex shape for this variety.

3. ***R. sutchuenense*** Franchet, J. Bot. (Morot) 9: 392 (1895).

Shrub, 1–6 m. Bark pinkish-brown. Last season's branches 1 cm diam. Young shoots densely tomentose, tomentum white, soon glabrescent. Petiole not winged, 18–35 mm, tomentose when young. Lamina oblanceolate or elliptic, 10–22 × 3–6.5 cm, base cuneate, apex cuspidate rarely acuminate, adaxial surface pale green, glabrous, abaxial surface pale green, glabrous at maturity or with long, simple and/or dendroid hairs along midrib, somewhat glabrescent, lamina sometimes with persisting juvenile tomentum. Inflorescence 8–12-flowered. Rhachis 9–14 mm. Pedicels 1.5–3 cm, glabrous, thick, bright red. Calyx minute, ca. 1 mm. Corolla 5(–6)-lobed, widely-campanulate to funnel-campanulate, puberulous for basal third on inner surface, white, or white flushed pale pink, or pale pink, 4–5 cm, with or without dark red blotch, always with some speckling. Stamens, 12–15, 2–3 cm, filaments puberulous for basal third. Ovary, 10–16-locular, 5–7 × 3–5 mm, glabrous, pale green with purple spotting. Style 35–45 mm, glabrous; stigma yellow and pink, capitate, 2.5–4 mm in diam. Capsule 25–45 × 8–12 mm.

Forests: 1,600–2,300 m. Guangxi, Guizhou, Hubei, Hunnan, Shaanxi, Sichuan Yunnan

Flowering time: April – May.

3a. *var.* ***sutchuenense***

Type: China, E. Sichuan, aux environs de Tchen-keou-tin, *Farges* (iso **E**).

Illust.:   Bot. Mag. 137: t. 8362 (1911) Millais, Rhododendrons ed. 1:16, t. (1917).

3b. var. ***praevernum*** (Hutchinson) H.P.Wilson[2] (Comb. Nov.)

Syn.:   *R. praevernum* Hutchinson, Gard. Chron. ser. 3, 67: 127 (1920).
  R. X geraldii (Hutchinson) Ivens, Gard. Chron. Ser.3, 101: 220 (1937).
  R. sutchuenense Franchet var. *geraldii* Hutchinson, Gard. Chron. ser. 3, 67: 127 (1920)

Type: China, W Hubei, *Wilson* 17, 1900 (holo. K; iso. **E**, iso. A).

Syntype: S Patung, *Henry* 5285 03/1889 (K), W Hubei, *Wilson* 509, 509A, 05/1907 (**E,** K)

Illust.:   Gard. Chron. ser. 3, 73: 159 (1923)

R. sutchuenense as circumscribed here is a highly variable species with a wide-ranging distribution. *R. sutchuenense* var. *praevernum* is always blotched and mostly occurs at low elevations of up to 1,800 m. Its leaves are generally glabrous or almost glabrous at maturity and are slightly smaller than those of the *R. sutchuenense*, which generally occurs at 1,400 – 2,300 m.
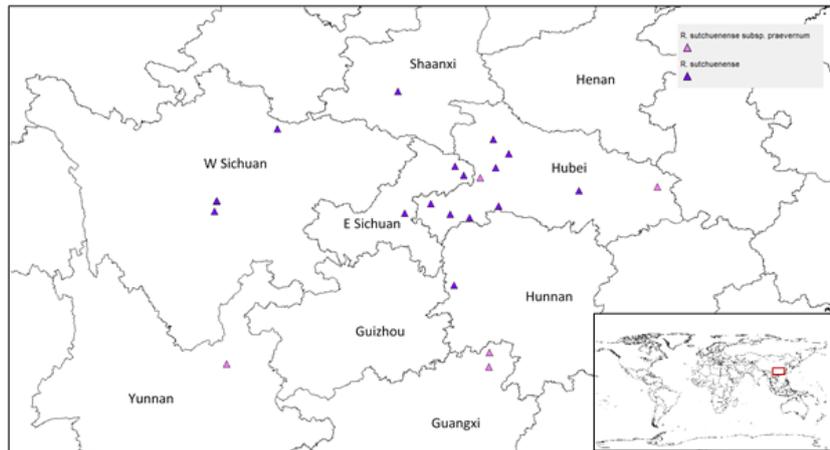
Figure 29: Distribution map of R. *sutchuenense* based on GBIF data. Purple triangles: R. *sutchuenense* var. *sutchuenense*. Pink triangles: R. *sutchuenense* var. *praevernum*

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[2] New combination suggested by author: H.P.Wilson merely authors initials, not official authority abbreviation.
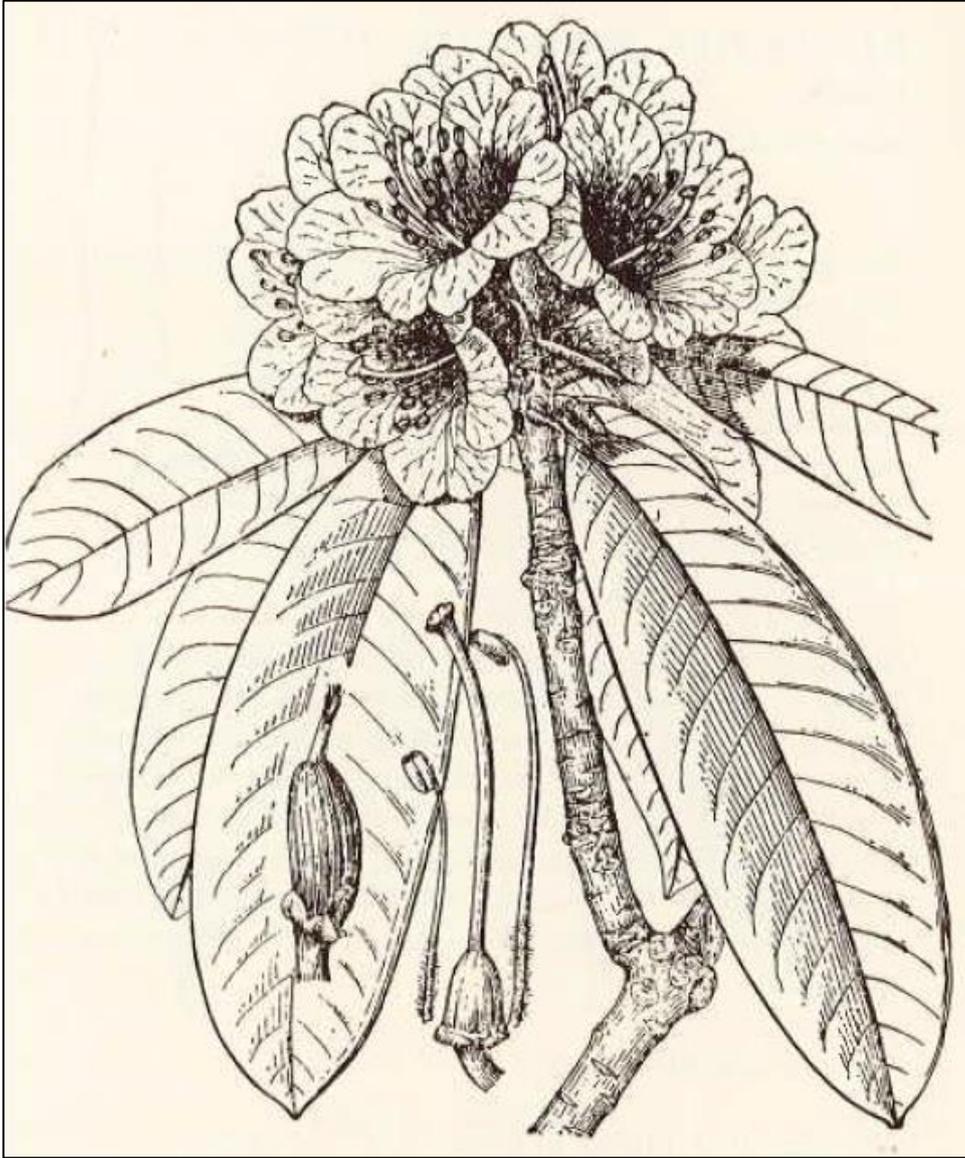
Figure 30: *R. sutchuenense,* showing the typical leaf shape and apex, and the capitate stigma.

Fortunea (s.s.)

Shrubs or trees to 15m. Bark rough, sometimes flaky. Young shoots with dense to sparse white to grayish floccose indumentum at emergence, soon glabrescent. Leaves oblanceolate, oblong, elliptic, rounded, ovate or orbicular, 5–30 × 2–10 cm, adaxial surface glabrous when mature, abaxial surface ± glabrous, sometimes with persistent punctulate hair bases. Inflorescence lax to dense, 5–30-flowered. Rhachis 3–70 mm, glandular. Pedicel usually glandular. Calyx minute to well-developed, 1–20 mm. Corolla (5–)6–8-lobed, funnel-campanulate to open-campanulate, white or pale pink to purple, nectar pouches usually absent (apparently present in *R. praeteritum*). Stamens 10–16. Ovary stipitate-glandular or glabrous. Style stipitate glandular. Stigma capitate.

Type species: *R. fortunei* Lindley, Gard. Chron., 868 (1859); Hook. f. Bot. Mag. t. 5596 (*Fortunei*).

Distribution: Bhutan, China, India, Myanmar, Nepal, Sikkim, Vietnam. Twenty-seven species.

Unresolved Taxa:

R. calophytum var. *pauciflorum* W.K.Hu, Acta Phytotax. Sin. 26: 304 (1988).

Figure 27, Figure 28 and Figure 30 are all taken from Young and Chong, "*Rhododendrons of China",* (1974).

9 Horticultural Implications

Rhododendrons are facultative out-breeders and widely interfertile (Chamberlain, 1982), (Milne and Abbott, 2008), (Zha, Milne and Sun, 2008). Hence, within a collection composed of species represented by low numbers, the likelihood of hybridisation is very high. Furthermore, the likelihood that the resultant offspring will be fertile is also very high (Cox & Cox, 1997), (Chamberlain & Hyam, 1998). Hybridisation is known to occur frequently in cultivation and has been commonly observed in collections, especially in long-established, large gardens such as those at Corrour.

The *Rhododendron* collection at Corrour was established by Sir John Stirling Maxwell, a renowned forester whose experiments in upland forestry greatly influenced the industry. His bold, orginal concept for a high-altitude (1,250ft – 1,650ft) *Rhododendron* woodland garden on the banks of Loch Ossian in the Scottish Highlands was rooted in his experiences establishing plantations at high elevations. Sir John planted hundreds of different species raised from seed from the plant-hunting expeditions of Wilson, Forrest, Kingdon Ward and Rock, among others. Some plants from Wilson's early collections were purchased from James Veitch & Sons of Coombe Wood in 1914, but the majority of wild origin seedlings planted out at the site between 1910 and the 1950's were raised at Sir John's Glasgow residence: Pollok. The seed was mostly acquired from R.B.G.E., as documented by regular letters of correspondence between Sir John and the Regius Keepers between 1914 and 1954. Unfortunately, Sir John's records of plantings at Corrour are believed to have been lost when a fire devastated Corrour Lodge in 1942. *R. sutchuenense* and *R. calophytum* are known to have been doing well at Corrour in the 1920s (Striling Maxwell, 1929), but with planting records lost and very few labels persisting until now, it has not been possible to verify that the plants selected for study from Corrour are of wild origin, despite their age being consistent with the early plantings on the estate. Most species present in the impressive collection at Corrour are represented by multiple plantings, often in large numbers, suggesting that the majority of seedlings raised from each seed pan were planted out together (Hammond, 2014).

Gardens such as Corrour create an artificial environment in which species with disjunct distributions that would never normally be able to hybridise with one another are grown side by side. The resultant hybrids contain a mixture of genes not found anywhere in the wild and are often inferior to the species and get weeded out, or ignored. However, occasionally, interesting plants establish themselves in a collection and are later noted for their striking flowers or unusual habit, and sent for identification, or propagated and distributed amongst other enthusiasts. Sometimes these chance hybrids are obviously just so. Often though, they are not noticed for several years, by which point it can be difficult to ascertain which of the plants in the garden were intentionally planted, and which have sown themselves. This can be especially problematic if the garden origin hybrid has arisen in among a patch of seedlings grown from wild collected seed and then planted out and left to their own devices for a few years to establish. Batches of wild-collected seeds are often not pure anyway, either through mixing up of seeds at the packaging stage, stray capsules at the point of collection, or sometimes because the collection was made from a recent natural hybrid in the wild.

So, it is quite possible for unnatural hybrids to be grown, circulated, and described under a collection number which in reality has no bearing on its true origin. To compound this problem, Rhododendron collectors have always been excited by the prospect of discovering new species, so odd and unusual plants may have been selected to illustrate the perceived differences between individuals of 'accepted' species, and highlight them as new forms, or even species, worthy of naming.

Accordingly, one of the main problems facing anyone wishing to study species from material in cultivation is identifying material that merits study in the first place. Even in well labelled gardens, there will be multiple specimens lacking labels, either missed, or forgotten, or else the label has been damaged or moved. Even when labels are present, how can one be sure the labels attached to the plants truly relate to the original introductions?

During the course of this project, the given identifications for numerous samples collected were questioned. Sometimes specimens were merely misidentified, keying out comfortably as another species. Some accessions though did not fit neatly into any species circumscription and were thus identified as putative hybrids. The individual cases are explored below.

Discussion of Anomalous or Doubtfully Identified Accessions included in Molecular Work

Of the 30 accessions sequenced for the molecular work, five were putative hybrids based on morphological characters.

The first anomalous accession was one of the samples collected from Dawyck (HANWIL5, henceforth Calo-op-1). An old label on the plant identified it as *R. calophytum* var. *openshawianum,* although in the garden database it is recorded as *R. brachycarpum* (*Pontica*). At the time of collection, its identity was questioned as the leaves were much thinner and less coriaceous than all other specimens collected that day; it was also flowering late in the season for this species (12th May). Calo-op-1 had the campanulate corolla and prominent discoid stigma typical of *R. calophytum*, but were notably small, and held in a rather compact truss. Detailed examination of morphological characters noted an array of abnormal characters in this accession; it lacked any sort of winged petiole, had some glandular hairs, the peduncle was densely tomentose, the calyx was indumented and the prominent, dark crimson blotch encircled the ovary instead of being restricted to the upper 1-3 lobes of the corolla. The accession could not be satisfactorily identified as *R. calophytum* var. *openshawianum* due to the suite of unusual characters listed above. However, it seemed much more similar to *R. calophytum* var. *openshawianum* than to *R. brachycarpum*, or indeed any other *Pontica* species, which are characterised by corolla markings ranging in colour from green, through yellow, to orange, but never red-black. Its morphological characters, coupled with its phylogenetic placement based upon cpDNA suggest Calo-op-1 is of recent hybrid origin, with *R. calophytum* as a likely recent paternal ancestor. Hybridisation in cultivation cannot be ruled out as the source of this plant is unknown

The second putative hybrid, specimen HANWIL23, henceforth Strig-X-sutch, was collected from Corrour estate. From afar the plant had the appearance of *R. sutchuenense*, but on closer inspection it was most definitely a hybrid, putatively identified as *R. strigillosum* X *R. sutchuenense.* It was

included in molecular analysis as *R. strigillosum* was included in the dataset so this hypothesis could be tested on molecular as well as morphological grounds. Strig-X-sutch was growing near a large stand of *R. strigillosum*. In the majority of angiosperm species, cpDNA is maternally inherited (McCauley et al., 2007) so the placement of this accession as sister to *R. strigillosum* supports the hypothesis that *R. strigillosum* was the maternal parent. Setose-glandular hairs matching those described for *R. strigillosum* were imaged using SEM alongside the long, simple, sinuous hairs characteristic of the indumentum patches along the midrib of *R. sutchuenense*, supporting the putative hybrid status of this specimen.

Finally, a group of specimens grown at Benmore under the name *R. calophytum* var. *pauciflorum* were of questionable descent: HANWIL12 (Calo-pau-1), HANWIL13 (Calo-pau-2 and HANWIL13 (Calo-pau-3). The three plants are of wild origin, collected under one number (AC1054) in Daguan County, Zhaotong Prefecture, but they have strikingly different morphologies. They were included since this was the only material under this name available for study as part of the project. Calo-pau-1 looks almost exactly like *R. oreodoxa*, a species with rounded, short, thin leaves and a narrow, wingless petiole also found in this region (E00247218). Calo-pau-2 has a thin, plasetered indumentum looks superficially like cross between *R. calophytum* and *R. farinosum* Lèveillè, of *Argyrophylla* which is also found in the locality. Calo-pau-3 is very similar to *R. calophytum* var. *openshawinaum* in leaf shape, but as no floral material was available for any of these plants, it is difficult to ascertain the most likely parents of the samples, a task beyond the scope of this project. All three plants are from the same collection so should be sister seedlings, and so assuming maternal inheritance of cpDNA, they should have come out neatly together in the phylogeny. However, Calo-pau-1 came out in H2 whilst the Calo-pau-2 and Calo-pau-3 were grouped in the *calophyta* clade, indicating that either they are closely related to one another but not to Calo-pau-1, or cpDNA is not maternally inherited in *Rhododendron*. Whilst it is possible cpDNA could be inherited paternally (McCauley et al., 2007), it is most likely Calo-pau-1 was a rogue seedling, perhaps by chance attached to the outside of a seed capsule (*R. oreodoxa* was collected on the same trip), or else accidentally mixed in during seed cleaning, or sowing.
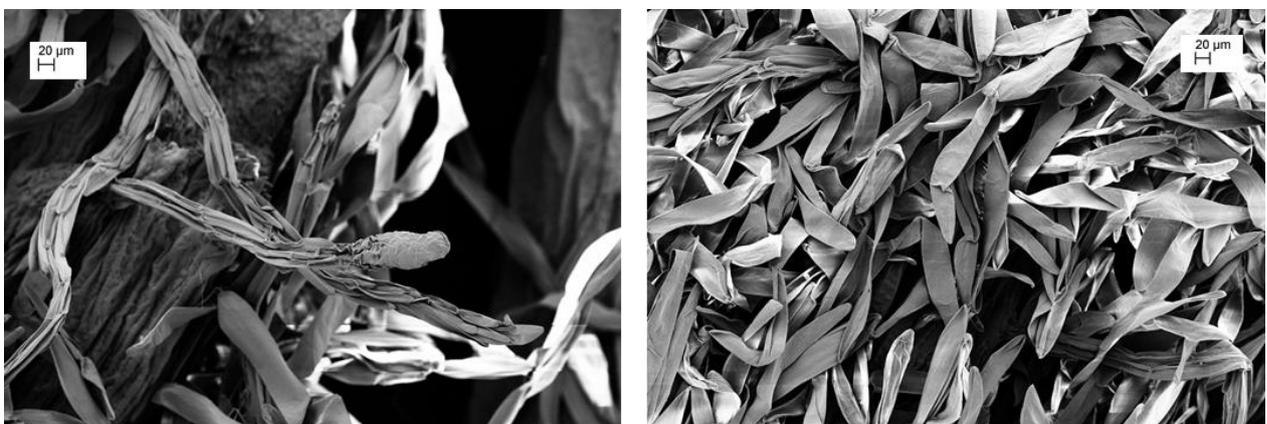
Figure 31: SEM images of trichomes on Strig-X-sutch. Left hand side: Glandular-tipped setose hair on leaf petiole-lamina. Image taken at 322x magnification with a working distance of 9.2 mm. Right hand side: Long, simple, sinuous hairs on midrib of Strig-X-sutch. Image taken at 300x magnification with a working distance of 9.8 mm

Two further accessions were identified as potentially mis-named, putative hybrids after constructing the cpDNA phylogeny; both specimens of wild origin collected as *R. × geraldii* in an attempt to explore the relationship between *R. praevernum* and *R. sutchuenense.* The accessions nested within the *Calophytum* Clade, in which all other taxa were varieties of *R. calophytum*. Upon inspecting the voucher specimens made, it became clear that despite affinities with *R. sutchuenense* in leaf shape, and indumentum characters, the leaves of these accessions were considerably larger than is typical for *R. sutchuenense* and, if anything, were keeled rather than recurved. They were also very sturdy, and quite thick. This combination of characters is consistent with *R. calophytum*, supporting the placement of the taxa within the phylogeny. Without floral material, and considering the plants were both very healthy and vigorous which can greatly affect leaf size and strength, it is difficult to determine their true identity. They have cautiously been dubbed *R. calophytum × praevernum* to reflect their affinities to each but a detailed study of floral material needs to be undertaken to before any level of confidence can be assigned to this putative hybrid hypothesis.

In conclusion, all seven accessions discussed above may be regarded as hybrids for the purpose of this study as they do not match the study group species in both molecular characters and morphological characters. These putative hybrids were excluded from this study for the taxonomic account, consideration of biogeography and relationship studies.

10 Conclusions and Suggested Further Work

Examination of morphological variation among *R. calophytum* and its immediate allies within subsection *Fortunea* found current species circumscriptions to be inadequate. A taxonomic account for the species in the *Calophyta* clade was composed from the morphological evidence, with due consideration of the biogeography of the group. The new combination *R. praevernum* var. *praevernum* (Hutchinson) H.P.Wilson is suggested, placing *R. praevernum* Hutchinson and *R. X geraldii* (Hutchinson) Ivens into synonymy. Other taxa in the group were maintained as per Chamberlain (1982). The variety *R. calophytum* var. *pauciflorum* was considered but not placed due to insufficient evidence to either retain or reject it. Additional fieldwork will need to be done to ascertain the status of this name.

The phylogenetic study presented here found *Rhododendron* subgenus *Hymenanthes* to be monophyletic for cpDNA with a clear division into two clades reflecting two disjoint biogeographic entities, consistent with previous studies. Subsection *Fortunea* was found to be polyphyletic for cpDNA across this division in *Hymenanthes*, suggesting the subsection as currently classified is composed of two distinct evolutionary lineages. The study group species form a monophyletic clade with *R. insigne* (*Argyrophylla*) within a clade composed of species in *Pontica* with a tertiary relict distribution.

The *Calophyta* group is able to be separated from all other species in *Fortunea* by a combination of morphological characters.

The morphological, molecular and biogeographical evidence provided is consistent with the hypotheses that a common ancestor of the study group species hybridised with a *Pontica* species after geographical split from other "proto-*Fortuneas*" resulting in chloroplast capture by introgression, followed by rapid speciation. *R. insigne* then gained its *Pontica* cpDNA type from one of the introgressed *Fortuneas.* However, evidence supporting this hypothesis is lacking. To be able to confidently accept this hypothesis the molecular study would need to be repeated using nuclear markers, and find a compatible topology, to be able to rule out homoploid hybrid speciation. It is also recommended that sampling is expanded to include more species from *Argyrophylla* so that the significance of the placement of *R.insigne* within the *Calophyta* clade may be explored.

If it is confirmed that both *Fortunea*(s.s.) (i.e. excluding the *Calophyta* group) and the *Calophyta* group are monophyletic, for both cpDNA and nDNA, then it is recommended that a new subsection be recognised within subgenus *Hymenanthes:* subsection *Calophyta*, to include only the species described in the taxonomic account.

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Appendices

Table 1: Exsiccatae studied. All specimens from E, PE were measured in detail for PCA analysis

Filed under	Det. H.Wilson	Collector	Coll. number	Date collected	Altitude (m)	Province	Institution	Barcode
R. asterochnoum	asterochnoum	H W Limpricht	1347	1914 04 27	3000	Sichuan	K	K000769348
R. asterochnoum	asterochnoum	H W Limpricht	1347	1914 04 27	3000	Sichuan	S	S08-1382
R. asterochnoum	asterochnoum	H W Limpricht	1347	1914 04 27	3000	Sichuan	WU	WU0042575
R. asterochnoum	R. asterochnoum	H W Limpricht	1347	1914 04 27	3000	Sichuan	E	E00327162
R. asterochnoum	R. calophytum var. calophytum	CEE	172	1991 09 16	2400	Sichuan	E	~
R. calophytum	R. ?calophytum var. openshawianum	W K Hu	8232	1946 11 04	~	Sichuan	E	~
R. calophytum	R. ?calophytum var. openshawianum	W K Hu	8235	1946 11 04	~	Sichuan	E	~
R. calophytum	R. calophytum	T T Yu	640	1932 05 05	2420	Sichuan	E	E00757363
R. calophytum	R. calophytum var. calophytum	W P Fang	2871	1928 08 13	2500-3000	Sichuan	E	~
R. calophytum	R. calophytum var. calophytum	W K Hu	8201	1946 11 04	~	Sichuan	E	~
R. calophytum	R. calophytum var. calophytum	W K Hu	8222	1946 11 04	~	Sichuan	E	~
R. calophytum	R. calophytum var. calophytum	W K Hu	8230	1946 11 04	~	Sichuan	E	~
R. calophytum	R. calophytum var. calophytum	W K Hu	8251	1946 11 04	~	Sichuan	E	~
R. calophytum	R. calophytum var. calophytum	W K Hu	8339	194611 08	~	Sichuan	E	~
R. calophytum	R. calophytum var. calophytum	A David	~	1870 -- --	4000	Sichuan	E	E00010422
R. calophytum	R. calophytum var. openshawianum	Z H Yang	81-0178	1981 04 22	1800-1900	Sichuan	E	~
R. calophytum var. calophytum	calophytum var. calophytum	T Yu	473	1932 04 21	2600	Sichuan	PE	~
R. calophytum var. calophytum	R. ?calophytum var. openshawianum	W K Hu	8286	1946 11 06	~	Sichuan	E	~
R. calophytum var. calophytum	R. calophytum var. calophytum	E H Wilson	1367	1908 11 --	~	Sichuan	E	~
R. calophytum var. calophytum	R. calophytum var. calophytum	W K Hu	8341	1946 11 08	~	Sichuan	E	~
R. calophytum var. openshawianum	calophytum var. openshawianum	E H Wilson	3414	1908 09 12	2300	Sichuan	A	A00015481
R. calophytum var. openshawianum	calophytum var. openshawianum	E H Wilson	3414	1908 09 12	2300	Sichuan	K	K000769349
R. calophytum var. openshawianum	R. ?calophytum var. openshawianum	K L Chu	2309	1936 04 09	1630	Sichuan	E	~
R. calophytum var. openshawianum	R. ?calophytum var. openshawianum	W K Hu	8356	1946 11 08	~	Sichuan	E	~
R. calophytum var. openshawianum	R. ?calophytum var. openshawianum	W K Hu	8705	1946 11 21	~	Sichuan	E	~
R. calophytum var. openshawianum	R. calophytum var. openshawianum	K L Chu	2310	1936 04 09	1360	Sichuan	E	~
R. calophytum var. openshawianum	R. calophytum var. openshawianum	E H Wilson	3414	1907	2300-2800	Sichuan	E	E00010427
R. calophytum var. openshawianum	R. calophytum var. openshawianum	P Cox & P Hutchison	7055	1995 09 27	2300	Sichuan	E	E00073206
R. calophytum var. openshawianum	R. calophytum var. openshawianum	E E Maire	~	1913 05 --	2300	Sichuan	E	~
R. calophytum var. openshawianum	R. calophytum var. openshawianum	E E Maire	32/1914	1914 05 --	3200	Sichuan	E	~
R. calophytum var. openshawianum	R. decorum	E H Wilson	1209A	1908 06-10	~	Sichuan	E	~
R. praevernum	praevernum	A Henry	5285	1889 03 --	~	Sichuan	K	K000769354
R. praevernum	praevernum	s.n.	s.n.	1919 04 19	~	Sichuan	K	K000789397
R. praevernum	praevernum	E H Wilson	17	1900 09	~	Sichuan	K	K000769353
R. praevernum	R. sutchuenense	E H Wilson	17	1901 09 --	~	W. Hupeh	E	E0001360
R. praevernum	R. sutchuenense	E H Wilson	17	1900 04 --	~	W. Hupeh	E	E00010419
R. sutchuenense	R. calophytum var. calophytum	CEE	172	1991 09 16	2400	Sichuan	E	E00079223
R. sutchuenense	R. sutchuenense	A N Steward & H C Cheo	1057	1933 09 18	~	~	E	~
R. sutchuenense	R. sutchuenense	Sino-Amer. Exped.	1231	1980 09 13	1780	Hubei	E	~
R. sutchuenense	R. sutchuenense	E H Wilson	2537	1907 08 --	~	Sichuan	E	~
R. sutchuenense	R. sutchuenense	R P Farges	~	1895	~	Sichuan	E	E00010418
R. sutchuenense	R. sutchuenense	R P Farges	~	1895	~	Sichuan	E	E0001359
R. X geraldii	R. sutchuenense	E H Wilson	509	1907 05 --	~	W. Hupeh	E	~
R. X geraldii	R. sutchuenense	E H Wilson	509A	1907 05 --	~	W. Hupeh	E	~
R. X geraldii	X geraldii	G Loder	s.n.	--	~		K	K000789404

Appendix 1: Specimens Studied

Table 2: Fresh specimens made as part of the project. To be added to the RBGE herbarium. DNA = yes, indicates included in molecular study. Measured = yes, indicates included in PCA analysis

Collected under	Det. H.Wilson	Location	Accession	Date collected	HANWIL #	DNA?	Measured?
R. asterochnoum	R. asterochnoum	Dawyck	20040714 A	14/05/2016	HANWIL2	Yes	Yes
R. asterochnoum	R. asterochnoum	Dawyck	20040714 B	14/05/2016	HANWIL4	Yes	Yes
R. asterochnoum	R. asterochnoum	Benmore	20040714 C	19/05/2016	HANWIL9	Yes	Yes
R. asterochnoum	R. asterochnoum	Glendoick	1	25/05/2016	HANWIL10	Yes	Yes
R. asterochnoum	R. asterochnoum	Glendoick	2	25/05/2016	HANWIL39	No	Yes
R. calophytum	R. calophytum var. calophytum	Dawyck	19952865 C	14/05/2016	HANWIL8	Yes	Yes
R. calophytum	R. calophytum var. calophytum	Corrour	389	09/06/2016	HANWIL21	Yes	Yes
R. calophytum	R. calophytum var. calophytum	Glendoick	Keith Rushforth	25/05/2016	HANWIL40	No	Yes
R. calophytum	R. calophytum var. calophytum	Cumbria	2	03/06/2016	HANWIL41	No	No
R. calophytum	R. calophytum var. calophytum	Cumbria	3	03/06/2016	HANWIL42	No	No
R. calophytum	R. calophytum var. calophytum	Cumbria	4	03/06/2016	HANWIL43	No	No
R. calophytum	R. calophytum var. openshawianum	Cumbria	5	03/06/2016	HANWIL44	No	No
R. calophytum var. calophytum	R. calophytum var. calophytum	Edinburgh	19960429 I	19/04/2016	HANWIL7	Yes	No
R. calophytum var. calophytum	R. calophytum var. calophytum	Benmore	19960422 A	07/06/2016	HANWIL25	Yes	No
R. calophytum var. calophytum	R. calophytum var. calophytum	Edinburgh	19724038 A	19/04/2016	HANWIL32	No	No
R. calophytum var. calophytum	R. calophytum var. calophytum	Edinburgh	19960429 H	19/04/2016	HANWIL33	No	No
R. calophytum var. openshawianum	R. calophytum var. openshawianum	Dawyck	19960770 A	14/05/2016	HANWIL1	Yes	Yes
R. calophytum var. openshawianum	R. calophytum var. openshawianum	Glendoick	C&H 7055	25/05/2016	HANWIL11	Yes	No
R. calophytum var. pauciflorum	R. oreodoxa	Benmore	19960655 A	19/05/2016	HANWIL12	Yes	No
R. calophytum var. pauciflorum	R. calophytum x argyrophylla ?	Benmore	19960655 C	19/05/2016	HANWIL13	Yes	No
R. calophytum var. pauciflorum	R. calophytum var. openshawianum	Benmore	19960655 D	19/05/2016	HANWIL14	Yes	No
R. facetum	R. facetum	Benmore	19962558 A	07/06/2016	HANWIL26	Yes	No
R. insigne	R. insigne	Edinburgh	19698662 I	08/07/2016	HANWIL31	Yes	No
R. planetum	R. planetum	Benmore	19270460 C	07/06/2016	HANWIL27	Yes	No
R. planetum	R. planetum	Benmore	~	07/06/2016	HANWIL46	No	No
R. praevernum	R. praevernum	Edinburgh	19698798 A	19/04/2016	HANWIL6	Yes	Yes
R. praevernum	R. praevernum	Dawyck	19795174 B	14/05/2016	HANWIL15	Yes	No
R. praevernum	R. calophytum var. calophytum	Corrour	387	09/06/2016	HANWIL20	Yes	Yes
R. praevernum	R. sutchuenense	Corrour	0404b	09/06/2016	HANWIL22	Yes	Yes
R. praevernum	R. praevernum	Edinburgh	19240357 A	19/04/2016	HANWIL34	No	No
R. praevernum	R. praevernum	Edinburgh	19240357 C	19/04/2016	HANWIL35	No	Yes
R. praevernum	R. praevernum	Edinburgh	19240357 D	19/04/2016	HANWIL36	No	Yes
R. praevernum	R. praevernum	Edinburgh	19240357 E	19/04/2016	HANWIL37	No	No
R. praevernum	R. praevernum	Edinburgh	19698798 B	19/04/2016	HANWIL38	No	Yes
R. sutchuenense	R. sutchuenense	Glendoick	Wilson original	25/05/2016	HANWIL16	Yes	Yes
R. sutchuenense	R. sutchuenense	Corrour	181	09/06/2016	HANWIL19	Yes	Yes
R. sutchuenense	R. sutchuenense	Corrour	595	09/06/2016	HANWIL24	Yes	Yes
R. sutchuenense	R. sutchuenense	Glenarn	1	09/06/2016	HANWIL28	Yes	Yes
R. sutchuenense	R. sutchuenense	Private	1	16/06/2016	HANWIL30	Yes	No
R. sutchuenense	R. sutchuenense	Cumbria	1	03/06/2016	HANWIL45	No	No
R. sutchuenense	R. sutchuenense	Private	2	16/06/2016	HANWIL47	No	No
R. sutchuenense aff	R. sutchuenense	Dawyck	19865006 B	14/05/2016	HANWIL3	Yes	No
R. x calophytum	R. X calophytum	Dawyck	19795452	14/05/2016	HANWIL5	Yes	Yes
R. X geraldii	R. praevernum x calophytum ?	Dawyck	19913262 B	14/05/2016	HANWIL17	Yes	No
R. X geraldii	R. praevernum x calophytum ?	Dawyck	19913262 A	14/05/2016	HANWIL18	Yes	No
R. X strigillosum	R. sutchuenense x strigillosum?	Corrour	559	09/06/2016	HANWIL23	Yes	Yes

Appendix 1: Specimens Studied (Continued)

Table 3: Georeferenced Specimens

Taxa	Latitude	Longitude	Collector	Ref. No.	Region	Additional Details
R. ?calophytum var. openshawianum	30.07	102.83	K L Chu	2309	Tein-chuan	Tien-Chuan, plantae szechuanense
R. calophytum var. calophytum	30.50	102.70	A David	s.n.	Sichuan: Moupin	Chine (Thibet oriental) - Provence de Moupin
R. calophytum var. calophytum	30.07	102.83	CEE	172	Tianchuan Xian	China, W Sichuan, Tianquan Xian, side of Erlang Shan, above Xinggou.
R. calophytum var. calophytum	30.07	102.83	CEE	172	Tianchuan Xian	China, W Sichuan, Tianquan Xian, side of Erlang Shan, above Xinggou.
R. calophytum var. openshawianum	30.07	102.83	K L Chu	2310	Tein-chuan	China, W Sichuan, Tianquan Xian, side of Erlang Shan, above Xinggou.
R. calophytum var. calophytum	30.38	102.83	E H Wilson	1367	Western Sichuan	China, W Sichuan, Tianquan Xian, side of Erlang Shan, above Xinggou.
R. decorum	30.38	102.83	E H Wilson	1209A	muping	muping=baoxing Exp recher and wilson
R. calophytum var. openshawianum	29.65	102.93	E H Wilson	3414	Yung-ching Hsien: Wa-wu-shan	Western szechuan, Yung-ching Hsien: Wa-shan
R. calophytum var. calophytum	29.25	103.00	T T Yu	640	Opien Hsien	Western szechuan, Yung-ching Hsien: Wa-shan
R. ?calophytum var. openshawianum	29.52	103.33	W K Hu	8232	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. ?calophytum var. openshawianum	29.52	103.33	W K Hu	8235	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. ?calophytum var. openshawianum	29.52	103.33	W K Hu	8286	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. ?calophytum var. openshawianum	29.52	103.33	W K Hu	8356	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. ?calophytum var. openshawianum	29.52	103.33	W K Hu	8705	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. calophytum var. calophytum	29.52	103.33	T T Yu	473	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. calophytum var. calophytum	29.52	103.33	W P Fang	2871	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. calophytum var. calophytum	29.52	103.33	W K Hu	8201	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. calophytum var. calophytum	29.52	103.33	W K Hu	8222	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. calophytum var. calophytum	29.52	103.33	W K Hu	8230	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. calophytum var. calophytum	29.52	103.33	W K Hu	8251	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. calophytum var. calophytum	29.52	103.33	W K Hu	8339	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. calophytum var. calophytum	29.52	103.33	W K Hu	8341	Omei-hsien: Mt. Omei	Western szechuan, Yung-ching Hsien: Wa-shan
R. calophytum var. openshawianum	28.50	103.60	P Cox & P Hutchison	7055	Sichuan: Liangshan Yi	Sichuan, S; Liangshan Yi Aut. Pref.: Liebo Co. Above Shuang He timber yard and camp
R. calophytum var. pauciflorum	27.83	103.67	A Clark	1054	Yunnan	North East Yunnan
R. calophytum var. openshawianum	27.75	104.25	Z H Yang	81-0178	Yiliang Xian: Xiao caoba	NE Yunnan, Yiliang Xian, Xiaocaoba
R. sutchuenense	31.50	110.50	Sino-Amer. Exped.	1231	Shennongjia: Zhushanyazi pass	Shennongjia Forest District, (31 30'N; 110 30'E) Zhushanyazi pass on the western side of the Dajiuhu basin.
R. asterochnoum	31.30	103.30	H W Limpricht	1347	Sichuan	Sichuan: Wen tschuan hsien
R. sutchuenense	30.70	102.60	E E Maire	s.n.	Sichuan	Mt. Zse-Tchou-pa
R. sutchuenense	31.40	110.20	E E Maire	32/1914	Western Sichuan	plants of western china, E. E. Maire, 32/1914. 'Near to mont Io-chan'
R. sutchuenense	109.80	31.30	E H Wilson	17	W. Hupeh	Western Hupeh, Veitch expedition

Appendix 1: Specimens studied (Continued)

Table 1: Genbank accessions used in molecular analysis

Species	Subgenus	Section	Subsection	Genbank trnL-F	Genbank matK	Accession
aberconwayi	Hymenanthes	Pontica	Irrorata	EU087392	EU087329	19370338
adenopodum	Hymenanthes	Pontica	Pontica	EU087363	EU087299	19620072
adenosum	Hymenanthes	Pontica	Glischra	EU087389	EU087326	19300433
aganniphum	Hymenanthes	Pontica	Taliensia	EU087412	EU087349	20052580
albiflorum	Candidastrum	n/a	n/a	AF394266	AB012731	n/a
alutaceum	Hymenanthes	Pontica	Taliensia	EU087410	EU087347	19913281
anhweiense	Hymenanthes	Pontica	Maculifera	EU087397	EU087334	19710038
praevernum	Hymenanthes	Pontica	Argyrophylla	EU087365	EU087302	19810820
atlanticum	Pentanthera	Pentanthera	Pentanthera	AY496924	AY494183	19730782
aureum	Hymenanthes	Pontica	Pontica	AY496918	AY494177	19450053
auriculatum	Hymenanthes	Pontica	Auriculata	EU087366	EU087303	19160027
barbatum	Hymenanthes	Pontica	Barbata	EU087367	EU087304	19370186
beanianum	Hymenanthes	Pontica	Nerriflora	EU087401	EU087338	19698404
beesianum	Hymenanthes	Pontica	Nerriflora	EU087411	EU087348	19491013
brachycarpum	Hymenanthes	Pontica	Pontica	AY496917	AY494176	19660135
bureavii	Hymenanthes	Pontica	Taliensia	EU087416	EU087353	19331022
calophytum	Hymenanthes	Pontica	Fortunea	EU087379	EU087316	19724038
campanulatum	Hymenanthes	Pontica	Campanulata	EU087369	EU087306	19720857
campylocarpum	Hymenanthes	Pontica	Campylocarpa	EU087370	EU087307	19832543
camtschaticum	Therorhodion	n/a	n/a	AB038898	AB012744	GAO2
canadense	Pentanthera	Rhodora	n/a	AF452212	AB012735	n/a
catacosmum	Hymenanthes	Pontica	Nerriflora	EU087402	EU087339	19698450
catawbiense	Hymenanthes	Pontica	Pontica	AY496915	AY494174	19340114
caucasicum	Hymenanthes	Pontica	Pontica	AY496916	AY494175	19521068
cerasinum	Hymenanthes	Pontica	Thomsonia	EU087418	EU087355	19291004
championae	Azaleastrum	Choniastrum	n/a	AF452188	AF454858	n/a
coryanum	Hymenanthes	Pontica	Argyrophylla	EU087364	EU087300	19698489
crinigerum	Hymenanthes	Pontica	Glischra	EU087391	EU087328	19950966
decorum	Hymenanthes	Pontica	Fortunea	EU087380	EU087317	19871529
degronianum	Hymenanthes	Pontica	Pontica	AY496920	AY494179	19341071
delavayi	Hymenanthes	Pontica	Arborea	DQ178247	KM606127	n/a
dichroanthum	Hymenanthes	Pontica	Nerriflora	EU087403	EU087340	19200011
eclecteum	Hymenanthes	Pontica	Thomsonia	EU087419	EU087356	19201020
edgeworthii	Rhododendron	Rhododendron	Edgeworthia	DQ999959	U61354	n/a
eudoxum	Hymenanthes	Pontica	Nerriflora	EU087404	EU087341	19794034
falconeri	Hymenanthes	Pontica	Falconera	EU087372	EU087309	19751301
fastigiatum	Rhododendron	Rhododendron	Lapponica	DQ999960	KM606131	n/a
faucium	Hymenanthes	Pontica	Thomsonia	EU087420	EU087357	19470106
ferrugineum	Rhododendron	Rhododendron	Rhododendron	AF394254	AB012741	n/a
floccigerum	Hymenanthes	Pontica	Nerriflora	EU087405	EU087342	19491018
forrestii	Hymenanthes	Pontica	Nerriflora	EU087408	EU087345	19250125
fortunei	Hymenanthes	Pontica	Fortunea	AF394247	AF454850	GAO1
fulgens	Hymenanthes	Pontica	Fulgensia	EU087377	EU087314	19371010
fulvum	Hymenanthes	Pontica	Fulva	EU087384	EU087321	19180010
galactinum	Hymenanthes	Pontica	Falconera	EU087373	EU087310	19913322
glischrum	Hymenanthes	Pontica	Glischra	EU087390	EU087327	19491014
grande	Hymenanthes	Pontica	Grandia	EU087385	EU087322	19698606
griersonianum	Hymenanthes	Pontica	Griersoniana	EU087388	EU087325	19320271
hongkongense	Azaleastrum	Azaleastrum	n/a	AF394260	U61338	n/a
hookeri	Hymenanthes	Pontica	Thomsonia	EU087421	EU087358	19291007
hyperythrum	Hymenanthes	Pontica	Pontica	AY496922	AY494181	19410106
insigne	Hymenanthes	Pontica	Argyrophylla	EU087425	EU087301	19698662
irroratum	Hymenanthes	Pontica	Irrorata	EU087393	EU087330	19812433
javanicum	Rhododendron	Vireya	Euvireya	AF394256	AB012742	n/a
kiusianum	Tsutsusi	Tsutsusi	n/a	AF394267	EU855891	n/a
lanatum	Hymenanthes	Pontica	Lanata	EU087395	EU087332	19810957
lanigerum	Hymenanthes	Pontica	Arborea	EU087362	EU087298	19291008
latoucheae	Azaleastrum	Choniastrum	n/a	AF394262	HQ427298	n/a
luteum	Pentanthera	Pentanthera	Pentanthera	AY496923	AY494182	19582084
macabeanum	Hymenanthes	Pontica	Grandia	EU087386	EU087323	19281023
macrophyllum	Hymenanthes	Pontica	Pontica	AY496914	AY494173	19734184
maculiferum	Hymenanthes	Pontica	Maculifera	EU087398	EU087335	19810812
mallotum	Hymenanthes	Pontica	Nerriflora	EU087406	EU087343	19201013
maximum	Hymenanthes	Pontica	Pontica	AY496912	AY494171	19800047
mengtszense	Hymenanthes	Pontica	Irrorata	EU087394	EU087331	19960617
molle	Pentanthera	Pentanthera	n/a	AF452211	U61356	n/a
moulmainense	Azaleastrum	Choniastrum	n/a	AF452194	AF454859	n/a
mucronulatum	Rhododendron	Rhododendron	Rhodorastra	AF394251	AF454855	n/a
nakotiltum	Hymenanthes	Pontica	Taliensia	EU087413	EU087350	179328170
neriiflorum	Hymenanthes	Pontica	Nerriflora	EU087407	EU087344	19200019
nipponicum	Pentanthera	Viscidula	n/a	AF452215	AB012739	n/a
occidentale	Pentanthera	Pentanthera	Pentanthera	AY496925	AY494184	19773072
orbiculare	Hymenanthes	Pontica	Fortunea	EU087378	EU087315	19460119
pentaphyllum	Pentanthera	Sciadorhodion	n/a	AB038840	AB012738	n/a
phaeochrysum	Hymenanthes	Pontica	Taliensia	EU087414	EU087351	19698781
ponticum	Hymenanthes	Pontica	Pontica	AY496913	AY494172	19773079
ponticum	Hymenanthes	Pontica	Pontica	AY496913	AY494172	AF452222
praevernum	Hymenanthes	Pontica	Fortunea	EU087381	EU087318	19240357
praevernum	Hymenanthes	Pontica	Fortunea	EU087382	EU087319	101715376
primuliflorum	Rhododendron	Pogonanthum	n/a	AF394255	AB012740	n/a
pseudochrysanthum	Hymenanthes	Pontica	Maculifera	EU087400	EU087337	19810864
pudorosum	Hymenanthes	Pontica	Grandia	EU087387	EU087324	19764021
racemosum	Rhododendron	Rhododendron	Scabrifolia	AF394250	AF454853	n/a
rothschildii	Hymenanthes	Pontica	Falconera	EU087374	EU087311	19764149
roxieanum	Hymenanthes	Pontica	Taliensia	EU087417	EU087354	19734059
santapaui	Rhododendron	Pseudovireya	n/a	AF452207	AB012743	n/a
selense	Hymenanthes	Pontica	Selensia	EU087409	EU087346	19812509
semibarbatum	Mumeazalea	n/a	n/a	AF452206	AB012733	n/a
semnoides	Hymenanthes	Pontica	Falconera	EU087375	EU087312	19301019
simsii	Tsutsusi	Tsutsusi	n/a	AF452216	AM296057	n/a
sinofalconeri	Hymenanthes	Pontica	Falconera	EU087376	EU087313	19960615
smirnowii	Hymenanthes	Pontica	Pontica	AY496921	AY494180	19698845
spinuliferum	Rhododendron	Rhododendron	Scabrifolia	AF452209	AF454854	n/a
stamineum	Azaleastrum	Choniastrum	n/a	AF394261	AB012730	n/a
strigillosum	Hymenanthes	Pontica	Maculifera	EU087399	EU087336	19754050
succothii	Hymenanthes	Pontica	Barbata	EU087368	EU087305	19902710
taliense	Hymenanthes	Pontica	Taliensia	EU087415	EU087352	19568652
thomsonii	Hymenanthes	Pontica	Thomsonia	EU087422	EU087359	19803353
trichocladum	Rhododendron	Rhododendron	Trichoclada	AF394253	AF454856	n/a
tsariense	Hymenanthes	Pontica	Lanata	EU087396	EU087333	19371016
ungernii	Hymenanthes	Pontica	Pontica	AY496919	AY494178	19623836
venator	Hymenanthes	Pontica	Venatora	EU087423	EU087360	19370170
vernicosum	Hymenanthes	Pontica	Fortunea	EU087383	EU087320	19141012
vialii	Azaleastrum	Azaleastrum	n/a	AF452205	KM606214	n/a
virgatum	Rhododendron	Rhododendron	Virgata	KC195978	AF440432	n/a
wadanum	Tsutsusi	Brachycalyx	n/a	AF452218	EU855909	n/a
wardii	Hymenanthes	Pontica	Campylocarpa	EU087371	EU087308	19698916
williamsianum	Hymenanthes	Pontica	Williamsiana	EU087424	EU087361	19320138

Appendix 2: Genbank Details.

Table 1: Discrete character states used for recording observations of qualitative characters (1 of 2)

				Leaves				Leaf indumentum				Stems: old							Foliage buds		Stems: new		New growth		
Health	Habit	Mature?	Petiole winged?	Leaf shape	Leaf apex	Leaf base	Leaf Margin	Location	Quantity	Colour	Hair type	Bark texture	Bark colour	Stem colour	Branchlet tomentum?	Branchlet tomentum	Leaf scars shape	Leaf scars arrangment	Shape	Colour	new growth stem colour	stem tomentum	Lower bud scale shape	Upper bud scale shape	Bud scale colour
very poor	shrub	no	no	lanceolate	acute	acute	slightly recurved	absent	absent	white	simple, short	smooth	brown	green	absent	thin, plastered	triangular	basal	egg shaped	pale green	absent	absent	ovoid	spathulate	yellow-pink
poor	tree	yes	yes, for entire length	oblanceolate	acuminate	acute-rounded	recurved	present on midrib only	occasional, solitary	brown	simple, long	rough	grey	pale green	sparse/minimal	white 'bloom'	flat based normal	evenly distributed	squat	dark green	pale green	dense white tomentum	semi-circular	narrowly spathulate	red
good	flat topped shrub	very	yes, for half length	lanceolate-oblanceolate	narrowly acuminate	rounded	not recurved	present on midrib and occasionally on lamina	occasional, clustered	yellowish	dendroid	flaky	red	reddish	some	dense hairs	curvy based normal	apical	teardrop	reddish	green	long hairs	bifid	lanceolate	green
very good	rounded shrub		yes for 1/3rd length	ovate	obtuse			present on midrib and common on lamina	sparse		multicullular dendroid		pinkish		lots	glandular	helmet		cone		red	sparse white tomentum	acute	linear	pale green
excellent	upright shrub		yes for 2/3rds length	elliptic	mucronate			present on midrib, secondary veins and occasially lamina	dense		stellate				lots for one year	sparse hairs	ovoid					dense yellow tomentum	acute with appendage	filliform	yellow
			laminar decurrent for entire length	elliptic-lanceolate	cuspidate			copious on midrib and secondary veins, occasional on lamina	very dense		simple and dendroid					lots, persistent							glandular hairs	mucronate, thick appendage		purple
			laminar partially decurrent								glandular					some,persistent								Long acuminate appendage		
			yes, very narrowly for entire length								cobwebby					some, persistent for one year									

Table 2: Discrete character states used for recording observations of qualitative characters (2 of 2)

		Inflorescence buds					Inflorescence														
Bud scale Indumentum?	Bud scale texture	Shape	Colour	Lower bud scale shape	Upper bud scale shape	Indumentum	Peduncle indumentum	Pedicel color	Pedicel texture	Calyx shape/lobes	Calyx texture	Calyx colour	Corolla shape	Corolla colour	Corolla colour outer	Markings?	Marking colour	Corolla texture	Filament hair type	Ovary color	Stigma colour
absent	waxy	egg shaped	pale green	deltoid	spathulate	absent	absent	red-purple above, green below, clear distintion	glabrous, smooth	annular	glabrous, smooth	green	funnel	white	white	absent	pale	puberulous inner to half way	absent	green	green
margin ciliate	silky	squat	dark green	semi-circular	narrowly spathulate	margin ciliate	some red/orange hairs	red-purple above, green below, speckled merging	glabrous, warty	pentagonal	glabrous, warty	pink	campanulate	pale pink	white flushed pink	blotch, no spots	dark	puberulous inner to 1/3rd	simple, short	pink	yellow
some short hairs	sticky	teardrop	reddish	bifid	lanceolate	some short hairs on inner, outer glabrous	white, simple hairs	green	indumented, smooth	lobes triangular	indumented, smooth	purple	funnel-campanulate	pink	pink	blotch and speckling	very dark	glabrous	simple, long	purple	green and pink
densely lanulose		cone		acute	linear	densely lanulose on inner, outer glabrous	densely tomented	red	indumneted, warty	lobes rounded	indumneted, warty	yellow	tubular-ventricose			speckling	minutely sparsely puberulous	complex	red	yellow and pink
slight tomentum on outside of lowers				acute with appendage	filliform	some short hairs all over		speckled red and yellow-green	glandular, smooth	lobes obtuse	glandular, smooth	red	widely-funneled			smear				green with purple spotting	pink
uppers with stripe of long simple hairs on outer, inner upper surface with long hairs, and lower outer surface with tomentum				ovoid, apex acuminate	spathulate, apex cuspidate	densely lanulose all over		yellow with light red speckling	glandular, warty		glandular, warty									green with extreme purple spotting becoming solid	
uppers with short stripe of long simple hairs on outer, inner upper surface with long hairs, no tomentum				ovoid					almost glabrous, few hairs near base, warty		smooth, occaisional short simple hairs near apex									green developing into purple	
minimal, only on inners				narrowly acuminate					indumentum of simple hairs, adaxially												
uppers densely lanulose inners, outers indumented, lowers																					
densely lanulose only on inners																					

Table 1: Reduced dataset for PCA 1

Det. H.Wilson	Specimen	Number of foliage flushes retained	Lamina length (cm)	Lamina width (mm)	a width/le	Petiole length (mm)	Petiole/lamina length	1 Seasons growth (cm)	# buds /branch apex
R. ?calophytum var. openshawianum	R. calophytum var. openshawianumK L Chu2309	2	14.67	37.00	0.063642	14.00	0.095455	1.500	1
R. ?calophytum var. openshawianum	R. calophytumW K Hu8232	1	25.00	39.50	0.024964	18.50	0.074	5.750	1
R. ?calophytum var. openshawianum	R. calophytumW K Hu8235	1	19.63	46.25	0.05554	19.33	0.098514	5.250	1.5
R. ?calophytum var. openshawianum	R. calophytum var. calophytumW K Hu8286	2	21.17	36.67	0.030008	14.00	0.066142	4.500	1
R. ?calophytum var. openshawianum	R. calophytum var. openshawianumW K Hu8356	2	12.00	28.00	0.054444	16.33	0.136111	2.833	2
R. ?calophytum var. openshawianum	R. calophytum var. openshawianumW K Hu8705	2	17.08	32.00	0.035088	18.75	0.109756	6.000	1
R. sutchuenense	R. sutchuenenseSino-Amer. Exped.1231	1	15.00	38.33	0.065309	23.33	0.155556	2.667	1
R. asterochnoum	R. asterochnoum 1	3	34.2	61.2	0.032119	40	0.116758	19.8	1.6
R. asterochnoum	R. asterochnoum 2	3	31	76.4	0.060948	39.8	0.128131	19.8	1.6
R. asterochnoum	R. asterochnoum 20040714 C	2	24.8	61.6	0.061776	27.2	0.111763	19.9	1.2
R. asterochnoum	R. asterochnoum 20040714 A	2	25.8	57.2	0.047489	27.8	0.109057	18.6	1
R. asterochnoum	R. asterochnoum 20040714 B	2	21.9	51	0.054387	28	0.127876	20	1.2
R. calophytum hybrid	R. calophytum var. openshawianum 19795452	2	21.6	39.4	0.033166	24.6	0.11429	4.9	1
R. calophytum var. calophytum	R. sutchuenenseCEE172	2	18.20	66.00	0.131506	20.20	0.110989	2.633	1
R. calophytum var. calophytum	R. asterochnoumCEE172	2	18.83	66.25	0.123742	19.00	0.100885	2.250	1
R. calophytum var. calophytum	R. calophytumT T Yu473	1	19.43	40.00	0.042388	16.50	0.084926	5.167	1
R. calophytum var. calophytum	R. calophytum var. calophytumE H Wilson1367	1	30.00	65.00	0.046944	21.75	0.0725	3.000	1
R. calophytum var. calophytum	R. calophytumW P Fang2871	2	27.00	56.25	0.043403	24.00	0.088889	4.833	1
R. calophytum var. calophytum	R. calophytumW K Hu8201	1	19.90	41.25	0.042968	16.67	0.083752	8.750	1
R. calophytum var. calophytum	R. calophytumW K Hu8222	1	19.17	41.67	0.047259	21.00	0.109565	2.875	1
R. calophytum var. calophytum	R. calophytumW K Hu8230	1	20.00	46.67	0.054444	19.67	0.098333	7.000	1
R. calophytum var. calophytum	R. calophytumW K Hu8251	1	12.00	30.00	0.0625	14.00	0.116667	2.000	1
R. calophytum var. calophytum	R. calophytumW K Hu8339	1	18.75	42.50	0.051378	19.50	0.104	3.833	1
R. calophytum var. calophytum	R. calophytum var. calophytumW K Hu8341	1	20.75	45.00	0.047031	23.00	0.110843	4.375	1
R. calophytum var. calophytum	R. calophytumT T Yu640	2	26.00	65.00	0.0625	20.25	0.077885	2.333	1
R. calophytum var. calophytum	R. calophytum Keith Rushforth ~	3	40.8	68	0.027576	32	0.078324	19.8	1
R. calophytum var. calophytum	R. calophytum 389	3	29.6	47.8	0.026239	25.4	0.086674	5.3	1
R. calophytum var. calophytum	R. calophytum 19952865 C	2	28	56	0.03986	27.8	0.099529	6.6	1
R. calophytum var. openshawianum	R. calophytum var. openshawianumK L Chu2310	1	13.75	33.17	0.058183	15.75	0.114545	3.750	1
R. calophytum var. openshawianum	R. calophytum var. openshawianumE H Wilson3414	3	14.00	37.00	0.069847	14.00	0.1	3.662	
R. calophytum var. openshawianum	lophytum var. openshawianumP Cox & P Hutchison	3	17.00	35.25	0.042995	18.75	0.110294	2.167	1
R. calophytum var. calophytum	R. calophytumA David~	2	24.83	65.00	0.06851	22.33	0.089933	4.500	1
R. calophytum var. openshawianum	. calophytum var. openshawianumE E Maire32/1914	1	15.56	36.43	0.054793	16.71	0.107401	3.200	1
R. calophytum var. openshawianum	R. calophytumZ H Yang81-0178	1	18.70	50.80	0.073798	19.00	0.101604	5.250	1
R. calophytum var. openshawianum	R. calophytum var. openshawianum 19960770 A	2	29.1	52	0.031994	28.4	0.097866	6.1	1
R. decorum	. calophytum var. openshawianumE H Wilson1209A	1	11.67	31.80	0.074295	17.25	0.147857	5.000	1
R. praevernum	R. praevernum 19240357 C	2	12.7	41.4	0.105765	21.4	0.170904	6.2	1
R. praevernum	R. praevernum 19698798 B	1	12.1	37.4	0.095896	21.4	0.17872	3.4	1
R. praevernum	R. praevernum 19698798 A	1	11	37.2	0.116	21.2	0.19419	2.5	1
R. praevernum	R. praevernum 19240357 D	2	15.1	44.4	0.087576	23	0.152917	8.1	1.6
R. praevernum	R. praevernum 387	2	26.8	70.4	0.069175	23.8	0.088449	10.8	1
R. praevernum	R. praevernum 0404b	2	16.5	51.6	0.103937	18.6	0.118709	4.4	1
R. strigillosum hybrid	R. X strigillosum 559	2	19.5	44.8	0.05247	13.6	0.069038	7.4	1
R. praevernum	R. praevernumE H Wilson17	1	12.75	40.00	0.098424	19.60	0.153725	4.867	1
R. praevernum	R. praevernumE H Wilson17	2	14.90	42.00	0.079456	22.80	0.15302	1.833	1
R. X geraldii	R. X geraldiiE H Wilson509	2	10.50	33.33	0.100781	23.00	0.219048	3.000	1
R. sutchuenense	R. sutchuenenseR P Farges~	1	12.00	43.75	0.132921	20.75	0.172917	0.000	1
R. sutchuenense	R. sutchuenenseA N Steward & H C Cheo1057	3	15.70	45.00	0.082153	18.40	0.117197	1.875	1
R. sutchuenense	R. sutchuenenseE H Wilson2537	1	17.83	45.00	0.063674	23.75	0.133178	5.000	1
R. sutchuenense	R. calophytum var. openshawianumE E Maire~	2	15.67	33.20	0.044908	19.20	0.122553	3.000	1
R. X geraldii	R. X geraldiiE H Wilson509A	1	15.75	43.33	0.075698	29.00	0.184127	2.250	1
R. sutchuenense	R. sutchuenense 595	2	19.1	54.8	0.082984	27.6	0.145498	8.6	1
R. sutchuenense	R. sutchuenense 181	2	19.7	51.6	0.06875	26.6	0.134897	3.3	1
R. sutchuenense	R. sutchuenense ~	2	21.4	57.8	0.072663	30.8	0.143581	4.6	1.8
R. sutchuenense	R. sutchuenense wilson original	1	20.7	66.6	0.103378	27.2	0.131276	8.4	1

Appendix 4: PCA Analysis

Table 2: Reduced dataset for PCA 2

| Det. H.Wilson	Specimen	Pedicel length (mm)	Peduncle length (mm)	Petiole length	Calyx size (mm)	# Foliage flushes retained	Lamina length (cm)	Lamina width (mm)	Lamina w/l	#flowers	Petiole/ lam L	1 Seasons growth (cm)	# per branch apex
R. ?calophytum var. openshawianum	R. calophytum var. openshawianumK L Chu2309	29.50	12.50	14.00	0.50	2	14.67	37.00	2.52	7.00	0.95	1.50	1
R. ?calophytum var. openshawianum	R. calophytumW K Hu8232	47.67	25.00	18.50	0.88	1	25.00	39.50	1.58	16.00	0.74	5.75	1
R. ?calophytum var. openshawianum	R. calophytumW K Hu8235	55.25	19.00	19.33	1.80	1	19.63	46.25	2.36	12.00	0.99	5.25	1.5
R. ?calophytum var. openshawianum	R. calophytum var. calophytumW K Hu8286	43.75	14.00	14.00	1.33	2	21.17	36.67	1.73	14.00	0.66	4.50	1
R. asterochnoum	R. asterochnoum 20040714 B	55.40	19.00	28.00	1.60	2	21.90	51.00	2.33	17.50	1.28	20.00	1.2
R. calophytum hybrid	R. calophytum var. openshawianum 19795452	28.60	12.00	24.60	1.90	2	21.60	39.40	1.82	18.20	1.14	4.90	1
R. calophytum var. calophytum	R. calophytumT T Yu473	48.83	19.00	16.50	0.50	1	19.43	40.00	2.06	12.00	0.85	5.17	1
R. calophytum var. calophytum	R. calophytum var. calophytumE H Wilson1367	54.67	17.00	21.75	1.00	1	30.00	65.00	2.17	15.00	0.73	3.00	1
R. calophytum var. calophytum	R. calophytumW P Fang2871	47.50	14.50	24.00	0.75	2	27.00	56.25	2.08	12.00	0.89	4.83	1
R. calophytum var. calophytum	R. calophytumW K Hu8201	39.80	21.00	16.67	1.25	1	19.90	41.25	2.07	16.00	0.84	8.75	1
R. calophytum var. calophytum	R. calophytumW K Hu8222	36.00	14.00	21.00	0.67	1	19.17	41.67	2.17	10.00	1.10	2.88	1
R. calophytum var. calophytum	R. calophytumW K Hu8230	48.43	20.00	19.67	1.38	1	20.00	46.67	2.33	22.00	0.98	7.00	1
R. calophytum var. calophytum	R. calophytumW K Hu8251	41.25	14.00	14.00	0.50	1	12.00	30.00	2.50	12.00	1.17	2.00	1
R. calophytum var. calophytum	R. calophytumW K Hu8339	38.83	14.00	19.50	1.25	1	18.75	42.50	2.27	15.00	1.04	3.83	1
R. calophytum var. calophytum	R. calophytum var. calophytumW K Hu8341	46.00	15.00	23.00	1.67	1	20.75	45.00	2.17	13.00	1.11	4.38	1
R. calophytum var. calophytum	R. calophytumT T Yu640	47.25	18.00	20.25	1.83	2	26.00	65.00	2.50	20.00	0.78	2.33	1
R. calophytum var. calophytum	R. calophytum Keith Rushforth ~	75.80	21.60	32.00	1.50	3	40.80	68.00	1.67	21.60	0.78	19.80	1
R. calophytum var. calophytum	R. calophytum 19952865 C	54.60	20.00	27.80	0.80	2	28.00	56.00	2.00	43.00	0.99	6.60	1
R. calophytum var. openshawianum	R. calophytum var. openshawianumK L Chu2310	42.00	12.00	15.75	0.38	1	13.75	33.17	2.41	8.00	1.15	3.75	1
R. calophytum var. openshawianum	R. calophytum var. openshawianumP Cox & P Hutchison7055	55.50	26.00	18.75	0.75	3	17.00	35.25	2.07	7.00	1.10	2.17	1
R. calophytum var. calophytum	R. calophytumA David~	34.00	14.00	22.33	0.50	2	24.83	65.00	2.62	12.00	0.90	4.50	1
R. calophytum var. openshawianum	R. calophytum var. openshawianumE E Maire32/1914	42.50	9.50	16.71	0.35	2	15.56	36.43	2.34	11.00	1.07	3.20	1
R. calophytum var. openshawianum	R. calophytumZ H Yang81-0178	47.43	15.00	19.00	0.50	1	18.70	50.80	2.72	14.00	1.02	5.25	1
R. calophytum var. openshawianum	R. calophytum var. openshawianum 19960770 A	53.00	15.20	28.40	1.90	2	29.10	52.00	1.79	18.20	0.98	6.10	1
R. decorum	R. calophytum var. openshawianumE H Wilson1209A	22.75	28.00	17.25	3.50	1	11.67	31.80	2.73	8.00	1.48	5.00	1
R. praevernum	R. praevernum 19240357 C	38.20	16.00	21.40	0.90	2	12.70	41.40	3.26	11.20	1.69	6.20	1
R. praevernum	R. praevernum 19698798 B	19.00	9.40	21.40	0.50	1	12.10	37.40	3.09	10.60	1.77	3.40	1
R. praevernum	R. praevernum 19698798 A	20.00	11.20	21.20	1.30	1	11.00	37.20	3.38	11.40	1.93	2.50	1
R. praevernum	R. praevernum 19240357 D	18.60	11.00	23.00	1.40	2	15.10	44.40	2.94	10.80	1.52	8.10	1.6
R. praevernum	R. praevernum 387	37.80	13.60	23.80	1.90	2	26.80	70.40	2.63	10.80	0.89	10.80	1
R. praevernum	R. praevernum 0404b	17.20	13.20	18.60	1.30	2	16.50	51.60	3.13	10.80	1.13	4.40	1
R. strigillosum hybrid	R. X strigillosum 559	18.60	8.60	13.60	3.10	2	19.50	44.80	2.30	11.40	0.70	7.40	1
R. praevernum	R. praevernumE H Wilson17	17.33	12.00	19.60	0.83	1	12.75	40.00	3.14	9.00	1.54	4.87	1
R. praevernum	R. praevernumE H Wilson17	18.00	10.00	22.80	0.75	2	14.90	42.00	2.82	4.00	1.53	1.83	1
R. X geraldii	R. X geraldiiE H Wilson509	12.25	12.50	23.00	1.00	2	10.50	33.33	3.17	5.50	2.19	3.00	1
R. sutchuenense	R. sutchuenenseR P Farges~	14.00	12.00	20.75	0.83	1	12.00	43.75	3.65	8.00	1.73	0.00	1
R. sutchuenense	R. sutchuenenseE H Wilson2537	21.20	25.00	23.75	2.00	1	17.83	45.00	2.52	11.00	1.33	5.00	1
R. sutchuenense	R. calophytum var. openshawianumE E Maire~	41.33	8.50	19.20	0.42	2	15.67	33.20	2.12	11.50	1.23	3.00	1
R. X geraldii	R. X geraldiiE H Wilson509A	17.00	9.00	29.00	0.75	1	15.75	43.33	2.75	5.00	1.84	2.25	1
R. sutchuenense	R. sutchuenense 595	20.80	15.40	27.60	1.90	2	19.10	54.80	2.87	13.20	1.45	8.60	1
R. sutchuenense	R. sutchuenense 181	21.80	14.60	26.60	1.30	2	19.70	51.60	2.62	10.20	1.35	3.30	1
R. sutchuenense	R. sutchuenense ~	15.80	11.00	30.80	1.50	2	21.40	57.80	2.70	10.40	1.44	4.60	1.8
R. sutchuenense	R. sutchuenense wilson original	24.00	18.80	27.20	0.90	1	20.70	66.60	3.22	17.60	1.31	8.40	1

Appendix 4: PCA Analysis (Continued)

Table 3: Reduced dataset for PCA 3

Det. H.Wilson	Specimen	# Foliage flushes retained	Lamina length (cm)	Lamina width (mm)	...a width/l...	Petiole length (mm)	Petiole/lamina length	1 Seasons growth (cm)	# per branch apex	#flowers	Rhachis length (mm)	Pedicel length (mm)	Calyx size (mm)	#lobes	#stamens	Style length (mm)	Style diameter (mm)	Stigma height (mm)	Stigma max diam (mm)
R. ?calophytum var. openshawianum	R. calophytum var. openshawianumK L Chu2309	2	14.67	37.00	0.25	14.00	0.10	1.50	1.00	7.00	12.50	29.50	0.50	5.00	20.00	26.75	2.00	2.00	6.50
R. asterochnoum	R. asterochnoum 20040714 B	2	21.90	51.00	0.23	28.00	0.13	20.00	1.20	17.50	19.00	55.40	1.60	5.00	17.80	29.80	1.20	1.10	5.60
R. calophytum hybrid	R. calophytum var. openshawianum 19795452	2	21.60	39.40	0.18	24.60	0.11	4.90	1.00	18.20	12.00	28.60	1.90	5.00	10.60	36.00	0.70	1.00	4.80
R. calophytum var. calophytum	R. calophytumT T Yu473	1	19.43	40.00	0.21	16.50	0.08	5.17	1.00	12.00	12.00	48.83	0.50	5.00	20.00	21.00	1.00	1.00	5.50
R. sutchuenense	R. sutchuenenseR P Farges~	1	12.00	43.75	0.36	20.75	0.17	0.00	1.00	8.00	12.00	14.00	0.83	5.00	13.00	46.00	1.00	1.00	2.50
R. calophytum var. calophytum	R. calophytum Keith Rushforth ~	3	40.80	68.00	0.17	32.00	0.08	19.80	1.00	21.60	21.60	75.80	1.50	5.00	20.00	32.80	0.90	1.90	7.20
R. calophytum var. calophytum	R. calophytum 19952865 C	2	28.00	56.00	0.20	27.80	0.10	6.60	1.00	43.00	20.00	54.60	0.80	5.40	19.80	27.00	1.10	2.00	7.40
R. calophytum var. openshawianum	R. calophytum var. openshawianumK L Chu2310	1	13.75	33.17	0.24	15.75	0.11	3.75	1.00	8.00	12.00	42.00	0.38	5.00	25.00	26.00	2.00	1.50	6.00
R. calophytum var. openshawianum	R. calophytumA David~	2	24.83	65.00	0.26	22.33	0.09	4.50	1.00	12.00	14.00	34.00	0.50	5.00	15.00	28.00	2.00	2.00	8.00
R. calophytum var. openshawianum	R. calophytum var. openshawianumE E Maire32/1914	1	15.56	36.43	0.23	16.71	0.11	3.20	1.00	11.00	9.50	42.50	0.35	5.00	25.00	24.50	1.75	1.50	6.50
R. calophytum var. openshawianum	R. calophytumZ H Yang81-0178	1	18.70	50.80	0.27	19.00	0.10	5.25	1.00	14.00	15.00	47.43	0.50	5.00	15.00	23.50	2.00	2.00	8.25
R. calophytum var. openshawianum	R. calophytum var. openshawianum 19960770 A	2	29.10	52.00	0.18	28.40	0.10	6.10	1.00	18.20	15.20	53.00	1.90	5.00	18.80	27.00	1.90	2.80	7.40
R. praevernum	R. praevernum 19240357 C	2	12.70	41.40	0.33	21.40	0.17	6.20	1.00	11.20	16.00	38.20	0.90	5.00	15.00	36.20	1.00	0.80	2.60
R. praevernum	R. praevernum 19698798 B	1	12.10	37.40	0.31	21.40	0.18	3.40	1.00	10.60	9.40	19.00	0.50	5.00	14.20	40.40	0.95	1.20	2.70
R. praevernum	R. praevernum 19698798 A	1	11.00	37.20	0.34	21.20	0.19	2.50	1.00	11.40	11.20	20.00	1.30	5.00	13.80	43.60	0.70	1.00	2.30
R. praevernum	R. praevernum 19240357 D	2	15.10	44.40	0.30	23.00	0.15	8.10	1.60	10.80	11.00	18.60	1.40	5.00	14.60	37.60	1.00	0.90	3.10
R. praevernum	R. praevernum 387	2	26.80	70.40	0.26	23.80	0.09	10.80	1.00	10.80	13.60	37.80	1.90	6.40	16.60	32.80	1.20	2.00	3.90
R. praevernum	R. praevernum 0404b	2	16.50	51.60	0.32	18.60	0.12	4.40	1.00	10.80	13.20	17.20	1.30	5.20	14.60	30.00	1.00	1.00	2.20
R. praevernum	R. praevernumE H Wilson17	1	12.75	40.00	0.31	19.60	0.15	4.87	1.00	9.00	12.00	17.33	0.83	5.00	13.50	33.50	1.12	1.00	3.50
R. X geraldii	R. X geraldiiE H Wilson509	2	10.50	33.33	0.32	23.00	0.22	3.00	1.00	5.50	12.50	12.25	1.00	5.00	14.00	35.00	1.00	1.00	4.00
R. calophytum var. calophytum	R. calophytumT T Yu640	2	26.00	65.00	0.25	20.25	0.08	2.33	1.00	20.00	18.00	47.25	1.83	5.00	17.00	30.33	2.00	1.83	7.50
R. sutchuenense	R. calophytum var. openshawianumE E Maire~	2	15.67	33.20	0.21	19.20	0.12	3.00	1.00	11.50	8.50	41.33	0.42	5.00	21.50	27.00	1.00	2.00	5.33
R. X geraldii	R. X geraldiiE H Wilson509A	1	15.75	43.33	0.28	29.00	0.18	2.25	1.00	5.00	9.00	17.00	0.75	5.00	14.50	41.50	1.50	1.00	3.50
R. sutchuenense	R. sutchuenense 595	2	19.10	54.80	0.29	27.60	0.15	8.60	1.00	13.20	15.40	20.80	1.90	5.80	14.80	33.40	1.00	0.80	2.70
R. sutchuenense	R. sutchuenense wilson original	1	20.70	66.60	0.32	27.20	0.13	8.40	1.00	17.60	18.80	24.00	0.90	5.00	15.20	42.40	1.00	1.10	3.30

Table 4: Reduced dataset for PCA 4

Det. H.Wilson	Specimen	# Foliage flushes retained	Lamina length (cm)	Lamina width (mm)	Lamina width/ length	Petiole length (mm)	petiole/ lamina length	1 Seasons growth (cm)	# buds per branch apex	# Flowers	Rhachis length (mm)	Pedicel length (mm)	Calyx size (mm)	AD-Fused corolla length (between lobes) (mm)	AB-Fused corolla length (between lobes) (mm)	Corolla length from longest corolla lobe (mm)	#lobes	Solid blotch height	Solid blotch width	area	w/l	Height of markings (mm)	Width of markings (mm)	Area	w/l	#stamens	Longest Filament length (mm)	Length of hairs up filament (mm) (of longest filment)	proportion hairy	Shortest Filament length	Length of hairs up filament (mm) (of shortest filment)	proportion hairy	Thecae length (mm)	Ovary length (mm)	Ovary width (mm)	Style length (mm)	Style diameter (mm)	Stigma height (mm)	Stigma max diam (mm)
R. praevernum	R. praevernum 19240357 C	2	12.70	41.40	0.33	21.40	0.17	6.20	1.00	11.20	16.00	38.20	0.90	38.20	34.00	53.60	5.00	18.60	19.60	#####	1.05	30.00	24.20	733.20	0.81	15.00	40.00	12.60	0.32	22.60	8.60	0.38	2.10	6.40	4.20	36.20	1.00	0.80	2.60
R. praevernum	R. praevernum 19698798 B	1	12.10	37.40	0.31	21.40	0.18	3.40	1.00	10.60	9.40	19.00	0.50	38.80	36.20	53.80	5.00	19.20	16.40	#####	0.85	34.00	20.60	698.20	0.61	14.20	39.20	11.80	0.30	21.60	8.40	0.39	2.60	6.60	3.80	40.40	0.95	1.20	2.70
R. praevernum	R. praevernum 19698798 A	1	11.00	37.20	0.34	21.20	0.19	2.50	1.00	11.40	11.20	20.00	1.30	39.00	34.40	52.20	5.00	17.40	17.00	#####	0.98	34.00	20.00	680.40	0.59	13.80	46.80	11.60	0.25	23.80	7.60	0.32	2.80	6.40	4.20	43.60	0.70	1.00	2.30
R. praevernum	R. praevernum 19240357 D	2	15.10	44.40	0.30	23.00	0.15	8.10	1.60	10.80	11.00	18.60	1.40	38.40	33.80	54.40	5.00	17.20	16.80	#####	0.98	30.60	21.20	656.00	0.69	14.60	40.20	12.00	0.30	22.40	7.40	0.33	2.50	6.80	4.20	37.60	1.00	0.90	3.10
R. praevernum	R. praevernum 387	2	26.80	70.40	0.26	23.80	0.09	10.80	1.00	10.80	13.60	37.80	1.90	28.60	24.20	38.20	6.40	12.20	9.80	#####	0.80	25.60	12.20	315.80	0.48	16.60	31.60	10.20	0.32	19.20	6.00	0.31	1.90	8.20	5.80	32.80	1.20	2.00	3.90
R. praevernum	R. praevernum 0404b	2	16.50	51.60	0.32	18.60	0.12	4.40	1.00	10.80	13.20	17.20	1.30	26.20	20.20	40.60	5.20	11.60	9.80	#####	0.84	19.00	14.60	276.40	0.77	14.60	29.40	10.00	0.34	16.80	6.00	0.36	1.90	8.60	5.20	30.00	1.00	1.00	2.20
R. sutchuenense	R. praevernumE H Wilson17	1	12.75	40.00	0.31	19.60	0.15	4.87	1.00	9.00	12.00	17.33	0.83	35.00	33.00	50.00	5.00	22.00	12.50	#####	0.57	48.00	30.00	#####	0.63	13.50	31.00	11.00	0.35	22.00	10.00	0.45	2.79	6.75	4.50	33.50	1.12	1.00	3.50
R. sutchuenense	R. X geraldiiE H Wilson509	2	10.50	33.33	0.32	23.00	0.22	3.00	1.00	5.50	12.50	12.25	1.00	29.50	27.00	49.25	5.00	20.00	14.00	420.00	0.70	28.00	15.00	420.00	0.54	14.00	35.00	12.00	0.34	24.50	8.00	0.33	2.83	6.50	3.75	35.00	1.00	1.00	4.00
R. sutchuenense	R. calophytumT T Yu640	2	26.00	65.00	0.25	20.25	0.08	2.33	1.00	20.00	18.00	47.25	1.83	40.00	32.33	56.00	5.00	17.70	16.00	#####	0.90	20.00	16.00	320.00	0.05	17.00	27.00	0.00	0.00	16.00	0.00	0.00	2.20	8.13	3.87	30.33	2.00	1.83	7.50
R. sutchuenense	R. calophytum var. openshawianumE E Maire	2	15.67	33.20	0.21	19.20	0.12	3.00	1.00	11.50	8.50	41.33	0.42	30.00	28.00	40.33	5.00	16.00	15.50	#####	0.97	16.00	15.50	248.00	0.06	21.50	23.00	9.00	0.39	15.00	7.00	0.47	2.08	7.67	3.50	27.00	1.00	2.00	5.33
R. sutchuenense	R. X geraldiiE H Wilson509A	1	15.75	43.33	0.28	29.00	0.18	2.25	1.00	5.00	9.00	17.00	0.75	31.50	29.00	49.33	5.00	22.50	13.50	#####	0.60	33.00	20.00	660.00	0.61	14.50	35.50	11.50	0.32	18.50	10.50	0.57	2.67	8.00	4.00	41.50	1.50	1.00	3.50
R. sutchuenense	R. sutchuenense 595	2	19.10	54.80	0.29	27.60	0.15	8.60	1.00	13.20	15.40	20.80	1.90	31.40	25.60	46.60	5.80	0.00	0.00	0.00	0.00	13.40	15.20	205.60	1.13	14.80	31.60	10.20	0.32	21.00	5.80	0.28	1.30	6.20	4.20	33.40	1.00	0.80	2.70
R. sutchuenense	R. sutchuenense wilson original	1	20.70	66.60	0.32	27.20	0.13	8.40	1.00	17.60	18.80	24.00	0.90	32.20	24.80	45.00	5.00	0.00	0.00	0.00	0.00	23.20	20.80	487.20	0.90	15.20	30.00	9.40	0.31	18.20	6.00	0.33	2.50	6.50	3.75	42.40	1.00	1.10	3.30

Appendix 4: PCA Analysis (Continued)

Phylogeny 1: Strict consensus tree from final maximum parsimony analysis
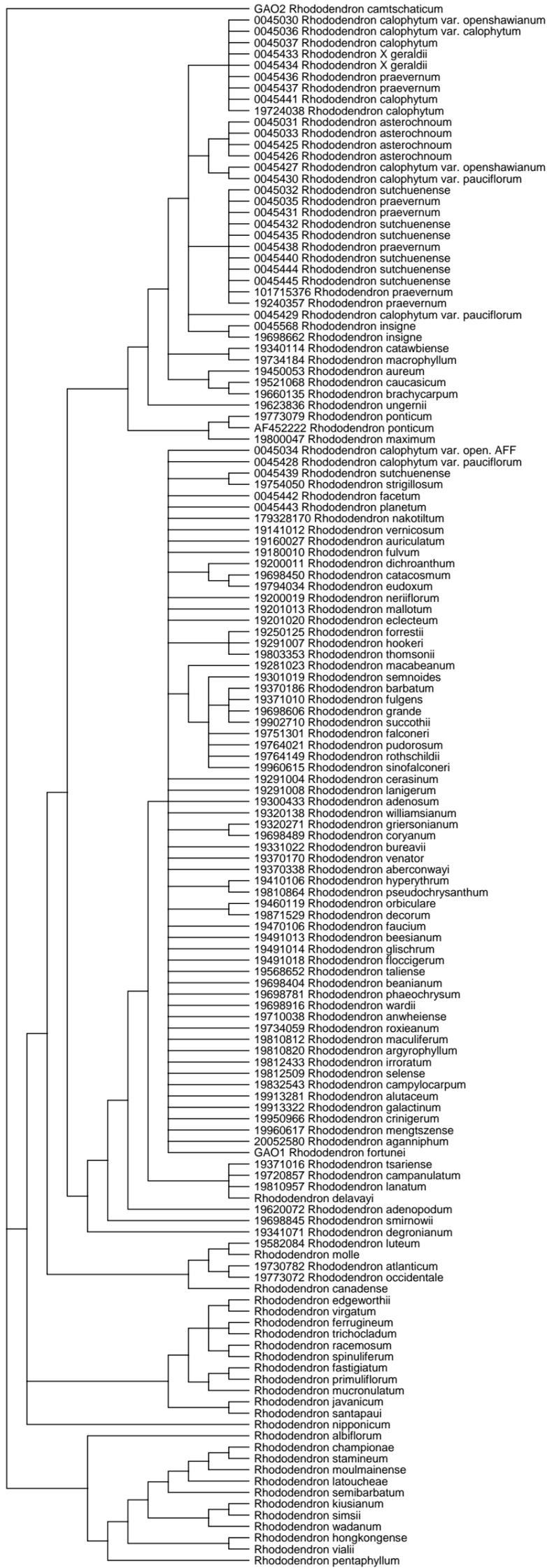
Phylogeny 2: Arbitrarily most parsimonious tree from final maximum parsimony analysis

Appendix 5: Phylogenies
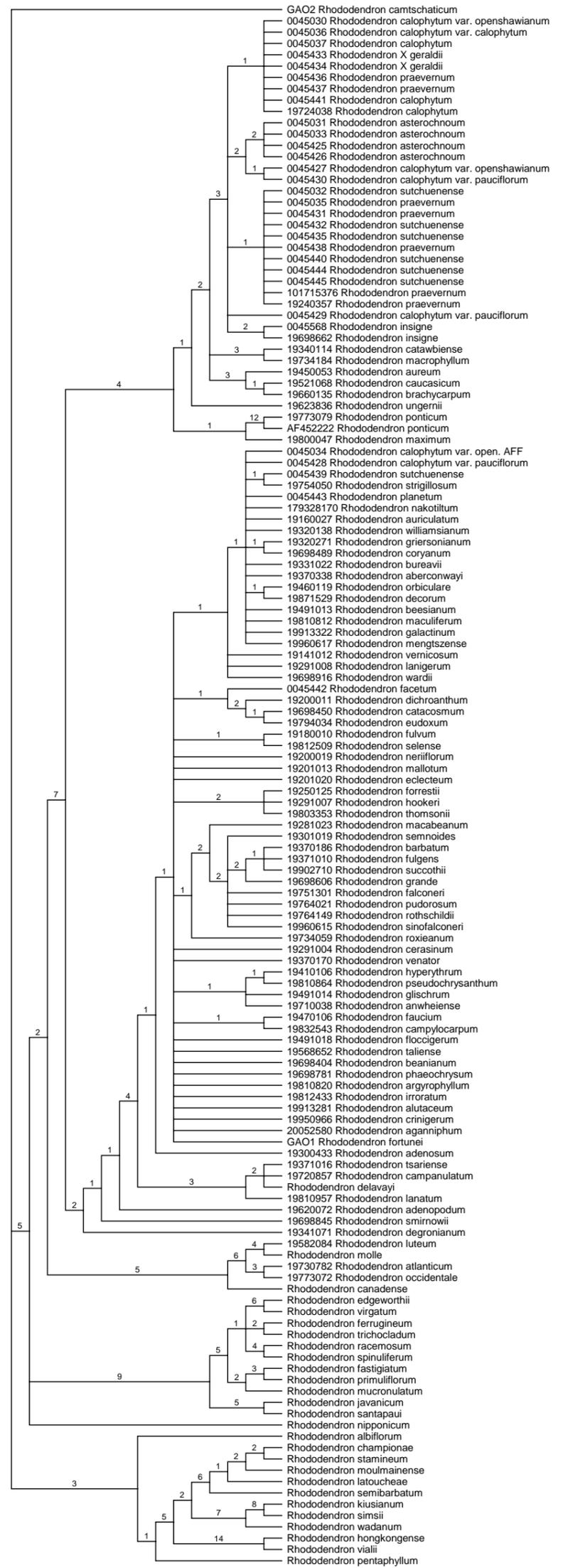
**Phylogeny 3: Majority rule consensus tree from final maximum parsimony analysis**
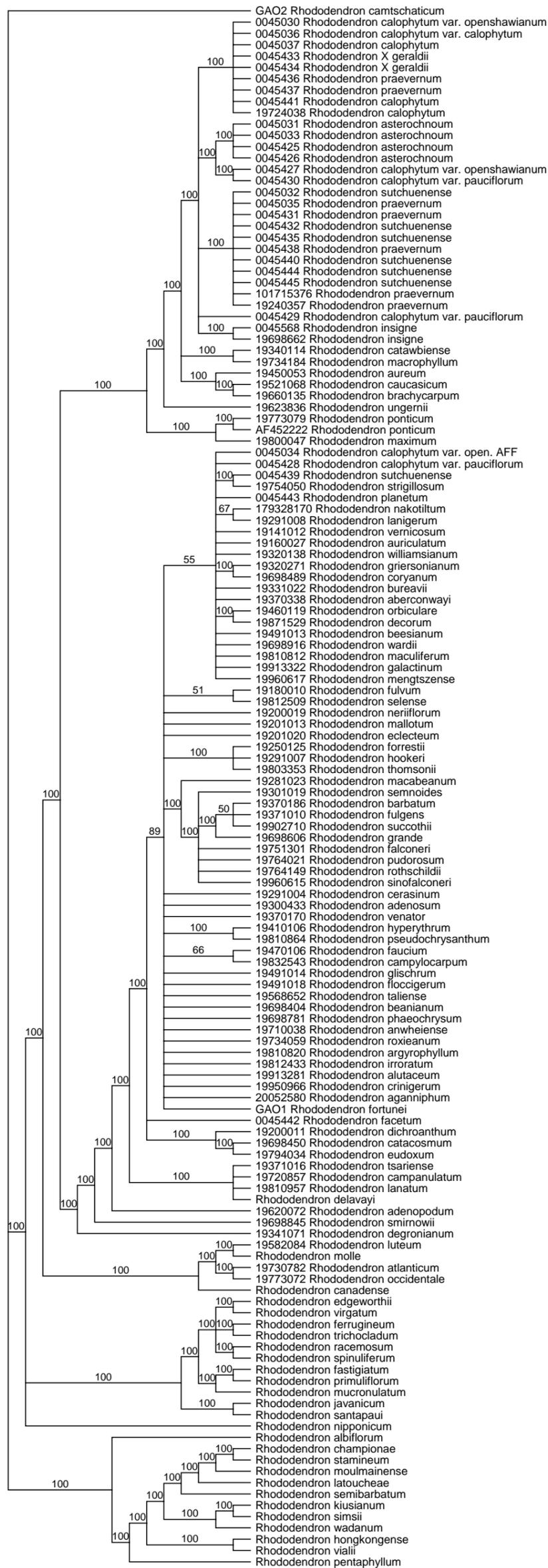
Figure 32: Consensus tree from Bayesian analysis and Bootstrap Maximum Parsimony. PP(%) above branches. BS(%) below branches.