Vireya *Rhododendron* in plant collections in New Zealand: potential for international conservation

A project for the Peter Skellerup Plant Conservation Scholarship

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Introduction

New Zealand contains significant living plant collections of *Rhododendron* subgenus *Vireya*, including wild-source species, species not known to be cultivated in other countries, and species that have been internationally red-listed[4]. These collections may have a role in international *ex-situ* conservation[5] of this genus, but any useful contribution depends on accurate identification and presence of sufficient diversity among accessions. Potential conservation action is also confounded by complex taxonomy, which raises questions about relationships between species and therefore conservation action. In this project molecular and conventional taxonomic methods were used to examine the range of *Rhododendron* subgenus *Vireya* in New Zealand, to explore relationships between red-list species and their near relatives, and to determine whether there is sufficient diversity present for New Zealand accessions to play a useful role in the forthcoming international conservation programmes for the group. Selected results are presented and implications for conservation are considered.

There are about 1250 species of *Rhododendron*, with about 850 being temperate species, largely from mainland Asia. The other approximately 350 species are the subtropical species of the *Vireya* subgenus, which is largely centred in Malesia, with 12 species from mainland Asia and two species from Australia. Malesia comprises the islands and archipelagos of Malaysia, New Guinea, Borneo, the Philippines and Indonesia and is a region that suffers habitat loss caused by palm oil production, forest clearance and urbanisation (McMorrow and Talip, 2001; Taylor et al., 1994; Woods, 1989). Many vireyas have restricted distribution, with frequent occurrence of point-endemics (i.e., those that are extremely restricted; Argent, 2006; Gibbs et al., 2011). One species, *R. retrosipilum,* is now extinct and many others are under some threat (Gibbs et al., 2011). Preliminary work on species in collections indicated that New Zealand has a significant diversity of vireyas, including wild-source material and conservation red-list species, suggesting our collections may be important in *ex-situ* conservation programmes (MacKay, 2008a, 2008b; Smith, 2009). Following the 2007 invitation for Marion MacKay to participate in the red-list assessment panel for *Rhododendron*, the New Zealand research team of Marion MacKay, Ahmed Fayaz, Claudia Wiedow, Graham Smith and Sue Gardiner, was formed and six objectives were set for the project.

In Objective One, we contributed to the red-list assessment and the completed red-list was used to focus our research around conservation species. In Objective Two, the Pukeiti inventory of vireya (Smith, 2009) and a survey of collections revealed the extent of collections in New Zealand and the range of material available for the research. In Objective Three, the taxonomic complexity of the subgenus was examined and this information was used in selection of species for testing. In Objective Four, tissue samples were collected from 340 accessions and several molecular methods were used to examine the samples. In Objective Five, herbarium samples were collected and examined for each species tested. Contribution to conservation planning, Objective Six, is ongoing.

Objective One: Research assessment and formation of the red-list

In July 2008, a workshop was held in Singapore to conduct the conservation red-list assessment for *Rhododendron*. Species were assessed against the International Union for Conservation of Nature (IUCN) red-list categories, with the red-list published by Gibbs et al. (2011). The Singapore workshop (and a subsequent one held in China to complete the assessment for the temperate species) was managed by Sara Oldfield from Botanic Gardens Conservation International (BGCI) in London, who manages Targets 2 and 8 of the Global Strategy for Plant Conservation (Sharrock, 2012) on behalf of the IUCN. Target 2 relates to conducting red-list assessments of all

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[4] Red-listed species are those that are threatened, endangered or locally extinct.
[5] *Ex-situ* plant conservation is the protection of threatened species by cultivating them outside of their natural habitat.

plant genera[6] and Target 8 relates to locating red-list species in cultivation, to determine the resource that might be available for conservation purposes.

Of the 355 species of vireyas that were assessed, 161 (45%) were red-listed (Gibbs et al., 2011). The numbers in each category are shown in Table 1. Compared with other red-list assessments, two features are noted. First is the number of Data Deficient (DD) ratings (57.7%), which is nearly twice the number in *Acer* (34.9%) or *Quercus* (29.7%) (MacKay et al., 2010). This high number of DD ratings indicates a marked gap in knowledge of those species, and a need for considerable research. For example, *Rhododendron maxwellii* (Fig. 1.) was rated Data Deficient.

Fig. 1 *Rhododendron maxwellii.*

Table 1 Number of species of vireya rhododendron in each red-list category (data from Gibbs et al., 2011).

Red-list category	Number of species
Extinct	1
Critical	12
Endangered	12
Vulnerable	38
Near Threatened	5
Data Deficient	93
Total	**161**

The second feature of the vireya red-list is that 97% of red-list species are from one geographic area (Malesia). This is in sharp contrast to other recent red-lists, where species were split about 50:50 between two geographic areas for Magnoliaceae, while in *Quercus* about 80% came from two areas and the rest were scattered over several areas (MacKay et al., 2010). When the geographic focus is combined with the number of species red-listed and the extent of Data Deficient ratings, vireya appears to have a conservation problem that is more acute than other recently assessed groups. Subsequent to the red-list process, the assessment was used to establish priorities for further work, focusing research on red-list species and their relatives. A second consequence was the need to determine the existence and diversity of rated species in cultivation (Target 8), which is within Objective Two of this project.

Objective Two: Range and distribution of *Rhododendron* subgenus *Vireya* species in New Zealand

Any useful contribution by New Zealand to *ex-situ* conservation of vireya depends on a reasonably diverse range of wild-source red-listed species being present. Preliminary data (MacKay, 2008a, 2008b; Smith, 2009) indicated presence of:

- (i) species that Argent (2006) considered to be “not in cultivation”
- (ii) an extensive range of species
- (iii) wild-source material, and
- (iv) red-list species.

However, these preliminary data were restricted to the Pukeiti collection and three trade sources, and did not cover other known collections or commercial growers in New Zealand. A survey of collections was undertaken in 2009 to determine presence of vireya species in other locations, but that survey had a limited response. Data have been collated on about seven collections; however, data could not be obtained for at least four more collections that are likely to be relevant. For comparison with New Zealand data, the records at Edinburgh Botanic Garden and Botanic Gardens Conservation International were examined, to determine the occurrence of species in overseas collections.

In total, 156 taxa of species or subspecies rank were found in New Zealand. The largest collection was at Pukeiti Gardens, which contains 150 taxa of species or subspecies rank, plus three natural hybrids. (Only 26 vireya species are listed on the Ministry of Primary Industries Plants Biosecurity Index database (http://www1.maf.govt.nz/cgi-bin/bioindex/bioindex.pl; searched 26 May 2012). Using the BGCI database as a measure of international frequency, 128 of the 156 taxa in New Zealand were found in three or fewer collections worldwide (note that this database does not cover the Pukeiti collection), indicating that there are limited collections of vireya worldwide.

The 156 taxa in New Zealand appear to be a significant collection. Of the 385 taxa recorded in total[7], 242 are in cultivation (taxa not listed in either the BGCI database or at Edinburgh were deemed as ‘not in cultivation’). Of the 242 in cultivation, 218 are held at Edinburgh, and the New Zealand collection of 156 appears to be the next largest.[8] In addition, New Zealand collections list several species that are not found at Edinburgh[9], such as *R. javanicum* subsp. *teysmannni* (Fig. 2).

Fig. 2 *Rhododendron javanicum* subsp. *teysmannni.*

Within the 156 taxa present in New Zealand are 66 that are ‘known wild-source’, for example *Rhododendron superbum* (Fig. 3). Material of ‘known wild-source’ is preferable for conservation programmes as it is a genuine representation of the wild type.

[6] In addition to the *Rhododendron* list, assessments have been performed on *Acer* (Gibbs and Chen, 2009), *Quercus* (Oldfield and Eastwood, 2007) and Magnoliaceae (Cicuzza et al., 2007), and these red-lists can be obtained from the BGCI website.

[7] The red-list assessment considered 355 species, but there are about 30 others that were not included in that exercise, making a total of about 385 species of vireya rhododendron.

[8] Using searches of online databases, Dublin Botanic Garden has 74 species, and the Rhododendron Species Foundation (USA) has 63 species. Kew Gardens has only about eight species.

[9] Species/subspecies found in New Zealand collections but not listed in the Edinburgh collection are: *R. asperum*, *R. bloembergenii*, *R. dianthosmum*, *R. inundatum*, *R. javanicum* subsp. *palawanense*, *R. javanicum* subsp. *teysmannii*, *R. pubigermen*, *R. radians* subsp. *pubitubum* and *R. williamsii*.

It is highly likely there is more wild-source material in New Zealand, but plant material is not currently documented as such.

Fig. 3 *Rhododendron superbum.*

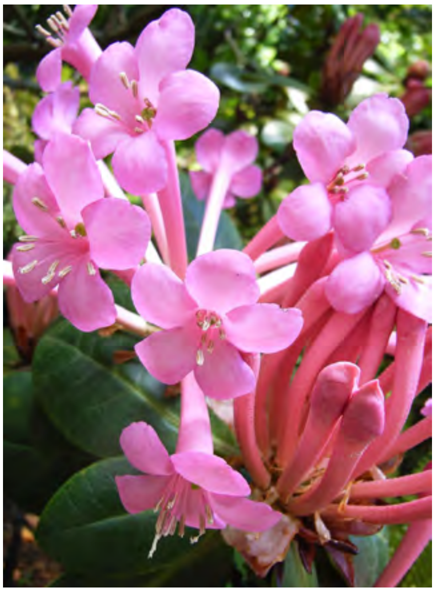

Fig 4 *Rhododendron archboldianum.*

Considering the 161 red-listed taxa only, 67 of them are in cultivation and therefore could be subject to conservation in collections; the remaining 94 have yet to be brought into cultivation. Of the 67 red-listed species in cultivation, 58 are held at Edinburgh while there are 28 in New Zealand[10] (e.g., *R. archboldianum*; Fig. 4). Of those 28, 11 are of 'known wild-source' origin and include *R. arenicola*, *R. bryophilum*, *R. ericoides*, *R. goodenoughii*, *R. leucogigas*, *R. luraluense* and *R. taxifolium*.

These data not only strongly reinforce the important role the Pukeiti collection plays as the primary collection of vireyas in New Zealand, but also provide a more detailed overall description of vireyas in New Zealand. The data also show that many species are represented in collections by a limited number of accessions, usually 1–3, perhaps 5–6. These limited numbers suggest restricted diversity, a problem that has also been observed in other plant collections (Maunder et al., 2001). The survey also highlights the potential perils of privately held collections; in two cases, the collection owner had passed away and the collections were not documented, so those collections may be lost unless extensive field work can be conducted.

Objective Three: Taxonomic complexity and identification of taxonomic issues

A vital difference between *Rhododendron* (particularly subgenus *Vireya*) and many other genera is that conservation is complicated by taxonomic issues. The morphologically based taxonomy is comprehensively described in Argent (2006), where every species (except some recently discovered species) is described in detail. Those descriptions include frequent queries about assignment to subgroups and distinctions between species. Recent molecular research further complicates the complexity by questioning previously accepted relationships among vireya species, and suggests some substantive changes to the taxonomy (Brown et al., 2006a, 2006b, 2006c; Craven et al., 2008; Goetsch et al., 2005). Such an altered view of relationships may have a marked influence on conservation action. For example, *R. jasminiflorum* subsp. *copelandii* (Fig. 5) was red-listed Vu D2 (vulnerable with a very restricted distribution); however, the other *R. jasminiflorum* subspecies were not – if the red-listed subspecies is not substantially different from the others, should the red-list status stand or be amended, and is conservation action necessary? Taxonomic complexity of this nature does not occur in many other genera – hence *Rhododendron* has a particular 'taxonomic dimension' in conservation issues and subsequent management.

Fig. 5 *Rhododendron jasminiflorum* subsp. *copelandii.*

The relationships between red-list species and their near relatives, and the consequent conservation issues, can be examined using molecular methods; however, first the species to be examined must be selected. To make this selection, existing literature was used to collate the known relationships and queries between red-list species and others (Argent, 2000; Argent, 2006; Argent et al., 2007; Cruttwell, 1988; Kores, 1978; Sarawak, 1988a; van Royen, 1984). A 'complexity chart' was used to give a visual representation of the relationships, and from these charts groups of red-list species and their associates were identified for molecular testing. A tabulated segment of this analysis shows the complexity of relationships (Table 2).

From the information in Table 2, the species for testing were selected. For example, *R. jasminiflorum*, its subspecies and the other species that are related to that group should be tested together, such as *R. suaveolens* (Fig. 6A) and *R. edanoi* subsp. *pneumonanthum* (Fig. 6B). Similarly, the difficulty in distinguishing *R. bryophilum* (red-listed) and *R. dielsianum* indicates that accessions of the two species should be compared. In the third example, the possible hybrid *R. archboldianum* (red-listed) and its proposed parents (*R. culminicola* and *R. herzogii*) should be compared, and because *R. inundatum* is closely related to *R. herzogii*, it could be included in this study group. In the final group, there

[10] The 28 red-list species found in New Zealand collections are: *R. abietifolium*, *R. acrophilum*, *R. alborugosum*, *R. album*, *R. archboldianum*, *R. arenicola*, *R. arfakianum*, *R. baconii*, *R. baenitzianum*, *R. bloembergenii*, *R. bryophilum*, *R. dianthosmum*, *R. ericoides*, *R. goodenoughii*, *R. intranervatum*, *R. lamrialianum*, *R. leucogigas*, *R. luraluense*, *R. maxwellii*, *R. mendumiae*, *R. nervulosum*, *R. notiale*, *R. pudorinum*, *R. rhodopus*, *R. rushforthii*, *R. santapaui*, *R. taxifolium* and *R. warianum*.

appears to be a series of relationships between *R. konori*, *R. superbum*, *R. dianthosmum* (red-listed) and *R. hellwigii*, and these species should be considered together. This approach was used to examine the whole subgenus and identify groups of species that were then considered in the next two stages of the project.

Fig. 6 **A**, *Rhododendron suaveolens* and **B**, *Rhododendron edanoi* subsp. *pneumonanthum*; both part of the broad *R. jasminiflorum* group of species.

Objective Four: Molecular studies
Molecular techniques can be used to investigate species diversity and relationships and relate this to conservation issues, and the Peter Skellerup Scholarship award for Plant Conservation contributed to this objective. Having prioritised species for testing, about 340 tissue samples were harvested from four collections and stored at Plant & Food Research in Palmerston North. An additional 18 samples were imported from the Rhododendron Species Foundation in America (kindly funded by the American Rhododendron Association). These samples were tested in several ways.

The first step was to extract DNA from the samples. There was limited prior work on *Rhododendron*, so after some trials in 2008 we found that a modified Kobayashi method (Kobayashi et al., 1998) was the most successful method for DNA extraction. Once DNA was extracted, the samples were then screened with Random Amplified Polymorphic DNA markers (known as RAPD markers), mainly to determine DNA quality, but in some instances the markers provided data which were then used to analyse the relationships among samples. Next, in 2009 and 2010, the samples were screened with 27 microsatellite markers[11]. Eight of those markers provided useful information and were analysed in various ways to extract information about the samples. In the final stages of the work, we sequenced segments of DNA, and about 130 samples were analysed with this method. These three data sets have been combined and discussion of the results is the focus of Ahmed Fayaz's PhD thesis.

In 2011 we were joined for six months by postgraduate student Sujana Reddy, who conducted RAPD and microsatellite screening on a set of 64 samples that had previously failed to give satisfactory results (due to difficulties of extraction from heavily scaly leaves), thus adding to the overall data set. She focused on a set of species from the Phaeovireya, Siphonovireya and Euvireya:solenovireya sections that contain several red-list species and have a series of interesting interconnections and relationships. She was able to obtain useful results for about 55 of those samples and her results will be published in due course.

Objective Five: Examination of herbarium samples
In conjunction with the molecular tests, a set of about 300 herbarium samples have been collected, and these were described, dissected, photographed and scanned. These samples were used to verify that the accession being tested matched its physical description, and to explore some of the physical features of the accessions in relation to the molecular results. For example, Fig. 7A–B shows two images of fresh material and a herbarium sample (Fig. 7C) for *R. superbum*, sample EK616. Fig. 7B clearly shows the key characteristic of this species, an ovary with scales but not hairs, and a style with no scales or hairs.

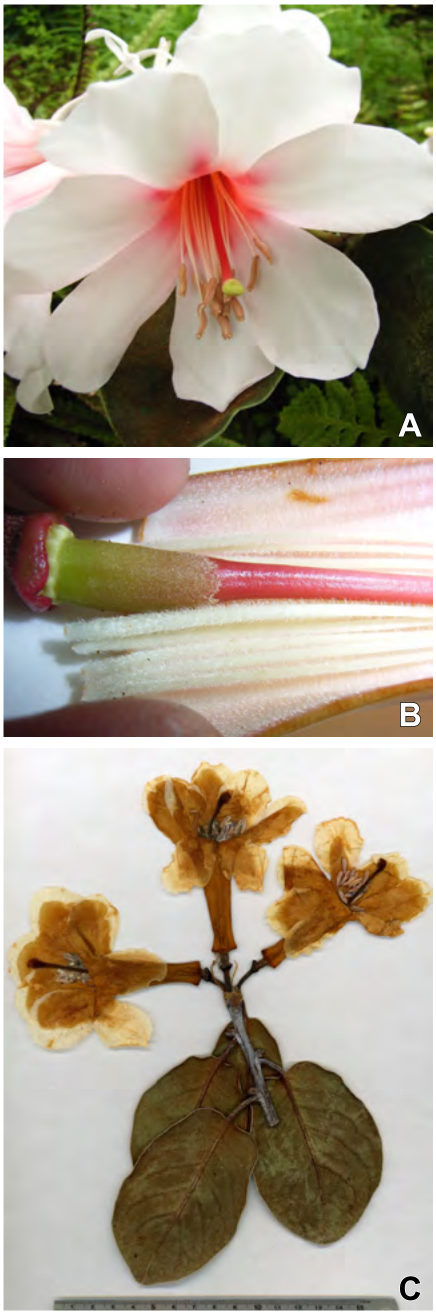

Fig. 7 *Rhododendron superbum* EK616. **A**, flower. **B**, close-up of pistil showing style and ovary. **C**, herbarium sample.

Selected results from the molecular and herbarium studies
With respect to relationships in the *R. jasminiflorum* group, molecular results support the proposition that there are distinct differences between the subspecies that were tested. For the RAPD, microsatellite marker data and DNA sequencing, a distinct difference was exhibited between *R. jasminiflorum* subsp. *jasminiflorum* and *R. jasminiflorum* subsp. *oblongifolium* (Fayaz, unpublished; MacKay et al., 2010). Clear physical differences were also evident in the herbarium samples. These results support the proposal that, for this

[11] Markers kindly provided by Frank Dunemann (Bundesforschungsinstitut für Kulturpflanzen, Dresden, Germany).

species, conservation of subspecies is warranted. In relation to the broader *R. jasminiflorum* group, sequence results support the relationship between that species and *R. ruttenii.* However, they do not support a relationship between *R. jasminiflorum* and *R. suaveolens* and *R. edanoi*, although the latter two appear to be related to each other using these data (Fayaz, unpublished).

With respect to the proposed relationship between *R. archboldianum*, *R. herzogii* and *R. culminicola*, some microsatellite data support a possible relationship between accessions of these three species (Fayaz, unpublished), but unfortunately the sequencing was unsuccessful for this set, so this could not be confirmed. The herbarium study also strongly indicated a relationship between *R. archboldianum* accession HF003 and *R. herzogii* accession EK639.

The relationships among *R. dianthosmum*, *R. superbum*, *R. hellwigii* and *R. konori* appear to be more complex. Unpublished microsatellite data (Fayaz, unpublished; Reddy and Wiedow, 2011) do not clearly separate accessions of *R. konori* and *R. superbum*, and examination of the herbarium samples shows inconsistent variation, suggesting a more variable group than has been previously proposed. In addition, *R. superbum* and *R. hellwigii*, two supposedly closely related species, do not group near each other according to the sequence data (Fayaz, unpublished), suggesting that they are not as closely related as previously proposed. It also transpires that accession EK565 (Fig. 8), labelled as *R. dianthosmum* (a red-list species), cannot be identified as this species by its physical characters but rather appears to be *R. superbum*, and this identification is supported by the microsatellite data. This result shows that this accession will not have any useful role in conservation for *R. dianthosmum*, and resources should be directed elsewhere.

Accessions of *R. bryophilum* (red-listed) and *R. dielsianum* also show some interesting results. Sample HF023 is labelled as *R. dielsianum* but it does not group with the American accession of the same name according to microsatellite or sequence data (Fayaz, unpublished). Examination of the herbarium sample shows that the features of the ovary do not match the description; the sample is neither *R. dielsianum* nor *R. bryophilum,* and is possibly a hybrid. Furthermore, sample EK649, which is labelled as *R. bryophilum*, does not have the hairs on the style that *R. bryophilum* should have, but is completely hairless, and it is likely that this sample is actually *R. dielsianum*, which indicates that accession EK649 is not useful for conservation.

Fig. 8 Accession EK565, which is labelled as *Rhododendron dianthosmum* but identifies as *Rhododendron superbum*.

The molecular work has generated a huge body of data; however, these data are not as complete as the authors would like. Of the 340 samples collected, only about 60% were successfully extracted and resulted in useable DNA. Identifying and resolving this extraction issue will be included in the discussion in Ahmed Fayaz's thesis. Of the samples that did yield DNA, many did not consistently yield results in both microsatellite and sequencing analysis, resulting in data for one set of tests but not the other set. These inconsistencies meant that data were patchy in some areas and that some questions could not be answered. Additional work will be needed to resolve some of these issues and we will continue with these aspects as funding permits.

Objective Six: Contribution to conservation planning

Conservation planning will be facilitated by several aspects of this project. The description of the range and frequency of vireya species in New Zealand, including those accessions that are of wild-source origin, provides a baseline set of data for this resource. The molecular data are generating a range of information on both relationships and diversity, and are revealing instances where New Zealand accessions may be useful for *ex-situ* conservation (as per the *R. jasminiflorum* example above). The herbarium study has identified accessions that are anomalous or of debatable identity, and which should not be used in conservation programmes (e.g., accessions EK565 and EK649). The full range of recommendations will be included in Ahmed Fayaz's thesis at the end of 2012 and further publications will be generated for 2013. Finally, we have developed a protocol for molecular studies that can be applied to other groups in the *Rhododendron* genus.

Conclusion

Ex-situ conservation through plant accessions in cultivated collections is part of a wider approach to plant conservation. Recent international initiatives to make red-list assessments and locate those species in cultivated collections are relevant to New Zealand because of the large resource of cultivated flora that is present in New Zealand (Dawson, 2010), including species of vireya rhododendron. This project has used a range of techniques to investigate vireya rhododendron in New Zealand and in due course, this work will contribute to our understanding of the *Rhododendron* subgenus *Vireya* and its conservation.

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Table 2 Examples of taxonomic queries in relation to red-list vireya rhododendron species and their associates.

Species and red-list category	Queries and associated species
Rhododendron jasminiflorum subsp. *copelandii* Vu D2	Part of the *R. jasminiflorum* complex. No particular relationships noted.
R. jasminiflorum Least Concern*	*R. jasminiflorum* is similar to *R. ruttenii* and *R. edanoi* subsp. *pneumonanthum* (Argent, 2006), and the latter is similar to *R. suaveolens* (Argent et al., 2007). *R. jasminiflorum* is replaced by *R. stapfianum* at higher altitudes in North Borneo (Argent, 2006), and *R. stapfianum* is similar to *R. suaveolens* (Argent et al., 2007). Argent (2000) reported molecular work in which *R. jasminiflorum* grouped with *R. suaveolens* and *R. stapfianum*. The same work showed that *R. jasminiflorum* subsp. *heusseri* was distinct from *R. jasminiflorum*, and this was supported by clear morphological differences (Argent, 2000).
R. archboldianum Data Deficient	Argent (2006) proposes that this species is a hybrid between *R. herzogii* and *R. culminicola*. Danet (2011) indicated that *R. herzogii* forms hybrids with several species, including *R. inundatum*. Stevens (1985) reported that *R. archboldianum* grew with several species, including *R. truncicola* in the south eastern mountains in New Guinea. Cruttwell (1988) noted a hybrid between *R. culminicola* and *R. dielsianum*.
R. dianthosmum Vu D2	Much material in cultivation is not true (Argent, 2006). Grows in the same region as *R. hellwigii*, *R. herzogii* and *R. superbum* (van Royen, 1984).
R. hellwigii Least Concern	Closely related to *R. superbum* and hybridises with that species (Argent, 2006). Kores (1978) observed *R. hellwigii* growing with *R. herzogii* on Mt Bangeta.
R. superbum Least Concern	Closely related to *R. hellwigii* and grows in the same area as *R. dianthosmum* and *R. hellwigii* (van Royen, 1984). Hybridises with *R. hellwigii*, and pink forms of *R. superbum* are likely to be such hybrids (Argent, 2006). Found in the same place as *R. gardenia*, and some plants of that species key to *R. superbum* (Argent, 2006). Has a much narrower geographic range than the closely related *R. konori* (Sarawak, 1988b).
R. konori Least Concern	Very variable in the wild and hybridises with several species. The only difference from *R. superbum* is the lack of hairs on ovary and style (Sarawak, 1988a). Highly variable (Headlam, 1979). Kores (1978) noted *R. konori* growing with *R. dielsianum* and *R. zoelleri*. Hybridises with *R. laetum* and *R. inundatum*, and *R. konori* subsp. *phaeopeplum* hybridises with *R. zoelleri* (Argent, 2006). Hybridises with *R. asperum* in the Arfak mountains and material in New Zealand may be this hybrid (Argent, 2006). Hybrids with *R. herzogii, R. aurigeranum* and *R. zoelleri* recorded (Cruttwell, 1988). A collection with dark red flowers was first thought to be *R. hellwigii*, but later identified as *R. konori* (Headlam, 1979).
R. bryophilum Data Deficient	Not distinct from *R. dielsianum* (Argent, 2006).
R. dielsianum Least Concern	Hybrids recorded between *R. dielsianum* and *R. macgregoriae*, *R. zoelleri*, *R. rarum*, and *R. culminicola* (Cruttwell, 1988). In turn, *R. dielsianum* is closely related to *R. phaeochitum* and *R. beyerinckianum* (Halliday, 1984). Kores (1978) noted *R. dielsianum* growing with *R. konori*, *R. zoelleri*, *R. rarum* and *R. macgregoriae*.

*'Least Concern' is the designation given when there is no conservation issue.