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... an extra *journal* for the plague year....



From the Chair: Gael Donaghy



Kia ora tatou

At the AGM earlier this year (in simpler times!) the committee was asked to look into the funding of native orchid research. We used an on-line discussion tool (Loomio), and seven out of twelve committee members contributed.

Having just read through the first article in this issue ("Wading through a 2-foot-deep river: resolution of the common leek or-

chid" by Jennifer Alderton-Moss and Carlos Lehnebach), I thought about bringing the Loomio discussion to the wider membership. I think this article is a fine example of a student's summer research project of the kind NZNOG might want to have some involvement in.

The discussion started with the request that NZNOG fund research into *Earina* taxa. Because any sponsorship NZNOG makes will be in the region of \$500 - \$1000, and Carlos is the main supervisor of orchid research, it makes sense to be guided by him. He suggested, as a priority, to start with orchids listed by DOC as being in a threatened species category. (See <u>https://www.doc.govt.nz/about-us/</u> <u>science-publications/conservation-publications/nz-threat-</u> <u>classification-system/</u> for orchids with a threat status - mainly *Corybas* species.) He also suggested two categories of student projects that we might like to consider; firstly, MSc student projects

(normally one year of field work) and secondly summer research projects (Dec, Jan, Feb). The latter, worth about \$6000, are supported half by the university and half by Te Papa. Grants could start for example from \$500 and be specified to be used as towards travel to collect or contribute to general lab expenses.

Carlos has three, possibly four, MSc students lined up for next year but he is hopeful that the summer research student projects could be every second year, so he can get a break over summer. The hope is to get students hooked enough for them to want to go further than a summer project, and enrol in Masters or PhD in orchidology.

As to the mechanics of advertising and awarding a grant, this is probably best left to Carlos, as he is in the best position to select the topic and the student. Funding research like this is within the constitution, and we have money in the bank to start the process, so the next step is to decide how much, and communicate our wish to sponsor a student to this amount to Carlos.

Let's hope we have the opportunity to meet face-to-face later in the year! The *Pterostlis trullifo lia* are up and in bud, so I'm hoping we can travel to the Coromandel soon to catch the early kauri orchids.

Enjoy J157 - there is so much to read and absorb in it. Thank you to Ian - this is a great contribution to keep us reading and learning in lock-down.

Original papers

Wading through a 2-foot-deep river, resolution of the common leek orchid

Jennifer Alderton-Moss^{1, 2}, Carlos Lehnebach¹ ¹Museum of New Zealand Te Papa Tongarewa, ²Victoria University of Wellington

Introduction

Prasophyllum colensoi Hook f. is as problematic as wading through a 2-foot-deep river in 1-foot-tall gumboots – that is, it's not an end-of-the-world type problem, it's just incredibly frustrating and leaves your spirits more wounded than anything else. Many people have attempted to understand and explain the variation that we find within this species, but there has been limited success to date.

P. colensoi, the common leek orchid, comes in various colours, shapes and sizes, has tiny upside-down flowers that need a microscope (or at the very least a magnifying glass) to dissect, and can be found throughout the country. Its initial description was only 103 words, and perhaps intentionally vague to encompass all of the variation observed [1].

To add some more confusion, no holotype (type specimen) was mentioned by Hooker in this initial description. Later, Molloy labelled one sheet held at Kew, containing 17 specimens, as the lectotype (Figure 1), and two further sheets as paralectotypes. However, despite references to a "forthcoming publication" featuring this lectotypification [2], we were unable to find this publication, suggesting that this lectotypification may not be valid. The problems here are two-fold: it is unknown if this collection is a valid lectotype and, with the suggestion that *P. colensoi* is actually made up of multiple species, it is unknown if the designated lectotype consists entirely of *P. colensoi*. With this "lectotypification" coming less than thirty years ago, it is no wonder that historically this species has caused much confusion. Now we suggest that we have the opportunity finally to resolve this.

The historic confusion

First described by J.D. Hooker in **1853** following his Antarctic voyage [1], this species has since struggled with taxonomic uncertainty. There have been multiple recurrences of new species being described and segregated from *P. colensoi*.

1885: Only 32 years after *P. colensoi's* initial description, William Colenso described the species "*P. pauciflorum*" from a single specimen collected West of Napier [3]. It was distinguished by its shorter stem, smaller perianth, acute dorsal sepal and more connate lateral sepals than *P. colensoi*. Colenso's new species is currently considered a synonym of *P. colensoi*.

1928 (published 2006): H.B. Matthews manuscript [4], published as part of the NZNOG's *Historical Series*, contained descriptions of



Figure 1: Specimens of *Prasophyllum colensoi* collected by W. Colenso stored at Kew Herbarium and labelled as lectotype by B. Molloy in 1991. Reproduced with permission of the Board of Trustees of the Royal Botanic Gardens, Kew.

two new *Prasophyllum* species. These two "rather slender" species, "*P. patentifolium*" and "*P. debile*", have likely only saved themselves from the status of synonym by not being taxonomically valid to begin with (due to publication without Latin translations or assigned type specimens). They were distinguished from each other and *P. colensoi* by bract shape (ovate v/s ovate-lanceolate v/s blunt), lateral sepals (1/3 fused v/s free), and phenology (flowering Jan-Feb v/s Oct-Nov).

1946: *P. rogersii*, an Australian species described by Rupp in 1928 [5], was suggested by Hatch [6] also to exist in New Zealand. He supported his claim with H. B. Matthews manuscript description of "*P. patentifolium*" and drawings made by H. B. Matthews and H. Carse from fresh material, which appeared to fit *P. rogersii's* description. However, the name *P. rogersii* sensu Hatch, is now considered to be a synonym of *P. colensoi.*

2001: The most recent attempt at sorting species boundaries within *P. colensoi* was the late Bruce Irwin with *Prasophyllum* "A" (Figure 2, above) and "B" (Figure 2, below) [7]. Features he noted as having differences include dorsal sepal (ovate v/s acuminate), floral bract (ovate v/s ovate-acuminate v/s blunt) and labellum length relative to lateral sepals (half v/s 5/6 v/s equal). It is worth noting that his description of *Prasophyllum* "B" has been aligned with Matthew's description of "*P. debile*", supporting the legitimacy of this distinction. This work, however, does not qualify as taxonomically valid descriptions.





Figure 2: Prasophyllum "A" and "B". Photos by Michael Pratt and Eric Scanlen.

Our Project

This summer I was recruited by Carlos Lehnebach (Curator of Botany at Te Papa) to help begin sorting out the common leek orchid, with thanks to a grant from the Australia & Pacific Science Foundation and a summer scholarship from Te Papa and Victoria University of Wellington. Like all good investigations I began in the library. The first line of the first paper on *Prasophyllum* that I read was as follows:

Prasophyllum is one of the most difficult of orchid genera to classify [6].

It is unclear if this was meant as a boast or a warning, but it left a rather unnerving impression on me. Despite the ominous introduction, this initial research helped to identify useful traits from previous taxonomic revisions, or features suggested as distinct in different *P. colensoi* specimens.

Following a note in this journal, several members sent us fresh *Prasophyllum* specimens (all of whom I am incredibly grateful to). We also spent a day in the Remutakas collecting samples, giving me some much-needed practice at spotting wild orchids (late-flowering onion orchids (*Microtis*), various sun orchids (*Thelymitra*) and two different forms of rabbit orchids (*Orthoceras*) – perhaps next year's project?). These, combined with access to Te Papa's herbarium (WELT), gave me all the specimens needed to get to work.

My aim was to investigate the physical and genetic features of this orchid. For the first portion I measured 25 features of 92 samples (22 fresh and 70 from the herbarium). For the genetics portion, we extracted DNA from 18 samples, of which 7 samples so far have had two regions of their DNA sequenced (*trnL-F* and ITS).

The preliminary results from the morphological analysis showed that specimens could be split into distinct groups based on the shape of the dorsal sepal. For example, a strongly acuminate dorsal sepal correlates with generally longer flower parts. Other features, such as plant colour, proved less useful.

Likewise, some grouping was seen in the preliminary results from the genetic analysis (Figure 3), although more genetic work is required.

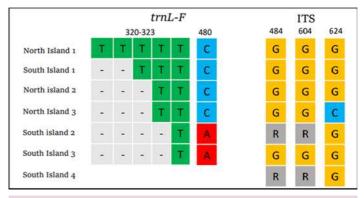


Figure 3: Differences in the chloroplast DNA and nuclear ITS sequences observed across North Island and South Island samples of *P. colensoi*.

Implications

Although this study is far from complete, the preliminary results of the investigation suggest that there may be two distinct groups within *Prasophyllum colensoi*. Further research is required to confirm this,

and our next move will be looking at more DNA sequences from a larger number of individuals. This study suggests the need for a revision of the conservation status of *P. colensoi* and segregated taxa as well. Taxonomy is imperative as a precursor to effective conservation. Without an understanding of the biodiversity that exists, prioritising conservation efforts is imprecise at best and realistically leaves many threatened taxa at risk of extinction. It is unclear if the suspected species within *P. colensoi* differ in population size and distribution, but this study has been another vital step in the long journey to understand our native orchids.

Acknowledge ments

This research was supported by the Australia & Pacific Science Foundation (grant APSF 19047) to Carlos Lehnebach and a Summer Research Scholarship from Te Papa and Victoria University of Wellington to Jennifer Alderton-Moss. We would also like to thank staff from AK, CHR, and WELT herbaria, the National Library of New Zealand, Andre Schuiteman from Kew for image permission and Ian St George for providing valuable literature.

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Thelymitra pulchella

By Bill Kosky, Melbourne.

Introduction

I have been following with interest discussion of New Zealand *Thelymitra* (sun) orchids in recent editions of *New Zealand Native Orchid Journal*. I was particularly fascinated by discussion of those forming part of what Jeff Jeanes (Jeanes, 2012) calls the *Thelymitra venosa* complex, in particular *T. pulchella* (Hook.) and those L.B. Moore included as its synonyms, viz: *T. concinna* (Col.), *T. fimbriata* (Col.), *T. pachyphylla* (Cheeseman) and *T. caesia* (Petrie).

It is useful to divide this complex into

(a) **Thelymitra cyanea** types: species with loose, relatively wellspaced, long, narrowly obovate tepals and a noticeably larger truncate (round ended) apron like labellum (often curled in), all, when fully open, thin/loose textured flat to obscurely concave with a short acute tip. The loose margins often wavy (undulate) and/or folded in, or under. Column arms (lateral lobes) longish, ends usually twisted in. Bifold tipped anther beak (middle lobe) protruding beyond column arms. *T. venosa* (R.Br.) and *T. cyanea* (Lind.) Benth.); and

(b) **Thelymitra erosa types:** species with overlapping, neatly arranged, shorter broadly obovate tepals and an only moderately larger labellum, all rigid concave (3-dimentionally spoon shaped), margins entire, with a short acute tip. Entire or emarginate anther beak more or less same length as column arms. *T. erosa* (Jones & Clem.), *T. incurva* (Jeanes), *T. alpicola* (Jeanes), and the similar New Zealand plants/species referred to below which in this instance it is useful to refer to collectively as **T. aff. erosa** NZ.



Fig.1: T. cyanea Rowan

Fig.2: *T. erosa* Arthur River, Tasmania

I am familiar with the Tasmanian endemic *Thelymitra erosa* and its Australian mainland relatives *T. alpicola* and *T. incurva*, ditto *T. cyanea* but only know the NSW endemic *T. venosa* by descriptions, specimens, and photographs.

Looking at the photos attributed to *Thelymitra pulchella* in *NOJ* 151 & 153 and photos sent to me by Kevin Matthews is like stepping into a parallel universe where some of



the orchids are much the same as Australian *T. erosa* types, and others, whilst bearing an uncanny resemblance to Australian species, are perhaps not quite the same. I have prepared some notes outlining my take on this subject that might be of interest to your readers.

For the record I should state I have a very limited knowledge of New Zealand sun orchids. That my interpretations of the various descriptions, specimens, and photos referred are to some extent speculative hypotheses, rather than based on any field, or scientific, studies.

The real difficulty faced by St George (2019) and others in trying to sort out New Zealand species in the *Thelymitra venosa* complex is that the most important diagnostic traits, such as column details, are not apparent on dried/pressed specimens. Further, in the past, specimens collected in the field, and sent to others for description, deteriorated by the time they arrived. Interpreting dried/pressed type and other *Thelymitra* specimens often involves reverse engineering. That is, fresh specimens used to aid interpretation of dried specimens—Jeanes (2012).

Hooker's description of Thelymitra pulchella

 Thelymitra *pulchella*, Hook. fil.; caule gracili 3-5-floro, folio anguste lineari, perianthii foliolis late obovatis acutis, labello obcuneato, staminodiis erectis ultra columnam porrectis apice fimbriatis dentatisve.

HAB. Northern and Middle Islands, Colenso. Otago, Lyall. Nat. name, "Maikaika," Lyall.

Tubers small, with long fibres at the base of the stem, on which other tubers are formed. Stem slender, 1 foot high. Leaf very narrow. Flowers glaucous, large, pale purple, very handsome; sepals and petals obovate, acute. Lip broadly obovate, truncate, or wedge-shaped. Column shorter than the erect toothed or fimbriate staminodia.— This is a handsome and very distinct form in the structure and length of the staminodia; I have fifteen very good specimens, but it is probable that it will be found to pass into T. Forsteri, through the following.





Fig.3: detail of holotype *T. pulchella* (K000827532) flowerheads with loose flowers.Fig.4: detail of upper right of sheet: open flower showing large labellum.Fig.5: detail of lower right: drawing of the column.

The type collection of *T. pulchella* comprises 4 specimens on one sheet: 2 specimens, Northern Island, Colenso, (K000827532) denoted (a) and includes a drawing of a column; 2 specimens Middle Island, Lyall (K000827531); and a second syntype comprising 4 specimens on a separate sheet (K000827533) also Middle Island, Lyall.

The note to the column drawing is *"Staminodia nuda, dentata"* (column arms bare, toothed). St George (2019) notes Hooker's description described the column arms as toothed <u>or fimbriate</u>. The latter descriptor odd because in no

way can the column drawing be described as depicting fimbriate column arms. St George attributes this addition to the subsequent interpretation of the Lyall specimens from Middle Island with fimbriate column arms, adding "Joseph Hooker saw them as a single species".

Mark Clements selected the Colenso collection denoted (a) including the drawing as the holotype. These specimens, in my opinion, are closest to Hooker's description of the petals, sepals and labellum. The texture of these is rather loosely structured with the labellum considerably larger than the tepals (similar to Thelymitra cyanea) as distinct from the overlapping rigid tepals, and only moderately larger labellum, seen on T. erosa. Hooker's description does not mention striped flowers and striping, as distinct from some veining, is not readily apparent on the holotype specimens. Whilst in some respects the holotype specimens may be likened to T. cvanea, the column illustration does not have the long, usually curled in, lateral lobes/arms of T. cvanea.

Clements denoted the Lyall collections as syntypes. These have shorter rather rigid overlapping striped flowers with a labellum on most only moderately larger than the tepals, and thus more like *T. erosa.* As noted above St George suggests these specimens had fimbriate column arms.



My interpretation based on Clements' selected holotype means that I consider Hooker's type collection comprises: the Colenso collection as described above with toothed column arms = T. *pulchella*; the other, the Lyall collection, as described, particularly if they had fimbriate column arms, may well be T. aff. *erosa* NZ but this conclusion is speculative given the lack of diagnostic detail exhibited by the specimens.

David Jones and Mark Clements (*AOR* 1998) in their description of *Thelymitra erosa* say that "an examination of the *T. pulchella type* specimens shows clearly that this species has [1] <u>striped flowers</u>, [2] <u>a greatly enlarged labellum</u> (similar to that of *T. cyanea and T. veno-*sa) and [3] <u>relatively short... column arms with deeply fringed or</u> <u>lobed margins.</u>" They say [4] <u>some NZ specimens and photos are</u> <u>closer to T. erosa</u>, and suggest that, [5] <u>at least 3 distinct entities</u> <u>shelter under the name T. pulchella</u>

[1] <u>striped flowers</u> are quite visible on the syntype flowers, but striping as distinct from some veining is not readily apparent on the holotype specimens. Hooker's description of the flowers as "*large, pale purple, very handsome*" doesn't mention stripes, which is a little odd. Even so, sometimes the distinction between what is indistinctly striped, or lightly veined, is not readily apparent on dried specimens.

[2] The greatly enlarged labellum (similar to that of T. cyanea and T. <u>venosa</u>) is as Hooker described *it "broadly ovate, truncate or wedge shaped*". The sepals and petals narrowly obovate, acute as seen on the enlarged holotype flower in Fig.4 above. This seems to be the principal trait by which Jones & Clements distinguished T. pulchella from T. erosa.

[3] The holotype drawing of the <u>column</u> does not show the particularly <u>deeply fringed or lobed margins</u>, as suggested by Jones & Clements, rather margins that are irregularly toothed. It is noted that

the lateral lobes in this drawing are quite different from the longer lateral lobes shown on the column drawing of *T. cyanea* on its holo-type sheet (below).

8 anthera opercularis incumbro apin triloba

Fig.8: Column drawing of *T. cyanea* holotype.

[4] some NZ specimens and photos are closer to T. erosa. I agree.

[5] *at least 3 distinct entities shelter under the name T. pulchaella*. Certainly, the name has been applied to a variety of forms of *Thelymitra*—see St George I. (2019), *NOJ* 156.

Based on my interpretation of the holotype specimens, I am of the opinion that *T. pulchella* can be distinguished from *T. erosa* and *T.* aff. *erosa* NZ by its relatively narrow almost naked irregularly notched column arms (more or less as drawn), its open flowers, relatively narrower tepals, a larger (relative to tepals) labellum, flat to slightly concave tepals and labellum, the texture of all thin/loose, more like *T. cyanea* and *T. venosa*. And from *T. cyanea* by its shorter lateral lobes more like *T. erosa*,

Does this indicate *T. pulchella* might be a hybrid, or of hybrid origin, that is, *T. cyanea* X *T. erosa* type? If that were so, it's odd that there are no Australian records of *T.cyanea* X *T. erosa* hybrids, as they often grow together.

The photo Kevin Matthews sent me as *Thelymitra pulchella*, Spain Road Wetland 17.11.10 (at right) might be a good match for *T. pulchella* based on my interpretation of Hooker's description, and the designated holotype. It follows that if my interpretation is correct the orchids in the photos on pages 21 & 22 of *NOJ* 156 are not *T. pulchella*.



Australian species in the Thelymitra venosa complex

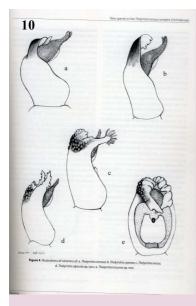


Fig.10: a, b T. cyanea types: a T. venosa; b T. cyanea; c, d, e T. erosa types: c T. erosa; d T. alpicola; e T. incurva. Note the large protruding bilobed tipped beak (anther) on a & b greatly reduced on c, d, & e. Illustration ex Jeanes (2012).

Fig.11: T. venosa endemic to NSW (Blue Mountains and nearby coastal areas). 25–70cm tall Flowers dark blue with darker blue longitudinal veining (rarely white or pink) opening freely in cold weather often remaining open at night. Lateral lobes (column arms) spirally twisted. Fig.12: T. cyanea, only faint veining, Mt Baw Baw, Vic, 1350m alt; in Australia morphology consistent across its wide range. Grows with T. alpicola, T. erosa and T. incurva but no hybrids with these or other Thelymitra species have, to my knowledge, been recorded. Widespread S-E Australia, Tasmania & NZ. In Australia in damp bogs, swampy plains and drainage lines, plentiful at higher altitudes, uncommon to rare lower altitudes. 15-30 (rarely to 55) cm tall. Flowers light to mid blue often with pink or purple hues, occasionally pink. Like striped flowers of T.

erosa types the dorsal sepals and petals usually with darker blue longitudinal striping; striping on lateral sepals slightly reduced; striping on the labellum often reduced to absent. Occasionally flowers with reduced or no striping. Lateral lobes incurved, most (but not all) with rolled in spirally twisted tips forming a partial or complete circle.

Jeanes (2012) described both *T. venosa* and *T. cyanea* as having a very short post anther lobe covered with beady glands. These glands are usually not visible but are on a NZ

photo of a pinkish purple flowered *T. cyanea* sent to me by Kevin Matthews. Some of Kevin's NZ *T. cyanea* photos have some lateral lobes without a spiral twist, which is not very common in Australia. Even so, the photos of NZ *T. cyanea* I have seen are a good match for Australian examples.





Australian Thelymitra erosa types compared to T. aff. erosa NZ

All my *Thelymitra alpicola* photo collection with stripes, all my *T.incurva* without, otherwise both have a reasonably consistent morphology. Both are rare, with disjunct populations. *T. erosa*, can be locally very common. Its populations contain a mix of plain and striped flowers, and lateral lobes (column arms) that are quite variable, but usually more or less with finger like ends. *T.* aff. *erosa* NZ

also appears to be quite variable, viz: with and without striping; the form of lateral lobes narrow shortly/sparsely toothed, to broad branching with fine filamentary tips; and the extent of the post anther lobe rudimentary to partially hooded, some with a central split; many in the following photo comparison difficult to distinguish from *T. erosa*. In that regard Moore is quoted as describing the column arms of plants she included in *T. pulchella* as "variously elaborated within one population of plants, …".



Fig.15: *T. incurva* Little River Swamp, E. Gippsland, Vic. 90m alt Fig.16: NZ Photo K. Matthews as *T. pulchella* var. *sansfrimbria* stripeless scented Gumfields 24.11.10 Fig.16a: Enlargement of Ahipara Gumfields as *T. pulchella* above. Post anther lobe partly hooded? Fig.17: *T. incurva* Wingan River, E. Gippsland, Vic. 90m alt



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Fig.22 , 23: both *T. erosa* Strahan, Tas.

Fig.24: NZ Photo K. Matthews as *T. pulchella* var. *sansfrimbria* stripeless scented Gumfields 24.11.10. Note branched lateral lobe.

Fig.25: *T. alpicola* Victorian High Country with well developed, split? post anther lobe.

Fig.26: *T. incurva*. E. Gippsland, near coastal grasstree plains









These are almost all the Tasmanian *T. erosa* plants on Colin and Mischa's web page. All with different column detail, and a mix of plain and striped flowers.



ie r.

C&MR ©2009 RetiredAussies.Com

Conclusions

- 1. My hypothesis is that *T. pulchella* has traits that distinguish it from what I have referred to as *T*. aff. *erosa* NZ. My hypothesis relies on both Hooker's description (and the column detail drawing) of *Thelymitra pulchella*, and Clements' selection of its holotype. It is an hypothesis that is difficult to prove, or disprove, given Hooker's short description and the lack of essential detail on the dried specimens in the holotype and syntypes.
 - a. If my hypothesis is correct, then it means that *T*. aff. *erosa* NZ needs to be named as one or more new species, or an appropriate existing name (or names), such as *T*. *fimbriata*, or even *T*. *erosa*, established as applicable.
 - b. If my hypothesis is wrong then prima facie T. aff. erosa NZ = T. pulchella subject to any subsequent synonyms being elevated to species level (or new species being identified) and perhaps a renewed need to distinguish T. erosa from T. pulchella.
- 2. The photos and descriptions included here establish that *T. erosa* in Tasmania is a plentiful, wide spread and variable species. That New Zealand plants I have included here as *T.* aff. *erosa* NZ share many of *T. erosa's* morphological characteristics and variability. This would seem to preclude the theory that *T.* aff. *erosa* NZ is a *T. longifolia* X *T. cyanea* hybrid, because *T. longifolia* does not occur in Tasmania.
- 3. The nub of the problem faced by St George and others in trying to identify and match plants seen in the field to named species, is that specimens relied on lack important morphological detail, early descriptions are short, subsequent descriptions often contradictory, and terminology may have different meanings for

different persons. Such an approach can only be taken so far, and cannot by its nature, produce a satisfactory result.

4. What is needed is for the historical descriptions to be put to one side to allow a fresh taxonomic study to take place based on fresh specimens, and the establishment of spirit and photo reference collections.

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Unearthing hidden alliances and unfaithful partners – exploring the diversity of mycorrhizal interactions across a selected group of New Zealand orchids.

By Carlos A. Lehnebach and Lara D. Shepherd. Museum of New Zealand Te Papa Tongarewa Email: <u>CarlosL@tepapa.govt.nz</u>

Orchids have extraordinary flowers. The diversity of shapes, sizes, colours, and aroma they exhibit seem endless. When people come across an orchid in full flower it is common to hear expressions of wonder or disbelief. These are usually follow by comments such as "it's beautiful" or "what a weird flower". After these striking flowers are pollinated, however, our fascination and interest slowly fades away. Over a few months, their amazing flowers have turned into an unremarkable green fruit. Technically, these fruits are a *capsule* but they are commonly referred to as a *pod* (a term which usually refers to the fruit produced by plants in the legume family, such as beans, peas, kōwhai or kākā beak).

Despite the simple external appearance of orchid fruits, they are as fascinating as the flowers. For example, the inside of *Earina autumnalis* capsules is covered by hundreds of coiled hairs (*elaters*). These hairs are humiditysensitive and they violently uncurl and twist when the air is dry. This quick and erratic movement aids seed dispersal because the elaters catapult the seeds out of the fruit. Some orchid fruits are also of great economic importance. Vanilla, which provides the flavour to many people's favourite ice -cream, is ranked as the world's second most expensive spice. Around 80% of the vanilla in the global market is produced in Madagascar, where the vanilla industry is worth over NZD 300 million.

On the other hand, apart from orchid growers, not many people have seen orchid seeds. But if you have, their minute size makes it impossible to appreciate their microscopic features (Figure 1).

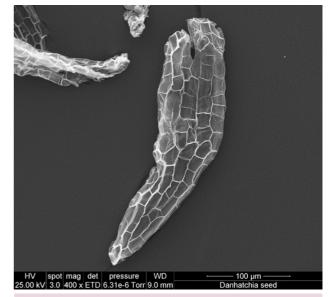
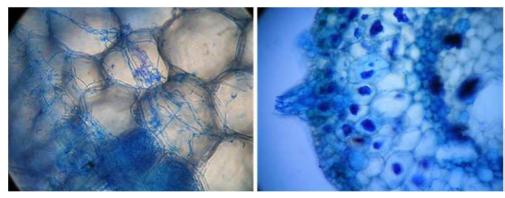


Fig.1: Seed of Danhatchia australis. This photograph was taken using a scanning electron microscope (SEM). The scale bar is 100 μ m; that is 0.1 millimetres.

Orchid seeds are very small and they have a dust-like appearance. Their size ranges from 0.2–0.75 mm wide x 0.3–5mm long (Arditti 1992, Dressler 1993). Their tiny size means thousands of seeds can be packed inside a single capsule. Seed numbers per capsule range from several hundred thousand to up to 4 million! When Charles Darwin was writing his book on the pollination of orchids (available from the Biodiversity Heritage Library, <u>https://doi.org/10.5962/bhl.title.37883</u>) he made a back-of-the-envelope calculation based on the number of seeds produced by a single plant of *Orchis maculata* (now *Dactylorrhiza maculata*). He concluded that if nearly all seeds from all capsules led to viable plants, the great-grandchildren of this plant would nearly cover the entire land area of the Earth! Impressive? Yes! But there's a catch. Unlike other plants, orchid seeds do not contain food resources for the embryo to feed from. Instead, orchids have packed their seeds with air. In fact, between 70–90% of the orchid seed consists of air. This helps with dispersal over long distances, for instance across the Tasman Sea (*ca.* 2,000 km).

Mycorrhiza; a plant-fungus alliance

Once orchid seeds reach their final destination, germination will only occur if the right species of fungus is present at its landing site. The fungus will help the embryo with the absorption of nutrients and minerals from the soil. The two organisms form an association (a *symbiosis*) that may last



for the entire life of the plant. This fungal-plant partnership occurs in most plants, and because it happens in the roots it has been termed *mycorrhiza* (from Greek, mýkēs: fungus, and rhiza: root). The term mycorrhiza was coined in 1855 by Albert Bernhard Frank (1839 –1900), a German botanist. Frank was commissioned to develop practical methods for truffle cultivation by the King of Prussia (Wilhelm I). Although Frank's truffle project was not successful, it helped him to understand how mycorrhizal interactions work (Arditti 1992).

There are two major types of mycorrhiza, *endomycorrhiza* (the fungus gets inside the cells of the roots and forms tree-like structures or vesicles inside them) and *ectomycorrhiza* (the fungus surrounds the root and creates a mantle around it, but it does not get inside the cells). Orchids have endomycorrhiza and the fungal "filaments" (*hyphae*) form coils inside the cells of the root (**Figure 2**).

Fig.2: Fungal hyphae (left) and pelotons (right) inside cells of the roots of *Microtis* sp. A blue stain has been added to the preparation to make the mycorrhiza visible.

These coils are commonly referred to as *pelotons*. Although the presence of pelotons inside young orchid seedlings (*protocorms*) had been observed in the mid-1800s their importance in seed germination was not fully understood until the French botanist Noël Bernard found the roots of seedlings of the Bird's-nest orchid (*Neottia nidus-avis*) filled with fungal pelotons. He published his discovery and observations in 1899 (here is a link to his work <u>http://isyeb.mnhn.fr/sites/isyeb/files/documents/tradbernard2017.pdf</u>) and concluded that mycorrhizae are essential for orchid seeds to germinate.

Pairing old-time buddies in the lab

Bernard's observations were the basis for the development of future orchid propagation protocols, including those seed germination techniques now used across the world to assist with the conservation of threatened orchids. Unlike in many other countries, there has been limited research into orchid-fungus interactions in New Zealand (NZ). Mycorrhizal associates have been investigated in only a few terrestrial orchids (Campbell 1962, 1982; Watkins 2012; Frericks 2014). Additionally, only one published study reports successful germination of a terrestrial orchid ex situ (in the lab) (Frericks et al. 2018). This study focused on the NZ ladies' tresses Spiranthes australis (previously known as S. novae-zelandiae), a species currently ranked "At Risk - Declining". Frericks et al. (2018) used mycorrhizal pelotons extracted from the lateral roots of an adult plant from a wild population to inoculate (infect) seeds collected from the same population (Figure 3). Because this method uses the orchid fungal partner to achieve germination it is termed symbiotic germination. Alternatively asymbiotic germination protocols can be used to germinate orchid seeds too. With this method the orchid fungal partner is not needed and the seed's fungal requirement is bypassed by adding a number of chemicals to the culture medium. Although this method is sometimes easier and more successful than symbiotic germination, excluding the mycorrhizal partner will limit many future conservation efforts. For example, if we want to reintroduce a species back to the wild or supplement/reinforce a small aging population, first we must confirm the right fungal partner is present at the site. This will not only help plants to establish

but also assist with germination and future recruitment. Nowadays, we can also "bank" the fungus in liquid nitrogen for years. So, both seeds and fungal partner can be stored as back-up. Knowing the identity of an orchid mycorrhizal partner is critical mostly because the interaction has been described as species-specific. This means it is a "one-to-one relationship" where only one particular species of fungus is capable of germinating the seeds of a specific species of orchid.



Fig.3: Close up view of an 81 day old seedling (protocorm) of *Spiranthes australis* growing under lab conditions. Seeds were inoculated with a *Tullasnella* fungus extracted from the roots of an adult plant from a wild population. (Photo by Jonathan Frericks).

Because we know so little about orchid-mycorrhizal interactions in NZ, attempts to propagate our threatened orchids for conservation purposes have been hindered. This is particularly alarming if we consider that more than 35% of our orchids are of conservation concern and, in extreme cases, some species are restricted to a single site with only a few hundred individuals. Other species, on the other hand, are widespread, abundant and co-exist with one or more different orchid species. Recent studies overseas have shown that coexisting orchid species have distinct mycorrhizal associates (Jacquemyn et al. 2014; Esposito et al. 2016). However, a contrasting scenario, where plants from mixed-species populations exhibit low specificity for fungal symbionts, has also been reported (Pellegrino et al. 2016). Here competition among orchid species is avoided by having a rather opportunistic approach to fungal partnerships. Whether similar patterns of mycorrhizal associations occur in NZ orchids is unknown.

Unearthing hidden alliances

During the last two years we have been investigating the diversity of mycorrhizal fungi associated with a group of terrestrial and epiphytic orchids growing at a conservation area near Wellington, in the southern North Island of New Zealand. More than 20 species of orchids are found at this site, mostly under black and hard beech forest. They represent seven orchid genera; two epiphytic (*Drymoanthus* and *Earina*) and seven terrestrial (*Acianthus, Caladenia, Corybas, Cyrtostylis, Gastrodia, Pterostylis* and *Thelymitra*). At this site, different species of orchid are found growing in close proximity, sometimes only a few centimetres apart, forming mixed-species populations.

To identify the fungal partner used by these orchids and find out whether they all have specialised to a distinct fungal partner we used DNA analyses. We collected lateral roots and stems of 12 species and confirmed the presence of pelotons by examining them under the microscope. DNA was extracted from the tissue containing the pelotons at Te Papa's genetics lab. For each orchid sample we made copies of a region of fungal DNA that is typically used for identifying fungal strains. These DNA copies were then sent to Massey University for sequencing. After processing our results we were able to identify the main fungal companions of these forest orchids. They belong to the families Ceratobasidiaceae, Mycenaceae, Physalacriaceae, Tullasnelaceae, Russulaceae, Sebacinaceae, and Serendepitaceae. The families Mycenaceae and Serendepitaceae were also detected in samples of Gastrodia and Microtis (respectively) growing in nearby street plantings. Overall, our results are quite exciting and show different scenarios. Interestingly, in some cases, these match with the ecology of the orchid in question. For instance, our data suggest that different species of orchids growing side by side form partnerships with different species of fungi, possibly to avoid competition (see Figure 4, Site 1 and Site 2). However, this does not apply to all the orchids we studied, and widespread and common species such as Thelymitra longifolia and Pterostylis alobula, rely on fungus of the same family (Ceratobasidiaceae). Second, not all orchid species are loyal to their fungal partner, and Gastrodia cunninghamii for instance, associates with fungus from three different families at three different sites; Sebacinaceae (genus Sebacina), Physalacriaceae (genus Armillaria) (Figure 4) and Mycenaceae (genus Mycena) (graph not shown). While others, such as *Caladenia chlorostyla*, are loyal to their partners (Figure 4, Sites 1 and 2, top). Third, the fungal community within the orchid root seems to change over time. A good example here is P. alobula in Figure 4, Site 3 or T. longifolia (Sites 1 and 2 v/s Site 3). The diversity of fungal families is higher in flowering/fruiting plants than in nonfertile plants. It is likely that the roots of these older plants are already in the process of rotting and this has promoted the colonisation by other nonmycorrhizal fungi. Alternatively, it could be that older plants had a longer time to be colonised by other fungi.

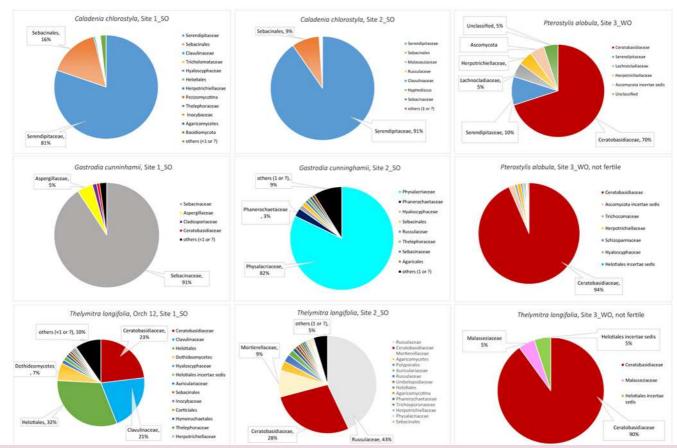


Fig.4: Fungal diversity found in plants of Caladenia chlorostyla, Gastrodia cunnighamii, Thelymitra longifolia (fertile and not fertile) and Pterostylis alobula (fertile and not fertile) at three different sites within the conservation area where this study was conducted.

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Findings from our study are a valuable contribution to the knowledge of orchid-mycorrhizal relationships in NZ. It has provided a point of reference for us to anticipate, based on NZ data, which group of fungi are likely found in other NZ orchids. But most importantly, our results will assist us with the implementation of propagation protocols and future orchid restoration projects. For instance, in the lab, this knowledge can guide us to adjust the culture medium to suit the specific requirements of some fungi. In the field, this new knowledge will help us to select the best plants to isolate fungal pelotons from or the most suitable site where to reintroduce a species. Site selection could be done by screening the soil for the right fungus using DNA analyses or by looking at what other orchid species is/are present at the site (and based on this avoid seedlings competing for the same fungal partner).

Our research on NZ orchids and their mycorrhizal partners is still far from complete. Our future projects should explore the distribution of orchid-mycorrhizal fungi in the soil and focus on the isolation, culture and long term storage of those fungi capable of stimulating seed germination and supporting seedling development. We are also interested in exploring orchid-mycorrhizal specificity in sub-alpine habitats and urban settings.

Acknowledgments

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BLACKWI

Pterostylis banksii

Laing and Blackwell's Plants of New Zealand,

Photo by Ellen or Frank Blackwell.

Fig. 32 (p.119) of the first edition of 1906.

A classic....

The inbox





letter from David McConachie,

They say that a picture is worth a thousand words, and the researchers at the Lankester Gardens in Costa Rica have put this into practice with the Lankester Composite Dissection (or Digital) Plate – LCDP. This is the digital equivalent of the Botanical Plate of yore.

They have taken advantage of the increasing availability of reasonable priced cameras with improved optics to take sharp macro (0.1x - 1x) range photos. They also developed a protocol for compiling the various elements photographed consistently.

The elements range from a whole growth photo, the flower and its various parts that are separated out as the flower is dissected. As well as being a work of art in its own right, an LCDP is visually informative. One online publication to embrace the use of LCDPs is "Species Orchidacearum" found at <u>https://</u> www.speciesorchidacearum.org/

LCDPs have several advantages over black and white illustrations and coloured drawings. The size, shape and colour of elements are more accurately and less subjectively displayed. Creating LCDPs of our orchids may make it easier to assess variation both between species and within species. This could help clarify the status of our tagnamed plants.

Just before the Lockdown I received a PDF from Adam Karremans from the Gardens explaining how to create a LCDP. It is in Spanish, but I managed to get the text translated. If you would like to have a go at creating your own LCDPs, please email me davpmac@gmail.com and I will send you a copy of both the pdf and translation.

David

The illustration at right is Vanilla costaricensis, from Adam Karremans, Lankester Botanical Garden, University of Costa Rica—Ed.



letter from East Gippsland ...

In East Gippsland all the T. pauciflora complex and a few other thelys are very tardy opening. Typically they open when there is a hot humid spell last week of October to first week of November lasting 1–3 days. The event is signalled by all the white ants flying the day before.

We have to run around and visit as many sites as we can fit in before they are all pollinated and collapse.

One year they were only open for about 2 hours on one day. The day was warm, humid and overcast. The clouds opened up, the sun came out and it became hot for 2 hours during which the orchids (mostly T. pauciflora, T. holmesii and T. brevifolia) opened. Then the clouds closed and a cool breeze came in. I couldn't believe it. During that short period hundreds of what appeared to be tiny native bees bombarded the open orchids, they were so quick. Next day all the T. pauciflora types were laxly open, which their fertilised flowers do before collapsing. These species are said to be self-pollinating (I don't doubt they can be) but from what I saw it only takes an hour of suitable conditions per year for all of them to be insect pollinated. Miss that and one might assume they never open before being fertilised.

The other phenomena with some of these is that they open in the morning and close by mid-day. My theory is that most are near coastal. The mornings still and warm, by midday a sea breeze comes in and most of the insects seek shelter in bushes or nooks and crannies of buildings. What adaptation: orchids that may open for just a few hours a year to be pollinated; and others that open in the mornings to best catch their pollinators' presence. In both cases they only open at the most opportune time to catch the flight of their insect pollinators.

Regards, Bill Kosky.

evin Matthews agreed,

Your observation of native bees visiting Thelymitra on what I call "the perfect Thelymitra window" in NZ is a case in point. This occurs rarely in the Far North of NZ when all the weather Gods have aligned. This gives our NZ Thelymitra an opportunity for allogamy cross pollination and therefore diversity in the genetics; otherwise our Thelymitra would fall back continually on autonomous self pollination. The one exception is our T. pulchella which does open freely and is therefore open to regular allogamy cross pollination. This in my opinion gives rise to the many variations within the T. pulchella complex.

nd another letter, from Melbourne...

I have the pleasant tasks of reading the Bulletins from other groups for our society. Your NZ bulletin is the most interesting to me.

Occasionally I pass a copy to somebody who I believe has an interest in the information contained. In this case it received an enthusiastic response.

I grow some thelymitra and also grow them, with permit, from seed for reintroduction in local council areas to supplement current populations or restart populations.

If I wish to collect seed from my nursery plants, I have to cover them with stocking before they flower, and then hand pollinate before they self. If not, they are heavily cross pollinated between species. And we have enough difficulty with species identification already.

Thank you for your most interesting journal and the keen observations of your contributors.

Good health. Richard Thomson.

o the editor from Georgina Upson,

In your "Mistaken identity" editorial you mention that Colenso said C. minor was "common in the North" but New Zealand is warmer now and the distribution of C. minor is now more extensive than in his time. I do not believe that Colenso had ever encountered C. minor (Hook) in the Northland area

Colenso sent both spirit, Bottle 7 Owae, and pressed specimens, no. 409 Tamateatai, from Northland. These, bar the questionable Lectotype of C. minor ostensibly sent by Edgerly, were all C. alata. No other Caladenia specimens were sent by Colenso from Northland. His specimen no.1945 from Castlepoint in Hawkes Bay for which Colenso proposed the name "glandulosus" is described by him as "very near the Bay of Islands specie". Colenso at the very least, implies that these plants were a different sp. from the northern ones hence the proposed name. This is supported by his statement when describing C. variegata, unless he had totally forgotten this collection in the intervening years. His "glandulosus" has been identified as a match for C. minor (at least not C. alata).

When Colenso described C. variegata many years later he noted, "C. minor which is so common at the north on clayey open hills among fern and leptospermum scrub (a quote from the Flora), I have never met with in these Southern parts". In saying this Colenso is of course referring to the Owae and Tamateatai C. alata he was familiar with in the belief that these were C. minor for which he had voiced his annoyance that he was not recognised in the name and claimed to have given specimens of to Dieffenbach. There is nothing in Colenso's letter 15 March 1839 to Cunningham that suggests he saw any of the Edgerly specimens Richard Day was accompanying. They would have been packed and stowed for travel. Colenso would not know whether they were common or not and if common and he had encountered them he would certainly have sent specimens to Kew himself. If Colenso had been aware of more than one species in the North he would have been asking Hooker what the other species was. The distribution of these orchids has not changed substantially since, with C. alata still confined to northern North Island.

—Georgina Upson

The photo on the next page is in Te Papa's collection. It is attributed to Henry Matthews, and titled "Three men at a campfire; 1917–1924". Kevin Matthews identified the men: "Harry Carse is centre with Henry Blencoe ("Blen") Matthews on the right. And the young fella is Henry's son Richard Blencoe Matthews (bom 1904)." Carse was a lifelong friend of the Matthews family.

Henry Blencoe "Blen" Matthews (1861–1934: think *Thelymitra matthewsii*) was four years younger than Harry Carse (1857–1930: think *Corybas carsei*) and they explored the Far North together, as well as the Central Volcanic Plateau. Eric Godley's biography tells us, "Blen joined Carse, and told Cheeseman on 5 February, 1920: 'Mr Carse and I were away 5 weeks. —We spent two nights on Hauhangatahi and a week at the "Haunted Whare", the balance of the time having been spent on the Waimarino Plains, Erua, and Raurimu'." This photograph was probably taken then.

Blen (at right) took the photo with a longish exposure in the evening light, movement causing some blurring; he is the only one paying attention (perhaps he was taking too long and the others' attention had wandered) and, having triggered the camera timer (I cant see a cable release), has dashed to an easily accessible seat. Sixtythree year old Carse is toying disconsolately with his watercrackers and sardines (the empty sardine tin is on the table); the kerosene tin with their dinner is steaming on the fire, the billy is ready to go on after the meal-judging by the cups, tea for two (Unity Tea, advertised in NZ newspapers from 1922) and "coffee" for one (Strang's Coffee & Chickory). Richard has the basin to do the dishes (his fist is clenched). There is a pipe on the table ready for an after-dinner smoke-perhaps Blen's, as that may be a packet of tobacco in his right weskit pocket; adjacent are gold-rimmed spectacles and a specimen jar that appears to contain an upper denture. The frying pan stands behind Blen's left leg. A candle in a tin by the fire will give them a little light later (sunset is at 2030 here in mid-December).

Nearby they found what Blen would name in manuscript, *Corybas* "aestivalis" (aff. *oblongus*), *Prasophyllum* "debile" (*P*. aff. *colensoi*), *Pterostylis* "pulchragalea" (possibly *P. speciosa* Col.), *P.* "rotundigalea" (*P. patens* Col.) and *Thelymitra* "carsei" (*T. form osa* Col.). He neglected Colenso's work on orchids, as Cheeseman did. G eorgina Upson emailed about *Gastrodia cooperae*, "Here are some photos showing the colour range to be found in *G. cooperae*. As you can see these are the same as for *G. cunning-hamii* demonstrating their close relationship. Plants can reach

over 90cm in height so take a closer look when out and about, *G. cooperae* may well be being overlooked. ►

"As a curiosity here is a 'twin' flower seen on a stem: the other flowers were normal." ▼







ssst! wanna read some sexy stuff about NZ native orchids? go to <u>https://www.nzgeo.com/stories/orchidelirium/?</u> utm_source=Facebook&utm_medium=Post&utm_campaign=Gre at_reads_orchideliriumCryptic&fbclid=IwAR3ztVrgDU4X0Qhn mYOBqq3cM59ji6tWDHG10dWZJPjhYM4sL4ZEY5FVu14 The unstriped *Thelymitra pulchella* photographed by Kathy Warburton and shown on the cover of this issue of the *journal*, raises the issue of stripes. Interestingly (as Bill Kosky notes in his paper) neither Hooker nor Cheeseman mentioned stripes in their formal descriptions. Hooker's original description was of pale mauve flowers sent by Colenso from Northland and others by Lyall from Otago.

C ontrol-click on <u>https://openresearch-repository.anu.edu.au/</u> <u>bitstream/1885/114724/2/b11729831.pdf</u> for a lovely book on the orchids of Papua New Guinea.

G o to <u>https://www.rbg.vic.gov.au/news/conserving-the-queen-of-sheba?fbclid=IwAR1HGiyqC240BSFLOf2HRC92jY0ue-PLZ-DgliTaMVuK5E3LgBmmVGo-XVs</u> to see the work being done to grow (and thus conserve) the Queen of Sheba orchid, *Thelymitra variegata*—close to our *T. matthewsii*.



A classic paper

Edwin Daniel Hatch, in Army training at Waiouru during WWII, was cycling about and finding orchids that didn't match the available descriptions, so he sent some to the Orchid Man, the Rev Herman Montague Rucker Riipp in Australia (bio at http://adb.anu.edu.au/biography/rupp-herman-montaguenucker-8298). Their collaboration was marked by the publication in 1945 of the jointly authored paper "Relation of the orchid flora of Australia to that of New Zealand" (read it in full at https://www.biodiversitylibrary.org/ page/34953404#page/113/mode/lup). Their "possible explanations" of the close relationships between the florae are still interesting...

... The hypothesis of a primeval antarctic continent... explains much, but it certainly does not explain everything. It provides a rational explanation for the development of such genera as Thelvmitra and Pterostvlis along similar lines in both countries; and if certain species were in process of being evolved into their present forms when the Palaeozelandic continent was broken up, no one can assert that there was anything in the new conditions to prevent the continuance of their development into identical forms. But if ancestral forms of Caladenia were also included in the Palaeozelandic orchid flora, why did they develop with such remarkable richness of colouring and great variety of form on the Australian side, and so poorly on the New Zealand side? Take the case of another genus-Chiloglottis. A common ancestral form in the ancient continent might well develop into C. comuta in New Zealand, and into C. Gunnii, C. Muelleri, and perhaps C. Pescottiana in Australia; their close affinity is obvious. But we cannot believe that in the far north of New Zealand they would evolve so different a form as C. formicifera, completely identical with a relatively rare New South Wales species. We have much to learn yet. This particular species is of special interest. In Australia it occurs nowhere in great abundance, but in considerable "colonies", from the Hunter River on the north to the Shoalhaven River on the south. It inhabits well-shaded forest gullies. How did it reach New Zealand? Was the seed carried across the 1,200 miles of the Tasman Sea by dust-storms? We know that in times of drought very considerable amounts of Australian dust are occasionally deposited on New Zealand: and orchid seeds are extremely minute. But is it likely that seeds of a dwarf terrestrial orchid from forest gullies of the coastal belt would be caught up and transported by a dust-storm from the dry interior? Another suggestion is that the seeds might have become attached to the feathers of migratory birds. They might; and that is all we can say at present. We do not deny that both birds and dust-storms may have been responsible for the appearance in New Zealand of certain orchid species. But is it not remarkable that, although New South Wales has nearly thirty species of Diuris on record, not a single species of this genus has been seen in New Zealand? Many species produce seeds guite freely, and some grow in almost any type of country. Or take the genus Cymbidium. The North Island of New Zealand should afford conditions suitable at least for C. canaliculatum and C. suave. Both produce immense quantities of fine, dust-like seeds, easily carried by wind from their arboreal homes. Yet neither occurs in New Zealand. Moreover, if birds and winds have transported orchid seeds successfully across the Tasman Sea, are they doing it still? We do not pretend to answer these questions, but we think they should be faced, and patient research and study will no doubt in time be rewarded by glimpses of the truth. As yet there is no complete explanation of the relation between the two orchid floras. They are explained in part if we accept the theory of the Palaeozelandic continent, for that allows us to believe in an inflow of allied ancestral forms both from north and south; in part perhaps by the agencies of birds and winds; more than this we cannot say. The distribution of orchids may not seem a subject of great importance in itself. But it must be remembered that the Orchidaceae now rank as the largest family of flowering plants; and, in the words of the late Dr. R. S. Rogers, facile princeps among Australasian orchidologists, "It is obviously desirable that such a matter as their distribution should be established as accurately and as early as possible. On it may depend, to some extent, the solution of much greater questions concerning the former disposition of land-masses, the origin of our flora, and the true relation of our continent" (and, we may add, of New Zealand) "to other portions of the globe". (Trans. Roy. Soc. S. Aust., xlvii, 1923, p. 322.)