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**A Taxonomic Review of *Corybas rivularis* (Orchidaceae) – Inferred from
Molecular and Morphological Analyses**

A thesis submitted in partial fulfilment
of the requirements for the degree
of
Master of Science in Biological Sciences

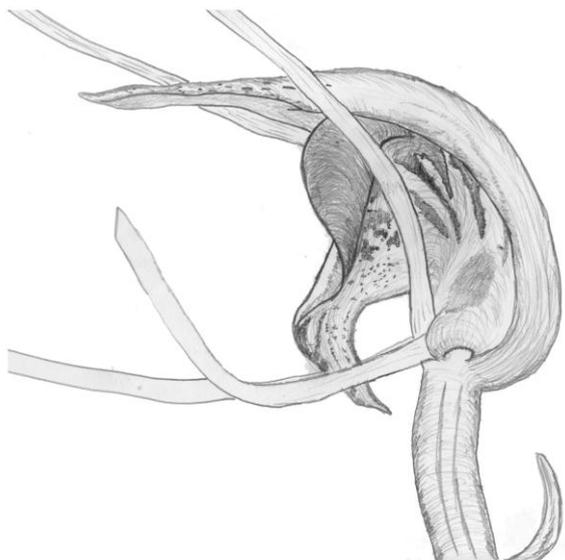
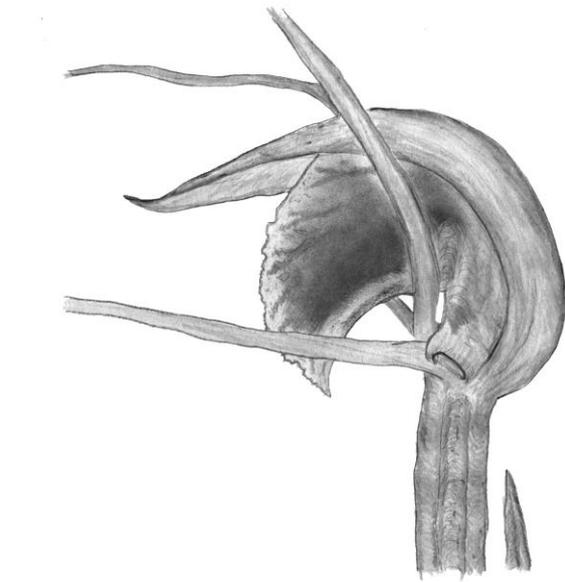
at
The University of Waikato

by
Abraham John Coffin



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Drawings of *Corybas rivularis* (top), *C. "kaimai"* (middle) and *C. "whiskers"* (bottom).

Illustrated by Abraham Coffin.

Abstract

This research has expanded the level of precision utilised in critically examining the morphology of *Corybas rivularis* Rchb.f (Orchidaceae), related species and undescribed populations. *Corybas rivularis* and related species have undergone taxonomic revisions, incorporating errors that took decades to discover. Utilising morphological and molecular analyses has provided insights into this problematic group. A new protocol for examining the morphological characteristics of *C. rivularis* has been developed, based on concepts of floral morphometrics, to determine the level of morphological variation within the species, closely related species and a range of undescribed populations, some of which have tag-names. The use of morphological techniques with multivariate statistics has not been previously used in this group.

A suite of precisely defined continuous characters is established, which are relevant to the four species *C. rivularis*, *C. iridescens* Irwin & Molloy, *C. orbiculatus* (Colenso) L.B.Moore and *C. papa* Molloy & Irwin, and five undescribed tag-named populations *C.* ‘veil’, *C.* ‘whiskers’, *C.* ‘kaimai’, *C.* ‘kaitarakihi’ and *C.* ‘pollok’, and a distance matrix collating all of the respective characters for each sample is generated. A Linear Discriminant Analysis (LDA) found the characters most capable of discriminating natural groups to be three aspects of the flower labellum; the length and width of the labellum bib, and the length of a furrow in the labellum formed from a developmental pinching of the labellum tube, which leads to the column. The LDA, along with a cluster analysis (UPGMA) allowed all species and tag-named populations studied to be determined as distinctive, except for two; *C. aff. rivularis* (AK251833; Kaitarakihi) and *C.* ‘veil’, which together form a distinct group. There are two morphological syndromes present in the studied species and tag-named populations; One group, allied to *Corybas iridescens*, tends to have a long and wide bib, with a small furrow and petiolate leaf, the other group, allied to *C. rivularis sensu stricto* tending to have a narrow and short bib, with a long furrow and sessile leaf.

Sequence variation of the nuclear ribosomal Internal Transcribed Spacer (ITS) region was used to reconstruct relationships. Consistent with other studies (Clements *et al.* 2007), many samples shared identical sequences. *C. iridescens*,

and tag-named populations *C. aff. rivularis* (AK251833; Kaitarakihi) and *C. “veil”* formed a highly supported clade. *Corybas rivularis* from the far North has variation from all other species and tag-named populations. The tag-named populations regarded as *C. aff. rivularis* (CHR 518313 “whiskers”), *C. aff. rivularis* (CHR 518025; Kaimai) and *C. “pollok”* shared identical sequences. Consistent with the morphological findings, there are two highly supported monophyletic groups present in the plants studied; one composed of *C. rivularis* and allied species, and another group composed of *C. iridescens* and allied species.

The evidence suggests a taxonomic revision is warranted however further research into this group is still required to further delimit species boundaries. Any taxonomic revision undertaken will have ramifications for conservation, both the threat classification status of some species, and the conservation management strategies.

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Table of Contents

Abstract	i
Acknowledgements	iii
Table of Contents	v
List of Figures	vii
List of Tables	xi
1 Chapter One: Thesis introduction	1
1.1 Research objectives.....	1
1.2 Thesis outline	2
References.....	4
2 Chapter Two: A literature review of <i>Corybas rivularis</i> (A.Cunn.) Rchb.f.; The natural history, taxonomy and ecology.	5
2.1 Natural history of New Zealand	5
2.2 Orchids of New Zealand	6
2.2.1 <i>Corybas</i>	8
2.2.1.1 <i>Corybas rivularis</i>	11
2.2.1.1.1 Taxonomy of <i>Corybas rivularis</i>	13
2.2.1.1.2 Natural history of <i>Corybas rivularis</i>	15
2.2.1.1.3 Ecology of <i>Corybas</i>	16
2.3 Conclusions	17
References.....	18
3 Chapter Three: A molecular and morphological analysis of <i>Corybas rivularis</i> (A.Cunn.) Rchb.f. (Orchidaceae), including tag-named entities and related species.	23
3.1 Abstract	23
3.2 Introduction	25
3.3 Materials and methods	31
3.3.1 Species and tag-named populations studied	31
3.3.2 Sampling methods.....	37
3.3.2.1 Morphology	37

3.3.2.2 DNA analyses	40
3.3.3 Analysis of data.....	42
3.3.3.1 Analysis of morphology data	42
3.3.3.2 Analysis of molecular data.....	42
3.4 Results	43
3.4.3 Results of morphological analysis.....	43
3.4.4 Results from phylogenetic analysis of ITS molecular sequences	46
3.5 Discussion	48
3.5.3 Morphological analysis of continuous characters	48
3.5.4 Molecular analysis of ITS sequences	51
3.6 Conclusions	52
References	54
4 Chapter Four: Synthesis	59
4.3 Conclusions	59
4.4 Recommendations.....	60
Appendix	63

List of Figures

- Figure 2.1; A bathymetric depiction of contemporary submerged continent of Zealandia outlined in red, composed of New Zealand and New Caledonia (Stagpoole 2002). 6
- Figure 2.2; Rough distribution (green) of *Corybas* across Asia, South East Asia, Papua New Guinea, Australia, New Zealand and the Pacific. Reproduced with the permission of the Trustees of the Royal Botanic Gardens, Kew (WCSP 2014). 8
- Figure 2.3; A cladogram of based on ITS sequences of *Corybas* sub-gen. *Corysanthes* sec. *Nematoceras*, demonstrating three clades within the monophyletic section. Numbers above branches indicate number of base pair changes supporting each node, numbers below branches are bootstrap support (Clements *et al.* 2007). 10
- Figure 2.4; Representatives of each of the three clades demonstrated by Clements *et al.* 2007 that compose *Corybas* sub-gen. *Corysanthes* sec. *Nematoceras sensu* Lyon 2014. A) *Corybas acuminatus* from Pirongia Forest Park, Waikato B) *Corybas* aff. *trilobus* from Pirongia Forest Park, Waikato, part of the “macranthum” clade C) *Corybas rivularis* from Puketia Forest Park, Northland, part of the ‘rivulare’ clade. 11
- Figure 2.5; *Corybas rivularis sensu stricto* and five tag-named populations of *Corybas* with affinities to *C. rivularis*; A) *C. rivularis* s.s from Puketia Forest Park, Northland. B) *C.* “pollok” *ex situ*, from near Pollok, Awhitu Peninsula. C) *C.* “kaimai” at Kauaeranga Valley, Coromandel Forest Park. D) *C.* “whiskers” at Pirongia Forest Park, Waikato. E) *C.* aff. *rivularis* “kaitarakihi” at Kaitarakihi Bay, Huia. F) *C.* “veil” at Waireinga/Bridal Veil Falls, Waikato. 12
- Figure 2.6; A dendrogram of recognised species of *Corybas* sub-gen. *Corysanthes* sect. *Nematoceras* with estimated dates of divergence (orange squares), with estimated ranges (blue bars), based on multiple regions of molecular variation (Lyon 2014). 15
- Figure 3.1; A) *Corybas cheesemanii* (Hook.f ex Kirk) Kuntze from Te Kauri Forest Park, Waikato. Arrows are pointing to closed labellum spurs. B) *Corybas* “whiskers” from Pirongia Forest Park. Open labellum auricle with arrows showing width of the aperture. 26
- Figure 3.2; A rough distribution (green) of *Corybas* across Asia, South-East Asia, Papua New Guinea, Australia, New Zealand and the Pacific. Reproduced with the permission of the Trustees of the Royal Botanic Gardens, Kew (WCSP 2014). 26
- Figure 3.3; Frontal view (top row) and side-on view (second row) of *Corybas rivularis*, A, F; *C.* “kaimai”, B,G; *C.* “whiskers”, C, H; *C.* “pollok”, D, I and *C. papa*, E, J. Frontal view (third row) and side view (bottom row) of *Corybas iridescens* K,O; *C.* “veil”, L,P; *C.*

“kaitarakihi”, M,Q and *C. orbiculatus*, N,R. The upper 1cm scale applies to images A-J, the lower 1cm scale applies to images K-R. ..32

- Figure 3.4; A map of the North Island of New Zealand, with colour coded labels for each of the populations included in this study.34
- Figure 3.5; Habitats and sample sites of *Corybas rivularis*, *C. “kaitarakihi”* and *C. “pollok”*; A) Habitat of *Corybas rivularis* at Puketi Forest, Northland. B) Study site of *C. rivularis*. C) Habitat of *C. “kaitarakihi”* at Kaitarakihi Bay, Huia. D) Study site of *C. “kaitarakihi”*. E) Habitat of *C. “pollok”* near Pollok on the Awhitu Peninsula. F) Study site of *C. “pollok”*. White arrows indicate location of colony.35
- Figure 3.6; Habitats and sample sites of *Corybas iridescens*, *C. “whiskers”*, *C. “kaimai”* and *C. “veil”*; A) Habitat of *C. iridescens* and *C. “whiskers”* in the lowlands of Pirongia Forest Park, Waikato. B) Study site of *C. iridescens* and *C. “whiskers”*. C) Study site and habitat of *C. “kaimai”* at Kauaeranga Valley, Coromandel Peninsula. D) Study site and habitat of *C. “veil”* at Waireinga/Bridal Veil Falls, Waikato. White arrows indicate location of colony.36
- Figure 3.7; A) Side view of longitudinal section of *C. “pollok”* from near Pollok, Awhitu Peninsula, with lateral sepals and petals excised: 1) Labellum furrow, 2) Labellum wing tube, 3) Labellum wing-bib transition, 4) Column chamber, 5) Inner flexure, 6) Outer flexure, 7) Auricle. B) Ovary and column of *C. “pollok”*: 8) Column length, 9) Column width.37
- Figure 3.8; A) Close frontal view of *C. “veil”*: 1) Dorsal sepal depth, 2) Dorsal sepal width, 3) Labellum furrow width, 4) Labellum furrow height, 5) Labellum width, 6) Labellum bib length. B) Profile of *C. “whiskers”* flower: 7) Flower height, 8) Labellum wing height, 9) Flower length to dorsal sepal, 10) Ovary length, 11) First floral bract length, 12) Second floral bract length. C) Frontal view of *C. iridescens*: 13) Lateral sepal length, 14) Lateral petal length, 15) Leaf lamina width, 16) Leaf lamina length. D) Side view of *C. iridescens*: 17) Flower length to labellum, 18) Flower peduncle length, 19) Leaf petiole length.....38
- Figure 3.9; Linear Discriminant Analysis plot of all samples using 9 characters; Labellum Width, Furrow Height, Bib Length, Leaf Length, Leaf Width, Lateral Sepal, Lateral Petal, Second Bract and Flower Petiole Length. Or = *C. orbiculatus*. Ir = *C. iridescens*. Kait = *C. “kaitarakihi”*. V = *C. “veil”*. R = *C. rivularis*. K = *C. “kaimai”*. W = *C. “whiskers”*. P = *C. papa*. Poll = *C. “pollok”*.....44
- Figure 3.10; A bar graph of the means, with standard deviations of the labellum furrow height, labellum width and bib length for the all studied entities and species. All measurements are in millimetres. n = sample sizes.44

Figure 3.11; UPGMA diagram of all *Corybas* species and entities studied using three characters: Labellum width, Bib length and Furrow height. Or = *C. orbiculatus*. Ir = *C. iridescens*. Kait = *C. “kaitarakihi”*. V = *C. “veil”*. R = *C. rivularis*. K = *C. “kaimai”*. W = *C. “whiskers”*. P = *C. papa*. Poll = *C. “pollok”*..... 45

Figure 3.12; A dendrogram of 36 samples of sect. *Nematoceras*, including species and tag-named populations, based on a BEAST analysis of ITS sequences. Node labels show posterior probabilities. Drawings aside samples depict morphological differences. 47

List of Tables

Table 2.1; A summary of the taxonomy for each of the members of <i>Corybas</i> sub-gen. <i>Corysanthes</i> sect. <i>Nematoceras</i> sensu Lyon 2014 (syn. <i>Nematoceras</i>).....	14
Table 3.1; A summary of the taxonomy for each of the members of <i>Corybas</i> sub-gen. <i>Corysanthes</i> sect. <i>Nematoceras</i> sensu Lyon 2014 (syn. <i>Nematoceras</i>).....	30
Table 3.2; A summary of the study site locations, basic morphological and habitat descriptions for each of the studied species and tag-named populations.	33
Table 3.3; A summary of character state averages (above) with minimum and maximum for each respective entity (in brackets below). Sample sizes (n) are the total for each of the entities, including those from multiple populations.	39
Table 3.4; A summary of the 36 samples used in the molecular analysis of ITS sequences. * These samples have provenance outside of New Zealand territory	41
Table 3.5; Summarised morphological differences observed between all species and tag-named populations studied in this research.	48

Chapter One: Thesis introduction

Corybas rivularis (A.Cunn.) Rchb.f (Orchidaceae) is a species with a number of closely related tag-named populations, some of which are recognised by New Zealand botanists (de Lange *et al* 2012). A complicated taxonomic history, and lack of a holistic and comprehensive study, has hindered the progress of resolving these forms, some of which have been purported to be distinctive for nearly a century (Scanlen 2010). Exactly which of the various populations was the type of *Corybas rivularis sensu stricto* was unknown, and a whole other species was considered to be *C. rivularis* for nearly a century (Clements & Hatch 1985). In addition to these quandaries, the generic ranking of New Zealand *Corybas* has undergone similar upheaval (Hooker, 1853, Hooker 1864, Cheeseman 1906, Jones *et al.* 2002), with many species being named within the genus *Nematoceras*. This has led to a suite of species without valid combinations under new classifications until recently (Lehnebach 2016). The threat classification status for these undescribed populations is difficult to establish without a formal assessment of their respective diversity (Mace 2004). *Corybas* “whiskers” and *C.* “kaimai” are classified as Data Deficient and *C.* “kaitarakihi” is Critically Endangered (de Lange *et al.* 2012, Townsend *et al.* 2008). The next step in resolving these issues is a close examination of the morphology, ecology, distribution and genetic variation. The era in which intuition with a brief morphological description was sufficient to describe a species is coming to an end, and a quantitative approach to resolving these taxa is necessary (Chase *et al.* 2015).

1.1 Research objectives

The core aim of this research was to examine the variation in the morphology and genetics of *Corybas rivularis* and closely related yet undescribed populations such that species can be accurately delimited and an appropriate classification developed. The specific questions I asked were; do *Corybas rivularis* and the undescribed populations constitute a single species as defined by morphological characters and DNA sequence variation? Are the tag-named populations indistinct from one another, or are they defined as distinct by morphological characters and DNA sequence variation?

These questions were addressed by:

1. Examining the history of the literature of the group, and current delimitations, including areas with outstanding issues.
2. Perform a detailed morphological analysis to assess if consistent informative characteristics are present.
3. Perform a molecular analysis to determine if variation exists and/or determine the phylogeny of the group.

1.2 Thesis outline

Chapter One: Thesis Introduction.

This chapter serves to provide an outline for the thesis content and research objectives.

Chapter Two: A literature review of *Corybas rivularis* (A.Cunn.) Rchb.f.; The natural history, taxonomy and ecology.

This chapter examines the natural history of the group, its origin, recent radiation, taxonomy, biogeography and phylogeny. Also examined are the historical species delimitations including those currently accepted and the tag-named populations with affinities to *Corybas rivularis*. This will provide a good overview of the current level of understanding of the group and highlight areas of future research that will be required.

Chapter Three: A molecular and morphological analysis of *Corybas rivularis* (A.Cunn.) Rchb.f. (Orchidaceae), including tag-named entities and related species.

This chapter examines the distinctiveness of *Corybas rivularis*, related species and undescribed tag-named populations using continuous morphological characters and multivariate statistical analyses, in addition to a molecular analysis of the Internal Transcribed Spacer (ITS) region. The morphological analysis seeks to determine if there are any consistent informative characteristics among the undescribed populations, and to test the characteristics used to describe currently accepted species to see if they applicable to a broader range of their respective subordinate entities.

Chapter Four: Synthesis.

This chapter will draw on all the findings from the research and discuss the potential impact and implications they will have, and recommend areas where future studies are still wanting.

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Chapter Two: A literature review of *Corybas rivularis* (A.Cunn.) Rchb.f.; The natural history, taxonomy and ecology.

2.1 Natural history of New Zealand

New Zealand lies around 1500 kilometres to the southeast of Australia. It is an archipelago that consists of many islands, with two large islands oriented north to south, a few relatively large islands, including Stewart Island, and numerous smaller islands. The islands of New Zealand range between latitudes 29°S and 53°S, giving New Zealand a temperate climate with the surrounding ocean providing a stabilising influence in inter-seasonal temperature variation. The Southern Hemisphere Westerly Winds contribute to the climate of New Zealand (Anderson *et al.* 2009), and have played a role in the long distance dispersal of plants and animals (Sanmartin & Ronquist 2004).

New Zealand forms part of the now mostly submerged continent Zealandia (Fig. 2.1), which was likely completely submerged during the late Oligocene (Landis *et al.* 2008). With land above water for at least the last 22 million years, New Zealand has an established flora that has many endemic species with at around 1735 (~78%), although few endemic genera with around 53 genera (Breitwieser *et al.* 2012).

Some suggest an entirely long-distance dispersal origin for the New Zealand flora (Pole 1994). There is a prevalence of small white flowers, dioecism and unspecialised pollination syndromes (Lloyd 1985). Non-endemic species and genera are more likely to be highly dispersible plants with small spores such as ferns, fern allies, or small seeds such as wetland species and orchids. This is likely due to selective immigration of species via long distance dispersal. (McGlone *et al.* 2001).

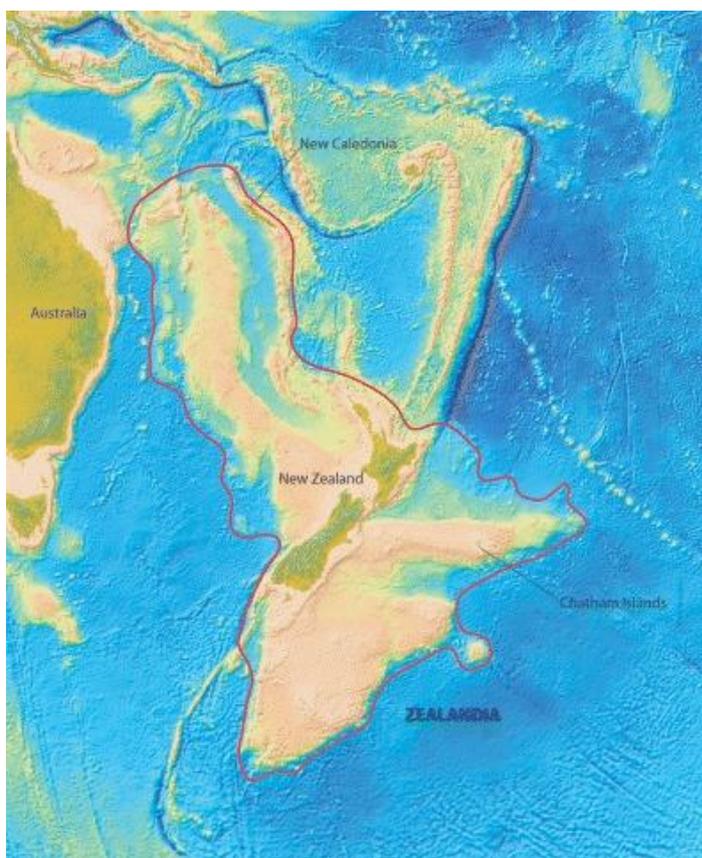


Figure 2.1; A bathymetric depiction of contemporary submerged continent of Zealandia outlined in red, composed of New Zealand and New Caldedonia (Stagpoole 2002).

2.2 Orchids of New Zealand

Orchidaceae Juss. comprises one of the world’s largest plant families with over twenty-five thousand species, and an innumerable number of hybrids and cultivars (Pridgeon *et al.* 2005). They have an almost cosmopolitan distribution, comprise the majority of known vascular epiphytes (Zotz 2013) and have peculiar floral characteristics including bilateral symmetry and highly modified sexual structure called a gynandrium, or column, and a modified petal called the labellum (Johnson & Edwards 2000).

Orchidaceae is comprised of five sub-families; Apostasioideae Horan., Cyripedioideae Kosteletzky., Vanilloideae (Lindley.) Szlachekto., Epidenroideae Kosteletzky. and Orchidoideae Eaton. (Angiosperm Phylogeny Group 2016). Although exact phylogeny has been difficult to resolve, Apostasioideae is a basal subfamily in which the column is improperly fused and still has three anthers present, making them triandrous (Kocyan *et al* 2004). It is represented by only a handful of species in two genera. Cyripedioideae has a relatively primitive

morphology, although molecular research indicates it is closer to monandrous orchids. This sub-family is commonly known as ‘slipper orchids’, and they possess two fertile anthers making them biandrous. Vanilloideae and the remaining two subfamilies Epidendroideae and Orchidoideae all possess only one fertile anther and are collectively known as the ‘monandrous orchids’. New Zealand has no representatives of Apostasioideae, Cyripedioideae or Vanilloideae.

Epidendroideae is the largest subfamily of orchids. Almost all are epiphytic, and with over 21 thousand species it is larger than all of the other sub-families combined (Govaerts *et al.* 2014). All of the epiphytic orchids of New Zealand belong to Epidendroideae, including one genus of terrestrial orchid *Gastrodia* R.Br. The sub-family Orchidoideae Lindl. is represented in New Zealand by nearly all species of terrestrial orchids, with the exception of *Gastrodia*. Members of this sub-family are characterised by a terrestrial habit and a tendency for producing subterranean storage organs, such as root tubers, as opposed to pseudobulbs.

Members of the Orchidaceae have been arriving in New Zealand for many millions of years, with fossil leaves dated to 23-20 million years before present representing early members of the largely epiphytic genera *Earina* Lindl, and *Dendrobium* Sw. respectively (Conran *et al.* 2009). Many of New Zealand orchids have arrived more recently, with some possibly arriving in the last million years from Australia (Lyon 2014). New Zealand has around 110 species of orchid, with 9 epiphytic species and 101 terrestrial species (de Lange *et al.* 2012).

All terrestrial species of orchid in New Zealand, except for members of *Gastrodia* belong to Orchidaceae sub-fam. Orchidoideae and the majority belong to the tribe Diurideae (Endl.) Lindl. ex Meisn. Diurideae in New Zealand is represented by 66 species across 17 genera in 8 sub-tribes, including sub-tribe Acianthinae Schltr. In New Zealand, Acianthinae has 21 species in 4 genera. One of these genera, *Corybas* Salisb., is represented globally by around 132 species, of which 17 are native to New Zealand (WCSP 2014).

2.2.1 *Corybas*

Corybas is a small terrestrial orchid and is recognised by its single, typically orbicular-cordate fleshy leaf, and a large single-flower that has a modified labellum with winged lobes and incurved margins that form a tube. The base margin of the labellum has protrusions that are either open, and are called auricles, or are closed and are called spurs. Plants do not form pseudobulbs but forms root tubers that act as a store over the dry or cold season, re-emerging the following season (Salisbury 1805, Edgar & Moore 1970, Lyon 2014).

Corybas is widely distributed, from Asia, South East Asia, Papua New Guinea, Australia, New Zealand and Pacific islands (Figure 2.2). Lyon's (2014) molecular studies suggest that the genus has an estimated crown age of about 15MYA, and has dispersed to New Zealand from Australia a number of times; the first was the ancestor of *Corybas oblongus* Hook.f. around 12.5MYA, followed by *C. cryptanthus* Hatch. 9MYA, followed by the common ancestor of *Corybas rivularis*, and related species 8MYA. There have also been four long distance dispersal events to New Zealand in the last 1MY (Lyon 2014).



Figure 2.2; Rough distribution (green) of *Corybas* across Asia, South East Asia, Papua New Guinea, Australia, New Zealand and the Pacific. Reproduced with the permission of the Trustees of the Royal Botanic Gardens, Kew (WCSP 2014).

Recent taxonomic research of *Corybas* suggests that five closely related genera should be amalgamated: *Nematoceras* (Hook.f) D.L.Jones & M.A.Clem., *Corysanthes* R.Br., *Singularybas* D.L.Jones & M.A.Clem., *Molloybas* D.L.Jones & M.A.Clem. and *Anzybas* D.L.Jones & M.A.Clem. (Lyon 2014). These genera were split from *Corybas* (Jones *et al.* 2002), but later rejected by Kew and various international and Australian institutions (Govaerts *et al.* 2003, Entwisle & Weston 2005, Chase *et al.* 2015). The genera are suggested to be recognised at ranks at sub-generic and sections levels respectively by Lyon (2014). *Nematoceras* is suggested to be described as a section, *Corybas* sub-gen. *Corysanthes* sec. *Nematoceras sensu* Lyon 2014. The rationale for using the names of genera for other ranks being that nomenclature requires stability without neglecting to recognise natural monophyletic groups (Entwisle & Weston 2005, Hopper 2009, Humphreys & Linder 2009, Lyon 2014). For this reason I use species names valid under *Corybas* in lieu of *Nematoceras* in this thesis, and refer to *Nematoceras* as sect. *Nematoceras*.

Corybas sub-gen. *Corysanthes* sect. *Nematoceras* is almost exclusively found in New Zealand, with the exception of two species, *Corybas sulcatus* (M.A.Clem. & D.L.Jones) G.N.Backh. and *C. dienemus* D.L.Jones., both found on Macquarie Island, which is geopolitically part of Australia. *Corybas* sub-gen. *Corysanthes* sec. *Nematoceras* is a monophyletic group with at least 12 species, and all have filiform lateral petals and sepals, open auricles, a column which leans back with respect to the ovary in varying degrees and a sharply deflexed labellum at the opening to the labellum wing tube.

Clements (2007) performed a molecular analysis of ITS sequences which showed there to be three highly supported clades within sect. *Nematoceras*; *Corybas acuminatus* M.A.Clem & Hatch. was resolved as sister to all other samples, and two clades referred to as the ‘macranthum’ and ‘rivulare’ clades respectively (Figure 2.3, Figure 2.4). The ‘macranthum’ clade is composed of at least *C. trilobus* (Hook.f) Rchb.f., *C. hypogaeus* (Hook.f) Lehnebach, *C. sulcatus* and *C. macranthus* (Hook.f) Rchb.f. The ‘rivulare’ clade is composed of at least six species; *C. rivularis* (A.Cunn) Rchb.f., *C. iridescens* Irwin & Molloy., *C. papa* Molloy & Irwin., *C. hatchii* (Hatch) Lehnebach, *C. orbiculatus* (Colenso) L.B.Moore and *C. dienemus*.

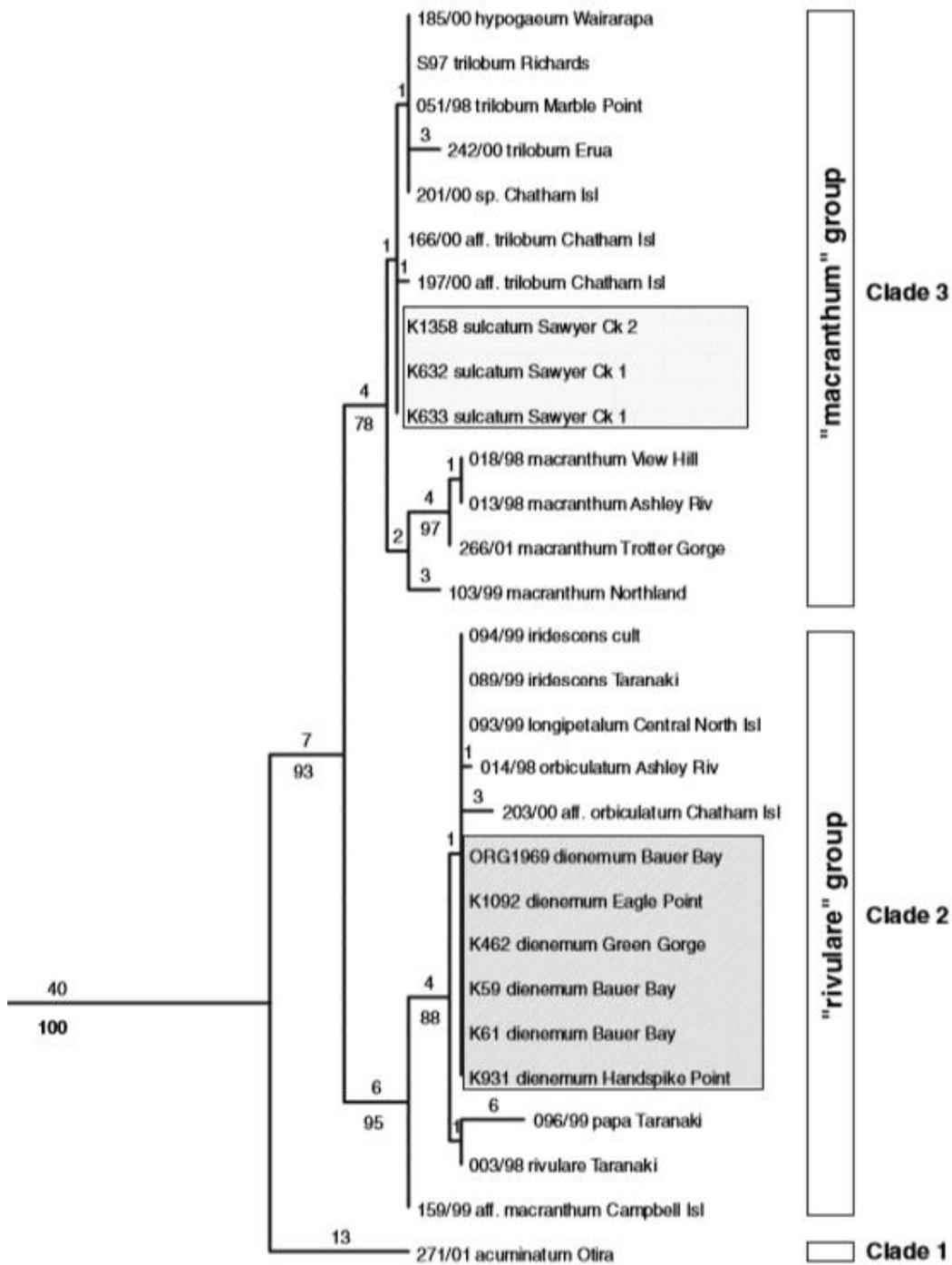


Figure 2.3; A cladogram based on ITS sequences of *Corybas* sub-gen. *Corysanthes* sec. *Nematoceras*, demonstrating three clades within the monophyletic section. Numbers above branches indicate number of base pair changes supporting each node, numbers below branches are bootstrap support (Clements *et al.* 2007).



Figure 2.4; Representatives of each of the three clades demonstrated by Clements *et al.* 2007 that compose *Corybas* sub-gen. *Corysanthes* sec. *Nematoceras sensu* Lyon 2014. A) *Corybas acuminatus* from Pirongia Forest Park, Waikato B) *Corybas* aff. *trilobus* from Pirongia Forest Park, Waikato, part of the “macranthum” clade C) *Corybas rivularis* from Puketiki Forest Park, Northland, part of the ‘rivulare’ clade.

2.2.1.1 *Corybas rivularis*

The members of the ‘rivulare’ clade all possess an acute-acuminate dorsal sepal, filiform lateral petals and sepals and a well developed v-shaped furrow at the labellum wing-tube opening. They have a fleshy leaf and occupy continuously wet habitats such as riversides, waterfalls and seeping banks. There are a number of undescribed tag-named populations, and collectively they form the focus of this research. There are four tag-named populations that are recognised by New Zealand’s Department of Conservation as having affinities to *C. rivularis*, *C. aff. rivularis* (AK 251833; Kaitarakihī), *C. aff. rivularis* (CHR 534752; “rest area”), *C. aff. rivularis* (CHR 518025; Kaimai) and *C. aff. rivularis* (CHR 518313 “whiskers”) (de Lange *et al.* 2012). These will be referred to in this thesis as *C. “kaitarakihī”*, *C. “rest area”*, *C. “kaimai”* and *C. “whiskers”* respectively. There are others recognised by the New Zealand Orchid Group (2016) including *C. “pollok”*, represented by specimen AK288095, and *C. “veil”* (Fig. 2.5).



Figure 2.5; *Corybas rivularis sensu stricto* and five tag-named populations of *Corybas* with affinities to *C. rivularis*; A) *C. rivularis s.s* from Puketi Forest Park, Northland. B) *C. “pollok” ex situ*, from near Pollok, Awhitu Peninsula. C) *C. “kaimai”* at Kauaeranga Valley, Coromandel Forest Park. D) *C. “whiskers”* at Pirongia Forest Park, Waikato. E) *C. aff. rivularis “kaitarakihi”* at Kaitarakihi Bay, Huia. F) *C. “veil”* at Waireinga/Bridal Veil Falls, Waikato.

1.1.1.1.1 Taxonomy of *Corybas rivularis*

Corybas rivularis was the first species to be described in New Zealand by Cunningham. The specimen was collected near Whangaroa, in the north of the north island in 1826, and was formally described as *Acianthus rivularis* A.Cunn. in *Precursor to Florae Insulare Novae Zealandiae* in 1837. In 1853 Joseph Hooker transferred this species to *Nematoceras* (*N. rivulare* (A.Cunn) Hook.f), and then to *Corysanthes* (*Corysanthes rivularis* (A.Cunn) Hook.f) (Hooker, 1853, Hooker 1864). Kirk in 1867 collected an undiscovered species but mistakenly ascribed it to *Corysanthes rivularis*. That undiscovered species is now known as *Corybas acuminatus* M.A.Clem. & Hatch. which has an acute leaf tip in flowering individuals. Confusion about these two species has been caused owing to an overly broad description of the leaf shape in The Flora of New Zealand handbook, which included “acuminate” (Hooker 1864, Scanlen 2010), a shape sometimes seen in juvenile, non flowering specimens. In 1906, Cheeseman circumscribed *C. rivularis* broadly enough to include the specimen found by Kirk (Cheeseman 1906). This established a precedent which lasted many decades, in which *C. rivularis sensu stricto*, and associated entities were collectively referred to as *C. orbiculatus*, and in which the improperly described *C. acuminatus* was referred to as *C. rivularis*. This was until Clements and Hatch discovered the origin of the confusion, and described the species *C. acuminatus* (Clements & Hatch 1985). *C. rivularis* and *C. orbiculatus* were recircumscribed and lectotypes were established, in the same paper that described *C. iridescens* and *C. papa* as distinctive from *C. rivularis sensu stricto* (Molloy & Irwin 1996). A summary of the taxonomic changes are in Table 2.1. The confusion created by the reshuffling of names has contributed to the tag-named populations being neglected in terms of study. This created a situation where purportedly distinct populations have been undescribed for considerable lengths of time. Little is known about them and various agencies have had considerable difficulty in establishing historical and contemporary records of distribution of these entities, particularly if the names have been applied inconsistently between observers, or between periods of time in which the naming conventions were different. There are as many tag-named populations awaiting description as there are currently accepted species within the ‘rivulare’ clade.

Table 2.1; A summary of the taxonomy for each of the members of *Corybas* sub-gen. *Corysanthes* sect. *Nematoceras* sensu Lyon 2014 (syn. *Nematoceras*).

<i>Actanthus</i> sensu A.Cunn. 1837	<i>Nematoceras</i> sensu Hook.f. 1853	<i>Corysanthes</i> sensu Hook.f. 1864	<i>Corybas</i> sensu Rchb.f. 1871	<i>Nematoceras</i> sensu Molloy, D.L.Jones & M.A.Clem. 2002	<i>Corybas</i> sub-gen. <i>Corysanthes</i> sec. <i>Nematoceras</i> sensu Lyon. 2014
			<i>Corybas rivularis</i> Cheeseman (1906) (nom. inv.)	<i>Nematoceras acuminatum</i> Molloy, D.L.Jones & M.A.Clem. 2002	<i>Corybas acuminatus</i> M.A.Clem & Hatch 1985
			<i>Corybas acuminatus</i> M.A.Clem & Hatch 1985		
			<i>Corybas dienemus</i> D.L.Jones 1993	<i>Nematoceras dienemus</i> D.L.Jones, & M.A.Clem. & Molloy 2002	<i>C. dienemus</i> D.L.Jones 1993
			<i>Corybas macranthus</i> var. <i>longipetalus</i> Hatch. 1947	<i>Nematoceras longipetalum</i> Molloy, D.L.Jones & M.A.Clem. 2002	<i>C. hatchii</i> (Hatch) Lehnebach. 2016
			<i>Corybas longipetalus</i> Hatch. 1993 (nom. illegit.)		
			<i>Corybas hatchii</i> (Hatch) Lehnebach. 2016		
		<i>Corysanthes hypogaea</i> Colenso. 1884	<i>Corybas hypogaeus</i> (Colenso.) Lehnebach 2016	<i>Nematoceras hypogaeum</i> (Colenso.) Molloy, D.L.Jones & M.A.Clem 2002	<i>C. hypogaeus</i> (Colenso.) Lehnebach 2016
			<i>Corybas iridescens</i> Irwin & Molloy 1996	<i>Nematoceras iridescens</i> (Irwin & Molloy) Molloy, D.L.Jones & M.A.Clem. 2002	<i>C. iridescens</i> Irwin & Molloy 1996
	<i>Nematoceras macranthum</i> Hook.f 1853	<i>Corysanthes macrantha</i> (Hook.f) Hook.f 1864	<i>Corybas macranthus</i> (Hook.f) Rchb.f 1871		<i>C. macranthus</i> (Hook.f) Rchb.f 1871
		<i>Corysanthes orbiculata</i> Colenso 1891	<i>Corybas orbiculatus</i> (Colenso) L.B.Moore 1970	<i>Nematoceras orbiculatum</i> (Colenso) Molloy, D.L.Jones & M.A.Clem 2002	<i>C. orbiculatus</i> (Colenso) L.B.Moore 1970
			<i>Corybas papa</i> Molloy & Irwin 1996	<i>Nematoceras papa</i> (Molloy & Irwin	<i>C. papa</i> Molloy & Irwin 1996
		<i>Corysanthes papillosa</i> Colenso 1884	<i>Corybas papillosus</i> (Colenso) Lehnebach 2016	<i>Nematoceras papillosum</i> (Colenso) Molloy, D.L.Jones & M.A.Clem 2002	<i>C. papillosus</i> (Colenso) Lehnebach 2016
<i>Actanthus rivularis</i> A.Cunn 1837	<i>Nematoceras rivulare</i> (A.Cunn.) Hook.f 1853	<i>Corysanthes rivularis</i> (A.Cunn.) Hook.f 1864	<i>Corybas rivularis</i> (A.Cunn.) Rchb.f. 1871	<i>Nematoceras paduratum</i> (Cheeseman) Molloy, D.L.Jones & M.A.Clem. 2002	<i>C. rivularis</i> (A.Cunn.) Rchb.f. 1871
			<i>Corybas orbiculatus</i> L.B.Moore. 1970		
			<i>Corybas sulcatus</i> (M.A.Clem. & D.L.Jones) G.N.Backh 2010	<i>Nematoceras sulcatum</i> M.A.Clem & D.L.Jones 2006	<i>C. sulcatus</i> (M.A.Clem. & D.L.Jones) G.N.Backh 2010
	<i>Nematoceras trilobum</i> Hook.f 1853	<i>Corysanthes triloba</i> (Hook.f) Hook.f 1864	<i>Corybas trilobus</i> (Hook.f) Rchb.f 1871		<i>C. trilobus</i> (Hook.f) Rchb.f 1871

An incentive to research this group is the conservation status of some of the constituents. A Kaitarakihi population (represented by specimen AK251833) and determined as “*C. aff. rivularis*” is listed as Critically endangered. Similarly, populations determined as (CHR 518313 ‘whiskers’) and Kaimai (CHR 518025) are listed as Data Deficient (de Lange *et al.* 2012). The taxonomic rank used to describe these currently undescribed populations will have an impact on the threat classification level, as broader concepts of species will tend to be classified as less threatened than narrowly defined species. Taxonomic ranking is a major component in the ability for conservation efforts to be focused, providing descriptions that allow the identification of entities from one another, to have a basic understanding of its distributional range and to allow governmental and organisational bodies that have an active role in implementing conservation efforts to make more informed decisions regarding the construction of conservation management strategies and delegation of resources (Mace 2004, Cameron 2010).

2.2.1.1.1 Natural history of *Corybas rivularis*

Corybas are known to have a high rate of endemism, and the species that compose the ‘rivulare’ clade are believed to have radiated within the last 1MY, however, they appear to have diverged from the ‘macranthum’ clade around 3MYA (Fig. 2.6, Lyon 2014). This rapid radiation following nearly 2MY of divergence raises the possibility of a genetic bottleneck after divergence and prior to subsequent radiation.

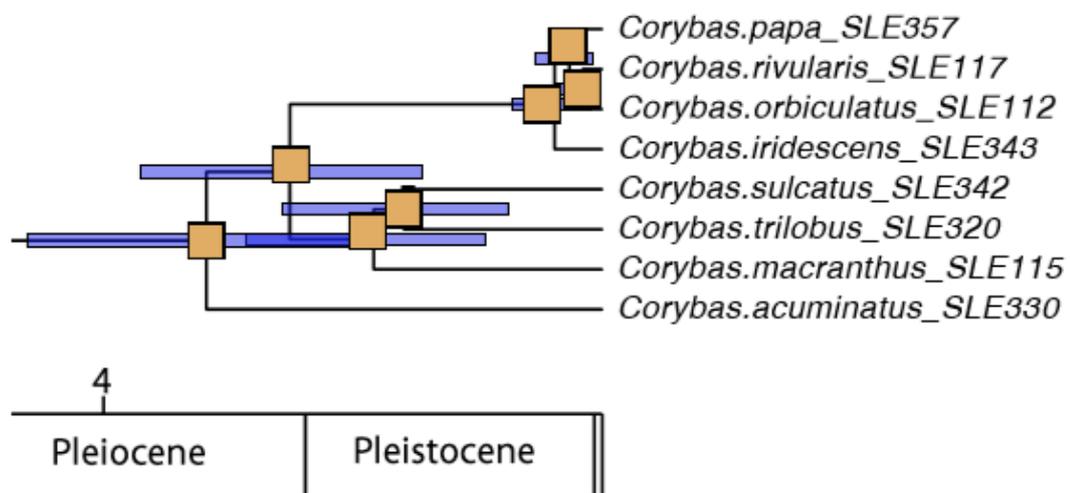


Figure 2.6; A dendrogram of recognised species of *Corybas* sub-gen. *Corysanthes* sect. *Nematoceras* with estimated dates of divergence (orange squares), with estimated ranges (blue bars), based on multiple regions of molecular variation (Lyon 2014).

2.2.1.1.2 Ecology of *Corybas*

All constituents of the ‘rivulare’ clade require almost permanently moist habitats, with preferences to high humidity. The habitat requirements seem precise, although there are number of suitable environments, such as streamsides, adjacent waterfalls, seawalls, seeping banks and roadside cuttings. Colonies face threats in the form of habitat destruction, with pulse floods scouring streamsides clean, the mossy verdure that clings to cliff faces adjacent waterfalls and seawalls can sluice off the rock faces, and road side banks are routinely cleared of most vegetation, which either destroys the colony directly, or exposes the colony to excessive light and heat (Watkins 2012). Excessive light is problematic with these orchids being preferable to conditions genial to mosses and bryophytes (Watkins 2002, Watkins 2012).

Orchid seeds typically require a fungal associate known as mycorrhiza (Bernard 1899, Burgeff 1936) in order to successfully germinate. Research into the mycorrhizal fungal associate of *C. iridescens* determined that *Tulasnella calospora* (Basidiomycetes) is present in the roots, and germinating seeds and plays a role in reducing the mortality of seedlings (Watkins 2012).

Corybas is pollinated by small insects in the Mycetophilidae family, commonly called fungus gnats (Jones 1971, Fuller 1979, Pridgeon 2001). Lacking nectaries, *Corybas* requires other mechanisms to attract pollinators (Lyon 2014). Brood site deception is thought to be a likely mechanism, operating possibly by fragrant volatiles and visual cues (Kelly *et al.* 2013). The long, filamentous lateral petals and sepals of the ‘rivulare’ clade are structured in a manner that in other plants is associated to sapromyophily, such that the plant may be transporting volatiles generated by the mycorrhizal associate then use the lateral petals and sepals as osmophores (Faegri & van der Pijl 1979, Vogel & Martens 2000). Alternatively they could be visual cues, functioning in conjunction with the maroon patterning. The auricles of all species and populations of sect. *Nematoceras* are pellucid-white, even in the deeply maroon flowered species such as *C. iridescens*. The function of these auricles is unclear, with some suggesting they may promote airflow through the flower and help distribute fragrant volatiles (Jones 1971), although the fact it is highly conserved among species to be white, it is likely at

least in part to act as a visual guide, allowing light into the column chamber, encouraging an insect into proper position.

A study suggests the related *Corybas cheesmanii* (Hook.f. ex Kirk) Kuntze undergoes autogamous pollination some of the time, but otherwise is typically pollinated by female fungus gnats. Visual mimicry of a brooding site, possibly assisted by fragrant volatiles was suspected to guide them into the flowers (Kelly *et al.* 2013), although in the cases of *C. cheesmanii*, ultra-violet light reflectance seems a more likely candidate than fragrant volatiles (Kelly & Gaskett 2014). Unlike *C. cheesmanii*, the tag-named populations of *C.* “whiskers” (CHR 518313) are noted to have a pungent scent in flower by Irwin (2009), which plays a role in brood site deception pollination syndromes (Urru *et al.* 2011, Jürgens *et al.* 2013). Whatever the mechanism by which the insects are attracted, there is no doubt that they are attracted to many of the species that make up this group, with observation of fungus gnat eggs being found in the flowers of *C. trilobus* (Scanlen 2006), observations of dead insects trapped in the column chamber of *C. macranthus*, and live insects visiting *C. iridescens* (St. George 2007) and personal observations of insect eggs found in the furrow of *C. rivularis*.

2.3 Conclusions

Until these entities are formally identified and or described, a consistent approach to cataloguing the distribution and number of these entities will remain difficult, and subsequently the approach to conserving them will not be as informed as it could be. It is therefore the objective of this study to determine which of the six different populations informally recognised as tag-named entities, each determined as *Corybas* aff. *rivularis*, merit formal taxonomic ranks. This will be achieved by; 1) multiple morphological analyses using precisely defined characters and character states taken from *in-situ* individuals and 2) determining if these taxa are genetically distinct based on molecular sequence variation of the nuclear ribosomal internal transcribed spacer region (ITS), isolated from desiccated leaf material. The results of these analyses will be used to inform a taxonomic revision.

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Chapter Three: A molecular and morphological analysis of *Corybas rivularis* (A.Cunn.) Rchb.f. (Orchidaceae), including tag-named entities and related species.

This chapter is written in the form of a manuscript for publication to be submitted to _____. As such some information from the previous chapters may be repeated herein.

Authors; Abraham Coffin – Collected data in the laboratory and field, performed molecular and morphological analyses and drafted manuscript.

Chrissen Gemmill – Chief supervisor, supervised research and molecular analyses, contributed to manuscript

Steven Miller – Co-supervisor and advisor for statistics used in morphological analyses.

3.1 Abstract

The undescribed tag-named populations with affinities to *Corybas rivularis* have long been awaiting a formalised approach to determining which, if any, are sufficiently distinctive morphologically and/or genetically to merit formal taxonomic description. To address this, we undertook a holistic approach combining molecular and morphological analyses of *C. rivularis*, segregate populations, and related species. Analysis of sequence variation of the nuclear ribosomal internal transcribed spacer (ITS) region revealed a low level of variation among taxa allied to *C. rivularis*, and no variation in taxa allied to *C. iridescens*, except for *C. orbiculatus*, which has a single base pair substitution. *Corybas acuminatus* was resolved as sister to two highly supported clades (Posterior Probability = 1), each with additional highly supported subclades. Morphological analyses were performed with multivariate statistical techniques. A Linear Discriminant Analysis (LDA) determined the three most informative characteristics to be the labellum width, bib length and furrow height. All species and tag-named populations formed groups in the LDA, and clusters in an UPGMA analysis, with the exception of individuals from Kaitarakihi Bay and

Bridal Veil Falls, which together formed a novel group. The morphological data were congruent with the molecular analyses. The clade composed of *C. iridescens*, allied species and tag-named populations have a comparatively large labellum bib length and width, with a small labellum furrow. The clade composed of *C. rivularis*, related species and tag-named populations, have a narrow labellum, short bib and a large labellum furrow. Taxonomic revisions of these taxa will follow in subsequent a publication.

3.2 Introduction

New Zealand lies around 1500 kilometres to the southeast of Australia. It is an archipelago that consists of many islands, with two large islands oriented north to south, a few relatively large islands, including Stewart Island, and numerous smaller islands. The islands of New Zealand sit between latitudes 29°S and 53°S, giving New Zealand a temperate climate. The surrounding ocean provides a stabilising influence with low inter-seasonal temperature variation. The Southern Hemisphere Westerly Winds also contribute to the climate of New Zealand (Anderson *et al.* 2009), and have played a role in the long distance dispersal of plants and animals to New Zealand (Sanmartin & Ronquist 2004).

New Zealand forms part of the now mostly submerged continent Zealandia, which was likely completely submerged during the late Oligocene (Landis *et al.* 2008). With land above water for at least the last 22 million years, New Zealand has an established flora that has many endemic species (1735, ~78%) (Breitwieser *et al.* 2012). Some suggest an entirely long-distance dispersal origin for the New Zealand flora (Pole 1994). There is a prevalence of small white flowers, dioecism and unspecialised pollination syndromes (Lloyd 1985). Non-endemic species and genera are more likely to be highly dispersible plants with small spores such as ferns, fern allies, or small seeds such as wetland species and orchids. This is likely due to selective immigration of species via long distance dispersal. (McGlone *et al.* 2001).

The family Orchidaceae has a fossil record in New Zealand dating to 23-20 million years before present (Conran *et al.* 2009) representing early members of the largely epiphytic genera *Earina* Lindl. and *Dendrobium* Sw. respectively. New Zealand has around 110 species of orchids (de Lange *et al.* 2012), with 9 epiphytic species and 101 terrestrial species. All but one of the terrestrial species belong to the sub-family Orchidoideae Lindl. and the majority belong to the tribe Diurideae (Endl.) Lindl. ex Meisn. The Diurideae in New Zealand is represented by 66 species across 17 genera in 8 sub-tribes, including Acianthinae Schltr. In New Zealand, the sub-tribe Acianthinae has 21 species in 4 genera. One of these genera, *Corybas* Salisb. is represented globally by around 132 species, of which 17 are native to New Zealand (World Checklist Selected Plant Families 2014).

Corybas is identified by its single, typically orbicular-cordate fleshy leaf, and a large solitary flower. *Corybas* flowers have a modified labellum with winged lobes, incurved margins that form a tube, and a labellum base margin that has protrusions that are either open, and thus called auricles, or are closed and are called spurs (Fig. 3.1). The plant does not form pseudobulbs but forms root tubers that act as a storage organ over the difficult season while the single leaf senesces, re-emerging the following season. (Salisbury 1805, Edgar & Moore 1970, Pridgeon & Chase 1995, Lyon 2014). *Corybas* is widely distributed, from Asia, South East Asia, Papua New Guinea, Australia, New Zealand, sub-Antarctic and Pacific islands (Figure 3.2).

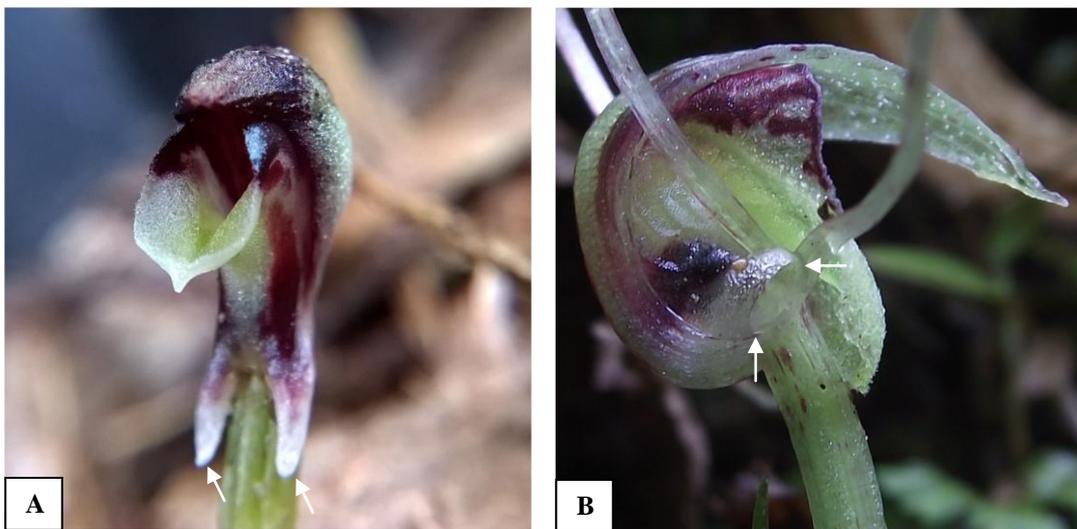


Figure 3.1; A) *Corybas cheesemanii* (Hook.f ex Kirk) Kuntze from Te Kauri Forest Park, Waikato. Arrows are pointing to closed labellum spurs. B) *Corybas* “whiskers” from Pirongia Forest Park. Open labellum auricle with arrows showing width of the aperture.



Figure 3.2; A rough distribution (green) of *Corybas* across Asia, South-East Asia, Papua New Guinea, Australia, New Zealand and the Pacific. Reproduced with the permission of the Trustees of the Royal Botanic Gardens, Kew (WCSP 2014).

A recent taxonomic study (Lyon 2014) on *Corybas* suggests that five closely related genera should be merged: *Nematoceras* (Hook.f) D.L.Jones & M.A.Clem., *Corysanthes* R.Br., *Singularybas* D.L.Jones & M.A.Clem., *Molloybas* D.L.Jones & M.A.Clem. and *Anzybas* D.L.Jones & M.A.Clem. These were split from *Corybas* (Jones *et al.* 2002), but later rejected by botanists at Kew and various international and Australian institutions (Govaerts *et al.* 2003, Entwisle & Weston 2005, Chase *et al.* 2015). The former genera will likely be attributed ranks at sub-generic and sections levels respectively. *Nematoceras* was used at the rank of Section, within subgenus *Corysanthes* within *Corybas* by Lyon 2014. The rationale for using generic names for other ranks being that nomenclature requires stability without neglecting to recognise natural monophyletic groups (Entwisle & Weston 2005, Hopper 2009, Humphreys & Linder 2009, Lyon 2014).

In this study we use *Corybas* in lieu of *Nematoceras* despite both being accepted by various institutions. *Corybas* sub-gen. *Corysanthes* sec. *Nematoceras* is almost exclusively found in New Zealand, with the exception of two species, *Corybas sulcatus* (M.A.Clem. & D.L.Jones) G.N.Backh. and *C. dienemus* D.L.Jones., found on Macquarie Island, which is geopolitically part of Australia yet floristically aligned with New Zealand. It is a monophyletic group with at least 12 species, and all have filiform lateral petals and sepals, open auricles, a column which leans back with respect to the ovary in varying degrees and a sharply deflexed labellum at the opening to the labellum wing tube (Lyon 2014). A previous molecular analysis of ITS sequences has shown there to be three distinct clades within sect. *Nematoceras*; a basal, monospecific clade composed of *Corybas acuminatus* M.A.Clem. & Hatch. and two clades referred to as the ‘macranthum’ and ‘rivulare’ clades respectively (Clements *et al.* 2007). The ‘macranthum’ clade is composed of at least *C. trilobus* (Hook.f) Rchb.f., *C. sulcatus* and *C. macranthus* (Hook.f) Rchb.f. The ‘rivulare’ clade is composed of at least six species; *C. rivularis* (A.Cunn) Rchb.f., *C. iridescens* Irwin & Molloy, *C. papa* Molloy & Irwin, *C. hatchii* (Hatch) Lehnebach, *C. dienemus* and *C. orbiculatus* (Colenso) L.B.Moore.

The members of the ‘rivulare’ clade *sensu* Clements *et al.* (2007) all possess an acute-acuminate dorsal sepal, filiform lateral petals and sepals and a well developed v-shaped furrow at the labellum wing-tube opening. They have a fleshy leaf and occupy continuously wet habitats such as riversides, waterfalls and

seeping banks. There are a number of populations that are similar to *C. rivularis*, yet remain undescribed tag-named populations, also referred to as segregates of *C. rivularis*, and collectively they form the focus of this research. There are four tag-named vouchered populations that are recognised by New Zealand's Department of Conservation (de Lange *et al.* 2012) as having affinities to *C. rivularis*. These are referred to by the herbarium and sheet number and that tag name: *Corybas* aff. *rivularis* known from Kaitarakiki (AK 251833; "Kaitarakihi"), *C. aff. rivularis* from National Park (CHR 534752; "rest area"), *C. aff. rivularis* from Kauaeranga Valley, Coromandel Forest Park (CHR 518025; "Kaimai") and *C. aff. rivularis* collected from Pirongia Forest Park and Waireinga/Bridal Veil Falls (CHR 518313 "whiskers"). These will be referred to as *C. "kaitarakihi"*, *C. "rest area"*, *C. "kaimai"* and *C. "whiskers"* respectively. There are others recognised by the New Zealand Orchid Group (2016) including *C. "pollok"* collected from near Pollok on the Awhitu Peninsula (AK288095), and *C. "veil"* collected from Waireinga/Bridal Veil Falls near Raglan.

In 1837, Cunningham described the first New Zealand species as *Acianthus rivularis* A.Cunn based on a collection made in 1826 near Whangaroa, in the north of the North Island. Joseph Hooker (1853) then transferred this species to *Nematoceras* (*N. rivulare* (A.Cunn) Hook.f), and then to *Corysanthes* (*Corysanthes rivularis* (A.Cunn) Hook.f) (Hooker 1864). Kirk (1868) made a collection that he described as *Corysanthes rivularis*, however this was not conspecific with *Corysanthes rivularis sensu* Hooker. Cunningham's species is now known as *Corybas acuminatus* M.A.Clem. & Hatch., which has an acute leaf tip in flowering individuals. According to Scanlen (2010), confusion about these two species has been due to an overly broad description of the leaf shape in The Flora of New Zealand handbook (Hooker 1864), which included "acuminate", a shape sometimes seen in juvenile, non flowering members of the 'rivulare' clade. In 1906, Cheeseman circumscribed *C. rivularis* broadly enough to include the specimen found by Kirk (Cheeseman 1906). Unfortunately, this established a precedent that lasted many decades, in which *C. rivularis sensu stricto* and associated entities were collectively referred to as *C. orbiculatus* (Col.) L.B. Moore, and in which the improperly described *C. acuminatus* was referred to as *C. rivularis*. This was until Clements and Hatch (1985) discovered the origin of the confusion, and described the species *C. acuminatus*, and, *C. rivularis* and *C.*

orbiculatus were recircumscribed and lectotypes were established, in the same paper that described *C. iridescens* and *C. papa* as distinctive from *C. rivularis sensu stricto* (Molloy & Irwin 1996). A summary of the taxonomic changes are in Table 3.1. The confusion created by the reshuffling of names has contributed to the tag-named populations being neglected in terms of study. This created a situation where purportedly distinct entities have been undescribed for considerable lengths of time. Little is known about them and various agencies have had considerable difficulty in establishing historical and contemporary records of distribution of these entities, particularly if the names have been applied inconsistently between observers, or between periods of time in which the naming conventions were different. There are currently as many tag-named populations awaiting description as there are currently accepted species within the ‘rivulare’ clade. One difficulty in examining this group is quantifying the character states that orchid enthusiasts and some botanists see that may differ among the respective populations. Determining the presence or absence of characters was sufficient for the species already described, and there are no species of *Corybas* subgen. *Corysanthes* sec. *Nematoceras* described in part on the basis of molecular variation. The traditional methods to determine the level of variation between the various entities has been insufficient in resolving the morphological variation of the remaining undescribed tag-named populations with affinities to *Corybas rivularis*, and a more comprehensive approach is required.

A further incentive to research this group is the listing of *Corybas* “kaitarakahi” as critically endangered, and *C.* “whiskers” and *C.* “kaimai” as data deficient (de Lange 2012). Until these populations are formally assessed for morphological and genetic distinctness, and hence taxonomic status, a consistent approach to cataloguing the distribution and number of these unknown populations will remain difficult, and subsequently the approach to conserving them will not be as informed as it could.

Table 3.1; A summary of the taxonomy for each of the members of *Corybas* sub-gen. *Corysanthes* sect. *Nematoceras sensu* Lyon 2014 (syn. *Nematoceras*).

<i>Actianthus sensu</i> A.Cunn. 1837	<i>Nematoceras sensu</i> Hook.f. 1853	<i>Corysanthes sensu</i> Hook.f. 1864	<i>Corybas sensu</i> Rchb.f. 1871	<i>Nematoceras sensu</i> Molloy, D.L.Jones & M.A.Clem. 2002	<i>Corybas</i> sub-gen. <i>Corysanthes</i> sec. <i>Nematoceras sensu</i> Lyon. 2014
			<i>Corybas rivularis</i> Cheeseman (1906) (<i>nom. inv.</i>)	<i>Nematoceras acuminatum</i> Molloy, D.L.Jones & M.A.Clem. 2002	<i>Corybas acuminatus</i> M.A.Clem & Hatch 1985
			<i>Corybas acuminatus</i> M.A.Clem & Hatch 1985		
			<i>Corybas dienemus</i> D.L.Jones 1993	<i>Nematoceras dienemum</i> D.L.Jones, & M.A.Clem. & Molloy 2002	<i>C. dienemus</i> D.L.Jones 1993
			<i>Corybas macranthus</i> var. <i>longipetalus</i> Hatch. 1947	<i>Nematoceras longipetalum</i> Molloy, D.L.Jones & M.A.Clem. 2002	<i>C. hatchii</i> (Hatch) Lehnebach. 2016
			<i>Corybas longipetalus</i> Hatch. 1993 (<i>nom. illegit.</i>)		
			<i>Corybas hatchii</i> (Hatch) Lehnebach. 2016		
		<i>Corysanthes hypogaea</i> Colenso. 1884	<i>Corybas hypogaeus</i> (Colenso.) Lehnebach 2016	<i>Nematoceras hypogaeum</i> (Colenso.) Molloy, D.L.Jones & M.A.Clem 2002	<i>C. hypogaeus</i> (Colenso.) Lehnebach 2016
			<i>Corybas iridescens</i> Irwin & Molloy 1996	<i>Nematoceras iridescens</i> (Irwin & Molloy) Molloy, D.L.Jones & M.A.Clem. 2002	<i>C. iridescens</i> Irwin & Molloy 1996
	<i>Nematoceras macranthum</i> Hook.f 1853	<i>Corysanthes macrantha</i> (Hook.f) Hook.f 1864	<i>Corybas macranthus</i> (Hook.f) Rchb.f 1871		<i>C. macranthus</i> (Hook.f) Rchb.f 1871
		<i>Corysanthes orbiculata</i> Colenso 1891	<i>Corybas orbiculatus</i> (Colenso) L.B.Moore 1970	<i>Nematoceras orbiculatum</i> (Colenso) Molloy, D.L.Jones & M.A.Clem 2002	<i>C. orbiculatus</i> (Colenso) L.B.Moore 1970
			<i>Corybas papa</i> Molloy & Irwin 1996	<i>Nematoceras papa</i> (Molloy & Irwin)	<i>C. papa</i> Molloy & Irwin 1996
		<i>Corysanthes papillosa</i> Colenso 1884	<i>Corybas papillosus</i> (Colenso) Lehnebach 2016	<i>Nematoceras papillosum</i> (Colenso) Molloy, D.L.Jones & M.A.Clem 2002	<i>C. papillosus</i> (Colenso) Lehnebach 2016
<i>Actianthus rivularis</i> A.Cunn 1837	<i>Nematoceras rivulare</i> (A.Cunn.) Hook.f 1853	<i>Corysanthes rivularis</i> (A.Cunn.) Hook.f 1864	<i>Corybas rivularis</i> (A.Cunn.) Rchb.f. 1871	<i>Nematoceras paduratum</i> (Cheeseman) Molloy, D.L.Jones & M.A.Clem. 2002	<i>C. rivularis</i> (A.Cunn.) Rchb.f. 1871
			<i>Corybas orbiculatus</i> L.B.Moore. 1970		
			<i>Corybas sulcatus</i> (M.A.Clem. & D.L.Jones) G.N.Backh 2010	<i>Nematoceras sulcatum</i> M.A.Clem & D.L.Jones 2006	<i>C. sulcatus</i> (M.A.Clem. & D.L.Jones) G.N.Backh 2010
	<i>Nematoceras trilobum</i> Hook.f 1853	<i>Corysanthes triloba</i> (Hook.f) Hook.f 1864	<i>Corybas trilobus</i> (Hook.f) Rchb.f 1871		<i>C. trilobus</i> (Hook.f) Rchb.f 1871

Morphometrics is an approach that uses landmarks to quantify continuous characters such they may be used in a contemporary statistical analysis. A landmark character is two or three precisely defined points on an organism. The benefit of morphometrics is the ability to retain information on the ‘form’ of an organism. The form is a manifestation of the size and shape, and it is this three dimensional aspect which has been difficult to describe and assess from pressed herbarium specimens. The shape is not measured explicitly, but is the aggregate of the absolute sizes of the various characters that describe the overall dimensions of the organ/organism being measured. As a result, size has a much larger influence alone than does shape in statistical analyses (Richtsmeier *et al.* 2002). The use of landmark characters in some instances is criticised for not encapsulating all aspects of shape, particularly curves and outlines, as it measures Euclidean distances in straight lines. An example is that landmark characters are unable to distinguish a diamond shape from an oval (Jensen 2003).

This research sought to determine whether *Corybas rivularis sensu stricto* and the undescribed populations are distinct entities or constitute a single species based on analysis of numerous precisely defined morphological characters measured *in situ*, in conjunction with DNA sequence variation. The results of these analyses will be used to inform a taxonomic revision in a subsequent publication.

3.3 Materials and methods

3.3.1 Species and tag-named populations studied

A front-on and side-view of each of the 9 studied species and tag-named populations allows the comparison of the flower anatomies (Fig. 3.3). The locations of the sampling sites, number of samples taken for molecular analysis, the number of specimens measured for the morphological analysis, habitats, and basic morphology for each of the species and tag-named populations are summarised in Table 3.2. The 9 species and tag-named populations assessed in this research were all sampled from populations in the North Island of New Zealand (Fig. 3.4). Photographs of the habitats and selected sample sites are shown in Fig. 3.5 and Fig. 3.6, and were all in either forested or coastal environments.



Figure 3.3; Frontal view (top row) and side-on view (second row) of *Corybas rivularis*, A,F; *C. "kaimai"*, B,G; *C. "whiskers"*, C,H; *C. "pollok"*, D,I and *C. papa*, E,J. Frontal view (third row) and side view (bottom row) of *Corybas iridescens* K,O; *C. "veil"*, L,P; *C. "kaitarakihi"*, M,Q and *C. orbiculatus*, N,R. The upper 1cm scale applies to images A-J, the lower 1cm scale applies to images K-R.

Table 3.2; A summary of the study site locations, basic morphological and habitat descriptions for each of the studied species and tag-named populations.

Species / tag-name	Locations	NZTM	# Leaves sampled for molecular analysis	# Specimens measured for morphological analysis	Morphology	Habitat
<i>Corybas rivularis</i>	Waipapa river track, Puketia Forest Park, Northland.	E1662529 N6097215	5	11	Small, maroon, forward projecting flower.	Rocky forest stream sides, wet banks, adjacent waterfalls.
<i>C. iridescens</i>	Pirongia Forest Park & Te Kauri Forest Park, Waikato	E1782221 N5799037, E1773731 N5784994	5	4	Large maroon flower with a small furrow and large bib with papillae.	Wet cliff faces, limestone seeps and forest stream sides.
<i>C. orbiculatus</i>	Waitaanga road, N.G. Tucker Scenic Reserve.	E1767453 N5697550	0	1	Large maroon flower with long bib, incurved margins.	Wet cliff faces and outcrops.
<i>C. papa</i>	Waitaanga road, N.G. Tucker Scenic Reserve.	E1767300 N5697651	0	5	Small green flower with double reflexed labellum, almost absent of papillae and with small furrow.	Wet cliff faces, banks, limestone seeps and forest stream sides.
<i>C. "whiskers"</i>	Pirongia Forest Park & Waireinga scenic reserve, Waikato.	E1782221 N5799037, E1782313 N5798931, E1766593 N5802552	15	11	Small green flower with double reflexed labellum with papillae.	Rocky stream sides, wet banks in forests.
<i>C. "kaimai"</i>	Kauaeranga Valley, Coromandel Forest Park, Coromandel.	E1837076 N5893878, E1837026 N5894011, E1836997 N5894027	15	15	Small-medium flower with a long furrow. Wing-tube is forward leaning.	On mossy rocks in streams, stream sides, wet forested banks and tree bases, wet well lit forest floor.
<i>C. "pollok"</i>	Near Pollok, Awhitu Peninsula.	E1742274 N5887888	5	3	Diminutive flower with maroon furrow.	Isolated forest relic in small coastal valley on limestone seep.
<i>C. "kaitarakahi"</i>	Kaitarakahi bay, Huia, Waitakere.	E1741164 N5903057	2	3	Medium sized maroon flower with entire labellum margins and small leaves on flowering plants.	Moss covered sea walls on limestone seep.
<i>C. "veil"</i>	Waireinga / Bridal Veil Falls scenic reserve, Waikato.	E1766660 N5802643	5	2	Medium-large sized maroon flower with entire labellum margins.	Moss covered cliff face with perpetual spray from waterfall.

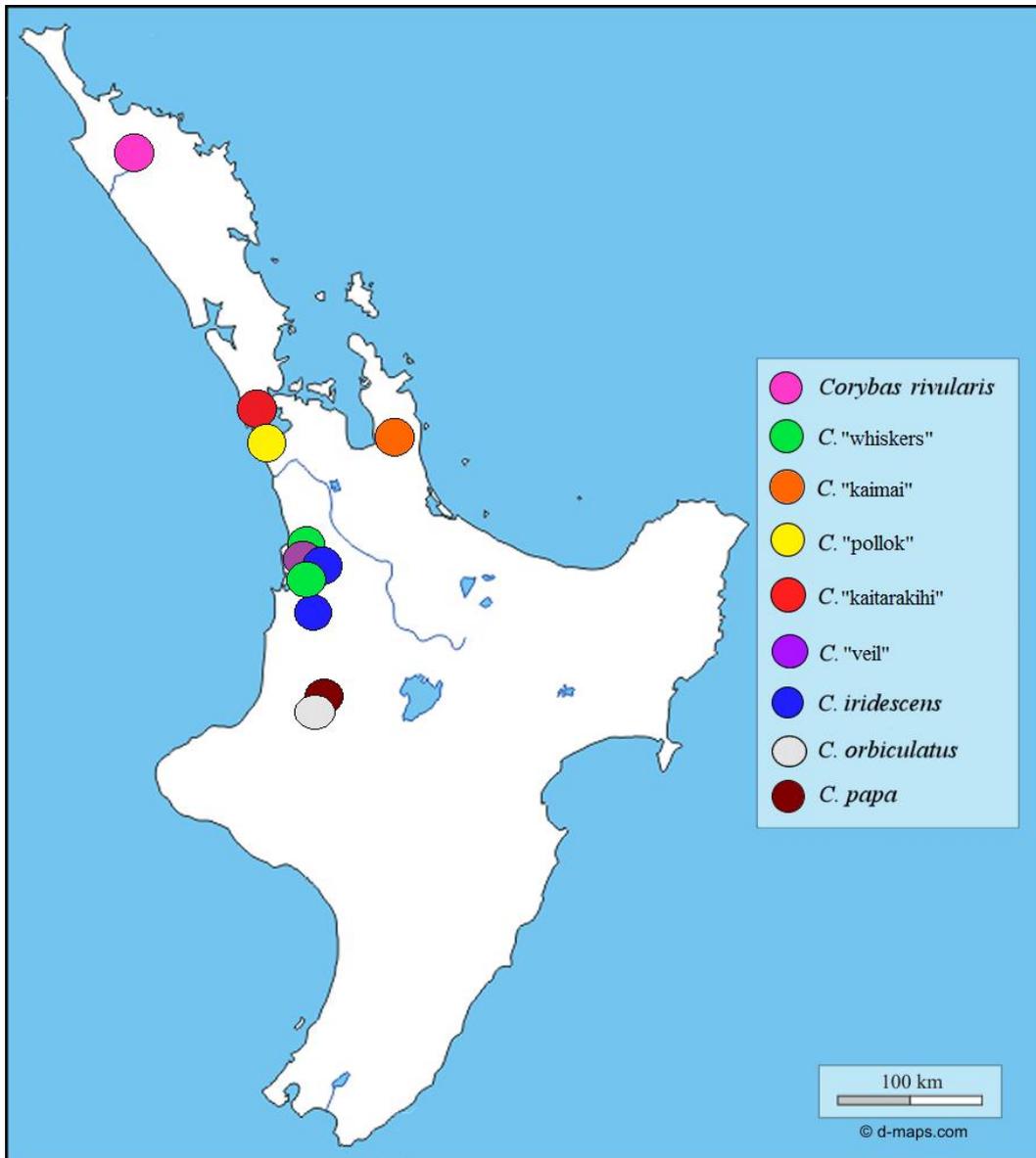


Figure 3.4; A map of the North Island of New Zealand, with colour coded labels for each of the populations included in this study.

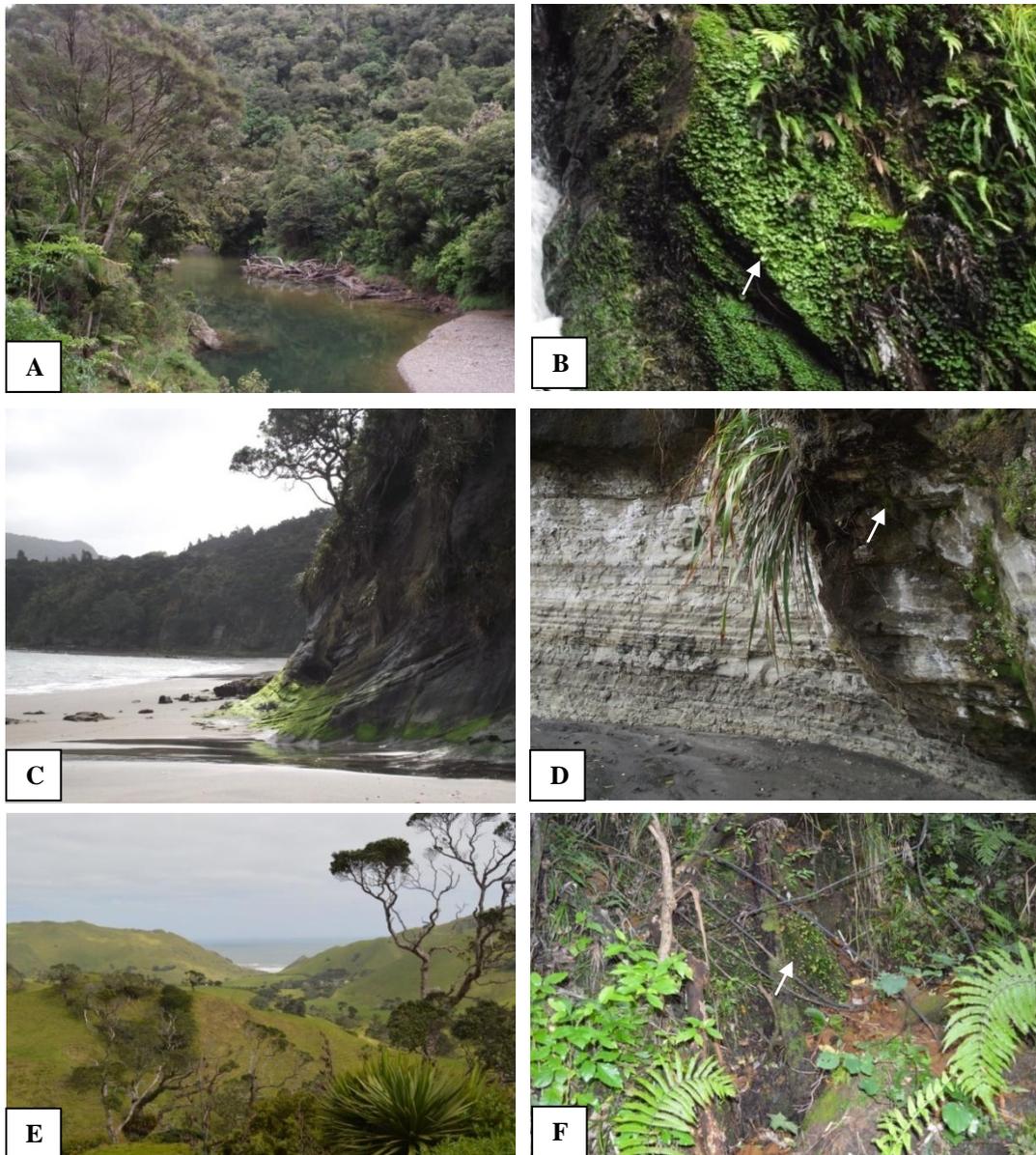


Figure 3.5; Habitats and sample sites of *Corybas rivularis*, *C.* “kaitarakihi” and *C.* “pollok”; A) Habitat of *Corybas rivularis* at Pukeki Forest, Northland. B) Study site of *C. rivularis*. C) Habitat of *C.* “kaitarakihi” at Kaitarakihi Bay, Huia. D) Study site of *C.* “kaitarakihi”. E) Habitat of *C.* “pollok” near Pollok on the Awhitu Peninsula. F) Study site of *C.* “pollok”. White arrows indicate location of colony.



Figure 3.6; Habitats and sample sites of *Corybas iridescens*, *C.* “whiskers”, *C.* “kaimai” and *C.* “veil”; A) Habitat of *C. iridescens* and *C.* “whiskers” in the lowlands of Pirongia Forest Park, Waikato. B) Study site of *C. iridescens* and *C.* “whiskers”. C) Study site and habitat of *C.* “kaimai” at Kauaeranga Valley, Coromandel Peninsula. D) Study site and habitat of *C.* “veil” at Waireinga/Bridal Veil Falls, Waikato. White arrows indicate location of colony.

3.3.2 Sampling methods

3.3.2.1 Morphology

Measurements of 55 *in situ* plants, summarised in Table 3.3, were made using digital callipers to make measurements of 24 precisely defined continuous characters (Fig. 3.7, Fig. 3.8, Appendix 2), with sub-millimetre precision for each flowering specimen, avoiding destructive sampling wherever possible. The measurements are made of continuous characters that intend to be as unambiguous as possible, by being precisely definable structural aspects of the plants, and are required to be applicable to all populations and species examined in this research. Flowering specimens approaching senescence were avoided. All photographs not otherwise attributed were taken for comparative analysis *in situ* with a Nikon S2500HD digital camera by Abraham Coffin. These photographs were taken in natural light, sometimes with the aid of a handheld lamp to ensure quality photographs.

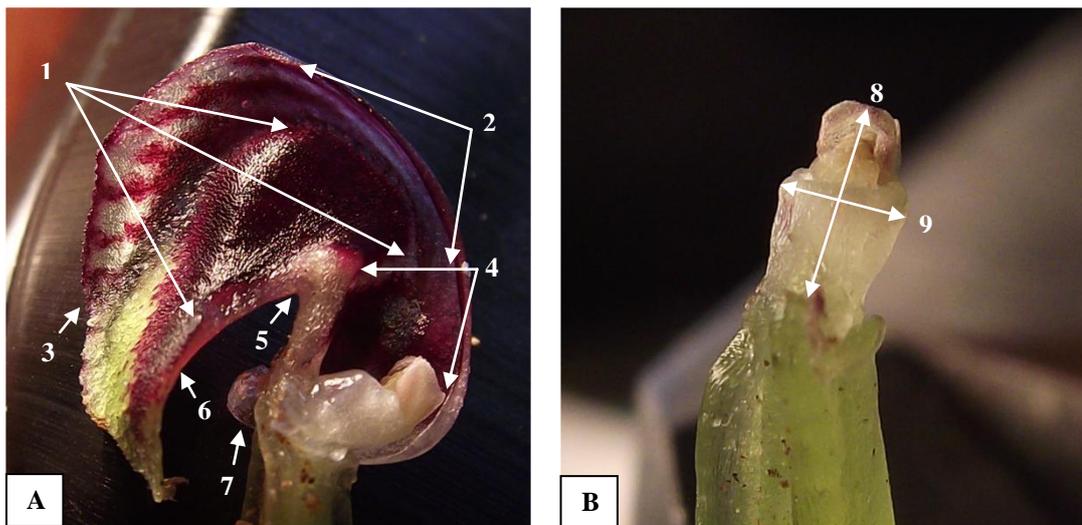


Figure 3.7; A) Side view of longitudinal section of *C. "pollok"* from near Pollok, Awhitu Peninsula, with lateral sepals and petals excised: 1) Labellum furrow, 2) Labellum wing tube, 3) Labellum wing-bib transition, 4) Column chamber, 5) Inner flexure, 6) Outer flexure, 7) Auricle. B) Ovary and column of *C. "pollok"*: 8) Column length, 9) Column width.

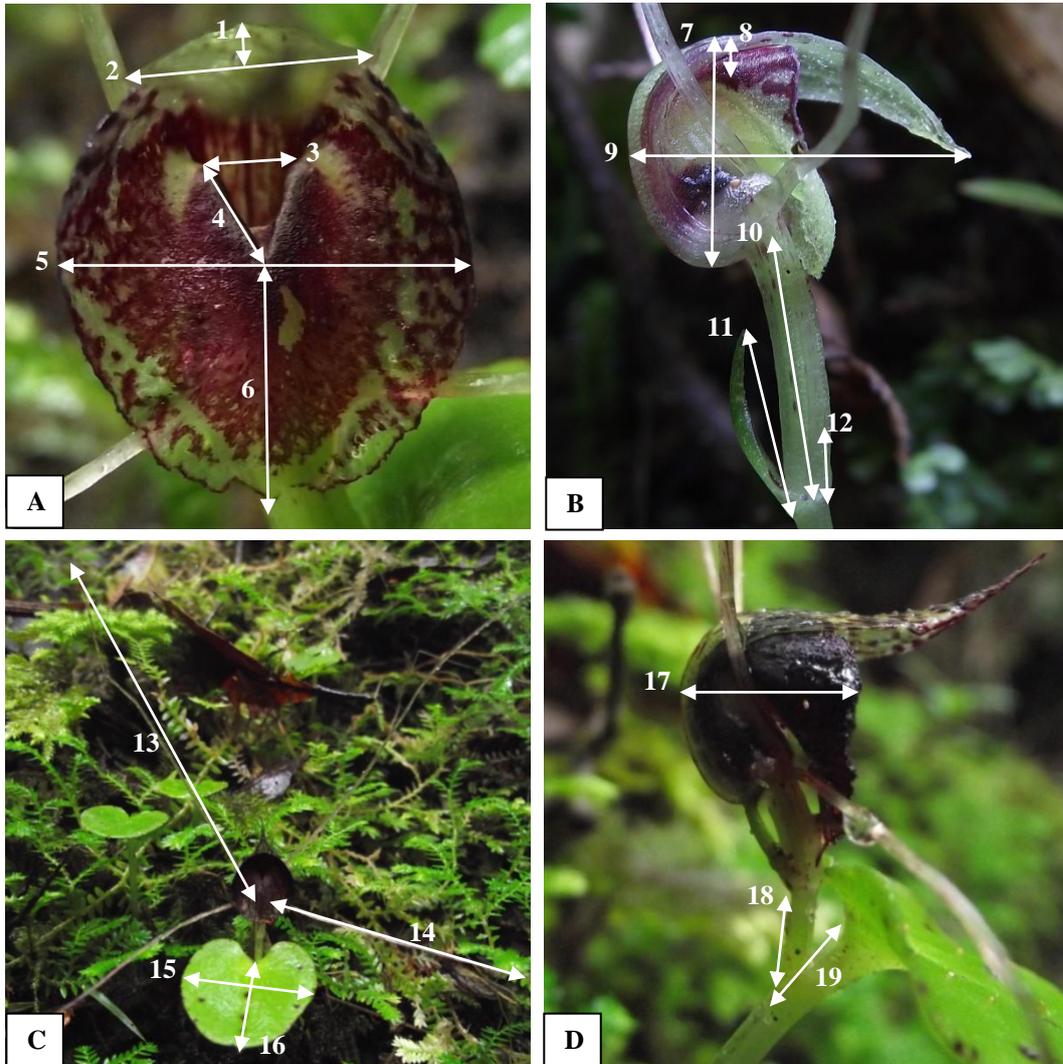


Figure 3.8; A) Close frontal view of *C. "veil"*: 1) Dorsal sepal depth, 2) Dorsal sepal width, 3) Labellum furrow width, 4) Labellum furrow height, 5) Labellum width, 6) Labellum bib length. B) Profile of *C. "whiskers"* flower: 7) Flower height, 8) Labellum wing height, 9) Flower length to dorsal sepal, 10) Ovary length, 11) First floral bract length, 12) Second floral bract length. C) Frontal view of *C. iridescens*: 13) Lateral sepal length, 14) Lateral petal length, 15) Leaf lamina width, 16) Leaf lamina length. D) Side view of *C. iridescens*: 17) Flower length to labellum, 18) Flower peduncle length, 19) Leaf petiole length.

**Table 3.3; A summary of character state averages (above) with minimum and maximum for each respective entity (in brackets below).
Sample sizes (n) are the total for each of the entities, including those from multiple populations.**

	Stem Length	Leaf Petiole Length	Peduncle Length	Ovary	First Bract	Second Bract	Leaf Length	Leaf Width	Basal lobe depth	Basal lobe width	Dorsal Sepal width	Dorsal sepal depth	Furrow height	Furrow width	Lateral sepal	Lateral petal	Labellum width	Bib length	Column length	Column width	Wing height	Flower length - Labellum	Flower height	Flower length - DS
<i>C. rivularis</i> n=11	20.35 (9.4-42.5)	3.33 (2.2-4.2)	5.42 (3.4-7.1)	7.7 (5.9-9.6)	6.92 (5.7-8)	2.98 (1.7-5.6)	20.62 (17.4-23)	21.19 (16.7-25.3)	3.44 (2.3-5.1)	8.49 (5.9-13)	4.81 (4.1-5.8)	1.55 (0.9-2.3)	5.61 (5.1-6.4)	2.07 (1.7-2.4)	35.28 (26.2-42)	28.8 (24.3-37)	7.52 (7.1-8)	3.28 (2.8-4)	2.32 (2-2.7)	1.35 (1-1.5)	1.69 (1.3-2.2)	14.11 (10.5-16.4)	9.83 (8.2-10.7)	9.57 (8-11.8)
<i>C. "whiskers"</i> n=11	22.8 (13.9-54.6)	1.72 (0.7-28)	3.64 (2.9-4.7)	6.66 (5.1-8.7)	6.26 (5.3-7.3)	2.36 (0.95-3)	17.57 (13.8-21.7)	20.98 (15.1-25.5)	4.35 (3.3-5.6)	5.94 (3.7-7.9)	5.24 (4.6-5.9)	2.28 (1.2-2.9)	4 (3.5-4.35)	1.59 (1.1-1.8)	35.41 (26-41.4)	25.15 (20.5-30.1)	5.72 (5.3-6.2)	3.74 (3.2-4.1)	2.11 (1.7-2.4)	1.24 (1.2-1.3)	1.47 (1.14-1.85)	11.46 (10.3-12.8)	8.49 (7.46-9.1)	7.85 (5.69-9.25)
<i>C. "kaimal"</i> n=15	16.26 (1.6-43.3)	1.82 (0.7-2.4)	4.41 (2.3-6.5)	6.39 (5.4-7.6)	5.38 (4.1-6.3)	2.81 (1.7-4.35)	20.05 (10.7-27)	22.59 (13.6-29.1)	4.3 (2.2-6.3)	9.07 (4.9-14.1)	4.31 (3.8-4.8)	1.76 (0.8-2.7)	5.16 (4.7-5.8)	2.11 (1.6-2.3)	38.61 (29.2-51.7)	27.94 (19.7-35.1)	6.02 (5.2-6.8)	3.26 (2.5-4.2)	2.29 (2-2.7)	1.28 (1.1-1.5)	2.06 (1.2-2.7)	12.97 (9.1-14.1)	9.84 (8-11.7)	7.66 (6.8-8.7)
<i>C. "pollok"</i> n=3	22.02 (20.2-22)	2.68 (2.5-2.8)	5.58 (4.5-7.2)	7.58 (6.9-8.7)	5.19 (3.8-6.2)	1.58 (0.7-3)	17.32 (17.8-18.1)	19.14 (17.4-20.6)	4.6 (4-4.9)	8.04 (7.3-8.7)	4.62 (3.8-5.4)	1.87 (1.7-2.1)	2.64 (2.6-2.7)	1.26 (0.8-1.6)	41.7 (32.4-48.5)	28.5 (22-38.4)	5.03 (4.2-5.8)	4.24 (3.9-4.7)	2.62 (2.2-3.1)	1.16 (0.9-1.45)	1.8 (1.7-2)	13.31 (-)	7.2 (-)	- (-)
<i>C. "katarakih"</i> n=3	2.81 (2.1-3.5)	1.99 (1.7-2.3)	1.81 (1.2-2.8)	5.83 (5.5-6.3)	4.1 (3.9-4.6)	1.52 (1-2)	9.47 (8.3-10.1)	11.66 (10.2-12.4)	1.91 (0-2.9)	2.85 (0.8-4.4)	3.48 (2.2-4.4)	1.63 (1-2)	3.06 (2.8-3.3)	2.07 (1.8-2.3)	32.79 (30.8-35.3)	23.79 (22.7-25.1)	8.04 (6.9-9)	5.53 (4.8-6.1)	1.67 (1.3-2.1)	1.18 (1-1.3)	1.27 (1.1-1.5)	14.59 (14-15.2)	10.08 (9.8-10.5)	7.73 (5.7-10.3)
<i>C. "veil"</i> n=2	14.62 (0.1-29.1)	4.14 (4-4.3)	2.44 (-)	5.85 (5.5-6.2)	3.64 (2.4-5)	1.3 (-)	14.41 (10.4-18.4)	17 (12.6-21.4)	4.3 (3-5.6)	7.16 (5.9-8.4)	5.06 (4.9-5.3)	1 (-)	3.53 (3.4-3.6)	2.73 (2.7-2.8)	32.62 (30.2-35)	23.03 (20.2-25.3)	8.15 (7.7-8.6)	6.3 (6.1-6.5)	2.33 (2.2-2.5)	1.48 (1.44-1.52)	3.6 (3.58-3.61)	14.15 (12.3-16)	11.35 (10.6-12.1)	- (-)
<i>C. iridescens</i> n=4	21.81 (-)	4.58 (3.1-6.3)	4.98 (4-6.3)	10.15 (9.6-10.8)	9.03 (9-9.1)	0 (-)	22.8 (18.9-30.3)	25.42 (19.8-30.3)	8.04 (7.7-8.4)	9.91 (9.5-10.3)	7.08 (6.7-7.9)	3.25 (3-3.5)	3.18 (3-3.5)	1.73 (1.7-1.8)	64.41 (55.6-74.1)	51.28 (42.7-57.7)	10.85 (10.3-11.5)	9.48 (8.3-10.9)	2.29 (2.25-2.33)	1.58 (1.51-1.65)	3.35 (3.34-3.36)	20.82 (20.1-21.8)	10.34 (9.6-11.1)	9.7 (9.4-10.1)
<i>C. orbiculatus</i> n=1	26.44 (-)	5.11 (-)	2.76 (-)	5.4 (-)	7.31 (-)	1 (-)	24.26 (-)	21.48 (-)	5.49 (-)	7.39 (-)	5.27 (-)	0 (-)	4.43 (-)	0 (-)	24.91 (-)	19.03 (-)	13.66 (-)	10.48 (-)	3.09 (-)	1.28 (-)	3.19 (-)	18.21 (-)	12.84 (-)	9.84 (-)
<i>C. papa</i> n=5	33.85 (29.5-39.9)	0.75 (0.4-1.2)	3.45 (2.3-3.9)	6.53 (5.2-8.3)	6.5 (6.1-7.1)	2.04 (1.8-2.2)	17.24 (16.2-18.2)	20.95 (19.1-23.4)	6.34 (5.1-7.2)	4.84 (3.4-7.7)	5.03 (4.4-5.6)	1.92 (1.7-2.2)	3.29 (2.9-3.7)	2.08 (1.9-2.2)	42.42 (32.8-58.2)	28.87 (25.9-32)	6.63 (6.2-7)	4.81 (4.7-5)	2.32 (-)	1.37 (-)	2.16 (1.3-2.8)	11 (10.2-11.8)	9.75 (8.9-10.7)	6.26 (5.4-7)

3.3.2.2 DNA analyses

Fresh leaf material was collected from flowering individuals, usually the same individuals that have been used for morphological measurements. A total of 52 leaf samples were obtained from 7 species and tag-named populations across 10 sites, with between 2-5 samples per species/tag-named population per site. Half a leaf of material was obtained and placed into labelled coffee filters then placed into a zip lock plastic bag with desiccant silica gel crystals in the field to ensure rapid drying. The number of leaves sampled from each of the populations is shown in Table 3.2 and a summary of all samples used in the analysis is shown in Table 3.4. Individuals sampled were photographed, as we were not permitted to collect herbarium vouchers under our Department of Conservation permit.

Total genomic DNA was extracted from the desiccated leaf material using a Bioline Isolate II Plant DNA Kit (Bioline, Alexandria, NSW, Australia) in accordance with the protocol provided by the manufacturer. To amplify the ITS region, PCR was performed in a total volume of 20 μ l consisting of 12.6 μ l of purified MQH₂O, 5 μ l of MyTaq Reaction Buffer (Bioline) at 10 μ M, 0.5 μ l of ITSHP5 primer at 10 μ M, 0.5 μ l of ITS4 primer at 10 μ M, 0.2 μ l of 1% Bovine Serum Albumin (BSA), 0.2 μ l of MyTaq polymerase and 1.0 μ l of total genomic DNA. The final concentrations in the 20 μ l volume were: primer concentrations were each 0.25 μ M, MyTaq Reaction Buffer was 2.5 \times , BSA was 0.01% and MyTaq polymerase was 0.05U. PCR was performed in an Eppendorf Mastercycler $\text{\textcircled{R}}$ pro thermocycler. The temperature profile was as follows: initial denaturing at 94 $^{\circ}$ C for 5 min, then 36 cycles of 94 $^{\circ}$ C for 30 sec, annealing at 51 $^{\circ}$ C for 30 sec and extension at 72 $^{\circ}$ C for 30 sec. The reactions are finalised with an extension for 10 minutes at 72 $^{\circ}$ C. To confirm amplification of the PCR products, a 1% agarose gel with 0.5X TBE was made using 1 μ l of RedsafeTM (iNtRON Biotechnology Inc.) and 3 μ l of PCR product from each of the samples were added to wells. A 100 base pair DNA ladder (InvitrogenTM) was also placed into a well. The gel was run for 55 minutes at 44v then imaged in UV light using an Innotech AlphaimagerTM. The PCR products were purified prior to sequencing with ExoSAP utilising the following protocol; 1 μ l Exonuclease I (Exo; Illustra), 1 μ l Shrimp Alkaline Phosphatase (SAP; Illustra) and 5 μ l of PCR product are all placed into 0.2ml reaction tubes then subjected to 37 $^{\circ}$ C for 15 min followed by

80°C for 15 min. The resulting purified PCR product containing the amplified sequences were sent to the Waikato DNA Sequencing Facility for sequencing in an Applied Biosystems™ 3130XL Genetic Analyzer that is fitted with 50cm capillary arrays. The sequencing reactions are performed utilising Applied Biosystems™ Big Dye v3.1 dye terminator chemistry.

Table 3.4; A summary of the 36 samples used in the molecular analysis of ITS sequences. * These samples have provenance outside of New Zealand territory

Taxa	Location	Genbank Accession #	Sample #
<i>Corybas acuminatus</i>	Kauaeranga Valley, Coromandel Forest Park	XXXXXXXXXX	Acu1
<i>Corybas acuminatus</i>	Otira, West Coast	DQ784551.1	
<i>Corybas dienemus</i>	Bauer Bay, Macquarie Island*	DQ784553.1	
<i>Corybas dienemus</i>	Handspike Point, Macquarie Island*	DQ422132.1	
<i>Corybas dienemus</i>	Bauer Bay, Macquarie Island*	DQ784552.1	
<i>Corybas dienemus</i>	Eagle Point, Macquarie Island*	DQ422134.1	
<i>Corybas dienemus</i>	Bauer Bay, Macquarie Island*	AF391770.1	
<i>Corybas dienemus</i>	Green Gorge, Macquarie Island*	DQ422133.1	
<i>Corybas hypogaeus</i>	Wairarapa	DQ784547.1	
<i>Corybas hatchii</i>	Central North Island	DQ584549.1	
<i>Corybas iridescens</i>	Kaniwhaniwha Valley, Pirongia Foerest Park.	XXXXXXXXXX	Ir1
<i>Corybas iridescens</i>	-	AF391772.1	
<i>Corybas iridescens</i>	Taranaki	DQ784548.1	
<i>Corybas macranthus</i>	Ashley River, Canterbury	AF348010.1	
<i>Corybas macranthus</i>	View Hill, Canterbury	DQ784554.1	
<i>Corybas macranthus</i>	Trotters Gorge, Otago	DQ784555.1	
<i>Corybas macranthus</i>	Northland, North Island	DQ784550.1	
<i>Corybas orbiculatus</i>	Ashley river, Canterbury	AF391775.1	
<i>Corybas papa</i>	Taranaki	AF391776.1	
<i>Corybas rivularis</i>	-	AF391778.1	
<i>Corybas rivularis</i>	Waipapa river track, Puketi Forest Park	XXXXXXXXXX	R1
<i>Corybas rivularis</i>	Waipapa river track, Puketi Forest Park	XXXXXXXXXX	R2
<i>Corybas</i> “whiskers”	Kaniwhaniwha Valley, Pirongia Forest Park	e	NCS1
<i>Corybas</i> “kaitarakihi”	Kaitarakihi Bay, Huia.	XXXXXXXXXX	Kait4
<i>Corybas</i> “kaimai”	Kauaeranga Valley, Coromandel Forest Park	XXXXXXXXXX	K7
<i>Corybas</i> “kaimai”	Kauaeranga Valley, Coromandel Forest Park	XXXXXXXXXX	S4-2
<i>Corybas</i> “pollok”	Pollok, Awhitu Peninsula	XXXXXXXXXX	AC4
<i>Corybas</i> “veil”	Waireinga / Bridal Veil Falls Scenic Reserve	XXXXXXXXXX	V1
<i>Corybas</i> “veil”	Waireinga / Bridal Veil Falls Scenic Reserve	XXXXXXXXXX	V2
<i>Corybas sulcatus</i>	Sawyer Creek Valley, Macquarie Island*	DQ422135.1	
<i>Corybas sulcatus</i>	Sawyer Creek Valley, Macquarie Island*	DQ784558.1	
<i>Corybas sulcatus</i>	Sawyer Creek Valley, Macquarie Island*	DQ422136.1	
<i>Corybas aff. trilobus</i>	Chatham Islands	DQ784561.1	
<i>Corybas trilobus</i>	North Island	DQ784559.1	
<i>Corybas trilobus</i>	Marble Point, Hamner Forest Park	AF391780.1	
<i>Corybas trilobus</i>	Erua, Erua Conservation Area	DQ784560.1	

3.3.3 Analysis of data

3.3.3.1 Analysis of morphology data

In order to factor in multiple measurements simultaneously, multivariate analyses are performed. To do this, the recorded measurements are collated into a single spreadsheet (Appendix Table A.1). A Linear Discriminant Analysis (LDA) is performed to determine if there are any characters that can discern natural groups. There were measurements made of 24 characteristics, however, only 9 characters were able to be measured in all 55 of the plants that were sampled, and LDA requires complete datasets. A distance matrix of informative characters as determined by the LDA was created using Dell Statistica version 12.5 (Dell Corporation), and was used to run a cluster analysis using Unweighted Pair-Group Method with Arithmetic mean (UPGMA), with Euclidean distances. These analyses were used owing to their use in other morphological and taxonomic studies and for the ease of implementing continuous characters for analysis (Fernández-Concha *et al* 2009, Shaiju & Omanakumari 2010, Anilkumar & Murugan 2014, Bunpha *et al.* 2014).

3.3.3.2 Analysis of molecular data

There were in total 11 bidirectional sequences of the complete ITS region, representing all of the study taxa, except for *C. papa* and *C. orbiculatus*. These were aligned and edited to amend sequencing call errors using Sequencher v.5.3 (Gene Codes Corporation). 25 sequences of *Corybas* (subgen. *Corysanthes*, sect. *Nematoceras*) taxa were obtained from NCBI GenBank® databases and along with the 11 molecular sequences obtained from field samples, all 36 sequences were compiled into a multiple sequence alignment matrix, at 675 base pairs in length. The optimum alignment was performed on the matrix in Seaview 4.5.4 (Galtier *et al.* 1996, Gouy *et al.* 2010) using the MUSCLE alignment algorithm (Edgar 2004). A BEAST xml file was created using BEAUti v.1.7.5 (Drummond & Rambaut 2007) with the parameters; Sites using the substitution model TN93, Trees using the Yule process with a random starting tree, and the Markov-chain length was set at 50 million generations. A Bayesian analysis was performed using Bayesian Analysis Sampling Trees (BEAST) ver. 1.7.5 (Drummond & Rambaut, 2007). TreeAnnotator ver. 1.7.5 was used to render a consensus tree

with a 10% burn-in. FigTree v.1.4.0 was used to format the final tree for publication.

3.4 Results

3.4.3 Results of morphological analysis

Linear Discriminant Analyses were performed to test the assumption the species and tag-named populations are distinct. The 9 characters that had no missing data across all 55 samples, labellum width, labellum furrow height, labellum bib length, leaf length, leaf width, lateral sepal length, lateral petal length, second bract and flower petiole length, were able to discriminate 50 into the correct assigned group (Fig 3.9). The analysis determined the most informative characters to be three aspects of the labellum; The labellum furrow height, labellum width and labellum bib length. These characters are summarised in Figure 3.10. An LDA performed with only these three characters was also able to correctly assign 50/55 samples, demonstrating the majority of the consistent variation between groups to be explained by these characters alone. In order to utilise all characters, missing data would need to be amended with group dataset averages. When this was done with 24 characters it was able to correctly discern 52/55 samples. However, the 21 extra characters were only able to provide a marginal increase in resolution while using group dataset averages indicates these extra characters are not particularly informative.

The UPGMA was performed using the three most informative characters; The labellum furrow height, labellum width and labellum bib length (Figure 3.11). All studied entities and species formed distinct, monophyletic clusters, with the exception of *C. "veil"* and *C. "kaitarakihi"*, which together have formed a distinct, monophyletic cluster. *C. papa* and *C. "pollok"* formed a cluster alongside a cluster consisting of *C. rivularis*, *C. "whiskers"* and *C. "kaimai"*, in which the latter two are subordinate to *C. rivularis*. *C. papa*, *C. "pollok"*, *C. "whiskers"*, *C. "kaimai"* and *C. rivularis* all form a greater cluster, subordinate to the cluster consisting of *C. "kaitarakihi"* and *C. "veil"*, which in turn is subordinate to a cluster consisting of *C. iridescens* and *C. orbiculatus* respectively.

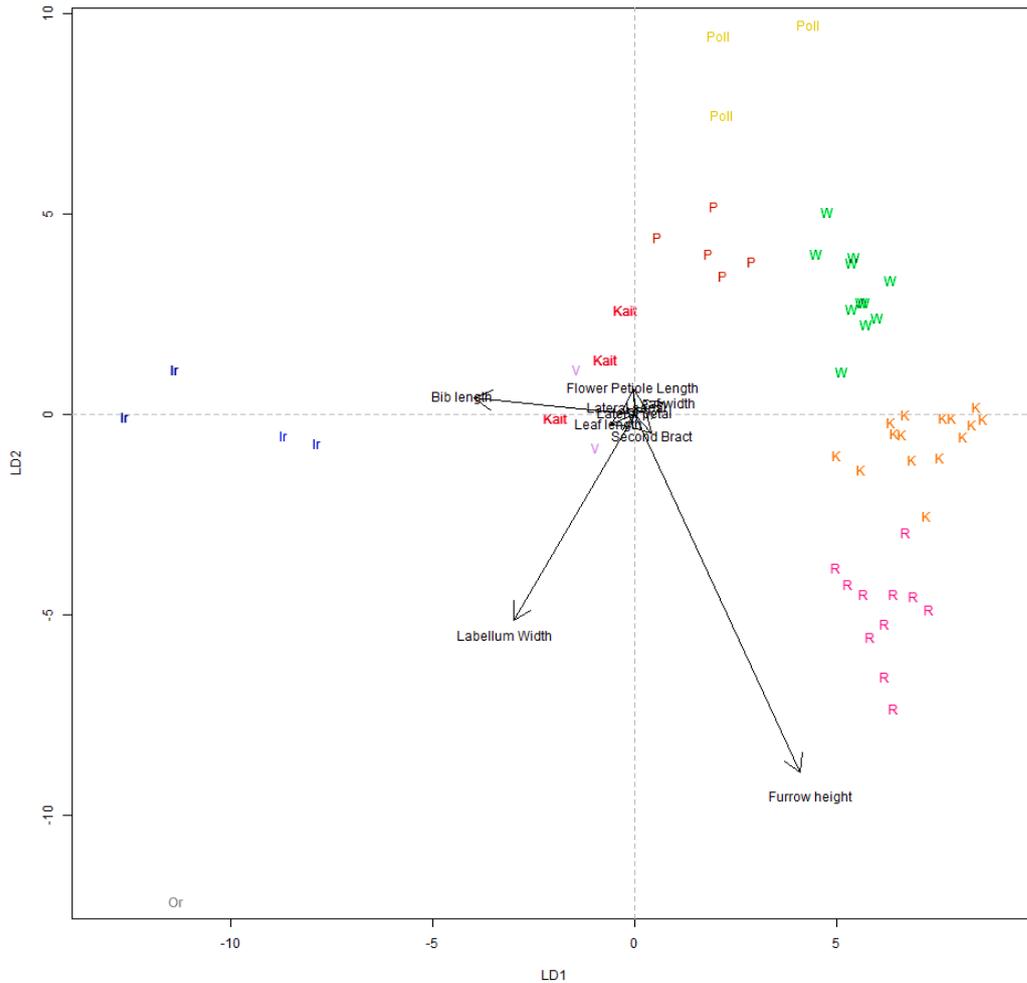


Figure 3.9; Linear Discriminant Analysis plot of all samples using 9 characters; Labellum Width, Furrow Height, Bib Length, Leaf Length, Leaf Width, Lateral Sepal, Lateral Petal, Second Bract and Flower Petiole Length. Or = *C. orbiculatus*. Ir = *C. iridescens*. Kait = *C. "kaitarakihi"*. V = *C. "veil"*. R = *C. rivularis*. K = *C. "kaimai"*. W = *C. "whiskers"*. P = *C. papa*. Poll = *C. "pollok"*.

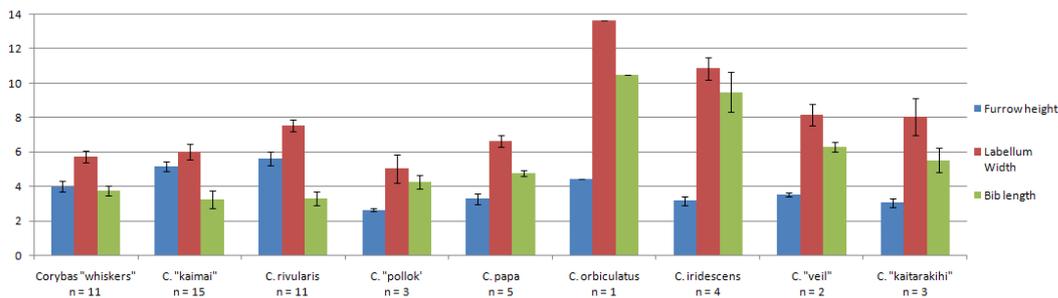


Figure 3.10; A bar graph of the means, with standard deviations of the labellum furrow height, labellum width and bib length for the all studied entities and species. All measurements are in millimetres. n = sample sizes.

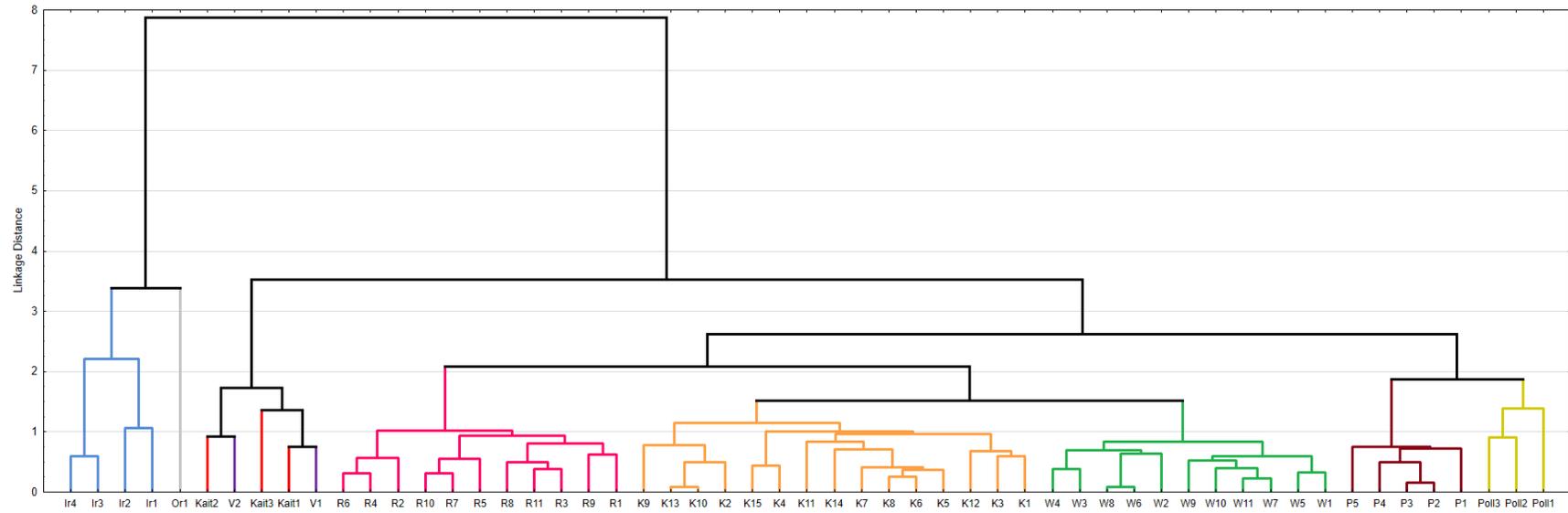


Figure 3.9; UPGMA diagram of all *Corybas* species and entities studied using three characters: Labellum width, Bib length and Furrow height. Or = *C. orbiculatus*. Ir = *C. iridescens*. Kait = *C. “kaitarakihi”*. V = *C. “veil”*. R = *C. rivularis*. K = *C. “kaimai”*. W = *C. “whiskers”*. P = *C. papa*. Poll = *C. “pollok”*.

3.4.4 Results from phylogenetic analysis of ITS molecular sequences

The tag-named populations *Corybas* “veil” and *C.* “kaitarakihi” have identical ITS sequences to *C. iridescens*, *C. dienemus*, and *C. hatchii*. *C. orbiculatus* was sister to these with a single base pair substitution at site #659 (Appendix Table A.2). All together these form a well supported clade with Posterior Probability (PP) = 1 (Figure 3.12).

Corybas “whiskers”, *C.* “kaimai” and *C.* “pollok” share identical sequences, while the two samples of *C. rivularis* from Puketi both share a single base pair substitution at site #653. *C. papa* appears sister to these, with 6 base pair substitutions at sites #27, #39, #117, #143, #598, #618. Together these form a well supported clade (PP 0.98).

The two aforementioned clades together form the ‘rivulare’ clade, or the broadly conceived *C. aff. rivularis* (Clements *et al.* 2007) which was found to be supported by this analysis (PP 0.84). The basal position of *C. acuminatus* is well supported (PP 1). The ‘macranthum’ clade is well supported (PP 1), and is comprised of two well supported clades. One is *C. macranthus* (PP 0.98), the other (PP 1) is composed of *C. trilobus*, *C. hypogaeus* and *C. sulcatus*. *C. sulcatus* is weakly supported (PP 0.74), sister to a well supported clade consisting of *C. trilobus* and *C. hypogaeus* (PP 0.97)

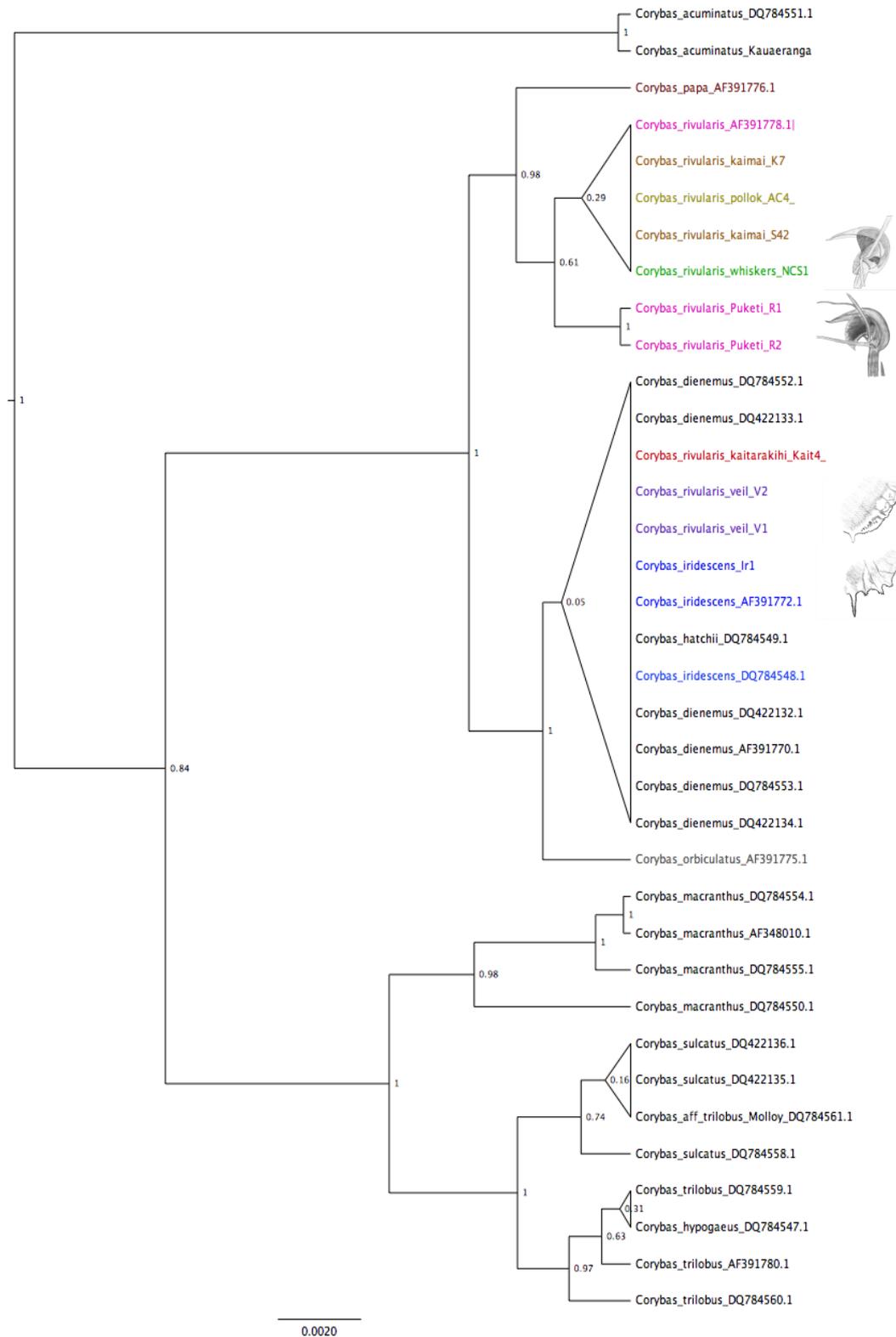


Figure 3.10; A dendrogram of 36 samples of sect. Nematoceras, including species and tag-named populations, based on a BEAST (Bayesian) analysis of ITS sequences. Node labels show posterior probabilities. Drawings aside samples depict morphological differences.

3.5 Discussion

Eight distinct groups were identified from the nine species and tag-named populations studied in the morphological analyses. *Corybas* “veil” and *C.* “kaitarakihi” are almost indistinguishable and together form a distinct cluster.

There was no fundamental incongruence between the molecular phylogeny and the morphological UPGMA cluster analysis except for *Corybas* “pollok” pairing with *C. papa* in the morphological analysis, but the single *C. papa* sample appears sister to *C. rivularis*, *C.* “kaimai”, *C.* “whiskers” and *C.* “pollok” in the molecular analysis of ITS sequences.

3.5.3 Morphological analysis of continuous characters

This theme is present in the results of this morphological analysis; Taxa aligned to *Corybas iridescens* in the molecular results tend to have a distinct, longer leaf petiole, proportionally small labellum furrow, and large bib length and labellum width. Conversely, taxa aligned to *C. rivularis* tend to have a leaf that is sessile or nearly so, a proportionally large furrow and a small bib and labellum width. The key differences are summarised in Table 3.5.

Table 3.5; Summarised morphological differences observed between all species and tag-named populations studied in this research.

Species / tag-name	Furrow height	Flower width /size	Bib length	Unique characters
<i>Corybas rivularis</i>	Large	Medium	Very Short	Singly-deflexed labellum, Dorsal sepal barely exceeds labellum
<i>C. iridescens</i>	Small	Large	Very Long	Large maroon flower with papillae, with irregularly lobed labellum
<i>C. orbiculatus</i>	Small-Medium	Large-Very Large	Very Long	Very long and wide maroon labellum with incurved margins.
<i>C. papa</i>	Small-Medium	Small-medium	Medium	Translucent green labellum, flower compressed front to back.
<i>C.</i> "whiskers"	Medium-Large	Small-medium	Short	Doubly reflexed labellum with profuse papillae on green bib.
<i>C.</i> "kaimai"	Large	Medium	Short	Forward leaning wing-tube, weakly doubly reflexed labellum
<i>C.</i> "pollok"	Medium-Small	Small	Short-medium	Small flower with maroon furrow with white labellum bib
<i>C.</i> "kaitarakihi"	Small	Medium-Large	Long	Crenate labellum margins, white stripe from furrow to column chamber
<i>C.</i> "veil"	Small	Medium-Large	Long	Crenate labellum margins, white stripe from furrow to column chamber

The tag-named populations *Corybas* “veil” and *C.* “kaitarakihi” together have formed a monophyletic clade. There are considerable similarities between the two entities in addition to the quantified continuous characters that are distinct from *C. iridescens*; A reduced column chamber, wing-tube, bib apicule, bib length and width. In addition; a common theme of maroon patterning on the labellum wings and bib. There is a well defined translucent stripe inset from the margin that runs either side of the bib apex up to the labellum wing lobes. The area between this stripe and the bib margins is peppered with blotchy maroon. There is also a white stripe that runs on either side from the furrow tops down to the column chamber. This stripe is visible from outside the flower on profile. The labellum wing lobes extend forward considerably less than *C. iridescens* and are blotchy maroon in a manner that resembles leopard spots. Superficially these entities appear to be essentially the same plant, and the combined molecular and morphological evidence suggests the same.

Corybas “whiskers”, *C.* “kaimai” and *C.* “pollok” have a distinctively recurved labellum wing-bib transition margin that is not present in *C. rivularis* or *C. papa*. *Corybas* “whiskers” and *C.* “kaimai” are distinct, but closely allied. They tend to have a similar habit, form and maroon patterning, however, there are distinctive differences; *C.* “kaimai” has a less developed wing-tube, which leans well forward, a longer furrow which extends beyond the ovary with a less acutely reflexed inner labellum flexure, longer, more narrow wing-lobes and a shorter labellum bib conversely to *C.* “whiskers”, which has a shorter, more steep furrow, a sharply reflexed inner labellum flexure, shorter, wider labellum wing lobes that terminate abruptly at the labellum wing to bib transition, and a longer bib with more profuse, longer papillae concentrated on the bib. *C.* “whiskers” has a distinctive column chamber when viewed in profile. *C.* “kaimai” tends to have a mildly recurved labellum wing-bib transition margin followed by a weakly incurved - flared bib margin that then recurves again for the recurved apex. This gives a mild appearance of an undulating labellum margin. The maroon patterning of *C.* “kaimai” tends to be more irregular and profuse, particularly on the dorsal sepal and bib. Although following a similar theme, *C.* “whiskers” tends to have considerably less variation in this regard, with maroon generally confined to the wing lobes, wing-tube and column chamber.

Corybas papa is a notably green flowered species that can tend to resemble *C. “whiskers”* superficially when not examined closely. In the analysis of continuous characters the samples of *C. papa* produced a cluster paired with *C. “pollok”*. The key features of *C. papa* are; a very small furrow, a near absence of papillae from bib, the flower is compressed front to back with a shorter flower length and a poorly defined column chamber. The maroon is quite distinctive and is generally confined to the furrow tops, leading down the wing-tube to the column chamber. It is notably absent from the wing and wing-tube margins. There is a tendency for a minute speckling of the furrow with maroon and rarely has a fully maroon furrow as in *C. “pollok”*. The pairing of *C. papa* and *C. “pollok”* may indicate similarities in the proportions of the characteristics used in the analysis, but there are differences between them that make it unlikely to be an inference of phylogeny; both *C. papa* and *C. “pollok”* have different habits and appearances to each other; *C. “pollok”* seems to have obtained its proportions by reducing to a diminutive form, and is quite anomalous. It has recurved wing-bib transitions in a position that resembles both *C. “whiskers”* and *C. “kaimai”*. The maroon is predominantly in the same places as *C. “whiskers”*, excepting for a ubiquitous maroon furrow. The usefulness of this completely maroon furrow is limited by the fact that *C. papa* and *C. “kaimai”* both sporadically can have the same maroon furrow.

There were a number of confounding factors that made vegetative characters less informative than floral characters. Phenotypic plasticity is the variability of the phenotype of an organism due to environmental factors, and if care is not taken during sampling and analysis, phenotypic plasticity can be taxonomically misleading (Pedersen 2010). A case study of orchid species *Liparis resupinata* Ridl. indicated large co-variation within the vegetative characters, and a relative independence between the vegetative and floral characters, with little co-variation. Further, the study found leaf characters to be more influenced by ecological parameters than the floral characters were (Tetsana *et al.* 2014, Price & Weitz 2012). A study examining the influence of sampling strategy on detecting morphological variation in orchids found the combined use of dried and preserved herbarium specimens with fresh material to be undesirable, and that a greater number of populations from a wide ecological and geographic range is preferable

to large sample sizes of fewer populations from a limited geographic area, where possible (Bunpha *et al.* 2014).

The morphological component of the study intended to examine the level of diversity among the entities more so than to determine the phylogeny. While the method employed in this study has been used for phylogenetic studies (Fernández-Concha *et al.* 2009, Shaiju & Omanakumari 2010, Anilkumar & Murugan 2014), it is more informative in determining the rank of species and below (Scotland *et al.* 2003).

3.5.4 Molecular analysis of ITS sequences

Corybas iridescens, *C.* “veil”, *C.* “kaitarakihi” and *C. hatchii* together form an unresolved group owing to identical ITS sequences, with *C. orbiculatus* sister owing to a single base pair substitution. *C.* “whiskers”, *C.* “kaimai” and *C.* “pollok” form an unresolved group also, owing to identical sequences. These three entities are sister to a well supported *C. rivularis* from Puketi Forest Park in Northland, which has a single base pair substitution. *C. rivularis* from Puketi Forest Park in Northland has shown to have a base pair substitution that is not present in the Genbank sample purportedly of *C. rivularis* from Taranaki. The Taranaki *C. rivularis* instead has an identical sequence to *C.* “whiskers”, *C.* “kaimai” and *C.* “pollok”. *C. papa* is sister to *C. rivularis*, *C.* “whiskers”, *C.* “kaimai” and *C.* “pollok”.

Molecular studies examining the ITS region are widespread, including research into orchids and taxonomy (Bateman *et al.* 2006, Clements *et al.* 2007, Fernández-Concha *et al.* 2009). It is quite clear however that analysis of the ITS region alone is insufficient for this group of orchids to resolve at the level required to demonstrate some individual entities. *Corybas dienemus* was described using ITS sequences despite having no molecular variation in this region from *C. iridescens*, but only in conjunction with a thorough morphological description. This region has proven most useful in this group at determining the broader phylogeny at what should be the sub-section and series levels.

The most useful discoveries of the molecular analysis were; there are two, non-intergrading clades of taxa within the broader ‘rivulare’ clade. The entities *C. “veil”* and *C. “kaitarakihi”* are demonstrably most closely allied to *C. iridescens* and *C. orbiculatus*.

3.6 Conclusions

The objectives this study sought to determine was whether *Corybas rivularis* is distinct from the undescribed tag-named populations based on morphological and molecular variation. All have been demonstrated to be distinct from a morphological basis, with the exception of *C. “kaitarakihi”* and *C. “veil”* which together have formed a novel distinct group.

Corybas “kaitarakihi” and *C. “veil”* are found to be indistinct with respect to each other both morphologically and molecularly, but together are morphologically distinct from all other entities. These together should be afforded a formal taxonomic rank. If these entities are regarded as a single species, there are implications for conservation. *C. “kaitarakihi”* is currently regarded as Nationally Critical, with the criteria that it occupy less than one hectare, and in one location. With no less than two disjunct populations a newly recognised species could possibly qualify as Nationally Endangered, provided that the combined population was stable and exceeded 250 individuals (Townsend *et al.* 2008). Defining individuals in clonal colonies is difficult however and it may be that there are fewer than 250 individuals that each has a unique, seed-derived genesis.

Corybas “whiskers” and *C. “kaimai”* are both morphologically distinctive, and are closely allied to *C. rivularis sensu stricto*. These are distinctive enough with well defined morphological boundaries that warrant a formal taxonomic rank and description. *C. “pollok”* is anomalous and appears distinct to *C. “whiskers”* mostly due to being diminutive. The sample size was only three flowers from one site and the molecular analysis does not show it to be particularly distinctive. It may have merit, but the analysis was not thorough enough to confirm. It may be best suited to a sub-specific rank, perhaps sub-ordinate to *C. “whiskers”* once it has been afforded a taxonomic rank.

Any future tag-named populations that have morphological characteristics closer to *Corybas iridescens* than to *C. rivularis* should be considered affinities to *C. iridescens*. e.g. *Corybas* aff. *iridescens*, and not be referred to as *C. aff. rivularis*.

There are some recommendations for future research into this group; The use of ITS molecular sequences is proving insufficient to account for the morphological diversity apparent among forms. A combination of molecular regions, or molecular fingerprinting methods such as AFLP need to be examined, with greater representation of all of the respective forms with as many samples from as many populations as possible.

More molecular sequences of *Corybas papa* need to be obtained to test the result of the basal position in the *C. aff. rivularis* clade of the one sample used in the analysis. Samples from the Te Henui *C. rivularis* should be examined in the same approach as in this study, to see if they align more closely with the far north *C. rivularis* than other populations. *C. hatchii* was not found in any of the three field trips to Waiouru, Kaimanawa and Ohakune, and *C. iridescens* was found instead. *C. hatchii* is not apparently the same as *C. "kaitarakihi"* or *C. 'veil'*, but it is allied with them, *C. iridescens* and *C. orbiculatus*. To form a more complete analysis of the tag-named populations with affinities to *C. iridescens*, this species would need to be included as part of the focus of a future study.

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Chapter Four: Synthesis

4.1 Conclusions

The main conclusions that can be drawn from this research are that all of the undescribed tag-named populations examined in these experiments are morphologically distinctive from pre-existing species. There is no longer a reliance on intuition that these taxa are distinct, nor is there a reliance on the maroon patterning that has been utilised in earlier delimitations. Instead, a quantitative approach has demonstrated that the morphology is more consistent within populations and entities, than amongst them, with the exception of *C. “kaitarakihi”* and *C. “veil”*, which are demonstrated to have a high degree of similarity to one another, enough to warrant them collectively as a new species.

The tag-named populations of *C. “kaimai”* and *C. “whiskers”* are distinct from each other and from *C. rivularis sensu stricto*, and each of them warrant a formal taxonomic rank. It is arguable however that based on the patterning of the clusters they form an intergrading complex, and are clearly a very closely related group.

The broader phylogeny of the ‘rivulare’ clade is nearly resolved, with most of the tag-named populations genetically assessed. The molecular analysis has determined the ‘rivulare’ clade to be comprised of two clades, one of which is composed of *C. iridescens*, *C. orbiculatus*, *C. hatchii*, *C. “kaitarakihi”* and *C. “veil”*. The other clade is composed of *C. rivularis s.s.*, *C. papa*, *C. “kaimai”*, *C. “whiskers”* and *C. “pollok”*. The evidence from the morphological assessment broadly comes to the same conclusion. These two clades are here morphologically characterised; The clade comprised of entities more genetically similar to *C. iridescens* than to *C. rivularis* has, on average, a smaller labellum furrow, a wider labellum width, longer labellum bib, and a tend to have short leaf petiole. Conversely, entities more genetically similar to *C. rivularis* than to *C. iridescens* have, on average, a taller labellum furrow, a less wide labellum width, short labellum bib and tend to have a sessile, rarely sub-sessile leaf.

4.2 Recommendations

A formal taxonomic ranking is appropriate for all of the entities studied. There are two ways in which this could be achieved. The first is to utilise the current framework for taxonomic convention employed by New Zealand botanists, which would be to designate all demonstrably distinctive entities at the rank level of species. The evidence in this research suggests that the pre-existing species are typically no more or less distinctive than the tag-named entities they are closely aligned to. This research has established a justification for a formal taxonomic rank that exceeds the evidence presented in the original descriptions of the currently delimited species. None of the currently delimited species were described on the basis of quantitative morphology, and although molecular evidence has been utilised in some of them, it was only used to demonstrate phylogeny as no molecular sequence variation was found in those instances.

The second way in which a new formal taxonomic ranking could be achieved is with a broader concept of species, combined with the intent to not disregard natural groups. The pending amalgamation of the genera that comprise *Corybas*, under condition that all natural sub-ordinate groups remain formally recognised establishes a new framework that could be carried into the considerations of how to determine an appropriate taxonomy at the species level. In such a framework, the two clades demonstrated to exist within the ‘rivulare’ clade could be considered species respectively. There could be *C. rivularis ssp. rivularis*, in addition to the attribution of several sub-species within *C. rivularis sensu lato*. Similarly *C. orbiculatus* could be composed of *C. orbiculatus ssp. orbiculatus*, *C. orbiculatus ssp. iridescens* and several others. This framework would reject the current species *C. papa*, *C. iridescens*, *C. hatchii* and *C. dienemus* and would likely be met with disagreement from some and agreement by others, depending on where they fit onto the ‘splitter-lumper’ continuum.

Given the second framework requires much upheaval and a step away from the current convention, it is likely to result in frustration, with all of the taxonomic changes that this group has been subject to. With this in mind it is the recommendation that these taxonomically indeterminate entities, demonstrated to be distinct, be afforded taxonomic rank at the level of species, until such a time in

which the framework for the entire complex, including currently delimited species, is changed.

Until such time, it is recommended that further research is done in this group, to bolster the sample sizes for some of the under- and non-represented taxa. In terms of morphology, the precisely defined continuous characters can be made even less ambiguous by avoiding using characters that do not have a precise structural aspect. The mapping of the bifurcating venation apices as landmarks could allow the use of this method in more members of *Corybas*, allowing three dimensional landmark models to be rendered and multivariate statistical analyses to be utilised in much the same ways that they were used in this research, but would provide a much greater resolution into the morphological variation for this group.

In terms of molecular variation, the use of ITS region markers has almost exhausted its usefulness. It was sufficient to determine the broader phylogeny of this group, but was unable to determine much in the way of variation between forms. Future research should try to use more, different markers, or use different molecular techniques altogether, such as molecular fingerprinting methods such as Amplified Fragment Length Polymorphism (AFLP) analyses. The use of such techniques should resolve the fine level phylogeny of the group, and could be used to test the findings of this study.

Appendix

This section contains the tables and figures relevant to but not included in the main section.

Table A. 1; The raw morphology data of all entities studied, for five continuous characters. Poll= *C. aff. rivularis* ‘pollok’, W= *C. aff. rivularis* ‘whiskers’, K= *C. aff. rivularis* ‘kaimai’, R = *C. rivularis s.s.*, P= *C. papa*, V= *C. aff. rivularis* ‘veil’, Kait= *C. aff. rivularis* ‘kaitarakihi’, Ir= *C. iridescens*, Or= *C. orbiculatus*.

	Leaf Petiole Length	Flower Petiole Length	Furrow height	Labellum Width	Bib length
Poll1	2.83	4.51	2.72	4.17	3.93
Poll2	2.67	7.24	2.64	5.13	4.1
Poll3	2.53	5	2.57	5.8	4.7
W1	2.8	3.18	4.24	5.28	3.68
W2	1.9	3.68	4.22	5.83	3.23
W3	0.68	4.49	4.35	6.12	4
W4	2.18	2.91	4.19	5.95	3.7
W5	2.16	3.5	4.33	5.53	3.88
W6	1.4	3.07	3.75	6.18	3.48
W7	2.22	3.73	3.87	5.6	3.99
W8	1.6	4.74	3.69	6.14	3.43
W9	1.62	3.78	3.51	5.42	3.69
W10	1.7	3.64	4.12	5.42	4.12
W11	0.7	3.32	3.69	5.47	3.94
K1	1.53	3.72	4.97	6.24	3.66
K2	2.34	3.08	4.8	5.83	2.68
K3	2.16	4.2	5.14	6.3	4.23
K4	1.23	2.32	4.96	5.39	3.82
K5	2.06	6.2	5.3	6.09	3.3
K6	1.95	6.55	5.23	6.1	2.98
K7	1.96	6.2	5.21	6.45	3.22
K8	1.9	5.1	5.44	6.24	2.95
K9	2.1	4.18	5.38	5.21	3.02
K10	2.33	5.43	5.22	5.71	2.55
K11	2.41	5.02	5.81	6.08	3.55
K12	1.04	4.03	4.81	6.81	3.77
K13	0.7	4.35	5.29	5.68	2.5
K14		3.44	4.68	6.49	3.04
K15		2.28	5.2	5.65	3.57
R1	2.95	4.78	5.07	7.3	3.02
R2	2.79	4.76	5.8	7.8	3.14
R3	4.17	7.1	5.75	7.41	2.93
R4	3.62	6.02	6.37	8	3.51
R5	2.61	5.19	5.41	7.67	4.02
R6	3.72	6.8	6.13	7.82	3.42
R7	2.2	5.23	5.49	7.21	3.7
R8	3.49	4.84	5.7	7.06	3.24
R9	4.03	3.44	5.06	7.9	2.84
R10	3.89	7.07	5.38	7.45	3.54
R11	3.19	4.42	5.54	7.13	2.76
P1	1.15	3.89	3.73	6.9	4.66
P2	0.7	3.84	3.26	6.53	4.95
P3	0.7	3.86	3.12	6.55	4.89
P4	0.84	3.38	3.42	6.18	4.69
P5	0.38	2.28	2.92	6.97	4.86
V1	4.25	2.44	3.61	8.6	6.1
V2	4.03	2.43	3.44	7.7	6.5
Kait1	2.02	2.81	3.09	8.94	5.67
Kait2	1.66	1.44	3.28	6.88	6.13
Kait3	2.28	1.19	2.8	8.3	4.78
Ir1	6.27	6.26	2.96	11.26	9.9
Ir2	4.2	4.17	2.99	11.53	10.93
Ir3	4.79	5.53	3.51	10.3	8.28
Ir4	3.07	3.95	3.25	10.32	8.82
Or1	5.11	2.76	4.43	13.66	10.48

Table A. 2 Expanded glossary of terms used in morphological assessments

Auricle: An aperture near the base of the flower made by an infolded labellum base margin that allows visual access to the column chamber.

Stem length: From lowest point of stem above the coleoptile to base of flower/leaf petiole.

Leaf petiole: From departure from stem (or flower petiole) to lamina base.

Flower petiole: From stem to First bract.

Ovary: From Second bract to flower base.

First bract: Leaf-like structure sheathing interface between flower petiole and ovary.

Second bract: Vestigial, pin like, often absent. Last node before ovary.

Leaf length: Measured from end of leaf petiole to end of apicule on leaf tip.

Leaf width: Widest part of leaf lamina. Usually near base.

Basal lobe depth: Basal lobes are rounded bases to the leaves that are longer on the leaf lamina than the leaf base. They are not included in the leaf length, even though they appear cordate/auriculate.

Basal lobe width: Measured distance between the two basal lobes at their apex.

Dorsal sepal width: Width of dorsal sepal at greatest width, typically around wing-bib interface.

Dorsal sepal depth: Dorsal sepal forms a hood over flower, and the hood surface is the abaxial side of the sepal. The adaxial side of the sepal is concave and clasps the wings of the labellum. Because of this, the middle-length of the abaxial surface is crested. The dorsal sepal depth is the vertical distance the dorsal sepal margins to the dorsal sepal abaxial crest.

Furrow: The adaxial surface of the labellum has bulges that resemble calli, however they are not calli as they are not thickened protrusions but a adaxially-convex, abaxially-concave part of the labellum that separates the bib-proper from a tube made from the labellum wings that leads down to the column. These bulges are either side of the midvein of the labellum, and form a narrow furrow between them which allows visual

access to the wing-tube. From outside, on the side of the flower, the abaxial surface of the furrow resembles a membrane similar to an ear drum.

Furrow height: From zone where furrow merges to form 'v' shape to the top of the furrow bulges. The furrow top is defined as the point of greatest curvature from furrow to the labellum wings. Sometimes delineated by clear stripe absent of maroon.

Furrow width: Distance between furrow tops.

Lateral sepal length: Lateral sepals are defined as the usually longer, upright filamentous sepals. Defined as the sepals as they arise lower down toward ovary than labellum/lateral petals. Abaxially concave portion of furrows seem to be 'making room' for lateral sepals to protrude vertically unimpeded.

Lateral petal length: Shorter, usually outward/forward facing, filamentous petals that arise near labellum base. Auricles seem to be 'making room' for the horizontal protrusion of the lateral petals.

Labellum width: Measured as the widest point on the labellum. Usually the wing-bib transition zone, otherwise stated.

Bib length: From bib apex/apicule to furrow base.

Column length: Length of column includes pollen flaps

Column width: Width is widest portion of column, typically the stigmatic surface and associated flanges.

Wing height: Measured as the vertical distance between the furrow tops to the wing margin. Helps determine narrowest point of wing-tube (excluding furrow itself).

Flower length: With ovary held vertical, measure from back of flower to dorsal sepal apex.

Flower height: Measure from ovary top/flower base to dorsal sepal crest. Stated if measured from dorsal sepal crest to bib apex and only done if greater than former method.

Flower length - Dorsal sepal: With ovary held vertical, measure from back of flower to whichever part of the labellum is the greatest extent.

Table A. 2 A quantitative breakdown of the morphology of the taxa studied. A dark circle denotes the character is present, a light circle denotes the character is not present

	Large petiole	Small petiole	Sessile leaf	Small peduncle	Medium peduncle	Long peduncle	Sepals vertical	Sepals forward	Sepals X
<i>C. rivularis</i>	○	●	○	○	●	○	●	●	○
<i>C. aff. rivularis</i> 'whiskers'	○	○	●	○	○	●	○	●	●
<i>C. aff. rivularis</i> 'kaimai'	○	○	●	○	○	●	○	●	●
<i>C. aff. rivularis</i> 'pollok'	○	○	●	○	○	○	●	●	○
<i>C. papa</i>	○	○	●	○	●	○	○	●	●
<i>C. iridescens</i>	●	○	○	○	●	○	○	○	●
<i>C. aff. rivularis</i> 'kaitarakihū'	●	○	○	●	○	○	●	●	○
<i>C. aff. rivularis</i> 'veil'	●	○	○	●	○	○	●	●	○
<i>C. orbiculatus</i>	●	○	○	○	●	○	●	○	○
	Sepals short	Sepals medium	Sepals long	Wing-bib transition	Wing bib transition recurved	Furrow short	Furrow medium	Furrow long	
<i>C. rivularis</i>	○	●	○	○	○	○	○	●	
<i>C. aff. rivularis</i> 'whiskers'	○	○	●	●	●	○	○	●	
<i>C. aff. rivularis</i> 'kaimai'	○	○	●	●	●	○	○	●	
<i>C. aff. rivularis</i> 'pollok'	○	●	○	●	●	●	○	○	
<i>C. papa</i>	○	●	○	●	○	●	○	○	
<i>C. iridescens</i>	○	○	●	○	○	●	○	○	
<i>C. aff. rivularis</i> 'kaitarakihū'	○	●	○	○	○	○	●	○	
<i>C. aff. rivularis</i> 'veil'	○	●	○	○	○	○	●	○	
<i>C. orbiculatus</i>	●	○	○	○	○	○	●	○	
	Furrow entrance leans back	Furrow entrance steep	Furrow entrance vertical	Furrow deep	Furrow shallow	Bib long	Bib medium	Bib short	
<i>C. rivularis</i>	●	●	○	●	○	○	○	●	
<i>C. aff. rivularis</i> 'whiskers'	○	●	○	○	●	○	○	●	
<i>C. aff. rivularis</i> 'kaimai'	○	●	○	○	●	○	○	●	
<i>C. aff. rivularis</i> 'pollok'	○	○	●	○	●	○	○	●	
<i>C. papa</i>	○	○	●	○	●	○	●	○	
<i>C. iridescens</i>	●	○	○	○	●	●	○	○	
<i>C. aff. rivularis</i> 'kaitarakihū'	●	●	○	○	●	●	○	○	
<i>C. aff. rivularis</i> 'veil'	●	●	○	○	●	●	○	○	
<i>C. orbiculatus</i>	●	○	○	○	●	○	○	○	
	Labelum wide	Labelum narrow	Column chamber horizontal	Column chamber curved up	Wing-tube back	Wing-tube upright	Wing-tube forward		
<i>C. rivularis</i>	○	●	○	●	○	○	●		
<i>C. aff. rivularis</i> 'whiskers'	○	●	●	○	●	○	○		
<i>C. aff. rivularis</i> 'kaimai'	○	●	○	●	○	○	●		
<i>C. aff. rivularis</i> 'pollok'	○	●	○	●	○	●	○		
<i>C. papa</i>	○	●	○	●	○	●	○		
<i>C. iridescens</i>	●	○	●	○	●	○	○		
<i>C. aff. rivularis</i> 'kaitarakihū'	●	○	○	●	○	●	○		
<i>C. aff. rivularis</i> 'veil'	●	○	○	●	○	●	○		
<i>C. orbiculatus</i>	●	○	○	●	○	●	○		

Table A. 4 The aligned sequence data of all samples used in the molecular analysis

1

Corybas iridescens DQ784548.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas dienumus DQ784552.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas rivularis veil V2	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas dienumus AF391770.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas iridescens Irl	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas rivularis veil V1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas dienumus DQ422132.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas macranthus DQ784555.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas macranthus DQ784550.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas macranthus DQ784554.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas sulcatus DQ422136.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas hypogaeus DQ784547.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas sulcatus DQ422135.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas sulcatus DQ784558.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas aff trilobus Molloy DQ784561.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas macranthus AF348010.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas trilobus DQ784560.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas trilobus AF391780.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas trilobus DQ784559.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas dienumus DQ784553.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas hatchii DQ784549.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas dienumus DQ422134.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas rivularis kaitarakihi Kait4	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas dienumus DQ422133.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas rivularis kaimai K7	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas rivularis whiskers NCS1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas rivularis AF391778.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas papa AF391776.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas acuminatus DQ784551.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas acuminatus Kauaeranga	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas iridescens AF391772.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas orbiculatus AF391775.1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas rivularis pollok AC4	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas rivularis Puketi R2	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas rivularis Puketi R1	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA
Corybas rivularis kaimai S42	ATCAITGTGG	AGACCCGAAA	GACCGAGCAA	TTTTGACAA

41

Corybas iridescens DQ784548.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas dienumus DQ784552.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas rivularis veil V2	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas dienumus AF391770.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas iridescens Irl	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas rivularis veil V1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas dienumus DQ422132.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas macranthus DQ784555.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas macranthus DQ784550.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas macranthus DQ784554.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas sulcatus DQ422136.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas hypogaeus DQ784547.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas sulcatus DQ422135.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas sulcatus DQ784558.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas aff trilobus Molloy DQ784561.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas macranthus AF348010.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas trilobus DQ784560.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas trilobus AF391780.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas trilobus DQ784559.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas dienumus DQ784553.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas hatchii DQ784549.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas dienumus DQ422134.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas rivularis kaitarakihi Kait4	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas dienumus DQ422133.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas rivularis kaimai K7	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas rivularis whiskers NCS1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas rivularis AF391778.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas papa AF391776.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas acuminatus DQ784551.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas acuminatus Kauaeranga	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas iridescens AF391772.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas orbiculatus AF391775.1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas rivularis pollok AC4	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas rivularis Puketi R2	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas rivularis Puketi R1	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA
Corybas rivularis kaimai S42	TTGTGAACAA	TCAATAAGGC	AACGGTGCAT	GCCGTTGCCA

81

Corybas_iridescens DQ784548.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_dienemus DQ784552.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_rivularis veil V2	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_dienemus AF391770.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_iridescens Ir1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_rivularis veil V1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_dienemus DQ422132.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_macranthus DQ784555.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_macranthus DQ784550.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_macranthus DQ784554.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_sulcatus DQ422136.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_hypogaeus DQ784547.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_sulcatus DQ422135.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_sulcatus DQ784558.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_aff_trilobus Molloy DQ784561.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_macranthus AF348010.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_trilobus DQ784560.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_trilobus AF391780.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_trilobus DQ784559.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_dienemus DQ784553.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_hatchii DQ784549.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_dienemus DQ422134.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_rivularis kaitarakihi_Kait4_	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_dienemus DQ422133.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_rivularis kaimai K7	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_rivularis whiskers NCS1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_rivularis AF391778.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_papa AF391776.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_acuminatus DQ784551.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_acuminatus Kauaeranga	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_iridescens AF391772.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_orbiculatus AF391775.1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_rivularis pollok AC4_	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_rivularis Puketi R2	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_rivularis Puketi R1	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT
Corybas_rivularis kaimai S42	AACACCCCTTC	TCFTTGTCTT	ATCTTCTTGA	ATGAGGTGGT

121

Corybas_iridescens DQ784548.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_dienemus DQ784552.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_rivularis veil V2	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_dienemus AF391770.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_iridescens Ir1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_rivularis veil V1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_dienemus DQ422132.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_macranthus DQ784555.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_macranthus DQ784550.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_macranthus DQ784554.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_sulcatus DQ422136.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_hypogaeus DQ784547.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_sulcatus DQ422135.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_sulcatus DQ784558.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_aff_trilobus Molloy DQ784561.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_macranthus AF348010.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_trilobus DQ784560.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_trilobus AF391780.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_trilobus DQ784559.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_dienemus DQ784553.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_hatchii DQ784549.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_dienemus DQ422134.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_rivularis kaitarakihi_Kait4_	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_dienemus DQ422133.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_rivularis kaimai K7	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_rivularis whiskers NCS1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_rivularis AF391778.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_papa AF391776.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_acuminatus DQ784551.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_acuminatus Kauaeranga	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_iridescens AF391772.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_orbiculatus AF391775.1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_rivularis pollok AC4_	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_rivularis Puketi R2	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_rivularis Puketi R1	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT
Corybas_rivularis kaimai S42	GGCATAGAGT	TTGGGTGGAA	AATAAAGCCG	GCGCAGTTAT

161

Corybas iridescens DQ784548.1	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas dienumus DQ784552.1	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas rivularis veil V2	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas dienumus AF391770.1	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas iridescens Irl	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas rivularis veil V1	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas dienumus DQ422132.1	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas macranthus DQ784555.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas macranthus DQ784550.1	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas macranthus DQ784554.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas sulcatus DQ422136.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas hypogaeus DQ784547.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas sulcatus DQ422135.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas sulcatus DQ784558.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas aff trilobus Molloy DQ784561.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas macranthus AF348010.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas trilobus DQ784560.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas trilobus AF391780.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas trilobus DQ784559.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas dienumus DQ784553.1	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas hatchii DQ784549.1	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas dienumus DQ422134.1	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas rivularis kaitarakihi Kait4	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas dienumus DQ422133.1	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas rivularis kaimai K7	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas rivularis whiskers NCS1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas rivularis AF391778.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas papa AF391776.1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas acuminatus DQ784551.1	GTGCCAAGGA	ATATG--CAA	TGTGAGTGA	CACGAGGCAAT
Corybas acuminatus Kauaeranga	GTGCCAAGGA	ATATG--CAA	TGTGAGTGA	CACGAGGCAAT
Corybas iridescens AF391772.1	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas orbiculatus AF391775.1	GTGCCAAGGA	ATATGTACAG	TGTGAGTGA	CACGACAAAT
Corybas rivularis pollok AC4	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas rivularis Puketi R2	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas rivularis Puketi R1	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT
Corybas rivularis kaimai S42	GTGCCAAGGA	ATATGTACAA	TGTGAGTGA	CACGACCAAT

201

Corybas iridescens DQ784548.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas dienumus DQ784552.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas rivularis veil V2	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas dienumus AF391770.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas iridescens Irl	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas rivularis veil V1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas dienumus DQ422132.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas macranthus DQ784555.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas macranthus DQ784550.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas macranthus DQ784554.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas sulcatus DQ422136.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas hypogaeus DQ784547.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas sulcatus DQ422135.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas sulcatus DQ784558.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas aff trilobus Molloy DQ784561.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas macranthus AF348010.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas trilobus DQ784560.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas trilobus AF391780.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas trilobus DQ784559.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas dienumus DQ784553.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas hatchii DQ784549.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas dienumus DQ422134.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas rivularis kaitarakihi Kait4	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas dienumus DQ422133.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas rivularis kaimai K7	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas rivularis whiskers NCS1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas rivularis AF391778.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas papa AF391776.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas acuminatus DQ784551.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas acuminatus Kauaeranga	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas iridescens AF391772.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas orbiculatus AF391775.1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas rivularis pollok AC4	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas rivularis Puketi R2	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas rivularis Puketi R1	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI
Corybas rivularis kaimai S42	CTTTCAACCT	TTCTAGGCTT	GATGGATTGA	CGTTGTCACI

241

Corybas_iridescens_DQ784548.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_dienemus_DQ784552.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_rivularis_veil_V2	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_dienemus_AF391770.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_iridescens_Irl	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_rivularis_veil_V1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_dienemus_DQ422132.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_macranthus_DQ784555.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_macranthus_DQ784550.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_macranthus_DQ784554.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_sulcatus_DQ422136.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_hypogaeus_DQ784547.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_sulcatus_DQ422135.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_sulcatus_DQ784558.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_aff_trilobus_Molloy_DQ784561.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_macranthus_AF348010.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_trilobus_DQ784560.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_trilobus_AF391780.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_trilobus_DQ784559.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_dienemus_DQ784553.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_hatchii_DQ784549.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_dienemus_DQ422134.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_rivularis_kaitarakihi_Kait4_	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_dienemus_DQ422133.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_rivularis_kaimai_K7	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_rivularis_whiskers_NCS1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_rivularis_AF391778.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_papa_AF391776.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_acuminatus_DQ784551.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_acuminatus_Kauaeranga	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_iridescens_AF391772.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_orbiculatus_AF391775.1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_rivularis_pollock_AC4_	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_rivularis_Puketi_R2_	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_rivularis_Puketi_R1	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG
Corybas_rivularis_kaimai_S42	TCTTATGGG	TGTAATGACT	TGGCAATGG	ATATCTTGG

281

Corybas_iridescens_DQ784548.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_dienemus_DQ784552.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_rivularis_veil_V2	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_dienemus_AF391770.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_iridescens_Irl	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_rivularis_veil_V1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_dienemus_DQ422132.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_macranthus_DQ784555.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_macranthus_DQ784550.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_macranthus_DQ784554.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_sulcatus_DQ422136.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_hypogaeus_DQ784547.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_sulcatus_DQ422135.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_sulcatus_DQ784558.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_aff_trilobus_Molloy_DQ784561.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_macranthus_AF348010.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_trilobus_DQ784560.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_trilobus_AF391780.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_trilobus_DQ784559.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_dienemus_DQ784553.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_hatchii_DQ784549.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_dienemus_DQ422134.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_rivularis_kaitarakihi_Kait4_	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_dienemus_DQ422133.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_rivularis_kaimai_K7	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_rivularis_whiskers_NCS1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_rivularis_AF391778.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_papa_AF391776.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_acuminatus_DQ784551.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_acuminatus_Kauaeranga	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_iridescens_AF391772.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_orbiculatus_AF391775.1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_rivularis_pollock_AC4_	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_rivularis_Puketi_R2_	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_rivularis_Puketi_R1	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG
Corybas_rivularis_kaimai_S42	TCTTGCATCG	ATGAGAGCG	CAGCGAATG	CGATACGTGG

321

Corybas_iridescens_DQ784548.1	TCGGAAATTGC	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_dienemus_DQ784552.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_rivularis_veil_V2	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_dienemus_AF391770.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_iridescens_Ir1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_rivularis_veil_V1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_dienemus_DQ422132.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_macranthus_DQ784555.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_macranthus_DQ784550.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_macranthus_DQ784554.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_sulcatus_DQ422136.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_hypogaeus_DQ784547.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_sulcatus_DQ422135.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_sulcatus_DQ784558.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_aff_trilobus_Molloy_DQ784561.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_macranthus_AF348010.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_trilobus_DQ784560.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_trilobus_AF391780.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_trilobus_DQ784559.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_dienemus_DQ784553.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_hatchii_DQ784549.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_dienemus_DQ422134.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_rivularis_kaitarakihi_Kait4_	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_dienemus_DQ422133.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_rivularis_kaimai_K7	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_rivularis_whiskers_NCS1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_rivularis_AF391778.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_papa_AF391776.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_acuminatus_DQ784551.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_acuminatus_Kauaeranga	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_iridescens_AF391772.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_orbiculatus_AF391775.1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_rivularis_pollock_AC4_	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_rivularis_Puketi_R2_	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_rivularis_Puketi_R1	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC
Corybas_rivularis_kaimai_S42	TGCGAATTGCG	AGAAATCCCGT	GAAACCATCGA	GTTTTTGAAC

361

Corybas_iridescens_DQ784548.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_dienemus_DQ784552.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_rivularis_veil_V2	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_dienemus_AF391770.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_iridescens_Ir1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_rivularis_veil_V1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_dienemus_DQ422132.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_macranthus_DQ784555.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_macranthus_DQ784550.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_macranthus_DQ784554.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_sulcatus_DQ422136.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_hypogaeus_DQ784547.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_sulcatus_DQ422135.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_sulcatus_DQ784558.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_aff_trilobus_Molloy_DQ784561.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_macranthus_AF348010.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_trilobus_DQ784560.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_trilobus_AF391780.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_trilobus_DQ784559.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_dienemus_DQ784553.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_hatchii_DQ784549.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_dienemus_DQ422134.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_rivularis_kaitarakihi_Kait4_	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_dienemus_DQ422133.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_rivularis_kaimai_K7	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_rivularis_whiskers_NCS1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_rivularis_AF391778.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_papa_AF391776.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_acuminatus_DQ784551.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_acuminatus_Kauaeranga	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_iridescens_AF391772.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_orbiculatus_AF391775.1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_rivularis_pollock_AC4_	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_rivularis_Puketi_R2_	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_rivularis_Puketi_R1	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG
Corybas_rivularis_kaimai_S42	GCAGGTTGGG	CCTGAGGCCA	TCTGGTTGAG	GGCAGGTCGG

481

Corybas_iridescens_DQ784548.1
Corybas_dienemus_DQ784552.1
Corybas_rivularis_veil_V2
Corybas_dienemus_AF391770.1
Corybas_iridescens_Ir1
Corybas_rivularis_veil_V1
Corybas_dienemus_DQ422132.1
Corybas_macranthus_DQ784555.1
Corybas_macranthus_DQ784550.1
Corybas_macranthus_DQ784554.1
Corybas_sulcatus_DQ422136.1
Corybas_hypogaeus_DQ784547.1
Corybas_sulcatus_DQ422135.1
Corybas_sulcatus_DQ784558.1
Corybas_aff_trilobus_Molloy_DQ784561.1
Corybas_macranthus_AF348010.1
Corybas_trilobus_DQ784560.1
Corybas_trilobus_AF391780.1
Corybas_trilobus_DQ784559.1
Corybas_dienemus_DQ784553.1
Corybas_hatchii_DQ784549.1
Corybas_dienemus_DQ422134.1
Corybas_rivularis_kaitarakihi_Kait4_
Corybas_dienemus_DQ422133.1
Corybas_rivularis_kaimai_K7
Corybas_rivularis_whiskers_NCS1
Corybas_rivularis_AF391778.1|
Corybas_papa_AF391776.1
Corybas_acuminatus_DQ784551.1
Corybas_acuminatus_Kauaeranga
Corybas_iridescens_AF391772.1
Corybas_orbiculatus_AF391775.1
Corybas_rivularis_pollok_AC4_
Corybas_rivularis_Puketi_R2_
Corybas_rivularis_Puketi_R1
Corybas_rivularis_kaimai_S42

521

Corybas_iridescens_DQ784548.1
Corybas_dienemus_DQ784552.1
Corybas_rivularis_veil_V2
Corybas_dienemus_AF391770.1
Corybas_iridescens_Ir1
Corybas_rivularis_veil_V1
Corybas_dienemus_DQ422132.1
Corybas_macranthus_DQ784555.1
Corybas_macranthus_DQ784550.1
Corybas_macranthus_DQ784554.1
Corybas_sulcatus_DQ422136.1
Corybas_hypogaeus_DQ784547.1
Corybas_sulcatus_DQ422135.1
Corybas_sulcatus_DQ784558.1
Corybas_aff_trilobus_Molloy_DQ784561.1
Corybas_macranthus_AF348010.1
Corybas_trilobus_DQ784560.1
Corybas_trilobus_AF391780.1
Corybas_trilobus_DQ784559.1
Corybas_dienemus_DQ784553.1
Corybas_hatchii_DQ784549.1
Corybas_dienemus_DQ422134.1
Corybas_rivularis_kaitarakihi_Kait4_
Corybas_dienemus_DQ422133.1
Corybas_rivularis_kaimai_K7
Corybas_rivularis_whiskers_NCS1
Corybas_rivularis_AF391778.1|
Corybas_papa_AF391776.1
Corybas_acuminatus_DQ784551.1
Corybas_acuminatus_Kauaeranga
Corybas_iridescens_AF391772.1
Corybas_orbiculatus_AF391775.1
Corybas_rivularis_pollok_AC4_
Corybas_rivularis_Puketi_R2_
Corybas_rivularis_Puketi_R1
Corybas_rivularis_kaimai_S42

561

Corybas iridescens DQ784548.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas dienemus DQ784552.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas rivularis veil V2	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas dienemus AF391770.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas iridescens Irl	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas rivularis veil V1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas dienemus DQ422132.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas macranthus DQ784555.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas macranthus DQ784550.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas macranthus DQ784554.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas sulcatus DQ422136.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas hypogaeus DQ784547.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas sulcatus DQ422135.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas sulcatus DQ784558.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas aff trilobus Molloy DQ784561.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas macFanthus AF348010.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas trilobus DQ784560.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas trilobus AF391780.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas trilobus DQ784559.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas dienemus DQ784553.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas hatchii DQ784549.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas dienemus DQ422134.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas rivularis kaitarakihi Kait4	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas dienemus DQ422133.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas rivularis kaimai K7	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas rivularis whiskers NCS1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas rivularis AF391778.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas papa AF391776.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas acuminatus DQ784551.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas acuminatus Kauaeranga	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas iridescens AF391772.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas orbiculatus AF391775.1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas rivularis pollok AC4	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas rivularis Puketi R2	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas rivularis Puketi R1	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT
Corybas rivularis kaimai S42	GGAAAGCTGCC	TTTTGGCCCA	CATTTTCATT	ATCGGCCAT

601

Corybas iridescens DQ784548.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas dienemus DQ784552.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas rivularis veil V2	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas dienemus AF391770.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas iridescens Irl	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas rivularis veil V1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas dienemus DQ422132.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas macranthus DQ784555.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAACGC
Corybas macranthus DQ784550.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAACGC
Corybas macranthus DQ784554.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAACGC
Corybas sulcatus DQ422136.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAACGC
Corybas hypogaeus DQ784547.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAACGC
Corybas sulcatus DQ422135.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAACGC
Corybas sulcatus DQ784558.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAACGC
Corybas aff trilobus Molloy DQ784561.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAACGC
Corybas macFanthus AF348010.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAACGC
Corybas trilobus DQ784560.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAACGC
Corybas trilobus AF391780.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAACGC
Corybas trilobus DQ784559.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAACGC
Corybas dienemus DQ784553.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas hatchii DQ784549.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas dienemus DQ422134.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas rivularis kaitarakihi Kait4	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas dienemus DQ422133.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas rivularis kaimai K7	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas rivularis whiskers NCS1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas rivularis AF391778.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas papa AF391776.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas acuminatus DQ784551.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas acuminatus Kauaeranga	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas iridescens AF391772.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas orbiculatus AF391775.1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas rivularis pollok AC4	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas rivularis Puketi R2	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas rivularis Puketi R1	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC
Corybas rivularis kaimai S42	TACATTGAGC	AATGCAGATT	AATATCCTAT	ANGGCCAATGC

Corybas_iridescens_DQ784548.1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_dienemus_DQ784552.1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_rivularis_veil_V2	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_dienemus_AF391770.1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_iridescens_Ir1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_rivularis_veil_V1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_dienemus_DQ422132.1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_macranthus_DQ784555.1	CATCTCCAT	GGA-TTGGT	GCGCCTTGAA	ATGGG
Corybas_macranthus_DQ784550.1	CATCTCCAT	GGA-TTGGT	GCGCCTTGAA	ATGGG
Corybas_macranthus_DQ784554.1	CATCTCCAT	GGA-TTGGT	GCGCCTTGAA	ATGGG
Corybas_sulcatus_DQ422136.1	TATCCTCCAT	GGAG-TGGT	GCGCCTTGAA	ATGGG
Corybas_hypogaeus_DQ784547.1	TATCCTCCAT	GGAG-TGGT	GCGCCTTGAA	ATGGG
Corybas_sulcatus_DQ422135.1	TATCCTCCAT	GGAG-TGGT	GCGCCTTGAA	ATGGG
Corybas_sulcatus_DQ784558.1	TATCCTCCAT	GGAG-TGGT	GCGCCTTGAA	ATGGG
Corybas_aff_trilobus_Molloy_DQ784561.1	TATCCTCCAT	GGAG-TGGT	GCGCCTTGAA	ATGGG
Corybas_macranthus_AF348010.1	CATCTCCAT	GGA-TTGGT	GCGCCTTGAA	ATGGG
Corybas_trilobus_DQ784560.1	TATCCTCCAT	GGAG-TGGT	GCGCCTTGAA	ATGGG
Corybas_trilobus_AF391780.1	TATCCTCCAT	GGAG-TGGT	GCGCCTTGAA	ATGGG
Corybas_trilobus_DQ784559.1	TATCCTCCAT	GGAG-TGGT	GCGCCTTGAA	ATGGG
Corybas_dienemus_DQ784553.1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_hatchii_DQ784549.1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_dienemus_DQ422134.1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_rivularis_kaitarakihi_Kait4_	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_dienemus_DQ422133.1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_rivularis_kaimai_K7	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_rivularis_whiskers_NCS1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_rivularis_AF391778.1	TATCCTCCA-	GGAGTTGGAT	GCGCCTT-AA	ATGGG
Corybas_papa_AF391776.1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_acuminatus_DQ784551.1	TATCCTCCAT	GGAGGTTGGT	GCGCCTTGAA	ATGGG
Corybas_acuminatus_Kauaeranga	TATCCTCCAT	GGAGGTTGGT	GCGCCTTGAA	ATGGG
Corybas_iridescens_AF391772.1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_orbiculatus_AF391775.1	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_rivularis_pollok_AC4_	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG
Corybas_rivularis_Puketi_R2_	TATCCTCCAT	GGTGTGGAT	GCGCCTTGAA	ATGGG
Corybas_rivularis_Puketi_R1	TATCCTCCAT	GGTGTGGAT	GCGCCTTGAA	ATGGG
Corybas_rivularis_kaimai_S42	TATCCTCCAT	GGAGTTGGAT	GCGCCTTGAA	ATGGG