Phytotaxa 270 (1): 001–024
http://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.270.1.1

Five new species of *Corybas* (Diurideae, Orchidaceae) endemic to New Zealand and phylogeny of the *Nematoceras* clade

CARLOS A. LEHNEBACH[1], ANDREAS J. ZELLER[1], JONATHAN FRERICKS[2] & PETER RITCHIE[2]
[1]*Museum of New Zealand Te Papa Tongarewa, PO BOX 467, Wellington. New Zealand; email: CarlosL@tepapa.govt.nz*
[2]*Victoria University of Wellington, PO BOX 600, Wellington, New Zealand*

Abstract

Five new species of *Corybas* endemic to New Zealand, *C. confusus*, *C. obscurus*, *C. sanctigeorgianus*, *C. vitreus*, and *C. wallii* are described. These species are segregated from the *Corybas trilobus* aggregate based on morphometric and DNA fingerprinting (AFLP) analyses. A key to the new species is also provided, and their distribution and conservation status are included. Phylogenetic results showed that, despite the great morphological and ecological diversity of these orchids, genetic divergence between species is low, suggesting recent diversification. We also found evidence for multiple dispersal events from New Zealand to several offshore and sub-Antarctic islands.

Key words: Brood-site deception, dispersal, islands, *Mycetophila*, speciation, sub-Antarctic, threatened flora

Introduction

Islands provide exceptional settings to investigate the evolution of biological diversity. Because of their discrete boundaries and isolated nature, the presence of a species can only be explained by dispersal from mainland sources or *in situ* speciation (Valente *et al*. 2014). Speciation may occur rapidly on these remote habitats, giving rise to species-rich lineages that may exhibit exceptional morphological and ecological diversification (Losos & Ricklefs 2009). Availability of resources, presence of novel unoccupied niches, low levels of competition, and evolution of key adaptations are some of the factors believed to trigger such radiations (Schluter 2000).

New Zealand (NZ) has been isolated from other landmasses for about 80 Myr after it broke away from Gondwana and drifted (Gibbs 2006). Although part of the biota has its origins in Gondwana, dispersal from other landmasses has occurred multiple times post-isolation (e.g. Lockhart *et al*. 2001, Winkworth *et al*. 2005, Lehnebach 2015). Some of these dispersal events have provided the ancestral stock for several plant radiations now found in sub-alpine and alpine areas of the country (Lockhart *et al*. 2001, Meudt *et al*. 2015). Mountain systems are of recent origin in NZ (4–2 Myr), and their formation created new habitats that facilitated diversification of several plant groups (Lockhart *et al*. 2001, Heenan & Mitchell 2003, Winkworth *et al*. 2005). Conversely, lowland habitats are geologically older, and species radiations are seemingly absent.

Orchids are a conspicuous and diverse element of the NZ flora with over 110 species (Breitwieser *et al*. 2012). Most NZ orchids are terrestrial and occupy coastal to alpine habitats of the North and South Island and Stewart Island. They are also found on many of the smaller offshore and sub-Antarctic islands (de Lange *et al*. 2007). Many NZ orchids belong to genera found also in Australasia, Melanesia and South Asia, but over 70% of the species occurring in NZ are endemic (St. George 1999).

Spider orchids are one of the most common and widespread terrestrial orchids in NZ. Unlike the Australian spider orchids, *Caladenia* Brown (1810: 323), NZ spider orchids are members of *Corybas* Hooker & Salisbury (1805: 83). The *Corybas* alliance is a group of ca 135 species distributed from the Himalayas to southern China, the Malay Archipelago, Polynesia, New Guinea and Australia (Jones *et al*. 2002, Lyon 2014). Generic classification of these orchids has changed several times over the years, and the NZ species have been place in *Nematoceras* Hooker (1853; 249), *Corysanthes* Brown (1810: 328) and *Corybas* (e.g. Hooker 1853, Hooker 1864, Moore 1976, Szlachetko & Rutkowski 2003). The latest taxonomic change occurred over a decade ago when Clements *et al.* (2002) and Jones *et al*. (2002) proposed a new classification based on morphological and ITS sequence data. These authors reinstated the

Accepted by Mark Chase: 25 Jul. 2016; published: 12 Aug. 2016

genus *Nematoceras* and included 11 NZ species in it along with two species from Macquarie Island (Australia). Our study focuses on this group of species, and here we referred to it as the *Nematoceras* clade of *Corybas* in line with recent studies and a revised orchid classification (Chase *et al.* 2015).

The remaining NZ species of *Corybas* were placed in three new genera *Anzybas* Jones *et al*. (2002: 442) (two species), *Molloybas* Jones *et al*. (2002: 448) (one species) and *Singularybas* Jones *et al*. (2002: 449) (one species). Only one species remained in *Corybas*. Australian members of the alliance, on the other hand, were regrouped under *Anzybas*, *Corybas* and *Corysanthes*. The last genus was reinstated to include a group of Australian endemics.

Lyon (2014) has recently reassessed subgeneric boundaries within *Corybas* using multiple DNA markers and including over 80 taxa from across *Corybas*. That study confirmed monophyly of the genus and revealed that *Corysanthes* and *Nematoceras* are sister clades. Furthermore, Lyon (2014) recommended that the names *Corysanthes* and *Nematoceras* be used for sections within *Corybas*. The *World checklist of selected plant families* (WCSP 2016) compiled by Kew Botanic Gardens has already accepted the views of Lyon (2014). Similarly, the *New Zealand plant names database* (http://nzflora.landcareresearch.co.nz; accessed 24 March 2016) has implemented this change, and all NZ *Nematoceras* are now listed under *Corybas.* This approach has been favoured as it maximises taxonomic stability and facilitates management of collections and data (see review by Hopper 2009).

Taxonomic uncertainty in the *Corybas* alliance also extends to the species level. *Corybas trilobus* (Hooker 1864: 265) Reichenbach (1871: 67) is one of the most widespread and morphologically variable spider orchids in NZ. Floral characters are particularly variable within this orchid, and it has been suggested that several taxa are probably included under this name (St. George 2006). Some of these variants are easily distinguished by qualitative characters (e.g. flower colour, plant size and leaf shape) and, although they are well known to orchid enthusiasts and local botanists, their taxonomic status and phylogenetic affinities are still unresolved. Furthermore, none of these variants was included in Lyon's (2014) work and their phylogenetic affinities remain unknown. Unfortunately, some of these "nameless" orchids face conservation issues and are included in the list of Threatened and Uncommon Plants of NZ (de Lange *et al*. 2013).

In this study, we assess species boundaries within the *Corybas trilobus* aggregate using multivariate analyses of morphometric data and DNA fingerprinting (AFLP) and investigate phylogenetic relationships and diversification within NZ spider orchids using sequences of nuclear and plastid markers.

Material and Methods

Sample collection and morphological characterisation:—We collected material of all NZ spider orchids previously included under *Nematoceras* and 11 variants of the *C. trilobus* aggregate from the North and South Islands of NZ. We considered as valid species only those species listed in the latest taxonomic inventory of NZ vascular plants (Breitweiser *et al*. 2012) and as accepted names those used by the *New Zealand plant names database* (http://nzflora.landcareresearch.co.nz).

The identification of *C. trilobus* s.s. specimens was confirmed by comparing them, under the stereomicroscope, with type specimens loaned from the Royal Botanic Gardens, Kew (K000364465!, K000364466!, K000364467!). Identification of *C. trilobus* variants and tag-name usage follow Scanlen & St. George (2011).

We included material and published sequences of species found on offshore islands such as the Chatham Islands (~ 680 km southeast of NZ) and Macquarie Island in the southern oceans. We used *C. oblongus* as an outgroup, a species previously regarded as the sole member of the NZ genus *Singularybas* (Clements *et al*. 2002, Jones *et al*. 2002) and outside the *Nematoceras* clade (Lyon 2014).

Samples were kept fresh until we returned from the field to the lab, where plants were photographed and dissected and 14 vegetative and floral characters (Table 1) were measured using a digital calliper. We measured 290 individuals: *C. hypogaeus* (n = 46), *C. trilobus* s.s (n = 29), "darkie" (n = 19), "eastern hills" (n = 42), "Rimutaka" (n = 16), "round leaf" (n = 45), "tribrive" (n = 4), "tricraig" (n = 5), "tridodd" (n = 13), "trisept" (n = 13), "triwhite" (n = 80) and "Trotters" (n = 7). We did not include "pygmy" in the morphometric analyses because only two fertile samples were found in the field, and these flowers were already wilting. This form is less than 10 mm tall so most of the plant was used for DNA analyses.

For each sampled population, we kept a voucher specimen comprising whole plants (i.e. comprising one flower and one leaf in *Corybas*) or only flowers for those samples included in DNA analyses. Herbarium specimens were deposited at WELT (Museum of New Zealand Te Papa Tongarewa). Voucher numbers are listed in the text, Table 2 and

and Appendix 1. Collection details and images (when available) are accessible online at http://collections.tepapa.govt.nz. Although initially we examined existing herbarium specimens, we did not include them in our study because floral features (e.g. size, colour, shape) are badly affected by pressing and drying. This makes it difficult, and sometimes impossible, to identify specimens to species. Previous researchers of *Corybas* have reported the same difficulties when working with herbarium material (e.g. Dransfield *et al*. 1986).

TABLE 1. Morphological characters measured from New Zealand spider orchids (*Corybas*) and their canonical correlation with the first (CV 1) and second axis (CV 2) obtained for each group comparison (group 1: *C. hypogaeus*, *C. obscurus*, “Rimutaka”, *C. wallii*, “Trotters”; group 2: *C. trilobus* s.s., *C. obscurus*, *C. wallii*; group 3: *C. trilobus* s.s., *C. sanctigeorgianus*, “tridodd”, “tricraig”, “tribrive”; group 4: *C. trilobus* s.s., *C. vitreus*, *C. confusus*). The most influential variables for each axis are shown in bold: 1, distance between the margin of the lateral leaf lobe and an imaginary line running from the petiole-leaf insertion point to the lateral margin of the leaf; 2, distance between the frontal and dorsal plane of the flower; 3, distance between the upper and lower margins of the labellum.

	Group 1		Group 2		Group 3		Group 4	
Character	CV 1	CV 2	CV 1	CV 2	CV 1	CV 2	CV 1	CV 2
Length of the petiole	**-0.300**	**0.632**	**0.930**	**-0.292**	**0.943**	0.256	**0.838**	**0.547**
Leaf width	-0.141	**0.750**	**0.523**	**-0.297**	**0.194**	**0.347**	**0.361**	**0.337**
Leaf length	0.015	0.502	**0.260**	-0.203	0.041	0.143	0.014	0.309
Lobe hight[1]	-0.051	0.203	0.176	-0.052	0.039	0.085	0.094	0.129
Acumen/mucro length	-0.038	-0.032	0.049	0.017	0.035	-0.079	0.072	-0.003
Length of the peduncule	0.155	0.046	-0.060	0.072	-0.020	0.218	0.056	-0.023
Length of the ovary	0.108	0.183	-0.026	-0.056	-0.072	**0.268**	0.013	-0.100
Length of floral bract	0.080	0.064	-0.060	-0.050	-0.118	-0.040	0.049	-0.006
Width of floral bract	0.039	0.045	-0.023	-0.012	-0.030	0.024	0.009	-0.002
Lenght of lateral sepals	**0.236**	0.521	0.038	-0.185	-0.170	0.155	**-0.463**	0.234
Length of petals	**0.936**	**0.722**	0.123	**0.836**	**-0.220**	**1.054**	0.177	**0.904**
Auricle (diameter)	0.017	0.015	0.014	0.012	0.017	0.031	0.007	0.015
Flower (depth)[2]	0.108	0.158	-0.012	0.003	0.013	0.237	-0.038	0.014
Flower (hight)[3]	0.032	0.306	-0.004	-0.228	-0.004	0.037	-0.003	0.038

DNA extraction, sequencing and fingerprinting:—Leaf tissue was placed in an Eppendorf® 2.5mL tube, frozen in liquid nitrogen and then finely crushed using a glass rod. This was then used for DNA extraction following Doyle & Doyle’s (1987) CTAB protocol. The internal transcribed spacer (ITS) and two plastid sequence markers recommended for low taxonomic level studies, i.e. *trnL* intron and *trnL-F* spacer combined and *psbJ-petA* spacer, were amplified using the primers of Baldwin (1992), Shaw *et al*. (2005) and Shaw *et al*. (2007), respectively. PCR was performed in a total volume of 20 μL that consisted of 1 μL of DNA, 4 μL betaine (5 M), 2.5 μL of dNTPs (2 mM), 2 μL 10 x buffer (Roche), 1 μL of each primer (10 nM), 0.2 μL of Taq (1 U, Roche) and 8.3 μL of water. PCRs were performed on a Biometra T1 Thermocycler (Whatman, Germany) and thermocycling conditions for ITS and plastid regions followed White *et al.* (1990) for ITS and Shaw *et al.* (2007) for the plastid regions. PCR products were purified using 2 μL Sap (2 U) and 1 μL Exo I (1 U), both from GE Healthcare Life Sciences, and sequenced in both directions using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kits (Applied Biosystems by Life Technologies) and run on an ABI 3730 Genetic Analyzer (Life Technologies). Consensus sequences were created from forward and reverse sequences using Geneious 5.6.5 (Biomatters Ltd.). Sequences were submitted to GenBank (Table 2).

Species boundaries among three members of the *C. trilobus* aggregate occurring sympatrically were further assessed using DNA fingerprinting. We generated AFLP profiles for *C. hypogaeus* (n = 15), “darkie” (n = 15), and “triwhite” (n = 15). We used five individuals of each taxon from three mixed-taxa populations located a few kilometres apart. The taxonomic status and morphological distinctiveness of *C. hypogaeus* is unquestionable, yet its sequences of ITS and plastid DNA are similar to “darkie” and “triwhite” (see phylogenetic results below). Therefore, we used *C. hypogaeus* as a control group. This allows us to confirm whether AFLP is capable of detecting species boundaries among these closely related orchids. We followed the protocol developed by Clarke & Meudt freely available from http://clarkeresearch.org/aflp_2012-01-26/aflp.html (accessed November 2014). Up to 250 ng of genomic DNA was digested for each sample using the restriction enzymes EcoRI (10U/1μL, Roche) and MseI (10U/1μL, NEB). A duplicate of each sample was digested and analysed simultaneously; this was done to reduce potential uncertainty around fragment sizes or any other technical artefacts that might affect our results. Based on recommendations from previous AFLP studies of orchids (Hedrén *et al.* 2001, Forrest *et al*. 2004, Fay *et al*. 2005, Mant *et al*. 2005, Janes *et al*. 2012) and the variability observed in preliminary tests on a subset of our samples the following selective primer combinations were chosen: Vic-EcoRI + ATA / MseI + CGTG, Pet-EcoRI + AGG / MseI + CAA and Ned-EcoRI + ACC / MseI + CAC (Sigma-Aldrich, except for ABI for the Vic, Ned, and Ted labelled primers). Selective PCRs were poolplexed in equal volumes (2 µL each) and genotyped on ABI 3730 Genetic Analyzer (Life Technologies) by the Massey Genome Service (Palmerston North, New Zealand).

TABLE 2. List of taxa included in the phylogenetic analyses with the voucher reference, tag name (when applicable) and GenBank accession numbers for DNA sequences. NI: North Island. SI: South Island. R: Reserve. RTBG: Royal Tasmanian Botanical Gardens, Hobart. Tasmania. s/n: no number.

Name	Tag name	Voucher number	Locality	Genbank accession numbers		
				nrITS	*trnL–trnF*	*psbJ–petA*
C. acuminatus	—	WELT SP104115	NI, Te Urewera National Park.	KX499849	KX523970	KX456183
C. confusus	round leaf	WELT SP104180	NI, Egmont/Taranaki National Park.	KX499839	KX523967	KX456173
C. confusus	round leaf	WELT SP104176	NI, Tongariro National Park.	KX499799	KX523963	KX456174
C. confusus	round leaf	WELT SP104126	SI, Kahurangi National Park.	KX499812	KX523961	KX456171
C. confusus	round leaf	WELT SP104143	SI, Arthur's Pass National Park.	KX499816	KX523964	KX456175
C. confusus	round leaf	WELT SP104160	SI, Mt Cook National Park.	KX499822	KX523966	KX456176
C. dienemus	—	—	Australia. Cultivated at RTBG.	KX499792	KX523950	KX456182
C. hatchii	—	WELT SP104118	NI, Ruahine Ranges Forest Park.	KX499795	KX523953	KX456181
C. hypogaeus	—	WELT SP104185	NI, Boundary Stream Mainland Island.	KX499800	KX523944	KX456141
C. hypogaeus	—	WELT SP104116	NI, Te Urewera National Park.	KX499802	KX523913	KX456132
C. hypogaeus	—	WELT SP104117	NI, Te Urewera National Park.	KX499829	KX523958	KX456133
C. hypogaeus	—	WELT SP104177	NI, Tongariro National Park.	KX499830	KX523943	KX456139
C. hypogaeus	—	WELT SP104121	SI, Lake Sumner Forest Park.	KX499809	KX523916	KX456144
C. hypogaeus	—	WELT SP104130	SI, Nelson Lakes National Park.	KX499806	KX523914	KX456131
C. hypogaeus	—	WELT SP104138	SI, Nelson Lakes National Park.	KX499803	KX523935	KX456128
C. iridescens	—	WELT SP104125	SI, Kahurangi National Park.	KX499796	KX523952	KX456177
C. macranthus	—	WELT SP104173	NI, East Harbour Regional Park.	KX499847	KX523955	KX456169
C. oblongus	—	WELT SP104170	NI, Tararua Forest Park.	KX499850	KX523971	—
C. obscurus	darkie	WELT SP104133	NI, Nelson Lakes National Park.	KX499840	KX523939	KX456147
C. obscurus	darkie	WELT SP104134	NI, Nelson Lakes National Park.	KX499819	KX523937	KX456145
C. obscurus	darkie	WELT SP104135	NI, Nelson Lakes National Park.	KX499837	KX523936	KX456146
C. obscurus	darkie	WELT SP104152	NI, Nelson Lakes National Park.	KX499827	KX523925	KX456134
C. orbiculatus	—	WELT SP104119	NI, Ruahine Ranges Forest Park.	KX499794	KX523951	KX456179
C. papa	—	WELT SP104168	NI, Whanganui, Atene.	KX499797	KX523949	KX456178
C. papillosus	—	WELT SP104161	SI, Fiordland National Park.	KX499846	KX523956	KX456168
C. rivularis	—	WELT SP104174	NI, Rimutaka Ranges.	KX499793	KX523954	KX456180
C. sanctigeorgianus	trisep	WELT SP104149	NI, Hunua Ranges Regional Park.	KX499834	KX523924	KX456149
C. sanctigeorgianus	trisep	WELT SP104166	NI, Hunua Ranges Regional Park.	KX499835	KX523934	KX456150
C. sanctigeorgianus	trisep	WELT SP104167	NI, Hunua Ranges Regional Park.	KX499836	KX523946	KX456151
C. sulcatus	—	—	Australia. Macquarie Island.	DQ784558	—	—
C. trilobus	—	WELT SP104184	NI, near Masterton.	KX499825	KX523930	KX456152
C. trilobus	—	WELT SP104163	NI, Ruahine Ranges Forest Park.	KX499813	KX523921	KX456160
C. trilobus	—	WELT SP104181	NI, Whanganui, Gordon Park.	KX499821	KX523919	KX456161
C. trilobus	—	WELT SP104182	NI, Whanganui, Gordon Park.	KX499798	KX523915	KX456158
C. trilobus	—	WELT SP104195	NI, Turakina Valley, Sutherland Bush.	KX499810	KX523929	KX456155
C. vitreus	eastern hills	WELT SP104186	NI, Boundary Stream Mainland Island.	KX499801	KX523969	KX456166

...continued on the next page

TABLE 2. (Continued)

Name	Tag name	Voucher number	Locality	Genbank accession numbers		
				nrITS	*trnL–trnF*	*psbJ–petA*
C. vitreus	eastern hills	Spirit collection (s/n)	NI, Ruahine Ranges Forest Park.	KX499815	KX523959	KX456163
C. vitreus	eastern hills	WELT SP104127	SI, Kahurangi National Park.	KX499811	KX523962	KX456164
C. vitreus	eastern hills	WELT SP104140	SI, Arthur's Pass National Park.	KX499818	KX523960	KX456167
C. vitreus	eastern hills	WELT SP104155	SI, Nelson Lakes National Park.	KX499824	KX523965	KX456165
C. wallii	triwhite	WELT SP104179	NI, Egmont/Taranaki National Park.	KX499845	KX523920	KX456140
C. wallii	triwhite	WELT SP104178	NI, Ruahine Forest Park.	KX499843	KX523945	KX456148
C. wallii	triwhite	WELT SP104175	NI, Tongariro National Park.	KX499844	KX523928	KX456135
C. wallii	triwhite	WELT SP104124	SI, Kahurangi National Park.	KX499842	KX523947	KX456130
C. wallii	triwhite	WELT SP104151	SI, Nelson Lakes National Park.	KX499841	KX523948	KX456136
C. aff. *sulcatus*	—	WELT SP104165	Chatham Island, Tuku Nature Reserve.	KX499828	KX523968	KX456172
Corybas sp.	Rimutaka	WELT SP104171	NI, East Harbour Regional Park.	KX499831	KX523933	KX456127
Corybas sp.	Rimutaka	WELT SP104172	NI, East Harbour Regional Park.	KX499808	KX523931	KX456129
Corybas sp.	Rimutaka	WELT SP104159	SI, Mt. Cook National Park.	KX499823	KX523922	KX456138
Corybas sp.	Rimutaka	WELT SP104129	SI, Nelson, Aniseed Valley.	KX499805	KX523940	KX456126
Corybas sp.	Trotters	WELT SP104197	NI, Cape Kidnappers.	KX499838	KX523918	KX456142
Corybas sp.	Trotters	WELT SP104196	NI, Cape Kidnappers.	KX499832	KX523942	KX456137
Corybas sp.	Trotters	WELT SP104187	NI, Boundary Stream Mainland Island.	KX499829	KX523917	KX456143
Corybas sp.	pygmy	No voucher	NI, Hunua Ranges Regional Park.	KX499817	KX523938	KX456156
Corybas sp.	pygmy	Spirit collection (s/n)	NI, Albany Scenic Reserve.	KX499814	KX523932	KX456153
Corybas sp.	tricraig	WELT SP104148	NI, Awhitu Peninsula.	KX499820	KX523926	KX456157
Corybas sp.	tridodd	WELT SP104146	NI, Awhitu Peninsula.	KX499833	KX523927	KX456154
Corybas sp.	tribrive	WELT SP104144	NI, Hunua Ranges Regional Park.	KX499807	KX523941	KX456162
Corybas sp.	tribrive	WELT SP104150	NI, Hunua Ranges Regional Park.	KX499804	KX523923	KX456159
Corybas sp.	—	WELT SP104162	SI, Fiordland National Park.	KX499848	KX523957	KX456170

Data analyses:—We used canonical variate analysis (CVA) as implemented in NTSYS-pc 2.2h1 (Applied Biostatistics Inc.) to analyse the morphometric data and detect quantitative characters that may support boundaries among each orchid groups otherwise distinguished by qualitative characters only. Unlike principal component analysis, CVA calculates variable weightings that help to explain differences among groups rather than individuals (Dytham 2003). Multiple accessions of *C. trilobus* s.s. were included in the CVA and compared against samples of all variants. Group comparisons among phylogenetically close variants were also performed.

AFLP profiles were visualised and analysed using GeneMarker V1.90 (Softgenetics). First, we used the default settings for fragment sizing and scoring. A panel of scorable fragments was created for each primer combination based on high quality profiles (score ≥94). This panel was then used for automatic scoring of fragments found between 60 and 250 bp, using a relative fluorescent unit threshold of 200. Scoring was performed using the following options on: local southern size call, peak saturation, baseline and enhanced baseline subtraction, smooth and enhanced smooth, spike removal, pull-up corrections, plus-A filter and stutter filter (10 %). Peak scores values of reject were set to reject = 1 and pass = 7. Profiles were then assessed manually, and dubious or low intensity peaks were excluded along with failed samples. After deleting duplicates, a presence/absence matrix including all primer combinations was exported and analysed by principal coordinate analyses (PCoA) using NTSYS-pc 2.2h1 (Applied Biostatistics Inc.). The matrix was also analysed by neighbor-joining (NJ), and support for main clusters determined by boostrap analyses (1000 replicates) using SplitsTree (Huson & Bryant 2006). A subset of these analyses was also performed on the unedited version of the data matrix so we could detect any effect manual scoring of alleles could have on our results.

We generated DNA sequences for three regions for 59 accessions of *Corybas*. Sequence alignments for each marker were performed with ClustalW (Thompson *et al*. 1994) and refined by eye as necessary. A partitioned Bayesian inference (BI) analysis including the three datasets was conducted using MrBayes (Huelsenbeck & Ronquist 2001). The models HKY rates equal, F81 rates equal and F81 + G were identified by jModelTest (Posada 2008) as the models that fit best each partition (i.e. ITS, *trnL-trnF* and *psbJ-petA*, respectively) under the Bayesian information criterion (BIC). Four Markov Monte Carlo chains were run simultaneously for five million generations and sampled every 1000 generations. Tracer ver. 1.5 (Rambaut & Drummond 2007) was use to examine convergence between the runs. After the runs reached convergence, analyses were stopped; 25% of the sampled trees were discarded as burn-in, and the remaining trees were used to generate a 50% majority rule consensus tree to establish posterior probabilities (PP). The effective sample size (ESS) at this stage was 1845.51, suggesting the analysis has run for enough generations and convergence was reached. Maximum parsimony (MP) analyses were conducted initially for each marker but we combined the data to produce greater levels of variation (partition homogeneity test not significant, $P = 0.403$) in PAUP* ver. 4.0b10 (Swofford 2003), using a heuristic search of 1000 replicates of random sequence addition, tree-bisection-reconnection (TBR) branch swapping and the MULTREES option on. All characters were weighted equally and gaps treated as missing data. Internal support was evaluated using 1000 bootstrap replicates (Felsenstein 1985) with TBR swapping and MULTREES on. Affinities among the *C. trilobus* aggregate were also explored by median network analyses using SplitsTree (Huson & Bryant 2006), which is useful for revealing contradicting signal among samples.

Results

Combined evidence from the morphometric analyses and DNA fingerprinting supports segregation of the variants "round leaf", "darkie", "trisept", "eastern hills" and "triwhite" from the *Corybas trilobus* aggregate and their recognition as species.

Canonical variate analysis (CVA):—CVA revealed that five of the 10 qualitatively determined variants can be discriminated from *C. trilobus* by using a set of quantitative characters. For instance, our first CVA showed that differences among *C. hypogaeus*, "triwhite", "darkie", "Rimutaka" and "Trotters", all members of the same clade (see phylogenetic results below) were significant (Wilk's $\Lambda = 0.042$, $F_{(56,\ 597.3)} = 13.381$, $P < 0.000$; Fig. 1). In this analysis the first and second axes accounted for most of the variation (95.8 %). *Corybas hypogaeus* and "triwhite" were fully separated along CV 1 (Fig. 1), whereas samples of "darkie" were separated from these two groups along CV 2 (only three individuals fell near *C. hypogaeus*). Separation of "Rimutaka" and "Trotters" was less evident, and most members of "Rimutaka" and "Trotters" overlapped with *C. hypogaeus* or fell within its morphospace. Variation along CV 1 correlated with petiole length, lateral sepal/petal length (Table 1, group 1). Variation along CV2 was correlated with petiole length, leaf width and petal length (Table 1, group 1). After samples of *C. hypogaeus*, "Rimutaka" and "Trotters" were removed from the analyses and replaced with those of *C. trilobus* s.s., separation of "triwhite" and "darkie" was still clear (Fig. 2, Wilk's $\Lambda = 0.1022$, $F_{(28,\ 226)} = 17.172$, $P < 0.000$). This time characters such as petiole length, leaf length and width (CVA 1) and the petals length (CVA 2) were the most important variables (Table 1, group 2).

CVA of *C. trilobus* s.s., "trisep", "tridodd", "tribrive" and "tricraig" was also statistically significant (Wilk's $\Lambda = 0.0082$, $F_{(56,\ 181.1)} = 7.861$, $P < 0.000$). In this case, the first and second axes accounted for 72.2% of the variation. Separation of some groups, however, was weak, and two mixed groups were recovered along CV1 (Fig. 3). The first group included members of "tridodd" and "tribrive" and the second members of *C. trilobus* s.s. and "tricraig". These two mixed groups were clearly separated from "trisept" along CV 2, which relates to leaf width and ovary and petal length (Table 1, group 3).

In our last CVA, we assessed morphological divergence among *C. trilobus* s.s., "eastern hills" and "round leaf" (the last two part of the same clade; see phylogenetic analysis below). The first and second axes accounted for the entire variation observed in the data, 72.9 and 27.1%, respectively. Among-group differences were also significant (Wilk's $\Lambda = 0.055$, $F_{(28,\ 202)} = 23.542$, $P < 0.000$), and separation of *C. trilobus* s.s. and "round leaf" along CV 1 was complete (Fig. 4). Both groups separated from "eastern hills" along CV 2, although four samples of the last fell near "round leaf". Variation along CV 1 was correlated to petiole length, leaf width and lateral sepal length (Table 1, group 4). Variation along CV2 was linked to the same leaf characters mentioned above and petal length (Table 1).

AFLP analyses:—The final AFLP data set consisted of 44 samples (one of the profiles of *C. hypogaeus* failed) and 138 bands. Of these, 101 bands were polymorphic and 77 potentially parsimony informative. The PCO analysis separates all samples into three clearly distinct clusters (Fig. 5A), each consisting of individuals of the same group identified *a priori* based on qualitative characters. The same groups were recovered in the NJ analyses, with two clusters receiving strong bootstrap support (Fig. 5B). Similar results were obtained when using automated scoring of the fragments (results not shown).

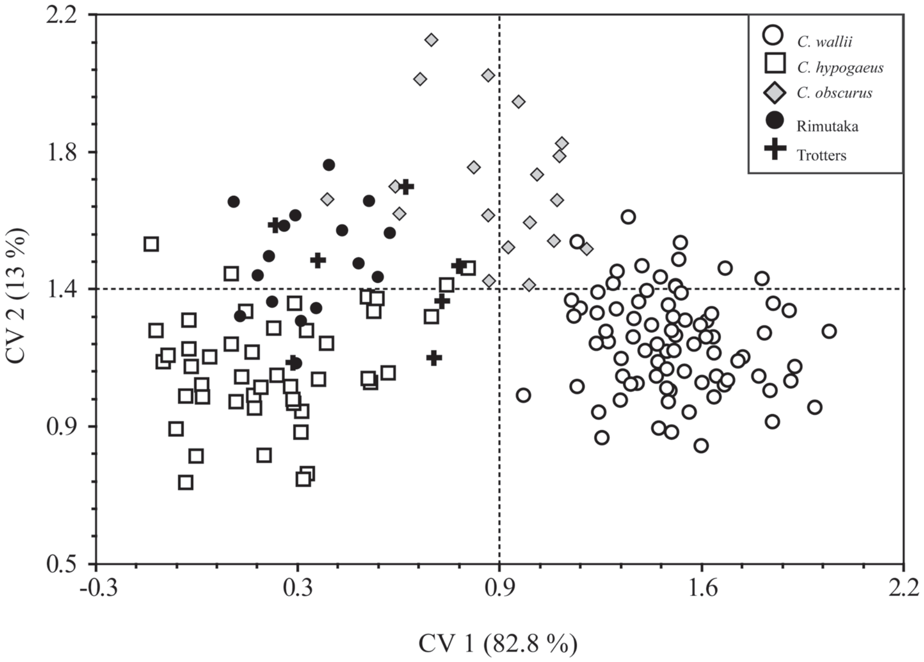

FIGURE 1. Canonical variate analysis of *Corybas hypogaeus*, *C. obscurus* ("darkie"), *C. wallii* ("triwhite") and two variants of the *C. trilobus* aggregate ("Rimutaka", "Trotters").

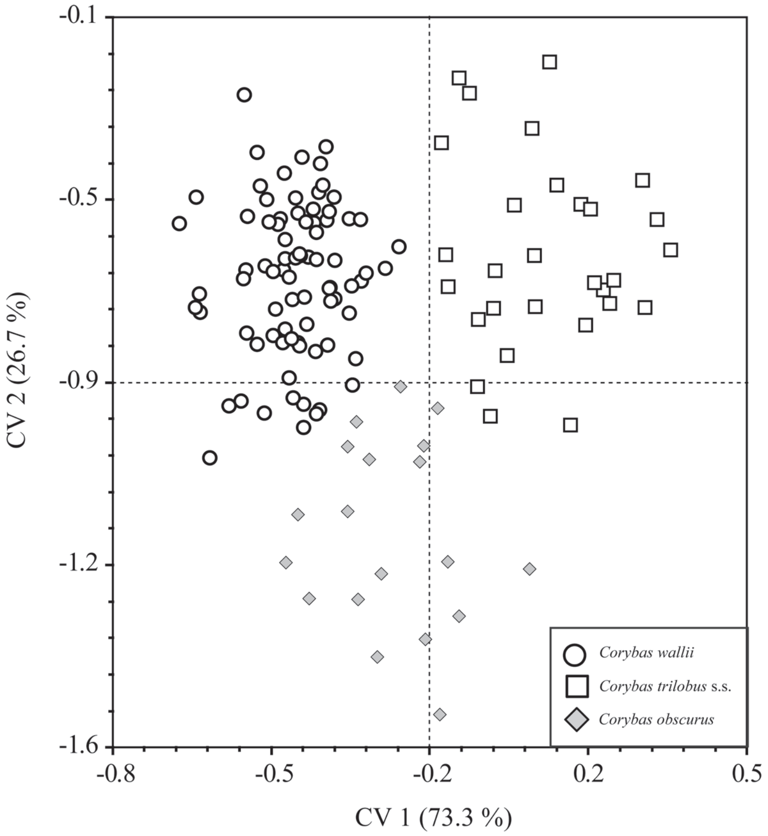

FIGURE 2. Canonical variate analysis of *Corybas trilobus* s.s., *C. obscurus* ("darkie") and *C. wallii* ("triwhite").

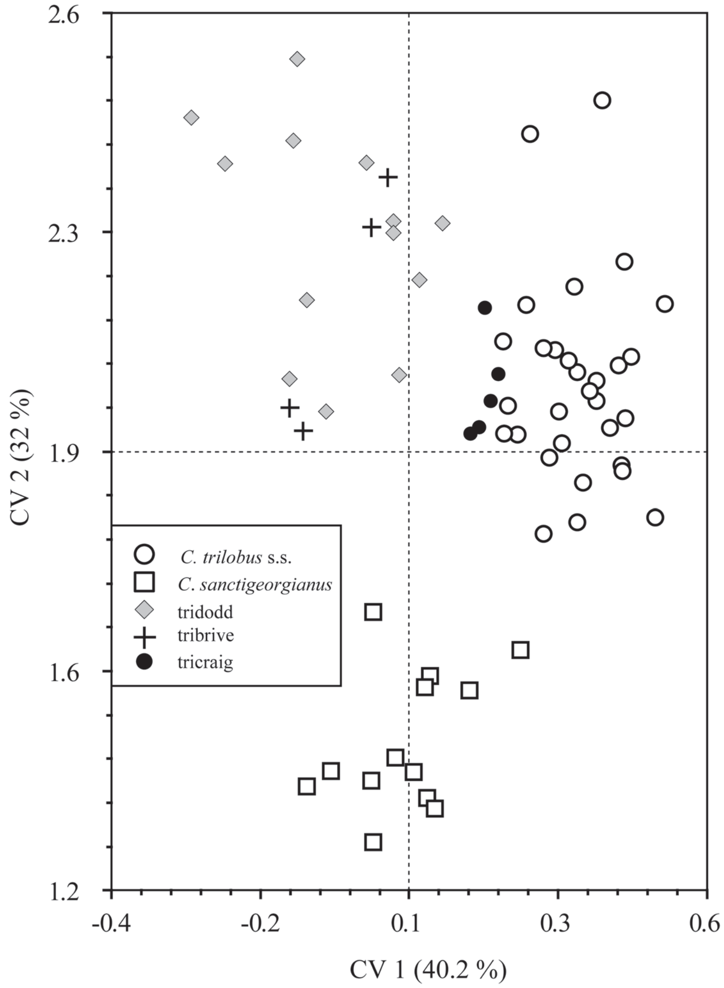

FIGURE 3. Canonical variate analysis of *Corybas trilobus* s.s., *C. sanctigeorgianus* ("trisept") and three variants of the *C. trilobus* aggregate ("tribrive", "tricraig", "tridodd").

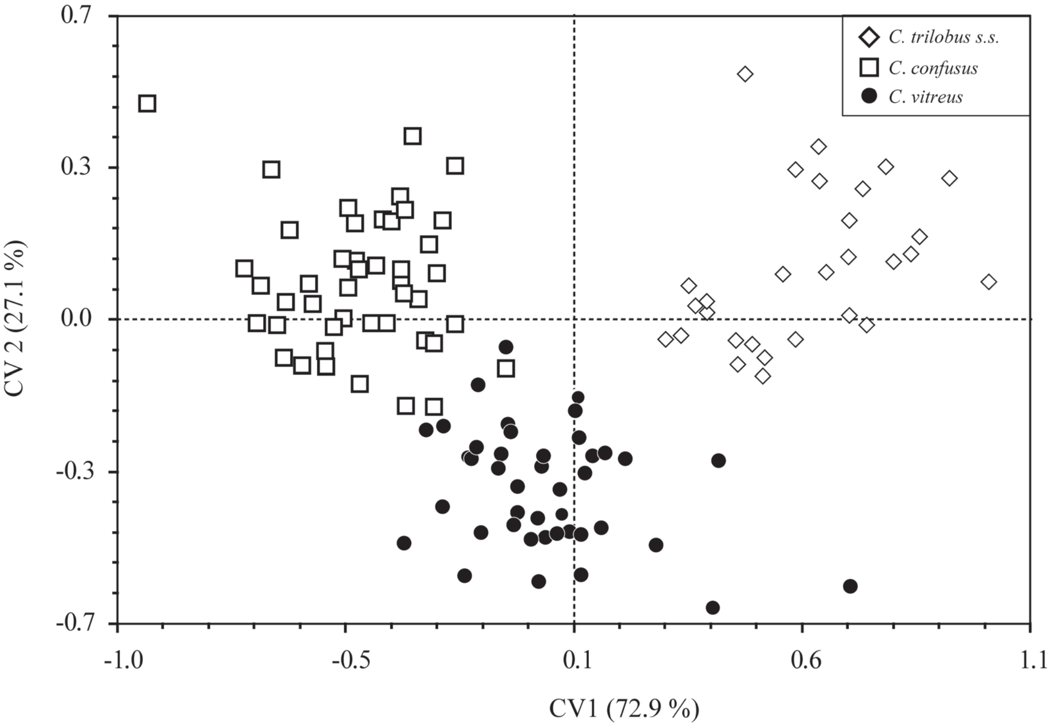

FIGURE 4. Canonical variate analysis of *Corybas trilobus* s.s., *C. confusus* ("round leaf") and *C. vitreus* ("eastern hills").

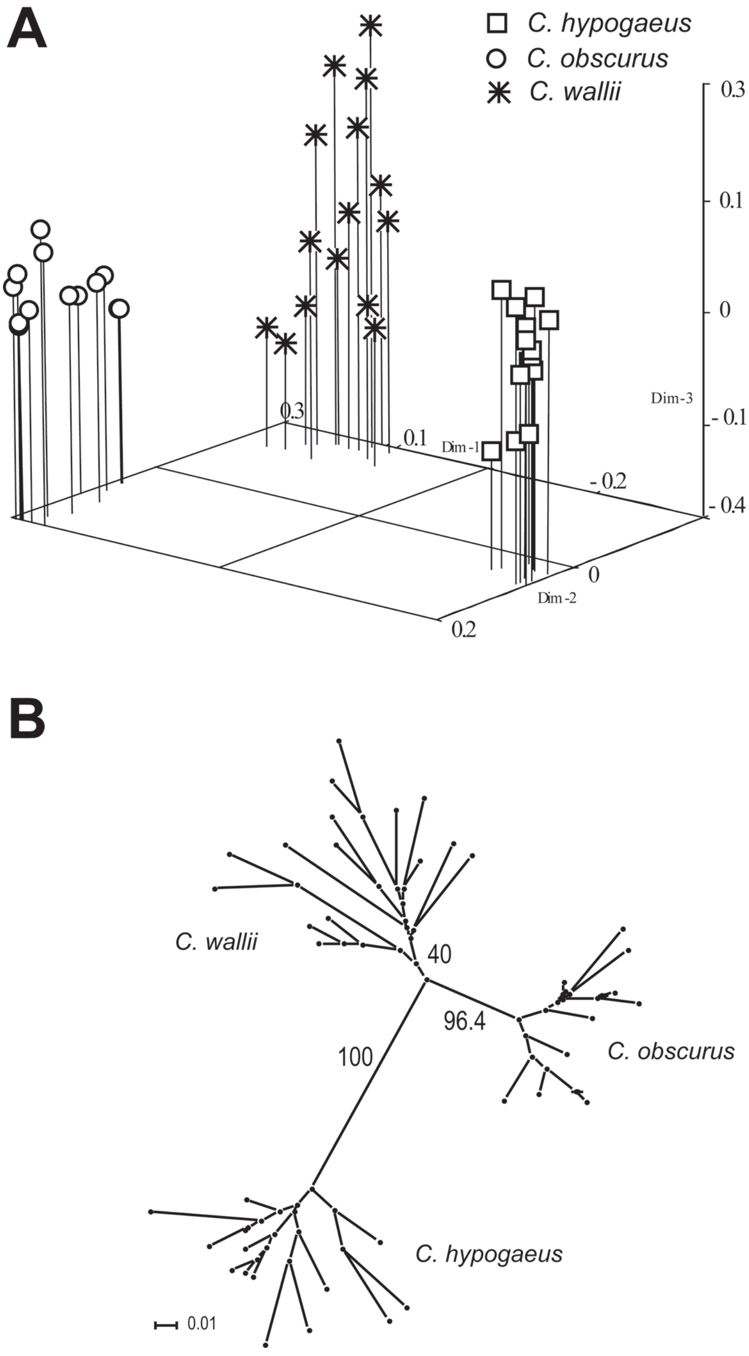

FIGURE 5. A. Principal coordinate analysis. B. Neighbor-Joining tree based on AFLP data from samples of *C. hypogaeus* (square), *C. obscurus* (circle) and *C. wallii* (asterisk) growing in sympatry. Numbers on NJ tree indicate bootstrap percentage.

Taxonomy

1. ***Corybas confusus*** Lehnebach, *sp. nov.* (Fig. 6A–C)

Type:—NEW ZEALAND. South Island: north-west Nelson, Kahurangi National Park, Salisbury Lodge, along the Potholes track, 16 November 2011, *Lehnebach, Frericks & Moorhouse SP104126* (holotype: WELT).

Diagnosis:—*C. confusus* resembles *C. obscurus* in the dark maroon colour of the labellum but differs by its dark to light green dorsal sepal with obtuse to slightly retuse apex that extends beyond the labellum. It differs from *C. trilobus* by its widely ovate to cordiform, distinctly mucronate leaf and dark maroon labellum.

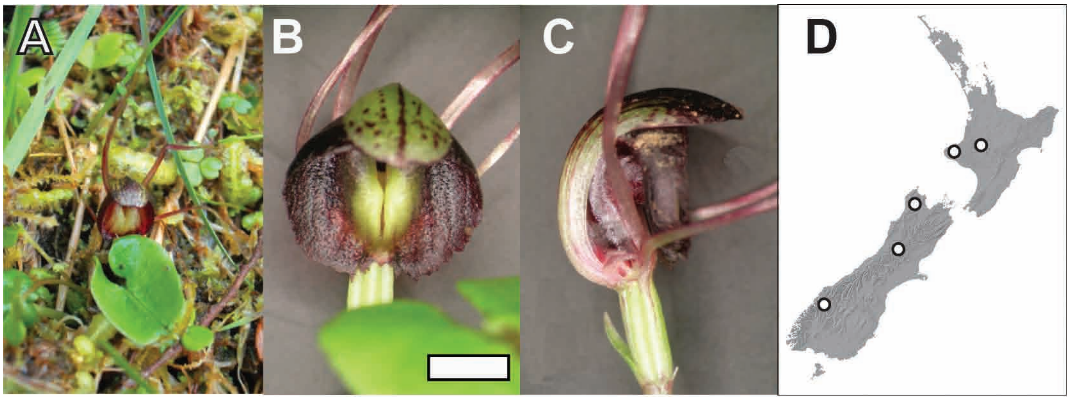

FIGURE 6. *Corybas confusus*, a new species of spider orchid endemic to New Zealand. A. Plant in the wild. B. Close up of the labellum. C. Side view of the flower. D. Distribution. Scale bar = 5 mm.

Terrestrial herbs, 12–30 mm tall at flowering. Leaf distinctly petiolate, petiole (2.8)7.5–12.5(31.4) mm long; lamina cordiform to widely ovate, (9.7)10.8–14.1(19.0) × (10.8)13.5–17.4(21.3) mm; margin entire; apex mucronate with mucro (0.1)0.8–1.5(2.2) mm long. Flower solitary, held erect on a peduncle (0.6)2.8–4.3(7.8) mm long; floral bract triangular to widely deltoid when flattened, (1)2.6–3.9(6.6) × (1.3)1.7–2.4(3) mm; dorsal sepal dark or light green, with red-maroon streaks and blotches towards the apex, arching over the labellum, concave to cucullate, narrow at the base and spathulate towards the tip, apex obtuse or slightly retuse; lateral sepals linear-filiform, whitish or red to maroon, (13.1)19.0–27.0(46.1) mm long; petals similar to the lateral sepals but longer, (27.0)36.9–49.7(75.0) mm long; labellum dark red to maroon with a pale green to yellowish centre, auriculate at base, aperture (0.8)1.8–2.3(3.0) mm in diameter; lamina deflexed, ca. 7–10 mm wide, with a central groove formed by an inwards folding of the lamina, extending downwards almost to the lower margin and sunken pit formed at the point where the lamina bends, upper margin incurving, mostly entire but finely erose or lacinate towards the lower margin, apex notched, inner surface covered with small hairs. Ovary (3.0)4.3–6.0(7.6) mm long. Column 2.1 mm long, straight, with truncate to emarginate wings at either side of the stigma.

Specimens examined:—NORTH ISLAND: Tongariro National Park, Whakapapa, sites along Whakapapanui stream, 16 October 2012, *McGlynn SP104176* (WELT); Tongariro National Park, Whakapapa, sample sites along Whakapapanui stream, 22 October 2011, *McGlynn SP104402* (WELT); Egmont/Taranaki National Park, Stratford plateau, 30 October 2012, *McGlynn SP104180* (WELT); Egmont National Park, Dawson falls, on Ridge track to Wilkies pools/Waingongoro hut, 30 October 2012, *McGlynn SP104403* (WELT). SOUTH ISLAND: Kahurangi National Park, at the intersection of Gordons Pyramid route and Potholes track, 16 November 2011, *Lehnebach, Frericks & Moorhouse SP104397* (WELT). Kahurangi National Park, along Gordons Pyramid route,16 November 2011, *Lehnebach, Frericks & Moorhouse SP104398* (WELT); Kahurangi National Park, along Potholes track, close to Sphinx Valley cave, 16 November 2011, *Lehnebach, Frericks & Moorhouse SP104399* (WELT); Arthur‘s Pass National Park, at the end of Otira Valley track, 12 December 2011, *Lehnebach & Zeller SP104143* (WELT); Arthur‘s Pass National Park, Kellys Creek, Carrol‘s hut track, 11 December 2011, Lehnebach & Zeller SP104400 (WELT); Arthur‘s Pass National Park, Otira Valley, 12 December 2011, *Lehnebach & Zeller SP104401* (WELT).

Distribution:—Endemic to NZ, both North and South Islands (Fig. 6D). It may also be present in the Chatham Islands, but further study of fresh fertile material is needed to confirm its presence there.

Habitat:—Found in leaf litter under southern beech [*Fuscospora* Heenan & Smissen (R.S.Hill & J.Read) (2013: 12–13) or *Lophozonia* Turczaninow (1858: 396), Nothofagaceae] forest, scrub or tussock in subalpine habitats, damp mossy subalpine open areas, 1000–1100 m.

Phenology:—Flowering October to early December; fruiting November to January.

Etymology:—The epithet *confusus* is a reference to the similarity between the flowers of *C. confusus* and *C. obscurus* and leaf shape to those of *C. macranthus* and *C. orbiculatus*.

Conservation status:—*Corybas confusus* is common in montane, sub-alpine and alpine areas of the North and South Islands. It is, therefore, regarded as 'Not Threatened' using the criteria of Townsend *et al*. (2008).

2. ***Corybas obscurus*** Lehnebach, *sp. nov*. (Fig. 7A–C)

Type:—NEW ZEALAND. South Island: Nelson Lakes National Park, Lake Rotoiti, along Lakeside track, near Whisky falls, 25 November 2011, *Lehnebach & Zeller SP104133* (holotype: WELT!).

Diagnosis:—Similar to *C. confusus* in lip colour but differs by its short dorsal sepal firmly appressed against the upper margins of the labellum and reniform to cordiform leaf. It differs from *C. trilobus* by the overall non-transparent, dark maroon to black flower.

FIGURE 7. *Corybas obscurus,* a new species of spider orchid endemic to New Zealand. A. Plant in the wild. B. Close up of the labellum. C. Side view of the flower. D. Distribution. Scale bar = 5 mm.

Terrestrial herbs, 14–30 mm tall at flowering. Leaf distinctly petiolate, petiole (5.1)14.2–23.1(40.8) mm long; lamina reniform to cordiform, (5.8)10.9–13.4(18.2) × (9.6)16–21.5(26.7) mm; margin entire; apex mucronate with mucro (1.1)15–2.2(2.5) mm long. Flower solitary, held erect on a peduncle (2.0)3.6–5.3(7.8) mm long, floral bract triangular to sub-triangular when flattened, (2.3)3.6–5.8(7.7) × (1.5)2.1–2.6(4.0) mm; dorsal sepal dark red to dark maroon to black, arching over the labellum, concave to cucullate, narrow at the base and widely spathulate towards the tip, apex retuse to emarginated; lateral sepal linear-filiform, crimson to dark red, (11.7)16.1–18.0(20.6) mm long; petals similar to the lateral sepals but longer, (27.3)35.7–43.6(57.2) mm long; labellum mostly dark crimson to almost black with a pale green centre, auriculate at base, aperture (1.6)1.7–2.1(2.4) mm in diameter; lamina deflexed, ca. 10 mm wide, with a central groove formed by the inwards folding of the lamina, extending downwards almost to the lower margin and sunken pit formed at the point where the lamina bends, margin incurving, mostly entire but erose at the lower edge, inner surface of the labellum covered glandular trichomes. Ovary (3.0)4.6–7.0(8.4) mm long. Column 1.6 mm long, curved, retuse column wings at either sides of the stigma.

Specimens examined:**—**SOUTH ISLAND: Nelson Lakes National Park, Cascade track. 26 November 2011, *Lehnebach & Zeller SP104134* (WELT); Nelson Lakes National Park, St Arnaud Range track, 27 November 2011, *Lehnebach & Zeller SP10413527* (WELT); Nelson Lakes National Park, Mt Robert road, 9 November 2012, *Lehnebach, Moorhouse & Upson SP104152* (WELT); Nelson Lakes National Park, Lake Rotoiti, Lakeside track from Mt Robert road to Coldwater hut, near Whiskey falls, 25 November 2011, *Lehnebach & Zeller SP104404* (WELT); Nelson Lakes National Park, Lake Rotoiti, Lakeside track from Mt Robert road to Coldwater hut, 25 November 2011, *Lehnebach & Zeller SP104405* (WELT).

Etymology:—A reference to the dark colour of the flower.

Distribution:—Endemic to NZ. Only known from Nelson Lakes National Park in the South Island and nearby forested areas (Fig. 7D).

Habitat:—Found in leaf litter under southern beech forest (*Fuscospora* or *Lophozonia,* Nothofagaceae] or Myrtaceae scrub, 600–1000 m.

Phenology:—Flowering October to early November; fruiting September to December.

Conservation status:—This species is common within the limits of Nelson Lakes National Park and nearby forested areas. According to Scanlen & St. George (2011) it also occurs in a few sites of the upper South Island, but we were not able to find any photographic or voucher material to confirm whether these sightings correspond to *C. obscurus*. Based on our field observations and following the New Zealand Threat Classification System (Townsend *et al*. 2008), we suggest *C. obscurus* should be ranked as Naturally Uncommon.

3. ***Corybas sanctigeorgianus*** Lehnebach, *sp. nov.* (Fig. 8A–C)

Type:—NEW ZEALAND. North Island, Hunua Regional Park, Wairoa Loop track, 10 August 2012, *Lehnebach & Zeller SP104149* (holotype: WELT!).

Diagnosis:—Similar to *C. hypogaeus* in flower and leaf shape but it can be distinguished by its straight ovary, labellum with a white centre and dorsal sepal extending beyond the labellum. It differs from *C. trilobus* by its long dorsal sepal and the white centre of the labellum.

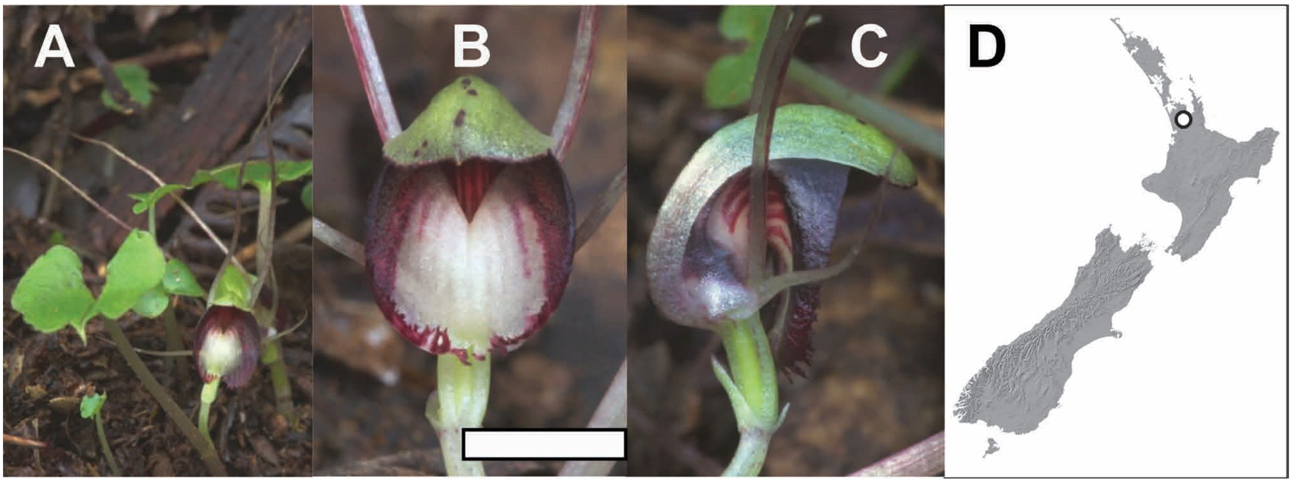

FIGURE 8. *Corybas sanctigeorgianus*, a new species of spider orchid endemic to New Zealand. A. Plant in the wild. B. Close up of the labellum. C. Side view of the flower. D. Distribution. Scale bar = 5 mm. Photos by P. Shearer.

Terrestrial herbs, 14–20 mm tall at flowering. Leaf distinctly petiolate, petiole (9.0)–9.8–12.7–(22.6) mm long; lamina reniform to cordiform, (8.5)10.0–13.2(13.8) × (12.6)14.0–19.0(20.3) mm; margin entire; apex acuminate (1.5)2.1–2.7(3.3) mm long. Flower solitary, held erect on a peduncle (2.5)3.3–3.9(4.4) mm long; floral bract ovate when flattened, (3.7)4.4–5.8(7.6) × (2.0)2.1–2.5(2.7) mm; dorsal sepal light green with a few blotches of dark red, arching over the labellum, concave to cucullate, narrow at the base and wide towards the tip, apex rounded or slightly mucronate; lateral sepals linear-filiform, white or translucent with dark red specks, (11.8)15.0–18.0(19.8) mm long; petals similar to the lateral sepals but longer, (32.8)38.9–47(53.3) mm long; labellum white in the centre, dark red towards the margins, and covered with short trichomes, auriculate at base; aperture (1.5)1.7–2.0(2.1) mm diameter; lamina deflexed, ca. 5 mm wide, with a central groove formed by the inward folding of the lamina, extending downwards half way the lamina and sunken pit formed at the point where the lamina bends, lateral margins incurve, mostly entire, lower margin laciniate to finally denticulate. Ovary (3.3)–4.0–4.4–(5.3) mm long. Column 1.8 mm long, straight, with truncate column wings on both sides of the stigma.

Specimens examined:—NORTH ISLAND: Auckland, Hunua Regional Park, September 2012, *Gaskett SP104166* (WELT); Auckland, Hunua Regional Park, September 2012, *Scanlen SP104167* (WELT).

Etymology:—This species is named in honour of Ian Saint George, who has greatly contributed to the knowledge of NZ orchids and promoted their study and conservation.

Distribution:—Endemic to NZ, known only from the Hunua Ranges in the North Island (Fig. 8D).

Habitat:—In leaf litter under scrub or podocarp-broadleaved forest, gravelly soil or decaying trunks of tree ferns, 140–330 m.

Phenology:—Flowering mid-August to September; fruiting October to early November.

Conservation status:—This species is only known from the type locality where a population of about hundred individuals occurs. Scanlen & St. George (2011) noted a second site existed in the 1960s, but this has now been lost. Based on these observations, our field observations and following the New Zealand Threat Classification System (Townsend *et al*. 2008), *C. sanctigeorgianus* should be ranked as "Nationally Critical".

4. ***Corybas vitreus*** Lehnebach, *sp. nov.* (Fig. 9A–C)

Type:—NEW ZEALAND. South Island: north-west Nelson, Kahurangi National Park, track to Gordon's Pyramid from Salisbury Lodge, 16 November 2011, *Lehnebach, Frericks & Moorhouse SP104127* (holotype: WELT!).

Diagnosis:—*C. vitreus* resembles *C. wallii* in the overall pale colour of its flower but differs by its translucent labellum lamina with a non-isometric, dark maroon to purple band along the lateral margin and straight translucent lower margin. It can be distinguished from *C. trilobus* by its broadly ovate flower in frontal view and emarginate to retuse dorsal sepal.

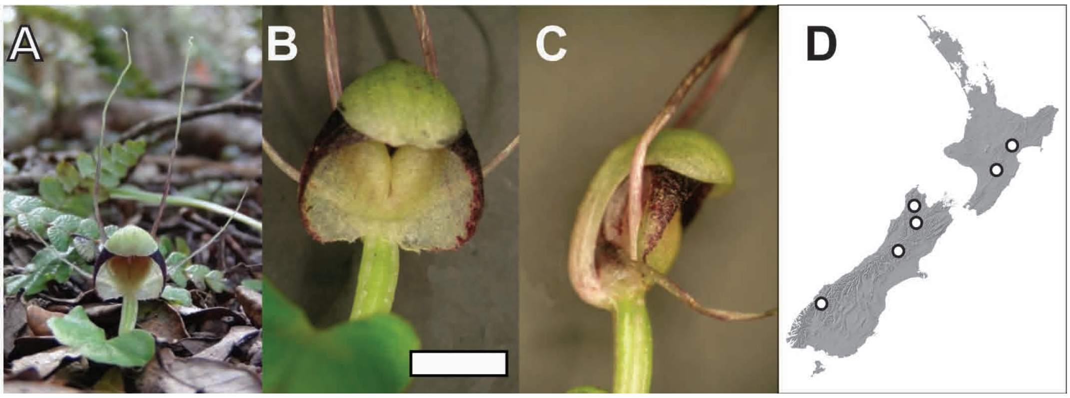

FIGURE 9. *Corybas vitreus*, a new species of spider orchid endemic to New Zealand. A. Plant in the wild. B. Close up of the labellum. C. Side view of the flower. D. Distribution. Scale bar = 5 mm.

Terrestrial herbs, 14–30 mm tall at flowering. Leaf distinctly petiolate, petiole (3.3)6.8–13(24.7) mm long; lamina cordiform, (3.3)8.5–11.3(14.1) × (7.8)12.3–16.0(19.6) mm; margin entire; apex mucronate with mucro (0.2)1.4–2(2.8) mm long. Flower solitary, held erect on a peduncle (0.7)2.7–5.5(8.1) mm long; floral bract triangular to widely deltoid when flattened, (1.8)3–4(6.8) × (1.3)1.8–2.4(3.2) mm; dorsal sepal pale green and less often with a few maroon streaks, concave to cucullate, arching over the labellum, narrow at the base and widely spathulate towards the tip, apex retuse to emarginated; lateral sepals linear-filiform, whitish to pale maroon, (12)15.4–21.6(26.7) mm long; petals are similar to the lateral sepals but longer, (21.3)33–43.6(56.5) mm long; labellum lamina mostly translucent, less often with a few streaks of maroon, covered with short trichomes, auriculate at base, aperture (1.2)1.8–2(2.6) mm in diameter; lamina deflexed, ca. 7 mm wide, with a central groove formed by the inwards folding of the lamina, extending downwards half way the labellum and sunken pit formed at the point where the lamina bends; lateral margin of the labellum incurving, entire, with a non-isometric dark maroon to purple band, lower margin translucent, straight and erose. Ovary (4.5)5.3–7.1(8.4) mm long. Column 1.8 mm long, straight, round wings flanking the stigma. Senescent flowers with apex of the dorsal sepal pressing against the labellum blocking access to the column.

Specimens examined:—NORTH ISLAND: Boundary Stream Mainland Island, Kamahi loop track, 25 September 2012, *Lusk SP104186* (WELT); Eastern Ruahine Ranges, slopes opposite to Waipawa river, 26 September 2011, Lehnebach, Zeller & Lusk *s/n*, spirit collection (WELT). SOUTH ISLAND: Kahurangi National Park, Salisbury track, 15 November 2011, *Lehnebach, Frericks & Moorhouse SP104390* (WELT); Kahurangi National Park, Gordons Pyramid route close to Salisbury track, 16 November 2011, *Lehnebach, Frericks & Moorhouse SP104391* (WELT); Kahurangi National Park, Gordons Pyramid route close to Salisbury track, 16 November 2011, *Lehnebach, Frericks & Moorhouse SP104392* (WELT); Kahurangi National Park, Gordons Pyramid route, 16 November 2011, *Lehnebach, Frericks & Moorhouse SP104396* (WELT); St Arnaud Range, road to Rainbow skifield, 10 November 2012, *Lehnebach, Moorhouse & Upson SP104155* (WELT); Arthur‘s Pass National Park, Avalanche Peak track, 10 December 2011, *Lehnebach & Zeller SP104140* (WELT); Mount Cook National Park, Governor‘s Bush track, 20 November 2012, *Lehnebach & Zeller SP104158* (WELT); Kinloch, Glacier Burn track, 23 November 2012; *Lehnebach & Zeller SP104394* (WELT); Routeburn track, track to Key Summit from the Divide, 25 November 2012, *Lehnebach & Zeller SP104395* (WELT).

Etymology:—The epithet reflecting the transparent nature of the labellum of this species.

Distribution:—Endemic to New Zealand. North and South Islands (Fig. 9D).

Habitat:—In leaf litter, under southern beech forest (*Fuscospora* or *Lophozonia*, Nothofagaceae) or Myrtaceae scrub, between 600 to 1300 m.

Phenology:—Flowering September to October; fruiting November to early January.

Conservation status:—*Corybas vitreus* is common in montane areas of the southern North Island and widespread in the South Island. It is, therefore, regarded as ‘Not Threatened’ using the criteria of Townsend *et al.* (2008).

5. ***Corybas wallii*** Lehnebach, *sp. nov*. (Fig. 10A–C)

Type:—NEW ZEALAND. North Island: Tongariro National Park, Whakapapa, along Whakapapanui stream, 21 October 2011, *McGlynn SP104210* (holotype: WELT!)

Diagnosis:—*C. wallii* resembles *C. vitreus* in the pale colour of its flower but it differs by its conspicuously yellow to pale green labellum lamina and margins, the presence of numerous glandular trichomes on the internal and external surface of the labellum and larger leaves. It differs from *C. trilobus* by its overall pale green to yellow flower.

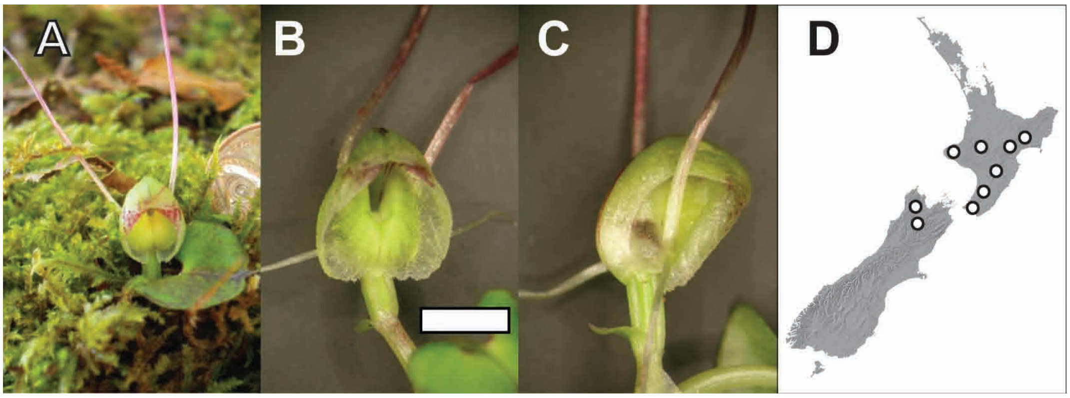

FIGURE 10. *Corybas wallii*, a new species of spider orchid endemic to New Zealand. A. Plant in the wild. B. Close up of the labellum. C. Side view of the flower. D. Distribution. Scale bar = 5 mm.

Terrestrial herbs, 13–34 mm tall at flowering. Leaf distinctly petiolate, petiole (5.5)9.3–15.1(28.8) mm long; lamina cordiform to sub-oblong or sub-hastate, (6.4)8.8–11.2(14) × (9.3)12.6–17.0(23.5) mm; margin entire; apex mucronate with mucro (0.2)1.5–2.0(2.6) mm long. Flower solitary, held erect on a peduncle (2.7)4.4–6.2(11.6) mm long, floral bract narrowly triangular to deltate when flattened, (2.6)4.2–5.5(10) × (1.4)2.2–2.8(3.7) mm, dorsal sepal pale yellow-green, arching over the labellum, concave to cucullate, narrow at the base and widely spathulate towards the tip and at times bearing trichomes on the upper surface, apex emarginated or rounded; lateral sepal linear-filiform, pale yellow-green, at times pale pink, (9.4)13.3–17.8(25) mm long; petals are similar to the dorsal sepals but longer, (24.5)39.5–51.2(62.2) mm long; labellum pale green or yellowish, less often with a few blotches of pink near the upper section, auriculate at base, aperture (1.2)1.8–2.1(2.6) mm in diameter; lamina deflexed, ca. 7 mm wide, with a central groove formed by the inwards folding of the lamina, extending downwards to the lower margin and sunken pit formed at the point where the lamina bends, margin incurve, mostly entire but erose at the lower margin, inner surface of the labellum covered with glandular trichomes, some also present in the outer surface. Ovary (3.0)4.7–6.5(9.0) mm long. Column 2.3 mm long, straight with deltate to shallowly deltate wings flanking the stigma.

Specimens examined:—NORTH ISLAND: Boundary Stream Main Island, Tumunako loop track, 19 October 2012, *Lusk SP104188* (WELT); Tongariro National Park, Whakapapa, sites along Whakapapanui river, 21 October 2011, *McGlynn SP104175* (WELT); Egmont/Taranaki National Park, about 75 m up Nature Loop track, behind camphouse, 14 November 2011, *McGlynn SP104179* (WELT); Egmont National Park, North Egmont visitor centre, Ngatoro loop walk, 14 November 2011, *McGlynn SP104409* (WELT); Egmont National Park, North Egmont Visitor Centre, Ngatoro loop walk, 29 October 2012, *McGlynn SP104410* (WELT); Egmont National Park, Stratford Plateau to ski field, 30 October 2012, *McGlynn SP104411* (WELT); Ruahine Forest Park, track to Rangiwahia hut, 24 September 2012, *McGlynn SP104178* (WELT); Eastern Ruahine Ranges, Sunrise trackt to Sunrise hut, 26 September 2011, *Lehnebach, Zeller & Lusk SP104406* (WELT); Eastern Ruahine Ranges, Swamp track, 26 September 2011, *Lehnebach, Zeller & Lusk SP104407* (WELT); Eastern Ruahine Ranges, Swamp track, 26 September 2011, *Lehnebach, Zeller & Lusk SP104408* (WELT); Tararua Forest Park, Otaki Forks, Pukeatua track, near the summit of Pukeatua, 25 October 2014, *Lehnebach & Zeller SP104164* (WELT); Rimutaka Range, Mount Climie, under scrub by the roadside, 02 Nov 2014, *Lehnebach & Zeller SP104169* (WELT). SOUTH ISLAND: Kahurangi National Park, track to Salisbury lodge, 15 November 2011, *Lehnebach, Frericks & Moorhouse SP104124* (WELT); Kahurangi National Park, Gordons Pyramid route, close to Potholes track, 16 November 2011, *Lehnebach, Frericks & Moorhouse SP104393* (WELT); Nelson Lakes National Park, Mt Robert road, 9 November 2012, *Lehnebach, Moorhouse & Upson SP104151* (WELT).

Etymology:—This species is named after Walli (Walburga) Zeller, mother of the second author of this article.

Distribution:—Endemic to New Zealand. North and South Islands (Fig. 10D).

Habitat:—In leaf litter under southern beech (*Fuscospora* or *Lophozonia* Nothofagaceae] or kamahi (*Weinmania racemosa* Linnaeus (1782: 227), Cunnoniaceae) forest, mossy seepages or gravelly soils, between 600 to 1150 m.

Phenology:—Flowering October to mid November, fruiting September to early January.

Conservation status:—*Corybas wallii* is common montane and sub-alpine areas of the North and South Islands. It is, therefore, regarded as 'Not Threatened' using the criteria of Townsend *et al*. (2008).

Key to the five species of *Corybas* segregated from *C. trilobus*

1	Labellum broadly expanded with lamina obtrullate in front view	clade D (Fig. 11)
	Labellum funnel- or dish-shaped with lamina orbicular to ovate in front view	2
2	Dorsal sepal extending past the labellum; apex free, sometimes reflexed	3
	Dorsal sepal shorter than the labellum; apex firmly pressing against its upper margin, not reflexed	6
3	Dorsal sepal apex acute	4
	Dorsal sepal apex retuse to obtuse	5
4	Labellum with a white centre, dark maroon towards the margins, leaf upper surface smooth	*C. macranthus*
	Labellum grading from dark red in the upper half to translucent-whitish in the lower half, leaf upper surface papillose	*C. papillosus*
5	Labellum dark red to maroon with a pale green to yellowish centre, lateral sepal > 19 mm, leaf cordiform to widely ovate	*C. confusus*
	Labellum dark red around the margins with a white centre, lateral sepal < 18 mm, leaf reniform to cordiform	*C. sanctigeorgianus*
6	Flowers translucent or mostly pale-coloured	7
	Flowers dark maroon with a pale green centre	9
7	Flowers pale green or yellowish with a few pink blotches	*C. wallii*
	Flowers with a mostly translucent labellum with dark red or maroon streaks or blotches	8
8	Flower broadly ovate in frontal view; apex of dorsal sepal emarginated to retuse; petals <43 mm long; petiole <13 mm long; leaf width <16 mm	*C. vitreus*
	Flower orbicular to widely elliptic in frontal view; apex of the dorsal sepal rounded; petals >43 mm long; petiole >13 mm long; leaf >18 mm width	*C. trilobus*
9	Flowers commonly < 6 mm diameter; dorsal sepal green; lateral sepals <13 mm long, petals <32 mm long; ovary curved backwards at *c* 45°	*C. hypogaeus*
	Flowers commonly >8 mm diameter; dorsal sepal dark maroon; lateral sepals >16 mm long; petals >35 mm long; ovary straight .	*C. obscurus*

Bayesian Analyses (BA):—Four clades were recovered within the NZ spider orchids. The first includes most members of the *C. trilobum* aggregate (Fig. 11, clade A). This is mostly unresolved and weakly supported (posterior probability/bootstrap percentage, PP/BP = 0.7/50) that consists of a subclade with accessions of *C. wallii* and *C. obscurus* forming three small clades, “Rimutaka”, “Trotters”, and North and South Island accessions of *C. hypogaeus*. The rest of clade includes accessions of *C. sanctigeorgianu*s that form the only well-supported group in this clade (Fig. 11, PP/BP = 1.0/75), and samples of *C. trilobus* s.s. and several variants from the northern North Island (e.g. “pygmy”, “tribrive”, “tricraig” and “tridodd). Also in this clade is *C. sulcatus*, a species considered endemic to Macquarie Island in the Southern Ocean.

The second clade comprises two subclades, one containing all accessions of *C. vitreus* and the other with all *C. confusus* (Fig. 11, clade B). Both species have been considered part of the *C. trilobus* aggregate, but here they fall in a different clade with moderate support. A sample collected in the Chatham Islands, identified as *C.* aff. *sulcatus* by its collector (WELT SP104165), falls within *C. confusus*.

The third and fourth clades are each strongly supported (Fig. 11, clade C, D; PP/BP = 1.0/100). Clade C includes *C. macranthus* and *C. papillosus* and a sample that needs further study (no fully developed flowers were available at the time for proper identification). The fourth clade (Fig. 11, clade D) includes six species for which genetic affinities are not fully resolved, except for the sister species *C. iridescens* and *C. papa*. A species from sub-Antarctic Macquarie Island, *C. dienemus*, also falls in this fourth clade.

Sister to these four clades is *Corybas acuminatus* (Fig. 11), a species widespread in NZ and also present in some of NZ off-shore islands (Chatham and Auckland) and Norfolk Island (Australia).

Maximum parsimony analyses (MPA):—The combined three-locus dataset included 1867 characters; 1646 characters were constant, 178 parsimony-uninformative and 43 potentially parsimony-informative. We recovered 1864 equally parsimonious trees, and the strict consensus tree obtained (not shown) is consistent with the BA tree (Fig. 11). The most significant difference between these trees is the presence in the MP tree of a weakly supported clade (BP <50) in clade A that only includes accessions of *C. wallii* and *C. obscurus*. Single-marker trees were poorly resolved, but the overall topology of each tree and clade content matched the combined tree (data not shown). For instance, clade A received 52, 56, 89 BP in the ITS, *trnL-trnF,* and *psbJ-petA* tree, respectively. Clade B received <50, 88 and 69 BS in the ITS, *trnL-trnF,* and *psbJ-petA* trees, respectively. Clade C received 84, 88 and <50 BP in the ITS, *trnL-trnF,* and *psbJ-petA* tree, respectively. Clade D received 99, 80 and 75 BP in the ITS, *trnL-trnF,* and *psbJ-petA* tree, respectively.

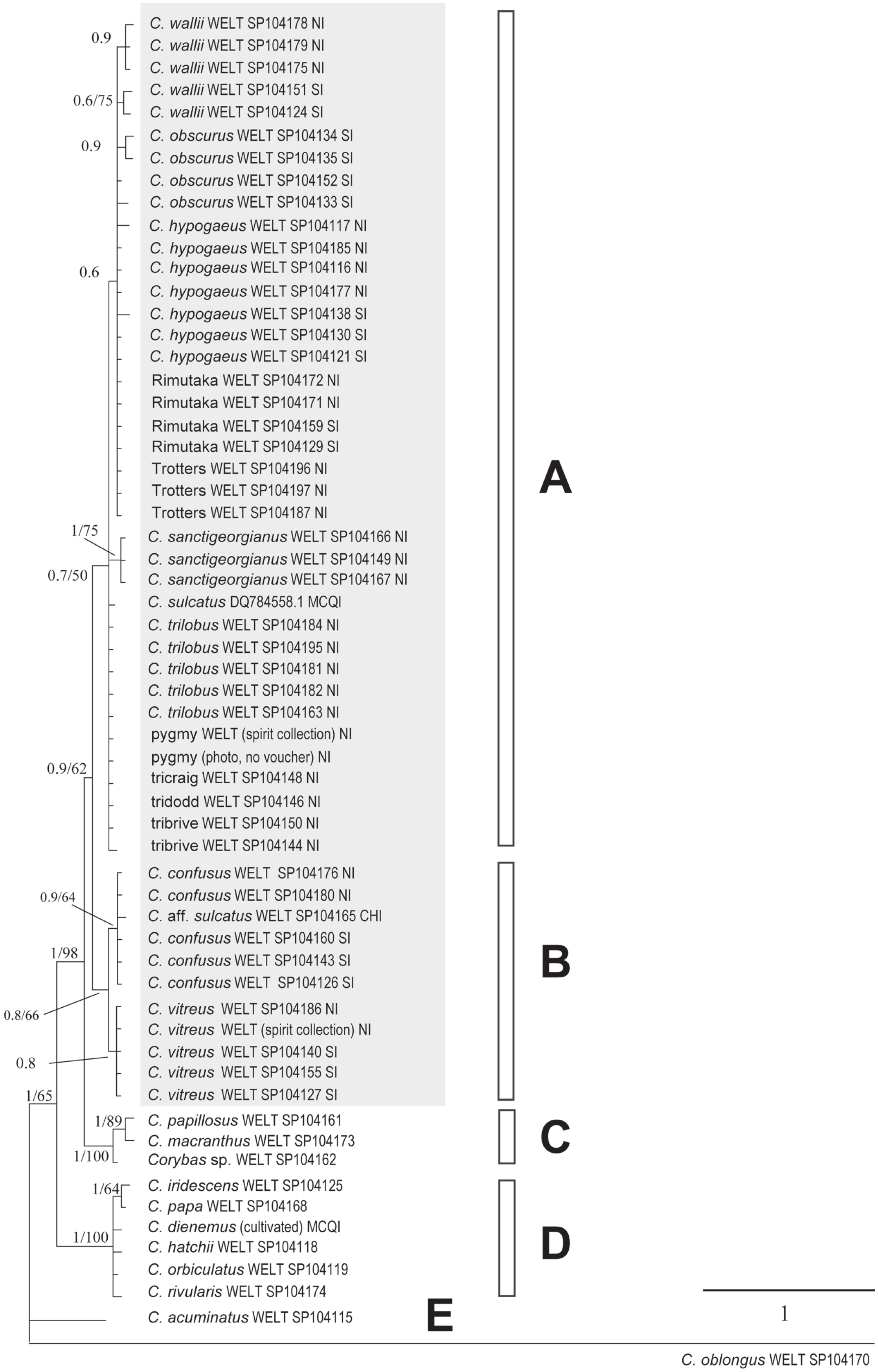

FIGURE 11. Bayesian tree of New Zealand spider orchids (*Corybas*) based on DNA sequence data from ITS, *trnL-trnF* and *psbJ-petA*. Major clades are indicated by open bars and capital letters, members of the *C. trilobus* aggregate are shaded, and posterior probabilities/ bootstrap percentages (≥50) indicated by numbers near each node. NI: North Island, SI: South Island, MCQI: Macquarie Island, CHI: Chatham Island.

Median network analysis (MNA):—All members of clade A (Fig. 11) except for *C. sulcatus*, for which only ITS sequences were available, were included in the MNA. Two groups are recovered in the network (Fig. 12, A and B), which are consistent in composition with those identified by BI (Fig. 11). Most samples fell in two polytomies except for *C. obscurus* and *C. wallii*, for which the relationship is represented as a complex network that connects accessions of *C. wallii* from the North and South Islands with samples of *C. obscurus*. Only one sample of *C. obscurus* (WELT SP104152) is not part of this section of the network, and it falls in a nearby polytomy that also includes all the accessions of *C. hypogaeus* (Fig. 12, arrow).

Discussion

New species and diversification of NZ spider orchids:—In New Zealand, orchids are a plant group requiring considerable systematic and taxonomic research (Lehnebach 2014). Numerous potentially new species have been reported over the years (e.g. de Lange *et al.* 1991, St. George 1999, de Lange *et al.* 2007, de Lange *et al.* 2013), yet their taxonomic status has remained unresolved. In this study, we formally described five new species of *Corybas* endemic to New Zealand, *C. confusus*, *C. obscurus*, *C. sanctigeorgianus*, *C. vitreus* and *C. wallii*. The distinctiveness of some of these orchids has been recognised for almost three decades (e.g. Gibbs 1987), and since then several qualitative vegetative and reproductive characters have been used to distinguish between them (Scanlen & St. George 2011). Our morphometric analysis has demonstrated these orchids are also distinguishable by quantitative characters. Features such as leaf petiole length and size, length of the lateral sepals and petals and the length of the ovary are the most informative characters. However, in the absence of fertile material, species identification is virtually impossible.

Taxonomic status of the other six variants is still unclear and requires further study to be solved. The morphometric analysis indicates some of these variants fall within the natural variation of widespread species, whereas other variants that appeared morphologically distinct do not form distinct genetic clusters in our analyses. For instance, the CVA analysis was unable to discriminate between samples of "Rimutaka", "Trotters" and *C. hypogaeus* (Fig. 1). Multiple accessions of these orchids were also indistinguishable from each other in their ITS and plastid DNA sequences. A similar result was obtained for "tricraig", which is found at single site on the northern North Island. All 'tricraig" samples grouped with *C. trilobus* s.s. in both morphometric and phylogenetic analyses. On the other hand, and in line with previous observations (e.g. Scanlen & St. George 2011), our morphometric analyses confirmed "tridodd" and "tribrive" are morphologically distinct from *C. trilobus* s.s. However, all three taxa are indistinguishable in their ITS and plastid DNA sequences (Fig. 11). As with "tricraig", these two entities are known only from a few, small, isolated populations on the northern North Island, and it is likely these phenotypic differences are merely local variants. To confidently decide whether these three variants are different species or simply local phenotypes of *C. trilobus*, we need to increase our sample sizes in future morphometric and DNA fingerprinting analyses. This will imply surveying remnants of native forest within private lands on the northern North Island, an area that has been highly deforested.

Our study showed that species boundaries between some sympatric species (i.e. *C. hypogaeus*, *C. obscurus* and *C. wallii*) are well defined, which supports operation of pre- or post-zygotic barriers, such as specific pollinators or hybrid incompatibility, respectively. Preliminary field observations suggest pollination of NZ spider orchids occurs by brood-site deception and is carried out by specific species of fungus gnats (*Mycetophyla*: Diptera; Lehnebach & Zeller 2015). Pollination takes place while the insects explore the flowers for a suitable place for oviposition. Further field observations and insect behavioural studies are still required to confirm specificity of this interaction and whether it has promoted diversification of these orchids in NZ.

Neither hybridisation in NZ spider orchids nor its contribution to species diversification can be ruled out. For instance, the net-like pattern observed on the MNA (Fig. 12) that connects *C. obscurus* with one North Island and two South Island accessions of *C. wallii* could indicate a potential hybrid origin for *C. obscurus*. Alternatively, such a pattern may reflect persistence of ancestral polymorphisms. Further assessment of our data confirmed that this pattern is caused by the ITS sequences from a single accession of *C. obscurus* (WELT SP104133; Fig. 12, star) from the South Island that groups with *C. wallii* (WELT SP104178; Fig. 12, star) from the North Island and not with the remaining samples of *C. obscurus*. Such non-monophyletic relationships are not unusual in NZ plants (Lockhart *et al.* 2001, Lockhart *et al.* 2014). A larger phylogeographic study including both orchid species using molecular markers with greater variability would be required to test whether past hybrisation has occurred in NZ *Corybas*.

Ecological and morphological diversification:—There is an unexpected mismatch between morphological and genetic divergence in these orchids. Genetic divergence was particularly low within clades with the most morphological diversity; i.e. *C. trilobus* aggregate and clade D (Fig. 11). These findings resemble those in *Ophrys*, a group of sexually deceptive orchids in which only a few clades are well supported or fully resolved (Soliva *et al*. 2001, Devey *et al.* 2007), despite using markers considered informative and suitable for studies at low taxonomic level (Shaw *et al*. 2007). Low genetic divergence within *Ophrys* was attributed to recent diversification (Devey *et al*. 2007) and a similar explanation may apply to NZ spider orchids.

Low genetic divergence in nuclear and plastid sequences has previously been reported in NZ plants, but mostly in species-rich alpine groups of recent origin (i.e. <10 Myr; Winkworth *et al*. 2005, Lehnebach 2015, Meudt *et al*. 2015). Although the NZ spider orchid is not an alpine group, it too seems to be of recent origin. Lyon (2014) suggests it diverged from its Australian sister clade, i.e. *Corysanthes*, and dispersed to NZ about 8 Myr ago (late Miocene). This was a time of significant climatic and geological changes in NZ; the temperature dropped, rainfall increased, uplift that would later create the NZ Alps began and cool-temperate elements such as southern beech dominated much of the vegetation (Fleming 1962, Heenan & McGlone 2013, Poole 2014). Nowadays, it is in the understory of these beech forests where many of these orchids grow, especially those within the *C. trilobus* aggregate. Plants belonging to less diverse clades such as C and D (Fig. 11) have colonised moist habitats such as stream or river banks, seepages and waterlogged peats (Clements & Jones 2007), both in lowland and sub-alpine areas. According to Heenan & McGlone (2013), such boggy habitats also have a recent origin in NZ and date from the late Miocene, about 6 Myr ago. Lyon's results (2014) suggest clades C and D radiated only 3.5–1.0 Myr ago (late Pleistocene).

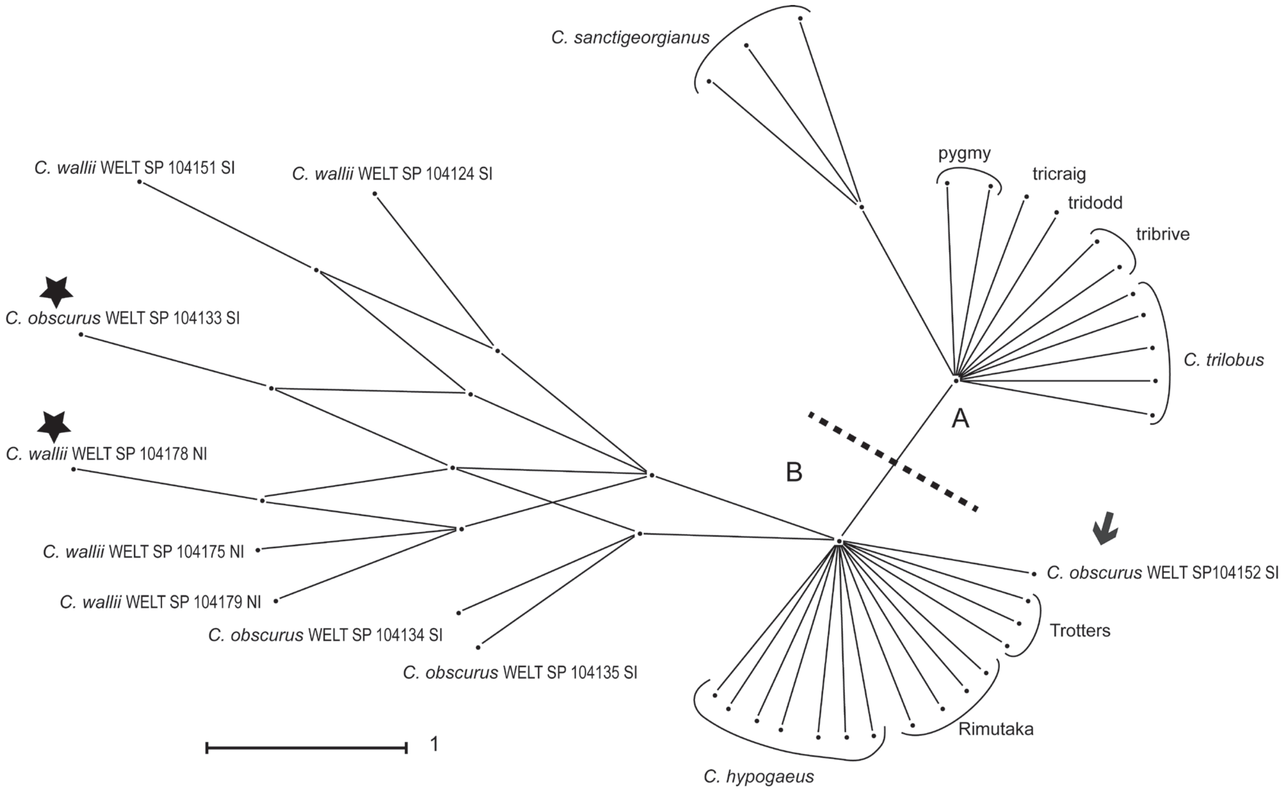

FIGURE 12. Median network analyses (MNA) of a subset of the *C. trilobus* aggregate (i.e. those in the clade A from Fig. 11) based on concatenated DNA sequence data from ITS, *trnL-trnF* and *psbJ-petA*. Stars and arrow indicate accessions discussed in the text. NI: North Island, SI: South Island.

Along with colonisation of different habitats, evolution of NZ spider orchids also involved diversification of breeding systems and floral morphology. These orchids are nectarless with dull-coloured flowers and long, filiform, forward- or upward-pointing lateral sepals and petals. This last feature is absent from the Australian sister clade *Corysanthes* (Hoffmann & Brown 1998), but it is present in some members of *Corybas* elsewhere (Lyon 2014). It is likely that this distinctive floral arrangement is involved in the attraction of pollinators (Lehnebach & Zeller 2015) as many of the NZ species are allogamous; however, this remains to be tested. Self-pollination, conversely, has evolved in only two species of the *Nematocera* clades, *C. dinemus* and *C. orbiculatus*, which both grow in damp habitats and belong to clade D (Fig. 11). These two species have undergone several structural changes; i.e. the rostellum is missing and the pollinia lack a viscidial disc and are in direct contact with the stigma, so self-pollination occurs as soon as the pollen matures and contacts the stigma. In *C. orbiculatus* the lateral sepals and petals are also distinctively short, shorter than in any other species in the *Nematoceras* clade. Since affinities within clade D are unresolved, we cannot determine if self-pollination has evolved once or twice in this group.

Further morphological shifts are observed in other floral and vegetative features. There are three types of labellum within NZ spider orchids, and these broadly coincide with the clades detected by BI (Fig. 11). For instance, species in clades A and B have flowers with a dish-shaped labellum with lateral margins slightly curved inwards (Fig. 13A). The labellum of species in clade C is funnel shaped, and its margins are extended and flared (Fig. 13B); species from clades D and *C. acuminatus* have a broadly flattened labellum with an obtrullate shape in frontal view (Fig. 13C). Also members of clade D and *C. acuminatus* lack a groove that runs longitudinally along the centre of the labellum where eggs of fungus gnats (*Mycetophila* sp., Diptera), their putative pollinators, are commonly found (Lehnebach & Zeller 2015).

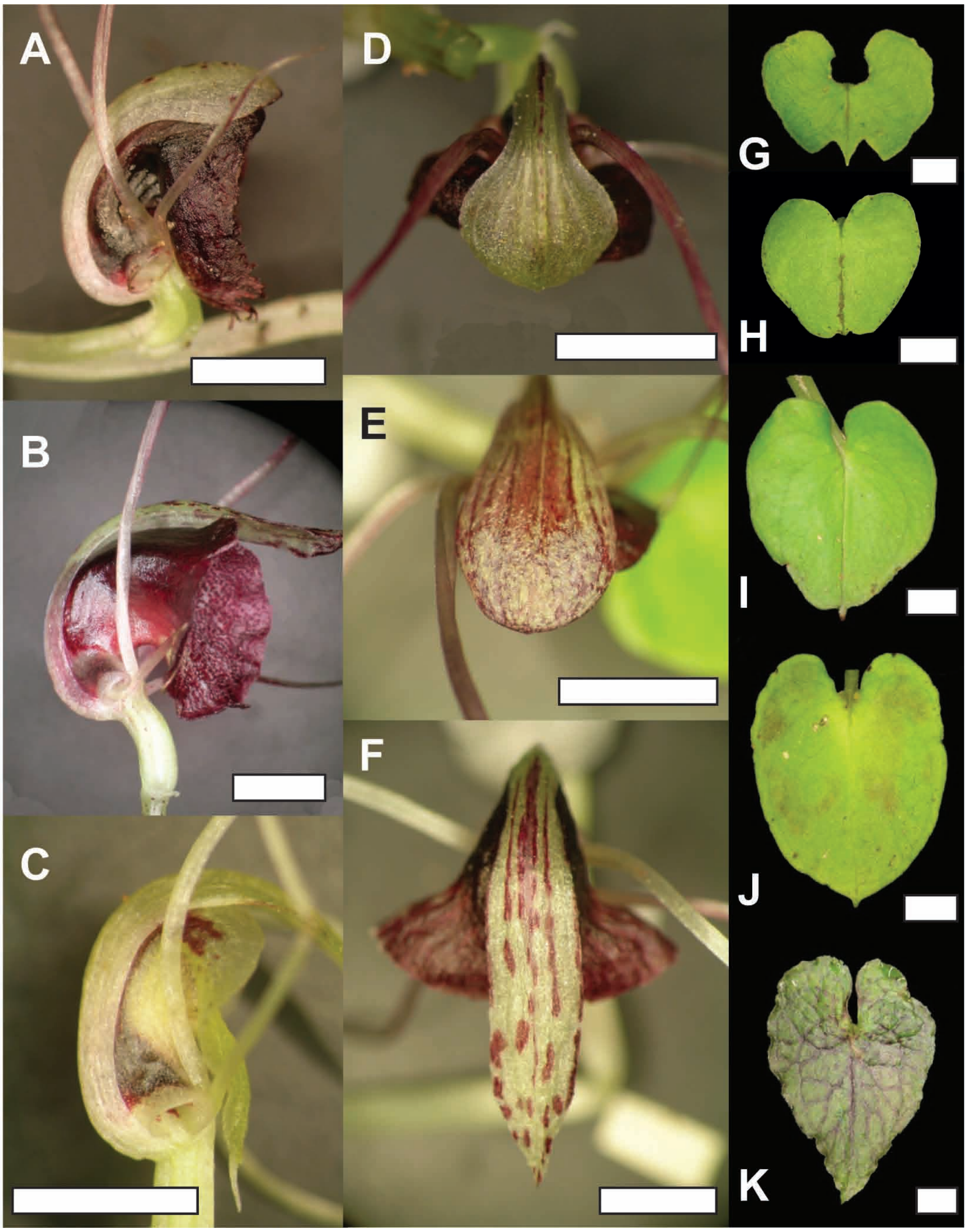

FIGURE 13. Morphological diversity of flowers and leaves in New Zealand spider orchids (*Corybas*). Labellum (A: *C. hypogaeus*, B: *C. macranthus*, C: *C. papa*); dorsal sepal (D: *C. hypogaeus*, E: *C. confusus*, F: *C. papillosus*), leaf (G: *C. hypogaeus*, H: "trotters", I: *C. confusus*, J: *C. orbiculatus*, K: *C. acuminatus*). Scale bar = 5 mm.

Shape and size of the dorsal sepals also vary between clades. Most members of clade A and *C. vitreus* (clade B) have a short dorsal sepal with a broad, rounded, retose or emarginate apex that firmly presses against the upper margins of the labellum (Fig. 13D). In *C. confusus* (clade B), the dorsal sepal is also relatively short, but it extends beyond the margins of the labellum and the apex is narrower to obtuse (Fig. 13E). In species of clade C, D and *C. acuminatus* on the other hand, the dorsal sepal is generally long, and the apex is free, acuminate and, in some species, strongly deflexed (Fig. 13F). Little is known about pollination of these orchids, and at this stage it is impossible to determine whether these floral changes are linked to shifts in pollination strategies.

As for leaf characters, species commonly found in leaf litter or moss under forests or scrub have thin, small, reniform to cordate leaves (Figs. 13G,H) whereas those in damp habitats have large orbicular leaves (Figs. 13I,J). Only *C. acuminatus* (Fig. 13K) from damp, shaded places has a deltoid-acuminate and thin leaf lamina.

Dispersal versus *in situ* speciation:—NZ spider orchids have dispersed from NZ to several offshore islands including those in the Southern Ocean, up to 700 km away. Evidence from our study, herbarium records (e.g. WELT SP018917, WELT SP018966) and results from Clements *et al.* (2007) indicate that dispersal has occurred south-eastwards and westwards, and members of all four clades have reached offshore islands. The Macquarie Island endemics, *C. dienemus* (Fig. 11, clade D) and *C. sulcatus* (Fig. 11, clade A), however, are believed to be the result of *in situ* speciation post colonization (Clements *et al.* 2007). If this is the case, diversification of these species has occurred in a short period of time because Macquarie Island is only 0.6 Myr old (Adamson *et al.* 1996) and with little genetic divergence between the resulting and other NZ relatives, e.g. *C. sulcatus* and *C. trilobus* differ only by 1-2 bp in their ITS sequences (Clements *et al.* 2007). The island endemic status of *C. sulcatus* and *C. dienemus*, in particular, is debatable. Clements *et al.* (2007) mentioned that plants similar to these species have been sighted in NZ (only photographic records were then available), but they considered them different from the Macquarie Island species. We disagree and, taking into account the widespread distribution of NZ spider orchids and recent collections of *C. dienemus*-like plants from montane, boggy habitats of the North Island of NZ (e.g. WELT SP104218), believe that the most likely explanation for these two orchids on Macquarie Island is long distance dispersal from NZ. Furthermore and because both species belong to different clades, we can infer that at least two long distance dispersal events to Macquarie Island have occurred. Further evidence of recent long distance dispersal is provided by the sample originally identified as *C.* aff. *sulcatus* (WELT SP104165) from the Chatham islands that falls within *C. confusus* (Fig. 11, clade B).

Future directions:—Our study has advanced the knowledge of orchid diversity in NZ and uncovered evidence of morphological and ecological diversification within a group where genetic divergence among species is low. Our results have also provided evidence of dispersal from NZ to offshore and sub-Antarctic islands. However, further work is needed to fully understand the processes that have triggered diversification of these orchids in NZ and evolution of their pollination system. Chromosome studies should also be encouraged as they could provide information on the role of potential polyploidy on the evolution of NZ spider orchids. Currently chromosomes numbers are available only for a few species, most of them diploid (2n = 36; Dawson *et al.* 2007).

Similarly, more taxonomic work is needed to assess the status of at least six variants of the *C. trilobus* aggregate. Along with the morphometric characterisation of fresh specimens, we recommend the use of fingerprinting techniques such as AFLP or microsatellites and a move away from traditional sequencing of nuclear and plastid markers, which are less informative. AFLP has proven useful to delimit species boundaries in our study and could be used to further explore phylogenetic structure within *Corybas* (e.g. Prebble *et al.* 2012). On the other hand, preliminary results of recently developed microsatellites are promising and could help to characterise genetic structure within uncommon, range-restricted species and mixed-species populations.

Acknowledgements

This work was supported by the Marsden Fund under Fast-Start Grant MNZ-1001 and the Australian Orchid Foundation under grant 298/2014 to CAL. We also thank the Department of Conservation, Greater Wellington Regional Council, Auckland Council and Forest & Bird Rangitikei for granting research/collection permits, P. Aspin, P. Dobbins, P. de Lange, A. Gaskett, P. Enright, M. Lusk, K. Luff, V. McGlynn, M. Moorhouse, C. Ogle, J. Rolfe, E. Scanlen, D, Steven, N. Swarts, G. Upson, for their assistance in the field or providing specimens to supplement our sampling, L. Perrie for advice on AFLP data analyses; P. Garnock-Jones, L. Shepherd and anonymous reviwers for comments on earlier versions of this manuscript.

References

Adamson, D.A., Selkirk, P.M., Price, D.M., Ward, N. & Selkirk, J.M. (1996) Pleistocene uplift and palaeoenvironments of Macquarie Island: evidence from palaeobeaches and sedimentary deposits. *Papers and Proceedings of the Royal Society of Tasmania* 130: 25–32.

Baldwin, B.G. (1992) Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics Evolution* 1: 3–16. http://dx.doi.org/10.1016/1055-7903(92)90030-K

Brawn, R. (1810) *Prodromus florae Novae Hollandiae et Insulae Van-Diemen*. Johnson, London, 460 pp.

Breitwieser, I., Brownsey, P.J., Garnock-Jones, P.J., Perrie, L.R. & Wilton, A.D. (2012) Phylum Tracheophyta: vascular plants. *In:* Gordon, D.P. (Ed.) *New Zealand inventory of biodiversity*. Canterbury University Press, Christchurch, pp. 411–459.

Chase, M.W., Cameron, K.M, Freudenstein, J.V., Pridgeon, A.M, Salazar, G., van den Berg, C. & Schuiteman, A. (2015) An updated classification of Orchidaceae. *Botanical Journal of the Linnean Society* 177: 151–174. http://dx.doi.org/10.1111/boj.12234

Clements, M.A. & Jones, D.L. (2007) A new species of *Nematoceras* and characterisation of *N. dienemum* (Orchidaceae), both from subantarctic Macquarie Island. *Telopea* 11: 405–411. http://dx.doi.org/10.7751/telopea20075739

Clements, M.A., Jones, D.L., Sharma, I.K., Nightingale, M.E., Garratt, M.J., Fitzgerald, K.J., Mackenzie A.M. & Molloy, B.P.J. (2002) Phylogenetic systematics of the Diurideae (Orchidaceae) based on the ITS and 5.8S coding region of nuclear ribosomal DNA. *Lindleyana* 17: 135–171.

Clements, M.A., Mackenzie, A.M., Copson G.R., Molloy, B.P.J., Carmichael, N., Skotnicki, M.L. & Selkirk P.M. (2007) Biology and molecular phylogenetics of *Nematoceras sulcatum*, a second endemic orchid species from subantarctic Macquarie Island. *Polar Biology* 30: 859–869. http://dx.doi.org/10.1007/s00300-006-0246-y

de Lange, P.J, Crowcroft, G.M. & Forester, L.J. (1991) *Thelymitra* “Ahipara” - an endangered orchid transferred, with notes on its taxonomic status, distributions and ecology. *Science & Research Internal Report 113*. Department of Conservation, Wellinton, 11 pp.

de Lange, P.J, Rolfe, J., St. George, I. & Sawyer, J. (2007) *Wild orchids of the lower North Island*. Department of Conservation, Wellington, 194 pp.

de Lange, P.J., Rolfe, J.R, Champion, P.D., Courtney, S.P., Heenan, P.B. Barkla, J., Cameron, E.K., Norton, D.A. & Hitchmough, R.A. (2013) Conservation status of New Zealand indigenous vascular plants, 2012. *New Zealand threat classification, series 3*. Department of Conservation, Wellington, 70 pp.

Dawson, M.I., Molloy, B.P.J. & Beuzenberg, E.J. (2007) Contributions to a chromosome atlas of the New Zealand flora - 39. Orchidaceae. *New Zealand Journal of Botany* 45: 611–684. http://dx.doi.org/10.1080/00288250709509743

Dransfield, J., Comber, J.B. & Smith, G. (1986) A synopsis of *Corybas* (Orchidaceae) in West Malaysia and Asia. *Kew Bulletin* 41: 575–613. http://dx.doi.org/10.2307/4103118

Devey, D.S., Bateman, R.M., Fay, M.F. & Hawkins, J.A. (2007) Friends or relatives? Phylogenetics and species delimitation in the controversial European orchid genus *Ophrys*. *Annals of Botany* 101: 385–402. http://dx.doi.org/10.1093/aob/mcm299

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Dytham, C. (2003) *Choosing and using statistics: a biologist’s guide*. Blackwell, Malden, 248 pp.

Fay, M.F., Cowan, R.S. & Leitch, I.J. (2005) The effects of nuclear DNA content (C-value) on the quality and utility of AFLP fingerprints. *Annals of Botany* 95: 237–246. http://dx.doi.org/10.1093/aob/mci017

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791. http://dx.doi.org/10.2307/2408678

Fleming, C.A. (1962) New Zealand biogeography - a palaeontologist’s approach. *Tuatara* 10: 53–104.

Forrest, A.D., Hollingsworth, M.L., Hollingsworth, P.M., Sydes, C. & Bateman, R.M. (2004) Population genetic structure in European populations of *Spiranthes romanzoffiana* set in the context of other genetic studies on orchids. *Heredity* 92: 218–227. http://dx.doi.org/10.1038/sj.hdy.6800399

Gibbs, G. (2006) *Ghosts of Gondwana: the history of the life in New Zealand*. Craig Potton Publishing, Nelson, 232 pp.

Gibbs, M. (1987) A *Corybas* species found at Iwitahi. *New Zealand Native Orchid Group Newsletter* 24: 6.

Hedrén, M., Fay, M.F. & Chase, M.W. (2001) Amplified fragment length polymorphisms (AFLP) reveal details of polyploid evolution in *Dactylorhiza* (Orchidaceae). *American Journal of Botany* 88: 1868–1880.
http://dx.doi.org/10.2307/3558363

Heenan, P.B. & McGlone, M.S. (2013) Evolution of New Zealand alpine and open-habitat plant species during the late Cenozoic. *New Zealand Journal of Ecology* 37: 105–113.

Heenan, P.B. & Mitchell, A.D. (2003) Phylogeny, biogeography and adaptive radiation of *Pachycladon* (Brassicaceae) in the mountains of South Island, New Zealand. *Journal of Biogeography* 30: 1737–1749.
http://dx.doi.org/10.1046/j.1365-2699.2003.00941.x

Heenan, P.B. & Smissen, R.D. (2013) Revised circumscription of *Nothofagus* and recognition of the segregate genera *Fuscospora*, *Lophozonia*, and *Trisyngyne* (Nothofagaceae) *Phytotaxa* 146 (1): 1–31.
http://dx.doi.org/10.11646/phytotaxa.146.1.1

Hoffmann, N. & Brown, A.P. (1998) *Orchids of south-west Australia*. University of Western Australia Press, Nedlands, 462 pp.

Hooper, S.D. (2009) Taxonomic turmoil down-under: recent developments in Australian orchid systematic. *Annals of Botany* 104: 447–455.
http://dx.doi.org/10.1093/aob/mcp090

Hooker, J.D. (1853) *Flora Novae-Zelandiae*. Reeve, London, 312 pp.

Hooker, J.D. (1864) *Handbook of the New Zealand flora*. Reeve, London, 392 pp.

Hooker, J.D. & Salisbury, R.A. (1805) *The paradisus Londinensis*. Shury, London, 83 pp.

Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.
http://dx.doi.org/10.1093/bioinformatics/17.8.754

Huson, D.H. & Bryant, D. (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23: 254–267.
http://dx.doi.org/10.1093/molbev/msj030

Janes, J.K., Steane, D.A. & Vaillancourt, R.E. (2012) What does population structure analysis reveal about the *Pterostylis longifolia* complex (Orchidaceae)? *Ecological Evolution* 2: 2631–2644.
http://dx.doi.org/10.1002/ece3.376

Jones, D.L., Clements, M.A., Sharma, I.K., Mackenzie, A.M. & Molloy, B.P.J. (2002) Nomenclatural notes arising from studies into the tribe Diurideae (Orchidaceae). *The Orchadian* 13: 437–468.

Lehnebach, C.A. (2014) New Zealand native orchids: current research and conservation efforts. *Orchids*: 73–78.

Lehnebach, C.A. (2015) A garden at the edge of the world; the diversity and conservation status of the New Zealand flora. *In*: Stow, A., Maclean, N. & Holwell, G.I. (Eds.) *Austral ark: the state of wildlife in Australia and New Zealand*. Cambridge University Press, Cambridge, pp. 240–258.

Lehnebach, C.A. & Zeller, A.J. (2015) Dissecting the smell of deception; the chemistry behind the pollination system on New Zealand spider orchids. *Orchids*: 65–70.

Linnaeus, C. (1782) *Supplementum plantarum systematis vegetabilium.* Orphanotrophe, Brunswig. 467 pp.

Lockhart, P., McLenachan, P.A., Havell, D., Glenny, D., Huson, D. & Jensen, U. (2001) Phylogeny, dispersal and radiation of New Zealand alpine buttercups: molecular evidence under split decomposition. *Annals of the Missouri Botanical Garden* 88: 458–477.
http://dx.doi.org/10.2307/3298586

Lockhart, P.J., Larkum, A.W.D., Becker, M. & Penny, D. (2014) We are still learning about the nature of species and their evolutionary relationships. *Annals of the Missouri Botanical Garden* 100: 6–13.
http://dx.doi.org/10.3417/2012084

Losos, J.B. & Ricklefs, R.B. (2009) Adaptation and diversification on islands. *Nature* 457: 830–836.
http://dx.doi.org/10.1038/nature07893

Lyon, S.P. (2014) Molecular systematics, biogeography, and mycorrhizal associations in the Acianthinae (Orchidaceae), with a focus on the genus *Corybas*. Doctoral thesis, University of Wisconsin-Madison, USA.

Mant, J., Peakall, R. & Schiestl, F.P. (2005) Does selection on floral odor promote differentiation among populations and species of the sexually deceptive orchid genus *Ophrys*? *Evolution* 59: 1449–1463.
http://dx.doi.org/10.1554/04-547

Meudt, H.M., Prebble, J.M. & Lehnebach, C.A. (2015) Native New Zealand forget-me-nots (*Myosotis*, Boraginaceae) comprise a Pleistocene species radiation with very low genetic divergence. *Plant Systematics and Evolution* 301: 1455–1471.
http://dx.doi.org/10.1007/s00606-014-1166-x

Moore, L.B. (1976) Orchidaceae. *In*: Moore, L.B. & Edgar, E. (Eds.) *Flora of New Zealand*, Volume II (102–167). Shearer Government

Printer, Wellington, pp. 102–167.

Poole, M. (2014) The Miocene climate in New Zealand: estimates from paleobotanical data. *Palaeontologia Electronica* 17: 1–79.

Posada, D. (2008) JModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256. http://dx.doi.org/10.1093/molbev/msn083

Prebble, J.M., Meudt, H.M. & Garnock-Jones, P. (2012) Phylogenetic relationships and species delimitation of New Zealand bluebells (*Wahlenbergia*, Campanulaceae) based on analyses of AFLP data. *New Zealand Journal of Botany* 50: 365–378. http://dx.doi.org/10.1080/0028825X.2012.698624

Rambaut, A. & Drummond, A.J. (2007) *Tracer*. Available from: http://beast.bio.ed.ac.uk/Tracer (accessed 1 August 2016)

Reichenbach, H.G. (1871) Beitr*ä*ge zur Systematischen Planzenkunde. Hamburg: Meissner, 73 pp.

Scanlen, E. & St. George, I. (2011) *Colour field guide to the native orchids of New Zealand.* New Zealand Native Orchid Group Inc., Wellington, 83 pp.

Schluter, D. (2000) *The ecology of adaptive radiation.* Oxford University Press, New York, 296 pp.

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E. & Small, R.L. (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142–166.
http://dx.doi.org/10.3732/ajb.92.1.142

Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. (2007) Comparison of whole chloroplast genome sequences to choose non-coding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* 94**:** 275–288. http://dx.doi.org/10.3732/ajb.94.3.275

Soliva, M., Kocyan, A. & Widmer, A. (2001) Molecular phylogenetics of the sexually deceptive orchid genus *Ophrys* (Orchidaceae) based on nuclear and chloroplast DNA sequences. *Molecular Phylogenetics and Evolution* 20: 78–88. http://dx.doi.org/10.1006/mpev.2001.0953

St. George, I. (1999) *The natural guide to New Zealand orchids.* Random House, Auckland, 176 pp.

St. George, I. (2006) The New Zealand orchids: the editor's annual list of the New Zealand orchid *taxa*. *New Zealand Native Orchid Group Journal* 101: 6–14.

Swofford, D.L. (2003) PAUP*. Phylogenetic analysis using parsimony (*and other methods, Version 4). Sinauer Associates, Sunderland.

Szlachetko, D. & Rutkowski, P. (2003) Quelques notes additionnelles sur la sous-famille Thelymitroideae (Orchidaceae). *Richardiana* 3: 90–100.

Thompson, J., Higgins, D. & Gibson, T. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4690. http://dx.doi.org/10.1093/nar/22.22.4673

Townsend, A., de Lange, P., Duffy, C., Miskelly, C., Molloy, J. & Norton, D. (2008) *New Zealand threat classification system manual.* Department of Conservation, Wellington, 30 pp.

Turczaninow, C.G.J. (1858) Animadversiones in secundam partem herbarii Turczaninowiani, nunc Universitatis Caesareae Charkowiensis. *Bulletin de la Société Imperiale des Naturalistes de Moscou* 31: 380–500.

Valente, L.M., Etienne, R.S. & Phillimore, A.B. (2014) The effects of island ontogeny on species diversity and phylogeny. *Proceedings of the Royal Society B: Biological Sciences* 281: 20133227.
http://dx.doi.org/10.1098/rspb.2013.3227

WCSP (2016) *World checklist of selected plant families*. Facilitated by the Royal Botanic Gardens, Kew. Available from: http://apps.kew.org/wcsp/ (accessed 8 January 2016) [Published on the Internet]

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*. Academic Press, New York, pp. 315–322.
http://dx.doi.org/10.1016/b978-0-12-372180-8.50042-1

Winkworth, R.C., Wagstaff, S.J., Glenny, D. & Lockhart. P.J. (2005) Evolution of the New Zealand mountain flora: origins, diversification and dispersal. *Organisms, Diversity and Evolution* 5: 237–247.
http://dx.doi.org/10.1016/j.ode.2004.12.001

APPENDIX 1. Voucher details for other specimens of *Corybas* included in the morphometric study.

C. trilobus *s.s.*
NORTH ISLAND: Whanganui, Gordon Park Scenic Reserve, 02 October 2012, *McGlynn SP104181* (WELT); Whanganui, Gordon Park Scenic Reserve, 02 October 2012, *McGlynn SP104182* (WELT); Whanganui, Gordon Park Scenic Reserve, 21 October 2012, *McGlynn SP104183* (WELT). Masterton, Upper Plain road, Highden Station, a QEII covenant, 15 September 2012, *Enright SP104184* (WELT); Masterton, Upper Plain road, Highden Station, a QEII covenant, 15 September 2012, *Enright SP104423* (WELT).Turakina Valley Road, Sutherland's Bush, a Forest and Bird Reserve, in Podocarp-broadleaf forest by river terrace, 19 November 2012, *McGlynn SP104195* (WELT).

Rimutaka
NORTH ISLAND: Wellington, Lower Hutt, East Harbour Regional Park, from Bus Barn track to Butterfly creek, 10 September 2012, *Lehnebach SP104171* (WELT). SOUTH ISLAND: Nelson, Aniseed Valley, Roding River, patch of forest by the road, 17 November 2011, Lehnebach, Frericks & Moorhouse *SP104129* (WELT); Mount Cook National Park, Governor's Bush track, 20 November 2012, Lehnebach & Zeller *SP104159* (WELT).

Trotters
NORTH ISLAND: Masterton, Upper Plain Road, Highden Station, a QEII covenant, 15 Sepember 2012, *Enright SP104422* (WELT).

Tribrive
NORTH ISLAND: Hunua Ranges Regional Park, Wairoa loop track, 26 August 2012, *Gaskett SP104421* (WELT).

Tricraig
NORTH ISLAND: Auckland, Awhitu Peninsula, 09 August 2012, *Lehnebach, Zeller & Aspin SP104148* (WELT)

Tridodd
NORTH ISLAND: Auckland, Awhitu Peninsula, 09 August 2012, *Lehnebach, Zeller & Aspin SP104146* (WELT); Auckland, Awhitu Peninsula, 09 August 2012, *Lehnebach, Zeller & Aspin SP104388* (WELT); Auckland, Awhitu Peninsula, 09 August 2012, *Lehnebach, Zeller & Aspin SP104389* (WELT).