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Two new species of *Gastrodia* (Gastrodieae, Orchidaceae) endemic to New Zealand

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Abstract

Two new species of *Gastrodia*, *G. cooperae* and *G. molloyi* are described. These species are endemic to New Zealand and can be distinguished from other New Zealand *Gastrodia* by the length of the column and the colour of the labellum tip. *Gastrodia cooperae* is known from only three localities and it is ranked as Threatened: Nationally Critical.

Keywords: AFLP, conservation, myco-heterotrophy, new species, orchid, threatened flora

Introduction

Gastrodia is one of the largest genera of non-photosynthetic myco-heterotrophic orchids. It comprises about 70 species (WCSP 2015) and although the highest diversity of species and endemism occurs in Asia (Hsu & Kuo 2011), *Gastrodia* is well represented across Africa, Oceania and islands of the Indian and Pacific Ocean (Cribb *et al*. 2010). The southernmost limit of distribution of *Gastrodia* is in New Zealand (NZ) where it reaches ca. 47°S.

Three species of *Gastrodia* are listed for NZ (Breitwieser *et al*. 2012); *G. cunninghamii* Hooker (1853: 251), *G. minor* Petrie (1893: 273) and *G. sesamoides* Brown (1810: 330). The latter is also native to Australia and has naturalised in South Africa (Martos *et al*. 2015). *G. cunninghamii* is the most common and widespread species in NZ. It occurs in the North, South and Stewart Islands and the Chatham Islands, about 680 km southeast from mainland NZ. *G. minor* has a similar distribution and abundance but it is not in the Chatham Islands. Both are autonomous self-pollinating species (Lehnebach *et al*. 2005, Macdonald *et al*. 2015). *G. sesamoides* is common in NZ too but it is restricted to the North Island and northern part of the South Island. The pollination of this species is unknown in NZ, but it is likely to be insect-pollinated in common with other *Gastrodia* species that have long columns (Jones 1991, Macdonald *et al*. 2015). The internal floral structure (i.e. anther and stigma not in close contact) and scented flowers also support this idea. Natural habitats of NZ *Gastrodia* include southern beech forest, shrublands dominated by Myrtaceae species and coastal wetlands but interestingly, all three species have colonised pine plantations and urban areas such as cemeteries, gardens, and traffic islands (de Lange *et al*. 2007).

The modest number of *Gastrodia* species in NZ has not prevented taxonomic confusion (Scanlen 2012), and for the last ca. 30 years the taxonomic status of at least three morphological variants has been debated by local field botanists and orchid enthusiasts. These three variants are presumed to have affinities with *G. sesamoides* (de Lange *et al*. 2007, Scanlen 2012) because, like that species, they share a long column. Currently, these orchids are known by "tag names" (i.e. vernacular names) (see Wilson 1982, Gibbs 2001, Scanlen 2005), and several photographs and notes discussing their distribution, origin, possible genetic affinity, overall morphology and diagnostic features have been published (e.g. Rolfe 2015), yet their taxonomic status has never been resolved.

The first morphological variant is locally known as *Gastrodia* "long column black", referring to its long column and the black labellum apex. The overall morphology of this plant resembles *G. cunninghamii* but, as the name suggests, the column is long whereas in *G. cunninghamii* it is very short. *Gastrodia* "long column black" was first considered to be a distinct entity by Cooper (1983) who had previously included it in *Gastrodia sesamoides*, based on a specimen from the southern Tararua Range in the North Island (Cooper 1981). The second form is known as *Gastrodia* "long column". It differs from *Gastrodia* "long column black" by its yellow to orange labellum tip, and its overall greater stature and usually paler colour. This form has been observed widely in the North Island, South Island, and Stewart

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Island, although it was reported as a distinct entity only as recently as 1982 (Wilson 1982). The third variant has only been found in urban settings such as traffic islands and public gardens, and it is known as *Gastrodia* "city". This variant is morphologically similar to *G. sesamoides* but it has charcoal grey sepal tips. It was first reported from traffic islands in Hamilton city in the North Island (Gibbs 2001).

In this study we investigate the taxonomic status of these three morphological variants and, based on evidence from morphological and DNA fingerprinting data, we describe two of them as new species. We also evaluate their conservation status and provide an identification key to the species of *Gastrodia* in NZ and photographs of their diagnostic features.

Methods

We collected several flowering specimens of the three species of *Gastrodia* listed for NZ and the three morphological variants. These samples represent populations from the North Island and South Island of NZ. Voucher specimens were deposited at the herbarium WELT (Museum of New Zealand Te Papa Tongarewa) and collection details and images (when available) are accessible online at http://collections.tepapa.govt.nz. Flowers at different stages of development were collected from representative specimens, preserved in 90% ethanol and used later to study internal floral features.

We examined and measured eight morphological characters (stem width, length of the lowest stem bract, number of flowers, length of lowest floral bract, length of the pedicel and ovary at anthesis, width and length of the perianth at anthesis) from 177 mature, flowering specimens deposited at WELT and other national herbaria (AK, CHR, NZFRI and OTA) (Appendix 1). Since many of these specimens represent historical collections; destructive sampling was not conducted and only external floral characters, along with vegetative characters, were measured using a digital calliper. Two floral characters were measured from the preserved flowers; ratio length to width of the petal, and length of the column. We conducted a one-way ANOVA to determine whether morphological differences between species were significant using the software SYSTAT 13.1 (SYSTAT Software Inc.).

We also studied the type specimens of *Gastrodia leucopetala* Colenso (1886: 268) (WELT SP 22658!, WELT SP 22659!, WELT SP 24288!) a name currently synonymised with *G. cunninghamii*. Type specimens of all Australian *Gastrodia* or their images (available from http://plants.jstor.org); *G. crebriflora* Jones (1991: 62) (BRI-AQ0521041!), *G. entomogama* Jones (1991: 63) (CBG 9004649!), *G. lacista* Jones (1991: 64) (CBG 8913934!), *G. procera* Carr (1991: 22) (MEL 223604!), *G. sesamoides* Brown (1810: 330) (BM000990493!), *G. surcula* Jones (2008: 554) (CBG 9219995!), *G. urceolata* Jones (1991: 64) (BRI-AQ0643810!) and *G. vescula* Jones (1991: 65) (CBG 9707621!) were also examined. Herbarium acronyms follow Thiers (2015).

Species distribution was collated from herbarium specimens, field observations, photographs that were submitted to us, and observations from the citizen science website NatureWatch New Zealand (www.naturewatch.org.nz, accessed 2 April 2016). We only used NatureWatch observations and submitted photographs that we could confidently identify (Appendix 2).

DNA extraction and fingerprinting (AFLP)

A whole flower was finely crushed and used for DNA extraction using a modified CTAB protocol (Doyle & Doyle 1987). AFLP profiles were generated for 20 samples of *Gastrodia* (*G. cunninghamii* n=4; *G. minor* n=4; *G. sesamoides* n=3; *G.* "long column black" n=5; *G.* "long column" n=3; *G.* "city" n=1). Fingerprinting 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 *Eco*RI (10 U/ 1 µL, Roche) and *Mse*I (10 U/ 1 µL, NEB). A duplicate of each sample was digested alongside 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 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: 6FAM-EcoRI + ACT / MseI + CAG, Vic-EcoRI + ATA / MseI + CGT, 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).

Data analyses

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. The following options were used for scoring the fragments: 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 %). We set peak scores values of reject to "reject = 1 and pass = 7". Profiles were then assessed manually and dubious or low intensity peaks were not scored and failed samples discarded. After excluding duplicates, a presence/absence matrix that included all primer combinations was created and analysed by Neighbour-joining (NJ) using SplitsTree (Huson & Bryant 2006). Bootstrap support for main clusters was calculated by boostrap analyses (1000 replicates) also using SplitsTree. Principal Coordinate Analyses (PCoA) using NTSYS-pc 2.2h1 (Applied Biostatistics Inc.) was also used to examine genetic affinity within a subset of the data.

Results

Our analyses provide evidence to describe *Gastrodia* "long column black" and *G.* "long column" as new species. For convenience and consistency throughout this article, these are referred to as *G. cooperae* and *G. molloyi* respectively. Their formal descriptions follow later in the article.

DNA fingerprinting (AFLP)

Our AFLP data set consisted of 20 samples and 289 bands; 279 of these bands were polymorphic. Samples of *Gastrodia cooperae* and *G. molloyi* formed two strongly supported groups (BS: 100 % and 97.6 %, respectively), distinct from each other and from other *Gastrodia* species (Fig. 1). *Gastrodia cooperae* is close to *G. cunninghamii* and both taxa formed a strongly supported clade in the NJ tree (BS: 100 %, Fig. 1). On the other hand, the sample of *G.* "city" fell within a strongly supported clade that includes only samples of *G. sesamoides* (Fig. 1).

Similar results were obtained by the PCoA of the reduced data set that excluded *Gastrodia minor* and *G. sesamoides* (Fig. 2). Here, samples of *G. cunninghamii* and *G. cooperae* clustered at the opposite end of the ordination space from *G. molloyi*. The affinity of *G. cunninghamii* with *G. cooperae* is again evident in the PCoA; as well as a North Island / South Island separation of the accessions of *G. cooperae* and *G. cunninghamii* (Fig. 2, dashed line).

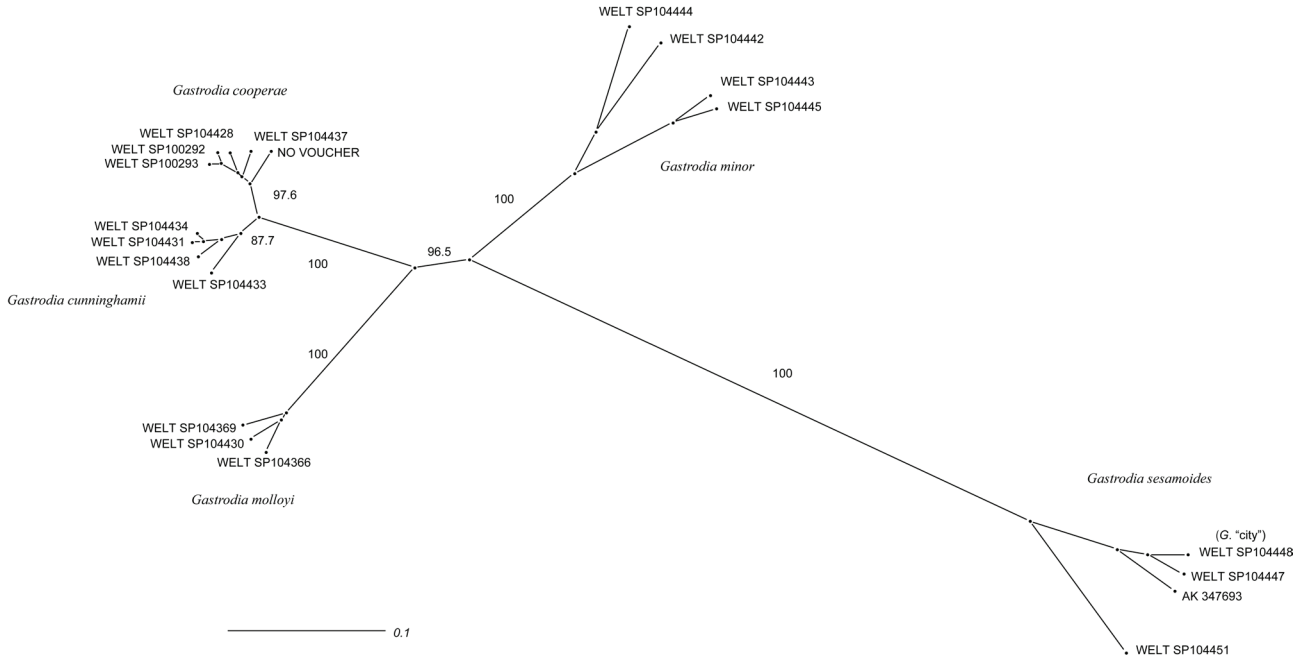

FIGURE 1. Neighbour-Joining tree based on AFLP data generated from New Zealand *Gastrodia*. Numbers on NJ tree indicate bootstrap support above 50 %. *Gastrodia cooperae* = *Gastrodia* "long column black"; *Gastrodia molloyi* = *Gastrodia* "long column". Sample labelled "no voucher" was collected from the same locality as specimen WEL SP104437.

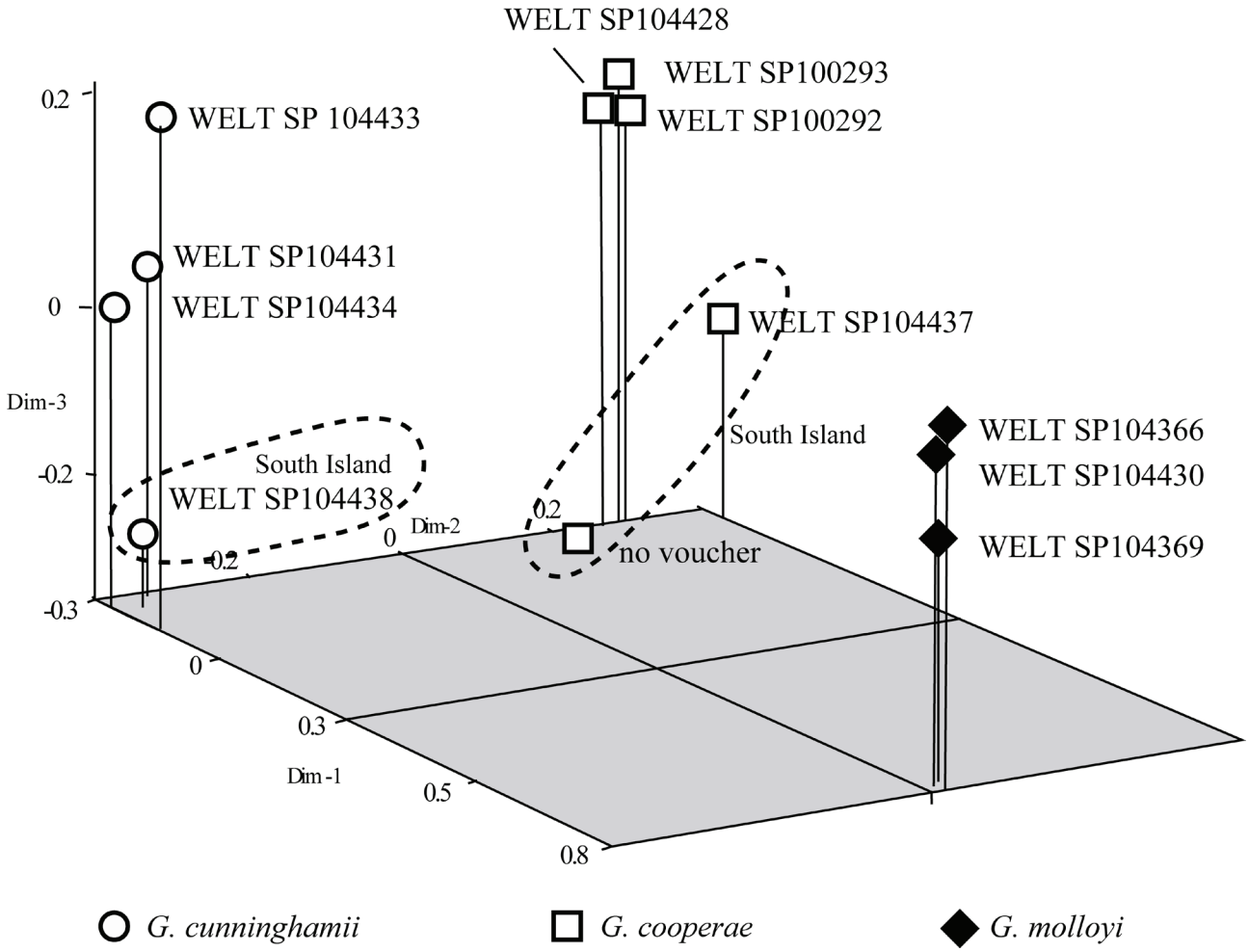

FIGURE 2. Principal Coordinate Analysis (PCoA) based on Jaccard's similarity coefficient calculated from AFLP data of *G. cooperae* (*Gastrodia* "long column black"), *G. molloyi* (*Gastrodia* "long column") and *G. cunninghamii*. Sample labelled "no voucher" was collected from the same locality as specimen WEL SP104437.

Morphology

Most of the vegetative and extra-floral characters we measured from *Gastrodia cunninghamii*, *G. cooperae*, *G. sesamoides*, and *G. molloyi* overlapped considerably (Fig. 3A–J). Characters such as the number of flowers, and length and width of the perianth differed significantly between some species (e.g. Fig 3D–F), but overlap between species was still considerable. Only intra-floral features such as the length of the column (Fig. 3I) and the petal shape (Fig. 3J) markedly differed between species. For instance, *G. cunninghamii* is the only one of these four species that has flowers with a short column. The type specimens of *G. leucopetala*, a heterotypic synonym of *G. cunninghamii*, also have short columns. As for the petals, in *G. sesamoides* they are mostly ovate, while in *G. molloyi* and *G. cooperae* petals tend to be oblong and widely oblong in *G. cunninghamii*. The colour and texture of the perianth, and the colour of the labellum tip (all features clearly distinguishable in fresh material) were also informative. For instance, *G. sesamoides* has pale brown to white flowers with a smooth perianth. Flowers of remaining species, however, are pale green or brown to golden-brown to dark green or brown, and the perianth surface is always tuberculate to a greater or lesser degree. The labellum tip is yellow in *G. sesamoides* and *G. molloyi* and brown to black in *G. cunninghamii* and *G. cooperae*.

Both new species are easily distinguishable from *G. minor*. Our data shows *G. minor* is distinctively slender, ca. 20 cm tall, and has only 1–6 flowers (morphometric data not shown but available on request), which have a short column. From the Australian species, only the type specimens of *G. entomogama*, *G. lacista* and *G. procera* show some resemblance with the new species. However, these three Australian endemics have larger and wider flowers with a pale-brown perianth, and columns almost twice as long as those found in *G. cooperae* and *G. molloyi*.

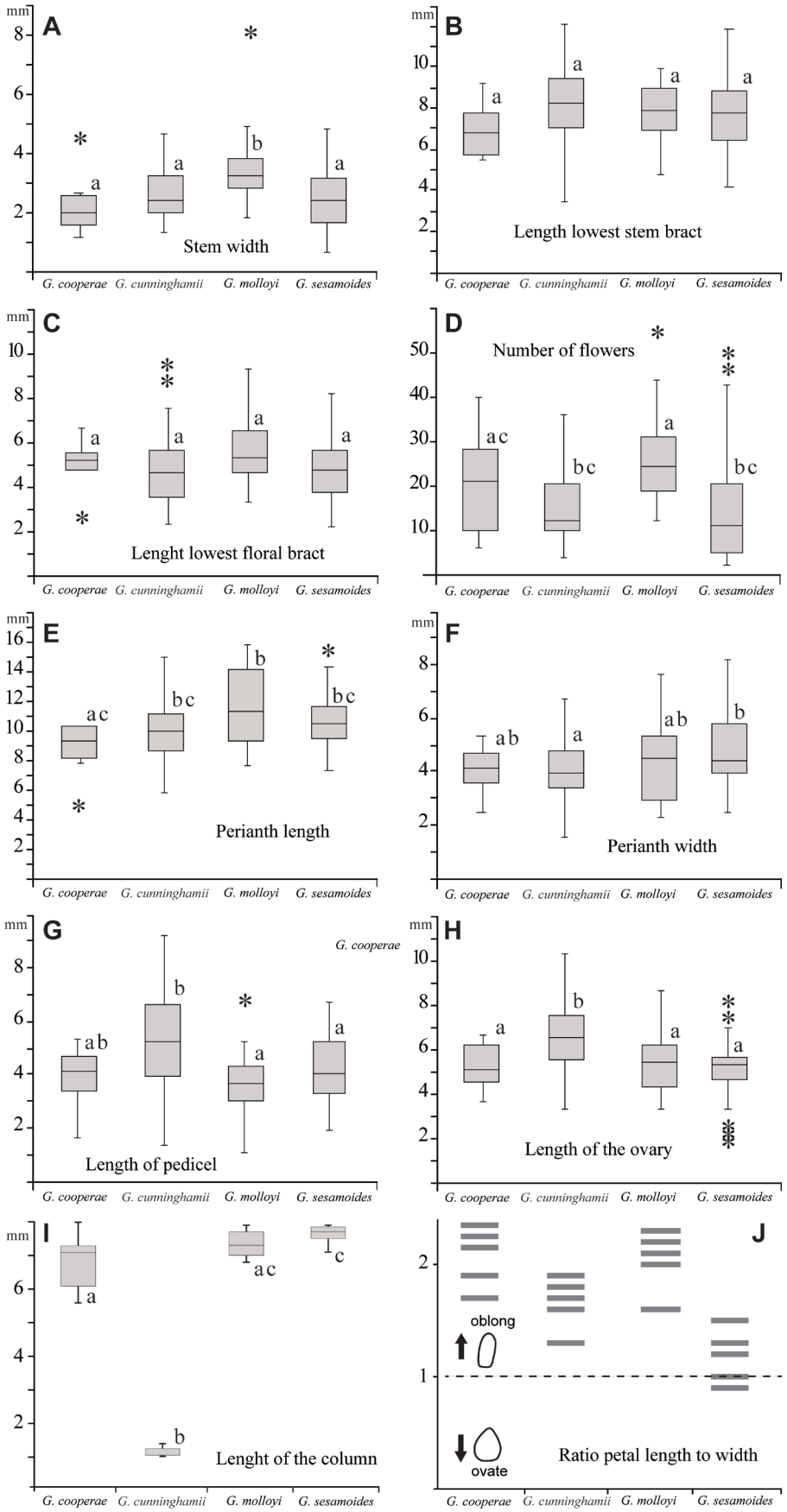

FIGURE 3. Box-plot (A–I) and scatter-plot (J) representation of the variation found within ten morphological characters measured from *Gastrodia cunninghamii*, *G. molloyi* (*Gastrodia* "long column"), *G. cooperae* (*Gastrodia* "long column black"), and *G. sesamoides*. Like letters indicate that differences between species are not significant, Tukey's HSD test, $P < 0.05$. Dashed line in the scatter-plot J indicates a ratio = 1; i.e. circular.

Taxonomy

Gastrodia cooperae Lehnebach & J.R.Rolfe, *sp. nov*. (Fig. 4)

Holotype:—NEW ZEALAND. North Island, Mangapakeha, Marangai Station, upper slopes of *Kunzea robusta* de Lange (2014: 155) forest, *J.R. Rolfe, P. Enright & B. Campbell*, 02 January 2013, WELT SP 100292 (Isotype: WELT SP 100293).

Diagnosis:—Similar to *G. cunninghamii* and *Gastrodia molloyi* in the colour and texture of the perianth but it differs from *G. cunninghamii* by its long column and from *Gastrodia molloyi* by its dark brown—black labellum tip. It differs from *G. sesamoides* by its dark brown—green verrucose perianth and dark brown—black labellum tip.

Description:—Terrestrial, leafless, achlorophyllous, deciduous herb. Rhizome tuberous, fusiform, pale brown. Plant (22)36–45.5(96) cm tall at flowering. Stem solitary, glabrous, dark to pale brown with greyish longitudinal streaks; (1.2)1.6–2.6(4.5) mm diameter. Stem bracts 4–5, papery, glabrous, sheathing. Inflorescence erect, terminal, flowers (6)12–27(40), non-resupinated, nectarless, scented, erect when developing and pendulous at anthesis. Floral bract papery, glabrous, deltoid to widely deltoid, apex acute, (2.1)4.7–5.4(6.6) × 1.6–2.2 mm. Pedicel (1.6)3.4–4.6(5.3) mm long. Perianth tube (4.9)8.4–10.4 × (2.5)3.6–4.6(5.3) mm, brown to dark brown, surface with pale green to gray wart-like elevations. Sepals reflexed, white to pale green abaxially; margin entire to slightly irregular towards the apex; apex acuminate. Petals adnate to the tube formed by the sepals, oblong, 3.0–3.4 × 1.3–1.8 mm, white on both sides; margin irregular, apex obtuse to round. Labellum 6.5–10.8 mm long; adnate to the base of the perianth, fleshy. Hypochile pale brown, covered on pseudo-pollen, margin entire. Mesochile strongly contracted; margins entire. Epychile trilobed, white at the base to pale yellow towards the apex. Lateral margins incurved, entire in the basal portion then irregularly crenate to cristate towards the apex. Labellum apex fleshy, dark brown to black. Two pale yellow ridges covered in pseudopollen extend from the mid-section of the epychile to the apex where they join. Column erect, 5.4–7.8 mm tall, narrow at base and wide towards the apex, 1.2–2 mm wide. One oblong or two deltoid wings flank the rostellum. Pollinia two, pollen joined into massulae; rostellum flap like, positioned under anther. Stigma at base of the column, ovate. Ovary (3.7)4.6–6.1(6.7) mm long, brown with a few greyish wart-like elevations. Capsules upright when mature; seeds pale brown.

Specimens studied:—NORTH ISLAND: Wairarapa, Marangai Station, *Rolfe GASlcb1320 & Gibbins*, 13 Dec 2013, WELT SP 104428. SOUTH ISLAND: Takaka, *Scott s.n.*, Dec 1952, CHR 79950A, B; Canaan Road, *Druce s.n.*, Jan 1969, CHR 192308; Mount Arthur, *Heine s.n.*, 12 Jan 1933, WELT SP 19098; Clarke Valley, *Rolfe 15001*, 6 Jan 2015, WELT SP 104437.

Etymology:—The epithet of the species is adopted to recognise Dorothy 'Dot' A. Cooper (1941–) who established the New Zealand Native Orchid Group and who authored the *Field Guide to New Zealand orchids* (Cooper 1981), which led to the recognition of this species as distinct.

Distribution:—Endemic to New Zealand. North Island, a single population in eastern Wairarapa. South Island, two populations in north-west Nelson (Fig. 5).

Habitat:—*G. cooperae* is today known only from a remnant patch of *Kunzea robusta* forest in the North Island, and southern beech forest in the South Island. Information on the habitat of *G. cooperae* at its historical locations is lacking, but it is presumed to have habitat requirements similar to the closely related *G. cunninghamii*. The two species are syntopic in the South Island and *G. cunninghamii* is present at North Island sites where *G. cooperae* historically occurred.

Phenology:—Flowering in December and January; fruiting in January and February.

Conservation status:—The earliest specimens of *Gastrodia cooperae* were collected between the 1930s and 1960s from Mount Arthur (WELT SP19098 as *G. cunninghamii*), Takaka (CHR 79950A, B) and Canaan Road (CHR 192308) (both as *G. sesamoides*) in the South Island. A small population of ca. 10 plants had also been reported near Whanganui in the North Island during the 1980s and 1990s but it has not been seen there since that time (M. Pratt pers. comm.). It also seems to be no longer extant at the site of Cooper's (1981, 1983) record in the southern Tararua Range in the North Island. *Gastrodia cooperae* is today known from only three locations. In eastern Wairarapa in the North Island, it is sparsely distributed across an area of ca. 0.2 ha within a ca. 10-ha forest. No formal census has been conducted there but it is unlikely that the population is greater than 100 individuals. In north-west Nelson, its area of occupancy is even smaller, and fewer than 20 plants have been observed there. Although no information is available to estimate population trends, the current situation of fewer than 250 mature individuals, enables *G. cooperae* to be assessed as Threatened: Nationally Critical A1 using the criteria of Townsend *et al*. (2008). Because of the lack of

trend data and the large extent of unsurveyed potential habitat in the north-west South Island, we recommend that the qualifier DP (Data Poor) be appended to the assessment.

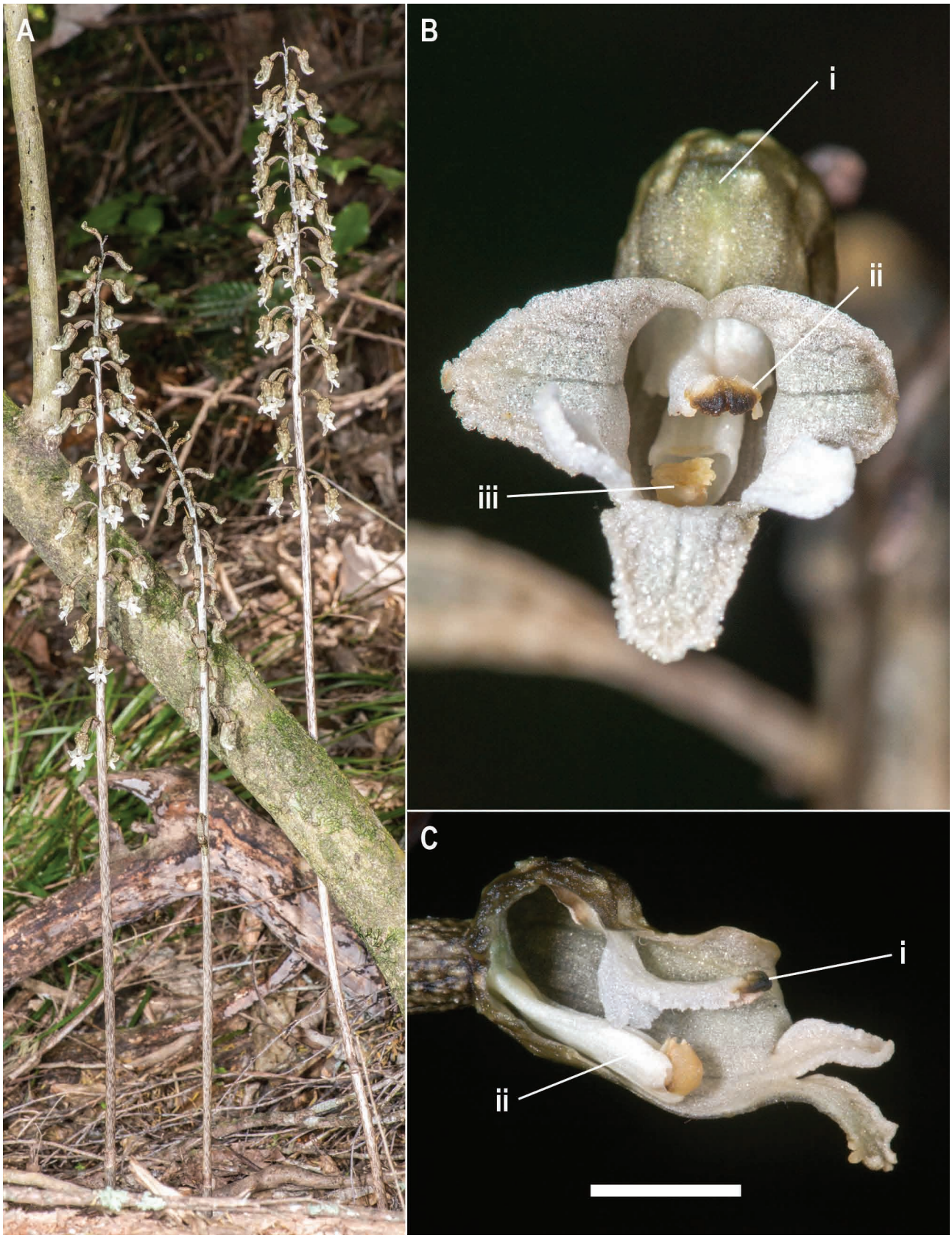

FIGURE 4. *Gastrodia cooperae* Lehnebach & J.R.Rolfe in the wild. A: Habit; B: Flower showing (i) tuberculate perianth surface, (ii) dark labellum apex, (iii) column apex; C: Longitudinal section of flower showing (i) dark labellum apex, (ii) column. Bar = 5 mm. Photos by J.R. Rolfe.

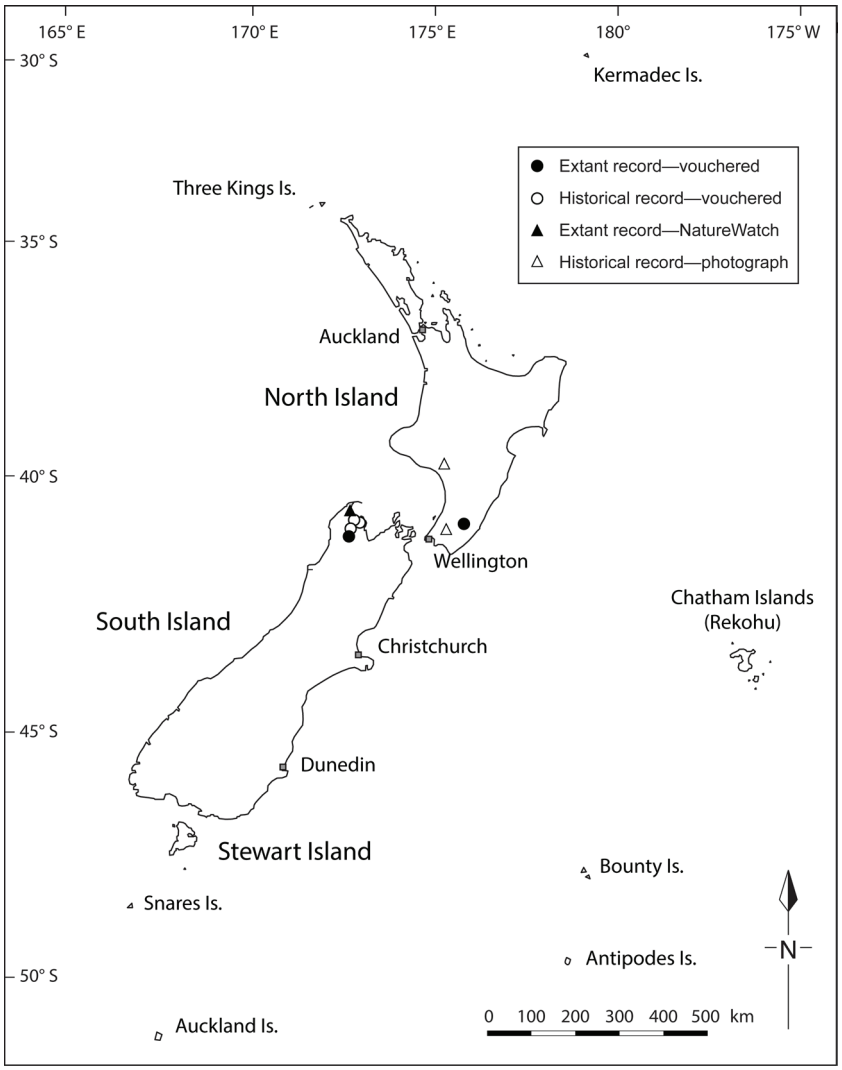

FIGURE 5. Extant and historical distribution of *Gastrodia cooperae* Lehnebach & J.R.Rolfe in New Zealand based on voucher specimens and verified photographic records.

Gastrodia molloyi Lehnebach & J.R.Rolfe, *sp. nov*. (Fig. 6)

Holotype:—NEW ZEALAND. North Island, Paraparaumu, Maungakotukutuku Valley, in *Beilschmiedia tawa* (Cunningham 1838: 379) Benth. & Hook.f. ex Kirk (1889: 257) forest, *J.R. Rolfe GASlc1327 & M. Ward*, 24 January 2014, WELT SP 104366 (Isotype: WELT SP 104369).

Diagnosis:—It resembles *G. cooperae* and *G. sesamoides* in the length of the column but it differs from *G. cooperae* by the yellow colour of the labellum tip and from *G. sesamoides* by the verrucose texture and golden-brown to dark green colour of the perianth.

Description:—Terrestrial, leafless, achlorophyllous, deciduous herb. Rhizome tuberous, fusiform, pale brown to blackish, often covered in papery scales. Plant (29.6)39.3–65.8(80) cm tall at flowering. Stem solitary, glabrous, golden brown to pale pink-greyish, with gray-whitish longitudinal streaks; (1.8)2.9–3.8(8.5) mm diameter. Stem bracts 3–5, papery, glabrous, sheathing. Inflorescence erect, terminal, flowers (12)20–31(55), densely arranged, non-resupinated, nectarless, scented, erect when developing and pendulous at anthesis. Floral bract papery, glabrous, deltoid, apex acute, (3.3)4.7–6.6(9.3) × 1.8–2.7 mm. Pedicel (1.1)3.1–4.3(6.6) mm long. Perianth tube (7.7)9.4–14.1 × (2.3)3–5.3(7.6) mm, greenish gold to golden brown, surface with pale green to gray wart-like elevations. Sepals slightly reflexed, white to pale green abaxially; margin entire to slightly irregular towards the apex; apex acuminate. Petals adnate to the tube formed by the sepals, oblong, 2.7–4.4 × 1.3–2 mm, white on both sides; margin irregular, apex obtuse. Labellum 7.8–10.5 mm long; adnate to the base of the perianth, fleshy. Hypochile yellow to orange, covered in pseudo-pollen, margin entire. Mesochile strongly contracted; margins entire. Epychile trilobed, white. Lateral margins incurved, entire in the basal portion then irregularly crenate to cristate towards the apex. Two bright yellow to orange, warty ridges

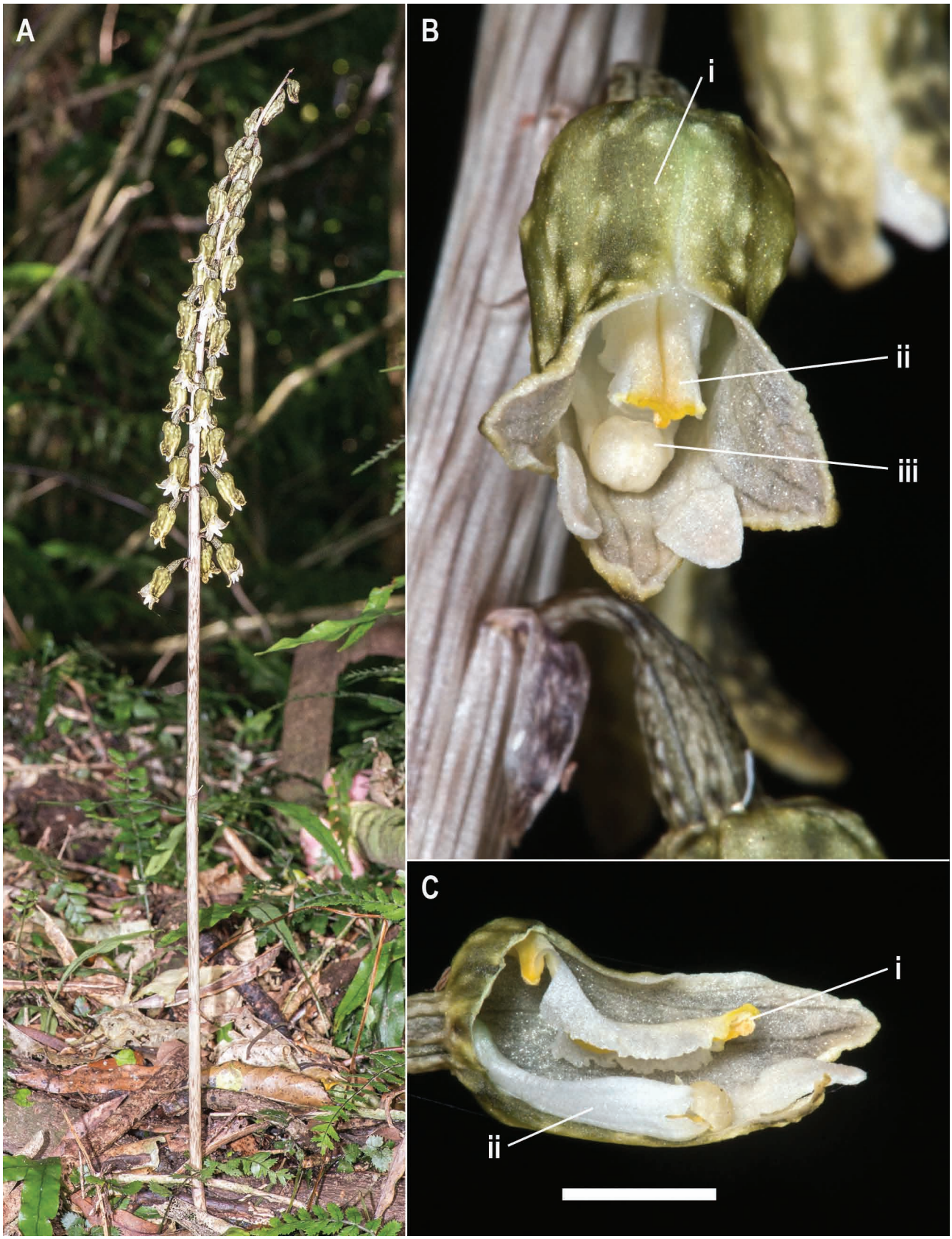

FIGURE 6. *Gastrodia molloyi* Lehnebach & J.R.Rolfe in the wild. A: Habit; B: Flower showing (i) tuberculate perianth surface, (ii) yellow labellum apex, (iii) column apex; C: Longitudinal section of flower showing (i) yellow labellum apex, (ii) column. Bar = 5 mm. Photos by J.R. Rolfe.

extend from the basal section of the epychile to the middle section, where they join and extend as one towards the apex. Pseudo-pollen accumulates on both sides of the ridge. Labellum apex fleshy, yellow to orange. Column erect, 6.8–7.9

mm tall, narrow at base and wide towards the apex, 2.5–3.7 mm wide. The rostellum is flanked by one oblong or two deltoid column wings. Pollinia two, pollen joined into massulae; rostellum flap like, positioned under anther. Stigma at base of the column, ovate. Ovary (3.4)4.3–6.2(8.7) mm long, brown with a few grayish wart-like elevations. Capsules upright when mature; seeds pale brown.

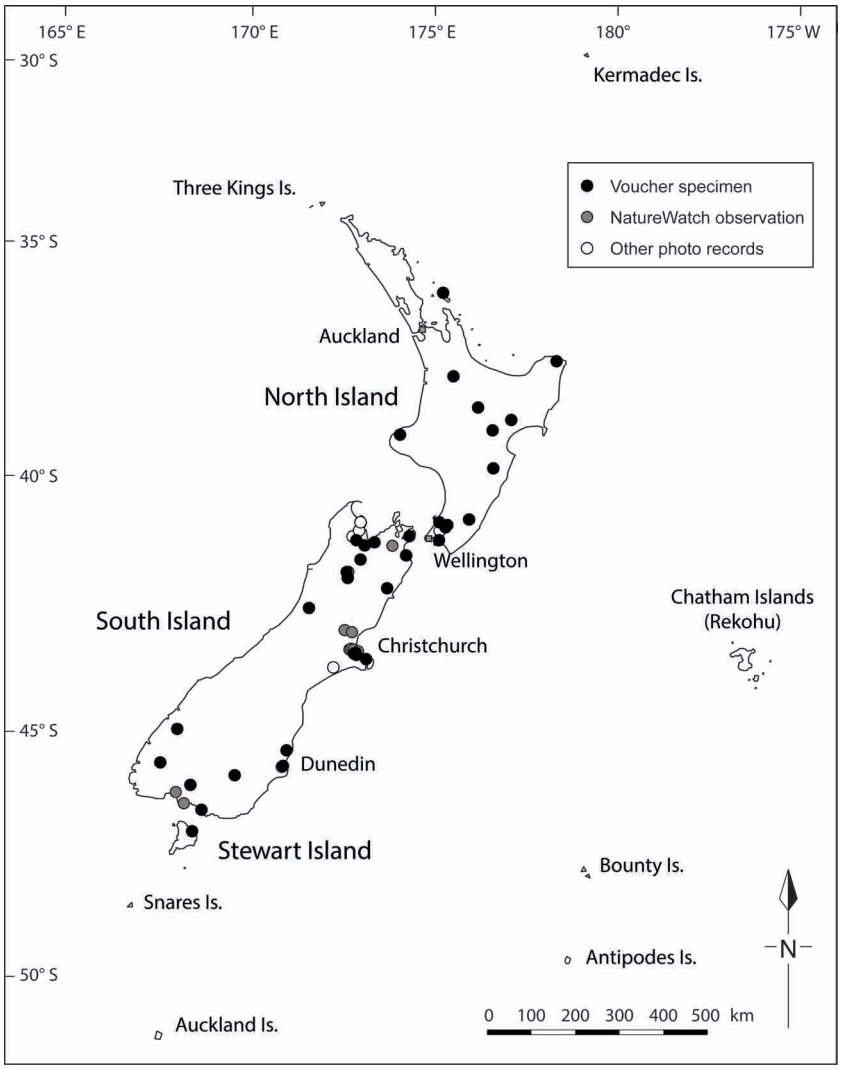

FIGURE 7. Distribution of *Gastrodia molloyi* Lehnebach & J.R.Rolfe in New Zealand based on voucher specimens, observations published online at naturewatch.org.nz, and verified photographic records.

Specimens studied:—NORTH ISLAND: Auckland, Great Barrier Island, Motairehe Stream, *Taylor s.n.,* 7 Jan 1983, AK 271500; Auckland, Great Barrier Island, Motairehe Stream, *Taylor s.n.*, 7 Jan 1983, AK 161005; Waikato, Maungatautari, *Petrie s.n.* , Jan 1917, WELT SP019066; Bay of Plenty, Kaingaroa Forest, *Purnell s.n.*, 28 Jan 1964, CHR 148365; East Coast, Gisborne, Awatere River, *Petrie s.n.*, Jan 1897, WELT SP 019086; Hawke's Bay, Puketitiri, Ball's Clearing, *Ecroyd s.n.*, 28 Jan 1989, NZFRI 17987; Hawke's Bay, Waipukurau, Tukituki Bush, *Andrew s.n.*, no date, WELT SP043709; Taranaki, New Plymouth, *Arden s.n.*, no date, WELT SP019097; Wairarapa, Marangai Station, *Rolfe GASlc1321 & Gibbins*, 13 Dec 2013, WELT SP 102797; Wellington, Tararua Range, Smith Creek, *Moore s.n.*, 7 Feb 1942, CHR 44681; Wellington, Tararua Range, Puffer Track, *Molloy*, 27 Nov 1989, CHR 584233; Wellington, Orongorongo River, Green Stream, *Huzziff s.n.*, 11 Jan 1971, CHR 586385. SOUTH ISLAND: Nelson, The Brook Waimarama Sanctuary, *Ecroyd s.n.*, 8 Feb 2014, WELT SP 104441; Nelson, Wairoa Gorge, *Kirk s.n.*, 18 Jan 1896, WELT SP43710; Marlborough, Queen Charlotte Sound, Resolution Bay, *Beever 9145*, *7* Jan 1992, AK 208132; Marlborough, Cloudy Bay, *Macmillan 95/25 & Aiken*, 20 Jan 1995, CHR 506634; Marlborough, Kaikoura, Mount Fyffe Road, *de Lange 3232*, 7 Feb 1997, AK 232679; Tasman, Kikiwa, *Allan*, 26 Jan 1929, CHR 1091; Tasman, Lake Rotoroa, *Godley s.n. & Lyon*, Jan 1974, CHR 285331; Tasman, Lake Rotoroa, D'Urville River delta, *Simpson 2502*, 1 Feb 1961, CHR 118811; West Coast, Otira, Kellys Creek, *Petrie*, Jan 1893, AK 3679; Canterbury, Christchurch, Banks Peninsula, Gebbies Pass, *Wilson BP902 & Robertson*, 10 Dec 1985, CHR 493283; Otago, Waikouaiti River, *Johnson*

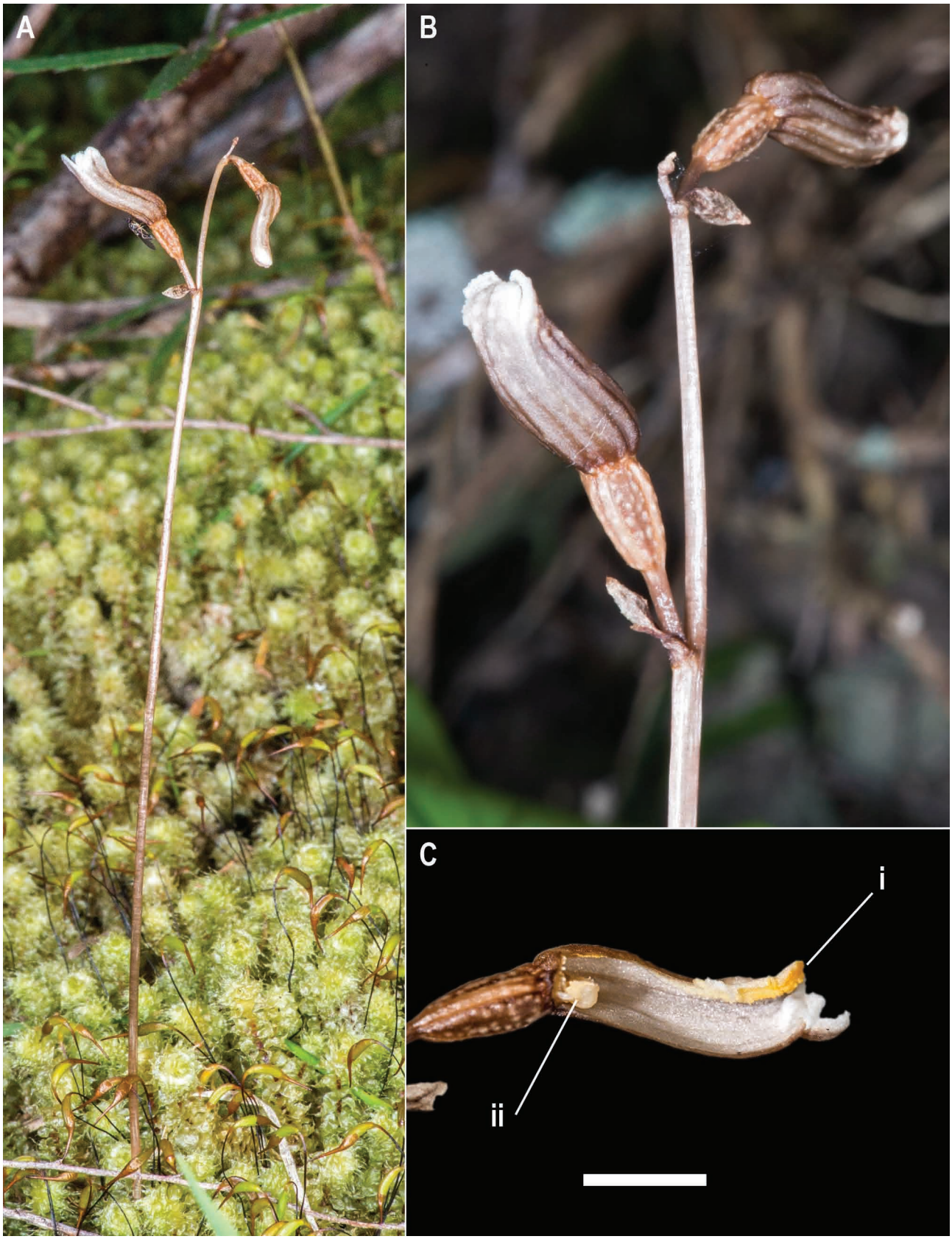

FIGURE 8. *Gastrodia minor* in the wild. A: Habit; B: Flower showing striped perianth; C: Longitudinal section of flower showing (i) yellow labellum apex, (ii) short column. Bar = 5 mm. Photos by J.R. Rolfe.

604, 11 Jan 1987, CHR 436735; Otago, Dunedin, Town Belt, *Bannister s.n.*, Mar 1985, OTA 042142; Southland, Tapanui, *Rolfe GASlc1329 & Lazare*, 4 Feb 2014, WELT SP 104429); Southland, Te Anau, Eglinton Valley, Knobs Flat, *Scott s.n.*, 11 Feb 1966, OTA 014949; Southland, Lake Manapouri, Shallow Bay, *Johnson s.n.*, 18 Jan 1970, OTA

028525; Southland, Otautau, Holt Park, *Rennell s.n.*, 16 Mar 2003, CHR 622449; Southland, Invercargill, *Esler s.n.*, 3 Feb 2014, WELT SP 104436. STEWART ISLAND: Halfmoon Bay, *Wilson 798-260 & Tindal*, 10 Feb 1980, CHR 368819.

Etymology:—The epithet of the species honours Brian P. J. Molloy (1930–) for his important contributions to the study and conservation of the New Zealand flora in general, especially orchids, and for his early insights about the distinctiveness of this species.

Distribution:—Endemic to New Zealand. North Island (south of Auckland), South Island and Stewart Island (Fig. 7).

Habitat:—*Gastrodia molloyi* is commonly found on disturbed sites including, for example, under *Salix viminalis* Linnaeus (1753: 1021) and *S. fragilis* Linnaeus (1753: 1017) alongside flood-prone waterways, amongst trackside exotic weeds and grasses including *Holcus lanatus* Linnaeus (1753: 1048), *Rubus armeniacus* Focke (1874: 183) and *Trifolium repens* Linnaeus (1753: 767) in forest dominated by *Lophozonia menziesii* (Hooker 1844: t. 852) Heenan & Smissen (2013: 16), and under *Pinus pinea* Linnaeus (1753: 1000) in a private garden. It is also known from indigenous forests dominated by *Beilschmiedia tawa*, *Kunzea robusta* or Nothofagaceae species and scrublands dominated by *Kunzea* species or *Leptospermum scoparium* Forster & Forster (1775: 72). As with other *Gastrodia* species it may be found singly, in groups of a few stems, or in large numbers.

Phenology:—Flowering in December to March; fruiting in January to April.

Conservation status:—*Gastrodia molloyi* is widespread throughout most of NZ south of Auckland. It is, therefore, regarded as 'Not Threatened' using the criteria of Townsend *et al*. (2008).

Identification key to species of *Gastrodia* found in New Zealand

1a	Column much shorter than labellum, column apex close to stigma, column apex not visible in entrance of perianth tube 2
1b	Column almost as long as labellum, column apex distant from stigma, column apex usually visible in entrance of perianth tube 3
2a	Flowering stem < 30 cm tall, flowers < 10 per stem; perianth < 10 mm long; labellum apex yellow..... *G. minor* (Fig. 8)
2b	Flowering stem usually > 30 cm tall, flowers usually > 10 per stem (stems often shorter and fewer-flowered on plants exposed to strong light); perianth > 15 mm long; labellum apex brown or black.....*G. cunninghamii* (Fig. 9)
3a	Perianth pale brown to cream or pale pink, surface smooth; labellum apex yellow .....*G. sesamoides* (Fig. 10)
3b	Perianth greenish gold, golden-brown to dark green or brown, surface tuberculate; labellum apex yellow to orange or dark brown to black 4
4a	Perianth dark brown to black; labellum apex dark brown to black.....*G. cooperae* (Fig. 5)
4b	Perianth greenish gold to golden brown; labellum apex yellow to orange .....*G. molloyi* (Fig. 6)

Discussion

Our study has gathered morphological and genetic evidence that supports the recognition of two new species of *Gastrodia* endemic to New Zealand—*G. cooperae* and *G. molloyi*. Floral characters, such as the colour of the labellum tip and the length of the column, are the most significant and diagnostic features that help to discriminate between these new species and other NZ *Gastrodia*. This is particularly true for *G. cooperae* and *G. cunninghamii*, which are morphologically very similar and may be found growing sympatrically. On the other hand, we did not find support to recognise *Gastrodia* "city" as a distinct species. The AFLP study and our unpublished nuclear and mitochondrial DNA sequences indicate *Gastrodia* "city" is conspecific with *G. sesamoides*. The gray charcoal sepal tips of this variant may represent a natural morphological variation within the species or a plastic response to local habitat conditions. Also, the presumed affinity between *G. sesamoides* and the two new species is not supported by our AFLP data.

In *Gastrodia*, the length of the column is a fair indicator of the mating system, and plants with a short column tend to be autogamous or cleistogamous, while those with a long column are allogamous and pollinator dependent (see Lehnebach *et al.* 2005, Hu *et al.* 2014, Suetsugu 2014, Macdonald *et al*. 2015 but also see Hsu *et al*. 2012, Suetsugu 2013). The syntopic occurrence of two morphologically similar species, such as *G. cooperae* and *G. cunninghamii*, with contrasting reproductive systems may be interpreted by some as a single species with a dimorphic mating system. In fact, it is not uncommon to find orchid species, or other flowering plants, with self-pollinating and out-crossing forms or races growing in sympatry (see by Gamisch *et al*. 2014 and citations therein). However, under this scenario our samples of *G. cooperae* and *G. cunninghamii* from the North Island and South Island would have grouped according to provenance and not by the length of their columns as they do in the PCoA (Fig. 2). Furthermore, transition between

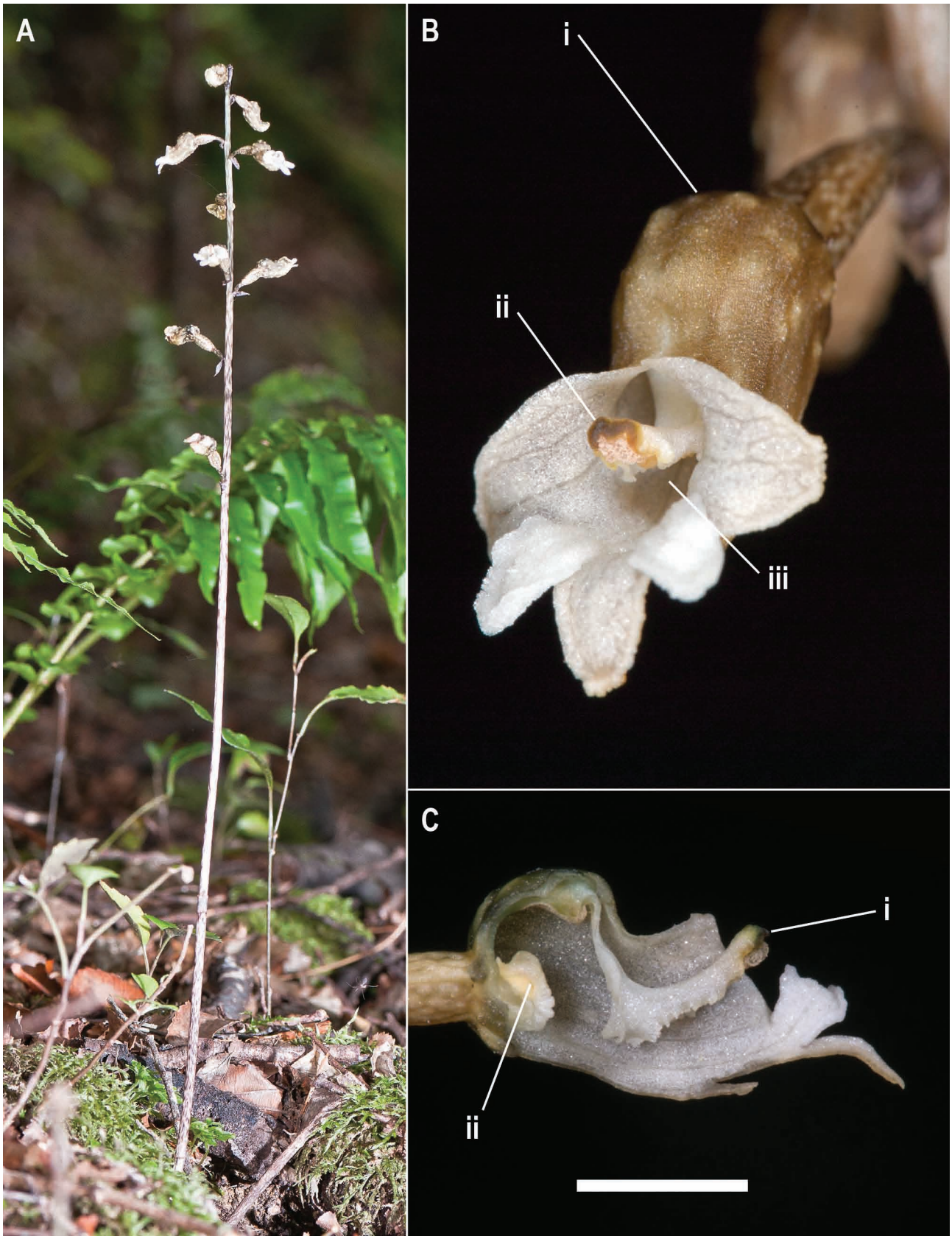

FIGURE 9. *Gastrodia cunninghamii* in the wild. A: Habit; B: Flower showing (i) tuberculate perianth surface, (ii) dark labellum apex, (iii) short column not visible; C: Longitudinal section of flower showing (i) dark labellum apex, (ii) short column. Bar = 5 mm. Photos by J.R. Rolfe.

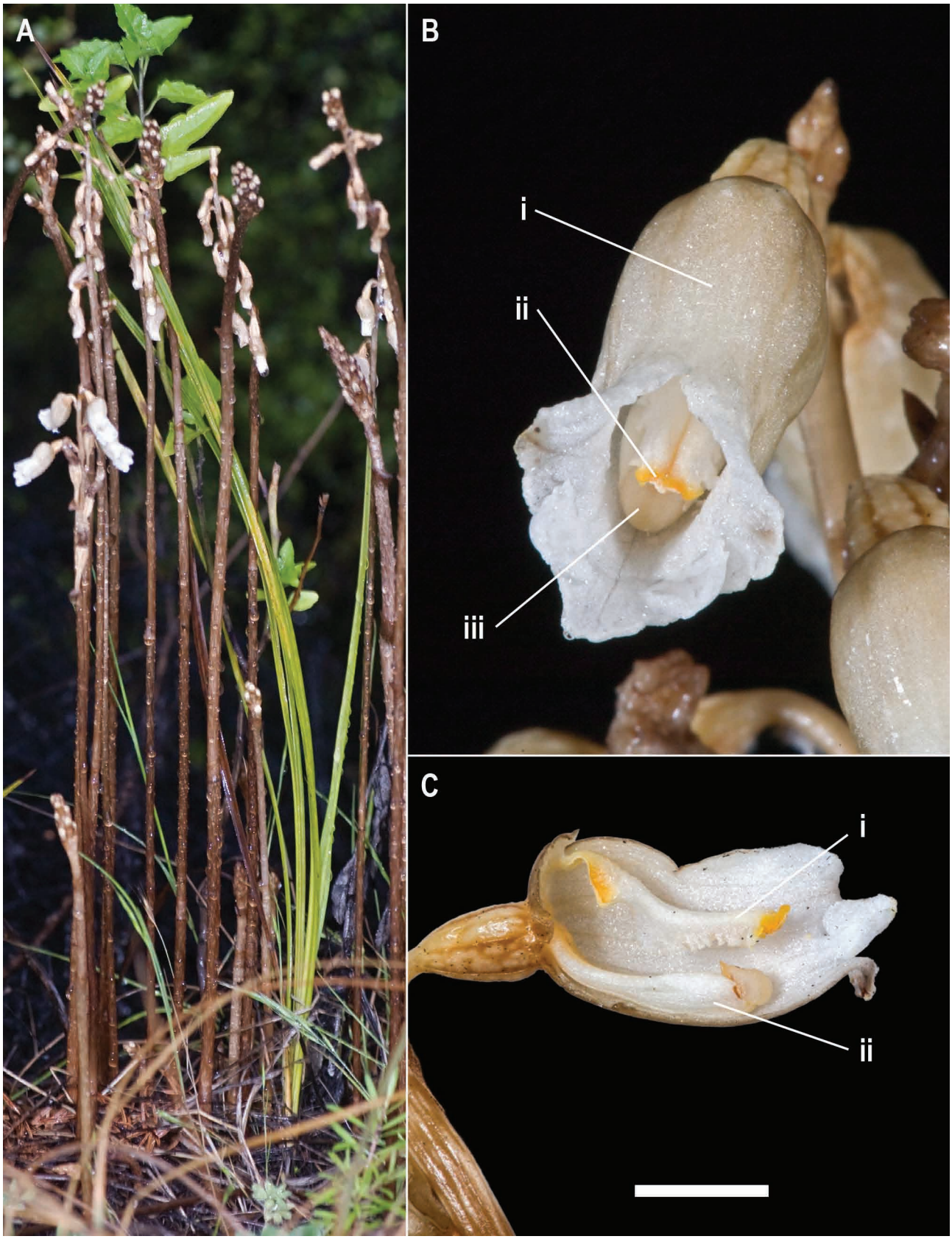

FIGURE 10. *Gastrodia sesamoides* in the wild. A: Habit; B: Flower showing (i) smooth perianth surface, (ii) orange labellum apex, (iii) column apex; C: Longitudinal section of flower showing (i) orange labellum apex, (ii) column. Bar = 5 mm. Photos by J.R. Rolfe.

the self-fertilisation and out-crossing state is common within *Gastrodia* and there are several species pairs in which the length of the column is the most diagnostic character (e.g. Tan *et al.* 2012, Hu *et al.* 2014, Suetsugu 2014). One of the most recent additions to *Gastrodia* is a species from China which has long- and short-column flowers within the same inflorescence (Huang *et al*. 2015). This has never been observed in *G. cooperae* or *G. molloyi*.

Now that the taxonomic status of these two NZ *Gastrodia* has been resolved, future studies should focus on their autecology and conservation. To secure the long-term survival of *G. cooperae* we need to assess its dependency on pollinators, measure reproductive success and recruitment, and identify and manage potential threats at each of the known sites. Population genetic studies using microsatellites could also provide an interesting insight into past population bottlenecks and provide a measure of the genetic diversity within each of these populations. At a broader scale, ongoing phylogenetic studies in collaboration with overseas colleagues are aiming to uncover the origin of NZ *Gastrodia* and resolve phylogenetic affinities within African and Australasian *Gastrodia*. The resulting phylogeny could unravel colonisation routes, dispersal events and provide a framework to study the evolution of mating systems within this enigmatic orchid genus.

Acknowledgements

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APPENDIX 1. List of specimens included in the morphological and AFLP analyses (*).

G. cooperae: AK 71359, CHR 192308, CHR7 9950A, CHR 79950B, WELT SP 019098, WELT SP 100292*, WELT SP100293*, WELT SP 104428*, WELT SP 104437*.

G. cunninghamii: AK 11221, AK 122039, AK 128982, AK 132334, AK 132336, AK 132355, AK 160883, AK 192726, AK 200471, AK 208144, AK 221543, AK 246014, AK 246015, AK 246601, AK 250080, AK 255600, AK 276917, AK 293239, AK 293917, AK 3681, AK 3682, AK 3684, CHR 110671, CHR 148922, CHR 168062, CHR 174511, CHR 181616, CHR 182108, CHR 221686, CHR 250396, CHR 260242, CHR 269268, CHR 278212, CHR 285331, CHR 289736, CHR 314685, CHR 50127, CHR 554409, CHR 586256, CHR 586297, CHR 586327, CHR 586416, CHR 69837, OTA 002911, OTA 009813, OTA 023927, WELT SP 007278, WELT SP 007279, WELT SP 019057, WELT SP 019073, WELT SP 019074, WELT SP 022658, WELT SP 022659, WELT SP 024288, WELT SP 039082, WELT SP 043719, WELT SP 076914, WELT SP 091345, WELT SP 099564, WELT SP 099572, WELT SP 099581, WELT SP 104432, WELT SP 104452, WELT SP 104431*, WELT SP 104433*, WELT SP 104434*, WELT SP 104438*.

G. minor: AK 108986, AK 108988, AK 151985, AK 24599, AK 24600, AK 24601, AK 264852, AK 271118, AK 339698, AK 3688, CHR 151172, CHR 183388, CHR 188910, CHR 208809, CHR 22540, CHR 235444, CHR 274698, CHR 289741, CHR 355439, CHR 388084, CHR 63271, OTA 062549, WELT SP 007281, WELT SP 019054, WELT SP 019055/A, WELT SP 019055/B, WELT SP 019063, WELT SP 019064, WELT SP 019081, WELT SP 067595, WELT SP 079238, WELT SP 099577, WELT SP 099579, WELT SP 104442*, WELT SP 104443*, WELT SP 104444*, WELT SP 104445*.

G. molloyi: AK 14700, AK 161005, AK 208132, AK 232679, AK 271500, AK 283908, AK 3677, AK 3679, CHR 118811, CHR 368819, CHR 436735, CHR 44681, CHR 490812, CHR 506634, CHR 586385, NZFRI 17987, OTA 014949, OTA 042142, WELT SP 019084, WELT SP 019085, WELT SP 019086, WELT SP 10097, WELT SP 19066, WELT SP 19342, WELT SP 43709, WELT SP 43710, WELT SP 104428*, WELT SP 104430*, WELT SP 104366*, WELT SP 104369*.

G. sesamoides: AK 347693*, AK119011, AK155155, AK159528, AK161369, AK173982, AK188385, AK221396, AK224770, AK225971, AK234302, AK234404, AK234409, AK234414, AK237280, AK245805, AK24593, AK246013, AK251847, AK253005, AK 2677, AK 286239, AK 294554, AK 296908, AK 329515, AK 3676, AK 3678, AK 3686, CHR 122712, CHR 22232, CHR 226319, CHR 285331, CHR 289735, CHR 289737, CHR 362280, CHR 437535, CHR 485466, CHR 524293, CHR 525849, WELT SP 019061, WELT SP 019062, WELT SP 019075, WELT SP 019077, WELT SP 019082, WELT SP 019083, WELT SP 019087, WELT SP 099567, WELT SP 099582, WELT SP 099584, WELT SP 104446, WELT SP 104447*, WELT SP 104448*, WELT SP 104451*.

APPENDIX 2. List of observations sourced from NatureWatch New Zealand (http://naturewatch.org.nz) included in this study.

Gastrodia cooperae: http://naturewatch.org.nz/observations/2533881

Gastrodia molloyi: http://naturewatch.org.nz/observations/0949156; http://naturewatch.org.nz/observations/0981727; http://naturewatch.org.nz/observations/0982391; http://naturewatch.org.nz/observations/1175931; http://naturewatch.org.nz/observations/1190130; http://naturewatch.org.nz/observations/1191440; http://naturewatch.org.nz/observations/1200430; http://naturewatch.org.nz/observations/1579940; http://naturewatch.org.nz/observations/248452