An investigation of large-leaved *Gunnera* L. (Gunneraceae) grown outside in Britain and Ireland

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Abstract

A molecular investigation of large-leaved *Gunnera* growing outside in Britain and Ireland was conducted. Two low-copy nuclear *CYCLOIDEA*-like genes (*CYC*-like 1 and *CYC*-like 2) and two chloroplast DNA regions (*matK* and *psbD-trnT*) were sequenced for 271 samples of *Gunnera*. While it was confirmed that genuine *G. tinctoria* is growing both in cultivation and in the wild, the results support recently published morphological and historical findings that the species *G. manicata* appears no longer to be present in Britain and Ireland. Instead, the plant under this name is *G.* × *cryptica*, a hybrid between *G. manicata* and *G. tinctoria*. The implication of this discovery for legislation on invasive non-native species where *G. manicata* and *G. tinctoria* are listed is explored.

Introduction

The genus *Gunnera* L. (Gunneraceae) comprises around sixty species in six subgenera: *Gunnera*; *Milligania* (Hook.f.) Schindl.; *Misandra* (Comm.) Schindl.; *Ostenigunnera* Mattf.; *Panke* (Molina) Schindl.; and *Pseudogunnera* (Oerst.) Schindl. (Mora-Osejo *et al.*, 2011). In Britain and Ireland (including the Channel Islands and the Isle of Man for the purpose of this paper), there are two commonly grown *Gunnera*, both of subgenus *Panke*, known as *G. manicata* Linden ex André, native to southern Brazil (Mora-Osejo *et al.*, 2011; Hassemer, 2020),

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and *G. tinctoria* (Molina) Mirb., native to Chile and Argentina (Mora-Osejo *et al*., 2011) (Fig. 1). They are spectacular in stature, with magnificent large leaves and inflorescences, which has made them popular plants in gardens.

Despite being such popular horticultural subjects, distinguishing these two species has long been considered difficult (Goldring, 1879; Elwes & Stapf, 1919; Clement, 2003; Grant, 2004). To aid identification, keys (for example, Grant, 2004; Stace, 2010) and guides (for example, Riches, 2008; National Biodiversity Data Centre, 2010) have been produced. However, their separation has remained often not straightforward and their identity subject to question in gardens and the horticultural trade. Variable seedlings, intermediates between *Gunnera manicata* and *G. tinctoria*, and suggestions of hybridisation have long been reported (Tallack, 1894; Smith, 1904; Burbidge, 1905; Nelson, 1986; Osborne *et al*., 1991; Nelson & Grills, 1998; Grant, 2004). At the Royal Horticultural Society (RHS) Garden, Wisley, UK, plants are grown as both *G. manicata* and *G. tinctoria* but among these there are also plants that have been thought to show intermediacy between the two species. A difference in the inflorescence, with *G. manicata* having slender branches and *G. tinctoria* having stout branches, has often been given as a distinguishing character (for example, Grant, 2004; Stace, 2010), yet at Wisley there are specimens that bear both sorts of inflorescence on the same plant. This was also reported in O'Rourke & O'Flynn (2014). Furthermore, Clement (2003) noted that descriptions of *G. manicata* in the wild in Brazil differ from plants of that name in cultivation in Britain and Ireland. Hybridisation between species of *Gunnera* subgenus *Panke* has been observed in the wild on several occasions (Johow, 1896; Skottsberg, 1922; Palkovic, 1978; Mora-Osejo, 1984; Pacheco *et al*., 1991; Mora-Osejo *et al*., 2011).

In an earlier paper from this study, based on morphological and historical investigation (Shaw *et al*., 2022), we reported that while genuine *Gunnera tinctoria* is growing in Britain and Ireland, it appears that *G. manicata* is no longer present, but instead has been replaced by a hybrid between *G. manicata* and *G. tinctoria*, named as *Gunnera* × *cryptica* J.M.H. Shaw (Fig. 1).

A detailed historical account was provided in Shaw *et al*. (2022), in which it was shown that the parental species of the hybrid were both introduced into cultivation in western Europe (Belgium) in the 19th century (*Gunnera tinctoria* from Chile just before 1839 (van Houtte, 1870) and *G. manicata* from Brazil around 1861) and that hybridisation took place thereafter, followed by a gradual and unnoticed disappearance of *G. manicata* from cultivation. By 1873 large plants of both species were reported to be growing alongside each other at the nursery of Louis van Houtte, Belgium (Jongkindt-Coninck, 1873). Since *Gunnera* are wind-pollinated (González & Bello, 2009), hybridisation could have taken place from that time onwards if the flowering of both species coincided. Subsequent cold winters (Tallack, 1894; McMillan Browse, 2007), combined with garden selection for size and vigour, are proposed by Shaw *et al*. (2022) to have led to the loss of true *G. manicata* in cultivation and its replacement by the hybrid. Other species of *Gunnera* recorded as having been in cultivation in Europe can be discounted from being involved in the hybrid from examination of their gross morphological features, as set out in Mora-Osejo *et al.* (2011). Evidence of hybrids between *G. manicata*

Fig. 1 Images of *Gunnera tinctoria* and *G.* × *cryptica* at RHS Garden Wisley, UK (photos: RHS), and *G. manicata* in Santa Catarina, Brazil (photo: G. Hassemer).

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and *G. tinctoria* in cultivation in Europe and New Zealand was also provided from the molecular investigation of van Valkenburg *et al.* (2023).

As well as being cultivated, plants known as *Gunnera manicata* and *G. tinctoria* are now present in natural and unmanaged areas in Britain and Ireland. Dines & Dehnen-Schmutz (2023a) report that *G. tinctoria* was first recorded from the wild in Guernsey in 1884. In Stace (2019) the species is described as now being naturalised in scattered places across much of the lowland areas of the British Isles, spreading vegetatively and often self-sown and invasive where long established. Invasive populations are present in the western areas of Ireland (Osborne *et al.*, 1991; Sheehy Skeffington & Hall, 2011; Gioria & Osborne, 2013), the Outer Hebrides (Gioria & Osborne, 2013) and Cornwall, UK (French, 2020). See also Dines & Dehnen-Schmutz (2023a). Elsewhere in the world it is problematic on São Miguel in the Azores (Silva *et al.*, 1996) and in New Zealand (Williams *et al.*, 2005).

In contrast, *Gunnera manicata*, which is reported in Dines & Dehnen-Schmutz (2023b) as being first recorded from the wild in Monmouthshire, Wales, in 1938, is recorded in Stace (2019) as now being persistent through much of the lowland parts of the British Isles and fertile, but not self-sown. McClintock (1975) and Dines & Dehnen-Schmutz (2023b) also report that it is not known to reproduce by seed, though in French (2020) it is considered to have self-sown at a couple of locations in Cornwall. Sheehy Skeffington & Hall (2011), in a pilot study, obtained some seed germination, but at a considerably lower rate (1.3 per cent) than for *G. tinctoria* (39.7 per cent). However, the same authors also reported that seedlings of around five years old had been observed in the Inagh Valley, Connemara, Ireland, and Williams *et al.* (2005) stated that seedlings had arisen near to some cultivated plants in New Zealand. Tallack (1894) described seedlings raised from seed collected in Cornwall. There have been no reports of it becoming problematic in the wild.

Gunnera tinctoria is listed in Schedule 9 of the amended UK Wildlife & Countryside Act, 1981 (England and Wales) and Schedule 9 of the Wildlife (Northern Ireland) Order (1985) as amended by the Wildlife and Natural Environment Act (Northern Ireland) 2011, making it illegal to plant or otherwise cause the species to grow in the wild. In addition, it is listed in Schedule 3 of the Republic of Ireland Statutory Instrument No. 477, European Communities (Birds and Natural Habitats) Regulations 2011. In August 2017 it was added to the EU List of Invasive Alien Species of Union Concern under EU Regulation 1143/2014 on Invasive Alien Species (IAS Regulation), which banned it from sale in the EU from August 2018. Following the UK's departure from the European Union, the IAS Regulation has been adopted into UK legislation. All the species listed as of Union Concern are now termed species of Special Concern. Despite *G. manicata* not recorded as being invasive, it is listed together with *G. tinctoria* in Schedule 3 of the Republic of Ireland Statutory Instrument No. 477, European Communities (Birds and Natural Habitats) Regulations 2011.

The aim of this paper is to provide molecular support for the earlier morphological and historical investigation of Shaw *et al.* (2022) into the correct identity of large-leaved *Gunnera* grown in Britain and Ireland. The conclusions arrived at from our two papers will assist both gardeners and field botanists by clarifying nomenclature and providing new distinguishing morphological characters between the taxa. Confirming the identification of large-leaved gunneras in Britain and Ireland is of particular importance as it enables scarce resources to be focused on the management of the invasive species *G. tinctoria*.

Materials and methods

A total of 271 samples were used for the molecular investigation. Two hundred and twenty-six fresh samples, thought to be *Gunnera manicata*, *G. tinctoria* or putative hybrids between the two species, were collected from Britain, Ireland and the Channel Islands, either from gardens or from the wild. Of these, three samples provided by British nurseries were reported to have been sourced from mainland Europe; one *G. tinctoria* sample, from Royal Botanic Garden Edinburgh (RBGE), Scotland, was from a plant that had been collected from the wild in Chile; and fifty-three were from seedlings growing at The Eden Project, Cornwall, from a concentrated patch where hybridisation was thought to be possibly occurring. Material was sought from gardens in mainland Europe, resulting in a further nine samples from three botanic gardens in Belgium. Nine samples of *G. manicata* were collected from five locations in Santa Catarina and Rio Grande do Sul, southern Brazil (under the Brazilian Sistema de Autorização e Informação em Biodiversidade – SISBIO permit number 51051-1 and SisGen permit number R50E5C3). Twenty-two *G. tinctoria* samples were collected from the Los Lagos region of Chile and a further two samples from the Aisén region of Chile. Other species of *Gunnera* subgenus *Panke* sourced from cultivation in Britain and Ireland were sampled for a broader representation where possible, including one sample each of *G. berteroi* Phil. and *G. insignis* (Oerst.) Oerst. *Gunnera magellanica* Lam., from subgenus *Misandra*, was sampled as an outgroup.

Leaf material from fresh collections was dried and stored in silica gel at room temperature and voucher specimens prepared. Voucher specimens were deposited at WSY apart from specimens collected at RBGE, which were deposited at E, and specimens collected in Brazil, which were deposited at HTL (Thiers, 2023). Details of the sampled taxa are given in Appendix 1.

Total genomic DNA was extracted from dried leaf material using a QIAGEN DNeasy Plant Mini Kit.[10] The quality of the DNA extractions was visualised on agarose gels. DNA concentrations were quantified with a Qubit™ 3.0 Fluorometer.[11] Aliquots of 2–2.5 ng/µl were prepared for PCR amplification.

Two chloroplast DNA regions, *matK* and *psbD-trnT*, were amplified. Both have been shown to be a good choice for molecular studies at low taxonomic levels (Shaw *et al.*, 2007; Könyves, 2014) and *matK* has been proposed as one of the core DNA barcodes for plants (Hollingsworth *et al*., 2011).

PCR reactions for *matK* were performed with primers X and 5 (Ford *et al*., 2009) in 25 µl volumes containing final concentrations of 1× Bioline BioMix™ Red[12], 0.35 µM of each primer, 0.2 mg/ml bovine serum albumin (BSA), 4 per cent v/v dimethyl sulfoxide (DMSO) and 10 ng DNA template. Cycling conditions were 94 °C (120 s), followed by 35 cycles of 94 °C (30 s), 48 °C (30 s), 72 °C (60 s), with a final extension step of 72 °C (7 min). PCR reactions for *psbD-trnT* were performed with primers psbD and trnT$^{(GGU)}$-R (Shaw *et al*., 2007) in 25 µl volumes containing final concentrations of 1× Bioline BioMix™ Red, 0.2 µM of each primer, 0.2 mg/ml BSA and

[10] https://www.qiagen.com
[11] https://www.thermofisher.com
[12] https://www.bioline.com

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10 ng DNA template. Cycling conditions were 80 °C (300 s), then 30 cycles of 95 °C (60 s) and 50 °C (60 s), followed by a ramp of 0.3 °C/s to 65 °C, 65 °C (250 s) and finally 65 °C (5 min).

Two low-copy nuclear *CYCLOIDEA*-like (*CYC*-like) genes (*CYC*-like 1 and *CYC*-like 2) were amplified using the primers developed for *Gunnera* by Citerne *et al*. (2013). Low-copy nuclear genes are alternatives to the most commonly used nuclear marker, the Internal Transcribed Spacer (ITS), in species-level studies (Nieto Feliner & Rosselló, 2007). *CYC*-like genes are fast evolving (Citerne, 2006) and have been isolated from many plant families (Citerne *et al*., 2000, 2017; Smith *et al.*, 2004; Damerval *et al*., 2007; Chapman *et al.*, 2008; Howarth *et al.*, 2011).

Attempts were made to sequence ITS following the protocol of Wanntorp *et al*. (2002), but the results were of very low quality, most likely due to length variation in the sequenced alleles as shown by van Valkenburg *et al*. (2023).

PCR reactions of the *CYC*-like genes were performed in 25 µl volumes containing final concentrations of 1× Bioline Biomix Red, 0.2 µM of each primer, 0.2 mg/ml BSA and 10 ng DNA template. Cycling conditions were 94 °C (120 s), then 35 cycles of 94 °C (30 s), 56 °C (30 s), 72 °C (60 s) and finally 72 °C (7 min).

PCR products were visualised on 1 per cent agarose gels in 1× TBE buffer stained with SYBR® Safe.[13] Gels were illuminated with UV light and photographs taken to record amplification success. The approximate size and concentration of the PCR products were determined using HyperLadder™ 1kb (200 bp to 10,000 bp) as a marker.[14]

Direct sequencing of PCR products in forward and reverse direction was carried out by Genewiz UK Ltd., Bishop's Stortford, UK (formerly Beckman Coulter (UK) Ltd.), using the amplification primers.[15] A subset of 24 samples which showed ambiguous base calls was resequenced with newly designed allele specific primers:

CYC1_SEQ_G R: 5′-CTAACTGGTATGAC-3′;
CYC1_SEQ_A R: 5′-CTAACTGGTATGAT-3′;
CYC2_SEQ_G F: 5′-GGTTTGACAAAGCG-3′;
CYC2_SEQ_C F: 5′-GGTTTGACAAAGCC-3′;
CYC2_SEQ_G R: 5′-ACCGTTGTCAATAC-3′;
CYC2_SEQ_A R: 5′-ACCGTTGTCAATAT-3′.

The primers were designed following the guidelines of Scheen *et al.* (2012). The two ambiguous bases in *CYC*-like 1 were close to the end of the sequence and the distance between them only 50 bp. Because the start of a sequencing read is often of lower quality and obtaining overlapping reads over a short distance is unlikely, ambiguous *CYC*-like 1 sequences were only resequenced with allele-specific reverse primers. Sequence data for all samples have been deposited in GenBank. Accession numbers are given in Appendix 1.

Sequence trace files were assembled and edited using SeqMan Pro 13[16] or Geneious Prime 2022.0.1.[17] Base pair positions where strong double peaks that were clearly discriminated from background noise were present were recorded using IUPAC ambiguity codes (Cornish-Bowden, 1985). Such strong double peaks can indicate hybridity (Edwards *et al*., 2015). Sequences were aligned with MUSCLE ver. 3.8. 425 (Edgar, 2004) implemented in Geneious Prime. The ends of the alignments were trimmed to the point

[13] https://www.thermofisher.com
[14] https://www.bioline.com
[15] https://www.genewiz.com
[16] https://www.dnastar.com
[17] https://www.geneious.com

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where all sequences were present and base calls were unambiguous.

To explore variation within the chloroplast and nuclear genomes, and relationships between the sampled taxa, statistical parsimony networks were constructed from the combined *matK* and *psbD-trnT* dataset, the *CYC*-like 1 and the *CYC*-like 2 alleles using the package *haplotypes* ver. 1.1.2 (Aktas, 2015) in the statistical program *R* ver. 4.1.2 (R Development Core Team, 2016) under the 95 per cent statistical parsimony criterion. Indels were coded according to the simple indel coding method (Simmons & Ochotorena, 2000). The 53 samples collected from seedlings at The Eden Project were not included in the statistical parsimony networks construction and their genotypes are reported separately.

Results

The total analysed length of the combined cpDNA regions was 2292 bp. The haplotype network analysis of the combined cpDNA regions recovered nine different chloroplast DNA haplotypes (Fig. 2). Excepting those of *Gunnera berteroi*, *G. insignis* and *G. magellanica*, the samples from Britain and Ireland, the Channel Islands and Belgium were found to have one of two different haplotypes (H1 and H4), separated by five single nucleotide polymorphisms and two indels of 7 bp and 2 bp (Appendix 2). All Chilean samples, including the Chilean-sourced *G. tinctoria* from RBGE, contained H1. The Brazilian *G. manicata* samples contained four haplotypes (H2, H3, H5, H6). Haplotype H4 was not found in samples from the wild but differed by just 1 bp from the *G. manicata* haplotype H3. The remaining three haplotypes were unique to *G. berteroi*, *G. insignis* and *G. magellanica*.

The length of the *CYC*-like 1 and *CYC*-like 2 alignments after excluding the beginning and the end were 802 bp and 590 bp respectively. Three alleles were found in homozygous individuals for both

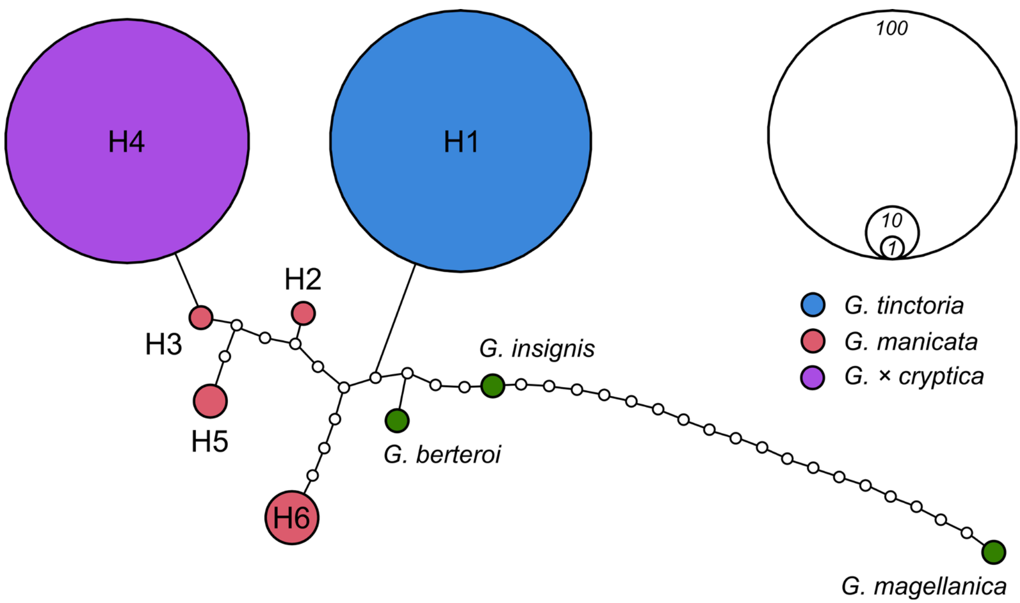

Fig. 2 Haplotype network of the combined cpDNA sequences. Coloured circles represent the observed haplotypes. Haplotype numbers correspond to those explained in text. Open circles indicate inferred haplotype; the length of lines does not have meaning. Diagram prepared by the authors.

CYC-like 1 and *CYC*-like 2 genes (Table 1). The homozygous *Gunnera tinctoria* individuals outside of Chile carried alleles 1 or 2 for both *CYC*-like regions. The Chilean samples carried both alleles 1 and 2 for *CYC*-like 1 but only allele 2 for *CYC*-like 2 (Table 2). The Brazilian *G. manicata* samples only contained allele 3 for *CYC*-like 1 and alleles 3 and 4 for *CYC*-like 2 (Table 2). However, allele 4 of *CYC*-like 2 (Table 1) was only present in a single heterozygous individual. Resequencing a subset of 24 samples that showed ambiguous base calls for *CYC*-like genes with allele specific primers confirmed the presence of all *CYC*-like alleles in Britain and Ireland. The alleles found in *G. berteroi*, *G. insignis* and *G. magellanica* were unique to these species (Table 1). The alleles found in *G. magellanica* could not be connected to the network in either of the nuclear regions under the 95 per cent statistical parsimony criterion (Fig. 3) and the species was omitted from the figure.

A total of 110 samples contained wild *Gunnera tinctoria* cpDNA haplotype H1 (Table 2, genotypes G1–G5). Of these, 97 samples were homozygous for the nuclear regions and contained either allele 1 (G1: N = 73) or allele 2 (G2: N = 24) for both *CYC*-like regions. Samples that had been thought to be hybrids due to the presence of both stout and slender inflorescence branches were shown to have either G1 or G2 genotypes. Two other samples with *G. tinctoria* H1 were heterozygous for the *CYC*-like 1 region between alleles 1 and 3 and homozygous for *CYC*-like 2 with allele 1 (G5). The *G. tinctoria* sample from RBGE originally sourced from Chile (with H1 and allele 2 for both *CYC*-like regions) had genotype G2. Seven samples had G4 (allele 1 in *CYC*-like 1, allele 2 in *CYC*-like 2). The remaining four samples were G3 (heterozygous in *CYC*-like 1 between alleles 1 and 2, and carried allele 2 for *CYC*-like 2).

Haplotype H4 was recovered in 96 samples (G6–G13). Most of these samples were heterozygous for both nuclear regions (G6: N = 79), showing perfect additivity of allele 1 and the Brazilian *Gunnera manicata* allele 3 for both *CYC*-like genes. Additionally, two samples showed a heterozygous pattern

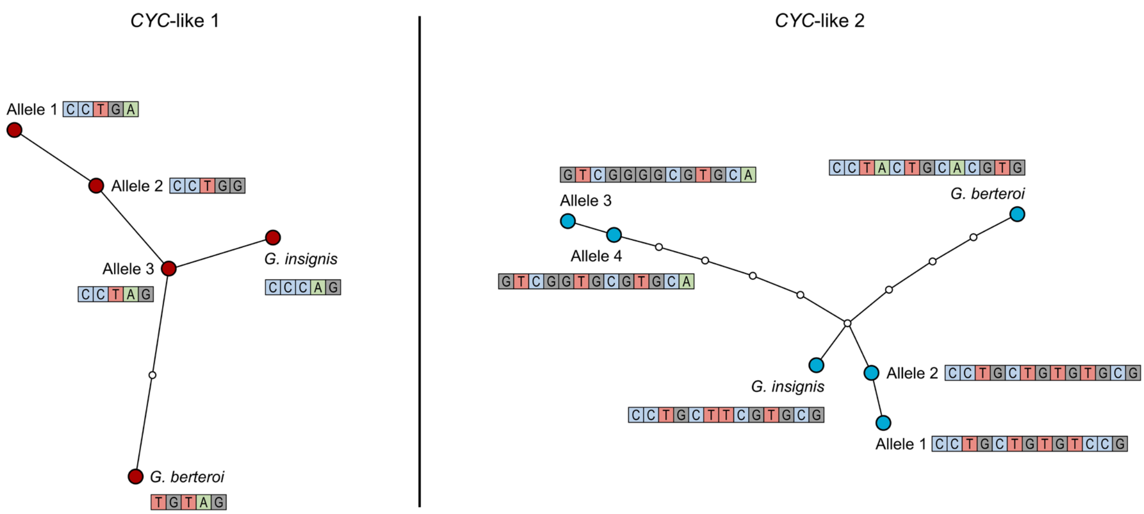

Fig. 3 *CYC*-like networks – haplotype networks of the *CYC*-like alleles for *CYC*-like 1 and *CYC*-like 2. Coloured circles represent the observed alleles. Allele numbers correspond to those found in Table 1. Open circles indicate inferred haplotype; the length of lines does not have meaning. Diagram prepared by the authors.

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Table 1 *CYC*-like gene alleles recovered from the *Gunnera* samples included in the project.

Gene: *CYC*-like 1

Species	Alignment position (bp)	24	148	184	205	250	352	378	458	538	594	612	621	624	633	634	635	636	637	638	661	713	777
G. magellanica	Allele magellanica	G	C	C	T	G	G	C	C	G	A	G	A	T	–	–	–	–	–	–	G	C	A
G. berteroi	Allele berteroi	C	T	G	T	A	C	A	G	A	G	A	T	G	C	G	C	T	T	G	G	G	G
G. insignis	Allele insignis	C	C	C	C	A	C	A	G	A	G	A	T	G	C	G	C	T	T	G	G	G	G
G. tinctoria	Allele 1	C	C	C	T	A	C	A	G	A	G	G	T	G	C	G	C	T	T	G	A	G	G
G. tinctoria	Allele 2	C	C	C	T	A	C	A	G	A	G	G	T	G	C	G	C	T	T	G	G	G	G
G. manicata	Allele 3	C	C	C	T	A	C	A	G	A	G	A	T	G	C	G	C	T	T	G	G	G	G

Gene: *CYC*-like 2

Species	Alignment position (bp)	24	117	156	163	164	228	232	241	246	250	281	369	381	386	404	413	421	428	454	471	473	484	501	510	542	544	563	575
G. magellanica	Allele magellanica	G	G	T	A	C	G	C	C	G	G	T	C	G	C	A	A	G	T	G	T	G	A	C	G	T	G	C	A
G. berteroi	Allele berteroi	A	C	C	G	T	A	T	G	C	A	T	A	A	A	T	G	A	A	G	C	A	C	C	A	C	G	T	G
G. insignis	Allele insignis	A	C	C	G	T	G	T	G	C	A	T	A	A	A	T	G	A	A	T	C	A	C	C	G	T	G	C	G
G. tinctoria	Allele 1	A	C	C	G	T	G	T	G	C	A	T	A	A	A	T	G	A	A	G	C	A	C	T	G	T	C	C	G
G. tinctoria	Allele 2	A	C	C	G	T	G	T	G	C	A	T	A	A	A	T	G	A	A	G	C	A	C	T	G	T	G	C	G
G. manicata	Allele 3	A	G	T	G	C	G	T	G	G	A	G	A	A	A	T	G	A	A	G	C	A	C	C	G	T	G	C	A
G. manicata	Allele 4 (derived)	A	G	T	G	C	G	T	G	G	A	T	A	A	A	T	G	A	A	G	C	A	C	C	G	T	G	C	A

Table 2 Genotypes recovered for *G. manicata*, *G. tinctoria* and putative hybrids between the two species from the combined chloroplast and nuclear DNA variation. cpDNA = haplotype variation recovered from the combined chloroplast *matK* and *psbD-trnT* sequences. *CYC*-like 1 and *CYC*-like 2 = sequence variation found in the two nuclear *CYC*-like regions. N = number of samples observed for each genotype. Ambiguity codes (bases other than A, C, G, T) indicate positions where *G. manicata* and *G. tinctoria* differ.

Species	Genotype	cpDNA	*CYC*-like 1		*CYC*-like 2								Britain, Ireland & the Channel Islands	Mainland Europe	Chile	Brazil
G. tinctoria	G1	H1	G	A	C	C	T	C	T	T	C	G	70	3		
	G2	H1	G	G	C	C	T	C	T	T	G	G	11		13	
	G3	H1	G	R	C	C	T	C	T	T	G	G			4	
	G4	H1	G	A	C	C	T	C	T	T	G	G			7	
G. × *cryptica*	G5	H1	R	R	C	C	T	C	T	T	C	G	2			
	G6	H4	R	R	S	Y	Y	S	K	Y	S	R	76	3		
	G7	H4	R	R	C	C	T	C	T	T	C	G	5	1		
	G8	H4	R	R	G	T	C	G	G	C	G	A		1		
	G9	H4	G	A	S	Y	Y	S	K	Y	S	R	3			
	G10	H4	A	G	S	Y	Y	S	K	Y	S	R	1			
	G11	H4	G	R	C	C	T	C	T	T	S	G	1			
	G12	H4	G	R	S	Y	Y	S	K	Y	G	R	1			
	G13	H4	G	A	C	C	T	C	T	T	C	G	3	1		
G. manicata	G14	H3	A	G	G	T	C	G	G	C	G	A				1
	G15	H5	A	G	G	T	C	G	G	C	G	A				2
	G16	H6	A	G	G	T	C	G	G	C	G	A				5
	G17	H2	A	G	G	T	C	G	K	C	G	A				1
	Alignment position (bp)		612	661	117	156	164	246	281	501	544	575				

containing alleles 1 and 2 for *CYC*-like 1, with one of the samples carrying alleles 1 and 2 (G11) and the other carrying alleles 2 and 3 (G12) for *CYC*-like 2. Six of the samples showed heterozygosity with alleles 1 and 3 in *CYC*-like 1, but carried allele 1 for *CYC*-like 2 (G7). One sample showed heterozygosity with alleles 1 and 3 in *CYC*-like 1, but contained allele 3 for *CYC*-like 2 (G8). G9 and G10 showed perfect additivity between alleles 1 and 3 for *CYC*-like 2, but were homozygous for alleles 1 and 3 respectively for *CYC*-like 1 (G9: N = 3 and G10: N = 1). The final four samples (G13) were homozygous in both nuclear regions with allele 1.

G14–G17 represent the Brazilian *Gunnera manicata* with unique haplotypes H2, H3, H5 and H6; all but one of the samples were homozygous with allele 3 in both nuclear regions. None of the samples collected in Britain and Ireland, the Channel Islands or Belgium carried the Brazilian *G. manicata* cpDNA alleles (H2, H3, H5, H6) and only two samples were homozygous with Brazilian *CYC*-like alleles (G10: allele 3 in *CYC*-like 1, G8: allele 3 in *CYC*-like 2).

Of the 53 seedling sample subset from The Eden Project, 51 contained the *Gunnera tinctoria* haplotype H1 (Table 3, G1, G2, G18, G19). Of these, 47 had genotypes G1 and G2,

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Table 3 Genotypes recovered from The Eden Project seedling sample subset from the combined chloroplast and nuclear DNA variation. cpDNA = haplotype variation recovered from the combined chloroplast *matK* and *psbD-trnT* sequences. *CYC*-like 1 and *CYC*-like 2 = sequence variation found in the two nuclear *CYC*-like regions. N = number of samples observed for each genotype. Ambiguity codes (bases other than A, C, G, T) indicate positions where *G. manicata* and *G. tinctoria* differ.

Species	Genotype	cpDNA	*CYC*-like 1		*CYC*-like 2								N
G. tinctoria seedling	G1	H1	G	A	C	C	T	C	T	T	C	G	45
	G2	H1	G	G	C	C	T	C	T	T	G	G	2
	G18	H1	G	R	C	C	T	C	T	T	S	G	3
	G19	H1	G	R	C	C	T	C	T	T	C	G	1
G. × *cryptica* seedling	G9	H4	G	A	S	Y	Y	S	K	Y	S	R	2
	Alignment position (bp)		612	661	117	156	164	246	281	501	544	575	

being homozygous for the nuclear regions and containing either allele 1 (G1: N = 45) or allele 2 (G2: N = 2) for both *CYC*-like regions. The remaining four samples (G18: N = 3 and G19: N = 1) were heterozygous in *CYC*-like 1 with alleles 1 and 2. For *CYC*-like 2 the three samples with G18 were heterozygous with alleles 1 and 2 and the sample with G19 was homozygous with allele 1. These two genotypes (G18 and G19) were only found in the seedling sample subset. Just two seedlings carried cpDNA haplotype H4. They had genotype G9 with a homozygous *CYC*-like 1, comprising allele 1, but showed perfect additivity with alleles 1 and 3 for *CYC*-like 2.

Discussion and conclusions

Gunnera tinctoria

The results of the molecular investigation have confirmed that material genetically matching Chilean *Gunnera tinctoria* grows in Britain and Ireland (genotype G2) and that there is another similar genotype in existence (G1), not recovered from Chilean samples but present in Britain and Ireland, the Channel Islands and mainland Europe.

Gunnera manicata *and* G. × cryptica

The conclusions from the morphological and historical investigation of Shaw *et al.* (2022) are supported by this molecular study. Together, they provide strong evidence that *Gunnera manicata* has been replaced by the hybrid between *G. manicata* and *G. tinctoria*, *G.* × *cryptica*, in Britain and Ireland, as well as in the Channel Islands and probably mainland Europe given the evidence also provided by van Valkenburg *et al.* (2023).

None of the samples collected from Britain and Ireland, the Channel Islands and Belgium was an exact match with Brazilian *Gunnera manicata*, though chloroplast sequence data for genotypes G6–G13 (H4, Fig. 2, Table 2) did group with *G. manicata*. Therefore, haplotype H4 most likely originated from an unsampled *G. manicata* population in Brazil. Furthermore, combined chloroplast and nuclear sequence data demonstrated the presence of plants genetically intermediate between *G. manicata* and *G. tinctoria* that appear to be of hybrid origin.

The majority of the intermediate samples (G6: N = 79) showed perfect additivity between the two species, with no variation at other base pair positions under study. A plausible explanation is that these plants are F1 hybrids between the species or part of a segregating population of hybrids for which none of the other possible intermediates were found. The lack of other possible intermediates is likely to be because, as reported above, the plant grown as *Gunnera manicata* (*G.* × *cryptica*) appears to be non-seeding or have very poor germination. The partial additivity seen in G7–G13 (N = 14 Britain and Ireland and the Channel Islands; N = 3 Belgium) suggest that crosses between hybrid individuals or backcrosses to *G. tinctoria* have occurred. G5 (N = 2) also appeared to be a hybrid, but with *G. tinctoria* as its seed parent (haplotype H1) and the hybrid as the pollen parent.

For the sample subset from The Eden Project, most of the samples were confirmed as *Gunnera tinctoria* (G1: N = 45 and G2: N = 2). Just two samples from the subset were shown to be *G.* × *cryptica* seedlings (G9: N = 2). Four seedlings showed genetic intermediacy between *G. tinctoria* genotypes (G18: N = 3 and G19: N = 1).

Plants with both stout and slender inflorescences were found to be *Gunnera tinctoria*, having genotypes G1 and G2 rather than any of the genetically intermediate genotypes G5–G13. This suggests that further studies are needed to understand the morphological variation in *G. tinctoria*.

The molecular data show that there is some gene flow between *Gunnera* × *cryptica* and *G. tinctoria* in both directions, albeit apparently on a very small scale. It is not understood how complex the processes of gene flow are in *Gunnera* and it is possible that plants of complex mixed parentage are represented in naturalised populations given that the plants are wind pollinated. As with other examples, such as *Quercus* × *rosacea* Bechst. and its parents *Q. petraea* (Matt.) Liebl. and *Q. robur* L., in which the whole range of characters can be observed (reviewed in Stace *et al*., 2015), such plants could be difficult to separate from *G. tinctoria* on morphology alone.

Implications for Gunnera *as an invasive plant*

As stated previously, *Gunnera tinctoria* has been listed in legislation as an invasive plant since 2010 (UK) and 2011 (Republic of Ireland) and subsequently, in 2017, became listed as being of Union Concern (now of Special Concern in the UK adopted legislation), attracting the highest level of restrictions for the whole of the European Union and in Britain. As our studies have shown that plants widely referred to as *G. manicata* in cultivation and in the wider environment in Europe are likely to be *G.* × *cryptica*, the listing in the relevant invasive non-native species legislation, such as Schedule 3 of the Republic of Ireland Statutory Instrument No. 477 European Communities (Birds and Natural Habitats) Regulations 2011, should be updated to reflect our improved understanding of the identity of the plant. There is, however, a more fundamental issue. As is commonly the case in biodiversity-related legislation, in Article 3 of the IAS Regulation an 'alien species' is defined as 'any live specimen of a species, subspecies or lower taxon … as well as any hybrids, varieties or breeds that might survive and subsequently reproduce'. This means that the Regulation applies not only to the listed species but to any hybrid containing that species. As a consequence, strict application of the Regulation would mean that *G.* × *cryptica* should be considered a taxon of Union or Special Concern with all that implies for trade and management of that taxon within gardens. However, much depends upon the degree to which any hybrid is able to reproduce itself. A similar case is the hybrid of *Lysichiton americanus* Hultén & H.St.John (listed as a species of Union/Special Concern) with *L. camtschatcensis* (L.) Schott (not listed), *L.* × *hortensis* J.D.Arm. & B.W.Phillips (Armitage

& Phillips, 2011). So far, this implication of the legislation has not been tested in the UK but those responsible for plant collections including such plants need to be aware of the potential risk that such hybrids could be treated as banned in future. Likewise, in the Republic of Ireland, hybrids are included in the definition of a plant in Statutory Instrument No. 477 (2011). This issue is discussed further in van Valkenburg *et al.* (2023).

When a naturalised population of gunneras growing in the presence of both *Gunnera tinctoria* and *G.* × *cryptica* was examined at The Eden Project, only two of fifty-three seedlings were found to contain *G. manicata* genes. Although *G.* × *cryptica* does appear to occasionally reproduce by seed, the invasive threat appears overwhelmingly to be *G. tinctoria*. If *G. tinctoria* is eradicated it appears very likely that *G.* × *cryptica* would not spread much by itself. However, although our observations suggest that *G.* × *cryptica* does not pose an invasive threat in Britain and Ireland, this study has not sampled populations of the hybrid densely enough to say for sure. Further sampling of populations in natural habitats should be undertaken to determine whether *G.* × *cryptica* represents a genuine threat of becoming invasive. In addition, the diagnostic morphological characters that have been provided in our earlier paper from this study (Shaw *et al*., 2022) can be used to strengthen confidence that existing recordings for the large-leaved *Gunnera* taxa growing in the wild in Britain and Ireland are correct.

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Appendix 1 Details of specimens included in the molecular analyses with the sample code, source details, voucher specimen information (herbarium code and, where available, barcode), the recovered genotype from the molecular analyses, the taxon name and the GenBank accession numbers (in the following order: *matK*, *psbD-trnT*, *CYC*-like 1, *CYC*-like 2). Herbaria codes: E, Royal Botanic Garden Edinburgh, Scotland; HTL, Universidade Federal de Mato Grosso do Sul, Brazil; WSY, Royal Horticultural Society Wisley, England. RBGE = Royal Botanic Garden Edinburgh; RHS = Royal Horticultural Society; SHHG = Sir Harold Hillier Gardens, England. Additional collection details are available on request from WSY.

Sample code	Source details	Voucher location/barcode	Genotype	Taxon name	GenBank accession number			
					matK	*psbD-trnT*	*CYC*-like 1	*CYC*-like 2
GUN001	Jersey	WSYP000008–011	G13	*G.* × *cryptica*	OR248875	OR249146	OR249417	OR249688
GUN002	Jersey	WSYP000012–014	G6	*G.* × *cryptica*	OR248876	OR249147	OR249418	OR249689
GUN003	Jersey	WSYP000015–017	G6	*G.* × *cryptica*	OR248877	OR249148	OR249419	OR249690
GUN004	Jersey	WSYP000018–020	G6	*G.* × *cryptica*	OR248878	OR249149	OR249420	OR249691
GUN005	Isle of Skye, Scotland	WSYP000021–023	G1	*G. tinctoria*	OR248879	OR249150	OR249421	OR249692
GUN006	Isle of Skye, Scotland	WSYP000024–027	G1	*G. tinctoria*	OR248880	OR249151	OR249422	OR249693
GUN007	Isle of Skye, Scotland	WSYP000028–031	G6	*G.* × *cryptica*	OR248881	OR249152	OR249423	OR249694
GUN008	Isle of Skye, Scotland	WSYP000032–033 and 035	G6	*G.* × *cryptica*	OR248882	OR249153	OR249424	OR249695
GUN009	Wiltshire, England	WSYP000040–045	G6	*G.* × *cryptica*	OR248883	OR249154	OR249425	OR249696
GUN010	Wiltshire, England	WSYP000046–049	G6	*G.* × *cryptica*	OR248884	OR249155	OR249426	OR249697
GUN011	Wiltshire, England	WSYP000050–057	G6	*G.* × *cryptica*	OR248885	OR249156	OR249427	OR249698
GUN012	Pembrokeshire, Wales	WSYP000036	G6	*G.* × *cryptica*	OR248886	OR249157	OR249428	OR249699
GUN013	Pembrokeshire, Wales	WSYP000037	G7	*G.* × *cryptica*	OR248887	OR249158	OR249429	OR249700
GUN014	Pembrokeshire, Wales	WSYP000038	G13	*G.* × *cryptica*	OR248888	OR249159	OR249430	OR249701
GUN015	Pembrokeshire, Wales	WSYP000039	G9	*G.* × *cryptica*	OR248889	OR249160	OR249431	OR249702
GUN016	N Lincs, England	WSYP000058–060	G6	*G.* × *cryptica*	OR248890	OR249161	OR249432	OR249703
GUN017	N Lincs, England	WSYP000061–063	G6	*G.* × *cryptica*	OR248891	OR249162	OR249433	OR249704
GUN018	N Lincs, England	WSYP000064–065	G1	*G. tinctoria*	OR248892	OR249163	OR249434	OR249705
GUN019	N Lincs, England	WSYP000066–067	G7	*G.* × *cryptica*	OR248893	OR249164	OR249435	OR249706
GUN020	Greater London, England	WSYP000068–075	G6	*G.* × *cryptica*	OR248894	OR249165	OR249436	OR249707
GUN021	North Uist, Scotland	WSYP000076–079	G1	*G. tinctoria*	OR248895	OR249166	OR249437	OR249708
GUN022	Benbecula, Scotland	WSYP000080–083	G1	*G. tinctoria*	OR248896	OR249167	OR249438	OR249709
GUN023	North Uist, Scotland	WSYP000084–085	G6	*G.* × *cryptica*	OR248897	OR249168	OR249439	OR249710
GUN024	South Uist, Scotland	WSYP000086–088	G1	*G. tinctoria*	OR248898	OR249169	OR249440	OR249711
GUN025	Argyll and Bute, Scotland	WSYP000089–090	G6	*G.* × *cryptica*	OR248899	OR249170	OR249441	OR249712
GUN026	Brecknockshire, Wales	WSYP000118–121	G7	*G.* × *cryptica*	OR248900	OR249171	OR249442	OR249713
GUN027	Brecknockshire, Wales	WSYP000122–124	G6	*G.* × *cryptica*	OR248901	OR249172	OR249443	OR249714
GUN028	Brecknockshire, Wales	WSYP000125–127	G1	*G. tinctoria*	OR248902	OR249173	OR249444	OR249715
GUN029	Kent, England	WSYP000129–132	G6	*G.* × *cryptica*	OR248903	OR249174	OR249445	OR249716
GUN030	Kent, England	WSYP000133–134	G6	*G.* × *cryptica*	OR248904	OR249175	OR249446	OR249717
GUN031	Kent, England	WSYP000135	G6	*G.* × *cryptica*	OR248905	OR249176	OR249447	OR249718
GUN032	E Sussex, England	WSYP000136–138	G6	*G.* × *cryptica*	OR248906	OR249177	OR249448	OR249719
GUN033	E Sussex, England	WSYP000139–141	G6	*G.* × *cryptica*	OR248907	OR249178	OR249449	OR249720
GUN034	Wester Ross, Scotland	WSYP000142	G1	*G. tinctoria*	OR248908	OR249179	OR249450	OR249721
GUN035	Wester Ross, Scotland	WSYP000143	G6	*G.* × *cryptica*	OR248909	OR249180	OR249451	OR249722
GUN036	Isles of Scilly, England	WSYP000150–158	G1	*G. tinctoria*	OR248910	OR249181	OR249452	OR249723
GUN037	Co. Fermanagh, Northern Ireland	WSYP000330–335	G6	*G.* × *cryptica*	OR248911	OR249182	OR249453	OR249724
GUN038	Dorset, England	WSYP000444–447	G6	*G.* × *cryptica*	OR248912	OR249183	OR249454	OR249725
GUN039	Dorset, England	WSYP000448–449	G6	*G.* × *cryptica*	OR248913	OR249184	OR249455	OR249726
GUN040	Dorset, England	WSYP000450–451	G1	*G. tinctoria*	OR248914	OR249185	OR249456	OR249727
GUN041	Dorset, England	WSYP000452	G1	*G. tinctoria*	OR248915	OR249186	OR249457	OR249728

Sample code	Source details	Voucher location/barcode	Genotype	Taxon name	GenBank accession number			
					matK	***psbD-trnT***	***CYC*-like 1**	***CYC*-like 2**
GUN042	Dorset, England	WSYP000453	G6	*G.* × *cryptica*	OR248916	OR249187	OR249458	OR249729
GUN043	Dorset, England	WSYP000454	G1	*G. tinctoria*	OR248917	OR249188	OR249459	OR249730
GUN044	Devon, England	WSYP000455	G9	*G.* × *cryptica*	OR248918	OR249189	OR249460	OR249731
GUN045	Devon, England	WSYP000456	G1	*G. tinctoria*	OR248919	OR249190	OR249461	OR249732
GUN046	Devon, England	WSYP000001	G1	*G. tinctoria*	OR248920	OR249191	OR249462	OR249733
GUN047	Essex, England	WSYP000002–003	G6	*G.* × *cryptica*	OR248921	OR249192	OR249463	OR249734
GUN048	Essex, England	WSYP000004–005	G1	*G. tinctoria*	OR248922	OR249193	OR249464	OR249735
GUN049	Essex, England	WSYP000485–486	G2	*G. tinctoria*	OR248923	OR249194	OR249465	OR249736
GUN052	Glasgow, Scotland	WSYP000489–490	G1	*G. tinctoria*	OR248924	OR249195	OR249466	OR249737
GUN054	Kent, England	WSYP000491–493	G6	*G.* × *cryptica*	OR248925	OR249196	OR249467	OR249738
GUN055	Kent, England	WSYP000494	G1	*G. tinctoria*	OR248926	OR249197	OR249468	OR249739
GUN056	E Sussex, England	WSYP000495	G6	*G.* × *cryptica*	OR248927	OR249198	OR249469	OR249740
GUN057	Greater London, England	WSYP000435–436	G13	*G.* × *cryptica*	OR248928	OR249199	OR249470	OR249741
GUN058	Greater London, England	WSYP000437–438	G1	*G. tinctoria*	OR248929	OR249200	OR249471	OR249742
GUN059	Myddelton House Garden, Greater London, England	WSYP000496–498	G6	*G.* × *cryptica*	OR248930	OR249201	OR249472	OR249743
GUN060	Myddelton House Garden, Greater London, England	WSYP000499–501	G1	*G. tinctoria*	OR248931	OR249202	OR249473	OR249744
GUN061	Myddelton House Garden, Greater London, England	WSYP000502–504	G1	*G. tinctoria*	OR248932	OR249203	OR249474	OR249745
GUN062	Myddelton House Garden, Greater London, England	WSYP000505–507	G1	*G. tinctoria*	OR248933	OR249204	OR249475	OR249746
GUN063	RHS Garden Hyde Hall, Essex, England, Acc. No. H20080141-A	WSYP000508–510	G1	*G. tinctoria*	OR248934	OR249205	OR249476	OR249747
GUN064	RHS Garden Hyde Hall, Essex, England, Acc. No. H19980100-A	WSYP000511–514	G1	*G. tinctoria*	OR248935	OR249206	OR249477	OR249748
GUN065	RHS Garden Hyde Hall, Essex, England, Acc. No. H19970024-B	WSYP000515–517	G1	*G. tinctoria*	OR248936	OR249207	OR249478	OR249749
GUN066	RHS Garden Hyde Hall, Essex, England, Acc. No. H19970024-B	WSYP000515–516, 518–519	G1	*G. tinctoria*	OR248937	OR249208	OR249479	OR249750
GUN067	RHS Garden Rosemoor, Devon, England, Acc. No. R906316K	WSYP000520–522	G6	*G.* × *cryptica*	OR248938	OR249209	OR249480	OR249751
GUN068	RBGE, Scotland, Acc. No. 19599789	E	G6	*G.* × *cryptica*	OR248939	OR249210	OR249481	OR249752
GUN069	RBGE, Scotland, Acc. No. 19961148	E	G2	*G. tinctoria*	OR248940	OR249211	OR249482	OR249753
GUN070	RBGE, Scotland, Acc. No. 20040236	E	N/A	*G. berteroi*	OR248941	OR249212	OR249483	OR249754
GUN071	SHHG, Hampshire, England, Acc. No. 1992.0527-B	WSYP000523–527	G2	*G. tinctoria*	OR248942	OR249213	OR249484	OR249755
GUN072	SHHG, Hampshire, England, Acc. No. 1977.8676-U	WSYP000528–534	G6	*G.* × *cryptica*	OR248943	OR249214	OR249485	OR249756
GUN073	SHHG, Hampshire, England, Acc. No. 76.43512	WSYP000535–538	G6	*G.* × *cryptica*	OR248944	OR249215	OR249486	OR249757
GUN074	Harris Garden, University of Reading, Berkshire, England	—	G6	*G.* × *cryptica*	OR248945	OR249216	OR249487	OR249758
GUN075	RHS Garden Wisley, Surrey, England	WSYP000677–678	G2	*G. tinctoria*	OR248946	OR249217	OR249488	OR249759
GUN076	Cornwall, England	—	G11	*G.* × *cryptica*	OR248947	OR249218	OR249489	OR249760
GUN077	Cornwall, England	—	G12	*G.* × *cryptica*	OR248948	OR249219	OR249490	OR249761
GUN078	RHS Garden Wisley, Surrey, England, Acc. No. W964777-A	WSYP000676	G2	*G. tinctoria*	OR248949	OR249220	OR249491	OR249762
GUN079	RHS Garden Wisley, Surrey, England, Acc. No. W20090672-A	WSYP000683–685	G2	*G. tinctoria*	OR248950	OR249221	OR249492	OR249763
GUN080	RHS Garden Wisley, Surrey, England, Acc. No. W853778-A	WSYP000679–680	G6	*G.* × *cryptica*	OR248951	OR249222	OR249493	OR249764
GUN081	RHS Garden Wisley, Surrey, England, Acc. No. W964777-B	WSYP000681–682	G2	*G. tinctoria*	OR248952	OR249223	OR249494	OR249765
GUN082	RHS Garden Wisley, Surrey, England, Acc. No. W963344-A	WSYP000671–672	G1	*G. tinctoria*	OR248953	OR249224	OR249495	OR249766
GUN083	RHS Garden Wisley, Surrey, England, Acc. No. W852298-B	WSYP000668–670	G6	*G.* × *cryptica*	OR248954	OR249225	OR249496	OR249767
GUN084	RHS Garden Wisley, Surrey, England, Acc. No. W20122001-A	WSYP000686	G2	*G. tinctoria*	OR248955	OR249226	OR249497	OR249768
GUN085	RHS Garden Wisley, Surrey, England, Acc. No. W852298-A	WSYP000663–665	G6	*G.* × *cryptica*	OR248956	OR249227	OR249498	OR249769
GUN086	RHS Garden Wisley, Surrey, England, Acc. No. W20022776-A	WSYP000666–667	G6	*G.* × *cryptica*	OR248957	OR249228	OR249499	OR249770
GUN087	RHS Garden Wisley, Surrey, England, Acc. No. W852298-C	WSYP000673–675	G6	*G.* × *cryptica*	OR248958	OR249229	OR249500	OR249771
GUN088	RHS Garden Wisley, Surrey, England	WSYP000659–662	G6	*G.* × *cryptica*	OR248959	OR249230	OR249501	OR249772
GUN091	RHS Garden Wisley, Surrey, England, Acc. No. W19970293-E	WSY0141177	N/A	*G. magellanica*	OR248960	OR249231	OR249502	OR249773
GUN093	Co. Mayo, Ireland	WSYP000313	G6	*G.* × *cryptica*	OR248961	OR249232	OR249503	OR249774

Sample code	Source details	Voucher location/barcode	Genotype	Taxon name	GenBank accession number			
					matK	***psbD-trnT***	***CYC*-like 1**	***CYC*-like 2**
GUN094	Co. Sligo, Ireland	—	G1	*G. tinctoria*	OR248962	OR249233	OR249504	OR249775
GUN095	Co. Sligo, Ireland	WSYP000276–277	G1	*G. tinctoria*	OR248963	OR249234	OR249505	OR249776
GUN096	Co. Sligo, Ireland	WSYP000336–341	G1	*G. tinctoria*	OR248964	OR249235	OR249506	OR249777
GUN097	Co. Donegal, Ireland	WSYP000282–285	G1	*G. tinctoria*	OR248965	OR249236	OR249507	OR249778
GUN098	Co. Donegal, Ireland	WSYP000358–361	G6	*G.* × *cryptica*	OR248966	OR249237	OR249508	OR249779
GUN099	Co. Donegal, Ireland	WSYP000320–324	G1	*G. tinctoria*	OR248967	OR249238	OR249509	OR249780
GUN100	Co. Donegal, Ireland	WSYP000286–297	G6	*G.* × *cryptica*	OR248968	OR249239	OR249510	OR249781
GUN101	Co. Donegal, Ireland	WSYP000278–281	G1	*G. tinctoria*	OR248969	OR249240	OR249511	OR249782
GUN102	Co. Donegal, Ireland	WSYP000387–388	G1	*G. tinctoria*	OR248970	OR249241	OR249512	OR249783
GUN103	Hampshire, England	WSYP000006	G1	*G. tinctoria*	OR248971	OR249242	OR249513	OR249784
GUN104	Hampshire, England	WSYP000007	G1	*G. tinctoria*	OR248972	OR249243	OR249514	OR249785
GUN105	Isle of Skye, Scotland	WSYP000181–189	G7	*G.* × *cryptica*	OR248973	OR249244	OR249515	OR249786
GUN106	Guernsey	WSYP000171–172	G1	*G. tinctoria*	OR248974	OR249245	OR249516	OR249787
GUN109	Ness Botanic Gardens, Cheshire, England	WSYP000539–544	G6	*G.* × *cryptica*	OR248975	OR249246	OR249517	OR249788
GUN110	Isle of Wight, England	WSYP000159	G1	*G. tinctoria*	OR248976	OR249247	OR249518	OR249789
GUN111	Isle of Wight, England	WSYP000160	G6	*G.* × *cryptica*	OR248977	OR249248	OR249519	OR249790
GUN112	Isles of Scilly, England	WSYP000150–158	G1	*G. tinctoria*	OR248978	OR249249	OR249520	OR249791
GUN113	Dublin Zoo, Ireland	WSYP000592	G5	*G.* × *cryptica*	OR248979	OR249250	OR249521	OR249792
GUN115	Cheshire, England	WSYP000545	G1	*G. tinctoria*	OR248980	OR249251	OR249522	OR249793
GUN116	Hampshire, England	WSYP000546	G1	*G. tinctoria*	OR248981	OR249252	OR249523	OR249794
GUN117	Hampshire, England	WSYP000547	G1	*G. tinctoria*	OR248982	OR249253	OR249524	OR249795
GUN118	Cornwall, England	WSYP000176	G6	*G.* × *cryptica*	OR248983	OR249254	OR249525	OR249796
GUN119	Cornwall, England	WSYP000175	G6	*G.* × *cryptica*	OR248984	OR249255	OR249526	OR249797
GUN120	Cornwall, England	WSYP000180	G6	*G.* × *cryptica*	OR248985	OR249256	OR249527	OR249798
GUN121	Cornwall, England	WSYP000173	G6	*G.* × *cryptica*	OR248986	OR249257	OR249528	OR249799
GUN122	Cornwall, England	WSYP000177	G6	*G.* × *cryptica*	OR248987	OR249258	OR249529	OR249800
GUN123	Cornwall, England	WSYP000179	G1	*G. tinctoria*	OR248988	OR249259	OR249530	OR249801
GUN124	Wester Ross, Scotland	WSYP000144–146	G6	*G.* × *cryptica*	OR248989	OR249260	OR249531	OR249802
GUN126	Isle of Arran, Scotland	WSYP000253–255	G6	*G.* × *cryptica*	OR248990	OR249261	OR249532	OR249803
GUN127	Isle of Arran, Scotland	WSYP000256–258	G1	*G. tinctoria*	OR248991	OR249262	OR249533	OR249804
GUN128	Isle of Arran, Scotland	WSYP000259–261	G6	*G.* × *cryptica*	OR248992	OR249263	OR249534	OR249805
GUN129	Isle of Arran, Scotland	WSYP000262–264	G6	*G.* × *cryptica*	OR248993	OR249264	OR249535	OR249806
GUN130	Isle of Arran, Scotland	WSYP000265–267	G1	*G. tinctoria*	OR248994	OR249265	OR249536	OR249807
GUN131	Co. Cork, Ireland	WSYP000431–434	G1	*G. tinctoria*	OR248995	OR249266	OR249537	OR249808
GUN132	Co. Cork, Ireland	WSYP000342–348	G10	*G.* × *cryptica*	OR248996	OR249267	OR249538	OR249809
GUN133	Co. Cork, Ireland	WSYP000349–357	G6	*G.* × *cryptica*	OR248997	OR249268	OR249539	OR249810
GUN134	Co. Kerry, Ireland	WSYP000399–405	G6	*G.* × *cryptica*	OR248998	OR249269	OR249540	OR249811
GUN135	Co. Kerry, Ireland	WSYP000298–303	G1	*G. tinctoria*	OR248999	OR249270	OR249541	OR249812
GUN136	Co. Kerry, Ireland	WSYP000314–319	G1	*G. tinctoria*	OR249000	OR249271	OR249542	OR249813
GUN137	Co. Kerry, Ireland	WSYP000421–422	G1	*G. tinctoria*	OR249001	OR249272	OR249543	OR249814
GUN138	Co. Kerry, Ireland	WSYP000411–420	G5	*G.* × *cryptica*	OR249002	OR249273	OR249544	OR249815
GUN139	Co. Kerry, Ireland	WSYP000406–410	G1	*G. tinctoria*	OR249003	OR249274	OR249545	OR249816
GUN142	Cornwall, England	WSYP000174	G6	*G.* × *cryptica*	OR249004	OR249275	OR249546	OR249817
GUN143	N Harris, Scotland	WSYP000091–093	G1	*G. tinctoria*	OR249005	OR249276	OR249547	OR249818
GUN144	Lewis, Scotland	WSYP000094–096	G1	*G. tinctoria*	OR249006	OR249277	OR249548	OR249819
GUN145	Lewis, Scotland	WSYP000097–098	G1	*G. tinctoria*	OR249007	OR249278	OR249549	OR249820
GUN146	Lewis, Scotland	WSYP000099–101	G1	*G. tinctoria*	OR249008	OR249279	OR249550	OR249821

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Sample code	Source details	Voucher location/barcode	Genotype	Taxon name	GenBank accession number			
					matK	***psbD-trnT***	***CYC*-like 1**	***CYC*-like 2**
GUN147	Lewis, Scotland	WSYP000102–103	G1	*G. tinctoria*	OR249009	OR249280	OR249551	OR249822
GUN148	Lewis, Scotland	WSYP000104	G1	*G. tinctoria*	OR249010	OR249281	OR249552	OR249823
GUN149	Lewis, Scotland	WSYP000105	G2	*G. tinctoria*	OR249011	OR249282	OR249553	OR249824
GUN151	N Harris, Scotland	WSYP000106	G1	*G. tinctoria*	OR249012	OR249283	OR249554	OR249825
GUN152	N Harris, Scotland	WSYP000107–109	G1	*G. tinctoria*	OR249013	OR249284	OR249555	OR249826
GUN153	N Harris, Scotland	WSYP000110–113	G1	*G. tinctoria*	OR249014	OR249285	OR249556	OR249827
GUN154	S Harris, Scotland	WSYP000114–117	G1	*G. tinctoria*	OR249015	OR249286	OR249557	OR249828
GUN155	Co. Waterford, Ireland	WSYP000190–192	G6	*G.* × *cryptica*	OR249016	OR249287	OR249558	OR249829
GUN156	Co. Waterford, Ireland	WSYP000193–205	G2	*G. tinctoria*	OR249017	OR249288	OR249559	OR249830
GUN157	Co. Waterford, Ireland	WSYP000206–211	G1	*G. tinctoria*	OR249018	OR249289	OR249560	OR249831
GUN158	Co. Waterford, Ireland	WSYP000212, 214–217	G6	*G.* × *cryptica*	OR249019	OR249290	OR249561	OR249832
GUN159	Co. Waterford, Ireland	WSYP000227–233	G6	*G.* × *cryptica*	OR249020	OR249291	OR249562	OR249833
GUN160	Co. Cork, Ireland	WSYP000234–241	G6	*G.* × *cryptica*	OR249021	OR249292	OR249563	OR249834
GUN161	Co. Wexford, Ireland	WSYP000243–252	G6	*G.* × *cryptica*	OR249022	OR249293	OR249564	OR249835
GUN162	Derbyshire, England	WSYP000161–164	G6	*G.* × *cryptica*	OR249023	OR249294	OR249565	OR249836
GUN163	Derbyshire, England	WSYP0000161–164	G6	*G.* × *cryptica*	OR249024	OR249295	OR249566	OR249837
GUN164	Co. Galway, Ireland	WSYP000380–386	G1	*G. tinctoria*	OR249025	OR249296	OR249567	OR249838
GUN165	Co. Galway, Ireland	—	G1	*G. tinctoria*	OR249026	OR249297	OR249568	OR249839
GUN166	Co. Galway, Ireland	WSYP000373–379	G1	*G. tinctoria*	OR249027	OR249298	OR249569	OR249840
GUN167	Co. Galway, Ireland	WSYP000362–372	G6	*G.* × *cryptica*	OR249028	OR249299	OR249570	OR249841
GUN168	Co. Galway, Ireland	WSYP000304–312	G1	*G. tinctoria*	OR249029	OR249300	OR249571	OR249842
GUN169	Co. Galway, Ireland	WSYP000268–275	G1	*G. tinctoria*	OR249030	OR249301	OR249572	OR249843
GUN170	Co. Galway, Ireland	WSYP000389–398	G6	*G.* × *cryptica*	OR249031	OR249302	OR249573	OR249844
GUN183	Co. Down, Northern Ireland	WSYP000165–166	G1	*G. tinctoria*	OR249032	OR249303	OR249574	OR249845
GUN184	Co. Down, Northern Ireland	WSYP000167–168	G6	*G.* × *cryptica*	OR249033	OR249304	OR249575	OR249846
GUN185	Co. Down, Northern Ireland	WSYP000169–170	G6	*G.* × *cryptica*	OR249034	OR249305	OR249576	OR249847
GUN186	Co. Cork, Ireland	WSYP000548–550	G7	*G.* × *cryptica*	OR249035	OR249306	OR249577	OR249848
GUN187	Co. Wexford, Ireland	WSYP000325–329	G6	*G.* × *cryptica*	OR249036	OR249307	OR249578	OR249849
GUN188	The Eden Project, Cornwall, England, Acc. No. 20002227A	WSYP000593	G6	*G.* × *cryptica*	OR249037	OR249308	OR249579	OR249850
GUN189	The Eden Project, Cornwall, England	WSYP000594	G1	*G. tinctoria*	OR249038	OR249309	OR249580	OR249851
GUN190	The Eden Project, Cornwall, England	WSYP000595	G2	*G. tinctoria*	OR249039	OR249310	OR249581	OR249852
GUN191	The Eden Project, Cornwall, England, Acc. No. 20040311A	WSYP000596	G1	*G. tinctoria*	OR249040	OR249311	OR249582	OR249853
GUN192	The Eden Project, Cornwall, England, Acc. No. 20040311A	WSYP000597	G1	*G. tinctoria*	OR249041	OR249312	OR249583	OR249854
GUN193	The Eden Project, Cornwall, England, Acc. No. 20020330A	WSYP000598	G6	*G.* × *cryptica*	OR249042	OR249313	OR249584	OR249855
GUN194	Co. Cork, Ireland	WSYP000551–552	G1	*G. tinctoria*	OR249043	OR249314	OR249585	OR249856
GUN195	Santa Catarina, Brazil	HTL	G14	*G. manicata*	OR249044	OR249315	OR249586	OR249857
GUN196	Santa Catarina, Brazil	HTL	G15	*G. manicata*	OR249045	OR249316	OR249587	OR249858
GUN197	Santa Catarina, Brazil	HTL	G15	*G. manicata*	OR249046	OR249317	OR249588	OR249859
GUN198	Co. Longford, Ireland	WSYP000423–430	G6	*G.* × *cryptica*	OR249047	OR249318	OR249589	OR249860
GUN200	The Eden Project, Cornwall, England	WSYP000599	G1	*G. tinctoria*	OR249048	OR249319	OR249590	OR249861
GUN201	The Eden Project, Cornwall, England	WSYP000600	G1	*G. tinctoria*	OR249049	OR249320	OR249591	OR249862
GUN202	The Eden Project, Cornwall, England	WSYP000601	G1	*G. tinctoria*	OR249050	OR249321	OR249592	OR249863
GUN203	The Eden Project, Cornwall, England	WSYP000602–603	G1	*G. tinctoria*	OR249051	OR249322	OR249593	OR249864
GUN204	The Eden Project, Cornwall, England	WSYP000604	G1	*G. tinctoria*	OR249052	OR249323	OR249594	OR249865
GUN205	The Eden Project, Cornwall, England	WSYP000605	G1	*G. tinctoria*	OR249053	OR249324	OR249595	OR249866
GUN206	The Eden Project, Cornwall, England	WSYP000606	G1	*G. tinctoria*	OR249054	OR249325	OR249596	OR249867
GUN207	The Eden Project, Cornwall, England	WSYP000607	G1	*G. tinctoria*	OR249055	OR249326	OR249597	OR249868

Sample code	Source details	Voucher location/barcode	Genotype	Taxon name	GenBank accession number			
					matK	***psbD-trnT***	***CYC*-like 1**	***CYC*-like 2**
GUN208	The Eden Project, Cornwall, England	WSYP000608	G1	*G. tinctoria*	OR249056	OR249327	OR249598	OR249869
GUN209	The Eden Project, Cornwall, England	WSYP000609	G1	*G. tinctoria*	OR249057	OR249328	OR249599	OR249870
GUN210	The Eden Project, Cornwall, England	WSYP000610	G9	*G.* × *cryptica*	OR249058	OR249329	OR249600	OR249871
GUN211	The Eden Project, Cornwall, England	WSYP000611	G1	*G. tinctoria*	OR249059	OR249330	OR249601	OR249872
GUN212	The Eden Project, Cornwall, England	WSYP000613–614	G1	*G. tinctoria*	OR249060	OR249331	OR249602	OR249873
GUN213	The Eden Project, Cornwall, England	WSYP000615	G1	*G. tinctoria*	OR249061	OR249332	OR249603	OR249874
GUN214	The Eden Project, Cornwall, England	WSYP000616	G1	*G. tinctoria*	OR249062	OR249333	OR249604	OR249875
GUN215	The Eden Project, Cornwall, England	WSYP000617	G2	*G. tinctoria*	OR249063	OR249334	OR249605	OR249876
GUN216	The Eden Project, Cornwall, England	WSYP000618	G2	*G. tinctoria*	OR249064	OR249335	OR249606	OR249877
GUN217	The Eden Project, Cornwall, England	WSYP000619	G1	*G. tinctoria*	OR249065	OR249336	OR249607	OR249878
GUN218	The Eden Project, Cornwall, England	WSYP000620	G1	*G. tinctoria*	OR249066	OR249337	OR249608	OR249879
GUN219	The Eden Project, Cornwall, England	WSYP000621	G1	*G. tinctoria*	OR249067	OR249338	OR249609	OR249880
GUN220	The Eden Project, Cornwall, England	WSYP000622	G1	*G. tinctoria*	OR249068	OR249339	OR249610	OR249881
GUN221	The Eden Project, Cornwall, England	WSYP000623	G1	*G. tinctoria*	OR249069	OR249340	OR249611	OR249882
GUN222	The Eden Project, Cornwall, England	WSYP000624	G1	*G. tinctoria*	OR249070	OR249341	OR249612	OR249883
GUN223	The Eden Project, Cornwall, England	WSYP000625	G1	*G. tinctoria*	OR249071	OR249342	OR249613	OR249884
GUN224	The Eden Project, Cornwall, England	WSYP000626	G1	*G. tinctoria*	OR249072	OR249343	OR249614	OR249885
GUN225	The Eden Project, Cornwall, England	WSYP000627	G1	*G. tinctoria*	OR249073	OR249344	OR249615	OR249886
GUN226	The Eden Project, Cornwall, England	WSYP000628	G1	*G. tinctoria*	OR249074	OR249345	OR249616	OR249887
GUN227	The Eden Project, Cornwall, England	WSYP000629	G1	*G. tinctoria*	OR249075	OR249346	OR249617	OR249888
GUN228	The Eden Project, Cornwall, England	WSYP000630	G18	*G. tinctoria*	OR249076	OR249347	OR249618	OR249889
GUN229	The Eden Project, Cornwall, England	WSYP000631	G1	*G. tinctoria*	OR249077	OR249348	OR249619	OR249890
GUN230	The Eden Project, Cornwall, England	WSYP000632	G1	*G. tinctoria*	OR249078	OR249349	OR249620	OR249891
GUN231	The Eden Project, Cornwall, England	WSYP000633	G1	*G. tinctoria*	OR249079	OR249350	OR249621	OR249892
GUN232	The Eden Project, Cornwall, England	WSYP000634	G1	*G. tinctoria*	OR249080	OR249351	OR249622	OR249893
GUN233	The Eden Project, Cornwall, England	WSYP000635	G1	*G. tinctoria*	OR249081	OR249352	OR249623	OR249894
GUN234	The Eden Project, Cornwall, England	WSYP000636	G1	*G. tinctoria*	OR249082	OR249353	OR249624	OR249895
GUN235	The Eden Project, Cornwall, England	WSYP000637	G1	*G. tinctoria*	OR249083	OR249354	OR249625	OR249896
GUN236	The Eden Project, Cornwall, England	WSYP000638	G1	*G. tinctoria*	OR249084	OR249355	OR249626	OR249897
GUN237	The Eden Project, Cornwall, England	WSYP000640	G1	*G. tinctoria*	OR249085	OR249356	OR249627	OR249898
GUN238	The Eden Project, Cornwall, England	WSYP000641	G1	*G. tinctoria*	OR249086	OR249357	OR249628	OR249899
GUN239	The Eden Project, Cornwall, England	WSYP000642	G1	*G. tinctoria*	OR249087	OR249358	OR249629	OR249900
GUN240	The Eden Project, Cornwall, England	WSYP000643–644	G1	*G. tinctoria*	OR249088	OR249359	OR249630	OR249901
GUN241	The Eden Project, Cornwall, England	WSYP000645	G1	*G. tinctoria*	OR249089	OR249360	OR249631	OR249902
GUN242	The Eden Project, Cornwall, England	WSYP000646	G1	*G. tinctoria*	OR249090	OR249361	OR249632	OR249903
GUN243	The Eden Project, Cornwall, England	WSYP000647	G1	*G. tinctoria*	OR249091	OR249362	OR249633	OR249904
GUN245	The Eden Project, Cornwall, England	WSYP000650	G18	*G. tinctoria*	OR249092	OR249363	OR249634	OR249905
GUN246	The Eden Project, Cornwall, England	WSYP000651	G9	*G.* × *cryptica*	OR249093	OR249364	OR249635	OR249906
GUN247	The Eden Project, Cornwall, England	WSYP000652	G1	*G. tinctoria*	OR249094	OR249365	OR249636	OR249907
GUN248	The Eden Project, Cornwall, England	WSYP000653	G1	*G. tinctoria*	OR249095	OR249366	OR249637	OR249908
GUN249	The Eden Project, Cornwall, England	WSYP000654	G1	*G. tinctoria*	OR249096	OR249367	OR249638	OR249909
GUN250	The Eden Project, Cornwall, England	WSYP000655	G1	*G. tinctoria*	OR249097	OR249368	OR249639	OR249910
GUN251	The Eden Project, Cornwall, England	WSYP000656	G1	*G. tinctoria*	OR249098	OR249369	OR249640	OR249911
GUN252	The Eden Project, Cornwall, England	WSYP000657	G19	*G. tinctoria*	OR249099	OR249370	OR249641	OR249912
GUN253	The Eden Project, Cornwall, England	WSYP000658	G18	*G. tinctoria*	OR249100	OR249371	OR249642	OR249913
GUN267	Caerhays Estate, Cornwall, England	WSYP000457–464	G6	*G.* × *cryptica*	OR249101	OR249372	OR249643	OR249914

Sample code	Source details	Voucher location/barcode	Genotype	Taxon name	GenBank accession number			
					matK	***psbD-trnT***	***CYC*-like 1**	***CYC*-like 2**
GUN268	Caerhays Estate, Cornwall, England	WSYP000465–475	G6	*G.* × *cryptica*	OR249102	OR249373	OR249644	OR249915
GUN269	Caerhays Estate, Cornwall, England	WSYP000476–484	G6	*G.* × *cryptica*	OR249103	OR249374	OR249645	OR249916
GUN270	Plantentuin Meise, Belgium, Acc. No. 19580470	WSYP000553–562	G6	*G.* × *cryptica*	OR249104	OR249375	OR249646	OR249917
GUN271	Plantentuin Meise, Belgium, Acc. No 19960009-28	WSYP000563–571	G1	*G. tinctoria*	OR249105	OR249376	OR249647	OR249918
GUN272	Jersey	—	G9	*G.* × *cryptica*	OR249106	OR249377	OR249648	OR249919
GUN273	Cornwall, England	WSYP00439–443	G6	*G.* × *cryptica*	OR249107	OR249378	OR249649	OR249920
GUN277	Arboretum Kalmthout, Belgium, Acc. No. 00007452A	WSYP000572–574	G1	*G. tinctoria*	OR249108	OR249379	OR249650	OR249921
GUN278	Arboretum Kalmthout, Belgium, Acc. No. 19960051A	WSYP000575–577	G6	*G.* × *cryptica*	OR249109	OR249380	OR249651	OR249922
GUN279	Arboretum Kalmthout, Belgium, Acc. No. 19880452A	WSYP000578–582	G6	*G.* × *cryptica*	OR249110	OR249381	OR249652	OR249923
GUN280	Arboretum Kalmthout, Belgium, Acc. No. 00007614A	WSYP000583–585	G1	*G. tinctoria*	OR249111	OR249382	OR249653	OR249924
GUN284	Arboretum Bokrijk, Belgium	—	G8	*G.* × *cryptica*	OR249112	OR249383	OR249654	OR249925
GUN285	Arboretum Bokrijk, Belgium	—	G13	*G.* × *cryptica*	OR249113	OR249384	OR249655	OR249926
GUN286	Arboretum Bokrijk, Belgium	—	G7	*G.* × *cryptica*	OR249114	OR249385	OR249656	OR249927
GUN293	Parque Katalapi, Chile	—	G4	*G. tinctoria*	OR249115	OR249386	OR249657	OR249928
GUN294	Parque Aiken, Chile	—	G2	*G. tinctoria*	OR249116	OR249387	OR249658	OR249929
GUN295	Parque Aiken, Chile	—	G2	*G. tinctoria*	OR249117	OR249388	OR249659	OR249930
GUN296	Region de Los Lagos, Chile	WSYP000588	G3	*G. tinctoria*	OR249118	OR249389	OR249660	OR249931
GUN297	Region de Los Lagos, Chile	WSYP000588	G4	*G. tinctoria*	OR249119	OR249390	OR249661	OR249932
GUN298	Region de Los Lagos, Chile	WSYP000588	G4	*G. tinctoria*	OR249120	OR249391	OR249662	OR249933
GUN299	Region de Los Lagos, Chile	WSYP000588	G2	*G. tinctoria*	OR249121	OR249392	OR249663	OR249934
GUN300	Region de Los Lagos, Chile	WSYP000588	G3	*G. tinctoria*	OR249122	OR249393	OR249664	OR249935
GUN301	Region de Los Lagos, Chile	WSYP000588	G4	*G. tinctoria*	OR249123	OR249394	OR249665	OR249936
GUN302	Region de Los Lagos, Chile	WSYP000588	G3	*G. tinctoria*	OR249124	OR249395	OR249666	OR249937
GUN303	Region de Los Lagos, Chile	WSYP000588	G4	*G. tinctoria*	OR249125	OR249396	OR249667	OR249938
GUN304	Region de Los Lagos, Chile	WSYP000588	G2	*G. tinctoria*	OR249126	OR249397	OR249668	OR249939
GUN305	Region de Los Lagos, Chile	WSYP000588	G2	*G. tinctoria*	OR249127	OR249398	OR249669	OR249940
GUN306	Region de Los Lagos, Chile	WSYP000588	G3	*G. tinctoria*	OR249128	OR249399	OR249670	OR249941
GUN307	Region de Los Lagos, Chile	WSYP000586–587	G2	*G. tinctoria*	OR249129	OR249400	OR249671	OR249942
GUN308	Region de Los Lagos, Chile	WSYP000586–587	G4	*G. tinctoria*	OR249130	OR249401	OR249672	OR249943
GUN309	Region de Los Lagos, Chile	WSYP000586–587	G2	*G. tinctoria*	OR249131	OR249402	OR249673	OR249944
GUN310	Region de Los Lagos, Chile	WSYP000586–587	G2	*G. tinctoria*	OR249132	OR249403	OR249674	OR249945
GUN311	Region de Los Lagos, Chile	WSYP000586–587	G4	*G. tinctoria*	OR249133	OR249404	OR249675	OR249946
GUN312	Region de Los Lagos, Chile	WSYP000586–587	G2	*G. tinctoria*	OR249134	OR249405	OR249676	OR249947
GUN313	Region de Los Lagos, Chile	WSYP000586–587	G2	*G. tinctoria*	OR249135	OR249406	OR249677	OR249948
GUN314	Region de Los Lagos, Chile	WSYP000586–587	G2	*G. tinctoria*	OR249136	OR249407	OR249678	OR249949
GUN315	Region de Los Lagos, Chile	WSYP000586–587	G2	*G. tinctoria*	OR249137	OR249408	OR249679	OR249950
GUN316	Region de Los Lagos, Chile	WSYP000586–587	G2	*G. tinctoria*	OR249138	OR249409	OR249680	OR249951
GUN317	Rio Grando do Sul, Brazil	HTL	G16	*G. manicata*	OR249139	OR249410	OR249681	OR249952
GUN318	Rio Grando do Sul, Brazil	HTL	G16	*G. manicata*	OR249140	OR249411	OR249682	OR249953
GUN319	Rio Grando do Sul, Brazil	HTL	G16	*G. manicata*	OR249141	OR249412	OR249683	OR249954
GUN320	Serra do Faxinal, Brazil	HTL	G16	*G. manicata*	OR249142	OR249413	OR249684	OR249955
GUN321	Serra do Faxinal, Brazil	HTL	G16	*G. manicata*	OR249143	OR249414	OR249685	OR249956
GUN322	Santa Catarina, Brazil	HTL	G17	*G. manicata*	OR249144	OR249415	OR249686	OR249957
GUN323	Tregrehan Garden, Cornwall, England	WSYP000589–591	N/A	*G. insignis*	OR249145	OR249416	OR249687	OR249958

Appendix 2 Pairwise sequence variation of cpDNA haplotypes indicating the number of base pair and indel differences between cpDNA haplotypes. Haplotype numbers correspond to those found in Fig. 2. SNPs = single nucleotide polymorphisms. Numbers in parentheses indicate base pair length of indels.

	H1	**H2**	**H3**	**H4**	**H5**
H1					
H2	3 SNPs + indel (2 bp)				
H3	6 SNPs + indel (7 bp)	3 SNPs + indel (7 bp)			
H4	5 SNPs + indels (2, 7 bp)	4 SNPs + indel (7 bp)	1 SNP		
H5	5 SNPs + indels (2, 7 bp)	4 SNPs + indel (7 bp)	3 SNPs	4 SNPs	
H6	4 SNPs + indels (8, 8 bp)	5 SNPs + indels (6, 8 bp)	6 SNPs + indels (6, 7, 8 bp)	7 SNPs + indels (6, 7, 8 bp)	7 SNPs + indels (6, 7, 8 bp)