SUPPORTING INFORMATION

Dense infraspecific sampling reveals rapid and independent trajectories of plastome degradation in a heterotrophic orchid complex

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Notes S1. A. Members of the *Corallorhiza striata* complex. From left to right: *C. striata* var. *striata* (Montana, USA); *C. striata* var. *vreelandii* (Arizona, USA); *C. striata* [Sierra Nevada, California, USA]; *C. bentleyi* (Virginia, USA); *C. involuta* (Morelos, México). **B**. List of accessions sequenced in this study. 'Taxon' follows Barrett & Freudenstein (2011). 'St. Dev. coverage (×)' = standard deviation of coverage depth as assessed in GENEIOUS; '% plastid reads' = normalized percentage of plastid reads for each accession mapping to reference; 'Incertae sedis' indicates unknown taxonomic affinities of coastal CA and western Oregonian samples; * = that accession was sequenced by genome skimming and sequence capture; [1] = accessions excluded due to low coverage. **C**. Source citations and GenBank accession numbers for the four-gene dataset used in divergence time estimation. **D**. Fossils used to calibrate divergence time estimates. **E**. Numbers of polymorphic sites and nucleotide diversity (π) within each taxon of the *C. striata* complex, and average number of differences between each taxon. **F**. Divergence time estimates and 95% HPD for the four-gene orchid dataset, focusing on Calypsoinae and *Corallorhiza*, based on four genes and fossil calibrations described above in D. **G**. Minimum spanning haplotype network among plastomes of the *C. striata* complex, constructed in PopART. Numbering along network edges represents the numbers of mutations between haplotypes.

Collections used in this study are presented below in **A**., and voucher specimens were published in two previous studies (Barrett and Freudenstein, 2009; 2011).

Divergence time estimation. We compiled a four-gene, 54-taxon dataset for the purpose of estimating divergence times among species of orchid subtribe Calypsoinae, genus *Corallorhiza*, and specifically the *C. striata* complex. We chose the nuclear Internal Transcribed Spacer (ITS), and the plastid genes *matK*, *rbcL*, and *psaB* based on their completeness in taxon sampling across the group, and based on the fact that each shows sufficient variation for phylogenetic analysis at both high (subfamily-level; Givnish et al., 2015) and low taxonomic levels (genus, species, and infraspecific levels; Freudenstein and Senyo, 2008; Barrett and Freudenstein, 2010; Freudenstein et al., 2017). Sequences were mined from orchid-wide plastome studies (*psaB*; Cameron, 2004; *matK*, *rbcL*, *psaB*; Givnish et al., 2015), and from a recently published study of subtribe Calypsoinae, to which *Corallorhiza* belongs (ITS, *matK*; Freudenstein et al., 2017). Remaining sequences were filled in from newly sequenced material for *Corallorhiza*, and the last remaining gaps were filled by searching in GenBank (mostly for ITS, *psaB*). In a few cases for the latter GenBank searches, if we were not able to find the same species for a particular locus, we used an alternative species of the same genus. No data were available for *psaB* in *Changnienia*, *Cremastra*, *Danxiaorchis*, *Oreorchis*, or *Yunorchis*. GenBank accessions are listed in **C**.

We used RAxML to produce a Maximum Likelihood tree based on a gene-partitioned GTR+GAMMA model, using ten replicate searches for the four-gene matrix. The resulting tree and dataset were used to determine divergence time estimates in BEAST v.2.4.7 (Bouckaert et al., 2014). Substitution models for each dataset were determined in MEGA v.7 (Kumar et al., 2016), under the corrected Akaike Information Criterion (AICc). The model with the lowest AICc score for each of the four datasets was GTR+I+G. We built the BEAST .xml command file in BEAUTI2 (part of the BEAST2 suite of tools). We chose to analyze the combined dataset under a GTR+I+G substitution model, with parameters estimated from the data. We estimated divergence times under the uncorrelated lognormal clock model, which treats each branch as having its own rate drawn from a distribution of discrete rates (Drummond et al., 2006).

Fossil calibrations were chosen based on two recent findings (**C**): *Dendrobium* from New Zealand (Conran et al., 2009), and a recently described member of subfamily Epidendroideae from Baltic amber (Poinar and Rasmussen, 2017). For each, fossils were used as minimum age estimates. We placed lognormal prior distributions on fossil calibration points, using the 'offset' function as a bound on the minimum age of each clade [20 my for the stem node of *Dendrobium* (mean = 22.7 mya, standard deviation = 2); Conran et al., 2009, based on the recommendation of Iles et al., 2015 in a review of monocot fossils; and 40 my as a minimum age for the stem node of Epidendroideae (mean = 48.05, standard deviation = 2) based on Poinar and Rasmussen et al. 2017). The means of the lognormal priors were set based on findings for those clades in Givnish et al. (2015), ± two standard deviations, to cast a wide range for the prior on each calibration node. A Yule speciation model was chosen as in Givnish et al. (2015), in order to be consistent with that study. BEAST was run three times from random starting seeds, for 2.0×10^8 generations of the Markov Chain Monte Carlo sampler, storing trees and parameters every 10^4 generations, and discarding the first 30% of trees/parameters as burn-in. Effective sample sizes of >200 for parameters were checked in TRACER v.1.6 (http://tree.bio.ed.ac.uk/software/tracer) to verify stationarity of each run, and to assess convergence on the same parameter values across independent runs. Post burn-in data were combined in LogCombiner, trees were constructed in TreeAnnotator (both part of the BEAST2 suite of tools), and visualized in FigTree v.1.4.3 (tree.bio.ed.ac.uk/software/figtree), displaying mean divergence estimates and 95% highest posterior densities to account for estimation error (**F**). In addition, branch widths were scaled by thickness based on rates, measured as $\text{substitutions}\cdot\text{site}^{-1}\cdot\text{year}^{-1}$ to assess shifts in substitution rate over time across the tree.

Patterns of plastid genome diversity in the *C. striata* complex. We assessed diversity among the five taxonomic entities of the *C. striata* complex by calculating GTR-corrected p-distances within and between species and varieties in MEGA v.7 (Kumar et al., 2016). We tested correlation of genetic vs. geographic distance (isolation by distance) by constructing pairwise genetic and geographic distance matrices, in MEGA and the Geographic Distance Matrix Generator (http://biodiversityinformatics.amnh.org/open_source/gdmg), respectively. We assessed correlation between these matrices via a Mantel test in GenAlEx v.6.5 (Peakall and Smouse, 2012). A minimum spanning haplotype network was created in PopART (Bandelt et al., 1999; Leigh and Bryant, 2015).

Results are listed in **F** and **G**.

A. Members of the *Corallorhiza striata* complex. From left to right: *C. striata* var. *striata* (Montana, USA); *C. striata* var. *vreelandii* (Arizona, USA); *C. striata* [Sierra Nevada, California, USA]; *C. bentleyi* (Virginia, USA); *C. involuta* (Morelos, México).

B. List of accessions sequenced in this study. 'Taxon' follows Barrett & Freudenstein (2011). 'St. Dev. coverage (×)' = standard deviation of coverage depth as assessed in Geneious; '% plastid reads' = normalized percentage of plastid reads for each accession mapping to reference; 'Incertae sedis' indicates unknown taxonomic affinities of coastal CA and western Oregonian samples; * = that accession was sequenced by genome skimming and sequence capture; [1] = accessions excluded due to low coverage. Voucher specimens for populations of each accession are listed in Barrett and Freudenstein (2009; 2011).

Taxon	Accession	Locality	Mean coverage depth (×)	St. Dev. coverage (×)	% plastid reads (or capture efficiency in sequence capture)	Length (bp)
Genome skimming						
Calypso bulbosa var. americana	349 OR	Josephine Co., Oregon, USA	154.5	52.2	0.98	149,313
Corallorhiza trifida	JVF 2763a MI	Mackinac Co, Michigan, USA	253.2	80.4	2.78	149,376
Corallorhiza bentleyi	JVF 2550 WV	Monroe Co., West Virginia, USA	95	36.8	0.21	124,482
Corallorhiza involuta	237c MEX	Mexico, Mexico	166.4	39.2	1.13	124,433
Corallorhiza striata* var. *striata	120b UT	Cache Co., Utah, USA	163.3	81.5	0.38	141,915
***Corallorhiza striata* Sierra Nevada**	242b CA	Placer Co., California, USA	202.7	38.9	1.33	137,068
Corallorhiza incertae sedis	350b OR	Jackson Co., Oregon, USA	263	45.4	1.55	141,202
Targeted sequence capture						
***Corallorhiza striata* Sierra Nevada**	5a CA	Calaveras Co., California, USA	1762	892.3	46.52	
***Corallorhiza striata* Sierra Nevada**	8 CA	El Dorado Co., California, USA	1041.2	508.9	57.23	
***Corallorhiza striata* Sierra Nevada**	9a CA	Nevada Co., California, USA	2192.2	1199.9	38.27	

***Corallorhiza striata* Sierra Nevada**	13a CA	Tehama Co., California, USA	424.8	216.4	31.79
***Corallorhiza striata* Sierra Nevada**	242b CA*	Placer Co., California, USA	1487.1	736.9	42.54
***Corallorhiza striata* Sierra Nevada**	248 CA	Madera Co., California, USA	921	418.9	28.43
***Corallorhiza striata* Sierra Nevada**	253c CA	Fresno Co., California, USA	1148.3	602.8	36.76
Corallorhiza incertae sedis	312a CA	Santa Cruz Co., California, USA	610.7	317.3	38.68
Corallorhiza incertae sedis	312c CA	Santa Cruz Co., California, USA	736.5	395.6	42.18
Corallorhiza striata* var. *striata	2b MI	Schoolcraft Co., Michigan, USA	816.9	427.9	42.04
Corallorhiza striata* var. *striata	29a OR	Lane Co., Oregon, USA	1062.6	586.5	37.31
Corallorhiza striata* var. *striata	39b WA	Skamania Co., Washington, USA	2853.6	1481.2	31.81
Corallorhiza striata* var. *striata	48a WA	Lewis Co., Washington, USA	2637.1	1256.7	44.08
Corallorhiza striata* var. *striata	DJ68 OR	Wallowa Co., Oregon, USA	569.4	317.4	30.43
Corallorhiza striata* var. *striata	120b UT*	Cache Co., Utah, USA	862.5	459.2	45.31
Corallorhiza striata* var. *striata	125e WY	Lincoln Co., Wyoming, USA	2954.7	1555.1	38.72
Corallorhiza striata* var. *striata	135a WY	Natrona Co., Wyoming, USA	1935.9	1042.6	37.10
Corallorhiza striata* var. *striata	200a MT	Glacier Co., Montana, USA	450.7	217.8	34.44
Corallorhiza striata* var. *striata	206c AB	Bow River, Alberta, Canada	1902.8	1012.9	35.62
Corallorhiza striata* var. *striata	219c BC	Okanagan-Similkameen, British Columbia, Canada	881.3	474.9	36.01
Corallorhiza striata* var. *striata	223c BC	Thompson-Nicola, British Columbia, Canada	1359.1	667.3	32.48
Corallorhiza striata* var. *striata	350b OR*	Jackson Co., Oregon, USA	852.8	460.2	40.08
Corallorhiza striata* var. *striata	HeshA MB	Winnipeg, Manitoba, Canada	1780.7	841.7	42.44
Corallorhiza striata* var. *striata	JH1b MN	Crow Wing Co., Minnesota, USA	1777.6	840.4	53.31
Corallorhiza striata* var. *striata	JH2c WI	Douglas Co., Wisconsin, USA	1072.5	720.9	32.02
Corallorhiza striata* var. *vreelandii	100b NM	Santa Fe Co., New Mexico, USA	1742.3	825.9	61.16

Corallorhiza striata* var. *vreelandii	103b NM	Otero Co., New Mexico, USA	1346.7	218.2	49.77
Corallorhiza striata* var. *vreelandii	110b AZ	Graham Co., Arizona, USA	3961.5	1654.9	64.89
Corallorhiza striata* var. *vreelandii	112c AZ	Pima Co., Arizona, USA	2386.3	979.6	80.41
Corallorhiza striata* var. *vreelandii	113b AZ	Gila Co., Arizona, USA	1612.4	78.4	46.79
Corallorhiza striata* var. *vreelandii	114c UT	Utah Co., Utah, USA	7632.2	2993	48.56
Corallorhiza striata* var. *vreelandii	116c UT	Tooele Co., Utah, USA	1692.3	808.7	48.058
Corallorhiza striata* var. *vreelandii	158b CO	Saguache Co., Colorado, USA	550	323.7	30.00
Corallorhiza striata* var. *vreelandii	163e CO	Ouray Co., Colorado, USA	839.9	421.3	55.82
Corallorhiza striata* var. *vreelandii	229a HID[1]	El Chico, Hidalgo, Mexico	***35.5***	19.4	42.51
Corallorhiza striata* var. *vreelandii	229b HID	El Chico, Hidalgo, Mexico	372	208.9	36.35
Corallorhiza striata* var. *vreelandii	LR5 NL[1]	Lomond River, Newfoundland, Canada	***33.6***	17.8	39.61
Corallorhiza striata* var. *vreelandii	RB3b NL	Corner Brook, Newfoundland, Canada	1807	954.3	45.46
Corallorhiza bentleyi	63a VA	Giles Co., Virginia, USA	600.8	321.4	27.54
Corallorhiza bentleyi	66a VA	Giles Co., Virginia, USA	777.7	420	33.54
Corallorhiza bentleyi	2550 WV*	Monroe Co., West Virginia, USA	854.7	487.6	37.17
Corallorhiza bentleyi	257i VA	Giles Co., Virginia, USA	641.5	341.8	42.00
Corallorhiza bentleyi	G18 VA	Giles Co., Virginia, USA	641	134.1	39.21
Corallorhiza involuta	228aR MOR[1]	Cuernavaca, Morelos, Mexico	***90.7***	50.9	25.88
Corallorhiza involuta	228b MOR	Cuernavaca, Morelos, Mexico	937.8	520.7	28.09
Corallorhiza involuta	237c MEX*	Toluca, Mexico, Mexico	2158.3	1273.5	42.80
Corallorhiza involuta	237cR MEX	Toluca, Mexico, Mexico	242	153.6	24.69
Corallorhiza involuta	237dR MEX	Toluca, Mexico, Mexico	347.8	215.4	21.78
Corallorhiza trifida	2767a MI	Schoolcraft Co., Michigan, USA	2265.2	1144.4	51.55
Corallorhiza trifida	225c BC	Thompson-Nicola, British Columbia, Canada	873.9	447.1	47.61

C. Source citations and GenBank accession numbers for the four-gene dataset used in divergence time estimation.

	matK	**ITS**	***rbcL***	***psaB***
Aa palacea	Givnish et al., 2015	FJ473308	Givnish et al., 2015	Givnish et al., 2015
Angraecum sesquipedale	Givnish et al., 2015	KX669265	Givnish et al., 2015	Givnish et al., 2015
Aplectrum hyemale	Freudenstein et al., 2017	Freudenstein et al., 2017	EU391356	AY380935
Calopogon tuberosus	Givnish et al., 2015	AF273395	AF074119	Givnish et al., 2015
Calypso bulbosa	This study	Freudenstein et al., 2017	Givnish et al., 2015	Givnish et al., 2015
Catasetum integerrinum	Givnish et al., 2015	C. saccatum EU441204	Givnish et al., 2015	Givnish et al., 2015
Cattleya aurantiaca	Givnish et al., 2015	KR149440	Givnish et al., 2015	Givnish et al., 2015
Changnienia amoena	Freudenstein et al., 2017	Freudenstein et al., 2017	KM873666	n/a*
Chloraea gavilu	Givnish et al., 2015	Freudenstein et al., 2017	FR831981	Givnish et al., 2015
Chysis bractescens	Givnish et al., 2015	EF079363	AF074126	Givnish et al., 2015
Codonorchis lessonii	Givnish et al., 2015	AF348005	Givnish et al., 2015	Givnish et al., 2015
Coelia triptera	Freudenstein et al., 2017	Freudenstein et al., 2017	Givnish et al., 2015	Givnish et al., 2015
Coelogyne flaccida	Givnish et al., 2015	AF029855	Givnish et al., 2015	Givnish et al., 2015
Corallorhiza bentleyi	This study	Freudenstein et al., 2017	This study	This study
Corallorhiza bulbosa	Barrett et al., 2014	Freudenstein et al., 2017	Barrett et al., 2014	Barrett et al., 2014
Corallorhiza involuta	This study	Freudenstein et al., 2017	This study	This study
Corallorhiza macrantha	Barrett et al., 2014	Freudenstein et al., 2017	Barrett et al., 2014	Barrett et al., 2014
Corallorhiza maculata maculata	Barrett et al., 2014	Freudenstein et al., 2017	Barrett et al., 2014	Barrett et al., 2014
Corallorhiza maculata mexicana	Barrett et al., 2014	Freudenstein et al., 2017	Barrett et al., 2014	Barrett et al., 2014
Corallorhiza maculata occidentalis	Barrett et al., 2014	Freudenstein et al., 2017	Barrett et al., 2014	Barrett et al., 2014
Corallorhiza mertensiana	Barrett et al., 2014	Freudenstein et al., 2017	Barrett et al., 2014	Barrett et al., 2014
Corallorhiza odontorhiza	Barrett et al., 2014	Freudenstein et al., 2017	Barrett et al., 2014	Barrett et al., 2014
Corallorhiza striata CA	This study	JF319707	This study	This study
Corallorhiza striata striata	This study	Freudenstein et al., 2017	This study	This study
Corallorhiza striata vreelandii	Barrett and Davis, 2012	Freudenstein et al., 2017	Barrett and Davis, 2012	This study

Corallorhiza trifida	Barrett et al., 2014	Freudenstein et al., 2017	Barrett et al., 2014	Barrett et al., 2014
Corallorhiza wisteriana	Barrett et al., 2014	Freudenstein et al., 2017	Barrett et al., 2014	Barrett et al., 2014
Coryanthes macrantha	Givnish et al., 2015	AF239359	Givnish et al., 2015	Givnish et al., 2015
Corycium carnosum	Givnish et al., 2015	AJ000123	Givnish et al., 2015	Givnish et al., 2015
Cremastra appendiculata	Freudenstein et al., 2017	Freudenstein et al., 2017	EU391354	n/a
Cymbidium lancifolium	Givnish et al., 2015	AF470520	Givnish et al., 2015	Givnish et al., 2015
Cyrtopodium paranaense	Givnish et al., 2015	C. saintlegerianum KY988620	Givnish et al., 2015	Givnish et al., 2015
Dactylostalix ringens	Freudenstein et al., 2017	Freudenstein et al., 2017	KM526772	KM526760
Danxiaorchis singchiana	Freudenstein et al., 2017	Freudenstein et al., 2017	JX293187	n/a
Dendrobium heterocarpum	Givnish et al., 2015	JN388593	Givnish et al., 2015	Givnish et al., 2015
Earina autumnalis	Givnish et al., 2015	AF260149	Givnish et al., 2015	Givnish et al., 2015
Eria rosea	Givnish et al., 2015	E. corneri KM025158	Givnish et al., 2015	Givnish et al., 2015
Eulophia petersii	Givnish et al., 2015	KF318906	Givnish et al., 2015	Givnish et al., 2015
Govenia superba	Freudenstein et al., 2017	Freudenstein et al., 2017	AF074175	AY381017
Iris tenax	JQ276413	AF488761	JQ273918	JQ276168
Liparis loeselii	Givnish et al., 2015	EF079387	Givnish et al., 2015	Givnish et al., 2015
Listera cordata	Givnish et al., 2015	KJ023678	Givnish et al., 2015	Givnish et al., 2015
Masdevalia coccinea	Givnish et al., 2015	AF262789	Givnish et al., 2015	Givnish et al., 2015
Maxillaria variabilis	Givnish et al., 2015	KP323349	Givnish et al., 2015	Givnish et al., 2015
Oreorchis patens	Freudenstein et al., 2017	Freudenstein et al., 2017	EU391355	n/a
Phaius tankervillieae	Givnish et al., 2015	KM025161	Givnish et al., 2015	Givnish et al., 2015
Pogonia ophioglossoides	Givnish et al., 2015	EU498161	Givnish et al., 2015	Givnish et al., 2015
Thelymitra cyanea	Givnish et al., 2015	AF348068	Givnish et al., 2015	Givnish et al., 2015
Tipularia discolor	Freudenstein et al., 2017	Freudenstein et al., 2017	AF074234	AY381084
Triphora trianthophora	Givnish et al., 2015	KM598428	Givnish et al., 2015	Givnish et al., 2015
Tropidia polystachya	Givnish et al., 2015	EU490674	Givnish et al., 2015	Givnish et al., 2015
Vanilla planifolia	Givnish et al., 2015	AF391786	Givnish et al., 2015	Givnish et al., 2015

Yunorchis	Freudenstein et al., 2017	Freudenstein et al., 2017	KM526774	n/a
Zygopetalum mackayi	Givnish et al., 2015	AF239322	Givnish et al., 2015	Givnish et al., 2015

*n/a = not available.

D. Fossils used to calibrate divergence time estimates.

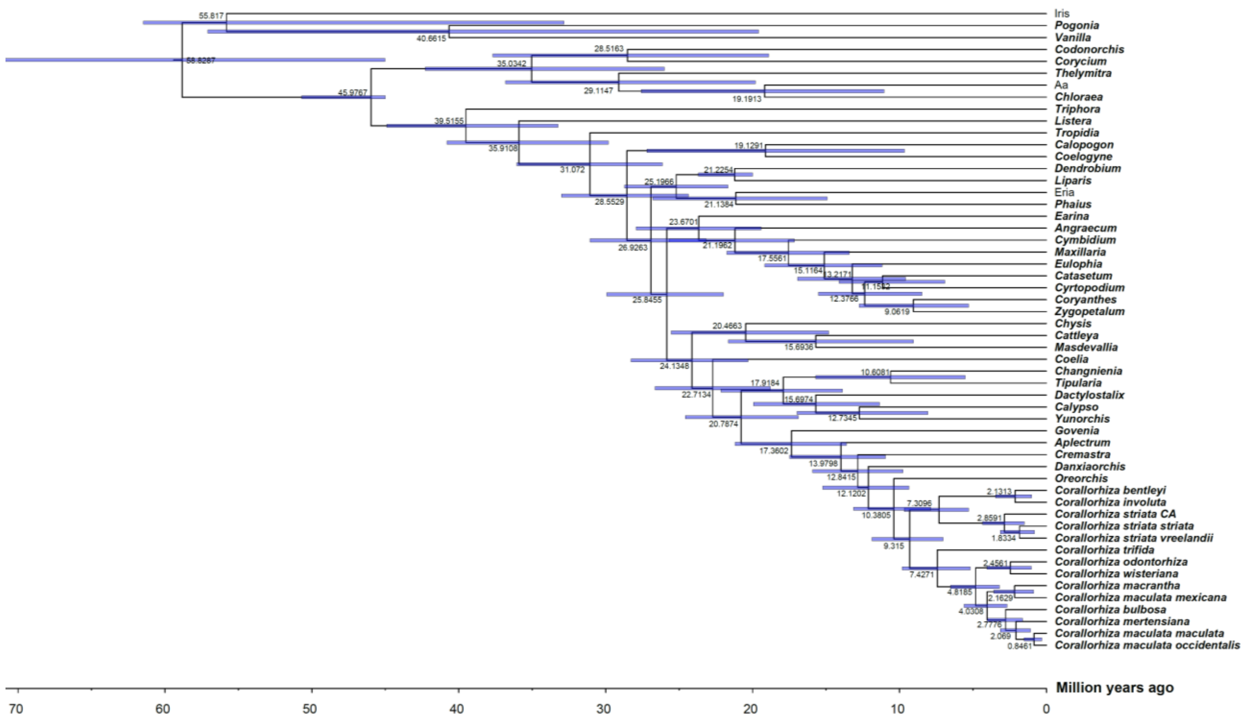
Species	**subfamily**	**Tribe**	**Age range of fossil**	**Minimum age, place in tree**	**Citation**
Succinanthera baltica	Epidendroideae	n/a	40-55 my	40 my for stem node of Epidendroideae	Poinar and Rasmussen, 2017
Dendrobium winikaphyllum	Epidendroideae	Dendrobiinae	20-23 my	20 my for stem node of *Dendrobium*	Conran et al., 2009

E. Numbers of polymorphic sites and nucleotide diversity (π) within each taxon of the *C. striata* complex (top), average number of differences between each taxon (middle), and result of Mantel correlation text (bottom).

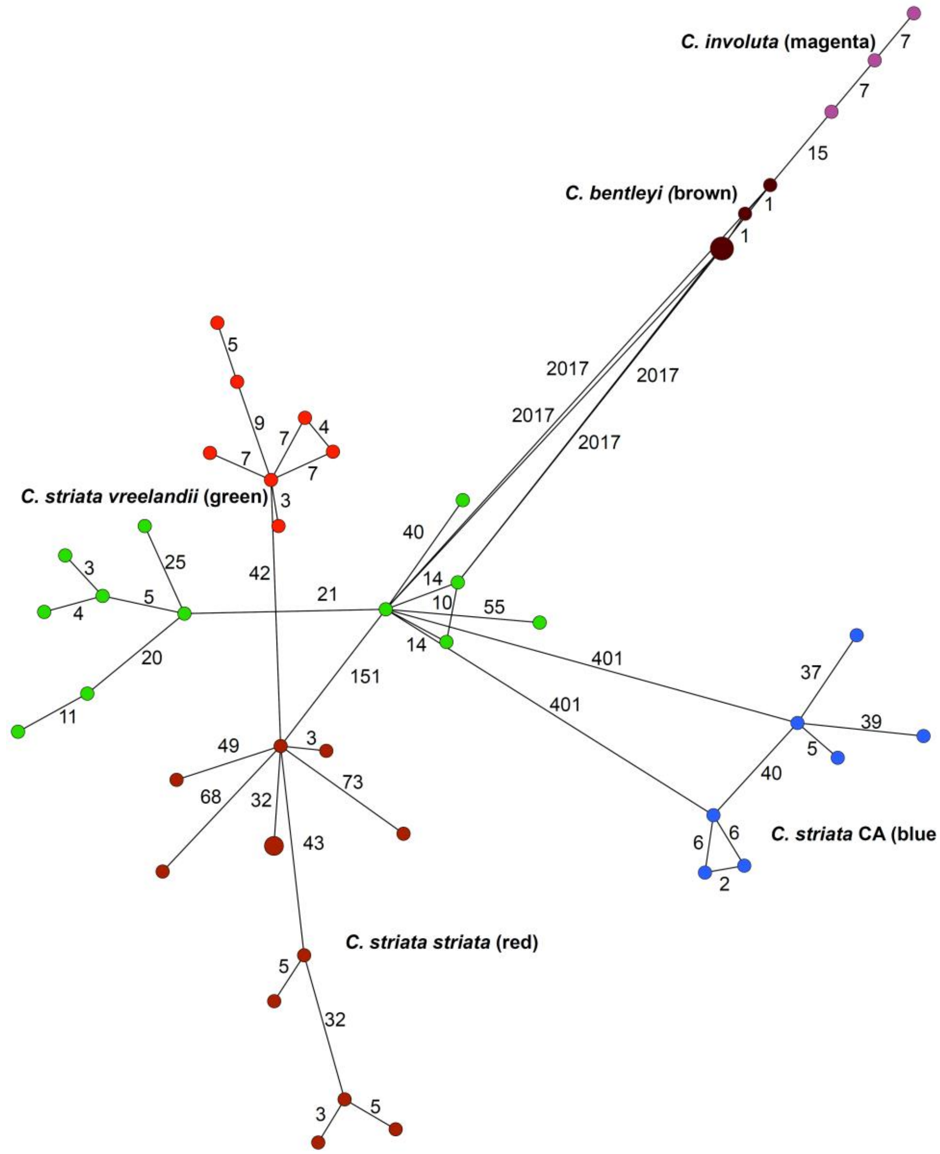
Taxon (# individuals)	**# polymorphic sites**	**nucleotide diversity (π)**
***C. striata* var. *striata* (18)**	364	0.00063
***C. striata* var. *vreelandii* (11)**	192	0.00039
***C. striata Sierra Nevada* (7)**	127	0.00048
***C. bentleyi* (5)**	2	0.00001
***C. involuta* (3)**	17	0.00012

C. striata* var. *striata				
C. striata* var. *vreelandii	222.5			
C. striata Sierra Nevada	620.8	556		
C. bentleyi	2237.9	2110.8	2292.6	
C. involuta	2251.7	2127.8	2405.0	24.2

Mantel	R = 0.54	p = 0.01



F. Divergence time estimates and 95% HPD for the four-gene orchid dataset, focusing on Calypsoinae and *Corallorhiza*, based on four genes and fossil calibrations described above in **D**.



G. Minimum spanning haplotype network among plastomes of the *C. striata* complex, constructed in PopART. Numbering along network edges represents the numbers of mutations between haplotypes.

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