

Supplementary Material

Plastid phylogenomics of the Eriostemon group (Rutaceae; Zanthoxyloideae): support for major clades and investigation of a backbone polytomy

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Table S1. Assembly statistics for plastome sequences of each sample in the genome-skimming dataset.

Taxon	Collector number	Total reads mapped	GC %	Mean read depth
<i>Acronychia laevis</i>	PIF33410	151370	0.368	142.23
<i>Asterolasia asteriscophora</i>	MJB2564	129955	0.372	122.34
<i>Asterolasia drummondii</i>	BJM330	110755	0.38	104.08
<i>Boronia edwardsii</i>	MJB1974	261059	0.421	251.36
<i>Boronia imlayensis</i>	MJB2005	619691	0.38	571.32
<i>Boronia ternata</i>	MJB1931	384624	0.372	364.05
<i>Brombya platynema</i>	PIF34088	254887	0.388	229.72
<i>Chorilaena anceps</i>	BJM475	63507	0.369	60.55
<i>Chorilaena euphemiae</i>	BJM424	681616	0.381	623.56
<i>Chorilaena quercifolia</i>	MJB1954	210902	0.371	200.34
<i>Correa alba</i>	MJB1876	167251	0.374	161.08
<i>Correa glabra</i>	MJB2476	32489	0.378	30.91
<i>Correa lawrenceana</i> var. <i>grampiana</i>	MJB1988	142325	0.372	127.93
<i>Correa lawrenceana</i> var. <i>latrobeana</i>	MJB2567	150816	0.376	140.44
<i>Crowea angustifolia</i> var. <i>platyphylla</i>	MJB1953	131807	0.368	126.12
<i>Crowea exalata</i> subsp. <i>exalata</i>	D Ohlsen s.n.	88272	0.372	82.40
<i>Crowea exalata</i> var. <i>revoluta</i>	MJB1992	176094	0.378	165.71
<i>Crowea saligna</i>	D Ohlsen s.n.	73379	0.376	68.31
<i>Cyanothamnus anemonifolius</i>	MJB2562	11657	0.376	11.22
<i>Diplolaena drummondii</i>	MJB1956	323773	0.369	310.46
<i>Diplolaena obovata</i>	MJB1908	16778	0.368	15.39
<i>Drummondita calida</i>	PIF22556	211112	0.368	194.97
<i>Drummondita fulva</i>	AMarkey6212	69438	0.372	64.41
<i>Drummondita hassellii</i>	MJB1925	79421	0.376	74.06
<i>Eriostemon australasius</i>	PIF34192	55082	0.367	51.80
<i>Eriostemon banksii</i>	PIF33960	58061	0.368	78.08
<i>Euodia pubifolia</i>	PIF25751	128623	0.373	120.40
<i>Geleznowia verrucosa</i>	MJB1910	85504	0.381	79.10
<i>Geleznowia verrucosa</i>	BM344	222976	0.369	208.93
<i>Halfordia kendack</i>	Sanko3019	37386	0.37	35.12
<i>Halfordia kendack</i>	PIF34073	50147	0.365	47.00
<i>Halfordia kendack</i>	DGF10829	126943	0.368	120.00
<i>Halfordia kendack</i>	PIF34090	187726	0.366	177.91
<i>Halfordia kendack</i>	PIF34580	273385	0.368	258.51
<i>Leionema beckleri</i>	PIF33439	63703	0.369	55.88
<i>Leionema ellipticum</i>	PIF25021	193942	0.37	220.07
<i>Leionema lamprophyllum</i> subsp. <i>obovatum</i>	MJB2563	253789	0.373	241.16
<i>Leionema rotundifolium</i>	PIF34469	48048	0.366	45.57
<i>Medicosma cunninghamii</i>	PIF33501	103895	0.369	96.70
<i>Melicope hayesii</i>	PIF36183	67494	0.368	63.32
<i>Muiriantha hassellii</i>	BJM474	574565	0.379	539.37
<i>Myrtopsis</i> sp.	Munz3458	107718	0.385	92.87
<i>Nematolepis phebaliooides</i>	AMarkey6215	218082	0.369	204.36
<i>Nematolepis squamea</i>	PIF34811	32152	0.37	33.13
<i>Nematolepis wilsonii</i>	MJB2568	48693	0.374	47.48

Taxon	Collector number	Total reads mapped	GC %	Mean read depth
<i>Neobyrnesia suberosa</i>	MJB1904	179925	0.373	170.12
<i>Neoschmidia pallida</i>	PHW3303	146185	0.365	136.47
<i>Phebalium clavatum</i>	BJM398	78434	0.366	74.08
<i>Phebalium elegans</i>	BJM403	33611	0.369	31.11
<i>Phebalium longifolium</i>	PIF25088	56140	0.376	51.69
<i>Phebalium multiflorum</i>	RB1280	25207	0.359	47.73
<i>Phebalium stenophyllum</i>	MJB2560	12155	0.367	14.18
<i>Phebalium tuberculosum</i>	BJM375	576767	0.378	519.30
<i>Phebalium whitei</i>	PIF34467	134112	0.372	126.22
<i>Philotheeca angustifolia</i> subsp. <i>angustifolia</i>	MJB1990	91721	0.374	86.37
<i>Philotheeca difformis</i> subsp. <i>smithiana</i>	MJB s.n.	18519	0.38	17.33
<i>Philotheeca fitzgeraldii</i>	MJB1942	116288	0.375	110.91
<i>Philotheeca gardneri</i>	MJB1949	36539	0.368	34.46
<i>Philotheeca myoporoides</i> subsp. <i>myoporoides</i>	MJB2565	71828	0.374	67.62
<i>Philotheeca nodiflora</i> subsp. <i>lasiocalyx</i>	MJB1962	107475	0.378	99.38
<i>Philotheeca pinoides</i>	MJB11	39589	0.365	35.56
<i>Philotheeca pungens</i>	MJB1872	121381	0.374	115.87
<i>Philotheeca spicata</i>	MJB1907	94192	0.378	92.89
<i>Philotheeca tomentella</i>	MJB1913	248796	0.373	237.79
<i>Philotheeca trachyphylla</i>	MJB1900	16916	0.374	15.78
<i>Picrella glandulosa</i>	MJB2104	92891	0.372	87.34
<i>Zieria arborescens</i> subsp. <i>arborescens</i>	MJB2566	164404	0.376	155.98

Table S2. Best-fit models for under all tested partition schemes.

Scheme		Maximum Likelihood Model (IQ-TREE)	Bayesian Inference Model (MrBayes)
Unpartitioned		TVM+F+R5	GTR+F+I+G4
CDS; non-coding		TVM+F+R3; TVM+F+R5	GTR+F+I+G4; GTR+F+I+G4
Codon position (1+2); codon position (3); non-coding		TVM+F+R2; TVM+F+R3; TVM+F+R5	GTR+F+I+G4; GTR+F+I+G4; GTR+F+I+G4
Amino acid		Q.bird+F+R3	-
Locus	1	accD CDS	TVM+F+R3
	2	accD gene-psaI gene spacer	K3Pu+F+R2
	3	atpA CDS	TIM+F+I+G4
	4	atpA gene-atpF gene spacer	K3Pu+F+G4
	5	atpB CDS	K3Pu+F+R2
	6	atpB gene-rbcL gene spacer	TVM+F+R2
	7	atpE CDS	K3P+G4
	8	atpF CDS	GTR+F+R3
	9	atpF gene-atpH gene spacer	K3Pu+F+R2
	10	atpH CDS	K2P+I
	11	atpH gene-atpI gene spacer	GTR+F+R3
	12	atpI CDS	K3Pu+F+G4
	13	atpI gene-rps2 gene spacer	K3Pu+F+R2
	14	ccsA CDS	TVM+F+R3
	15	ccsA gene-ndhD gene spacer	TVM+F+R2
	16	cemA CDS	TVM+F+R2
	17	cemA gene-petA gene spacer	K3Pu+F+G4
	18	clpP1 CDS	K3P+I
	19	clpP1 gene-psbB gene spacer	K3Pu+F+R3
	20	matK CDS	TVM+F+R3
	21	matK gene-rps16 gene spacer	K3Pu+F+R4
	22	ndhA CDS	TVM+F+R3
	23	ndhB CDS	K3Pu+F+I
	24	ndhB gene-rps7 gene spacer	F81+F
	25	ndhC CDS	TVM+F+R2
	26	ndhC gene-trnV-UAC gene spacer	K3Pu+F+R3
	27	ndhD CDS	GTR+F+R2
	28	ndhD gene-psaC gene spacer	K3Pu+F+R3
	29	ndhE CDS	TVM+F+G4
	30	ndhE gene-ndhG gene spacer	K3Pu+F+G4
	31	ndhG CDS	GTR+F+R2
	32	ndhG gene-ndhI gene spacer	TVM+F+R2
	33	ndhH CDS	TVM+F+R2
	34	ndhH gene-rps15 gene spacer	K3Pu+F+G4
	35	ndhI CDS	TPM3+F+G4
	36	ndhI gene-ndhA gene spacer	K3Pu+F
	37	ndhJ CDS	K3Pu+F+R2
	38	ndhJ gene-ndhK gene spacer	TPM2+F+I
	39	ndhK CDS	TPM3+F+R2
	40	ndhK gene-ndhC gene spacer	TIM+F
	41	petA CDS	K3Pu+F+R2
	42	petA gene-psbJ gene spacer	TVM+F+R3
	43	petB CDS	K3Pu+F+R2
	44	petB gene-petD gene spacer	TVM+F+R2
	45	petD CDS	K3Pu+F+R2
	46	petD gene-rpoA gene spacer	K3Pu+F+R2
	47	petG CDS	HKY+F
	48	petG gene-trnW-CCA gene spacer	K3P+G4
	49	petL CDS	K3P
	50	petL gene-petG gene spacer	K3Pu+F+R2
	51	petN CDS	K2P
	52	petN gene-psbM gene spacer	TVM+F+R3
	53	psaA CDS	K3Pu+F+R2
	54	psaA gene-ycf3 gene spacer	K3Pu+F+R2
	55	psaB CDS	TVM+F+R2
	56	psaB gene-psaA gene spacer	K2P+I
	57	psaC CDS	K2P+R2
	58	psaC gene-ndhE gene spacer	K3Pu+F+G4

59	psaI CDS	HKY+F	-
60	psaI gene-ycf4 gene spacer	K3Pu+F+G4	-
61	psaJ CDS	K2P+G4	-
62	psaJ gene-rpl33 gene spacer	TIM+F+R2	-
63	psbA CDS	HKY+F+I+G4	-
64	psbA gene-trnK-UUU gene spacer	K3Pu+F+R3	-
65	psbB CDS	K3Pu+F+R2	-
66	psbB gene-psbT gene spacer	K3Pu+F+G4	-
67	psbC CDS	K3Pu+F+R2	-
68	psbC gene-trnS-UGA gene spacer	TVMe+R3	-
69	psbD CDS	K3Pu+F+R2	-
70	psbE CDS	K3P+I	-
71	psbE gene-petL gene spacer	TVM+F+R3	-
72	psbF CDS	K3P+I	-
73	psbF gene-psbE gene spacer	K2P+I	-
74	psbH CDS	K2P+G4	-
75	psbH gene-petB gene spacer	K3Pu+F+R2	-
76	psbI CDS	K2P+I	-
77	psbI gene-trnS-GCU gene spacer	K3Pu+F+G4	-
78	psbJ CDS	TIM3+F	-
79	psbJ gene-psbL gene spacer	TPM2+F+G4	-
80	psbK CDS	TPM3+F+G4	-
81	psbK gene-psbI gene spacer	TVM+F+G4	-
82	psbL CDS	HKY+F	-
83	psbL gene-psbF gene spacer	K3P	-
84	psbM CDS	HKY+F+G4	-
85	psbM gene-trnD-GUC gene spacer	TVM+F+R2	-
86	psbN CDS	K2P+G4	-
87	psbN gene-psbH gene spacer	K3Pu+F+G4	-
88	psbT CDS	HKY+F+I	-
89	psbT gene-psbN gene spacer	JC+G4	-
90	psbZ CDS	HKY+F+G4	-
91	rbcL CDS	K3P+R3	-
92	rbcL gene-accD gene spacer	TVM+F+R2	-
93	rpl14 CDS	HKY+F+G4	-
94	rpl14 gene-rpl16 gene spacer	TPM2+F+G4	-
95	rpl16 CDS	K3Pu+F+R2	-
96	rpl16 gene-rps3 gene spacer	K3Pu+F+G4	-
97	rpl2 CDS	K3Pu+F	-
98	rpl2 gene-rpl23 gene spacer	K3P+I	-
99	rpl20 CDS	K3Pu+F+R2	-
100	rpl20 gene-rps12 gene spacer	TIM+F+R3	-
101	rpl22 gene-rps19 gene spacer	JC+I	-
102	rpl23 CDS	F81+F	-
103	rpl23 gene-trnM-CAU/trnI-CAU gene spacer	TPM2+F+R2	-
104	rpl32 CDS	TPM2+F+R2	-
105	rpl32 gene-trnL-UAG gene spacer	TVM+F+G4	-
106	rpl33 CDS	GTR+F+G4	-
107	rpl33 gene-rps18 gene spacer	TVM+F+R2	-
108	rpl36 CDS	K2P+G4	-
109	rpl36 gene-rps8 gene spacer	K3Pu+F+G4	-
110	rpoA CDS	TVM+F+R2	-
111	rpoA gene-rps11 gene spacer	TPM3+F+I	-
112	rpoB CDS	TVM+F+R3	-
113	rpoB gene-trnC-GCA gene spacer	K3Pu+F+R3	-
114	rpoC1 CDS	TVM+F+R3	-
115	rpoC1 gene-rpoB gene spacer	HKY+F+R2	-
116	rpoC2 CDS	TVM+F+R3	-
117	rpoC2 gene-rpoC1 gene spacer	K3P	-
118	rps11 CDS	TIMe+I	-
119	rps11 gene-rpl36 gene spacer	TVM+F+R2	-
120	rps12 CDS	K2P+I	-
121	rps12 gene-clpP1 gene spacer	K3P+G4	-
122	rps14 CDS	K3Pu+F+R2	-
123	rps14 gene-psaB gene spacer	K3P+G4	-
124	rps15 CDS	K3Pu+F+G4	-
125	rps15 gene-ycf1 gene spacer	TIM+F+R2	-
126	rps16 CDS	K3Pu+F+G4	-
127	rps16 gene-trnQ-UUG gene spacer	K3Pu+F+R2	-
128	rps18 CDS	TN+F+R3	-
129	rps18 gene-rpl20 gene spacer	TPM3+F+G4	-
130	rps19 CDS	K3Pu+F+G4	-
131	rps19 gene-rpl2 gene spacer	K3Pu+F+I	-

132	rps2 CDS	K3Pu+F+G4	-
133	rps2 gene-rpoC2 gene spacer	K3Pu+F+G4	-
134	rps3 CDS	TPM3+F+G4	-
135	rps4 CDS	K3Pu+F+G4	-
136	rps4 gene-trnT-UGU gene spacer	TVM+F+R2	-
137	rps7 CDS	K3Pu+F+I	-
138	rps7 gene-trnV-GAC gene spacer	TIM+F+R2	-
139	rps7 gene-trnV-GAC gene spacer 2	K3Pu+F+R2	-
140	rps8 CDS	TVM+F+G4	-
141	rps8 gene-rpl14 gene spacer	K3Pu+F+G4	-
142	rrn16 gene-trnE-UUC/trnI-GAU gene spacer	K3P+R2	-
143	rrn23 gene-rrn4.5 gene spacer	JC	-
144	rrn4.5 gene-rrn5 gene spacer	K2P+R2	-
145	rrn5 gene-trnR-ACG gene spacer	JC+G4	-
146	trnA-UGC gene-rrn23 gene spacer	JC+I	-
147	trnC-GCA gene-petN gene spacer	TVM+F+R2	-
148	trnD-GUC gene-trnY-GUA gene spacer	TVM+F+R2	-
149	trnE-UUC gene-trnT-GGU gene spacer	K3Pu+F+R3	-
150	trnE-UUC/trnI-GAU gene-trnA-UGC gene spacer	JC	-
151	trnF-GAA gene-ndhJ gene spacer	TVM+F+R2	-
152	trnfM-CAU gene-rps14 gene spacer	HKY+F+G4	-
153	trnG-UCC gene-trnR-UCU gene spacer	K3Pu+F+R2	-
154	trnH-GUG gene-psbA gene spacer	TVM+F+R3	-
155	trnL-CAA gene-ndhB gene spacer	K3Pu+F+G4	-
156	trnL-UAA gene-trnF-GAA gene spacer	K3Pu+F+R2	-
157	trnL-UAG gene-ccsA gene spacer	TPM3+F+R2	-
158	trnM-CAU gene-atpE gene spacer	TVM+F+R2	-
159	trnM-CAU/trnI-CAU gene-ycf2 gene spacer	JC	-
160	trnN-GUU gene-ycf1/ycf1-fragment gene spacer	K3Pu+F+G4	-
161	trnP-UGG gene-psaJ gene spacer	K3Pu+F+R3	-
162	trnQ-UUG gene-psbK gene spacer	K3Pu+F+R3	-
163	trnR-ACG gene-trnN-GUU gene spacer	F81+F+R2	-
164	trnR-UCU gene-atpA gene spacer	TIM+F+R2	-
165	trnS-GCU gene-trnG-UCC gene spacer	GTR+F+R2	-
166	trnS-GGA gene-rps4 gene spacer	K3Pu+F+G4	-
167	trnS-UGA gene-psbZ gene spacer	GTR+F+G4	-
168	trnT-GGU gene-psbD gene spacer	TVM+F+R3	-
169	trnT-UGU gene-trnL-UAA gene spacer	TVM+F+R3	-
170	trnV-GAC gene-rrn16 gene spacer	K2P+I	-
171	trnV-UAC gene-trnM-CAU gene spacer	K3Pu+F	-
172	trnW-CCA gene-trnP-UGG gene spacer	K3Pu+F+G4	-
173	trnY-GUA gene-trnE-UUC gene spacer	K3P	-
174	ycf1 CDS	TVM+F+R4	-
175	ycf1/ycf1-fragment gene-rpl32 gene spacer	TVM+F+R3	-
176	ycf15 CDS	F81+F	-
177	ycf15 gene-trnL-CAA gene spacer	K3Pu+F+R2	-
178	ycf2 CDS	K3Pu+F+R2	-
179	ycf2 gene-ycf15 gene spacer	JC	-
180	ycf3 CDS	TPM2+F+R3	-
181	ycf3 gene-trnS-GGA gene spacer	K3Pu+F+R2	-
182	ycf4 CDS	TVM+F+R2	-
183	ycf4 gene-cemA gene spacer	K3Pu+F+R2	-

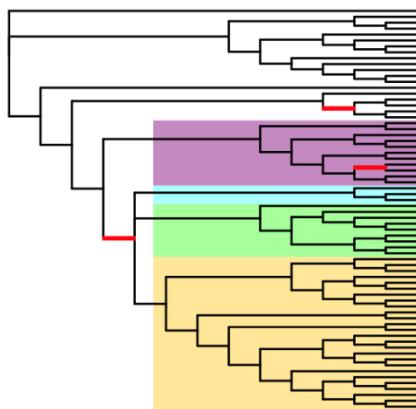
The amino acid and locus schemes were not analysed with Bayesian Inference, and thus no model search was performed. The amino acid scheme was also analysed under a fixed Q.plant model, which produced results consistent with those of the Q.bird model.

Table S4. Results of topology tests on possible resolutions of the polytomy.

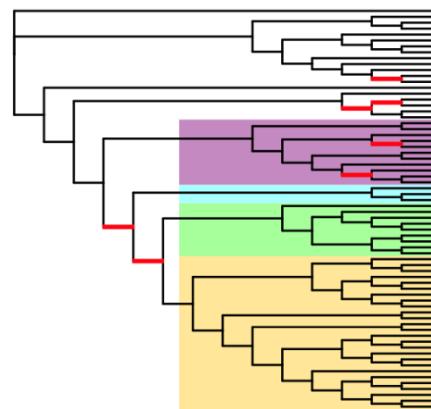
Tree	logL	deltaL	bp-RELL	p-SH	p-WKH	p-WSH	c-ELW	p-AU						
Most likely	-494970.83	1.16E-10	0.13	+	0.934	+	0.639	+	0.163	+	0.764	+		
Hard polytomy	-494975.27	4.446	9.00E-05	-	0.225	+	0.124	+	0.648	+	0.00808	-	0.0218	-
1	-494974.05	3.2185	0.0496	+	0.381	+	0.216	+	0.688	+	0.047	+	0.347	+
2	-494975.18	4.3504	0.00198	-	0.24	+	0.132	+	0.59	+	0.0102	-	0.0701	+
3	-494973.93	3.1049	0.0546	+	0.407	+	0.221	+	0.686	+	0.0486	+	0.334	+
4	-494974.38	3.5497	0.0222	+	0.34	+	0.188	+	0.658	+	0.0283	+	0.191	+
5	-494971.58	0.7469	0.161	+	0.782	+	0.313	+	0.786	+	0.129	+	0.606	+
6	-494974.77	3.9455	0.0096	-	0.287	+	0.161	+	0.64	+	0.0192	+	0.109	+
7	-494975.48	4.6497	0.00076	-	0.207	+	0.0982	+	0.585	+	0.0067	-	0.00179	-
8	-494974.6	3.7669	0.0141	-	0.316	+	0.169	+	0.616	+	0.0215	+	0.119	+
9	-494975.38	4.5493	0.00112	-	0.216	+	0.119	+	0.643	+	0.00739	-	0.0387	-
10	-494971.85	1.0255	0.0809	+	0.746	+	0.255	+	0.719	+	0.0884	+	0.163	+
11	-494974.25	3.418	0.043	+	0.364	+	0.197	+	0.684	+	0.0373	+	0.353	+
12	-494974.89	4.0631	0.00958	-	0.278	+	0.149	+	0.592	+	0.0158	-	0.0444	-
13	-494971.96	1.1339	0.133	+	0.758	+	0.31	+	0.744	+	0.11	+	0.499	+
14	-494972.09	1.2629	0.109	+	0.742	+	0.284	+	0.72	+	0.0953	+	0.441	+
15	-494970.83	0	0.179	+	1	+	0.687	+	0.979	+	0.163	+	0.798	+

“+” and “-” apply to the test in the column to the left. The SH and AU tests return P -values, thus a tree is rejected if $P < 0.05$ (denoted by “-” if rejected, or “+” if accepted). The bp-RELL and c-ELW return posterior weights, and “+” is assigned to those largest values which accumulate above 0.95 (thus forming the confidence set of trees).

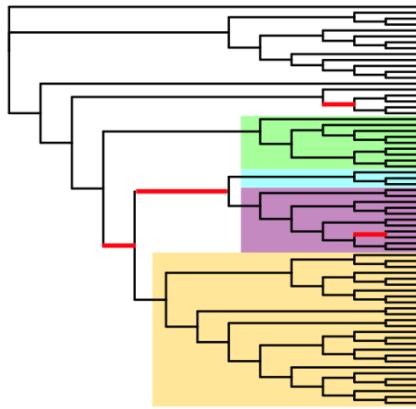
MrBayes 50% majority-rule consensus tree, unpartitioned



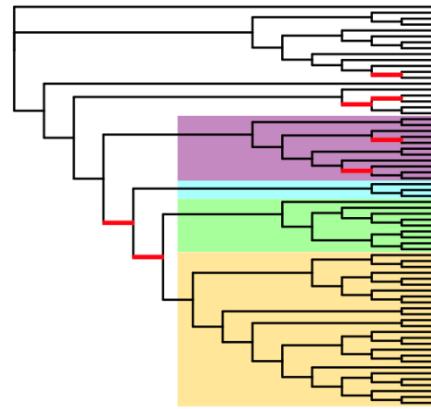
IQ-TREE single most likely tree, unpartitioned



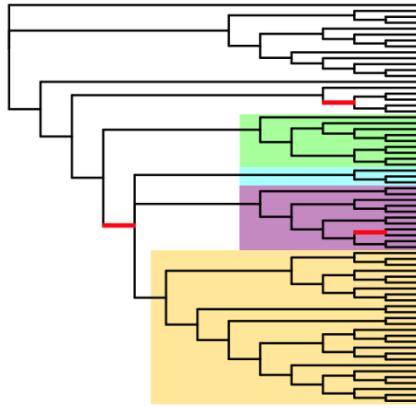
MrBayes 50% majority-rule consensus tree, partitioned by region (CDS, Non-coding)



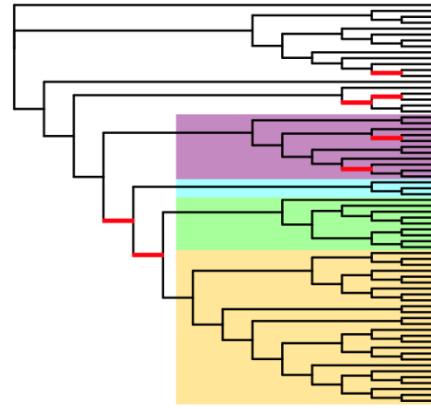
IQ-TREE single most likely tree, partitioned by region (CDS, Non-coding)



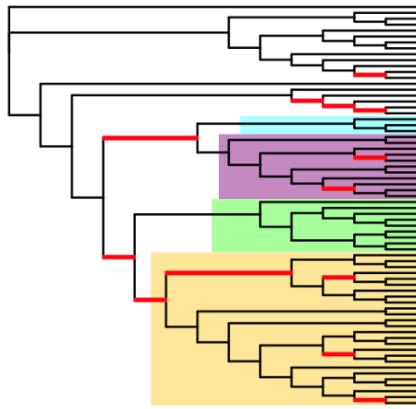
MrBayes 50% majority-rule consensus tree, partitioned by codon position (Codons 1 & 2, Codon 3, Non-coding)



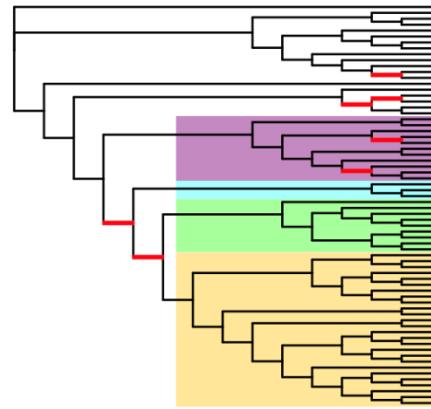
IQ-TREE single most likely tree, partitioned by codon position (Codons 1 & 2, Codon 3, Non-coding)



ASTRAL



IQ-TREE single most likely tree, partitioned by locus



█ *Muiiantha*, *Philotheca* sect. *Cyanochlamys*
█ *Myrtopsis*, *Correa*, *Leionema*
█ *Philotheca* sect. *Eriomena*, *Philotheca* sect. *Philotheca*, *Drummondia*, *Geleznowia*
█ *Crowea*, *Eriostemon*, *Philotheca* sect. *Corynonema*, *Asterolasia*, *Diplorena*, *Nematolepis*, *Chorilaena*, *Phebalium*

Fig. S1. Comparison of phylogenies (as cladograms) produced by various analyses of the basic alignment. Red branches indicate posterior probability < 1 in MrBayes trees, local posterior probability < 1 in the ASTRAL tree and < 95 % UFBoot and SH-aLRT in IQ-TREE trees.

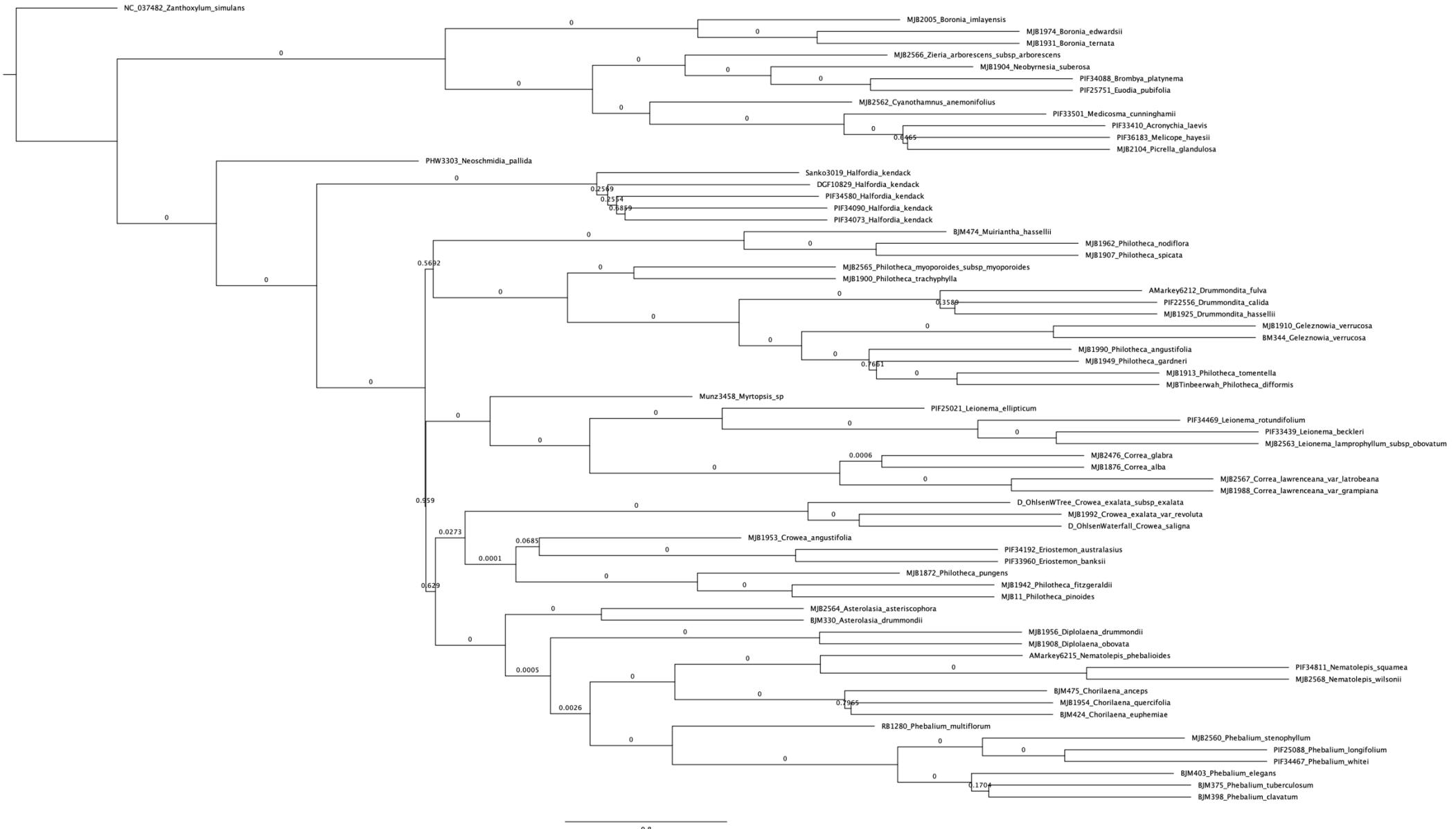


Fig. S2. ASTRAL polytomy test. Branch support values are p-values, where $i > 0.05$ for a given branch corresponds with a failure to reject the null hypothesis that the branch is a true (hard) polytomy.

Cluster 1

Cluster 2

Cluster 3

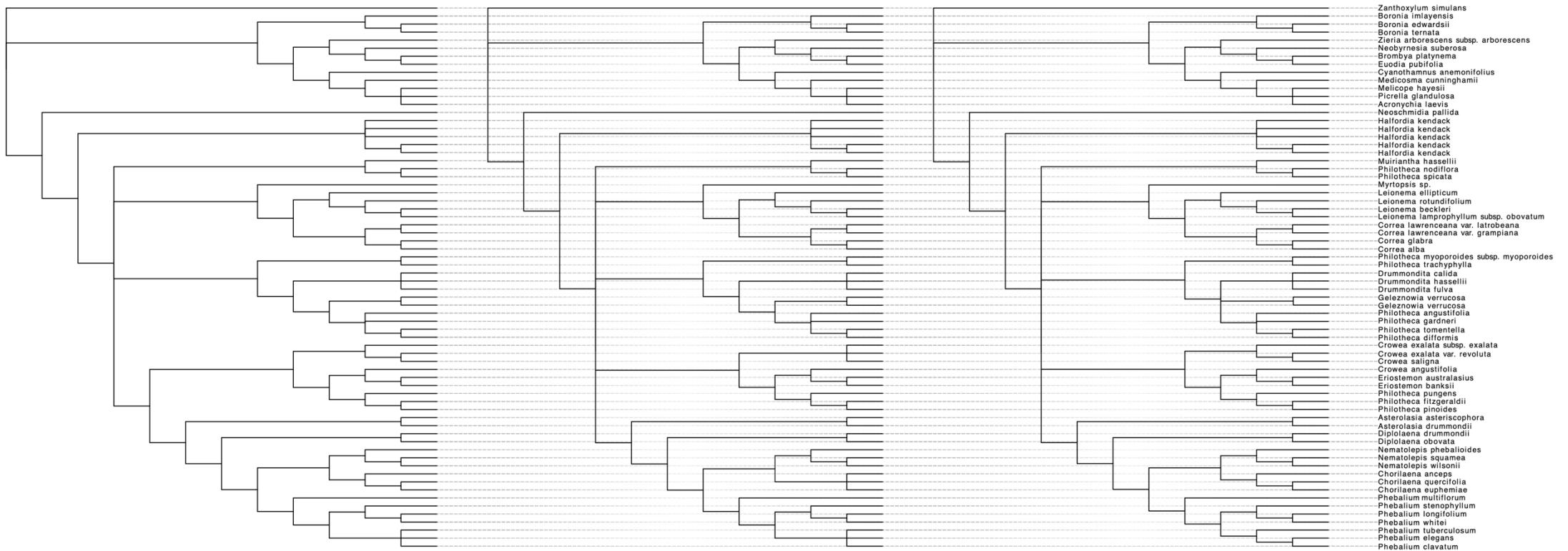


Fig. S3. Comparison of phylogenies produced by IQ-TREE analysis of concatenated genes in Clusters 1, 2 and 3. Branches with less than 95% UFBoot support are collapsed.

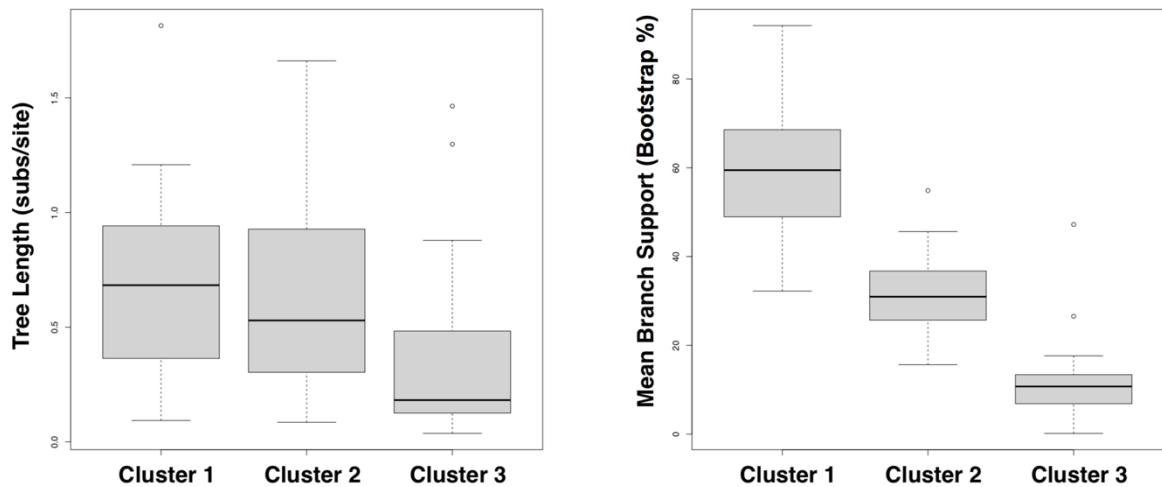


Fig. S4. Boxplots comparing length and branch support of RAxML gene trees across three topology clusters. Horizontal lines represent medians, boxes show interquartile ranges (IQR) and whiskers show the full range excluding outliers (denoted by open circles; these are values that fall outside whiskers, i.e. greater than $1.5 \times \text{IQR} + \text{Q3}$ and less than $1.5 \times \text{IQR} - \text{Q1}$).

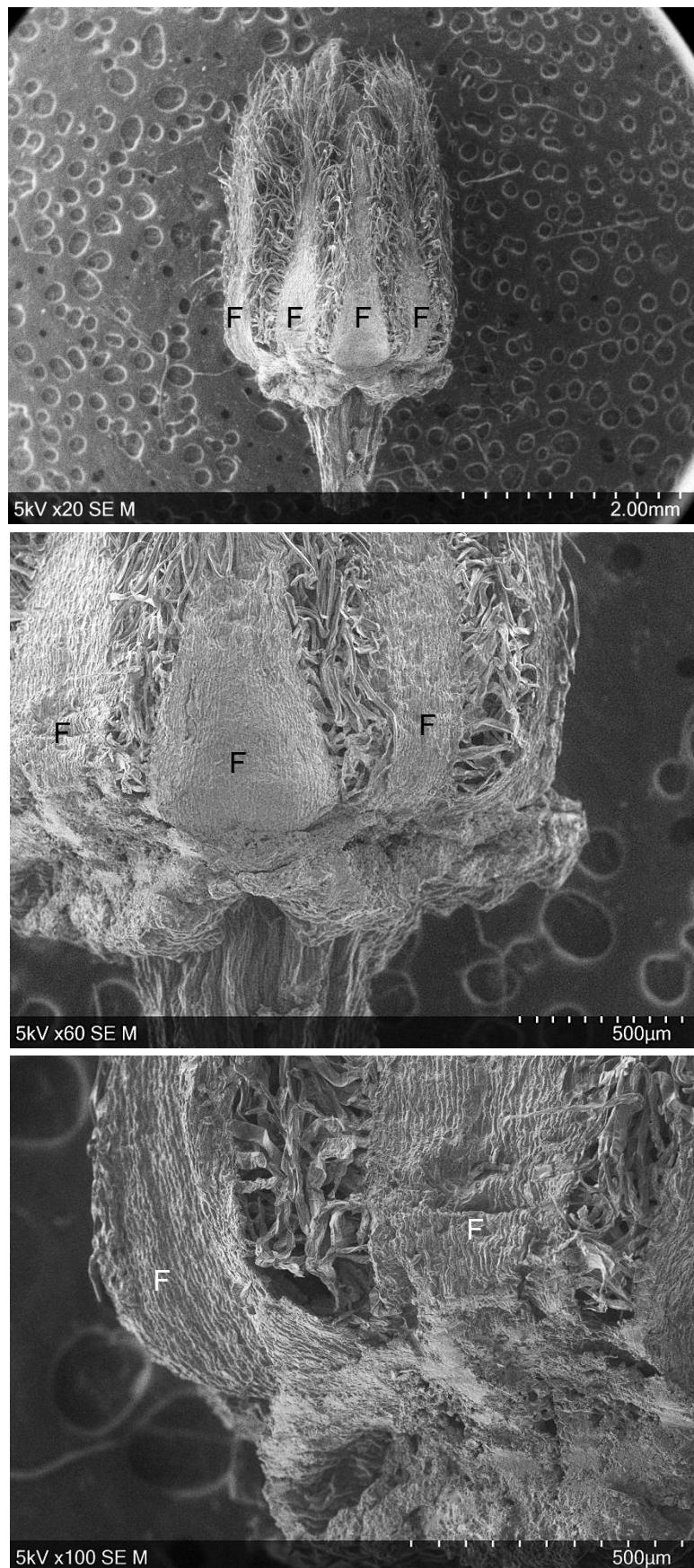


Fig. S5. Scanning Electron Micrographs of staminal filaments in *Philotheca coateana* (K.H. Coate 292; MEL 2278579A) at 20 \times , 60 \times and 100 \times magnification. Images show a mature flower with sepals and petals removed. The filaments (F) are free from each other at their bases.

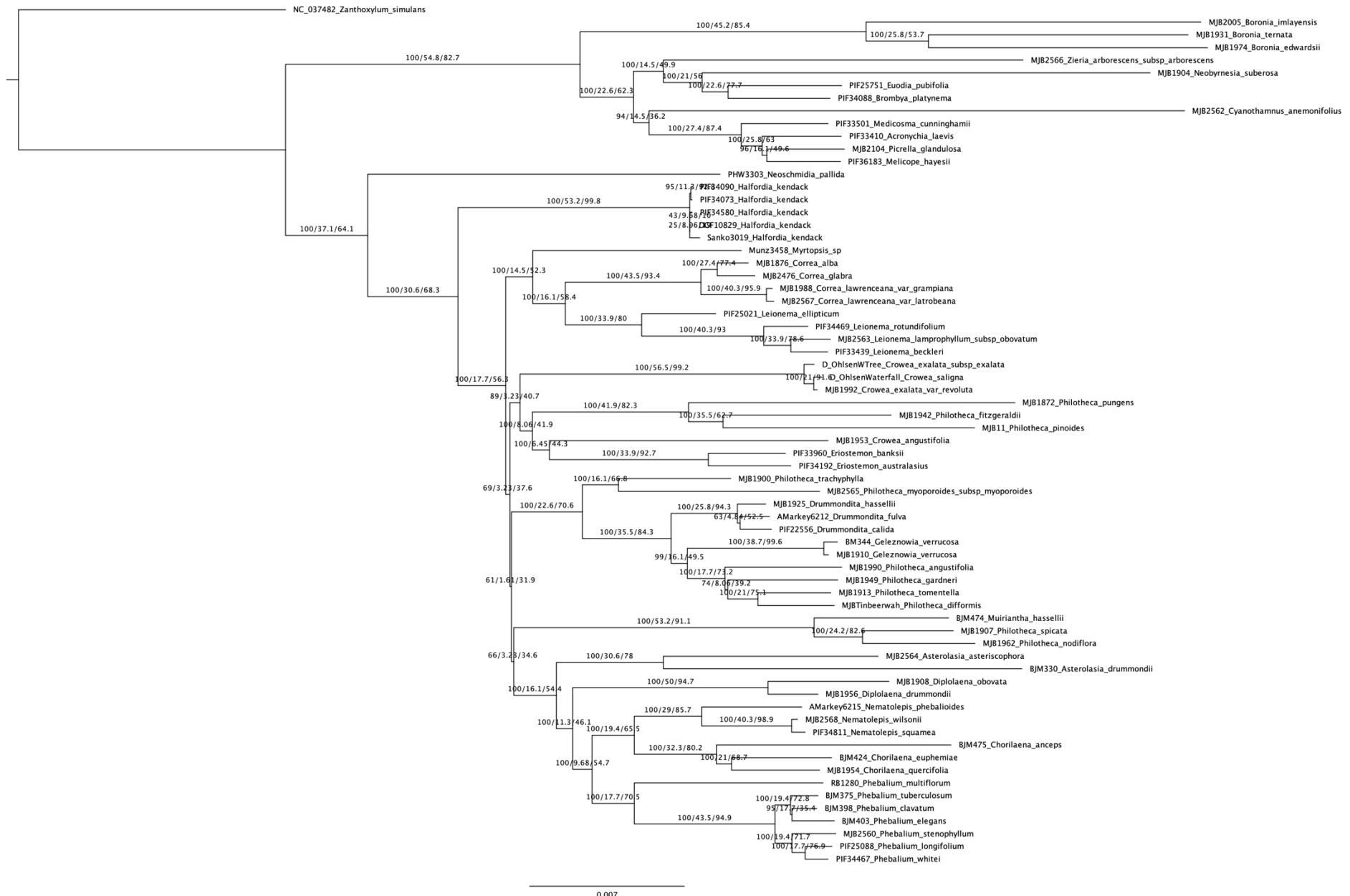


Fig. S6. Phylogeny produced from IQ-TREE analysis of the CDS loci from the basic alignment, translated to amino acids. Branch support is quantified by UFBoot values, gene concordance (gCF) or site concordance (sCF).

Methods and results of analyses of 18S–5.8S–26S nuclear ribosomal cistron sequences from shotgun-sequenced samples

Sequence assembly, editing and alignment

Raw Illumina reads were *de-novo* assembled into contigs with CLC Genomics Workbench (ver. 9.5.1, Qiagen) using default settings. Contigs and raw reads were imported into Geneious Prime (ver. 2021.1.1, see <http://www.geneious.com>) and plastome sequences for all samples were assembled in this software. Initially, the draft cistron sequence of *Acronymia laevis* was constructed using the nrDNA cistron sequence of *Eremophila macdonnellii* (GenBank: MN411575.1) as a reference. Following this, reads were re-mapped to the draft assembly multiple times with manual checking and corrections to produce a final cistron sequence. To assemble the remaining cistron sequences we adopted an approach of using a closely related, already-assembled sequence as the reference for the next assembly in the same manner as that used to assemble plastome sequences (beginning with *A. laevis* cistron sequence). Assemblies were carried out following the same protocol and settings used for the plastome sequences. Sequences were then aligned using MAFFT (ver. 7.450) with default settings, and to reduce the potential for inclusion of non-homologous sites, sites with more than 30% gaps were removed to produce a final alignment 5,810 bp long.

Phylogenetic analyses

The nrDNA sequence alignment was analysed using Maximum Likelihood and Bayesian Inference approaches in IQ-TREE (ver. 2.1.3) and MrBayes (ver. 3.2.7a). For both analyses, the alignment file was partitioned by marker, resulting in a total of five partitions for the markers: 18S rRNA, 5.8S rRNA, 26S rRNA, ITS1, ITS2. Best-fit models were estimated in IQ-TREE using ModelFinder, with models for MrBayes selected using the ‘-mset’ option to restrict model searches to only those implemented in MrBayes. The MrBayes analysis was run for 100,000 generations, with a 25% burn-in and manual checking to ensure that the standard deviation of split frequencies reached below 0.01. We assessed convergence in Tracer (ver. 1.7.2) to ensure effective sample sizes (ESS) were above 200 for all parameters. Support for branches in the IQ-TREE phylogeny was estimated using ultrafast bootstrap (UFBoot) and SH-aLRT with each set to 1000 replicates. Branch support for the MrBayes phylogeny was assessed using posterior probability.

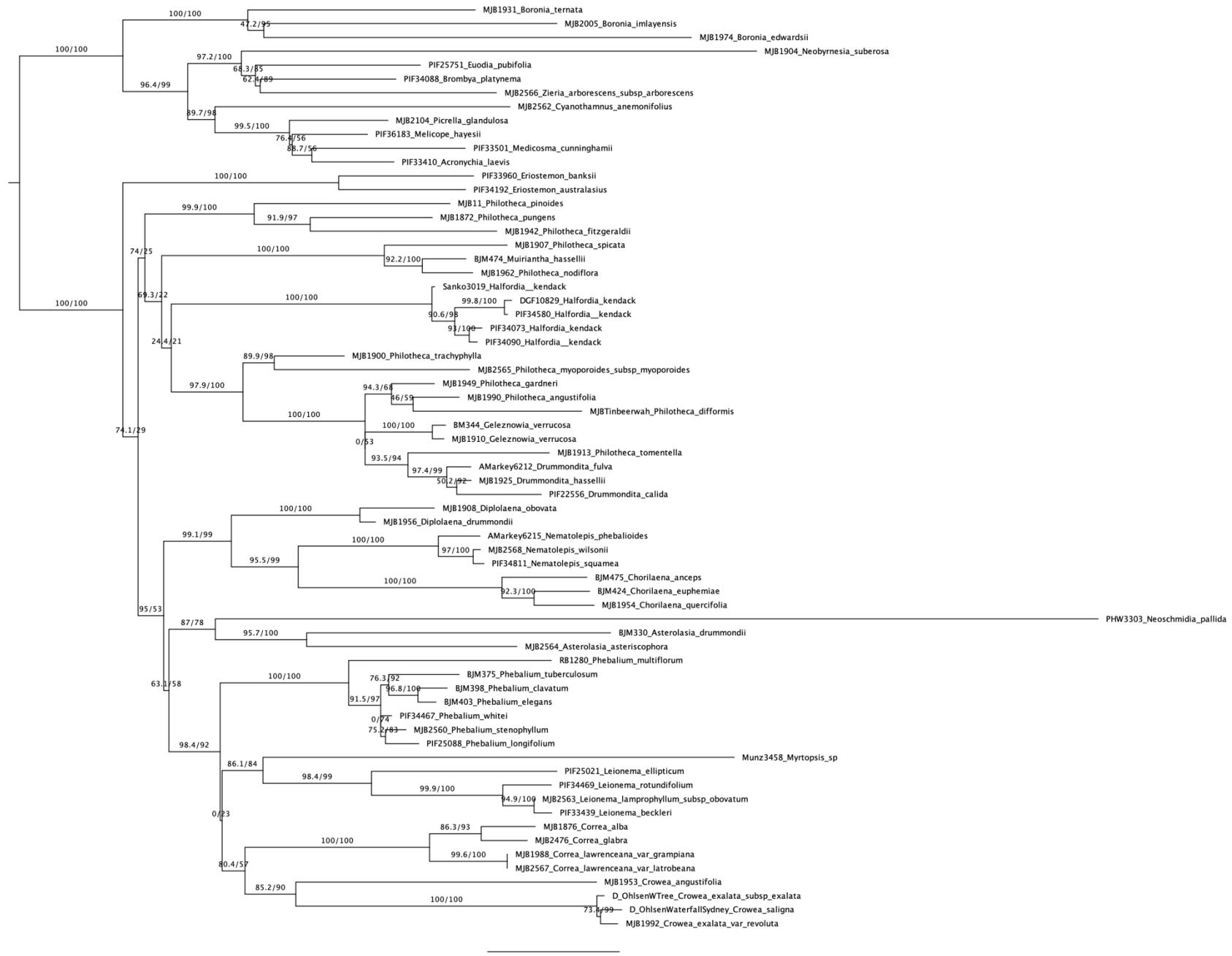


Figure S7. Maximum likelihood phylogeny from IQ-TREE analysis of nrDNA cistron sequences. Branch support values are SH-aLRT/UFBoot.

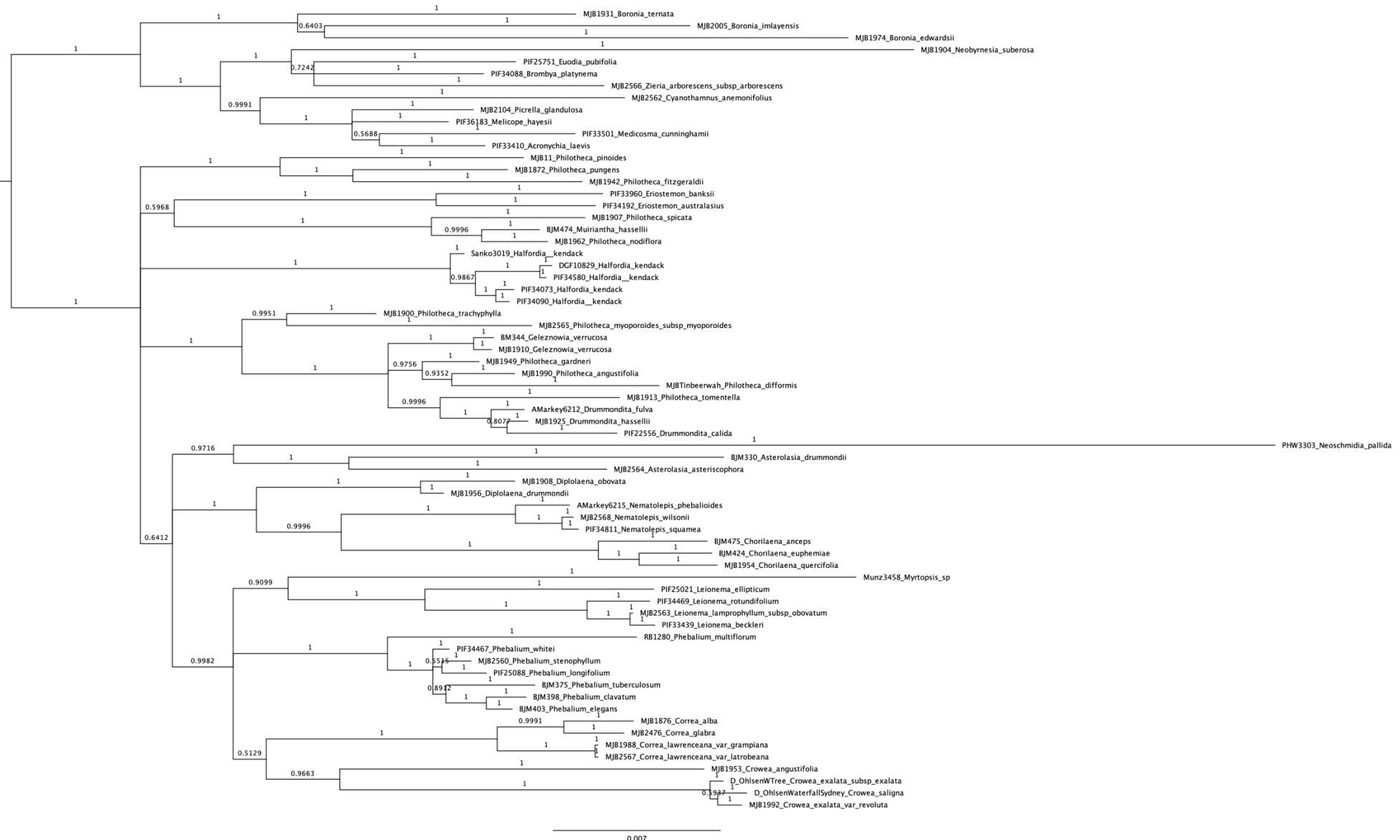


Figure S8. Bayesian inference 50% majority-rule consensus phylogeny from MrBayes analysis of nrDNA cistron sequences. Branch support values are posterior probabilities.