

Supplementary Material

The *Crinum flaccidum* (Amaryllidaceae) species complex in Australia

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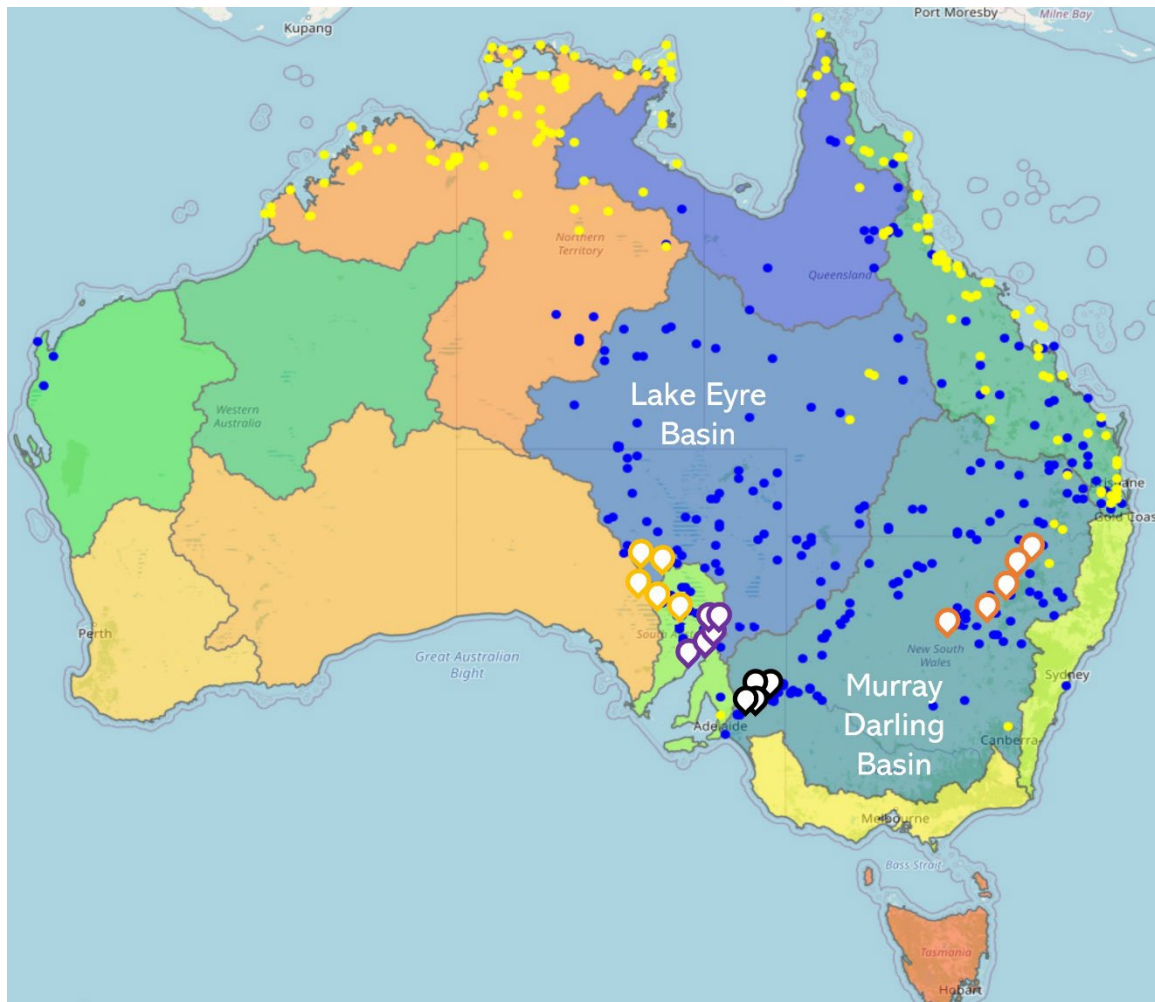


Fig. S1. Sampling locations of *Crinum* in the *C. flaccidum* species complex: *C. luteolum* 'northern' morph (gold); *C. luteolum* 'southern' morph (purple); *C. flaccidum* South Australia River Murray (black); and *C. flaccidum* New South Wales (orange) populations. The small dots are the AVH known localities for *C. flaccidum* (blue), including *C. luteolum*, v. *C. arenarium* (yellow). Major Australian drainage systems are also shown (Data from CHAH, see <https://avh.ala.org.au/>).

Table S1. Sample metadata: sample localities used in molecular and morphological analyses. *Crinum luteolum* and *C. flaccidum* samples are identified by morphotypes. Additional *C. flaccidum* New South Wales samples were obtained from the NCW Beadle Herbarium (NE) and outgroup species from the Botanic Gardens of Adelaide and State Herbarium of South Australia (AD).

Species	Region/morphotype	Code	Collector	Collection number	Latitude	Longitude	Herbarium code	Herbarium accession number	Methodology (DNA,morphology)
<i>Crinum luteolum</i>	Southern	L1	JC Simpson & JG Conran	JGC-4261-A	-32.58327	137.76026	-	-	DNA, morphology
<i>Crinum luteolum</i>	Northern	L2	JC Simpson & JG Conran	JGC 4262-B	-30.438956	137.222715	-	-	DNA, morphology
<i>Crinum luteolum</i>	Northern	L3	JC Simpson & JG Conran	JGC 4264-C	-30.292112	136.947131	-	-	DNA, morphology
<i>Crinum luteolum</i>	Northern	L4	JC Simpson & JG Conran	JGC 4265-D	-31.141832	136.856946	-	-	DNA, morphology
<i>Crinum luteolum</i>	Northern	L5	JC Simpson & JG Conran	JGC 4266-E	-31.411407	136.989633	-	-	DNA, morphology
<i>Crinum luteolum</i>	Northern	L6	JC Simpson & JG Conran	JGC 4267-F	-31.655202	137.155365	-	-	DNA, morphology
<i>Crinum luteolum</i>	Southern	L7	JC Simpson & JG Conran	JGC 4268-G	-32.154454	138.041123	-	-	DNA, morphology
<i>Crinum luteolum</i>	Southern	L8	JC Simpson & JG Conran	JGC 4269-H	-32.189678	138.015731	-	-	DNA, morphology
<i>Crinum luteolum</i>	Southern	L9	JC Simpson & JG Conran	JGC 4270-I	-32.44159	137.977304	-	-	DNA, morphology
<i>Crinum luteolum</i>	Southern	L10	JC Simpson & JG Conran	JGC 4271-J	-32.47624	137.932285	-	-	DNA, morphology
<i>Crinum flaccidum</i>	South Australia	F1	JC Simpson & JG Conran	JGC 4273-K	-34.342902	139.624562	-	-	DNA, morphology
<i>Crinum flaccidum</i>	South Australia	F2	JC Simpson & JG Conran	JGC 4274-L	-34.314114	140.365978	-	-	DNA, morphology
<i>Crinum flaccidum</i>	South Australia	F3	JC Simpson & JG Conran	JGC 4276-M	-34.862212	139.43946	-	-	DNA, morphology

<i>Crinum flaccidum</i>	South Australia	F4	JG Conran	JGC 4277-N	-34.88014	139.517861	-	-	DNA, morphology
<i>Crinum flaccidum</i>	New South Wales	-	RL Palsson	RP241	-28.893527	150.143333	-	-	DNA
<i>Crinum flaccidum</i>	New South Wales	-	RL Palsson	RP263	-28.65702	150.33153	-	-	DNA
<i>Crinum flaccidum</i>	New South Wales	F5	RL Palsson	RP 280	-30.247296	149.885985	NE	110349	DNA, morphology
<i>Crinum flaccidum</i>	New South Wales	F6	RL Palsson	RP 283	-30.726315	149.523018	NE	110350	DNA, morphology
<i>Crinum flaccidum</i>	New South Wales	F7	RL Palsson	RP 303	-30.943353	148.033334	NE	110477	DNA, morphology
<i>Crinum americanum</i>	-	Ameri	ULM	-	-	-	AD	98507166	DNA, morphology
<i>Crinum angustifolium</i> (C. arenarium)	-	Arena	DE Symon	-	-	-	AD	98666518	DNA, morphology
<i>Crinum bulbispermum</i>	-	Bulb	R Hill	-	-	-	AD	99511068	DNA, morphology
<i>Crinum moorei</i>	-	Moor-1	CJ Brodie	-	-34.9488	138.668611	AD	269702	DNA, morphology
<i>Crinum moorei</i>	-	Moor-2	R Swinbourne	-	-	-	AD	99511057	DNA, morphology
<i>Crinum moorei</i>	-	-	CJ Brodie	-	-37.014722	139.739166	AD	242965	DNA
<i>Crinum uniflorum</i>	-	Uni	DE Symon	-	-13.483333	134.516666	AD	98666554	DNA, morphology
<i>Crinum yemenense</i> (C. album)	-	Album	DE Symon	-	-	-	AD	98728017	DNA, morphology
<i>Crinum</i> × <i>powellii</i>	Adelaide Botanic Gardens	-	JG Conran	ADBG G875892	-	-	-	-	DNA
<i>Crinum pedunculatum</i>	Adelaide Botanic Gardens	-	JG Conran	ADBG G875897	-	-	-	-	DNA
<i>Crinum mauritianum</i>	Adelaide Botanic Gardens	-	JG Conran	ADBG G874319	-	-	-	-	DNA
<i>Crinum arenarium</i>	-	-	DE Symon	-	-	-	AD	98666519	DNA
<i>Allium ampeloprasum</i>	-	Al-Amp-1	CJ Brodie	-	-37.683611	140.62777	AD	242217	Morphology
<i>Allium ampeloprasum</i>	-	Al-Amp-2	PJ Lang	-	-37.85694	140.693055	AD	242218	Morphology
<i>Allium ampeloprasum</i>	-	-	DJ Duval	-	-37.628333	140.794444	AD	243653	DNA
<i>Allium neapolitanum</i>	-	Al-Nea-1	B Copley	-	-	-	AD	96647043	Morphology
<i>Allium neapolitanum</i>	-	Al-Nea-2	T Jacques	-	-35.37222	136.89	AD	186822	Morphology

<i>Allium</i>	-	-	DE Murfet	-	-35.525555	138.676666	AD	216581	DNA
<i>neopolitanum</i>	-	-		-					
<i>Amaryllis</i>	-	Am-	CJ Brodie	-			AD	234663	Morphology
<i>belladonna</i>	-	Bel		-					
<i>Amaryllis</i>	-	-	CJ Brodie	-			AD	280651	DNA
<i>belladonna</i>	-			-					
<i>Calostemma</i>	-	C-Abd	PJ Lang	-	-27.0325	132.494444	AD	200210	DNA, morphology
<i>abdicatum</i>	-			-					
<i>Calostemma</i>	-	C-Lut	DE Murfet	-	-30.647222	145.009444	AD	200226	Morphology
<i>luteum</i>	-			-					
<i>Calostemma</i>	-	C-Pur	PJ Lang	-	-34.070555	140.771944	AD	200213	DNA, morphology
<i>purpureum</i> ×	-			-					
<i>luteum</i>	-			-					
<i>Calostemma</i>	-	-	PJ Lang	-	-34.070555	140.771944	AD	200214	DNA
<i>purpureum</i> ×	-			-					
<i>luteum</i>	-			-					
<i>Calostemma</i>	-	-	TS Te	-	-35.538055	138.630277	AD	214055	DNA
<i>purpureum</i>	-			-					
<i>Calostemma</i>	-	-	CJ Brodie	-	-35.018611	138.586111	AD	273808	DNA
<i>purpureum</i>	-			-					

Table S2. Raw morphology data for *Crinum* taxa and outgroup taxa.

Sample	Character															
	F-No	Pedi	F-Tube	F-Sym	F-Shape	F-Col	Peri-L	Peri-W	Pet-No	Peri-Lobe	Anth-C	Fila-C	Fila-L	S-No	S-Fused	Sty-L
L1	13.5	1	1	0	1	2	1.778151	1.308209	6	1	1	2	1.556303	6	0	1.596597
L2	13.5	1	1	0	1	2	?	?	?	?	1	2	1.425969	?	?	1.718778
L3	11	1	1	0	1	2	1.672098	1.185637	6	2	1	2	1.441957	6	0	1.535716
L4	14.5	1	1	0	1	1	1.724276	1.308209	6	2	1	2	1.409369	6	0	1.694605
L5	9	1	1	0	1	2	1.690196	1.213075	6	2	1	2	1.477121	6	0	1.568202
L6	14	1	1	0	1	2	?	?	6	?	1	2	?	?	?	?
L7	17	1	1	0	1	1	1.71321	1.348954	6	3	1	2	1.431364	6	0	1.64836
L8	12.5	1	1	0	1	2	1.69897	1.238882	6	2	1	2	1.486667	6	0	1.64673
L9	14	1	1	0	1	2	1.750765	1.361728	6	2	1	2	1.50515	6	0	1.659599
L10	13	1	1	0	1	2	1.773299	1.271067	6	2	1	2	1.548185	6	0	1.70757
F1	11	1	1	0	1	3	1.876987	1.278754	6	1	1	3	1.70757	6	0	1.816241
F2	11	1	1	0	1	3	1.838849	1.230449	6	1	1	3	1.60206	6	0	1.81068
F3	13.5	1	1	0	1	3	1.745595	1.255273	6	1	1	3	1.6163	6	0	1.80956
F4	?	1	1	0	?	3	?	?	?	?	1	3	?	?	?	?
F5	12	1	1	1	1	3	1.815135	1.217484	6	?	1	4	1.583577	6	0	1.826075
F6	8	1	1	1	1	3	1.701856	?	6	?	1	4	1.568202	6	0	1.556303
F7	6	1	1	1	1	3	1.681241	1.166331	6	1	1	3	1.544068	6	0	1.724276
Yemen	20	1	1	0	1	6	2.064458	1.525045	6	4	2	4	1.922552	3	0	1.963788
Moor-1	?	1	1	0	1	6	1.959041	1.441957	6	1	2	3	1.745595	6	0	1.869232
Bulb	8	1	1	0	1	5	1.915576	1.322219	6	5	3	5	1.822822	6	0	1.816241
Ameri	?	1	1	0	1	3	1.915576	1.079181	6	5	2	4	1.70757	6	0	1.877947
Al-Nea-1	26	1	0	0	2	3	0.954243	0.726999	6	6	4	3	?	6	0	?

Al-Nea-2	30.5	1	0	0	2	3	0.970037	0.753328	6	6	4	3	?	6	0	?
Al-Amp-1	100	1	0	0	2	8	0.778151	0.477121	6	5	3	7	0.753328	6	0	0.90309
Al-Amp-2	100	1	0	0	2	7	0.726999	0.564271	6	5	3	7	0.753328	6	0	0.69897
C-Abd	6.5	1	1	0	3	7	1.355388	0.778151	6	1	1	6	1.255273	6	1	1.290035
C-Lut	20	1	1	0	3	1	1.39211	0.778151	6	1	1	1	1.217484	6	1	1.290035
C-Pur	15	1	1	0	3	5	1.217484	0.726999	6	1	1	6	1.113943	6	1	1.290035
Uni-1	1	0	1	1	1	3	1.750765	0.726999	6	5	?	?	1.531479	6	0	1.653213
Moor-2	5.5	1	1	0	1	3	2.028029	1.656418	6	1	?	?	1.899456	6	0	1.960629
Angus	4	1	1	0	1	3	1.992701	0.801632	6	6	?	?	1.675167	6	0	1.799341
Am-Bel	8.5	1	1	1	3	4	1.880814	1.322219	6	1	2	4	1.701856	6	1	1.826075

Sample	Character							
	Sty-C	Leaf-Shape	Leaf-L	Pedu-L	Leaf-W	Pedi-L	Leaf-Mar	Leaf-Apex
L1	2	1	2.739572	2.824776	1.500602	1.876987	1	1
L2	2	1	2.567026	2.4843	1.30103	1.665894	1	1
L3	2	1	2.577109	2.644439	1.255273	1.890235	1	1
L4	2	?	2.793325	2.716838	1.335792	1.939519	1	?
L5	2	1	2.655779	2.702431	1.20412	1.912045	1	1
L6	2	1	2.669317	2.717671	1.263241	1.869232	1	1
L7	2	1	2.755367	2.91169	1.409369	1.906694	1	?
L8	2	1	2.733465	2.622214	1.355388	1.875061	1	?
L9	2	?	2.470557	2.71265	1.054358	1.845098	1	?
L10	2	?	2.677303	2.781037	1.335792	1.997095	1	1
F1	3	1	2.615248	2.719331	1.579784	2.075547	1	2
F2	4	1	2.364864	2.663701	1.20412	1.954243	1	?
F3	3	?	2.64575	2.731589	1.409369	2.09575	1	?
F4	3	?	?	?	?	?	?	?

F5	7	1	3.082307	2.581495	1.271067	2.065704	1	? 13
F6	7	1	2.841985	2.568202	1.130334	1.960629	1	? 14
F7	6	?	2.801747	2.604226	1.20412	1.840942	2	?
Yemen	7	2	3.086004	2.580925	1.908485	2.159367	2	?
Moor-1	3	2	2.849726	2.683497	1.804821	2.170262	2	3
Bulb	7	2	2.874192	2.728083	1.788875	2.11171	2	?
Ameri	5	1	2.900094	?	1.278754	2.056905	2	4
Al-Nea-1	3	1	2.287055	2.457125	1.041393	1.355388	2	3
Al-Nea-2	3	1	2.303196	2.354749	1.194977	1.342423	2	3
Al-Amp-1	8	1	2.607991	3.114944	1.041393	1.469822	2	3
Al-Amp-2	8	1	2.731589	2.982271	1.230449	1.452298	2	3
C-Abd	6	1	2.514548	2.467855	0.954243	1.409369	2	3
C-Lut	1	1	2.291147	2.457125	0.778151	1.355388	2	4
C-Pur	6	1	2.408805	2.541995	0.954243	1.185637	2	4
Uni-1	?	1	2.311754	2.296665	0.425969	0	2	4
Moor-2	?	2	3.071882	?	2.021189	2.045323	2	4
Angus	?	3	2.610128	2.552668	1.556303	2.11059	2	4
Am-Bel	5	1	?	2.808886	?	1.544068	?	?

Table S3. Morphological characters. Shaded characters were invariable within the species complex samples.

Character	State	Code
Anther Colour	deep-yellow – 1, pale-yellow – 2, white – 3, pale-purple – 4	Anth-C
Filament Colour*	yellow – 1, pale-yellow – 2, white – 3, pink-white – 4, dark-pink – 5, pale-pink – 6, pale-purple – 7	Fila-C
Filament Length (mm)	-	Fila-L
Floral Tube	absent – 0, present – 1	F-Tube
Floral Tube Length (mm)	-	Tube-L
Flower Colour^	yellow – 1, pale-yellow – 2, white – 3, pink-white – 4, pink – 5, pale-pink – 6, purple-pink – 7, purple – 8	F-Col
Flower Shape	lilaceous – 1, campanulate – 2, funnel shaped – 3	F-Shape
Flower Symmetry	actinomorphic – 0, zygomorphic – 1	F-Sym
Leaf Apex ^Δ	obtuse – 1, sub-obtuse – 2, acuminate – 3, acute – 4	Leaf-Apex
Leaf Length (mm)	-	Leaf-L
Leaf Margin	serrulate – 1, entire – 2	Leaf-Mar
Leaf Shape	linear – 1, ensiform – 2, subulate – 3	Leaf-Shape
Leaf Width (mm)	-	Leaf-W
Number of Flowers in Umbel	-	F-No
Number of Petals	-	Pet-No
Pedicel	absent – 0, present – 1	Pedi
Peduncle Length (mm)	-	Pedu-L
Perianth Lobe Length (mm)	-	Peri-L
Perianth Lobe Shape	oblanceolate – 1, elliptic – 2, oval – 3, lanceolate – 4, linear – 5, ovate – 6	Peri-Lobe
Perianth Lobe Width (mm)	-	Per-W
Stamen Fused	no – 0, yes – 1	S-Fused
Stamen Number	-	S-No
Style Colour [#]	yellow – 1, pale-yellow – 2, white – 3, pale-pink (at apex)-white – 4, pink (at apex)-white – 5, pale-pink – 6, dark-pink – 7, pale-purple – 8	Sty-C
Style Length (mm)	-	Sty-L

*Filament Colour



Predominantly white,
distally tinged pink (3) –
Crinum flaccidum (South
Australia)



Pink-White (4) – *Crinum
flaccidum* (New South
Wales)



Pale Pink (6) – *Calostemma
purpureum*

^Flower Colour



White (3) – *Crinum
flaccidum* (South
Australia)



Pink-White (4) –
*Amaryllis
Belladonna*

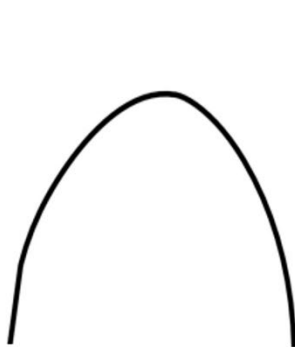


Pink (5) –
*Calostemma
purpureum*



Pale Pink (6) –
Crinum moorei

^Leaf Apex



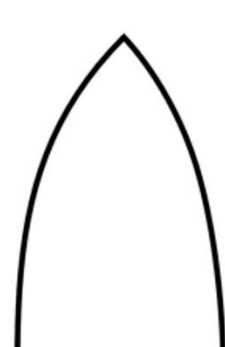
Obtuse



Sub-obtuse



Acuminate



Acute

#Style Colour



White (3) – *Crinum flaccidum* (South Australia)



Pale Pink (at apex) -
White (4) – *Crinum flaccidum* (South Australia)



Pink (at apex) -
White (5) –
Amaryllis belladonna



Pale Pink (6) – *Crinum flaccidum* (New South Wales)

Table S4. Gene regions.

Chloroplast genes	Aligned length (bp)	chloroplast genes	Aligned length (bp)
<i>psbA</i>	1085	<i>ycf4</i>	798
<i>matK</i>	1666	<i>cemA-petA</i>	1967
<i>psbK</i>	397	<i>psbE-psbF-psbL-psbJ</i>	987
<i>trnG-UCC</i>	918	<i>rps18</i>	530
<i>atpA-atpF</i>	2138	<i>rpl12</i>	572
<i>atpH</i>	320	<i>psbB-psbT-psbN-psbH</i>	2443
<i>rps2-atpI</i>	1866	<i>petB</i>	746
<i>rpoC1</i> (part.)	1739	<i>petD</i>	471
<i>rpoB-rpoC1</i> (partial)	3868	<i>rps11-rpoA</i>	1759
<i>psbD-psbC</i>	1367	<i>rps8</i>	602
<i>psbC</i>	1123	<i>rpl16</i> (part.)- <i>rpl14</i>	1576
<i>psaA-psaB-rps14</i>	4885	<i>rps3-rpl16</i> (part.)	1301
<i>ycf3</i>	302	<i>rpl22</i>	300
<i>rps4</i>	729	<i>ndhF</i>	1421
<i>trnL-UAA</i>	853	<i>psaC-ndhD-cssA</i>	3185
<i>ndhC-ndhK-ndhJ</i>	1845	<i>ndhG-ndhE</i>	1164
<i>trnV-UAC</i>	783	<i>ndhA-ndhI</i>	1421
<i>atpB-atpE</i>	1867	<i>rps15-ndhH-ndhA</i>	2437
<i>rbcL</i>	1550		

Expanded Phylogenies

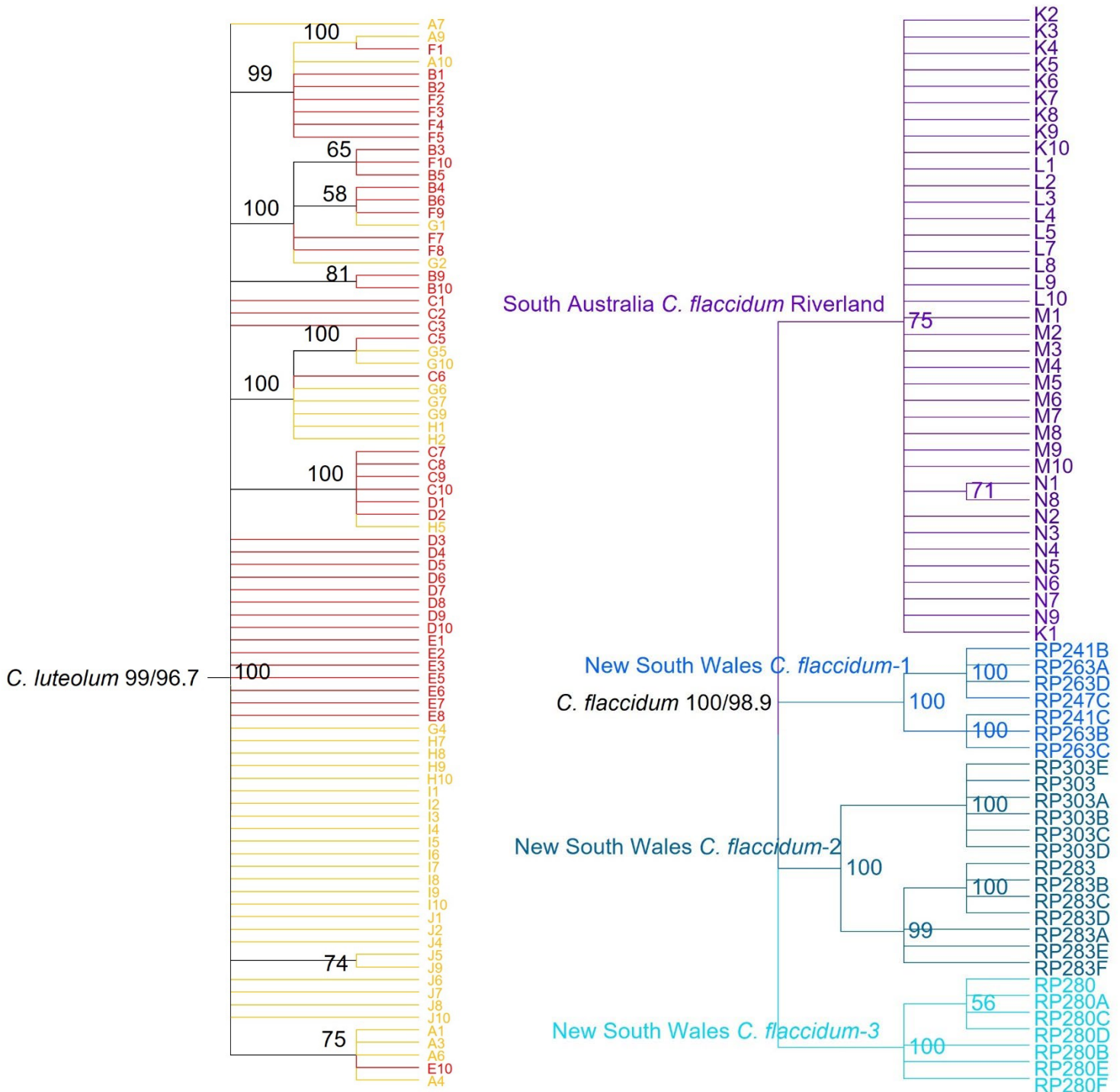


Fig. S2. *Crinum luteolum* and *C. flaccidum* clades expanded (from Fig. 1). For *C. luteolum* red indicates species belonging to the northern morphotype, and orange the southern morphotype. The letter at the start of South Australian samples indicates the population (Table S1) whereas the number is the sample number in that population. New South Wales populations are identifiable by RP (3 numbers) and the specific sample in that population is the letter. Node support values MrBayes/UFBoot2 with single values belonging to MrBayes.

NMDS

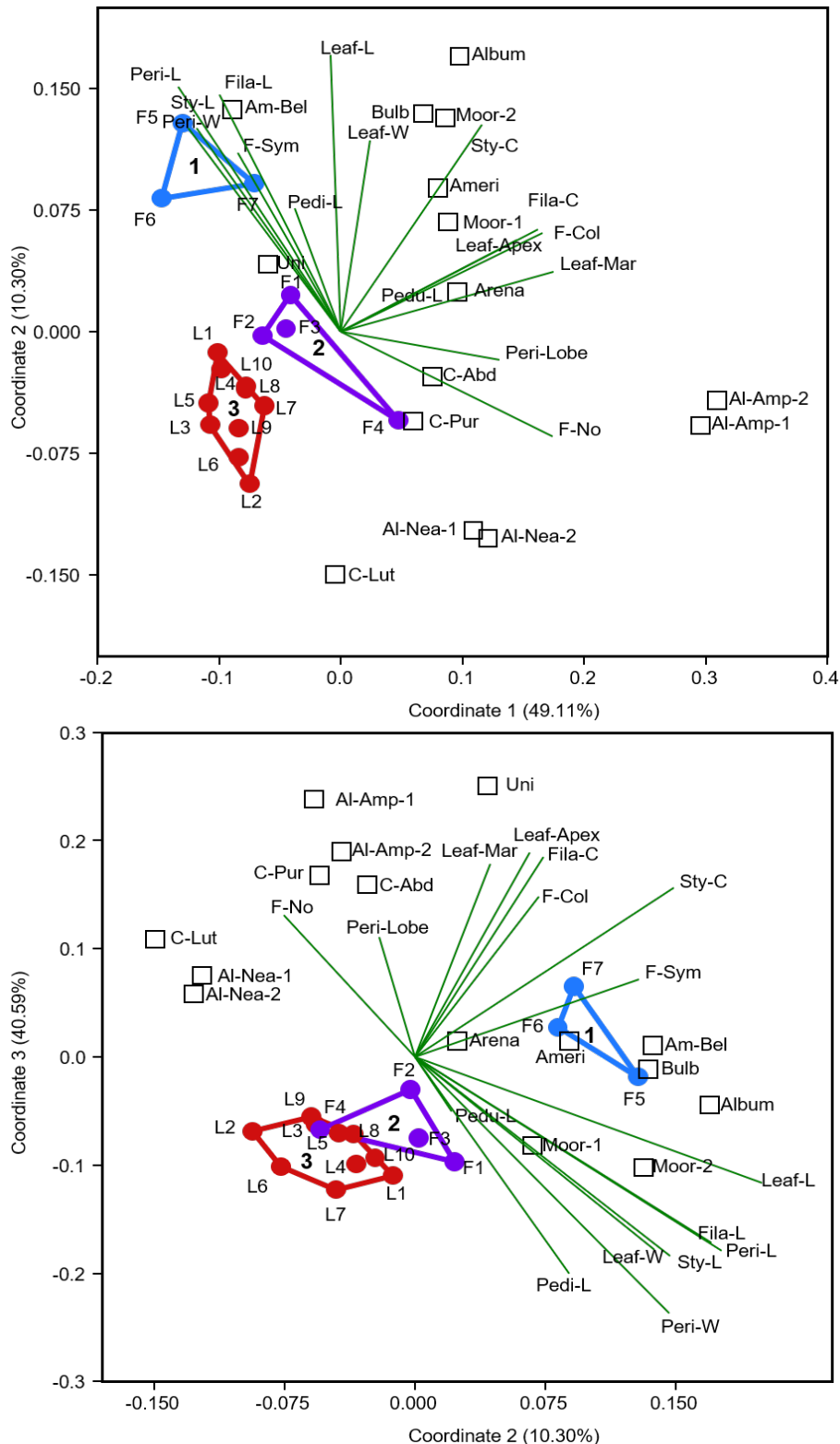


Fig. S3. Three-dimensional NMDS with biplot analysis of the morphological data for all sampled taxa, using Gower similarity. The vectors are the biplot analysis of the 24 morphological characters. The ordination plots have a STRESS score of 12.78%. Polygons represent the area covered by each group; 1, New South Wales *Crinum flaccidum*; 2, South Australian *C. flaccidum*; and 3, *C. luteolum*. Sample and character codes can be found in Tables S1 and S3 respectively.