

SUPPLEMENTARY DATA

TABLE S1. Origin of plant material of *Disa* and *Brownleea* examined. Order of taxa and species follows Bytebier *et al.* (2008). Abbreviations for geographical regions follow Brummitt (2001). Herbarium codes: BOL = Bolus Herbarium, University of Cape Town, South Africa, NU = Bews Herbarium, University of KwaZulu-Natal, South Africa. s.n. = no collection number available.

Taxon	Origin	Collector, collection number, herbarium, accession number
Brownleea		
<i>Brownleea galpinii</i> ssp. <i>major</i>	NAT, Bushman's Nek	Hobbhahn, 005, BOL
<i>Brownleea macroceras</i>	CPP-EC, Naude's Nek	Hobbhahn, 006, NU 0037314
<i>Brownleea parviflora</i>		Unknown, s.n., NU 0037322
Disa		
<i>Disa caulescens</i>	CPP-WC, Bain's Kloof	Hobbhahn, 007, BOL
<i>Disa filicornis</i>		Linder, s.n., BOL 312
<i>Disa racemosa</i>	CPP-WC, Cape Point	Hobbhahn, s.n., BOL 2726
<i>Disa tripetaloides</i>		Linder, 1750, BOL 538
<i>Disa uniflora</i>	CPP-WC, Table Mountain	Hobbhahn, s.n., BOL 2713
<i>Disa uniflora</i>	CPP-WC, Grootwinterhoek Wilderness Area	Hobbhahn, 033, NU 0037293
Atromaculiferae		
<i>Disa glandulosa</i>	CPP-WC, Table Mountain	Hobbhahn, 008, BOL
<i>Disa vaginata</i>	CPP-WC, Table Mountain	Bruyns, 1238, BOL 392
<i>Disa vaginata</i>	CPP-WC, Table Mountain	Hobbhahn, 032, NU 0037294
Phlebidia		
<i>Disa longicornu</i>	CPP-WC, Table Mountain	Hobbhahn, 009, NU 0037307
Pardoglossa		
<i>Disa rosea</i>	CPP-WC, Table Mountain	Linder, s.n., BOL 427
Ovalifoliae		
<i>Disa ovalifolia</i>		Linder, 5871, BOL 2289; Burger, s.n., BOL 2100
Schizodium		
<i>Disa flexuosa</i>	CPP-WC, Villiersdorp	Hobbhahn, 010, BOL
<i>Disa obliqua</i> ssp. <i>obliqua</i>	CPP-WC, Table Mountain	Hobbhahn, 035, NU 0037295
<i>Disa satyrioides</i>		Linder, s.n., BOL 1432
Vaginaria		
<i>Disa fasciata</i>	CPP-WC, Hottentots Holland Nature Reserve	Hobbhahn, 028, NU 0037301; 036, NU 0037290
Coryphaea		
<i>Disa rungweensis</i>	TVL-MP, Long Tom Pass	SD Johnson, s.n., NU 0037312
<i>Disa sagittalis</i>		Unknown, s.n., NU 0037299
<i>Disa triloba</i>		Esterhuysen, 34157, BOL 1378
Disella		
<i>Disa bodkinii</i>		Bruyns, 1544, BOL 203
<i>Disa elegans</i>		Oliver, s.n., BOL 320
<i>Disa elegans</i>	CPP-WC, Grootwinterhoek	Hobbhahn, 012, NU 0037309
<i>Disa obtusa</i> ssp. <i>hottentotica</i>	CPP-WC, Hottentots Holland Nature Reserve	Hobbhahn, 013, BOL
<i>Disa uncinata</i>		Linder, 1689, BOL 551

Monadenia

<i>Disa atrorubens</i>		Linder, 1129, BOL 918
<i>Disa bolusiana</i>	CPP-WC, Swartberg Nature Reserve	Bytebier, 2572, NU
<i>Disa bracteata</i>		Chesselet, 13, BOL 2035
<i>Disa bracteata</i>	CPP-WC, Villiersdorp	Hobbhahn, 011, BOL
<i>Disa brevicornis</i>	NAT, Mount Gilboa, Midlands	Hobbhahn, 029, NU 0037298
<i>Disa comosa</i>	CPP-WC, Middelburg Pass	Hobbhahn, 023, NU 0037313
<i>Disa comosa</i>	CPP-WC, Table Mountain	Hobbhahn, 022, NU 0037310
<i>Disa cylindrica</i>		Kurzweil, 904, BOL 1568
<i>Disa cylindrica</i>	CPP-WC, Hottentots Holland Nature Reserve	Hobbhahn, 024, BOL
<i>Disa ophrydea</i>		Kurzweil, 923, BOL 1336
<i>Disa ophrydea</i>	CPP-WC, Hottentots Holland Nature Reserve	Hobbhahn, 037, NU 0037248
<i>Disa rufescens</i>	CPP-WC, Hottentots Holland Nature Reserve	Hobbhahn, 025, BOL
<i>Disa sabulosa</i>	CPP-WC, Betty's Bay	Hobbhahn, 014, NU 0037306

Reticulibractea

<i>Disa harveiana</i> ssp. <i>longicalcarata</i>	CPP-WC, Dasklip Pass	Hobbhahn, s.n., BOL 2728
<i>Disa karooica</i>	CPP-NC, Leliefontein	Hobbhahn, 015, NU 0037302

Repandra

<i>Disa cornuta</i>	CPP-WC, Cape Point	Hobbhahn, 034, NU 0037291
<i>Disa tysonii</i>	CPP-EC, Bastervoetpad	Hobbhahn, 016, BOL
<i>Disa tysonii</i>		Linder, 806, BOL 516

Trichochila

<i>Disa graminifolia</i>		Linder, 1763, BOL 819
<i>Disa hians</i>		Linder, 1731, BOL 849
<i>Disa salteri</i>		Langley, s.n., BOL; Langley, s.n. BOL 2541
<i>Disa salteri</i>	CPP-WC, Safraansrivier	Hobbhahn, 017, BOL
<i>Disa tenuis</i>		Linder, s.n., BOL 394

Stenocarpa

<i>Disa aristata</i>		Davidson, 3270, BOL 406
<i>Disa cephalotes</i> ssp. <i>cephalotes</i>	NAT, Witsieshoek	Van der Niet, s.n., NU 0037297
<i>Disa gladioliflora</i> ssp. <i>gladioliflora</i>	CPP-EC, Tierkop, Langeberg	Hobbhahn, s.n., BOL 2729
<i>Disa nivea</i>	CPP-EC, Bastervoetpad	Hobbhahn, 018, BOL
<i>Disa saxicola</i>		Unknown, s.n., NU 0037311
<i>Disa stricta</i>		Unknown, s.n., NU 0037316
<i>Disa vigilans</i>	TVL-MP, Mokobulaan	Bellstedt, 827, NU

Emarginatae

<i>Disa nervosa</i>	NAT, Hella Hella	Hobbhahn, s.n., BOL 2730
<i>Disa patula</i> var. <i>transvaalensis</i>	NAT, Mount Gilboa, Midlands	Hobbhahn, s.n., BOL 2721
<i>Disa stachyoides</i>	NAT, Mount Gilboa, Midlands	Hobbhahn, s.n., BOL 2724

Spirales

<i>Disa brachyceras</i>	CPP-WC, Shaw's Pass	Bytebier, 2607, NU
<i>Disa tenella</i> ssp. <i>tenella</i>	CPP-WC, Mamre Road Station	Linder, 1124, BOL 520

Aconitoideae

<i>Disa aconitoides</i> ssp. <i>aconitoides</i>		Linder, 1920, BOL 211
<i>Disa aconitoides</i> ssp. <i>aconitoides</i>	NAT, Howick	Unknown, 49, NU
<i>Disa similis</i>		Unknown, 165, NU 0037318
Micranthae		
<i>Disa chrysostachya</i>	NAT, Mount Gilboa, Midlands	Hobbhahn, s.n., BOL 2723
<i>Disa chrysostachya</i>	CPP-EC, Coldstream	Hobbhahn, 020, NU 0037296
<i>Disa cooperi</i>	NAT, Himeville Nature Reserve	Hobbhahn, 021, NU 0037305
<i>Disa crassicornis</i>	CPP-EC, Bastervoedpad	Hobbhahn, 030, NU 0037304
<i>Disa erubescens</i> ssp. <i>erubescens</i>	MLW, Mt. Mlanje	Kurzweil, 1404, BOL 1920
<i>Disa fragrans</i> ssp. <i>fragrans</i>	NAT, Witsieshoek	Hobbhahn, s.n., BOL 2727
<i>Disa galpinii</i>	CPP-EC, Naude's Nek	Hobbhahn, 026, BOL
<i>Disa hircicornis</i>	NAT, Table Mountain	Unknown, 160, NU 0037319
<i>Disa polygonoides</i>	NAT, Vernon Crookes Nature Reserve	Hobbhahn, 027, BOL
<i>Disa rhodantha</i>	TVL-MP, Graskop	SD Johnson, s.n., NU 0037303
<i>Disa sanguinea</i>		Unknown, s.n., NU 0037315
<i>Disa sanguinea</i>	NAT, Sani Pass	McMurtry, s.n., NU 0037320
<i>Disa sankeyi</i>	NAT, Bushman's Nek	Cozien & Van der Niet, s.n., NU 0037308
<i>Disa scullyi</i>	CPP-EC, Bastervoedpad	Hobbhahn, s.n., BOL 2714
<i>Disa thodei</i>	CPP-EC, Naude's Nek	Hobbhahn, 031, NU 0037300
<i>Disa versicolor</i>	NAT, Mount Gilboa, Midlands	Hobbhahn, s.n., BOL 2725
<i>Disa woodii</i>		Unknown, s.n., NU 0037321; NU 003717
<i>Disa zuluensis</i>	TVL-MP, Dullstroom district	SD Johnson, s.n., NU 0037292

TABLE S2. Mean (\pm standard error) stomata and spur characteristics of *Disa* species with uncertain nectar status that were examined by scanning electron microscopy, but excluded from analysis. Order of taxa and species follows Bytebier *et al.* (2008). Sample sizes for number of stomata are given as ($N_{\text{Specimens}}$), those for stomata size and spur-wall thickness as ($N_{\text{Measurement}}$, $N_{\text{Specimens}}$).

Taxon	Number of stomata	Stoma area (mm ²)	Spur wall thickness (cell layers)
Ovalifoliae			
<i>Disa ovalifolia</i>	0 (3)	-	7 \pm 0.8 (5, 2)
Schizodium			
<i>Disa satyrioides</i>	0 (1)	-	
Coryphaea			
<i>Disa triloba</i>	0 (1)	-	4 \pm 0.3 (4, 1)
Monadenia			
<i>Disa atrorubens</i>	32 (1)	1.76 \pm 0.085 (5, 2)	7 \pm 0.3 (3, 1)
Spirales			
<i>Disa brachyceras</i>	0 (2)	-	5 \pm 0 (2, 1)
Stenocarpa			
<i>Disa aristata</i>	0 (1)	-	7 \pm 0.3 (4, 1)
Aconitoideae			
<i>Disa similis</i>	0 (1)	-	7 \pm 0.4 (4, 1)
Micranthae			
<i>Disa sanguinea</i>	0 (3)	-	6 \pm 0.3 (3, 1)

TABLE S3. Reconstructed states from parsimony analyses of stomata occurrence ($n = 60$ species) and nectary type ($n = 103$ species) in *Disa*. For each tree that contains a given node, the state that was reconstructed as uniquely best for the node is identified, and the number of trees in which the uniquely best state occurred is summarized. Reconstruction of stomata occurrence: Optimal state: 0 = stomata absent, 1 = stomata present, - = no optimal state. Nectary-type reconstruction: 0 = nectary absent, 1 = stomatal nectary, 2 = secretory epidermis.

Occurrence of stomata				Nectary type							
Node	Number of trees containing node	Optimal state	Number of trees with optimal state	Node	Number of trees containing node	Optimal state	Number of trees with optimal state	Node	Number of trees containing node	Optimal state	Number of trees with optimal state
2	1000	-	0	2	1000	0	1000	127	1000	1	1000
3	1000	-	0	3	1000	0	1000	128	1000	1	1000
4	1000	0	1000	4	1000	2	1000	131	349	0	349
7	1000	-	0	5	1000	0	1000	132	465	0	465
9	840	-	0	6	1000	0	1000	133	1000	0	1000
10	1000	-	0	9	1000	0	1000	135	1000	0	1000
13	1000	-	0	12	998	0	998	139	999	0	999
16	1000	-	0	14	1000	0	1000	140	1000	0	1000
18	1000	0	145	16	967	0	967	143	1000	0	1000
19	1000	0	1000	19	1000	0	1000	144	1000	0	1000
21	1000	0	1000	20	840	0	840	145	1000	0	1000
24	1000	0	145	21	1000	0	1000	146	1000	0	1000
25	1000	0	1000	24	1000	0	1000	149	1000	0	1000
27	1000	0	1000	26	1000	0	1000	151	1000	0	1000
30	1000	0	145	28	1000	0	1000	154	899	0	899
		1	145	31	1000	0	1000	155	1000	0	1000

31	994	0	144	34	1000	0	1000	156	579	0	579
32	1000	0	146	35	1000	0	1000	159	940	0	940
34	1000	0	1000	38	998	0	998	162	985	0	985
36	1000	0	1000	41	1000	0	1000	165	1000	0	1000
39	994	0	994	42	1000	0	1000	166	1000	0	1000
41	1000	0	1000	43	1000	0	1000	170	1000	0	1000
42	1000	0	1000	44	1000	0	1000	171	1000	0	1000
45	232	0	232	45	1000	0	1000	172	1000	2	1000
47	882	0	882	49	1000	0	1000	173	891	0	891
48	961	0	961	50	994	0	993	175	1000	0	1000
51	1000	0	1000	51	1000	0	1000	178	1000	0	1000
54	999	0	145	53	694	0	694	181	1000	0	1000
		1	145	56	995	0	995	183	937	0	937
55	366	0	10	58	962	0	962	185	152	0	152
		1	58	60	1000	0	1000	186	189	0	189
56	465	0	465	62	1000	0	1000	189	191	0	191
58	1000	0	1000	66	1000	0	1000	192	985	0	985
61	1000	0	1000	67	1000	0	1000	193	724	0	724
62	1000	0	1000	68	994	1	763	196	1000	0	1000
63	1000	0	1000	70	1000	0	1000	199	1000	0	1000
66	1000	0	1000	73	232	1	232	200	1000	0	1000
69	1000	0	1000	75	882	1	882	203	1000	0	1000
71	1000	0	1000	76	961	1	961	206	1000	0	1000
73	1000	0	1000	79	1000	1	1000				
75	1000	0	1000	82	1000	0	1				
77	899	0	899				763				
80	349	0	12	83	999	0	999				

		1	62	84	366	0	366
81	568	0	3	85	568	0	568
		1	183	87	1000	0	1000
83	1000	0	234	88	1000	0	1000
85	1000	0	234	90	698	0	698
88	115	0	23	93	1000	0	1000
90	1000	0	1000	96	115	0	115
92	1000	0	1000	98	1000	0	1000
93	1000	0	1000	99	1000	0	1000
95	1000	0	1000	100	1000	1	1000
97	1000	0	1000	102	910	1	910
100	946	0	946	104	1000	1	1000
102	999	0	999	109	1000	1	1000
103	910	0	910	110	1000	1	1000
105	1000	0	1000	111	1000	1	1000
107	1000	0	1000	113	997	1	997
109	1000	0	1000	118	1000	1	1000
112	1000	0	1000	119	1000	1	1000
114	997	0	997	120	999	1	999
116	1000	0	1000	121	946	1	946
118	1000	0	1000	124	1000	1	1000

FIG. S1. Ancestral state reconstruction of the occurrence of stomata in *Disa* by parsimony. Results from 1000 chronograms summarized on the maximum-clade credibility chronogram containing 60 species. Species highlighted in yellow produce nectar. Node colours: white = stomata absent, black = stomata present, grey = equivocal. For support values of all nodes (Fig. S2) see Table S3.

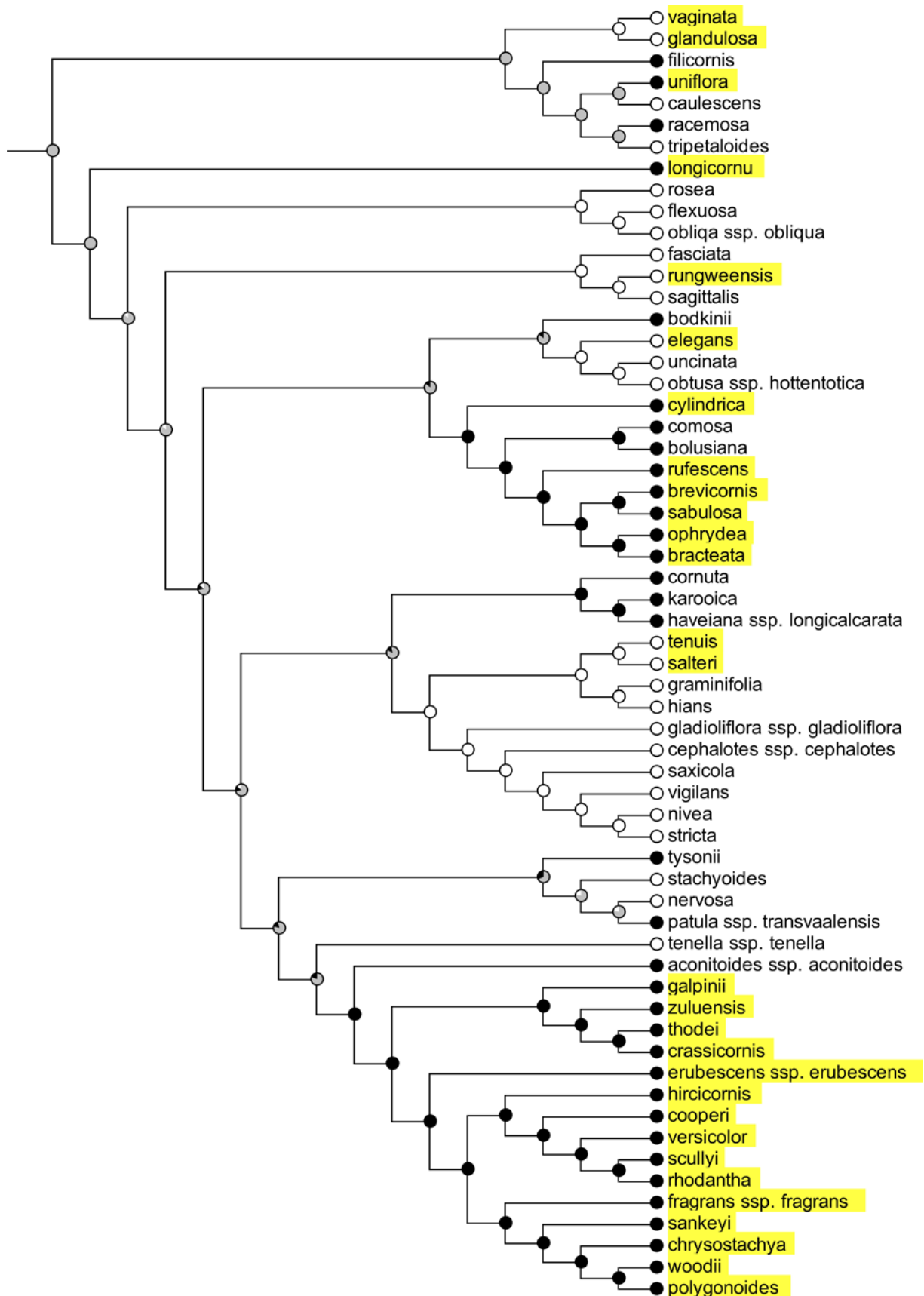


FIG. S2. Node numbers associated with ancestral state reconstruction of stomata occurrence on the maximum-clade credibility chronogram containing 60 species (Fig. S1), see Table S3 for support values for each node. Branch lengths are not representative of clade age. Species highlighted in yellow produce nectar.

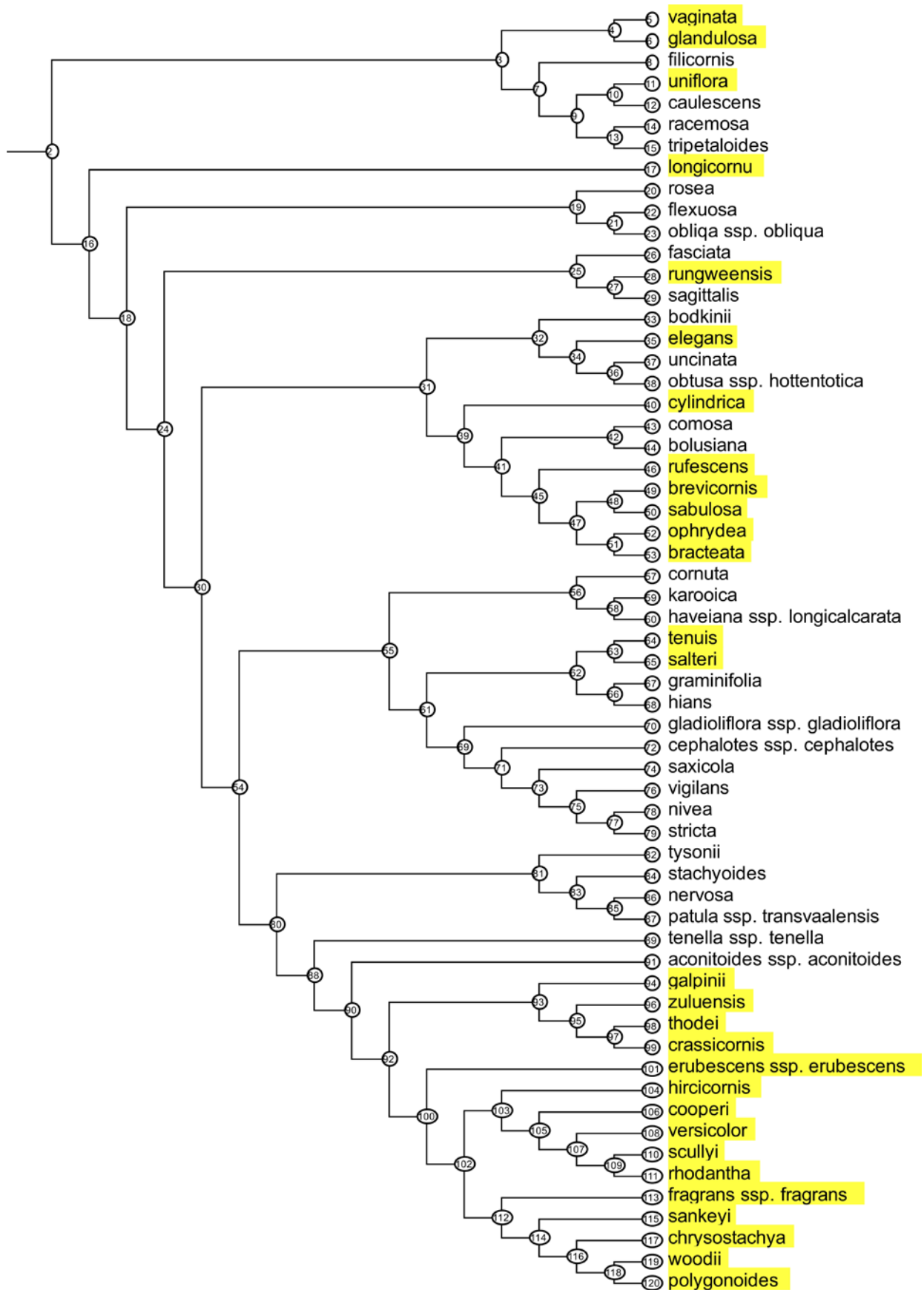


FIG. S3. Node numbers associated with ancestral state reconstruction of nectary type by parsimony on the maximum-clade credibility chronogram containing 103 species (Fig. 5), see Table S3 for support values for each node. Branch lengths are not representative of clade age. Species highlighted in yellow produce nectar.

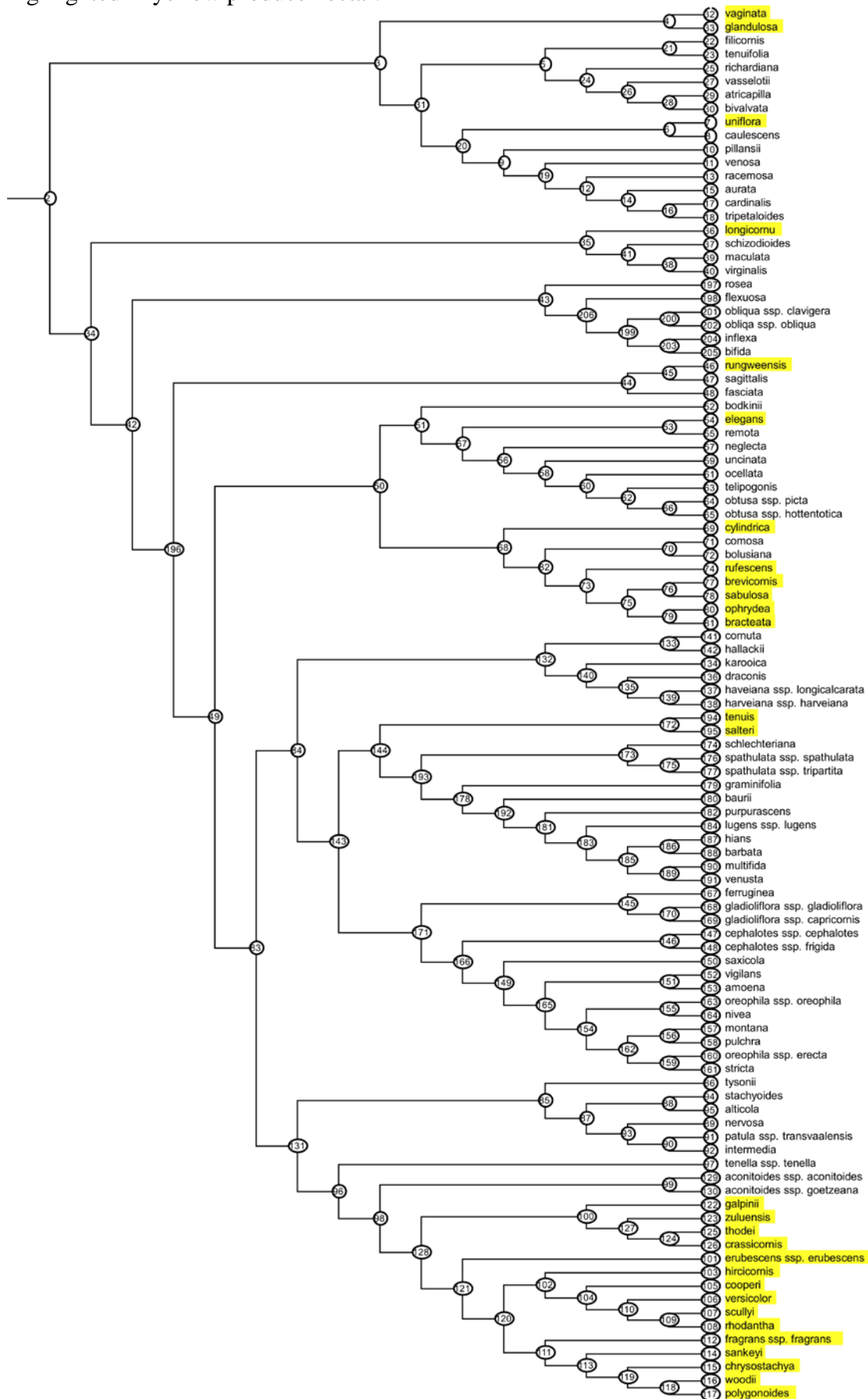


FIG. S4: Frequency distributions of P values obtained from 1000 chronograms used to account for phylogenetic uncertainty in tests for phylogenetic signal in continuous traits, and in comparisons using phylogenetic generalized estimating equations (pGEEs). (A-C) Tests for phylogenetic signal of stomata number (A) and size (B), and spur-wall thickness (C). (D-H) Comparisons between nectarless and nectar-producing species of stomata number on all examined flower parts (D) and in spurs only (E), of stoma area on all examined flower parts (F) and in spurs only (G), and of spur-wall thickness including (light grey bars) and excluding (dark grey bars) *Disa uniflora* (H). (I-J) Comparisons between sections *Monadenia* and *Micranthae* of stomata number (I) and size (J), and of spur-wall thickness (K). (L) Comparison of spur-wall thickness between species with secretory epidermis and stomatal nectaries. Dotted lines indicate $P = 0.05$.

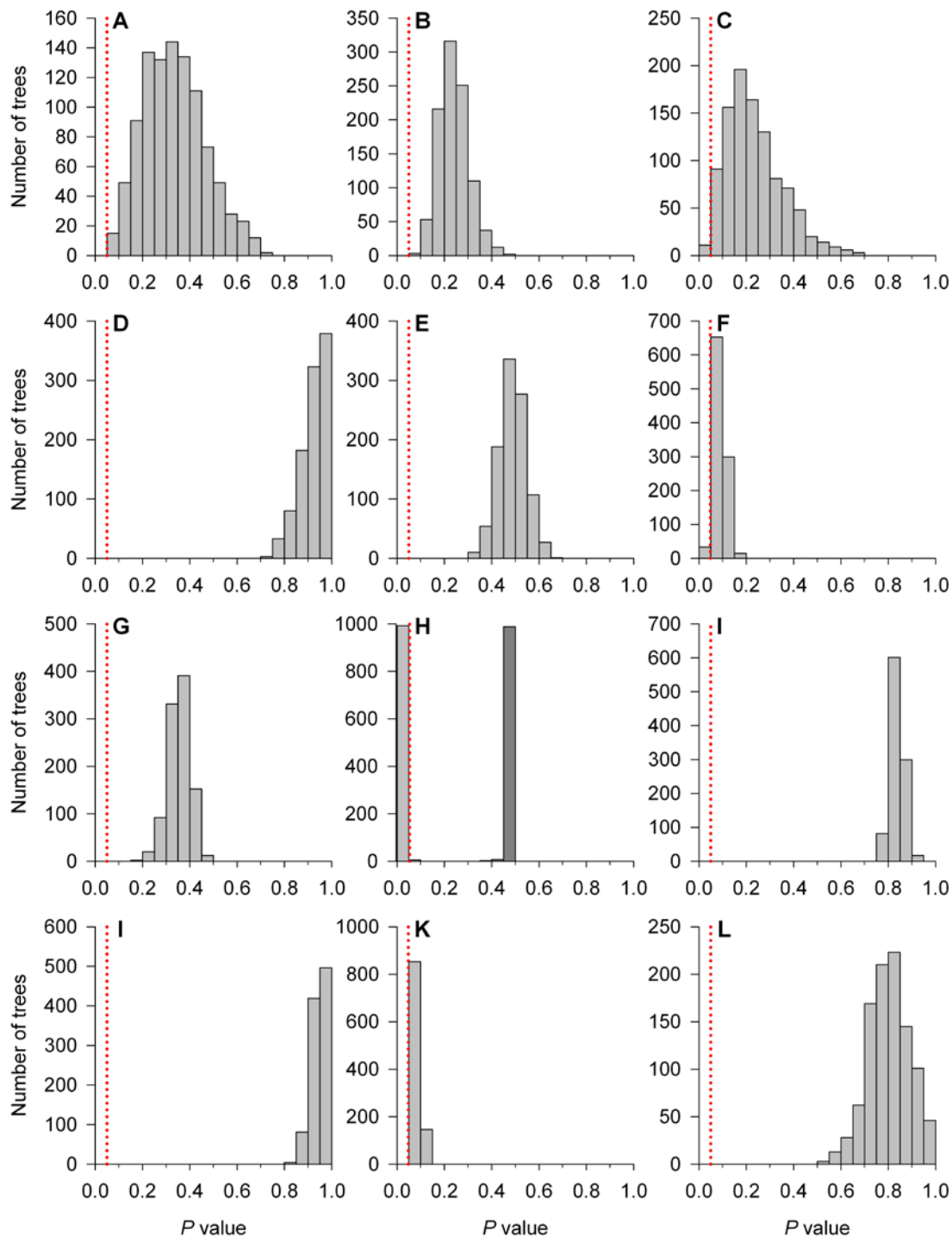
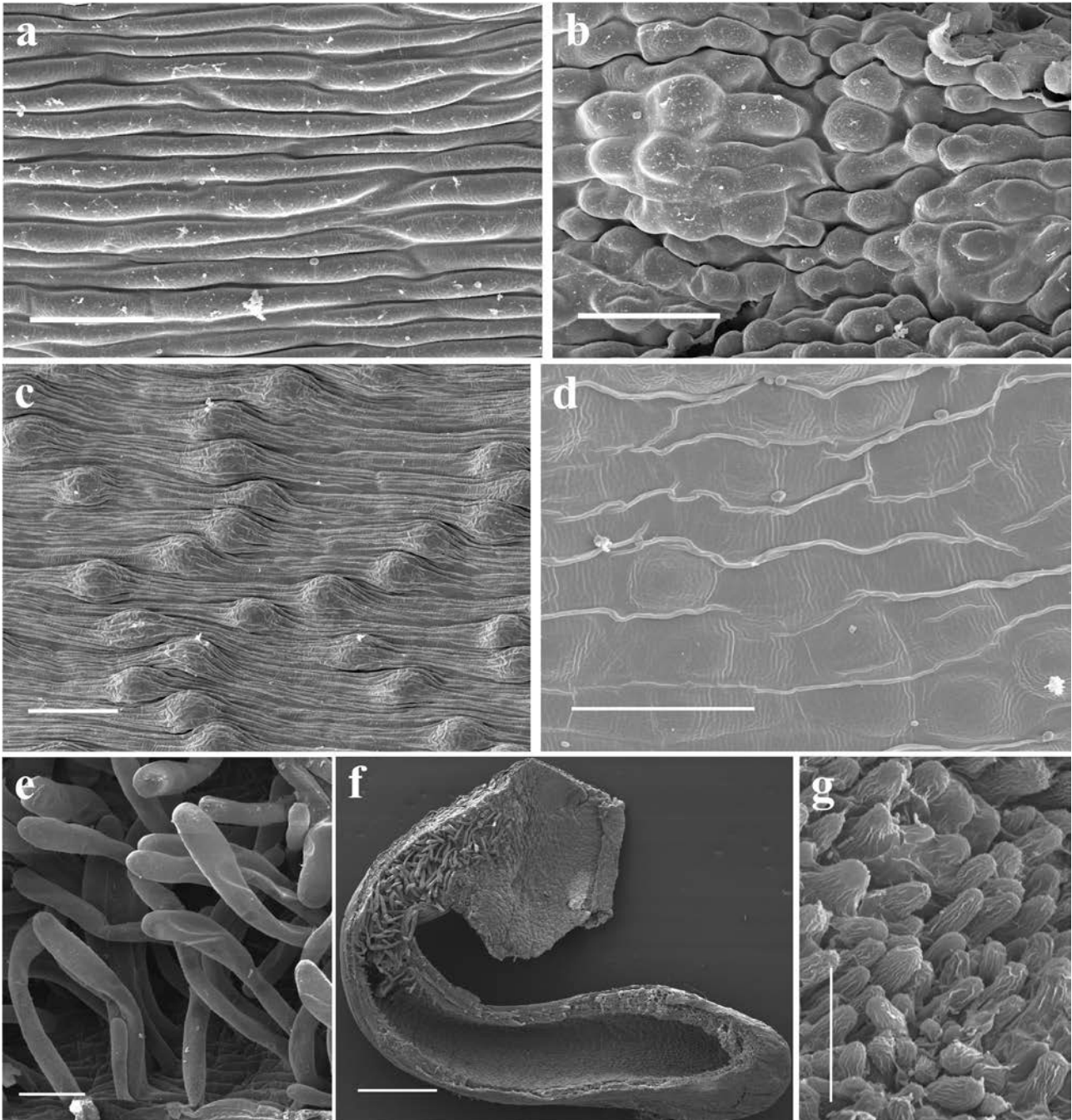


FIG. S5: Scanning electron micrographs of spur tissues of the examined *Brownleea* species. a, b: *Brownleea galpinii*, representative epidermis cells of spur (a) and spur tip (b). c, d: *B. macroceras*, representative epidermis cells of spur (c) and spur tip (d). Cells in (d) collapsed during critical-point drying, causing prominent lateral cell walls. e-f: *B. parviflora*, unicellular trichomes in spur entrance (e), spur overview (f), representative spur epidermis cells (g). Scale bars: a, b, d, 50 μm ; c, 100 μm ; e, g, 75 μm ; f, 1 mm.



LITERATURE CITED

- Brummitt RK. 2001.** World geographical scheme for recording plant distributions. *International Working Group on Taxonomic Plant Sciences*. 2 ed. Pittsburgh, Hunt Institute for Botanical Documentation.
- Bytebier B, Bellstedt DU, Linder HP. 2008.** A new phylogeny-based sectional classification for the large African orchid genus *Disa*. *Taxon* **57**: 1233–1251.