

Supplementary Material

Taxonomy of the *Nicotiana megalosiphon* species complex (Solanaceae; *Nicotiana* section *Suaveolentes*): analyses of RADseq data identifies a new cryptic species

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Table S1. Voucher numbers, herbaria and provenance of the plant material.

Species name	Voucher number (<i>Chase & Christenhusz</i>)	Latitude; longitude	Provenance (brief locality name, all Australia)
<i>megalosiphon</i>	17005	-31°3'16.4"S; 147°56'3.6"E	New South Wales, Gibson Way, Macquarie Marshes
<i>megalosiphon</i>	17009	-29°34'5.2"S; 149°24'22.9"E	New South Wales, Telleraga-Millie Road, bridge over Mehi River
<i>megalosiphon</i>	17010	-29°19'20.1"S; 150°12'22.1"E	New South Wales, County Boundary Road, just north of Milguy
<i>megalosiphon</i>	21009 <i>Palsson 442</i> (NE)	-28°21'0.76"S; 148°58'54.18"E	Queensland, Maranoa, between Weengallon and Nindigully
<i>megalosiphon</i>	20013 <i>Palsson 416</i> (NE)	-30°15'43"S; 149°42'27"E	New South Wales, Narrabri, Mollee Weir
<i>megalosiphon</i>	18161	-24°16'44"S; 145°20'34"E	Queensland, Blackall, Douglas Ponds Creek
<i>megalosiphon</i>	18175	-20°37'42"S; 143°4'4"E	Queensland, Richmond–Woolgar Road, Double Barrell Creek
<i>palssonae</i>	20011 <i>Palsson 410</i> (NE)	-30°57'14"S; 145°53'51"E	New South Wales, south of Bourke, Kidman Way
<i>palssonae</i>	20014 <i>Palsson 419</i> (NE)	-31°53'30"S; 141°25'58"E	New South Wales, Broken Hill, Living Desert State Park
<i>palssonae</i>	20012 <i>Palsson 412</i> (NE)	-30°34'18"S; 145°42'47"E	New South Wales, Gundabooka NP, Ben Lomond Gorge
<i>palssonae</i>	20005 <i>Palsson 321</i> , (<i>NE 110374</i>)	-29°44'43"S; 147°33'13.2"E	New South Wales, Narran Lake NR, Kurrajong Road
<i>palssonae</i>	18016 <i>Prendergast 354</i> (<i>BRI AQ0501528</i>)	-26°52'24.9"S; 144°35'33.3"E	Queensland, Warrego, 15.5 km SE of Wynburn turn-off, old Quilpie–Charleville road
<i>latzii</i>	18074 <i>Latz 21442</i> (<i>NT A0110082</i>)	-23°51'15"S; 138°30'50"E	Queensland, 5 km east of Ethabuka Station Homestead
<i>latzii</i>	18124 <i>Jobson 3235</i> (<i>NSW</i>)	-22°53'43"S; 139°2'21"E	Queensland, Anabranche of the Georgina River, Donohue Hwy, 24 k east of Glenormiston Homestead
<i>simulans</i>	<i>Conran 3552</i> (<i>SA</i>)	-29°14'57"S; 135°7'56"E	South Australia, 17 km east of Stuart Highway
<i>simulans</i>	<i>Conran 3559</i> (<i>SA</i>)	-29°14'11"S; 134°52'56"E	South Australia, 24 km west of Stuart Highway
<i>simulans</i>	<i>Conran 3560</i> (<i>SA</i>)	-29°7'57"S; 134°34'21"E	South Australia, Mount Clarence Station
<i>simulans</i>	<i>Conran 3564</i> (<i>SA</i>)	-28°24'20"S; 134°59'17"E	South Australia, Coober Pedy–Oodnadatta Road, Algebullcullia Creek
<i>simulans</i>	16023 <i>B</i>	-23°57'47"S; 134°3'9"E	Northern Territory, Santa Teresa Road, 40 km SE of Alice Springs
<i>simulans</i>	16092	-25°10'39"S; 133°24'1"E	Northern Territory, Idracowra Station
<i>simulans</i>	18055 <i>Schubert 572</i> (<i>NT D0269780</i>)	-29°27'24"S; 133°6'32"E	South Australia, Tallaringa Conservation Reserve
<i>simulans</i>	18058 <i>Latz 25743</i> (<i>NT D0199155</i>)	-25°58'16"S; 135°2'29"E	Northern Territory, New Crown Homestead
<i>sessilifolia</i>	16016	-22°7'54"S; 133° 24'15"E	Northern Territory, Nturiya Rd, 2 km west of Ti-Tree
<i>sessilifolia</i>	16017	-22°48'22"S; 133°24'55"E	Northern Territory, Native Gap, Hann Range
<i>sessilifolia</i>	16025	-23°40'37"S; 133°43'9"E	Northern Territory, Simpson's Gap
<i>sessilifolia</i>	16027	-23°59'23"S; 133°26'9"E	Northern Territory, Lawrence Gorge, Waterhouse Range
<i>sessilifolia</i>	16051	-23°44'58"S; 132°58'45"E	Northern Territory, Serpentine Gorge
<i>sessilifolia</i>	16064	-24°2'45"S; 132°42'28"E	Northern Territory, Palm Valley
<i>sessilifolia</i>	16069	-23°41'11"S; 132°40'27"E	Northern Territory, Glen Helen Gorge
<i>sessilifolia</i>	16215	-23°44'48"S; 134°0'54"E	Northern Territory, Jessie Gap
<i>latifolia</i>	18054 <i>Forster 37332</i> (<i>BRI AQ0816103</i>)	-23°1'21"S; 138°13'12"E	Queensland, Cravens Peak
<i>latifolia</i>	18176	-20°42'16"S; 140°29'47"E	Queensland, Ramsay Street, Cloncurry
<i>latifolia</i>	18191	-20°40'57"S; 139°29'45"E	Queensland, Mount Isa, Mondarra Drive
<i>walpa</i>	16116	-25°17'6"S; 130°43'36"E	Northern Territory, Kata-Tjuta, Valley of the Winds Trail
<i>walpa</i>	16105	-25°17'12"S; 130°44'53"E	Northern Territory, Kata-Tjuta, Valley of the Winds Trail
<i>walpa</i>	16056	-23°49'3"S; 132°18'57"E	Northern Territory, Gosse's Bluff

Unless otherwise indicated; vouchers deposited at PERTH, NSW or BRIS, depending on the state in which they were collected. Accessions raised from seeds retrieved from herbarium material are documented by secondary vouchers at RBG Kew (K); for these, the original collector, number, and herbarium accession number in Australia are also provided.

Table S2. *Rdr1* presence or absence, seed sizes and germination, flowering and capsule maturation times for a selection of relevant accessions and species of *N.* sect. *Suaveolentes*.

Accession	Group	<i>Rdr1</i> insertion	Seed size (microns)	Days to germination	Days to flowering	Days to capsule maturation
LAB	NT	present	790–805	3	55	87
<i>Chase & Christenhusz 16006</i>	NT	absent	650–680	4	53	87
<i>Chase & Christenhusz 16009</i>	NT	present	715–755	4	54	>87
<i>Cowie 13343^A (18082)</i>	NT	present	720–780	5	54	>90
<i>Chase & Christenhusz 18190^A</i>	QLD	absent	530–590	4	62	>90
<i>Chase & Christenhusz 18183^A</i>	QLD	absent	530–585	4	58	>90
<i>Latz 22902 (18040)</i>	eWA	absent	510–565	5	50	>87
<i>Goods 1145^A (18033)</i>	eWA	absent	505–555	5	64	>90
<i>Bean 25412 (18039)</i>	WA2	absent	520–585	5	60	>90
<i>Chase & Christenhusz 68174</i>	WA2	absent	520–580	5	60	>90
<i>Chase & Christenhusz 68199</i>	WA1	absent	530–580	5	58	>90
<i>McMaster 25736 (18085)</i>	WA1	absent	535–585	6	58	>90
<i>N. simulans^A</i>	n/a	absent	580–620	3	35	72
<i>N. exigua^A</i>	n/a	absent	450–485	3	37	75

These data were collected for all 36 accessions of the *N. benthamiana* species group studied here, but we do not include all these due to their highly redundant nature. Note: *Nicotiana simulans* and *N. exigua* are distantly related to the *N. benthamiana* complex and do not carry the *Rdr1* insert, but they develop and mature much more quickly compared to the species of the *N. benthamiana* complex.

^ACleistogamous flowers produced before normal (chasmogamous) flowers.

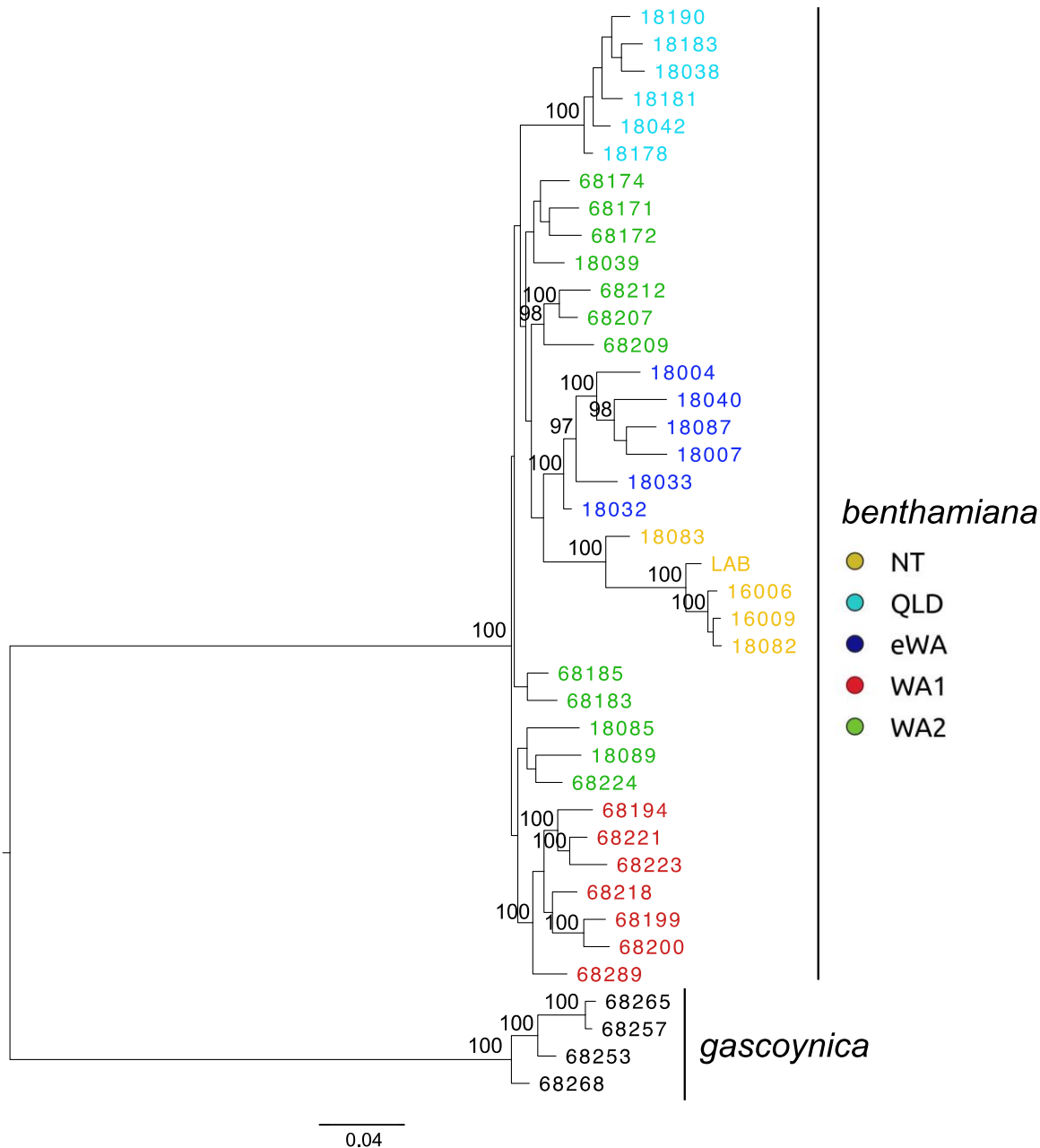


Fig. S1. *RaxML* tree based on 14,818 SNPs. The color of the clusters represents their geographical provenances: yellow – Northern Territory and north-eastern-most Western Australia (NT); dark blue – the deserts of eastern Western Australia (EWA); light blue – Queensland and western-most northern Territory (QLD); red - Pilbara coast of north-western Western Australia (WA1) and; green - the Pilbara Craton of Western Australia (WA2), the last unresolved but the interrelations of these accessions and their relationships to the other groups are not well supported.

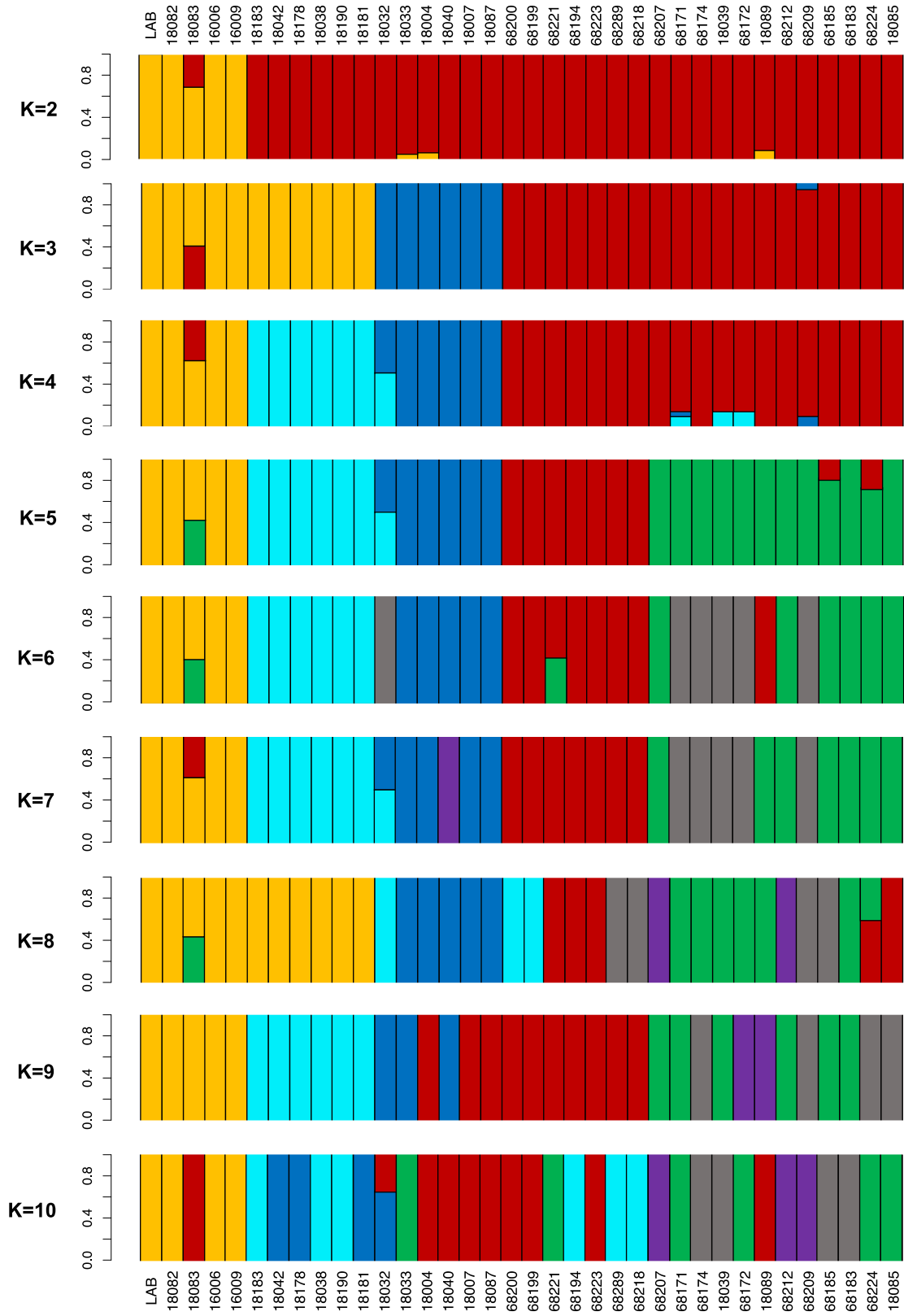


Fig. S2. Structure plots of *N. benthamiana* groups obtained in *NGSadmix*. The y values represent admixture proportion.

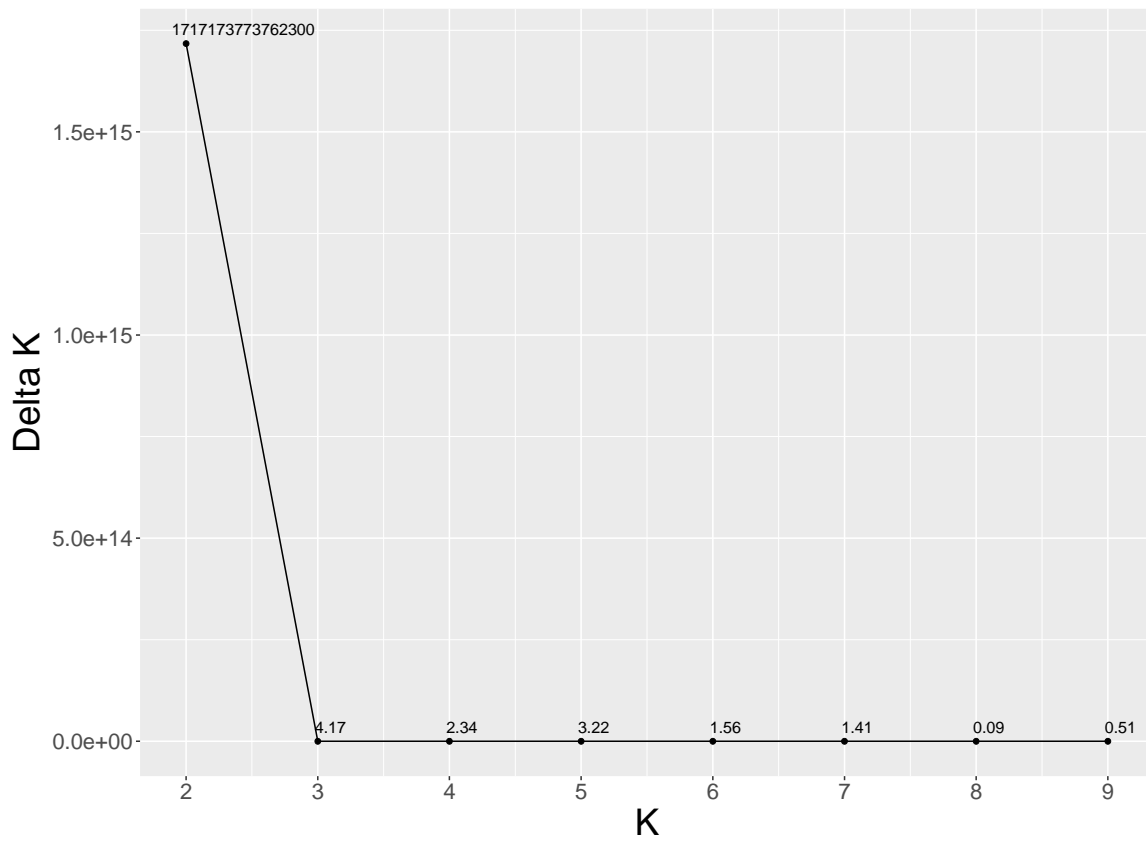


Fig. S3. Delta K values for the best K model obtained in *NGSadmix*. The best K was estimated in accordance with the Evanno method (<http://clumpak.tau.ac.il/bestK.html>).

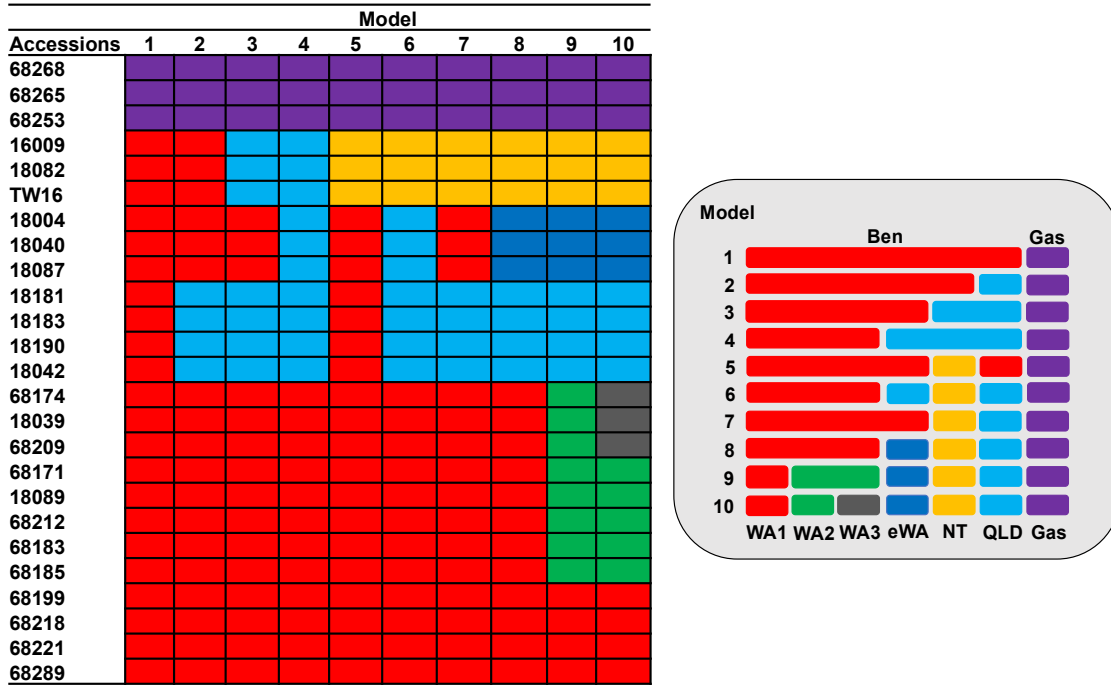


Fig. S4. Models and accessions used in the Bayesian species delimitation analysis in *SNAPP*.