**Supporting Information**

Article title: Reticulate evolution, ancient chloroplast haplotypes, and rapid radiation of the Australian plant genus – *Adenanthos* (Proteaceae)

Francis J. Nge1,2,4, Ed Biffin1,2, Kevin R. Thiele3, Michelle Waycott1,2

1School of Biological Sciences, Faculty of Science, The University of Adelaide, Adelaide, South Australia, 5005, Australia

2State Herbarium of South Australia, G.P.O. Box 1047, Adelaide, South Australia 5001

3School of Biological Sciences, University of Western Australia, 35 Stirling Hwy, Crawley (Perth), WA 6009, Australia

Author for correspondence: Francis Nge

4Corresponding author Email: francis.nge@adelaide.edu.au

**Supplementary Results**

**Phylogenetic relationships from nuclear loci**

*Adenanthos* sect. *Eurylaema* is monophyletic in both the ML and coalescent ASTRAL analyses with high support (BS = 100) (Figs. 2, 3). *Adenanthos* subsect. *Anaclastos* is polyphyletic with respect to *A.* subsect. *Adenanthos,* with *A. dobagii* and *A. drummondii* from *A.* subset. *Anaclastos* nested within clades B and F respectively in the ML analysis, and in clades B and C in the ASTRAL analysis (Figs. 2, 3). *Adenanthos apiculatus* from *A.* subset *Anaclastos* is also nested amongst species from *A.* subset *Adenanthos* in clade C of the ASTRAL tree (Fig. 3). *Adenanthos detmoldii* is shown to be nested amongst *A. barbigera* in both analyses. The southeastern Australian *Adenanthos* species are monophyletic in both analyses with high support (BB = 90; local branch support LBS = 53). The cultivated *A. sericeus* is sister to *A. dobagii, A. oreophila,* and the two subspecies of *A. sericeus* in the ML tree with high support (BS = 88) (Fig. 2), whereas it is shown to be sister to *A. oreophila* in the ASTRAL tree (LBS = 37) (Fig. 3). The putative hybrid *A. cuneatus* x *ellipticus* is sister to *A. ellipticus* in both ML and ASTRAL analyses (BB = 95, LBS = 45). *Adenanthos* x *cunninghamii* is sister to *A. pungens, A. ellipticus, A. cuneatus,* and *A. cuneatus* x *ellipticus* in both ML and ASTRAL analyses (BB = 67, LBS = 41). Lower support values at the backbone of the ASTRAL tree were the result of conflict from individual gene trees. The ML topologies of phased and unphased nuclear datasets were largely consistent, with majority of taxa have monophyleic alleles including the two putative hybrids (*A.* x *cunninghamii, A. cuneatus x ellipticus*) (Fig. S7).

**Phylogenetic relationships and conflict in plastid data**

In the plastid ML tree, none of the sections or subsections of *Adenanthos* are monophyletic in the plastid tree (Fig. 2). Clades D’ and E’ are resolved with high support (BS = 100/100) (Fig. 2). *Adenanthos obovatus* from *A.* sect. *Eurylaema* is nested in Clade D’ (BS = 100) with species belonging to *A.* sect. *Adenanthos,* whereas *A. barbigera* and *A. detmoldii* from *A.* sect. *Eurylaema* form Clade E’ (BS = 94) (Fig. 2). In contrast, all three species belonging to *A.* sect. *Eurylaema* are resolved as monophyletic in Clade D of the nuclear topology (Fig. 2). Similar to the nuclear dataset, *A. detmoldii* is nested within *A. barbigera.* The three species (*Adenanthos apiculatus, A. drummondii,* and *A. dobagii*)from *A.* subsect. *Anaclastos* are nested within clades containing species from *A.* subsect. *Adenanthos* (Clades D’, C’, A’ respectively) (Fig. 2).

The two southeastern *Australian* species (*A. macropodianus* and *A. terminalis*) are recovered as monophyletic (BS = 100) and sister to *A. cygnorum* and *A. stictus* in Clade D’ of the plastid topology (Fig. 2). However the relationship between the two taxa in the plastid tree is in conflict with the nuclear topology, as the Kangaroo Island endemic *A. macropodianus* is nested in *A. terminalis* in the plastid tree, being sister to the Kangaroo Island sample of *A. terminalis* (TST 1226) whereas the two samples of *A. terminalis* are recovered as monophyletic in the nuclear tree (Fig. 2).

The two subspecies of *Adenanthos glabrescens* are resolved as monophyletic and sister to *A. dobsonii* in the nuclear topology (Clade A; BS = 99), whereas the two subspecies are paraphyletic in the plastid topology (Clade C’) (Fig. 2).

*Adenanthos pungens* and allies (*A. cuneatus, A. ellipticus*) are shown to be monophyletic in Clade D (BS = 82) of the nuclear tree. In contrast, the subspecies of *A. pungens,* individuals of *A. cuneatus* and alliesare paraphyletic in the plastid tree (Fig. 2). *Adenanthos pungens* subsp. *pungens, A. cuneatus* FN403, and *A. linearis* are shown to be sister to *A. obovatus* with high support (BS = 98) in Clade D’ of the plastid tree. Whereas the other subspecies of *Adenanthos pungens* (subsp. *effusus*)and its allies (*A. cuneatus* x *ellipticus, A. ellipticus*, and *A. cuneatus* FN281) are in Clade B’ (BS = 100). The hybrid *A.* x *cunninghamii* is found to be sister to *A. pungens* and allies in the nuclear dataset but sister to the cultivated *A. sericeus* in the plastid dataset (Fig. 2).

**Supplementary material tables and figures**

**Table S1a** Newly sequenced *Adenanthos* samples used in this study, from freshly collected material, and associated State Herbarium of South Australia (AD) voucher id, along with other relevant information associated with each sample – date collected, and locality coordinates.

**Table S1b** Newly sequenced *Adenanthos* samples used in this study, sourced from herbarium specimens, living collections at Botanic Gardens, or South Australian Seed Centre (TST).

**Table S2a)** Proteaceae family-wide calibration scheme with six fossil calibration constraints following Sauquet *et al.* 2009, used in the BEAST dating analyses. Highlighted rows indicate calibration coonstraints. Y = monophyletic constraint on a group. d. = default parameters; **b)** Monophyletic constraints used in the Proteaceae family-wide calibration scheme.

**Table S3** Proteoideae subfamily calibration scheme with calibration constraints following Sauquet *et al.* 2009, used in the **a)** *matK*, and **b)** ITSBEAST dating analyses.

**Table S4** Secondary calibrations applied on NGS *Adenanthos* data using divergence-age estimates obtained from *matK* BEAST dating analysis.

**Table S5** Proteaceae outgroup taxa with associated GenBank accessions for sequences included in this study.

**Table S6** MCMC runs and ESS convergence for the BEAST analyses based on different calibration schemes.

**Table S7** *Adenanthos* taxa included in each subset JML analyses.

**Table S8** Divergence-age estimates derived from BEAST analyses of *Adenanthos* based on different datasets and calibration schemes.

**Table S9** Pairwise chloroplast distance comparisons with non-significant p-values (p > 0.1) for **a)** the 23 taxa full dataset; and significant p-values (p < 0.1) for **b)** subsets A, B, C.

**Table S1a** Newly sequenced *Adenanthos* samples used in this study, from freshly collected material, and associated State Herbarium of South Australia (AD) voucher id, along with other relevant information associated with each sample – date collected, and locality coordinates.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Collector's no. | voucher | Taxa | Date collected | Latitude | Longitude |
| FN232 | n/a | *Adenanthos terminalis* | Sep-17 | -36.576 | 140.519 |
| FN241 | n/a | *Adenanthos terminalis*  | Sep-17 | -36.353 | 140.353 |
| FN262 | AD280026 | *Adenanthos barbiger* | Sep-17 | -32.326 | 116.215 |
| FN270 | AD280008 | *Adenanthos apiculata* | Sep-17 | -34.589 | 118.500 |
| FN274 | AD280007 | *Adenanthos flavidiflorus* | Sep-17 | -34.339 | 118.735 |
| FN280 | AD280021 | *Adenanthos venosus* | Sep-17 | -33.923 | 120.031 |
| FN281 | n/a | *Adenanthos cuneatus* | Sep-17 | -33.930 | 120.016 |
| FN282 | AD280020 | *Adenanthos ellipticus* | Sep-17 | -33.931 | 120.014 |
| FN284 | AD280024 | *Adenanthos cuneatus* x *ellipticus* | Sep-17 | -33.931 | 120.014 |
| FN287 | AD280019 | *Adenanthos oreophilus* | Sep-17 | -33.888 | 119.923 |
| FN290 | AD279995 | *Adenanthos glabrescens* subsp. *exaperatus* | Sep-17 | -33.707 | 120.469 |
| FN298 | AD280032 | *Adenanthos glabrescens* subsp. *glabrescens* | Sep-17 | -33.271 | 119.650 |
| FN300 | AD280014 | *Adenanthos barbigera* | Sep-17 | -33.783 | 115.428 |
| FN304 | AD280016 | *Adenanthos meisneri* | Sep-17 | -33.749 | 115.492 |
| FN305 | AD280006 | *Adenanthos obovatus* | Sep-17 | -33.749 | 115.492 |
| FN308 | AD280003 | *Adenanthos detmoldii* | Sep-17 | -34.202 | 115.267 |
| FN310 | AD280004 | *Adenanthos barbiger* | Sep-17 | -33.509 | 115.749 |
| FN311 | AD280005 | *Adenanthos obovatus* | Sep-17 | -34.202 | 115.267 |
| FN312 | AD280001 | *Adenanthos meisneri* | Sep-17 | -34.202 | 115.267 |
| FN319 | AD280011 | *Adenanthos cygnorum* | Sep-17 | -32.022 | 115.981 |
| FN325 | n/a | *Adenanthos cygnorum* subsp. *cygnorum* | Sep-17 | -30.794 | 115.582 |
| FN326 | AD280010 | *Adenanthos stictus* | Sep-17 | -29.944 | 116.060 |
| FN353 | n/a | *Adenanthos obovatus* | Sep-17 | -32.135 | 115.949 |
| FN403 | n/a | *Adenanthos cuneatus* | Sep-17 | -34.426 | 117.741 |
| FN430 | n/a | *Adenanthos sericeus* cultivated | 15-May-18 | -34.920 | 138.605 |

**Table S1b** Newly sequenced *Adenanthos* samples used in this study, sourced from herbarium specimens, living collections at Botanic Gardens, or South Australian Seed Centre (TST).

|  |  |  |
| --- | --- | --- |
| Collector's no. | Taxa | Voucher |
| FN330 | *Adenanthos cygnorum* subsp. *chamaephyton* | Kings Park living collection |
| FN327 | *Adenanthos dobagii* | Kings Park living collection |
| FN329 | *Adenanthos linearis*  | Kings Park living collection |
| FN328 | *Adenanthos* x *cunninghamii*  | Kings Park living collection |
| FN338 | *Adenanthos ileticos*  | MEL 2019219 |
| FN333 | *Adenanthos cacomorphus* | Newbey 2779 |
| FN341 | *Adenanthos velutinus* | PERTH 03487059 |
| FN331 | *Adenanthos acanthophyllus* | PERTH 05272742 |
| FN343 | *Adenanthos sericeus* subsp. *sphalma* | PERTH 0576248 |
| FN334 | *Adenanthos dobsonii* | PERTH 07703791 |
| FN342 | *Adenanthos sericeus* subsp. *sericeus* | PERTH 07730225 |
| FN335 | *Adenanthos filifolius* | PERTH 08014620 |
| FN336 | *Adenanthos forrestii* | PERTH 08067791 |
| FN339 | *Adenanthos pungens* subsp. *effusus* | PERTH 08422419 |
| FN332 | *Adenanthos argyreus* | PERTH 08568766 |
| FN340 | *Adenanthos pungens* subsp. *pungens* | PERTH 08632782 |
|  | *Adenanthos gracilipes* | PERTH 08802041 |
| TST 1222 | *Adenanthos macropodianus* | AD voucher pending |
| TST 1226 | *Adenanthos terminalis* | AD voucher pending |

**Table S2a** Proteaceae family-wide calibration scheme with six fossil calibration constraints following Sauquet *et al.* 2009, used in the BEAST dating analyses. Highlighted rows indicate calibration constraints. Y = monophyletic constraint on a group. d. = default parameters.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **All Proteaceae calibration** | **monophyletic** | **Prior** | **M** | **S** | **Offset** | **age (Ma)** |
| a) Prot stem prior |  | normal | 110 | d. | d. | 110 |
| a) Prot mono  | **y** |  |  |  |  |  |
| b) Banksia mono | **y** |  |  |  |  |  |
| b) Banksieae stem |  | uniform | 55.8–100 | d. | d. | 55.8 |
| c) pan-Musgravieae stem |  | uniform | 33.9–100 | d. | d. | 33.9 |
| d) Macadamia crown prior (mono) | y | uniform | 70.6–100 | d. | d. | 70.6 |
| e) Embotrium stem | y | uniform | 35.4–100 | d. | d. | 35.4 |
| e) Embo mono | **y** |  |  |  |  |  |
| f) Franklandia stem (mono) | y | uniform | 70.6–100 | d. | d. | 70.6 |
| f) Adenanthos mono | **y** |   |   |   |   |   |

**Table S2b** Monophyletic constraints used in the Proteaceae family-wide calibration scheme.

|  |  |
| --- | --- |
| **All Proteaceae calibration** | **monophyletic constraint** |
| a) Proteaceae stem  | mono constraint (all except *Platanus*) |
|  |  |
| b) *Banksieae* stem | *Austromullera* |
|  | *Banksia, Dryandra* |
|  | *Musgravea* |
|  | *Sphalmium* |
|  |  |
| d) *Macadamia* crown  | *Malagasia\_alticola\_* |
|  | *Athertonia\_diversifolia\_* |
|  | *Macadamia\_claudiensis\_* |
|  | *Brabejum\_stellatifolium\_* |
|  | *Panopsis\_* |
|  | *Panopsis\_yolombo\_* |
|  | *Cardwellia\_sublimis\_* |
|  | *Gevuina\_avellana\_* |
|  | *Hicksbeachia\_pinnatifolia\_* |
|  | *Euplassa\_duquei\_* |
|  | *Euplassa\_occidentalis\_* |
|  |  |
| e) *Embotrium* stem | *Embothrium\_coccineum\_* |
|  | *Alloxylon\_flammeum\_* |
|  | *Oreocallis\_mucronata\_* |
|  |  |

**Table S2b** cont.

|  |  |
| --- | --- |
| **All Proteaceae calibration** | **monophyletic constraint** |

|  |  |
| --- | --- |
| e) *Embotrium* mono. | Alloxylon\_flammeum\_ |
|  | Oreocallis\_mucronata\_ |
|  |  |
| f) *Franklandia* stem  | *Franklandia\_fucifolia\_* |
|  | *Isopogon\_* |
|  | *Adenanthos\_sericeus\_* |
|  | *Leucadendron\_* |
|  | *Leucadendron\_uniflorum\_* |
|  | *Paranomus\_bracteolaris\_* |
|  | *Vexatorella\_alpina\_* |
|  | *Serruria\_longipes\_* |
|  | *Serruria\_trilopha\_* |
|  | *Leucospermum\_pedunculatum\_* |
|   | *Diastella\_divaricata\_* |

**Table S3a** Proteoideae subfamily calibration scheme with one fossil calibration (*Franklandia*) and secondary calibrations following Sauquet *et al.* 2009, used in the *matK* BEAST dating analyses.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Proteoideae calibration** | **monophyletic** | **Prior** | **M** | **S** | **Offset** | **age (Ma)** |
| *Synaphea* | y | log-normal | 1.8 | 0.3 | 76.5 | 82 |
| *Franklandia* | y | log-normal | 1 | 1.2 | 70.6 | 70.6 |
| *Adenanthos* | y |  |  |  |  |  |

**Table S3b** Proteoideae subfamily calibration scheme with secondary calibrations constraints following Sauquet *et al.* 2009, used in the ITSBEAST dating analyses.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Proteoideae calibration** | **monophyletic** | **Prior** | **M** | **S** | **Offset** | **age (Ma)** |
| *Synaphea* | y | log-normal | 1.8 | 0.3 | 76.5 | 82 |
| *Protea, Faurea* | y | log-normal | 1.8 | 0.2 | 68 | 74 |
| Proteaceae max constraint |   | uniform | 0–120 |   |   | 120 |

**Table 4** Secondary calibrations applied on NGS *Adenanthos* data using divergence-age estimates obtained from *matK* BEAST dating analysis.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Adenanthos* calibration** | **monophyletic** | **Prior** | **M** | **S** | **Offset** | **age (Ma)** |
| *Isopogon* stem |  | log-normal | 1 | 0.23 | 41.5 | 44 |
| *Adenanthos* crown | y | log-normal | 1 | 0.23 | 16 | 18.5 |

**Table S5** Proteaceae outgroup taxa with associated GenBank accessions for sequences included in this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Number** | **Taxa** | **ITS** | ***matK*** | ***rbcL*** |
| 1 | *Acidonia\_microcarpa\_* | EU676080 |  |  |
| 2 | *Adenanthos\_sericeus\_*Genbank |  | EU169606 | DQ875820 |
| 3 | *Agastachys\_odorata\_* |  | EU169607 | DQ875821 |
| 4 | *Alloxylon\_flammeum\_* | EU676081 | EU169608 | DQ875822 |
| 5 | *Athertonia\_diversifolia\_* |  | EU169609 |  |
| 6 | *Aulax\_* |  | EU169610 | DQ875823 |
| 7 | *Austromuellera\_trinervia\_* |  |  | DQ875824 |
| 8 | *Banksia\_* | EU676082 | EU169611 | DQ875825 |
| 9 | *Beauprea\_* |  | EU169612 | DQ875826 |
| 10 | *Bellendena\_montana\_* |  | EU169613 | DQ875827 |
| 11 | *Brabejum\_stellatifolium\_* |  |  | DQ875828 |
| 12 | *Buckinghamia\_ferruginiflora\_* |  | EU169614 | DQ875829 |
| 13 | *Cardwellia\_sublimis\_* |  | EU169615 | DQ875830 |
| 14 | *Carnarvonia\_araliifolia\_* |  | EU169616 | DQ875831 |
| 15 | *Cenarrhenes\_nitida\_* |  |  | DQ875832 |
| 16 | *Conospermum\_* |  | EU169617 | DQ875833 |
| 17 | *Darlingia\_darlingiana\_* | EU676083 | EU169618 |  |
| 18 | *Diastella\_divaricata\_* |  | EU169619 |  |
| 19 | *Dilobeia\_thouarsii\_* |  | EU169620 |  |
| 20 | *Dryandra\_* | EU676084 | EU169621 |  |
| 21 | *Eidothea\_zoexylocarya\_* |  | EU169622 | DQ875834 |
| 22 | *Embothrium\_coccineum\_* | EU676085 |  | DQ875835 |
| 23 | *Eucarpha\_deplanchei\_* | EU676086 |  |  |
| 24 | *Euplassa\_duquei\_* |  | EU169623 |  |
| 25 | *Euplassa\_occidentalis\_* |  | EU169624 |  |
| 26 | *Faurea\_forficuliflora\_* |  | EU169625 |  |
| 27 | *Floydia\_praealta\_* |  | EU169626 | DQ875836 |
| 28 | *Franklandia\_fucifolia\_* |  | EU169627 | DQ875837 |
| 29 | *Garnieria\_spathulifolia\_* | EU676087 | EU169628 |  |
| 30 | *Gevuina\_avellana\_* |  |  | DQ875838 |
| 31 | *Grevillea\_* |  | EU169629 | DQ875839 |
| 32 | *Grevillea\_juniperina\_* |  | EU169630 |  |
| 33 | *Grevillea\_robusta\_* |  | EU169631 |  |
| 34 | *Hakea\_* | EU676088 | EU169632 |  |
| 35 | *Hakea\_*sp.\_1-Anderson\_7\_ |  | EU169633 |  |
| 36 | Hakea\_sp.\_2-Anderson\_8b\_ |  | EU169634 |  |
| 37 | *Helicia\_* | EU676089 | EU169635 | DQ875840 |
| 38 | *Hicksbeachia\_pinnatifolia\_* |  |  |  |
| 39 | *Hollandaea\_riparia\_* | EU676090 |  | DQ875841 |
| 40 | *Isopogon\_* |  | EU169636 | DQ875842 |
| 41 | *Knightia\_excelsa\_* | EU676091 | EU169637 | DQ875843 |
| 42 | *Lambertia\_* | EU676092 | EU169638 | DQ875844 |
| 43 | *Lasjia\_hildebrandii\_* |  | EU169639 |  |
| 44 | *Leucadendron\_* |  | EU169640 | DQ875845 |

**Table S5** cont.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Number** | **Taxa** | **ITS** | ***matK*** | ***rbcL*** |
| 45 | *Leucadendron\_uniflorum\_* |  | EU169641 |  |
| 46 | *Leucospermum\_pedunculatum\_* |  | EU169642 |  |
| 47 | *Lomatia\_* | EU676093 | EU169643 | DQ875846 |
| 48 | *Lomatia\_silaifolia\_* |  | EU169644 |  |
| 49 | *Macadamia\_claudiensis\_* |  |  | DQ875847 |
| 50 | *Malagasia\_alticola\_* |  | EU169645 |  |
| 51 | *Megahertzia\_amplexicaulis\_* | EU676094 |  |  |
| 52 | *Mimetes\_hirtus\_* |  | EU169646 |  |
| 53 | *Musgravea\_heterophylla\_* | EU676095 |  | DQ875848 |
| 54 | *Neorites\_kevediana\_* | EU676096 |  | DQ875849 |
| 55 | *Opisthiolepis\_heterophylla\_* | EU676097 | EU169647 | DQ875850 |
| 56 | *Oreocallis\_mucronata\_* | EU676098 | EU169648 |  |
| 57 | *Orites\_* |  | EU169649 |  |
| 58 | *Orites\_myrtoidea\_* | EU676099 |  | DQ875851 |
| 59 | *Panopsis\_* |  | EU169650 | DQ875852 |
| 60 | *Panopsis\_yolombo\_* |  | EU169651 |  |
| 61 | *Paranomus\_bracteolaris\_* |  | EU169652 |  |
| 62 | *Persoonia\_linearis\_* |  | EU169653 |  |
| 63 | *Petrophile\_* |  | EU169654 | DQ875853 |
| 64 | *Placospermum\_coriaceum\_* | EU676100 |  | DQ875854 |
| 65 | *Platanus\_* |  | EU169655 | DQ875855 |
| 66 | *Platanus\_wrightii\_* |  | EU169656 |  |
| 67 | *Protea\_* |  | EU169657 | DQ875856 |
| 68 | *Protea\_neriifolia\_* |  | EU169658 |  |
| 69 | *Protea\_susannae\_* |  | EU169659 |  |
| 70 | *Roupala\_montana\_* | EU676101 | EU169660 |  |
| 71 | *Serruria\_longipes\_* |  | EU169661 |  |
| 72 | *Serruria\_trilopha\_* |  | EU169662 |  |
| 73 | *Sphalmium\_racemosum\_* |  |  | DQ875857 |
| 74 | *Stenocarpus\_salignus\_* | EU676102 | EU169663 | DQ875858 |
| 75 | *Stenocarpus\_sinuatus\_* |  | EU169664 |  |
| 76 | *Stenocarpus\_trinervis\_* |  |  |  |
| 77 | *Stirlingia\_latifolia\_* |  | EU169665 | DQ875859 |
| 78 | *Strangea\_linearis\_* | EU676103 |  |  |
| 79 | *Symphionema\_montanum\_* |  | EU169666 | DQ875860 |
| 80 | *Synaphea\_polymorpha\_* |  | EU169667 |  |
| 81 | *Synaphea\_spinulosa\_* |  | EU169668 | DQ875861 |
| 82 | *Telopea\_* | EU676104 |  | DQ875862 |
| 83 | *Telopea\_speciosissima\_* |  | EU169669 |  |
| 84 | *Toronia\_toru\_* |  |  | DQ875863 |
| 85 | *Triunia\_*sp.\_Lundberg\_44\_ |  | EU169670 |  |
| 86 | *Triunia\_youngiana\_* | EU676105 | EU169671 | DQ875864 |
| 87 | *Vexatorella\_alpina\_* |  | EU169672 |  |
| 88 | *Xylomelum\_* |  | EU169673 |  |
| 89 | *Xylomelum\_pyriforme\_* | EU676106 |   | DQ875865 |

**Table S6** MCMC runs and ESS convergence for the BEAST analyses based on different calibration schemes.

|  |  |  |
| --- | --- | --- |
| **Dataset** | **MCMC (million)** | **ESS convergence** |
| **Proteaceae-wide cal.** |  |  |
| NGS chloroplast data | 300 | 3536 |
| NGS nuclear data | 300 | 441 |
| ITS, *matk, rbcL*  | 100 | 310 |
| ITS | 200 | 808 |
|  |  |  |
| **Secondary NGS cal.** |  |  |
| NGS chloroplast data | 200 | 13702 |
| NGS nuclear data | 200 | 366 |
| NGS combine data | 600 | 31900 |
|  |  |  |
| **Proteoideae cal.** |  |  |
| ITS | 10 | 340 |
| *matK* | 10 | 1494 |

**Table S7** *Adenanthos* taxa included in each subset JML analyses.

|  |  |  |
| --- | --- | --- |
| **Subset A** | **Subset B** | **Subset C** |
| *A. obovatus* (3) | *A. cygnorum* (3) | *A. pungens* (2) |
| *A. barbigera* (3) | *A. sericeus* (2) | *A. cuneatus x ellipticus* |
| *A. linearis*  | *A. stictus* | *A. ellipticus* |
|  |  | *A. cuneatus* (2) |
|  |  | *A. meisneri*  |
|  |  | *A. stictus* |
|  |  | *A. linearis* |

**Table S8** Divergence-age estimates derived from BEAST analyses of *Adenanthos* based on different datasets and calibration schemes.

|  |  |  |  |
| --- | --- | --- | --- |
|   | **Proteaceae-wide calibration** | **Proteoideae calibration** | **NGS secondary calibration** |
|   | **chl NGS** | **nu NGS** | **combine ITS, *matK, rbcl*** | **ITS** | ***matK\**** | **nu NGS** | **chl NGS** | **combine NGS** |
| *Adenanthos* taxa no. | 39 | 42 | 43 | 36 | 43 | 43 | 39 | 39 |
| basepairs | 43945 | 39172 | 12266 | 867 | 4634 | 26900 | 34218 | 65549 |
|  |  |  |  |  |  |  |  |  |
| ***Adenanthos* crown** | 24 | 32.7 | 25.1 | 18.7 | **18.7** | **18.7\*** | **18.5\*** | **18.7\*** |
| ***Adenanthos* stem** | 36.1 | 39.3 | 38.4 | - | 44.4 | - | - | - |
| *Franklandia* stem | 73.4 | 73.2 | 73.9 | - | 72.3 | - | - | - |
| *Isopogon* stem | 47.6 | 49 | 49.4 | - | 44.4 | - | - | - |
| South Australian Adenanthos crown (*A. macropodianus, A. terminalis*) | 1.3 | 15.3 | 3.35 | 8.4 | 1.2 | 9.4 | 1.62 | 7.3 |
| South Australian Adenanthos stem (*A. macropodianus, A. terminalis*) | 16.59 | 21.6 | 20.8 | 9.71 | 16.5 | 12.6 | 15 | 17.4 |
|  |   |   |   |   |   |   |   |   |
| *A. obovatus* – *A. barbigera* divergence | 20.52 | 22 | 21.97 | 9.08 | 15.4 | 13.74 | 18.7\* | 18.7\* |
|  |   |   |   |   |   |   |   |   |
| *A. obovatus* crown | 3.5 | 11.7 | - | 1.27 | 2.9 | 7.5 | 2.8 | 5.7 |
| *A. barbigera* crown | 5.4 | 11.9 | - | 1.14 | 5.7 | 7.1 | 4.7 | 5.9 |
|  |   |   |   |   |   |   |   |   |
| *A. acanthophyllus* | 24 | 14.8 | - | - | 18.5 | - | - | - |

**\****Adenanthos* crown derived from *matK* BEAST divergence analysis.

**Table S9a** Pairwise chloroplast distance comparisons with non-significant p-values (p > 0.1) for the 23 taxa full dataset, all other pairwise combinations (244/253) were signficant (p < 0.1).

|  |  |
| --- | --- |
| **23 taxa dataset** | **p value > 0.1** |
| *A. cuneatus*57 – *A. ellipticus*54 | 0.79 |
| *A. argyreus*69 – *A. drummondii*47 | 0.452 |
| *A. barbigera*44 – *A. detmoldii*42 | 0.426 |
| *A. cygnorumC*67 – *A. cygnorumC*66 | 0.206 |
| *A. detmoldii*42 –*A. barbigera*2 | 0.181 |
| *A. terminalis*78 – *A. cygnorumC*67 | 0.143 |
| *A. terminalis*78 – *A. cygnorumCh*68 | 0.134 |
| *A. argyreus*69 – *A. linearis*52 | 0.125 |
| *A. cygCh*68 – *A. cygnorumC*67 | 0.101 |

**Table S9b** Pairwise chloroplast distance comparisons with significant p-values (p < 0.1) for subsets A, B, C.

|  |  |
| --- | --- |
| **Subset A** | **p value < 0.1** |
| *A. linearis – A. obovatus* | 0.092 |
|  |  |
| **Subset B** |  |
| *A. stictus – A. sericeus* | 0.048 |
|  |  |
| **Subset C** |  |
| *A. meisneri – A. cuneatus x ellipticus* | 0.075 |
| *A. meisneri – A. ellipticus* | 0.056 |
| *A. pungens – A. linearis* | 0.051 |
| *A. pungens* (1,2) *– A. meisneri* | 0.098, 0.098 |
| *A. stictus – A. cuneatus x ellipticus* | 0.083 |
| *A. stictus – A. cuneatus*  | 0.084 |
| *A. stictus – A. meisneri* | 0.094 |
| *A. stictus – A. pungens* (1,2) | 0.09, 0.029 |