

Phylogenetic Relationships of Andromonoecious and Dioecious Australian Species of *Solanum* subgenus *Leptostemonum* section *Melongena*: Inferences from ITS Sequence Data

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ABSTRACT. This is the first extensive molecular phylogeny for any group of *Solanum* in Australia. A total of 64 specimens representing 29 taxa were sampled, including 18 endemic Australian members of subgenus *Leptostemonum* (the “spiny solanums”) section *Melongena* sensu Symon. Data from the rDNA ITS region were analyzed using parsimony, maximum likelihood, and Bayesian methods to test the hypothesis that the dioecious and andromonoecious Australian species of section *Melongena* constitute a monophyletic group. Analyses showed support for the recognition of five clades among the Australian species of the section, but little support for the monophyly of the section itself. Australian dioecious *Solanum* species form two distinct clades and a number of enigmatic and previously unplaced dioecious taxa are here placed within a single clade. Three groups of andromonoecious species are also each monophyletic and are nested in a polytomy with three clusters of species from outside section *Melongena*. Furthermore, the phylogeny indicates that dioecy has evolved either once or twice in Australian *Solanum*, possibly from andromonoecious ancestry.

KEYWORDS: andromonoecy, breeding system, dioecy, inaperturate pollen, Kimberley Plateau, Solanaceae.

Nearly two decades ago, Anderson and Symon (1989) confirmed that all of the *Solanum* species in Australia presumed to be androdioecious (Symon 1979) were in fact cryptically dioecious. Extensive morphological and crossing studies led to the discovery that the pollen grains produced by the hermaphroditic flowers of the nine dioecious species were inaperturate (Zavada and Anderson 1997; Zavada et al. 2000) and non-germinable, rendering the individuals that bear them functionally pistillate. These species are thus functionally dioecious even though individual plants appear to be either staminate or hermaphroditic. As such, functionally pistillate plants (with normal-appearing anthers that produce a large quantity of ingerminal pollen) are still able to attract (via the anthers) and reward (the pollen itself) bees as pollinators and avoid the potential costs (e.g., inbreeding depression) of self-fertilization in small, isolated populations (Holsinger 1993, 2000). This discovery corroborated previous work on dioecy in *Solanum* (Anderson 1979; Anderson and Levine 1982; Levine and Anderson 1986; Anderson and Symon 1988), and supported the contention that true androdioecy is rare or absent in the angiosperms (Charlesworth 1984).

The reproductive biology and crossing studies Anderson and Symon (1989) carried out with 19 species of spiny solanums yielded a functional classification with 10 species in “Group I” (andromonoecious) and nine in “Group II” (dioecious). Aside from an additional andromonoecious species (*S. campanulatum*), these two groups constitute the Australian members of *Solanum* subgenus *Leptostemonum* section *Melongena* as treated by Symon (1981) in his comprehensive mono-

graph of the genus in Australia. These two groups also constitute the “dioicum group” as circumscribed by Whalen (1984) and later used by Bean (2004). Because of uncertainty about whether these species truly are closely related (Symon 1981; Whalen 1984; Anderson and Symon 1989), it has not been possible to conclude much about the evolution of dicliny in Australian *Solanum*. For example, what are the relationships of the dioecious species to each other and to the andromonoecious species, and how many times did dioecy and andromonoecy arise? In particular, it has been difficult to conclude whether dicliny in subgenus *Leptostemonum* has followed the hypothetical path (Bawa 1980; Charlesworth 1984; Anderson and Symon 1989) from hermaphroditic-flowered ancestors (plesiomorphic in subgenus *Leptostemonum*), through andromonoecious-flowered intermediate stages (plesiomorphic in section *Melongena*), to an ultimate culmination in one or more lineages of dioecious-flowered species.

These questions are of interest not only because of the rarity of dioecy in general (present in only 6–10% of angiosperm species [Yampolsky and Yampolsky 1922; Renner and Riklefs 1995]) and in Solanaceae (<1% of 2300 species [Anderson 1979; Symon 1979; Anderson and Levine 1982; Levine and Anderson 1986; Anderson and Symon 1989; Knapp et al. 1998; Sawyer and Anderson 2000]), but also because few examples exist of taxonomic groups in which hermaphroditism, andromonoecy, and the likely derived condition of dioecy all are present. Because all three of these breeding systems (as per the definition of Neal and Anderson 2004) are found in the genus *Solanum*, it has been an ideal group in which to study breeding

system evolution (Anderson and Stebbins 1984; Anderson and Symon 1989). However, until robust phylogenies exist for the *Solanum* clades where dioecy occurs, it is not possible to trace the evolutionary history of dioecy in the genus. The group of species constituting the Australian members of Symon's (1981) section *Melongena* is an appropriate group to use for such a study. Of the 15 known occurrences of dioecy in *Solanum*, 10 (including a recently described species [Brennan et al. in press]) occur in this particular group of species distributed across the sub-arid tropical region of northwestern Australia. In particular, the dioecious Australian solanums are split between two subregions: the Kimberley Plateau of far northern Western Australia and the Kakadu National Park area of the Northern Territory's "Top End."

Solanum is among the largest genera of flowering plants, comprising approximately 1500 species (Levin et al. 2006). Systems of infrageneric classification have been proposed by Dunal (1852), Seithe (1962), Danert (1970), and D'Arcy (1972). All four treatments recognized subgenus *Leptostemonum* (Dunal) Bitter (the so-called "spiny solanums"), with D'Arcy (1972) splitting the subgenus into 22 sections, including section *Melongena*. Morphological characterization of *Leptostemonum* is based primarily on the presence of stellate pubescence, prickly stems and leaves, and attenuate anthers, all considered to be derived characters in *Solanum* (Whalen 1984). More than 450 species are included in the subgenus, with the highest diversity of species found in tropical America, Africa, and Australia (Whalen 1984). The only taxonomic monograph of *Leptostemonum* was published by Whalen (1984). He included 33 species groups rather than the 22 sections that D'Arcy (1972) recognized, with many of the differences representing further splitting of comparable groupings. Recent molecular phylogenetic work (Bohs and Olmstead 1997; Olmstead and Palmer 1997; Levin et al. 2006) supports the monophyly of *Leptostemonum*, as well as the monophyly of the Old World species within the subgenus, but the studies also call into question some of the affiliations of both D'Arcy's sections and Whalen's species groups, including the monophyly of section *Melongena*.

Symon's (1981) taxonomic treatment of species in section *Melongena* was not intended to support phylogenetic monophyly (Anderson and Symon 1989). He distinguished the Australian members of the section primarily on the basis of their mostly woody habit, large yellow berries, and dark seeds. His concept of the group is narrower than that of D'Arcy (1972), as illustrated by Symon moving a number of Australian species to section *Torvum* that had been previously included by D'Arcy in section *Melongena*. Symon also placed the andromonoecious *S. campanulatum* of south-eastern Australia (a species incorrectly identified as di-

oecious by Knapp et al. [1998]) in the monotypic section *Campanulata* Symon. Symon (1981) considered the dioecious species as a group, but considered them "discordant" and without obvious andromonoecious progenitors in Australia. However, the working hypothesis of Anderson and Symon (1989), as well as of this study, is that all or some of the andromonoecious species (i.e., the Group I cited previously, excluding *S. campanulatum*) share a most recent common ancestor with all or some of the dioecious species (i.e., Group II).

We present the results of analyses using the internal transcribed spacer region (ITS) of the nuclear ribosomal DNA in parsimony, likelihood, and Bayesian frameworks. The purpose of this study was to conduct a molecular analysis of Australian andromonoecious and dioecious *Solanum* species in order to test: 1) the monophyly of section *Melongena* sensu Symon (1981), 2) the monophyly of Anderson and Symon's (1989) Groups I and II, and 3) whether an evolutionary pathway from andromonoecy to dioecy can be identified in *Solanum*. By examining the evolution of these species, we hope to determine the best strategy for partitioning the group into natural units for further study.

MATERIALS AND METHODS

Taxon Sampling and Outgroup Selection. Herbarium specimens were acquired through loans from AD (300+ sheets selected by D. Symon to represent geographic and morphological diversity) and NY. Difficulties experienced with extracting clean DNA from many of the older specimens made a new collecting expedition an immediate priority. In May–June 2004, field expeditions were made in the Kakadu region (C. Martine, D. Symon, K. Brennan, and H. Toelken) and the Northern Kimberley (C. Martine and W.R. Barker). More than 250 additional *Solanum* vouchers were made during these trips, including a number of collections representing new localities as well as key collections used to describe a new dioecious species of *Solanum* (Brennan et al. in press). Collection, voucher, and GenBank information is listed in Appendix 1.

Leaf samples from all field-collected specimens were placed immediately in NaCl-cetyltrimethylammonium bromide (CTAB) gel preservative (Rogstad 1992) in 30 ml Nalgene HDPE bottles, a technique that allows for indefinite storage of "fresh" leaf material for later use in molecular studies. Multiple voucher specimens were made for each individual plant from which leaf samples were taken, and all vouchers were annotated by D. Symon at AD. Herbarium vouchers for new collections are accessioned at CONN and AD, with regionally appropriate specimens also deposited at PERTH and DNA (Darwin).

Outgroups were selected based on inferences from Symon (1981), Whalen (1984), Levin et al. (2006), and preliminary analyses using different combinations of available candidate species from subgenus *Leptostemonum*, eliminating those species that displayed very long branches and large pairwise distances from the ingroup taxa (e.g., *S. oligandrum*, *S. pugionuliferum*, and *S. sturtianum*). Thus, *S. aculeastrum* of Africa and *S. torvum* of the New World were selected as suitable outgroup taxa.

DNA Extraction and Purification. Total DNA was extracted from gel-preserved specimens using the CTAB method for fresh material of Doyle and Doyle (1987, 1990). Extractions from selected herbarium specimens were made using the CTAB protocol as modified for dried specimens by Lockermann and Jansen (1996). For particularly difficult dried specimens, the modified protocol

of Witzell (1999), including a three-day isopropanol precipitation at -20°C (Drabkova et al. 2002) was followed.

Amplification and Sequencing. Double-stranded DNA was generated using PCR to amplify the ITS-1, ITS-2, and 5.8s nuclear ribosomal DNA regions using the following primer combinations: a) ITS-5 and ITS-4 (White et al. 1990); b) ITS-leu1 and ITS-4 (Bohs and Olmstead 2001); and c) ITS-5 and ITS-2 in combination with ITS-3 and ITS-4. Cycle sequencing was done using the ITS-5 and ITS-4 primers. PCR amplification reactions, which were made up to 25 μL , contained 15.37 μL of ddH_2O , 2.0 μL of 50mM MgCl_2 , 2.5 μL 10X NH_4 buffer, 2.5 μL DMSO, 0.5 μL of each primer, 0.5 μL of dNTPs, 0.13 μL of Taq polymerase, and 1 μL of DNA template. The PCR amplification program for the ITS region was: a single denature step at 94°C for 3 minutes, followed by 30 cycles of 94°C for 1 minute, an annealing step at 50°C for 30 seconds, and a 1 minute extension at 72°C . PCR amplifications were cleaned using the QIAquick PCR Purification Kit columns (Qiagen) following the manufacturer's protocol. Forward and reverse sequencing reactions of the purified PCR templates were made up to 10 μL using 3 μL of ddH_2O , 2 μL of Big Dye terminator mix, 0.5 μL DMSO, 0.5 μL of the primer, and 4 μL of the template. Sequence cycling settings were 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes.

Forward and reverse sequences were analyzed using an ABI 3100 DNA automated sequencer (Applied Biosystems). Clean ITS sequences were acquired from a total of 68 accessions representing 18 Australian species included in section *Melongenae* by Symon, two previously undescribed species, and six related species included as outgroup taxa. The sequences were aligned manually and analyzed for polymorphisms and/or variable sites using Sequencher (Gene Codes, Ann Arbor, MI) and MacClade 4 (Maddison and Maddison 2000). Ambiguous leading and trailing regions of sequence were excluded from the analysis. All new sequences were submitted to GenBank (Appendix 1) and the data sets and representative trees deposited in TreeBASE [study accession number S1441, matrix accession number M2593].

Maximum Likelihood Analysis. We used DTModSel (Minin et al. 2003) to choose a best-fit model of nucleotide substitution. This program uses the Bayesian Information Criterion (BIC) (Schwarz 1978) in a decision theory framework to make simultaneous comparisons of non-nested models. The method is performance-based, using relative branch length estimation error as a measure of performance.

The portable version of PAUP* version 4b10 (Swofford 2002) was used for all maximum likelihood analyses. Forty heuristic searches were performed using starting trees obtained from random stepwise sequence addition. These were swapped using the tree-bisection-reconnection (TBR) branch-swapping algorithm. Parameter estimates were obtained through a series of refinement steps (Sullivan and Swofford 1997) in which a starting tree was estimated using parsimony. Parameters for the model selected by DTModSel were first estimated then fixed for this starting topology. This tree was then swapped using TBR for 200 rearrangements. Parameters were then re-estimated on the new topology, fixed, and swapped for an additional 200 rearrangements. Parameters were estimated one final time and fixed for the likelihood search. Resulting trees were viewed using TreeViewX (Page 1996). Maximum likelihood bootstrap analysis was performed using the model and parameter estimates obtained for the maximum likelihood search; 235 bootstrap pseudo-replicates were performed with one random addition sequence replicate. The resulting starting trees were swapped using TBR up to a limit of 15,000 rearrangements. The bootstrap analysis was spread out over 10 different files with 20 pseudo-replicates per file, which were executed on a 16 node Apple G5 Xserve cluster with each node running dual 2.3 Ghz processors. The resulting trees were imported into the graphical user interface version of PAUP* version 4b10 (Swofford 2002) with the "store tree weights" option in effect. These were combined as a majority rule consensus tree with the "use tree weights" option selected.

Parsimony Analysis. An equally weighted maximum parsimony analysis was performed with PAUP* version 4b10. Starting trees from 500 random addition sequence replicates were swapped

using TBR. For each random addition sequence replicate, 1,000 trees were saved and swapped to completion using the "nchuck" and "chuckscore" commands in PAUP*. A maximum limit of 500,000 trees was set using the "maxtrees" option. These settings were selected based on preliminary analyses in which each heuristic search recovered the same most parsimonious tree island but would swap indefinitely on equally parsimonious resolutions.

A parsimony bootstrap analysis was performed in the following manner. 200 bootstrap pseudo-replicate data sets were generated. For each pseudo replicate, 100 random addition sequence replicates were performed, saving at most 1,000 trees per replicate. Bootstrap replicates were separated into two files of 100 and executed separately on the computing cluster described previously. The resulting trees were combined in PAUP* as described previously for the maximum likelihood analysis.

Bayesian Analysis. Model selection for the Bayesian analysis is restricted somewhat by the number of models that are implemented in MrBayes 3vb4. As a result, the same model selected by DTModSel could not be used in the Bayesian analysis. MrModel selected the General-Time-Reversible (GTR) (Tavaré 1986) with invariant sites (Gu et al. 1995) and a discrete gamma parameter (Yang 1994a) for modeling among-site-rate-variation (ASRV). However, given the nature of the data (i.e., an alignment of sequences consisting of regions that are invariable or nearly invariable, punctuated by regions of extreme variability), we elected to perform additional analyses under the GTR model with the auto-correlated, discrete gamma approximation (Γ_{AD}) (Yang 1995) to account for ASRV. Two runs, 2,000,000 generations in length were performed under each modeling scenario. Each run consisted of three heated chains (with temperatures set to the default settings in MrBayes) and one cold chain. Samples were drawn from the cold chain every 100 generations. Runs were checked for convergence of the $-\text{LnL}$ and all parameter estimates using Tracer v1.2.1 (Rambaut and Drummond 2003). When convergence between runs was established, trees sampled from stationarity in both runs were combined and a majority rule consensus tree was constructed using the "sumt" command implemented in MrBayes. All runs were observed to reach stationarity by 100,000 generations and a conservative "burn-in" of 200,000 generations (2,000 trees) was discarded for each analysis.

RESULTS

Sequencing of the ITS region resulted in an alignment of 752 bp in length. Of these, 108 characters were excluded from the end regions as they were uninformative and only present in a few species. The remaining 644 characters consisted of 469 constant characters and 175 variable characters. Sixty-four variable characters were parsimony uninformative leaving 111 parsimony informative characters. A chi-square test of base homogeneity indicated no significant heterogeneity in base composition among taxa (Chi-square=20.882, $\text{df}=189$, $P=1.000$).

DTModSel indicated the TIM+I+ Γ was the best-fit model of nucleotide substitution for the maximum likelihood analysis. This model consists of two rates for transversions and two rates for transitions. The maximum likelihood search yielded a single most likely tree ($-\text{LnL}=3021.8056$) presented as Fig. 1. Characteristic of small datasets with few variable characters, the topology has many short internal branches.

The parsimony search resulted in 303,036 most parsimonious trees that were 366 steps in length with a consistency index (CI) of 0.578 and a retention index (RI) of 0.774 (Fig. 2). These were combined in PAUP to

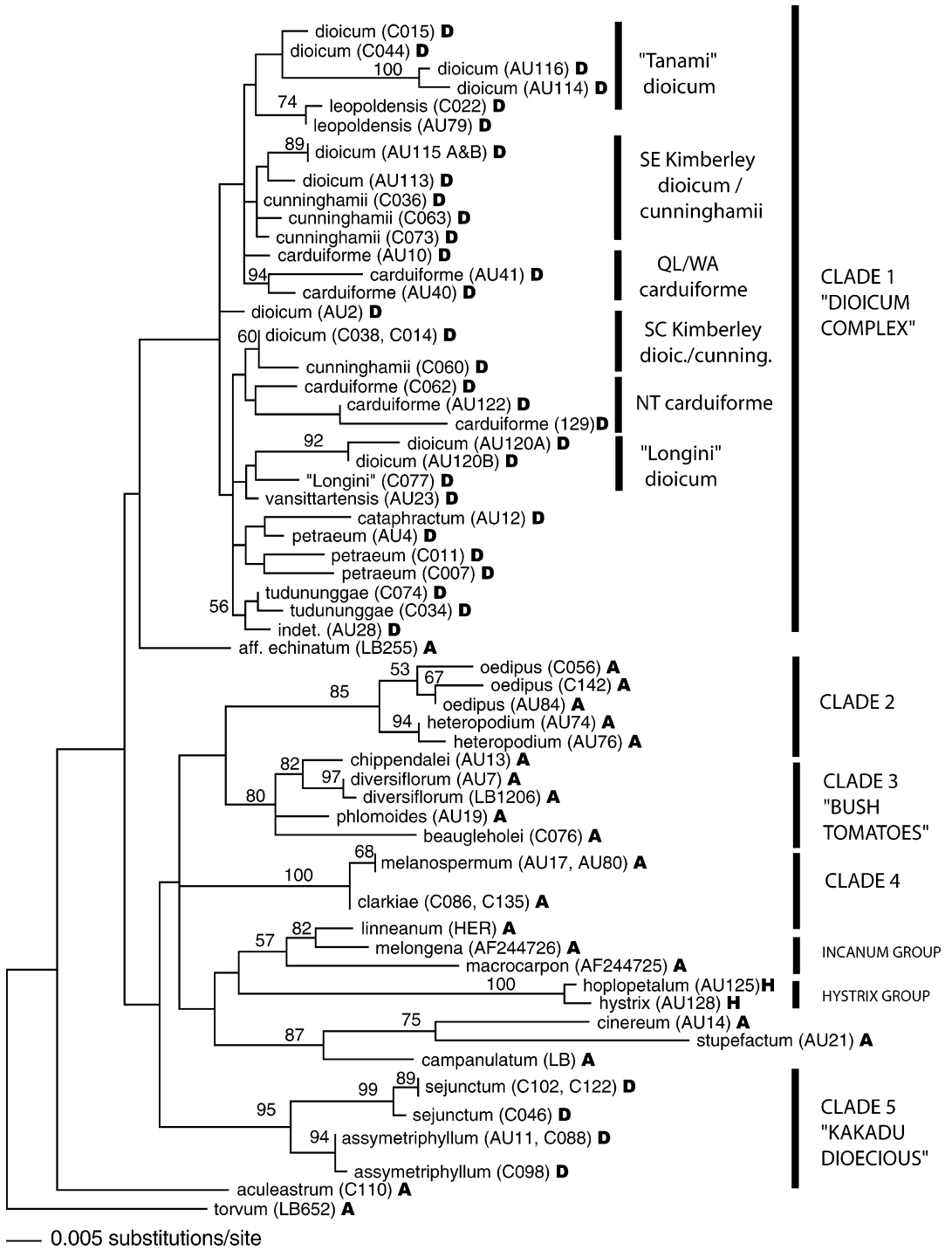


FIG. 1. Maximum likelihood tree generated with ten random addition sequence replicates. Support values above branches are 50% majority rule ML bootstrap support values from a consensus of 235 bootstrap replicates. Breeding systems are noted as follows for each accession: D—dioecy, A—andromonoecy. The species in Clades 1–5 constitute the members of Symon's (1981) section *Melongena*. For visual clarity, more than one accession number may be listed at a given branch tip in cases where sequences from separate accessions are identical.



FIG. 2. Strict consensus tree of 303,039 most parsimonious trees with 500 random addition sequence replicates. Support values above branches are 50% majority rule parsimony bootstrap support values from a consensus of 20,000 trees using 200 random addition sequence replicates. Breeding systems are noted as follows for each accession: D—dioecy, A—andromonoecy. The species in Clades 1–5 constitute the members of Symon's (1981) section *Melongenina*.

yield the single strict consensus topology shown in Fig. 2.

Figure 3 shows the majority rule consensus topology that resulted from the combination of the two independent Bayesian analyses.

The trees produced by the parsimony, maximum

likelihood, and Bayesian analyses are highly congruent (Figs. 1–3) in the placement the focal 20 species into five main clades, including two groups of dioecious species. The composition of these five main clades was the same in all three analyses. Differences among trees lie in the resolution of and support for internal nodes.

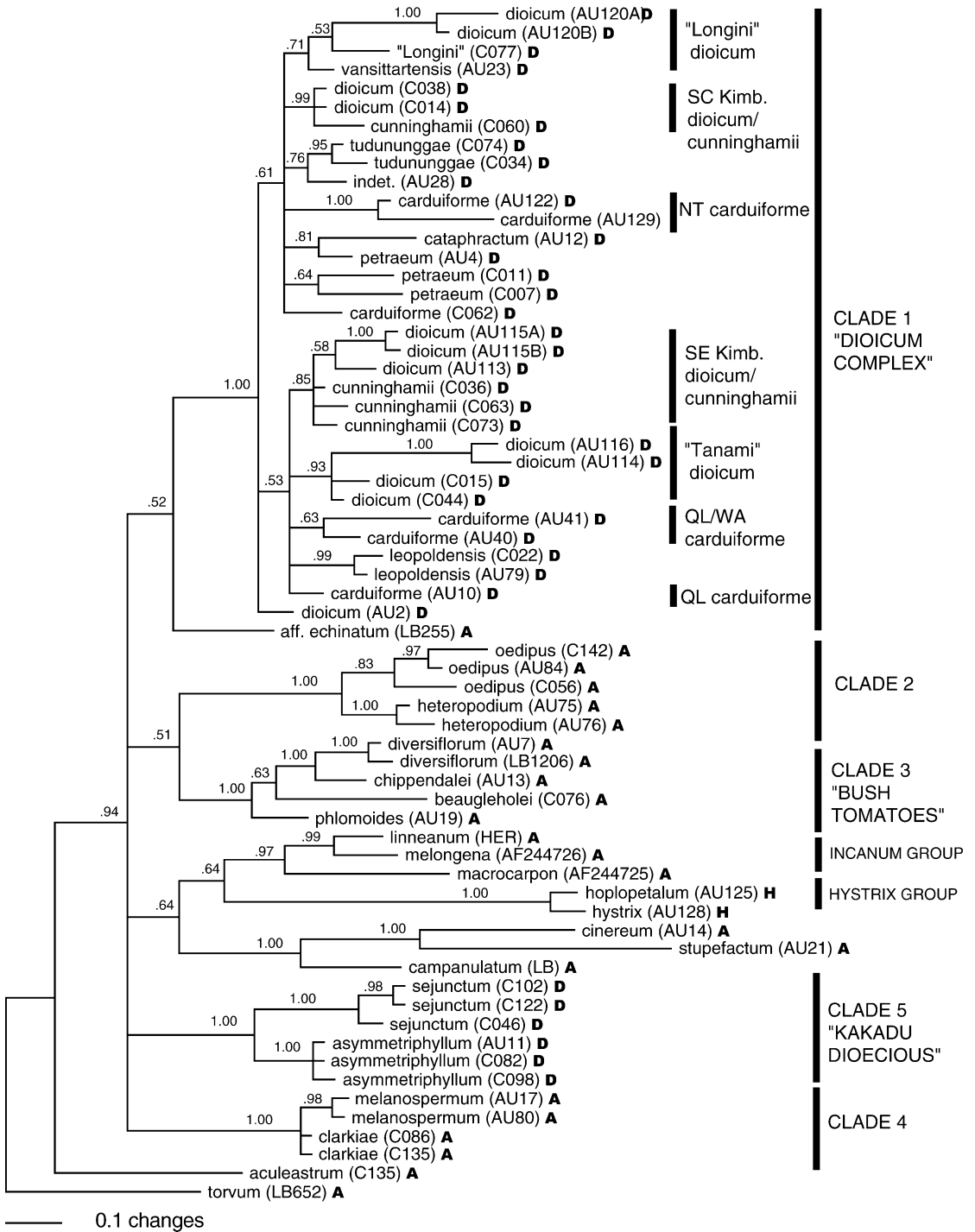


FIG. 3. Tree resulting from the Bayesian analysis from the combined runs of 4,000,000 generations with a burn-in of 4,000 trees. Support values above branches are Bayesian posterior probabilities. Breeding systems are noted as follows for each accession: D—dioecy, A—andromonoecy. The species in Clades 1–5 constitute the members of Symon’s (1981) section *Melongena*.

Although support is strong for the five main clades and the relationships within them, the relationships among the clades, as well as their placement among outgroup species/clades, are not well supported by ITS data. As an example, the highest Bayesian posterior probability for a sister relationship between any two of the five clades is 0.24. Clade 1 (the "dioicum complex") consists of the nine Kimberley dioecious taxa while another dioecious clade (Clade 5) consists of only the two Kakadu species: *S. asymmetriphyllum* and *S. sp.nov.* (Brennan et al. in press). This result renders Anderson and Symon's (1989) Group II, the dioecious species, non-monophyletic. The three remaining clades (2, 3, and 4) consist of andromonoecious species (all Australian andromonoecious species of section *Melongenina*). Section *Melongenina* in Australia is non-monophyletic, as evidenced by the inclusion of a number of other Australian species used as outgroup species (*S. echinatum*, *S. hoplopetalum*, and *S. hystrix* [Symon's section *Oliganthes*]; *S. stupefactum* [unplaced]; *S. cinereum* [unplaced] and *S. campanulatum* [section *Campanulata*]) as well as the cultivated eggplant (*S. melongena*) and two African species (*S. linneanum* and *S. macrocarpon*). The Bayesian posterior probability for a monophyletic group representing Symon's (1981) Australian section *Melongenina* is less than 0.05.

Clade 1 includes the highly variable and problematic species *S. dioicum*, *S. cunninghamii*, and *S. carduiforme*, plus five other described species: *S. tudumunggae*, *S. vantsittartensis*, *S. petraeum*, *S. cataphractum*, and *S. leopoldensis*. Accessions that may represent a new Western Australia species also fall into the dioicum complex (identified here as "Longini"). Additionally, specimens of a form of *S. dioicum* with a blue cast, often identified on herbarium sheets as *S. dioicum* "Tanami," group together in the complex and may represent a separate species.

The Australian dioecious species of the Kakadu Region of the Northern Territory, *S. asymmetriphyllum* and *S. sp. nov.*, are a monophyletic group (Clade 5). There is morphological and molecular support for the recognition of *S. sp. nov.* as a separate entity (Brennan et al. in press).

Clade 3 consists of the "bush tomatoes" *S. chippendalei*, *S. phlomoides*, *S. beaugleholei*, and *S. diversiflorum*. The group is monophyletic and probably includes *S. eburneum*, a species relatively similar in morphology to *S. diversiflorum*, but for which sequence data could not be acquired.

The remainder of the andromonoecious species fall into two clades: Clade 2, consisting of *S. oedipus* (from Kalumburu) and *S. heteropodium* (from the Mitchell Plateau), both isolated species of the far northern Kimberley Region; and Clade 4, consisting of *S. clarkiae* (of the Kakadu region) and *S. melanospermum* (of the

northeastern corner of the Northern Territory), both isolated species of the far northern Northern Territory.

Solanum campanulatum, considered by Symon to be the lone member of its own section, does not ally with any of the Group I andromonoecious species, and instead occurs in a strongly supported group with *S. cinereum* and *S. stupefactum*, two other andromonoecious species considered difficult to place (Symon 1981; Whalen 1984; Symon 1995). *Solanum hystrix* and *S. hoplopetalum*, placed by Symon in section *Oliganthes* and by Whalen in the "hystrix group," are sister taxa and are not allied with either *S. leopoldensis* or *S. oedipus*, a possibility proposed by Whalen (1984).

DISCUSSION

Circumscription and Monophyly of Section *Melongenina*. The results of these phylogenetic analyses are for the most part congruent with the species relationships inferred by Symon (1981) in his monograph of Australian *Solanum*. The five clades recovered here are easily aligned with Symon's interpretations, with slight modifications/clarifications. However, the ITS data do not allow for robust resolution of deeper branches. In particular, the resolution is not sufficient to allow strong conclusions about the hypothesis that dioecy evolved through andromonoecy in Australian *Solanum*. The data do call to question whether all 20 endemic diclinous species studied by Anderson and Symon (1989) form a monophyletic group (i.e., Australian members of section *Melongenina*), because a number of "outgroups" included in the analysis are nested within this group. We briefly outline the five recovered clades below and interpret these relationships with respect to breeding system evolution in Australian *Solanum*.

CLADE 1: "DIOICUM COMPLEX". (*S. dioicum* sensu Symon, *S. cunninghamii* sensu Symon, *S. petraeum*, *S. carduiforme* sensu Symon, *S. vantsittartensis*, *S. tudumunggae*, *S. cataphractum*, *S. leopoldensis*, *S. 'Longini'*, *S. 'Tanami'*)—Symon (1981) defined a complex of three of these species (*S. dioicum* [and variants], *S. cunninghamii*, and *S. petraeum*) on the basis of their similar morphology and the apparent tendency for the taxa to intergrade where ranges overlap. The species tend to be rather prickly (densely so on the calyces that envelop the fruits) with unlobed and generally rusty-tomentose leaves, although localized variants occur in each species. Various attempts have been made to sort the groups out on geographical and geological bases with little satisfaction (D. Symon, pers. comm.). The molecular analyses reported here confirm the conclusions of Symon (1981; pers. comm.) that these three species, as currently defined, may not be distinct. Accessions of these three species do not form a clade consistent with nomenclatural conventions, although they are all part of a redefined "dioicum complex." Hybridization is a

possible cause for the ambiguity of relationships, although hybrids are apparently rare in *Solanum* subgenus *Leptostemonum* (L. Bohs, pers. comm.). Whatever the cause, based on ITS data, at least three of the species names used to identify dioecious solanums in the Kimberley region represent non-monophyletic groups. Nevertheless, the populations associated with these polyphyletic name-groups all fall into the monophyletic group identified here as the “dioicum complex”.

A number of dioecious species previously considered problematic or at least poorly understood fall into the “dioicum complex.” *Solanum cataphractum*, a rare and little known species of the northwestern Kimberley coast, is apparently allied with *S. petraeum*. In fact, the sister accession to *S. cataphractum* (Figs. 1–3) is a narrow-leaved collection (“AU4”) of *S. petraeum* species from Bachsten Creek, Western Australia. Two other rare species with localized ranges in the northern Kimberley, *S. tudununggae* and *S. vansittartensis*, are placed similarly in the dioicum complex, each of them forming a clade with collections of unknown identity (one, “Longini” is a diminutive, undescribed dioecious species collected on several occasions from the Longini area near Kalumburu [at the far-northern tip of the Kimberley coast] Western Australia). Another rare species, *S. carduiforme*, belongs in the “dioicum complex” clade, although the accessions of this widely disjunct species (including collections from a new locality discovered in May 2004 by C. Martine and W.R. Barker within the Keep River National Park, Northern Territory) do not form a clade based on ITS data. Taxonomic problems associated with the widely disjunct range of *S. carduiforme* sensu Symon (i.e., there are fewer than 10 isolated populations known from Western Australia, the Northern Territory, and Queensland) are being explored by C. Martine and D. Symon. Also of note is the inclusion in this complex of *S. leopoldensis*, a species considered by both Symon and Whalen to have obscure relationships. Whalen (1984) proposed that *S. leopoldensis* and the andromonoecious *S. oedipus* were more closely related to the hermaphroditic *S. hystrix* and should be included in his “hystrix group.” Our findings show all three of these species are distantly related (Fig. 3). Further work is needed to delimit the species in the “dioicum complex” and to clarify the relationships among them, but the monophyly of the complex itself is apparent. The support for the relationship between the “dioicum complex” and the weakly andromonoecious *S. echinatum* (section *Oliganthes* sensu Symon [1981]), seen in the likelihood (Fig. 1) and Bayesian (Fig. 3) analyses, is not well supported and should be treated with skepticism until further studies are done. It is noteworthy, however, that Whalen (1984) recognized a similarity between close relatives of *S. echinatum* (which he treated in his “ellipticum group”) and *S. dioicum*.

CLADE 2: *S. OEDIPUS* + *S. HETEROPODIUM*. *Solanum oedipus*, found only in the Kakadu region, and *S. heteropodium*, of the northwestern coast and off-shore islands, are both poorly known and little collected. Symon (1981) inferred a possible relationship between these two rare andromonoecious species based on their similar fruiting characteristics. Each of the species has a small, weakly bilobed berry borne on a basally broadened pedicel that is enclosed and exceeded by a prickly calyx of distinct linear lobes. Symon also recognized vegetative similarities between *S. oedipus* and the dioecious *S. leopoldensis* (green aspect, minute pubescence, and prickly nature), but discounted their importance in defining an evolutionary relationship. Whalen (1984) later used this same set of characters to formulate the hypothesis that these two species might be better placed in his “hystrix group.” Our data support a sister relationship between *S. oedipus* and *S. heteropodium* (Figs. 1–3), two andromonoecious species of the far northern Kimberley. We find no support for the close relationship proposed by Whalen (1984) among *S. oedipus*, *S. leopoldensis* (of Clade 1), and the members of the “hystrix group” (Figs. 1–3).

CLADE 3: “ANDROMONOECIOUS BUSH TOMATOES”. (*S. chippendalei*, *S. diversiflorum*, *S. phlomoides*, *S. beaugleholei*, probably also *S. eburneum*)—Symon (1981) considered this group of species to be closely allied with similar species of sub-arid Africa and used this alliance as the link between the Australian species in his section *Melongena* and the African species previously placed there by D’Arcy (1972). Like their relatives in the African “incanum group” recognized by Whalen (1984), the Australian species are andromonoecious with inflorescences characterized by a basal flower that is a heavily-armed hermaphrodite and later yields a globose, relatively large, yellow-skinned, and usually mucilaginous berry (Symon 1981). The African species often bear additional hermaphrodite flowers in the distal part the inflorescence (Miller and Diggle 2003; Anderson and Martine, unpubl. data), a flowering sequence that never occurs in the Australian relatives where only a single basal hermaphrodite is formed (Symon 1981; Anderson and Symon 1989; Anderson et al. in prep). Our results (Fig. 3) show a relationship among the “andromonoecious bush tomatoes” (Clade 3), as previously proposed by Symon. *Solanum diversiflorum* is the most different of the group, but, as noted by Symon (1981), may also be closely related to *S. eburneum* (from the area of Gregory National Park, Northern Territory). Unlike the rest of the clade, these two species share a smaller habit and lobed leaves.

CLADE 4: *S. MELANOSPERMUM* + *S. CLARKIAE*. Symon (1981) considered *S. melanospermum* to be most closely related to *S. chippendalei* (Fig. 1, Clade 3), but remarked that some aspects of their morphology appeared to vary continuously. However, Symon (1981) also rec-

ognized the affinity of *S. clarkiae* to *S. melanospermum*. Our data support a sister relationship between the andromonoecious *S. clarkiae* and *S. melanospermum*, even though *S. clarkiae* differs from *S. melanospermum* by its biennial habit, lack of rusty tomentum, and larger, nearly entire leaves (Symon 1981; Barker 2005). Both of these species are isolated geographically from other related species. The range of *S. melanospermum* (vicinity of the McArthur and Robinson Rivers of northwestern Northern Territory) does not overlap with any related species. *Solanum clarkiae* is sympatric only with the dioecious *S. asymmetriphyllum* (Fig. 1, Clade 5) in the Kakadu region, although they occupy different habitats.

CLADE 5: "KAKADU DIOECIOUS". This clade of species from the Kakadu region of Northern Territory is the second of two dioecious clades of *Solanum* in Australia. It consists of *S. asymmetriphyllum* and the recently described *S. sp. nov.* (Brennan et al. in press), and is the second of two dioecious clades of *Solanum* in Australia. Both morphology and ITS data clearly distinguish these two species. The species share a lightly armed appearance, except for the berries that are almost wholly enclosed in a connate calyx scattered with conical prickles. The species also exhibit some preference for sandstone outcrops.

Breeding System Evolution. Previous interpretations of relationships in Australian *Solanum* have led to hypotheses on the evolution of breeding systems that lack a phylogenetic framework. Notably, andromonoecy has often been postulated as a precursor to dioecy in *Solanum* (Symon 1981; Anderson and Stebbins 1984; Anderson and Symon 1989; Knapp et al. 1998), although no phylogenetic data have been used to test this presumption. The least robust branches in our phylogenies are in the deeper areas of the topologies. Nevertheless, the extent of resolution and support provided by this study allows us to formulate several phylogenetically based hypotheses for breeding system evolution in the group. Definitive conclusions may not be possible until analysis of additional gene regions and morphological characters provide additional resolution and higher levels of internal support (C. Martine, in progress).

Based on the ITS data, dioecy has evolved either once or twice in Australian *Solanum*. If twice, independent origins of dioecy would have occurred in the ancestor of the "dioicum complex" (Clade 1) and again in the ancestor of the "Kakadu dioecious" group (Clade 5). We cannot dismiss the hypothesis that dioecy arose from andromonoecy in Australian *Solanum*, particularly because the dioecious clades are nested in a largely andromonoecious lineage.

Anderson and Stebbins (1984) recognized three characteristics of dioecious solanums that may have contributed to the evolution of dioecy: a) the popula-

tions are small (few ramets) and widely separated; b) the populations are effectively even smaller because "individuals" are often ramets of only one or a few genets; and c) the pollinator fauna consists mainly of small-bodied *Nomia* and *Trigona* bees with relatively limited foraging areas (Anderson and Symon 1988). These three factors characterize the species in both of the dioecious Australian clades and may have played the roles suggested in the Kimberley (for Clade 1) and the Kakadu regions (Clade 5). These factors also may function to maintain the dioecious condition, especially when considering the recent and rapid radiation that has taken place in the "dioicum complex." Our phylogenetic hypotheses indicate that as many as a dozen separate species have arisen after the origin of dioecy in Australian *Solanum*. Fieldwork indicated that the dioecious species typically are found in very small, isolated groups, with many populations apparently consisting of a single individual. This situation was especially apparent in *S. sp. nov.* from Kakadu, which was only collected from two sandstone boulders supporting 9–10 clonal stems in each case. Had ancestral populations (likely andromonoecious) of Clade 5 (Figs. 1–3) encountered the same spatial limitations, they would have faced a high likelihood of self-fertilization and its associated inbreeding costs (Holsinger 1993, 2000).

Ongoing work using additional gene regions as well as morphological characters hopefully will clarify the relationships among the clades recognized here and provide further clarification of the pathways involving hermaphroditism, andromonoecy, and dioecy in these solanums. An improved understanding of phylogenetic relationships should provide the basis for a revised classification of the non-monophyletic species groups represented by Symon's (1981) section *Melongenina*. Our work also shows a need for further study of the "dioicum complex" where the species boundaries indicated by molecular data do not reflect the nomenclature and taxonomy currently in use.

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APPENDIX 1

Collection, voucher, and GenBank accession information. Numbers in parentheses following taxon names are CTM extraction numbers. Codes beginning with 'AU' are from sheets held at AD. ITS sequences provided by L. Bohs are marked with an *. Sequences obtained from GenBank are marked with a \$. Nijmegen accession numbers refer to the Solanaceae collection at the University of Nijmegen, The Netherlands.

Australian *Solanum* of section *Melongena* sensu Symon: *S. asymmetriphyllum* Specht. (AU11) Weber 9816 (AD), NT: Kakadu: Ubirr Rock (DQ364747). *S. asymmetriphyllum* (C088) Martine 739 (CONN), NT: Kakadu: E. of Jabaluka (DQ364748). *S. asymmetriphyllum* (C098) Martine 723 (CONN), NT: Kakadu: E. Alligator River (DQ364749). *S. beagleholei* Symon (C076) Martine 804 (CONN), WA: Kalumburu Rd. (DQ364737). *S. carduiforme* F. Mueller (C062) Martine 773 (CONN), NT: Keep River: Jarrarn Escarp. (DQ364711). *S. carduiforme* (AU129) Latz 10132 (AD), NT: Sculthorpe Pond (DQ364715). *S. carduiforme* (AU122) Latz 15043 (AD), NT: Nathan River Homestead (DQ364714). *S. carduiforme* (AU10) Symon 15790 (AD), QL: Lawn Hill Gorge (DQ364712). *S. carduiforme* (AU40) Thomas MRS614 (AD), QL: Musselbrook Gorge (DQ364716). *S. carduiforme* (AU41) Kenneally (AD), WA: Bungle Bungles (DQ364713). *S. cataphractum* A. Cunningham ex Bentham (AU12) Mitchell 3469 (AD), WA: Sandstone Country (DQ364717). *S. chippendalei* Symon (AU13) Symon 15796 (AD), QL: Thornton Station (DQ364734). *S. clarkiae* Symon (C086) Martine 748 (CONN), NT: Gunlom Falls (DQ364740). *S. clarkiae* (C135) Martine 734 (CONN), NT: Kakadu: Koongarra Saddle (DQ364741). *S. cunninghamii* W.V. Fitzgerald (C036) Martine 891 (CONN), WA: Highway 1: nr. Bidalanga (DQ364725). *S. cunninghamii* (C063) Martine 893 (CONN), WA: Great N. Hwy.: Deep River (DQ364727). *S. cunninghamii* (C073) Martine 888 (CONN), WA: Highway 1: nr. Bidalanga (DQ364728). *S. cunninghamii* (C060) Martine 884 (CONN), WA: Highway 1: nr. Bidalanga (DQ364726). *S. dioicum* W.V. Fitzgerald (AU116) Muir 5006 (AD), WA: Northern Australia (DQ364705). *S. dioicum* (AU2) Latz 8608 (AD), NT: Pargee Range (DQ364702). *S. dioicum* (AU120) Symon 10185 (AD), WA: Longini (DQ364704). *S. dioicum* (AU113) Mitchell 2727 (AD), WA: Shelemar Farm (DQ364707). *S. dioicum* (AU115) Willis s.n. (AD), WA: Winnama Spring; SE Kimberley (DQ364706). *S. dioicum* (AU114) Kenneally 11311 (AD), WA: Berkeley River mouth (DQ364708). *S. dioicum* (C014) Martine 879A (CONN), WA: Great Northern Hwy (DQ364709). *S. dioicum* (C038) Martine 879B (CONN), WA: Great Northern Hwy (DQ364703). *S. dioicum* (C044) Martine 772 (CONN), NT: Keep River NP (DQ364701). *S. dioicum* (C015) Martine 843 (CONN), WA: W. Gibb Riv. Rd. (DQ364700). *S. diversiflorum* F. Mueller (AU7) Mollemans 2448 (AD), WA: Pilbarra (DQ364735). *S. diversiflorum** (LB1206) Bohs 2854 (UT) Nijmegen #914750131 (AY996505). *S. heteropodium* Symon (AU74) Kenneally 10788 (AD), WA: Mt. Talbot (DQ364732). *S. heteropodium* (AU76) Mitchell 3506 (AD), WA: Wilson's Head (DQ364733). *S. leopoldensis* Symon (C022) Martine 860 (CONN), WA: Lennard River Gorge (DQ364720). *S. leopoldensis* (AU79) Symon 12071 (AD), WA: Fern Creek (DQ364721). *S. melanospermum* non F. Mueller (AU80) Muir 5007 (AD), NT (DQ364739). *S. melanospermum* (AU17) Murfet 2395 (AD), NT: Batten Road (DQ364738). *S. oedipus* Symon (C056) Martine 814A (CONN), WA: Longini (DQ364729). *S. oedipus* (C142) Martine 814B (CONN), WA: Longini (DQ364730). *S. oedipus* (AU84) Symon 12120 (AD), WA: Longini (DQ364731). *S. petraeum* Symon (AU4) Handasyde TH01/176b (AD), WA: Bachsten Creek (DQ364723). *S. petraeum* (C011) Martine 830 (CONN), WA: Mitchell Falls (DQ364722). *S. petraeum* (C007) Martine 857 (CONN), WA: Beverley Springs (DQ364724). *S. phlomooides* A. Cunningham ex Bentham (AU19) Symon 10048 (AD), WA: Wittenoom (DQ364736). *S. sp. nov.* (C102) Martine 730 (CONN), NT: Koongarra Saddle: Nourlangie (DQ364744). *S. sp. nov.* (C046) Martine 735 (CONN), NT: Koongarra Saddle: Baroalba Creek (DQ364746). *S. sp. nov.* (C122) Brennan s.n. (AD), NT: Koongarra Saddle (DQ364745). *S. tudumunggae* Symon (C034) Martine 826 (CONN), WA: Kalumburu (DQ364719). *S. tudumunggae* (C074) Martine 823 (CONN), WA: Kalumburu (DQ364718). *S. vantsittartensis* Gardner (AU23) Mitchell 3421 (AD), WA: Krait Bay (DQ364742). *S. indet.* (AU28) Fryxell 4802 (AD), WA: Vantsittart Bay (DQ364743). *S. 'Longini'* (C077) Martine 805 (CONN), WA: Longini (DQ364710).

Outgroups: *S. aculeastrum* Dunal (C110) Martine 637 (CONN), US: UConn Conservatory Greenhouse #20020004 (DQ364755). *S. campanulatum* R. Br. (LB) DNA from RGO 245, Olmstead S-78 (WTU) from seed accession BIRM S.0387 (AY996488). *S. cinereum* R. Brown (AU14) Bates 49377 (AD), SA: Broadview Gorge (DQ364756). *S. aff. echinatum** (LB255) Bohs 2727 (UT) Nijmegen #954750052 (DQ364751). *S. hoplopetalum* Bitter & Summerhayes (AU125) Dodd 196 (AD), WA: Northam (DQ364752). *S. hystrix* R. Brown (AU128) Lepshi 1735 (AD), SA: Kimba (DQ364753). *S. linneanum* Hepper & Jaeger (HER) Martine 630 (CONN), US: UConn Conservatory Greenhouse, Nijmegen #904750164 (DQ364750). *S. macrocarpon* (AF244725). *S. melongena*§ (AF244726). *S. stupefactum* (AU21) Beane 16060 (AD), QL: Mt. Binga: Moreton (DQ364757). *S. torvum** (LB652) Cipollini 64 (UT), Nijmegen #814750073 (DQ364754).