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PHYLOGENY AND EVOLUTION OF ANOMALOUS ROOTS IN *DAVIESIA* (FABACEAE: MIRBELIEAE)

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The phylogeny of the Australian legume genus *Daviesia* was estimated using sequences of the internal transcribed spacers of nuclear ribosomal DNA. Partial congruence was found with previous analyses using morphology, including strong support for monophyly of the genus and for a sister group relationship between the clade *D. anceps* + *D. pachyloma* and the rest of the genus. A previously unplaced bird-pollinated species, *D. epiphyllum*, was well supported as sister to the only other bird-pollinated species in the genus, *D. speciosa*, indicating a single origin of bird pollination in their common ancestor. Other morphological groups within *Daviesia* were not supported and require reassessment. A strong and previously unreported sister clade of *Daviesia* consists of the two monotypic genera *Erichsenia* and *Viminaria*. These share phyllode-like leaves and indehiscent fruits. The evolutionary history of cord roots, which have anomalous secondary thickening, was explored using parsimony. Cord roots are limited to three separate clades but have a complex history involving a small number of gains (most likely 0–3) and losses (0–5). The anomalous structure of cord roots (adventitious vascular strands embedded in a parenchymatous matrix) may facilitate nutrient storage, and the roots may be contractile. Both functions may be related to a postfire resprouting adaptation. Alternatively, cord roots may be an adaptation to the low-nutrient lateritic soils of Western Australia. However, tests for association between root type, soil type, and growth habit were equivocal, depending on whether the variables were treated as phylogenetically dependent (insignificant) or independent (significant).

Keywords: trait evolution, phylogenetically independent contrasts, parsimony, maximum likelihood, Bayesian inference, ITS sequences, legumes.

Introduction

By comparison with the rest of Australia, the southwest of Western Australia is a region of high floristic diversity and endemism across a wide range of families (Lamont et al. 1984; Boden and Given 1995; Hopper et al. 1996; Crisp et al. 2001), including the legumes (Fabaceae). Its diversity is similar to that of certain regions of the world with Mediterranean climates, such as Greece, the California Floristic Province, and the Cape Floristic Region of South Africa (Lamont et al. 1984; Cowling et al. 1992; Ornduff 1996). The highest Western Australian diversity occurs in a major land system known as “Kwongan” or simply “sandplain” (Pate and Beard 1984; Fox 1999). Kwongan soils are mostly derived from laterite; they are often sandy and are highly deficient in nutrients (Bettenay 1984; Fox 1999). The vegetation is a sclerophyll heathland, often with emergent taller shrubs. The high species diversity of the Kwongan and comparable Mediterranean regions is considered to be driven by a combination of climate, topography, poor soils, and fire (Lamont et al. 1984; Cowling et al. 1992). In particular, it has been hypothesized that climatic fluctuation with the onset of Late Cenozoic aridity in the Southern Hemisphere may have led to rapid speciation by fragmentation of popu-

lations, both in South Africa and Western Australia (Hopper 1979; Richardson et al. 2001).

Plants of the rich heathlands (Kwongan) of southwest Western Australia have evolved a diversity of growth and life forms (Pate et al. 1984), probably as adaptations to environmental stresses such as heat, seasonality, drought, nutrient deficiency, and fire (Lamont et al. 1984; Pate and Dixon 1996). Some of the most specialized adaptations are found in underground organ systems, e.g., microstilts, woody water-storing structures, sand-binding roots, and schizogenous roots (Dodd et al. 1984; Pate and Dixon 1996). More than 40% of species included in a broad survey of taxa ($n = 429$) had some form of unusual modification of their roots (Pate et al. 1984).

In one highly anomalous root modification found in this ecosystem, secondary thickening occurs by the successive production of adventitious vascular cambia outside the current outermost layer of phloem (Pate et al. 1984, 1989; Pate and Dixon 1996). In cross section, these cambia form concentric rings, while longitudinally they anastomose, giving rise to a complex network of interlinking vascular strands. In external morphology, the roots develop either flanges or a plaited rope-like appearance, hence the name “cord roots.” To date, cord roots have been reported only in species of the legume tribe Mirbelieae, where this trait appears to be restricted to a number of species of *Daviesia* and single species in each of the related genera *Euchilopsis* and *Phyllota* (Pate et al. 1989; Pate and Dixon 1996). Cord roots have not been reported previously in species of these genera occurring outside the Kwongan,

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but species of *Daviesia* with normal (unistelar) secondary thickening in their roots coexist with cord-rooted congeners (Pate and Dixon 1996). No unique function has been found for cord-root anatomy. However, coexistence of species with and without cord-root anatomy does not rule out differential responses in the two root types to a common environmental stress or adaptation to different microhabitats in the same community.

Daviesia has a significant presence in the Kwongan. Of 125 recognized species in the genus (Crisp 1995), 92 occur in southwest Western Australia (all but one are endemic), and, of these, 84 are found in the Kwongan. At many sites, up to five species coexist. The occurrence of a number of species of *Daviesia* in other parts of Australia allows the evolution of traits such as cord roots to be put into a biogeographic and ecological context. Have cord roots evolved only in southwest Western Australia (or the Kwongan) under a localized selection regime, or are they phylogenetically constrained to particular clades that coincidentally occur in this region?

The evolutionary origin of cord roots has been examined previously using a phylogenetic tree derived from morphological data and a sample of 53 species of *Daviesia* and related genera whose root type was known (Pate et al. 1989). Most cord-rooted species formed a single clade, but two grouped with unistelar species, indicating that cord roots had evolved twice within the genus. However, Pate et al. (1989) included cord roots as a character in the analysis and did not test the placement of cord-rooted species by excluding the root-type character. Thus, there was the possibility of circularity in using the tree to examine the evolutionary origins of the trait. In addition, critical branches on the tree were not tested.

This study presents the first estimate of the phylogeny of *Daviesia* using DNA sequence data. The resulting trees are used to investigate the evolutionary history of cord roots in the context of geographic distribution and habitat. Additionally, the monophyly of the genus is tested by using a number of outgroups, and the pattern of major clades within the genus is compared with groups previously recognized from morphology.

Material and Methods

Specimens and DNA Extraction

To represent the phylogenetic diversity in *Daviesia*, 46 exemplars (table 1) were sampled from 16 of 18 recognized morphological groups (M. D. Crisp, unpublished manuscript). The eight outgroups (table 1) include exemplars of *Erichsenia*, *Gompholobium*, *Sphaerolobium*, and *Viminaria*. These are the other four genera in the *Daviesia* group, which is characterized by embryo sacs with giant antipodal cells (Crisp and Weston 1995; Crisp et al. 2000). On the basis of the results of previous phylogenetic analyses (Crisp et al. 2000; Crisp and Cook 2003), the trees were rooted using *Gompholobium*. Total genomic DNA was extracted from fresh NaCl/CTAB-stored specimens (Rogstad 1992) or dry specimens using CTAB/chloroform extraction. Vouchers for all accessions are lodged in CANB or PERTH and are cited by collector's name and number in table 1.

PCR and Sequencing

The internal transcribed spacer (ITS) region (incorporating ITS-1, 5.8S rRNA, and ITS-2) was amplified using primers P1L and P2R (Crisp et al. 1999). Each 25 μ L PCR reaction contained 5 pmol each primer, 3 mM MgCl₂, 2.5 μ L 10 \times PCR buffer (Perkin Elmer), 0.2 mM each dNTP, and 1 unit of AmpliTaq DNA polymerase (Perkin Elmer). Standard PCR conditions with an annealing temperature of 55°C were used.

Sequence Editing, Alignment, and Partitions

Sequences were assembled and edited using Sequencher 3.0 (Gibbs and Cockerill 1995) and aligned by eye in PAUP* (Swofford 2002), taking account of predicted secondary structures for ITS-2 (Mai and Coleman 1997). The ITS region was partitioned into ITS-1, 5.8S rDNA, and ITS-2 to check for congruence among partitions. Two contiguous sequences (28 characters in ITS-1 and 78 characters in ITS-2) were excluded from analyses because they could not be unambiguously aligned among all taxa.

Sequence Variation and Phylogenetic Analysis

Base composition differences among taxa (nonstationarity) were tested using a χ^2 test as implemented in PAUP* for each of the partitions. Phylogenetic analyses were performed by maximum parsimony (MP) and maximum likelihood (ML) using PAUP* and by Bayesian inference using MrBayes (Huelsenbeck et al. 2001).

Maximum parsimony. Heuristic searches comprised 100 random addition sequence starting trees with TBR branch swapping and saving only 10 MP trees followed by searching from the resulting trees with MAXTREES set to 20,000. This strategy was run several times, and the resulting trees were compared. Several weighting schemes were applied, using the same search strategy, to test for robustness of the MP strategy: (1) equal weighting of all positions, (2) transition : transversion weighting (1 : 2), and (3) downweighting loops and single-stranded regions relative to stems (1 : 2).

Nonparametric bootstrap (BS) tests (Felsenstein 1985a) were conducted using 1000 replicates, each with 10 random addition starting sequences saving no more than 100 MP trees and with taxa having identical sequences removed. Partition homogeneity tests as implemented in PAUP* were performed among ITS-1 and ITS-2. These partitions were subsequently combined for further analyses because they were not incongruent ($P = 0.87$).

Bayesian inference. Bayesian analyses were performed using MrBayes (Huelsenbeck et al. 2001). A GTR+ Γ model (six substitution types and a gamma distribution of site-specific rates) was used because of the pattern of base frequencies and among-site rate differences observed in the data. Modeltest (Posada and Crandall 1998) also indicated GTR+ Γ as the most appropriate model. Each run comprised four Markov chains starting from random trees and 500,000 generations with trees saved at each 10. Five runs were performed to check for convergence among runs. The majority rule consensus tree was calculated after the removal of trees saved during the "burn-in" period of each run (<30,000 generations).

Maximum likelihood. The parameters for input into the

Table 1
Taxa Sampled with Voucher Collector Codes and GenBank
Accession Numbers for Sequences

Species	Collector's code ^a	Accession number
Outgroup taxa:		
<i>Gompholobium minus</i> Sm.	MDC9153	AY233086
<i>G. obcordatum</i> Turcz.	MDC9031	AF287659
<i>G. villosum</i> (Meisn.) Crisp	MDC8951	AF287658
<i>Sphaerolobium medium</i> R. Br.	MDC8942	AF287660
<i>S. minus</i> Labill.	MDC9154	AF518101
<i>S. nudiflorum</i> (Meisn.) Benth.	RB891	AF518102
<i>Erichsenia uncinata</i> Hemsl.	MDC8524	AF287663
<i>Viminaria juncea</i> (Schrad.) Hoffmanns.	MDC8935	AF287664
Ingroup taxa:		
<i>Daviesia acicularis</i> Sm.	MDC9119	AY233087
<i>D. alternifolia</i> Endl.	MDC8967	AY233088
<i>D. anceps</i> Turcz.	MDC8992	AY233089
<i>D. angulata</i> Benth.	MDC9241	AY233090
<i>D. arborea</i> W. Hill	MDC9167	AY233091
<i>D. articulata</i> Crisp	MDC8999	AY233092
<i>D. benthamii</i> Meisn. subsp. <i>benthamii</i>	MDC8995	AY233093
<i>D. brachyphylla</i> Meisn.	MDC9181	AY233095
<i>D. buxifolia</i> Benth.	MDC9136	AY233096
<i>D. chapmanii</i> Crisp	MDC9247	AY233097
<i>D. cordata</i> Sm.	MDC8908	AY233098
<i>D. corymbosa</i> Sm.	MDC9124	AY233099
<i>D. daphnoides</i> Meisn.	MDC9013	AY233100
<i>D. decurrens</i> Meisn.	MDC9008	AY233101
<i>D. dilatata</i> Crisp	MDC8989	AY233102
<i>D. divaricata</i> Benth.	MDC8941	AY233103
<i>D. elliptica</i> Crisp	MDC9051	AF518099
<i>D. emarginata</i> (Miq.) Crisp	MDC8962	AY233104
<i>D. epiphyllum</i> Meisn.	MDC9245	AY233105
<i>D. flexuosa</i> Benth.	MDC8976	AY233106
<i>D. genistifolia</i> Cunn. ex Benth.	MDC9142	AY233107
<i>D. gracilis</i> Crisp	MDC8953	AY233108
<i>D. incrassata</i> Sm. subsp. <i>incrassata</i>	MDC9174	AY233109
<i>D. latifolia</i> R. Br.	MDC9130	AY233110
<i>D. leptophylla</i> A. Cunn. ex G. Don	MDC9145	AY233111
<i>D. megacalyx</i> Crisp	MDC8986	AY233112
<i>D. mimosoides</i> R. Br.	MDC9151	AY233113
<i>D. mollis</i> Turcz.	MDC8988	AY233114
<i>D. nematophylla</i> F. Muell. ex Benth.	MDC8984	AY233115
<i>D. nova-anglica</i> Crisp	MDC9137	AY233116
<i>D. nudiflora</i> Meisn. subsp. <i>amplectens</i> Crisp	MDC9034	AY233117
<i>D. obovata</i> Turcz.	MDC8948	AY233118
<i>D. pachyloma</i> Turcz.	MDC9025	AF287662
<i>D. pachyphylla</i> F. Muell.	MDC8985	AY233119
<i>D. physodes</i> A. Cunn. ex G. Don	MDC8940	AY233094
<i>D. podophylla</i> Crisp	MDC9244	AY233120
<i>D. rhombifolia</i> Meisn.	MDC8913	AY233121
<i>D. rubiginosa</i> Crisp	MDC9001	AY233122
<i>D. smithiorum</i> Crisp	MDC9029	AY233123
<i>D. speciosa</i> Crisp	MDC9017	AY233124
<i>D. spiralis</i> Crisp	MDC9024	AY233125
<i>D. teretifolia</i> R. Br. ex Benth.	MDC8987	AY233126
<i>D. ulicifolia</i> subsp. <i>ruscifolia</i> (A. Cunn. ex Benth.) G. Chandler & Crisp	MDC9149	AY233127
<i>D. ulicifolia</i> subsp. <i>stenophylla</i> G. Chandler & Crisp	MDC9117	AY233128
<i>D. ulicifolia</i> Andrews subsp. <i>ulicifolia</i>	MDC9115	AF518100
<i>D. wyattiana</i> F. M. Bailey	MDC9160	AY233129

Note. Vouchers are lodged in the Australian National Herbarium (CANB) and Western Australian Herbarium (PERTH).

^a MDC = Mike Crisp; RB = Rhyonon Butcher.

GTR+ Γ model used in the ML analysis were estimated from the Bayesian inference tree. The heuristic search using the ML criterion used the 114 trees derived from MP analyses as starting trees and the TBR branch swapping algorithm.

Evolution of Root Traits

Root anatomy (cord or unistellar) was scored from field observation for all 54 taxa. The putative origins of root type were assessed by MP optimization using ACCTRAN and DELTRAN. Topology-dependent permutational probability tests (Faith 1991) were run and compared different constraint trees of cord-rooted and unistellar taxa.

Tree-based ML modeling (Pagel 1994, 1999; Cunningham 1999; Mooers and Schluter 1999) of the evolution of root traits was not used in this study because part of the tree was poorly resolved, with very short basal internodes and a mixture of root types. This would imply a very high rate of evolutionary change along the very short internodes, which seems unrealistic for a trait with discrete states such as root type. Moreover, different resolutions among the many possible for the unresolved nodes would imply different rates of change. By contrast, in the well-resolved parts of the tree, the root traits appear to have rarely changed. No single likelihood model would accurately model change in a tree showing such internal inconsistency.

Relationships of Root Type to Other Variables

The possibility that root type (cord vs. unistellar) is associated with soil type or growth habit was assessed using χ^2 tests. The sample included all *Daviesia* species for which these variables could be scored unambiguously: 95 for root type and soil type and 106 for root type and growth habit (data not shown).

Soil types recorded on herbarium sheets of each species were classified into laterite-derived types (the dominant type in the Kwongan) versus other types (in general, of higher nutrient status; Bettenay 1984). For all taxa included in the phylogenetic analysis, these data were checked in the field. Because both lateritic soils and cord-rooted species are restricted mainly to the southwest of Western Australia, the test was repeated including only species from that area (75 species).

Growth habit was classified into plants with stems (or rhizomes) arising from a buried root crown and plants with stems arising at or above ground level. Plants of the first type usually have a well-developed tap root and multiple fine stems and continue to produce new stems from the root crown (Dodd et al. 1984, fig. 8.7B1). Plants of the second type usually have one or few stems and tend to grow by the branching of aerial stems. In shrubs in fire-prone ecosystems such as the Kwongan, burial of the root crown or other perennating structures such as rhizomes is a growth form associated with survival and resprouting following fire, especially if these structures are 5 cm or more below the soil (Pate 1994; Whelan 1995). Shrubs with their shoot buds held above ground are more likely to be killed by fire and to depend on seeds to regenerate following fire.

By assuming independence of all data points, the χ^2 tests may have failed to take account of phylogenetic constraint in the evolution of the traits (Felsenstein 1985b). However, phy-

logenetic contrasts do not necessarily perform better than those treating species as separate data points (Price 1997; Harvey and Rambaut 2000). Moreover, in a tree with very short internal internodes ("starburst"), such as those obtained in part of the tree in this study, no difference is expected between the two approaches (Price 1997). We repeated the χ^2 tests based on the tree (fig. 1) and treated each supported clade (both BS and Bayes posterior probability [PP] > 60) as a single data point if all species in the clade had the same state for both variables. In parts of the tree with short, unsupported nodes, each species was treated as a separate data point.

Results

Sequence Variation and Phylogenetic Analysis

The ITS alignment comprised 798 characters of which 242 (112 within *Daviesia*) were parsimony informative (table 2). These included nine indel positions (two in ITS-1, seven in ITS-2), each less than three base pairs in length. There was no base composition bias (nonstationarity) among taxa. Within *Daviesia*, there was 0%–13% divergence in ITS-1 and 0%–16% in ITS-2. Distances to outgroups were 21%–34% (ITS-1) and 14%–26% (ITS-2). No evidence was found for multiple copies of ITS within individuals.

The MP search of the ITS data found 114 trees (length excluding uninformative positions = 587, consistency index = 0.68, retention index = 0.82). The strict consensus tree (not shown) was almost identical to the trees found by Bayesian inference (fig. 1) and ML (fig. 2) and differed only in some poorly resolved parts. Only a single ML tree was found ($-\ln$ likelihood = 4281.57809). MP weighting schemes did not identify any alternative supported topologies.

Phylogenetic Relationships

There was strong support for monophyly of *Daviesia* (BS = PP = 100); however, its sister group relationship was unclear because ITS placed *Sphaerolobium* as the sister group with only weak support (BS = 58, PP = 72) (fig. 1).

Four well-supported, nonoverlapping clades were identified within *Daviesia* (figs. 1, 2) and have been labeled according to morphological features of each: Terminal Inflorescence, Regular Calyx, Lipped Calyx, and Thick Pod. The remaining species comprised a poorly resolved Residual (Residual group) with very short internal nodes (fig. 2).

The Terminal Inflorescence clade was the sister group to the rest of the genus, within which the Regular Calyx clade was sister group to a well-supported clade of the rest (BS = PP = 100). The Thick Pod clade formed a weak clade with the Residual group (BS = 62, PP = 92). Among the latter, *D. epiphyllum* and *D. speciosa* were weakly supported as sister taxa (BS = 65, PP = 70). Both have similarly large red flowers with a standard that does not reflex.

Evolution of Root Traits

Cord roots were observed in 20 of the 46 taxa of *Daviesia* sampled. Contrary to a previous report (Pate et al. 1989), *D. anceps*, *D. epiphyllum*, *D. obovata*, *D. pachyloma*, *D. pachy-*

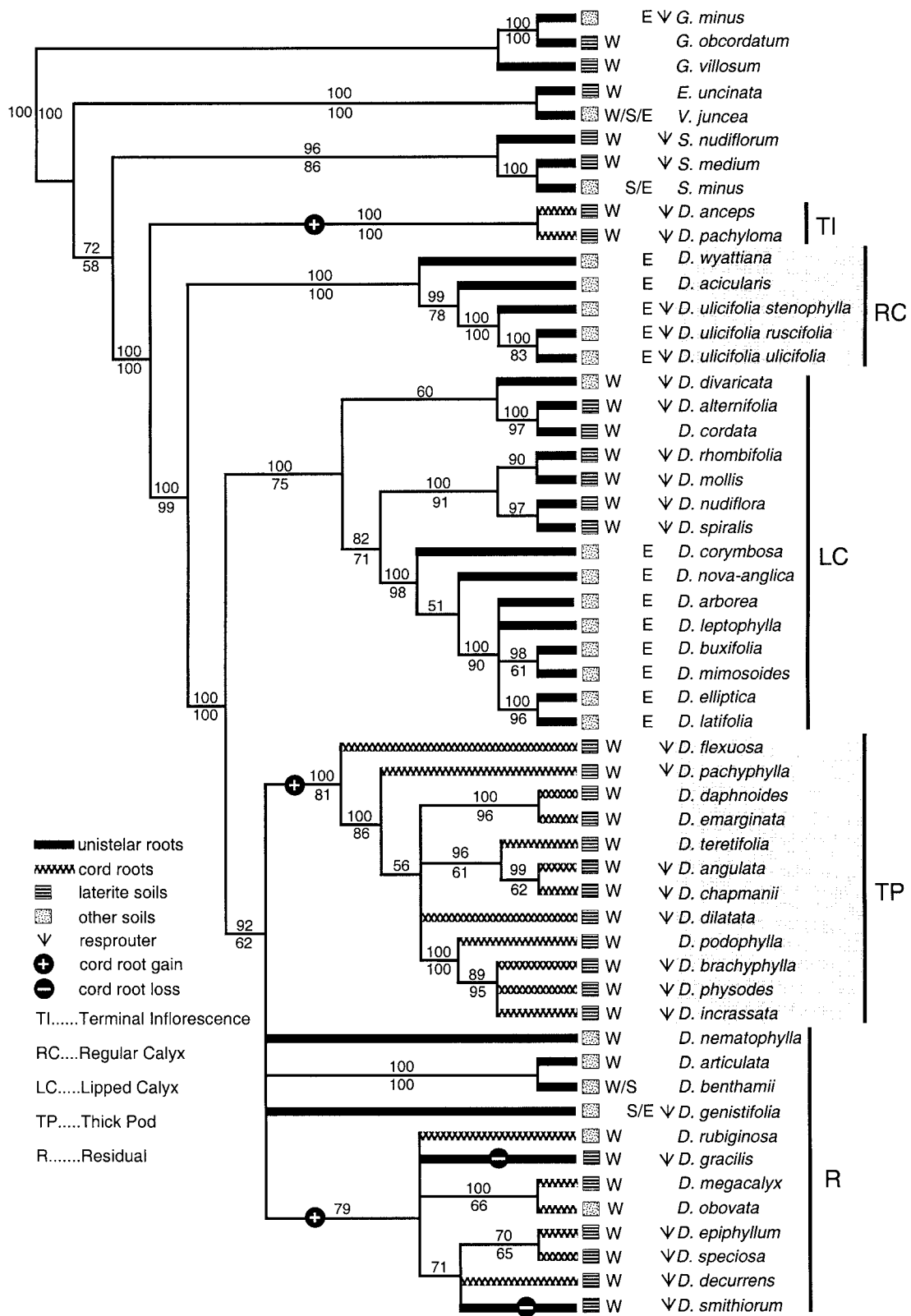


Fig. 1 Majority rule consensus from a Bayesian search (GTR+ Γ) of internal transcribed spacer sequences from *Daviesia* and related genera. Major groups, root types, growth habits, and soils are mapped as indicated in the key at left. Evolutionary transitions in root types inferred by parsimony are mapped on the branches. Geographic distribution is indicated for each terminal: W = southwest Western Australia; S = South Australia; E = eastern Australia. Bayesian posterior probabilities are shown above branches, and bootstrap values >50 are shown below branches. For full taxon names with authors, see table 1.

Table 2
Base Composition and Distribution of Parsimony-Informative Sites among Internal Transcribed Spacer (ITS) Partitions

Region	A	C	G	T	No. BP	No. parsimony informative	
						All taxa	<i>Daviesia</i>
ITS (total including indels)	0.24	0.22	0.26	0.28	670	242	112
ITS-1	0.23	0.23	0.24	0.3	239	132	49
5.8S rRNA	0.26	0.24	0.27	0.23	164	8	4
ITS-2	0.24	0.19	0.25	0.32	217	92	50

phylla, and *D. teretifolia* were observed to have cord roots and *D. gracilis* unistelar roots.

Root type was uniform within clades Terminal Inflorescence and Thick Pod (cord roots) and within Regular Calyx and Lipped Calyx (unistelar roots) but was mixed within Residual (figs. 1, 2). Constraining cord-rooted species to be a monophyletic group required trees a minimum of 29 steps longer than the MP trees ($\Delta = 29$) and was rejected (T-PTP = 0.01). Therefore, a complex pattern of cord-root gain and loss needed to be invoked.

MP optimization of root type gave the same results with both ACCTRAN and DELTRAN (fig. 1). It showed three independent “originations” (gains) of cord roots: one in each of the hypothetical ancestors of clades Terminal Inflorescence, Thick Pod, and Residual (fig. 1). There were two implied reversals (losses) from cord to unistelar roots in *D. gracilis* and *D. smithiorum* within Residual, which gave a total of five steps.

If cord roots had a single origin in the ancestor of *Daviesia*, a loss would be implied in each of Regular Calyx and Lipped Calyx plus either five losses or one loss and four regains within Residual. This requires a total of nine evolutionary steps, which is four more than in the MP reconstruction. Given the poor support for the structure in Residual (fig. 1), plausible alternative topologies could give more parsimonious reconstructions of root evolution. For example, monophyly of all cord-rooted species in Thick Pod + Residual would minimally imply two gains and no losses over the whole tree (rejected: $\Delta = 8$, T-PTP = 0.01). Similarly, monophyly of unistelar species in Lipped Calyx + Residual would imply one gain and two losses (rejected: $\Delta = 10$, T-PTP = 0.01). Monophyly of the cord-rooted species in Residual, which would imply three gains and no losses, was not rejected ($\Delta = 2$, T-PTP = 0.13). Monophyly of the unistelar-rooted species in Residual, implying one gain and three losses, was barely rejected ($\Delta = 7$, T-PTP = 0.05).

Relationships of Root Type to Other Variables

The χ^2 tests including all species indicated that root type was significantly associated with soil type ($P = 4 \times 10^{-7}$, $n = 95$, $df = 1$), even when the test was restricted to southwest Western Australia ($P = 0.005$, $n = 75$, $df = 1$). A separate test showed root type to be significantly associated with growth habit ($P = 0.00004$, $n = 106$, $df = 1$). However, the tree-based χ^2 tests found no significant association among any of the variables. None of the results was changed by restricting the tests to Western Australian species or by exclusion of the unresolved group.

Discussion

Phylogenetic Relationships

This study has identified a strong sister group relationship between *Erichsenia* and *Viminaria* that has not been recognized previously. The only previous systematic studies including both taxa were based on morphology and placed *Viminaria* as sister to *Daviesia*, whereas *Erichsenia* was placed in the sister group to those genera (Crisp and Weston 1987, 1995). All three genera were grouped by shared presence of phyllodes (leaves derived from petioles; Blackmore and Tootill 1984). In *Viminaria* and *Erichsenia*, the adult leaves have a similar terete shape, and their petiolar homology is evident from transitional forms in the seedling. In *Daviesia*, no transitional types are seen in seedlings, and interpretation of the leaves as phyllodes depends on their (convergent) resemblance to the classic “phyllodes” of *Acacia* (Crisp and Weston 1987).

A character shared by *Erichsenia* and *Viminaria* that was not scored in the earlier morphological analysis is indehiscent fruits. This feature is not found in the related genera *Daviesia*, *Gompholobium*, and *Sphaerolobium*. Despite these similarities, *Erichsenia* and *Viminaria* are very different in general appearance, perhaps because they are adapted to different habitats. *Erichsenia* is a low, wiry shrub growing in sandy soils in a semiarid region of the Kwongan, whereas *Viminaria* is a tall shrub or small tree in waterlogged heaths and forest along the eastern, southern, and western coasts of Australia. These ITS data and a previous (Crisp et al. 2000) study placed *Sphaerolobium* sister to *Daviesia*; however, this relationship is not well supported.

Monophyly of *Daviesia* was strongly supported in this study, as it has been in all previous systematic studies. However, the infrageneric relationships of *Daviesia* estimated from these molecular data are only partially congruent with those derived from morphology. The analysis by Pate et al. (1989) also placed *D. anceps* + *D. pachyloma* as sister to the rest of the genus. Therefore, it appears likely that the absence of derived morphological features in *D. anceps* and *D. pachyloma* reflects their divergence before the origin of these features, which include dimorphic stamens with compressed filaments and a determinate unit inflorescence with involucre bracts.

This study has identified a previously unrecognized sister species relationship between *D. epiphyllum* and *D. speciosa*. Although the two taxa differ remarkably in vegetative habit, both species have large red flowers and are the only putatively bird-pollinated taxa in the genus. Their close relationship im-

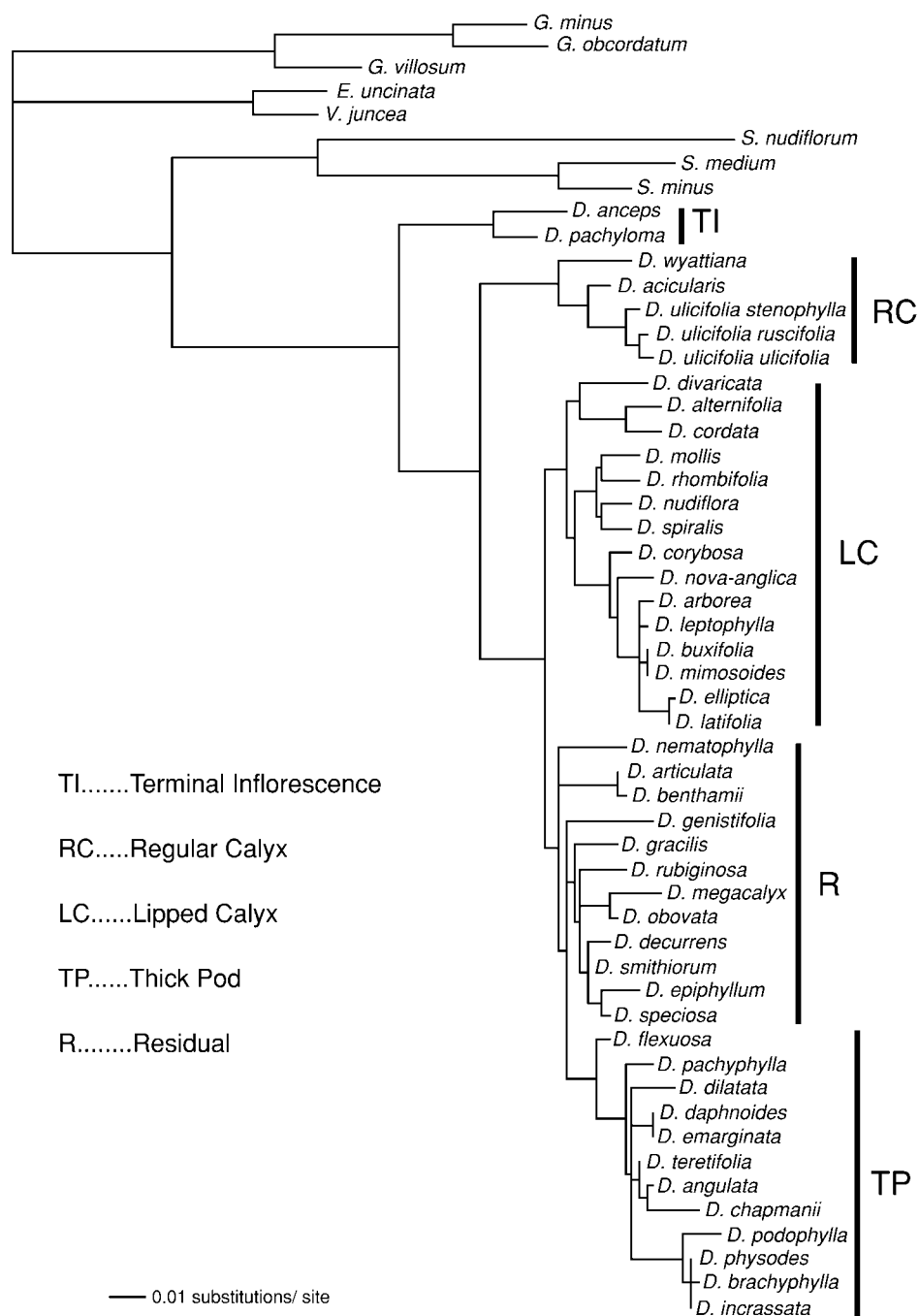


Fig. 2 Phylogram of single tree resulting from maximum likelihood search of internal transcribed spacer sequences from *Daviesia* and related genera (GTR+I; $\alpha = 0.549491$; substitution rate matrix = 1.172614, 2.624805, 1.556172, 0.479229, 4.966558, 1; base frequencies A = 0.23293, C = 0.22770, G = 0.25351, T = 0.28586; $-\ln$ likelihood = 4281.57809). Group labels are as those in fig. 1.

plies a single origin of bird pollination from a bee-pollinated ancestor.

The well-supported clades within *Daviesia* show a strong geographic pattern that may indicate a regional basis for recent radiation in the genus. The Terminal Inflorescence and Thick Pod clades are strictly Western Australian and may be products

of two separate diversifications of cord-rooted taxa in the Kwongan. Lipped Calyx is a clade of eastern species nested within and possibly originating from a group of western species. All the taxa in Regular Calyx are eastern, but this is an artifact of sampling because unsampled taxa in this group occur in central and Western Australia. With comprehensive sam-

pling, this group may be useful for investigating the biogeographic relationships across southern and central Australia.

Evolution of Cord Roots

It appears from this study that evolutionary change of root type in *Daviesia* has occurred only occasionally. Root type was conserved within each of the major clades but not in the Residual group. However, the hypothesis that cord-rooted species within Residual form a single clade could not be rejected. The Lipped Calyx clade comprised only unistellar taxa, which suggests a lack of change from the ancestral root type, even though the taxa differ in growth habit and occur in disparate regions and on different soil types.

The likely number of evolutionary gains of cord roots within *Daviesia* was three or fewer. MP favored three separate origins in Terminal Inflorescence, Thick Pod, and Residual, followed by two losses within Residual. Alternatively (though less parsimoniously), there may have been a single origin in the ancestor of the genus (or earlier) followed by losses in Regular Calyx, Lipped Calyx, and Residual. This scenario is considered because some authors have argued that it is easier to lose complex traits (such as cord roots) in evolution than it is to gain or regain them (Dollo 1893; Farris 1977; Donoghue and Sanderson 1994; Doyle et al. 1995; Cunningham 1999). However, both gains and losses of complex types of inflorescences and flowers have been demonstrated as a result of small mutations in genes that control development (Coen 1991; Cubas et al. 1999; Wang et al. 1999; Cronk 2001).

If cord roots originated independently in the clades Terminal Inflorescence, Thick Pod, and, possibly, Residual (fig. 1), they are not homologous. In this case, root anatomy may differ between clades in structure, function, or development. The only cord-rooted species from this data set whose anatomy and development have been studied in detail are *D. incrassata* (Thick Pod clade) and *D. decurrens* (Residual group). In *D. incrassata*, the roots are externally braided, whereas in *D. decurrens*, they are flanged (Pate et al. 1989), thereby indicating that cord structure may differ between clades. This is consistent with the hypothesis of independent origin of cord roots in each clade.

Developmental studies of roots in *Daviesia* have shown that all species initiate normal secondary thickening and that cord roots develop later if they develop at all (Pate et al. 1989). In some species, such as *D. incrassata*, the central unistellar meristem is suppressed early in development, and cord roots continue as the only form of secondary thickening in the adult plant (Pate et al. 1989). In other species such as *D. obovata*, *D. epiphyllum*, and *D. dilatata*, we have observed both types of secondary thickening operating in parallel in the mature plant. A specimen of *D. dilatata* has a central stele 2 cm in diameter, with many seasonal growth rings, and outside it seasonal rings of cord roots. Both types are seen in *D. pachyphylla*, too, but seedlings appear to delay initiation of cord roots for several seasons. Therefore, any evolutionary loss of cord roots may have been the result of neoteny, i.e., an indefinite delay in their initiation.

Cordlike roots may have originated not in the ancestral species of *Daviesia* but earlier, because such roots have been reported in *Phyllota* and *Euchilopsis* (Pate et al. 1989; Pate and

Dixon 1996). If these were homologous with the cord roots of *Daviesia*, then a single origin of the trait would trace back to the nearest common ancestor of both genera, at or close to the base of the tribe Mirbelieae (Crisp et al. 2000). In this case, cord roots would have been lost in most genera of Mirbelieae. However, preliminary observations indicate that the cordlike roots of *Phyllota* differ anatomically and developmentally from those of *Daviesia*. Therefore, it seems most likely that cord roots have multiple origins, although phylogenetic constraint in an ancestral member of Mirbelieae may have predisposed the parallel evolution of cordlike types. In this case, their evolution may have been favored by certain adaptive pressures.

Adaptive Significance of Cord Roots

No function has been proposed for cord roots. One possibility is that they are an adaptation to the lateritic soils of the Kwongan, which are deficient in nutrients and water-holding capacity (Bettenay 1984; Pate and Dixon 1996). However, cord-rooted and unistellar-rooted species of *Daviesia* coexist in the same habitats on lateritic soils (Pate and Dixon 1996; M. D. Crisp and L. G. Cook, personal observations). The statistical relationship between gross soil type and root type was equivocal, depending on whether all species were treated as independent. Perhaps the two root types are alternative adaptations that allow closely related species of *Daviesia* to coexist on Kwongan soils by niche differentiation.

Another possibility is that cord roots have a storage function that aids the survival and resprouting of plants following fire. Many cord-rooted species have thick tap roots, with the root crown buried by several centimeters, and the plants appear to resprout after fire. There is a high proportion of parenchyma in cord roots, both within and between the vascular strands, and there is evidence of high quantities of starch (Pate and Dixon 1996, fig. 29; M. D. Crisp and L. G. Cook, personal observations). We found a significant association between this growth habit and cord roots only when the species were treated as independent. Therefore, this possible relationship requires further investigation.

Some cord roots may be contractile. Such roots have evolved independently in disparate taxa, such as ferns (H. Schneider, personal communication), *Oxalis* (Oxalidaceae), and a number of monocot groups (Putz and Froebe 1995; Putz et al. 1995) including *Xanthorrhoea* (Xanthorrhoeaceae) (Staff and Waterhouse 1981). They are commonly associated with storage organs (e.g., bulbs and corms) and roots that either do not undergo secondary thickening or are anomalously thickened. Two mechanisms have been identified that move the plant into the soil: (i) radial expansion (pushing) that opens a channel in the soil and lowers resistance to downward movement and (ii) pulling work by lateral or distal roots (Putz et al. 1995). Underground organs with strands of vascular tissue embedded in a parenchymatous matrix can expand and contract to form channels in the soil (Putz et al. 1995). Some cord roots have this kind of anatomy, at least in the juvenile stages (Pate et al. 1989). (This is not true of late-developing cord anatomy in a few species such as *D. pachyphylla*.) In *Xanthorrhoea*, contractile roots draw the crown of seedlings underground and protect the plants at a life stage when they are

vulnerable to fire (Gill and Ingwersen 1976). Cord-rooted seedlings of some species of *Daviesia*, having established after fire, rapidly develop several vascular layers in their tap roots, e.g., one to two in the first season and one to four in each of the next three seasons (Pate et al. 1989). If cord roots are contractile, this may be a mechanism to enable postfire survival by protecting the crown, especially in the vulnerable seedling stage.

Conclusion

Cord roots are limited to three separate clades within *Daviesia* but have a complex history involving a small number of gains (most likely 0–3) or losses (0–5). The hypothesis that cord roots may be an adaptation to the low-nutrient lateritic soils of Western Australia appeared to be supported by a significant association between root type, geographic region, and

soil type but only when these variables were treated as independent. Tests were insignificant when they treated occurrence of the same root trait in all species of a lineage as nonindependent. This difference in result underlines the importance of taking phylogenetic constraint into account when testing comparative hypotheses. Therefore, the restriction of most cord-rooted species of *Daviesia* to the Kwongan soils of southwestern Australia may be a coincidence in which the trait occurs only in particular clades that are geographically restricted to that region.

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