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HISTORICAL BIOGEOGRAPHY AND THE ORIGIN OF STOMATAL DISTRIBUTIONS IN *BANKSIA* AND *DRYANDRA* (PROTEACEAE) BASED ON THEIR CPDNA PHYLOGENY[1]

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Banksia and *Dryandra* have undergone extensive speciation and adaptive radiation, especially in Australia's isolated Southwest Botanical Province. We derive a phylogeny for these groups based on cpDNA sequences and use it to reconstruct their historical biogeography and evolution of leaf traits thought to be adapted to drought and/or nutrient poverty. Slowly evolving regions (*trnL* intron, *trnL*/*trnF* spacer) are used to resolve large-scale relationships; faster evolving regions (*rp116* intron, *psbA*/*trnH* and *trnT*/*trnL* spacers) are used to resolve relationships among closely related species. *Banksia* is paraphyletic with respect to *Dryandra*. The lineage underwent a basal split into two clades (here named /*Cryptostomata* and /*Phanerostomata*), and four infrageneric taxa supported by morphological cladistic analyses (series *Spicigerae*, *Abietinae*, *Tetragonae*, and *Banksia*) are not monophyletic. Dispersal-vicariance analysis resolves a southwestern Australian origin for the lineage, with two later expansions to the east followed by vicariance events. Stomatal crypts arose with the /*Cryptostomata*, which is characterized by tough, long-lived leaves and common in southwestern Australia. Sequestering of stomata also arose multiple times in /*Phanerostomata*, which is characterized by softer, short-lived leaves and common in moister coastal areas, via inrolling of the margins of narrow leaves and restricting stomata to shallow pits. The hypothesis that sclerophylly preadapted the plants to xeromorphy is supported in the case of shallow stomatal pits and deep stomatal crypts, but not narrow, needle-like leaves.

Key words: Australia's Southwest Botanical Province; *Banksia*; *Dryandra*; historical biogeography; molecular systematics; preadaptation; sclerophylly; xeromorphy.

Phylogenetic study of the closely related Australian genera *Banksia* L.F. (79 spp.) and *Dryandra* R.Br. (93 spp.) of Proteaceae is compelling for at least three reasons. First, these genera are among the most striking examples of adaptive radiation in the flowering plants (Carlquist, 1974). Species range from prostrate woody mats to trees >20 m tall. Their variously colored inflorescences are elongate (*Banksia*) or capitate (*Dryandra*) and are pollinated by birds, bats, bees, and marsupials (Taylor and Hopper, 1988). They survive frequent fires by resprouting from underground lignotubers or by releasing seed from massive woody follicles (Lamont and Markey, 1995). Perhaps most remarkably, some species hold soft, entire leaves for only 1–2 yr, while others maintain tough, often viciously serrate ones for over a decade (e.g., see Witkowski et al., 1992), and striking parallelisms occur in sclerophyllous and xeromorphic characters associated with this leaf persistence. Thus, their value to comparative ecology is great and, arguably, would be best understood in a phylogenetic context.

Second, 80% of *Banksia* and all members of *Dryandra* are restricted to Australia's Southwest Botanical Province, one of the world's great centers of floristic endemism with 75% of 8000 native species found nowhere else (Hopper, 1992). Most taxa have quite limited ranges, and none of the eastern Australian members of *Banksia* are also found in the isolated southwest. It is thought that floristic interchange between the regions was obstructed by climatic and edaphic conditions as early as the Eocene, first by marine flooding of the large Eucla Basin on the south-central coast, then by aridity in the central deserts and by calcareous substrates on the Nullarbor Plain (Crisp, West, and Linder, 1999; Fig. 1). The phylogeny of *Banksia* and *Dryandra* provides a framework for determining the importance of these and other vicariance events in generating such staggering diversity in Australia's southwest corner.

Finally, the ancient and extensive macrofossil record of *Banksia* and *Dryandra* provides direct evidence of ancestral leaf traits in these groups and critical insights into the origin of sclerophylly and xeromorphy in the Australian flora during the continent's Tertiary aridification (Hill, 1990, 1992, 1994, 1998; Hill and Merrifield, 1993; Hill, Scriven, and Jordan, 1995; Hill et al., 1999). *Banksieaephyllum taylorii* R.J.Carpenter, G.J.Jordan, & R.S.Hill from the Late Paleocene represents the earliest known macrofossil of *Banksia* and/or *Dryandra* (Carpenter, Jordan, and Hill, 1994). This taxon has sclerophyllous features (sensu Hill, 1990: i.e., thick, fibrous leaves and dense venation), but it lacks clearly xeromorphic ones and is thought to have been part of a rainforest assemblage. Taxa displaying the extreme adaptations to aridity seen in the two genera today (including stomata restricted to deep crypts and needle-like leaves with strongly revolute margins; see fig. 16.6 of Hill, 1994) are seen at the earliest in the Late Eocene (McLoughlin and Hill, 1996). Hill (1990, 1992, 1994; Hill and Merrifield,

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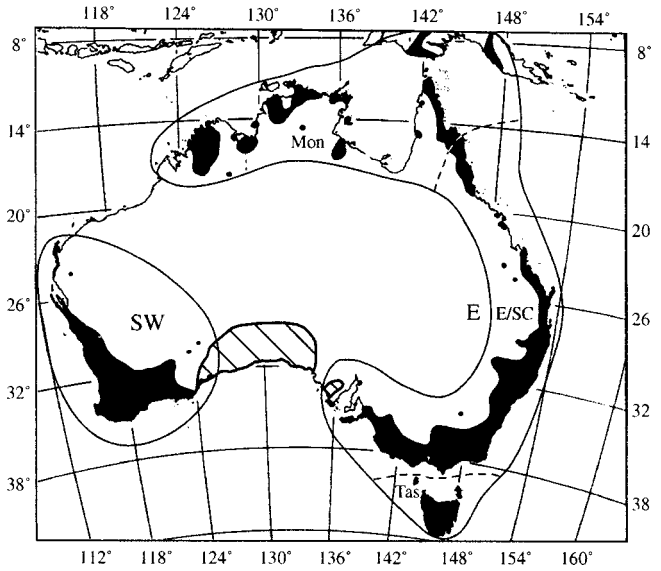

Fig. 1. Distribution of *Banksia* in Australia and New Guinea. Our study considers the disjunction between species from the southwest (SW) and east (E). The ''east,'' as recognized in our study, is a composite of Crisp, Linder, and Weston's (1995) ''east/south coast group'' of areas (E/SC), ''monsoon group'' of areas (Mon), and Tasmania (Tas). Fifteen of the sixteen eastern species of *Banksia* are primarily distributed in the ''east/south coast group'' of areas. Only one, *B. dentata*, is primarily distributed in the ''monsoon group'' of areas, and only two, *B. marginata* and *B. serrata*, have populations in the ''east/south coast group'' of areas and Tasmania. *Dryandra* is endemic to the southwest. The Eucla basin is shown with hatchmarks. The distribution of *Banksia* is redrawn from George (1981), and the position of the Eucla Basin is redrawn from Kemp (1978).

Figure Abbreviations: SW = southwestern Australia; E = eastern Australia; Mon = monsoon group of areas from Australia and New Guinea; E/SC = east/south coast group of areas from Australia; Tas = Tasmania; M = subtribe Musgraveinae; /*Crypto* = /*Cryptostomata*; /*Phanero* = /*Phanerostomata*.

1993) concludes from this evidence that sclerophylly was present in the early Tertiary as a response to nutrient poor soils and that it ''preadapted'' the ancestral lineages to the later onset of aridity. Reconstruction of sclerophyllous and xeromorphic character states on the phylogeny can provide a test of these hypotheses independent of the fossil record.

Systematics and previous phylogenetic studies—*Banksia* and *Dryandra* constitute subtribe Banksiinae. With two small genera from the rainforests of Queensland (*Austromuellera* C.T.White and *Musgravea* F.Muell.; subtribe Musgraveinae; four species), they constitute tribe Banksieae. Morphological synapomorphies support the monophyly of subtribe Banksiinae and tribe Banksieae (Johnson and Briggs, 1975; Thiele and Ladiges, 1996). Recent authors (Carpenter, Jordan, and Hill, 1994; Thiele and Ladiges, 1996) suggested that morphological synapomorphies have yet to be described for subtribe Musgraveinae. However, Johnson and Briggs (1975, p. 111) believed that the three hypogynous glands of flowers in Musgraveinae represent a reduction from the primitive condition of four for the group in support of its monophyly. An extremely condensed, capitate inflorescence and an involucre of conspicuous bracts support the monophyly of *Dryandra* (Thiele and Ladiges, 1996). The monophyly of *Banksia* is tentatively supported by the presence of a ''banksioid cortical mesh'' in the inflorescence (Venkata Rao, 1964; Thiele and Ladiges, 1996). However, this vascular feature could have been secondarily lost in *Dryandra* during an extreme reduction of its inflorescence axis (Thiele and Ladiges, 1996).

Thiele and Ladiges (1996) performed cladistic analyses of *Banksia* at multiple levels using 90 qualitative and 14 quantitative morphological and anatomical characters. From their results, they derived an infrageneric classification for the genus (see supplementary material at the *American Journal of Botany's* website, http://ajbsupp.botany.org/v89). Unfortunately, attempts to use *Dryandra* or subtribe Musgraveinae as outgroups were frustrated by the high frequency of parallelisms in the former and the difficulty of interpreting homologous structures in the latter. Their cladogram thus remained unrooted.

Two molecular studies with limited sampling have considered phylogenetic relationships within tribe Banksieae. Mast (1998) sampled the nuclear ribosomal internal transcribed spacer (ITS) regions and the chloroplast *trnL* intron, *trnL* 3′ exon, and *trnL*/*trnF* spacer for up to 22 taxa in the tribe. Sequence comparisons with taxa outside the tribe could be made only with the more conserved cpDNA data. Those data support the monophyly of tribe Banksieae, subtribe Banksiinae, and subtribe Musgraveinae. Within subtribe Banksiinae, however, neither *Banksia* nor *Dryandra* are resolved as monophyletic, though *Dryandra* could be, given the placement of its taxa in a single polytomy. The nuclear ITS results resolve a paraphyletic *Banksia* with respect to a monophyletic *Dryandra* and unambiguously support the rooting of Thiele and Ladiges' (1996) cladogram along its second longest branch (Mast, 1998).

Maguire et al. (1997) sampled randomly amplified polymorphic DNA (RAPD) markers for 37 taxa and *trnL*/*trnF* spacer sequences for 14 taxa in the tribe; taxa outside the tribe were not sampled. They judged their RAPD results uninformative when compared across the breadth of the subtribe. Their *trnL*/*trnF* strict consensus tree resolves a polyphyletic *Banksia* and *Dryandra*. However, in comparison with the sequences generated by Mast (1998) and this study, their sequences are frequently missing random fragments one to three nucleotides (nts) in length. Such fragmented data affects alignment and phylogeny reconstruction in unpredictable ways, and so we will not consider their *trnL*/*trnF* strict consensus tree further.

Objectives—Our objectives in this study are thus to develop a well-resolved, well-supported phylogenetic hypothesis for *Banksia* and *Dryandra* with which we can then (1) test the monophyly of these two genera and the infrageneric taxa recognized by Thiele and Ladiges (1996), (2) determine the area of origin for the lineage and the history of floristic interchange between eastern Australia and the Southwest Botanical Province, and (3) reconstruct the origins of sclerified leaves and stomatal distributions in the group to assess the hypothesis of sclerophylly pre-adapting the lineage to xeromorphy.

MATERIALS AND METHODS

Sampling—We sampled 84 of the 91 taxa of *Banksia*, 5 of the 93 species of *Dryandra*, 1 of the 2 genera of subtribe Musgraveinae, and 11 additional genera of the 44 in subfamily Grevilleoideae. From each of these taxa, two to five cpDNA regions were sampled (see supplementary material at http://ajbsupp.botany.org/v89), depending on each taxon's function in the following four sampling strategies.

Strategy I sampled the greatest taxonomic breadth with reduced taxonomic density and used the more slowly evolving *trnL* and *rp116* introns and *trnL*/*trnF* spacer. The sampling included (1) a single taxon from each of Thiele and Ladiges' (1996) series of *Banksia*, (2) all taxa of uncertain placement in *Banksia*, (3) 5 species of *Dryandra*, (4) 1 genus in subtribe Musgraveinae, and (5) 11 additional genera from subfamily Grevilleoideae.

Strategy II included the greatest number of taxa within subtribe Banksiinae but did not include taxa outside of it. For this, the *rp116* intron and *psbA*/*trnH* spacer were sequenced from 84 taxa of *Banksia* and 5 species of *Dryandra*. Genera outside of the subtribe were not included because large stretches of their *psbA*/*trnH* spacer sequences could not be aligned with the ingroup. This strategy forms an important bridge between strategy I and strategies III and IV, for it tests the adequacy of placeholders in the former and defines subsamples for the latter two.

Strategies III and IV separate the 89 taxa from strategy II into the two clades formed by the basal split of the subtribe according to the results of strategy I. Strategy III added *trnT*/*trnL* spacer data to that from the *rp116* intron and *psbA*/*trnH* spacer for 45 taxa (one clade). Sequencing the *trnT*/*trnL* spacer of the remaining 44 taxa (the other clade) proved difficult because of a long AT-rich region in their *trnT*/*trnL* spacer. Thus, strategy IV sampled these 44 taxa without new DNA regions (with only *rp116* and *psbA*/*trnH* data). Reducing the number of taxa considered at one time from 89 to, at most, 45 facilitated maximum likelihood (ML) analyses.

DNA extraction, amplification, and sequencing—The first author collected leaf tissue and vouchers for 80 of the 101 taxa included here during field trips to Australia in 1995 and 1996. The remaining leaf tissue came from other individuals, herbarium specimens, or botanical gardens (see supplementary material at http://ajbsupp.botany.org/v89). We extracted total genomic DNA from 1–1.5 g of frozen leaf material, 0.25–0.5 g of silica dried material, or 0.1–0.3 g of herbarium material following the same procedure as Mast (1998).

Each cpDNA region was amplified using the polymerase chain reaction (PCR; Mullis and Faloona, 1987): the *trnL* intron and the *trnL*/*trnF* spacer with primers ''c'' and ''f'' of Taberlet et al. (1991), the *rp116* intron with primers ''F71'' of Jordan, Courtney, and Neigel (1996) and ''R1516'' of Baum, Small, and Wendel (1998), the *psbA*/*trnH* spacer with ''psbAF'' and ''trnHR'' of Sang, Crawford, and Stuessy (1997), and the *trnT*/*trnL* spacer with primers ''a'' and ''b'' of Taberlet et al. (1991). For this, the most effective thermal cycling program proved to be 28–32 cycles of 0.5 min at 94°C, 1 min at 48–52°C, and 1.5 min at 72°C, with an extension of 7 min at 72°C after the last cycle. To detect successfully amplified DNA and the possible contamination of negative controls, we examined PCR products on 1% agarose gels. We purified successful PCR reactions with the QIAquick Spin PCR Purification Kit (QIAgen, Valencia, California, USA).

We prepared cycle-sequencing reactions with the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA). The primers that we used to sequence included those used to amplify the DNA, as well as primers ''d'' and ''e'' of Taberlet et al. (1991) for the *trnL* intron, and the *trnL*/*trnF* spacer, respectively. A Gene Amp PCR System 2400 (Applied Biosystems) performed all thermal cycling. We precipitated sequenced product in ethanol and sodium acetate to remove excess dye terminators before running it out on an ABI Prism 377 DNA Sequencer (Applied Biosystems). To detect mistakes and correct uncertainties in the computer-generated sequence, we compared aligned trace-files in Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA).

Defining substitution and insertion and deletion (indel) characters—The manually aligned sequence matrices are available at TreeBase (http://www.treebase.org/treebase/index.html). Though most of the alignments were straightforward, length changes and nucleotide substitutions occasionally combined to make the alignment of a section ambiguous. The largest of these regions occurred in the *rp116* intron (involving a repeated motif about 19 nts long; positions 19–123) and the *trnT*/*trnL* spacer (involving a hypervariable AT-rich region; positions 135–288). We excluded these two long regions and five short regions from all analyses. We observed three inversions in the data sets: (1) an inversion 2 nts long in the *trnL* intron (positions 846–847); (2) one 24 nts long in the *psbA*/*trnH* spacer (positions 64–113; the two inversion types are offset in the alignment), and (3) one 3 nts long in the *trnT*/*trnL* spacer (positions 113–115). The regions bordering these inversions are the reverse complement of one another and likely form a stem structure when the DNA is single stranded. These short inversions proved to be highly homoplasious and were excluded from all analyses, as recommended by Sang, Crawford, and Stuessy (1997).

Indels were coded as additional, unordered characters for maximum parsimony (MP) analyses if they were bordered by stretches of unambiguously aligned nts and were potentially informative. We chose not to code single base pair indels if they were adjacent to strings of the same nt (e.g., four As present versus five As). Other researchers have similarly excluded this type of indel because it may arise due to experimental error (e.g., Downie et al., 1998; McDade and Moody, 1999), or they have noted its evolutionary lability (e.g., Small et al., 1998). We coded indels as the same state if they were the same size and did not vary by more than 1 nt substitution (when greater than 6 nts long). We excluded regions from the analysis that appeared to contain insertions of different origin because the positions involved were already coded as single events.

Phylogenetic reconstruction—Sequence data were analyzed using MP and ML approaches, as implemented in PAUP* 4.04ba (Swofford, 2000). We explored the effect of alternative weighting combinations on the MP results. The first weighted transitions and transversions equally (ti : tv = 1 : 1) and nts and indels equally (nt : indel = 1 : 1). The second excluded the indels (nt : indel = 1 : 0) and weighted the transitions and transversions at the inverse of the empirical ti : tv. We estimated this ratio within each sampling framework during the ML analyses of the data using the Hasegawa-Kishino-Yano (HKY; Hasegawa, Kishino, and Yano, 1985) substitution model. A third weighting of ti : tv = 1 : 1 and the nts and indels weighted the inverse of the empirical nt : indel always produced the same topology as the first combination (Mast, 2000) and is not presented here. The MP analysis employed tree-bisection-reconnection branch swapping with ten random addition replicates; only potentially informative characters were included.

To ascertain the relative degree of support for branches in the MP topologies, bootstrap (Felsenstein, 1985) and decay indices (Bremer, 1988, 1994) were computed in PAUP* 4.0b4a. The bootstrap function resampled the data 100 times; MP analyses of each replicate employed nearest-neighbor-interchange branch swapping with the maximum number of saved trees set at 5000. We determined the decay indices by progressively saving trees one step longer than the previous search, starting at one step longer than the shortest tree. When this method became impractical, we constrained the searches, in turn, to trees that did not have each of the branches that remained. For these reverse-constraint searches, PAUP* 4.0b4a performed ten random addition replicates.

The adequacy of eight ML models were assessed, in turn, for strategies I, III, and IV by Mast (2000). The significance of improvement in ML scores was determined by calculating a likelihood-ratio test statistic and comparing it with a chi-squared approximation of the null distribution (Goldman, 1993). Only the results with the ML models determined to be adequate for these three strategies are reported here.

Biogeography—The southwestern/eastern biogeographic disjunction was examined at a coarse level by mapping the subcontinental distributions of extant taxa onto the cpDNA strict consensus for tribe Banksieae (strategy II with the Musgraveinae grafted as sister). With three exceptions, ''eastern'' Australia, as the term is used here to describe the distribution of *Banksia*, corresponds to the ''east/south coast group'' of areas from the cladistic biogeographic analysis of nine plant groups from Australia and New Guinea by Crisp, Linder, and Weston (1995). One exception involves *Banksia dentata* L.f., which is found primarily outside of the ''east/south coast group,'' in Crisp and colleagues' ''monsoon group'' of areas from northern Australia and New Guinea. The other two exceptions involve the distribution of two widespread taxa, *B. marginata* Cav. and *B. serrata* L.F., in the ''east/south coast group'' of areas and Tasmania, which was considered to be separate from them. ''Southwestern'' Australia, as it is used here to describe the distribution

TABLE 1. Sequence descriptions. Outgroup taxa are those outside of tribe Banksieae. The raw length for the *psbA*/*trnH* spacer that is given in parentheses represents an outlier (*Dryandra calophylla*). See text for criteria used to exclude aligned positions. The mean guanine and cytosine (GC) content is given as a percentage of the sequence lengths. Some cells of the table are not applicable (—).

Region	Ingroup raw length (range)	Outgroup raw length (range)	Aligned length	Number of positions excluded	Mean GC content
trnL intron	457–463	457–475	526	2	35.2%
trnL/*trnF* spacer	376–389	266–395	422	2	32.4%
rp116 intron	937–1010	923–961	1131	178	34.2%
psbA/*trnH* spacer	360 (177)–405	—	499	24	27.0%
trnT/*trnL* spacer	469–526	—	625	157	29.7%

of *Banksia* and *Dryandra*, is equivalent to Beard's (1980) Southwest Botanical Province, with the exception of a few species that are also found in adjacent parts of the Southwestern Interzone and Eremaean Province.

We applied two methods to determine the distribution of ancestral taxa. The first, the dispersal-vicariance method in DIVA 1.1a (Ronquist, 1997), reconstructs the most recent common ancestor of allopatric daughter clades as widespread, with allopatry arising by vicariance. The second, Fitch optimization in MacClade 3.05 (Maddison and Maddison, 1992), does not allow widespread (polymorphic) ancestral taxa and reconstructs dispersal and cladogenetic events as co-occurring.

Origin of sclerophyllous and xeromorphic characters—We tested the hypothesis of sclerophylly pre-adapting plants to xeromorphy by reconstructing the evolutionary history of ''vertically transcurrent plates'' of sclerenchyma associated with the leaf vasculature (Cookson and Duigan, 1950) and stomatal distributions in the lineage. Strategy II was again used, with the Musgraveinae grafted as sister, as in the biogeographic analysis. Character-state data for subtribe Banksiinae are from Cookson and Duigan (1950) and Thiele and Ladiges (1996), whereas stomatal positions for subtribe Musgraveinae are from Carpenter (1994). G. Jordan determined that plates of sclerenchyma in association with the vasculature are present in the leaves of *Musgravea heterophylla* L.S.Sm. (University of Tasmania, personal communication); we are unaware of studies that have examined the remaining three species in the subtribe. The reconstructions implement Fitch optimization in MacClade 3.05 (Maddison and Maddison, 1992) with the polytomies resolved as ''hard.''

The three states that we recognize for the distribution of stomata differ slightly from those recognized by Cookson and Duigan (1950). They recognized the stomata of *Banksia* to be (1) superficial, (2) in longitudinal grooves on either side of the midrib, or (3) in pits. Thiele and Ladiges (1996) pointed out that the second distribution type is really a special condition of the first, for the superficial stomata are in grooves formed by the revolute leaf margins of narrow, linear leaves. Further, Thiele and Ladiges (1996) observed that the ''pits'' of series *Salicinae* Meissner, *Grandes* A.S.George, and *Coccineae* A.S.George are actually shallow depressions, rather than the more crypt-like structures with constricted entrances seen elsewhere. The three stomatal positions thus recognized here are (1) superficial, (2) in shallow pits, and (3) in deep crypts. The evolution of needle-like leaves with revolute margins was reconstructed separately. This trait, and the sequestering of stomata in crypts and pits, were considered unambiguous xeromorphic adaptations by Hill (1998).

RESULTS

Nt and indel variation—In total, 3203 aligned nt positions are considered in these analyses (Table 1). Eleven percent of these (363 nts) are excluded from analyses due to alignment ambiguities, inversions, or multiple insertion events. The low guanine and cytosine (GC) content (27–35.2%) is similar to that reported for cpDNA in general (Palmer, 1985).

As a percentage of aligned (and included) nt positions, the most informative regions are the *rp116* intron in strategy I, the *psbA*/*trnH* spacer in strategy II, the *trnT*/*trnL* spacer in strategy III, and the *rp116* intron (for nts) or the *psbA*/*trnH* spacer (for indels) in strategy IV (Table 2). We coded 29 indel characters (Table 3): 9 characters that are most parsimoniously reconstructed as deletions, 15 that are reconstructed as insertions, and 5 that are of uncertain reconstruction. Two of the insertion characters were coded as having three states, each of which are shared between two or more taxa: two dissimilar insertions of 9 nts long at position 419 of the *psbA*/*trnH* spacer

TABLE 2. DNA character descriptions. Informative nucleotide (nt) positions and insertions or deletions (indels) are given (in parentheses) as a percentage of the total aligned positions for each region. The *psbA*/*trnH* region in STRATEGY IV contains different numbers of informative nt positions depending on whether or not the transitions and transversions are weighted equally. The empirical transition to transversion ratio (ti:tv) is estimated using a Hasegawa-Kishino-Yano (HKY; Hasegawa, Kishino, and Yano, 1985) substitution model on one of the most parsimonious trees found using only substitution data. Divergence was measured assuming an HKY substitution model. Some cells of the table are not applicable (—).

Strategy	Region	Informative nt positions	Informative indels	Empirical ti : tv	Divergence within subtribe	Divergence including outgroups
I	*trnL* intron	20 (3.8%)	3 (0.6%)	1.71 : 1	0–1.12%	0–5.50%
	trnL/*trnF* spacer	29 (6.9%)	3 (0.7%)	1.38 : 1	0–2.49%	0–8.04%
	rp116 intron	71 (7.5%)	8 (0.8%)	2.03 : 1	0–1.98%	0–7.14%
	Combined	120 (6.3%)	14 (0.7%)	1.77 : 1	0–1.55%	0–6.59%
II	*rp116* intron	46 (4.8%)	9 (0.9%)	2.60 : 1	0–2.10%	—
	psbA/*trnH* spacer	25 (5.3%)	6 (1.3%)	0.31 : 1	0–4.40%	—
	Combined	71 (5.0%)	15 (1.1%)	0.96 : 1	0–2.17%	—
III	*rp116* intron	21 (2.2%)	4 (0.4%)	3.43 : 1	0–1.52%	—
	psbA/*trnH* spacer	15 (3.2%)	0 (0.0%)	0.52 : 1	0–3.18%	—
	trnT/*trnL* spacer	17 (3.6%)	4 (0.9%)	1.34 : 1	0–2.62%	—
	Combined	53 (2.8%)	8 (0.4%)	1.44 : 1	0–1.41%	—
IV	*rp116* intron	23 (2.4%)	5 (0.5%)	1.71 : 1	0–1.38%	—
	psbA/*trnH* spacer	11, 12 (2.3%)	6 (1.3%)	0.22 : 1	0–2.92%	—
	Combined	34, 35 (2.4%)	11 (0.8%)	0.61 : 1	0–1.73%	—

TABLE 3. Insertion and deletion (indel) characters. The alphabetical character code is used when mapping indel character changes onto cladograms. Uncertainties (?) in whether a character is an insertion or deletion arise when the character is variable in taxa outside tribe Banksieae.

Region	Code	Starting position	Approximate length (nts)	Insertion (I) or deletion (D)
trnL intron	A	169	5	D
	B	199	5	I
	C	385	4	D
trnL/*trnF* spacer	D	18	94	?
	E	191	1	?
	F	203	21	?
rp116 intron	G	8	8	?
	H	263	1	D
	I	332	2	D
	J	347	2	D
	K	415	1	I
	L	527	1	?
	M	711	5	I
	O	773	1	I
	P	775	8	I
	Q	834	7	I
	R	947	1	D
	S	961	8	D
	T	1033	5	D
psbA/*trnH* spacer	U	161	11	I
	V	235	1	I
	W	317	8	I
	X	389	7	I
	Y	419	9	I
	Z	450	9, 23	I
trnT/*trnL* spacer	AA	30	8	I
	AB	99	10	I
	AC	524	4	D
	AD	585	4	I

and two insertions of unequal size (9 vs. 23 nts) at position 450 of the *psbA*/*trnH* spacer. Taking these into account, the mean length of the informative deletions is 3.6 nts, and the mean length of the informative insertions is 7.4 nts.

Phylogenetic reconstruction—When the relationships within subtribe Banksiinae are compared on strict consensus trees derived from different strategies, there is no topological conflict. Additionally, Mast (2000) determined that all pairwise comparisons of data sets within each strategy support homogeneity at a level of $P = 0.05$ with the incongruence length difference test (Mickevich and Farris, 1981; Farris et al., 1995).

Strategy I—Equally weighted (ti : tv = 1 : 1, nt : indel = 1 : 1) MP analysis of the *trnL* intron, the *trnL*/*trnF* spacer, and the *rp116* intron results in 720 shortest trees (229 steps, consistency index [CI] = 0.681, retention index [RI] = 0.870; all reported MP indices with uninformative characters excluded). The strict consensus of these trees (Fig. 2) supports the monophyly of tribe Banksieae (87% bootstrap, 2 steps decay), subtribe Banksiinae (100% bootstrap, 15 steps decay), and genus *Dryandra* (91% bootstrap, 2 steps decay). *Dryandra* is nested in *Banksia* by three branches of 94, 79, and 62% bootstrap and 3, 1, and 1 steps decay, respectively. The alternative weighting scheme (ti : tv = 1 : 1.8, nt : indel = 1 : 0) produces a strict consensus tree that only differs from Fig. 2 in the collapse of three branches (arrows in Fig. 2) and the resolution

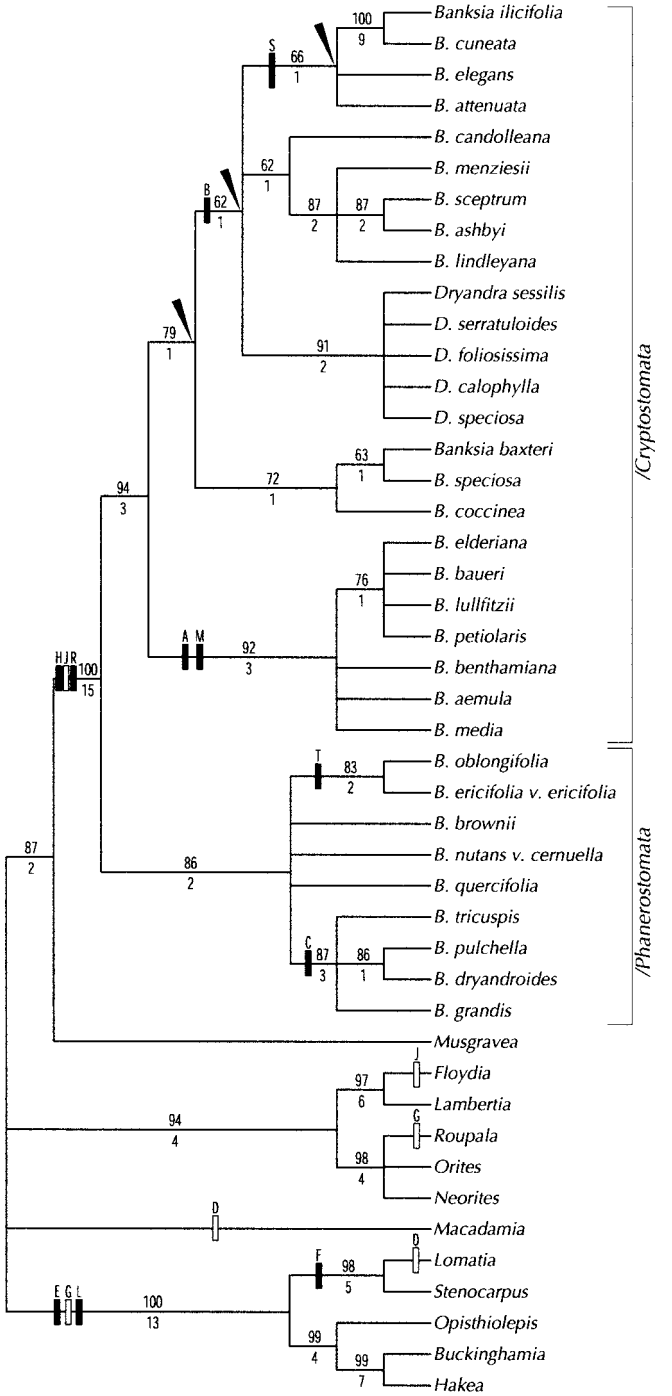

Fig. 2. Strict consensus of 720 shortest trees (229 steps; consistency index [CI] = 0.681, retention index [RI] = 0.870) found in maximum parsimony (MP) analysis using data from the *trnL* intron, *trnL*/*trnF* spacer, and *rp116* intron (STRATEGY I) with a transition to transversion weighting (ti : tv) = 1 : 1 and nt : indel = 1 : 1. Bootstrap values are shown above the branches and decay values are shown below. Unique indel changes are shown with black boxes and changes that occur in parallel are shown with empty boxes. Coding of indels is found in Table 3. Arrows refer to branches that collapse with different character weightings or in maximum likelihood analysis (see text).

of (1) *Neorites* L.S.Sm. as sister to *Orites* R.Br. and (2) *Macadamia* F.Muell., as sister to a clade composed of *Lomatia* R.Br., *Stenocarpus* R.Br., *Opisthiolepis* L.S.Sm., *Buckinghamia* F.Muell., and *Hakea* Schrad.

Of the ML models considered, the general time-reversible (GTR; Lanave et al., 1984; Tavare, 1986; Barry and Hartigan, 1987; Rodriguez et al., 1990) substitution model with discrete approximations of the gamma distribution (Yang, 1994) best describes the data (Mast, 2000). The single tree found with this model ($-\ln L = 5422.35$) is identical to the strict consensus of the MP trees found in the weighting that excluded indels, with the exception of an additional sister relationship between *Banksia petiolaris* F.Muell. and a clade composed of *B. elderiana* F.Muell. & Tate, *B. baueri* R.Br., and *B. lullfitzii* C.A.Gardner.

We here name the two clades formed by the basal split in subtribe Banksiinae the /*Cryptostomata* and /*Phanerostomata*, using the ''clademark'' convention of Baum, Alverson, and Nyffeler (1998) for naming clades. The two clades are reciprocally defined using a stem-based definition (de Queiroz, 1994). /*Cryptostomata* is the most inclusive clade that contains *Banksia ilicifolia* R.Br. but not *B. oblongifolia* Cav. The reverse is the case for /*Phanerostomata*. As will become apparent (see below), these names reflect differences in stomatal distributions, with stomata often occurring superficially or in shallow, open pits in /*Phanerostomata* and in crypts with constricted entrances in /*Cryptostomata*.

Strategy II—Equally weighted MP analysis of the *rp116* intron and the *psbA*/*trnH* spacer results in 40 shortest trees (130 steps, CI = 0.785, RI = 0.961). Of the twelve series recognized by Thiele and Ladiges (1996), six (*Banksia*, *Tetragonae* A.S.George, *Prostratae* A.S.George, *Ochraceae* K.Thiele, *Spicigerae* A.S.George, and *Abietinae* Meisn.) are not resolved as monophyletic in the strict consensus tree (Fig. 3). The alternative weighting scheme (ti : tv = 1 : 1, nt : indel = 1 : 0) produces a strict consensus tree that only differs from Fig. 3 in the collapse of six branches (arrows in Fig. 3).

The ML analyses were not performed for this sampling strategy because of computational limitations. The 89 taxa of this strategy were grouped into two clades of nearly equal size (strategies III and IV) according to the basal split inferred from strategy I.

Strategy III—Equally weighted MP analysis of the *rp116* intron, *psbA*/*trnH* spacer, and *trnT*/*trnL* spacer results in 12 shortest trees (79 steps, CI = 0.823, RI = 0.956). Eight new branches are resolved in the strict consensus of these trees (Fig. 4). Four of the six series that are not monophyletic in the MP trees of strategy II are sampled in strategy III. Of these, one (series *Ochraceae*) is resolved as monophyletic, with the modified taxonomic and character sampling. The alternative weighting scheme (ti : tv = 1 : 1.4, nt : indel = 1 : 0) produces a strict consensus tree that only differs from Fig. 4 in the collapse of four branches (arrows in Fig. 4).

The ML model that adequately describes the data is the HKY substitution model with equal substitution rates (Mast, 2000). The tree found with this model ($-\ln L = 3300.54$) is identical to the strict consensus of the MP trees found in the weighting that excluded indels except that branches iii and iv (Fig. 4) are resolved and series *Prostratae* is monophyletic.

Strategy IV—Equally weighted MP analysis of the *rp116* intron and *psbA*/*trnH* spacer results in a single shortest tree (64 steps, CI = 0.812, RI = 0.950) which is topologically identical to the clade's resolution with the taxonomic sampling of strategy II (Fig. 3). The alternative weighting scheme (ti : tv = 1 : 1.4, nt : indel = 1 : 0) produces a strict consensus tree that only differs from Fig. 3 in the collapse of branches iv and v (arrows in Fig. 3).

The ML model that adequately describes the data is the HKY substitution model with equal substitution rates (Mast, 2000). The tree found with this model ($-\ln L = 2467.83$) is identical to Fig. 3 except that it does not resolve branches v and vi (Fig. 3), but does resolve a sister relationship between *Banksia dolichostyla* (A.S.George) K.Thiele and *B. violacea* C.A.Gardner.

Biogeography—The biogeographic relationships among the taxa (Fig. 3) can be summarized in a tree with nine operational taxonomic units (OTUs) and one polytomy (Fig. 5A). Two alternative resolutions of the polytomy in Fig. 5A are relevant here: one that resolves the eastern taxa as sister to the southwestern taxa and an alternative that resolves a southwestern lineage sister to the eastern and remaining southwestern taxa. The dispersal-vicariance method resolves three widespread ancestral taxa that experienced vicariance events and identifies the origin of subtribe Banksiinae as having occurred in southwestern Australia (Fig. 5B). Fitch parsimony resolves the distribution of the most recent common ancestor of subtribe Banksiinae as southwestern (if a southwestern clade is resolved as sister to the remaining clades in /*Phanerostomata*) or as ambiguous (if the eastern clade is resolved as sister to the remaining clades). It also resolves the distribution of the most recent common ancestor of tribe Banksieae as ambiguous in both relevant resolutions of the polytomy of /*Phanerostomata*.

Origin of sclerophyllous and xeromorphic characters—Vertically transcurrent plates of sclerenchyma associated with the leaf vasculature are reconstructed as present in the most recent common ancestor of tribe Banksieae, with either multiple losses of plates in /*Phanerostomata* or a single loss and multiple new gains of plates (Fig. 6). Five origins of needle-like leaves with strongly revolute margins are reconstructed; four of these origins (all in /*Phanerostomata*) are in clades that do not have the plates of sclerenchyma. In three of these four instances, loss of the plates is unambiguously resolved as occurring prior to the inrolling of the margins. In one instance (the clade that includes *Banksia tricuspis* Meisn.), ordering the loss of plates and the origin of revolute margins is problematic because of ambiguity in reconstructing the former character. Stomata are reconstructed as positioned superficially in the most recent common ancestors of tribe Banksieae and subtribe Banksiinae, with two gains of stomata in deep crypts and four gains of stomata in shallow pits (Fig. 6). In each of those cases where stomata are sequestered in a depression, plates of sclerenchyma are present. However, only in those cases of *Musgravea* and /*Cryptostomata* are the plates reconstructed as present prior to the invagination of the leaves; the ordering of character state changes in /*Phanerostomata* is uncertain again because of ambiguity in reconstructing plates in that lineage.

DISCUSSION

Paraphyly of Banksia with respect to Dryandra—The position of *Dryandra* in the cpDNA phylogeny more than dou-

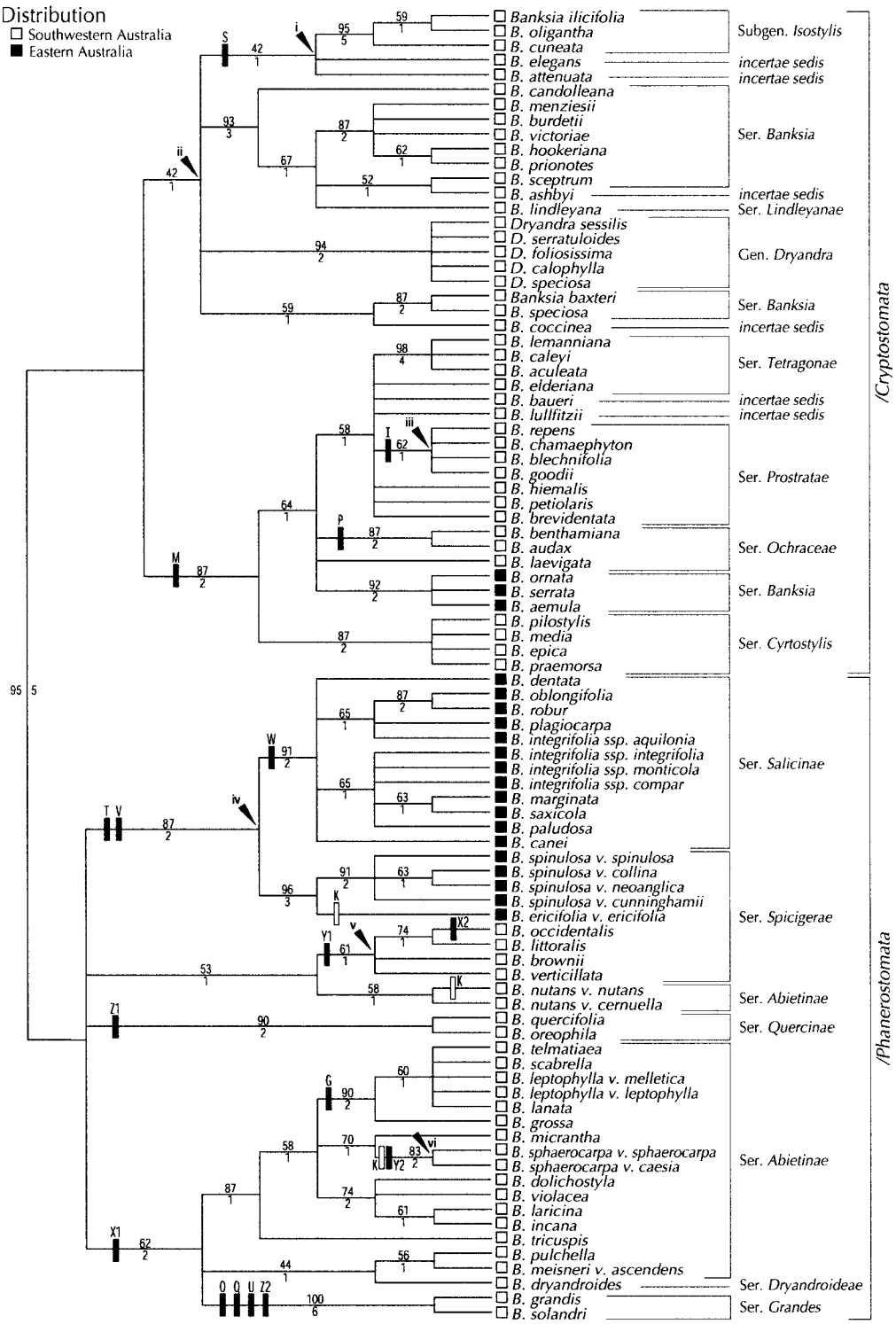

Fig. 3. Strict consensus of 40 shortest trees (130 steps; CI = 0.785, RI = 0.961) found in MP analysis using data from the *rp116* intron and *psbA*/*trnH* spacer (STRATEGY II) with ti : tv = 1 : 1 and nt : indel = 1 : 1. Branch support as in Fig. 2. Boxes between the branch tips and taxon names indicate the distribution of each taxon in the southwest (empty box) or the east (black box; Fig. 1). Infra-generic taxa to the right of the taxon names are from Thiele and Ladiges' (1996) classification (see supplementary material at http://ajbsupp.botany.org/v89).

bles the number of descendents of the most recent common ancestor of all banksias. The two clades formed by the basal split in the lineage of *Banksia* and *Dryandra* in the cpDNA phylogeny (Fig. 2) correspond to those seen previously in the nuclear ITS phylogeny of Mast (1998). As noted by Mast (1998), this basal split roots the unrooted morphological cladogram of *Banksia* by Thiele and Ladiges (1996) along its second longest branch.

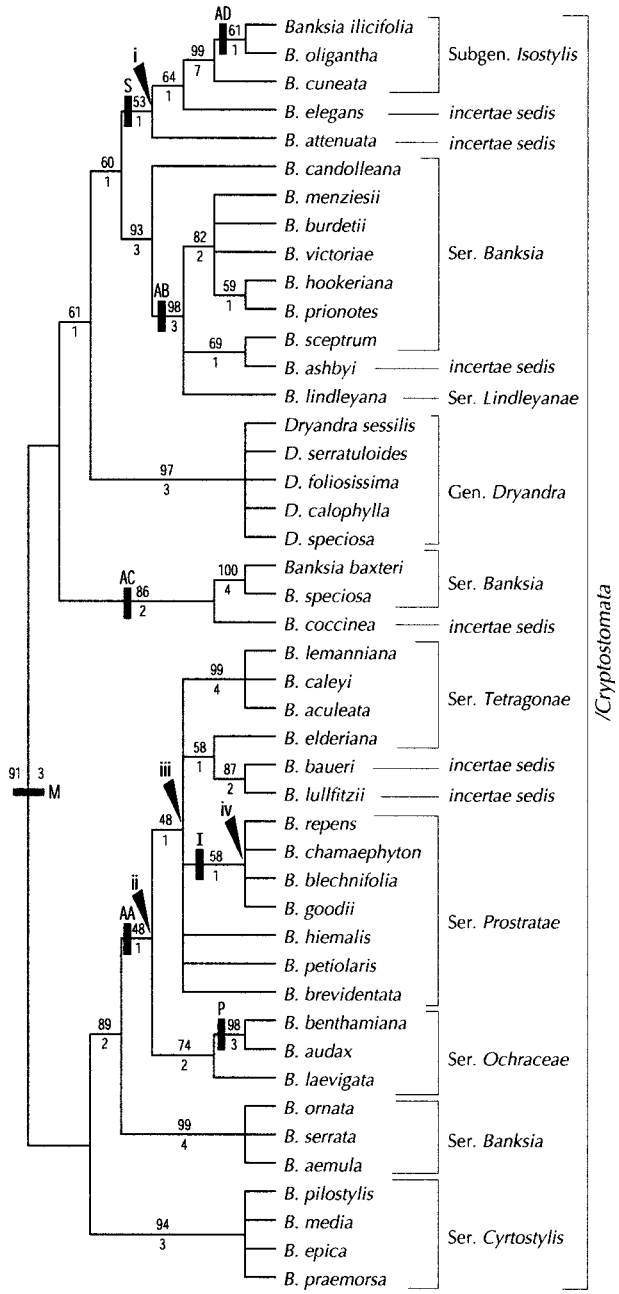

Fig. 4. Strict consensus of 12 shortest trees (79 steps, CI = 0.823, RI = 0.956) found in MP analysis using data from the *rp116* intron, *psbA*/*trnH* spacer, and *trnT*/*trnL* spacer (STRATEGY III) with ti : tv = 1 : 1 and nt : indel = 1 : 1. Branch support as in Fig. 2. Infrageneric taxa as in Fig. 3.

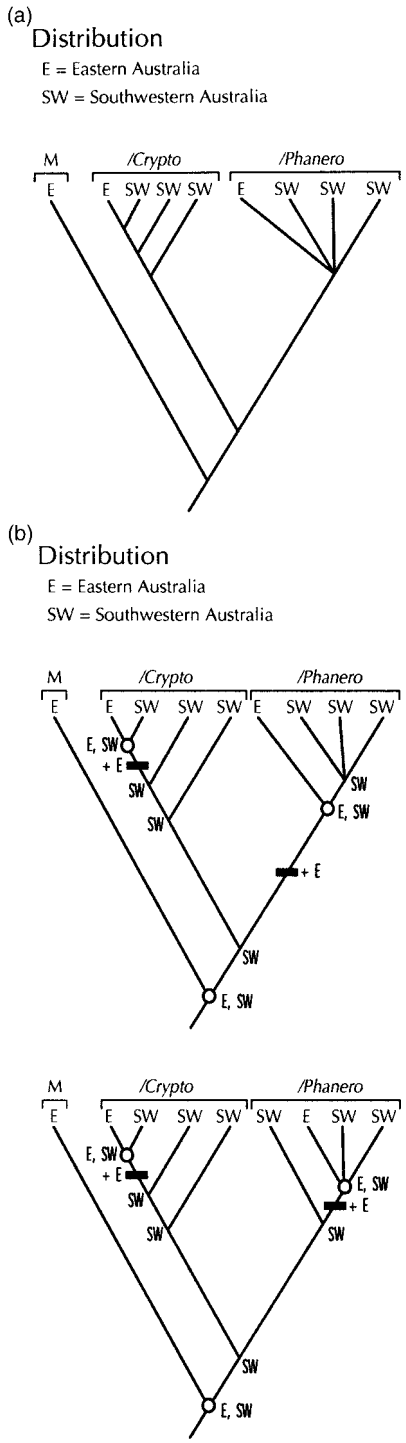

Fig. 5. Biogeographic relationships in tribe Banksieae. (A) Simplified representation of area relationships in Fig. 3 with subtribe Musgraveinae (M) grafted as sister to subtribe Banksiinae. (B) Dispersal-vicariance reconstruction of vicariance events on the simplified cladogram with both relevant resolutions of the polytomy in */Phanerostomata* (*/Phanero*) considered. For figure abbreviations, see Fig. 1.

Four morphological characters change state uniquely along that branch (Thiele and Ladiges, 1996): (1) a distinct quadrangular neck present below the pollen-presenter (banksias in */Cryptostomata*) vs. absent (*/Phanerostomata*), (2) follicle valves beaked (banksias in */Cryptostomata*) vs. unbeaked (*/Phanerostomata*), (3) raphe trace facial (banksias in */Cryptostomata*) vs. marginal (*/Phanerostomata*), and (4) cotyledons flabellate (banksias in */Cryptostomata*) vs. spathulate (*/Phanerostomata*). Thiele and Ladiges (1996) only sampled *Banksia*, and the state of these characters in *Dryandra* have yet to be thoroughly documented.

Each of these four characters should provide a synapomorphy for one or the other clade depending on the assessment of which states are primitive. Within the sister lineage (subtribe Musgraveinae), there appear to be flabellate coty-

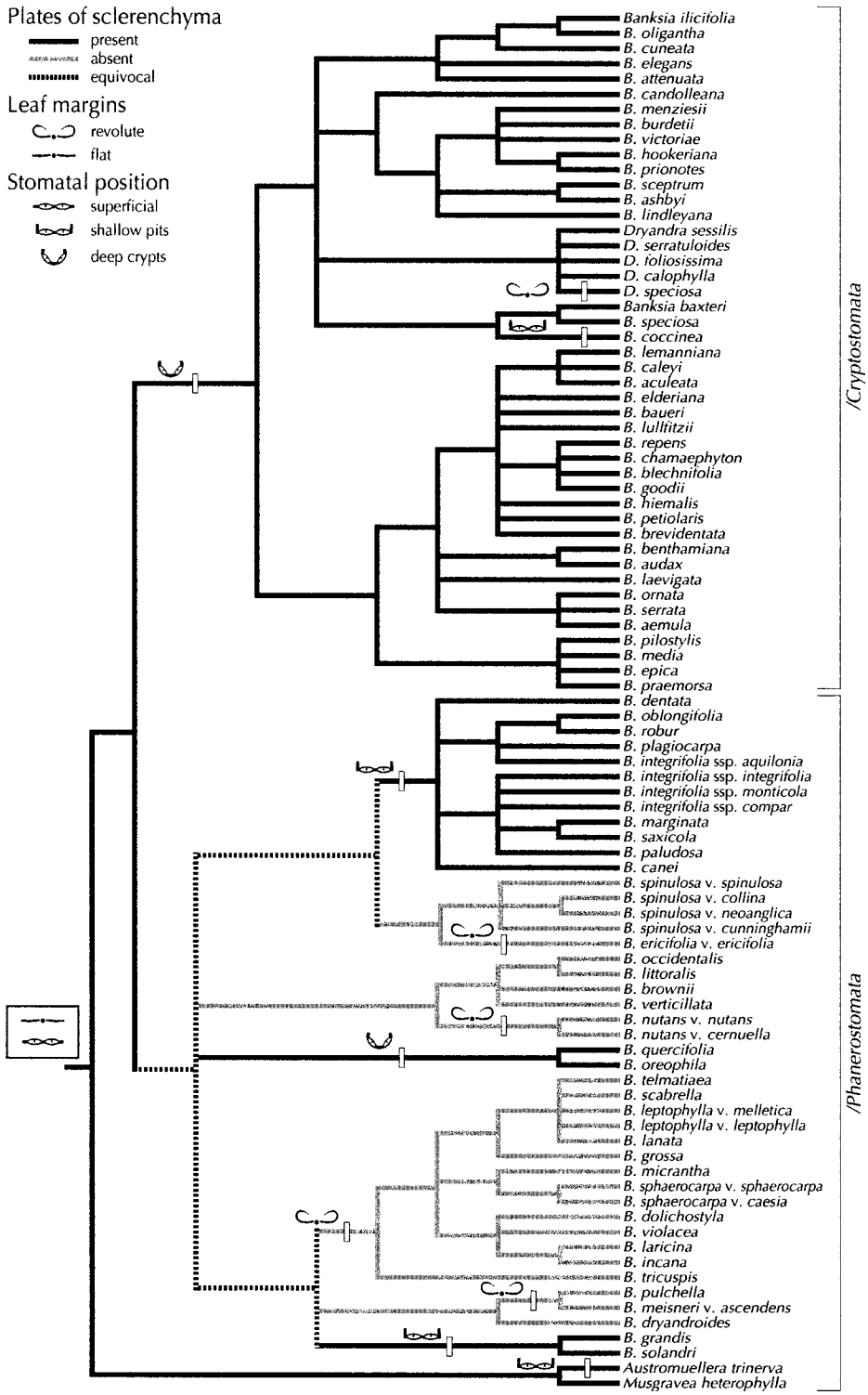

Fig. 6. Fitch reconstruction of the evolutionary history of sclerophyllous and xeromorphic character states. Tracing is the presence/absence of plates of sclerenchyma associated with the leaf vasculature. Origins of revolute leaf margins and stomatal positions are mapped onto branches using hollow bars. Phylogeny is taken from Fig. 3 with subtribe Musgraveinae grafted as sister to subtribe Banksiinae.

ledons (fig. 24 of Hyland, 1999b) and unbeaked follicle valves (fig. 24 of Hyland, 1999b and fig. 23 of Hyland, 1999a). These conditions support beaked follicles as a synapomorphy for /*Cryptostomata* and spathulate cotyledons as a synapomorphy for /*Phanerostomata*. *Dryandra* commonly has beaked follicles (A. George, personal communication), which supports its close relationship with the banksias of /*Cryptostomata*. We are unaware of observations for the other two characters in subtribe Musgraveinae.

Detailed anatomical study of the ''banksioid cortical mesh''

(Venkata Rao, 1964) previously described as a synapomorphy for *Banksia* (Thiele and Ladiges, 1996) could provide new insights into the evolution of this character. Venkata Rao (1964) examined just three species of *Banksia* and five of *Dryandra*.

Nonmonophyly of infrageneric taxa in Banksia—Four of the series resolved as monophyletic in Thiele and Ladiges' (1996) morphological cladistic study are resolved as para- or polyphyletic in one or more of the cpDNA cladograms (Figs. 2–4). These are series *Banksia*, *Tetragonae*, *Spicigerae*, and *Abietinae*. We will consider each of these in turn below. Additional infrageneric taxa from George's (1981, 1999) treatment are also resolved as nonmonophyletic here, including subgenus *Banksia*, sections *Banksia* and *Oncostylis* Benth., and series *Crytostylis* (Benth.) A.S.George and *Banksia*. However, the nonmonophyly of these groups has already been considered elsewhere (Thiele and Ladiges, 1996; Mast, 1998) and so will not be discussed further here.

The monophyly of series *Banksia* is supported in the morphological analysis of Thiele and Ladiges (1996) by a state change to the Orthostylis-type first seedling leaves (elliptic or spathulate leaves with 3–6 deep teeth on a side); a second character-state change on the branch (to a strongly ribbed pollen-presenter surface) later reverses. The leaf character is a complex one, with ten states. The Orthostylis type grades into the Isostylis type, which generally has more pungent teeth apices, and the Prostratae type, which is not so deeply incised. All but seven of the species of *Banksia* from /*Cryptostomata* have one of these three leaf types, and shifts between these similar states appear to have been common based on the cpDNA phylogeny. The series is one of two (the other being series *Spicigerae*) that contain members from both eastern and southwestern Australia. Series *Banksia* fragments in a geographically coherent manner, based on the cpDNA phylogeny (Figs. 3 and 4). The clades formed are from the east coast (*B. ornata* F.Muell. ex Meisn., *B. serrata*, and *B. aemula* R.Br.), the Esperance Plains of the southwest (*B. baxteri* R.Br. and *B. speciosa* R.Br.) and the Northern Sandplains of the southwest (the remaining taxa).

The monophyly of series *Tetragonae* is supported morphologically by a state change to pendent inflorescences, which is paralleled elsewhere (*Banksia nutans* R.Br.) and might be under selection from pollinators. However, the single branch disrupting this group's monophyly is not strongly supported (58% bootstrap; Fig. 4), and we await additional character sampling before commenting further on this result.

Series *Spicigerae* is supported as monophyletic in Thiele and Ladiges' (1996) cladograms by three changes: one change (to terminal buds of flowering stems that transform into inflorescences after a resting period) that is paralleled in series *Salicinae* and two changes (to 180° style torsion and to a nonyellow style color) that later reverse. Like series *Banksia*, the series splinters in a geographically coherent fashion. In this case, it forms an eastern clade (*B. spinulosa* Sm. and *B. ericifolia* L.f.) that is sister to the eastern series *Salicinae* and a southwestern clade (the remaining species of *Spicigerae*) that is sister to an enigmatic member of the southwestern series *Abietinae* (*B. nutans*).

The polyphyly of the *Abietinae* is, upon first comparison, a point of strong disagreement between the molecular (our cpDNA cladogram and the ITS cladogram of Mast [1998]) and the morphological (Thiele and Ladiges, 1996) results. The morphological analysis that included only members of the *Abietinae* and three outgroups (fig. 21 of Thiele and Ladiges, 1996) suggests that four unique character-state changes occur along the branch leading to the *Abietinae*. These are shifts to (1) hypogynous scales that are rectangular and often emarginate, (2) styles that bow through the perianth dorsally, (3) follicle valve walls that are largely soft and fibrous, and (4) first seedling leaves that are entire and strongly revolute (the Abietinae type). The first of these is not, in fact, a unique change, for the character reverses to a triangular state in *B. nutans* (fig. 6 of Thiele and Ladiges, 1996). The second condition is perhaps not homologous in *B. nutans*, for all *Abietinae* have erect inflorescences except that species. Dorsal bowing (relative to the vertical) might thus be something quite different in *B. nutans*, as it is opposite that seen in the other *Abietinae* relative to the base of the inflorescence. We have no empirical basis to critique the remaining two characters, though we observe that the seed leaf character is complex (as discussed above) and may be under significant selection in the dry, infertile sandplains occupied by these species. Three of the five independent origins of needle-like leaves in tribe Banksieae are due to the polyphyly of series *Abietinae*, which is characterized by such juvenile and adult foliage.

Biogeography—The vicariance approach to the data reconstructs three ancestral taxa of tribe Banksieae as widespread in the southwest and east prior to vicariance events. These include the most recent common ancestor of tribe Banksieae, as well as one ancestor in each of /*Cryptostomata* and /*Phanerostomata* (Fig. 5B). The three vicariance events require the origin of either two or three barriers, such as those that likely arose for the group during Tertiary flooding of the Eucla Basin in south-central Australia and aridification of the continent.

At least one fossil taxon of subtribe Banksiinae, *Banksieaephyllum taylorii* (Carpenter, Jordan, and Hill, 1994) (and possibly also *B. praefastigatum* Valada & Drinnan; Vadala and Drinnan, 1998) is known from the Late Paleocene in the southeast, prior to the flooding of the Eucla Basin. Flooding of the basin has been documented from the Eocene and Miocene (McGowran, 1989; Taylor, 1994), but the extent and duration of marine incursions during the Oligocene is uncertain (Quilty, 1994). Marchant (1973) hypothesized a vegetation corridor north of the flooded area before widespread aridity struck the continent, and an intermittent corridor may have occurred south of the basin, on the Great Australian Bight, during drops in sea level (Nelson, 1981). Seasonal dry periods are thought to have arisen in central Australia by the middle Miocene (Bowler, 1982; Quilty, 1994) and would have disrupted any vegetation corridor that existed north of the Eucla Basin at that time (Nelson, 1981).

The two vicariance events that are reconstructed in /*Cryptostomata* and /*Phanerostomata* could have been the result of a single obstruction (e.g., the beginning of the marine incursion in the Eocene), or these two events could have resulted separately. Disruption in the typically coastal /*Phanerostomata*, characterized by a less drought-adapted, superficial stomatal position and occasionally by stomatal sequestering via inrolling of leaf margins or restriction of stomata to shallow pits, might have occurred at the beginning of the marine incursions. Disruption in the better drought-adapted /*Cryptostomata*, which is characterized by a stomatal position in deep crypts and which typically possesses tougher, longer-lived, more strongly armored foliage, may have occurred when their

populations to the north of the basin were extirpated with increasing aridity in central Australia.

The origin of subtribe Banksiinae in the southwest is, at present, incongruent with the distribution of the earliest fossils of the subtribe (*Banksieaephyllum taylorii*) in New South Wales (Carpenter, Jordan, and Hill, 1994). Not until the Middle or Late Eocene are fossils with affinities to subtribe Banksiinae also found in the southwest (McNamara and Scott, 1983). This incongruence can be viewed as either a prediction that earlier fossils will someday be uncovered in the southwest (very few Paleocene floras have been described from Australia; Christophel, 1994) or, perhaps, as a problem with our choice of a vicariance framework. The earliest occurrence of fossil Musgraveinae in the late Middle Eocene of southeastern Australia (Christophel, 1984) narrows the gap between the southwest and the current distribution of the sister group of Banksiinae in the Atherton area of Queensland.

Under some conditions, the alternative dispersalist framework resolves equally optimal reconstructions of the area of origin for subtribe Banksiinae in the east and southwest. However, the vicariance, rather than the dispersalist, explanation for the distribution of taxa in the subtribe seems more reasonable to us for several reasons. The propagules of *Banksia* and *Dryandra* are only slightly anemochorous; their small seed wings disperse them over distances quite short of the hundreds of kilometers occupied by the Nullarbor Plain (on the order of tens of meters; B. Lamont, Curtin University, personal communication). Crossing the limestone mantle of the Nullarbor Plain or the deserts of central Australia by small-scale dispersion events over many generations also seems unlikely. The species are principally calcifuge, though three species (*Banksia epica* A.S.George, *B. media* R.Br., and *B. speciosa* R.Br.) persist on the western margin of the Nullarbor at Point Culver (Taylor and Hopper, 1988). Further, none of the species are known from Australia's arid center, though one, *Banksia elderiana*, occupies desert margins in the southwest (Taylor and Hopper, 1988).

Origin of sclerophyllous and xeromorphic characters—Sclerophylly, as measured by the presence or absence of vertically transcurrent plates of sclerenchyma, is reconstructed as the primitive condition for tribe Banksieae (Fig. 6). This is consistent with their appearance in the fossil record, for the plates are known from the earliest well-preserved leaf fossils of the group (from the Oligocene; Cookson and Duigan, 1950).

However, reconstruction of the history of stomatal distributions and its relationship to the presence/absence of the plates offers mixed support for hypotheses generated from their order of appearance in the fossil record. Hill (1990, 1992, 1994, 1998; Hill and Merrifield, 1993; Hill, Scriven, and Jordan, 1995; Hill et al., 1999) observed that the fossil taxa of *Banksia* and *Dryandra* demonstrate early sclerophylly (by Late Paleocene) and late xeromorphy (by Late Eocene) and suggested that the early response of the plants to the oligotrophic soils of the rainforests in which they grew (sclerophylly) ''preadapted'' them to the arid conditions that struck Australia in the mid-Tertiary.

Recent reviews have highlighted confusion over the definition of ''sclerophylly'' (e.g., Seddon, 1974; Read et al., 2000) and its evolutionary advantage (e.g., Turner, 1994; Salleo and Nardini, 2000). We consider the presence/absence of the plates of sclerenchyma in the leaves to be congruent with Hill's (1990, 1992, 1994, 1998; Hill and Merrifield, 1993; Hill, Scriven, and Jordan, 1995; Hill et al., 1999) use of the term and to be a significant determinant of biomechanical properties typically associated with sclerophylly (as suggested by Read et al. [2000] in their study of *Banksia marginata*). For the moment, let us also accept Hill's view that sclerophylly is an adaptation to infertile soils and that stomata in pits/crypts and narrow, needle-like leaves with revolute margins are unambiguous xeromorphic adaptations to reduce water loss, through increases in the length of the diffusive pathway from the chloroplasts to the atmosphere or decreases in leaf boundary-layer resistance and, hence, transpiration. What then do our results imply about Hill's (1990, 1992, 1994; Hill and Merrifield, 1993) hypothesis that sclerophylly ''preadapts'' plants to xeromorphy?

In the absence of an explicit definition by Hill, we take ''preadaptation'' to mean the same as Gould and Vrba's (1982) ''preaptation'': a character that was previously shaped by natural selection for a particular function, but which has since been co-opted for a new use. The strongest support that ancestral-state reconstructions could provide for the co-opting of primitive sclerophyllous traits by later lineages to reduce water loss would be the multiple independent origins of xeromorphic traits in, and only in, primitively sclerophyllous lineages.

On the one hand, this does not appear to have been the case for the lineages of /*Phanerostomata* that evolved needle-like leaves with strongly revolute margins. Instead, a *loss* of the plates of sclerenchyma appears to have occurred prior to the inrolling of margins in three or four of the four cases. Such a loss, perhaps related to the frequent distribution of /*Phanerostomata* in relatively moist coastal areas, might have provided a reduction in the rigidity of the leaf lamina that otherwise would have precluded its rolling. Contradictory to this hypothesis is the reported presence of plates in the needle-leaved *Dryandra speciosa* Meisn. (Cookson and Duigan, 1950) of /*Cryptostomata*, and future re-examination of this apparent exception might prove particularly informative.

On the other hand, evolutionary patterns and leaf anatomy associated with the sequestering of stomata in shallow pits or deep crypts lends support to a preaptation hypothesis in a way not considered by Hill. The deep crypts and shallow pits of tribe Banksieae, as well as the superficial stomata of *Austromuellera*, form in interveinal regions of the leaf (Carpenter, 1994; Carpenter, Jordan, and Hill, 1994). In all of these cases, vertical plates of sclerenchyma are associated with the vascular bundles (Cookson and Duigan, 1950; Thiele and Ladiges, 1996) and these form, with the adaxial epidermis and (when present) hypodermis, a rigid box around each lacuna (Thiele and Ladiges, 1996; Read et al., 2000). These boxes appear to provide critical structural reinforcement to the deeply perforated leaves (Thiele and Ladiges, 1996). Thus, this particular sclerophyllous trait could represent a preaptation, having been co-opted several times for structural support as the lineage evolved in response to the later onset of aridity. However, the pre-existence of plates in these lineages prior to the invaginations of their leaf for stomata is unambiguous in only two of the five cases. Support for the hypothesis from the remaining three cases will depend on future resolution of the polytomy in /*Phanerostomata*.

Any hypothesis of preaptation for the plates of sclerenchyma requires qualification, though, for it ultimately rests upon the assumed functions of the trait today and in the past. Over the years, sclerophylly has been described not only as an adaptation to nutrient poverty (e.g., Loveless, 1961, 1962; Beadle, 1966), but also to seasonal water deficits (e.g., Schimper, 1898; Oertli,

Lips, and Agami, 1990). In a recent study, Cunningham, Summerhayes, and Westoby (1999) tried to resolve this long-standing debate by examining trends in various aspects of leaf morphology and physiology along gradients of rainfall vs. soil fertility in southeastern Australia. Using phylogenetically independent contrasts, they found that leaf thickness and density increased in similar ways as moisture and soil fertility decreased. However, vascular sclerification increased more consistently with drought than with decreasing soil fertility. They conclude from this that sclerification is likely to be important in maintaining the structural integrity of the leaves during negative turgor pressure. Their hypothesis deserves further consideration in future explanations for the origin of sclerenchymatous plates in the leaves of tribe Banksieae.

Conclusions—The paraphyly of *Banksia* with respect to *Dryandra*, as now supported by nrDNA (Mast, 1998), cpDNA (this study), and morphology (the synapomorphy of beaked follicles), more than doubles the number of descendants from the ancestor of all banksias, from 79 species to 172. Recognizing this relationship explicitly in the taxonomy by sinking *Dryandra* into *Banksia* would make it the seventh largest genus of vascular plants in Australia, behind *Acacia*, *Eucalyptus*, *Grevillea*, *Melaleuca*, *Eromophila*, and *Leucopogon* (Orchard, 1999). We await the results from our sampling of single- or low-copy nuclear DNA regions to make realignments, however, for they are best made with detailed morphological, chloroplast DNA, and nuclear DNA phylogenies in hand. A prior attempt by the first author to use ITS data for phylogeny reconstruction at low levels in *Banksia* and *Dryandra* was frustrated by divergent ITS paralogues in individuals that were not resolved as monophyletic when considered with data from other species.

Future integration of the historical biogeography, ecology, and fossil record for *Banksia* and *Dryandra* would benefit from descriptions of additional Paleocene floras at sites in southwestern Australia, as well as thorough explorations of environmental correlates to the distribution of extant plants with alternative traits. The basal split in subtribe Banksiinae appears to have produced two lineages with largely distinct ecologies that offer fertile ground to comparative ecological studies. The extant members of /*Cryptostomata* are typically short-statured shrubs that occupy dry, infertile sandplains, especially in southwestern Australia, and produce long-lived leaves that are thick, tough, serrate, and bearing deep stomatal crypts reinforced by sclerenchymatous boxes. The extant members of /*Phanerostomata* are typically tall shrubs and trees that occupy moister coastal mountains, especially in eastern Australia, and produce short-lived leaves that are thin, soft, and less strongly toothed. Multiple invasions of the Southwest Australian sandplains by members of /*Phanerostomata* are correlated to the loss of sclerenchymatous reinforcement and the evolution of narrow, revolute leaves from broad-leaved ancestors. Future studies will use the molecular phylogenies presented here to analyze patterns of adaptive radiation in growth form and leaf structure and physiology, as well as detailed patterns of geographic speciation at much smaller, regional scales.

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