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Podocarp Evolution: A Molecular Phylogenetic Perspective

Edward Biffin, John G. Conran, and Andrew J. Lowe

ABSTRACT. Phylogenetic reconstructions of the relationships among extant taxa can be used to infer the nature of the processes that have generated contemporary patterns of biotic diversity. In this study, we present a molecular phylogenetic hypothesis for the conifer family Podocarpaceae based upon three DNA fragments that have been sampled for approximately 90 taxa. We use Bayesian relaxed-clock methods and four fossil constraints to estimate divergence times among the lineages of Podocarpaceae. Our dating analyses suggest that although the family is old (Triassic–Jurassic), the extant species groups are of recent evolutionary origin (mid- to late Cenozoic), a pattern that could reflect a temporal increase in the rate lineage accumulation or, alternatively, a high and constant rate of extinction. Our data do not support the hypothesis that Podocarpaceae have diversified at a homogeneous rate, instead providing strong evidence for a three- to eightfold increase in diversification associated with the Podocarpoid–Dacrydioid clade, which radiated in the mid- to late Cretaceous to the earliest Cenozoic, around 60–94 MYA. This group includes a predominance of taxa that develop broad leaves and/or leaf-like shoots and are distributed predominantly throughout the tropics. Tropical podocarps with broad leaves may have experienced reduced extinction and/or increased speciation coincident with the radiation of the angiosperms, the expansion of megathermal forests, and relatively stable tropical climates that were widespread through the Tertiary.

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INTRODUCTION

Patterns of species diversity reflect the balance of speciation and extinction over the evolutionary history of life. These, in turn, are parameters influenced by extrinsic factors, such as environmental condition and long-term processes of geological and climatic change, and intrinsic attributes of organisms, such as morphological innovations that increase the propensity for speciation or reduce the risk of extinction. The key aims of evolutionary biologists are to

explain patterns of diversity and, foremost, to determine whether there is evidence for significant heterogeneity in the "per lineage" patterns of diversity that require explanation (Sanderson and Wojciechowski, 1996; Magallón and Sanderson, 2001; Davies et al., 2004; Moore and Donoghue, 2007; Rabosky et al., 2007). Phylogenetic reconstructions of evolutionary relationships provide an indirect record of the speciation events that have led to extant species. Because evolutionary rates can be estimated from phylogenetic data, such reconstructions can help to elucidate the significance and drivers of biotic diversity patterns (Moore and Donoghue, 2007; Ricklefs, 2007).

Molecular phylogenetics has revolutionized the field of evolutionary biology. For instance, the molecular clock hypothesis predicts that the level of genetic divergence between any two lineages will be proportional to the time since divergence from a most recent common ancestor. Therefore, using external calibrations (e.g., timing of vicariance events, fossils of known age, and phylogenetic affinity) or known mutation rates, it is possible to estimate the age of all of the splits in a molecular phylogenetic tree (which often comprise a majority of splits with no associated fossil data to directly estimate the age). There has been justified criticism of the molecular clock hypothesis. In particular, there is strong evidence that in most lineages, the constancy of mutation rates in proteins or DNA sequences cannot be assumed (e.g., Ayala, 1997; Bromham and Penny, 2003). Recently developed methods, which attempt to incorporate heterogeneity into phylogenetic analysis by specifying a model of rate variation among lineages (referred to as relaxed-clock methods), are believed to provide more realistic estimates of divergence times in the absence of rate constancy (for recent reviews see Bromham and Penny, 2003; Rutschmann, 2006). Furthermore, there have been promising developments in methods to account for uncertainty inherent in the fossil record; Bayesian methods, in particular, can incorporate fossil calibrations because parametric prior probability distributions make fewer assumptions (relative to "fixed"-point calibrations) concerning the nodal placement of a given fossil datum on a phylogenetic tree (Yang and Rannala, 2006; Sanders and Lee, 2007). With improved confidence in hypotheses, there has been a diversification of questions and associated methodologies developed around molecular clock phylogenies. These include the examination of vicariance versus dispersal explanations for diversity patterns (e.g., Crisp and Cook, 2007), the timing of evolutionary radiations and/or extinctions and coincidence with environmental change (e.g., Davis et al., 2005), and estimation of the tempo of diversification using a statistical framework to contrast phylogenetic data with null expectations (e.g., Rabosky et al., 2007; Rabosky and Lovette, 2008).

The conifer family Podocarpaceae comprises approximately 173 species and 18 genera (Farjon, 1998) distributed primarily in the Southern Hemisphere, although extending northward as far as subtropical China and Japan and to Mexico and the Caribbean (see Enright and Jaffré, this volume; Dalling et al., this volume). The podocarps have a rich fossil record that suggests an origin in the Triassic and a distribution in both the Northern and Southern hemispheres through the Mesozoic, although by the Cenozoic the fossil record of the family is overwhelmingly southern (Hill and Brodribb, 1999). Currently, the Podocarpaceae comprise a majority of species-poor (Figure 1.1), range-restricted genera (e.g., *Acmopyle*, *Lagarostrobos*, *Microcachrys*, *Microstrobos*, *Saxegothaea*) that are presumably relictual, as evidenced by the fossil record, which indicates broader distributions and greater species diversities in the past (Hill and Brodribb, 1999). Relatively few genera are species rich and widely distributed, although *Podocarpus* comprises approximately 105 species (Figure 1.1) and occurs on all continents except Antarctica and Europe.

"Nearest living relative" comparisons of Podocarpaceae suggest the conservation of morphology, community associations, and ecological response over evolutionary time (Brodribb and Hill, 2004). From this perspective, the Podocarpaceae provide an outstanding opportunity to explore the influences of organism–environment interactions in the context of large-scale geological and climatic change in shaping patterns of extant distribution and diversity. For example, the majority of podocarp species presently occur within angiosperm-dominated humid forests. This pattern is of considerable interest to paleobotanists, ecologists, and biogeographers (e.g., Brodribb and Hill, 2004; Brodribb and Feild, 2008), given that conifers in general have been considered less competitive than angiosperms in productive environments (Bond, 1989). Using the ecophysiological tolerances of extant species as representative of closely allied extinct fossil taxa, it has been argued that characteristics such as leaf flattening and associated physiologies have promoted the persistence of Podocarpaceae in the face of angiosperm competition (Brodribb and Hill, 1997; Brodribb and Feild, 2008). However, within Australia, these characteristics may also be associated with range contraction and at least local extinction of several lineages as a consequence of increasing aridity in the Miocene–Pliocene (Hill and Brodribb, 1999; Brodribb and Hill, 2004).

Here we use molecular (DNA sequence) data, first, to assess phylogenetic relationships among Podocarpaceae and, second, using a relaxed molecular clock approach, to estimate the timing of diversification events for the major

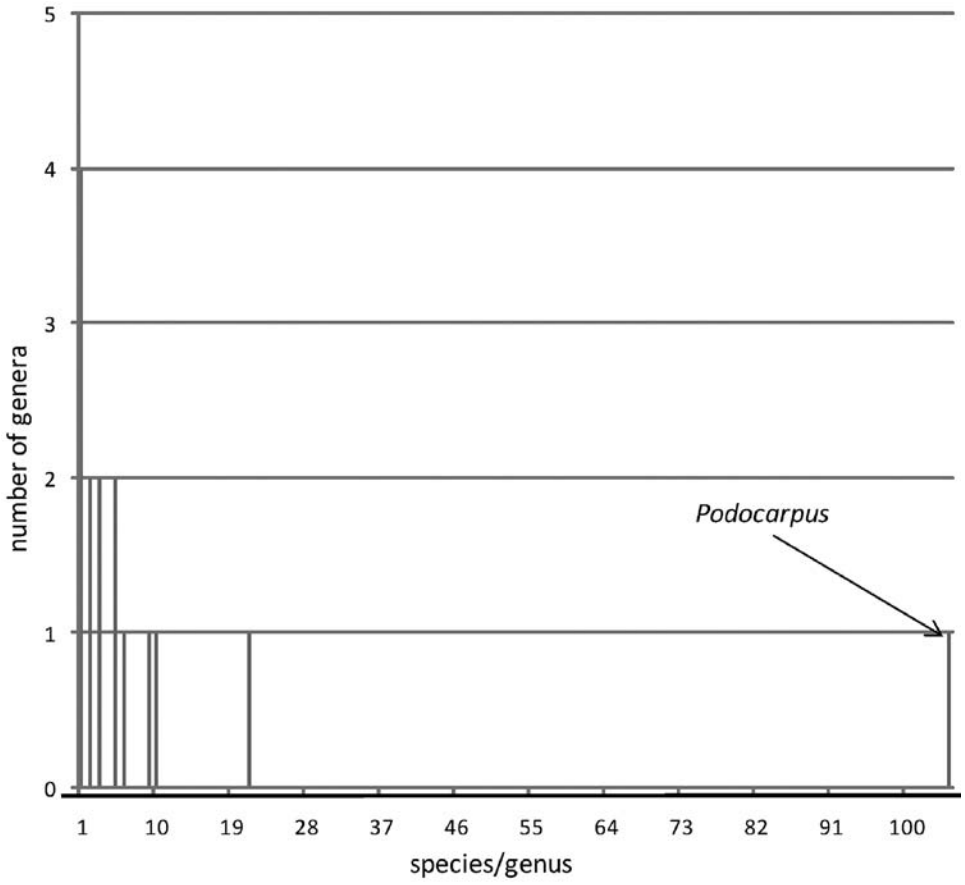

FIGURE 1.1. Frequency distribution of species/genus for the Podocarpaceae (estimates according to Farjon, 1998).

lineages. From this perspective we explore macroevolutionary patterns within the family and specifically use the dated molecular phylogeny to test whether it is necessary to invoke among-lineage variation in diversification rates to explain the disparities in extant diversity among major groups of Podocarpaceae. Our results indicate a highly significant shift in diversification rates corresponding to approximately the Cretaceous–Tertiary boundary. The significance of this diversification rate shift is briefly explored in the context of the angiosperm radiation, biogeography, and ecophysiology.

PHYLOGENETIC RELATIONSHIPS IN THE PODOCARPACEAE

Previous Studies

There have been several previous phylogenetic studies of the Podocarpaceae, including those based upon morphology (Hart, 1987; Kelch, 1997, 1998) and molecular (DNA sequence) data (Kelch, 1998, 2002; Conran et al., 2000; Sinclair et al., 2002). A key focus of these studies has been the assessment of relationships for classification; for instance, the status of *Phyllocladus* has been controversial, although the elevation of this taxon to family level as Phyllocladaceae (e.g., Page, 1990a; Bobrov et al., 1999) is not supported by phylogenetic analyses to date (Conran et al., 2000; Quinn et al., 2002; Sinclair et al., 2002; Wagstaff, 2004; Rai et al., 2008). More generally, there have been conflicting results from morphological versus molecular data; for example, the morphological analyses reported in Kelch (1997) suggest that gross leaf morphology is a reasonable predictor of evolutionary relationships in the Podocarpaceae, whereas analyses of DNA sequences found that scalelike leaves were polyphyletic on the estimated topologies (Kelch, 1998; Conran et al., 2000; Sinclair et al., 2002). In this instance, there are, perhaps, reasonable grounds to favor the molecular over the morphological data, given the generally poor support

for relationships from morphology, and in contrast to the molecules, the morphological data are not entirely independent of the conclusions (i.e., leaf morphologies were included as characters). From a wide range of studies, issues with morphology include fewer variable characters compared to DNA sequences and homoplasy, which may be a consequence of the choice of characters and character construction (i.e., homology assessment) as much as convergent or parallel evolution (Givnish and Sytsma, 1997; Scotland et al., 2003). Nevertheless, morphological data will continue to be important in reconstructing phylogeny; for instance, they provide the only readily sourced information on extinct fossil taxa (Wiens, 2004).

Phylogenetic studies to date have focused predominantly on generic relationships among members of the Podocarpaceae, but Hart (1987) and Kelch (1997) included, at best, a single taxon per genus, with a view to resolving deeper branches of the podocarp phylogeny. The studies of Conran et al. (2000), Kelch (2002), and Sinclair et al. (2002) included denser taxon sampling, so that generic monophyly could be assessed. Encouragingly, the evidence from both chloroplast and nuclear DNA is consistent with contemporary generic schemes (e.g., Page, 1988, 1990b; Farjon, 1998), although there are a few minor exceptions. For example, *Sundacarpus* is nested within *Prumnopitys* with strong statistical support, according to Sinclair et al. (2002).

Data and Phylogenetic Methods

There is a range of evolutionary questions that are best addressed using complete, or near complete, sampling of species, which is the eventual aim of the authors. In the present context, we present a preliminary phylogenetic analysis of the Podocarpaceae using a data set comprising 89 taxa (including two Araucariaceae as an out-group) that have been sequenced for two chloroplast genes (*matK* gene and the *trnL–trnF* intron and spacer region) and internal transcribed spacer 2 of nuclear ribosomal DNA (ITS2). Data not sourced from GenBank were sequenced de novo (Table 1.1). For sequencing methods, the reader is referred to Quinn et al. (2002; *matK*) and Sinclair et al. (2002; *trnL–trnF* and ITS2) and to Table 1.2, which details the primer combinations used for each fragment. Sequence alignment was performed using ClustalW (Thompson et al., 1994) and manual ("by eye") adjustment. The aligned data matrix is available from the authors upon request. The molecular data were analyzed using Bayesian phylogenetic methods (as implemented in MrBayes version 3.1.2; Ronquist and Huelsenbeck, 2003). In the first instance, each of the *matK*, *trnL–trnF* intron and spacer, and ITS2 sequence alignments was analyzed separately, assuming a general time reversible (GTR) model of sequence evolution with Γ distributed rate variation among sites, and a proportion of sites were considered invariant (I) (run conditions as below). The topologies from each of the separate analyses were visually inspected to identify well-supported (posterior probability (PP) ≥ 0.95; i.e., the grouping is found in $\geq$95% of the topologies sampled from the PP distribution) but conflicting resolutions among individual data sets (none found), and the data were concatenated and analyzed in combination using partitioned Bayesian analyses (i.e., topologies were derived by allowing each of the separate data partitions to evolve its best-fit set of GTR model parameters). Topologies were estimated from four independent runs of 1×10^6 generations, sampling topology, and parameter values every hundredth generation, each with four starting chains (one cold, three heated). Convergence was assessed relative to the variance in parameter estimates between independent runs and by inspection of the convergence diagnostics that are summarized using the "sump" command in MrBayes. Majority rule consensus trees were generated using the "sumt" command, discarding trees generated during the burn-in, with the burn-in proportion determined by inspection of the convergence diagnostics.

Some studies have reported high Bayesian posterior probability values corresponding to relatively weaker clade support from nonparametric bootstrapping (BS) for the same data set (see Alfaro et al., 2003, and references therein). In addition to Bayesian analyses, we used the maximum likelihood (ML) implementation GARLI (Zwickl, 2006) to estimate support for podocarp relationships. For these analyses, we used the concatenated alignment, a GTR + I + Γ model of sequence evolution with parameter values estimated from the data, and we performed 100 BS pseudoreplicates to estimate clade support.

Phylogenetic Relationships of the Podocarpaceae

The Bayesian majority rule consensus topology from the concatenated data analyses is presented in Figure 1.2. We recovered generally consistent topologies from Bayesian and ML analyses in terms of both resolution and statistical support: clades receiving a PP > 0.95 also had ML BS values of >80%, and there were no strongly supported conflicting resolutions among criteria. As with previous molecular phylogenies of the Podocarpaceae, the conventionally recognized genera are strongly supported as monophyletic, with the exception of *Prumnopitys*, which includes

TABLE 1.1. Taxon sampling for DNA sequences. GenBank accession numbers are listed. An asterisk (*) indicates de novo sequencing; a dash (-) indicates missing data; and ITS2 = internal transcribed spacer 2.

Taxon	*matK*	*trnL–trnF*	ITS2
Podocarpaceae			
Acmopyle pancheri (Brong. & Gris) Pilger	*	AY083141/AY083097	AY083057
A. sahniana Buchholz & N. E. Gray	*	*	*
Afrocarpus falcatus (Thunb.) C. N. Page	AF457111	*	*
A. gaussenii (Woltz) C. N. Page	-	AY083145/AY083101	AY083061
A. gracilior (Pilg.) C. N. Page	-	*	*
A. mannii (Hooker f.) C. N. Page	*	*	*
Dacrycarpus cinctus (Pilger) de Laub.	*	*	*
D. compactus (Wasscher) de Laub.	*	*	AY083055
D. dacrydioides (A. Rich) de Laub.	*	*	*
D. imbricatus (Blume) de Laub.	*	*	*
D. veillardii (Parl.) de Laub.	*	*	*
Dacrydium araucarioides Brogn. & Gris	-	AY083138/AY083094	AY083054
D. balanse Brogn & Gris	*	*	*
D. cupressinum Soland. ex Lamb.	AF457112	AY03136/AY083092	AY083052
D. guillauminii Buchholz	*	*	*
D. lycopodioides Brongniart et Grisebach	*	*	*
D. nausoriense de Laub.	*	*	*
D. nidulum de Laub.	*	*	*
Falcatifolium falciforme (Parl.) de Laub.	*	*	*
F. gruezoi de Laub.	-	AY083144/AY083100	AY083060
F. taxoides (Brongn. & Gris) de Laub.	-	AY083143/AY083099	AY083059
Halocarpus bidwillii (Hook. f. ex Kirk) Quinn	*	AY083128/AY083084	AY083044
H. biformis (Hook.) Quinn	*	AY083129/AY083085	AY083045
H. kirkii (F. Muell ex Parl.) Quinn	AF457117	AY083130/AY083086	AY083046
Lagarostrobos franklinii (Hook. f.) Quinn	*	AY083132/AY083088	AY083048
Lepidothamnus fonkii Phil. S. Wagstaff	-	AY083119/AY083075	AY083035
L. laxifolius (Hook. f.) Quinn	AF457114	AY083120/AY083076	AY083036
Manoao colensoi (Hook.) Molloy	*	*	*
Microcachrys tetragona (Hook.) Hook. f.	*	AY083134/AY083090	AY083050
Microstrobos fitzgeraldii (F. Muell.) J. Garden & L. A. S. Johnson	*	AY083135/AY083091	AY0835051
M. niphophilus J. Garden & L. A. S. Johnson	*	*	*
Nageia fleuryi (Hickel) de Laub.	*	*	*
N. formosensis (Dummer) C. N. Page	*	*	*
N. nagi (Thunb.) O. Kuntze H. Katsurada	AF228112	AY083147/AY083103	AY083063
N. wallichiana (Presl.) O. Kuntze	*	*	*
Parasitaxus ustus (Veillard) de Laub.	*	AY083131/AY083087	AY083047
Phyllocladus alpinus Hook. f. Wardle	AY442146	-	AY442160
P. aspleniifolius (Labill.) Hook. f.	AY442147	AY083117/AY083073	AY442167
P. hypophyllus Hook. f. J. Read	AY442148	AY083116/AY083072	AY442156
P. toatoa Molloy	AY442149	-	AY442163
P. trichomanoides D. Don ex Cunn.	AY442150	AY083118/AY083074	AY442165
Podocarpus affinis Seem.	*	*	*
P. alpinus R. Br. ex Hook. f.	*	*	*
P. annamiensis N. E. Gray	-	*	*
P. aristulatus Parl.	*	*	*

continued

TABLE 1.1. (*Continued*)

Taxon	*matK*	*trnL–trnF*	ITS2
Podocarpaceae			
P. brassii Pilger in Engler	*	*	-
P. chinensis (Roxb.) Wall. ex Forbes	*	*	*
P. costalis C. Presl.	*	*	*
P. cunninghamii Colenso	-	*	*
P. dispermus White	*	*	*
P. drouynianus F. Muell.	*	*	*
P. elatus R. Br. ex Endl.	AF457113	*	*
P. elongatus (Aiton) L'Herit. ex Persoon	*	*	-
P. gnidioides Carrière	*	*	*
P. guatemalensis Standl.	-	AY083151/AY083107	AY083067
P. henkelii Stapf	*	*	AY845209
P. lambertii Klotzsch ex Endl.	*	*	*
P. latifolius (Thunb.) R. Br. ex Mirb.	-	*	AY845215
P. lawrencei Hook. f.	*	*	*
P. lawrencei	*	*	*
P. lawrencei	*	*	*
P. lawrencei	*	*	*
P. longifoliolatus Pilger in Engler	*	AY083149/AY083105	AY083065
P. macrophyllus (Thunb.) Sweet	AF228111	*	*
P. matudae Lundell	*	*	*
P. neriifolius D. Don in Lamb.	*	*	*
P. nivalis Hook. f.	*	*	*
P. nubigenus Lindley	*	*	*
P. polystachyus R. Br. ex Endl.	*	*	*
P. rumphii Blume	*	*	*
P. salignus D. Don	*	AY083148/AY083104	AY083064
P. smithii de Laub.	-	*	*
P. spinulosus (Smith) R. Br.	*	*	*
P. sylvestris J. Buchholz	*	AY083152/AY083108	AY083068
P. totara D. Don	*	*	*
Prumnopitys andina (Poepp. ex Endl.) de Laub.	*	AY083124/AY083080	AY083040
P. ferruginea (D. Don) de Laub.	AF457115	AY083127/AY083083	AY083043
P. ferruginoides (Compton) de Laub.	*	AY083126/AY083082	AY083042
P. ladei (Bailey) de Laub.	*	AY083125/AY083081	AY083041
P. taxifolia (Soland. ex D. Don) de Laub.	*	AY083123/AY083079	AY083039
Retrophyllum comptonii (Buchh.) C. N. Page	-	*	*
R. vitiense (Seeman) C. N. Page	*	*	*
Saxegothaea conspicua Lindl.	AF457116	AY083121/AY083077	AY083037
Sundacarpus amarus (Blume) C. N. Page	*	AY083122/AY083078	AY083038
Araucariaceae			
Agathis australis (D. Don) Loudon	EU025980	AY083115/AY083071	AY083031
Araucaria heterophylla (Salisb.) Franco	AF456374	-	*
Araucaria biramulata Buchholz	-	AY083114/AY083070	-

TABLE 1.2. PCR and sequencing primers.

Region	Name	Sequence 5'-3'	Reference
matK	matkF1	AAYAARCATAGATCTTGGCARCAAT	This study
matK	matkF2	TGYGAATCCATTHTAGTTCCYCTT	This study
matK	matKR1	AGSRATCTTTCBCSRTATCTCACATA	This study
matK	matKR2	TTAGCRCATGAAAGTAGAAGTA	This study
trnL–trnF	TabC	CGAAATCGGTAGACGCTACG	Taberlet et al. (1991)
trnL–trnF	TabF	TI'TGAACTGGTGACACGAG	Taberlet et al. (1991)
ITS2	ITS3P	GCCACGATGAAGAACGTAGCGA	Modified from White et al. (1990)
ITS2	ITS4P	CCGCTTATTGATATGCTTAAGCTCA	Modified from White et al. (1990)

Sundacarpus (*Prumnopitys* sensu lato). The relationships among genera are also largely well supported. Strongly supported groupings include a "Podocarpoid" clade (*Afrocarpus*, *Nageia*, *Podocarpus*, *Retrophyllum*), a "Dacrydioid" clade (*Dacrydium*, *Dacrycarpus*, *Falcatifolium*), and a "Prumnopityoid" clade (*Halocarpus*, *Lagarostrobos*, *Manoao*, *Parasitaxus*, *Prumnopitys* sensu lato) (Figure 1.2). These groupings were previously recovered by Conran et al. (2000), although with relatively weak support. At a lower taxonomic level, resolutions include the pairing of *Manoao* and *Lagarostrobos*, with *Parasitaxus* sister to these, a relationship that was suggested, but not statistically supported, in the analyses of Sinclair et al. (2002). Given the level of divergence, the segregation of *Manoao* from *Lagarostrobos* is reasonable on the basis of the present data, as is that of *Falcatifolium* from *Dacrydium* sensu stricto (cf. Conran et al., 2000). The deepest branches in the phylogeny are not strongly supported, although a group including *Lepidothamnus*, *Phyllocladus*, and the Prumnopityoid clade has a PP of 0.91 and a BS and ML bootstrap support of 60%, and the pairing of *Lepidothamnus* and *Phyllocladus* is also weakly supported (PP = 0.79, BS = 68%).

Although genera, and most intergeneric relationships, receive strong support from these data, relationships among species are relatively ambiguous. For instance, all resolutions within *Afrocarpus*, *Dacrycarpus*, *Nageia*, and *Phyllocladus* are, at best, weakly supported (PP ≤ 0.9), whereas within *Dacrydium* and *Podocarpus* the majority of nodes receive low levels of statistical support. On one hand, poor resolution may be a consequence of data conflict (i.e., individual data sets support conflicting resolutions), leading to topological ambiguity. Alternatively, there may be insufficient evidence to adequately resolve relationships, analogous to sampling error in small data sets (Graham et al., 1998). As noted above, there were no strongly supported conflicting resolutions noted in the visual inspection of topologies derived from individual data partitions, suggesting that the latter is a reasonable hypothesis. Furthermore, as the data are not uninformative per se, it could be argued that poor resolution among species is a consequence of relatively recent radiations, such that lineages have had insufficient time to accrue informative mutations. Typically, this scenario is associated with a broomlike topology (as discussed by Crisp et al., 2004), which is evident in the present data. Low-level studies of *Afrocarpus* (Barker et al., 2004), *Phyllocladus* (Wagstaff, 2004), and *Retrophyllum* (Herbert et al., 2002) report similarly poor resolution among species, consistent with relatively recent origins (see Wagstaff, 2004, who explicitly considers divergence time estimates). The timing of radiations within the Podocarpaceae is addressed further in the next section.

MOLECULAR DATING ANALYSES

In the present study, we use the Bayesian relaxed-clock implementation in BEAST (Drummond and Rambaut, 2007) to estimate divergence times among lineages of the Podocarpaceae. BEAST uses a probabilistic model to describe the pattern of change in molecular rates through time and Markov chain Monte Carlo (MCMC) simulation sampling substitution rates, branch lengths, the individual parameters of the substitution model, and tree priors to derive the posterior probability of divergence time estimates (Drummond et al., 2006; Rutschmann, 2006). In contrast to other currently available molecular dating implementations, BEAST samples both tree topology and branch lengths, allowing the user to coestimate phylogeny and substitution rates. Departures from the molecular clock

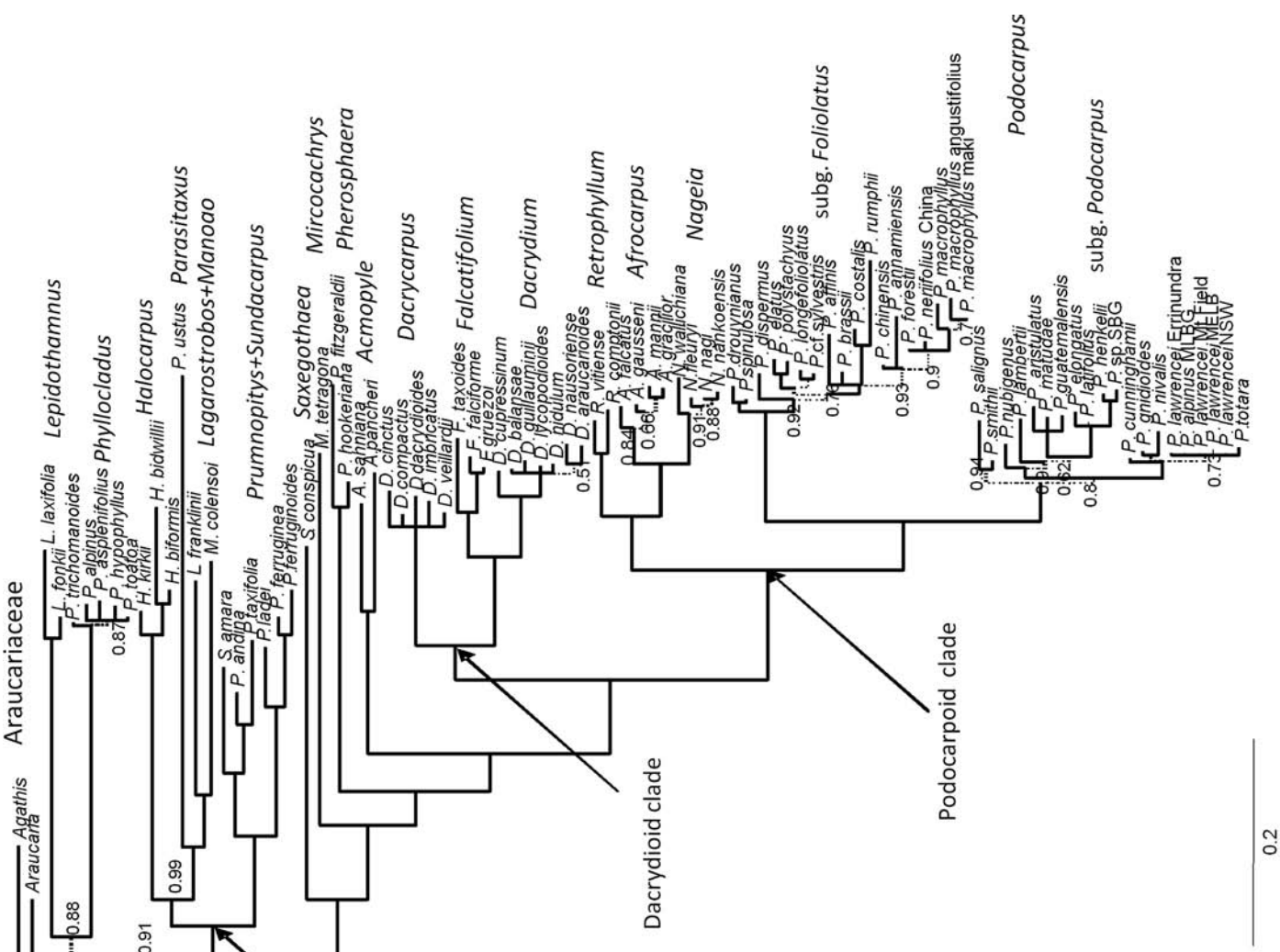

FIGURE 1.2. Phylogenetic relationships (Bayesian analysis, 50% majority rule) in the Podocarpaceae inferred from a *matK*, *trnL–trnF* chloroplast intron and spacer, and ITS2 data set. Branch lengths are proportional to the inferred number of changes; dashed branches have a posterior probability (PP) < 0.95, and unless otherwise indicated, solid branches have a PP of 1.0. Genera are according to Farjon (1998), and higher-order groupings are according to Conran et al. (2000).

assumption are estimated from the data, with completely clocklike (i.e., a single rate across the tree) to highly heterogeneous (i.e., numerous rate changes among branches) models representing the opposite extremes of a spectrum of rate variation (Drummond et al., 2006).

In BEAST, fossil calibrations are incorporated as prior probability distributions, including parametric distributions such as normal, lognormal, or exponential prior probabilities on the distribution of node ages. Given that many (if not most) fossil placements have a degree of uncertainty, this approach has advantages over the incorporation of fossil data as "fixed"-point calibrations on a particular node (see, e.g., Yang and Rannala, 2006; Benton and Donoghue, 2007; Sanders and Lee, 2007). For instance, a prior probability distribution can be designed with a peak probability corresponding to the age of a fossil and with decreasing, but nonzero, probabilities that the calibration node is either older or younger than the fossil age distributed according to a normal distribution (Sanders and Lee, 2007).

Divergence time estimates were derived from a chloroplast data set comprising the *matK* coding region and *trnL–trnF* spacer and intron for 92 taxa (90 representatives of the Podocarpaceae and 2 representatives of the Araucariaceae). Four fossil-derived constraints were used to calibrate molecular rates (Table 1.3). These were selected from the literature (e.g., Hill and Brodribb, 1999) on the basis of the oldest reasonably placed macrofossil age for the associated lineage. The incompleteness of the fossil record usually requires that a fossil assigned to a particular node provides the minimum age for that node (i.e., older fossils may yet be found), although fossil age constraints are often applied to the node that subtends the crown group, which may be significantly younger than the actual age of the fossil lineage (Magallón and Sanderson, 2001; Magallón, 2004; Renner, 2005). Where the accuracy of fossil placement is uncertain the most objective calibration method may be to fix the stem group (age) as the minimum age for the diversification of the descendant crown group (given that the fossil in question is correctly assigned to a lineage; Renner, 2005). In other words, the stem group node must be at least as old or older than a fossil belonging to that lineage. Maximum (upper) constraints are more difficult to establish (Benton and Donoghue, 2007), although a "soft" upper bound (i.e., with nonzero probabilities associated with all reasonable values) can be defined using an appropriate parametric distribution (Yang and Rannala, 2006; Sanders and Lee, 2007).

In the present study, the fossil-derived dates were used to provide the minimum age for the most recent common ancestor of the corresponding stem group. Uncertainty in the association between the calibration node and the fossil

TABLE 1.3. Fossil calibration points used for divergence time estimation. Calibration node numbers correspond to Figure 1.3. Abbreviations: CI, confidence interval; HPD, highest posterior density.

Calibration node	Reference	Fossil age	Translated lognormal prior (median, 95% CI)	Posterior (median, 95% HPD of node age)
1. Dacrydioid clade (*Dacrycarpus, Dacrydium*, *Falcatifolium*)	*Dacrycarpus linifolius* Wells and Hill emend. Hill and Carpenter (Hill and Carpenter, 1991) *D. mucronatus* Wells and Hill emend. Hill and Carpenter (Wells and Hill, 1989; Hill and Carpenter, 1991)	Early Eocene	60 (50–102)	62 (51–76)
2. Podocarpoid clade (*Afrocarpus*, *Nageia*, *Podocarpus*, *Retrophyllum*)	*Podocarpus strzeleckianus* Townrow (Townrow, 1965)	Early Eocene	60 (50–102)	57 (49–67)
3. Prumnopityoid clade (*Halocarpus*, *Lagarostrobos*, *Manoao*, *Parasitaxus*, *Prumnopitys*)	*Prumnopitys limaniae* Pole *Prumnopitys* sp. Mt Somers (Pole, 1998) *Prumnopitys opihiensis* Pole (Pole, 1997)	Paleocene	67 (55–98)	101 (73–135)
4. Podocarpaceae (Podocarpaceae, Araucariaceae)	*Mataia podocarpoides* (Ettingshausen) Townrow *Nothodacrium warrenii* Townrow *Rissikia* Townrow	Upper Triassic–Jurassic	197 (178–257)	193 (177–223)

record was accommodated by providing a parametric (lognormal) prior probability distribution for the age of the node. The details of the fossil calibration priors are presented in Table 1.3 and are illustrated in Figure 1.3. In each instance, the estimated fossil age was used to define the zero offset of the lognormal calibration prior, thereby imposing a minimum age constraint approximating the fossil age on the relevant stem node. In addition to the above constraints, an upper age constraint of 300 MYA was placed upon the age of the root. Although several extant lineages have been associated with microfossils extending back before the earliest known macrofossils (see Morley, this volume), we preferred the macrofossil evidence because of its greater complexity of characters. This allows greater confidence in the assignment of fossil material to extant lineages as the problems of homoplasy in the fossil record tend to increase with clade age (Wagner, 2000), which can readily mislead inferences when there are few characters for comparison (e.g., Willyard et al., 2007). Note that our calibration approach does not rule out much older ages (i.e., consistent with the microfossil dates) a priori as the calibration prior includes dates approximately twice as old as the macrofossil age in the 95% confidence interval (Table 1.3). We expect, however, that a detailed assessment of the fossil record of the Podocarpaceae is needed to identify synapomorphies uniting fossil and extant taxa (e.g., Saquet et al., 2009), rather than postulated relationships based upon gross morphological similarity.

For the analyses in BEAST, a GTR + I + Γ model of sequence evolution was assumed with the substitution model parameters unlinked across data partitions. An uncorrelated lognormal model of rate variation among branches in the tree and a Yule prior on branch rates was also assumed a priori. Four independent MCMC runs, each of 5×10^6 steps, were performed and subsequently pooled (after excluding an appropriate burn-in fraction, as determined using Tracer version 1.4; Rambaut and Drummond, 2007) to derive the 95% highest posterior density of topology and parameter estimates. The topology presented in Figure 1.3 is the maximum clade credibility tree derived from the sample of 20,000 trees, with clade posterior probability and 95% highest posterior density (i.e., 95% of topologies sampled from the posterior have values within this range) of divergence times indicated. As with the nonclock analyses, the genera and most of the deeper internal branches are strongly statistically supported. Furthermore, the relationships inferred among lineages are generally consistent among the clock and nonclock phylogenetic analyses (compare Figures 1.2 and 1.3).

ANALYSES OF DIVERSITY

Temporal Patterns of Diversity

Although the family appears to be of ancient origin (mid-Mesozoic, 95% highest posterior density 177–223 MYA), the molecular dating analyses suggest that the majority of extant genera have arisen relatively recently (Upper Cretaceous to Cenozoic; Figure 1.3), whereas the extant crown groups of these genera are estimated to have diversified from predominantly the mid- to late Cenozoic. In Figure 1.4, the number of species in the phylogeny from the origination of the clade to the present (log scale) is plotted against the relative timing of inferred speciations (proportion of time since origination of the clade). This lineages-through-time plot shows a gradual increase in the rate of lineage accumulation and then an upturn at approximately 40 MYA, reflecting the estimated recent timing of the origination of most extant lineages.

Lineages-through-time plots have been widely used to infer macroevolutionary patterns; for instance, comparison of the data to a Yule (or pure birth) speciation model can be used to make inferences regarding the tempo of evolution (e.g., Harmon et al., 2003; Ricklefs, 2007; Rabosky and Lovette, 2008). Under a Yule model, there is an instantaneous rate of per lineage speciation and no extinction, giving an exponential increase in the number of lineages through time. Significant departures from this null model are indicative of temporal variation in the diversification rate (i.e., the per lineage rate of speciation and/or extinction; Harmon et al., 2003).

In Figure 1.5, the expectation under a Yule model was generated by connecting the point representing the first node in the phylogeny with the point representing the number of extant podocarp taxa (173) on the log-linear lineages-through-time plot. This resulting straight line provides the null hypothesis of exponential growth of lineages. To provide a confidence interval on the expectation of exponential growth, 100 phylogenies were simulated under a Yule model, each giving rise to 173 extant lineages. Clearly, the podocarp data show significant departure from the null model of exponential diversification, the comparison being consistent with the hypothesis that diversification rates in the Podocarpaceae have increased toward the present. However, there are other plausible hypotheses that can be assessed using more complex models of diversification.

In Figure 1.6, the lineages-through-time plot is compared with a constant birth-death model, which includes

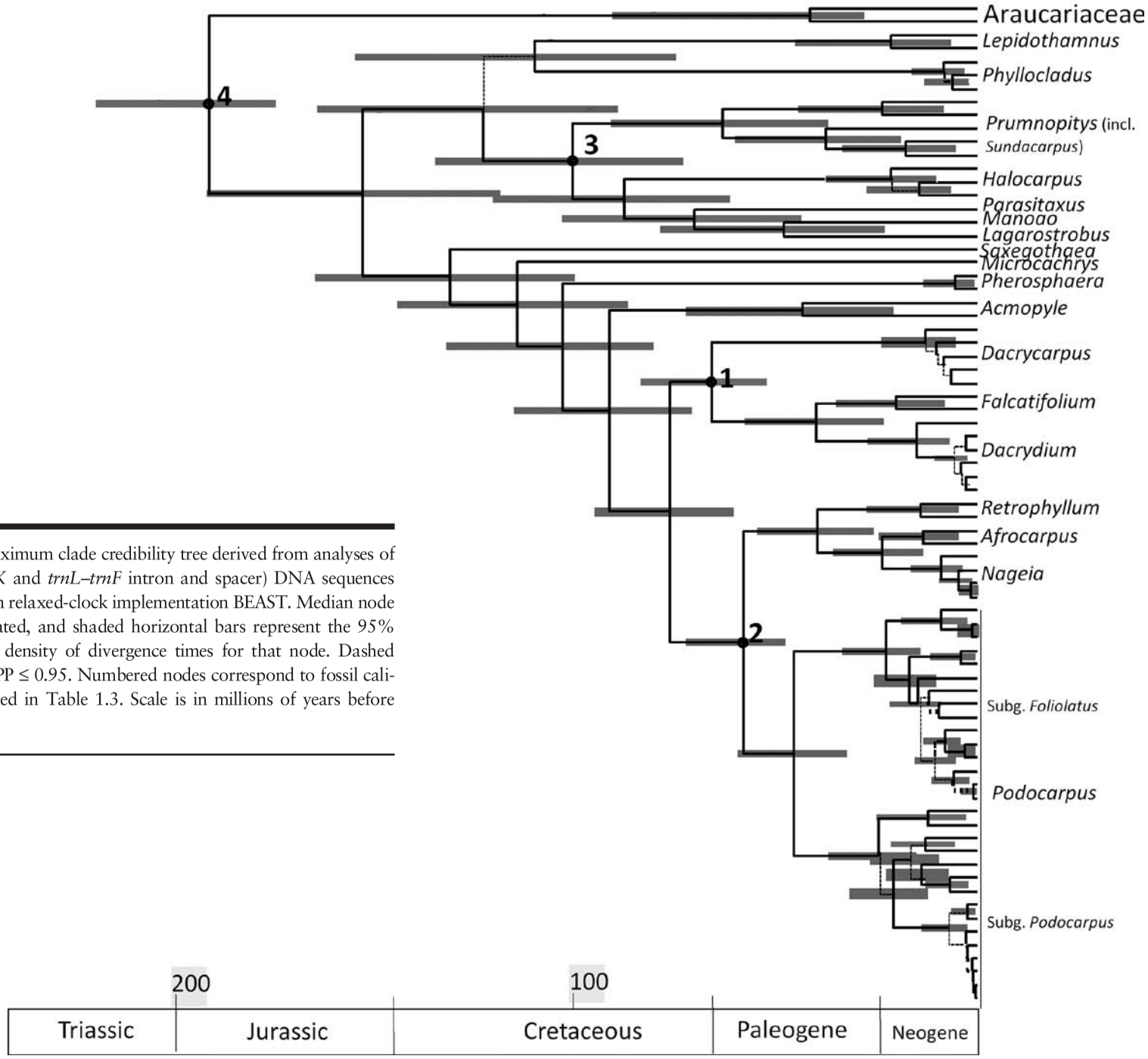

FIGURE 1.3. Maximum clade credibility tree derived from analyses of chloroplast (*matK* and *trnL–trnF* intron and spacer) DNA sequences using the Bayesian relaxed-clock implementation BEAST. Median node heights are indicated, and shaded horizontal bars represent the 95% highest posterior density of divergence times for that node. Dashed branches have a PP ≤ 0.95. Numbered nodes correspond to fossil calibrations as detailed in Table 1.3. Scale is in millions of years before present.

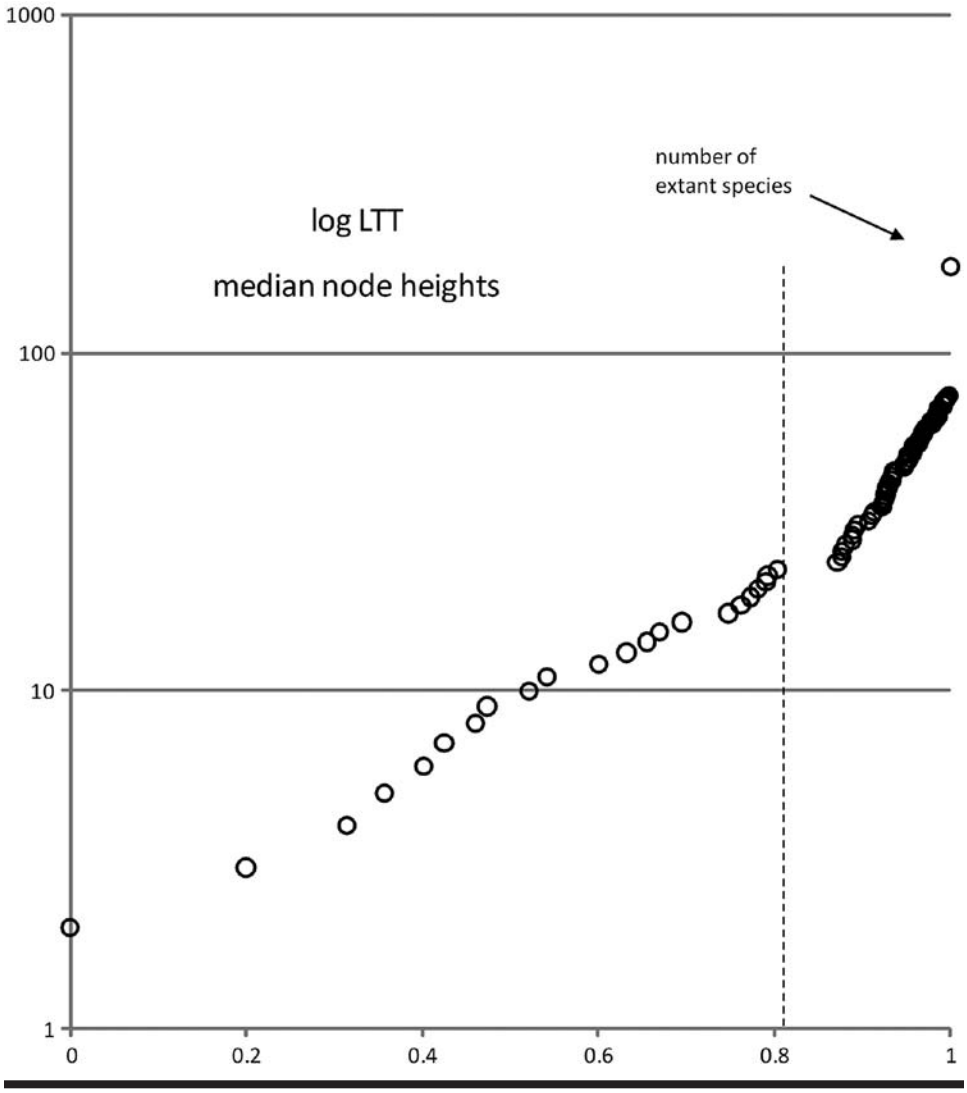

FIGURE 1.4. Lineages-through-time plot (log scale) for the Podocarpaceae based upon the relaxed-clock analyses of chloroplast data. Sampling is reasonably complete for approximately 80% of the time since origination (dashed vertical line).

an instantaneous rate of speciation and extinction. For the birth–death model, the extinction fraction was set to 0.95 (i.e., lineages have a 5% chance of survival to the present), and a 95% confidence interval was generated from 100 simulated birth-death topologies, as described above. As with the actual data, the simulated topologies show a sharp upturn toward the present and provide a close approximation to the data across the full depth of the Podocarpaceae phylogeny. In this instance, the sharp upturn in the rate of lineage accumulation may be ascribed to the "pull of the present" (Nee et al., 1994). That is, at a high relative extinction rate, the probability of a lineage persisting into the present decreases with the age of the lineage, and therefore, recently evolved lineages are more likely to be observed in studies using only extant taxa. Recent simulation studies demonstrate that a similar upturn in lineages-through-time plots can also be generated under models with large declines in diversification rate, when the decline is mediated by a temporally increasing extinction rate (Rabosky and Lovette, 2008). Failure to consider extinction can lead to potentially spurious inferences of evolutionary tempo.

Rabosky (2008) provides a method by which a relative extinction rate can be approximated from phylogenetic data, which is implemented in the LASER package (Rabosky, 2006) for the R programming language. This

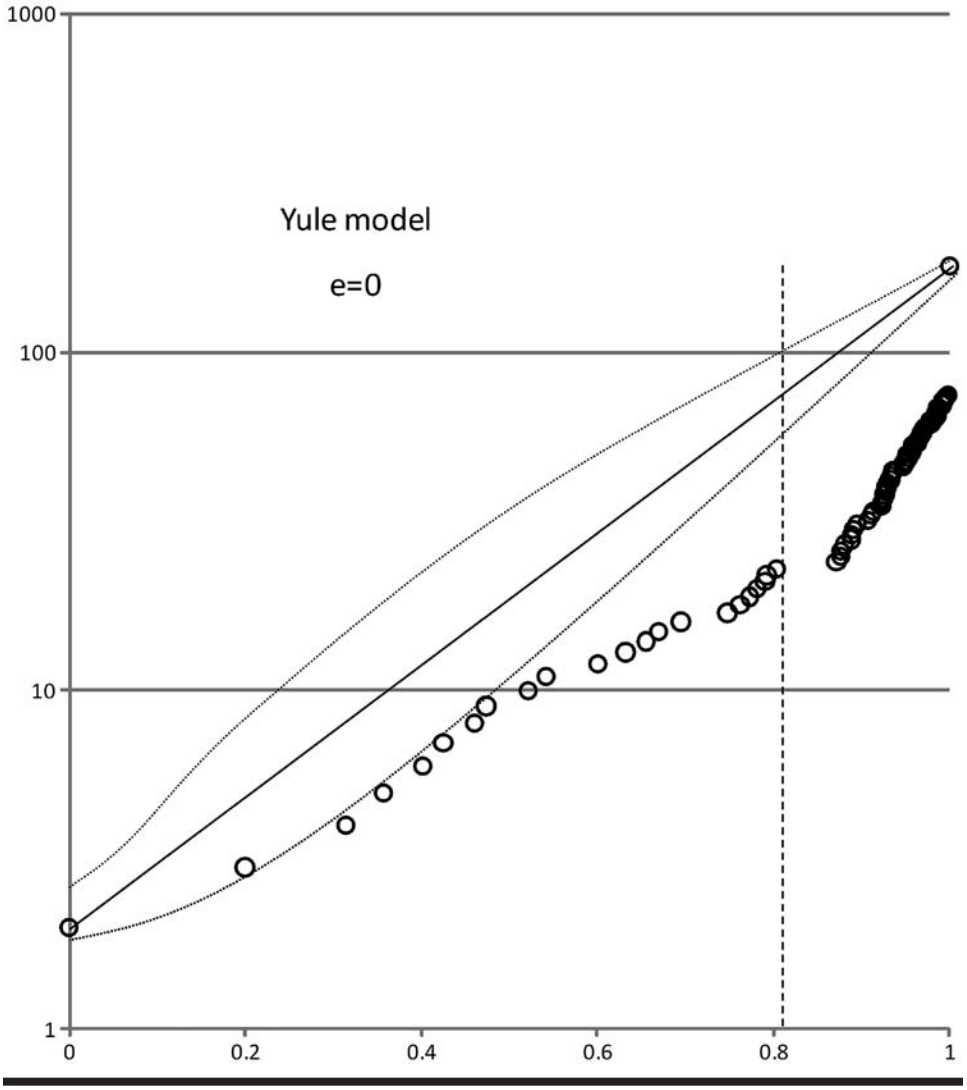

FIGURE 1.5. Lineages-through-time plot (log scale) for the Podocarpaceae compared to the expectation of exponential growth of lineages (Yule model; solid line). A 95% confidence interval on this expectation (dashed lines) was generated from 100 phylogenies simulated under a Yule model, each giving rise to 173 extant species.

method uses (ultrametric) branch length estimates and standing diversity estimates of terminal taxa to derive a maximum likelihood estimate of diversification rate, which varies with the relative extinction fraction (*e*). The likelihood surface can be visualized across the range of values of *e* to determine the value that returns the maximum likelihood estimate of diversification rate. For the podocarp data, this analysis was performed on an ultrametric topology (median node heights estimated from the relaxed-clock analyses, above) sampled to generic level by pruning all but one representative per genus, with generic species richness estimates assigned to the terminals according to Farjon (1998) (see Figure 1.8). Figure 1.7 plots the likelihood surface for relative extinction fractions ranging from zero (no extinction) to 0.99 (99% of lineages go extinct) and suggests that (given the model) a relative extinction rate somewhat in excess of 0.9 (i.e., lineages have a <10% chance of surviving to the present) provides a reasonable approximation for these data. Although there are no direct estimates of an extinction rate for the Podocarpaceae derived from fossils, the inference of a high relative extinction rate seems reasonable in light of the levels of Cenozoic diversity of the Podocarpaceae in the Southern Hemisphere fossil record (Hill and Brodribb, 1999).

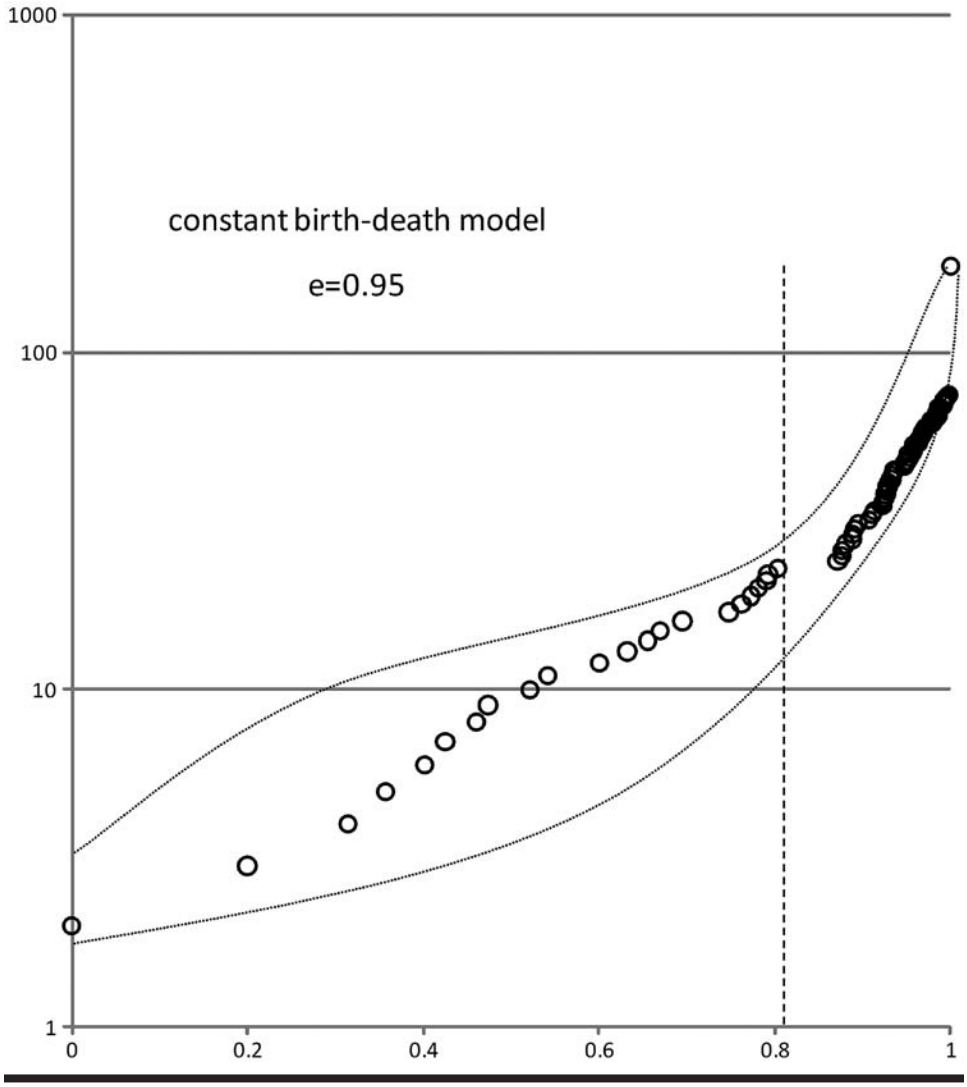

FIGURE 1.6. Lineages-through-time plot (log scale) for the Podocarpaceae compared to the expectation under a constant birth–death speciation model. The dashed lines represent a 95% confidence interval generated from 100 phylogenies simulated under a time-homogenous extinction rate of 0.95.

Shifts in Diversification Rate

The above estimate assumes that the podocarp phylogeny was generated under a constant rate of lineage diversification, an assumption that can be tested by contrasting the likelihood of a model that fits a homogeneous diversification rate to the data with one in which an ancestral diversification rate shifts at some point to a new diversification rate (Sanderson and Wojciechowski, 1996.; Rabosky et al., 2007). These analyses were performed in LASER, using the branch length and per genus diversities as above, and were repeated for 100 topologies sampled from the 95% highest posterior density of the BEAST runs to assess the robustness of the conclusion to variations in topology and branch length estimates. In the first instance, the relative extinction rate was set to 0.95, but subsequent analyses were performed using $e = 0$ to test that the conclusions were robust to the model assumptions. For both relative extinction rates and all sampled topologies, comparison of standing diversities with those expected under a uniform diversification rate rejects the null hypothesis of a homogeneous diversification rate for the Podocarpaceae ($p < 1 \times 10^{-5}$, $e = 0.95$). That is, the observed levels of diversity among the podocarpaceous lineages are better

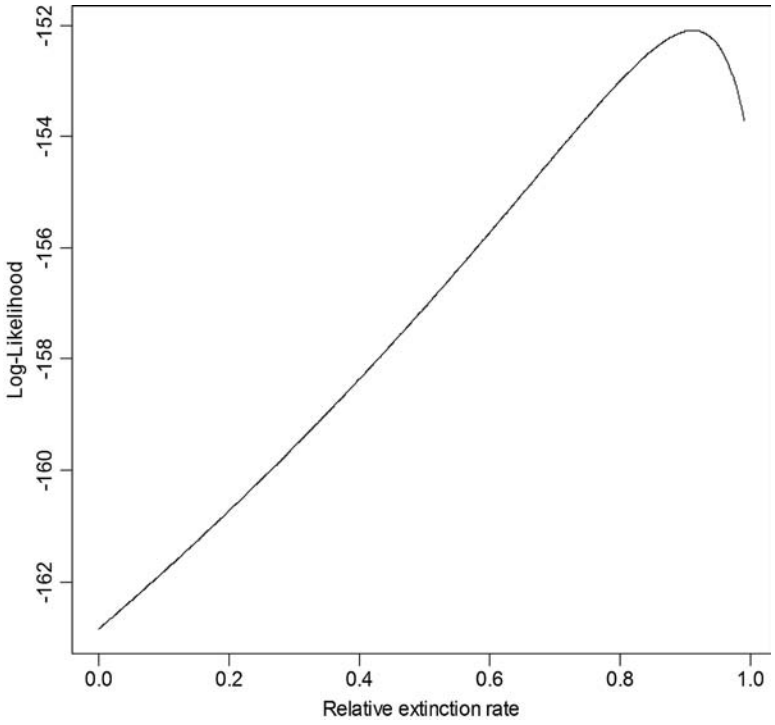

FIGURE 1.7. The likelihood of diversification rate estimates plotted against the relative extinction fraction under a model that assumes a homogenous diversification rate that varies with the extinction fraction. Analyses performed in LASER (Rabosky, 2006) using branch length and species richness data as in Figure 1.8.

accounted for by a model in which diversification rates have increased or decreased significantly at some point in the evolution of family.

For the variable diversification rate model, the phylogenetic tree is sequentially split at each node, and a diversification rate is optimized onto each descendant lineage. The maximum likelihood diversification shift point is the node with the highest combined likelihood obtained by summing the lineage-specific likelihood estimates from each bipartite tree (Rabosky et al., 2007). For the podocarp data, the maximum likelihood shift point is located on the most recent common ancestor (MRCA) of the Dacrydioid and Podocarpoid clades (Figure 1.8). The extent of this shift ranges from an approximately threefold ($e = 0$) to an eightfold increase in diversification rate at $e = 0.95$. At $e = 0.95$, other nodes with a likelihood (L) approaching the inferred maximum likelihood shift include the immediate ancestor of the Podocarpoid–Dacrydioid clade and the successively deeper node ($\Delta L = 3.5$ and 1.9, respectively, compared to the maximum likelihood shift point), the *Podocarpus* crown node ($\Delta L = 3.53$), and the MRCA of *Lepidothamnus*, *Phyllocladus*, and the Prumnopityoid clade ($\Delta L = 4.9$) (Figure 1.8). The latter is the largest diversification rate *decrease* inferred from these data. Among these, the two successive nodes immediately below the maximum likelihood shift point are perhaps a consequence of "trickle down," that is, potentially spurious inference of rate shifts stemming from the nested nature of phylogenetic data and the high diversity of immediately more nested nodes (Moore et al., 2004). Similarly, the Podocarpoid–Dacrydioid clade not only includes *Podocarpus* but also unites other, relatively species rich, clades (e.g., the Dacrydioid clade with 35 species) and has a likelihood score exceeding the immediately more nested nodes. The identified maximum likelihood shift point was robust to variations in the modeled extinction fraction.

Timing and Correlates of Shifts in Diversification Rate

A major event in the evolution of land plants was ecological radiation and taxonomic diversification of flowering plants, which is concomitantly associated with declining diversities among other plant groups, including conifers (e.g., Crane, 1987; Crane and Lidgard, 1989; McElwain et al., 2005). The major diversification of angiosperms is believed to have occurred in the mid- to Late Cretaceous (middle Albian to early Cenomanian, approximately 100–94 mya); for instance, there is a dramatic increase in the representation of angiosperms in regional palynofloras (from approximately <5% to >40%) over a 40 million year period from the mid-Cretaceous, consistent with rapid radiation (Crane, 1987). Although the timing is debated, angiosperm-dominated megathermal

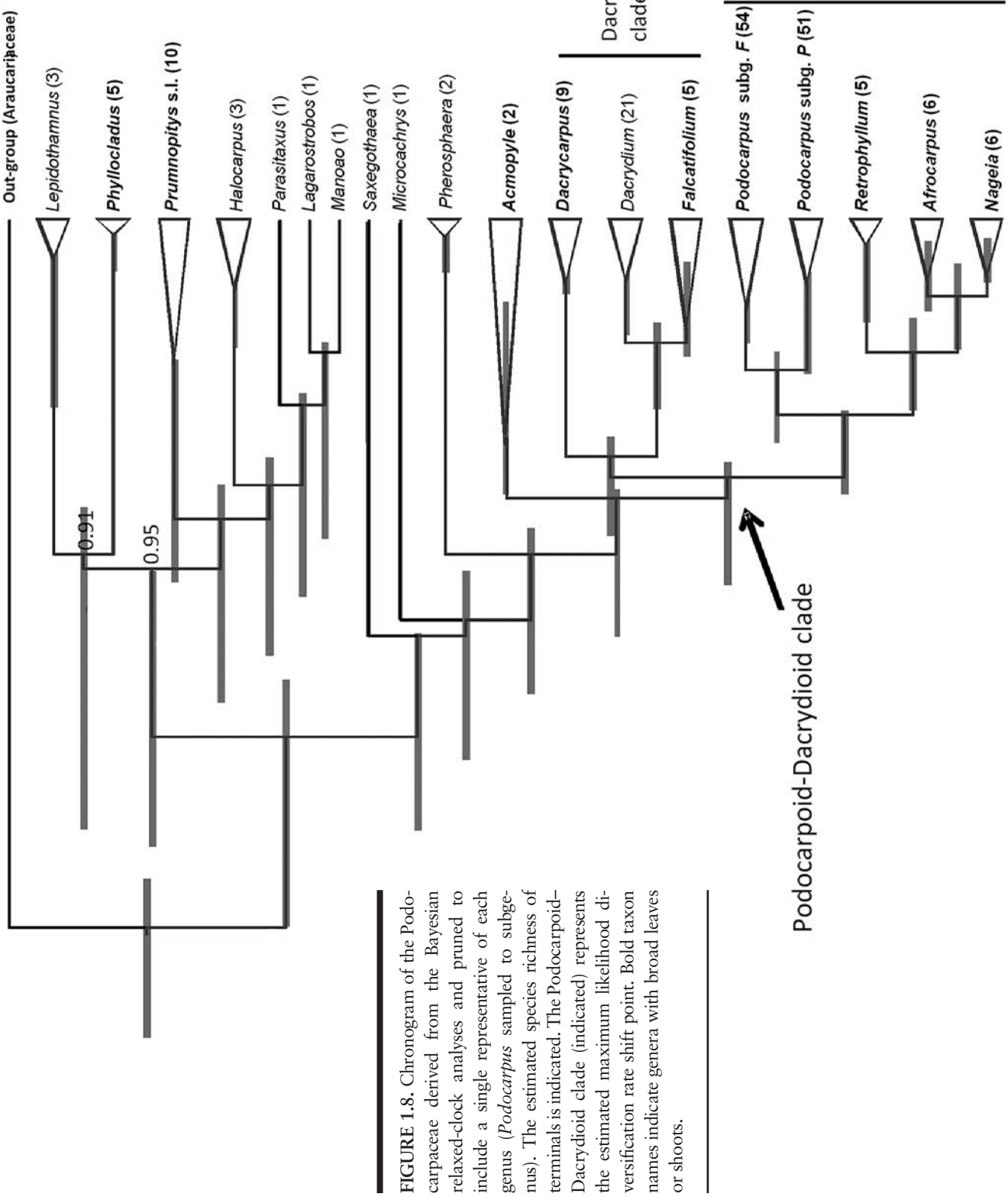

FIGURE 1.8. Chronogram of the Podocarpaceae derived from the Bayesian relaxed-clock analyses and pruned to include a single representative of each genus (*Podocarpus* sampled to subgenus). The estimated species richness of terminals is indicated. The Podocarpoid–Dacrydioid clade (indicated) represents the estimated maximum likelihood diversification rate shift point. Bold taxon names indicate genera with broad leaves or shoots.

forests appear to have expanded principally from the Albian (e.g., Davis et al., 2005) to the Cretaceous–Tertiary boundary (e.g., Morley, 2000). Interestingly, these dates accord with the inferred timing of the radiation of the Podocarpoid–Dacrydioid clade (Figure 1.3), which on the strength of the evidence presented here, has experienced higher speciation and/or lower extinction rates relative to other podocarp lineages (Figure 1.8).

Among conifers, the podocarps are unusual in that an overwhelming majority of taxa are restricted to humid environments, including angiosperm-dominated forests extending into the tropics. Explanations for this pattern have been sought from comparative ecophysiology and from the paleobotanical and paleoclimatic data (reviewed by Hill and Brodribb, 1999; Brodribb and Hill, 2004; Brodribb, this volume). Briefly, the relative success of the Podocarpaceae in wet forests has been ascribed to morphological/physiological traits, such as leaf flattening, and life history characteristics, including the longevity of individuals, which are believed to facilitate regeneration of the podocarps among the dense shade of broad-leaved angiosperms (Brodribb and Hill, 1997, 2004; see also Brodribb and Feild, 2008). However, the physiological mechanism related to persistence of podocarps in wet forest environments appears to be associated negatively with drought tolerance (Brodribb and Hill, 1998, 2004; Hill and Brodribb, 1999), and the demise of several Podocarpaceae genera in Australia has been linked to decreasing rainfall, increasingly seasonal rainfall regimes, and increased fire frequency and intensity through the Cenozoic (Hill and Brodribb, 1999; Brodribb and Hill, 2004).

The Podocarpoid–Dacrydioid clade comprises a predominance of taxa with broad leaves and shoots (*Afrocarpus*, most *Dacrycarpus* spp., *Falcatifolium*, *Nageia*, *Podocarpus*, *Retrophyllum*; Figure 1.8). As suggested elsewhere (e.g., Brodribb and Hill, 2004), leaf/shoot flattening among podocarps probably arose prior to the major expansion of flowering plants but subsequently contributed to the persistence of those lineages in low-light conditions beneath angiosperm-dominated canopies. Furthermore, the species included in the Podocarpoid–Dacrydioid clade are, for the main part, concentrated in the tropics (cf. Kelch's "tropical clade"; Kelch, 1997) and particularly the paleotropics. An exaptation to rainforests may have buffered those taxa from the extremes of historical climatic change relative to those experienced at higher latitudes (e.g., Dynesius and Jansson, 2000; Jansson and Davies, 2008) and facilitated northward expansion of "Gondwanan" lineages with the close proximity of the Australian and Sunda plates from the mid- to late Tertiary (Morley, 2003). Thus, a combination of ecophysiological adaptation/exaptation and the past and present distribution of suitable climates may have reduced the probability of extinction relative to imbricate-leaved lineages and facilitated range expansion and speciation.

Contingency, Convergence, and Key Traits

In light of the above results, the fact that some of the genera with broad shoots (*Acmopyle*, *Prumnopitys* sensu lato, and *Saxegothaea*; Figure 1.8) or phylloclades (*Phyllocladus*) have failed to radiate at a rate comparable to the Podocarpoid–Dacrydioid clade requires explanation. Of these, *Acmopyle*, *Phyllocladus*, and *Prumnopitys* sensu lato have at least some representation within tropical regions. One possible interpretation is provided by historical contingency; that is, when the influence of a particular sort of character (for instance, on rates of speciation or extinction) is dependent on the proximity of other factors (de Queiroz, 2002). Fleshy fruits, for example, are associated with high rates of diversification among tropical rainforest understory angiosperms (Smith, 2001) but imperfectly in other contexts (Herrera, 1989). Certain putative "key traits" of angiosperms (vessels, reticulate venation, closed carpels) may have only achieved significance upon transition from the understory into high-light environments (Feild et al., 2004). Therefore, an imperfect correlation between a trait (or traits) and a particular mechanistic hypothesis to explain elevated diversification rates suggests the need to carefully consider other potentially significant associations (Donoghue, 2005). The long-term evolution of geographic range of the Podocarpaceae in the context of historical climatic/geological scenarios (e.g., Yesson and Culham, 2006; Moore and Donoghue, 2007) would be a fruitful avenue for further investigation given that it is "easier to move than evolve" (Donoghue, 2008:11551) and species–area effects can strongly influence past and present diversities (Jaramillo et al., 2006).

Furthermore, there can be different ways to construct an outwardly similar organ, and the various pathways can have different outcomes in terms of rates of lineage accumulation (Donoghue, 2005). In this context, the difference between parallel and convergent evolution may be significant. Given that leaf flattening is associated with several evolutionarily distant lineages in the Podocarpaceae (Figure 1.8), it is probable that similarities are convergent (i.e., constructed from different starting points). This appears to be the case for *Phyllocladus*, which develops broad phyllodes rather than true leaves, the latter being the otherwise general condition within the family. Similarly, the development

of leaflike shoots of small distichous, flattened leaves in several divergent lineages may represent an adaptation to catch light on the rainforest floor, but these shoots are lost when trees reach the canopy. Detailed studies of morphological variation, ideally including fossil taxa, would help distinguish parallel from convergent evolution in candidate traits and refine mechanistic hypotheses.

SUMMARY

We have presented a preliminary hypothesis of evolutionary relationships among the Podocarpaceae using molecular phylogenetic data. On this basis, we have incorporated fossil constraints to estimate molecular evolutionary rates and divergence times for lineages of the Podocarpaceae. In general, the molecular phylogeny is largely in agreement with conventionally (morphology) based classifications for the family, although relative to previous hypotheses there is a high level of confidence in most intergeneric relationships. However, there is weak support for the majority of relationships within genera, and we present evidence that the majority of species are of recent evolutionary origin. Although this could be taken to indicate an upturn in diversification rates toward the present, a similar pattern could be inferred, for example, under a homogenous rate of speciation and a high but constant rate of extinction. Although extinction rates appear to have been high among members of the Podocarpaceae, a major shift in diversification rate is estimated to be of mid- to Late Cretaceous age, which could reflect a response, in terms of reduced extinction and/or increased speciation rates, to the radiation and expansion of angiosperm-dominated forests. Although further work is needed, the results of this study highlight the potential of molecular phylogenetic approaches to develop and test a range of hypotheses in the context of evolutionary biology and ecology.

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REFERENCES

Alfaro, M. E., S. Zoller, and F. Lutzoni. 2003. Bayes or Bootstrap? A Simulation Study Comparing the Performance of Bayesian Markov Chain Monte Carlo Sampling and Bootstrapping in Assessing Phylogenetic Confidence. *Molec. Biol. Evol.* 20: 255–266.

Ayala, F. J. 1997. Vagaries of the molecular clock. *Proc. Natl. Acad. Sci. U.S.A.* 94: 7776–7783.

Barker, N. P., E. M. Muller, and R. R. Mill. 2004. A Yellowwood by Any Other Name: Molecular Systematics and the Taxonomy of *Podocarpus* and the Podocarpaceae in Southern Africa. *S. African J. Sci.* 100: 630–632.

Benton, M. J., and P. C. J. Donoghue. 2007. Paleontological Evidence to Date the Tree of Life. *Molec. Biol. Evol.* 24: 26–53.

Bobrov, A. V., A. P. Melikian, and E. Y. Yembaturova. 1999. Seed Morphology, Anatomy and Ultrastructure of *Phyllocladus* L. C. & A. Rich. ex Mirb. (Phyllocladaceae (Pilg.) Bessey) in Connection with the Generic System and Phylogeny. *Ann. Bot.* (*London*) 83: 601–618.

Bond, W. J. 1989. The Tortoise and the Hare: Ecology of Angiosperm Dominance and Gymnosperm Persistence. *Biol. J. Linn. Soc.* 36: 227–249.

Brodribb, T. J. 2011 (this volume). A Functional Analysis of Podocarp Ecology. In *Ecology of the Podocarpaceae in Tropical Forests,* B. L. Turner and L. A. Cernusak, eds., pp. 165–175. Smithsonian Contributions to Botany, No. 95. Smithsonian Institution Scholarly Press, Washington, D.C.

Brodribb, T. J., and T. S. Feild. 2008. Evolutionary Significance of a Flat-Leaved *Pinus* in Vietnamese Rainforest. *New Phytol.* 178: 201–209.

Brodribb, T. J., and R. S. Hill. 1997. The Light Response Characteristics of a Morphologically Diverse Group of Southern Hemisphere Conifers. *Oecologia* 110: 10–17.

Brodribb, T. J., and R. S. Hill. 1998. The Photosynthetic Drought Physiology of a Diverse Group of Southern Hemisphere Conifer Species Is Correlated with Minimum Seasonal Rainfall. *Funct. Ecol.* 12: 465–471.

Brodribb, T. J., and R. S. Hill. 2004. The Rise and Fall of the Podocarpaceae in Australia—A Physiological Explanation. In *The Evolution of Plant Physiology*, A. R Hemsley and I. Poole, eds., pp. 379–381. Academic Press, London, UK.

Bromham, L., and D. Penny. 2003. The Modern Molecular Clock. *Nat. Rev. Genet.* 4: 216–224.

Conran, J. G., G. G. Wood, P. G. Martin, J. M. Dowd, C. J. Quinn, P. A. Gadek, and R. A. Price. 2000. Generic Relationships within and between the Gymnosperm Families Podocarpaceae and Phyllocladaceae Based on an Analysis of the Chloroplast Gene *rbc*L. *Austral. J. Bot.* 48: 715–724.

Crane, P. R. 1987. Vegetational Consequences of the Angiosperm Diversification. In *The Origins of Angiosperms and Their Biological Consequences*, E. M. Friis, W. G. Chaloner, and P. R. Crane, eds., pp. 107–144. Cambridge University Press, Cambridge, UK.

Crane, P. R., and S. Lidgard. 1989. Angiosperm Diversification and Paleolatitudinal Gradients in Cretaceous Floristic Diversity. *Science* 246: 675–678.

Crisp, M. D., and L. G. Cook. 2007. A Congruent Molecular Signature of Vicariance across Multiple Plant Lineages. *Molec. Phylogen. Evol.* 43: 1106–1117.

Crisp, M. D., L. G. Cook, and D. A. Steane. 2004. Radiation of the Australian Flora: What Can Comparisons of Molecular Phylogenies across Multiple Taxa Tell Us about the Evolution of Diversity in Present-Day Communities? *Philos. Trans. Roy. Soc. London, Ser. B* 359: 1551–1571.

Dalling, J. W., P. Barkan, P. J. Bellingham, J. R. Healey, and E. V. J. Tanner. 2011 (this volume). Ecology and Distribution of Neotropical Podocarpaceae. In *Ecology of the Podocarpaceae in Tropical*

Forests, B. L. Turner and L. A. Cernusak, eds., pp. 43–56. Smithsonian Contributions to Botany, No. 95. Smithsonian Institution Scholarly Press, Washington, D.C.

Davies, T. J., T. G. Barraclough, M. W. Chase, P. S. Soltis, D. E. Soltis, and V. Savolainen. 2004. Darwin's Abominable Mystery: Insights from a Supertree of the Angiosperms. *Proc. Natl. Acad. Sci. U.S.A.* 101: 1904–1909.

Davis, C. C., C. O. Webb, K. J. Wurdack, C. A. Jaramillo, and M. J. Donoghue. 2005. Explosive Radiation of Malpighiales Supports a Mid-Cretaceous Origin of Modern Tropical Rain Forests. *Amer. Naturalist* 165: E36–E65.

de Queiroz, A. 2002. Contingent Predictability in Evolution: Key Traits and Diversification. *Syst. Biol.* 51: 917–929.

Donoghue, M. J. 2005. Key Innovations, Convergence, and Success: Macroevolutionary Lessons from Plant Phylogeny. *Paleobiology* 31: 77–93.

Donoghue, M. J. 2008. A Phylogenetic Perspective on the Distribution of Plant Diversity. *Proc. Natl. Acad. Sci. U.S.A.* 105: 11549–11555.

Drummond, A. J., S. W. Y. Ho, M. J. Phillips, and A. Rambaut. 2006. Relaxed Phylogenetics and Dating Confidence. *PLoS Biol.* 4: e88, doi:10.1371/journal.pbio.0040088.

Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian Evolutionary Analysis by Sampling Trees. *BMC Evol. Biol.* 7: 214, doi:10.1186/1471-2148-7-214.

Dynesius, M., and R. Jansson. 2000. Evolutionary Consequences of Changes in Species' Geographical Distributions Driven by Milankovitch Climate Oscillations. *Proc. Natl. Acad. Sci. U.S.A.* 97: 9115–9120.

Enright, N. J., and T. Jaffré. 2011 (this volume). Ecology and Distribution of the Malesian Podocarps. In *Ecology of the Podocarpaceae in Tropical Forests,* B. L. Turner and L. A. Cernusak, eds., pp. 57–77. Smithsonian Contributions to Botany, No. 95. Smithsonian Institution Scholarly Press, Washington, D.C.

Farjon, A. 1998. *World Checklist and Bibliography of Conifers.* Royal Botanical Gardens, Kew, Richmond, Surrey, UK.

Feild, T. S., N. C. Arens, J. A. Doyle, T. E. Dawson, and M. J. Donoghue. 2004. Dark and Disturbed: A New Image of Early Angiosperm Ecology. *Paleobiology* 30: 82–107.

Givnish, T. J., and K. J. Sytsma. 1997. Homoplasy in Molecular vs. Morphological Data: The Likelihood of Correct Phylogenetic Inference. In *Molecular Evolution and Adaptive Radiation*, T. J. Givnish and K. J. Sytsma, eds., pp. 55–102. Cambridge University Press, Cambridge, UK.

Graham, S. W., J. R. Kohn, B. R. Morton, J. E. Eckenwalder, and S. C. H. Barrett. 1998. Phylogenetic Congruence and Discordance among One Morphological and Three Molecular Data Sets from Pontederiaceae. *Syst. Biol.* 47: 545–567.

Harmon, L. J., J. A. Schulte, A. Larson, and J. B. Losos. 2003. Tempo and Mode of Evolutionary Radiation in Iguanian Lizards. *Science* 301: 961–964.

Hart, J. A. 1987. A Cladistic Analysis of Conifers: Preliminary Results. *J. Arnold Arbor.* 68: 269–307.

Herbert, J., P. M. Hollingsworth, M. F. Gardner, R. R. Mill, and P. I. Thomas. 2002. Conservation Genetics and Phylogenetics of New Caledonian *Retrophyllum* (Podocarpaceae) Species. *New Zealand J. Bot.* 40: 175–188.

Herrera, C. M. 1989. Seed Dispersal by Animals: A Role in Angiosperm Diversification? *Amer. Naturalist* 133: 309–322.

Hill, R. S., and T. J. Brodribb. 1999. Southern Conifers in Time and Space. *Aust. J. Bot.* 47: 639–696.

Hill, R. S., and R. J. Carpenter. 1991. Evolution of *Acmopyle* and *Dacrycarpus* (Podocarpaceae) Foliage as Inferred from Macrofossils in South-eastern Australia. *Austral. Syst. Bot.* 4: 449–479.

Jansson, R., and T. J. Davies. 2008. Global Variation in Diversification Rates of Flowering Plants: Energy vs. Climate Change. *Ecol. Letters* 11: 173–183.

Jaramillo, C., M. J. Rueda, and G. Mora. 2006. Cenozoic Plant Diversity in the Neotropics. *Science* 311: 1893–1896.

Kelch, D. G. 1997. Phylogeny of the Podocarpaceae Based on Morphological Evidence. *Syst. Bot.* 22: 113–131.

Kelch, D. G. 1998. Phylogeny of Podocarpaceae—Comparison of Evidence from Morphology and 18s rDNA. *Amer. J. Bot.* 85: 986–996.

Kelch, D. G. 2002. Phylogenetic Assessment of the Monotypic Genera *Sundacarpus* and *Manoao* (Coniferales: Podocarpaceae) Utilising Evidence from 18S rDNA Sequences. *Austral. Syst. Bot.* 15: 29–35.

Magallón, S. 2004. Dating Lineages: Molecular and Paleontological Approaches to the Temporal Framework of Clades. *Int. J. Pl. Sci.* 165: S7–S21.

Magallón, S., and M. J. Sanderson, 2001. Absolute Diversification Rates in Angiosperm Clades. *Evolution* 55: 1762–1780.

McElwain, J. C., K. J. Willis, and R. Lupia. 2005. Cretaceous CO_2 Decline and the Radiation and Diversification of Angiosperms. In *A History of Atmospheric CO_2 and Its Effects on Plants, Animals and Ecosystems*, J. R Ehrlinger, T. E. Cerling, and M. D. Dearing, eds., pp. 133–165. Ecological Studies, No. 177, Springer-Verlag, Berlin.

Moore, B. R., M. A. K. Chan, and M. J. Donoghue. 2004. Detecting Diversification Rate Variation in Supertrees. In *Phylogenetic Supertrees: Combining Information to Reveal the Tree of Life*, O. R. P. Bininda-Emonds, ed., pp. 487–533. Kluwer Academic Press, Dordrecht, Netherlands.

Moore, B. R., and M. J. Donoghue. 2007. Correlates of Diversification in the Plant Clade Dipsacales: Geographic Movement and Evolutionary Innovation. *Amer. Naturalist* 170: S28–S55.

Morley, R. J. 2000. *The Origin and Evolution of Tropical Rain Forests.* John Wiley and Sons, Chichester, UK.

Morley, R. J. 2003. Interplate Dispersal Routes for Megathermal Angiosperms. *Perspect. Pl. Ecol. Evol. Syst.* 6: 5–20.

Morley, R. J. 2011 (this volume). Dispersal and Paleoecology of Tropical Podocarps. In *Ecology of the Podocarpaceae in Tropical Forests,* B. L. Turner and L. A. Cernusak, eds., pp. 21–41. Smithsonian Contributions to Botany, No. 95. Smithsonian Institution Scholarly Press, Washington, D.C.

Nee, S., R. M. May, and P. H. Harvey. 1994. The Reconstructed Evolutionary Process. *Philos. Trans. Roy. Soc. London, Ser. B* 344: 305–311.

Page, C. N. 1988. New and Maintained Genera in the Conifer Families Podocarpaceae and Pinaceae. *Notes Roy. Bot. Gard. Edinburgh* 45: 377–396.

Page, C. N. 1990a. Phyllocladaceae. In *Volume 1: Pteridophytes and Gymnosperms*, K. Kubitzki, ed., pp. 317–319, *The Families and Genera of Vascular Plants.* Springer-Verlag, Berlin.

Page, C. N. 1990b. Podocarpaceae. In *Volume 1: Pteridophytes and Gymnosperms*, K. Kubitzki, ed., pp. 332–336, *The Families and Genera of Vascular Plants*. Springer-Verlag, Berlin.

Pole, M. S. 1997. Paleocene Plant Macrofossils from Kakahu, South Canterbury, New Zealand. *J. Roy. Soc. New Zealand* 27: 371–400.

Pole, M. S. 1998. Paleocene Gymnosperms from Mt Somers, New Zealand. *J. Roy. Soc. New Zealand* 28: 375–403.

Quinn, C. J., R. A. Price, and P. A. Gadek. 2002. Familial Concepts and Relationships in the Conifers Based on *rbcL* and *matK* Sequence Comparisons. *Kew Bull.* 57: 513–531.

Rabosky, D. L. 2006. LASER: A Maximum Likelihood Toolkit for Detecting Temporal Shifts in Diversification Rates. *Evol. Bioinform.* 2: 257–260.

Rabosky, D. L. 2008. Testing for Diversification Rate Shifts on Phylogenetic Trees Using Combined Phylogenetic and Taxonomic Data. http://www.eeb.cornell.edu/Rabosky/dan/software_files/Rhelp_Rabosky2007.pdf (accessed June 2008).

Rabosky, D. L., S. C. Donnellan, A. L. Tallaba, and I. J. Lovette. 2007. Exceptional Among-Lineage Variation in Diversification Rates during the Radiation of Australia's Most Diverse Vertebrate Clade. *Proc. Roy. Soc. London, Ser. B, Biol. Sci.* 274: 2915–2923.

Rabosky, D. L., and I. J. Lovette. 2008. Explosive Evolutionary Radiations: Decreasing Speciation or Increasing Extinction through Time? *Evolution* 62: 1866–1875.

Rai, H. S., P. A. Reeves, R. Peakall, R. G. Olmstead, and S. W. Graham. 2008. Inference of Higher-Order Conifer Relationships from a Multi-locus Plastid Data Set. *Botany* 86: 658–669.

Rambaut, A., and A. J. Drummond. 2007. Tracer. Version 1.4. http://beast.bio.ed.ac.uk/Tracer (accessed 9 Oct 2009).

Renner, S. S. 2005. Relaxed Molecular Clocks for Dating Historical Plant Dispersal Events. *Trends Pl. Sci.* 10: 550–558.

Ricklefs, R. E. 2007. History and Diversity: Explorations at the Intersection of Ecology and Evolution. *Amer. Naturalist* 170: S56–S70.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian Phylogenetic Inference under Mixed Models. *Bioinformatics* 19: 1572–1574.

Rutschmann, F. 2006. Molecular Dating of Phylogenetic Trees: A Brief Review of Current Methods That Estimate Divergence Times. *Diversity Distrib.* 12: 35–48.

Sanders, K. L., and M. S. Y. Lee. 2007. Evaluating Molecular Clock Calibrations Using Bayesian Analyses with Soft and Hard Bounds. *Biol. Lett.* 3: 275–279.

Sanderson, M. J., and M. F. Wojciechowski. 1996. Diversification Rates in a Temperate Legume Clade: Are There "So Many Species" of *Astragalus* (Fabaceae)? *Amer. J. Bot.* 83: 1488–1502.

Saquet, H., P. H. Weston, C. L. Anderson, N. P Barker, D. J. Cantrill, A. R. Mast, and V. Savolainen. 2009. Contrasted Patterns of Hyperdiversification in Mediterranean Hotspots. *Proc. Natl. Acad. Sci. U.S.A.* 106: 221–225.

Scotland, R. W., R. G. Olmstead, and J. R. Bennett. 2003. Phylogeny Reconstruction: The Role of Morphology. *Syst. Biol.* 52: 539–548.

Sinclair, W. T., R. R. Mill, M. F. Gardner, P. Woltz, T. Jaffré, J. Preston, M. L. Hollingsworth, A. Ponge, and M. Moller. 2002. Evolutionary Relationships of the New Caledonian Heterotrophic Conifer, *Parasitaxus usta* (Podocarpaceae), Inferred from Chloroplast *trn*L–F Intron/Spacer and Nuclear rDNA ITS2 Sequences. *Pl. Syst. Evol.* 233: 79–104.

Smith, J. F. 2001. High Species Diversity in Fleshy-Fruited Tropical Understory Plants. *Amer. Naturalist* 147: 646–653.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal Primers for Amplification of Three Non-coding Regions of Chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignments through Sequence Weighting, Position Specific Gap Penalties and Weight Matrix Choice. *Nucl. Acids Res.* 22: 4673–4680.

Townrow, J. A. 1965. Notes on Tasmanian Pines. I. Some Lower Tertiary Podocarps. *Pap. Proc. Roy. Soc. Tasmania* 99: 87–107.

Wagner, P. J. 2000. Exhaustion of Morphological Character States among Fossil Taxa. *Evolution* 54: 365–386.

Wagstaff, S. J. 2004. Evolution and Biogeography of the Austral Genus *Phyllocladus* (Podocarpaceae). *J. Biogeogr.* 31: 1569–1577.

Wells, P. M., and R. S. Hill. 1989. Fossil imbricate-leaved Podocarpaceae from Tertiary sediments in Tasmania. *Austral. Syst. Bot.* 2: 387–423.

White, T. J., T. Burns, S. Lee, and J. Taylor. 1990. Amplification and Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In *PCR Protocols. A Guide to Methods and Applications*, M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds., pp. 315–322. Academic Press, San Diego.

Wiens, J. J. 2004. The Role of Morphological Data in Phylogeny Reconstruction. *Syst. Biol.* 53: 653–661.

Willyard, A., J. Syring, D. S. Gernandt, A. Liston, and R. Cronn. 2007. Fossil Calibration of Molecular Divergence Infers a Moderate Mutation Rate and Recent Radiations for *Pinus*. *Molec. Biol. Evol.* 24: 90–101.

Yang, Z., and B. Rannala. 2006. Bayesian Estimation of Species Divergence Times under a Molecular Clock Using Multiple Fossil Calibrations with Soft Bounds. *Molec. Biol. Evol.* 23: 212–226.

Yesson, C., and A. Culham. 2006. Phyloclimatic Modeling: Combining Phylogenetics and Bioclimatic Modeling. *Syst. Biol.* 55: 785–802.

Zwickl, D. J. 2006. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequences Datasets under Maximum Likelihood Criterion. Ph.D. diss., The University of Texas at Austin, Austin.