The phylogenetic history and biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data

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Abstract

Generalized hypotheses for the vicariant, Gondwanan origin of pantropically distributed eudicotyledon families must be refined to accommodate recently revised dates that indicate major continental rifting events predate the evolution of many tricolpate angiosperm clades. Here, we use molecular phylogenies of an eudicotyledon family previously hypothesized to have a Gondwanan origin, the Burseraceae, to test this and other alternative biogeographical hypotheses in light of recalibrated geological events. Phylogenies based on nuclear and chloroplast data were reconstructed for 13 of the 18 genera (50 spp. total) of Burseraceae using parsimony, maximum likelihood, and Bayesian methods. Ages of all lineages were estimated using penalized likelihood and semiparametric rate smoothing [Bioinformatics 2003 (19) 301], which allows the user to calibrate phylogenies based on non-clock-like DNA sequence data with fossil information. Biogeographical hypotheses were tested by comparing ages of species and more inclusive lineages with their extant and most parsimonious ancestral distributions. Our data support a North American Paleocene origin for the Burseraceae followed by dispersal of ancestral lineages to eastern Laurasia and Southern Hemisphere continents.

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1. Introduction

The Burseraceae are best known for producing fragrant resins of economic, medicinal, and cultural value, such as frankincense, myrrh, and copal (Langenheim, 2003). The family comprises 18 genera (ca. 700 spp.) and is divided into three tribes (Canarieae, Protieae, and Bursereae) each of which is distributed pantropically across a broad range of low-elevation, frost-free habitats including rainforest, dry deciduous forest, and desert. The Burseraceae and the order to which it belongs, the Sapindales, are part of the monophyletic group of flowering plants that bear tricolpate pollen, the eudicotyledons (Doyle and Hotton, 1991). Molecular phylogenetic studies of the Sapindales (APG II, 2003; Gadek et al., 1996) place the Burseraceae sister to the Anacardiaceae, a family that closely resembles the Burseraceae and with which it has been traditionally allied. The Burseraceae are distinguished morphologically from the Anacardiaceae by the presence of fragrant non-allergenic resin (vs. sometimes allergenic), pulvinulate leaflets, induplicate-valvate (vs. usually imbricate) petals, and two epitropous ovules (vs. one apotropous) per carpel.

The pantropical distribution of the tribes and their component genera poses a challenge to interpreting the biogeographical history of the Burseraceae. All tribes are represented in the American, African, and Indo-Asian tropics, and generic diversity is highest in the Southern
Hemoglobin. Additionally, each tribe contains a cosmopolitan genus distributed across all three tropical regions: *Dacryodes* (Canaricae), *Protium* (Protieae), and *Commiphora* (Bursereae). On the basis of this information and the fact that the Bursereae cannot survive freezing temperatures at higher latitudes, Raven and Axelrod (1974) proposed that the Bursereae had a tropical Gondwanan origin followed by vicariance of its ancestral lineages. Yet, one can only hypothesize a scenario involving Gondwanan vicariance if the Bursereae originated prior to the division of South America from Africa, which is the last division of Gondwanan landmasses at tropical, frost-free latitudes. Recently, published dates indicate that the division of South America and Africa took place between 105 and 119 Ma (McLoughlin, 2001). To place this event in context, the earliest known fossils attributable to the eudicotyledon lineage and the Sapindales date to ca. 125 Ma (Doyle and Hotton, 1991; Magallon et al., 1999) and ca. 65 Ma (Knobloch and Mai, 1986), respectively. The early eudicot fossils are not attributable to any extant family but were found in Gabon, a former Gondwanan terrane. By contrast, the Sapindales fossil attributed to the Rutaceae was found in Germany, which is a former Laurasian terrane. A model in which extant pantropical distributions of the Bursereae are due solely to strict Gondwanan vicariance requires that the Bursereae originated either in Gondwana before proto-south east Asia rifted from the supercontinent (300–152 Ma; Hall, 1995; McLoughlin, 2001; Metcalfe, 1995) or originated before the division of Africa and the Madagascar–India subcontinent (165 Ma; McLoughlin, 2001), events that are unlikely to have subdivided any eudicotyledon lineage, much less the Bursereae.

Incorporating these data, we find the assumptions of the generalized vicariant Gondwanan biogeographic model as proposed by Raven and Axelrod (1974) require reassessment for the Bursereae, as well as for many other extant eudicotyledon taxa that are distributed among American, African, and south east Asian tropics. McLoughlin (2001) suggested associating vicariant biogeographies of Southern Hemisphere angiosperms with more recent episodes of continental rifting, such as the division of Madagascar and India (88 Ma; Storey et al., 1995) or India’s collision with the Asian continent (43 Ma) with subsequent dispersal to other Southern and Northern Hemisphere regions. To understand the evolution and diversification of the Bursereae and to help explain the development of this pantropical distribution pattern, the biogeography of the family was examined in a three-step process. First, a molecular phylogeny of the family was generated to test the monophyly of the family and allows the user to estimate the ages of clades in the phylogeny by applying fossil-based calibration points to nodes within the phylogeny. Lastly, possible instances of ancestral continental vicariance or long distance dispersal were determined with respect to the ages of lineages by mapping the extant distributions of species onto the phylogeny using parsimony as a criterion.

### 1.1. Tribal taxonomy and phylogeny of the Bursereae

Tribal groups were delimited by Engler (1913) on the basis of pyrene structure and subsequently revised by Lam (1932), who established two subtribes within the most polymorphic tribe of the family, the Bursereae (Table 1). Most recently, Harley and Daly (1995; see for detailed taxonomic history of family) revised the subtribes of the Bursereae, Burserinae, and Boswelliinae, to reflect newly collected morphological data and to

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>List of genera in the Bursereae, approximate number of species, tribal and subtribal placement, and geographic range</td>
</tr>
<tr>
<td>Bursereae</td>
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<tr>
<td>Burserinae</td>
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<tr>
<td><em>Bursera</em> Jacq. ca. 100 spp. Caribbean, Mexico, Central, and S. America</td>
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<tr>
<td>Canaricae</td>
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<tr>
<td><em>Canarium</em> L. ca. 105 spp. SE Asia, Malaysia, Africa</td>
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<tr>
<td><em>Dacryodes</em> Vahl 66 spp. Caribbean, Mexico, C. and S. America, SE Asia, Africa</td>
</tr>
<tr>
<td><em>Haplolobus</em> H.J. Lam 22 spp. E. Malaysia</td>
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<tr>
<td><em>Pseudodacryodes</em> R. Pierlot 1 sp. Central Africa</td>
</tr>
<tr>
<td><em>Scutinanthe</em> Thwaites 2 spp. Sri Lanka, S. Myanmar, Celebes, Sumatra, Malay Peninsula, Borneo</td>
</tr>
<tr>
<td><em>Trianna</em> Hook. f. 1 sp. E. Malaysia</td>
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<tr>
<td>Protieae</td>
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<tr>
<td><em>Crepodiscus</em> Hook. f. 6 spp. S. America</td>
</tr>
<tr>
<td><em>Protium</em> Burm. f. 130 spp. Mexico, C. and S. America, Africa, SE Asia</td>
</tr>
<tr>
<td><em>Tetragastris</em> Gaertn. 9 spp. Central and S. America</td>
</tr>
<tr>
<td>Garuga was placed informally within the Bursereae by Harley and Daly (1995).</td>
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</tbody>
</table>
include the recently discovered Mexican genus, *Beiselia*. The Burseraeinae are distinguished from the Boswelliinae by having a leathery exocarp that splits open upon dehiscence, an oily pseudaril, 1 (2–3) pyrenes, and no axial intrusion between the pyrenes. The Boswelliinae have a dry fruit that shatters on dehiscence, three or more pyrenes separated by axial intrusions from a central column, and wing-like projections on each pyrene-fruit segment. Harley and Daly informally accommodated the enigmatic genus, *Garuga*, within the Burseraceae, although they did not name its subtribe.

A recent molecular phylogenetic investigation of the Burseraceae tested the monophyly of the three tribes but did not address their historical biogeographies. Bootstrap support for all nodes is greater than 75% with the exceptions of the two nodes indicated by asterisks (*).

### 2. Materials and methods

#### 2.1. Taxon sampling

The rps 16 intron and ETS were sequenced from 47 and 48 species of Burseraceae, respectively, and are
available as GenBank Accession Nos. AY314997–AY315150. Sequences of the chloroplast rps 16 intron from two additional Burseraceae genera were downloaded from GenBank (Garuga floribunda AJ41479, Triomma malaccensis AJ416487) and were added to the data matrix, yielding 50 species of Burseraceae representing 13 of the 18 genera in the family. Five species of Anacardiaceae were used to root the analyses including three rps 16 sequences downloaded from GenBank (Gluta renghas AJ416487, Schinus molle AJ416488, Semecarpus sp. AJ416482). Information regarding voucher specimens and geographic origin for all species sampled is listed in Table 2. Genera within the Burseraceae not available for this study are Scutinanthe, Haplolobus, Rossella, Pseudodacryodes, and Aucoumea. The following sequences are missing from the data matrix: rps 16 of

Fig. 2. Strict consensus of 24 most parsimonious trees (L = 1331, CI = 0.597, RI = 0.783, RC = 0.467). Bootstrap support values based on 1000 pseudoreplicates are listed above branches, decay indices are listed below branches. Tribe, subtribe, and section names are indicated by bars to the right of the taxon names.
Burseraceae

Beisella mexicana Forman Pell. s.n. Mexico: Jalisco, cultivated at New York Botanical Garden, USA

Boswellia carterii Birdw. Weeks 01-X-08-3 North East Africa; plant from Rare Plant Research, Oregon USA

Boswellia frereana Birdw. Somalia: Candala, seed grown progeny of *Thuja and Warfa* 5599 (UPS)

Boswellia neglecta S. Moore *Weeks 00-VIII-29-1* Ethiopia; plant from Great Penthalama Greenhouse, California USA

Bursera biflora Standl. *Weeks 99-VII-17-7* Mexico: Oaxaca, plant from Rare Plant Research, Oregon USA

Bursera copalifera (Sessé and Moc. ex DC.) Engl. *Weeks 00-X-24-1* Mexico; plant from Rare Plant Research, Oregon USA

Bursera cineata (Schltdl.) Engl. *Weeks 99-VII-17-1* Mexico; plant from Rare Plant Research, Oregon USA


Bursera fagaroides (H.B.K.) Engl. *Weeks 01-X-08-1* Mexico; cultivated at University Texas at Austin Living Greenhouse Collection

Bursera hindiana Engl. *Weeks 00-VI-14-1* Mexico: Baja California Sur, plant from Rare Plant Research, Oregon, USA


Bursera microphylla A. Gray *Weeks 01-X-08-2* USA: Arizona, Waterman Mountains

Bursera sarukhanii Guevara and Rzed. *Weeks 00-VIII-18-6* Mexico: Michoacan, plant from Rare Plant Research, Oregon USA

Bursera simaruba (L.) Sarg. Goldman s.n. (BH) USA: Florida

Bursera spinosissima Urb. and Ekman *Weeks 01-VII-23-1* Dominican Republic: Barahona

Bursera texomana (DC) Standl. *Weeks 02-IV-23-1* Mexico: Guerrero, plant from Rare Plant Research, Oregon, USA

Canarium indicum L. Lai s.n. (BH) Malaysia; cultivated at Singapore Botanical Garden

Canarium littorale Blume Lai s.n. (BH) Malaysia; cultivated at Singapore Botanical Garden

Canarium pilosum A.W. Benn. Bogler s.n. Malaysia: Perlis, cultivated at Fairchild Tropical Garden, no. FTG 961086A, Florida, USA

Canarium vulgare Leenh. Lai s.n. (BH) Malaysia; cultivated at Singapore Botanical Garden

Canarium zeylanicum Blume Lai s.n. (BH) Malaysia; cultivated at Singapore Botanical Garden

Commiphora angolensis Engl. *Rad and Rad 801* South Africa: Transvaal

Commiphora edulis (Klotzsch) Engl. *Weeks 00-VI-14-3* Zimbabwe; plant grown from seed provided by Silverhill Seeds Nursery, RSA

Commiphora eminii subsp. zimmermannii (Engl.) J.B. Gillett *Mvandoka and Shangai* 595 (MO) Tanzania: Tanga District (T3)

Commiphora franciscana Capuron *Labat 2082* (BH) Madagascar: Tulear

Commiphora kau (R. Br. ex Royle) K. Vollesen Gilbert et al. *7629* (MO) Ethiopia: Sidamo

Commiphora leptophloeos (Mart.) J.B. Gillett *Abbott 16293* Boliva: Santa Cruz


Commiphora schimperi Engl. *Weeks 00-VIII-18-8* Republic of South Africa; plant grown from seed provided by Silverhill Seeds Nursery, RSA

Commiphora uugogensis Engl. *Loret 1626* (MO) Tanzania: Iringa District

Commiphora wightii (Arn.) Bhandari *Weeks 00-VIII-18-3* India; plant from Arid Lands Greenhouse, Arizona, USA

Crepidospermum goudotianum Triana and Planch. *de Michel and Capra* 2439 (NY) Bolivia: Beni

Crepidospermum prancei Daly *Vasquez and Jaramillo* 6232 (MO) Peru: Prov. Maynas

Crepidospermum rhoifolium (Benth.) Triana and Planch. *Delgado 759* (NY) Venezuela: Amazonas

Dactyodes buettneri H. J. Lam *Caravello 5748* Guinea equatorial: Bata-Niefang

Dactyodes edulis (G. Don) H.J. Lam Wilks 2552 (NY) Gabon: Woleu-Ntem, Mission Otouma

Dactyodes klaineana (Pierre) H.J. Lam *Merello et al. 1615* (MO) Guinea equatorial: Bata-Niefang

Garuga floribunda Decne. *Chase 2088* (K) Sulawesi; cultivated at Kebun Raya, Bogor, Indonesia *AJ41479* (tps 16 intron)

Protium copal (Schlecht. et Chamisso) Engl. *Killeen et al. 3136* (MO) Mexico: Campeche

Protium guianense (Aubl.) Marchand *Miller and Hauck* 9391 (MO) Suriname: Sipaliwini


Protium pilosum (Cuartrec.) Daly *Gentry 49069* (MO) Brazil: Para


Santriria apiculata A.W. Benn. Lai s.n. (BH) Malaysia; cultivated at Singapore Botanical Garden

Santriria griffithii Engl. Lai s.n. (BH) Malaysia; cultivated at Singapore Botanical Garden

Tetragastris altissima (Aubl.) Swart *Polak 616* (MO) Guyana: Waraputo

Tetragastris mucronata (Rusby) Swart *Killeen et al. 3136* (MO) Bolivia: Beni


Trattinnickia glaziowii Swart. *Gentry and Revilla 69488* (tps 16 intron)

Trionna malaccensis Hook. *Chase 2091* (K) Maluku; cultivated at Kebun Raya, Bogor, Indonesia *AJ416487* (tps 16 intron)

Anacardiaceae (out-groups)

*Pistacia microcarpa* H.B.K. *Weeks 01-X-08-5* USA: Texas, cultivated at the University Texas at Austin

*Rhus trilobata* Nutt. *Weeks 01-X-08-4* USA: Texas, cultivated at the University Texas at Austin

*Gluta renghas* L. *Chase 2066* (K) Java; cultivated at Kebun Raya, Bogor, Indonesia *AJ416487* (tps 16 intron)

*Schinus molle* L. Anderson *J3602* (MICH) Argentina *AJ416478* (tps 16 intron)

*Semecarpus* sp. *Chase 2070* (K) Indonesia; cultivated at Kebun Raya *AJ416482* (tps 16 intron).

All vouchers are deposited in TEX unless otherwise noted. Sequences downloaded from GenBank are noted.

Table 2

Taxon information including voucher information and locality.

2.2. Tissue collection and DNA extraction

Provenance of leaf material for DNA extraction is divided between fresh material from greenhouse-grown...
plains, silica-dried leaf tissue prepared in the field, and
dried herbarium specimens (MO, NY, TEX; with per-
mission). Whole genomic DNA was extracted from fresh
or dried leaf material following the 2× CTAB protocol
of Doyle and Doyle (1987) with 1% (w/v) polyvinylprop-
ylene added to the extraction buffer to reduce polysac-
charide compounds. All Bursera DNA extracts required
further purification due to secondary compounds and
were cleaned using cesium gradient centrifugation
(Palmer, 1986) at 20°C at 60 K rpm for 10 h.

2.3. PCR amplification and sequencing

ETS was amplified using the Bur-ETS1F primer (5’-
TTC GGT ATC CTG TGT TGC TTA C-3’) and a previ-
ously published 18S primer (Baldwin and Markos,
1998). The region encompasses approximately 330 bp
of the 3’ end of the ETS within the Burseraceae; the anneal-
ing site of the Bur-ETS1F primer within Arnica mollis
(GenBank Accession No. AF06900) is 152 bp upstream
from the 18S gene. PCR volumes of 25μl included: 10–
100 ng of template DNA, 2× PCR buffer (Epícentre),
1 mM MgCl2, 400 μM each dNTP, 0.4 mM of each
primer, and 1 U of Taq polymerase. This region was
amplified using an initial denaturation of 95°C (5 min;
after which Taq was added to each reaction), 35 cycles of
denaturation at 94°C (3 min), 56°C (1 min), 72°C (1 min 20 s + 3 s/cycle), and a final extension period of 72°C
(7 min). PCR products of ETS from each taxon were
cloned to test whether multiple copies of ETS were pres-
ent and whether they were orthologous. PCR products
were cloned using the TOPO-TA Cloning kit (Invitro-
gen) using one-third the recommended reaction volumes.
ETS was amplified directly from positively trans-
formed colonies using the same PCR chemistry. One to
three cloned ETS copies were sequenced bi-directionally
for each taxon. The chloroplast rps 16 intron was ampli-
fied using the primer pair rpsF and rpsR2 (Oxelman
et al., 1997). PCR chemistry and temperature profiles of
the PCR used to amplify rps 16 were identical to those
for the ETS region.

All PCR products were verified prior to sequencing
using agarose gel electrophoresis and were cleaned using
Qiaquick columns (Qiagen) or Centri-Sep columns
(Princeton Separation) packed with G-50 Sephadex.
Each region was prepared for bidirectional sequencing
following a cycle sequencing protocol that included the
original amplification primers, Big Dye (Perkin-Elmer)
fluorescent dye-terminator reagent mix and 20-40 ng of
template DNA. The cycle sequencing PCR protocol
used a temperature ramping rate of 1°C/s and included
an initial denaturation of 96°C (1 min) followed by 25
cycles of 96°C (10 s), 50°C (5 s), and 60°C (4 min) with a
final extension period of 72°C (7 min). Samples were
cleaned with Centri-Sep columns and sequenced either
at the Institute of Cellular and Molecular Biology DNA
Core Facility, The University of Texas at Austin using
an ABI Prism 3700 Automated Sequencer (Perkin-
Elmer) or by the first author using an MJ Research Bas-
estation automated sequencer.

2.4. Sequence alignment

Raw sequence data were trimmed in EditView (Per-
kin-Elmer) and the complementary bi-directional
sequence strands were assembled and edited in Sequen-
cher v. 3.0–4.0 (Gene Codes). The sequences were aligned
automatically in Clustal X (Thompson et al., 1997),
and then adjusted by hand in SeqApp (Gilbert, 1992)
and MacClade v. 4.0 (Maddison and Maddison, 2000).

2.5. Parsimony analysis

Initial parsimony analyses of ETS data showed that
all clones coalesced by species and were orthologous, so
a single consensus sequence of ETS for each taxon was
constructed from the clones to combine and compare it
with the rps 16 intron dataset. All subsequent analyses of
ETS involve these “consensus” sequences. Aligned data-
sets of ETS and rps 16 were tested for contrasting phylo-
getic signal using the incongruence length difference
test (Farris et al., 1994) as implemented by PAUP* v. 4.0
beta v. 10 (Swofford, 2002). Parsimony analysis was con-
ducted in PAUP* for individual and combined datasets
using a heuristic search including 1000 random addition
replicates, tree-bisection reconnection branch swapping
with multrees in effect, ACCTRAN character optimiza-
tion and gaps treated as missing data. Non-parametric
bootstrap tests of the data used 1000 pseudoreplicates
within PAUP*. Decay indices were determined using
AutoDecay v. 4.0 (Eriksson, 1998).

2.6. Maximum likelihood

The best fitting model of sequence evolution was
determined using the maximum likelihood ratio test
(Goldman, 1993) as implemented by ModelTest v. 3.06
(Posada and Crandall, 1998) and this model was incor-
porated within PAUP* during heuristic searches using
the maximum likelihood criterion. Heuristic searches
used one random addition starting tree and tree bisection-
reconnection branch swapping with multrees in effect.

2.7. Bayesian analysis

Bayesian inference of the combined datasets was con-
ducted using the Metropolis Coupled Markov Chain
Monte Carlo simulation program, MrBayes v. 3.0b4
(Huelsenbeck and Ronquist, 2001). The combined data-
set was partitioned by gene region to incorporate the
best-fitting model of sequence evolution for each region.
Based on results from ModelTest, the best fitting models for either datasets could not be accommodated by MrBayes (MrBayes can incorporate only six models differing by the number of rate change categories and type of base frequencies) and new models were selected by rerunning likelihood ratio tests using only the available models. Four chains were run at the default level of heating (0.2) and additionally, the proposal parameters for updating the state of the chains were adjusted to ensure 20–60% acceptance for each of the estimated parameters (with proposal rates \( \geq 1 \)) and time-efficient convergence of all chains. The point of stationarity was determined by examining the posterior probability distribution for each estimated parameter among replicate analyses. Two million generations were analyzed and 20,000 trees were saved (one tree per 100 generations). All trees saved during the first four million generations (10,000) that contained trees from the burn-in period (1000) as well as trees at stationarity (9000) were discarded. Majority rule consensus trees were constructed from the remaining 10,000 trees at stationarity.

2.8. Parametric bootstrap tests of hypothetical phylogenetic relationships

We tested whether the two other alternative, unobserved relationships between the major in-group clades, ((Canarieae plus Boswelliinae, Proteiaceae), Burserinae) and ((Canarieae plus Boswelliinae, Burserinae), Proteiaceae), were significantly worse than the most parsimonious solution, ((Proteiaceae, Burserinae), Canarieae plus Boswelliinae), and whether both subgenera of Bursera could be sister to Commiphora (B. subg. Bursera, B. subg. Elaphrium), and Commiphora given that our data support another hypothesis, ((B. subg. Bursera, Commiphora), and B. subg. Elaphrium). We tested these hypotheses using the parametric bootstrap approach as described by Goldman et al. (2000). We searched our original dataset using a constraint tree representing our hypothesized phylogenetic outcome, ranked the resultant longer trees based on their log likelihood scores under the best fitting model of evolution, and then simulated 1000 datasets using Seq-Gen v. 1.2.5 (Rambaut and Grassly, 1997) based on the most likely topology corresponding to our hypothesized phylogenetic outcome. We analyzed each simulated dataset to find the most parsimonious tree, then constrained the analysis to find the most parsimonious trees corresponding to our hypothesized phylogenetic outcome, and then determined the tree length difference between the two results. The test statistic is the tree length difference between the original tree and most parsimonious tree(s) corresponding to the unobserved phylogenetic outcome. To reject the null hypothesis (the unobserved phylogenetic outcome), the test statistic must greater than or equal to 95% of the observed distribution of tree length differences.

2.9. Dating of evolutionary divergence events

Based on results from the maximum likelihood ratio test that indicated our combined sequence data did not evolve in a clock-like manner, we estimated divergence times of all nodes by applying fossil calibration to our maximum likelihood tree using r8s version 1.50 (Sanderson, 2003), which incorporates algorithms to smooth uneven rate variation across lineages. We pruned the Anacardiaceae out-groups from the maximum likelihood phylogeny to eliminate the arbitrary zero-length root branches generated by PAUP* during the rooting process as recommended by the r8s manual (Sanderson, August 2002), and we collapsed all zero length branches within the in-group. This maximum likelihood phylogeny was based on the original dataset that incorporated 3.5% missing data due to the three species (Trattinickia cf. burserifolia, G. floribunda, and T. malaccensis) for which we had only one of the two markers.

To choose time constraints for particular nodes based on fossils, we followed the selection parameters outlined by Doyle and Donoghue (1993) with one exception. They recommend that fossils should be assigned to lineages if the fossil contains an apomorphy of the entire clad and a synapomorphy of a more derived sub-clade. We chose five fossil dates to apply to the most recent common ancestor (MRCA) of the following clades: *Canarium* (23 Ma), Proteiaceae (50 Ma), *Bursera* subg. *Elaphrium* (50 Ma), *Bursera* subg. *Bursera* (35 Ma), and Burseraceae subtribe Burserinae (50 Ma). The *Canarium* fossil is applied to calibrate the genus rather than the tribe Canarieae. The reason for this less conservative approach is based on the fact that if this relatively young fossil were used to constrain an entire tribe, it would force one-third of the family to evolve later than the Oligocene, which seems unrealistic when compared to other fossil evidence for the family. Although *Bursera* subg. *Elaphrium* cannot be the same age as the Burserinae (50 Ma), we chose to include all fossil data to observe the interaction of node depth and branch lengths on the age estimation of other nodes within the phylogeny. Fixing the node of Burseraceae subtribe Burserinae at 50 Ma would be an incorrect assumption when derived lineages are indicated to be at least as ancient.

Although r8s allows the user to constrain MRCA nodes to minimum and maximum ages, it usually requires that the user fix one node at an absolute, precise age. We tested each of the five fossil calibration points individually as absolute ages rather than minimal constraints (Table 3). Based on the range of estimated ages from the five experiments across the topology, we then chose one fossil calibration point whose effects on the estimation of all node ages was the most conservative. In addition to this one fixed node calibration point, we assigned minimum ages to the remaining four fossil-node calibration points for our final analysis. These were
The effects of each experiment on the age estimates for other nodes are listed in rows.

### 3. Results

The aligned matrices of ETS and rps 16 sequence data totaled 429 bp (mean G + C content = 60.8%) and 985 bp (mean G + C content = 33.3%), respectively. Alignment was unambiguous for most in-group taxa; however, in some areas *Beiselia mexicana* proved more challenging to align than the Anacardiaceae due to sequence divergence. Partition homogeneity tests of the combined dataset yielded a tree with a score of 9277.16224 and the GTR + I + F model was indicated (TVM, also called TIM; Rodriguez et al., 1990) that incorporated one transition type and four transversion types plus Γ. When ETS and the rps 16 intron were analyzed together, the model GTR + I + Γ best fit the data. Maximum likelihood analysis of the combined dataset yielded a tree with a score of −ln L = 9277.16224 and −ln L = 9417.90930, when a molecular clock was enforced. We rejected clock-like sequence evolution at the < 0.01 level (df = 53, observed value = 140.75, \( \chi^2 \) critical value = 79.84). The maximum likelihood tree (Fig. 3) differed from the parsimony strict consensus tree by having *Daecryodes* sister to the clade containing *Canarium*, *Sanitaria*, and *Trattinnickia* and by having several different species relationships within *Commiphora*.

### 3.2. Maximum likelihood analyses

Maximum likelihood ratio tests indicated that ETS sequence evolution was best described by a submodel of the general time reversible model (GTR; Rodriguez et al., 1990) that incorporated 1 transversion rate and 2 transition rates (TrN; Tamura and Nei, 1993), rate heterogeneity (\( \Gamma \)), and a proportion of invariant sites (\( I \)). For the rps 16 intron data, another submodel of the GTR model was indicated (TVM, also called TIM; Rodriguez et al., 1990) that incorporated one transition type and four transversion types plus Γ. When ETS and the rps 16 intron were analyzed together, the model GTR + I + Γ best fit the data. Maximum likelihood analysis of the combined dataset yielded a tree with a score of −ln L = 9277.16224 and −ln L = 9417.90930, when a molecular clock was enforced. We rejected clock-like sequence evolution at the < 0.01 level (df = 53, observed value = 140.75, \( \chi^2 \) critical value = 79.84). The maximum likelihood tree (Fig. 3) differed from the parsimony strict consensus tree by having *Daecryodes* sister to the clade containing *Canarium*, *Sanitaria*, and *Trattinnickia* and by having several different species relationships within *Commiphora*.

### 3.3. Bayesian inference

When models of sequence evolution compatible with MrBayes were tested on the ETS and rps 16 datasets, the GTR + I + Γ and the GTR + Γ best fit the datasets, respectively. Only the Dirichlet parameter for changing the state frequencies needed to be increased from 300 to 900 to ensure 20–60% acceptance rates for proposals on all the estimated parameters. Comparison of the means and standard deviations for the estimated parameters in the original and replicate Bayesian analyses confirmed that MrBayes was consistent in the analysis of the data and that stationarity was reached within the first 100,000 of the 2 million generations analyzed. The 50% majority rule consensus tree from the 10,000 trees saved during
the last 1 million generations at stationarity is shown (Fig. 4). In general, the Bayesian tree differed from the parsimony and maximum likelihood topology by having more unresolved nodes throughout the phylogeny. Notably, the relationship of the major basal clades is unresolved as is the relationship between the subgenera of *Bursera* and *Commiphora*, between the genera of the Protieae, and between genera of the Canarieae.
### 3.4. Parametric bootstrap tests

The test that constrained species of *Bursera* to monophyly rejected this alternative hypothesis (test statistic $D_3$, $p < 0.001$). Alternative resolutions of the basal clades within the family could not be rejected in parametric bootstrap tests for ((Canarieae plus Boswelliinae, Protieae), Burserinae) (test statistic $D_2$, $p = 0.165$) and for ((Canarieae plus Boswelliinae, Burserinae), Protieae) (test statistic $D_2$, $p = 0.232$).

### 3.5. Fossil calibration of maximum likelihood topology

Cross-validation of smoothing rates within r8s indicated that a rate of 1 returned the most predictive estimates of branch lengths across the tree. Age profiles for

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*Fig. 4. The 50% majority rule consensus tree from Bayesian inference based on the last 10,000 trees saved during stationarity. Posterior probability values for all clades $\geq 50\%$ are listed above branches.*
every node in millions of years are listed on the maximum likelihood tree (Fig. 3). The mean age estimates with standard deviations from the parametric bootstrap procedure are shown above the branches. The five absolute and minimum age constraints are noted by closed and open circles, respectively. The most parsimonious reconstruction of geographic distributions for every lineage is shown in Fig. 5.

4. Discussion

4.1. Phylogenetic relationships within the Burseraceae

All phylogenetic analyses indicate that the Burseraceae is monophyletic group and that *Beiselia mexicana*, a monotypic genus from south western Mexico, is the basal most taxon with 95–100% bootstrap support (BS).
and Bayesian posterior probability (BPP), respectively. The relationship of the next three, most basal clades comprising the Proteae, Burserae subtribe Burserinae, and the clade combining a paraphyletic grade of the remaining genera of the Boswelliinae and a monophyletic Canarieae is not strongly supported. The most parsimonious relationship, ((Proteae, Burserinae), Canarieae plus Boswelliinae), is weakly supported (69% BS), and the maximum likelihood and Bayesian analyses find no support for this or any other nested relationship. Additionally, parametric bootstrap tests cannot reject alternative relationships among these basal clades.

While our results do not reveal a robust hypothesis of relationships among the major basal clades within the family, the observed sister relationship of the Proteae and Burserae subtribe Burserinae is tenable from a morphological standpoint. Harley and Daly (1995) first outlined a close relationship between these two groups but did not consider the possibility that the Burserae could represent an evolutionarily divergent, paraphyletic assemblage, although they did recognize the Boswelliinae as morphologically distinct from the Burserinae. They argued that the Proteae and the Burserae, as a whole, could not be maintained as separate tribes because they lacked distinctive suites of morphological characters. These two tribes both have (3) 4–5 merous flowers, fleshy fruits, pseudarils, columnellate axes separating the pyrenes, and unilocular (not compound) pyrenes. The paraphyly of the Burserae clarifies some of these “uniting” characters. Sisters Proteae and Burserinae share the presence of fleshy fruit with pseudarils and unwinged pyrenes, whereas the Boswelliinae have dry, winged pyrenes and no pseudaril. The columnellate axis separating the pyrenes, present in both the Proteae and Boswelliinae but not the Burserinae, is an apparent plesiomorphic character because the most basal member of the family, Beisella, also shares this feature.

Burserae subtribe Burserinae is a well-supported monophyletic group with 95% BS and 100% BPP. Historically, the close evolutionary relationship between the component genera of the subtribe, Commiphora and Bursera, has never been disputed. A taxonomist of Bursera who visited tropical East Africa, A.A. Bullock, once remarked that Commiphora would be considered Bursera if it grew in Mexico (Gillett, 1980). Commiphora is a genus of ca. 190 species native to Africa, the Middle East, Madagascar, and India; two species native to South America, one of them is undescribed. Bursera is an entirely New World genus. Species of both genera form prominent components of the woody vegetation in dry deciduous forest and desert regions across their ranges.

The two genera are maintained as separate taxa on the basis of only one morphological character: whether the sepals expose the petals in bud (Bursera) or obscure them (Commiphora). Bursera (2n = 24) and Commiphora (2n = 26) have different chromosome numbers (Bawa, 1973; Gillett, 1980); 3 and 4 species counted, respectively. Palynological investigations have found that both Bursera and Commiphora species can have “cross-over characters” (Harley and Clarkson, 1999). Pollen from 5 of the 26 Commiphora species examined possess the lobed triangular polar outline typical for Bursera and, conversely, two Bursera species (the only two presented) have the spinulose exine that is characteristic of the majority of Commiphora species.

In our phylogeny, Bursera is either paraphyletic and divided into two well supported monophyletic groups corresponding to its subgenera, B. subg. Bursera (83% BS, 100% BPP) and B. subg. Elaphrium (98% BS, 100% BPP) or its subgenera are reduced to a polytomy with the clade of Commiphora, which has 96% BS and 100% BPP. In parsimony analysis, B. subg. Elaphrium is the basal most clade of the Burserinae and subtends the sister pair of B. subg. Bursera and Commiphora with 69% BS. Parametric bootstrap tests reject the monophyly of Bursera. In the maximum likelihood topology, the paraphyly of Bursera is supported by a zero-length branch, whereas Bayesian inference reduces these three clades to a polytomy.

The paraphyly of Bursera is not unexpected given the artificial boundaries between this genus and Commiphora. Rzedowski and Kruse (1979) proposed two possible evolutionary relationships between the subgenera of Bursera and Commiphora. The first scenario suggested that B. subg. Bursera gave rise to B. subg. Elaphrium. The second scenario implicated a diphyletic origin of B. subg. Bursera and B. subg. Elaphrium, with B. subg. Elaphrium giving rise to Commiphora. Although the nested relationships of the subgenera we currently observe are reversed from Rzedowski’s predictions (he was swayed by the fact B. subg. Elaphrium and Commiphora share 4-merous flowers and bicarpellate ovaries), they indicate that morphological distinctions between the subgenera of Bursera are great enough to hypothesize separate evolutionary histories for them. For instance, B. subg. Elaphrium is distinguished from B. subg. Bursera by having (mostly) non-exfoliating bark, cataphylls, 4- to 5-merous flowers, and a bicarpellate ovary, whereas B. subg. Bursera has exfoliating bark, no cataphylls, 3-, 4-, and 5-merous flowers, and a tricarpellate ovary. Based on our phylogeny, B. subg. Bursera and Commiphora share exfoliating bark, the lack of cataphylls, and pseudarils that completely cover the pyrene (always in Bursera but only in some species of Commiphora). Interestingly, the basal most member of B. subg. Elaphrium in our analyses, B. tecomaeca, was transferred to Commiphora by Rzedowski and Palacios-Chávez (1985) on the basis of what we can now interpret as the presence of plesiomorphic pollen characters of Bursera and Commiphora. Previously published molecular phylogenetic studies of Bursera using nrDNA sequence data (Becerra, 1997; Becerra and Venable, 1999; Becerra, 2003) that suggest that Bursera is a strongly supported monophyletic group sampled Commiphora.
sparsely (3–6 spp.) and, consequently, do not capture the range of genetic variation within *Commiphora* as compared to that sampled for *Bursera*. The only published family phylogeny (Clarkson et al., 2002) sampled only one species each of *Bursera* and *Commiphora*.

The divergent evolutionary relationships of the Burserinae and the Boswelliinae is understandable in light of their many morphological differences, yet the well supported placement of the Boswelliinae as a paraphyletic clade basal to the monophyletic Canarieae necessitates novel hypotheses about the morphological evolution of the family. This is a surprising result that mirrors the findings of Clarkson et al. (2002). *Garuga* and the Canarieae share indehiscent fruits, and all taxa within the Canarieae plus Boswelliinae clade lack pseudarils. Both *Boswellia* and *Triomma* have dry schizocarpic fruits. *Garuga* and *Boswellia* have similarly shaped prolate-spheroidal pollen, a finely perforate tectum, and pronounced endexine thickenings in the pollen wall (Harley and Daly, 1995).

The Proteae comprise a well supported monophyletic group (100% BS, 100% BPP). *Protium* is paraphyletic with Old World *Protium madagascariense* sister to the New World *Crepidospermum* clade. This species, unlike New World *Protium*, has a red (vs. white) pseudaril and a unique pollen type. Harley and Daly (1995) have identified nine natural but non-exhaustive groupings of *Protium*. Our sampling includes representatives of only one New World group, the *P. trifoliolatum*-group, in addition to our sampling of the Old World group that contains *P. madagascariense*, *Protium* section *Marignia*. While our data suggest that Old and New World *Protium* may constitute separate lineages, ongoing phylogenetic studies of *Protium* (Fine et al., 2003) will provide additional tests of this finding. The placement of *Tetragastris* sister to the rest of *Protium* corresponds to existing hypotheses about its origin, which consider it a segregate genus and evolutionary “offshoot” of *Protium*.

4.2. Historical biogeography of the Burseraceae

Our dating experiments using fossil calibration of the molecular phylogeny suggest that the Burseraceae diverged from the Anacardiaceae 60 ± 1.9 Ma. This Paleocene age is consistent with the hypothetical minimum age of the Sapindales based on fossil evidence (65 Ma; Knobloch and Mai, 1986) and fossil calibrations of angiosperm phylogeny (80–84 Ma; Wikström et al., 2001). This age also corresponds with the dates of first appearance for numerous other angiosperms families in the fossil record (ca. 60–80 Ma; Magallón and Sanderson, 2001). Two generalizations can be made regarding the early diversification of the Burseraceae. The earliest divergence of *Beiselia* from the rest of the family and the next most basal bifurcation dates to the Paleocene and were located in North America, Mexico, or the Caribbean (Boxes A and B, Figs. 3 and 5). These ages postdate all major vicariant episodes of the Gondwanan supercontinent with the exception of the division of the southern tip of South American from Antarctica (35 Ma) and the collision of India with Asia (43 Ma). For the purposes of further discussion, the biogeographical pattern of each major clade will be discussed individually. A map of the Early Eocene continents and their relative positions helps illustrate the range expansion of the Burseraceae (Fig. 6).

The Early Eocene age of the Burserinae and its broadly North American origin (Box C, Figs. 3 and 5) implicates at least one migration event to the Old World to explain the African, Madagascar, and Indian distributions of *Commiphora* species. Migration from western

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Fig. 6. Early Tertiary (53 Ma) map of the world, based on Smith et al. (1994) with permission. Arrows represent routes of range expansion for Burseraceae following its North American origin and basal diversification. BLB = area corresponding to Boreotropical Land Bridge during the Early to Middle Eocene. Stars represent localities of Burseraceae fossils.
Laurasia (North America) to eastern Laurasia (Europe and Asia) may have been facilitated by the boreotropical land bridge (Tiffney, 1985; Tiffney and Manchester, 2001), which spanned the North Atlantic during the Early to Middle Eocene. Global temperatures during the Eocene were highest during this time period and tropical vegetation is known to have occurred in this land corridor (Wolfe, 1978; Zachos et al., 2001). Cooler temperatures during the Middle Eocene extinguished frost-intolerant taxa in this region and the physical land connections disappeared sometime afterward. This vicariant scenario for Commiphora is indirectly supported by the localities of Burserinae fossils from the Early Eocene at high northern latitudes in Utah, Colorado, and England. The extant distributions of Bursera subgenera within the New World also indirectly support this northern route. Bursera subgenus Bursera, which is sister to Commiphora (Box D, Figs. 3 and 5), includes extant species that have a more northerly distribution than those of B. subg. Elaphrium. All of the Bursera species endemic to the Greater and Lesser Antilles (ca. 15 spp.) and all species distributed in United States (2 spp.) are in B. subg. Bursera, whereas the basal most member of Elaphrium is distributed in south west Mexico (B. tecomaca). Within Mexico, those species distributed in more northerly latitudes are predominantly in B. subg. Bursera, whereas the few Bursera species in Central and South America (<10 spp.) are predominantly members of B. subg. Elaphrium.

Following the migration of ancestral Commiphora into the Old World, Commiphora appears to have dispersed and radiated within continental Africa during the Middle Eocene (44 ± 7.6 Ma; Box E, Figs. 3 and 5). The Oligocene origin of the Malagasy species, C. franciscana, is consistent with the age of the hypothesized Mozambique Channel land bridge connecting Africa to Madagascar (McCall, 1997). The Oligocene origin of the South American species, C. leptophrallaeos, from an African ancestor may be due to long distance dispersal or migration through the boreotropical land bridge (Box F, Figs. 3 and 5). However, since its date of divergence is near the closure of the boreotropical corridor (Tiffney, 1985; Tiffney and Manchester, 2001; Zachos et al., 2001) and its South American distribution would require a very long overland migration that left no other Commiphora species at higher latitudes, trans-Atlantic long distance dispersal seems more probable (Renner, 2004). The spread of Commiphora to India appears to have occurred in relatively recent geologic time (5.0 ± 4.4 Ma; Box G, Figs. 3 and 5), perhaps due to a northeasterly range expansion of Commiphora coincident with the establishment of arid habitat in East Africa (deMenocal, 1995; Potts and Behrensmeier, 1992).

The Canarieae plus Boswelliinae clade may have evolved from a southeast Asian ancestor (Box H, Figs. 3 and 5). However, the basal species of this clade (Boswellia, Garuga, Triomma) are African, Indian or south east Asian in distribution, whereas the majority of extant species in the Canarieae span eastern Asia including the Malaysian Archipelago to Melanesia, the Philippines, and to Northern Queensland, Australia. The progressive eastward expansion of this clade combined with its Early Eocene age suggests the Canarieae plus Boswelliinae clade originated closer to western Laurasia, and then spread eastward over time. Fossil evidence of Canarium from the Czech Republic during the Late Oligocene (23 Ma) indicates that Canarium once had a distribution closer to the trans-Atlantic boreotropical corridor than its current distribution pattern would suggest. Migration of taxa to southeast Asia during the Eocene may have followed the northern margin of the Tethys Sea, the southern and most tropical region of Laurasia during the Eocene (Tiffney, 1985). Alternatively, global cooling during the Middle and Late Tertiary may have forced the range of the Canarieae and Boswelliinae at higher latitudes towards more tropical, equatorial regions in Africa and Asia. Unfortunately, our sampling is lacking some representative species of the widespread genera, such as African Santiria, South American and southeast Asian Dacryodes, and African Canarium. Dacryodes within South America consists of two morphologically distinct lineages and may represent evolutionarily divergent clades when compared to Old World species of Dacryodes. If these species were sampled, similar patterns of species-area relationships might be discovered among the different genera, such as southeast Asian clades basal to African and South American sister groups. Particularly intriguing is the sister group relationship of South American Trattinnickia and southeast Asian Santiria (Box I, Figs. 3 and 5). Without further sampling Santiria, the disjunction of these two genera may best be explained by migration of ancestral Trattinnickia from southeast Asia across the Beringia into North America during the Late Oligocene (26 ± 8.5 Ma), although at this time temperatures may have been too cool to support tropical vegetation (Tiffney and Manchester, 2001). Long distance dispersal of Trattinnickia ancestors from either Africa or southeast Asia to South America is also a possibility.

Our most parsimonious geographic reconstruction indicates that the Proteae are derived from a South American ancestor (Box J, Figs. 3 and 5). The vast majority of Proteae species are South American in distribution and only Proteum has a handful of Old World species (7–9 spp. of 147 spp. worldwide). Additionally, the Old World species of Proteum are considered morphological relicts (Harley and Daly, 1995) and our phylogeny suggests African and New World Proteum may constitute separate, distantly related genera. Despite this, the relative isolation of South America after its separation from Africa (105–119 Ma; McLoughlin, 2001) and the Antarctic Peninsula (30–35 Ma) poses a problem for interpreting the historical biogeography of geologically
old South American taxa. A South American origin of the Protieae is highly unlikely due to the long isolation of this continent from the North America, the area of origin for the family. The distribution of Middle Eocene Protieae fossils in England also does not support a South American origin for the tribe (Chandler, 1961). The English fossils are distributed in a geographic location that during the Early to Middle Eocene was the midway point between eastern and western portions of Laurasia, which suggests ancestors of genera within the Protieae were located near the trans-Atlantic boreotropical land bridge during the time of the Eocene thermal maxima. The basal Protieae lineages may have originated in North America, with the lineage leading to Old World Protium (Box K, Figs. 3 and 5) migrating east to Africa and Asia along the Tethys seaway during the Late Eocene (37 ± 10 Ma). Within North America, more northern populations may have become extinct during the cooling of the Late Tertiary and Quaternary (Boxes L and M; Figs. 3 and 5). The tribe could have then spread to South America by stepping stone dispersal via the islands of the Caribbean (MacPhee and Iturralde-Vinent, 1995) during the Eocene or more recently through the Isthmus of Panama. Few species of Protium are distributed in the Caribbean, which does not lend support to the Caribbean scenario of MacPhee and Iturralde-Vinent. Long-distance dispersal may have introduced the Protieae to South America prior to the establishment of land connections between the American continents.

4.3. Vicariant Laurasian biogeographies of other predominantly tropical angiosperm families

The role of the trans-Atlantic boreotropical land bridge is key to interpreting the migration of ancestral Burseraceae lineages from North America into the Old World during the Tertiary; the other land connection that existed during this time, Beringia, did not support tropical vegetation (Tiffney and Manchester, 2001). Similar biogeographic patterns involving Laurasian origins and boreotropical, trans-Atlantic exchanges have been hypothesized for other tropical angiosperm families using methods similar to those of this project (cf., Lauraceae; Chanderbali et al., 2001; Melastomataceae; Renner et al., 2001; Malpighiaceae, Davis et al., 2002a,b). Other families such as the Anacardiaceae, Chloranthaceae, Fabaceae, Illiciaceae, Malvaceae, Menispermaceae, Musaceae, Rutaceae, and Sterculiaceae have extant genera restricted to southeast Asia, Africa and/or South America and also have North American Tertiary fossils (Lavin and Luckow, 1993; Lavin et al., 2000; Manchester, 1999). It will be instructive to determine whether the historical biogeographies of these additional families corroborate the Laurasian biogeographic scenario shared by the Burseraceae and others.

4.4. Dispersal methods of Burseraceae fruits

Although Laurasian vicariance can account for the distribution patterns among the basal lineages in the Burseraceae, long distance dispersal needs to be invoked to explain much younger, geographically disjunct relationships among certain groups. With the exception of Beiselia, Boswellia, and Trionna, which have dry schizocarpic fruits adapted for wind-dispersal, genera of the Burseraceae have fruits that are adapted for endozoochoric dispersal, a mode that has been shown to be important in the establishment of isolated oceanic island floras (Carlquist, 1974). These fruits have fleshy mesocarp, pseudarils, and/or seeds high in fat and protein (24–73% fat, 2.7–25.9% protein; Marcone et al., 2002; Greenberg et al., 1995; Omoti and Okiy, 1987; Snow, 1962) as well as thick endocarps that protect the seed from being crushed. Fruits of the Burseraceae are known to be consumed by hornbills (Buceros bicornis; Kannan, 1994; Ceratogyna atrata, C. cylindricus; Poulsen et al., 2002; Penelopides panini; Hamman and Curio, 1999), oilbirds (Steatornis caripensis; Snow, 1962), fruit pigeons (Hamman and Curio, 1999; Webb et al., 1999) as well as multiple species of warblers, vireos, orioles, flycatchers, tanagers, woodpeckers, and loeries (Clark et al., 2001; Greenberg et al., 1995; Trainer and Will, 1984). Other vertebrate consumers of Burseraceae fruit include primates (Cercopithecus spp., Lophocebus albigena; Clark et al., 2001; Poulsen et al., 2002), lemurs (Varecia variegata subsp. variegata; Goodman and Sterling, 1996), and sun bears (Helarcos malayanus; McConkey and Galetti, 1999). All of these animal species either discard the endocarp immediately after eating the mesocarp or pseudaril and/or void some of the endocarps intact later. None of the fruits has morphologies associated with epizoochoric dispersal such as sticky mucilage or hooks. To date, no known studies have examined the water dispersal capability of Burseraceae fruits.

5. Conclusions

Basal phylogenetic resolutions for the Burseraceae mirror those found by Clarkson et al. (2002) with several important caveats for higher-level relationships. Like Clarkson et al., we find that the most basal lineage of the Burseraceae is Beiselia mexicana, a monotypic genus native to south west Mexico. We also do not find significant support for basal relationships among the three major clades that comprise the rest of the family, the Canarieae plus Boswelliinae clade, the Protieae, and the Burserinae. However, with our expanded species and generic sampling, we find that Bursera is paraphyletic with Commiphora embedded within it, Old and New World Protium constitute separate, distantly related lineages, and that New World Trattinickia is most closely
related to southeast Asian Santiria. Calibration of the molecular phylogeny with available fossil evidence implicates a vicariant Laurasian origin for the family with secondary dispersal to southern Hemisphere continents rather than a vicariant Gondwanan origin. Future molecular phylogenetic studies of the Burseraceae must include those genera not available for this study as well as all continental representatives of the more widespread genera such as Dacryodes and Santiria.

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