Evolution, 56(1), 2002, pp. 42–57

PHYLOGENY OF *GAERTNERA* LAM. (RUBIACEAE) BASED ON MULTIPLE DNA MARKERS: EVIDENCE OF A RAPID RADIATION IN A WIDESPREAD, MORPHOLOGICALLY DIVERSE GENUS

SIMON T. MALCOMBER[1,2,3]
[1]*Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299*
[2]*Program of Evolutionary and Population Biology, Department of Biology, Washington University, St. Louis, Missouri 63130*

Abstract.—Phylogenetic relationships among 28 of the 68 species of the paleotropical genus *Gaertnera* (Rubiaceae) and two related genera were inferred from nucleotide sequence variation in four nuclear DNA (nDNA) markers: the internal transcribed spacers of nuclear rDNA (ITS), the large and small copies of phosphoenolpyruvate carboxylase (*PepC*-large and *PepC*-small), and triose phosphate isomerase (*Tpi*). Phylogenetic analysis of the combined nDNA dataset suggested that *Gaertnera* is monophyletic, but genetic variation among species was insufficient to reconstruct well-supported relationships within the genus. This was counter to expectations based on the very distinct morphologies and widespread distribution of the genus (West Africa to Sulawesi). Molecular clock analyses suggested variable dates of origin for *Gaertnera* depending upon the calibration method used. The most plausible calibration implies that *Gaertnera* migrated to Africa during the early Tertiary, possibly via a boreotropical land bridge and suggests that *Gaertnera* started to radiate 5.21 ± 0.14 million years ago. This implies that range expansion in the group has occurred via a number of long-distance dispersal events rather than vicariance. The molecular clock estimate in turn estimated an unusually rapid lineage diversification rate within the radiation of 0.717–0.832 species/million years, comparable to those estimated for radiations on oceanic islands. Although low interspecific competition levels may have contributed to the diversification of *Gaertnera* on Mauritius, the mechanisms driving the rapid radiation of the group in other parts of its range remain elusive.

Key words.—Biogeography, lineage diversification, Madagascar, Mauritius, molecular clock, Southeast Asia, Sri Lanka.

Received January 23, 2001. Accepted September 27, 2001.

Clades that are morphologically diverse and cover a large geographical area are generally expected to exhibit more genetic variation than clades whose members are morphologically similar and restricted in range. Geographically restricted exceptions to this generalization include the cichlids in the African Rift Lakes (Meyer et al. 1990); Darwin's finches in the Galapagos Islands (Lack 1947; Grant 1986); *Arygyranthemum* in Macronesia (Francisco-Ortega et al. 1997); and silverswords (Carr and Kyhos 1981; Baldwin and Sanderson 1998), honeycreepers (Freed et al. 1987), and fruit flies (Carson 1970) in Hawaii. The limited sequence variation recovered among species in these examples is considered indicative of a rapid radiation, often hypothesized to have been driven by low levels of competition in new habitats (Liem 1990). Geographically widespread, morphologically diverse clades that have radiated rapidly are reported only infrequently. The temperate herb *Aquilegia* (Ranunculaceae) is currently the best-documented example, with limited genetic variation in noncoding nrDNA (ITS) and cpDNA (*atpB-rbcL* spacer) despite diverse flowers and a distribution that encompasses the entire Northern Hemisphere (Hodges and Arnold 1994, 1995). Given the difficulties in applying traditional competition-based hypotheses of rapid lineage diversification in *Aquilegia*, Hodges and Arnold (1994, 1995) proposed instead that the diversification was driven by the evolution of nectar spurs, which were inferred to be key innovations. Documenting other geographically widespread, morphologically diverse clades that have radiated rapidly in other parts of the world will improve understanding of the tempo and mode of evolutionary change during adaptive radiations.

Gaertnera Lam. (Rubiaceae) is a genus of 68 species of small trees and shrubs distributed throughout the wet tropics of Western and Central Africa, Madagascar, Mascarenes (Mauritius and Réunion), Sri Lanka, and Southeast Asia (from central Thailand through Peninsular Malaysia to Sulawesi). All *Gaertnera* species are regional endemics. Madagascar has the highest species diversity (25 species) followed by Southeast Asia (16 species), Africa (12 species), Mascarenes (10 species), and Sri Lanka (five species; Malcomber 2000). The genus is morphologically diverse (see Fig. 1). Inflorescences are most commonly corymbiform (Fig. 1a), but can also be thyrsiform (Fig. 1c), capituliform (Fig. 1d), few-flowered (Fig. 1b), or reduced to a single flower (Fig. 1e). Interpetiolar stipules are either calyptrate (Fig. 1f); cylindrical (Figs. 1g, h); or funnel-shaped, with a combination of wings and/or ridges on the tube and around the petiole (Figs. 1g, h). Stipule apices can be truncate (Fig. 1g), lobed (Fig. 1f), or lobed with many setae (Fig. 1h). Calyx lobes are either absent, small and deltoid-linear, or expanded into large colored petaloid structures (calycophylls). Flowers are either white, pink, or red and range in size from 2 to 30 mm long. All *Gaertnera* species have either a dioecious or distylous breeding system (Malcomber 2000).

A sister relationship between *Gaertnera* and the South American genus *Pagamea* is supported by cpDNA analyses (*rbc*L: Bremer 1996; *rps*16: Andersson and Rova 1999) and numerous morphological characters, including sheathing stipules, secondarily superior ovaries, xylem with parenchyma bands, and compound pollen apertures with crescent-

[3] Present address: Department of Biology, University of Missouri–St. Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121; E-mail: malcombers@msx.umsl.edu.

TABLE 1. List of sequences analyzed. *Gaertnera* taxonomy based on Malcomber (2000). Herbarium voucher information is included within Genbank accession numbers. Different Genbank accession numbers represent different cloned sequences for the same individual. An asterisk identifies sequences used in combined analysis.

Taxon	ITS	*PepC*-L	*PepC*-S	*Tpi*	ITS/ *PepC*-L	ITS/ *PepC*-S	ITS/ *Tpi*	*PepC*-L/ *PepC*-S	*PepC*-L/ *Tpi*	*PepC*-S/ *Tpi*
G. acuminata Benth.	AF333816*	AF33384*	AY046338* AY046339		x	x		x		
G. aphanodioica S. T. Malcomber	AF333817*	AY046371* AY046372	AF333876*	AY046381* AY046382	x	x	x	x	x	x
G. belemutensis S. T. Malcomber	AF333818*	AF333849*	AF333877*	AF333907*	x	x	x	x	x	x
G. brevipedicellata S. T. Malcomber	AF333819*	AY046373 AY046374*	AY046344* AY046345	AY046383* AY046384	x	x	x	x	x	x
G. cooperi Hutch. et M. B. Moss	AF333820*	AF333851*	AF333879*		x	x		x		
G. cuneifolia Bojer	AF333821*	AY046375 AY046376*	AY046346 AY046347*	AY046385* AY046386	x	x	x	x	x	x
G. drakeana Aug. DC.	AF333822*	AF333853*	AY046348 AY046349*	AF333910*	x	x	x	x	x	x
G. edentata Bojer	AF333823*	AF333854*	AF333882*	AF333911*	x	x	x	x	x	x
G. fractiflexa Beusekom	AF333824*	AF333855*	AY046357* AY046358	AF333912*	x	x	x	x	x	x
G. globigera Beusekom	AF333825*	AF333856*	AY046333* AY046334	AF333913*	x	x	x	x	x	x
G. hispida Aug. DC.	AF333826*	AF333857*	AF333885*	AF333914*	x	x	x	x	x	x
G. inflexa Baill.	AF333827*	AF333858*	AY046335 AY046336*	AF333915*	x	x	x	x	x	x
G. junghuhniana Miq.	AY046325 AY046326*	AY046377* AY046378	AY046365* AY046366	AY046387* AY046388	x	x	x	x	x	x
G. longifolia Bojer	AF333829*	AF333860*	AY046337* AY046340 AY046363	AY046389 AY046390*	x	x	x	x	x	x
G. lowryi S. T. Malcomber	AF333830*	AY046379* AY046380	AY046341* AY046364	AF333918*	x	x	x	x	x	x
G. macrostipula Baker	AF333831*	AF333862*	AY046342 AY046343*		x	x		x		
G. madagascariensis (Hook. f.) S. T. Malcomber & A. P. Davis	AF333832*	AF333863*	AF333891*	AF333919*	x	x	x	x	x	x
G. oblanceolata King & Gamble	AF333833*	AF333864*	AF333892*		x	x		x		
G. paniculata Benth.	AY046327 AY046328 AY046329* AY046330	AF333865*	AF333893*		x	x		x		
G. pauciflora S. T. Malcomber	AF333835*	AF333866*	AF333894*	AF333920*	x	x	x	x	x	x
G. psychotrioides Baker	AF333836*	AF333867*	AY046362* AY046368	AF333921*	x	x	x	x	x	x
G. rosea Thw. ex Benth.	AF333837*		AF333896*	AY046391* AY046392		x	x			x
G. schatzii S. T. Malcomber	AF333838*	AF333868*	AF333897*		x	x		x		
G. schizocalyx Bremek.	AF333839*	AF333869*	AY046355* AY046367		x	x		x		
G. ternifolia Thw.	AY046331* AY046332	AF333870*	AY046360 AY046361*	AF333923*	x	x	x	x	x	x
G. vaginans (DC.) Merr.	AF333841*		AY046350* AY046351 AY046352	AY046393* AY046394		x	x			x
G. viminea Hook. f. ex C. B. Clarke	AF333842*	AF333871*	AY046353 AY046354*		x	x		x		
G. walkeri Wight	AF333843*	AF333872*	AY046356* AY046359	AF333925*	x	x	x	x	x	x
M. citrifolia L.	AF333844*	AF333873*	AF333903*		x	x		x		
M. royoc L.	AF333845*	AF333874*	AY046369 AY046370*		x	x		x		
P. guianensis Aubl.	AF333846*		AF333905*	AF333926*		x	x			x

shaped costae (Igersheim et al. 1994; Jansen et al. 1996). The *Gaertnera* clade is supported by a morphological synapomorphy of glabrous corolla lobes (Malcomber 2000).

In this paper I estimate a phylogeny for *Gaertnera* using four noncoding nDNA regions. I first assess the statistical congruence between different molecular datasets and then perform combined maximum-parsimony (MP) and maximum-likelihood (ML) phylogenetic analyses. Using the resulting molecular phylogenies, I infer the pattern of geographical and morphological evolution within the genus, test for clocklike evolution, and estimate the divergence time of the *Gaertnera* clade using several calibration methods.

Materials and Methods

Up to 28 *Gaertnera* species, encompassing the entire distribution and much of the morphological variation of the genus, were sampled (Table 1). *Pagamea guianensis* Aubl. was included to represent the sister genus of *Gaertnera*, and two *Morinda* species, *M. citrifolia* L. and *M. royoc* L., were chosen as outgroups based on results of family level molecular analyses within the Rubiaceae (*rbc*L: Bremer 1996; *rps*16: Andersson and Rova 1999). The four noncoding regions of the nuclear genome include the internal transcribed spacer (ITS), the fourth intron of the two gene copies of phosphoenolpyruvate carboxylase (*PepC*-large and *PepC*-small, named for the intron size), and the fourth intron of triose phosphate isomerase (*Tpi*). Although it would have been desirable to include a cpDNA marker, the most variable region of the chloroplast, the *trnL-F* spacer/intron region (Taberlet et al. 1991), did not show enough variation to be useful in *Gaertnera* (data not shown).

Laboratory Procedures

DNA extraction.—Leaf material was collected in the field and dried immediately in silica gel. DNA was isolated using 6×CTAB buffer and the CTAB miniprep protocol of Doyle and Doyle (1987). For extractions that would not amplify, total DNA extractions were further purified using an additional sodium acetate/ethanol precipitation or by using Nucleon phytopure resin (Amersham Pharmacia Biotech Inc., Piscataway, NJ). Negative controls were used in all polymerase chain reactions (PCRs).

Polymerase chain reaction amplification, cloning, and sequencing.—All gene fragments were amplified from genomic DNA using PCR. The 50-μl reactions contained 0.5 μl (2.5 units) of *Taq* polymerase (Promega Corp., Madison, WI), 5 μl of 10× reaction buffer, 5 μl of 25 mM $MgCl_2$, 0.5 μl dNTP (10 mM stock solution), 1 μl each of the primers (10 mM stock solution), 0.6–1.5% (by volume) dimethyl sulfoxide, and 4 μl of undiluted template DNA. In the case of ITS, the reactions were initially denatured for 4 min at 94°C, followed by 30–40 cycles of 30 sec at 94°C, 60 sec at 48°C, and 90 sec at 72°C, followed by 7 min at 72°C. In the case of *PepC* and *Tpi* a hot-start PCR was employed with the *Taq* being added after the initial 4 min at 94°C. Subsequent reaction conditions consisted of 40 cycles of 30 sec at 94°C, 60 sec at 55°C, and 90 sec at 72°C, followed by 7 min at 72°C.

Double-stranded PCR products were amplified for the entire ITS region, using primers ITSLEU1 (5′-GTCCA CTGAA CCTTA TCATT TAG-3′, designed by L. E. Urbatsch and provided by M. Nepokroeff) and ITS4 (5′-TCCTT CCGCT TATTG ATATG C-3′; White et al. 1990). PCR products were purified using either differential centrifugation with Qiaquick columns (Qiagen, Inc., Valencia, CA) or polyethylene glycol (PEG) precipitation (Sambrook et al. 1989). Cleaned PCR products were then either sequenced directly or were cloned using pGEM-T easy vector systems (Promega Corp.). When sequenced directly, forward- and reverse-strand sequences were generated using primers ITSLEU1, ITS2 (5′-GCTGC GTTCT TCATC GATGC-3′; White et al. 1990), ITS3B (5′-GCATC GATGA AGAAC GTAGC-3′; Baum et al. 1994), and ITS4. When cloned, plasmid DNA was cleaned using an alkaline lysis/PEG precipitation protocol (Sambrook et al. 1989) prior to sequencing using plasmid primers T7 and SP6, and internal primers ITS2 and ITS3B. ITS sequences are distributed as tandem arrays within the nuclear genome. Concerted evolution is known to act between paralogous copies, but multiple copies are possible (Wendel et al. 1995; Buckler et al. 1997). To check for intraspecific variation among ITS paralogs, two to six clones were sequenced for each species and DMSO was used in both the PCR and sequencing reactions, following the recommendations of Buckler et al. (1997).

Phosphoenolpyruvate carboxylase (*PepC*) is an enzyme involved in the photosynthetic pathway of C4 plants and in the production of Krebs cycle acids of both C3 and C4 plants. Double-stranded PCR products were amplified for the fourth intron of *PepC* using primers PEPCX4F (5′-ACTCC ACAGG ATGAG ATGAG-3′) and PEPCX5R (5′-GCAGC CATCA TTCTA GCCAA-3′), both designed and provided by J. Gaskin. Two distinct PCR products of approximately 450 bp and 900 bp were amplified for most taxa. PCR products were gel purified using Qiaex II gel extraction system (Qiagen Inc.) and then ligated into the pGEM-T easy vector, as for ITS. Two to six clones of each PCR band were sequenced in both directions using plasmid primers T7 and SP6 for the 450-bp product and primers T7, PEPCINTF (5′-YGGAC GTAGA CTTGT TT-3′), PEPCINTR (5′-TGCAG GGRCA ATACA GA-3′), and SP6 for the 900-bp product.

Triose phosphate isomerase (*Tpi*) is an enzyme involved in the conversion of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate during glycolysis. Double-stranded PCR products were amplified for the fourth intron of *Tpi* using primers TPIX4FN (5′-AAGGT CATTG CATGT GTTGG-3′) and TPIX6RN (5′-TTTA CCAGT TCCAA TAGCC A-3′) developed by Strand et al. (1997). PCR products were cleaned using PEG precipitation and then ligated into the pGEM-T easy vector. Two to four clones were sequenced in both directions using primers T7, TPI307F (5′-AGCCA GCTCA TTAGA TA-3′), TPIINTR (5′-CATCT CYAAG CCTRA GC-3′), and SP6.

Dideoxy sequencing was conducted using the BigDye dye terminator cycle sequencing protocol (from Applied Biosystems, Foster City, CA). Sequencing reactions were analyzed on an ABI 377 (Applied Biosystems) automated DNA sequencer. Sequences were submitted to Genbank (Table 1).

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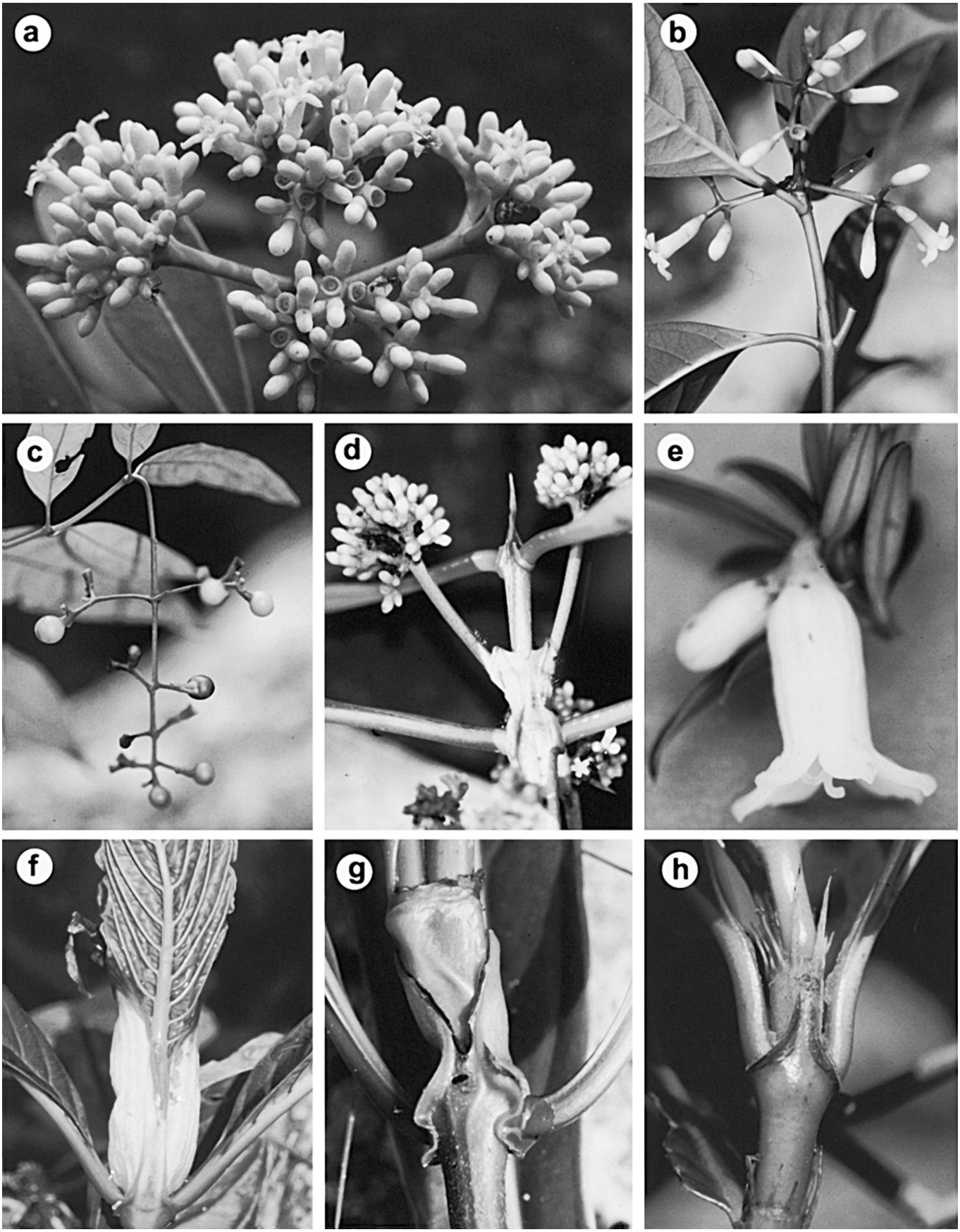
FIG. 1. Some of the morphological variation within *Gaertnera*. Inflorescence forms (a) corymbiform cyme, *G. aphanodioica* (Brunei Darussalam); (b) few-flowered cyme, *G. acuminata* (Malaysia); (c) thyrsiform cyme, *G. fractiflexa* (Malaysia); (d) capituliform cyme, *G. oblanceolata* (Malaysia); (e) cyme reduced to a single (or few) flowers, *G. ternifolia* (Sri Lanka). Stipule forms: (f) calyptrate, *G. obovata* var. *sphaerocarpa* (Madagascar); (g) cylindrical stipules with longitudinal ridges, *G. globigera* (Malaysia); (h) cylindrical stipules with lobes and setae at the apex, *G. hirtiflora* (Mauritius).


a
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Phylogenetic Analyses

Alignment.—Chromatograms and contiguous alignments were edited using either Sequencher version 3.1 (Gene Codes Corp., Ann Arbor, MI) or Seqman version 4.00 (DNASTAR Inc., Madison, WI). Preliminary alignment of the individual data sets was performed using Clustal W (Thompson et al. 1994), and subsequently manipulated using Se-Al version 1.d1 (Rambaut 1996, available via ftp://evolve.zo.ox.ac.uk/packages/Se-Al/Se-Al10a1.hqx).

Preliminary parsimony analysis.—Unless specified, phylogenetic analyses were conducted using PAUP* 4 (Swofford 2000). Equal weight parsimony analyses were performed with heuristic search options as follows: tree-bisection-reconnection (TBR) branch swapping, 1000 random addition sequence replicates, gaps treated as missing data, and MAXTREES set to 10,000. A converse-constraint heuristic search, with the strict consensus from the first analysis loaded as a constraint, was then performed to check for additional equally or more parsimonious trees. The shortest trees for each dataset recovered from the different searches were pooled and used to generate a strict consensus tree. This converse-constraint search strategy was repeated until no additional most-parsimonious trees were found. Bootstrap support (Felsenstein 1985) for each clade within the strict consensus tree was estimated using 300 replications of simple addition sequence, TBR searches with MAXTREES set to 100.

Partitions.—To test for conflict between the different nDNA datasets, I conducted an incongruence length difference (ILD) test (Farris et al. 1994) with all invariant characters removed (Cunningham 1997) and heuristic search options set as follows: simple addition sequence, TBR branch swapping, and MAXTREES set to 500. For each of the pairwise data partitions 500 random partitions were analyzed as recommended by Johnson and Soltis (1998).

To localize the sources of conflict in datasets identified by the ILD tests as incongruent ($P < 0.05$), relationships supported by one but rejected by the other dataset were coded as constraints. The length of the most-parsimonious trees satisfying a constraint was determined using a heuristic search, with 1000 random additions and TBR branch swapping. Differences between the constrained and unconstrained tree lengths were tested with Wilcoxon sign-rank tests (Templeton test: Templeton 1983; Larson 1994) and corrected for multiple tests with sequential Bonferroni analyses (Rice 1989).

Combined molecular analyses.—MP and ML analyses were performed on the 31 species combined molecular dataset using PAUP*. As in the preliminary parsimony analyses, the combined parsimony analyses consisted of an initial heuristic search with 1000 random addition sequences and TBR branch swapping, followed by a converse-constraint search with the strict consensus tree from the initial search loaded as constraint. Bootstrap support was estimated from 300 bootstrap replicates with simple sequence addition, TBR branch swapping, and MAXTREES set to 500. A randomly chosen MP tree was evaluated using PAUP* and Modeltest (Posada and Crandall 1998) to identify which model of molecular evolution is most appropriate for these data. Once identified, the model and parameters were fixed and used in subsequent ML analyses. The ML heuristic search strategy consisted of 10 random addition sequence replicates with TBR branch swapping to completion. Aligned matrices and trees were submitted to TREEBASE (http://www.herbaria.harvard.edu/treebase/).

ML and MP trees were loaded as a constraint and differences in length or likelihood score between the constrained and unconstrained trees were evaluated using the Templeton and Shimodaira-Hasegawa tests (Templeton 1983; Shimodaira and Hasegawa 1999; Goldman et al. 2000).

Morphology.—Twenty-four morphological characters were scored for each of the 31 terminal taxa from herbarium material at Missouri Botanical Garden (see Appendix 1). A set of most-parsimonious trees from the morphological dataset was generated using a heuristic search with 1000 random addition-sequences, TBR branch swapping, and MAXTREES set to 6000. To test for conflict between the morphological and combined molecular datasets, I performed an ILD test with identical settings to those described above for the individual molecular datasets (see *Partitions*).

To examine levels of homoplasy within individual morphological characters, each character was tested for the hypothesis that it had a CI = 1. A series of constraint trees was constructed where all taxa with a particular morphological character were constrained to form a partition. A Templeton test was then conducted to test whether the most parsimonious molecular tree satisfying this constraint was significantly longer than the unconstrained tree.

Molecular clock analyses.—To estimate the age of diversification within *Gaertnera* a temporal dimension was added to the phylogeny. Molecular clock estimations of branching time are controversial and should be treated with caution (Baum et al. 1998; Sanderson 1998), but when paleontological data are lacking, molecular clock estimates provide the only means of inferring lineage ages and diversification rates (Li and Graur 1991). Molecular clock analyses were conducted with the ITS dataset (with gaps and ambiguous sites removed) only because of the paucity of informative characters and/or missing data within the other datasets. Rate constancy in ITS was tested within the *Gaertnera, Gaertnera* + *Pagamea*, and *Gaertnera* + *Pagamea* + *Morinda* datasets within the context of the combined phylogeny using the likelihood-ratio test (Felsenstein 1988) as discussed by Sanderson (1998) using the HKY85 (Hasegawa et al. 1985) model of sequence evolution with gamma-distributed rates (HKY + G). The ML tree obtained under each of the different models of evolution in the absence of molecular clock was then reanalyzed in the presence of the molecular clock with trees rooted at the midpoint. Significance was determined by comparison with a χ^2 table using $n - 2$ degrees of freedom (Felsenstein 1988). All likelihoods were estimated using PAUP*. Absolute divergence times were then estimated using three calibration dates: (1) the origin of the Mauritian lineage is assumed to have coincided with Mauritius emerging from the Indian Ocean (8 million years ago: MacDougal and Chauman 1969); (2) *Pagamea* is assumed to have diverged from *Gaertnera* as South America separated from Africa (95 million years ago: Parrish 1993); and (3) the divergence of *Pagamea* from *Gaertnera* is assumed to be coincident with the first occurrence of Rubiaceae fossils during the Eocene (54

TABLE 2. Alignment statistics, statistical significance of nonrandom structure and, equally weighted parsimony analyses of the four individual nDNA and combined datasets. g_1 was calculated from 10,000 random trees. bp, base pairs; mpt, most parsimonious trees; CI, consistency index; RI, retention index; RC, rescaled consistency index. CI, RI, and RC all calculated without uninformative sites.

Dataset	ITS	*PepC*-L	*PepC*-S	*Tpi*	Combined
Number of taxa	36	33	51	28	31
Aligned length (bp)	649	937	458	1032	3076
Length of individual *Gaertnera* sequences (bp)	623–626	892–896	451–455	975–1018	1970–2989
Length of individual *Morinda* sequences (bp)	620–626	915–920	455–456	—	1989–1991
Length of *Pagamea* sequence (bp)	636	—	455	938	2029
Kimura two-parameter sequence divergence among *Gaertnera* sequences	0.0–3.3%	0.0–3.8%	0.0–4.6%	0.5–2.9%	0.3–2.2%
Inferred number of indels	23	37	8	16	65
Length of indels (bp)	1–8	1–11	1–2	1–43	1–43
Total no. parsimony-informative sites	93	121	63	30	259
No. parsimony-informative sites within *Gaertnera*	32	36	17	26	58
g_1	3.945	−5.838	5.244	−1.355	−3.760
No. mpts	1816	>30,000	4191	>30,000	5746
Tree length	334	290	151	221	875
CI	0.685	0.789	0.809	0.739	0.748
RI	0.731	0.816	0.868	0.745	0.760
RC	0.501	0.643	0.702	0.550	0.569

million years ago: Roth and Dilcher 1979). Standard errors of the divergence times were estimated by a three-step nonparametric bootstrap procedure as described by Baldwin and Sanderson (1998). First, 100 resampled ITS data matrices were generated using Seqboot within Phylip 3.57 (Felsenstein 1995). Second, these character matrices were imported into PAUP* and branch lengths estimated on the rooted combined phylogeny using the HKY + G model of sequence evolution with a molecular clock enforced. Third, branch lengths were converted to time and the mean and standard errors calculated using JMP ver. 3.2.6 (SAS Institute, Inc., Cary, NC).

Lineage diversification rates were estimated using the formula rate = ln N/t, where N = standing diversity and t = age (Sanderson and Donoghue 1996).

RESULTS

Alignment

The four datasets lacked hypervariable regions, and alignment was unambiguous (see Table 2 for alignment statistics). Intraspecific variation was 0.0–2.4%, but was not considered significant because all sequences from a single species formed a single cluster (Fig. 2). In those species with intraspecific variation, the sequence with the shortest patristic distance to the base of the species cluster was used to represent the species in subsequent analyses.

Preliminary Parsimony Analysis

All datasets had significant nonrandom structure as determined by g_1 statistic (Hillis and Huelsenbeck 1992; Table 2). Equal-weight parsimony analyses of the separate datasets indicated a well-supported *Gaertnera* clade, but very little phylogenetic structure within the genus (Fig. 2). High consistency, retention, and rescaled-consistency indices (Table 2) seen in all but the ITS data suggest that the lack of resolution within the *Gaertnera* clade results from insufficient sequence divergence among the terminal taxa rather than homoplasy.

Partitions

Only the ITS versus *PepC*-large data partitions were determined to be significantly incongruent using the ILD test ($P = 0.032$). The ITS data suggest two patterns in conflict with the *PepC*-large data: (1) The Sri Lankan and Southeast Asian species are monophyletic; and (2) the African species *G. cooperi* and *G. paniculata* are part of an otherwise Sri Lankan–Southeast Asian clade. Similarly, the *PepC*-large data suggest two patterns that disagree with the ITS data: (1) the Malagasy species *G. lowryi* and *G. schatzii* are part of an otherwise Sri Lankan–Southeast Asian clade; and (2) *G. cooperi* is sister to all other *Gaertnera* species.

Wilcoxon sign-rank tests reveal that none of these individual constraints are significant at the $P < 0.05$ level (Table 3). When multiple constraints were imposed, only the ITS dataset with constraints 1 + 2 significantly rejected the *PepC*-large constraint at $P < 0.05$, but this significant result disappeared after sequential Bonferroni correction (Table 3).

Combined Maximum-Parsimony Analysis

An equal-weighted MP analysis of the 31 taxon combined molecular dataset recovered 5746 most-parsimonious trees of 875 steps (CI = 0.748, RI = 0.760, RC = 0.569, calculated without uninformative characters). The combined 3076 bp matrix contained 259 parsimony informative characters, 58 of which were informative within *Gaertnera*. Monophyly of the *Gaertnera* clade was supported (100% bootstrap), and the branch subtending the *Gaertnera* clade was estimated to be at least 35 unambiguous changes long using ACCTRAN optimization. These changes reflect only data from ITS (23 changes) and *PepC*-small (12 changes) datasets because no *PepC*-large sequence exists for *Pagamea* and no *Tpi* sequence exists for *Morinda*. A phylogram of a randomly chosen most-parsimonious tree indicates very short internal branches within the *Gaertnera* clade (zero to eight unambiguous changes using ACCTRAN optimization). Although only a few relationships are supported by bootstrap >70%, the separate

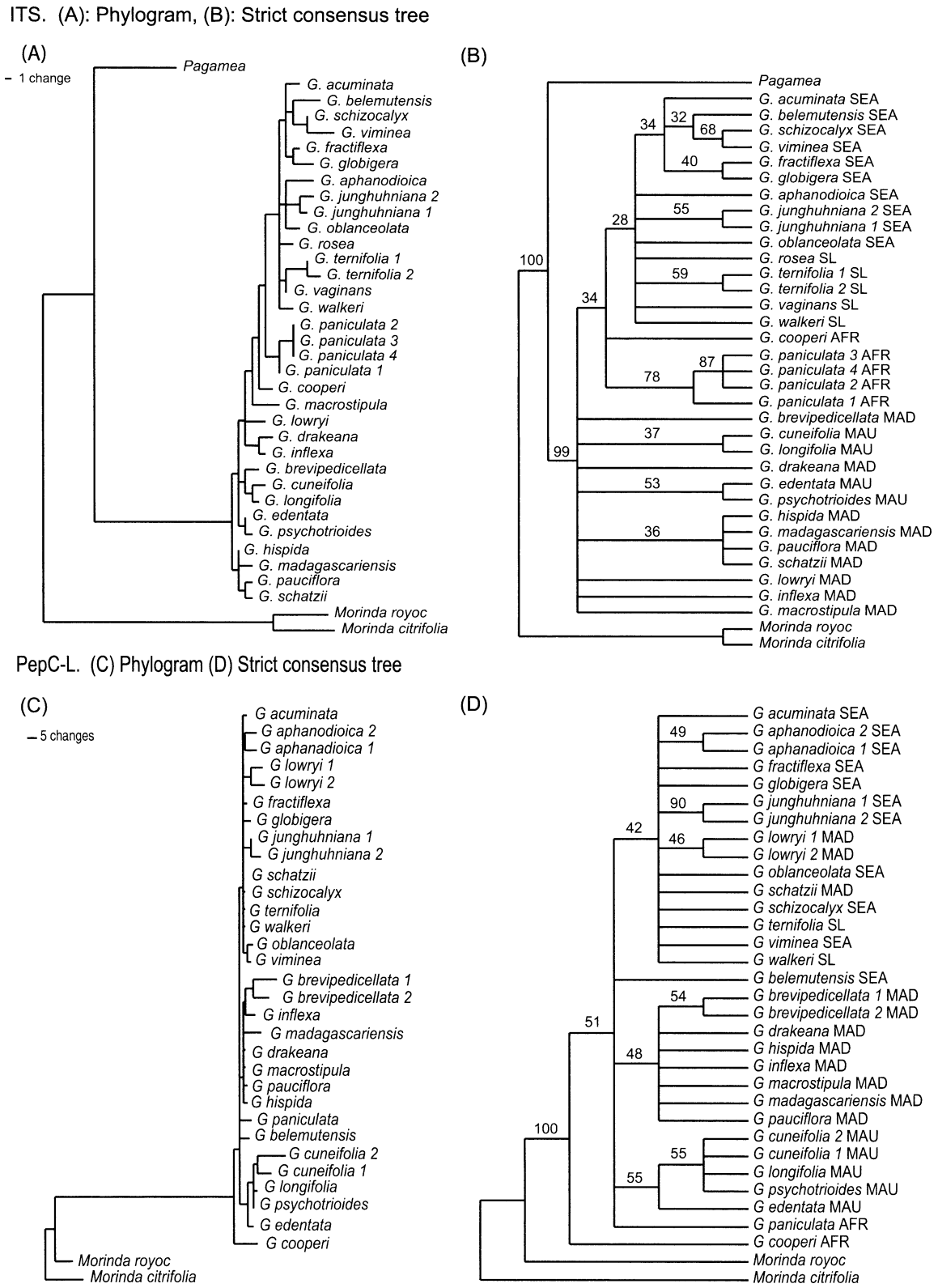

FIG. 2. Relationships among 21–28 *Gaertnera* species, *Pagamea*, and two *Morinda* species as outgroups. The numbers 1, 2, 3, or 4 after species name refer to clone sequence number. Clone sequence 1 was used in subsequent combined analyses. Bootstrap values included above branches in strict consensus trees. AFR, Africa; MAD, Madagascar; MAU, Mauritius; SEA, Southeast Asia; SL, Sri Lanka. ITS dataset: (A) randomly chosen phylogram from set of 1816 most parsimonious trees (mpts); (B) strict consensus of 1816 mpts. *PepC*-large dataset: (C) randomly chosen phylogram from set of 30,000 mpts; (D) strict consensus of 30,000 mpts; *PepC*-small dataset: (E) randomly chosen phylogram from set of 4191 mpts; (F) strict consensus of 4191 mpts. *Tpi* dataset: (G) randomly chosen phylogram from set of 30,000 mpts; (H) strict consensus of 30,000 mpts.

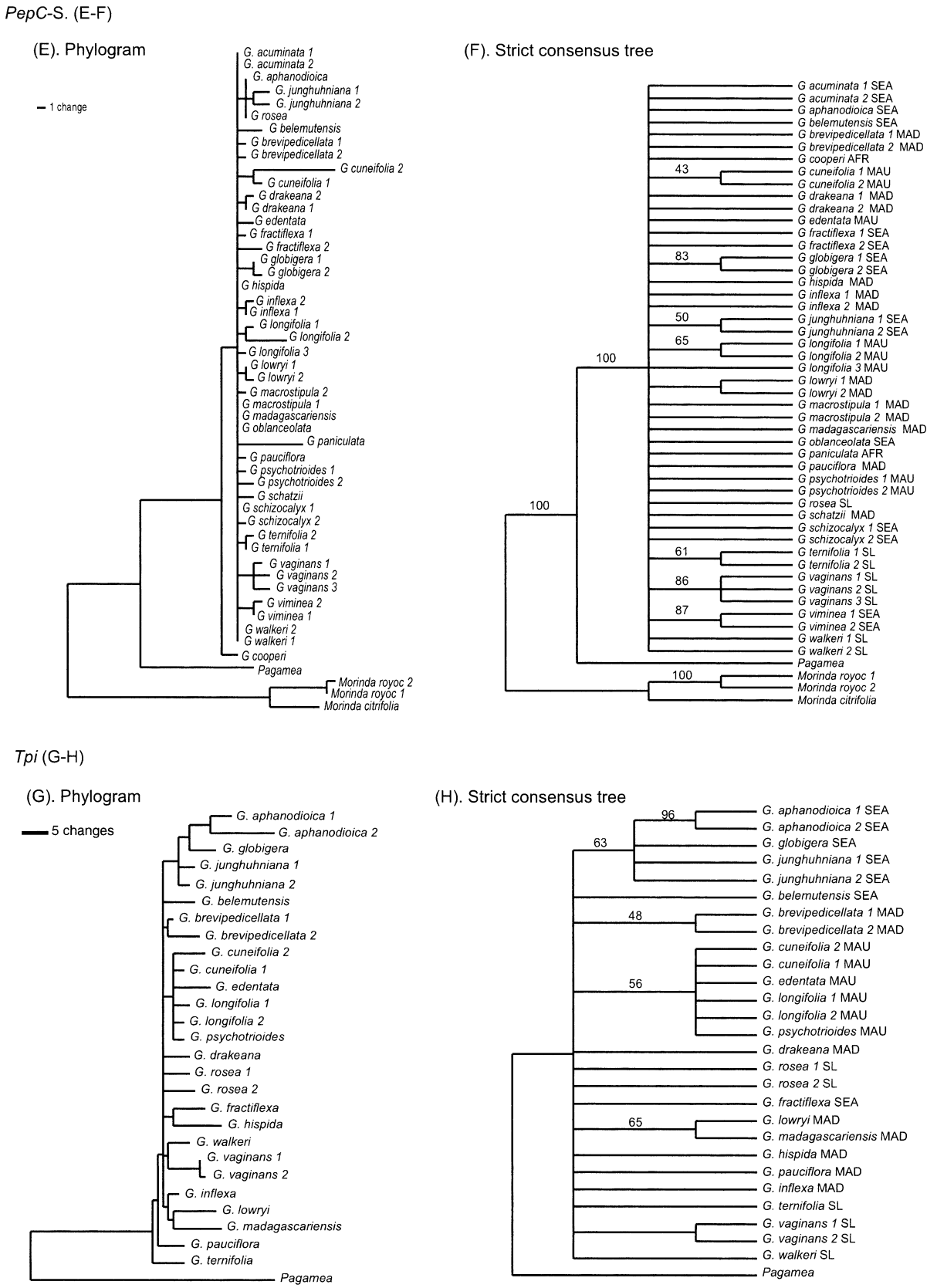

FIG. 2. Continued.

TABLE 3. Templeton test results of imposing constraints suggested by the ITS and *PepC*-large datasets on the other data partition. ITS: Constraint 1. Malagasy *Gaertnera lowryi* and *G. schatzii* are part of an otherwise Sri Lankan–Southeast Asian clade = (*acum apha frac glob jung lowr obla scha schi tern vimi walk*); Constraint 2. *G. cooperi* is sister to all other *Gaertnera* species = (*cooperi* (all other *Gaertnera* species)). *PepC*-large: Constraint 1. Sri Lankan–Southeast Asian species form a clade = (*acum apha bele frac glob jung schi vimi obla rose tern vagi walk*); Constraint 2. African *G. cooperi* and *G. paniculata* are sister to the Sri Lankan–Southeast Asian clade = (*coop pani* (*acum apha frac glob jung schi vimi obla rose tern vagi walk*)). Taxon abbreviations are for first four letters of the species epithet (see Appendix 2 for full names).

Dataset	Constraint	Difference in length	Gain	Loss	*N*	Ts	*P* (two-tailed)
ITS	1	8	11	3	11	15	0.0883
	2	2	6	4	7	11	0.6029
	1 + 2	10	13	3	12	15	0.0480
PepC-L	1	1	1	0	1	0	0.3173
	2	2	9	7	15	52.5	0.6374
	1 + 2	2	9	7	15	52.5	0.6374

clades of the strict consensus tree are largely geographically congruent (Fig. 3). The West African *G. cooperi* is sister to all other *Gaertnera* species, whereas the West African *G. paniculata* is unresolved relative to the Malagasy species. Two other major clades are recognized within the parsimony strict consensus tree, a well-supported Mauritian clade (84% bootstrap) and a combined Sri Lankan–Southeast Asia clade (56% bootstrap).

Maximum-Likelihood Analysis

The best-fitting likelihood model for the equal-weighted maximum parsimony tree was Hasegawa-Kishino-Yano (HKY, Hasegawa et al. 1985), with gamma-distributed rates (i.e., HKY + G). This tree differed from the MP strict consensus tree in three respects: (1) the Mauritian clade was sister to all other *Gaertnera* species; (2) the Malagasy *G. lowryi* was sister to a combined Sri Lankan–Southeast Asian clade; and (3) the African *G. cooperi* and *G. paniculata* were unresolved relative to the combined *G. lowryi*–Sri Lankan–Southeast Asian clade (Fig. 4).

The most-parsimonious phylogenetic estimate compatible with the ML tree was 876 steps long and was not statistically longer than the unconstrained tree (Templeton test, cost = 1, $P = 0.7963$). Similarly, a ML heuristic search with the parsimony strict consensus tree loaded as constraint was not significantly different when tested using the Shimodaira-Hasegawa test (difference in $-\ln L = 0.98$, $P = 0.42$).

Morphology

The parsimony analysis of the morphological matrix recovered more than 6000 trees 64 steps long. The ILD test suggested that the molecular and morphological data partitions were significantly incongruent ($P = 0.002$).

Only two informative morphological characters, breeding system morphology (dioecious versus distylous) and drupe shape (ellipsoid versus globose), were compatible with the unconstrained MP strict consensus tree and the MP tree constrained by relationships recovered in the ML analysis. Of the other informative morphological characters, only those trees constrained to lack homoplasy in corolla color (cost = 6, $P = 0.1533$) were not significantly longer than the most parsimonious molecular trees. Trees constrained to lack homoplasy in the presence of ridged stipules (cost = 12, $P = 0.0512$), the texture of stipule (cost = 10, $P = 0.1233$), and the color of the corolla (cost = 5, $P = 0.3711$) were not significantly longer than the parsimony trees constrained by the ML analysis. All other morphological characters showed high levels of homoplasy, and constraint trees were significantly longer than unconstrained trees. For example, Fitch parsimony optimizations indicate that the reduction of the inflorescence to a dense, capitulum evolved at least three times using the MP tree (Fig. 3) and at least three times using the ML tree, whereas the reduction to a single-flowered cyme has evolved at least twice using the MP tree (Fig. 3) and three times using the ML tree. Stipule shape was also homoplastic, with calyptrate stipules having evolved on at least three occasions in Africa, Madagascar, and Mauritius (Fig. 3) as were several aspects of flower morphology.

Molecular Clock

Likelihood ratio tests indicate that a molecular clock could not be rejected with either the *Gaertnera* or *Gaertnera* + *Pagamea* ITS datasets with either the ML or MP estimates of relationships (Table 4). Estimated ages, ITS substitution rates, and diversification rates, are presented in Table 5.

DISCUSSION

The phylogenetic estimates presented here represent the first phylogenetic analysis of *Gaertnera* and are based on separate and combined analysis of four nDNA datasets: ITS, *PepC*-large, *PepC*-small, and *Tpi*. All four datasets support the monophyly of *Gaertnera*, but none has sufficient variation to convincingly reconstruct relationships within the genus. This lack of genetic variation among the sampled species is indicative of either a rapid radiation or slow molecular evolution, although the molecular-clock analysis suggests the former. The rapid radiation of *Gaertnera* is counter to expectations based on the morphological variation and geographical range of the genus.

Maximum-Parsimony and Maximum-Likelihood Analyses Estimate Different Relationships within Gaertnera.

Attempts to recover a well-supported, bifurcating estimate of phylogenetic relationships within *Gaertnera* were confounded by the limited sequence divergence among sampled species. Although the ML and MP analyses both suggest a monophyletic *Gaertnera* clade, the separate analyses differ in their estimates of relationships within *Gaertnera*. The MP strict consensus tree estimates the West African species, *G. cooperi*, to be sister to all other *Gaertnera* species and *G. paniculata*, the other African species included in the analysis, as unresolved relative to the Malagasy species. Otherwise, the MP analysis suggests a well-supported Mauritian clade (84% bootstrap) and a Sri Lankan–Southeast Asian clade, (Fig. 3). The ML phylogeny, in contrast, estimates the Maur-

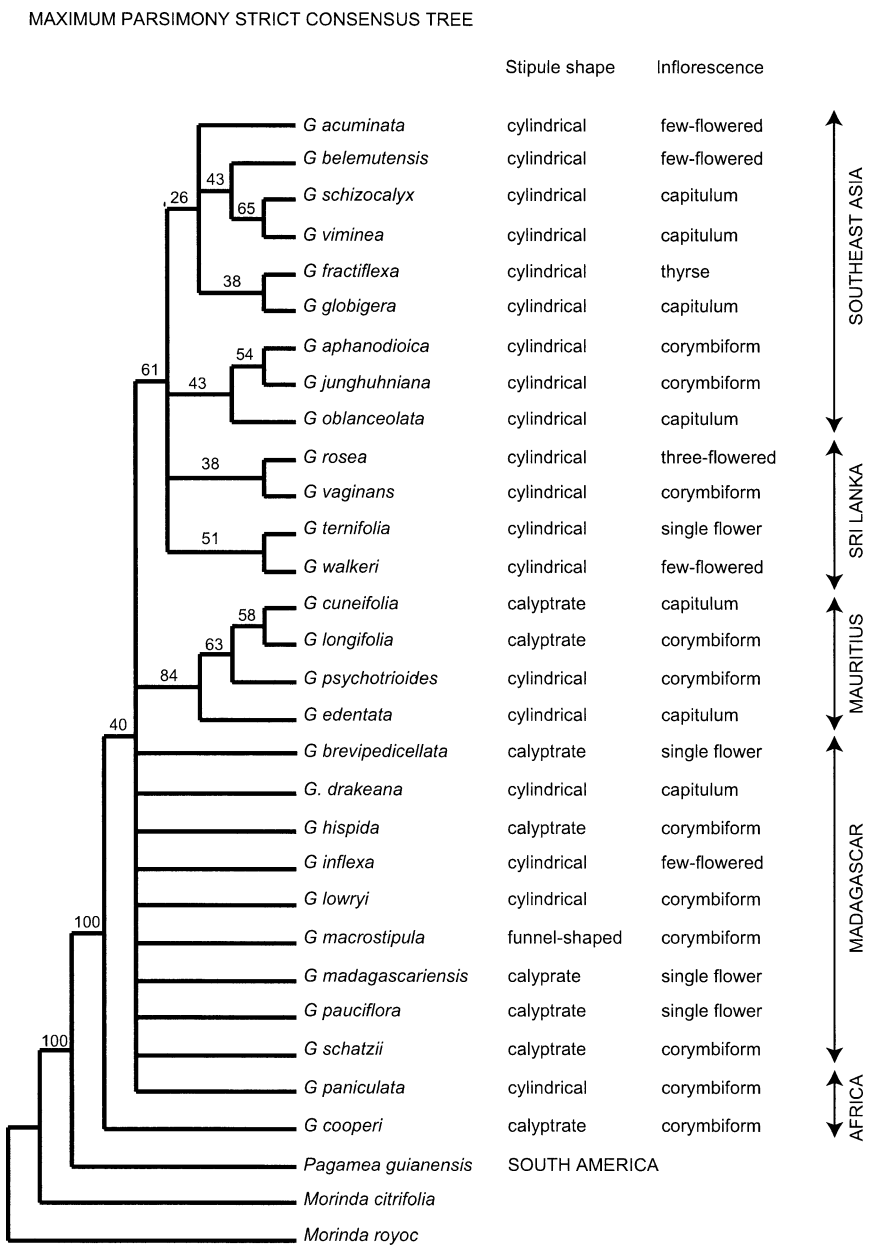

FIG. 3. Maximum-parsimony strict consensus of 5746 most parsimonious trees (CI = 0.748, RI = 0.760, RC = 0.569), bootstrap support included above the branches. Stipule shape, inflorescence morphology, and geographical distribution are associated with the terminal taxa.

itian clade to be sister to all other *Gaertnera* species, the Malagasy *G. lowryi* to be sister to the Sri Lankan–Southeast Asian clade, and the African *G. cooperi* and *G. paniculata* to be unresolved relative to the *G. lowryi*–Sri Lankan–Southeast Asian clade (Fig. 4). Neither hypothesis is significantly suboptimal when tested against the other topology.

The MP estimate of *G. cooperi* being sister to all other *Gaertnera* species is supported by the presence of ruminate endosperm in the drupe (the plesiomorphic condition that is only known from one other *Gaertnera* species, *G. aurea* S. T. Malcomber *ined.*, also from West Africa) and incised corolla tube, a condition intermediate between *Pagamea* and other *Gaertnera* species. Placing *G. cooperi* in a clade sister to a *G. lowryi*–Sri Lankan–Southeast Asian clade, as estimated by the ML phylogeny, would require reversals in both of these characters when reconstructed using Fitch parsimony. Biogeography also favors the MP tree because it requires fewer dispersal and extinction events. The MP trees suggest an African origin for *Gaertnera* species and require one dispersal each to Madagascar, Mauritius, Sri Lanka, and Southeast Asia. In contrast, the ML tree cannot be explained without several additional dispersal and extinction events. In conclusion, if one wished to select either the MP or ML tree as being the best estimate of the group's phylogeny, the MP analysis trees are more compatible with the morphological and biogeographic data.

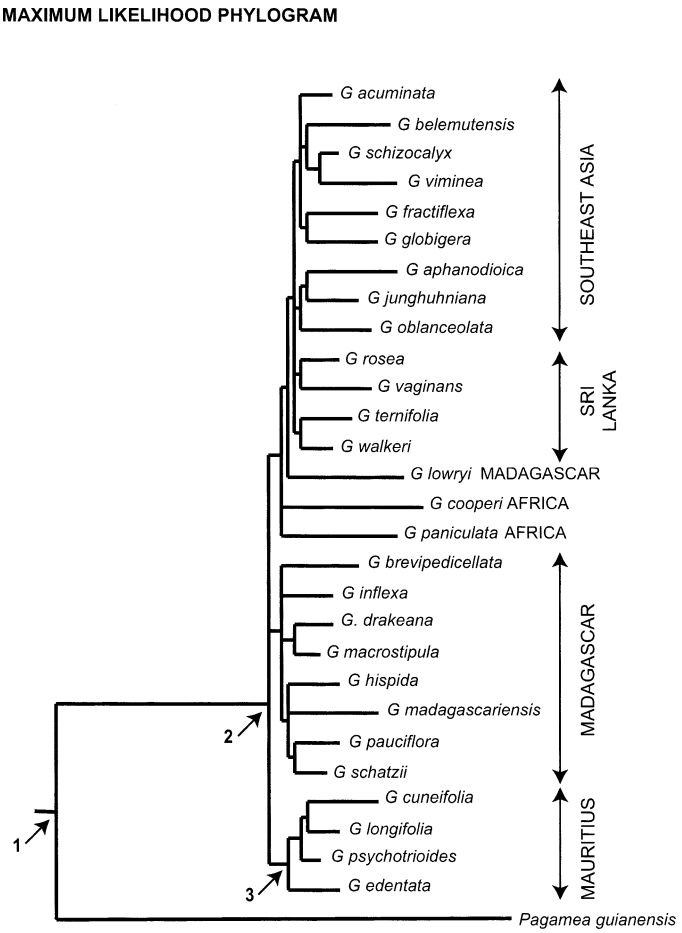

FIG. 4. Maximum-likelihood tree recovered using HKY model with gamma distributed rates (HKY + G) − ln = 9308.85. Geographical distribution is associated with terminal taxa. 1, node marking separation of *Gaertnera* from *Pagamea*; 2, *Gaertnera* radiation node; 3, Mauritian node.

Both MP and ML analyses suggest several long-distance dispersal events in *Gaertnera*. All *Gaertnera* species have dark blue drupes of 5–15 mm diameter; this fruit morphology suggests birds may serve as long-distance dispersal vectors. As yet, however, there are no reports of birds with transoceanic distributions feeding on *Gaertnera* species. Other drupaceous Rubiaceae genera show similar transoceanic distributions and have also been hypothesized as bird dispersed (Bremer and Eriksson 1992). Efficient long-distance dispersal within *Gaertnera* is, however, complicated by the distylous or dioecious breeding system of the species. Distyly and dioecy require two individuals for fertile seed production—a pin and thrum morph in the case of the distylous species and a male and female individual in the case of the dioecious species. Thus, one plant of either morph is required to establish a population after the long-distance dispersal event, unless a breakdown in self-incompatibility systems permits self-pollination in the distylous species. A breakdown in self-incompatibility systems has been reported in *G. vaginata* (Pailler and Thompson 1997) on Réunion, but it is unknown how many of the distylous *Gaertnera* species in Africa, Madagascar, Mauritius, and Sri Lanka are partially self-compatible.

Morphological Character Evolution

The *Gaertnera* + *Pagamea* clade is supported by multiple morphological characters including a secondarily derived superior ovary, sheathing stipules, and wood and pollen characters (Igersheim et al. 1994; Jansen et al. 1996), and the *Gaertnera* clade is supported by a morphological synapomorphy of glabrous corolla lobes (Malcomber 2000). Of the morphological characters examined in this study, only drupe shape (ellipsoid vs. globose) is congruent with the *Gaertnera* phylogenies. Drupe shape is ellipsoid-obovoid only in Mascarene species, elsewhere it is (sub)globose (Malcomber 2000). Breeding system morphology (dioecy vs. distyly) is compatible with the ML and strict consensus MP phylogenies, but is only congruent in 1325 of the 5746 most-parsimonious trees where the Southeast Asian species form a clade. Distyly, plesiomorphic within *Gaertnera*, is found within all African, Malagasy, Mascarene, and Sri Lankan species, whereas dioecy is restricted to Southeast Asian species (van Beusekom 1968; Malcomber 2000). In the other 4421 most parsimonious trees dioecy is estimated to have evolved at least twice using Fitch parsimony.

The high levels of homoplasy in the other morphological characters included in this analysis, as indicated by Templeton tests, suggest that similar morphological structures have evolved repeatedly in different geographical areas as the lineage has dispersed and diversified. Although the basis for this homoplasy is currently unknown, few genetic loci have been shown to effect large changes in morphological characters in both monocots (Doebley and Stec 1991) and dicots (Bradshaw et al. 1995). In particular, inflorescence morphology, hypothesized to be under the control of few genetic

TABLE 4. Comparison of likelihood scores for different phylogenetic estimates using the HKY + G model of sequence evolution. MP tree refers to the likelihood tree compatible with the maximum-parsimony estimate. ML tree refers to the maximum-likelihood tree. The likelihood-ratio test is based on difference between the log-likelihoods for the two models. *P*-values are based on a χ^2-distribution with degrees of freedom equal to $(n - 2)$. $^{**}P < 0.01$. Other values $P > 0.05$.

Taxa included (degrees of freedom)	Tree	−log L1 (nonclock)	−log L2 (clock)	−2(log L1/L2)
Gaertnera (27 df)	MP tree	1582.283	1597.759	30.954
	ML tree	1582.283	1597.759	30.954
Gaertnera + *Pagamea*	MP tree	1914.533	1930.196	31.326
(28 df)	ML tree	1912.218	1931.965	39.494
Gaertnera + *Pagamea* + *Morinda*	MP tree	2481.908	2529.957	96.098**
(30 df)	ML tree	2478.123	2533.481	110.716**

TABLE 5. Summary of age estimates (million years ago ± two standard errors) based on different molecular clock calibrations: (1) separation of *Gaertnera* from *Pagamea*; (2) age of *Gaertnera* radiation; (3) age of Mauritius node; (4) ITS substitution rate within the *Gaertnera* radiation; (5) net diversification rate within the *Gaertnera* radiation (where rate = ln 68/age of radiation).

	Ages calibrated on age of Mauritian node at 8 million years ago, as Mauritius emerged from Indian Ocean		Ages calibrated on separation of *Gaertnera* from *Pagamea* at 95 million years ago, as Africa separated from South America		Ages calibrated on the separation of *Gaertnera* from *Pagamea* at the first occurrence of Rubiaceae in fossil record during the Eocene (54 million years ago)	
	MP tree	ML tree	MP tree	ML tree	MP tree	ML tree
1. Time (million years ago)	229 ± 35.68	196 ± 32.68	95		54	
2. Time (million years ago)	23.63 ± 3.79	19.11 ± 3.40	10.00 ± 0.36	10.41 ± 0.36	5.68 ± 0.21	5.21 ± 0.14
3. Time (million years ago)	8		4.64 ± 0.38	5.26 ± 0.49	2.53 ± 0.22	2.99 ± 0.28
4. ITS substitution rate (substitutions/site/year)	4.136×10^{-10}–5.716×10^{-10}	4.527×10^{-10}–6.486×10^{-10}	1.094×10^{-9} – 1.176×10^{-9}	9.458×10^{-10}–1.017×10^{-9}	1.926×10^{-9} – 2.071×10^{-9}	1.904×10^{-9} – 2.010×10^{-9}
5. Net diversification rate (species/million years)	0.154–0.217	0.187–0.269	0.407–0.438	0.392–0.420	0.717–0.771	0.789–0.832

loci (Kellogg 2000), may change rapidly given an appropriate selective environment.

Molecular Clock

Estimates of the age of the *Gaertnera* radiation vary greatly depending on the calibration date used; however, the different topologies within *Gaertnera* do not affect estimates of age (Table 5). Sampling additional *Gaertnera* species might improve the accuracy of divergence estimates; it is unlikely, however, to increase clade age because *G. cooperi*, considered the most plesiomorphic of species in terms of morphology, is already included within the analysis.

Calibrating the molecular clock phylogeny by assuming the age of the Mauritian clade to be 8 million years old implies that *Gaertnera* was an early colonizer of the volcanic island, whereas species today are restricted to late-successional, high-altitude moss forest. Thus, ages using this calibration are likely overestimates. This is confirmed because this calibration would date the divergence of *Gaertnera* from *Pagamea* at 229 ± 35.7 million years ago (MP tree) or 196 ± 32.3 million years ago (ML tree), at least 38.7 million years before the first eudicot pollen appeared in the fossil record at the Barremian-Aptian boundary of the Cretaceous (Doyle and Donoghue 1993).

The alternative analyses calibrated the molecular clock by assuming that *Gaertnera* diverged from *Pagamea* either as Africa separated from South America 95 million years ago (Parrish 1993) or at the time Rubiaceae pollen first enters the fossil record during the Eocene (54 million years ago: Roth and Dilcher 1979). Using the former, geological calibration date estimates that *Gaertnera* started to radiate between 10.00 ± 0.36 million years ago (MP tree) and 10.41 ± 0.36 million years ago (ML tree). However, this calibration date is also likely an overestimate because it requires a hidden fossil record for the family, predating the current oldest fossil, by at least 41 million years.

Using a biological calibration date, based on the time when Rubiaceae pollen first enters the fossil record, implies that *Gaertnera* migrated to Africa in the early Tertiary, possibly via the boreotropical land bridge (Tiffney 1985) and began to radiate between 5.68 ± 0.2 million years ago (MP tree) and 5.21 ± 0.1 million years ago (ML tree; Table 5). Subsequent migration of the lineage from Africa to Southeast Asia occurred via long-distance dispersal.

The biological calibration implies an ITS substitution rate within the *Gaertnera* radiation of 1.904×10^{-9}–2.071×10^{-9} substitutions/site/year, which is toward the upper end of the published rates (highest: 5.3×10^{-9} substitutions/site/year, Wendel et al. 1995; lowest: 3.5×10^{-10} substitutions/site/year, Suh et al. 1993). However, if the stem node is used instead of the crown node (i.e., node 1 instead of node 2 in Fig. 4), then ITS substitution rate for the genus is estimated at 1.95×10^{-10} substitutions/site/year, which is toward the lower end of published rates. Similarly, lineage diversification rates vary greatly depending on whether the stem or crown node is used. Using the crown node estimate, the net lineage diversification rate within the *Gaertnera* radiation is inferred to be 0.717–0.832 species/million years, comparable to the 0.56 ± 0.17 species per million years lineage diversification estimate for Hawaiian silverswords (Baldwin and Sanderson 1998), whereas using the stem node estimates a genus rate of only 0.078 species/million years (where $N = 68$ and $t = 54$).

The dispersal(s) of *Gaertnera* to Southeast Asia from Sri Lanka is correlated with a switch from distylous to dioecious breeding system. Assuming monophyly of the Southeast Asian taxa, the molecular-clock analyses of the MP tree estimates the age of the dioecious clade at 4.10 ± 0.17 million years ago and a lineage diversification rate of 0.65–0.71 species/million years (where $N = 16$ and $t = 3.93$ or 4.27). In contrast, the hermaphroditic Sri Lankan sister taxon is estimated to have diversified at a rate of 0.37–0.41 species/million years (where $N = 5$ and $t = 3.93$ or 4.27). The higher rate of diversification in the dioecious lineage is counter to the findings of Heilbuth (2000), who reported lower speciation (or increased extinction rates) in dioecious clades relative to hermaphroditic sister taxa.

The Mechanism Driving the Rapid Radiation in Gaertnera

A central question in evolutionary biology is the mechanism driving lineage diversification. The classic examples of rapid species radiations are all geographically restricted, with high levels of sympatry. In these cases, authors have frequently focused on the importance of low interspecific competition levels in driving lineage diversification in species depauperate habitats (Jensen 1990; Liem 1990). Apart from competition levels, key innovations are purported to enable the rapid ra-

diation of a lineage by increasing the rate at which stable new species arise (Liem 1990; Hodges and Arnold 1994).

Pagamea, the sister taxon of *Gaertnera*, comprises 24 distylous species and is restricted to northeast South America (Steyermark 1965; Boom 1989), whereas *Gaertnera* comprises 68 species and is distributed throughout the wet tropics of Western and Central Africa, Madagascar, Mascarenes, Sri Lanka, and Southeast Asia (Malcomber 2000). Most *Gaertnera* species are distylous, but 16 species in Southeast Asia are dioecious (Malcomber 2000). The long branch subtending the *Gaertnera* clade could indicate that the group was not diversifying very fast in Africa with extensive species turnover due to extinction. The rapid radiation of the *Gaertnera* lineage only occurred after the group began colonizing Madagascar, Mauritius, Sri Lanka, and Southeast Asia. The number of sympatric congeners is not uniform across the distributional range of *Gaertnera*. In Madagascar and Mauritius up to eight *Gaertnera* species can be found within the same forest, whereas in Southeast Asia species are generally allopatric. This difference suggests that different mechanisms may be driving the rapid diversification of *Gaertnera* in different parts of the range. The volcanic island of Mauritius is only 8 million years old (MacDougal and Chauman 1969) and *Gaertnera* is estimated to have colonized the island 2.53 $\pm$ 0.2 million years ago (MP tree) or 2.99 $\pm$ 0.3 million years ago (ML tree). It is unknown how species depauperate Mauritius was at that time, but it is conceivable that reduced interspecific competition at the time of colonization may have favored the diversification of the lineage. However, low interspecific competition is unlikely to have contributed to lineage diversification elsewhere in the range, particularly in Southeast Asia, which is regarded as one of the most species-rich and oldest tropical forests (Richards 1996).

It is noteworthy, however, that the rapid diversification of *Gaertnera* is correlated with a change in corolla morphology. The corolla in all *Pagamea* species is characterized by a short tube and long spreading lobes. In most *Gaertnera* species, the lobes are more erect and fused to form a longer tube than that in *Pagamea*. In *G. cooperi*, the most plesiomorphic species, the corolla is deeply incised and intermediate between these forms. Based on the preferred MP phylogeny, the change in corolla morphology coincided with the rapid diversification of *Gaertnera*. Therefore, this change potentially qualifies as a key innovation by allowing a shift in pollinators. Paradoxically, the flower morphology of most of these *Gaertnera* species is typical of plants that are pollinated by small generalist insects (Bawa et al. 1985); thus, it is unlikely that this shift in corolla morphology promoted pollinator specialization.

The lack of genetic variation among the sampled *Gaertnera* species is counter to expectations based on the morphological variation and geographical range of the genus. I interpret this lack of genetic variation within the four molecular datasets to indicate not a slow rate of molecular evolution but a relatively recent and rapid radiation of *Gaertnera* on an intercontinental scale. *Gaertnera* provides the first example of a widespread rapid radiation in a genus characterized by a tropical distribution. Although low interspecific competition may be a factor in the diversification of *Gaertnera* species on Mauritius, the mechanisms driving the radiation elsewhere remain elusive. This study illustrates that in evolutionary studies of widespread taxa much is still to be discovered about the mechanisms of lineage diversification and morphological change.

Acknowledgments

This research was supported by National Science Foundation grant 9701008, Missouri Botanical Garden, American Society of Plant Taxonomists, Andrew W. Mellon Foundation, Washington University Division of Biology and Biomedical Sciences, and University of Missouri, St. Louis. I thank J. Barber, H. Cota, A. Doust, C. Hemingway, E. Kellogg, the Kellogg laboratory group, A. Larson, W. Lewis, J. Losos, M. Nepokroeff, P. Raven, M. Richardson, P. Stevens, R. Sussman, K. Sytsma, and T. Verboom for discussion and comments. D. Baum and two anonymous reviewers provided helpful comments on earlier versions of this manuscript. I thank P. Ashton, P. Berry, R. Gereau, D. Harder, C. Jongkind, D. Lorence, G. McPherson, Pacific Tropical Botanic Garden, G. Schatz, and C. Taylor for material. I also gratefully acknowledge the following herbaria and their staff for providing loans of herbarium material, access to collections, and/or assistance in the field: A, BM, BR, BRUN, CGE, E, FHO, GH, G, G-DC, K, KEP, L, MAU, MO, P, PDA, SAN, SAR, SING, TAN, TEF, UBD, W, WAG, WU (acronyms according to Holmgren et al. 1990). This research would not have been possible without support of Association National pour la Gestion des Aires Protégées (Madagascar), National Parks and Conservation Service (Mauritius), Economic Planning Unit (Malaysia), Ministère de la Recherche Scientifique Centre National de Recherche Appliquée au Développement Rural (Madagascar), Ministère des Eaux et Forêts (Madagascar), Universiti Brunei Darussalam, and the National Botanic Garden Peredeniya, (Sri Lanka).

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Corresponding Editor: D. Baum

Appendix 1

Morphological characters and character states. All multistate characters are treated as unordered.

(1) Presence of ridged internodes: 0, absent (internodes smooth); 1, present

(2) Presence of pubescence on underside of leaves: 0, absent, underside of leaves glabrous; 1, present, underside of leaves pubescent

(3) Presence of fused interpetiolar stipules: 0, absent (stipules not fused into a tube); 1, present

(4) Presence of lobes at apex of interpetiolar stipules: 0, absent (apex truncate); 1, lobes present, deltate in shape; 2, lobes present, filiform in shape

(5) Texture of interpetiolar stipules: 0, stipules charactaceous; 1, stipules membranous

(6) Interpetiolar stipule shape: 0, lobes free, elliptic-ovate; 1, lobes fused, cylindrical; 2, fused, calyprate; 3, fused, laciniate

(7) Presence of incisions at stipule apex: 0, absent, no incisions; 1, present

(8) Cyme shape: 0, globose; 1, domed; 2, conical; 3, cylindrical

(9) Inflorescence position. 0, terminal; 1, supra-axillary (borne at apex of supra-axillary branches); 2, axillary (borne in leaf axils or at apices of supra-axillary branches)

(10) Inflorescence branching: 0, absent, cymes reduced to a solitary flower; 1, lax inflorescence branching; 2, inflorescence branching congested at apex, but otherwise lax; 3, inflorescence branching congested

(11) Number of flowers per inflorescence: 0, one (cymes reduced to a single flower); 1, 3–8 flowers; 2, $>$ 15 flowers

(12) Presence of a pedicel: 0, absent, flowers sessile; 1, lateral flowers pedicellate, central flower in cyme sessile; 2, present, all flowers pedicellate

(13) Flower merosity: 0, 6-merous; 1, 4-merous; 2, 5-merous

(14) Corolla color: 0, corolla white; 1, corolla pink or red

(15) Presence of villous hairs in the corolla throat: 0, absent; 1, present

(16) Corolla tube length: 0, $<$ 4 mm long; 1, $>$ 5 mm long

(17) Breeding system morphology: 0, flowers bisexual, plants distylous 1, flowers unisexual, plants dioecious

(18) Position of ovary in fruit: 0, inferior (below the calyx); 1, superior (above the calyx)

(19) Presence of a syncarpous fruit: 0, present; 1, absent, fruit not fused

(20) Drupe shape: 0, globose; 1, ellipsoid

(21) Presence of an entire endosperm: 0, absent, endosperm ruminate; 1, present

(22) Position of preformed gemination slits: 0, 2 basal and 1 ventral germination slits; 1, 2 basal marginal slits

(23) Pyrene surface texture: 0, smooth; 1, finely rugose; 2, distinctly striated

(24) Endosperm type: 0, endosperm starchy; 1, endosperm oily

APPENDIX 2
Morphological Matrix

G. acuminata	0	0	1	1	0	1	0	1	0	1	1	1	2	0	1	1	1	1	1	0	1	0	1	0
G. aphanodioica	0	0	1	1	0	1	02	1	0	2	2	1	2	0	0	1	1	1	1	0	1	0	1	0
G. belemutensis	0	0	1	1	0	1	02	1	0	3	1	0	2	0	0	1	1	1	1	0	1	0	1	0
G. brevipedicellata	1	0	1	1	1	2	1	?	0	0	0	0	2	0	0	0	0	1	1	0	1	0	1	0
G. cooperi	0	2	1	1	0	2	1	1	0	2	2	1	2	0	0	1	0	1	1	0	0	0	1	0
G. cuneifolia	0	0	1	1	1	2	1	0	0	3	2	0	2	0	1	1	0	1	1	1	1	0	1	0
G. drakeana	0	0	1	1	0	1	12	1	0	3	2	0	2	0	1	1	0	1	1	0	1	0	1	0
G. edentata	0	0	1	1	0	1	0	1	0	2	2	1	2	0	0	1	0	1	1	1	1	0	1	0
G. fractiflexa	0	0	1	1	0	1	0	2	0	1	2	1	1	0	0	0	1	1	1	0	1	0	1	0
G. globigera	0	0	1	1	0	1	12	0	0	3	2	0	2	?	0	?	?	1	1	0	1	0	1	0
G. hispida	0	2	1	1	1	2	1	1	0	2	2	1	2	0	0	1	0	1	1	0	1	0	1	0
G. inflexa	0	0	1	1	0	1	0	2	01	1	2	1	1	0	0	0	0	1	1	0	1	0	1	0
G. junghuhniana	0	0	1	1	0	1	012	1	0	2	2	1	2	0	0	0	1	1	1	0	1	0	1	0
G. longifolia	0	01	1	1	0	2	1	1	0	2	2	1	2	0	0	1	0	1	1	1	1	0	1	0
G. lowryi	0	0	1	1	0	1	1	12	0	2	2	1	2	0	1	1	0	1	1	0	1	0	1	0
G. macrostipula	0	0	1	1	0	1	0	1	0	2	2	1	2	1	0	1	0	1	1	0	1	0	1	0
G. madagascariensis	0	0	1	1	0	1	1	?	0	0	0	2	2	0	1	1	0	1	1	0	1	0	1	0
G. oblanceolata	0	0	1	1	0	1	02	02	12	3	2	1	2	0	0	0	1	1	1	0	1	0	1	0
G. pauciflora	1	0	1	1	1	2	1	?	0	0	0	2	12	0	1	1	0	1	1	0	1	0	1	0
G. paniculata	0	0	1	1	0	1	0	1	0	2	2	1	2	0	0	0	0	1	1	0	1	0	1	0
G. psychotrioides	0	0	1	1	0	1	02	1	0	2	2	1	2	0	0	1	0	1	1	1	1	0	1	0
G. rosea	1	0	1	1	0	1	0	01	0	3	1	0	1	1	1	1	0	1	1	0	1	0	1	0
G. schatzii	0	1	1	1	0	1	1	1	0	2	2	1	2	0	0	1	0	1	1	0	1	0	1	0
G. schizocalyx	0	2	1	1	0	1	02	0	0	3	2	1	2	0	0	0	1	1	1	0	1	0	1	0
G. ternifolia	1	0	1	1	0	1	3	?	0	0	0	2	02	0	0	1	0	1	1	0	1	0	1	0
G. vaginans	0	0	1	1	0	1	012	12	0	2	2	1	2	0	0	0	0	1	1	0	1	0	1	0
G. viminea	0	0	1	1	0	1	0	12	0	1	2	1	1	0	0	0	1	1	1	0	1	0	1	0
G. walkeri	0	0	1	1	0	1	0	1	0	1	1	1	2	0	0	1	0	1	1	0	1	0	1	0
M. citrifolia	0	0	0	0	0	0	2	0	0	3	2	0	2	0	0	1	0	0	0	0	0	1	0	1
M. royoc	0	0	0	0	0	0	2	0	0	3	2	0	2	0	0	1	0	0	0	0	0	1	0	1
P. guianensis	0	0	1	3	0	1	0	2	2	2	2	0	2	0	0	0	0	1	1	0	0	0	2	0