Supporting Information

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SI Text

Expanded Materials and Methods. *Phylogenetic analyses.* We reconstructed phylogenetic relationships using DNA sequences from 5 different markers: the *mat*K and *rbc*L genes, the ITS1-5.8S–ITS2 and *trn*L–F regions, and the *rps*16 intron. Most of these sequences were previously obtained by using the methodology published in Andersson and Antonelli (1), except for 6 additional species of *Isertia* (*I. haenkeana*, *I. hypoleuca*, *I. parviflora*, *I. pittieri*, *I. rosea*, and *I. spiciformis*) that were newly sequenced for this study to increase taxon sampling within tribe Isertieae. Accession numbers and source of all sequences included in this study are listed in Table S3. Following Andersson and Antonelli (1), we used representative genera of 4 families of Gentianales as outgroup for the analysis, based on evidence that the Rubiaceae are the sister group to the rest of Gentianales (e.g., ref. 2). The dataset also included representatives from the 2 other Rubiaceae subfamilies Rubioideae and Ixoroideae, as well as genera representing all tribes in subfamily Cinchonoideae (Fig. 1). In addition, an extra outgroup species (*Nicotiana tabacum*) was included in the phylogenetic analysis to determine the position of the root within the basal branch, as required by the molecular dating method described below, but this taxon was subsequently excluded from the results. The complete dataset was realigned by using MAFFT v. 5.64 (3) and adjusted manually. Gaps were coded as present/absent (0/1) following the principles outlined by Antonelli (4) and included in the phylogenetic analysis. The final aligned data matrix comprised 5,894 characters, of which 1,600 derived from *mat*K, 1,398 from *rbc*L, 1,254 from *trn*L-F, 908 from *rps*16, 676 from the ITS region, and 58 from gap codings. Of 2,153 variable characters, 1,165 were parsimony informative.

Parsimony jackknife support values (5) were estimated in PAUP v. 4.0b10 (6) by running 10,000 replicates with 37% deletion, 10 random addition sequence replicates, using TBR branch swapping and saving up to 30 trees per replicate. Following recent works (7, 8), the Akaike Information Criterion, implemented in MrModelTest v. 2.2 (9), was used to choose the optimal model of sequence evolution for each DNA marker. Bayesian analyses were subsequently implemented in MrBayes v. 3.1.2 (10) using the GTR+G model for *mat*K and *rps*16, and the GTR+I+G model for ITS, *rbc*L, and *trn*L-F. Gap-codings were analyzed as a separate partition under the Restriction Site (Binary) Model. Two simultaneous analyses with 8 Metropolis-Coupled MCMC chains each were run for 2.5 million generations, sampling every 500th generation. Each analysis was started from different, randomly sampled topologies and let to run until the average standard deviation of split frequencies became <0.01, indicating convergence of trees and model parameters across the runs (11). The burn-in value was set in all cases to 1,000,000 generations after visual inspection of the split (clade) frequencies using the software AWTY (12). Final results were based on the pooled values from the 2 independent analyses. The parsimony jackknife consensus tree was virtually congruent with the Bayesian tree (Fig. S3), and both were very similar to the tree of Andersson and Antonelli (1). Nearly 80% of all tree nodes were strongly supported (Bayesian posterior probability values, pp ≥95% or jackknife ≥85%). *Kerianthera* was placed with strong support as the sister to *Isertia*, and within the latter there were 3 strongly supported clades of sister species (Fig. S3). The Bayesian and jackknife trees, as well as the aligned dataset used for the analyses, are available from TreeBase (www.treebase.org), accession numbers S2334 and M4437.

Molecular dating. Divergence times were estimated on the tree topology with the highest posterior probability from the Bayesian analysis, but using mean branch lengths calculated from 1,000 post-burn-in trees from the Bayesian sample (see below). A likelihood ratio test (13) strongly rejected the hypothesis of a molecular clock for the tree ($P < 0.0001$). The clock-independent algorithm Penalized Likelihood (14) implemented in the software r8s v. 1.70 (15) was then used for estimating divergence times in the tree.

Fossils have been assigned to some 10 genera of extant Rubiaceae in our phylogeny (ref. 17; the Paleobiology Database, http://paleodb.org). However, in the majority of cases, a careful evaluation of the original fossil descriptions made evident that almost none of these could be deemed fully reliable in terms of taxonomic placement and/or geologic age. Approximately more than half of these records are fragmentary traces of leaves, and the rest are pollen grains examined with light microscopy. Considering the great convergence of leaf and pollen forms among angiosperms, which render even fresh material very difficult to identify in the absence of various other vegetative and floral parts, we have therefore opted for not using any of these 2 types of fossils as calibration points.

An important exception among Rubiaceae fossils exists in *Cephalanthus*. The oldest fossil of the genus is *Cephalanthus kireevskianus* from the Late Eocene of Germany (18), which appears to be both correctly identified (E. M. Friis, personal communication) and dated (S. Schultka, personal communication). Additional findings indicate that this species was common and widespread from the Late Eocene onwards in Europe (19) and in Western Siberia, where the species was originally described (20, 21). Another fossil species of the genus, *C. pusillus*, was described from the Middle Miocene of Denmark (22). Fossil fruits of *Cephalanthus* possess several morphological features that make them taxonomically recognizable by means of overall similarity. These include fruit type, a schizocarp; mericarp obovoid, slightly dorsoventrally flattened; ventral face flat with a shallow median furrow; dorsal face convex, apically truncate; placentation apical; seed strophiolate, apotropous pendulous, obovoid, slightly dorsoventrally flattened; strophiole apical, sickle-shaped; hilum dorsal, marked by a narrow slit (ref. 22; Fig. S4*A Inset*).

The continuous fossil records in every single geologic epoch from the Late Eocene to the Pliocene in almost 20 fossil sites (Fig. S4 *B and C*), combined with the reliability of their taxonomic placement thanks to a high quality of preservation, make *Cephalanthus* an ideal calibration point for the Rubiaceae phylogeny. The oldest fossil finding in *Cephalanthus* was therefore used to place a minimum age constraint of 33.9 Ma on the first split in tribe Naucleeae, separating *Cephalanthus* and *Hallea + Nauclea*. The stratigraphical age of this fossil was converted to an absolute age by using the ending point of the geological epoch to which it was assigned, using the time scale of Gradstein et al. (23). In addition, a maximum age constraint of 78 Ma was independently enforced for the basal node in the tree based on the crown age of Gentianales, as estimated from a well-sampled analysis of the asterids calibrated with multiple fossils (16).

Although our approach of using a single fossil calibration may seem too conservative (it could be that some other Rubiaceae fossils are correctly identified and dated) most fossils described in the literature (including the *Posoqueria* and *Cosmibuena* fossils cited in ref. 17) are too young to produce any influence in the molecular dating results obtained by using the *Cephalan-*

thus and root calibrations ([Fig. S4](#)). The only potential exceptions are the following 2 fossils, which may have been correctly dated, but that we do not consider reliable for the reasons specified:

1) Assigned name: *Remijia tenuiflorafolia*
Type of fossil: Leaf traces
Minimum age: 47.46
Country: Argentina
Primary reference: ref. 24.

Comments: In our judgment, the leaf characters originally used for placing these fossils in *Remijia* (size 9.5 × 2.25 cm, secondary veins 10–13, camptodromous venation, equally acute apex and base), as well as the illustrations provided, indicate that these fossil leaves could equally well belong to several other species of Rubiaceae (e.g., *Agouticarpa curviflora*, *Kutchubaea surinamensis*, or *Alibertia bertierifolia*, in tribe Gardenieae) or even to other plant families. Moreover, this taxon was described from southwestern Argentina, which is some 3,000 km south of the southernmost border of the present-day range of the genus ([Fig. S1*D*](#)).

2) Assigned name: *Psychotria eogenica*
Type of fossil: Seeds
Minimum age: 28.4
Country: Peru
Primary reference: ref. 25.

Comments: As traditionally circumscribed, *Psychotria* is one of the most species-rich genera among angiosperms, at most comprising some 1,950 species (26, 27). Phylogenetic analyses have shown, however, that "*Psychotria* is broadly paraphyletic and defined by lack of characters used to define other genera in the tribe" (26). It is thus not surprising that the placement of this fossil in *Psychotria* was originally done "with some hesitation for the reason that in all seeds of that genus that I have seen they are smaller than the fossils and the ribbing is more continuous and is also more pronounced, as is also true in the case of related genera such as *Phialanthus* Grisebach and *Ixora* Linné" (25).

All other fossils listed in the Paleobiology Database and the literature for the genera sampled in this study would have been far too recent to reach the lower bound of the age intervals calculated using the 2 calibration points we rely on (i.e., *Cephalanthus* and the root). In sum, we argue that it is a better approach to base our dating analysis on a single (but by all means reliable) fossil, than using several more or less dubious records.

Finally, to account for topological and branch length uncertainty in our age estimates, 1,000 randomly chosen trees from the Bayesian stationary sample were independently dated and results summarized to obtain the median value and 95% credibility intervals of node ages ([Fig. S4](#) and [Table S2](#)), by using the softwares TreeAnnotator and FigTree v. 1.4 (28).

Biogeographic Analysis. *Distribution and altitudinal data.* Except for *Remijia*, all larger genera of Cinchoneae and Isertieae have been the subject of recent taxonomic revisions (29–32), and fairly detailed distribution data are available for all major taxa (29–39). Distribution data on the remaining Neotropical Rubiaceae and the paleotropical genera were obtained from Andersson (40), Bridson and Verdcourt (41), Mabberley (42), Puff (43), and Smith (44). In addition, we searched for recent collections retrieved from various herbaria at the Global Biodiversity Information Facility (www.gbif.org), but such datapoints were only used when they were deemed reliable (e.g., identifications by experts).

Operational areas used in the biogeographic analysis. The delimitation of areas for the biogeographic analysis was based on the extant distribution patterns of Rubiaceae taxa (i.e., congruent distributional ranges shared by 2 or more species) and on geological history, i.e., areas historically isolated from one another by dispersal barriers (45, 46). In South America, we also tried to maximize congruence with other biogeographic studies in the

region by selecting similarly defined areas (47–50). However, our altitudinal boundaries were usually lower than it is commonly adopted, reflecting ecological constraints in our taxon distributions (many Rubiaceae are premontane species). In all, 11 areas were defined:

A: Central America. From southern Mexico (Veracruz, Oaxaca, Tabasco, Campeche, Yucatán and Quintana Roo) south to Panama. Although this region has had a complex geologic history and its land and island connections to South America are still prone to discussion, there has been a long-term isolation from South America until the uplift of the Panama Isthmus at 3.5 Ma (45, 51).

B: West Indies. Excluding Trinidad and Tobago, which are geologically and biologically more closely related to South America than to the other Caribbean islands. Although the Greater and the Lesser Antilles have different geologic histories, they have been long isolated by water from other American landmasses.

C: Northern Andes (10° N–5° S). From Venezuela and Colombia south to northernmost Peru (Piura, Cajamarca, and Amazonas), from elevations >500 m. This area is roughly the same as the Páramo recognized in other biogeographic studies (e.g., refs. 48–50), except that occurrences in this area are arbitrarily coded beginning at altitudes somewhat inferior to the ones generally adopted (due to the distributional patterns shown by our study taxa). Our delimitation of the Northern Andes is slightly different from that of Taylor (45) ranging from 10°N to 3°S. The reason for this is that we wanted the boundary between the Northern and the Central Andes to coincide with the Western Andean Portal (Guayaquil Gap), following Hoorn (52) and Hurgerbühler et al. (53). This region seems to be a major biogeographic barrier for many groups of Neotropical plants (see ref. 54). Also, many Cinchoneae species endemic to area C extend their range down to 5–6°S.

D: Central Andes (5°S–18°S). From Peru (San Martin and La Libertad) southwards to the Tropic of Capricorn, from elevations higher than 500 m. Similarly to the operational area defined for the Northern Andes, this area approximately corresponds to the Puna or Altiplano commonly recognized (e.g., refs. 48–50) but is used here as if occurring from a lower altitudinal limit.

E: The Chocó area. Comprises areas west of the Andes and below 500-m altitude in Colombia (Chocó, El Valle, Cauca, and Nariño), Ecuador, and Peru (Tumbes, Piura). This area is usually recognized by bird biogeographers as a center of endemism (e.g., refs. 47 and 55).

F: Amazonia. Comprises the lowland (<500 m) vegetation in Colombia, Ecuador, Peru, Bolivia, Brazil, Venezuela, Guyana, Suriname, and French Guiana and includes the islands immediately off the South American coast.

G: The Guiana Shield. Includes the elevated (≥500 m) areas in northeastern South America, comprising parts of Venezuela, Guyana, Suriname, French Guiana, and Brazil. It corresponds to the Guianan Bedrock region.

H: Southeastern South America. Mostly comprised of the Brazilian Shield, but also including the lowlands in eastern Brazil and the Rio Paraná drainage. The area corresponds to the pre-Cambrian Brazilian Bedrock formation.

I: Temperate North America. From the Tropic of Cancer northwards. This area is ecologically separated from Central America by the current occurrence of arid and semiarid habitats in northern Mexico.

J: Africa. Separated from South America at about 110–100 Ma (56, 57).

K: Australasia. Includes also Madagascar and the Seychelles, which were separated from Africa together with India already at ≈121 Ma (58).

Dispersal-vicariance analysis. Dispersal-vicariance analysis (DIVA, refs. 59 and 60) was used to infer ancestral distributions at

internal nodes in the phylogeny of Rubiaceae and to identify the biogeographic events involved in the history of the group. DIVA has the advantage over more cladistically oriented methods that it makes no a priori assumptions about the shape or existence of general biogeographic patterns, making it very useful in regions where area relationships have varied greatly through time such as the Neotropics (47). However, like most biogeographic inference methods (61, 62), DIVA requires completely resolved, fully bifurcated trees. This can be a problem because unresolved trees are a common feature of Andean radiations, presumably due to rapid diversification rates. To account for phylogenetic uncertainty in our biogeographic reconstructions, we used here a method that averages DIVA reconstructions over a Bayesian sample of trees (in this case $n = 1,000$) reflecting credibility values on each clade (63). Moreover, it has been observed that integrating over the posterior distribution of trees often reveals preference for a single or more restricted set of solutions, thus reducing the uncertainty in DIVA optimizations (63). DIVA analyses were run unconstrained, i.e., with no constraint in the maximum number of areas applied to ancestral area optimizations (59).

Altitudinal optimization. To infer ancestral altitudinal ranges, Maximum Parsimony (Fitch) optimizations were performed in the software Mesquite v. 2.0.1 (64). Independent optimizations were run on 5,000 Bayesian trees from a stationary tree sample and plotted on the 50% majority-rule consensus tree (compatible groups added) of the Bayesian analysis.

Expanded Results and Discussion. Diversity patterns in Neotropical Rubiaceae show that although it is cosmopolitan in distribution, the highest diversity in family Rubiaceae is distinctly confined to the tropics. Subfamily Rubioideae is pantropically distributed and comprises some highly diverse groups in the Neotropics (e.g., Palicoureeae and Spermacoceae) but is otherwise concentrated to the Old World where it probably originated (65, 66). The majority of species in the subfamily Ixoroideae (except for the "Condamineeae–Calycophylleae" alliance) are also found in the Paleotropics, whereas the subfamily Cinchonoideae, with the exception of tribe Naucleeae, is predominantly Neotropical. There are several tribes (Hamelieae, Hillieae, Chiococceae, Rondeletieae, and Guettardeae) distributed in Central America and the West Indies, where they inhabit wet lowlands and cloud forests, with some species extending their range to southern North America, and others (such as in the genus *Hillia*) confined to South America.

Cinchonoideae is represented in tropical South America by the sister tribes Cinchoneae and Isertieae (1). A list of all currently recognized genera is shown in Table S1 and their species listed in Table S4. Figs. S1–S2 show detailed distribution maps for the main genera in Cinchoneae and Isertieae still not depicted by earlier studies (drawn by Lennart Andersson).

The tribe Isertieae is more or less continuously distributed from Guatemala to central Bolivia and the Amazon mouth (Fig. 1*A*). The greatest species diversity (3–4 species per grid square) is found in eastern Guiana and adjacent parts of the Amazon basin, between 68°W and 74°W, but there is not a very pronounced center of diversity (the highest number of species per grid square is only 5). The range west of 72°W is occupied by *I. haenkeana*, *I. rosea*, and species of the subgenus *Cassupa*, which do not occur east of 66°W. The high species richness in the central Amazon basin is mainly due to the overlap of the ranges of widespread species, whereas the species richness in eastern Guiana and easternmost Amazonia depends mainly on the presence of narrowly restricted endemics.

The distribution range of Cinchoneae (Fig. S1*B*) comprises most of the Neotropical region. Within this range, two distinct centers of diversity can be found, one along the Andes and another in central Amazonia. The Andean center is formed mainly by species of *Cinchona*, *Joosia*, and *Ladenbergia*, whereas the eastern Amazonian center comprises a large number of endemic *Remijia* species. The genera *Ciliosemina*, *Cinchonopsis*, *Maguireocharis*, and *Pimentelia* have too few species to clearly show any center of diversity. Cinchoneae is also present in the Brazilian Shield through two species of *Ladenbergia* (*L. chapadensis* and *L. cujabensis*). There is one species of *Remijia* (*R. ferruginea*) isolated on the southeastern fringe of the Brazilian Shield as well as scattered occurrences of *Ladenbergia hexandra* in the Atlantic coast of southeastern Brazil.

The distribution range of *Ladenbergia* (Fig. S1*C*) covers most of the range of Cinchoneae, but species diversity is highest in the Andes. Most species have relatively restricted ranges (31), with only a few species extending further than 10° in direction north to south and no species further than a few degrees west to east except for *L. oblongifolia*. The majority of Andean species occur at relatively low altitudes, mainly <1,500 m (31). The wide range of *Ladenbergia* outside the Andean area is entirely attributable to a small cluster of species, which appeared to be closely related with each other according to morphology (31). The only exceptions are two species that are not related to these: *Ladenbergia oblongifolia*, which has its main range in the Andes, and also has a disjunct occurrence in the Serra da Neblina, and *L. hexandra*, which is endemic to the mountains of southeastern Brazil.

The distribution range of *Cinchona* (Fig. S2*A*) is Andean, except for *C. pubescens*, which extends its range into southern Central America and the coastal mountains of Venezuela. Like *Ladenbergia*, *Cinchona* has a predominantly premontane to montane range. All records outside this range are probably introductions and, occasionally, subsequent naturalization (32).

The distribution range of *Ciliosemina* (Fig. S2*B*) falls largely within the Northern Andes, but reaches marginal parts of the Amazon basin.

Maguireocharis neblinae is a montane species growing at considerable altitude in the Serra da Neblina (Fig. S2*A*). In the absence of sequence data, and because of its unrevealing morphology, its relationships are obscure (1).

The monotypic genus *Pimentelia* (Fig. S2*B*) occurs on the eastern slopes of the Andes in southern Peru and northern Bolivia, whereas *Stilpnophyllum* (Fig. S2*D*) occurs in the Eastern Cordillera of northern Peru and southern Ecuador, and in the Central Cordillera of northern Colombia. *Pimentelia* could not be sequenced, but morphology indicates that it is the sister group of *Stilpnophyllum* (1). These 6 related species are all confined to the Andes. *Stilpnophyllum* appears to have a wide distribution gap between southern Ecuador and Colombia. This is likely to be real, because montane forests of eastern Ecuador are fairly well explored. Montane forests of northern and central Peru, however, are poorly explored, so the number of localities shown in this region is probably an underestimate.

The range of *Joosia* (Fig. S2*C*) extends over most of the tropical Andean chain, and it is almost fully confined to it. One species, *J. umbellifera*, extends throughout the range of the genus and is the only species occurring north of 1°N. Except for the single locality of *J. multiflora* in southernmost Peru, *J. umbellifera* is also the only species occurring south of 11°S. The largest species diversity of *Joosia* occurs in Ecuador and northern Peru, although species richness is not great anywhere. The slight drop seen in northernmost Peru is almost certainly an artifact caused by poor exploration of this region. Most species occur in the premontane to lower montane life zones, but 3 have only been found in the lowlands, and only 1 in the montane zone (30).

The distribution range of *Cinchonopsis amazonica* (Fig. S1*E*), the only species of the genus, comprises most of the western half of the Amazon Basin, and adjacent parts of the Orinoco drainage. Scattered information on herbarium labels suggests that it grows mainly in upland forest on white sand, which also agrees with the single field observation made by one of us (A.A.).

The range of *Remijia* (Fig. S1*D*) comprises mainly the central parts of the Amazon basin (between ≈55°W and 74°W) and adjacent parts of the Guiana Shield. In addition, there are 2 apparently disjunct areas, 1 in the western Amazonia and 1 in the Serra do Espinhaço in southeastern Brazil. Each of these exclaves has but a single endemic species (*R. chelomaphylla* and *R. ferruginea*, respectively). *Remijia* has a very pronounced center of diversity in central Amazonia, mainly in the Rio Negro, Rio Branco, and Rio Madeira basins, but extending into the Guiana Highlands. This seems to be related to an ecological preference of *Remijia* species for savanna and Amazonian caatinga, biomes that are particularly common in these regions (e.g., ref. 67). However, this is not the only explanation, because some central Amazonian species seem to occur in the rain forest. The phylogeny of Andersson and Antonelli (1) showed 2 sister groups, *R. chelomaphylla*–*R. macrocnemia* and *R. pacimonica*–*R. ulei*. The first 2 species occur only west of the diversity center, *R. chelomaphylla* in the western exclave and *R. macrocnemia* in a relatively small range west and northwest of the Trapecio Amazónico, where it is the only species. The other 2 species occur within the region of high species richness and are morphologically very similar. The apparent east–west sister group relationship may be an artifact, however, because many *Remijia* species have not yet been sampled.

Effects of Taxon Sampling on Biogeographic Reconstruction and Age Estimates. *Biogeographic reconstruction.* Barber and Bellwood (68) showed that biogeographical optimization methods such as dispersal-vicariance analysis could be highly sensitive to incomplete taxon sampling, especially if the missing taxa occupy a basal position in the phylogeny and/or occur in areas that are underrepresented in the analysis.

Although taxon sampling may be a problem in a large family such as Rubiaceae, spanning nearly all continents, we have tried to overcome this problem by using very encompassing areas outside the Neotropics, where most of the Rubioideae and Ixoroideae are found, as well as the first diverging clade in Cinchonoideae, the tribe Naucleeae. Although the small tribes Hamelieae, Hillieae, and Chiococceeae are poorly represented in number of taxa, the distribution of the species included here are representative of their tribes, which are all predominantly distributed in Central America and the Caribbean region. A possible source of "noise" in the optimizations may be caused by *Guettarda speciosa*, an Australasian species that alone represents the tribe Guettardeae. Except for that species, the tribe has a Neotropical distribution (mainly in Central America and the West Indies; ref. 69). To estimate the effect of this bias in ancestral area optimizations, we carried out a new analysis coding the *Guettarda* lineage as restricted to Central America, the West Indies, and both. Except for the ancestor of the clade comprising *Guettarda* and *Rogiera*, the new coding did not affect any other ancestral area optimizations.

For tribes Cinchoneae and Isertieae, which are the focus of this article and upon which our main biogeographic conclusions depend, the key areas are the Northern and Central Andes (areas C and D in Fig. 1 *Inset*). In special, the presence of Central Andean species in a basal position within these tribes could potentially alter the conclusions drawn based on the empirical sequences analyzed.

To test the sensitivity of DIVA reconstructions to incomplete taxon sampling, we first estimated the geographic bias in our sample for each genus included in tribes Isertieae and Cinchoneae. We compiled distribution data for all species, genera, and tribes (Table S4) and compared the representation of each area in the complete inventory against that of our molecular sample. Fig. S6 (left column) shows that the geographical distribution of species in our sample is fairly representative of the actual distribution of the specie. For tribes, area C is slightly under-

represented in Cinchoneae and area D in Isertieae. In contrast, area F (Amazonia) is fairly represented in both tribes. For genera, the geographical bias is largest in *Joosia*, *Stilpnophyllum*, and *Cinchona*, underrepresented in area D, and *Remijia*, which is missing all 8 species endemic to the Guiana Shield and the Brazilian Planalto (areas G and H). Some of these genera are also poorly represented in botanical collections: Most species of *Stilpnophyllum*, and many of *Joosia*, are known from the type specimen only (Fig. S6, right column). The paucity of specimens in botanical collections directly reflects the difficulty to find these species in the field, as evidenced by numerous botanical expeditions undertaken from our research institute in the last decades, as well as by the experience of many colleagues working locally. Although this paucity may sometimes be attributed to poor botanical exploration of some inaccessible regions, most species seem to be naturally rare. In addition, many species are only minimally distinct, which calls into question the species concept applied within each genus and suggests that some of the rarest species could in fact be variations of a more common species, or hybrids (e.g., ref. 32).

Based on this survey (Fig. S6), we then followed a similar approach to Barber and Bellwood (68). We performed a series of heuristic analyses in which a hypothetical missing taxon was added, one at a time, at certain points in the phylogeny. Instead of random addition, we kept the basic backbone of the phylogeny (all major nodes for Cinchonoideae genera) as we did for the temporal simulations (see below). The phylogenetic position of the missing taxa within each genus was chosen following morphological cladograms whenever available (e.g., for genera *Joosia*, *Ladenbergia*). When no morphological phylogeny was available (e.g., genus *Remijia*) or did not include any additional species (genus *Isertia*), the missing taxon was added, alternatively, to the most basal or the most distal node within the genus. The distribution of the missing (hypothetical) taxon was determined following the geographical bias estimated for each genus (see Fig. S6).

Fig. S7 shows the results of these simulations. Somewhat surprisingly, DIVA ancestral area reconstructions proved to be very stable to the addition of missing taxa. Adding hypothetical taxa from underrepresented areas to the phylogeny did not alter the original ancestral area reconstructions (with no taxon addition) for the key nodes in the Isertieae/Cinchoneae phylogeny. The only exception was node 51: The addition of a taxon from areas G or H to the base of the *Remijia* clade (J6, Fig. S7*A*) resulted in the ancestor of *Remijia*, *Ciliosemina*, and *Ladenbergia* being originally distributed in areas G and/or H (J6, Fig. S7*B*). However, this has no consequences for our biogeographic scenario, because ancestral area reconstructions for the rest of the nodes remained unaltered (Fig. S7*B*). That is, *Remijia* would have reached the Guiana Shield and Southeastern South America (areas G and H, respectively) earlier than we postulate in our scenario, but biogeographical inferences concerning the Western Andean Portal and Lakes Pebas would not change. Moreover, these changes in the ancestral distribution of *Remijia* would only occur if the missing taxon really is sister to the rest of the genus: A distal position among the remaining ≈40 species would instead produce the same outcome as in our original reconstruction (J7, Fig. S7).

The only scenario that could significantly alter our hypotheses is if the 2 species from area D (Central Andes) in genus *Joosia* (*J. multiflora* and *J. dichotoma*) and the 2 species from area D in genus *Isertia* (*I. krausei* and *I. reticulata*) came to occupy the 2 most basal positions within the phylogeny of each of these genera (simulations not shown). This would result in area D being inferred as the ancestral area of *Joosia* and of *Isertia*, and area CD as the ancestral area of tribe Cinchoneae. In other words, tribe Cinchoneae would have been originally present in the North and Central Andes instead of dispersing to the Central

Andes from the north after the uplift of the WAP as suggested by our original reconstruction. However, the probability that the 2 Central Andean species of *Joosia* would occupy the 2 most basal positions in the phylogeny of the genus is low. In Andersson's (30) morphological phylogeny, these 2 species are not phylogenetically close: *Joosia multiflora* occupies the most basal position in the cladogram, whereas *J. dichotoma* is "firmly nested within the genus" (p. 28 in ref. 30). Both *Joosia multiflora and J. dichotoma* are known from the type collection only, and we have been unable to find them in the field for sequencing. In the case of *Isertia*, Andersson's (70) morphological phylogeny of Isertieae did not include any Central Andean species. In the only published revision of the genus, Boom (29) included *Isertia krausei* and *I. reticulata* within section *Cassupa*, which is separated from section *Isertia* on the basis of the presence of fruits with fleshy endocarp. However, our molecular phylogeny does not support Boom's infrageneric classification: *Isertia pittieri* and *I. laevis* from section *Cassupa* appear nested within a clade composed exclusively by species from section *Isertia*. Moreover, *Isertia krausei* is known from the type collection only, whereas *I. reticulata* is "a seldom collected and imperfectly known species" that may occur also in Colombia (29).

Out of the eight species of *Cinchona* endemic to the Central Andean region, only one (*C. calisaya*) was possible to include in our analysis. Our molecular phylogeny places *Cinchona calisaya* as the sister group to the rest of the genus, and DIVA infers the Northern and Central Andes (area CD) as the geographic origin of *Cinchona* (node 46). Even if the other 7 Central Andean species in the genus come out as a basal grade in the phylogeny—an unlikely inference according to Andersson's (32) morphological cladogram, which places all Central Andean species in different clades—and D is inferred as the ancestral area of *Cinchona* (with later dispersal to C), this would not affect the ancestral reconstructions for the backbone nodes in Cinchoneae (nodes 44, 45, and 51), which still is inferred as having originated in the Northern Andes, area C.

All things considered, we believe that the possibility of missing taxa from area D to have a significant influence in our scenario is low and that the DIVA results presented here are stable to incomplete taxon sampling.

Age estimates. Linder et al. (71) demonstrated that limited taxon sampling may influence age estimates, generally resulting in younger ages for nodes than those obtained with a denser sample. To investigate these effects in the Rubiaceae, we followed a similar approach to Linder et al. (71): first, we identified 14 "core" nodes across the phylogeny corresponding to all genera in tribes Cinchoneae and Isertieae, main clades in Cinchonoideae including the *Cephalanthus* calibration point, subfamilies Rubioideae and Ixoroideae, and outgroup (nodes 2, 7, 13, 22, 25, 27, 32, 33, 41, 43, 46, 52, 55, and 56 in Fig. S4); second, we generated reduced matrices comprising 30%, 40%, 60%, and 80% of the original dataset by randomly deleting terminal taxa, while still keeping at least 2 species from each core node in order to keep the backbone of relationships in the phylogeny (the basic structure of the phylogeny); third, we performed Bayesian phylogenetic analyses and computed mean-length consensus phylograms for each of the reduced matrices, using the same settings as for the original dataset; and last, we estimated divergence times for each of those phylograms using the same methodology as previously described.

The results of these analyses are summarized in Fig. S8. Although there is a slight tendency to underestimation of ages when taxon sampling decreases (Fig. S8A), for all core nodes in the phylogeny the ages obtained for the reduced matrices fall within the 95% confidence intervals estimated from complete dataset (Fig. S8B). Similarly, even a 2-fold increase in the number of taxa (trend lines in Fig. S8A) is not expected to result in node ages outside the confidence intervals of particular nodes.

The only exceptions are nodes 20, 31, and 33 in the dataset comprising only 30% of the original taxa: their ages are underestimated by 1.3, 0.7, and 0.53 Ma from the original confidence intervals, respectively. It seems that 30% represents a threshold level for those nodes, after which ages stabilize.

These results indicate that the addition of more taxa in Rubiaceae is not expected to significantly influence the empirical results presented, nor their temporal interpretation. Moreover, they support the finding by Linder et al. (71) that Penalized Likelihood is largely insensitive to taxon sampling, as compared with other dating methods such as nonparametric rate smoothing and Bayesian dating.

Generality of the Biogeographic Patterns Found. *Western Andean Portal (WAP).* Although it has never been properly analyzed, it is particularly noteworthy that several studies have demonstrated a biogeographic disjunction between the Northern and Central Andes, roughly corresponding to the latitude used in this study for separating these 2 operational areas (≈5°S). However, the dispersal barrier between these regions has received many different denominations in the literature, which seem to refer to approximately the same area: Western Portal, Andean Gate, Marañón Portal, Guayaquil Gap, Huancabamba Depression, Huancabamba Deflexion, Northern Peruvian Low, and Pirua Divide.

In plants, the pattern has been demonstrated in many families, such as Campanulaceae (72), Calceolariaceae (73), Tropaeolaceae (74), Loasaceae, Passifloraceae, Grossulariaceae (54, 75), and Alstroemeriaceae (76). In animals, Vuilleumier (77) showed such a disjunction for populations of the bird superspecies *Asthenes flammulata* (Furnariidae), Nores (78) defined the region north of this barrier (northwestern Andes) as a general area of bird endemism, and Cortés-Ortiz (79) defined this barrier as the southern limit of the monkey *Alouatta palliata*. Moreover, this region (the Río Marañon in Peru) has long been recognized as the turnover point between the Northern and Central Andean regions of bird endemism (80), further emphasizing the role of the WAP as a major biogeographic barrier. We have not attempted to compile an exhaustive account of taxa whose distribution is affected by the barrier, but anticipate that many other groups may show similar patterns.

Although our results demonstrate how and when the WAP influenced the distribution of a modern group of plants, they cannot provide evidence to its exact nature, geographic extension, and duration. The few paleontological studies in the area strongly support the existence a marine incursion that reached the western part of present-day Amazonia at least during the Late Eocene (81, 82) and Middle Miocene (53), but how long marine settings dominated the region before and after these epochs is a matter of debate. Moreover, contrary to several earlier studies (e.g., ref. 83), new marine geophysical data suggest that global sea levels may have been relatively stable from the Early Eocene onwards (ref. 84 and references therein). Therefore it seems plausible that a marine incursion dominated the WAP during most of the Eocene–Middle Miocene, ending with the uplift of the Eastern Cordillera.

The geographic extent of the WAP has also been under debate. From the Pacific coast, marine settings have been demonstrated to reach as far east as ≈75° W in the Late Eocene (82) and ≈79° W in the Middle Miocene (53). An aquatic connection between the Pacific and Amazonia has long been hypothesized (e.g., 53, 85, 86), but no conclusive evidence seems yet to support such a long connection (F. P. Wesselingh and C. Hoorn, personal communication). However, there is some evidence that intermittent connections existed between Lake Pebas in western Amazonia and the Pacific through the Ecuadorean Andes during the Middle Miocene (87). Even if the WAP would not have been filled by sea water during its entire existence

and even if marine incursions did not reach as far east as Amazonia, it could still have acted as a dispersal barrier to montane organisms: either by being lowland corridors or by imposing ecological constraints (e.g., very dry climates were present in southern Ecuadorean Andes in the Miocene; ref. 87). We make here a plea for more studies that address these important issues.

Lake Pebas. The biogeographic scenario proposed here implies that all plant species adapted to dry land conditions (terra firme) in present-day western Amazonia have gained their current distribution after Lake Pebas drained (i.e., in the last ≈11 Ma; ref. 88). Similarly, in situ speciation in those terrestrial groups could not have taken place before that event.

These results may seem controversial given the fact that western Amazonia is characterized today by an outstanding number of endemic plant species, of which many belong to long-lived woody families not expected to speciate fast. In a recent study using large molecular chronograms from 5 angio-sperm families, Smith and Donoghue (89) showed that there is a strong correlation between growth mode and speciation rates: Herbs generally diversify much more rapidly than shrubs and trees. However, the few molecular dating studies that have dealt with a representative number of Amazonian trees have presented very contrasting results. Two of the largest genera of Neotropical trees have been inferred to have diversified recently: *Inga* (Fabaceae, 300 spp), which is estimated to have started its diversification either 9.8 Ma (based on cpDNA) or 1.6 Ma (based on nrDNA; ref. 90); and *Guatteria* (Annonaceae, 265 spp), where the crown age for all Neotropical taxa (including 2 large Amazonian subclades) is estimated at 7.4 ± 1.4 Ma (91). Among animals, a recent study (92) suggested that the diversification of Amazonian poison frogs (Dendrobatidae) also occurred recently, with radiations in the last 10 Ma. These results fit well into the paleogeographic scenario for Amazonia proposed here and suggest that Amazonian organisms may have evolved at an unusually fast rate after the end of Lake Pebas.

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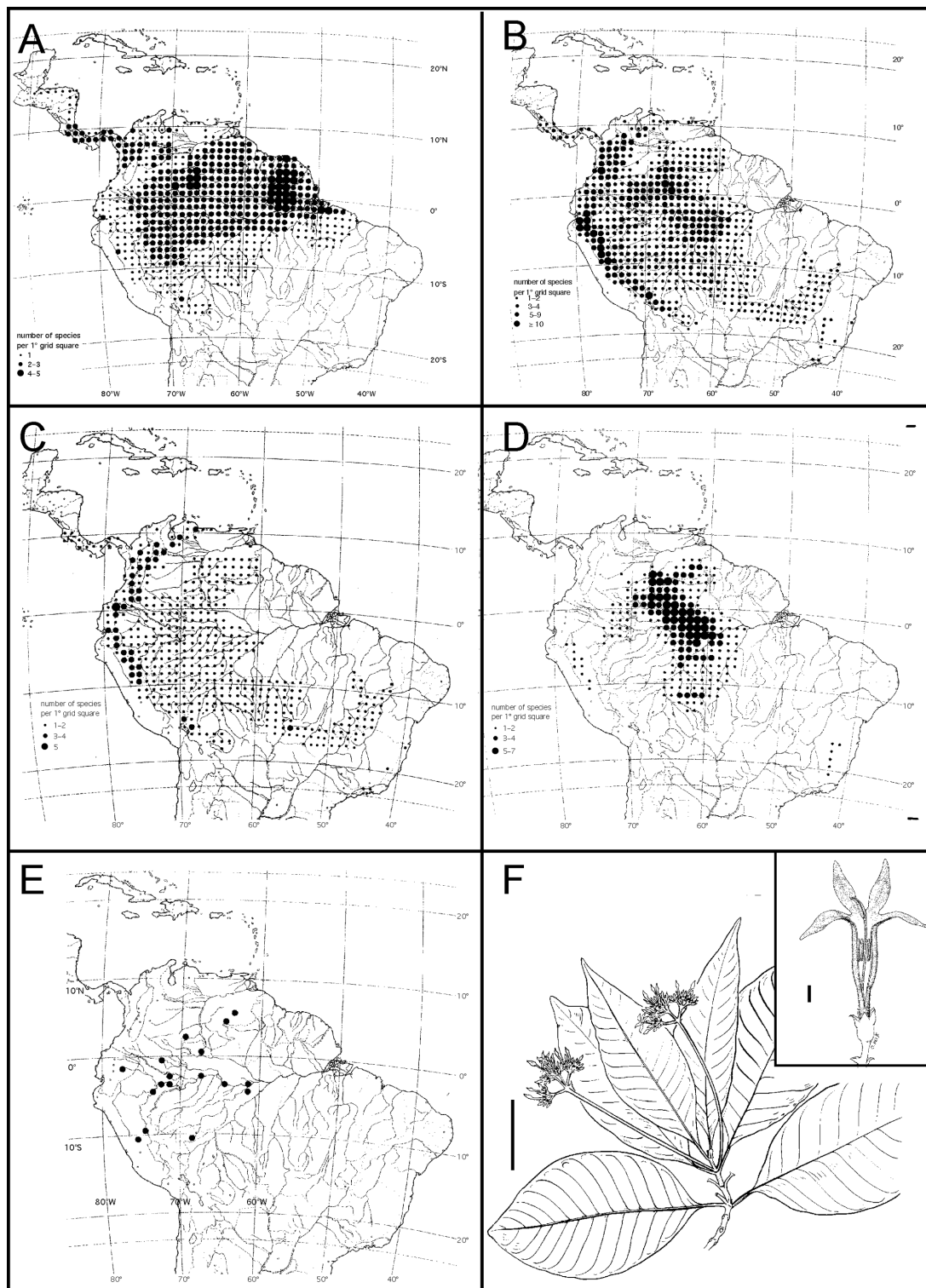
Fig. S1. Distribution patterns in Neotropial Rubiaceae: tribes Isertieae and Cinchoneae. (*A*) Geographical range and species richness of Rubiaceae tribe Isertieae s. str. (based on maps in ref. 29, specimen citations in ref. 38, and localities cited by ref. 36). (*B*) Geographical range and species richness of Rubiaceae tribe Cinchoneae. (*C*) Geographical range and species richness of the genus *Ladenbergia* (based on data from ref. 31). (*D*) Geographical range and species richness of the genus *Remijia* (based on Lennart Andersson's unpublished notes). (*E*) Recorded localities of *Cinchonopsis amazonica* (based on Lennart Andersson's unpublished notes). (*F*) Branch tip with inflorescences of *Ciliosemina pedunculata* and detail of a dissected flower (branch based on herbarium specimen Lleras et al. in Prance 16958, S and flower detail on St. John and Arcila 20627, GB. [Flower image in *F* reproduced with permission from ref. 1 (Copyright 2005, Olof Helje). Branch image in *F* reproduced with permission from ref. 35 (Copyright 1994, University of Gothenburg).]

Fig. S2. Distribution patterns in tribe Cinchoneae. (*A*) Geographical range and species richness of the genus *Cinchona* (based on data from ref. 32), and localities of *Maguireocharis neblinae* recorded by ref. 33. (*B*) Recorded localities of *Ciliosemina* and *Pimentelia* (based on Lennart Andersson's unpublished notes). (*C*) Recorded occurrences of the genus *Joosia* (based on data from ref. 30). (*D*) Geographical range and species richness of the genus *Stilpnophyllum* (based on specimen citations in ref. 35, and Lennart Andersson's unpublished notes).

Fig. S3. Summary of the Bayesian phylogenetic analysis (50% majority-rule consensus tree with compatible groups added). A thick-lined branch indicates that the branch was also present in the majority-rule consensus tree of the parsimony-jackknife analysis. Numbers above branches indicate the posterior probability of the clade. Numbers below branches show jackknife support values, whenever applicable. Brackets identify subfamilies and tribes (CHI, Chiococceae s. lat.; CIN, Cinchoneae s. str.; GUE, Guettardeae; HAM, Hamelieae; HIL, Hillieae; ISE, Isertieae s. str.; NAU, Naucleeae s. lat.; RON, Rondeletieae).
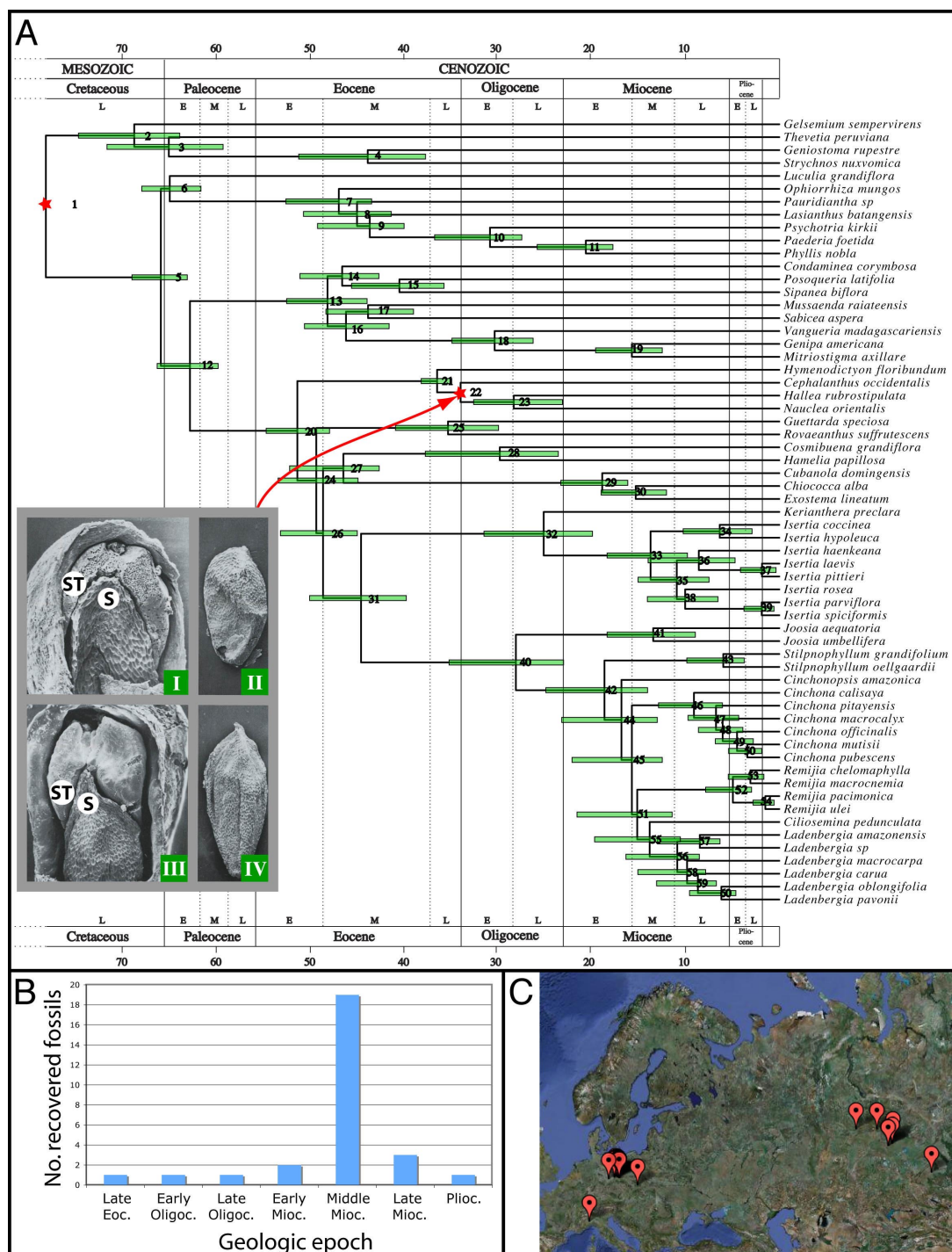
Fig. S4. Molecular dating analysis of the Rubiaceae. (*A*) Chronogram showing 95% confidence intervals of node ages (indicated by bars; *n* = 1,000). Tree topology is the 50% majority-rule Bayesian consensus tree with all compatible groups added. Node numbers refer to Table S2. Red stars indicate calibration points based on molecular and fossil evidence [Node 1: crown group age of Gentianales as determined in the large-scale analysis of Bremer et al. (16); node 22: fruits of *Cephalanthus kireevskianus* from the Late Eocene of Germany reported by Mai and Walther (18)]. (*Inset*) SEM pictures depicting fossil (I–II) and extant (III–IV) fruits of *Cephalanthus*. I, III: Apical part of mericarp with germination valve detached showing characteristic strophiole (ST) and seed (S). II, IV: seed. [Reproduced with permission from ref. 22 (Copyright 1985, The Royal Danish Academy of Sciences and Letters).] (*B*) Number of recovered *Cephalanthus* fruit fossils per geologic epoch. (*C*) Locations of all 19 records of *Cephalanthus* fossilized fruits.
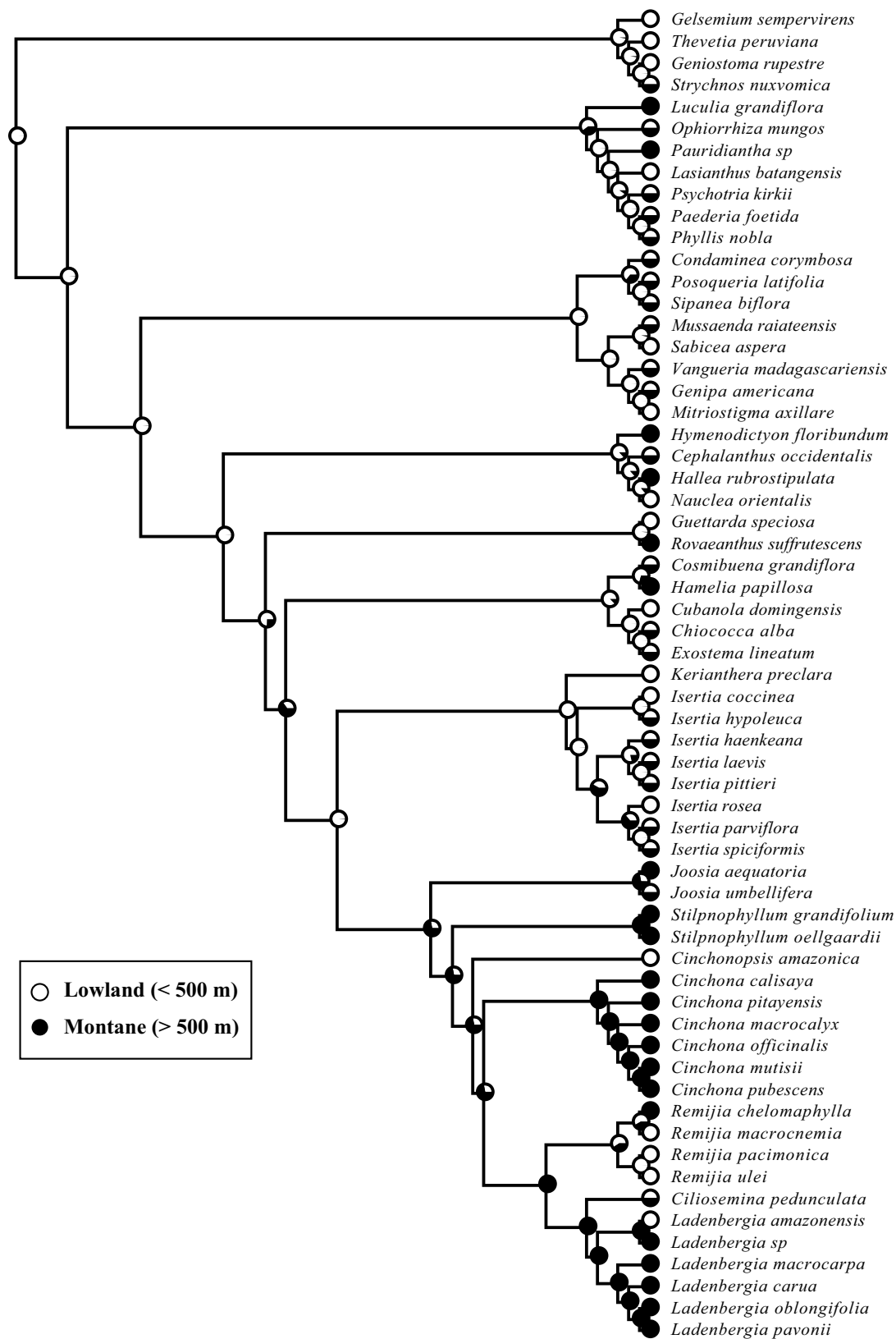
Fig. S5. Altitudinal optimization on ancestral nodes. Pie charts represent relative frequencies of optimizations, based on 5,000 Bayesian trees from a stationary sample. Optimizations performed in the software Mesquite under the Maximum Parsimony (Fitch) criterion, and plotted on the 50% majority-rule consensus tree (compatible groups added) of Fig. 1. Only trees where the relevant node is present were considered in calculating the relative frequencies.

Fig. S6. Estimate of geographic bias on taxon sampling for tribes Isertieae and Cinchoneae. (*Left*) Geographic distribution of all species in the main genera and tribes of subfamily Cinchonoideae, compared with the distribution of species sampled in our phylogeny. The number of species for each chart is given immediately after it. Area codings as in Fig. 1. (*Right*) Number of collected specimens for each species in a genus/tribe. Note that these are minimal numbers, because only specimens verified in taxonomic revisions have been accounted for (additional specimens may have been collected recently and deposited at various herbaria).

A)

Kerianthera praeclara
Isertia coccinea
Isertia hypoleuca
Isertia haenkeana
Isertia laevis
Isertia pittieri
Isertia rosea
Isertia parviflora
Isertia spiciformis
Joosia umbellifera
Joosia aequatoria
Stilpnophyllum grandifolium
Stilpnophyllum oellgaardii
Cinchonopsis amazonica
Cinchona calisaya
Cinchona pitayensis
Cinchona macrocalyx
Cinchona officinalis
Cinchona mutisii
Cinchona pubescens
Remijia chelomaphylla
Remijia macrocnemia
Remijia pacimonica
Remijia ulei
Ciliosemina pedunculata
Ladenbergia amazonensis
Ladenbergia sp.
Ladenbergia macrocarpa
Ladenbergia carua
Ladenbergia oblongifolia
Ladenbergia pavonii

B)

Taxon addition	Ancestral area reconstructions for key nodes													
	31	32	33	40	41	42	43	44	45	46	51	52	55	56
(none)	CF	F	F	C	C	C	C	CF	C	CD	C CF	F CF CDF	C	CD
J_0:D	CF	F	F	C	C	C	C	CF	C	CD	C CF	F CF CDF	C	CD
J_1:D	CF	F	F	C	C	C	C	CF	C	CD	C CF	F CF CDF	C	CD
J_2:D	CF	F	F	C	C	C	C	CF	C	CD	C CF	F CF CDF	C	CD
J_3:D	CF	F	F	C	C	C	C	CF	C	CD	C CF	F CF CDF	C	CD
J_4:D	CF	F	F	C	C	C	C	CF	C	CD	C CF	F CF CDF	C	CD
J_5:D	CF	F	F	C	C	C	C	CF	C	CD	C CF	F CF CDF	C	CD
J_6:G	CF	F	F	C	C	C	C	CF	C	CD	C CF CG CFG	F CF CDF	C	CD
J_6:H	CF	F	F	C	C	C	C	CF	C	CD	C CF CH CFH	F CF CDF	C	CD
J_7:G	CF	F	F	C	C	C	C	CF	C	CD	C CF	F CF CDF	C	CD
J_7:H	CF	F	F	C	C	C	C	CF	C	CD	C CF	F CF CDF	C	CD
J_8:H	CF	F	F	C	C	C	C	CF	C	CD	C CF	F CF CDF	C	CD
J_9:A	CF	F	F	C	C	C	C	CF	C	CD	C CF	F CF CDF	C	CD
J_9:H	CF	F	F	C	C	C	C	CF	C	CD	C CF	F CF CDF	C	CD

Fig. S7. Effect of missing taxa on ancestral area reconstructions. (*A*) Phylogeny of tribes Isertieae and Cinchoneae, as estimated under the Bayesian analysis (50% majority-rule consensus tree with all compatible groups added). J_0–J_9 indicate branches where missing taxa might be expected to attach, as suggested by morphological evidence (e.g., morphology-based cladograms, accounts in taxonomic revisions); when no morphological evidence was available, the missing taxon was attached to the most basal or most distal node of the genus. Node numbers as in Fig. S4. (*B*) Results from DIVA simulations after the addition of missing taxa at each intersection node (J_0–J_9) outlined above. The distribution of the missing (hypothetical) taxon added is given after each intersection node, according to the area codings in Fig. 1 (*Inset*) and based on the geographic bias estimated for each genus (Fig. S6). Ancestral area distributions differing from the original reconstruction (no taxon addition) are marked in yellow.

A)

B)

Node No.	Percentage of taxa included					Ages based on 1000 trees (all taxa included)			Fit of trend line
	30	40	60	80	100	Mean	95% Confidence Interval		R²
							Lower bound	Upper bound	
20	46.5	48.9	49.0	50.3	51.3	51.3	47.8	54.6	0.92
31	39.0	44.1	40.2	41.9	44.5	45.2	39.7	50.0	0.29
32	20.1	22.6	22.7	24.3	25.1	25.0	19.9	31.4	0.93
33	9.25	10.7	12.6	12.8	13.7	13.1	9.78	18.3	0.98
40	24.3	26.7	25.3	22.3	28.0	28.6	22.9	35.1	0.02
42	15.8	17.8	16.7	17.9	18.6	19.0	14.0	24.8	0.62
44	14.3	15.7	14.8	15.8	16.8	17.2	14.0	24.8	0.60
45	13.5	14.7	14.1	14.9	15.7	16.2	12.5	22.0	0.68
46	6.45	6.95	6.92	7.16	9.08	9.00	6.05	12.9	0.57
52	3.63	4.09	4.30	4.59	4.94	5.19	2.96	7.84	0.96
55	12.7	13.8	13.1	13.9	13.8	14.4	10.5	19.7	0.47
56	10.9	11.6	11.1	11.4	10.9	11.8	8.51	16.3	0.00

Fig. S8. Effect of missing taxa on divergence time estimations. (*A*) Ages of key nodes estimated from randomly reduced datasets (including 30%, 40%, 60%, and 80% of the original number of species) and their expected development with the addition of missing taxa (projection curve calculated following a second-degree logarithmic function). Node numbers as in Fig. S4. (*B*) Ground data for the diagram above, compared with age node statistics calculated from the original dataset (all taxa included). Boxes marked in yellow fall outside the 95% confidence interval calculated from 1,000 Bayesian trees inferred from the whole dataset. The last column shows the statistical fit of the trend line to the data points in the diagram.

Table S1. Genera and number of species currently recognized in tribes Cinchoneae and Isertieae

Tribe	Genus	No. of species
Cinchoneae	*Ciliosemina*	2
	Cinchona	23
	Cinchonopsis	1
	Joosia	11
	Ladenbergia	34
	Maguireocharis	1
	Pimentelia	1
	Remijia	41
	Stilpnophyllum	4
Isertieae	*Isertia*	14
	Kerianthera	1

A complete list of the species in each genus is given in Table S4.

Table S2. Crown group ages of all nodes in the phylogeny

	Consensus phylogram	Estimates based on 1000 Bayesian trees		
			95% credibility interval	
Node	Age	Median Age	Lower	Upper
1	78.0	78.0	–	–
2	68.6	68.8	63.8	74.5
3	64.9	65.4	59.2	71.5
4	43.8	44.0	37.6	51.1
5	65.8	66.1	63.0	68.8
6	64.8	65.1	61.5	67.8
7	46.8	47.9	43.4	52.5
8	44.9	46.0	41.3	50.6
9	43.6	44.2	39.9	49.1
10	30.8	31.8	27.4	36.7
11	20.6	21.5	17.7	25.8
12	62.7	62.7	59.7	66.2
13	48.1	48.1	43.8	52.4
14	46.5	46.9	42.6	51.0
15	40.4	41.0	35.7	45.5
16	46.1	45.9	41.5	50.5
17	43.7	43.7	38.9	48.2
18	30.3	30.3	26.2	34.8
19	15.7	15.8	12.5	19.5
20	51.3	51.3	47.8	54.6
21	36.4	36.3	34.8	38.1
22	33.9	33.9	–	–
23	28.2	28.3	23.1	32.5
24	49.2	49.4	44.8	53.3
25	35.2	35.3	29.9	40.8
26	48.5	49.2	44.9	53.1
27	46.4	47.3	42.5	52.1
28	29.7	30.0	23.5	37.6
29	18.8	19.2	16.1	23.2
30	15.3	15.6	12.0	18.9
31	44.5	45.2	39.7	50.0
32	25.1	25.0	19.9	31.4
33	13.7	13.1	9.78	18.3
34	6.34	5.76	2.90	10.2
35	10.9	10.8	7.47	15.0
36	8.56	8.80	4.71	14.0
37	1.85	1.91	0.35	4.12
38	10.0	10.3	6.52	14.0
39	1.87	1.94	0.57	3.76
40	28.0	28.6	22.9	35.1
41	13.4	13.7	8.93	18.3
42	18.6	19.0	14.0	24.8
43	5.97	6.05	3.73	9.83
44	16.8	17.2	14.0	24.8
45	15.7	16.2	12.5	22.0
46	9.08	9.00	6.05	12.9
47	6.74	6.67	4.32	9.71
48	6.02	5.98	3.88	8.60
49	4.50	4.51	2.77	6.80
50	3.40	3.42	1.87	5.39
51	15.1	15.8	11.4	21.5
52	4.94	5.19	2.96	7.84
53	3.08	3.27	1.65	5.43
54	1.49	1.54	0.56	2.80
55	13.8	14.4	10.5	19.7
56	10.9	11.8	8.51	16.3
57	8.46	9.07	6.31	13.0
58	9.81	10.6	7.83	15.0
59	8.66	9.42	6.69	13.1
60	6.16	6.68	4.62	9.55

Statistics based on 2 calculations: Consensus phylogram refers to ages calculated from mean branch lengths of trees sampled by a Bayesian MCMC analysis (*n* = 6,000); Estimates based on 1,000 Bayesian trees refer to median age estimates and 95% credibility intervals calculated by independently dating trees from a random subsample of the Bayesian MCMC analysis (*n* = 1,000). Node numbers refer to Fig. S4.

Table S3. Sequences used in the phylogenetic and biogeographic analyses, together with source and GenBank accession numbers

Taxon				GenBank accession number			
Outgroup	Origin	Voucher	ITS	*mat*K	*rbc*L	*rps*16	*trn*L–F
Gelsemium sempervirens (L.) J. St.-Hil.	GenBank	—	—	Z70195	L14397	AF004092	AF159696
Geniostoma rupestre J.R. Foster & G. Foster	GenBank	—	—	Z70194	Z68828		
Strychnos nux-vomica L.	GenBank	—	—	Z70193	L14410	AF004094	AF102484
Thevetia peruviana K. Schum.	GenBank	—	—	Z70188	X91773	—	
Ingroup							
Cephalanthus occidentalis L.	GenBank	—	—	AY538377	X83629	AF004033	AF152692
Chiococca alba (L.) Hitchc.	GenBank	—	—	AY538378	L14394	AF004034	AF102400
Cinchona calisaya Wedd.	GenBank	—	AY538352	AY538379	AY538478	AF242927	AY538447
C. macrocalyx Pav. ex DC.	GenBank	—	—	AY538380	AY538479	AF538425	AY538448
C. mutisii Lamb.	GenBank	—	AY538353		—	—	AY538426	AY538449
C. officinalis L.	GenBank	—	AY538354	AY538381	AY538480	AY538427	AY538450
C. pitayensis Wedd.	GenBank	—	AY538355	AY538382	AY538481	—	—
C. pitayensis Wedd.	GenBank	—	—	—	—	AF242928	AF152684
C. pubescens Vahl	GenBank	—	AY538356	—	—	—	AY538451
C. pubescens Vahl	GenBank	—		Z70197	X83630	AF004035	
Cinchonopsis amazonica (Standl.) L. Andersson	GenBank	—	AY538357	AY538383	AY538482	AY538428	AY538452
Condaminea corymbosa (Ruiz & Pav.) DC.	GenBank	—	—	AY538384	Y18713	AF004039	AF102406
Cosmibuena grandiflora (Ruiz & Pav.) Rusby	GenBank	—	—	AY538385	AY538483	AF242929	AF152686
Cubanola domingensis (Britton) Aiello	GenBank	—	—	AY538386	X83632	AF004044	AF152701
Exostema lineatum (Vahl) Roem. & Schult.	GenBank	—	—	AY538387	AY538484	AF242944	AF152698
Genipa americana L.	GenBank	—	—	AY538388	Z68839	AF200997	AF201045
Guettarda speciosa L.	GenBank	—	—	AY538389	AY538485	AF246924	AF152725
Hallea rubrostipulata (K. Schum.) J.-F. Leroy	GenBank	—	—	AY538390	AY538486	AF538429	AY538453
Hamelia papillosa Urb.	GenBank	—	—	AY538391	AY538487	AF004053	AF102439
Hymenodictyon floribundum Robinson	GenBank	—	—	AY538392	AY538488	AF004058	AY538454
Isertia coccinea (Aubl.) J.F. Gmel.	GenBank	—	AY538358	AY538393	AY538489	AY538430	AY538455
I. laevis (Triana) Boom	GenBank	—	AY538359	AY538394	AY538490	AY538431	AY538456
I. haenkeana DC.	Colombia	Alzate et al. 203 (GB),	DQ448607*	—	DQ448595*	DQ448601*	—
I. hypoleuca Benth.	Colombia	Andersson et al. 2173 (GB)	DQ448608*	—	DQ448596*	DQ448602*	—
I. parviflora Vahl	French Guiana	Andersson et al. 1969 (GB),	DQ448609*	—	DQ448597*	DQ448603*	—
I. pittieri (Standl.) Standl.	Colombia	Andersson et al. 2099 (GB)	DQ448610*	—	DQ448598*	DQ448604*	—
I. rosea Spruce ex K. Schum.	Ecuador	Hekker & Hekking 10.147 (GB)	DQ448611*	—	DQ448599*	DQ448605*	—
I. spiciformis DC.	French Guiana	Andersson et al. 1905 (GB	DQ448612*	—	DQ448600*	DQ448606*	—
Joosia aequatoria Steyerm.	GenBank	—	AY538360	AY538395	AY538491	AY538432	AY538457
J. umbellifera H. Karst.	GenBank	—	AY538361	AY538396	AY538492	AY538433	AY538458
Kerianthera preclara Kirkbr.	GenBank	—	AY538362	AY538397	AY538493	AF242970	AY538459
Ladenbergia amazonensis Ducke	GenBank	—	AY538363	AY538398	AY538494	AY538434	AY538460
L. carua (Wedd.) Standl.	GenBank	—	AY538364	AY538399	AY538495	AY538435	AY538461
L. macrocarpa (Vahl) Klotzsch	GenBank	—	AY538365	AY538400	AY538496	AF242971	AF152683
L. oblongifolia (Mutis) L. Andersson	GenBank	—	AY538366	AY538401	AY538497	AY538436	AF538462
L. pavonii (Lamb.) Standl.	GenBank	—	AY538367	AY538402	Z68801	AY538437	AY538463
L. sp. (prob. nova)	GenBank	—	AY538368	AY538403	AY538498	AY538438	AY538464
Lasianthus batangensis K. Schum.	GenBank	—	—	AY538404	AY538499	AY538439	AY538465
Luculia grandiflora Ghose	GenBank	—	—	Z701999	X83648	AF242974	AF102453
Mitriostigma axillare Hochst.	GenBank	—	—	AY538405	X83650	AF201006	AF201054
Mussaenda raiateensis J.W. Moore	GenBank	—	—	AY538406	AY538500	AF242983	AY538466
Nauclea orientalis L.	GenBank	—	—	AY538407	AY538501	AY538440	AY538467
Ophiorrhiza mungos L.	GenBank	—	—	AY538408	X83656	AF004064	AF152610
Paederia foetida L.	GenBank	—	—	AY538409	AF332373	AF004065	AF152619
Pauridiantha sp.	GenBank	—	—	AY538410	AY538502	AF004068	AF102467
Phyllis nobla L.	GenBank	—	—	AY538411	Z68814	AF003613	AY538468
Posoqueria latifolia (Rudge) Roem. & Schult.	GenBank	—	—	AY538412	Z68850	AF242998	AF152680
Psychotria kirkii Hiern	GenBank	—	—	AY538413	X83663	AF410728	AY538469
Remijia chelomaphylla G.A. Sullivan	GenBank	—	AY538369	AY538414	AY538503	AY538441	AY538470
R. macrocnemia (Mart.) Wedd.	GenBank	—	AY538371	AY538415	AY538504	AY538442	AY538471
R. pacimonica Standl.	GenBank	—	AY538372	AY538416	AY538505	AY538443	AY538472
R. pedunculata (H. Karst.) Flueck.	GenBank	—	AY538373	AY538417	AY538506	AY538444	AY538473
R. ulei K. Krause	GenBank	—	AY538374	AY538418	AY538507	AY538445	AY538474
Rovaeanthus suffrutescens (Brandeg.) Borhidi	GenBank	—	—	AY538419	X83665	AF243003	AF152738
Sabicea aspera Aubl.	GenBank	—	—	AY538420	AY538508	AF004079	AY538475
Sipanea biflora (L.f.) Cham. & Schltdl.	GenBank	—	—	AY538421	AY538509	AF004085	AF152675
Stilpnophyllum grandifolium L. Andersson	GenBank	—	AY538375	AY538422	AY538510	AY538446	AY538476
S. oellgaardii L. Andersson	GenBank	—	AY538376	AY538423	AY538511	AF243026	AY538477
Vangueria madagascariensis J.F. Gmel.	GenBank	—	—	AY538424	X83670	AF243033	AF152654

Data on origin and voucher are given only for sequences added in this study (indicated by an asterisk).

Table S4. Checklist of species and genera in the sister tribes Cinchoneae and Isertieae, their distribution, and number of collections verified in recent taxonomic revisions (see *SI Text* for references)

Species count in tribes	Species count in genera	Genus	Species	Distribution	No. of collections
1	**1**	***Ciliosemina***	***pedunculata***	**CF**	**>6**
2	2	*Ciliosemina*	*purdieana*	F	2
3	1	*Cinchona*	*antioquiae*	C	>6
4	2	*Cinchona*	*asperifolia*	D	2
5	3	*Cinchona*	*barbacoensis*	CE	>6
6	**4**	***Cinchona***	***calisaya***	**D**	**>6**
7	5	*Cinchona*	*capuli*	C	>6
8	6	*Cinchona*	*fruticosa*	C	2
9	7	*Cinchona*	*glandulifera*	D	5 or 6
10	8	*Cinchona*	*hirsuta*	D	4
11	9	*Cinchona*	*krauseana*	D	5 or 6
12	10	*Cinchona*	*lancifolia*	C	>6
13	11	*Cinchona*	*lucumifolia*	C	>6
14	**12**	***Cinchona***	***macrocalyx***	**CD**	**>6**
15	13	*Cinchona*	*micrantha*	CD	>6
16	**14**	***Cinchona***	***mutisii***	**C**	**>6**
17	15	*Cinchona*	*nitida*	D	5
18	**16**	***Cinchona***	***officinalis***	**C**	**>6**
19	17	*Cinchona*	*parabolica*	CD	6
20	**18**	***Cinchona***	***pitayensis***	**C**	**>6**
21	**19**	***Cinchona***	***pubescens***	**ACD**	**>6**
22	20	*Cinchona*	*pyrifolia*	D	3
23	21	*Cinchona*	*rugosa*	C	>6
24	22	*Cinchona*	*scrobiculata*	C	1 (type only)
25	23	*Cinchona*	*villosa*	C	2
26	**1**	***Cinchonopsis***	***amazonica***	**F**	**>6**
27	**1**	***Isertia***	***coccinea***	**F**	**>6**
28	**2**	***Isertia***	***haenkeana***	**ABCEF**	**>6**
29	**3**	***Isertia***	***hypoleuca***	**ACFG**	**>6**
30	4	*Isertia*	*krausei*	D	1 (type only)
31	**5**	***Isertia***	***laevis***	**ACDE**	**>6**
32	6	*Isertia*	*longifolia*	F	>6
33	**7**	***Isertia***	***parviflora***	**FG**	**>6**
34	**8**	***Isertia***	***pittieri***	**EC**	**>6**
35	9	*Isertia*	*reticulata*	D	5
36	**10**	***Isertia***	***rosea***	**F**	**>6**
37	11	*Isertia*	*scorpioides*	A	5
38	**12**	***Isertia***	***spiciformis***	**FG**	**>6**
39	13	*Isertia*	*verrucosa*	F	5
40	14	*Isertia*	*wilhelminensis*	G	3
41	**1**	***Joosia***	***aequatoria***	**C**	**2**
42	2	*Joosia*	*dichotoma*	D	>6
43	3	*Joosia*	*dielsiana*	C	>6
44	4	*Joosia*	*longisepala*	C	2
45	5	*Joosia*	*macrocalyx*	C	1 (type only)
46	6	*Joosia*	*multiflora*	D	1 (type only)
47	7	*Joosia*	*obtusa*	C	1 (type only)
48	8	*Joosia*	*oligantha*	C	1 (type only)
49	9	*Joosia*	*pulcherrima*	CD	>6
50	10	*Joosia*	*standleyana*	C	3
51	**11**	***Joosia***	***umbellifera***	**ACDF**	**>6**
52	**1**	***Kerianthera***	***preclara***	**F**	**4**
53	1	*Ladenbergia*	*acutifolia*	D	4
54	**2**	***Ladenbergia***	***amazonensis***	**DFG**	**>6**
55	3	*Ladenbergia*	*brenesii*	A	>6
56	4	*Ladenbergia*	*bullata*	D	2
57	5	*Ladenbergia*	*buntingii*	C	3
58	**6**	***Ladenbergia***	***carua***	**D**	**>6**
59	7	*Ladenbergia*	*chapadensis*	H	1 (type only)
60	8	*Ladenbergia*	*cujabensis*	H	>6
61	9	*Ladenbergia*	*discolor*	D	6
62	10	*Ladenbergia*	*dwyeri*	A	>6
63	11	*Ladenbergia*	*epiphytica*	C	1 (type only)
64	12	*Ladenbergia*	*ferruginea*	D	1 (type only)
65	13	*Ladenbergia*	*graciliflora*	DFH	>6
66	14	*Ladenbergia*	*heterophylla*	AC	>6
67	15	*Ladenbergia*	*hexandra*	H	>6
68	16	*Ladenbergia*	*klugii*	D	1 (type only)
69	17	*Ladenbergia*	*lambertiana*	FG	>6
70	18	*Ladenbergia*	*laurifolia*	A	3

Species count in tribes	Species count in genera	Genus	Species	Distribution	No. of collections
71	19	*Ladenbergia*	*lehmanniana*	C	3
72	**20**	***Ladenbergia***	***macrocarpa***	**C**	**>6**
73	21	*Ladenbergia*	*magdalenae*	C	>6
74	22	*Ladenbergia*	*moritziana*	C	>6
75	23	*Ladenbergia*	*muzonensis*	CEF	>6
76	24	*Ladenbergia*	*nubigena*	A	5
77	**25**	***Ladenbergia***	***oblongifolia***	**CDEFG**	**>6**
78	26	*Ladenbergia*	*obovata*	C	3
79	27	*Ladenbergia*	*paraensis*	F	1 (type only)
80	28	*Ladenbergia*	*pauciflora*	C	1 (type only)
81	**29**	***Ladenbergia***	***pavonii***	**CE**	**>6**
82	30	*Ladenbergia*	*pittieri*	C	>6
83	31	*Ladenbergia*	*riveroana*	C	>6
84	32	*Ladenbergia*	*rubiginosa*	CE	2
85	33	*Ladenbergia*	*stenocarpa*	C	>6
86	34	*Ladenbergia*	*undata*	C	>6
87	1	*Maguireocharis*	*neblinae*	G	2
88	1	*Pimentelia*	*glomerata*	CD	>6
89	1	*Remijia*	*amazonica*	F	not revised
90	2	*Remijia*	*amphithrix*	F	not revised
91	3	*Remijia*	*aracamuniensis*	G	not revised
92	4	*Remijia*	*argentea*	FG	not revised
93	5	*Remijia*	*asperula*	F	not revised
94	6	*Remijia*	*berryi*	F	not revised
95	7	*Remijia*	*bracteata*	F	not revised
96	**8**	***Remijia***	***chelomaphylla***	**CD**	**not revised**
97	9	*Remijia*	*delascioi*	F or G	not revised
98	10	*Remijia*	*densiflora*	G	not revised
99	11	*Remijia*	*duckei*	F	not revised
100	12	*Remijia*	*ferruginea*	H	not revised
101	13	*Remijia*	*firmula*	FG	not revised
102	14	*Remijia*	*globosa*	G	not revised
103	15	*Remijia*	*glomerata*	F	not revised
104	16	*Remijia*	*hispida*	F	not revised
105	17	*Remijia*	*involucrata*	F	not revised
106	18	*Remijia*	*leiocalyx*	E	not revised
107	19	*Remijia*	*longifolia*	F	not revised
108	**20**	***Remijia***	***macrocnemia***	**F**	**not revised**
109	21	*Remijia*	*macrophylla*	C	not revised
110	22	*Remijia*	*maguirei*	G	not revised
111	23	*Remijia*	*marahuacensis*	F	not revised
112	24	*Remijia*	*megistocaula*	F	not revised
113	25	*Remijia*	*morilloi*	F	not revised
114	**26**	***Remijia***	***pacimonica***	**F**	**not revised**
115	27	*Remijia*	*paniculata*	H	not revised
116	28	*Remijia*	*peruviana*	F	not revised
117	29	*Remijia*	*physophora*	F	not revised
118	30	*Remijia*	*pilosinervula*	G	not revised
119	31	*Remijia*	*prismatostylis*	C	not revised
120	32	*Remijia*	*reducta*	F	not revised
121	33	*Remijia*	*roraimae*	FG	not revised
122	34	*Remijia*	*sessilis*	F	not revised
123	35	*Remijia*	*sipapoensis*	G	not revised
124	36	*Remijia*	*steyermarkii*	G	not revised
125	37	*Remijia*	*tenuiflora*	F	not revised
126	38	*Remijia*	*trianae*	F	not revised
127	**39**	***Remijia***	***ulei***	**F**	**not revised**
128	40	*Remijia*	*vaupesiana*	F	not revised
129	41	*Remijia*	*wurdackii*	F	not revised
130	**1**	***Stilpnophyllum***	***grandifolium***	**C**	**1 (type only)**
131	2	*Stilpnophyllum*	*lineatum*	D	1 (type only)
132	**3**	***Stilpnophyllum***	***oellgaardii***	**C**	**2**
133	4	*Stilpnophyllum*	*revolutum*	C	1 (type only)

Species in bold type were included in the molecular phylogeny. Distribution codings are according to Fig. 1 *Inset*.