

**MONOPHYLY OF *KELLOGGIA* TORREY EX BENTH.  
(RUBIACEAE) AND EVOLUTION OF ITS  
INTERCONTINENTAL DISJUNCTION BETWEEN WESTERN  
NORTH AMERICA AND EASTERN ASIA<sup>1</sup>**

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*Kelloggia* Torrey ex Benth (Rubiaceae) consists of two species disjunctly distributed in western North America (*K. galioides* Torrey) and the western part of eastern Asia (*K. chinensis* Franch.). The two species exhibit a high level of morphological divergence. To test its monophyly and to infer its biogeographic history, we estimated the phylogeny of *Kelloggia* and its relatives from sequences of three chloroplast DNA regions (*rbcL* gene, *atpB-rbcL* spacer, and *rps16* intron). The monophyly of *Kelloggia* was strongly supported, and it forms a sister relationship with the tribe Rubieae. The divergence time between the two disjunct species of *Kelloggia* was estimated to be  $5.42 \pm 2.32$  million years ago (mya) using the penalized likelihood method based on *rbcL* sequence data with fossil calibration. Our result does not support the Madrean-Tethyan hypothesis, which assumes an earlier divergence time of 20–25 mya. Ancestral area analysis, as well as dispersal-vicariance (DIVA) analysis, suggests the Asian origin of *Kelloggia* and the importance of Eurasia in the diversification of its close relatives in the Rubieae-Theligoneae-Paederieae group. The intercontinental disjunction in *Kelloggia* is suggested to have evolved via long-distance dispersal from Asia into western North America.

**Key words:** disjunction; eastern Asia; *Kelloggia*; monophyly; Rubiaceae; western North America.

The disjunct distribution of plants between eastern Asia and North America has attracted much attention from botanists, zoologists, mycologists, and biogeographers since the time of Linnaeus (Boufford and Spongberg, 1983; Wen, 1999; Mueller et al., 2001; Sanmartín et al., 2001). Most studies have focused on the eastern Asian and eastern North American subset of this disjunct pattern (Wen, 1999). Another common pattern between the two continents is the eastern Asian and western North American disjunction (Li, 1952; Thorne, 1972; Wu, 1983; Hong, 1993; Wen, 1999), which has been rarely examined using modern phylogenetic and biogeographic methods. The floristic migrations between eastern Asia and North America have been hypothesized to be via either the Bering land bridge across the northern Pacific (Hopkins, 1967) or the North Atlantic land bridge indirectly across the Tethys Seaway, southwestern Europe, and the Thulean land bridge (Tiffney, 1985a). Both migration pathways perhaps allowed the wide distribution of many disjunct plants in the Northern Hemisphere in the Tertiary. The modern disjunction of plants in eastern Asia and North America has largely resulted from the range restrictions of formerly widespread mesophytic forests (Leopold et al., 1972; Wood, 1972; Axelrod, 1975; Tiffney, 1985b; Boulter, 1994; Collinson, 1994; Manchester, 1999;

Wen, 1999; Tiffney and Manchester, 2001; Weber, 2003). The mesophytic forest became widely discontinuous in distribution as late as the middle Miocene in eastern Asia and eastern and western North America because of orogenies and subsequent development of a xeric and higher-elevation coniferous flora in western North America and the gradual cooling of the climate in the Northern Hemisphere, which finally brought heavy glaciations in Europe and in other regions of higher northern latitudes (Graham, 1972; Wolfe, 1972; Hoey and Parks, 1991; Wen, 1999).

Another important disjunct pattern in the Northern Hemisphere is the disjunction among western North America, the Mediterranean region, and the Tibetan Plateau (Graham, 1999; Sun and Li, 2003). Evidence from fossil floras across the Northern Hemisphere indicates the presence of a rich, subtropical or warm temperate forest as the north-Tethyan floristic belt that extended across Laurasia from California in the west through southeastern North America, Europe, and Mediterranean to eastern Asia in the east, even to north China and Japan from the Cretaceous to the Eocene (Takhtajan, 1969; Axelrod, 1975; Raven and Axelrod, 1978; Kubitzki and Krutzsch, 1996). Recent studies on Malpighiaceae also show that this belt is a conduit of Laurasian migration between the Old and the New Worlds (Davis et al., 2002). Sun and Li (2003) suggest that the disjunction between eastern Himalayas and western North America may be related to the pattern between Mediterranean and western North America.

At present, molecular phylogenetic data have been generated only for two genera with an eastern Asian and western North American disjunction: *Oplopanax* (Miq.) Miq. (Lee and Wen, 2002) of Araliaceae and *Glehnia* Schmidt ex Miq. of Apiaceae (Sun et al., 2004). *Oplopanax* was suggested to have diverged between the two continents in the Pliocene about 5.6

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TABLE 1. Morphological and ecological differences between *Kelloggia galioides* and *K. chinensis* (Rubiaceae).

Character	<i>K. galioides</i>	<i>K. chinensis</i>
Stem	15–60 cm tall, shrub-like herb, glabrous	5–20 cm tall, slender herb, hairy
Leaf	15–50 mm long and 2–15 mm wide, glabrous	6–15 mm long and 2–5 mm wide, hairy
Stipule	ca. 1 mm in length and width; usually entire or bifid	2–4 mm long, 2–3 mm wide; fimbriate, 3–7-fid
Flower	4–5-merous, 4–8 mm long, corolla pink or white	5-merous, 3–6 mm long, corolla red
Pedicel	10–25 mm long, swelling at the tip, without obvious elongation after anthesis	1–3 mm long, no swelling at the tip, elongated up to 20 mm at fruiting
Fruit	obovoid in shape, 3–4 mm long; the two halves readily separable	nearly globose in shape, 2–3 mm long, the two halves not readily separable
Ecology	open places in coniferous forests, wooded or open slopes in the mountains, among rocks, or along streams	alpine meadows or open places in shrublands, or along streams
Elevation	1100–3000 m	about 3000 m
Distribution	western USA: California, Nevada, Iowa, Washington, Wyoming, Utah, Arizona	southwestern China: in the Hengduan Mountains of northwestern Yunnan, western Sichuan, and eastern Tibet

million years ago (mya). The two species of *Glehnia* were found to be non-monophyletic. The times of divergence and the migration history of congeners between eastern Asia and western North America need to be analyzed in many more plant groups before any generalizations can be made on the evolution of such a disjunction.

*Kelloggia* Torrey ex Bentham of Rubiaceae shows such a disjunction between eastern Asia and western North America. It consists of only two species: *K. chinensis* Franch. (Franchet, 1892) from the Hengduan Mountains of southwestern China (Smith, 1921; Handel-Mazzetti, 1936; Dempster, 1975; Chen, 1999; Fang, 2003) and *K. galioides* Torrey from the western United States (Bentham and Hooker, 1873; Torrey, 1874; Hitchcock and Cronquist, 1973; Hickman, 1993). *Kelloggia chinensis* is distributed at the western edge of eastern Asia, close to central Asia and the Mediterranean region. Many close relatives of *Kelloggia* (such as *Putoria* Pers. and *Plocama* Aiton in the tribe Paederieae) also occur in the Mediterranean region (Puff, 1982). The biogeographic diversification of *Kelloggia* and its close relatives needs to be examined in a broader phylogenetic framework including taxa across the Northern Hemisphere.

*Kelloggia* is characterized by a combination of characters including well-developed creeping rhizomes, opposite, entire, and sessile leaves with small interpetiolar stipules, perfect and four- to five-merous flowers with short funnel-shaped corolla tubes, and small indehiscent fruits covered with hooked bristles. In addition, the whole plant becomes black after drying, which is similar to plants in Paederieae, but in contrast to those in Rubieae. It was recognized by some workers as a monotypic genus, consisting of *K. galioides* from western North America only (Davis, 1952; Munz and Keck, 1959; Abrams and Ferris, 1960; Munz, 1974; Dempster, 1975). The taxonomic and phylogenetic status of the eastern Asian *K. chinensis* has not been examined since its original description by Franchet in 1892. The two widely disjunct species of *Kelloggia* are morphologically highly distinct (Table 1 and Fig. 1). Ecologically *K. chinensis* occurs in alpine meadows or open places or shrublands along the stream of high mountains (about 3000 m a.s.l.), whereas *K. galioides* grows in somewhat open places in coniferous forests (1100–3000 m a.s.l.), sometimes among rocks or along streams (Hickman, 1993).

The phylogenetic position of *Kelloggia* within Rubioideae has long been controversial. Hooker (1873) originally placed it in the tribe Anthospermeae (also see Baillon, 1879; Schumann, 1891). Puff (1982) placed all the wind-pollinated genera

in the tribe Anthospermeae and insect-pollinated genera in the tribe Paederieae and transferred the insect-pollinated *Kelloggia* to the tribe Paederieae. Bremer and Manen (2000) did not include *Kelloggia* in their broad molecular analysis of Rubioideae, yet they still recognized *Kelloggia* in the paraphyletic tribe Paederieae.

Because the two species of *Kelloggia* exhibit a high level of morphological differentiation and are widely disjunct in two highly isolated biogeographic regions, we attempt to: (1) test the monophyly of *Kelloggia* using sequences of three DNA markers, (2) if they form a sister species relationship, estimate the divergence time of the two species, and (3) discuss the evolution of the disjunct pattern between eastern Asia and western North America in *Kelloggia*.

## MATERIALS AND METHODS

**Taxa and sequencing**—Six populations representing both species of *Kelloggia* and one of *Paederia scandens* (Lour.) Merr. were sequenced (Appendix 1; see Supplemental Data accompanying the online version of this article). To evaluate the monophyly of genus and its phylogenetic position, sequences of the close relatives of *Kelloggia* in Rubiaceae, especially tribes Paederieae and Rubieae (Puff, 1982; Andersson and Rova, 1999; Bremer and Manen, 2000), were obtained from GenBank (Appendix 2; see Supplemental Data accompanying the online version of this article). The outgroups were chosen in agreement with results from earlier investigations (Bremer and Manen, 2000); three taxa (*Hillia*, *Faramea*, and *Mitchella*) were selected from groups outside of tribes Anthospermeae, Paederieae, and Rubieae in Rubiaceae (Fig. 2).

DNAs were extracted with the CTAB method of Doyle and Doyle (1987). Three chloroplast markers including *rps16* intron, *atpB-rbcL* spacer, and *rbcL* gene were selected because many sequences of these markers are already available for Rubiaceae (e.g., Bremer et al., 1995; Manen and Natali, 1996; Andersson and Rova, 1999; Bremer and Manen, 2000). The DNA regions were amplified following the protocols in Bremer et al. (1995) for *rbcL*, Andersson and Rova (1999) for the *rps16* intron, and Bremer and Manen (2000) for the *atpB-rbcL* spacer. DNA amplifications were performed in 20  $\mu$ L reactions with approximately 10–50 ng of total DNA, 20 mmol/L Tris buffer (pH 8.3, with 50 mmol/L KCl, 1.5 mmol/L MgCl<sub>2</sub>, and 0.1% Tween 20), 0.15 mmol/L of each dNTP, 5  $\mu$ mol/L of each primer, 2  $\mu$ L of *Taq* polymerase. The polymerase chain reactions (PCR) were run on a Peltier Thermal Cycler DNA engine DYAD (MJ Research, Watertown, Massachusetts, USA) for 37 cycles starting at 94°C for 2 min, followed by denaturation (94°C for 40 s), annealing (50°C for 2 min), and extension (72°C for 2 min). A 5-min final extension at 72°C followed the 37th cycle to ensure the completion of novel strands. To be cleaned for sequencing, the subsequent PCR products were then run on a 1% low-melting agarose gel, and the bands excised and gelled, using the GELase (Epicentre Technologies, Madison, Wisconsin, USA) method. Cycle sequencing was conducted using BigDye version 3 re-

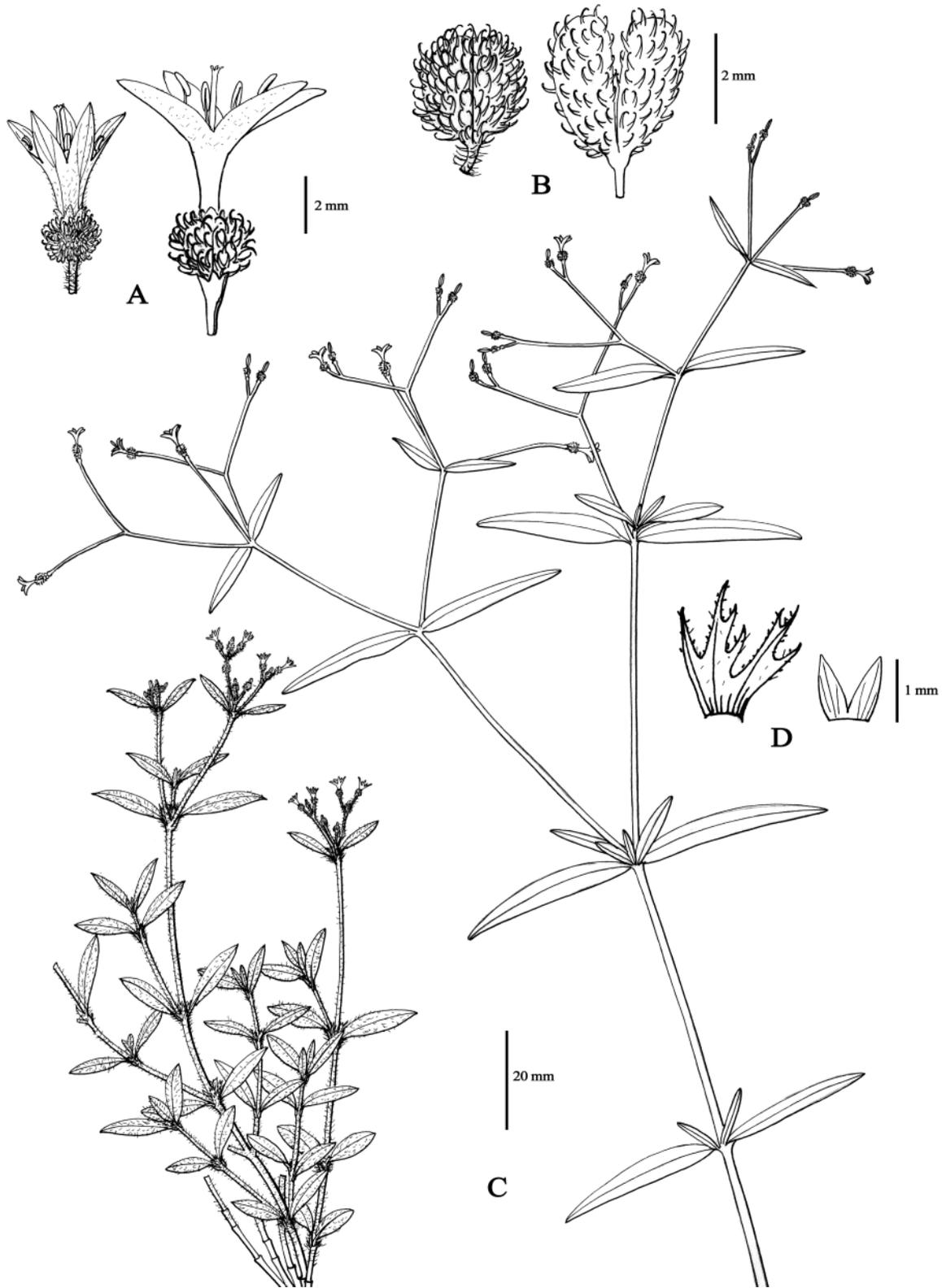


Fig. 1. Morphological comparisons of *Kelloggia chinensis* (left) and *K. galioides* (right). (A) flower, (B) fruit, (C) habit, (D) stipule.

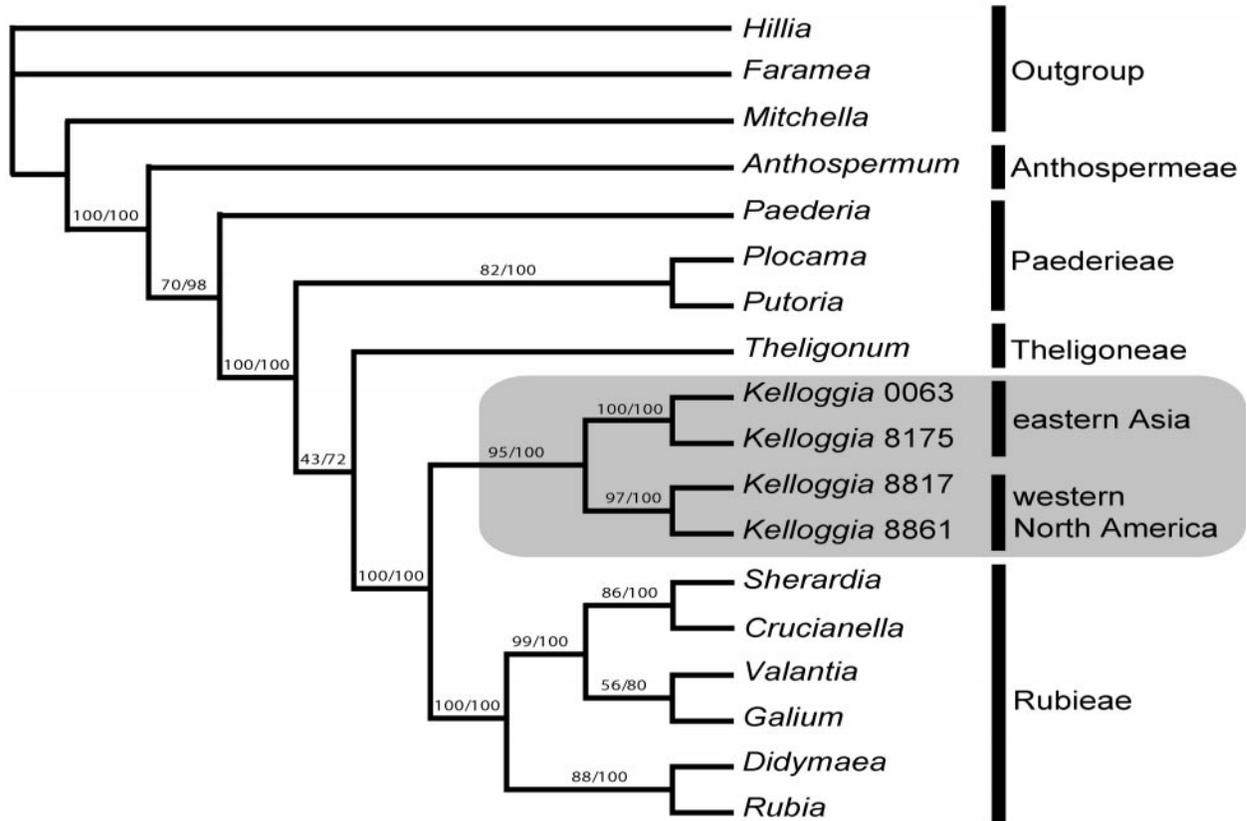


Fig. 2. Single most parsimonious tree of *Kelloggia* and its close relatives based on the combined data set of *rps16* intron, *atpB-rbcL* spacer, and *rbcL*, treating gaps as missing data (tree length = 981 steps, consistency index excluding uninformative characters = 0.6221, retention index = 0.7211, and rescaled consistency index = 0.5361). Numerals above lines are bootstrap values in 1000 replicates (left), and the Bayesian Markov chain Monte Carlo (mcmc) posterior probabilities (right).

agents (Applied Biosystems, Foster City, California, USA) with an ABI model 3100 automated sequencer (Applied Biosystems). Cycling conditions included an initial denaturing step at 94°C for 5 min, followed by 30 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Each sample was sequenced in the sense and antisense direction. The resulting sequences were assembled using Sequencher 4.1.10 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Sequences obtained in this study have been assigned GenBank accession numbers (Appendix 1). They were then aligned by ClustalX version 1.83 (PC version) (Thompson et al., 1997), followed by manual adjustments.

**Phylogenetic analyses**—Phylogenetic analyses were performed with PAUP\* 4.0b10 (Swofford, 2003) using maximum parsimony (MP) (Farris et al., 1970; Fitch, 1971) and maximum likelihood (ML) (Felsenstein, 1981) methods. The heuristic searches for MP analysis with 100 random taxon addition replicates, tree bisection-reconnection (TBR) branch swapping, and collapse of zero-length branches were conducted, and character state changes were equally weighted in the analysis. Gaps were treated either as missing data or as new characters. The amount of support for the clades revealed in the maximally parsimonious tree(s) (MPTs) was examined with 1000 bootstrap replicates with the heuristic search options using parsimony. The appropriate model of DNA substitution for the maximum likelihood analysis was determined using Modeltest version 3.06 (Posada and Crandall, 1998). Once the best-fit model was determined, maximum likelihood searches were performed for the data set (Felsenstein, 1981).

Bayesian analyses (Rannala and Yang, 1996; Mau et al., 1999) were carried out using MrBayes version 3.0b3 (Huelsenbeck and Ronquist, 2001, available at <http://morphbank.ebc.uu.se/mrbayes/>) with the same model parameters as the ML searches. Bayesian analyses started from random trees and employed four Markov chain Monte Carlo (mcmc) runs, monitored over  $1 \times 10^6$  gen-

erations, resampling trees every 100 generations. Runs were repeated twice to confirm results. The resulting log likelihood and number of generations were plotted to determine the point after which the log likelihoods had stabilized. After discarding the trees saved prior to this point as burn-in, the remaining trees were imported into PAUP\*, and a 51% majority-rule tree was produced to obtain posterior probabilities of the clades. Internodes with posterior probabilities  $\geq 95\%$  were considered statistically significant.

Using the MP and ML methods as described, a phylogenetic analysis was also performed using *rbcL* sequences to test the monophyly of *Kelloggia* with a broader sampling of taxa from Rubiaceae, because a large number of *rbcL* sequences are available in GenBank. A total of 46 *rbcL* sequences were included in the analysis, encompassing the three main subfamilies (Rubioidae, Ixoroideae, and Cinchonoideae) of Rubiaceae and two outgroups from *Kopsia* Blume of Apocynaceae and *Ligustrum* L. of Oleaceae, respectively (Appendix 2; Fig. 3). The ML tree of this broader analysis was also used for estimating the divergence times and inferring biogeographic ancestral areas.

**Historical biogeography**—Estimates of divergence times using *rbcL* sequences—A likelihood ratio test (Felsenstein, 1988) was carried out to test whether the *rbcL* sequence evolved in a clocklike fashion. This test resulted in  $P < 0.005$ , suggesting that rate constancy in this data set was not supported. The penalized likelihood (PL) analysis (Sanderson, 2002, 2003) was then used for estimating divergence times. The PL method is a semiparametric approach that uses rate smoothing to allow for robust estimation of node ages in the presence of rate variation between lineages (Sanderson, 2002). Ages of nodes in the tree were estimated using penalized likelihood rate smoothing under a truncated newton algorithm with the program r8s version 1.60 (Sanderson, 2003; available at <http://ginger.ucdavis.edu/r8s/>) on a Linux platform. A cross-

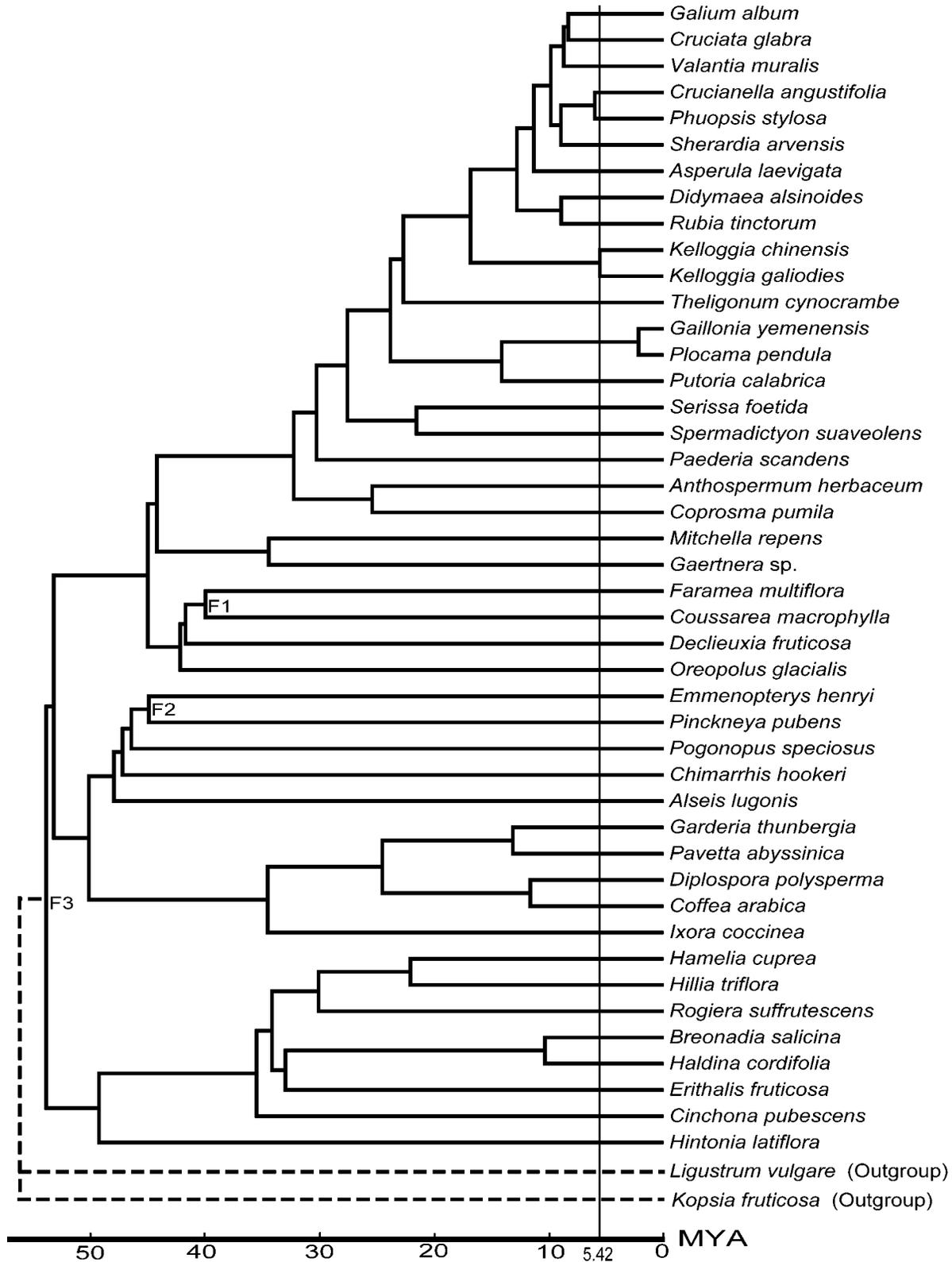


Fig. 3. Cladogram showing divergence times among *Kelloggia* and taxa of the Rubiaceae based on the *rbcL* maximum likelihood tree. Divergence times were estimated with the penalized likelihood method using the truncated newton algorithm with internal age constraints enforced (mya = million years ago). F1 and F2 were constrained to 40 and 45 mya, respectively, and F3 was set to be 54 mya, based on fossil evidence.

validation analysis was performed to obtain the most likely smoothing parameter.

The fossils of Rubiaceae are rather poorly known, with only a few unequivocal records in the Northern Hemisphere (Manchester, 1994; Robbrecht, 1996). *Emmenopterys* Oliver is the earliest record of Rubiaceae fruits from the middle Eocene (Manchester, 1994, 1999). *Pinckneya* Michx. fossil is a seed known from the Oligocene (Meyer and Manchester, 1997). Pollen fossils of *Faramea* Aublet are known from late Eocene to recent (Graham, 1985). Some other fossil records in this family were assigned to the family or certain genera on a tentative basis and are thus not used in our analysis (Muller, 1981; Manchester, 1999). These include *Mitchella* L. (Tiffney, 1985b) and *Galium* L. (Muller, 1981; Tiffney, 1985b). Based on the available fossil data, nodes of the *Faramea-Coussarea* clade (node F1 in Fig. 3) and the *Emmenopterys-Pinckneya* clade (node F2 in Fig. 3) were constrained with minimum ages of 40 and 45 mya, respectively. Because of the first record of Rubiaceae fossils is from the early Eocene (Roth and Dilcher, 1979), the root (node F3 in Fig. 3) of Rubiaceae was set to be 54 mya (also see Magallón-Puebla et al., 1999; Malcomber, 2002).

To estimate the standard errors associated with divergence times, we used a parametric bootstrapping strategy similar to that in Baldwin and Sanderson (1998) and Davis et al. (2002): (1) 100 data sets were simulated on the maximum likelihood tree with the computer program Seq-Gen version 1.2.7 (Rambaut and Grassly, 1997), (2) the divergence times were estimated on the original tree with the sequence model and parameters estimated from the original data, and (3) the resulting simulated data sets were imported into PAUP\*, and maximum likelihood trees were generated. The divergence times were estimated on each tree using r8s version 1.60, and the resulting ages of the nodes from the simulated data sets were used to calculate the variance in divergence time estimates.

**Ancestral area and DIVA analyses**—We used the ancestral area analysis of Bremer (1992) and dispersal-vicariance analysis (DIVA; Ronquist, 1996, 1997) to infer the biogeographic origin of *Kelloggia* and its close relatives based on their *rbcL* data (Fig. 4). Three areas of endemism were circumscribed according to the main geographic distribution of *Kelloggia* and its close relatives (Fig. 4), as well as results of previous biogeographic analysis of the Northern Hemisphere (Wen, 1999; Donoghue et al., 2001; Fritsch, 2001; Sanmartín et al., 2001; Sanmartín, 2003). Because our main interests lie in the construction of dispersal/vicariance history of *Kelloggia* between eastern Asia and western North America, subdividing Eurasia is not necessary in our case. Such subdivision will be useful to gain important insights into the diversification history of Rubiaceae, which is beyond the scope of the present analysis. These three areas of endemisms in our analysis are North America, Eurasia, including the Mediterranean region, and Africa.

Ancestral area analysis is a cladistic procedure to approximate ancestral areas of individual groups from the topological and distributional data without assumptions about the mechanism of speciation. Each area was treated as a single character, which may be optimized onto the taxon cladogram using either the forward or reverse Camin-Sokal parsimony. The areas inhabited by each taxon were treated as binary characters with two states, present or absent. The areas were optimized on the cladogram, and the number of gains or presences (*G*) and losses or absences (*L*) for each individual area were computed. The *G/L* ratio for each area was then estimated, and the individual area with the highest *G/L* ratio was hypothesized to be the ancestral area (AA).

Dispersal-vicariance analysis (Ronquist, 1997) is implemented in the computer program DIVA version 1.1 (Ronquist, 1996). The program optimizes distributions for each node of the tree by favoring vicariance events and minimizing the number of assumed dispersals and extinctions under a parsimony criterion. In DIVA, vicariance events (allopatric speciation) and duplication events (sympatric speciation, i.e., speciation within the area) carry a cost of zero, whereas dispersal and extinction events cost one per unit area added or deleted from the distribution (Ronquist, 1996). Optimizations minimizing the cost are favored in this analysis.

## RESULTS

**Phylogenetic analysis**—The alignment of 20 *rps16* intron sequences of *Kelloggia* and its close relatives generated a data matrix of 928 positions, 35 of which are indel positions. A total of 111 positions were parsimony-informative. The *atpB-rbcL* spacer matrix of 19 taxa comprised 769 aligned positions, 58 of which were parsimony-informative, and 26 of which were indels. The 18 aligned *rbcL* sequences contained 1331 positions without positional homology ambiguities or gaps. Of these, 99 characters were parsimony-informative. Because there is no recombination within the chloroplast genome, we combined all three cpDNA data sets (*rps16* intron, *atpB-rbcL* spacer, and *rbcL*) of *Kelloggia* and its close relatives of the tribes Rubieae and Paederieae. The combined data set of 18 taxa comprised 3028 positions, 265 of which were parsimony-informative.

With gaps as missing data, the MP analyses of the combined data set generated one MPT with a total length of 981 steps, a consistency index (CI) excluding uninformative characters of 0.6221, a retention index (RI) of 0.7211, and a rescaled consistency index (RC) of 0.5631. Among the multiple populations sampled for *Kelloggia*, two clades were resolved with robust support, corresponding to the Asian clade of *K. chinensis* (100% bootstrap value) and the North American clade of *K. galioides* (97% bootstrap value). The monophyly of *Kelloggia* was strongly supported with a 95% bootstrap value and a 100% mcmc posterior probability value in the Bayesian analysis. Analysis of treating gaps as new characters did not change the topology of the trees. Modeltest suggested that the transversion model (TVM + I + G) was the best-fitted model ( $-\ln$  likelihood score = 9744.8818). The base frequencies were A = 0.3074, C = 0.1704, G = 0.1887, T = 0.3336, and the substitution rate matrix was as follows, A/C = 1.1424, A/G = 2.1375, A/T = 0.4323, C/G = 1.3059, C/T = 2.1375, G/T = 1.0000. The gamma distribution shape parameter was 0.7901. The maximum likelihood tree had an identical topology to the MPT.

The 46 *rbcL* sequences of Rubiaceae generated a data matrix of 1331 positions, 212 of which were parsimony informative. The MP analyses generated eight MPTs with a total length of 842 steps, a CI of 0.5107, an RI of 0.7578, and an RC of 0.3870. general time reversible (GTR + I + G) was suggested by Modeltest as the best-fitted model ( $-\ln$  likelihood score = 6795.6343) for the ML analysis. The base frequencies were A = 0.2687, C = 0.1913, G = 0.2459, T = 0.2941, and the substitution rate matrix was as follows, A/C = 1.0248, A/G = 2.1070, A/T = 0.2963, C/G = 1.2865, C/T = 3.0423, G/T = 1.0000. The gamma distribution shape parameter was 0.7038. Both the MPTs and MLT also supported the monophyly of *Kelloggia*, and MLT topology is presented in Fig. 3.

**Sequence divergence**—The pairwise distances between the two species and among populations of *Kelloggia* were estimated (Table 2). The highest divergence (1.722–1.825%) was between populations of *K. chinensis* and *K. galioides* of *rps16* intron sequences. The lowest value (0.00%) was between populations of *K. galioides* of *rps16* intron and *atpB-rbcL* spacer. The sequence divergence between the two species is much larger than that within the species. The Chinese species showed a higher level of infraspecific sequence variation.

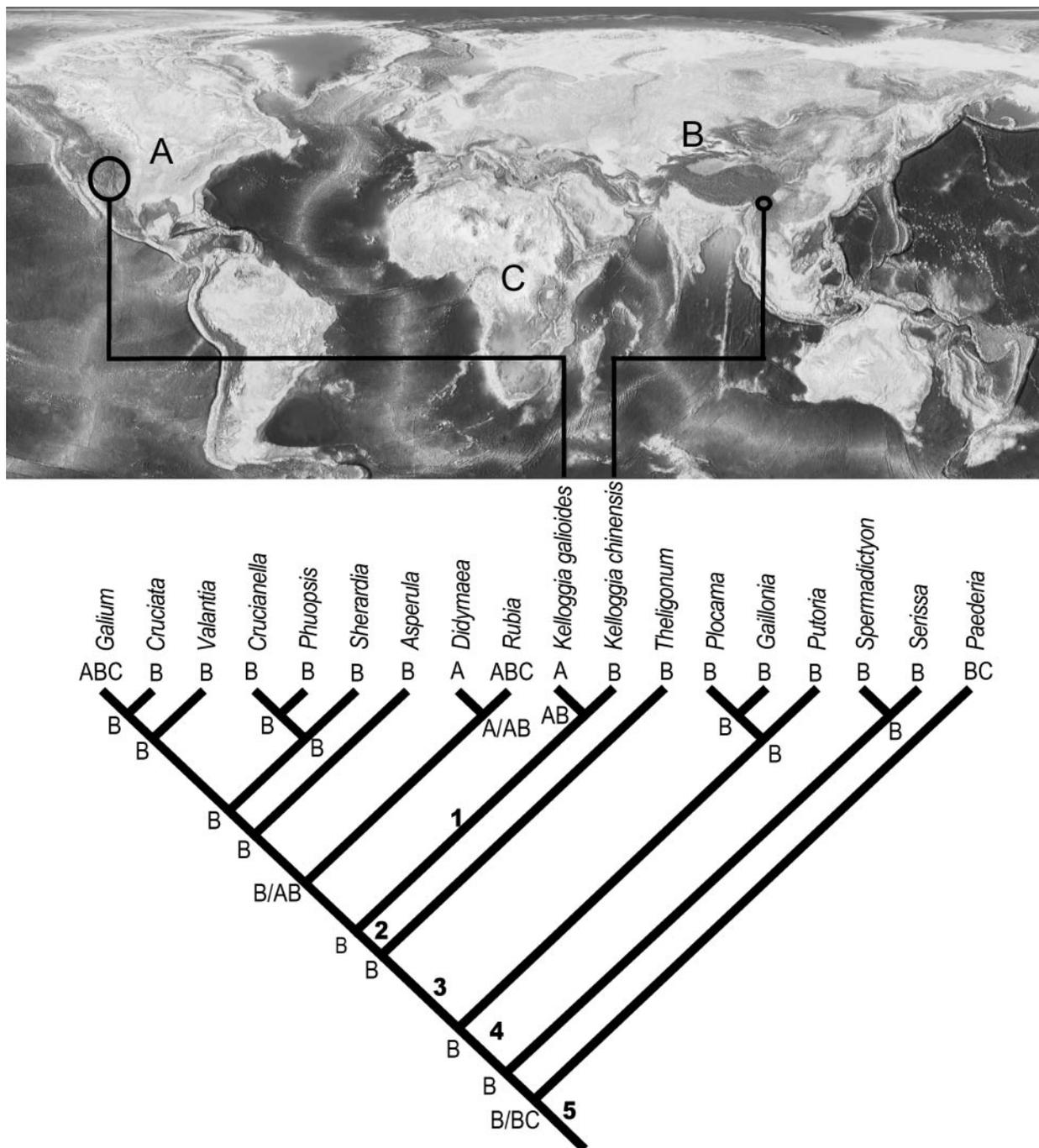


Fig. 4. The *rbcL* maximum likelihood tree for *Kelloggia* and its close relatives in the Rubieae-Theligoneae-Paederieae clade from Fig. 3 with the distributional areas (A–C) for the ancestral areas and DIVA analyses. A = North America, B = Eurasia, and C = Africa. 1, 2, 3, 4, and 5 are labeled nodes for which ancestral areas were inferred in Table 3. At these five nodes, the optimal distributions are also given; alternative, equally optimal distributions are separated with slash marks.

TABLE 2. Sequence characteristics of *Kelloggia* species. Sequence divergence values were estimated with the pairwise distance.

Characteristic	<i>rps16</i>	<i>atpB-rbcL</i>	<i>rbcL</i>	Combined data
No. populations of <i>Kelloggia</i> sequenced	6	5	4	4
Sequence size (bp)	816–840	552	1331	2699–2723
Divergence among populations of <i>Kelloggia chinensis</i>	0–0.735%	0.184%	0.15%	0.334%
Divergence among populations of <i>K. galioides</i>	0	0	0.15%	0.074%
Divergence between <i>K. chinensis</i> and <i>K. galioides</i>	1.722%–1.825%	0.551%–0.735%	0.376%–0.601%	0.856%–0.964%

TABLE 3. Gains (*G*) and losses (*L*) for the geographic areas of taxa in different clades in the cladogram of *Kelloggia* and its close relatives (see Fig. 4). The most probable ancestral area (AA) for the group corresponds to the one with the highest *G/L* quotient.

Area	<i>G</i>	<i>L</i>	<i>G/L</i>	AA
Clade 1				
North America	1	1	1.00	1.00
Eurasia	1	1	1.00	1.00
Clade 2				
North America	3	5	0.60	0.30
Eurasia	4	2	2.00	1.00
Africa	2	6	0.33	0.17
Clade 3				
North America	3	6	0.50	0.20
Eurasia	5	2	2.50	1.00
Africa	2	7	0.29	0.11
Clade 4				
North America	3	7	0.43	0.14
Eurasia	6	2	3.00	1.00
Africa	2	8	0.25	0.08
Clade 5				
North America	3	9	0.33	0.10
Eurasia	8	2	3.50	1.00
Africa	3	9	0.33	0.10

**Biogeographic analysis**—Using a smoothing value of 100 as obtained from the cross-validation procedure, the PL analysis suggested that the divergence time between the two intercontinental disjunct species of *Kelloggia* was  $5.42 \pm 2.32$  mya at the Miocene and the Pliocene boundary (Fig. 3).

The ancestral area analysis employed the *rbcL* ML tree based on the broad sampling of *Kelloggia* relatives from Fig. 3. Eurasia was suggested to have played an important role in the diversification of the following major clades of *Kelloggia* relatives: clade 2 (*Kelloggia*-Rubiaceae), clade 3 (*Kelloggia*-Rubiaceae-*Theligonum*), clade 4 (*Kelloggia*-Rubiaceae-*Putoria*-*Plocama*-*Gaillonia*), and clade 5 (*Kelloggia*-Rubiaceae-Paederieae), with the highest *G/L* values of 2.00, 2.50, 3.00, and 3.50, respectively (Table 3). As shown in Fig. 4, DIVA suggests that Eurasia was the ancestral area of clades 2–4, when the possible ancestral area was constrained to be two areas (the minimum allowable option). For clade 5, Eurasia and Eurasia plus Africa were possible ancestral areas.

## DISCUSSION

**Monophyly and phylogenetic position of *Kelloggia***—Our results based on the combined analysis of three chloroplast markers support the monophyly of the genus comprising these two widely disjunct species: *Kelloggia galioides* from western North America and *K. chinensis* from southwestern China (95% bootstrap value, Fig. 2). This *Kelloggia* clade is also supported by the *rbcL* analysis alone with a broader sampling (Fig. 3). Nevertheless, the two species have shown a high level of morphological divergence (Table 1; Fig. 1). They occupy highly distinct ecological habitats. *Kelloggia galioides* from western North America occurs in dry areas and has relatively tall stems and large leaves and is shrublike, whereas *K. chinensis* of western China is a high montane species in wet meadows of the eastern Himalayas and Hengduan Mountains. The low stems and small hairy leaves of the Chinese species are perhaps adaptations to the habitat of high altitudes. Ad-

aptation of each species to its different habitats after the formation of the disjunction may have largely contributed to the high level of morphological differentiation.

Our analyses of the chloroplast DNA data show that *Kelloggia* is sister to the Rubieae clade (e.g., *Rubia* L. and *Galium* L.) with a bootstrap value of 100% (Fig. 2). Fruits of *Kelloggia* are similar to those of *Galium*, but the latter usually has verticillate leaves (four or more leaves on each node). The morphological similarity of *Kelloggia* and *Galium* led Smith (1921) to describe *Galium abbreviatus* Smith, which is actually a synonym of *Kelloggia chinensis* (Handel-Mazzetti, 1936). The tribe Rubieae has been suggested to be monophyletic in previous molecular analysis (Manen et al., 1994; Manen and Natali, 1995; Natali et al., 1995) and pollen morphological studies (Huysmans et al., 2003). It is characterized by a specific 50-bp deletion of the remaining *atpB* promoter sequence (Natali et al., 1995). However, we found that such a deletion was absent in the *atpB-rbcL* sequences of *Kelloggia*. The pollen morphology of *K. galioides* is similar to that of taxa in the tribe Paederieae in having three-colpate, spherical to prolate grains (Robbrecht, 1982). The relatively medium- to large-sized three-colpate pollen of *Kelloggia* and Paederieae is different from that of Rubieae, which is characterized by small pollen with 5–13 colpi (Huysmans et al., 2003). *Kelloggia* thus perhaps diverged early from the common ancestor with Rubieae. *Kelloggia* may be best recognized as comprising a separate tribe, but a more comprehensive analysis of Paederieae is needed before a new formal taxonomic treatment of *Kelloggia* at the tribal level is proposed.

**Intraspecific variation**—Both species of *Kelloggia* exhibit intraspecific sequence variation (Table 2). The Asian *Kelloggia chinensis* has a higher level of intraspecific divergence than the North American *K. galioides*. In Asia, *K. chinensis* is distributed in the eastern Himalayan region of the Hengduan Mountains, which have undergone a rapidly changing environment corresponding to the uplift of the Himalayas after the collision between the Eurasian and the Indian plates in the Paleogene (Tao, 2000). The region is characterized by rich biodiversity, with many regional evolutionary radiations, and diversification of many species groups associated with the many isolated habitats and mountains (Wu, 1988; Li and Li, 1993; Sun, 2002). *Kelloggia galioides* is distributed in California and extends to Arizona, Idaho, Nevada, Oregon, Utah, Washington, and Wyoming. This region also experienced drastic geologic changes in the Tertiary after the collision between the Pacific and the North American plates (Axelrod, 1973; Wilken, 1993). The geographic isolation of populations of both species in relatively high montane habitats is perhaps responsible for the observed intraspecific sequence variation.

**Historical biogeography**—Although the intercontinental distributional pattern in *Kelloggia* was briefly mentioned in Hong (1993), Wu (1983), and Ying (1983), the evolution of the disjunction has never been critically examined. Our molecular study, together with fossil data, suggests the divergence time between the eastern Asian and western North American species to be about  $5.42 \pm 2.32$  mya (in late Miocene to early Pliocene). The estimated time is similar to that (in the late Miocene) in *Datisca* (Datisceae), which is disjunct between western North America and southwest-central Asia (Liston et al., 1992). Our estimate does not support vicariance via the North Atlantic land bridge as the explanation for the evolution

of the disjunction in *Kelloggia*. The North Atlantic land bridge was no longer viable by the middle Miocene (Parks and Wendel, 1990; Tiffney and Manchester, 2001). Axelrod (1975) employed the Madrean-Tethyan hypothesis to explain the many disjunctions between western North America and the Mediterranean region in the Old World. He suggested that the age of the disjunct elements was about 20–25 mya. Several recent studies of taxa with representatives in western North America and the Mediterranean region have demonstrated various levels of support for the Madrean-Tethyan hypothesis (Liston et al., 1989a, b, 1992; Fritsch, 1996, 2001; Liston, 1997; Hileman et al., 2001). However, Raven (1971, 1973) and Wolfe (1975) argued that plants common to the arid regions of western North America and Eurasia were the products of convergent evolution to arid climates from once more widespread mesophytic ancestors or were the result of long-distance dispersal (Köhler and Brückner, 1989; Ray, 1995; Manos et al., 1999). Our estimate in *Kelloggia* was also apparently much more recent than the age of the Madrean-Tethyan elements. Long-distance dispersal is perhaps the most reasonable explanation for the intercontinental disjunction in *Kelloggia*. Fruits of *Kelloggia* possess hooked bristles similar to those in *Galium*. These hooked bristles may facilitate long-distance dispersal via birds and other animals. But why each of the two species of *Kelloggia* has so restricted distribution remains an intriguing enigma.

Both the ancestral area and DIVA analyses supported Eurasia as the most likely ancestral area of *Kelloggia* together with its close relatives (clades 2–4) (Table 3; Fig. 4). Of clade 2, Rubieae is centered in temperate and tropical mountain regions (Natali et al., 1995; Devesa and Ortega-Olivencia, 2003), especially highly diverse and rich in endemics in the Mediterranean region and Europe (Mabberley, 1997). Eurasia thus seemed to be more important for the early diversification of the *Kelloggia*-Rubieae clade than North America. However, our current analysis included only nine genera of Rubieae with *rbcL* sequences available in GenBank. Additional biogeographic analysis is needed to further understand the diversification history of Rubieae and *Kelloggia*.

Clades 3–4 include all the close relatives of *Kelloggia* including *Putoria*, *Gaillonia*, and *Plocama* in tribe Paederieae and the monotypic tribe Theligoneae (*Theligonum*) (Rutishauser et al., 1998; Bremer and Manen, 2000). As pointed out by Puff (1982), *Putoria*, *Gaillonia*, and *Plocama* were more closely related to *Kelloggia* than to other genera of the tribe. This argument was also supported by the molecular data (clade 4 in Fig. 4). *Theligonum* was sister to the *Kelloggia*-Rubieae clade in our analyses (clade 3) and disjunct between the Mediterranean region and eastern Asia (Rutishauser et al., 1998). Eurasia was also hypothesized to be the most likely ancestral areas for these clades by both the ancestral area and DIVA analyses.

The combined results of biogeographic evidence presented thus supported that Eurasia was highly important in the origin of *Kelloggia* and its close relatives in the Rubieae-Theligoneae-Paederieae group. Our data suggest that the ancestor of *Kelloggia galioides* was dispersed from the Old World to western North America at  $5.42 \pm 2.32$  mya (Figs. 3 and 4). Recent biogeographic and phylogenetic analyses have also shown that dispersal was important in the evolution of Rubiaceae (Eriksson and Bremer, 1991; Bremer and Eriksson, 1992; Anderson et al., 2001). A phylogenetic and biogeographic study of *Gaertnera* (Malcomber, 2002) of Rubiaceae suggested long-

distance dispersal from Africa to southeastern Asia about 5.21 mya ago. The current disjunct distribution of *Kelloggia* is best explained to be via long-distance dispersal from the Old World into North America after the uplift of the Himalayan Mountains and the western cordillera of North America.

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