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Molecular Phylogeny and Biogeography of *Ribes* (Grossulariaceae), with an Emphasis on Gooseberries (subg. *Grossularia*)

LISA M. SCHULTHEIS[1,2,3] and MICHAEL J. DONOGHUE[1]

[1]Department of Ecology and Evolutionary Biology, Yale University, P.O. Box 208105, New Haven, Connecticut 06520;
[2]The Arnold Arboretum of Harvard University, 125 Arborway, Jamaica Plain, Massachusetts 02130;
[3]Present address: Biological and Health Sciences Division, Foothill College, 12345 El Monte Road, Los Altos Hills, California 94022

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ABSTRACT. Gooseberries are often distinguished from currants as a distinct genus (*Grossularia*) or subgenus (*Ribes* subg. *Grossularia*), but recent molecular phylogenetic analyses of chloroplast and nuclear data disagree as to the monophyly of this group. We report new sequence data from the 18–26S nuclear rDNA ITS and ETS regions and from the chloroplast *psbA-trnH* intergenic spacer that, in combination with previously reported data, suggest subg. *Grossularia* is monophyletic and nested within *Ribes*. Two main lineages are evident within subg. *Grossularia*, corresponding to the true gooseberries (subg. *Grossularia* sect. *Grossularia*) and a clade of glabrous-styled western North American gooseberries (subg. *Grossularia* sect. *Robsonia*, subg. *Hesperia*, *Lobbia*). Biogeographic analyses based on DIVA optimizations suggest a western North American origin for subg. *Grossularia*, with subsequent dispersal to east Asia giving rise to a well-supported clade of Asian gooseberry species in sect. *Grossularia*. This example contrasts with the well-documented pattern of dispersal from Asia to North America, and highlights the need to investigate additional groups distributed widely through the Northern Hemisphere.

Ribes L. comprises approximately 150 species of shrubby plants with strikingly diverse floral and fruit features. The infrageneric classification for *Ribes* varies among the primary treatments (e.g., Janczewski 1907; Coville and Britton 1908; Berger 1924; Rehder 1940; Sinnott 1985), creating a difficult taxonomy (reviews in Spongberg 1972; Sinnott 1985). One of the more notable and consistent divisions within the genus is between the currants (subg. *Ribes*) and the gooseberries (subg. *Grossularia*). Currants are mostly spineless shrubs bearing multi-flowered racemes with jointed pedicels, whereas gooseberries have nodal spines and sometimes bristly stems, bear few-flowered racemes with non-jointed pedicels, and have highly reflexed sepals (Senters and Soltis 2003). The differences between gooseberries and currants have led many researchers to recognize gooseberries at the subgeneric (subg. *Grossularia*) (Janczewski 1907; Sinnott 1985) or generic (*Grossularia*) (e.g., Coville and Britton 1908; Berger 1924) level. Previous phylogenetic analyses, however, are at odds regarding the status of the gooseberries as a natural group (Fig. 1). Sequence data from the nuclear 18S–26S rDNA internal transcribed spacer (ITS) region indicated monophyly of subg. *Grossularia* (Senters and Soltis 2003), while restriction site data from the 18S–26S nuclear rDNA region (Messinger et al. 1993) and from two chloroplast regions indicated poly- or paraphyly (Fig. 1; Messinger et al. 1999). The chloroplast data strongly resolved the morphologically intermediate spiny currants (subg. *Ribes* sect. *Grossularioides*) as a paraphyletic grade subtending only the true gooseberries (subg. *Grossularia* sect. *Grossularia*), while a clade of glabrous-styled gooseberries (subg. *Hesperia*, *Lobbia*, and *Grossularia* sect. *Robsonia*) were weakly placed along a separate lineage. A second study using ITS sequence data also supported the monophyly of the true gooseberries (sect. *Grossularia*), but did not sample glabrous-styled gooseberries or spiny currants (Fenton et al. 2000). Given the long tradition of recognizing the gooseberries as a distinct genus (e.g., Coville and Britton 1908; Berger 1924) or subgenus (Janczewski 1907; Sinnott 1985), one goal of this study was to clarify the monophyly of subg. *Grossularia*.

A first step towards addressing the monophyly of the gooseberries (subg. *Grossularia*) is to combine the available chloroplast (Messinger et al. 1999) and ITS (Senters and Soltis 2003) datasets, and to obtain additional data to resolve apparent conflicts evident in these previous studies. Additional data may result in a convergence onto similar topologies, indicating that the apparent conflict was weak or due to insufficient data, or alternatively, may reinforce the apparent conflict between the chloroplast and nuclear genomes, indicating possible hybridization and introgression events in the history of the group (Rieseberg and Wendel 1993; Avise 1994). Additional datasets reported here include sequence data from the nuclear encoded 18S–26S rDNA external transcribed spacer region (ETS) and from the chloroplast encoded *psbA-trnH* intergenic spacer region. Previous studies reported levels of variation in the ETS similar to or greater than those typical of the ITS regions (Baldwin and Markos 1998; Bena et al. 1998; Linder et al. 2000). Studies have also reported sufficient variation within the *psbA-trnH* intergenic spacer region for phylogenetic reconstruction within genera (e.g., Sang et al. 1997; Mast and Givnish 2002; Mort et al. 2002).

A second goal of this study was to consider *Ribes*

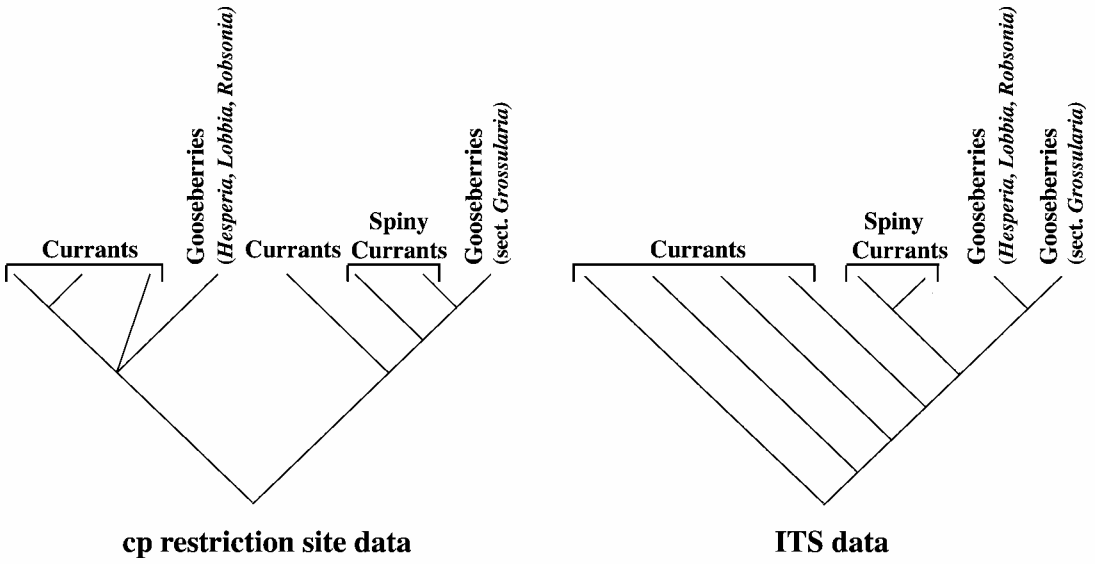

FIG. 1. A simplified representation of prior phylogenetic hypotheses for *Ribes*. Chloroplast restriction site data from Messinger et. al.(1999) suggest that gooseberries (subg. *Grossularia*) are non-monophyletic. ITS data from Senters and Soltis (2003) support the monophyly of gooseberries.

in the context of Northern Hemisphere biogeography. The Northern Hemisphere problem has recently attracted attention from a phylogenetic perspective, by both zoologists (e.g., Sanmartín et al. 2001) and botanists (e.g., Manos and Donoghue 2001). It has become clear that floristic similarities among the major areas of endemism, such as eastern Asia and eastern North America (see Boufford and Spongberg 1983; Wen 1999), were established in several ways (e.g., movement through Beringia or the North Atlantic) and at different times in different groups (e.g., Tiffney 1985a; Manchester 1999; Wen 1999; Xiang et al. 1998, 2000; Donoghue et al. 2001; Fritsch et al. 2001; Manos and Stanford 2001; Tiffney and Manchester 2001; Xiang and Soltis 2001). Several recent studies have highlighted plant groups that appear to have diversified initially in Asia and subsequently moved to North America via the Bering Land Bridge, apparently at several different times (e.g., Donoghue et al. 2001; Xiang and Soltis 2001). This iterative trans-Beringian movement resembles the pattern described for mammals earlier in the Tertiary (Beard 1998), and generally suggests that Asia has been a primary source area for Northern Hemisphere diversity. However, this may reflect the sample of taxa that has been examined to date, and other patterns, including movement from North America to Asia, have also been described (see examples in Sanmartín et al. 2001). It is noteworthy that Northern Hemisphere plant groups that are especially diverse today in western North America have seldom been the focus of phylogenetic biogeographic analyses.

Ribes is very broadly distributed around the Northern Hemisphere, and extends south in the mountains of South America, but is especially diverse in western North America, both in terms of the number of species and the representation of major subclades. Likewise, subg. *Grossularia*, which is the focus of our analysis, occurs around the Northern Hemisphere, but is most diverse in western North America. Specifically, numerous glabrous-styled gooseberries (subg. *Hesperia*, *Lobbia*, and sect. *Robsonia*) are distributed in western North America, while the true gooseberries (sect. *Grossularia*) are found throughout North America and Asia (including Taiwan and Japan), with one species in Europe. Better knowledge of phylogenetic relationships in *Ribes*, and especially in *Grossularia*, would make it possible to assess geographic patterns of diversification and directions of movement in the group.

MATERIALS AND METHODS

Samples. New data reported here include 12 ITS sequences, 73 ETS sequences (from 57 species), and 53 *psbA-trnH* sequences. Previous studies provided an additional 67 ITS sequences (Senters and Soltis 2003) and restriction site data from two amplified chloroplast regions (*rbcL* to *accD* and *rpoC1* to *rpoC2*) for 32 species (Messinger et. al. 1999). Our sampling was weighted towards subg. *Grossularia*, the focus of this study. We included all of the true gooseberry species (sect. *Grossularia*) recognized by Sinnott (1985), all of the Asian true gooseberry species recognized by Berger (1924), representatives from each of the three glabrous-styled gooseberry taxa (sect. *Robsonia*, subg. *Hesperia*, *Lobbia*), and both spiny currant species (sect. *Grossularioides*). In total, 82 of the approximately 150 species of *Ribes* were included in this study (Table 1), representing all of the infrageneric taxa recognized by Berger (1924), but with nomenclature following Sinnott (1985). *Itea*, represented by *I. virginica* and *I. ilicifolia*, was chosen as the outgroup based on previously published studies (Soltis et al. 1990, 1993; Morgan and Soltis 1993; Soltis and Soltis 1997), in which *Ribes*, *Itea*, and Saxifragaceae s.s. appeared to be closely related members of Saxifragales.

DNA Isolation. Total DNA was isolated from fresh, silica-desiccated, and herbarium material. All extractions were performed using QiaGen DNeasy Plant Mini kits, following manufacturer's instructions, except that plant material was ground in warmed AP1 buffer (provided in QiaGen kit) rather than liquid nitrogen. Some DNA aliquots were provided by Senters and Soltis (University of Florida), as indicated in Table 1.

Amplification and Sequencing. PCR and sequencing reactions were performed using a Perkin-Elmer Corporation GeneAmp 9600 or MJ Research Inc. DNA Engine™ Thermal Cycler. PCR products were cleaned using QiaGen QiaQuick PCR Purification kits. Sequencing reactions were performed with ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kits, using half or full reactions, and were cleaned using either EtOH/NaOAc precipitation, following recommendations in BigDye kits, or Edge Biosystems Performa DTR™ Gel Filtration systems. In some cases, sequences were improved with the addition of DMSO to sequencing reactions (approximately 6% final volume). Sequencing reactions were resolved on 5% polyacrylamide gels with an Automated Biosystems (ABI) 377 sequencer, loaded with standard or Rapid Load membrane combs (The Gel Company). Sequencher version 3.1.1 was used to examine and edit sequence chromatograms.

ITS amplification products for the 12 new sequences reported in this study were generated using primers ITS4 (White et al. 1990) and ITS-I (5′-GTCCACTGAACCTTATCATTTAG-3′; designed by L. E. Urbatsch, Louisiana State University) with the following parameters: initial denaturation (97°C, 1 min), followed by 35 cycles of denaturation (97°C, 10 sec), annealing (48°C, 30 sec), extension (72°C, 20 sec increasing 4 sec with each cycle), and concluding with a final extension (72°C, 7 min). PCR reactions contained 2.5μL 10X AmpliTaq buffer (Perkin-Elmer), 2.5μL 10mM dNTPs, 2.5μL 25mM $MgCl_2$, 1.25μL BSA, 1.25μL 10μM ITS4 primer, 1.25μL 10μM ITS-I primer, 0.1μL AmpliTaq DNA polymerase (Perkin-Elmer), and 1–10ng DNA, to a total volume of 25μL. Amplification products were sequenced with ITS4, ITS2 (White et al. 1990), ITS-I and ITS3B (the reverse complement of ITS3; White et al. 1990). Most the length of ITS 2 was sequenced in both directions. Sequencing primers for ITS 1 often produced short sequences with minimal overlap, such that sequences were largely based on a single strand. A portion of the 5.8S region could not be recovered from *R. cynosbati* (41 bp) or *R. echinellum* (77 bp), and was thus coded as N's in the matrix. Of the 67 ITS sequences obtained from Senters and Soltis (2003), five were missing a large portion of ITS 1 (approx. 100bp for *R. giraldii* and *R. montigenum*; approx. 240 for *R. missouriense*; approx. 130 for *R. watsonianum*; approx. 230 for *R. rubrum*).

Sequences from the external transcribed spacer of 18–26S nuclear rDNA (ETS) were obtained following the strategy of Baldwin and Markos (1998). *Ribes odoratum* (subg. *Ribes* sect. *Symphocalyx*) and *R. hirtellum* (subg. *Grossularia* sect. *Grossularia*) were chosen for initial long-distance PCR of the inter-genic spacer region (IGS) in order to design an internal primer conserved across *Ribes*. Long-distance PCR of the IGS was conducted with the following parameters: initial denaturation (94°C, 1 min), followed by 35 cycles of denaturation (94°C, 30 sec), and combined annealing and extension (72°C, 6 min). PCR reactions contained the following components: 2.5μL 10X KlenTaq LA Polymerase Mix (Clontech Laboratories, Inc.), 2.5μL 10mM dNTPs, 2.5μL 10μM 18S-IGS primer (Baldwin and Markos 1998), 2.5μ L10μM 26S-IGS primer (Baldwin and Markos 1998), 0.5μL KlenTaq LA DNA polymerase, and 1–10 ng DNA, to a total volume of 25 μL. Amplification products were sequenced in one direction using primer 18S-E (Baldwin and Markos 1998), yielding approximately 480bp of readable sequence. Primer ETS-Rib1 (5′ GAACTGTTGTCGCGTGCGTCGT 3′) was designed 5′ of the 18S-E priming site, from a conserved region within ETS. Attempts were made to sequence further into the ETS region using primer ETS-Rib2 (5′ ACGACGCACGCGACAACAGTTC 3′), the reverse complement of ETS-Rib1, but readable sequences were only obtained from *R. hirtellum*, hindering efforts to identify a conserved site further 5′ of ETS-Rib1.

Short-distance PCR of the 3′ portion of the ETS region was performed using the ETS-Rib1 primer in conjunction with the 18S-ETS primer (Baldwin and Markos 1998) under the following parameters: initial denaturation (94°C, 2 min), followed by 35 cycles of denaturation (94°C, 30 sec), annealing (65°C, 1 min), extension (72°C, 1.5 min), and concluding with a final extension (72°C, 7 min). PCR reactions contained 2.5μL 10X AmpliTaq buffer (Perkin-Elmer), 2.5μL 10mM dNTPs, 2.5μL 25mM $MgCl_2$, 0.5μL 10μM ETS-Rib1 primer, 0.5μL 10μM 18S-ETS primer, 0.1μL AmpliTaq DNA polymerase (Perkin-Elmer), and –10ng DNA, to a total volume of 25μL. Cleaned amplification products were sequenced in both directions with primers ETS-Rib1 and 18S-ETS. *Itea ilicifolia* is a partial sequence, missing the first 107 bp.

Primers psbAF and trnHR (Sang et al. 1997) were used for both amplification and sequencing of the *psbA-trnH* intergenic spacer region. PCR reaction parameters were: initial denaturation (94°C, 2 min), followed by 35 cycles of denaturation (94°C, 30 sec), annealing (61°C, 30 sec), extension (72°C, 1 min), and concluding with a final extension (72°C, 7 min). PCR reactions contained 2.5μL 10X AmpliTaq buffer (Perkin-Elmer), 2.5μL 10mM dNTPs, 2.5μL 25mM $MgCl_2$, 1.25μL 10μM psbAF primer, 1.25μL 10μM trnHR primer, 0.1μL AmpliTaq DNA polymerase (Perkin-Elmer), and1–10ng DNA, to a total volume of 25μL. For some samples, amplification was aided by the addition of 2.5μL BSA to the PCR reaction. *Ribes viburnifolium* was not successfully sequenced, possibly due to homopolymer strings close to both the 5′ and 3′ primer sites. A central portion of approximately 159 bp could not be recovered from *Itea virginica* and was coded as N's in the matrix.

Sequence identities were verified by performing BLAST searches (National Center for Biotechnology Information, National Institutes of Health) using sequences from *Ribes burejense*.

Alignment. Sequences were initially aligned in Clustal X Multiple Sequence Alignment Program version 1.81 under the default settings, then adjusted by eye using MacClade 4 sequencing editor features (Maddison and Maddison 2000). All *Ribes* sequences were readily alignable by eye, as were *Itea* sequences, but alignment between *Ribes* and *Itea* was often difficult. Ambiguous regions were aligned so that the number of informative sites was minimized. Base pairs that could not be assigned with confidence due to weak or noisy signal were coded with ''N'' or with IUPAC-IUB ambiguity symbols. Gaps inserted for alignment in regions of inferred indels were coded as ''–'' and treated as missing data. Seven large indels coded as present (1) or absent (0) were added to the *psbA-trnH* matrix. The seven coded indels included three insertions in *Itea* (positions 79–88, 199–207, 418–434), two deletions in *Itea* (positions 110–114, 359–372), an insertion consisting primarily of TA repeats in members of sect. *Grossularia* (positions 279–295), and a deletion in *R. himalense*, *R. manshuricum*, and *R. rubrum* (positions 111–116). Datasets are available at TreeBASE (study accession number s1001).

Dataset Combinability. Overlap among the four datasets (ITS, ETS, *psbA-trnH,* restriction sites) was not complete. Of the 84 species included in this study, 50 were represented in at least three of the four datasets (Table 1). A series of partition homogeneity tests (Farris et al. 1995) were performed in PAUP* ver. 4.0 (Swofford 2001) to assess dataset combinability. Each test consisted of 100 replicates employing heuristic searches with simple taxon addition, TBR branch swapping, maxtrees set to 1000, and invariant characters excluded.

Initial partition homogeneity tests indicated that the *psbA-trnH* and restriction site datasets were combinable ($p=0.66$), while all other dataset combinations were incongruent ($p \leq 0.01$). To explore potential sources of incongruence, we conducted additional homogeneity tests with individual taxa or groups of taxa excluded based on differential and well-supported (bootstrap >70%) placement in trees, or on differential placement regardless of support levels (deQueiroz et al. 1995). For the ETS and ITS comparison, taxa were excluded one at a time and in groups if they represented composites of multiple accessions (Table 1) and exhibited differential placements in ETS versus ITS trees. Samples of *R. oxyacanthoides* subsp. *oxyacanthoides* were also excluded in ETS versus ITS comparisons because there was divergence between the two included ETS sequences (sequenced for this study), thus it was not clear which should be combined with the ITS sequence (obtained

TABLE 1. Taxa included in this study, with GenBank accession numbers. Following Messinger et al. (1999), taxonomy corresponds to Sinnott (1985) with *Grossularia* subg. *Hesperia* and subg. *Lobbia* treated under *Ribes*. ITS sequences noted in boldface are from Senters and Soltis (2003). Senters and Soltis provided the DNA for italicized sequences. Restriction site data are from Messinger et al. (1999). Voucher information is cited as per Senters and Soltis (2003) or Messinger et al. (1999) when the data or DNA came from those sources. Herbarium codes follow the Index Herbariorum, Eighth Edition. NPGR refers to the USDA-ARS National Plant Germplasm Repository. UCBG refers to the University of California Botanical Garden. RBGE refers to the Royal Botanic Garden, Edinburgh.

Subg. Ribes L.

Sect. *Berisia* Spach (Alpine Currants)

R. acuminatum (Hook. F.&Thomson) Jancz. *Chase 3585* (K): ITS **AF426376.**

R. alpinum L. Arnold Arboretum 678–80E: ETS AY138021; *psbA-trnH* AY138094. *Chase 3587* (K): ITS **AF426378.** NPGR 6640: restriction sites.

R. diacanthum Pallas Arnold Arboretum 1852–81B: ETS AY138022; *psbA-trnH* AY138095; ITS AY138047. NPGR 34 (*Messinger 315*; OSC): restriction sites.

R. giraldii Janczewski Arnold Arboretum 609–74B: ETS AY138023; *psbA-trnH* AY138096. *M. Mort s.n.* (WS): ITS **AF426381.**

R. glaciale Wall. UCBG 91.0285: ETS AY138024; *psbA-trnH* AY138097; ITS AY138048.

R. komarovii Pojark. UCBG 91.078: ETS (1): AY138025. Arnold Arboretum 1094–82A: ETS (2): AY138026; *psbA-trnH* AY138098.

R. maximowiczii Batalin *T. S. Elias 10940* (RSA): ITS **AF426380.** NPGR 267: restriction sites.

R. orientale Desf. *Boufford et al. 28317* (A): ETS AY138027; *psbA-trnH* AY138099.

R. tenue Janczewski *Boufford et al. 27604* (A): ETS AY138028; *psbA-trnH* AY138100. *Chase 3610* (K): ITS **AF426377.**

R. vilmorini Janczewski *Chase 3612* (K): ITS **AF426379.**

Sect. *Calobotrya* (Spach) Jancz. (Ornamental Currants)

R. affine H.B.K. *M. Medina 2517* (NY): ITS **AF426326.**

R. brandegeei Eastwood s.c. 350568 (WS): ITS **AF426331.**

R. cereum Douglas UCBG 93.1213: ETS AY138013; *psbA-trnH* AY138087. NPGR 237.001: restriction sites. *Ross 3425* (NY): ITS **AF426328.**

R. ceriferum Coville and Rose s.c. 05017194 (MO): ITS **AF426333.**

R. ciliatum H. and B. *Diggs 2625* (NY): ITS **AF426327.** NPGR 670.001 (*Messinger 311*; OSC): restriction sites.

R. dugesii Greenman *Siplivinsky 3939* (WS): ITS **AF426329.**

R. glutinosum Benth. *Chase 3594* (K): ITS **AF426340.**

R. indecorum Eastwood UCBG 86.0903: ETS AY138014; *psbA-trnH* AY138088. *Mort 1370* (WS): ITS **AF426336.**

R. malvaceum Smith UCBG 91.1481: ETS AY138015; *psbA-trnH* AY138089. *Johnson s.n.* (WS): ITS **AF426338.**

R. mogollonicum Greene NPGR 294.001: ITS **AF426332**; restriction sites.

R. neglectum Rose *Villarrea 4940* (NY): ITS **AF426330.**

R. nevadense Kellogg UCBG 89.1635: ETS AY138016; *psbA-trnH* AY138090. *Mort 1373* (WS): ITS **AF426339.**

R. sanguineum Pursh UCBG 90.0193: ETS AY138017; *psbA-trnH* AY138091. *Mort 1372* (WS): ITS **AF426335.** NPGR 46: restriction sites.

R. tortuosum Benth *Breedlove 62230* (MO): ITS **AF426325.**

R. viscosissimum Pursh *Grimes 1878* (NY): ITS **AF426334.** NPGR 281.001 (*Fredricks 394*; OSC): restriction sites.

R. wolfii Rothrock *Siplivinsky 4587* (NY): ITS **AF426341.**

Sect. *Coreosma* (Spach) Jancz. (Black Currants)

R. americanum Mill. NPGR 93: restriction sites. *Nee 24196* (NY): ITS **AF426375.**

R. bracteosum Douglas UCBG 89.1645: ETS AY138033; *psbA-trnH* AY138103; ITS AY138049.

R. fragrans Pallas s.c. 4378976 (MO): ITS **AF426373.**

R. hudsonianum Richardson *B. Ertter 3807* (NY): ITS **AF426372.**
var. *petiolare* (Douglas) Jancz. NPGR 278 (*Fredricks 390*; OSC): restriction sites.

R. janczewskii Pojark. *Chase 3597* (K): ITS **AF426370.**

R. nigrum L. NPGR 215.001 (OSC): ITS **AF426374.**

R. viburnifolium A. Gray UCBG 65.1431: ETS AY138034. NPGR 762.001: ITS **AF426371**; restriction sites.

Sect. *Grossularioides* (Jancz.) Rehd. (Spiny, or Gooseberry-stemmed Currants)

R. lacustre (Pers.) Poiret *Lesica 4710* (NY): ETS (1): AY138018; ITS **AF426366.** Arnold Arboretum 777–93A: ETS (2): AY138019; *psbA-trnH* AY138092. NPGR 45: restriction sites.

R. montigenum McClatchie *Bugham & Miller s.n.* (WS): ETS *AY138020*; *psbA-trnH AY138093*; ITS **AF426367.** NPGR 864.001 (*Messinger 254*; OSC): restriction sites.

Sect. *Heritiera* Jancz. (Dwarf Currants)

R. erythrocarpum Coville and Leiberg NPGR 860.001 (*Messinger 249*; OSC): ITS **AF426342**; restriction sites.

R. howellii Greene NPGR 449.001 (*Messinger 333*; OSC): ITS **AF426343**; restriction sites.

R. glandulosum Grauer UCBG 89.0750: ETS (1): AY138035. *Schultheis 589–00* (YU): ETS (2): AY138036; *psbA-trnH* AY138105. NPGR 231: restriction sites. *Chase 3605* (K) (= *R. prostratum* L'Her.): ITS **AF426345.**

R. laxiflorum Pursh NPGR 439: restriction sites. *Goodrich 19052* (WS): ITS **AF426344.**

Sect. *Parilla* Jancz. (Andine Currants)

R. andicola Jancz. *Friere-Fierro 2577* (NY): ITS **AF426368.**

R. fasciculatum Sieb. & Zucc. UCBG 88.0615: ETS (1): AY138043; *psbA-trnH* AY138104. Arnold Arboretum 1879: ETS (2): AY138044. *Chase 3592* (K): ITS **AF426346.**

R. ovalifolium Jancz. *Gentry s.n.* (MO): ITS **AF426369.**

R. valdivianum Phil. *Messinger 314* (OSC): restriction sites.

Sect. *Ribes* L. (Red Currants)

TABLE 1. Continued.

R. himalense Decaisne *Boufford et al. 28903* (A): ETS AY138037; *psbA-trnH* AY138106. s.c. 04714258 (MO): ITS **AF426369.**
R. manshuricum Komarow Arnold Arboretum 67–7: ETS AY138038; *psbA-trnH* AY138107. *Chase 3599* (K): ITS **AF426320.**
R. petraeum Wulfen *Chase 3604* (K): ITS **AF426318.**
R. rubrum L. Arnold Arboretum 1119–78A: ETS (1): AY138039; *psbA-trnH* AY138108. Arnold Arboretum 214–96: ETS (2): AY138040. *Schulwerk 7039* (NY): ITS **AF426321.**
R. sativum (Rehbeh) Syme Missouri Botanical Garden: ITS **AF426323.**
cv. 'Diploma' NPGR 747 (Thompson 46; OSC): restriction sites.
R. spicatum Robs. *Chase 3609* (K): ITS **AF426322.**
R. triste Pallas UCBG 94.1114: ETS (1): *AY138041.* Arnold Arboretum 407–94: ETS (2): AY138042; *psbA-trnH* AY138109. *Messinger 314* (OSC): restriction sites. *Baldwin 2269* (WS): ITS **AF426319.**
Sect. *Symphocalyx* Berland. (Golden Currants)
R. aureum Pursh UCBG 56.0948: ETS (1): AY138030. Arnold Arboretum 460–81A: ETS (2): AY138029; *psbA-trnH* AY138101. NPGR 769: restriction sites. *Clement 26* (WS): ITS **AF426382.**
R. odoratum Wendl. UCBG 86.0126: ETS (1): AY138032. Arnold Arboretum 1192–74A: ETS (2): AY138031; *psbA-trnH* AY138102. NPGR 691: restriction sites.
Subg. *Grossularia* (Mill.) Pers.
Sect. *Grossularia* (Mill.) Nutt.
R. aciculare Sm. *A.K. Skvortsov sn* (A): ETS AY137976; *psbA-trnH* AY138060; ITS AY138050.
R. alpestre Wall. ex Decne. *Boufford et al. 28437* (A): ETS AY137975; *psbA-trnH* AY138059. *Chase 3586* (K): ITS **AF426349.**
R. burejense F. Schmidt NPGR 259.001: ETS AY137977; *psbA-trnH* AY138061; restriction sites. *Messinger 260* (OSC): ITS **AF426355.**
R. curvatum J.K. Small *R. Kral 53094* (GH): ETS AY137979.
R. cynosbati L. Arnold Arboretum 1108–78A: ETS (1): AY137981; *psbA-trnH* AY138063; ITS AY138051. *Schultheis 590–00* (YU): ETS (2): AY137980.
R. divaricatum Douglas UCBG 85.1596: ETS (1): AY137982; *psbA-trnH* AY138064. *Chase 3591* (K): ETS (2): AY137983; ITS **AF426347.**
R. echinellum (Coville) Rehd. Arnold Arboretum 234–96: ETS AY137984; *psbA-trnH* AY138065; ITS AY138052.
R. formosanum Hayata *Wang 1096* (MO): ETS (1): *AY137985*; ITS **AF426354.** RBGE 19934301: ETS (2): AY137986; *psbA-trnH* AY138066.
R. grossularioides Maxim. RBGE 19592035: ETS AY137987; *psbA-trnH* AY138067; ITS AY138053.
R. hirtellum Michx. UCBG 86.0125: ETS (1): AY137988. *Schultheis 588–00* (YU): ETS (2): AY137989; *psbA-trnH* AY138068. *Chase 3595* (K): ITS **AF426353.**
R. inerme Rydb. *Layser 1770* (UW): ETS (1): *AY137990*; ITS **AF426356.** Arnold Arboretum 225–80A: ETS (2): AY137991; *psbA-trnH* AY138069.
R. missouriense Nutt. *W.R. Smith 11396* (GH): ETS AY137993; *psbA-trnH* AY138071. *Solomon 859* (WS): ITS **AF426348.**
R. niveum Lindl. *Messinger 226* (OSC): ETS *AY137994*; *psbA-trnH AY138072*; ITS **AF426351.** NPGR 777.001 (*Messinger 226*; OSC): restriction sites.
R. oxyacanthoides L.
subsp. *oxyacanthoides* NPGR 139.001: ETS (1): AY137996. *Alverson 1645* (WIS): ETS (2): AY137995; *psbA-trnH* AY138073. *Peterson & Annable 4440* (WS): ITS **AF426383.**
subsp. *cognatum* (Greene) Sinnott *Woodland s.n.* (WS): ETS *AY137978*; *psbA-trnH AY138062*; ITS **AF426357.**
subsp. *irriguum* (Douglas) Sinnott RBGE 19794064: ETS AY137992; *psbA-trnH* AY138070; ITS AY138054. NPGR 773.001 (*Messinger 221*; OSC): restriction sites.
subsp. *setosum* (Lindl.) Sinnott Arnold Arboretum 1395–83A: ETS AY137998; *psbA-trnH* AY138075. *Lackschewitz 7845* (UW): ITS **AF426352.**
R. rotundifolium Michx. *Mitchell & Barbour 10,112* (NYS): ETS AY137997; *psbA-trnH* AY138074; ITS AY138055.
R. stenocarpum Maxim. RBGE 19698970: ETS AY137999; *psbA-trnH* AY138076; ITS AY138056.
R. uva-crispa L. UCBG 87.0719: ETS (1): AY138001. Arnold Arboretum 1404–80B: ETS (2): AY138000; *psbA-trnH* AY138077. *Hill 20953* (NY) (= *R. grossularia* L.): ITS **AF426350.**
Subg. *Hesperia* A. Berger
R. amarum McClatchie UCBG 89.1081: ETS AY138002; *psbA-trnH* AY138078.
R. californicum Hook. and Arn. UCBG 82.1692: ETS AY138004; *psbA-trnH* AY138080; ITS AY138057.
R. menziesii Pursh *Messinger 233* (OSC): ETS *AY138006*; *psbA-trnH AY138082*; ITS **AF426364.** NPGR 769.001 (*Messinger 233*; OSC): restriction sites.
R. roezli Regel *Nelson s.n.* (WS): ETS *AY138007*; *psbA-trnH AY138083*; ITS **AF426365.**
var. *cruentum* (Greene) Regel NPGR 772.001 (*Messinger 217*; OSC): restriction sites.
Subg. *Lobbia* A. Berger
R. binominatum A.Heller *Messinger 260* (OSC): ETS *AY138003*; *psbA-trnH AY138079*; ITS **AF426359.** NPGR 867.001 (*Messinger 260*; OSC): restriction sites.
R. lobbii A. Gray UCBG 85.1496: ETS AY138005; *psbA-trnH* AY138081. *Smith Jr. 7443* (WS): ITS **AF426361.**
R. velutinum Greene *Annable 2503* (NY): ETS *AY138011*; *psbA-trnH AY138085*; ITS **AF426358.** NPGR 865 (*Messinger 255.1*; OSC): restriction sites.
R. watsonianum Koehne *Patrick s.n.* (UW): ETS *AY138012*; *psbA-trnH AY138086*; ITS **AF426360.**
Sect. *Robsonia* Berland.
R. speciosum Pursh UCBG 84.0004: ETS (1): AY138008; *psbA-trnH* AY138084. *Mort 1371*: WS: ETS (2): *AY138009*; ITS **AF426362.** NPGR 901.001: restriction sites.
R. thacherianum Johnson s.n. (WS): ETS *AY138010*; ITS **AF426363.**

TABLE 1. Continued.

Outgroups

Itea virginica L. New York Botanic Garden 76/98C: ETS AY138045; *psbA-trnH* AY138110. *Messinger 337* (OSC): restriction sites. *Ware 94* (WS): ITS **AY231368.**

I. ilicifolia Oliver UCBG 86.0414: ETS AY138046; *psbA-trnH* AY138111; ITS AY138058.

from Senters and Soltis 2003). It is possible that incongruence between ETS and ITS datasets was due to intraspecific variation, an as yet unexplored issue within *Ribes* that may limit future use of composite accessions. The presence of ETS and ITS regions on the same transcript, similar GC contents (ETS= mean 0.49; ITS=mean 0.58), similar proportions of invariant characters (ETS= 297/462; ITS=438/700), and similar levels of rate heterogeneity across sites (ETS: gamma = 0.65; ITS: gamma= 0.57) argued against different evolutionary processes of sequence evolution as an explanation for incongruence.

In order to maximize taxon sampling and data, tests were also used to assess combinability of taxa represented in three of the four available datasets. While this required the inclusion of taxa for which one dataset was coded as missing, the decrease in phylogenetic accuracy was expected to be insignificant (Weins and Reeder 1995).

Phylogenetic Analysis of Sequence Data. Analyses of single (ITS, ETS, and *psbA-trnH*) and combined datasets were performed using a parsimony criterion in PAUP* 4.0. All sites and all changes were equally weighted, in contrast to the 1.3 gains:1.0 loss weighting scheme used by Messinger et al. (1999) in their analyses of restriction site data. *psbA-trnH* data were analyzed both with indels treated as missing data, and with seven large indels included as binary characters. Positions 309–327 and 448–465 of the aligned *psbA-trnH* dataset were excluded from all analyses due to ambiguous alignments.

Analyses of the ITS, ETS, and *psbA-trnH* datasets consisted of heuristic searches with 100 replicates of random addition, and TBR branch swapping. No more than 400 trees greater than a specified length (234 for ETS; 556 for ITS; 137 for *psbA-trnH*) were saved per replicate, or in the case of *psbA-trnH* with indels, no more than 250 trees greater than length 144. The limiting tree length was determined based on the shortest trees found in prior, incomplete searches, and was imposed to prevent computational overload searching sub-optimal trees. Analyses of all datasets combined or of combined chloroplast datasets were conducted with the branch-and-bound algorithm, with taxa excluded based on partition homogeneity test results. For datasets combining ETS+ITS, ETS+*psbA-trnH*, *psbA-trnH*+rDNA, or all data for those taxa represented in three of the four datasets, analyses consisted of heuristic searches with 100 replicates of random addition, TBR branch swapping, and maxtrees set to 40,000.

Clade support was assessed using bootstrap analyses (Felsenstein 1985) as implemented in PAUP* 4.0 with 300 replicates of heuristic searches, each with 5 replicates of random taxon addition and Nearest Neighbor Interchange (NNI) branch swapping. For the combination of all four datasets, and for the combined chloroplast datasets, clade support was assessed using 100 bootstrap replicates each with branch and bound searches.

Kishino-Hasegawa Tests. The parsimony implementation of the Kishino-Hasegawa test (Kishino and Hasegawa 1989) in PAUP* 4.0 was used to compare the hypothesis of subg. *Grossularia* monophyly to that of subg. *Grossularia* non-monophyly, in which sect. *Grossularioides* and sect. *Grossularia* form a clade. The parsimony version of the test was used because comparisons included restriction site data. As recommended by Goldman et al. (2000), comparisons were between a priori hypotheses based on previously published phylogenies (Messinger et al. 1999; Senters and Soltis 2003).

Analyses were run enforcing each of two constraint trees. The first constraint tree resolved subg. *Grossularia* as monophyletic, but left relationships within subg. *Grossularia* and among all remaining species unresolved. The second constraint tree enforced resolution of a clade containing sect. *Grossularioides* plus sect. *Grossularia*, but relationships within this clade were left unresolved, as were relationships among all remaining species. Comparisons were between trees from constrained branch and bound searches for the chloroplast data, and from constrained heuristic searches for the rDNA data.

Ancestral Area Reconstruction. Ancestral areas were inferred using DIVA, which assigns areas to internal nodes such that dispersal and extinction events are minimized (Ronquist 1996, 1997). Default costs were used (vicariance = 0, dispersal = 1, extinction =1), and the number of inferred areas per node was left unconstrained. These analyses used trees from the combined *psbA-trnH* and rDNA datasets (Fig. 8), with species assigned to one or more of four areas (eastern North America, western North America, eastern Eurasia, and western Eurasia). Geographic distributions followed Berger (1924), Janczewski (1907), and Sinnott (1985). Sect. *Calobotrya*, *R. aureum*, *R. bracteosum*, *R. watsonianum*, and the clade containing the remaining glabrous-styled gooseberries (*Hesperia*, *Lobbia*, *Robsonia*) were all coded with western North American distributions. Sect. *Grossularioides* was coded for both North America and eastern Eurasia. Subg. *Berisia* was coded for both eastern and western Eurasia. *Ribes triste* was coded for all areas except western Eurasia. *Itea* was coded for both eastern North America and eastern Eurasia.

Because DIVA analyses do not permit polytomies, we treated *R. aureum* and *R. bracteosum* as sister taxa. Within sect. *Grossularia*, members of the Asian gooseberry clade were all coded as eastern Eurasia. The remaining species of the large polytomy all live in western or eastern North America. We resolved this polytomy in several different ways, so as to explore some of the possibilities. For example, in some analyses we created one eastern North American and one western North American clade. In others, the North American species were arranged pectinately in relation to the Asian clade.

RESULTS

Sequence Characteristics and Analyses. The aligned 18–26S rDNA ITS dataset, including 5.8S(positions 283–452), was 700 bp (282bp=ITS1; 248bp=ITS2) with 2.8% of the data matrix coded as missing data. For the region as a whole, sequences ranged from 438–662bp (variation due in part to missing terminal sequence data), uncorrected pairwise distances within *Ribes* ranged from 0.0–0.08, distances with *Itea* ranged from 0.27 (with *R. missouriense*) to 0.32 (with *R. malvaceum*), and mean GC content was 0.58. Of the 700 aligned base-pairs, 528 were constant within *Ribes*, and 183 were parsimony informative. ITS1 displayed higher levels of divergence and a higher proportion of informative characters than did ITS2 (ITS1 = 0.0–0.15, 65/282 informative; ITS2 = 0.0–0.11, 25/248 informative). Included ITS1 sequences ranged from 34–258bp, with the lower limit due to taxa for which much of ITS1 sequence data was missing. ITS2 sequences ranged from 216–241bp. 5.8S ranged from 161–164bp, but was only 142bp in *R. sanguineum* due to a large deletion.

Lengths and GC contents fell within the ranges reported for a survey of angiosperm taxa (Baldwin et al. 1995). Analyses of the ITS and 5.8S regions generated 36,400 trees (length=556; CI=0.65, 0.58 excluding uninformative characters; RI=0.84).

Amplified products from the 18–26S rDNA intergenic spacer region were approximately 2kb in *R. odoratum* (sect. *Symphocalyx*) and 3kb in *R. hirtellum* (sect. *Grossularia*), the two species used to design internal primers for amplifying 3′ ETS. Only 406bp of the 3′ end of the ETS region and 56bp of the 5′ end of 18S were sequenced in this study. Included sequences ranged from 299–462bp in size, with some variation due to missing terminal sequence data, and had a mean GC content of 0.49. Uncorrected pairwise distances among ETS sequences within *Ribes* ranged from 0.0 to 0.07. Pairwise distances between *Ribes* and *Itea* ranged from 0.26 (with *R. oxyacanthoides*, accession 2) to 0.32 (with *R. triste*, accession 1). Of the 462 aligned basepairs, 388 were constant within *Ribes*, 60 were parsimony informative, and 2% were coded as missing data. ETS analyses generated 34,005 trees (length=234; CI=0.80, 0.74 excluding uninformative characters; RI=0.93; Fig. 2).

The aligned *psbA-trnH* intergenic spacer dataset was 502bp, including a 3′ portion of *psbA* (positions 1–37) and a 5′ portion of *trnH* (position 484–502). One percent of the matrix was coded as missing data. Included sequences ranged from 355–445bp in length, with some variation due to missing terminal sequence data, and had a mean GC content of 0.29. The spacer region alone ranged from 352–378bp within *Ribes*, and was 369bp in *Itea*, based on complete sequences. Uncorrected pairwise distances (including *psbA* and *trnH* terminals) within *Ribes* ranged from 0.0 to 0.04. Distances between *Ribes* and *Itea* ranged from 0.15 (with *R. odoratum*, accession 2) to 0.21 (with *R. bracteosum*). Within *Ribes*, 405/465 included characters were constant, and 34/465 were parsimony informative. Excluded characters represented a polyA region ranging from 4–13bp, and a 16–18bp insertion in *Itea* (relative to *Ribes*) with which 5–6bp segments of *Ribes* could be aligned in various ways. Analyses of sequence data from the *psbA-trnH* intergenic spacer produced 34,000 trees with little homoplasy (length=137; CI=0.92, 0.88 excluding uninformative characters; RI=0.94; Fig. 3). Analyses including indels coded as binary characters produced 18,001 trees (length=144; CI=0.92, 0.90 excluding uninformative characters; RI=0.95).

Trees Generated by Analyses of Individual Datasets. Results from the expanded ITS dataset concur with those presented by Senters and Soltis (2003) and are not illustrated here. Subg. *Grossularia* was resolved as monophyletic in ITS and ETS trees, with weak to moderate bootstrap support (Figs. 1, 2). *psbA-trnH* trees showed little resolution, but did not conflict with a monophyletic subg. *Grossularia* (Fig. 3; trees from analyses including indels are not shown).

Two main lineages may be identified within subg. *Grossularia*, the true gooseberries (sect. *Grossularia*) and the glabrous-styled gooseberries (*Hesperia*, *Lobbia*, *Robsonia*). The glabrous-styled gooseberries are resolved as a clade in ITS, restriction site, and *psbA-trnH* trees (Figs. 1, 3; Messinger et al. 1999). ETS trees also resolve the glabrous-styled gooseberries (*Hesperia*, *Lobbia*, *Robsonia*) as a clade with the exception of *R. watsonianum* (subg. *Lobbia*), placed with strong support in the true gooseberry clade (sect. *Grossularia*) (Fig. 2). The integrity of the individual sections comprising the glabrous-styled gooseberries (*Hesperia*, *Lobbia*, *Robsonia*) is unclear. The true gooseberries (sect. *Grossularia*) are resolved as a clade in restriction site, ITS and ETS trees (including *R. watsonianum* in the latter), and in *psbA-trnH* trees when indels are included (Figs. 1, 2; Messinger et al. 1999; Senters and Soltis 2003). If indels are ignored, *psbA-trnH* trees resolve the majority of true gooseberries as a clade, and are consistent with a monophyletic sect. *Grossularia* (Fig. 3). Within sect. *Grossularia*, the ETS dataset provided strong support for an Asian clade, including the European *R. uvacrispa*. ITS trees were consistent with an Asian clade, but excluded *R. uvacrispa*. *psbA-trnH* trees were also consistent with an Asian clade, with the weakly supported inclusion of the North American *R. oxyacanthoides* subsp. *irriguum*. Restriction site trees had limited sampling in sect. *Grossularia*, but were consistent with an Asian versus North American divergence (Messinger et al. 1999).

All datasets except the restriction sites resolved a clade comprising sect. *Calobotrya*, sect. *Grossularioides*, and subg. *Grossularia* (sect. *Grossularia*, sect. *Robsonia*, subg. *Lobbia*, *Hesperia*). Members of this clade are largely from western North America, with sect. *Grossularia* having a wider distribution. ETS trees included the black currant *Ribes bracteosum* (sect. *Coreosma*) within this clade, in an unresolved position (Fig. 2). ITS trees included the South American sect. *Parilla* in this clade, in an unresolved position (Senters and Soltis 2003). In all trees, sect. *Calobotrya* was resolved as monophyletic, including some members of sect. *Heritiera* in restriction site and ITS trees (only one species of sect. *Heritiera* was included in ETS and *psbA-trnH* analyses, and falls elsewhere). Section *Grossularioides* was resolved with strong support in ETS and ITS trees (Figs. 1, 2; Senters and Soltis 2003), but is unresolved or strongly paraphyletic in the *psbA-trnH* and restriction site trees respectively (Figs. 1, 3; Messinger et al. 1999).

The clade containing sects. *Calobotrya*, *Grossularioides* and subg. *Grossularia* was clearly nested within *Ribes* in ITS, ETS and *psbA-trnH* trees (Figs. 2, 3; Senters and Soltis 2003). Among the remaining sections of *Ribes*, all datasets provided strong support for sect. *Berisia*,

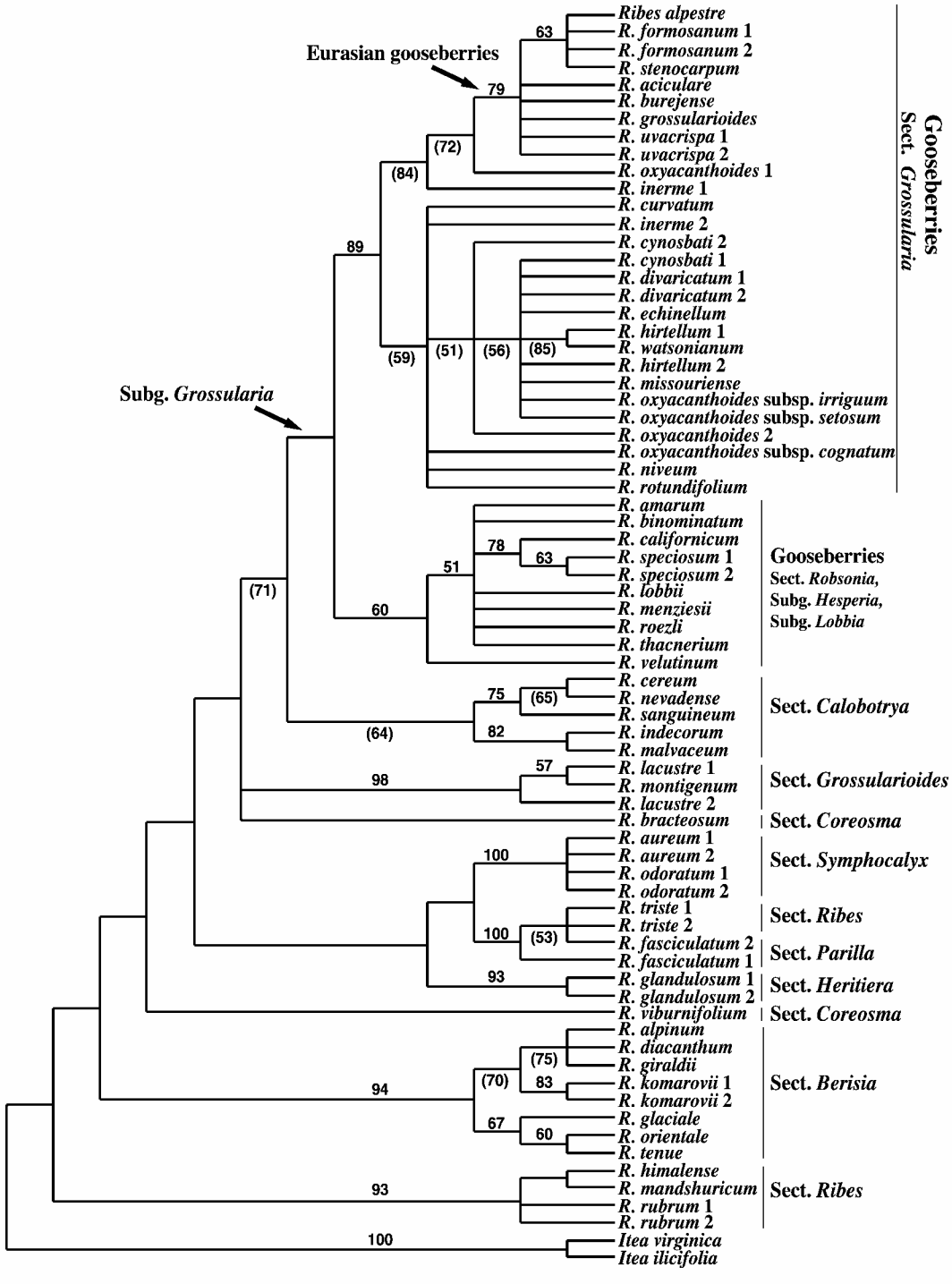

FIG. 2. The majority-rule consensus of 34,005 trees resulting from analyses of ETS sequence data (CI=0.80, 0.74 excluding uninformative characters; RI=0.93). Numbers above the branches indicate the percentage of bootstrap replicates in which the clade appeared. Numbers in parentheses below the branches indicate the percentage of trees in which the clade appeared, when less than 100%.

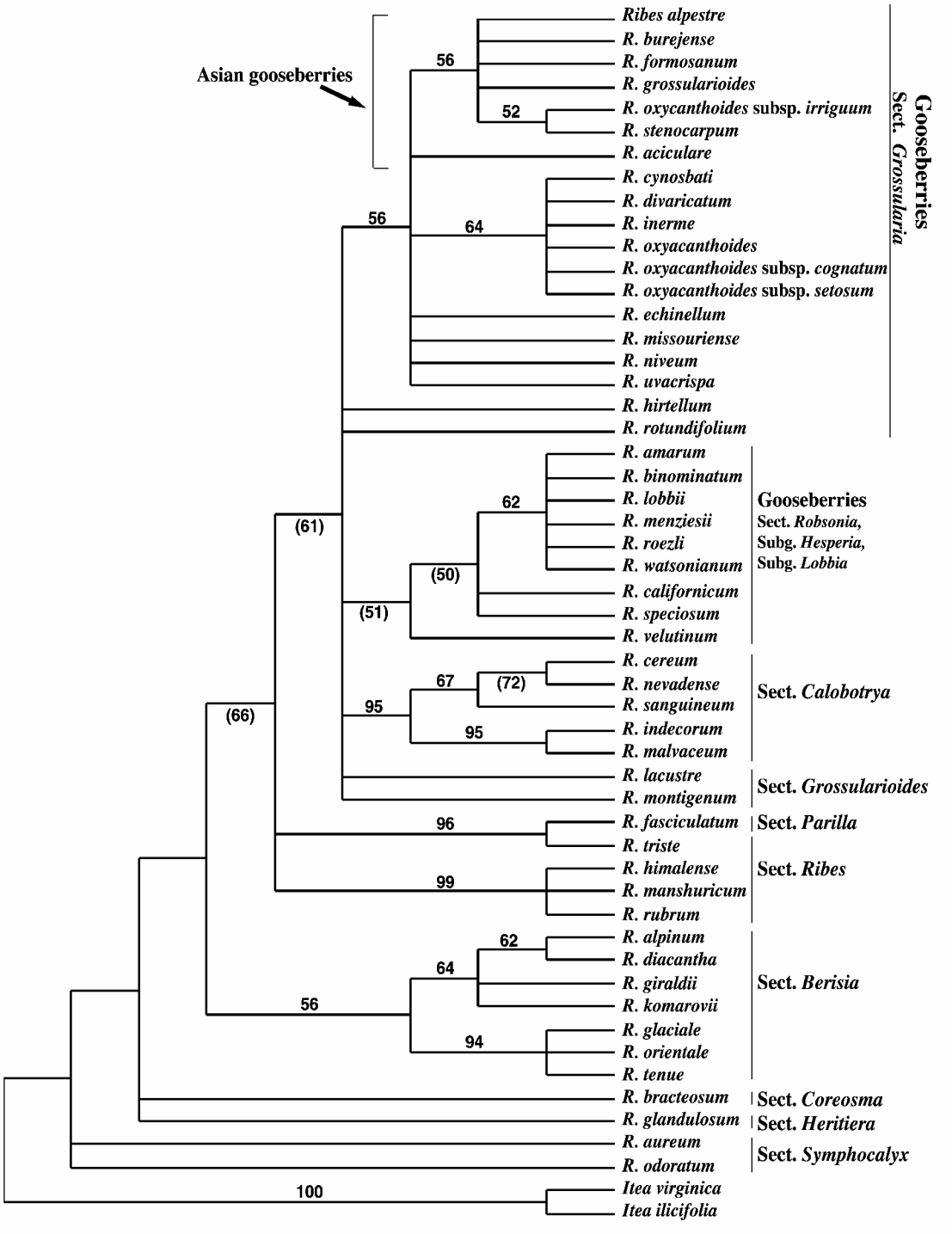

FIG. 3. The majority-rule consensus of 34,000 trees resulting from analyses of *psbA-trnH* sequence data (CI=0.92, 0.88 excluding uninformative characters; RI=0.94). Numbers above the branches indicate the percentage of bootstrap replicates in which the clade appeared. Numbers in parentheses below the branches indicate the percentage of trees in which the clade appeared, when less than 100%.

with the inclusion of some species of sect. *Ribes* in ITS trees (Senters and Soltis 2003). Section *Symphocalyx* was strongly resolved in ETS and restriction site trees (Fig. 2; Messinger et al. 1999), but was unresolved in *psbA-trnH* trees (Fig. 3) (ITS trees included only one species). Section *Heritiera* appeared to be polyphyletic in ITS and restriction site trees (only one species of sect. *Heritiera* is included in ETS or *psbA-trnH* analyses) (Messinger et al. 1999; Senters and Soltis 2003). The monophyly of sects. *Ribes* and *Coreosma* remain unclear. For those species sampled, sect. *Ribes* was resolved as monophyletic in restriction site trees (Messinger et al. 1999), as monophyletic excluding *R. triste* in ETS and *psbA-trnH* trees (Figs. 2, 3), and as polyphyletic in ITS trees, with some species clearly related to sect. *Berisia* (Senters and Soltis 2003). Section *Coreosma* was resolved as polyphyletic in ETS and restriction site trees (Fig. 2, Messinger et al. 1999), and as para- or polyphyletic in ITS trees (Senters and Soltis 2003) (only one species was included in the *psbA-trnH* dataset). Previous restriction site analyses of 18–26S rDNA suggested that members of sect. *Coreosma* were divergent from one another and from other *Ribes* species (Messinger et al. 1993). Sampling from sect. *Parilla* was too limited to draw strong conclusions, but the ITS trees supported its monophyly to the exclusion of *R. fasciculatum*, the only non-South American member of the section (Senters and Soltis 2003). Relationships among the sections outside of the predominantly western North American clade (sects. *Calobotrya*, *Grossularioides*, subg. *Grossularia*) varied markedly among datasets, with little support for any one resolution (Figs. 2, 3).

Trees Generated from Analyses of Combined Datasets. Dataset combinability is typically based on a critical value of $p = 0.05$ (i.e., datasets are incongruent when $p < 0.05$). However, studies suggest that a critical value of $p = 0.05$ may be too strict such that the null hypothesis of dataset congruence will be falsely rejected (Huelsenbeck et al. 1996; Sullivan 1996; Cunningham 1997), and that a value such as $p = 0.01$ may be more appropriate (Cunningham 1997). Many of the datasets reported here were not combinable based on intitial partition homogeneity tests, but the incongruence was generally eliminated when problematic taxa were excluded.

Analyses of the readily combinable chloroplast *psbA-trnH* and restriction site data ($p=0.66$) generated six trees (length 144; CI=0.90, 0.79 excluding uninformative characters; RI=0.87). In agreement with analyses of restriction site data alone, true gooseberries (sect. *Grossularia*) were not placed as sister to the remaining glabrous-styled gooseberries (*Hesperia*, *Lobbia*, *Robsonia*), suggesting that subg. *Grossularia* is not monophyletic (Figs. 1, 4). The true gooseberries were nested in a paraphyletic sect. *Grossularioides*, as in the restriction site trees, with sect. *Calobotrya* weakly placed as sister.

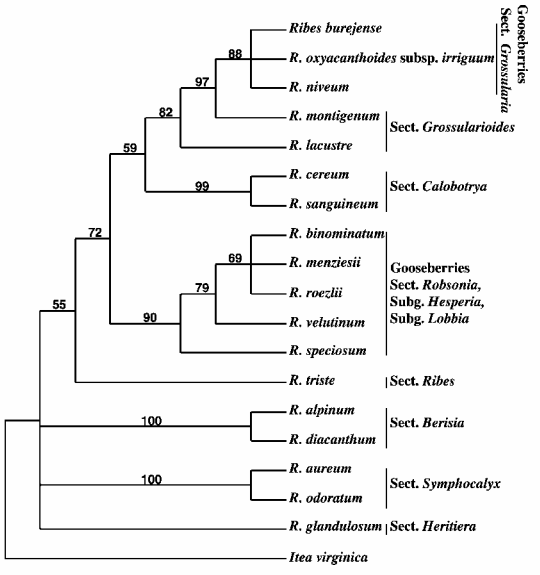

FIG. 4. The strict consensus of six trees resulting from analyses of *psbA-trnH* sequence data combined with chloroplast restriction site data (Messinger et al. 1999) (CI=0.90, 0.79 excluding uninformative characters; RI=0.87). Data combinability determined by partition homogeneity tests ($p=0.66$). Numbers above the branches indicate the percentage of bootstrap replicates in which the clade appeared.

Support for sect. *Grossularia*, sect. *Calobotrya*, and the glabrous-styled gooseberries (*Hesperia*, *Lobbia*, *Robsonia*) increased relative to analyses of either dataset alone (Fig. 3; Messinger et al. 1999). Support for a paraphyletic sect. *Grossularioides* decreased slightly relative to the restriction site analyses (Fig. 4; Messinger et al. 1999).

Analyses of the combined ETS and ITS rDNA data ($p=0.04$; excluded *R. fasciculatum*, *R. glandulosum*, *R. himalense*, *R. manshuricum*, *R. nevadense*, *R. rubrum*, *R. oxyacanthoides* subsp. *oxycanthoides*, *R uvacripsa*) generated 150 trees (length 614; CI=0.77, 0.70 excluding uninformative characters; RI=0.85) (Fig. 5). Subg. *Grossularia* was monophyletic, as were the true gooseberries (sect. *Grossularia*) (Fig. 5). The glabrous-styled gooseberries (*Hesperia*, *Lobbia*, *Robsonia*) were strongly resolved as monophyletic with the exception of *R. watsonianum* (sect. *Lobbia*), placed strongly as sister to the true gooseberries. Within sect. *Grossularia*, only the Asian clade was resolved with strong support. The clade containing subg. *Grossularia*, sect. *Grossularioidies*, and sect. *Calobotrya* was again evident, with sect. *Grossularioides* resolved as sister to subg. *Grossularia*. Support for the glabrous-styled gooseberries (*Hesperia*, *Lobbia*, *Robsonia*), sect. *Calobotrya*, sect. *Grossularioides*, and the Asian clade within sect. *Grossularia* increased relative to support from ETS or ITS datasets analyzed individually (Figs. 2, 5; Senters and Soltis 2003).

The most evident difference between chloroplast and

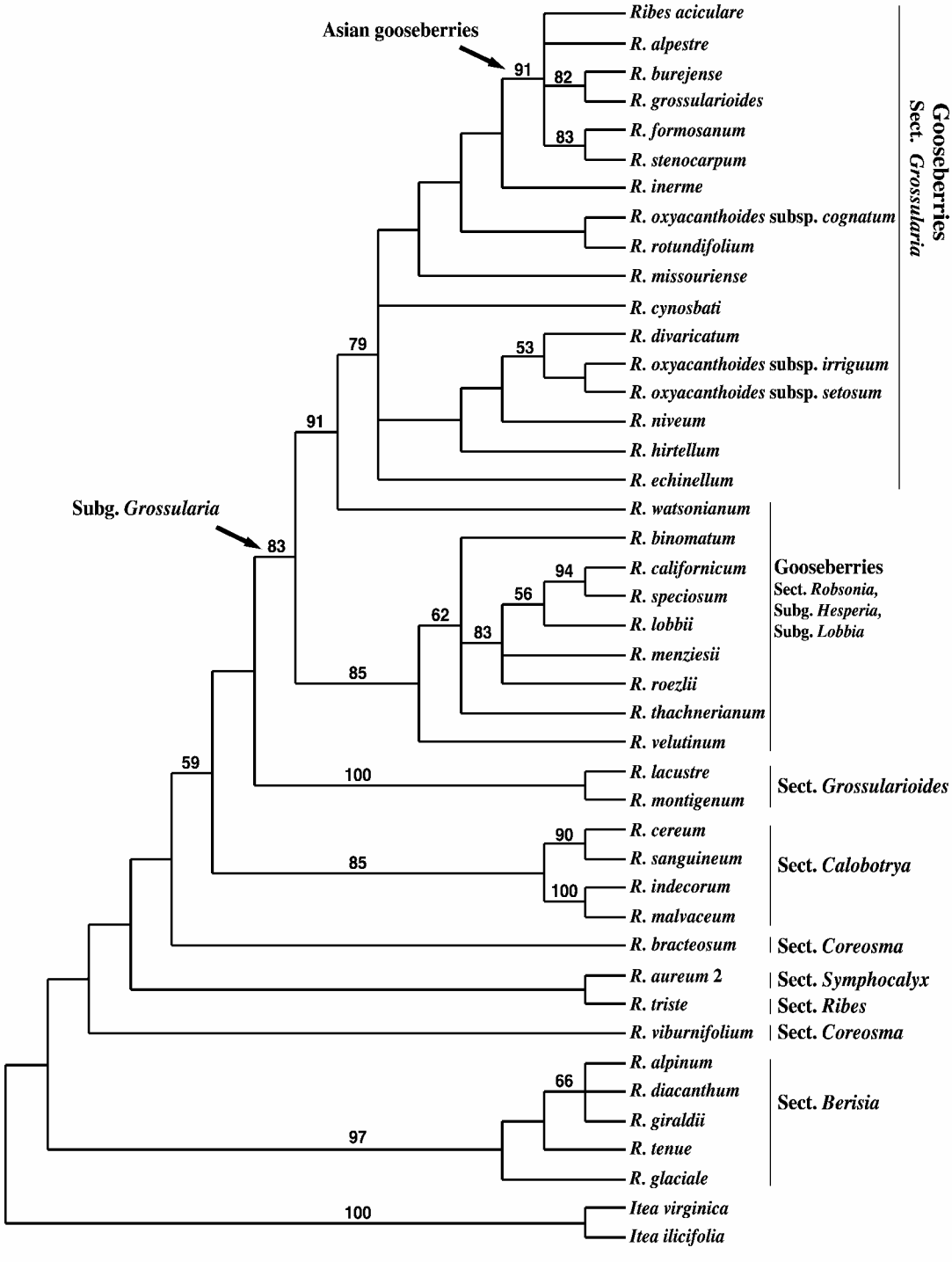

FIG. 5. The strict consensus of 150 trees resulting from analyses of combined ETS and ITS sequence data (CI=0.77, 0.70 excluding uninformative characters; RI=0.85). Data combinability determined by partition homogeneity tests (p=0.04). Numbers above the branches indicate the percentage of bootstrap replicates in which the clade appeared.

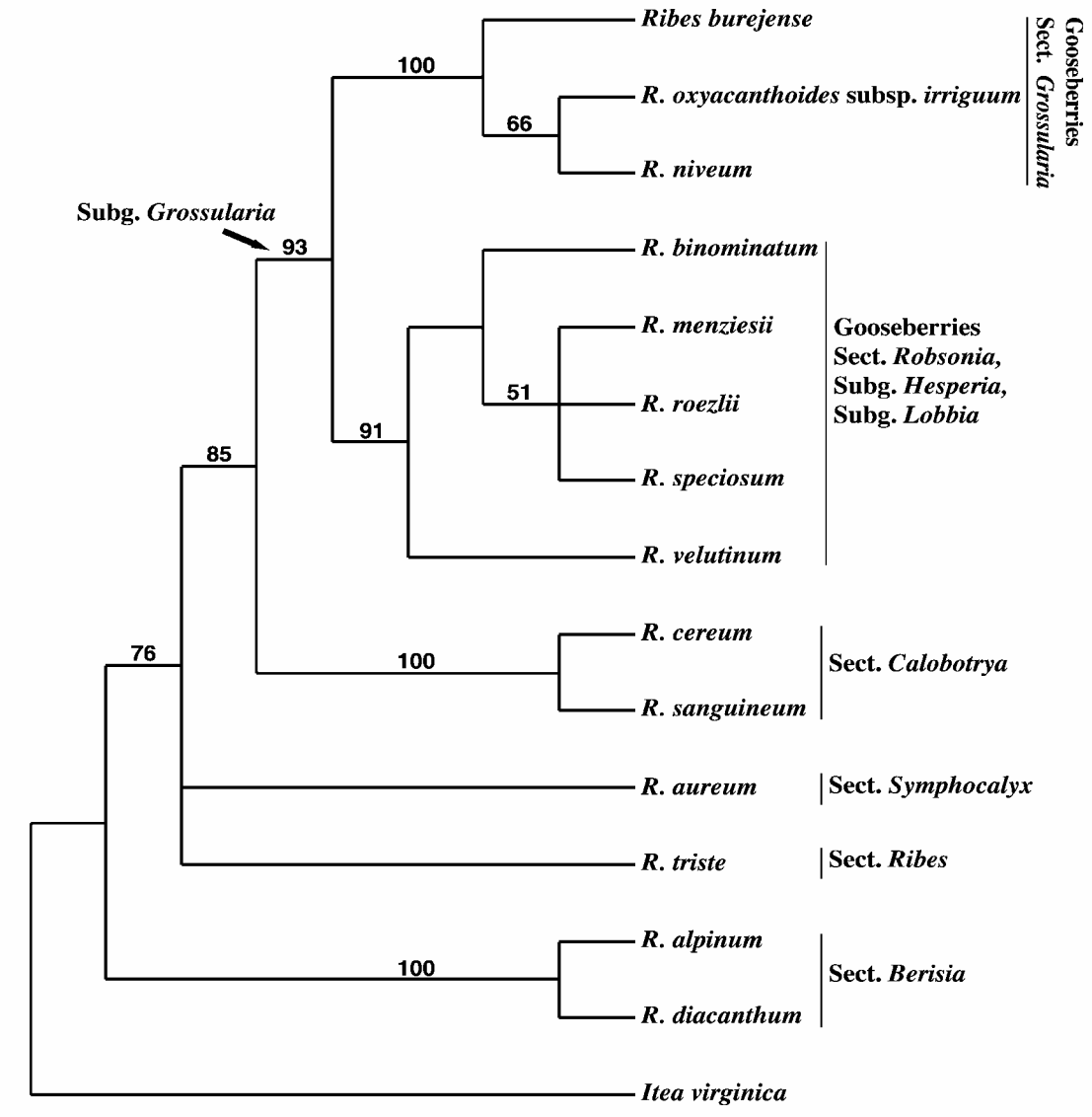

FIG. 6. The strict consensus of four trees resulting from analyses of the four datasets combined (ETS, ITS, *psbA-trnH*, chloroplast restriction sites). Data combinability was determined by partition homogeneity tests (p=0.75). Numbers above the branches indicate the percentage of bootstrap replicates in which the clade appeared.

rDNA trees was the position of sect. *Grossularioides*. Subgenus *Grossularia* is monophyletic in rDNA trees, with sect. *Grossularioides* placed weakly as sister (Fig. 5). In contrast, subg. *Grossularia* is polyphyletic in chloroplast trees, with sect. *Grossularia* nested within sect. *Grossulariodes*, and sect. *Calobotrya* sister to that grouping (Fig. 4). Analyses of the combined chloroplast and rDNA datasets (p=0.75; excluded *R. glandulosum*, *R. lacustre*, *R. montigenum*) generated four trees (length 556; CI=0.88, 0.69 excluding uninformative characters; RI=0.76) (Fig. 6). Taxon sampling was limited, due to the limited overlap among datasets, but trees showed strong resolution of sect. *Calobotrya*, sect. *Grossularia*, and the glabrous-styled gooseberries (*Hesperia*, *Lobbia*, *Robsonia*). These groups again formed a well-supported clade, nested within *Ribes* (Fig. 6). The strongly supported sister-group relationship between the true and glabrous-styled gooseberries (Fig. 6) is consistent with the monophyly of subg. *Grossularia*, but could change with the inclusion of sect. *Grossularioides*.

ETS and *psbA-trnH* combined analyses (p=0.04; excluded *R. oxyacanthoides* subsp. *irriguum*) generated 329 trees (length 372; CI=0.84, 0.77 excluding uninformative characters; RI=0.90) (Fig. 7). Subgenus *Grossularia* was resolved as monophyletic, within which both the glabrous-styled and the true gooseberries were strongly supported, the latter including *R. watsonianum* (subg. *Lobbia*). The Asian clade was well supported

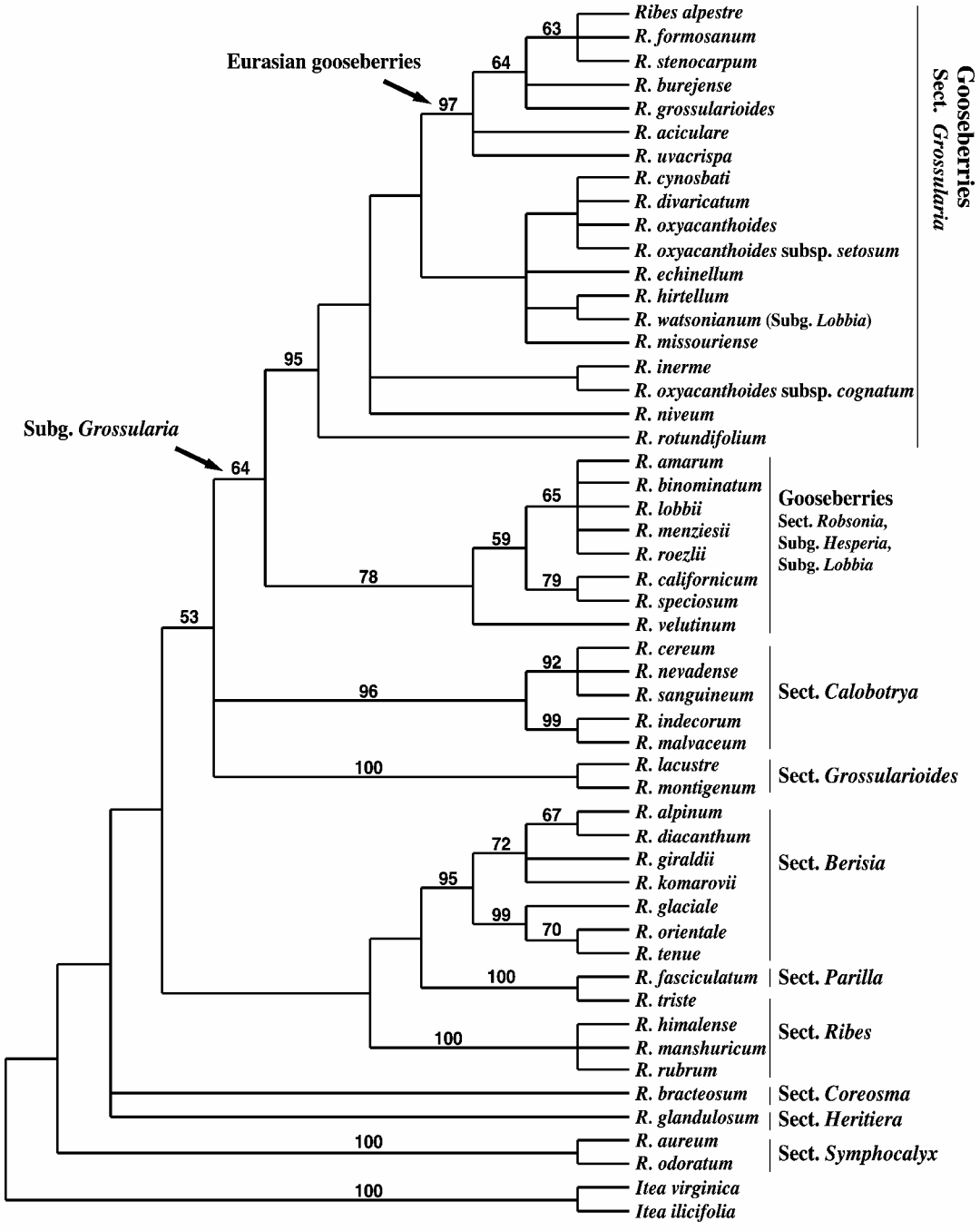

FIG. 7. The strict consensus of 329 trees resulting from analyses of combined ETS and *psbA-trnH* data (CI=0.84, 0.77 excluding uninformative characters; RI=0.91). Dataset combinability was determined by partition-homogeneity tests (p= 0.04). Numbers above the branches indicate the percentage of bootstrap replicates in which the clade appeared.

within sect. *Grossularia*, including the European *R. uva-crispa*. Both sect. *Calobotrya* and sect. *Grossularioides* were strongly supported, but neither was resolved as sister to subg. *Grossularia*. The clade comprising subg. *Grossularia*, sect. *Grossularioides*, and sect. *Calobotrya* was again evident, nested within *Ribes*.

Combined analyses of *psbA-trnH* and rDNA datasets (p=0.02; excluded *R. oxyacanthoides* subsp. *irriguum* and taxa excluded from ETS+ITS analysis) generated 3080 trees (length = 719; CI=0.80, 0.74 excluding uninformative characters; RI=0.85). Sects. *Grossularioides*, *Calobotrya*, *Grossularia*, and the glabrous-styled gooseberries

(*Hesperia*, *Lobbia*, *Robsonia*) were each strongly supported, and again formed a strongly supported clade nested in *Ribes* (Fig. 8). Support for the monophyly of subg. *Grossularia* was increased relative to support seen with any individual dataset (Figs. 2, 3, 8).

Combinability (p=0.12) of all datasets for those taxa represented in three of the four datasets required exclusion of composite taxa problematic within the rDNA dataset, as well as exclusion of taxa problematic for the chloroplast versus rDNA datasets. Combined analyses generated 4,802 trees (length=768; CI=0.80, 0.74 excluding uninformative characters; RI=0.85). Section *Calobotrya*, sect. *Grossularia*, and the glabrous-styled gooseberries (*Hesperia*, *Lobbia*, *Robsonia*) excluding *R. watsonianum* were each well supported, and together formed a strongly supported clade (Fig. 9). Subgenus *Grossularia* was resolved as monophyletic, bearing in mind that sect. *Grossularioides* was excluded from the analyses.

While all combined datasets resolved the clade containing sects. *Calobotrya*, *Grossularioides*, and subg. *Grossularia* as nested within *Ribes* (Figs. 4–9), relationships among other lineages were unclear, as in the analyses of individual datasets (Figs. 2, 3). Basal relationships were unresolved in the analyses of combined chloroplast data (Fig. 4). The combined ETS and *psbA-trnH* analyses suggested basal positions for golden currants (sect. *Symphocalyx*) (Fig. 7). All remaining analyses suggested basal positions for sects. *Berisia* and *Ribes* (Figs. 5, 6, 8, 9). Basal relationships were not well-supported in any analysis.

Kishino-Hasegawa Tests. Trees generated from analyses constraining the monophyly of subg. *Grossularia* versus trees constrained to resolve a sister relationship between sect. *Grossularioides* and sect. *Grossularia* were marginally, but significantly different in both the chloroplast (p=0.03) and the rDNA (p=0.05) datasets.

Ancestral Area Reconstructions. DIVA reconstructions of ancestral areas support a western North American origin for a large segment of *Ribes*, beginning with node 2 in Fig. 8. Within this clade it is also the case that subg. *Grossularia* is inferred to have diversified first within western North America (Fig. 8, node 6). Subsequent movement to eastern Asia is inferred to have occurred somewhere within sect *Grossularia*, but the ancestral area for the true gooseberries is equivocal, and can include virtually any combination of areas depending on the exact resolution of the large polytomy at the base of this clade (Fig. 8, node 8). Importantly, our results indicate that the Asian species within sect. *Grossularia* form a clade, implying a single migration from North America to Asia. The ancestral area for *Ribes* is reconstructed to be western North America and western Eurasia, or western North America and all of Eurasia (Fig. 8, node 1). However, this result hinges on resolution at the base, which remains highly uncertain and requires more intensive sampling of *Ribes* lineages outside of subg. *Grossularia*.

Discussion

Gooseberry Monophyly. Results from previous phylogenetic analyses based on nuclear 18S–26S rDNA ITS data (Senters and Soltis 2003) and chloroplast restriction site data (Messinger et al. 1999) differed regarding gooseberry monophyly. Adding the additional nuclear 18S–26S rDNA ETS and chloroplast *psbA-trnH* datasets could increase support and resolution in resulting topologies, or could reinforce the differences between the nuclear and chloroplast topologies, with each reflecting accurate but separate histories.

Initial examination of the trees produced with the additional datasets reinforced the apparent conflict between nuclear and chloroplast genomes. ITS, ETS, and combined rDNA datasets supported the monophyly of subg. *Grossularia* (Figs. 1, 2, 5). In contrast, the combined chloroplast datasets (restriction sites plus *psbA-trnH*) suggested a closer relationship between the true and glabrous-styled gooseberries than did the restriction site data alone (Fig. 1; Messinger et al. 1999), but still resolved subg. *Grossularia* as non-monophyletic, with sect. *Grossularioidies* forming a grade at the base of sect. *Grossularia* (Fig. 4). The increase in tree length when combined chloroplast data was constrained to resolve a monophyletic subg. *Grossularia* (p=0.03) or when combined rDNA data was constrained to resolve sects. *Grossularioides* and *Grossularia* as a clade (p=0.05) also indicated dataset incongruence.

The discrepancy between nuclear and chloroplast topologies primarily involved the restriction sites dataset and not the *psbA-trnH* dataset. The *psbA-trnH* dataset provided little resolution (Fig. 3), conflicting with neither the rDNA nor the restriction sites topologies. Analyses combining the *psbA-trnH* data with either the ETS data (p=0.04; Fig. 7) or the rDNA data (p=0.02; Fig. 8) produced trees resolving subg. *Grossularia* as monophyletic with increased support relative to ETS or rDNA data alone (Figs. 2, 5). Section *Grossularioides* was either sister to subg. *Grossularia* (*psbA-trnH* + rDNA; Fig. 8) or was unresolved in a polytomy with subg. *Grossularia* and sect. *Calobotrya* (*psbA-trnH* + ETS; Fig. 7). The monophyly of subg. *Grossularia* was also supported by analyses of all datasets combined, whether including taxa represented in all four datasets (p=0.75; Fig. 6) or in three of the four datasets (p=0.12; Fig. 9). Analyses combining all datasets required exclusion of sect. *Grossularioides*, thus leaving subg. *Grossularia* monophyly uncertain. However, including sect. *Grossulariodes* in spite of dataset incongruence (p=0.01) produced trees resolving a monophyletic subg. *Grossularia* sister to a monophyletic sect. *Grossularioides* (not shown).

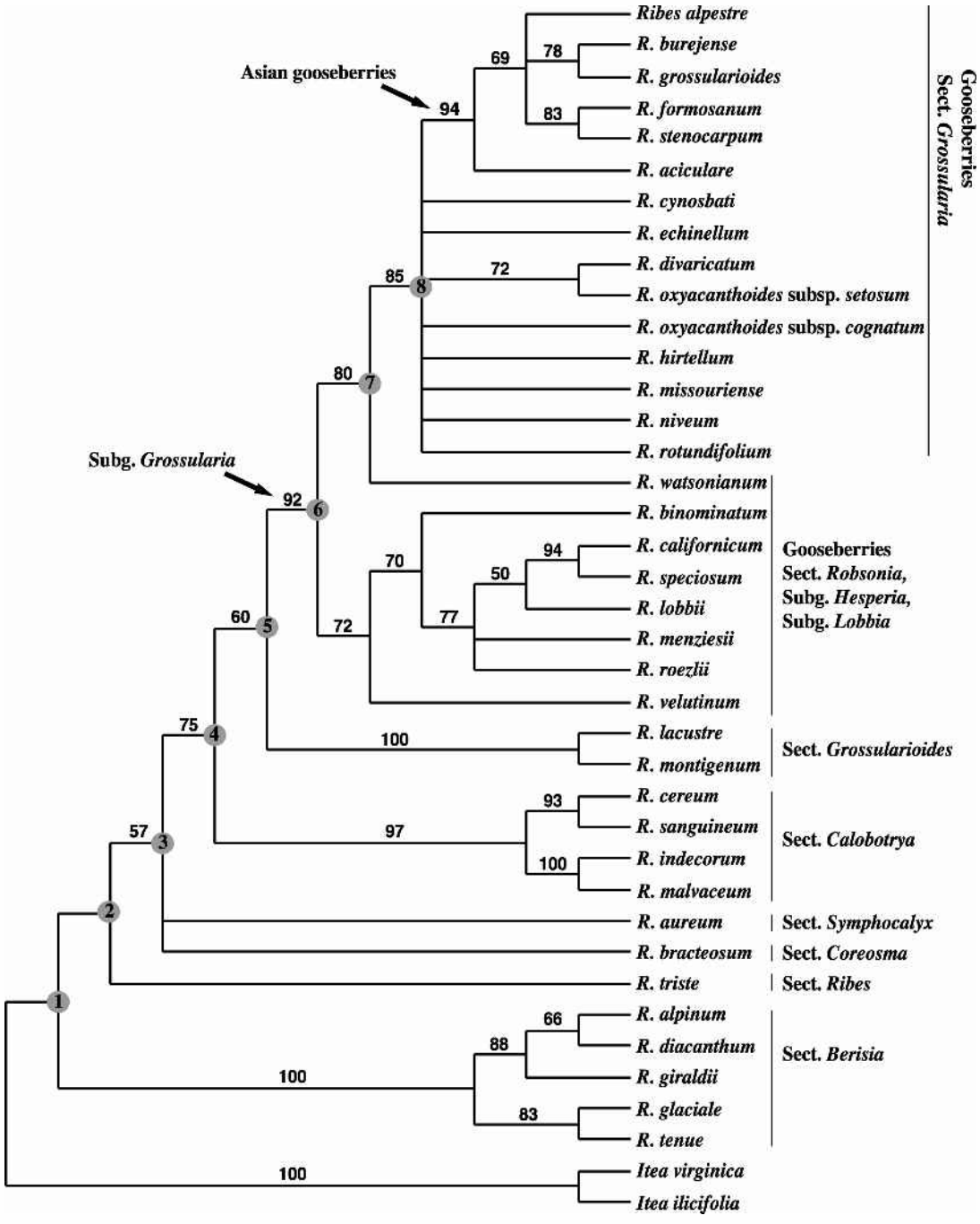

FIG. 8. The strict consensus of 3,080 trees resulting from analyses of combined *psbA-trnH* and rDNA data (CI = 0.80, 0.74 excluding uninformative characters; RI = 0.85). Dataset combinability was determined by partition homogeneity tests (p=0.02). Numbers above the branches indicate the percentage of bootstrap replicates in which the clade appeared. DIVA (Ronquist 1996) was used to infer ancestral areas at the numbered nodes. The ancestral areas at nodes two through seven were reconstructed as western North America. Node one was reconstructed as western North America plus either western Eurasia or all of Eurasia. There were multiple possible reconstructions at node 8, including (1) western North America and eastern Eurasia, (2) North America and eastern Eurasia, (3) North America, or (4) western North America.

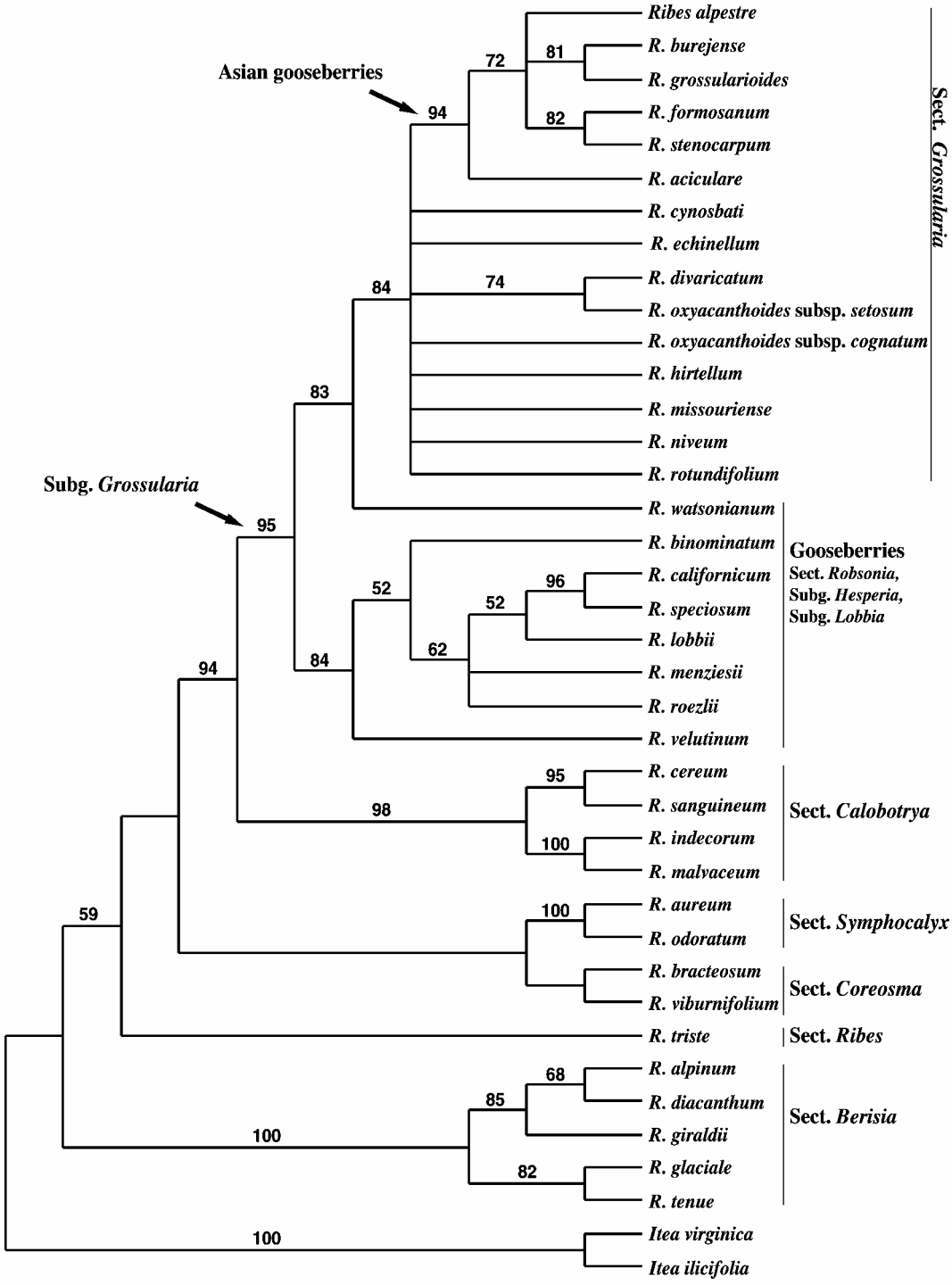

FIG. 9. The strict consensus of 4,802 trees resulting from analyses of all four datasets (ITS, ETS, *psbA-trnH*, chloroplast restriction sites), including taxa represented in at least three datasets (CI=0.80, 0.74 excluding uninformative characters; RI=0.85). Dataset combinability was determined using partition homogeneity tests (p=0.12). Numbers above the branches indicate the percentage of bootstrap replicates in which the clade appeared.

The general picture that emerges from these analyses suggests that subg. *Grossularia* is monophyletic (Figs. 2, 5–9). rDNA data supported gooseberry monophyly (Figs. 2, 5). *psbA-trnH* data, while lacking sufficient variation to resolve gooseberry monophyly or non-monophyly, supported gooseberry monophyly in combination with rDNA data (Figs. 7, 8). Finally, analyses of all datasets combined supported gooseberry monophyly, although these analyses excluded the spiny currants (sect. *Grossularioides*) (Figs. 6, 9). What requires further investigation is the role hybridization may have played in the history of the spiny currants (sect. *Grossularioides*) and the true gooseberries (sect. *Grossularia*). Messinger et al. (1999) encouraged further exploration of this possibility in *Ribes*, in which they noted potential chloroplast capture of the sect. *Grossularia* chloroplast type by sect. *Grossularioides*. Crosses have not been successful between species of sect. *Grossularioides* and other sections within *Ribes*, and are usually unsuccessful between sections or subgenera (Keep 1962). Confirmation of the pattern seen in trees generated from the chloroplast restriction site data (Messinger et al. 1999) is needed from additional chloroplast datasets that provide greater resolution than did the *psbA-trnH* dataset.

Relationships Within the Gooseberries. Subgenus *Grossularia* comprises two main lineages (Figs. 2, 5–9), the true gooseberries (sect. *Grossularia*) and a clade of glabrous-styled gooseberries (*Hesperia*, *Lobbia*, *Robsonia*). The four gooseberry sections are traditionally distinguished from each other by basally pubescent styles in sect. *Grossularia* (with the exception of some Asian species), by four versus five parted flowers in sect. *Robsonia*, by anthers broader at the base in subg. *Hesperia*, and by the absence of the above set of features in subg. *Lobbia*. The glabrous-styled gooseberry lineage, comprising 27 species distributed through western North America into Mexico (Berger 1924), was well-supported given our sampling, but sections within this lineage were not resolved. *Ribes watsonianum* (subg. *Lobbia*) was surprisingly placed outside of the glabrous-styled gooseberry lineage, as sister to the true gooseberries (Figs. 2, 5, 7–9). ITS data resolved *R. watsonianum* within the glabrous-styled gooseberry lineage (Senters and Soltis 2003), but ETS data strongly resolved the species within the true gooseberry lineage (sect. *Grossularia*) (Fig. 2). This conflict within the rDNA data may reflect hybridization between *R. watsonianum* and a member of sect. *Grossularia* followed by fixation within the *R. watsonianum* genome of a recombinant rDNA repeat type. Accounts of *Ribes* hybrids in the field are relatively rare (Messler et al. 1991) and do not implicate *R. watsonianum*, but fertile hybrids can be obtained from artificial crosses between glabrous-styled gooseberries and true gooseberries (Keep 1962, citing *R. lobbii* × *R. divaricatum*). Alternatively, *R. watsonianum* may posses multiple rDNA repeat types, with different repeat types sampled in our ITS versus ETS datasets. Each of these possibilities has been reported in other plant taxa (Wendel et al. 1995a; Campbell et al. 1997 for potential recombinant rDNA repeat types: Suh et al. 1993; Wendel et al. 1995b for incomplete homogenization of rDNA repeat types).

The true gooseberries (sect. *Grossularia*) have a broad range throughout the northern hemisphere, with nine species in North America (Sinnott 1985) and seven species in Eurasia (Berger 1924). In his treatment of North American gooseberries, Sinnott (1985) divided his nine species into five groups based on phenetic analyses. The groups consisted of (1) *R. cynosbati*, with spiny fruits, (2) *R. niveum*, *R. curvatum*, and *R. missouriense* with highly exserted stamens and long sepals, (3) *R. rotundifolium* and *R. divaricatum*, with medium length stamens and purple sepals, (4) *R. inerme* and *R. hirtellum*, with intermediate length stamens and small floral features, and (5) the five subspecies of *R. oxyacanthoides*, with short stamens. Sinnott (1985) recommended that *R. echinellum* be excluded from the true gooseberries and aligned with the glabrous-styled gooseberries of western North America (*Hesperia*, *Lobbia*, *Robsonia*). The analyses presented here showed strong support for the inclusion of *R. echinellum* as well as the Eurasian species within the true gooseberry lineage (Figs. 2–3, 5, 7–9). Resolution among the North American species was lacking or not well-supported (Figs. 2–9) and thus neither supported nor conflicted with Sinnott's hypothesized relationships.

The seven Eurasian species of sect. *Grossularia* (Berger 1924) include *R. uvacrispa* in Europe, *R. alpestre* and *R. aciculare* in the Himalayan region, *R. stenocarpum* and *R. formosanum* in southern and eastern Asia, and *R. burejense* and *R. grossularioides* in northern and eastern Asia. A clade of Asian species is well-supported (Figs. 2, 5, 7–9), with possible inclusion of *R. uvacrispa* (Figs. 2, 7). The *psbA-trnH* dataset includes *R. oxyacanthoides* subsp. *irriguum* within the Asian clade (Fig. 3), but this is weakly supported (Fig. 3) and requires further substantiation, particularly since *R. oxyacanthoides* subsp. *irriguum* is restricted to northwestern North America and may itself be of hybrid origin from crosses between *R. oxyacanthoides* subsp. *setosum* and *R. inerme* (Sinnott 1985), both distributed in western North America. Resolution within the clade of Asian species suggests a correspondance to geographic and floristic regions within Asia. Whether the Asian and North American true gooseberries represent divergent lineages or a monophyletic Asian clade nested within a North American grade is unclear given the available data.

***Other Groups within* Ribes.** Since this study focused on the gooseberries, sampling within other sections of *Ribes* was sometimes limited. Nevertheless, the

data did support the monophyly of sects. *Calobotrya*, *Parilla*, *Symphocalyx*, and *Berisia*. Section *Calobotrya* is a group of 21 western North American species (Berger 1924) known as the ornamental currants. The monophyly of sect. *Calobotrya* was well-supported, with the inclusion of some dwarf currants (sect. *Heritieria*) (Figs. 2–9; Messinger et al. 1999; Senters and Soltis 2003). Sampling was limited in sect. *Parilla*, a group of 41 dioecious South American species (Janczewski 1907), but the group was supported in ITS trees, excluding the east Asian *R. fasciculatum*, the section's only non-South American species (Senters and Soltis 2003). A possible sister relationship between sect. *Parilla* and sect. *Calobotrya* (unpubl. data cited in Weigend and Binder 2001) is consistent with the position of sect. *Parilla* seen with ITS data (Senters and Soltis 2003). Section *Symphocalyx*, the golden currants, includes five species distributed through northern Mexico, and central and western North America (Berger 1924). The golden currants were well-supported given the available sampling (Fig. 2, 4, 9). The alpine currants, sect. *Berisia*, are a group of 17 dioecious species distributed through Eurasia (Janczewski 1907). The monophyly of sect. *Berisia* was well-supported (Figs. 2–9; Messinger et al. 1999), but with the inclusion of some red currants (sect. *Ribes*) in ITS trees (Senters and Soltis 2003).

The monophyly of sects. *Ribes* and *Coreosma* was questionable (Figs. 2, 3, 5). The red currants (sect. *Ribes*) are a group of 15 Eurasian species, with one species, *R. triste*, also distributed in North America. Section *Coreosma*, the black currants, includes six species in North America and six in Eurasia (Berger 1924). Section *Heritiera*, the dwarf currants, appeared to be polyphyletic, as seen in Messinger et. al. (1999) and Senters and Soltis (2003). This group of six species (Berger 1924) is defined primarily by a prostrate habit, and, as suggested by Messinger et al. (1999), is likely a case of convergence.

Biogeography. The spread of taxa around the Northern Hemisphere has been facilitated by the availability at various times of two major migration routes—the Beringian and the North Atlantic land bridges (Tiffney 1985a, 1985b; Donoghue et al. 2001; Sanmartín et al. 2001). Especially in large and relatively old clades, which have become widespread around the Northern Hemisphere, it is possible (perhaps even likely) that both pathways were used. Information on the timing of key divergences and on the direction of movement within subclades will be critical in sorting out the possibilities in particular cases. *Ribes* provides an excellent example of such a group, being represented in Eurasia by six sections (*Berisia*, *Ribes*, and some species from *Heritiera*, *Coreosma*, *Grossularioides*, and *Grossularia*), in western North America by nine (*Symphocalyx*, *Calobotrya*, *Robsonia*, *Hesperia*, *Lobbia*, and some species of *Coreosma*, *Heritiera*, *Grossularioides*, and *Grossularia*), and in eastern North America by three (some species of *Heritiera*, *Ribes*, and *Grossularia*). One approach for sorting through a complex biogeographic history, as might be expected in *Ribes*, is to examine phylogenetic patterns in component clades and, ideally, to date divergences.

Our analyses indicate that subg. *Grossularia* is nested within a predominantly western North American clade (Figs. 2, 5–9), and that it diversified initially in that region (Fig. 8, node 6). Subsequently, within sect. *Grossularia*, it appears that movement occurred from western North America to eastern Asia, presumably through Beringia, followed by vicariance and the origin and diversification of the well-supported Asian gooseberry clade. Donoghue et al. (2001) and Xiang and Soltis (2001) highlighted cases of movement out of Asia into North America through the Bering Land Bridge. *Ribes* appears to provide a case of movement in the opposite direction. Movement in both directions is, of course, to be expected. What remains to be determined in future studies of disjunct taxa is exactly which groups moved in which directions, and whether there are any significant generalizations that can be made about these different patterns.

Elsewhere in *Ribes* there are other possible cases of movement between North America and Asia. One case involves *R. lacustre* of sect. *Grossularioides*, which is distributed in eastern Asia as well as in North America. Other possible cases relate to resolution at the base of *Ribes*, where relationships remain uncertain. The basal positions of sect. *Symphocalyx* in trees resulting from *psbA-trnH* data and from the combination of *psbA-trnH* and ETS data suggest a western North American origin for the entire clade (Figs. 2, 7). This would imply early dispersion to Asia, giving rise to sects. *Berisia* and *Ribes*. However, all other datasets and dataset combinations suggested a broader Eurasian plus western North American distribution at the base (Fig. 8, node 1), owing to the basal positions of sects. *Berisia* and *Ribes*. This would imply early vicariance involving Asia and North America, and movement back to Asia within the western North American clade. Future studies should include more sampling of sects. *Berisia*, *Ribes*, *Coreosma* and *Symphocalyx* to help resolve basal relationships and biogeographic patterns in *Ribes*.

The fossil record for *Ribes* consists largely of leaves, with few reports of seeds (Kremenetski 1998), fruits (Cevallos-Ferriz 1995), and flowers (Gandolfo et al. 1998). The leaf record for *Ribes* in North America may extend from approximately 2 mya (Hannibal 1911; Dorf 1930; Axelrod 1966) to at least 34.5 mya (MacGinitie 1953; Manchester 2001), and possibly to 45 or 50 mya (Axelrod 1998; Wehr and Hopkins 1994). Although leaf features are seldom used to distinguish extant taxa of *Ribes*, Wolfe (1964) used leaf serration and venation features to distinguish between fossil

leaves of subg. *Ribes* and subg. *Grossularia* from Nevada. If these features are reliable, this dates subg. *Grossularia* to at least 14 mya (Fig. 9, node 6). A critical next step in understanding *Ribes* biogeography will be to accurately assign *Ribes* fossils to particular subclades so as to infer the timing of intercontinental divergences.

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