Phylogenetics of the Caprifolieae and Lonicera (Dipsacales) Based on Nuclear and Chloroplast DNA Sequences

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Abstract—Recent phylogenetic analyses of the Dipsacales strongly support a Caprifolieae clade within Caprifolieae including Leycesteria, Triosteum, Symphoricarpos, and Lonicera. Relationships within Caprifolieae, however, remain quite uncertain, and the monophyly of Lonicera, the most species-rich of the traditional genera, and its subdivisions, need to be evaluated. In this study we used sequences of the ITS region of nuclear ribosomal DNA and five chloroplast non-coding regions (rpoB–trnC spacer, atpB–rbcL spacer, trnS–trnG spacer, petN–psbM spacer, and psbM–rps16 spacer) to address these problems. Our results indicate that Heptacodium is sister to Caprifolieae, Triosteum is sister to the remaining genera within the tribe, and Leycesteria and Symphoricarpos form a clade that is sister to a monophyletic Lonicera. Within Lonicera, the major split is between subgenus Caprifolium and subgenus Lonicera. Within subgenus Lonicera, sections Coelxylosteum, Isokosteu, and Nintooa are nested within the paraphyletic section Isika. Section Nintooa may also be non-monophyletic. Our analysis of the genus Lonicera highlights instances of homoplasy in several morphological characters (e.g. hollow stems in subgenus Caprifolium and section Coelxylosteum in subgenus Lonicera). Furthermore, our data indicate possible instances of hybridization in section Nintooa and biogeographic disjunctions between the Old and New Worlds (e.g. subsections Distegiae and Alpigenae).

Keywords—chloroplast non-coding region, Heptacodium, honeysuckle, Kolkwitzia, Weigela, Zabelia.

Recent phylogenetic studies have resolved many relationships within Dipsacales (Donoghue et al. 2001, 2003; Bell and Donoghue 2005), the most important exception being within the tribe Caprifolieae of Caprifolieae (sensu Donoghue et al. 1998, 2003; = Caprifolieae s. str. sensu Backlund and Pyck 1998; AFG 1998). This clade contains Leycesteria Wallich. (6 species), Lonicera L. (ca. 200 species), Symphoricarpos Duhamel. (ca. 15 species), and Triosteum L. (6 species), and is well-supported in all recent analyses based on morphology (Judd et al. 1994) and DNA sequences (reviewed in Donoghue et al. 2003). However, none of the previous analyses have adequately tested the monophyly of each of the included genera, or confidently resolved relationships among them.

Caprifolieae includes genera that are among the most speciose in the Northern Hemisphere (i.e. Lonicera) and genera that contain few species (i.e. Triosteum and Leycesteria, Hsu and Wang 1988). With the exception of Leycesteria, the genera span the New and Old Worlds. Triosteum, with just six species, has both North American and Asian representatives (China and Japan) while Symphoricarpos has just one species in China, the remainder being native to North and Central North America. Lonicera is mainly distributed in temperate and subtropical areas, with several species extending their range into tropical areas of India, Malaysia, and the Philippines (Rehder 1903; van Steenis 1946). Many species within Caprifolieae have medicinal properties. Species of Symphoricarpos contain alkaloids and saponins used medicinally by several Native American tribes, while extracts from species of Lonicera have long been used in some traditional Chinese medicines for inflammation relief. A number of species have been bred as ornamentals worldwide, including several Lonicera species which have escaped from cultivation in the United States and have become costly invasives, including Japanese honeysuckle (L. japonica Thunb.) and the bush honeysuckles Amur (L. maackii Rupr.), Tartarian (L. tatarica L.), and Morrow’s honeysuckle (L. morrowii Gray), and their hybrid Bell’s honeysuckle (L. × bella Zabel).

Historically, Lonicera has received the most extensive taxonomic evaluation. Rehder (1903) published a synopsis of Lonicera, wherein he reviewed the taxonomic literature in detail and proposed a classification system. This was the first and most comprehensive taxonomic published treatment of Lonicera and has since been adopted widely with only minor changes. Rehder (1903) recognized two subgenera in Lonicera, the first of which, Chamacerasus (= Lonicera), with more than 150 species, consisted of four sections (Coelxylosteum, Isika, Isokosteu, and Nintooa) and 20 subsections. Later, Rehder combined subsections within Chlamydocarpus and Viscaritae (Rehder 1909, 1913). The second subgenus, Periclymenum (= Caprifolkium), with approximately 22 species, was composed of four subsections. Subgenus Lonicera is characterized by two-flowered cymes and free leaves, whereas subgenus Caprifolkium has three-flowered cymes in whorls and perfoliate leaves subtending the inflorescences.

In his treatment of the Japanese species of Lonicera, Nakai (1938) proposed many sections and subsections, including sections Bracteae, Fragrantissimae, Monanthae, Pararhodan-thae, Praeflorentes, Ramoissimae, Rhodantheae, Rotatae, and subsections Cerasinae, Euchranthae, Subsessilifora, and Tetramesae. However, these groups have rarely been accepted with the exception of section Monanthae, which has a single species endemic to Japan (L. gracilipes) and was treated as a subsection within section Isika by Hara (1983). In their treatment of Chinese Lonicera species, Hsu and Wang (1988) largely adopted Rehder’s classification system, but rejected several of Rehder’s subsections, including Spinoseae in section Isoxy-losteum, Tataricae in section Coelxylosteum, Distegiae in section Isika, and Brevisiliorae and Longiflorae in section Nintooa. Rehder’s four sections, therefore, have been generally agreed upon by most botanists who have considered the problem. Nevertheless, phylogenetic relationships among and within
these sections have never been explicitly addressed. Furthermore, the circumscriptions and evolutionary relationships of the numerous subsections have not been subjected to phylogenetic analysis.

The primary objectives of the present study were to resolve relationships within Caprifoliaceae, and test the monophyly of Lonicera and its subgenera, sections, and subsections using sequences from nuclear and chloroplast DNA regions that have been used widely in resolving relationships of closely related genera, including the ITS region of nuclear ribosomal DNA (Baldwin et al. 1995) and five chloroplast non-coding regions (rpoB-trnC spacer, atpB-rbcL spacer, trnS-trnG spacer, petN-psbM spacer, and psbM-trnD spacer; Shaw et al. 2005).

Materials and Methods

Plant Material—Fifty-one individuals of Lonicera were sampled, representing 47 species, 21 of the 24 subsections, all four sections, and both subgenera (Appendix 1). From other Caprifoliaceae we included two species of Leucesteria and three species each of Symphoricarpos and Triosteum and the monospecific genus Heptacodium. From Caprifoliaceae, two species of Weigela (Dierveillea) and one species each of Kolkwitzia and Zabelia (Linnaeae) were included in the analysis. The latter were used as outgroups for rooting purposes based on Donoghue et al. (2003) and Zhang et al. (2003). ITS sequences of Leucesteria and Triosteum were obtained from GenBank (Gould and Donoghue 2000); all others were newly obtained for this study.

Molecular Techniques—DNAs were extracted from silica-gel dried leaves using a DNeasy Plant Extract Mini kit following the manufacturer’s protocol (Qiagen, Valencia, California). All amplification was performed using either Eppendorf or MJ) Research thermal cyclers in 25–50 µL volumes with the following reaction components: 1–5 µL template DNA (10–100 ng), 1–2 µL primer, 10× buffer (Invitrogen Corporation, Carlsbad, California), 200 µM each dNTP, 1.5–2.0 mM MgCl2, 0.4–0.6 µM/L each primers, and 2.5 units Taq. Some reactions included bovine serum albumin with a final concentration of 0.2 µg/µL to improve amplification. ITS reactions included 10% dimethylsulfoxide. The sequencing primers and parameters for amplification are given in Table 1.

For most of the accessions, PCR products for ITS were cloned using the T-A tail cloning technique, and pPSM plasmid system (Promega, Madison, Wisconsin) and XLI blue competent E. coli cells (Stratagene, La Jolla, California) following manufacturer’s instructions. Two to five clones were sequenced for representative species to check for sequence heterogeneity among ITS repeats. Cycle sequencing reactions were carried out using a BigDye Terminator V3.1 kit (Applied Biosystems Inc., Foster City, California). Sequences were analyzed using an ABI automated DNA Sequencer 3700 (Perkin-Elmer/ABI), and edited using Sequencer (version 4.1, Gene codes Corporation, Ann Arbor, Michigan).

Phylogenetic Analysis—The edited sequences were aligned by eye using Se-Al v2.0a8 (Rambaut 1996). Matrices have been submitted to TreeBASE (study number S2161). Sequence limits of the ITS-1, 5.8S, and ITS-2 geneity among ITS repeats. Cycle sequencing reactions were carried out using a BigDye Terminator V3.1 kit (Applied Biosystems Inc., Foster City, California). Sequences were analyzed using an ABI automated DNA Se- quencer 3700, ABI Prism 3100 or 3730 (Perkin-Elmer/ABI), and edited using Sequencer (version 4.1, Gene codes Corporation, Ann Arbor, Michigan).

Phylogenetic Analysis—The edited sequences were aligned by eye using Se-Al v2.0a8 (Rambaut 1996). Matrices have been submitted to TreeBASE (study number S2161). Sequence limits of the ITS-1, 5.8S, and ITS-2 were determined by comparing them to a published sequence in Gen-

Table 1. PCR parameters for the ITS and five chloroplast noncoding regions.

<table>
<thead>
<tr>
<th>Regions</th>
<th>Initial denaturing step, time</th>
<th>Denaturing temperature, time</th>
<th>Primers annealing temperature, time</th>
<th>Chain extension temperature, time</th>
<th>Final extension temperature, time</th>
<th>Primer source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITS</td>
<td>94°C, 3 min 34x</td>
<td>94°C, 30 sec</td>
<td>50°C, 1 min</td>
<td>72°C, 1 min</td>
<td>72°C, 10 min</td>
<td>Baldwin et al. (1995)</td>
</tr>
<tr>
<td>rpoB-trnC</td>
<td>80°C, 5 min 35x</td>
<td>96°C, 1 min</td>
<td>55°C, 2 min</td>
<td>72°C, 3 min</td>
<td>72°C, 5 min</td>
<td>Shaw et al. (2005)</td>
</tr>
<tr>
<td>atpB-rbcL</td>
<td>94°C, 2 min 34x</td>
<td>94°C, 1 min</td>
<td>53°C, 1 min</td>
<td>72°C, 90 sec</td>
<td>72°C, 5 min</td>
<td>Zurawski et al. (1984), Crayn and</td>
</tr>
<tr>
<td>trnS-trnG</td>
<td>94°C, 3 min 34x</td>
<td>94°C, 45 sec</td>
<td>59°C, 1 min</td>
<td>72°C, 90 sec</td>
<td>72°C, 7 min</td>
<td>Quinn (2000)</td>
</tr>
<tr>
<td>petN-psbM</td>
<td>94°C, 3 min 34x</td>
<td>94°C, 1 min</td>
<td>55°C, 1 min</td>
<td>72°C, 2 min</td>
<td>72°C, 7 min</td>
<td>Hamilton (1999)</td>
</tr>
<tr>
<td>psbM-trnD</td>
<td>94°C, 3 min 34x</td>
<td>94°C, 1 min</td>
<td>55°C, 1 min</td>
<td>72°C, 2 min</td>
<td>72°C, 7 min</td>
<td>Lee and Wen (2004)</td>
</tr>
</tbody>
</table>
spacer, and 2.5% for the psbM–trnD intergenic spacer region. Pairwise distances are reported between Lonicera and representatives of the other included genera in Table 2.

**Congruence of Data Sets**—Results of the ILD test comparing ITS to all 5 cpDNA data sets taken together suggest that they are incongruent ($p < 0.01$). Comparison of tree topologies indicated that the incongruence was evident in two clades. In the Nintooa clade of the chloroplast tree, Lonicera henryi is sister to L. japonica (BP = 100%), while in the ITS tree, L. henryi is sister to L. giraldii (BP = 84%). From section Isika, L. fragrantissima is sister to L. standishii (BP = 98%) in the chloroplast tree rather than to L. hensleyana as is the case in the ITS tree (BP = 97%). The ILD tests were repeated with these taxa excluded, but the partitions remained incongruent ($p < 0.01$). The cause of the remaining heterogeneity is unclear, though this could reflect differences in the number of parsimony informative (PI) characters (Table 2) and substitution rates between markers (Dolphin et al. 2000; Darlu and Lecointre 2002). Therefore, we proceeded to combine the data sets because the conflicts appear to be localized (Yoder et al. 2001, Hipp et al. 2004, Levin and Miller 2005).

**Phylogenetic Relationships**—The data set from the chloroplast DNA had 5,883 characters, 740 of which were parsimony informative, over 200,000 trees were retained with 1,882 steps. The nuclear DNA data set contained 653 characters, of which 132 were parsimony informative. This analysis yielded 1,915 trees with 504 steps. The combined data set of chloroplast and nuclear DNA regions had 6,536 characters, 872 of which were parsimony informative. MP analyses generated 2,136 trees of 2,433 steps; the strict consensus tree is shown in Figs. 1, 2 (CI = 0.77, RI = 0.82). Heptacodium was sister to the Caprifoliaceae clade and Triosteum species formed a clade that split from the remaining Caprifoliaceae (BP = 95%; DI = 6). Symphoricarpos and Lecesteria formed a clade (BP = 84%, DI = 2) that was sister to the monophyletic Lonicera (BP = 100%; DI = 20). Within Lonicera there were two major clades corresponding to the two subgenera: Caprifolium and Lonicera. Within Lonicera, section Coeloxyleostem formed a clade as did section Isokylosteum. However, species of section Nintooa were in two separate clades and section Isika was paraphyletic, within subgenus Lonicera. All subsections within section Isika formed monophyletic clades except for subsections Pilcatae, and Vescicariae.

Bayesian analyses of the partitioned data set resulted in stabilization of likelihood scores by 100,000 generations; all prior generations were discarded as burn-in. Many nodes were resolved with posterior probabilities of 100% (Fig. 1). As in the MP analyses, Lonicera sections Coeloxyleostem and Isokylosteum were monophyletic, whereas sections Isika and Nintooa were not. However in the MP analyses, L. etrusca and L. hispida were not sister species within subgenus Caprifolium; although, bootstrap support for this topology was less than 50%. Overall there was not strong disagreement between the Bayesian and MP analyses for the placement of any taxa, but there was higher support for the topology of the tree from the Bayesian analysis. Most notably there was support for many of the deeper nodes in the tree. However, there was relatively weak support for the placement of Triosteum as the basal taxa to Caprifoliaceae (PP = 75).

**Discussion**

**Intergeneric Relationships Within Caprifoliaceae**—In previous phylogenetic analyses of Dipsacales there is a well-supported but poorly resolved Caprifoliaceae clade containing Lonicera, Lecesteria, Symphoricarpos, and Triosteum (e.g. Backlund and Donoghue 1996; Pyck et al. 1999; Zhang et al. 2003; Donoghue et al. 2003). The precise relationships of this clade to other Caprifoliaceae s. l. have not been strongly supported. For rooting purposes we included Zabelia and Kolkwitzia from Linnaeaceae and two species of Weigela from Diervillaceae. Recent molecular analyses have suggested that the monospecific Heptacodium may be the sister group of Caprifoliaceae (Pyck and Smets 2000; Bell et al. 2001; Donoghue et al. 2003). Although our sampling of the remainder of Dipsacales is limited, our analyses add support to the placement of Heptacodium with Caprifoliaceae (Figs. 1, 2). Nevertheless, Heptacodium is morphologically unique with its strongly 3-nerved leaves and dense cymes. This uniqueness is reflected in the molecular data also, with an exceptionally long branch leading to Heptacodium (Fig. 2).

Conflicting topologies and poor resolution have characterized analyses of the relationships within Caprifoliaceae. Donoghue et al. (2001) found Lecesteria to be sister to a clade containing Triosteum and the sister taxa Lonicera and Symphoricarpos, but with bootstrap support <50%. Zhang et al. (2003) obtained the following relationships: (Triosteum (Symphoricarpos (Lecesteria Lonicera))), all with bootstrap support <65%. In our trees (Fig. 1), Triosteum (BP = 96%) is sister to a clade containing the remaining genera, followed by a clade (BP=88%) of Lecesteria and Symphoricarpos, which is sister to Lonicera (BP = 100%). Triosteum is the only herbaceous group and has been considered to be in its own tribe (Hsu 1983). A direct connection between Lecesteria and Symphoricarpos was suggested in an early phenetic analysis of morphological characters by Hsu (1983). Both genera have terminal inflorences, but we know of no clear synapomorphies for this clade. While this topology is consistent with the Bayesian result (Fig. 1), support for the placement of Triosteum is not as high (PP = 75%). Although our analyses provide stronger results for relationships within Caprifoliaceae than previous studies, additional studies, including a broader sample of other Dipsacales, are necessary to assess the placement of Triosteum and the relationship between Lecesteria and Sym-
**Fig. 1.** The combined majority rule consensus tree from the Bayesian analysis and the strict consensus tree from the parsimony analysis based on sequences of nrDNA (ITS), and cpDNA (rpoB–trnC spacer, atpB–rbCL spacer, trnS–trnG spacer, petN–psBM spacer, and psbM–trnD spacer). Dashed lines represent conflict between the chloroplast and nuclear data (BS > 84%) in the parsimony analysis. Numbers above the branches are posterior probabilities and below the branches are bootstrap percentages/decay indices. The asterisk indicates the different topology of the *Caprifolium* subgenus as determined by the parsimony analysis (left corner). Acronyms on the right indicate taxonomic groups following Rehder (1903, 1909, 1913), Hara (1983) and Hsu and Wang (1988). Genus: *Lonicera*. Subgenera: *Caprifolium* and *Lonicera*. Sections: *Nintooa* (*Nin*), *Isoxylosteum* (*Iso*), and *Coeloxylosteum* (*Coe*) indicated in shaded boxes all others in the section *Isika*. Subsections: *Alpigenae* (*alp*), *Bracteatae* (*bra*), *Breviflorae* (*bre*), *Calcaratae* (*cal*), *Chlamydocarpi* (*chl*), *Coeruleae* (*cae*), *Cupulae* (*cup*), *Cypholeae* (*cyh*), *Distogetae* (*dis*), *Eucaprifolium* (*euc*), *Fragrantissimae* (*fra*), *Longiflorae* (*lon*), *Monanthae* (*mon*), *Ochranthae* (*ocr*), *Phenianthi* (*phe*), *Pileatae* (*pil*), *Purpurascentes* (*pur*), *Pyrenaicae* (*pyr*), *Rhodanthae* (*rho*), *Spinusae* (*spi*), *Tataricae* (*tar*), *Vesicariae* (*ves*).
phoricarpos suggested here. All of the species of Lonicera sampled in our study form a well supported clade in both MP and Bayesian analyses.

Subgenus Delimitations—Rehder (1903) divided Lonicera into two subgenera: Lonicera and Caprifolium. With just over 20 species, Caprifolium is the smaller subgenus in the genus Lonicera, though its geographical distribution is broad with a range throughout Europe and North America, Afghanistan, and Central China. Morphologically, the subgenera are distinctive, most significantly by the inflorescence, with the former having two-flowered cymes, while the latter has three-flowered cymes. In both MP and Bayesian trees (Fig. 1), species of subgenus Caprifolium form a clade that is sister to subgenus Lonicera. This provides the first phylogenetic support for the recognition of these two taxa.

Sections of Subgenus Caprifolium—Based on the corolla symmetry and the fusion of bractlets, Rehder (1903) recognized four subsections in subgenus Caprifolium: Phenianthi (5 spp.), Cyphoeales (10 spp.), Eucaprifolia (7 spp.), and Thoracianthae (1 sp.). However, in our phylogenetic trees (Fig. 1), subgroups with 2 or more species sampled (L. carnosifolia, L. hispidula and L. dioica of Cyphoeales, and L. tragophylla and L. etrusca of Eucaprifolia) do not form monophyletic groups, indicating that it may be inappropriate to separate the subgenus into smaller groups. Nevertheless, more species of the subgenus are needed to further test the monophyly of subsections.

Sections of Subgenus Lonicera—Four sections are generally recognized in subgenus Lonicera: Coeloxylosteum, Isoxylosteum, Nintooa, and Isika (Rehder 1903; Hara 1983; Hsu and Wang 1988). Each of these sections is marked by a unique combination of morphological characters. For example, section Isoxylosteum is characterized by branches with solid pith, five nectaries, and the absence of accessory buds. Section Coeloxylosteum is characterized by flowers with two-lipped corolla and hollow branches. Both of these characters are shared with Nintooa and we can infer from our trees that hollow branches evolved more than once within Lonicera (Fig. 2). Section Nintooa differs from others in its twining or creeping habits, combined with cymes forming terminal panicles, and corollas with long, slender tubes. Section Isika is the largest and most diverse section. These plants have solid branches, as in section Isoxylosteum, but differ in having both zygomorphic and nearly actinomorphic corollas with 1–3 nectaries, and in having accessory buds. In our trees (Fig. 1), Coeloxylosteum and Isoxylosteum each form a clade, supporting their monophyly, whereas Nintooa is divided into two separate clades, suggesting that it is non-monophyletic. Furthermore, all three of these sections are nested within section Isika. Therefore, section Isika, as traditionally circumscribed, is paraphyletic.

Lonicera Section Isoxylosteum—This section consists of eight species that are distributed in eastern Himalayas and southwestern China. These plants are compact shrubs with solid, white pith, small leaves, and flowers with nearly actinomorphic corollas that are non-gibbous at the base. Rehder (1903) recognized two subsections: Microstylae (= Cupulae Hsu and Wang 1988) and Spinosa. These differ in whether the stamens and styles are exerted from the corolla tube or not. Hsu and Wang (1988), however, combined the two subsections and treated L. spinosa, the only species of subsection Spinosa, as a variety of L. myrtillus. In our combined tree, L. spinosa is embedded within section Microstylae, supporting Hsu and Wang’s merge of these two sections.

Lonicera Section Nintooa—All 30 species in this section are vines and their upper leaves are reduced or even bract-like. There are three subsections, including the monotypic subsection Calcaratae, which is unique in having a long nectar spur, conuate ovaries, and bracteoles. Interestingly, although support for the placement of L. calcarata is low, it does not form a clade with other representative species of Nintooa: L. japonica (subsection Breviflorae), L. giraldii, and L. henryi (subsection Longiflorae). When we forced all four species of Nintooa to form a clade, six more steps were needed. This is not significantly longer than the unconstrained phylogeny as

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**Fig. 2.** Phylogram of the strict consensus tree based on a parsimony analysis of the combined data of chloroplast and nuclear DNA regions, showing branch lengths. Dashed lines indicate shortened lines to improve visualization of the remaining branches; above those branches is showing branch lengths. Dashed lines indicate shortened lines to im-
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judged by the one-tailed nonparametric Shimodaira-Hasegawa test (P > 0.5). Thus, more data are needed to test the monophyly of \textit{Nintroa}. Within \textit{Nintroa} our chloroplast data offer strong support for the sister relationship of \textit{L. henryi} and \textit{L. japonica}; however, the nuclear rDNA data recognize the closer relationship of \textit{L. henryi} with \textit{L. giraldii}. \textit{Lonicera henryi} is a hexaploid (2N = 54), while the other two species are diploid species (Rüdenberg and Green 1966). Thus, hybridization may be the cause of the phylogenetic incongruence among the species. Therefore, it is desirable to further examine the evolutionary history of the section with an extensive taxon sampling.

It has been postulated that \textit{Nintroa} links the two subgenera because its members have hollow branches and climbing habit, traits that are also found in subgenus \textit{Caprifolium}, though the leaves subtending the inflorescence are not fused (Rehder 1903). However, in our phylogenetic trees section \textit{Nintroa} appears to have arisen from within section \textit{Isika} of subgenus \textit{Lonicera}. Therefore, twining habit and hollow branches appear to have arisen more than once in \textit{Lonicera}.

\textbf{Lonicera Section Coeloxysteum}—This section contains about 14 species from eastern and Central Asia, north and west of the Himalayas, Europe, and North Africa. It is a rather homogeneous group, characterized by evanescent pith, distinctly two-lipped corollas, and the tendency to have distinct ovaries, an upright habit, and red fruits. The two subsections, \textit{Ochranthae} and \textit{Tataricae}, are distinguished only by minor morphological differences. Members of \textit{Tataricae} are more glabrous throughout, and their flowers are pink to white, and not fading to yellow. \textit{Ochranthae} species, in contrast, are rarely glabrous, and their flowers are white or yellowish white, rarely tinged with red, and fading to yellow. In addition, there are numerous hybrids between their members, though all species are diploid (2N = 18; Rehder 1903; Green 1966). Sequences from both chloroplast and nrDNA suggest that neither of the two subsections formed a clade, owing to the placement of \textit{L. morrowii} (subsection \textit{Ochranthae}) within \textit{Tataricae}, supporting the merger of the two subsections (Hsu and Wang 1988). It is also noteworthy that accesses of \textit{L. xylosteum} and of \textit{L. koehneana} do not form clades in either the chloroplast or nuclear tree. Instead, we recovered two clades containing one accession of each species (Fig. 1); clearly these species are closely related, and might even be conspecific. Hybridization, as an explanation for the topology we found is unlikely since trees based on nrDNA and cpDNA were concordant. However, more accessions are needed to critically evaluate this result.

\textbf{Lonicera Section Isika}—This is the largest section in \textit{Lonicera}, with about 75 species, and it is distributed throughout the range of the genus. This group is also most diverse in habit, in the size and shape of the bracts and bracteoles, in the outer scales of the winter buds, and in the shape of the corolla. Accordingly, Rehder (1903) recognized 12 subsections within \textit{Isika}, half of these with only one or two species. When he proposed \textit{Isika}, Rehder pointed out that some species in this section were related to section \textit{Isoxylosteum} and others to section \textit{Coeloxysteum}, while still others formed clearly defined groups that exhibited no clear affinity to other \textit{Lonicera} species (Rehder 1903). Our trees support the paraphyly of \textit{Isika}-species groups assigned to this section form a grade of lineages, with some being closely allied with the other sections (Fig. 1). For example, subsection \textit{Rhodanthae} as a well supported clade appears to be more closely related to sections \textit{Nintroa}, \textit{Isoxylosteum}, and \textit{Coeloxysteum} than it is to other species of \textit{Isika}. Similarly, \textit{L. gymnochlamydea} of subsection \textit{Piletae} forms a clade with the other three sections and is distantly related to other species of the subsection.

Other subsections within section \textit{Isika} each form their own clades. The proposed merge of \textit{Chlamydocarpus} with \textit{Vesicularia} (Rehder 1909, 1913) is upheld by our data and would result in yet another well supported subsection. \textit{L. hispida} of subsection \textit{Bracteatae} forms a robust clade with species of subsection \textit{Fragrantissimae}. This is supported by morphology; plants of the subsections are often setosely hispid and have branches terminated by two axillary winter buds (Rehder 1903). In the ITS tree, however, \textit{L. hemsleyana} of subsection \textit{Alpigenae} is grouped with subsection \textit{Fragrantissimae}, while our chloroplast data is consistent with morphology in placing \textit{L. hemsleyana} together with other species of subsection \textit{Alpigenae}. We suspect that sequence heterogeneity might play a role here, but it is necessary to do a thorough analysis of the DNA region to test this speculation. Subsections \textit{Distegiae} and \textit{Alpigenae} are grouped in a well supported clade, supporting Rehder’s (1903) observations that \textit{Distegiae} is similar to \textit{Alpigenae} in general habit and pubescence. \textit{Distegiae} has two species, represented in our analysis by \textit{L. involucrata}, native to northern and western North America, which is sister to members of subsection \textit{Alpigenae}, represented here by two of the 14 species (\textit{L. alpigena} and \textit{L. hemsleyana}) is restricted to Europe and Asia. Therefore, our data indicate an interesting biogeographic disjunction between the New and the Old Worlds. A critical appraisal of the status of these subsections as well as their biogeography will require the inclusion of additional species. Our results for section \textit{Isika}, along with other sections set the stage for more detailed phylogenetic analyses within the major clades that we have identified, which will surely provide many further insights into character evolution, hybridization, and the biogeographic history of \textit{Lonicera}.

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L. inconspicua Batal., Rehd., cultivated at Quarryhill Botanical Garden, Elsik, Erwin and Hofstetter 1792-80, USA (A), EU265547, EU265611.
L. koehneana Rehd. (1), cultivated at Arnold Arboretum, Youngerman and Driskill 815-84, China (A), EU240715, EU265581, EU265645.
L. koehneana Rehd. (2), cultivated at Arnold Arboretum, Youngerman and Driskill 684-85 C, China (A), EU240718, EU265584, EU265502, EU265501, EU265655, EU265576, EU265640.
L. lonicerae, cultivated at Smith College, Theis 001, Vermont, USA (A), EU240713, EU265579, EU265543, EU265507, EU265563.
L. lonicerae Maxim., L. kochiana (1), cultivated at Arnold Arboretum, Youngerman and Driskill 8836-70, USA (A), EU265508, EU265572, EU265563.
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L. orientalis Lam., cultivated at Arnold Arboretum, Heffter, Hardy-Brown, and Heja 607-87 A (A), EU240718, EU265384, EU265448, EU265512, EU265576, EU265640.
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