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A PHYLOGENY OF ALL SPECIES OF *ARCEUTHOBIUM* (VISCACEAE) USING NUCLEAR AND CHLOROPLAST DNA SEQUENCES[1]

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The genus *Arceuthobium* (dwarf mistletoes, Viscaceae) comprises 42 species that parasitize hosts in Pinaceae and Cupressaceae in the Old and New Worlds. Maximum parsimony analyses were conducted on two data partitions (separately and combined): nuclear ribosomal internal transcribed spacer (ITS) sequences for all 42 currently recognized species and chloroplast *trnT-L-F* sequences for 34 New World species. The Old and New World species were phylogenetically distinct using ITS, thus making subgenus *Arceuthobium* paraphyletic. *Arceuthobium pendens* and *A. guatemalense* comprise the basalmost clade of subgenus *Vaginata*, characterized by the presence of flabellate secondary branching. The *trnT-L-F* sequences, which vary widely in length depending upon taxon, contain three times less phylogenetic signal than ITS, although homoplasy for this partition is lower. Several of the clades obtained from analysis of nuclear ITS sequences are also recovered using trnT-L-F sequences such as *A. guatemalense* and *A. pendens*, the *A. rubrum* group, the *A. vaginatum* group, and the *A. campylopodum* group. The ITS + *trnT-L-F* tree is well resolved except for four internal nodes. A revised classification of the genus is discussed that recognizes only monophyletic species that are well differentiated by molecular data.

Key words: chloroplast DNA; dwarf mistletoe; ITS; nuclear ribosomal DNA; parasitic plant; phylogenetic species; *trnT-L-F*.

Arceuthobium, commonly called dwarf mistletoe, is one of seven genera in the family Viscaceae and comprises 42 species (Hawksworth and Wiens, 1996). From an economic perspective, it includes species that are the most damaging pathogens of commercially important conifers in North and Central America. This well-defined genus is unique in the family owing to its bicolored, explosively dehiscent fruits, and a geographic distribution that includes both the New and Old Worlds. Centers of diversity include the western United States, Mexico, and China. Although the generic name implies diminutive plants, shoot height varies from ca. 1 cm (*A. minutissimum*) to ca. 90 cm (*A. globosum* subsp. *grandicaule*). The genus is dioecious and both male and female plants have leaves reduced to squamate scales, and secondary branching is either flabellate or verticillate (when branches are present). Morphological features that are useful in distinguishing species are generally of a quantitative nature, and considerable morphometric and color variability exists. Moreover, the evolutionary trend towards morphological reduction or loss in floral and vegetative features renders identification at the species level difficult.

For these reasons, concepts of relationships among the species of *Arceuthobium* have changed through time. The first taxonomic monograph of the United States species was by Gill (1935) who recognized the value of flowering period and described several host forms of *A. campylopodum* and *A. vaginatum* based upon which host species was infected. A resurgence of discovery began in 1963 when Hawksworth and Wiens described five species new to science between Durango and El Salto in Mexico (Hawksworth and Wiens, 1965). The first comprehensive monograph that included all known species of *Arceuthobium* worldwide was published in 1972 (Hawksworth and Wiens, 1972). Continuing through the 1980s, Hawksworth, Wiens, and collaborators named or recircumscribed additional taxa, thus resulting in 22 new taxa in the genus, 13 of which were from Mexico and/or Central America (Hawksworth and Wiens, 1977, 1980).

Isozymes were first used to determine species relationships among dwarf mistletoes from the United States by Nickrent (1986) who found high levels of genetic diversity, surprising given the overall morphological similarity among species. Isozymes supported the segregation of the genus into two subgenera, *Arceuthobium* with verticillate secondary branching and *Vaginata* with flabellate secondary branching (Hawksworth and Wiens, 1970, 1972). The close relationship among one section of subgenus *Vaginata*, *Campylopoda*, was also supported. In contrast, isozymes did not indicate a close relationship between two diminutive species, *A. pusillum* and *A. douglasii*. In an additional isozyme analysis Nickrent (1996) examined 24 species, including seven previously unsampled Mexican species, such as *A. abietis-religiosae* and *A. verticilliflorum*. These latter two species were shown to be related to *A. americanum* of subgenus *Arceuthobium*. This study showed that most members of section *Vaginata* occur in Mexico whereas section *Campylopoda* predominates in the USA. Two parasites of pinyon pines, *A. divaricatum* and *A. pendens*, were

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distinct from one another and were not a component of section *Campylopoda*. While advancing knowledge of overall species relationships, the isozyme data did not place dwarf mistletoe relationships in a phylogenetic context nor were any Old World taxa included in the study.

The first molecular phylogeny of *Arceuthobium* (Nickrent et al., 1994) utilized sequences of the internal transcribed spacer (ITS) and 5.8S ribosomal DNA from 22 species, all but one (*A. oxycedri*) being from North America. As implied with isozymes, genetic distances between dwarf mistletoe species were 2–5 times higher than ITS distances among other green plants sampled at that time (cf. Baldwin, 1993). In agreement with isozyme data, *A. rubrum*, *A. strictum*, and *A. douglasii* were shown to be components of section *Vaginata*, and *A. douglasii* was not related to the eastern dwarf mistletoe *A. pusillum*. A surprising result was the relationship between the eastern dwarf mistletoe and *A. bicarinatum* from Hispaniola, the latter formerly considered part of section *Campylopoda* (Hawksworth and Wiens, 1972). A new clade, basalmost in subgenus *Vaginata*, was also recognized, composed of two rare endemics from Mexico and Central America, *A. pendens* and *A. guatemalense*.

Although that ITS rDNA study provided new information that advanced our knowledge of dwarf mistletoe phylogenetic relationships, a number of questions remained that stemmed mainly from the absence of sequence data from various species. Only one Old World species had been sampled (*A. oxycedri*); however, seven additional Old World species remained to be sequenced to allow phylogenetic inferences about the genus worldwide: *A. juniperi-procerae*, *A. azoricum*, *A. chinense*, *A. minutissimum*, *A. pini*, *A. sichuanense*, and *A. tibetense*. Moreover, the following New World taxa were not included in Nickrent et al. (1994): *A. aureum* subsp. *aureum*, *A. aureum* subsp. *petersonii*, *A. globosum* subsp. *grandicaule*, *A. oaxacanum*, *A. hawksworthii*, *A. yecorense*, and *A. hondurense*, as well as six of the 13 species placed in section *Campylopoda* sensu stricto (Nickrent, 1996). Thus, our goal has been to obtain genomic DNA from all 42 extant species of *Arceuthobium* and reconstruct a molecular phylogeny of these species using DNA sequences. We also wished to supplement the ITS data set with one from the chloroplast genome to serve as an independent test of the nuclear phylogeny.

Chloroplast DNA has been extensively utilized in plant molecular phylogenetic investigations (Chase and Albert, 1998). Restriction fragment length polymorphisms have seen wide use in addressing interspecific relationships (Jansen et al., 1998), and more recently noncoding regions of the chloroplast have been used because they evolve at a higher rate than more conservative coding regions. Three such regions surrounding chloroplast tRNA genes (*trnT, trnL, trnF*) have been shown to be useful in addressing species-level questions (Taberlet et al., 1991), and primers have been designed in the flanking conservative sequences. These ''Taberlet'' primers have been employed in numerous species-level plant phylogenetic studies and indeed were used for two viscaceous mistletoes: *Korthalsella* (Molvray et al., 1999) and *Phoradendron* (Ashworth, 1999). The *trnT-L-F* region (hereafter referred to as the *trnL* region) includes seven parts: a small portion of the 3′ end of *trnT*, the *trnT-L* spacer, the 5′ exon of *trnL*, the *trnL* intron, the 3′ exon of *trnL*, the *trnL-F* spacer, and a small portion of *trnT*. In this paper we compare phylogenetic trees resulting from analyses of nuclear ITS rDNA and the chloroplast *trnL* region sequences among the same suite of *Arceuthobium* species.

MATERIALS AND METHODS

Voucher information for the *Arceuthobium* collections used in this study are listed in the Appendix (see Supplemental Data accompanying the online version of this article). DNA samples were obtained from tissues existing in a variety of conditions: frozen (−80°C) seeds, frozen shoots, and herbarium samples (shoots). All genomic DNA extractions followed the 2× cetyltrimethylammonium bromide miniprep protocol given in Nickrent (1994) or the E.Z.N.A. Plant MiniPrep Kit (Omega-Biotech, Doraville, USA). The primer pair 18S 1830for (5′-AAC AAG GTT TCC GTA GGT GA-3′) and 26S 40rev (5′-TCC TCC GCT TAT TGA TAT GC-3′) was used for polymerase chain reaction (PCR) amplification and sequencing of the ITS region. The PCR amplification of the *trnL* region was accomplished using the six primers described in Taberlet et al. (1991). The PCR reaction mixture contained (final concentrations in a 100-μL reaction): 50 mmol/L KCl, 10 mmol/L Tris pH 8.8, 0.1% Triton X-100, 2.5 mmol/L $MgCl_2$, 1.25 mmol/L of each dNTP, 1 μL of each primer at 125 μg/mL (ca. 20 pmol), 2.5 units of Promega (Madison, Wisconsin, USA) Taq polymerase (M166) and ca. 10–30 ng of genomic DNA. Some ITS and all *trnL* region PCR reactions utilized Ready-to-Go PCR Beads (Amersham Biosciences, Piscataway, New Jersey, USA). The cycling parameters were as follows: 5 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, followed by 33 cycles at 94°C for 30 s, 48°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. Before the cycling parameters were initiated, an initial denaturation at 94°C for 5 min was done when using PCR beads. Controls, lacking genomic DNA, were run for each experiment to check for DNA contamination of the reagents. When light PCR products were visualized on agarose gels, cloning was conducted using either the TOPO TA cloning kit (Invitrogen, Carlsbad, California, USA) or pGEM-T easy-vector II cloning kit (Promega). Amplification products were cleaned using the QIAquick PCR purification kit (QIAGEN, Valencia, California, USA) or E.Z.N.A. Clean kit (Omega Biotech, Doraville, Georgia, USA). Cycle sequencing reactions were conducted in a GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, California, USA) with the BigDye terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase (Applied Biosystems), using primers specific to ITS and *trnL* region or universal primers specific to the plasmid (M13for, M13rev). The sequence reactions were purified by the ethanol/sodium acetate precipitation method suggested within the ABI Prism Big Dye kit. Electrophoresis was conducted on an ABI Prism 377 DNA Sequencer (Applied Biosystems). The ABI Prism Sequencing Analysis software (Applied Biosystems) was used to edit the resulting electropherograms and to assemble contiguous sequences. Sequences were then imported into SeqApp (Gilbert, 1993) and aligned manually. Boundaries of the ITS region were previously determined (Nickrent et al., 1994) and those of the *trnL* region by comparison to the complete chloroplast genome sequences of *Nicotiana* (Shinozaki et al., 1986) and *Arabidopsis* (Sato et al., 1999). The ITS and *trnL* alignments are available as supplemental data with the online version of this article.

The ITS and *trnL* sequences have been deposited with the NCBI (Genbank) database with the accession numbers indicated in Appendix 1. Twenty-six of the total 68 ITS sequences used have previously been published (Nickrent et al., 1994; Mathiasen et al., 2001, 2002a, b), whereas all 49 of the *trnL* sequences are being reported here for the first time. Of the 42 *Arceuthobium* species (four with subspecific taxa), 21 are represented by at least two collections, thus allowing assessment of the monophyly of these species. For the combined ITS and *trnL* analysis, placeholders were required for three taxa: *A. abietinum* f. sp. *concoloris* (ITS 2159, *trnL* 2154), *A. americanum* (ITS 1911, *trnL* 1918), and *A. vaginatum* subsp. *cryptopodum* (ITS 1978, *trnL* 1979). For the remaining species, the same genomic DNA was used for both ITS and *trnL* sequences.

Gaps, including indels, were coded as missing. Both the ITS and *trnL* alignments were inspected for phylogenetically informative indels that can be coded and appended to the data matrices. For ITS, coded gaps would contribute only nine additional characters that simply supported Old World vs.

New World clades, i.e., ones that already have strong support. Coding gaps for the *trnL* region alignment was not done (1) because this would only add synapomorphies to already well-supported clades and (2) because the presence and absence of gaps is clearly homoplastic in several taxa (see later).

Minimum length Fitch trees were constructed with maximum parsimony (MP) using heuristic searches with TBR branch-swapping and MULPARS options in PAUP* 4.0b10 (Swofford, 2002). Search for multiple islands was done using 100 random taxon additions (five trees saved per replicate). Branch robustness was estimated by bootstrap analysis (Felsenstein, 1985) using 100–1000 heuristic replicates, depending upon the data set. Separate analyses of ITS and *trnL*-F as well as combined analyses were conducted. Matrices containing sequences from all available taxa are referred to as ''all taxa'' and abbreviated ''AT.'' Because sequences of both ITS and *trnL* were identical or nearly identical among a group of 14 taxa related to *A. campylopodum*, taxon combinations (using TAXSETS option in PAUP*) were constructed that removed all but three placeholders (*A. blumeri*, *A. campylopodum*, *A. tsugense*) to permit more efficient parsimony analyses. These ''reduced *Campylopoda*'' taxon suites were abbreviated ''RC.'' Also, sequences were sometimes available for more than one representative per species, which, given their similarity, also extended tree searches. Removal of all but one placeholder per species resulted in the ''no duplicates'' taxon suite, abbreviated ''ND.''

Finally, both the ReC and ND taxon combinations were constructed and called ''ReC + NDReC.'' Homogeneity between the ITS and *trnL* region data partitions was tested using the partition homogeneity test (Farris et al., 1995) as implemented by PAUP* 4.0b10. One hundred replicate data partitions were run (heuristic search, simple addition sequence, TBR branch swapping).

As discussed in Nickrent et al. (1994), ITS sequences among various viscaceous genera are too divergent to allow unambiguous alignment. In their study of *Korthalsella*, Molvray et al. (1999) also attempted intergeneric alignment using CLUSTAL with various gap penalties, but came to the same conclusion. More conservative 18S rDNA sequences have been obtained for all genera of Viscaceae including three *Arceuthobium* species (Nickrent, 1996). Although relationships among the genera were unresolved, thus not allowing one to choose the sister genus to *Arceuthobium*, the topology of the *Arceuthobium* clade placed *A. oxycedri* basal and sister to *A. verticilliflorum* and *A. pendens*. Thus, Old World taxa will be used herein to root phylogenetic trees.

RESULTS

For all 68 dwarf mistletoe accessions (42 species), PCR amplifications of ITS were successful. In contrast, none of the *trnL* region amplifications of the Old World *Arceuthobium* (seven species) were successful, thus 50 accessions of 35 New World species were sequenced for this gene. Sequence length, the degree of variability, proportion of parsimony informative sites, tree lengths, and other statistics associated with the parsimony analyses of ITS and the *trnL* region are shown in Table 1.

Characteristics of ITS—Alignment of ITS (685 characters or positions, range 600–655 bp) was generally straightforward, but differed from the one reported in Nickrent et al. (1994) that included only one Old World species (*A. oxycedri*). The sequence of *A. abietis-religiosae* (number 2010) reported in that publication was found to be erroneous; the corrected sequence is reported here. The addition of all Old World taxa allowed for a better alignment, particularly in the highly variable portions of ITS-2. The ITS-1 and 5.8S rDNA junction occurred at position 220 and the 5′ end of ITS-2 began at position 390. The ITS sequences for Old World *Arceuthobium* averaged ca. 640 base pairs (bp) in length, whereas New World taxa averaged 601 bp. This increase can partly be attributed to a 24-bp insertion at position 432 and a 28-bp insertion at position 648 in ITS-2 in these taxa. For the AT and ReC taxon combinations, ca. 50% of the sites were parsimony informa-

TABLE 1. Sequence length, variability, and parsimony-based tree parameters for *Arceuthobium* ITS and *trnL* region sequences.

	No. taxa	No. characters	Constant characters	Variable uninformative characters	Parsimony-informative characters	Parsimony-informative characters/length	No. trees	Tree length	CI	HI	CI−	HI−	RI	RC
ITS AT	68	685	260	76	349	0.5095	3960	938	0.647	0.353	0.614	0.386	0.917	0.596
ITS ReC	57	685	263	73	349	0.5095	440	928	0.651	0.349	0.619	0.381	0.908	0.591
ITS ND	46	685	281	91	313	0.4569	27	849	0.677	0.323	0.634	0.366	0.878	0.595
ITS NDReC	35	685	282	90	313	0.4569	3	846	0.681	0.319	0.638	0.362	0.85	0.579
trnL AT	50	1441	1127	100	214	0.1485	>10000	439	0.802	0.198	0.743	0.257	0.9	0.721
trnL ReC	39	1441	1131	96	214	0.1485	6279	435	0.8	0.2	0.743	0.257	0.867	0.694
trnL ND	38	1441	1134	171	136	0.0944	1495	428	0.804	0.196	0.663	0.337	0.835	0.671
trnL NDReC	27	1441	1138	167	136	0.0944	1495	424	0.802	0.198	0.663	0.337	0.728	0.584
ITS + *trnL*	46	2096	1472	231	393	0.1875	54	1051	0.727	0.273	0.644	0.356	0.853	0.620
ITS + *trnL* + OW	54	2126	1410	225	491	0.2309	36	1315	0.703	0.297	0.637	0.363	0.874	0.614

Abbreviations: CI = consistency index, HI = homoplasy index, CI− = consistency index minus uninformative sites, HI− = homoplasy index minus uninformative sites, RI = retention index, RC = rescaled consistency index. AT = all taxa, ReC = reduced *Campylopoda*, ND = no duplicate accessions.

tive. For the ND and NDReC taxon combinations, the percentage of parsimony informative sites dropped to 45%.

Characteristics of the trnL region—The *trnL* region was extremely variable in length across the various New World *Arceuthobium* species (range, 263–1237 bp) with an aligned length of 1441 characters (including 17 bp of *trnT* and 51 bp of *trnF*). The mean length of the *trnL* region for members of subgenus *Arceuthobium* (*A. abietis-religiosae*, *A. americanum*, *A. verticilliflorum*) as well as various species of subgenus *Vaginata* such as *A. pusillum*, *A. aureum*, *A. globosum*, and *A. pendens* was 1220 bp. These species all had intact *trnL* spacers, exons, and intron. The lengths of the *trnL* region segments for *A. abietis-religiosae*, a representative taxon with a full-length sequence are: *trnT-L* spacer (510 bp), *trnL* (UAA) 5′ exon (33 bp), *trnL* intron (500 bp), *trnL* 3′ exon (50 bp), and *trnL-F* spacer (144 bp) totaling 1237 bp. The total length is somewhat shorter than many published dicot sequences (e.g., *Nicotiana* 1690, *Arabidopsis* 1894), mainly because of a short *trnL-F* spacer. Most members of subgenus *Vaginata* had significantly shorter *trnL* regions, averaging 490 bp. The 14 taxa related to *A. campylopodum* had *trnL* regions identical in length (350 bp). Both of these groups entirely lacked the *trnL* exons and intron as well as portions of the flanking spacers. The most unusual *trnL* region was seen in *A. douglasii*. After only 18 bp into the trnT-L spacer, a large deletion begins that ends ca. 110 bp into the *trnL* intron. Three different accessions of this species were sequenced and all had identical boundaries. After resuming in the intron, the sequence continued and was fully intact through *trnF*. Thus, *A. douglasii* lacked the *trnL* 5′ exon but retains the 3′ exon, thereby resulting in a *trnL* ''pseudogene.'' In all three accessions of *A. americanum*, a 43-bp portion of the *trnL* 3′ exon and the adjacent *trnL-F* spacer is the reverse complement of this region in other taxa. The reverse complement of the *A. americanum* sequences were used in phylogenetic analyses. For the AT and ReC taxon combinations, ca. 14% of the sites were parsimony informative, i.e., three times less than with ITS. For the ND and NDReC taxon combinations, the percentage of parsimony-informative sites drops to 9%.

Phylogenetic analyses of ITS—Parsimony analyses of ITS resulted in successively smaller numbers of trees with the AT, ReC, ND, and NDReC data sets. With all accessions included (AT, 68 taxa), 3960 trees were recovered. This high number stems from inclusion of 13 *Campylopodum* taxa that have identical or nearly identical sequences. When these are reduced (ReC, 57 taxa), the number of trees drops to 440; the strict consensus is shown in Fig. 1. This tree allows an assessment of the monophyly of various species represented by more than one accession (18 taxa, including subspecies of *A. aureum*, *A. globosum*, and *A. vaginatum*.) Seven species were monophyletic: *A. durangense*, *A. oaxacanum*, *A. pusillum*, *A. douglasii*, *A. divaricatum*, *A. abietis-religiosae*, *A. azoricum*, and *A. minutissimum*. Five other species formed polytomies: *A. hondurense*, *A. vaginatum*, *A. gillii*, *A. aureum*/*A. aureum* subsp. *petersonii*, and *A. juniperi-procerae*. The remaining five species were not monophyletic: *A. nigrum*, *A. globosum*/*A. globosum* subsp. *grandicaule*, *A. sichuanense*, *A. oxycedri*, and *A. chinense*. The ITS sequences of *A. oxycedri* 4335 (Morocco) and 4236 (Turkey) were genetically divergent from the three other accessions of this species that all originated from the Iberian peninsula. Two accessions of *A. chinense* (4239 and 4240) are quite divergent, despite both being from Yunnan Province, China, and both parasitizing the same host species.

Because of the large number of trees recovered, bootstrap (BS) values were not obtained for the ReC tree (Fig. 1). By removing duplicate accessions of the same species (giving 46 taxa), 27 trees were obtained; the strict consensus phylogram is shown in Fig. 2. Bootstrapping (100 replications) was possible with this data set, and BS values are plotted on the ITS ND tree. Rooted with *A. oxycedri* accession 2832 from Spain, the Old World taxa corresponding to section *Arceuthobium* (*A. oxycedri*, *A. tibetense*, and *A. juniperi-procerae*) occur as a grade at the base of the tree. Bootstrap support is 72% for a clade containing *A. pini*, *A. sichuanense*, *A. chinense*, and *A. minutissimum* (section *Chinense*). *Arceuthobium azoricum* occurs as sister to the New World taxa but with low BS support. In analyses using distance measures, *A. azoricum* is associated with sections *Arceuthobium* and *Chinense*. Over 120 changes on the ITS ND tree separate the Old World from the New World clades. Sister to the remaining New World members (subgenus *Vaginata*) is a strongly supported (100% BS) clade composed of the three verticillately branched species, *A. abietis-religiosae*, *A. americanum*, and *A. verticilliflorum* that have traditionally been classified in subgenus *Arceuthobium*. The next well-supported clade (100% BS) is composed of *A. guatemalense* and *A. pendens*, followed by another well-supported (97% BS) clade of all remaining species. The next two clades, although present on the strict consensus tree, represent an unresolved portion of the tree that appears in all analyses. These two nodes, plus the next that is also unresolved, produces a polytomy composed of seven relatively well-defined taxa. The first is a well-supported clade composed of the species and subspecies of *A. globosum* and *A. aureum*. Two taxa, *A. douglasii* and *A. divaricatum* do not seem to have strong associations with any of the other clades and emerge as part of the large polytomy. The next clade is composed of 14 species related to *A. campylopodum*, with *A. blumeri* strongly supported as basal to this clade. A clade composed of *A. strictum*, *A. hondurense*, *A. hawksworthii*, *A. vaginatum*, *A. vaginatum* subsp. *cryptopodum*, and *A. durangense* received 94% BS support. Lower support (79% BS) is obtained for the clade composed of *A. gillii*, *A. nigrum*, *A. yecorense*, *A. oxacanum*, and *A. rubrum*. The surprising relationship between *A. pusillum* of eastern North America and *A. bicarinatum* of Hispaniola is seen in this analysis with strong (100% BS) support.

Phylogenetic analyses of the trnL region—The *trnL* region contains three times less phylogenetic signal than ITS, although homoplasy for this partition is lower (Table 2). The *trnL* AT (50 taxa) and ReC (39 taxa) samplings gave similar results upon parsimony analysis, both yielding over 6200 trees of length 439 and 435, respectively. The majority rule consensus tree resulting from analysis of the *trnL* AT data set is shown in Fig. 3. The tree is rooted with *A. abietis-religiosae*, which from previous analyses of ITS appears as basalmost among the New World members of subgenus *Arceuthobium*. A clade composed of *A. americanum* and *A. verticilliflorum* appears next and is sister to the remaining species. Several of the clades obtained from analysis of nuclear ITS sequences are recovered (with identical topologies) using the *trnL* region: the *A. campylopodum* group (13 species including a basal *A. blumeri*), *A. guatemalense*/*A. pendens*, *A. yecorense*/*A. oxacanum*/*A. rubrum*, and *A. vaginatum*/*A. vaginatum* subsp. *cryptopodum*/*A. durangense*. The *A. aureum* and *A. globosum*

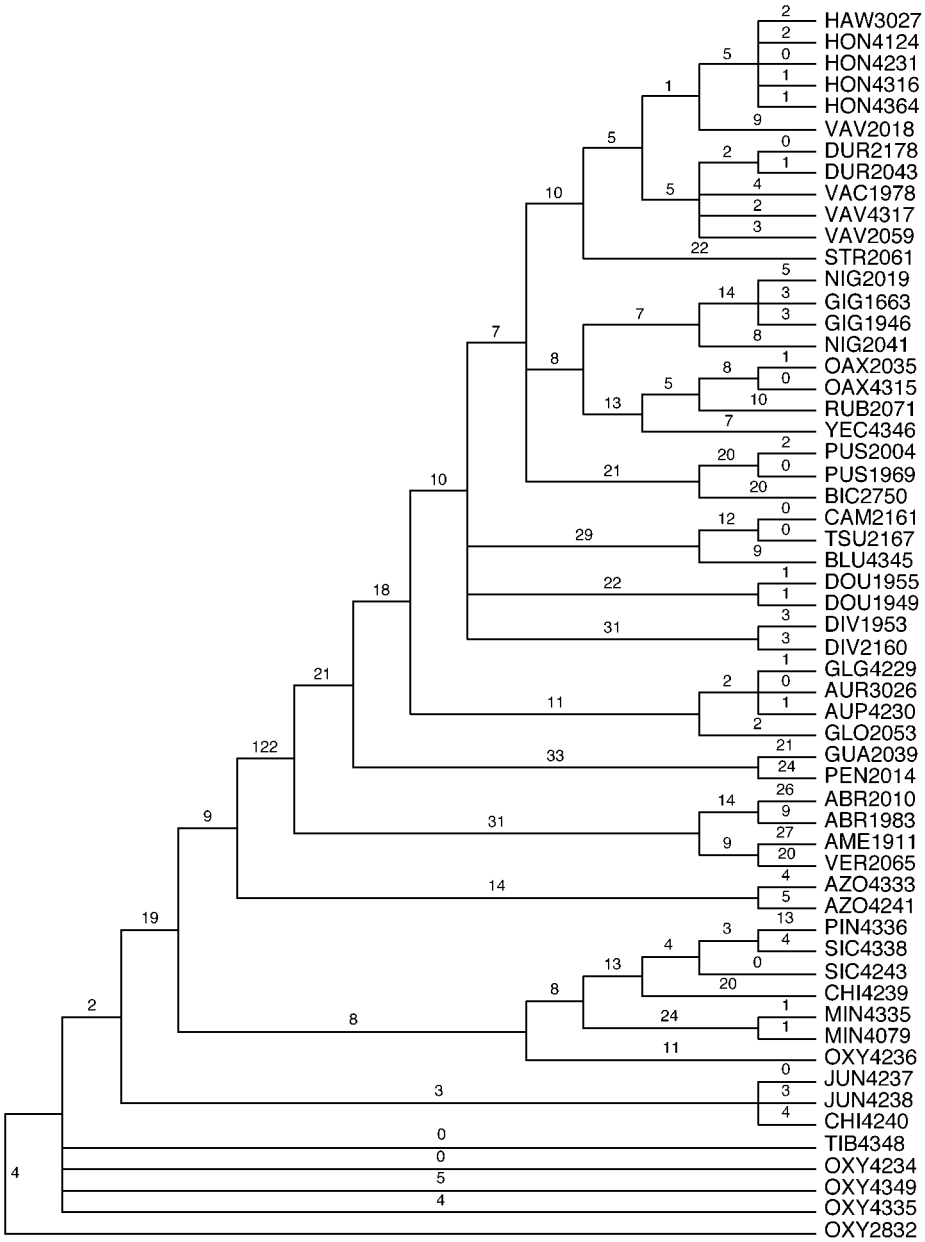

Fig. 1. Relationships among 34 *Arceuthobium* species (57 accessions) using nuclear ITS sequences. Strict consensus of 440 shortest trees generated using maximum parsimony on the ''RC'' data set (reduced number of section *Campylopodum* taxa, see text). Numbers above branches are branch lengths (number of changes). See Appendix (supplemental data accompanying the online version of this article) for code to taxon abbreviations and Table 1 for tree statistics.

clade is also obtained, albeit with a different topology compared with ITS. *Arceuthobium hondurense* and *A. hawksworthii* form a clade but are here sister to *A. gillii* and *A. nigrum*. These latter two taxa were part of the *A. rubrum* clade using ITS. *Arceuthobium divaricatum* and *A. douglasii* are components of a polytomy that includes the *A. campylopodum* group taxa.

Test of conflict between the data partitions—The partition homogeneity test for both the ITS + *trnL* ND and the ITS + *trnL* NDReC taxon suites indicated that the partitions were significantly different than random partitioning ($P < 0.01$). In trees obtained from the separate partitions, the majority of the topological conflict comes from *A. gillii*, *A. nigrum*, *A. douglasii*, and *A. divaricatum*. When these taxa are pruned from the matrix, the partition homogeneity test indicates that the ITS and *trnL* partitions were not significantly different at the 0.05 level. The topic of whether separate data partitions should be combined is a matter of debate (de Queiroz et al., 1995; Johnson and Soltis, 1998). Aside from the position of the above four species, the *trnL* region and ITS trees share a number of clades and the topology of the former partition is generally a less-resolved version of the latter. It has also been argued that simultaneous analyses of combined data provides the greatest possible explanatory power in parsimony analyses (Nixon and Carpenter, 1996); hence, we combined the data partitions to explore whether resolution would be improved by increasing the amount of sequence data.

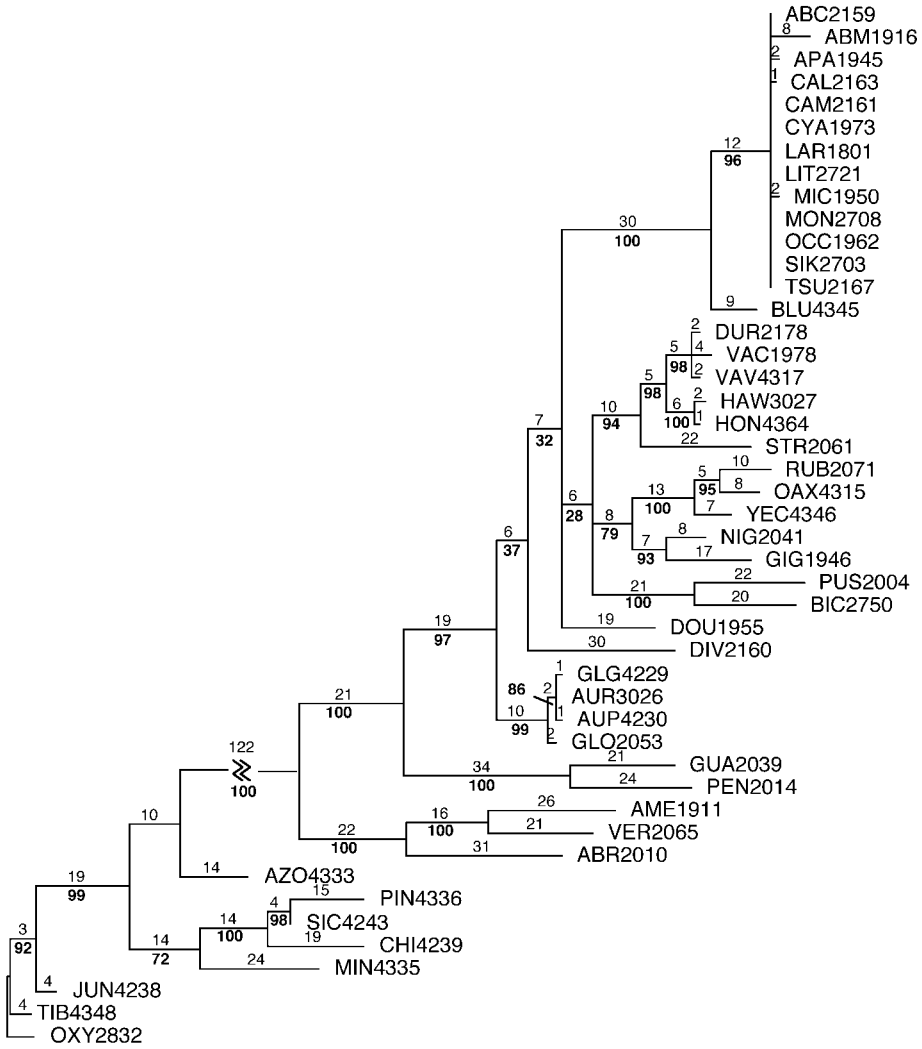

Fig. 2. Relationships among all 42 Old and New World species of *Arceuthobium* using nuclear ITS sequences. Strict consensus of 27 shortest trees generated using maximum parsimony on the ''ND'' data set (no duplicate accessions, see text). Numbers above branches are branch lengths (number of changes), numbers below are bootstrap percentages from 100 replications. See Appendix (supplemental data accompanying the online version of this article) for code to taxon abbreviations and Table 1 for tree statistics.

Phylogenetic analyses of the combined data sets—Given that *trnL* region sequences could not be obtained from any Old World taxa, two approaches were used. The first used all New World taxa for which both ITS and *trnL* region sequences were available (ITS + *trnL* data set). The second added to that matrix ITS sequences from one representative each of the eight Old World species and coded the *trnL* region for them as missing data (ITS + *trnL* + OW). The topologies of trees resulting from parsimony analyses of the two data sets were identical for the New World taxa (see the ITS + *trnL* + OW tree in Fig. 4). This tree shares many features with the ITS tree (Fig. 2) with some degree of added resolution. Like the ITS tree, nearly all section *Vaginata* taxa emerge from a large polytomy involving three nodes labeled A, B and C, all occurring in less than 50% of the BS replications. The major difference in this tree compared to Fig. 2 is the position of *A. divaricatum* and *A. douglasii*. Here, these species are weakly supported (58%) as sister to the *Campylopodum* group. These relationships derive from the *trnL* region partition, as can be seen on the majority rule consensus tree (Fig. 3).

DISCUSSION

Over 5000 (full or partial) angiosperm rDNA internal transcribed spacer sequences and 7000 *trnL* region sequences are now in the NCBI database. Combined ITS and *trnL* region data sets have frequently been used to address intergeneric and infraspecific phylogenetic questions in angiosperms (Gielly et al., 1996; Molvray et al., 1999; Bortiri et al., 2001; Roalson et al., 2001; Hodkinson et al., 2002; Razafimandimbison and Bremer, 2002; Ronsted et al., 2002; Sinclair et al., 2002; Zimmer et al., 2002; Klak et al., 2003). These sequences vary widely in terms of their utility for resolving phylogenetic questions. In some cases, the number of trees recovered from the ITS partition is greater and the consistency index less than with the *trnL* region (Bortiri et al., 2001; Roalson et al., 2001). In other cases, the number of trees recovered from the ITS partition and the consistency index is less than with *trnL* region (Sinclair et al., 2002; Zimmer et al., 2002). In the study of the *Lampranthus* group of Aizoaceae (Klak et al., 2003), both partitions produced thousands of equally parsimonious

TABLE 2. Phylogenetic classification of *Arceuthobium* M. Bieb.

Subgenus *Arceuthobium*

Section *Arceuthobium*

1. *A. juniperi-procerae* Chiovenda
2. *A. oxycedri* (DC) Bieb.
3. *A. tibetense* H. S. Kiu & W. Ren

Section Chinense Nickrent

4. *A. chinense* Lecomte
5. *A. minutissimum* J.D: Hooker
6. *A. pini* Hawksw. & Wiens
7. *A. sichuanense* (H.S. Kiu) Hawksw. & Wiens

Section Azorica Nickrent

8. *A. azoricum* Hawksw. & Wiens

Subgenus *Vaginata* Hawksw. & Wiens

Section *Americana* Nickrent

9. *A. abietis-religiosae* Hiel
10. *A. americanum* Nutt. Ex Engelm.
11. *A. verticilliflorum* Engelm.

Section *Penda* Nickrent

12. *A. guatemalense* Hawksw. & Wiens
13. *A. pendens* Hawksw. & Wiens

Section *Globosa* Nickrent

14. *A. globosum* Hawksw. & Wiens [including: *A. globosum* subsp. *grandicaule* Hawksw. & Wiens, *A. aureum* Hawksw. & Wiens subsp. *aureum*, *A. aureum* subsp. *petersonii* Hawksw. & Wiens]

Section *Pusilla* Nickrent

15. *A. bicarinatum* Urban.
16. *A. pusillum* Peck.

Section *Rubra* Hawksw. & Wiens

17. *A. gillii* Hawksw. & Wiens [including *A. nigrum* Hawksw. & Wiens]
18. *A. rubrum* Hawksw. & Wiens [including: *A. oaxacanum* Hawksw. & Wiens]
19. *A. yecorense* Hawksw. & Wiens

Section *Vaginata* Hawksw. & Wiens

20. *A. hondurense* Hawksw. & Wiens [including: *A. hawksworthii* Wiens & Shaw]
21. *A. strictum* Hawksw. & Wiens
22. *A. vaginatum* (Willd.) Presl. [including: *A. vaginatum* subsp. *cryptopodum* (Engelm.) Hawksw. & Wiens, *A. durangense* (Engelm.) Hawksw. & Wiens]

Section *Minuta* Hawksw. & Wiens

23. *A. divaricatum* Engelm.
24. *A. douglasii* Engelm.

Section *Campylopoda* Hawksw. & Wiens

25. *A. blumeri* A. Nelson
26. *A. campylopodum* Engelm. [including: *A. abietinum* Hawksw. & Wiens, *A. apachecum* Hawksw. & Wiens, *A. californicum* Hawksw. & Wiens, *A. cyanocarpum* (A. Nelson ex Rydberg) Coulter & Nelson, *A. laricis* (Piper) St. John, *A. littorum* Hawksw., Wiens & Nickrent, *A. microcarpum* (Engelm.) Hawksworth & Wiens, *A. monticola* Hawksw., Wiens & Nickrent, *A. occidentale* Engelm., *A. siskiyouense* Hawksw., Wiens & Nickrent, *A. tsugense* (Rosendahl) G.N. Jones]

trees resulting in poor resolution overall. All studies surveyed combined these two partitions irrespective of the results of partition homogeneity tests. From such work, it is apparent that the level of phylogenetic resolution achieved with ITS and *trnL* region sequences is highly dependent upon the taxonomic group being studied.

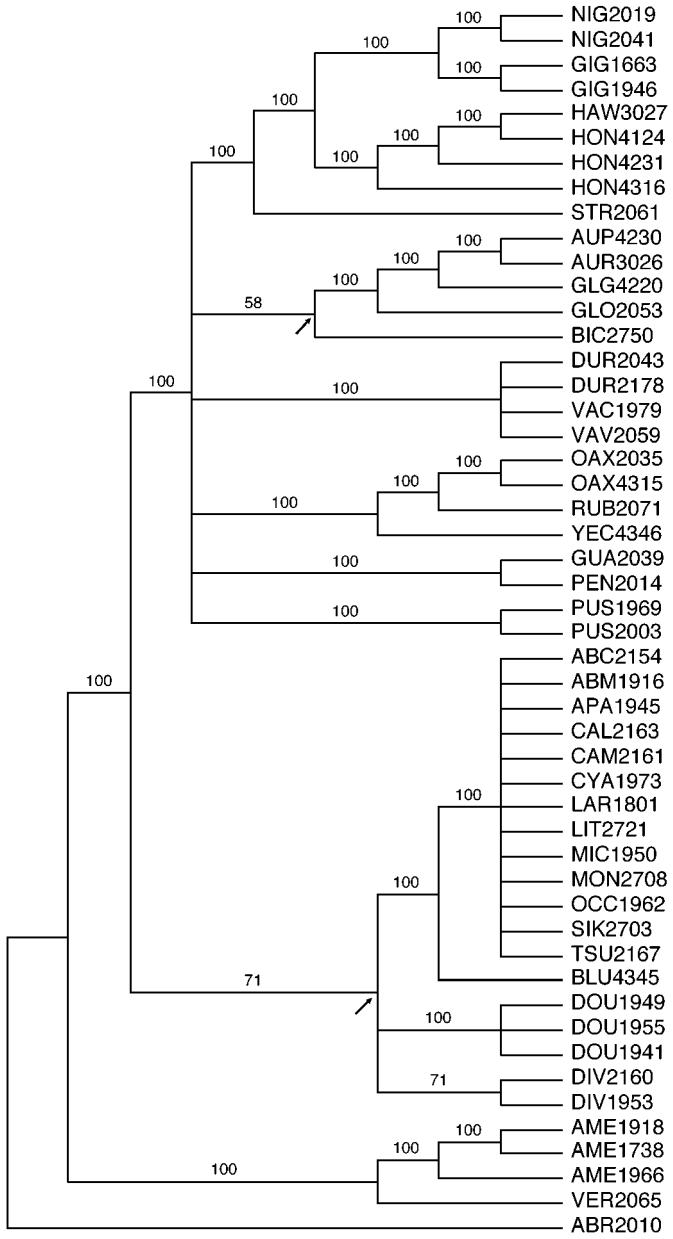

Fig. 3. Relationships among 34 New World *Arceuthobium* species (50 accessions) using chloroplast *trnL* region sequences. Majority rule consensus of 6280 shortest trees generated using maximum parsimony on the ''AT'' data set (all New World taxa included, see text). Numbers above branches are the percentage of trees showing that particular clade. Nodes indicated by arrowheads collapse in the strict consensus tree. See Appendix (supplemental data accompanying the online version of this article) for code to taxon abbreviations and Table 1 for tree statistics.

Conflict between the ITS and *trnL* region trees stemmed mainly from the positions of *A. gillii*, *A. nigrum*, *A. douglasii*, and *A. divaricatum*. Gene trees may differ significantly from organism trees for a number of reasons (Wendel and Doyle, 1998), including chloroplast capture, lineage sorting and introgression, hybridization, intragenic recombination, or lack of concerted evolution giving multiple different copies of ITS in the plant genome (see Soltis and Soltis, 1998). Although it has been repeatedly emphasized that there exists no evidence of hybridization in *Arceuthobium* (Hawksworth and Wiens, 1972,

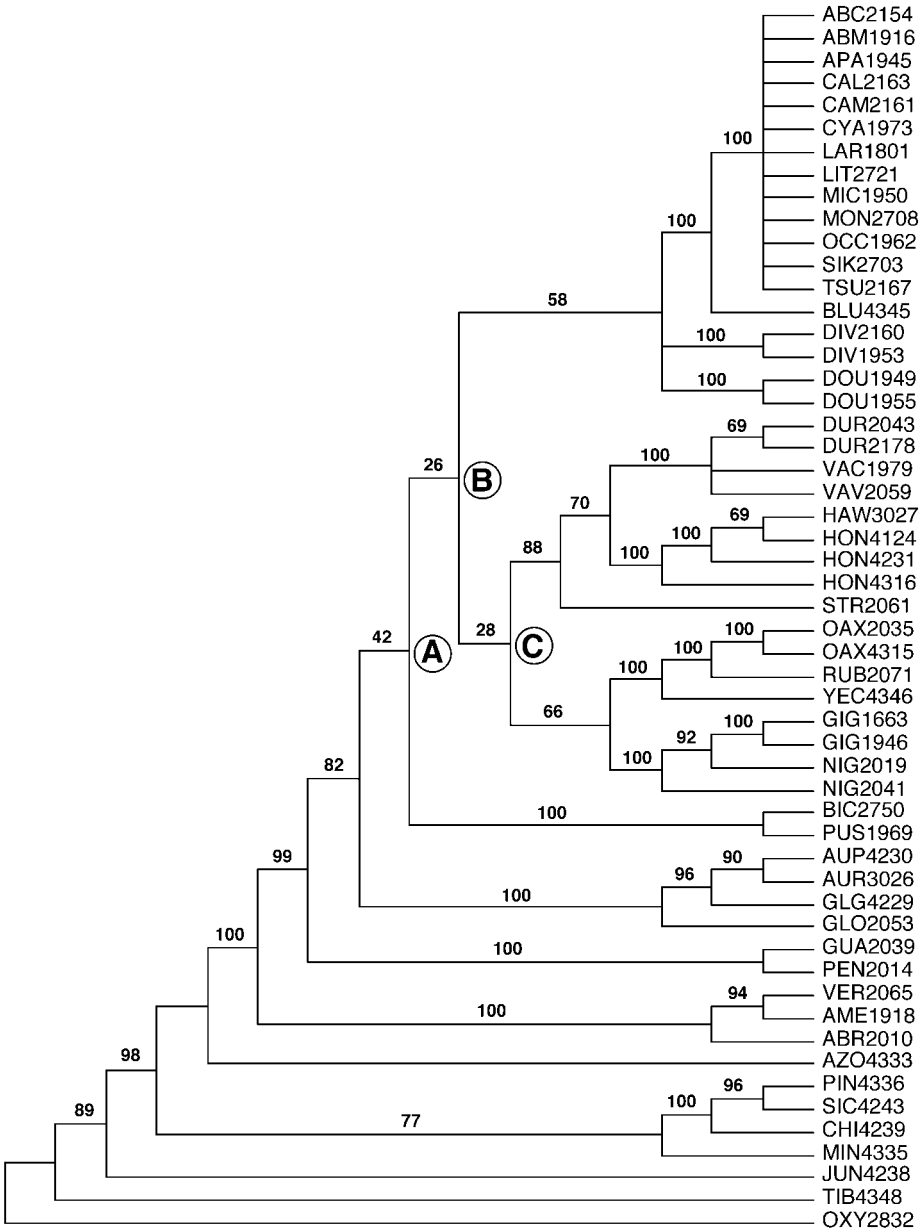

Fig. 4. Relationships among all 42 Old and New World *Arceuthobium* species (54 accessions) using combined nuclear ITS and chloroplast *trnL* region sequences. Only ITS sequences were obtained for Old World species (OXY to AZO). Strict consensus of 36 shortest trees generated using maximum parsimony on the ''ND'' data set (no duplicate accessions, see text). Numbers above branches are bootstrap percentages from 100 replications. Nodes indicated by A–C have BS values below 50%. See Appendix (supplemental data accompanying the online version of this article) for code to taxon abbreviations and Table 1 for tree statistics.

1996), it remains to be empirically demonstrated that hybrids do not occur. When the species in question are morphologically and genetically nearly identical, how could one identify a hybrid if it were formed (Kuijt, 1973)?

Molecular evolution of trnL region—Our inability to obtain *trnL* region sequences from any Old World members of *Arceuthobium* likely stems from divergence at PCR priming sites, caused by either substitutional mutations or deletions. It is curious, however, that sequences from the three New World members of subgenus *Arceuthobium* were amplified, that these were full length (with fewer indels), and that these taxa are the closest relatives of the Old World members of that subgenus. Given that the Old World taxa are phylogenetically basal in the genus and that other genera in Viscaceae can be PCR amplified, it would be assumed that these taxa similarly could be amplified. The *trnT* ''a'' and *trnF* ''f'' primers (Taberlet et al., 1991) are therefore not ''universal,'' despite working on diverse plants including algae to angiosperms. Attempts to amplify the *trnL* region using other combinations of the six Taberlet primers were also unsuccessful, thus suggesting major reorganization of the chloroplast genome in Old World *Arceuthobium*. Loss of tRNA genes in parasitic angiosperms is well documented in Orobanchaceae (Wolfe et al., 1992; Lohan and Wolfe, 1998) but is usually associated with the loss of photosynthesis in holoparasites.

Deletions in the *trnL* region sometimes appear to follow phylogenetic lines (e.g., all *A. campylopodum* group taxa), whereas in other cases, the deletions are present in only one member of a clade strongly supported by ITS data. Two curious examples of this can be discussed: *A. bicarinatum*/*A. pusillum* and *A. guatemalense*/*A. pendens*. *Arceuthobium pusillum* has a *trnL* region sequence that is full length, as is seen in *A. abietis-religiosae*, *A. americanum*, and *A. verticilliflorum*. The *trnL* region in *A. bicarinatum*, however, is truncated to the length typically seen in many members of section *Vaginata*. Upon parsimony analysis of the *trnL* region, *A. pusillum* and *A. bicarinatum* are associated with a group of section *Vaginata* taxa, not at the base of the tree with *A. abietis-religiosae*. Although these taxa did not appear as sister in Fig. 3, they share a unique insertion in the *trnT-L* spacer (TTCATAATTAGA) and a unique deletion (TTCGGAAA) that contributes further evidence of their relationship. The second example involves *A. pendens* and *A. guatemalense* where the former species has a full-length *trnL* region but the latter is truncated to the length seen in section *Vaginata* members. But once again, parsimony analysis does not separate these two taxa but places them as sister within the *Vaginata* group (Fig. 3). From these examples, one can speculate about the utility of *trnL* region sequences as phylogenetic markers in *Arceuthobium*. The presence of large deletions in some taxa removes large amounts of potentially phylogenetically informative sites. For example, the *A. campylopodum* group taxa have only 30% of the sequence present in taxa such as *A. abietis-religiosae*, *A. americanum*, and *A. aureum* whose sequences are ''full length.'' Parsimony can only group taxa based upon synapomorphic sites in the sequence that are still present in both taxa, thus it is surprising that despite major differences in lengths, taxa such as *A. guatemalense* and *A. pendens* emerge as sister. This indicates that sufficient phylogenetic signal remains in the sequence regions they share. However, in the case of the *A. campylopodum* taxa, *A. divaricatum*, and *A. douglasii*, their position as part of a large polytomy at the base of the *trnL* tree (Fig. 3) suggests that their short sequences may be compromising resolution.

Although we argued earlier that indels support an association between *A. bicarinatum* and *A. pusillum*, we feel that using such evidence in phylogenetic analyses requires extreme caution. As mentioned, we did not score such indels as additional characters because interpreting their presence and absence appears to be highly homoplastic. Examples of intraspecific variation in the presence/absence of indels include *A. americanum*, *A. divaricatum*, *A. douglasii*, and *A. globosum*. Similar cases of homoplastic indels (and inversions) have been shown in *Phoradendron trnL* region sequences (Ashworth, 1999). In that genus, a 59 bp long inversion is present in the *trnL-F* spacer in nine species that are not closely related based upon morphology or nuclear rDNA sequences. For these mistletoes, the presence/absence of indels among taxa are likely to reflect both shared ancestry and events unique to particular clades and even populations. In this study of *Arceuthobium*, we interpret indels to be homologous only if they are composed of sequences identical in composition and length. Certain regions appear more predisposed for indels (mutational ''hotspots''). For example, in the case of the *trnT-L* spacer insertion shared by *A. guatemalense* and *A. pendens*, *A. divaricatum* and *A. gillii* also have insertions in this region but of different length, thus they are interpreted as being nonhomologous.

Old World subgenus Arceuthobium taxa—In this study, the *Arceuthobium* trees were not rooted with another genus in Viscaceae; however, the Old World dwarf mistletoes are very likely the most primitive members of the genus (see 18S rDNA and rbcL evidence in Materials and Methods). From ITS data, this group of eight verticillately branched species is separated by very long branches from the New World members. Given the topology of the gene trees (Figs. 1, 2, 4), subgenus *Arceuthobium*, traditionally defined to include both Old and New World mistletoes with verticillate secondary branching (Hawksworth and Wiens, 1972, 1996; Mark and Hawksworth, 1981), is paraphyletic. Following strict phylogenetic nomenclature, the Old World species could be classified based upon the existence of three clades (sections): *Arceuthobium* (*A. oxycedri*, *A. juniperi-procerae*, *A. tibetense*), *Chinense* (*A. chinense*, *A. minutissimum*, *A. sichuanense*, *A. pini*), and *Azorica* (*A. azoricum*). These groupings are similar to the proposed sections presented by Nickrent (1996) with the following differences: section *Arceuthobium* does not include any New World members and *A. tibetense* is included in this section, not in section *Chinense*. We are skeptical about this position of *A. tibetense*, a parasite of *Abies forrestii*, because the four Old World species that are parasitic on Pinaceae occur in a clade (section *Chinense*). The other three Old World species are all parasites of Cupressaceae (*Juniperus*). Similarly questionable is the position of *A. chinense* number 4240 (Fig. 1).

Arceuthobium azoricum was classified in its own section in Nickrent (1996) owing to morphological characters that distinguish it from other species: base of shoots up to 1 cm in diameter (as compared with thinner shoots in other taxa) and the frequency of four-merous staminate flowers. This distinctiveness is supported by molecular data. In strict and majority rule consensus trees using parsimony analyses, *A. azoricum* appears as the Old World taxon most similar to the New World taxa, but with low BS support (Fig. 4). Distance methods, however, place this taxon with sections *Arceuthobium* and *Chinense*. This taxon is important from a biogeographic perspective given its isolated position in the Azore islands. Only one other dwarf mistletoe, *A. bicarinatum*, is endemic to an oceanic island (Hispaniola). Hawksworth and Wiens (1976) suggested that *A. azoricum* and its host (*Juniperus brevifolia*) might be Tertiary relics, restricted to volcanic islands that formed along the mid-Atlantic ridge (McKenna, 1972). The phylogenetic position of *A. azoricum* is crucial in understanding the migrational history of the genus. If *A. azoricum* is a component of the Old World clades, this is not in conflict with the proposal that *Arceuthobium* entered the New World in the early Tertiary from Asia via a Beringian land bridge (Hawksworth and Wiens, 1972, 1996). However, if *A. azoricum* is sister to all New World *Arceuthobium*, entry of the genus into North America from western Europe is a viable hypothesis. Resolving each of these two hypotheses depends upon rooting with an outgroup genus.

As stated, the five accessions of *A. oxycedri* did not emerge as monophyletic using ITS sequence data. The two collections from Spain and the one from Portugal were part of the section *Arceuthobium* clade but were not monophyletic. The number of steps in parsimony trees (and number of changes on neighbor-joining trees) separating these accessions from each other and from *A. tibetense* and *A. juniperi-procerae* is low. This could be caused by insufficient phylogenetic signal present in ITS to differentiate these taxa. The most genetically divergent *A. oxycedri* accessions are 4236 from Turkey and 4335 from

Morocco. Given that the sequences were obtained from PCR products amplified from degraded genomic DNA obtained from herbarium specimens, the first consideration is that these may have resulted from contamination. The sequences obtained, however, are from an *Arceuthobium*, and they are not similar to any of the other taxa, thus ruling out cross contamination from DNA of other *Arceuthobium* species present in adjacent tubes during the PCR setup. If the sequences of the various accessions are real, this suggests a high degree of genetic differentiation in *A. oxycedri* across its range. This species has a distribution from Spain and Morocco to the Himalayas of China and is reported to parasitize a wide range of hosts (Hawksworth and Wiens, 1996, table 16.8). Two allopatric taxa have already been segregated from *A. oxycedri* (*A. azoricum* and *A. juniperi procerae*), thus the possibility exists that additional genetically distinct, yet morphologically cryptic, taxa exist.

New World subgenus Arceuthobium taxa—The three New World subgenus *Arceuthobium* taxa (*A. abietis-religiosae*, *A. americanum*, *A. verticilliflorum*) were strongly supported as monophyletic and as sister to the remaining species in the genus. As mentioned, the tree topology makes subgenus *Arceuthobium* paraphyletic and the defining character for this subgenus (verticillate secondary branching) symplesiomorphic. Indeed, verticillate branching is observed in members of subgenus *Vaginata* such as *A. campylopodum* and *A. occidentale*. Thus, the statement by Mark and Hawksworth (1981) that subgenus *Vaginata* should be defined by the presence of flabellate secondary branching, not the absence of verticillate branching, is accurate.

In the previous ITS study of this genus (Nickrent et al., 1994), the sequences of *A. abietis-religiosae* and a single accession of the Old World species *A. oxycedri* were found to be similar, thereby resulting in a clade. This relationship is now known to be erroneous owing to a PCR artifact. The correct sequence of *A. abietis-religiosae* shows that it is clearly related to the New World members of subgenus *Arceuthobium*. *Arceuthobium verticilliflorum* is unique in the genus because of its extremely large fruits whose pedicels do not elongate and curve downward during maturation. Moreover, it is also the only species that lacks explosive seed dehiscence. Hawksworth and Wiens (1996, p. 256) state that this ''species is perhaps the most distinctive and primitive in the genus.'' They also state that ''the primitive morphological features associated with this species indicate that birds are likely the original mode of dispersal and not a derived system.'' The phylogenetic trees presented here clearly indicate that this mode of seed dispersal is not plesiomorphic but represents a loss of explosive dehiscence and a ''reacquisition'' of bird dispersal, a feature common to all other Viscaceae. This example can thus serve as a demonstration of the use of explicit phylogenetic data to confirm or refute hypotheses derived from other fields.

Three accessions of *A. americanum* were sequenced for the *trnL* region, and the two from California (1918 and 1738) formed a clade that was sister to the accession from Colorado (1966). The *A. americanum* clade was sister to *A. verticilliflorum* from Mexico. Recently (Jerome and Ford, 2002a, b) reported results of a population genetic study of *A. americanum* using AFLPs. Their UPGMA dendrogram showed three distinct races that generally followed geographic distribution and host: *Pinus banksiana*, *P. contorta* var. *latifolia*, and *P. contorta* var. *murrayana*. Although their studies were not phylogenetic, their phenogram placed the parasites from *P. contorta* var. *murrayana* at the base. Our results suggest that these dwarf mistletoes should be rooted with the parasites on *Pinus contorta* var. *latifolia*.

Arceuthobium guatemalense and A. pendens—The sister-group relationship between *A. guatemalense* and *A. pendens*, first reported by Nickrent et al. (1994), remains well-supported, now with chloroplast as well as nuclear sequence data. A parasite of *Pinus ayacahuite*, *A. guatemalense*, is found only in Guatemala and the states of Chiapas and Oaxaca in Mexico. *Arceuthobium pendens* is also a rare endemic, being found only in San Luis Potosí, Veracruz, and Puebla, Mexico. Because this latter species parasitizes pinyon pines, as does *A. divaricatum*, Hawksworth and Wiens (1980) first suggested they were related, but this was not supported using isozymes (Nickrent, 1996) nor ITS sequences (Nickrent et al., 1994). Thus, *A. guatemalense* and *A. pendens* represent the first clade of subgenus *Vaginata*, a group that evolved flabellate secondary branching from ancestors with the plesiomorphic verticillate secondary branching character. Among these two species, only *A. pendens* retains a full-length *trnL* region sequence, thus linking it to more primitive members of *Arceuthobium*. Mathiasen et al. (2000) attempted to relocate several populations of *A. guatemalense* in Guatemala but were unsuccessful. Apparently extensive logging has extirpated these populations, thus making *A. guatemalense* one of the rarest and most endangered dwarf mistletoe species.

The Arceuthobium globosum group—This well-supported clade is composed of two species, each with two named subspecies. These taxa were all formerly considered part of an *A. globosum* complex (Hawksworth and Wiens, 1972) but were subsequently subdivided into species and subspecies by Hawksworth and Wiens (1977). *Arceuthobium aureum* is restricted to Guatemala, whereas *A. aureum* subsp. *petersonii* occurs in Chiapas and Oaxaca, Mexico. *Arceuthobium globosum* occurs in the Sierra Madre Occidental from Chihuahua through Durango to northern Jalisco. *Arceuthobium globosum* subsp. *grandicaule* is allopatric, occurring from southern Jalisco to Oaxaca, Mexico, to Guatemala and Honduras. Although relatively few accessions were examined here, the *A. aureum*/*A. aureum* subsp. *petersonii* clade is sister to the more southern subspecies, *A. globosum* subsp. *grandicaule*, thus making *A. globosum* paraphyletic. Branch lengths separating these taxa, however, are very short, even when using the combined ITS and *trnL* region data set. Thus, the molecular data employed here do not provide sufficient numbers of substitutional differences to support recognition of these subspecific taxa.

Arceuthobium bicarinatum and A. pusillum—This clade was discussed at length in Nickrent et al. (1994) mainly owing to the intriguing biogeographic implications that stem from this phylogenetic relationship. These two taxa have few morphological synapomorphies, and their populations are currently separated by over 2000 km (eastern North America and Hispaniola). Hawksworth and Wiens (1972) suggested that *A. bicarinatum* was derived from *A. hondurense*, a taxon of Honduras and Mexico and that this migration occurred in the late Tertiary via a Central American land bridge. Our molecular data clearly show that *A. bicarinatum* is not related to *A. hondurense*. Results from ITS and *trnL* region sequence analysis are not congruent as to the relationship between *A. bicarina-*

tum and *A. pusillum*. Ribosomal ITS sequences strongly support a sister-group relationship between these species (Figs. 1, 2), whereas the *trnL* region is less conclusive. These species share unique indel characters for the *trnL* region, but because their sequences are quite different in length (owing to deletions in *A. bicarinatum*) they are not placed as sister in Fig. 3. Additional accessions of *A. bicarinatum*, as well as other gene sequences, are needed to further test the phylogenetic relationships of this taxon to *A. pusillum* as well as the *A. globosum* complex with which it has weak affinity using *trnL* region sequences.

The Arceuthobium rubrum group—Three species, *A. rubrum*, *A. oaxacanum*, and *A. yecorense*, occur on a well-supported clade in analyses using ITS, *trnL* region, and combined partitions. *Arceuthobium rubrum* occurs primarily in Durango and adjacent Sinaloa, *A. oaxacanum* is restricted to Oaxaca, and *A. yecorense* is known from disjunct populations in Chihuahua, Sonora, and Durango. *Arceuthobium oaxacanum* was first considered a disjunct population of *A. rubrum* but was later described as a distinct species (Hawksworth and Wiens, 1989). *Arceuthobium yecorense* was also first described in Hawksworth and Wiens (1989), but based on morphology, it was considered to be most closely related to *A. aureum*. Both ITS and *trnL* region sequence analyses result in a clade of the two *A. oaxacanum* accessions (2035 and 4315, Oaxaca) and this clade is sister to *A. rubrum* (2071, Durango) followed by *A. yecorense* (4346, Sonora). Molecular analyses do not support a close relationship between *A. yecorense* and *A. aureum*. The fruits of *A. rubrum* and *A. oaxacanum* are shiny, a unique and distinctive morphological synapomorphy. Using ITS, this clade of three species receives moderate support (79% BS) as sister to *A. gillii* and *A. nigrum*, whereas with the *trnL* region, the latter two species are sister to *A. hawksworthii* and *A. hondurense*. The topology in the ITS trees is also recovered in the combined analyses, but BS support is reduced for this clade owing to conflict between the two partitions.

The Arceuthobium vaginatum group—Three taxa have previously been considered subspecies within the *A. vaginatum* complex: *A. vaginatum* subsp. *cryptopodum*, *A. vaginatum* subsp. *vaginatum*, and *A. durangense*. The latter was named as a distinct species by Hawksworth and Wiens (1989). *Arceuthobium vaginatum* sensu lato has a wide distributional range, from Utah and Wyoming in the United States (*A. vaginatum* subsp. *cryptopodum*) to Oaxaca, Mexico (*A. vaginatum* subsp. *vaginatum*). This complex also has the broadest host range in the genus, parasitizing at least 20 different pine species in the United States and Mexico. Populations of both subspecies of *A. vaginatum* occur near each other in west-central Chihuahua where a ''gradation in characters'' has been observed (Hawksworth and Wiens, 1996). Ribosomal ITS sequences show that these three taxa are very closely related; all are separated by four or fewer substitutional changes. One accession, *A. vaginatum* 2018 from Veracruz, appears more closely related to *A. hondurense* than to other accessions of this species (Fig. 1). Although no male plants were collected, the female vouchers have swollen lower nodes typical of *A. hondurense*, thus this accession could be misidentified.

Two other species in this complex, *A. hawksworthii* from Belize and *A. hondurense* from Honduras and Mexico (Chiapas and Oaxaca) are shown to be very closely related according to both ITS and *trnL* region sequences. *Arceuthobium hondurense* was described in Hawksworth and Wiens (1972, 1996) as occurring only in Honduras and was thought to be one of the rarest dwarf mistletoes in the New World, possibly in danger of extinction. Subsequent field work has expanded the range of this species to southern Mexico (Mathiasen et al., 2001, 2002b). Plants from Chiapas were previously classified as *A. nigrum* (Hawksworth and Wiens, 1972, 1996). Hawksworth and Wiens (1996, p. 222) state that this species is closely related to *A. bicarinatum*; however, as detailed earlier, molecular evidence does not support a close relationship of *A. hondurense* to either *A. bicarinatum* or *A. nigrum*. This species appears most closely related to *A. hawksworthii*, a dwarf mistletoe that was only recently described as a species distinct from *A. globosum* and *A. aureum* (Wiens and Shaw, 1994). This mistletoe is extremely rare, primarily occupying only ca. 250 km^2 in area in the Mountain Pine Ridge east of Augustine, Belize (Mathiasen et al., 1999). Recently discovered in Honduras, its distribution there is poorly known (Mathiasen et al., 2002). The sixth taxon in this complex, *A. strictum*, was previously classified in section *Campylopoda* (Hawksworth and Wiens, 1972, 1996), but both ITS and *trnL* region sequence data show that it is related to section *Vaginata* taxa. This species is restricted to Durango, Mexico, and is distinctive in that the staminate plants are unbranched and the staminate flowers have up to seven perianth parts.

Arceuthobium divaricatum and A. douglasii—Interestingly, the positions of both *A. divaricatum* and *A. douglasii* remained unresolved using either ITS or *trnL* region sequences. Both partitions place these species as part of a polytomy that is basal to most subgenus *Vaginata* taxa. The combined analysis (Fig. 4) places these species as part of a polytomy involving the *Campylopodum* group, but with weak BS support (58%). *Arceuthobium divaricatum* was classified in section *Campylopoda* by Hawksworth and Wiens (1972), but isozyme analysis first suggested an association with *A. douglasii* (Nickrent, 1986, 1996). Isozymes also did not support the classification of *A. douglasii* and *A. pusillum* together in section *Minuta* (Hawksworth and Wiens, 1970); the diminutive size and systemic broom formation seen in these two species is almost certainly a result of convergence. As discussed by Nickrent et al. (1994), when ITS-2 sequences of *A. divaricatum* and *A. douglasii* are analyzed separately, these taxa occur on a clade with high BS support. The *trnL* region majority rule consensus tree shows that 71% of the trees associate *A. divaricatum* and *A. douglasii* with the *Campylopodum* group, but these nodes collapse on the strict consensus tree. Taking all evidence together, we still favor a clade composed of *A. divaricatum* and *A. douglasii*.

The Arceuthobium campylopodum group—Section *Campylopoda* as defined by Hawksworth and Wiens (1972) has been significantly revised during the past 30 years. The following taxa have been removed from the group following isozyme and DNA sequence analyses: *A. divaricatum*, *A. guatemalense*, *A. bicarinatum*, *A. hondurense*, *A. rubrum*, and *A. strictum*. In the classification proposed by Nickrent (1996), section *Campylopoda* was composed of 13 very closely related, mainly United States species, and the results presented here further support this composition, both from ITS as well as *trnL* region sequences. All share a unique deletion of 156 bp in the *trnT-L* spacer. Among this complex, sequences are either identical or differ by only a few substitutions, thus calling into

question the validity of naming these taxa at the species level. All of these species have previously been considered conspecific with or forms of *A. campylopodum*. Hawksworth and Wiens (1970) were the first to elevate these taxa, considered by Gill (1935) to be ''host forms,'' to the rank of species. Their justification was that these taxa were morphologically diagnosable and that their morphological integrity was maintained when occurring on different hosts. Although genetic differentiation between members of this complex is not as great as comparisons between species from different sections, isozymes have demonstrated some degree of genetic distinctiveness (often allele frequency differences) in various taxa in the complex (Nickrent, 1986, 1996; Nickrent and Butler, 1990, 1991; Nickrent and Stell, 1990). Among the 13 species, all are endemic to the United States except *A. abietinum* f. sp. *concoloris*, *A. apachecum*, and *A. blumeri* whose distributions extend from the United States into northern Mexico. One of these, *A. blumeri*, occupies a basal position on the section *Campylopoda* clade with ITS sequences, *trnL* region sequences, and isozymes. From this, it appears that *A. blumeri* could be considered a ''transitional'' species between the mainly Mexican and central American species of subgenus *Vaginata* and the mainly United States section *Campylopoda*.

Species delimitation in Arceuthobium—The goals of this study have been to identify phylogenetic units (clades) within *Arceuthobium* to infer its evolutionary history. When comparing these results to previous investigations of the genus, specifically the monographic treatments by Hawksworth and Wiens (1972, 1996), it becomes apparent that the methods and goals differ. Hawksworth and Wiens (1996, p. 141) state: ''the monographer's charge is to define taxa by whatever taxonomically valid characteristics are available.'' In addition to morphological features (most of which were quantitative), they examined cytogenetic characters, host specificity, and life cycle characteristics such as time of anthesis, time of meiosis, and period of seed dispersal. Their early work utilized a phenetic approach to grouping taxa into hierarchical units (Hawksworth et al., 1968), and these results provided the foundation for the first subgeneric classification of the genus (Hawksworth and Wiens, 1972). Since that time, systematic biology has undergone two major transformations: the acceptance of monophyly as a criterion for defining taxa above the species level (Hennig, 1966) and the broad utilization of macromolecular data in phylogenetic inference (Davis, 1995). Whether monophyly is a meaningful concept below the level of species is contentious (because of tokogeny), but for *Arceuthobium*, the supposed lack of interspecific hybridization suggests that species trees should be divergent, not reticulate. Thus, barring any such gene-tree/species-tree issues, cases of paraphyletic species relationships such as *A. gillii* and *A. nigrum* or *A. rubrum* and *A. oaxacanum* might be resolved by considering them to be conspecific.

Molecular phylogenetic analysis of *Arceuthobium* DNA sequences shows that branch lengths leading to different taxa vary widely. Many species are separated from other species by 20 or more substitutions in ITS (Fig. 2), such as *A. minutissimum*, *A. azoricum*, *A. abietis-religiosae*, *A. americanum*, *A. verticilliflorum*, *A. guatemalense*, *A. pendens*, and *A. douglasii*. In contrast, species in sections *Globosa*, *Vaginata*, and *Campylopoda* are not well differentiated, stemming from either lack of a sufficient number of substitutional differences, paraphyletic topologies, or both. Molecular data (combined ITS and *trnL* region tree, not shown) show at least an order of magnitude difference in branch lengths across a range of taxa, a result inconsistent with past species circumscriptions (e.g., Hawksworth and Wiens, 1972). Although the taxonomic goals of a forest pathologist may be to recognize any differences that exist among parasites on economically important hosts, such ''splitting'' results in numerous taxa that are essentially impossible to identify, particularly when examined apart from host information (Kuijt, 1973). From the perspective of a biological species concept, the level of reproductive isolation of the species should be addressed; however, cross pollination experiments have only rarely been conducted (Mathiasen, 1982). It is of interest to note that this experiment involving *A. apachecum* and *A. blumeri* demonstrated incompatibility in cross-pollinations and that the results reported herein (Fig. 4) document that among all the section *Campylopoda* taxa, *A. blumeri* is the most genetically distinct. Cross-inoculation experiments on alternate hosts are needed to test the assertion that morphological integrity of each species is maintained, particularly among section *Campylopoda*. Additional molecular work using more rapidly evolving markers, such as the AFLP analysis of *A. americanum* (Jerome and Ford, 2002a, b), should be conducted on the section *Globosa*, *Vaginata*, and *Campylopoda* species complexes.

It is instructive to compare and contrast the *Arceuthobium* results with those of *Korthalsella* where named species are grossly polyphyletic (Molvray et al., 1999). Morphological variation does not correlate with clades recovered from molecular analyses of either nuclear or chloroplast genes nor with previous classifications. These authors did not propose a revision of the genus based on their molecular results in the interest of avoiding generating numerous ''microspecies'' (morphologically undiagnosable species recognizable only by their DNA sequences). For *Arceuthobium*, the opposite condition exists in some cases, such as the *Campylopoda* complex. Here morphological ''microspecies'' have been named that cannot be diagnosed with the sequence-based DNA markers utilized to date. In other cases, such as *A. bicarinatum*, *A. divaricatum*, *A. guatemalense*, and *A. pendens*, morphological features suggest alliance with the *Campylopodum* complex, yet sequence data clearly differentiate these evolutionarily distinct species.

A revised classification of Arceuthobium—A classification of *Arceuthobium* based upon molecular evidence was first presented by Nickrent et al. (1994); however, for the Old World taxa, only *A. oxycedri* was included and several New World taxa were missing. The classification in Nickrent (1996) included all species in the genus, but molecular data were still lacking for a number of taxa, hence their placement was provisional. The present study obtained nuclear ITS rDNA sequences from all 42 extant species of *Arceuthobium* and *trnL* region sequences from all 34 New World species. These sequences included representatives of both subspecies for *A. aureum*, *A. globosum*, and *A. vaginatum* as well as multiple accessions from the same species for 18 taxa. Although additional accessions from some taxa would be desirable (e.g., *A. azoricum*, *A. bicarinatum*, *A. chinense*, and *A. tibetense*), we believe that our degree of taxon sampling is sufficient to preclude major artifacts that are often associated with low taxon density in molecular phylogenetic investigations (Wheeler, 1992; Kim, 1996).

A monophyletic (phylogenetic) species concept has been

adopted by the majority of botanists conducting molecular (DNA) analyses (Luckow, 1995). Following this philosophy, we present a revised classification of *Arceuthobium* that reduces the number of species from 42 to 26 (Table 2). The purpose of this classification is to present only those species that are distinct based upon the sequence data employed herein. We recognize that the application of other genetic markers (e.g., AFLPs) may provide additional resolution useful in addressing species-level relationships, particularly in sections *Globosa*, *Rubra*, *Vaginata*, and *Campylopoda*. If the taxa we have ''lumped'' with this approach are truly deserving of species status, we expect that morphological discontinuities could also be demonstrated through comprehensive multivariate analyses. Documentation of incompatibility (reproductive isolation) among sympatric taxa should be attempted by cross-pollination experiments.

In the artificial key to species in the Hawksworth and Wiens (1996) monograph, the majority of couplets involve geographic location and host (no ''natural'' key is provided). Thus, without such information, many of the species in section *Campylopoda* cannot be identified with certainty. Essentially all of the species in that section parasitize different primary host species, but significant overlap occurs when tabulating secondary hosts. Although cross-inoculation experiments would be valuable for all taxon pairs in section *Campylopoda*, it is likely that genetically determined preferences have evolved among some taxa. The question then to be asked is whether these differences are sufficient to classify those taxa as species. As discussed, genetic races patterned around three mainly allopatric pine species have been discovered in *A. americanum* (Jerome and Ford, 2002b). In *Arceuthobium vaginatum*, genetic differentiation can be maintained among proximal subpopulations parasitizing primary and secondary hosts (Linhart et al., 2003); thus, these could be considered host races as have been described in *Phoradendron tomentosum* (May, 1972; Clay et al., 1985), *P. californicum* (Glazner et al., 1988), and *P. serotinum* (Spooner, 1983). For taxa within sections *Globosa*, *Rubra*, *Vaginata*, and *Campylopoda* the situation might best be described as ''incipient speciation'' whereby the earliest stages of reproductive isolation and genetic differentiation are taking place among various population complexes. Over time, geographical and phenological discontinuities may arise that reinforce the genetic differentiation, thereby producing evolutionary entities that can be considered species.

Conclusions—Sequences of nuclear ITS have been obtained from representatives of all 42 species of *Arceuthobium* and chloroplast *trnT-L-F* region from 34 New World species. Gene trees from the two partitions were generally congruent; however, conflict was seen that derived from four taxa (*A. douglasii*, *A. divaricatum*, *A. gillii*, and *A. nigrum*). Analyses combining the two partitions were generally most similar to ITS analyzed separately. The *trnT-L-F* region has undergone extensive molecular evolution involving large deletions in many New World taxa, some of which appear to be homoplastic. A high degree of genetic differentiation exists in ITS sequences between Old and New World taxa, thus subgenus *Arceuthobium*, as traditionally defined by the presence of verticillate secondary branching, is paraphyletic. The phylogenetic (monophyletic) species concept employed here suggests a revised classification of *Arceuthobium* that includes 26 species.

LITERATURE CITED

ASHWORTH, V. E. T. M. 1999. Phylogenetic relationships in Phoradendreae (Viscaceae) inferred from DNA sequence data. Ph.D. dissertation, Claremont Graduate University, Claremont, California, USA.

BALDWIN, B. G. 1993. Molecular phylogenetics of *Calycadenia* (Compositae) based on its sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. *American Journal of Botany* 80: 222–238.

BORTIRI, E., S. OH, J. JIANG, S. E. BAGGETT, A. C. WEEKS, M. BUCKINGHAM, D. POTTER, AND D. PARFITT. 2001. Phylogeny and systematics of *Prunus* (Rosaceae) as determined by sequence analysis of ITS and the chloroplast *trnL-trnF* spacer DNA. *Systematic Botany* 26: 797–807.

CHASE, M., AND V. A. ALBERT. 1998. A perspective on the contribution of plastid *rbcL* DNA sequences to angiosperm phylogenetics. *In* D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants II, 488–507. Kluwer Academic, Boston, Massachusetts, USA.

CLAY, K., D. DEMENT, AND M. REJMANEK. 1985. Experimental evidence for host races in mistletoe (*Phoradendron tomentosum*). *American Journal of Botany* 72: 1225–1231.

DAVIS, J. I. 1995. Species concepts and phylogenetic analysis—introduction. *Systematic Botany* 20: 555–559.

DE QUEIROZ, A., M. J. DONOGHUE, AND J. KIM. 1995. Separate versus combined analysis of phylogenetic evidence. *Annual Review of Ecology and Systematics* 26: 657–681.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1995. Testing significance of incongruence. *Cladistics* 10: 315–319.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

GIELLY, L., Y.-M. YUAN, P. KÜPFER, AND P. TABERLET. 1996. Phylogenetic use of noncoding regions in the genus *Gentiana* L.: chloroplast *trnL* (UAA) intron versus nuclear ribosomal internal transcribed spacer sequences. *Molecular Phylogenetics and Evolution* 5: 460–466.

GILBERT, D. G. 1993. SeqApp, version 1.9a157. Biocomputing Office, Biology Department, Indiana University, Bloomington, Indiana, USA.

GILL, L. S. 1935. *Arceuthobium* in the United States. *Connecticut Academy of Arts and Science Transactions* 32: 111–245.

GLAZNER, J. T., B. DEVLIN, AND N. C. ELLSTRAND. 1988. Biochemical and morphological evidence for host race evolution in desert mistletoe, *Phoradendron californicum* (Viscaceae). *Plant Systematics and Evolution* 161: 13–21.

HAWKSWORTH, F. G., G. F. ESTABROOK, AND D. J. ROGER. 1968. Application of an information theory model for character analysis in the genus *Arceuthobium* (Viscaceae). *Taxon* 17: 605–619.

HAWKSWORTH, F. G., AND D. WIENS. 1965. *Arceuthobium* in Mexico. *Brittonia* 17: 213–238.

HAWKSWORTH, F. G., AND D. WIENS. 1970. New taxa and nomenclatural changes in *Arceuthobium* (Viscaceae). *Brittonia* 22: 265–269.

HAWKSWORTH, F. G., AND D. WIENS. 1972. Biology and classification of dwarf mistletoes (*Arceuthobium*). Forest Service, USDA, Washington, D.C., USA.

HAWKSWORTH, F. G., AND D. WIENS. 1976. *Arceuthobium oxycedri* and its segregates *A. juniperi-procerae* and *A. azoricum* (Viscaceae). *Kew Bulletin* 31: 71–80.

HAWKSWORTH, F. G., AND D. WIENS. 1977. *Arceuthobium* (Viscaceae) in Mexico and Guatemala: additions and range extensions. *Brittonia* 29: 411–418.

HAWKSWORTH, F. G., AND D. WIENS. 1980. A new species of *Arceuthobium* (Viscaceae) from Central Mexico. *Brittonia* 32: 348–352.

HAWKSWORTH, F. G., AND D. WIENS. 1989. Two new species, nomenclatural changes, and range extensions in Mexican *Arceuthobium* (Viscaceae). *Phytologia* 66: 3–11.

HAWKSWORTH, F. G., AND D. WIENS. 1996. Dwarf mistletoes: biology, pathology, and systematics. Forest Service, USDA, Washington, D.C., USA.

HENNIG, W. 1966. Phylogenetic systematics. University of Illinois Press, Urbana, Illinois, USA.

HODKINSON, T. R., M. W. CHASE, C. TAKAHASHI, I. J. LEITCH, M. D. BENNETT, AND S. A. RENVOIZE. 2002. The use of DNA sequencing (ITS and *trnL*-F), AFLP, and fluorescent in situ hybridization to study allopolyploid *Miscanthus* (Poaceae). *American Journal of Botany* 89: 279–286.

JANSEN, R. K., J. L. WEE, AND D. MILLIE. 1998. Comparative utility of

chloroplast DNA restriction site and DNA sequence data for phylogenetic studies of plants. *In* D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants, II. DNA sequencing, 87–100. Kluwer Academic, Boston, Massachusetts, USA.

JEROME, C. A., AND B. A. FORD. 2002a. Comparative population structure and genetic diversity of *Arceuthobium americanum* (Viscaceae) and its *Pinus* host species: insight into host–parasite evolution in parasitic angiosperms. *Molecular Ecology* 11: 407–420.

JEROME, C. A., AND B. A. FORD. 2002b. The discovery of three genetic races of the dwarf mistletoe *Arceuthobium americanum* (Viscaceae) provides insight into the evolution of parasitic angiosperms. *Molecular Ecology* 11: 387–405.

JOHNSON, L. A., AND D. E. SOLTIS. 1998. Assessing congruence: empirical examples from molecular data. *In* D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants, II. DNA sequencing, 297–348. Kluwer Academic, Boston, Massachusetts, USA.

KIM, K.-J. 1996. General inconsistency conditions for maximum parsimony: effects of branch lengths and increasing numbers of taxa. *Systematic Biology* 45: 363–374.

KLAK, C., T. A. HEDDERSON, AND H. P. LINDER. 2003. A molecular systematic study of the *Lampranthus* group (Aizoaceae) based on the chloroplast *trnL-trnF* and nuclear ITS and 5S NTS sequence data. *Systematic Botany* 28: 70–85.

KUIJT, J. 1973. Review: biology and classification of dwarf mistletoes (*Arceuthobium*) by F. G. Hawksworth and D. Wiens. U.S. Govt. Printing Office, Washington, DC. 1972. *Madroño* 22: 34–35.

LINHART, Y., L. ELLWOOD, J. KARRON, AND J. GEHRING. 2003. Genetic differentiation in the dwarf mistletoes *Arceuthobium vaginatum* and *Arceuthobium americanum* on their principal and secondary hosts. *International Journal of Plant Sciences* 164: 61–69.

LOHAN, A., AND K. WOLFE. 1998. A subset of conserved tRNA genes in plastid DNA of nongreen plants. *Genetics* 150: 425–433.

LUCKOW, M. 1995. Species concepts: assumptions, methods, and applications. *Systematic Botany* 20: 589–605.

MARK, W. R., AND F. G. HAWKSWORTH. 1981. Taxonomic implications of branching patterns in the dwarf mistletoes (*Arceuthobium*). *Phytologia* 47: 441–444.

MATHIASEN, R. 1982. Taxonomic studies of dwarf mistletoes parasitizing *Pinus strobiformis*. *Great Basin Naturalist* 24: 455–461.

MATHIASEN, R., J. MELGAR, AND B. HOWELL. 2002a. First report of *Arceuthobium hawksworthii* in Honduras. *Plant Disease* 86: 568.

MATHIASEN, R., D. NICKRENT, AND C. DAUGHERTY. 2002b. First report of *Arceuthobium hondurense* in Oaxaca, Mexico. *Plant Disease* 86: 72.

MATHIASEN, R., D. NICKRENT, C. PARKS, J. BEATTY, AND S. SESNIE. 2001. First report of *Arceuthobium hondurense* in Mexico. *Plant Disease* 85: 444.

MATHIASEN, R., C. G. PARKS, B. W. GEILS, AND J. S. BEATTY. 1999. Notes on the distribution, host range, plant size, phenology, and sex ratio of two rare dwarf mistletoes from Central America: *Arceuthobium hawksworthii* and *A. hondurense*. *Phytologia* 84: 154–164.

MATHIASEN, R., C. PARKS, D. NICKRENT, J. BEATTY, AND B. GEILS. 2000. Status of dwarf mistletoes in Central America. *In* P. Angwin [ed.], Proceedings of the forty-eighth western international forest disease work conference, 78–92. Forest Service, USDA, Redding, California, USA.

MAY, D. S. 1972. Morphological and physiological differentiation of *Phoradendron* populations in Texas. *American Journal of Botany* 59: 12–22.

MCKENNA, M. C. 1972. Was Europe connected directly to North America prior to the Middle Eocene? *Evolutionary Biology* 6: 179–189.

MOLVRAY, M., P. J. KORES, AND M. W. CHASE. 1999. Phylogenetic relationships within *Korthalsella* (Viscaceae) based on nuclear ITS and plastid *trnL-F* sequence data. *American Journal of Botany* 86: 249–260.

NICKRENT, D. L. 1986. Genetic polymorphism in the morphologically reduced dwarf mistletoes (*Arceuthobium*, Viscaceae): an electrophoretic study. *American Journal of Botany* 73: 1492–1502.

NICKRENT, D. L. 1994. From field to film: rapid sequencing methods for field collected plant species. *BioTechniques* 16: 470–475.

NICKRENT, D. L. 1996. Molecular systematics. *In* F. G. Hawksworth and D. Wiens [eds.], Dwarf mistletoes: biology, pathology, and systematics, 155–170. USDA Forest Service Agricultural Handbook 709.

NICKRENT, D. L., AND T. L. BUTLER. 1990. Allozymic relationships of *Arceuthobium campylopodum* and allies in California. *Biochemical Systematics and Ecology* 18: 253–265.

NICKRENT, D. L., AND T. L. BUTLER. 1991. Genetic relationships in *Arceuthobium monticola* and *A. siskiyouense* (Viscaceae): new dwarf mistletoe species from California and Oregon. *Biochemical Systematics and Ecology* 19: 305–313.

NICKRENT, D. L., K. P. SCHUETTE, AND E. M. STARR. 1994. A molecular phylogeny of *Arceuthobium* based upon rDNA internal transcribed spacer sequences. *American Journal of Botany* 81: 1149–1160.

NICKRENT, D. L., AND A. L. STELL. 1990. Electrophoretic evidence for genetic differentiation in two host races of hemlock dwarf mistletoe (*Arceuthobium tsugense*). *Biochemical Systematics and Ecology* 18: 267–280.

NIXON, K. C., AND J. M. CARPENTER. 1996. On simultaneous analysis. *Cladistics* 12: 221–241.

RAZAFIMANDIMBISON, S. G., AND B. BREMER. 2002. Phylogeny and classification of Naucleeae s.l. (Rubiaceae) inferred from molecular (ITS, *rbcL*, and *trnT-F*) and morphological data. *American Journal of Botany* 89: 1027–1041.

ROALSON, E., J. COLUMBUS, AND E. FRIAR. 2001. Phylogenetic relationships in Cariceae (Cyperaceae) based on ITS (nrDNA) and *trnT-L-F* (cpDNA) region sequences: assessment of subgeneric and sectional relationships in *Carex* with emphasis on section *Acrocystis*. *Systematic Botany* 26: 318–341.

RONSTED, N., M. W. CHASE, D. C. ALBACH, AND M. A. BELLO. 2002. Phylogenetic relationships within *Plantago* (Plantaginaceae): evidence from nuclear ribosomal ITS and plastid *trnL*-F sequence data. *Botanical Journal of the Linnean Society* 139: 323–338.

SATO, S., Y. NAKAMURA, T. KANEKO, E. ASAMIZU, AND S. TABATA. 1999. Complete structure of the chloroplast genome of *Arabidopsis thaliana*. *DNA Research* 6: 283–290.

SHINOZAKI, K., ET AL. 1986. The complete nucleotide sequence of the tobacco chloroplast genome. *Plant Molecular Biology Reporter* 4: 110–147.

SINCLAIR, W. T., R. R. MILL, M. F. GARDNER, P. WOLTZ, T. JAFFRE, J. PRESTON, M. L. HOLLINGSWORTH, A. PONGE, AND M. MOLLER. 2002. Evolutionary relationships of the New Caledonian heterotrophic conifer, *Parasitaxus usta* (Podocarpaceae), inferred from chloroplast *trn*L-F intron/spacer and nuclear rDNA ITS2 sequences. *Plant Systematics and Evolution* 233: 79–104.

SOLTIS, D. E., AND P. S. SOLTIS. 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. *In* D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants, II. DNA sequencing, 1–42. Kluwer Academic, Boston, Massachusetts, USA.

SPOONER, D. M. 1983. The northern range of eastern mistletoe, *Phoradendron serotinum* (Viscaceae), and its status in Ohio. *Bulletin of the Torrey Botanical Club* 110: 489–493.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (* and other methods). Sinauer, Sunderland, Massachusetts, USA.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

WENDEL, J. F., AND J. J. DOYLE. 1998. Phylogenetic incongruence: window into genome history and molecular evolution. *In* D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants, II. DNA sequencing, 265–296. Kluwer Academic, Boston, Massachusetts, USA.

WHEELER, W. C. 1992. Extinction, sampling, and molecular phylogenetics. *In* M. J. Novacek and Q. D. Wheeler [eds.], Extinction and phylogeny, 205–215. Columbia University Press, New York, New York, USA.

WIENS, D., AND C. G. I. SHAW. 1994. *Arceuthobium hawksworthii* (Viscaceae), a new species of dwarf mistletoe from Belize. *Journal of the Idaho Academy of Science* 30: 25–32.

WOLFE, K. H., C. W. MORDEN, S. C. EMS, AND J. D. PALMER. 1992. Rapid evolution of the plastid translational apparatus in a nonphotosynthetic plant: loss or accelerated sequence evolution of tRNA and ribosomal protein genes. *Journal of Molecular Evolution* 35: 304–317.

ZIMMER, E. A., E. H. ROALSON, L. E. SKOG, J. K. BOGGAN, AND A. IDNURM. 2002. Phylogenetic relationships in the Gesnerioideae (Gesneriaceae) based on nrDNA ITS and cpDNA *trnL-F* and cpDNA *trnL-F* and *trnE-T* spacer region sequences. *American Journal of Botany* 89: 296–311.