Aliso: A Journal of Systematic and Floristic Botany

Volume 19 | Issue 1 Article 5

2000

Phylogenetic Relationships in Phoradendreae (Viscaceae) Inferred from Three Regions of the Nuclear Ribosomal Cistron. II. The North American Species of Phoradendron

Vanessa E. T. M. Ashworth
Rancho Santa Ana Botanic Garden; Claremont Graduate University

Follow this and additional works at: https://scholarship.claremont.edu/aliso

 Part of the Botany Commons

Recommended Citation

Ashworth, Vanessa E. T. M. (2000) "Phylogenetic Relationships in Phoradendreae (Viscaceae) Inferred from Three Regions of the Nuclear Ribosomal Cistron. II. The North American Species of Phoradendron," *Aliso: A Journal of Systematic and Floristic Botany*: Vol. 19: Iss. 1, Article 5.
Available at: https://scholarship.claremont.edu/aliso/vol19/iss1/5

Aliso, 19(1), pp. 41–53
© 2000, by The Rancho Santa Ana Botanic Garden, Claremont, CA 91711-3157

PHYLOGENETIC RELATIONSHIPS IN PHORADENDREAE (VISCACEAE) INFERRED FROM THREE REGIONS OF THE NUCLEAR RIBOSOMAL CISTRON. II. THE NORTH AMERICAN SPECIES OF *PHORADENDRON*

VANESSA E. T. M. ASHWORTH[1]

Rancho Santa Ana Botanic Garden and Claremont Graduate University
Claremont, California 91711-3157 USA
e-mail: vanessaa@citrus.ucr.edu

ABSTRACT

A parsimony analysis based on sequences from the ITS region and two partitions of the 26S subunit of nuclear ribosomal DNA was used to infer phylogenetic relationships among the North American species of *Phoradendron*. A strongly supported clade united all but one of the species typically lacking cataphylls, a character used previously to distinguish the northern species from those of Central and South America. The divergent placement of *P. californicum* relative to the members of this "northern" clade confirmed the hypothesis that species lacking cataphylls are polyphyletic. Four of five species parasitic on conifers formed a well-supported clade. However, a strongly supported relationship between *P. rhipsalinum* and *P. brachystachyum*, the former a parasite of conifers, renders conifer parasitism homoplastic. A sister group relationship between these two species is not apparent from morphological evidence. A clade uniting *P. serotinum, P. tomentosum,* and *P. velutinum* was strongly supported. A broad host range characterized two of the three lineages of the basal tritomy in the northern clade, whereas the third lineage united species specialized in parasitism of oaks or conifers.

Key words: divergent domains, flowering period, host range, host specificity, internal transcribed spacer (ITS), nuclear ribosomal DNA, *Phoradendron*, phylogeny, Viscaceae, 26S.

INTRODUCTION

The two mistletoe genera *Phoradendron* Nutt. and *Dendrophthora* Eichler together compose the New World tribe *Phoradendreae* Engler. Both genera occur in Central America, the West Indies, and tropical latitudes of South America. However, only *Phoradendron* extends into the subtropical and temperate latitudes of North and South America. *Phoradendron* species parasitize a wide range of angiosperms and less commonly conifers. Trelease (1916) distinguished 240 species, but more recent estimates fluctuate between some 100 species or less (Kuijt 1982) and over 200 species (J. Kuijt pers. comm.). This discrepancy is due to the continual discovery of new species, but above all to the difficulty in circumscribing species, reflecting an unusual morphology in which few characters can be scored as discrete and many cannot be scored at all in a substantial subset of species. This paper represents the second of two contributions on phylogenetic relationships in Phoradendreae, the first dealing with major clades and the second focusing on Wiens's revision (1964) of *Phoradendron* species in North America.

Morphology

Phoradendreae are characterized by an articulated spike, whereby successive internodes of the inflorescence are arranged in an opposite decussate pattern (Fig. 1). Inflorescence internodes vary in number from one (Fig. 2) to ca. 10. Each internode consists of two flower areas on opposite sides of the inflorescence axis (Fig. 1). These flower areas are studded with tiny, sessile, mostly trimerous flowers arranged in vertical rows (series), the apical flower either remaining the only flower in its row (biseriate inflorescence; Fig. 3) or crowning a central row (triseriate inflorescence; Fig. 4). The number of series cannot be discerned in species where each side of the inflorescence internode holds an insufficient number of flowers (one to four; e.g., Fig. 2). Among the *Phoradendron* species in North America, triseriate inflorescences are the rule, the biseriate type predominating in species farther south.

Vegetatively, the North American species of *Phoradendron* are characterized by the predominant absence of cataphylls (Fig. 5). These small leaflike or bractlike structures are inserted in pairs on the first internode of lateral branches (Fig. 6) and are typical of tropical species. However, occasionally they are formed in several North American species said to lack cataphylls. Usually, cataphylls are not associated with floral organs (Fig. 6), but some species produce fertile cataphylls that subtend inflorescences. Cataphylls differ from foliage leaves by their much reduced size. In some species, however, foliage leaves are reduced to scales. The distinction between scalelike foliage leaves and cataphylls is then often difficult to diagnose.

[1] Current address: Department of Botany and Plant Sciences-072, University of California, Riverside, Calif. 92521-0124 USA.

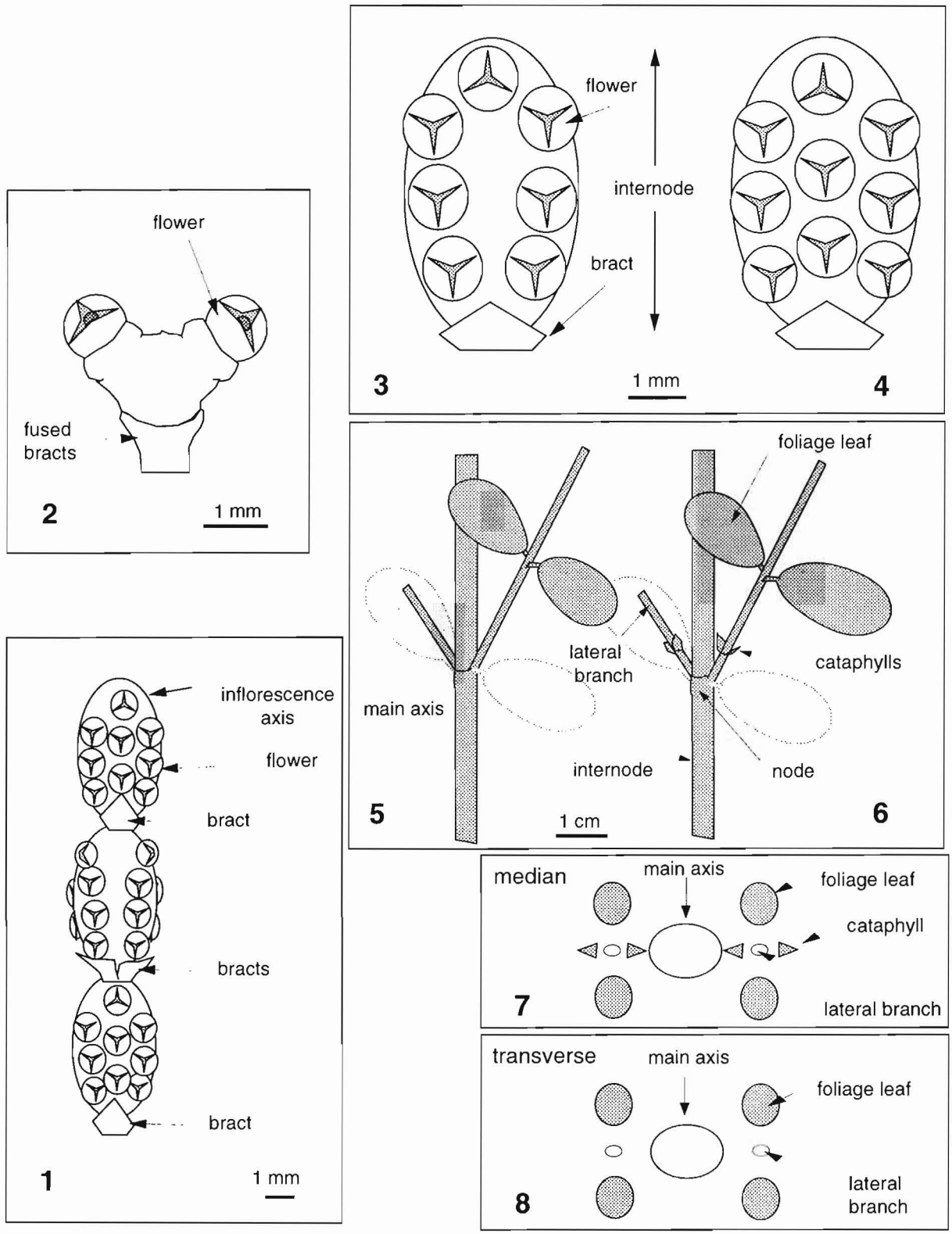

Fig. 1–8. Morphological characters in *Phoradendron*.—1–4 Inflorescences.—1–2. Degrees of elaboration of an articulated spike.—1. Spike consisting of three internodes in opposite decussate orientation. Each internode contains two flower areas on opposite sides of the inflorescence axis. Individual internodes are subtended by paired bracts.—2. Inflorescence in its simplest form, consisting of a single internode with one flower per flower area.—3–4. Inflorescence seriation, showing one flower area of an inflorescence internode and the subtending bract. The number of vertical flower rows (series) determines the inflorescence type.—3. Biseriate inflorescence.—4. Triseriate inflorescence.—5–6. Cataphylls.—5. Cataphylls absent.—6. Cataphylls located near the base of a lateral branch (basal cataphylls).—7–8. Orientation of the lowermost foliar organs on lateral branches in relation to the main branch axis (primary orientation).—7. Transectional view of the branch axis just above a node, showing a median orientation of the cataphylls.—8. Transectional view, showing a transverse orientation of the foliage leaves.

Primary orientation refers to the orientation of the lowermost (primary) foliar organs (cataphylls, if present, otherwise leaves) on lateral branches in relation to the main branch axis. In the majority of tropical species and several species in North America, the primary orientation is consistently median (Fig. 7), whereas most North American species have a consistently transverse orientation (Fig. 8). Two species, however, exhibit both orientation types.

Taxonomy

Two taxonomic treatments (Trelease 1916; Wiens 1964) cover the species of *Phoradendron* in North America. Trelease's monograph of the entire genus places the majority of North American species in "Boreales," an informal group comprising 57 taxa (50 species). Its members are characterized by the absence of cataphylls. However, the monophyly of the North American species based on the absence of cataphylls has been questioned, in that certain predominantly acataphyllous species occasionally develop cataphylls (Kuijt 1996), while the acataphyllous *P. californicum* shares other morphological traits with southern species (Kuijt 1959, 1996; Wiens 1964). Trelease placed *P. californicum,* a scale-leaved species, together with two other scale-leaved species in "*Aphyllae,*" a subdivision of section *Pauciflorae* Engler.

Wiens's revision (1964) is confined to the acataphyllous species of *Phoradendron* in North America. It covers the same suite of taxa as Trelease's "Boreales," whereby Wiens's 24 taxa (18 species) are a result of placing many of Trelease's taxa in synonymy. Wiens retained *P. californicum* in his revision, but placed it in monotypic section *Phoradendron.* The two taxonomic treatments also differ in the placements of *P. longifolium, P. robinsonii,* and *P. scaberrimum* in *Pluriseriales* Engler by Trelease (1916), but in *Calyculatae* Trelease by Wiens (1964).

Here, I develop a phylogenetic hypothesis for the North American species of *Phoradendron* using molecular sequences of the nuclear rDNA. The phylogenetic framework is then used to compare the taxonomic treatments by Trelease and Wiens in a phylogenetic context. The distribution of several morphological and life history characters reported in the literature (Wiens 1964; Kuijt 1996) on the molecular phylogeny is used to infer additional phylogenetic structure.

MATERIALS AND METHODS

Of the 43 taxa sampled, 34 belong to *Phoradendron,* six to *Dendrophthora* sensu Kuijt (1961) and three are outgroup species belonging to Viscaceae (*Korthalsella latissima*) and Santalaceae (*Acanthosyris asipapote* and *Thesium carinatum*). *Dendrophthora* species are considered part of the ingroup because recent evidence suggests that *Phoradendron* may not be monophyletic unless *Dendrophthora* is included within it (Nickrent and Duff 1996; Ashworth 2000). The 34 *Phoradendron* taxa include 19 North American representatives of Wiens's and Trelease's treatments, one species (*P. rhipsalinum*) that was unknown to Trelease and Wiens, and 14 other species, primarily from Central and/or South America and the West Indies (Table 1). All plants collected by the author in Mexico were sent to J. Kuijt for identification. For convenience, nomenclature follows Wiens (1964) for the North American acataphyllous species and Trelease (1916) for the remainder, even though some nomenclatural modifications have been made since then (e.g., *P. serotinum* is now *P. leucarpum*; Reveal and Johnston 1989). In most cases, a species is represented by a single sequence and for multiply sequenced species nucleotide polymorphisms at a particular site are encoded in terms of standard ambiguity codes. However, multiple sequences were retained for several species in order to represent separate subspecies (*P. bolleanum, P. juniperinum, P. tomentosum,* and *P. villosum*) and geographic differentiation (*P. tomentosum* from San Luis Potosí, Mexico, and Texas and *P. velutinum* from San Luis Potosí and Michoacán, Mexico).

The sequences used in this study comprise the ITS region and two partitions of the 26S nuclear rDNA (Fig. 9). Rapid sequence evolution in Viscaceae (Nickrent and Franchina 1990; Nickrent and Starr 1994; Nickrent et al. 1994; Nickrent and Soltis 1995; Nickrent and Duff 1996; Molvray et al. 1999) prompted the use of a composite gene region in which slower-evolving sequences enabled alignment with outgroups and the more divergent ingroup species, while the faster-evolving sequences resolved relationships between closely related taxa. A detailed account of the rationale dictating taxon sampling, outgroup selection, and choice of an appropriate gene region is given elsewhere (Ashworth 2000).

Methodology pertaining to DNA extraction followed the 2X CTAB buffer protocol by Doyle and Doyle (1987), with DNA extracts diluted to a final concentration of 10 *ng*/μl. Double-stranded DNA template was amplified by the polymerase chain reaction (Mullis et al. 1986) using amplification conditions detailed in Ashworth (2000). The three DNA regions were amplified and cycle sequenced using various combinations of forward and reverse primers ITS2, ITS3, ITS4, ITS4i, ITS5, ITS5i (White et al. 1990), 1643f (CCGCCCGTCGCTCCTACCG) and ITS4–172 (CCCAAACAACCCGACTCACCAACAG) (Ashworth 2000) for the ITS region, and 641r, 1715f and 2134r (D.L. Nickrent), S1' and S2 (Bult and Zimmer 1993) for 26S rDNA. The two regions of 26S rDNA (D2 and D8; Fig. 9) are delimited by primer pairs S2 + 641r and 1715f + 2134f, respectively. They corre-

Table 1. Specimens used in the study. Unless otherwise stated, all are deposited at RSA. Nomenclature follows Wiens (1964) for species from North America and Trelease (1916) for the remainder. All species are Viscaceae, except for *Acanthosyris asipapote* and *Thesium carinatum* (Santalaceae).

Taxon	Origin	Voucher/specimen	GenBank accession number
Acanthosyris asipapote M. Nee	Bolivia: Santa Cruz	*Nickrent 4051*, SIU	AF181776 (D2), AF181819 (D8)
Dendrophthora clavata (Benth.) Urban	Columbia: Boyaco	*Nickrent 2182*, SIU	AF178742, AF181770, AF181813
D. costaricensis Urban	El Salvador	*Villacorta 1288*, MO	AF178743 (ITS1), AF178744 (ITS2), AF181771 (D2), AF181814 (D8)
D. domingensis (Spreng.) Eichler	Cuba	*Hermann 7131*	AF178741 (ITS2), AF181769 (D2), AF181812 (D8)
D. guatemalensis Standley	Mexico: Chiapas	*Breedlove 23390*	AF178726, AF181759, AF181801
D. opuntioides (L.) Eichler	Jamaica	*Crosby et al. 285*	AF178739 (ITS1), AF178740 (ITS2), AF181768 (D2), AF181811 (D8)
D. squamigera (Benth.) Kuntze	Costa Rica	*Schmid 1979-18*	AF178745, AF181772, AF181815
Korthalsella latissima (Tiegh.) Dans.	USA: Hawaii	*Molvray 309*, MO	AF181775 (D2), AF181818 (D8)
Phoradendron bolleanum (Seem.) Eichler subsp. *bolleanum*	Mexico: Sonora	*Columbus 2753*	AF178708 (ITS)
P. bolleanum subsp. *densum* (Torr.) Wiens	USA: California	*Ashworth 59*	AF178705, AF181740, AF181782
P. bolleanum subsp. *pauciflorum* (Torr.) Wiens	USA: California	*Ashworth 42*	AF178706, AF181741, AF181783
P. brachystachyum (DC) Nutt.	Mexico: Michoacán	*Ashworth 273*	AF178720, AF181753, AF181795
P. brevifolium Oliver	Mexico: Puebla	*Ashworth 282*	AF178724, AF181757, AF181799
P. californicum Nutt.	USA: Arizona	*Ashworth 76*	AF178728, AF181761, AF181803
P. capitellatum Torr. ex Trel.	USA: Arizona	*Ashworth 84*	AF178702, AF181737, AF181779
P. carneum Urban	Mexico: Michoacán	*Ashworth 271*	AF178732, AF181765, AF181807
P. crassifolium (Pohl ex DC) Eichler	Peru	*Young & Jaramillo 2176*, MO	AF178746 (ITS1), AF178747 (ITS2), AF181773 (D2), AF181816 (D8)
P. forestierae Robinson & Greenman	Mexico: Querétaro	*Ashworth 254*	AF178723, AF181756, AF181798
P. galeottii Trel.	Mexico: Oaxaca	*Lorence 4621*	AF178716, AF181750, AF181792
P. heydeanum Trel.	Honduras	*Forstreuter 9156*, MB	AF178729, AF181762, AF181804
P. juniperinum Engelm. ex A. Gray subsp. *juniperinum*	USA: Arizona	*Ashworth 77*	AF178703, AF181738, AF181780
P. juniperinum subsp. *libocedri* (Engelm.) Wiens	USA: California	*Ashworth 37*	AF178704, AF181739, AF181781
P. longifolium Eichler	Mexico: Querétaro	*Ashworth 257*	AF178717 (ITS)
P. minutifolium Urban	Mexico: Puebla	*Ashworth 280*	AF178707, AF181742, AF181784
P. nervosum Oliver	Bolivia: La Paz	*Lewis 8869*, MO	AF178734 (ITS1), AF178735 (ITS2), AF181766 (D2), AF181808 (D8)
P. piperoides (Kunth) Trel.	Puerto Rico	*Miller & Sherman 6439*, MO	AF178748 (ITS1), AF178749 (ITS2), AF181774 (D2), AF181817 (D8)
P. reichenbachianum (Seem.) Oliver	Mexico: Puebla	*Ashworth 285*	AF178725, AF181758, AF181800
P. rhipsalinum Rzed.	Mexico: Jalisco	*Ashworth 270*	AF178719, AF181752, AF181794
P. robinsonii Urban	Mexico: San Luis Potosí	*Ashworth 260*	AF178718, AF181751, AF181793
P. robustissimum Eichler	Honduras	*Forstreuter 9135*, MB	AF178733 (ITS)

Table 1. Continued.

Taxon	Origin	Voucher/specimen	GenBank accession number
P. scaberrimum Trel.	Mexico: Chihuahua	*Ashworth 100*	AF178715, AF181749, AF181791
P. serotinum (Raf.) M.C. Johnston	USA: Illinois	*Ashworth 308*, SIU	AF178711, AF181745, AF181787
P. sulfuratum Rizzini	Venezuela: Bolivar	*Steyermark 131692*, MO	AF178737 (ITS1), AF178738 (ITS2), AF181767 (D2), AF181810 (D8)
P. tamaulipense (Kunth) Krug & Urban	Mexico: San Luis Potosí	*Ashworth 261*	AF178730, AF181763, AF181805
P. tomentosum (DC) Engelm. ex A. Gray subsp. *macrophyllum* (Engelm.) Wiens	USA: California	*Ashworth 58*	AF178709, AF181743, AF181785
P. tomentosum subsp. *tomentosum*	Mexico: San Luis Potosí	*Ashworth 263*	AF178710, AF181744, AF181786
P. tomentosum subsp. *tomentosum*	USA: Texas	*Roalson 1225*	AF178712, AF181746, AF181788
P. cf. *tonduzii* Trel.	Honduras	*Forstreuter 9154*, MB	AF178736, AF181778, AF181809
P. trinervium (Lam.) Griseb.	Puerto Rico	*Struwe 1108*, NY	AF178731, AF181764, AF181806
P. velutinum (DC) Nutt.	Mexico: San Luis Potosí	*Ashworth 262*	AF178721, AF181754, AF181796
P. velutinum	Mexico: Michoacán	*Ashworth 275*	AF178722, AF181755, AF181797
P. vernicosum Greenman	Honduras	*Forstreuter 9141*, MB	AF178727, AF181760, AF181802
P. villosum (Nutt.) Nutt. subsp. *coryae* (Trel.) Wiens	USA: Arizona	*Ashworth 82*	AF178714, AF181748, AF181790
P. villosum subsp. *villosum*	USA: California	*Ashworth 62*	AF178713, AF181747, AF181789
Thesium carinatum A. DC.	South Africa	*Nickrent 4094*, SIU	AF181777 (D2), AF181820 (D8)

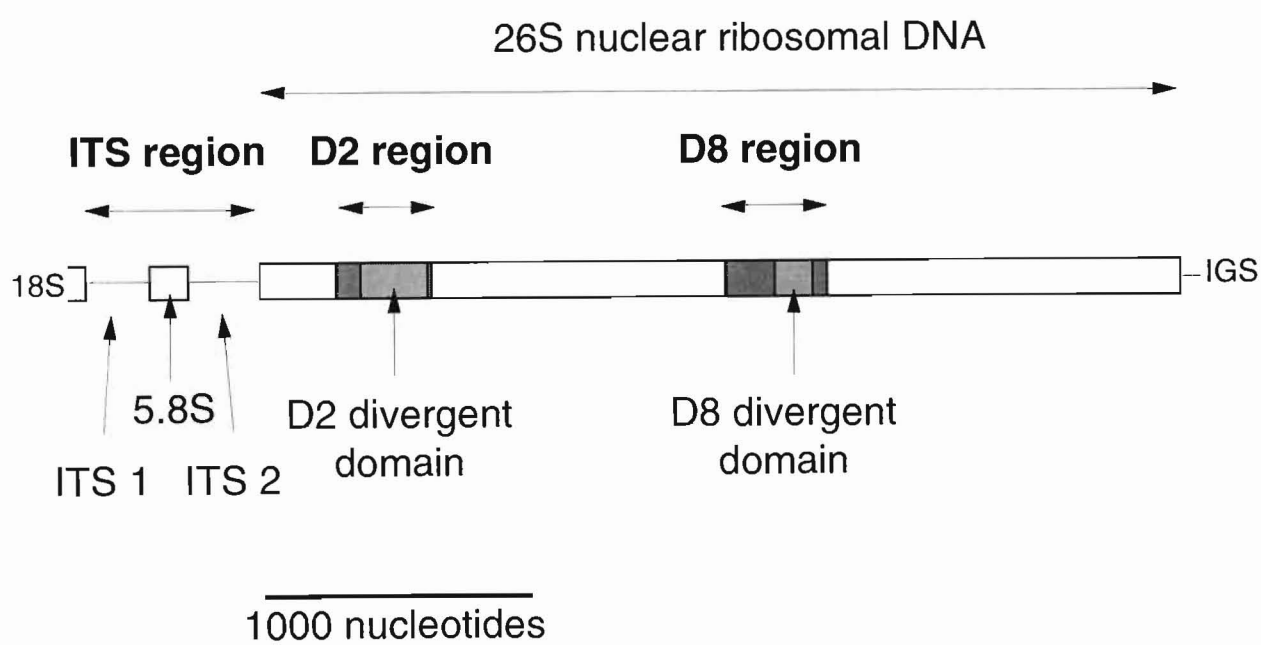

Fig. 9. Diagram of the ITS region and 26S large subunit of nuclear rDNA drawn to scale. Gene regions sequenced in this study (ITS1 and ITS2 spacers, the 5.8S portion of the small-subunit nuclear rDNA, and the D2 and D8 regions) are printed in bold. The D2 region is bounded by primers S2 and 641r and the D8 region by primers 1715f and 2134r. The D2 and D8 divergent domains (light gray) are regions with increased numbers of nucleotide substitution relative to the more conserved flanking sequences of the 26S nuclear rDNA. They are located within the D2 and D8 regions (dark gray), respectively.

spond to positions 1128–1495 and 2583–2983 in the 26S sequence of *Arabidopsis thaliana* (GenBank X52320; Unfried and Gruendler 1990).

Sequences were generated on an Applied Biosystems 373A automated DNA sequencer using a Sequagel-6 polyacrylamide gel (National Diagnostics). The sequences were assembled, edited, and combined into a consensus sequence using Sequencher 3.0 (Gene Codes Corporation, Inc., Ann Arbor, MI). All sequences are available from GenBank and accession numbers are provided in Table 1. Aligned sequences were submitted to parsimony analysis using PAUP* version 4.0 β1 (Swofford 1998). Several alternative alignments, including computer-assisted (ClustalW version 1.4; Thompson et al. 1994) and visual alignments, were tested. The visually aligned matrix is archived in Ashworth (1999; Ph.D. thesis) and posted on the Internet at the website: http://www.science.siu.edu/parasitic-plants/Alignments/Phor.align.html. Parsimony analyses used heuristic searching with accelerated transformation optimization and TBR branch swapping. Starting trees were obtained by simple addition or 100 cycles of random addition, and branch swapping restricted to the best trees only. During branch swapping, the STEEPEST DESCENT option was switched off. All optimal trees were saved (MULPARS).

Branch support was calculated using 1000 bootstrap replicates (Felsenstein 1985), as implemented on PAUP* version 4.0 β1 in the "Fast stepwise addition" function, and using the decay approach (Bremer 1988; Donoghue et al. 1992). Bootstrap values of at least 70% are taken to indicate strong branch support (Hillis and Bull 1993; but see Efron et al. 1996). Homoplasy of the sequence data was calculated using the consistency index (Kluge and Farris 1969; Sanderson and Donoghue 1989) and the retention index (Farris 1989). Gaps were coded as missing data.

RESULTS

The total length of the aligned data matrix was 1602 nucleotides, of which 813 nucleotides resided in the ITS region, 382 in the D2 region and 407 in the D8 region. Of 800 variable sites, 594 were potentially parsimony-informative. Missing data accounted for 3.96% of the data matrix. Lengths of sequences of the ingroup taxa (excluding partially sequenced taxa and gaps) ranged from 1380 to 1496 nucleotides, with an average of 1412.

Sequence alignment revealed the presence of 36 indels. Fourteen are relevant in the context of this study. Eight support branches on the strict consensus tree (Fig. 10) and six are homoplastic (e, g′, n, y, I, J), of which one (n) is shared by two of three branches in a tritomy. Ten of the indels are 1–2 nucleotides in length (five each), three are 3 nucleotides long and one is four nucleotides long. All but two are located in the ITS region.

Figure 10 depicts the strict consensus of four equally most parsimonious trees (MPTs) of 2234 steps. Of a total of 37 ingroup nodes, 27 have bootstrap values of ≥70%, 28 have decay values of ≥3, and 25 have both. A bootstrap value of 88% and a decay value of 10 support a clade, henceforth called the northern clade (Fig. 10), including *P. rhipsalinum* and all members of Trelease's "Boreales" and Wiens' revision except *P. californicum*. *Phoradendron californicum* appears as sister to a clade that includes not only members of the northern clade, but several other species (*Dendrophthora guatemalensis, P. brevifolium, P. forestierae, P. reichenbachianum,* and *P. vernicosum*) that have cataphylls. The precise placement of *P. californicum* is uncertain owing to weak bootstrap support (67%; Fig. 10) and a long branch (146 nucleotide substitutions; Fig. 11). Interestingly, the branch decays only when all trees more than five steps longer than the most parsimonious solution are included in the analysis.

Within the northern clade, several clades receive strong support (Fig. 10). The first of these combines *P. brachystachyum* and *P. rhipsalinum,* the second *P. serotinum, P. tomentosum,* and *P. velutinum* (henceforth the *P. serotinum* clade), the third *P. galeottii* and *P. longifolium,* the fourth *P. villosum* subsp. *coryae* and *P. scaberrimum,* and the fifth *P. bolleanum* subsp. *bolleanum* to *P. capitellatum,* seven taxa parasitic on conifers occurring in western North America (henceforth the conifer-parasite clade). In the *P. serotinum* clade, *P. serotinum* and *P. tomentosum* form a clade whose sister species is *P. velutinum.* Within the conifer-parasite clade, three clades each unite (a) the two subspecies of *P. juniperinum,* (b) two of the three subspecies of *P. bolleanum* (subspp. *densum* and *pauciflorum),* and (c) *P. minutifolium* and *P. bolleanum* subsp. *bolleanum.*

DISCUSSION

Phoradendron californicum

The divergent position of *P. californicum* argues strongly against the monophyly of "Boreales" sensu Trelease (1916) or a monophyletic acataphyllous lineage sensu Wiens (1964) (Fig. 11). Instead, it agrees with morphological evidence (shared biseriate inflorescences) that suggests a closer relationship of *P. californicum* with cataphyllous tropical species (Kuijt 1959, 1996). Regrettably, *P. olae,* its putatively closest relative (Kuijt 1997), is not part of this analysis. The data also suggest two independent origins of scale leaves (Fig. 10). Wiens (1964) placed *P. californicum* in a monotypic section *Phoradendron.* Marked sequence divergence evidenced by the long branch (Fig.

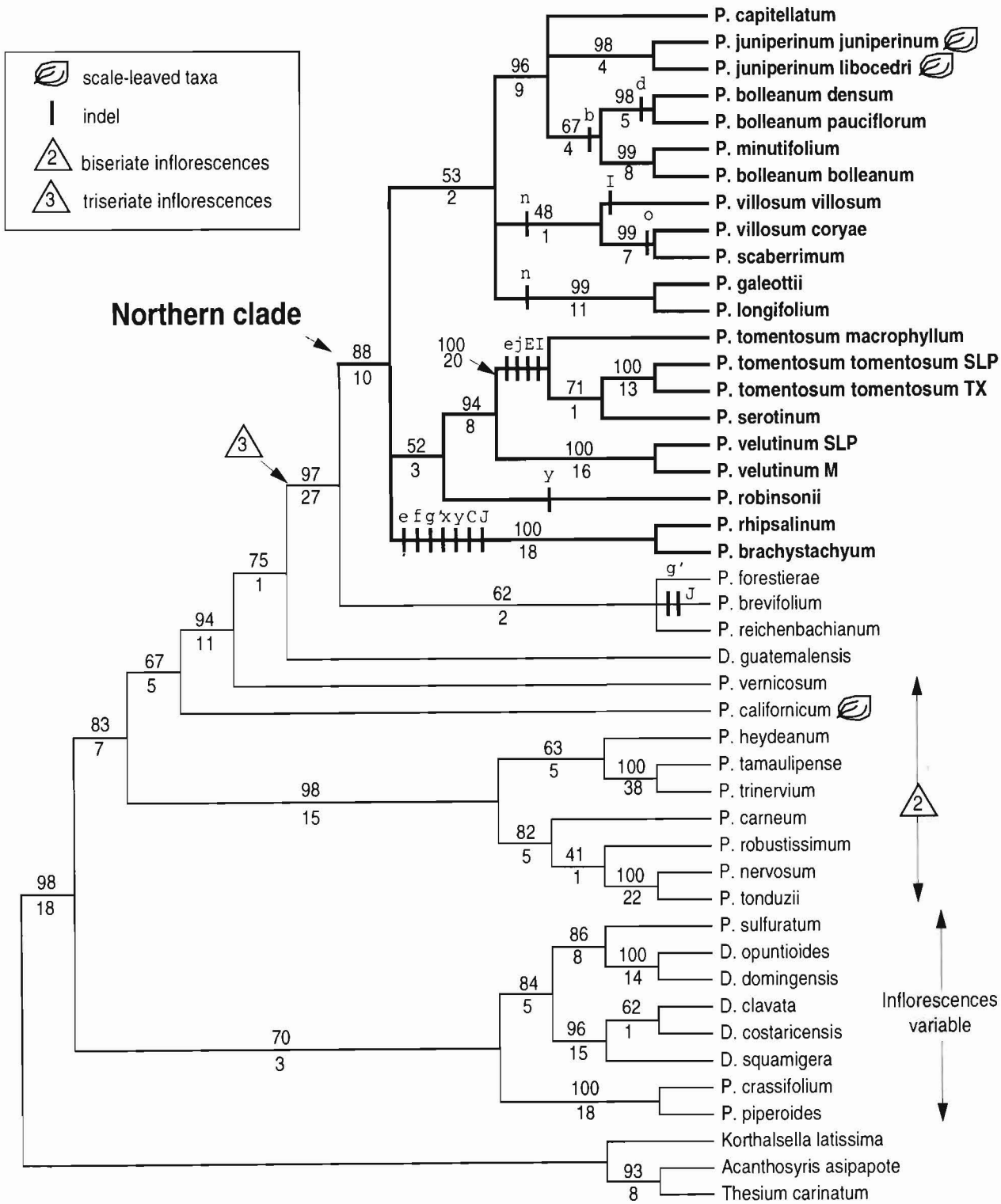

Fig. 10. Strict consensus tree (CI = 0.56, RI = 0.75) of four equally most parsimonious trees of length 2234 steps from a maximum parsimony analysis of three regions of the nuclear rDNA. Bootstrap and decay values are located above and below each branch, respectively. *Phoradendron tomentosum* and *P. velutinum* are each represented by two accessions from Texas (TX) and the Mexican states of San Luis Potosí (SLP) and Michoacán (M). Branches and species in bold face indicate the members of the northern clade, as defined in the text. Triseriate inflorescences characterize all members of the northern clade and its sister clade. Biseriate inflorescences are found in *P. vernicosum, P. californicum,* and the clade that is sister to *P. californicum.*

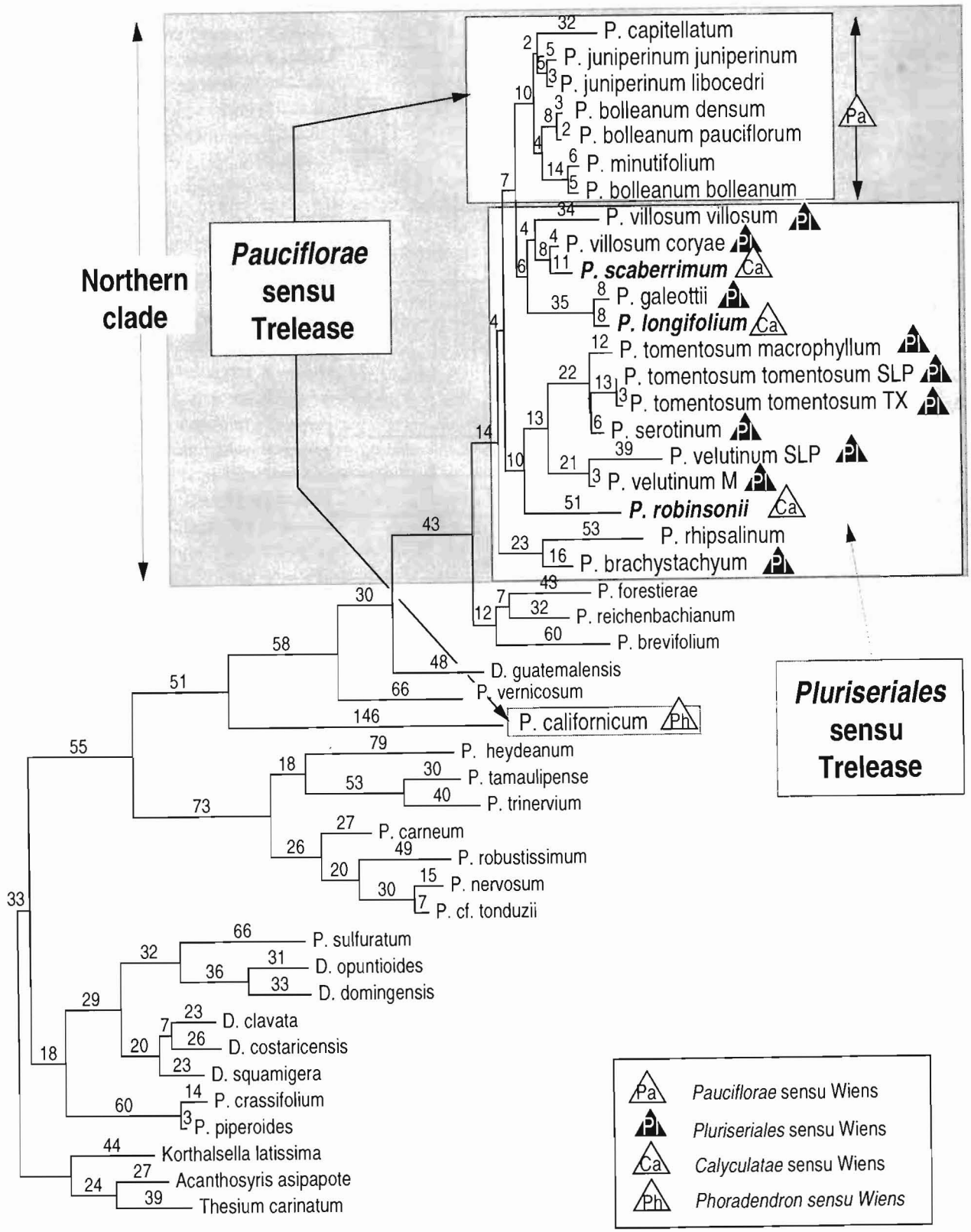

Fig. 11. Phylogram of one of four equally most parsimonious trees of length 2234 steps from a maximum parsimony analysis of three regions of the nuclear rDNA, showing the contrasting sectional delimitations by Trelease (1916) and Wiens (1964). *Pauciflorae* and *Pluriseriales* sensu Trelease are enclosed in a white and pale gray box, respectively, while *Pauciflorae, Pluriseriales,* and *Calyculatae* sensu Wiens are indicated next to each taxon inside a triangle. Taxon names of the members of Wiens's section *Calyculatae* are additionally italicized and bolded. The northern clade is enclosed in a dark gray box. Numbers above branches denote branch lengths. *Phoradendron californicum* is separated from the remaining members of *Pauciflorae* sensu Trelease. *Phoradendron rhipsalinum* was not known to either Trelease or Wiens. Species outside Trelease's "Boreales" and Wiens's revision are printed in a smaller font size.

11) may reflect accelerated sequence evolution, extinctions that eliminated close relatives, or incomplete sampling. Incomplete sampling is perhaps the simplest explanation, in that this analysis includes less than half of *Phoradendron* species and ca. 10% of *Dendrophthora* species.

Parasites of Conifers

Pauciflorae sensu Wiens (excluding *P. californicum*; Fig. 11) is monophyletic, and its members form a fairly uniform group in terms of morphology (small to scalelike leaves and few-flowered spikes), host preference (conifers, usually forming highly specific associations; Fig. 12) and nucleotide sequence data (bootstrap 96%, decay of 9). Strongly supported sister group relationships for three species pairs suggest that (1) *P. juniperinum* is monophyletic, but (2) *P. bolleanum* is not. Further evidence for the polyphyly of *P. bolleanum* comes from indel data. Although node support is weak (bootstrap of 67%, decay of 4), the clade uniting the three *P. bolleanum* subspecies and *P. minutifolium* is supported by insertion b. Insertion d unites *P. bolleanum* subspp. *densum* and *pauciflorum* but not *P. bolleanum* subsp. *bolleanum* (Fig. 10, 12). *Phoradendron bolleanum* is monophyletic only if *P. minutifolium* is placed in synonymy or subspp. *densum* and *pauciflorum* are treated as species. Interestingly, plants presumed to have originated by hybridization between *P. bolleanum* subsp. *densum* and *P. juniperinum* resemble *P. minutifolium* (Vasek 1966; Wiens and DeDecker 1972).

The sister group relationship between the two subspecies of *P. juniperinum* is strongly supported. *Phoradendron juniperinum* subsp. *libocedri* differs from the typical subspecies by a more pendulous habit and longer internodes that mimic the morphology of its host, *Calocedrus decurrens* (Torr.) Florin. The typical variety is a parasite of *Juniperus* and *Cupressus* species. Three MPTs (not shown) support a sister group relationship between *P. juniperinum* and *P. capitellatum*. The latter is the most divergent within *Pauciflorae*, by virtue of numerous autapomorphic nucleotide substitutions (Fig. 11) and a deletion of 44 nucleotides in the ITS1 region. *Phoradendron capitellatum* is also the only species in the conifer-parasite clade to flower in winter (December–February; Wiens 1964), although reports of summer flowering do exist (J. Kuijt pers. comm.). Only one of the four MPTs (not shown) shows *P. capitellatum* as sister to a clade composed of the three *P. bolleanum* subspecies and *P. minutifolium*. Fosberg (1941) placed *P. capitellatum* as subspecies under *P. bolleanum* whilst retaining *P. minutifolium* at the species level.

Phoradendron rhipsalinum

Pauciflorae sensu Wiens (1964) includes all but one species that parasitize conifers (primarily Cupressaceae s.l.). The discovery of *P. rhipsalinum* (Calderón de Rzedowski and Rzedowski 1972) occurred after Wiens's revision, but the authors suggested its placement in *Pluriseriales* sensu Wiens (1964). By contrast, Kuijt (1996) suggested it "may be a distant relative of *P. bolleanum*." Unlike the members of *Pauciflorae*, however, *P. rhipsalinum* has elongated inflorescences with several internodes, occasionally develops cataphylls (Kuijt 1996), and has both median and transverse primary orientation. Both morphological and molecular data thus agree that *P. rhipsalinum* does not neatly fit into *Pauciflorae*.

Far more surprising is the strongly supported relationship between *P. rhipsalinum* and *P. brachystachyum*, the latter a parasite of angiosperms (Fig. 12). Three indels (f, x, and C) are uniquely shared by these two species (Fig. 10). Interestingly, *P. brachystachyum* also occasionally produces cataphylls (Kuijt 1996). *Phoradendron rhipsalinum* is an endemic of central Mexico where it is found exclusively on *Taxodium mucronatum* Ten. Like *P. juniperinum* subsp. *libocedri*, it appears to mimic the pendulous branches of its host by means of long internodes and, in this case, linear leaves. Little is known about the biology of this species. *Phoradendron brachystachyum*, by contrast, is widely distributed in Mexico, grows on a diverse array of angiosperms (especially Fabaceae) and exhibits considerable variation in leaf shape, without apparent host mimicry. Vegetative mimicry (leaves) is well documented among certain Australian Loranthaceae (Barlow and Wiens 1977).

Parasites of Angiosperms

Within the *P. serotinum* clade, *P. serotinum* and *P. tomentosum* are sister species (Fig. 10). They share medium-sized (ca. 25–30 mm long × 12–20 mm wide), orbicular to obovate leaves having somewhat obtuse apices and inconspicuous venation and sometimes a tomentum that tends to diminish or disappear with maturity (Wiens 1964). They parasitize a wide range of angiosperms, including Betulaceae, Fabaceae, Fagaceae, Oleaceae, and Platanaceae. Two unique synapomorphic insertions (j and E) support the sister group relationship between *P. serotinum* and *P. tomentosum* (Fig. 10 and 12). Sister species to this clade is *P. velutinum*. This species differs from the preceding two by a yellower color and more elongate leaves with acute tips and tapered bases. Both *P. velutinum* specimens sampled were found growing on Rosaceae and not oak, the primary host reported by Wiens (1964), although a survey of herbarium specimens suggests that *P. velutinum* has a wide host range. A patristic

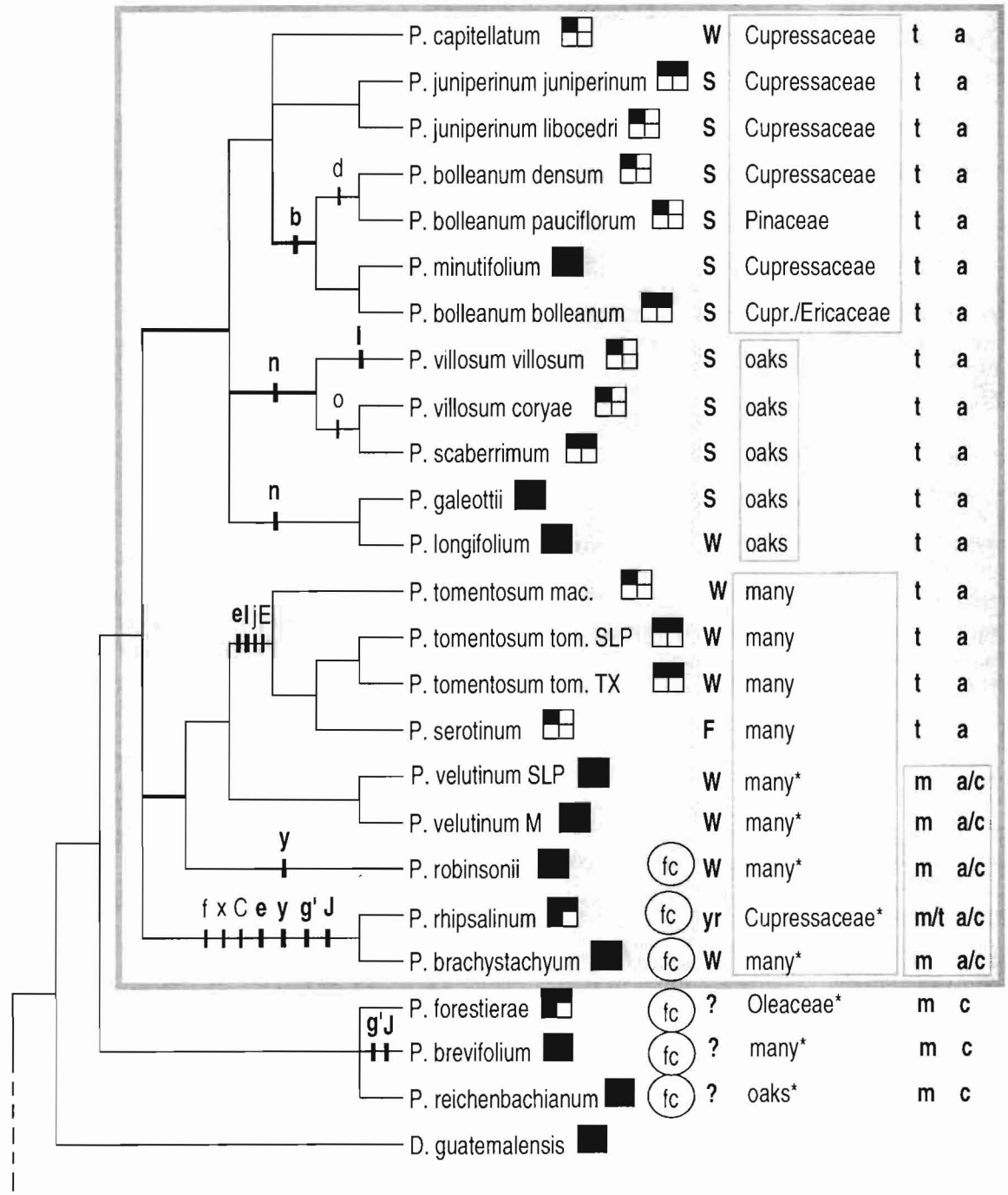

Fig. 12. Excerpt of the strict consensus tree from a maximum parsimony analysis of three regions of nuclear rDNA. Geographic distribution, indels, morphological traits (Kuijt 1996), life history characters (Wiens 1964), and preferred hosts (Wiens 1964 and personal observations) are indicated for the members of the northern clade (gray box) and adjacent parts of the tree. Thickened branches have weak bootstrap support (<70%). Only indels pertaining to species in the northern clade are mapped onto the tree. Homoplastic indels are indicated in bold. Abbreviations: fc: fertile cataphylls; W: winter flowering, S: summer flowering, F: fall flowering, yr: flowering year-round; t: transverse orientation, m: median orientation, m/t: both types of orientation; a: cataphylls absent, c: cataphylls present, a/c: cataphylls sometimes present. Asterisks denote host ranges gathered from surveys of herbarium specimens; all other host ranges are from Wiens (1964). Geographic distribution is indicated by a boxed grid, in which one to three blackened squares respectively indicate a distribution that extends no farther south than 26°, 22°, and 18° N latitude and four blackened squares represent a distribution extending south of—or occurring exclusively south of—18° N latitude. All distribution ranges are from Wiens (1964). The three latitudes correspond approximately to northern Sinaloa, central Aguascalientes, and southern Puebla, Mexico.

distance of 42 nucleotide substitutions between the two *P. velutinum* sequences (Fig. 11) perhaps reflects a high degree of polymorphism of this species (Trelease 1916).

The strongly supported association between *P. scaberrimum* and *P. villosum* subsp. *coryae* renders *P. villosum* polyphyletic. Indel o further strengthens the sister group relationship between these two species (Fig. 10). Indel n is present in *P. galeottii, P. longifolium, P. scaberrimum,* and *P. villosum* subspp. *villosum* and *coryae* that are almost exclusively parasites of oaks (Fig. 12). Insertion I uniting *P. villosum* subsp. *villosum* with the *P. serotinum* clade is homoplastic.

Sister species *P. galeottii* and *P. longifolium* share the same hosts, have overlapping distribution ranges, attain a fairly large size, and have a pendulous habit. Their leaves are linear-(ob)lanceolate, although those of *P. longifolium* are longer (a mean of 52 mm, compared with 29 mm in *P. galeottii*; Wiens 1964) However, they differ in time of anthesis (Fig. 12), *P. galeottii* sharing summer flowering (ca. July to September; Wiens 1964) with members of the conifer-parasite clade (except *P. capitellatum)* and with *P. villosum,* whereas *P. longifolium* flowers from December to February (Wiens 1964). Wiens (1964) placed *P. galeottii* in *Pluriseriales* Engler, a section including species with shorter internodes and smaller leaves than section *Calyculatae.* However, *P. galeottii* often has flattened and twisted stems with dilated nodes, characteristic of section *Calyculatae* (Wiens 1964). Because members of *Pluriseriales* sensu Wiens (*P. galeottii* and *P. villosum* subsp. *coryae)* each are sister species to a member of *Calyculatae* sensu Wiens (*P. longifolium* and *P. scaberrimum,* respectively), *Pluriseriales* and *Calyculatae* are polyphyletic. *Pluriseriales* sensu Wiens and Trelease is paraphyletic also because *Pauciflorae* is nested within it (Fig. 11). Trelease's section *Calyculatae* is monotypic and its sole representative, *P. calyculatum,* was not included in this study.

Phylogenetic Structure Within the Northern Clade

Figure 12 shows a portion of the strict consensus tree, with geographic distribution, morphological characters, flowering period and host range (Wiens 1964; Kuijt 1996) indicated for each member of the northern clade. Several members of the clade occasionally exhibit morphological characters characteristic of species occurring farther south. Specifically, *P. brachystachyum, P. rhipsalinum, P. robinsonii,* and *P. velutinum* occasionally develop cataphylls and have median orientation of the lowermost foliar organs. Fertile cataphylls and indel y are shared by *P. brachystachyum, P. rhipsalinum,* and *P. robinsonii.* Indels g′ and J are shared by *P. brachystachyum, P. rhipsalinum,* and *P. brevifolium.* This suggests that the initial branching event within the northern clade may have involved the divergence of the *P. brachystachyum* clade first, followed by the *P. serotinum* clade with its sister species *P. robinsonii.*

Flowering period suggests a switch from predominant winter (or fall) flowering in members of the *P. brachystachyum* and *P. serotinum* clades and in *P. robinsonii,* to summer flowering in the poorly supported clade (bootstrap value of 53%, decay value of 2; Fig. 10) that includes conifer and oak parasites. Exceptions are the winter-flowering *P. capitellatum* and *P. longifolium.* Calderón de Rzedowski and Rzedowski (1972) report year-round flowering and fruiting for *P. rhipsalinum,* a common feature of species in Central and South America. Host specificity shows a correlation with flowering period, in that most of the winter-flowering taxa form generalist host associations, whereas summer flowering tends to accompany narrow host specificity. *Phoradendron bolleanum* subsp. *bolleanum* is unusual by parasitizing not only Cupressaceae s.l. but also Ericaceae. Conversely, *P. rhipsalinum* is highly host-specific, despite its phylogenetic proximity to a host generalist.

CONCLUSIONS

The molecular sequence data presented in this study supports Kuijt's (1959, 1996) view that cataphyll presence/absence and the orientation of the lowermost foliar organs should not be used to circumscribe two major lineages in *Phoradendron.* However, a record of the variability for these two characters in some species (Kuijt 1996) has here been used to infer additional structure along some of the weakly supported branches of the molecular phylogeny. The role of temperature or day length in influencing cataphyll formation cannot be ruled out, in that acataphyllous species, including *P. californicum,* are typically found at higher latitudes.

A latitudinal influence on host specificity and flowering period is likely, in that almost all tropical species are known to be host generalists with year-round flowering and fruiting. However, within the northern clade, host specificity seems to follow a phylogenetic pattern of distribution.

Interestingly, the host-specific associations among the conifer parasites are almost always associated with vegetative mimicry. Diagnosing leaf mimicry is subjective in that it is more readily recognized in mistletoes growing on hosts with distinctive (scalelike or needlelike or linear) leaf shapes (i.e., conifers) than on hosts having (ob)ovate leaves. Barlow and Wiens (1977) hypothesize that high host specificity is a condition for mimicry. The adaptive role of mimicry is speculative, although camouflage from extant of ancient (vertebrate) herbivores is likely. A preference for mistletoes has been documented for white-tailed deer

feeding on a range of plants including *Phoradendron* spp. (Gallina 1988), and for other vertebrate herbivores (e.g., Choate et al. 1987). Regardless of mimicry, high host specificity is thought to be an advantage in open habitats dominated by one or only a few host species, as is true of many conifer habitats (e.g., pinyon-juniper woodland).

Of the sections delimited by Trelease and Wiens, only *Pauciflorae* sensu Wiens is monophyletic. However, poor resolution at the base of the northern clade discourages the retention of sectional boundaries. Additional evidence confirming the paraphyly of *P. tomentosum, P. villosum,* and *P. bolleanum* needs to precede nomenclatural adjustments.

ACKNOWLEDGMENTS

This paper represents a portion of the author's Ph.D. thesis. Anezka, Molly and Nigel Boyle, Rosa Cerros, Travis Columbus, Oscar Dorado, Dan Nickrent, and Maria-Elena Siqueiros facilitated field work. Specimens were collected and/or DNA samples provided by Travis Columbus, Wolfgang Forstreuter, Dan Nickrent, Eric Roalson, Maria-Elena Siqueiros, and Lena Struwe. Funding was provided by the American Society of Plant Taxonomists, Claremont Graduate University Graduate Student Council, the Goldhamer Award, the Hardman Award for Evolutionary and Conservation Research, the A. W. Mellon Foundation, Sigma Xi, the Scientific Research Society, Southern California Botanists, and the Year-Round Garden Club. Leigh Johnson set up the decay index analysis in PAUP. Curtis Clark, Travis Columbus, Dan Nickrent, and Roy Taylor provided helpful comments on the manuscript, and Elizabeth Friar, Elizabeth Kellogg, Job Kuijt, Mark Porter, Eric Roalson, and Delbert Wiens valuable discussions.

LITERATURE CITED

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

———. 2000. Phylogenetic relationships in Phoradendreae (Viscaceae) inferred from three regions of the nuclear ribosomal cistron. I. Major lineages and paraphyly of *Phoradendron*. *Syst. Bot.* **25**: 349–370.

BARLOW, B. A., AND D. WIENS. 1977. Host-parasite resemblance in Australian mistletoes: the case for cryptic mimicry. *Evolution* **31**: 69–84.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**: 795–803.

BULT, C. J., AND E. A. ZIMMER. 1993. Nuclear ribosomal RNA sequences for inferring tribal relationships within Onagraceae. *Syst. Bot.* **18**: 48–63.

CALDERÓN DE RZEDOWSKI, G., AND J. RZEDOWSKI. 1972. Dos especies nuevas de la familia Loranthaceae del centro de México. *Cact. Suc. Mex.* **17**: 99–104.

CHOATE, J. H., R. H. ANDREWS, AND B. S. BARLOW. 1987. Herbivory and cryptic mimicry in Australian Loranthaceae, pp. 127–135. *In* Proceedings of the 4th International Symposium on Parasitic Flowering Plants. Marburg, Germany.

DONOGHUE, M. J., R. G. OLMSTEAD, J. F. SMITH, AND J. D. PALMER. 1992. Phylogenetic relationships of Dipsacales based on *rbc*L sequences. *Ann. Missouri Bot. Gard.* **79**: 333–345.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* **19**: 11–15.

EFRON, B., E. HALLORAN, AND S. HOLMES. 1996. Bootstrap confidence levels for phylogenetic trees. *Proc. Natl. Acad. Sci. U.S.A* **93**: 13429–13434.

FARRIS, J. S. 1989. The retention index and the rescaled consistency index. *Cladistics* **5**: 417–419.

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

FOSBERG, F. R. 1941. Notes on Mexican plants. *Lloydia* **4**: 274–250.

GALLINA, S. 1988. Importancia del injerto (*Phoradendron* sp.) para el venado. *Southwestern Naturalist* **33**: 21–25.

HILLIS, D. M., AND J. J. BULL. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* **42**: 182–192.

KLUGE, A. G., AND J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* **18**: 1–32.

KUIJT, J. 1959. A study of heterophylly and inflorescence structure in *Dendrophthora* and *Phoradendron* (Loranthaceae). *Acta Bot. Neerl.* **8**: 506–546.

———. 1961. A revision of *Dendrophthora* (Loranthaceae). *Wentia* **6**: 1–145.

———. 1982. The Viscaceae in the southeastern United States. *J. Arnold Arbor.* **63**: 401–410.

———. 1996. Cataphylls and taxonomy in *Phoradendron* and *Dendrophthora* (Viscaceae). *Acta Bot. Neerl.* **45**: 263–277.

———. 1997. *Phoradendron olae* Kuijt, a new species from Mexico pivotal in the taxonomy of the genus, with comments on *P. californicum* Nutt. *Brittonia* **49**: 181–188.

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. *Amer. J. Bot.* **86**: 249–260.

MULLIS, K., F. FALOONA, S. SCHARF, R. SIAKI, G. HORN, AND H. ERLICH. 1986. Specific enzymatic amplification of DNA *in vitro*: The polymerase chain reaction. *Cold Spring Harbor Symp. Quant. Biol.* **51**: 263–273.

NICKRENT, D. L., AND R. J. DUFF. 1996. Molecular studies of parasitic plants using ribosomal RNA, pp. 28–52. *In* M. T. Moreno, J. I. Cubero, D. Berner, D. Joel, L. J. Musselman, and C. Parker [eds.], Advances in Parasitic Plant Research. Junta de Andalucia, Dirección General de Investigación Agraria, Cordoba, Spain.

———, AND C. R. FRANCHINA. 1990. Phylogenetic relationhips of the Santalales and relatives. *J. Molec. Evol.* **31**: 294–301.

———, K. P. SCHUETTE, AND E. M. STARR. 1994. A molecular phylogeny of *Arceuthobium* (Viscaceae) based on nuclear ribosomal internal transcribed spacer sequences. *Amer. J. Bot.* **81**: 1149–1160.

———, AND D. E. SOLTIS. 1995. A comparison of the angiosperm phylogenies from nuclear 18S rDNA and *rbc*L sequences. *Ann. Missouri Bot. Gard.* **82**: 208–234.

———, AND E. M. STARR. 1994. High rates of nucleotide substitution in nuclear small-subunit (18S) rDNA from holoparasitic flowering plants. *J. Molec. Evol.* **39**: 62–70.

REVEAL, J. L., AND M. C. JOHNSTON. 1989. A new combination in *Phoradendron* (Viscaceae). *Taxon* **38**: 107–108.

SANDERSON, J., AND M. J. DONOGHUE. 1989. Patterns of variation in levels of homoplasy. *Evolution* **43**: 1781–1795.

SWOFFORD, D. L. 1998. *PAUP*. Phylogenetic analysis using parsimony (*and other methods)* [computer program]. *Ver. 4.* Sinauer, Sunderland, Massachusetts.

THOMPSON, J. D., D. G. HIGGINS, AND T. J. GIBSON. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence

alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucl. Acids Res.* **22**: 4673–4680.

TRELEASE, W. 1916. The genus *Phoradendron.* A monographic revision. 224 p. University of Illinois Press, Urbana.

UNFRIED, I. AND P. GRUENDLER. 1990. Nucleotide sequence of the 5.8S and 25S rRNA genes and of the internal transcribed spacers from *Arabidopsis thaliana.* Nucl. Acids Res. **18**: 4011.

VASEK, F. 1966. The distribution and taxonomy of three western junipers. *Brittonia* **18**: 350–372.

WHITE, T. J., T. BRUNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal genes for phylogenetics, pp. 315–322. *In* M. A. Innis, D. J. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, California.

WIENS, D. 1964. Revision of the acataphyllous species of *Phoradendron. Brittonia* **16**: 11–54.

———, AND M. DEDECKER. 1972. Rare natural hybridization in *Phoradendron* (Viscaceae). *Madroño* **21**: 395–402.