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The Molecular Systematics of *Rhododendron* (Ericaceae): A Phylogeny Based Upon *RPB2* Gene Sequences

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Abstract. Classification of *Rhododendron* species based on morphology has led to a consensus taxonomy recognizing the major subgenera *Azaleastrum*, *Hymenanthes*, *Pentanthera*, *Rhododendron*, *Tsutsusi*, and three minor ones. To determine whether these subgenera are monophyletic and to infer phylogenetic relationships between *Rhododendron* sections and species, we carried out a cladistic analysis using molecular data, including all groups within the genus. For this purpose, we sequenced a large part of the nuclear gene *RPB2-I,* encoding a major RNA Polymerase II subunit, from 87 species and analyzed the data by maximum parsimony, maximum likelihood, and Bayesian methods. The resulting phylogenies show subgenera *Azaleastrum* and *Pentanthera* to be polyphyletic and group all *Rhododendron* species (except the two in section *Therorhodion*) into three large clades. Based upon these results, modifications in *Rhododendron* classification are proposed, which consolidate minor subgenera and recognize monophyletic subgenera and sections.

More than 90% of the 1,025 *Rhododendron* species described prior to 1996 (Chamberlain et al. 1996) belong to the predominately Asian subgenera *Hymenanthes*, *Rhododendron*, and *Tsutsusi*. The first two of these have many species in the Himalayan-Southwest China region, and the 300 species of section *Vireya* in subgenus *Rhododendron* are distributed mainly through the islands of the Malay Archipelago (Sleumer 1966), extending from their probable origin on the Asian mainland to northern Australia. The geologically recent juxtaposition (< 10 million years ago) of the eastern and western halves of this archipelago (Hall 1998) raises interesting biogeographic questions for future phylogenetic study of *Vireya* species, as does the Himalayan orogeny (Irving and Hebda 1993) for *Hymenanthes* and *Rhododendron* species of the Sino-Himalayan area. In addition to these species-rich areas, subgenera *Rhododendron* and *Hymenanthes* and section *Pentanthera* are represented in the montaine flora of eastern and western North America and western Eurasia. Rhododendrons of subgenus *Tsutsusi* have a mainly east Asian maritime distribution (Japan, Korea, Taiwan, and east China) with no species in either western Eurasia or North America.

Systematic studies that encompassed all sections and subgenera of *Rhododendron* were initiated by Sleumer (1949) who proposed a comprehensive system of *Rhododendron* classification in the form of a key to the subgenera and sections (Table 1). Subsequently, the conclusions of a number of more narrowly focused morphological taxonomic studies (Sleumer 1966; Cullen 1980; Chamberlain 1982; Philipson and Philipson 1986; Judd and Kron 1995) were incorporated into an alternative *Rhododendron* classification (Table 1; Chamberlain et al. 1996). This taxonomic system is now generally accepted by *Rhododendron* specialists (Cox and Cox 1997) because it embodies the findings of substantially all morphology-based *Rhododendron* systematic studies since 1980.

Significant differences between the Sleumer (1949, 1980) and Chamberlain et al. (1996) taxonomic systems concern subgenus *Therorhodion*, which Sleumer placed outside the genus *Rhododendron*, and placement of the four species of section *Sciadorhodion* (Table 1). Based on studies by Judd and Kron (1995), Chamberlain et al. (1996) assigned these species to subgenus *Pentanthera*, while Sleumer (see discussion and Table 3) merged them with section *Brachycalyx* in subgenus *Anthodendron*, equivalent to subgenus *Tsutsusi* (Chamberlain and Rae 1990). An interesting feature of Sleumer's taxonomic key is the proximity of the deciduous section *Pentanthera* to the evergreen subgenus *Hymenanthes*. These taxa both lack lepidote scales and, for both, the new leafy shoots emerge from the axils of shoots from the previous year's growth (Table 1). Lepidote scales, unique to subgenus *Rhododendron*, are modified hairs on both leaf surfaces that consist of a flat polygonal scale attached by a stalk. Scale shape, color, size, spacing, and stalk length are all useful characters for designating species (Cullen 1980). The leaves of rhododendrons in subgenus *Hymenanthes* are generally thick and have, in many species, a thick coating of fuzzy hairs (indumentum) on the lower surface (Cox and Cox 1997).

In subgenus *Pentanthera*, the Chamberlain et al. (1996) classification system includes the major section *Pentanthera*, comprising 15 species from the southeastern United States plus three from other regions: section *Sciadorhodion* and the smaller sections *Rhodora* (2 spp., North America) and *Viscidula* (1 sp., Japan). Other than having deciduous leaves covered in hairs and terminal rather than axillary inflorescences, few morphological attributes link these four sections together (Cox and Cox 1997).

TABLE 1. *Rhododendron* subgenera and sections proposed by Sleumer (1949) and by Chamberlain et al. (1996). Authorities for taxonomic names in the Chamberlain et al. (1996) classification system are in Appendix 1.

Character	Sleumer (1949)	Chamberlain et al. (1996)	No. species	No. sampled
I. Inflorescence buds terminal				
A. Presence of leaf scales	subg. *Lepidorrhodium* Koehne	subg. *Rhododendron*		
	sect. *Pogonanthum* G. Don	sect. *Pogonanthum*	13	3
	sect. *Lepipherum* G. Don	sect. *Rhododendron*	149	28
	sect. *Vireya* Copel.f.	sect. *Vireya*	300	9
B. Absence of leaf scales				
1. New leafy shoots from axils of buds of last year's shoots				
a. Leaves evergreen	subg. *Eurhododendron* Maxim.	subg. *Hymenanthes*		
		sect. *Pontica*	224	21
b. Leaves deciduous	subg. *Pseudanthodendron* Sleumer	subg. *Pentanthera*		
	sect. *Pentanthera* G. Don	sect. *Pentanthera*	15	5
	sect. *Rhodora* G. Don	sect. *Rhodora*	2	2
	sect. *Viscidula* Matsum. & Nakai	sect. *Viscidula*	1	1
2. New foliage shoots from axils of lowest scaly-leaves	subg. *Anthodendron* Rehder & Wilson			
	sect. *Brachycalyx* Sweet	sect. *Sciadorhodion*	4	2
		subg. *Tsutsusi*		
	sect. *Brachycalyx* Sweet	sect. *Brachycalyx*	15	3
	sect. *Tsutsusi* Sweet	sect. *Tsutsusi*	65	2
II. Inflorescence buds lateral				
A. Leaves evergreen	subg. *Azaleastrum* Planch.	subg. *Azaleastrum*		
	sect. *Euazaleastrum* Sleumer	sect. *Azaleastrum*	5	2
	sect. *Choniastrum* Franch.	sect. *Choniastrum*	11	3
B. Leaves deciduous	sect. *Candidastrum* Sleumer	subg. *Candidastrum*	1	1
	sect. *Mumeazalea* Sleumer	subg. *Mumeazalea*	1	1
	(outside *Rhododendron*)	subg. *Therorhodion*	2	1

Historically, the most taxonomically problematic rhododendrons have been the subgenera *Azaleastrum*, *Mumeazalea*, and *Candidastrum* (Table 1). Both classification systems place sections *Azaleastrum* and *Choniastrum*, which share the lateral inflorescence character, in subgenus *Azaleastrum* even though they differ consistently in number of stamens (5 vs. 10) and other characters (Philipson and Philipson 1986). Because of distinctive floral and seed characteristics, the deciduous taxa *R. semibarbatum* Maxim. (Japan) and *R. albiflorum* Hook.f. (North America), were placed, respectively, in separate monotypic subgenera *Mumeazalea* and *Candidastrum*.

A broad-scale cladistic analysis of *Rhododendron* was carried out by Kron and Judd (1990) using 14 leaf and floral characters. They concluded that, for *Rhododendron* to be monophyletic, species from the related genera *Ledum* L. and *Menziesia* Smith must be included. Moreover, their cladistic analysis showed subgenus *Therorhodion* to be sister to all other rhododendrons. Molecular data, both in this paper and elsewhere (Kron 1997; Kurashige et al. 2001) support these conclusions.

Two studies of molecular systematics across the genus *Rhododendron* have previously been published. The first used sequences from the chloroplast *matK* and *trnk* genes (Kurashige et al. 2001) and the second used nuclear ITS sequences (Gao et al. 2002). As detailed below, several of the contradictions between morphology-based *Rhododendron* taxonomy and the *RPB2-I* phylogeny determined in this paper are also evident in the plastid and ITS phylogenies, although those publications did not emphasize the contradictions.

RNA Polymerase II is the multisubunit enzyme that transcribes pre-mRNA from nuclear genes (Weinmann et al. 1974). The *RPB2-I* gene of *Rhododendron* and of all *Ericales* studied encodes one of two genes for the 140kd second-largest RNA Polymerase II subunit. Between the *RPB2-I* and *RPB2-d* paralogs, there is 80% exon sequence similarity. Although the 24 intron sequences occupy perfectly homologous positions in the coding sequences of the two genes, they are totally non-alignable (Oxelman et al. 2004). Because these two genes have evolved as separate lineages, they differ in exon sequences sufficiently to be separately amplified by the polymerase chain reaction (PCR). Of the RPB2-I DNA analyzed in this paper, approximately 80% consists of intron sequences which, largely lacking functional constraints, evolve rapidly, facilitating resolution of closely-related taxa.

In this investigation, we recovered, sequenced and computationally analyzed sequences of *RPB2-I* from 87 *Rhododendron* species (Appendix 1) in order to address several related issues. First, we set out to test whether the morphology-based sections and subgenera of *Rho-*

dodendron proposed by the taxonomic systems of Sleumer (1949, 1980) and Chamberlain et al. (1996) are monophyletic. A second objective was to resolve, irrespective of these and other taxonomic proposals, the relationships between all *Rhododendron* sections, including subsection *Ledum* and genus *Menziesia* (Kron and Judd, 1990). The monophyletic groups so identified, together with morphological information, provide the basis for a revised classification system for *Rhododendron*, which we describe briefly.

Materials and Methods

Taxon Sampling, Voucher Specimens, and Sequence Data. Representative taxa were chosen from all sections and subgenera of *Rhododendron* (Table 1; Appendix 1). Except for species native to Washington, all samples were obtained from the Rhododendron Species Foundation Botanical Garden (RSF), Federal Way, Washington, USA. RSF accessions are grown from wild-collected seed. Also listed in Appendix 1 are the RSF accession numbers for all species, herbarium accession numbers for vouchers deposited in the University of Washington Herbarium (WTU), and GenBank accession numbers.

DNA Extraction, Amplification, and Sequencing. Total DNA was extracted from young leaves or floral tissue using the DNeasy Plant Minikit (Qiagen, Valencia, California, USA) or a modified CTAB method (Doyle and Doyle 1987). Target regions were PCR amplified in 30 μl with 10–20 ng of genomic DNA, 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 100 μM dNTPs, 2.5 pmols of each primer, and 1.5 units Taq polymerase (Invitrogen, Carlsbad, California, USA). Reactions were carried out on a PTC-100 Programmable Thermal Controller (MJ Research Inc., Waltham, Massachusetts, USA) under the following conditions: (1) initial denaturation at 94°C for 4 min; (2) 35 cycles of denaturation at 94°C for 45 sec, annealing at 57°C for 45 sec, slope rate of 1°C per 5 sec, and extension at 72°C for 45 sec− 1 min 20 sec; (3) final extension at 72°C for 10 min. Most PCR fragments were sequenced directly after purification with the QIAquick PCR Purification Kit (Qiagen). Multiple or weak amplification products were cloned using the TOPO TA Cloning Kit (Invitrogen). Three clones were subsequently PCR screened, purified as described above, and sequenced in both directions using the BigDye Terminator v1.1 Cycle Sequencing Kit (PE Applied Biosystem, Foster City, California, USA). Sequence analysis was performed on ABI 3700 or ABI 3730XL (PE Applied Biosystem) automated sequencers.

Sequence Alignment and Phylogenetic Analysis. Sequences were assembled into contigs and edited using Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Contigs were subsequently aligned under the default cost matrix using CLUSTALX (Thompson et al. 1997) and modified by hand as needed using Se-Al v2.0 (Rambaut 2003). Manual adjustments were performed to minimize the number of gaps. All indels were coded as missing data for parsimony, maximum likelihood, and Bayesian analyses. All sequences are complete for the gray regions in Fig. 1 except for *R. micranthum*, for which intron 23 was not sequenced, therefore these positions in the alignment were coded as missing data. Ambiguous regions composed of homopolymer sequences were excluded from all analyses (aligned positions: 652–668, 979–982, 2,724–2,731, 2,738–2,743, 3,021–3,032, 3,085–3,101, 3,892–3,907, 6,534–6,537, 7,225–7,228). Our complete alignment and phylogenetic trees can be located in TreeBASE (study accession: S1244; matrix accession: M2277).

Phylogenetic analyses were conducted using PAUP* 4.0b10 (Swofford 2001) with *Empetrum nigrum* as an outgroup (Kron 1997). Trees were constructed using heuristic searches with all characters equally weighted, tree bisection and reconnection (TBR) branch swapping, and a modified search algorithm (DeBry and Olmstead 2000). We retained two trees per replicate across 500 random addition replicates and condensed all most parsimonious trees into a strict consensus tree. The heuristic search was repeated, using the strict consensus tree as an inverse constraint, until search efforts did not discover additional optimal trees. Non-parametric bootstrap values (Felsenstein 1985) were determined from 1,000 replicates of the heuristic search option with 50 random addition replicates, retaining a single tree per replicate and employing TBR branch swapping.

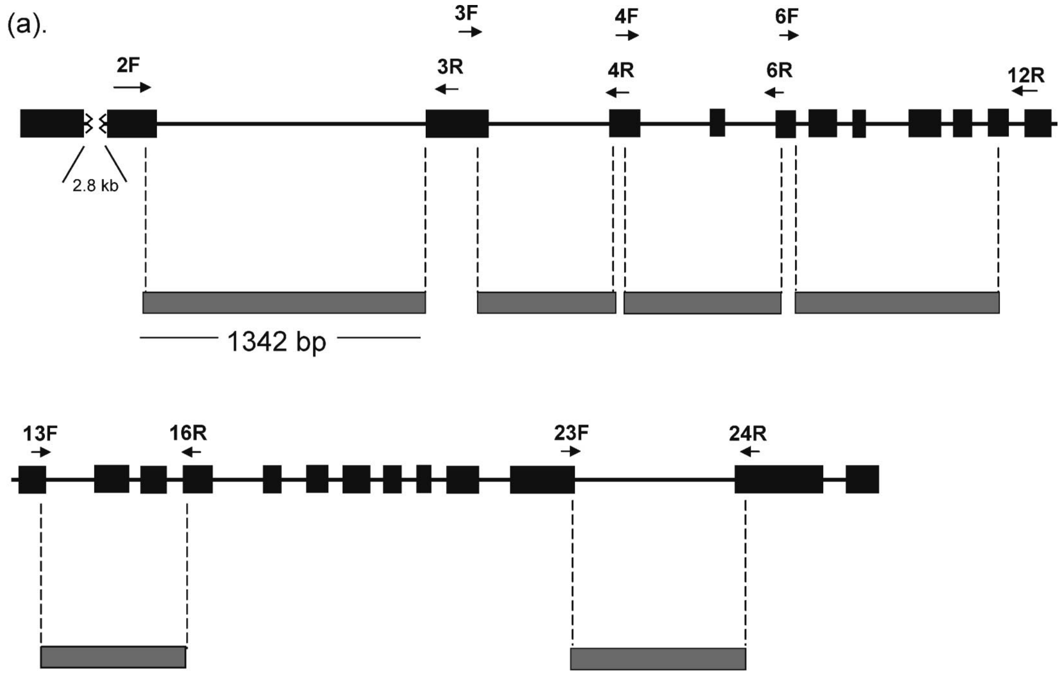
Maximum likelihood analyses were also conducted using PAUP* 4.0b10 under an HKY85 plus gamma distributed rates among sites (G) model of DNA sequence evolution. A hierarchical likelihood ratio test, as performed in ModelTest 3.04, was used to identify the most optimal model for the data (Posada and Crandall 2000). Trees were constructed using a heuristic search with TBR and simple addition of taxa.

Bayesian analyses were conducted with Mr. Bayes 3.0b4 using the best-fit model of sequence evolution from the maximum likelihood analysis (Huelsenbeck and Ronquist 2003). Analyses were run for 1×10^6 generations with the default priors and five chains sampled at every 100th generation from five random starting points. A burn-in of 10% (n = 5,000) of the resulting 50,000 trees was discarded; to obtain posterior probabilities for each node, the remaining 45,000 trees were imported into PAUP* 4.0b10 and condensed into a strict consensus tree. For comparison to non-parametric bootstrap support values, posterior probabilities are reported on a percent scale (posterior probability × 100).

Results

DNA sequence data for *Rhododendron* species were obtained from six regions of the *RPB2-I* gene; three are sequences of large introns and three are contiguous gene sequences containing both introns and exons (Fig. 1). Together, the six regions account for 5.2 of the total 12 kb of *RPB2-I* sequence present in *R. macrophyllum*, the reference taxon. In the strict consensus phylogeny we inferred from these data (number of trees = 995, tree length = 2,691 steps, CI = 0.762, RI = 0.836), all *Rhododendron* species except *R. camtschaticum* fall into three large clades, designated A, B, and C (Fig. 2), each with 100% bootstrap support and posterior probability. With equally strong support, monophyletic groups comprising the major subgenera *Rhododendron* and *Hymenanthes* are nested, respectively within clades A and B. Vertical bar symbols on major branches of the MP phylogeny (Fig. 2) show the phylogenetic positions of indels that provide additional support for the adjacent node. The positions of these are, for clade A (aligned position: 6,547), for clade B (aligned positions: indel 1 = 627–1,017; indel 2 = 1,125–1,132; indel 3 = 3,770), and for clade C (aligned positions: indel 1 = 1,336–1,341; indel 2 = 5,503–5,657; indel 3 = 7,267–7,273). We attribute the high degree of phylogenetic resolution and statistical support to the large aggregate size of the DNA regions sequenced and also to the substantial and well-distributed phylogenetic signal in *RPB2-I* sequences, resulting in 767 parsimony-informative sites.

Major features of the maximum likelihood phylogeny (Fig. 3; −ln L = 26,935.221) are the same as for parsimony analysis, with resolution of a few additional branches that were weakly supported in the parsimony tree. The longest branches (Fig. 3) are in clade C and in the *Pentanthera* azaleas of clade B. The very short



(b).

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2F  GAA CCT GAC CTA CTC ATC TCC ATT
3R  TCG GAT ACA TAA GCA TAT TTG TTG G
3F  CCA ACA AAT ATG CTT ATG TAT CCG AG
4F  CCG TGC ACT GGG ATT TGT TGC AGA
4R  TCT GCA ACA AAT CCC AGT GCA CGG
6F  CAC GGG GGA ATA TTG TGA AAC T
6R  AGT TTC ACA ATA TTC CCC CGT G
12R CTC CTC CAG AAA CTC CAA TAT G
13F TTC ATC GTA ACC CTG AGC TC
16R TGG GCA GAG ACG TGC AGA TG
23F AAT TGA GGG CAT CTG TCC AGA CAT C
24R TCG TAT AAG TCA GCG GAC GCC CTG
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FIG. 1. *RPB2-I* gene structure, PCR amplification, and sequencing. (a) Intron (thin line) and exon (solid bar) lengths are shown to scale, with the exception of intron one. The scale below intron two applies elsewhere. Regions sequenced are in gray. The approximate position and polarity of each PCR primer is shown by an arrow. (b) Sequences of the PCR primers used.

branch at the node common to clades A and B is consistent with its relatively low bootstrap support (Fig. 2).

In the *RPB2-I* phylogeny (Figs 2, 3), *R. camtschaticum* is sister to all remaining rhododendrons, including the *Menziesia* species. Subgenus *Rhododendron*, encompassing sections *Rhododendron*, *Pogonanthum*, and *Vireya*, is monophyletic with 100% bootstrap support and posterior probability. Both subgenus *Azaleastrum* (Philipson and Philipson 1986) and subgenus *Pentanthera* (Kron 1993; Judd and Kron 1995) are polyphyletic. The three strongly supported clades in the *RPB2-I* phylogeny group *Rhododendron* sections differently than either of the morphology-based classification systems (Table 1) would have predicted.

DISCUSSION

Within existing subgenus *Azaleastrum* (Table 1), section *Choniastrum* is sister to subgenus *Rhododendron* in clade A, while section *Azaleastrum* occupies a position within clade C. From the former subgenus *Pentanthera*, section *Sciadorhodion*, section *Viscidula* and *R. vaseyi* are also found within clade C, while section *Pentanthera*,

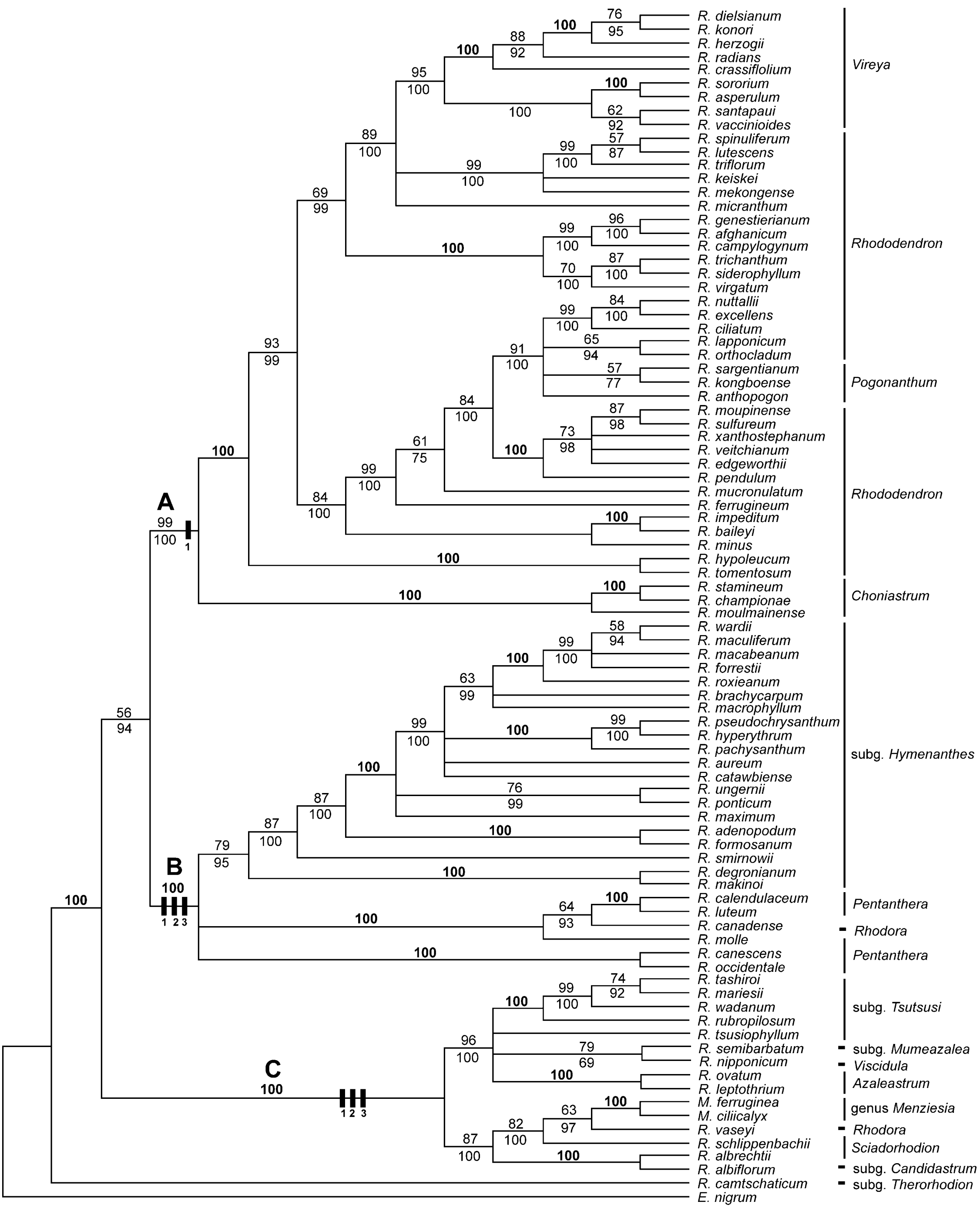

FIG. 2. Maximum parsimony strict consensus tree based upon *RPB2-I* gene sequences. Numbers above the branches give the bootstrap support for 1,000 replicates. Only those bootstrap values >50% are shown. Bayesian posterior probabilities (× 100) are shown below the branches or, when equal to bootstrap values, as a single number (bolded) above the branch. Taxon names on the extreme right refer to sections (Table 1) unless otherwise indicated. The vertical bars represent unambiguous synapomorphic indels.

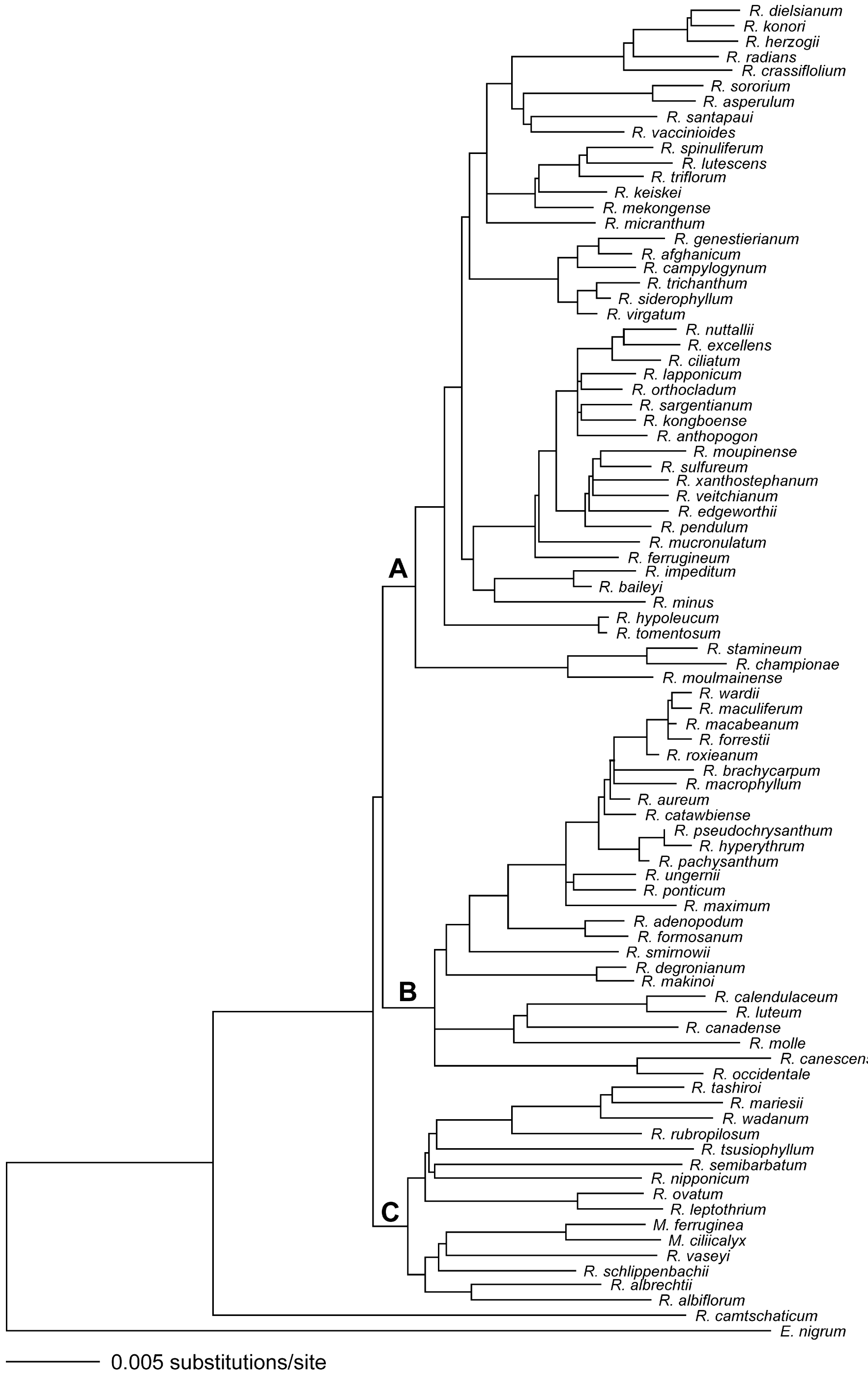

FIG. 3. Maximum likelihood phylogram. The topology is identical to the parsimony-based topology in Fig. 2.

along with *R. canadense* (in section *Rhodora*) falls within an expanded *Hymenanthes* clade B. To summarize the major differences between the relationships inferred from Figs. 2 and 3 and the existing *Rhododendron* taxonomic systems (Table 1), subgenera *Azaleastrum* and *Pentanthera* need to be conceptually disassembled and the clades containing subgenera *Rhododendron*, *Hymenanthes* and *Tsutsusi* correspondingly expanded.

Phylogenies Inferred from Various Data Sets. The *RPB2-I* phylogeny inferred for *Rhododendron* (Fig. 2) shares certain features with *Rhododendron* phylogenies determined using other genes (Kurashige et al. 2001;

Gao et al. 2002) but there are several significant differences. As regards the circumscription of *Rhododendron*, these results agree with and augment the conclusions from morphological and molecular analyses (Kron and Judd 1990; Kurashige et al. 2001; Gao et al. 2002), that the species of *Menziesia* and *Ledum* should be included within genus *Rhododendron*. In our phylogeny (Fig. 2), the two *Menziesia* species are in a clade entirely composed of deciduous taxa (*R. albiflorum*, section *Sciadorhodion*, and *R. vaseyi*) and the *Ledum* species (*R. tomentosum* and *R. hypoleucum*) fall within subgenus *Rhododendron*. The *RPB2-I* phylogeny places *R. camtschaticum*, representing subgenus *Therorhodion*, sister to all taxa of the expanded *Rhododendron* clade as do the analyses based on plastid (Kurashige et al. 2001) and ITS (Gao et al. 2002) sequences. In both the *RPB2-I* and plastid phylogenies, there is weak support for a sister relationship between clades containing, respectively, subgenus *Rhododendron* and subgenus *Hymenanthes* plus section *Pentanthera*. Sister to the preceding assemblage is a clade (clade C in Fig. 2) consisting of subgenus *Tsutsusi*, section *Azaleastrum* and a group of deciduous taxa that includes *Menziesia*.

The *RPB2-I* phylogeny, like those for ITS (Gao et al. 2002) and *matK* + *trnK* (Kurashige et al. 2001), strongly supports monophyletic subgenus *Rhododendron* and monophyletic subgenus *Hymenanthes*. Both *RPB2-I* (Fig. 2) and ITS (Gao et al. 2002) placed *Ledum* within the lepidote clade with 99–100% bootstrap support and posterior probability, while the plastid DNA phylogeny (Kurashige et al. 2001) has *Ledum* (*R. tomentosum*) plus *R. albrechtii* as a weakly supported (20%) sister group to subgenera *Rhododendron* and *Hymenanthes*.

A point of substantial agreement between the *RPB2-I* and plastid DNA analyses concerns the relationship between species of subgenus *Hymenanthes* and those of section *Pentanthera* (deciduous azaleas, mainly from North America). In Fig. 2, strongly supported clade B contains all species of section *Pentanthera* and subgenus *Hymenanthes*, while in the plastid DNA phylogeny representatives of these taxa make up a clade with 69% bootstrap support. In the ITS phylogeny, the positions of the section *Pentanthera* and subgenus *Hymenanthes* clades are unresolved.

Comparison with Morphology-based Systematics and Cladistics. Derivation of the two systems of *Rhododendron* taxonomy based upon morphology (Table 1) came about in different ways. The taxonomic key of Sleumer (1949), which predates cladistics, reflects many detailed observations across the entire genus, made both in the field and in herbaria, that were processed and interpreted by a single experienced botanist (Sleumer 1980). On the other hand, the taxonomic system outlined by Chamberlain et al. (1996) is, in effect, a summation of the work of many individual systematists (Sleumer 1966; Cullen 1982; Chamberlain 1984; Philipson and Philipson 1986; Chamberlain and Rae 1992; Kron 1993; Judd and Kron 1995). In the research supporting this system, limited attention was given to critically testing the hypothesized relationships between sections and subgenera and greater effort was devoted to placement of species in sections and subsections (Chamberlain 1996; Cox and Cox 1997). The only assessment of higher order relationships made using modern phylogenetic methods was the cladistic study of Kron and Judd (1990). Because just 14 characters were used in their analysis and several of the characters exhibited homoplasy, four cladograms were equally parsimonious. Therefore, this cladistic study neither strongly supported nor refuted the taxonomic system presented in Chamberlain et al. (1996).

The *Rhododendron* phylogenies inferred from molecular data differ greatly from predictions based upon the *Rhododendron* classification system of Chamberlain et al. (1996) with regard to subgenus *Pentanthera* (Judd and Kron 1995). Both the plastid DNA analysis (Kurashige et al. 2001) and the present study place section *Pentanthera* within the same clade as *Hymenanthes* (Fig. 2). *Rhodora*, *Sciadorhodion*, and *Viscidula*, the other sections of subgenus *Pentanthera* (Judd and Kron 1995), are deciduous azaleas for which the classification has been exceptionally labile over time. In our study, these taxa are represented by the species *R. canadense*, *R. vaseyi*, *R. nipponicum*, *R. albrechtii*, and *R. schlippenbachii* (Table 2). The monograph of Wilson and Rehder (1921) placed *R. schlippenbachii* and *R. quinquefolium* Bisset & S. Moore in section *Sciadorhodion*, together with the cohesive group *R. farrerae* Tate, *R. reticulatum* D. Don, *R. mariesii*, and *R. weyrichii* Maxim. on the basis of two shared traits: flowers and leaves both developing from the same terminal bud and leaves occurring in whorls of 3 to 5 at the ends of branchlets. The basis for this grouping was preserved in the taxonomic system of Sleumer (1949), who renamed the section *Brachycalyx*, retaining it within subgenus *Anthodendron*, while moving sections *Pentanthera*, *Rhodora*, and *Viscidula* to a new subgenus, *Pseudoanthodendron* (Table 2). Subsequently, Philipson (1980) proposed that *R. schlippenbachii* and *R. quinquefolium* be removed from *Brachycalyx* and combined with *R. albrechtii* and *R. pentaphyllum* Maxim. in section *Sciadorhodion* of subgenus *Pentanthera*.

The cladistic analysis of morphological characters by Judd and Kron (1995) bore directly on the two contrasting views of deciduous azalea classification (Table 2) and seemed to support Philipson's (1980) proposal for subgenus *Pentanthera*, comprising sections *Pentanthera*, *Rhodora*, *Sciadorhodion*, and *Viscidula*. Judd and Kron's (1995) analysis, however, included no taxa from the *R. farrerae*-*R. reticulatum* segment of *Sciadorhodion* (sensu Wilson) and used *Menziesia* as an outgroup. For

TABLE 2. Deciduous azalea species sampled in this study and their designations by various authors.

	Clade (Fig. 2)	Wilson and Rehder (1921)		Sleumer (1949, 1980)		Philipson (1980), Chamberlain et al. (1996)	
		Subgenus	Section	Subgenus	Section	Subgenus	Section
R. luteum (type) plus four additional spp.	B	*Anthodendron*	*Pentanthera*	*Pseudoanthodendron*	*Pentanthera*	*Pentanthera*	*Pentanthera*
R. canadense	B	*Anthodendron*	*Rhodora*	*Pseudoanthodendron*	*Rhodora*	*Pentanthera*	*Rhodora*
R. vaseyi	C	*Anthodendron*	*Rhodora*	*Pseudoanthodendron*	*Rhodora*	*Pentanthera*	*Rhodora*
R. nipponicum	C	*Anthodendron*	*Rhodora*	*Pseudoanthodendron*	*Viscidula*	*Pentanthera*	*Viscidula*
R. albrechtii	C	*Anthodendron*	*Rhodora*	*Anthodendron*	*Brachycalyx*	*Pentanthera*	*Sciadorhodion*
R. schlippenbachii	C	*Anthodendron*	*Sciadorhodion*	*Anthodendron*	*Brachycalyx*	*Pentanthera*	*Sciadorhodion*
R. mariesii (farrerae alliance)	C	*Anthodendron*	*Sciadorhodion*	*Anthodendron*	*Brachycalyx*	*Tsutsusi*	*Brachycalyx*
R. wadanum (reticulatum alliance)	C	*Anthodendron*	*Sciadorhodion*	*Anthodendron*	*Brachycalyx*	*Tsutsusi*	*Brachycalyx*

these reasons, its support for subgenus *Pentanthera* is not convincing. The molecular phylogenies (Fig. 2; Kurashige et al. 2001) clearly show subgenus *Pentanthera* to be polyphyletic, with section *Pentanthera* plus *R. canadense* in clade B within or sister to subgenus *Hymenanthes*, while the remaining taxa of subgenus *Pentanthera* occupy various positions within clade C, together with genus *Menziesia* and sections *Brachycalyx* and *Tsutsusi*.

One consequence of the polyphyly of subgenus *Pentanthera* is the emergence of a strongly supported new grouping: clade B, consisting of subgenus *Hymenanthes* (*Eurhododendron*) together with section *Pentanthera*. The affinity of these two taxa was foreshadowed in Sleumer's taxonomic key (Table 1). His subgenera *Eurhododendron* and *Pseudoanthodendron* uniquely share two characters (Table 1; Sleumer 1949): absence of lepidote scales on the leaves and prolepsis, the emergence of new leafy shoots from the axils of leaves of last year's shoots. The latter character, which also applies to subgenus *Rhododendron* but not to clade C, is therefore a synapomorphy (Fig. 2; Sleumer 1949). The past controversy (Table 2) regarding the positions of *R. albrechtii*, *R. pentaphyllum*, *R. quinquefolium*, and *R. schlippenbachii* is also resolved by the molecular data (Fig. 2) in accord with the taxonomies of Sleumer (1949) and Rehder and Wilson (1921). Their placement of these four species together with section *Tsutsusi* in subgenus *Anthodendron* is consistent with the positions of *R. schlippenbachii* and *R. albrechtii* in clade C in the *RPB2-I* phylogeny (Fig. 2). For the species *R. vaseyi* and *R. nipponicum* (Table 2), the molecular phylogeny of Fig. 2 is inconsistent with all three taxonomic treatments (Table 2). Both occupy positions in clade C, rather than, as these systems would predict, ones close to the species of section *Pentanthera*.

Morphological Traits and Geographic Distribution. *Rhododendron* species in sections *Azaleastrum*, *Choniastrum*, *Candidastrum*, and *Mumeazalea* (Table 1) have inflorescence buds that are lateral, rather than terminal. While Sleumer (1949) placed all of these in subgenus *Azaleastrum*, Philipson and Philipson (1968) proposed that section *Choniastrum* be taxonomically separated from others in this group, based on petiole and nodal structure. In a later paper, Philipson and Philipson (1986) described subgenus *Azaleastrum*, containing both sections *Azaleastrum* and *Choniastrum*, and this revised viewpoint has been incorporated into the classification system of Chamberlain et al. (1996). However, subgenus *Azaleastrum* is polyphyletic (Fig. 2; Kurashige et al. 2001), implying that the change from terminal to lateral inflorescence occurred independently in sections *Choniastrum* and *Azaleastrum*.

Clades B and C have analogous composition in two respects. Each contains an evergreen subgenus that is largely or completely Asian (*Hymenanthes* and *Tsutsusi*

TABLE 3. Proposed changes in *Rhododendron* classification.

New designation		
Subgenus	Section	Present names of constituent taxa (Table 1; Chamberlain et al. 1996)
Choniastrum (Franchet) Drude		sect. *Choniastrum*
Hymenanthes K. Koch		
	Ponticum G. Don	sect. *Ponticum*
	Pentanthera G. Don	sect. *Pentanthera* & *R. canadense*
Azaleastrum Planch.		
	Tsutsusi Sweet	subg. *Tsutsusi*, subg. *Mumeazalea*, sect. *Viscidula*, & sect. *Azaleastrum*
	Sciadorhodion Rehder & Wilson	*Menziesia*, subg. *Candidstrum*, sect. *Sciadorhodion*, & *R. vaseyi*

respectively), as well as a deciduous section (*Pentanthera*) or species assemblage (*Menziesia*, *R. albiflorum*, *R. vaseyi*, section *Sciadorhodion*) that is wholly or substantially North American.

Morphological correlates for the position of the three *Choniastrum* species in clade A are difficult to find since, unlike other taxa in this clade, their leaves lack lepidote scales. Several species in section *Choniastrum* do, however, have bristles (setose hairs) on the leaves (Cox and Cox 1999). According to the formal scheme proposed by Seithe (1980), who made comprehensive studies of leaf scales, hairs, and glands throughout *Rhododendron*, bristles are homologs of lepidote scales and glands.

Classification. The results of this investigation clarify the phylogeny of *Rhododendron* and indicate that several changes in the infrageneric systematics of *Rhododendron* are warranted. Based upon the molecular data that we and others have obtained, a revised taxonomic system is proposed (Table 3). Because of minor differences between the *RPB2* and plastid phylogenies (Fig. 2; Kurashige et al. 2001), no change is proposed at this time in the sectional designations within subgenus *Rhododendron*, even though both phylogenies show section *Rhododendron* to be paraphyletic. For taxa outside of subgenus *Rhododendron*, our classification eliminates three subgenera and two sections that are present in the taxonomic system of Chamberlain et al. (Table 1). Inclusion of section *Pentanthera* within subgenus *Hymenanthes* reflects the strong support for clade B (Fig. 2). Sections *Sciadorhodion* and *Viscidula* and *R. vaseyi* (section *Rhodora*) from the discontinued subgenus *Pentanthera* are combined with sections *Azaleastrum*, *Tsutsusi*, and *Brachycalyx* to form an expanded and revised subgenus *Azaleastrum*. Sister groups in this subgenus are the sections *Tsutsusi* (largely evergreen) and *Sciadorhodion* (entirely deciduous). While the *RPB2-I* phylogeny places section *Choniastrum* in clade A, as sister taxon to subgenus *Rhododendron*, *Choniastrum* lacks the attribute most characteristic of this subgenus, lepidote scales on the leaves. For this reason, we propose that *Choniastrum* be considered a separate subgenus.

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LITERATURE CITED

CHAMBERLAIN, D. F. 1982. A revision of *Rhododendron* II. subgenus *Hymenanthes*. *Notes from the Royal Botanic Garden Edinburgh* 39: 209–486.

——— and S. J. RAE. 1990. A revision of *Rhododendron* IV. Subgenus *Tsutsusi*. *Edinburgh Journal of Botany* 47: 89–200.

———, R. HYAM, G. ARGENT, G. FAIRWEATHER, and K. S. WALTER. 1996. *The genus* Rhododendron *its classification and synonymy.* Edinburgh: Royal Botanic Garden.

COX, P. A. and K. N. E. COX. 1997. *The encyclopedia of* Rhododendron *species.* Perth: Glendoick Publishing.

CULLEN, J. 1980. A revision of *Rhododendron* I. subgenus *Hymenanthes*. *Notes from the Royal Botanic Garden Edinburgh* 39: 1–207.

DEBRY, R. and R. G. OLMSTEAD. 2000. A simulation study of reduced tree-search effort in bootstrap resampling analysis. *Systematic Biology* 49: 171–179.

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

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 137–195.

GAO, L. M., D. Z. LI, C. Q. ZHANG, and J. B. YANG. 2002. Infrageneric and sectional relationships in the genus *Rhododendron* (Ericaceae) inferred from ITS sequence data. *Acta Botanica Sinica* 44: 1351–1356.

HALL, R. 1998. The plate tectonics of Cenozoic Southeast Asia and the distribution of land and sea. Pp. 99–132. in *Biogeography and Geological Evolution of SE Asia.* eds. R. Hall and J. D. Holloway. Leiden: Backhuys Publishers.

HUELSENBECK, J. P. and F. RONQUIST. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

IRVING, E. and R. HEBDA. 1993. Concerning the origin and distribution of rhododendrons. *Journal of the American Rhododendron Society* 47: 139–162.

JUDD, W. S. and K. A. KRON. 1995. A revision of *Rhododendron* VI. Subgenus *Pentanthera* (sections *Sciadorhodion*, *Rhodora* and *Viscidula*). *Edinburgh Journal of Botany* 52: 1–54.

KRON, K. A. 1993. A revision of *Rhododendron* section *Pentanthera*. *Edinburgh Journal of Botany* 50: 249–364.

———. 1997. Phylogenetic relationships of *Rhodendroideae* (Ericaceae). *American Journal of Botany* 84: 973–980.

——— and W. S. JUDD. 1990. Phylogenetic relationships within the *Rhodoreae* (*Ericaceae*) with specific comments on the placement of *Ledum*. *Systematic Botany* 15: 57–68.

KURASHIGE, Y., J. I. ETOH, T. HANDA, K. TAKAYANAGI, and T. YUKAWA. 2001. Sectional relationships in the genus *Rhododendron* (*Ericaceae*): evidence from *matK* and *trnK* intron sequences. *Plant Systematics and Evolution* 228: 1–14.

OXELMAN, B., N. YOSHIKAWA, B. L. MCCONAUGHY, J. LUO, A. L. DENTON, and B. D. HALL. 2004. *RPB2* gene phylogeny in flowering plants, with particular emphasis on asterids. *Molecular Phylogenetics and Evolution* 32: 462–479.

PHILIPSON, W. R. 1980. Problems in the classification of the azalea complex. Pp.53–62 in *Contributions toward a classification of* Rhododendron, eds. J. L. Luteyn and M. E. O'Brien. New York: New York Botanical Garden.

——— and M. N. PHILIPSON. 1968. Diverse nodal types in *Rhododendron*. *Journal of the Arnold Arboretum* 49: 193–224.

——— and ———. 1986. A revision of *Rhododendron* III. Subgenera *Azaleastrum*, *Mumeazalea*, *Candidastrum* and *Therorhodion*. *Notes from the Royal Botanical Garden Edinburgh* 44: 1–23.

POSADA, D, and K. A. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

SEITHE, A. 1980. *Rhododendron* hairs and taxonomy. Pp. 89–115. in *Contributions toward a classification of Rhododendron.* eds. J. L. Luteyn and M. E. O'Brien. New York: New York Botanical Garden.

SLEUMER, H. 1949. Ein System der Gattung *Rhododendron* L. *Botanische Jahrbuch Systematic* 74: 511–553.

———. 1966. An account of *Rhododendron* in Malesia. *Flora Malesiana, Series I*, 6: 469–674.

———. 1980. Past and present taxonomic systems of *Rhododendron* based on macromorphological characters. Pp. 19–26 in *Contributions toward a classification of* Rhododendron, eds. J. L. Luteyn and M. E. O'Brien. New York: New York Botanical Garden.

SWOFFORD, D. 2001. PAUP*: Phylogenetic analysis using parsimony (and other methods). Version 4.0b10. Sunderland: Sinauer Associates, Inc.

WEINMANN, R., H. J. RASKAS, and R. G. ROEDER. 1974. Role of DNA-dependent RNA polymerase II and III in transcription of the adenovirus genome late in productive infection. *Proceedings of the National Academy of Sciences USA* 71: 3426–3430.

WILSON, E. H. and A. REHDER. 1921. A monograph of azaleas. *Rhododendron* subgenus *Anthodendron*. Cambridge: Publications of the Arnold Arboretum. no. 9. The University Press.

APPENDIX 1

Accession numbers for rhododendron species, grouped by the taxonomic system of Chamberlain et al. (1996), collected at the Rhododendron Species Foundation Botanical Garden (RSF), giving the collection localities for seeds planted at the RSF, RSF accession number, number of the voucher at the University of Washington Herbarium (WTU), and the GenBank accession numbers are listed as the set of all RPB2-I DNA sequence fragments recovered from each species. Contact information for the RSF is available upon request.

Rhododendron L.

subg. *Azaleastrum* Planch.

sect. *Azaleastrum*—*R. leptothrium* Balf.f. & Forrest, Yunnan Province, China; RSF 66/601, WTU 357224, AY765914–AY765919. *R. ovatum* (Lindl.) Maxim., Hebei Province, China; RSF 82/012, WTU 357235, AY765920–AY765925.

sect. *Choniastrum* Franch.—*R. championae* Hook.f., Guangdong Province, China; RSF79/048, WTU 357225, AY765716–AY765721. *R. moulmainense* Hook.f., Burma; RSF 75/040, WTU 357234, AY765722–AY765727. *R. stamineum* Franch., Royal Botanic Gardens, Kew, UK; RSF 76/380, WTU 357191, AY765728–AY765733.

subg. *Candidastrum* Franch.—*R. albiflorum* Hook., Washington, USA; RSF 187sd97, WTU 357195, AY765974–AY765979.

subg. *Hymenanthes* (Blume) K. Koch

sect. *Ponticum* G. Don—*R. adenopodum* Franch., Sichuan Province, China; RSF 76/142, WTU 357198, AY765842–AY765847. *R. aureum* Georgi, Japan; RSF 82/160, WTU 357232, AY765770–AY765775. *R. brachycarpum* D. Don ex G. Don, South Korea; RSF 96/231, WTU 357233, AY765758–AY765763. *R. catawbiense* Michx., North Carolina, USA; RSF 99/183, WTU 357179, AY765806–AY765811. *R. degronianum* subsp. *degronianum* Carrière, Japan; RSF 65/250, WTU 357231, AY765830–AY765835. *R. formosanum* Hemsl., Taiwan; RSF 73/108, WTU 357214, AY765848–AY765853. *R. forrestii* Balf.f. ex Diels, Yunnan Province, China; RSF 98/205, WTU 357173, AY765746–AY765751. *R. hyperythrum* Hayata, Taiwan; RSF 69/884, WTU 357217, AY765800–AY765805. *R. macabeanum* Watt ex Balf.f., India; RSF 96/016, WTU 357238, AY765740–AY765745. *R. macrophyllum* D. Don ex G. Don, Washington, USA; WTU 351265, WTU 351265, AY765764–AY765769. *R. maculiferum* Franch., UBC Botanical Garden, British Columbia, Canada; RSF 65/253, AY765776–AY765781; WTU 357164,. *R. makinoi* Tagg, Japan; RSF 74/131, WTU 357180, AY765836–AY765841. *R. maximum* L., Virginia, USA; RSF 89/023, WTU 357194, AY765818–AY765823. *R. pachysanthum* Hayata, Taiwan; RSF 76/064, WTU 357258, AY765794–AY765799. *R. ponticum* L., Turkey; RSF 76/411, WTU 357248, AY765812–AY765817. *R. pseudochysanthum* Hayata, Taiwan; RSF 73/410, WTU 357246, AY765788–AY765793. *R. roxieanum* Forrest, Yunnan Province, China; RSF 312sd97, WTU 357228, AY765752–AY765757. *R. smirnowii* Trautv., Turkey; RSF 2003/337, WTU 357196, AY765824–AY765829. *R. ungernii* Trautv., Turkey; RSF 77/358, WTU 357183, AY765782–AY765787. *R. wardii* W. W. Sm., Tibet; RSF 69/096, WTU 357175, AY765734–AY765739.

subg. *Mumeazalea* (Sleumer) W. R. Philipson & M. N. Philipson—*R. semibarbatum* Maxim., Japan; RSF 98/649, WTU 357216, AY765932–AY76937.

subg. *Pentanthera* (G. Don) Pojarkova

sect. *Pentanthera*—*R. calendulaceum* (Michx.) Torr., North Carolina, USA; RSF SEH-1016, AY765866–AY765871. *R. canescens* (Michx.) Sweet, Florida, USA; RSF 76/278, WTU 357189, AY765854–AY765859. *R. luteum* Sweet, Republic of Georgia; RSF 88/065, WTU357241, AY765872–AY765877. *R. molle* (Blume) G. Don, China; RSF 80/091, WTU 357229, AY765878–AY765883. *R. occidentale* (Torr. & A. Gray) A. Gray, California, USA; RSF 77/388, WTU 357230, AY765860–AY765865.

sect. *Rhodora* (L.) G. Don—*R. canadense* (L.) Torr, Nova Scotia, Canada; RSF 307sd95, WTU 357205, AY765884–AY765889. *R. vaseyi* A. Gray, North Carolina, USA; RSF 2000/223, WTU 357188, AY765956–AY765961.

sect. *Sciadorhodion* Rehder & Wilson—*R. albrechtii* Maxim., Japan; RSF 99/105, WTU 357199, AY765968–AY765973. *R. schlippenbachii* Maxim., Korea; RSF 77/364, WTU 357261, AY765962–AY765967.

sect. *Viscidula* Matsum. & Nakai—*R. nipponicum* Matsum., Japan; RSF 76/048, WTU 357244, AY765938–AY765943.

subg. *Rhododendron*

sect. *Pogonanthum* Aitch. & Hemsl.—*R. anthopogon* D. Don, Bhutan; RSF 66/588, WTU 357240, AY765542–AY765547. *R.*

kongboense Hutch., Tibet; RSF 74/078, WTU 357259, AY765536–AY765541. *R. sargentianum* Rehder & E. H.Wilson, Sichuan Province, China; RSF 77/721, WTU 357247, AY765530–AY765535.

sect. *Rhododendron*—*R. afghanicum* Aitch. & Hemsl., Afghanistan; RSF 80/083, WTU 357227, AY765656–AY765661. *R. baileyi* Balf.f., Bhutan; RSF 94/393, WTU 357181, AY765572–AY765577. *R. campylogynum* Franch., Yunnan Province, China; RSF 95/076, WTU 357215, AY765632–AY765637. *R. ciliatum* Hook.f., Sikkim, India; RSF 2000/064, WTU 357237, AY765512–AY765517. *R. edgeworthii* Hook.f., Yunnan Province, China; RSF 98/sd324, WTU 357186, AY765482–AY765487. *R. excellens* Hemsl. & E. H. Wilson, Vietnam; RSF 94/380, WTU 357236, AY765506–AY765511. *R. ferrugineum* L., Austria; RSF 98/752, WTU 357174, AY765554–AY765559. *R. genestierianum* Forrest, Burma; RSF 77/690, WTU 357208, AY765638–AY765643. *R. hypoleucum* (Kom.) Harmaja, Japan; RSF 98/702, WTU 357226, AY765704–AY765709. *R. impeditum* Balf.f. & W. W. Sm., Yunnan Province, China; RSF CCHH #8253, WTU 357219, AY765566–AY765571. *R. keiskei* Miq., Japan; RSF 66/624, WTU 357242, AY765686–AY765691. *R. lapponicum* (L.) Wahlenb., Japan; RSF 78/066, WTU 357176, AY765518–AY765523. *R. lutescens* Franch., Sichuan Province, China; RSF 84/061, WTU 357245, AY765674–AY765679. *R. mekongense* Franch., Yunnan Province, China; RSF 98/sd/441, WTU 357210, AY765698–AY765703. *R. micranthum* Turcz., South Korea; RSF 98/191, WTU 357260, AY772485–AY772489. *R. minus* var. *chapmanii* (A. Gray) W. H. Duncan & Pullen, Florida, USA; RSF 98/173, WTU 357201, AY765560–AY765565. *R. moupinense* Franch., Sichuan Province, China; RSF 79/131, WTU 357243, AY765464–AY765469. *R. mucronulatum* Turcz., South Korea; RSF 76/127, WTU 357184, AY765518–AY765523. *R. nuttallii* Booth, Yunnan Province, China; RSF 2001/315, WTU 357218, AY765500–AY765505. *R. orthocladum* Balf.f. & Forrest, Yunnan Province, China; RSF 83/141, WTU 357207, AY765524–AY765529. *R. pendulum* Hook.f., Bhutan; RSF 93/053, WTU 357203, AY765494–AY765499. *R. siderophyllum* Franch., China; RSF 99/396, WTU 357206, AY765662–AY765667. *R. spinuliferum* Franch., Yunnan Province, China; RSF 84/058, WTU 357197, AY765650–AY765655. *R. sulfureum* Franch., Yunnan Province, China; RSF 2000/113, WTU 357172, AY765488–AY765493. *R. tomentosum* (Stokes) Harmaja, South Siberia, Russia; RSF 99/225, WTU 357165, AY765710–AY765715. *R. trichanthum* Rehder, Sichuan Province, China; RSF 76/059, WTU 357182, AY765644–AY765649. *R. triflorum* subsp. *triflorum* Hook.f., Sikkim, India; RSF 99/278, WTU 357204, AY765680–AY765685. *R. veitchianum* Hook.f., Thailand; RSF 2002/012, WTU 357202, AY765476–AY765481. *R. virgatum* Hook.f., UBC Botanical Gardens, British Columbia, Canada; RSF 65/404, WTU 357220, AY765668–AY765673. *R. xanthostephanum* Merr., Yunnan Province, China; RSF 77/666, WTU357353, AY765470–AY765475.

sect. *Vireya* (Blume) Copel.f.—*R. asperulum* Hutch. & Kingdon–Ward, Yunnan Province, China; RSF SEH1519, WTU 357187, AY765614–AY765619. *R. crassifolium* Stapf, Borneo; RSF 88/055, RSF 73, AY765602–AY765607. *R. dielsianum* Schltr., New Guinea; RSF 83/060, RSF 95, AY765578–AY765583. *R. herzogii* Warb., New Guinea; RSF 89/004, WTU 357185, AY765590–AY765595. *R. konori* Becc., New Guinea; RSF 70/036, WTU 357257, AY765596–AY765601. *R. radians* J. J. Sm., Sulawesi, Indonesia; 97/063, WTU 357163, AY765584–AY765589. *R. santapaui* Sastry et al., India; RSF 98/020, WTU 357211, AY765620–AY765625. *R. sororium* Sleumer, Vietnam; RSF 96/057, WTU 357221, AY765608–AY765613. *R. vaccinioides* Hook.f., Yunnan Province, China; 96/56 BASE No., WTU 357212, AY765626–AY765631.

subg. *Therorhodion* (Maxim.) A. Gray—*R. camtschaticum* Pall., Alaska, USA; RSF 77/080, WTU 357178, AY765980–AY765985.

subg. *Tsutsusi* (Sweet) Pojarkova

sect. *Brachycalyx* Sweet—*R. mariesii* Hemsl. & E. H. Wilson, Taiwan; RSF 76/079, WTU 357222, AY765902–AY765907. *R. wadanum* Makino, Japan; RSF 279sd98, WTU 357190, AY765896–AY765901.

sect. *Tsutsusi*—*R. rubropilosum* Hayata, Taiwan; RSF 96/080, WTU 357223, AY765908–AY765913. *R. tashiroi* Maxim., Japan; RSF 77/227, WTU 357213, AY765890–AY765895. *R. tsusiophyllum* Sugim., Japan; RSF 76/353, WTU 357177, AY765926–AY765931.

Menziesia Smith—*M. ciliicalyx* Maxim., Japan; RSF 94/075, WTU 357162, AY765950–AY765955. *M. ferruginea* Smith, Washington, USA; WTU 357209, AY765944–AY765949.

Empetrum L.—*E. nigrum* L., Washington, USA; RSF 92/5036, WTU 357192, AY765692–AY765697.