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 subg enera *Azaleastrum*, *Hymenanthes*, *Pentanthera*, *Rhododendron*, and *Tsutsusi*. The first two of these have many species in the Himalayan-Southwest China region, and the 300 species of section *Vireya* within 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 *Rhodo- dendron* and *Hymenanthes* and section *Pentanthera* are represented in the montane 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 *Therorhodium*, 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 *Anthodron*; 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).
Historically, the most taxonomically problematic rhododendrons have been the subgenera *Azaleastrum*, *Mumeazalea*, and *Candidastrum* (Table 1). Both classification systems place sections *Azaleastrum* and *Candidastrum*, 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 these 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 MgCl₂, 100 µM dNTPs, 2.5 pmol 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 weakly amplified 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 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. mucranthum, 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° generations with the default priors and five chains sampled at every 100° 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. macrophylum, 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
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 RPB2-1 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. albidiflorum, section Sciadorhodion, and R. valseyi) 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 Azaloeastrum 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 Therorhodion 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. valseyi, 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. farrerac Tate, R. reticulatum D. Don, R. nariesii, 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. farrerac-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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Clade (Fig. 2)</th>
<th>Subgenus</th>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. luteum (type) plus four additional spp.</td>
<td>B</td>
<td>Pentanthera</td>
<td>Endodendron</td>
</tr>
<tr>
<td>R. canadense</td>
<td>B</td>
<td>Pentanthera</td>
<td>Rhodora</td>
</tr>
<tr>
<td>R. vaseyi</td>
<td>C</td>
<td>Pentanthera</td>
<td>Rhodora</td>
</tr>
<tr>
<td>R. nipponicum</td>
<td>C</td>
<td>Pentanthera</td>
<td>Viscidula</td>
</tr>
<tr>
<td>R. albrechtii</td>
<td></td>
<td>Anthodendron</td>
<td>Rhodora</td>
</tr>
<tr>
<td>R. schlippenbachii</td>
<td></td>
<td>Anthodendron</td>
<td>Brachycalyx</td>
</tr>
<tr>
<td>R. mariesii (farrerae alliance)</td>
<td></td>
<td>Chillmay padre</td>
<td>Choniastrum</td>
</tr>
<tr>
<td>R. wadanum</td>
<td></td>
<td>Anthodendron</td>
<td>Brachycalyx</td>
</tr>
</tbody>
</table>

largely or completely Asian (Hymenanthes and Tsutsusi)

in two respects. Each contains an evergreen subgenus that is

Sleumer (1949) placed all of these in subgenus Eurhododendron, 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.

<table>
<thead>
<tr>
<th>Subgenus</th>
<th>Section</th>
<th>Present names of constituent taxa (Table 1; Chamberlain et al. 1996)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choniastrum (Franchet)</td>
<td>sect. Choniastrum</td>
<td></td>
</tr>
<tr>
<td>Drude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hymenanthes K. Koch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ponticum G. Don</td>
<td>sect. Ponticum</td>
<td></td>
</tr>
<tr>
<td>Pentanthera G. Don</td>
<td>sect. Pentanthera &amp; R. canadense</td>
<td></td>
</tr>
<tr>
<td>Azaleastrum Planch.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 sections in species Choniastrum do, however, have bristles (setose hairs) on the leaves (Cox and Cox 1999). According to the formal scheme proposed by Seihe (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 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


IRVING, E. and R. HERDA. 1993. Concerning the origin and distri-

Judd, W. S. and K. A. Kron. 1995. A revision of Rhododendron VI. Subgenus Pentanthera (sections Scandrorhod, Rhodora and Vis-


—— and W.S. Judd. 1990. Phylogenetic relationships within the Rhododendron (Ericaceae) group: evidence from mitK and trnK intron sequenc-


Sleumer, H. 1949. Ein System der Gattung Rhododendron L. Botan-
siche Jahrbuch Systematik 74: 511–553.

——. 1966. An account of Rhododendron in Malesia. Flora Male-
siana, Series I, 6: 469–674.


Swofford, D. 2001. PAUP*: Phylogenetic analysis using parsi-
mony (and other methods). Version 4.0b10. Sunderland: Sin-
auer Associates, Inc.

Weinmann, R., H. J. Rascas, and R.G. Roeder. 1974. Role of DNA-dependent RNA polymerase II and III in transcription of the adeno virus genome late in productive infection. Pro-
cedings of the National Academy of Sciences USA 71: 3436–3440.


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-DNA sequence fragments recovered from each species. Contact information for the RSF is available upon request.

Rhododendron L.

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


R. sargentianum ... 357209, AY765944–AY765949.