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Sectional relationships in the genus *Rhododendron* (Ericaceae): evidence from *mat*K and *trn*K intron sequences

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Abstract. Phylogenetic relationships among all eight subgenera and 12 sections in *Rhododendron* as well as its related genera were inferred from *mat*K and *trn*K intron sequences. The results of this study provided the following insights: (1) *Rhododendron* is paraphyletic because *Menziesia* is nested within *Rhododendron.* (2) Subgenus *Therorhodion* forms a basal lineage of tribe Rhodoreae. (3) Subgenera *Hymenanthes* and *Tsutsusi* are monophyletic. (4) Subgenera *Azaleastrum* and *Pentanthera* are polyphyletic. (5) Subgenus *Rhododendron* is monophyletic, if section *Rhododendron* subsection *Ledum* is excluded.

Key words: Ericaceae, Rhodoreae, *Rhododendron*, molecular systematics, cladistics, *mat*K gene sequences.

The genus *Rhododendron* L. (Ericaceae), which comprises over 1,000 species (Chamberlain et al. 1996), has developed predominantly in East to Southeast Asia. Since Linnaeus (1753) established *Rhododendron*, this large genus has posed systematic problems in terms of infrageneric circumscription and ranks (Don 1834; Planchon 1854; Maximowicz 1870; Wilson and Rehder 1921; Copeland 1943; Sleumer 1949, 1980). Such unstable circumstances are caused by the great diversity of vegetative organs and the relatively uniform floral morphology. Recent revisions of the genus on the basis of Sleumer's systems (1949, 1980) recognized the following eight subgenera: *Rhododendron* (Sleumer 1966, Cullen 1980); *Hymenanthes* (Chamberlain 1982); *Azaleastrum*, *Mumeazalea*, *Candidastrum*, and *Therorhodion* (Philipson and Philipson 1986); *Tsutsusi* (Chamberlain and Rae 1990); and *Pentanthera* (Kron 1993, Judd and Kron 1995). In addition, Kron and Judd (1990) reduced *Ledum* (tribe Rhodoreae), which has been widely recognized at a generic rank (e.g. Stevens 1971, Sleumer 1980, Yamazaki 1989), to a subsectional rank of section *Rhododendron* based on morphological synapomorphies of the two taxa. These results were compiled by Chamberlain et al. (1996). Under this system, tribe Rhodoreae comprises the genera *Rhododendron* and *Menziesia*; in contrast, Stevens (1971) recognized five genera in this tribe: *Rhododendron*, *Menziesia*, *Ledum*, *Tsusiophyllum*, and *Therorhodion.* We will follow the system proposed by Chamberlain et al. (1996) as reference, because this work took account of recent results.

Recent phylogenetic studies of the genus *Rhododendron* using macromolecular data (Kron 1997, Kurashige et al. 1998, Chamberlain and Hyam 1998) have clarified many systematic problems at subgeneric and sectional levels. The results of these studies showed nested positions of the genera *Ledum* and *Menziesia* in *Rhododendron* as well as a sister group relationship of subgenus *Therorhodion* to the remaining members of tribe Rhodoreae. At the subgeneric level of *Rhododendron*, the monophyly of subgenera *Tsutsusi* and *Rhododendron* were indicated. Subgenera *Azaleastrum* and *Pentanthera*, however, were considered to be polyphyletic; and the relationships of these two subgenera in the genus *Rhododendron* have not been clarified.

In our previous comparison of *mat*K sequences (Kurashige et al. 1998), we elucidated a substantial part of the phylogenetic relationships in *Rhododendron*; however, several relationships in this large, elusive genus still remain unresolved. Therefore, in this study, we compare *mat*K and *trn*K intron sequences with increased sampling to investigate infrageneric relationships in *Rhododendron*.

Materials and methods

Within tribe Rhodoreae, we chose 51 species representing all eight subgenera and 12 sections of *Rhododendron* recognized by Chamberlain et al. (1996) and a single species from the remaining genus, *Menziesia*. Among subfamily Rhododendroideae, we chose one species each from the genera *Elliottia* (tribe Cladothamneae), *Loiseleuria* (tribe Phyllodoceae), and *Phyllodoce* (tribe Phyllodoceae). A single species of *Cassiope* (subfamily Vaccioideae tribe Cassiopeae) was selected as the outgroup based on the results of analyses of *mat*K sequences (Kron 1997), 18s rDNA (Kron 1996), and *rbc*L (Kron and Chase 1993). Table 1 shows the materials examined. All voucher specimens were deposited at TNS.

Total DNA was extracted from fresh tissue following the methods of Kobayashi et al. (1998). Sequences were determined by first PCR-amplifying the *mat*K gene and its flanking *trn*K introns from a total DNA extract by use of the primers shown in Fig. 1 and Table 2. Single-stranded DNA for dideoxy sequencing was produced in a second round of amplification using the double-stranded product as a template. Both the forward and reverse strands were sequenced for all taxa.

All parsimony analyses were conducted with PAUP, Phylogenetic Analysis Using Parsimony, Version 3.1 (Swofford 1993). The heuristic search option with 100 random replicates (Maddison 1991) was used to perform Fitch parsimony analyses (Fitch 1971). Branch lengths for trees were calculated by ACCTRAN optimization (Swofford and Maddison 1987). For assessment of the relative robustness for clades found in each Fitch parsimony analysis, the bootstrap method (Felsenstein 1985) was used on 1,000 replicates (saving 100 trees per replicate).

Results

Our *mat*K and its flanking *trn*K sequences provided a matrix of 2430 bp. The complete matrix can be obtained by e-mail from Y. Kurashige (see addresses). A total of 214 nucleotide positions were phylogenetically informative. Of the 22 indels identified from the aligned sequences, nine were informative and unambiguous. These indels were not used to construct the phylogenetic trees shown here, because identical indels may have multiple origins in unrelated taxa (e.g. Golenberg et al. 1993). The phylogenetic analysis resulted in 267 most parsimonious trees, each of 744 steps. These trees had a consistency index (CI) excluding uninformative characters of 0.625 and a retention index (RI) of 0.805. The strict consensus tree and one of the most parsimonious trees are shown in Figs. 2 and 3, respectively.

All of the most parsimonious trees indicated that the genera *Elliottia* (tribe Cladothamneae), *Loiseleuria* (tribe Phyllodoceae), and *Phyllodoce* (tribe Phyllodoceae) were not nested within tribe Rhodoreae. *Rhododendron* subgenus *Therorhodion* diverged early from the rest of the members of tribe Rhodoreae with a bootstrap value of 94% (12 apomorphic mutations). *Menziesia*, the remaining genus in tribe Rhodoreae, fell into the genus *Rhododendron*, and was grouped with a part of subgenus *Pentanthera* section *Sciadorhodion*. In the core of tribe Rhodoreae, two major clades were apparent. The first clade com-

Table 1. Species of *Rhododendron* and its related genera used for *mat*K and *trn*K sequencing. Subdivision of *Rhododendron* is based on Chamberlain et al. (1996). Classification of all other taxa is based on Stevens (1971)

Species	Subgenus	Section	Voucher
Subfamily Rhododendroideae			
Tribe Cladothamneae			
Elliottia paniculata (Siebold & Zucc.) Benth. & Hook.f.			Kurashige 256
Tribe Phyllodoceae			
Loiseleuria procumbens (L.) Desv.			Kurashige & Yukawa 332
Phyllodoce nipponica Makino			Kurashige & Yukawa 339
Tribe Rhodoreae			
Menziesia multiflora Maxim.			Kurashige 356
Rhododendron leptothrium Balf. f. & Forrest	*Azaleastrum* Planch.	*Azaleastrum* (Planch.) Maxim.	Rcherry 194
Rhododendron ovatum (Lindl.) Maxim.	*Azaleastrum* Planch.	*Azaleastrum* (Planch.) Maxim.	Wilson 1391
Rhododendron championae Hook.f.	*Azaleastrum* Planch.	*Choniastrum* Franch.	AKAGI NATURE PARK 92/0220
Rhododendron stamineum Franch.	*Azaleastrum* Planch.	*Choniastrum* Franch.	AKAGI NATURE PARK 94/0084
Rhododendron albiflorum Hook.	*Candidastrum* Franch.		AKAGI NATURE PARK 95/0359
Rhododendron arboreum Sm.	*Hymenanthes* (Blume) K.Koch	*Ponticum* G.Don	Howick & McNamara 1923
Rhododendron barbatum Wall. ex G.Don	*Hymenanthes* (Blume) K.Koch	*Ponticum* G.Don	Beer, Lancaster & Morris 325
Rhododendron campanulatum D.Don	*Hymenanthes* (Blume) K.Koch	*Ponticum* G.Don	Stainton, Sykes & Williams 9107
Rhododendron campylocarpum Hook.f.	*Hymenanthes* (Blume) K.Koch	*Ponticum* G.Don	Rushforth 1768
Rhododendron fortunei Lindl.	*Hymenanthes* (Blume) K.Koch	*Ponticum* G.Don	AKAGI NATURE PARK 85/0052
Rhododendron falconeri Hook.f.	*Hymenanthes* (Blume) K.Koch	*Ponticum* G.Don	Cox, Hutchinson & McDonald 3072
Rhododendron griersonianum Balf.f. & Forrest	*Hymenanthes* (Blume) K.Koch	*Ponticum* G.Don	AKAGI NATURE PARK 90/0100
Rhododendron irroratum Franch.	*Hymenanthes* (Blume) K.Koch	*Ponticum* G.Don	SBEC 64
Rhododendron maculiferum Franch.	*Hymenanthes* (Blume) K.Koch	*Ponticum* G.Don	GUIZ 121

Table 1 (continued)

Species	Subgenus	Section	Voucher
Rhododendron ponticum L.	*Hymenanthes* (Blume) K.Koch	*Ponticum* G.Don	Apold, Cox & Hutchinson 205
Rhododendron taliense Franch.	*Hymenanthes* (Blume) K.Koch	*Ponticum* G.Don	CLD 1281
Rhododendron thomsonii Hook.f.	*Hymenanthes* (Blume) K.Koch	*Ponticum* G.Don	Rushforth 1655
Rhododendron semibarbatum Maxim.	*Mumeazalea* (Sleumer) Philipson & M.N.Philipson		Kurashige 264
Rhododendron luteum Sweet	*Pentanthera* (G.Don) Pojark.	*Pentanthera* G.Don	AKAGI NATURE PARK 89/0177
Rhododendron molle (Blume) G.Don	*Pentanthera* (G.Don) Pojark.	*Pentanthera* G.Don	Rock 11316
Rhododendron periclymenoides (Michx.) Shinners	*Pentanthera* (G.Don) Pojark.	*Pentanthera* G.Don	AKAGI NATURE PARK 89/0031
Rhododendron viscosum (L.) Torr.	*Pentanthera* (G.Don) Pojark.	*Pentanthera* G.Don	AKAGI NATURE PARK 90/0206
Rhododendron canadense (L.) Torr.	*Pentanthera* (G.Don) Pojark.	*Rhodora* (L.) G.Don	AKAGI NATURE PARK 93/0150
Rhododendron albrechtii Maxim.	*Pentanthera* (G.Don) Pojark.	*Sciadorhodion* Rehder & E.H.Wilson	Kurashige 349
Rhododendron quinquefolium Bisset & Moore	*Pentanthera* (G.Don) Pojark.	*Sciadorhodion* Rehder & E.H.Wilson	Kurashige 586
Rhododendron pentaphyllum Maxim.	*Pentanthera* (G.Don) Pojark.	*Sciadorhodion* Rehder & E.H.Wilson	Kurashige 166
Rhododendron schlippenbachii Maxim.	*Pentanthera* (G.Don) Pojark.	*Sciadorhodion* Rehder & E.H.Wilson	AKAGI NATURE PARK 92/0280
Rhododendron nipponicum Matsum.	*Pentanthera* (G.Don) Pojark.	*Viscidula* Matsum. & Nakai	Kurashige 241
Rhododendron primuliflorum Bureau & Franch.	*Rhododendron* L.	*Pogonanthum* Aitch. & Hemsl.	SICH 143
Rhododendron camelliiflorum Hook.f.	*Rhododendron* L.	*Rhododendron* L.	EMAK 730
Rhododendron campylogynum Franch.	*Rhododendron* L.	*Rhododendron* L.	Farrer 1046
Rhododendron dauricum L.	*Rhododendron* L.	*Rhododendron* L.	Kurashige 200
Rhododendron edgeworthii Hook.f.	*Rhododendron* L.	*Rhododendron* L.	CLD 1430

Table 1 (continued)

Rhododendron ferrugineum L.	*Rhododendron* L.	*Rhododendron* L.	AKAGI NATURE PARK 90/0001
Rhododendron hypoleucum (Kom.) Harmaja	*Rhododendron* L.	*Rhododendron* L.	Kurashige 256
Rhododendron maddenii Hook.f.	*Rhododendron* L.	*Rhododendron* L.	Rushforth 1695
Rhododendron micranthum Turcz.	*Rhododendron* L.	*Rhododendron* L.	AKAGI NATURE PARK 90/0461
Rhododendron saluenense Franch.	*Rhododendron* L.	*Rhododendron* L.	Forrest 21772
Rhododendron scabrifolium Franch.	*Rhododendron* L.	*Rhododendron* L.	SBEC – K 160
Rhododendron fallacinum Sleumer	*Rhododendron* L.	*Vireya* (Blume) H.F.Copel.	Kurashige & Yukawa 59
Rhododendron javanicum (Blume) Benn.	*Rhododendron* L.	*Vireya* (Blume) H.F.Copel.	AKAGI NATURE PARK 86/0004
Rhododendron konori Becc.	*Rhododendron* L.	*Vireya* (Blume) H.F.Copel.	AKAGI NATURE PARK 86/0006
Rhododendron retusum (Blume) Benn.	*Rhododendron* L.	*Vireya* (Blume) H.F.Copel.	AKAGI NATURE PARK 86/0019
Rhododendron santapaui Sastry et al.	*Rhododendron* L.	*Vireya* (Blume) H.F.Copel.	Cox & Hutchinson 459
Rhododendron camtschaticum Pall.	*Therorhodion* (Maxim.) A.Gray		Kurashige 458
Rhododendron farrerae Tate	*Tsutsusi* (Sweet) Pojark.	*Brachycalyx* Sweet	AKAGI NATURE PARK 89/0021
Rhododendron wadanum Makino	*Tsutsusi* (Sweet) Pojark.	*Brachycalyx* Sweet	Kurashige 168
Rhododendron indicum (L.) Sweet	*Tsutsusi* (Sweet) Pojark.	*Tsutsusi* Sweet	Kurashige 195
Rhododendron kaempferi Planch.	*Tsutsusi* (Sweet) Pojark.	*Tsutsusi* Sweet	Ki 520-1
Rhododendron tashiroi Maxim.	*Tsutsusi* (Sweet) Pojark.	*Tsutsusi* Sweet	Kurashige 100
Rhododendron tsusiophyllum Sugim.	*Tsutsusi* (Sweet) Pojark.	*Tsutsusi* Sweet	Kurashige 771
Subfamily Vaccioideae			
Tribe Cassiopeae			
Cassiope lycopodioides D.Don			Kurashige 1124B

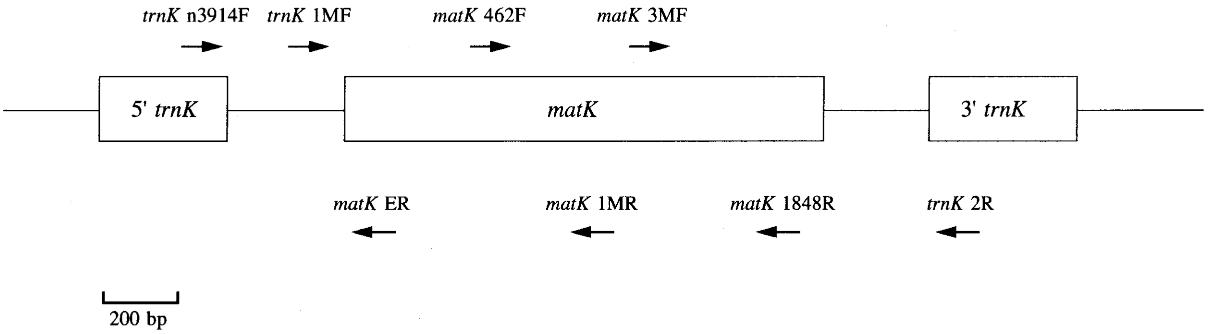


Fig. 1. Relative position of the PCR amplification and sequencing primers used for *mat*K and *trn*K introns. Arrows indicate the direction of strand synthesis. Boxed areas represent coding regions

Table 2. Location and base composition of amplification and sequencing primers used for *mat*K and *trn*K introns

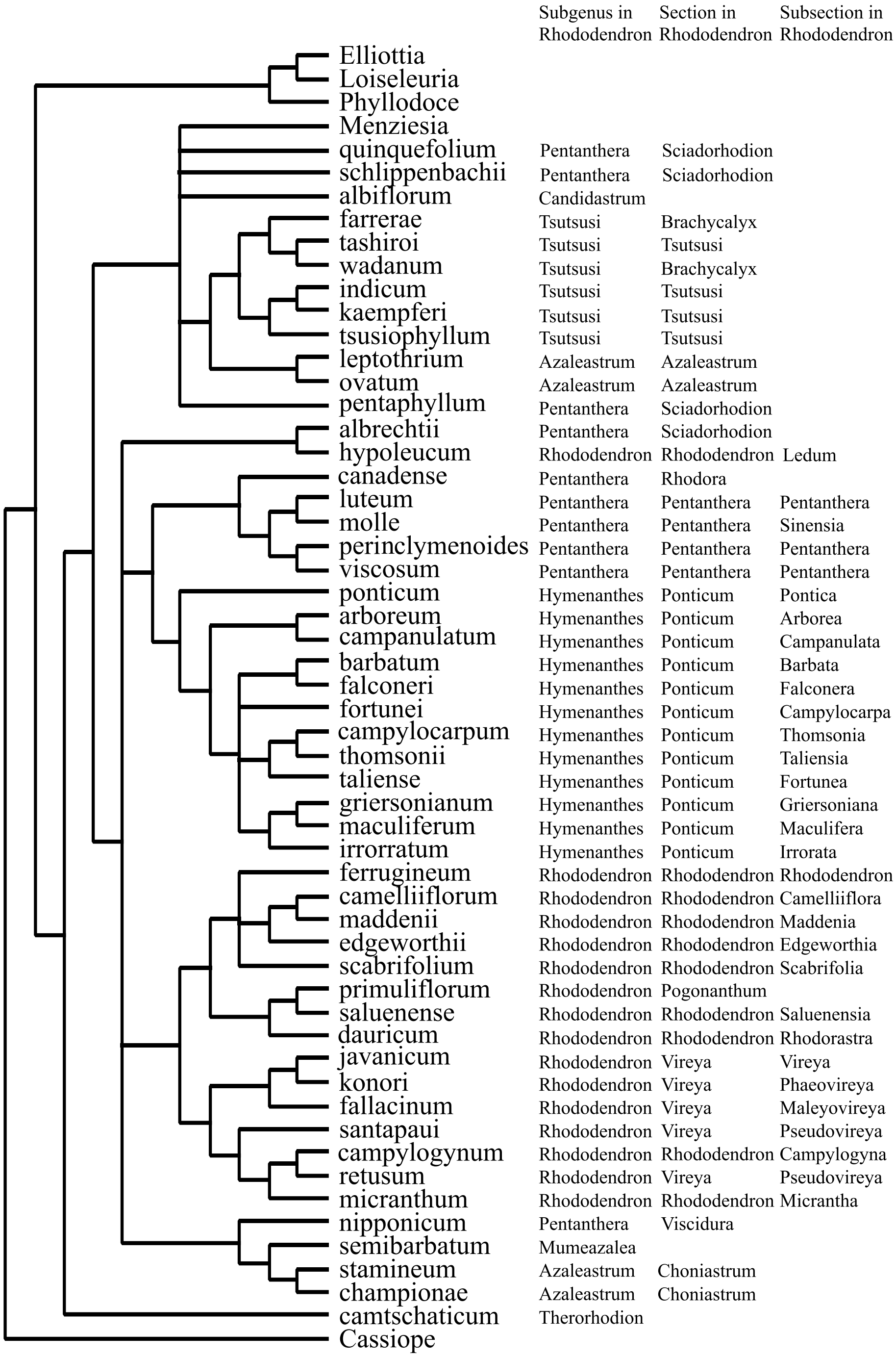
Primer	5′ sequence 3′	Designed by
*trn*K n3914F	GGG GTT GCT AAC TCA AC	Yukawa
*trn*K 1MF	GAT AAG TTT ACC GAG GTA GC	Yukawa
*mat*K 462F	AAT ACC CTA [C/T]CC C[A/G]T [C/T]CA TC	Chase
*mat*K 3MF	GTG GTC TCA ACC AAG AAG G	Yukawa
*mat*K ER	TTT TGG GGT TAT CAA ATC AT	Etoh
*mat*K 1MR	GTA GAA AAA ATC GTA ATA GC	Yukawa
*mat*K 1848R	TAT CGA ACT TCT TAA TAG C	Johnson/Soltis
*trn*K 2R	AAC TAG TCG GAT GGA GTA G	Steele

prised *Rhododendron* subgenera *Candidastrum*, *Tsutsusi*, *Azaleastrum* (in part), *Pentanthera* (in part), and genus *Menziesia* (Clade 1); and the second clade consists of *Rhododendron* subgenera *Pentanthera* (in part), *Rhododendron*, *Hymenanthes*, *Azaleastrum* (in part), and *Mumeazalea* (Clade 2).

Subgenus *Tsutsusi* formed a monophyletic group with a 99% bootstrap value in Clade 1. Within this clade, *R. tashiroi* (section *Tsutsusi*) was grouped with members of section *Brachycalyx* (100% bootstrap value; 6 apomorphic mutations), and the rest of section *Tsutsusi* formed a clade with a 95% bootstrap value.

The monophyly of subgenus *Azaleastrum* was not supported in this study. Section *Azaleastrum* showed a sister group relationship to subgenus *Tsutsusi* in Clade 1 (64% bootstrap value), and section *Choniastrum* formed a clade with subgenus *Mumeazalea* in Clade 2 (100% bootstrap value). However, the monophyly of both sections *Azaleastrum* and *Choniastrum* in themselves were strongly supported (100%, 95% bootstrap values, respectively). The monotypic subgenus *Candidastrum* showed a sister group relationship to the common ancestor of subgenus *Tsutsusi* and subgenus *Azaleastrum* section *Azaleastrum* in Clade 1, but this relationship was only weakly supported (48% bootstrap value).

The polyphyly of subgenus *Pentanthera* was suggested in all of the most parsimonious trees. A part of section *Sciadorhodion* fell into Clade 1: *R. schlippenbachii* and *R. quinquefolium* were grouped with genus *Menziesia*; *R. pentaphyllum* showed a sister group relationship to the common ancestor of three groups, namely, subgenus *Candidastrum*, subgenus *Tsutsusi*, and subgenus *Azaleastrum* section *Azaleastrum*, but this relationship was not supported by a high bootstrap value (31%). On the other hand, *R. albrechtii*, another member of section *Sciadorhodion*, was grouped with section *Rhododendron* sub-



	Subgenus in Rhododendron	Section in Rhododendron	Subsection in Rhododendron
Elliottia			
Loiseleuria			
Phyllodoce			
Menziesia			
quinquefolium	Pentanthera	Sciadorhodion	
schlippenbachii	Pentanthera	Sciadorhodion	
albiflorum	Candidastrum		
farrerae	Tsutsusi	Brachycalyx	
tashiroi	Tsutsusi	Tsutsusi	
wadanum	Tsutsusi	Brachycalyx	
indicum	Tsutsusi	Tsutsusi	
kaempferi	Tsutsusi	Tsutsusi	
tsusiophyllum	Tsutsusi	Tsutsusi	
leptothrium	Azaleastrum	Azaleastrum	
ovatum	Azaleastrum	Azaleastrum	
pentaphyllum	Pentanthera	Sciadorhodion	
albrechtii	Pentanthera	Sciadorhodion	
hypoleucum	Rhododendron	Rhododendron	Ledum
canadense	Pentanthera	Rhodora	
luteum	Pentanthera	Pentanthera	Pentanthera
molle	Pentanthera	Pentanthera	Sinensia
perinclymenoides	Pentanthera	Pentanthera	Pentanthera
viscosum	Pentanthera	Pentanthera	Pentanthera
ponticum	Hymenanthes	Ponticum	Pontica
arboreum	Hymenanthes	Ponticum	Arborea
campanulatum	Hymenanthes	Ponticum	Campanulata
barbatum	Hymenanthes	Ponticum	Barbata
falconeri	Hymenanthes	Ponticum	Falconera
fortunei	Hymenanthes	Ponticum	Campylocarpa
campylocarpum	Hymenanthes	Ponticum	Thomsonia
thomsonii	Hymenanthes	Ponticum	Taliensia
taliense	Hymenanthes	Ponticum	Fortunea
griersonianum	Hymenanthes	Ponticum	Griersoniana
maculiferum	Hymenanthes	Ponticum	Maculifera
irroratum	Hymenanthes	Ponticum	Irrorata
ferrugineum	Rhododendron	Rhododendron	Rhododendron
camelliiflorum	Rhododendron	Rhododendron	Camelliiflora
maddenii	Rhododendron	Rhododendron	Maddenia
edgeworthii	Rhododendron	Rhododendron	Edgeworthia
scabrifolium	Rhododendron	Rhododendron	Scabrifolia
primuliflorum	Rhododendron	Pogonanthum	
saluenense	Rhododendron	Rhododendron	Saluenensia
dauricum	Rhododendron	Rhododendron	Rhodorastra
javanicum	Rhododendron	Vireya	Vireya
konori	Rhododendron	Vireya	Phaeovireya
fallacinum	Rhododendron	Vireya	Maleyovireya
santapaui	Rhododendron	Vireya	Pseudovireya
campylogynum	Rhododendron	Rhododendron	Campylogyna
retusum	Rhododendron	Vireya	Pseudovireya
micranthum	Rhododendron	Rhododendron	Micrantha
nipponicum	Pentanthera	Viscidura	
semibarbatum	Mumeazalea		
stamineum	Azaleastrum	Choniastrum	
championae	Azaleastrum	Choniastrum	
camtschaticum	Therorhodion		
Cassiope			

Fig. 2. Strict consensus of 267 most parsimonious Fitch trees based on *mat*K and *trn*K intron sequences, length = 744; consistency index (excluding uninformative characters) = 0.625; retention index = 0.805

section *Ledum* in Clade 2 (83% bootstrap value; 2 apomorphic mutations). The monotypic section *Viscidula* formed a clade with the common ancestor of subgenus *Mumeazalea* and subgenus *Azaleastrum* section *Choniastrum* in Clade 2 (59% bootstrap value). Besides, in clade 2, the monophyly of section *Pentanthera* (100% bootstrap value; 9

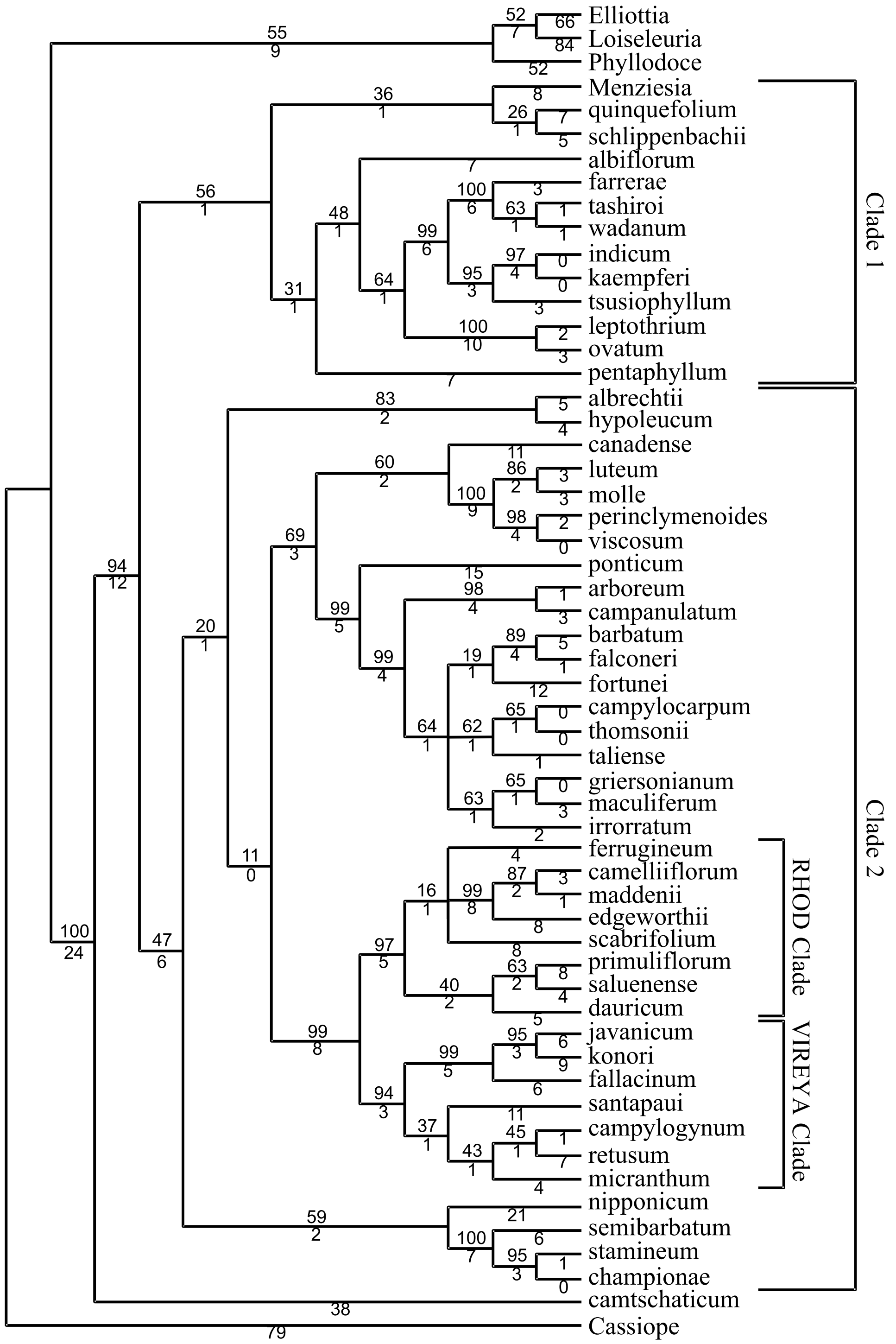


Fig. 3. One of 267 most parsimonious Fitch trees based on *mat*K and *trn*K intron sequences. Numbers above internodes indicate bootstrap values from 1,000 replicates and numbers below internodes suggest the number of mutations supporting each monophyletic group (ACCTRAN optimization)

apomorphic mutations) as well as a sister group relationship of section *Pentanthera* to section *Rhodora* (60% bootstrap value) was indicated. Within section *Pentanthera*, the monotypic subsection *Sinensia* was nested within subsection *Pentanthera* with a bootstrap value of 86%.

The monophyletic subgenus *Hymenanthes* (bootstrap value of 99%; 5 apomorphic mutations) formed a sister group relationship with the common ancestor of subgenus *Pentanthera* sections *Rhodora* and *Pentanthera* in Clade 2 (69% bootstrap value). Among members of subgenus *Hymenanthes*, a sister group relationship between subsection *Pontica* and the rest of subgenus *Hymenanthes* (99% bootstrap value), monophyly of subsections *Arborea* and *Campanulata* (98% bootstrap value), and monophyly of subsections *Barbata* and *Falconera* (89% bootstrap value) were strongly suggested.

Members of subgenus *Rhododendron* excluding section *Rhododendron* subsection *Ledum* formed a clade with a 99% bootstrap value (8 apomorphic mutations). Within this clade, two major groups were apparent. The first group, supported by a 97% bootstrap value, included section *Pogonanthum* and a part of section *Rhododendron* (RHOD Clade). Within the RHOD Clade, subsections *Camelliiflora*, *Maddenia*, and *Edgeworthia* of section *Rhododendron* formed a monophyletic group (99% bootstrap value). On the other hand, the remaining members of section *Rhododendron* were nested within a clade made up by members of section *Vireya* with a bootstrap value of 94% (VIREYA Clade). Within this clade, the monophyly of three groups comprising subsections *Vireya*, *Phaeovireya*, and *Malayovireya* of section *Vireya* was strongly supported (99% bootstrap value).

Discussion

Paraphyly of the genus *Rhododendron*. Our results do not support the monophyly of *Rhododendron*, because the genus *Menziesia* falls among members of *Rhododendron*. If *Menziesia* is included within *Rhododendron*, the latter genus becomes monophyletic. This was also revealed by *mat*K sequences analyses of subfamily Rhododendroideae (Kron 1997) and tribe Rhodoreae (Kurashige et al. 1998) and by an analysis of ITS sequences of tribe Rhodoreae (Chamberlain and Hyam 1998).

The recognition of *Menziesia* at generic rank is mostly due to the following unique combination of characters: longitudinal dehiscence of anthers and lack of viscin threads (Stevens 1971). The former is a shared character for *Menziesia* and *R. tsusiophyllum* (subgenus *Tsutsusi* section *Tsutsusi*), which has been often treated as a separate genus, *Tsusiophyllum* (e.g. Stevens 1971, Sleumer 1980, Yamazaki 1989). Kron and Judd (1990) also suggested a close relationship between the two taxa based on the results of a cladistic study using morphological characters. However, our results did not reveal this relationship, because *R. tsusiophyllum* was grouped with members of subgenus *Tsutsusi*. An analysis of ITS sequences also supports a distant position of *Menziesia* to *R. tsusiophyllum*, whereas the placement of *Menziesia* was different from our results (Chamberlain and Hyam 1998). Therefore, it seems best to conclude that the vertical dehiscing anthers evolved twice within tribe Rhodoreae and that the loss of viscin threads in *Menziesia* represents an autapomorphy of this taxon. Although the exact placement of *Menziesia* in *Rhododendron* is uncertain, we are convinced that *Menziesia* should be treated as an infrageneric taxon of *Rhododendron*.

Infrageneric relationships in *Rhododendron*: (1) Subgenus *Therorhodion*. This subgenus is characterized by its elongated inflorescence axis with leaf-like bracts and bracteoles as well as by its base chromosome number ($x = 12$), characters are not found in the remaining members of tribe Rhodoreae. Therefore, this subgenus has been widely recognized as genus *Therorhodion* (Copeland 1943, Sleumer 1949, Stevens 1971, Yamazaki 1989). On the other hand, Philipson and Philipson (1986) reduced this genus to subgeneric rank within *Rhodo-*

dendron, because both genera have a similar type of racemose inflorescence. Our results strongly suggest that subgenus *Therorhodion* forms the basal lineage of Rhodoreae (94% bootstrap value; 12 apomorphic mutations). A cladistic analysis using morphological characters of tribe Rhodoreae (Kron and Judd 1990), *mat*K sequences analyses of subfamily Rhododendroideae (Kron 1997) and tribe Rhodoreae (Kurashige et al. 1998), and an ITS sequences analysis of Rhodoreae (Chamberlain and Hyam 1998) also support the basal position of *Rhododendron* subgenus *Therorhodion*.

(2) Subgenus *Tsutsusi*. Its monophyly was definitely supported by a 99% bootstrap value (6 apomorphic mutations). Within this clade, *Rhododendron tsusiophyllum* was strongly grouped with members of subgenus *Tsutsusi* section *Tsutsusi* (95% bootstrap value; 3 apomorphic mutations). Some taxonomists (e.g. Stevens 1971, Sleumer 1980, Yamazaki 1996) treated this species as a separate genus, *Tsusiophyllum*, with emphasis upon its vertically dehiscing anthers and the three locules of its ovary, a unique combination of characters in genus *Rhododendron*. Our results, however, do not support a separate generic status of this species. Although intersectional hybrids are scarcely known in this genus, Takahashi et al. (1998) revealed that *R. koudzumontanum* is a hybrid between *R. kaempferi* (section *Tsutsusi*) and *R. tsusiophyllum* based on the results of allozyme analyses, anther morphology, and pollen fertility. Moreover, the number of ovary locules varies within a single section of genus *Rhododendron*; i.e., 5–10 locules in section *Rhododendron* (Cullen 1980) and 5–20 locules in subgenus *Hymenanthes* section *Pontica* (Chamberlain 1982). These facts also indicate that *R. tsusiophyllum* is not recognizable as a separate genus. Moreover, a couple of synapomorphic morphological characters, namely, inflorescence and leaf buds enclosed by terminal bud scales and dimorphic leaves, also suggest the monophyly of section *Tsutsusi* including *R. tsusiophyllum*.

Rhododendron tashiroi was nested within members of subgenus *Tsutsusi* section *Brachycalyx*: the position of this species makes both sections *Tsutsusi* and *Brachycalyx* para-/polyphyletic. Chamberlain and Rae (1990) stressed the taxonomic importance of its persistent leaves and flattened hairs on young stems rather than its monomorphic leaves in pseudowhorls of three; and they recognized *R. tashiroi* as a member of section *Tsutsusi*. However, flattened hairs on young stems are also found in *R. farrerae* of section *Brachycalyx* (Kurashige, unpubl.). Furthermore, there are several natural hybrids between *R. tashiroi* and species of section *Brachycalyx* (Yamazaki 1996), whereas Yamaguchi et al. (1985) reported low cross compatibilities between *R. tashiroi* and the members of section *Tsutsusi*. Therefore, we conclude that *R. tashiroi* is a member of section *Brachycalyx*. The monomorphic leaves in pseudowhorls of three at the shoot apex represent a primary diagnostic character of section *Brachycalyx* rather than the persistence or deciduousness of leaves.

(3) Subgenera *Azaleastrum*, *Candidastrum*, and *Mumeazalea*. A close affinity among these subgenera with lateral inflorescences was not supported in this study. Subgenus *Azaleastrum* was shown to be polyphyletic, because section *Azaleastrum* showed a sister group relationship to subgenus *Tsutsusi* in Clade 1 and section *Choniastrum* formed a clade with subgenus *Mumeazalea* in Clade 2. The monophyly of both sections *Azaleastrum* and *Choniastrum*, however, was strongly supported. An analysis of ITS sequences (Chamberlain and Hyam 1998) also supported a segregate position of sections *Azaleastrum* and *Choniastrum*, whereas a close relationship between section *Choniastrum* and subgenus *Mumeazalea* was not recognized.

The monotypic subgenus *Candidastrum* formed a clade with the common ancestor of subgenus *Tsutsusi* and subgenus *Azaleastrum* section *Azaleastrum* in Clade 1, but this relationship was not strongly supported by the bootstrap value (48%). A distant position of subgenus *Candidastrum* from subgenera *Azaleastrum* and *Mumeazalea* was also indicated by its isolated distribution in North

America and morphological characters such as its actinomorphic flowers and inflorescences developed from buds in leaf axils of the previous year's growth. Although the relationships among lateral-flowered taxa were not completely elucidated in this study, it is likely that the character of lateral inflorescence evolved more than once in *Rhododendron*.

(4) Subgenus *Pentanthera*. This was shown to be polyphyletic in our study because its members were scattered among clades 1 and 2. The monophyly of section *Pentanthera*, however, was strongly supported by a high bootstrap value (100%; 9 apomorphic mutations) as well as by the following synapomorphies: pubescent indument with unicellular hairs on the abaxial surface of the corolla and five stamens. In section *Pentanthera*, Kron (1993) recognized two subsections, namely, *Pentanthera* and *Sinensia*. She placed *R. molle* in the monotypic subsection *Sinensia* on the basis of its broadly funnel-shaped corolla, lack of multicellular hairs on the abaxial surface of the corolla, spots on the upper corolla lobes, and stamens not exserted from the corolla. Although our results suggested a sister group relationship between a part of subsection *Pentanthera* (*R. luteum*) and subsection *Sinensia*, few morphological characters unite these two taxa. To resolve paraphyly of subsection *Pentanthera*, three alternatives are available: (1) to include subsection *Sinensia* in subsection *Pentanthera*; (2) to move *R. luteum* to subsection *Sinensia*; (3) to establish a new subsection for *R. luteum*.

The monophyly of section *Sciadorhodion* as well as its close relationship to other sections in subgenus *Pentanthera* were not supported in this study. A part of this section, namely, *R. schlippenbachii* and *R. quinquefolium*, were grouped with the genus *Menziesia* in Clade 1, but this clade had a low bootstrap value (36%). The remaining members of the section showed the following relationships: *R. pentaphyllum* fell within Clade 1; and *R. albrechtii* formed a clade with section *Rhododendron* subsection *Ledum* in Clade 2. The polyphyly of section *Sciadorhodion* was also suggested by the ITS sequences analysis of tribe Rhodoreae (Chamberlain and Hyam 1998). The composition of leaf and flower buds is inconsistent among the members of this section. *R. quinquefolium* and *R. schlippenbachii* have mixed buds. This condition is also found in subgenus *Tsutsusi*. On the other hand, *R. albrechtii* and *R. pentaphyllum* develop an inflorescence from a terminal bud and a vegetative shoot from a lateral bud. The monotypic section *Viscidula* formed a clade with the common ancestor of subgenus *Mumeazalea* and subgenus *Azaleastrum* section *Choniastrum*, but there are no morphological characters uniting these taxa. Although relationships among subgenus *Pentanthera* were partially unresolved in this study, we are convinced that sections *Sciadorhodion* and *Viscidula* should be segregated from subgenus *Pentanthera* and that the former section is polyphyletic. Further anatomical and morphological studies, particularly those of inflorescence and vegetative buds, should make an exact placement of the species in sections *Sciadorhodion* and *Viscidula* possible.

(5) Subgenus *Hymenanthes*. This subgenus with the single section *Ponticum* was shown to be monophyletic with a bootstrap value of 99% (5 apomorphic mutations). The following characters unite this subgenus: complex dendritic hairs, complex nodal anatomy, and presence of caryatin in the leaves (Chamberlain and Hyam 1998). Among members of section *Ponticum*, the most widespread distribution of subsection *Pontica* in Europe, Asia, and North America implies the early divergence of this subsection from the remaining subsections. Subsectional relationships proposed by Chamberlain (1982) are not well supported in this study, as shown in the following two clades: subsection *Arborea* with subsection *Campanulata*, and subsection *Barbata* with subsection *Falconera* (Figs. 2 and 3). He stressed the taxonomic importance of the following morphological characters in this subgenus: corolla shape, number of corolla lobes, presence or absence of depressed nectar pouches at the base of the ovary, and characteristics of hairs on the abaxial surface of the leaf. However, the following combination of character states are found: corolla with nectar pouches in subsection *Arborea*; corolla without nectar pouches in subsection *Campanulata*; 5-lobed corolla with nectar pouches in subsection *Barbata*; *ca.* 10-lobed corolla without nectar pouches and cup-shaped hairs on the abaxial surface of the leaf in subsection *Falconera*. Consequently, characters used by Chamberlain (1982) to subdivide subgenus *Hymenanthes* at a subsectional level are not useful. At the same time, we have failed to find synapomorphies for the *Arborea* – *Campanulata* clade and the *Barbata* – *Falconera* clade. Therefore, re-examination of subsectional relationships in subgenus *Hymenanthes* is required.

(6) Subgenus *Rhododendron*. The monophyly of subgenus *Rhododendron* was not supported in this study because section *Rhododendron* subsection *Ledum* was grouped with *R. albrechtii* (subgenus *Pentanthera* section *Sciadorhodion*) with a moderately high bootstrap value of 83% (2 apomorphic mutations). Although subsection *Ledum* has been recognized at a generic rank by several taxonomists (e.g. Stevens 1971, Sleumer 1980, Cullen 1980, Yamazaki 1989), Kron and Judd (1990) reduced this genus to a subsectional rank of section *Rhododendron* with emphasis upon the presence of a similar type of scales on the abaxial surface of leaves. They also suggested a close relationship of *Ledum* to subsection *Edgeworthia* (section *Rhododendron*) based on the following synapomorphies: long crisped multicellular trichomes on the abaxial surface of leaves and stems; indumentum with unicellular hairs on the abaxial surface of leaves; and revolute vernation. However, our results do not support the placement of subsection *Ledum* in subgenus *Rhododendron* as proposed by Kron and Judd (1990). Although Sinclair (1937) and Philipson (1985) noted revolute leaves in the bud of *R. pendulum* (of subsection *Edgeworthia*), Yamazaki (1996) reported involute vernation of the species of subgenus *Rhododendron*. Consequently, it seems best to conclude that subsection *Ledum* should be segregated from subgenus *Rhododendron*. To reinvestigate distinct characters of subsection *Ledum*, such as a choripetalous corolla, a capsule dehiscing from the base at maturity, lepidote scales on the abaxial surface of the leaf, and revolute vernation, relationships between subsection *Ledum* and *R. albrechtii* have to be assessed.

The members of subgenus *Rhododendron* excluding subsection *Ledum* formed a monophyletic clade with a bootstrap value of 99% (8 apomorphic mutations). Cullen (1980) recognized three sections, namely, *Pogonanthum*, *Rhododendron*, and *Vireya*, in subgenus *Rhododendron*. Our result, however, did not support Cullen's subdivision of this subgenus. A part of section *Rhododendron* formed a clade with section *Pogonanthum* (RHOD Clade), the rest of the former section was grouped with section *Vireya* (VIREYA Clade). The clear-cut division between the RHODO clade and the VIREYA clade is inconsistent with the following diagnostic characters proposed by Cullen (1980): sharply deflexed or straight style, length of seed appendages, type of upper leaf epidermis, presence or absence of foliar sclereids, and habit. Within the RHOD clade, subsections *Camelliiflora*, *Maddenia*, and *Edgeworthia* formed a robust subclade with a bootstrap value of 99%. The following synapomorphies are also found in this subclade: winged seeds, multiple-layered leaf upper epidermis, foliar sclereids, and epiphytic habit. Among the members of the VIREYA clade, three subsections of section *Vireya*, i.e., *Vireya*, *Phaeovireya*, and *Malayovireya*, formed a particularly well supported group with a bootstrap value of 99%. This grouping is also supported by the following synapomorphies: more or less distinctly lobed scales on the abaxial surface of leaves (Sleumer 1966) and clearly larger flowers (2–19 cm long, 3–15 cm wide) than those of the remaining species of this clade. On the other hand, the rest of the VIREYA clade, section *Vireya* subsection *Pseudovireya* and section *Rhododendron* subsections *Campylogyna* and *Micrantha*, are characterized by their entire lepidote scales

(Hedegaard 1980, Sleumer 1966) and smaller flowers (0.5–2.5 cm long, 1–2 cm wide). Although we failed to find any synapomorphic character for both the RHODO and VIREYA clades, re-examination of lepidote scales may support our results.

In conclusion, this study indicates the following taxonomic inferences: (1) *Rhododendron* subgenus *Therorhodion* is the basal clade of tribe Rhodoreae; (2) genus *Menziesia* is included in the genus *Rhododendron*; (3) *R. tashiroi* is a member of subgenus *Tsutsusi* section *Brachycalyx* rather than of subgenus *Tsutsusi* section *Tsutsusi*; (4) *R. tsusiophyllum* is a member of subgenus *Tsutsusi* section *Tsutsusi*; (5) sections *Sciadorhodion* and *Viscidula* should be separated from subgenus *Pentanthera*; (6) section *Rhododendron* subsection *Ledum* should be excluded from subgenus *Rhododendron*.

Overall, poor resolution of the basal lineages indicates radiation early in the history of the genus *Rhododendron*. Therefore, the necessity of phylogenetic analyses utilizing more rapidly evolving DNA regions and of further morphological and anatomical studies is quite evident.

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