THE PHYLOGENY, MORPHOLOGICAL EVOLUTION AND BIOGEOGRAPHY OF THE GAULTHERIEAE (ERICACEAE)

By

CATHERINE MARY BUSH

A Dissertation Submitted to the Graduate Faculty of
WAKE FOREST UNIVERSITY GRADUATE SCHOOL OF ARTS AND SCIENCES
In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY
in the Department of Biology
May 2010
Winston-Salem, NC

Approved by:

Kathleen A. Kron, Ph.D., Advisor ____________________

Examining Committee:

Zack Murrell, Ph.D., Chair ____________________

William K. Smith, Ph.D. ____________________

Peter D. Weigl, Ph.D. ____________________

Clifford Zeyl, Ph. D. ____________________

ACKNOWLEDGEMENTS

This dissertation would absolutely not have been possible without the support, mentoring and patience of many people. I leaned on my family and fiancé to get through this process, at times wonderful and at times overwhelming. I had their support as I spent months doing field work in six countries. The distance was never easy for either side, but they knew my work made me happy and they were behind me even when it was difficult. I would like to thank my advisor, Kathleen A. Kron, who allowed me to become an independent scientist. Kathy's door was always open when I needed a push in the right direction and I have learned a tremendous amount from being in her lab. Thanks also to my advisory committee, Dr. Zack Murrell, Dr. William Smith, Dr. Peter D. Weigl and Dr. Clifford Zeyl for their support and guidance.

I would like to thank Dr. Gerald L. Smith, my undergraduate advisor who led me with his enthusiasm and kindness on the road to botanical exploration and research. He is a colleague but also a dear friend and I treasure the times we have spent together in the lab or in the field, trying to understand the ever-delightful and elusive spider lilies. I also want to thank the entire biology department faculty at High Point University. My alma mater professors have kept in touch as I progressed in this degree and they are like an extended family to me.

I absolutely could not have made it through this degree without the empathy and shared laughter of my labmates past and present – Ann Powell, Emily Gillespie, Adriana Sanchez, Tanja Schuster and Sabrina Setaro.

I want to thank Dr. Peter W. Fritsch for taking a chance on me and inviting me on my first major field expedition to China. I am very grateful for the collaboration and friendship that grew from that first trip. Peter has been constant source of encouragement and guidance throughout this journey.

One of the unexpected gifts of this degree was Lu Lu, my Chinese collaborator and dear friend. She has taught me so much, about both science and life.

Finally, I want to thank the generous and kind people who hosted me around the world: Darren Crayn, João Luiz M. Aranha Filho, Angela B. Martins, Ted Oliver, Karen Rea, Rimi Repin, Gisela Sancho, Ross Turner and Steve Wagstaff. Your hospitality will not be forgotten.

Chapter-specific acknowledgements

Chapter 2 - We thank the anonymous reviewers for their helpful suggestions. We would also like to acknowledge the financial support from Wake Forest University and the National Science Foundation (DEB-0717724, DEB-0717711).

Chapter 3 - We thank the anonymous reviewers for their helpful suggestions. We would also like to acknowledge the financial support from Wake Forest University and the National Science Foundation (DEB-0717724; DEB-0717711).

Chapter 4 - We acknowledge the financial support from Wake Forest University and the National Science Foundation (DEB-0717724; DEB-0717711). We thank the curators of the Allan Herbarium, the National Herbarium of New South Wales and the Tasmanian Herbarium for access to the databases and collections as well as the New Zealand Department of Conservation and Biodiversity Conservation Branch (Department of

Primary Industries, Water and Environment; Tasmania) for their helpful advice and for administering our collection permits. We also thank Darren Crayn for help in the field, and two anonymous reviewers for their helpful manuscript suggestions.

Chapter 5 - We would like to thank the curators of the following herbaria for loaning us the material needed for this morphological revision and/or for their expert advice regarding type specimens: AK, B, BM, CHR, E, GOET, HO, K, L, LINN, LIV, MELU, MO, MPN, MW, NSW, NZFRI, P, PH, UNSW, UPS, W, WAIK and WELT. We thank Dr. Gerald L. Smith for his knowledgeable critiques of this manuscript. We acknowledge the financial support from Wake Forest University and the National Science Foundation (DEB-0717724; DEB-0717711).

TABLE OF CONTENTS

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

CHAPTER VI

CHAPTER VII

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Bush, Catherine M.

THE PHYLOGENY, MORPHOLOGICAL EVOLUTION AND BIOGEOGRAPHY OF THE GAULTHERIEAE (ERICACEAE)

Dissertation under the direction of Kathleen A. Kron, Ph.D.,
Associate Professor

The tribe Gaultherieae (Ericaceae: subfamily Vaccinioideae) comprises *Chamaedaphne* Moench, *Diplycosia* Blume, *Eubotrys* Nutt., *Eubotryoides* (Nakai) Hara, *Gaultheria* Kalm ex L., *Leucothoë* D. Don, and *Tepuia* Camp., with a total of approximately 250 species. The tribe is defined by its four-appendaged anthers and a base chromosome number of 11, although both of these characters also occur in a closely related genus, *Zenobia* D. Don (tribe Andromedeae; Kron et al. 2002). The group exhibits an amphi-Pacific distribution, that is, temperate and tropical regions of the Americas, eastern Asia, Southeast Asia, and New Guinea, Australia, and New Zealand. The informally named wintergreen group (*Diplycosia*, *Gaultheria* and *Tepuia*) is thought to be diagnosable by the presence of methyl salicylate, although this compound has apparently been lost in many species (or has not been detected).

Phylogenetic analyses using DNA sequence data have shown that the Gaultherieae and the wintergreen group are monophyletic. Optimization of morphological characters emphasized in classifications of the Gaultherieae onto the molecular phylogeny revealed that (1) fleshy calyces evolved either early in the lineage leading to the wintergreen group or later in two separate clades; (2) capsular fruits are plesiomorphic and berry fruits have evolved independently in *Diplycosia* and *Tepuia* and

once or twice within a large clade containing all sampled *Gaultheria* sect. *Pernettya* members and *Gaultheria* species not exhibiting a berry fruit; and (3) A racemose inflorescence is the ancestral state for both the Gaultherieae and wintergreen group. Solitary-flowered inflorescences have evolved at least eight times within the Gaultherieae, with several changes back to racemose or fascicular inflorescences.

The genus *Leucothoë* s.l. has been split into as many as three genera in current taxonomic work involving morphological data. Based on phylogenetic data, *Leucothoë* s.l. is divided into three genera: *Eubotryoides* (*E. grayana*), *Eubotrys* (*E. racemosa* and *E. recurva*), and *Leucothoë* s.s. (*L. axillaris, L. davisiae, L. fontanesiana, L. griffithiana,* and *L. keiskei*).

Several strongly supported clades within the wintergreen group (i.e., *Gaultheria* s.s.) include members from a particular geographic region. Phylogenetic relationships within *Gaultheria* L. from Australia and New Zealand were examined by using DNA sequence data and it was found that all Australia/New Zealand species form a clade that is sister to a clade of temperate South American species. A historical biogeographical analysis that included the temperate southern hemisphere element in *Gaultheria* suggests a South American origin of the Australia/New Zealand clade, followed by three dispersal events from New Zealand to Australia. Whether the origin is from temperate or tropical South America is ambiguous in the analysis. The species of *Gaultheria* from Australia and New Zealand were analyzed using morphological data and the species number for this region was reduced from fifteen to ten; the results were corroborated by the molecular phylogeny.

Finally, the disjunct Brazilian species of *Gaultheria* were analyzed in a molecular phylogeny. Six species of *Gaultheria* are endemic to the *Mata Atlantica* (Atlantic rainforest) in Brazil, several of which exhibit unique morphological characters within *Gaultheria*. A strongly supported clade of five Brazilian endemics was recovered (corresponding to the currently recognized *G*. ser. *Myrtilloideae* clade) and is sister to a clade of *Gaultheria* from temperate South America. *Gaultheria serrata*, another endemic *Gaultheria* species and two other *Gaultheria* that exhibit distributions in Brazil and the Andes are closely related to each other and other species from the Andes/Mexico. These results support the hypothesis that some Brazilian species of *Gaultheria* are derived from Andean ancestors. However, the *G*. ser. *Myrtilloideae* clade is imbedded within a clade containing species from temperate South America, indicating that the Andes may not have served as the only source area for the species in the *Mata Atlantica* of Brazil.

CHAPTER I

AN INTRODUCTION TO THE GAULTHERIEAE

The tribe Gaultherieae (Ericaceae: subfamily Vaccinioideae) comprises *Chamaedaphne* Moench, *Diplycosia* Blume, *Eubotrys* Nutt., *Eubotryoides* (Nakai) Hara, *Gaultheria* Kalm ex L., *Leucothoë* D. Don and *Tepuia* Camp. with a total of approximately 250 species. A potential synapomorphy for the tribe is its 4-appendaged anthers and a base chromosome number of 11, although both of these characters also occur in a closely related genus, *Zenobia* D. Don (tribe Andromedeae; Kron et al. 2002). The group exhibits an amphi-Pacific distribution, i.e., temperate and tropical regions of the Americas, the Himalayan/Indo-China region, Southeast Asia, and the Pacific (New Guinea, Australia and New Zealand).

The phylogenetic relationships of the genera in the Gaultherieae have been investigated as part of a comprehensive study of the Ericaceae based on morphological and molecular data (Kron et. al., 2002). This study found the Gaultherieae to be monophyletic and sister to the tribe Andromedeae (*Andromeda* L. and *Zenobia*) in the Vaccinioideae although strong support for the Gaultherieae was low [bootstrap support (bt) < 50%]. The wintergreen group within the Gaultherieae (*Diplycosia*, *Gaultheria* and *Tepuia*; five species were sampled in total from among these genera) was recovered (bt = 92) as sister (bt < 50) to a clade comprising *Chamaedaphne calyculata* + *Eubotrys racemosa* (bt = 63). Sister to the entire clade was *Leucothoë fontanesiana* (bt < 50).

Chamaedaphne calyculata is the only species in *Chamaedaphne* and it has numerous distinct morphological characters including: terminal inflorescences with leafy bracts, anthers with tubules, unique floral and embryological anatomy, and peltate scales (Palser 1951, 1952; Kron et al. 1999). Also, along with *Leucothoe racemosa* and *L. recurva*, this species exhibit a "*L. racemosa*-type" fall inflorescence where the reproductive shoot resembles a vegetative shoot and has long, green, leaf-like bracts not enclosing the floral buds. The floral buds themselves are protected by the thick sepals of the individual flowers (Waselkov and Judd 2008). The species is widespread in the Northern Hemisphere (Kron et al. 1999).

The most recent, unpublished monograph of the genus *Leucothoe* (Melvin 1980) contains eight species split into groups based on geography and the character of deciduous versus evergreen leaves. *Leucothoë racemosa* A. Gray and *L. recurva* A. Gray are North American species that possess deciduous leaves. *Leucothoë grayana* Maxim. is a morphologically distinct species that also exhibits the deciduous habit but occurs in Japan. The remaining species are evergreen: three are North American [*L. axillaris* D. Don (type of the genus), *L. fontanesiana* (Steud.) Sleumer, and *L. davisiae* Torr. & A. Gray] and two are Asian (*L. griffithiana* C.B. Clarke, Himalayas; *L. keiskei* Miq., Japan). Based on morphological characters, Melvin (1980) divided the genus into three sections: *Leucothoë* (composed of all five evergreen species), *Eubotrys* Nuttall (containing the two North American deciduous species), and *Eubotryoides* (T. Nakai) H. Hara (containing only the deciduous Japanese species, *L. grayana*).

The morphological characters that have been described as uniting *Leucothoë* s.l. are variable and occur in other closely related genera. These characters include: serrate leaf margins, axillary racemes formed from buds that overwinter (i.e., develop in the autumn; *L. grayana* is the only exception, with its racemes forming in the spring), dry calyces (shared with many other Ericaceae), awned anthers (shared most notably with many species of *Gaultheria*), superior ovaries with numerous ovules and seeds with marginal cells that form degrees of a winged appearance (a wing is lacking in *L. racemosa*; Sleumer 1959; Stevens 1969, 1971; Luteyn et al. 1996).

The informally named wintergreen group (*Diplycosia*, *Gaultheria* and *Tepuia*) is thought to be diagnosable by the presence of methyl salicylate, although this compound has apparently been lost in many species (or has not been detected) and it is found in other ericads such as *Cavendishia* Lindl. and *Pyrola* L. as well as in unrelated families (*Betula* L.: Betulaceae; *Spiraea* L.: Rosaceae; Powell and Kron, 2001). The wintergreen genera have historically been recognized as closely related, but morphological characters have kept them taxonomically separate (Cox, 1948).

The genus *Diplycosia* contains epiphytic and terrestrial shrubs and it identified by their superior or occasionally half-inferior ovaries, flowers solitary or in fascicles, two apical bracteoles connate into a calyculus and anthers with tubules (Sleumer 1967). *Diplycosia* also has free fibers in the mesophyll, although Middleton has shown that the latter can be seen in some *Gaultheria* species (1993). The fruits are typically capsules surrounded by a fleshy calyx (Sleumer 1967). The monotypic genus *Pernettyopsis* King and Gamble had been delimited from *Diplycosia* by its berry (versus capsule; both have a fleshy accrescent calyx; Sleumer, 1967). Argent (1989), however, noted that *D. acuminata* also exhibits a berry fruit and on this basis suggested that *Pernettyopsis* should be included within *Diplycosia*. There are approximately 100 species of *Diplycosia* and they are endemic to southeast Asia (Sleumer 1967).

Tepuia is a small genus of only 8 species which occur in the tepuis of Venezuela (Luteyn, Neotropical Tepuia). The fruit of *Tepuia* is a berry with an unchanged calyx and it was considered a distinct genus due to its caducous calyx, long-pilose filaments, terminal anther tubules and pustular glands on the petiole bases (Luteyn, 1995c).

Powell and Kron (2001) analyzed sequence data from three gene regions (*matK*, nrITS and *atpB-rbcL* spacer) for 34 wintergreen group representatives and 19 outgroups and obtained the following results: 1) the wintergreen group was recovered as monophyletic with strong support (bt = 95); 2) *Tepuia* was recovered as the first-diverging lineage within the wintergreen group but with low support (bt < 50); 3) *Diplycosia* was found to be monophyletic and nested within *Gaultheria*; and 4) the former genus *Pernettya* was found nested within *Gaultheria*, with *P. tasmanica* Hook. f. grouping in a different clade than the other three species of *Pernettya* sampled.

The genus *Gaultheria* L. contains approximately 130 species that occur in New Zealand, Australia, eastern Asia, the Indomalaya region, and both tropical and temperate regions of the Americas (Middleton 1991). The genus is characterized by having superior ovaries, fruits that are either capsules or berries with either a fleshy accrescent calyx or an unchanged calyx (± dry at maturity), straight filaments and 4-awned anthers (Lutyen 1995a, 199b; Middleton, 1991). Species of *Gaultheria* typically have dry capsular fruits surrounded by a fleshy, often brightly colored (e.g. red, white, or blue) accrescent calyx (Middleton 1991). In some treatments *Pernettya* has been separated from *Gaultheria* because its fruit is a berry with an unchanged calyx (versus a capsule with typically a persistent fleshy accrescent calyx; e.g., Luteyn, 1995a, 1995b). As noted by Middleton and Wilcock (1990), however, some species of *Pernettya* have a fruit with a fully accrescent and fleshy calyx (i.e., *G. tasmanica*; Australia), or at least swollen calyx bases (i.e., *G. macrostigma* and *G. parvula*; New Zealand) in addition to the fleshy mature ovary wall. Furthermore, some species of *Gaultheria* have a capsule with a completely unchanged calyx (i.e., *G. crassa*, *G. colensoi*, *G. oppositifolia*, *G. paniculata* and *G. rupestris*; New Zealand). On this basis, Middleton and Wilcock (1990) subsumed *Pernettya* into *Gaultheria*. *Pernettya* has continued to be recognized by some authors (Luteyn 1995b; Stace *et al*. 1997).

Middleton (1991) divided *Gaultheria* (including *Pernettya*) into ten sections primarily on the character of inflorescence structure. Seven sections (50 species total) comprise solitary-flowered species, whereas the remaining sections (85 species total) comprise racemose inflorescences (Middleton, 1991). As noted by Middleton (1991), some species placed into the solitary-flowered sections can also exhibit few-flowered racemes, and one of the racemose sections includes one solitary-flowered species (*G. schultesii*; Mexico) whose sectional placement is based on other morphological characters (Middleton, 1991). Middleton (1991) agreed with Airy-Shaw (1940) that the solitary-flowered species were likely to have been derived from ancestors with racemose inflorescences, but neither author clarified the number of times this may have occurred.

There are 14 currently recognized species of *Gaultheria* in Australasia (here defined as Australia and New Zealand, with New Guinea excluded). Although the Australasian species of *Gaultheria* constitute merely 11% of the species diversity of the genus, together they encompass the extremes of variation across the genus in several morphological characters, as reflected in their distribution among four different sections (*G. nubicola*: *Chamaephyta* D. J. Middleton; *G. antipoda*, *G. depressa*: *Monoanthemona* D. J. Middleton; *G. lanceolata, G. macrostigma*, *G. parvula*, *G. tasmanica*: *Pernettya* (Gaud.) D. J. Middleton; *G. appressa*, *G. hispida, G. colensoi*, *G. crassa*, *G.*

oppositifolia, *G. paniculata*, *G. rupestris*: *Brossaea* (L.) D. J. Middleton; Middleton, 1991). *Gaultheria appressa* is endemic to New South Wales and Victoria, *G. hispida*, *G. lanceolata*, and *G. tasmanica* are endemic to Tasmania, and *G. antipoda*, *G. colensoi*, *G. crassa*, *G. macrostigma*, *G. nubicola*, *G. oppositifolia*, *G. paniculata*, *G. parvula*, and *G. rupestris* are endemic to New Zealand. *Gaultheria depressa* is the only species of *Gaultheria* to occur in both Australia (Tasmania) and New Zealand (Burtt and Hill 1935; Franklin 1962; Middleton 1991).

Notable variable characters in this group include a fleshy versus non-fleshy fruiting calyx, capsular versus baccate fruit, and a solitary-flowered inflorescence versus one that is racemose or paniculate. Only thirteen species of *Gaultheria* possess berries: *G. insana* plus all species of sect. *Pernettya*. Four of these (*G. lanceolata*, *G. macrostigma*, *G. parvula*, and *G. tasmanica*) occur in Australasia, whereas the remainder occur in the American tropics and temperate South America.

Gaultheria antipoda, *G. appressa*, *G. depressa*, and *G. hispida* all have the typical capsule/fleshy calyx fruit. Most species of Ericaceae have a capsule with a non-fleshy calyx. Although this condition occurs only rarely in *Gaultheria*, it is found in five New Zealand species (*G. colensoi*, *G. crassa*, *G. oppositifolia*, *G. paniculata*, and *G. rupestris*); it otherwise occurs only in *G. itatiaiae* (Wawra ex Drude) Sleumer and *G. sleumeriana* Kin.-Gouv. (southeastern Brazil), and *G. nubigena* B. L. Burtt & Sleumer (temperate South America; Middleton 1991).

Inflorescences of the Australasian species of *Gaultheria* are solitary-flowered in *G. antipoda*, *G. depressa, G. macrostigma, G. parvula* and *G. tasmanica* and racemose or paniculate in the remaining species. Some otherwise solitary-flowered species, such as

G. antipoda, can sometimes have inflorescences where the subtending leaves for each flower become smaller near the growing tip until the structure resembles a raceme (also referred to as a pseudoraceme), whereas others (e.g., *G. pyrolifolia* Hook. f. ex C.B. Clarke; eastern Asia) consistently exhibit pseudoracemes. This indicates that inflorescence structure may not be a reliable character for infrageneric classification in *Gaultheria*, unlike as implied in the literature.

Despite such divergent morphologies, the New Zealand species of *Gaultheria* readily hybridize, with mixed species populations often forming apparent hybrid swarms. Burtt & Hill (1935) described six putative hybrid combinations among the species with a dry capsule (treated as *Gaultheria* in the narrow sense), and five putative hybrids between capsular- and berry-fruited species (treated as *Pernettya*). Franklin (1962) expanded the number of parental species combinations to 14 among the capsular-fruited species, in almost every combination possible, and eight capsular-fruited × berry-fruited hybrids. Hybrid formation is apparently restricted to disturbed habitats, as in road cuts or braided river systems (Parsons and Hermanutz 2006; C. Bush, S. Wagstaff, pers. obs.) and thus the New Zealand species can still easily be discerned as distinct in ecologically stable environments. Furthermore, although hybrids are documented as occurring in other areas of the world besides New Zealand (Luteyn 1995*a*, Luteyn 1995*b*), in the majority of cases they appear to originate from parents from the same series or section [exceptions include *Gaultheria reticulata* Kunth X *G. myrsinoides* Kunth (sects. *Brossaea* and *Pernettya*) and *G. erecta* Vent. X *G. myrsinoides, G. anastomosans* Kunth and *G. vaccinioides* Weddell (sects. *Brossaea*, *Pernettya* and *Monoanthemona*)]. Thus, the apparent ease with which hybridization occurs between species from different sections of

Gaultheria in New Zealand suggests that these sections may be closely related or that the species within may actually form a monophyletic group. No detailed phylogenetic data for the Australia and New Zealand species of *Gaultheria* yet exist, however, that could be used to test this idea.

In tropical America, there is a prominent disjunct distribution of *Gaultheria* between the Andes and southeast Brazil. Forty-three species of *Gaultheria* occur in Latin America (primarily in the Andes) and of these only eight occur in Brazil (Luteyn 1991). Six species are endemic to Brazil while the remaining also occur in other parts of tropical America.

The endemic species in Brazil are morphologically very distinctive. Four species have the typical *Gaultheria* fruit condition of a fleshy calyx surrounding a capsule: *G. bradeana*, *G. myrtilloides*, *G. serrata* and *G. ulei* (Luteyn 1991). *Gaultheria bradeana* is a small shrub that can be found nested among mosses in boggy areas in cloud forest zones (Rio de Janeiro, Minas Gerais, São Paulo and Santa Catarina; Luteyn 1991). This species has the unique character of fleshy pedicels and bracteoles (pers. obs., C. Bush and P. Fritsch) as well as awnless anthers (Luteyn 1991). *Gaultheria myrtilloides* is a small shrub that occurs in higher elevations in the states of Minas Gerais, Rio de Janeiro and Rio Grande do Sul (Luteyn 1991; pers. obs., C. Bush and P. Fritsch). The plant is densely hirsute, persistent on the leaves, stems and calyces (Luteyn 1991). *Gaultheria serrata* is a widespread and common species, occurring in Espirito Santo/Minas Gerais, Rio de Janeiro, São Paulo, Santa Catarina and Parana at elevations from 1000 – 2800 m (Luteyn 1991). It has two varieties (*G. serrata* var. *serrata* and *G. serrata* var. *organensis*), both of which are found only in Brazil. This species has bright pink corollas in racemes and dark blue accrescent fleshy calyces (Luteyn 1991; pers. obs., C. Bush and P. Fristch). Of the two varieties recognized in the literature only *Gaultheria serrata* var. *serrata* is included in this study. Finally, *G. ulei* is endemic to Santa Catarina at elevations of 1200-1400 m (Luteyn 1991). This is the only endemic species not included in the present study because fresh material was unavailable.

Two species endemic to Brazil have dry calyces; a feature rarely found within *Gaultheria*. *Gaultheria itatiaiae* occurs in the cloud forest zone in the Minas Gerais/Rio de Janeiro border region (Itatiaiae National Park) south to Rio Grande do Sul (Luteyn 1991). It is distinct due to the combination of pseudoracemes, long calyx lobes and awnless anthers (Luteyn 1991). Previously, this species was thought to be without close relatives in *Gaultheria* and instead was considered closely related to *Agarista* (Luteyn 1991). *Gaultheria sleumeriana* is found in disturbed habitats on Serra da Bocaina in São Paulo. *Gaultheria sleumeriana* shares with *G. itatiaiae* dry calyces, long, acuminate calyx lobes, awnless anthers (in *G. sleumeriana* a short tubule is present) and capsules with slightly ridged sutures (Luteyn 1991). A unique morphological character in *G. sleumeriana* is the presence of a prominent submarginal nerve on both sides of the leaf (Luteyn 1991).

Data from a new species of *Gaultheria* formerly described as *"Gaylussacia corvensis"* has also been included in this study. The formal transfer of this species to *Gaultheria* is pending (pers. comm. Gérson Ramão). *"Gaylussacia corvensis"* has only been found on a man-made, steep, moist road cut at Serra do Corvo Branco near Grao Pará in Santa Catarina (pers. obs., C. Bush and P. Fritsch). Its fruit is a capsule surrounded by a slightly fleshy calyx (pers. obs., C. Bush and P. Fritsch).

There are two species of *Gaultheria* in Brazil that are also found in other areas of tropical South America. *Gaultheria erecta* is very common in the Andes, from Venezuela to northern Argentina (Luteyn 1991). It is rarely seen in Brazil and is known only from five collections (Amazonas, Minas Gerais, Santa Catarina; Luteyn 1991). The species is a large plant with puberulent and long-glandular hairs on the twigs and inflorescences (Luteyn 1991). Its mature fruit has a blue-black fleshy calyx (Luteyn 1991). *Gaultheria eriophylla* has two recognized varieties: *G. eriophylla* var. *eriophylla* is common in the montane forests of Rio de Janeiro, Minas Gerais, Espirito Santo and São Paulo while *G. eriophylla* var. *mucronata* is found in southern Peru and northern Bolivia (Luteyn 1991). The species is quite distinctive with tomentose-lanate leaves, rachises, pedicels and flowers (Luteyn 1991). Its fruit is a blue-black fleshy accrescent calyx (Luteyn 1991). Only the Brazilian variety of *G. eriophylla* (var. *eriophylla*) is sampled in this study.

Finally, *G. setulosa* was included by Luteyn (1991) in the Brazilian *Gaultheria*; however, this species occurs on the tepuis of the Guyana Highland in Venezuela and has currently not been collected in Brazil (Luteyn 1991). The species has a dwarf habit, small, thick leaves, prominent tertiary venation and a fleshy blue-black calyx at fruit maturity (Luteyn 1991). It is not included in the present study.

There are several other vascular and non-vascular plant groups that have distributions in the Andes as well as in southeast Brazil (Brade 1942; R. Tyron 1944; Smith 1962; A. Tryon 1962). It has been hypothesized that this distribution is the result of long-distance dispersal from the south-central Andes to southeast Brazil (Luteyn 1991; Gradstein and Reiner-Drehwald 2007).

Although the study of Powell and Kron (2001) represents a significant step forward in our understanding of the phylogeny and evolution of the wintergreen group and its close relatives, low taxon sampling precluded the resolution of some of the major issues regarding the classification of the tribe. The goals of this project are: (1) to test the monophyly of the Gaultherieae and the wintergreen group and to use molecular-based trees to determine the putative origins of the clades within the wintergreen group and evolution of morphological characteristics traditionally used to classify the genera (including fruit, calyx and inflorescence type), (2) to reconstruct the relationships within the wintergreen group, particulary in respect to the placement of *Diplycosia* and *Tepuia*, (3) to increase support and resolution of the phylogeny of *Leucothoe s.l.* + *Chamaedaphne* with the addition of molecular data, (4) to explore the relationships in the Australian/New Zealand species as well as their biogeographic origin and determine a new classification of the species based on molecular and morphological data and (5) to determine the closest relatives of the endemic and non-endemic species of *Gaultheria* in Brazil and test hypotheses of their origin.

Literature Cited

Airy-Shaw HK 1940 Studies in the Ericales. IV. Classification of the Asiatic species of *Gaultheria*. Kew Bull 1940:304–330.

Argent GCG 1989 *Diplycosia* in Borneo. Notes R Bot Gard Edinb 46:17–26.

Brade AC (1942) A composição da flora pteridófita do Itatiaia. *Rodriguésia* **6**, 26–43.

Britton, N.L. and A. Brown. 1913. An illustrated flora of the northern United States and Canada. Ed. 2. New York: Charles Scribner's Sons.

Burtt BL, Hill AW (1935) The genera *Gaultheria* and *Pernettya* in New Zealand, Tasmania, and Australia. *Journal of the Linnean Society: Botany* 49, 611–644.

Cox HT 1948 Studies in the comparative anatomy of the Ericales. II. Ericaceae—subfamily Arbutoideae. Am Midl Nat 40:493–516.

Franklin DA (1962) The Ericaceae in New Zealand (*Gaultheria* and *Pernettya*). *Transactions of the Royal Society of New Zealand* 1, 155–173.

Gradstein SR, Reiner-Drehwald ME (2007) The status of *Neopotamolejeunea* (Lejeuneaceae) and description of a new species from Ecuador and southern Brazil. *Systematic Botany* **32**, 487–492.

Hara, H. 1935. *Eubotryoides*. *Journal of Japanese Botany* 11: 626-627.

Honda, M. 1949. *Paraleucothoë*. *Journal of Japanese Botany* 24: 29.

Kron KA, WS Judd, DM Crayn 1999 Phylogenetic analyses of Andromedeae (Ericaceae subfam. Vaccinioideae). Am J Bot 86:1290– 1300.

Kron KA, WS Judd, F Stevens, DM Crayn, AA Anderberg, PA Gadek, CJ Quinn, JL Luteyn 2002 Phylogenetic classification of Ericaceae: molecular and morphological evidence. Bot Rev 68:335–423.

Luteyn, J.L., W.S. Judd, S.P. Vander Kloet, L.J. Dorr, G.D. Wallace, K.A. Kron, P.F. Stevens, and S.E. Clemants. 1996. Ericaceae of the southeastern United States. *Castanea* 61: 101-144.

Luteyn JL 1995a *Gaultheria*. Pages 384–488 in JL Luteyn, ed. Flora Neotropica monograph. Vol 66. Ericaceae, pt II. The superior-ovaried genera. N Y Bot Gard, New York.

——— 1995b *Pernettya*. Pages 365–383 in JL Luteyn, ed. Flora Neotropica monograph. Vol 66. Ericaceae, pt II. The superior-ovaried genera. N Y Bot Gard, New York.

——— 1995c *Tepuia*. Pages 351–364 in JL Luteyn, ed. Flora Neotropica monograph. Vol 66. Ericaceae, pt II. The superior-ovaried genera. N Y Bot Gard, New York.

Luteyn JL 1991 The genus *Gaultheria* in Brazil. *Bol. Mus. Para. Emílio Goeldi, sér. Bot.* 7, 309–333.

Melvin NC 1980 A systematic investigation of the genus *Leucothoë* (Ericaceae). PhD diss. University of Miami, Coral Gables, FL.

Middleton DJ 1991a Infrageneric classification of the genus *Gaultheria* L. Ericaceae. Bot J Linn Soc 106:229–258.

——— 1991b Ecology, reproductive biology and hybridisation in the genus *Gaultheria* L. (Ericaceae). Edinb J Bot 48:81–89.

Middleton DJ, Wilcock CC 1990 A critical examination of the status of *Pernettya* Gaud. as a genus distinct from *Gaultheria* L. Edinb J Bot 47:291–301.

——— 1993 A systematic survey of leaf and stem anatomical characters in the genus *Gaultheria* and related genera (Ericaceae). Bot J Linn Soc 113:199–215.

Nuttall, T. 1843. Description and notices of new or rare plants in the natural orders Lobeliaceae, Campanulaceae, Vaccinieae, Ericaceae, collected in a journey over the continent of North America, and during a visit to the Sandwich Islands, and Upper California. *Transactions of the American Philosophical Society* 8: 251-272.

Palser, B.F. 1951. Studies of floral morphology in the Ericales. I. Organography and vascular anatomy in the Andromedeae. *Botanical Gazette* 112: 447–485.

Palser, B.F. 1952. Studies of floral morphology in the Ericales. II. Megagametophyte development in the Andromedeae. *Botanical Gazette* 114: 33–52.

Parsons K, Hermanutz L 2006 Conservation of rare, endemic braya species (Brassicaceae): breeding system variation, potential hybridization and human disturbance. *Biological Conservation* 128, 201–214.

Powell EA, KA Kron 2001 An analysis of the phylogenetic relationships in the wintergreen group (*Diplycosia, Gaultheria, Pernettya, Tepuia*; Ericaceae). Syst Bot 26:808–817.

Sleumer, H. 1959. Studien uber die Gattung *Leucothoë* D. Don. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 78: 435-480.

Sleumer H 1967 Ericaceae. Flora Malesiana ser I, 6:669–914.

Small, J.K. 1914. Ericaceae. *North American Flora* 29: 33-102.

Smith LB (1962) Origins of the flora of southern Brazil. *Contr. U.S. Natl. Herb.* **35**, 215–249.

Stace HM, Chapman AR, Lemson KL, Powell JM (1997) Cytoevolution, phylogeny and taxonomy in Epacridaceae. *Annals of Botany* 79**,** 283–290.

Stevens, P.F. 1969. *Taxonomic studies in the Ericaceae*. Unpubl. Ph.D. thesis. Edinburgh, Scotland: University of Edinburgh.

Stevens, P.F. 1971. A classification of the Ericaceae: subfamilies and tribes. *Botanical Journal of the Linnean Society* 64: 1-53.

Tryon AF (1962) A monograph of the fern genus *Jamesonia*. *Contri. Gray Herb.* **191**, 109–197.

Tryon RM (1944) Dynamic phytogeography of *Doryopteris*. *Am. J. Bot.* **31**, 470–473.

Waselkov, K. and W.S. Judd. 2008. A phylogenetic analysis of *Leucothoë* s.l. (Ericaceae; tribe Gaultherieae) based on phenotypic characters. *Brittonia* 60: 382-397.

Wood, C.E. 1961. The genera of Ericaceae in the Southeastern United States. *Journal of the Arnold Arboretum* 42: 10-80.

CHAPTER II

PHYLOGENY OF GAULTHERIEAE (ERICACEAE: VACCINIOIDEAE) BASED ON DNA SEQUECE DATA FROM *MATK*, *NDHF* AND nrITS

Catherine M. Bush, Lu Lu, Peter W. Fritsch, De-Zhu Li, and Kathleen A. Kron

The following manuscript was published in *International Journal of Plant Sciences*, volume 170, pages 355-364, 2009, and is reprinted with permission. Stylistic variations are due to the requirements of the journal. C.M. Bush performed the field work, data analysis and prepared the manuscript. L. Lu and D. Li contributed sequence data to the publication. P.W. Fritsch was also present for the the field work and P.W. Fritsch and K.A. Kron provided logistical support, guidance and editorial assistance.

Abstract

Phylogenetic relationships within the tribe Gaultherieae (*Chamaedaphne*, *Diplycosia*, *Eubotrys*, *Gaultheria*, *Leucothoë*, and *Tepuia*) were examined with DNA sequence data from *matK*, *ndhF*, and nrITS. Parsimony analyses were performed in PAUP* for each gene region, combined chloroplast data, and total combined data. A Bayesian analysis was performed in MrBayes on the chloroplast and total combined data sets. The total combined parsimony and Bayesian analyses both strongly support the monophyly of the Gaultherieae and the wintergreen group (*Diplycosia*, *Gaultheria*, and *Tepuia*). *Diplycosia* is monophyletic and nested within part of *Gaultheria*. In the Bayesian total combined analysis, *Tepuia* is strongly supported as sister to a clade containing *Gaultheria procumbens*, *Gaultheria hispidula*, *Gaultheria leucocarpa* var. *cumingiana*, *Gaultheria leucocarpa* var. *leucocarpa*, and all sampled *Diplycosia* species. Optimization of morphological characters emphasized in classifications of the Gaultherieae onto the molecular phylogeny revealed that (1) fleshy calyces evolved either early in the lineage leading to the wintergreen group or later in two separate clades; (2) capsular fruits are plesiomorphic and berry fruits have evolved independently in *Diplycosia* and *Tepuia* and once or twice within a large clade containing all sampled sect. *Pernettya* members and *Gaultheria* species not exhibiting a berry fruit; and (3) the ancestral state for both the Gaultherieae and wintergreen group is a racemose inflorescence; solitary-flowered inflorescences have evolved at least eight times within the Gaultherieae, with several changes back to racemose or fascicular inflorescences.

Introduction

The tribe Gaultherieae (Ericaceae: subfamily Vaccinioideae) comprises *Chamaedaphne* Moench, *Diplycosia* Blume, *Eubotrys* Nutt., *Eubotryoides* (Nakai) Hara, *Gaultheria* Kalm ex L., *Leucothoë* D. Don, and *Tepuia* Camp., with a total of approximately 250 species. The tribe is defined by its four-appendaged anthers and a base chromosome number of 11, although both of these characters also occur in a closely related genus, *Zenobia* D. Don (tribe Andromedeae; Kron et al. 2002). The group exhibits an amphi-Pacific distribution, that is, temperate and tropical regions of the Americas, eastern Asia, Southeast Asia, and the Pacific (New Guinea, Australia, and New Zealand).

The phylogenetic relationships of the genera in the Gaultherieae have been investigated as part of a comprehensive study of the Ericaceae based on morphological and molecular data (Kron et al. 2002). This study found the Gaultherieae to be monophyletic and sister to the tribe Andromedeae (*Andromeda* L. and *Zenobia*) in the Vaccinioideae, although support for the Gaultherieae was low (bootstrap support [bt] < 50%). The wintergreen group within the Gaultherieae (*Diplycosia*, *Gaultheria*, and *Tepuia*; five species were sampled in total from among these genera) was recovered (bt = 92) as sister (bt < 50) to a clade comprising *Chamaedaphne calyculata* + *Eubotrys racemosa* (bt = 63). Sister to the entire clade was *Leucothoë fontanesiana* (bt < 50).

The wintergreen group is thought to be diagnosable by the presence of methyl salicylate, although this compound has apparently been lost in many species (or has not been detected) and is found in other ericads such as *Cavendishia* Lindl. and *Pyrola* L. as well as in unrelated families (*Betula* L.: Betulaceae; *Spiraea* L.: Rosaceae; Powell and

Kron 2001). Powell and Kron (2001) analyzed sequence data from three gene regions (*matK*, nrITS, and the *atpB-rbcL* spacer) for 34 wintergreen group representatives and 19 outgroups and obtained the following results: (1) the wintergreen group was recovered as monophyletic with strong support (bt = 95); (2) *Tepuia*, a small genus that is found only on the tepuis of Venezuela, was recovered as the first-diverging lineage within the wintergreen group but with low support (bt < 50); (3) *Diplycosia*, a predominantly epiphytic, Southeast Asian group, was found to be monophyletic and nested within *Gaultheria*; and (4) *Pernettya* Gaud., a genus often segregated from *Gaultheria* by its berry fruit (vs. a capsule), was found nested within *Gaultheria*, with *Pernettya tasmanica* Hook. f. grouping in a different clade than the other three species of *Pernettya* sampled.

The wintergreen genera historically have been recognized as closely related, but morphological characters have kept them taxonomically separated (Cox 1948). *Diplycosia* was considered unique due to the terminal tubules on its anthers, whereas *Gaultheria* members have awned anthers (Stevens 1995). *Diplycosia* has bracteoles that are conspicuously fused at the top of the pedicel (Stevens 1995) and free fibers in the mesophyll, although Middleton and Wilcock (1993) showed that the latter can be seen in some *Gaultheria* species. *Tepuia* exhibits unique long-pilose filaments as well as terminal anther tubules and pustular glands on the petiole bases (Luteyn 1995c).

Although the study of Powell and Kron (2001) represents a significant step forward in our understanding of the phylogeny and evolution of the wintergreen group and its close relatives, low taxon sampling precluded the resolution of some of the major issues regarding the classification of the tribe. Among these are the implications of inflorescence architecture evolution for infrageneric classification. Middleton (1991a) divided *Gaultheria* (including *Pernettya*) into ten sections primarily on this character. Seven sections (50 species total) comprise solitary-flowered species, whereas the remaining sections (85 species total) comprise racemose inflorescences (Middleton 1991a). As noted by Middleton (1991a), some species placed into the solitary-flowered sections can also exhibit few-flowered racemes, and one of the racemose sections includes one solitary flowered species (*Gaultheria schultesii*; Mexico) whose sectional placement is based on other morphological characters (Middleton 1991a). Middleton (1991a) agreed with Airy-Shaw (1940) that the solitary-flowered species were likely to have been derived from ancestors with racemose inflorescences, but neither author clarified the number of times this may have occurred.

Inflorescence architecture also varies among the other genera of the Gaultherieae. *Chamaedaphne* exhibits solitary-flowered inflorescences, whereas *Leucothoë*, *Eubotrys*, and *Tepuia* have racemes (Melvin 1980; Luteyn 1995c; Kron et al. 1999; Waselkov and Judd 2008). The inflorescences of *Diplycosia* can be solitary flowered or occur in fascicles of two to many flowers (Sleumer 1967). The fascicular condition seen in some species of *Diplycosia* and *Zenobia* is somewhat ambiguous because fascicles can also be described merely as reduced racemes (Judd et al. 2007), as has been done, for example, in *Gaultheria dumicola* and *Gaultheria wardii* (Middleton 1991a). Some species have one or two flowers per leaf axil (Sleumer 1967). Some species in *Gaultheria* section *Brossaeopsis* series *Dumicolae* exhibit extremely short inflorescence rachises that are similar to the fascicles commonly seen in *Diplycosia* (Airy-Shaw 1940; Middleton 1991a). Airy-Shaw (1940) based his hypothesis that these two groups are closely related on this similarity. No species of *Gaultheria* ser. *Dumicolae* were available to Powell and Kron (2001) with which to test this hypothesis.

Another major issue unresolved by Powell and Kron (2001) is the full impact of fruit evolution in the classification of the Gaultherieae. Whereas *Chamaedaphne*, *Eubotrys*, and *Leucothoë* have a capsule subtended by a persistent but unchanged calyx (i.e., the calyx is relatively the same shape, size, and thickness in both flower and fruit; Kron et al. 1999), the genera of the wintergreen group can have a capsule or a berry, with either an unchanged calyx or one that is accrescent and fleshy. *Pernettya* has been delimited from *Gaultheria* by a berry with an unchanged calyx (vs. a capsule with typically a persistent fleshy accrescent calyx; e.g., Luteyn 1995a, 1995b). As noted by Middleton and Wilcock (1990), however, some species of *Pernettya* have a fruit with a fully accrescent and fleshy calyx (cf. *Gaultheria tasmanica*; Australia), or at least swollen calyx bases (cf. *Gaultheria macrostigma* and *Gaultheria parvula*; New Zealand) in addition to the fleshy mature ovary wall. Furthermore, some species of *Gaultheria* have a capsule with a completely unchanged calyx (e.g., *G. crassa*, *G. colensoi*, *G. oppositifolia*, *G. paniculata*, and *G. rupestris*; New Zealand). On this basis, Middleton and Wilcock (1990) subsumed *Pernettya* into *Gaultheria*. Similarly, the monotypic genus *Pernettyopsis* King and Gamble has been delimited from *Diplycosia* by its berry (vs. a capsule; both have a fleshy accrescent calyx; Sleumer 1967). Argent (1989), however, noted that *Diplycosia acuminata* also exhibits a berry fruit and on this basis suggested that *Pernettyopsis* should be included within *Diplycosia*. Although *Tepuia* is similar to *Pernettya* in its berry with an unchanged calyx, it is unique within the Gaultherieae in that its calyx is also caducous and has thus been retained as a distinct genus (Luteyn 1995c).

The objective of this article is to build on the initial molecular study of the Gaultherieae conducted by Powell and Kron (2001) in the following ways: (1) generate a more inclusive and better-resolved phylogenetic estimate of the Gaultherieae by increasing taxon sampling and adding additional sequence data from the *ndhF* gene; (2) more accurately estimate the closest relative of *Diplycosia* by including a member of *Gaultheria* ser. *Dumicolae*; and (3) explore the evolution of fruit fleshiness and inflorescence architecture, two of the more prominent, traditionally important taxonomic characters within the Gaultherieae, in a phylogenetic context.

Material and Methods

DNA Extraction and Taxon Sampling

Total DNA was extracted from silica-dried fresh or herbarium material by using a modified CTAB extraction protocol (Doyle and Doyle 1987). Taxa sampled were two species of *Tepuia*, seven species of *Diplycosia*, 34 species of *Gaultheria* (*Gaultheria leucocarpa* is represented by two varieties), four species of *Leucothoë* , two species of *Eubotrys*, and the single *Chamaedaphne* species (51 terminals total; table A1, available in the online edition of the International Journal of Plant Sciences). The representative *Gaultheria* species are diverse in both their geographic location and inflorescence and calyx morphology. Brazil and New Guinea are two significant geographic regions that have not been sampled in this analysis yet whose inclusion could alter our results, particularly in the morphological evolution analyses. However, the majority of these taxa exhibits the typical capsule fruit condition with a fleshy accrescent calyx and therefore should not alter the results found in this study (Middleton 1991a). The included species represent eight of the 10 sections of Middleton (1991a), including 16 representatives from the most taxon-rich section (*Brossaea*). The outgroup comprises *Andromeda polifolia*, *Zenobia pulverulenta* (both Andromedeae), and *Vaccinium macrocarpon* (Vaccinieae; table A1). All taxa are included in every analysis (54 terminals in total; table A1). All analyses are rooted with *V. macrocarpon* because the Vaccinieae have been demonstrated to be sister to the Andromedeae + Gaultherieae clade (Kron et al. 2002).

Gene Regions and DNA Sequencing

Two chloroplast gene regions (*ndhF* and *matK*) and one nuclear gene region (nrITS) were sampled. The regions were amplified by using standard PCR techniques, with primers provided by Johnson and Soltis (1994) and Steele and Vilgalys (1994) for *matK*, White et al. (1990) for nrITS, and Olmstead and Sweere (1994) and Alverson et al. (1999) for *ndhF*. Additional primers were designed specifically for amplifying and sequencing the 3' *ndhF* end of *Gaultheria* (1036F: TTA GGA GCT ACT TTA GCG C; 1824R: CC AAA CCC ATT ACG GAT TGA TCG) because the traditional primers for the 3' end of *ndhF* failed for many taxa. These new primers amplify from position 1036 to position 1824 of the 2200 total base pairs for *ndhF* (Olmstead and Sweere 1994; Alverson et al. 1999). Due to this truncation as well as the removal of ambiguous alignment regions and areas that are incomplete due to the placement of primers in those locations, the total *ndhF* gene portion for this study is 1609 base pairs. Some sequences used in analyses were generated previously (Powell and Kron 2001); GenBank numbers for all taxa are listed in table A1. Bold GenBank numbers in table A1 represent new data generated for this study. PCR-amplified fragments were cleaned by using the Qiagen Gel Isolation Kit (Qiagen Sample and Assay Technologies, Valencia, CA). DNA was sequenced at the Wake Forest University Bowman Gray Technical Center DNA sequencing facility on an ABI 377 automated sequencer. Sequences were edited in Sequencher 3.1.1 (1988; Gene Codes) and aligned manually. Significant missing data in this analysis includes that of *Diplycosia apoensis* (3' end of ndhF), *Tepuia venusta* (~600 bp of *matK*), *Gaultheria foliolosa* (5' end of ndhF), *G. leucocarpa* var. *cumingiana* (3' end of *matK*; 5' end of *ndhF*), *G. leucocarpa* var. *leucocarpa* (3' end of *matK*), *Gaultheria pyroloides* (~600 bp of *matK*), and *Leucothoë griffithiana* (5' end of *ndhF*).

Phylogenetic Analyses

Parsimony analyses were performed first on single-gene matrices; if no strong conflict (>80% bt support) was seen between single-gene matrices, then the matrices were combined into a single matrix and analyzed as total-evidence data. Separate parsimony analyses were performed on the *ndhF*, *matK*, nrITS, combined chloroplast, and total combined data sets. Phylogenetic analyses were performed with PAUP* 4.0b2 by using the maximum parsimony settings (Swofford 1999). All characters were unordered, gaps were treated as missing data, and any areas exhibiting ambiguous alignments were excluded from the analyses. All characters were equally weighted, and only those that were parsimony-informative were included. Tree construction was performed by using a heuristic search with 1000 replicates, tree bisection and reconnection (TBR) branch swapping, and random stepwise addition. Clade support was estimated with bt analyses (Felsenstein 1985). For the total combined parsimony bt analysis, 1000 replicates of TBR branch swapping with 100 replicates in the heuristic search were performed. For the other four parsimony analyses (*ndhF*, *matK*, nrITS, combined chloroplast), "fast" bootstrap replicates were performed due to the long time required for a complete heuristic search. The values produced by fast bootstrapping will typically be lower than those produced by bootstrapping that involves heuristic searches, so the bootstrap values for these analyses is a conservative estimate (Mort et al. 2000). The fast bootstrap analysis consisted of 100,000 replicates for the *ndhF*, *matK*, nrITS, and combined chloroplast analyses.

The combined chloroplast and total combined data sets were analyzed in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). ModelTest 3.7 (Posada and Crandall 1998) was used to determine the best substitution types (Nst) and rate distribution models (rates) for the data sets. Both Bayesian analyses ran for 1,000,000 generations and were sampled every 100 generations. The burn-in period for both was the first 25% of the sampled generations. The model used for the chloroplast and combined analysis was GTR + I + G and TVM + I + G, respectively. Posterior probability values (pP) were calculated in MrBayes. The *ndhF*, *matK*, and nrITS individual analyses were not completed with Bayesian analyses because the algorithm did not reach an acceptable standard deviation of split frequencies (i.e., <0.01); results from each were predominantly unresolved in the parsimony analysis.

Morphological Evolution

For all analyses of morphological character evolution, the character states were optimized onto the single total combined Bayesian tree with MacClade 4.08 with all reconstructions shown (Maddison and Maddison 2005). All included taxa were scored.

Both persistent calyx fleshiness and fruit type were scored in the analysis of fruit evolution. The character states of the calyx are “calyx fleshy” and “calyx unchanged”. An unchanged calyx here refers to a calyx that does not enlarge or become fleshy by the time of fruit maturation. When an unchanged calyx becomes older (i.e., fruits from a previous year), the calyx can become dry and brittle (personal observation). For the purpose of this study, this condition is still considered an unchanged calyx because we are interested in the calyx condition at peak maturity only. The character states of fruit type are “capsule” and “berry”.

Inflorescence type was divided into solitary-flowered inflorescences, racemes, and fascicles. Sleumer (1967) was consulted for the species of *Diplycosia* sampled in the analysis. *Diplycosia barbigera* and *Diplycosia apoensis* usually have solitary-flowered inflorescences but sometimes have two-flowered inflorescences. These species were scored as having solitary flowers because this is the predominant condition in the two species (Sleumer 1967). All sampled species in the predominantly solitary-flowered sections of *Gaultheria* (i.e., sects. *Gaultheria*, *Chiogenopsis*, *Amblyandra*, *Chamaephyta*, *Gymnocaulos*, *Monoanthemona*, and *Pernettya*) were scored as solitary-flowered even though some species can also have few-flowered inflorescences. *Andromeda*, *Chamaedaphne*, *Leucothoë*, *Tepuia*, and *Zenobia* were scored by using relevant monographs and other publications (Melvin 1980; Luteyn 1995c; Kron et al. 1999, 2002). The caducous calyx condition in *Tepuia* is autapomorphic within the Gaultherieae (Luteyn 1995c) and therefore was not used in this analysis.

Results

Phylogenetic Analysis

Due to the low amount of resolution in the nrITS and separate and combined chloroplast data parsimony analyses, only the total combined data analyses (parsimony and Bayesian) will be discussed in detail.

The aligned nrITS data set is 525 nucleotide positions long and includes 81 parsimony-informative characters (15.4%). Areas of ambiguous alignment in the original matrix (characters 1–257; ITS 1) were excluded. There were 16,776 most parsimonious trees found (length [L] = 201; consistency index [CI] = 0.49; retention index [RI] = 0.78).

The aligned matK data set comprises 1540 nucleotide positions and includes 168 parsimony-informative characters (10.9%). No ambiguous areas in the alignment were present. The analysis recovered 2138 most parsimonious trees (L = 334; CI = 0.65; RI = 0.82). The aligned ndhF data set comprises 1569 nucleotide positions and 197 parsimony-informative characters (12.6%). Ambiguous alignment areas in the original matrix (959–1008) were excluded. The analysis recovered 646 most parsimonious trees (L = 452; CI = 0.61; RI = 0.81). Results of the separate *ndhF* and *matK* parsimony analyses were not well resolved, and there were no strongly supported clades that were in conflict between the two data sets; therefore, these data were combined. The combined chloroplast data set comprises 3109 nucleotide positions and 365 parsimony informative characters (11.7%). The analysis recovered nine most parsimonious trees (L = 792; CI = 0.62; RI = 0.81). There were only a few differences between the Bayesian chloroplast analysis and the Bayesian total combined data analysis. In the chloroplast analysis, *Gaultheria procumbens* is sister to *Tepuia* (pP = 0.87) whereas in the total combined Bayesian analysis, *Tepuia* is sister to the larger clade of *G. procumbens* + *Gaultheria hispidula* + *Gaultheria leucocarpa* var. *cumingiana* + *G. leucocarpa* var. *leucocarpa* + *Diplycosia* (pP = 1.00). Also, in the chloroplast analysis, *Gaultheria foliolosa* is sister to *Gaultheria insipida* + *Gaultheria strigosa* (pP = 1.00), but in the total combined data, the taxon is in a polytomy with *Gaultheria antarctica* (pP = 1.00). However, because the majority of the Bayesian chloroplast analysis is identical to that of the Bayesian total data analysis, only the latter will be discussed in detail.

There was a single point of conflict between the parsimony analyses of the chloroplast and nuclear data. In the *ndhF* analysis, *Gaultheria hookeri* was strongly supported as being sister to *Gaultheria fragrantissima* (bt = 95) whereas in the nrITS analysis *Gaultheria griffithiana* was sister to *G. fragrantissima* with moderate support (bt = 88; in the *matK* analyses, these clades were predominantly unresolved). Because the species involved in this conflict do not restrict our ability to make inferences about the general phylogeny and morphological evolution of the Gaultherieae in this analysis, all three data sets were still combined in order to create a total data phylogenetic evidence tree. The total combined parsimony analysis contained 3634 base pairs and 446 parsimony informative characters (12.3%). The analysis resulted in six most parsimonious trees (L = 1,021; CI = 0.58; RI = 0.79). Relationships in the total combined Bayesian analysis are nearly identical to that from the strict consensus of the parsimony analysis (figs. 2.1, 2.2). The Gaultherieae and wintergreen group are both monophyletic (bt = 100, 100; pP = 1.00 and 1.00, respectively). The species of *Eubotrys* and *Leucothoë* each form clades (bt = 98, 100; pP = 1.00, 1.00, respectively). *Leucothoë* forms the first-diverging clade within the Gaultherieae (bt = 100; pP = 1.00), and *Eubotrys* + *Chamaedaphne* (bt = 100; pP = 1.00) form the second-diverging clade (bt = 68; pP = 1.00).

Within the wintergreen group, the species of *Diplycosia* form a clade (bt = 99; pP = 1.00) that is sister to *Gaultheria leucocarpa* var. *cumingiana* + *G. leucocarpa* var. *leucocarpa* (bt = 100; pP = 1.00). The two species of *Tepuia* form a clade (bt = 100; pP = 1.00). In the Bayesian tree, *G. hispidula* is sister to the *G. leucocarpa* var. *cumingiana* + *G. leucocarpa* var. *leucocarpa* + *Diplycosia* clade (pP = 0.66), and *G. procumbens* is sister to this clade (pP = 0.63). The *Tepuia* clade is sister to this larger clade (pP = 1.00). In the parsimony consensus, the *Tepuia* clade is sister to *G. procumbens* (bt < 50) and this clade is in turn sister (bt = 60) to a clade containing *G. hispidula* + *G. leucocarpa* var. *cumingiana* + *G. leucocarpa* var. *leucocarpa* + *Diplycosia*.

The remaining species of the wintergreen group, all species of *Gaultheria*, form a clade (bt = 81; pP = 1.00) divided into two subclades (bt = 99, 52; pP = 1.00, 0.97, respectively). In the first of these, a South American clade (SA-1 in figs. 2.1, 2.2; bt = 99; pP = 1.00) is sister to *Gaultheria shallon* (bt = 100; pP = 1.00), and this larger clade is in turn sister (bt = 99; pP = 1.00) to a clade comprising *Gaultheria adenothrix* + *Gaultheria ovatifolia* (bt = 100; pP = 1.00). In the second, a South American clade (SA-2 in figs. 2.1, 2.2; bt = 91; pP = 1.00) is sister to *Gaultheria tasmanica*, the only sampled Australian taxon (bt = 70; pP = 0.99). Another South American clade (SA-3 in figs. 2.1, 2.2; bt = 100; pP = 1.00) is sister to SA-2 + *G. tasmanica* (bt = 100; pP = 1.00). An eastern Asian clade of *Gaultheria borneensis* and *Gaultheria pyroloides* (EA-1 in figs. 2.1, 2.2; bt =

100; pP = 1.00) is sister to the clade SA-2 + SA-3 + *G. tasmanica* (bt = 97; pP = 1.00). Another eastern Asian clade (EA-2 in figs. 2.1, 2.2; bt = 100; pP = 1.00) is sister to the clade SA-2 + SA-3 + G. tasmanica + EA-1 (bt = 52; pP = 0.97).

Morphological Evolution

There are two equally optimal reconstructions of calyx evolution over the total combined Bayesian tree, and each has four steps (fig. 2.3). In both optimizations, the most recent common ancestor (MRCA) of the Gaultherieae is inferred to have had an unchanged calyx (fig. 2.3). In one optimization, calyx fleshiness is gained along the branch to the wintergreen group, with reversals to an unchanged calyx along the branches to the *Tepuia* clade, the SA-3 clade, and *Gaultheria wardii*. In the other optimization, calyx fleshiness is gained along the branches to the *Diplycosia* + *G. leucocarpa* var. *cumingiana* + *G. leucocarpa* var. *leucocarpa* + *G. procumbens* + *G. hispidula* + *Tepuia* clade and the clade comprising SA + EA + *G. shallon* + *G. adenothrix* + *G. ovatifolia* + *G. tasmanica*, with reversals to an unchanged calyx along the branches to the SA-3 clade, *Tepuia*, and *G. wardii*.

There are four equally optimal reconstructions of fruit evolution over the total combined Bayesian tree, and each has five steps (fig. 2.4). In all optimizations, the MRCAs of both the Gaultherieae and the wintergreen group are inferred to have had a capsule. Independent gains to a berry occur along the branch to the *Tepuia* clade and in *Diplycosia acuminata* in all optimizations. The fruit changes to a berry along the branch to the SA-2 + SA-3 + *G. tasmanica* clade, followed by a reversal to a capsule in the branch leading to the SA-2 clade or the fruit changes to a berry along the branch leading to the SA-3 clade and along the branch to the SA-2 + *G. tasmanica* clade, followed by a reversal to a capsule in the clade SA-2.

There are six equally optimal reconstructions of inflorescence evolution over the total combined Bayesian tree, and each has 15 steps (fig. 2.5). In all optimizations, the MRCAs of the Gaultherieae and the wintergreen group are both inferred to have had a raceme. Within the Gaultherieae, there are eight independent gains of the solitary-flowered inflorescences: *Chamaedaphne*, *Gaultheria amoena*, *Gaultheria nummularioides*, *Gaultheria schultesii*, and along the branches to *Gaultheria sinensis* + *Gaultheria thymifolia*, SA-2 + SA-3 + *Gaultheria tasmanica*, *Gaultheria ovatifolia* + *Gaultheria adenothrix*, and *Diplycosia* + *G. leucocarpa* var. *cumingiana* + *G. leucocarpa* var. *leucocarpa* + *G. hispidula* + *G. procumbens*. In addition, there are reversals back to a raceme along the branch to *Gaultheria sclerophylla* + *Gaultheria megalodonta* + *G. insipida* + *G. strigosa* and in *G. leucocarpa* var. *cumingiana* + *G. leucocarpa* var. *leucocarpa* (fig. 2.5). Inflorescence type is inferred to be solitary flowered along the branch to Diplycosia, but within the Diplycosia clade, the changes between solitary flowered and fascicular inflorescences are equivocal.

Disscussion

The monophyly of the Gaultherieae was previously supported with DNA sequence data from *matK* and *rbcL* but with less than 50% bootstrap support (Kron et al. 2002). Our analyses recover a monophyletic Gaultherieae with strong support (bt = 100; pP = 1.00). Our analyses also strongly support a monophyletic wintergreen group (bt = 100; pP = 1.00), as found previously with combined *matK*, *atpB-rbcL* spacer, and nrITS sequence data (bt = 95–99; Powell and Kron 2001).

As in a previous study (Powell and Kron 2001), *Diplycosia* was found to be monophyletic, and its sister clade was found to be *Gaultheria leucocarpa* var. *cumingiana* (= *Gaultheria cumingiana* from Powell and Kron 2001) + *G. leucocarpa* var. *leucocarpa* (bt = 100, pP = 1.00: our results; bt = 98, Powell and Kron 2001 [with combined *matK*, *atpB-rbcL*, and nrITS data, as *G. cumingiana*; *G. leucocarpa* var. *leucocarpa* was not sampled in Powell and Kron 2001]). The "*Gaultheria leucocarpa*" that was sister to *Gaultheria nummularioides* in the *matK* analysis (100% bt) of Powell and Kron (2001) was determined later to be an accession of *Gaultheria hookeri*. Morphological studies had suggested that *Gaultheria dumicola* (Himalayan distribution) is closely related to *Diplycosia* due its extremely shortened raceme, which is similar to the fascicular inflorescence of *Diplycosia* (Sleumer 1967; Middleton 1991a). In our study, *G. dumicola* never groups with *Diplycosia*; instead, it is recovered in the EA-2 clade with strong support (bt = 94; pP = 0.98).

The two members of *Tepuia* sampled consistently form a clade in our analyses. In the parsimony analysis, the *Tepuia* clade is weakly supported as sister to *Gaultheria procumbens*, but in the Bayesian analysis, it is sister to the *G. procumbens* + *Gaultheria* *hispidula* + *G. leucocarpa* var. *cumingiana* + *G. leucocarpa* var. *leucocarpa* + *Diplycosia* clade with strong support (pP = 1.00). This is the first published phylogenetic analysis that reveals the potential closest relatives of *Tepuia*. Increased taxon sampling in this group is difficult due to their range in the tepuis of Venezuela, but more extensive sampling is desired to further explore the relationships and evolution of this clade within the wintergreen group.

In our study, two varieties of *G. leucocarpa* were included: *G. leucocarpa* var. *cumingiana* (Philippines; = *G. cumingiana* in Powell and Kron 2001) and *G. leucocarpa* var. *leucocarpa* (Malaysian Peninsula; not sampled in Powell and Kron 2001). Our results for this widespread taxon corroborate those found in Powell and Kron (2001). *Gaultheria leucocarpa* var. *cumingiana* and *G. leucocarpa* var. *leucocarpa*, both species from Southeast Asia, are recovered with strong support as the sister clade to *Diplycosia*, a predominantly Southeast Asian group. In Powell and Kron (2001), *G. cumingiana* is sister to the *Diplycosia* clade, with strong support (bt = 98 in the combined analysis of *matK*, *atpB-rbcL* spacer, and nrITS). The widespread species occurs from 500 to 3300 m altitude in 10 provinces of China, including Taiwan, and also occurs in Cambodia, Indonesia, Laos, Malaysia, the Philippines, Thailand, and Vietnam (Fang and Stevens 2005).

Our study offers an overall improvement in both clade resolution and support values as compared to that of Powell and Kron (2001). In general, shared taxa between the studies are recovered in the same clades, although resolution at the deeper nodes is substantially higher in ours. For example, in the parsimony analyses of Powell and Kron (2001), *G. hispidula* (once placed in the monotypic genus *Chiogenes* due to the presence of a semi-inferior ovary; Airy-Shaw 1940) forms a polytomy with *Tepuia* and two other clades of the wintergreen group. In both the parsimony and Bayesian analyses from our study, however, *G. hispidula* is sister to *G. leucocarpa* var. *cumingiana* + *G. leucocarpa* var. *leucocarpa* + *Diplycosia* with low support (bt = 51; pP = 0.75). Also, in Powell and Kron (2001), *Gaultheria shallon* was sister to *Gaultheria eriophylla* with moderate support (bt = 79), whereas in our study, it is sister to clade SA-1 with strong support (bt = 100; pP = 1.00).

The analysis of calyx evolution indicates that the ancestral condition for the tribe Gaultherieae is probably a dry fruit (fig. 2.3). The ancestral condition for the wintergreen group, however, is equivocal (fig. 2.3). Dry calyces evolved at least twice within the wintergreen group (fig. 2.3). The data for calyx fleshiness in *Gaultheria wardii* was taken from Middleton (1991a); however, in the Flora of China series, Fang and Stevens (2005) report that *G. wardii* has a fleshy calyx. Personal observation indicates that *G. wardii* has a semifleshy, thin, mealy calyx. If *G. wardii* is scored as having a fleshy calyx (vs. unchanged), this would indicate that unchanged calyces only arose twice within the wintergreen group: in clade SA-3 and Tepuia. Based on our current observations, it appears that dry calyces usually co-occur with a berry fruit. Future field work and analyses will test this observation.

All the sampled temperate South American *Pernettya* members (i.e., *Gaultheria mucronata*, *Gaultheria poeppigii*, and *Gaultheria pumila*) are found in clade SA-3, and all possess berries. The only other sampled *Pernettya* member, *Gaultheria tasmanica*, is sister to the dry-capsule SA-2 clade, and it exhibits a berry with a completely accrescent fleshy calyx (Middleton 1991a). Berries also independently arose in *Diplycosia acuminata* and *Tepuia* (fig. 2.4).

The calyx and fruit analyses have significant implications for the classification of the wintergreen group. Based on phylogenies that strongly support a monophyletic wintergreen group (i.e., our study; Powell and Kron 2001), *Diplycosia*, *Gaultheria*, and *Tepuia* should be included in *Gaultheria* s.l. The alternative of dividing the group into multiple smaller genera is suboptimal because it may be difficult or impossible to determine morphological synapomorphies for some of these clades, (e.g., *Diplycosia* + *G. leucocarpa* var. *cumingiana* + *G. leucocarpa* var. *leucocarpa* + *G. hispidula* + *G. procumbens*), whereas the presence of methyl salicylate is a potential synapomorphy for *Gaultheria* s.l. The infrageneric classification of *Gaultheria* s.l. could use calyx and fruit characteristics to determine sectional divisions within the genus.

A solitary-flowered inflorescence was traditionally thought to be a derived condition within *Gaultheria* (Airy-Shaw 1940). Although both Airy-Shaw (1940) and Middleton (1991a) appear to have been accurate in their assertion that racemose inflorescences are ancestral in *Gaultheria*, the high number of times solitary-flowered inflorescences appear in the evolution of the group suggests that inflorescence type is not an appropriate basis for the higher-level classification of the tribe. Even at the lower levels of classification, the use of inflorescence type may not be useful; for example, the large section *Brossaea* is highly paraphyletic based on our results (fig. 2.5).

There is evidence that the evolution of inflorescence type is affected by several factors, such as the rate of self-fertilization and resource availability (Schoen and Dubuc 1990). Studies have shown that *Gaultheria myrsinoides*, *G. hispidula*, *G. shallon*, *Gaultheria domingensis*, *Gaultheria gracilis*, *Gaultheria odorata*, *G. procumbens*, *G. nummularioides*, *G. leucocarpa*, and *Gaultheria punctata* tend to have low insect visitation and high self-compatibility (Sleumer 1967; Mirick and Quinn 1981; Middleton 1991b). When self-compatibility is frequent and inbreeding depression is high, the stable size of an inflorescence can decrease (Schoen and Dubuc 1990). The homoplasious characteristic of inflorescence type demonstrated here may be dependent on these complex factors.

This study confirms the monophyly of the Gaultherieae and the wintergreen group with strong support. Internal relationships within the tribe, the wintergreen group, and Gaultheria are more resolved than those from previous studies. Many questions remain about the evolutionary factors that may have led to the pattern of fruit and inflorescence structure exhibited in *Gaultheria*. This phylogenetic work will form the basis for a future biogeographical analysis as well as the eventual reclassification of the genera within the Gaultherieae.

Literature Cited

Airy-Shaw HK 1940 Studies in the Ericales. IV. Classification of the Asiatic species of *Gaultheria*. Kew Bull 1940:304–330.

Alverson WS, BA Whitlock, R Nyffeler, C Bayer, DA Baum 1999 Phylogeny of the core Malvales: evidence from ndhF sequence data. Am J Bot 86:1474–1486.

Argent GCG 1989 *Diplycosia* in Borneo. Notes R Bot Gard Edinb 46:17–26.

Cox HT 1948 Studies in the comparative anatomy of the Ericales. II. Ericaceae—subfamily Arbutoideae. Am Midl Nat 40:493–516.

Doyle JJ, JL Doyle 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15.

Fang RZ, PF Stevens 2005 *Gaultheria*. Pages 464–475 in ZYWu, PH Raven, DY Hong, eds. Flora of China. Vol 14 (Apiaceae through Ericaceae). Science, Beijing; Mo Bot Gard, St. Louis.

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

Johnson LA, DE Soltis 1994 matK DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. Syst Bot 19:143–156.

Judd WS, CS Campbell, EA Kellogg, PF Stevens, MJ Donoghue 2007 Plant systematics: a phylogenetic approach. Sinauer, Sunderland,MA.

Kron KA, WS Judd, DM Crayn 1999 Phylogenetic analyses of Andromedeae (Ericaceae subfam. Vaccinioideae). Am J Bot 86:1290– 1300.

Kron KA, WS Judd, F Stevens, DM Crayn, AA Anderberg, PA Gadek, CJ Quinn, JL Luteyn 2002 Phylogenetic classification of Ericaceae: molecular and morphological evidence. Bot Rev 68:335–423.

Luteyn JL 1995a *Gaultheria*. Pages 384–488 in JL Luteyn, ed. Flora Neotropica monograph. Vol 66. Ericaceae, pt II. The superior-ovaried genera. N Y Bot Gard, New York.

——— 1995b *Pernettya*. Pages 365–383 in JL Luteyn, ed. Flora Neotropica monograph. Vol 66. Ericaceae, pt II. The superior-ovaried genera. N Y Bot Gard, New York.

——— 1995c *Tepuia*. Pages 351–364 in JL Luteyn, ed. Flora Neotropica monograph. Vol 66. Ericaceae, pt II. The superior-ovaried genera. N Y Bot Gard, New York.

Maddison DR, WP Maddison 2005 MacClade 4.08: an analysis of phylogeny and character evolution. Sinauer, Sunderland, MA.

Melvin NC 1980 A systematic investigation of the genus *Leucothoë* (Ericaceae). PhD diss. University of Miami, Coral Gables, FL.

Middleton DJ 1991a Infrageneric classification of the genus *Gaultheria* L. Ericaceae. Bot J Linn Soc 106:229–258.

——— 1991b Ecology, reproductive biology and hybridisation in the genus *Gaultheria* L. (Ericaceae). Edinb J Bot 48:81–89.

Middleton DJ, Wilcock CC 1990 A critical examination of the status of *Pernettya* Gaud. as a genus distinct from *Gaultheria* L. Edinb J Bot 47:291–301.

——— 1993 A systematic survey of leaf and stem anatomical characters in the genus *Gaultheria* and related genera (Ericaceae). Bot J Linn Soc 113:199–215.

Mirick S, JA Quinn 1981 Some observations on the reproductive biology of *Gaultheria procumbens* (Ericaceae). Am J Bot 68:1298– 1305.

Mort ME, PS Soltis, DE Soltis, ML Mabry 2000 Comparison of three methods for estimating internal support on phylogenetic trees. Syst Biol 49:160–171.

Olmstead RG, JA Sweere 1994 Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. Syst Biol 43:467–481.

Posada D, KA Crandall 1998 Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

Powell EA, KA Kron 2001 An analysis of the phylogenetic relationships in the wintergreen group (*Diplycosia, Gaultheria, Pernettya, Tepuia*; Ericaceae). Syst Bot 26:808–817.

Ronquist F, JP Huelsenbeck 2003 MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Schoen DJ, M Dubuc 1990 The evolution of inflorescence size and number: a gamete-packaging strategy in plants. Am Nat 135:841– 857.

Sleumer H 1967 Ericaceae. Flora Malesiana ser I, 6:669–914.

Steele KP, R Vilgalys 1994 Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene matK. Syst Bot 19: 126–142.

Stevens P 1995 Familial and infrafamilial relationships. Pages 1–12 in JL Luteyn, ed. Flora Neotropica monograph. Vol 66. Ericaceae, pt II. The superior-ovaried genera. N Y Bot Gard, New York.

Swofford DL 1999 PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.01. Sinauer, Sunderland, MA.

White TJ, T Bruns, S Lee, J Taylor 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 in M Innis, D Gelfand, J Sninsky, T White, eds. PCR protocols: a guide to methods and applications. Academic Press, San Diego, CA.

Figure 2.1

Strict consensus of the six most parsimonious trees (L = 1,021, CI = 0.58, RI = 0.79) from the total combined data parsimony analysis (*matK*, *ndhF*, and nrITS). Bootstrap values are indicated above the branches. SA = South America; EA = eastern Asia. Species placed within Gaultheria section *Pernettya* (Middleton 1991a) are shown in bold.

Figure 2.1

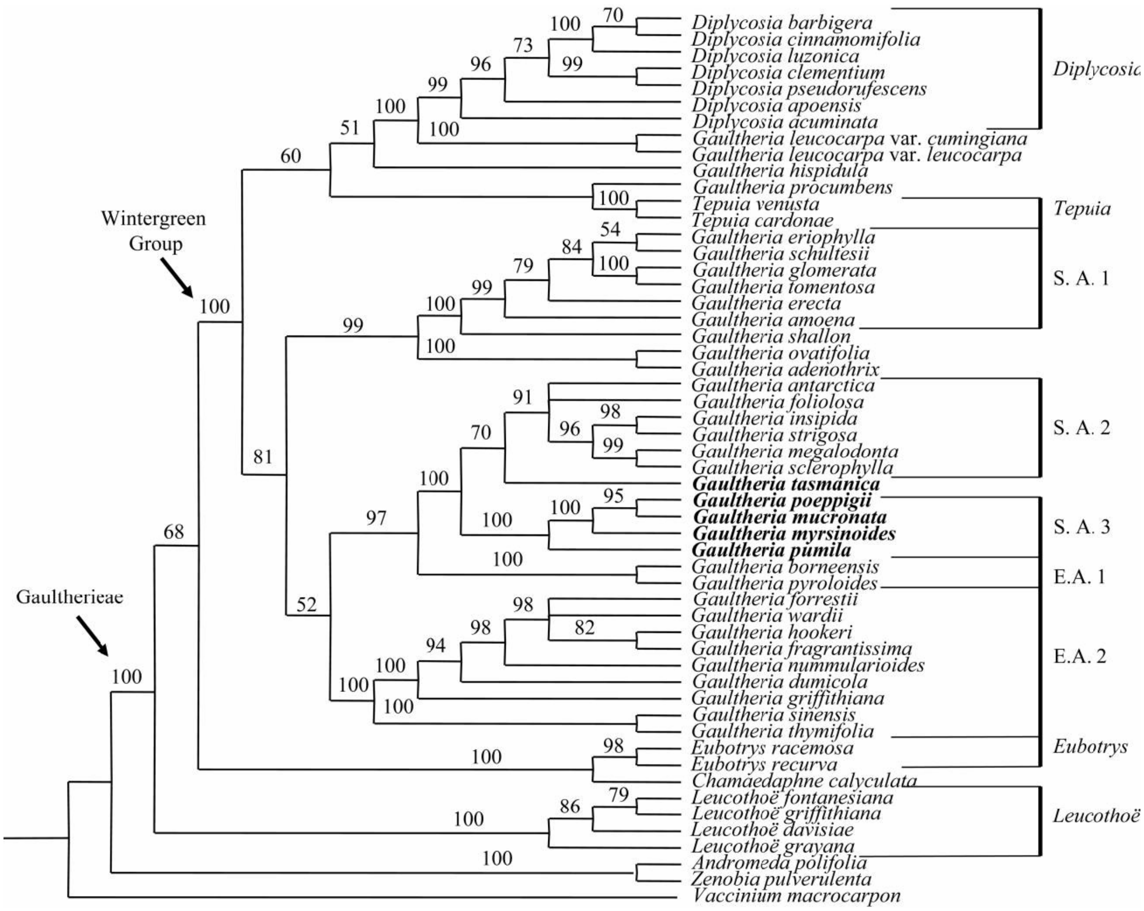

Diplycosia barbigera
Diplycosia cinnamomifolia
Diplycosia luzonica
Diplycosia clementium
Diplycosia pseudorufescens
Diplycosia apoensis
Diplycosia acuminata
Gaultheria leucocarpa var. cumingiana
Gaultheria leucocarpa var. leucocarpa
Gaultheria hispidula
Gaultheria procumbens
Tepuia venusta
Tepuia cardonae
Gaultheria eriophylla
Gaultheria schultesii
Gaultheria glomerata
Gaultheria tomentosa
Gaultheria erecta
Gaultheria amoena
Gaultheria shallon
Gaultheria ovatifolia
Gaultheria adenothrix
Gaultheria antarctica
Gaultheria foliolosa
Gaultheria insipida
Gaultheria strigosa
Gaultheria megalodonta
Gaultheria sclerophylla
Gaultheria tasmanica
Gaultheria poeppigii
Gaultheria mucronata
Gaultheria myrsinoides
Gaultheria pumila
Gaultheria borneensis
Gaultheria pyroloides
Gaultheria forrestii
Gaultheria wardii
Gaultheria hookeri
Gaultheria fragrantissima
Gaultheria nummularioides
Gaultheria dumicola
Gaultheria griffithiana
Gaultheria sinensis
Gaultheria thymifolia
Eubotrys racemosa
Eubotrys recurva
Chamaedaphne calyculata
Leucothoë fontanesiana
Leucothoë griffithiana
Leucothoë davisiae
Leucothoë grayana
Andromeda polifolia
Zenobia pulverulenta
Vaccinium macrocarpon
Diplycosia
Tepuia
S. A. 1
S. A. 2
S. A. 3
E.A. 1
E.A. 2
Eubotrys
Leucothoë
Wintergreen Group
Gaultherieae
70
100
73
99
96
99
100
100
51
60
100
54
84
100
79
99
100
99
100
91
98
96
99
70
100
95
100
100
97
100
81
98
82
98
94
100
100
100
52
100
68
100
98
79
86
100
100
100

Figure 2.2

Single consensus tree from a Bayesian analysis of the total combined data set (*matK*, *ndhF*, and nrITS). Posterior probabilities are indicated above the branches (in percent probability). SA = South America; EA = eastern Asia. Species placed within *Gaultheria* section *Pernettya* (Middleton 1991a) are shown in bold.

Figure 2.2

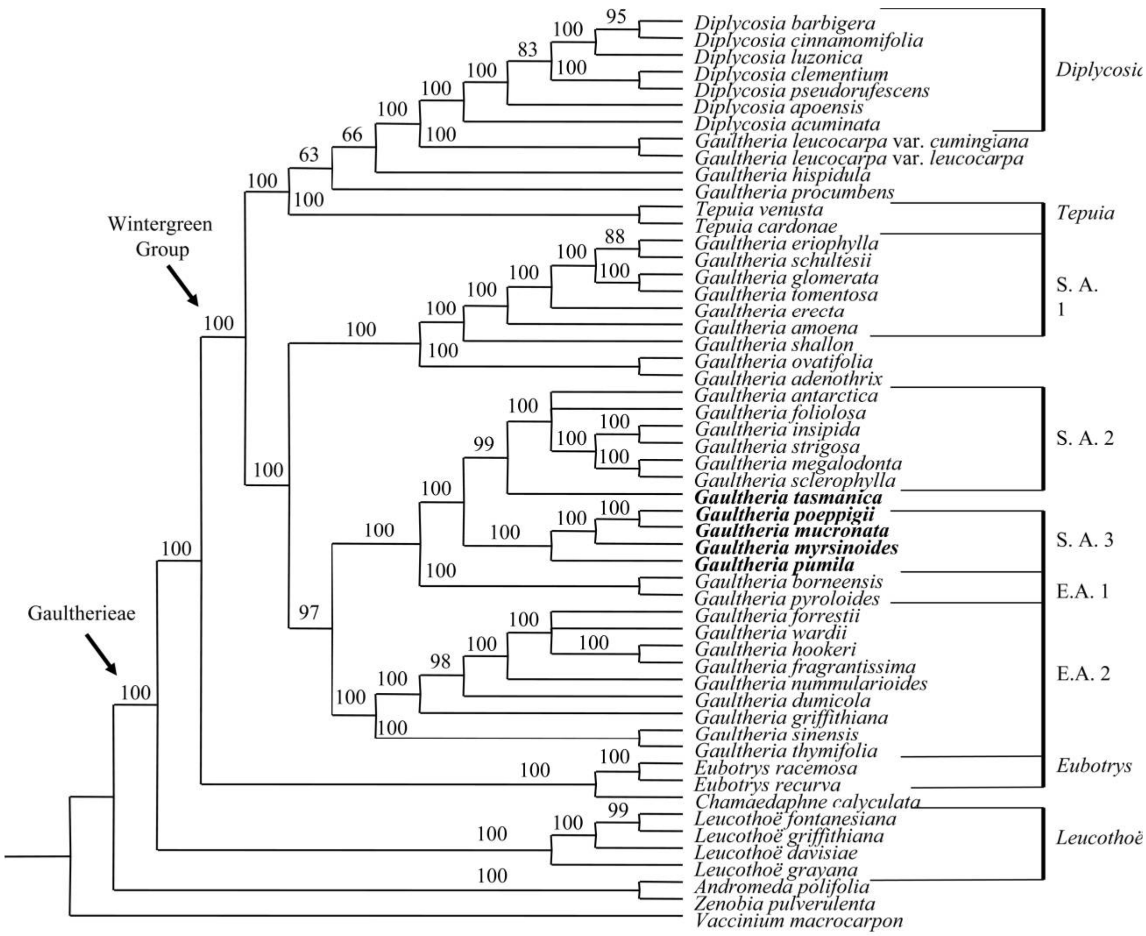

Diplycosia barbigera
Diplycosia cinnamomifolia
Diplycosia luzonica
Diplycosia clementium
Diplycosia pseudorufescens
Diplycosia apoensis
Diplycosia acuminata
Gaultheria leucocarpa var. cumingiana
Gaultheria leucocarpa var. leucocarpa
Gaultheria hispidula
Gaultheria procumbens
Tepuia venusta
Tepuia cardonae
Gaultheria eriophylla
Gaultheria schultesii
Gaultheria glomerata
Gaultheria tomentosa
Gaultheria erecta
Gaultheria amoena
Gaultheria shallon
Gaultheria ovatifolia
Gaultheria adenothrix
Gaultheria antarctica
Gaultheria foliolosa
Gaultheria insipida
Gaultheria strigosa
Gaultheria megalodonta
Gaultheria sclerophylla
Gaultheria tasmanica
Gaultheria poeppigii
Gaultheria mucronata
Gaultheria myrsinoides
Gaultheria pumila
Gaultheria borneensis
Gaultheria pyroloides
Gaultheria forrestii
Gaultheria wardii
Gaultheria hookeri
Gaultheria fragrantissima
Gaultheria nummularioides
Gaultheria dumicola
Gaultheria griffithiana
Gaultheria sinensis
Gaultheria thymifolia
Eubotrys racemosa
Eubotrys recurva
Chamaedaphne calyculata
Leucothoë fontanesiana
Leucothoë griffithiana
Leucothoë davisiae
Leucothoë grayana
Andromeda polifolia
Zenobia pulverulenta
Vaccinium macrocarpon
Diplycosia
Tepuia
S. A. 1
S. A. 2
S. A. 3
E.A. 1
E.A. 2
Eubotrys
Leucothoë
Wintergreen Group
Gaultherieae
95
100
83
100
100
100
100
66
100
63
100
100
88
100
100
100
100
100
100
100
100
100
100
100
99
100
100
100
100
100
100
100
97
100
100
98
100
100
100
100
100
100
100
99
100
100
100

Figure 2.3

Calyx morphology traced onto the single consensus tree from a Bayesian analysis of the total combined data set (*matK*, *ndhF*, and nrITS). Character states: gray branches = fleshy accrescent fruiting calyx; black branches = calyx unchanged in fruit; and branches with vertical lines = equivocal state. SA = South America; EA = eastern Asia. Species placed within *Gaultheria* section *Pernettya* (Middleton 1991a) are shown in bold.

Figure 2.3

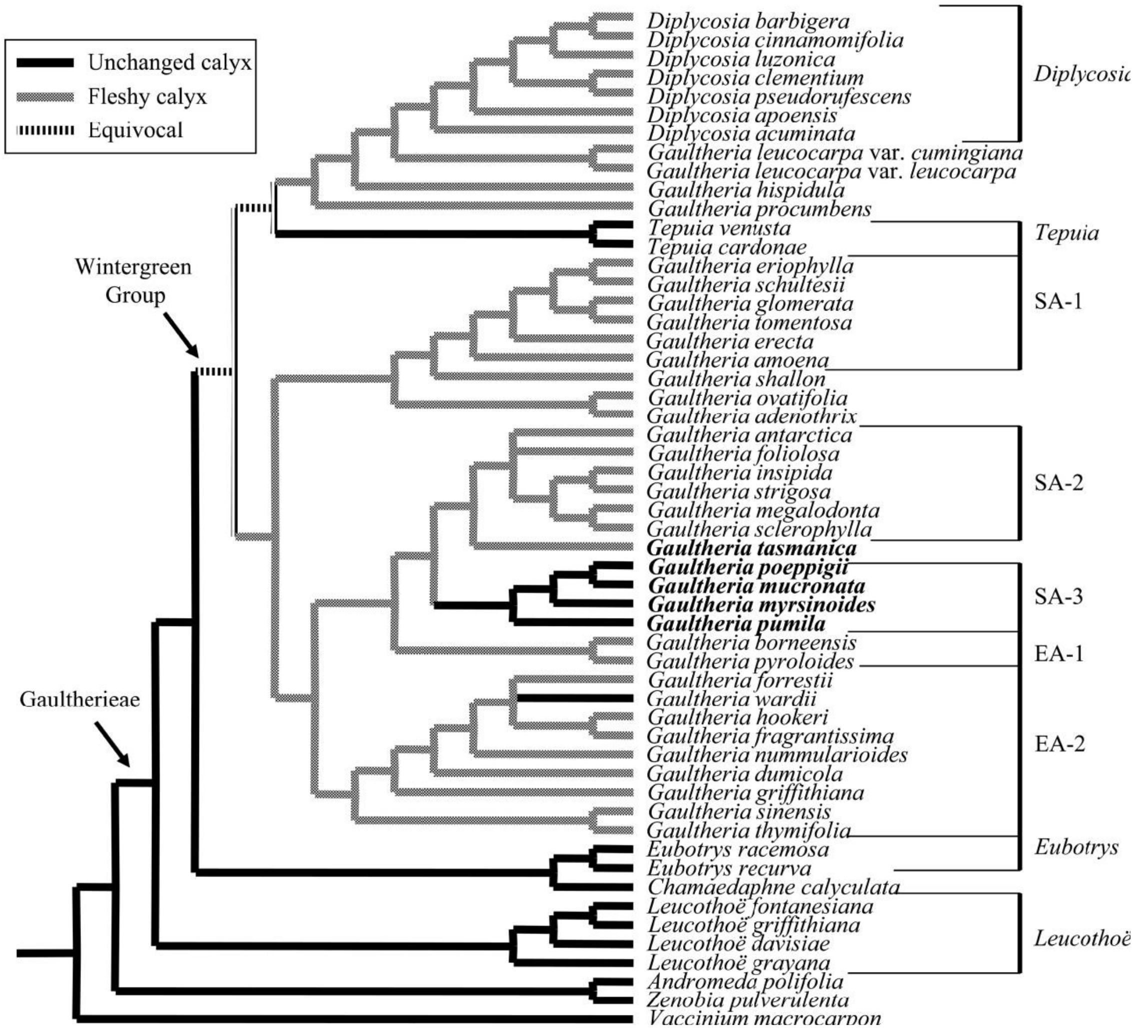
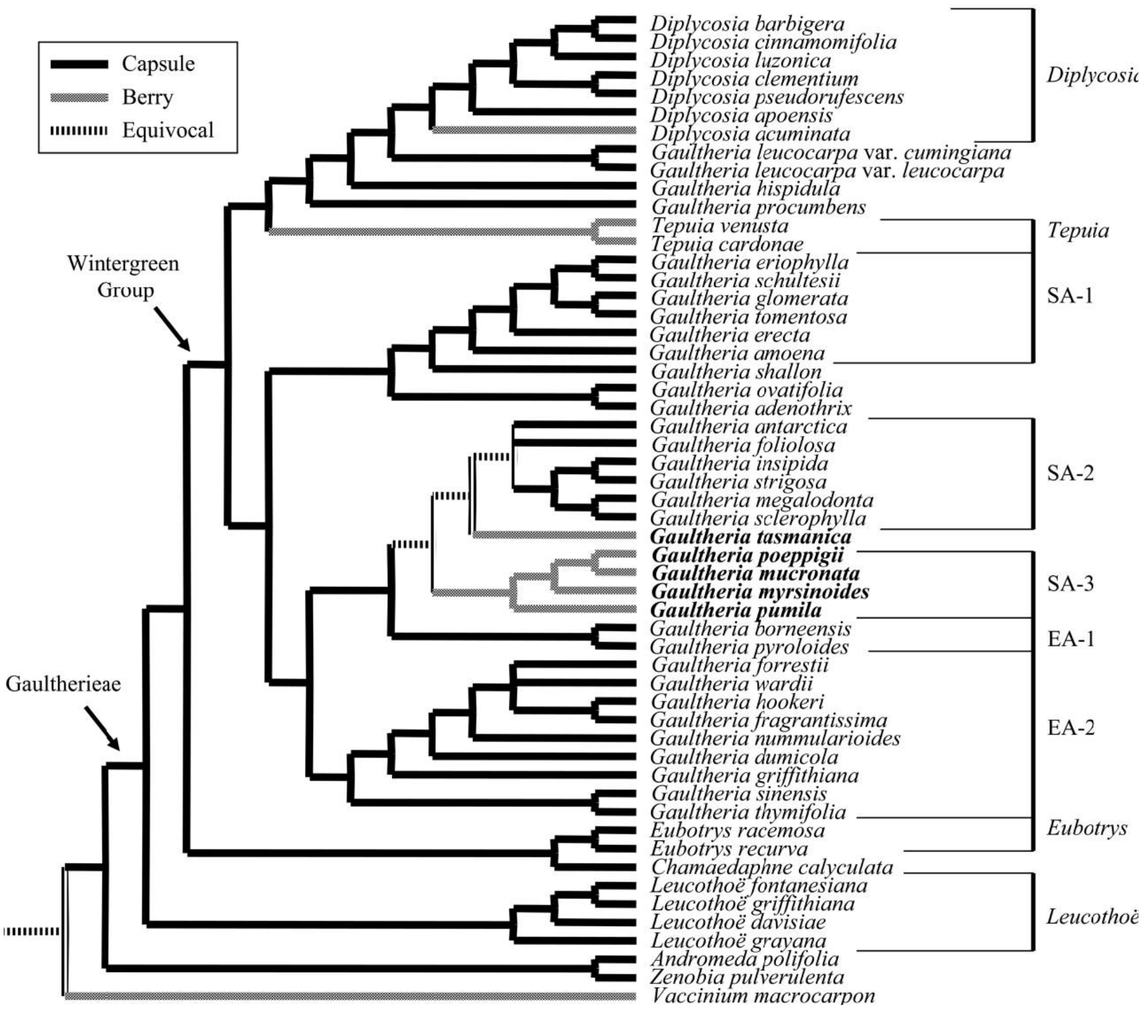

Unchanged calyx
Fleshy calyx
Equivocal
Wintergreen Group
Gaultherieae
Diplycosia barbigera
Diplycosia cinnamomifolia
Diplycosia luzonica
Diplycosia clementium
Diplycosia pseudorufescens
Diplycosia apoensis
Diplycosia acuminata
Gaultheria leucocarpa var. cumingiana
Gaultheria leucocarpa var. leucocarpa
Gaultheria hispidula
Gaultheria procumbens
Tepuia venusta
Tepuia cardonae
Gaultheria eriophylla
Gaultheria schultesii
Gaultheria glomerata
Gaultheria tomentosa
Gaultheria erecta
Gaultheria amoena
Gaultheria shallon
Gaultheria ovatifolia
Gaultheria adenothrix
Gaultheria antarctica
Gaultheria foliolosa
Gaultheria insipida
Gaultheria strigosa
Gaultheria megalodonta
Gaultheria sclerophylla
Gaultheria tasmanica
Gaultheria poeppigii
Gaultheria mucronata
Gaultheria myrsinoides
Gaultheria pumila
Gaultheria borneensis
Gaultheria pyroloides
Gaultheria forrestii
Gaultheria wardii
Gaultheria hookeri
Gaultheria fragrantissima
Gaultheria nummularioides
Gaultheria dumicola
Gaultheria griffithiana
Gaultheria sinensis
Gaultheria thymifolia
Eubotrys racemosa
Eubotrys recurva
Chamaedaphne calyculata
Leucothoë fontanesiana
Leucothoë griffithiana
Leucothoë davisiae
Leucothoë grayana
Andromeda polifolia
Zenobia pulverulenta
Vaccinium macrocarpon
Diplycosi
Tepuia
SA-1
SA-2
SA-3
EA-1
EA-2
Eubotrys
Leucothoë

Figure 2.4

Fruit morphology traced onto the single consensus tree from a Bayesian analysis of the total combined data set (*matK*, *ndhF*, and nrITS). Character states: gray branches = berry fruit; black branches = capsular fruit; and branches with vertical lines = equivocal state. SA = South America; EA = eastern Asia. Species placed within *Gaultheria* section *Pernettya* (Middleton 1991a) are shown in bold.

Figure 2.4

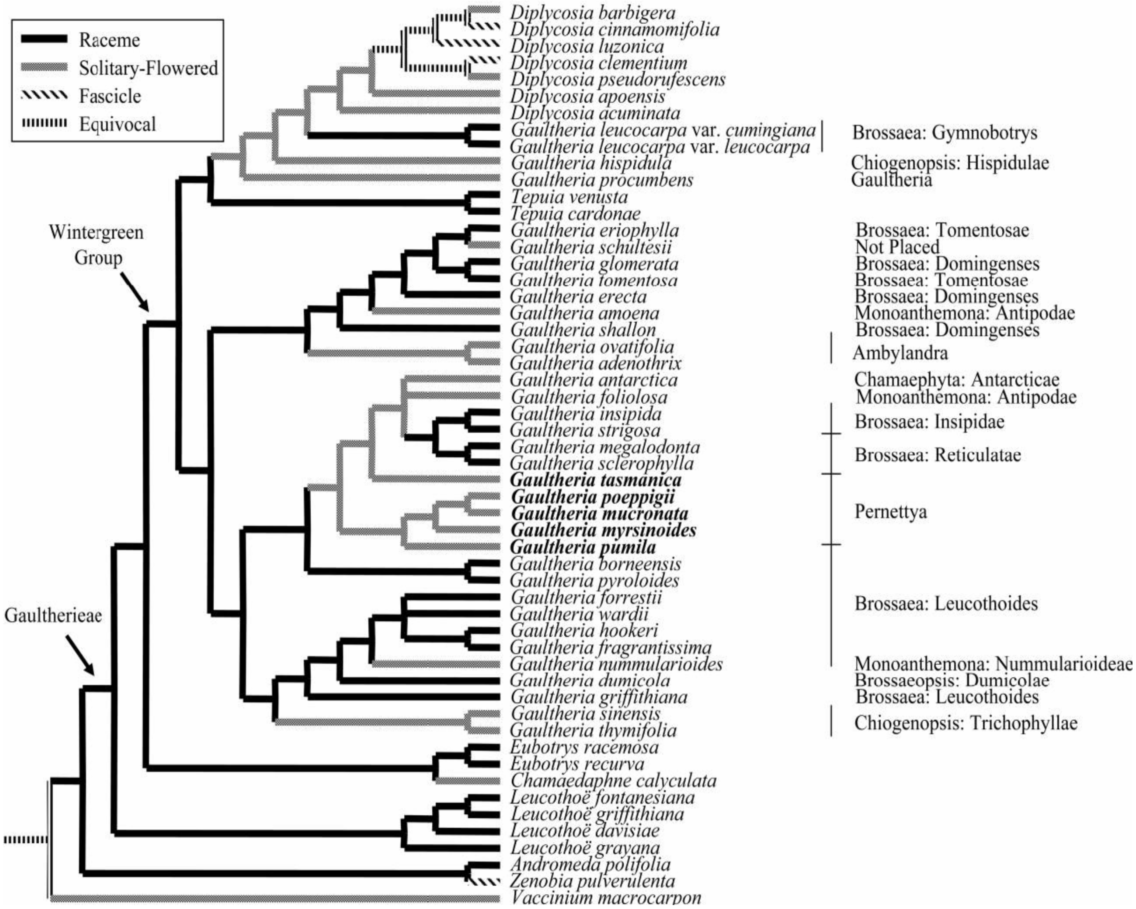


Capsule
Berry
Equivocal
Wintergreen Group
Gaultherieae
Diplycosia barbigera
Diplycosia cinnamomifolia
Diplycosia luzonica
Diplycosia clementium
Diplycosia pseudorufescens
Diplycosia apoensis
Diplycosia acuminata
Gaultheria leucocarpa var. cumingiana
Gaultheria leucocarpa var. leucocarpa
Gaultheria hispidula
Gaultheria procumbens
Tepuia venusta
Tepuia cardonae
Gaultheria eriophylla
Gaultheria schultesii
Gaultheria glomerata
Gaultheria tomentosa
Gaultheria erecta
Gaultheria amoena
Gaultheria shallon
Gaultheria ovatifolia
Gaultheria adenothrix
Gaultheria antarctica
Gaultheria foliolosa
Gaultheria insipida
Gaultheria strigosa
Gaultheria megalodonta
Gaultheria sclerophylla
Gaultheria tasmanica
Gaultheria poeppigii
Gaultheria mucronata
Gaultheria myrsinoides
Gaultheria pumila
Gaultheria borneensis
Gaultheria pyroloides
Gaultheria forrestii
Gaultheria wardii
Gaultheria hookeri
Gaultheria fragrantissima
Gaultheria nummularioides
Gaultheria dumicola
Gaultheria griffithiana
Gaultheria sinensis
Gaultheria thymifolia
Eubotrys racemosa
Eubotrys recurva
Chamaedaphne calyculata
Leucothoë fontanesiana
Leucothoë griffithiana
Leucothoë davisiae
Leucothoë grayana
Andromeda polifolia
Zenobia pulverulenta
Vaccinium macrocarpon
Diplycosi
Tepuia
SA-1
SA-2
SA-3
EA-1
EA-2
Eubotrys
Leucothoë

Figure 2.5

Inflorescence type traced onto the single consensus tree from a Bayesian analysis of the total combined data set (*matK*, *ndhF*, and nrITS). Character states: diagonally hatched branches = fascicle; black branches = racemes; gray branches = solitary-flowered; and branches with vertical lines = equivocal state. SA = South America; EA = eastern Asia. Species placed within *Gaultheria* section *Pernettya* (Middleton 1991a) are shown in bold. Infrageneric classification of *Gaultheria* species taken from Middleton (1991a; Section: Series).

Figure 2.5


Raceme
Solitary-Flowered
Fascicle
Equivocal
Diplycosia barbigera
Diplycosia cinnamomifolia
Diplycosia luzonica
Diplycosia clementium
Diplycosia pseudorufescens
Diplycosia apoensis
Diplycosia acuminata
Gaultheria leucocarpa var. cumingiana
Gaultheria leucocarpa var. leucocarpa
Gaultheria hispidula
Gaultheria procumbens
Tepuia venusta
Tepuia cardonae
Gaultheria eriophylla
Gaultheria schultesii
Gaultheria glomerata
Gaultheria tomentosa
Gaultheria erecta
Gaultheria amoena
Gaultheria shallon
Gaultheria ovatifolia
Gaultheria adenothrix
Gaultheria antarctica
Gaultheria foliolosa
Gaultheria insipida
Gaultheria strigosa
Gaultheria megalodonta
Gaultheria sclerophylla
Gaultheria tasmanica
Gaultheria poeppigii
Gaultheria mucronata
Gaultheria myrsinoides
Gaultheria pumila
Gaultheria borneensis
Gaultheria pyroloides
Gaultheria forrestii
Gaultheria wardii
Gaultheria hookeri
Gaultheria fragrantissima
Gaultheria nummularioides
Gaultheria dumicola
Gaultheria griffithiana
Gaultheria sinensis
Gaultheria thymifolia
Eubotrys racemosa
Eubotrys recurva
Chamaedaphne calyculata
Leucothoë fontanesiana
Leucothoë griffithiana
Leucothoë davisiae
Leucothoë grayana
Andromeda polifolia
Zenobia pulverulenta
Vaccinium macrocarpon
Wintergreen Group
Gaultherieae
Brossaea: Gymnobotrys
Chiogenopsis: Hispidulae
Gaultheria
Brossaea: Tomentosae
Not Placed
Brossaea: Domingenses
Brossaea: Tomentosae
Brossaea: Domingenses
Monoanthemona: Antipodae
Brossaea: Domingenses
Ambylandra
Chamaephyta: Antarcticae
Monoanthemona: Antipodae
Brossaea: Insipidae
Brossaea: Reticulatae
Pernettya
Brossaea: Leucothoides
Monoanthemona: Nummularioideae
Brossaeopsis: Dumicolae
Brossaea: Leucothoides
Chiogenopsis: Trichophyllae

CHAPTER III

THE PHYLOGENY OF *LEUCOTHOE S.L.* (ERICACEAE: VACCINIOIDEAE) BASED ON MORPHOLOGICAL AND MOLECULAR (*NDHF*, *MATK* AND nrITS) DATA

Catherine M. Bush, Walter S. Judd, Louis Frazier, and Kathleen A. Kron

The following manuscript is in press in *Systematic Botany*. C.M. Bush performed the laboratory work, data analysis and prepared the manuscript. J. Frazier provided some sequence data. W.S. Judd provided the diagnostic key for the genera and K.A. Kron and W.S. Judd provided logistical support, guidance and editorial assistance.

Abstract

Leucothoë s.l. has been split into as many as three genera in current taxonomic work involving morphological data. In this paper, phylogenetic relationships within *Leucothoë* s.l. were examined using DNA sequence data from *matK*, *ndhF*, nrITS, and morphological characters. The total combined data indicate that *Leucothoë* s.l. species are distributed between two clades. *Leucothoë racemosa* and *L. recurva* are strongly supported as being sister taxa and are in turn sister to *Chamaedaphne calyculata*. These two *Leucothoë* species are deciduous and they exhibit "*L. racemosa*"-type autumn inflorescences (as does *Chamaedaphne*). The other six *Leucothoë* s.l. species (*L. axillaris, L. davisiae, L. fontanesiana, L. grayana, L. griffithiana,* and *L. keiskei*) form a strongly supported clade. *Leucothoë grayana*, a morphologically distinctive taxon, is sister to the other five species in this latter clade. Based on these data, it is proposed that *Leucothoë* s.l. should be divided into three genera: *Eubotryoides* (*E. grayana*), *Eubotrys* (*E. racemosa* and *E. recurva*), and *Leucothoë* s.s. (*L. axillaris, L. davisiae, L. fontanesiana, L. griffithiana,* and *L. keiskei*).

Introduction

The genus *Leucothoë* D. Don is currently in a state of taxonomic flux. The most recent, unpublished monograph of the genus (Melvin 1980) contains eight species split into groups based on geography and the character of deciduous versus evergreen leaves. *Leucothoë racemosa* A. Gray and *L. recurva* A. Gray are North American species that possess deciduous leaves. *Leucothoë grayana* Maxim. is a morphologically distinct species that also exhibits the deciduous habit but occurs in Japan. The remaining species are evergreen: three are North American [*L. axillaris* D. Don (type of the genus), *L. fontanesiana* (Steud.) Sleumer, and *L. davisiae* Torr. & A. Gray] and two are Asian (*L. griffithiana* C.B. Clarke, Himalayas; *L. keiskei* Miq., Japan). Based on morphological characters, Melvin (1980) divided the genus into three sections: *Leucothoë* (composed of all five evergreen species), *Eubotrys* Nuttall (containing the two North American deciduous species), and *Eubotryoides* (T. Nakai) H. Hara (containing only the deciduous Japanese species, *L. grayana*).

A useful review of the complex taxonomic history of *Leucothoë* is presented in Waselkov and Judd (2008). In previous revisions of the genus, species now included in *Leucothoë* s.l. had been placed in several genera, including *Eubotrys* (*L. racemosa* and *L. recurva*; Nuttall 1843; Britton and Brown 1913), *Oreocallis* Small (*Leucothoë davisiae*; Small 1914), *Eubotryoides* (*L. grayana*; Hara 1935), and *Paraleucothoë* (T. Nakai) M. Honda (*L. keiskei*; Honda 1949). Sleumer (1959) and Stevens (1969) maintained *Leucothoë* as a broadly circumscribed genus with six sections. Wood (1961) included all eight species concerned in *Leucothoë* s.l. but only recognized three sections that are identical to those later proposed by Melvin (1980).

The morphological characters that have been described as uniting *Leucothoë* s.l. are variable and occur in other closely related genera. These characters include: serrate leaf margins, axillary racemes formed from buds that overwinter (i.e., develop in the autumn; *L. grayana* is the only exception, with its racemes forming in the spring), dry calyces (shared with many other Ericaceae), awned anthers (shared most notably with many species of *Gaultheria*), superior ovaries with numerous ovules and seeds with marginal cells that form degrees of a winged appearance (a wing is lacking in *L. racemosa*; Sleumer 1959; Stevens 1969, 1971; Luteyn et al. 1996).

The recent morphological study by Waselkov and Judd (2008) is the most current analysis of *Leucothoë* s.l. In this paper, 69 phenotypic characters, including morphology, anatomy, chromosome counts, and secondary chemistry, were scored for the eight species of *Leucothoë* s.l. and for eight closely related species in the Gaultherieae, Andromedeae, Vaccinieae, and Lyonieae of the Vaccinioideae (Ericaceae; Waselkov and Judd 2008; Kron et al. 2002). A parsimony analysis of these characters showed that *Leucothoë* s.l. as defined by Melvin (1980) is polyphyletic, with three separate clades of *Leucothoë* s.l. members (Fig. 3.1; Waselkov and Judd 2008). *Leucothoë racemosa* and *L. recurva* were strongly supported as being sister taxa and in turn sister to *Chamaedaphne calyculata* Moench (Fig. 3.1; Waselkov and Judd 2008). This clade was sister to the wintergreen group, represented by *Diplycosia clementium* Sleumer, *Gaultheria procumbens* L., and *G. domingensis* Urb. (Fig. 3.1; Waselkov and Judd 2008). There was a clade including all of the evergreen members of *Leucothoë*, although with bootstrap support <50% (Fig. 3.1; Waselkov and Judd 2008). *Leucothoë grayana* was placed in neither of these clades; instead it was sister to *Andromeda polifolia* var. *latifolia* (= *A. glaucophylla*) (Fig. 3.1;

Waselkov and Judd 2008). Based on these results, the authors redefined the genus *Leucothoë* by splitting it into three genera: *Leucothoë* s.s. (all five evergreen species), *Eubotrys* (*E. racemosa* and *E. recurva*), and *Eubotryoides* (*E. grayana*; Waselkov and Judd 2008).

Molecular evidence to date supports the polyphyly of *Leucothoë*. Earlier studies (Kron et al. 1999; Powell and Kron 2001; Kron et al. 2002) had poor taxon sampling in *Leucothoë* s.l. but the frequently sampled taxa *L. fontanesiana* and *L. racemosa* were never resolved as being monophyletic in these analyses. Bush et al. (2009) presented a phylogeny of the entire Gaultherieae tribe based on *matK, ndhF,* and nrITS data and sampled six members of *Leucothoë* s.l. *Leucothoë racemosa* and *L. recurva* (as *Eubotrys* in Waselkov and Judd 2008) were found to be sister taxa and in turn sister to *Chamaedaphne*, which corresponds to the results/conclusions of Waselkov and Judd (Fig. 3.1 in this paper; 2008). Also, the three evergreen species sampled (*Leucothoë* s.s.) form a strongly supported clade (Bush et al., 2009). Finally, *L. grayana* (as *Eubotryoides* in Waselkov and Judd 2008) is strongly supported as being sister to the clade of evergreen species, a novel relationship (Bush et al., 2009). In Waselkov and Judd (2008), however, the Gaultherieae were not found to be monophyletic whereas in Bush et al. (2009) both the wintergreen group (*Diplycosia* Blume, *Gaultheria* L., and *Tepuia* Camp) and the Gaultherieae were found to be strongly supported. In Bush et al. (2009), the wintergreen group clade (*Diplycosia, Gaultheria,* and *Tepuia*) was sister to *Chamaedaphne calyculata + Leucothoë racemosa + L. recurva* with strong support (70% bt; 1.00 pP).

The goals of this paper are to determine the phylogeny of *Leucothoë* s.l. using molecular (*matK, ndhF,* and nrITS) and morphological data; specifically to determine with strong support and resoluton, 1.) the number of clades in *Leucothoë* s.l., and 2.) the placement of the morphologically distinct *L. grayana*.

Materials and Methods

DNA Extraction and Taxon Sampling

Total DNA was extracted from silica dried, fresh or herbarium material by using a modified CTAB extraction protocol (Doyle and Doyle 1987). Taxa sampled were: all eight recognized species of *Leucothoë* s.l. (including *L. axillaris, L. davisiae, L. fontanesiana, L. grayana, L. griffithiana, L. keiskei, L. racemosa,* and *L. recurva*), *Chamaedaphne calyculata, Diplycosia clementium, Gaultheria domingensis, G. procumbens* (Gaultherieae), *Andromeda polifolia* (Andromedeae), *Satyria warszewiczii* Klotzsch, and *Vaccinium meridionale* Sw. (Vaccinieae; Kron et al. 2002) All analyses were rooted with *Agarista populifolia* (Lyonieae) because the clade containing this species was strongly supported as being sister to the Gaultherieae + Andromedeae + Vaccinieae tribes (Kron et al. 2002). This taxon sampling is identical to that of Waselkov and Judd (2008) except in the molecular analysis *Andromeda polifolia* var. *polifolia* was included and in the morphological analysis the closely related (and intergrading) *Andromeda polifolia* var. *latifolia* Aiton (= *A. glaucophylla*) was examined (Appendix 1).

Gene Regions and DNA Sequencing

Two chloroplast gene regions (*ndhF* and *matK*) and one nuclear gene region (nrITS) were sampled. The regions were amplified by using standard PCR techniques with primers as published in Johnson and Soltis (1994) and Steele and Vilgalys (1994) for *matK*, Olmstead and Sweere (1994) and Alverson et al. (1999) for *ndhF*, and White et al. (1990) for nrITS. Additional primers were designed specifically for amplifying and sequencing the 3' *ndhF* end of *Gaultheria* and related genera (1036F: TTA GGA GCT

ACT TTA GCG C; 1824R: CC AAA CCC ATT ACG GAT TGA TCG) because the traditional primers for the 3' end of *ndhF* failed for many taxa. These new primers amplify from position 1036 to 1824 of the 2200 total base pairs for *ndhF* (Olmstead and Sweere 1994; Alverson et al. 1999). Due to this truncation as well as the removal of ambiguous alignment regions and areas that are incomplete due to the placement of primers in those locations, the total *ndhF* gene portion for this study is 1592 base pairs. Bold-faced GenBank numbers in Appendix 1 represent data submitted for this study. Data matrices and trees are deposited in TreeBASE (study accession S2384; matrix accession M4520). PCR-amplified fragments were cleaned by using the Qiagen Gel Isolation Kit (Qiagen Sample and Assay Technologies, Valencia, California, U.S.A). DNA was sequenced at the Wake Forest University Bowman Grey Technical Center DNA sequencing facility on an ABI 377 automated sequencer. Sequences were edited in Sequencher 3.1.1 (Gene Codes Corp., Inc.1998) and aligned using MAFFT v6.602b (Katoh et al. 2002). Significant missing data in this analysis includes *Diplycosia clementium* (*matK* 5' end), *Satyria warszewiczii* (*matK* 5' end), *Gaultheria domingensis* (*matK* 3' end), and *Leucothoë keiskei* (*matK* 3' end).

Phylogenetic Analyses

Parsimony analyses were performed first on single gene matrices; if no strong conflict [>80 percent bootstrap (bt) support for conflicting clades] was seen between single gene matrices, then the matrices were combined and analyzed as total-evidence data. Separate parsimony analyses were performed on the *ndhF*, *matK*, nrITS, combined chloroplast, and total combined molecular data sets. The morphological data from the study by Waselkov and Judd (2008) were combined with the molecular data and analyzed using parsimony. All parsimony analyses were performed using PAUP* 4.0b2 (Swofford 1999). Characters were unordered, gaps were treated as missing data and any areas exhibiting ambiguous alignments were excluded from the analyses. All characters were equally weighted and only those that were parsimony-informative were included. Tree construction was performed by using a heuristic search with 1000 replicates, TBR branch swapping, and random step-wise addition. Clade support was estimated with bootstrap (bt) analyses (Felsenstein 1985). For all bt analysis, 1000 replicates of TBR branch swapping with 100 replicates in the heuristic search were performed.

The maximum likelihood analyses for the combined molecular data set and the total combined data set (molecular and morphological data) were run in RAxML 7.0.4 (Stamatakis 2006). A fast bootstrap analysis (1000 replicates) was performed simultaneously with the ML analysis (option "-f a"). The model used was GTRMIX (as calculated in RAxML), which infers the initial tree topology under GTRCAT and then analyzes the final tree topology with GTRGAMMA until stable likelihood values are reached (Stamatakis 2006).

Results

The aligned *matK* data set is 1470 nucleotide positions long and includes 86 parsimony-informative characters (5.9%). Areas of ambiguous alignment in the original matrix (characters 1-64 and 1535-1763) were excluded. Nine most parsimonious trees were found (L = 146; C.I. = 0.73; R.I. = 0.75). The aligned *ndhF* data set comprises 1592 nucleotide positions with 127 parsimony-informative characters (8.0%). Areas of ambiguous alignment in the original matrix (characters 1-48 and 1640-2060) were excluded. The analysis recovered two most parsimonious trees (L = 206; C.I. = 0.73; R.I. = 0.80). There were no strongly supported clades that were in conflict between the *matK* and *ndhF* data sets; therefore, these data were combined. The combined chloroplast data set comprises 3062 nucleotide positions and 213 parsimony-informative characters (7.0%). The analysis recovered one most parsimonious tree (data not shown; L = 356; C.I. = 0.72; R.I. = 0.77).

The aligned nrITS data set comprises 678 nucleotide positions and 81 parsimony-informative characters (12.0%). Ambiguous alignment areas in the original matrix (1-117 and 796-874) were excluded. The analysis recovered 12 most parsimonious trees (data not shown; L = 182; C.I. = 0.57; R.I. = 0.62). There were no strongly supported clades (>80% bt) that were in conflict between the chloroplast and nrITS data sets, so they were combined into a total molecular data analysis. This analysis contained 3740 nucleotide positions and 294 parsimony-informative characters (7.9%). The analysis recovered one tree (data not shown; L = 543; C.I. = 0.66; R.I. = 0.72).

The results of the morphological data analysis in Waselkov and Judd (2008) were not in conflict with the total molecular data analysis so these two data sets were combined in a ‘total-evidence’ analysis. Together, the data set comprises 3814 characters and includes 349 parsimony-informative characters (9.2%). The parsimony analysis recovered one most parsimonious tree (Fig. 3.2; L = 689; C.I. = 0.62; R.I. = 0.67).

ML analyses of the combined molecular data and the total combined data (molecular + morphology) both show the exact same topology that is presented in Fig. 3.2 except for the placement of *Diplycosia clementium, Gaultheria domingensis*, and *G. procumbens* (see below).

The topology and support of the individual analyses (*matK, ndhF,* chloroplast, nrITS, morphology, and combined molecular data) differed only in a single clade. In the nrITS, combined molecular, and ML analysis of the total combined data, *Diplycosia clementium* was sister to *Gaultheria procumbens* with *G. domingensis* sister to the pair (greater than 80% bt for *D. clementium* + *G. procumbens* in nrITS and combined molecular analyses). However, in the *ndhF*, combined chloroplast, morphological, and MP analysis of the total combined data, *D. clementium* was sister to *G. domingensis* with *G. procumbens* sister to the pair (<80% bt for *D. clementium* + *G. domingensis* in all analyses; Fig. 3.2). In the *matK* analysis, the three taxa occur in a polytomy. This discrepancy in the data could be due to poorly supported chloroplast vs. nuclear discordance or from significant missing data in this clade (including the 5' end of *matK* in *D. clementium* and the 3' end of *matK* in *G. domingensis*).

Due to the similarity in topology between the individual analyses, only the total combined data analyses (morphological and molecular data; parsimony and ML analyses) will be discussed in detail (Fig. 3.2). In the total combined data analyses, the sampled representatives of the wintergreen group (*Diplycosia* and *Gaultheria*) form a strongly supported clade (Fig. 3.2). Sister to the wintergreen group is *Chamaedaphne calyculata + Leucothoë racemosa + L. recurva*. *Leucothoë racemosa* is supported as being sister to *L. racemosa*. The remaining *Leucothoë* members form a clade that is sister to this larger clade. Within this latter *Leucothoë* clade, *L. axillaris* is sister to *L. fontanesiana* which in turn is sister to *L. keiskei + L. griffithiana*. *Leucothoë davisiae* is sister to these two clades and *L. grayana* is sister to all other taxa in the clade. *Andromeda polifolia* is sister to the monophyletic Gaultherieae members (*Chamaedaphne, Diplycosia, Gaultheria*, and *Leucothoë*). *Satyria warszewiczii* is sister to *Vaccinium meridionale* and this clade is at the deepest node of the tree.

Discussion

This molecular study included all *Leucothoë* s.l members and largely corroborates the of the molecular phylogeny of Gaultherieae by Bush et al. (2009) as well as the *Leucothoë* s.l. phylogeny based on morphological characters of Waselkov and Judd (2008). In all analyses, *Leucothoë* s.l. is polyphyletic with members found in two or three clades (Bush et al., 2009; Waselkov and Judd 2008). Therefore, *Leucothoë*, as broadly circumscribed, cannot be maintained. *Leucothoë racemosa* and *L. recurva* consistently are sister taxa and are in turn sister to *Chamaedaphne calyculata* (see Figs. 3.1, 3.2). This clade is sister to representatives of the wintergreen group (*Diplycosia* and *Gaultheria*) in all studies (70% bt, 1.00 pP in Bush et al., 2009; <50% bt in Waselkov and Judd 2008; 91% bt MP, 100% ML in the present study). In Bush et al. (2009) and the current study, this larger clade is sister to the remaining sampled members of *Leucothoë* s.l with strong support (86% bt MP, 98% bt ML). In Waselkov and Judd (2008), deeper nodes were unresolved; the evergreen species of *Leucothoë* s.l. formed a clade (<50% bt) and *L. grayana* was sister to *Andromeda polifolia* var. *latifolia* (<50% bt), a novel relationship.

The clade containing *Leucothoë racemosa* and *L. recurva* has several morphological synapomorphies (Waselkov and Judd 2008). The two species are deciduous, a condition shared only with *L. grayana* within *Leucothoë* s.l. Also, along with *Chamaedaphne*, these species exhibit a *“L. racemosa*-type” fall inflorescence where the reproductive shoot resembles a vegetative shoot and has long, green, leaf-like bracts not enclosing the floral buds. The floral buds themselves are protected by the thick sepals of the individual flowers (Waselkov and Judd 2008). These two species also have leaves with unicellular hairs scattered on the adaxial side of the leaf (versus unicellular hairs present only on the adaxial midrib or the hairs absent) as well as megagametophytes with antipodals dividing (versus not dividing; Waselkov and Judd 2008). Synapomorphies for the clade of *Leucothoë* s.s. (excluding *L. grayana*) include: winged seeds and *"L. axillaris*-type" inflorescences where the floral buds are protected by overlapping bracts (Waselkov and Judd 2008). The former character is also shared with *Leucothoë recurva* (Waselkov and Judd 2008). The evergreen condition of *Leucothoe* s.s. likely is a retained plesiomorphy.

The sister relationship between *Leucothoë racemosa* + *L. recurva* and *Chamaedaphne* was not suggested in any non-cladistic treatment of *Leucothoë* and its relatives. This may be due to the numerous distinct morphological characters *Chamaedaphne* exhibits, including: terminal inflorescences with leafy bracts, anthers with tubules, unique floral and embryological anatomy, and peltate scales (Palser 1951, 1952; Kron et al. 1999). However, as previously mentioned, the *Leucothoë racemosa* + *L. recurva* + *Chamaedaphne* clade does possess the morphological synapomorphy of "*L. racemosa*-type" inflorescences (Waselkov and Judd 2008), a unique feature. Other homoplasious synapomorphies within Ericaceae include filaments with papillae, placentae with a bare spot adaxially, and campylotropous ovules. The last character is nearly unique among the species analyzed, occurring also in *Andromeda* alone. Because *Leucothoë racemosa* and *L. recurva* form a well supported clade that is morphologically very divergent from *Chamaedaphne*, *L. racemosa* and *L. recurva* are best recognized as *Eubotrys* (see also Nuttall 1843; Gleason and Cronquist 1991; Stevens et al. 2004) instead of included within an expanded *Chamaedaphne*.

In our study, the evergreen species of *Leucothoë* s.l (*L. axillaris, L. fontanesiana, L. keiskei, L. griffithiana*, and *L. davisiae*) are sister with strong support to the deciduous species from Japan, *L. grayana* (Fig. 3.2). *Leucothoë grayana* has many unique morphological characters within the genus, including its spring-emerging inflorescences with vegetative leaf-like bracts (versus autumn-emerging like all other *Leucothoë* species), the floral bract basally adnate to the pedicel, bracts of the reproductive shoots large and indistinguishable from leaves proximally, strongly urceolate corolla, awnless anthers, and capsules with thin-walled valves (Waselkov and Judd 2008). This species also evolved the deciduous habit independently of *L. racemosa* and *L. recurva*. Due to these numerous morphological distinctions and the fact that the *L. grayana* + *Leucothoë* s.s. clade is not easily diagnosed, we support the segregation of *L. grayana* as the genus *Eubotryoides* (Hara 1935).

These analyses revealed two distantly related sister pairs that exhibit similar geographic patterns in the southeastern United States. *Leucothoë fontanesiana* (southern Appalachian mountains) is sister to *L. axillaris* (Atlantic coast and lower Piedmont) and *Eubotrys recurva* (Southern Appalachian mountains) is sister to *E. racemosa* (Atlantic coast and lower Piedmont). By way of contrast, the two species from Japan are not sister to each other: *Leucothoë keiskei* (Japan; Honshu Island) is sister to *L. griffithiana* from the Himalayas and *L. grayana* (Japan; Honshu and Hokkaido Islands) is sister to all *Leucothoë* s.s. members. *Leucothoë davisiae*, from the Sierra Nevada mountains in the western United States, is sister to *L. fontanesiana* + *L. axillaris* + *L. griffithiana* + *L. keiskei*.

The taxonomy of *Leucothoë* s.l. previously included from one to three genera for these eight species. The current study confirms the polyphyly of *Leucothoë* s.l., as these species are members of two distinct lineages. Our cladograms (see Fig. 3.2), along with an assessment of the pattern of morphological variation, result in our decision to recognize three monophyletic and morphologically diagnostic genera for the group: *Eubotryoides*, *Eubotrys*, and *Leucothoë* s.s. These are distinguished in the following key.

Key to the Genera of Leucothoë s.l.

1. Leaves evergreen; the inflorescence autumn-emerging, having the appearance of an elongate bud, the individual floral buds covered by overlapping, non-leafy bracts; the bracteoles basal. . . . ……………………………….*Leucothoë*
1. Leaves deciduous; the inflorescence autumn-emerging, resembling a vegetative shoot with long, green, leaf-like bracts not enclosing floral buds, and the bracteoles apical, OR the inflorescence spring-emerging, with bracts of reproductive shoots large and indistinguishable from leaves proximally, reduced distally, not enclosing floral buds, and the bracteoles ± basal……....2
2. Inflorescences autumn-emerging; pedicel free from subtending bract and with apical bracteoles; corolla elongate-urceolate, white; anthers with 2 or 4 awns; filaments without unicellular hairs. . .. .….. . . . . *Eubotrys*
2. Inflorescences spring-emerging; pedicel ± adnate to basal portion of subtending bract and with ± basal bracteoles; corolla strongly and shortly urceolate, pale green; anthers awnless; filaments with unicellular hairs. . . . . ……………... . . . . *Eubotryoides*

Literature Cited

Alverson, W.S., B.A. Whitlock, R. Nyffeler, C. Bayer, and D.A. Baum. 1999. Phylogeny of the core Malvales: evidence from *ndh*F sequence data. *American Journal of Botany* 86: 1474-1486.

Britton, N.L. and A. Brown. 1913. An illustrated flora of the northern United States and Canada. Ed. 2. New York: Charles Scribner's Sons.

Bush, C.M., L. Lu, P.F. Fritsch, D. Li, and K.A. Kron. 2009. Phylogeny of Gaultherieae (Ericaceae: Vaccinioideae) based on DNA sequence data from *matK*, *ndhF*, and nrITS. *International Journal of Plant Sciences* 170: 355-364.

Doyle, J. and J. 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: 783-791.

Gleason, H.A. and A. Cronquist. 1991. Manual of vascular plants of northeastern United States and adjacent Canada. Ed. 2. New York: New York Botanical Garden.

Hara, H. 1935. *Eubotryoides*. *Journal of Japanese Botany* 11: 626-627.

Holmgren, P. K. and N. H. Holmgren. 1998 [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/

Honda, M. 1949. *Paraleucothoë*. *Journal of Japanese Botany* 24: 29.

Johnson, L.A. and D.E. Soltis. 1994. *mat*K DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. *Systematic Botany* 19: 143-156.

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059-3066.

Kron, K.A., W.S. Judd, and D.M. Crayn. 1999. Phylogenetic analyses of Andromedeae (Ericaceae subfam. Vaccinioideae). *American Journal of Botany* 86: 1290-1300.

Kron, K.A., W.S. Judd, P.F. Stevens, D.M. Crayn, A.A. Anderberg, P.A. Gadekëë, C.J. Quinn, and J.L. Luteyn. 2002. Phylogenetic classification of Ericaceae: molecular and morphological evidence. *The Botanical Review* 68: 335-423.

Luteyn, J.L., W.S. Judd, S.P. Vander Kloet, L.J. Dorr, G.D. Wallace, K.A. Kron, P.F.

Stevens, and S.E. Clemants. 1996. Ericaceae of the southeastern United States. *Castanea* 61: 101-144.

Melvin, N.C. 1980. *A systematic investigation of the genus Leucothoë (Ericaceae).* Unpubl. Ph.D. thesis. Coral Gables, Florida: University of Miami.

Nuttall, T. 1843. Description and notices of new or rare plants in the natural orders Lobeliaceae, Campanulaceae, Vaccinieae, Ericaceae, collected in a journey over the continent of North America, and during a visit to the Sandwich Islands, and Upper California. *Transactions of the American Philosophical Society* 8: 251-272.

Olmstead, R.G. and J.A. Sweere. 1994. Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. *Systematic Biology* 43: 467- 481.

Palser, B.F. 1951. Studies of floral morphology in the Ericales. I. Organography and vascular anatomy in the Andromedeae. *Botanical Gazette* 112: 447–485.

Palser, B.F. 1952. Studies of floral morphology in the Ericales. II. Megagametophyte development in the Andromedeae. *Botanical Gazette* 114: 33–52.

Powell, E.A. and K.A. Kron. 2001. An analysis of the phylogenetic relationships in the wintergreen group (*Diplycosia*, *Gaultheria*, *Pernettya*, *Tepuia*; Ericaceae). *Systematic Botany* 26: 808-817.

Sleumer, H. 1959. Studien uber die Gattung *Leucothoë* D. Don. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 78: 435-480.

Small, J.K. 1914. Ericaceae. *North American Flora* 29: 33-102.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.

Steele, K.P. and R. Vilgalys. 1994. Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *mat*K. *Systematic Botany* 19: 126-142.

Stevens, P.F. 1969. *Taxonomic studies in the Ericaceae*. Unpubl. Ph.D. thesis. Edinburgh, Scotland: University of Edinburgh.

Stevens, P.F. 1971. A classification of the Ericaceae: subfamilies and tribes. *Botanical Journal of the Linnean Society* 64: 1-53.

Stevens, P.F., J.L. Luteyn, E.G.H. Olilver, T.L. Bell, E.A. Brown, R.K. Crowden, A.S. George, G.J. Jordan, P. Ladd, K. Lemson, C.B. McLean, Y. Menadue, J.S. Pate, H.M. Stace, and C.M. Weiller. 2004. Ericaceae. Pp. 145-194 in *The families and genera of vascular plants*. vol. 6, ed. K Kubitzki. Berlin: Springer-Verlag.

Swofford, D.L. 1999. PAUP* Phylogenetic analysis using parsimony (*and other methods), v. 4.01. Sunderland: Sinauer Associates.

Waselkov, K. and W.S. Judd. 2008. A phylogenetic analysis of *Leucothoë* s.l. (Ericaceae; tribe Gaultherieae) based on phenotypic characters. *Brittonia* 60: 382-397.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 in *PCR protocols: a guide to methods and applications* eds. M. Innis, D. Gelfand, J. Sninsky, and T. White. San Diego: Academic Press.

Wood, C.E. 1961. The genera of Ericaceae in the Southeastern United States. *Journal of the Arnold Arboretum* 42: 10-80.

Appendix 3.1

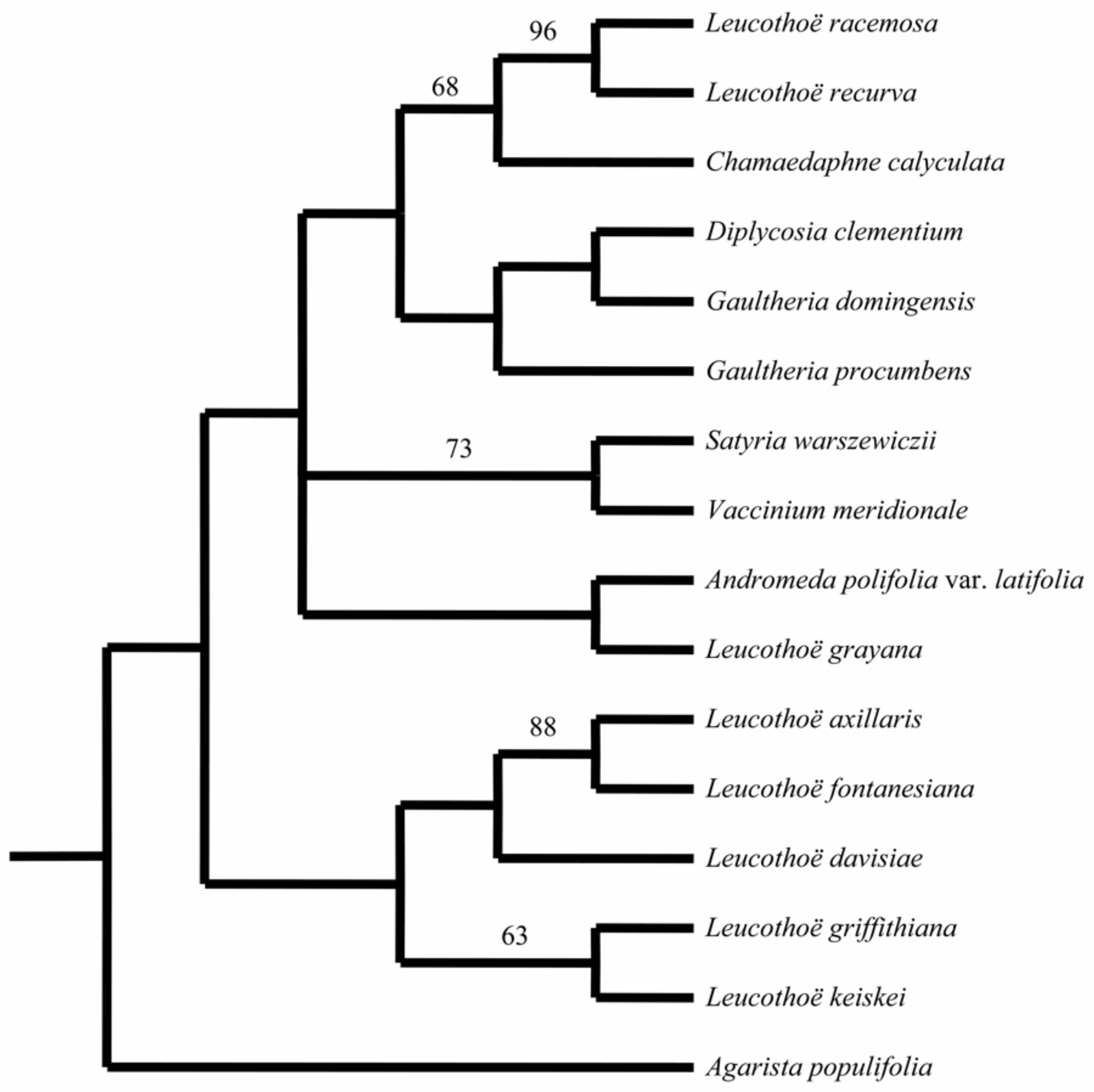
Species, Genbank accession numbers (*matK*, *ndhF*, nrITS), collection locality and voucher information is presented here. Genbank numbers obtained from previous studies are in plain font; bold Genbank numbers represent sequences submitted from this study. Herbarium acronyms follow *Index Herbariorum* (Holmgren and Holmgren, 1998).

Agarista populifolia (Lam.) Judd; U61306, **GQ179746**, **GQ179751**; Florida, USA, *Judd 8034* (FLAS). *Andromeda polifolia* L. var. *polifolia*; AF124569, AF419722, AF358872; *no voucher* (1976-6099; K). *Chamaedaphne calyculata* Moench; AF015630, FJ008930, AF358873; Canada, *A. Evans, s.n.* (E). *Diplycosia clementium* Sleumer; AF366624, **GQ179747**, AF358877; Malaysia, *Fuller s.n.* (WFU). *Gaultheria domingensis* Urb.; **GQ179743**, **GQ179748**, **GQ179752**; Dominican Republic, *Judd 8157* (FLAS). *Gaultheria procumbens* L.; AF366643, FJ008949, AF358895; Canada, *Powell s.n.* (WFU). *Leucothoë axillaris* D. Don; **GQ179744**, **GQ179749**, **GQ179753**; North Carolina, USA, *Huffstetler 33* (HPU). *Leucothoë davisiae* Torr. & A. Gray; FJ010617, FJ008972, FJ010599; *no voucher* (Rhododendron Species Foundation). *Leucothoë fontanesiana* (Steud.) Sleumer; AF124570, FJ008957, AF358903; North Carolina, USA, *Kron 1876* (WFU). *Leucothoë grayana* Maxim.; FJ010621, FJ008976, FJ010603; Japan, *DGH 486* (Rhododendron Species Foundation). *Leucothoë griffithiana* C.B. Clarke; FJ010616, FJ008971, FJ010598; China, *GLGS 32580* (CAS). *Leucothoë keiskei* Miq.; **GQ179745**, **GQ179750**, **GQ179754**; Japan, *T.Takahashi et al., 1354 A* (GH). *Leucothoë racemosa* A. Gray; AF124564, FJ008958, AF358904; North Carolina, USA, *Kron & Powell s.n.* (WFU). *Leucothoë recurva* A. Gray; FJ010620, FJ008975, FJ010602; North Carolina, USA, *Moretz s.n.* (WFU). *Satyria warszewiczii* Klotzsch; U61314, AY331956, AF382698; Costa Rica, *G. A. C. Herklots, s.n.* (E). *Vaccinium meridionale* Sw.; U89759, AF419756, AF382731; Jamaica, *VanderKloet s.n.* (WFU).

Figure 3.1

Strict consensus of the two most parsimonious trees (L = 155, CI = 0.56, RI = 0.53) based on morphological/non-molecular data. Bootstrap values are indicated above the lines for clades with bootstrap support above 50%. Modified from Waselkov and Judd (2008).

Figure 3.1

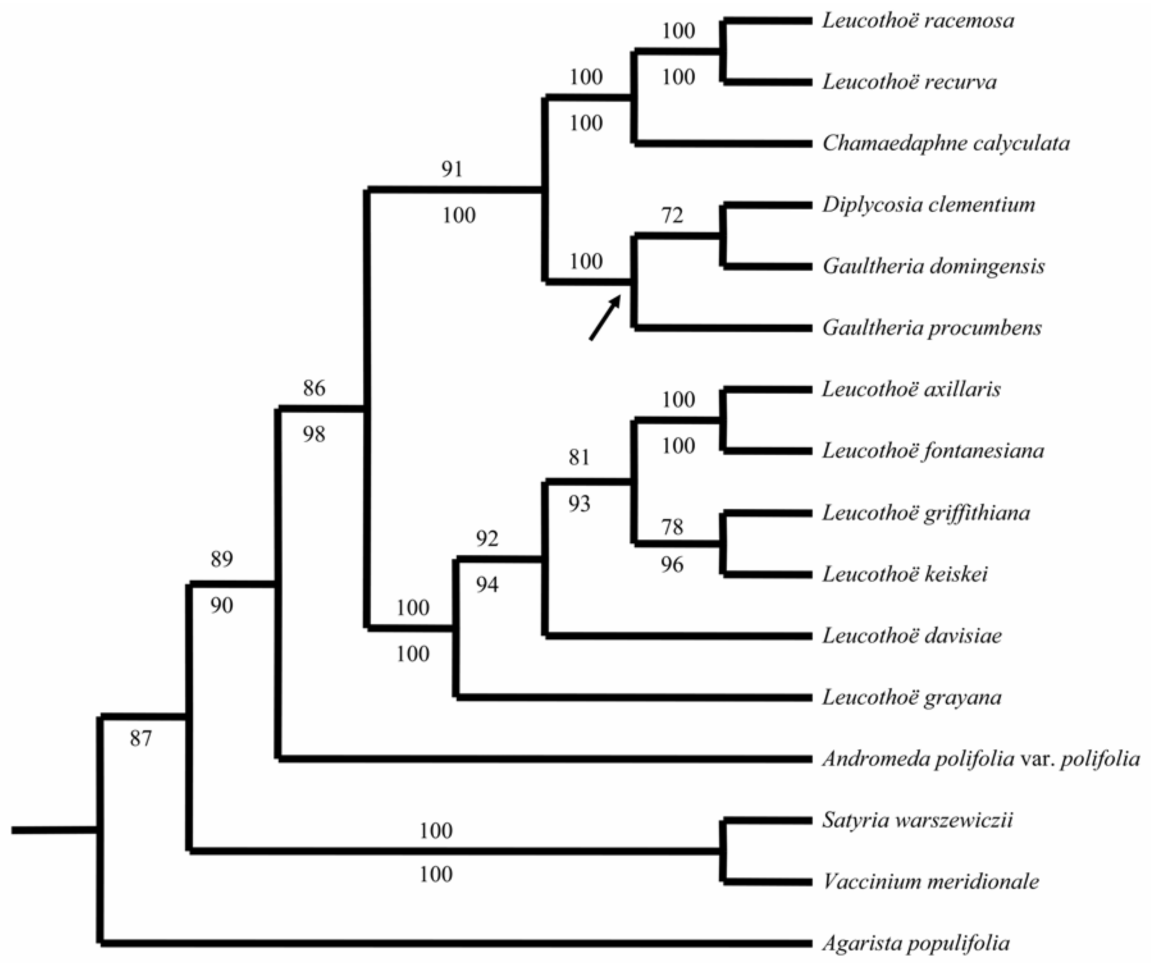


96
68
Leucothoë racemosa
Leucothoë recurva
Chamaedaphne calyculata
Diplycosia clementium
Gaultheria domingensis
Gaultheria procumbens
73
Satyria warszewiczii
Vaccinium meridionale
Andromeda polifolia var. latifolia
Leucothoë grayana
88
Leucothoë axillaris
Leucothoë fontanesiana
Leucothoë davisiae
63
Leucothoë griffithiana
Leucothoë keiskei
Agarista populifolia

Figure 3.2

The one most parsimonious tree (L = 689, CI = 0.62, RI = 0.67) from the total combined data parsimony analysis (*matK, ndhF*, nrITS, morphology). Bootstrap values from the parsimony analysis are indicated above the branches; bootstrap values from the RAxML analysis are shown below the branches. The arrow indicates where topological incongruities are located when compared to the total evidence ML analysis (see text).

Figure 3.2


Leucothoë racemosa
Leucothoë recurva
Chamaedaphne calyculata
Diplycosia clementium
Gaultheria domingensis
Gaultheria procumbens
Leucothoë axillaris
Leucothoë fontanesiana
Leucothoë griffithiana
Leucothoë keiskei
Leucothoë davisiae
Leucothoë grayana
Andromeda polifolia var. polifolia
Satyria warszewiczii
Vaccinium meridionale
Agarista populifolia
100
100
100
100
91
100
72
100
86
98
100
100
81
93
78
96
92
94
89
90
100
100
87
100
100

CHAPTER IV

THE PHYLOGENY, BIOGEOGRAPHY AND MORPHOLOGICAL EVOLUTION OF *GAULTHERIA* (ERICACEAE) FROM AUSTRALIA AND NEW ZEALAND

Catherine M. Bush, Steven J. Wagstaff, Peter W. Fritsch and Kathleen A. Kron

The following manuscript was published in *Australian Systematic Botany*, volume 22, 229-242, and is reprinted with permission. Stylistic variations are due to the requirements of the journal. C.M. Bush performed the laboratory and field work, data analysis and prepared the manuscript. S.J. Wagstaff and P.W. Fritsch also participated in the field work for this study. S.J. Wagstaff, P.W. Fristch and K.A. Kron were involved in editorial assistance.

Abstract

Phylogenetic relationships within *Gaultheria* L. from Australia and NewZealand were examined by using DNA sequence data from *matK*, *ndhF*, nrITS, *waxy* and *lfy*. In the combined parsimony and maximum likelihood analyses, all Australia/New Zealand species form a clade that is sister to a clade of temperate South American species. Optimisation of morphological characters that have been emphasised in classifications of *Gaultheria* onto the molecular phylogeny revealed that, within the Australia/New Zealand clade, non-fleshy fruiting calyces, berries and solitary-flowered inflorescences each evolved twice, from fleshy fruiting calyces, capsules and multiple-flowered inflorescences, respectively. A historical biogeographical analysis that included the temperate southern hemisphere element in *Gaultheria* supports a South American origin of the Australia/New Zealand clade, followed by three dispersal events from New Zealand to Australia. Whether the origin is from temperate or tropical South America is ambiguous in our analysis.

Introduction

The genus *Gaultheria* L. contains ~130 species that occur in New Zealand, Australia, eastern Asia, the Indomalaya region, and both tropical and temperate regions of the Americas (Middleton 1991). There are 14 currently recognised species of *Gaultheria* in Australasia (here defined as Australia and New Zealand, with New Guinea excluded). *G. appressa* is endemic to New South Wales and Victoria, *G. hispida*, *G. lanceolata* and *G. tasmanica* are endemic to Tasmania, and *G. antipoda*, *G. colensoi*, *G. crassa*, *G. macrostigma*, *G. nubicola*, *G. oppositifolia*, *G. paniculata*, *G. parvula* and *G. rupestris* are endemic to New Zealand. *G. depressa* is the only species of *Gaultheria* to occur in both Australia (Tasmania) and New Zealand (Burtt and Hill 1935; Franklin 1962; Middleton 1991).

Although the Australasian species of *Gaultheria* constitute merely 11% of the species diversity of the genus, together they encompass the extremes of variation across the genus in several morphological characters, as reflected in their distribution among four different sections (*G. nubicola*: *Chamaephyta* D.J.Middleton; *G. antipoda*, *G. depressa*: *Monoanthemona* D.J.Middleton; *G. lanceolata*, *G. macrostigma*, *G. parvula*, *G. tasmanica*: *Pernettya* (Gaud.) D.J.Middleton; *G. appressa*, *G. hispida*, *G. colensoi*, *G. crassa*, *G. oppositifolia*, *G. paniculata*, *G. rupestris*: *Brossaea* (L.) D.J.Middleton; Middleton 1991). Notable among these are a fleshy *v.* non-fleshy fruiting calyx, capsular *v.* baccate fruit, and a solitary-flowered inflorescence *v.* one that is racemose or paniculate.

Species of *Gaultheria* typically have dry capsular fruits surrounded by a fleshy, often brightly coloured (e.g. red, white, blue) accrescent calyx (Middleton 1991). *G.*

antipoda, *G. appressa*, *G. depressa* and *G. hispida* all have this type of fruit. Most species of Ericaceae have a capsule with a non-fleshy calyx. Although this condition occurs only rarely in *Gaultheria*, it is found in five New Zealand species (*G. colensoi*, *G. crassa*, *G. oppositifolia*, *G. paniculata*, *G. rupestris*); it otherwise occurs only in *G. itatiaiae* (Wawra ex Drude) Sleumer and *G. sleumeriana* Kin.-Gouv. (south-eastern Brazil), and in *G. nubigena* B.L.Burtt & Sleumer (temperate South America; Middleton 1991).

Gaultheria fruits can also be berries, with or without a fleshy calyx. The berry-fruited species were traditionally segregated from *Gaultheria* as *Pernettya* Gaud. (Baas 1985; Sleumer 1985); however, Middleton and Wilcock (1990) found no basis for maintaining distinct genera and thus subsumed *Pernettya* into *Gaultheria*. Middleton subsequently (1991) erected *Gaultheria* sect. *Pernettya* for these species in an infrageneric classification. Nonetheless, *Pernettya* has continued to be recognised by some authors (Luteyn 1995*b*; Stace *et al*. 1997). Only 13 species of *Gaultheria* possess berries, namely *G. insana* plus all species of sect. *Pernettya*. Four of these (*G. lanceolata*, *G. macrostigma*, *G. parvula*, *G. tasmanica*) occur in Australasia, whereas the remaining species occur in the American tropics and temperate South America.

Inflorescences of the Australasian species of *Gaultheria* are solitary-flowered in *G. antipoda*, *G. depressa*, *G. macrostigma*, *G. parvula* and *G. tasmanica* and racemose or paniculate in the remaining species. Middleton (1991) relied heavily on such variation for the infrageneric classification of *Gaultheria*, although nonetheless acknowledged the occurrence of widespread intermediacy between these two conditions. Some otherwise solitary-flowered species, such as *G. antipoda*, can sometimes have shortened or congested racemes (‘pseudoracemes’) near the growing tip, whereas others (e.g. *G. pyrolifolia* Hook.f. ex C.B.Clarke; eastern Asia) consistently exhibit pseudoracemes. This indicates that inflorescence structure may not be a reliable character for infrageneric classification in *Gaultheria*, even though the character has been used extensively in taxonomic treatments of the genus.

Despite such divergent morphologies, the New Zealand species of *Gaultheria* readily hybridise, with mixed-species populations often forming apparent hybrid swarms. Burtt and Hill (1935) described six putative hybrid combinations among the species with a dry capsule, and five putative hybrids between capsular- and berry-fruited species. Franklin (1962) expanded the number of parental species combinations to 14 among the capsular-fruited species, in almost every combination possible, and to eight in capsular-fruited X berry-fruited hybrids. Hybrid formation is apparently restricted to disturbed habitats, such as road cuts or braided river systems (Parsons and Hermanutz 2006; C. Bush, P. Fritsch, S. Wagstaff, pers. obs.) and thus the New Zealand species can still easily be discerned as distinct in ecologically stable environments.

The presence of four sections of *Gaultheria* in Australia and New Zealand, each with a starkly contrasting suite of morphological characters, would by itself suggest independent spatiotemporal origins of this group. For example, a reasonable biogeographical scenario might involve at least two biogeographical events, one involving the dry-fruited species of New Zealand and South America, the other the berry-fruited species of New Zealand, Australia and South America. Additional events might also involve solitary-flowered species from within and outside of the region. Nonetheless, recent phylogenetic work based on DNA sequence data indicates that all three of these characters are, to various extents, homoplasious within the genus (Bush *et al*. 2009). Furthermore, although hybrids are documented as occurring in other areas of the world besides New Zealand (Luteyn 1995*b*, 1995*c*), in the majority of cases they appear to originate from parents from the same series or section (exceptions include *G. reticulate* Kunth_*G. myrsinoides* Kunth (sections *Brossaea* and *Pernettya*) and *G. erecta* Vent. *G. myrsinoides*, *G. anastomosans* Kunth and *G. vaccinioides* Weddell (sections *Brossaea*, *Pernettya* and *Monoanthemona*)). Thus, the apparent ease with which hybridisation occurs between species from different sections of *Gaultheria* in New Zealand suggests a closer phylogenetic relationship among the species than morphology might otherwise predict. Since no detailed phylogenetic data for the Australian and New Zealand species of *Gaultheria* exist, sectional monophyly for these species is undetermined.

The goals of the present study are to (1) reconstruct the phylogeny of the Australian and New Zealand species of *Gaultheria* and reveal their closest relatives with molecular data, (2) assess the current infrageneric classification of these species by tracing the evolution of three characters emphasised in the differentiation of taxa (calyx type, fruit type and inflorescence structure) on a molecular-based tree and (3) use the tree to infer the number of dispersal events that have taken place in *Gaultheria* among Australia, New Zealand and other areas.

Materials and Methods

DNA extraction and taxon sampling

Total DNA was extracted from silica-dried, fresh or herbarium material by using a modified CTAB extraction protocol (Doyle and Doyle 1987). Every currently defined species of *Gaultheria* present in Australia and New Zealand is represented in the analysis except for *G. nubicola*. We could not relocate this species from the type locality with confidence, and it may represent merely a high-elevation representative of *G. depressa*. Taxa sampled in the present analysis include 13 species of *Gaultheria* from Australia and New Zealand. The additional inclusion of both varieties of *G. depressa* and one unpublished form of *G. appressa* (‘*viridicarpa*’) result in 16 terminals. Because the New Zealand species can readily form hybrids and hybrid swarms in areas of overlapping range, care was taken to sample from pure populations of species, i.e. samples possessed the standard morphological features of species, and no other species were located in the immediate vicinity of the collection site. Also included were 12 species of *Gaultheria* encompassing the geographical, taxonomic and morphological range of the genus, and one species each of *Diplycosia* and *Tepuia. Leucothoë griffithiana* (Gaultherieae) was included to root the tree because previous studies place it as sister to all other Gaultherieae (Bush *et al*. 2009). GenBank numbers for all taxa are listed in Table 1. Some sequences used in our analyses have been generated previously (Powell and Kron 2001; Bush *et al*. 2009). There were 108 sequences newly generated for this study.

Gene regions, DNA sequencing and cloning

Two chloroplast gene regions (*ndhF*, *matK*) and three nuclear gene regions (nrITS, *waxy*, *lfy*) were sampled. The GBSSI gene (*waxy*) functions in the synthesis of amylose and has 13 translated exons with a total length of ~4500 base pairs (Mason-Gamer *et al*. 1998; Peralta and Spooner 2001). The present study utilises only the region between exons 9–11 of the *waxy* gene. The *lfy* gene (*LEAFY*) functions in flower development (Frohlich and Meyerowitz 1997) and is currently being investigated for its phylogenetic signal in members of the Ericaceae (Kron laboratory, Wake Forest University, Winston–Salem, NC). The present study utilises only the intron between Exons 2 and 3 (*2i3*). The regions were amplified by standard PCR techniques with primers provided by Johnson and Soltis (1994) and Steele and Vilgalys (1994) for *matK*, Olmstead and Sweere (1994) and Alverson *et al*. (1999) for *ndhF*, White *et al*. (1990) for nrITS, Mason-Gamer *et al*. (1998) and Peralta and Spooner (2001) for *waxy*, and Frohlich and Meyerowitz (1997) for *lfy2i3* (*lfy*). The gene regions *ndhF* and *matK* were amplified in two different sections (5' and 3', respectively); alignment ambiguity in the region of primer overlap was therefore excluded (see below). Additional primers were designed specifically for amplifying and sequencing the 3' *ndhF* end of *Gaultheria* (1036F: 5'-TTA GGA GCTACTTTAGCGC- 3'; 1824R: 3'-CCAAACCCATTACG GAT TGA TCG-5') because the traditional primers for the 3' end of *ndhF* failed for many taxa. These new primers amplify from positions 1036 to 1824 of the 2200 total base pairs for *ndhF* (Olmstead and Sweere 1994; Alverson *et al*. 1999). Owing to this truncation as well as the exclusion of primer binding sites and areas of ambiguous alignment, the total *ndhF* gene portion for the present study is 1543 base pairs.

PCR-amplified fragments were cleaned with the Qiagen Gel Isolation Kit (Qiagen Sample and Assay Technologies, Valencia, CA). DNA was sequenced at the DNA sequencing facility at the Bowman Grey Technical Center, Wake Forest University, NC, USA, on an ABI 377 automated sequencer. Sequences were edited in Sequencher 3.1.1 (Gene Codes Corp. Inc., Ann Arbor, MI) and aligned with MAFFT v6.602b (Katoh and Kuma 2002). Extensive missing data (i.e. most or all of the gene region is missing because of failed amplification or cycle sequencing) in this analysis includes *Diplycosia barbigera* (*lfy*), *G. appressa* (*matK* 3', *lfy*), *G. depressa* var. *depressa* (Tasmania; *waxy*), *G. depressa* var. *depressa* (New Zealand; *waxy*), *G. depressa* var. *novae-zealandiae* (*waxy*), *G. hispida* (*matK* 3'), *G. insana* (*ndhF* 5'), *G. insipida* (*lfy*), *G. lanceolata* (*matK* 3', *lfy*), *G. macrostigma* (*matK* 3'), *G. nubigena* (*matK* 3'), *G. oppositifolia* (*matK* 3'), *G. parvula* (*matK* 3') and *Tepuia venusta* (*matK* 5', *lfy*).

PCR fragments from all sampled taxa for the gene region *lfy* and *waxy* were cloned with the Topo Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA). Up to five clones were sequenced for each taxon. Several studies have shown that *waxy* is a single-copy gene in most dicots (Mérida *et al*. 1999; Wang *et al*. 1999). Double copies are notably present in the Rosaceae (Smedmark *et al*. 2003) and evidence of multiple copies exists in some taxa of Ericaceae (K. Kron, pers. obs.). Separate maximum likelihood (ML) analyses that included all clones were run in RAxML 7.0.4 (Stamatakis 2006) with all *lfy* and *waxy* sequences. If all clones from a species formed a clade (or a least were not strongly supported as being located in separate clades), a single clone was randomly chosen to represent that species in subsequent analyses (i.e. *waxy* analysis, *lfy* analysis, nuclear and total combined data analyses). If clones of a species were recovered in two or more separate clades, that species was excluded from the terminal *waxy* or *lfy* analyses, and its *waxy* or *lfy* sequence was subsequently excluded from subsequent combined data analyses.

Phylogenetic analyses

Parsimony analyses were performed first on single-gene matrices; if no strong conflict (>80 percent bootstrap [bt] support) was observed between single gene matrices, then the matrices were combined into a single matrix and analysed as total-evidence data. Separate parsimony analyses were performed on the *ndhF*, *matK*, nrITS, *waxy*, *lfy*, combined chloroplast, combined nuclear and total combined datasets. Phylogenetic analyses were performed with PAUP* 4.0b2 (Swofford 2000). All characters were unordered, gaps were treated as missing data, and any areas exhibiting ambiguous alignments were excluded from the analyses. All characters were equally weighted and only parsimony-informative characters were included. Tree construction was performed by using a heuristic search with 1000 replicates, tree-bisection-reconnection (TBR) branch swapping and random stepwise addition. Clade support was estimated with bootstrap analyses (Felsenstein 1985). For the total combined parsimony bt analysis, 1000 replicates of TBR branch swapping with 100 replicates in the heuristic search were performed. For the other seven parsimony analyses (*ndhF*, *matK*, nrITS, *waxy*, *lfy*, combined chloroplast and combined nuclear), however, 'fast' bootstrap replicates were performed because of the long time required for a complete heuristic search. The values from fast bootstrapping will typically be lower than those produced by bootstrapping that involves heuristic searches; thus, they are a conservative estimate of clade support (Mort *et al*. 2000). The fast bootstrap analysis consisted of 100 000 replicates for the *ndhF*, *matK*, nrITS, *waxy*, *lfy*, combined chloroplast and combined nuclear analyses.

The ML analysis for the total combined dataset (as well as the *lfy* and *waxy* clone analyses) was run in RAxML. A fast bootstrap analysis (1000 replicates) was performed simultaneously with the ML analysis (option '-f a'). The GTRMIX model was used, which infers the initial tree topology under GTRCAT and then analyses the final tree topology with GTRGAMMA until stable likelihood values are reached (Stamatakis 2006).

Morphological evolution

All character states were optimised onto the fully resolved combined ML tree with MacClade 4.08 (Maddison and Maddison 2005) with all reconstructions shown. All of the included taxa were scored.

Both persistent calyx fleshiness and fruit type were scored in the analysis of fruit evolution. The character states of the fruiting calyx are 'calyx fleshy' and 'calyx non-fleshy'. A non-fleshy fruiting calyx here refers to one that does not enlarge or become fleshy by the time of fruit maturation; however, when a non-fleshy calyx becomes older (i.e. fruits from a previous year), the calyx can become dry and brittle (C. Bush, P. Fritsch, K. Kron, S. Wagstaff, pers. obs.). The character states of fruit type are 'capsule' and 'berry'. Data are from Burtt and Hill (1935), Franklin (1962), Sleumer (1967), Melvin (1980) and Luteyn (1995*a*, 1995*b*, 1995*c*) and were confirmed by us through field work (Australia and New Zealand: November–December 2007; New Zealand: March–April 2008) and herbarium study.

Inflorescence type was divided into solitary-flowered inflorescences and those that have racemes or panicles as their inflorescence structure. Sleumer (1967) was consulted for the single species of *Diplycosia* sampled in the analysis. *Diplycosia barbigera* usually has solitary-flowered, although sometimes two-flowered, inflorescences (Sleumer 1967). The species was scored as solitary-flowered because this is the predominant condition in the species. All sampled species in the predominantly solitary-flowered sections of *Gaultheria* (i.e. sections *Amblyandra*, *Chamaephyta*, *Chiogenopsis*, *Gaultheria*, *Gymnocaulos*, *Monoanthemona* and *Pernettya*) were scored as solitary-flowered, even though some species can occasionally exhibit few-flowered inflorescences (i.e. pseudoracemes). *Leucothoë* and *Tepuia* were scored by using monographic treatments and other publications (Melvin 1980; Luteyn 1995*a*; Waselkov and Judd 2008).

Historical biogeography

Biogeographical regions were mapped onto the tree generated from the combined-data ML analysis. We defined geographic regions in accordance with a study on the historical biogeography of the southern hemisphere by Sanmartín and Ronquist (2004). The biogeographical regions of Australia (including both mainland Australia and Tasmania), New Zealand, temperate South America and the Indomalaya region are identical to those in Sanmartín and Ronquist (2004). To accommodate members of the Gaultherieae or outgroup taxa located in the northern hemisphere, we included the regions temperate North America and eastern Asia, and expanded Sanmartín and Ronquist's (2004) northern South America region to include all of tropical America.

Fossil data for *Gaultheria* are scarce and in most cases the findings are so recent they are not useful for estimating divergence times within the genus. Fossil records for *Rhododendron* are more plentiful, and seeds of this genus have been found in strata that date to the early Paleocene (~65 million years ago; Collinson and Crane 1978). *Rhododendron* is in subfamily Ericoideae of the Ericaceae, however, and this group originated much earlier than the Vaccinioideae, in which *Gaultheria* and its relatives belong (Kron *et al*. 2002). It would therefore be conservative to assume that *Gaultheria* evolved less than 80 million years ago (after the land mass that would become New Zealand split from Australia) and that dispersal events played the defining role in shaping the current distribution of *Gaultheria* in Australia and New Zealand. This is consistent with Pole (1994), who noted the high probability that most of the flora in the forests of New Zealand arrived via long-distance dispersal. From this reasoning, we used Fitch parsimony to infer the pattern of historical biogeography in *Gaultheria* because it is an exclusively dispersal-based model, unlike e.g. DIVA or other methods (Ronquist 1996, 1997).

Results

Phylogenetic analysis

The *lfy* clone analysis comprised 26 taxa and 79 cloned sequences. There were between two and five clones per taxon. This analysis did not reveal strongly supported conflict in any taxon (data not shown). All clones from a single species either form clades or are only weakly supported as being in separate clades, suggesting that only single copies of *lfy* are present.

The *waxy* clone analysis comprised 28 taxa and 95 cloned sequences. There were between two and five clones per taxon. In the ML analysis, support values were low although there was one taxon that exhibited multiple copies of the *waxy* gene, with separate copies in more than one location in the tree (data not shown). One *G. tasmanica* clone was sister to a pair of *G. parvula* clones (77% bt), whereas the other two were sister to *G. macrostigma* clones (<50% bt). Because of the disparate location of the *G. tasmanica* clones, the *waxy* sequences for this taxon were removed from all subsequent data analyses. No other taxa exhibited discordant *waxy*.

The number of nucleotide positions, parsimony-informative characters, number of most parsimonious trees, length, CI and RI for all individual (*matK*, *ndhF*, ITS, *waxy*, *lfy*) and combined (chloroplast, nuclear, total) maximum parsimony (MP) analyses can be found in Table 2.

The resulting trees from both the parsimony and ML analyses for the total combined dataset were very similar (Figs 4.1 and 4.2, respectively). In all analyses, *Gaultheria* is not monophyletic, consistent with the results of previous studies (Powell and Kron 2001; Bush *et al*. 2009). In both the MP and ML analyses, the Australian +

New Zealand taxa form a clade (84% bt and 99% bt, respectively). *G. antipoda* and the accessions of *G. depressa* form an internally unresolved clade in the MP analysis (80% bt). In the ML analysis, *G. antipoda* is sister to this clade (97% bt). In both analyses, *G. oppositifolia* is sister to *G. antipoda* + *G. depressa* (55% bt, 56% bt). This large clade is in turn sister (64% bt, 86% bt) to an Australian clade (51% bt, 79% bt) of *G. lanceolata* + *G. hispida* + *G. appressa*. Within the Australian clade, *G. lanceolata* is sister to *G. hispida* (100% bt, 100% bt), this clade is sister to *G. appressa* (69% bt, 73% bt) and the clade of these three is sister to *G. appressa* '*viridicarpa*'. The clade of all species above is sister to the remaining clade of Australian/New Zealand *Gaultheria*. Within the latter, *G. rupestris* + *G. crassa* (91% bt, 99% bt) is sister to *G. paniculata* (64% bt, 51% bt). The clade of these taxa are in turn sister to *G. colensoi* (53% bt,; 56% bt). Sister to this clade (<50%bt, 74%bt) is a clade of *G. macrostigma*, *G. tasmanica* and *G. parvula* (96% bt, 100% bt).

In the MP analysis, the clade that includes *G. mucronata*, *G. poeppigii* and *G. pumila* (all of sect. *Pernettya*), *G. nubigena* of sect. *Brossaea*, and *G. insana* of sect. *Pseudogaultheria* (81%) is sister to the New Zealand/Australian clade (56% bt). Within this clade, *G. mucronata* + *G. poeppigii* (85% bt), *G. pumila* (86% bt), *G. nubigena* (81% bt) and *G. insana* (81% bt) are successive sister taxa. The temperate South American species *G. antarctica* of sect. *Chamaephyta* is sister to all previously mentioned species (87% bt). In the ML analysis, however, all of the sampled temperate South American species (*G. poeppigii*, *G. mucronata*, *G. pumila*, *G. nubigena*, *G. insana* and *G. antarctica* = Temperate South America (TSA) clade) are monophyletic (<50% bt). In both analyses, a clade containing tropical American taxa (55% bt, 77% bt) is sister (<50% bt, 89% bt) to the Australia/New Zealand clade + temperate South American taxa.

Morphological evolution

There are three equally optimal reconstructions of calyx evolution over the total combined ML tree of Fig. 4.2, each of five steps (Fig. 4.3). In all optimisations, the calyx of the most recent common ancestor of the Australia/New Zealand clade is inferred to have been fleshy. The calyx changed to non-fleshy along the branches to both *G. oppositifolia* and the *G. colensoi* + *G. crassa* + *G. paniculata* + *G. rupestris* clade. In the TSA clade, non-fleshy calyces arose once along the branch to the *G. poeppigii* + *G. mucronata* + *G. pumila* + *G. nubigena* clade.

There are two equally optimal reconstructions of fruit evolution over the total combinedMLtree, each of five steps (Fig. 4.4). A dry, capsular fruit is plesiomorphic in theAustralia/NewZealand clade. Within this clade, berries have evolved once along the branches to both the *G. parvula* + *G. macrostigma* + *G. tasmanica* clade and *G. lanceolata*. Within the TSA clade, berries have evolved either alongthebranchto*G.mucronata* + *G.poeppigii* + *G.pumila*, + *G. nubigena* + *G. insana*, with a reversal to capsular fruit in *G. nubigena*, or along the branches to *G. insana* and *G. mucronata* + *G. poeppigii* + *G. pumila*.

There is one optimal reconstruction of inflorescence evolution over the total combined ML tree, of seven steps (Fig. 4.5). Within the Australia/New Zealand clade, solitary flowers arose along the branches to the *G. tasmanica* + *G. macrostigma* + *G.*

parvula clade and the *G. antipoda* + *G. depressa* clade. Within the TSA clade, they arose along the branches to *G. antarctica* and *G. mucronata* + *G. poeppigii* + *G. pumila*.

Historical biogeography

There are nine equally optimal reconstructions of biogeographical regions over the total combined ML tree, each of seven steps (Fig. 4.6). The most recent common ancestor of the Australasian clade is inferred to be from New Zealand, with three dispersals to Australia from New Zealand (i.e. along the branches leading to *G. depressa* var. *depressa* (Tasmania), *G. appressa* + *G. appressa* '*viridicarpa*' + *G. hispida* + *G. lanceolata* and *G. tasmanica*). The initial dispersal to New Zealand could have occurred from either tropical South America (with a concomitant dispersal from tropical to temperate South America), or temperate South America.

Discussion

Phylogenetic relationships

Despite the diverse morphological variation among the Australasian *Gaultheria* species, they form a monophyletic group, with the Australian members imbedded within a clade of New Zealand *Gaultheria* species. *G. antipoda* from New Zealand and *G. depressa* are both members of sect. *Monoanthemona* and they are the only New Zealand species of *Gaultheria* that have a dry capsule surrounded by a fleshy calyx (Middleton 1991). Our results strongly support the sister-species relationship of these taxa. The Australasian species of section *Pernettya*, which are distinguished by the possession of a berry fruit, is paraphyletic in our analyses. The three mat-forming species (*G. macrostigma*, *G. parvula*, *G. tasmanica*) form a strongly supported clade, and *G. lanceolata* is sister to the capsular-fruited species *G. hispida* of sect. *Brossaea. G. lanceolata* resembles *G. hispida* in its relatively robust size and large, lanceolate leaves. Thus, vegetative characters rather than fruit morphology seem to be tracking phylogenetic relationships in these two species.

The remaining section containing Australasian species of *Gaultheria*, the racemose, dry-fruited sect. *Brossaea* (ser. *Rupestres*), is also paraphyletic. Four of the five Australasian species (*G. colensoi*, *G. crassa*, *G. paniculata*, *G. rupestris*) form a clade, with *G. crassa* strongly supported as sister to *G. rupestris* and *G. colensoi* forming the first-diverging lineage in the clade. Both *G. colensoi* and *G. crassa* have been considered varieties of *G. rupestris* (Franklin 1962). Our data unambiguously reject this varietal designation for *G. colensoi*. The other Australasian species of sect. *Brossaea*, *G. oppositifolia*, is sister to the *G. antipoda* + *G. depressa* clade. This placement of *G.*

oppositifolia may merely be a consequence of homoplasy, because the support values for this placement are low. There is nonetheless someevidence to indicate, if not necessarily a specific relationship with *G. antipoda* + *G. depressa*, at least a relatively distant relationship with the rest of the Australasian species of sect. *Brossaea. G. oppositifolia* is perhaps the most unusual New Zealand species of *Gaultheria*. It exhibits opposite or subopposite leaves, a condition very rare in the Ericaceae. Moreover, the leaves are cordate and sessile or short-petiolate, whereas in the other dry-fruited species the leaves are elliptic and distinctly petiolate. Finally, most *Gaultheria* species in New Zealand tend to be upright or mat-forming, although *G. oppositifolia* is arching-pendent over road cuts or cliffs.

The phylogenetic analyses strongly indicate that *G. tasmanica* is closely related to both *G. parvula* and *G. macrostigma*. The phylogenetic placement of *G. tasmanica* is in conflict between the nuclear and chloroplast datasets (sister to *G. parvula* in nrITS and nuclear combined analyses yet sister to *G. macrostigma* in the chloroplast combined analysis; data not shown). These results may indicate that there has been genetic exchange between these species in relatively recent times.

Character evolution

Our data support the evolution of non-fleshy calyces in two distinct lineages (i.e. in *G. oppositifolia* and *G. colensoi* + *G. crassa* + *G. paniculata* + *G. rupestris*; Fig. 4.3). They also support the evolution of berries in two distinct Australasian lineages (i.e. in *G. lanceolata* and the clade containing *G. tasmanica* + *G. parvula* + *G. macrostigma*); berries may also have evolved once or twice within the TSA clade (Fig. 4.4). Smith (2000) explored the evolution of capsules and berries in the Neotropical tribes Beslerieae and Napeantheae (Gesneriaceae) and found that dry capsular fruits appear to evolve into berries via an intermediate step of a fleshy dehiscent capsule. Semi-fleshy capsules that partially dehisce can be seen in the range of variation within some New Zealand species of *Gaultheria* (or putative hybrid specimens; C. Bush, P. Fritsch, S. Wagstaff, pers. obs.). Clausing *et al*. (2000) used comparative anatomical data to determine that the traditional classification of Melastomataceae was based on similar although nonhomologous characters of the fruit (capsule *v*. berry). Our results suggest that similar data on *Gaultheria* fruits would be insightful for assessing potential homologies within the genus; however, no such data have been documented. A useful first step would be the careful documentation of the potentially large morphological continuum that marks the fruits of this group.

Knapp (2002) observed that species of Solanaceae with capsular fruits (plesiomorphic in Solanaceae as they are in Ericaceae) tend to occur in dry areas with shrubby vegetation. Specialised habitat types similarly may be driving the apparently rapid changes from capsules to berries in *Gaultheria*. In New Zealand, the capsular-fruited species *G. colensoi*, *G. crassa*, *G. paniculata* and *G. rupestris* tend to occur in dry, shrubby areas. The tendency for the dry-fruited species *G. oppositifolia* to hang over banks or stone cliffs suggests wind-dispersal. In contrast, the species that exhibit dry capsules and fleshy calyces (*G. antipoda*, *G. appressa*, *G. depressa*, *G. hispida*) or berry fruits (*G. macrostigma*, *G. parvula*, *G. tasmanica*) tend to occur in forested areas or forest margins and may depend on brightly coloured and fleshy calyces and berries to attract animal dispersal agents. More work on seed-dispersal agents as well as the frequency and distance of dispersal may reveal relationships between fruit type and biogeographic pattern in *Gaultheria*.

The evolution of inflorescences within Australasian *Gaultheria* is the most homoplasious of the three morphological characters, requiring seven steps within the tree (Fig. 4.5). Within the Gaultherieae, solitary flowers likely arose from racemes at least seven times (Bush *et al*. 2009). Furthermore, variation within a single species can be extensive. For example, in the New Zealand species *G. antipoda*, flowers tend to be solitary although they can sometimes appear in a shortened or congested raceme near the growing tip (Middleton 1991). Our results corroborate the conclusions of Bush *et al*. (2009) that inflorescence architecture is likely to be of little utility for delimiting major taxonomic groups in *Gaultheria*.

Historical biogeography

Opinion on the biogeographical history of the Australasian flora is extensive. Early debate centred on the relative influence of long-distance dispersal *v*. various hypothesised land bridges (see review in Pole 1994). In the 1970s, after the general acceptance of continental drift, the distribution of the Australasian flora came to be predominantly considered the result of large-scale vicariant events related to the stepwise break-up of Gondwana (e.g. Raven and Axelrod 1972). Dispersal was considered to be rarer, and so random that no discernable patterns would result from these events (Craw 1982), although even strong advocates for vicariance acknowledged a role for long-distance dispersal for various elements of the flora (e.g. Raven and Axelrod 1972, 1974).

More recent data from palynological and phylogenetic analyses are nonetheless supporting long-distance dispersal as a general explanation for the southern hemisphere distribution patterns of plants (e.g. Pole 1994, 2001; Richardson *et al*. 2004; Sanmartín and Ronquist 2004; de Queiroz 2005), mainly because many plant lineages are now thought to be too young to have been affected by the separation of landmasses owing to continental drift. Paleogeographic evidence for landmass relationships from multiple sources currently supports Australia as sister to New Guinea, and this clade is sister to southern South America; New Zealand + New Caledonia is then sister to the rest (Sanmartín and Ronquist 2004). In stark contrast, Sanmartín and Ronquist (2004) recovered the general area relationship (southern South America (Australia, New Zealand)) through the analysis of 19 phylogenies. The authors interpret this result as evidence for the prevalent dispersal of plants between Australia and New Zealand (Sanmartín and Ronquist 2004).

In our study, the sister group of the Australasian *Gaultheria* clade comprises taxa sampled from temperate South America. These results are the same as those from several other studies, including Sanmartín and Ronquist (2004). For example, Seberg (1991) used area cladograms from plant, insect and fungal representatives to obtain a general area cladogram that placed southern South America as sister to a clade containing New Zealand, south-eastern Australia and Tasmania. Within the latter clade, south-eastern Australia + Tasmania is sister to New Zealand. Similarly, a study of *Nothofagus* by Linder and Crisp (1995) showed that Australia and New Zealand are more closely related to each other than either is to southern South America.

Our data supports an initial dispersal of *Gaultheria* to New Zealand from South America. Whether the South American origin is temperate or tropical is, however, ambiguous from our results. Sanmartín and Ronquist (2004) found that in their plant dataset, the dispersal frequency from temperate South America to New Zealand was one of the highest (5.90, $P < 0.05$) observed in the data. The dispersal frequency from New Zealand to temperate South America was only 1.10 (Sanmartín and Ronquist 2004). These results suggest that dispersal of *Gaultheria* from temperate South America to New Zealand is more likely than that from New Zealand to temperate South America.

Our results indicate that *Gaultheria* dispersed from New Zealand to Australia/Tasmania at least three times (Fig. 4.6). Traditionally, dominant west winds were thought to bias dispersal from Australia to New Zealand rather than *vice versa* (Winkworth *et al*. 2002*b*). However, several studies support dispersal from New Zealand to Australia (Wagstaff and Garnock-Jones 2000; Lockhart *et al*. 2001; von Hagen and Kadereit 2001; Winkworth *et al*. 2002*a*). Sanmartín and Ronquist (2004) found that dispersal frequencies were higher for dispersals from Australia to New Zealand (6.59) but that routes from New Zealand to Australia were still common (3.82).

Literature Cited

Alverson WS, Whitlock BA, Nyffeler R, Bayer C, Baum DA (1999) Phylogeny of the core Malvales: evidence from *ndhF* sequence data. *American Journal of Botany* **86**, 1474–1486. doi: 10.2307/2656928

Baas P (1985) Comparative leaf anatomy of *Pernettya* Gaud. (Ericaceae). *Botanische Jahrbucher* **105**, 481–495.

Burtt BL, Hill AW (1935) The genera *Gaultheria* and *Pernettya* in New Zealand, Tasmania, and Australia. *Journal of the Linnean Society: Botany* **49**, 611–644. doi: 10.1111/j.1095-8339.1935.tb01342.x

Bush CM, Lu L, Fritsch PF, Li D, KronKA(2009) Phylogeny of Gaultherieae (Ericaceae: Vaccinioideae) based on DNA sequence data from *matK*, *ndhF* and nrITS. *International Journal of Plant Sciences* **170**, 355–364. doi: 10.1086/596330

Clausing G, Meyer K, Renner SS (2000) Correlations among fruit traits and evolution of different fruits within Melastomataceae. *Botanical Journal of the Linnean Society* **133**, 303–326. doi: 10.1111/j.1095-8339.2000.tb01548.x

Collinson ME, Crane PR (1978) *Rhododendron* seeds from the Paleocene of southern England. *Botanical Journal of the Linnean Society* **76**, 195–205. doi: 10.1111/j.1095-8339.1978.tb02361.x

Craw R (1982) Phylogenetics, areas, geology and the biogeography of Croizat: a radical view. *Systematic Zoology* **31**, 304–316. doi: 10.2307/2413237

de Queiroz A (2005) The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology & Evolution* **20**, 68–73. doi: 10.1016/j.tree.2004.11.006

Doyle J, Doyle J (1987)ArapidDNAisolation 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**, 783–791. doi: 10.2307/2408678

Franklin DA (1962) The Ericaceae in New Zealand (*Gaultheria* and *Pernettya*). *Transactions of the Royal Society of New Zealand* **1**, 155–173.

Frohlich MW, Meyerowitz EM (1997) The search for flower homeotic gene homologs in basal angiosperms and Gnetales: a potential new source of data on the evolutionary origin of flowers. *International Journal of Plant Sciences* **158**, S131–S142. doi: 10.1086/297513

Johnson LA, Soltis DE (1994) *matK* DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. *Systematic Botany* **19**, 143–156. doi: 10.2307/2419718

Katoh M, Kuma M (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**, 3059–3066. doi: 10.1093/nar/gkf436

Knapp S (2002) Tobacco to tomatoes: a phylogenetic perspective on fruit diversity in Solanaceae. *Journal of Experimental Botany* **53**, 2001–2022. doi: 10.1093/jxb/erf068

Kron KA, Judd WS, Stevens PF, Crayn DM, Anderberg AA, Gadek PA, Quinn CJ, Luteyn JL (2002) Phylogenetic classification of Ericaceae: molecular and morphological evidence. *Botanical Review* **68**, 335–423. doi: 10.1663/0006-8101(2002)068[0335:PCOEMA]2.0.CO;2

Linder HP, Crisp MD (1995) *Nothofagus* and Pacific biogeography. *Cladistics* **11**, 5–32. doi: 10.1111/j.1096-0031.1995.tb00002.x

Lockhart PJ, McLenachan PA, Havell D, Glenny D, Huson D, Jensen U (2001) Phylogeny, dispersal and radiation of New Zealand alpine buttercups: molecular evidence under split decomposition. *Annals of the Missouri Botanical Garden* **88**, 458–477. doi: 10.2307/3298586

Luteyn JL (1995*a*) *Tepuia*. In 'Flora Neotropica monograph. Vol 66. Ericaceae, part II – the superior-ovaried genera'. (Ed. JL Luteyn) (New York Botanical Garden Press: New York)

Luteyn JL (1995*b*) *Pernettya*. In 'Flora Neotropica monograph. Vol 66. Ericaceae, part II – the superior-ovaried genera'. (Ed. JL Luteyn) (New York Botanical Garden Press: New York)

Luteyn JL (1995*c*) *Gaultheria*. In 'Flora Neotropica monograph. Vol 66. Ericaceae, part II – the superior-ovaried genera'. (Ed. JL Luteyn) (New York Botanical Garden Press: New York)

Maddison DR, Maddison WP (2005) 'MacClade 4.08: an analysis of phylogeny and character evolution.' (Sinauer Associates, Inc.: Sunderland, MA)

Mason-Gamer RJ, Weil CF, Kellogg EA (1998) Granule-bound starch synthase: structure, function, and phylogenetic utility. *Molecular Biology and Evolution* **15**, 1658–1673.

Melvin NC (1980) A systematic investigation of the genus *Leucothoë* (Ericaceae). PhD Thesis Dissertation, University of Miami, Coral Gables, FL.

Mérida A, Rodríguez-Galán JM, Vincent C, RomeroJM (1999) Expression of the granule-bound starch synthase I (*Waxy*) gene from snapdragon is developmentally and circadian clock regulated. *Plant Physiology* **120**, 401–410. doi: 10.1104/pp.120.2.401

Middleton DJ (1991) Infrageneric classification of the genus *Gaultheria* L. Ericaceae. *Botanical Journal of the Linnean Society* **106**, 229–258. doi: 10.1111/j.1095-8339.1991.tb02293.x

Middleton DJ, Wilcock CC (1990) A critical examination of the status of *Pernettya* Gaud. as a genus distinct from *Gaultheria* L. *Edinburgh Journal of Botany* **47**, 291–301.

Mort ME, Soltis PS, Soltis DE, Mabry ML (2000) Comparison of three methods for estimating internal support on phylogenetic trees. *Systematic Biology* **49**, 160–171. doi: 10.1080/10635150050207456

Olmstead RG, Sweere JA (1994) Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. *Systematic Biology* **43**, 467–481. doi: 10.2307/2413546

Parsons K, Hermanutz L (2006) Conservation of rare, endemic braya species (Brassicaceae): breeding system variation, potential hybridization and human disturbance. *Biological Conservation* **128**, 201–214. doi: 10.1016/j.biocon.2005.09.029

Peralta IE, Spooner DM(2001) Granule-bound starch synthase (*GBSSI*) gene phylogeny of wild tomatoes (*Solanum* L. section *Lycopersicon* [Mill.] Wettst. subsection *Lycopersicon*). *American Journal of Botany* **88**, 1888–1902. doi: 10.2307/3558365

Pole M (1994) The New Zealand flora – Entirely long distance dispersal? *Journal of Biogeography* **21**, 625–635. doi: 10.2307/2846036

Pole M (2001) Can long-distance dispersal be inferred from the New Zealand plant fossil record? *Australian Journal of Botany* **49**, 357–366. doi: 10.1071/BT00022

Powell EA, Kron KA (2001) An analysis of the phylogenetic relationships in the wintergreen group (*Diplycosia*, *Gaultheria*, *Pernettya*, *Tepuia*; Ericaceae). *Systematic Botany* **26**, 808–817.

Raven PH, Axelrod DI (1972) Plate tectonics and Australasian paleobiogeography. *Science* **176**, 1379–1386. doi: 10.1126/science.176.4042.1379

Raven PH, Axelrod DI (1974) Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden* **61**, 539–673. doi: 10.2307/2395021

Richardson JE, Chatrou LW, Mols JB, Erkens RHJ, Pirie MD (2004) Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and

Rhamnaceae. *Philosophical Transactions of the Royal Society B Biological Sciences* **359**, 1495–1508. doi: 10.1098/rstb.2004.1537

Ronquist F (1996) 'DIVA 1.1: dispersal-vicariance analysis.' Available at ftp. uu.se [Verified 1996].

Ronquist F (1997) Dispersal–vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology* **46**, 195–203. doi: 10.2307/2413643

Sanmartín I, Ronquist F (2004) Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology* **53**, 216–243. doi: 10.1080/10635150490423430

Seberg O (1991) Biogeographic congruence in the South Pacific. *Australian Systematic Botany* **4**, 127–136. doi: 10.1071/SB9910127

Sleumer H (1967) Ericaceae. *Flora Malesiana Ser. I* **6**, 669–914.

Sleumer H (1985) Taxonomy of the genus *Pernettya* Gaud. (Ericaceae). *Botanische Jahrbucher* **105**, 449–480.

Smedmark JE, Eriksson T, Evans RC, Campbell CS (2003) Ancient allopolyploid speciation in Geinae (Rosaceae): evidence from nuclear granule-bound starch synthase (GBSSI) gene sequences. *Systematic Biology* **52**, 374–385.

Smith JF (2000) Aphylogenetic analysis of tribes Beslerieae and Napeantheae (Gesneriaceae) and evolution of fruit types: parsimony and maximum likelihood analyses of *ndhF* sequences. *Systematic Botany* **25**, 72–81. doi: 10.2307/2666674

Stace HM, Chapman AR, Lemson KL, Powell JM (1997) Cytoevolution, phylogeny and taxonomy in Epacridaceae. *Annals of Botany* **79**, 283–290. doi: 10.1006/anbo.1996.0333

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690. doi: 10.1093/bioinformatics/btl446

Steele KP, Vilgalys R (1994) Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *matK. Systematic Botany* **19**, 126–142. doi: 10.2307/2419717

Swofford DL (2000) 'PAUP* Phylogenetic analysis using parsimony (*and other methods). Version 4.01.' (Sinauer Associates, Inc.: Sunderland, MA)

von Hagen KB, Kadereit JW (2001) The phylogeny of *Gentianella* (Gentianaceae) and its colonization of the southern hemisphere as revealed by nuclear and chloroplast DNA sequence variation. *Organisms, Diversity & Evolution* **1**, 61–79. doi: 10.1078/1439-6092-00005

Wagstaff SJ, Garnock-Jones PJ (2000) Patterns of diversification in *Chionohebe* and *Parahebe* (Scrophulariaceae) inferred from ITS sequences. *New Zealand Journal of Botany* **38**, 389–407.

Wang S, Yeh K, Tsai C (1999) Molecular characterization and expression of starch granule-bound starch synthase in the sink and source tissues of sweet potato. *Physiologia Plantarum* **106**, 253–261. doi: 10.1034/j.1399-3054.1999.106301.x

Waselkov K, Judd WS (2008) A phylogenetic analysis of *Leucothoë* s.l. (Ericaceae; tribe Gaultherieae) based on phenotypic characters. *Brittonia* **60**, 382–397.

White TJ, Bruns T, Lee S, Taylor J (1990)Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In 'PCR protocols: a guide to methods and applications'. (Eds M Innis, D Gelfand, J Sninsky, T White) (Academic Press: San Diego, CA)

Winkworth RC, Grau J, Robertson AW, Lockhart PJ (2002*a*) The origins and evolution of the genus *Myosotis* L. (Boraginaceae). *Molecular Phylogenetics and Evolution* **24**, 180–193. doi: 10.1016/S1055-7903(02)00210-5

Winkworth RC, Wagstaff SJ, Glenny D, Lockhart PJ (2002*b*) Plant dispersal N.E.W.S. from New Zealand. *Trends in Ecology & Evolution* **17**, 514–520. doi: 10.1016/S0169-5347(02)02590-9

Table 4.1

Taxa sampled in this study of the *Gaultheria* from Australia and New Zealand. GenBank accession numbers are listed under the appropriate gene region. Herbarium acronyms follow Holmgren and Holmgren (www.nybg.org/bsci/ih). GenBank numbers in bold represent new data obtained for this study

Table 4.1

Taxon	Voucher information (accession/collection number, institution)	Section according to Middleton (1991) *Gaultheria species only	Approximate geographic distribution (see text)	*matK*	*ndhF*	ITS	*waxy*	*lfy*
Diplycosia barbigera Sleumer	Fuller s.n.; WFU	n.a.	Indomalaya region	AF366622	FJ008932	AF358875	**FJ665760**	n.a.
Gaultheria antarctica Hook.f.	Kron s.n.; WFU	Chamaephyta	Temperate South America	AF366627	FJ008937	AF358880	**FJ665761**	**FJ665717**
Gaultheria antipoda G.Forst.	Bush, Wagstaff and Fritsch 424; WFU	Monoanthemona	New Zealand	**FJ665743**	**FJ665787**	**FJ665699**	**FJ665762**	**FJ665718**
Gaultheria appressa A.W.Hill	Crayn 1131; WFU	Brossaea	New South Wales, Australia	**FJ665744**	**FJ665788**	**FJ665700**	**FJ665763**	n.a.
Gaultheria appressa 'viridicarpa' informally named	Crayn 1132; WFU	Brossaea	New South Wales, Australia	**FJ665745**	**FJ665789**	**FJ665701**	**FJ665764**	**FJ665719**
Gaultheria colensoi Hook.	Bush and Wagstaff 487; WFU	Brossaea	New Zealand	**FJ665746**	**FJ665790**	**FJ665702**	**FJ665765**	**FJ665720**
Gaultheria crassa Allen	Bush, Wagstaff and Fritsch 416; WFU	Brossaea	New Zealand	**FJ665747**	**FJ665791**	**FJ665703**	**FJ665806**	**FJ665721**
Gaultheria depressa Hook.f. var. *depressa* (Tasmania)	Bush 415; WFU	Monoanthemona	Tasmania, Australia	**FJ665748**	**FJ665792**	**FJ665704**	n.a.	**FJ665722**
Gaultheria depressa var. *depressa* (NZ)	Bush, Wagstaff and Fritsch 420; WFU	Monoanthemona	New Zealand	**FJ665749**	**FJ665793**	**FJ665705**	n.a.	**FJ665723**
Gaultheria depressa var. *novae-zealandiae* D.A.Franklin (NZ)	Bush, Wagstaff and Fritsch 417; WFU	Monoanthemona	New Zealand	**FJ665750**	**FJ665794**	**FJ665706**	n.a.	**FJ665724**
Gaultheria hispida R.Br.	Bush 414; WFU	Brossaea	Tasmania, Australia	**FJ665751**	**FJ665795**	**FJ665707**	**FJ665767**	**FJ665725**
Gaultheria humifusa Rybd.	Fritsch 1837; CAS	Amblyandra	Temperate North America	**FJ665752**	**FJ665796**	**FJ665708**	**FJ665768**	**FJ665726**
Gaultheria insana (Molina) D.J.Middleton	19924312; RBGE	Pseudogaultheria	Temperate South America	**FJ665753**	**FJ665797**	**FJ665709**	**FJ665769**	**FJ665727**
Gaultheria insipida Benth.	Luteyn 15328; NY	Brossaea	Tropical America	AF366636	FJ008944	AF358889	**FJ665770**	n.a.

Table 4.1 Continued

Gaultheria lanceolata Hook.f.	Bush 403; WFU	Pernettya	Tasmania, Australia	**FJ665754**	**FJ665798**	**FJ665710**	**FJ665771**	n.a.
Gaultheria macrostigma (Colenso) D.J.Middleton	Bush and Wagstaff 456; WFU	Pernettya	New Zealand	**FJ665755**	**FJ665799**	**FJ665711**	**FJ665772**	**FJ665728**
Gaultheria mucronata Hook. & Arn.	Cubey 54 825 #7; RBGE	Pernettya	Temperate South America	FJ010622	FJ008977	FJ010604	**FJ665773**	**FJ665729**
Gaultheria nubigena B.L.Burtt & Sleumer	C.R.Villamil 10407; NY	Brossaea	Temperate South America	**FJ665808**	**FJ665800**	**FJ665712**	**FJ665774**	**FJ665730**
Gaultheria nummularioides D.Don	vander Kloet 2241092; ACAD	Monoanthemona	Eastern Asia	AF366641	FJ008947	AF358893	**FJ665775**	**FJ665731**
Gaultheria oppositifolia Hook.f.	Bush and Fritsch 440; WFU	Brossaea	New Zealand	**FJ665756**	**FJ665801**	**FJ665713**	**FJ665776**	**FJ665732**
Gaultheria paniculata B.L.Burtt & A.W.Hill	Bush and Fritsch 442; WFU	Brossaea	New Zealand	**FJ665757**	**FJ665802**	**FJ665714**	**FJ665777**	**FJ665733**
Gaultheria parvula D.J.Middleton	Bush, Wagstaff and Fritsch 433; WFU	Pernettya	New Zealand	**FJ665758**	**FJ665803**	**FJ665715**	**FJ665778**	**FJ665734**
Gaultheria poeppigii DC.	Kron s.n.; WFU	Pernettya	Temperate South America	AF366642	FJ008948	AF358894	**FJ665779**	**FJ665735**
Gaultheria procumbens L.	Powell s.n.; WFU	Gaultheria	Temperate North America	AF366643	FJ008949	AF358895	**FJ665780**	**FJ665736**
Gaultheria pumila (L.f.) D.J.Middleton	Kron s.n.; WFU	Pernettya	Temperate South America	AF366644	FJ008950	AF358896	**FJ665781**	**FJ665737**
Gaultheria rupestris (G.Forst.) R.Br.	Bush, Wagstaff and Fritsch 426; WFU	Brossaea	New Zealand	**FJ665759**	**FJ665804**	**FJ665716**	**FJ665782**	**FJ665738**
Gaultheria schultesii Camp	ex garden; Rhododendron Species Foundation	Not placed by Middleton (1991)	Tropical America	FJ010619	FJ008974	FJ010601	**FJ665783**	**FJ665739**
Gaultheria sclerophylla Cuatrec.	Luteyn 15331; NY	Brossaea	Tropical America	AF366646	FJ008951	AF358898	**FJ665784**	**FJ665740**
Gaultheria tasmanica (Hook.f.) D.J.Middleton	1977-5050; RBGE	Pernettya	Tasmania, Australia	AF124568	FJ008954	AF358901	n.a.	**FJ665741**
Leucothoë griffithiana C.B.Clarke	GLGS 32580; CAS	n.a.	Eastern Asia	FJ010616	FJ008971	FJ010598	**FJ665785**	**FJ665742**
Tepuia venusta Camp in Gleason & Killip	Michelangeli 535; NY	n.a.	Tropical America	AF366649	**FJ665807**	AF358905	**FJ665786**	n.a.

Table 4.2

Statistics for individual and combined data maximum parsimony analyses. The total combined dataset was run in the Mafft v6.602b (Katoh and Kuma 2002) alignment program so the total number of base pairs in this aligned dataset is not equal to the sum of the separate datasets. PIC = parsimony-informative characters, CI = consistency index, RI = retention index

Table 4.2

Gene region	Number of taxa	PIC	No. base pairs	Percentage informative	No. trees	Length	CI	RI	Bootstrap analysis
matK	31	39	1411	2.8	479 900 (ran out of memory)	64	0.70	0.79	100 000 fast
ndhF	31	59	1543	3.8	125	105	0.69	0.82	100 000 fast
Chloroplast	31	98	2954	3.3	1039	173	0.68	0.80	100 000 fast
ITS	31	38	649	5.9	18	69	0.64	0.71	100 000 fast
lfy	26	151	1347	11.2	396	369	0.55	0.55	100 000 fast
waxy	27	33	642	5.1	691 700 (ran out of memory)	51	0.69	0.80	100 000 fast
Nuclear	31	222	2638	8.4	540	520	0.54	0.56	100 000 fast
Total data	31	318	5578	5.7	5	733	0.54	0.59	1000/100 full heuristic

Figure 4.1

Strict consensus of the five most parsimonious trees (L = 733, CI = 0.54, RI = 0.59) from a parsimony analysis of the total combined data (*matK*, *ndhF*, nrITS, *lfy*, *waxy*). Bootstrap values are indicated above the branches.

Figure 4.1

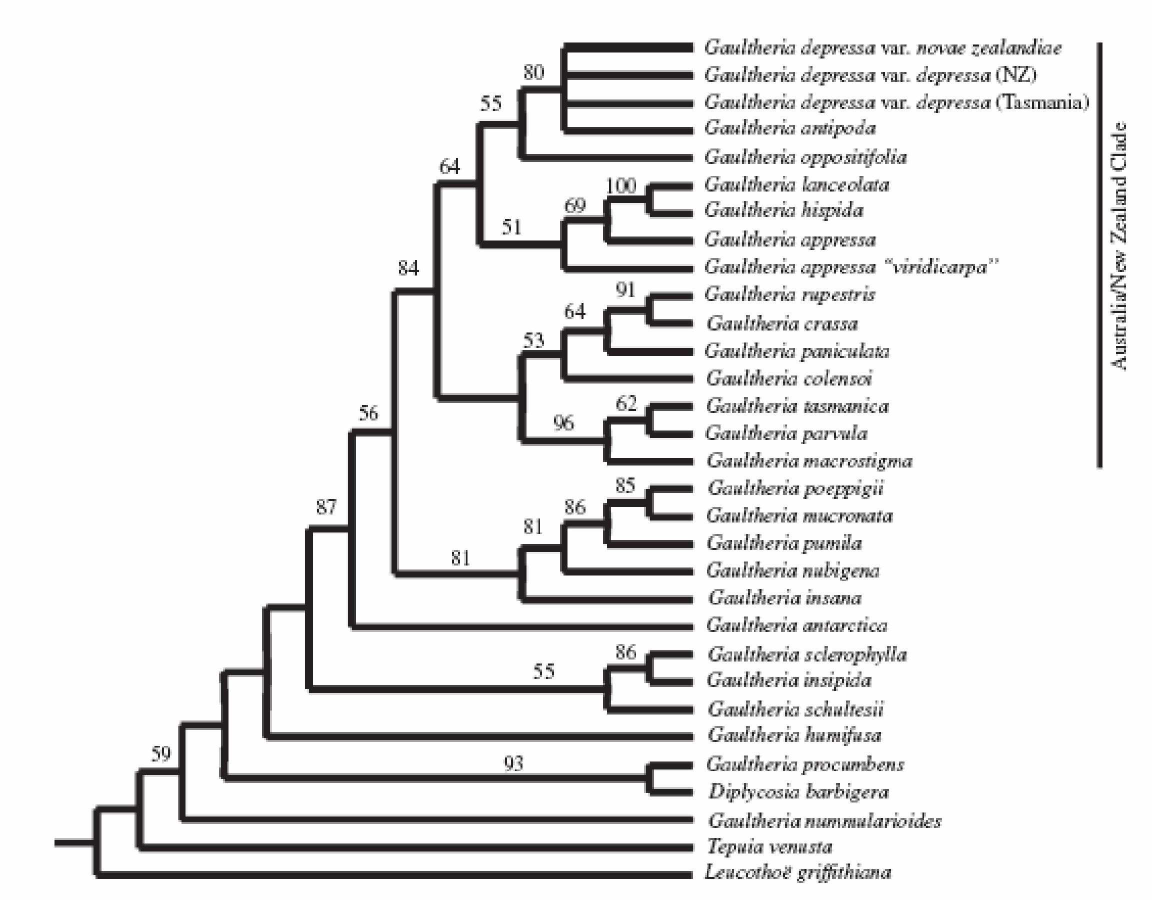

Gaultheria depressa var. novae zealandiae
Gaultheria depressa var. depressa (NZ)
Gaultheria depressa var. depressa (Tasmania)
Gaultheria antipoda
Gaultheria oppositifolia
Gaultheria lanceolata
Gaultheria hispida
Gaultheria appressa
Gaultheria appressa "viridicarpa"
Gaultheria rupestris
Gaultheria crassa
Gaultheria paniculata
Gaultheria colensoi
Gaultheria tasmanica
Gaultheria parvula
Gaultheria macrostigma
Gaultheria poeppigii
Gaultheria mucronata
Gaultheria pumila
Gaultheria nubigena
Gaultheria insana
Gaultheria antarctica
Gaultheria sclerophylla
Gaultheria insipida
Gaultheria schultesii
Gaultheria humifusa
Gaultheria procumbens
Diplycosia barbigera
Gaultheria nummularioides
Tepuia venusta
Leucothoë griffithiana
Australia/New Zealand Clade
80
55
64
100
69
51
84
91
64
53
62
96
56
85
86
87
81
81
86
55
93
59

Figure 4.2

The single tree from a maximum likelihood analysis of the total combined dataset (*matK*, *ndhF*, nrITS, *lfy*, *waxy*). Bootstrap values are indicated above the branches.

Figure 4.2

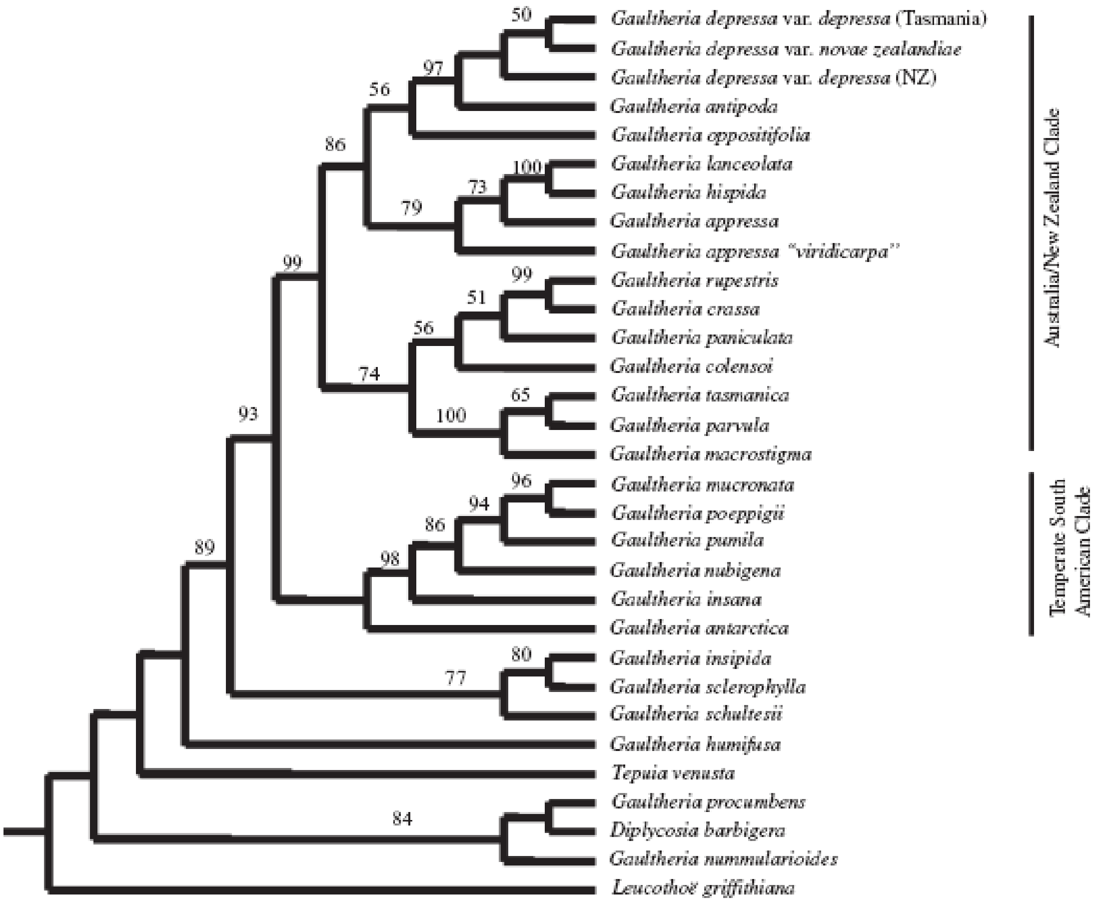

50
97
56
86
100
73
79
99
99
51
56
74
65
100
93
96
94
86
98
89
80
77
84
Gaultheria depressa var. depressa (Tasmania)
Gaultheria depressa var. novae zealandiae
Gaultheria depressa var. depressa (NZ)
Gaultheria antipoda
Gaultheria oppositifolia
Gaultheria lanceolata
Gaultheria hispida
Gaultheria appressa
Gaultheria appressa "viridicarpa"
Gaultheria rupestris
Gaultheria crassa
Gaultheria paniculata
Gaultheria colensoi
Gaultheria tasmanica
Gaultheria parvula
Gaultheria macrostigma
Gaultheria mucronata
Gaultheria poeppigii
Gaultheria pumila
Gaultheria nubigena
Gaultheria insana
Gaultheria antarctica
Gaultheria insipida
Gaultheria sclerophylla
Gaultheria schultesii
Gaultheria humifusa
Tepuia venusta
Gaultheria procumbens
Diplycosia barbigera
Gaultheria nummularioides
Leucothoë griffithiana
Australia/New Zealand Clade
Temperate South American Clade

Figure 4.3

Calyx morphology traced onto the tree from the maximum likelihood tree in Fig. 4.2.

Figure 4.3

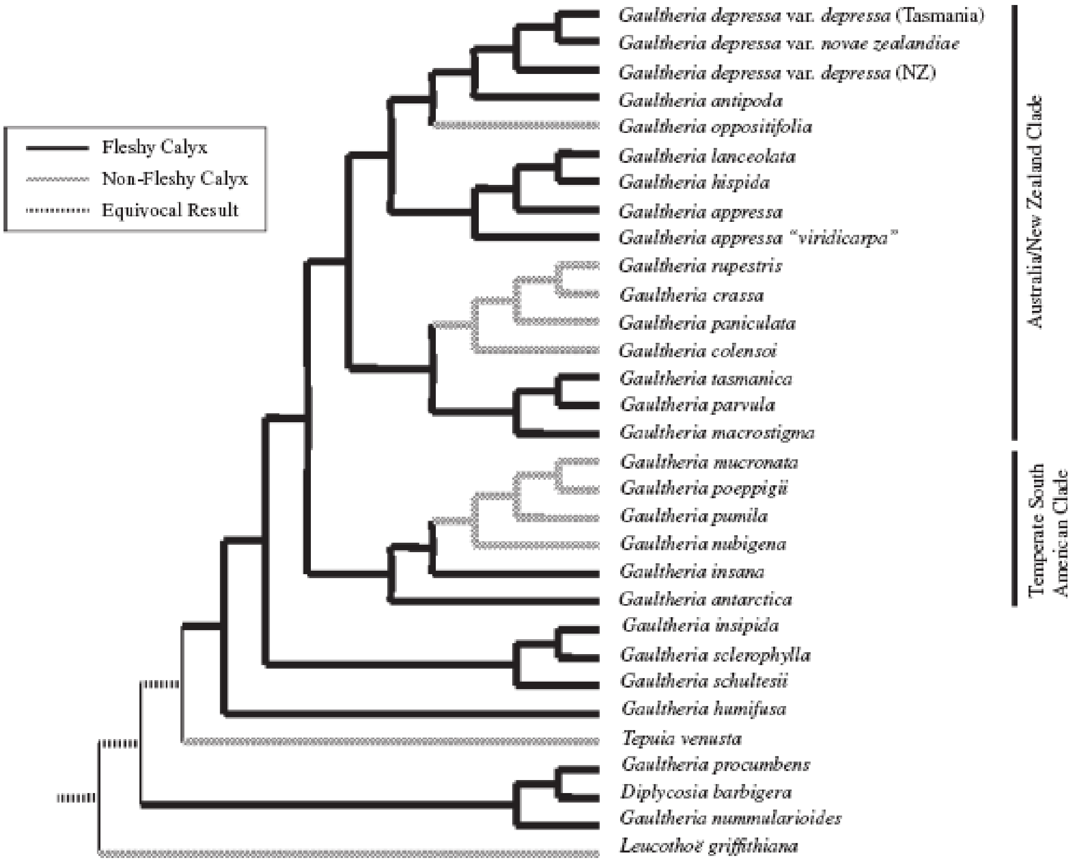
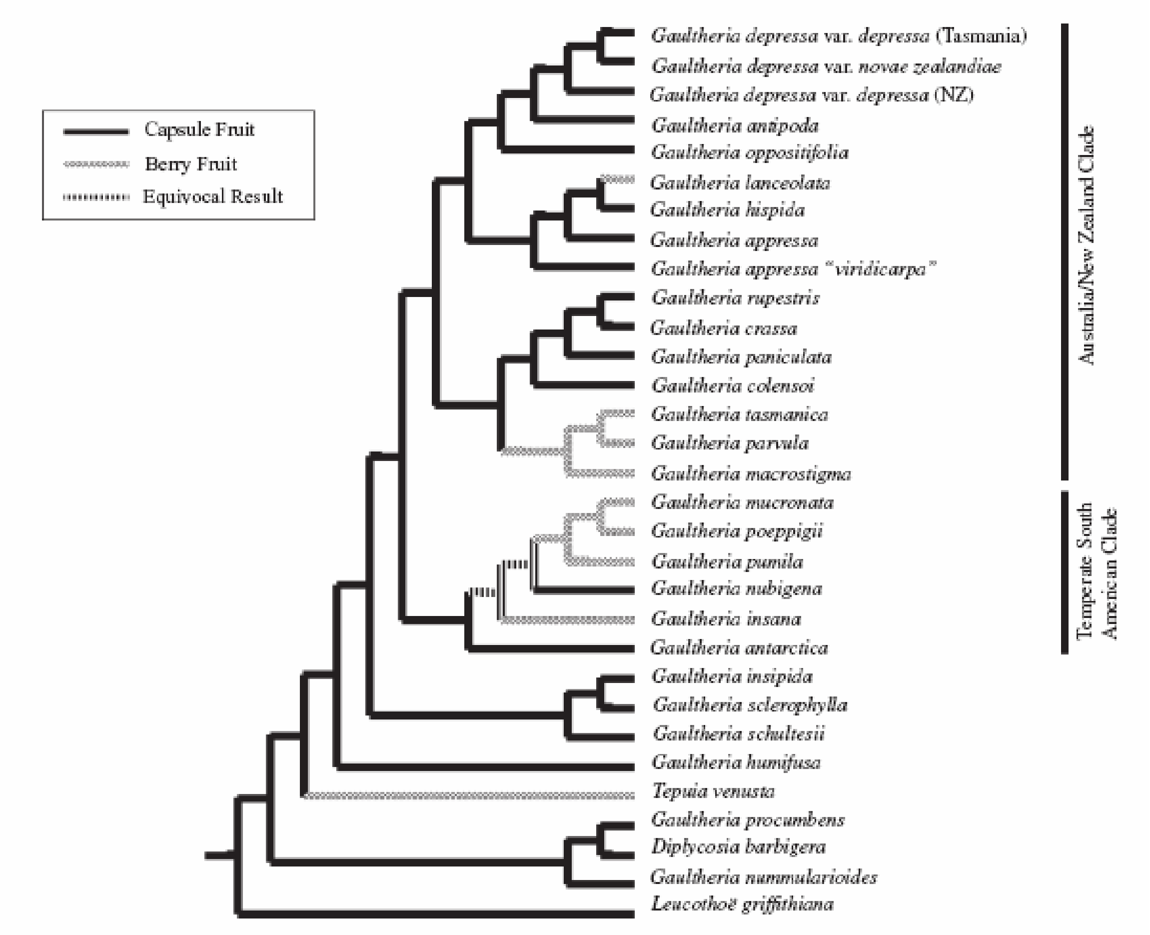

Fleshy Calyx
Non-Fleshy Calyx
Equivocal Result
Gaultheria depressa var. depressa (Tasmania)
Gaultheria depressa var. novae zealandiae
Gaultheria depressa var. depressa (NZ)
Gaultheria antipoda
Gaultheria oppositifolia
Gaultheria lanceolata
Gaultheria hispida
Gaultheria appressa
Gaultheria appressa "viridicarpa"
Gaultheria rupestris
Gaultheria crassa
Gaultheria paniculata
Gaultheria colensoi
Gaultheria tasmanica
Gaultheria parvula
Gaultheria macrostigma
Gaultheria mucronata
Gaultheria poeppigii
Gaultheria pumila
Gaultheria nubigena
Gaultheria insana
Gaultheria antarctica
Gaultheria insipida
Gaultheria sclerophylla
Gaultheria schultesii
Gaultheria humifusa
Tepuia venusta
Gaultheria procumbens
Diplycosia barbigera
Gaultheria nummularioides
Leucothoë griffithiana
Australia/New Zealand Clade
Temperate South American Clade

Figure 4.4

Fruit morphology traced onto the tree from the maximum likelihood tree in Fig. 4.2.

Figure 4.4



Capsule Fruit
Berry Fruit
Equivocal Result
Gaultheria depressa var. depressa (Tasmania)
Gaultheria depressa var. novae zealandiae
Gaultheria depressa var. depressa (NZ)
Gaultheria antipoda
Gaultheria oppositifolia
Gaultheria lanceolata
Gaultheria hispida
Gaultheria appressa
Gaultheria appressa "viridicarpa"
Gaultheria rupestris
Gaultheria crassa
Gaultheria paniculata
Gaultheria colensoi
Gaultheria tasmanica
Gaultheria parvula
Gaultheria macrostigma
Gaultheria mucronata
Gaultheria poeppigii
Gaultheria pumila
Gaultheria nubigena
Gaultheria insana
Gaultheria antarctica
Gaultheria insipida
Gaultheria sclerophylla
Gaultheria schultesii
Gaultheria humifusa
Tepuia venusta
Gaultheria procumbens
Diplycosia barbigera
Gaultheria nummularioides
Leucothoë griffithiana
Australia/New Zealand Clade
Temperate South American Clade

Figure 4.5

Inflorescence type traced onto the tree from the maximum likelihood tree in Fig. 4.2.

Figure 4.5

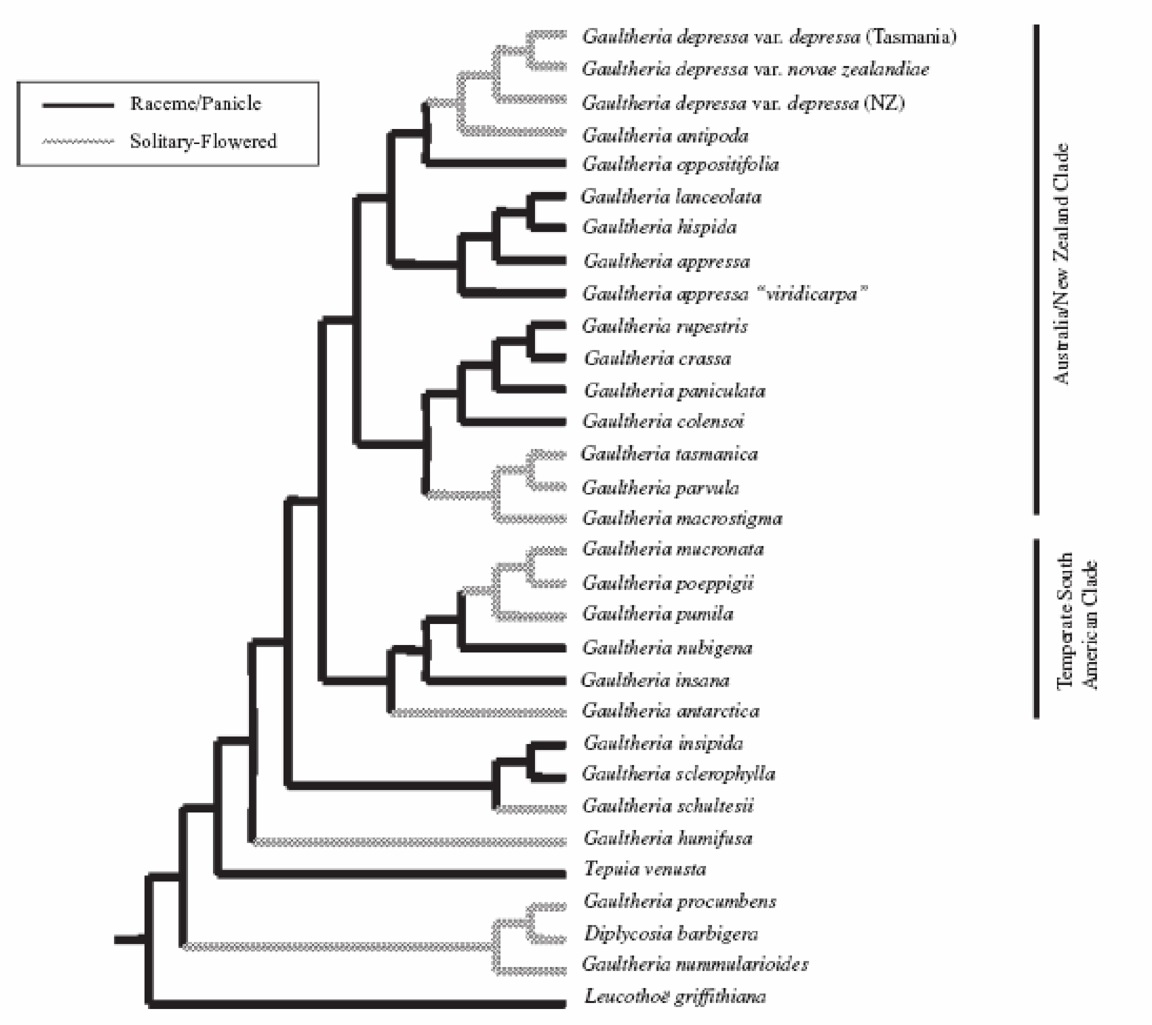
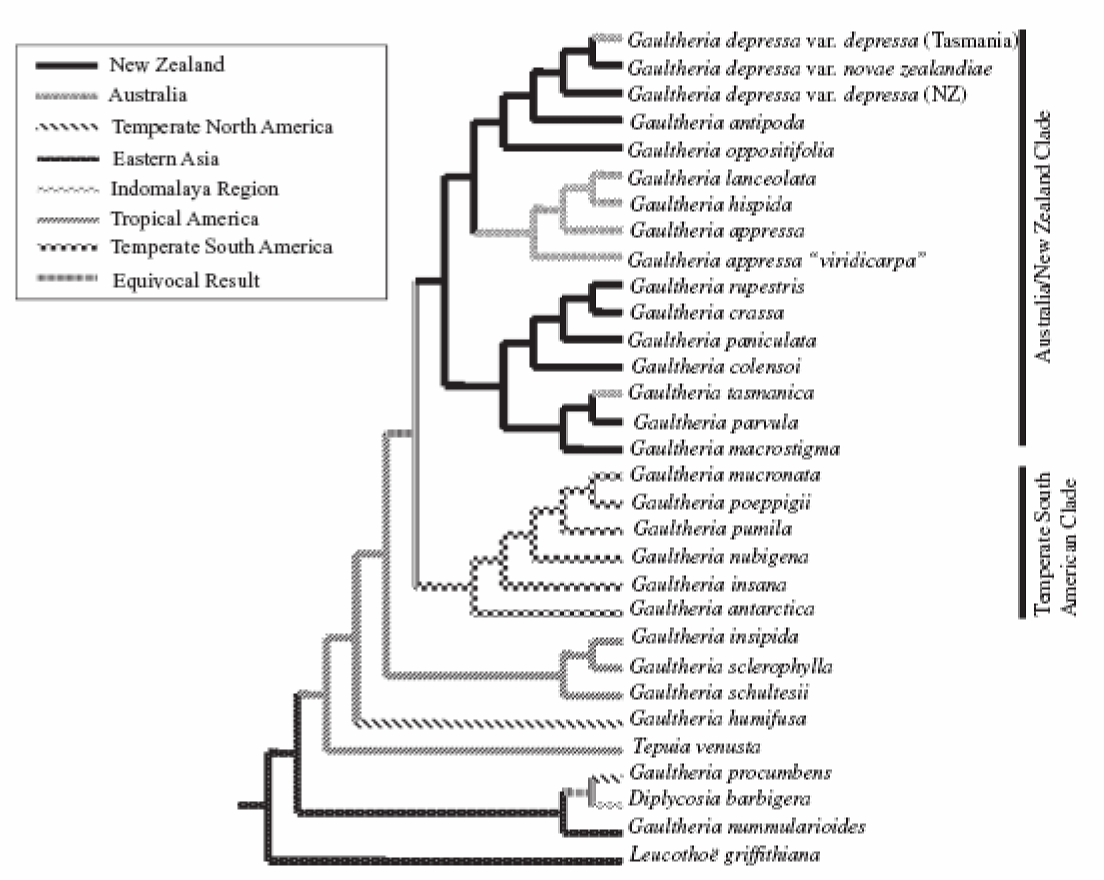

Raceme/Panicle
Solitary-Flowered
Gaultheria depressa var. depressa (Tasmania)
Gaultheria depressa var. novae zealandiae
Gaultheria depressa var. depressa (NZ)
Gaultheria antipoda
Gaultheria oppositifolia
Gaultheria lanceolata
Gaultheria hispida
Gaultheria appressa
Gaultheria appressa "viridicarpa"
Gaultheria rupestris
Gaultheria crassa
Gaultheria paniculata
Gaultheria colensoi
Gaultheria tasmanica
Gaultheria parvula
Gaultheria macrostigma
Gaultheria mucronata
Gaultheria poeppigii
Gaultheria pumila
Gaultheria nubigena
Gaultheria insana
Gaultheria antarctica
Gaultheria insipida
Gaultheria sclerophylla
Gaultheria schultesii
Gaultheria humifusa
Tepuia venusta
Gaultheria procumbens
Diplycosia barbigera
Gaultheria nummularioides
Leucothoë griffithiana
Australia/New Zealand Clade
Temperate South American Clade

Figure 4.6

Biogeographical regions traced onto the maximum likelihood tree from Fig. 4.2 (*matK*, *ndhF*, nrITS, *lfy*, *waxy*). The biogeographic regions are New Zealand, Australia, temperate North America, eastern Asia, the Indomalaya region, tropical America and temperate South America.

Figure 4.6


New Zealand
Australia
Temperate North America
Eastern Asia
Indomalaya Region
Tropical America
Temperate South America
Equivocal Result
Gaultheria depressa var. depressa (Tasmania)
Gaultheria depressa var. novae zealandiae
Gaultheria depressa var. depressa (NZ)
Gaultheria antipoda
Gaultheria oppositifolia
Gaultheria lanceolata
Gaultheria hispida
Gaultheria appressa
Gaultheria appressa "viridicarpa"
Gaultheria rupestris
Gaultheria crassa
Gaultheria paniculata
Gaultheria colensoi
Gaultheria tasmanica
Gaultheria parvula
Gaultheria macrostigma
Gaultheria mucronata
Gaultheria poeppigii
Gaultheria pumila
Gaultheria nubigena
Gaultheria insana
Gaultheria antarctica
Gaultheria insipida
Gaultheria sclerophylla
Gaultheria schultesii
Gaultheria humifusa
Tepuia venusta
Gaultheria procumbens
Diplycosia barbigera
Gaultheria nummularioides
Leucothoë griffithiana
Australia/New Zealand Clade
Temperate South American Clade

CHAPTER V

A REVISION OF *GAULTHERIA* (ERICACEAE) IN AUSTRALIA AND NEW ZEALAND.

Abstract

A taxonomic revision of the genus *Gaultheria* in Australia and New Zealand is presented. Ten species are recognized, all of which only occur in Australia and/or New Zealand. A recently described taxon, *G. viridicarpa*, is maintained as a distinct species. Several species have been synonomized: *G. parvula* (= *G. tasmanica*); *G. colensoi*, *G. crassa* and *G. paniculata* (= *G. rupestris*) and *G. nubicola* (= *G. depressa*). Lectotypes are designated for *Gaultheria appressa*. The treatment includes PCA analyses of nine morphological quantitative characters, a key to the species, descriptions, distributions and specimen citations.

Introduction

The genus *Gaultheria* L. (including *Diplycosia*, *Pernettya* and *Tepuia*) contains approximately 130 species that occur in New Zealand, Australia, eastern Asia, the Indomalaya region, and both tropical and temperate regions of the Americas (Middleton 1991; Bush et al. 2009). There are 14 currently recognized species of *Gaultheria* in Australasia (here defined as Australia and New Zealand, with New Guinea excluded; Middleton 1991). *Gaultheria appressa* is endemic to New South Wales and Victoria, *G. hispida*, *G. lanceolata*, and *G. tasmanic*a are endemic to Tasmania, and *G. antipoda*, *G. colensoi*, *G. crassa*, *G. macrostigma*, *G. nubicola*, *G. oppositifolia*, *G. paniculata*, *G. parvula*, and *G. rupestris* are endemic to New Zealand. *Gaultheria depressa* is the only species of *Gaultheria* to occur in both Australia (Tasmania) and New Zealand (Burtt and Hill 1935; Franklin 1962; Middleton 1991). The formal recognition of *Gaultheria viridicarpa* is currently in press; this taxon was considered to be a variety of *G. appressa* (var. *glabra*; Franklin 1962). Representatives of this taxon are included in the morphological analyses. Phylogenetic studies have confirmed that the Australasian clade of *Gaultheria* is monophyletic with strong support (Bush et al. 2009). In that study the Australian species are not monophyletic; there are at least three dispersal events from New Zealand to Australia hypothesized indicated (Bush et al. 2009).

Although the Australasian species of *Gaultheria* constitute merely 11% of the species diversity of the genus, together they encompass the extremes of variation across the genus in several morphological characters, as reflected in their distribution among four different sections (*G. nubicola*: sect. *Chamaephyta* D. J. Middleton; *G. antipoda*, *G.*

depressa: sect. *Monoanthemona* D. J. Middleton; *G. lanceolata, G. macrostigma*, *G. parvula*, *G. tasmanica*: sect. *Pernettya* (Gaud.) D. J. Middleton; *G. appressa*, *G. hispida, G. colensoi*, *G. crassa*, *G. oppositifolia*, *G. paniculata*, *G. rupestris*: sect. *Brossaea* (L.) D. J. Middleton; Middleton, 1991). Notable features among these species are a fleshy versus non-fleshy fruiting calyx, capsular versus baccate fruit, and a solitary-flowered inflorescence versus one that is racemose or paniculate.

Species of *Gaultheria* typically have dry capsular fruits surrounded by a fleshy, often brightly colored (e.g. red, white, or blue) accrescent calyx (Middleton 1991). *Gaultheria antipoda*, *G. appressa*, *G. viridicarpa*, *G. depressa*, and *G. hispida* all have this type of fruit. Most species of Ericaceae have a capsule with a non-fleshy calyx (i.e. dry). Although this condition occurs only rarely in *Gaultheria*, it is found in five New Zealand species (*G. colensoi*, *G. crassa*, *G. oppositifolia*, *G. paniculata*, and *G. rupestris*); it otherwise occurs only in *G. itatiaiae* (Wawra ex Drude) Sleumer and *G. sleumeriana* Kin.-Gouv. (southeastern Brazil), and *G. nubigena* B. L. Burtt & Sleumer (temperate South America; Middleton 1991). The fruit characteristics of *G. nubicola* have never been confirmed and there are very few collections of this species (pers. obs., C. Bush).

Gaultheria fruits can also be berries, with or without a fleshy calyx. The berry-fruited species have traditionally been segregated from *Gaultheria* as *Pernettya* Gaud. (Baas 1985; Sleumer 1985), but Middleton and Wilcock (1990) found no basis for maintaining distinct genera and thus subsumed *Pernettya* into *Gaultheria*. Middleton subsequently (1991) erected *Gaultheria* sect. *Pernettya* for these species in an infrageneric classification of *Gaultheria*. This is supported by molecular analyses (Bush et al. 2009) that showed the paraphyly of "*Pernettya*" Only thirteen species of *Gaultheria* possess berries: *G. insana* plus all species of sect. *Pernettya*. Four of these (*G. lanceolata*, *G. macrostigma*, *G. parvula*, and *G. tasmanica*) occur in Australasia, whereas the remainder occur in the American tropics and temperate South America.

Bush et al. (2009) showed that dry calyces, berries and solitary-flowered inflorescences each likely evolved twice within the Australasian clade (in *G. oppositifolia* and *G. rupestris + G. crassa + G. colensoi + G. paniculata*; in *G. lanceolata* and *G. parvula + G. macrostigma + G. tasmanica*; and in *G. antipoda + G. depressa* and *G. parvula + G. macrostigma + G. tasmanica*, respectively).

Despite such divergent morphologies, the New Zealand species of *Gaultheria* readily hybridize, with mixed species populations often forming apparent hybrid swarms. Burtt & Hill (1935) described six putative hybrid combinations among the species with a dry capsule (treated as *Gaultheria* in the narrow sense), and five putative hybrids between capsular- and berry-fruited species (treated as *Pernettya*). Franklin (1962) expanded the number of parental species combinations to 14 among the capsular-fruited species, in almost every combination possible, and eight capsular-fruited × berry-fruited hybrids. Hybrid formation is apparently restricted to disturbed habitats, as in road cuts or braided river systems (Parsons and Hermanutz 2006; C. Bush, S. Wagstaff, pers. obs.) and thus, the New Zealand species can still easily be discerned as distinct in ecologically stable environments.

Bush et al. (2009) determined that the Australasian clade was sister to a clade of temperate South American species of *Gaultheria*. The origin of the Australasian clade was determined to have been from South America, although the results were ambiguous as to whether the origin was from temperate or tropical South America (Bush et al. 2009).

The Diagnosable Species Concept is used in this revision, where a species is defined with a unique combination of morphological characters (Nixon and Wheeler 2008). The common Biological Species Concept (Mayr 1942) is not applicable in this situation because evidence for spatial or genetic reproductive isolation has not been adequately studied for the proposed species. Species concepts based on shared ancestral lineage is not applicable because multiple accessions of the same species were not tested for monophyly (Evolutionary or Phylogenetic Species Concepts; Nixon and Wheeler 2008).

The phylogenetic analysis by Bush et al. (2009) sampled species as they were presented in Franklin (1962) and Burtt and Hill (1935), the most recent and thorough treatments of New Zealand and Australian Gaultheria respectively. Extensive field work and study of herbarium material were performed before the formal revision of these species was begun. Therefore, there was great familiarity with the previously determined species and their evolutionary relationships, which may have affected the determination of species in this revision. However, great care was taken to identify distinct morphological entities (species), regardless of previous circumscription.

The purpose of this study is to re-examine the species boundaries provided by Burtt and Hill (1935) and Franklin (1962) of the currently recognized taxa of *Gaultheria* from Australia and New Zealand by performing morphometric measurements on vegetative and reproductive characters on herbarium sheets. Observations made and recorded during field work will also contribute to the final species descriptions.

Materials and Methods

A total of 1205 herbarium sheets of *Gaultheria* from Australia and New Zealand were examined. Material was obtained via loan from a total of thirteen institutions (AK, CHR, E, HO, K, MELU, MPN, NSW, NZFRI, P, UNSW, WAIK, WELT). A total of 272 herbarium sheets were selected for the morphometric analysis based on the presence of fruiting and vegetative material. These sheets represent every currently named and/or morphological distinct group of *Gaultheria* from Australia and New Zealand (Table 5.1). A total of 78 characters were measured or observed and then recorded (Table 5.2).

Principle component analyses were performed in PAST (Hammer et al., 2001). Two separate data sets were analyzed. Nine vegetative and reproductive characters were included in the first analysis with a total of 105 individuals (henceforth referred to as "Vegetative + Reproductive" data set). The characters included in this data set are: average (of 3) leaf length, average (of 3) leaf width, petiole length, peduncle length, calyx lobe length (in fruit), calyx lobe width (in fruit), calyx length (in fruit), fruit width and fruit length (= height; Table 5.2). The other data set included only the first three vegetative charaters [average (of 3) leaf length, average (of 3) leaf width, petiole length] that were also included in the Vegetative + Reproductive data set (Table 5.2). This data set will be referred to as "Vegetative Only" and it includes 258 individuals. Both data sets (Vegetative + Reproductive and Vegetative Only) have no missing data. Individuals that were clearly noted as being putative hybrids were excluded.

Within each data set, five individual PCA analyses were run. The first analysis included all the individuals (258 for Vegetiative Only data set or 105 individuals for Vegetative + Reproductive data set). The next analyses included subsets of these individuals based on species groupings derived from the previous PCA analysis as well as field observations. These groups were supported by the PCA analyses and morphology.

For the species descriptions, a single measurement range indicates length. Leaf width was measured at the widest part of the leaf. All measurements were taken from herbarium material. Measurements in species descriptions primarily come from the efforts of this study; in only a few instances measurements were taken from the literature (Middleton 1991). Anthers were measured from the base to the terminal pore and did not include the length of any appendages such as awns.

Results

The results from the Vegetative Only and Vegetative + Reproductive analyses are quite similar (Fig. 5.1, 5.2). The additional individuals in the Vegetative Only data set compared to the Vegetative + Reproductive data set allowed the groupings to be visualized more clearly.

In both sets of analyses, individuals sampled form distinct clusters (Figs. 5.1, 5.2). One cluster corresponds to individuals currently assigned to *Gaultheria antipoda*, *G. depressa* and *G. nubicola* (absent in the 9 character data set). Other individuals form three groups corresponding to several currently recognized species each 1.) *G. macrostigma*, *G. parvula* and *G. tasmanica*; 2.) *G. crassa, G. rupestris* and *G. paniculata*; and 3.) *G. appressa* and *G. hispida*. Individuals sampled that do not associate with the clusters named above correspond to *G. viridicarpa*, *G. colensoi, G. oppositifolia*, and *G. lanceolata*.

The individuals corresponding to the species *Gaultheria macrostigma*, *G. parvula* and *G. tasmanica* grouped together in both the Vegetative Only and Vegetative + Reproductive data sets. Individuals of *Gaultheria macrostigma* were found to form a distinct separate cluster in the Vegetative Only data set; in the Vegetative + Reproductive data set, the separation is not as evident (Fig. 5.3, 5.4). Individuals of sampled *Gaultheria parvula* and *G. tasmanica*, however, could not be differentiated from one another in either PCA analysis.

In the analysis of the New Zealand taxa with dry calyces and capsules, *Gaultheria oppositifolia* individuals were distinct and isolated entities (Figs 5.5, 5.6). Individuals that corresponded to *Gaultheria rupestris*, *G. paniculata*, *G. colensoi* and *G. crassa* could not be differentiated from each other in the Vegetative Only data set. In the Vegetative + Reproductive data set, *G. colensoi* appears to be set apart from the rest of the group but there are only three representatives of the taxon included in this analysis (Fig. 5.6).

In the *G. antipoda + G. depressa* + *G. nubicola* subset analyses, individuals corresponding to *G. antipoda* and *G. depressa* can generally be separated out by PCA analysis (Figs. 5.7, 5.8). Individuals of *Gaultheria nubicola* group with *G. depressa* in the large PCA analysis (Fig. 5.1). In the three gene data set PCA analysis of just *G. appressa, G. depressa* and *G. nubicola*, *G. nubicola* is not distinct from *G. depressa* (Fig. 5.7; the species is not included in the Vegetative + Reproductive date set due to missing data). An additional analysis was performed with the same Vegetative Only on the two proposed varieties of *Gaultheria depressa.* Nine individuals of *G. depressa* var. *depressa* and thirteen representatives of *G. depressa* var. *novae-zealandiae* were included in this PCA analysis (Fig 5.9). The results indicate that there is no discernable difference between individuals representing the two varieties (Fig. 5.9).

The Vegetative Only and Vegetative + Reproductive PCA analyses for *Gaultheria appressa, G. hispida, G. lanceolata* and *G. viridicarpa* reveals that *G. lanceolata* and *G. viridicarpa* form very distinct entities (Figs. 5.10, 5.11). *Gaultheria appressa* and *G. hispida* are slightly mixed in the Vegetative + Reproductive data set PCA with fewer representatives but are clearly separate in the Vegetative Only data set (Fig. 5.11).

Discussion

The results indicate that *G. macrostigma* is distinct from *G. tasmanica* + *G. parvula*. Burtt and Hill (1935) dealt with the *Gaultheria* from both Australia and New Zealand so a direct dichotomous key comparison between the two taxa (*Pernettya nana* = *Gaultheria parvula* and *P. tasmanica* = *G. tasmanica*) reveals that the only differences between the species are slightly smaller leaves in *G. parvula* and filaments that are ribbon-like in *G. parvula* whereas *G. tasmanica* filaments exhibit a distinct swelling at the base. The key states that *G. parvula* has short awns on its anthers whereas *G. tasmanica* lacks awns (Burtt and Hill 1935). However, under the description of *Pernettya tasmanica*, it is noted (Burtt and Hill 1935) that some specimens do have minute awns. Both species have pink to red berries with a fleshy calyx subtending the fleshy ovary, often with the free distal parts of the calyx lobe not becoming fleshy and instead remaining somewhat dry and green (Burtt and Hill 1935; pers. obs, C. Bush). *Gaultheria macrostigma* consistently has longer leaves, a wiry habit with a relatively long distance between leaf nodes and a propensity for riverine or boggy habitats; *G. tasmanica* + *G. parvula* has shorter leaves and a small, dense habit (pers. obs., C. Bush, P. Fritsch, S. Wagstaff). The phylogenetic analysis done by Bush et al. (2009) supports the designation of two species for this group: *G. macrostigma* and *G. tasmanica* s.l (including *G. parvula*). The combined data analysis shows *G. tasmanica* as sister to *G. parvula* (65% bt) with *G. macrostigma* being sister to the pair of species (100% bt).

In the PCA analyses, *G. crassa*, *G. colensoi*, *G. paniculata* and *G. rupestris* cannot be separated from each other. These four taxa will be synonomized under the name *G. rupestris*. *Gaultheria rupestris*, therefore, is a widespread and variable species, which can range from a subshrub in alpine habitats (i.e., *"G. crassa"*) to a small tree at lower elevations (*"G. paniculata"*). Phylogenetic analyses support these findings (Bush *et al.* 2009). In the total combined, five gene maximum likelihood analysis of the Australasian clade, *G. rupestris* is sister to *G. crassa* with strong support (99% bt); these two species are in turn sister to *G. paniculata* (51% bt; Bush *et al*. 2009). Finally, *G. colensoi* is sister to the previous three taxa (56% bt; Bush *et al*. 2009). *Gaultheria colensoi* is a narrow endemic taxa found only in Tongariro National Park in the North Island of New Zealand (Franklin 1962). It was considered distinct from *Gaultheria rupestris* s.l. by having terminal inflorescences, but morphological observations show that *G. colensoi* can have terminal and axillary infloresences and it is morphologically similar to *G. crassa*. *Gaultheria oppositifolia* was sister to the *G. antipoda* + *G. depressa* clade (56% bt) in the phylogenetic analysis, and not closely related to species from New Zealand species with dry calyces (Bush *et al*., 2009). This taxon is distinct morphologically due to its opposite or sub-opposite leaves (a very rare character in the Ericaceae), that are cordate and sessile and its pendent habit. Based on PCA analyses, field observations and molecular data, two species in this group are recognized: *G. oppositifolia* and *G. rupestris*.

Although it could be maintained that *G. depressa* is a high-altitude form of *G. antipoda* (both have similar leaf shape and overlapping leaf length and width measurements; pink, red or white fleshy calyces surrounding a capsule and can occasionally exhibit gynodioecy), these two species have been seen growing together at Arthur's Pass (South Island) as well as other localities in New Zealand and this supports their continued separation as distinct species (pers. obs.; C. Bush, P. Fritsch, S.

Wagstaff). There are several other distinct morphological distinctions between the species. *Gaultheria antipoda* is an erect shrub 30 cm – 2 m tall and *G. depressa* is procumbent and only 8 -15 cm high. The adaxial leaf surface of *G. antipoda* is smooth with very faint secondary venation while *G. depressa* has prominent, sulcate veins. Also, *G. antipoda* has thin-coriaceous leaves whose margins are serrate; *G. depressa* has thick leaves with rounded teeth. The thin leaves of *G. antipoda* often curl or appear undulatory at the edges in the field or when pressed on herbarium sheets (pers. obs., C. Bush). Finally, the pedicel length is generally longer in *G. antipoda* (1.7 – 4.3 mm) and the anthers are awned compared to *G. depressa* (0.8 – 1.5 mm) where awns on the anthers are absent or are only minute projections.

The type of *Gaultheria nubicola* lacks any fruiting material and intensive field work has failed to obtain and/or confidently identify this poorly collected and described species. Originally named by D. A. Franklin (1962) as *Pernettya alpina*, the species was placed as *Gaultheria nubicola* in its own series by Middleton (1991). The name *Gaultheria alpina* was already in the literature as a synonym of *G. foliolosa* (Sleumer 1936). The original placement in *Pernettya* (Franklin 1962) implies the fruit would be a berry; however, this has not been confirmed. *Pernettya alpina* (= *Gaultheria nubicola*) was thought by Franklin (1962) to be closely related to *Pernettya nana* (= *G. parvula*); both species have predominantly entire leaf margins with only a few teeth on each edge (as opposed to *G. depressa* which has serrate leaf margins). Without fruit characteristics present on the type material, this taxon is extremely ambiguous. Vegetatively, it is most similar to *G. depressa* and PCA analyses support its inclusion in the taxon. Both species also exhibit anthers that are awnless (Franklin 1962). Also, a combined analysis using sequence data (nrITS, 5' *matK* and *ndhF*) revealed that an accession of *G.* cf. *nubicola* collected in New Zealand (Bush, Fritsch, Wagstaff; November 2007) was sister to an unresolved clade containing three different accessions of *G. depressa* (< 50% bt; data not shown). Based on the morphological and molecular evidence, *Gaultheria nubicola* is synonomized with *G. depressa*.

Gaultheria depressa var. *depressa* occurs in both Tasmania and New Zealand and is traditionally defined as having persistent, distinct hairs extending from the teeth of the leaf margin. *Gaultheria depressa* var. *novae-zealandiae* is found only in New Zealand and it lacks these long persistent hairs along the leaf margin. Also, morphological study revealed that some leaves of *G. depressa* var. *novae-zealandiae* did have persistent hairs; the taxon also has very prominent nubs on the teeth of its leaf margin (pers. obs; C. Bush). Because of this, no varietal ranks are recognized in *Gaultheria depressa.* This result is also supported in the phylogeny generated by Bush *et al.* (2009). In the total data (five gene) maximum likelihood analysis of the Australasian species of *Gaultheria*, *G. depressa* var. *depressa* from Tasmania was sister to *G. depressa* var. *novae-zealandiae* [50% bootstrap (bt) support] and these two taxa were in turn sister to *G. depressa* var. *depressa* from New Zealand (< 50% bt support). These results indicate that the three taxa are very closely related.

Gaultheria appressa and *G. hispida* are similar morphologically, although *G. appressa* is found in mainland Australia and *G. hispida* is found in Tasmania (Burtt and Hill 1935). According to the key written by Burtt and Hill (1935), the main morphological difference between the two species is the consistent presence of spreading hairs on the branchlets of *G. hispida* versus appressed hairs in *G. appressa*. *Gaultheria* *lanceolata* is very morphologically isolated since it vegetatively resembles *G. hispida* but it exhibits a berry fruit as opposed to the capsular one seen in *G. hispida* and *G. appressa*. *Gaultheria viridicarpa*, which is known only from a few populations, is remarkably distinct from *G. appressa*. *Gaultheria viridicarpa* has aborted racemes which make the inflorescence look like solitary flowers with several bracteoles along the pedicel. Also, the species has no hairs on the stem, a character very prominent in *G. appressa* and *G. hispida*.

In Bush *et al*. 2009, *G. lanceolata* is strongly supported as being sister to *G. hispida* (100% bt; both species are from Tasmania) while *G. appressa* is sister to these two taxa (73% bt) and *G. viridicarpa* is sister to the former three taxa (79% bt). The authors maintain the species designations for all four of these taxa.

Taxonomic Treatment of the *Gaultheria* from Australia and New Zealand

Gaultheria L. Sp. Pl. 1: 395, 1753. Type species: *Gaultheria procumbens* L.

Brossaea L. Sp. Pl. 1: 1190, 1753.

Gualteria Duhamel. Traite arbr. arbust. 1: 285, 1755 (orth. var.).

Brossea Cothenius, Disp. veg. meth. 11, 1790 (orth. var.).

Chiogenes Salisb. Trans. Hort. Soc. London. 2: 94, 1817.

Shallonium Raf. Amer. Month Mag. 2: 266, 1818.

Glyciphylla Raf. Amer. Monthly Mag. 4: 192, 1819.

Pernettya Gaud. Ann. Sci. Nat. (Paris) 5: 102, 1825.

Phalerocarpus G. Don. Gen. Hist. 3: 841, 1834.

Epigaea L. sect. *Brossaea* de Candolle, Prodr. 7: 591, 1838.

Lasierpa Torrey. Geol. Report New York 152, 1839.

Shrubs evergreen, procumbent to erect, to 3.5 m tall. Branchlet indumentum absent or of small unicellular or larger multicellular appressed or erect hairs; often caducous. Leaves 3.0 – 185.0 X 2.0 – 110.0 mm, alternate and spirally arranged (although opposite/sub-opposite in one species), coriaceous, serrate, crenulate or entire, venation pinnate or melastomataceous, base cordate to cuneate, apex rounded to acuminate, glabrous or with multicellular erect hairs particularly on midrib of abaxial leaf surface. Inflorescence axillary, (rarely terminal), in racemes, panicles or solitary-flowered (occasionally aggregating towards the tips of the branches as the subtending leaves become smaller, resembling bracts: i.e. pseudoraceme). When in racemes and panicles two or more bracteoles are present with one larger subtending bract. Bract 1.3

– 2.0 X 1.0 – 1.7 mm, reddish tan, broadly ovate, with a maximum length of approximately 14.0 mm, persistent, glabrous or with multicellular appressed or erect hairs, margin has tan fringe and/or short white hairs, apex obtuse/acute. Bracteoles 0.6 – 1.5 X 0.6 – 1.5 mm, often paired and opposite each other (rarely several and then alternate along pedicle), persistent, glabrous, margin with small white hairs and/or tan fringe, apex obtuse (occasionally bracteoles become fleshy, obscuring the pedicel). Flowers hermaphroditic, dioecious or gynodioecious. Pedicels 1.6 – 4.3 mm long, sometimes with short white hairs and/or long erect brown hairs present, occasionally becoming fleshy with fruit maturity. Calyx in flower 1.2 – 4.2 X 2.0 – 6.3 mm, synsepalous, apex acute. Corolla 2.0 – 15.0 mm long, urceolate, cylindrical or campanulate, glabrous or with multicellular or unicellular hairs adaxially and abaxially. Stamens 10, slightly dimorphic. Filament papillose, rarely smooth, straight, widest at base. Two anther locules opening by terminal pores, rarely with a terminal tubule extending beyond pore; awned, awnless or with minute projections; awns usually 2 (rarely 1) per anther locule when awns present. Ovary 0.7 – 1.6 X 1.3 – 2.0 mm, 5-lobed, superior or semi-inferior, usually glabrous. Style stout and columnar. Stigma irregularly capitate. Fruit a globose capsule dehiscing loculicidally or a thin-walled berry; mature calyx in fruit often swollen, fleshy, accrescent and colored; occasionally, the floral axis becomes fleshy. Seeds tiny, to 1.3 mm long, unwinged.

Approximately 136 species occurring in South, Central and North America, temperate east Asia, India, Sri Lanka, southeast Asia, southeast Australia, Tasmania and New Zealand.

Key to the species of *Gaultheria* from Australia and New Zealand

1. Fruit consistently with a fleshy ovary (berry) ……………………………………………………………………**2**
  2. Leaves 2.8 – 13.2 mm X 1.0 – 4.2 mm…………………………………………………………………………**3**
    3. Habit < 4 cm, matted, dense shrub with short leaf internodes (generally < 4 mm)………..***G. tasmanica***
    3. Habit to 30 cm, procumbent wiry shrub with long leaf internodes (generally 5 mm or greater)…………………………………………………………………………..***G. macrostigma***
  2. Leaves 15.2 – 20.2 mm X 3.8 – 5.4 mm…………………………………………………………***G. lanceolata***
1. Fruit consistently a dry capsule……………………………………………………………………………………**4**
  4. Calyx dry and non-fleshy at fruit maturity…………………………………………………………………**5**
    5. Leaves opposite or sub-opposite. Habit a pendent shrub………………………………***G. oppositifolia***
    5. Leaves alternate. Habit an erect shrub or small tree…………………………………………***G. rupestris***
  4. Calyx fleshy, accrescent and colored (including white) at fruit maturity……………………………………**6**
      6. Leaves 4.7 – 14.0 mm X 3.0 – 10.0 mm……………………………………………………………....**7**
        7. Shrub 30 cm – 2.0 m tall. Adaxial leaf venation not prominent, leaf margins deeply serrate, jagged. Pedicel length 1.7 – 4.3 mm…………………………….***G. antipoda***
        7. Shrub 8.0 cm – 15.0 cm tall. Adaxial leaf venation prominent and sulcate, leaf margins crenulate, teeth rounded. Pedicel length 0.8 – 1.5 mm……………………***G. depressa***
      6. Leaves 27.8 – 61.0 mm X 6.3 – 21.2 mm………………………………………………………………**8**
        8. Stems glabrous, Corolla size large, 5.5 – 6.1 mm X 4.4 – 4.6 mm. Inflorescences aborted racemes, with apparently solitary flowers on long pedicel with many bracteoles…………………………………………………………***G. viridicarpa***
        8. Stems with scattered long brown hairs. Corolla size 3.2 – 5.1 mm X 2.4 – 4.2 mm. Inflorescence is a raceme with only two bracteoles on the pedicel…………………....**9**
          9. Hairs on stem erect…………………………………………………***G. hispida***
          9. Hairs on stem appressed……………………………………………***G. appressa***

Species Descriptions

1. ***Gaultheria tasmanica*** (Hook. f.) D. J. Middleton. Edinburgh J. Bot. 47(3): 298, 1990. *Pernettya tasmanica* Hook. f. Hook. Lond. Journ. Bot. vi: 268, 1847. Type. Australia. Tasmania, Port Arthur. Backhouse s.n. (holotype, K).

Gaultheria parvula D.J.Middleton. Edinburgh J. Bot. 47(3): 298, nom. nov. 1990. Type. New Zealand. South Island, near Mount Cook Hermitage. January 1890. H. Suter s.n. (holotype, WELT).

Pernettya nana Colenso Trans. N.Z. Inst. xxiii, 389 1891. Type. New Zealand. South Island. near Mount Cook Hermitage. January 1890. H. Suter s.n. (holotype, WELT!).

Pernettya tasmanica var. novae-zelandica Kirk Trans. N.Z. Inst.

xxvii: 351, 1895. Type. New Zealand. South Island, Porter River, Broken River Basin, 2000 ft. J. D. Enys s.n. (holotype, WELT).

Shrub occurring in mats, < 4 cm tall. Branchlets reddish tan, sometimes with short white hairs or scattered long brown hairs. Petiole 0.1 – 1.1 mm, red, glabrous. Leaves 2.8 – 8.6 X 1.0 – 2.7 mm, elliptic, adaxially glossy dark green with light green prominent veins, occasionally with short white hairs, particularly near junction of midrib and petiole; abaxially light green and covered with round glands, venation obscure; base acute, margin with few serrations, each tooth tipped with a stout black/red hair, apex acute. Inflorescence axillary, solitary-flowered, bracts absent. Pedicel 0.9 – 3.1 mm, glabrous; bracteoles 0.6 – 1.5 X 0.6 – 1.5 mm, several, basal and along pedicel, persistent, glabrous, margin with short white hairs and/or tan fringe, apex obtuse. Flowers hermaphroditic or gynodioecious. Calyx 2.1 – 2.7 mm; lobes 1.8 – 2.4 X 0.8 – 1.5 mm, 5 , broadly ovate, ciliate, reddish along margin, adaxially with short white hairs, not overlapping at base, apex acute/acuminate. Corolla 3.2 – 4.4 X 2.6 – 5.4 mm, white/cream to pink and red, urceolate, glabrous adaxially and abaxially; lobes 0.8 – 1.9 mm long, 5, oblong. Staminodes (in female flowers) 10, ca. 1.3 mm long, occasionally with filament tip forked. Stamens 10, filaments 0.8 – 2.2 mm, widest at base, occasionally tan papillae present; anthers 0.6 – 0.7 mm, 4-awned, minute projections or no projections on anthers. Ovary 1.2 – 1.5 X 1.6 – 1.7 mm, glabrous; style 1.0 – 1.9 mm, glabrous. Fruit a capsule, 3.1 – 9.4 mm in diam., glabrous. Fruit a berry with a fleshy accrescent calyx (particularly toward the base),bright pink to red, tips of calyx lobes green and typically not fleshy and do not entirely surround the capsule.

Distribution and Ecology: South Island of New Zealand and the state of Tasmania (Australia). Provinces in New Zealand: Canterbury and Otago. Localities include: Lake Ohau (Canterbury), Castel Hill (Canterbury), Upper Waimakarira (Canterbury), Tasman Valley River (Canterbury), Lake Pukaki (Canterbury), South Crittel Range (Otago) and Hyde Rock (Old Man Range; Otago). In Tasmania, localities include: Ben Lomond National Park, Mt. Rufus, Breton Rivulet (Mother Lords Plains), Billop Bluff north of Arthurs Lake on Western Tiers, Lagoon Plain (Central Highlands), Molly Yorks Night Cap (Central Highlands), Paradise Plains, Ironstone Mountain, Dunning Rivulet, Little Split Rock, Gunns Lake (Central Highlands), Alma Pass (between Interlaken and The Steppes), Sandbanks Tier (Great Lake), Flagstaff Creek (Waddamana Road). Found matted in short tussock grasslands, in rock crevices, among alpine short heath and cushion plants or dolerite boulder valleys or in mosses around moist heath areas. 520 – 1570 m. Flowering September – November.

Selected specimens examined: AUSTRALIA. Tasmania: Alma Pass (between Interlaken and The Steppes), 970 m, 17 May 2006 (fr), *M. Visoiu 197* (HO); Little Split Rock, 1240 m, March 1984 (fr), *A. Moscal 6650* (HO), Molly Yorks Night Cap, Central Highlands, 990 m, 21 February 1990 (fr), *A. Moscal 18885* (HO); Lagoon Plain 6 km north of Lake Sorell, Central Highlands, 1040 m, 24 February 1989 (fr), *A. Moscal 17041* (HO); Mt Rufus, near Hobart Walking Club, 1150 m, 13 January 1983 (fl), *A.M. Buchanan 1074* (HO). **NEW ZEALAND. Canterbury:** Lake Pukaki, Irishman Creek Station, 1625 ft, 4 March 1970 (fr), *B.H. Macmillan and A.R. Mitchell s.n.* (CHR); near

L. Lillian, Harper/Avoca Rv's, Rakaia Catchment, 700 m, 28 November 1986 (fl), *K.H. Platt s.n.* (CHR).

2. ***Gautheria macrostigma*** (Colenso) D.J. Middleton. Edinburgh J. Bot. 47(3): 298, 1990. *Pernettya macrostigma* Colenso. Trans. N.Z. Inst. 21: 92, 1888. Type. New Zealand. North Island, Glenross Station, County of Hawke's Bay. December 1887. D.P. Balfour s.n. (holotype, WELT; isotype, K).

Gaultheria antipoda var. *microphylla* Hook. f. Fl. N.Z., 1: 161, 1854. Type. Lost or destroyed. (K, N.V.)

Gaultheria depressa var. *microphylla* (Hook. f.) Cheeseman, Man. N.Z. Fl. ed. 2: 690, 1925. Type. Lost or destroyed. (K, N.V.)

Gaultheria perplexa Kirk Trans. N.Z. Inst. xxix: 538, 1896, nom. nud. Type. New Zealand. South Island, South Otago, Bluff Hill. Nov 1883. Kirk 1028 (holotype, WELT).

Shrub procumbent, wiry, to 30 cm tall. Branchlets reddish-brown with scattered long brown and short white hairs, particularly on newer stems. Petiole 0.5 – 1.5 mm, typically glabrous but occasionally with scattered long brown hairs; leaf blade 5.6 – 13.2 X 1.3 – 4.2 mm, linear, adaxially glossy, dark green, occasionally with short white hairs, venation not prominent; abaxial surface covered in small round glands, light green, venation not prominent, glabrous, base acute, margin serrate with several setulose-tipped teeth per side, apex acute. Inflorescence solitary-flowered or in a *pseudoraceme with one flower in the axils of increasingly smaller leaves, these becoming bract-like. Pedicel 1.2 – 3.4 mm, with some short white hairs; bracteoles 0.6 – 1.4 X 0.5 – 1.1 mm, several, basal or nearly so, broadly ovate, persistent, glabrous, margin with short white hairs/tan fringe, apex acute/obtuse. Flowers hermaphroditic or gynodioecious. Calyx 1.4 – 3.1 mm; lobes 0.9 – 2.1 X 0.6 – 1.1 mm, 5, ovate, short white hairs adaxially and on edge of calyx lobes, not overlapping at base, apex acute/acuminate. Corolla 2.5 – 4.1 X 1.9 – 2.5 mm, white, urceolate, occasionally short white hairs adaxially; lobes 0.7 – 0.9 mm, 5, oblong. Staminodes (in female flowers) 10, ca. 0.9 mm. Stamens 10; filaments 0.9 – 1.2 mm, widest near base, white/tan papillae present on filament; anthers 0.6 – 0.8 mm, 4-awned. Ovary 0.7 – 0.8 X 1.0 – 1.3 mm, glabrous; style 0.9 – 1.7 mm, glabrous. Fruit a berry, 3.3 – 7.9 mm in diam., glabrous. with a fleshy calyx (particularly at the base), white, pink, bright pink or red, tips of the sepal lobes may not be fleshy (lobes do not surround berry).*

Distribution and Ecology: On both North and South Islands of New Zealand. DOC provinces include: Tongariro/Lake Taupo, Nelson/Marlborough, Westland, Canterbury, Otago and Southland. Localities include: Mt. Pureora summit (Tongariro/Lake Taupo), shore of Lake Roto Aira, South Lake Taupo (Tongariro/Lake Taupo), Waimarino Plain near Ruapehu (Tongariro/Lake Taupo), Red Hills (Nelson), Rahu Scenic Reserve (Nelson), Peter’s Pool at Franz Joseph Glacier (Westland), Waiho Valley (Westland), Crater’s Mill, near Okuru (Westland), Potters Creek (Westland), Avoca River at the foot of Mt. Fitzwilliam (Canterbury), Mt. Peel at Raoul Creek (Canterbury), Flagstaff Hill near Dunedin (Otago), Swampy Hills near Dunedin (Otago), Signal Hill, Dunedin

(Otago), Mokoreta (Southland) and Waituna Bog (Southland). Found in: snow-tussock grasslands, sphagnum bogs or along shady riverine banks. 223 – 1067 m. Flowering November to December.

Selected specimens examined: NEW ZEALAND. Nelson/Marlborough: nr. Rahu Scenic reserve Nelson LD, 410 m, May 1973 (fr), *D. and G.C. Kelly s.n.* (CHR). **Canterbury:** Avoca River, foot of Mt Fitzwilliam, 650 m, 17 April 1985 (fr), *K.H. Platt s.n.* (CHR). **Otago:** The Bluff Hill, 27 November 1884 (fl, fr), *T.K. s.n.* (WELT); Swampy Hill, 4 miles N of Dunedin, Taieri County, 2000 ft, 14 April 1962 (fr), *R. and E.F. Melville 6926 and G. Baylis* (K). **Southland:** Headwaters of Otapiri stream, Glenure Station, 6 mls. E of Dipton, Southland Co., 950 ft, 22 February 1962 (fr), *R. and E.F. Melville 6484* (K).

3. ***Gaultheria lanceolata*** Hook. f. Hook. Lond. Journ. Bot. vi: 267, 1847. *Brossaea lanceolata* (Hook. f.) Kuntze. Rev. Gen. 388, 1891. *Pernettya lanceolata* (Hook. f.) B. L. Burtt & A. W. Hill, Journal of the Linnean Society, Botany 49: 638, 1935. Type. Australia. Tasmania, Summit of Western Mountains. 17 May 1845 (fr.). R. C. Gunn 515 (holotype K; isotypes L, NSW!).

Shrub to 30 cm tall. Branchlets reddish-brown, particularly new stems, short white hairs and scattered erect long brown hairs present. Petiole 0.7 – 1.6 mm, sometimes with short white hairs and/or scattered long brown hairs; leaf blade 15.2 –

20.2 X 3.8 – 5.4 mm, lanceolate, adaxially glossy, glabrous except for occasional small white hairs on midrib near petiole; abaxially light green surface covered with small round glands, occasionally long brown hairs along midrib, venation prominent; base obtuse, margin serrate with reddish nubs at the tip of each tooth, apex acute/acuminate. Inflorescences solitary-flowered or in pseudoracemes with one flower in the axils of increasingly smaller leaves, these becoming bract-like. Pedicel 3.8 – 4.8 mm, with scattered short white hairs and/or long brown hairs; bracteoles 1.4 – 1.8 X 1.0 – 1.1 mm, several, overlapping, basal or sometimes along pedicel, ovate, persistent, glabrous, with tan/white fringe along margin, apex obtuse. Flowers hermaphroditic. Calyx ca. 2.1 mm; lobes ca. 2.3 X 1.5 mm long,5, ovate, not overlapping at base , short white hairs along margin and adaxially, particularly near tip, apex acute/acuminate. Corolla white, urceolate; lobes 5, oblong. Stamens 10; anthers 4-awned. Ovary glabrous; style glabrous. Fruit a berry, 4.3 – 6.1 mm in diam., glabrous with a fleshy calyx, lobes not surrounding the fruit.

Distribution and Ecology: Australia. State: Tasmania. Localities include: Ben Lomond, Drys Bluff and Mt. Arrowsmith. Occurs as community shrub on the forest floor, typically with *Eucalyptus delegatensis* R.T. Baker. 980 – 1010 m.

Selected specimens examined: AUSTRALIA. Tasmania: Tasmania, 5 February 1905 (fr), *R..C. Gunn s.n.* (P); Mt. Arrowsmith, 1 March 1946 (fr), *H. Huxley s.n.* (HO); Drys Bluff (fr), *W.M. Curtis s.n.* (HO); Drys Bluff, NW Slope, 1000 m, 20 December 1984 (fr), *A.M. Buchanan 5044* (HO); Ben Lomond, February 1895 (fr), *L. Rodway 486* (HO).

4. ***Gaultheria oppositifolia*** Hook. f. Fl. N.Z. 1: 162, t. 43, 1854. *Brossaea oppositifolia* (Hook, f.) Kuntze, Rev. Gen. 388, 1891. Type. New Zealand. North Island, Mt. Egmont. Bidwill s.n. (K, N.V.)

Gaultheria multibracteolata Colenso *Trans. N. Z. Inst.* 24: 389, 1891. Type. New Zealand. North Island, interior hilly country near Taupo. 1889. H. Hill s.n. (holotype, WELT!).

Pendent shrub 30 cm – 2 m tall. Branchlets reddish brown, sometimes with scattered strigose hairs. Petiole 0.5 – 2.7 mm, occasionally with long brown hairs or short white pubescence; leaf blade 24.1 – 45 X 12.6 – 23.3 mm, broadly ovate, adaxially glossy with reticulate venation; abaxially, light green with many small round glands and prominent darker green reticulate venation, sometimes with scattered brown/red hairs or nubs, particularly on midrib; base cordate, margin serrate with short black nubs at the teeth, apex obtuse/acute. Inflorescence a raceme or panicle; bract 1.3 – 2.0 X 1.0 – 1.7 mm, single, basal, persistent, reddish tan, broadly ovate, glabrous, margin with tan fringe and/or short white hairs, apex obtuse/acute. Pedicel 1.6 – 3.0 mm, glabrous; bracteoles 0.8 – 1.9 X 0.6 – 1.0 mm, 2, broadly ovate, persistent, reddish tan, basal, glabrous, margin has tan fringe and/or short white hairs, apex obtuse/acute. Flowers hermaphroditic. Calyx 1.4 – 2.2 mm; lobes 0.8 – 1.6 X 0.7 – 1.2 mm, 5, ovate, short white hairs along margin and adaxially, apex acuminate. Corolla 2.7 – 4 X 1.9 – 3 mm, white, urceolate, occasionally white hairs adaxially; lobes 0.7 – 1.3 mm, 5, oblong. Stamens 10; filaments 0.6 – 1.3 mm, widest at base, with white papillae; anthers 0.5 – 0.8 *mm, 4-awned. Ovary 1.0 – 1.1 X 1.3 – 1.6 mm, glabrous; style 1 – 2 mm, glabrous. Fruit a capsule, 1.1 -1.5 mm in diam with a dry calyx.*

Distribution and Ecology: Only occurs on North Island of New Zealand. DOC Provinces: Bay of Plenty, Tongariro/ Lake Taupo, Wanganui. Localities include: Mayor Island (Bay of Plenty), Mt. Putauaki/Mt. Edgecumbe (Bay of Plenty), Rainbow Mt. (Bay of Plenty), Rotorua/Mt. Tarawera (Bay of Plenty), Whakatane (Bay of Plenty), Eastern Volcanic Plateau (Tongariro/Lake Taupo), Lake Taupo (Tongariro/Lake Taupo), Kauarapaoa Rd. (Wanganui), Mangapurua Rd. (Wanganui), Messenger Tunnel (Wanganui), Taumaranni (Wanganui). Occurs in leeched clay hills and banks, roadside banks with thin loam/mudstone, limestone banks, amongst rocky boulders, and particularly on pumiceous slopes. 43 – 1097 m. Flowering November – January.

Selected specimens examined: NEW ZEALAND. Bay of Plenty: Whakarewarewa, Rotorua, *T.F. Cheesman s.n.* (NSW); Ohinemuri County, Mayor Island, growing in rock crevices along base of inland bluffs NE of Opuahau Trig., 20 November 1981 (fl), *A.E. Wright 4341* (WELT). **Tongariro/Lake Taupo:** Eastern Volcanic Plateau, Eastern Kaingaroa plains, 2600 ft, December 1950 (fr), *R.T. Fenton RF65* (NZFRI). **Wanganui:** Off Highway 3 near Messenger Tunnel, 43 m, 5 December 2007 (fr), *Fritsch and Bush 441* (WFU, CHR); Taumarunui, December 1908 (fl, fr), *D. Petrie s.n.* (WELT).

5. ***Gaultheria rupestris*** (G. Forst.) R. Br. Prodr. Fl. Nov. Holland. 559, 1810. *Andromeda rupestris* G. Forst. Fl. Ins. Austr. 195, 1786. *Brossaea rupestris* (G. Forst.) Kuntze, Rev. Gen. 388, 1891. Type. New Zealand. “Middle Island”, in swamps and on wet rocks. 1773. G. Forster s.n. (BM, N.V.)

Gaultheria calycina Colenso Trans. & Proc. New Zealand Inst. xxxi: 274, 1898. Type. New Zealand. North Island, Ruahine mountain range, east side. 1898. A. Olsen, s.n.

Gaultheria colensoi Hook. f. Fl. N.Z. 1: 162, 1854. Type. New Zealand. Base of Mount Tongariro. June 1850. Colenso 2410 (holotype, K).

Gaultheria crassa Allan Fl. New Zealand i: 508, 1961. Type. New Zealand. Nelson Mountains. Bidwill 60 (holotype, K).

Gaultheria divergens Colenso Trans. N.Z. Inst. 20: 198, 1887. Type. New Zealand. North Island, Tongariro. H. Hill s.n. (holotype, WELT!).

Gaultheria glandulosa Colenso Trans. & Proc. New Zealand Inst. xxviii: 600, 1896. Type. New Zealand. North Island, Ruahine mountain range, east side. 1894. H. Hill s.n. (lectotype here designated from syntypes, WELT).

Gaultheria paniculata B.L. Burtt & A.W. Hill Journ. Linn. Soc. 49: 614, t. 1, 1935. Type. New Zealand. Near summit of Mount Messenger road. Nov. 1928. Allan 5505 A, B, C, D (lectotype here designated from syntypes).

Gaultheria rupestris var. δ Hook. f. Handbook. N.Z. Fl. 1: 175, 1864.

Gaultheria rupestris var. *humilis* Simps. Trans. Royal Soc. N.Z. 79: 423,

1952. Type. New Zealand. South Island, Flagstaff Hill, Dunedin, in Plant Research Bureau, Wellington.

Gaultheria rupestris var. *parviflora* β Hook. f. Fl. N.Z. 1: 161, t. 42, 1854.

Gaultheria rupestris var. *subcorymbosa* (Colenso) B.L. Burtt & A.W. Hill. Journ. Linn. Soc. 49: 614, t. 1, 1935. Type. New Zealand. North Island, on eastern slope of Ruahine Mountain range, County of Waipawa. H. Hill s.n.

Gaultheria subcorymbosa Colenso Trans. N.Z. Inst. 22: 476, 1889. Type. New Zealand. North Island, on eastern slope of Ruahine Mountain range, County of Waipawa, H. Hill s.n. (holotype, WELT!)

Shrubs to small trees, 15 cm – 3 m tall. Branchlets tan to reddish brown, long brown hairs present, short white hairs occasional. Petiole 0.7 – 4.2 mm, commonly red, glabrous or with scattered long brown hairs and/or short white hairs; leaf blade 8.5 – 43.6 X 5.2 – 22.2 mm, elliptic or oblong-orbicular, coriaceous, adaxially glossy with reticulate venation, glabrous or with short white hairs and/or black or brown nubs/hairs, particularly on midrib; abaxially light green with round glands and prominent dark green venation, glabrous or with red/brown hairs; midrib or tip of leaf can be red, base obtuse to acute, margin prominently serrate, teeth with black or brown nub, apex acute to obtuse. Inflorescences axillary or occasionally in terminal racemes; bracts 1.4 – 3.9 X 0.8 – 2.3 mm, 1, basal, broadly ovate, persistent, glabrous, margin with tan/white fringe, apex obtuse/acute. Pedicel 2 – 5.2 mm, short white hairs and/or scattered long brown *hairs present; bracteoles 1.0 – 2.2 X 0.6 – 1.5 mm, 2-3, basal or proximate, broadly ovate, glabrous, margin with tan/white fringe , apex obtuse/acute. Flowers hermaphroditic or gynodioecious. Calyx 1.2 – 3.4 mm; lobes 1.1 – 2.6 X 0.6 – 1.9 mm, 5, not overlapping at base, ovate, short white hairs adaxially, margin with short white hairs and/or tan or red fringe, apex acuminate. Corolla 2.6 – 4.7 X 1.9 – 3.6 mm, white or cream, urceolate, small white hairs adaxially; lobes 0.5 – 1.6 mm, 5, tips occasionally red, oblong. Staminoides (in female flowers) 10, filaments 1.2 – 1.9 mm. Stamens 10; filaments 0.7 – 1.6 mm, widest at base, tan/white papillae present; anthers 0.4 – 1.2 mm, 4-awned. Ovary 0.7 – 1.6 X ~ 1.6 mm, glabrous, style 1.4 – 2.9 mm, glabrous. Fruit a capsule, 1.8 – 3.7 mm in diam., glabrous with a dry calyx.*

Distribution and Ecology: Found on both the North and South Islands of New Zealand. DOC provinces include: Bay of Plenty, Tongariro/Lake Taupo, Wanganui, Wellington, Nelson/Marlborough, Westland, Canterbury, Otago and Southland. Localities include: Rainbow Mountain (Bay of Plenty), Urewera National Park (Bay of Plenty), Mt. Tarawera (Bay of Plenty), Mangeitepopo National Park (Tongariro/Lake Taupo), Mt. Pukeonake (Tongariro/Lake Taupo), Pureora, west Taupo (Tongariro/Lake Taupo), Waihohonu Hut (Tongariro/Lake Taupo), Holdsworth Lookout, Tararua (Wanganui), Mount Hector, Tararua Range (Wanganui), Whangamomomona Hotel Road (Wanganui), Messenger Tunnel (Wanganui), Wilton’s Bush (Wellington), Mt. Robert (Nelson), Boulder Lake/Aorere Valley (Nelson), Cobb Ridge (Nelson), Denniston (Westland), Styx River/Lake Kaniere (Westland), Greymouth (Westland), Upper Olira Valley (Westland), Whitcomb River Valley (Westland), Porter’s Pass (Canterbury), Bealy River Valley

Track (Canterbury), Lake Ohau and Lake Pukaki (Canterbury), Mount Cook National Park (Canterbury), Hooker Valley near Hermitage (Canterbury), Governor's Bush, Mt. Cook (Canterbury), Craigeburn Range (Canterbury), Arthur's Pass (Canterbury), Cardrona Valley (Otago), Ben Lomond (Otago), Port Craig (Southland), Milford Track, south Fiordland (Southland), Mt. George, Fiordland (Southland). Found in: open tussock grasslands, rocky outcrop slopes, roadside or river banks, subalpine open rocky volcanic montane environments with pumice, subalpine or alpine scrub, disturbed/succesional vegetation or in silver beech forest margins. 61 – 1650 m. Flowering November to January.

Selected specimens examined: NEW ZEALAND. Bay of Plenty: Mt. Tarawera, plateau near airstrip, 28 November 1981 (fl), *D.O. Bergin s.n.* (NZFRI). **Tongariro/Lake Taupo:** 0.5 km before Waituhi Lookout on road to Turangi, 849 ft, 5 December 2007 (fl), *C.M. Bush and P. W. Fritsch 443* (WFU, CHR); Tongariro Ecological Region, track to Tukino skifield on eastern slope of Mt Ruapehu, 1450 m, 4 February 1998 (fr), *Enzat 163* (CHR); Haimarino, Ruapehu, 21 January 1921 (fl, fr), *H.B. Matthews s.n.* (AK); Kaimanawa Mts., 5000 ft, January 1947 (fl), *A.P. Druce s.n.* (CHR). **Wanganui:** Southern North Island, Mangapurua Trig., 30 January 1983 (fr), *A.E. Wright 5531* (AK). **Nelson/Marlborough:** Molesworth Station, Bert's Creek, off Wairau-Hanmer Springs, hydro road, 1100 m, 28 December 1999 (fl), *C.M. Beard and C. Jones s.n.* (WAIK). **Westland:** Kokatahi River Valley, Crawford Junction, terrace alongside the hut, 440 m, 26 November 2001 (fl), *PJ Bellingham 1688* (CHR). **Canterbury:** Craigieburn Range, Craigieburn Stream, 18 November 1987 (fl), *W.R. Sykes 373/87* (CHR); Mount Cook

National Park, Glencoe Fan., November 1962 (fl), *J.M. Wilson 435* (CHR). **Otago:** Cardrona Valley, 8 January 1987 (fr), *P. Douglass and P. Garnock-Jones 8704* (HO).

6. ***Gaultheria antipoda*** G. Forst. Fl. Ins. Austr. 34 (196), 1786. *Brossaea antipoda* (G. forst.) Kuntze, Rev. Gen. 388, 1891. Type. New Zealand "Middle Island", 1773. G. Forster s.n. (isolectotype, MW).

Gaultheria epiphyta Colenso. Trans. N.Z. Inst. 22: 474, 1889. Type. New Zealand. North Island, wood, south of Dannervirke, County of Waipawa. 1887-89. *W. C. s.n.* (holotype, K!; isotype, AK!).

Gaultheria erecta Banks & Sol. ex Hook. f. nom. illeg.

Gaultheria fluviatilis A. Cunn. ex DC. Ann. Nat. Hist. 2(49), 419, 1839. Type. New Zealand. North Island, in the pebbly bed of the Keri-Keri River near the Great Fall, Bay of Islands. 1834. R. Cunningham s.n. (holotype, K!)

Shrub to small tree, 30 cm – 2 m tall. Branchlets densely strigose, also sometimes with a white puberlence. Petiole 0.8 – 2.5 mm, reddish strigose hairs present as well as white purberlence occasionally; leaf blade 7 – 14 mm X 5 - 10 mm, sub-orbicular to broadly elliptic, thin-coriaceous, adaxial side, glabrous, glossy and dark green with prominent venation; abaxial surface glabrous, covered with round glands, pale green with translucent prominent venation; base rounded to obtuse, margin prominently serrate, sometimes reddish in color, teeth setulose-tipped, apex rounded to obtuse. Inflorescence solitary-flowered or in a pseudoraceme with single flowers in the axils of increasingly smaller leaves, these becoming bract-like. Pedicel 1.7 – 4.3 mm, sparsely *strigose and also with short white puberulence; bracteoles, 0.6 – 1.7 mm X 0.5 – 1.0 mm, several basal or occasionally proximate, broadly ovate, persistent, glabrous, margin with short white hairs and/or tan fringe, apex obtuse to acute. Flowers hermaphroditic, occasionally gynodioecious. Calyx 1.9 – 2.5 mm long; lobes 1.5 – 1.7 mm X 1.1 – 1.5 mm, not overlapping at the base, 5, ovate, glabrous, short white hairs along margin and adaxially, apex acuminate. Corolla 2.4 – 3.7 mm, white, urceolate, glabrous abaxially, sometimes with short white hairs adaxially; lobes 0.8 – 1.1 mm, 5, oblong,. Staminodes (when present in female flowers) 10. Stamens 10; filaments 1.0 – 1.3 mm, widest at base, white/tan papillae present; anthers ca. 0.4 mm, 4-awned. Ovary ~ 1.0 X 1.3 mm, glabrous; style 1.1 – 2.2 mm, glabrous. Fruit a capsule 2.6 – 5.2 mm with a fleshy calyx, colored white, pink or red; lobes slightly incurved, occasionally with lobes completely surrounding the capsule.*

Distribution and ecology: On both North and South Islands of New Zealand. DOC Provinces include: Northland, East Coast/Hawke’s Bay, Tongariro/Lake Taupo, Wanganui, Wellington, Nelson/Marlborough, Westland, Canterbury, Otago, Southland. Commonly collected areas include: Bay of Islands (Northland), Waikaremoana (East Coast/Hawke’s Bay), Tongariro National Park (Tongariro/Lake Taupo), Ruahines (Wanganui), Taranaki (Wanganui), Taurewa mountains (Wanganui), Arthur’s Pass (Canterbury), Jack’s stream (Lake Pukaki; Canterbury), Ben Lomond (Otago), and Stewart Island (Southland). Found in a variety of habitats: open tussock grassland, steep scrubby banks, open rock outcrop areas, inside and along the edge of *Nothofagus* forests, coastal scrubs, subalpine forest, and regenerating and disturbed open areas. 100 – 1219 m. Flowering November – January.

Selected specimens examined: NEW ZEALAND. East Coast/Hawke's Bay: Glengarry Road, near Rissington, HB, 24 Oct. 1966 (fl), *F.B. Knowles s.n.* (NZFRI). **Tongariro/Lake Taupo:** Tongariro National Park, at junction of Highway 47 and Whakapapanui Stream, 24 May 1972 (fr), *A.E. Orchard 3334* (AK). **Wanganui:** Edge of subalpine forest, Taurewa, 830 m, 30 May 1985 (fr), *A.M. McCarrison 730233* (AK). **Wellington:** Ruakokoputuna V., Aorangi Range, 1500 ft, Feb. 1972 (fr), *A.P. Dunce s.n.* (CHR); Holdsworth Lookout, Taraua Mountains, 1934 ft, 8 April 2008 (fr), *Bush and Wagstaff 474* (CHR, WFU). **Canterbury:** Arthur's Pass, trail leading from parking lot of DOC office, 2481 ft, 1 April 2008 (fr), *Bush and Wagstaff 448* (CHR, WFU); North Canterbury, Organ Range, lower slopes above Organ Stream, Islands Hills Station, 960 m, 12 March 1991 (fr), *E.H. Woods and B.H. Macmillan 18/91* (K). **Otago:** Lindis Pass, 2000 ft, 2 March 1971 (fr), *B. Molloy s.n.* (CHR).

7. ***Gaultheria depressa*** Hook. f. Hook. Lond. Journ. Bot. vi: 267, 1847. Type. Australia. Tasmania, Claybank, Ben Lomond (fr.). Gunn 516 (holotype, K!).

Gaultheria antipoda var. *depressa* Hook. f. Fl. N.Z. i: 161. 1854. Type. New Zealand.

Gaultheria depressa var. *novae-zelandiae* D.A.Franklin Trans. Roy. Soc. New Zealand 1 (13): 163, 1962. Type. New Zealand. North Island, Renata Peak, Southern Tararuas, 3000 ft. November 21 1958. D.A. Franklin s.n. (holotype, WELT!).

Gaultheria nubicola D.J. Middleton Edinburgh J. Bot. 47(3): 298, 1990. Type. New Zealand. South Island, Frew's Saddle, Hokitika River, Westland. January 8 1958 (fl.) D. A. Franklin s.n. (holotype, WELT!).

Pernettya alpina D.A. Franklin Trans. Roy. Soc. New Zealand, Bot. Trans. Roy. Soc. New Zealand 1 (13): 164, 1962. Type. New Zealand. South Island, Frew's Saddle, Hokitika River, Westland, 4000 ft. January 8 1958 (fl.), D. A. Franklin s.n. (holotype, WELT!).

Shrub, mat-forming and/or prostrate, 8 – 15 cm tall. Branchlets light brown with short white hairs and long brown hairs present. Petiole 0.7 – 2.0 mm, often red, occasionally with scattered long brown hairs present; leaf blade 4.7 – 9.6 X 3.0 – 7.0 mm, orbicular – orbicular/oblong, coriaceous, adaxially glabrous and occasionally with red patches, glossy, reticulate venation prominently indented, veins leading to teeth; abaxially glabrous, light green, with red color near petiole and/or near teeth, surface covered in small round glands, venation darker green, prominent, veins leading to teeth; base obtuse, margin prominently serrate (rounded teeth) with black nubs or long brown hairs extending from teeth (to 0.1 mm long), apex rounded to obtuse. Inflorescences axillary, solitary, bracts absent. Pedicel 0.8 – 1.5 mm, some short white hairs/long brown hairs present; bracteoles 0.6 – 2.0 X 0.5 – 1.8 mm, several, basal or somewhate proximal along pedicel, ovate, persistent, glabrous, tan/white fringe along the margin, apex obtuse. Flowers hermaphroditic occasionally gynodioecious. Calyx 2.0 – 2.2 mm long; lobes 1.3 – 1.6 X 1.1 – 1.2 mm, 5, ovate, not overlapping at base, short white hairs along margin and adaxially, particularly near apex; apex acuminate. Corolla 2.3 – 2.5 X

1.6 – 2.1 mm, white, urceolate; lobes 0.6 – 1.2 mm, 5, oblong. Stamens 10; filaments 0.7 – 0.9 mm; anthers 0.3 – 0.6 mm, not awned nor with minute projections. Ovary ~1.2 X 1.6 mm, glabrous; style ca. 1 mm, glabrous. Fruit a capsule, 2.6 – 6.0 mm in diam. with a fleshy calyx, white to pink to red, glabrous. Calyx lobes sometimes nearly surrounding the capsule.

Distribution and Ecology: South and North Island of New Zealand and the state of Tasmania (Australia). Provinces in New Zealand: Waikato, Tongariro/Lake Taupo, Nelson/Marlborough, Westland, Canterbury, Otago, Southland. Localities in New Zealand include: Hauhungaroa Range (Waikato), Waimarino Plain near Ruapehu (Tongariro/Lake Taupo), Tongariro National Park (Tongariro/Lake Taupo), Waihohonu Hut (Tongariro/Lake Taupo), Iron Hill (Nelson/Marlborough), Bealy River Valley Track (Canterbury), Porter’s Pass (Canterbury), Temple Basin (Canterbury), Ben Ohau Range, Twin Stream catchment (Canterbury), Arthur’s Pass (Canterbury), Inverary, Mt. Somers (Canterbury), Mingha Valley (Canterbury), Ben Lomond (Otago), Flagstaff Hill (Otago), Lake District (Otago), Upper Spey Valley, Spey Hut, Fiordland (Southland), Lake Harris Saddle (Southland), Brokey River (Southland). Localities in Tasmania include: Frenchman’s Cap, Mt. Rufus (northern ridge Cradle Mountain, Lake St. Clair National Park), Nevada Peak, Mt. Inglis (Central Highlands), Bain Bluff and Mt. Field National Park. Found in: tussock grasslands, rock crevices, subalpine low shrubbery, alpine herbfields, summit areas and mossy *Nothofagus* forest edges. 457 – 1463 m. Flowering October to December.

Selected specimens examined: AUSTRALIA. Tasmania: Frenchman's Cap, summit area, 1420 m, 31 March 2007 (fr), *AM Buchanan 16676* (HO); 1 km E NE of Nevada Peak, 1190 m, 25 February 1990 (fr), *P Collier 4554* (HO). **NEW ZEALAND. Tongariro/Lake Taupo:** Whenuakura Clng. Road, 200 m from Keepa Rd. gate, Taupo region, 700 m, 11 March 1995 (fr), *Anne Fraser s.n.* (WAIK); Desert Road, S of the Waihohonu Track, opp the Ruapehu-Ngauruhoe Saddle, Taupo Co., 3250 ft, 15 March 1962 (fr), *R., E.F. Melville 6712B and E.J. Godley* (K). **Nelson/Marlborough:** Iron Hill, 4800 ft, February 1974 (fr), *A.P. Dunce s.n.* (CHR). **Westland:** Near Westport, Stockton Plateau, lower slopes of Mt. Augustus, 1000 m, 8 January 1985 (fr), *M.J.A. Simpson 8538* (CHR). **Southland:** Slopes of Mount Burns, 5 January 1997 (fl), *W.R. Sykes 16/97* (CHR).

8. ***Gaultheria viridicarpa*** J.B.Williams *nom. nov. Gaultheria appressa* var. *glabra* B. L. Burtt & A. W. Hill J. Linn. Soc., Bot. 49: 1935.Type. Australia. New South Wales, top of mountains at heads of Macleay and Bellinger Rivers, a very pretty shrub growing among rocks frequently covered with snow. C.Moore 319 (lectotype here designated from syntypes, MEL; isolectotype, K, BM).

Shrub, 30 – 40 cm tall. Branchlets reddish tan, with very few scattered long brown hairs. Petiole 2.7 – 4.2 mm, glabrous; leaf blade 31.5 – 38.7 X 10.2 – 13.6 mm, elliptic, thin-coriaceous, adaxially dark green, glossy, with scattered dark nubs on *surface, venation prominent; abaxially light green and covered with small round glands, venation prominent; base acute, margin serrate with black nubs at teeth, apex acute. Inflorescence axillary, flowers appear solitary due to aborted racemes, bracts absent. Pedicel 6.6 – 7.7 mm, glabrous; bracteoles 1.9 – 2.6 X 1.1 – 1.3 mm, several, basal and somewhat proximal along pedicel, persistent broadly ovate, margin with tan fringe, apex acute. Flowers hermaphroditic. Calyx 3.5 – 4.2 mm; lobes 2.5 – 2.9 X 1.2 – 1.5 mm, 5, not overlapping at base, ovate, small white hairs adaxially and on margin, apex acute/acuminate. Corolla 5.5 – 6.1 X 4.4 – 4.6 mm, white, urceolate, small white hairs adaxially; lobes 1.4 – 1.8 mm 5, oblong. Stamens 10; filaments 1.8 – 2.4 mm, widest at base, tan/white papillae present; anthers ca. 1.1 mm, 4-awned. Ovary 1.4 -1.5 X ~2.0 mm, glabrous; style ca. 3.6 mm, glabrous. Fruit a capsule ca. 3.4 mm in diam. with a fleshy calyx, color not known, glabrous. Calyx lobes sometimes nearly surrounding the capsule.*

Distribution and Ecology: Australia. State: New South Wales (NSW). Locality: North Tablelands, New England National Park, Point Lookout. Found in open eucalypt woodlands in rock crevices. Ca. 1500 – 1600 m. Flowering November – December.

Selected specimens examined: AUSTRALIA. New South Wales: Point Lookout, on southern side of loop walking track to lookout, New England national park, 1570 m, 18 November 1998 (fl), *G. Errington 630 and J. Whyte* (NSW); Northern Tablelands, New England National Park, Point Lookout, lookout walking track, 1563 m, 20 November

2000 (fl), *J.J. Bruhl 2004 and I.R. Telford* (NSW); Point Lookout, 23 May 1977 (fr), *J.T. Waterhouse and M.M. Hindmarsh 5784* (UNSW).

9. ***Gaultheria hispida*** R. Br. Prodr. Fl. Nov. Holland. 558, 1810. Type. Australia. Tasmania, Doubtless and probably Mount Wellington. May 1802 (fr.). R. Brown s.n. (holotype, E; isotype, NSW!).

Shrub, 50 cm – 2 m tall. Branchlets reddish-brown with erect, long brown hairs. Petiole 1.4 – 3.8 mm, with long brown hairs; leaf blade 27.8 – 61 X 6.3 – 11.9 mm, elliptic, coriaceous, adaxially glossy with reticulate venation and occasionally scattered long brown hairs/brown nubs, particularly along the midrib; abaxially light green, covered with round glands between the veins, scattered long brown hairs present, especially along the midrib; base acute, margin serrate with black nubs at teeth, apex acute. Inflorescence axillary, racemes; Bract 1.9 – 3.8 X 1.5 – 2.5 mm, 1, at base of pedicel, broadly ovate, persistent, glabrous, margin with tan/reddish fringe, apex obtuse/acute. Pedicel 2.2 – 7.4 mm, short white hairs and long brown hairs present; bracteoles, 1.5 – 2.4 X 1.1 – 2.0 mm, 2, mostly basal, broadly ovate, persistent, glabrous, margin with tan/red fringe, apex obtuse/acute. Flowers hermaphroditic. Calyx 2.6 – 3.5 mm; lobes 2.1 – 3.0 X 1.2 – 1.7 mm, 5, not overlapping at base, ovate, short white hairs along margin and adaxially, apex acuminate. Corolla 3.6 – 5.1 X 2.5 – 4.2 mm, white to pale yellow/green, urceolate, short white hairs on inside of corolla; lobes 0.9 – 1.5 mm, 5, oblong. Stamens 10; filaments 1.1 – 1.6 mm, widest at base, tan papillae present;

anthers 0.6 – 0.8 mm, 4-awned. Ovary 1.0 – 1.1 X 1.4 – 1.5 mm, glabrous; style 1.5 – 2.3 mm, glabrous. Fruit a capsule 3.9 – 4.9 mm in diam., glabrous with a fleshy calyx, white, lobes incurved, sometimes nearly surrounding the capsule.

Distribution and Ecology: Australia. State: Tasmania. Localities include: Mt. Barrow, Mt. Wellington, Drys Bluff, Argent Tunnel, Tararina Tasman Penninsula, Wylds Craig Central Highlands, Mount Field National Park (Lake Dobson Road, Lake Fenton), 24 km from Strahan on road to Queenstown, Myrtle Forest Creek, Riawunna Spur (Geeveston District). Found in wet sclerophylla forest edges, or in *Nothofagus* forests. 320 – 1273 m. Flowering September – early January.

Selected specimens examined: AUSTRALIA. Tasmania: 24 km from Strahan on road to Queenstown, 8 February 1981 (fr), *A.E. Orchard 5379* (AK); Mt. Wellington, February 1959 (fr), *R.K. Crowden s.n.* (UNSW); Myrtle Forest Creek, 1.5 km SSW of Springdale, 14 km WNW of Hobart, 580 m, April 1978 (fr), *J.R. Busby 1* (HO); Riawunna Spur, Geeveston District, 26 October 1981 (fl), *T.J. Wardlaw s.n.* (HO); Mt Arthur Track near Mt. Wellington, 2600 ft, 13 December 1931 (fl), *F.H. Long 1071* (HO).

10. ***Gaultheria appressa*** A. W. Hill J. Linn. Soc., Bot. 49: 1935. Type. Australia. Victoria, Craven State Forest. July 1917 (fr.). W.A.W. de Beuzeville s.n. (lectotype here designated from syntypes: NSW!)

Shrub, densely spreading, 70 cm – 1.5 m tall. Branchlets reddish-brown with copious appressed long brown hairs with red bases. Petiole 2.4 – 4.7 mm, with long brown hairs; leaf blade 42.1 – 58.5 X 11.3 – 21.2 mm, elliptic, coriaceous, adaxially glossy green with reticulate venation; abaxial surface light green with round glands, reticulate veins dark green and prominent, black nubs/long hairs scattered on surface, particularly along midrib; base acute, margin serrate with black hair nubs at teeth, apex acute. Inflorescence axillary racemes; bract 1.9 – 4.4 mm X 1.1 – 2.6 mm, 1, basal, broadly ovate, persistent, glabrous, margin with tan fringe, apex obtuse/acute. Pedicel 3.3 – 4.2 mm, short white hairs and twisted long brown hairs present; bracteoles 1.6 – 1.8 X 0.9 – 1.6 mm, 2, mostly basal, broadly ovate, persistent, glabrous, margin with tan fringe, apex obtuse/acute. Flowers hermaphroditic. Calyx 2.5 – 3.1 mm; lobes 1.9 – 2.2 X 1.0 – 1.7 mm, 5, not overlapping at the base, ovate, short white hairs along margin and adaxially, apex acuminate. Corolla 3.2 – 4.1 X 2.4 – 3.2 mm, white, urceolate, white hairs adaxially; lobes 0.9 – 1.1 mm, 5, oblong. Stamens 10; filaments 0.9 – 1.4 mm, widest at base, with tan papillae; anthers 0.6 – 0.7 mm, 4-awned. Ovary 1.1 – 1.6 X 1.8 - 1.9 mm, glabrous; style 1.1 – 2.6, occasionally long brown hairs present. Fruit a capsule 2.0 – 5.5 mm in diam., glabrous with a fleshy calyx, white or pink, lobes incurved and nearly surrounding the capsule.

Distribution and Ecology: Australia. States: New South Wales (NSW) and Victoria (VIC). Localities include: Barrington Tops National Parks (NSW), Polblue Swamp (NSW), Southern Tablelands (NSW), Baw Baw Plateau (VIC), Eastern Highlands, Emu

Plain, 3 km E of Mt. Wombargo (VIC), Upper Delegase River (VIC) and Upper Yarra (VIC). Found in open, wet sclerophyll forest dominated by *Eucalyptus*. 680 – 1300 m.

Selected specimens examined: AUSTRALIA. Victoria: Nuniong road, ca 13 km from Tongio-Bindi road turnoff, which is a turning off the Omeo Highway, 23 March 1975 (fr), *A.E. Orchard 4589* (AK); Rocky Valley, Bogong, 680 m, 19 April 1987 (fr), *P. Collier 2396* (HO); Two km NE from Moscow Villa, Bentley's Plain area, 26 February 1982 (fr), *N.G. Walsh 878* (HO); Eastern Highlands, Emu Plain, 3 km E of Mt Wombargo, 1300 m, December 1987 (fl), *I.R. Telford 10513 and M.D. Crisp* (HO).

Literature Cited

Baas P (1985) Comparative leaf anatomy of *Pernettya* Gaud. (Ericaceae). *Botanische Jahrbucher* **105**, 481–495.

Burtt BL, Hill AW (1935) The genera *Gaultheria* and *Pernettya* in New Zealand, Tasmania, and Australia. *The Journal of the Linnean Society: Botany* **49**, 611–644.

Bush CM, Wagstaff SJ, Fritsch PW, Kron KA (2009) Phylogeny, biogeography and morphological evolution of *Gaultheria* (Ericaceae) from Australia and New Zealand. *Australian Systematic Botany* **22**, 229-242.

Franklin DA (1962) The Ericaceae in New Zealand (Gaultheria and Pernettya). Transactions of the Royal Society of New Zealand 1, 155-173.

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* 4(1): 9pp.

Luteyn JL (1995*b*) *Pernettya* In 'Flora Neotropica Monograph Vol 66. Ericaceae, part II the superior-ovaried genera.' (Ed. JL Luteyn) (New York Botanical Garden Press: New York).

Mayr E (1942) Systematics and the origin of species (Columbia University Press: New York).

Middleton DJ, Wilcock CC (1990) A critical examination of the status of *Pernettya* Gaud. as a genus distinct from *Gaultheria* L. *Edinburgh Journal of Botany* **47**, 291–301.

Middleton DJ (1991) Infrageneric classification of the genus *Gaultheria* L. Ericaceae. *Botanical Journal of the Linnean Society* **106**, 229–258.

Nixon KC, Wheeler QD (2008) An amplification of the phylogenetic species concept. *Cladistics* **6**, 211-223.

Parsons K, Hermanutz L (2006) Conservation of rare, endemic braya species (Brassicaceae): breeding system variation, potential hybridization and human disturbance. *Biological Conservation* **128**, 201–214.

Sleumer H (1936) Ericaceae americanae novae vel minus cognitae, III. Notizbl. Bot. Gart. Berlin-Dahlem **13**, 206-214.

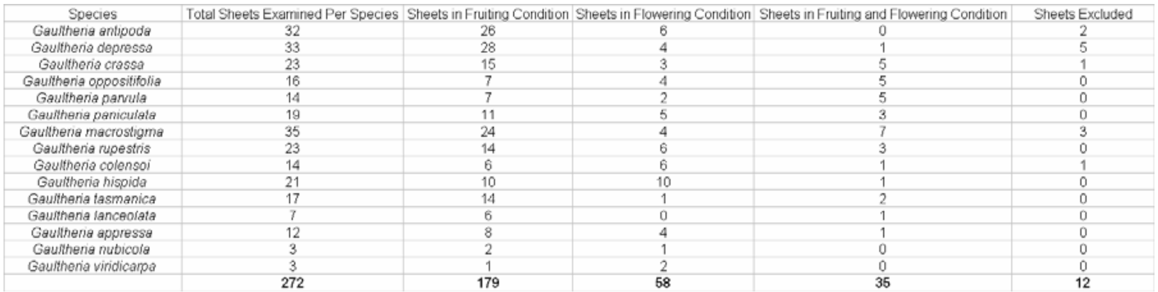
Sleumer H (1985) Taxonomy of the genus *Pernettya* Gaud. (Ericaceae). *Botanische Jahrbucher* **105**, 449–480.

Stace HM, Chapman AR, Lemson KL, Powell JM (1997) Cytoevolution, Phylogeny and Taxonomy in Epacridaceae. *Annals of Botany* **79**, 283–290.

Table 5.1

Total number of herbarium sheets examined per species.

Table 5.1



Species	Total Sheets Examined Per Species	Sheets in Fruiting Condition	Sheets in Flowering Condition	Sheets in Fruiting and Flowering Condition	Sheets Excluded
Gaultheria antipoda	32	26	6	0	2
Gaultheria depressa	33	28	4	1	5
Gaultheria crassa	23	15	3	5	1
Gaultheria oppositifolia	16	7	4	5	0
Gaultheria parvula	14	7	2	5	0
Gaultheria paniculata	19	11	5	3	0
Gaultheria macrostigma	35	24	4	7	3
Gaultheria rupestris	23	14	6	3	0
Gaultheria colensoi	14	6	6	1	1
Gaultheria hispida	21	10	10	1	0
Gaultheria tasmanica	17	14	1	2	0
Gaultheria lanceolata	7	6	0	1	0
Gaultheria appressa	12	8	4	1	0
Gaultheria nubicola	3	2	1	0	0
Gaultheria viridicarpa	3	1	2	0	0
	272	**179**	**58**	**35**	**12**

Table 5.2

Characters Measured/Observed for Morphometric Study. Flr = Flower; fr = fruit. Bold entries indicate which characters were included in the Vegetative + Reproductive PCA analyses. Italicized entries indicate which characters were included in the Vegetative Only PCA analyses.

Table 5.2

Vegetative Characters Measured/Observed	Flowering Characters Measured/Observed	Fruiting Characters Measured/Observed
general habit	solitary/raceme	**length of peduncel**
hair on stem	axillary/terminal	**calyx lobe length (fr)**
color of stem	bracts/pedicel (base)	**calyx lobe width (fr)**
leaf width (widest)	bracteoles/pedicel (on)	**calyx length (fr)**
leaf width	length of pedicel (flowering)	]calyx width (fr)
leaf width	length of bract	hairs on calyx
avg leaf width	width of bract	calyx color (fr)
leaf length	length of bracteole	calyx fleshy (fr)
leaf length	width of bracteole	fruit type
leaf length	hairs on bract (base ped)	fruit color
avg leaf length	hairs on bracteoles	**fruit width (without calyx)**
leaf thickness	color of bract/bracteoles	**fruit length (height)**
hair on top surface	hair on pedicel	
hair on bot surface	color of pedicel	12
hair on midrib - top	# of bracts (base raceme)	
hair on midrib - bot	length of bract (base race)	
length of petiole	width of bract (base race)	
hair on petiole	hairs on bracts	
color of petiole	hair on raceme	
margin edge	color of raceme	
hair on margin	flower color	
veination impressed	flower shape	
color of leaves	petal lobe descript	
	merosity	
23	filament length	
	hair on filament	
	male sterility	
	anther length	
	style length	
	projections on anther	
	nectar disc present	
	hair on corolla	
	corolla lobe length	
	corolla lobe width	
	corolla length	
	corolla width	
	hair on ovary	
	calyx lobes fused?	
	calyx lobe length (flr)	
	calyx lobe width (flr)	
	calyx length (flr)	
	calyx width (flr)	
	calyx color (flr)	
	43	

Figure 5.1

Principal Component Analysis of the Vegetative Only data set with 258 individuals, representing 15 taxa of *Gaultheria*. Symbols are coded as in legend.

Figure 5.1

Component 2
Component 1
6
3
-3
-6
-9
-12
-15
-18
-16
-8
8
16
24
32
40
48
Gaultheria antipoda
Gaultheria crassa
Gaultheria depressa
Gaultheria oppositifolia
Gaultheria parvula
Gaultheria paniculata
Gaultheria rupestris
Gaultheria colensoi
Gaultheria hispida
Gaultheria tasmanica
Gaultheria lanceolata
Gaultheria appressa
Gaultheria nubicola
Gaultheria viridicarpa
Gaultheria macrostigma

Figure 5.2

Principal Component Analysis of the Vegetative + Reproductive data set with 105 individuals, representing 14 taxa of *Gaultheria*. Symbols are coded as in legend.

Figure 5.2

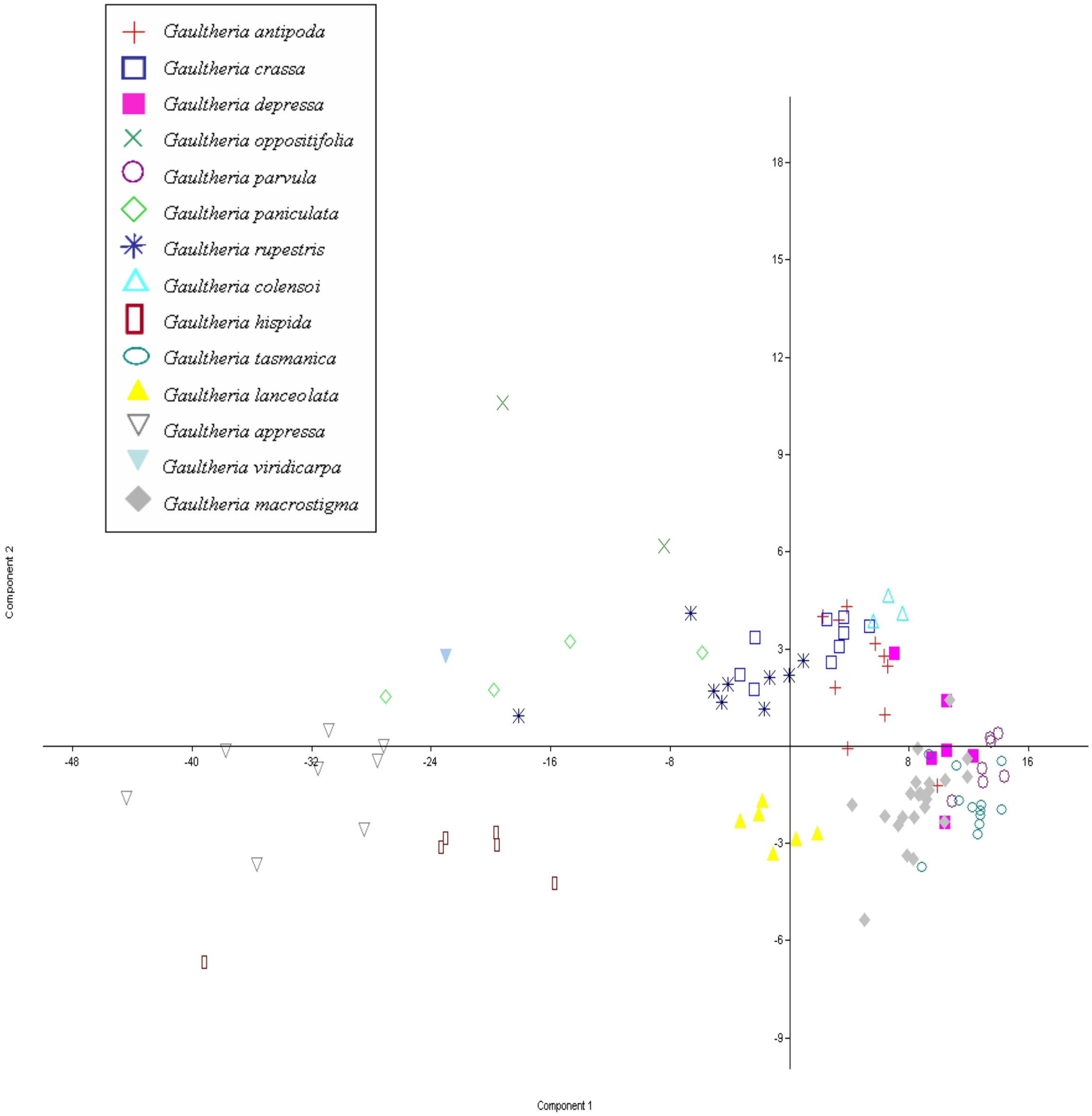

Gaultheria antipoda
Gaultheria crassa
Gaultheria depressa
Gaultheria oppositifolia
Gaultheria parvula
Gaultheria paniculata
Gaultheria rupestris
Gaultheria colensoi
Gaultheria hispida
Gaultheria tasmanica
Gaultheria lanceolata
Gaultheria appressa
Gaultheria viridicarpa
Gaultheria macrostigma
18
15
12
9
6
3
-3
-6
-9
-48
-40
-32
-24
-16
-8
8
16
Component 2
Component 1

Figure 5.3

Principal Component Analysis of the Vegetative Only data set with 63 individuals, representing three taxa of *Gaultheria*. Symbols are coded as in legend.

Figure 5.3

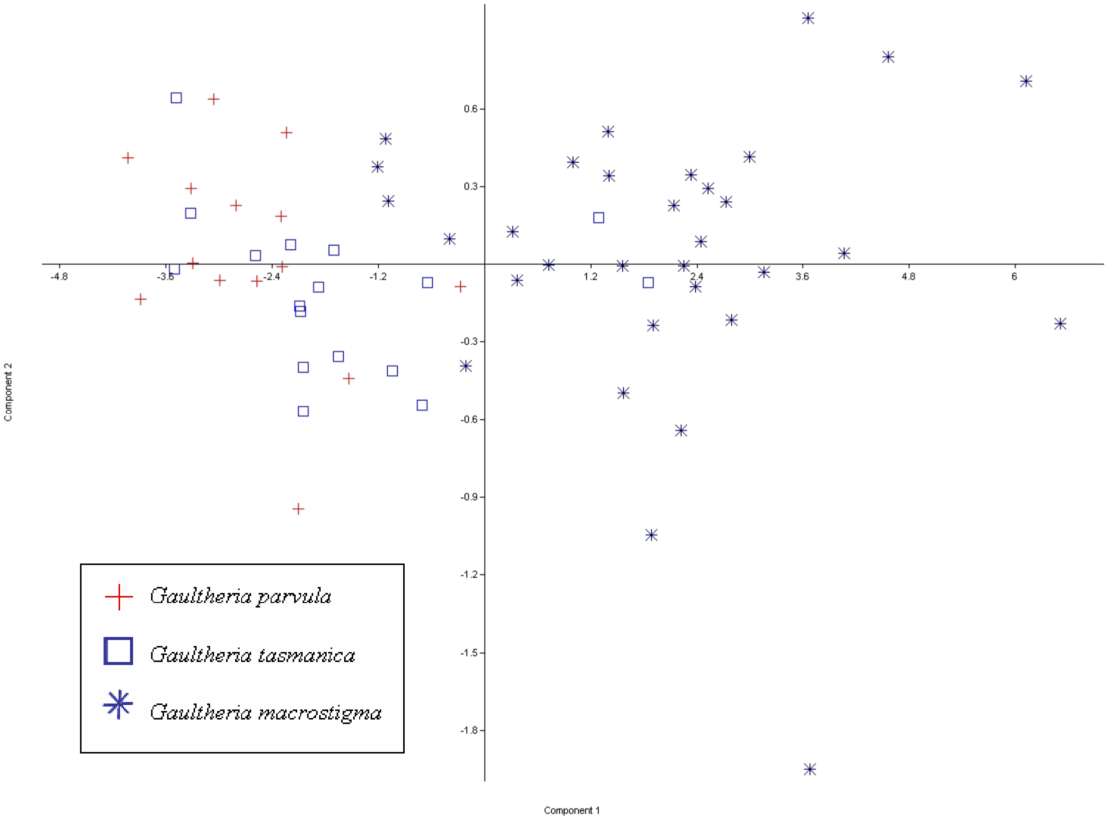
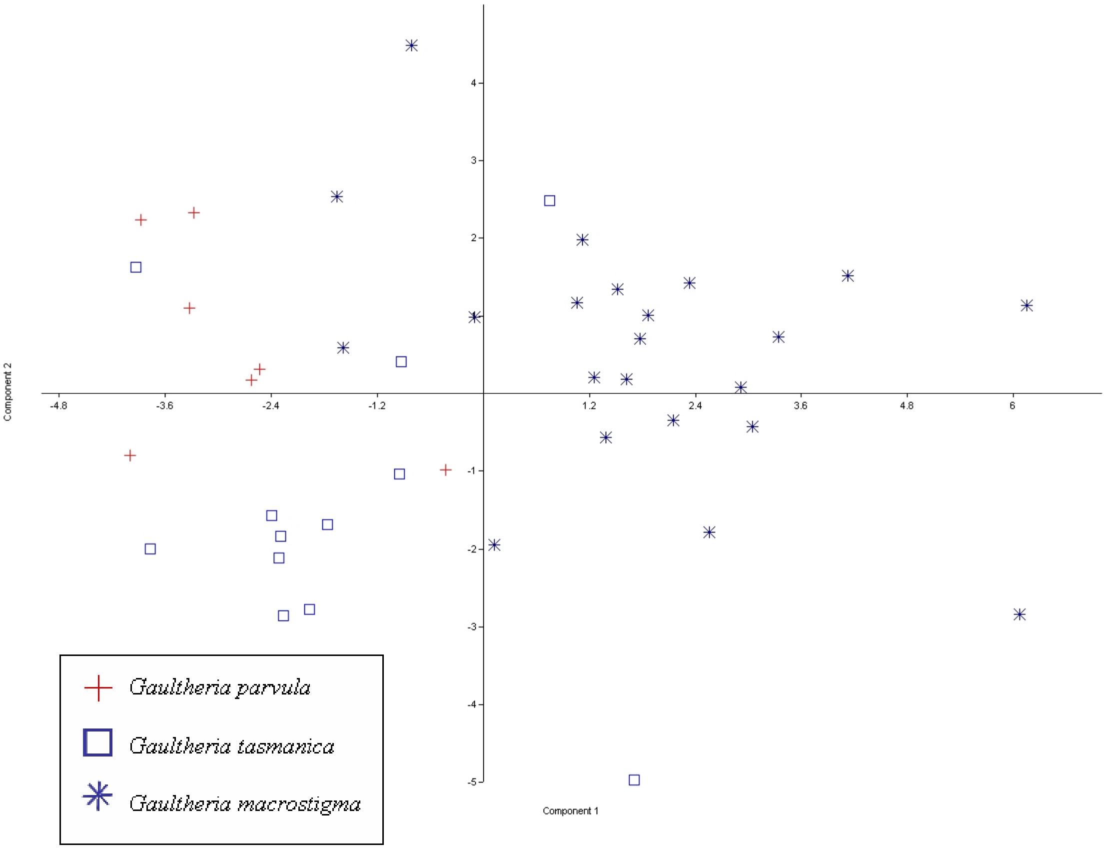

0.6
0.3
-0.3
-0.6
-0.9
-1.2
-1.5
-1.8
-4.8
-3.6
-2.4
-1.2
1.2
2.4
3.6
4.8
6
Component 2
Component 1
Gaultheria parvula
Gaultheria tasmanica
Gaultheria macrostigma

Figure 5.4

Principal Component Analysis of the Vegetative + Reproductive data set with 41 individuals, representing three taxa of *Gaultheria*. Symbols are coded as in legend.

Figure 5.4



Component 2
Component 1
-4.8
-3.6
-2.4
-1.2
1.2
2.4
3.6
4.8
6
4
3
2
-1
-2
-3
-4
-5
Gaultheria parvula
Gaultheria tasmanica
Gaultheria macrostigma

Figure 5.5

Principal Component Analysis of the Vegetative Only data set with 92 individuals, representing five taxa of *Gaultheria*. Symbols are coded as in legend.

Figure 5.5

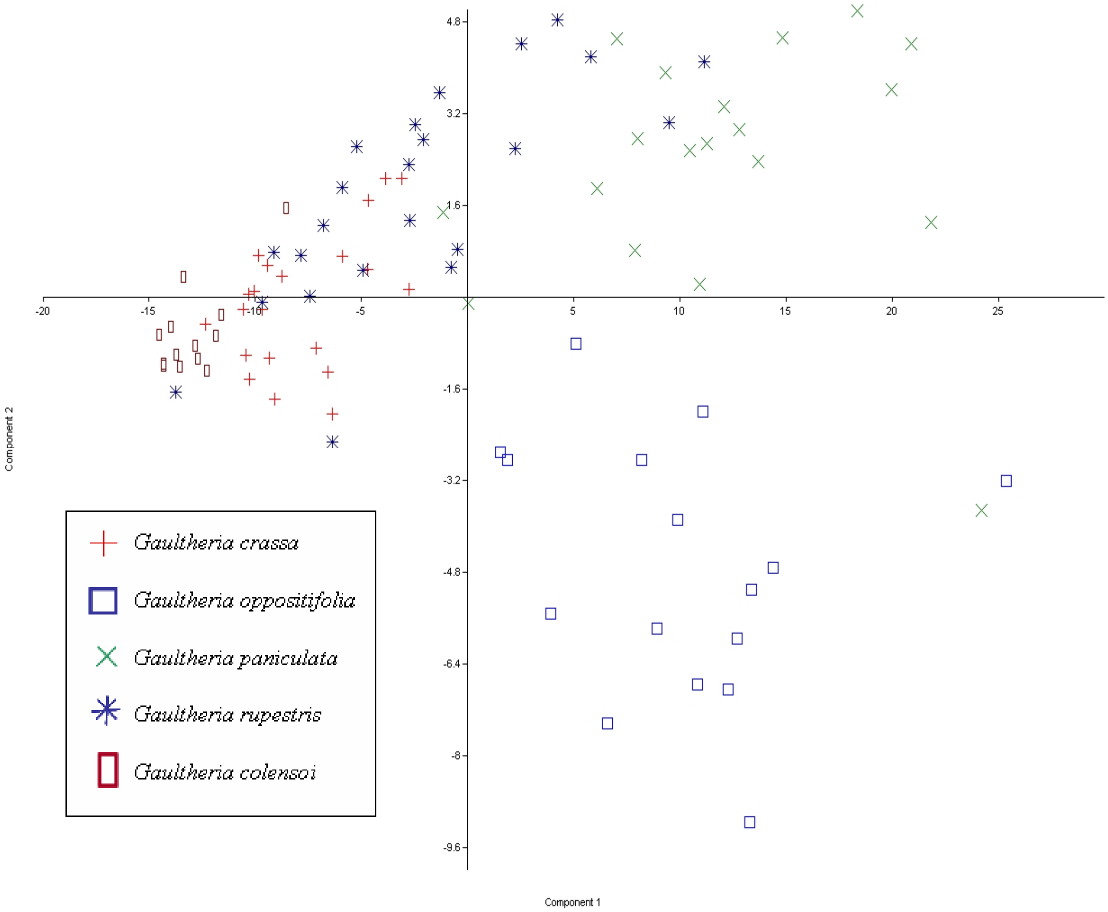
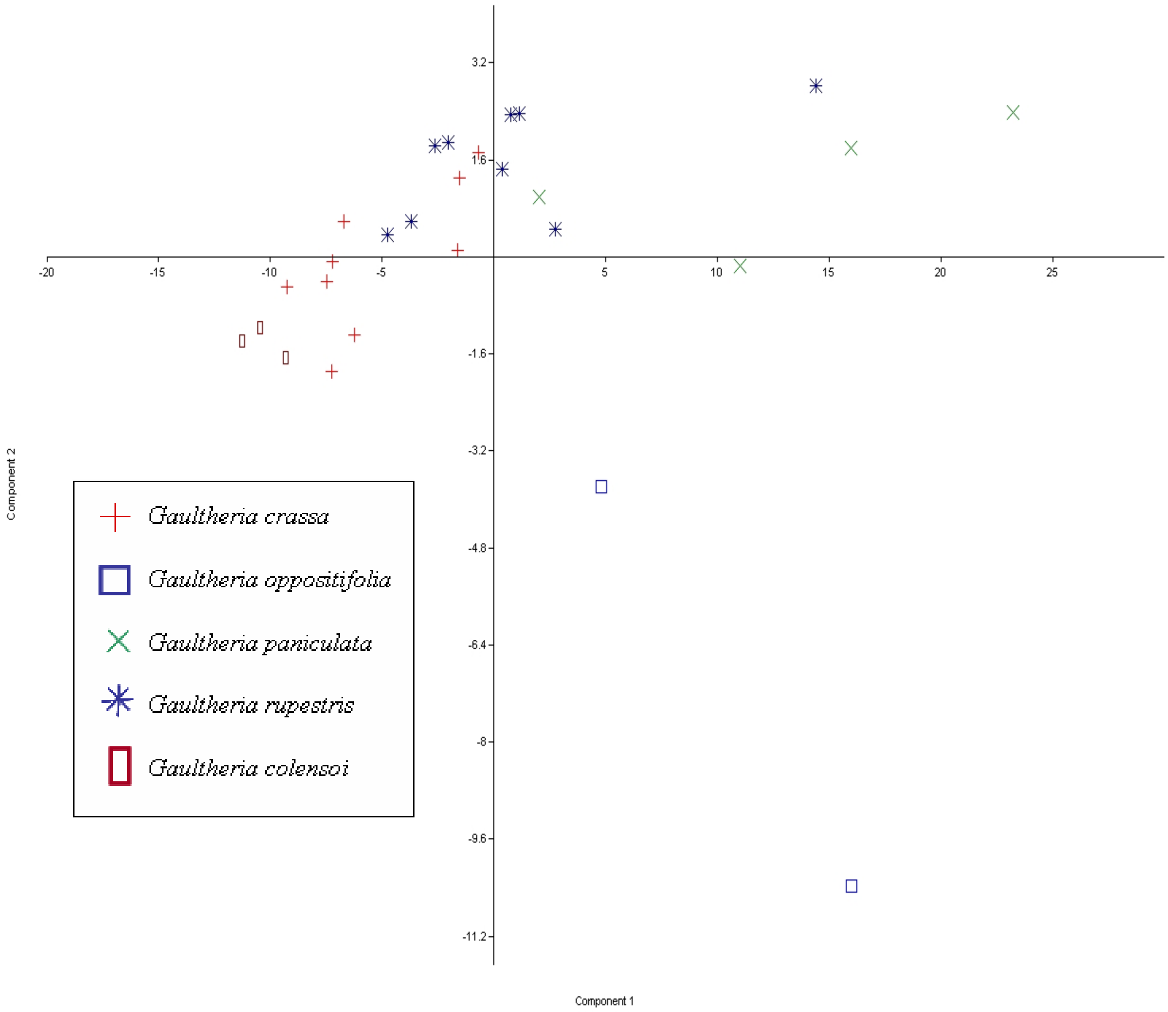

4.8
3.2
1.6
-1.6
-3.2
-4.8
-6.4
-8
-9.6
-20
-15
-10
-5
5
10
15
20
25
Component 2
Component 1
Gaultheria crassa
Gaultheria oppositifolia
Gaultheria paniculata
Gaultheria rupestris
Gaultheria colensoi

Figure 5.6

Principal Component Analysis of the Vegetative + Reproductive data set with 27 individuals, representing five taxa of *Gaultheria*. Symbols are coded as in legend.

Figure 5.6



3.2
1.6
-1.6
-3.2
-4.8
-6.4
-8
-9.6
-11.2
-20
-15
-10
-5
5
10
15
20
25
Component 2
Component 1
Gaultheria crassa
Gaultheria oppositifolia
Gaultheria paniculata
Gaultheria rupestris
Gaultheria colensoi

Figure 5.7

Principal Component Analysis of the Vegetative Only data set with 60 individuals, representing three taxa of *Gaultheria*. Symbols are coded as in legend.

Figure 5.7

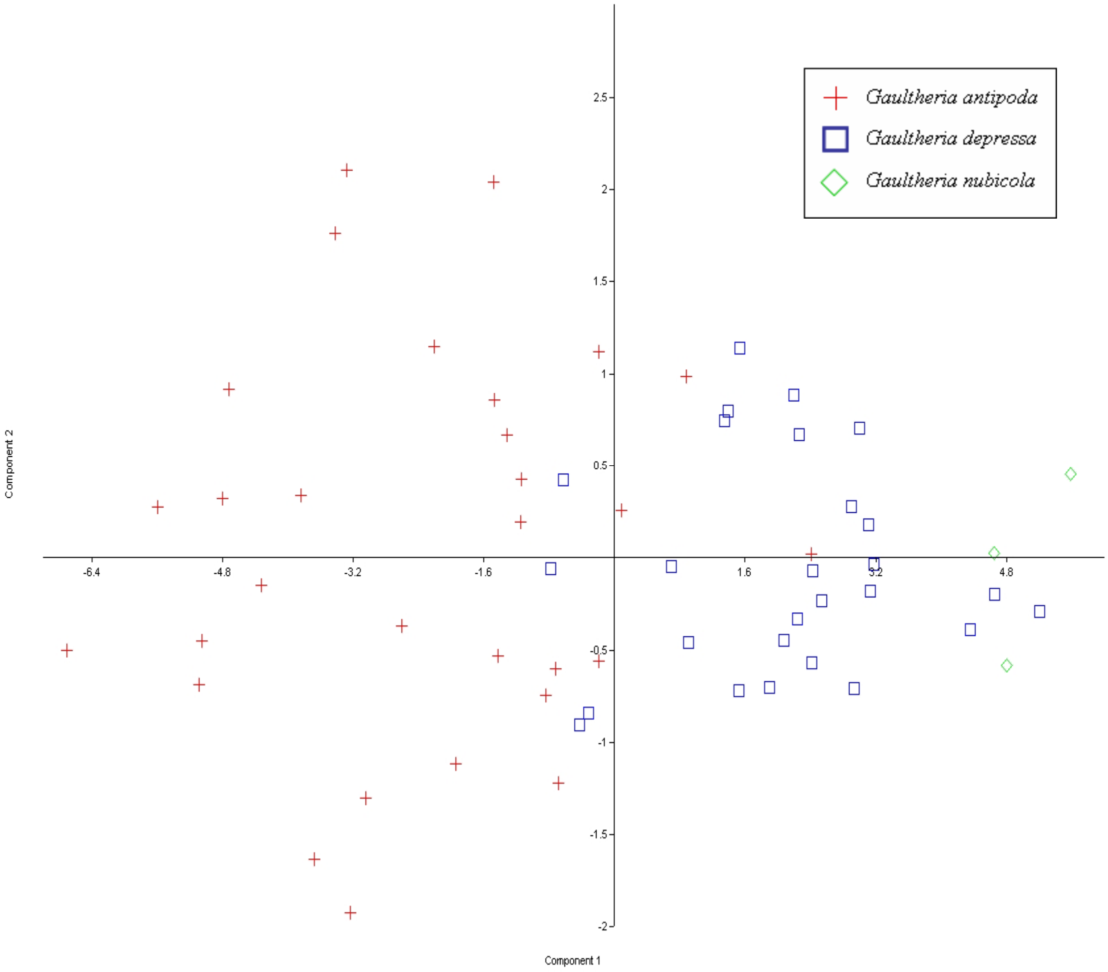

Gaultheria antipoda
Gaultheria depressa
Gaultheria nubicola
2.5
2
1.5
1
0.5
-0.5
-1
-1.5
-2
-6.4
-4.8
-3.2
-1.6
1.6
3.2
4.8
Component 2
Component 1

Figure 5.8

Principal Component Analysis of the Vegetative + Reproductive data set with 16 individuals, representing two taxa of *Gaultheria*. Symbols are coded as in legend.

Figure 5.8

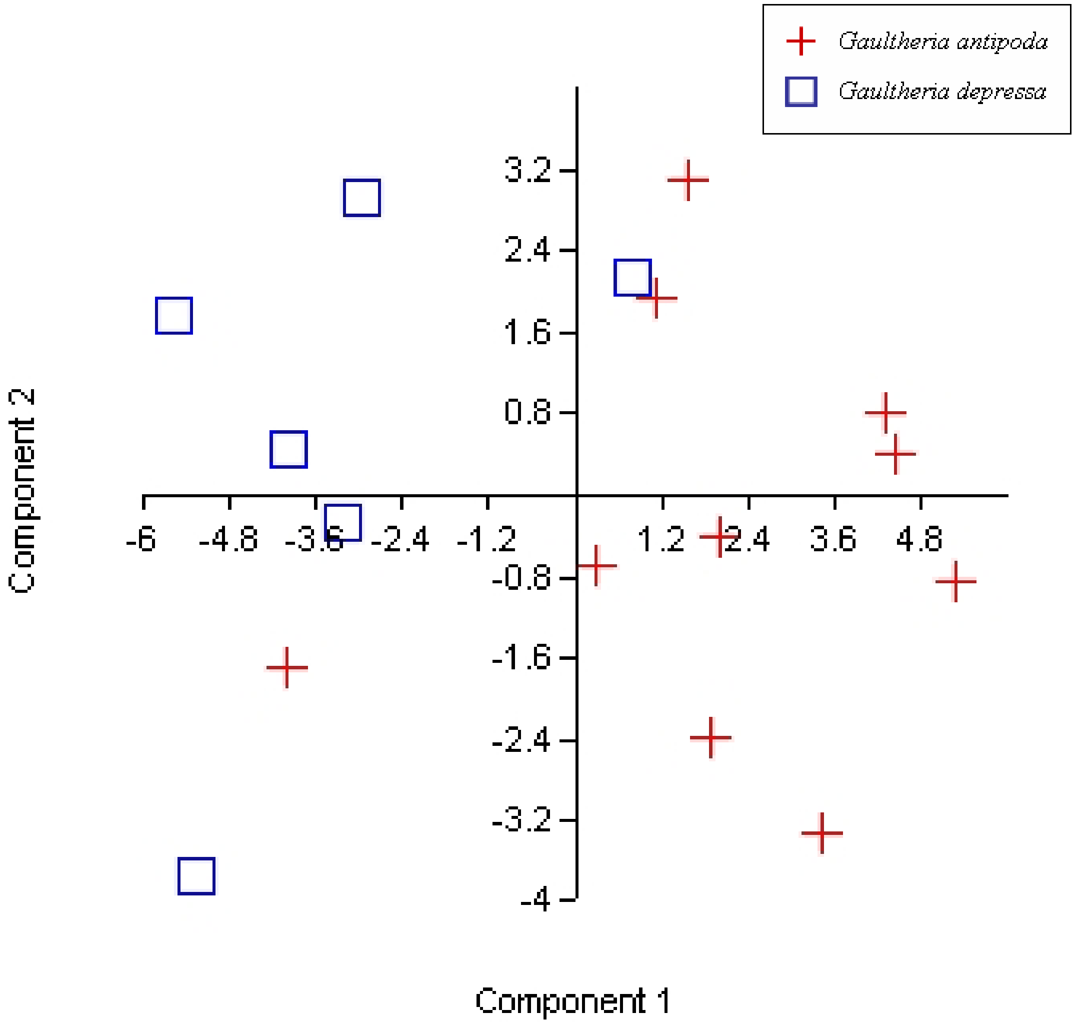

Gaultheria antipoda
Gaultheria depressa
3.2
2.4
1.6
0.8
-0.8
-1.6
-2.4
-3.2
-4
-6
-4.8
-3.6
-2.4
-1.2
1.2
2.4
3.6
4.8
Component 2
Component 1

Figure 5.9

Principal Component Analysis of the Vegetative Only data set with 22 individuals, representing two taxa of *Gaultheria*. Symbols are coded as in legend.

Figure 5.9

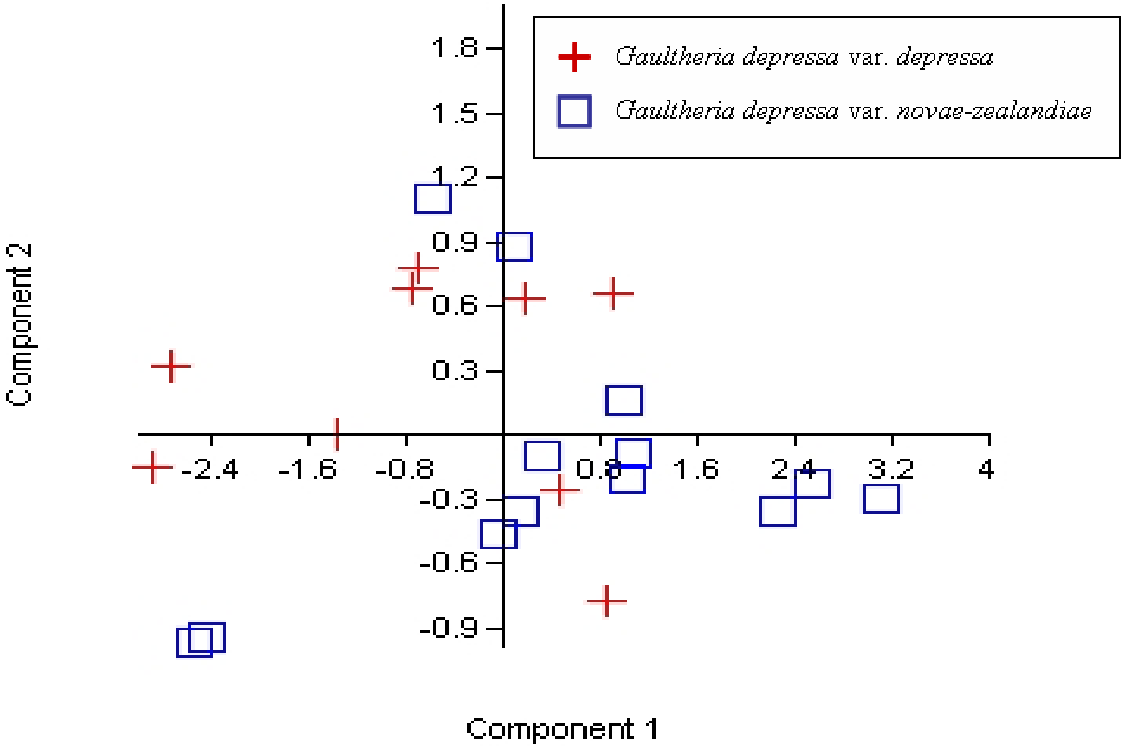

Gaultheria depressa var. depressa
Gaultheria depressa var. novae-zealandiae
1.8
1.5
1.2
0.9
0.6
0.3
-0.3
-0.6
-0.9
-2.4
-1.6
-0.8
0.8
1.6
2.4
3.2
4
Component 2
Component 1

Figure 5.10

Principal Component Analysis of the Vegetative Only data set with 43 individuals, representing four taxa of *Gaultheria*. Symbols coded as in legend.

Figure 5.10

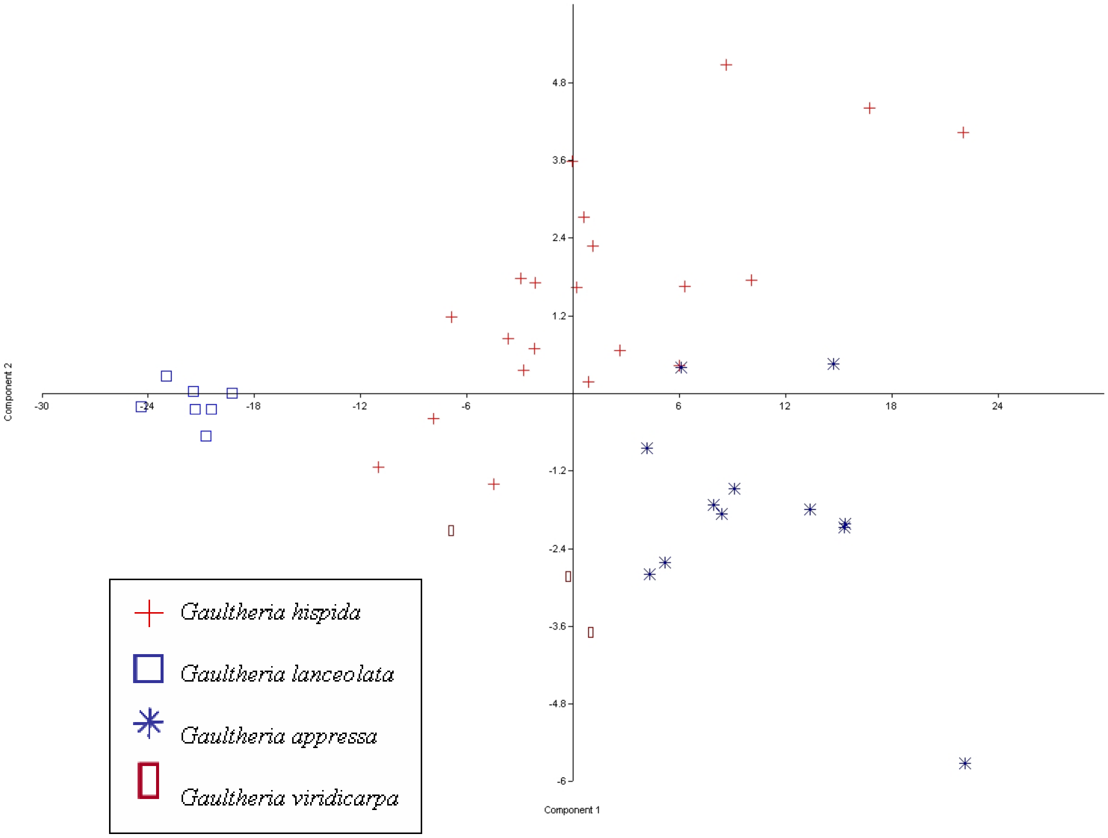
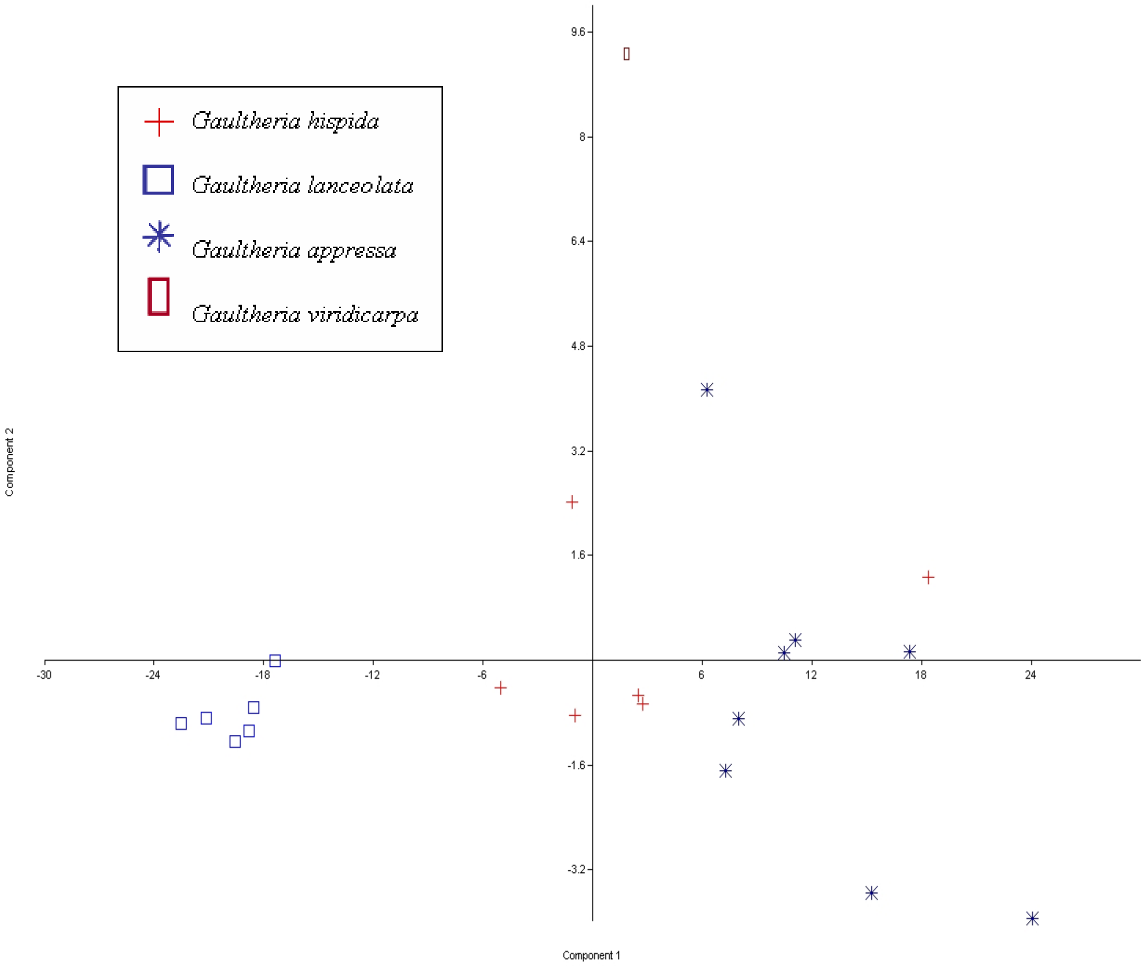

4.8
3.6
2.4
1.2
-1.2
-2.4
-3.6
-4.8
-6
-30
-24
-18
-12
-6
6
12
18
24
Component 1
Component 2
Gaultheria hispida
Gaultheria lanceolata
Gaultheria appressa
Gaultheria viridicarpa

Figure 5.11

Principal Component Analysis of the Vegetative + Reproductive data set with 21 individuals, representing four taxa of *Gaultheria*. Symbols are coded as in legend.

Figure 5.11


Gaultheria hispida
Gaultheria lanceolata
Gaultheria appressa
Gaultheria viridicarpa
9.6
8
6.4
4.8
3.2
1.6
-1.6
-3.2
-30
-24
-18
-12
-6
6
12
18
24
Component 2
Component 1

CHAPTER VI

THE PHYLOGENY AND MORPHOLOGY OF THE BRAZILIAN *GAULTHERIA* (ERICACEAE) SPECIES

Catherine M. Bush, Peter W. Fritsch, Boni C. Cruz, Angela B. Martins, Lu Lu and
Kathleen A. Kron

The following manuscript has been submitted for publication in *Systematic Botany*. Stylistic variations are due to the requirements of the journal. C.M. Bush performed laboratory and field work, data analysis and prepared the manuscript. P.W. Fritsch and A.B. Martins also partook in the field work. B.C. Cruz and L. Lu provided some sequence data. P.W. Fritsch and K.A. Kron provided logistical support and editorial assistance.

Abstract

Six species of *Gaultheria* are endemic to the *Mata Atlantica* (Atlantic rainforest) in Brazil, several of which exhibit unique morphological characters within *Gaultheria*. *Gaultheria bradeana, G. myrtilloides, G. serrata* and *G. ulei* all have the typical *Gaultheria* fruit morphology (capsular fruit surrounded by a colored fleshy calyx). However, *G. itatiaiae* and *G. sleumeriana* have capsular fruits and dry calyces which are otherwise present only in some species of *Gaultheria* in New Zealand (*G. colensoi, G. crassa, G. oppositifolia, G. paniculata* and *G. rupestris*) as well as one species in temperate South America (*G. nubigena*). *Gaultheria bradeana* has the unique characters of fleshy pedicels and bracteoles. *Gaylussacia corvensis*, recently understood to belong to *Gaultheria*, exhibits capsular fruits with only slightly fleshy calyx lobes. The endemic Brazilian species of *Gaultheria* have never been examined in a phylogenetic analysis. Analyses of a total combined dataset based on data (*matK, ndhF, trnL-F, trnS-G, rpl16*, nrITS, *waxy* and *leafy* DNA sequence data) including all *Gaultheria* species in Brazil except *G. ulei* yielded a clade of five Brazilian endemics (*G.* ser. *Myrtilloideae* clade: *Gaultheria bradeana, "G. corvensis", G. itatiaiae, G. myrtilloides* and *G. sleumeriana*). *Gaultheria serrata* var. *serrata,* a common endemic species, is sister to a clade of tropical South American *Gaultheria* species. *Gaultheria erecta* and one variety of *G. eriophylla* that occur disjunctly between southeastern Brazil and the Andes form a clade that is sister to *G. schultesii* from Mexico. The *G.* ser. *Myrtilloideae* clade is imbedded within a clade containing species from temperate South America, contradicting the hypothesis that the Andes have served as the only source area for the species in the *Mata Atlantica* of Brazil.

Introduction

The genus *Gaultheria* L. (Ericaceae: tribe Gaultherieae) contains approximately 130 species that occur in both tropical and temperate regions of the land masses bordering the Pacific Rim, i.e., the Americas, eastern Asia, the Indomalaya region, Australia and New Zealand (Middleton 1991). Forty-three species of *Gaultheria* occur in tropical America. These occur primarily in the Andes but eight occur disjunctly in Brazil (Luteyn 1991). Six of the Brazilian species are endemic, whereas the remaining two also occur in other parts of tropical America.

Middleton (1991) placed the Brazilian species among two of the ten sections of *Gaultheria* recognized in his comprehensive classification of the genus. Five of the Brazilian species are currently recognized as comprising *G*. ser. *Myrtilloideae* of section *Monoanthemona*: *G. bradeana*, *G. itatiaiae*, *G. myrtilloides*, *G. sleumeriana* and *G. ulei* (Middleton 1991). All of these species were originally characterized by having cylindrical corollas and anthers that are longer than is typical for the genus. Because of their relatively long length, Middleton (1991) interpreted the anthers of these species as having tubules, a condition otherwise known among the members of the tribe Gaultheriaeae only in *Diplycosia* and *Tepuia*. *Gaultheria itatiaiae* and *G. sleumeriana* clearly have anthers with this morphology, but Middleton (1991) was not able to examine reproductive material for the other species of *G*. ser. *Myrtilloideae*, and Luteyn (1991) states that these do not have "long anthers narrowing apically" (i.e., anther tubules). Of the species in *G*. ser. *Myrtilloideae*, only *G. myrtilloides* has additional appendages, in the form of awns, on its anthers (Middleton 1991). Also, there is great variation in corolla shape in this series from cylindric to cylindric-campanulate and cylindric-urceolate; cylindrical corollas are also present in many other species of *Gaultheria* (Luteyn 1991).

Despite their placement in the same series, the species in *Gaultheria* ser. *Myrtilloideae* exhibit wide variation in both inflorescence structure and texture of the mature calyx, characters that otherwise were employed heavily to define sections and other series in Middleton's (1991) treatment. Infloresence type in *Gaultheria* includes solitary flowers, racemes and pseudoracemes (Luteyn 1991). Pseudoracemes occur when axillary solitary flowers are subtended by leaf-like bracts that become smaller towards the tip of the branchlet, resembling a terminal raceme (Luteyn 1991). All three conditions occur among the species of *G.* ser. *Myrtilloides*. Both *G. bradeana* and *G. myrtilloides* have solitary flowers; those of *G. bradeana* are subtended by exclusively normal leaves, whereas those of *G. myrtilloides* are subtended by normal or slightly reduced leaves (Luteyn 1991). *Gaultheria sleumeriana* has racemes, and both *G. itatiaiae* and *G. ulei* have pseudoracemes (Luteyn 1991).

Gaultheria bradeana, *G. myrtilloides* and *G. ulei* exhibit the typical *Gaultheria* fruit condition of a fleshy calyx surrounding a capsule (Luteyn 1991). *Gaultheria bradeana*, a prostrate shrub that grows in boggy areas among cloud forests in Rio de Janeiro, Minas Gerais, São Paulo and Santa Catarina states (Luteyn 1991), also has fleshy pedicels and bracteoles (Luteyn 1991), a character that appears to be unique within *Gaultheria* (pers. obs., C. Bush and P. Fritsch). *Gaultheria myrtilloides*, a small erect shrub occurring at high elevations in the states of Minas Gerais, Rio de Janeiro and Rio Grande do Sul (Luteyn 1991; pers. obs., C. Bush and P. Fritsch), is persistently and densely hirsute on the calyx, an uncommon feature in *Gaultheria* (Luteyn 1991).

Gaultheria ulei (endemic to Santa Catarina state) occurs at elevations of 1200-1400 m (Luteyn 1991), is poorly characterized and known from only few collections. It is considered to most resemble *G. bradeana* in morphology (Luteyn 1991; Middleton 1991), differing mainly in having two bracteoles like all other species in *G*. ser. *Myrtilloideae* (versus five or six in *G. bradeana*; Luteyn 1991).

The other two species of *G*. ser. *Myrtilloideae* have dry calyces, a feature only rarely found within *Gaultheria*. *Gaultheria itatiaiae* occurs in cloud forests of the Minas Gerais/Rio de Janeiro border region (Itatiaia National Park) south to Rio Grande do Sul (Luteyn 1991). *Gaultheria sleumeriana* is found in disturbed habitats in the Serra da Bocaina of São Paulo. Other features shared by these two species are long, acuminate calyx lobes, awnless anthers, the presence of tubules and capsules with slightly ridged sutures (Luteyn 1991). A character in *G. sleumeriana* unique in *Gaultheria* is the presence of a distinct submarginal nerve on both sides of the leaf (Luteyn 1991).

The other three species of *Gaultheria* in Brazil are included in the largest and most variable section of *Gaultheria*, *G.* sect. *Brossaea* (Middleton 1991). The three Brazilian species of this section are all large shrubs with racemose inflorescences and blue-black fleshy calyxes. *Gaultheria serrata* is a widespread and common endemic of southeastern and southern Brazil, occurring in the states of Espirito Santo/Minas Gerais, Rio de Janeiro, São Paulo, Santa Catarina and Parana at elevations from 1000 – 2800 m (Luteyn 1991). *Gaultheria erecta* is common in the Andes, from Venezuela to northern Argentina (Luteyn 1991) but it is only known from five collections in Brazil (Amazonas, Minas Gerais, Santa Catarina; Luteyn 1991). *Gaultheria eriophylla*, like *G. lanigera* and *G. tomentosa* of Andean South America, exhibits tomentose-lanate leaves, rachises, pedicels and flowers (Luteyn 1991). *Gaultheria eriophylla* var. *eriophylla*, endemic to Brazil, is common in the montane forests of Rio de Janeiro, Minas Gerais, Espirito Santo and São Paulo whereas *G. eriophylla* var. *mucronata* is found in southern Peru and northern Bolivia (Luteyn 1991). Middleton (1991) placed *G. serrata* and *G. erecta* in *G.* ser. *Domingenses* and *G. eriophylla* in *G.* ser. *Tomentosae*.

A species endemic to the Serra do Corvo Branco in Santa Catarina state that grows in cloud forests on steep canyon walls has been described as *Gaylussacia corvensis* (Silva and Cervil 2003). In contrast to the inferior ovary and distinctly fleshy fruit of *Gaylussacia*, however, *G. corvensis* has a superior ovary, a capsular fruit and a slightly fleshy calyx, characters that are instead consistent with members of the Gaultherieae. The formal transfer of *Gaylussacia corvensis* to *Gaultheria* is pending (pers. comm. Gérson Ramão). The distribution of "*Gaultheria corvensis*" lies within that of *G.* ser. *Myrtilloideae* and we hypothesize that it may be closely related to one or more species of *G.* ser. *Myrtilloideae*.

Many other plant groups have distributions that span both the Andes and southeastern Brazil (Brade 1942; R. Tryon 1944; Rambo 1951; Smith 1962; A. Tryon 1962; Clark 1992; Safford 1999). For example, Safford (1999) found that up to one-third of the plant genera represented in the Serra do Itatiaia are also found in the parámos of Colombia. It has been hypothesized that these distributions are the result of long-distance dispersal from the south-central Andes to southeastern Brazil (Luteyn 1991; Gradstein and Reiner-Drehwald 2007). However, very few phylogenetic analyses have tested the biogeographic patterns of this distribution in plants (Sánchez-Baracaldo 2004).

Previous phylogenetic studies (Bush et al. 2009a, b) show that the characters traditionally used to define sections and series in *Gaultheria* (i.e., inflorescence structure, fruit type and mature calyx texture) have evolved repeatedly within the Gaultherieae and are unreliable in diagnosing the major clades of this group. In this context, it is uncertain whether the morphologically diverse species of *G*. ser. *Myrtilloideae* are monophyletic. Here we reconstruct the phylogeny of the Brazilian species of *Gaultheria* with DNA sequence data to reveal their interspecific relationships, test the monophyly of *G*. ser. *Myrtilloideae*, and use the molecular-based tree to estimate the putative origin of the Brazilian clade(s). We also will test our hypothesis that "*Gaultheria corvensis*" is related specifically to one or more members of *G.* ser. *Myrtilloideae*.

Materials and Methods

DNA extraction and taxon sampling

Total DNA was extracted from silica dried, fresh or herbarium material by using a modified CTAB extraction protocol (Doyle and Doyle 1987) or with the DNEasy Plant Extraction Kit (Qiagen Sample and Assay Technologies, Valencia, California, U.S.A). Every currently defined species of *Gaultheria* present in Brazil is represented in this analysis except for *G. ulei*, which is the only Brazilian endemic species not collected during field work by Bush, Fritsch, and Martins (March – April 2009). A sample of "*Gaultheria corvensis*" is also included. The total number of terminals from Brazil is seven. Also included were 23 species of *Gaultheria* encompassing the geographical, taxonomic and morphological range of the genus, and one species each of *Diplycosia* and *Tepuia*, two other genera of the Gaultherieae. The sample of *G. erecta* included in this study was collected in Panama. *Leucothoë griffithiana* (Gaultherieae) was used to root the tree because previous studies place *Leucothoë* (sensu Waselkov and Judd 2008) as sister to all other Gaultherieae (Bush *et al.* 2009a). GenBank numbers for all taxa are listed in Table 1. Some sequences used in our analyses have been generated previously (Powell and Kron 2001; Bush *et al* 2009a; Bush *et al* 2009b). There were 150 sequences newly generated for this study.

Gene regions, DNA sequencing and cloning

Five chloroplast gene regions (*matK, ndhF, rpl16, trnL-F* and *trnS-G*) and three nuclear gene regions (nrITS, *waxy* and *leafy*) were sampled. The gene regions *ndhF* and *matK* were amplified in two different sections (5' and 3' respectively); alignment ambiguity in the region of primer overlap was therefore excluded (see below). Due to this truncation as well as the exclusion of primer binding sites and areas of ambiguous alignment, the total *ndhF* gene portion for this study is 1539 base pairs. PCR amplification follows the methods of Bush *et al*. (2009b) by using the primer combinations in Bush *et al.* (2009b) for *ndhF, matK,* nrITS*, waxy* and *leafy*, Fritsch *et al.* (2008) for *rpl16* and *trnL-trnF* and Yao *et al.* (2008) for *trnS-trnG*.

PCR-amplified fragments were cleaned, DNA was sequenced and sequences were edited and aligned according to Bush *et al*. (2009b). Extensive missing data (i.e., that due to failed gene region amplification or cycle sequencing) in this analysis occurs in *Diplycosia barbigera* (*trnL-F* large gap 5'; *leafy*), *Gaultheria appressa* (*leafy*), *G. eriophylla* var. *eriophylla* (*leafy,* 5' *ndhF*), *G. insana* (5' *ndhF*), *G. insipida* (*leafy*), *G. itatiaiae* (*leafy*), *G. lanceolata* (*leafy*), *G. myrtilloides* (5' *ndhF*), *G. procumbens* (3' *trnS-G*), *G. sclerophylla* (*rpl16*), *G. serrata* var. *serrata* (5' *ndhF*; *leafy*), *G. sleumeriana* (nrITS), and *Tepuia venusta* (*leafy*).

PCR fragments from all sampled taxa for the gene region *leafy* and *waxy* were cloned with the Topo Cloning Kit for Sequencing by Invitrogen. Up to five clones were sequenced for each taxon. *Waxy* clones from *Gaultheria eriophylla* var. *eriophylla* could not be successfully obtained so the sequence included in the gene analysis for this taxon was sequenced directly. Clones were analyzed as in Bush *et al*. (2009b).

Phylogenetic analyses

Parsimony analyses were performed first on single gene matrices; if no strong conflict [(>80 percent bootstrap (bt) support)] was observed between single gene trees, then the matrices were combined into a single matrix and analyzed as total-evidence data. Separate parsimony analyses were performed on the *matK*, *ndhF*, *trnL-F, trnS-G, rpl16,* nrITS, *waxy*, *leafy*, combined chloroplast, combined nuclear and total combined data sets. Phylogenetic analyses were performed with PAUP* 4.0b2 (Swofford 2000). All characters were unordered, gaps were treated as missing data, and any areas exhibiting ambiguous alignments were excluded from the analyses. All characters were equally weighted and only parsimony-informative characters were included. Tree construction was performed by using a heuristic search with 1000 replicates, TBR branch swapping, and random step-wise addition. Clade support was estimated with bootstrap analyses (Felsenstein 1985). For the eleven parsimony analyses (*matK*, *ndhF*, *trnL-F*, *trnS-G*, *rpl16*, nrITS, *waxy*, *leafy*, combined chloroplast, combined nuclear and total data) "fast" bootstrap replicates were performed due to the long time required for a complete heuristic search. The values from fast bootstrapping will typically be lower than those produced by bootstrapping that involves heuristic searches and thus are a conservative estimate of clade support (Mort *et al.* 2000). The fast bootstrap analysis consisted of 100,000 replicates for all parsimony analyses.

The ML analysis for the total combined data set (as well as the *leafy* and *waxy* clone analyses) was run in RaxML. A fast bootstrap analysis (1000 replicates) was performed simultaneously with the ML analysis (option "-f a"). The GTRMIX model was used, which infers the initial tree topology under GTRCAT and then analyzes the final tree topology with GTRGAMMA until stable likelihood values are reached (Stamatakis 2006).

Results

The *leafy* clone analysis comprised 25 taxa and 71 cloned sequences while the *waxy* clone analysis comprised all 33 taxa and 94 cloned sequences. There were between one to six clones per taxon. Neither *leafy* nor *waxy* clone analyses revealed strongly supported conflict in any taxon (data not shown). All clones from a single species either form clades or are only weakly supported as being in separate clades, suggesting that only a single copy of *leafy* and *waxy* is present in each species sampled.

The number of nucleotide positions, parsimony-informative characters, number of most parsimonious trees, length, C.I. and R.I. for all individual (ITS, *leafy, matK*, *ndhF*, *rpl16, trnL-F, trnS-G* and *waxy*) and combined (chloroplast, nuclear and total) maximum parsimony analyses can be found in Table 2. Individual chloroplast and nuclear gene parsimony analyses, respectively, were not in conflict and were thus combined into a chloroplast matrix and nuclear analysis. Analysis of each data set indicated strongly supported conflict between the chloroplast and nuclear analysis data (data not shown). The combined nuclear parsimony analysis was neither well resolved nor supported but there was 86% bootstrap support (bt) for the relationship of *Gaultheria erecta + G. eriophylla* var. *eriophylla + G. schultesii + G. sclerophylla + G. insipida* and in the chloroplast analysis, *G. insipida + G. sclerophylla* is sister to *G. antarctica* from temperate South America (86% bt). For this reason, *G. insipida* and *G. sclerophylla* were excluded from the combined total data analysis.

The resulting trees from both the parsimony and ML analyses for the total combined data set were very similar with no conflict between them (data not shown). The

ML analysis is more resolved and will be the only tree presented and described here for the combined data (Fig. 6.1).

In the combined data ML analysis, the four species of *Gaultheria* ser. *Myrtilloideae* plus “*Gaultheria corvensis*” form a clade (100% bt; Fig. 6.1). *Gaultheria itatiaiae* is sister to *G. myrtilloides* (100% bt) and this pair is in turn sister to “*Gaultheria corvensis*” + *Gaultheria sleumeriana* (97% bt; sister relationship 82%; Fig. 6.1). *Gaultheria bradeana* is sister to all other *G*. ser. *Myrtilloideae* members (100% bt; Fig. 6.1). Sister to the *G*. ser. *Myrtilloideae* clade is a clade of temperate South American taxa (100% bt for the clade; <50% bt for the sister relationship; TSA Clade I in Fig. 6.1). Sister (100% bt) to the *G*. ser. *Myrtilloideae* clade + TSA Clade I is a clade of species from New Zealand, Australia and temperate South America (81% bt; New Zealand/Australia Clade + TSA Clade II in Fig. 6.1). *Gaultheria serrata* is in a clade of tropical South American/Mexican species, including *G. eriophylla* var. *eriophylla, G. erecta* and *G. schultesii* (100% bt; Fig. 6.1). Sister to this clade is the North American species represented by *G. humifusa* (82% bt; Fig. 6.1).

Discussion

Despite striking differences in both inflorescence architecture and calyx texture (and disregarding for the moment the placement of "*Gaultheria corvensis*"), the species of *G*. ser. *Myrtilloideae* form a strongly supported clade in this study, thus corroborating the classification of Middleton (1991). *Gaultheria ulei*, although not sampled in this study, would most likely also fall into the *G*. ser. *Myrtilloideae* clade based on both its original inclusion in this series by Middleton (1991) and its hypothesized close relationship with *G. bradeana* (Sleumer 1952; Luteyn 1991). Nonetheless, a clear synapomorphy has not yet been detected for the series. Middleton's (1991) assertion that the group has anther tubules is true only for two of the species; three of them do not have such tubules (i.e., *G. bradeana*, *G. myrtilloides* and *G. ulei*; Luteyn 1991).

"*Gaultheria corvensis*" was described after the publication of Middleton's (1991) *Gaultheria* classification, and also after the publication of the revisions by Luteyn on Neotropical *Gaultheria* (1991; 1995*a*, *b*) and *Gaylussacia* (Sleumer 1967). Thus, these authors were not aware of this species and no one other than the original author of the species has proposed a taxonomic placement for it. "*Gaultheria corvensis*" is strongly supported as being placed within the *Gaultheria* ser. *Myrtilloides* clade in our study and its relationship with *G. sleumeriana* is consistent with their shared coriaceous leaves and dry to slightly fleshy mature calyx.

Previous taxonomic treatments considered *Gaultheria itatiaiae* to be closely related to *G. sleumeriana* due to the shared characters of dry calyx lobes, anthers with tubules and capsules with slightly ridged sutures (Middleton 1991; Luteyn 1991). However, in this study *G. itatiaiae* is strongly supported instead as sister to *G.*

myrtilloides. Reported morphological characters shared between these two taxa are a thin-stemmed habit, strigose stems, leaves and pedicels, ovate leaves, and long-acuminate calyx lobes (Luteyn 1991). Additionally, *G. itatiaiae* was characterized by having a "pseudoraceme" (Luteyn 1991) but both species have solitary flowers that can be in the axils of reduced leaves. Bush et al. (2009a) showed that the inflorescence structure in the Gaultherieae is evolutionarily labile and that the "pseudoraceme" term as applied to this group is ambiguous.

Our results suggest that the ancestor of the *Gaultheria* ser. *Myrtilloideae* (henceforth also including "*Gaultheria corvensis*") clade did not originate from the south-central Andes, as has been previously hypothesized for species with geographic distributions shared between the Andes and southeastern Brazil (Luteyn 1991). The species of *G.* ser. *Myrtilloideae* are most closely related to those from temperate South America and New Zealand/Australia rather than those of tropical Andean South America (Fig. 6.1). Phylogenetic analyses of the New Zealand/Australian species of *Gaultheria* have suggested that a long-distance dispersal event from temperate South America to New Zealand likely introduced *Gaultheria* to Australasia (Bush et al. 2009b). The current study suggests that the ancestor of *G.* ser. *Myrtilloideae* also dispersed from temperate South America, or possibly vice-versa.

In contrast, the endemic Brazilian taxa of *G.* sect. *Brossaea*, i.e., *Gaultheria serrata* and *G. eriophylla* var. *eriophylla*, are clearly most closely related to species from Andean South America and Mexico. This phylogenetic pattern is thus at least consistent with the south-cenral Andean origin of the Brazilian species, although the precise origin of the endemic species of *G.* sect. *Brossaea* cannot be determined on the basis of the current data.

One of the few other phylogenetic analyses to examine biogeographic relationships between the southeastern Brazil and Andean species of a plant group found that the sister taxon to a large Andean radiation of neotropical ferns (*Jamesonia*/*Eriosorus* complex) was a Brazilian species, *Eriosorus myriophyllus* (Sánchez-Baracaldo 2004). The author states that this result suggests that the Andean group had Brazilian ancestors (Sánchez-Baracaldo 2004). Additionally, the analyses found that *Jamesonia brasiliensis* from Itatiaia National Park, Brazil was nested within a clade of Andean species, suggesting that a long distance dispersal event from the Andes (specifically the Eastern Cordillera of Colombia) to Brazil occurred (Sánchez-Baracaldo 2004). Together, this study and ours suggests that the biogeographic patterns including the Andes, the *Mata Atlantica* of Brazil and temperate South America are complex and may include multiple dispersal events from different source areas, but the data are yet too sparse to make general conclusions on the biogeographical relationships among these areas.

Literature Cited

Brade AC (1942) A composição da flora pteridófita do Itatiaia. *Rodriguésia* **6**, 26–43.

Bush CM, Lu Lu, Fritsch PW, Li D, Kron KA (2009a) Phylogeny of Gaultherieae (Ericaceae: Vaccinioideae) based on DNA sequence data from *matK*, *ndhF* and nrITS. *International Journal of Plant Sciences* **170**, 355–464.

Bush CM, Wagstaff SJ, Fritsch PW, Kron KA (2009b) The Phylogeny, Biogeography and Morphological Evolution of *Gaultheria* (Ericaceae) from Australia and New Zealand. *Australian Systematic Botany* **22**, 229-242.

Clark LG (1992) *Chusquea* sect. *Swallenochloa* (Poaceae: Bambosoideae) and allies in Brazil. *Brittonia* **44**, 387–422.

Doyle J, Doyle J (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**, 783–791.

Fritsch PW, Kelly LM, Wang Y, Almeda F, Kriebel R (2008) Revised infrafamilial classification of Symplocaceae based on phylogenetic data from DNA sequence and morphology. *Taxon* **57**, 823-852.

Gradstein SR, Reiner-Drehwald ME (2007) The status of *Neopotamolejeunea* (Lejeuneaceae) and description of a new species from Ecuador and southern Brazil. *Systematic Botany* **32**, 487–492.

Luteyn JL (1991) The genus *Gaultheria* in Brazil. *Bol. Mus. Para. Emílio Goeldi, sér. Bot.* **7**, 309–333.

Luteyn JL (1995*b*) *Gaultheria* In 'Flora Neotropica Monograph Vol 66. Ericaceae, part II the superior-ovaried genera.' (Ed. JL Luteyn) (New York Botanical Garden Press: New York).

Luteyn JL (1995*a*) *Pernettya* In 'Flora Neotropica Monograph Vol 66. Ericaceae, part II – the superior-ovaried genera.' (Ed. JL Luteyn) (New York Botanical Garden Press: New York).

Mort ME, Soltis PS, Soltis DE, Mabry ML (2000) Comparison of three methods for estimating internal support on phylogenetic trees. *Systematic Biology* **49**, 160–171.

Powell EA, Kron KA (2001) An analysis of the phylogenetic relationships in the wintergreen group (*Diplycosia*, *Gaultheria*, *Pernettya*, *Tepuia*; Ericaceae). *Systematic Botany* **26**, 808–817.

Rambo B (1951) O elemento andino no pinhal riograndese. *Anais Bota^nicos do Herba´rio Barbosa Rodriguez* **3**, 7–39.

Safford HD (1999) Brazilian Paramos II. Macro and mesoclimate of the campos de altitude and affinities with high mountain climates of the tropical Andes and Costa Rica. *Journal of Biogeography* **26**, 713–737.

Sánchez-Baracaldo P (2004) Phylogenetics and biogeography of the neotropical fern genera *Jamesonia* and *Eriosorus* (Pteridaceae). *American Journal of Botany* **91**, 274-284.

Silva RR, Cervil AC (2003) *Gaylussacia corvensis* R.R. Silva and Cervi (Ericaceae) a new species from austral Brazil. *Sellowia* **53-55**, 23-27.

Sleumer H (1952) Die Arten der Gattung *Gaultheria* L. in Brasilien. *Botanische Jahrbücher* **75**, 443-450.

Sleumer H (1967) Die Gattung *Gaylussacia* H.B.K. Botanische Jahrbucher **86**:309–384.

Smith LB (1962) Origins of the flora of southern Brazil. *Contr. U.S. Natl. Herb.* **35**, 215–249.

Stamatakis A (2006) RaxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.

Swofford DL (2000) PAUP* Phylogenetic analysis using parsimony (*and other methods). Version 4.01. (Sinauer Associates, Inc.: Massachusetts).

Tryon AF (1962) A monograph of the fern genus *Jamesonia*. *Contri. Gray Herb.* **191**, 109–197.

Tryon RM (1944) Dynamic phytogeography of *Doryopteris*. *Am. J. Bot.* **31**, 470–473.

Waselkov K, Judd WS (2008) A phylogenetic analysis of *Leucothoë* s.l. (Ericaceae; tribe Gaultherieae) based on phenotypic characters. *Brittonia* **60**: 382-397.

Yao X, Ye Q, Fritsch PW, Cruz BC, Huang H (2008) Phylogeny of *Sinojackia* (Styacaceae) based on DNA sequence and microsatellite data: implications for taxonomy and conservation. *Annals of Botany* **101**, 651-659.

Table 6.1

Taxa sampled in this study of the Gaultheria from Brazil. GenBank accession numbers are listed under the appropriate gene region. Herbarium acronyms follow Holmgren and Holmgren (www.nybg.org/bsci/ih). GenBank numbers in bold represent new data obtained for this study.

Table 6.1

			GenBank numbers							
Taxon	Voucher information (accession/collection number; institution)	Approximate Collection Locality	*matK*	*ndhF*	*rpl16*	*trnL-F*	*trnS-G*	ITS	*lfy*	*waxy*
Diplycosia barbigera Sleumer	Fuller s.n.; WFU	Mount Kinabalu, Malaysia	AF366622	FJ008932	g	g	g	AF358875	n/a	FJ665760
Gaultheria antarctica Hook.f.	Kron s.n.; WFU	Temperate South America	AF366627	FJ008937	g	g	g	AF358880	FJ665717	FJ665761
Gaultheria antipoda G. Forst.	Bush, Wagstaff and Fritsch 424; WFU	New Zealand	FJ665743	FJ665787	g	g	g	FJ665699	FJ665718	FJ665762
Gaultheria appressa A.W. Hill	Crayn 1131; WFU	New South Wales, Australia	FJ665744	FJ665788	g	g	g	FJ665700	n/a	FJ665763
Gaultheria bradeana Sleumer	Almeda 9785; CAS	Southeast Brazil	g	g	g	g	g	g	g	g
Gaultheria caespitosa Poepp. & Endl.	Sancho and Bush 114; LP	Temperate South America	g	g	g	g	g	g	g	g
Gaultheria erecta Vent.	Luteyn 114813; NY	Panama	g	g	g	g	g	g	g	g
Gaultheria eriophylla (Pers.) Sleumer ex Buret var. *eriophylla*	Almeda 8795; CAS	Southeast Brazil	g	g	g	g	g	g	n/a	g
Gaultheria humifusa Rybd.	Fritsch 1837; CAS	Temperate North America	FJ665752	FJ665796	g	g	g	FJ665708	FJ665726	FJ665768
Gaultheria insana (Molina) D.J. Middleton	19924312; RBGE	Temperate South America	FJ665753	FJ665797	g	g	g	FJ665709	FJ665727	FJ665769
Gaultheria insipida Benth.	Luteyn 15328; NY	Tropical America	AF366636	FJ008944	g	g	g	AF358889	n/a	FJ665770
Gaultheria itatiaiae Wawra	Almeda 9790; CAS	Southeast Brazil	g	g	g	g	g	g	n/a	g
Gaultheria lanceolata Hook. f.	Bush 403; WFU	Tasmania, Australia	FJ665754	FJ665798	g	g	g	FJ6650710	n/a	FJ665771
Gaultheria mucronata Hook. & Arn.	Cubey 54 825 #7; RBGE	Temperate South America	FJ010622	FJ008977	g	g	g	FJ010604	FJ665729	FJ665773
Gaultheria myrtilloides Chamiss & Schlechtendal	Almeda 9757; CAS	Southeast Brazil	g	g	g	g	g	g	g	g
Gaultheria nubigena B.L. Burtt & Sleumer	C.R. Villamil 10407; NY	Temperate South America	FJ665808	FJ665800	g	g	g	FJ665712	FJ665730	FJ665774
Gaultheria nummularioides D.Don	vander Kloet 2241092; ACAD	Eastern Asia	AF366641	FJ008947	g	g	g	AF358893	FJ665731	FJ665775
Gaultheria oppositifolia Hook. f.	Bush and Fritsch 440; WFU	New Zealand	FJ665756	FJ665801	g	g	g	FJ665713	FJ665732	FJ665776
Gaultheria paniculata B.L. Burtt & A.W. Hill	Bush and Fritsch 442; WFU	New Zealand	FJ665757	FJ665802	g	g	g	FJ665714	FJ665733	FJ665777
Gaultheria parvula D.J. Middleton	Bush, Wagstaff and Fritsch 433; WFU	New Zealand	FJ665758	FJ665803	g	g	g	FJ665715	FJ665734	FJ665778
Gaultheria phillyreifolia (Pers.) Sleumer	Sancho and Bush 110; LP	Temperate South America	g	g	g	g	g	g	g	g
Gaultheria poeppigii DC.	Kron s.n.; WFU	Temperate South America	AF366642	FJ008948	g	g	g	AF358894	FJ665735	FJ665779
Gaultheria procumbens L.	Powell s.n.; WFU	Temperate North America	AF366643	FJ008949	g	g	g	AF358895	FJ665736	FJ665780
Gaultheria pumila (L.f.) D.J.Middleton	Kron s.n.; WFU	Temperate South America	AF366644	FJ008950	g	g	g	AF358896	FJ665737	FJ665781
Gaultheria rupestris (G. Forst.) R. Br.	Bush, Wagstaff and Fritsch 426; WFU	New Zealand	FJ665759	FJ665804	g	g	g	FJ665716	FJ665738	FJ665782
Gaultheria schultesii Camp	ex garden; Rhododendron Species Foundation	Tropical America	FJ010619	FJ008974	g	g	g	FJ010601	FJ665739	FJ665783
Gaultheria sclerophylla Cuatrec.	Luteyn 15331; NY	Tropical America	AF366646	FJ008951	n/a	g	g	AF358898	FJ665740	FJ665784
Gaultheria serrata (Vell.) Sleumer ex Kin.-Gouv. var. *serrata*	Almeda 8793; CAS	Southeast Brazil	g	g	g	g	g	g	n/a	g
Gaultheria sleumeriana Kin.-Gouv.	Almeda 9829; CAS	Southeast Brazil	g	g	g	g	g	n/a	g	g
Gaultheria tenuifolia (Phil.) Sleumer	Sancho and Bush 111; LP	Temperate South America	g	g	g	g	g	g	g	g
"Gaylussacia corvensis" R.R. Silva & Cervi	Almeda 9854; CAS	Southeast Brazil	g	g	g	g	g	g	g	g
Leucothoë griffithiana C.B.Clarke	GLGS 32580; CAS	Eastern Asia	FJ010616	FJ008971	g	g	g	FJ010598	FJ665742	FJ665785
Tepuia venusta Camp in Gleason & Killip	Michelangeli 535; NY	Tropical America	AF366649	FJ665807	g	g	g	AF358905	n/a	FJ665786

Table 6.2

Statistics for individual and combined data maximum parsimony analyses. The total combined dataset was run in the Mafft v6.602b (Katoh et al., 2002) alignment program independently so the total number of base pairs in this aligned dataset is not equal to the sum of the separate datasets. P.I.C. = Parsimony Informative Characters; C.I. = Consistency Index; R.I. = Retention Index.

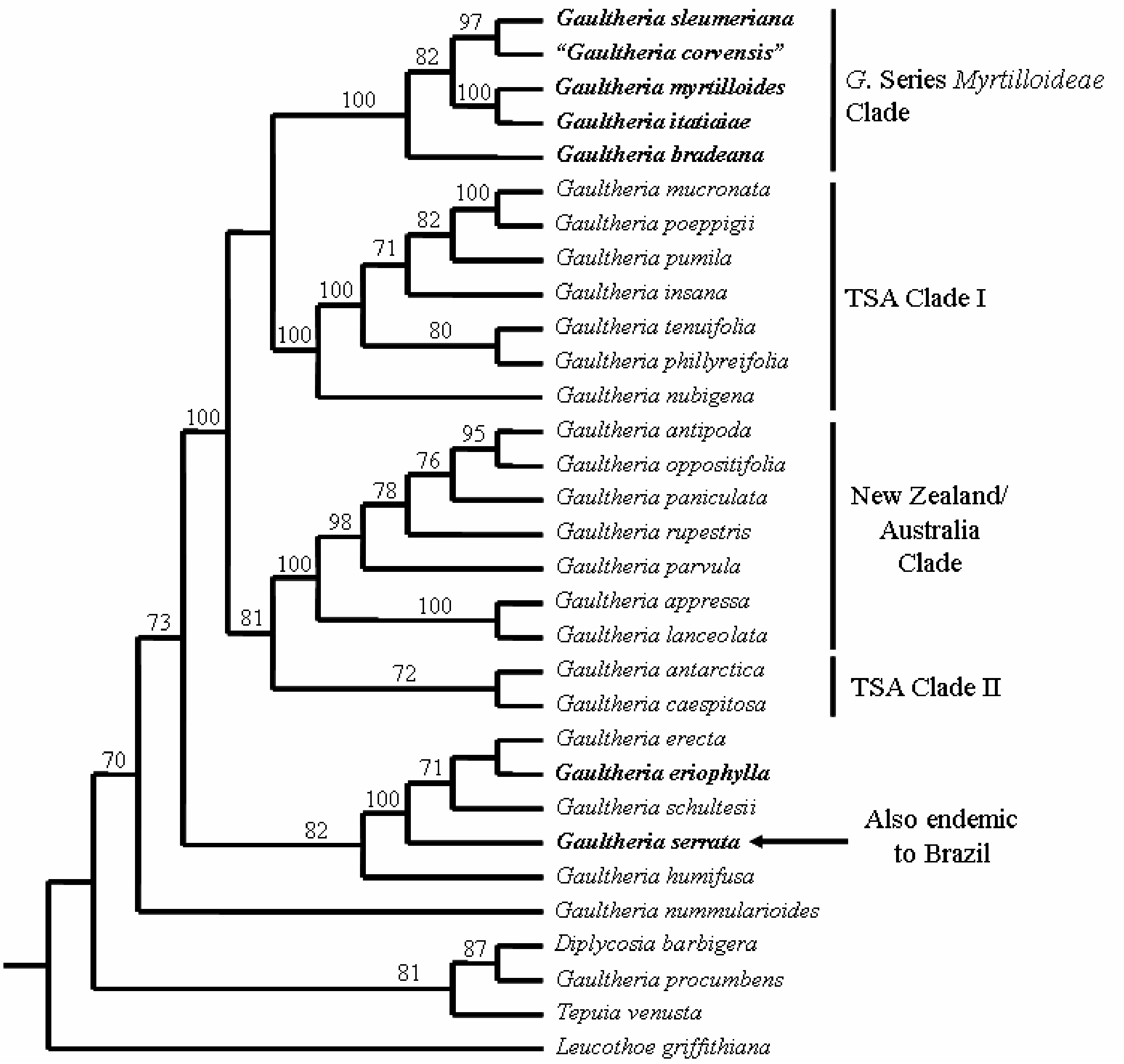
Table 6.2

Gene region	Number of taxa	P.I.C.	Number of base pairs	Percent informative	Number of trees	Length	C.I.	R.I.	Bootstrap analysis
matK	33	51	1521	3.40%	864	85	0.71	0.85	100,000 fast
ndhF	33	85	1539	5.50%	6	158	0.70	0.83	100,000 fast
rpl16	32	26	1021	2.50%	168	40	0.78	0.90	100,000 fast
trnL-F	33	26	885	2.90%	4180	39	0.74	0.88	100,000 fast
trnS-G	33	42	1593	2.60%	146	55	0.78	0.93	100,000 fast
Chloroplast	33	230	6559	3.50%	144	389	0.70	0.86	100,000 fast
ITS	32	38	656	5.80%	105	74	0.60	0.74	100,000 fast
leafy	25	151	1911	7.90%	12	364	0.55	0.55	100,000 fast
waxy	33	28	572	4.90%	15120	46	0.70	0.82	100,000 fast
Nuclear	33	217	3139	6.90%	295	508	0.55	0.58	100,000 fast
Total Data	31	433	9698	4.50%	1	908	0.59	0.71	100,000 fast

Figure 6.1

The single tree from a maximum likelihood analysis of the total combined data set (*matK*, *ndhF*, *rpl16*, *trnL-F*, *trnS-G*, nrITS, *leafy*, *waxy*). Bootstrap values are indicated above the branches. TSA = Temperate South America. Bold taxa represent species collected in Brazil.

Figure 6.1


97
82
100
100
Gaultheria sleumeriana
"Gaultheria corvensis"
Gaultheria myrtilloides
Gaultheria itatiaiae
Gaultheria bradeana
G. Series Myrtilloideae Clade
100
82
71
100
100
80
Gaultheria mucronata
Gaultheria poeppigii
Gaultheria pumila
Gaultheria insana
Gaultheria tenuifolia
Gaultheria phillyreifolia
Gaultheria nubigena
TSA Clade I
100
95
76
78
98
100
100
81
Gaultheria antipoda
Gaultheria oppositifolia
Gaultheria paniculata
Gaultheria rupestris
Gaultheria parvula
Gaultheria appressa
Gaultheria lanceolata
New Zealand/ Australia Clade
72
73
Gaultheria antarctica
Gaultheria caespitosa
TSA Clade II
71
100
82
70
Gaultheria erecta
Gaultheria eriophylla
Gaultheria schultesii
Gaultheria serrata
Gaultheria humifusa
Also endemic to Brazil
Gaultheria nummularioides
87
81
Diplycosia barbigera
Gaultheria procumbens
Tepuia venusta
Leucothoe griffithiana

CHAPTER VII

CONCLUSION

The monophyly of the Gaultherieae had been supported previously with DNA sequence data from *matK* and *rbcL*, but with less than 50% bootstrap support (Kron et al., 2002). Our analyses recover a monophyletic Gaultherieae and wintergreen group with strong support, as found previously with combined *matK*, *atpB-rbcL* spacer, and nrITS sequence data (Powell and Kron, 2001). As in a previous study (Powell and Kron, 2001), *Diplycosia* was found to be monophyletic and the two members of *Tepuia* sampled consistently form a clade in our analyses. The genus *Gaultheria* is paraphyletic and is only monophyletic with the inclusion of both *Diplycosia* and *Tepuia*.

The analysis of calyx evolution indicates that the ancestral condition for the tribe Gaultherieae is likely a dry fruit. The ancestral condition for the wintergreen group, however, is equivocal. Dry calyces evolved at least twice within the wintergreen group Based on our current observations it appears that dry calyces usually co-occur with a berry fruit.

All the sampled temperate South American *Pernettya* members (i.e., *Gaultheria mucronata, G. poeppigii* and *G. pumila*) possess berries. The only other sampled *Pernettya* member, *G. tasmanica*, is sister to the dry-capsule clade and it exhibits a berry with a completely accrescent fleshy calyx (Middleton, 1991). Berries also independently arose in *Diplycosia acuminata* and *Tepuia*.

The calyx and fruit analyses have significant implications for the classification of the wintergreen group. Based on phylogenies that strongly support a monophyletic wintergreen group (i.e., our study; Powell and Kron, 2001), *Diplycosia*, *Gaultheria* and *Tepuia* should be included in *Gaultheria* s.l. The alternative of dividing the group into multiple smaller genera is suboptimal because it may be difficult or impossible to determine morphological synapomorphies for some of these clades, whereas the presence of methyl salicylate is a potential synapomorphy for *Gaultheria* s.l. The infrageneric classification of *Gaultheria* s.l. could use calyx and fruit characteristics to determine sectional divisions within the genus.

A solitary-flowered inflorescence was traditionally thought to be a derived condition within *Gaultheria* (Airy-Shaw, 1940). Although both Airy-Shaw (1940) and Middleton (1991) appear to have been accurate in their assertion that racemose inflorescences are ancestral in *Gaultheria*, the high number of times solitary-flowered inflorescences appear in the evolution of the group suggests that inflorescence type is not an appropriate basis for the higher-level classification of the tribe. Even at the lower levels of classification, the use of inflorescence type may not be useful, e.g., the large section *Brossaea* is highly paraphyletic based on our results.

Biogeographic analyses of the Gaulltherieae tribe indicate that the tribe and the wintergreen group originated in the Nothern Hemisphere (i.e., North America, Japan and China/Himalayas). Dispersal events from the Northern Hemisphere include Venezuela (*Tepuia*), southeast Asia (*Diplycosia* and other *Gaultheria* species) and tropical South America.

Leucothoë s.l. was found to be polyphyletic with members found in two or three clades (Bush et al., in press; Waselkov and Judd 2008). Therefore, *Leucothoë*, as broadly circumscribed, cannot be maintained. *Leucothoë racemosa* and *L. recurva* consistently are sister taxa and are in turn sister to *Chamaedaphne calyculata*. Because *Leucothoë racemosa* and *L. recurva* form a well supported clade that is morphologically very divergent from *Chamaedaphne*, *L. racemosa* and *L. recurva* are best recognized as

Eubotrys (see also Nuttall 1843; Gleason and Cronquist 1991; Stevens et al. 2004) instead of included within an expanded *Chamaedaphne*.

In our study, the evergreen species of *Leucothoë* s.l (*L. axillaris, L. fontanesiana, L. keiskei, L. griffithiana*, and *L. davisiae*) are sister with strong support to the deciduous species from Japan, *L. grayana. Leucothoë grayana* has many unique morphological characters within the genus, including its spring-emerging inflorescences with vegetative leaf-like bracts (versus autumn-emerging like all other *Leucothoë* species), the floral bract basally adnate to the pedicel, bracts of the reproductive shoots large and indistinguishable from leaves proximally, strongly urceolate corolla, awnless anthers, and capsules with thin-walled valves (Waselkov and Judd 2008). This species is also deciduous, like *L. racemosa* and *L. recurva*, and this apomorphic feature likely evolved in parallel in *L. grayana* and the common ancestor of *L. racemosa* and *L. recurva*. Due to these numerous morphological distinctions and the fact that the *L. grayana* + *Leucothoë* s.s. clade is not easily diagnosed, we support the segregation of *L. grayana* as the genus *Eubotryoides* (Hara 1935). *Leucothoe s.s.* will be maintained as a separate genus sister to *Eubotryoides.* Our cladograms, along with an assessment of the pattern of morphological variation, result in our decision to recognize three monophyletic and morphologically diagnostic genera for the group: *Eubotryoides*, *Eubotrys*, and *Leucothoë* s.s.

Despite the diverse morphological variation occurring among the Australasian *Gaultheria* species, they form a monophyletic group, with the Australian members imbedded within a clade of New Zealand *Gaultheria* species. The sister group of the Australasian *Gaultheria* clade comprises taxa sampled from temperate South America.

These results are the same as those from several other studies, including Sanmartin and Ronquist (2004). Our data unequivocally support an initial dispersal of *Gaultheria* to New Zealand from South America. Whether the South American origin is temperate or tropical is, however, ambiguous from our results. Sanmartin and Ronquist (2004) found that in their plant data set, dispersal frequencies from temperate South America to New Zealand was one of the highest observed in the data (5.90; results significant, $P<0.05$). The dispersal frequency from New Zealand to temperate South America was only 1.10 (Sanmartin and Ronquist 2004). These results suggest that dispersal of *Gaultheria* from temperate South America to New Zealand is more likely than from New Zealand to temperate South America.

Our results indicate that *Gaultheria* dispersed from New Zealand to Australia/Tasmania at least three times. Traditionally, dominant west winds were thought to bias dispersal from Australia to New Zealand rather than vice versa (Winkworth *et al*. 2002*b*). However, several studies support dispersal from New Zealand to Australia (von Hagen and Kaderit 2001; Lockhart *et al*. 2001; Wagstaff and Garnock-Jones 2000; Winkworth *et al*. 2002*a*). Sanmartin and Ronquist (2004) found that dispersal frequencies were higher for dispersals from Australia to New Zealand (6.59) but that routes from New Zealand to Australia were still common (3.82).

PCA analyses of 272 herbarium sheets and either the Vegetative Only or Vegetative + Reproductive data sets resulted in the reduction of the fifteen currently defined species of *Gaultheria* in Australia and New Zealand to ten. Four closely related species, *G. crassa, G. colensoi, G. paniculata* and *G. rupestris*, are combined into one species, *G. rupestris*. *Gaultheria tasmanica* and *G. parvula* were determined to be the same species. *Gaultheria depressa* had previously been divided into two varieties in New Zealand and one in Tasmania, but morphological evidence suggests that these varieties are undistinguishable. Finally, *G. nubicola* was a taxon named without reproductive material; the vegetative material available suggests it belongs to the species *G. depressa.*

Data show that the core Brazilian clade of *Gaultheria* (containing five of the six endemic *Gaultheria* species) did not originate from the south-central Andes as previously hypothesized (Luteyn 1991). Instead, these species are most closely related to species from temperate South America and New Zealand/Australia. The endemic Brazilian taxa *Gaultheria serrata* and *Gaultheria eriophylla* var. *eriophylla*, however, are clearly most closely related to species from South America or Mexico. Morphologically, these species are more similar to each other than to those species in the core Brazilian clade. The results clearly indicate that there are at least two introductions of *Gualtheria* into southeast Brazil – one from temperate South America and one from tropical South America.

These cumulative results have generated a much greater understanding of the phylogeny, morphological evolution and world-wide biogeography of the Gaultherieae tribe and the genera included within: *Chamaedaphne*, *Eubotryoides, Eubotrys, Gaultheria s.l.* and *Leucothoe*.

Literature Cited

Airy-Shaw HK 1940 Studies in the Ericales. IV. Classification of the Asiatic species of *Gaultheria*. Kew Bull 1940:304–330.

Gleason, H.A. and A. Cronquist. 1991 Manual of vascular plants of northeastern United States and adjacent Canada. Ed. 2. New York: New York Botanical Garden.

Hara, H 1935 *Eubotryoides*. *Journal of Japanese Botany* 11: 626-627.

Kron KA, WS Judd, F Stevens, DM Crayn, AA Anderberg, PA Gadek, CJ Quinn, JL Luteyn 2002 Phylogenetic classification of Ericaceae: molecular and morphological evidence. Bot Rev 68:335–423.

Lockhart PJ, McLenachan PA, Havell D, Glenny D, Huson D, Jensen U 2001 Phylogeny, dispersal and radiation of New Zealand alpine buttercups: molecular evidence under split decomposition. *Annals of the Missouri Botanical Garden* 88, 458–477.

Luteyn JL 1991 The genus *Gaultheria* in Brazil. *Bol. Mus. Para. Emílio Goeldi, sér. Bot.* 7, 309–333.

Middleton DJ 1991 Infrageneric classification of the genus *Gaultheria* L. Ericaceae. Bot J Linn Soc 106:229–258.

Nuttall, T. 1843 Description and notices of new or rare plants in the natural orders Lobeliaceae, Campanulaceae, Vaccinieae, Ericaceae, collected in a journey over the continent of North America, and during a visit to the Sandwich Islands, and Upper California. *Transactions of the American Philosophical Society* 8: 251-272.

Powell EA, KA Kron 2001 An analysis of the phylogenetic relationships in the wintergreen group (*Diplycosia, Gaultheria, Pernettya, Tepuia*; Ericaceae). Syst Bot 26:808–817.

Sanmartín I, Ronquist F 2004 Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology* 53, 216–243.

Stevens, P.F., J.L. Luteyn, E.G.H. Olilver, T.L. Bell, E.A. Brown, R.K. Crowden, A.S. George, G.J. Jordan, P. Ladd, K. Lemson, C.B. McLean, Y. Menadue, J.S. Pate, H.M. Stace, and C.M. Weiller. 2004 Ericaceae. Pp. 145-194 in *The families and genera of vascular plants.* vol. 6, ed. K Kubitzki. Berlin: Springer-Verlag.

von Hagen KB, Kadereit JW 2001 The phylogeny of *Gentianella* (Gentianaceae) and its colonization of the southern hemisphere as revealed by nuclear and chloroplast DNA sequence variation. *Organisms, Diversity & Evolution* 1, 61–79.

Waselkov, K. and W.S. Judd. 2008 A phylogenetic analysis of *Leucothoë* s.l. (Ericaceae; tribe Gaultherieae) based on phenotypic characters. *Brittonia* 60: 382-397.

Winkworth RC, Grau J, Robertson AW, Lockhart PJ 2002*a* The origins and evolution of the genus *Myosotis* L. (Boraginaceae). *Molecular Phylogenetics and Evolution* 24, 180–193.

Winkworth RC, Wagstaff SJ, Glenny D, Lockhart PJ 2002*b* Plant dispersal N.E.W.S. from New Zealand. *Trends in Ecology & Evolution* 17, 514–520.

Wagstaff SJ, Garnock-Jones PJ 2000 Patterns of diversification in *Chionohebe* and *Parahebe* (Scrophulariaceae) inferred from ITS sequences. *New Zealand Journal of Botany* 38, 389–407.

Catherine M. Bush
Wake Forest University
Department of Biology
Winston-Salem, NC 27109
(336) 403 2261
Email: bushcm3@wfu.edu

EDUCATION

B.S. Biology, 2003, High Point University
M.S. Biology, 2005, Wake Forest University
Advisor: Dr. Kathleen A. Kron
Ph.D. Candidate, Biology, Wake Forest University
Expected graduation: May 2010
Advisor: Dr. Kathleen A. Kron

TEACHING

Laboratory Classes Taught:

Comparative Physiology (2005); Wake Forest University
Cell Biology (2006); Wake Forest University

Guest Lecturer:

Plant Systematics, High Point University (2004)
Plant Systematics, High Point University (2006)
Plant Systematics, High Point University (2008)

Mentoring:

Advised four undergraduate students; High Point University (2006-2009)

RESEARCH

Publications:

Bush CM, Kron KA. 2008. A phylogeny of *Bejaria* (Ericaceae: Ericoideae) based on molecular data. *Journal of Botanical Research Institute of Texas* 2: 1193–1205.

Bush CM, Lu L, Fritsch PW, Li D, Kron KA. 2009. Phylogeny of Gaultherieae (Ericaceae: Vaccinioideae) based on DNA sequence data from *mat*K, *ndh*F and ITS. *International Journal of Plant Sciences* 170: 355-364.

Bush CM, Wagstaff SJ, Fritsch PW, Kron KA. 2009. The Phylogeny, Biogeography and Morphological Evolution of *Gaultheria* (Ericaceae) from Australia and New Zealand. *Australian Systematic Botany* 22: 229-242.

Bush CM, Rollins D, Smith GL. 2009. The Phylogeny of the Southeastern US *Hymenocallis* (Amaryllidaceae) based on ISSR Fingerprinting and Morphological Data. *Castanea* (in press).

Bush CM, Judd WS, Frazier L, Kron KA. 2009. The Phylogeny of *Leucothoë* s.l. (Ericaceae: Vaccinioideae) based on Morphological and Molecular Data (*ndhF*, *matK,* and nrITS). *Systematic Botany* (in press).

Lu L, Fritsch PW, Dong L, Bush CM, Wang H, Li D. 2009. Systematic implications of seed coat diversity in the Gaultherieae (Ericaceae). *Botanical Journal of Linnean Society* (accepted).

Manuscripts in Preparation

Bush CM, Fritsch PW, Cruz BC, Martins AB, Lu L, Kron KA. The phylogeny and morphology of the Brazilian *Gaultheria* (Ericaceae) species.

Lu L, Bush CM, Fritsch PW, Cruz BC, Sanchez A, Kron KA. The historical biogeography of Gaultherieae (Ericaceae).

Paper Presentations

Bush CM, Smith GL. "Morphological Cladistic Analysis of *Hymenocallis*". *North Carolina Academy of Science*, oral presentation (Wilmington, NC 2003).

Bush CM, Kron KA. "The phylogeny of *Bejaria*". *Botanical Society of America*, oral presentation (Snowbird, UT 2004).

Bush CM, Kron KA. "The phylogeny of *Bejaria*". *Wake Forest University departmental seminar* (2004).

Bush CM, Kron KA. "The Phylogeny of the Wintergreen Group". *Botanical Society of America*, oral presentation (Chico, CA 2006).

Bush CM, Fritsch PW, Kron KA. "The Phylogeny of the Wintergreen Group". *Association of Southeast Biologists*, oral presentation (Colombia, SC 2007).

Bush CM, Fritsch PW, Kron KA. "The phylogeny, biogeography and morphological evolution of the Gaultherieae". *Invited seminar* (Sydney, Australia 2007).

Bush CM, Fritsch PW, Kron KA. "The phylogeny, biogeography and morphological evolution of the Gaultherieae". *Invited seminar* (Christchurch, New Zealand 2007).

Bush CM, Wagstaff SJ, Fritsch PW, Kron KA. "The phylogeny of *Gaultheria* species from New Zealand and Australia". *Invited semimar* (Christchurch, New Zealand 2008).

Bush CM, Fritsch PW, Kron KA. "The Morphological Evolution of the Wintergreen Group". *Botanical Society of America*, oral presentation (Vancouver, B.C. 2008).

Bush CM. "Ground Zero: Global Biodiversity and Conservation". *Invited seminar: Sierra Club* (Winston-Salem, NC 2009).

Bush CM, Wagstaff SJ, Fritsch PW, Kron KA. "The phylogeny, biogeography and morphological evolution of the *Gaultheria* species from Australia and New Zealand". *Botanical Society of America*, oral presentation (Snowbird, UT 2009).

Field Research:

Georgia and Florida, U.S.A. *Hymenocallis.* 2003.

Loja and Zamorae, Ecuador. *Bejaria*. 2004.

Capetown, South Africa. *Erica*. 2004.

Tarapota, Peru. Systematic Tree Inventory of Lowland Plants. 2005.

Yunnan Province, China. Biodiversity Inventory of the Gaolgongshan Mountains. 2006.

Sydney, Australia. *Gaultheria*. 2007.

Christchurch, New Zealand. *Gaultheria*. 2007.

Christchurch, New Zealand. *Gaultheria.* 2008.

Florida, U.S.A. *Hymenocallis*. 2008.

Campinas, Brazil. *Gaultheria* and Melastomataceae. 2009.

Bariloche, Argentina. *Gaultheria*. 2009.

Sabah, Malaysia. *Diplycosia* and *Gaultheria*. 2009.

Awards:

Presidential Scholar Award (Full-Tutition)
-- High Point University (1999-2003)

John Rowley Derieux Award
-- North Carolina Academy of Science (2003)

John E. Ward Award for Outstanding Biology Seniors
-- High Point University (2003)

Graduated Summa Cum Laude
-- High Point University (2003)

Grants and Funding:

Vecellio Award
-- Wake Forest University (2004, 2006, 2007, 2008)
Coca Cola Foundations Grant for Studies of Latin American Biodiversity
-- Wake Forest University (2004)
Elton C. Cocke Travel Award

-- Wake Forest University, Biology Department (2004, 2006, 2008, 2009)

Alumni Travel Award
-- Wake Forest University (2008, 2009)

Professional Affiliations:

American Society of Plant Taxonomists (ASPT)

Botanical Society of America (BSA)

Association of Southeastern Biologists (ASB)