Phylogeny of Ericameria, Chrysothamnus and related genera (Asteraceae : Astereae) based on nuclear ribosomal DNA sequence data

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PHYLOGENY OF *ERICAMERIA*, *CHRYSOITHAMNUS* AND RELATED GENERA (ASTERACEAE: ASTEREEAE) BASED ON NUCLEAR RIBOSOMAL DNA SEQUENCE DATA

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

In

The Department of Biological Sciences

by

Roland P. Roberts
B.S.Ed., Southwest Texas State University, 1991
M.S., Southwest Texas State University, 1996
December, 2002
DEDICATION

I dedicate this dissertation to my son Roland H. Roberts, my mother Rosetta Roberts and my niece Colleen Roberts, for being a continued source of mutual love and respect.
ACKNOWLEDGMENTS

This dissertation was developed under the direction of my advisor, Dr. Lowell E. Urbatsch, Director of the Louisiana State University Herbarium and Associate Professor in the Department of Biological Sciences. I thank him for his guidance and advice throughout this project.

I am deeply grateful to the members of my dissertation committee: Dr. Meredith Blackwell, Dr. Michael Hellberg, Dr. Dominique Homberger, Dr. John Larkin, Dr. Frederick Sheldon, and Dr. Robert Strongin for their critical reviews of the manuscript, as well as their encouragement and interest taken in my overall development. I gratefully acknowledge Dr. L. C. Anderson (University of Florida) for his assistance during the initial phase of this project. I also acknowledge the assistance of Dr. Tom Wendt (Plant Resource Center, University of Texas, Austin), and curators of other herbaria for providing research specimens.

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Phylogenetic relationships and classification of *Chrysothamnus*, *Ericameria*, *Xylothamia* and related genera were investigated. The internal transcribed spacer and 3′ external transcribed spacers (ITS and ETS) of the nuclear ribosomal (nr) DNA were analyzed separately and combined employing different optimality criteria. These analyses indicated that the previous classifications and hypotheses of relationships were not monophyletic. *Chrysothamnus*, *Ericameria*, *Xylothamia*, and related genera were placed in separate lineages irrespective of data set and optimality criteria. *Chrysothamnus* species, as traditionally delimited, were resolved in four, not necessarily closely related lineages affiliated with the Solidagininae. Previous sectional classification of *Chrysothamnus* based primarily on morphology was not supported by the present molecular data. *Ericameria* was placed in a clade separate from both *Chrysothamnus* and *Xylothamia*. Associated with, but basal to, the *Ericameria* lineage was a clade composed of *Pentachaeta*, *Rigiopappus*, and *Tracyina*. Prior infrageneric classification of *Ericameria* was in part consistent with the results of this investigation. Species were placed in three, rather than four, lineages within the genus. The three annual genera and *Ericameria* represent a lineage separate from the Solidagininae and Hinterhuberinae. Species of *Xylothamia* were not monophyletic but were placed in at least five separate lineages. Four species were aligned with *Gundlachia*, while the others were strongly supported in a separate clade. Within that clade, however, the other species were usually in distinct, but unresolved lineages. *Xylothamia* and its relatives were resolved in a clade distinct from other Solidagininae and merits recognition of their distinctiveness. Both *Stenotus* and *Tonestus* were
polyphyletic. Type species of both genera were associated with other clades, and the relationship of most of the other species remains unclear. These results suggest a reclassification of these taxa into novel, distinct genera. In general, the results of this study were incongruent with relationships inferred from morphology.
CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

The Asteraceae (Sunflower family) is one of the largest families of flowering plants with approximately 23,000 species and has a worldwide distribution (Bremer, 1994). Asteraceae macrofossils are extremely rare, but palynological data are relatively abundant (Graham, 1996). Despite the paucity of the fossil record, the geographic origin of the family has been hypothesized to be located in South America, specifically the northern Andes (Raven and Axelrod, 1974; Turner, 1977). Bremer (1994) agreed with South America, excluding the Amazon basin, being the ancestral center of Asteraceae, based on his estimates of ancestral areas of a taxon from the topology of area cladograms (Bremer 1993).

Although there is some consensus with regards to the geographic origin of Asteraceae, there is little agreement with respect to its age (Graham, 1996), a problem attributed to the paucity of macrofossils. Despite this, there have been numerous attempts at estimating the age of the family. Most estimates rely on three sources of information: the distribution of families believed to be allied to Asteraceae, tectonic histories, and the available fossil record, particularly pollen. Turner (1977) argued a Cretaceous (approximately 100 MYA) age of the family. This estimate allows enough time for continental drift to promote the distribution of Asteraceae around the world. In contrast, Raven and Axelrod (1974) and Muller (1981) proposed an Oligocene to mid-Oligocene age (approximately 35 MYA). More recently, Bremer (1994) proposed an early Tertiary split of Asteraceae from its sister group, the Calyceraceae, an estimate that is inclusive of the latter. In a discussion of the origin and age of the Asteraceae, Böhm and Stuessy
(2001) hypothesized an origin of the Asteraceae in southern South America, and divergence of the Asteraceae from the Calyceraceae by the late Eocene or early Oligocene (ca. 38 MYA).

According to Bremer (1994), the Asteraceae comprises the subfamilies Barnadesioideae, Cichorioideae and Asteroideae. Bremer (1994) argued that Barnadesioideae and Asteroideae are monophyletic, whereas Cichorioideae is paraphyletic and contains the sister group to the Asteroideae. He noted, however, that the data, at that time, did not justify the break-up of the Cichorioideae into monophyletic subgroups, and that a combination of the Cichorioideae with the Asteroideae would result in a loss of systematic information (Bremer, 1994). Within the Asteroideae, Astereae appears to be well circumscribed and monophyletic (Bremer, 1994). Worldwide, the tribe Astereae includes approximately 3000 species in nearly 200 genera distributed among 14 subtribes (Nesom, 1994, 2000). North and South America are its centers of greatest diversity, although Africa and Australia also contain significant numbers of taxa (Nesom, 2000). In his recent account of mainly North and Central American Astereae, Nesom (2000) provides generic synonymy, description, useful historical taxonomic highlights, and statements of relationship for each of the 91 genera recognized in this region. The subtribal synopsis (Nesom, 2000) updates the worldwide treatment of Nesom (1994). The taxa investigated in this dissertation study are among an enormous assemblage of Astereae representatives in the southwestern United States.

**SYSTEMATICS OF *CHRYSOTHAMNUS* NUTT**

This genus is endemic to North America and occurs primarily in the western United States. Its range also extends short distances into Mexico and Canada (Nesom, 2000).
Species of *Chrysothamnus* vary widely in their distribution pattern. Some, such as *C. nauseosus* (Pallas ex Pursh) Britton [*Ericameria nauseosa* (specific author citations are given in Table 1.1)] and *C. viscidiflorus* are widespread and, along with *Artemisia* L., often dominate the landscape in vast shrubland communities of the intermountain west (McArthur and Welch, 1986). These shrubland communities, often referred to as sagebrush ecosystems, are estimated to cover 38 million ha., making it the largest range ecosystem in the United States (Whisenant, 1986). Species of *Chrsothamnus* tend to grow in openings in the sagebrush community or become more abundant after fire has destroyed *Artemisia* (Whisenant, 1986). Other species of *Chrysothamnus* are more restricted in distribution. For example, *Chrysothamnus eremobius* is restricted to southwestern Nevada, *C. gramineus* to southern Nevada and adjacent California, and *C. molestus* to Arizona. All three species are known from but a few populations (Anderson, 1986).

The taxonomy of *Chrysothamnus* has varied greatly throughout its history. The genus was established by Nuttall (1841) to include taxa previously placed in *Chrysocoma* (Pursh, 1814), *Crinitaria* (Hooker, 1834), and *Bigelowia* [*Bigelovia*] (DeCandolle, 1836). Subsequently, Torrey and Gray (1842) treated the species circumscribed by Nuttall as members of the genus *Linosyris* Cass. Bentham (1873) reasserted the name *Chrysothamnus* and widened its definition to include DeCandolle’s *Bigelovia* ignoring the priority of *Bigelovia*. Gray (1873) essentially adopted Bentham’s (1873) circumscription of *Chrysothamnus*, except that the priority of *Bigelovia* was asserted. Eventually, Greene (1895b) restored *Chrysothamnus* to generic rank and defended its segregation by Nuttall (1841) from the herbaceous species of eastern North America, on
Table 1.1. Taxa sampled in this study and their sources, voucher and GenBank data. Relevant literature citations for published sequences are at the end of the table.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Source localities and voucher data</th>
<th>ITS Genbank</th>
<th>ETS Genbank</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acamptopappus shockleyi</em> A. Gray</td>
<td>Nevada: Clark Co., Lane 3072 (RM)</td>
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<td>AY169723</td>
</tr>
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<td><em>Acamptopappus sphaerocephalus</em> (Harv. &amp; A. Gray in A. Gray) A. Gray</td>
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<td>AY169724</td>
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<td><em>Amellus strigosus</em> (Thunb.) Less.</td>
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<td><em>Amphiachyris dracunculoides</em> Nutt.</td>
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<td>AF477626 clone 1</td>
<td>AF477690 clone 1</td>
</tr>
<tr>
<td><em>Amphipappus fremontii</em> Torr. &amp; A. Gray var. fremontii</td>
<td>California: Inyo Co., Kurzius 874 (UNLV)</td>
<td>AF477627 clone 2</td>
<td>AF477691 clone 2</td>
</tr>
<tr>
<td><em>Aphanostephus ramosissimus</em> DC.</td>
<td>Mexico: Guanajuato, Ventura 7924 (MO)</td>
<td>AF046990 ²</td>
<td>–</td>
</tr>
<tr>
<td><em>Aster amellus</em> L.</td>
<td>Russia: N. Caucasus, Skvortsov s. n. (MO)</td>
<td>AF046961 ²</td>
<td>–</td>
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<tr>
<td><em>Astranthium integrifolium</em> (Michx.) Nutt.</td>
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<td>AF046958 ²</td>
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<td><em>Batopilasia byei</em> (S.D. Sundb. &amp; G.L. Nesom) G.L. Nesom &amp; Noyes</td>
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<td>AF477693 clone 1</td>
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<td>Chrysothamnus humilis Greene</td>
<td>Oregon: Grant Co., Urbatsch 1368 (LSU)</td>
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<tr>
<td><em>Chrysothamnus linifolius</em> Greene</td>
<td>Utah: Uinta Co., Urbatsch 7068 (LSU)</td>
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<td><em>Chrysothamnus molestus</em> (S. F. Blake) L.C. Anderson</td>
<td>Arizona: Coconino Co., Anderson 3146 (CAS)</td>
<td>AY170941 AY169738</td>
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<td><em>Chrysothamnus pulchellus</em> (A. Gray) Greene</td>
<td>New Mexico: Lincoln Co., Urbatsch 7977 (LSU)</td>
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<td><em>Chrysothamnus spathulatus</em> L.C. Anderson</td>
<td>New Mexico: Otero Co., Urbatsch 7983 (LSU)</td>
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<td><em>Chrysothamnus vaseyi</em> (A. Gray) Greene</td>
<td>Colorado: Ouray Co., Rollins 1987 (DS)</td>
<td>AY170944 AY169741</td>
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<td><em>Chrysothamnus viscidiflorus</em> (Hook.) Nutt. ssp. <em>viscidiflorus</em></td>
<td>California: Lassen Co., Urbatsch 7712 (LSU)</td>
<td>AY170945 AY169742</td>
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<tr>
<td><em>Columbiadoria hallii</em> (A. Gray) G. Nesom</td>
<td>Oregon: Wasco Co., Urbatsch 7692 (LSU)</td>
<td>AY170948 AY169745</td>
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<td><em>Conyza bonariensis</em> (L.) Cronquist</td>
<td>USA: Alabama, Noyes 1182 (IND)</td>
<td>AF118513 AF046949</td>
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<td><em>Crinitaria linosyris</em> (L.) Less.</td>
<td>Russia: Saratov, Skvortsov s. n. (MO)</td>
<td>AF046949 AF251576 AF251634</td>
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<td><em>Croptilon divaricatum</em> (Nutt.) Raf.</td>
<td>Texas: Nesom 7470 (UC)</td>
<td>AF477625 AF477754</td>
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<td><em>Diplostepheium rupestre</em> (H. B. K.) Wedd.</td>
<td>Ecuador: Napo, Holm-Nielsen 28233 (MO)</td>
<td>AF046962 AF251576</td>
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<td><em>Doellingeria umbellata</em> Nees</td>
<td>Michigan: Chippewa Co., Schmidt &amp; Merello 1060 (TEX)</td>
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<td><em>Eastwoodia elegans</em> Brandegee</td>
<td>California: Kern Co., Sanders 20427 (CAS)</td>
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<td><em>Ericameria albida</em> (M. E. Jones ex A. Gray) L. C. Anderson</td>
<td>Nevada: Nye Co., Urbatsch 1459 (LSU)</td>
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<td><em>Ericameria arborescens</em> (A. Gray) Greene</td>
<td>California: San Luis Obispo Co., Keil K14219 (TEX)</td>
<td>AY170951 AY169748</td>
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<td><em>Ericameria bloomeri</em> (A. Gray) J. F. Macbr.</td>
<td>California: Alpine Co., AY171006 Urbatsch &amp; Karaman 7719 (LSU)</td>
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<td><em>Ericameria brachylepis</em> (A. Gray) H. M. Hall</td>
<td>Mexico: Baja California, Burgess 6106 (TEX)</td>
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<td><em>Ericameria cervina</em> (S. Watson) Rydb. 1</td>
<td>Arizona: Coconino Co., Brian 98-291 (ASC)</td>
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<td><em>Ericameria cervina</em> (S. Watson) Rydb. 2</td>
<td>Arizona: Mohave Co., Gierisch 4486 (ASC)</td>
<td>AY171009 AY170977</td>
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<td><em>Ericameria compacta</em> (H. M. Hall) G. L. Nesom</td>
<td>Nevada: Clark Co., Urbatsch &amp; Roberts 7940 (LSU)</td>
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<td><em>Ericameria cooperi</em> H.M. Hall</td>
<td>California: San Bernardino Co., Helmkamp s.n. (TEX)</td>
<td>AF477640 AF477704</td>
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<td><em>Ericameria crispa</em> (L.C. Anderson) G. L. Nesom</td>
<td>Utah: Washington Co., Baird &amp; Warick 3196 (BRY)</td>
<td>AY171011 AY170979</td>
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<td><em>Ericameria cuneata</em> (A. Gray) McClatchie</td>
<td>California: Inyo Co. Urbatsch &amp; Roberts 7957 (LSU)</td>
<td>AF477641 AF477705</td>
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<td><em>Ericameria discoidea var. discoidea</em> (Nutt.) G. L. Nesom</td>
<td>Utah: Utah Co., Thompson 9067 (TEX)</td>
<td>AY171012 AY170980</td>
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<td><em>Ericameria discoidea var. linearis</em> (Ryd.) G. L. Nesom</td>
<td>Idaho: Bear Lake Co., Winward s. n. (BRY)</td>
<td>AY171013 AY170981</td>
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<td><em>Ericameria ericoides</em> (Less.) Jepson</td>
<td>California: Monterey Co. Sunberg 2646 (TEX)</td>
<td>AF477642 AF477706</td>
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<td><em>Ericameria fasciculata</em> (Eastw.) J.F. Macbr.</td>
<td>California: Monterey Co., Griffin 3968 (LSU)</td>
<td>AY171014 AY170982</td>
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<td><em>Ericameria gilmanii</em> (S. F. Blake) G. L. Nesom</td>
<td>California: Inyo Co., Urbatsch &amp; Roberts 7948 (LSU)</td>
<td>AY171015 AY170983</td>
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<td><em>Ericameria greenei</em> (A. Gray) G. L. Nesom</td>
<td>California: Trinity Co., AY171016 Urbatsch &amp; Karaman 7706 (LSU)</td>
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<td><em>Ericameria juarezensis</em> (Moran) Urbatsch</td>
<td>Mexico: Baja California, Moran 22986 (LSU)</td>
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<tr>
<td><em>Ericameria laricifolia</em> (A. Gray) Shinners</td>
<td>Texas: El Paso Co., Carr 10230 (TEX)</td>
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<td><em>Ericameria lignumviridis</em> (S. L. Welsh) G. L. Nesom</td>
<td>Utah: Sevier Co., Greenwood 5566 (BRY)</td>
<td>AY171019 AY170987</td>
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<td><em>Ericameria linearifolia</em> (DC.) Urbatsch &amp; Wussow</td>
<td>California: Inyo Co., Schramm 743 (UNLV)</td>
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<td><em>Ericameria martirensis</em> Wiggins</td>
<td>Mexico: Baja California, Thorne 61445 (TEX)</td>
<td>AY171021 AY170989</td>
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<td><em>Ericameria nana</em> Nutt.</td>
<td>Utah: Esmeralda Co., Urbatsch &amp; Roberts 7946 (LSU)</td>
<td>AY171022 AY170990</td>
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<td><em>Ericameria nauseosa</em> (Pall. ex Pursh) G. L. Nesom &amp; G. I. Baird</td>
<td>California: Inyo Co., Morefield 4336 (TEX)</td>
<td>AY170952 AY169749</td>
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<td><em>Ericameria obovata</em> (Rydb.) G. L. Nesom</td>
<td>Nevada: Elko Co., Urbatsch &amp; Karaman 7669 (LSU)</td>
<td>AY171024 AY170992</td>
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<td><em>Ericameria palmeri</em> (A. Gray) H. M. Hall</td>
<td>California: San Bernardino Co., Sanders 14215 (TEX)</td>
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<td><em>Ericameria paniculata</em> (A. Gray) Rydb.</td>
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<td><em>Ericameria parishii</em> (Greene) H. M. Hall</td>
<td>California: San Diego Co., Urbatsch 7082 (LSU)</td>
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<td><em>Ericameria parryi</em> (A. Gray) G. L. Nesom &amp; G. I. Baird</td>
<td>California: Kern Co., Helmikamp SN (TEX)</td>
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<td><em>Ericameria pinifolia</em> (A. Gray) H. M. Hall</td>
<td>California: San Diego Co., Urbatsch 7084 (LSU)</td>
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<td><em>Ericameria resinosa</em> Nutt.</td>
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**Ericameria suffruticosa** (Nutt.) G. L. Nesom  
Nevada: Humboldt Co., Tiehm 9999  
(TEX)  
AY171032  
AY171000

**Ericameria teretifolia** (Durand & Hilg.) Jeps.  
California: Inyo Co., Morefield 3130 (TEX)  
AY170954  
AY169751

**Ericameria watsonii** (A. Gray) G. L. Nesom  
Nevada: White Pine Co., Tiehm 11446 (CAS)  
AY171034  
AY171002

**Ericameria zionis** (L.C. Anderson) G. L. Nesom  
Utah: Garfield Co., Urbatsch & Roberts 7922 (LSU)  
AY171035  
AY171003

**Erigeron bellidiastrum** Nutt.  
Texas: Roberts Co. Karaman 8 (LSU)  
AF477644  
AF477708

**Erigeron procumbens** (Houstoun ex P. Miller) G.L. Nesom  
Louisiana: Jefferson Parish. Westphal 2121 (LSU)  
AF477645 clone 1  
AF477646 clone 2  
AF477709 clone 1  
AF477710 clone 2

**Erigeron subtrinervis** Rydb. ex Porter & Britton  
Colorado: Archuleta Co. Karaman 29 (LSU)  
AF477647  
AF477711

**Eurybia hemispherica** (Alexander) G. L. Nesom  
USA: Urbatsch s.n. (LSU)  
Unpublished  
Unpublished

**Eurybia wasatchensis** (M. E. Jones) G. L. Nesom  
Utah: Iron Co., Urbatsch & Karaman 7645 (LSU)  
Unpublished  
Unpublished

**Euthamia leptocephala** (Torr. & A. Gray) Greene  
Louisiana: Acadia Parish. Pellerin s.n. (LSU)  
AF477648  
AF477712

**Euthamia leptocephala** (Torr. & A. Gray) Greene  
Louisiana: West Feliciana Parish. Urbatsch 7989 (LSU)  
AF477649  
AF477713

**Euthamia occidentalis** Nutt.  
Mississippi: Wilkinson Co. Urbatsch 7990 (LSU)  
AF477650  
AF477714

**Euthamia tenuifolia** (Pursh) Nutt.  
Florida: Wakulla Co. Urbatsch 7585 (LSU)  
AF477652  
AF477716

**Euthamia tenuifolia** (Pursh) Nutt.  
Louisiana: St. Tammany Parish. Ferguson 246 (LSU)  
AF477653  
AF477717

**Felicia aethiopica** (Lees.) Grau  
South Africa: Cape, Rourke 1918 (MO)  
AF046941  
–

**Geisssolepis suaedaeolia** B. L. Robinson  
Mexico: San Luis Potosi, Nesom 6634 (MO)  
AF046995  
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**Grangea maderaspatana** (L.) Poir.  
Thailand: Chiang Mai, Maxwell 90-218 (MO)  
AF046951  
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Grenella ramulosa Greene  
Mexico: Baja California, Powell & Turner 2226 (LSU)  
Unpublished  
Unpublished

Grindelia lanceolata Nutt.  
Texas: Travis Co., Morgan 2114 (WWB)  
U97609  
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Gundlachia corymbosa (Urb.) Britton ex Bold  
Dominican Republic: de Montecristi. Veloz 2609 (LSU)  
AY173397 clone 1  
AY173398 clone 2

Gundlachia corymbosa (Urb.) Britton ex Bold.  
West Indies: Caicos Islands. Pine Cay. Correll 43104 (LL)  
AF477654  
AF477718

Gundlachia corymbosa (Urb.) Britton ex Bold.  
Puerto Rico: Quebradillas. Axelrod 11957 (LSU)  
AF477655 clone 1  
AF477656 clone 2

Gutierrezia sarothrae (Pursh) Britton & Rusby  
Colorado: Mesa Co. Urbatsch & Roberts 7896 (LSU)  
AF477657  
AF477721

Gutierrezia texana (DC.) Torr. & Gray  
Texas: Eastland Co. Urbatsch & Roberts 7826 (LSU)  
AF477658  
AF477722

Gymnosperma glutinosum Less.  
Texas: Frio Co. Urbatsch 2772 (LSU)  
AF477765  
AF477723

Haploppappus foliosus DC.  
CHILE: Rundel, s.n. UCBG 80.0298  
AF251577  
AF251635

Haploppappus glutinosus Cass.  
CHILE: Spare & Constance 17927 (UC)  
AF251578  
AF251636

Haploppappus marginalis Phil.  
CHILE: DeVore 1326 (UC)  
AF251580  
AF251638

Haploppappus paucidentatus Phil.  
CHILE: DeVore 1261 (UC)  
AF251581  
AF251639

Hazardia brickelliioides  
Nevada: Nye Co.: Bostick 5216 (DS)  
Unpublished  
Unpublished

Hazardia detonsa Greene  
California: Santa Cruz Island. UCBG 95.0527  
AF251582  
AF251640

Hazardia squarrosa Greene  
California: Los Angeles Co., Ross 5908 (UC)  
AF251583  
AF251641

Hesperodoria salicina (S. F. Blake) G. L. Nesom  
Arizona: Coconino Co., Scott 880 (ASC)  
AY170955  
AY169752

Hesperodoria scopulorum (M. E. Jones) Greene  
Utah: Washington Co., AY170956  
Shultz 5382 (CAS)  
AY169753

Heterotheca villosa (Pursh) Shinners  
USA: Colorado, Stein 1823 (MO)  
AF046994  
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<td><em>Ionactis lineatifolia</em> (L.) Greene</td>
<td>Louisiana: Rapides Parish. Bruser 357 (LSU)</td>
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<td>AF477724</td>
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<td><em>Isocoma acranenia</em> Greene</td>
<td>California: Riverside Co. Thorne 55404 (UC)</td>
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<td>AF251630</td>
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<td><em>Isocoma menziesii</em> (Hook. &amp; Arn.) G.L. Nesom</td>
<td>California: Los Angeles Co. Bartholomew 535 UCBG 78.0157</td>
<td>AF251571</td>
<td>AF251629</td>
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<td><em>Kalimeris integrifolia</em> Turcz. Ex DC.</td>
<td>China: Jiangsu, Wei 6003a (MO)</td>
<td>AF046960</td>
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<td><em>Kippistia suaeofolia</em> F.Muell.</td>
<td>Australia: New South Wales, Pickard 3657(NSW)</td>
<td>AF247071</td>
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<td><em>Lagenifera panamensis</em> S. F. Blake</td>
<td>Panama: Chiriqui, Schmalzel 1731 (MO)</td>
<td>AF046965</td>
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<td><em>Lessingia glandulifera</em> A.Gray</td>
<td>California: San Luis Obispo Co. Markos 169 (JEPS)</td>
<td>AF251602</td>
<td>AF251660</td>
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<td><em>Lessingia virgata</em> A.Gray</td>
<td>California: Tehama Co. Markos 152 (JEPS)</td>
<td>AF251624</td>
<td>AF251682</td>
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<td><em>Machaeranthera parviflora</em> A.Gray</td>
<td>Texas: Turner &amp; Powell 6094 (UC)</td>
<td>AF251568</td>
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<td><em>Machaeranthera tanacetifolia</em> Nees</td>
<td>New Mexico: Sanders 3065 (UC)</td>
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<td>AF251625</td>
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<td><em>Machaeranthera tanacetifolia</em> Nees</td>
<td>New Mexico: Colfax Co. Karaman 18 (LSU)</td>
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<td><em>Minuria integerrima</em> (DC.) Benth.</td>
<td>Australia: Queensland, Lowrey 1754 (UNSW)</td>
<td>AF247074</td>
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<td><em>Monoptilon bellidoides</em> (A. Gray) H. M. Hall</td>
<td>USA: Arizona, Yatskievych 93-06 (MO)</td>
<td>AF046981</td>
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<td><em>Olearia pannosa</em> Hook.</td>
<td>Australia: South, 24061 (UNSW)</td>
<td>AF247065</td>
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<td><em>Oligoneuron nitidum</em> (Torr. &amp; A. Gray) Small</td>
<td>Louisiana: Lasalle Parish, Urbatsch 5735 (LSU)</td>
<td>AY170957</td>
<td>AY169754</td>
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<td><em>Oligoneuron nitidum</em> (Torr. &amp; A. Gray) Small</td>
<td>Louisiana: Natchitoches Parish. Urbatsch 7581 (LSU)</td>
<td>AF477662</td>
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<td><em>Oligoneuron rigidum</em> (L.) Small</td>
<td>Louisiana: Winn Parish, Urbatsch 5219 (LSU)</td>
<td>AF477663</td>
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<td><em>Oreochrysum parryi</em> (A. Gray) Rydb.</td>
<td>Colorado: Lake Co., Urbatsch 7887 (LSU)</td>
<td>AY170958</td>
<td>AY169755</td>
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<td><em>Oreostemma alpigenum</em> (Torr. &amp; A.Gray) Greene</td>
<td>USA: Oregon, Merello 819 (MO)</td>
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<td><em>Oritrophium hircioides</em> (Wedd.) Cuatr.</td>
<td>Bolivia: La Paz, Solomon 16570 (MO)</td>
<td>AF046946</td>
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<td><em>Pentachaeta exilis</em> (A. Gray) A. Gray</td>
<td>California: Monterey Co., Keil 17085 (TEX)</td>
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<td><em>Perileura bicolor</em> (N.T.Burb.) G.L.Nesom</td>
<td>Australia: Queensland, Lowrey 1765 (UNSW)</td>
<td>AF247078</td>
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<td><em>Podocoma notobellidiastrum</em> (Griseb.) G.L.Nesom</td>
<td>Paraguay: Caazapa, Zardini 3009 (MO)</td>
<td>AF046963</td>
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<td><em>Prionopsis ciliata</em> Nutt.</td>
<td>Texas: Sutton Co., Morgan 2084 (TEX)</td>
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<td><em>Petradoria pumila</em> (Nutt.) Greene</td>
<td>Colorado: Mesa Co., Urbatsch 7889 (LSU)</td>
<td>AY170959</td>
<td>AY169756</td>
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<td><em>Psilactis tenuis</em> S.Watson</td>
<td>Texas: Jeff Davis Co., Morgan 2196 (WWB)</td>
<td>U97643</td>
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<td><em>Pteronia incana</em> (Burm.) DC.</td>
<td>South Africa: Cape, Joffe 850 (MO)</td>
<td>AF046947</td>
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<td><em>Pyrrocoma apargioides</em> (A. Gray) Greene</td>
<td>California: Plumas Co. Schoolcraft 2072 (UC)</td>
<td>AF251573</td>
<td>AF251631</td>
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<td><em>Pyrrocoma lanceolata</em> (Hook.) Greene</td>
<td>Utah: Neese 17626 (UC)</td>
<td>AF251574</td>
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<td><em>Rayjacksonia phyllocephala</em> (DC.) R.L.Hartman &amp; M.A.Lane</td>
<td>Texas: Chambers Co., Morgan 2032 (TEX)</td>
<td>U97645</td>
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<td><em>Rigiopappus leptocladus</em> A. Gray</td>
<td>California: Modoc Co., 6575 Bartholomew (TEX)</td>
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<td>AY171005</td>
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<td><em>Sericocarpus tortifolius</em> Nees</td>
<td>Florida: Wakulla Co. Urbatsch 7599 (LSU)</td>
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<td><em>Solidago canadensis</em> L.</td>
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which Bigelowia was based. From that time on Chrysothamnus was recognized as a genus distinct from its former congers and relatives.

Hall and Clements’ (1923) monograph of Chrysothamnus provided the most inclusive classification of the genus at that time and was followed for several decades thereafter. Their treatment provided a clearer delimitation of the genus based on morphological features of the capitulum. They also proposed an infrageneric classification, in which species were grouped as primitive or derived. Specific relationships were also extensively discussed. They considered their infrageneric classification as being more natural than preceding treatments because of its reliance on a more extensive assortment of morphological characters (Hall and Clements, 1923).

Anderson (1986) presented an infrageneric classification, also based on morphology, that was essentially similar to that of Hall and Clements (1923).

With regards to intergeneric relationships, Hall and Clements (1923) noted that the characteristics used to define Chrysothamnus were not unique to this genus. Features of the capitulum that are used to distinguish Chrysothamnus from species of Haplopappus Cass. are also displayed in some taxa of the latter genus. These similarities were not considered sufficient to merit congeneric treatment but were alluded to as probable indicators of convergent evolution (Hall and Clements, 1923). Despite this, Chrysothamnus was depicted on Hall’s (1928) phylogenetic diagram as originating from within, or close to, Haplopappus, section Ericameria. In the same publication, Hall (1928) alternatively suggested that the Chrysothamnus – Haplopappus connection might be through section Macronema.
Hall and Clements’ (1923) and Hall’s (1928) work stimulated investigations designed to explore the relationships between *Chrysothamnus* and *Haplopappus* for several decades thereafter. The resulting accumulation of cytological, anatomical, chemical and macromolecular data for *Chrysothamnus* and other taxa that are thought to be related (Anderson, 1963, 1964, 1970; Anderson and Fisher, 1970; Anderson and Weberg, 1974; Anderson et al., 1974; Anderson and Creech, 1975; Urbatsch et al., 1975; Suh, 1989; Morgan, 1990; Nesom, 1994; Lane et al., 1996) often provided conflicting views about the composition and boundaries of these taxa. Nesom and Baird (1995), like Hall and Clements (1923), attributed this confusion to apparent convergent evolution. Furthermore, Nesom and Baird (1995) suggested that morphological data, in particular, might not be good indicators of distance or affinity among these taxa because of convergent evolution.

Within the last decade, investigators set a course that dramatically altered the paradigm established by Hall and Clements (1923). Nesom and Baird (1993), using morphological criteria and certain cpDNA data accumulated by Suh (1989) and Morgan (1990), transferred four species, which had long been regarded as *Chrysothamnus*, to *Ericameria*. *Chrysothamnus, sensu* Nesom and Baird (1993), was left with 12 species. Subsequently, Anderson (1995) treated all species remaining in *Chrysothamnus* as *Ericameria*. His justification for uniting all taxa within a single genus was the occurrence of hybridization between *C. albidus* Greene, a core *Chrysothamnus sensu* Nesom and Baird (1993), and *E. nauseosa*, a recent transfer from *Chrysothamnus* by Nesom and Baird (1993), as well as the high degree of morphological similarity between the two sets of taxa (Anderson and Reveal, 1966). Contrary to Anderson’s (1995)
hypothesis, DNA sequence data for representative Astereae, including one species each from traditional *Chrysothamnus* and *Ericameria* indicated that these two are not congeneric and probably are not closely related (Noyes and Rieseberg, 1999), thus supporting Hall and Clements’ (1928) and Nesom and Baird’s (1995) suggestions that the morphological similarities are a case of convergence between the two.

Previous investigations using morphological and chloroplast DNA restriction data suggested that, in addition to *Ericameria*, several other taxa are possibly related to *Chrysothamnus*. *Xylothamia* G.L.Nesom, Y.B.Suh, D.R.Morgan & B.B.Simpson, a recent segregate from *Ericameria*, was shown by Lane et al. (1996) to be related to *Chrysothamnus* and *Ericameria* based on cpDNA restriction data. In contrast, Nesom (1993) indicated that *Xylothamia* was allied to *Chrysoma* Nutt., *Gundlachia* A. Gray, *Bigelowia* DC. and *Euthamia* (Nutt.) Cass. and a few other genera. Nesom (1993) also depicted *Chrysothamnus* as a sister group to *Stenotus* Nutt. However, he indicated that the relationship of the *Chrysothamnus*- *Stenotus* lineage to other genera in the subtribe Solidagininae was uncertain. *Petradoria* Greene has also been discussed as a close relative of *Chrysothamnus* (Hall, 1916; Anderson, 1963, 1986). *Hesperodoria* Greene, a genus of two species, and the monospecific *Vanclevea* Greene may be related to *Chrysothamnus* through *Petradoria* (Anderson and Weberg, 1974; Nesom, 1991, 2000). Nesom (2000) also noted that *Tonestus microcephalus* (Cronquist) G. L. Nesom & D. R. Morgan is possibly a *Chrysothamnus* species as suggested by the cpDNA investigations of Lane et al. (1996). *Tonestus* A. Nelson, although reconstituted as a distinct genus of nine species, is regarded as being heterogeneous and possibly polyphyletic by its proponents (Nesom and Morgan, 1990; Nesom, 2000). Also, Lane et al. (1996) and

This investigation (see Chapter 2) seeks to test the following hypotheses using sequence data of the nuclear ribosomal DNA (nrDNA) internal transcribed spacer region (ITS 1 and ITS 2, including the 5.8s) and the 3’ portion of the external transcribed spacer (3’ ETS): (1) whether *Chrysothamnus*, sensu lato, is monophyletic; (2) whether the molecular data support the sectional classification of Anderson (1986); (3) the relationship of *Chrysothamnus* to other North American genera of the Astereae.

**SYSTEMATICS OF ERICAMERIA NUTT**

Nuttall (1841) proposed the genus *Ericameria* and accommodated three species within it, noting that they cannot be, in any respect, congeners of *Haplopappus ciliatus* DC., “a genuine Chilean species.” Following its circumscription, several researchers followed Nuttall (1841) and accorded generic rank to *Ericameria* (Torrey and Gray, 1842; Hall, 1907; Wiggins, 1933). However, Gray, (1865) and Hall (1928) relegated it to sectional status in *Haplopappus*. Some species of *Ericameria* have also been considered congeneric with *Bigelowia* (Gray, 1873) and *Chrysoma* (Greene, 1895a).

Within the past 10 years, two different classifications have been proposed for *Ericameria* Nutt. The first was initiated with the circumscription of *Xylothamia* G. L. Nesom, Y. B. Suh, D. R. Morgan & B. B. Simpson (Nesom et al., 1990), followed by the
transfer of *Haplopappus* sections *Asiris* and *Macronema* to *Ericameria* and a redefinition of the section Stenotopsis (Nesom, 1990). It culminated in the transfer of four species from *Chrysothamnus* Nutt. to *Ericameria* (Nesom and Baird, 1993). *Xylothamia* was established to accommodate seven species of *Ericameria* from northern Mexico and southern Texas. Nesom (1994, 2000) did not regard *Ericameria* as closely related to *Chrysothamnus*, having placed them in different subtribes, the Hinterhuberinae and Solidagininae, respectively. In contrast, Bremer (1994), in his cladistic analysis of Astereae using mainly morphological features placed *Ericameria* in the subtribe Solidagininae as a sister group of *Chrysothamnus*.

The second classification was the result of Anderson’s (1995) disagreement with Nesom and Baird’s (1993) transfer of the four species of *Chrysothamnus* to *Ericameria*. Anderson’s (1995) argument was based primarily on the occurrence of hybridization between *C. albidus* and *E. nauseosa* (Anderson, 1973), the former being a core *Chrysothamnus* sensu Nesom & Baird (1993) and the latter being a recent transfer from *Chrysothamnus* to *Ericameria*. As a consequence, Anderson (1995) incorporated all remaining species of *Chrysothamnus* in *Ericameria*, thus increasing its size from the 32 species placed in that genus by Nesom (1990) and Nesom and Baird (1993, 1995) to 44. Anderson’s (1995) action not only combined what were considered two distinct genera, but members of two distinct subtribes sensu Nesom (1994, 2000). Nesom and Baird (1995) opposed Anderson’s treatment, arguing that the evidence implicating *C. albidus* in the parentage of the purported hybrid was insufficient.

Prior to the classifications discussed above, the most inclusive monograph of the species, which are now included in *Ericameria*, was that by Hall (1928). Hall (1928)
accorded *Ericameria* and its present congener *Asiris*, *Macronema* and *Stenotopsis* sectional rank in *Haplopappus*. His phylogenetic diagram depicted *Asiris* and *Ericameria* as terminal sister taxa in one of two major lineages of North American species, while the other two sections, *Macronema* and *Stenotopsis*, were placed in the other lineage. Both *Macronema* and *Stenotopsis* were portrayed as related to the sections *Oreochrysum* (Rydb.) H. M. Hall, *Stenotus* (Nutt.) H. M. Hall, and *Tonestus* (A. Nelson) H. M. Hall but not to *Ericameria* and *Asiris*. Hall’s (1928) treatment rather than ending the controversy regarding the composition and rank of *Ericameria* spurred more investigations into its relationship to other sections of *Haplopappus sensu lato*. Eventually, *Haplopappus* came to be recognized by most researchers as being polyphyletic as summarized by Lane and Hartman (1996).

Following Hall’s (1928) monograph, several researchers, exemplified by Johnston (1970), recognized *Ericameria* at the rank of genus rather than as a section in *Haplopappus*. Urbatsch (1975, 1976, 1978) followed this trend, but other researchers (Anderson, 1983; Welsh, 1993) continued to describe new species as *Haplopappus*. Section *Stenotopsis* was transferred to the genus *Ericameria* and redefined by Urbatsch and Wussow (1979) to accommodate *E. linearifolia* and *E. cooperi*, the latter being a core species within *Haplopappus* section *Ericameria sensu* Hall (1928). They cited natural hybridization between the two species as evidence supporting their close affinity. Their decision was based on the study of herbarium specimens. Following this, field investigations by Cody and Thompson (1986) confirmed the observations of Urbatsch and Wussow (1979), but also suggested *E. laricifolia* as an alternative parental species. *Ericameria laricifolia* and *H. linearifolia* are sympatric in the area of interest. However,
Cody and Thompson (1986) noted that differences in phenology, in that, \textit{E. cooperi} flowers in the spring while \textit{E. laricifolia} blooms in the late fall, and habitat preference preclude \textit{E. laricifolia} as a possible parent.

What has emerged since Hall’s (1928) monograph for \textit{Ericameria} is a greatly expanded genus wherein the species are classified into four sections (Nesom, 1990; Nesom and Baird, 1993, 1995). However, hypotheses regarding \textit{Ericameria}’s relationship to other putative congeners and its sister and phylogenetic relationships to other \textit{Astereae} are diverse. This investigation (see Chapter 3) seeks to test the following hypotheses using sequence data from nrDNA, specifically the internal transcribed spacer (ITS 1 and ITS 2 including the 5.8s region) and the 3’ end of the external transcribed spacer (ETS): (1) whether the specific composition of \textit{Ericameria sensu} Nesom (1990), Nesom and Baird (1993, 1995) or Anderson (1995) are supported by ITS and ETS sequence data; (2) whether the molecular data support the sectional classification recently proposed by Nesom (1990) and Nesom & Baird (1993, 1995); (3) the relationship of \textit{Ericameria} to other taxa of North American \textit{Astereae}; (4) the extent of congruence between relationships based on sequence data with those implied using morphological characters.

\textbf{SYSTEMATICS OF \textit{XYLOTHAMIA} NESOM, SUH, MORGAN & SIMPSON}

The genus \textit{Xylothamia} was established in 1990 to accommodate seven taxa previously placed in \textit{Ericameria} Nutt. or \textit{Haplopappus} Cass. Nesom \textit{et al.} (1990) argued that while these taxa superficially resemble other species of \textit{Ericameria}, they also displayed some key differences from typical \textit{Ericameria}. As circumscribed, \textit{Xylothamia} species are primarily Chihuahuan Desert endemics that form a clearly defined natural group (Nesom
et al., 1990). In addition to geography, taxa placed in *Xylothamia* are unified by their zygomorphic disc corollas with long lobes and phyllaries with an apical glandular patch but without a prominent midvein. Urbatsch (1978) also highlighted the uniqueness of taxa that were later placed in *Xylothamia* in comparison to the Californian species of *Ericameria*. He noted that as a group they differed from other species of *Ericameria* in their unique flavonoid chemistry and extraordinary morphology. Of the five species investigated by Urbatsch (1978), *E. laricifolia* (Gray) Shinners was proposed to be more closely related to *E. pinifolia* (Gray) Hall and *E. brachylepis* (Gray) Hall than to other Chihuahuan Desert species. The four other species are included among the seven segregates of Nesom et al. (1990). Urbatsch (1978) proposed the possibility of a common ancestor for the Chihuahuan Desert species and the Californian species or, that the Chihuahuan Desert species represented a relatively recent evolutionary lineage undergoing rapid evolution in response to changing environmental conditions. He also noted the close alliance of *Ericameria* (the California group) to *Chrysothamnus*. In addition to the four species treated in the Chihuahuan Desert assemblage by Urbatsch (1978), Nesom *et al.* (1990) transferred, to *Xylothamia*, three other species and described one new species, *X. johnstonii*. The three additional species transferred include *Ericameria diffusa* Benth. (*X. diffusa*), *Ericameria austrotexana* M.C. Johnston (*X. palmeri*) and *Ericameria riskindii* B. Turner & Langford (*X. riskindii*). Of these, *Ericameria diffusa* was originally described as a species of *Haplopappus* (De Candolle, 1836) and subsequently moved to various other genera, including its placement in section *Ericameria* of *Haplopappus* (Hall, 1928). *Ericameria palmeri* was transferred from the genus *Aster* (Johnston, 1967) to the genus *Ericameria* and *E. riskindii* was described by
Turner and Langford (1982). Prior to Johnston’s (1967) placement of *E. palmeri* in *Ericameria*, Shinners (1950) treated the species as an *Isocoma* Nutt. Since the circumscription of the genus, one new species, *X. truncata* (Nesom, 1992), was described, resulting in a total of nine species in *Xylothamia*.

In addition to delimiting the boundaries of *Xylothamia*, Nesom *et al.* (1990) discussed possible relationships to other genera in the Astereae. Based on morphology, *Xylothamia* appears to be allied to *Euthamia* Nutt. ex Cass. with which it shares narrow, resinous-punctate leaves, phyllaries strongly graduated into several series, deeply lobed and reflex-coiling disc corollas, and insertion of the filaments at the tube-throat junction of the corolla (Kapoor and Beaudry, 1966; Nesom *et al.*, 1990). Johnston (1970) also noted the resemblance between the two taxa and alluded to the possibility of this being more than superficial.

Morphological investigations conducted by Nesom (1990, 1991a, 1991b, 1993, 1994) to a large extent supported the relationships hypothesized by Suh (1989), Suh and Simpson (1990), Morgan (1990), and Morgan and Simpson (1992) based on cpDNA restriction site variation data. While lacking extensive taxon sampling, the investigations of Suh (1989) indicated that *Xylothamia*, represented by *X. palmeri*, is more closely allied to *Euthamia* Nutt. ex Cass., *Gutierrezia* Lag., *Gymnosperma* Less., *Amphiachrys* Nutt. and *Bigelowia* DC. than to species of *Ericameria* Nutt. or, *Chrysothamnus* Nutt. In addition, Suh and Simpson (1990) reported *Thurovia* Rose (*Gutierrezia triflora*) sister to *Amphiachyris* Nutt., and this lineage is basal to one containing species of *Gutierrezia* Lag. Basal to this assemblage is a grade consisting in part of *Gymnosperma*, *Amphipappus* and *Acamptopappus*. Furthermore, Suh and Simpson (1990) highlighted
the low level of nucleotide divergence among the taxa of the Astereae in their study with special emphasis on the extremely low level of sequence divergence between *Thurovia* (*G. triflora*) and *Amphiachyris*. Suh and Simpson’s observations placed some doubt on Lanes (1985) transfer of *Thurovia* to the genus *Gutierrezia*. While these studies represent important first steps in elucidating the relationship of *Xylothamia* based on molecular data, they lack the extensive sampling necessary to construct more rigorous hypotheses of relationships. In addition to taxa sampled by Suh (1989) and Suh and Simpson (1990), the present investigation includes *Gundlachia* Gray. Lane (1996) suggested that *Gundlachia* is probably allied to *Gymnosperma*, a species distributed in the southwestern United States and Mexico. In contrast, Nesom (1993, 1994) proposed an alliance to *Chrysoma*, *Bigelowia* and *Euthamia*, genera distributed primarily in the southeastern United States. At present, the position of *Greenella*, like that of *Thurovia*, is also ambiguous, Lane (1982, 1985) placed *Greenella* in *Gutierrezia* however, Suh and Simpson (1990) reported that *Thurovia* is sister to *Amphiachyris* but *Greenella* appears to be part of *Gutierrezia*. This investigation (see Chapter 4) aims to sample extensively among taxa, including all species of *Xylothamia*, in an effort to test the following hypotheses: (1) whether *Xylothamia* is a monophyletic group; (2) whether *Xylothamia* is closely related to *Ericameria* and *Chrysothamnus* or to other genera of the Astereae.

**SUBTRIBAL AFFINITIES OF CHRYSOTHAMNUS, ERICAMERIA AND XYLOTHAMIA**

Subtribal classification for the three genera has also been controversial. Within the past decade, two different subtribal classifications have been proposed. Bremer (1994) presented a classification, based to a large extent on the work of Zhang and Bremer (1993), in which species were classified into three subtribes: Granginae, Solidagininae,
and Asterinae. The assessment of relationships within and among subtribes was based primarily on a cladistic analysis of traditional morphological characters utilized in Astereae classification.


Prior to the proposals of Bremer (1994) and Nesom (1994), other researchers presented alternative subtribal classifications for the Astereae. Of note is that of Cassini (1819) who, in addition to circumscribing the tribe Astereae, classified its species into four subtribes. According to Bremer (1994), Cassini’s classification and the two that immediately followed, that of Lessing (1832) and DeCandolle (1836), were essentially artificial. Bentham (1873) offered a new classification of the Astereae, in which he organized the species into six subtribes. This classification was based primarily on the color of the ray florets and, like those of the previous researchers, is artificial because the color of ray florets is known to vary even within genera. The subtribal classification presented by Hoffmann (1890), except for a few changes in subtribal names, was in most
parts similar to that of Bentham (Bremer, 1994). Subsequently one additional subtribe, the Hinterhuberinae, was described (Cuatrecasas, 1969).

The cladistic treatment of Xhang and Bremer (1993) arranged Astereae genera into 23 informal groups. Taxa were subsequently sampled from among those groups for cladistic analyses. The investigation resulted in combining four of the seven recognized subtribes with the Asterinae. The other two subtribes were confirmed by the cladistic evaluation of Xhang and Bremer (Bremer, 1994). Bremer (1994) classified *Chrysothamnus*, *Ericameria*, and *Xylothamia* in the Solidagininae. Within the Solidagininae, *Chrysothamnus* and *Ericameria* are placed in the *Ericameria* group, while *Xylothamia* was aligned with the *Solidago* group. Other taxa included in this investigation represent all of Bremer’s subtribes. In contrast, Nesom (1994) classified *Ericameria* in the subtribe Hinterhuberinae, noting its affinity to South American representatives of that subtribe. Both *Chrysothamnus* and *Xylothamia* were placed in the Solidagininae (Nesom, 1994, 2000). Prior to this, Nesom (1990), Nesom et al. (1990) and Nesom and Baird (1993), argued for the recognition of the three genera as distinct from each other. Thus, Anderson’s (1995) transfer of all remaining *Chrysothamnus* to *Ericameria* crossed what other researchers considered both distinct generic and subtribal boundaries.

Noyes and Rieseberg (1999) using ITS data, demonstrated that the *Ericameria* representative in their study, *Ericameria cooperi*, is a sister taxon to a clade consisting of three genera of annuals: *Pentachaeta*, *Rigiopappus*, and *Tracyina*. These three had previously been regarded as Feliciinae by Nesom (1994), but given uncertain status in Nesom (2000). Bremer (1994) placed all three genera in the subtribe Asterineae nested in
the Chaetopappas group. Rigiopappus, however, was at one time placed in the tribe Heleniumae. However, based on cytological (Raven & Kyhos, 1961; Ornduff & Bohm, 1975), chemical (Ornduff & Bohm, 1975) and morphological evidence (Robinson & Brettell, 1973; Van Horn, 1973), Rigiopappus seems unequivocally related to taxa in the Astereae. Both Stenotus and Tonestus were placed in the Petradoria group of the Solidagininae (Bremer, 1994). However, Nesom (2000) placed Stenotus in the Solidagininae while Tonestus is among eight other genera of uncertain affinity and placed in a group coined “‘Primitive” Asters’.

A fairly thorough comparison of the two recent subtribal classifications has been discussed by Noyes and Rieseberg (1999) and is therefore not part of this current investigation. The gene trees of Noyes and Rieseberg (1999) also includes several Hinterhuberinae that are distributed among a basal grade of predominantly southern hemisphere taxa representing various subtribes. Therefore, Hinterhuberinae failed their monophyly test and provided evidence that Ericameria may be derived from North American ancestors.

Comparison of the conclusions of previous researchers with regards to relationships and subtribal classification in the Astereae is difficult, because most projects include only exemplar taxa and that researchers’ focus differed. For example, several taxa investigated by Lane et al (1996) were not included in the study of Noyes and Rieseberg (1999), hence limiting the number of comparisons that can be made for the taxa in the present study. One major incongruity is the lack of affinity of Chrysothamnus, sensu stricto for Solidago in the restriction site studies (Lane et al., 1996), suggesting that it is not Solidagininae. Nesom and Baird (1995) suggested widening the definition of
Chrysothamnus to include Hesperodoria and Petradora. They also highlighted that as a group they are apparently closely related to Stenotus, a member of the Solidagininae.

The goal here is not a comprehensive assessment of subtribal relationships in the Astereae, but to assess the relationships of the taxa here investigated based on the available nrDNA sequence data. By so doing, answers to the following hypotheses are tested: (1) whether Chrysothamnus, Ericameria and Xylothamia display the subtribal affinities as hypothesized by Nesom (1994, 2000) or Bremer (1994); (2) the placement of Chrysothamnus, Ericameria and Xylothamia in the larger subtribal classification of the Astereae.

LITERATURE CITED


Nesom, G. L. 1993. Taxonomic infrastructure of *Solidago* and *Oligoneuron* (Asteraceae : Astereae) and observations on their phylogenetic position. Phytologia 75: 1–44


CHAPTER 2: MOLECULAR PHYLOGENY OF *CHRYSOTHAMNUS* AND RELATED GENERA (ASTERACEAE: ASTEREAE) BASED ON NUCLEAR RIBOSOMAL 3’ ETS AND ITS SEQUENCE DATA

INTRODUCTION

*Chrysothamnus* Nutt., commonly known as rabbitbrush (Asteraceae, Astereae), is endemic to North America and occurs primarily in the western United States with small range extensions into Mexico and Canada (Nesom, 2000). Some species such as *C. nauseosus* (Pallas ex Pursh) Britton [*Ericameria nauseosa* (specific author citations are given in Table 2.1)] and *C. viscidiflorus* are widespread and, along with *Artemisia* L., often dominate the landscape in vast shrubland communities of the intermountain west (McArthur and Welch, 1986). The sagebrush ecosystem is estimated to cover 38 million ha, making it the largest range ecosystem in the United States (Whisennant, 1986). *Chrysothamnus* spp. tend to grow in openings in the sagebrush community or become more abundant after fire has destroyed *Artemisia* (Whisennant, 1986). Not all *Chrysothamnus* species are abundant and widespread: *Chrysothamnus eremobius* is restricted to southwestern Nevada, *C. gramineus* to southern Nevada and adjacent California, and *C. molestus* to Arizona where they are known from but a few populations (Anderson, 1986).

Concepts of *Chrysothamnus* have varied greatly throughout its history. The genus was established by Nuttall (1841) to include taxa previously placed in *Chrysocoma* (Pursh, 1814), *Crinitaria* (Hooker, 1834) and *Bigelowia* [*Bigelovia*] (DeCandolle, 1836). Torrey and Gray (1842) subsequently treated the species circumscribed by Nuttall as species of *Linasyris* Cass. The taxonomy of *Chrysothamnus* was again modified when Bentham and Hooker (1873) reasserted the name and widened its definition to include DeCandolle’s *Bigelowia* without respect to *Bigelowia* having priority. Bentham and
Hooker’s circumscription was essentially accepted by Gray (1873) except that the priority of *Bigelowia* was recognized. Greene (1895) restored *Chrysothamnus* to generic rank and defended its segregation by Nuttall (1841) from the herbaceous species of eastern North America on which *Bigelowia* was based.

Hall and Clements’ (1923) monograph of *Chrysothamnus* provided a taxonomy of the genus that was essentially followed for several decades. They provided clearer delimitation of the genus based on features of the capitulum, asserting that narrow cylindrical heads and vertically ranked phyllaries are diagnostic. They also proposed an infrageneric classification, a discussion of inter- and infra-specific relationships, and a phylogeny relative to primitive and derived species. In addition, they redefined the sectional classification of Gray (1873) and Hall (1919). Gray’s two sections were defined to include species in the genus *Bigelowia* whose circumscription at the time also included *Chrysothamnus* whereas Hall (1919) recognized five natural groups within *Chrysothamnus*. The sectional hypotheses of Hall and Clements (1923) combined Hall’s (1919) sections *Parryani* with *Nauseosi*. Furthermore, the sections and species within were presented in the order of primitive to most derived with section *Punctati* being the more primitive and *Nauseosi* more derived (Hall and Clements, 1923). This classification was considered more natural than preceding treatments because of its reliance on a more extensive assortment of morphological characters.

Regarding intergeneric relationships, Hall and Clements (1923) noted that characteristics used to define *Chrysothamnus* are not unique. The capitular characters used to distinguish between *Chrysothamnus* and species of *Haplopappus* Cass., are also displayed in some taxa of the latter genus. Apparently such similarities were never regarded as sufficient to merit congeneric treatment but were proposed as probable indicators of convergent evolution (Hall and Clements, 1923). *Chrysothamnus* is
depicted as originating from within or close to *Haplopappus* section *Ericameria* (Hall, 1928) with Section *Punctati*, characterized by resin pits, as the transitional link between the two. In the same publication, Hall alternatively suggested that the *Chrysothamus* – *Haplopappus* connection might be through section *Macronema*.

Hall and Clements’ (1923) and Hall’s (1928) work stimulated investigations for several decades thereafter designed to explore the relationships between *Chrysothamnus* and *Haplopappus*. The resulting accumulation of cytological, anatomical, chemical, and macromolecular data for *Chrysothamnus* and taxa thought to be related (Anderson, 1963; Anderson, 1964; Anderson, 1970; Anderson and Fisher, 1970; Anderson and Weberg, 1974; Anderson et al., 1974; Anderson and Creech, 1975; Urbatsch et al., 1975; Suh, 1989; Morgan, 1990; Nesom, 1994; Lane et al., 1996) often provided conflicting views about the composition and boundaries for these taxa. Nesom and Baird (1995), as was done by Hall and Clements (1923), attributed this confusion to apparent convergent evolution. Furthermore, the former suggested that morphological data, in particular, may not be good indicators of distance or affinity among these taxa.

Within the last decade investigators set a course that dramatically altered the paradigm established by Hall and Clements (1923). Nesom and Baird (1993) using morphological criteria and cpDNA data accumulated by Suh (1989) and Morgan (1990) transferred four species, long regarded as *Chrysothamnus*, to *Ericameria*. The former genus, *sensu* Nesom and Baird, was left with 12 species while *Ericameria* was experiencing considerable growth having earlier received species from *Haplopappus* sections *Asiris* and *Macronema* (Nesom, 1990). Subsequently, Anderson (1995) treated all species remaining in *Chrysothamnus* as *Ericameria*. His justification for uniting all taxa within a single genus was hybridization between *C. albidus* Greene, a core *Chrysothamnus sensu* Nesom and Baird, and *E. nauseosa*, a recent transfer from
*Chrysothamnus* by Nesom and Baird (1993), and the high degree of morphological similarity between the two sets of taxa (Anderson and Reveal, 1966). Contrary to Anderson’s hypothesis, DNA sequence data for representative Astereae including one species each from traditional *Chrysothamnus* and *Ericameria*, indicated that these two are not congeneric and probably are not closely related (Noyes and Rieseberg, 1999) thus supporting Hall and Clement’s (1928) and Nesom and Baird’s (1995) suggestions of convergence between the two.

Besides *Ericameria*, several other genera have often been suggested as being related to *Chrysothamnus*. *Xylothamia* G.L.Nesom, Y.B.Suh, D.R.Morgan & B.B.Simpson, a recent segregate from *Ericameria*, was shown by Lane et al. (1996) based on cpDNA restriction data to be related to *Chrysothamnus* and *Ericameria*. In contrast, Nesom (1993) indicated that *Xylothamia* was allied to *Chrysoma* Nutt., *Gundlachia* A. Gray, *Bigelowia* DC. and *Euthamia* (Nutt.) Cass. In the same publication, Nesom depicted *Chrysothamnus* as sister to *Stenotus* Nutt. However, he indicated that the relationship of the *Chrysothamnus-Stenotus* lineage to other genera in the subtribe Solidagininae was uncertain. *Petradoria discoidea* has at times been accommodated in *Chrysothamnus* (Hall, 1916; Anderson, 1963, 1986). *Hesperodoria* Greene, a genus of two species, the monospecific *Vancelevea* Greene, and *Stenotus* may be related to *Chrysothamnus* through *Petradoria* Greene (Anderson and Weberg, 1974; Nesom, 1991, 2000). Nesom (2000) also suggested that *Tonestus microcepthalus* (Cronquist) G. L. Nesom & D. R. Morgan is possibly a *Chrysothamnus*. *Tonestus* A. Nelson, although reconstituted as a distinct genus of nine species, is regarded as heterogeneous and possibly not monophyletic by its proponents (Nesom and Morgan, 1990; Nesom, 2000). Also included are representatives of *Acamptopappus* A. Gray, *Amphipappus* Torr. & A. Gray, *Brintonia* Greene, *Eastwoodia* Brandegee, *Oligoneuron* Small, *Oreochrysum*
Rydb., and *Solidago* L. because Lane et al. (1996) indicate their probably affinity to *Chrysothamnus*. Nesom (1993, 2000) also hypothesized that these taxa may be related to *Chrysothamnus*. The convoluted taxonomic history of *Chrysothamnus* together with the inconclusiveness of recent investigations makes necessary a more comprehensive investigation of the composition and relationships of the genus.

As a result, using sequence data of the nuclear ribosomal DNA internal transcribed spacer region (ITS 1 and ITS 2 including the 5.8s) and the 3′ portion of the external transcribed spacer (3′ ETS), this investigation sought to answer the following questions: (1) is *Chrysothamnus*, *sensu lato*, monophyletic? (2) do molecular data support the sectional classification of Anderson (1986)? (3) what are the relationships of *Chrysothamnus* to other North American Astereae?

**MATERIALS AND METHODS**

**Sampling.** Sixty sequences spanning the ITS-1, ITS-2 and the intervening 5.8S, and the 3′ ETS region of nuclear ribosomal DNA (nrDNA) were analyzed from samples representing 53 species in 20 genera of Astereae. The sample included members of subtribes Solidagininae, Hinterhuberinae, and primitive asters *sensu* Nesom (2000). All species and as many infraspecific taxa of *Chrysothamnus*, *sensu lato*, as possible were sampled. Their identifications were based on the keys and distributional data of Anderson (1986), and by comparison to specialist-annotated herbarium specimens. Because of its hypothesized relationship to *Chrysothamnus*, (Hall and Clements, 1923; Hall, 1928; Anderson, 1995; Nesom and Baird, 1995), representatives of *Ericameria* Nutt. and 19 other genera were also included in this study.

*Doellingeria* Nees was implicated as a suitable outgroup based on Noyes and Rieseberg (1999) and on preliminary analysis of a larger data set containing worldwide
representatives of Astereae. Collection and voucher deposition data are provided in Table 2.1.

In order to insure sequence fidelity, at least two individuals per taxon were sampled, or for rare plants, at least two independent DNA extractions and amplifications of the DNA of interest were performed. Ultimately, 49 ITS and 53 ETS sequences obtained were excluded to facilitate statistical comparison of data sets and resulting trees. However, the final data set contained at least one sequence for each taxon.

**DNA Isolation, Polymerase Chain Reaction (PCR) and Sequencing.** Total genomic DNA was isolated from approximately 20–500 mg of leaf material. Where possible fresh leaf tissue samples were obtained in the field, placed immediately in liquid nitrogen, and subsequently stored at –80°C. When fresh plant tissue was unobtainable samples from herbarium specimens were used. In preparation for DNA extraction, frozen tissue was ground with a mortar and pestle in liquid nitrogen while dried tissue was ground using a small amount of sterile sand. Later in the project, both types of plant tissue were pulverized in a Mini-BeadBeater-8™ (BioSpec Products, Inc. Bartlesville, Oklahoma) for 30-60 seconds. Fresh samples were kept frozen during pulverization. Dried samples were processed at ambient temperature and the lysis time was increased to 60 min rather than the 10 min recommended by the manufacturer. Initially, the 2X CTAB (hexadecyltrimethylammonium bromide) extraction protocol of Doyle and Doyle (1987) was employed. Later, DNA extraction was accomplished from the ground tissue using the Qiagen DNeasy™ Plant Mini Extraction Kit and protocol.

Double stranded DNA for sequencing was initially generated in 50 µl and later in 25 µl PCR reactions. The latter reaction size used 0.5 unit *Tth* DNA polymerase (Epicentre Technologies, Madison, Wisconsin), 8 µl H₂O, 12.5 µl premix buffer G (Epicentre Technologies), 1 µl each of 10nM forward and reverse primers, and 2 µl of
Table 2.1. Taxa sampled for this investigation, collection localities, voucher numbers, and genbank accession numbers.

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<th>ETS Genbank Number</th>
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Chrysothamnus viscidiflorus (Hook.) Nutt. ssp. viscidiflorus (LSU) California: Lassen Co., Urbatsch 7712 AY170947 AY169744


*Doellingeria umbellata* Nees Michigan: Chippewa Co., Schmidt AF477625 AF477754 1060 (TEX)

Eastwoodia elegans Brandegee California: Kern Co., Sanders 20427 AY170949 AY169746 (CAS)

Ericamia albida (M. E. Jones ex A. Gray) L. C. Anderson (LSU) Nevada: Nye Co., Urbatsch 1459 AY170950 AY169747

Ericameria arborescens (A. Gray) Greene California: San Luis Obispo Co., Keil AY170951 AY169748

Ericameria ericoides (Less.) Jeps. California: Monterey Co., Sundberg AF477642 AF477706 2646 (TEX)

Ericameria nauseosa (Pall. ex Pursh) G. L. Nesom & G. I. Baird (TEX) California: Inyo Co., Morefield 4336 AY170952 AY169749

Ericameria paniculata (A. Gray) Rydb. Nevada: Nye Co., Reveal 2014 (TEX) AY170953 AY169750

Ericameria teretifolia (Durand & Hilg.) Jepson (TEX) California: Inyo Co., Morefield 3130 AY170954 AY169751

Gundlachia corymbosa (Urb.) Britton ex Bold West Indies: Caicos Islands. Pine Cay, Correll 43104 (LL)

Hesperodoria salicina (S. F. Blake) G. L. Nesom (ASC) Arizona: Coconino Co., Scott 880 AY170955 AY169752

Hesperodoria scopulorum (M. E. Jones) Greene Utah: Washington Co. Shultz 5382 AY170956 AY169753

(Table 2.1 cont’d.)
**Oligoneuron nitidum** (Torr. & A. Gray) Small
Louisiana: Lasalle Parish, Urbatsch 5735 (LSU)

**Oligoneuron rigidum** (L.) Small
Louisiana: Winn Parish, Urbatsch 5219 AF477663 Unpub. (LSU)

**Oreochrysum parryi** (A. Gray) Rydb.
Colorado: Lake Co., Urbatsch 7887 (LSU)

**Petradoria pumila** (Nutt.) Greene
Colorado: Mesa Co., Urbatsch 7889 (LSU)

**Sericocarpus tortifolius** (Michx.) Nees
Florida: Wakulla Co., Urbatsch 7599 AF477664 AF477728 (LSU)

**Solidago canadensis** L.
Louisiana: W. Feliciana Parish, Lievens 3347 (LSU)

**Solidago fistulosa** Mill.
Florida: Gulf Co., Urbatsch 7587 AF477666 AF477730 (LSU)

**Solidago sempervirens** L.
Florida: Wakulla Co., Urbatsch 7590 AF477668 AF477732 (LSU)

**Stenotus acaulis** (Nutt.) Nutt.
Utah: Garfield Co., Davidson 129 (UNLV)

**Stenotus armerioides** Nutt.
Wyoming: Sublette Co., Cramer 8671 (RM)

**Stenotus lanuginosus** (A. Gray) Greene
Oregon: Baker Co., Brooks SN (CAS) AY170962 AY169759

**Stenotus macleanii** A. Heller
Canada: YukonTerr., Porsild 9556 (ALTA)

**Stenotus pulvinatus** (Moran) G. L.Nesom
Mexico: Baja California, Rebman 4176 AY170964 AY169761 (SD)

**Stenotus stenophyllus** (A. Gray) Greene
Oregon: Harney Co., Cutright 1122 AY170965 AY169762

(Table 2.1 cont’d.)
*Tonestus alpinus* (L. C. Anderson & Goodrich) G. L. Nesom & D. R. Morgan  
Nevada: Lander Co., Goodrich 12126  
AY170966  AY169763

*Tonestus eximius* (H. M. Hall) A. Nelson & J. F. Macbr.  
California: Alpine Co., Taylor 4174  
AY170967  AY169764

*Tonestus graniticus* (Tiehm & L. M. Shultz) G. L. Nesom & D. R. Morgan  
Nevada: Esmeralda Co., Tiehm 8252  
AY170968  AY169765

*Tonestus lyallii* (A. Gray) A. Nelson  
Canada: Alberta, Mc Calla 4540  
AY170969  AY169766

*Tonestus microcephalus* (Cronquist) G. L. Nesom & D. R. Morgan  
New Mexico: Rio Arriba Co., Fletcher 7145 (TEX)  
AY170970  AY169767

*Tonestus peirsonii* (D. D. Keck) G. L. Nesom & D. R. Morgan  
California: Inyo Co., Anderson 4326  
AY170971  AY169768

*Tonestus pygmaeus* (Torr. & A. Gray) A. Nelson  
Colorado: Lake Co., Urbatsch 7887.2  
AY170972  AY169769

*Vanclevea stylosa* (Eastw.) Greene  
Utah: Kane Co., Urbatsch 7625 (LSU)  
AY170973  AY169770

*Xylothamia triantha* (S. F. Blake) G. L. Nesom  
Texas: Brewster Co., Poewll 3542  
AY170974  AY169771

*Xylothamia truncata* G. L. Nesom  
Mexico: Coahuila. Nesom 5254 (TEX)  
AY170975  AY169772
DNA template usually diluted $10^{-2}$. Reactants in the 50 µl reactions were doubled. The initial 10 thermal cycles each consisted of 1 min of denaturation at 95°C, 1 min of annealing at 55°C and, 1 min of extension at 72°C with a 4 sec extension per cycle. The following 20 cycles were similar except for an annealing temperature reduction to 50°C, extension time increased to 1 min 40 sec plus 0.4 sec per cycle, and ending with a 7 min extension at 72°C. After completion of the 30 cycles, reactions were kept at 4°C until removed from the thermal cycler. This protocol proved adequate for the amplification of both the ITS and ETS regions. All reactions were performed using a PTC-100™ Thermal Cycler (MJ Research, Inc., Watertown, Massachusetts).

For the amplification of the ITS region, primers ITS-20 and ITS-262 (Urbatsch et al., 2000) were used in equimolar concentrations. When amplicon production was inadequate, products (2 µl) from the above reactions were used as templates and reamplified in subsequent PCR reactions using a set of nested primers, ITS-I.1, (5’-3’: TTCCACTGAACCTTATCA) modified from primer ITS-I (Urbatsch et al., 2000), and ITS4 (White et al., 1990), in order to increase yield. The ETS region was amplified using the primers 18S-ETS (Baldwin and Markos, 1998) and Ast-8 (Markos and Baldwin, 2001). Because of amplification failure with the previous primer pair for certain taxa, another reverse primer was designed. This primer was designated 18SR1 (5’-3’: CAAGCATATGACTACTGGCAG) and is located approximately 93 bp from the 5’ end of 18S-ETS. This primer was paired with Ast-1 (Markos and Baldwin, 2001) as a nested pair when amplification yield with Ast-8 and 18S-ETS proved inadequate.

Two PCR purification kits, QIAquick™ PCR Purification Kit (Avenue Stanford, Valencia, California) and Novagen SpinPrep™ (Novagen, Madison, Wisconsin) were used for the purification of amplicons. The concentration of purified amplicons was
estimated visually on agarose gels using Low DNA Mass Ladder (Life Technologies, Inc. Rockville, Maryland). Purified amplicons were sequenced using ABI PRISM® BigDye™ Terminator cycle sequencing (Applied Biosystems, Foster City, California.) and run on an ABI 377 automated DNA sequencer. The cycle sequence reactions were 10 µl total volume and included 2 µl BigDye™ terminators, 2 µl of 10nM primer, 15–45ng purified amplicons, and water when necessary. The reactions were run for 25 cycles; 96°C for 10 sec denaturation, 50°C for 5 sec annealing and 60°C for 4 min extension in the thermal cycler previously noted.

Several samples yielded polymorphic sequences. When this occurred the amplicons were cloned using the TOPO TA Cloning® System (Invitrogen, Carlsbad, California) or the Perfectly Blunt™ Cloning Kit (Novagen, Inc., Madison, Wisconsin). Inserts were amplified directly from bacterial colonies using the ITS and ETS primers discussed above. The PCR protocol was modified to include an initial 10 min at 94°C to lyse the bacterial cells. The amplicons were purified as described above in preparation for cycle sequencing reactions. Typically, two or more clones were amplified and sequenced for each polymorphic sample.

**Sequence Analysis.** Sequences were edited with the aid of Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan). Initial alignments were performed using Clustal W (Thompson et al., 1994) by submitting data to the Baylor College of Medicine sequence launcher (http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html), and manual sequence adjustments to these alignments were made as judged necessary. Sequences subsequently obtained were aligned by manual comparison to the existing data matrix. Boundaries of the ITS and ETS regions were determined by comparison with published sequences of the Heliantheae (Urbatsch et al., 2000) and Astereae (Baldwin and Markos, 1998; Markos and Baldwin, 2001). Cloned sequences were entered in the
matrix as individual OTUs following Urbatsch et al. (2000). Pairwise sequence divergence estimates were obtained using PAUP* 4.0b10 (Swofford, 2002). MacClade version 4.0 (Maddison and Maddison, 2000) was used to generate transversion/transition \((t_v/t_i)\) substitution ratios and for examination of sequence alignment. All ITS and ETS sequences analyzed in this study were deposited in GenBank under the accession numbers provided in Table 2.1.

**Phylogenetic Analysis.** The ITS and ETS data sets were first analyzed separately to investigate phylogenetic congruence between the two loci. Congruence was determined using the partition-homogeneity test (Farris et al., 1994, 1995) in PAUP* 4.0b10 (Swofford, 2002). Data analyses employed several optimality criteria: maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference. Bayesian inference was performed using MrBayes 2.01 (Huelsenbeck and Ronquist, 2001). All other analyses were performed using PAUP* 4.0b10 including the independent parsimony analysis of the ITS data for which PAUPRat (Sikes and Lewis, 2001) was also used. Models of sequence evolution required for ML and Bayesian estimations of phylogeny were obtained using Modeltest 3.04 (Posada and Crandall, 1998). The size of the data set dictated that heuristic search strategies be implemented for all phylogenetic estimates using PAUP* 4.0b10.

**Maximum Parsimony.** Equally weighted MP searches with 100 sequence addition replicates and tree bisection-reconnection (TBR) were conducted. All MP analyses were performed with MULTREES on, ACCTRAN optimization and gaps treated as missing data using all potentially phylogenetically informative characters. Heuristic parsimony analysis of the ITS data in PAUP* 4.0b10 failed due to exhaustion of computer memory compromising the rigor of the analysis. As a result, heuristic analysis of the ITS data set was accomplished with PAUPRat. Analysis of the ITS data
employed 25 runs each of 500 iterations where 25% of the characters were perturbed per iteration. Nonparametric bootstrap analyses (Felsenstein, 1985) with 1000 pseudoreplicates and 100 random sequence additions were conducted on all data sets. For bootstrap analyses MULTREES was turned off and 100 trees of a specified length, which varied with the input data, were retained for each pseudoreplicate.

**Maximum Likelihood.** The general time reversible model with some sites assumed to be invariable and variable sites assumed to follow a discrete gamma distribution (GTR + I + Γ; Yang, 1994a) was selected as the best-fit model of nucleotide substitution for the combined data, using Modeltest. The gamma distribution was separated into six discrete rate categories to better accommodate rate heterogeneity (Yang, 1994b). Model parameter estimates were initially calculated on a neighbor joining tree (uncorrected “p” distances). Initially, ML searches were conducted via heuristic search and TBR branch-swapping procedures. Maximum likelihood parameters for resulting topologies were recalculated using the ML tree scores command in PAUP* v4.0b10. The resulting parameter estimates were used to perform further ML analyses on the data set beginning with the topology for which the estimates were obtained (Swofford et al., 1996). This iterative procedure was repeated until no significant topological differences (Shimodaira and Hasegawa, 1999) were observed among resulting topologies. Finally, ML bootstrap analyses were performed using 100 pseudoreplicates, 10 random sequence additions and subtree pruning-regrafting (SPR) branch swapping beginning with the topology resulting from the ML heuristic search. Due to time constraints, bootstrap ML analysis was performed on the combined data only.

**Bayesian Inference.** Bayesian phylogenetic analyses were conducted using MrBayes 2.01 (Huelsenbeck and Ronquist, 2001). The GTR + I + Γ model was used in all
Bayesian analyses. Specific nucleotide substitution model parameter values were not defined a priori. Instead, model parameters were treated as unknown variables with uniform prior probabilities and were estimated as part of the analysis. Bayesian analyses were initiated with random starting trees and were run for $1.5 \times 10^6$ generations. Markov Chains were sampled every 100 generations. This resulted in 15,000 sampled trees and parameter estimates.

A critical aspect of Bayesian analysis is to ensure that the Markov Chain has reached stationarity. All sample points prior to stationarity are essentially random and are discarded as “burn-in” samples because they do not contain useful parameter estimates. Achievement of stationarity was presumed when log likelihood scores plotted against generation time exhibited stable equilibrium values (Huelsenbeck and Ronquist, 2001). Since stationary samples collectively form approximations of the posterior probability distribution, a conservative approach was taken for determining burn-in whereby some useful samples were discarded to avoid unknowingly retaining burn-in samples in posterior probability estimations (Leache and Reeder, 2002).

Analyses were repeated several times as a precaution against entrapment on local optima. Entrapment on local optima was evaluated by superimposing the log likelihood versus generation time of each independent analysis to determine if the mean log likelihood values were similar for each run. In addition, the search strategy implemented in MrBayes helps to avoid entrapment on local optima through the use of incrementally heated Markov chains (Metropolis-coupled MCMC) enabling a more thorough exploration of parameter space (Marinari and Parisi, 1992; Geyer and Thompson, 1995). This is accomplished via the random exchange of parameter values between heated chains effectively decreasing the distance between optimal peaks in parameter space.
This search algorithm enables movement between local optima, thus preventing entrapment. Analyses in this study used four incrementally heated Markov chains.

Bayesian searches yielded phylograms on which the posterior probabilities were later plotted. Posterior probabilities were estimated through the construction of a 50% majority rule consensus tree that provided estimates of the sampled trees containing a particular clade and that clade’s posterior probability (Huelsenbeck and Ronquist, 2001). Unlike nonparametric bootstrap support values, these posterior probabilities are interpreted as true probabilities for each clade under the assumed model (Rannala and Yang, 1996). Consequently, clades with probabilities of 95% or greater were considered significantly supported.

**Congruence of Methods and Hypothesis Testing.** The congruence of MP and ML trees with respect to that obtained via Bayesian inference was evaluated by assessing the number of shared nodes, topological congruence, and congruence between the estimated measures of support (bootstrap versus posterior probabilities). The Shimodaira-Hasegawa (SH) test statistic was used to compare alternative phylogenetic hypotheses (Shimodaira and Hasegawa, 1999; Goldman et al., 2000). SH tests were conducted using PAUP* v4.0b10, with full optimization.

**RESULTS**

**Independent Phylogenetic Analysis of ITS and ETS Data.** The length of the aligned ITS region (including the 5.8S unit) in the taxa investigated was 672 base pairs (bp). ITS-1 without gaps was approximately the same length (252 to 253 bp) for all samples, ITS-2 ranged from 198 to 213 bp, while the 5.8S unit was nearly constant at 164 bp except for one taxon, *Brintonia discoidea*, having 165 bp. An 11 bp insertion was observed in the ITS-2 region for all but the species of *Xylothamia*. Other indels in the ITS were two or fewer bp long. Among the 672 bp that constitute the ITS data set; 441
(65.6%) were invariable, 88 (13.1%) were variable but uninformative, and 143 (21.3%) were parsimony informative. The aligned 3’ ETS region investigated was 558 bp including an 81 bp insertion present only in species of *Xylothamia*. All other indels in the ETS region ranged from 1-3 bp. Without gaps, the length of the 3’ ETS region ranged from 409 bp in *Chrysothamnus depressus* to 525 bp in *Gundlachia corymbosa*. The aligned ETS data set contained 424 (76%) invariable sites, 74 (13.3%) variable, uninformative sites, and 60 (10.7%) parsimony informative sites. The range of uncorrected pairwise divergences among *Chrysothamnus* species for the 3’ ETS region ranged from 0.0 to 2.4%, and for ITS the range was 0.0 to 3.6%.

Parsimony analysis of the ETS resulted in 4,154 most parsimonious trees 223 steps long with a retention index (RI) of 0.831 and a consistency index (CI) of 0.753 in one tree island. Bootstrap analysis of the ETS data resulted in four nodes with ≥70% support. Nodes with ≥70% bootstrap support are indicated on the ETS Bayesian phylogram (Fig. 2.1). Among these are three clades of interest (Table 2.2) the fourth is subordinate to one of the three major clades. Parsimony ratchet analysis of the ITS resulted in 12,500 minimum length trees. The 50% majority-rule consensus tree for the parsimony ratchet (not shown) was 311 steps long with a RI of 0.822 and a CI of 0.723. Bootstrap analysis of the ITS data resulted in eight nodes with ≥70% support (Fig. 2.2). Six of the clades supported represented major lineages (Table 2.2) while the other two included all taxa above *Sericocarpus* and a subclade within the *Xylothamia/Gundlachia* lineage (Fig. 2.2). Both data sets support a monophyletic *Ericameria* and *Xylothamia-Gundlachia* with bootstrap support ≥ 93%. The trees shared no other clades with ≥ 70% support. However, among taxa considered ingroup, one ETS and five ITS clades
displayed bootstrap values ≥ 70% (Figs. 2.1, 2.2). Parsimony analyses where *Chrysothamnus*, *Stenotus*, and *Tonestus* were each constrained as monophyletic clades were compared with unconstrained topologies. The constrained ITS topology was less parsimonious and significantly different from the unconstrained tree \( p=0.000 \). The unconstrained ETS topology was not significantly different from the constrained tree \( p=0.559 \).

Bayesian analyses, for both data sets, converged on stable likelihood scores before 100,000 generations (Fig. 2.3). As a result, all sample points derived from the first 100,000 generations were discarded as burn-in. The resulting ETS and ITS topologies both support a monophyletic *Ericameria* and *Xylothamia/Gundlachia* with posterior support ≥0.95 (Figs. 2.1, 2.2; Table 2.2). Among taxa considered ingroup, two ETS and ten ITS clades displayed posterior probability values ≥0.95. However, the only ingroup clade supported by both data sets was that containing the two *Acamptopappus* species (Figs. 2.1, 2.2). Topological congruence of the ETS and ITS trees evaluated with the SH test indicated that they were significantly different \( p = 0.00 \).

Phylogenetic trees resulting from independent Bayesian and parsimony analyses of both data sets were evaluated for topological support and congruence. On the ITS topology the *Xylothamia-Gundlachia* clade had 93% bootstrap support. In all other instances monophyly of both *Ericameria* and *Xylothamia-Gundlachia* were supported by maximum posterior probabilities and bootstrap support. Including the two former clades, six nodes on the ETS phylogeny had posterior Bayesian probabilities ≥0.95 and four nodes had bootstrap support ≥70% (Fig. 2.1). Thirteen nodes on the ITS topology displayed posterior probabilities ≥0.95 and eight displayed bootstrap support ≥70% (Fig. 2.2). All nodes on the parsimony trees (not shown) with bootstrap support ≥70% also had
posterior probability support of $\geq 0.95$ (Figs. 2.1, 2.2). In addition to *Ericameria* and *Xylothamia-Gundlachia*, both data sets also support a monophyletic *Acamptopappus*. However, only the *Ericameria* and *Xylothamia-Gundlachia* clades were supported with both posterior Bayesian probability $\geq 0.95$ and bootstrap support $\geq 70\%$ (Figs. 2.1, 2.2; Table 2.2).

The ITS phylogeny displayed species of *Chrysothamnus sensu lato* in four lineages. *Chrysothamnus viscidiflorus*, the generitype and six other species comprise one lineage. It also includes *Amphipappus fremontii* var. *fremontii*, *Hesperodoria scopulorum*, and *Vanclevea*. Sister to this lineage are two unresolved clades containing *Amphipappus fremontii* var. *spinosus* and *Acamptopappus* spp. with *Tonestus lyallii* basal. Bayesian posterior probability of 1.0 and bootstrap of 76% support this clade (Fig. 2.2). In the ETS phylogram the position of *Amphipappus fremontii* var. *spinosus*, *Tonestus lyallii*, and *Tonestus graniticus* differed (Fig. 2.1) from that observed in the ITS tree. This clade though weakly supported with bootstrap support less than 50% and posterior Bayesian probability = 0.75, is identical in composition to that observed in the ITS tree (Fig. 2.2).

The second lineage containing species of *Chrysothamnus* in the ITS-based reconstruction consists of *C. baileyi*, *C. pulchellus*, *C. linifolius*, and *C. spathulatus*. Also contained in this lineage, having posterior Bayesian probability of 1.0 and bootstrap of 84%, are *Hesperodoria salicina*, *Tonestus microcephalus*, and *T. peirsonii* (Fig. 2.2). In the ETS tree, this lineage, though weakly supported, also contains *Eastwoodia elegans*, *Oreochrysum parryi*, *Stenotus pulvinatus*, and *Tonestus pygmaeus* (Fig. 2.1).

*Chrysothamnus gramineus* (*Petradoria discoidea*) in both ETS and ITS phylogenies is basal to the *Sericocarpus* clade and very distant from its traditional
congeners (Figs. 2.1, 2.2). Four other traditionally recognized chrysothamni, *C. albidus*, *C. nauseosus*, *C. paniculatus*, and *C. teretifolius* are placed in the *Ericameria* clade that has maximum support in both Bayesian (Figs. 2.1, 2.2) and MP (not shown) analyses. The maximally supported *Xylothamia-Gundlachia* lineage is distinct from all clades containing *Chrysothamnus* and from the *Ericameria* lineage (Figs. 2.1, 2.2).

Evident thus far in the ETS and ITS gene trees is the polyphyly of *Stenotus* and *Tonestus* (Figs. 2.1, 2.2). *Stenotus acaulis* and *S. amerioides* appear to be closely related to one another, as do *S. macleanii* and *S. stenophyllus*. The former two species constitute a clade in the ITS tree (Fig. 2.2) and along with *Tonestus alpinus* comprise a polytomy in the ETS tree basal to the *Solidago*-containing lineage (Fig. 2.1). The latter two form a clade in both the ITS and ETS trees distinct from other *Stenotus* (Figs. 2.1, 2.2). *Stenotus pulvinatus* is weakly affiliated with the *Chrysothamnus pulchellus* clade in the ETS phylogeny (Fig. 2.1) or it and *S. lanuginosus* are unresolved along with many other taxa in the polytomy above *Sericocarpus* in the ITS gene tree (Fig. 2.2).

As previously noted, five of the seven species of *Tonestus* included in this study are positioned within or near two of the *Chrysothamnus*-containing clades. Concerning the other two, *T. alpinus* shows affinities for *Petradoria pumila* and two species of *Stenotus* (Figs. 2.1, 2.2). Whereas, *T. eximeus*, *Columbiadoria*, representative species of *Solidago* and other taxa centered about that genus also contribute to the polytomy above the *Sericocarpus* clade (Figs. 2.1, 2.2).

**Analysis of the Combined Data.** Partition homogeneity testing indicated no significant conflict in phylogenetic signal ($P = 0.162$) between the ETS and ITS data, thus permitting their combined use (Huelsenbeck et al., 1996) in phylogenetic reconstruction of *Chrysothamnus*. The aligned, combined data set is 1230 bp long.
Maximum Parsimony. Parsimony analysis of the combined data resulted in 4,407 most parsimonious trees 533 steps long with a RI of 0.814 and a CI of 0.726 in two tree islands. Bootstrap analysis of these data resulted in 13 nodes with ≥70% support (Fig. 2.4). The monophyly of the *Ericameria*, including three of the four species transferred from *Chrysothamnus* by Nesom and Baird (1993) plus *E. albida* (*Chrysothamnus albidus*) transferred by Anderson (1995), is supported by maximum bootstrap. While Anderson (1995) actually made the *E. albida* combination, his transfer involved all *Ericameria*. Nuclear ribosomal DNA data confirm Nesom and Baird’s (1993) four species transfer, *C. nauseoa, C. paniculata*, and *C. teretifolia* here included and *C. parryi* (A. Gray) Greene (Chapter 3). In addition, these data provide support for the transfer of *C. albidus* to *Ericameria*. The *Xylothamia-Gundlachia* clade is also resolved and garners 100% bootstrap support. *Chrysothamnus gramineus* [*Petradoria discoidea*] was resolved as observed in the independent analyses (Figs. 2.1, 2.2). The other two major lineages containing species of *Chrysothamnus*, i.e., *C. viscidiflorus* and *C. pulchellus* lineages have moderate bootstrap support with 79% and 88%, respectively. *Stenotus* and *Tonestus* are not monophyletic but are resolved similar to the independent analyses of the separate data sets (Figs. 2.1, 2.2).

Maximum Likelihood. ML analysis was performed using the GTR + I + Γ model as previously described and resulted in a single tree (not shown). The tree derived from ML bootstrap analysis (Fig. 2.5; \(lnL = -5012.376\)) displayed 15 ingroup nodes with ≥70% bootstrap support, and the monophyly of *Ericameria*, including three of the recent transfers from *Chrysothamnus*, and *Xylothamia-Gundlachia* is supported. *Chrysothamnus parryi* [*E. parryi*] and *C. albidus* [*E. albida*] were not included in the ML analysis. Likewise, the monophyly of the *C. gramineus, C. viscidiflorus*, and *C. pulchellus*
Fig. 2.1. The 50% majority rule consensus tree from the Bayesian analysis of the ETS data. Mean \( \ln L: -2043.494117 \), variance: 82.678536, 95% CI: -2061.81, -2026.68. Numbers above the nodes represent parsimony changes along the branches. Numbers below the nodes represent posterior probability values and MP bootstrap support, respectively. Branches and taxon names in bold indicate species considered *Chrysothamnus* by Anderson (1986).
Table 2.2. Summary of clade support for the various data sets by optimality criteria. Shaded values indicate moderate to strong support. BI = Bayesian inference, ML = maximum likelihood, MP = maximum parsimony.

<table>
<thead>
<tr>
<th>Clade</th>
<th>Independent data analyses</th>
<th>Combined ETS and ITS data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MP-ETS</td>
<td>MP-ITS</td>
</tr>
<tr>
<td>Acamptopappus</td>
<td>&lt;70</td>
<td>94</td>
</tr>
<tr>
<td>Acamptopappus/T. graniticus</td>
<td>&lt;70</td>
<td>&lt;70</td>
</tr>
<tr>
<td>C. gramineus</td>
<td>&lt;70</td>
<td>&lt;70</td>
</tr>
<tr>
<td>C. pulchellus</td>
<td>&lt;70</td>
<td>85</td>
</tr>
<tr>
<td>C. viscidiflorus</td>
<td>&lt;70</td>
<td>76</td>
</tr>
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<td>Ericameria</td>
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<td>100</td>
</tr>
<tr>
<td>Petradoria</td>
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<td>&lt;70</td>
</tr>
<tr>
<td>Petradoria/Solidago</td>
<td>&lt;70</td>
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</tr>
<tr>
<td>Sericocarpus</td>
<td>&lt;70</td>
<td>&lt;70</td>
</tr>
<tr>
<td>Solidago</td>
<td>84</td>
<td>&lt;70</td>
</tr>
<tr>
<td>Stenotus macleanii/S.</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>stenophyllus</td>
<td>&lt;70</td>
<td>100</td>
</tr>
<tr>
<td>Xylothamia/Gundlachi</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Fig. 2.2. The 50% majority rule consensus tree from the Bayesian analysis of the ITS data. Mean lnL: -2894.763159, variance: 86.101492, 95% CI: -2913.94, -2877.30. Numbers above the nodes represent parsimony changes along the branches. Numbers below the nodes represent significant posterior probability values and MP bootstrap support, respectively. Branches and taxon names in bold indicate species regarded as Chrysothamnus by Anderson (1986).
Fig. 2.3. Burn-in plots for the independent analyses of the ETS and ITS data. Two independent runs for each data set are superimposed.
lineages were supported in this analysis. In addition, ML analysis confirmed the polyphyly of *Stenotus* and *Tonestus* (Fig. 2.5) as is evident in previous analyses (Figs. 2.1, 2.2, 2.4).

**Bayesian Analysis.** Bayesian analysis of the combined data for $1.5 \times 10^6$ generations resulted in a posterior probability distribution of $1.5 \times 10^4$ samples. Two independent analyses of the data both attained stationarity before 100,000 generations (Fig. 2.6). As a result, the initial 1,000 trees for each analysis were discarded as burn-in. Those remaining were combined yielding $2.8 \times 10^4$ sample points. The 50% majority rule consensus tree displayed 23 nodes with significance values $\geq 0.95$ (Fig. 2.7). Affiliation of *Chrysomthamus* was with the same four clades, as observed in the MP and ML analyses, supported with posterior probability values $\geq 0.96$. The polyphyly of *Stenotus* and *Tonestus* was again evident in this analysis.

Comparison of the MP and Bayesian topologies based on the combined data using the SH test failed to reject either phylogenetic hypothesis resulting from the different search algorithms ($p=0.178$). Both analytical methods together with ML similarly support the monophyly of *Ericameria* and *Xylothamia-Gundlachia* representatives. Comparison of all trees (MP, ML, and Bayesian) revealed thirteen nodes with bootstrap support of $\geq 70\%$ and posterior probabilities of $\geq 95\%$. All lineages of *Chrysothamnus* except the *Chrysothamnus gramineus* clade are well supported in all analyses of the combined data (Figs. 2.4, 2.5, 2.7; Table 2.2). The lineage including *C. viscidiflorus* had posterior probability support of 1.0, and MP and ML bootstrap support of 79% and 81%, respectively. The lineage containing *C. pulchellus* had posterior Bayesian support of 1.0, MP bootstrap support of 88% and ML bootstrap support of 87%. In all instances *Chrysothamnus gramineus* is basal to the *Sericocarpus* clade. The core of all
Fig. 2.4. Phylogenetic tree resulting from bootstrap parsimony analysis of the combined ETS and ITS data. Bootstrap percentages appear above the nodes. Tree length: 525 steps, CI: 0.69, RI: 0.77. *Chrysothamnus* lineages are indicated by thickened branches and bold type.
Fig. 2.5. Maximum likelihood estimate of phylogeny based on the combined ETS and ITS data. Numbers above the nodes represent bootstrap percentages. Taxon names in bold and thicker branches indicate species included in Chrysothamnus by Anderson (1986).
chrysothammi lineages is identical except that in the Bayesian topology (Fig. 2.7)

*Eastwoodia elegans*, *Oreochrysum parryi* and *Tonestus pygmaeus* are weakly aligned
with the *C. pulchellus* clade whereas they are unresolved in trees obtained via other
optimality criteria (Figs. 2.4, 2.5).

**Phylogenetic Relationships.** *Chrysothamnus*. Species of *Chrysothamnus sensu lato*, based primarily on analyses of the combined data, are represented by four distinct
lineages (Fig. 2.7). The type-containing clade with *C. viscidiflorus* contains six additional
species of *Chrysothamnus*. Support is also strong for the inclusion of both varieties of
*Amphipappus fremontii, Hesperodoria scopulorum, Tonestus lyallii, and Vanclevea stylosa* (Figs. 2.4, 2.5, 2.7). This clade, though weakly supported by the ETS independent
analysis (Fig. 2.1), is supported in all other analyses by bootstrap values ≥ 70% and
posterior Bayesian probabilities of ≥ 95%. The composition of this clade is identical in
all analyses.

The second lineage contains four species of *Chrysothamnus, Hesperodoria salicina, Tonestus microcephalus*, and *T. peirsonii*. This clade is weakly supported by
independent analysis of each data set (Figs. 2.1, 2.2) and in the combined data analyses
by bootstrap ≥ 84% and posterior Bayesian probabilities ≥0.95 (Figs. 2.4, 2.5, 2.7).
Bayesian analysis of the ETS and combined data weakly support the inclusion of
*Eastwoodia elegans, Oreochrysum parryi* and *Tonestus pygmaeus* in this clade (Figs. 2.1,
2.7). Finally, *Chrysothamnus gramineus* is basal to *Sericocarpus* in all analyses and not
aligned with any of its traditional congers (Figs. 2.1, 2.2, 2.4, 2.5, 2.7) while *C. albidus*, *C. nauseosus, C. paniculatus* and *C. teretifolius* are strongly aligned (bootstrap
support=100%, posterior Bayesian probability=1) with the *Ericameria* clade (Figs. 2.1,
2.2, 2.4, 2.5, 2.7). *Chrysothamnus parryi*, although not included in this study, was
Fig. 2.6. Burn-in plots for the two independent Bayesian analyses of the combined ETS and ITS data. Results of the analyses are superimposed, indicating that the log-likelihood scores converged on similar values.
Fig. 2.7. The 50% majority rule consensus tree from the Bayesian analysis of the combined ETS and ITS data. Mean lnL: -4997.686312, variance: 89.183883, 95% CI: -5017.04, -4980.03. Numbers above the nodes represent parsimony changes along the branches. Numbers below the nodes represent posterior probability values. Only posterior probability values \( \geq 95\% \) are shown. Lineages indicated by thicker branches and bold print were considered *Chrysothamnus* (Anderson, 1986).
robustly supported in *Ericameria* as proposed by Nesom and Baird (1993) in the molecular investigations of that genus (Chapter 3).

**Stenotus and Tonestus.** All analyses indicate that *Stenotus* and *Tonestus* as presently defined (Nesom and Morgan, 1990; Morse, 1998) are polyphyletic. *Stenotus stenophyllus* and *S. macleanii* are sisters in all trees. Also, *S. acaulis* and *S. armerioides* are sister and placed in a clade with *Tonestus alpinus* and *Petradoria pumila* (Figs. 2.1, 2.2, 2.4, 2.5, 2.7). Except for the ETS analysis, support for this clade is relatively strong (bootstrap ≥ 70%, Bayesian probability ≥ 0.95). *Stenotus lanuginosus* is unresolved in the clade above *Sericocarpus* in all topologies (Figs. 2.1, 2.2, 2.4, 2.5) except the one resulting from Bayesian analysis of the combined data where it is basal to a clade containing the *C. viscidiflorus/C. pulchellus* lineages (Fig. 2.7). *Stenotus pulvinatus* is placed in the same polytomy as *S. lanuginosus* in all but the ETS gene tree where it is weakly associated with the *C. pulchellus* lineage (Fig. 2.1).

The phylogenetic distribution of *Tonestus* in the present gene trees is somewhat more diverse. Preliminary analyses indicated that *Tonestus aberrans* and *T. kingii* are more closely related to *Eurybia* than to any taxa in the Solidagininae. As a result, these taxa were excluded from this investigation of *Chrysothamnus*. Topologies resulting from analyses of the ETS, ITS and combined data are not congruent in the placement of all species of *Tonestus*. *Tonestus lyallii* in all analyses is allied with the *C. viscidiflorus* lineage while *T. peirsonii* and *T. microcephallus* are included to the *C. pulchellus* clade. In addition, *Tonestus alpinus* is part of the *Solidago* lineage where it is closely aligned with *Petradoria pumila*, *Stenotus acaulis* and *S. armerioides* (Figs. 2.1, 2.2, 2.4, 2.5, 2.7). The other three species of *Tonestus* vary in their position depending on data set and optimality criteria. *Tonestus eximius* and *T. graniticus* are part of a grade basal to the *C.
*viscidiflorus* lineage in both the ML and Bayesian analyses of the combined data (Figs. 2.4, 2.7). In all other analyses, except the independent ETS, the position of these taxa is unresolved within the clade above *Sericocarpus* (Figs. 2.2, 2.5). In the ETS tree (Fig. 2.1) *T. graniticus* shows some affinity for the *Acamptopappus* clade (MP bootstrap=51%, posterior probability =0.97). Finally, *Tonestus pygmaeus* is weakly allied to the *C. pulchellus* clade in both the independent ETS and Bayesian analysis of the combined data (Figs. 2.1, 2.7) whereas, its position is unresolved above *Sericocarpus* in all other instances (Figs. 2.2, 2.4, 2.5).

**Other Taxa.** As noted previously, *Eastwoodia* is weakly aligned with the *C. pulchellus* clade in the topology resulting from Bayesian analyses of the ETS and combined data (Figs. 2.1, 2.7). In all other analyses the position of this taxon is unresolved in the clade above *Sericocarpus* (Figs. 2.2, 2.4, 2.5). *Columbiadoria* displayed no affinity to any other taxon however it was consistently placed in the clade above *Sericocarpus* (Figs. 2.1, 2.2, 2.4, 2.5, 2.7). *Oreochrysum parryi* occupied a similarly unresolved position except in the combined ETS + ITS Bayesian tree where it is placed basal to the *C. pulchellus* clade but not with significant support. The uniqueness of these two taxa has long been recognized. They have been singled-out as separate monotypic genera or infrageneric taxa in other genera throughout their taxonomic histories. The position of *Brintonia, Chrysoma, Oligoneuron* and *Solidago* was congruent in all but the ITS tree (Fig. 2.2) where *Brintonia* and *Chrysoma* were unresolved. In all but the ITS and combined data MP trees (Figs. 2.2, 2.4), the *Solidago* lineage was sister to the *Petradoria* clade (Figs. 2.1, 2.5, 2.7).

**DISCUSSION**

*Chrysothamnus.* The present gene-based phylogenies fail to support the recent concepts for *Chrysothamnus* of Anderson (1986; 1995) or of Nesom and Baird (1993).
The present investigation and that of *Ericameria* (Chapter 3) support Nesom and Baird’s (1993) transfer of *Chrysothamnus nauseosus*, *C. paniculatus*, *C. parryi*, *C. teretifolius* to *Ericameria*. *Chrysothamnus paniculatus* and *C. teretifolius* had both been considered *Ericameria* in the past by Rydberg (1917) and Jepson (1925), respectively, and support for this was also presented by Urbatsch (1975). *Chrysothamnus nauseosus* and *C. parryi* constituted section *Nauseosi* in Anderson’s (1986) rendition of the genus. Chloroplast DNA restriction site studies using representative species indicated that *C. nauseous* and *Ericameria ericoides* form a robustly supported clade (Morgan and Simpson, 1992). Investigation of phenolic compounds by McArthur et al. (1978) showed that *C. nauseous* and *C. parryi* were similar to each other but different from other *Chrysothamnus* examined as discussed by Nesom and Baird (1993). *Chrysothamnus albidus* was part of the wholesale transfer of all *Chrysothamnus* to *Ericameria* by Anderson (1995). Putative natural and garden hybrids (Anderson, 1973) between *C. albidus*, as a core *Chrysothamnus* and *E. nauseosa*, a recent transfer from *Chrysothamnus* (Nesom and Baird, 1993) was the basis for treating existing *Chrysothamnus* as *Ericameria*. Nesom and Baird (1995), however, maintained that *C. albidus* is an extraneous element within *Chrysothamnus*, a genus they maintained as distinct from *Ericameria*. Furthermore, they noted that evidence for such hybrids was not compelling. The DNA evidence, however, supports the inclusion of *C. albidus* in *Ericameria*. This species is similar to other *Ericameria* in its shrubby habit, resinous, punctate leaves, and flowers organized in many small heads. The white corollas of *E. albidus* are not unusual in *Ericameria* since this feature also occurs in *E. gilmanii* and *E. resinosa*.

These sequence-based analyses indicate that *Chrysothamnus sensu* Nesom and Baird (1993), except for *C. albidus* as discussed previously, represents three well supported lineages. A fourth lineage supports the transfer by Nesom and Baird (1993) of
four species from *Chrysothamnus* to *Ericameria*. Two of these also include taxa not previously defined as *Chrysothamnus*. One lineage consists of taxa clustered around the generitype *C. viscidiflorus*. Five other species of *Chrysothamnus* plus *Amphipappus*, *Acapmtopappus*, *Hesperodoria scopulorum*, *Tonestus lyallii* and *Vanclevea* comprise this clade. The apparent affinity of *T. lyallii* for the *C. viscidiflorus* clade is well supported in all but the ETS phylogeny. The position of *Amphipappus* and *Acamptopappus* within the *C. viscidiflorus* lineage supports, in part, the hypothesis of relationship proposed by Lane et al. (1996) who suggested their affinity for the *Chrysothamnus/Ericameria/Macronema* alliance. This investigation and the *Ericameria* study (Chapter 3) show, as had been suggested in the Noyes and Rieseberg (1999) investigations, that *Chrysothamnus* and *Ericameria* are not close relatives. While there is little resolution within the *C. viscidiflorus* lineage, the sister relationship of *Amphipappus* to *Acamptopappus* showed by Lane et al. (1996) seems unlikely. Furthermore, the two varieties of *Amphipappus*, while in the same lineage, are not sister. Nesom (2000) highlighted the differences between these two geographically distinct varieties, therefore, their lack of affinity might be expected. *Vanclevea* was considered to be related to *Acamptopappus* and *Amphipappus* by Lane (1988) as well as to *Chrysothamnus* and other genera, although few morphological synapomorphies were found to support this hypothesis. The present investigations offer little support for the purported relationship of *Eastwoodia* to *Acamptopappus*, *Amphipappus*, and *Vanclevea* suggested by Lane (1988). Rather they somewhat parallel the cpDNA findings (Lane et al., 1996) where *Eastwoodia* shows some affinity for the *Solidago/Oligoneuron* group. However, neither their cpDNA data set nor the present ETS + ITS sequence data provides robust phylogenetic resolution of taxa in this part of the tree.
Concerning the taxonomy of this lineage, I propose that *Chrysothamnus* be redefined to include the widespread *C. viscidiflorus* complex and the taxa beginning at the node with *Tonestus lyallii*. The genus would encompass these two taxa plus *Acamptopappus*, the two varieties of *Amphipappus fremontii*, *Hesperodoria scopulorum*, *Vanclevea*, and six additional species of *Chrysothamnus* (see Figs. 2.2, 2.4, 2.5, 2.7).

Most of these species are adapted to arid regions in western North America including the Great Basin and surrounding arid habitats, the Mojave and Sonoran Deserts, and for some species their ranges extend into juniper woodlands. Most species are shrubs with white bark. *Tonestus lyallii* is unlike the other desert-adapted shrubs in this clade. It is a diminutive constituent of alpine sedge land communities of the northern Rocky Mountains and the Olympic Mountains in western Washington where it tends to grow in protected, moist areas (Hall, 1928). A separate study is underway to investigate the morphology and other features in more detail for the taxa in this clade. The taxonomy and nomenclature will be considered elsewhere. *Tonestus graniticus*, a narrow endemic of the desert mountains of southern Nevada, and *T. eximeus* that is restricted to alpine and subalpine sites in the northern high Sierra Province of California (Brown, 1993), show some affinities for this lineage, but support is not robust. Additional molecular plus morphological investigations may help to resolve more definitively these latter suggested relationships.

The second lineage resolved with core *Chrysothamnus* includes *C. pulchellus*, *C. spathulatus*, *C. linifolius*, *C. baileyi*, *Hesperodoria salicina*, *T. microcephalus*, and *T. peirsonii*. A close relationship of these two *Chrysothamnus* lineages to one another is not supported in the present analyses. Sequence data support the affinity of *T. microcephalus* for *Chrysothamnus* as suggested by Lane et al. (1996) and Nesom (2000). *Hesperodoria sensu* Nesom (1991) appears to be biphyletic with the generitype, *H. scopulorum*, aligned
with the *C. viscidiflorus* lineage while the other species, *H. salicina*, is placed in the *C. pulchellus* clade. Nesom (2000) noted that the two species shared certain features, but they also differed in many ways. The ETS + ITS data support this suggestion of differences and offer no support for merging *Hesperodoria sensu lato* with *Petradoria* and *Vanclevea* into a single genus as discussed by Nesom (2000). *Tonestus peirsonii*’s placement in this clade is unexpected since its appearance is quite different due to its having toothed leaves, stalked glandular hairs, and large heads. In order to check whether its placement in this clade is due to contaminated DNA samples, several sequences for this taxon were run at different times from independent extractions of the sample. The results, however, proved to be the same.

Although the taxonomy for this group will be considered elsewhere, it appears that this clade constitutes a distinct genus consisting of *C. baileyi*, *C. linifolius*, *C. pulchellus*, *C. spathulatus*, *Hesperodoria salicina*, *Tonestus microcephalus*, and *T. peirsonii*. Most of these species are shrubs or subshrubs with somewhat restricted geographic ranges. Except for *T. peirsonii*, which occurs in the high central Sierra Nevada province in California (Brown, 1993), the center of diversification for this lineage appears to be the southern Rocky Mountains. The range for *C. baileyi* also extends eastward in the plains of eastern New Mexico and the western Texas panhandle. This lineage shows certain parallels with the *C. viscidiflorus* clade in that most species are adapted to arid, mid-elevation habitats with one or two outliers adapted to high elevations. In this lineage both *T. microcephalus* and *T. peirsonii* are perennials with a branching caudex and for the latter species fewer, larger capitula.

The third *Chrysothamnus* lineage consists of one species, *C. gramineus*. Anderson (1963) based on anatomical similarities transferred *C. gramineus* to *Petradoria* as *P. discoidea*. However, he later rescinded this action with his discovery of *C.
eremobius (Anderson, 1983, 1986) and subsequently treated the two species as
Chrysothamnus section Gramini. The sequence-based trees are unequivocal about the
distinctness of C. gramineus. Its placement outside of the Sericocarpus clade offers no
support for previous hypotheses of relationship. Chrysothamnus gramineus should
therefore be recognized as a monotypic genus.

The sequence-based phylogenies present a very different view of Petradoria.
Although generally maintained as a monospecific genus, Anderson (1963), as noted
previously, had included C. gramineus [P discoidea] in Petradoria. In the gene trees
presented here, taxa occurring in the same clade as P. pumila are Tonestus alpinus and
two species of Stenotus. This group shows some affinity for Solidago and relatives rather
than any lineage containing Chrysothamnus. Lane et al. (1996) who included only these
two species of Stenotus, S. acaulis and S. armerioides, in their cpDNA survey of North
American Astereae, provided strong support for their affinity. Petradoria pumila,
however, was shown to be a couple nodes removed from Stenotus in their reconstruction.
Because their data matrix out-sized computer memory, they implemented MacClade’s
“merge taxa” function to produce “merged” and “twice merged” matrices. In the
investigation of the Xylothamia lineage (Chapter 4) it was shown that this methodology
resulted in topologies that differed significantly from robust sequence-based ones. This
observation suggests that the merge taxa function when used on matrices with low levels
of variation may be ineffective in recovering accurate phylogenies.

In habit, all four taxa in the Petradoria lineage are similar in having much
branched, woody caudices crowned with numerous annual, aerial stems bearing capitula.
Petradoria pumila, S. acaulis, and S. armerioides are similar in having multiple clusters
of persistent leaves arising from the caudices. The leaves in these three species are
similar in being narrow, elongate, often linear with prominent veins. Tonestus alpinus
differs in having much broader leaves with more obscure veins. Capitulescence form
offers the most conspicuous differences between *P. pumila* which has numerous, narrow,
cylindric heads in dense terminal, corymiform clusters and the other three species whose
individual shoots are monocephalous. Noteworthy is that *S. acaulis* is the type species
for *Stenotus* and it has priority over *Petradoria*. Thus, if the sequence-based
reconstructions withstand further scrutiny, *Petradoria* would reside in the synonymy of
*Stenotus* whose concept would also differ dramatically from present ones. The pattern of
morphological variation in the *Petradoria* clade, where lower elevation taxa have
numerous small heads compared to higher elevations taxa with fewer, larger heads, is
parallel to that observed in the *C. viscidiflorus* and the *C. pulchellus* lineages.

With reference to infrageneric relationships, the present molecular data do not
support the specific, sectional composition in *Chrysothamnus* as proposed by Anderson
(1986). Molecular, phylogenetic analyses consistently place *C. linifolius* and *C.
spathulatus*, members of Anderson’s section *Chrysothamnus*, with *C. pulchellus*, a
member of his section *Pulchelli*. Furthermore, in all analyses *C. depressus*, *C. vaseyi* and
*C. molestus* are consistently associated with species in Anderson’s section
*Chrysothamnus* rather than with section *Pulchelli*. Section *Gramini* lacks molecular
support in that *C. eremobius* is associated with the *C. viscidiflorus* group whereas *C.
gramineus* is but distantly related to any *Chrysothamnus*, *sensu lato* Traditionally used
morphological features of the capitulum and other organs do not seem to be useful as
characters for defining generic boundaries of *Chrysothamnus* and associated taxa as had
been envisioned by previous workers (Hall and Clements, 1923; Anderson, 1986).

The present sequence data provide no support for the traditional relationships of
*Columbiadoria*, *Eastwoodia* and *Oreochrysum* (Lane, 1988, Lane et al., 1996; Nesom,
1991, 1993, 1994). Instead all three taxa are for the most part unresolved in a clade above
Sericocarpus. All three taxa typically have been recognized as distinct genera or infrageneric taxa.

**Stenotus and Tonestus.** Neither a monophyletic Stenotus nor Tonestus is supported by the results of this investigation. Stenotus is represented in this investigation by all six species recognized by Morse (1998). In his primarily morphological-based treatment, he maintained the monophyly of Stenotus. However, analyses of ETS and ITS data, independently and combined, do not support such a concept. *Stenotus pulvinatus*, a diminutive, moss-like plant known from only a few sites in Baja California, does not appear to be related to other Stenotus. Nesom (1989) noted that this species is a geographically isolated member of the genus that is also morphologically unique in having eradiate heads. In this study it is one of many taxa contributing to the polytomy basal to the *C. pulchellus*, *C. viscidiflorus*, and *Petradoia* clades.

Most other species of Stenotus appear associated with at least one other of their congeners except for *S. lanuginosus*, which is of unresolved position in all but the tree resulting from the Bayesian analysis of the combined data (Fig. 2.7). On this tree it appears basal to a lineage composed of the *C. viscidiflorus* and *C. pulchellus* clades, although Bayesian probability support for this relationship is <0.95, the value considered significant. *Stenotus macleanii* and *S. stenophyllus* form a well-supported clade in all but the tree resulting from independent analysis of the ETS data. In fact, the branch supporting this clade is one of the longest on the gene trees. *Stenotus macleanii* is restricted to the Yukon and *S. stenophyllus* occurs in the northwestern United States. *Stenotus acaulis*, the generitype, and *S. armerioides* are included in the well-supported *Petradoria* clade as noted previously. *Stenotus acaulis* and *S. armerioides* are the only species of *Stenotus* that associate with *Petradoria*. Lane et al. (1996) and Nesom (2000) presumably assuming the monophyly of *Stenotus* hypothesized a close relationship for
these two genera. This investigation offers no support for a close relationship of *Stenotus* to any clade containing *Chrysothamnus* as suggested by Neson and Baird (1993).

*Tonestus* was reinstated as a genus (Nesom and Morgan, 1990) but its composition seems to be as eclectic as it was when attributed sectional status in *Haplopappus*. Nesom (2000) commented on the morphological heterogeneity of the genus and concluded that the group as defined may not be monophyletic. This investigation revealed no species of *Tonestus* that were consistently more closely aligned to each other than to species presently placed in other genera. Lane et al. (1996) and Nesom (2000) hypothesized a relationship between *T. microcephalus* and *Chrysothamnus*. The sequence data support this suggestion in that the species placed in a clade with a subset of taxa formerly regarded as *Chrysothamnus*, i.e. the *C. pulchellus* lineage that also contains *T. peirsonii*. The diverse association of the species in *Tonestus* with other taxa requires a redefinition of the genus. The generitype, *T. lyallii*, is placed in the *C. viscidiflorus* clade. *Tonestus alpinus* is aligned with the *Petradoria* lineage while *T. eximius*, *T. graniticus* and *T. pygmaeus* are of unresolved or exhibit only weakly supported affinities in most analyses. The molecular data partially support the proposed relationship of some species of *Tonestus* to *Chrysothamnus* (Lane et al., 1996; Nesom and Morgan, 1990). However, it also supports the hypothesis of a polyphyletic *Tonestus*.

This study also offers caution in the use of morphological similarity in assessing affinity among this group of taxa. Characteristics of habit, leaf size and position used to distinguish between *Stenotus* and *Tonestus* are possibly the result of convergent evolution and therefore may not be indicative of evolutionary affinity. If the sequence-based reconstructions represent true relationships for the taxa investigated, a similar evolutionary scenario where taxa are evolving in a convergent manner in at least three parallel lineages identified in the present study. The *C. viscidiflorus*, the *C. pulchellus*
clade, and the *Petradoria* clades all contain shrubs, large ones in some cases, with numerous small capitula adapted for arid habitats at relatively lower elevations, and diminutive taxa with annual, herbaceous, aerial stems with larger leaves and capitula that are adapted to alpine or subalpine habitats.

**LITERATURE CITED**


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CHAPTER 3. MOLECULAR PHYLOGENY OF ERICAMERIA (ASTERACEAE: ASTEREEAE) BASED ON NUCLEAR RIBOSOMAL 3’ ETS AND ITS SEQUENCE DATA

INTRODUCTION

Within the past 10 years two different classifications have been proposed for Ericameria Nutt., a genus of shrubs distributed throughout western North America. Generic remodeling was initiated with the circumscription of Xylothamia G. L. Nesom, Y. B. Suh, D. R. Morgan & B. B. Simpson (Nesom et al., 1990) and the subsequent transfer of four species from Chrysothamnus Nutt. to Ericameria by Nesom and Baird (1993). Xylothamia was established to accommodate seven species of Ericameria from northern Mexico and southern Texas. The four species of Chrysothamnus placed in Ericameria include C. nauseosus (Pallas ex Pursh) Britton, C. paniculatus (A. Gray) H. M. Hall, C. parryi (A. Gray) Greene and C. teretifolius (Dur. & Hilg.) H. M. Hall. Subsequent to establishing Xylothamia, Ericameria was expanded to include Haplopappus Cass. sections Asiris (H. M. Hall) G. L. Nesom and Macronema (Nutt.) G. L. Nesom (Nesom, 1990). Nesom also accepted, with modification, the transfer of Stenotopsis (Rydb.) Urbatsch & Wussow to Ericameria (Urbatsch and Wussow, 1979). Chrysothamnus, however, was maintained as a genus distinct from Ericameria albeit reduced in size (Nesom, 1990; Nesom and Baird, 1995). Nesom (1994, 2000) did not regard Ericameria as closely related to Chrysothamnus, having placed them in different subtribes, the Hinterhuberinae and in the Solidagininae, respectively. In contrast, Bremer (1994) in his cladistic analysis of Astereae using mainly morphological features treated Ericameria in subtribe Solidagininae sister to Chrysothamnus.

(Anderson, 1973), the former a core Chrysothamnus sensu Nesom & Baird (1993) and
the latter a recent transfer from Chrysothamnus to Ericameria. As a consequence
Anderson (1995) incorporated all remaining species of Chrysothamnus in Ericameria
thus increasing its size from the 32 species placed in that genus by Nesom (1990) and
Nesom and Baird (1993, 1995) to 44. This action not only combined what were
considered two distinct genera, but members of two distinct subtribes sensu Nesom
(1994, 2000). Nesom and Baird (1995) opposed Anderson’s treatment, arguing that the
morphological similarity used as evidence implicating C. albidus in the parentage of the
purported hybrid was insufficient. However, phylogenetic reconstruction based on
nuclear ribosomal DNA (nrDNA) sequence data indicated that C. albidus and the species
with which it purportedly hybridizes, E. nauseosa (formerly C. nauseosus (Pall. ex
Pursh) Britton), are both robustly supported within the Ericameria lineage (Chapter 2).

Nuttall (1841) proposed the genus Ericameria and accommodated three species
within it noting that they cannot be, in any respect, congeners of [H]aplopappus ciliatus
DC., “a genuine Chilian species.” Following its circumscription, several researchers
followed Nuttall and accorded generic rank to Ericameria (Torrey and Gray, 1842; Hall,
1907; Wiggins, 1933) while others relegated it to sectional status in Haplopappus (Gray,
1865; Hall, 1928). Certain species in Ericameria have also been considered congeneric
with Bigelowia (Gray, 1873) and Chrysoma (Greene, 1895).

The most inclusive treatment for Ericameria sensu Nesom (1990) was Hall’s
monograph (1928). Hall accorded Ericameria and its present congeners Asiris,
Macronema, and Stenotopsis sectional rank in Haplopappus. His phylogenetic diagram
depicted Asiris and Ericameria as terminal sister taxa in one of two major lineages of
North American species while the other two sections, Macronema and Stenotopsis, were
placed in the other lineage. Both *Macronema* and *Stenotopsis* were portrayed as related to sections *Oreochrysum* (Rydb.) H. M. Hall, *Stenotus* (Nutt.) H. M. Hall, and *Tonestus* (A. Nelson) H. M. Hall and not to *Ericameria* and *Asiris*. Hall’s treatment, rather than ending the controversy regarding the composition and rank of *Ericameria*, spurred more investigations into its relationship to *Haplopappus sensu lato* that came to be recognized by most researchers as polyphyletic as summarized by Lane and Hartman (1996).

Johnston (1970) recognized *Ericameria* at the rank of genus rather than as a section in *Haplopappus*. Urbatsch (1975, 1976, 1978) followed this trend, despite the fact that other researchers continued to describe new species as *Haplopappus* (Anderson, 1983; Welsh, 1993). Section *Stenotopsis* was transferred to the genus *Ericameria* and redefined by Urbatsch and Wussow (1979) to accommodate *E. linearifolia* and *E. cooperi*, the latter a core species within *Haplopappus* section *Ericameria sensu* Hall (1928). They cited natural hybridization between the two species as evidence supporting their close affinity. Field investigations by Cody and Thompson (1986) confirmed the observations of Urbatsch and Wussow (1979), but also suggested *E. laricifolia* as an alternative parental species. It and *H. linearifolia* are sympatric in the area where the reported hybrids between *E. linearifolia* and *E. cooperi* occur. However, noted differences in phenology, *E. cooperi* and *E. laricifolia* flower in the spring and late fall, respectively, and in habitat preference make this hypothesis less probable. *Stenotopsis* contained two species when treated as a section in *Haplopappus* (Hall, 1928), *H. linearifolius* DC. (*E. linearifolia*) and *H. parrasanus* S. F. Blake (*Xylothamia parrasana* (S. F. Blake) G. L. Nesom). These two now appear but distantly related (Nesom et al., 1990; Chapter 4).
What has emerged since Hall’s (1928) monograph for *Ericameria* is a greatly expanded genus wherein the species are classified into four sections shown in Table 3.1 (Nesom, 1990; Nesom and Baird, 1993, 1995). However, hypotheses regarding *Ericameria’s* relationship to other putative congeners and its sister and phylogenetic relationships to other *Astereae* are diverse. This investigation seeks to answer the following questions using sequence data of nuclear ribosomal DNA (nrDNA), specifically the internal transcribed spacer (ITS 1 and ITS 2 including the 5.8s region) and the 3′ end of the external transcribed spacer (ETS): (1) What is the specific composition of *Ericameria*? (2) Do molecular data support the sectional classification recently proposed by Nesom (1990) and Nesom & Baird (1993, 1995)? (3) What is the relationship of *Ericameria*, based on molecular data, to other taxa of North American *Astereae*? (4) Is there congruence between relationships based on sequence data with those implied using morphological characters?

**MATERIALS AND METHODS**

**Sampling.** Fifty sequences spanning the ITS-1, ITS-2 and the intervening 5.8S, and the 3′ ETS region of nuclear ribosomal DNA (nrDNA) were analyzed from samples representing 48 species in 12 genera of Astereae. Subtribes sampled include Solidagininae, Hinterhuberinae, and primitive asters *sensu* Nesom (2000). All known species of *Ericameria* were sampled. Identifications were based on the keys of Welsh et al. (1987) and of Brown and Keil (1993), and by comparison to specialist-annotated herbarium specimens. Because of its hypothesized relationship to *Ericameria*, (Hall and Clements, 1923; Hall, 1928; Anderson, 1995; Nesom and Baird, 1995), representatives of *Chrysothamnus* Nutt. and 10 other genera were also included in this study. *Doellingeria* Nees was implicated as a suitable outgroup based on Noyes and Rieseberg (1999) and on

<table>
<thead>
<tr>
<th>Section</th>
<th>Species</th>
</tr>
</thead>
</table>
| *Asiris* | *E. cervina*  
|         | *E. nana*  
|         | *E. obovata*  
|         | *E. resinosa*  
|         | *E. watsonii*  
| *Ericameria* | *E. arborescens*  
|         | *E. brachylepis*  
|         | *E. cooperi*  
|         | *E. cuneata*  
|         | *E. ericoides*  
|         | *E. fasciculata*  
|         | *E. juarezensis*  
|         | *E. laricifolia*  
|         | *E. martirensis*  
|         | *E. palmeri*  
|         | **E. paniculata**  
|         | *E. parishii*  
|         | *E. pinifolia*  
|         | **E. teretifolia**  
| *Macronema* | *E. bloomeri*  
|         | *E. compacta*  
|         | *E. crispa*  
|         | *E. discoidea*  
|         | *E. gilmanii*  
|         | *E. greenei*  
|         | *E. lignumviridis*  
| *E. nauseosa* | *E. ophtidis*  
| *E. parryi* | *E. suffraticosa*  
| *Stenotopsis* | *E. zionis*  
|         | *E. linearifolia*  

preliminary analysis of a larger data set containing worldwide representatives of Astereae (see Chapter 5). Collection, voucher deposition data and author citations are provided in Table 3.2.

In order to insure sequence fidelity, at least two individuals per taxon were sampled, or for rare plants, at least two independent extractions and amplifications of the DNA of interest were performed. Ultimately, 46 ITS and 66 ETS sequences obtained were excluded to facilitate statistical comparison of data sets and resulting trees. However, the final data set contained at least one sequence for each taxon.

**DNA Isolation, Polymerase Chain Reaction (PCR) and Sequencing.** Total genomic DNA was isolated from approximately 20–500 mg of leaf material. Where possible, fresh leaf tissue samples were obtained in the field, placed immediately in liquid nitrogen, and subsequently stored at –80°C. When fresh plant tissue was unobtainable, samples from herbarium specimens were used. In preparation for DNA extraction, frozen tissue was ground with a mortar and pestle in liquid nitrogen while dried tissue was ground using a small amount of sterile sand. Later in the project, both types of plant tissue were pulverized in a Mini-BeadBeater-8™ (BioSpec Products, Inc. Bartlesville, Oklahoma) for approximately 30-60 seconds. Fresh samples were kept frozen during pulverization while dried samples were processed at ambient temperature. For the latter, the lysis time was increased to 60 min rather than the 10 min recommended by the manufacturer. Initially, the 2X CTAB (hexadecyltrimethylammonium bromide) extraction protocol of Doyle and Doyle (1987) was employed. Later, DNA extraction was accomplished from the ground tissue using the Qiagen DNeasy™ Plant Mini Extraction Kit and protocol.
Table 3.2. Taxa sampled for this investigation, collection localities, voucher numbers, and Genbank accession numbers.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Source localities and voucher information</th>
<th>ITS GenBank Number</th>
<th>ETS GenBank Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chrysoma pauciflosculosa</em> (Michx.) Greene</td>
<td>Florida: Gulf Co., Urbatsch 7609 (LSU)</td>
<td>AF477637</td>
<td>AF477701</td>
</tr>
<tr>
<td><em>Chrysothamnus depressus</em> Nutt.</td>
<td>Colorado: Mesa Co., Urbatsch 1313 (LSU)</td>
<td>AY170932</td>
<td>AY169729</td>
</tr>
<tr>
<td><em>Chrysothamnus gramineus</em> H. M. Hall</td>
<td>Nevada: Clark Co., Alexander 457 (UNLV)</td>
<td>AY170936</td>
<td>AY169733</td>
</tr>
<tr>
<td><em>Chrysothamnus linifolius</em> Greene</td>
<td>Utah: Uinta Co., Urbatsch 7068 (LSU)</td>
<td>AY170940</td>
<td>AY169737</td>
</tr>
<tr>
<td><em>Chrysothamnus pulchellus</em> (A. Gray) Greene</td>
<td>New Mexico: Lincoln Co., Urbatsch 7977 (LSU)</td>
<td>AY170942</td>
<td>AY169739</td>
</tr>
<tr>
<td><em>Chrysothamnus viscidiflorus</em> (Hook.) Nutt. ssp. viscidiflorus</td>
<td>California: Lassen Co., Urbatsch 7712 (LSU)</td>
<td>AY170947</td>
<td>AY169744</td>
</tr>
<tr>
<td><em>Doellingeria umbellata</em> Nees</td>
<td>Michigan: Chippewa Co., Schmidt 1060 (TEX)</td>
<td>AF477625</td>
<td>AF477754</td>
</tr>
<tr>
<td><em>Ericameria albida</em> (M. E. Jones ex A. Gray) L.C. Anderson</td>
<td>Nevada: Nye Co., Urbasch 1459 (LSU)</td>
<td>AY170950</td>
<td>AY169747</td>
</tr>
<tr>
<td><em>Ericameria arborescens</em> (A. Gray) Greene</td>
<td>California: San Luis Obispo Co., Keil K14219 (TEX)</td>
<td>AY170951</td>
<td>AY169748</td>
</tr>
<tr>
<td><em>Ericameria brachylepis</em> (A. Gray) H. M. Hall</td>
<td>Mexico: Baja California, Burgess 6106 (TEX)</td>
<td>AY171007</td>
<td>AY170975</td>
</tr>
<tr>
<td><em>Ericameria cervina</em> (S. Watson) Rydb. 1</td>
<td>Arizona: Coconino Co., Brian 98-291 (ASC)</td>
<td>AY171008</td>
<td>AY170976</td>
</tr>
<tr>
<td><em>Ericameria cervina</em> (S. Watson) Rydb. 2</td>
<td>Arizona: Mohave Co., Gierisch 4486 (ASC)</td>
<td>AY171009</td>
<td>AY170977</td>
</tr>
<tr>
<td><em>Ericameria compacta</em> (H. M. Hall) G. L. Nesom</td>
<td>Nevada: Clark Co., Urbatsch 171010 &amp; Roberts 7940 (LSU)</td>
<td>AY171010</td>
<td>AY170978</td>
</tr>
<tr>
<td><em>Ericameria cooperi</em> (A. Gray) H. M. Hall</td>
<td>California: San Bernardino Co., Helmkamp s. n. (TEX)</td>
<td>AF477640</td>
<td>AF477704</td>
</tr>
<tr>
<td><em>Ericameria crispa</em> (L.C. Anderson) G. L. Nesom</td>
<td>Utah: Washington Co., Baird &amp; Warick 3196 (BRY)</td>
<td>AY171011</td>
<td>AY170979</td>
</tr>
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</table>

(Table 3.2 cont’d.)
<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Accession Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ericameria cuneata</strong> (A. Gray) McClatchie</td>
<td>California: Inyo Co., Urbatsch &amp; Roberts 7957 (LSU)</td>
<td>AF477641 AF477705</td>
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<tr>
<td><strong>Ericameria discoidea var. discoidea</strong> (Nutt.) G. L. Nesom</td>
<td>Utah: Utah Co., Thompson 9067 (TEX)</td>
<td>AY171012 AY170980</td>
</tr>
<tr>
<td><strong>Ericameria discoidea var. linears</strong> (Ryd.) G. L. Nesom</td>
<td>Idaho: Bear Lake Co., Winward s. n. (BRY)</td>
<td>AY171013 AY170981</td>
</tr>
<tr>
<td><strong>Ericameria ericooides</strong> (Less.) Jeps.</td>
<td>California: Monterey Co., Sundberg 2646 (TEX)</td>
<td>AF477642 AF477706</td>
</tr>
<tr>
<td><strong>Ericameria fasciculata</strong> (Eastw.) J. F. Macbr.</td>
<td>California: Monterey Co., Griffin 3968 (LSU)</td>
<td>AY171014 AY170982</td>
</tr>
<tr>
<td><strong>Ericameria gilmanii</strong> (S. F. Blake) G. L. Nesom</td>
<td>California: Inyo Co., Urbatsch &amp; Roberts 7948 (LSU)</td>
<td>AY171015 AY170983</td>
</tr>
<tr>
<td><strong>Ericameria greenei</strong> (A. Gray) G. L. Nesom</td>
<td>California: Trinity Co., Urbatsch &amp; Karaman 7706 (LSU)</td>
<td>AY171016 AY170984</td>
</tr>
<tr>
<td><strong>Ericameria juarezensis</strong> (Moran) Urbatsch</td>
<td>Mexico: Baja California, Moran 22986 (LSU)</td>
<td>AY171017 AY170985</td>
</tr>
<tr>
<td><strong>Ericameria laricifolia</strong> (A. Gray) Shinners</td>
<td>Texas: El Paso Co., Carr 10230 (TEX)</td>
<td>AY171018 AY170986</td>
</tr>
<tr>
<td><strong>Ericameria lignumviridis</strong> (S. L. Welsh) G. L. Nesom</td>
<td>Utah: Sevier Co., Greenwood 5566 (BRY)</td>
<td>AY171019 AY170987</td>
</tr>
<tr>
<td><strong>Ericameria linearifolia</strong> (DC.) Urbatsch &amp; Wussow</td>
<td>California: Inyo Co., Schramm 743 (UNLV)</td>
<td>AY171020 AY170988</td>
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<tr>
<td><strong>Ericameria martirensis</strong> Wiggins</td>
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<td><strong>Ericameria nana</strong> Nutt.</td>
<td>Utah: Esmeralda Co., Urbatsch &amp; Roberts 7946 (LSU)</td>
<td>AY171022 AY170990</td>
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<tr>
<td><strong>Ericameria obovata</strong> (Ryd.) G. L. Nesom</td>
<td>Nevada: Elko Co., Urbatsch &amp; Karaman 7669 (LSU)</td>
<td>AY171024 AY170992</td>
</tr>
<tr>
<td><strong>Ericameria palmeri</strong> (A. Gray) H. M. Hall</td>
<td>California: San Bernardino Co., Sanders 14215 (TEX)</td>
<td>AY171026 AY170994</td>
</tr>
</tbody>
</table>

(Table 3.2 cont’d.)
Ericameria paniculata (A. Gray) Rydb.  
Nevada: Nye Co., Reveal 2014 (TEX)  
AY170953 AY169750

Ericameria parishii (Greene) H. M. Hall  
California: San Diego Co., Urbatsch 7082 (LSU)  
AY171028 AY170996

Ericameria parryi (A. Gray) G. L. Nesom & G. I. Baird  
California: Kern Co., Helmkamp SN (TEX)  
AY171029 AY170997

Ericameria pinifolia (A. Gray) H. M. Hall  
California: San Diego Co., Urbatsch 7084 (LSU)  
AY171030 AY170998

Ericameria resinosa Nutt.  
Washington: Klickitat Co., Brooks 20195 (RM)  
AY171031 AY170999

Ericameria suffruticosa (Nutt.) G. L. Nesom  
Nevada: Humboldt Co., Tiehm 9999 (TEX)  
AY171032 AY171000

Ericameria teretifolia (Durand & Hilg.) Jeps.  
California: Inyo Co., Morefield 3130 (TEX)  
AY170954 AY169751

Ericameria watsonii (A. Gray) G. L. Nesom  
Nevada: White Pine Co., Tiehm 11446 (CAS)  
AY171034 AY171002

Ericameria zionis (L.C. Anderson) G. L. Nesom  
Utah: Garfield Co., Urbatsch & Roberts 7922 (LSU)  
AY171035 AY171003

Gundlachia corymbosa (Urb.) Britton ex Bold.  
West Indies: Caicos Islands. Pine Cay, Correll 43104 (LL)  
AF477654 AF477718

Pentachaeta exilis (A. Gray) A. Gray  
California: Monterey Co., Keil 17085 (TEX)  
AY171036 AY171004

Rigiopappus leptocladus A. Gray  
California: Modoc Co., Bartholomew 6575 (TEX)  
AY171037 AY171005

Sericocarpus tortifolius (Michx.) Nees  
Florida: Wakulla Co., Urbatsch 7599 (LSU)  
AF477664 AF477728

Solidago canadensis L.  
Louisiana: W. Feliciana Parish, Lievens 3347 (LSU)  
AF477665 AF477729

Tracyina rostrata S. F. Blake  
California: Humboldt Co., Ornduff 6348 (US)  
AF477673 AF477737

Xylothamia triantha (S. F. Blake) G. L. Nesom  
Texas: Brewster Co., Poewll 3542 (TEX)  
AF477687 AF477751

Xylothamia truncata G. L. Nesom  
Mexico: Coahuila. Nesom 5254 (TEX)  
AF477688 AF477752
Double-stranded DNA for sequencing was initially generated in 50 µl and later in 25 µl reactions. The latter reaction size used 0.5 unit Taq DNA polymerase (Epicentre Technologies, Madison, Wisconsin), 8 µl H2O, 12.5 µl premix buffer G (Epicentre Technologies), 1 µl each of 10nM forward and reverse primers, and 2 µl of DNA template usually diluted 10⁻². Reactants in the 50 µl reactions were doubled. The initial 10 thermal cycles each consisted of 1 min of denaturation at 95°C, 1 min of annealing at 55°C and, 1 min of extension at 72°C plus 4 sec per cycle. The following 20 cycles were similar except for an annealing temperature reduction to 50°C, extension time increased to 1 min 40 sec plus 0.4 sec per cycle, and ending with a 7 min extension at 72°C. After completion of the 30 cycles, reactions were kept at 4°C until removed from the thermal cycler. This protocol proved adequate for the amplification of both the ITS and ETS regions. All reactions were performed using a PTC-100™ Thermal Cycler (MJ Research, Inc., Watertown, Massachusetts).

For the amplification of the ITS region, primers ITS-20 and ITS-262 (Urbatsch et al., 2000) were used in equimolar concentrations. When amplicon production was inadequate, products from the above reactions were used as templates and reamplified in subsequent PCR reactions using a set of nested primers, –ITS-I.1 (Chapter 2) modified from primer ITS-I (Urbatsch et al., 2000), and ITS4 (White et al., 1990), in order to increase yield. The ETS region was amplified using the primers 18S-ETS (Baldwin and Markos 1998) and Ast-8 (Markos and Baldwin 2001). Because of amplification failure with the previous primer pair, products from the above reactions were used as templates and reamplified in subsequent PCR reactions using a set of nested primers 18SR1 (Chapter 2) and Ast-1 (Markos and Baldwin, 2001) to increase amplicon yield.
Two PCR purification kits, QIAquick™ PCR Purification Kit (Avenue Stanford, Valencia, California) and Novagen SpinPrep™ (Novagen, Madison, Wisconsin) were used for the purification of amplicons. The concentration of purified amplicons was estimated visually on agarose gels using Low DNA Mass Ladder (Life Technologies, Inc. Rockville, Maryland). Purified amplicons were sequenced using ABI PRISM® BigDye™ Terminator cycle sequencing (Applied Biosystems, Foster City, California.) and run on an ABI 377 automated DNA sequencer. The cycle sequence reactions were 10 µl total volume and included 2 µl BigDye™ terminators, 2 µl of 10nM primer, 15–45ng purified amplicons, and water when necessary. The reactions were run for 25 cycles; 96°C for 10 sec denaturation, 50°C for 5 sec annealing and 60°C for 4 min extension in the thermal cycler previously noted.

Several samples yielded polymorphic sequences. When this occurred the amplicons were cloned using the TOPO TA Cloning® System (Invitrogen, Carlsbad, California) or the Perfectly Blunt™ Cloning Kit (Novagen, Inc., Madison, Wisconsin). Inserts were amplified directly from bacterial colonies using the ITS and ETS primers discussed above. The PCR protocol was modified to include an initial 10 min at 94°C to lyse the bacterial cells. The amplicons were purified as described above in preparation for cycle sequencing reactions. Typically, two or more clones were amplified and sequenced for each polymorphic sample.

**Sequence Analysis.** Sequences were edited with the aid of Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan). Initially, alignments were performed using Clustal W (Thompson et al., 1994) by submitting data to the Baylor College of Medicine sequence launcher (http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html). Additionally, manual sequence adjustments were made as judged necessary. Sequences
subsequently obtained were aligned by manual comparison to the existing data matrix. Boundaries of the ITS and ETS regions were determined by comparison with published sequences of the Heliantheae (Urbatsch et al., 2000) and Astereae (Baldwin and Markos, 1998; Markos and Baldwin, 2001). Cloned sequences were entered in the matrix as individual OTUs following Urbatsch et al., (2000). Pairwise sequence divergence estimates were obtained using PAUP* 4.0b10 (Swofford, 2002). MacClade version 4.0 (Maddison and Maddison, 2000) was used for examination and editing of sequence alignment. All ITS and ETS sequences analyzed in this study were deposited in GenBank under the accession numbers provided in Table 3.2.

**Phylogenetic Analysis.** Analyses were conducted individually on the ITS and ETS data sets and on a combined ITS/ETS matrix. Data analyses employed several optimality criteria: maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference. Bayesian inference was performed using MrBayes 2.01 (Huelsenbeck and Ronquist, 2001). All other analyses were performed using PAUP* 4.0b10 including the independent parsimony analysis of the ITS data for which PAUPRat (Sikes and Lewis, 2001) was also used. Models of sequence evolution required for ML and Bayesian estimations of phylogeny were obtained using Modeltest 3.04 (Posada and Crandall, 1998). The size of the data set dictated that heuristic search strategies be implemented for all phylogenetic estimates using PAUP* 4.0b10.

**Maximum Parsimony.** Equally weighted MP searches with 100 sequence addition replicates and tree bisection-reconnection (TBR) were conducted. All MP analyses were performed with MULTREES on, ACCTRAN optimization and gaps treated as missing data using all potentially, phylogenetically informative characters. Heuristic parsimony analysis of the ITS data in PAUP* 4.0b10 failed due to exhaustion
of computer memory compromising the rigor of the analysis. As a result, heuristic analysis of the ITS data set was accomplished with PAUPRat. Analysis of the ITS data employed 25 runs each of 500 iterations with 25% of the characters perturbed each iteration. Nonparametric bootstrap analyses (Felsenstein, 1985) with 1000 pseudoreplicates and 100 random sequence additions were conducted on all data sets. For bootstrap analyses MULTREES was turned off and 100 trees of a specified length, which varied with the input data, were retained for each pseudoreplicate.

**Maximum Likelihood.** The best-fit model of nucleotide substitution for the ETS data was the Transversion model with some sites assumed to be invariable and variable sites assumed to follow a discrete gamma distribution (TVM + I + . Posada and Crandall, 1998), The Symmetrical model with variable sites following a discrete gamma distribution (SYM + . Zharkikh, 1994) was selected as the best-fit model for the ITS data, Finally, the general time reversible model with some sites assumed to be invariable and variable sites assumed to follow a discrete gamma distribution (GTR + I + ; Yang, 1994a) was selected as the best-fit model of nucleotide substitution for the combined data using Modeltest. In all instances, the gamma distribution was separated into six discrete rate categories to better accommodate rate heterogeneity (Yang, 1994b). Heuristic ML searches were implemented with a starting tree obtained via neighbor joining (uncorrected “p” distances). Model parameters were optimized on this tree. An iterative approach was used in which model parameters were re-estimated on the resulting tree and were then used in a subsequent heuristic search (Swofford et al., 1996). This iterative procedure was repeated until no significant topological differences (Shimodaira and Hasegawa, 1999) were observed among resulting topologies. Finally, ML bootstrap analyses were performed (on the combined data only) using 100 pseudoreplicates, 10
random sequence additions and subtree pruning-regrafting (SPR) branch swapping
beginning with the topology resulting from the ML heuristic search.

**Bayesian Inference.** Bayesian phylogenetic analyses were conducted using
MrBayes 2.01 (Huelsenbeck and Ronquist, 2001). The models indicated above for the
ETS, ITS and combined data were used in the Bayesian analyses. Specific nucleotide
substitution model parameter values were not defined a priori. Instead, model parameters
were treated as unknown variables with uniform prior probabilities and were estimated as
part of the analysis. Bayesian analyses were initiated with random starting trees and
were run for $2.0 \times 10^6$ generations. Markov Chains were sampled every 100 generations.
This resulted in 20,000 sampled trees and parameter estimates.

A critical aspect of Bayesian analysis is to ensure that the Markov Chain has
reached stationarity. All sample points prior to stationarity are essentially random and are
discarded as “burn-in” samples because they do not contain useful parameter estimates.
Achievement of stationarity was presumed when log likelihood scores plotted against
generation time exhibited stable equilibrium values (Huelsenbeck and Ronquist, 2001).
Since stationary samples collectively form approximations of the posterior probability
distribution, a conservative approach was taken for determining burn-in whereby some
useful samples were discarded to avoid unknowingly retaining burn-in samples in
posterior probability estimations (Leache and Reeder, 2002).

Analyses were repeated several times as a precaution against entrapment on local
optima. Entrapment on local optima was evaluated by superimposing the log likelihood
versus generation time of each independent analysis to determine if the mean log
likelihood values were similar for each run. In addition, the search strategy implemented
in MrBayes helps to avoid entrapment on local optima through the use of incrementally
heated Markov chains (Metropolis-coupled MCMC) enabling a more thorough exploration of parameter space (Marinari and Parisi, 1992; Geyer and Thompson, 1995). This is accomplished via the random exchange of parameter values between heated chains, effectively decreasing the distance between optimal peaks in parameter space. This search algorithm enables movement between local optima, thus preventing entrapment. Analyses in this study used four incrementally heated Markov chains.

Bayesian searches yielded phylograms on which the posterior probabilities were later plotted. Posterior probabilities were estimated through the construction of a 50% majority rule consensus tree that provided estimates of the sampled trees containing a particular clade and that clade’s posterior probability (Huelsenbeck and Ronquist, 2001). Unlike nonparametric bootstrap support values, these posterior probabilities are interpreted as true probabilities for each clade under the assumed model (Rannala and Yang, 1996). Consequently, clades with probabilities of 95% or greater were considered significantly supported.

**Congruence of Methods and Hypothesis Testing.** The congruence of MP and ML trees with respect to that obtained via Bayesian inference was evaluated by assessing the number of shared nodes, topological congruence, and congruence between the estimated measures of support (bootstrap versus posterior probabilities). The Shimodaira-Hasegawa (SH) test statistic was used to statistically compare alternative phylogenetic hypotheses (Shimodaira and Hasegawa, 1999; Goldman et al., 2000). SH tests were conducted using PAUP* v4.0b10, with full optimization.
RESULTS

Independent Phylogenetic Analysis of ITS and ETS Data. The final aligned 3’ ETS data matrix consisted of 50 sequences, each 505 bp long, representing 48 species in 12 genera of the Astereae. The 3’ ETS region sequence length without gaps varied from 406 bp in Rigiopappus leptocladus to 482 bp in Gundlachia corymbosa. Pairwise distances for the ETS data ranged from 0.0% between seven pairs of taxa (Chrysothamnus depressus/C. viscidiflorus, Ericameria pinifolia/E. ericoides, E. bloomeri/E. compacta, E. lignumviridis/E. cervina, E. watsonii/E. cervina, E. suffruticosa/E. greenei, E. watsonii/E. lignumviridis) to 12.6% between Pentachaeta exilis and Doellingeria umbellata. The C. depressus/C. viscidiflorus species pair separated by 0.0% distance is among the outgroup taxa while the others are composed of ingroup taxa, i.e., species of Ericameria. Based on existing sectional classification (see Table 3.1, Nesom, 1990; Nesom and Baird, 1993, 1995), one species pair consists of taxa placed in section Ericameria. Of the five remaining pairs of species, two are aligned with section Macronema and one with section Asiris. The remaining two both contain representatives of sections Asiris and Macronema. Of 505 total characters in the aligned 3’ ETS matrix 68 (13.5%) characters were parsimony-informative, 362 (71.7%) were constant, and 75 (14.8%) were variable but uninformative. A 50 bp insertion characterized the two species of Xylothamia and Gundlachia corymbosa. A 3 bp insertion characterizes all species of Chrysothamnus, Solidago canadensis and Chrysoma pauciflosculosa. All other indels scored in the ETS region involved one or two base pairs.

The complete aligned ITS data set is 657 bp in length and similar in sequence number, species composition and generic representation to the ETS data set. ITS region
sequence length without gap alignment insertions varied from 569 bp in *Ericameria pinifolia* to 633 bp in *Ericameria cuneata*. With the exception of *E. parryi* having 163 bps in the 5.8S region, all other taxa exhibited 164 bps for that region. ITS 1 sequence length ranged from 247 bp in *Gundlachia corymbosa* to 253 bp in most other taxa sampled. The longest ITS 2 sequence, 216 bp, was observed in *E. cuneata* while the shortest, 152 bp, was seen in *E. pinifolia*. Pairwise distances between species as determined in PAUP* v4.0b10 from the uncorrected ("p") distance matrix ranged from 0.16% between two species pairs *E. crispa/E. compacta* and *E. nana/E. lignumviridis* to 11.0% between *Rigiopappus leptocladus* and *Gundlachia corymbosa*. Of 657 total characters in the aligned matrix, 88 (13.4%) were parsimony-informative, 468 (71.2%) were constant, and 101 (15.4%) were variable but parsimony-uninformative. A total of 28 indel events characterized the ITS region. The majority of these involved 1 or 2 bps. The largest indel, a 12 bp deletion near the end of ITS 2, was exhibited by both species of *Xylothamia*. Other indel events characterized individual species and did not represent synapomorphies for any group of taxa.

Parsimony analysis of the ETS resulted in 576 most parsimonious trees 236 steps long with a retention index (RI) of 0.823 and a consistency index (CI) of 0.737 in one tree-island. Parsimony bootstrap analysis of the ETS data resulted in five nodes with ≥70% support including four outgroup clades and one ingroup clade. Nodes with ≥70% bootstrap support are indicated on the ETS Bayesian phylogram (Fig. 3.1). Parsimony ratchet analysis of the ITS resulted in 12,000 minimum length trees. The 50% majority-rule consensus tree for the parsimony ratchet (not shown) was 305 steps long with a RI of 0.769 and a CI of 0.734. Bootstrap analysis of the ITS data resulted in 11 nodes.
with ≥70% support including six ingroup nodes and five outgroup nodes. Nodes with parsimony bootstrap scores ≥70% are indicated on the Bayesian phylogram for the ITS data (Fig. 3.2).

A maximum likelihood heuristic search was performed using the TVM + I + model (Posada and Crandall, 1998) for the ETS data set as previously described and resulted in a single tree (not shown). The tree derived from this analysis of the ETS data had a log-likelihood score (lnL) of -2068.845 and with a few exceptions was similar in topology to that resulting from Bayesian analysis of the data set (Fig. 3.1). *Tracyina rostrata* was not included in a clade with the two other annual species but was basal to the *Ericameria* lineage on the ETS Bayesian tree (Fig. 3.1). On the tree resulting from ML analyses of the ETS data *T. rostrata* was included in the *Ericameria* clade in a polytomy with clades comprised of section *Ericameria*, *Stenotopsis*, and six species of section *Macronema*. Another difference is in the position of the two varieties of *E. discoidea*. On the ETS Bayesian phylogeny, the two varieties are very weakly supported as sister taxa (Fig. 3.1). However, on the tree resulting from ML analysis the taxa are part of a polytomy including a clade composed of *E. nauseosa* and *E. parryi*. Heuristic ML analysis of the ITS data set was performed similarly to that of the ETS data set utilizing the SYM + model (Zharkikh, 1994). The tree derived from ML analysis of the ITS data (not shown) had a log-likelihood score (lnL) of -2713.319 and displayed greater resolution than that resulting from Bayesian analysis of the data set (Fig. 3.1). On both topologies, *Pentachaeta*, *Rigiopappus* and *Tracyina* are within the *Ericameria* lineage. However, these taxa are within a clade consisting of representative species of sections *Ericameria* and *Stenotopsis* on the ML tree whereas they are in a polytomy with all species of *Ericameria* on the Bayesian tree (Fig. 3.2).
Finally, a subclade comprised of *E. arborescens*, *E. palmeri* and *E. parishii* is either unresolved or aligned with species of section *Ericameria* (Figs. 3.1, 3.2).

Bayesian analysis, for the ETS data set, converged on stable likelihood scores before 100,000 generations. As a result, all sample points derived from the first 100,000 generations were discarded as burn-in. Fourteen nodes on the resulting phylogram displayed posterior Bayesian probability support of ≥0.95 (Fig. 3.1). Bayesian analysis, for the ITS data set, attained stationarity before 200,000 generations. All sample points derived from the first 200,000 generations were discarded as burn-in. The resulting phylogram displayed twelve nodes with posterior Bayesian probabilities ≥0.95 (Fig. 3.2).

The greatest resolution obtained for independent analysis of the data sets was observed for the Bayesian analysis of the ETS data. Both data sets strongly support a clade composed of *Ericameria*, *Pentachaeta*, *Rigiopappus* and *Tracyina* irrespective of optimality criteria implemented (Figs. 3.1, 3.2). This clade received maximum Bayesian posterior probability support and parsimony bootstrap support of 97% and 93% on the ETS and ITS trees, respectively. Among outgroup taxa, the *Xylothamia/Gundlachia* lineage was maximally supported except on the topology resulting from parsimony analysis of the ITS data where support was 89% (Fig. 3.2). *Chrysothamnus gramineus* is not closely related to any other *Chrysothamnus* lineage. A clade including *Sericocarpus* is present on all trees but received significant support (0.96 posterior probability) only from Bayesian analysis of the ETS data (Fig. 3.1). Within this lineage, a clade composed of *C. viscidiflorus* and *C. depressus* is strongly supported independent of optimality criteria. In addition, *C. pulchellus* and *C. linifloius* are supported in a clade distinct from the previous clade on all but the tree resulting from analysis of the ETS data set (Fig. 3.1). Finally, a clade composed of *Solidago canadensis* and *Chrysoma pauciflosculosa* is
supported (parsimony bootstrap 99%, posterior Bayseian probability 1.0) on trees resulting from analysis of the ETS data only (Fig. 3.1).

Among ingroup taxa, independent analyses of the data do not agree with the placement of the annual taxa *Pentachaeta, Rigiopappus* and *Tracyina*. On trees resulting from analyses of the ETS data, *Pentachaeta* and *Rigiopappus* are resolved as sister taxa while *Tracyina* is either basal to the *Ericameria* lineage (Fig. 3.1) or in a polytomy with species of *Ericameria* (tree not shown). *Pentachaeta, Rigiopappus* and *Tracyina* are resolved with maximum bootstrap and posterior probability support on trees resulting from independent analyses of the ITS data set. However, this clade, while resolved as sister to the *Ericameria* lineage in the parsimony bootstrap analysis (not shown) is unresolved from *Ericameria* in the Bayesian phylogram (Fig. 3.2).

*Ericameria* is resolved as a distinct lineage in the trees resulting from Bayesian analysis of the ETS data (Fig. 3.1) and the parsimony analysis of the ITS data (not shown). Within the *Ericameria* lineage, three distinct though weakly supported clades are observed (Fig. 3.1). These lineages are centered around *E. ericoides* and *E. nana* both with posterior Bayesian probability support of 0.91, and *E. suffruticosa* with Bayesian posterior probability of 0.94 (Fig. 3.1). The *E. ericoides* lineage contains the typical representatives of section *Ericameria* (Table 3.1) along with *E. linearifolia* the lone member of section *Stenotopsis*, and *E. paniculata* and *E. teretifolia* two species recently transferred to *Ericameria*. The *E. nana* lineage contains most members of section *Asiris* (Table 3.1) along with *E. lignumviridis*, *E. discoidea*, *E. nauseosa* and *E. parryi* (posterior probability = 0.95). *Ericameria nauseosa* and *E. parryi* are recent transfers from *Chrysothamnus* and form a subclade with *E. discoidea* that receives maximum posterior Bayesian probability support (Fig. 3.1). The last subclade with affinity for the
Asiris lineage consists of *E. resinosa*, generally treated as an *Asiris*, plus *E. gilmanii* and *E. ophitidis* of section *Macronema*. This subclade forms a polytomy with the *Asiris/E. nauseosa* lineage and *E. albida* (Fig. 3.1). The final lineage in *Ericameria* is clustered around *E. suffruticosa* and contains six species typically classified in section *Macronema* (Fig. 3.1). None of the preceding clades is strongly supported in its entirety on trees resulting from parsimony analysis of the ETS data (tree not shown) or any analysis of the ITS data (Fig. 3.2). On the tree resulting from Bayesian analysis of the ITS data four clades are resolved in *Ericameria*. Two are composed of species classified in section *Ericameria sensu* Nesom (1990), one clade is composed of three species of section *Macronema*, and the last encompasses five species placed by Nesom in sect. *Asiris* plus *E. gilmanii*, *E. lignumviridis*, and *E. ophitidis* (Fig. 3.2). In each case posterior probability support was <0.95 and the relationship among these clades and to other ingroup species is unresolved. On the ITS bootstrap tree (not shown) two clades both containing species of section *Ericameria* were supported by bootstrap values ≥70%. One clade is comprised of *E. ericoides*, *E. pinifolia* and *E. fasciculata* and the other of *E. arborescens* and *E. parishii*. A clade comprised of *E. bloomeri*, *E. greenei* and *E. suffruticosa* and one composed of *E. lignumviridis*, *E. nana*, *E. obovata* and *E. watsonii* were supported by bootstrap values <70%.

**Analysis of the Combined Data.** Maximum Parsimony. The aligned, combined data set contains 1216 bp for 50 taxa. Parsimony analysis of the combined data resulted in 27,663 most parsimonious trees 554 steps long with a RI of 0.792 and a CI of 0.732 in four tree islands. Bootstrap analysis of these data resulted in 18 nodes with ≥70% support (Fig. 3.3). Parsimony bootstrap support for the nodes is indicated on the 50% majority rule tree resulting from the heuristic search (Fig. 3.3).
Fig. 3.1. The 50% majority rule consensus tree from the Bayesian analysis of the ETS data. Mean lnL: -2134.382, variance: 77.476, 95% CI: -2152.32, -2117.88. Numbers above the nodes represent parsimony bootstrap support. Numbers below the nodes represent Bayesian posterior probability values. Sectional classification \textit{sensu} Nesom (1990) is indicated as follows; \textit{Asiris} =*, \textit{Ericameria} =**, \textit{Macronema} =***, and \textit{Srenotopsis} =****. Taxa without asterisk have not been classified to section in \textit{Ericameria}. 
Fig. 3.2. The 50% majority rule consensus tree from the Bayesian analysis of the ITS data. Mean lnL: -2781.457, variance: 79.482, 95% CI: -2799.68, -2765.15. Numbers above the nodes represent parsimony bootstrap support. Numbers below the nodes represent Bayesian posterior probability values. Sectional classification sensu Nesom (1990) is indicated as follows; *Asiris =*, **Ericameria =**, ***Macronema =***, and ****Srenotopsis =****. Taxa without asterisk have not been classified to section in Ericameria.
Outgroup resolution is similar to that observed for the independent data analyses. All lineages among outgroup taxa except that indicating sister relationship between *Chrysothamnus pulchellus/C. linifolius* and *Solidago canadensis/Chrysoma pauciflosculosa* were supported by parsimony bootstrap values >70%. The *Ericameria* lineage receives stronger support in this analysis with an 84% bootstrap value. Sister to this clade is one containing the annuals *Pentacheata, Rigiopappus* and *Tracyina* with maximum bootstrap support. Within the *Ericameria* lineage two large weakly supported clades are observed. These lineages are supported by bootstrap values of 56% and 57%. One of these clades is similar in composition and resolution to the *Asiris* clade observed on the tree resulting from Bayesian analysis of the ETS data (Fig. 3.1). The other lineage combines the *E. ericoides* and *E. suffruticosa* lineages (Fig. 3.3).

**Maximum Likelihood.** ML heuristic search was performed using the GTR + I + model as previously described and resulted in a single tree (*lnL* = -4900.23, not shown). Topologically, this tree was similar to that obtained via Bayesian analysis of the combined data. There were four topological differences between the trees. On the ML tree (not shown) *E. brachylepis, E. juarezensis* and *E. martirensis* form a clade whereas they are unresolved on the Bayesian topology (Fig. 3.4). Also, on the ML phylogeny *E. crispa* and *E. zionis* are in a clade with *E. greenei* and *E. suffruticosa* whereas on the Bayesian tree *E. bloomeri* and *E. compacta* are also included in this clade. *Ericameria lignumviridis, E. obovata* and *E. watsonii* form a distinct clade in ML but are unresolved in a clade that also includes two samples referred to as *E. cervina* and *E. nana*. Finally, *E. cooperi* and *E. laricifolia* are weakly supported as a basal grade to six other species of *Ericameria* (Fig. 3.4).
Fig. 3.3. Phylogenetic tree resulting from heuristic parsimony analysis of the combined ETS and ITS data. Bootstrap percentages appear below the nodes. Tree length: 544 steps, CI: 0.73, RI: 0.79. Sectional classification *sensu* Nesom (1990) is indicated as follows; *Asiris =*, *Ericameria =**, *Macronema =***, and *Srenotopsis =****. Taxa without asterisk have not been classified to section in *Ericameria*. 
The tree derived from ML bootstrap analysis (Fig. 3.5; $lnL = -4938.304$) displayed 17 nodes with $\geq 70\%$ bootstrap support. Resolution of the outgroup taxa and the two major ingroup lineages is identical to that observed in the Bayesian tree (Fig. 3.4). Bootstrap support for these major lineages is $\geq 74\%$. The support value for *Ericameria sensu stricto* is weak to moderate at 61%. All sublineages beginning with *E. albida* that were resolved in the Bayesian tree were also identified with moderate to strong bootstrap values in the ML tree (Fig. 3.5). The ML tree differs from the Bayesian in not supporting the relationship between *E. arborescens, E. palmeri*, and *E. parishii*. Likewise, *E. cuneata* is not supported within the *E. paniculata* and *E. teretifolia* clade(Fig. 3.5). The clade strongly supported in the Bayesian tree (0.96) that includes all species identified as section *Ericameria* (Table 3.1) and *E. linearifolia* partially collapses to a polytomy of seven subclades that also contains several species previously regarded as section *Macronema* (Fig. 3.5).

**Bayesian Analysis.** Bayesian analysis of the combined data for $2.0 \times 10^6$ generations resulted in a posterior probability distribution of $2.0 \times 10^4$ samples. Two independent analyses of the data both attained stationarity before 100,000 generations. As a result, the initial 1,000 trees for each analysis were discarded as burn-in, those remaining were combined yielding $3.8 \times 10^4$ sample points. The 50% majority rule consensus tree displayed 24 nodes with significance values $\geq 0.95$ (Fig. 3.4). Resolution of outgroup taxa is similar to that observed in most previous analyses (Figs. 3.1, 3.3, 3.5). Except for one node, all Bayesian posterior probability support values for outgroup nodes are $\geq 0.97$. The clade composed of the annuals and *Ericameria* is maximally supported. The clade of annuals, *Pentachaeta, Rigiopappus* and *Tracyina*, is strongly supported as a
Fig. 3.4. The 50% majority rule consensus tree from the Bayesian analysis of the combined ETS and ITS data. Mean lnL: -4961.086, variance: 65.739, 95% CI: -4977.75, -4946.21. Numbers above the nodes represent the fraction of total trees sampled containing the indicated node. Numbers below the nodes represent Bayesian posterior probability values. Sectional classification \textit{sensu} Nesom (1990) is indicated as follows; \textit{Asiris} =*, \textit{Ericameria} =**, \textit{Macronema} =***, and \textit{Srenotopsis} =****. Taxa without asterisk have not been classified to section in \textit{Ericameria}. 
Fig. 3.5. Maximum likelihood phylogram based on the combined ETS and ITS data ($lnL=4938.304$). Numbers above the nodes represent bootstrap percentages. Sectional classification sensu Nesom (1990) and Nesom & Baird (1993) is indicated as follows; *Asiris*, **Ericameria**, ***Macronema***, and ****Srenotopsis***. Taxa without asterisk have not been classified to section in *Ericameria*. 
monophyletic lineage sister to *Ericameria* (Fig. 3.4). Having posterior probability values =0.80 indicates weak support for *Ericameria*. However, 13 nodes within this clade have significant posterior probability support. Resolution of taxa above *E. albida* is similar to that seen in previous analyses (Figs. 3.1, 3.3, 3.5). Other species of *Ericameria* form a grade that has 0.86 posterior probability support. Within this grade, five species centered around *E. suffruticosa* are resolved with maximum support. Other species of *Ericameria* are aligned in a clade that includes *E. ericoides* and *E. linearifolia*, the sectional types of *Ericameria* and *Stenotopsis*, respectively, with posterior probability support of 0.96 (Fig. 3.4). Comparison of the MP, ML and Bayesian topologies based on the combined data using the SH test failed to reject any of these alternative phylogenetic hypotheses (p=0.05).

**Phylogenetic Relationships. Outgroup Taxa.** Species included among outgroup taxa are resolved similarly to that seen in analysis designed to investigate relationships among those taxa (Chapter 2; Chapter 4). *Doellingeria* appears basal in all topologies irrespective of optimality criteria (Figs. 3.1-3.5). The *Xylothamia/Gundlachia* clade is well supported. It is often unresolved from *Doellingeria* (Figs. 3.1, 3.4, 3.5), or sometimes it is weakly associated with the *Chrysothamnus* and *Solidago* lineages (Figs. 3.2, 3.3) and in PAUP Ratchet analyses results of the ITS data (tree not shown). *Chrysothamnus gramineus* is consistently basal to the *Sericocarpus* clade. In our analyses *Sericocarpus* is always basal the lineages containing *Chrysoma*, *Chrysothamnus*, and *Solidago*. Above *Sericocarpus*, *Chrysothamnus viscidiflorus/C. depressus*, *Chrysothamnus pulchellus/C. linifolius*, and *Solidago canadensis/Chrysoma pauciflosculosa* mostly appear as three often unresolved lineages (Fig. 3.1, 3.3-3.5).
Ericameria and Its Sister Clade. There is strong support for a monophyletic *Pentachaeta, Rigiopappus* and *Tracyina* (Figs. 3.2-3.5). Within this clade, *Rigiopappus* is strongly supported as sister to *Tracyina* with *Pentachaeta* basal. This clade of annuals is consistently supported as sister to the *Ericameria* lineage. Within the *Ericameria* clade, *E. ericoides* is in a sublineage associated with *E. fasciculata, E. pinifolia, E. brachylepis, E. juarezensis,* and *E. martirensis.* The former three are usually strongly supported while the latter three are typically unresolved, basal elements. Below this clade is a grade consisting of other species of section *Ericameria.* *Ericameria linearifolia,* the lone member of section *Stenotopsis,* is never resolved as a lineage distinct from species in section *Ericameria.* The lineage composed of *E. suffruticosa* and several other species of section *Macronema* is generally resolved (Figs. 3.1, 3.3-3.5). However, robust support for this clade is provided only by Bayesian analysis of the combined data (Fig. 3.4). All analyses indicate that *E. nana* is related to *E. cervina, E. lignumviridis, E. obovata* and *E. watsonii* (Figs. 3.1-3.5). The *E. nana* subclade is sister to one composed of *E. discoidea, E. nauseosa* and *E. parryi.* Together they form a polytomy with *E. albida* and a subclade consisting of *E. gilmanii, E. ophitidis* and *E. resinosa* (Figs. 3.1-3.5).

**DISCUSSION**

*Ericameria.* The present DNA-based phylogenies favor the narrower circumscription of *Ericameria* sensu Nesom (1990) and Nesom and Baird (1993, 1995). Except for one species, *E. albida,* Anderson’s (1995) transfer of all species of *Chrysothamnus* to *Ericameria* is unsupported. Molecular investigations by Morgan and Simpson (1992) provided evidence that previous treatments of *Ericameria* were, at least, paraphyletic. *Ericameria ericoides, Macronema discoidea,* and *C. nauseosus* comprised a strongly supported clade in their chloroplast DNA (cpDNA) restriction site based
phylogenies. Alignment of the nine species of *Xylothamia* (Nesom et al., 1990; Nesom, 1992) with the *Gutierrezia* group (Nesom, 1991) rather than with *Ericameria* is generally supported (Chapter 4). Thus, *Ericameria* was also polyphyletic when encompassing *Xylothamia*. However, the relationship of some species of *Xylothamia* to *Gundlachia* was robustly supported while the remaining ones showed affinity for other genera within the *Gutierrezia* group (Chapter 4).

The present study supports Nesom’s (1990) inclusion of species generally treated as *Haplopappus* sections *Asiris* and *Macronema* in *Ericameria*. Chloroplast DNA-based investigations by Lane et al. (1996) who included certain representative species of these two sections, also supported this transfer. In addition, their data did not support Nesom’s treatment of *E. linearifolia* as a distinct section within *Ericameria*.

Except for *E. albida*, the inclusion in *Ericameria* of all other species of *Chrysothamnus* (Anderson, 1995) is unsupported by the gene-based trees. However, the earlier transfer of *C. nauseosus*, *C. paniculatus*, *C. parryi*, and *C. teretifolius*, by Nesom and Baird (1993) is supported. *Chrysothamnus* as perceived by these investigators has, in fact, been found to consist of three additional, distinct lineages more closely related to other Astereae (Chapter 2). Thus the number of species traditionally treated as *Chrysothamnus* is reduced to seven. In addition to these seven species, molecular data suggest redefining *Chrysothamnus* to include five taxa previously treated in other genera (Chapter 2).

Four of the five species of section *Asiris sensu* Nesom, (see Table 3.1) consistently form a clade in the analyses presented here. The other species, *Ericameria resinosa*, is supported as having a closer relationship to *E. gilmanii* and *E. ophitidis*. *Ericameria cervina* is represented in this study by two morpho-types that have different
habitat preferences. *Ericameria cervina* 1 has narrower, longer leaves and grows on limestone soils of the Grand Canyon of Arizona while *E. cervina* 2 has leaves that are shorter and broader and is representative of a more widespread taxon that often occurs on granitic outcrops. Thus appears to represent an undescribed species. Also included in this clade is *E. lignumviridis*, a species referred to section *Macronema* by Nesom and Baird (1995). Their suggestion of a close relationship of *E. lignumviridis* to *E. crispa* in section *Macronema* is unsupported by the present investigation.

Although *E. resinosa* has been accommodated in section *Asiris* by Hall (1928) and by Nesom (1990), it is allied with *E. gilmanii* and *E. ophitidis* in this investigation. All three species are characterized by very pale to white corollas. *Ericameria gilmanii* and *E. ophitidis* are restricted to California while *E. resinosa* is more widely distributed in Idaho, Oregon, and Washington. The position of *E. resinosa* is similar to that indicated by Lane et al. (1996) where it emerged as a basal element of the *Ericameria* clade and very distant from *Macronema watsonii* (A. Gray) Greene (*E. watsonii*), the other representative of section *Asiris* included in that investigation.

Also associated with the *Asiris* lineage, but in a separate clade, are *E. discoidea*, traditionally assigned to *Macronema*, and *E. nauseosa* and *E. parryi*, species recently transferred from *Chrysothamnus* to *Ericameria* section *Macronema*. The two varieties of *E. discoidea* included in this investigation appear as sister taxa only on the tree derived from independent analysis of the ETS data (see Fig. 3.1). Otherwise, when resolved in this clade they form a polytomy with the *E. nauseosa/E. parryi* lineage. The nrDNA sequence data suggest that these three species are more closely related to taxa in section *Asiris* rather than to species in section *Macronema*. In contrast, the chloroplast restriction site data of Lane et al. (1996) supported *C. nauseosus* (*E. nauseosa*) and *E. ericoides*,
type for section *Ericameria*, terminal in a grade that also includes *E. discoidea* and *E. watsonii*, representatives of traditional sections *Macronema* and *Asiris*, respectively, with *C. parryi* (*E. parryi*) basal. Nesom and Baird (1995) stated that “the relationship *E. parryi* clearly is with sect. *Macronema*; we also placed *E. nauseosa* in sect. *Macronema* but noted that it has similarities to *Ericameria* sect. *Asiris.*” Morgan and Simpson (1992) in their cpDNA restriction site investigation of certain Astereae presented support for a lineage wherein *Macronema discoidea* (*E. discoidea*) and *Chrysothamnus nauseosus* (*E. nauseosa*) are sister with *E. ericoides* basal. This latter investigation is more congruent with the findings of the present investigation. Hybridization between *E. discoidea* and *E. nauseosa* also provides additional evidence for this close relationship. *Ericameria xbolanderi* (Gray) Nesom & Baird [*E. discoidea* (*Haplopappus macronema*) x *E. nauseosa* (*Chryothamnus nauseosus*)] was reported by Anderson and Reveal (1966) to be a natural intergeneric hybrid that they viewed as exemplifying the arbitrary nature of these genera. The similarity of species in section *Macronema* to *E. parryi* (*C. parryi*) has also been noted by Cronquist (1955).

*Ericameria albida* (*Chrysothamnus albidus*) is associated with the three previous clades in a polytomous lineage. Despite its punctate, resinous leaves, and similarity in habit to *Ericameria*, most investigators except Anderson (1995) referred this species to *Chrysothamnus*. Nesom and Baird (1995) stated that this taxon is a “phylogenetically extraneous element within *Chrysothamnus,***” noting that “…its peculiar morphology… makes it difficult to discern the nature of its relationship…” Apparently they derived support for retaining the species in *Chrysothamnus* by concluding that the purported hybrids with *E. nauseosa* (Anderson, 1973) as the other parent was, in fact, that species
(Nesom and Baird, 1993). No explicit sectional placement for *E. albida* in *Ericameria* has been proposed. In this investigation *E. albida* is associated with a clade containing the only other species in *Ericameria* with white or pale yellow corollas (*E. gilmanii, E. ophitis,* and *E. resinosa*) and with species mostly found in the Great Basin of the western United States.

The current DNA sequence-based analyses suggest that section *Macronema* is more restricted than previously conceived (Nesom, 1990; Nesom and Baird, 1993). Six of their 12 species have been shown in this study to be more closely allied to *Asiris* and associated lineages. Four of the remaining taxa have traditionally been classified in section *Macronema* (Hall, 1928). Two additional species, *E. crispa* and *E. zionis*, have been described since Hall’s treatment. The six species are consistently aligned in a clade in all but the independent ITS phylogeny (Fig. 3.2). Although not characteristic of all species and not unique to this group, several species feature stems that become shiny and reddish-brown with age, herbaceous outer phyllaries, and long-exserted style branches with collecting appendages longer than the stigmatic portion. All species in this clade lack punctate leaves but have some form of pubescence. *Ericameria suffruticosa* of this clade and *E. watsonii*, of the *Asiris* clade, are both characterized by biseriate-stalked, glandular trichomes. Since these species are not closely allied in the gene-based phylogenies, this character appears to have arisen independently at least twice within *Ericameria*.

Species of section *Ericameria* and *Stenotopsis* when resolved as a clade constitute a grade above the *E. suffruticosa* lineage (see Figs. 3.3, 3.4). Significant posterior probability support is provided in the Bayesian analysis of the combined data (Fig. 3.4), but not by parsimony bootstrap where support is less than 50%. Except for *E.*
Ericameria linearifolia’s placement in this clade we have found its composition to be identical to Nesom’s (1990) and Nesom and Baird’s (1993) section Ericameria. There also is considerable agreement between the specific composition of our gene-derived clade with the treatment of section Ericameria by Hall (1928). However, he had included in this section two taxa, Haplopappus sonoriensis and H. vernicosus, now regarded as belonging to other genera. The former was treated as Xylothamia, (Nesom, 1990), and more recent investigations support its affinity to Gundlachia (Chapter 4), while the latter has been transferred to the genus Hazardia (Clark, 1979). Additionally, two species, E. juarezensis and E. maritirensis were not known to Hall at that time and two others, E. paniculata and E. teretifolia, were then treated as Chrysothamnus.

Ericameria linearifolia is placed within the Ericameria lineage in this investigation. Nesom (1990) had maintained it as the sole member of sect. Stenotopsis. However, Lane et al. (1996) in their cpDNA based phylogeny showed E. linearifolia basal to the clade consisting of representative species of sect. Ericameria. Hall (1928) regarded section Stenotopsis to be distantly related to Ericameria, and the other species placed in it, H. parrasnas, is now supported in the Gutierrezia group (Chapter 4).

Ericameria linearifolia is not supported as being sister to E. cooperi as suggested by Urbatsch and Wussow (1979) although both species are placed in sect. Ericameria wherein species relationships are not fully resolved.

The most consistently supported clade in sect. Ericameria consists of E. ericoides, E. fasciculata, and E. pinifolia. These three occur mainly on dunes or sandy soils on or near the Pacific coast from central California southward to northern Baja California. Hall recognized this lineage as well and also suggested that E. palmeri is related to this trio, a hypothesis not supported by the present investigation. Two species
restricted to the mountains of Baja California, *E. juarezensis* and *E. martirensis*, are often resolved with the *E. ericoides* clade. Another species with a primarily Baja California distribution, *E. brachylepis*, is also allied with these taxa in the present study. Thus Blake’s (1935) suggestion that the latter is most closely related to *E. martirensis* receives some support by the DNA sequence data. The resemblance noted by Moran (1969) between *E. martirensis* and *E. juarezensis* may be indicative of close relationship. He also noted certain similarities between *E. cuneata* and the latter, but he found no hybrids where the two co-occur in Baja California. The present study offers no strong support for such affinity suggesting that the resemblance may be due to convergence.

*Ericamria cooperi* and *E. laricifolia* are also often resolved in the nrDNA sequence-based phylogenies as part of the section *Ericameria* grade. Hall (1928) suggested that the two constitute a lineage with the former being more derived, an assertion that can neither be supported nor refuted based on the results of this investigation. Furthermore, he regarded *E. laricifolia* as being representative of the *Ericameria* ancestral type. This study suggests that it is less derived within the section, but not necessarily the most basal.

Maximal Bayesian support for the clade of *E. arborescens* and *E. parishii* and strong bootstrap support in the ML and MP trees based on combined data reinforces the long-held view that these two taxa are closely related (Hall, 1928). In fact, Moran (1969) had reduced the two to subspecies of *E. arborescens* when describing *E. a. subsp. peninsularis*. Nesom (1989) in noting the distinctiveness of *E. arborescens* and *E. parishii* maintained them as separate species while designating *E. a. subsp. peninsularis* a variety of the latter. The nrDNA sequence divergence (1.3%) observed between *E. arborescens* and *E. parishii* is approximately the same seen for other well-defined
species of *Ericameria*. *Ericameria palmeri* is the only other species that shows some affinity for this clade albeit below the desired level of support. Otherwise, the relationship of *E. palmeri* is unresolved.

Basal within the section *Ericameria* grade is a subclade consisting of *E. cuneata*, *E. paniculata* and *E. teretifolia* (Figs. 3.3, 3.4), although support for it is weak or not significant. The sister relationship of *E. paniculata* to *E. teretifolia* is supported by posterior Bayesian probability but not by strong ML or MP bootstrap percentages. *Ericameria paniculata* and *E. teretifolia* have had a long history of association to one another having been treated as sect. *Punctati* in *Chrysothamnus* (Hall and Clements, 1923; Anderson, 1986). Hall and Clements (1923) regarded *E. teretifolia* as the more “advance.” Although support for *E. cuneata’s* association with this clade is less than the level desired, this is its strongest alliance based on the nrDNA sequence data. Hall (1928) noted that *E. cuneata*, though nested within section *Ericameria*, shows no obvious connection to any other species. Based on the nrDNA sequence data, similarities between *E. cuneata* and *E. juarezensis* as noted by Moran (1969) do not seem indicative of a close relationship. Leaf shape, large number of phyllaries, and often scaly peduncles in *E. cuneata* are unique within the section *Ericameria* clade. Morphological features uniting it with *E. paniculata* to *E. teretifolia* also are not evident. The latter two species are desert shrubs found in southeastern California and adjoining areas, while *E. cuneata* occupies rocky ledges surrounding the desert floor (Urbatsch, 1976; Brown and Keil, 1993).

**Pentachaeta, Rigiopappus and Tracyina.** Species in these genera are annuals, unlike most other taxa included in this investigation, which are shrubs. *Pentachaeta, Rigiopappus* and *Tracyina* are strongly supported in a monophyletic clade basal to the *Ericameria* lineage. All three genera occupy similar habitats though *Rigiopappus* is
relatively widespread in the western United States while *Pentachaeta* and *Tracyina* are restricted to California (Bohm and Stuessy, 2001). Blake (1937) attributed resemblances in habit and foliage, among these genera to adaptations to similar habitat. He viewed similarities between *Tracyina* and *Rigiopappus* as superficial and that in its ‘technical characters’ *Tracyina* is closely related to *Pentachaeta*. The DNA sequence based phylogenies presented here do not agree with that conclusion, but they are congruent with the ITS phylogeny of Noyes and Rieseberg (1999) where a closer relationship of *Tracyina* to *Rigiopappus* than to *Pentachaeta* is shown. The relationships evident from the DNA-based phylogenies are also supported by morphological (Robinson and Brettell, 1973; Van Horn, 1973), cytological (Raven and Kyhos, 1961; Ornduff and Bohm, 1975) and chemical evidence (Ornduff and Bohm, 1975). As indicated on the present phylograms, these taxa are represented by relatively long branches. This may be due to their relatively shorter generation time, in comparison to the shrubs in *Ericameria*, resulting in the accumulation of more mutations per unit time (Li, 1997).

**Outgroup Taxa.** Relationships among outgroup taxa presented here are similar to those discussed in Chapters 2 and 4. *Doellingeria* is consistently supported as a basal element of North American Astereae as reported by Noyes and Rieseberg (1999) based solely on ITS sequence data. Representative species of *Xylothamia* included in the present study are strongly aligned with *Gundlachia*. The relationship of these taxa to *Chrysothamnus* and *Ericameria* are detailed in Chapters 2 and 4. In Chapter 2 it was noted that only one of the 12 species remaining in *Chrysothamnus sensu* Nesom and Baird (1993) is supported in *Ericameria*. The other species in *Chrysothamnus* are resolved in three distinct lineages. The species of *Chrysothamnus* included in this investigation display similar alignment. *Chrysothamnus gramineus* is basal to the
Sericocarpus clade and not aligned with any of its congeners. The four other species of Chrysothamnus are resolved in two clades one of which may be closely related to the Solidago lineage. In general, outgroup taxa are strongly supported in lineages distinct from Ericameria.

The DNA-based phylogenetic hypotheses presented here support the recognition of Ericameria as a lineage distinct from both Chrysothamnus and Xylothamia. The composition of Ericameria presented here is congruent with that of Nesom (1990) and Nesom and Baird (1993) and, for the most part, is unsupportive of Anderson’s (1995) proposal. The Ericameria lineage in this investigation is resolved as three clades with moderate support that might be treated as three sections. Section Asiris is here expanded to encompass 14 species. Two of these are new within Ericameria, one newly described and the other newly elevated in rank. Appropriate nomenclatural changes will be treated elsewhere. Ericameria albida, regarded as Chrysothamnus by Nesom and Baird (1995), is also allied to Asiris. Section Macronema is here reduced to six species centered about E. suffruticosa. Section Ericameria is similar in composition to that proposed by Nesom (1990) except for including E. linearifolia that he accommodated in section Stenotopsis. The sister relationship of Pentachaeta, Rigiopappus and Tracyina to Ericameria is strongly supported by this investigation. Within that lineage Tracyina and Rigiopappus are closely allied with Pentachaeta sister.

LITERATURE CITED


Sikes, D. S. and Lewis, P. O. 2001. PAUPRat: A tool to implement parsimony ratchet searches using PAUP*. Distributed by the authors. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, USA.


CHAPTER 4. MOLECULAR PHYLOGENY OF XYLOTHAMIA, GUNDLACHIA, AND RELATED GENERA (ASTERACEAE: ASTEREAE) BASED ON 3’ ETS AND ITS nrDNA SEQUENCE DATA *

INTRODUCTION

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_Xylothamia_ consists of nine species of shrubs found in the Chihuahuan and Sonoran Deserts of northeastern Mexico and southern Texas (Nesom et al., 1990; Nesom, 1992). Reduced, resin-coated leaves, reduced capitulescences, indurate phyllary bases, and shortened corolla tubes characterize most species and appear to be adaptations to the arid environments where most species occur. Investigators’ varied interpretations of relationships for this small group of taxa demonstrate its taxonomic difficulty. Convergent evolution in morphology has been suggested to explain this situation (Hall, 1928; Hall and Clements, 1923; Nesom and Baird, 1995), although no independent test for this hypothesis has been conducted.

The three earliest species discovered and later attributed to _Xylothamia_ were described as _Ericameria_ Nutt., _E. diffusa_ Bentham (1844) from southern Baja California and western Sonora, _E. purpusii_ Brandegee (1911), and _E. parrasana_ Blake (1917) from Coahuila, Mexico. Subsequently, these three species and about 150 others were treated as _Haplopappus_, a diverse assemblage of mainly western North and South American taxa accommodated among 21 sections (Hall, 1928). _Ericameria_ was recognized as one of the sections in _Haplopappus_, but it contained only one of the three species, _E. diffusa_, destined to become _Xylothamia_. The other two, _E. parrasana_ and _E. purpusii_, were placed in _Haplopappus_ sections _Stenotopsis_ and _Asiris_, respectively. Hall (1928) hypothesized two major evolutionary lineages for North American species of

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Haplopappus. Sections Asiris and Ericameria terminated one of the lines in Haplopappus while Stenotopsis occupied a midpoint position in the other lineage.

During the next several decades, accrual of cytological, palynological, hybridization, and additional morphological data demonstrated a polyphyletic Haplopappus which is based on the South American *H. glutinosus* (Hall, 1928). North American species are now treated as genera other than Haplopappus as summarized by Lane and Hartman (1996).

Subsequent to Hall’s (1928) treatment, Ericameria was restored to generic rank and expanded by reinstating species originally described in that genus and by the addition of others mainly from northern Mexico and southern Texas (Johnston, 1967, Urbatsch, 1978, 1989; Turner and Langford, 1982). Core species of *Ericameria, sensu* Hall (1928), are shrubs of arid habitats found mostly in California’s chaparral, creosote-bush scrub, coastal dune, and rocky outcrop communities. Species from northern Mexico and southern Texas are much like their mainly Californian counterparts in being shrubs adapted to arid habitats. Morphologically, the taxa are evergreen shrubs, often with punctate, resin-coated leaves, small, usually radiate capitula with multiseriate, graduated phyllaries, and generally corymbiform capitulescences. All taxa have a base chromosome number of \( x = 9 \). Despite the apparent similarities among the *Ericamerias sensu lato*, differences between the species of California and the ones in northern Mexico and Texas were noted in addition to similarities to other genera such as *Euthamia* (Johnston, 1970, Urbatsch, 1978).

Chloroplast DNA restriction site studies by Suh (1989), Morgan (1990), and Morgan and Simpson (1992), which included representatives of *Ericameria*, indicated
that species from northern Mexico and southern Texas were but distantly related to the California species. These data provided evidence for establishing the genus *Xylothamia* by Nesom et al. (1990) who noted similarities of the new genus to *Euthamia* in base chromosome number and in leaf and capitular morphology. The name *Xylothamia* selected for the new genus emphasizes its distinctive woody nature while also drawing attention to its *Euthamia*-like qualities. Furthermore, Nesom et al. (1990) noted that chloroplast DNA studies suggested that *Xylothamia* along with *Amphiachyris*, *Euthamia*, *Gutierrezia*, and *Gymnosperma* constituted one “strongly defined” group while *Ericameria sensu stricto, Chrysothamnus* and *Macronema* formed another. In contrast to Nesom’s work, evidence presented by Lane et al. (1996), in a more comprehensive cpDNA restriction site survey of North American Astereae, maintained *Xylothamia* in the *Ericameria/Chrysothamnus* clade while *Amphiachyris, Biglowia, Euthamia, Gutierrezia, Gymnosperma*, and *Thurovia* defined another distinct lineage. Chloroplast DNA restriction site investigations by Suh (1989) and Morgan (1990) had also identified the distinctive *Gutierrezia* lineage, the so called “*Gutierrezia group*” (Nesom, 1991).

*Gundlachia*, an endemic West Indian genus, has been hypothesized as sister to *Gymnosperma* based on morphological and cytological comparisons (Lane, 1996). Earlier investigations based on morphology suggested *Gundlachia’s* alliance to the *Gutierrezia* group (Nesom, 1991). In that study *Chrysoma*, a monospecific genus of the Atlantic and Gulf Coastal plains of the southeastern United States was discounted as a close relative of *Gundlachia* despite its shrubby habit and otherwise superficial resemblance and adaptation to coastal habitats. *Chrysoma* has an isolated, basal position relative to *Solidago* and its allies (Nesom, 1991). Subsequently, it was regarded as sister
to *Solidago, Oligoneuron, Oreochrysum* by Nesom (2000) where the molecular data reported by Semple et al. (1999) is credited in support of this hypothesis. Nesom et al. (1990) asserted that the *Gutierrezia* and *Solidago* lineages together were clearly definable as subtribe Solidagininae.

Although concepts in recent synoptical treatments have varied greatly for Solidagininae, *Xylothamia*’s placement has generally been in that subtribe. Bremer (1994) and Zhang and Bremer (1993), who employed mainly cladistic analyses of morphology, recognized Solidagininae and two other subtribes in Astereae. They further subdivided Solidagininae into nine generic groups. *Xylothamia* together with *Bigelowia, Chrysoma, Euthamia, Oreochrysum, Sericocarpus,* and *Solidago* constituted the “*Solidago* group.” *Gundlachia* was suggested as a member of the *Gutierrizia* group (Bremer 1994). More recently, Nesom (1994, 2000) recognized 14 subtribes and four groups of uncertain affinity in Astereae, based largely on available molecular and morphological data. *Xylothamia, Gundlachia,* and 21 other North American genera are placed in subtribe Solidagininae in that treatment.

Investigations designed to explore phylogenetic relationships among *Ericameria* and other Astereae (Chapters 3), based on nrDNA sequence data, showed that *Gundlachia* is sister to a clade composed of species of *Xylothamia*. Expanded investigations showed that the other species in *Xylothamia* constituted several distinct, non-sister clades (Chapter 5). Consequently, many presumed close relatives were sampled to test phylogenetic hypotheses of relationship for *Xylothamia*. Specifically, this investigation sought to; (1) test the monophyly and circumscription of *Xylothamia* and learn more precisely its relationship to *Gundlachia.* (2) Explore the affinities of these taxa
to others thought to be related. (3) Evaluate the congruence of relationships based on
sequence data with those hypothesized from morphological and cytological features.

MATERIALS AND METHODS

**Taxa.** Samples for analysis were obtained from collections of natural populations
or from specimens deposited in various herbaria (LSU, TEX/LL, and elsewhere, Table
4.1). All species of *Xylothamia* and three accessions of *Gundlachia*, plus representatives
of taxa thought to be related to these genera, were included (Nesom et al., 1990, 2000;
Lane et al., 1996). Fourteen of 21 genera of Solidagininae and species representing nine
additional subtribal or generic groupings with uncertain affinities recognized by Nesom
(2000) were also sampled. Noyes and Rieseberg (1999), in their ITS-based phylogenetic
study, demonstrated that North American taxa in Astereae comprise a clade with
*Doellingeria* at the base. Data for several other genera listed among the “primitive
Asters” by Nesom (2000), i.e. *Batopilasia, Boltonia, Chloracantha*, and *Ionactis*, along
with *Doellingeria* were also incorporated in the present study to test potential hypotheses
of relationships.

ITS and ETS sequence data have been employed in this investigation. ITS
sequence data have an established record for providing useful phylogenetic insights at
the generic and specific levels (Baldwin et al., 1995; Baldwin and Wessa, 2000;
Clevinger and Panero, 2000; Urbatsch et al., 2000; Fernandez et al., 2001; Francisco et
al., 2001). ETS sequence data has been shown to be equal to or more useful than ITS data
for recently evolved lineages. ETS sequences evolve as much as 1.4 times faster by
nucleotide substitution and they provide a somewhat higher level of phylogenetically
informative characters than the ITS region (Markos and Baldwin, 2001). ETS and ITS
data sets have been shown to be congruent and combinable resulting in better resolved phylogenies with higher character and statistical support (Baldwin and Markos, 1998; Clevinger and Panero, 2000; Markos and Baldwin, 2001).

The 102 ITS-region sequences (ITS-1, ITS-2, plus the 5.8S) represent 72 species in 38 genera, and the 86 3′-ETS sequences represent 65 species in 33 genera. Sixty-seven ITS and 68 ETS sequences have not previously been reported. Thirty-four ITS and 17 ETS sequences, respectively, were obtained from GenBank. One unpublished ETS sequence for *Xanthocephalum* was kindly supplied by D. Morgan, Western Washington Univiversity, Bellingham. Table 4.1 lists the taxa sampled, sources of the material, voucher documentation, and GenBank accession numbers.

**DNA Isolation, PCR, and Sequencing.** For each sample of field-collected leaf tissue (kept on ice or frozen in liquid nitrogen and subsequently stored at -80°C), approximately 100 mg was ground using the Mini Beadbeater™ 8 (BioSpec Products, Inc., Bartlesville, Oklahoma) in sterile 2 ml screw cap tubes. Tissue was kept frozen during this procedure by alternating cycles of grinding and freezing by placing tubes in an ultra-cold freezer or in liquid nitrogen. Total genomic DNA was subsequently isolated and purified from these samples using the Qiagen DNeasy® Plant Kit following the manufacturer’s protocol. Herbarium specimen samples were treated similarly except that 20-30 mg of leaf tissue was ground and left in the extraction buffer for 0.5-1 hour at 65°C instead of the recommended 10 minutes.

In order to optimize PCR conditions in 25 µL reactions, various samples were subjected to a series of 12 premix buffers in the FailSafe™ PCR System (Epicentre Technologies, Madison, Wisconsin.). The most efficient premix buffer was used in
Table 4.1. Taxa sampled in this study and their sources, voucher and Genbank data, and relevant literature citations for published sequences. DNA was extracted from fresh collected, frozen leaves for taxa marked with an asterisk. For unmarked taxa DNA was taken from leaves on the herbarium specimen.

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<th>TAXON</th>
<th>Source localities plus voucher data</th>
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<th>ETS GenBank Locus Nos.</th>
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<td>AF477693 clone 1</td>
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<td>AF477630 clone 3</td>
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Small

Pyrrocoma apargioides  California: Plumas Co. AF251573 AF251631
(A. Gray) Greene Schoolcraft 2072 (UC)

Pyrrocoma lanceolata  Utah: Neese 17626 (UC) AF251574 AF251632
(Hook.) Greene

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subsequent reactions. A typical 25 µL PCR reaction incorporated 0.5-1 units of \textit{Tfl} polymerase (Epicentre Technologies) and premix buffer “G,” which contained dNTPs, buffer, MgCl\(_2\), and other reagents, approximately 0.3 µM of each primer, and \(\approx\)50 ng of template DNA. The protocol for DNA amplification consisted of 3 min at 95\(^{\circ}\)C denaturation cycle followed by 10 thermal cycles of 1 min of denaturation at 95\(^{\circ}\)C, 1 min of annealing at 55\(^{\circ}\)C, and 1 min of extension at 72\(^{\circ}\)C with a 4 s per cycle extension. Except for using an annealing temperature of 50\(^{\circ}\)C, the next 20 cycles proceeded as before followed by a final extension phase of 7 min at 72\(^{\circ}\)C. ETS and ITS amplifications used the same reaction conditions and thermocycler protocols.

The ITS region (ITS1, ITS2, and the 5.8s subunit) was routinely amplified using primers 20 and 262 (Urbatsch et al., 2000). If that primer pair failed, attempts were made using primers 18 or 350 or ITS-I (Urbatsch et al., 2000) and ITS-4 (White et al., 1990). In some instances, modifications to primer ITS-I designated ITS-I.2 (5′-3′ sequence: \textit{GTCCACTGAACCTTATCATTTAG}) and ITS-I.3 (5′-3′ sequence: \textit{TCCACTGAACCTTATCATTTAG}) improved amplification results. When PCR reactions contained insufficient concentrations of product for cycle sequencing, several rounds of PCR reactions were performed initially using diluted DNA as template followed by sequential amplifications of subsequent PCR products using nested primers. Nested primer pairs generally used were 18/350, 20/262, and ITS-I/ITS-4, but other combinations were also attempted as the situation dictated. Removing unincorporated
dNTPs and primers with QIAquick Spin PCR purification columns (QUIAGEN Corporation) between successive PCR reactions generally resulted in better yields and a cleaner product.

Approximately 400-600 bp of the 3′ region of the External Transcribed Spacer (ETS) were amplified using primers 18S-ETS and Ast-1 and Ast-8 (Baldwin and Markos, 1998; Markos and Baldwin, 2001). One additional primer designated 18S-R1 (Chapter 2) gave better results with some templates. Primers were obtained from GeneLab in the School of Veterinary Medicine, Louisiana State University, Baton Rouge.

Prior to sequencing, PCR products were purified using QIAquick Spin PCR Purification columns. Quantification of PCR product was performed visually on agarose gels using Low DNA Mass Ladder (Life Technologies, Inc. Rockville, Maryland) as the standard. Both strands of PCR products were directly sequenced in 10 µL reactions mainly using ITS-I and ITS-4 for the ITS region and 18S-ETS or 18S-R1 and either Ast-1 or Ast-8 for the 3′ ETS region. Cycle sequencing was conducted using BigDye™ Terminators Cycle Sequencing reagents (Applied Biosystems, Foster City, California) for 25 cycles in the PTC-100 where each cycle consisted of 10 sec denaturation at 96ºC, 5 sec annealing at 50ºC, and 4 min extension at 60ºC. Electrophoretic separation and analysis of the labeled DNA molecules were accomplished with the ABI PRISM® 377 DNA Sequencer (also Applied Biosystems). Assigned GenBank accession numbers for sequences obtained in this study are given in Table 4.1.

When sequence quality was poor, amplified copies of the ITS and ETS regions were cloned using the TOPO™ TA Cloning® Kit (Invitrogen Corporation) or the pSTBlue-1 Perfectly Blunt™ Cloning Kit (Novagen, Darmstadt, Germany) according to
manufacturers protocols. The cloned ITS and ETS regions were re-amplified directly from plate transformed colonies using M13 primers, or for the ITS region primers ITS-I and ITS-4. The same pair of ETS primers used in the original amplification was often used for re-amplification of the cloned colonies. Amplification conditions used were the same as discussed previously except that cells were lysed at 94 °C for 10 min prior to the PCR run. Typically, two or three cloned PCR products per sample were directly sequenced as previously described.

Sequence Analysis. Sequence fragments were edited and assembled with the aid of Sequencer 3.0 software (Gene Codes, Ann Arbor, Michigan). Boundaries of the spacer regions were determined by comparison to some of the many published studies (Urbatsch et al., 2000; Baldwin and Markos, 1998; Markos and Baldwin, 2000; Clevinger and Panero, 2000). Sequences resulting from cloned amplicons were entered into the data matrix as individual OTUs. Edited sequences were aligned with Clustal W 1.8 (Baylor College of Medicine sequence launcher; http://searchlauncher.bcm.tmc.edu/multialign/multi-align.html). Manual adjustments were made when judged necessary. Also, sequences subsequently obtained were aligned by manual comparison to the existing data matrix. MacClade version 4.0 (Maddison and Maddison, 2000) was used to examine and edit sequence alignments. Pairwise sequence divergence estimates were obtained using the distance matrix option in PAUP* 4.0b10 (Swofford, 2002).

Phylogenetic Analyses. Maximum parsimony and Baysian analysis were conducted to test the monophyly of Xylothamia and to estimate phylogenetic relationships among all taxa investigated. Doellingeria was designated as an outgroup based on the Noyes and Rieseberg (1999) study. Phylogenetic analyses were conducted
individually on the ITS and ETS data sets and on a combined ITS/ETS data. Individual sequence lengths varied greatly in the ETS matrix due to the success of primers Ast-1 and Ast-8 and the presence of an ≈84 bp indel. Approximately 125 5’ bp were missing in 16 of 84 taxa in the ETS matrix, and substantial data were also missing from the 3’ end. To test whether missing data affected tree topologies, phylogenetic analyses were performed on the ETS matrix with all characters, then with 125 characters excluded from the 5’ end, and finally with an additional 141 characters excluded from the 3’ end. Analyses of the combined ITS/ETS matrix was also performed on the matrix with all characters, with 125 5’ ETS bp excluded, and finally with the additional 141 3’ ETS bp excluded.

The use of the PAUPRat (Sikes and Lewis, 2001) enabled parsimony analysis of the individual ITS and ETS since such heuristic searches often failed when using PAUP* 4.0b10 (Swofford, 2002). due to tree storage limitations. PAUPRat was useful for analysis of other data sets as well. Because the clade containing Xylothamia was robustly resolved in all analyses, unweighted parsimony was performed on this reduced data set using PAUP* 4.0b10 (Swofford, 2002). In two separate series of analyses, Doellingeria was used individually as an outgroup followed by its combined use with Ericameria and Sericocarpus based on the studies of Lane et al. (1996) and Noyes and Rieseberg (1999). Heuristic parameters for all searches included using at least 100–500 RANDOM sequence additions with TBR branch swapping, MULPARS on, and STEEPEST DESCENT off. Gaps were treated as missing data. Parsimony analyses were performed initially with all potentially informative characters and subsequently by excluding 5’ and 3’ regions of sequence as previously described. Internal branch support was evaluated by bootstrap analysis on reduced data sets (Felsenstein, 1985) with 100 replicate heuristic
analyses using 10 RANDOM addition sequence replicates, MULPARS on, STEEPEST DESCENT off, and TBR branch swapping. Bootstrap analyses were conducted using all informative characters.

Bayesian analyses were performed with MrBayes 2.01 (Huelsenbeck and Ronquist, 2001) on the separate and combined ETS and ITS data sets and on data representing the *Xylothamia* clade, *sensu lato* Bayesian analyses consist of maximum likelihood (ML) comparisons of trees where the tree topology and ML parameters were permuted using a Markov chain Monte Carlo method and sampled periodically. The sample trees are drawn from a posterior probability distribution, and thus the frequency with which they are sampled indicates their probability. Similarly, the posterior probability of any clade is the sum of the posterior probabilities of all trees that contain that clade (Huelsenbeck and Ronquist, 2001). ModelTest (Posada and Crandall, 1998) indicated that the general time reversible substitution model best fits the model of DNA evolution. The Markov chain Monte Carlo process was set so that four chains ran simultaneously for 2,000,000 generations, with trees being sampled every 100 generations for a total of 20,000 trees and parameter estimates in the initial sample. Visualization of variation of the ML scores using scatter-plots showed that “stationarity” was achieved by the 3,000th tree. Therefore, the first 3,000 trees were discarded and the posterior probability of the phylogeny and its branches was determined from the remaining 17,000 trees. Multiple (usually 2-3) two-million generation runs of the same data set were performed in MrBayes to test whether trees with improved ML scores would be discovered and to learn whether consensus trees computed from such additional runs resulted in topological differences.
A separate data matrix was constructed to take advantage of potential phylogenetic information in inferred insertion/deletion (indel) mutations. Inferred indels were recoded as additional binary characters for all sequences. Indels in the same aligned position and of the same length were scored as homologous.

RESULTS

The aligned ITS data set is 672 bp in length and contains 86 sequences representing 65 species in 32 genera of Astereae. The ITS region sequence length without gap alignment insertions varied from 617 bp in *Xylothamia triantha* and one of the two *X. truncata* sequences to 635 bp in *Chaetopappa ericoides*. With the exception of one sequence each for *X. purpursii* and *X. truncata* having 165 bps in the 5.8S, all other taxa exhibited 164 bps for that region. ITS 1 sequence length ranged from 226 to 260 bps in *Amphiachyris* and *X. palmeri*, respectively. The longest ITS 2 sequence, 217 bp, was observed in *C. ericoides* while the shortest, 201 bp, was seen in *X. triantha* and *X. truncata*. Pairwise distances between species as determined in PAUP* from the uncorrected ("p") distance matrix ranged from 0.16% between *X. johnstonii* and *X. palmeri* to 14.6% between *Symphiotrichum subulatum* and *Tracyina rostrata*. Of 672 total characters in the aligned matrix, 261(38.8%) were parsimony-informative, 356 (53%) were constant, and 55(8.2 %) were variable but parsimony-uninformative.

A total of 79 indel events were scored for the ITS region. The majority of these involved 1 or 2 bps. The largest indel, a 33 bp deletion near the beginning of ITS 1, was exhibited by *Amphiachyris*. A 5 bp insertion was observed in the clade designated [AX] in Fig. 4.1, but it was absent from taxa from the clade [Xd] containing *Gundlachia* and four species of *Xylothamia* and all other taxa investigated.
Phylogenetic trees based on heuristic analysis of the combined 3'ETS + ITS + indel data matrices. Numbers above the branches represent support values. Branches in bold highlight species of \textit{Xylothamia}. The dash lines highlight samples of \textit{Gundlachia}. Labels in brackets identify major clades referred to in the text. Taxon names can correlated with samples in Table 4.1 by name, clone number, and last three digits of the ITS GenBank Locus number. (A) 50\% majority rule consensus tree of 8533 most parsimonious trees from PAUPRatchet analysis. (B) 50\% majority rule consensus tree of 2592 most parsimonious trees from the PAUP* heuristic searches.
Its presence could not be determined in \textit{Amphiachyris} because the 33 bp deletion spanned the 5 bp. Other indel events characterized individual genera, species, or samples.

Due to the absence of a conserved primer region, the use of various primer pairs in the ETS region resulted in sequences of various lengths. From the 5' and 3' ends, 125 and 72 bps were deleted, respectively, in the original matrix to reduce substantially the amount of missing data. The final data matrix consisted of 502 characters for the 86 samples. Pairwise distances were somewhat greater for ETS data compared to the ITS, and ranged from 0.2\% between \textit{Xylothamia triantha} and \textit{X. truncata} to 22.0\% between \textit{Machaeranthera parviflora} and \textit{Erigeron bellidiastrum}. 204 of 502 (40.6\%) characters were parsimony-informative, 244 (48.6\%) were constant, and 54 (10.8\%) were variable but uninformative. An 84 bp insertion characterized the clade labeled [X] and a nearly identical one was seen in \textit{Erigeron prostrata}. A 5 bp insertion is clade specific for the taxa sampled in the \textit{Chrysoma/Solidago} lineage. In total 34 indels were scored in the ETS region; most involved one or two bps.

The most resolved phylogeny resulted from the combined analysis of the ITS, ETS, and indel matrices. Both heuristic and parsimony ratchet analyses produced trees of 1857 steps, excluding uninformative characters, having consistency indices (CI) = 0.496 and a retention indices (RI) = 0.801. Two hundred random entries of the data in a heuristic search yielded 2592 maximally parsimonious trees while the parsimony ratchet produced an additional 5,941 minimum length trees. Except for two nodes, the parsimony ratchet 50\% majority rule consensus tree was fully dichotomized while the heuristic tree contained six polytomous nodes (Fig. 4.1).
*Xylothamia*, *Gundlachia*, and representatives of six other genera comprised a robustly supported clade, labeled [X], in analyses of all data sets (Figs. 4.1-4.3). Results based on sequence data clearly support two subclades designated [AX] and [Xd], with species of *Xylothamia* being divided between the two. *Xylothamia triantha*, the type for the genus, *Gundlachia, X. diffusa*, and two other *Xylothamia* constituted lineage [Xd] with maximum support in all analyses. The second clade designated [AX] had 100% support in all analyses and contained the other five species of *Xylothamia*.

In some cases, the sister relationship of *Gundlachia* and to other taxa in [Xd] was supported (Figs. 4.1, 4.2). However, this result was not supported by PAUP* heuristic and parsimony ratchet analyses of the ITS data alone and the heuristic search of ETS + ITS without indels (Fig. 4.3). *Gundlachia* likewise was internal in the ETS parsimony ratchet phylogeny and sister to all [Xd] clade members except for *X. diffusa* which was basal (results not shown). *Gundlachia, X. diffusa, X. riskindii*, and the *X. triantha/X. truncata* lineage are unresolved in the Baysian analysis of the ETS matrix (results not shown). The position of *X. riskindii* was variable depending on the data and method of analysis. It was basal to other *Xylothamia sensu stricto* in heuristic searches based on ETS + ITS + indel data (Fig. 4.1). In the Baysian tree *X. riskindii* represented one branch of a trichotomy involving three other species of *Xylothamia sensu lato* Posterior Bayseian probability support for this clade at 0.81 is not significant (Fig. 4.2). *Xylothamia riskindii* was basal in clade [Xd] based on combined sequence data minus the indels (Fig. 4.3).

The other five species of *Xylothamia* were placed in a distinct sister clade, [AX]. *Xylothamia johnstonii* and *X. palmeri*, clade [Xj], received maximum support as sister
Fig. 4.2. The 50% majority rule consensus tree derived from Bayesian analysis of the combined ITS and ETS data sets. Mean lnL: -10338.967, variance: 138.581, 95% CI: -10362.7 – 10316.52. Numbers above branches represent posterior probability values. Labels in brackets are designations for major lineages referred to in the text.
taxa in all analyses (Figs. 4.1-4.3). In the PAUP* heuristic and parsimony ratchet analyses clade [Xj] represented one of two basal sublineages in clade [AX] containing species of *Xylothamia sensu lato* (Fig. 4.1). A similar relationship was indicated in the parsimony ratchet ETS phylogeny (results not shown). In the Bayesian consensus tree based on ITS data (results not shown) and in the ETS + ITS tree clade [Xj] and *Thurovia* were sisters, but support was very low (Fig. 4.2). In other analyses [Xj] was but one of many unresolved branches in a large polytomy in clade [AX] (Fig. 4.3).

The three remaining species of *Xylothamia* constitute a third clade, [Xp], sister to the *Bigelowia*/*Thurovia* lineage in the heuristic topologies (Fig. 4.1). Clade [Xp] was usually not defined in the other analyses. *Xylothamia pseudobaccharis* was excluded from [Xp] in the Bayesian phylogeny (Fig. 4.2), whereas, in the heuristic tree based on combined ETS + ITS data *X.parrisana* and *X.pseudobaccharis/X.purpusii* were part of a large polytomy with many other taxa (Fig. 4.2). *Xylothamia pseudobaccharis* likewise was part of a polytomous clade distinct from other [Xp] in analyses of the ETS matrix (results not shown). In the Bayesian ITS phylogeny (results not shown) clade [Xp] was unresolved with all three *Xylothamia* species participating in a large polytomy.

Other clades resolved in lineage [AX] include *Amphiachyris, Gutierrezia,* and *Gymnosperma* [AG]. The latter two were sisters in parsimony ratchet, but in PAUP* heuristic *Amphiachyris* and *Gymnosperma* were sisters and *Gutierrezia* was basal (Fig. 4.1). *Amphiachyris* and *Thurovia* are sisters in PAUP* ETS + ITS heuristic and unresolved from the *Gutierrezia/Gymnosperma* clade (Fig. 4.3). The three genera constituted a trichotomy in the Bayesian phylogeny (Fig. 4.2). *Euthamia* was sister to [AG] with 58% support in parsimony ratchet (Fig. 4.1A). In the PAUP* heuristic
Fig. 4.3. The 50% majority rule consensus tree derived from 9035 most parsimonious from PAUP*Ratchet analysis of the combined ETS + ITS data sets without indels. Each tree had a CI of 0.5271 and a RI of 0.8102. Bold and dash branches highlight Xylothamia and Gundlachia OTUs, respectively. Fractional number designations “indicate branch support/branch length.”
and the PAUPRatchet trees *Bigelowia/Thurovia* clade was sister to [Xp] (Fig. 4.1). In the tree resulting from PAUPRatchet clade [Xp] and the *Bigelowia/Thurovia* clade are resolved from the [AG] and *Euthamia* clades (Fig. 4.1A), whereas in the PAUP* heuristic tree clades [AG], [Xp] and the *Bigelowia/Thurovia* clade form a trichotomy with *Euthamia* (Fig. 4.1B). The clade consisting of [AG], *Euthamia*, *Bigelowia/Thurovia*, and [Xp] exhibited 74% and 67% support in the PAUPRatchet and PAUP* heuristic analyses, respectively (Fig. 4.1). This lineage collapsed as part of a polytomy including clade [Xj] in all other analyses (Figs. 4.2, 4.3).

Among outgroup taxa, identical lineages were resolved in PAUP* and Bayes analyses although their relationships to one another differed somewhat. Clade [SEC] composed of *Ericameria/Traycina* was sister to a grade of taxa with *Sericocarpus* basal that also includes *Chrysothamnus, Chrysoma, Oligoneuron, and Solidago* (Figs. 4.1-4.3). The sister relationship of the *Boltonia* containing lineage [BBC] to the Symphyotrichinae and Machaerantherinae [SM] clade was resolved in all cases. *Ionactis* was resolved as basal to the [BBC]/[SM] grade in the heuristic analyses (Fig. 4.1). In the Bayesian tree it was an unresolved, basal taxon (Fig. 4.2). The remaining outgroup lineage, [CE], consisted of *Chaetopappa* sister to *Croptilon/Erigeron*. Its relationship to other outgroup taxa varied with optimality criteria and data type (Figs. 4.1-4.3).

**DISCUSSION**

Species of *Xylothamia* and *Gundlachia* are contained in clade [X] with strong character support that is confirmed with maximum bootstrap and posterior Bayesian probability values. An 84 bp insertion near the 3′ end of the ETS region is unique, lending further evidence for the group’s monophyly. The ITS + 3′ ETS sequence-based
phylogenies clearly fail to support the monophyly of \textit{Xylothamia} and offer no support for previous taxonomic hypotheses, i.e. its inclusion in \textit{Ericameria} or affiliation with other \textit{Haplopappus sensu} Hall (1928). Lineage [X] conforms in generic composition most closely to the “\textit{Gutierrezia} group” proposed by Nesom (1991) who credited the chloroplast restriction investigations by Suh (1989) and Morgan (1990) for its definition. Besides \textit{Gutierrezia}, this group included \textit{Amphiachyris, Bigelowia, Euthamia, Gundlachia, Gymnosperma, Thurovia and Xylothamia}. \textit{Gundlachia} was not among the taxa sampled by Suh or Morgan; it was included on the basis of its having leaf storage parenchyma like that of \textit{Euthamia} as reported by Anderson and Creech (1975). Except for \textit{Xylothamia} being part of the \textit{Ericameria-Chrysothamnus} alliance, chloroplast restriction studies by Lane et al. (1996) supported the concept of the \textit{Gutierrezia} group. \textit{Gundlachia} was not included in their study either. Later Nesom (1993) extended group membership to encompass \textit{Chrysoma} and \textit{Sericocarpus} referring to this constellation of genera as the “\textit{Gutierrezia} lineage” which he subdivided into the \textit{Euthamia} and \textit{Gutierrezia} groups. \textit{Bigelowia, Chrysoma, Euthamia, Gundlachia, Sericocarpus, and Xylothamia} made up the former while \textit{Amphiachyris, Gutierrezia, Gymnosperma, and Thurovia} the latter.

Although Nesom et al. (1990) noted “an extreme degree of differentiation among species” in \textit{Xylothamia}, their distribution between the two clades recognized herein was unexpected in light of previous morphology-based assessments of relationship. Patterns of similarities and differences observed in \textit{Xylothamia}, especially in leaf and capitular structure, do not coincide with the molecular-based clades. Apparently, convergence has played a much larger role than previously hypothesized in shaping the appearance of each
species. Previous investigators used one or two representative species of *Xylothamia* in performing higher-level phylogenetic assessments. Presumably, they assumed monophyly for constituent genera in their investigations.

**Clade [Xd].** Phylogenetic analyses of the 3′ ETS + ITS sequence data provides maximum support for subclade [Xd]. With regard to *Gundlachia*, Lane et al. (1996) stated that it and *Gymnosperma* are morphologically more similar to each other than either is to *Gutierrezia* or other genera [in the *Gutierrezia* lineage]. Therefore, *Gundlachia*’s placement in subclade [Xd] with a subset of species of *Xylothamia* was unexpected, despite Anderson and Creech’s (1975) provision of anatomical evidence for its similarity for taxa in the *Gutierrezia* lineage.

Except for the sister relationship of *X. triantha* and *X. truncata* and their association (with moderate support) with *X. diffusa*, as suggested by Nesom et al. (1990) and Nesom (1992), relationships in [Xd] were unresolved or variably resolved with weak support irrespective of optimality criteria and data used. When the four species of *Xylothamia* resolved as a clade sister to *Gundlachia*, support was weak to moderate. Bayesian support for this *Xylothamia* lineage is much less than the 0.95 posterior probability value considered significant. Heuristic searches of the combined ETS and ITS data sets without indels produced a topology where *Gundlachia* and *X. diffusa* are sisters and terminal, *X. riskindii* is basal, and *X. triantha/X.truncata* is an intermediate grade (Fig. 4.3). Results from the combination of sequence data with indels are no more compelling. Bootstrap analysis of the combined sequence data places *X. riskindii* basal to a clade comprised of *Gundlachia* sister to the other three species of *Xylothamia*. In these alternative topologies, all internal nodes were weakly supported. The trichotomy
consisting of *Gundlachia, X. riskindii*, and the remaining three species of *Xylothamia* in clade [Xd] also indicates that these two traditionally recognized genera are not monophyletic.

Perhaps [Xd] evolved from a *Gundlachia*-like ancestor that extended into Mexico during the late Tertiary or at some time during the Pleistocene when less arid conditions prevailed. As aridity increased, adaptations such as smaller stature and reduced leaves evolved in *X. diffusa, X. triantha, and X. truncata*. The former occurs primarily in near-coastal areas in sandy to gravelly soils in Baja California and Sonora associated with such xeric vegetation as *Larrea, Prosopis, Yucca, and Pachycereus*. *Xylothamia triantha* and *X. trucata* grow in the Chihuahuan Desert also in association with mesquite, creosote bush, and other xerophytes typical of the flora. *Xylothamia riskindii*, on the other hand, grows at higher elevations in more mesic habitats associated with pine-fir-oak woodland in southeastern Coahuila and adjacent Nuevo Leon. This species, as suggested by its less reduced leaves and habitat preferences, may be a relict from a more mesic past. Available paleofloras from Cuba, Panama, and northeastern Mexico of Eocene to Miocene epochs predominantly show North rather than South American affinities (Graham et al., 2000). Species of *Gundlachia* and *Xylothamia* may, however, have had a more recent origin suggesting that seed dispersal and climate changes may have been major factors in their evolution rather than plate tectonics.

Lane (1996), who last investigated the taxonomy of *Gundlachia*, recognized two species, *G. domingingensis* and *G. corymbosa*. Of the six varieties in the latter, five had been treated as distinct species until their status was reduced (Lane, 1996). Branch lengths for the two populations of *G. corymbosa var. corymbosa* from different islands in
the present study are as great or greater than that observed for taxa recognized as distinct species and indicate significant genetic differentiation and possibly cryptic species. Typically, conspecific samples show little or no difference in base pair composition. Investigation of genetic variation in Gundlachia could serve as an important model for understanding evolution of the Caribbean flora.

In order to make the taxonomic nomenclature for clade [Xd] more consistent with phylogeny, Gundlachia, based on priority, warrants expansion to include the four Xylothamia based on the strength of support for the clade’s monophyly. Geographic separation of Gundlachia and Xylothamia might be used for distinguishing the two groups, but this seems arbitrary since the data at hand fail to otherwise confidently resolve species relationships within [Xd]. Because the type for Xylothamia, X. diffusa, is among these species to be transferred, Xylothamia is to be placed in synonymy and unavailable for further use. New combinations will be made according to traditional, hierarchical, taxonomic protocol in a separate paper with full taxonomic treatment.

Clade [AX]. This is also robustly supported by the gene trees. Possession of a four bp insertion in [AX] (except for Amphiachyris which has an overlapping deletion) and its absence in [Xd] also strengthens support for the lineage’s monophyly. The five other species of Xylothamia are placed in sister clade [AX] based on the nrDNA data but not as a monophyletic lineage. Xylothamia johnstonii and X. palmeri are consistently

resolved as sister taxa mostly with maximal support that is congruent with previous assessments of relationship based on morphology, geographic distribution, and seasonal reproductive isolation (Nesom et al., 1990). This clade’s relationship to Thurovia in the Bayesian tree is not statistically significant, and the clade’s relationship to the other three
Xylothamia is essentially unresolved. Foliar similarities between *X. johnstonii* and *X. palmeri*, taken as indications of a closer relationship to *Euthamia* by Nesom et al. (1990), are supported in part since these two species are closer to *Euthamia* than *X. diffusa* and other [Xd] taxa, but apparently not closer than *Bigelowia* or most other [AX] clade members.

Placement of the remaining three species of *Xylothamia* is ambiguous because relationships are weakly supported. The heuristic searches of the sequence data plus indels provide the strongest support for a clade composed of *X. parrasana*, *X. pseudobaccharis*, and *X. purpursii*, clade [Xp] (Figs. 4.1). The monophyletic relationship among the three *Xylothamia*, however, is not supported in other analyses. In the Bayesian tree *X. pseudobaccharis* joins the polytomy in [AX] while the clade *X. parrasana*/*X. purpursii* receives less than significant support.

Phyllary features such as the obscure costae, induate bases, apical patches, and glands used to define *Xylothamia sensu lato* appear to be plesiomorphic since they are also seen in *Bigelowia*, most *Euthamia*, and many *Gutierrezia*. The reduced leaves of *Xylothamia* in [AX] may result from convergence assuming that their progenitors were adapted to more mesic conditions. Investigators have long recognized that morphological convergence is frequently observed in plants adapted to dry habitats (Small, 1973).

*Xylothamia purpursii* is the most unusual species of *Xylothamia* in [AX] and it appears to represent a new model for xeric adaptation in this clade. Unlike the other taxa investigated, it has non-punctate, needle-like leaves. *Xylothamia parrasana* and *X. pseudobaccharis* are each defined by a number of morphological apomorphies based on leaf size and spacing, pubescence, presence of ray flowers, and capitulescence type as
noted by Nesom et al. (1990). Their relationships are not robustly resolved in the gene
trees. Therefore, their immediate common ancestors could not been determined and the
role of convergence in fashioning their similarities remains a matter of speculation.

All subclades in [AX] represent an apparent radiation into mostly xeric habitats of
northern Mexico, the western United States, and the Gulf Coast region of the Southeast.
The short branch lengths might be indicative of the relatively short time frame during
which these events occurred. Also, the shrubby, long-lived nature of many species
especially of Xylothamia would no doubt slow the relative pace of genetic change due to
their longer generation times.

Integrity for other genera besides Xylothamia in clade [AX] is supported by the
molecular data. *Bigelowia* is a genus of two species where one is adapted to dry, rocky
outcrops and the other to seasonally dry, sandy, coastal, pine savannas in the Gulf and
Atlantic Coast regions. Anderson (1970, 1972, 1977) investigated anatomical and
karyological details for these taxa, and his suggestions of affinity to *Euthamia* is
generally supported by nrDNA although relationships are often not fully resolved.
Nesom’s (1994) placement of *Bigelowia* close to *Chrysoma, Euthamia, Gundlachia,* and
Xylothamia within the Solidagininae is incompletely supported by DNA evidence. Its
relationship is with *Euthamia* and certain species of *Xylothamia sensu lato Gundlachia* is
not contained within the same lineage as *Bigelowia,* and *Chrysoma* is even more distant
(Chapter 2).

*Euthamia* is a genus of approximately eight species of herbaceous perennials
widespread in the eastern and central United States with one species widely distributed in
western North America (Sieren, 1981). The molecular data support its monophyly. Its
treatment at one time within *Solidago* is a relationship that now appears very distant. Leaf anatomy also shows the very distinct nature of *Euthamia* and *Solidago* (Anderson and Creech, 1975). *Euthamia*’s placement, the sole representative of the *Gutierrezia* lineage in Noyes and Rieseberg (1999), basal to *Chaetopappa/Monoptilon* is incongruous with the present findings and may be an artifact of sampling.

Support for *Amphiachyris*, as a lineage distinct from *Gutierrezia* and *Xanthocephalum*, is congruent with earlier hypotheses of relationship (Solbrig, 1960; Lane, 1979). Lane (1982) and DeJong and Beaman (1963) considered, based on chromosome number and certain morphological traits, *Xanthocephalum* a closer ally of *Grindelia* and relatives than of the *Gutierrezia* complex. Several subsequent molecular studies support this hypothesis (Suh and Simpson, 1990; Morgan and Simpson, 1992; Lane et al., 1996; Markos and Baldwin, 2001) and show it as part of the *Machaerantha* alliance. Lane (1985) expanded *Gutierrezia* to include several species previously treated as *Xanthocephalum*, and the monotypic genera *Greenella* and *Thurovia*. The latter two taxa are narrow endemics of central Baja California and the Texas Gulf Coast, respectively. Her hypothesis of relationship for *Greenella* is strengthened by cpDNA data (Suh and Simpson, 1990) and by the results of this investigation. *Thurovia*’s placement sister to *Amphiachyris* suggests that its relationship to *Gutierrezia* is more distant than indicated by Lane (1985). Two cpDNA investigations also corroborate this finding (Suh and Simpson, 1990; Lane et al., 1996). Features shared by *Thurovia* and *Amphiachyris* include annual habit, reduced or scale-like pappus, and reduced chromosome numbers. *Thurovia* is sister to [Xj] in the Bayesian tree although support for its position is not significant. When indel and sequence data are analyzed heuristically, *Thurovia* is sister to
Bigelowia. Gutierrezia as treated by Lane (1985), except for Thurovia’s exclusion, remains the most species rich genus of this complex. Its monophyly is affirmed by cpDNA (Suh and Simpson, 1990; Lane et al., 1996) and by the present nrDNA data. The ITS and ETS-based trees further confirm the cpDNA-based close relationship to the monotypic Gymnosperma (Suh and Simpson, 1990; Lane et al., 1996). Its considerable morphological similarity to Gundlachia, as indicated by Lane (1996), as support for their shared ancestry is discounted in the present study.

Nomenclature for the five species of Xylothamia in [AX] is problematic since the type species, X. diffusa, is placed in a distinct, well-supported clade. Furthermore, only X. johnstonii and X. palmeri are supported as a monophyletic lineage among the five former Xylothamia in [AX] while the other three are unresolved. Additional work will be attempted to further assess their relationships. However, based on the present results and standard taxonomic practice, four new genera will have to be established to accommodate these taxa.

Other Outgroup Taxa. Topological constancy is seen in lineages [BBC] and [SM] in the present study. With the addition of many other representative Astereae and the use of additional analytical methods, the composition but not the precise topology of [SM] as presented by Markos and Baldwin (2001) is maintained. Its derivation from a Symphiotrichoid ancestry is suggested in this investigation and is consistent with the ITS-based investigations of Noyes and Rieseberg (1999). This observation of relationship may change with increased sampling in the large, diverse subtribe Symphiotrichiinae.

Clade [BBC] has maximal support in the present analyses. The close relationship of Boltonia and Batopilasia [as Erigeron byei] discovered in the ITS study by Noyes and
Rieseberg (1999) has been confirmed herein. Because of expanded sampling, the sister relationship of *Batopilasia* is with *Chloracantha* rather than with *Boltonia*—a hypothesis favored by Nesom (2000). *Boltonia*, having flattened, winged, epappose achenes or with a pappus of reduced awns, has diverged considerably from its sister clade where terete to slightly flattened achenes and a pappus of barbellate bristles are characteristic. *Boltonia*-like achene features in Old World *Kalimeris* once were used as evidence for combining the two into a single genus as discussed by Gu and Hoch (1997) who concluded that such similarities are superficial. The nrDNA data and that of Noyes and Rieseberg (1999) support the premise of Gu and Hoch and indicate that a close phylogenetic relationship of *Boltonia* and *Kalimeris* is unlikely and that similarities in achene morphology might be due to convergence. The basal placement of clade [BBC] to the Symphiotrichiinae is stable in all analyses.

Representative taxa of Chrysopsidinae and Conyzinae (*sensu* Nesom 2000) are supported, in part, as monophyletic lineages in the present nrDNA sequence-based studies. *Croptilon*, the sole representative of subtribe Chrysopsidinae, is allied with [CE]—a result consistent with the Noyes and Rieseberg (1999) phylogeny. *Chaetopappa*’s basal position in [CE], in general, approximates the findings of Noyes and Rieseberg (1999) who showed it positioned within a graded series that steps up through clades represented by taxa of the *Townsendia* group, Chrysopsidinae, and Conyzinae (*sensu* Nesom, 2000). The large insertion in ETS of *Erigeron prostrata* similar to the one in clade [X] appears to have been derived independently.

The position of *Ionactis* in the present study is unstable. It is basal to the [SM]/[BBC] lineage some distance from *Doellingeria* in the heuristic analyses, whereas,
it is an unresolved basal element in the Bayesian phylogeny. There is no support for

Ionactis’ alliance with Symphiotrichum though at one time it was thought to be an Aster
sensu lato (Jones and Young, 1983). Xiang and Semple (1996) in their cpDNA
investigations show some affinity of Ionactis for Oclema, a taxon not included in the
present study. Nesom (2000) had placed Ionactis among his “Incertae sedis” in the group
of primitive asters.

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CHAPTER 5. SUBTRIBAL AFFINITIES OF *CHRYSOTHAMNUS*, *ERICAMERIA* AND, *XYLOTHAMIA* BASED ON nrDNA SEQUENCE DATA

INTRODUCTION

Astereae occur on all continents except Antarctica. Generally, approximately 135 to 189 genera and 2,500 to 3,020 species are recognized in this tribe (Grau, 1977; Bremer, 1994; Nesom, 1994; Bohm and Stuessy, 2001). Astereae are most diversified in the Americas and southern Africa (Grau, 1977). Within the past decade, two different subtribal classifications have been proposed. Bremer (1994), based to a large extent on the work of Zhang and Bremer (1993), recognized three subtribes, Granginae, Solidagininae and Asterinae. Their assessment of relationships within and among subtribes was based primarily on cladistic analysis of traditional morphological characters. Nesom (1994) in his worldwide treatment of Astereae proposed recognizing 14 subtribes. Unlike the classification of Bremer (1994), Nesom’s employed a wider array of morphological characters in addition to results of chloroplast DNA (cpDNA) restriction site investigations of Suh (1989), Suh and Simpson (1990), Morgan (1990) and Morgan and Simpson (1992).

Prior to the proposals of Bremer (1994) and Nesom (1994, 2000), other researchers tendering subtribal classifications of note for Astereae are Cassini (1819), Lessing (1832), DeCandolle (1836), and Bentham (1873). Cassini (1819), in addition to circumscribing and naming tribe Astereae, classified its species into four subtribes. Bremer (1994) regarded Cassini’s classification and the two that immediately followed, Lessing (1832) and DeCandolle (1836), essentially artificial. Bentham’s (1873) offering differed in that he organized species into six subtribes. This classification emphasized ray floret color and, like those of the previous researchers, is artificial because this character is known to vary within and among genera. The subtribal classification presented by
Hoffmann (1890), except for a few changes in subtribal names, was similar to that of Bentham (Bremer, 1994). Subsequently one additional subtribe, Hinterhuberinae, was described (Cuatrecasas, 1969).

The cladistic treatment of Xhang and Bremer (1993) arranged Astereae genera into 23 informal groups. Taxa were subsequently sampled from among those groups for cladistic analyses. The investigation resulted in combining four of the seven recognized subtribes with the Asterinae. The other two subtribes survived cladistic evaluation of Xhang and Bremer (Bremer, 1994).

*Chrysothamnus, Ericameria* and *Xylothamia* are placed in the Solidagininae by Bremer (1994). Within the subtribe, *Chrysothamnus* is sister to *Ericameria* in the *Ericameria* group while *Xylothamia* is associated with the *Solidago* group. Other taxa included in the present investigation represent all three subtribes proposed by Bremer (1994). In contrast, Nesom (1994) classified *Ericameria* in subtribe Hinterhuberinae, noting its affinity to South American representatives of that subtribe. Both *Chrysothamnus* and *Xylothamia* were placed in the Solidagininae along with 21 other genera (Nesom, 1994, 2000). Nesom (2000) also presented modifications to the previously proposed classification (Nesom, 1994). The 14 subtribes proposed by Nesom (1994) were maintained, with some reassignment of genera, and four groups of uncertain affinity were created. Prior to this, Nesom (1990), Nesom et al. (1990), and Nesom and Baird (1993) argued for recognizing *Chrysothamnus, Ericameria, and Xylothamia* as distinct from each other. Thus, Anderson’s (1995) transfer of all remaining *Chrysothamnus* to *Ericameria* crossed what other researchers considered both distinct generic and subtribal boundaries. Nesom’s classification, because of its extensive taxon sampling and use of nontraditional characters (both morphological and molecular)
presents a more natural grouping of genera within subtribes and serves as the benchmark for further evaluating subtribal relationships. As a result, discussion of subtribal relationships of genera included in this investigation will be based primarily on comparisons to Nesom (1994, 2000) rather than on Bremer (1994). Representatives of all 14 of Nesom’s (1994, 2000) subtribes and four groups of uncertain affinity are included in this dissertation project.

The relationships hypothesized by Noyes and Rieseberg’s (1999) ITS-based phylogeny investigating the origins of North American Astereae are more congruent with Nesom’s classification than with Bremer’s. A comparison of Nesom’s and Bremer’s subtribal classifications was also presented by Noyes and Rieseberg (1999). Of note is the revelation that several taxa representing subtribe Hinterhuberinae were distributed in a basal grade of predominantly southern hemisphere taxa separate from *Ericameria*. Therefore, Hinterhuberinae failed their monophyly test and provided evidence that *Ericameria* may be derived from North American ancestors.

The *Ericameria* representative included in the Noyes and Rieseberg (1999) study is sister to a clade consisting of three genera of annuals, *Pentachaeta*, *Rigiopappus*, and *Tracyina*. The latter three had previously been regarded as Feliciinae by Nesom (1994) but given uncertain status in Nesom 2000. Bremer (1994) placed all three genera in the subtribe Asterineae nested in the *Chaetopappa* group. *Rigiopappus*, however, was at one time placed in the tribe Helenieae. Cytological (Raven and Kyhos, 1961; Ornduff and Bohm, 1975), chemical (Ornduff and Bohm, 1975) and morphological evidence (Robinson and Brettell, 1973; Van Horn, 1973) all show that *Rigiopappus* is unequivocally related to taxa in Astereae. Both *Stenotus* and *Tonestus* were placed in the *Petradoria* group of the Solidagininae (Bremer, 1994). However, Nesom (2000) placed
Stenotus in the Solidagininae while Tonestus is among eight other genera of uncertain affinity within a group coined “Primitive” Asters.

Comparison of the conclusions of previous researchers with regards to relationships and subtribal classification in the Astereae is difficult because most projects include only exemplar taxa and researchers’ focus was different. For example, several taxa investigated by Lane et al. (1996) were not included in the Noyes and Rieseberg (1999) study, limiting the number of comparisons to be made for the taxa in the present study. One major incongruity is the lack of affinity of Chrysothamnus, sensu stricto for Solidago examplars in the restriction site studies by Lane et al. (1996) suggesting that it is not Solidagininae. Nesom and Baird (1995) suggested widening the definition of Chrysothamnus to include Hesperodoria and Petradora. They also highlighted that, as a group, the previous three genera are apparently closely related to Stenotus a member of the Solidagininae.

The goal here is not a comprehensive assessment of subtribal relationships in the Astereae, but to assess the relationships of the taxa here investigated based on the available DNA sequence data. By so doing, answers to the following questions are sought; 1. Do Chrysothamnus, Ericameria and Xylothamia display the subtribal affinities hypothesized by Nesom (1994, 2000)? 2. How do these taxa fit in to the larger subtribal classification based on molecular data?

MATERIALS AND METHODS

Taxa. Samples for analysis were obtained from field collections of natural populations or from specimens deposited in various herbaria (Table 5.1). For Chrysothamnus, species representing the three lineages identified by this study (Chapter
Table 5.1 Taxa sampled in this study, their sources, voucher and Genbank data, and relevant literature citations for published sequences.

<table>
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<th>Taxon</th>
<th>Locality and voucher information</th>
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<th>ETS-GenBank numbers</th>
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<td><em>Acamptopappus sphaerocephalus</em> (Harv. &amp; A. Gray in A. Gray) A. Gray</td>
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<td><em>Amellus strigosus</em> (Thunb.) Less.</td>
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<td><em>Amphiachyris dracunculoides</em> Nutt.</td>
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<td><em>Amphipappus fremontii</em> Torr. &amp; A. Gray var. fremontii A. Gray</td>
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<td><em>Aphanostephus ramosissimus</em> DC.</td>
<td>Mexico: Guanajuato, Ventura 7924 (MO)</td>
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<td><em>Aster amellus</em> L.</td>
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<td><em>Astranthium integrifolium</em> (Michx.) Nutt.</td>
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<td><em>Baccharis dracunculifolia</em> DC.</td>
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<td><em>Batopilasia byei</em> (S.D. Sundb. &amp; G.L. Nesom) G.L. Nesom &amp; Noyes</td>
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<td><em>Bellis perennis</em> L.</td>
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<td><em>Bigelowia nuttallii</em> L.C. Anderson</td>
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<td><em>Boltonia asteroides</em> L'Her.</td>
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<td><em>Chloracantha spinosa</em> (Bentham) G.L. Nesom</td>
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<td><em>Chrysothamnus eremobius</em> L. C. Anderson</td>
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<td><em>Chrysothamnus viscidiflorus</em> (Hook.) Nutt. ssp. puberulus_ (D. Urbatsch) H. M. Hall &amp; Clem.</td>
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<td><em>Ericameria albida</em> (M. E. Jones ex A. Gray) L. C. Anderson</td>
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<td><em>Ericameria bloomeri</em> (A. Gray) J. F. Macbr.</td>
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<td><em>Felicia aethiopica</em> (Lees.) Grau</td>
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<td><em>Geissolepis suaedefolia</em> B. L. Robinson</td>
<td>Mexico: San Luis Potosi, Nesom 6634 (MO)</td>
<td>AF046995 AF046996</td>
</tr>
<tr>
<td><em>Grangea maderaspatana</em> (L.) Poir.</td>
<td>Thailand: Chiang Mai, Maxwell 90-218 (MO)</td>
<td>AF046951 AF046952</td>
</tr>
<tr>
<td><em>Grenella ramulosa</em> Greene</td>
<td>Mexico: Baja California, Powell &amp; Turner 2226 (LSU)</td>
<td>Unpublished Unpublished</td>
</tr>
<tr>
<td><em>Grindelia lanceolata</em> Nutt.</td>
<td>Texas: Travis Co., Morgan 2114 (WWB)</td>
<td>U97609</td>
</tr>
<tr>
<td><em>Gundlachia corymbosa</em> (Urb.) Britton ex Bold.</td>
<td>West Indies: Caicos Islands. Pine Cay. Correll 43104 (LL)</td>
<td>AF477654 AF477718</td>
</tr>
<tr>
<td><em>Gundlachia corymbosa</em> (Urb.) Britton ex Bold.</td>
<td>Puerto Rico: Quebradillas. Axelrod 11957 (LSU)</td>
<td>AF477655 AF477719</td>
</tr>
<tr>
<td><em>Gutierrezia sarothrae</em> (Pursh) Britton &amp; Rusby</td>
<td>Colorado: Mesa Co. Urbatsch &amp; Roberts 7896 (LSU)</td>
<td>AF477657 AF477721</td>
</tr>
<tr>
<td><em>Gymnosperma glutinosum</em> Less.</td>
<td>Texas: Frio Co. Urbatsch 2772 (LSU)</td>
<td>AF477765 AF477723</td>
</tr>
<tr>
<td><em>Haplopappus folius</em> DC.</td>
<td>CHILE: Rundel, s.n. UCBG 80.0298</td>
<td>AF251577 AF251635</td>
</tr>
<tr>
<td><em>Haplopappus glutinosus</em> Cass.</td>
<td>CHILE: Spare and Constance 17927 (UC)</td>
<td>AF251578 AF251636</td>
</tr>
<tr>
<td><em>Hazardia brickellioides</em></td>
<td>Nevada: Nye Co.: Bostick 5216 (DS)</td>
<td>Unpublished Unpublished</td>
</tr>
<tr>
<td><em>Hazardia detonsa</em> Greene</td>
<td>California: Santa Cruz Island. UCBG 95.0527</td>
<td>AF251582 AF251640</td>
</tr>
<tr>
<td><em>Hesperodoria salicina</em> (S. F. Blake) G. L. Nesom</td>
<td>Arizona: Coconino Co., Scott 880 (ASC)</td>
<td>AY170955 AY169752</td>
</tr>
</tbody>
</table>

Table 5.1 cont’d.
Hesperodoria scopulorum (M. E. Jones) Greene
Utah: Washington Co. Shultz 5382 (CAS)
AY170956 AY169753

Heterotheca villosa (Pursh) Shinners
USA: Colorado, Stein 1823 (MO)
AF046994

Ionactis lineariifolia (L.) Greene
Louisiana: Rapides Parish. Bruser 357 (LSU)
AF477660 AF477724

Isocoma menziesii (Hook. & Arn.) G.L. Nesom
California: Los Angeles Co. Bartholomew 535 UCBG 78.0157
AF251571 AF251629

Kalimeris integrifolia Turcz. Ex DC.
China: Jiangsu, Wei 6003a (MO)
AF046960

Kippistia suaedifolia F. Muell.
Australia: New South Wales, Pickard 3657 (NSW)
AF2470715

Lagenifera panamensis S. F. Blake
Panama: Chiriqui, Schmalzel 1731 (MO)
AF046965

Lessingia virgata A. Gray
California: Tehama Co. Markus 152 (JEPS)
AF251624 AF251682

Machaeranthera parviflora A. Gray
Texas: Turner & Powell 6094 (UC)
AF251568 AF251626

Minuria integerrima (DC.) Benth.
Australia: Queensland, Lowry 1754 (UNSW)

Monoptilon belliioides (A. Gray) H. M. Hall
USA: Arizona, Yatskievych 93-06 (MO)
AF046981

Olearia pannosa Hook.
Australia: South, 24061 (UNSW)
AF247065

Oligoneuron nitidum (Torr. & A. Gray) Small
Louisiana: Lasalle Parish, Urbatsch 5735 (LSU)
AY170957 AY169754

Oligoneuron rigidum (L.) Small
Louisiana: Winn Parish, Urbatsch 5219 (LSU)
AF477663 Unpublished

Oonopsis wardii (A. Gray) Greene
Wyoming: Albany Co., Brown 2797 (RM)
U97638

Oreochoysum parryi (A. Gray) Rydb.
Colorado: Lake Co., Urbatsch 7887 (LSU)
AY170958 AY169755

Oreostemma alpigenum (Torr. & A. Gray) Greene
USA: Oregon, Merello AF046978 819 (MO)

Oritrophium hieracioides (Wedd.) Cuatr.
Bolivia: La Paz, Solomon 16570 (MO)
AF046946

Pentachaeta exilis (A. Gray) A. Gray
California: Monterey Co., Keil 17085 (TEX)
AY171036 AY171004

Table 5.1 cont’d.
| **Peripleura bicolor** (N.T.Burb.) G.L.Nesom | Australia: Queensland, Lowrey 1765 (UNSW) | – |
| **Podocoma notobellidiastrum** (Griseb.) G.L.Nesom | Paraguay: Caazapa, Zardini 3009 (MO) | AF046963 |
| **Prionopsis ciliata** Nutt. | Texas: Sutton Co., Morgan 2084 (TEX) | U97644 |
| **Petradoria pumila** (Nutt.) Greene | Colorado: Mesa Co., Urbatsch 7889 (LSU) | AY170959 AY169756 |
| **Psilactis tenuis** S.Watson | Texas: Jeff Davis Co., Morgan 2196 (WWB) | U97643 |
| **Pteronia incana** (Burm.) DC. | South Africa: Cape, Joffe 850 (MO) | AF046947 |
| **Pyrocoma apargioides** (A. Gray) Greene | California: Plumas Co. Schoolcraft 2072 (UC) | AF251631 |
| **Rayjacksonia phyllocephala** (DC.) R.L.Hartman & M.A.Lane | Texas: Chambers Co., Morgan 2032 (TEX) | U97645 |
| **Rigiopappus leptoclados** A. Gray | California: Modoc Co., Bartholomew 6575 (TEX) | AY171005 |
| **Sericocarpus tortifolius** Nees | Florida: Wakulla Co., Urbatsch 7599 (LSU) | AF477764 AF477728 |
| **Solidago canadensis** L. | Louisiana: West Feliciana Parish. Lievens 3347 (LSU) | AF477665 AF477729 |
| **Solidago fistulosa** Mill. | Florida: Gulf Co. Urbatsch 7587 (LSU) | AF477666 AF477730 |
| **Stenotus acaulis** (Nutt.) Nutt. | Utah: Garfield Co., Davidson 129 (UNLV) | AY170960 AY169757 |
| **Stenotus armerioides** Nutt. | Wyoming: Sublette Co., Cramer 8671 (RM) | AY170961 AY169758 |
| **Stenotus lanuginosus** (A. Gray) Greene | Oregon: Baker Co., Brooks SN (CAS) | AY170962 AY169759 |
| **Stenotus macleanii** A. Heller | Canada: YukonTerr., Porsild 9556 (ALTA) | AY170963 AY169760 |
| **Stenotus pulvinatus** (Moran) G. L.Nesom | Mexico: Baja California, Rebman 4176 (SD) | AY170964 AY169761 |
| **Stenotus stenophyllus** (A. Gray) Greene | Oregon: Harney Co., Cright 1122 (OSC) | AY170965 AY169762 |
| **Symphyotrichum subulatum** (Michx.) G.L. Nesom | Louisiana: Calcasieu Parish. Neyland 1616 (LSU) | AF477670 AF477734 |
| **Symphyotrichum tenuifolium** (L.) G.L. Nesom | Louisiana: Terrebonne Parish. Buras 413 (LSU) | AF477669 AF477733 |

Table 5.1 cont’d.
Tetramolopium pumilum Mattf. New Geinea, Lowrey 1546 (UNM) AF2470925 –

Thurovia triflora J.N. Rose Texas: Maragorda Co. Carr 17925 (TEX) AF477735


Tonestus alpinus (L. C. Anderson & Goodrich) G. L. Nesom & D. R. Morgan Nevada: Lander Co., Goodrich 12126 (UTC) AY170966 AY169763

Tonestus eximius (H. M. Hall) A. Nelson & J. F. Macbr. California: Alpine Co., Taylor 4174 (CAS) AY170966 AY169764

Tonestus lyrallii (A. Gray) A. Nelson Canada: Alberta, Mc Calla 4540 AY170969 AY169766

Tonestus micropus (Cronquist) G. L. Nesom & D. R. Morgan New Mexico:Rio Arriba Co., Fletcher 7145 (TEX) AY170970 AY169767

Tonestus pygmaeus (Torr. & A. Gray) A. Nelson Colorado: Lake Co., Urbatsch 7887.2 (LSU) AY170972 AY169769

Townsendia florifer (Hook.) A. Gray USA: Oregon, Merello 773 (MO) AF0469852 –

Tracyina rostrata S.F. Blake California: Ornduff 6348 (US) AF477737

Vanclevea stylosa (Eastw.) Greene Utah: Kane Co., Urbatsch 7625 (LSU) AY170973 AY169770

Vittadinia sulcata N.T.Burbidge Australia: Western, Lowrey 1727 (UNSW) AF2471125 –

Xanthocephalum gymnospermoides (A. Gray) Benth. & Hook. f. Texas: Jeff Davis Co. Morgan 2200 (TEX) U976503 D. Morgan, unpublished

Xylorhiza tortifolia (Torr. & A. Gray) Greene California: Inyo Co., Wisura 4770 (UC) AF2515704 AF2516284

Xylothamia diffusa (Benth.) G.L. Nesom Mexico: Sonora, Frisbein 1983a (TEX) AF477674 AF477738

Xylothamia johnstonii G.L. Nesom Mexico: Hidalgo. Vilchis 379 (TEX) AF477677 AF477741

Xylothamia palmeri (A.Gray) G.L. Nesom Texas: Mcmullen Co. Carr 10906 (TEX) AF477679 AF477743

Table 5.1 cont’d.
Xylothamia parrasana (S.F.Blake) G.L. Nesom
Mexico: Zacatecas.
Johnston 11542 (TEX)
AF477680 AF477744

Xylothamia pseudobaccharis (S.F.Blake) G.L. Nesom
Mexico: Coahuila.
Nesom 7688 (TEX)
AF477682 AF477746

Xylothamia purpusii (Brandegee) G.L. Nesom
Mexico: Durango.
Chiang et al. 9984 (LL)
AF477684 AF477748

Xylothamia riskindii (B.Turner & G. Langford) G.L. Nesom
Mexico: Nuevo Leon.
Nesom 7697 (TEX)
AF477686 AF477750

Xylothamia triantha (S.F. Blake) G.L. Nesom
Texas: Brewster Co.
Powell 3542 (TEX)
AF477687 AF477751

Xylothamia truncata G.L. Nesom
Mexico: Coahuila.
Nesom 5254 (TEX)
AF477688 AF477752

2) are included. All species of *Xylothamia* and two accessions of *Gundlachia*, plus representatives of taxa thought to be related to these genera, are included (Chapter 4; Nesom, 2000; Nesom et al., 1990; Lane et al., 1996). Also, several species representing the lineages in the *Ericameria* (Chapter 3) are included in this study. In addition, taxa identified as related to *Chrysothamnus* and *Ericameria* in this investigation were sampled. With reference to subtribal sampling, *Ericameria* and five other genera representing the Hinterhuberinae *sensu* Nesom (1994, 2000) are included. The Solidagininae, including *Chrysothamnus* and *Xylothamia*, is represented by 21 genera. Five subtribes are each represented by one genus. All other subtribes and groups of uncertain affinity are represented by at least two genera. Worldwide Astereae included in the Noyes and Rieseberg (1999) investigation employing ITS sequence data indicated that *Amellus* and *Felicia* are basal to all other taxa. As a result, representative species of these genera were designated outgroup for the analysis of the ITS data. External transcribed spacer sequence data for several taxa were not obtained. As a result, the rooting of analyses of the ETS data set was with *Doellingeria*. As discussed in previous chapters, the Noyes and Rieseberg (1999) ITS-based phylogenetic study demonstrated that North American taxa in Astereae comprise a clade with *Doellingeria* at the base.

Both ITS and ETS sequence data have been employed in this investigation. Results of previous research established that both the ITS and ETS regions provide useful phylogenetic information at the genus and species levels (Baldwin et al., 1995; Baldwin and Wessa, 2000; Clevinger and Panero, 2000; Urbatsch et al., 2000; Fernandez et al., 2001; Francisco et al., 2001). However, ETS sequence data has been shown to be equal to or more useful than ITS data for recently evolved lineages. The ETS region evolves as much as 1.4 times faster and provides a somewhat higher level of phylogenetically
informative characters than the ITS region (Markos and Baldwin, 2001). In addition, ETS and ITS data sets have been shown to be congruent and combinable resulting in better resolved phylogenies with higher character and statistical support (Baldwin and Markos, 1998; Clevinger and Panero, 2000; Markos and Baldwin, 2001).

The 137 ITS-region sequences (ITS-1, ITS-2, plus the 5.8S) represent 134 species in 81 genera, and the 102 3′-ETS sequences represent 99 species in 46 genera. Sequences from GenBank were added to both data sets. Accession numbers, publication and author citations are provided in Table 5.1. As noted previously (Chapter 4), one unpublished ETS sequence was kindly supplied by D. Morgan, Western Washington University, Bellingham.

**DNA Isolation, PCR, and Sequencing.** For each sample of field-collected leaf tissue approximately 100 mg was ground using the Mini Beadbeater™ 8 (BioSpec Products, Inc., Bartlesville, Oklahoma) in sterile 2 ml screw cap tubes. Tissue was kept frozen during this procedure by alternating cycles of grinding and freezing. Total genomic DNA was isolated and purified from these samples using the Qiagen DNeasy® Mini Plant Kit following the manufacturer’s protocol, or the 2X CTAB (hexadecyltrimethylammonium bromide) extraction protocol of Doyle and Doyle (1987). Herbarium specimen samples were treated similarly except that 20-30 mg of leaf tissue was ground and left in the extraction buffer for 0.5-1 hour at 65°C instead of the recommended 10 minutes.

Double stranded DNA for sequencing was initially generated in 50µl and later in 25µl reactions. The latter reaction size used 0.5 unit *Tfl* DNA polymerase (Epicentre Technologies, Madison, Wisconsin), 8µl H₂O, 12.5µl premix buffer G (Epicentre Technologies), 1µl each of 10nM forward and reverse primers, and 2µl of DNA template
usually diluted $10^{-2}$. Reactants in the 50µl reactions were doubled. The protocol for DNA amplification consisted of 3 min at 95°C denaturation cycle followed by 10 thermal cycles of 1 min of denaturation at 95°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C with a 4 s per cycle extension. Except for using an annealing temperature of 50°C, the next 20 cycles proceeded as before followed by a final extension phase of 7 min at 72°C. This protocol proved adequate for the amplification of both the ITS and ETS regions.

For amplification of the ITS region primers ITS-20 and ITS-262 (Urbatsch et al., 2000) were used in equimolar concentrations. When amplicon production was inadequate, products from the above reactions were used as templates and reamplified in subsequent PCR reactions using a set of nested primers, ITS-I.1 (Chapter 3) modified from primer ITS-I (Urbatsch et al., 2000), and ITS4 (White et al., 1990), in order to increase yield. Removing unincorporated dNTPs and primers with QIAquick Spin PCR purification columns (QUIAGEN Corporation) between successive PCR reactions generally resulted in better yields and a cleaner product.

Approximately 400-600 bp of the 3′ region of the External Transcribed Spacer were amplified using primers 18S-ETS and Ast-1 and Ast-8 (Baldwin and Markos, 1998; Markos and Baldwin, 2001). One additional primer designated 18S-R1 (Chapter 2) gave better results with some templates when paired with Ast-1. Primers were obtained from GeneLab in the School of Veterinary Medicine, Louisiana State University, Baton Rouge.

Prior to sequencing, amplicons were purified using QIAquick Spin PCR Purification columns or Novagen SpinPrep™ columns (Novagen, Madison, Wisconsin). Quantification of PCR product was performed visually on agarose gels using Low DNA
Mass Ladder (Life Technologies, Inc. Rockville, Maryland) as the standard. Both strands of DNA were directly sequenced in 10µL reactions using ITS-I and ITS-4 for the ITS region and 18S-ETS or 18S-R1 and either Ast-1 or Ast-8 for the 3′ ETS region. Cycle sequencing was conducted using BigDye™ Terminators Cycle Sequencing reagents (Applied Biosystems, Foster City, California) for 25 cycles in the PTC-100 where each cycle consisted of 10 sec denaturation at 96°C, 5 sec annealing at 50°C, and 4 min extension at 60°C. Electrophoretic separation and analysis of the labeled DNA molecules were accomplished with the ABI PRISM® 377 DNA Sequencer (Applied Biosystems). Assigned GenBank accession numbers for sequences obtained in this study are given in Table 5.1.

When sequence quality was poor, amplified copies of the ITS and ETS regions were cloned using the TOPO™ TA Cloning® Kit (Invitrogen Corporation) or the pSTBlue-1 Perfectly Blunt™ Cloning Kit (Novagen, Darmstadt, Germany) according to manufacturers’ protocols. The cloned ITS and ETS regions were re-amplified directly from plate transformed colonies using primers ITS-I and ITS-4. The same pair of ETS primers used in the original amplification was often used for re-amplification of the cloned colonies. Amplification conditions used were the same as discussed previously except that cells were lysed at 94 °C for 10 min prior to the PCR run. Typically, two or three cloned PCR products per sample were sequenced.

**Sequence Analysis.** Sequence fragments were edited and assembled with the aid of Sequencer version 3.0 (Gene Codes, Ann Arbor, Michigan). Boundaries of the spacer regions were determined by comparison to some of the many published studies (Urbatsch et al., 2000; Baldwin and Markos, 1998; Markos and Baldwin, 2000; Clevinger and Panero, 2000). Sequences resulting from cloned amplicons were entered into the data
matrix as individual operational taxonomic units. Edited sequences were aligned with Clustal W 1.8 (Baylor College of Medicine sequence launcher; http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html). Manual adjustments were made when judged necessary. Also, sequences subsequently obtained were aligned by manual comparison to the existing data matrix. MacClade version 4.0 (Maddison and Maddison, 2000) was used to examine and edit sequence alignments. Pairwise sequence divergence estimates were obtained using the distance matrix option in PAUP* 4.0b10 (Swofford, 2002).

**Phylogenetic Analyses.** Maximum parsimony and Bayesian analysis were conducted to estimate phylogenetic relationships among all taxa investigated. *Amellus* and *Felicia* were designated outgroups for the ITS analyses while *Doellingeria* was the outgroup for the ETS and combined data analyses based on the Noyes and Rieseberg (1999) study. Phylogenetic analyses were conducted individually on the ITS and ETS data sets and on the combined ITS/ETS data.

PAUPRat (Sikes and Lewis, 2001) enabled parsimony analysis of all data sets since heuristic parsimony searches often failed when using PAUP* 4.0b10 (Swofford, 2002) due to tree storage limitations. Nixon (1999) demonstrated the efficiency of this search algorithm in exploring tree space and resulting with more parsimonious topologies than obtained from PAUP* 4.0b10. Analysis of all data sets employed 25 runs each of 500 iterations where 25% of the characters were perturbed per iteration. Nonparametric bootstrap analyses (Felsenstein, 1985) with 1000 pseudo-replicates and 100 random sequence additions were conducted on all data sets. For bootstrap analyses MULTREES was turned off and 1000 trees of a specified length, which varied with the input data, were retained for each pseudo-replicate.
Bayesian analyses were performed with MrBayes 2.01 (Huelsenbeck and Ronquist, 2001) on the separate and combined ETS and ITS data sets. The best-fit model of nucleotide substitution for the ITS data was the Transversion model with some sites invariant and variable sites assumed to follow a discrete gamma distribution (TVM + I + Γ; Posada and Crandall, 1998). The Hasegawa-Kishino-Yano model (HKY; Hasegawa et al., 1985) with variable sites following a discrete gamma distribution (HKY + Γ; Posada and Crandall, 1998) was selected as the best-fit model for the ETS data. The general time reversible model with some sites invariant and variable sites assumed to follow a discrete gamma distribution (GTR + I + Γ; Yang, 1994a) was selected as the best-fit model of nucleotide substitution for the combined data, using Modeltest (Posada and Crandall, 1998). The gamma distribution was separated into six discrete rate categories to better accommodate rate heterogeneity (Yang, 1994b). The Markov chain Monte Carlo process was set so that four chains ran simultaneously for 1,500,000 generations for the independent data analyses and 2,000,000 generations for the analysis of the combined data. Trees and parameter estimates were sampled every 100 generations for a total of 15,000 and 20,000 trees and parameter estimates. Visualization of variation of the ML scores using scatter-plots showed that “stationarity” was achieved by the 3,000th tree for all analyses. Therefore, the first 3,000 trees were discarded and the posterior probability of the phylogeny and its branches was determined from the remaining 12,000 and 17,000 trees for the independent analyses and combined analyses, respectively. Multiple (usually 2-3) two-million generation runs of the same data set were performed in MrBayes to test whether trees with improved ML scores would be discovered and to learn whether consensus trees computed from such additional runs resulted in topological differences.
RESULTS

The aligned ITS data set is 688 bp in length and contains 137 sequences representing 134 species in 81 genera of Astereae. ITS region sequence length without gap alignment insertions varied from 560 bp in *Astranthium integrifolium* to 638 bp in *Monoptilon bellioides*. With the exception of one sequence each for *X. purpursii* and *X. truncata* having 165 bps in the 5.8S, all other taxa exhibited 164 bps for that region. ITS 1 sequence length ranged from 226 to 260 bps in *Amphiachyris* and *X. palmeri*, respectively. The longest ITS 2 sequence, 222 bp, was observed in *M. bellioides* while the shortest, 143 bp, was observed in *Astranthium integrifolium*. Pairwise distances between species as determined in PAUP* from the uncorrected ("p") distance matrix ranged from 0.16% between in two species pairs *Ericameria nana/E. lignumviridis* and *Xylothamia johnstonii/X. palmeri* to 18.8% between *Erigeron procumbens* and *Chiliotrichum diffusum*. Of 688 total characters in the aligned matrix, 287(41.7%) were parsimony-informative, 297 (43.2%) were invariant, and 104(15.1 %) were variable but parsimony-uninformative.

A total of 60 indel events were scored for the ITS region. The majority of these involved one or two bps. The largest indel, a 33 bp deletion near the beginning of ITS 1, was exhibited by *Amphiachyris*. A four bp insertion in the ITS 1 region was observed in some taxa in a sublineage of the *Gutierrezia* lineage [EGX]. Within this lineage the insertion was absent in *Grenella* and its presence could not be determined in *Amphiachyris* because the 33 bp deletion spanned the four bp. This insertion was absent from all other taxa in the [EGX] lineage (Figs. 5.1-5.4). Representative species of *Chrysopsis* and *Croptilon* exhibited a five bp insertion in the ITS 2 region. Also,
*Monoptilon* and *Chaetopappa* were characterized by a three bp insertion in this region. Other indel events in the ITS region were two bp or fewer.

The final data matrix for the ETS consisted of 521 characters for the 102 samples. Pairwise distances ranged from 0.0 % between eight pairs of taxa to 21.0% between three species pairs. In the ETS region 199 of 521 (38.2%) characters were parsimony-informative, 235 (45.1%) were constant, and 87 (16.7%) were variable but uninformative. A 53 bp insertion characterized clade [EGX] and a nearly identical one was seen in *Erigeron prostrata*. A seven bp insertion characterized *Chaetopappa ericoides* and a 3 bp insertion is clade specific for the taxa sampled in the [CSS] lineage composed of *Chrysothamnus*, *Sericocarpus*, and *Solidago* above *Chrysothamnus gramineus*. *Croptilon divaricatum* is characterized by a six bp deletion in the ETS region. In total 22 indels were scored in the ETS region most involved one or two bp.

The major lineages represented in this study are resolved in all analyses of the separate ITS and ETS data sets (Figs. 5.1, 5.2; ETS trees not shown). Support for and resolution within these lineages are generally greater when the data sets are combined irrespective of optimality criteria (Figs. 5.3, 5.4). Parsimony ratchet analyses of the ITS data resulted in 10,379 minimum length trees of 1,734 steps having consistency indices (CI) = 0.378 and a retention indices (RI) = 0.692 (Fig. 5.1), whereas, analysis of the combined data resulted in 9,908 minimum length trees. Trees were 1,739 steps long with CI = 0.496 and RI = 0.753 (Fig. 5.4). On all topologies, a clade [PC] is resolved with parsimony bootstrap support of 67% and 82% for the ITS and combined analyses, respectively (Figs 5.1, 5.4). Posterior Bayesian probability support for this clade was not significant (Figs. 5.2, 5.3). Within this clade the [CSS] lineage is resolved with bootstrap support >70% on the topology resulting from analysis of the combined data (Fig. 5.4).
However, posterior Bayesian probability support for the [CSS] clade was not significant for any data set. The [CS] clade is supported with parsimony bootstrap scores of 93% and 99% on the ITS and combined trees, respectively (Figs. 5.1, 5.4) and maximum Bayesian support (Figs. 5.2, 5.3). The lineage [ER] containing *Ericameria, Pentachaeta, Rigiopappus* and *Tracyina* receives strong bootstrap and maximum posterior probability support on all trees. It is sister to the [PC] lineage on all trees except for the one based on parsimony ratchet of ITS where it is basal to other North American clades (Fig. 5.1). Clade [EGX] is sister to the [PC] and [ER] clades in trees based on combined data but bootstrap support for this relationship is less than 50% and Bayesian posterior probability is not significant (Fig. 5.3, 5.4).

A clade designated [SM] consisting of the Symphyotrichinae [Sy] and Machaerantherinae [ME] lineages is resolved on trees resulting from both data sets with bootstrap support >70% and maximum posterior Bayesian probability support. Sister to [SM] is the *Batophilasia, Boltonia* and *Chloracantha* clade [BBC] with moderate bootstrap support and strong (0.99) Bayesian support in the ITS-based trees (Figs 5.1, 5.2). However, in the combined ITS/ETS trees support for this placement of [BBC] is week (Figs. 5.3). On the tree resulting from parsimony ratchet of the combined data clade [BBC] emerges basal to most other lineages of North American Astereae included in this study.

Two species of *Tonestus, T. aberrans* and *T. kingii*, are variably aligned with [ME] and not associated with other members of this genus as classically defined by Nesom and Morgan (1990) (Figs. 5.1-5.4). The other species of *Tonestus* are included in the Solidagininae clade. Details of their relationships are presented in chapter 2.
Fig. 5.1. The 50% majority rule consensus tree of 10,379 most parsimonious trees from PAUPRatchet analysis of the ITS data. The tree was 1,734 steps with a CI of 0.378 and RI of 0.692. Numbers above the nodes represent percent of total trees sampled containing the indicated node followed by bootstrap values from 1000 bootstrap replications. Branches labeled with a single value are supported by less than 50% bootstrap. Labels in brackets are designations for major lineages referred to in the text. The positions of *Tonestus abberans* is indicated by the ‘#’ and *T. kingii* is indicated by a ‘*’.
Fig. 5.2. The 50% majority rule consensus tree derived from Bayesian analysis of the ITS data set. Mean lnL: -10322.864, variance: 192.869, 95% CI: -10351.8 – 10297.6. Numbers above branches represent posterior probability values. Labels in brackets are designations for major lineages referred to in the text. The positions of *Tonestus abberans* is indicated by the ‘#' and *T. kingii* is indicated by a ‘*’. 
Fig. 5.3. The 50% majority rule consensus tree derived from Bayesian analysis of the combined ITS and ETS data sets. Mean lnL: -11464.52, variance: 152.73, 95% CI: -11489.9 –11442.0. Numbers above branches represent posterior probability values. Labels in brackets are designations for major lineages referred to in the text. The positions of Tonestus aberrans is indicated by the ‘#’ and T. kingii is indicated by an asterisk ‘*’.
Fig. 5.4. The 50% majority rule consensus tree of 9,908 most parsimonious trees from PAUPRatchet analysis of the combined data. The tree was 1,739 steps with a CI of 0.496 and RI of 0.753. Numbers above the nodes represent percent of total trees sampled containing the indicated node followed by bootstrap values from 1000 bootstrap replications. Branches labeled with a single value are supported by less than 50% bootstrap. The positions of *Tonestus aberrans* is indicated by the ‘#’ and *T. kingii* is indicated by a ‘*’.
DISCUSSION

Recent attempts at subtribal classification in the Astereae (Nesom 1994, 2000; Bremer 1994) present conflicting views with respect to the placement of *Chrysothamnus, Ericameria* and *Xylothamia*. Both treatments place *Chrysothamnus* and *Xylothamia* in subtribe Solidagininae but differed in the subtribal classification of *Ericameria*. While Bremer (1994) placed *Ericameria* in the Solidagininae, Nesom (1994, 2000) placed it in subtribe Hinterhuberinae. Noyes and Rieseberg (1999) in investigating the origins of North American Astereae indicated that *Chrysothamnus* is aligned with *Sericocarpus* and *Solidago* while *Ericameria* was supported in a clade with three genera of annuals, *Pentachaeta, Rigiopappus,* and *Tracyina* and not with any representative of the Hinterhuberinae included in that study. These relationships, especially the latter, were unexpected since the shrubby *Chrysothamnus* and *Ericameria* had generally been considered as closely related (Hall and Clements, 1923; Hall, 1928; Anderson, 1995) and *Pentachaeta, Rigiopappus* and *Tracyina* were placed in the Asterinae and Feliciinae by Bremer and Nesom, respectively. *Xylothamia* was not represented in the investigation of Noyes and Rieseberg (1999).

**Subtribe Solidaginineae.** Nesom (1994) proposed that the Solidagininae is composed of the *Amphipappus* group, the *Chrysothamnus* group, the *Gutierrezia* lineage which is composed of the *Euthamia* and *Gutierrezia* groups, and *Solidago* and its relatives. This study provides support for two alternative hypotheses on the composition of subtribe Solidaginineae depending on the methods of analysis and data used. One treatment may recognize this subtribe in the broad sense as including clades [PC], [ER] and [EGX]. Another, narrower definition of the Solidagininae may include only clade [PC].
Clade [EGX] appears distant from Solidago and its relatives in parsimony analysis of the ITS data, suggesting that it represents an independently derived lineage, whereas in analyses of the combined data, clade [EGX] is basal to both the Solidago/Chrysothamnus and Ericameria lineages. Bayesian analyses of the ITS data set places clade [EGX] in a lineage with subtribes Chrysopsidinae, Conyzinae, Machaerantherinae, Symphyotrichinae and the [BBC] lineage, though relationships within the lineage are unresolved. Inclusion of the Ericameria lineage in the Solidaginae is also open to question. All analyses of the combined data and Bayesian analysis of the ITS data show Ericameria sister to the Solidago lineage [PC]. However, parsimony analysis of the ITS data shows Ericameria associated in a clade distantly related to the Solidago lineage. All lineages of Chrysothamnus sensu lato appear to be nested within the Solidaginae. In addition, six of the eight species of Tonestus, a genus designated of uncertain affinity by Nesom (2000), are aligned with the Solidaginae. The other two species were associated with the Machaerantherinae clade in the present sequence-based trees.

Apparent differences in the relationship of the Ericameria [ER] and Gutierrezia [EGX] lineages may be due to taxon sampling. The ITS data set contains a more representative sample of worldwide Astereae than the ETS data set. Sampling for the combined data set was limited by the unavailability of ETS data for representative Astereae outside North America. Increased taxon sampling has been shown to reduce phylogenetic error (Zwickl and Hillis, 2002)

Nesom’s (1994, 2000) treatment of Ericameria in the primarily South American Hinterhuberiinae is a hypothesis discounted by Noyes and Rieseberg (1999). The present study also suggests that such a relationship is unlikely. Ericameria is not associated with
any other representatives of the Hinterhuberinae included in this investigation. Instead, it is aligned with three genera of western North American herbs. All three, *Pentachaeta*, *Rigiopappus* and *Tracyina* are designated of uncertain affinity by Nesom (2000), probably related to the subtribe Feliciinae. *Rigiopappus* was at one time considered a member of the tribe Helenieae a relationship now viewed as unfounded. While the position of *Ericameria* is equivocal in this investigation, this taxon appears but distantly related to other members of the Hinterhuberinae. However, sampling of South American taxa is still very meager and additional relationships undoubtedly remain to be discovered.

Nesom (2000) proposed that *Euthamia*, *Gundlachia*, *Gutierrezia* and *Xylothamnia* are members of the Solidigininae. The present study indicates that all four genera and taxa closely related to them comprise a clade distinct from Solidagininae. Distinctiveness of this lineage is supported not only by sequence data but also by a 53 bp insertion in the ETS region. Increased resolution of relationships within Astereae might be accomplished through the accumulation of additional sequence and morphological data and more extensive taxon sampling.

**LITERATURE CITED**


Sikes, D. S., and Lewis, P. O. 2001. Beta software, version PAUPRat: PAUP* implementation of the parsimony ratchet. Distributed by the authors. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, USA.


CHAPTER 6. SUMMARY AND CONCLUSIONS

CHRYSOTHAMNUS AND RELATED GENERA

My analysis of nrDNA sequences from 195 taxa suggests that species traditionally classified in *Chrysothamnus sensu* Nesom are instead grouped in four distinct, well-supported lineages. The generic type, *C. viscidiflorus*, is associated with six other species of *Chrysothamnus sensu lato*, *C. depressus*, *C. eremobius*, *C. greenei*, *C. humilis*, *C. molestus* and *C. vaseyi*. *Acamptopappus*, represented by two species, *Hesperodoria scopulorum*, the type species of the apparently biphyletic genus, and the monospecific *Vanclevea*, are also included in this lineage. The two varieties of *Amphipappus*, that based on molecular and morphological data, merit elevation in rank to species, are placed in this clade as non-sister taxa. *Tonestus lyallii*, the type species for *Tonestus*, is also associated with this lineage. Its inclusion in *Chrysothamnus sensu stricto* renders the name *Tonestus* in synonymy with *Chrysothamnus* and unavailable for further use.

The second lineage resolved with core *Chrysothamnus* includes *C. baileyi*, *C. linifolius*, *C. pulchellus*, and *C. spathulatus*. *Hesperodoria salicina*, the other species of *Hesperodoria* included in this investigation, *T. microcephalus*, a taxon previously reported as having affinity for *Chrysothamnus*, and *T. peirsonii* complete this lineage. Type species for all taxa represented in this clade are aligned with the *C. viscidiflorus* lineage. It is therefore recommended that this lineage be recognized as a new genus, *Lorandersonia*, in recognition of Dr. Loran C. Anderson, Florida State University, a long-time avid student of *Chrysothamnus* and related taxa. Nomenclature and taxonomic treatment are to be considered elsewhere.
*Chrysothamnus gramineus* is not allied to any other lineage containing species of *Chrysothamnus*. Instead, it is basal to a clade consisting in part of the previous two lineages plus, *Brintonia, Chrysoma, Columbiadoria, Eastwoodia, Oligoneuron, Oreochrysum, Petradoria pumila, Sericocarpus*, the *Solidago* lineage, and several species of *Stenotus* and *Tonestus sensu lato* This taxon appears basal to the *Solidago* lineage and does not appear to be closely related to any lineage of *Chrysothamnus* or to *Petradoria* as previously proposed. This taxon will be treated as a newly described monotypic genus in another study. Preliminary analyses indicate that this entity is basal in a redefined subtribe Solidagininae.

Five species previously regarded as *Chrysothamnus* are supported in the genus *Ericameria*. Four of these (*C. nauseosus, C. paniculatus, C. parryi, and C. teretifolius*) had been transferred earlier based, in part, on DNA restriction site analyses. The other, *C. albidus*, is also supported as *Ericameria* in the present sequence-based study.

A close relationship of these four *Chrysothamnus* lineages to one another is not supported in the present analyses. There is little agreement between the composition of molecular based lineages containing *Chrysothamnus* and the classically derived sectional classifications proposed by various earlier workers.

*Solidago* is consistently associated with *Brintonia, Chrysoma* and *Oligoneuron*. Affiliation of *Stenotus stenophyllus* with *S. mcleanii* is robustly supported. Also, *Petradoria pumila* is strongly aligned with *Stenotus acaulis, S. armerioides*, and *Tonestus alpinus*. With the accumulation of more data, the latter clade may be recognized as the genus *Stenotus* given the priority of this name and the inclusion of its type *S. acaulis*. *Sericocarpus* is basal to all other genera above *C. gramineus*. Additional investigation is
needed to clearly resolve the relationships of *Columbiadoria*, *Eastwoodia*, *Oreochrysum* to other genera included in this investigation. Also, resolution of the relationship of *Stenotus* and *Tonestus sensu lato* requires the accumulation of more data. However, it appears that with the inclusion of the type species of both genera in other lineages the remaining taxa may be incorporated in other genera or treated within several novel genera.

With reference to infrageneric classification and relationships, the present molecular data do not support the sectional compartmentalization of *Chrysothamnus* proposed by previous researchers. Molecular phylogenetic analyses consistently place *C. linifolius* and *C. spathulatus*, members of section *Chrysothamnus*, with *C. pulchellus*, a member of section *Pulchelli*. Furthermore, in all analyses *C. depressus*, *C. vaseyi* and *C. molestus* are consistently associated with species in section *Chrysothamnus* rather than with section *Pulchelli*. Section *Gramini* lacks molecular support in that *C. eremobius* is associated with the *C. viscidiflorus* lineage whereas *C. gramineus* is but distantly related to any *Chrysothamnus, sensu lato* Traditionally used morphological features of the capitulum and other organs do not seem to be useful as characters for defining generic boundaries of *Chrysothamnus* and associated taxa in contrast to what had been envisioned by previous workers. Finally, all species in *Chrysothamnus sensu lato*, including all taxa above and *C. gramineus*, are aligned with subtribe Solidagininae of the Astereae.

**ERICAMERIA AND RELATED GENERA**

The DNA-based phylogenetic hypotheses presented here support the recognition of *Ericameria* as a lineage distinct from both *Chrysothamnus* and *Xylothamia*. Also, the
treatment of *Ericameria* to include the four species of *Chrysothamnus* (*C. nauseosa*, *C. paniculatus*, *C. parryi* and *C. teretifolius*) transferred to that genus, and sections *Asiris*, *Macronema* and *Stenotopsis* is supported by the results of this investigation. Except for *C. albida*, the transfer of all remaining *Chrysothamnus* to *Ericameria* is unsupported. The *Ericameria* lineage in this investigation is resolved as three clades that might be treated as three sections. Section *Asiris* is here expanded to encompass 14 species. Two of these are new within *Ericameria*, one newly described and the other newly elevated in rank. Appropriate nomenclatural changes will be treated elsewhere. *Ericameria albida*, a species with no previous sectional classification, is allied with section *Asiris*. Section *Macronema* is here reduced to six species centered around *E. suffruticosa*. Section *Ericameria* is similar in composition to that previously proposed, except for including *E. linearifolia* that was earlier accommodated in section *Stenotopsis*. The sister relationship of *Pentachaeta*, *Rigiopappus* and *Tracyina* to *Ericameria* is strongly supported by this investigation. Within that lineage *Tracyina* and *Rigiopappus* are closely allied with *Pentachaeta* as the basal taxon.

The clade consisting of *Ericameria* and the three annual genera is well supported in all analysis and is distinct from all other lineages attributed to the Hinterhuberinae. *Pentachaeta*, *Rigiopappus* and *Tracyina* are strongly aligned with *Ericameria* in the Astereae and may merit recognition as a subtribe distinct from and apparently not closely affiliated with either Hinterhuberinae or Solidagininae.

**XYLOTHAMIA AND RELATED GENERA**

The hypotheses of relationships resulting from nrDNA sequence data indicate that *Xylothamia* as presently defined is polyphyletic. In order to make the taxonomic
nomenclature and classification of *Xylothamia sensu lato* more consistent with results of this phylogenetic study, *Gundlachia*, based on priority, warrants expansion to include four species of *Xylothamia* based on the strength of support for the clade’s monophyly. Because the type for *Xylothamia*, *X. triantha*, is among these species to be transferred, *Xylothamia* is to be placed in synonymy and unavailable for further use. New combinations will be made according to traditional, hierarchical, taxonomic protocol in a separate paper with full taxonomic treatment.

The five remaining species of *Xylothamia sensu lato* are placed in a separate clade but are not resolved as sister taxa. Only *X. johnstonii* and *X. palmeri* are supported as a monophyletic lineage among the five former *Xylothamia*. Nomenclature for the five remaining species of *Xylothamia sensu lato* needs to be addressed since the type species, *X. triantha*, is placed in a separate, well-supported clade. Additional work will be attempted to further assess their relationships. However for the present, using standard taxonomic practice, four new genera will have to be established to accommodate the four lineages that they represent. Former *Xylothamia* and related taxa are strongly supported in a clade distinct from both *Ericameria* and *Chrysothamnus*. The distinctiveness of this group merits its recognition as a lineage possibly related to but separate from Solidagininae. Based on priority of generic names included in this lineage, the most appropriate name for this clade would be based on the genus *Gutierrezia*.

**OTHER TAXA**

Two species of *Tonestus* included in this investigation are not aligned in the *Solidago* lineage with other species of *Tonestus*. Both *Tonestus aberrans* and *T. kingii* appear to be closely associated with *Eurybia*. *Tonestus aberrans* is probably closely
related to *E. hemispherica* while *T. kingii* is weakly associated with or unresolved close to *E. wasatchensis*. Both species of *Tonestus* and the species of *Eurybia*, representing the eastern and western North American distribution of the genus, are basal within the subtribe Machaerantherinae *sensu lato*.

Topological constancy is seen in the lineage containing *Batopilasia*, *Boltonia* and *Chlorocantha* in the present study. This clade has maximal support in the present analyses. The close relationship of *Boltonia* and *Batopilasia* [as *Erigeron byei*] discovered in a previous study based on ITS has been confirmed herein. The sister relationship of *Batopilasia* is with *Chloracantha* rather than with *Boltonia*. Results of this investigation are congruent with previous proposals. Although this clade’s placement basal to the Symphyotrichinae and Machaerantherinae is somewhat equivocal, it is consistently resolved with strong support across data sets and optimality criteria. The distinctiveness of this lineage suggests its being given subtribal recognition.
APPENDIX: LETTER OF PERMISSION

American Journal of Botany
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This transmission consists of one page

October 17, 2002

Mr. Roland P. Roberts
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Dear Mr. Roberts:

This letter provides you with authorization from the American Journal of Botany to use any and all material necessary from:


in your doctoral dissertation to be submitted to the Graduate School of Louisiana State University.

Sincerely,

Karl Niklas
Karl Niklas (Editor-in-Chief)
VITA

Roland Roberts was born in St. David’s, Grenada, on 6 October, 1960. He completed his pre-college education at the Grenada Boys Secondary School in St. George’s, Grenada. In 1983, he attended the Grenada Teachers’ College, where he earned a Teaching Certificate from the University of the West Indies, Cave Hill, Barbados. In 1988, he attended Southwest Texas State University in San Marcos, where he earned the degree of Bachelor of Science in Education in 1991. Subsequently, he began his graduate studies, at the same institution, under the supervision of Dr. David Lemke and obtained his Master of Science in Biology in 1996 specializing in plant taxonomy. In the fall of 1996, Roland joined the doctoral program of the Department of Plant Biology, Louisiana State University and Agricultural and Mechanical College in Baton Rouge, under the direction of Dr. L. Urbatsch, in order to continue his education in systematics and evolutionary biology. He will receive the degree of Doctor of Philosophy in December 2002.