Université de Montréal

La phylogénie moléculaire du genre nord-américain *Eurybia* (Asteraceae : Astereae) et ses proches parents (*Oreostemma*, *Herrickia*, *Triniteurybia*)

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Mémoire présenté à la Faculté des études supérieures
en vue de l'obtention du grade de
maîtrise en sciences biologiques

Octobre, 2009

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Université de Montréal
Faculté des études supérieures

Ce mémoire intitulé :

La phylogénie moléculaire du genre nord-américain *Eurybia* (Asteraceae : Astereae) et ses proches parents (*Oreostemma*, *Herrickia*, *Triniteurybia*)

présenté par :
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a été évalué par un jury composé des personnes suivantes :

Denis Barabé, président-rapporteur
Luc Brouillet, directeur de recherche
Christopher Cameron, membre du jury

Résumé

Eurybia et ses proches parents *Oreostemma*, *Herrickia* et *Triniteurybia* sont appelés le grade des eurybioïdes. Comprenant 31 espèces vivaces, ce grade appartient au clade Nord-américain de la tribu des Astereae. Les analyses moléculaires antérieures ont montré que ce groupe est à la fois paraphylétique aux Machaerantherinae et un groupe frère aux Symphyotrichinae. Les relations infragénériques partiellement résolues et faiblement supportées empêchent d'approfondir l'histoire évolutive des groupes et ce, particulièrement dans le genre principal *Eurybia*. Le but de cette étude est de reconstruire les relations phylogénétiques au sein des eurybioïdes autant par l'inclusion de toutes les espèces du grade que par l'utilisation de différents types de régions et de méthodes d'inférence phylogénétique. Cette étude présente des phylogénies basées sur l'ADN ribosomal nucléaire (ITS, ETS), de l'ADN chloroplastique (*trn*L-F, *trn*S-G, *trn*C-*ycf6*) et d'un locus du génome nucléaire à faible nombre de copie (CNGC4). Les données sont analysées séparément et combinées à l'aide des approches de parcimonie, bayesienne et de maximum de vraisemblance. Les données ADNnr n'ont pas permis de résoudre les relations entre les espèces polyploïdes des *Eurybia*. Les analyses combinées avec des loci d'ADNnr et d'ADNnr+cp ont donc été limitées à des diploïdes. Les analyses combinées ont montré une meilleure résolution et un meilleur support que les analyses séparées. La topologie de l'ADNnr+cp était la mieux résolue et supportée. La relation phylogénétique de genres appartenant au grade des eurybioïdes est comme suit : *Oreostemma* (*Herrickia* s.str. (*Herrickia kingii* (*Eurybia* (*Triniteurybia* - *Machaerantherinae*)))). Basé sur la topologie combinée de l'ADNnr+cp, nous avons effectué des analyses de biogéographie à l'aide des logiciels DIVA et LaGrange. Ces analyses ont révélé une première radiation des eurybioïdes dans l'Ouest de l'Amérique du Nord, suivi de deux migrations indépendantes dans l'Est de l'Amérique du Nord chez les *Eurybia*. Due au relatif manque de variabilité de

l'ADNnr, l'ADNcp et CNGC4, où le triage de lignés incomplet était dominant, l'origine du grade est interprétée comme récente, possiblement du Pliocène. La diversification du groupe a été probablement favorisée par les glaciations Pléistocènes.

Mots-clés : ITS, ETS, *trn*C-*ycf6*, *trn*S-G, *trn*L-F, ADNcp, ADNnr, Machaerantherinae

Abstract

Eurybia and it relatives, *Oreostemma*, *Herrickia*, and *Triniteurybia*, are collectively called the eurybioid grade. Comprising 31 perennial species, this grade belongs to the North American clade of the tribe Astereae. Early molecular analyses had inferred that this group is paraphyletic to the Machaerantherinae and sister to the Symphyotrichinae. The partially resolved and poorly supported relationships at the infrageneric level within the group, particularly within the core genus *Eurybia*, is preventing further insights into the evolutionary history of the group. The aim of this study is to reconstruct the phylogenetic relationships among the eurybioids by including all species of the grade and by using both different types of regions and multiple phylogenetic inference methods. The present study provides phylogenies based on nuclear ribosomal DNA (ITS, ETS), chloroplastic DNA (*trn*L-F, *trn*S-G, *trn*C-*ycf6*), and a low-copy nuclear locus (CNGC4), in separate and combined datasets analyzed using maximum parsimony, Bayesian and maximum likelihood approaches. In a separate analysis of the nrDNA dataset, the relationships of polyploids in *Eurybia* proved to be impossible to resolve. The nrDNA and nr+cpDNA combined analyses therefore were restricted to diploids. The combined analyses provided greater resolution and support than separate analyses. The nr+cpDNA phylogeny was the best resolved and supported. The phylogenetic relationship of genera belonging to the eurybioid grade is as follows: *Oreostemma* (*Herrickia* s.str. (*Herrickia kingii* (*Eurybia (Triniteurybia* – Machaerantherinae)))). Based on the nr+ cpDNA combined topology, we performed biogeographical analyses using DIVA and LaGrange. These analyses revealed

an initial radiation of the eurybioids in western North America, with two independent migrations to eastern North America within *Eurybia*. Based on the relative lack of variation in nrDNA, cpDNA and CNGC4, where incomplete lineage sorting was dominant, the origin of the grade is interpreted as recent, probably from the Pliocene. Diversification of the group was probably favored by the Pleistocene glaciations.

Keywords: ITS, ETS, *trn*C-*ycf6*, *trn*S-G, *trn*L-F, cpDNA, nrDNA, CNGC4, Machaerantherinae.

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ADN: acide désoxyribonucléique

ADNr: ADN ribosomal

ADNnr: ADN nucléaire ribosomal

a.s.l.: au dessus du niveau de la mer (‘above sea level’)

bp: paire de bases (‘base pair’)

B.P. : avant le présent (‘before present’)

CTAB: bromure de cétyltriméthylammonium

DMSO: diméthyl sulfoxyde

ITS: espaceur interne transcrit

ETS: espaceur externe transcrit

e.g. *exempli gratia* (Latin) veut dire ‘par exemple’

FNA: Flore de l’Amérique du Nord (‘Flora North America’)

i.e.: *id est* (Latin) veut dire ‘c’est à dire’

L: litre

LB: Luria-Bertani

m : mètre

min : minute

ML: maximum de vraisemblance (‘maximum likelihood’)

mmol: millimolaire

myr: million d’années (‘million years’)

n: niveau de ploidie

NA: Amérique du Nord (‘North American’)

S: sous-unité

s.l.: *sensu lato* veut dire ‘sans large’

spp: espèces

x: nombre de chromosome à la base

x^2: Chi-carré

µ: micro

g: gramme

°: degré

%: pourcentage

>: plus grand

≥: plus grand ou égal

<: plus petit

x: hybride

&: et

Remerciements

D'abord, un sincère remerciement à mon superviseur de recherche, Luc Brouillet, pour m'avoir fait confiance, donné l'opportunité et le plaisir de travailler auprès de lui. Tout au long de ces dernières années, il a su me guider, me conseiller, me soutenir et m'orienter vers une prochaine aventure. Sans aucun doute, au travers de ces années, il a su surpasser son rôle de superviseur à mon égard pour donner le meilleur de lui-même !

Merci à Anne pour m'avoir considérée comme l'un des ses étudiants ainsi que pour la correction du premier chapitre. Un grand merci aux membres du jury, à Christopher Cameron et Denis Barabé, pour leur disponibilité et leurs commentaires pertinents sur ce mémoire. Je dois également remercier mes collègues de laboratoire Bruneau- Brouillet: Jamil, Simon, Élyse, Marie-Pierre, Stacy et Vincent, pour m'avoir familiarisée aux techniques de laboratoire, pour nos conversations entre deux réactions de PCR et les services rendus. Je lève mon chapeau aussi aux autres étudiantes du laboratoire (Marjorie, Mariannick, Carole) qui ont participé à la bonne ambiance du laboratoire. Durant mes années à l'IRBV, plusieurs personnes ont grandement contribué à agrémenter mon entourage : les étudiants des laboratoires voisins tout autant les anciens finissants (i.e., Martin, Hugo, Sier Ching, Stéphanie, Eric) que ceux encore présents comme Firas, Youssef, Julie, Jonathan et Eric. Mes remerciements vont également à certains professeurs (M. Hijri, M. Barabé, M. Rivoal et Mme. Sonia) et au personnel de l'IRBV (Stuart, Denis, les trois dames à l'administration!), les agents de sécurité de même que le personnel du

jardin botanique, spécialement Raymond Archambault. J'aimerais remercier mes amis (Vincent, Eva, Eric, Yann, Taïca, Oleh, Jérome et Kit) pour avoir partagé leurs expériences et leurs univers, pour avoir enrichi les soirées et fait le plein d'énergie lorsque celui-ci était nécessaire! Finalement, ma famille (mes parents, mes frères et ma soeur) pour m'avoir fait confiance et encouragée tout le long de ce périple.

Introduction générale

Incluses au sein de la sous-famille des Asteroideae (Asteraceae) et considérées comme la deuxième plus grande tribu de la famille, les Astereae Cassini comptent quelque 3100 espèces groupées en 222 genres. La tribu comprend des herbacées annuelles ou vivaces et des arbustes, ainsi que quelques vignes ou arbres (Nesom & Robinson 2007, Nesom 2009). Généralement, les représentants de cette tribu occupent des habitats ouverts et leur aire de répartition géographique s'étendent à la majorité des régions du monde, tant dans les zones alpines de l'Arctique que dans les sous-bois des zones tropicales. Ils sont surtout présents dans les milieux xériques à humides des régions tempérées (Brouillet et al. 2009). L'Amérique du Nord est l'un des plus importants centres de diversification de cette tribu (Brouillet et al. 2009). Cette région, qui va du Mexique à l'Arctique, comprend approximativement 25% des espèces et 33% des genres de la tribu. Dans une récente revue de littérature des Astereae (Brouillet et al. 2009), les auteurs ont confirmé que l'origine des Astereae sur le continent nord-américain serait monophylétique, un fait auparavant souligné par Noyes & Rieseberg (1999). Elle résulterait d'un événement unique de dispersion à longue distance à partir de l'Amérique du Sud. Une fois arrivées en Amérique du Nord, les Astereae auraient connu une radiation rapide. D'après Brouillet et al. (2009) et Funk et al. (2009), la plupart des genres qui s'y trouvent sont endémiques au continent. Toutefois, la date d'origine de la tribu sur le contient nord-américain, un événement d'importance majeure, n'a pu être déterminée (chapitre 2).

La première classification du groupe nord-américain avait été réalisée uniquement à l'aide des caractères morphologiques (Bremer 1994, Nesom 1994, Nesom 2000). À cause

de l'uniformité des caractères, de la difficulté d'interprétation et du faible nombre de caractères variables, la position taxonomique de nombreux genres et espèces demeurait problématique. De surcroît, certains genres de la tribu étaient devenus des genres « fourre-tout » dans lesquels étaient incluses un grand nombre d'espèces superficiellement semblables mais non apparentées. Ces genres ont souvent accumulé un grand nombre d'espèces, comme le genre *Aster* sensu lato (s. l.) qui comptait jusqu'à 400 espèces à la fin des années 1980, faisant de lui l'un des principaux genres de la tribu et l'un des plus répandus à travers le continent nord américain.

Récemment, une meilleure compréhension du genre *Aster* s. l. est venue des travaux morphologiques de Nesom (1994) et de la phylogénie moléculaire (sites de restriction) de Xiang & Semple (1996) utilisant l'ADN chloroplastique. Ces études ont contribué à la reconnaissance de plusieurs genres distincts.

En plus, les travaux de systématique moléculaire méritent d'être soulignés pour leur contribution à l'amélioration de la connaissance de l'histoire évolutive des Astereae nord-américaines. Ainsi, depuis la première utilisation de l'ADN chloroplastique (i.e. analyses de restriction, RFLP) (Suh & Simpson 1990) et de l'ADN nucléaire ribosomal chez les Asteraceae (espaceur interne transcrit (ITS), Baldwin 1992; espaceur externe transcrit (ETS), Markos & Baldwin 2001), de nombreux taxons nord-américains ont été repositionnés tant au sein de la famille qu'au niveau des espèces (Brouillet et al. 2009). Par exemple, en se basant sur l'ITS, Noyes & Rieseberg (1999) furent les premiers à démontrer que les *Aster* nord-américaines formaient un groupe distinct des *Asters* sensu stricto, une hypothèse préalablement proposée par Nesom (1994). D'après leurs résultats, les Astereae nord-américaines seraient d'origine récente comparativement à celles de l'Amérique du

Sud. Malgré ces efforts, il n'en demeure pas moins qu'il subsiste beaucoup de questions d'ordre évolutif au sein de différents groupes qui demandent des études à différents niveaux phylogénétiques, de la sous-tribu à l'espèce ou aux variétés.

Dans la présente étude, la majorité des genres d'intérêt traités étaient initialement compris au sein du genre *Aster* s. l (Jones 1980*a*, 1980*b*; Semple & Brouillet 1980a, 1980b ; Semple et al. 2002). En effet *Oreostemma*, compris dans la section *Oreastrum* du genre *Aster* L. (Cronquist 1948), a été restauré comme un clade par Nesom (1993). Également, dans un traitement basé sur des caractères morphologiques et des distributions géographiques publié l'année suivante, Nesom (1994) reconnaît formellement *Eurybia* comme un genre distinct des *Aster* s.l.. Dans cet ouvrage, il inclut *Herrickia* comme une section du sous-genre *Eurybia* et il délimite le genre *Oreostemma* à trois espèces. En se basant sur des résultats moléculaires, Brouillet et al. (2004) ont restauré les espèces *Triniteurybia aberrans* et *Herrickia kingii* anciennement classifiées comme *Tonestus aberrans* (Nesom & Morgan 1990) et *Tonestus kingii* (Nesom 1991) respectivement. Ces mêmes auteurs reconnaissent *Oreostemma*, *Herrickia*, *Eurybia* et *Triniteurybia* comme quatre genres distincts. En référence à ces genres, ils proposent l'utilisation de « grade des eurybioïdes » (du nom du genre principal, *Eurybia*). Cependant, dans une récente publication, Nesom (2009) soutient qu'il n'y a aucun caractère morphologique diagnostique justifiant la séparation des genres *Herrickia*, *Eurybia* et *Triniteurybia* en trois différents clades. Comme solution alternative, il suggère une extension de la section *Herrickia* du genre *Eurybia*.

Les études antérieures traitant tant de la classification (Nesom 1994, Semple 2005) que des relations phylogénétiques des eurybioïdes (Brouillet et al. 2004, Lamboy & Jones

1988, Lamboy et al. 1991, Semple 2005, Semple et al. 2002) sont incomplètes puisque ces dernières étaient restreintes à une fraction du groupe. Afin d'obtenir un portrait de l'ensemble et de reconstruire une phylogénie robuste, la présente étude inclut les 31 espèces appartenants aux quatre genres: *Eurybia* (Cass.) S.F. Gray (23 sp.), *Herrickia* Wooton & Standley (4 sp.), *Oreostemma* Greene (3 sp.) et *Triniteurybia* Brouillet, Urbatsch, & R.P. Roberts (1 sp.) (Brouillet et al. 2004) (Appendice 1).

L'objectif général de notre étude est de reconstruire l'histoire évolutive des eurybioïdes (*sensu* Brouillet et al. 2004) à l'aide de régions génomiques pour ainsi parvenir à la délimitation du groupe et donner une interprétation de la biogéographie du groupe. Pour y parvenir, nous allons évaluer l'utilité de plusieurs régions d'ADN considérées potentiellement utiles aux niveaux inter- et intra-génériques, et les analyser à l'aide de différentes méthodes d'analyse phylogénétique. Une fois de plus, par souci de se rapprocher de la phylogénie du groupe (et non des gènes individuels, Doyle 1992), nous allons explorer des marqueurs provenant de deux compartiments de la cellule: les génomes chloroplastique et nucléaire. Nous n'avons pas utilisé le génome mitochondrial car contrairement aux animaux, le taux de substitution nucléotidique chez les plantes est plus faible que dans les deux autres génomes (Wolfe et al. 1987, Palmer et al. 1988).

Dans un premier temps, notre objectif est de tenter de résoudre l'histoire évolutive des eurybioïdes à l'aide de deux régions: l'ITS et l'espaceur externe transcrit (ETS) de l'ADN ribosomal (Chapitre 1). Plus précisément, la reconstruction phylogénétique des eurybioïdes basée sur l'ITS et d'ETS nous permettra d'évaluer les hypothèses de classification précédemment émises par Nesom (1994) et Semple (2005). De plus, nous serons en mesure de valider les hypothèses de relations entre les espèces de la section

Eurybia proposées par Lamboy et Jones (1988) et Lamboy et al. (1991). Grâce au clonage de l'ITS, nous tenteront de déterminer la parentalité des espèces polyploïdes du genre *Eurybia*. Enfin, avec l'ensemble des données morphologiques et phylogénétiques, nous proposerons une hypothèse concernant à la fois la biogéographie et l'écologie du genre *Eurybia*.

Le second objectif est d'examiner l'utilité de certaines régions du génome chloroplastique pour reconstruire la phylogénie des eurybioïdes (Chapitre 2). Ceci sera fait dans le souci de valider l'histoire du grade telle que suggérée par le génome nucléaire ribosomal. Nous espérons aussi retracer le parent maternel des espèces polyploïdes d'*Eurybia*. Finalement, nous testerons l'histoire biogéographique hypothétique du chapitre précédent (chapitre 1) à l'aide de deux méthodes de reconstruction biogéographique.

Le troisième et dernier objectif sera d'évaluer une région nucléaire à faible nombre de copies afin d'accroître la résolution de la phylogénie et d'approfondir notre connaissance de l'histoire du groupe (Chapitre 3).

Chapitre 1

Molecular phylogeny of the North American eurybioid asters (Asteraceae, Astereae) based on the nuclear ribosomal internal and external transcribed spacers

Sugirthini Selliah and Luc Brouillet

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Received 28 February 2008. Accepted 21 May 2008. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 1 August 2008.

Publié dans ***Botany*** (anciennement ***Canadian Journal of Botany***) août 2008. Volume 86 (8) : 901-915.

Il s'agit d'un article parmi une sélection de papiers publiés dans l'un des deux numéros spéciaux, intitulé '*la systématique des plantes* ', ayant pour but de faire valoir la recherche dans le domaine de la systématique des plantes au Canada.

Molecular phylogeny of the North American eurybioid asters (Asteraceae, Astereae) based on the nuclear ribosomal internal and external transcribed spacers

Abstract

The eurybioid asters *Oreostemma*, *Herrickia*, *Eurybia*, and *Triniteurybia* form a complex that is part of the North American clade of tribe Astereae. They comprise 31 species of perennial herbs that are widely distributed on the continent. Previous analyses had shown poor resolution among the four genera and among the species, particularly within *Eurybia* (23 spp.), which includes diploids and polyploids. We investigated phylogenetic relationships within the group using the nuclear ribosomal ITS and ETS regions, in separate and combined parsimony and Bayesian analyses. We detected incongruence between the ITS and ETS regions when polyploids were included, and so only diploids were considered in the combined analyses. *Eurybia pygmaea* (Lindl.) G.L. Nesom is confirmed as a member of *Symphyotrichum*; *Eurybia* is monophyletic once this species is re-classified. The eurybioids form a paraphyletic grade with *Oreostemma*, sister to the remaining taxa, followed in succession by *Herrickia*, *Eurybia*, and *Triniteurybia*, and with the latter genus sister to subtribe Machaerantherinae. Thus the xeric Machaerantherinae (x = 6, 5, 4) is nested within the grade of mesic eurybioids (x = 9). Although largely grouping together, the polyploid taxa of *Eurybia* apparently do not constitute a clade and their relationships to the diploid taxa and to each other could not be assessed further. Among the diploids, two clades emerge: one including the western *Eurybia integrifolia* (Nutt.) G.L. Nesom and *Eurybia sibirica* (L.) G.L. Nesom, and the southeastern *Eurybia eryngiifolia* (Torr. & A. Gray) G.L. Nesom; and a second including the western *Eurybia radulina* (A. Gray) G.L. Nesom, the eastern cordate-leaved (sect. *Eurybia*) and the narrow-leaved, mostly coastal plain species of *Eurybia*. Our analyses, therefore, do not support the current classifications of *Eurybia*.

Introduction

The Astereae is the second largest tribe of the Asteraceae (Funk et al. 2005), with over 3000 species and 222 genera (emended from Nesom and Robinson 2007). One of the major diversifications in the tribe is the North American clade (Noyes and Rieseberg 1999), with 77 genera and 719 species (north of Mexico) (FNA Editorial Committee 2006). The North American asters are one of the most diverse groups within this clade. Most of these are embedded within a group that comprises the subtribes Boltonieae, Symphyotrichinae, and Machaerantherinae (subtribal delimitation of Nesom and Robinson 2007). Brouillet et al. (2004) showed that *Eurybia* (Cass.) S.F. Gray (23 spp.) and its relatives, *Herrickia* Wooton & Standley (4 spp.), *Oreostemma* Greene (3 spp.), and *Triniteurybia* Brouillet, Urbatsch, & R.P. Roberts (1 spp.), collectively comprising the eurybioid grade (Brouillet et al. 2004), are paraphyletic to subtribe Machaerantherinae. These 31 species are widely distributed across the continent: *Eurybia* is present in both eastern and western North America, usually in mesic habitats; *Herrickia* inhabits mesic to semixeric habitats of the intermountain ranges of the western United States; *Oreostemma* usually grows in dry to wet meadows and fens at mid to high elevations of the western United States ranges; and *Triniteurybia* is restricted to rocky, montane habitats of Idaho and Montana. The eurybioids share numerous morphological characters (Nesom 1994; Semple et al. 2002). Leaf shape has played a major role in subgeneric classification. It varies from linear to lanceolate, oblanceolate or widely elliptic, and to cordate; the margin is entire or serrate, teeth

sometimes becoming indurate spines. The base chromosome number for all the genera is *x* = 9, and the ploidy level ranges from diploid to high polyploid (2n = 18–122) (Table 1; Lamboy et al. 1991; Semple et al. 2002; Brouillet 2006*a*, 2006*b*, 2006*c*; Nesom 2006*a*).

Oreostemma, called *Aster* L. section *Oreastrum* by Cronquist (1948), was reinstated by Nesom (1993). Long included within *Aster* s. l. (e.g., Jones 1980a, 1980b; Semple and Brouillet 1980a, 1980b; Semple et al. 2002), *Eurybia* was considered distinct by Nesom (1994). In this treatment, based on morphological characters and geographic distributions, Nesom included three species within *Oreostemma* and 28 within *Eurybia*. He subdivided the latter into two subgenera, *Eurybia* and *Heleastrum*, and eight sections (Table 1). *Herrickia*, originally described for the single species *Herrickia horrida* Wooton & Standley (which was never placed in Aster), was incorporated as a section of subgenus *Eurybia*. Nesom and Morgan (1990) and Nesom (1991), respectively, classified *Triniteurybia aberrans* (A. Nelson) Brouillet, Urbatsch, & R.P. Roberts (*Macronema aberrans* A. Nelson) and *Herrickia kingii* (D.C. Eaton) Brouillet, Urbatsch, & R.P. Roberts (Aster *kingii* D.C. Eaton) within *Tonestus*. Recently, in their tribal classification, Nesom and Robinson (2007) noted *Herrickia* and *Triniteurybia* as distinct genera; all eurybioid genera were unplaced as to subtribe. Because of discordant basic chromosome number (*x* = 7) and morphology (basal rosette leaves and paniculiform capitulescences; Semple (1982)), and following an ITS-based molecular phylogenetic analysis (Brouillet et al. 2001), Semple et al. (2002) moved *Eurybia chapmanii* to *Symphyotrichum* Nees, as *Symphyotrichum chapmanii* (Torr. & A. Gray) Semple & Brouillet. Brouillet and Selliah (2005), on the basis of a preliminary phylogenetic analysis of ITS data, transferred *Eurybia pygmaea* (Lindl.)

G.L. Nesom to *Symphyotrichum*, as *Symphyotrichum pygmaeum* (Lindl.) Brouillet & S. Selliah.

Table 1. Comparison of recent classifications of the eurybioid genera, with known chromosome numbers for each species.

		Nesom (1994)	Semple (2005)
Species	2*n*	*Eurybia*	*Eurybia*
		Subg. *Heleastrum*	Subg. *Heleastrum*
E. avita (Alexander) G.L. Nesom	18	Sect. *Heleastrum*	Sect. *Heleastrum*
E. hemispherica (Alexander) G.L. Nesom	18	Sect. *Heleastrum*	Sect. *Heleastrum*
E. paludosa (Aiton) G.L. Nesom	36	Sect. *Heleastrum*	Sect. *Heleastrum*
E. spinulosa (Chapman) G.L. Nesom	?	Sect. *Eryngiifolii*	Sect. *Eryngiifolii*
E. eryngiifolia (Torrey & A. Gray) G.L. Nesom	18	Sect. *Eryngiifolii*	Sect. *Eryngiifolii*
Symphyotrichum chapmanii (Torrey & A. Gray) Semple & Brouillet	14	Sect. *Chapmaniani*	(*Symphyotrichum* subg. *Chapmaniani*[a])
		Subg. *Eurybia*	Subg. *Eurybia*
Eurybia radula (Ait.) G.L. Nesom	18	Sect. *Radulini* subsect. *Radulini*	Sect. *Calliastrum*
E. saxicastelli (J.J.N. Campbell & Medley) G.L. Nesom	54	Sect. *Radulini* subsect. *Radulini*	Sect. *Calliastrum*
E. radulina (A. Gray) G.L. Nesom	18	Sect. *Radulini* subsect. *Radulini*	Sect. *Calliastrum*
E. conspicua (Lindl.) G.L. Nesom	108, 122	Sect. *Radulini* subsect. *Radulini*	Sect. *Calliastrum*
E. sibirica (L.) G.L. Nesom	18	Sect. *Radulini* subsect. *Sibiricae*	Sect. *Calliastrum*
E. merita (A. Nelson) G.L. Nesom	36	Sect. *Radulini* subsect. *Sibiricae*	Sect. *Calliastrum*
Symphyotrichum pygmaeum (Lindl.) Brouillet & S. Selliah	?	Sect. *Radulini* subsect. *Sibiricae*	(*Symphyotrichum* subg. *Virgulus*[a])
E. compacta G.L. Nesom	18	Sect. *Calliastrum*	Sect. *Calliastrum*
E. spectabilis (Aiton) G.L. Nesom	72	Sect. *Calliastrum*	Sect. *Calliastrum*
E. surculosa (Michx.) G.L. Nesom	36	Sect. *Calliastrum*	Sect. *Calliastrum*
E. mirabilis (Torrey & A. Gray) G.L. Nesom	18	Sect. *Eurybia*	Sect. *Calliastrum*
E. macrophylla (L.) Cass.	72	Sect. *Eurybia*	Sect. *Eurybia*
E. schreberi (Nees) Nees	54	Sect. *Eurybia*	Sect. *Eurybia*
E. jonesiae (Lamboy) G.L. Nesom	54	Sect. *Eurybia*	Sect. *Eurybia*
E. chlorolepis (E. S. Burgess) G.L. Nesom	36, 39, 45	Sect. *Eurybia*	Sect. *Eurybia*
E. divaricata (L.) G.L. Nesom	18	Sect. *Eurybia*	Sect. *Eurybia*
E. furcata (E.S. Burgess) G.L. Nesom	18	Sect. *Eurybia*	Sect. *Eurybia*
E. integrifolia (Nutt.) G.L. Nesom	18	Sect. *Integrifoliae*	Sect. *Integrifoliae*
			Herrickia
Herrickia horrida Wooton & Standley	18	Sect. *Herrickia*	
H. glauca (Nutt.) Brouillet	18	Sect. *Herrickia*	
H. wasatchensis (M.E. Jones) Brouillet	18	Sect. *Herrickia*	
H. kingii (D.C. Eaton) Brouillet, Urbatsch & R.P. Roberts	18	*Tonestus*[a]	
Triniteurybia aberrans (A. Nelson) Brouillet, Urbatsch & R.P. Roberts	18	*Tonestus*[a]	*Triniteurybia*
		Oreostemma	*Oreostemma*
Oreostemma peirsonii (Sharsmith) G.L. Nesom	18		
O. elatum (Greene) Greene	18		
O. alpigenum (Torrey & A. Gray) Greene	18		

Note: *Chromosome numbers from Nesom 2006; Brouillet 2006*a*, 2006*b*, 2006c

[a]Not classified in eurybioids.

Based on morphological and available phylogenetic data, Semple (2005) proposed an alternate classification of the eurybioids. He included 23 species within *Eurybia*, with an infrageneric taxonomy similar to that of Nesom (1994), except that section *Radulini* was merged with section *Calliastrum*, resulting in five recognized sections (*Integrifolia*e, *Eurybia*, *Calliastrum*, *Eryngiifolii*, and *Heleastrum*; Table 1). Species descriptions for all eurybioid genera were published recently (Nesom 2006*a*; Brouillet 2006*a*, 2006*b*, 2006*c*). Few studies have addressed the classification and evolution of *Eurybia* and the eurybioids. Using isozyme similarities, Lamboy et al. (1991) proposed a hypothesis of relationships within the polyploid complex of *Eurybia* section *Eurybia* (as *Aster* sect. *Biotia*). According to this study, *Eurybia divaricata* (L.) G.L. Nesom (2n = 18), *Eurybia chlorolepis* (Burgess) G.L. Nesom (2n = 36), and *Eurybia macrophylla* (L.) Cass. (2n = 72) of the eastern deciduous forests were closely related. This group shared affinities with *Eurybia mirabilis* (Torrey & A. Gray) G.L. Nesom (2n = 18) and *Eurybia jonesiae* (Lamboy) G.L. Nesom (2n = 54) of the Piedmont. The mid-western *Eurybia furcata* (Burgess) G.L. Nesom (2n = 18) was considered most distinct. A restriction fragment length polymorphism (RFLP) analysis of plastid DNA of the North American asters suggested an affinity of eurybioids with *Aster amellus* L. (Xiang and Semple 1996), apparently contradicting the classification of Nesom (1994).

The development of molecular markers from the plastid and nuclear genomes has allowed systematists to re-assess the position of many taxa within tribe Astereae (e.g., Brouillet et al. 2004). One of the most widely used molecular markers in phylogenetic studies at the inter- and sub-generic levels within the tribe has been the nuclear ribosomal DNA internal transcribed spacers ITS1 and ITS2 (e.g., Noyes and Rieseberg 1999; Markos

and Baldwin 2001; Brouillet et al. 2004), including the 5.8S rDNA locus (collectively referred to here as "ITS"). More recently, data from part of the 3′-end of the external transcribed spacer (ETS) between the 26S and 18S rDNA genes of the nuclear ribosomal repeat units, was added in the hope of providing better resolution and stronger support to resulting phylogenetic trees (e.g., Markos and Baldwin 2001; Morgan 2003; Roberts and Urbatsch 2003, 2004; Urbatsch et al. 2003; Brouillet et al. 2004). Within the eurybioids, a preliminary combined phylogenetic analysis of ITS and of the plastid transfer RNA genes *trn*L–UAA and *trn*F–GAA and associated intron and spacer regions (*trn*L–F) hinted at the potential monophyly of *Eurybia* (Bastien and Brouillet 2002). Using ITS and ETS data, Brouillet et al. (2004) showed that *Tonestus kingii* (D.C. Eaton) G.L. Nesom and *Tonestus aberrans* (A. Nelson) G.L. Nesom & D.R. Morgan are related to *Eurybia* and not to *Tonestus* as previously hypothesized (Nesom and Morgan 1990, Nesom 1991, 1994). These authors reinstated *Herrickia*, expanded it to incorporate *Herrickia kingii*, and created the new genus *Triniteurybia* to accommodate *T. aberrans*. In this study, however, it was unclear whether *Herrickia* formed a single or two distinct clades, with *H. kingii* separate from *Herrickia* s. str. Although *Oreostemma* and *Herrickia* appeared to be early diverging lineages, the relationships among the eurybioid genera were little resolved: *Oreostemma–Herrickia–Eurybia* was resolved as sister to a clade comprising *Triniteurybia* and subtribe Machaerantherinae, suggesting that the eurybioids were paraphyletic. The Machaerantherinae include mainly taprooted taxa of xeric habitats with purple, white, or yellow rays, and base chromosome numbers of x = 6, 5, 4. The relationship between *Machaeranthera* and the core asters (including *Eurybia*) was discussed by Cronquist and

Keck (1957) when they segregated the former from the latter on the basis of various characters, including the taproot.

Several phenomena increase the difficulty of establishing phylogenetic relationship among eurybioid species. Hybridization and polyploidy (Lamboy et al. 1991), phenotypic variability, and the interpretation of morphological characters all contribute to this. Reconstructing the evolutionary history of reticulate polyploid complexes may be particularly challenging (Skala and Zrzavy 1994). Thus, the relationships among species of the eurybioid complex remain uncertain. Furthermore, the existing sectional classifications of *Eurybia* are partly contradictory.

The objectives of our study are thus to elucidate the phylogenetic relationships among the eurybioid genera and species using nuclear ribosomal ITS and 3′ ETS sequence data, and to evaluate the hypotheses of relationships reflected in current classifications. Elucidating relationships will allow us to investigate the evolution of certain traits and propose hypotheses on the ecological and biogeographic history of the group.

Materials and methods

Taxon sampling

We included all species of *Oreostemma*, *Eurybia*, *Herrickia*, and *Triniteurybia*, as defined by Nesom (1994, 2006) and Brouillet (2006*a*, 2006*b*, 2006*c*; Brouillet et al. 2004), in addition to representatives of genera belonging to the North American clade of Astereae (Noyes and Rieseberg 1999). Samples were obtained from herbarium specimens or as fresh-collected material preserved in silica gel. We performed an analysis on the larger ITS dataset (hereinafter, ITS analysis), which comprised 52 new samples and 25 sequences from GenBank, for a total of 25 genera and 68 species (supplementary data, Appendix 2); *Doellingeria infirma* (Michx.) Greene was used as the outgroup. In the ETS analysis, we included 42 sequences (9 from GenBank and 33 newly sequenced for this study, supplementary data, Appendix 2); for the combined ITS and ETS analyses (hereinafter, combined analyses), only the 25 diploid species of the eurybioid and two species of Machaerantherinae genera were included (supplementary data, Appendix 2); *Chlorocantha spinosa* (Benth.) G.L. Nesom was used as the outgroup. We used a single individual per species, except for seven *Eurybia* species (three individuals for *Eurybia compacta* G.L. Nesom and two each for *Eurybia spectabilis* (Aiton) G.L. Nesom, *Eurybia surculosa* (Michx.) G.L. Nesom, *Eurybia merita* (A. Nelson) G.L. Nesom, *Eurybia eryngiifolia* (Torrey & A. Gray) G.L. Nesom, *Eurybia integrifolia* (Nutt.) G.L. Nesom, and E. *chlorolepis*). To explore the extent of intra-individual ITS variation within *Eurybia* species, we generated 59 cloned sequences from 8 of the 12 polyploid species; 11 sequences of diploid species and 1 of *Machaeranthera tanacetifolia* (Kunth) Nees were added for a total of 70 sequences to carry out a network analysis (supplementary data, Appendix 2). Three polyploid species, *Eurybia spectabilis*, *Eurybia spinulosa* (Chapman) G.L. Nesom, and

Eurybia conspicua (Lindl.) G.L. Nesom, were not included in the latter analysis because we were unable to clone them. All new sequence accessions were deposited in GenBank.

DNA extraction, PCR amplification, cloning, and sequencing

DNA extraction was done using a modified CTAB protocol (Joly et al. 2006) or with the QIAgen DNeasy Plant Mini Kit (QIAGEN, Mississauga, Ont.), following the manufacturer instructions. Amplifications of the ITS and ETS regions were performed using the primers AB101 and AB102 for ITS (Douzery et al. 1999), and Ast-8 (Markos and Baldwin 2001) and 18S-2L (Linder et al. 2000) for ETS. A number of ITS sequences also were obtained using primers optimized for the Astereae (J. Vaezi, Institut de recherche en biologie végétale, Université de Montréal, personal commication, 2007): ITSvR (5′ GATATGCTTAAACTCAGCGG) and ITSvF (5′AGGAAGGAGAAGTCGTAACAAGG). The PCR amplification reaction mix contained 10× PCR Buffer with 1.5 mmol/L $MgCl^2$, (Roche Diagnostics, Laval, Que.), 100 μmol/L of each dNTP, 0.3 mmol/L of each primer, 2.5%–5% DMSO and glycerol, one unit of *Taq* DNA polymerase, and 70 ng genomic DNA, in a final reaction volume of 25 μL. For some samples, we added 1.5 mmol/L of $MgCl^2$, 0.05% Tween 20, or 2.5 μg BSA. For ETS amplification, the reaction mix was the same, but without the addition of BSA or Tween 20.

Because we observed double peaks during direct sequencing of the polyploid species, which suggests alleles or potential paralogues, we cloned the PCR products of the ITS region for seven species in the hope of detecting the parental signal for these species. Only one or two double peaks were observed in ETS polyploid sequences and cloning was not performed on these. This did not affect the combined analyses, as they are based on diploid taxa only. Amplification conditions and purification procedures were kept the same except that each PCR was done in triplicate and combined after amplification (during purification) to minimize PCR recombination artifacts (Joly et al. 2006). We cloned the PCR product using the pGEM-T vector (Promega Corp., Madison, Wisc.) and grew positive colonies overnight in LB broth. The vector-specific primers SP6–T7 were used for

amplification and sequencing. Each sequenced clone was compared with the others and with the initial sequence obtained from direct sequencing, to eliminate PCR-artifact clones caused by *Taq* DNA polymerase errors and to detect putative paralagous variants.

With few exceptions, *Taq* was added after the first 2 min of the initial denaturation phase of the reaction (hot start PCR). Conditions for PCR amplification of ITS were: initial denaturation for 3 min at 94 °C, 35 cycles 30 s at 94 °C, 30 s annealing at 53 °C, 30 s at 72 °C, and lastly 7 min extension at 72 °C. The amplification conditions for ETS were similar, except that the annealing temperature was 50 °C. PCR products were purified according to PEG purification (see modified protocol of Joly et al. 2006). Sequencing cycles were performed by adding "Big Dye" Terminator chemistry version 1.1 kit (Applied Biosystems, Foster City, Calif.) following the manufacturer instructions, except that 0.25 μL of dye terminator were used in a total mix volume of 10 μL. For each polyploid individual, 5–11 clones were sequenced. Approximately 60 ng of PCR sequencing products were precipitated using a sodium acetate solution and ethanol (70%). For each amplicon, double-stranded sequences were generated using an ABI 3100–Avant automated DNA sequencer (Applied Biosystems).

Phylogenetic analyses

We assembled, edited, and base-called sequences using Sequencher version 4.1 (Genecodes Corp., Ann Arbor, Mich.). New ITS sequences were incorporated into an aligned matrix of Astereae ITS sequences using BioEdit version 7.0.5.3 (Hall 1999) and aligned manually; the original alignment was based on the ITS secondary structure published for Asteraceae by Goertzen et al. (2003).

For the ITS data matrix, the number of unaligned ITS characters was 619–629 bp. Five sequences were incomplete; the shortest sequence was 502 bp (E. *compacta*). Once aligned, the ITS matrix of 639 characters was partitioned into three regions: ITS1 (259 bp), 5.8S rDNA (164 bp), and ITS2 (216 bp). To select the substitution model for each partition,

the hierarchical Likelihood Ratio Test (hLRT) and the Akaike information criterion (AIC) were performed as implemented in MrModeltest version 2.0 (Nylander 2004). Both tests proposed models that, after analysis, produced identical topologies with similar support values. According to Posada and Buckley (2004), the AIC offers several advantages over the hLRT, such as assessing model selection uncertainty, allowing comparison of multiples models simultaneously and model averaging, and not relying on a subjective significance level. Therefore, only the analyses based on models selected using the AIC are presented here. The most appropriate models suggested by the AIC for the ITS1, 5.8S, and ITS2 partitions, respectively, were, for the ITS analysis, the SYM + G, K80, and SYM + G models, and for the combined analysis, the SYM + I, K80, and SYM + I models (Kimura 1980; Zharkikh 1994).

The ETS unaligned matrix comprised 504 characters and included two incomplete sequences, the shortest with a length of 488 bp (*E. mirabilis*). The best-fit model for the ETS partition in the combined analysis was the GTR + G model. In the combined analyses, a total of 1128–1138 bp nucleotides were included before alignment; five sequences were incomplete, the shortest having 1080 bp (*E. compacta*).

For each data set, both Bayesian and parsimony analyses were done. Bayesian analysis was performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) on a shared-memory multiprocessor computer (Altix 4700, Réseau québécois de calcul de haute performance, Université de Montréal, Que.) with two independent runs and 16 Markov chains in each run; 15 heated chains and 1 cold chain were used, starting from a random tree; the chains were run simultaneously for 10 000 000 generations. Parameter estimation for each partition was rendered independent using the unlink option. In each run, trees were sampled every 1000th generation for a total of 20 002 trees. In the ITS analysis, the first 5000 trees per run prior to apparent stationarity were discarded using Tracer version 1.4 (Rambaut and Drummond 2007). The remaining 10 002 trees were used to compute the 50% majority-rule consensus tree and to obtain posterior probability values (PP). In the combined analysis, the consensus-computing strategy was similar; the number of retained

trees after a burnin of 3000 trees was 14 002. Because support values estimated by posterior probabilities generally tend to be overestimations when compared to bootstrap values (Hillis and Bull 1993; Wilcox et al. 2002; Douady et al. 2003), only values ≥ 95% were considered to be well supported.

Parsimony analyses were conducted with PAUP* version 4.b10 (Swofford 2002) using a heuristic search with the following options: ACCTRAN, MulTrees, tree bisection reconnection (TBR) branch swapping with 100 random addition sequence replicates, maximum of 100 000 trees retained. Characters were weighted equally and unordered. Since indels (insertion–deletion mutations) may provide useful phylogenetic information (e.g., Kelchner 2000), they were coded separately as binary (presence/absence) characters following the simple indel coding method of Simmons and Ochoterena (2000), as implemented in Gapcoder (Young and Healy 2003). To evaluate clade support, a bootstrap analysis (boostrap support, BS) was performed with 1000 replicates in a heuristic search with random taxon addition and TBR branch swapping, and a maximum of 100 trees retained per replicate. To assess the combinability of the ITS and ETS data sets, incongruence length differences (ILD) tests were performed, as implemented in the partition homogeneity test of PAUP* with 1000 replicates. As there is controversy concerning the use of the ILD test (e.g., high type I error rate as a congruence test, not powerful enough to detect heterogeneity in lineage-specific or site-specific cases (see Barker and Lutzoni 2001; Darlu and Lecointre 2002)), a direct tree and support value comparison was used as a complementary test of incongruence.

To ascertain the putative parents of the polyploid species, a network analysis (hereinafter ITS clone analysis) of the ITS clones was carried out. All cloned sequences from the polyploids and direct sequences of the diploid species were included in this matrix. Twelve identical clones within species were identified and removed using Collapse version 1.2 (Posada 2004). The Recombination Detecting Program version 2.0 (Martin et al. 2005) was used to detect potential recombinant sequences, parental sequences, and recombination breakpoints on sequence alignments. This package includes 10

recombination detection methods with different characteristics. According to Martin et al. (2005), using a combination of methods is more efficient than using a single one. In our analyses, we used six: RDP (Martin and Rybicki 2000), GENECONV (Sawyer's runs test; Padidam et al. 1999), Maximum $\chi 2$ (Maynard-Smith 1992), BootScan (Salminen et al. 1995), Chimaera (Posada and Crandall 2001), and Sister Scanning (Gibbs et al. 2000). They were applied with the default settings, using a Bonferroni corrected p-value cutoff of 0.05 and a window size of 20. The network analysis was done with SplitsTree version 4.1 (Huson and Bryant 2006) using the Neighbor-Net algorithm (Bryant and Moulton 2004) with default settings (i.e., characters transformation: uncorrected P-distances; splits transformation: equal angle; maximum dimensions: four).

The geographical distribution and two leaf morphology characters, blade shape and margin, were mapped onto one of the 20 equally most parsimonious trees of the combined analysis using MacClade version 4.07 (Maddison and Maddison 2005) to explore hypotheses about biogeography and character evolution in the eurybioid grade. The leaf characters were coded as multistate and unordered (margin: 0, entire; 1, serrate; 2, spinose; 3, lobed; blade shape: 0, lanceolate to oblanceolate (including oblong, ovate-oblanceolate, etc.); 1, cordate (ovate with cordate base); 2, linear).

Results

The aligned ITS matrix comprised 639 characters, with 0.01% missing data, of which 181 (28.3%) were parsimony informative. MP analysis yielded 100 000 trees of 529 steps with a consistency index (CI, excluding uninformative characters) of 0.52 and a retention index (RI) of 0.81. The ETS matrix included 513 characters plus three indels, with 0.002% missing data, of which 73 (14.2%) were parsimony informative. The MP analysis recovered 108 shortest trees of 212 steps (CI = 0.66; RI = 0.85). The combined data matrix comprised 1142 characters plus seven indels, with 0.006% missing data, of which 135 (11.8%) were parsimony informative. MP analysis resulted in 20 shortest trees of 382 steps (CI = 0.67; RI = 0.83). Results of the ETS analysis are not shown because the ITS and ETS trees were similar. No significant incongruence was detected between the diploid ITS and ETS data sets using the ILD test (p = 0.659) and direct tree comparison. The combined analysis was more resolved than the separate ITS and ETS analyses; therefore, the combined matrix was used in subsequent analyses.

For the ITS analysis, the consensus topology obtained with Bayesian (Fig. 1) and parsimony (not shown) analyses showed identical relationships (BS and PP values are given on Fig. 1). In the ITS (Fig. 1) and combined (Fig. 2) topologies, the following relationships are moderately supported by Bayesian inference within the eurybioid grade: (Symphyotrichinae (*Oreostemma–Herrickia* s. str. (*H. kingii* (*Eurybia* (*Triniteurybia* (Machaerantherinae)))))). All genera except *Herrickia* appear to be monophyletic, once *Symphyotrichum pygmaeum* (=*E. pygmaea*) is excluded from *Eurybia*. On the ETS tree (not shown), species group within their respective genus but relationships among and within genera are unresolved.

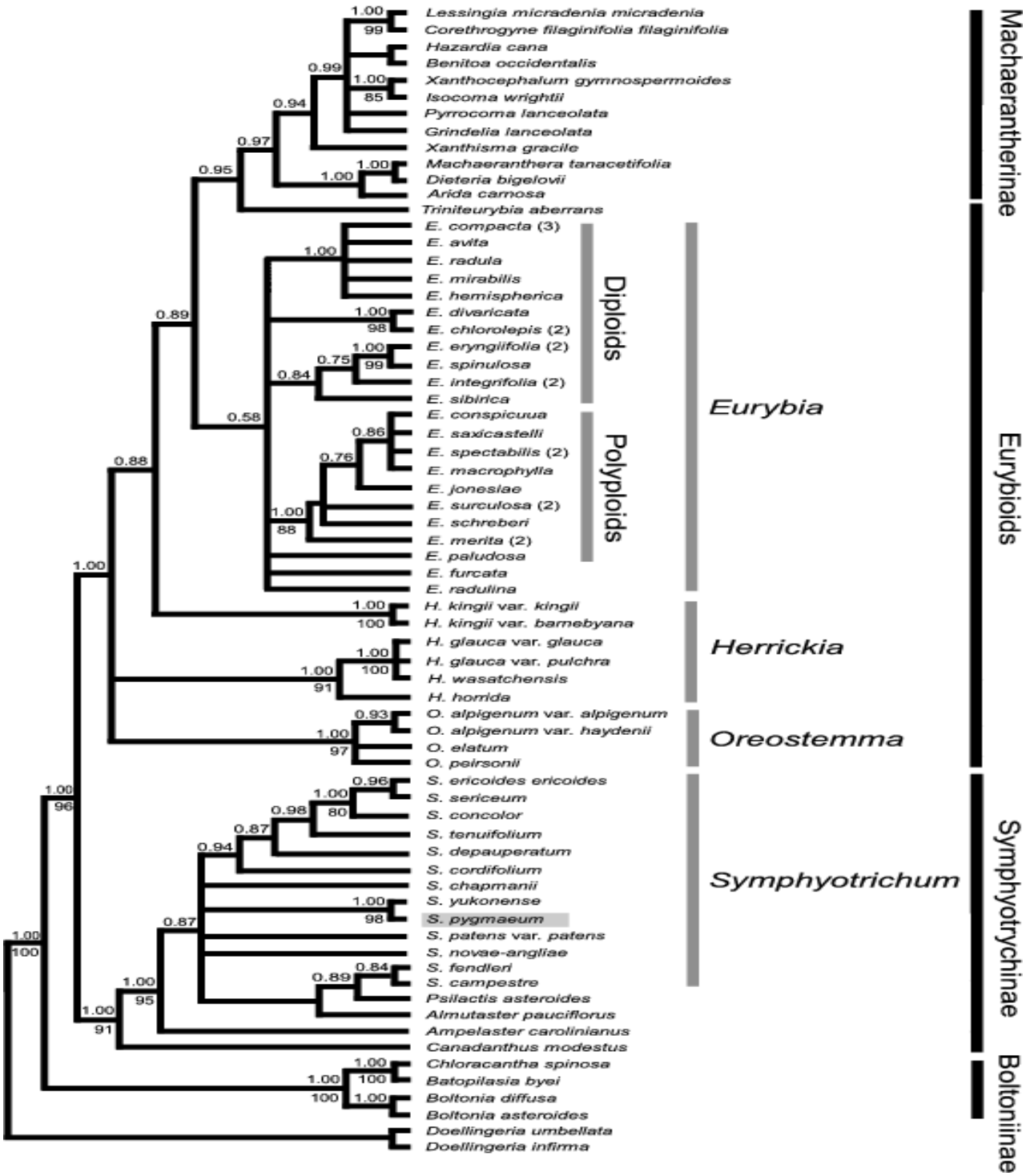
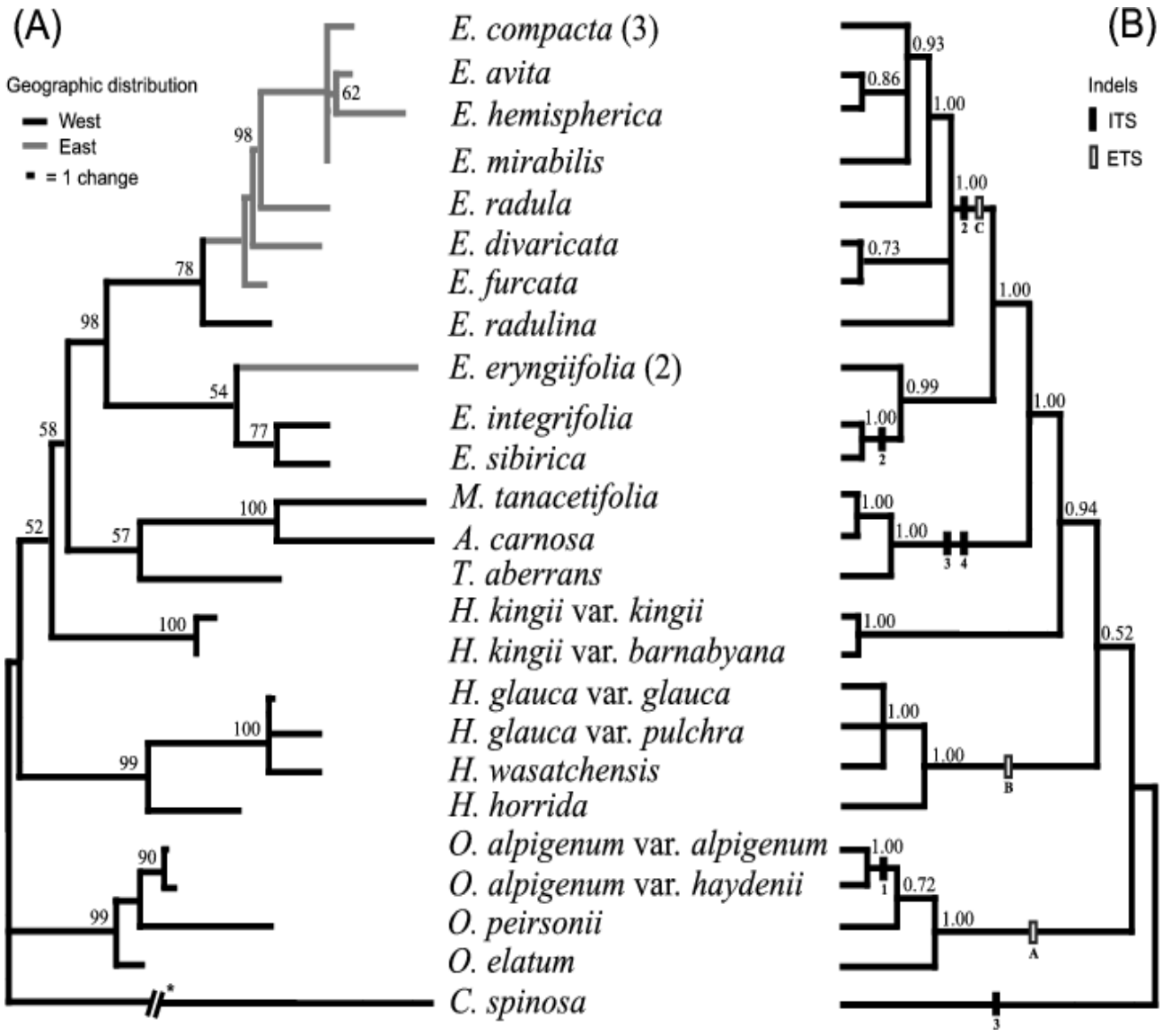

Lessingia micradenia micradenia
Corethrogyne filaginifolia filaginifolia
Hazardia cana
Benitoa occidentalis
Xanthocephalum gymnospermoides
Isocoma wrightii
Pyrrocoma lanceolata
Grindelia lanceolata
Xanthisma gracile
Machaeranthera tanacetifolia
Dieteria bigelovii
Arida carnosa
Triniteurybia aberrans
E. compacta (3)
E. avita
E. radula
E. mirabilis
E. hemispherica
E. divaricata
E. chlorolepis (2)
E. eryngiifolia (2)
E. spinulosa
E. integrifolia (2)
E. sibirica
E. conspicuua
E. saxicastelli
E. spectabilis (2)
E. macrophylla
E. joneslae
E. surculosa (2)
E. schreberi
E. merita (2)
E. paludosa
E. furcata
E. radulina
H. kingii var. kingii
H. kingii var. barnebyana
H. glauca var. glauca
H. glauca var. pulchra
H. wasatchensis
H. horrida
O. alpigenum var. alpigenum
O. alpigenum var. haydenii
O. elatum
O. peirsonii
S. ericoides ericoides
S. sericeum
S. concolor
S. tenuifolium
S. depauperatum
S. cordifolium
S. chapmanii
S. yukonense
S. pygmaeum
S. patens var. patens
S. novae-angliae
S. fendleri
S. campestre
Psilactis asteroides
Almutaster pauciflorus
Ampelaster carolinianus
Canadanthus modestus
Chloracantha spinosa
Batopilasia byei
Boltonia diffusa
Boltonia asteroides
Doellingeria umbellata
Doellingeria infirma
Diploids
Polyploids
Eurybia
Herrickia
Oreostemma
Symphyotrichum
Machaerantherinae
Eurybioids
Symphyotrychinae
Boltoniinae

Figure **1**. (Previous page) Majority rule consensus of 14 002 trees from the Bayesian phylogenetic analysis of North American Astereae, based on nrDNA ITS sequence data. *Doellingeria infirma* is the outgroup; numbers above branches are Bayesian posterior probabilities, below are bootstrap support values obtained by parsimony analysis of 100 000 trees (only those above 70% are noted); the number of individuals included for each species is noted in parentheses if more than one. The gray box highlights a species previously classified in *Eurybia*. The predominantly diploid species of *Eurybia* (marked) include the polyploid *E. chlorolepis* and *E. spinulosa*.

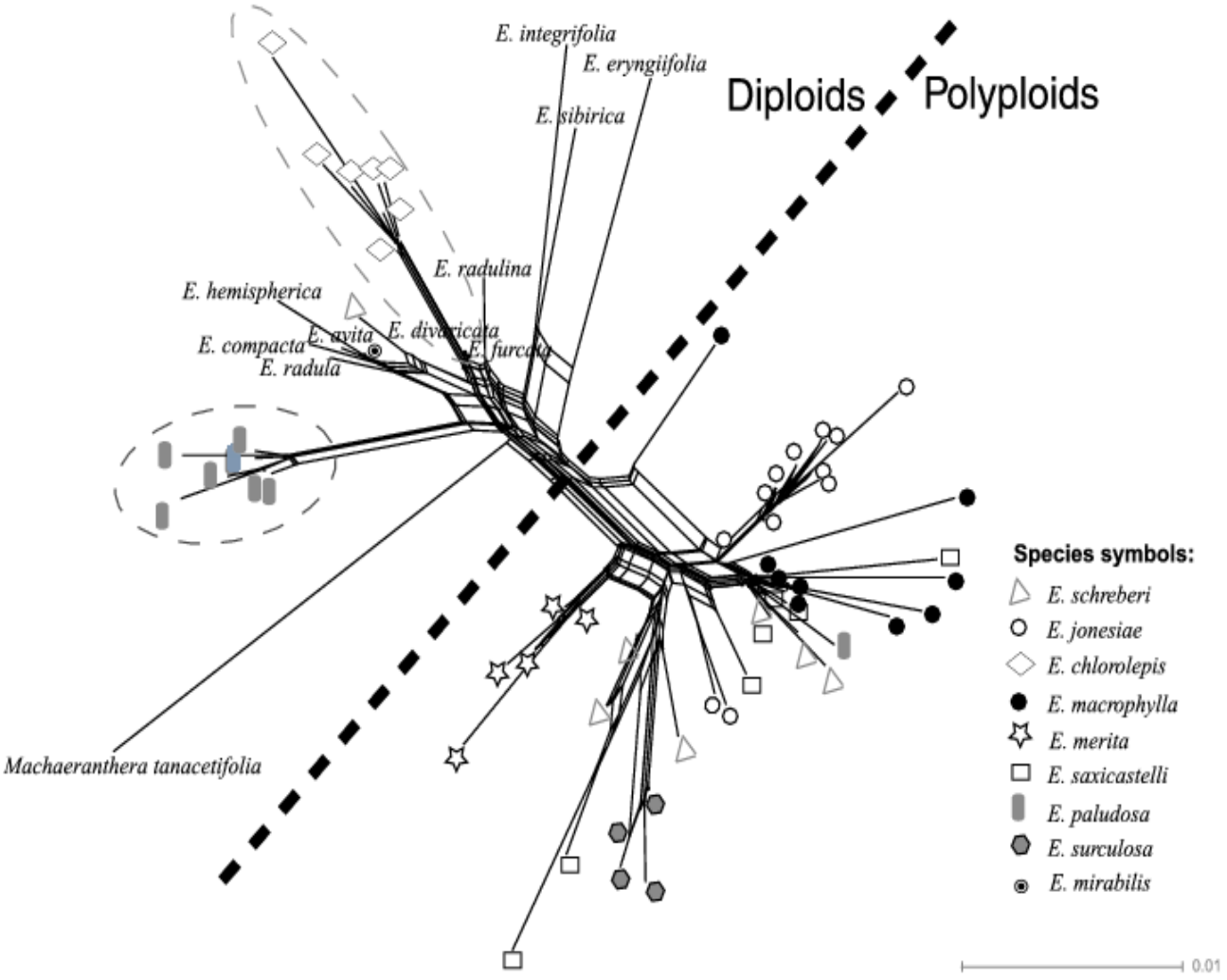
Figure 2. (Following page) (**A**) Phylogram of one of the 20 shortest trees from a parsimony analysis of the combined nrDNA ITS and ETS sequence data of diploid species of eurybioid genera and representative Machaerantherinae; *Chloracantha spinosa* is the outgroup (tree length = 76; *, branch length not to scale); numbers above branches are bootstrap support values (>50%); the number of individuals included for each species is noted in parentheses if more than one; the geographic distribution of each species is mapped onto the tree (western vs. eastern North America). (**B**) Majority-rule consensus tree of 14 002 trees from a Bayesian analysis of combined nrDNA ITS and ETS data from the same species. Posterior probability values are noted above the branches; synapomorphic indels are mapped on the tree: full bars are ITS indels (1, 112 bp; 2, 126 bp; 3, 414 bp; 4, 440 bp); open bars are ETS indels (A, 198 bp; B, 251 bp; C, 370 bp).


(A)
Geographic distribution
West
East
= 1 change
E. compacta (3)
E. avita
E. hemispherica
E. mirabilis
E. radula
E. divaricata
E. furcata
E. radulina
E. eryngiifolia (2)
E. integrifolia
E. sibirica
M. tanacetifolia
A. carnosa
T. aberrans
H. kingii var. kingii
H. kingii var. barnabyana
H. glauca var. glauca
H. glauca var. pulchra
H. wasatchensis
H. horrida
O. alpigenum var. alpigenum
O. alpigenum var. haydenii
O. peirsonii
O. elatum
C. spinosa
(B)
Indels
ITS
ETS

MP and Bayesian analyses of the combined data for diploids (Fig. 2) show similar relationships except between *E. divaricata*, *E. furcata* and *Eurybia radulina* (A. Gray) G.L. Nesom, where relationships are contradictory but poorly supported. On the ITS tree (Fig. 1), *E. divaricata* is sister to the polyploid *E. chlorolepis* with strong support (no polyploids were included in the combined analyses).

Within the ITS clone matrix, a single putative recombinant clone in *E. macrophylla* was detected by two of the programs used (Max $\chi 2$ and Chimaera); this sequence was discarded. It is useful to remember that clones for each species originated from a single individual. The number of splits in the ITS network was 227, with a total weight of 0.321. The network (Fig. 3) shows two major groups, one mainly of diploid and one of polyploid species, with *M. tanacetifolia* attaching to the diploids. This division corresponds to the clades in the ITS topology (Fig. 1) and is the most significant split on the network. Two tetraploid species, *E. chlorolepis* and *Eurybia paludosa* (Aiton) G.L. Nesom, cluster with the diploids. In general, clones from each polyploid species are somewhat variable but tend to group together. One clone of *E. paludosa* groups with the polyploids. The hexaploid *Eurybia schreberi* (Nees) Nees has three clusters: one variant is with diploid *Eurybia hemispherica* (Alexander) G.L. Nesom, *E. compacta*, *Eurybia avita* (Alexander) G.L. Nesom, *E. mirabilis*, and *Eurybia radula* (Ait.) G.L. Nesom, adjacent to the cluster including diploid *E. divaricata* and tetraploid *E. chlorolepis*, one set with *Eurybia saxicastelli* (Campbell & Medley) G.L. Nesom and *E. surculosa*, and a third with *E. macrophylla*, *E. saxicastelli*, and *E. paludosa*. In addition to grouping with the latter cluster, the octoploid *E. macrophylla* has one clone that is clearly distinct from this cluster. In addition to its presence in the two clusters mentioned above, hexaploid *E. saxicastelli* has one clone asssociated with *E.* jonesiae.

Figure 3. Network of nrDNA ITS sequence data of the *Eurybia* species; polyploid species clones are represented by symbols; diploid sequences are represented by species names, except *E. mirabilis*, which is represented by a symbol; *Machaeranthera tanacetifolia* represents the Machaerantherinae; the broken line divides the clones into groups that are primarily diploids vs. polyploids (note a single clone of the polyploid *E. schreberi* and two clusters of polyploid clones from *E. paludosa* and *E. chlorolepis* among the diploid taxa).



Discussion

As in other inter- or sub-generic studies (e.g., Widmer and Baltisberger 1999; Whittall et al. 2000; Markos and Baldwin 2001; Rauscher et al. 2002; Obbard et al. 2006; Siripun and Schilling 2006) where ITS and ETS were considered useful in resolving phylogenetic relationships with good support, our phylogenetic trees were mostly resolved and well supported, although more poorly so in parsimony analyses. The Bayesian ITS analysis (Fig. 1) shows that the eurybioid genera are well supported as members of the clade comprising the subtribes Symphyotrichinae and Machaerantherinae, as suggested by Brouillet et al. (2004). Relationships among these genera also are similar to those found by these authors (see below). As discussed by Brouillet and Selliah (2005), *S. pygmaeum* clearly belongs to *Symphyotrichum* rather than *Eurybia* (as in Nesom 1994). This species appears to be closely related to *Symphyotrichum yukonense* (Cronq.) G.L. Nesom, a member of subgenus *Virgulus* section *Grandiflori* (Brouillet et al. 2006), as initially hypothesized by Hultén (1968) and Porsild and Cody (1980). This relationship has been confirmed by a morphometric analysis of *S. pygmaeum* and *S. yukonense* versus *Eurybia sibirica* (C. Wattier, Institut de recherche en biologie végétale, Université de Montréal, personal communication, 2007). The delimitation of genera and the classification within eurybioids proposed by Nesom (1994, modified in Nesom and Robinson 2007) and Semple (2005) are not fully supported in our molecular phylogenetic study.

Oreostemma

Genus *Oreostemma* is strongly supported (Fig. 1: PP = 1.00, BS = 97; Fig. 2: PP = 1.00, BS = 99; unique indel in ETS, Fig. 2B) as a monophyletic group that includes three species, *Oreostemma alpigenum* (Torrey & A. Gray) Greene, *Oreostemma peirsonii* (Sharsmith) G.L. Nesom, and *Oreostemma elatum* (Greene) Greene. The position of *Oreostemma* in the ITS analysis is partly unresolved (Fig. 1). Species of *Oreostemma* grow

in open, montane to alpine habitats. *Oreostemma elatum* occurs in fens and other wet habitats at lower elevations (1000–1500 m a.s.l.), *O. alpigenum* grows in wet to moist areas, lake edges, clearings, alpine meadows or tundra (1200–3300 m a.s.l.), and *O. peirsonii* is found on dry alpine slopes, meadows and ridges (3000–3800 m a.s.l.). *Oreostemma alpigenum* is more widespread than *O. elatum* and *O.* peirsonii, both of which are restricted to small areas of the Sierra Nevada – Cascades of California (Nesom 2006*a*). Within the genus, *O. elatum* is sister to *O. peirsonii* and *O.* alpigenum. This suggests that adaptation from low elevation montane wetlands to high altitude alpine habitats occurred during speciation in the genus.

Herrickia

This genus had been subsumed as a section of *Eurybia* by Nesom (1994); originally monospecific, Nesom had expanded it to include *Herrickia glauca* (Nutt.) Brouillet (plus var. *pulchra* as *Eurybia pulchra* (S.F. Blake) G.L. Nesom) and *Herrickia wasatchensis* (Jones) Brouillet, the two species morphologically distinct. Our current analyses confirm previous results (Brouillet et al. 2004) with one exception (Figs. 1 and 2). In both sets of phylogenetic analyses, the genus is divided into two clades: *H. kingii* (with two subspecies) versus *Herrickia* s. str., the latter including *H.* horrida, *H. glauca* (two varieties) and *H. wasatchensis*. In the ITS (Fig. 1) and ETS (not shown) analyses, *Oreostemma*, *Herrickia* s. str., and the remaining eurybioids plus Machaerantherinae form a trichotomy. *Herrickia kingii* is then sister to *Eurybia–Triniteurybia*–Machaerantherinae with high support in the ITS and combined Bayesian analyses (respectively Fig. 1, PP = 0.88; Fig. 2B, PP = 0.94). In the combined analyses (Fig. 2), *Herrickia* s. str. is characterized by a unique ETS indel and is weakly supported as sister to *H. kingii–Eurybia–Triniteurybia*–Machaerantherinae, which may suggest that *H. kingii* belongs to a separate genus. In our current combined analyses, support for the position of *Herrickia* s. str. is low (PP = 0.52, BS ≤ 50; Fig. 2), and its relationships will remain unresolved until more data are obtained. *Herrickia* is mostly distributed in the mountains of the Intermountain region of western North America, usually in relatively open, mesic to dry habitats within an arid landscape. In the *Herrickia* s.

str. clade, *H. horrida* is found along canyons and hillsides at the Colorado – New Mexico border (Brouillet 2006*a*); it is sister to the three other taxa, *H. glauca* var. *glauca* and var. *pulchra*, and *H. wasatchensis*. Both varieties of *H. glauca* appear to be able to inhabit drier habitats than *H. wasatchensis*; var. *glauca* is widespread while the other two taxa are restricted in distribution; this variety is also the sole member of the whole clade to be eglandular (Brouillet 2006*a*). These three taxa currently form a polytomy and it is not possible to determine their interrelationships. Both *Herrickia* lineages include restricted, canyon-inhabiting species, which would suggest that this type of habitat may have played a key role in the early evolution of the group.

Triniteurybia

Both the ITS and combined trees show a sister relationship between *T. aberrans* and subtribe Machaerantherinae, as noted by Brouillet et al. (2004), but this inference is strongly supported only in the Bayesian analyses (Fig. 1: PP = 0.95; Fig. 2B: PP = 1.00). *Triniteurybia* was never included in Aster or *Eurybia*, probably in part due to its lack of ray florets. *Triniteurybia* is a rhizomatous perennial whereas the Machaerantherinae often are taprooted perennials or annuals. The possibility that the xeric-adapted subtribe Machaerantherinae (x = 6, 5, 4) is derived from the mostly mesic eurybioid grade (x = 9) raises interesting avenues in the study of the evolution of this species-rich, western North American group. A reduction in chromosome number correlated with a migration to xeric habitats within a lineage was suggested for *Brachyscome* Cass. (Astereae) (Field et al. 2006) and for tribes Gnaphalieae and Inuleae (Asteraceae) (Watanabe et al. 1999) in Australia.

Eurybia

Eurybia is the largest genus among the eurybioids and includes both diploid and polyploid species. In both analyses, it is sister to the *Triniteurybia*–Machaerantherinae clade and, as circumscribed here, is monophyletic, as noted by Brouillet et al. (2004).

Eurybia polyploid species occur at different ploidy levels: most are tetraploid but some are hexaploid or octoploid, and one is deca- to dodeca-ploid (Brouillet 2006*b*). Usually, each species has a single ploidy level. These polyploids probably have undergone a complex reticulate evolution and could be autopolyploid, allopolyploid, or the result of retrogressive–progressive polyploidization (Lamboy and Jones 1988; Lamboy et al. 1991).

Eurybia polyploids

The ITS topology (Fig. 1) divides the *Eurybia* species into two distinct groups, diploids and polyploids, except for two tetraploid species, *E. paludosa* and *E. chlorolepis*, which group primarily with the diploids. These two species may prove to be autotetraploids. A similar phenomenon was observed in the ITS clone network analysis (Fig. 3) except that the presence of a locus of *E. paludosa* within the polyploid complex might suggest recent allotetraploidy instead. In the network analysis (Fig. 3), clones of the cordate-leaved *E. divaricata* (2*x*) and *E. chlorolepis* (4*x*) are adjacent to the cluster in which one clone of *E. schreberi* (6*x*) is found. This may indicate a partial relationship of these three taxa. The split between the two clusters and the disjunct cluster of *E. schreberi* clones appear to contradict the hypothesis of Lamboy et al. (1991) who suggested that *E. schreberi* might be an autopolyploid derivative of *E. divaricata*, in which case all *E. schreberi* clones would be expected to group with *E. divaricata* and *E.* chorolepis, as does the latter. Lamboy et al. (1991) alternately hypothesized that *E. macrophylla* and *E. divaricata* were the parents of the hybrid (having contributed to *E. schreberi* by retrogressive polyploidy) or that *E. schreberi* may have been one of the parents of *E. macrophylla*. Both hypotheses may be supported by our data. In the network, we were unable to confirm the putative parents of the polyploid species inferred from previous studies (Lamboy et al. 1991; Lamboy 1992) because, aside from the cases mentioned above, none of the polyploid clones provided clues about putative parents.

There is no morphological basis for a phylogenetic split between diploid and polyploid species. For instance, polyploid species of sect. *Eurybia* do not cluster with the

diploid species of the section (Fig. 1) even though they share morphological features, notably their cordate leaves. In the ITS clone analysis, even if the ancestors of section *Eurybia* polyploids originated partly from outside the section, we would have expected to find at least some of the polyploid clones to group with diploid sequences, which is not the case. Processes such as concerted evolution, gene loss, homoeologous recombination, genome rearrangement, and the presence of pseudogenes may obscure the phylogenetic signal; this also may lead to the loss of the repeat type of one of the parents within the polyploid (Wendel 2000; Bailey et al. 2003; Slotte et al. 2006). An examination of the clones of the *Eurybia* polyploids showed that they do not have an obvious pseudogene signature nor a signal characteristic of straightforward chimeric or recombinant sequences. We tentatively interpret the pattern obtained as a rapid recombination of sequences after polyploidization, followed by concerted evolution within each locus, with subsequent divergence through point mutations at distinct loci, which would indeed prevent the reconstruction of phylogenetic relationships. Although in many studies the putative hybrid origin of polyploids was confirmed using ITS data (e.g., *Eupatorium* L. (Asteraceae): Siripun and Schilling 2006; *Mercurialis* L. (Euphorbiaceae): Obbard et al. 2006; *Glycine* Willd. (Leguminosae): Rauscher et al. 2002; *Sidalcea* A. Gray (Malvaceae): Whittall et al. 2000; *Draba* L. (Brassicaceae): Widmer and Baltisberger 1999), in our study, the nrDNA markers turned out to be inappropriate to infer the evolutionary history of the polyploids, as noted by Mavrodiev et al. (2005) in *Tragopogon* L. (Asteraceae) and Slotte et al. (2006) in *Capsella* Medik. (Brassicaceae).

At the present time, it is difficult to interpret the evolutionary and biogeographic history of the polyploid members of *Eurybia*, the nrDNA dataset being inadequate to untangle their relationships. Nonetheless, it is notable that all section *Eurybia* polyploid species are part of the deciduous and mixed forests of eastern North America, whereas most members of sections *Calliastrum* and *Heleastrum* are restricted to the coastal plain and adjacent areas.

Eurybia diploids

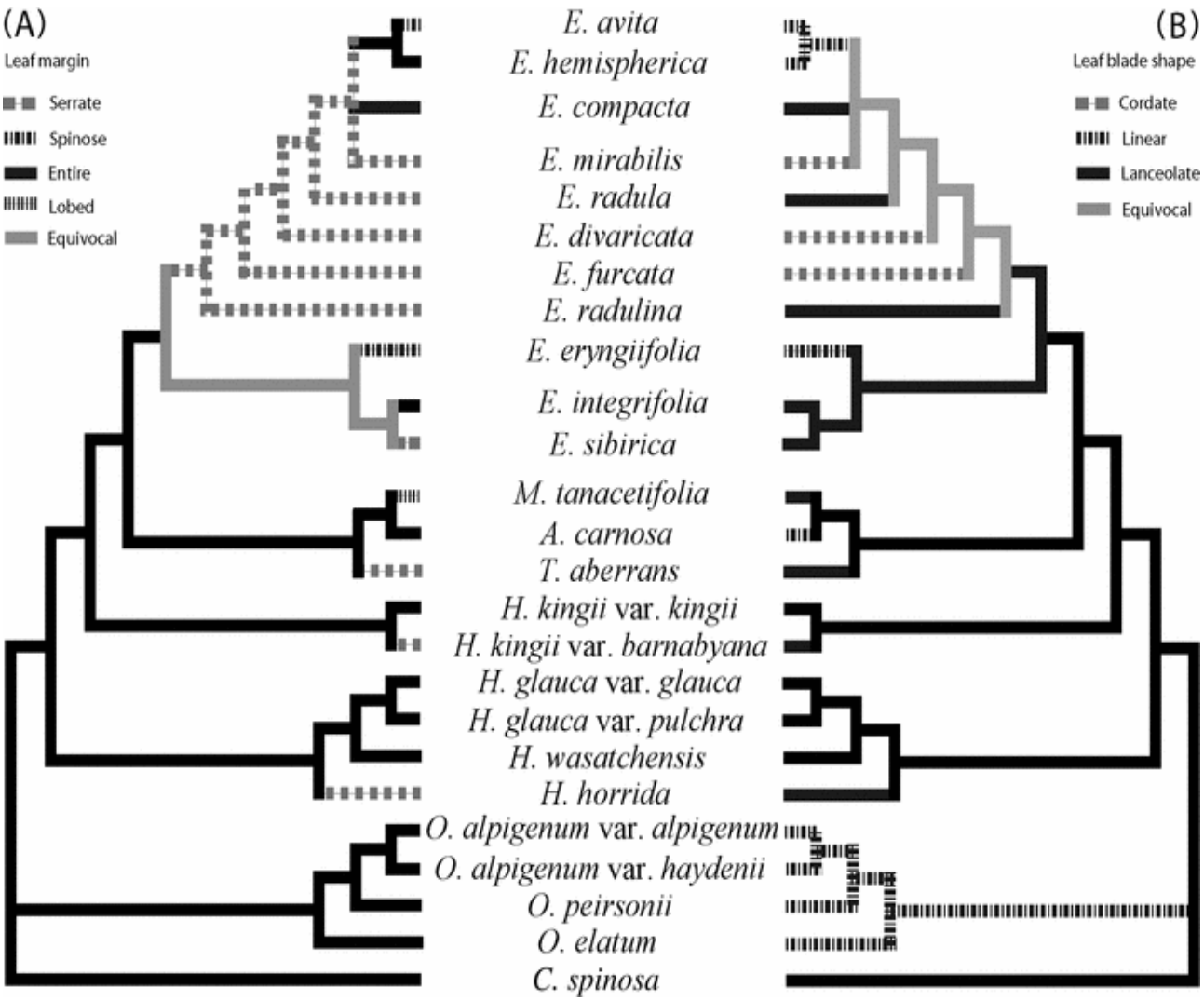
There have been several modifications to the composition of *Eurybia* and the eurybioids since the genus was reinstated by Nesom (1994): *Herrickia* was recognized as distinct (Brouillet et al. 2004; Semple 2005), and two species were transferred to *Symphyotrichum* on the basis of ITS and morphological data, *S. chapmanii* (Semple et al. 2002) and S. *pygmaeum* (Brouillet and Selliah 2005).

Neither the subgenera nor sections recognized by Nesom (1994) and Semple (2005) within *Eurybia* are supported. In our study (Fig. 2), two clades are resolved: a clade of mainly eastern species (the western *E. radulina* excepted), and a clade comprising *E. eryngiifolia* from Florida and the western *E. integrifolia* and *E. sibirica*. The latter clade groups species that are morphologically and ecologically distinct from each other.

According to Nesom (1994) and Semple (2005), *E. eryngiifolia* belongs to section *Eryngiifolii* (see Table 1) along with *E. spinulosa*, as shown in the ITS analysis (the latter is polyploid, but could not be included in the ITS clone analysis). In their classifications, section *Eryngiifolii* is sister to section *Heleastrum*, which includes the diploid *E. avita*, the diploid-autotetraploid *E. hemispherica* (Semple 1982), and the tetraploid *E. paludosa*. These two sections form subgenus *Heleastrum*, characterized by grass-like, more or less coriaceous, sometimes marginally spinose leaves and phyllaries. The molecular phylogeny indicates that the subgenus is polyphyletic and that the leaf characters currently used to group the species (Figs. 4A and 4B) might be the result of convergent adaptation to the conditions of the southeastern coastal plain of North America. *Eurybia eryngiifolia* may represent a first migration to eastern North America, with adaptation to the fire-prone, seasonally wet pine flatlands of the southern coastal plain. Various morphological characteristics of this species are indeed typical of such ecosystems, such as woody rootstocks and grass-like, coriaceous, spinose leaves. The original inclusion of *S. chapmanii* within this group (e.g., Nesom 1994) was also based on its grass-like leaves, a

further example of convergent evolution. *Eurybia eryngiifolia*, with its unique large, hemispheric capitula bearing numerous phyllaries and florets (Brouillet 2006*b*) likely will remain taxonomically isolated, except for a possible close relationship to *E. spinulosa* (Fig. 1). All other *Heleastrum* species (Table 1) have smaller heads with a relatively low number of phyllaries and florets; they are all most likely part of the eastern clade.

Figure 4. Reconstruction of morphological character evolution on one of 20 equally most parsimonious combined trees. (A) Leaf margin (9 steps). (B) Leaf blade shape (6 steps).



The apparent relationship between *E. integrifolia* , a montane, western United States species, and *E. sibirica* from northwestern North America and Eurasia, cannot be readily explained on the basis of morphology or ecology. Our ITS analysis does not allow us to determine whether they are indeed sister to each other among diploids (Fig. 1: PP = 0.84, BS ≤ 50) or whether one or the other could be sister to the remainder of the genus; however, the combined analyses suggests they are sister species (Fig. 2, PP = 1.00, BS = 77). In both classifications (Nesom 1994; Semple 2005), *E. integrifolia* was placed in its own section *Integrifolia* e of subgenus *Eurybia* (Table 1), indicating an isolated position. As pointed out by Nesom (1994; see also Brouillet 2006*b*), its morphology is unique within *Eurybia*, with persistent, entire basal leaves and racemo-corymbiform capitulescences; it is a species of dry to moist montane meadows and open forests (Brouillet 2006*b*). In contrast, *E. sibirica* has cauline, serrate leaves and corymbiform capitulescences, and inhabits stream banks, lakeshores, and other relatively humid, disturbed habitats of the boreal forest and the low Arctic, from sea level to the montane zone (Brouillet 2006*b*).

The placement of *E. sibirica* in our phylogeny (Fig. 2) does not concur with that in either the Nesom (1994) or Semple (2005) classifications. Nesom placed this species in subsection *Sibiricae* (with *E. merita* and *S. pygmaeum*) of section *Radulini*, while Semple included it within his larger section *Calliastrum* (Table 1). Its morphological similarity to *E. radulina*, a species of the coastal ranges from British Columbia to California and sole western member of the eastern clade, could result from symplesiomorphies, since the latter is part of a basal polytomy within the eastern clade (Figs. 1 and 2).

The eastern clade, supported by one indel each in ITS and ETS (Fig. 4B), is the most diverse group in the genus and may represent an example of radiation following the invasion of a new area by a taxon, in this case eastern North America. The eastern clade includes both wide, cordate-leaved species adapted to lower-light habitats (deciduous forests and forest edges), such as *E. furcata*, *E. divaricata*, and *E. mirabilis* (section *Eurybia*), and narrow-leaved species, including *E. radula* and the Atlantic and Gulf coastal

plain *E. compacta*, *E. avita*, and *E. hemispherica* (sections *Calliastrum* and *Heleastrum*, Nesom 1994) (Fig. 4A).

Our analysis does not allow us to resolve the relationships among *E. radulina*, *E. divaricata*, and *E. furcata*, because support is low in both analyses (Fig. 2). The association of the western *E. radulina* with eastern species is supported by both parsimony and Bayesian analyses (Fig. 2). This relationship may suggest a single colonization event of eastern North America within this clade, and a second migration of the genus to eastern North America. As there is little resolution in the relevant part of the tree, however, settling this issue will require further data.

The three diploid species of the cordate-leaved section *Eurybia*, *E. furcata*, *E. divaricata*, and *E. mirabilis*, do not form a monophyletic group (Figs. 2 and 4B). The former two are part of the basal polytomy, while the third groups with the narrow-leaved clade (Fig. 2A: BS < 50; Fig. 2B: PP = 0.93). This may be due to homoplasy or low variation in the nrDNA dataset. Section *Eurybia* may either be the sister group to the eastern narrow-leaved species (if it turns out to be monophyletic upon further study) or represent a paraphyletic basal grade to this group; current data do not allow us to resolve this issue. Section *Eurybia* species are usually assumed to form a monophyletic group because of their wide cordate leaves, a unique feature in *Eurybia*. For instance, Lamboy et al. (1991) restricted their isozyme analysis of species relationships solely to this section, without considering that eastern species from other sections might have been involved, despite the existence of intersectional hybridization (e.g., *Eurybia* ×*herveyi* = *E. macrophylla* × *E. spectabilis*; Uttall 1962). The ITS (Fig. 1) and ITS clone (Fig. 3) analyses show that the tetraploid *E. chlorolepis* may be sister to *E. divaricata*, as was observed also in the analysis of ETS data (not shown). Our data thus appear to support the hypothesis of Lamboy (1992) that *E. chlorolepis* may be either an autopolyploid derivative of *E. divaricata*, or an allopolyploid with *E. divaricata* as one of its parents.

The eastern species morphologically most similar to the western *Eurybia radulina* is *E. radula*, which is sister to the other narrow-leaved species. This northeastern species reaches well into the boreal zone of eastern Canada. It grows on river banks and poor fens, habitats that are more or less open, often nutrient poor, and wet. It is morphologically similar to the polyploid *E. saxicastelli* and both were placed in section *Radulini* (Nesom 1994) or *Calliastrum* (Semple 2005).

A last diploid diversification in the genus occurred on the Atlantic and Gulf coastal plain, an invasion of this ecosystem by members of a second lineage of *Eurybia* (the first lineage that of *E. eryngiifolia*). This clade is well-supported in the Bayesian analysis (Fig. 2A: BS = 54; Fig. 2B: PP = 0.99). These species share the thick woody rootstocks and grass-like, sometimes more or less coriaceous, sometimes also marginally spinose, leaves encountered in *E. eryngiifolia* (Fig. 4A). We interpret this as a case of convergent evolution due to selection in this particular ecosystem.

Our data support Cronquist's (1980) hypothesis of an affinity between *E. compacta* and *E. avita*. The relationship of *E. hemispherica* and *E. avita* also confirms the hypotheses of Nesom (1994) and Semple (2005). In both classifications, they are included within section *Heleastrum* (Table 1). *Eurybia compacta*, however, was placed by Nesom (1994) and Semple (2005) in section *Calliastrum* (Table 1). Thus members of both subgenera *Eurybia* and *Heleastrum* are united within a single well-supported clade in the phylogeny, as are members of sections *Calliastrum* and *Heleastrum*. Therefore, our results do not support the phylogenetic relationships among *Eurybia* species suggested by current classifications (Nesom 1994; Semple 2005). In contrast, they indicate that all the narrow-leaved species of eastern North America, except *E. eryngiifolia* and possibly *E. spinulosa*, are closely related, which would support their inclusion within a single section.

Conclusion

Eurybia origin

Our nrDNA-based molecular phylogeny (Fig. 2) allows us to present an overall biogeographical and ecological hypothesis of the history of *Eurybia* diploids in North America. Among North American Astereae, subtribe Boltonieae is sister to both the subtribe Symphyotrichinae and the eurybioids-subtribe Machaerantherinae lineage (Fig. 1), as shown also in wider analyses of the tribe (e.g., Semple et al. 2002). The genera included within this subtribe are present in Mexico and the southern United States. Within Symphyotrichinae, *Canadanthus*, *Almutaster*, and *Psilactis* are western in origin, *Ampelaster* is south-eastern, while *Symphyotrichum* is distributed across the continent. *Oreostemma*, *Herrickia* (both lineages), and *Triniteurybia* are western in distribution. Members of the Machaerantherinae are western North American. All groups sister to *Eurybia* are western taxa of humid to mesic or semi-dry habitats. Given the outgroup relationships described above, it is most likely that the origin of the genus was western and mesic.

Even though the markers used in this study provided limited resolution, we were able to detect significant phylogenetic signal within the eurybioid grade. Our results do not support previous classifications of *Eurybia*. It is however too early to propose a new classification of the group, particularly within *Eurybia*.

Aknowledgements

The authors thank J.C. Semple (University of Waterloo, Waterloo, Ont.) for having provided leaf material for this study, and the curators of ALA, BRIT, KANU, MT, NMC, RM, TEX, VDB, and WAT herbaria for having allowed us to take samples from herbarium specimens. The authors also thank reviewers and G.A. Allen (University of Victoria, Victoria, B.C.) for constructive comments on this manuscript. Bayesian analyses were carried out with the help of the Réseau québécois de calcul de haute performance (RQCHP), Université de Montréal. This project was supported by a Natural Sciences and Engineering Research Council of Canada Discovery grant to L.B.

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Chapitre 2

Phylogeny and biogeography of the intracontinally disjunct North American eurybioid asters (Asteraceae: Astereae) inferred from combined ribosomal and plastid DNA data

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Ce chapitre sera sujet d'un deuxième article. Pour publication, ce dernier sera soumis à la revue scientifique *Systematic Botany*.

Phylogeny and biogeography of the intracontinally disjunct North American eurybioid asters (Asteraceae: Astereae) inferred from combined ribosomal and plastid DNA data

Abstract

The eurybioid asters comprise four North American genera *Oreostemma*, *Herrickia*, *Eurybia* and *Triniteurybia*. Earlier nrDNA-based studies showed that this group is sister to Symphyotrichinae and paraphyletic to Macharantherinae, whereas species relationships were only partly resolved and poorly supported, and it proved impossible to ascertain the relationships of polyploids. The current analysis is restricted to diploid taxa. In an attempt to increase resolution and support, we investigated non-coding regions from cpDNA (*trn*C-*ycf6*, *trn*S-G, *trn*L-F), in addition to ITS and ETS nrDNA data. Both separate and combined analyses of cp and nrDNA data were performed using Maximum parsimony and Bayesian analyses; the combined analyses are presented here. For biogeographic analyses, DIVA and LaGrange were used on the combined dataset. The combined phylogenetic analyses brought more resolution and support than the phylogenies based on nrDNA data, confirming the following relationships: *Oreostemma* (*Herrickia* (*Herrickia kingii* (*Eurybia* (*Triniteurybia*-Machaerantherinae))). *Herrickia* is confirmed as paraphyletic, with *Herrickia kingii* well supported as sister to the crown eurybioids. Within *Eurybia*, the western *E. integrifolia* is sister to the whole genus, followed by the divergence of the Florida panhandle *E. eryngiifolia*. Next are two western species, *E. sibirica* and *E. radulina*, the latter sister to an eastern North American (NA) clade. Both biogeographic analyses confirmed a radiation in the western United States for the eurybioids, with two

independent migrations to eastern North America within *Eurybia*. The group evolution was considered recent, probably not older than Pliocene in age and therefore strongly influenced by the Pleistocene glaciations.

Introduction

The eurybioid grade (Selliah & Brouillet 2008) consists of perennial herbs that are widely distributed within North America, except in the Prairies or in xeric habitats. The grade mostly includes diploid members with a chromosomal base number of $x = 9$; polyploids are restricted to *Eurybia* (Brouillet 2006*a, b, c*). The grade is part of the NA clade of tribe Astereae (Asteraceae). The eurybioids comprises 31 species in four genera: *Oreostemma* Greene (3 spp.), *Herrickia* Wooton & Standley (4 spp.), *Eurybia* (Cass.) S. F. Gray (23 spp.) and *Triniteurybia* Brouillet, Urbatch & R. P. Roberts (1 sp.) (Brouillet et al. 2004, Selliah & Brouillet 2008). This group is sister to subtribe Symphyotrichinae and is paraphyletic to subtribe Macharantherinae (Selliah & Brouillet 2008). Current taxonomic and phylogenetic knowledge of the eurybioids is based on morphology (Nesom 1994, Semple et al. 2002, Nesom & Robinson 2007), isoenzymes (Lamboy & Jones 1988, Lamboy et al. 1991), chloroplastic DNA (cpDNA) restriction sites (Xiang & Semple 1996), and nuclear ribosomal DNA (nrDNA) data, including ITS and ETS (Brouillet et al. 2004, Selliah & Brouillet 2008). In the most recent molecular studies based on nrDNA data, relationships among genera were only partly resolved and not strongly supported. In both analyses (Brouillet et al. 2004, Selliah & Brouillet 2008), genus *Herrickia* tended to segregate into two clades, *Herrickia* s. str and *H. kingii*, but without support in parsimony analyses. Genus *Eurybia* also appeared to split into two clades, the first mainly eastern and the second mainly western and consisting of *Eurybia integrifolia* (Nutt.) G.L. Nesom, *Eurybia eryngiifolia* (Torrey & A. Gray) G.L. Nesom, and *Eurybia sibirica* (L.) G.L.

Nesom, the latter not supported in this group by morphological or ecological data (chapter 1). The *Eurybia* polyploids species relationships were little resolved. Thus, the nrDNA study did not enable us to establish the relationships of the polyploid *Eurybia* species to their diploid progenitors or to each other. Nearly all polyploid ribotypes clustered together in these analyses, without relation to morphological affinities (possibly due to concerted evolution after recombination or other mutational events). All species of the grade are found in humid to semi-xeric habitats, all in the western North America except for the eastern species of *Eurybia*. Our previous analysis (Selliah & Brouillet 2008) led us to hypothesize that within *Eurybia*, two independent migrations had occurred from western to eastern North America.

To date, no extensive study has used cpDNA sequence data to establish a phylogeny of the NA clade (Noyes & Rieseberg 1999), even though chloroplastic regions are widely used in phylogenetic studies due to their ease of amplification with universal primers, availability in high copy number, nonrecombination, and uniparental inheritance (Sears 1980, Taberlet et al. 1991, Small et al. 2005). Therefore, we explored the phylogenetic utility of several noncoding cpDNA regions for the eurybioid grade. After preliminary tests, three plastids makers were retained.

The objectives of this study are to improve our understanding of the phylogenetic relationships of the eurybioids (sensu Brouillet et al. 2004) using cpDNA regions, combining them with nrDNA regions in order to obtain a better resolved phylogeny, and to test our hypotheses (Selliah & Brouillet 2008) concerning the biogeographic history of the group. In the present study, we would like to verify two hypotheses: whether the eurybioids

originated in western North America and whether two independent migrations occurred in the west within *Eurybia*.

Materials and methods

Taxon sampling

Thirty one species and six varieties from the four genera of the eurybioid grade (Selliah & Brouillet 2008) were considered as the ingroup in this study. In general, each species was represented by a single sample. In addition, two representatives of subtribe Machaerantherinae and three of subtribe Symphyotrichinae were added to the data sets. *Doellingeria infirma* (Michx.) Greene and *Chlorocantha spinosa* (Benth.) G.L. Nesom were used as outgroups in the analyses. All sequence accessions were deposited in Genbank (Appendix 3).

DNA extraction

Samples were obtained from herbarium specimens or as fresh-collected material dried in silica gel. Total genomic DNA was extracted using a modified CTAB protocol (Joly et al. 2006) or with the QIAgen DNeasy Plant Mini Kit (QIAGEN, Mississauga, Ontario, Canada).

Choice of cpDNA regions

In order to determine the utility of cpDNA regions, we evaluated the variability of selected regions by examining the variability (nucleotide substitutions, indels, and

inversions) among sequences (Shaw et al. 2005, M. Lauzé, pers. comm. 2006). We screened 18 genes and non-coding regions proposed in previous review papers (Taberlet et al. 1991; Panero and Crozier 2003; Shaw et al. 2005) including commonly used regions; *trn*L-*trn*F and *trn*L intron, *trn*H-*psb*A, *mat*K and *trn*K introns, *rps*16, *rpl*16, *ycf6*-*psb*M, *trn*S-*trn*G, *ndh*F, and *ndh*D, and less commonly used regions; *ndh*J-*ndh*C-*ndh*K, *ndh*I, *ndh*I-*ndh*G and *trn*C-*ycf6*. Whenever possible, one representative from each genus of the ingroup and *Doellingeria infirma*, an outgroup (or if the latter was not available, one of the Symphyotrichinae representatives) were selected for comparison. The primers used for amplification and sequencing are as mentioned in the papers cited above.

PCR amplification and sequencing

Among the investigated regions (Table 2), three non-coding chloroplastic regions were considered as potential phylogenetic regions: i) *trn*C-*ycf6*, ii) *trn*S-G including the *trn*G intron (hereafter *trn*S-G), and iii) *trn*L(UAA)-*trn*F(GAA) including the *trn*L intron (hereafter *trn*L-F). The selected cpDNA regions were amplified and sequenced using the $TrnC^{GCA}F$- ycf6R and $trnS^{GCU}$- $trnG^{UUC}$ (Shaw et al. 2005), and *trnL*c- *trnL*f (Taberlet et al. 1991) pairs of primers, respectively.

For all regions, the 25 µl PCR amplification reaction mix contained 40-70 ng genomic DNA, one unit of *Taq* DNA polymerase, 10x Roche Buffer with 1.5 mM $MgCl_2$ (Roche Diagnostics, Laval, Quebec, Canada), 100 µM of each dNTP, 0.3 mM of each primer, 2.5-5 % dimethyl sulfoxide (DMSO) and glycerol, and 1.5 mM of Mg Cl_2 was added when necessary. Prior to *Taq* polymerase incorporation, PCR reactions were heated

for 2 min of denaturation at 94°C (hot start PCR). PCR amplification conditions for the chloroplastic regions were: initial denaturation of 3 min at 94°C, 35 cycles of 30 sec at 94°C, annealing of 30 sec at 48°C, 30 sec at 72°C, and 10 min at 72°C. The annealing temperature for *trn*C-*ycf6* was 58°C, however.

We also included the nuclear ribosomal DNA (ITS and ETS) data from our previous study (Selliah & Brouillet, 2008) in the analyses in order to reach better resolution and support in the phylogeny. Information about amplification, purification, cloning, sequencing protocols and the ribosomal data sets may be found in that study.

Phylogenetic analyses

All DNA sequences were edited manually and assembled into contiguous sequences using Sequencher v.4.7 (Genecodes Corp., Ann Arbor, Michigan). Matrices were aligned initially in BioEdit v.7.0.5.3 (Hall 1999) using Clustal X (Thompson et al. 1997), and subsequently edited manually.

Insertions and deletions (indels) were observed in all alignments. For *trn*L-F, *trn*S-G and *trn*C-*ycf6*, we noted one, two, and seven indels, respectively. Indels were coded as binary characters in Gapcoder (Young & Healy 2003) using the simple indel coding method of Simmons & Ochoterena (2000).

We carried out separate analyses of each data set as well as concatenated analyses, with and without partitioning, under maximum parsimony (MP) and Bayesian inference (BI; Yang & Rannala 1997). The concatenated cpDNA matrices, containing polyploid

Eurybia members, were partitioned by region. The ribosomal data (Selliah & Brouillet 2008), containing only diploid *Eurybia* species sequences, were added to the concatenated chloroplastic of diploid data sets to produce a cpDNA+ nrDNA combined data set (hereafter refered to as the combined analysis). Internal transcribed spacer (ITS) data were partitioned into ITS1, 5.8S, ITS2; external transcribed spacer (ETS) was not partitioned; information concerning individual and combined analyses of the ribosomal ITS and ETS data is provided in Selliah & Brouillet (2008). Indels were mapped on the combined topology. Analyses were carried out on the concatenated-partitioned cpDNA and combined-partitioned cpDNA + nrDNA data. To evaluate the combinability of the chloroplastic and ribosomal data sets, we performed an ILD_bionj test, an adaptation of the ILD test (Farris et al. 1994) that uses the bionj algorithm (Gascuel 1997), as implemented in ILD bionj v1.0 (Zelmer & Daubin 2004).

MP analyses were performed with PAUP* v 4.0b10 (Swofford 2002) using heuristic searches with the MulTrees option in effect and tree-bisection-reconnection (TBR) branch swapping with 500 random taxon addition replicates. Characters were equally weighted and unordered. Gaps were considered as missing data. Clade support was determined by a bootstrap analysis with 1000 heuristic search replicates (as above) and random taxon addition, with a maximum of 100 trees retained per replicate. A bootstrap support (BS) value $\geq$ 75% was considered significant for MP.

For the Bayesian analyses, each cpDNA and the nrDNA were partitioned into spacer and exon-intron regions and assigned to a sequence evolution model. The cpDNA introns were included within the intergenic spacer partition because of their low variation.

To assess the best-fitting model of sequence evolution for each region, we used MrModeltest v2.0 (Nylander 2004) with the Akaike information criterion (AIC) for BI. The results of selected models for these tests are shown in table 3.

Bayesian analyses were carried out using MrBayes 3.1.2. (Huelsenbeck & Ronquist 2001) on a shared memory multiprocessor computer (Altix 4700). Two independent runs with 16 Markov chains each were conducted simultaneously for 20 million generations, except for ten million for the distinct cpDNA data sets. Analyses started from a random neighbour-joining tree. The parameter estimation for each partition was made independent using the unlink option. For each run, one tree per 1,000 generations was sampled, resulting in 40,002 trees. Using the programs AWTY (Wilgenbusch et al. 2004) and Tracer v1.4 (Rambaut & Drummond 2007), we assessed the convergence of runs, the first trees of each run in the Bayesian analyses corresponding to the burnin phase were removed. Table 3 summarizes the number of trees removed during burnin and the number of trees used to compute the consensus tree in each analysis. A 50% majority-rule consensus tree was computed with the remaining trees in order to determine the posterior probability values (PP). A PP value ≥ 0.95 was considered evidence of strong clade support and $\geq 0.85 \leq 0.94$ as intermediate support.

Biogeographic analyses

To test hypotheses on the biogeographic history of the eurybioids, we used two biogeographic reconstruction methods. Dispersal-vicariance analysis (DIVA v.1.1; Ronquist 1996) is based on the parsimony approach and minimizes the dispersal, vicariance

and extinction events. LaGrange (v.2 Ree & Smith 2008) is a maximum likelihood (ML) method that calculates and assigns relative probabilities for each node using likelihood analysis of geographic range evolution. Both analyses were conducted on a single, fully-resolved tree retained after the burnin phase, resulting from the 28 002 trees of the combined datasets from the BI analysis. In order to reduce noise (e.g., having a large number of distributions or having the entire terminal at the root node) and to increase the accuracy of the ancestral area reconstruction, the representatives of the two sister clades (Machaerantherinae and *Symphyotrichum*), as well as *Chlorocantha spinosa* were pruned from the tree using Phyutility v.2.2 (Smith & Dunn 2008). We used *Canadanthus modestus*, a sister group, as the outgroup. We assumed that the eurybioids are a recently diverged group (Noyes & Rieseberg 1999, Brouillet et al. 2008), plausibly of Pliocene age (5 million years before present (myr B.P.)). Most of the northern half of North America (most of Canada and parts of Alaska) was glaciated during the Pleistocene glaciations (Mann & Hamilton 1955), while the southern half (essentially the conterminous United States) was not, though it was affected. The flora of glaciated areas is the result of recent (less than 18 000 yr B.P.) species migrations into the area. Although members of the eurybioid grade are currently present across much of North America, their distributions in Canada and Alaska reflect postglacial migrations that occurred well after speciation. *Eurybia sibirica* is present in the northern United States Rockies, in glaciated western North America, in unglaciated parts of Alaska-Yukon, as well as across Eurasia, and it may have reached unglaciated Beringia before the last glaciation, crossing into Eurasia at the earliest during the last glaciation; we are postulating that its arrival in Beringia was after its origin in the Northern Rockies, given the proximity of the latter area to other areas of

speciation in other eurybioids. The presence of eurybioids in glaciated areas thus has little bearing on the speciation and the early biogeographic history of the grade. Therefore, we excluded the northern half of the continent from our modelization. The circumscription of the geographic areas used here took into consideration the limitations of LaGrange (Ree & Smith 2008), which allows a maximum of four or five areas. Firstly, we performed a global analysis; we divided North America into four areas: (A) Pacific western United States, (B) Intermountain region and Rocky Mountains, (C) southeastern coastal plain, and (D) eastern deciduous forest. A second analysis at a finer scale in eastern North America was carried out: (E) Appalachian Mountains (F) Atlantic coastal plain, (G) Piedmont and Blue Ridge, and (H) Gulf coastal plain. This subdivision is coarse compared with the distribution of individual species and species ranges may spill over onto adjacent areas. We tested the distribution of ancestral areas using the default option settings of the programs; in DIVA analyses, however, the maximum number of areas was constrained to two using the maxareas option as recommended by Ronquist (2006).

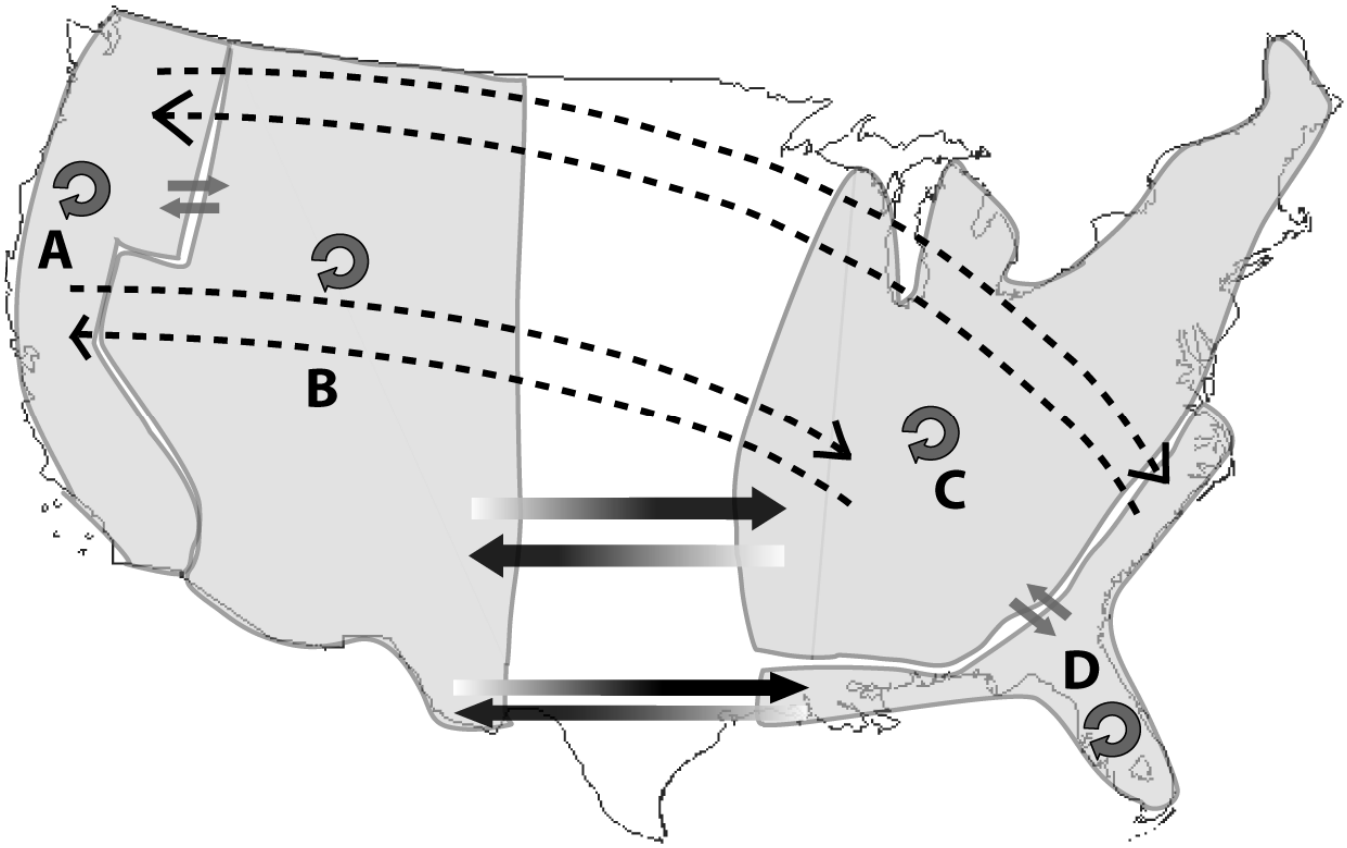
Combined with the species distribution ranges, macrofossil data and reliable documentation about the origin of the NA Astereae could contribute to more accurate biogeographical interpretations of the group (Moore et al. 2007), whereas a lack of such data would restrict interpretation. Currently, the divergence time of the Asteraceae has been estimated at around the mid Eocene (42- 47 myr B.P.) (Kim et al. 2005) but the ranges vary between dates as old as 43-50 myr B.P., (Funk et al. 2009) and as young as 40 myr B.P. (Schmidt & Schilling 2000). Similarly, the origin of tribe Astereae, based on pollen, also is uncertain: Graham (1996) placed it around the Eocene (35-50 myr B.P.), while

Karaman (2006) suggested that most of the Asteraceae tribe were present at the end of the Oligocene (22-25 myr B.P.) but without specifying a date of origin for the Astereae. The accuracy of this estimate is uncertain, however, given the lack of fossils for the Astereae. The eurybioid asters suffer from a similar limitation due to lack of fossils, both for validating distribution assumptions and as internal calibration points in a molecular dating approach. Estimates of ITS molecular evolutionary rates exist for other Asteraceae lineages (e.g., *Dendroseris,* Sang et al. (1994); *Robinsonia*, Sang et al.(1995); Hawaiian silversword alliance, Baldwin & Sanderson (1998); *Eupatoria,* Schmidt & Schilling (2000); *Chaetanthera,* Hershkovitz et al. (2006); *Abrotanella*, Wagstaff et al. (2006); *Pleurophyllum*, Wagstaff et al. (2007); *Ainsliaea,* Mitsui et al. (2008)). In these studies, calibration of the ITS evolutionary rate was indirect and based on dates estimated from studies of cpDNA, themselves without solid calibration points. A transposition of such rates to our group would appear susceptible to large bias in age estimation (Kay et al. 2006). Even if a molecular dating approach is commonly used to address phylogeographic and biogeographic concerns, the accuracy of age estimations and the use of derived estimates of evolutionary rates are controversial (Renner 2005, Kay et al. 2006). Moreover, we considered that the past five million years is a relatively short time period compared to nrDNA region substitution rates (here relatively low) and are therefore not useful to estimate the divergence time of the eurybioids. Finally, the use of apparently correlated geological events to calibrate phylogenies may be improper due to potential circularity (Renner 2005). Given the lack of firm fossil evidence, we abstained from dating the phylogeny.

For the spatial reconstruction of ancestral ranges, assumptions about the dispersal opportunity of the eurybioids between the four areas defined above were evaluated using constraints in LaGrange (Ree & Smith 2008). Assumptions about the dispersal connections between western and eastern areas were established based on a general model of glacial-interglacial episodes. Although the probability of bidirectional dispersal events between the western and eastern parts of the continent is non-null, we hypothesized that dispersal for an anemochorous group such as the eurybioids must have been more frequently from west to east given prevailing winds at mid-latitudes, as exemplified by the disjunct cordilleran elements in the flora of the Gulf of St. Lawrence region (e.g. *Cirsium scariosum*, Golden et al. 2008). Thus for the global analysis, we assigned four relative dispersal opportunity probabilities of connections between these areas during the warm periods of the glacial (cooling) - interglacial (warming) cycles: maximal (1.0), equivalent (0.5), minimal (0.1), and zero (0) probability of dispersal events. Dispersal within any area (A, B, C or D) was assumed to be maximum (1.0) due to lower barriers to dispersal within versus between areas, though we recognize that migration within the Intermountain- Rocky Mountains area may have been limited between mountain ranges depending on the time during the glaciations. We considered that dispersal events between adjacent areas (western "A-B", and eastern "C-D") had equal probabilities (0.5) because, a priori, there appeared to be limited barriers between them. Dispersal between extremely disjunct areas ("A-D" and "A-C") was assigned a probability of 0, considering that the large geographic gap and intervening obstacles created a nearly absolute barrier (except for very rare long distance dispersals) (Fig. 5). We tested the four scenarios, with constraints from west to east, respectively of 0, 0.1, 0.5 and 1.0, and compared the global maximum likelihood values

calculated for each. For instance, with a constraint of 0.1, connections between the western and eastern United States were assigned a probability of dispersal success of 0.5, and a probability of 1.0 within the four areas, while the connections between Rockies-Intermountains and the eastern deciduous forest or the southern coastal plain were assigned a 0.1 probability.

Figure 5. Dispersal opportunity model between four major North-American areas implemented in LaGrange. The alphabetic letters represent the defined geographic areas (A: Pacific western United States, B: Intermountain region and Rocky Mountains, C: eastern deciduous forest, D: southeastern coastal plain). Arrows are associated with unique spatial scales and specific dispersal probabilities. Circled arrows: dispersal probability of 1 within areas; grey arrows: dispersal probability of 0.5 between adjacent areas; shaded arrows: dispersal probability of 0.1 to 1 between disjunct areas; and dotted arrows: null probability between extremely disjunct areas.



Results

Results for the screened cpDNA regions are provided in table 2. A majority of these regions was easily amplified; *rps16* and *rpl16* were amplified only in a few species and the chromatograms proved unreadable; amplification of *ycf6- psb*M was unsuccessful. The *trn*C- *ycf6* and *trn*S-G regions proved to be the most variable. They both consist of relatively short (Table 2) and easily amplified fragments. The *trn*L-F region was selected because it is among the most widely used chloroplastic regions. Noncoding chloroplast regions sequenced for this paper showed little variation within our group. Commonly used regions, useful at lower phylogenetic levels in other studies (e. g., *Coreopsis* (Asteraceae) Crawford & Mort 2005, *Townsendia hookeri* (Asteraceae) Thompson & Whitton 2006), appeared to be less variable here. The *mat*K-*trn*K introns region, though more variable than the *trn*L-F region, was not used here but appears promising. For *trn*C-*ycf6*, *trn*S-G, and *trn*L-F, the total potential informative characters (PICs) value and the percentage of variability were 18 (4.1%), 14 (1.7%), and 8 (0.9%), respectively.

Table 2. General data about the genes and non-coding *cp*DNA regions tested in this study. One Asterisk (*) indicates a lack of sampling; for one out of four genera, two asterisks (**) designate the potential informative characters (PIC) calculated only within the *Eurybia* genus; three asterisks (***) indicate missing data due to amplification failure.

Region	Primers	Litterature	Total aligned lenth (excluding outgroup)	PIC	PIC (excluding outgroup)
***trn* L^{UAA} intron+ *trn* F^{GAA} *trn* L^{UAA} spacer**	*trnL* c- *trnL* f	Taberlet et al. 1991	917	0.0098	0.0087
***Atp* B- *rbc* L spacer**	1G2-1G5	Panero & Crozier 2003	930	***	***
***ndh* J-C-K genes**	ndhJF-ndhK2R		810	0.0099*	0.0099*
	ndhK1F-ndhCR		870	0.0057*	0.0057*
***ndh* D**	Ycf5F-732R		800	0.0088	0.0088
	672F-psacR		900	0.0078	0.0033
***mat* K gene + *trn* K introns**	3914F-1254R		1220	0.0057	0.0033
	816F-1857R		960	0.0156	0.0115
	1755F-trnK2R		810	0.0099*	0.0062*
***ndh* I + *ndh* I-*ndh* G spacer**	ndhGF-ndhAexon2R		745	0.0081*	0.0054*
***ndh* F**	52-1212R		1200	0.0083	0.0075
	972F-607		1390	5.833*	0.0129*
23S-*trn* A spacer + *trn* A intron	23SF-trnIR		1120	0.0027	0.0018
***trn* C-*ycf6* spacer**	TrnC^{GCA}F-ycf6R	Shaw et al. 2005	469	0.0405	0.0414
***trn* S-G spacer**	trnS^{GCU}-trnG^{UUC}		838	0.0167	0.0158
***trn* H-*psb* A**	psbAF- trnHR		300	0.0033**	0.0033**
***rpS* 16**	rpS16F-rpS16R		NA	***	***
***rpL* 16**	rpL16F71-rpL16R1516		1150	***	***
***ycf6* - *psb* M spacer**	psbMR- ycf6F		490	***	***

Data on the MP and BI analyses are provided in table 3 and figure 6. Preliminary analyses of individual chloroplast data sets provided little resolution due to a lack of variable characters. Therefore, we present only the results of analyses based on the concatenated cp DNA data sets (Fig.6). All the indels observed within these three chloroplastic regions are mapped on the cpDNA topology (Fig. 6). The ITS and ETS indels were mapped in our previous paper (Selliah & Brouillet 2008). The non-coding region *trn*C-*yf6r* is more variable than *trn*S-G, while *trn*L-F is the least variable, respectively with eight, two and one indel. In the combined analysis, the polyploid species of *Eurybia* were excluded (for details, see in Selliah & Brouillet 2008). Although they show identical relationships, topologies resulting from partitioned matrices exhibit greater support values than those from unpartitioned ones (ln values respectively -4265.60 and -4306.74). Therefore, only topologies from partitioned analyses are shown here. MP and BI topologies are identical with respect to intergeneric relationships among eurybioids in the combined datasets; the grade tends to form a polytomy in the MP reconstruction but is resolved in BI (Fig. 6).

The overall percentage of variable sites of the three cpDNA chloroplastic regions was 1.54% versus 11.83% for nrDNA (ITS+ETS). The ILD_bionj test rejected the congruency of cp and nrDNA datasets with 5000 iterations (p= 0.024 with p-value of 0.05). However, after examination of the incongruence, we decided to combine the two datasets nonetheless in order to reach increased resolution and node support. Due to higher node support values than those observed in separate topologies (e.g., Fig.1 and 2 in Selliah & Brouillet 2008), we retained the combined topology. The cpDNA (Fig.6) and combined (Fig.7) BI topologies highly support intergeneric relationships, except for the position of

Eurybia integrifolia. An examination of the concatenated chloroplastic regions reveals that this specie does not share the indels typical of other *Eurybia* species (A, C, H and J, Fig. 6). In the cpDNA topology, Machaerantherinae, *Triniteurybia abberans* and *E. integrifolia* appear to group with *Herrickia*. This grouping is supported by one insertion (I) and a single nucleotide substitution from G to A in *trn*C-*ycf6*. In the combined topology, however, *E. integrifolia* is positioned strongly as sister to all other members of *Eurybia*.

Table 3. Summary of the results provided by parsimony and Bayesian analyses. Asterisks (*) represent values obtained after excluding uninformative characters

DNA region	**cpDNA +ITS +ETS**	**cpDNA**	**trnC-ycf6**	**trnS-G**	**trnL-trnF**
Taxa included (including outgroup)	28	42	42	38	34
Lenth variation (bp)	3370-3566	2157-2224	435-469	824-838	916-917
Missing data (%)	8,5	3,8	0,7	7,3	1,6
Variable characters	176	57	18	27	12
Parsimony-informative characters	208	47	23	14	10
Indels	19	10	7	2	1
Tree lenth	590	126	49	47	23
number of parsimonious tree	6	50 000	50 000	50 000	6
CI*	0.6262	0.7353	0.8333	0.75	0.9091
HI*	0.3738	0.2647	0.1 667	0.25	0.0909
RI	0.794	0.9294	0.9709	0.881	0.9756
AIC Model	((GTR+G)+ (GTR+I)+ (GTR+I)) +((SYM+G)+ (JC)+(SYM+G)) + (HKY+G)	((GTR+G)+(GTR+I) +(GTR+I))	GTR+G	GTR+I	GTR+I
Burnin trees	10 000	10 000	4 000	4 000	4 000
Trees retained	30 002	30 002	16 002	16 002	16 002

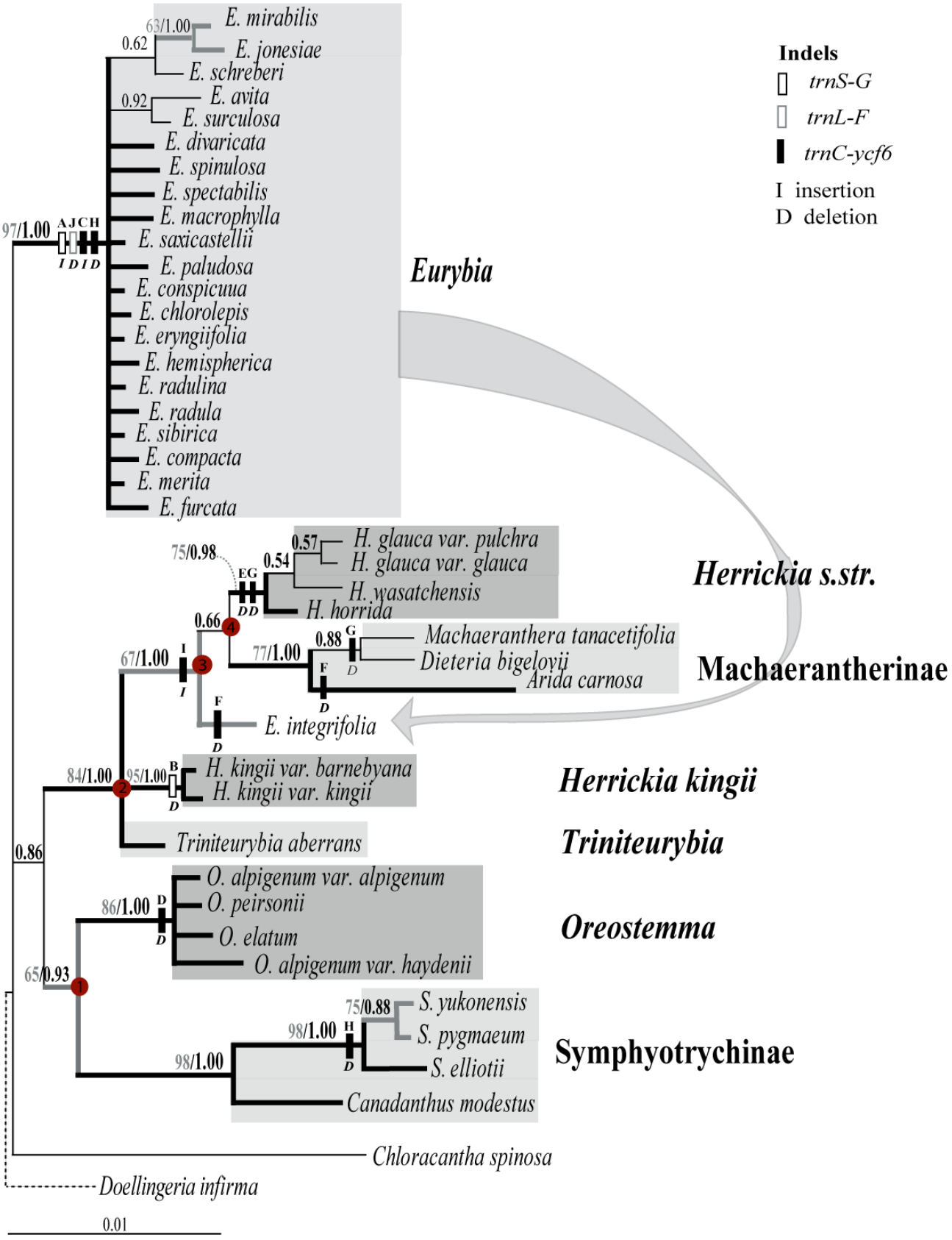
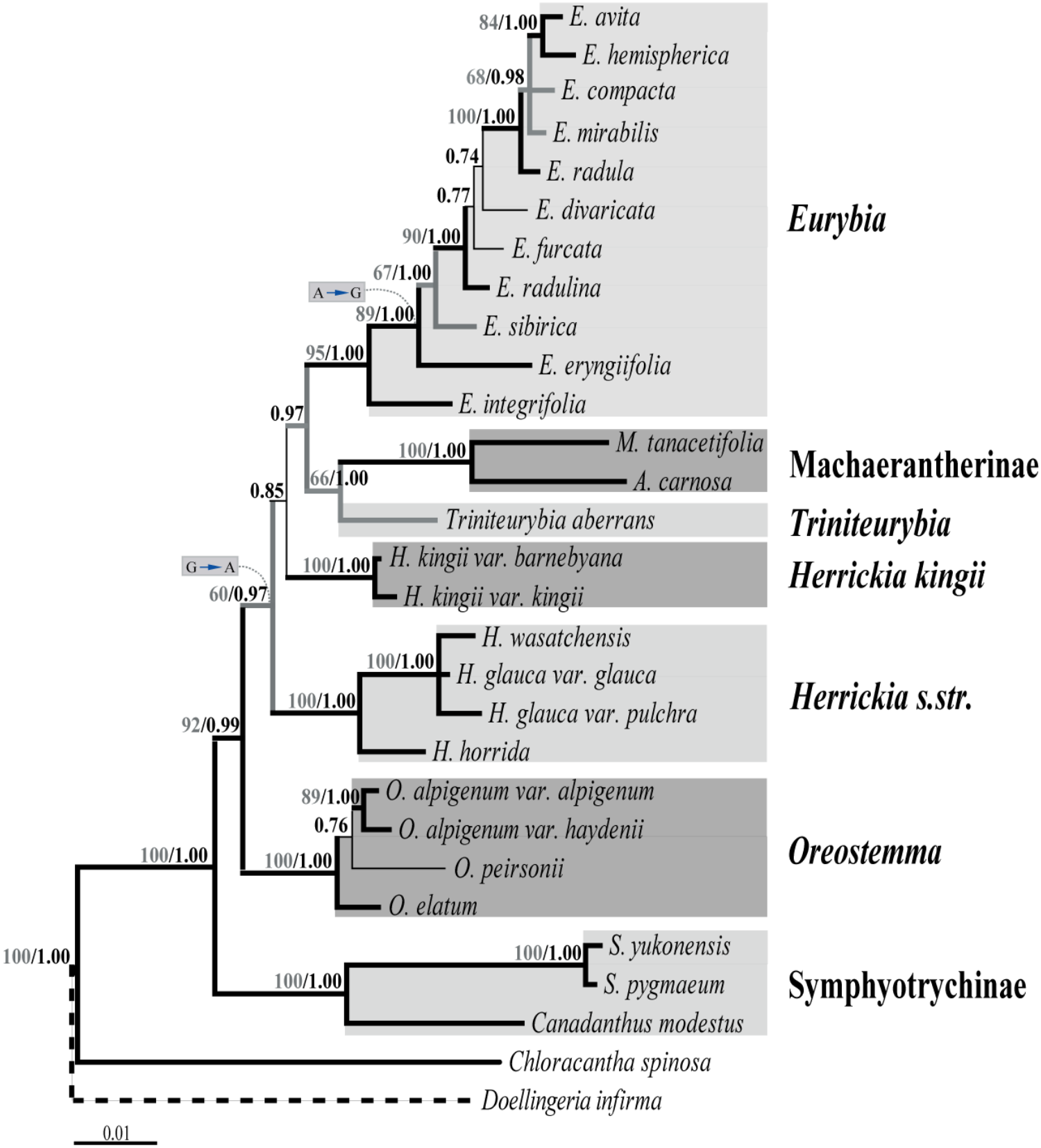

Indels
trnS-G
trnL-F
trnC-ycf6
I insertion
D deletion
E. mirabilis
E. jonesiae
E. schreberi
E. avita
E. surculosa
E. divaricata
E. spinulosa
E. spectabilis
E. macrophylla
E. saxicastellii
E. paludosa
E. conspicuua
E. chlorolepis
E. eryngiifolia
E. hemispherica
E. radulina
E. radula
E. sibirica
E. compacta
E. merita
E. furcata
Eurybia
H. glauca var. pulchra
H. glauca var. glauca
H. wasatchensis
H. horrida
Herrickia s.str.
Machaeranthera tanacetifolia
Dieteria bigelovii
Arida carnosa
Machaerantherinae
E. integrifolia
H. kingii var. barnebyana
H. kingii var. kingii
Herrickia kingii
Triniteurybia aberrans
Triniteurybia
O. alpigenum var. alpigenum
O. peirsonii
O. elatum
O. alpigenum var. haydenii
Oreostemma
S. yukonensis
S. pygmaeum
S. elliotii
Canadanthus modestus
Symphyotrychinae
Chloracantha spinosa
Doellingeria infirma
0.01

Figure 6. (Previous page) Combined and partitioned *trn*L-F, *trnS*-G and *trn*C-*ycf6 cp*DNA phylogram based on Bayesian analyses rooted with *Doellingeria infirma* (dotted line) including bootstrap value supports inferred by parsimony analysis. Bold black branches represent significant support by parsimony (≥75%) and Bayesian (≥0.95) analyses. Grey branches indicate support by only one of the two analyses. Grey numbers above branches are support values obtained from parsimony analysis, followed by Bayesian posterior probabilities in black. Synapomorphic indels are indicated by bars; open black bars are *trn*S-G indels (A: 5 bp, B: 2 bp), the open grey bar is a *trn*L-F indel (9bp) and solid black bars are *trn*C-*ycf6* indels (C: 6 bp, D: 5 bp, E: 4 bp, F: 2 bp, G: 1 bp, H: 1 bp, I: 26 bp). The insertion and deletion events are respectively represented by 'D' and 'I', in slanting character below bars. The node numbers one to four (in circles) refer to the relationships that are found to be different from the *nr*DNA analyses (see section 'The taxonomy of the eurybioids', in discussion).

Figure 7. (Following page) Combined and partitioned phylogram of cpDNA and nrDNA based on Bayesian analysis rooted with *Doellingeria infirma* (dashed line) including bootstrap support values inferred by parsimony analysis. Bold black branches represent significant support by parsimony (≥75%) and Bayesian (≥0.95) analyses. Grey branches indicate support by only one of the two analyses. Grey numbers above branches are support values obtained from parsimony analysis, followed by Bayesian posterior probabilities in black. Numbers within squares at each node correspond to nodes that are referred in the text (sections results and discussion, chapter 2).


84/1.00
E. avita
E. hemispherica
68/0.98
E. compacta
100/1.00
E. mirabilis
0.74
E. radula
0.77
E. divaricata
90/1.00
E. furcata
67/1.00
E. radulina
A → G
89/1.00
E. sibirica
95/1.00
E. eryngiifolia
E. integrifolia
Eurybia
0.97
100/1.00
M. tanacetifolia
A. carnosa
Machaerantherinae
66/1.00
0.85
Triniteurybia aberrans
Triniteurybia
G → A
100/1.00
H. kingii var. barnebyana
60/0.97
H. kingii var. kingii
Herrickia kingii
H. wasatchensis
100/1.00
H. glauca var. glauca
100/1.00
H. glauca var. pulchra
92/0.99
H. horrida
Herrickia s.str.
89/1.00
O. alpigenum var. alpigenum
0.76
O. alpigenum var. haydenii
100/1.00
100/1.00
O. peirsonii
O. elatum
Oreostemma
100/1.00
S. yukonensis
100/1.00
S. pygmaeum
100/1.00
Canadanthus modestus
Symphyotrychinae
Chloracantha spinosa
Doellingeria infirma
0.01

The ancestral distributions (Fig.8, Table 4) resulting from DIVA optimization are single optimal unit areas, except at a few nodes where sets of two or three areas and alternate optimal areas are proposed. When the number of areas is reduced to four, the presence of all four units was noted only once in the optimal distribution list (Fig.8, Table 4). The DIVA reconstruction required nine dispersal events for all constrained unit areas tested. The maximal number of ancestral ranges ('splits') for each node inferred by ML within 2 log-likelihood units was five. When several possibilities were listed, only the scenarios with the two highest relative probabilities were considered (except where probability differences between splits were not significant). The ln value of the ML reconstruction was -35.87 (Ree et al. 2005).

Although some biogeographic scenarios proposed by LaGrange are more restrictive than those in DIVA, the results from both analyses were generally similar (Fig.8, Table 4). Overall, the ancestral distribution(s) proposed by the parsimony reconstruction at each node corresponded to that with the highest probability in the ML inference, except at the outgroup, *E. eryngiifolia-E. hemispherica* node (node 7) and *E. radula -E. hemispherica* (node 12) nodes (Fig. 8, Table 4). At the outgroup node, MP analysis suggests three combinations: 'A, B or AB' while ML analyses propose 'B' as the highest (0.32), then 'A' (0.21), 'A|AB' (0.12) and 'B|AB' (11%), etc. At node 7, MP suggests 'BC, ABC or BCD' as a combination of the optimal ancestral areas, while ML indicates 'C|ABD' (0.77) or 'C|AB' (0.11). At node 12, MP indicates 'FG or FH', while ML proposes ' F|EG', 'F|EGH', 'F|G', 'F|F', 'F|E' or 'F|FG' (respectively with 0.13, 0.12, 0.09, 0.09, 0.07 and 0.04).

With respect to hypotheses of dispersal success (connection) within the eurybioids, the highest ML values were obtained with the most constrained scenario (0.1) with -29.03, followed by the 0.5 constraint (-32.31), the defaults settings (-35.87), and the maximum probability scenario (1) (-37.39).

Figure 8. (Following page) Reconstruction of the optimal biogeographical scenarios suggested by DIVA (circles) and LaGrange (squares). Grey and black color outlines indicate, respectively, global and finer scale analyses. Full circles or squares represent optimal distribution areas retained. Alphabetic letters represent geographic areas: A, Pacific western United States; B, Intermountain region and Rocky Mountains; C, eastern deciduous forest; D, southeastern coastal plain; E, Appalachian Mountains; F, Atlantic coastal plain; G, Piedmont and Blue Ridge; H, Gulf coastal plain.

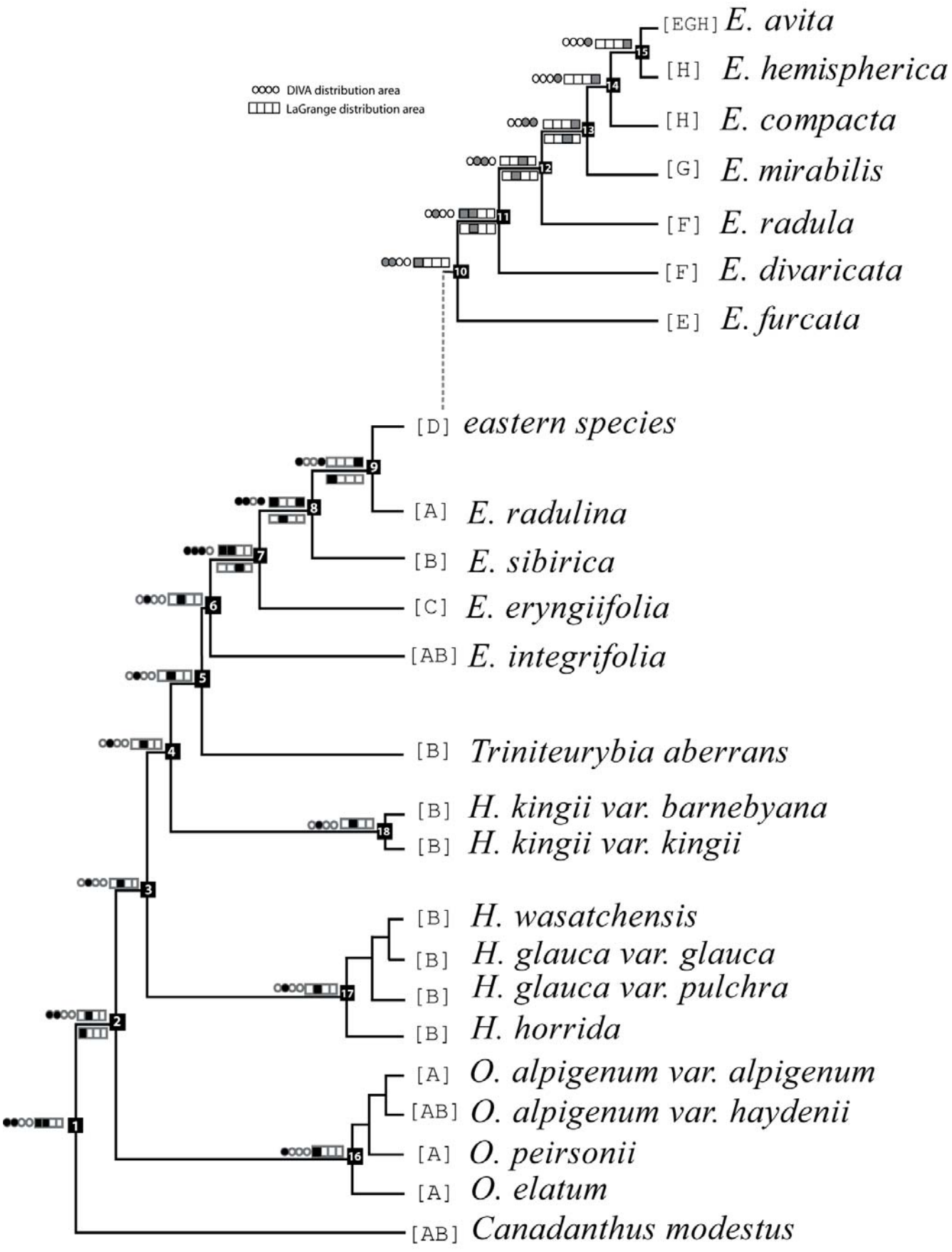

DIVA distribution area
LaGrange distribution area
[EGH] E. avita
[H] E. hemispherica
[H] E. compacta
[G] E. mirabilis
[F] E. radula
[F] E. divaricata
[E] E. furcata
[D] eastern species
[A] E. radulina
[B] E. sibirica
[C] E. eryngiifolia
[AB] E. integrifolia
[B] Triniteurybia aberrans
[B] H. kingii var. barnebyana
[B] H. kingii var. kingii
[B] H. wasatchensis
[B] H. glauca var. glauca
[B] H. glauca var. pulchra
[B] H. horrida
[A] O. alpigenum var. alpigenum
[AB] O. alpigenum var. haydenii
[A] O. peirsonii
[A] O. elatum
[AB] Canadanthus modestus

Table 4: Summary of the ancestral and optimal distributions listed by DIVA and LaGrange analyses. Bold ancestral ranges were those retained in this study when numerous optimal ranges were suggested.

	Ancestral range	
Node number	**LaGrange** splits ([|]) with relative probability	**DIVA**
1	**[B|B] 0.3235** **[A|A] 0.2099** [A|AB] 0.1161 [B|AB] 0.111 [A|B] 0.05636	A / B / **AB**
2	**[A|B] 0.6722** [B|B] 0.178 [AB|B] 0.09633	**AB**
3	**[B|B]** 0.945	B
4	**[B|B]** 0.9373	B
5	**[B|B]** 0.8399	B
6	**[B|B] 0.2533** [A|AB] 0.06707 [B|AB] 0.06149 [A|ABDC] 0.06047 [B|ABDC] 0.05293 [AB|B] 0.05239 [B|BC] 0.05178	B
7	[C|ABD] 0.7688 **[C|AB] 0.112**	BC/ **ABC** / BCD
8	**[B|AD]** 0.889	AB / BD / **ABD**
9	**[A|D]** 0.8846	**AD**
10	**[E|E]** 1	**EF**
11	**[F|EF] 0.37** [E|E] 0.33 [EF|F] 0.09 [F|E] 0.06715	**F**
12	[F|EG] 0.1315 [F|EGH] 0.12 **[F|G] 0.09** [F|F] 0.09183 [F|E] 0.06911	**FG** / FH
13	**[G|H]** 0.18 [G|EG] 0.1023	GH
14	**[H|H]** 0.31	H
15	**[H|H]** 0.32	H
16	**[A|A]** 0.6842 [AB|A] 0.2219 [A|B] 0.09633	A
17	**[B|B] 1**	B
18	**[B|B] 1**	B

Discussion

Utility of cpDNA regions

The three chloroplast DNA regions individually showed limited variability and proved of little use to resolve relationships among the closely related species of the eurybioid complex. The variability of the concatenated cpDNA chloroplast regions was less than that of the nrDNA (ITS+ETS). Nonetheless, these regions provided phylogenetic signal distinct from that of the nrDNA regions. When information from both genomes was combined, the resulting topologies provided relationships consistent with those found in our previous study (Selliah & Brouillet 2008), based only on the nrDNA regions. Despite a few topological incongruences between the distinct cpDNA and nrDNA datasets (see below), the resulting combined topology was better resolved and the relationships more highly supported than in separate analyses (Fig. 6).

The topological conflict encountered between the cpDNA and nrDNA regions, underlined using the ILD_bionj test, may have resulted from (a) a relative lack of variation, (b) intergeneric hybridization, (c) chloroplast capture, (d) sampling error, or (e) a parallel gain or loss of characters during evolution (Soltis and Kuzoff 1995, Maddison 1997, Sang & Zhong 2000, Fehrer et al. 2007). The low variation of the chloroplast sequences may have caused some incongruence, notably the grouping of *Herrickia*, *Triniteurybia* and the Machaerantherinae (Fig. 6), which appears to be based on symplesiomorphies. Hybridization is one of the main processes leading to plastid and nuclear genome incongruence. Intergeneric hybridization has been reported, particularly within recently

radiated groups where reproductive isolation is imperfect (e.g., *Heuchera* group of Saxifragaceae, Soltis et al. (1991); in the Asteraceae: *Achillea* (Anthemideae), Guo et al. (2004); *Pilosella- Andryala* (Cichorieae), Fehrer et al. (2007); in the Astereae: between *Oclemena* and *Doellingeria*, Gerdes (1998) and Nesom (2001), and within the Machaerantherinae, Morgan (2003, 1997)). Interspecific hybridization may have played a significant evolutionary role within *Eurybia*. The numerous allopolyploid species of different ploidy levels within this genus reflect a complex history of reticulate evolution (e.g., Lamboy et al. 1991). Uttal (1962) also documented the hybrid origin of *Eurybia* x*herveyi* from the octoploids *E. macrophylla* and *E. spectabilis*. If it were the case within the eurybioids, species relationships based on various datasets may prove to be different or ambiguous, resulting in a polytomy.

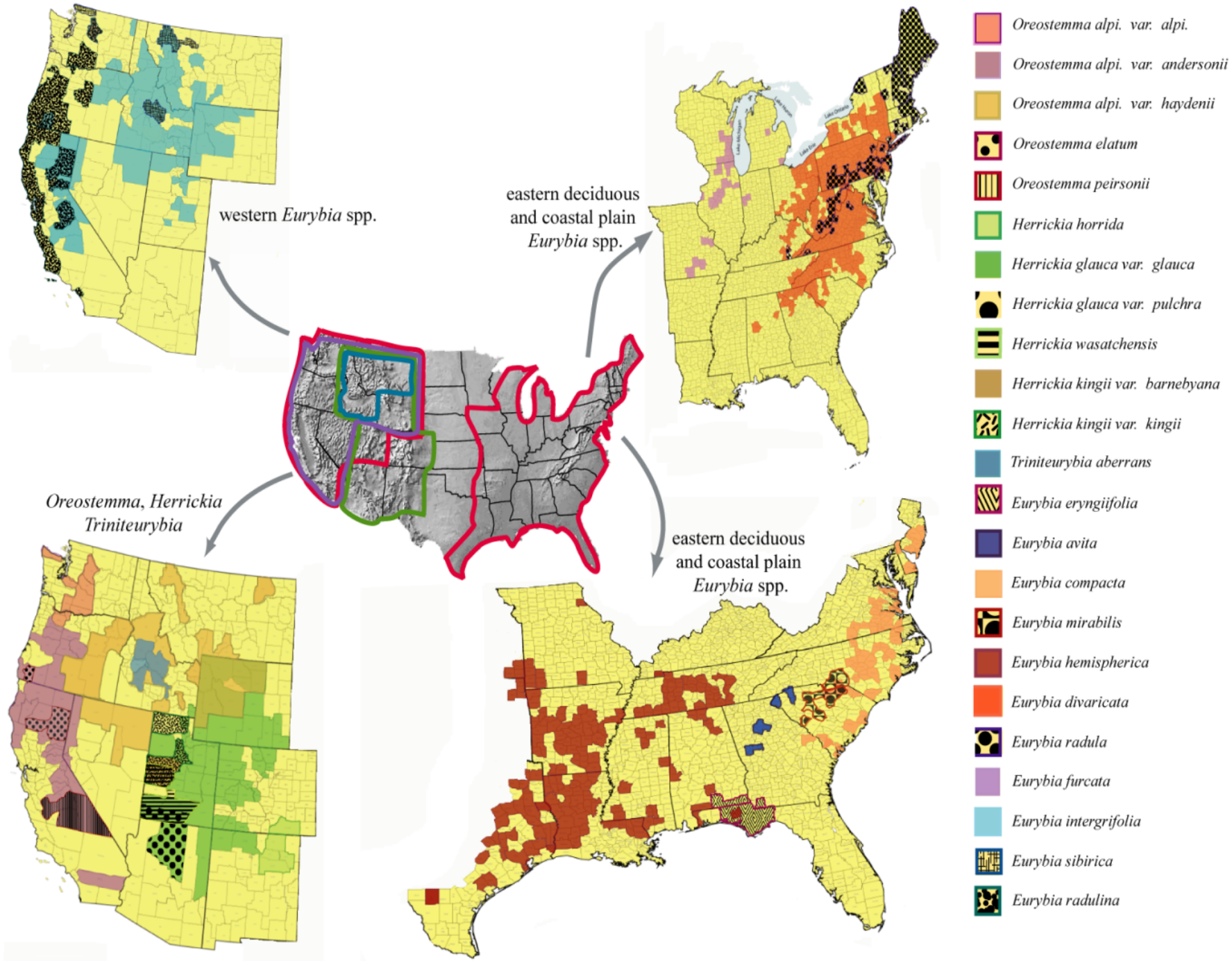
In the cpDNA topology (Fig. 6), two insertions and two deletions are shared by all *Eurybia* species except *E. integrifolia*. This is combined with one insertion shared by *E. integrifolia* with *Herrickia* s. str. and the Machaerantherinae (node 3 in Fig. 6). Chloroplast data therefore would appear to indicate a closer affinity of *E. integrifolia* with the latter than with *Eurybia*. An affinity of *E. integrifolia* to *Herrickia* was also observed with the low copy nuclear data [chapter 3], but was not suggested in the nrDNA topology (Selliah & Brouillet 2008). If intergeneric hybridization is possible, chloroplast capture (Rieseberg & Soltis 1991) between *Eurybia* and *Herrickia* or *Triniteurybia* might explain this pattern, as was found in the *Verbena* complex (Vebenaceae; Yuan & Olmstead 2008), *Penstemon* (Plantaginaceae; Wolfe et al. 2006), *Graptopelatum* (Crassulaceae; Acevedo-Rosas et al. 2004), between *Hazardia* and *Lessingia* (Astereae) Morgan (2003), and

between *Oönopsis* and *Machaeranthera tanacetifolia* (Astereae) (Morgan & Simpson 1992).

The possibility of sampling error was minimized as much as possible in this study. The same individual was used to sequence the different regions, several individuals were sequenced when relationships appeared doubtful (e.g., three individuals of *E. integrifolia*), and different methods of phylogeny reconstruction were used. Among all hypotheses mentioned above to explain conflicting results, the sampling error appears the least likely.

Although multiple gain or loss events are not most parsimonious, they may still occur during evolution and obscure relationships within the cpDNA topology, notably the affinity between *Eurybia integrifolia*, the Machaerantherinae and *Herrickia* (3 in Fig. 6). This may provide an alternate explanation of the incongruence pattern observed here. Of ten mapped chloroplastic indels (Fig. 6), six were autapomorphic and four, all from *trn*C-*ycf6,* appeared to be homoplasious. Three are deletions, F (2bp), G (1bp) and H (1bp), and may have occurred twice independently, and the insertion I (26bp) apparently was gained three times, in *Herrickia* s. str., the Machaerantherinae, and *E. integrifolia*. This could explain the grouping of these taxa in the cp DNA topology. The nucleotide substitution of G to A observed in *trn*C-*ycf6*, could be interpreted as having occurred below the split between *Herrickia* s. str. and the remaining eurybioids-Machaerantherinae; it could have been followed by a reversal of A to G, the initial state, after the emergence of *E. integrifolia* (Fig. 6), therefore characterizing all other *Eurybia* species.

Figure 9. (Following page) Area distribution map of eurybioids across the southern region of North America. The global distribution of each genus is color coded (i.e., *Oreostemma* in purple, *Herrickia* in green, *Eurybia* in red and *Triniteurybia* in blue) and represented in the global map. Species distribution is also detailed by county, for each state. The geographic distribution information sources are Flora of the Southeast (from University of North Carolina herbarium, UNC: http://www.herbarium.unc.edu/seflora/firstviewer.htm, last modified: 27 Aug. 2007), Natural Resources Conservation Service (from United States Departement of the Agriculture, USDA: http://plants.usda.gov/, last modified: 26 Oct. 2009) and Flora of North America (Flora of North America Association, FNA: http://www.fna.org/, last modified: 28 Oct. 2009).


western *Eurybia* spp.
eastern deciduous and coastal plain *Eurybia* spp.
Oreostemma, *Herrickia* *Triniteurybia*
eastern deciduous and coastal plain *Eurybia* spp.
Oreostemma alpi. var. alpi.
Oreostemma alpi. var. andersonii
Oreostemma alpi. var. haydenii
Oreostemma elatum
Oreostemma peirsonii
Herrickia horrida
Herrickia glauca var. glauca
Herrickia glauca var. pulchra
Herrickia wasatchensis
Herrickia kingii var. barnebyana
Herrickia kingii var. kingii
Triniteurybia aberrans
Eurybia eryngiifolia
Eurybia avita
Eurybia compacta
Eurybia mirabilis
Eurybia hemispherica
Eurybia divaricata
Eurybia radula
Eurybia furcata
Eurybia intergrifolia
Eurybia sibirica
Eurybia radulina

Taxonomy of the eurybioids

The current phylogenetic reconstructions (cpDNA and combined) generally confirm previous findings (Brouillet et al. 2004, Selliah & Brouillet 2008). The better-resolved combined topology provides more detailed information about evolutionary relationships within the grade. *Oreostemma* and *Eurybia* are monophyletic, and *Triniteurybia aberrans* appears to be sister to subtribe Machaerantherinae. *Herrickia* s. str. (excluding *H. kingii*) is characterized by two synapomorphic deletions; it diverges after *Oreostemma* and is sister to the *H. kingii*-*Eurybia*-*Triniteurybia*-Machaerantherinae clade, a relationship that is strongly supported only by the Bayesian analysis (PP = 0.97). In both cpDNA (Fig. 6) and combined (Fig. 7) topologies, *Herrickia kingii* is shown to be distinct from *Herrickia* s. str. and sister to the clade comprising *Eurybia, Triniteurybia* and the Machaerantherinae, as suggested by Selliah & Brouillet (2008). This relationship is well supported (PP = 0.85) and is further strengthened by a synapomorphic deletion (B, Fig.6) in the *trn*S-G region. *Eurybia integrifolia* is the earliest diverging member of genus *Eurybia*, a position supported by a deletion (F) and the lack of two cpDNA insertions (A and C) and two deletions (J and H), synapomorphic for all other *Eurybia* species (Fig. 6). The second earliest diverging species of the genus is *E. eryngiifolia* (Fig. 7); it has the longest branch. Contrary to the nrDNA topology (Selliah & Brouillet 2008), *E. sibirica* is not sister to *E. integrifolia,* but diverges next and is sister to all remaining *Eurybia* species, as was hypothesized by Selliah & Brouillet (2008).

The concatenated cpDNA dataset added few but valuable phylogenetic regions to the unique indels of the nrDNA dataset. The chloroplastic regions provided each eurybioid clade with at least one synapomorphic indel. The cpDNA topology (Fig. 6) offers little infrageneric resolution, except within *Herrickia* and among some *Eurybia* species. In this topology, however, *E. integrifolia* does not cluster with *Eurybia* but forms a polytomy with *Herrickia* s.str., *H. kingii*, *Triniteurybia*, and the Machaerantherinae (node 2 in Fig. 6). Contrary to nrDNA analyses (Brouillet et al. 2004, Selliah & Brouillet 2008), *Oreostemma* groups with the Symphyotrichinae (node 1 in Fig.6) and the Machaerantherinae are sister to *Herrickia* s.str. (node 4 in Fig.6), rather than *Triniteurybia*. Within *Eurybia*, the cpDNA reconstruction indicates a close relationship between *E. schreberi*, *E. jonesiae* and *E. mirabilis*, as reported by Lamboy et al. (1991) based on phenetic and isosyme evidences. Such a close connection already had been noted using ITS (Selliah & Brouillet 2008). This may support the hypothesis that the hexaploid *E. jonesiae* arose from the diploid *E. mirabilis* (Lamboy et al. 1991) as one of its parents. Contrary to the hypothesis latter authors, *E. schreberi* does not appear to group with *E. divaricata* but with *E. mirabilis* and *E. jonesiae*. All these species are members of section *Biotia* (Nesom 1991; Semple 2005). The topology also infers a close relationship between *E. avita* and *E. surculosa*, a tetraploid eastern species, and would support the affinities previously hypothesized by Kral (1983) based on comparisons of leaves and rootstocks morphology. Such a relationship appears unexpected based on overall morphology, given that the two species were classified in different subgenera respectively *Heleastrum* (in section *Heleastrum*) and *Eurybia* (in section *Calliastrum*) by Nesom (1994) and Semple (2005). Despite a lack of distinguishing morphological features with respect to other eastern narrow-leaved species, these two

species share geographical and ecological features: both are known from Georgia, South Carolina, and North Carolina, and require open, sandy or granitic substrates.

The relationships suggested by the combined topology are similar to those provided by the nrDNA topology of Selliah & Brouillet (2008), with a few exceptions. Nearly all relationships in the combined tree (Fig. 7) are resolved and strongly supported, particularly the positions of *Oreostemma, Herrickia* s. str., and *Eurybia* species such as *E. integrifolia*, *E. eryngiifolia*, and *E. sibirica*. The taxonomic affinities of *Herrickia kingii* have long been debated (Cronquist & Keck 1957, Nesom 1991, Cronquist 1994, Brouillet et al. 2004, Robinson and Nesom 2007, Selliah & Brouillet 2008). The combined phylogeny shows a segregation of *Herrickia* (sensu Brouillet et al. 2004) into two distinct clades, *Herrickia* s. str. and *Herrickia kingii*, a highly supported segregation suggested earlier by Selliah & Brouillet (2008). In our tree (Fig. 7) *H. kingii* is sister to the *Eurybia*-*Triniteurybia*-Machaerantherinae clade. It may deserve the rank of genus. Further morphological and molecular studies are needed before a firm decision can be made.

Within the combined phylogeny, the *Eurybia* species no longer form two subdivisions (Selliah & Brouillet 2008) but rather constitute a grade. Adding the cpDNA to the nrDNA dataset helped elucidate relationships among species of *Eurybia*, particularly concerning the early diverging members of the genus, which were difficult to interpret in the nrDNA topology (Selliah & Brouillet 2008). Indeed, *E. integrifolia*, *E. eryngiifolia*, and *E. sibirica*, the earliest diverging members of the clade, do not segregate as a distinct subclade, and *E. integrifolia* and *E. sibirica* do not form a clade. Here, *E. integrifolia* is confirmed as the earliest diverging species of *Eurybia.* This relationship is supported by

two independent nucleotide substitutions in *trn*C-*ycf6*. Both events appear to have occurred after the divergence of *E. integrifolia* (Fig. 7).

The *Eurybia* subgenera and sections proposed in the classifications of Nesom (1994) and Semple (2005) are not supported by topologies in the present study (Fig. 7). For instance, according to these authors, *E. hemispherica* and *E. avita* belongs to subgenus *Heleastrum* section *Heleastrum*, while *E. mirabilis* and *E. compacta* are classified in subgenus *Eurybia* section *Calliastrum*, respectively [chapter 1]. The combined analysis shows a close affinity between all these species. This would support Brouillet's (2006*b*) questioning of the taxonomic value of the subgenera and sections proposed by Nesom (1994) and Semple (2005).

Biogeography

In the past, biogeographic studies of major NA discontinuous ranges mainly involved intercontinental range disjunctions (as described in Thorne 1972; see for instance in Coleman et al. 2003, Nie et al. 2006, Spalik & Downie 2007, Guzman & Vargas 2009); few specifically addressed intracontinental disjunctions. The taxonomic groups treated were either intergeneric or infraspecific level (Wood 1971, Fontanella et al. 2007), the taxa treated were considered as Arcto- Tertiary relict origin (Wood 1971, Huck et al. 1989) nor instead of considering the whole range only a geographic portion of the latter was mainly invoked (e.g., Lewis & Crawford 1995). With the exception of Wood (1971; and references therein: McVaugh 1943, Sharp 1951, Braun 1955), few closely related species have been documented with similar ranges in recent works (*Packera,* Senecioneae; Bain &

Golden 2000; *Cirsium scariosum*, Golden et al. 2008). Although several western-eastern NA disjunctions have been noted within the NA Astereae (e.g., *Eucephalus*- *Doellingeria*, Brouillet et al. 2001; *Ionactis*, Nesom 2006*b*; *Sericocarpus*; Brouillet et al. 2009), no biogeographical works have been undertaken. Thus, the Southwestern and Southeastern North America range disjunction is poorly understood (Wood 1971, Thorne 1972).

During the Pliocene-Pleistocene age, major geologic events considerably affected the development of the NA vegetation and flora (Pielou 1991, Hewitt 1993, Hewitt 1996, Comes & Kadereit 1998, Cox & Moore 2000, Knowles 2001, Winkworth et al. 2005). The series of glacial-interglacial cycles of the Pleistocene had a significant impact on environmental and climatic changes which shaped the distribution of the Northern Hemisphere temperate regions. Indeed, the advance and retreat of glacial ice sheets with others climatic changes have influenced biodiversity dynamics such as migration, diversification, extinction and isolation of taxa into refugia (Hewitt 1996, Avise et al. 1998, Starkey et al. 2003, Hoffman & Blouin 2004). This is the historical environment in which the eurybioids evolved.

Brouillet et al. (2009) showed that the NA clade (Noyes and Rieseberg 1999) of the Astereae is sister to South American clades, all well nested within the crown Astereae. The relatively short branches within the NA clade would indicate that it is recently derived and that it underwent an explosive radiation after reaching North America. Arrival of the NA clade in North America is most likely to have been late Miocene or early Pliocene, though solid dating data are lacking for dating it. Nonetheless, this provides a timeframe in which to interpret the biogeographic history of the eurybioids, which was influenced strongly by

late Pliocene and Pleistocene climatic changes and orogeny, as hypothesized for other NA groups (e.g., Wood 1971, Noyes 2000, Drovetski 2003). Current molecular evidence concerning the eurybioids, including the limited variability of both nrDNA and cpDNA regions, as well as incomplete lineage sorting in the low-copy nuclear gene CNGC4 [chapter 3], also would support recent divergence of the group (Winkworth et al. 2005), the latest speciation events nonetheless most likely predating the end of the Wisconsinan glaciation (18, 000 yr B.P.).

Eurybioids

The biogeographic framework resulting from both methods of analysis, DIVA and LaGrange, appears to indicate that the common ancestor of the eurybioids originated in western North America (Fig. 8, node 1). The most probable ancestral areas suggested by ML (Table 4) is 'AB'; the outgroup is also distributed over both areas. It is not possible, however, to determine whether one of the two western areas, A or B, may have been the area of origin for the group. Western North America is considered to have been an important center of origin for numerous taxa (e.g., Stebbins 1952; Axelrod, 1958; Marlowe & Hufford, 2007). This common ancestor gave rise to two sister lineages (Fig. 8, node 2): *Oreostemma* in the Cascade-Sierra Nevada ranges, the second in the Intermountain Region and Rocky Mountains, where it eventually diverged into *Herrickia* s. str., *Herrickia kingii*, *Triniteurybia*, *Eurybia*, and subtribe Machaerantherinae (Fig. 9). At the probable time of the split, in the Plio-Pleistocene, uplifting was occurring throughout mountainous western North America (see below). Uplift also caused drier conditions to the east of ranges, and

the Great Basin became rapidly dominated by grass and herbaceous species and the midcontinent by prairies (Axelrod 1958, Tidwell et al. 1972). As habitats became drier, grasslands became more widespread (Graham 1993) and created a barrier between western and eastern NA biotas. At the same time, the mesic Californian forests and woodlands became confined to coastal or mountain areas, while lowlands became dominated by grasslands and shrubs (Axelrod, 1959) before developing into deserts. The conjunction of mountain uplift and lowland drying may have contributed to the split in the range of the eurybioid ancestor.

Oreostemma

Oreostemma occupies humid to mesic habitats of the Sierra Nevada-Cascades. The phylogeny shows two branches (Fig. 8, node 16): *O. elatum* at relatively low elevations, and *O. alpigenum* and *O. peirsonii* at higher ones (Nesom 2006*a*). One interpretation would be that adaptation to higher altitudes coincided with the episode of mountain uplift and erosion that occurred during the Plio-Pleistocene in the Sierra Nevada-Cascades Mountains (Axelrod 1958, Tidwell et al. 1972, Raven & Axelrod 1978, Brouillet & Whetstone 1993, Delcourt & Delcourt 1993; Graham 1993), a period of great disruption for western floras. Mitchell et al. (1966) likewise documented the migration of alpine plant species from neighbouring areas into the California-Nevada alpine vegetation zone only after alpine habitats became available during the Pleistocene. The varieties of *O. alpigenum* may have spread to the northern Rocky Mountains region during subsequent glacial phases of the Pleistocene.

The ancestral range of the remaining eurybioids appears to be the Intermountain Region and Rocky Mountains (node 3, 4, 5, 6 and 17, Fig.8 and Table 4), an area where islands of mesic or semi-xeric habitats, in the form of disjunct mountain ranges, are scattered within a more xeric landscape. The evolution of the group was therefore influenced by the vertical and horizontal displacements that resulted from the environmental fluctuations that occurred during glacial-interglacial episodes in the region (Axelrod 1959, Mitchell 1973, Knowles 2001, Winkworth et al. 2005). Such displacements were also used by Critchfield (1984) to account for the genetic diversity of NA boreal and temperate conifer populations during the glacial -interglacial cycles. The current, broad mountain distribution of *Oreostemma alpigenum*, *Herrickia glauca*, and *Eurybia integrifolia* is likely a consequence of such movements, as was reported for many plant species (Wolfe 1987, Huntley & Webb 1989, Pielou 1991, Elias 1996). Latitudinal and altitudinal zonation shifts of perennial plants communities in the western United States during the last full-glacial period is well documented (Mitchell 1973, Van Devender et al. 1987, Thompson 1988, Wells 1983 cited in Delcourt and Delcourt 1993, Thorne 1993). Notably, Spaulding et al. (1983) recorded a shift in elevational ranges of southwestern NA plants on calcareous substrates. We assume that, during the Pleistocene, the mesic, mid to high altitude taxa were subject to such vertical shifts in elevation, along with their whole plant communities. Moreover, the montane distribution of eurybioids in the area means that range fragmentation or endemism (local restriction) occurred during drier, warmer interglacials, and that populations were restricted to higher, smaller habitats, subjecting species to range contractions. During cooler, wetter glacial episodes, populations move down the mountains, expanding into wider areas toward their bases and sometimes being

able to migrate among ranges, depending upon the suitability of habitats in lowlands. This may have promoted range expansion.

Herrickia s.str., H. kingii* and *Triniteurybia

The second earliest diverging genus is *Herrickia* s.str. Peripheral to the Great Basin, it usually grows at the edges of semi-xeric to mesic woodlands or in open plant communities, at elevations ranging from 800 to 3700 m. The genus is relatively widespread in the eastern Intermountain and adjacent Rockies and populations are often disjunct between mountain ranges. Radiocarbon-dated deposits of plant communities from the Grand Canyon in northern Arizona similar to those in which *Herrickia* s.str. species are found today, showed an elevational shift upward during the last 24,000 years (Delcourt & Delcourt 1993). The next diverging lineage is *Herrickia kingii*, which is restricted to canyons and ridges of the Wasatch and Canyon mountains in Utah, at elevations of 1700-3300 m. Finally, the monotypic *Triniteurybia* inhabits granite cliffs and drier coniferous forests of the Sawtooth and Bitterroot Mountains of Idaho and Montana, at 1300- 2500 m. The biogeographic analyses show that all the earliest diverging lineages are from the Intermountain and Rockies Region (B), which implies that Pleistocene glaciations may have played a key role on the distribution, both horizontal and vertical, of the species, as with the previous genus, and it probably also contributed to speciation within *Herrickia* s. str.

Eurybia

Eurybia is the only genus of the group that comprises both western and eastern NA diploid members: there are three western (*E. integrifolia*, *E. sibirica*, *E. radulina*) and eight eastern (*E. eryngiifolia*, *E. furcata*, *E. divaricata*, *E. radula*, *E. mirabilis*, *E. hemispherica*, *E. avita* and *E. compacta*) species (Fig. 9). Apart from *E. eryngiifolia*, all western species originated before eastern species in the tree (Fig. 8).

Eurybia integrifolia

The earliest diverging species, *Eurybia integrifolia*, currently occupies mesic to wet habitats at elevations of 1600 to 3200 m in the western mountains and is relatively widespread. Both biogeographic reconstructions (Fig. 8) indicate that the ancestral distribution area of both the species and the genus was in the Rockies and Intermountains (B, node 6). This reconstruction suggests that the presence of this species in the Sierra Nevada and Cascades Ranges may be due to secondary expansion into that region during the Pleistocene.

Eurybia eryngiifolia

The next diverging member of *Eurybia* is the eastern *E. eryngiifolia*, which is found in the Florida Panhandle and southern Georgia and Alabama within the Gulf part of the Atlantic and Gulf Coastal Plain floristic province (Fig. 9). The species has a distinctive morphology, adapted to the fire-prone, periodically wet or poorly drained, acidic, sandy areas of pine flatwoods and barrens (UNC herbarium database, http://www.herbarium.unc.edu/seflora/firstviewer.htm, last modified: 27 Aug. 2007). The

Appalachicola Region of the Florida Panhandle, where the species resides, is one of six centers of endemism in the southeastern United States (Estill & Cruzan 2001). As with many herbaceous communities of the Florida flatwood vegetation (Wunderlin & Hansen 2000, Sorries & Weakley 2006), frequent fires may be important for the maintenance of *E. eryngiifolia* (UNC herbarium database, Beckage et al. 2006). Biogeographically, the common ancestral distribution with the highest probability for *E. eryngiifolia* was ' C|ABD' (0.77, Table 4; Fig. 8, node 7). This hypothesis is not retained, however, because there is no parsimonious explanation for the inclusion of the eastern deciduous forest (D) within the ancestral range of this taxon. It is more parsimonious to retain the hypothetical range with the second highest probability ('C|AB', Fig. 8; 0.11, Table 4), which would have involved a direct migration of the *E. eryngiifolia* ancestor from western North America to the Gulf Coastal Plain, either via long distance dispersal (Sorrie & Weakley 2001) or through corridors in the south of North America, most likely during a glacial period (see below). This strongly supports the hypothesis of an early, isolated migration of the genus into eastern North America (Selliah & Brouillet 2008). Our topology, and the resulting biogeographic hypothesis, would suggest a relatively early dispersal event to the east from western North America and Pleistocene survival of *E. eryngiifolia* in the region. The flora of the Gulf and Atlantic Coastal Plain Provinces is relatively young and results from migrations from various directions (Sorrie & Weakley 2006), though certain areas contain older elements, like the Florida Panhandle, one of the floristically richest glacial refugia of the coastal plain (Estill & Cruzan 2001, Soltis et al. 2006). This area was not affected by the repeated Pleistocene erosions and inundations cycles (Thorne 1993, Sorrie & Weakley

2001). In addition, this species is present in Pike County, Alabama, the location of Goshen Springs, one of three long -recognised southeastern refugia (Delcourt et al. 1980).

Eurybia sibirica

The next species to diverge (Fig. 7) is the western *E. sibirica*, the most northern, geographically widespread, and morphologically variable member of the genus. It is encountered in the northern Rocky Mountains and ranges northwards to Alaska and the Northwest Territories, and from there westward into Eurasia to northern Europe. It grows mostly in open, sandy or gravelly areas along rivers and in mountain meadows. Our analysis determined that the ancestral areas of this species were the Intermountain region and Rocky Mountains (B, node 8). The part of the range north of the NA southern glacial limit (Canada, Alaska and Eurasia) was not taken into account in the biogeographic analysis since it was not critical to understanding biogeographic history among the diploid species of the genus. Nonetheless, we cannot rule out the possibility of an Alaskan origin for the species, in the case where its ancestor had moved there during one of the earlier interglacial episodes. The current range over glaciated territory in North America was acquired during the Holocene. The westward migration through the Bering land bridge must have occurred during a glacial period (Wisconsinan or older) when sea levels were lower, as was noted for *Erigeron* (Astereae subtribe Conyzinae; Noyes 2000). This also implies that migration to Alaska, largely unglaciated during the Pleistocene (Brouillet & Whetstone 1993, Mann & Hamilton 1995), would have occurred at the latest during the interglacial that preceded the Wisconsin glaciation, even if the species did not originate

there. The lack of datation in our biogeographic analyses prevents us from choosing between alternate hypotheses. A phylogeographic study of this species would be required to resolve its biogeographic and evolutionary Pleistocene history.

Eurybia radulina

The fourth species to diverge in the phylogeny (Fig.7) is *Eurybia radulina*, which inhabits the Coastal Ranges (southern Vancouver Island to California) and the central Sierra Nevada, down to the chaparral of Orange County, California. The ancestral range suggested is 'A|D' (0.89), where 'A' corresponds to the Pacific western United States; it is the westernmost (at temperate latitudes) and last diploid western member of the genus. The ancestor of this species also gave rise to all remaining eastern NA species 'D' (Fig. 8), the second migration eastward for the genus.

An initial survey by Wood (1971) revealed frequent floristic relationships between eastern and western North America at the levels of genera, species or varieties. In particular, a significant number of southern Appalachian taxa have at least one disjunct member in western North America. Due to the climatic fluctuations during the Pliocene and Quaternary, floras migrated thousands of kilometers to track their ecological niche (Grichuk 1984, Delcourt & Delcourt 1993). Such migrations may have occurred along corridors or by long distance dispersal. Long distance dispersal played a major role in the colonization of new lands; it has been well documented and frequently hypothesized as a scenario to explain range expansion in numerous tribes of the Asteraceae, notably the Vernonieae (Keeley et al. 2007) and Astereae (Brouillet et al. 2009, Liu et al. 2002, Eastwood et al. 2004). We are hypothesizing that the common ancestor of the eastern clade

arrived in the east by a relatively more northern path compared to *E. eryngiifolia*, this dispersal probably having occurred before the Wisconsinan glaciation. If migration occurred during a glacial episode, the prairie vegetation of the Great Plains, a major barrier during interglacials, would have been greatly reduced. Hence, migration of the common ancestor of the eastern *Eurybia* species would have been facilitated and may have occurred either by a direct passage or through shorter long distance dispersal. The possibility that a long distance dispersal event occurred during an interglacial, when conditions were much less favorable since distances from the western source to the eastern forests would have been greater, and a chance dispersal event less probable, cannot be ruled out. The eurybioids have propagules that lend themselves well to long-distance dispersal by anemochory. *Eurybia* cypselae are relatively small (1.7-4.7 mm) with pappus bristles measuring 2.7 to 8.2 mm (Brouillet 2006*b*). Westerlies currently predominate at temperate latitudes and similar conditions may have prevailed during the Quaternary, which would have favored an eastward anemochorous dispersal. When we tested models concerning the probability of dispersal in LaGrange, the model favored was that of lowest dispersal probability (0.01), implying that dispersal events were indeed rare. This result is consistent with our observation of the dispersal of only two lineages into eastern North America (Fig. 8). As for the number of dispersal event estimated by DIVA (i.e., 9), the latter seems to be unrealistic and overestimated. First, it is difficult to evaluate and distinguish the 'true' dispersal from the gradual vicariance because compared to most of the biogeographic studies (i.e., intercontinental disjunction), this present study imply an intracontinental disjunction. According to the results, there is strong evidence of two dispersal events. We may interpret the remaining dispersal events suggested by DIVA as vicariance events. For

instance, a continuing proximal dispersal due to western climatic instability followed by a physical barrier establishment which may modify a taxa distribution and lead to an isolation of the latter.

Eurybia furcata, E. divaricata* and *E. radula

Once in eastern North America, *Eurybia* underwent a rapid radiation that gave birth to seven diploid species (Fig. 8). The first three diverging taxa are successively *E. furcata*, *E. divaricata*, and *E. radula*. Globally, the reconstruction (Fig. 8) proposes the ancestral area as the eastern deciduous forest 'D' (1.0 relative probability, data not shown). However in the finer analysis, the ancestral distribution for *E. furcata* corresponds to the Appalachian Mountains 'E' (node 10, Fig. 8; Table 4; Fig. 9) and for the latter two species the area is the Atlantic coastal plain with respectively 0.37 (node 11) and 0.09 relative probabilities (node 12). This is consistent with the hypothesis of Braun (1955) that the coastal plain plants may have arisen in the Appalachian uplands. *Eurybia furcata* is a Midwest endemic that grows in relatively open, limestone habitats, such as north-facing slopes, seepy bluffs and deciduous woods, particularly along streams (Brouillet 2006*b*). *Eurybia divaricata* is widespread in the eastern deciduous forest. *Eurybia radula* is distributed along the Appalachian range (Fig. 9) and extends northward to Labrador and James Bay in open or wet areas such as fens, wet meadows, and along streams. The latter speciation may have resulted from an ecological shift from mesic forests to the edges of relatively open wetlands, accompanied by a shift in morphology from cordate to lanceolate leaves (Selliah & Brouillet 2008).

Eurybia mirabilis, E. avita* and *E. hemispherica

The remaining four diploid species are all located east of the Appalachians, on the Piedmont and the Atlantic coastal plains, the most probable location of their common ancestor range (D, Fig. 8; Fig. 9). The short branch lengths of the members of this clade in the phylogram (Fig. 7) implies a rapid radiation in the area, most likely from a Piedmont common ancestor shared between the cordate-leaved *E. mirabilis* and the narrow-leaved species. *Eurybia mirabilis* is endemic to the lower Piedmont Plateau (Fig. 8 node 13) of North and South Carolina. It occupies the edges of deciduous or mixed deciduous forests, notably moist stream bluffs and slopes. There are three diploid, narrow- leaved species: *E. compacta*, *E. avita* and *E. hemispherica*, and their ancestral range is located on or around the Gulf coastal plain with 0.31 and 0.32 (node 14 and 15, Fig. 8; Table 4; Fig. 9). As for *E. compacta*, it is known from seasonally dry, frequently disturbed, acid areas such as sandy soils, pine savannas, bogs and barrens of the Atlantic coastal plains and outer Piedmont. *Eurybia hemispherica* range is in the Atlantic Coastal Plain in open, dry to mesic areas such as bottomlands, prairies, pastures, and roadsides. It expanded into similar habitats in the Gulf coastal plain and southern Appalachian areas. *Eurybia avita* grows on shallow sandy soils at the edges of granite flatrock outcrops of Georgia and Carolina. This species lives within the southern Appalachian, a center of endemism (Estill & Cruzan 2001). Speciation of the narrow-leaved species occurred within specific habitats. For instance, *E. avita* grows

only on granite flatrocks, possibly as a consequence of periodical Pleistocene glaciations and inundations, as was observed for other taxa (Leblond 2001). The distribution and the habitat requirements of *E. compacta* and *E. mirabilis*, species endemic to North and South Carolina and adjacent regions, also appear to be correlated to the Pleistocene constraints. These two species are recent arrivals to the Cape Fear Arch region, uplifted between 5-3.5 myr B.P. and 85 000 yr B.P. (Colquhoun et al. 1981). According to Leblond (2001), the current range of plant endemic (or nearly endemic) to the Arch region, suggest that it may have constituted a refugia and a speciation center during the most recent glacial period.

Conclusion

This study is the first published that tests the utility of cpDNA makers both within the eurybioids and within the NA Astereae clade (Noyes and Rieseberg 1999). The cpDNA variability encountered in the study, even though limited, is greatly due to the presence of indels. The combined datasets tree reconstruction (Fig. 7) resulted in better resolution and supports than the analyses of the separated datasets. The limited utility of cpDNA regions at the infrageneric level within NA Astereae emphasizes the difficulty of finding suitable phylogenetic characters for the whole tribe. The results of this study contradict previous classifications (Nesom 1994 and Semple 2005). Future work should study cpDNA intergeneric spacers, potentially known to be more variable at low taxonomic levels than those tried here, as proposed by Shaw et al. (2007) and Timme et al. (2007). Even though the eurybioids are widely distributed on both sides of the continent, the poor divergence between sequences among closely related species suggest that these latter may have undergone a recent diversification with a rapid distribution. The grade seems to have arisen in the western North America around the Plio-Pleistocene. Among them, *Eurybia* seems to have encountered two independent dispersal events across the east side of the continent; the first in the southeastern coastal plain and the second in the eastern deciduous forest then, in to the coastal plain.

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Chapitre 3

Insights into the evolutionary relationships of the North American genera *Oreostemma*, *Herrickia*, *Eurybia* and *Triniteurybia* (Asteraceae, Astereae) based on a low-copy nuclear DNA region, CNGC4

Sugirthini Selliah and Luc Brouillet

Ce chapitre présente les résultats et certaines conclusions préliminaires obtenus lors de l'évaluation de l'utilité phylogénétique de régions à faible nombres de copies au sein des eurybioids, particulièrement chez le genre *Eurybia*. Les données de séquences manquantes pour certaines espèces seront complétées avant une éventuelle soumission à une revue scientifique (non-déterminée) pour publication.

Insights into the evolutionary relationships of the North American genera *Herrickia*, *Eurybia* and *Triniteurybia* (Asteraceae, Astereae) based on a low-copy nuclear DNA region, CNGC4

Abstract

The eurybioid grade is paraphyletic to subtribe Machaerantherinae, and is sister group to subtribe Symphyotrichinae. Previous works showed that the combined nuclear ribosomal and chloroplastic DNA topology provided phylogenetic resolution and strong support at the generic level within the grade. However, resolution at the species level is still limited, particularly among the diploid and polyploid members of *Eurybia*, the core genus. Attempts at inferring relationships within this genus based on nuclear ribosomal and chloroplastic DNA regions proved to be ineffective when polyploid species were included. Therefore previous analyses were restricted to diploid taxa. Low-copy nuclear loci seem suitable candidates for resolving low-level phylogenetic relationships. After examining the phylogenetic utility of several low-copy nuclear regions within the eurybioids, we are presenting preliminary results on the most promising region, CNGC4. Part of this region was directly sequenced or cloned and sequenced for nearly all *Eurybia* species. Alignment and preliminary analyses showed two distinct types of sequences for the region, presumably paralogous. A single type was retained for further analysis. The sequences were partitioned into exons and intron and analyzed using Bayesian and maximum likelihood approaches. Despite recovering a few phylogenetic relationships, the CNGC4 topologies generally resulted in unresolved relationships and low support values at the

infrageneric level and, in some cases, clones from a single individual did not group. Topologies exhibited a high degree of incomplete lineage sorting, which supports the hypothesis that the group is of recent origin.

Introduction

Our previous work (Selliah & Brouillet 2008) indicated that the nuclear ribosomal (nr) DNA regions provide limited phylogenetic resolution within the eurybioids, particularly among species. The inference of relationships was particularly problematic when polyploid species of genus *Eurybia* were included. Species of the latter tended to cluster into two groups, with diploid species in one and polyploids in the other, without support from morphology for the groupings. With few exceptions, cloning of ITS failed to retrace the parents of polyploids, and analysis of clones showed the same segregation into two distinct clades as direct sequences had. A phylogenetic study of three chloroplastic (cp) DNA regions (chapter 2), both separate or concatenated, was unable to resolve relationships among the eurybioid taxa because of insufficient variability, even though some of these regions are known to be usually fast-evolving (Demeure et al. 1996, Chiang et al. 1998, Shaw et al. 2005, Shaw et al. 2007, Mort et al. 2004). Within *Eurybia*, the core genus of the group, nearly all species fell into a vast polytomy, preventing the identification of maternal parents. Furthermore, the cpDNA genome is insufficient for retracing the parentage of the polyploids since it recovers only the maternal history of the grade. Combining nrDNA and cpDNA sequences provided higher resolution and support at the generic level within the grade, but resolution at the species level remained limited, particularly among the diploid and polyploid members of *Eurybia*. Loci of the nuclear genome may provide greater among-species phylogenetic resolution (Small et al. 2004) and might prove more useful in detecting reticulations (Sang 2002, Mort & Crawford 2004). Low-copy nuclear loci are attractive in this respect because of their biparental inheritance, low number of copies when

compared to nrDNA, and generally greater evolutionary rates than plastid or ribosomal regions (Clegg et al.1991, Choi et al. 2006). Therefore, we examined the potential phylogenetic utility of low-copy nuclear regions to help resolve relationships among eurybioid species.

After an initial study of several low-copy nuclear loci, the most promising proved to be the low-copy nuclear gene Cyclic Nucleotide Gated Channel-like protein (CNGC4, Choi et al. 2006). It was first identified during a comparative genomic mapping study of two *Medicago* (Fabaceae) species (Choi et al. 2004). Included within the nuclear CNGC family, this gene is apparently involved in plant defense responses (Clough et al. 2000). Except for a few phylogenetic studies within the Fabaceae (Scherson et al. 2005, Choi et al. 2004, 2006; McMahon 2005), the utility of this marker has not been evaluated. These studies reported that the CNGC4 region was potentially useful from the tribal to the species levels and was evidentally a single copy gene. The purpose of this paper is to evaluate the phylogenetic utility of CNGC4 to study phylogenetic relationships within *Eurybia* at the species level, in order to retrace the origin and parentage of polyploids and to confirm the phylogeny provided by nrDNA and cpDNA within eurybioids.

We expected that using several unlinked loci from the plastid and nuclear genomes may lead to an accurate interpretation of the phylogenetic history of the group. To do so, we compare the topologies obtained with CNGC4 to those based on nrDNA and cpDNA (Selliah & Brouillet 2008, chapter 2). This chapter presents preliminary results and an early conclusion of the study, as more extensive sampling will be needed before we are able to draw firm conclusion.

Materials and methods

Taxon sampling

Since this is a preliminary work and is focused on *Eurybia*, sampling includes more species from this genus and only a few representatives of other genera. It comprises nine out of eleven diploid species, and seven out of eleven polyploid species of *Eurybia*. *Herrickia horrida* and *Triniteurybia aberrans* were included as representatives of other genera of the eurybioids. Three *Symphyotrichum* species were included as an immediate outgroup, and as external outgroup, *Chlorocantha spinosa* was used. Each species was represented by a single sample. All nuclear sequence accession numbers were deposited in Genbank (Appendix 4).

DNA extraction

See chapter 2.

Choice of the low-copy nuclear makers

Prior to considering CNGC4, we screened and tested nine low-copy nuclear genes for this study: Glyceraldehyde-3-phophate-dehydrogenase (G3PHD; Howarth & Baum 2005), Malate Synthase (MS; Lewis & Doyle 2001), Phosphastase1 (PP1; Choi et al. 2004), Ferredoxin-NADP reductase precursor (FENR; Scherson et al. 2005), Triose phosphate isomerase (Tpi; Zhang & Chinnappa 1994), *myo*-Inositol-1-phosphate synthase (MIPS;

Majumder et al., 2003), Nitrate reductase (NIA; Howarth & Baum 2002), and Phosphoribulokinase (PRK; Petersen et al. 2006). They proved unsuitable for diverse reasons: insufficient sequence variation (PP1), amplification and sequencing difficulties (G3PDH, MS, NIA, PRK, Tpi), or possible multiple copies (FENR, MIPS).

PCR amplification and sequencing

Amplification of the partial exons five and six with the complete intervening intron of the nuclear gene CNGC4 was carried out using primers redesigned from the original sets of Scherson et al. (2005). In order to obtain these specific primers, we aligned and compared few representative sequences of *Eurybia* which were sequenced with the original primers, with sequence-tag site (STS) of species suggested by Choi et al. (2004) using BioEdit 7.0.5.3 (Hall 1999). Then, the new primers were optimized for the eurybioids using Amplify v.1.2 (Genetics Dep., Wisconsin, Madison). The new primers are CNGC4-Rs (AACTATGGATGTCTCATCGC) and CNGC4-Fs (TCGTCCACTGGATCTCCYTCT). Direct sequencing often resulted in unclear and ambiguous (overlapped or unreadable) readings in the poly-T region of the intron. In such cases, we cloned the amplicon as described in Selliah & Brouillet (2008). Within *Eurybia*, all polyploids and seven out of 11 diploid species were cloned; two diploid direct sequences were also included in the matrix.

For detail on PCR amplification reaction mix and sequencing protocols, refer to chapter 2. The amplification conditions for CNGC4 were identical to those for the chloroplastic regions (chapter 2) except that the annealing temperature was 50°C for 40 cycles. Due to amplification difficulties, six *Eurybia* species were excluded from the present study, two diploids, *E. divaricata* and *E. sibirica*, and four polyploids, *E. spinulosa*,

E. chlorolepis, *E. merita*, and *E. jonesiae*. Three to 11 different clones were sequenced for each polyploid individual.

Phylogenetic analyses

For sequence editing and assembling, see chapter 2.

Within the CNGC4 alignment, a poly-T region between 5 to 25 bp in length was removed from the intron since it could not be aligned properly. A few sequences were eliminated: identical sequences, sequences with stop codons (as detected after translation into amino acids), and sequences that had two or more different amino acids in order to minimize the possible inclusion of paralogous sequences. In the CNGC4 alignment, six indels were detected, all within the intron; they were coded and treated as in chapter 2.

The regions were partitioned into exons and intron. After partitioning, a sequence evolution model was determined. Introns of the cpDNA regions were incorporated with the intergenic spacer because of their lack of variation. To assess the best-fitting model of sequence evolution for each region, we used MrModeltest v2.0 (Nylander 2004) with the Akaike information criterion (AIC) for BI analysis. The results of selected models for BI test was (GTR+ G) for the intron and (K80+ I) and (HKY +I +G) for the first and second exons, respectively. Bayesian analysis was performed on a parallel version of MrBayes 3.1.2. (Huelsenbeck & Ronquist 2001) with two independent runs with 16 Markov chains. For each run, the tree were sampled every 1000 generations for a total of 10 million generations, resulting in 20,002 trees. The parameter estimation for each partition was made independent. To assess the convergence of runs, we used the programs AWTY (Wilgenbusch et al. 2004) and Tracer v1.4 (Rambaut & Drummond 2007), the first 2,000

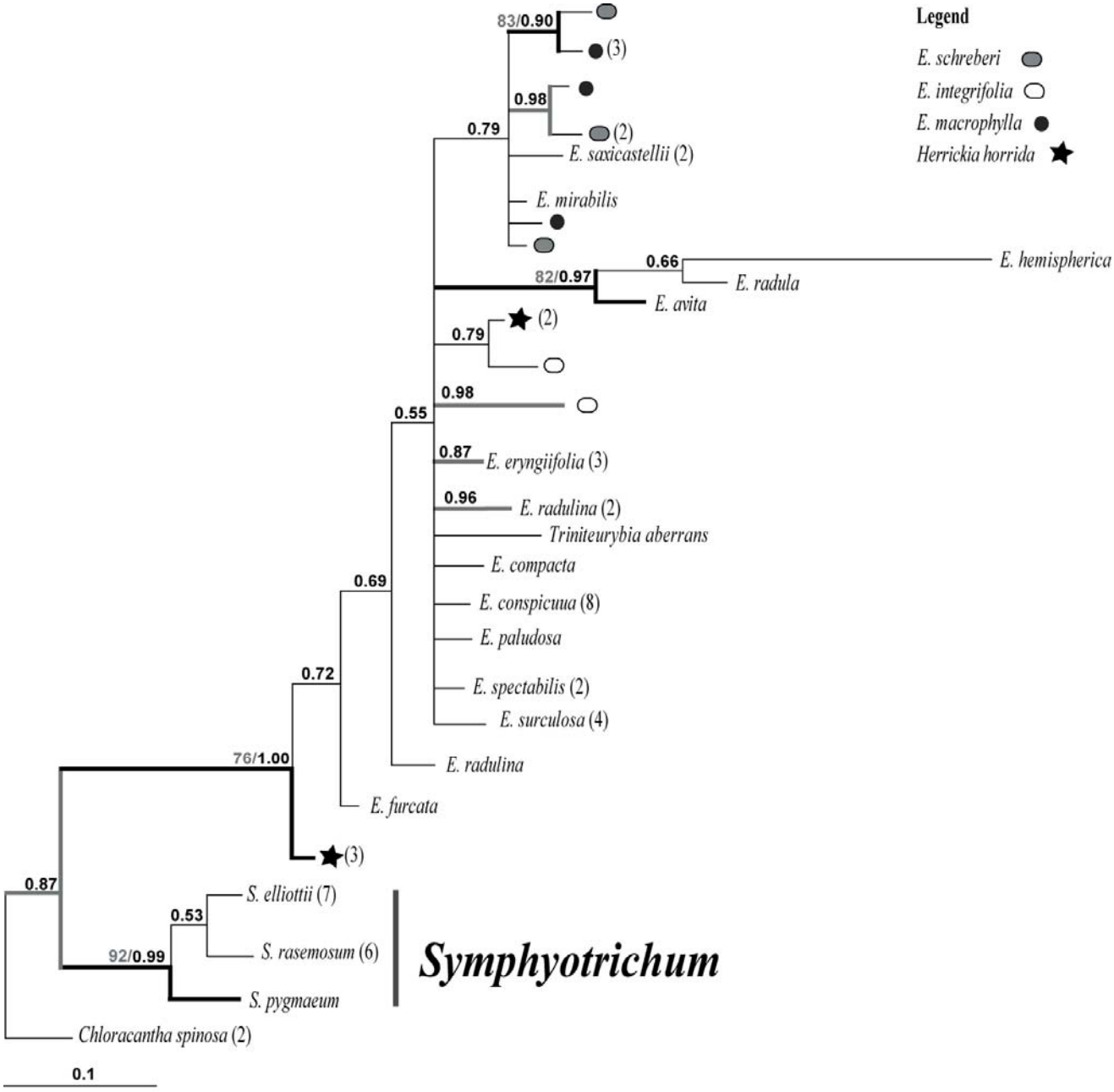
trees of each run in the Bayesian analyses corresponding to the burnin phase were removed and the remaining 18,002 trees were used to compute a 50% majority-rule consensus tree. A probability (PP) value ≥ 0.95 was considered evidence of strong clade support and ≥ 0.85 ≤ 0.94 as intermediate support.

The ML analysis was conducted using TREEFINDER v.June 2008 (Jobb 2008). The best-fit model estimation was determined using the 'proposed model' option of the program. The estimation of the proposed evolutionary model comprises three parts, two of which are optional: the substitution model (including rate and frequency parameters), the rate heterogeneity among sites (optional), and the number of rate categories (optional). For both partial exon parts, the substitution model was the HKY model with a listed rate and an 'empirical' frequency (HKY {3, 1, 1, 1, 1, 3}, Empirical). For the intron, the suggested model was J3 with an 'optimization' of the rate and 'empirical' frequency parameters; it included an 'optimal' heterogeneity model with the number of rate category corresponding to five (J3[Optimum, Empirical]: G[Optimum]: 5). To improve analyses, we generated and defined ten different starting trees prior to the global tree search. ML bootstrap analyses were performed with 500 replicates of the ten starting trees. A bootstrap (BS) value of ≥ 75% was considered statistically significant support for ML.

Results

Even though the CNGC4 intron has greater variability than both exons, the tree resulting from this region shows many unresolved relationships at the infrageneric level and in some cases, clones from a single individual do not group (Fig. 10). Support values in both analyses were low, the values for ML being the lowest (Fig. 10). Despite this low support, the phylogeny suggests that *Eurybia* is monophyletic and that *Triniteurybia* has an unresolved position within *Eurybia*. In contrast to this, *Symphyotrichum* is highly supported as monophyletic (MS = 92%; PP = 0.99) and appears sister to the eurybioids. *Herrickia horrida*, the sole representative of its genus, was represented by five clones: three are sister to *Eurybia* and *Triniteurybia* (MS =76%; PP = 1.00), and two groups with an *E. integrifolia* clone. Although support is low, *Eurybia furcata* and one of three clones of *E. radulina* are sisters to the large polytomy. Some clones of *E. macrophylla* cluster with those of *E. schreberi* (MS= 83%; PP=0.90), while the latter are grouped with *E. mirabilis* and *E. saxicastelli*. *Eurybia hemispherica*, has the longest branch and groups with *E. radula* and *E. avita* clones.

Figure 10. CNGC4 phylogram based on Bayesian rooted with *Chloracantha spinosa* (dash line) including posterior probability and bootstrap support values inferred by ML analysis analysis. Bold black branches represent significant support by ML (≥75%) and Bayesian (≥95%) analyses. Grey branches indicate support by only one of the two analyses. Number of different sequence clones for each species is noted in parentheses.



Discussion

Utility of low-copy nuclear markers

For CNGC4, our preliminary analysis showed that almost all individuals, whether diploid or polyploid, had sequences that clustered into two groups. We translated the exon regions into amino acids and aligned them against the complete reference sequence of *Arabidopsis thaliana* (GenBank accessions number: NM_124805). The alignment revealed two different sequence types, each characterized by two unique amino acids within the first exon: the first (here referred as clade 'A') had [...] KERVRR [...] NLPKDLRRD [...], and the second (clade 'B') [...] KEHVRR [...] NLPKYLRRD [...]. To ensure that these sequences were from the same CNGC family, we blasted both sequence types in GenBank: the two types proved more similar to each other than to other sequences available in GenBank. Among the latter, the CNGC4 sequences appeared to be the closest analogues.

In contrast to the studies of Choi et al. (2004) and Scherson et al. (2005), the eurybioids tended to possess two paralogous copies of the CNGC4-like gene. We have been unable to determine whether both are functional because only a short segment of the gene was sequenced. This segment includes two exons out of six and one intron out of five, for a total of 31% of the whole gene length. These copies may have arisen from a duplication event sometime before the origin of the eurybioids, the exact time yet undetermined. Alternately, even if this hypothesis is less plausible, the copies may represent different members of the CNGC gene family that were not present in the GenBank dataset. Even though extensive gene sampling is needed to support a hypothesis of genome or

chromosome segment duplication, as suggested for *Symphyotrichum* (Vaezi & Brouillet pers. comm.) and *Kalimeris* (Nishino & Morita 1994), we could not ignore such a possibility. Preliminary analyses of the separate copies, based on a reduced sampling of the clone sequences, revealed that sequence position appeared random within clade B and did not reflect relationships derived from previous morphological and molecular analyses. Relationships within clade A showed monophyly of the major genera. Based on these results, we included only clade A sequences in further analyses.

The little-resolved CNGC4 topology (Fig. 10) reflects the poor variability of the region in the study group. With the exception of a few species such as *E. eryngiifolia*, incomplete lineage sorting was clearly observed within the group and obscures our understanding of relationships within the eurybioids. For instance, alleles from several species (e.g., *H. horrida*, *E. radulina*, *E. integrifolia*, *E. macrophylla*, and *E. schreberi*) do not form monophyletic groups. This evidence supports the hypothesis of a recent and explosive radiation of the Astereae (Brouillet et al. 2009). This type of difficulty, added to allelic recombination and hybridization are some of the challenges that can be encountered when using the nuclear genome (Sang 2002).

Taxonomy of the eurybioids

A comparison of the relationships resulting from previous phylogenetic analyses (e.g., separate (ITS: Fig. 1. in Selliah & Brouillet 2008; cpDNA: Fig. 1. in chapter 2) and combined regions (nrDNA: Fig. 2. in Selliah & Brouillet 2008; nr+cpDNA, Fig. 2. in chapter 2)) with that obtained using low-copy nuclear data is crucial to resolve the eurybioids phylogeny. The major source of inconsistency among the three datasets involves

the position of *Eurybia integrifolia*, which was the earliest diverging lineage of *Eurybia* in the nrDNA tree but associated with *Herrickia* s. str. and the Machaerantherinae in the cpDNA and CNGC4 datasets. Moreover, the CNGC4 allele topology (Fig.10), uniquely grouped *E. integrifolia* and *H. horrida*, the only representative of its genus. Given that the eurybioids appear derivated, it is possible that the CNGC4 haplotypes have not been completely sorted, the presence of the CNGC4 ancestral polymorphism therefore obscuring phylogenetic relationships. This incomplete sorting phenomenon was also noticed with the cpDNA (chapter 2). This needs to be explored further before any conclusion can be reached.

Despite the limited variability of the cpDNA and nrDNA regions, and the presence of incomplete lineage sorting in CNGC4, the topology derived from the low-copy nuclear dataset (Fig. 10) suggests some phylogenetic relationships. A potential affinity between *E. schreberi*, *E. macrophylla* and *E. mirabilis*, partially recovered in the cpDNA topology, is indicated. This would support the Lamboy et al. (1991) hypothesis. This hypothesis could not be verified using ITS (Selliah & Brouillet 2008). The hexaploid *E. saxicastelli* seems to be associated with the species mentioned above. This species may be the 'Hypothetical Aster' evoked by Lamboy et al. (1991), which may have contributed, along with other species, to the origin of the octoploid *E. macrophylla*. The close relationship between *E. avita* and *E. hemispherica*, both of section *Heleastrum* in Nesom (1991) and Semple (2005), is confirmed, as it was with the nrDNA regions. However *E. radula*, included within section *Radulini* by Nesom (1994) and section *Calliastrum* by Semple (2005), appears to show an unpredicted affinity to members of section *Heleastrum*, characterized by their narrow leaves. Single allele of *Eurybia furcata* allele and *E. radulina* are sister to

the polytomy. A phylogenetic relationship between these two species was also noticed in the concatenated topology (Selliah & Brouillet 2008, Fig. 2). The monophyly of the *E. eryngiifolia* alleles, where several clones were represented by only one, would support its distinct position within the genus (Nesom 1991, Semple 2005). Most of the relationships mentioned above were consistent and support the finding based on ITS clone data (chapter 1) and some congruence with the cpDNA reconstruction (chapter 2).

Conclusion

The preliminary finding of this study was that the phylogenetic utility of the partiel CNGC4 sequence to resolve *Eurybia* relationships is limited. The presence of two paralogous copies in the preliminary alignment and analysis of the CNGC4 sequence region may have arisen from a duplication event prior to the eurybioids origin. Moreover, the topology of further analyses indicated a high level of incomplete lineage sorting, which would support the hypothesis of a recent origin of the group prior to rapid radiation.

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Conclusion générale

Présentement, certains systématiciens classifient les eurybioïdes parmi les groupes taxonomiquement méconnus (Nesom & Robinson 2007), d'autres encore se questionnent sur leur position phylogénétique exacte au sein des Astereae (Nesom 2009), sachant que *Oreostemma*, *Herrickia*, *Eurybia* et *Triniteurybia* sont proches parents et groupe-frère de la sous-tribu des Machaerantherinae (Brouillet et al. 2004). En plus d'inférer un scénario biogéographique, cette étude est parvenue à approfondir davantage l'histoire évolutive du groupe à l'aide de plusieurs types de régions : l'ADN ribosomal, l'ADN chloroplastique et l'ADN nucléaire à faible nombre de copies.

Le point sur les marqueurs

Parmi les types de régions phylogénétiques évalués, les régions ribosomales ITS et ETS (chapitre 1) étaient les plus variables, suivies des régions chloroplastiques (chapitre 2) et du gène nucléaire CNGC4 (chapitre 3). Les topologies résultant des analyses combinées sont mieux résolues et mieux supportées que les analyses séparées. Ainsi, la phylogénie résultant de la combinaison des données chloroplastiques et ribosomales offre la meilleure résolution aux niveaux inter -et infragénériques au sein du groupe (chapitre 2). Bien que la variabilité des régions chloroplastiques soit plus limitée que celle des régions ribosomales, la topologie obtenue à partir de *trn*L-F, *trn*S-G et *trn*C-*ycf6r* révèle des relations similaires, dont quelque-unes inattendues ou parfois complémentaires à celle de l'ADN ribosomal. Par exemple, la division de *Herrickia* s. str. et de *H. kingii* comme clades distincts est soutenue par au moins un caractère dérivé unique. Nous interprétons certaines relations insoupçonnées comme étant due à de l'homoplasie (e.g., le partage d'un caractère ancestral entre *E. integrifolia* et les autres genres des eurybioïdes).

Les 16 régions chloroplastiques évaluées ont toutes une variabilité limitée et se sont montrées inadéquates pour détecter le parent maternel des espèces polyploïdes et résoudre les relations interspécifiques au sein du groupe (chapitre 2). Quant à l'ITS, même après clonage, aucune relation entre diploïdes et polyploïdes n'a pu être mise à jour sauf

exceptions, car les séquences clonales se groupaient ensemble, masquant les relations entre espèces (chapitre 1). Donc, notre étude ne nous a pas permis d'établir les liens entre les espèces diploïdes et polyploïdes du genre *Eurybia*.

L'exploration de gènes nucléaires à faible nombre de copies a une fois de plus montré que les eurybioïdes constituent un groupe ardu. En somme, neuf régions à faible nombre de copie provenant de l'ADN nucléaires ont été évaluées avant de sélectionner la portion d'ADN appelée CNGC4. La recherche de régions suffisamment variables pour refléter l'évolution du groupe ne fut pas évidente. Comme le montre CNGC4, ces régions sont difficiles à amplifier (parfois à cause d'une duplication ou de la présence de copies multiples), peu variables, ou donnent une topologie non résolue à cause de phénomènes qui tendent à obscurcir l'histoire du groupe comme le triage de lignées incomplet, ou les événements de duplication, ou de recombinaison de séquences.

Histoire évolutive des eurybioïdes

Cette étude a permis de dresser un premier portrait évolutif global des eurybioïdes, tout en confirmant certaines relations déjà soulignées dans l'étude de Brouillet et al. (2004), où l'échantillonnage était plus restreint. Les eurybioïdes forment un grade évolutif paraphylétique à la sous-tribu des Machaerantherinae, l'ensemble étant groupe-frère de la sous-tribu des Symphyotrichinae. Tel que constaté antérieurement, le genre *Oreostemma* forme un groupe monophylétique, frère des autres eurybioïdes. Le genre *Herrickia* se sépare en deux groupes distincts, *Herrickia* s. str. et *H. kingii*. *Herrickia* s. str. se positionne comme frère du clade *H. kingii*-*Eurybia*-*Triniteurybia*-Machaerantherinae, le premier étant frère des trois derniers, confirmant le caractère paraphylétique du groupe soulevé par Brouillet et al. (2004). *Triniteurybia aberrans* se place comme groupe-frère des Machaerantherinae. Finalement, *Eurybia* paraît former un clade. Notre étude apporte cependant des précisions autant aux relations interspécifiques des diploïdes qu'au sein du genre. Nos résultats ne confirment pas les classifications sous-génériques du genre *Eurybia* proposées par Nesom (1994) et Semple (2005), mais confirment certaines hypothèses de relations avancées par Lamboy et al. (1991). *Eurybia pygmaea*, une espèce souvent

associée à l'*E. sibirica*, est exclue du genre pour être placée dans *Symphyotrichum* (Brouillet & Selliah 2005), un genre de la sous-tribu voisine, les Symphyotrichinae.

Histoire biogéographique

D'après nos analyses phylogénétiques et les données écologiques, le berceau des eurybioïdes se situerait dans l'Ouest de l'Amérique du Nord, dans des habitats plutôt mésiques. Cette hypothèse est renforcée par la présence de la plupart des clades basaux des Astereae nord-américains et des eurybioïdes dans l'ouest. L'ancêtre commun des eurybioïdes s'est initialement divisé en deux groupes, l'un à l 'ouest dans les Cascades – Sierra Nevada ayant donnée naissance à *Oreostemma,* l'autre à l'est dans la "Intermountain Region" et les Rocheuses, qui donnera naissance successivement à cinq lignées: *Herrickia* s. str., *H. kingii*, *Eurybia*, *Triniteurybia* et les Machaerantherinae. Les Machaerantherinae représenteraient une radiation du groupe dans les milieux arides de l'ouest américain. Au sein du genre *Eurybia*, *E. integrifolia*, une espèce occidentale, est la première à avoir divergé. Par la suite, une première migration vers l'est américain a donné naissance à l'*E. eryngiifolia.* Puis, il y eut spéciation successive de deux membres occidentaux, *E. sibirica* et *E. radulina*. Ce dernier partagerait un ancêtre avec l'ancêtre commun ayant effectué la deuxième migration vers l'est du continent. Une fois établie à l'est, l'espèces ancestrale a donné naissance à une radiation évolutive donnant *E. furcata* et *E. divaricata*, des espèces associées forêts mésiques, et *E. radula* une espèce préférant les rivages et les tourbières. Enfin, une deuxième radiation s'en est suivie, cette fois davantage vers l'est des Appalaches, probablement due aux conditions particulières du Piedmont par *E. mirabilis* et de la plaine côtière par *E. compacta*, *E. hemispherica* et *E. avita*. Selon notre étude, les caractères morphologiques utilisés dans les classifications précédentes (Nesom 1994, Semple 2005) notamment la forme des feuilles (e.g., sous-genre *Heleastrum* regroupent des espèces à feuilles étroites comme *E. eryngiifolia*, *E. hemispherica* et *E. avita*) résultent d'une convergence d'adaptation aux milieux de la plaine côtière.

Sans fossiles, nous sommes incapables de dater précisément l'origine du groupe et les radiations successives. Nous nous sommes donc restreints à proposer une hypothèse générale sur la divergence des eurybioïdes sans préciser de date. La relativement faible variabilité des séquences ribosomales, chloroplastiques et du CNGC4, et le polymorphisme

ancestral de ce dernier suggèrent une origine récente des eurybioïdes. Le groupe aurait évolué entre 5 millions d'années (fin Pliocène), époque de soulèvement accru des Cascades, de la Sierra Nevada et des chaînes montagneuses plus à l'est, et 18 000 ans, la fin de la glaciation Wisconsinienne. Nous estimons que la diversification et la migration des eurybioïdes ont été grandement influencées par les changements climatiques survenus durant les glaciations du Pléistocène. Ainsi, l'adaptation entre chaînes de montagnes, la disjonction entre l'ouest et l'est du continent ainsi que l'endémisme à des conditions particulières sont des exemples de résultat de migrations engendrées par les glaciations.

À l'avenir

Cette étude a permis de recueillir des informations importantes sur l'évolution des eurybioïdes. Néanmoins, il reste encore des éléments à préciser, notamment éclaircir les relations interspécifiques au sein des genres. Premièrement, il faudrait déterminer les parents des espèces polyploïdes du genre *Eurybia* en poursuivant l'exploration de microsatellites et de régions d'ADN chloroplastiques et nucléaires à faible nombre de copies qui seraient plus variables. Deuxièmement, inclure des membres des eurybioïdes dans un projet de séquençage génomique à grande échelle serait une avenue prometteuse pour faciliter la sélection de régions phylogénétiques appropriées pour le groupe. Troisièmement, il faut approfondir notre connaissance de *Herrickia kingii* et déterminer si l'on doit en faire un nouveau genre. Quatrièmement, il faudrait réaliser une étude morphologique exhaustive des espèces et variétés des eurybioïdes afin d'identifier des caractères synapomorphiques qui confirmeront la phylogénie moléculaire. Dans les études à venir, il serait intéressant d'élargir l'échantillonnage à plusieurs populations de régions différentes et d'approfondir l'histoire de chacun des genres sous un angle phylogéographique.

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Appendix 1

As a reference, few representatives of the eurybioids

Eurybia spectabilis

Triniteurybia aberrans

Eurybia eryngiifolia

Oreostemma alpigenum

Oreostemma elatum

Eurybia integrifolia

Herrickia glauca

Herrickia wasatchensis

Photos :

- Semple, J. [Online]. Available from: http://www.jcsemple.uwaterloo.ca/Eurybia.htm, [accessed 27 October 2009].
- Monroe, G.A. . [Online]. Available from: http://plants.usda.gov/java/profile?symbol=ORAL4&photoID=oral4_002_ahp.tif, [accessed 27 October 2009].
- Brouillet and Selliah: personnel photos

voucher data (with herbarium acronym) or literature source (data available in GenBank). ETS data are not provided for taxa not used in the combined analysis; the absence of an ETS accession number is indicated by a dash. GenBank accession numbers of *Eurybia* polyploid species for which ITS clones were sequenced are indicated in parentheses after the direct ITS sequence accession number, along with the number of clones; for a single *E. surculosa* specimen, only ITS clones were sequenced.

Data available in GenBank for Chapter 1

Batopilasia byei (Sundberg & G.L. Nesom) G.L. Nesom & Noyes, AF046974, --, Noyes and Rieserberg (1999); *Benitoa occidentalis* H. M. Hall, AF251585, --, Markos and Baldwin (2001); *Boltonia asteroides* (L.) L'Héritier, AF046975, --, Noyes and Rieserberg (1999); *Boltonia diffusa* Elliott, AF477633, --, Urbatsch et al. (2003); *Canadanthus modestus* (Lindl.) G.L. Nesom, AY772432, --, Brouillet et al. (2004); *Dieteria bigelovii* (A.Gray) Morgan & Hartman, AY772419, --, Brouillet et al. (2004); *Doellingeria umbellata* Nees, AF477625, --, Urbatsch et al. (2003); *Eurybia divaricata* (L.) G.L. Nesom, AY772423, AY772437, Brouillet et al. (2004); *Eurybia eryngiifolia* (Torrey & A. Gray) G.L. Nesom, AY772420, AY772434, Brouillet et al. (2004); *Eurybia sibirica* (L.) G.L. Nesom, AY772421, AY772435, Brouillet et al. (2004); *Eurybia surculosa* (Michx.) G.L. Nesom, AY772422, AY772436, Brouillet et al. (2004); *Grindelia lanceolata* Nutt., AF046976, --, Noyes and Rieserberg (1999); *Hazardia cana* (A. Gray) Greene, U97612, --, Morgan (1997); *Herrickia glauca* (Nutt.)Brouillet, AY772424, AY772438, Brouillet et al. (2004); *Herrickia horrida* Wooten & Standley, AY772425, AY772439, Brouillet et al. (2004); *Herrickia kingii var. kingii* (D.C. Eaton) Brouillet, Urbatsch & R.P. Roberts, AY772428, AY772442, Brouillet et al. (2004); *Isocoma wrightii* Rydb., U97617, --, Morgan (1997); *Lessingia micradenia* Greene var. *micradenia*, AF251615, --, Markos and Baldwin (2001); *Oreostemma alpigenum* (T.C. Porter) G.L. Nesom var. *haydenii* (T. C. Porter) G. L. Nesom, AY772430, AY772444, Brouillet et al. (2004); *Psilactis asteroides* A. Gray, U97640, --, Morgan (1997); *Pyrrocoma lanceolata* (Hook.) Greene, AF251574, --, Markos and Baldwin (2001); *Triniteurybia aberrans* (A. Nelson) Brouillet, Urbatsch & R. P. Roberts, AY772426, AY772440, Brouillet et al. (2004); *Xanthisma gracile* (Nuttall) Morgan & Hartman, U97625, --, Morgan (1997); *Xanthocephalum gymnospermoides* (A. Gray)

Bentham & Hooker f., U97650, --, Morgan (1997); *Corethrogyne filaginifolia* (Hooker & Arnott) Nuttall, AF251594, --, Markos and Baldwin (2001).

New sequences for Chapter 1

Almutaster pauciflorum (Nutt.) Á. Löve & D. Löve, EU200184, --, USA, Utah, Duschene Co. Mytan, Semple 5763 (WAT); *Ampelaster carolinianum* (Walter) G.L. Nesom, EU200185, --, USA, Fla., Osceola Co., Davenport, Semple 5354 (WAT); *Arida carnosa* (A. Gray) D.R. Morgan & R.L. Hartman, EU200186, EU196510, USA, Calif., Inyo Co., Lone Pine, Semple 8668 (WAT); *Chloracantha spinosa* (Benth.) G.L. Nesom, EU200187, EU196511, USA, N. Mex., Las Cruces, Spellenberg & Brouillet s.n. (MT); *Doellingeria infirma* (Michx.) Greene, EU200188, --, USA, W.Va., Mill Point, Semple 10716 (WAT); *Eurybia avita* (Alexander) G.L. Nesom, EU200189, EU196478, USA, Ga., Dekalb Co., Stone Mt, Semple 10573 (WAT); *Eurybia furcata* (E.S. Burgess) G.L. Nesom, EU200195, EU196482, Montreal Botanical Garden, Brouillet 00-551 (MT); *Eurybia jonesiae* (Lamboy) G.L. Nesom, EU200201 (12 clones: EU625621, EU625622, EU625623, EU625624, EU625625, EU625626, EU625627, EU625628, EU625629, EU625630, EU625631, EU625632), EU196493, USA, Ga., Heard Co., Franklin, Semple and Semple 11207 (WAT); *Eurybia macrophylla* (L.) Cass., EU200202 (9 clones: EU625600, EU625601, EU625602, EU625603, EU625604, EU625605, EU625606, EU625607, EU625608), EU196496, Montreal Botanical Garden, Brouillet 2002-01 (MT); *Eurybia merita* (A. Nelson) G.L. Nesom, EU200203, EU196501, USA, Wyo., Beartooth Pass, Semple and Brouillet 4438 (WAT); *Eurybia chlorolepis* (E.S. Burgess) G.L. Nesom, EU200190 (7 clones: EU625639, EU625640, EU625641, EU625642, EU625644, EU625645, EU625647), EU196490, USA, N. Car., Yancey Co., Semple and Suripto 9694 (WAT); *Eurybia chlorolepis* (E.S. Burgess) G.L. Nesom, EU200191, EU196491, USA, Tenn., Carter Co., Churchill 90-503 (VDB); *Eurybia compacta* G.L. Nesom, EU200192, EU196480, USA, N. J., Chatsworth, Semple 10365 (WAT); *Eurybia compacta* G.L. Nesom, EU200196, EU196483, USA, Va., Regina, Weldy & Showacre 940 (BRIT); *Eurybia compacta* G.L. Nesom, EU200197, EU196484, USA, N. J., Chatsworth, Semple & Suripto 9512 (WAT); *Eurybia conspicua* (Lindl.) G.L. Nesom, EU200193, EU196492, Can., Alta., Semple and Semple 9694 (WAT); *Eurybia eryngiifolia* (Torrey & A. Gray) G.L. Nesom, EU200194, EU196481, USA, Fla., Telogia, Kral 82815 (VDB); *Eurybia*

hemispherica (Alexander) G.L. Nesom, EU200198, EU196485, USA, Miss., Franklin, Semple and Semple 11186 (WAT); *Eurybia integrifolia* (Nutt.) G.L. Nesom, EU200199, EU196486, USA, Utah, Rich Co., Semple and al. 9259 (WAT); *Eurybia integrifolia* (Nutt.) G.L. Nesom, EU200200, --, USA, Wyo., Teton Co., Semple 11288 (WAT); *Eurybia merita* (A. Nelson) G. L. Nesom, EU200204 (5 clones: EU625609, EU625610, EU625611, EU625612, EU625613), --, USA, Wyo., Carbon Co., Semple & Zhang 10431 (WAT); *Eurybia mirabilis* (Torrey & A. Gray) G.L. Nesom, EU200205, EU196487, USA, S. Car., Richland Co., Broad Riv., Creel s.n. (WAT); *Eurybia paludosa* (Aiton) G.L. Nesom, EU200206 (8 clones: EU625648, EU625649, EU625650, EU625651, EU625652, EU625653, EU625654, EU625655), EU196495, USA, N. Car. Brunswick Co., Semple & Suripto 9767 (WAT); *Eurybia radula* (Ait.) G.L. Nesom, EU200207, EU196488, Can., Nfld., Gulch Pond, Brouillet 00-113 (MT); *Eurybia radulina* (A. Gray) G.L. Nesom, EU200208, EU196489, USA, Oreg., Douglas Co., Drew, Semple 7146 (WAT); *Eurybia saxicastellii* (J. J. N. Campbell & Medley) Nesom (7 clones: EU625614, EU625615, EU625616, EU625617, EU625618, EU625619, EU625620), EU196502, USA, Ky., Laurel Co., Semple & Suripto 9858 (WAT); *Eurybia schreberi* (Nees) Nees, EU200210 (7 clones: EU625656, EU625657, EU625658, EU625659, EU625660, EU625661, EU625662), EU196494, USA, Vt., Orange Co., Bradford, Semple & Brouillet 3494 (WAT); *Eurybia spectabilis* (Aiton) G.L. Nesom, EU200211, EU196499, USA, Mass., Ellisville, Semple & Brouillet 3556 (WAT); *Eurybia spectabilis* (Aiton) G.L. Nesom, EU200212, EU196500, USA, Mass., Plymouth Co., Semple & Semple 11028 (WAT); *Eurybia surculosa* (Michx.) G.L. Nesom, EU200214, EU196498, USA, Tenn., Royal Blue, Semple & Semple 11178 (WAT); *Eurybia surculosa* (Michx.) G.L. Nesom, [no direct ITS] (4 clones: EU625633, EU625634, EU625636, EU625637), --, USA, N. Car., Cherokee Co., Ranger, Semple 10527 (WAT); *Eurybia spinulosa* (Chapman) G. L. Nesom , EU200213, EU196497, USA, Fla., Beacon Hill, Anderson 11508 (MO); *Herrickia kingii* (D. C. Eaton) Brouillet, Urbatsch & R. P. Roberts var. *barnebyana* (S. L. Welsh & Goodrich) Brouillet, Urbatsch & R. P. Roberts, EU200215, EU196503, USA, Utah, Logan Riv., Morse 990 (KANU); *Herrickia glauca* (Nuttall) Brouillet var. *pulchra* (S. F. Blake) Brouillet, EU200216, EU196504, USA, Utah. Rockville, Welsh et al. 23851 (RM); *Herrickia wasatchensis* (M.E. Jones) Brouillet, EU200217, EU196505, USA, Utah, Cedar Canyon, Welch et al. 20619 (RM); *Machaeranthera tanacetifolia* (Kunth) Nees, EU200218, EU196509, USA, Tex., Borden Co., Post, Semple 8835 (WAT); *Oreostemma alpigenum* (Torrey & A. Gray) Greene var. *alpigenum*, EU200219, EU196506,

USA, Oreg., Mt. Hood, Semple 10271 (WAT); *Oreostemma elatum* (Greene) Greene, EU200220, EU196507, USA, Calif., Chico, Oswald & Ahart 8120B (CHSC); *Oreostemma peirsonii* (Sharsmith) G.L. Nesom, EU200221, EU196508, USA, Calif., Tulare Co., Raven 8379 (JEPS); *Symphyotrichum chapmanii* (Torrey & A. Gray) Semple & Brouillet, EU200223, --, USA, Fla., Choctawhatchee Riv., Semple 10560 (WAT); *Symphyotrichum novae-angliae* (L.) G.L. Nesom, EU200229, --, USA, Ga., Dade Co., Tenton, Semple 11001 (WAT); *Symphyotrichum pygmaeum* (Lindl.) Brouillet & S. Selliah, EU200231, --, USA, Alaska, Deadhorse, Parker 8207 (ALA); *Symphyotrichum campestre* (Nuttall) G.L. Nesom, EU200222, --, USA, Mont., Beaverhead Co., Semple and Brouillet 7019 (WAT); *Symphyotrichum concolor* (L.) G.L. Nesom var. *concolor*, EU200224, --, USA, Ga., Dade Co., Trenton, Semple 10992 (WAT); *Symphyotrichum cordifolium* (L.) G.L. Nesom, EU200225, --, USA, Me., Guilford, Semple 4639 (WAT); *Symphyotrichum depauperatum* (Fern.) G.L. Nesom, EU200226, --, USA, Pa., Nottingham, Semple 7681 (WAT); *Symphyotrichum ericoides* (L.) G.L. Nesom var. *ericoides*, EU200227, --, USA, S. Dak., Mound City, Semple 6664 (WAT); *Symphyotrichum fendleri* (A. Gray) G.L. Nesom, EU200228, --, USA, Kansas, Ford Co., Semple 7302 (WAT); *Symphyotrichum patens* (Ait.) G.L. Nesom var. *patens*, EU200230, --, USA, Ky., Red River Gorge, Semple & Suripto 9864 (WAT); *Symphyotrichum sericeum* (Vent.) G.L. Nesom, EU200232, --, Can., Ont., Rainy River, Semple 8787 (WAT); *Symphyotrichum tenuifolium* (L.) G.L. Nesom, EU200233, --, USA, N. J., Ocean Co., Cedar Run, Semple 9519 (WAT); *Symphyotrichum yukonense* (Cronq.) G.L. Nesom, EU200234, --, Can., Yukon, Kluane Lake, Semple 10624 (WAT).

References

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Morgan, D. R. 1997. Reticulate evolution in *Machaeranthera* (Asteraceae). Syst. Bot. 22: 599-531.

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Urbatsch, L. E., Roberts, R. P., and Karaman, V. 2003. Phylogenetic evaluation of *Xylothamia*, *Gundlachia*, and related genera (Asteraceae, Astereae) based on ETS and ITS nrDNA sequence data. Am. J. Bot. 90: 634-649.

Appendix 3

List of species of eurybioid asters and outgroups included in the Chapter 2. For each taxon, arranged by alphabetically, the taxonomic name, the Genbank accession numbers and the voucher data (with herbarium acronym are respectively listed. Data are listed in the following ordre: *trn*C-*ycf6*, *trn*S-G and *trn*L-F. For ITS and ETS data; see Appendix 2, chapter 1. A dash (-) indicates the absence of a sequence accession number.

New sequences for Chapter 2

Arida carnosa (A. Gray) D.R. Morgan & R.L. Hartman, GU480694, -, GU480728, USA. Calif. Inyo Co., Lone Pine, Semple 8668 (WAT), *Canadanthus modestus* (Lindl.) G.L. Nesom, GU480691, GU480763, GU480725, Idaho, Blaune Co., 31 July 2006, Semple et Semple 11359 ((WAT)), *Chloracantha spinosa* (Benth.) G.L. Nesom, GU480692, GU480764, GU480726, USA. N. Mex., Las Cruces, Spellenberg & Brouillet s.n. (MT), *Dieteria bigelovii* (A.Gray) Morgan & Hartman, GU480689, -, GU480723, X, Semple 10468 (WAT), *Doellingeria infirma* (Michx.) Greene, GU480693, GU480765, GU480727, USA. W.Va. Mill Point, Semple 10716 (WAT), *Eurybia avita* (Alexander) G.L. Nesom, GU480653, -, -, USA. Ga. Dekalb Co., Stone Mt, Semple 10573 (WAT), *Eurybia chlorolepis* (E.S. Burgess) G.L. Nesom, GU480664, GU480739, -, USA. N.C. Yancey Co., Semple and Suripto 9694 (WAT), *Eurybia compacta* G.L. Nesom, GU480657, GU480732, GU480698, USA. N. J. Chatsworth, Semple 10365 (WAT), *Eurybia conspicua* (Lindl.) G.L. Nesom, GU480665, GU480740, GU480705, Can. Alta., Semple and Semple 9694 (WAT), *Eurybia divaricata* (L.) G.L. Nesom, GU480654, GU480729, GU480695, X, Semple 10710 (WAT), *Eurybia eryngiifolia* (Torrey & A. Gray) G.L. Nesom, GU480663,

GU480738, GU480704, USA. Calif. Inyo Co., Lone Pine, Semple 8668 (WAT), *Eurybia furcata* (E.S. Burgess) G.L. Nesom, GU480655, GU480730, GU480696, USA. N. Mex., Las Cruces, Spellenberg & Brouillet s.n. (MT), *Eurybia hemispherica* (Alexander) G.L. Nesom, GU480661, GU480736, GU480702, USA. W.Va. Mill Point, Semple 10716 (WAT), *Eurybia integrifolia* (Nutt.) G.L. Nesom, GU480674, GU480748, GU480710, Montreal Botanical Garden, Brouillet 00-551 (MT), *Eurybia jonesiae* (Lamboy) G.L. Nesom, GU480670, GU480745, GU480707, USA. Ga. Heard Co., Franklin, Semple and Semple 11207 (WAT), *Eurybia macrophylla* (L.) Cass., GU480669, GU480744, -, Montreal Botanical Garden, Brouillet 2002-01 (MT), *Eurybia merita* (A. Nelson) G.L. Nesom, GU480656, GU480731, GU480697, USA. N.C. Yancey Co., Semple and Suripto 9694 (WAT), *Eurybia mirabilis* (Torrey & A. Gray) G.L. Nesom, GU480662, GU480737, GU480703, USA. Tenn., Carter Co., Churchill 90-503 (VDB), *Eurybia paludosa* (Aiton) G.L. Nesom, GU480666, GU480741, -, USA. N. J. Chatsworth, Semple 10365 (WAT), *Eurybia radula* (Ait.) G.L. Nesom, GU480659, GU480734, GU480700, USA. Va. Regina, Weldy and Showacre 940 (Brit.), *Eurybia radulina* (A. Gray) G.L. Nesom, GU480660, GU480735, GU480701, USA. N. J. Chatsworth, Semple and Suripto 9512 (WAT), *Eurybia saxicastellii* (J. J. N. Campbell & Medley) Nesom , GU480667, GU480742, GU480706, Can. Alta., Semple and Semple 9694 (WAT), *Eurybia schreberi* (Nees) Nees, GU480671, GU480746, GU480708, USA. Fla. Telogia, Kral 82815 (VDB), *Eurybia sibirica* (L.) G.L. Nesom, GU480658, GU480733, GU480699, USA. Miss. Franklin, Semple and Semple 11186 (WAT), *Eurybia spectabilis* (Aiton) G.L. Nesom, GU480672, GU480747, GU480709, USA. Utah,T. Rich Co., Semple and al. 9259 (WAT), *Eurybia spinulosa* (Chapman) G. L. Nesom , GU480673, -, -, USA. Wyo, Teton Co., Semple 11288

(WAT), *Eurybia surculosa* (Michx.) G.L. Nesom, GU480668, GU480743, GU480714, USA. Wyo. Carbon Co., Semple and Zhang 10431 (WAT), *Herrickia glauca* (Nutt.)Brouillet, GU480680, GU480754, -, Can. NC. Brunswick Co., Semple and Suripto 9767 (WAT), *Herrickia glauca* (Nuttall) Brouillet var. *pulchra* (S. F. Blake) Brouillet , GU480679, GU480753, -, Montreal Botanical Garden, Brouillet 00-113 (MT), *Herrickia horrida* Wooten & Standley, GU480678, GU480752, GU480713, USA. Or. Douglas Co., Drew, Semple 7146 (WAT), *Herrickia kingii* (D. C. Eaton) Brouillet, Urbatsch & R. P. Roberts var. *barnebyana* (S. L. Welsh & Goodrich) Brouillet, Urbatsch & R. P. Roberts, GU480675, GU480749, GU480711, USA. Ky. Laurel Co., Semple and Suripto 9858 (WAT) , *Herrickia kingii* var. *kingii* (D.C. Eaton) Brouillet, Urbatsch & R.P. Roberts , GU480676, GU480750, -, USA. Vt. Orange Co., Bradford, Semple and Brouillet 3494 (WAT), *Herrickia wasatchensis* (M.E. Jones)Brouillet, GU480677, GU480751, GU480712, USA. Mass. Ellisville, Semple and Brouillet 3556 (WAT), *Machaeranthera tanacetifolia* (Kunth) Nees, GU480688, GU480761, GU480722, USA. Tenn. Royal Blue, Semple and Semple 11178 (WAT), *Oreostemma alpigenum* (T.C. Porter) G.L. Nesom var. *haydenii* (T. C. Porter) G. L. Nesom, GU480681, -, GU480715, USA. Fla. Beacon Hill, Anderson 11508 (MO), *Oreostemma alpigenum* (Torrey & A. Gray) Greene var. *alpigenum*, GU480682, GU480755, GU480716, USA. Or. Mt. Hood, Semple 10271 (WAT), *Oreostemma elatum* (Greene) Greene, GU480683, GU480756, GU480717, USA. Calif. Chico, Oswald and Ahart 8120B (CHSC), *Oreostemma peirsonii* (Sharsmith) G.L. Nesom, GU480684, GU480757, GU480718, USA. Utah. Logan Riv., Morse 990 (KANU), *Symphyotrichum elliottii* (Torrey & A. Gray) G. L. Nesom , GU480685, GU480758, GU480719, X, *Symphyotrichum pygmaeum* (Lindl.) Brouillet & S. Selliah, GU480687,

GU480760, GU480721, USA. Utah. Rockville, Welsh et al. 23851 (RM), *Symphyotrichum yukonense* (Cronq.) G.L. Nesom, GU480686, GU480759, GU480720, USA. Tex. Borden Co., Post, Semple 8835 (WAT), *Triniteurybia aberrans* (A. Nelson) Brouillet, Urbatsch & R. P. Roberts, GU480690, GU480762, GU480724, USA. Idaho,Blaine Co. Alturas Lake, Morse and Smith 818 (KANU).

Appendix 4

List of species of eurybioid asters and outgroups included in the Chapter 3. For each taxon, arranged by alphabetically, the taxonomic name, the Genbank accession numbers and the voucher data (with herbarium acronym) are respectively listed. Each different clone sequence of a taxon is assigned to a GenBank accession number and indicated in parentheses, along with the number of clones if this is more than one. Only *E. hemispherica* and *E. compacta* are direct sequences.

New sequences for Chapter 3

Chloracantha spinosa (Benth.) G.L. Nesom, USA. N. Mex., (2:GU480645, GU480646), Las Cruces, Spellenberg & Brouillet s.n. (MT), *Eurybia avita* (Alexander) G.L. Nesom, (GU480651), USA. Ga. Dekalb Co., Stone Mt, Semple 10573 (WAT), *Eurybia compacta* G.L. Nesom, (GU480647), USA. N. J. Chatsworth, Semple 10365 (WAT), *Eurybia conspicua* (Lindl.) G.L. Nesom, (8:GU480587, GU480588, GU480589, GU480590, GU480612, GU480613, GU480614, GU480615), Can. Alta., Semple and Semple 9694 (WAT), *Eurybia eryngiifolia* (Torrey & A. Gray) G.L. Nesom, (3:GU480591, GU480592, GU480593), USA. Calif. Inyo Co., Lone Pine, Semple 8668 (WAT), *Eurybia furcata* (E.S. Burgess) G.L. Nesom, (GU480649), USA. N. Mex., Las Cruces, Spellenberg & Brouillet s.n. (MT), *Eurybia hemispherica* (Alexander) G.L. Nesom, (GU480648), USA. W.Va. Mill Point, Semple 10716 (WAT), *Eurybia integrifolia* (Nutt.) G.L. Nesom, (2:GU480625, GU480626), Montreal Botanical Garden, Brouillet 00-551 (MT), *Eurybia macrophylla* (L.) Cass.,(5:GU480607, GU480608, GU480609, GU480610, GU480611), Montreal Botanical Garden, Brouillet 2002-01 (MT), *Eurybia mirabilis* (Torrey & A. Gray) G.L. Nesom, (GU480650), USA. Tenn., Carter Co., Churchill 90-503 (VDB), *Eurybia paludosa* (Aiton) G.L. Nesom, (GU480600), USA. N. J. Chatsworth, Semple 10365 (WAT), *Eurybia radula* (Ait.) G.L. Nesom, (GU480652), USA. Va. Regina, Weldy and Showacre 940 (Brit.), *Eurybia radulina* (A. Gray) G.L. Nesom, (3:GU480616, GU480617,

GU480618), USA. N. J. Chatsworth, Semple and Suripto 9512 (WAT), *Eurybia saxicastellii* (J. J. N. Campbell & Medley) Nesom, (2:GU480601, GU480602), Can. Alta., Semple and Semple 9694 (WAT), *Eurybia schreberi* (Nees) Nees, (4:GU480603, GU480604, GU480605, GU480606), USA. Fla. Telogia, Kral 82815 (VDB), *Eurybia spectabilis* (Aiton) G.L. Nesom, (2:GU480598, GU480599), USA. Utah,T. Rich Co., Semple and al. 9259 (WAT), *Eurybia surculosa* (Michx.) G.L. Nesom, (4:GU480594, GU480595, GU480596, GU480597), USA. Wyo. Carbon Co., Semple and Zhang 10431 (WAT), *Herrickia horrida* Wooten & Standley, (5:GU480619, GU480620, GU480621, GU480622, GU480623), USA. Or. Douglas Co., Drew, Semple7146 (WAT), *Symphyotrichum elliottii* (Torrey & A. Gray) G. L. Nesom, (7:GU480637, GU480638, GU480639, GU480640, GU480641, GU480642, GU480643), X, *Symphyotrichum pygmaeum* (Lindl.) Brouillet & S. Selliah, (GU480644), USA. Utah. Rockville, Welsh et al. 23851 (RM), *Symphyotrichum rasemosum* (Elliot.) G.L. Nesom, (6:GU480631, GU480632, GU480633, GU480634, GU480635, GU480636), X, Semple 9895(WAT), *Triniteurybia aberrans* (A. Nelson) Brouillet, Urbatsch & R. P. Roberts, (GU480624), USA. Idaho,Blaine Co. Alturas Lake, Morse and Smith 818 (KANU).