Université de Montréal

Origin of *Symphyotrichum anticostense* (Asteraceae: Astereae), an endemic species of the Gulf of St. Lawrence

par

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Origin of *Symphyotrichum anticostense* (Asteraceae: Astereae), an endemic species of the Gulf of St. Lawrence

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Abstract

This thesis discusses the origin of *Symphyotrichum anticostense,* a decaploid species restricted to the Gulf of St. Lawrence region (Quebec, New Brunswick, and Maine). Two closely related species, *S. novi-belgii*, a hexaploid and *S. boreale*, a putatively tetraploid species have been proposed to be putative parents of *S. anticostense. Symphyotrichum novi-belgii* is distributed in the eastern coastal region of North America and *S. boreale* is widespread throughout the northern part of North America from west to east. These three species belong to the genus *Symphyotrichum,* which comprises ca. 91 species of which more than 50% are polyploids. The evolutionary history of the genus is poorly known even at the diploidy level. Before investigating the origin of *S. anticostense*, it seems necessary in the first step to elucidate phylogenetic relationships within the genus. The objectives of this thesis are therefore to delimit species boundaries excluding polyploid species and to investigate the origin of *S. anticostense*. The ITS data set nearly supports previous classifications based on morphological and cytological approaches, while the GAPDH nuclear gene is in poor agreement with the ITS results. Incomplete lineage sorting within *Symphyotrichum* is shown by stochastic sorting of ancestral polymorphisms among the GAPDH alleles. This evidence, in combination with the insufficient resolution among ribotypes, suggests recent evolutionary radiation of the genus. Univariate and multivariate morphological analyses as well as the ITS results roughly support *S. anticostense* to be a hybrid derivative of *S. novi-belgii* and *S. boreale*. The ITS-based network analysis suggests three independent geographical origins for *S. anticostense*: 1) Lake St. John; 2) Gaspé Peninsula- New Brunswick- Maine; 3) Anticosti Island. The results of two nuclear genes (GAPDH and MIPS) demonstrate relative incongruence between the phylogenetic trees. Although the results obtained from the

latter markers support those obtained from the morphological and ITS data sets, incomplete lineage sorting strongly affected the phylogenetic relationships among species.

Key words: Asteraceae, Astereae, *Symphyotrichum*, *Symphyotrichum anticostense*, polyploidy, hybridization, phylogeny, incongruence, incomplete lineage sorting, radiation.

Résumé

Cette thèse porte sur l'origine de *Symphyotrichum anticostense*, une espèce décaploïde de la région du golfe du Saint-Laurent (Québec, Nouveau Brunswick, et Maine). Il a été proposé que *S. anticostense* serait une allopolyploïde dérivée de l'hybride entre *S. novi-belgii*, une hexaploïde et *S. boreale*, une espèce putatifment tetraploïde. *S. novi-belgii* est distribuée dans la région côtière d'est d'Amérique du Nord et *S. boreale* est répandue à travers de la partie du nord d'Amérique du Nord de l'ouest à l'est. Ces trois espèces appartient au genre *Symphyotrichum* qui comprend 91 espèce. Plus que 50% de celles-ci sont polyploïdes. Les relations phylogénétiques au sein du genre sont aussi inconnues même au niveau diploïdie. Avant d'examiner l'origine de *S. anticostense*, il semble nécessaire dans la première étape d'expliquer les relations phylogénétiques du genre. Les objectifs de cette thèse sont donc de délimiter des espèces excluant des polyploïdes et examiner l'origine de *S. anticostense*. Le résultat d'analyse de donnée de l'ITS supporte presque des classifications auparavant basées sur les approches morphologiques et cytologiques. Le résultat du gène nucléaire GAPDH est faiblement en accord avec le résultat obtenu de l'ITS. Le triage des lignées incomplet dans *Symphyotrichum* est souligné par le triage au hasard des polymorphismes ancestraux parmi les alleles de GAPDH. Cette preuve ainsi que la résolution insuffisante parmi ribotypes, suggèrent une évolution récente du genre. Des analyses univariées et multivariées de données morphologiques ainsi que le résultat de marqueur d'ITS supportent la position taxonomique de *S. anticostense* comme un hybride dérivée de *S. novi-belgii* et *S. boreale*. L'analyse basée sur réseau des ribtypes suggère trois origines géographiques pour *S. anticostense*: 1) Lac Saint-Jean ; 2) Gaspèsie- Nouveau Brunswick- Maine ; 3) l'Ile d'Anticosti. Les résultats de deux gènes nucléaires (GAPDH

et MIPS) démontrent relativement l'incongruence entre les arbres phylogenetiques. Bien que les résultats obtenus de ces marqueurs supportent ceux-là obtenus des données morphologiques et l'ITS, le triage des lignées incomplet a affecté extrêmement les relations phylogenetiques parmi des espèces.

Mots clés : Asteraceae, Astereae, *Symphyotrichum*, *Symphyotrichum anticostense*, polyploïdie, hybridation, phylogénie, incongruence, triage des lignées incomplet, radiation.

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List of abbreviations

AFLP	amplified fragment length polymorphism
AIC	akaike information criterion
ANOVA	analysis of variance
ca.	approximately
CDA	canonical discriminant analysis
cpDNA	chloroplast DNA
CTAB	cetyltrimethylammonium bromide
DNA	deoxyribonucleic acid
GAPDH	glyceraldehydes 3-phosphate dehydrogenase
ILD	incongruence length difference
ITS	internal transcribed spacer
LRT	likelihood ratio test
MCMC	markov chain monte carlo
MIPS	myoinositol 1-phosphate synthase
mM	mili molar
NOR	nucleolus organizer region
nrDNA	nuclear ribosomal DNA
PC	principal components
PCA	principal component analysis
PCR	polymerase chain reaction
PP	posterior probability
RAPD	random amplification of polymorphic DNA
SNP	single nucleotide polymorphism
TBR	tree bisection and reconnection

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CHAPTER 1

Introduction

The Astereae is the second largest tribe of Asteraceae, with over 170 genera and 3000 species worldwide (Bremer, 1994). The North American clade of tribe Astereae is well defined phylogenetically (Noyes and Rieseberg, 1999; Brouillet et al., 2001). Some genera of this clade were previously classified within *Aster*, including *Doellingeria*, *Chloracantha*, *Eucephalus*, *Eurybia*, *Ionactis*, *Oclemena*, *Oreostemma*, *Sericocarpus*, and the *Symphyotrichum* clade (Nesom, 1994b).

The *Symphyotrichum* clade is monophyletic (Brouillet et al., 2001). It includes a number of morphologically distinct taxa that have been treated as separate genera or as subgenera, sections, subsections or series within *Aster* sensu lato. Sometimes, these have been treated as a single broadly circumscribed genus *Symphyotrichum* s. l. (Semple et al., 1996), or as a number of separate but closely related genera, including *Canadanthus*, *Ampelaster*, *Almutaster* with x = 9, *Psilactis* with x = 9, 5, 4, and *Symphyotrichum* s. str. (hereafter *Symphyotrichum*) (Nesom, 1994b, 2000; Semple et al., 2002). The *Symphyotrichum* clade has been named subtribe Symphyotrichinae by Nesom (1994a) and Nesom and Robinson (2007).

Interspecific relationships within *Symphyotrichum* have been controversial (Semple and Brouillet, 1980a; Jones, 1980a; Jones and Young, 1983; Nesom, 1994a, b, 2000; Semple et al., 1996; Semple et al., 2002). Two main classifications have been proposed by Nesom (1994b) and Semple (2005). The former has cytologically and morphologically divided the genus into two subgenera: *Symphyotrichum* (x = 13, 8, 7, and 5), and *Virgulus*

(x = 5, 4), whereas Semple (2005) has classified the genus into five subgenera: *Symphyotrichum* (x = 8, 7), *Ascendentes* (x = 13), *Virgulus* (x = 5, 4), *Astropolium* (x = 5), and *Chapmaniani* (x = 7) based on cytological, morphological, and molecular data.

Hybridization is a phenomenon that frequently occurs within a group of closely related species (e.g., Rieseberg, 1991, 1995; Grundt et al., 2004; Popp et al., 2005; Talent and Dickinson, 2005; Kao, 2008). Both interspecific and intersectional hybridization have been reported within *Symphyotrichum* (Jones, 1977; Semple and Brammall, 1982; Allen et al., 1983; Dean and Chambers, 1983; Allen, 1984, 1985, 1986; Brouillet and Labrecque, 1987; Allen and Eccleston, 1998; Semple et al., 2002). In addition, in most cases, auto- and allopolyploidy are the products of intra- and interspecific hybridization, respectively (Semple et al., 2002). These two processes, particularly at high ploidy levels, may have resulted in reticulation that could have complicated the delimitation of species boundaries and may be been misleading when studying the origin of hybrid species (Abbott, 1992; Rieseberg, 1998; Comes and Abbott, 2001; Linder and Rieseberg, 2004). In subtribe Machaerantherinae, which is closely related to subtribe Symphyotrichinae, reticulation has been documented using molecular data (Morgan, 1997). Poor phylogenetic resolution obtained from previous molecular-based studies (Noyes and Rieseberg, 1999; Brouillet et al., 2001), in combination with high levels of interspecific hybridization and morphological similarities (Jones and Young, 1983; Labrecque and Brouillet, 1996; Owen et al., 2006) within *Symphyotrichum*, may lead to considering a recent evolution for the genus.

Incomplete lineage sorting may also be present in a recently radiated group of taxa. In this process, a group of alleles from a single locus coalesce below the speciation event (Pamilo and Nei, 1988). This can lead to incorrect phylogenetic inferences. Both reticulation and incomplete lineage sorting contribute to the difficulty of determining phylogenetic relationships within *Symphyotrichum*.

Moreover, "multiple origins" is another evolutionary feature that may affect the evolutionary history of polyploid species (Soltis and Soltis, 1995). In this process, species

of hybrid origin may have originated independently several times from different populations of the same pair of parental species. Although a recurrent origin may increase reticulation among phylogenetic lineages, it may also increase the genetic diversity and, possibly the ecological adaptability of the hybrid species to different habitats (Soltis and Soltis, 1999; Soltis et al., 2004).

Symphyotrichum anticostense (Fern.) G. L. Nesom (synonyms: *Aster anticostensis* Fern., *A. gaspensis* Victorin) was first described by Fernald (1915) from a specimen of Anticosti Island. It is endemic to the Baie des Chaleurs (Gaspé Peninsula), Anticosti Island, Lake St. John, and the St. John river basin. Because *S. anticostense* populations are few in number and have a limited range, Labrecque and Brouillet (1990) suggested giving the species a "Threatened" status, a status adopted by the Committee On the Status of Endangered Wildlife In Canada (COSEWIC, 2007).

According to Brouillet and Labrecque (1987), *S. anticostense* has been proposed to be a deca-alloploid species ($2n = 80 = 10x$) derivative of the hybrid between tetraploid ($2n = 32 = 4x$) individuals of *S. boreale* (T. and G.) Á. Löve & D. Löve and members of *S. novi-belgii* (L.) G. L. Nesom, a hexaploid ($2n = 48 = 6x$) species. *Symphyotrichum boreale* is distributed throughout the boreal region of North America and experienced autopolyploidy ($2n = 16$ [2x] to 64 [8x]) in its evolutionary history. In the hypothesis, the tetraploid lineages have been suggested to be involved in the hybridization giving rise to *S. anticostense*. *Symphyotrichum novi-belgii* is distributed in coastal northeastern North America and overlaps with the entire range of *S. anticostense*. It is known from a single ploidy level ($2n = 48 = 6x$) throughout its range (Labrecque and Brouillet, 1996). The proposed origin of *S. anticostense* is based on cytological ($4x + 6x = 10x$) and morphological evidence, particularly the number of heads and leaf width which are intermediate in *S. anticostense* compared to its putative parents. However, this hypothesis has not been documented by other evidence.

Furthermore, as outlined above, the putative parents of *S. anticostense* are polyploid, which may increase the effects of reticulation in a phylogenetic framework.

Therefore, the present study investigates a possible example of hybrid speciation within *Symphyotrichum* which may simultaneously involve hybridization, polyploidization, and recurrent formation.

1.1 Taxonomic history of *Symphyotrichum anticostense*

Until 1987, *Symphyotrichum anticostense* was an enigmatic species recognized solely from its type specimen (Fernald, 1915, 1950). The morphological and cytological investigations by Brouillet and Labrecque (1987) and Labrecque and Brouillet (1990) shed light on our understanding of the probable taxonomic situation of this species within *Symphyotrichum*. The nomenclatural history of the species is summarized in Table 1.1.

Table 1.1 Taxonomic treatments of *Symphyotrichum anticostense*

Fernald (1915)	*Aster anticostensis* Fernald
Marie-Victorin (1932)	*A. gaspensis* Victorin
Fernald (1950)	*A. johannensis* Fernald p. p.
	A. anticostensis Fernald
Cronquist (1952)	*A. gaspensis* Victorin
Cronquist (1958)	*A. johannensis* Fernald p. p.
Boivin (1966)	*A. novi-belgii* L. var. *villicaulis* (Torrey and Gray) Boivin
Boivin (1972)	*A. hesperius* var. *gaspensis* (Marie-Victorin) Boivin
	A. hesperius var. *gaspensis* f. *albiflora* (Marie-Victorin)
Scoggan (1979)	*A. novi-belgii* L. var. *novi-belgii*
Jones (1987)	*A. novi-belgii* L. var. *tardiflorus* (L.) A. G. Jones
Nesom (1994b)	*Symphyotrichum anticostense* (Fernald) G. L. Nesom

The holotype of *S. anticostense* was collected by John Macoun on the shores of the Jupiter River on Anticosti Island in 1983 and deposited at the Gray Herbarium. In 1901, the species was collected at Fort Fairfield, Maine (USA) by Williams, Robinson, Fernald, and Churchill, and identified as *Aster junceus*. The species was described by Fernald (1915) as *Aster anticostensis* from the holotype collected by Macoun. In 1921, Marie-Victorin found the species on calcareous shores of Lake St. John, Quebec, and identified it as *A. longifolius*. In 1930 and 1931, Marie-Victorin and his colleagues collected it on

the strand of the rivers Bonaventure, Grande Rivière, and Petit Pabos. These specimens were identified as a new species, *A. gaspensis* Marie-Victorin (Marie-Victorin, 1926). In 1945, the species was collected in Woodstock (New Brunswick) along the River St. John by Dore and Gorham. The specimens were identified as *A. foliaceus*. During the decades of 1960 and 1970, the species was collected at the sites already recognized, but was identified as *A. novi-belgii*. Locations were revisited and confirmed by Brouillet and Labrecque (1987), Labrecque and Brouillet (1988, 1990, 1999), Coursol et al. (2000), Vaezi, Brouillet, and Zargarbashi (during sampling period of the current study in 2004 and 2005), and in New Brunswick by Hinds and Blaney (personal communication). After nearly a century, the species was recollected on the shores of the Aroostook River (Maine) by Haines (2000).

After examination of specimens in 12 herbaria (CAN, DAO, GH, ISC, MO, MT, NEBC, NY, TRT, UNB, WAT, and WIS), Labrecque and Brouillet (1990), established the species as *Aster anticostensis*. Due to priority, *A. gaspensis* was reduced to synonymy. When the genus *Aster* was revised by Nesom (1994b), he transferred all taxa having the base chromosome number of x = 8 to genus *Symphyotrichum*. Therefore, *Aster anticostensis* is now known as *Symphyotrichum anticostense* (Fernald) G. L. Nesom.

1.2 Biological characteristics

1.2.1 Taxonomy

The classification of tribe Astereae (Asteraceae) has recently benefited from data on chromosome numbers and morphology and from molecular studies (Semple, 1972, 1978, 1979, 1982, 1984, 1985, 1992, 1995; Jones, 1980a, b, 1983; Semple and Brouillet, 1980b; Semple and Ford, 1981; Semple and Brammall, 1982; Chmielewski and Semple, 1983, 1985, 1989; Semple and Chmielewski, 1983, 1985, 1987, 1991; Semple et al., 1983, 1989, 1993, 2001, 2002; Suh, 1989; Morgan, 1990; Nesom, 1994b; Xiang and Semple,

1996; Allen et al., 2001; Brouillet et al., 2001; Semple and Cook, 2004; Nesom and Robinson, 2007).

Nesom (1994a) divided the northern hemisphere Astereae into five subtribes (Asterinae, Solidagininae, Machaerantherinae, Chrysopsidinae and Hinterhuberinae) and hypothesized that they form a monophyletic group that is distinct from those of the southern hemisphere. According to this author, the New World subtribe Asterinae comprises 15 genera, including *Almutaster, Ampelaster, Aster, Chloracantha, Canadanthus, Doellingeria, Eucephalus, Eurybia, Ionactis, Oclemena, Oreostemma, Psilactis, Sericocarpus, Symphyotrichum* and *Tonestus*. This subtribe was not confirmed as a monophyletic group based on the nrDNA (ITS) sequence data including the North American and Old World species (Brouillet et al., 2001; Semple et al., 2002). *Almutaster, Ampelaster, Canadanthus, Psilactis,* and *Symphyotrichum* formed a well-supported group, called the *Symphyotrichum* clade (Brouillet et al., 2001). This clade was considered as subgroup (Nesom 1994b) or subtribe Symphyotrichinae (Nesom, 1994a, 2000; Nesom and Robinson, 2007).

Genus *Symphyotrichum,* established by Nees (1832), was described based on *S. unctusom* Nees (synonym: *S. novi-belgii* (L.) G. L. Nesom). In Brouillet et al. (2006), the genus is characterized as follows:

> "**Annuals or perennials**, 2-200 cm (colonial or cespitose, usually ± strongly heterophyllous, usually eglandular, sometimes stipitate-glandular; rhizomatous, with woody caudices, or taprooted). **Stems** ascending to erect, rarely vinelike, usually simple, sometimes branched distally, seldom proximally, usually hairy in decurrent lines at least distally, proximally often glabrous, sometimes hairy, sometimes stipitate-glandular distally. **Leaves** basal (sometimes persistent to flowering) and cauline; petiolate (often basal and proximal, sometimes distal, petioles often ± winged, clasping, ciliate) or sessile; blades (often purplish abaxially, 1, sometimes to 3-nerved) cordate to elliptic, oblanceolate, or spatulate (basal), ovate, elliptic, lanceolate, oblanceolate, or linear (cauline, usually progressively, sometimes abruptly or little reduced distally), margins serrate, crenate, or entire, scabrous or ciliate, faces glabrous or hairy, sometimes stipitate-glandular. **Heads** radiate or disciform (sect. *Conyzopsis*), usually in paniculiform, sometimes in racemiform or subcorymbiform arrays, sometimes borne singly. **Involucres** cylindric or campanulate to hemispheric, 4-22 mm

diam. **Phyllaries** 20-84 in (3-)4-6(-9) series, 1(-3)-nerved (not keeled), oblong-lanceolate or oblanceolate or spatulate (outer and mid) to linear (innermost), unequal to subequal, outer sometimes foliaceous, bases usually indurate, margins usually scarious, erose, hyaline or not, (apices usually with a well-defined green zone, sometimes ± foliaceous), faces glabrous or hairy, sometimes stipitate-glandular. **Receptacles** flat to slightly convex, pitted, epaleate. **Ray florets** (8-)12-35(-75+); usually in 1 series, in 2-5 series, rarely in 4-5+ series in *S. frondosum*, pistillate, fertile; corollas white, pink, blue, or purple (rays 0, peripheral pistillate florets in 2-5+ series, corollas lacking laminae in sect. *Conyzopsis*). **Disc florets** (7-)15-50(-110), bisexual, fertile; corollas yellow to white, becoming purplish to reddish or pinkish at maturity, ± ampliate, tubes usually shorter than funnelform (cylindric in sect. *Conyzopsis*) throats, lobes 5, erect, spreading, or reflexed, delate, triangular, or lanceolate; style-branch appendages lanceolate. **Cypselae** usually obovoid or obconic, sometimes fusiform, ± compressed, nerves (2-)3-5(-10, sometimes dark-translucent), faces glabrous or strigillose, eglandular (sparsely stipitate-glandular in *S. novae-angliae*); **pappi** persistent, of (20-)25-40(-55) white to brownish, ± equal barbellate, apically attenuate bristles in 1(-3) series, x = 8, 7, 5, 13, 18, 21."

Genus *Symphyotrichum* has been classified as *Aster* subgenus *Symphyotrichum* by Jones (1980a), but it has been considered a distinct genus of Astereae by other botanists (Nesom, 1994b, 2000; Brouillet et al., 2001; Semple et al., 2002). Two major classifications for the genus were proposed by Nesom (1994b) and Semple (2005) (Table 1.2). Nesom (1994b), divided the genus into two subgenera, *Symphyotrichum* with eight sections and *Virgulus* with four, based on morphological and cytological evidence. Semple (2005) subdivided the genus into five subgenera, *Symphyotrichum*, *Ascendentes*, *Virgulus*, *Astropolium*, and *Chapmaniani*, based on morphological, cytological, and to some extent, molecular (ITS) data; subgenera *Symphyotrichum* and *Virgulus* were subdivided into three and five sections, respectively. A major difference between the two classifications is the position of subg. *Chapmaniani*, which is classified by Nesom as a section of subg. *Heleastrum* of genus *Eurybia*, whereas in the Semple classification, it is segregated from *Eurybia* and transferred to genus *Symphyotrichum*. The details of both classifications are provided in Table 1.2. The position of *S. anticostense* and its putative parents based on the Semple classification is shown in Table 1.3.

The following morphological key proposed by Labrecque and Brouillet (1990) distinguishes *S. anticostense* from its putative parents:

1- Plant slender; rhizomes filiform, less than 2 mm in diameter; leaves linear, recurved at the margins, sessils, non-clasping; large but few heads (often monocephalous) on very slender peduncles .....*S. boreale*

- Plant robust; rhizomes thick, more than 2 mm in diameter; leaves different; plant usually not monocephalous 2

2- Leaves lanceolate, slightly fleshy, clasping at base; capitulescence highly branched, with numerous heads on short (usually less than 3 cm) peduncles.....*S. novi-belgii*

- Leaves linear-lanceolate, little or not narrowing at the base, sessile or almost sessile; capitulescence paniculiform, open, heads borne on long (usually more than 3 cm) peduncles 3

3- Leaves arched, coriaceous, rigid, persisting; capitulescence made of strongly ascending branches ..... *S. anticostense*

- Leaves slightly fleshy, not rigid, deciduous at the base at anthesis; capitulescence branches not strongly ascending .....*S. novi-belgii var. villicaule* (=*Aster longifolius*)

Table 1.2 Two main classifications proposed by Nesom (1994b) and Semple (2005) for genus *Symphyotrichum*.

Nesom 1994

Subgenus	Section	Subsection	Chromosome base
Symphyotrichum	*Symphyotrichum*	—	8
	Cordifolii	—	
	Concinni	*Concinni*	8
		Turbinelli	48
	Dumosi	*Dumosi*	8
		Divergentes	
		Porteriani	
	Oxytripolium	—	5
	Conyzopsis	—	7
	Occidentales	—	8
	Ascendentes	—	13
Virgulus	*Grandiflori*	*Grandiflori*	5
		Polyligulae	
		Mexicanae	
		Brachyphylli	
	Ericoidei	—	
	Patentes	—	
	Concolores	—	4, 5

Semple 2005

Subgenus	Section	Subsection	Chromosome base
Symphyotrichum	*Symphyotrichum*	*Symphyotrichum*	8
		Heterophylli	
		Occidentales	
		Dumosi	
		Porteriani	
	Conyzopsis	—	7
	Turbinelli	—	48
Ascendentes	—	—	13
Virgulus	*Concolores*	—	4, 5
	Grandiflori	*Grandiflori*	5
		Mexicanae	
	Patentes	*Patentes*	
		Brachyphylli	
	Polyliguli	—	
	Ericoidei	—	
Astropolium	—	—	5
Chapmaniani	—	—	7

Table 1.3 Taxonomic position of *S. anticostense* and its two putative parents based on the classification of Semple (2005).

Subgenus	Section	Subsection	Series	Species	Chromosome number
Symphyotrichum	*Symphyotrichum*	*Symphyotrichum*	*Symphyotrichum*	*S. anticostense*	2n= 10x= 80
				S. novi-belgii	2n= 6x= 48
		Dumosi	—	*S. boreale*	2n= 2x, 4x, 6x, 8x= 16, 32, 48, 64

1.2.2 Distribution

Symphyotrichum anticostense is an endemic species found in the Gulf of St. Lawrence (Baie des Chaleurs, Anticosti Island, and Lake St. John) (Québec), and along the St. John, Restigouche (New Brunswick) and Aroostook (Maine; Haines, 2000)) rivers (Fig. 1.1).

Distribution of the putative parents is important to detect the origin of *S. anticostense* and hybrid zone(s). *Symphyotrichum novi-belgii* is distributed in eastern North America (Fig. 1.2) and overlaps with the whole range of *S. anticostense*. In some regions where *S. anticostense* and *S. novi-belgii* are sympatric, they may hybridize and produce morphologically intermediate individuals. Brouillet and Labrecque (1987) collected hybrids (2n = 64) between the two taxa. Labrecque and Brouillet (1990) also reported introgression between them. In our sampling periods 2004 and 2005, we also encountered such hybrids. In contrast, *S. boreale* is distributed throughout boreal North America (Fig. 1.3). This species usually grows in fens and marshy areas, particularly beside streams.

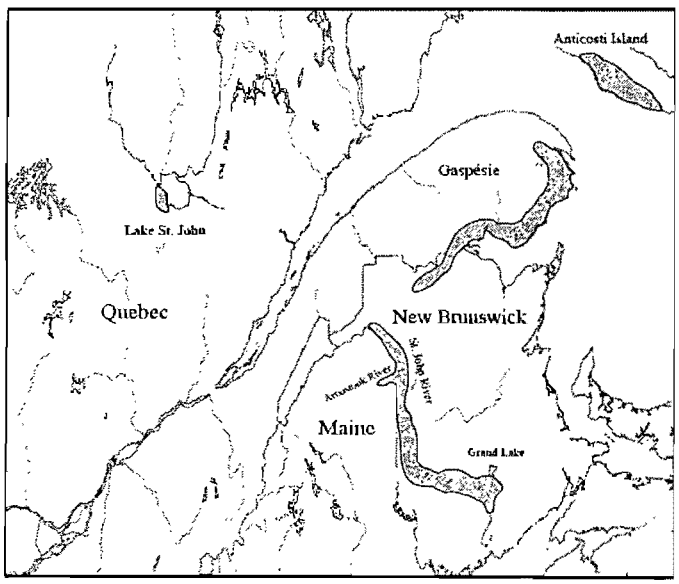

Figure 1.1 Present distribution of *Symphyotrichum anticostense* indicated by shaded regions.

Figure 1.2 Geographical distribution of *Symphyotrichum novi-belgii*, a putative parent of *Symphyotrichum anticostense*, in the eastern part of the North America indicated by shaded region.

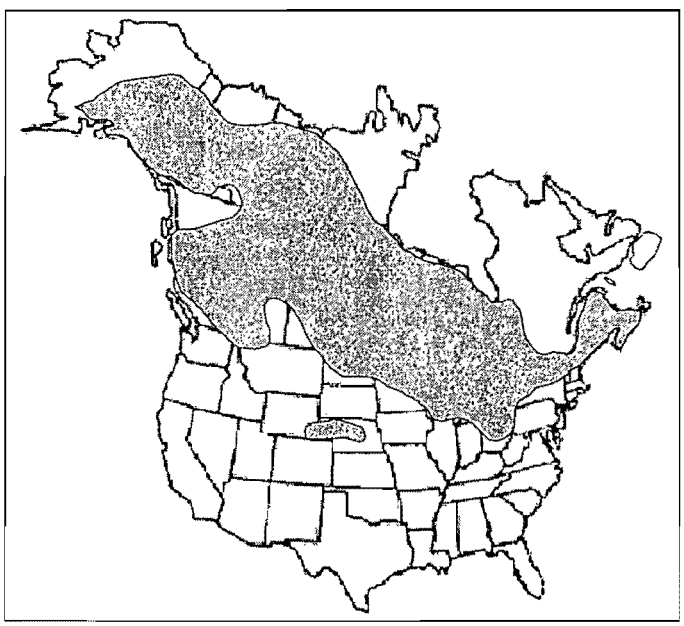

Figure 1.3 Geographical distribution of *S. boreale*, a putative parent of *S. anticostense*, in the nothern part of the North America indicated by shaded regions.

1.2.3 Ecology

Symphyotrichum anticostense colonizes the geolittoral zone of rivers with significant volume and flow (at least in the spring), and is maintained in pioneer habitats on a calcareous substrate (outcrops or gravel) by the action of high spring water and ice (Coursol et al., 2000). The species was not found in areas where the substrate was different (acidic or sandy) (Labrecque and Brouillet, 1990). Loose soil or sand (as on the Grand-Pabos River) apparently prevents establishment of *S. anticostense*. Substrate mobility can result in the burial of rhizomes or plantlets, or that type of soil may favor better-adapted species (Labrecque and Brouillet, 1990).

The species most frequently associated with *S. anticostense* in the field are of three types, as reported in Coursol et al. (2000).

1. Ubiquitous species of open areas, of which several are introduced, such as *Fragaria virginiana* Dcne., *Leucanthemum vulgare* Lam., *Plantago major* L., *Prunella vulgaris* L., *Silene vulgaris* (Moench) Garcke, *Taraxacum officinale* Weber, *Tussilago farfara* L., and *Vicia cracca* L.;
2. Species of wet habitats with wide distributions such as *Agrostis stolonifera* L., *Bromus ciliatus* L., *Cornus sericea* L., *Doellingeria umbellata* (Miller) Nees, *Elymus trachycaulus* (Link) Gould, *Eutrochium maculatum* (L.) Lam., *Muhlenbergia glomerata* (Willd.) Trinius, *Prunus pumila* L. var. *depressa* (Pursh) Bean, and *Rosa blanda* Ait.;
3. Calcicolous species such as *Hedysarum alpinum* L., *Lobelia kalmii* L., *Muhlenbergia richardsonis* (Trin.) Rydb., *Parnassia glauca* Raf., *Dasyphora fruticosa* L., and *Trisetum melicoides* (Michx.) Vasey.

Flowering occurs from late July to September. Achenes were not observed during our sampling periods in August 2004 and 2005 as we were too early in the season; however, the species was able to invade locally disturbed habitats (such as roadsides)

near established populations, indicating the ability of the species to perpetuate itself by seed (Labrecque and Brouillet, 1990; Vaezi, personal observation).

Symphyotrichum anticostense is apparently unable to hold its own against adventitious plants in an environment that is not regularly disturbed. In areas where it grows along with *S. cordifolium*, *S. ciliolatum* and particularly *S. novi-belgii*, hybrids may be produced between them (Labrecque and Brouillet, 1988, 1990). During August 2004 and 2005, we observed *S. anticostense* and *S. novi-belgii* growing together in several regions (Florenceville, Restigouche River, Bonaventure River, Grand River, and Petit-Pabos River). The presence of species like *S. novi-belgii*, *S. cordifolium,* and *S. ciliolatum* on a large scale may eventually interfere with the genetic integrity of *S. anticostense* populations and complicate our ability to detect the origin of the proposed hybrid (Labrecque and Brouillet, 1990).

1.3 Objectives

This dissertation has two objectives that are organized in three chapters. These objectives are as follows:

1) To reconstruct the evolutionary history of the diploid species of genus *Symphyotrichum*

It is necessary to investigate the phylogenetic relationships among diploid species before considering the origin of *S. anticostense*. This helps us to examine the possible involvement of diploid species in the origin of *S. anticostense* and its putative paretnts in the next two chapters. With the exception of a taxonomically incomplete nrDNA (ITS) phylogeny (Brouillet et al., 2001), no molecular study has attempted to do so at the diploid level. This objective is addressed in chapter 2. I am investigating the evolutionary relationships among diploid species of *Symphyotrichum* using two nuclear markers with

probably independent evolutionary histories: 1) nrDNA ITS (internal transcribed spacer), with a high-copy number with a potential to undergo concerted evolution, potentially leading to uniformity of ITS sequences at the individual, population, and species levels (Liao, 2003; Kovarik et al., 2005); and 2) GAPDH (glyceraldehyde 3-phosphate dehydrogenase), a low-copy nuclear gene, which is unlikely to have undergone concerted evolution.

2) To investigate the origin of *Symphyotrichum anticostense*

The second objective is to investigate the origin of *S. anticostense* by testing the hypothesis of Brouillet and Labrecque (1987) that the polyploid species *S. boreale* and *S. novi-belgii* are involved. Chapter 3 addresses this objective using morphometric (univariate and multivariate analyses) and molecular (nrDNA ITS) studies. In addition, in the molecular study, all diploid species used in chapter 2 are included to evaluate their possible involvement in the hybridization. I am also investigating the possibilityof multiple geographic origins for *S. anticostense*. In chapter 4, I am addressing this objective using two further nuclear markers, GAPDH marker, also used in chapter 2, and MIPS (myoinositol 1-phosphate synthase), used for the first time in a phylogenetic framework.

To achieve the objectives of the present project, it is important to elaborate on some notions that are pertinent to the study.

1.4 Polyploidy

A major and well-known form of genomic change is the duplication of an entire genome or polyploidy (Kellogg and Bennetzen, 2004). Polyploidy is a prevalent feature in the plant kingdom (Stebbins, 1950; Otto and Whitton, 2000), being found abundantly in algae (Nicholas, 1980), mosses (Crosby, 1980), ferns (Wagner and Wagner, 1980), and flowering plants (Stebbins, l. c.; Grant, 1981). In flowering plants, depending upon the method of estimation, estimates of the numbers of polyploid taxa range from 35% (Stebbins, 1971) to 70% (Averett, 1980; Goldblatt, 1980). Thus, polyploidy appears to have played a major role in the evolution, diversification (Bretagnolle and Thompson, 1995), and speciation (Otto and Whitton, 2000) of the plant kingdom. Botanists have long recognized the importance of polyploid hybrid speciation in plant evolution (Stebbins, 1950, 1971; Grant, 1981; Soltis et al., 1992; Soltis and Soltis, 1993; Masterson, 1994). Otto and Whitton (2000) suggested that polyploidization may be the most common mechanism of sympatric speciation in plants.

Polyploids can be classified on the basis of their origin. Autopolyploids (i.e., intraspecific polyploids; Lewis, 1980) are considered to have originated from one individual, from crosses between different individuals within the same parental population, or from different parental populations of the same species (Bretagnolle and Thompson, 1995; Ramsey and Schemske, 1998). Allopolyploids (i.e., interspecific polyploids, Lewis, 1980) are derived from the combination of distinct genomes followed by doubling (Stebbins, 1950; Soltis and Soltis, 2000). Allopolyploids derive from hybrids between species, where species are defined according to their degree of pre- and/or post-zygotic isolation (biological species concept). The analysis of allopolyploidization, including all of the intermediates between the extreme categories of auto- and allopolyploidy, suggests that the production of 2n gametes may have played a role in the creation of new polyploids by hybridization (Briggs and Walters, 1984; Eckenwalder and Brown, 1986; Bretagnolle and Thompson, 1995).

One of the problems in the interpretation of phylogenetic results sometimes is a consequence of gene duplications. If the genes being analyzed are duplicated, researchers must distinguish between orthologous and paralogous sequences as well as lineage sorting (see following section) among alleles of each gene (Linder and Rieseberg, 2004). Three main possibilities have been suggested for the evolutionary fate of duplicated genes: (1) functional diversification, i.e. acquisition of novel function after duplication (Allendorf, 1979; Li, 1985; Ohta, 1991; Clegg et al., 1997); (2) decay through mutation leading to 'silencing' of one of the two duplicated copies (Stephens, 1951; Ohno, 1970; Soltis and Soltis, 1989; Gastony, 1991; Shoemaker et al., 1996a); (3) retention of the original function (Ferris and Whitt, 1979; Hughes and Hughes, 1993; Cook et al., 1997). Of these, the latter is a frequent evolutionary outcome of duplication for both gene copies (Wendel, 2000).

1.5 Hybridization

1.5.1 Detection of hybrid speciation

Although hybridization was mostly ignored in early phylogenetic studies, several approaches were suggested for the treatment of hybrids. Most frequently, it was proposed that hybrids should be detected by other biosystematic tools and then excluded from the phylogenetic study (e.g., Wagner, 1983). Another common suggestion was to include all taxa in the initial phylogenetic analyses, followed by searches for phylogenetic signatures of hybridization such as character conflict and polytomies (e.g., Funk, 1985). Unfortunately, analyses of the placement of known hybrids in phylogenetic trees failed to reveal predictable hybrid phylogenetic patterns, at least for morphological characters, leading McDade (1992) to predict that phylogenetic approaches were unlikely to be an effective tool for detecting hybrids. In contrast, early molecular phylogenetic studies were more successful at detecting the footprint of hybridization. The first studies comparing biparental nuclear and uniparental plastid phylogenies revealed discrepancies that were

interpreted as resulting from hybridization (Palmer et al., 1983; 1985), and a few years later, Rieseberg and Soltis (1991) were able to compile 36 such examples.

A variety of molecular techniques has been used to detect ancient or more recent hybridization events and to identify parental taxa. Isozyme analysis (Urbanska et al., 1997; Warwick and Anderson, 1997; Bleeker et al., 1999; Bleeker and Hurka, 2001; Eschmann-Groupe et al., 2003), nrDNA (ITS) sequencing (Alvarez and Wendel, 2003; Siripun and Schilling, 2006), cpDNA (Dobeš et al., 2004; Bleeker and Matthies, 2005; Lihová et al., 2006), and fingerprinting methods such as RAPD, AFLP (Neuffer and Jahncke, 1997; Bleeker and Matthies, 2005) are amongst the most widely used. The nrDNA (ITS) region may retain traces of past hybridizations in spite of the fact that concerted evolution can erase nucleotide additivity (Marhold and Lihová, 2006). For instance, this marker has significantly contributed to the identification of hybrids or species of hybrid origins in *Cardamine* (Franzke and Mummenhoff, 1999), *Draba* (Widmer and Baltisberger, 1999), and *Eupatorium* (Siripun and Schilling, 2006). But in order to resolve the evolutionary history of allopolyploid hybrids, a phylogenetic study using low-copy nuclear genes is required, because such markers reflect better biparental lineages and thus are powerful tools in reconstructing reticulate histories of plant groups (Lee et al., 2002). Alternatively, studies of cpDNA haplotype diversity can identify the maternal parent, as well as demonstrate multiple hybrid origins (Bleeker and Hurka, 2001; Lihová et al., 2006).

Three lines of evidence might be employed to detect and reconstruct hybrid speciation. First, detection of hybrid speciation could be as simple as looking for sets of incongruent trees from separate analyses of independent data sets, each representing a different parent of the hybridization (Maddison, 1997; Nakhleh et al., 2004). In theory, reconstruction of each hybrid speciation events could be accomplished accurately with one or a set of biparentally inherited markers that evolved at the appropriate rate, but in reality incongruence due to retention of ancestral polymorphisms, particularly in a recently evolved group of taxa, may mislead the detection of hybrid speciation (Linder and Rieseberg, 2004; Maddison and Knowles, 2006).

The second way to detect hybridization would be to combine DNA sequences from multiple independent loci into a single analysis and look for phylogenetic signals that indicate a set of two or more histories, by doing splits decomposition for example (Bandelt and Dress, 1992; Huson, 1998; Bryant and Moulton, 2002), or using statistical tests such as the ILD test (Farris et al., 1995; Zelwer and Daubin, 2004; Chang et al., 2007). A third approach would involve searching for associations among genetically linked markers. The expectation is that tightly linked markers in a hybrid species are significantly more likely to come from the same parent and therefore to display linkage disequilibrium (Linder and Rieseberg, 2004).

1.5.2 Reticulate evolution and multiple origins

Reticulation complicates reconstruction of evolutionary relationships and makes phylogeny inferences a challenging undertaking (Marhold and Lihová, 2006). Linder and Rieseberg (2004) and Vriesendrop and Bakker (2005) pointed out that the evolutionary history of many plant groups does not follow divergent evolutionary patterns and hardly can be unraveled using a tree-building procedure. Rather, it is network-like and displays a number of reticulate evolutionary events, particularly hybrid speciation. As shown in figure 1.4, a tree node has one ancestral branch (K) and two or more descendant branches (F and E) but a network node has two ancestral branches (X and Y) and only one descendant branch (B).

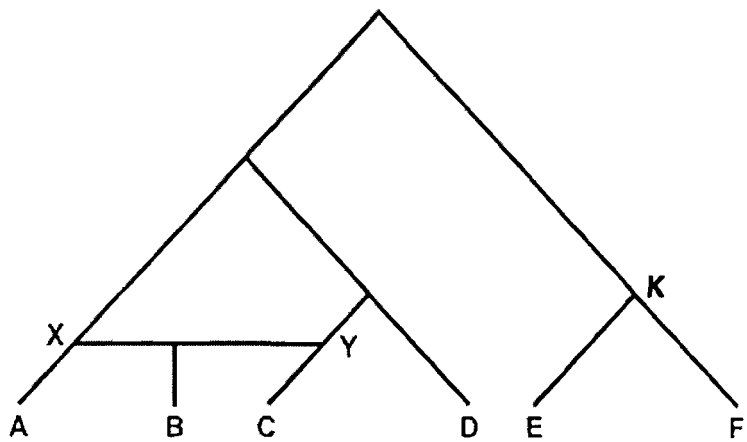

Figure 1.4 Example of a phylogenetic network with a single hybrid species B (see the text).

Multiple or recurrent origins (independent origins from different genotypes of the same progenitor species) is an important feature of most taxonomically recognized polyploid species (Soltis and Soltis, 1993, 1995, 1999; Lee et al., 2002; Saito et al., 2006; Thompson and Whitton, 2006). Single and multiple origins of polyploids are illustrated in figures 1.5 and 1.6, respectively. According to these scenarios, a single origin would result in genetic uniformity (at least initially) across all individuals of a species (Soltis and Soltis, 1995), whereas multiple origins in space and time would result in increased genetic diversity and complexity (Soltis and Soltis, 1999; Soltis et al., 2004).

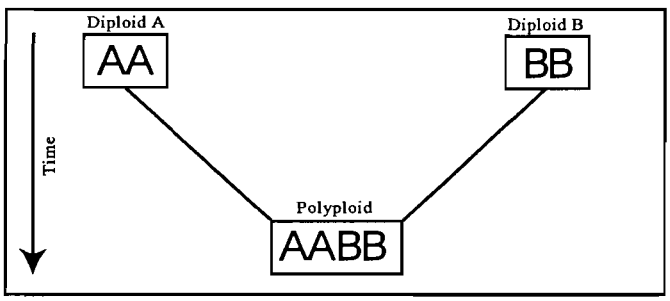

Figure 1.5 The traditional view (single origin) envisioned each polyploid species forming only once, resulting in a new species that was genetically uniform (or nearly so).

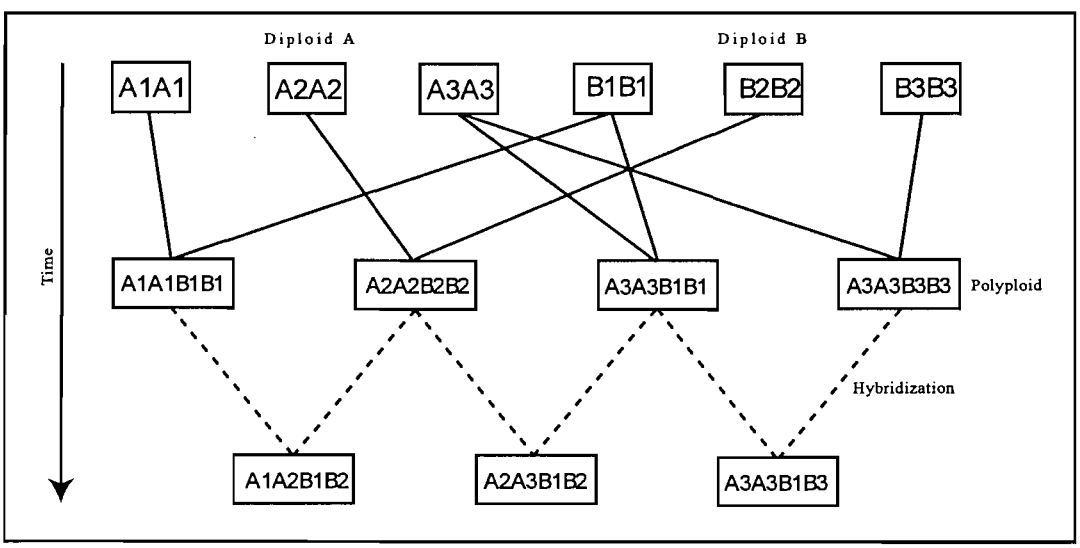

Figure 1.6 The new view (multiple origins) suggests that each polyploid species forms over and over again from different parental genotypes generating a diverse array of polyploid genotypes. Subsequent hybridization among these polyploid genotypes and recombination result in additional genetic variability.

1.6 Lineage sorting

In the construction of phylogenetic trees, it is important to distinguish between a species tree and a gene tree. The former refers to a tree of a group of species that reflects the actual evolutionary pathways, whereas the latter is a tree of a group of homologous (orthologous) genes each sampled from a different species (Tateno et al., 1982; Nei, 1987). When there is allelic polymorphism within a species, a tree reconstructed from DNA sequences for a given gene may be quite different from the species tree (Tajima, 1983; Takahata and Nei, 1985; Neigel and Avise, 1986). Consider, for instance, the case where ancestral alleles are polymorphic (Fig. 1.7) and a particular polymorphism is maintained through one or more speciation events. The daughter lineages arising from this speciation event each have two alleles, and these are shared across the species boundary. Accordingly, each of the two alleles is older than the species to which they belong. Extending this process of organismal divergence through time, and introducing allele extinction through stochastic or selective means and genesis through mutation, it is possible to envision how gene trees might fail to reflect the true phylogeny of a species (Wendel and Doyle, 1998). Incongruence among evolutionary trees can result when the loci sampled have undergone lineage sorting that is independent of speciation events, and relationships among sequences distributed among species may therefore not reflect species phylogeny.

One important manifestation of lineage sorting is that alleles from different species may be more closely related to each other than are alleles within the same species (Wendel and Doyle, 1998). Incomplete lineage sorting is likely when the time between nodes is short, because newly acquired neutral mutations can take considerable time to become fixed (Nei, 1987), particularly when the effective population size is large (Pamilo and Nei, 1988), because alleles in small populations reach fixation or are lost more rapidly than alleles from large populations, and hence the time to coalescence is less in small populations. In general, lineage sorting is only expected to be a source of incongruence at lower taxonomic ranks (Wendel and Doyle, 1998).

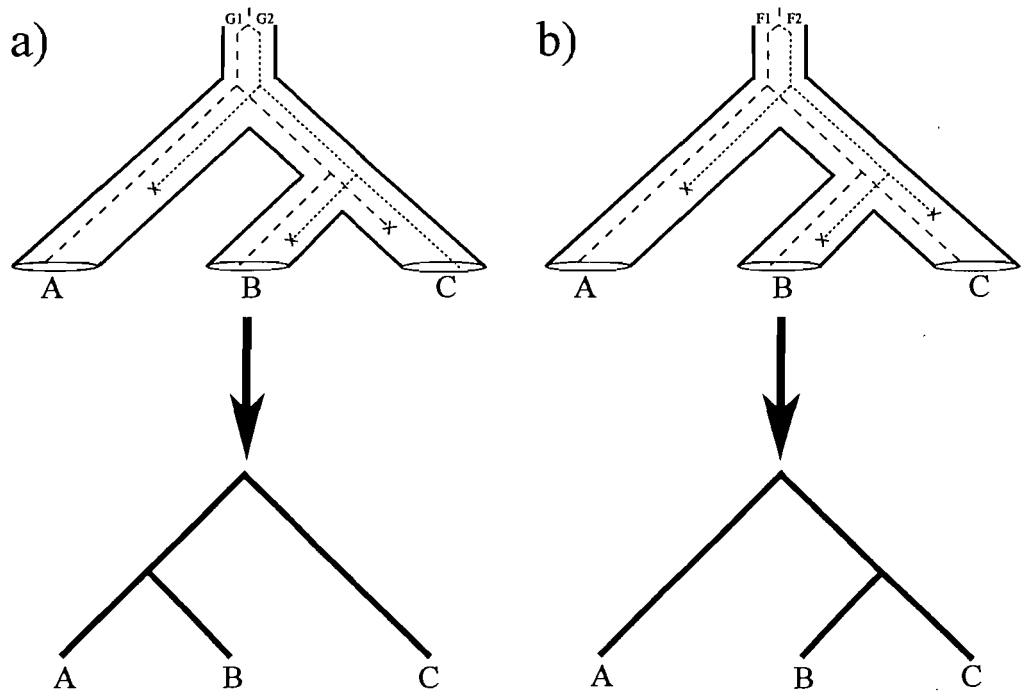

Figure 1.7 An example of the gene tree/species tree problem. The species phylogeny is represented by pipe-like lines. The gene trees are represented by black lines. (a) Prior to the root of the ABC clade, a gene (G_1, dashed line) is either duplicated or mutated to produce a new allele (G_2, pointed line). Both versions of G are inherited at the two speciation events, but G_2 is lost in the lineages leading to species A and B, and G_1 is lost in the lineage leading to species C. The tree that would be reconstructed from the paralogous versions of G would incorrectly indicate that species A is the sister species of B. (b) A second gene (F) from the ABC clade where only F_2 is lost. Using the F_1 orthologs produces the correct set of species relationships. Note that the two genes produce incongruent trees, which indicates the possibility that B is a hybrid of A and C (from Linder and Rieseberg, 2004).

CHAPTER 2

Phylogenetic relationships among diploid species of *Symphyotrichum* (Asteraceae: Astereae) based on two nuclear markers: ITS and GAPDH[1]

2.1 Résumé

La sous-tribu des Symphyotrichinae (Asteraceae: Astereae) comprend les genres *Canadanthus*, *Ampelaster*, *Psilactis*, *Almutaster*, et *Symphyotrichum*. Les relations intergeneriques et interspecifiques au sein de la sous-tribu ont été examinées dans le passé, notamment par Nesom (1994) et Semple (2005), en utilisant des approches morphologiques et cytologiques surtout. *Symphyotrichum* est le plus grand et le plus complexe genre de la sous-tribu et inclut quatre sous-genres: *Symphyotrichum* (x = 7, 8), *Virgulus* (x = 4, 5), *Astropolium* (x = 5), et *Chapmaniani* (x = 7) (Semple 2005). Dans cette étude nous avons utilisé deux marqueurs nucléaires, les espaceurs transcrits des gènes ribosomiques (ITS) et le gène nucléaire glycéraldéhyde 3-phosphate déshydrogénase (GAPDH), pour inférer les relations intergénériques et interspécifiques de la sous-tribu au niveau diploïde, et pour déterminer si ces phylogénies supportent les classifications de Nesom et de Semple. Nos résultats confirment que *Canadanthus* et *Ampelaster* sont des genres indépendants, tandis que *Psilactis* et *Almutaster* forment des polytomies avec *Symphyotrichum* et leur position s'avère ambigue. Chez *Symphyotrichum*, le sous-genre *Virgulus* est un groupe monophylétique selon l'ITS et apparaît polyphylétique dans le cas du gène GAPDH. L'ITS et le gène GAPDH ne soutiennent pas

[1] Vaezi, Jamil and Luc Brouillet. Paper to be submitted.

un statut distinct pour le sous-genre *Astropolium*, qui se groupe avec le sous-genre *Symphyotrichum*. En général, les relations interspécifiques ne sont pas résolues. Ce manque de résolution chez *Symphyotrichum* pourrait être interprété comme le résultat d'une évolution récente et rapide.

2.2 Abstract

Subtribe Symphyotrichinae (Asteraceae: Astereae) comprises *Canadanthus*, *Ampelaster*, *Psilactis*, *Almutaster*, and *Symphyotrichum*. Intergeneric and interspecific relationships within the subtribe have been investigated in the past, particularly by Nesom (1994b) and Semple (2005), using mostlhy morphological and cytological approaches. *Symphyotrichum* is the largest and most complex genus within the subtribe and includes four subgenera: *Symphyotrichum* (x= 7, 8), *Virgulus* (x= 4, 5), *Astropolium* (x= 5), and *Chapmaniani* (x= 7) (Semple 2005). In this study we used two nuclear markers, the internal transcribed spacer (ITS) of nrDNA and the low-copy nuclear gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH), to resolve intergeneric and interspecific relationships within the subtribe at the diploid level, and to determine whether our phylogenies validate the classifications of Nesom or Semple. Our results confirm the status of *Canadanthus* and *Ampelaster* as distinct genera, whereas *Psilactis* and *Almutaster* are part of polytomy with *Symphyotrichum* species and their position is equivocal. Within *Symphyotrichum*, subg. *Virgulus* is monophyletic based on ITS and appears polyphyletic based on GAPDH. Neither the ITS nor the GAPDH analyses support a distinct status for subg. *Astropolium*, which groups within subg. *Symphyotrichum*. In general, interspecific relationships within *Symphyotrichum* are unresolved. Lack of resolution in *Symphyotrichum* may be interpreted as a case of recent evolutionary radiation.

2.3 Introduction

With 215 genera including more than 3000 species worldwide, Astereae is the second largest tribe of the Asteraceae (Funk et al., 2005). Nesom and Robinson (2007) divided the tribe into 18 subtribes based in part on the nrDNA (ITS) phylogenetic study of Noyes and Rieseberg (1999).

One of these subtribes, the Symphyotrichinae, is defined as monophyletic (Xiang and Semple, 1996; Brouillet et al., 2001), and includes five genera: *Symphyotrichum* Nees (x = 4, 5, 7, 8), *Canadanthus* G. L. Nesom (x= 9), *Ampelaster* G. L. Nesom (x = 9), *Almutaster* Á. Löve & D. Löve (x = 9), and *Psilactis* A. Gray (x = 3, 4, 9). Phylogenetic analyses of the nrDNA (ITS) and of cpDNA restriction sites have shown that the last four genera form a grade from which *Symphyotrichum* is derived (Morgan, 1993, 1997, 2003; Lane et al., 1996; Xiang and Semple, 1996; Semple et al., 2001). Genus *Symphyotrichum* is mostly distributed in North and South America. More than half of the 91 species belonging to this genus are polyploid (Semple, 1985; Semple and Chmielewski, 1987; Semple et al., 1989, 1992, 1993, 2001; Semple and Cook, 2004).

The taxonomic history of *Symphyotrichum* has been complex. Two major classifications have been proposed recently for the genus by Nesom (1994b) and Semple (2005) (Table 2.1). Nesom subdivided the genus into two subgenera, *Symphyotrichum* and *Virgulus*, and 12 sections, based on morphological and cytological evidence. Semple subdivided the genus into five subgenera, *Symphyotrichum, Virgulus, Ascendentes, Astropolium,* and *Chapmaniani,* and eight sections, based on morphological, cytological, and, to some extent, nrDNA ITS phylogenetic data.

Table 2.1 Comparison of two classifications proposed by Nesom (1994b) and Semple (2005) for genus *Symphyotrichum*.

Nesom 1994		Semple 2005	
Subgenus	Section	Section	Subgenus
Symphyotrichum	*Symphyotrichum*	*Symphyotrichum*	*Symphyotrichum*
	Cordifolii		
	Dumosi		
	Occidentales		
	Concinni	*Turbinelli* (in part)	
	Conyzopsis	*Conyzopsis*	
	Oxytripolium		*Astropolium*
	Ascendentes		*Ascendentes*
Virgulus	*Grandiflori*	*Grandiflori*	*Virgulus*
		Polyliguli (in part)	
	Patentes	*Patentes*	
	Concolores	*Concolores*	
	Ericoidei	*Ericoidei*	
Genus *Eurybia* (subg. *Heleastrum*)	*Chapmaniani*		*Chapmaniani*

The study of interspecific relationships within *Symphyotrichum* has been limited to morphometric and cytological approaches (Allen et al., 1983; Jones and Young, 1983; Labrecque and Brouillet, 1996; Owen et al., 2006). High levels of morphological plasticity and extensive interspecific hybridization have resulted in difficulties in delimiting species within the genus in both diploids and polyploids (Jones and Young, 1983; Brouillet and Labrecque, 1987; Labrecque and Brouillet, 1996; Allen and Eccleston, 1998; Semple et al., 2002; Owen et al., 2006). Previous ITS-based studies of representatives of Astereae and *Symphyotrichum* resulted in unresolved phylogenies (Noyes and Rieseberg, 1999; Brouillet et al., 2001). This lack of resolution in combination with a high number of hybrid species within the genus agrees with models of recent adaptive radiation (Kim et al., 1996; Baldwin, 1997; Seehausen, 2004; Al-Shehbaz et al., 2006; Wiens et al., 2006).

Recent progress in the development of molecular markers has facilitated inferring the evolutionary history of a set of taxa and representing it in a phylogenetic tree (Takahata, 1996; Cann, 2001; Beilstein et al., 2006). Despite the fact that molecular markers may provide powerful tools for delimitating species boundaries (e.g., Brouat et al., 2004; Joly et al., 2006), the different modes of inheritance of these markers may cause phylogenetic incongruence between cytoplasmic and nuclear DNA in both plants and animals (Soltis and Kuzoff, 1995; Sota and Vogler, 2001; Okuyama et al., 2005).

The ITS region is among the most widely used molecular markers for inferring phylogenetic history at different taxonomic levels (Baldwin et al., 1995; Soltis and Soltis, 1998; Volkov et al., 2007). Despite its biparental mode of inheritance, easy amplification, and availability of universal primers, which explains the popularity of the ITS region as a phylogenetic marker, concerted evolution, which may cause homogenization of sequences within individuals or species, and insufficient resolution at low taxonomic levels, in particular, have sometimes limited the use of this marker in species delimitation and hybrid detection (Brochmann et al., 1996; Grundt et al., 2004; Volkov et al., 2007).

Therefore, single- or low-copy nuclear markers with relatively rapid evolutionary rates appear necessary to infer phylogenetic history of species groups such as *Symphyotrichum* that were probably subject to recent radiation (Maddison, 1997; Funk and Omland, 2003; Buckley et al., 2006). In a group of recently evolved taxa, such molecular markers present other difficulties in phylogeny reconstruction, however, such as the retention of ancestral polymorphisms (Doyle et al., 1999; Lihová et al., 2006), which may lead to a lack of concordance between gene trees derived from different nuclear markers (Page and Charleston, 1997; McCracken and Sorenson, 2005; Buckley et al., 2006; Lihová et al., 2006; Fehrer et al., 2007).

In the current study, our objectives are to investigate the phylogenetic relationships among diploid species of *Symphyotrichum*, and to determine whether our phylogenetic inferences validate the classification of Nesom (1994b) or Semple (2005). To address these goals we used the ITS region and the glyceraldehyde 3-phosphate dehydrogenase

(GAPDH) nuclear marker. The latter is a low-copy nuclear gene (Strand et al., 1997) that has been widely used in molecular studies (e.g., Olsen and Schaal, 1999; Camara et al., 2002; Joly et al., 2006).

2.4 Materials and Methods

2.4.1 Taxon sampling

One individual per diploid species for a total of 32 species of *Symphyotrichum* were included in the phylogenetic analyses (Table 2.2). These are representative of all subgenera and sections of the genus based on the classification of Semple (2005). Among these, we included 19 species of sect. *Symphyotrichum*, all three species of sect. *Conyzopsis*, seven species of subg. *Virgulus*, two species of subg. *Astropolium*, and *S. chapmanii* of subg. *Chapmaniani*. We did not include representatives of sections *Concinni* (sensu Nesom), *Turbinelli* (sensu Semple), and subgenus (sensu Semple) /section (sensu Nesom) *Ascendentes*. Sections *Concinni* and *Turbinelli* comprise only polyploid species and *Ascendentes* includes two species (*S. ascendens* and *S. defoliatum*) with a hybrid nature (Allen and Eccleston, 1998). To investigate phylogenetic relationships among the genera of Symphyotrichinae (*Ampelaster*, *Almutaster*, *Psilactis*, *Canadanthus,* and *Symphyotrichum*), we included one species for the first four genera in the phylogenetic analyses, although three genera, *Almutaster, Ampelaster,* and *Canadanthus* are monotypic. We rooted our phylogenetic trees with one closely and one distantly related genus, *Oreostemma* and *Heterotheca,* respectively (Xiang and Semple, 1996; Noyes and Rieseberg, 1999; Brouillet et al., 2001).

Table 2.2 Specimens included in the study of Symphyotrichum and related genera. The base chromosome number (x) for each species and Genbank accessions are indicated in the last three columns. Taxonomic nomenclature of the taxa is based on the classification of Semple (2005).

Taxon	Locality	Collector(s)	x	GenBank accession	
				ITS [a]	GAPDH
OUTGROUPS:					
Heterotheca monarchensis D.A. York, Shevock & Semple	Kern Co./ Calif.	Shwock & York, 109 (WAT)	9	-	EU708562-3
Oreostemma alpigenum (T. & G.) Greene	Mt. Hood/ Oreg.	Semple, 10271 (WAT)	9	EU200219 [c]	EU708564-5
Canadanthus modestus (Lind.) G.L. Nesom	Swift Current/ Sask.	Hudson, 3997 (Sask)	9	EU781457	EU708567
Ampelaster carolinianus (Walter) G.L. Nesom	Davenport/ Fla.	Semple, 5354 (WAT)	9	EU200185 [c]	EU708566
Almutaster pauciflorus (Nuttall) Á. Löve & D. Löve	Oakburn/ Man.	Marchand, 1983 (Sask)	9	EU781462	EU708569
Psilactis tenuis S. Watson	JeffDavis Co./ Tex.	Semple, 8201 (WAT)	9	-	EU708568
SUBG. *SYMPHYOTRICHUM*:					
Sect. *Symphyotrichum*					
Subsect. *Symphyotrichum*					
S. elliottii (T. & G.) G.L. Nesom	Onslow Co./ N.C.	Semple, 10538 (WAT)	8	EU853710 [b]	EU708514
S. puniceum (L.) Á. Löve & D. Löve	Marion Co./ N.C.	Semple, 10853 (WAT)	8	EU781142-3	EU708512-3
S. firmum (Nees) G.L. Nesom	Lake Co./ Mont.	Gerdes, 4945 (NM)	8	EU781250	EU708540-1
Subsect. *Heterophylli*					
S. anomalum (Engel. ex T. & G.) G.L. Nesom	Carroll Co./ Ark.	Semple & Suripto, 9950 (WAT)	8	EU781321-6	EU708547
S. cordifolium (L.) G.L. Nesom	Carleton Co./ N.B.	Semple & Keir, 4670 (MT)	8	EU781411-2	EU708552-3
S. drummondii (Lind.) G.L. Nesom	Newton Co./ Tex.	Semple, 10049 (WAT)	8	EU781140-1	EU708554
S. shortii (Lind.) G.L. Nesom	Adair Co./ Ky.	Semple & Suripto, 9449 (MT)	8	EU781413-4	EU708555-6
S. undulatum (L.) G.L. Nesom	Orangeburg Co./ S.C.	Semple & Chmielewski, 6133 (MT)	8	EU781415-6	EU708557-8
S. urophyllum (Lind. ex de Cand.) G.L. Nesom	Elgin Co./ Ont.	Semple, 10594 (WAT)	8	EU781138-9	EU708510-1
Subsect. *Occidentales*					
S. foliaceum (Lind. ex de Cand.) G.L. Nesom	Missoula Co./ Mont.	Semple, 10310 (WAT)	8	EU781463-4	EU708545-6
Subsect. *Dumosi*					
S. dumosum (L.) G.L. Nesom	Amite Co./ Miss.	Semple & Suripto, 10102 (MT)	8	EU781402-6	EU708560-1
S. lateriflorum (L.) Á. Löve & D. Löve	Prince Edward/ Ont.	Brouillet & Brammall, 587 (MT)	8	EU781418	EU708537-8
S. nahanniense (Cody) Semple	Nahanni N.P.R./ N.W.T.	Semple, 11161 (WAT)	8	EU781252-3	EU708543-4
S. racemosum (Elliott) G.L. Nesom	Wayne Co./ Miss.	Semple, 9895 (WAT)	8	EU853715 [b]	EU708533-4
S. tradescantii (L.) G.L. Nesom	Lévis/ Que.	Bouchard & Cuerrier, K-11 (MT)	8	EU853717 [b]	EU708548-9
S. welshii (Cronquist) G.L. Nesom	Lake Co./ Mont.	Semple, 11374 (WAT)	8	EU781407-8	EU708542
Subsect. *Porteriani*					
S. depauperatum (Fern.) G.L. Nesom	Nottingham/ Pa.	Semple, 7681 (WAT)	8	EU200226 [c]	EU708531-2
S. parviceps (E.S. Burgess) G.L. Nesom	Adams Co./ Ill.	Semple & Brouillet, 7378 (MT)	8	EU781417	EU708559

Table 2.2 *continued ...*

Taxon	Locality	Collector(s)	x	GenBank accession	
				ITS [a]	GAPDH
S. porteri (A. Gray) G.L. Nesom	Clear Creek Co./ Colo.	Semple, 10470 (WAT)	8	EU853714 [b]	EU708529-30
Sect. *Conyzopsis*					
S. frondosum (Nuttall) G.L. Nesom	Lake Co./ Oreg.	Houle & Legault, 45 (MT)	7	EU853711 [b]	EU708525-6
S. ciliatum (Ledeb.) G.L. Nesom	Manitoulin/ Ont.	Morton & Venn, 9942 (MT)	7	EU781410	EU708528
S. laurentianum (Fern.) G.L. Nesom	Ile de la Madeleine/ Que.	Houle & Brouillet, 81 (MT)	7	EU853712 [b]	EU708527
SUBG. *VIRGULUS*					
Sect. *Concolores*					
S. plumosum (Small) Semple	Franklin Co./ Fla.	Semple, 10929 (WAT)	4	EU853713 [b]	EU708517-8
S. concolor (L.) G.L. Nesom	Laurens Co./ Ga.	Semple, 4040 (MT)	4	EU781460-1	EU708515-6
S. sericeum (Ventenat) G.L. Nesom	Rainy river/ Ont.	Semple & Heard, 8787 (WAT)	5	EU200232 [c]	EU708519-20
Sect. *Grandiflori*					
S. oblongifolium (Nuttall) G.L. Nesom	Webster Co./ Nebr.	Semple & Brouillet, 7337 (MT)	5	EU781459	EU708521
S. yukonense (Cronquist) G.L. Nesom	Kluane Lake/ Yukon	Semple, 10624 (WAT)	5	EU200234 [c]	EU708524
Sect. *Polyliguli*					
S. novae-angliae (L.) G.L. Nesom	Tenton/ Ga.	Semple, 11001 (WAT)	5	EU200229 [c]	EU708539
Sect. *Ericoidei*					
S. ericoides (L.) G.L. Nesom	Mound City/ S.Dak.	Semple, 6664 (WAT)	5	EU200227 [c]	EU708522-3
SUBG. *ASTROPOLIUM*					
S. subulatum (Michaux) G.L. Nesom	Marengo Co./ Ala.	Semple & Chmielewski, 6362 (MT)	5	EU781409	EU708535
S. tenuifolium (L.) G.L. Nesom	Cedar Run/ N.J.	Semple, 9519 (WAT)	5	EU200233 [c]	EU708536
SUBG. *CHAPMANIANI*					
S. chapmanii (T. & G.) Semple & Brouillet	Choctawhatchee R./ Fla.	Semple, 10560 (WAT)	7	EU200223 [c]	EU708550-1

a. Accession numbers of *Heterotheca fulcrata* (Greene) Shinners (U97615, Morgan, 1997) and *Psilactis asteroides* A. Gray (U97640, Morgan, 1997), available in GenBank, are used in this study.
b. Accession numbers submitted by Brouillet et al., (in preparation).
c. Accession numbers submitted by Selliah & Brouillet, 2008.

2.4.2 Molecular methods

2.4.2.1. DNA extraction

Silica-dried and herbarium leaves of all species were used for DNA extraction using the Doyle and Doyle (1987) CTAB protocol followed by a modification (Joly et al., 2006), or with the QIAgen DNeasy Plant Mini Kit (QIAGEN, Mississauga, Ontario, Canada), following the instructions of the manufacturer.

2.4.2.2 Primer design

In a preliminary phase of this study, the ITS region was amplified using the universal primers 17SE-26SE (Sun et al., 1994), also named AB101-AB102 (Douzery et al., 1999). The amplified band on the electrophoresis gel was unique but after sequencing, chromatograms were often difficult to read; it was not always possible to distinguish single nucleotide polymorphisms (SNPs) from noise. Therefore, the PCR products of three individuals were cloned (see below) and the aligned sequences of the most conserved 5′and 3′ end regions were used to design a new set of more internal primers: ITSvF (5′-AGGAAGGAGAAGTCGTAACAAGG-3′) and ITSvR (5′-GATATGCTTAA ACTCAGCGG-3′). Direct sequencing using the more specific primers showed clearly the singleton polymorphisms and noise was eliminated.

For the GAPDH gene, we designed a primer pair by blasting partial mRNA sequence of *Scaevola procera* (Genbank accession number: AY894500) with similar sequences of Asteridae followed by alignment with a complete GAPDH sequence of *Arabidopsis thaliana* (Genbank accession number: AC068324) using MegAlign software package (Lasergene, DNASTAR Inc). The alignment was refined manually. Subsequently, two conserved 5′→ 3′ regions between 3rd and 6th exons were selected as a primer pair: GAPDHx3F (5′-TTGAGGGTCTTATGACTACAGT-3′) and GAPDHx6R (5′-GGTGTATCCCAAGATACCCTTGAGC-3′). After amplification and sequencing (see below) we obtained ambiguous and unreadable chromatograms. Cloning (see below) was applied to identify alleles. After aligning the alleles with the complete sequence, we identified some pseudogenes which lacked the 4th exon. To prevent amplification of these

pseudogenes, we designed a new primer within the 4th exon (GAPDHx4F: 5′-AGGACTG GAGAGGTGGAAGAGC-3′). The primers were designed using the Amplify program version 3.1.4 (Engels, 2005).

2.4.2.3 PCR amplification, Sequencing and Cloning

Amplification of the ITS1-5.8S-ITS2 region was done in 25µl reactions containing 2.5µl 10×PCR reaction buffer (Roche Diagnostics, Indianapolis, IN, USA), 0.5 µl $MgCl_2$ (25 mM, Promega, Madison, WI, USA), 100 µmol/L of each dNTP, 1 µl DMSO, two units of *Taq* Polymerase, ca. 200 ng genomic DNA and 1 mmol/L of each primer. An initial denaturation step at 94 °C for 3 min was followed by 35 cycles of denaturation (30 s at 94 °C), annealing at 52 °C for 30 s, elongation at 72 °C for 2 min, and final extension at 72 °C for 10 min. A long elongation step (2 min) was used to reduce putative PCR recombinants (Judo et al., 1998; Cronn et al., 2002). The GAPDH gene was amplified using the same conditions as for the ITS region with the exception that the amplification was performed in 40 cycles and the annealing temperature was set at 64 °C. The PCR-product purification and sequencing steps were the same as in Joly et al. (2006).

Direct sequences with two or more SNPs were cloned using the pGEM-T vector (Promega Corporation, WI, USA) transformed into competent E. coli DH5-α at 42°C. The transformed bacteria were screened on a selective and solid LB Petri dish media containing 50 mg/ml kanamycin, 100 mg/ml ampicilin, 50 mg/ml X-gal, and 0.5 M of IPTG (Isopropyl β-D-1-thiogalactopyranoside) at 37°C overnight. Twelve to 15 positive colonies were selected and cultivated overnight in 1.5 ml eppendorf containing LB liquid as well as the two antibiotics. Positive cultures were amplified and sequenced using the same protocol as for direct sequencing.

2.4.3 Data analyses

2.4.3.1 Phylogenetic analyses

The ITS and GAPDH sequences were aligned using Clustal W (Thompson et al., 1994) as implemented in BioEdit Sequence Alignment Editor (Hall, 1999); the results were manually modified to maximize the numbers of homologous characters and minimize the numbers of insertions and deletions (indels). After aligning the sequences, almost half of the GAPDH sequences had three stop codons at the 5′ end of the 5th exon. These sequences were considered pseudogenes and were removed from the matrix. Pseudogenes were found in most of the diploid species. Repeated intraindividual sequences were removed using the Collapse program v.1.2. (Posada, 2004). Indels were coded using the simple gap-coding method (Simmons and Ochoterena, 2000) as implemented in SEQSTATE (Müler, 2005).

Before determining the best-fit substitution model of sequence evolution, the ITS and GAPDH sequences were partitioned as follows: ITS: ITS1, 5.8S, and ITS2; GAPDH: 4th intron, 5th exon, and 5th intron. For each data partition, the Akaike Information Criterion (AIC; Akaike, 1973) and the Likelihood Ratio Test (LRT; Felsenstein, 1988) were used to identify best-fitting models as implemented in MrModeltest 2.2 (Nylander, 2004) with executable MrModelblock file in PAUP* version 4.10b (Swofford, 2002). The choice of the two model selection criteria has been controversial in phylogenetic studies (Savill et al., 2001; Posada and Buckley, 2004; Domonicus et al., 2006). Some authors prefer to use both criteria in phylogenetic inferences (Beilstein et al., 2006; Fehrer et al., 2007), whereas others apply either LRT or AIC (e.g., Goldman et al., 2000; Rabosky, 2006). We used Bayes factors (Kass and Raftery, 1995) to evaluate which competing criterion (LRT or AIC) provided the best phylogenetic estimation. In previous studies, this approach has been applied to select single versus multiple DNA data partitions (Nylander et al., 2004; McGuire et al., 2007). Many methods have been proposed (reviewed in Nylander et al., 2004) to calculate Bayes factors. We used the method proposed by Newton and Raftery (1994), which applies the harmonic mean of

likelihood values as provided by MrBayes from MCMC analysis of the posterior distribution after burn-in. We accepted Bayes factors greater than two (considered to be "positive" according to the Kass and Raftery (1995) rule) in support of a criterion with higher harmonic mean log likelihood.

Bayesian analyses were performed for two million generations for both data sets, each with two replicates (LRT and AIC) using MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001). We used four Markov Chains at default temperature setting for each run and trees were sampled every 100 generations. To assess convergence of the topologies, we compared the posterior probabilities of different splits between pairs of identical runs using TRACER version 1.3 (Rambaut and Drummond, 2003). After excluding the 2000 trees found in the burn-in phase, the 50% majority-rule consensus trees were computed. Tree visualization was carried out using Tree View version 1.6.6 (Page, 2001).

2.4.3.2 Incongruence length difference test

One of the most frequently used strategies to infer species phylogeny from multiple genes is to combine data (Kluge, 1989), although many authors have indicated that this strategy is not suitable for genes with different histories (Bull et al., 1993; DeQueiroz et al., 1995). To determine whether the genes under study have had similar evolutionary histories, we used the incongruence length difference (ILD) test (Farris et al., 1994) coupled with the BIONJ algorithm (ILD-BIONJ) (Gascuel, 1997) to detect the presence of strongly supported character conflict among individual data sets within a combined analysis. Cunningham (1997) concluded that the ILD test performed the best in predicting when data should be combined, compared with the tests of Templeton (1983) and Rodrigo et al. (1993). This test needs two matrices of identical sizes. The numbers of sequences in the two matrices differ (44 for the ITS and 62 for the GAPDH, Table 2.2). To combine the two matrices, for a species when a single ITS ribotype corresponded to two alleles of GAPDH or vice versa, a sequence duplication was performed. One hundred replications with heuristic searches and TBR branch swapping were used to assess the

congruence between the two data sets. The test was implemented in PAUP* version 4b 10 (Swofford, 2002) using the partition homogeneity test.

2.4.3.3. Gene trees in a species tree: a simulation approach

A gene tree which is constructed from nucleotide variation is an illustrational method to represent not only the evolutionary history of a gene but also to more or less reflect the evolutionary history of species, particularly when a single allele is sampled from each species. In contrast, when two or more alleles from each species are sampled, inconsistency between a gene tree and the species tree is likely because of a retention and arbitrary sorting of ancestral polymorphisms at shallow time depths. This may lead to incorrect inferences about the relationships among species (Pamilo and Nei, 1988; Doyle, 1992; Lyons-Weiler and Milinkovitch, 1997; Maddison and Knowles, 2006).

To consider a gene tree as evidence of the species tree we can embed the gene tree within the species tree. An optimal species tree is that in which the correspondent gene tree can be embedded with the least cost (Page, 1998). The numbers of duplications, losses, and deep coalescences are the estimators by which we can evaluate the cost (Maddison, 1997). A straightforward use of this strategy here is to topologically compare the classifications of Nesom (1994b) and Semple (2005). We do not have species tree representing the interspecific relationships of *Symphyotrichum* species based on the classifications of Nesom and Semple. Thus, we traced two species trees each according to these classifications using Mesquite version 1.11 (Maddison and Maddison, 2006). The ITS and GAPDH phylogenetic trees are embedded into the species trees using deep coalescence criterion (Maddison, 1997).

2.5 Results

2.5.1 ITS analysis

The ITS matrix comprises 44 sequences and 633 aligned characters excluding coded indels. The Bayes factors positively supported the evolutionary models suggested by the AIC criterion (Table 2.3). The phylogenetic tree (Fig. 2.1) supported (Posterior probability (PP) = 0.84) the monophyly of subtribe Symphyotrichinae, which is discriminated by two transitions and two transversions from the outgroups. Two genera, *Canadanthus* and *Ampelaster*, are positioned as sister to *Symphyotrichum*, *Psilactis*, and *Almutaster*. Genus *Symphyotrichum* does not form a monophyletic group. Genera *Almutaster* and *Psilactis* constitute polytomous branches with *Symphyotrichum*. Subgenus *Virgulus* forms a well-supported monophyletic group (PP = 1.00) with one transition and three indels as discriminating characters. All other subgenera are grouped together as a strongly supported clade (PP = 0.93). *Symphyotrichum chapmanii* is sister to the subgenera *Symphyotrichum* and *Astropolium* with strong support (PP = 0.95). The representatives of subg. *Astropolium* are grouped within subg. *Symphyotrichum*. Within subg. *Symphyotrichum*, neither sect. *Conyzopsis* (x = 7) nor sect. *Cordifolii* (x = 8) form a monophyletic group.

Table 2.3 Statistical information of each partition for two molecular markers, ITS and GAPDH, and selected DNA substitution models by Bayes factors.

Marker	Length	Variable sites		Informative sites		Evolutionary model (LRT/AIC)	-HML (LRT)	-HML (AIC)	2 ln Bayes factors
		No.	%	No.	%				
ITS									
ITS1	255	76	30	31	12	K80+G/SYM+G			
5.8S	164	3	1.8	1	0.6	JC/JC			
ITS2	214	72	34	30	14	SYM+G/SYM+G			
Total	633	141	22	55	8.7		2428.73	2427.19	3.08
GAPDH									
Intron 4	369	130	35	77	21	HKY+G/HKY+G			
Exon 5	151	33	22	11	7.3	K80/K80			
Intron 5	171	61	36	34	20	HKY/HKY+I			
Total	691	224	32	122	17.7		3336.24	3328.85	14.78

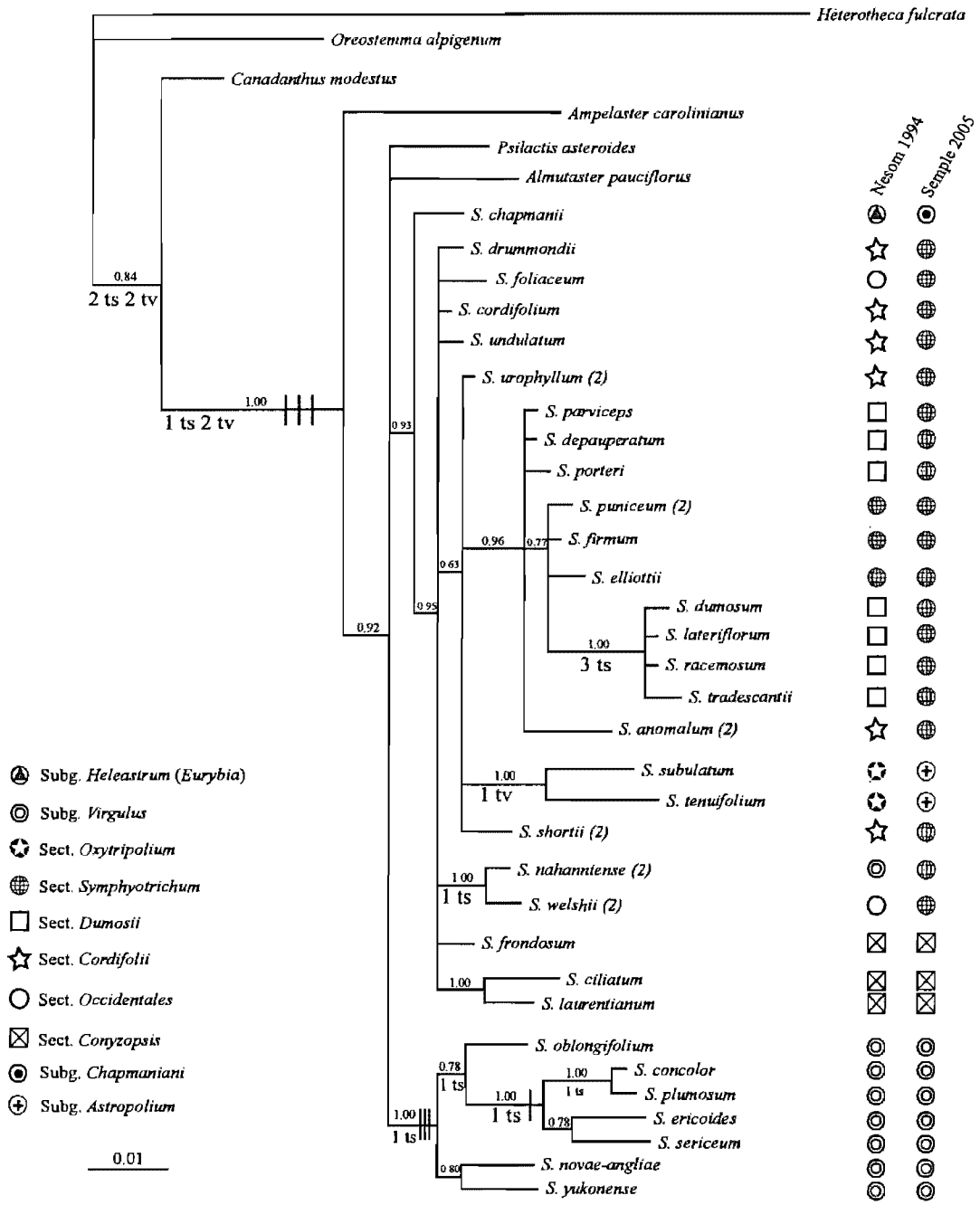

Figure 2.1 Bayesian 50% majority rule consensus phylogram of the ITS data set. The classifications of Nesom (1994b) and Semple (2005) are compared using symbols for each subgenus or section. Posterior probabilities are provided above the branches. The number and type of substitutions (ts= transition and tv= transversion) are given below the branches. The bars on the branches indicate the indels. The number of the ribotypes (if more than one) found for each species is indicated in the parentheses.

2.5.2 GAPDH analysis

The GAPDH data set includes 60 sequences and 691 aligned characters excluding coded gaps. The Bayes factors very strongly supported the evolutionary models suggested by the AIC criterion (Table 2.3). The phylogenetic tree (Fig. 2.2) supports the monophyly of subtribe Symphyotrichinae as a highly supported clade (PP = 1.00) which is discriminated by one indel and two substitutions from the outgroups. *Canadanthus modestus* is the sister species to other genera of Symphyotrichinae with moderately strong support (PP = 0.72). Genus *Symphyotrichum* does not form a monophyletic group. *Ampelaster carolinianus* and *Psilactis asteroides* together are sister to *Symphyotrichum* and *Almutaster pauciflorus*. The latter species is grouped with members of subg. *Virgulus*. Within *Symphyotrichum*, none of the subgenera and sections (sensu Semple, 2005) form distinct clades. In general, infraspecific variation appears to be greater than interspecific: the two alleles of a species are not monophyletic, but instead each groups with homologous alleles from other species. For instance, the two alleles of *S. urophyllum* are grouped with their homologs of *S. cordifolium* in two independent clades.

2.5.3 ILD test

The ILD-BIONJ congruence test rejected the null hypothesis of congruence (P-value = 0.01) between the ITS and GAPDH data sets.

2.5.4 Nesting the gene trees within the species tree

Under the criterion of minimizing deep coalescences, 43, 14, and 59 deep coalescences, duplications, and losses, respectively, are needed to fit the ITS phylogenetic tree within the species tree derived from the classification of Semple (2005), whereas these values are 51, 19, and 77, respectively, for embedding the ITS tree within the species tree derived from the classification of Nesom (1994b). Similarly, 151, 34, and 178 deep coalescences, duplications, and losses, are respectively needed to fit the

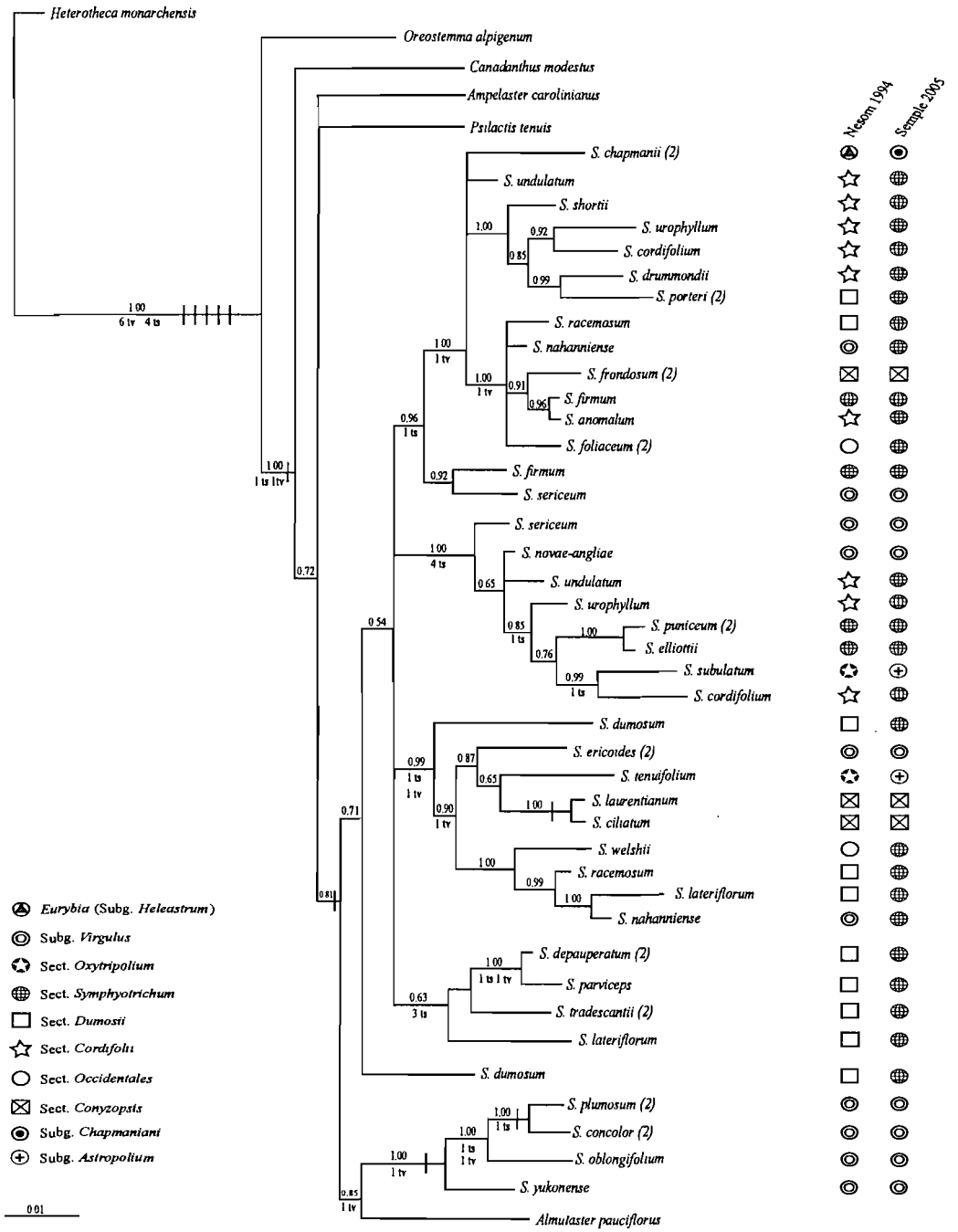

Figure 2.2 Bayesian 50% majority rule consensus phylogram of the GAPDH data set. The classifications of Nesom (1994b) and Semple (2005) are compared using symbols indicated for each subgenus and section. Posterior probabilities are provided above the branches. The number and type of substitutions (ts= transition and tv= transversion) are given below the branches. The bars on the branches indicate the indels. The number of the alleles (if more than one) found for each species is indicated in the parentheses.

GAPDH phylogenetic tree within the species tree derived from the classification of Semple, whereas these values are 208, 38, and 235, respectively for the species tree derived from the classification of Nesom. We illustrated the incongruence between the two classifications by symbols corresponding to the subgenera and sections of each classification using both phylogenetic trees (Figs. 2.1 and 2.2). The inferred gene trees show conspicuous incongruences with respect to both classifications in the placement of some taxa.

2.6 Discussion

The ITS phylogenetic tree fits better within both species trees than the GAPDH tree based on the number of deep coalescences, which is higher in the GAPDH tree (151 and 208) than in the ITS tree (43 and 51) in both classifications. The ILD test confirms that the two data sets could not be combined. It is not surprising to observe incongruence between two unrelated data sets, one with high-copy numbers and probably concerted copies, and the other with low-copy numbers that are unlikely to have undergone concerted evolution. However, the ILD result is in agreement with the different number of deep coalescences when embedding the gene trees within the species trees.

Based on the number of deep coalescences (see the results), which is higher in the case of the classification of Nesom (1994b), it appears that both phylogenetic trees generally fit better with the classification of Semple (2005).

Both the ITS and GAPDH phylogenetic trees support the monophyly of subtribe Symphyotrichinae as seen in previous studies (Xiang and Semple, 1996; Brouillet et al., 2001). The position of monospecific, *Canadanthus* and *Ampelaster* as independent genera proposed first by Nesom (1994b), is more resolved and supported in the ITS tree than in the GAPDH tree. In contrast, the position of *Almutaster* and *Psilactis* as independent genera appears to be equivocal in both phylogenetic trees. The status of these two genera has not been stable in previous studies. Sundberg (1986) hypothesized that the monospecific, *Almutaster* is an alloploid taxon derivative of a cross between

members of subg. *Virgulus* and *Psilactis* based on morphological similarities (see also Nesom, 2000). Though this hypothesis had not been confirmed in previous studies based on isozyme loci (Gottlieb, 1981) and karyologic data (Stucky and Jackson, 1975), placement of *Almutaster* within subg. *Virgulus* (apart from retention of ancestral polymorphisms) in the GAPDH tree (Fig. 2.2) may revive the hypothesis. Another possible origin of *Almutaster* was proposed by Xiang and Semple (1996) and Lane et al. (1996) who suggested an allopolyploid origin for the genus as a hybrid derivative from *Psilactis* and subg. *Astropolium* based on morphological and molecular (cpDNA) approaches. This hypothesis is not supported based on our phylogenetic trees. However, by increasing the number of individuals and species of *Almutaster* and *Psilactis*, respectively, phylogenetic support may be obtained for the independence of these two genera.

Subgenus *Virgulus* is morphologically (Nesom, 1994b) and cytologically (Semple and Brouillet, 1980b) distinct relative to the remaining subgenera of *Symphyotrichum*. It has had a controversial position. Semple and Brouillet (1980a) treated it as an independent genus, *Lasallea*, close to the *Machaeranthera* lineage based on morphological and cytological similarities. Nesom (1994b) rejected this hypothesis and placed it within *Symphyotrichum* based on morphological similarities and natural hybridization between them (subg. *Ascendentes*, Jones, 1977; Allen, 1986; Allen and Eccleston, 1998).The distinctive status of this subgenus as a monophyletic group based on the ITS analysis with one substitution and three indels (Fig. 2.1) supports results from previous studies (Jones, 1980a; Semple and Brouillet, 1980a; Nesom, 1994b). It is polyphyletic based on the GAPDH result. Our results also indicate no close relationship between the members of subg. *Virgulus* and *Ampelaster* as suggested by Xiang and Semple (1996) based on cpDNA restriction site analyses. Within subg. *Virgulus*, *S. concolor* and *S. plumosum*, which were recently segregated by Semple et al. (2002) as two independent species, are strongly supported as closely related species in both phylogenetic analyses. Placement of *S. nahanniense* within subg. *Virgulus* as a synonym to *S. falcatum* var. *commutatum*, as proposed by Nesom (1994b), was strongly rejected based on both phylogenetic analyses (see also Owen et al., 2006). The ITS result

indicates that within subg. *Virgulus*, *S. novae-angliae* (the single member of sect. *Polyliguli*, sensu Semple) is closely related to *S. yukonense* (a member of sect. *Grandiflori*). This evidence is in agreement with the classification of Nesom, rather than splitting the aggregate sect. *Grandiflori* as done by Semple (Table 2.1). Moreover, both classifications placed *S. sericeum* (x = 5) within sect. *Concolores* (x = 4, 5). Based on the ITS analysis, this species is sister to *S. ericoides*. This suggests that *S. sericeum* might be a member of sect. *Ericoidei* (x = 5 exclusively).

Subgenus *Chapmaniani* (Semple, 2005) is a monotypic taxon (*S. chapmanii*) with a base chromosome number of x = 7. Jones and Young (1983) hypothesized a hybrid origin for the species, derivative from subg. *Heleastrum* (x = 9) of genus *Eurybia* and subg. *Astropolium* (x = 5) based on cytological and morphological evidence. Nesom (1994b) treated it within subg. *Heleastrum* based on similarities in leaves and capitulescences; the different chromosome numbers were interpreted as a reduction from x = 9 to x = 7. However, based on both phylogenetic analyses, we confirm the taxonomic treatment of subg. *Chapmaniani* within *Symphyotrichum* as suggested by Semple. *Symphyotrichum chapmanii* is sister to the remaining subgenera based on the ITS analysis, while this position is not supported based on the GAPDH tree.

Section *Oxytripolium* (x = 5) which was placed within subg. *Symphyotrichum* (x = 7, 8) by Nesom (1994b), was upgraded to subgeneric level as subg. *Astropolium* by Semple (2005) based on the different base chromosome numbers. Semple and Brouillet (1980a) suggested a close relationship between this taxon and subg. *Virgulus* due to the similar base chromosome number of x = 5, though they are distinct in NOR chromosome morphology (Semple and Brouillet, 1980b). Our results, especially the ITS tree, reject this idea. This taxon, represented by *S. subulatum* and *S. tenuifolium*, forms a well-supported monophyletic group (PP = 1.00) within subg. *Symphyotrichum* based on the ITS tree and it is characterized by a single transversion. The position of this taxon as sect. *Oxytripolium* within *Symphyotrichum* appears to fit better with our results and no support was obtained to upgrade it to the subgeneric level as suggested by Semple.

Nesom (1994b) and Semple (2005) placed sect. *Conyzopsis* (x = 7) within subg. *Symphyotrichum* based on their similarities in morphological traits (reduced vestiture, lack of glands, and unkeeled phyllaries, Nesom, 1994b) and NOR morphology (Semple and Brouillet, 1980b). Nesom (1994b) hypothesized that the base chromosome number of x = 7 is derived from ancestors with x = 8. This section with three members, *S. ciliatum*, *S. laurentianum*, and *S. frondosum,* is distinguished from other members of subg. *Symphyotrichum* by having 2-3 series of ray florets, pappi longer than disc florets, and its base chromosome number. Both phylogenetic analyses strongly supported (PP = 1.00) close relationships between *S. ciliatum* and *S. laurentianum*. These species have a single deletion of 152 nucleotides in length within the 4th intron of the GAPDH gene. In contrast, two GAPDH alleles obtained from *S. frondosum* do not have this long deletion. However, despite its distinct features outlined above, our phylogenetic results do not support the section as a monophyletic group within subg. *Symphyotrichum* as suggested in previous studies (Houle and Brouillet, 1985; Houle and Haber, 1990; COSEWIC, 2004).

Section *Symphyotrichum* (sensu Semple, 2005) comprises the majority of the species (ca. 52) of the genus. A high proportion of these species is polyploid (Semple and Brammall, 1982; Allen et al., 1983; Dean and Chambers, 1983; Brouillet and Labrecque, 1987; Nesom, 1994c; Semple and Cook, 2004 and references therein). In both phylogenetic analyses, the sections defined by Nesom (1994b) were not supported as monophyletic. Our results (Figs. 2.1 and 2.2) support merging these sections into one (sect. *Symphyotrichum*) as suggested by Semple (2005) (Table 2.1).

Lack of sufficient phylogenetic resolution within *Symphyotrichum* based on the ITS analysis (8.7% informative sites including outgroup ribotypes, Table 2.3) and abundant interspecific hybridization, particularly within subg. *Symphyotrichum* (Brouillet and Labrecque, 1987; Allen and Eccleston, 1998; Semple et al., 2002) may be interpreted as the occurrence of a recent radiation in the evolutionary history of the genus. Despite the greater amount of variation observed (Table 2.3) among the GAPDH alleles (32%) relative to the ribotypes (22%), a major proportion of this variation appears to be intraspecific rather than interspecific. For instance, the GAPDH alleles of *S. sericeum*, *S.*

firmum, *S. dumosum*, *S. undulatum*, and *S. cordifolium* are grouped with their homologs from other species. Moreover, based on the GAPDH tree, none of the ITS-based clades within genus *Symphyotrichum* (e.g., subg. *Virgulus*) is distinct or monophyletic. These evidences indicate that two processes might be involved: introgression or incomplete lineage sorting (Lyons-Weiler and Milinkovitch, 1997; Page and Charleston, 1997; Wendel and Doyle, 1998). Introgression arises when a similar allelic position occurs non-randomly in introgressed species in the topologies, whereas stochastic sorting of ancestral polymorphisms into descendant species results from lineage sorting. Incomplete lineage sorting potentially contributes to phylogenetic incongruence at lower taxonomic levels (Rieseberg and Soltis, 1991; Soltis and Kuzoff, 1995) and occurs when allelic divergence preceded speciation. Many recent studies have shown that lineage sorting is prevalent in recent and rapidly radiating species with short evolutionary terminal branches that reflect a relatively recent diversification (e.g., Ballard et al., 2002; Shaw, 2002; Broughton and Harrison, 2003; Hughes and Volger, 2004; Buckley et al., 2006).

Several studies have demonstrated that concerted evolution may homogenize the multiple copies of nrDNA organized in tandemly repeated units and quickly eliminate the traces of introgression (e.g., Franzke and Mummenhoff, 1999; Fuertes-Aguilar et al., 1999). On the other hand, the GAPDH marker which has been recognized as a low-copy nuclear marker is unlikely to have experienced concerted evolution. Though incongruence among a group of closely related but allopatric diploid species is more likely to be interpreted as incomplete lineage sorting rather than introgression (Comes and Abbott, 2001; Fehrer et al., 2007), it appears to be difficult here to discriminate between these events using two molecular markers with possibly two different evolutionary histories that are difficult to compare.

To resolve the phylogenetic relationships among a group of recently diverged species it seems we need markers evolving with high mutation rates in the terminal branches of the phylogeny corresponding to species. Using these markers would increase the probability of fixing polymorphisms within each branch leading to species rather than these being shared by related species, and thus they would be useful to obtain a correct

species delimitation. Incomplete sampling of alleles is another aspect that increases the effects of incomplete lineage sorting. Sampling more individuals per species could increase the probability of sampling more alleles within a species and decrease the frequency of incomplete lineage sorting events (Linder and Rieseberg, 2004; Maddison and Knowles, 2006).

CHAPTER 3

Origin of *Symphyotrichum anticostense* (Asteraceae: Astereae), an endemic, high polyploid species of the Gulf of St. Lawrence, based on morphological and nrDNA evidence[2]

3.1 Résumé

Symphyotrichum anticostense (Fernald) G. L. Nesom (Asteraceae: Astereae), une espèce endémique et rare de la région du Golfe de St. Laurent (Québec, Nouveau Brunswick, et Maine), est un haut allopolyploïde (2n = 10x = 80). On a proposé qu'il soit dérivé de l'hybride entre des individus tetraploïdes (2n = 4x = 32) du *S. boreale* (Torr. & A. Gray) Löve et Löve et l'hexaploïde (2n = 6x = 48) *S. novi-belgii* (L.) G. L. Nesom. Nous avons examiné cette hypothèse en utilisant des données morphologique et moléculaire (séquences de l'ITS ribosomique) pour vérifier la relation entre *S. anticostense* et ses parents potentiels, et afin de déterminer le nombre potentiel d'origines géographiques du taxon. Toutes les espèces diploïdes disponibles du genre *Symphyotrichum* ont été incluses dans l'analyse moléculaire afin d'examiner l'implication possible de ces espèces dans l'hybridation. L'analyse morphologique univariée montre que 67,5% des caractères de *S. anticostense* sont parentaux, 43,5% de ceux-ci du *S. novi-belgii* et 13% du *S. boreale*, le reste ne différant pas statistiquement des parents ; 23,5% sont intermédiaires; et 9% seraient transgressifs. Les analyses multivariées (ACP et ACD) montrent généralement que le *S. anticostense* est intermédiaire entre les parents proposés.

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[2] Vaezi, Jamil and Luc Brouillet. Paper to be submitted.

À cause de la résolution insuffisante des ribotypes sur l'arbre phylogénétique, une analyse de réseau a été construite. Les résultats moléculaires paraissent soutenir les résultats morphologiques, mais en raison de la résolution insuffisante des ribotypes, un marqueur évolutivement plus rapide serait nécessaire pour vérifier l'origine du *S. anticostense*. Selon l'étude moléculaire, trois origines indépendantes seraient possibles pour le *S. anticostense* ; 1) Ile d'Anticosti, 2) Lac Saint-Jean, et 3) Gaspésie et Nouveau Brunswick-Maine.

3.2 Abstract

Symphyotrichum anticostense (Fernald) G. L. Nesom (Asteraceae: Astereae), an endemic and rare species of the Gulf of St. Lawrence region (Quebec, New Brunswick, and Maine), is a high allopolyploid (2n = 10x = 80). It has been hypothesized to be derived from the hybrid between tetraploid (2*n* = 4x = 32) individuals of *S. boreale* (Torr. & A. Gray) Löve and Löve and the hexaploid (2*n* = 6x = 48) *S. novi-belgii* (L.) G. L. Nesom. We investigated this hypothesis using morphological and molecular (nrDNA ITS sequence data) data to ascertain the relationship of *S. anticostense* to its putative parents, as well as to determine the potential number of geographic origins of the taxon. All available diploid species of *Symphyotrichum* were included in the molecular analysis to investigate the possible involvement of these species in the hybridization. Univariate morphological analyses show that 67.5% of the *S. anticostense* characters are parent-like, 43.5% from *S. novi-belgii* and 13% from *S. boreale*, the remainder not differing statistically from either parent; 23.5% are intermediate; and 9% appear to be transgressive. Multivariate analyses (PCA and CDA) generally show that *S. anticostense* is intermediate between its putative parents. Due to lack of resolution among the ITS ribotypes in the phylogenetic tree, a network phylogenetic analysis was done. The molecular results support the morphological ones, but due to the insufficient resolution among ribotypes on the tree, a more rapidly evolving marker will be needed to ascertain more reliably the origin of *S. anticostense*. Based on the

molecular study, three independent origins might be suggested for *S. anticostense*; 1) Anticosti Island, 2) Lake St. John, and 3) Gaspé Peninsula and New Brunswick-Maine.

3.3 Introduction

The importance of hybridization and polyploidy in plant evolution has often been emphasized (e.g., Stebbins, 1971; Rieseberg and Ellstrand, 1993; Soltis and Soltis, 2000; Wu, 2001; Perný et al., 2005; Wissemann, 2007). Reticulation is a common outcome of these phenomena and takes place at the chromosomal, genomic and species levels (Lee et al., 2002; Levy and Feldman, 2004; Linder and Rieseberg, 2004; Marhold and Lihová, 2006; Sušnik et al., 2007; Timme et al., 2007). In groups where hybridization and polyploidy are frequent, complex reticulations at high ploidy levels may occur. Such groups have been called polyploid pillar complexes by Stebbins (1971), who cited the North American asters, most of which belong to genus *Symphyotrichum* Nees (Nesom, 1994a; Brouillet et al., 2006), as a prime example. Furthermore, complex polyploid reticulations result in evolutionary histories that can be detected with difficulty by divergent evolutionary patterns, i.e. evolutionary relationships of a high polyploid species with its close relatives may not be revealed by building phylogenetic trees. Therefore, to unravel reticulate evolutionary events, it might be necessary to use network rather than tree-building procedures (Linder and Rieseberg, 2004; Vriesendorp and Bakker, 2005).

Symphyotrichum belongs to subtribe Symphyotrichinae of the Astereae (Asteraceae), which also includes *Almutaster*, *Ampelaster*, *Canadanthus*, and *Psilactis* (Nesom and Robinson, 2007); the subtribe is a well-supported monophyletic group (Brouillet et al., 2001; Chapter 2). With more than 90 species (Brouillet et al., 2006), *Symphyotrichum* is the most speciose genus of this clade. In *Symphyotrichum*, ploidy levels range from diploid to duodecaploid (Semple and Brouillet, 1980b; Semple et al., 2002), and both auto- and allopolyploidy have been reported (Jones, 1977; Semple and Brammall, 1982; Allen, 1986; Brouillet and Labrecque, 1987; Nesom, 1994b).

Interspecific, including intersectional and intersubgeneric, hybrids have been reported frequently in *Symphyotrichum* and hybridization has been hypothesized as often followed by polyploidization (Jones, 1977; Semple and Brammall, 1982; Allen, 1986; Brouillet and Labrecque, 1987; Nesom, 1994b; Labrecque and Brouillet, 1996; Semple et al., 2002). An example of a high polyploid with a potentially complex evolutionary history is *Symphyotrichum anticostense* (Fern.) G. L. Nesom, an endemic species of northeastern of North America.

Brouillet and Labrecque (1987) hypothesized that *S. anticostense* might be an allodecaploid ($2n$ = 10x = 80) derivative of a hybrid between tetraploid ($2n$ = 4x= 32) populations of *S. boreale* (Torr. & A. Gray) Á. Löve & D. Löve (other ploidy levels are encountered in this species, from diploid, $2n$ = 16, to octoploid, $2n$ = 64; Owen et al., 2006) and members of *S. novi-belgii* (L.) G. L. Nesom, a hexaploid ($2n$ = 6x= 48) species. *Symphyotrichum anticostense* is distributed in the Lake St. John, Anticosti Island in the Gulf of St. Lawrence, the Baie des Chaleurs region, Gaspé Peninsula (Quebec), and the Restigouche (Quebec-New Brunswick), St. John (New Brunswick), and Aroostook (Maine) river basins (Fig. 3.1). *Symphyotrichum boreale* is distributed throughout the boreal part of North America (Owen et al., 2006). *Symphyotrichum novi-belgii* inhabits coastal regions of eastern North America (Labrecque and Brouillet, 1996), and its range completely overlaps that of *S. anticostense* (Fig. 3.1). The hybrid origin for *S. anticostense* has been proposed based on cytological and morphological evidence, but has not been tested using other approaches.

Ecologically, *S. boreale* colonizes calcareous, moist substrates near streams and in fens, whereas *S. novi-belgii* grows on sandy soils with sufficient moisture, such as sand dunes, along rivers on the geolittoral zone, as well as in disturbed areas such as roadsides. It is also tolerant of saline environments and is present in river estuaries throughout its distribution. *Symphyotrichum anticostense* grows on calcareous and coarsely sandy soil, preferentially of gravel texture, rather than acidic or finely sandy substartes. In addition, this species is absent from river estuaries, indicating its intolerance of saline environments. It also invades locally disturbed habitats.

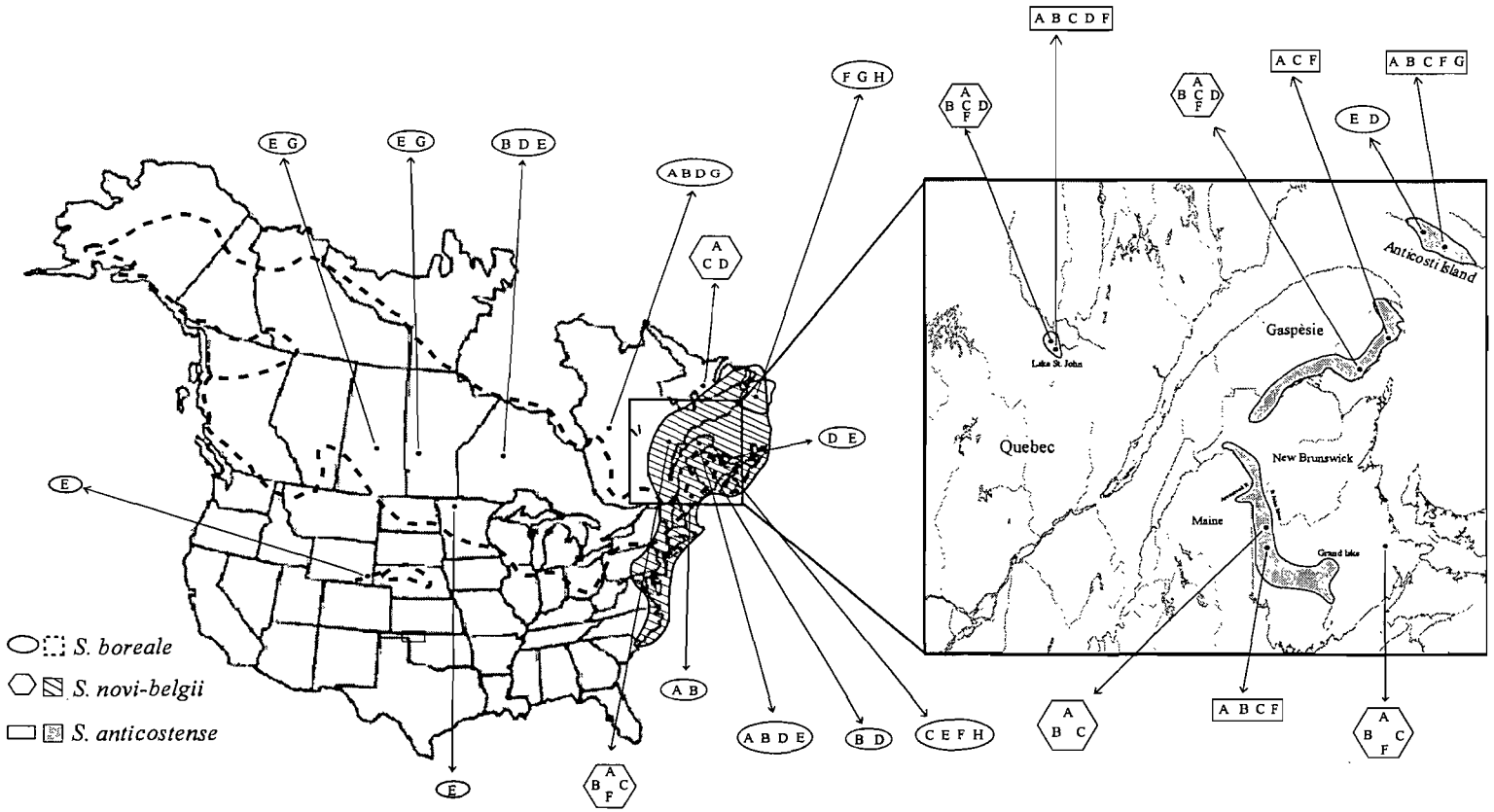

Figure 3.1 Geographical and ribotype distribution of the three species under study. Dashed lines, diagonal lines and darked-gray regions indicate the distribution of *S. boreale, S. novi-belgii* and *S. anticostense*, respectively. Ellipse, hexagon and rectangle forms indicate ribotype symbols of *S. boreale, S. novi-belgii* and *S. anticostense,* respectively. The letters inside the forms correspond to the ribotype groups indicated in the Table 3.1.

Past glaciations in the northern hemisphere had enormous effects on the history of genealogical lineages (Comes and Kadereit, 1998; Dobeš et al., 2004). Using molecular markers, several studies have demonstrated the recent origin for polyploid species that arose after the last Pleistocene glaciation in North America and Europe, e.g. *Saxifraga svalbardensis* (Brochmann and Håpnes, 2001), *Cardamine amporitana* (Lihová et al., 2004), *Capsella bursa-pastoris* (Slotte et al., 2006), and *Biscutella laevigata* (Marhold and Lihová, 2006). It seems postglacial distribution areas had a major role as favorable environments for sympatric speciation via hybridization and polyploidization (Comes and Abbott, 2001; Bolnick and Fitzpatrick, 2007, and references therein). Owen et al. (2006) suggested that the range of *S. boreale* was affected during the last glaciation as it most likely migrated from western North American refugial areas into eastern North America. Meanwhile, *S. novi-belgii* may have been widespread in eastern coastal areas (Labrecque and Brouillet, 1996) where its habitats (moist, sandy soils) were available (Webb, 1989). Thus, the data appear to imply postglacial hybridization in areas where the putative parents became sympatric.

The slightly disjunct distribution of *S. anticostense* populations raises the question of whether this allopolyploid may not have had multiple origins. The recurrent formation of polyploids at various locations over a short period of time has been well documented (Soltis and Soltis, 1993, 1999, 2000). Molecular markers have revealed that recurrent formation is usual rather than exceptional in polyploid evolution (Haugen et al., 2003; Mabuchi et al., 2005; Alon et al., 2006; Pillon et al., 2007).

Many approaches have been proposed to detect species of hybrid origin, including analysis of morphological characters (reviewed in Rieseberg and Ellstrand, 1993), isozymes (Urbanska et al., 1997; Bleeker and Hurka, 2001; Eschmann-Groupe et al., 2003), the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (e.g., Alvarez and Wendel, 2003; Siripun and Schilling, 2006; Volkov et al., 2007), AFLP or RAPD (Neuffer and Jahncke, 1997; Bleeker and Matthies, 2005), and cpDNA markers (Dobeš et al., 2004; Lihová et al., 2006).

Multivariate morphometric approaches have been commonly used in the study of species complexes (e.g., Labrecque and Brouillet, 1996; Gengler-Nowak, 2002; Otieno et al., 2006; Owen et al., 2006; Cron et al., 2007), and in hybrid detection (e.g., Marhold et al., 2002; Carine et al., 2007; Segarra-Moragues et al., 2007). In recent years, these studies have been combined with molecular data, notably using the ITS region (Lihová et al., 2004; Perný et al., 2005; Ishikawa et al., 2006; Segarra-Moragues et al., 2007). It has been demonstrated that there is often a positive correlation between morphological features and nrDNA (ITS) sequence data in reflecting true phylogenetic species relationships (Okuyama et al., 2005; Fehrer et al., 2007).

The use of the ITS region has been popular in phylogenetic analyses (Baldwin et al., 1995; Volkov et al., 2007), because of its biparental inheritance, universality and simplicity of amplification, although concerted evolution may constitute a disadvantage when using this marker. Alvarez and Wendel (2003) indicated three possible consequences of concerted evolution that may occur after hybridization: (i) maintenance of divergent copies; (ii) presence of chimeric ITS sequences due to recombination; and (iii) dominance of one ITS parental type in a hybrid species. Of these, the first outcome may help demonstrate hybrid origin using phylogenetic analysis. The second may give rise to misleading phylogenetic results. The third may detect one of the parents involved in the hybridization, while all traces of the other are lost. Nevertheless, previous studies have shown that co-occurrence of parental ribotypes, detected as ribotype polymorphisms in hybrids, may be more likely in recent hybrids, particularly those that originated following the last glaciation (Marhold et al., 2002; Koch et al., 2003; Perný et al., 2005; Lihová et al., 2006).

The objectives of this study are to investigate the origin of *S. anticostense*, examine the relative contribution of each proposed parent to the genomic constitution of the allopolyploid, and investigate the possible number of geographic origin(s) of *S. anticostense*. We use sequence data from the ITS region as well as morphological data to address these questions.

3.4 Materials and Methods

3.4.1 Taxon sampling

Herbarium specimens (including molecular vouchers) of *S. anticostense*, *S. novi-belgii* and *S. boreale* were included in the morphometric study (Table 3.1). Silica-dried leaves of *S. anticostense* and *S. novi-belgii* were field-collected in 2004 and 2005, including13 populations and 16 individuals representing the entire range of *S. anticostense* except Anticosti Island. Seven herbarium specimens of *S. anticostense* from two populations of Anticosti Island were used to represent this region. For *S. novi-belgii*, 23 individuals belonging to 17 populations were included in the molecular study. Samples from herbarium specimens of *S. boreale* from its entire range were included in the molecular study (Table 3.1). Thirty-four diploid species representing the four subgenera of *Symphyotrichum* (Semple, 2005) were included in the molecular study; material was obtained from cytological vouchers. Four individuals from three closely related genera belonging to the Symphyotrichinae (*Ampelaster*, *Almutaster* and *Canadanthus*) were included as outgroups (Table 3.1).

Table 3.1 Accessions included in the morphological and molecular study including the species of genus *Symphyotrichum* as well as four outgroups. Individuals used for the morphological or molecular studies are indicated by asterisk. The Genbank accession and corresponding clade per individual indicated in two last columns.

Species	Locality/ Province	Collector(s)	Mor.	Mol.	ITS GenBank Acc. [a]	Clade
S. novi-belgii (L.) G.L. Nesom	Grand-Rivière/ Que.	Germain, 8206 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Mont St. Pièrre/ Que.	Victorin & Germain, 49405 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	R. Port-Daniel/ Que.	Victorin et al. 44284 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Lac Monroe/ Que.	Germain, 3165 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Thetford Mines/ Que.	Hamel, C 66233 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	R. aux Canards/ Que.	Brouillet & Brouillet, 890 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	R. du Loup/ Que.	Brouillet, 964 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Maria Chapdelaine/ Que.	Brouillet & Brouillet, 841 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Back Lake/ Que.	Blais et al. 10776 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Tuckers Head/ Nfld.	Bouchard & Hay, 73119 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Mollichicgneck Brook/ Nfld.	Rouleau, 6489 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Serpentine Lake/ Nfld.	Rouleau, 4007 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Porter Island/ N.S.	Sampson, 278 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Brackley Beach/ P.E.I.	Erskine, 1656 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Rivière Ste. Anne/ Que.	Victorin et al. 3849 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	R. Ste. Marguerite/ Que.	Coyouette & Brisson, 64747 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	R. Petit-Pabos/ Que.	Victorin et al. 44276 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Région Côte Nord/ Que.	Goyette, A 38 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Ste. Adelaide/ Que.	Germain, 8266 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Betchouane/ Que.	Victorin & Germain, 21369 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Ile Cap aux meules/ Que.	Samuel, 5461 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Ilets Jeremie/ Que.	Brisson, 909 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Cap-Jaseux/ Que.	Brisson, 5024 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Beauceville/ Que.	Labr. & Cour. 21 L/2650173 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Matapedia/ Que.	Le Gallo, 202 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Upsalquitch/ N.B.	Labrecque et al. 88-20 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Hab and Loc/ Que.	Rousseau, 32359 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Pointe à la Frégate/ Que.	Brouillet, 984 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Carleton/ Que.	Victorin et al. 33567 (MT)	x	-	-	-
S. novi-belgii (L.) G.L. Nesom	Labrador/ Nfld. and Labr.	Bay, 229 (MT)	-	x	EU781172-4	A, C, D

Table 3.1 *Continued ...*

Species	Locality/ Province	Collector(s)	Mor.	Mol.	ITS GenBank Acc. [a]	Clade
S. novi-belgii (L.) G.L. Nesom	Labrador/ Nfld. and Labr.	Bay, 233 (MT)	-	x	EU781175-7	A, C
S. novi-belgii (L.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 238 (MT)	-	x	EU781178-82	A, B, C, F
S. novi-belgii (L.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 242 (MT)	-	x	EU781183-6	A, B, C, D
S. novi-belgii (L.) G.L. Nesom	Saguenay river/ Que.	Vaezi & Brouillet, 250 (MT)	-	x	EU781187-90	A, B, C
S. novi-belgii (L.) G.L. Nesom	Saguenay river/ Que.	Vaezi & Brouillet, 252 (MT)	-	x	EU781191	B
S. novi-belgii (L.) G.L. Nesom	Porteneuf river/ Que.	Vaezi & Brouillet, 253 (MT)	-	x	EU781192-5	A, B, C, F
S. novi-belgii (L.) G.L. Nesom	Porteneuf river/ Que.	Vaezi & Brouillet, 257 (MT)	-	x	EU781196-201	A, B, C
S. novi-belgii (L.) G.L. Nesom	Bic/ Que.	Vaezi & Brouillet, 258 (MT)	-	x	EU781202-9	A, B, C, F
S. novi-belgii (L.) G.L. Nesom	Bic/ Que.	Vaezi & Brouillet, 259 (MT)	-	x	EU781210-11	B, C
S. novi-belgii (L.) G.L. Nesom	Les Escoumins/ Que.	Vaezi & Brouillet, 263 (MT)	-	x	EU781212-5	A, B, C, F
S. novi-belgii (L.) G.L. Nesom	Les Escoumins/ Que.	Vaezi & Brouillet, 266 (MT)	-	x	EU781216-22	A, B, C
S. novi-belgii (L.) G.L. Nesom	Paspébiac/ Que.	Vaezi & Brouillet, 267 (MT)	-	x	EU781223-6	B, D, F
S. novi-belgii (L.) G.L. Nesom	Grand rivière/ Que.	Vaezi & Brouillet, 282 (MT)	-	x	EU781231-3	A, C
S. novi-belgii (L.) G.L. Nesom	R. petit pabos/ Que.	Vaezi & Brouillet, 302 (MT)	-	x	EU781240-3	A, B, C, F
S. novi-belgii (L.) G.L. Nesom	R. Bonaventure/ Que.	Vaezi & Brouillet, 315 (MT)	-	x	EU781247-9	A, B, F
S. novi-belgii (L.) G.L. Nesom	Limestone/ N.B.	Vaezi & Zargarbashi, 407 (MT)	-	x	EU781254-9	A, C
S. novi-belgii (L.) G.L. Nesom	Hartland/ N.B.	Vaezi & Zargarbashi, 450 (MT)	-	x	EU781277-82	A, B, C
S. novi-belgii (L.) G.L. Nesom	St. John/ N.B.	Vaezi & Zargarbashi, 510 (MT)	-	x	EU781295-8	B, C
S. novi-belgii (L.) G.L. Nesom	Alma/ N.B.	Vaezi & Zargarbashi, 521(MT)	-	x	EU781299-301	A, C
S. novi-belgii (L.) G.L. Nesom	Moncton/ N.B.	Vaezi & Zargarbashi, 555 (MT)	-	x	EU781302-3	B
S. novi-belgii (L.) G.L. Nesom	Renton/ N.B.	Vaezi & Zargarbashi, 564 (MT)	-	x	EU781304-7	A, F
S. novi-belgii (L.) G.L. Nesom	Miramichi/ N.B.	Vaezi & Zargarbashi, 567 (MT)	-	x	EU781308-9	A, C
S. boreale (T. & G.) Á. Löve & D. Löve	Bay of Islands/ Nfld.	Djan-chékar et al., 1461(MT)	x	x	EU781316-20	F, G
S. boreale (T. & G.) Á. Löve & D. Löve	McAdam lake/ N.S.	Smith et al., 5472 (MT)	x	x	EU781353-6	C, E, F
S. boreale (T. & G.) Á. Löve & D. Löve	Lake Bog/ Ohio	s.n., 530926-0256 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	St. Quentin/ N.B.	Haber & Bristow, 3601 (MT)	x	x	EU781314-5	B, D
S. boreale (T. & G.) Á. Löve & D. Löve	Raspberry lake/ Sask.	Harms, 24704 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Birch river/ Man.	Semple & Brouillet, 4143 (MT)	x	x	EU781312-3	E
S. boreale (T. & G.) Á. Löve & D. Löve	Lac Labelle/ Que.	Plourde, 240 (MT)	x	x	EU781372-3	B
S. boreale (T. & G.) Á. Löve & D. Löve	Lac au saumon/ Que.	LeGallo, 841 (MT)	x	x	EU781344-8	B, D, E
S. boreale (T. & G.) Á. Löve & D. Löve	Dundee station/ P.E.I.	Smith, 335 (MT)	x	x	EU781349-52	D, E
S. boreale (T. & G.) Á. Löve & D. Löve	Longlac/ Ont.	Baldwin & Breitung, 3376 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	R. Eastmain/ Que.	Gagnon & Barabé, 74510 (MT)	x	x	EU781364-7	B, D

Table 3.1 *Continued ...*

Species	Locality/ Province	Collector(s)	Mor.	Mol.	ITS GenBank Acc. [a]	Clade
S. boreale (T. & G.) Á. Löve & D. Löve	Rupert House/ Que.	Spafford, 149 (MT)	x	x	EU781368-71	D, G
S. boreale (T. & G.) Á. Löve & D. Löve	Meadow lake/ Sask.	Breitung, 8350 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Sutherland/ Sask.	Boivin & Breitung, 6697 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Summit lake/ B.C.	Weber, 2603 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Smith/ Alta.	Boivin & Perron, 12762 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Bay James/ Que.	Gagnon & Hay, 75013 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Manitoulin/ Ont.	Brouillet, 550 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Swanson's Creek/ Man.	Cody & Wojtas, 25154 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Lake Winnipeg/ Man.	Scoggon, 5046 (MT)	x	x	EU781390	G
S. boreale (T. & G.) Á. Löve & D. Löve	Big river/ Sask.	Tisdale,117980 (Sask)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Beaver river/ Sask.	Looman, 117975(Sask)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Hills Park/ Sask.	Breitung, 64469(Sask)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Big river/ Sask.	Tisdale, 117979(Sask)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	North Sask R./ Sask.	Lepage, 90360(Sask)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Lake Road/ Sask.	Jeglum, 39461(Sask)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Sutherland/ Sask.	Fraser, 117983(Sask)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Batchawana/ Ont.	Taylor, 1546 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Fitzwilliam/ Ont.	Morton, 8463 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Thunder Bay/ Ont.	Garton, 18432 (MT)	x	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Kenora/ Ont.	Oldham & Sutherl., 24500 (WAT)	-	x	EU781144-6	B
S. boreale (T. & G.) Á. Löve & D. Löve	Albany Co./ Wyo.	Semple, 11233 (WAT)	-	x	EU781251	E
S. boreale (T. & G.) Á. Löve & D. Löve	Albert Park/ Sask.	Harms, 43283 (MT)	-	x	EU781310	E
S. boreale (T. & G.) Á. Löve & D. Löve	M. Creek/ Sask.	Harms, 38132 (MT)	-	x	EU781311	E
S. boreale (T. & G.) Á. Löve & D. Löve	R. Bonaventure/ Que.	LePage, 3704 (MT)	-	x	EU781327	A
S. boreale (T. & G.) Á. Löve & D. Löve	Anticosti Island/ Que.	Victorin et al., 21341 (MT)	-	x	EU781328	E
S. boreale (T. & G.) Á. Löve & D. Löve	Lac au saumon/ Que.	LeGallo, 886 (MT)	-	x	EU781329	B
S. boreale (T. & G.) Á. Löve & D. Löve	Anticosti Island/ Que.	Victorin & Germain, 27595 (MT)	-	x	EU781330-1	E
S. boreale (T. & G.) Á. Löve & D. Löve	St. Hippolyte/ Que.	Hébert, 72-124-1 (MT)	-	x	EU781332-6	A, B
S. boreale (T. & G.) Á. Löve & D. Löve	Anticosti Island/ Que.	Victorin & Germain, 21338 (MT)	-	x	EU781337	E
S. boreale (T. & G.) Á. Löve & D. Löve	Anticosti Island/ Que.	Victorin & Germain, 27594 (MT)	-	x	EU781338-43	D, E
S. boreale (T. & G.) Á. Löve & D. Löve	Grand Rapids/ Minn.	Wheeler & Glaser, 2342 (MT)	-	x	EU781357	E
S. boreale (T. & G.) Á. Löve & D. Löve	Vermont	Seymour, 29703 (MT)	-	x	EU781358-62	A, B
S. boreale (T. & G.) Á. Löve & D. Löve	Gold smith lake/ N.B.	Malte, 1019/29 (MT)	-	x	EU781363	B

Table 3.1 *Continued ...*

Species	Locality/ Province	Collector(s)	Mor.	Mol.	ITS GenBank Acc. [a]	Clade [b]
S. boreale (T. & G.) Á. Löve & D. Löve	Oka/ Que.	Beaudry & Love, 58-202 (MT)	-	x	EU781374-8	B, D
S. boreale (T. & G.) Á. Löve & D. Löve	Chibougamau/ Que.	Hustich, 772 (MT)	-	x	EU781379-80	B
S. boreale (T. & G.) Á. Löve & D. Löve	La Trappe/ Que.	Beaudry & L.-Marie, 55-249 (MT)	-	x	EU781381-2	B
S. boreale (T. & G.) Á. Löve & D. Löve	Nominingue/ Que.	Robert, 872 (MT)	-	x	EU781383-5	B, D
S. boreale (T. & G.) Á. Löve & D. Löve	Kingston/ Ont.	Garwood & Zavitz, 1987 (MT)	-	x	EU781386	B
S. boreale (T. & G.) Á. Löve & D. Löve	Sandstone lake/ Ont.	Garton, 1687 (MT)	-	x	EU781387-8	E
S. boreale (T. & G.) Á. Löve & D. Löve	Kapiskau river/ Ont.	Ringius et al., 872 (MT)	-	x	EU781389	D
S. boreale (T. & G.) Á. Löve & D. Löve	Anglin lake/ Sask.	Harms, 24741 (Sask)	-	x	EU781447-50	E, G
S. boreale (T. & G.) Á. Löve & D. Löve	Nipawin park/ Sask.	Argus & Hudson, 4419 (Sask)	-	x	EU781451-6	E, G
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Victorin et al., 4036 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	Matapedia/ Que.	Labrecque et al., 88-64 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	Woodstock/ N.B.	Victorin et al., 44844 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	Lac St. Jean/ Que.	Victorin, 15408 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Petit-Pabos/ Que.	Victorin et al., 44273 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Brouillet & Labrec., 1432 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	Grande R./ Que.	Labrecque, AM-002 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Petit-Pabos/ Que.	Labrecque et al., 88-184 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	Grande R./ Que.	Victorin et al., 44256 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	Aroostook R./ Maine	Williams et al., 18 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	Grande R./ Que.	Labrecque et al., 88-162 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Victorin et al., 44265 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Victorin et al., 4047 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Victorin et al., 4051 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Petit-Pabos/ Que.	Labrecque, 165-90 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	Grande R./ Que.	Labrecque et al., 88-132 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Victorin et al., 4039 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Labrecque et al., 88-99 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	Matapedia/ Que.	Cayouette, J85-151 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Petit-Pabos/ Que.	Victorin et al., 44279 (MT)	x	-	-	-
S. anticostense (Fern.) G.L. Nesom	Four Falls/ N.B.	Vaezi & Zargarbashi, 422 (MT)	x	x	EU781260-5	A, B
S. anticostense (Fern.) G.L. Nesom	Hartland/ N.B.	Vaezi & Zargarbashi, 445 (MT)	x	x	EU781273-6	A, C
S. anticostense (Fern.) G.L. Nesom	Grand lake/ N.B.	Vaezi & Zargarbashi, 472 (MT)	-	x	EU781283-4	B
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrecque & Jean, 377274 (LM)	x	x	EU781425-30	C, F

Table 3.1 *Continued ...*

Species	Locality/ Province	Collector(s)	Mor.	Mol.	ITS GenBank Acc. [a]	Clade
S. anticostense (Fern.) G.L. Nesom	Grande R./ Que.	Vaezi & Brouillet, 275 (MT)	-	x	EU781227-30	A, F
S. anticostense (Fern.) G.L. Nesom	Oakpoint/ N.B.	Vaezi & Zargarbashi, 486 (MT)	x	x	EU781291-4	B, C
S. anticostense (Fern.) G.L. Nesom	Grand lake/ N.B.	Vaezi & Zargarbashi, 473 (MT)	x	x	EU781285-90	B, C
S. anticostense (Fern.) G.L. Nesom	Florenceville/ N.B.	Vaezi & Zargarbashi, 436 (MT)	x	x	EU781266-8	A, F
S. anticostense (Fern.) G.L. Nesom	Connell/ N.B.	Vaezi & Zargarbashi, 437 (MT)	x	x	EU781269-72	A, C, F
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrecque & Jean, 377273 (LM)	x	x	EU781420-4	A, C, F
S. anticostense (Fern.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 208 (MT)	-	x	EU781148-53	A, B, C, F
S. anticostense (Fern.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 212 (MT)	-	x	EU781154-7	B, A, C
S. anticostense (Fern.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 217 (MT)	-	x	EU781158-63	A, B, C, F
S. anticostense (Fern.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 218 (MT)	-	x	EU781164-8	A, B, D, F
S. anticostense (Fern.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 222 (MT)	-	x	EU781169-71	C, D, F
S. anticostense (Fern.) G.L. Nesom	Grande R./ Que.	Vaezi & Brouillet, 287 (MT)	-	x	EU781234-7	A, C, F
S. anticostense (Fern.) G.L. Nesom	R. Petit-Pabos/ Que.	Vaezi & Brouillet, 295 (MT)	-	x	EU781238-9	A
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Vaezi & Brouillet, 310 (MT)	-	x	EU781244-6	A, C
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrec. & Jean, 696 (MT)	-	x	EU781391-6	A, B, C, F
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrec. & Jean, 697 (MT)	-	x	EU781397-401	A, B, C, G
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrec. & Jean, 377275 (LM)	-	x	EU781431-6	B, C, F
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrec. & Jean, 377276 (LM)	-	x	EU781437-41	A, F
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrec. & Jean, 377242 (LM)	-	x	EU781442-6	A, C, F
S. urophyllum (Lind. ex de Cand.) G.L. Nesom	Elgin Co./ Ont.	Semple, 10594 (WAT)	-	x	EU781138-9	F
S. drummondii (Lind.) G.L. Nesom	Newton Co./ Tex.	Semple, 10049 (WAT)	-	x	EU781140-1	-
S. puniceum (L.) Á. Löve & D. Löve	Van Zandt Co./ Tex.	Nixon & Ward, 12663 (SMU)	-	x	EU781147	G
S. puniceum (L.) Á. Löve & D. Löve	Marion Co./ N.C.	Semple, 10853 (WAT)	-	x	EU781142-3	G
S. nahanniense (Cody) Semple	Nahanni N.P.R./ N.W.T.	Semple, 11161 (WAT)	-	x	EU781252-3	E
S. dumosum (L.) G.L. Nesom	Amite Co./ Miss.	Semple & Suripto, 10102 (MT)	-	x	EU781402-6	E
S. welshii (Cronquist) G.L. Nesom	Lake Co./ Mont.	Semple, 11374 (WAT)	-	x	EU781407-8	E
S. oblongifolium (Nuttall) G.L. Nesom	Webster Co./ Nebr.	Semple & Brouillet, 7337 (MT)	-	x	EU781459	-
S. ciliatum (Ledeb.) G.L. Nesom	Manitoulin/ Ont.	Morton & Venn, 9942 (MT)	-	x	EU781410	-
S. cordifolium (L.) G.L. Nesom	Carleton Co./ N.B.	Semple & Keir, 4670 (MT)	-	x	EU781411-2	-
S. shortii (Lind.) G.L. Nesom	Adair Co./ Ky.	Semple & Suripto, 9449 (MT)	-	x	EU781413-4	F
S. lateriflorum (L.) Á. Löve & D. Löve	Prince Edward/ Ont.	Brouillet & Brammall, 587 (MT)	-	x	EU781418	-
S. spathulatum (Lind.) G.L. Nesom	Mono Co./ Calif.	Semple & Heard, 8715 (MT)	-	x	EU781419	E
S. depauperatum (Fern.) G.L. Nesom	Nottingham/ Pa.	Semple, 7681 (WAT)	-	x	EU200226 [c]	G

Table 3.1 *Continued ...*

Species	Locality/ Province	Collector(s)	Mor.	Mol.	ITS GenBank Acc. [a]	Clade
S. elliottii (T. & G.) G.L. Nesom	Onslow Co./ N.C.	Semple, 10538 (WAT)	-	x	EU853710 [b]	G
S. frondosum (Nuttall) G.L. Nesom	Lake Co./ Oreg.	Houle & Legault, 45 (MT)	-	x	EU853711 [b]	-
S. laurentianum (Fern.) G.L. Nesom	Ile de la Madeleine/ Que.	Houle & Brouillet, 81 (MT)	-	x	EU853712 [b]	-
S. racemosum (Elliott) G.L. Nesom	Wayne Co./ Miss.	Semple, 9895 (WAT)	-	x	EU853715 [b]	-
S. tradescantii (L.) G.L. Nesom	Lévis/ Que.	Bouchard & Cuerrier, K-11 (MT)	-	x	EU853717 [b]	-
S. firmum (Nees) G.L. Nesom	Lake Co./ Mont.	Gerdes, 4945 (NM)	-	x	EU781250	G
S. anomalum (Engel. ex T. & G.) G.L. Nesom	Carroll Co./ Ark.	Semple & Suripto, 9950 (WAT)	-	x	EU781321-6	-
S. sericeum (Ventenat) G.L. Nesom	Rainy river/ Ont.	Semple & Heard, 8787 (WAT)	-	x	EU200232 [c]	-
S. concolor (L.) G.L. Nesom	Laurens Co./ Ga.	Semple, 4040 (MT)	-	x	EU781460-1	-
S. undulatum (L.) G.L. Nesom	Orangeburg Co./ S.C.	Semple & Chmielewski, 6133 (MT)	-	x	EU781415-6	-
S. parviceps (E.S. Burgess) G.L. Nesom	Adams Co./ Ill.	Semple & Brouillet, 7378 (MT)	-	x	EU781417	G
S. ericoides (L.) G.L. Nesom	Mound City/ S.Dak.	Semple, 6664 (WAT)	-	x	EU200227 [c]	-
S. novae-angliae (L.) G.L. Nesom	Tenton/ Ga.	Semple, 11001 (WAT)	-	x	EU200229 [c]	-
S. patens (Aiton) G.L. Nesom	Red River Gorge/ Ky.	Semple & Suripto, 9864 (WAT)	-	x	EU200230 [c]	-
S. yukonense (Cronquist) G.L. Nesom	Kluane Lake/ Yukon	Semple, 10624 (WAT)	-	x	EU200234 [c]	-
S. plumosum (Small) Semple	Franklin Co./ Fla.	Semple, 10929 (WAT)	-	x	EU853713 [b]	-
S. porteri (A. Gray) G.L. Nesom	Clear Creek Co./ Colo.	Semple, 10470 (WAT)	-	x	EU853714 [b]	G
S. subulatum (Michaux) G.L. Nesom	Ocean Co./ N.J.	Semple, 9525 (WAT)	-	x	EU853716 [b]	-
S. subulatum (Michaux) G.L. Nesom	Marengo Co./ Ala.	Semple & Chmielewski, 6362 (MT)	-	x	EU781409	-
S. tenuifolium (L.) G.L. Nesom	Cedar Run/ N.J.	Semple, 9519 (WAT)	-	x	EU200233 [c]	-
S. chapmanii (T. & G.) Semple & Brouillet	Choctawhatchee R./ Fla.	Semple, 10560 (WAT)	-	x	EU200223 [c]	-
Almutaster pauciflorus (Nuttall) Á. Löve & D. Löve	Oakburn/ Man.	Marchand, 1983 (Sask)	-	x	EU781462	-
Ampelaster carolinianus (Walter) G.L. Nesom	Davenport/ Fla.	Semple, 5354 (WAT)	-	x	EU200185 [c]	-
Canadanthus modestus (Lind.) G.L. Nesom	Swift Current/ Sask.	Hudson, 3997 (Sask)	-	x	EU781457	-
Canadanthus modestus (Lind.) G.L. Nesom	Yukon	Cody, s.n. (Sask)	-	x	EU781458	-

a. Accession number of *Symphyotrichum falcatum* (EF017389, Allen et al., 2007), available in GenBank, is used for this study.
b. Accession numbers submitted by Brouillet et al., (in preparation).
c. Accession numbers submitted by Selliah and Brouillet 2008.

3.4.2 Morphological analysis

3.4.2.1 Measurements

Thirty-four quantitative morphological characters, as detailed in Labrecque and Brouillet (1996), were measured (Table 3.2). Of these, 19 were vegetative and 15 reproductive. Eighty seven individuals, including 29 of *S. novi-belgii*, 28 of *S. anticostense*, and 30 of *S. boreale*, were scored. One systematically chosen (on axis 1, see Table 3.2) flower per individual was removed and measured after wetting. All measures were taken with a ruler (precision 1 mm) for the vegetative characters, and with a micrometric slide with a precision of 0.1 mm (WILD; Heerbrugg, Switzerland) using a dissection microscope for floral characters.

3.4.2.2 Univariate analyses

To visualize the variation and mean of the morphological characters of each species, box-plot diagrams were produced. Univariate analyses were used to determine which characters most effectively discriminated the three species and to evaluate the mode of expression (parental, intermediate, transgressive) of each character in *S. anticostense*. All characters were tested for normality using the Kolmorogov-Smirnov test. The Box-Cox transformation was applied to normalize variables that were not normally distributed. Homogeneity of variances was tested using Levene's statistic. ANOVA (analysis of variance) was used where the two assumptions (normality and homogeneity) were met. For normally distributed characters with unequal variances, ANOVA was applied using the Games-Howell post-hoc test. To determine the mode of expression of each character, a post-hoc Tukey-Kramer HSD test was used following the ANOVA. The Kruskal-Wallis non-parametric test was used for the characters in which, after applying transformations, normality of residuals was rejected. The Kolmorogov-Smirnov test and Box-Cox transformation were implemented using the R Package (Casgrain and Legendre, 2001). Other tests were performed using SPSS release 11.5.0 (SPSS Inc., Chicago, U.S.A.).

Table 3.2 Description of the morphological characters used in the current study to determine the morphological situation of *S. anticostense* related to *S. novi-belgii* and *S. boreale*. Measured units are indicated in the parenthesis. The floral characters are specified as asterisk.

No	Abbreviation	Character
1	HAUTOT	Stem height from the base up to the highest inflorescence (mm)
2	DIATIG	Basal stem diameter (mm)
3	NBRCAP*	No. of inflorescent heads (count)
4	NBENTG	No. of internodes from the base up to the first inflorescent ramification (count)
5	NBENIF	No. of inflorescent-axis internodes from the first inflorescent ramification toward the uppermost excluding the terminal peduncle (count)
6	LONINF	Length of inflorescent axis (mm)
7	LOBRIF	Bract length of inflorescent axis; the bract situated in the middle of the inflorescent axis (character 5 divided by 2) (mm)
8	LGBRIF	Bract width of the inflorescent axis (as character 7) (mm)
9	LMAX1F	Distance between apex and maximum bract width of the inflorescent axis (as character 7) (mm)
10	NBAXE1	No. of axes 1; number of inflorescent ramification carrying more than one head (count)
11	NBENA1	Internode no. of axis 1; the axis situated in the middle of the inflorescent axis (count)
12	LOAXA1	Length of axis 1 (as character 11) (count)
13	LOBRA1	Bract length of axis 1; the bract situated in the middle of axis 1 (character 12 divided by 2) (mm)
14	LGBRA1	Bract width of axis 1 (as character 13) (mm)
15	LMAXA1	Distance between apex and maximum bract width of axis 1 (as character 12) (mm)
16	LONPED*	Peduncle length; from the last ramification of axis 1 to phyllary base of the terminal inflorescence (mm)
17	LOFCAU	Length of stem leaf; the leaf situated in the middle of the stem (character 4 divided by 2) (mm)
18	LGFCAU	Width of the stem leaf (as character 17) (mm)
19	LMAXCA	Distance between apex and maximum width of the stem leaf (mm)
20	NBDENT	Teeth no. of the stem leaf; measure taken on one side of the leaf (count)
21	LGBASE	Basal width of the stem leaf (mm)
22	HAUINV*	Involucre height of terminal head of the axis 1 (mm)
23	LOTGEX*	Length of the external phyllary (as character 22) (mm)
24	LOTGIN*	Length of the internal phyllary (mm)
25	LGTGEX*	Width of the external phyllary (mm)
26	LGTGIN*	Width of the internal phyllary (mm)
27	NBRAYO*	No. of ray florets (count)
28	LORAYO*	Ray length; length mean of three rays (mm)
29	NBFLEU*	No. of disk florets (count)
30	LOTUBE*	Tube length; length mean of three tubes (mm)
31	LOLIMB*	Limb length; length mean of three limbs (mm)
32	LOLOBE*	Lobe length; length mean of three lobes (mm)
33	LOANTH*	Anther length;total length including terminal appendix;length mean of three florets (mm)
34	LOSTIG*	Stigma length; length of one branch; length mean of three florets (mm)

3.4.2.3 Multivariate analyses

Multivariate analyses were performed on the 34 morphological characters of the raw matrix after standardization. Characters were standardized by dividing the centered variables by their standard deviation. Inter-variable correlations were applied in the analysis due to the presence of two different units of measurements (Legendre and Legendre, 1998). Principal Component Analysis (PCA) was used to ordinate the individuals on the reduced space without *a priori* knowledge of species identity. The PCA was performed using CANOCO (CanoDraw, Microsoft Corp.).

Canonical Discriminant Analysis (CDA) is a classification method that serves to identify functions that will discriminate *a priori* identified groups. This analysis was carried out to demonstrate the separation among the three species (*S. novi-belgii*, *S. anticostense* and *S. boreale*) and to verify the position of *S. anticostense* with respect to its putative parents in two-dimensional space. A Box-Cox transformation was applied to normalize the variables, though normality is not required when CDA is used as an ordination procedure (Pimentel, 1981). CDA was implemented in SPSS release 11.5.0 (SPSS Inc., Chicago, U.S.A.).

3.4.3 Molecular analysis

3.4.3.1 DNA extraction, PCR amplification, sequencing and cloning

One individual per population of *S. anticostense, S. novi-belgii,* and *S. boreale,* as well as one (rarely two) herbarium specimen(s) for each outgroup species were included in the study (Table 3.1). DNA was extracted following a modification of the Doyle and Doyle (1987) CTAB protocol (Joly et al., 2006) or with the QIAgen DNeasy Plant Mini Kit (QIAGEN, Mississauga, Ontario, Canada), following the instructions of the manufacturer. The ITS (ITS1-5.8S-ITS2) region was amplified using the primers ITSvF and ITSvR designed in chapter 2. Amplification conditions are detailed in chapter 2. PCR purification and sequencing were performed as described by Joly et al. (2006). PCR-

direct sequences with unreadable chromatograms or including more than two SNPs were cloned following the protocol explained in chapter 2.

3.4.3.2 Data analyses

Ribotypes were aligned using ClustalW (Thompson et al., 1994) with full multiple alignment implemented in BioEdit Sequence Alignment Editor (Hall, 1999), followed by manual corrections. To remove redundant intraindividual ribotypes, the aligned ribotypes were collapsed using the program COLLAPSE version1.2 (Posada, 2004). To detect potentially recombinant ribotypes between those of the putative parents within the ribotypic pool of *S. anticostense*, six methods, RDP (Martin and Rybicki, 2000), GENECONV (Padidam et al., 1999), Bootscanning (Salminen et al., 1995), MaxChi (Maynard-Smith, 1992), Chimaera (Posada and Crandall, 2001) and SiScan (Gibbs et al., 2000) were applied as implemented in the RDP2 program (Martin et al., 2005). Because few gaps were present in the aligned sequences, they were treated as missing data.

To estimate the gene genealogy of the ITS marker, a maximum likelihood approach was applied using the PhyML program (Guindon and Gascuel, 2003). To determine the evolutionary model that best fitted the sequence data, the Akaike Information Criterion (AIC; Akaike, 1973) and the Likelihood Ratio Test (LRT; Felsenstein, 1988) were computed using MrModeltest 2.2 (Nylander, 2004) with executable MrModelblock file in PAUP* version 4.10b (Swofford, 2002). Among the 24 available models, the GTR substitution model was chosen by both criteria with fixed invariable sites (p-invar= 0), and a gamma shape parameter (α= 0.57). A Neighbor Joining tree was used as a default starting tree. One hundred replicates of the original data were generated to bootstrap the data set. The resulting tree showed insufficient resolution within some clades including ribotypes of the three species under study in particular. To determine phylogenetic relationships among and within these clades, a network was constructed using statistical parsimony as implemented in the TCS program version 1.21 (Clement et al., 2000). The ribotype network was produced with the 95% probability limit of parsimonious connections and gaps treated as missing data.

3.5 Results

3.5.1 Morphometric analyses

3.5.1.1 Univariate analyses

The distribution of the morphological variables that discriminate among *S. anticostense* and its putative parents is represented by box plots in figure 3.2. The Kolmorogov-Smirnov and Levene's statistic tests showed that eight characters (indicated by the symbol † in Table 3.3) were normally distributed with uniform variances; therefore the Tukey-HSD test was implemented to determine which of these were suitable to discriminate between paired species. Eighteen variables (indicated by the symbol ‡ in Table 3.3) were normally distributed but not homogeneous; therefore, the Games-Howell post-hoc test was applied. The remaining variables (showed by the symbol Ω in Table 3.3) were not normally distributed after transformation, and the non-parametric Kruskal-Wallis test was implemented on these.

Examination of box plots (Fig. 3.2) and of statistical analysis (Table 3.3) show that ten variables, HAUTOT, LOAXA1, LMAXA1, LONPED, LORAYO, LOTUBE, LOLIMB, LOLOBE, LOANTH, and LOSTIG, are not significantly different among the three taxa. Their variance in *S. anticostense* overlaps that of the parents. These characters are presumed to be parent-like in expression.

Eight variables, DIATIG, NBENTG, LOBRIF, NBAXE1, LGBASE, HAUINV, LOTGIN, and NBRAYO, are significantly different between the species pairs *S. novi-belgii- S. boreale* and *S. boreale- S. anticostense,* but not *S. novi-belgii- S. anticostense* (Table 3.3). These characters appear to be *novi-belgii*-like. LONINF and LMAXIF significantly discriminate *S. boreale* from *S. anticostense* but not *S. anticostense* from *S. novi-belgii* or the latter from *S. boreale*; these characters also appear to be *novi-belgii*-like. LGBRIF, NBENIF, and NBFLEU have similar variances in *S. anticostense* and *S. boreale*, and their expression appears to be *boreale*-like.

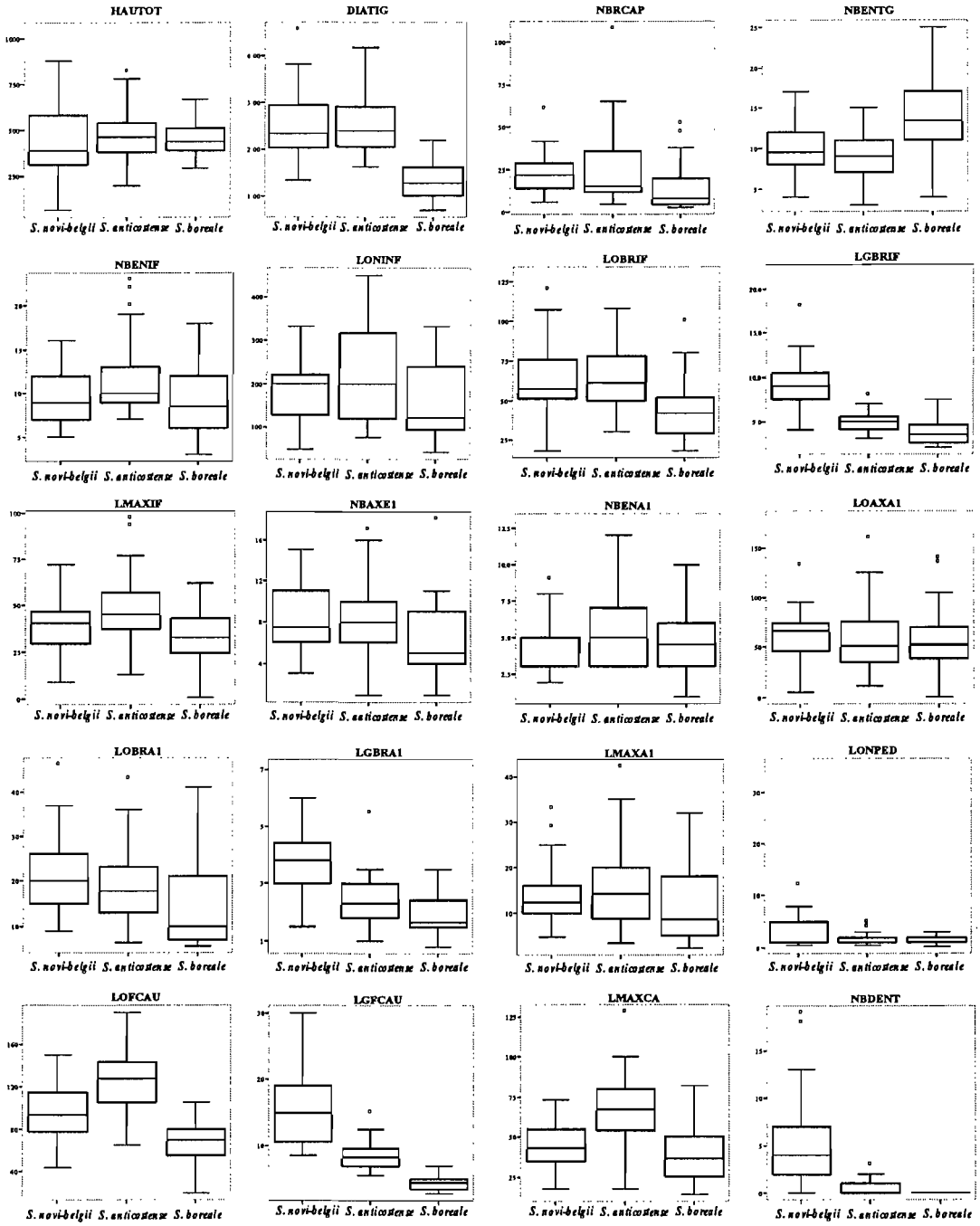

Figure 3.2 Boxplots of character variation using 34 morphological variables of the three species under study. Eighty seven specimens including 30, 29, and 28 specimens of *S. boreale*, *S. novi-belgii*, and *S. anticostense*, respectively were used in this representation. Abbreviations are explained in Table 3.2.

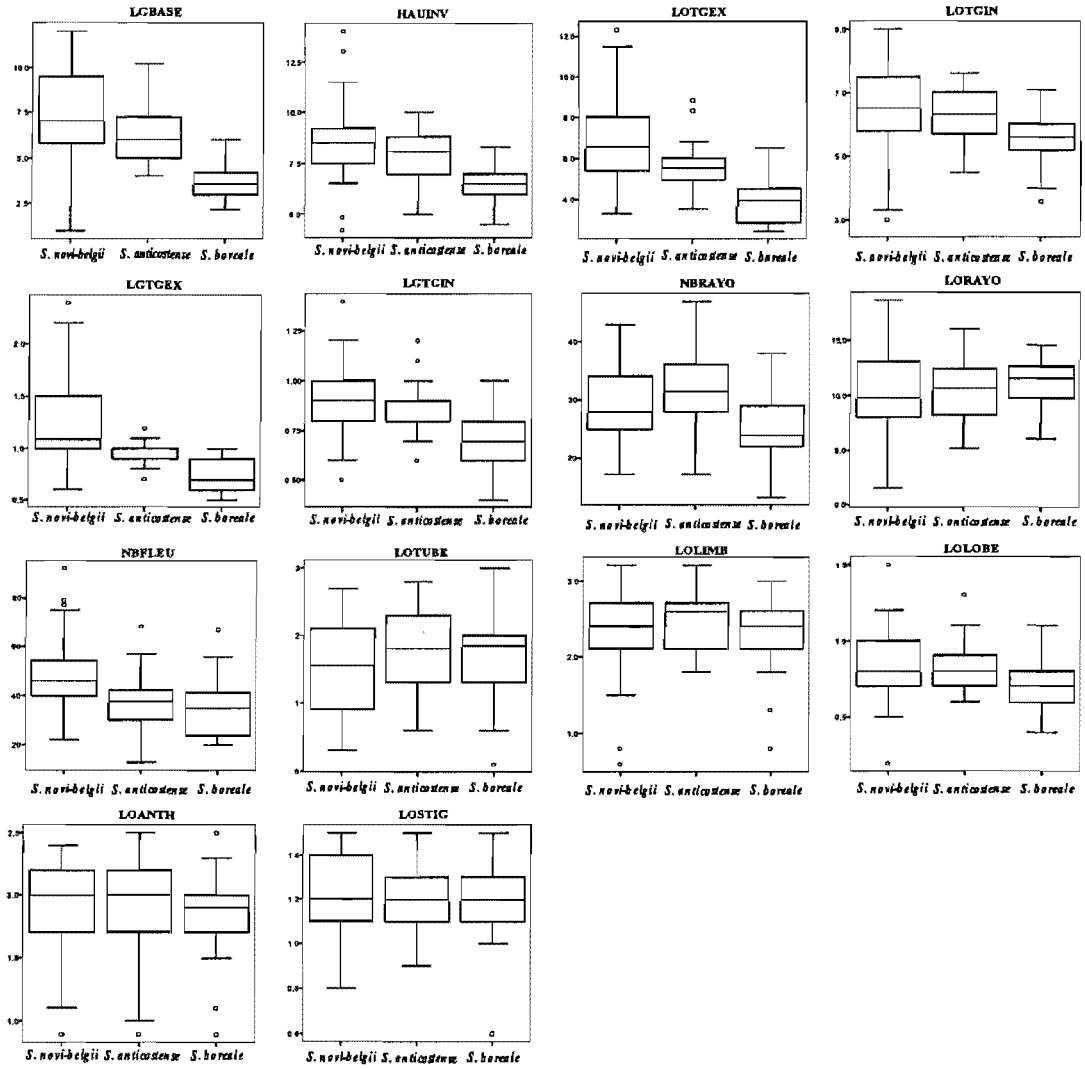

Figure 3.2 *continued...*

Table 3.3 Results of the univariate analyses using 34 morphological characters and 87 accessions of the three species in the study. The symbol (†) specified for the normal and uniform characters, the symbol (‡) for the normal but non-uniform characters, the symbol (Ω) for non-normal characters, and the asterisk symbol (*) for the floral characters. Parental-like characters are specified as "N" for *S. novi-belgii*-like or "B" for *S. boreale*-like characters. The character abbreviations are described in Table 3.2.

	Mean			ANOVA	Multiple Comarison (*P-Value*)						Kruskal-Wallis test		
					novi.- bor.		*novi.- anti.*		*bor.- anti.*				
Character	*novi.*	*bor.*	*anti.*	*p-Value*	TH	GH	TH	GH	TH	GH	Chi-Sq.	*p-Value*	Expression mode
HAUTOT†	411.67	459.67	480.55	0.23	0.47	-	0.22	-	0.87	-	-	-	Parental
DIATIG†	2.54	1.31	2.55	<0.0001	<0.0001	-	0.99	-	<0.0001	-	-	-	Parental ^{N}
NBRCAP‡*	28.03	15.63	26.03	0.04	-	0.03	-	0.93		0.09	-	-	Intermediate
NBENTG‡	9.77	13.93	8.90	<0.0001	-	<0.0001	-	0.54	-	<0.0001	-	-	Parental ^{N}
NBENIF‡	9.47	9.37	11.87	0.03	-	0.99	-	0.04	-	0.08	-	-	Parental ^{B}
LONINF†	179.63	157.33	221.67	0.03	0.63	-	0.20	-	0.03	-	-	-	Parental ^{N}
LOBRIF‡	63.73	43.25	65.50	<0.0001	-	<0.0001	-	0.94	-	<0.0001	-	-	Parental ^{N}
LGBRIF†	9.50	3.63	4.96	<0.0001	<0.0001	-	<0.0001	-	0.08	-	-	-	Parental ^{B}
LMAXIF‡	39.43	33.97	48.78	<0.0001	-	0.33	-	0.09	-	<0.0001	-	-	Parental ^{N}
NBAXE1‡	8.40	5.97	8.40	0.01	-	0.02	-	0.97	-	0.04	-	-	Parental ^{N}
NBENA1Ω	4.23	4.63	5.40	-	-	-	-	-	-	-	6.37	0.04	Transgressive
LOAXA1‡	64.67	57.59	61.63	0.72	-	0.67	-	0.94	-	0.89	-	-	Parental
LOBRA1‡	21.37	15.11	19.14	0.03	-	0.03	-	0.60	-	0.23	-	-	Intermediate
LGBRA1‡	3.65	1.85	2.49	<0.0001	-	<0.0001	-	<0.0001	-	0.04	-	-	Intermediate
LMAXA1‡	13.88	11.24	15.84	0.09	-	0.34	-	0.64	-	0.10	-	-	Parental
LONPEDΩ*	3.15	3.11	1.81	-	-	-	-	-	-	-	1.27	0.53	Parental
LOFCAU‡	95.40	69.07	126.87	<0.0001	-	<0.0001	-	<0.0001	-	<0.0001	-	-	Transgressive
LGFCAU†	15.13	4.37	8.44	<0.0001	<0.0001	-	<0.0001	-	<0.0001	-	-	-	Intermediate
LMAXCA‡	44.40	38.68	65.17	<0.0001	-	0.34	-	<0.0001	-	<0.0001	-	-	Transgressive
NBDENTΩ	5.40	0.27	1.03	-	-	-	-	-	-	-	40.46	<0.0001	Intermediate
LGBASE†	7.15	3.73	6.15	<0.0001	<0.0001	-	0.13	-	<0.0001	-	-	-	Parental ^{N}
HAUINV†*	8.52	6.50	7.92	<0.0001	<0.0001	-	0.24	-	<0.0001	-	-	-	Parental ^{N}
LOTGEX†*	6.86	3.84	5.61	<0.0001	<0.0001	-	<0.0001	-	<0.0001	-	-	-	Intermediate
LOTGIN‡*	6.53	5.50	6.25	<0.0001	-	0.01	-	0.63	-	0.01	-	-	Parental ^{N}
LGTGEXΩ*	1.23	0.73	1.00	-	-	-	-	-	-	-	35.55	<0.0001	Intermediate
LGTGINΩ*	0.89	0.70	0.85	-	-	-	-	-	-	-	17.14	<0.0001	Intermediate
NBRAYO‡*	29.10	25.27	32.20	-	-	0.05	-	0.23	-	<0.0001	-	-	Parental ^{N}
LORAYO‡*	10.22	10.72	10.68	0.81	-	0.85	-	0.86	-	0.99	-	-	Parental
NBFLEU‡*	48.97	34.63	37.63	<0.0001	-	<0.0001	-	0.01	-	0.60	-	-	Parental ^{B}
LOTUBEΩ*	1.52	1.72	1.74	-	-	-	-	-	-	-	1.33	0.51	Parental
LOLIMB‡*	2.30	2.26	2.47	0.27	-	0.95	-	0.43	-	0.21	-	-	Parental
LOLOBEΩ*	0.83	0.72	0.82	-	-	-	-	-	-	-	5.76	0.06	Parental
LOANTH‡*	1.89	1.85	1.91	0.83	-	0.92	-	0.98	-	0.81	-	-	Parental
LOSTIGΩ*	1.20	1.20	1.20	-	-	-	-	-	-	-	0.58	0.75	Parental

LOBRA1 and NBRCAP significantly (ANOVA, *p-value*=0.03) discriminate the two parents but their variation in *S. anticostense* overlaps that of both parents. The mean values for *S. anticostense* are between those of the parents and these characters are assumed to be intermediate. LGTGEX, NBDENT, LGTGIN, LGBRA1, LOTGEX, and LGFCAU discriminate the three species; their expression is intermediate in *S. anticostense*. LOFCAU and NBENA1 differentiate completely the three species. The mean values of these traits in *S. anticostense* are extreme with regard to both parents; these variables are presumed to be transgressive. LMAXCA does not discriminate between the two parents but discriminates *S. anticostense* from them; this trait also seems to be transgressive (Table 3.3).

Overall, 23 of 34 (67.5%) characters are presumed to be parent-like, eight (23.5%) intermediate, and three (9%) transgressive. Moreover, 84% of the vegetative and 53% of the floral characters significantly contributed to discriminate the three species.

3.5.1.2 Principal Component Analysis

Figure 3.3 provides the results of the PCA analysis of morphometric data. The PC1 and PC2 axes together account for 41.9% of the total variation. Such a value is usual when many characters are analyzed (Rosenthal et al., 2002). The position of most individuals of *S. anticostense* in the reduced-space graph is intermediate between the two parents. PC1 is the most effective axis in discriminating between *S. novi-belgii* and *S. boreale*. In the case of *S. anticostense* and *S. novi-belgii*, infraspecific variation appears greater than the interspecific one; for instance, three accessions of *S. anticostense* are grouped with accessions of *S. novi-belgii* (Fig. 3.3). PC2 does not discriminate among the three species. The majority of characters have a high loading towards the accessions of *S. novi-belgii* and *S. anticostense* which all have negative PC1 scores (see inset in Fig. 3.3). NBENTG is the only variable with a significant positive loading on axis 1 towards *S. boreale* (Fig. 3.3); the mean of this trait is highest in *S. boreale* (Table 3.3). PC2 has low eigenvector for LONPED and high eigenvector values for the floral traits. In general, the PCA of all variables did not clearly discriminate the three species.

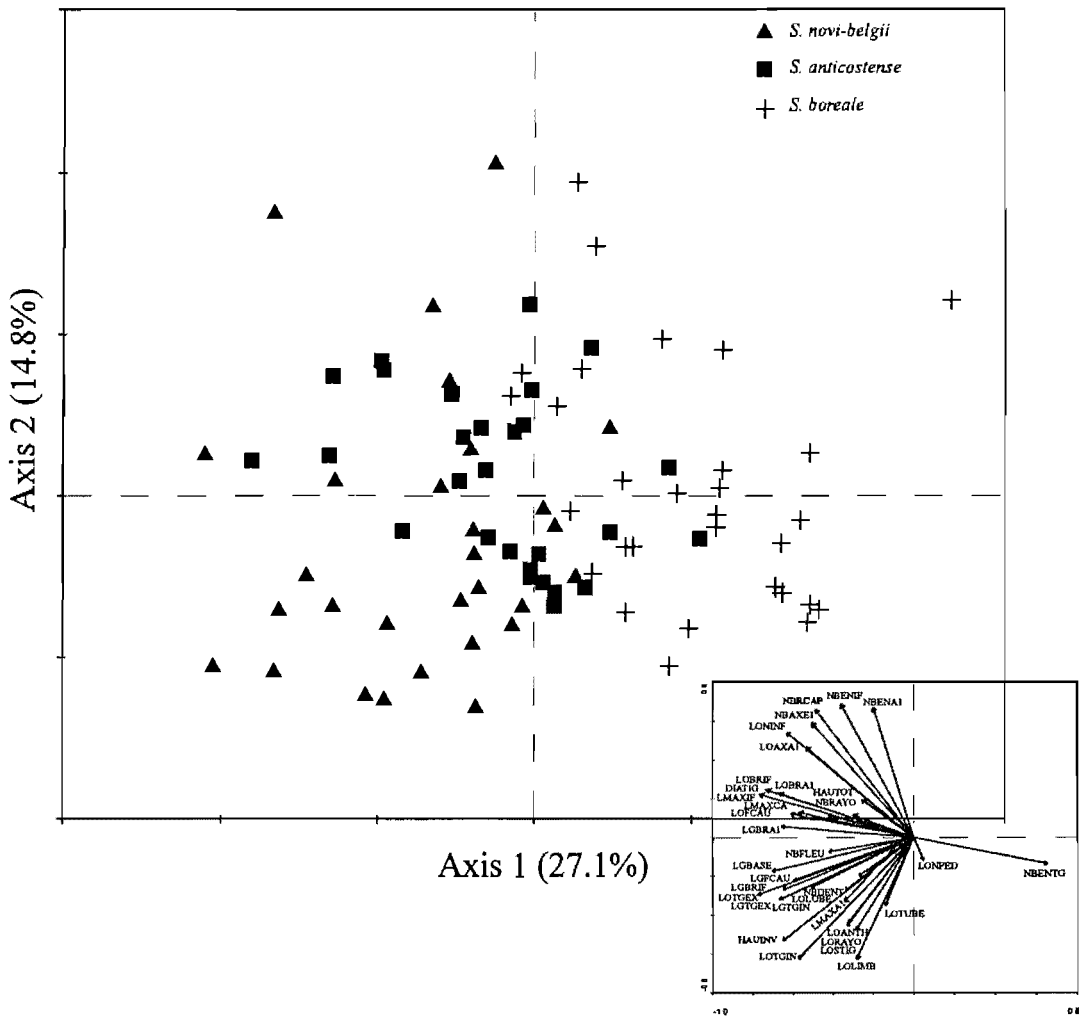

Figure 3.3 Principal Component Analysis (PCA) of the morphological data comprising 28, 29, and 30 accessions of *S. anticostense, S. novi-belgii*, and *S. boreale,* respectively. The inset represents the character vector, which was scaled to 1 in this analysis. Abbreviations used in inset graph are explained in Table 3.2.

3.5.1.3 Canonical Discriminant analysis

The first canonical axis of the CDA significantly supports (Wilk's $\lambda = 0.041$, df = 64) the *a priori* species classification, whereas the second does not (Wilk's $\lambda = 0.297$, df = 31). The first function accounts for 72.4% of the total variation. The traits contributing the most to the separation of the three species along this axis are, in descending order:

LGFCAU, LGBRIF, LOTGEX, NBDENT, LGBRA1, LGBASE, LGTGEX, HAUINV, NBFLEU, LGTGIN, and LOTGIN. The following traits contribute the most to the total variation along the second canonical axis, in descending order: LOFCAU, DIATIG, LMAXCA, NBENTG, NBRAYO, LMAXIF, and LOBRIF. In addition, 96.6% of the *S. novi-belgii* specimens were correctly classified (one accession is classified with *S. anticostense*), 92.9% of *S. anticostense* individuals (one accession is classified between the accessions of *S. boreale* and *S. novi-belgii*, and one with *S. novi-belgii*), and 100% of *S. boreale* individuals (Table 3.4, Fig. 3.4). The CDA of the *a priori* groups shows a nearly complete separation of the three species along the first function. Most *S. anticostense* accessions are intermediate between the parents along this axis. The second function could not discriminate the two parents, but separates *S. anticostense* from them (Fig. 3.4).

3.5.2 Molecular analyses

3.5.2.1 Phylogenetic analysis

After removal of 14 repeated intraindividual ribotypes, 343 ribotypes (Table 3.1) were included in the analysis, including 630 aligned characters, of which 455 were constant and 82 parsimony informative. No recombinant ribotypes were detected.

The maximum likelihood analysis generated a single tree (-ln = 3431.6494) (Fig. 3.5). The ingroup (all species of *Symphyotrichum* included in the analysis) forms a well-supported clade (bootstrap proportion = 84) within Symphyotrichinae. In the phylogenetic tree, insufficient resolution was found among the ribotypes indicated by clades A to G. All the ribotypes of *S. anticostense* and *S. novi-belgii* are found within these groups.

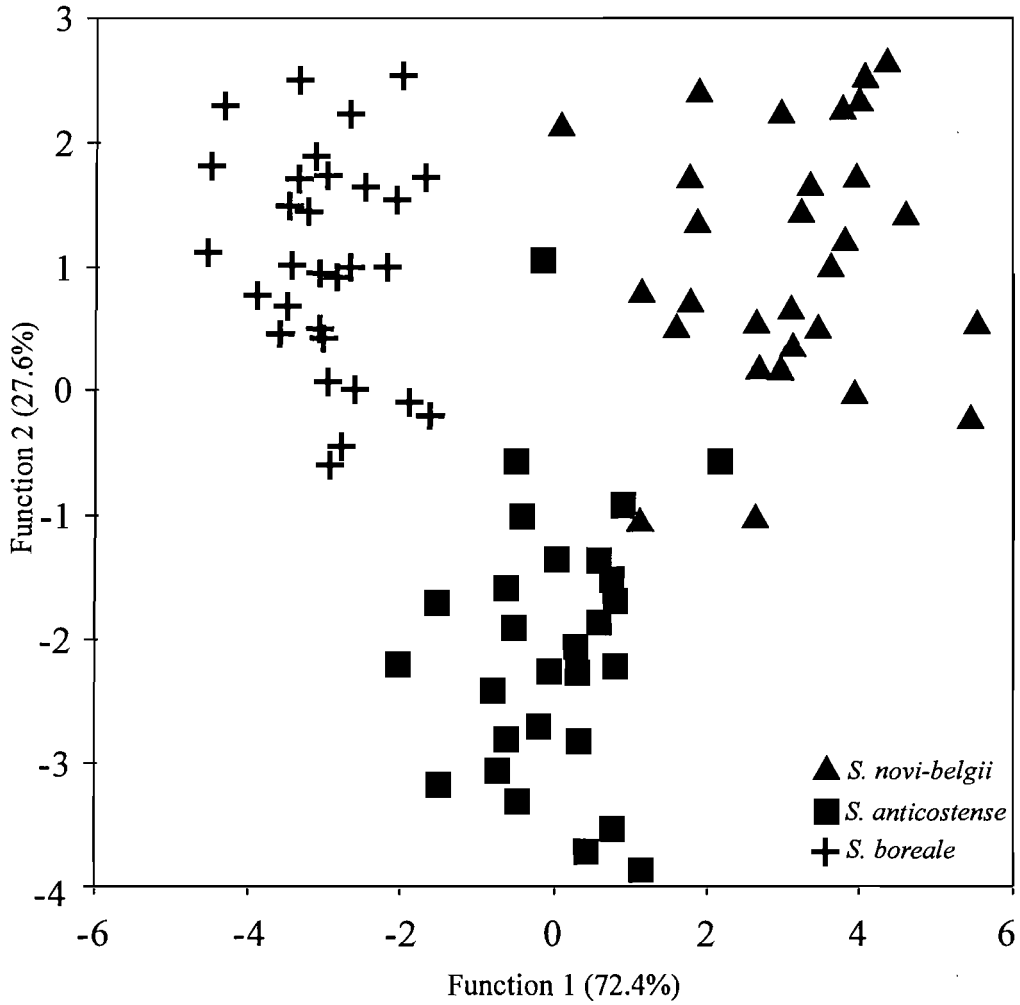

Figure 3.4 Canonical Discriminant Analysis (CDA) of the morphological characters including 28, 29, and 30 accessions of *S. anticostense, S. novi-belgii*, and *S. boreale,* respectvely. Three groups were defined as a priori groups in the analysis.

Table 3.4 Results of an a posteriori classification using canonical discriminant analysis based on 87 accessions and 34 morphological characters.

		A posteriori group			Percentage correctly
A priori group	N	*S. novi-belgii*	*S. anticostense*	*S. boreale*	classified
S. novi-belgii	29	28	1	0	96.6
S. anticostense	28	1	26	1	92.9
S. boreale	30	0	0	30	100
Total	87	29	27	31	96.5

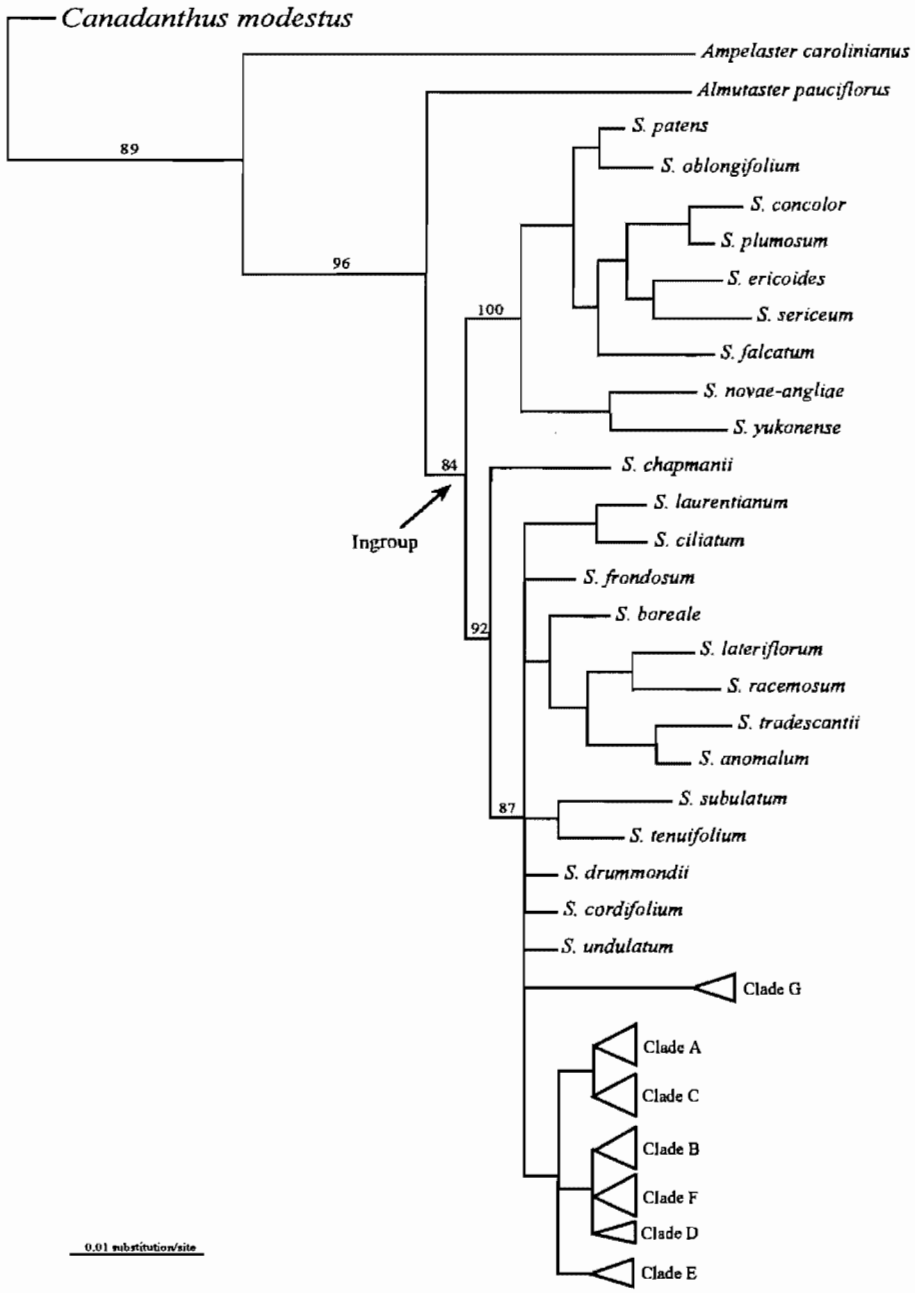

Figure 3.5 Phylogenetic tree of the ITS data set resulting from ML analysis using the three species under study, 34 diploid species as well as 3 genera as outgroups. The clades A, B, C, D, E, F, and G are comprised of the non-resolved ribotypes that are expanded in figure 3.6 and appendix 1. The numbers indicated above the branches are bootstrap support values.

3.5.2.2 Haplotype network

Details concerning the number and GenBank number of ribotypes of each accession are summarized in Table 3.1. A simplified network is shown in figure 3.6 (the complete network is provided in Appendix 1). Clade A, excluding its derivatives, is the most frequent (30 occurrences) followed by groups B (29), C (24), D (15), E (12), F (9), and G (6). Clades A, B, and D (excluding their derivatives) comprise ribotypes found exclusively in *S. anticostense*, *S. novi-belgii*, and *S. boreale* accessions. Clades C and F (excluding their derivatives) include ribotypes belonging to *S. anticostense* and *S. novi-belgii* accessions.

Derivatives of clades A, B, C, and D include ribotypes that belong exclusively to *S. anticostense*, *S. novi-belgii*, and *S. boreale*. Derivatives of clade F include ribotypes of the three species under study as well as those of diploid species. Clade E which is connected to clade F via three mutational steps, includes ribotypes shared by diploid species *S. spathulatum*, *S. dumosum*, *S. welshii*, *S. nahanniense*, and *S. boreale*. No shared or derived ribotype of *S. anticostense* was detected within this clade. Clade G, which is connected to clade F through 12 mutational steps, includes ribotypes shared by the diploid species *S. firmum*, *S. anomalum*, *S. elliottii*, *S. porteri*, *S. parviceps*, *S. depauperatum*, *S. puniceum*, and *S. boreale*. One derived ribotype in this clade is found among ribotypes of *S. anticostense* (accession 697, Table 3.1, Fig. 3.6). No ribotype of *S. novi-belgii* was found within clades E and G. In summary, seven ribotypes are shared exclusively by *S. anticostense* and *S. novi-belgii*, one by *S. anticostense* and *S. boreale*, one by *S. boreale* and *S. novi-belgii*, and four by all three species.

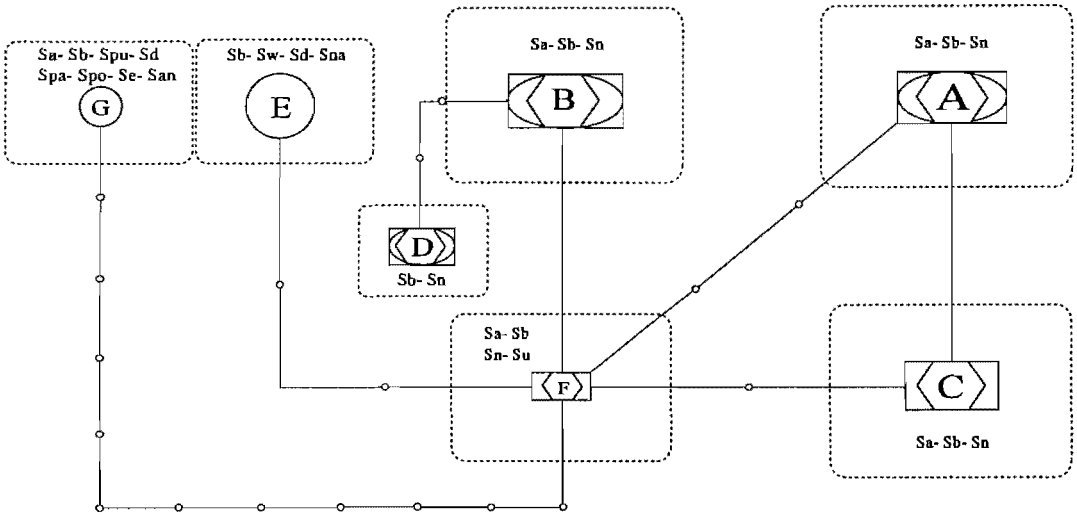

Figure 3.6 Simplified ribotype network of the ITS data set using all ribotypes of the clades A to G of the phylogenetic tree in figure 3.5. The ellipse, hexagon and rectangle indicate the ribotypes of *S. boreale, S. novi-belgii* and *S. anticostense,* respectively. The three forms are collapsed when the species share same ribotype. Large circles indicate share ribotype among *S. boreale* and the other diploid species (see the Text). The small circles indicate unsampled ribotypes. The size of the symbols is proportionate to the number of shared ribotype. Derivatives of each clade are indicated as their abbreviations (Sa: *S. anticostense*; Sb: *S. boreale*; Sn: *S. novi-belgii*; Spu: *S. puniceum*; Sd: *S. dumosum*; Spa: *S. parviceps*; Spo: *S. porteri*; Se: *S. elliottii*; San: *S. anomalum*; Sw: *S. welshii*; Sna: *S. nahanniense*; Su: *S. uropliyllum*) in the areas designated by the broken-line rectangles. The complete network is given in appendix 1.

3.6 Discussion

3.6.1 Morphological evidence

In general, the ANOVA results have shown that 16 of 19 (84%) vegetative traits significantly differentiate the three species, in contrast to 7 of 15 (53%) floral characters (Table 3.3). The three species differ more in their vegetative than reproductive features, as was shown by Labrecque and Brouillet (1996). During field studies, the most discriminating feature between *S. anticostense* and *S. novi-belgii* was leaf shape: stem leaves of *S. anticostense* are longer and narrower than those of *S. novi-belgii*, in addition to being more rigid and arched (a feature neither easily measurable nor visible on dried

specimens). This is reflected in the statistically significant differences observed in the length (LOFCAU) and width (LGFCAU) of stem leaves between these two species (Table 3.3 and CDA results).

The proportion of parental characters (traits for which the value in *S. anticostense* does not significantly differ from one or the other parent) reported here for *S. anticostense* (67.5%) is higher than that reported on average (48.7%) for hybrid species (Rieseberg and Ellstrand, 1993). One explanation for this difference is that in Rieseberg and Ellstrand (l.c.), polyploid species were excluded from their compilation as it is difficult to compare these values. Ten of 23 (43.5%) parental traits of *S. anticostense* are *novi-belgii*-like, and three (13%) are *boreale*-like. The mean values of the remaining parental traits do not differ significantly among the three taxa. The greater similarity of *S. anticostense* to *S. novi-belgii* may be explained by the fact that *S. novi-belgii*, a hexaploid, contributed six copies to the genome of *S. anticostense*, and *S. boreale*, putatively tetraploid, four. This may indicate a dosage effect in which morphological variables controlled by quantitative genes would lean more toward *S. novi-belgii* values because of its greater genomic contribution (Uijtewaal et al., 1987; Guo et al., 1996; Aagaard et al., 2005).

Of the eight intermediate characters, number of heads per inflorescence (NBRCAP), stem leaf teeth number (NBDENT), and stem leaf width (LGFCAU) are the traits most discriminating *S. anticostense* from its putative parents.

Stem leaf length (LOFCAU), number of internodes on axis 1 (NBENA1), and distance between the apex and the point of maximum width of the stem leaf (LMAXCA) appear to be transgressive in *S. anticostense* relative to the values in its parents. Transgressive traits could result from mutated alleles, an interaction between the parental genomes resulting in a new trait, or the sum of the effects of the parental genes (reviewed in Rieseberg et al., 1999). Our results do not allow us to distinguish which scenario generated the transgressive traits. The low percentage of transgressive characters (9%) obtained in the current study for *S. anticostense* concords with the results reported by

Rieseberg et al. (1999) in which transgressive traits appear to be more frequent in intraspecific crosses than interspecific ones.

Both PCA (Fig. 3.3) and CDA (Fig. 3.4) demonstrate the intermediacy of *S. anticostense* with respect to its putative parents. In the PCA, three accessions of *S. anticostense* are grouped with *S. novi-belgii*. These accessions were collected in the Gaspé Peninsula (Table 3.1; accessions 1432, 44256, and 44279) where the two species are sympatric and often in ecological contact. It is possible that such individuals were introgressed with *S. novi-belgii*, since hybrids (2n = 64) between the two taxa have been collected (Brouillet and Labrecque, 1987). The high proportion of correct a priori classification of the *S. anticostense* accessions (92.9%) indicates that the species can be readily identified from its parents.

3.6.2 Molecular evidence

There was neither bootstrap support (> 50%) nor sufficient phylogenetic resolution among the clades A to G in the phylogenetic tree. Lack of resolution among and within these clades and abundant interspecific hybridization including auto-and allopolyploidy (Jones, 1977; Semple and Brammall, 1982; Allen, 1986; Brouillet and Labrecque, 1987; Labrecque and Brouillet, 1996; Semple et al., 2002) within *Symphyotrichum* probably indicate that the genus is a group of recently diverged species. The recent evolution of the genus and the considerable allelic variation found within each individual of the polyploid species (see Table 3.1 for the number of alleles found per individual) may indicate that concerted evolution alone is unlikely to be responsible for the low resolution among ribotypes.

Shared ribotypes within clades A, B, C, D, and F among the three species and the absence of ribotypes belonging to the diploid species in these clades could highlight the occurrence of hybridization between the proposed parents. Within the ribotypic pool of *S. anticostense*, one ribotype (accession 697, Table 3.1 and Fig. 3.6) is found within clade G, which forms an isolated clade relative to the others. This ribotype is present in *S. boreale*

but absent in *S. novi-belgii*. Therefore, this may be considered as an indicator of the contribution of *S. boreale* to the hybridization.

Ribotypes within clades E and G belong exclusively to the diploid species and *S. boreale*. It is possible that this pattern resulted from the retention of ancestral polymorphisms, possibly due to the recency of the radiation of the genus (Pamilo and Nei, 1988), rather than introgression among this group of species which are often allopatric (Brouillet et al., 2006). The absence of shared ribotypes between *S. anticostense* and clade E suggests that ribotypes of this clade did not participate in the hybridization. Ribotypic distribution (Fig. 3.1) shows that clade E is frequent in the western part of the range of *S. boreale,* where clades A to D are absent. This evidence may raise a question as to whether the western populations of *S. boreale* participated in the hybridization, though Owen et al. (2006) suggested that these populations possibly migrated from west to east. Our molecular data do not reject this hypothesis, but indicate that the migrated populations carrying ribotype E may not have contributed to the hybridization.

The total number of ribotypes of *S. anticostense* compared to *S. novi-belgii* appears to be proportional to genome dosage (68 vs 44 ≈ 10: 6; each for 23 accessions), whereas such a theoretical ratio is not respected in the case of *S. boreale* (58 ribotypes for 33 accessions) vs *S. anticostense* (≈ 8: 5 instead of the expected 10: 4). This difference could be explained by: 1) the number of accessions used for *S. boreale* being greater than those of *S. novi-belgii* and *S. anticostense*, therefore more ribotypes generally might have been detected (Table 3.1); 2) the distribution range of *S. boreale* being much wider than those of *S. novi-belgii* and *S. anticostense* (Fig. 3.1) and greater genetic differentiation may have occurred; 3) the ploidy level of *S. boreale* ranging from 2x to 8x, ploidy level being unknown for the herbarium specimens used in our study; the greater number of ribotypes could be due to this variability.

3.6.3 Geographic origins

An examination of the spatial distribution of the *S. anticostense* ribotypes (Fig. 3.1) would indicate that there were possibly three independent geographic origins for *S. anticostense*: 1) Anticosti Island, 2) Gaspé Peninsula and New Brunswick, 3) Lake St. John. Firstly, ribotype G from *S. anticostense* occurs only on Anticosti Island. Anticosti populations of *S. boreale* involved in the hybridization have not been sampled and the present populations of this species (accessions 21341, 27595, and 21338, but not 27594; Table 3.1) included in the study do not appear to have contributed to this hybridization (Fig. 3.1). Moreover, Anticosti Island has never been connected to the mainland since deglaciation (Brouillet and Wheststone, 1993; Josenhans and Lehman, 1999; Lavoie and Filion, 2001). Though long distance dispersal cannot be precluded in a wind-dispersed group such as *Symphyotrichum*, it appears more likely that populations of *S. anticostense* arose independently on the island and remained isolated after their inception.

The second plausible origin may be in the Gaspé Peninsula and New Brunswick, where neither ribotypes D nor G are present in populations of *S. anticostense*. Ribotype D is present in populations of both parents on both sides of the St. Lawrence River. This ribotype is only present in the western populations of *S. anticostense* (Lake St. John), not in the eastern ones (Gaspé Peninsula and New Brunswick). This could be explained by the fact that eastern populations of both parents carrying ribotype D, established themselves after the hybridization occurred or else, they simply never contributed to the event.

The third probable origin of *S. anticostense* is on Lake St. John, where the unique ribotype D is found in the *S. anticostense* population. This population is confined and probably never migrated out of its area. The absence of significant calcareous substrates between Lake St. John and the Gaspé Peninsula (along the Saguenay River), and the long distance between them, would appear to preclude the hypothesis of a dispersal of *S. anticostense* between the two regions.

Alternately to the multiple origins hypothesis, it would be possible to consider a single origin for *S. anticostense*. The current geographic distribution of *S. anticostense* could be interpreted as an initial origin at a single locale followed by subsequent long-distance dispersal; the observed ribotype variation could then be explained by introgression with its parents or local mutations. It is difficult to determine the place of origin of *S. anticostense* in such a scenario with the current data. A comprehensive sampling strategy should be designed to investigate these hypotheses.

It seems that *S. anticostense* inherited its habitat (calcareous, disturbed geolittoral) from both its parents: it grows on calcareous substrates in association with fresh water (never in saline areas), similarly to *S. boreale*, as well as on slightly sandy soil and disturbed areas, as does *S. novi-belgii* (Labrecque and Brouillet, 1990, 1996; Owen et al., 2006). *Symphyotrichum anticostense* tends to settle in habitats ecologically closer to those of *S. novi-belgii* than to those of *S. boreale*. This may help explain why *S. anticostense* has not been found in mixed populations with *S. boreale* and no hybrid has been reported between them. The intermediate ecological traits inherited from both parents, in combination with the morphologically intermediate characters and shared ribotypes, are all indicators of the allopolyploid origin of *S. anticostense*.

CHAPTER 4

Inferring parental lineages of a high allopolyploid, *Symphyotrichum anticostense* (Asteraceae: Astereae) using two nuclear markers[3]

4.1 Résumé

Le processus d'hybridation est un événement fréquent chez *Symphyotrichum*. *Symphyotrichum anticostense* (Fern.) G. L. Nesom est une espèce néo-endémique et rare du nord-est de l'Amérique du Nord. On a proposé qu'il s'agirait d'une espèce allo-décaploïde (2n = 10x = 80), dérivée d'un hybride entre les *S. boreale* (Torr. & A. Gray) Á. Löve & D. Löve, probablement tetraploïde (2n = 4x = 32), et *S. novi-belgii* (L.) G. L. Nesom, un hexaploïde (2n = 6x = 48). Nous utilisons deux marqueurs moléculaires du génome nucléaire à faible nombre de copies, la glycéraldéhyde-3-phosphate déshydrogénase (GAPDH) et la myo-inositol 1-phosphate synthase (MIPS), pour démontrer la nature hybride du taxon et ses relations aux parents potentiels. Les arbres phylogénétiques des gènes GAPDH et MIPS sont incongruents. À cause de l'évolution récente du genre *Symphyotrichum*, ce conflit pourrait résulter du polymorphisme ancestral des allèles par rapport aux événements de spéciation. Néanmoins, des allèles des deux parents sont détectés chez l'allopolyploïde. Cependant, ceci n'est pas la preuve définitive du parentage de l'allopolyploïde, parce que certains alleles de *S. anticostense* sont groupées avec ceux d'espèce diploïdes.

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[3] Vaezi, Jamil and Luc Brouillet. Paper to be submitted.

4.2 Abstract

Hybridization is a common process in *Symphyotrichum*. *Symphyotrichum anticostense* (Fern.) G. L. Nesom is a rare neo-endemic species from northeastern North America. It has been hypothesized to be an allo-decaploid species (2n = 10x = 80), derived from a hybrid between *S. boreale* (Torr. & A. Gray) Á. Löve & D. Löve, possibly tetraploid (2*n* = 4x = 32), and *S. novi-belgii* (L.) G. L. Nesom, a hexaploid (2*n* = 6x = 48). We are using two low-copy nuclear genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and myo-inositol 1-phosphate synthase (MIPS), to investigate the hybrid nature of the taxon and its relationships to the putative parents. The GAPDH and MIPS phylogenetic trees show incongruence. Given the recent divergence of genus *Symphyotrichum,* this conflict may result from the incomplete lineage sorting of alleles with respect to speciation events. Nonetheless, alleles from both parents are detected in the allopolyploid. This is not definite proof, however, of the parentage of the allopolyploid because some alleles of *S. anticostense* are grouped with those of diploid species of the genus.

4.3 Introduction

Polyploidy has occurred not only in angiosperms (Stebbins, 1950; Masterson, 1994), but also apaprently in the lineages leading to yeast, insects, reptiles, and fish (Lewis, 1980; Spring, 1997; Postlethwait et al., 1998; Simon et al., 2003). Allo-polyploidy, a form of hybrid speciation, has been shown to have played an important role in the evolution and speciation of both plants and animals (Arnold, 1992, 1997). Polyploid origin of species has been well studied in recent decades (e.g., Grant, 1966; Ingram and Noltie, 1984; Rieseberg, 1991; Popp and Oxelman, 2001; Kadereit et al., 2005; Fehrer et al., 2007). Molecular markers of both maternally and biparentally inherited DNA sequences have proven to be powerful tools for identifying hybrid origins in many plant taxa (Sang and Zhang, 1999; Small and Wendel, 2000; Doyle et al., 2003; Kadereit et al., 2005; Popp et al., 2005; Fortune et al., 2008; Kao, 2008). The use of markers with different evolutionary histories, however, may lead to incongruence in

phylogenetic inferences, particularly when one or more alloploid species are included (Wendel and Doyle, 1998; Lihová et al., 2006). Gene duplication, introgression (a form of horizontal gene transfer), and incomplete lineage sorting can all possibly explain incongruence (Lyons-Weiler and Milinkovitch, 1997; Slowinski et al., 1997; Buckley et al., 2006; Maddison and Knowles, 2006). It is difficult to distinguish among these different historical phenomena because they generate similar phylogenetic traces (Holder et al., 2001). Nevertheless, incomplete lineage sorting accounts well for incongruence among gene genealogies in recently radiated groups of species in which terminal branches of the species tree are short and where coalescence of alleles belonging to a single locus precedes the common ancestor of the group (Pamilo and Nei, 1988; Doyle, 1992; Maddison, 1997; Rosenberg, 2003). Incomplete lineage sorting, which is a challenge to phylogenetic inference, has contributed to the evolution of recently diverged taxa such as *Senecio* (Comes and Abbott, 2001), *Arabis* (Dobeš et al., 2004), *Maoricicada* (Buckley et al., 2006), *Pardosa astrigera* (Chang et al., 2007), and *Dactylorhiza* (Pillon et al., 2007).

Furthermore, in a group of closely related species where hybridization is frequent, introgression may cause further reticulation and produce incongruence among lineages (Abbott, 1992; Rieseberg, 1998; Comes and Abbott, 2001; Linder and Rieseberg, 2004). It then becomes more difficult to determine with certainty whether shared alleles among species reflect introgression or ancestral polymorphisms.

Despite the potential complexity due to introgression and incomplete lineage sorting, it is possible to investigate allopolyploid origin including the putative parental species in a phylogenetic framework using low-copy nuclear genes and other molecular markers (e.g., nrDNA ITS, RAPD) (Popp and Oxelman, 2001; Ingram and Doyle, 2003; Grundt et al., 2004; Fehrer et al., 2007). Using molecular markers with repetitive sequences such as tandemly repeated nrDNA increases the possibility that concerted evolution may have homogenized the copies towards one parent and deleted traces of the other (Zimmer et al., 1980; Wendel et al., 1995). In contrast, using low-copy nuclear gene markers probably decreases the possibility of interlocus homogenization and increases

the probability of detecting allopolyploid origin (Popp and Oxelman, 2001; Ingram and Doyle, 2003).

Symphyotrichum Nees (Asteraceae: Astereae) comprises approximately 91 species that are predominantly North, Central and South American, with one species native in Eurasia. The base chromosome numbers within the genus are x = 4, 5, 7, and 8 (Nesom, 2000; Brouillet et al., 2006; Nesom and Robinson, 2007). *Symphyotrichum* and four closely related genera, *Canadanthus*, *Ampelaster*, *Almutaster*, and *Psilactis,* form a monophyletic group (Xiang and Semple, 1996; Brouillet et al., 2001; chapter 2) defined as subtribe Symphyotrichinae (Nesom, 2000). Hybridization is widespread within the genus wherever two or more species co-occur (Semple et al., 2002). More than 50 species (>50%) have experienced polyploidization during their evolutionary history (Brouillet et al., 2006).

Symphyotrichum anticostense (Fern.) G. L. Nesom is a high-polyploid species (2n = 10x = 80) distributed mainly in the Gulf of St. Lawrence region (Quebec, New Brunswick, and Maine; Fig. 4.1). It grows on calcareous and coarsely sandy soils with a preferentially gravelly texture, on the geolittoral of rivers. The species has been hypothesized to be an allo-polyploid species derived from *S. boreale* (2n = 4x = 32) and *S. novi-belgii* (2n = 6x =48) (Brouillet and Labrecque, 1987). *Symphyotrichum boreale* also has other ploidy levels (2x, 6x, and 8x) and is distributed throughout boreal North America (Owen et al., 2006). It grows on calcareous, moist habitats near streams and in fens. *Symphyotrichum novi-belgii* is distributed in the eastern coastal regions of North America and overlaps the entire range of *S. anticostense* (Labrecque and Brouillet, 1996; Semple et al., 2002). It grows on sandy soils (on serpentine soil in Newfounland) with sufficient moisture along rivers, especially on the geolittoral zone, on sand dunes, in salt marshes, and in disturbed areas such as roadsides.

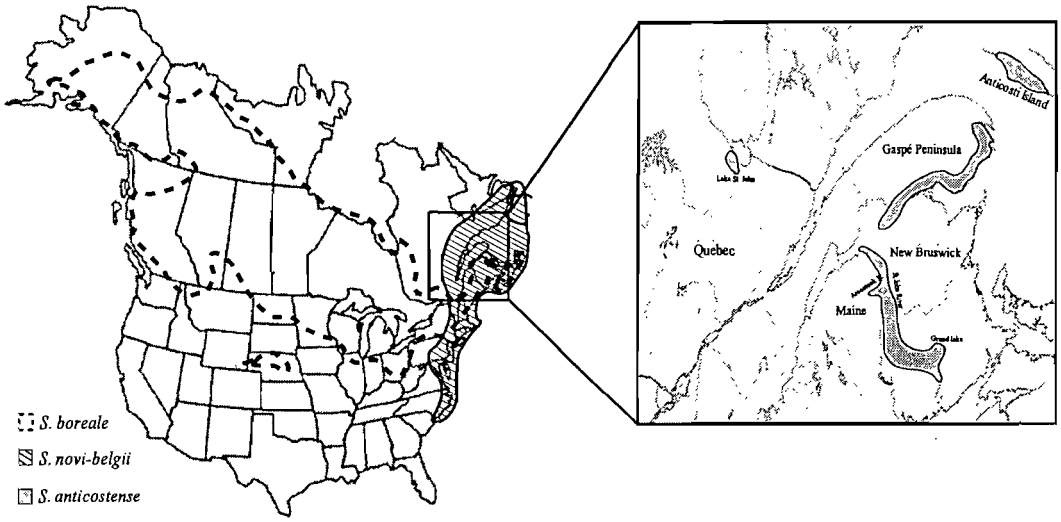


Figure 4.1 Geographical distribution of *S. anticostense* (darked-gray regions of inset), *S. novi-belgii* (diogonal lines), and *S. boreale* (broken lines) in northern North America.

In chapter three the origin of *S. anticostense* was investigated using morphological and molecular (nrDNA) evidence. The analyses which included the hypothesized parents as well as most diploid species within the genus, showed that the proposed parents were probably involved in the hybridization event. Due to very similar morphologies, mainly in floral but also in vegetative traits among species of subgenus *Symphyotrichum* (Labrecque and Brouillet, 1996) where *S. anticostense* is placed, and due to the little resolution among the tandemly repeated ITS ribotypes (chapter 3), uncertainty still remains as to the origin of *S. anticostense*.

Here we use sequence data from two biparentally inherited nuclear genes: glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and myo-inositol 1-phosphate synthase (MIPS). The GAPDH marker has been used in previous studies and is recognized as a low-copy nuclear marker (Olsen and Schaal, 1999; Camara et al., 2002; Joly et al., 2006; chapter 2). MIPS plays a critical role in the biosynthesis of inositol as it catalyses glucose 6-phosphate to myo-inositol-1-phosphate (Stein and Geiger, 2002). This marker is used for the first time in phylogenetic analysis in the current study.

The main objective of the present study is to investigate the hypothesis of allopolyploid hybrid origin of *S. anticostense* by including the putative parents and diploid species within a phylogenetic framework, using low-copy nuclear genes.

4.4 Materials and Methods

4.4.1 Plant materials and DNA isolation

We sampled leaf material from one or rarely two individuals per population throughout the entire range of *S. anticostense* and *S. novi-belgii* (Fig. 4.1, Table 4.1) in 2004 and 2005. The leaf material was dried in silica gel. Herbarium specimens were used as a source for *S. boreale* from its entire range. A total of 20 and 17 individuals of *S. anticostense*, 19 and 12 individuals of *S. novi-belgii*, as well as 25 and 29 individuals of *S. boreale* were included in the GAPDH and MIPS phylogenetic analyses, respectively. In addition, one or rarely two individuals per diploid species and a total of 22 species representing the four subgenera *Symphyotrichum*, *Virgulus*, *Astropolium*, and *Chapmaniani*, were included in the study to investigate the possible involvement of these species in the hybridization. We rooted our phylogenetic trees with three closely related species, *Canadanthus modestus*, *Ampelaster carolinianus*, and *Psilactis tenuis*, and a distantly related species, *Heterotheca monarchensis*, as outgroups. Cytological vouchers were used for diploid species. For most samples, DNA was extracted using the CTAB method (Doyle and Doyle, 1987) as modified by Joly et al. (2006); for samples difficult to amplify, the QIAgen DNeasy Plant Mini Kit (QIAGEN, Mississauga, Ontario, Canada) was used following the instructions of the manufacturer.

Table 4.1 List of plant material and Genbank accession numbers for the three molecular markers used in the study.

(sub)genus/species	Locality/ Province	Collector(s)	GenBank Accession No.		
			GAPDH	MIPS paralog 1	MIPS paralog 2
subg. *Sympyotrichum*					
S. novi-belgii (L.) G.L. Nesom	Labrador/ Nfld. and Labr.	Bay, 233 (MT)	EU732786-8	EU754268-74	EU754611-3
S. novi-belgii (L.) G.L. Nesom	Labrador/ Nfld. and Labr.	Bay, 236 (MT)	-	-	EU754614
S. novi-belgii (L.) G.L. Nesom	Lac St. John/ Que.	Vaezi & Brouillet, 242 (MT)	EU732789-91	EU754275-84	-
S. novi-belgii (L.) G.L. Nesom	Saguenay river/ Que.	Vaezi & Brouillet, 250 (MT)	EU732792	-	-
S. novi-belgii (L.) G.L. Nesom	Saguenay river/ Que.	Vaezi & Brouillet, 252 (MT)	EU732793-4	EU754285-90	-
S. novi-belgii (L.) G.L. Nesom	Porteneuf river/ Que.	Vaezi & Brouillet, 253 (MT)	EU732795-6	EU754291-4	EU754615-7
S. novi-belgii (L.) G.L. Nesom	Bic/ Que.	Vaezi & Brouillet, 259 (MT)	EU732797-9	EU754295-300	EU754618-21
S. novi-belgii (L.) G.L. Nesom	Les Escoumins/ Que.	Vaezi & Brouillet, 263 (MT)	EU732800-3	EU754301-5	-
S. novi-belgii (L.) G.L. Nesom	Paspébiac/ Que.	Vaezi & Brouillet, 267 (MT)	EU732804-9	EU754306-12	EU754622
S. novi-belgii (L.) G.L. Nesom	Grand Rivière/ Que.	Vaezi & Brouillet, 282 (MT)	EU732817-23	-	-
S. novi-belgii (L.) G.L. Nesom	Rivière petit pabos/ Que.	Vaezi & Brouillet, 302 (MT)	EU732837-41	EU754327-33	-
S. novi-belgii (L.) G.L. Nesom	Rivière Bonaventure/ Que.	Vaezi & Brouillet, 315 (MT)	EU732851-3	EU754340-6	-
S. novi-belgii (L.) G.L. Nesom	Restigouche R./ N.B.	Vaezi & Brouillet, 347 (MT)	-	EU754358-64	-
S. novi-belgii (L.) G.L. Nesom	Limestone/ N.B.	Vaezi & Zargarbashi, 407 (MT)	EU732864-5	EU754408-11	-
S. novi-belgii (L.) G.L. Nesom	Hartland/ N.B.	Vaezi & Zargarbashi, 450 (MT)	EU732886-91	-	-
S. novi-belgii (L.) G.L. Nesom	St. John R./ N.B.	Vaezi & Zargarbashi, 510 (MT)	EU732904-5	-	-
S. novi-belgii (L.) G.L. Nesom	Moncton/ N.B.	Vaezi & Zargarbashi, 555 (MT)	EU732906-10	-	-
S. novi-belgii (L.) G.L. Nesom	Renton/ N.B.	Vaezi & Zargarbashi, 564 (MT)	EU732911-5	-	-
S. novi-belgii (L.) G.L. Nesom	Batibog river/ N.B.	Vaezi & Zargarbashi, 572 (MT)	EU732916-21	-	-
S. novi-belgii (L.) G.L. Nesom	Rivière Matapedia/ Que.	Vaezi & Zargarbashi, 580 (MT)	EU732922	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Botton Brook/ Nfld.	Brouillet, s.n. (MT)	EU732770-3	EU754252,54-6	-
S. boreale (T. & G.) Á. Löve & D. Löve	Albany Co./ Wyo.	Semple, 11233 (WAT)	EU732863	EU754391-4	EU754631-5
S. boreale (T. & G.) Á. Löve & D. Löve	Moose Mountains Creek/ Sask.	Harms, 38132 (MT)	EU732923	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Eirch river/ Man.	Semple & Brouillet, 4143 (MT)	EU732924	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	St. Quentin/ N.B.	Haber & Bristow, 3601 (MT)	EU732925	EU754429-35	EU754638
S. boreale (T. & G.) Á. Löve & D. Löve	Bay of Islands/ Nfld.	Djan-chékar et al., 1461 (MT)	EU732926-8	EU754436-41	EU754639-40
S. boreale (T. & G.) Á. Löve & D. Löve	Lac au saumon/ Que.	s.n., 845 (MT)	EU732929	EU754450-3, EU754500-1	-
S. boreale (T. & G.) Á. Löve & D. Löve	Rivière Bonaventure/ Que.	LePage, s.n. (MT)	-	EU754457-8	EU754645-9

Table 4.1 *Continued ...*

(sub)genus/species	Locality/ Province	Collector(s)	GenBank Accession No.		
			GAPDH	MIPS paralog 1	MIPS paralog 2
S. boreale (T. & G.) Á. Löve & D. Löve	Anticosti Island/ Que.	Victorin et al., 21341 (MT)	-	EU754454	EU754650-4
S. boreale (T. & G.) Á. Löve & D. Löve	St. Hippolyte de Kilkenny/ Que.	Hébert, 72-124-1 (MT)	-	EU754455-6, EU754459-61	EU754655
S. boreale (T. & G.) Á. Löve & D. Löve	Anticosti Island/ Que.	Victorin & Germain, 27594 (MT)	EU732930-1	EU754462-8	-
S. boreale (T. & G.) Á. Löve & D. Löve	Lac au saumon/ Que.	LeGallo, 841 (MT)	EU732932-7	EU754469-75	-
S. boreale (T. & G.) Á. Löve & D. Löve	Dundee station/ P.E.I.	Smith, 335 (MT)	EU732938-9	EU754476-9	-
S. boreale (T. & G.) Á. Löve & D. Löve	McAdam lake/ N.S.	Smith et al., 5472 (MT)	EU732940	EU754480-1	-
S. boreale (T. & G.) Á. Löve & D. Löve	Grand Rapids/ Minn.	Wheeler & Glaser, 2342 (MT)	EU732941-4	EU754482-6	-
S. boreale (T. & G.) Á. Löve & D. Löve	Vermont	Seymour, 29703 (MT)	EU732945-6	EU754487-92	-
S. boreale (T. & G.) Á. Löve & D. Löve	Restigouche R./ N.B.	s.n., 3601 (MT)	EU732947-52	EU754493-9	-
S. boreale (T. & G.) Á. Löve & D. Löve	Gold smith lake/ N.B.	Malte, 1019/29 (MT)	EU732953-7	EU754502-4	EU754656-60
S. boreale (T. & G.) Á. Löve & D. Löve	Rivière Eastmain/ Que.	Gagnon & Barabé, 74510 (MT)	EU732958	EU754505-10	-
S. boreale (T. & G.) Á. Löve & D. Löve	Rupert House/ Que.	Spafford, 149 (MT)	EU732959-63	-	EU754661-4
S. boreale (T. & G.) Á. Löve & D. Löve	Lac Labelle/ Que.	Plourde, 240 (MT)	EU732964-7	EU754511-6	-
S. boreale (T. & G.) Á. Löve & D. Löve	Oka/ Que.	Beaudry & Love, 58-202 (MT)	-	EU754517-9	EU754665
S. boreale (T. & G.) Á. Löve & D. Löve	Chibougamau/ Que.	Hustich, 772 (MT)	-	EU754520-3	-
S. boreale (T. & G.) Á. Löve & D. Löve	La Trappe/ Que.	Beaudry & Louis-Marie, 55-249 (MT)	EU732968-70	EU754524-8	-
S. boreale (T. & G.) Á. Löve & D. Löve	Lac aux Nymphes/ Que.	s.n. (MT)	-	EU754529-32	-
S. boreale (T. & G.) Á. Löve & D. Löve	Nomininguе/ Que.	Robert, 872 (MT)	-	EU754533-5	-
S. boreale (T. & G.) Á. Löve & D. Löve	Lac Mistassini/ Que.	s.n., 1525 (MT)	EU732971-3	EU754536-8	EU754666-9
S. boreale (T. & G.) Á. Löve & D. Löve	Oka/ Que.	s.n., 1925 (MT)	-	EU754539-43	-
S. boreale (T. & G.) Á. Löve & D. Löve	Kingston/ Ont.	Garwood & Zavitz, 1987 (MT)	-	EU754544-50	EU754670
S. boreale (T. & G.) Á. Löve & D. Löve	Longlac/ Ont.	Baldwin & Breitung, 3376 (MT)	-	EU754551-5	EU754671
S. boreale (T. & G.) Á. Löve & D. Löve	Lake Winnipeg/ Man.	Scoggan, 5046 (MT)	-	EU754556-60	EU754672
S. boreale (T. & G.) Á. Löve & D. Löve	Anglin lake/ Sask.	Harms, 24741 (Sask)	EU733004-8	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Nipawin provincial park/ Sask.	Argus & Hudson, 4419 (Sask)	EU733009-14	-	-
S. anticostense (Fern.) G.L. Nesom	Lac St. John/ Que.	Vaezi & Brouillet, 212 (MT)	EU732774-8	-	-
S. anticostense (Fern.) G.L. Nesom	Lac St. John/ Que.	Vaezi & Brouillet, 215 (MT)	-	EU754257-9	-
S. anticostense (Fern.) G.L. Nesom	Lac St. John/ Que.	Vaezi & Brouillet, 222 (MT)	EU732779-85	EU754260-7	-
S. anticostense (Fern.) G.L. Nesom	Grande rivière/ Que.	Vaezi & Brouillet, 275 (MT)	EU732810-6	EU754313-6	EU754623
S. anticostense (Fern.) G.L. Nesom	Grande rivière/ Que.	Vaezi & Brouillet, 287 (MT)	EU732824-30	EU754317-22	EU754624-6
S. anticostense (Fern.) G.L. Nesom	Rivière Petit-Pabos/ Que.	Vaezi & Brouillet, 295 (MT)	EU732831-6	EU754323-6	EU754627
S. anticostense (Fern.) G.L. Nesom	Rivière Bonaventure/ Que.	Vaezi & Brouillet, 310 (MT)	EU732842-50	EU754334-8	EU754628-9
S. anticostense (Fern.) G.L. Nesom	Restigouche R./ N.B.	Vaezi & Brouillet, 335 (MT)	-	EU754347-50	-
S. anticostense (Fern.) G.L. Nesom	Restigouche R./ N.B.	Vaezi & Brouillet, 343 (MT)	EU732854-7	EU754351-7	-

Table 4.1 *Continued ...*

(sub)genus/species	Locality/ Province	Collector(s)	GenBank Accession No.		
			GAPDH	MIPS paralog 1	MIPS paralog 2
S. anticostense (Fern.) G.L. Nesom	Restigouche R./ N.B.	Vaezi & Brouillet, 349 (MT)	-	EU754365-70	EU754630
S. anticostense (Fern.) G.L. Nesom	St. John R.-Bath/ N.B.	Vaezi & Brouillet, 361 (MT)	EU732858-62	EU754371-8	-
S. anticostense (Fern.) G.L. Nesom	St. John R.-Bristol/ N.B.	Vaezi & Brouillet, 370 (MT)	-	EU754379-84	-
S. anticostense (Fern.) G.L. Nesom	St. John R.-Connell/ N.B	Vaezi & Brouillet, 382 (MT)	-	EU754385-90	-
S. anticostense (Fern.) G.L. Nesom	Four Falls/ N.B.	Vaezi & Zargarbashi, 422 (MT)	-	EU754412-7	EU754636-7
S. anticostense (Fern.) G.L. Nesom	Florenceville/ N.B.	Vaezi & Zargarbashi, 436 (MT)	EU732866-71	EU754418-20	-
S. anticostense (Fern.) G.L. Nesom	Connell/ N.B.	Vaezi & Zargarbashi, 437 (MT)	EU732872-9	EU754421-8	-
S. anticostense (Fern.) G.L. Nesom	Hartland/ N.B.	Vaezi & Zargarbashi, 445 (MT)	EU732880-5	-	-
S. anticostense (Fern.) G.L. Nesom	Grand lake/ N.B.	Vaezi & Zargarbashi, 472 (MT)	EU732892-4	-	-
S. anticostense (Fern.) G.L. Nesom	Grand lake/ N.B.	Vaezi & Zargarbashi, 473 (MT)	EU732895-900	-	-
S. anticostense (Fern.) G.L. Nesom	Oakpoint/ N.B.	Vaezi & Zargarbashi, 486 (MT)	EU732901-3	-	-
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrecque & Jean, 696 (MT)	EU732974-9	EU754224-5	EU754673-7
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrecque & Jean, 697 (MT)	EU732980-4	EU754226-30	EU754678-80
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrecque & Jean, 377273 (LM)	EU732989-90	-	-
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrecque & Jean, 377274 (LM)	EU732991-6	-	-
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrecque & Jean, 377275 (LM)	EU732997-3002	-	-
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrecque & Jean, 377242 (LM)	EU733003	-	-
S. urophyllum (Lind. ex de Cand.) G.L. Nesom	Elgin Co./ Ont.	Semple, 10594 (WAT)	EU708510-1	-	EU754567-70
S. drummondii (Lind.) G.L. Nesom	Newton Co./ Tex.	Semple, 10049 (WAT)	EU708554	EU754237-40	-
S. puniceum (L.) Á. Löve & D. Löve	Marion Co./ N.C.	Semple, 10853 (WAT)	EU708512-3	EU754242	EU754571-6
S. nahanniense (Cody) Semple	Nahanni N.P.R./ N.W.T.	Semple, 11161 (WAT)	EU708543-4	EU754404-7	-
S. dumosum (L.) G.L. Nesom	Amite Co./ Miss.	Semple & Suripto, 10102 (MT)	EU708560-1	-	EU754712-7
S. welshii (Cronquist) G.L. Nesom	Garfield Co./ Utah	Semple, 11249 (WAT)	EU708542	EU754395-403	-
S. ciliatum (Ledeb.) G.L. Nesom	Manitoulin/ Ont.	Morton & Venn, 9942 (MT)	EU708528	-	EU754699-705
S. foliaceum (Lind. ex de Cand.) G.L. Nesom	Missoula Co./ Mont.	Semple, 10310 (WAT)	EU708545-6	EU754442-5	EU754641-4
S. cordifolium (L.) G.L. Nesom	Guilford/ Maine	Semple, 4639 (WAT)	-	-	EU754563-6
S. cordifolium (L.) G.L. Nesom	Carleton Co./ N.B.	Semple & Keir, 4670 (MT)	EU708552-3	-	-
S. lateriflorum (L.) Á. Löve & D. Löve	Henderson Co./ N.C.	Semple, 10823 (WAT)	EU732768-9	EU754253	EU754605-10
S. depauperatum (Fern.) G.L. Nesom	Nottingham/ Pa.	Semple, 7681 (WAT)	EU708531-2	-	-
S. depauperatum (Fern.) G.L. Nesom	Granville Co./ N.C.	Semple, 11607 (WAT)	-	EU754231,33	EU754684-9
S. laurentianum (Fern.) G.L. Nesom	Ile de la Madeleine/ Que	Houle & Brouillet, 81 (MT)	EU708527	-	EU754706-11
S. racemosum (Elliott) G.L. Nesom	Wayne Co./ Miss.	Semple, 9895 (WAT)	EU708533-4	-	-
S. racemosum (Elliott) G.L. Nesom	Virginia	Semple, 11620 (WAT)	-	-	EU754681-3
S. tradescantii (L.) G.L. Nesom	Botton Brook/ Nfld.	Brouillet, 03-56 (MT)	EU708548-9	EU754448-9	-
S. anomalum (Engel. ex T. & G.) G.L. Nesom	Carroll Co./ Ark.	Semple & Suripto, 9950 (WAT)	EU708547	EU754446-7	-

Table 4.1 *Continued ...*

(sub)genus/species	Locality/ Province	Collector(s)	GenBank Accession No.		
			GAPDH	MIPS paralog 1	MIPS paralog 2
subg. *Virgulus*					
S. ericoides (L.) G.L. Nesom	Mound City/ S.Dak.	Semple, 6664 (WAT)	EU708522-3	-	EU754581-3
S. oblongifolium (Nuttall) G.L. Nesom	Webster Co./ Nebr.	Semple & Brouillet, 7337 (MT)	EU708521	-	EU754584-7
S. novae-angliae (L.) G.L. Nesom	Tenton/ Ga.	Semple, 11001 (WAT)	EU708539	-	EU754588-91
S. plumosum (Small) Semple	Franklin Co./ Fla.	Semple, 10929 (WAT)	EU708517-8	-	EU754577-80
subg. *Astropolium*					
S. subulatum (Michaux) G.L. Nesom	Ocean Co./ N.J.	Semple, 9525 (WAT)	EU732767	-	-
S. subulatum (Michaux) G.L. Nesom	Marengo Co./ Ala.	Semple & Chmielewski, 6362 (MT)	EU708535	EU754246-51	-
S. tenuifolium (L.) G.L. Nesom	Cedar Run/ N.J.	Semple, 9519 (WAT)	EU708536	EU754241,43-5	-
subg. *Chapmaniani*					
S. chapmanii (T. & G.) Semple & Brouillet	Walton Co./ Fla.	Semple & Suripto, 10136 (MT)	EU732985-8	-	EU754692-8
Ampelaster carolinianus (Walter) G.L. Nesom	Davenport/ Fla.	Semple, 5354 (WAT)	EU708566	-	EU754561-2
Canadanthus modestus (Lind.) G.L. Nesom	Blaune Co./ Idaho	Semple, 11359 (WAT)	EU708567	EU754232,34-6	EU754690-1
Psilactis tenuis S. Watson	JeffDavis Co./ Tex.	Semple, 8201 (WAT)	EU708568	-	EU754592-6
Heterotheca monarchensis D.A. York, Shevock & Semple	Kern Co./ Calif.	Shwock & York, 109 (WAT)	EU708562-3	-	EU754597-604

4.4.2 Primer design, DNA amplification, sequencing and cloning

For the GAPDH gene the primers GAPDHx4F and GAPDHx6R were used (chapter 2). After amplification, cloning, sequencing, and alignment (see below) of GAPDH sequences, we identified pseudogene sequences that had three stop codons at the 5′ end of 5th exon of the gene. These sequences were removed from final analysis. For MIPS, we blasted the partial mRNA sequence of *Aster tripolium* L. (Genbank accession: AB090886) with some similar Asteridae Genbank sequences. The resulting sequences were aligned with a complete sequence of *Arabidopsis thaliana* (L.) Heynh. (Genbank accession: AT2G22240). Subsequently, two conserved 5′→3′ regions between 4th and 6th exons were selected to design the primer pair: MIPSx4F (5′-AACCARGGGTCACGTGCAGA TAACG-3′) and MIPSx6R (5′-CCTCTTGATGGCCAATTCAATAACC-3′).

The PCR for both genes was performed in a 25 μL-master mix containing 2.5 μL of 10x PCR reaction buffer (Roche Diagnostics, Indianapolis, IN, USA), 0.5 μL Mgcl2 (25 mM, Promega, Madison, WI, USA), 100 μmol/L of each dNTP, 1 μL dimethylsulphoxide (DMSO), two units of *Taq* polymerase, about 200 ng of genomic DNA, and 1 mmol/L of each primer. Thermal cycling started with a denaturation step at 95 °C lasting 5 min, followed by 40 cycles each comprising 30s at 94 °C, 30s at 64 °C (GAPDH) 62 °C (MIPS), and 2 min at 72 °C, with a final extension of 10 min at 72 °C. In addition, two strategies were applied to reduce potential PCR recombinants (Judo et al., 1998; Cronn et al., 2002): firstly, all reactions were performed in triplicate and secondly, a long extension time (10 min) was used (Joly et al., 2006). The purification and sequencing steps are as detailed in Joly et al. (2006).

Direct sequences of both genes were unreadable, particularly in the case of *S. anticostense* and its putative parents, apparently due to the superimposition of homoeologous DNA sequences. Therefore, purified PCR products were ligated into PGEM-T vector (Promega Corporation) and transformed into competent E. coli DH5-α at 42 °C according to the manufacturer's instructions. Transformed bacteria were grown overnight at 37 °C on LB plates containing 50 mg/ml kanamycin, 100 mg/ml ampicilin,

50 mg/ml X-gal, and 0.5 M of IPTG (Isopropyl β-D-1-thiogalactopyranoside). Subsequently, fifteen to 20 white colonies were picked and cultivated in 1.5 ml eppendorf containing LB liquid as well as the two antibiotics outlined above. All positive cultures were amplified and sequenced using the vector primers T7 and SP6 (Promega Corporation).

4.4.3 Phylogenetic analyses

The GAPDH and MIPS sequences were aligned using Clustal W (Thompson et al., 1994) as implemented in BioEdit (Hall, 1999), followed by manual adjustments. Collapse version 1.2 (Posada, 2004) was used to identify repetitive alleles within each individual. Before determining the best-substitution evolutionary model, GAPDH sequences were partitioned in three: 4th intron, 5th exon, and 5th intron, respectively. Indels were coded using the simple gap-coding method (Simmons and Ochoterena, 2000) as implemented in SEQSTATE (Müler, 2005).

After alignment, we visually detected two distinct groups of sequences within the MIPS data set. To determine whether these were orthologs or paralogs, and hence to avoid affecting our phylogenetic analysis with a gene tree/species tree problem (Page and Charleston, 1997), we used split-based methods (e.g., Bryant and Moulton, 2002) that computes networks to visually detect events such as hybridization, recombination, and gene loss/duplication (Huson and Bryant, 2006). In addition, Zmasek and Eddy (2001) showed that the bootstrap value can be used as a sampling method to confidently infer duplication events. The network was built using the NeighbourNet algorithm (Bryant and Moulton, 2004) on all MIPS sequences, coupled with bootstrapping splits implemented in the SplitsTree4 program (Huson and Bryant, 2006). The network identified two groups (Paralogs 1 and 2, hereafter MIPS1 and MIPS2, respectively; Fig. 4.2) with a bootstrap value of 76%. The groups are recognized by ten SNPs which are mapped on the network. No stop codon was found within the exon of both paralogs. Moreover, within MIPS2 we identified two subgroups that are distinguished by four SNPs with a bootstrap value of 39% (Fig. 4.2). Due to the low confidence value, we did not separate the two subgroups

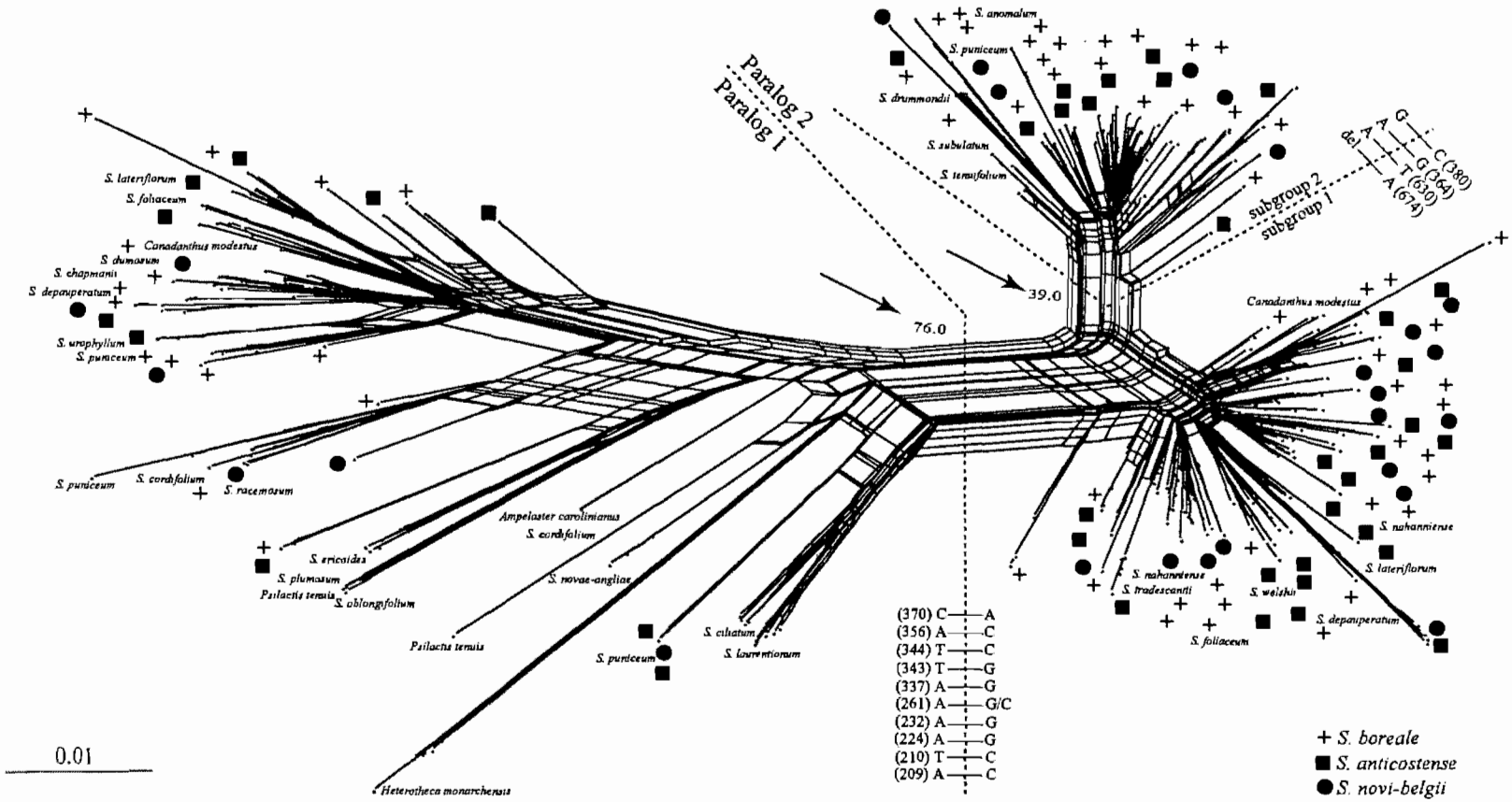


Figure 4.2 Bootstraping NeighbourNet network of Myo-Inositol 1-Phosphate Synthase (MIPS) alleles derived from all species included in the study. Two different types of MIPS gene indicated by paralogs 1 and 2 are separated by ten single nucleotide polymorphisms. Two subgroups within paralog 2 are discriminated by three substitutions and one indel. The arrows show the bootstrap supports for splits resulting from separation of the paralogs and subgroups.

in subsequent analyses. In the final phylogenetic analyses, we separated the two paralogs as independent data sets. The same partitioning strategy as for GAPDH was applied to both paralogs. After comprehensive sampling, we did not succeed in obtaining MIPS2 allele(s) from *H. monarchensis*. Thus, we rooted the MIPS2 phylogenetic tree using *Canadanthus modestus* as the outgroup.

The three data sets were examined to detect the possibility of recombination between sequences, particularly between those of the putative parents. Six recombination detection algorithms were used as implemented in the program RDP2 (Martin et al., 2005): RDP (Martin and Rybicki, 2000), GENECONV (Sawyer, 1989), Bootscanning (Salminen et al., 1995), MaxChi (Maynard-Smith, 1992), Chimaera (Posada and Crandall, 2001), and SiScan (Gibbs et al., 2000).

To ascertain genealogical relationships among the taxa investigated, we performed Bayesian phylogenetic analyses using MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist, 2001), after selecting the best-fitting evolutionary models. We identified best-fitting models for each data partition using the Akaike Information Criterion (AIC; Akaike, 1973) and the Likelihood Ratio Test (LRT; Felsenstein, 1988) as implemented in MrModeltest 2.2 (Nylander, 2004) with executable MrModelblock file in PAUP* (version 4.10b; Swofford, 2002) (Table 4.2). No difference was found between substitution models suggested by the LRT and AIC criteria for the MIPS1 data set. For the GAPDH and MIPS2 data sets, we used Bayes factors (Kass and Raftery, 1995) to evaluate which competing criterion (LRT or AIC) fits better with our data sets (see chapter 2 for more details). To calculate Bayes factors, we applied the harmonic mean likelihood values as provided by MrBayes using the posterior distribution after burn-in. According to Kass and Raftery (1995), we accepted the values greater than two as a positive support in favor of a criterion with higher harmonic mean log likelihood (Table 4.2). Bayesian analyses were run for 5.5 and 8 million generations for GAPDH and MIPS2, respectively, each with two replicates (LRT and AIC), and four million generations with one replicate for MIPS1. Trees were sampled at every 100 generations.

We confirmed convergence and the burn-in phases using the program TRACER version 1.3; (Rambaut and Drummond, 2003). For the GAPDH and MIPS2 data sets, the Bayes factors strongly supported the AIC criterion. Therefore, we performed subsequent steps, including consensus tree computation and posterior probabilities, based on the AIC criterion.

4.5 Results

4.5.1 GAPDH analysis

A total of 305 GAPDH alleles including 694 characters were analyzed (Table 4.2). No recombined alleles were detected among sequences of *S. anticostense*. The phylogenetic tree (Fig. 4.3) obtained here supports the monophyly of subtribe Symphyotrichinae with strong posterior probability (PP = 1.00). Apart from *Canadanthus*, which was early diverging within Symphyotrichinae, the genera *Ampelaster* and *Psilactis* are positioned within *Symphyotrichum*.

Collapsing sequences showed that one allele of *S. anticostense* was identical to an allele of *S. novi-belgii*. Similarly, one allele of *S. anticostense* was identical to one allele of *S. boreale*. In eight clades (A1-A8; Fig. 4.3), alleles of *S. anticostense* are grouped with those of *S. novi-belgii*, in six (B1-B6) with those of *S. boreale*, and in five (C1-C5) with those of both putative parents. No shared allele was found between *S. anticostense* and diploid species included in the analysis.

Figure 4.3 Majority rule consensus tree from the Bayesian analysis based on the GAPDH sequences data from the accessions indicated in Table 4.1. Posterior probabilities are given above the branches. Clades with abbreviation letters which are indicated in circles are discussed in the text. Species with identical sequences are listed sequentially. Diploid species are indicated in bold.
→

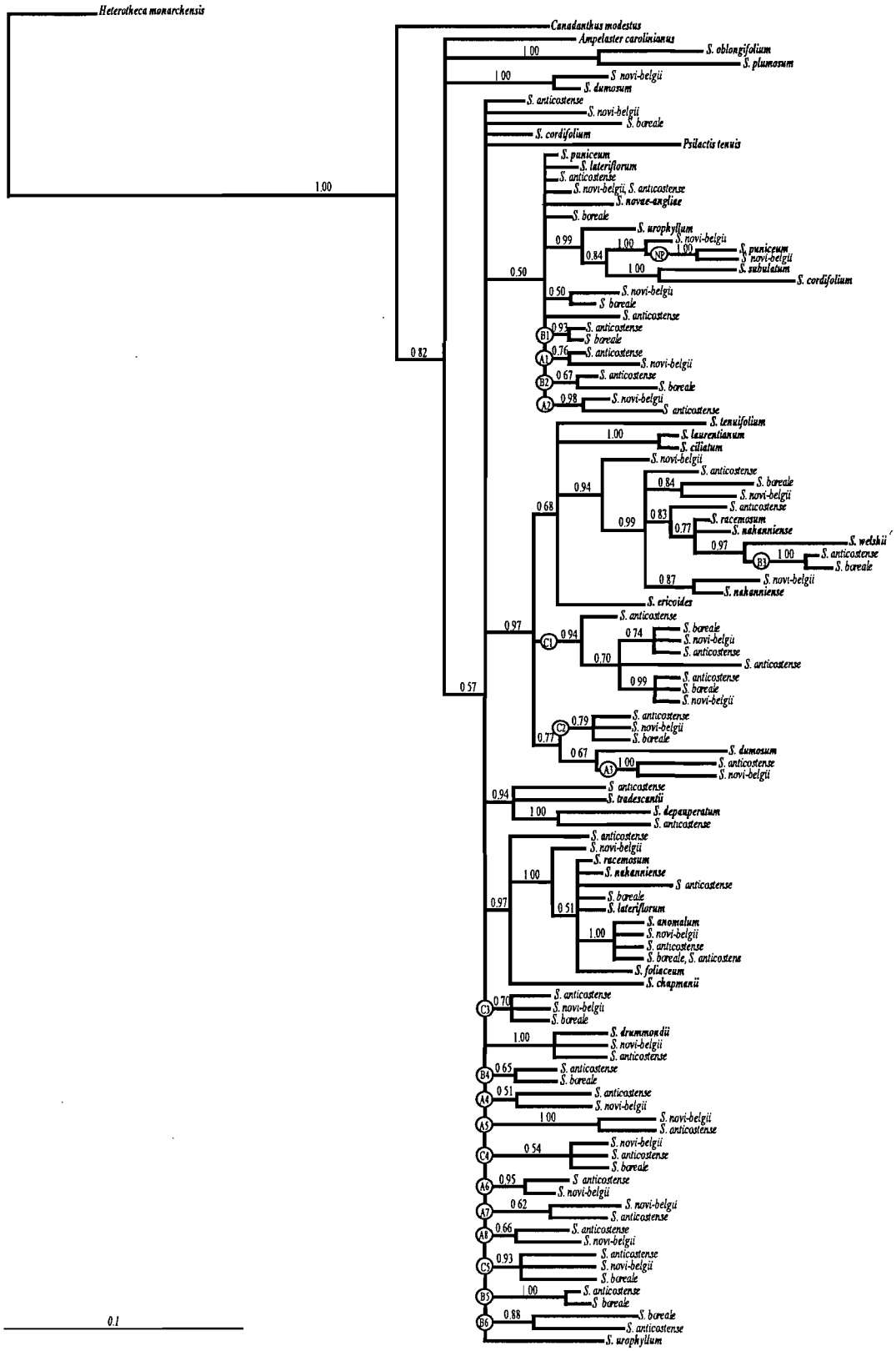

Heterotheca monarchensis
Canadanthus modestus
Ampelaster carolinianus
S. oblongifolium
S. plumosum
S. novi-belgii
S. dumosum
S. anticostense
S. novi-belgii
S. boreale
S. cordifolium
Psilactis tenuis
S. puniceum
S. lateriflorum
S. anticostense
S. novi-belgii, S. anticostense
S. novae-angliae
S. boreale
S. urophyllum
S. novi-belgii
S. puniceum
S. novi-belgii
S. subulatum
S. cordifolium
S. novi-belgii
S. boreale
S. anticostense
S. anticostense
S. boreale
S. anticostense
S. novi-belgii
S. anticostense
S. boreale
S. novi-belgii
S. anticostense
S. tenuifolium
S. laurentianum
S. ciliatum
S. novi-belgii
S. anticostense
S. boreale
S. novi-belgii
S. anticostense
S. racemosum
S. nahanniense
S. welshii
S. anticostense
S. boreale
S. novi-belgii
S. nahanniense
S. ericoides
S. anticostense
S. boreale
S. novi-belgii
S. anticostense
S. anticostense
S. anticostense
S. boreale
S. novi-belgii
S. boreale
S. novi-belgii
S. dumosum
S. anticostense
S. novi-belgii
S. anticostense
S. tradescantii
S. depauperatum
S. anticostense
S. anticostense
S. novi-belgii
S. racemosum
S. nahanniense
S. anticostense
S. boreale
S. lateriflorum
S. anomalum
S. novi-belgii
S. anticostense
S. boreale, S. anticostens
S. foliaceum
S. chapmanii
S. anticostense
S. novi-belgii
S. boreale
S. drummondii
S. novi-belgii
S. anticostense
S. anticostense
S. boreale
S. anticostense
S. novi-belgii
S. novi-belgii
S. anticostense
S. novi-belgii
S. anticostense
S. novi-belgii
S. anticostense
S. novi-belgii
S. boreale
S. anticostense
S. boreale
S. boreale
S. anticostense
S. urophyllum
0.1

Table 4.2 Statistical information and DNA substitution models including number of parameters, Harmonic Mean Log-Likelihood (-HML) for both AIC and LRT criteria, and 2ln Bayes factors of each partition applied for the three molecular markers used in the current study.

Partitions	Length	Variable sites No.	%	Model comparison (LRT/AIC)	No. of parameters LRT	AIC	-HML (LRT)	-HML (AIC)	2 ln Bayes factor
GAPDH									
4th intron	367	196	53	HKY+G/HKY+G	5	5			
5th exon	151	59	39	K80+G/K80+G	2	2			
5th intron	176	97	55	HKY+G/GTR+G	5	9			
Total	694	352	51		12	16	9185.96	9135.77	100.38
MIPS pararolg1									
4th intron	378	200	53	GTR+G/GTR+G	9	9			
5th exon	227	78	34	SYM+G/SYM+G	6	6			
5th intron	90	40	44	HKY+G/HKY+G	5	5			
Total	695	318	46		20	20	5881.8	5881.8	0
MIPS pararolg2									
4th intron	377	175	46	HKY+G/GTR+G	5	9			
5th exon	227	111	49	K80+G/SYM+G	2	6			
5th intron	90	60	67	K80/HKY	1	4			
Total	694	346	50		8	19	7970.1	7943.2	53.84

4.5.2 MIPS analyses

4.5.2.1 MIPS1

A subset of 157 MIPS1 sequences resulted in the alignment of 695 nucleotides included in the Bayesian analysis (Table 4.2). The tree provided strong support (PP = 1.00) for the monophyly of subtribe Symphyotrichinae. No recombination was detected between parental sequences within the allelic pool of *S. anticostense*. The phylogenetic tree (Fig. 4.4) provided moderate resolution and statistical support for most clades. The closely related *Canadanthus modestus*, *Ampelaster carolinianum*, and *Psilactis tenuis* are grouped within *Symphyotrichum*. Within sampled alleles of *S. anticostense,* 17 different MIPS1 alleles were found. Of these, one allele was identical to that of *S. boreale.*

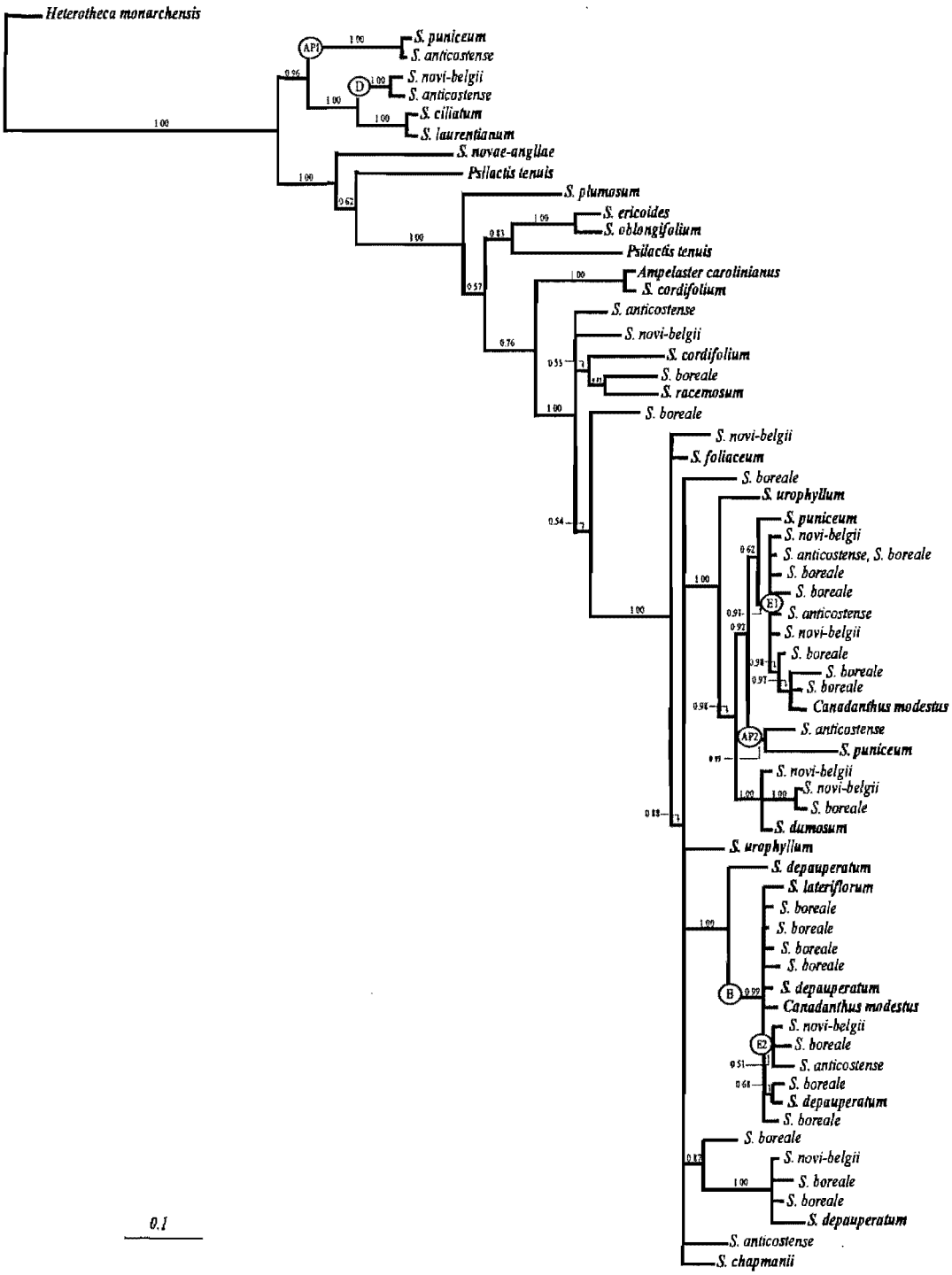


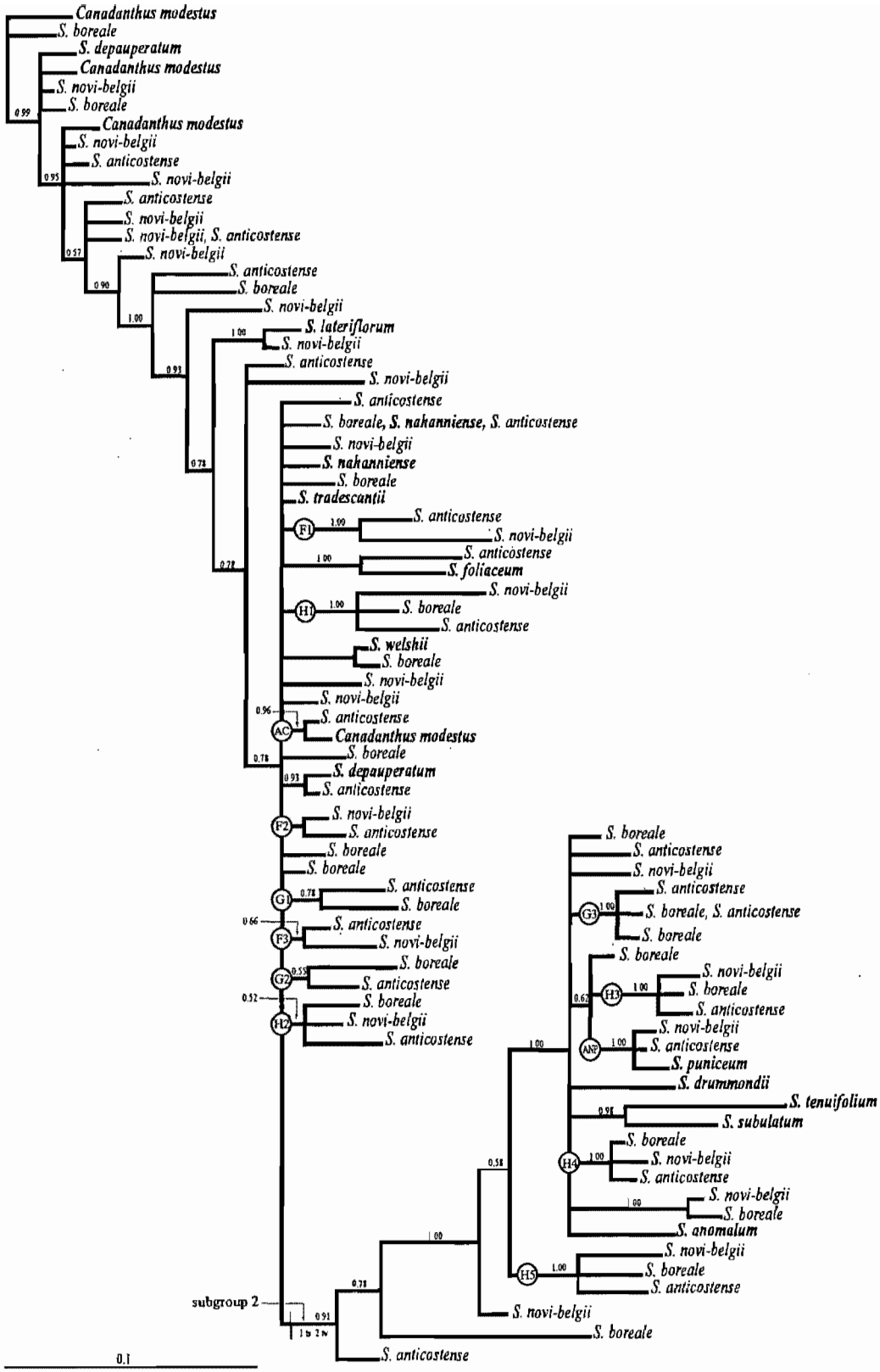
Figure 4.4 Majority rule consensus tree from the Bayesian analysis based on MIPS1 sequences data from the accessions indicated in Table 4.1. Posterior probabilities are given above the branches. Clades with abbreviation letters which are indicated in circles are discussed in the text. Species with identical sequences are listed sequentially. Diploid species are indicated in bold.

No allele was found to be identical between *S. anticostense* and *S. novi-belgii* or between the former and diploid species. In subclade D (Fig. 4.4), one accession of *S. anticostense* is grouped with one of *S. novi-belgii*. In clade E1, alleles of *S. anticostense* are grouped with those of both parents as well as of *C. modestus*. In clade E2, one allele of *S. anticostense* is grouped with alleles of both putative parents. Moreover, in two clades (AP1 and AP2), alleles of *S. anticostense* and *S. puniceum* are grouped together.

4.5.2.2 MIPS2

n the MIPS2 data set, a total of 337 alleles including 694 aligned bases were analysed (Table 4.2). Due to the lack of MIPS2 alleles from *Heterotheca monarchensis*, we are unable to conclude on the monophyly of subtribe Symphyotrichinae. No putative recombination event was found among alleles of *S. anticostense*. As in the network (Fig. 4.2), the phylogenetic tree (Fig. 4.5) distinguished two subgroups within paralog 2 identified by an indel, a transition, and two transversions. One allele of *S. novi-belgii* was identical to one of *S. anticostense*. In addition, in subgroup1, one allele of *S. boreale* was identical to one of *S. anticostense*, as well as one of *S. nahanniense*. Within six resolved clades (indicated by F1-F3 and G1-G3, Fig. 4.5), *S. anticostense* is grouped exclusively with either parent and in five clades (H1-H5, Fig. 4.5) with both parents. Apart from grouping with its parents, five accessions of *S. anticostense* are also grouped with diploid species included in the analysis.

Figure 4.5 Majority rule consensus tree from the Bayesian analysis based on the MIPS2 sequences data from the accessions of Table 4.1. The position of subgroup 2 within MIPS2 indicated by arrow which is separated from subgroup 1 by one transition, two transversions and one indel (bar line). Posterior probabilities are given above the clades. Clades with abbreviation letters which are indicated by circles are discussed in the text. Species with identical sequences are listed sequentially. Diploid species are indicated in bold. ⟶


Canadanthus modestus
S. boreale
S. depauperatum
Canadanthus modestus
S. novi-belgii
S. boreale
Canadanthus modestus
S. novi-belgii
S. anticostense
S. novi-belgii
S. anticostense
S. novi-belgii
S. novi-belgii, S. anticostense
S. novi-belgii
S. anticostense
S. boreale
S. novi-belgii
S. lateriflorum
S. novi-belgii
S. anticostense
S. novi-belgii
S. anticostense
S. boreale, S. nahanniense, S. anticostense
S. novi-belgii
S. nahanniense
S. boreale
S. tradescantii
F1
S. anticostense
S. novi-belgii
S. anticostense
S. foliaceum
S. novi-belgii
H1
S. boreale
S. anticostense
S. welshii
S. boreale
S. novi-belgii
S. novi-belgii
AC
S. anticostense
Canadanthus modestus
S. boreale
S. depauperatum
S. anticostense
F2
S. novi-belgii
S. anticostense
S. boreale
S. boreale
G1
S. anticostense
S. boreale
F3
S. anticostense
S. novi-belgii
G2
S. boreale
S. anticostense
H2
S. boreale
S. novi-belgii
S. anticostense
S. boreale
S. anticostense
S. novi-belgii
S. anticostense
G3
S. boreale, S. anticostense
S. boreale
S. boreale
H3
S. novi-belgii
S. boreale
S. anticostense
S. novi-belgii
ANP
S. anticostense
S. puniceum
S. drummondii
S. tenuifolium
S. subulatum
S. boreale
H4
S. novi-belgii
S. anticostense
S. novi-belgii
S. boreale
S. anomalum
S. novi-belgii
H5
S. boreale
S. anticostense
S. novi-belgii
S. boreale
S. anticostense
subgroup 2
0.1

4.6 Discussion

4.6.1 Origin of *Symphyotrichum anticostense*

The majority of *S. anticostense* accessions in the three phylogenetic trees were grouped with those of its putative parental species. Disregarding incomplete lineage sorting, shared alleles between a hybrid and its putative parents appear to be a reliable indicator of recent genetic exchange between parental populations (Koopman et al., 2007). In our molecular data sets, two and three identical alleles were detected between *S. anticostense* and *S. novi-belgii* and between *S. anticostense* and *S. boreale*, respectively. This may indicate the occurrence of hybridization between the proposed parents.

In investigating the evolutionary origin of a polyploid species, alleles sampled from an allopolyploid are expected usually to form two clades; one set of alleles forms a clade with one parent and another with the second. In our phylogenetic trees (Figs. 4.3, 4.4, and 4.5), alleles of *S. anticostense* are exclusively grouped with those of *S. novi-belgii* in 12 clades (GAPDH: A1-A8, MIPS1: D, MIPS2: F1-F3), with those of *S. boreale* in nine (GAPDH: B1-B6, MIPS2: G1-G3), and with those of both parents in 12 (GAPDH: C1-C5, MIPS1: E1-E2, MIPS2: H1-H5). The allelic contribution of the three species in these clades appears to be a signature of hybridization. In contrast, in clades where accessions of the three species are grouped together with those of diploid species, it becomes more difficult to discriminate between incomplete lineage sorting and introgression. A shared allele between *S. anticostense*, *S. boreale*, and *S. nahanniense* in the MIPS2 tree (Fig. 4.5) provides evidence for a possible shared ancestor between *S. boreale* and *S. nahanniense* (see Owen et al., 2006).

In a recently diverged group of species, inferring the phylogenetic origin of a hybrid species with a high ploidy level may be coupled with the retention of ancestral polymorphisms and introgression at all ploidy levels. As outlined above, these processes may be identified through incongruence between two or more molecular markers with different evolutionary histories. However, ancestral alleles affected by incomplete lineage

sorting are expected to be randomly distributed in species, whereas alleles introgressed from one species into another should be more systematically distributed. Incongruence is expected to be stochastic in the first case and nonrandom in the second (Buckley et al., 2006; McGuire et al., 2007). For instance, in our GAPDH phylogenetic tree (Fig. 4.3), *S. anticostense* alleles are grouped with those of diploid species such as *S. dumosum*, *S. tradescantii*, *S. anomalum*, and *S. drummondii*, but such relationships are not confirmed by the two other phylogenetic trees (Figs. 4.4 and 4.5) or by ITS sequence data (chapter 3). Therefore, retention of ancestral alleles could explain these relationships rather than introgression or hybridization. This scenario may be complicated when two of the three phylogenetic trees show similar traces. For instance, in our phylogenetic analyses of the MIPS1 and MIPS2 data sets (but not GAPDH), the *S. anticostense* accessions are grouped with *Canadanthus modestus* (clades E2 and B, Fig. 4.4; clade AC, Fig. 4.5) and *S. puniceum* (clades AP1 and AP2, Fig. 4.4; ANP, Fig. 4.5). We interpret this as evidence of:1) molecular convergence; or 2) involvement of diploid species in *S. anticostense* origin. Recent studies have shown the occurrence of evolutionary convergence with molecular data (e.g., Hermsen and Hendricks, 2008 and references therein). This may occur in distantly related organisms that share similar habitats (Wiens et al., 2003). For instance, *C. modestus* and *S. boreale* inhabit similar habitats (stream or lake shores on wet calcareous soils) and are grouped together in dichotomous or polytomous branches in both the MIPS1 and MIPS2 phylogenetic trees; it is possible that the apparent molecular convergence was inherited by *S. anticostense* from *S. boreale*. Alternately, diploid species may have been involved in the origin of *S. anticostense* not as immediate common ancestors but through introgression with either parent. For instance, *S. puniceum* grows sympatrically in the same habitats (sandy soils with sufficient moisture and disturbed areas) and regions (Brouillet et al., 2006) as *S. novi-belgii*. They are morphologically closely related species that sometimes are confused in the field (Labrecque and Brouillet, 1996). In addition, in the GAPDH tree, *S. puniceum* is sister to *S. novi-belgii* (clade NP, Fig. 4.3). *S. puniceum* might be a one of the putative parents of *S. novi-belgii*. Thus, the most parsimonious explanation here is that *S. puniceum* may have been involved in the origin of *S. anticostense* through *S. novi-belgii*.

Based on the three phylogenetic trees, *S. anticostense* is also grouped with *S. depauperatum*, an endemic species of inland serpentine barrens of the eastern United States. It is difficult to infer direct contact between these geographically and ecologically distinct species. It could be speculated that introgression has occurred between *S. depauperatum* and either *S. novi-belgii* or *S. boreale* during the last glaciation in the southern regions of the glacial maximum where they were in refugia. This hypothesis could be rejected based on the ecological requirements of *S. depauperatum*, which differ from those of both *S. novi-belgii* and *S. boreale*. Therefore, in this case, mutational convergence or retention of ancestral alleles appears to be more likely than introgression or a close relationship.

The evolution of *Symphyotrichum* and of *S. anticostense* in particular seems to have been affected strongly by Pleistocene glaciations, especially the most recent (Wisconsinan). This event contributed to the evolution of other taxa such as *Dryas integrifolia* (Tremblay and Schoen, 1999), *Silene* (Popp et al., 2005), and *Solidago* (Semple and Cook, 2006). Due to shared alleles (current study) and ribotypes (chapter 3), insufficient ribotypic resolution, as well as morphological similarities, particularly in floral characters (chapter 3), between *S. anticostense* and its parents, we suggest that the origin of this hybrid species occurred during the last glaciation (18000-10000 YBP) as it has not had sufficient time to accumulate new mutations. During the postglacial period and the retreat of glaciers, new habitats arose. This was associated with secondary contact and range expansion of previously allopatric species resulting from postglacial migration from refugia. Thus, these new habitats provided suitable conditions for the occurrence of interspecific hybridization (Carman, 2001; Marshall et al., 2002). This scenario may have occurred for *S. novi-belgii* and *S. boreale* where they were in contact in newly disturbed and probably favorable areas during northward migration in eastern regions of North America following ice retreat.

4.6.2 Gen(om)e duplication

Gene and/or genome duplications are frequent events, particularly in allopolyploids with unbalanced chromosome numbers to facilitate sexual reproduction through normal meiotic chromosome pairing (Ramsey and Schemske, 1998; Wendel, 2000). This phenomenon has also been demonstrated in diploid-like plants such as *Arabidopsis* (Arabidopsis Genome Initiative, 2000), which underwent an ancient polyploidization event. In the present study, both nuclear markers appear to be duplicated not only in polyploids but also in diploids. In nearly all diploid species included in the GAPDH analysis, two paralogs were sampled, one functional and one pseudogene. The latter was excluded from our matrix. In the MIPS data sets (Fig. 4.2), both paralogs were detected in five (*S. puniceum*, *S. depauperatum*, *S. lateriflorum*, *S. foliaceum*, *Canadanthus modestus*) out of 19 diploid species. Insufficient sampling or loss of one paralog may explain why both were not found in all diploid species. Three evolutionary fates have been suggested for duplicate genes: i) novel function for one copy (Clegg et al., 1997); ii) silencing of one of the duplicated copies (Shoemaker et al., 1996b); iii) retention of similar functions for both paralogs (Cook et al., 1997). It seems the second scenario might explain the evolutionary history of GAPDH in our group, whereas the third might explain the evolution of MIPS paralogs. However, based on our results in combination with preliminary results for the triose phosphate isomerase (TPI) nuclear gene, which is also duplicated in diploid species (results not shown), we suggest that the genome may have been duplicated before the origin of subtribe Symphyotrichinae. Further investigations using other molecular methods are needed to verify this hypothesis.

CHAPTER 5

Conclusion

The main objective of this thesis was to determine the origin of *Symphyotrichum anticostense* using morphological and molecular evidence. Although finding the origin of this species at first glance may seem straightforward, especially as two species had been proposed as putative parents, processes such as reticulation at high polyploidy levels and incomplete lineage sorting prevented us from confirming its origin with absolute certainty. Previous studies based on morphological and cytological evidence had not succeeded in establishing clear phylogenetic relationships among species of *Symphyotrichum* even at the diploid level (e.g., Nesom, 1994b and references therein). Thus, firstly, I attempted to determine the phylogenetic relationships among diploid species of subtribe Symphyotrichinae using nuclear markers. Secondly, I investigated the origin of *S. anticostense*. I showed that the nrDNA (ITS) evidence appears to be a more reliable indicator in not only delimiting diploid species but also in identifying the origins of *S. anticostense*.

5.1 Phylogenetic relationships within Symphyotrichinae

ITS or GAPDH: Which gene tree reflects best the species tree?

In chapter two, phylogenetic relationships among diploid species of subtribe Symphyotrichinae were investigated. The ITS marker confirmed somewhat the taxonomic relationship based on morphological and cytological studies, at least at the generic and subgeneric levels. In contrast, in the GAPDH data set, despite providing

more variation relative to the ITS data set (Tables 2.3), the absence of fixed allelic variation within each species caused by incomplete lineage sorting resulted in misleading phylogenetic estimates (Fig. 1.7). Similarly, in chapter four, the phylogenies based on the MIPS data sets (Figs. 4.4 and 4.5) showed the occurrence of incomplete lineage sorting among the diploid taxa. In some cases, these phylogenies provided stronger support for the stochastic sorting of ancestral polymorphisms among the species (e.g., *Canadanthus modestus*). As outlined in chapter 2, the lack of resolution among ribotypes, the non-monophyly of GAPDH alleles within each species, and the high rate of interspecific hybridization within *Symphyotrichum*, suggest that this genus radiated recently. Thus, it appears that morphological, cytological, and to some extent, the ITS tree provide better resolution and confidence in the phylogeny of *Symphyotrichum* and a better overview of the relationships within this group, despite their limitations.

5.2 Origin of *Symphyotrichum anticostense*

In chapters 3 and 4, the origin of *S. anticostense* was investigated using morphological and molecular evidence. Univariate and multivariate (PCA and CDA) methods were used to document the intermediacy of the *S. anticostense* phenotype, which putatively arose by hybridization. Of 67.5% parental characters expressed in *S. anticostense*, 43.5% and 13% were *novi-belgii*-like and *boreale*-like, respectively. These proportions indicate the relative contributions of each parental genome to the genomic pool of *S. anticostense*. This evidence, in combination with 23.5% intermediate characters, may provide morphological support for the hypothesis of hybridization between *S. novi-belgii* and *S. boreale*. Moreover, the high percentages of a priori correctly identified accessions of *S. anticostense*, *S. novi-belgii*, and *S. boreale* (92.9%, 96.6%, and 100%, respectively) indicate that the accessions included in the morphological analyses had been correctly determined.

Morphological characters alone are of limited value in detecting hybridization, and additional data are needed to prove hybrid origin (Lihovà et al., 2007). Three nuclear markers (ITS, GAPDH, and MIPS) were used to evaluate the morphological results and

to further investigate the origin of *S. anticostense*. The phylogenetic tree obtained from the ITS data set (Fig. 3.5) provided weak resolution among ribotypes. The parsimony network was able to identify the clades in which the *S. anticostense* ribotypes were grouped with those of either parent (Fig. 3.6). The network results confirmed those of morphological data.

It is intriguing to note that despite the high ribotypic variation in *S. boreale* from eastern populations relative to western ones (Fig. 3.1), morphological variation was sligth between these populations (Figs. 3.3 and 3.4; see also Owen et al., 2006). Despite the comprehensive coverage in chromosome counts by Semple and colleagues for *S. boreale* (summarized in Owen et al., 2006), the authoploid or alloploid origin of the cytotypes reported for the species (2n =16, 32, 48, 64) remains to be investigated. Morphological uniformity among different cytotypes would support an autoploid origin, while ribotypic variation may appear to confirm allopolyploidy. Interestingly, the different cytotypes reported are not restricted geographically and there is no concordance between ribotypic variation and geographic distribution of cytotypes.

On the other hand, *S novi-belgii* is known solely as a hexaploid (2n = 48) from its whole geographical range. Due to its high infraspecific morphological and ribotypic variation (Labrecque and Brouillet, 1996; chapter 3), it appears that *S. novi-belgii* may have had a hybrid origin. No shared ribotype was detected between this species and diploid species included in the ITS analysis, however. The GAPDH and MIPS analyses (chapter 4) suggest that *S. puniceum* may probably be a putative parent of *S. novi-belgii*. The lack of shared ribotrype between these species could be explained by the erasure of the traces of *S. puniceum* in the nrDNA through concerted evolution. These species are morphologically similar, though they are distinguishable morphometrically (Labrecque and Brouillet, 1996).

Molecular phylogenies are powerful tools for identifying likely cases of hybrid origin, but when studying a group of recently evolved species, two evolutionary processes need to be taken into account: 1) reticulation, that is, extensive hybridization or

introgression within the group; and 2) incomplete lineage sorting. Genetic data may not always be able to distinguish between introgression and incomplete lineage sorting, particularly in a recently diverged group such as *Symphyotrichum*. The hypothesis of incomplete lineage sorting assumes that the original ancestor of *Symphyotrichum* was highly polymorphic, that some of this variation became locally extinct randomly following speciation, and that the remainder assorted randomly among lineages. Traces of introgression and incomplete lineage sorting are retained through successive rounds of speciation (Wendel and Doyle, 1998). Incongruence between the GAPDH and MIPS phylogenetic trees is evidence of occurring introgression, incomplete lineage sorting or both together during the evolution of *Symphyotrichum*. Although determining whether incongruence between the trees results from introgression/hybridization or incomplete lineage sorting is difficult, similar topological patterns among taxa may support introgression rather than incomplete lineage sorting (Buckley et al., 2006). No topologically identical pattern was found among the diploid species included in the phylogenetic trees. For instance, *S. cordifolium* is grouped with *S. subulatum* on the GAPDH tree (Fig. 4.3), whereas in the MIPS1 tree (Fig. 4.4), it is grouped with *S. racemosum* and *S. boreale*. Therefore, it seems that stochastic sorting of ancestral polymorphisms fits better with these patterns than introgression among diploid species. In light of the above evidence, it appears that introgression is a less parsimonious explanation than incomplete lineage sorting for the occurrence of incongruence among diploid species, but further assays of nuclear genes are necessary to confirm this.

5.3 Gen(om)e duplication

Whole-genome duplication followed by extensive loss, rearrangement, and degradation of homoelogous (duplicated) regions, has been postulated as a powerful mechanism of evolutionary innovation (Ohno, 1970). It has been shown that genome duplication is ubiquitous in plants (Soltis and Soltis, 1999). Therefore, it is not surprising to add another taxon to the list of genome-duplicated plants. As outlined in chapter 4, visual inspection of the two nuclear matrices (GAPDH and MIPS) indicated that they are

subject to gene duplication. In figures 5.1 and 5.2, the GAPDH and MIPS sequences were analyzed including diploid species alone to avoid the impact of polyploidy on gene duplication.

Our results indicate that probably both genes are duplicated at the diploid level. This gave rise to the question as to whether the gene duplication is restricted to these two randomly selected genes or whether it arose via whole-genome duplication. To investigate the latter hypothesis, the triose phosphate isomerase (TPI) nuclear gene was sequenced for a few diploid species. The ambiguous and unreadable sequences were cloned and alleles were analyzed with the SplitsTree4 program (Huson and Bryant, 2006) (Fig. 5.3). The GenBank accession numbers are given in appendix 2.

TheTPI analysis (Fig. 5.3) would indicate the occurrence of a duplication of the gene as two paralogs have been identified within *S. shortii* and *S. drummondii*, each represented by a single individual. The absence of one or the other paralog within other species may be explained by the fact that one paralog has been randomly deleted from the whole genome by gene loss or by incomplete sampling. By increasing the number of individuals per species and the number of diploid species, we may overcome sampling bias. Therefore, as suggested in chapter 4, the results outlined above would indicate that the whole genome was duplicated independently from ploidy level before the divergence of subtribe Symphyotrichinae.

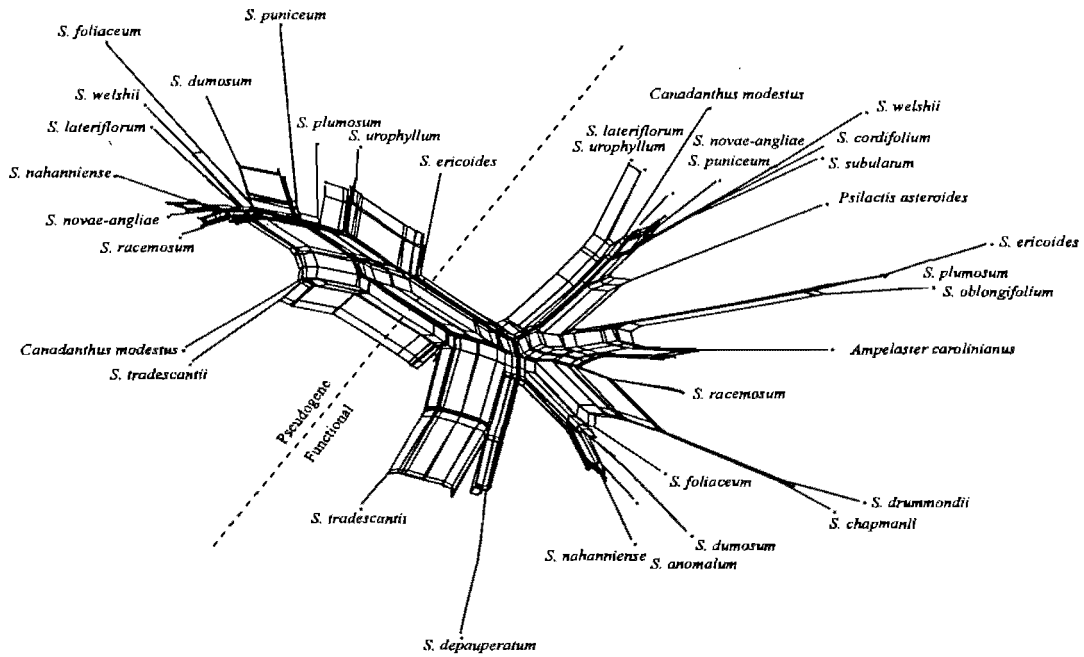


Figure 5.1 Network analysis of the GAPDH sequences of diploid species of subtribe Symphyotrichinae to investigate gene duplication. One individual per species was included in the analysis. Within most species, two paralogs were identified. One paralog included three stop codons and is considered a pseudogene.

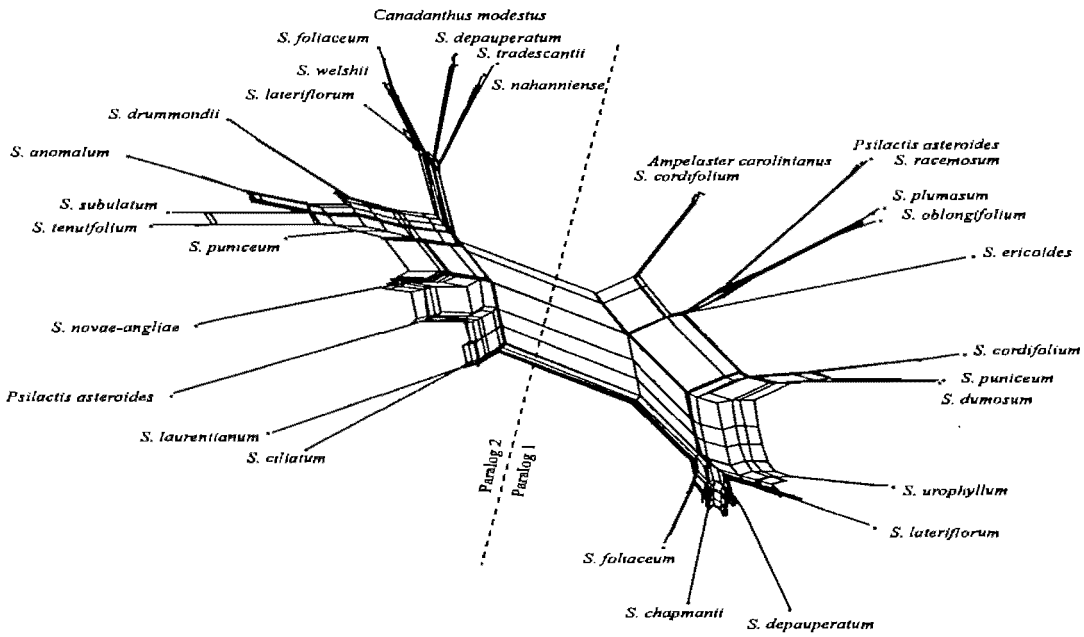


Figure 5.2 Network analysis of the MIPS sequences of diploid species of subtribe Symphyotrichinae to investigate gene duplication. One individual per species was included in the analysis. Within some species, two paralogs were identified.

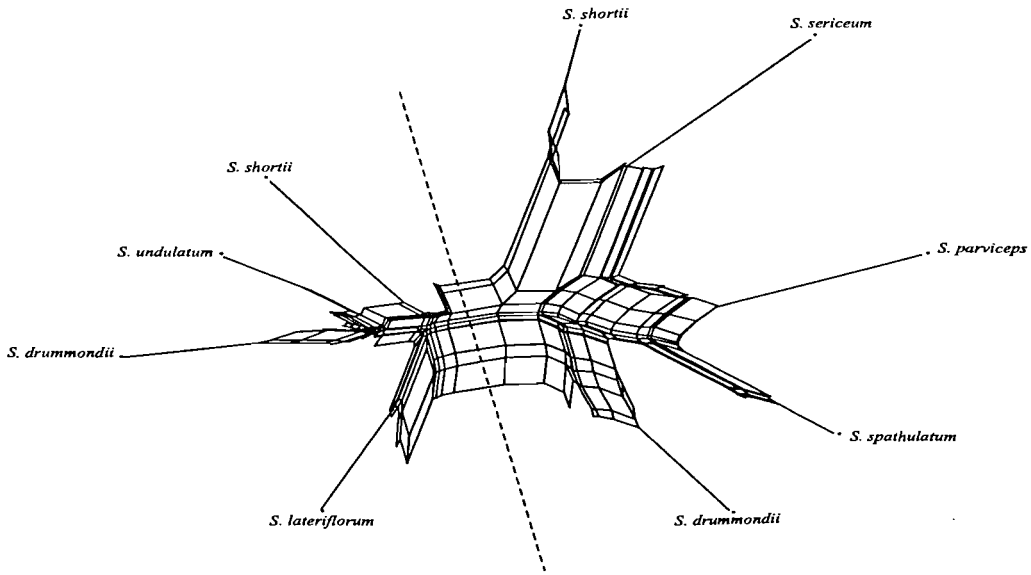


Figure 5.3 Network analysis of the TPI sequences of some diploid species of *Symphyotrichum* to investigate gene duplication. One individual per species was included in the analysis. Alleles within each species were collapsed where they positioned in a split. In *S. shortii* and *S. drummondii*, two paralogs were identified.

5.4 The future ...

Based on the figure 1.6, to investigate the origin of a high polyploid species, it appears to be necessary, first, to consider phylogenetic relationships among diploid species of the genus to which the polyploid species belongs. In chapter 2, I tried to investigate the phylogenetic relationships among the diploid species of *Symphyotrichum* before considering the origin of *S. anticostense*, but possibly due to the relative recency of *Symphyotrichum*, it appears that the evolutionary rate of both nuclear markers (ITS and GAPDH; also the MIPS marker in chapter 4) is too low and ancestral polymorphisms were still retained in terminal branches, i.e., species. Thus, in the future, I would recommend selecting molecular markers with higher evolutionary rates, to increase the chance that monophyly will have been achieved within terminals. Complete sampling both at the species and population levels should also be considered.

In this dissertation, I proposed that the whole genome at the diploid level is duplicated. To test this hypothesis, I suggest using pertinent methods that are able to find out this event, such as southern blot techniques.

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Appendix 1

Complete ribotype network of the ITS data set using all ribotypes of the clades A to G of the phylogenetic tree in figure 3.5. The ellipse, hexagon and rectangle indicate the ribotypes of *S. boreale, S. novi-belgii* and *S. anticostense,* respectively. The three forms are collapsed when the species share same ribotype. Large circles indicate share ribotype among *S. boreale* and the other diploid species. The small circles indicate unsampled ribotypes. The accession numbers are given in Table 3.1. The complete names for some abbreviations are; wel: *S. welshii*, nah: *S. nahanniense*, dum: *S. dumosum*, ell: *S. elliottii*, pun: *S. puniceum*, ano: *S. anomalum*, uro: *S. urophyllum*, sho: *S. shortii.* When two or more accessions (Table 3.1) share identical ribotype, subscript letters are used as follows:

A1 = 238, 721241, 422

A2 = 721241, 29703

A3 = 275, 218, 377276

A4 = 437, 295

B1 = 257, 555

B2 = 258, 267, 721241

B3 = 258, 217

B4 = 450, 218

B5 = 208, 74510

C1 = 258, 263, 266, 377242

C2 = 473, 486

C3 = 377273, 377275

D1 = 335, 872

E1 = 4143, 335

F1 = 258, 263

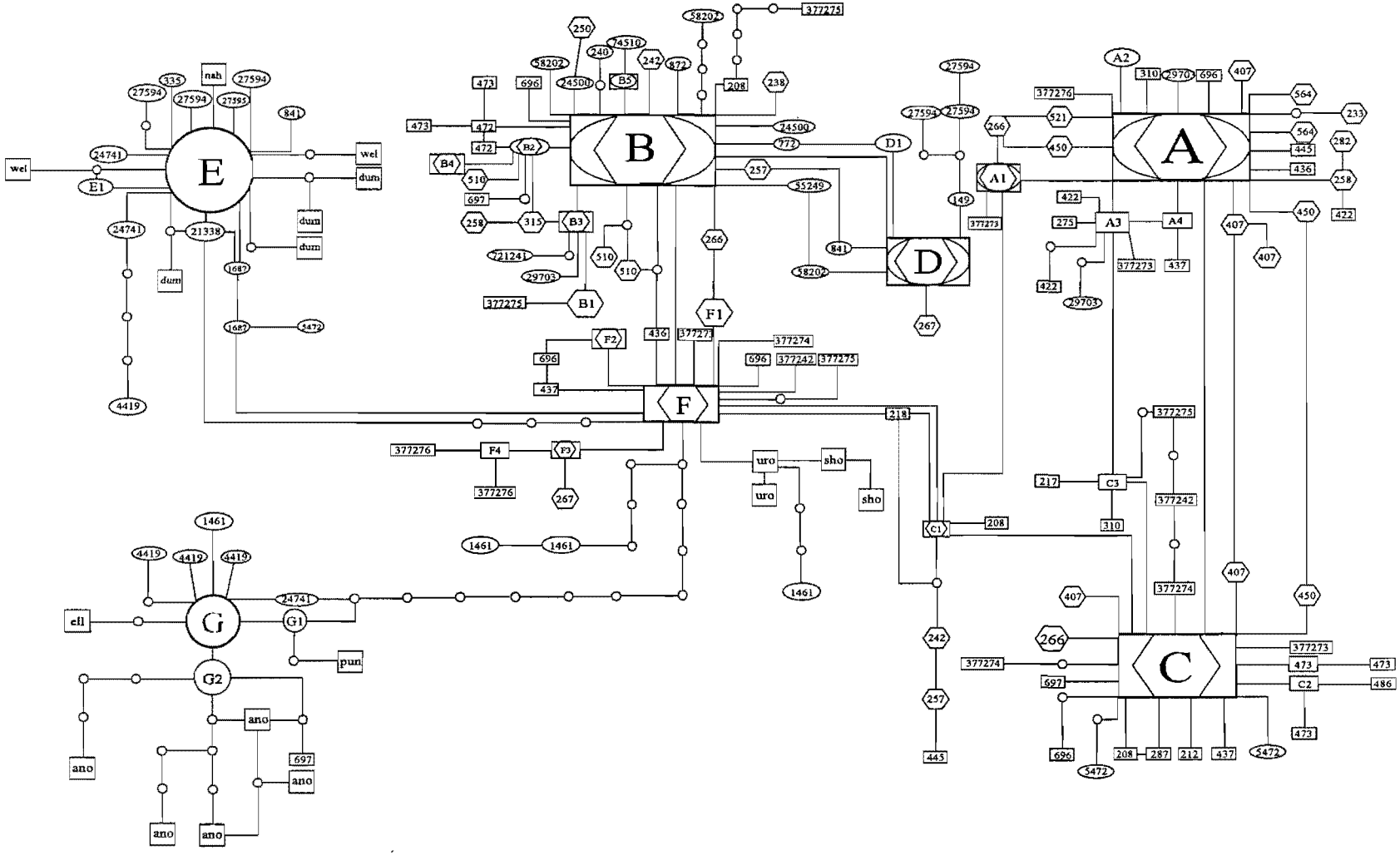
F2 = 302, 436, 564

F3 = 217, 287, 315

F4 = 377274, 377275

G1 = 1461, 12663

G2 = 149, 761, 7378, 10470


A
A1
A2
A3
A4
B
B1
B2
B3
B4
B5
C
C1
C2
C3
D
D1
E
E1
F
F1
F2
F3
F4
G
G1
G2
wel
dum
nah
pun
ell
ano
uro
sho

Appendix 2

The accessions of genus *Symphyotrichum* included in the TPI analysis to investigate the duplication event. The GenBank accessions are given in the last column (see chapter 5).

Taxon	Locality	Collector(s)	TPI GenBank Acc.
S. sericeum (Ventenat) G.L. Nesom	Rainy river/ Ont.	Semple & Heard, 8787 (WAT)	EU847464-70
S. drummondii (Lind.) G.L. Nesom	Newton Co./ Tex.	Semple, 10049 (WAT)	EU847471-6
S. shortii (Lind.) G.L. Nesom	Adair Co./ Ky.	Semple & Suripto, 9449 (MT)	EU847477-82
S. undulatum (L.) G.L. Nesom	Orangeburg Co./ S.C.	Semple & Chmielewski, 6133 (MT)	EU847483-8
S. parviceps (E.S. Burgess) G.L. Nesom	Adams Co./ Ill.	Semple & Brouillet, 7378 (MT)	EU847489-96
S. lateriflorum (L.) Á. Löve & D. Löve	Prince Edward/ Ont.	Brouillet & Brammall, 587 (MT)	EU847497-502
S. spathulatum (Lind.) G.L. Nesom	Mono Co./ Calif.	Semple & Heard 8715 (MT)	EU847503-7