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

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Abstract

Symphyotrichum anticostense (Fernald) G.L.Nesom (Asteraceae: Astereae), a rare endemic of the Gulf of St. Lawrence region is a high allopolyploid (2*n*=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 & Löve and the hexaploid (2*n*=6x=48) *S. novi-belgii* (L.) G.L.Nesom. We investigated this hypothesis using morphological and molecular ITS-sequence data, and we tried to determine the potentially geographic origins of the taxon. 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% transgressive. Multivariate analyses show that *S. anticostense* is intermediate between its putative parents. The molecular results support the morphological data, 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*. Besides the hypothesis of genetic drift and allele fixation following long-distance dispersals, at least three independent geographic origins may be suggested for *S. anticostense*; Anticosti Island, Lake St. John, and Gaspé Peninsula-New Brunswick-Maine.

Keywords: Asteraceae, hybridization, internal transcribed spacer (ITS), morphometric, polyploidy, *Symphyotrichum anticostense*

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; Peterson et al. 2009; Goulet et al. 2017 and references therein; Mitchell et al. 2019). 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; Mallet et al. 2015; Goulet et al. 2017). 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 the genus *Symphyotrichum* Nees (Nesom 1994a; Brouillet et al. 2006; Vaezi and Brouillet 2009), 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; Huson and Bryant 2006; Morrison 2014).

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; Brouillet et al. 2009). 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 1980; 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; Vaezi and Brouillet 2009). An example of a high polyploid with a potentially complex evolutionary history is *Symphyotrichum anticostense* (Fern.) G. L. Nesom, a species endemic to northeastern 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. According to Brouillet and Labrecque (1987) *S. boreale* experienced autopolyploidy in its evolutionary history. On the other hand, Hoffman (1995) stated that cultivated and wild populations of *S. novi-belgii* are mostly hybrids (≈ allopolyploid) in Europe. Geographically, *Symphyotrichum anticostense* is distributed in the Lake St. John area, on Anticosti Island in the Gulf of St. Lawrence, in the Baie des Chaleurs region of

the Gaspé Peninsula (Quebec), and in the Restigouche (Quebec-New Brunswick), St. John (New Brunswick), and Aroostook (Maine) river basins (Fig. 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. 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 substrates. In addition, this species is absent from river estuaries, suggesting an intolerance to saline environments. It also invades locally disturbed habitats (Brouillet and Labrecque 1987; Labrecque and Brouillet 1990; Coursol et al. 2000).

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 of polyploid species that arose after the last Pleistocene glaciation in North America and Europe, e.g. *Saxifraga svalbardensis* (Brochmann and Håpnes 2001), *Larrea tridentata* (Hunter et al. 2001) *Cardamine amporitana* (Lihová et al. 2004), *Capsella bursa-pastoris* (Slotte et al. 2006),

Biscutella laevigata (Marhold and Lihová 2006), *Draba* spp. (Jordon-Thaden and Koch 2008), *Primula marginata* (Casazza et al. 2012), *Arabidopsis* spp. (Novikova et al. 2018), *Campanula rotundifolia* polyploid complex (Sutherland and Galloway 2018). It appears that postglacial distribution areas played 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 by the last glaciation as it most likely migrated from western North American pro-glacial refugia into boreal eastern North America during deglaciation. Meanwhile, *S. novi-belgii* may have been widespread in eastern coastal areas (Labrecque and Brouillet 1996) where its habitats (coastal, moist, sandy soils) were available (Webb 1989). Thus, the data appear to imply postglacial hybridization in areas where the putative parents became sympatric.

The disjunct distribution of *Symphyotrichum anticostense* populations raises the question of whether or not this allopolyploid may have had either multiple origins or it has presumably been colonized through genetic drift followed by founder effect. The recurrent formation of polyploids at various locations over a short period of time has been well documented (Soltis and Soltis 1993, 1999, 2000; Jang et al. 2018). 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; Jang et al. 2018).

Many approaches have been proposed to detect species of hybrid origin, including analysis of morphological characters (reviewed in Rieseberg and Ellstrand 1993; Kaplan

and Fehrer 2004; Vaezi et al. 2019), of isozymes (Urbanska et al. 1997; Bleeker and Hurka 2001; Eschmann-Groupe et al. 2003; Blackstock and Ashtom 2010), of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (e.g., Alvarez and Wendel 2003; Siripun and Schilling 2006; Volkov et al. 2007; Kaplan and Fehrer 2004; Vaezi et al. 2019), of AFLP or RAPD (Neuffer and Jahncke 1997; Bleeker and Matthies 2005; Pepó 2006; Rajesh et al. 2014; Gobert et al. 2002), low-copy nuclear markers (Sang 2002; Kikuchi and Osone 2021), and of cpDNA markers (Dobeš et al. 2004; Lihová et al. 2006; Shimizu et al. 2016; Vaezi et al. 2019).

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; Pinheiro and De Barros 2009; Farsi et al. 2013), and in hybrid detection (e.g., Marhold et al. 2002; Carine et al. 2007; Segarra-Moragues et al. 2007; Vaezi et al. 2019). 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; Vaezi et al. 2019). 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; Cruz et al. 2011; Vaezi et al. 2019).

The use of the ITS region has been popular in phylogenetic analyses (Baldwin et al. 1995; Volkov et al. 2007; Kim et al. 2015; Olvera-Mendoza et al. 2020), because of its biparental inheritance, and 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; Casazza et al. 2012).

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 geographic origin(s) of *S. anticostense*. We use sequence data from the ITS region as well as morphological data to address these questions.

Materials and Methods

Taxon sampling

Herbarium and/or field-collected specimens of *S. anticostense* (28 individuals), *S. novi-belgii* (29 individuals), and *S. boreale* (30 individuals) were included in the morphometric study (Table 1). Herbarium and/or silica-dried leaves of *S. anticostense* (23 individuals), *S. novi-belgii* (23 individuals), and *S. boreale* (33 individuals) from their

entire range were incorporated in the molecular study (Table 1). Some specimens overlap between the morphometric and the molecular studies (see Table 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 to ensure knowledge of ploidy level. Four individuals from three closely related, diploid genera belonging to the Symphyotrichinae (*Ampelaster*, *Almutaster* and *Canadanthus*) were included as outgroups (Table 1).

Morphological measurements

Thirty-four quantitative morphological characters, as detailed in Labrecque and Brouillet (1996), were measured (Table 2). Of these, 19 were vegetative and 15 reproductive. One systematically chosen flower (on axis 1, see Table 2) 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.

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, USA).

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). The CDA was implemented in SPSS release 11.5.0 (SPSS Inc., Chicago, USA).

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 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.

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 (Fisher Biotech), two units of *Taq* Polymerase, ca. 200 ng genomic DNA and 1 mmol/L of each primer. The primers ITSvF and ITSvR (Vaezi and Brouillet 2009) were used. An initial denaturation step at 94 ºC for 3 min was followed by 35 cycles of denaturation (30 s at 94 ºC), annealing at 52 ºC for 30 s, elongation step at 72 ºC for 2 min, with a final extension at 72 ºC for 10 min. A long elongation step (2 min) was used to reduce the number of putative PCR recombinants (Judo et al. 1998; Cronn et al. 2002). The purification and sequencing steps are the same as those explained in Joly et al. (2006).

PCR-direct sequences including two or more single-nucleotide polymorphisms (SNPs) were cloned using pGEM-T vector (Promega Corporation) 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. All the positive cultures were amplified and sequenced using the same protocol as for the direct amplification.

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). The ribotype compositions and sequence divergence (two-parameter method, Kimura 1980) were estimated separately using MEGA version X (Kumar et al. 2018). To detect potential 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.

Results

Univariate analyses

The distribution of the morphological variables that discriminate among *S. anticostense* and its putative parents is represented by box plots (Supplementary data). 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 (47%) floral characters (Table 3). The Kolmorogov-Smirnov and Levene's statistic tests showed that eight characters (indicated by the symbol † in Table 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) were normally distributed but not homogeneous; therefore, the

Games-Howell post-hoc test was applied. The remaining variables (shown by the symbol Ω in Table 3) were not normally distributed after transformation, and the non-parametric Kruskal-Wallis test was implemented on these.

Examination of box plots (Supplementary data) and of statistical analyses (Table 3) show that ten variables, HAUTOT, LOAXA1, LMAXA1, LONPED, LORAYO, LOTUBE, LOLIMB, LOLOBE, LOANTH, and LOSTIG, do not significantly differ 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). 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.

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*. 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) and number of internodes on axis 1 (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. Distance between the apex and the point of maximum width of the stem leaf (LMAXCA) does not discriminate between the two parents but discriminates *S. anticostense* from them; this trait also seems to be transgressive (Table 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 in discriminating the three species.

Principal Component Analysis

Figure 2 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 to be greater than the interspecific one; for instance, three accessions of *S. anticostense* are grouped with accessions of *S. novi-belgii* (Fig. 2). 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. 2).

NBENTG is the only variable with a significant positive loading on axis 1 towards *S. boreale* (Fig. 2); the mean of this trait is highest in *S. boreale* (Table 3). PC2 has a low eigenvector value for LONPED and high values for the floral traits. In general, the PCA of all variables did not clearly discriminate the three species.

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 decreasing 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 decreasing order: LOFCAU, DIATIG, LMAXCA, NBENTG, NBRAYO, LMAXIF, and LOBRIF. In addition, 96.6% of the *S. novi-belgii* specimens were correctly classified (one accession classified with *S. anticostense*), 92.9% of *S. anticostense* individuals (one accession classified between the accessions of *S. boreale* and *S. novi-belgii*, and one with *S. novi-belgii*), and 100% of *S. boreale* individuals (Table 4, Fig. 3). 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).

Phylogenetic analysis

After removal of 14 repeated intraindividual ribotypes, 343 ribotypes (Table 1) were included in the analysis, including 630 aligned characters, of which 455 were constant and 82 parsimony informative. No recombinant ribotypes were detected. From the total number of ribotypes included in the analysis, 68, 44, and 58 belonged to *S. anticostense*, *S. novi-belgii*, and *S. boreale*, respectively. Intra-individual sequence divergence values of *S. anticostense*, *S. novi-belgii*, and *S. boreale* range from 0.0011 to 0.0138, 0 to 0.0125, and 0.0011 to 0.0161, respectively. Intra-specific ITS variations of *S. anticostense*, *S. novi-belgii*, and *S. boreale* range from 0.004 to 0.0183, 0.0024 to 0.011, and 0.0007 to 0.0117, respectively. Moreover, inter-specific sequence variations between species-pairs, *S. anticostense*-*S. novi-belgii*, *S. anticostense*-*S. boreale*, and *S. novi-belgii*-*S. boreale* are 0.0067, 0.0097, and 0.0085, respectively.

The maximum likelihood analysis generated a single tree (-ln = 3431.6494) (Fig. 4). 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.

Haplotype network and geographic origin

Details concerning the number and GenBank number of ribotypes of each accession are summarized in Table 1. 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 (Fig. 5). A simplified ribotype network is

shown in figure 6. Clade A, excluding its derivatives, is the most frequent (30 occurrences) followed by clades 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 five mutational steps, includes ribotypes shared by the 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 1, Fig. 6). No ribotype of *S. novi-belgii* was found within clades E and G.

Ribotypes of *S. anticostense* within the clades A, B, C, and F are found in the populations of all of its distribution areas, whereas the ribotypes of the clades D and G are distributed in the populations of Lake St. John and Anticosti island, respectively. Also, ribotypes of *S. novi-belgii* within the clades A, B, C, D, and F are found in the sampled populations of its entire distribution range (Fig. 1). Moreover, ribotypes of *S.*

394 *boreale* from all the clades are distributed in eastern North America, while mostly
395 ribotypes of clades E and G are found in western North America.

396 **Discussion**

397 **Morphological evidence**

398 The three species differ more in their vegetative than reproductive features, as was
399 shown by Labrecque and Brouillet (1996). During field studies, the most discriminating
400 feature between *S. anticostense* and *S. novi-belgii* was leaf shape: stem leaves of *S.*
401 *anticostense* are longer and narrower than those of *S. novi-belgii*, in addition to being
402 more rigid and arched (a feature neither easily measurable nor visible on dried
403 specimens). This is reflected in the statistically significant differences observed in the
404 length (LOFCAU) and width (LGFCAU) of stem leaves between these two species
405 (Table 3 and CDA results).

406 The proportion of parental characters (traits for which the value in *S. anticostense*
407 does not significantly differ from that of one or the other parent) reported here for *S.*
408 *anticostense* (67.5%) is higher than that reported on average (48.7%) for hybrid species
409 (Rieseberg and Ellstrand 1993). One explanation for this difference is that in Rieseberg
410 and Ellstrand's compilation, polyploid species were excluded as it is difficult to compare
411 these values. The greater similarity of *S. anticostense* to *S. novi-belgii* may be explained
412 by the fact that *S. novi-belgii*, a hexaploid, contributed six copies to the genome of *S.*
413 *anticostense,* and *S. boreale*, putatively tetraploid, four. This may indicate a dosage effect
414 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).

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. 2) and CDA (Fig. 3) 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 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.

Molecular evidence

There was neither bootstrap support (> 50%) nor sufficient phylogenetic resolution among the clades A to G in the phylogenetic tree (Fig. 4). 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 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 1) 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*. This may be considered as an indicator of the contribution of *S. boreale* to the hybridization.

Geographic origins

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; Vaezi and Brouillet 2009), 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. 1) shows that clade E is

459 frequent in the western part of the range of *S. boreale,* where clades A to D are absent.
460 This evidence may raise a question as to whether the western populations of *S. boreale*
461 participated in the hybridization, though Owen et al. (2006) suggested that these
462 populations possibly migrated from west to east. Our molecular data do not reject this
463 hypothesis, but indicate that the migrated populations carrying ribotype E may not have
464 contributed to the hybridization.

465 The total number of ribotypes of *S. anticostense* compared to that of *S. novi-belgii*
466 appears to be proportional to genome dosage (68 vs 44 ≈ 10: 6; each for 23 accessions),
467 whereas such a theoretical ratio is not respected in the case of *S. boreale* (58 ribotypes for
468 33 accessions) vs *S. anticostense* (≈ 8: 5 instead of the expected 10: 4). This difference
469 could be explained by: 1) the number of accessions used for *S. boreale* was greater than
470 those of *S. novi-belgii* and *S. anticostense*, therefore more ribotypes might have been
471 detected (Table 1); 2) the distribution range of *S. boreale* is wider than those of *S. novi-*
472 *belgii* and *S. anticostense* (Fig. 1) and greater genetic differentiation may have occurred;
473 or 3) the ploidy level of *S. boreale* ranges from 2x to 8x, and ploidy level was unknown
474 for the herbarium specimens used in our study; the greater number of ribotypes could be
475 due to this variability.

476 An examination of the spatial distribution of the *S. anticostense* ribotypes (Fig. 1)
477 would indicate that there were possibly three independent geographic origins for *S.*
478 *anticostense* in favor of multiple origins hypothesis: 1) Anticosti Island, 2) Gaspé
479 Peninsula and New Brunswick, and 3) Lake St. John. Firstly, ribotype G from *S.*
480 *anticostense* occurs only on Anticosti Island. Anticosti populations of *S. boreale* involved

in the hybridization have not been sampled and the populations of this species (accessions 21341, 27595, and 21338, but not 27594; Table 1) included in the study do not appear to have contributed to this hybridization (Fig. 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 disfavor the hypothesis of a dispersal of *S. anticostense* between the two regions.

Alternately to the multiple origins hypothesis mentioned above, 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 local followed by subsequent long-distance dispersals; the observed ribotype variations could then be explained by an initial colonization of a variant in each geographic range following genetic drift and subsequent selection/fixation of the new variant. However, 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 possible indicators of the allopolyploid origin of *S. anticostense*. Nonetheless, greater sampling and more experimental data particularly molecular ones would be needed to adequately confirm the origin of *S. anticostense*.

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Table1. Accessions included in the morphological and molecular study, including the species of genus *Symphyotrichum* as well as four outgroups. The number and name of ribotypes sequenced per individual indicated in the two last columns. The names of ribotypes correspond to those used in figures 4 and 5.

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)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Mont St. Pièrre/ Que.	Victorin & Germain, 49405 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	R. Port-Daniel/ Que.	Victorin et al. 44284 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Lac Monroe/ Que.	Germain, 3165 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Thetford Mines/ Que.	Hamel, C 66233 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	R. aux Canards/ Que.	Brouillet & Brouillet, 890 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	R. du Loup/ Que.	Brouillet, 964 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Maria Chapdelaine/ Que.	Brouillet & Brouillet, 841 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Back Lake/ Que.	Blais et al. 10776 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Tuckers Head/ Nfld.	Bouchard & Hay, 73119 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Mollichicgneck Brook/ Nfld.	Rouleau, 6489 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Serpentine Lake/ Nfld.	Rouleau, 4007 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Porter Island/ N.S.	Sampson, 278 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Brackley Beach/ P.E.I.	Erskine, 1656 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Rivière Ste. Anne/ Que.	Victorin et al. 3849 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	R. Ste. Marguerite/ Que.	Coyouette & Brisson, 64747 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	R. Petit-Pabos/ Que.	Victorin et al. 44276 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Région Côte Nord/ Que.	Goyette, A 38 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Ste. Adelaide/ Que.	Germain, 8266 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Betchouane/ Que.	Victorin & Germain, 21369 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Ile Cap aux meules/ Que.	Samuel, 5461 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Ilets Jeremie/ Que.	Brisson, 909 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Cap-Jaseux/ Que.	Brisson, 5024 (MT)	√	-	-	-

S. novi-belgii (L.) G.L. Nesom	Beauceville/ Que.	Labr. & Cour. 21 L/2650173 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Matapedia/ Que.	Le Gallo, 202 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Upsalquitch/ N.B.	Labrecque et al. 88-20 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Hab and Loc/ Que.	Rousseau, 32359 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Pointe à la Frégate/ Que.	Brouillet, 984 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Carleton/ Que.	Victorin et al. 33567 (MT)	√	-	-	-
S. novi-belgii (L.) G.L. Nesom	Labrador/ Nfld. and Labr.	Bay, 229 (MT)	-	√	EU781172-4	A, C, D
S. novi-belgii (L.) G.L. Nesom	Labrador/ Nfld. and Labr.	Bay, 233 (MT)	-	√	EU781175-7	A, C
S. novi-belgii (L.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 238 (MT)	-	√	EU781178-82	A, B, C, F
S. novi-belgii (L.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 242 (MT)	-	√	EU781183-6	A, B, C, D
S. novi-belgii (L.) G.L. Nesom	Saguenay river/ Que.	Vaezi & Brouillet, 250 (MT)	-	√	EU781187-90	A, B, C
S. novi-belgii (L.) G.L. Nesom	Saguenay river/ Que.	Vaezi & Brouillet, 252 (MT)	-	√	EU781191	B
S. novi-belgii (L.) G.L. Nesom	Porteneuf river/ Que.	Vaezi & Brouillet, 253 (MT)	-	√	EU781192-5	A, B, C, F
S. novi-belgii (L.) G.L. Nesom	Porteneuf river/ Que.	Vaezi & Brouillet, 257 (MT)	-	√	EU781196-201	A, B, C
S. novi-belgii (L.) G.L. Nesom	Bic/ Que.	Vaezi & Brouillet, 258 (MT)	-	√	EU781202-9	A, B, C, F
S. novi-belgii (L.) G.L. Nesom	Bic/ Que.	Vaezi & Brouillet, 259 (MT)	-	√	EU781210-11	B, C
S. novi-belgii (L.) G.L. Nesom	Les Escoumins/ Que.	Vaezi & Brouillet, 263 (MT)	-	√	EU781212-5	A, B, C, F
S. novi-belgii (L.) G.L. Nesom	Les Escoumins/ Que.	Vaezi & Brouillet, 266 (MT)	-	√	EU781216-22	A, B, C
S. novi-belgii (L.) G.L. Nesom	Paspébiac/ Que.	Vaezi & Brouillet, 267 (MT)	-	√	EU781223-6	B, D, F
S. novi-belgii (L.) G.L. Nesom	Grand rivière/ Que.	Vaezi & Brouillet, 282 (MT)	-	√	EU781231-3	A, C
S. novi-belgii (L.) G.L. Nesom	R. petit pabos/ Que.	Vaezi & Brouillet, 302 (MT)	-	√	EU781240-3	A, B, C, F
S. novi-belgii (L.) G.L. Nesom	R. Bonaventure/ Que.	Vaezi & Brouillet, 315 (MT)	-	√	EU781247-9	A, B, F
S. novi-belgii (L.) G.L. Nesom	Limestone/ N.B.	Vaezi & Zargarbashi, 407 (MT)	-	√	EU781254-9	A, C
S. novi-belgii (L.) G.L. Nesom	Hartland/ N.B.	Vaezi & Zargarbashi, 450 (MT)	-	√	EU781277-82	A, B, C
S. novi-belgii (L.) G.L. Nesom	St. John/ N.B.	Vaezi & Zargarbashi, 510 (MT)	-	√	EU781295-8	B, C
S. novi-belgii (L.) G.L. Nesom	Alma/ N.B.	Vaezi & Zargarbashi, 521(MT)	-	√	EU781299-301	A, C
S. novi-belgii (L.) G.L. Nesom	Moncton/ N.B.	Vaezi & Zargarbashi, 555 (MT)	-	√	EU781302-3	B
S. novi-belgii (L.) G.L. Nesom	Renton/ N.B.	Vaezi & Zargarbashi, 564 (MT)	-	√	EU781304-7	A, F
S. novi-belgii (L.) G.L. Nesom	Miramichi/ N.B.	Vaezi & Zargarbashi, 567 (MT)	-	√	EU781308-9	A, C
S. boreale (T. & G.) Á. Löve & D. Löve	Bay of Islands/ Nfld.	Djan-chékar et al., 1461(MT)	√	√	EU781316-20	F, G

S. boreale (T. & G.) Á. Löve & D. Löve	McAdam lake/ N.S.	Smith et al., 5472 (MT)	√	√	EU781353-6	C, E, F
S. boreale (T. & G.) Á. Löve & D. Löve	Lake Bog/ Ohio	s.n., 530926-0256 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	St. Quentin/ N.B.	Haber & Bristow, 3601 (MT)	√	√	EU781314-5	B, D
S. boreale (T. & G.) Á. Löve & D. Löve	Raspberry lake/ Sask.	Harms, 24704 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Birch river/ Man.	Semple & Brouillet, 4143 (MT)	√	√	EU781312-3	E
S. boreale (T. & G.) Á. Löve & D. Löve	Lac Labelle/ Que.	Plourde, 240 (MT)	√	√	EU781372-3	B
S. boreale (T. & G.) Á. Löve & D. Löve	Lac au saumon/ Que.	LeGallo, 841 (MT)	√	√	EU781344-8	B, D, E
S. boreale (T. & G.) Á. Löve & D. Löve	Dundee station/ P.E.I.	Smith, 335 (MT)	√	√	EU781349-52	D, E
S. boreale (T. & G.) Á. Löve & D. Löve	Longlac/ Ont.	Baldwin & Breitung, 3376 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	R. Eastmain/ Que.	Gagnon & Barabé, 74510 (MT)	√	√	EU781364-7	B, D
S. boreale (T. & G.) Á. Löve & D. Löve	Rupert House/ Que.	Spafford, 149 (MT)	√	√	EU781368-71	D, G
S. boreale (T. & G.) Á. Löve & D. Löve	Meadow lake/ Sask.	Breitung, 8350 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Sutherland/ Sask.	Boivin & Breitung, 6697 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Summit lake/ B.C.	Weber, 2603 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Smith/ Alta.	Boivin & Perron, 12762 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Bay James/ Que.	Gagnon & Hay, 75013 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Manitoulin/ Ont.	Brouillet, 550 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Swanson's Creek/ Man.	Cody & Wojtas, 25154 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Lake Winnipeg/ Man.	Scoggon, 5046 (MT)	√	√	EU781390	G
S. boreale (T. & G.) Á. Löve & D. Löve	Big river/ Sask.	Tisdale,117980 (Sask)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Beaver river/ Sask.	Looman, 117975(Sask)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Hills Park/ Sask.	Breitung, 64469(Sask)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Big river/ Sask.	Tisdale, 117979(Sask)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	North Sask R./ Sask.	Lepage, 90360(Sask)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Lake Road/ Sask.	Jeglum, 39461(Sask)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Sutherland/ Sask.	Fraser, 117983(Sask)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Batchawana/ Ont.	Taylor, 1546 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Fitzwilliam/ Ont.	Morton, 8463 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Thunder Bay/ Ont.	Garton, 18432 (MT)	√	-	-	-
S. boreale (T. & G.) Á. Löve & D. Löve	Kenora/ Ont.	Oldham & Sutherl., 24500 (WAT)	-	√	EU781144-6	B

S. boreale (T. & G.) Á. Löve & D. Löve	Albany Co./ Wyo.	Semple, 11233 (WAT)	-	√	EU781251	E
S. boreale (T. & G.) Á. Löve & D. Löve	Albert Park/ Sask.	Harms, 43283 (MT)	-	√	EU781310	E
S. boreale (T. & G.) Á. Löve & D. Löve	M. Creek/ Sask.	Harms, 38132 (MT)	-	√	EU781311	E
S. boreale (T. & G.) Á. Löve & D. Löve	R. Bonaventure/ Que.	LePage, 3704 (MT)	-	√	EU781327	A
S. boreale (T. & G.) Á. Löve & D. Löve	Anticosti Island/ Que.	Victorin et al., 21341 (MT)	-	√	EU781328	E
S. boreale (T. & G.) Á. Löve & D. Löve	Lac au saumon/ Que.	LeGallo, 886 (MT)	-	√	EU781329	B
S. boreale (T. & G.) Á. Löve & D. Löve	Anticosti Island/ Que.	Victorin & Germain, 27595 (MT)	-	√	EU781330-1	E
S. boreale (T. & G.) Á. Löve & D. Löve	St. Hippolyte/ Que.	Hébert, 72-124-1 (MT)	-	√	EU781332-6	A, B
S. boreale (T. & G.) Á. Löve & D. Löve	Anticosti Island/ Que.	Victorin & Germain, 21338 (MT)	-	√	EU781337	E
S. boreale (T. & G.) Á. Löve & D. Löve	Anticosti Island/ Que.	Victorin & Germain, 27594 (MT)	-	√	EU781338-43	D, E
S. boreale (T. & G.) Á. Löve & D. Löve	Grand Rapids/ Minn.	Wheeler & Glaser, 2342 (MT)	-	√	EU781357	E
S. boreale (T. & G.) Á. Löve & D. Löve	Vermont	Seymour, 29703 (MT)	-	√	EU781358-62	A, B
S. boreale (T. & G.) Á. Löve & D. Löve	Gold smith lake/ N.B.	Malte, 1019/29 (MT)	-	√	EU781363	B
S. boreale (T. & G.) Á. Löve & D. Löve	Oka/ Que.	Beaudry & Love, 58-202 (MT)	-	√	EU781374-8	B, D
S. boreale (T. & G.) Á. Löve & D. Löve	Chibougamau/ Que.	Hustich, 772 (MT)	-	√	EU781379-80	B
S. boreale (T. & G.) Á. Löve & D. Löve	La Trappe/ Que.	Beaudry & L.-Marie, 55-249 (MT)	-	√	EU781381-2	B
S. boreale (T. & G.) Á. Löve & D. Löve	Nominingue/ Que.	Robert, 872 (MT)	-	√	EU781383-5	B, D
S. boreale (T. & G.) Á. Löve & D. Löve	Kingston/ Ont.	Garwood & Zavitz, 1987 (MT)	-	√	EU781386	B
S. boreale (T. & G.) Á. Löve & D. Löve	Sandstone lake/ Ont.	Garton, 1687 (MT)	-	√	EU781387-8	E
S. boreale (T. & G.) Á. Löve & D. Löve	Kapiskau river/ Ont.	Ringius et al., 872 (MT)	-	√	EU781389	D
S. boreale (T. & G.) Á. Löve & D. Löve	Anglin lake/ Sask.	Harms, 24741 (Sask)	-	√	EU781447-50	E, G
S. boreale (T. & G.) Á. Löve & D. Löve	Nipawin park/ Sask.	Argus & Hudson, 4419 (Sask)	-	√	EU781451-6	E, G
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Victorin et al., 4036 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	Matapedia/ Que.	Labrecque et al., 88-64 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	Woodstock/ N.B.	Victorin et al., 44844 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	Lac St. Jean/ Que.	Victorin, 15408 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Petit-Pabos/ Que.	Victorin et al., 44273 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Brouillet & Labrec., 1432 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	Grande R./ Que.	Labrecque, AM-002 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Petit-Pabos/ Que.	Labrecque et al., 88-184 (MT)	√	-	-	-

S. anticostense (Fern.) G.L. Nesom	Grande R./ Que.	Victorin et al., 44256 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	Aroostook R./ Maine	Williams et al., 18 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	Grande R./ Que.	Labrecque et al., 88-162 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Victorin et al., 44265 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Victorin et al., 4047 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Victorin et al., 4051 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Petit-Pabos/ Que.	Labrecque, 165-90 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	Grande R./ Que.	Labrecque et al., 88-132 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Victorin et al., 4039 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Labrecque et al., 88-99 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	Matapedia/ Que.	Cayouette, J85-151 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	R. Petit-Pabos/ Que.	Victorin et al., 44279 (MT)	√	-	-	-
S. anticostense (Fern.) G.L. Nesom	Four Falls/ N.B.	Vaezi & Zargarbashi, 422 (MT)	√	√	EU781260-5	A, B
S. anticostense (Fern.) G.L. Nesom	Hartland/ N.B.	Vaezi & Zargarbashi, 445 (MT)	√	√	EU781273-6	A, C
S. anticostense (Fern.) G.L. Nesom	Grand lake/ N.B.	Vaezi & Zargarbashi, 472 (MT)	-	√	EU781283-4	B
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrecque & Jean, 377274 (LM)	√	√	EU781425-30	C, F
S. anticostense (Fern.) G.L. Nesom	Grande R./ Que.	Vaezi & Brouillet, 275 (MT)	-	√	EU781227-30	A, F
S. anticostense (Fern.) G.L. Nesom	Oakpoint/ N.B.	Vaezi & Zargarbashi, 486 (MT)	√	√	EU781291-4	B, C
S. anticostense (Fern.) G.L. Nesom	Grand lake/ N.B.	Vaezi & Zargarbashi, 473 (MT)	√	√	EU781285-90	B, C
S. anticostense (Fern.) G.L. Nesom	Florenceville/ N.B.	Vaezi & Zargarbashi, 436 (MT)	√	√	EU781266-8	A, F
S. anticostense (Fern.) G.L. Nesom	Connell/ N.B.	Vaezi & Zargarbashi, 437 (MT)	√	√	EU781269-72	A, C, F
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrecque & Jean, 377273 (LM)	√	√	EU781420-4	A, C, F
S. anticostense (Fern.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 208 (MT)	-	√	EU781148-53	A, B, C, F
S. anticostense (Fern.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 212 (MT)	-	√	EU781154-7	B, A, C
S. anticostense (Fern.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 217 (MT)	-	√	EU781158-63	A, B, C, F
S. anticostense (Fern.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 218 (MT)	-	√	EU781164-8	A, B, D, F
S. anticostense (Fern.) G.L. Nesom	Lac St. Jean/ Que.	Vaezi & Brouillet, 222 (MT)	-	√	EU781169-71	C, D, F
S. anticostense (Fern.) G.L. Nesom	Grande R./ Que.	Vaezi & Brouillet, 287 (MT)	-	√	EU781234-7	A, C, F
S. anticostense (Fern.) G.L. Nesom	R. Petit-Pabos/ Que.	Vaezi & Brouillet, 295 (MT)	-	√	EU781238-9	A
S. anticostense (Fern.) G.L. Nesom	R. Bonaventure/ Que.	Vaezi & Brouillet, 310 (MT)	-	√	EU781244-6	A, C

S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrec. & Jean, 696 (MT)	-	√	EU781391-6	A, B, C, F
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrec. & Jean, 697 (MT)	-	√	EU781397-401	A, B, C, G
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrec. & Jean, 377275 (LM)	-	√	EU781431-6	B, C, F
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrec. & Jean, 377276 (LM)	-	√	EU781437-41	A, F
S. anticostense (Fern.) G.L. Nesom	Anticosti Island/ Que.	Labrec. & Jean, 377242 (LM)	-	√	EU781442-6	A, C, F
S. urophyllum (Lind. ex de Cand.) G.L. Nesom	Elgin Co./ Ont.	Semple, 10594 (WAT)	-	√	EU781138-9	F
S. drummondii (Lind.) G.L. Nesom	Newton Co./ Tex.	Semple, 10049 (WAT)	-	√	EU781140-1	-
S. puniceum (L.) Á. Löve & D. Löve	Van Zandt Co./ Tex.	Nixon & Ward, 12663 (SMU)	-	√	EU781147	G
S. puniceum (L.) Á. Löve & D. Löve	Marion Co./ N.C.	Semple, 10853 (WAT)	-	√	EU781142-3	G
S. nahanniense (Cody) Semple	Nahanni N.P.R./ N.W.T.	Semple, 11161 (WAT)	-	√	EU781252-3	E
S. dumosum (L.) G.L. Nesom	Amite Co./ Miss.	Semple & Suripto, 10102 (MT)	-	√	EU781402-6	E
S. welshii (Cronquist) G.L. Nesom	Lake Co./ Mont.	Semple, 11374 (WAT)	-	√	EU781407-8	E
S. oblongifolium (Nuttall) G.L. Nesom	Webster Co./ Nebr.	Semple & Brouillet, 7337 (MT)	-	√	EU781459	-
S. ciliatum (Ledeb.) G.L. Nesom	Manitoulin/ Ont.	Morton & Venn, 9942 (MT)	-	√	EU781410	-
S. cordifolium (L.) G.L. Nesom	Carleton Co./ N.B.	Semple & Keir, 4670 (MT)	-	√	EU781411-2	-
S. shortii (Lind.) G.L. Nesom	Adair Co./ Ky.	Semple & Suripto, 9449 (MT)	-	√	EU781413-4	F
S. lateriflorum (L.) Á. Löve & D. Löve	Prince Edward/ Ont.	Brouillet & Brammall, 587 (MT)	-	√	EU781418	-
S. spathulatum (Lind.) G.L. Nesom	Mono Co./ Calif.	Semple & Heard, 8715 (MT)	-	√	EU781419	E
S. depauperatum (Fern.) G.L. Nesom	Nottingham/ Pa.	Semple, 7681 (WAT)	-	√	EU200226 [c]	G
S. elliottii (T. & G.) G.L. Nesom	Onslow Co./ N.C.	Semple, 10538 (WAT)	-	√	EU853710 [b]	G
S. frondosum (Nuttall) G.L. Nesom	Lake Co./ Oreg.	Houle & Legault, 45 (MT)	-	√	EU853711 [b]	-
S. laurentianum (Fern.) G.L. Nesom	Ile de la Madeleine/ Que.	Houle & Brouillet, 81 (MT)	-	√	EU853712 [b]	-
S. racemosum (Elliott) G.L. Nesom	Wayne Co./ Miss.	Semple, 9895 (WAT)	-	√	EU853715 [b]	F
S. tradescantii (L.) G.L. Nesom	Lévis/ Que.	Bouchard & Cuerrier, K-11 (MT)	-	√	EU853717 [b]	-
S. firmum (Nees) G.L. Nesom	Lake Co./ Mont.	Gerdes, 4945 (NM)	-	√	EU781250	G
S. anomalum (Engel. ex T. & G.) G.L. Nesom	Carroll Co./ Ark.	Semple & Suripto, 9950 (WAT)	-	√	EU781321-6	-
S. sericeum (Ventenat) G.L. Nesom	Rainy river/ Ont.	Semple & Heard, 8787 (WAT)	-	√	EU200232 [c]	-
S. concolor (L.) G.L. Nesom	Laurens Co./ Ga.	Semple, 4040 (MT)	-	√	EU781460-1	-
S. undulatum (L.) G.L. Nesom	Orangeburg Co./ S.C.	Semple & Chmielewski, 6133 (MT)	-	√	EU781415-6	-

S. parviceps (E.S. Burgess) G.L. Nesom	Adams Co./ Ill.	Semple & Brouillet, 7378 (MT)	-	√	EU781417	G
S. ericoides (L.) G.L. Nesom	Mound City/ S.Dak.	Semple, 6664 (WAT)	-	√	EU200227 [c]	-
S. novae-angliae (L.) G.L. Nesom	Tenton/ Ga.	Semple, 11001 (WAT)	-	√	EU200229 [c]	-
S. patens (Aiton) G.L. Nesom	Red River Gorge/ Ky.	Semple & Suripto, 9864 (WAT)	-	√	EU200230 [c]	-
S. yukonense (Cronquist) G.L. Nesom	Kluane Lake/ Yukon	Semple, 10624 (WAT)	-	√	EU200234 [c]	-
S. plumosum (Small) Semple	Franklin Co./ Fla.	Semple, 10929 (WAT)	-	√	EU853713 [b]	F
S. porteri (A. Gray) G.L. Nesom	Clear Creek Co./ Colo.	Semple, 10470 (WAT)	-	√	EU853714 [b]	G
S. subulatum (Michaux) G.L. Nesom	Ocean Co./ N.J.	Semple, 9525 (WAT)	-	√	EU853716 [b]	-
S. subulatum (Michaux) G.L. Nesom	Marengo Co./ Ala.	Semple & Chmielewski, 6362 (MT)	-	√	EU781409	-
S. tenuifolium (L.) G.L. Nesom	Cedar Run/ N.J.	Semple, 9519 (WAT)	-	√	EU200233 [c]	-
S. chapmanii (T. & G.) Semple & Brouillet	Choctawhatchee R./ Fla.	Semple, 10560 (WAT)	-	√	EU200223 [c]	-
Almutaster pauciflorus (Nuttall) Á. Löve & D. Löve	Oakburn/ Man.	Marchand, 1983 (Sask)	-	√	EU781462	-
Ampelaster carolinianus (Walter) G.L. Nesom	Davenport/ Fla.	Semple, 5354 (WAT)	-	√	EU200185 [c]	-
Canadanthus modestus (Lind.) G.L. Nesom	Swift Current/ Sask.	Hudson, 3997 (Sask)	-	√	EU781457	-
Canadanthus modestus (Lind.) G.L. Nesom	Yukon	Cody, s.n. (Sask)	-	√	EU781458	-

Notes: a. Accession number of *Symphyotrichum falcatum* (EF017389, Chapman and Burke 2007), available in GenBank, is used for this study.

b. Accession numbers submitted by Brouillet et al. 2008 (direct submission).

c. Accession numbers submitted by Selliah and Brouillet 2008.

Table 2. Description of the morphological characters used in the current study. Measured units are indicated in parentheses. Floral characters are indicated by an 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)

Table 3. Results of the univariate analyses using 34 morphological characters and 87 accessions of the three species in the study. The symbol (†) indicates normal and uniform characters, (‡) normal but non-uniform characters, (Ω) non-normal characters, (-) not applicable, and the asterisk (*) floral characters. Parental-like characters are specified as "N" for *S. novi-belgii*-like or "B" for *S. boreale*-like characters. Post-hoc Tukey-Kramer HSD test as "TH" and Games-Howell post-hoc test as "GH". Character abbreviations are provided in Table 2.

	Mean			ANOVA	Multiple Comparison (*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	<0.000 1	-	0.99	-	<0.000 1	-	-	-	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	-	<0.000 1	-	0.54	-	<0.000 1	-	-	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	-	<0.000 1	-	0.94	-	<0.000 1	-	-	Parental [N]
LGBRIF†	9.50	3.63	4.96	<0.0001	<0.000 1	-	<0.000 1	-	0.08	-	-	-	Parental [B]
LMAXIF‡	39.43	33.97	48.78	<0.0001	-	0.33	-	0.09	-	<0.000 1	-	-	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	-	<0.000 1	-	<0.000 1	-	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	-	<0.000 1	-	<0.000 1	-	<0.000 1	-	-	Transgressive
LGFCAU†	15.13	4.37	8.44	<0.0001	<0.000 1	-	<0.000 1	-	<0.000 1	-	-	-	Intermediate
LMAXCA‡	44.40	38.68	65.17	<0.0001	-	0.34	-	<0.000 1	-	<0.000 1	-	-	Transgressive
NBDENTΩ	5.40	0.27	1.03	-	-	-	-	-	-	-	40.46	<0.000 1	Intermediate
LGBASE†	7.15	3.73	6.15	<0.0001	<0.000 1	-	0.13	-	<0.000 1	-	-	-	Parental [N]
HAUINV†*	8.52	6.50	7.92	<0.0001	<0.000 1	-	0.24	-	<0.000 1	-	-	-	Parental [N]
LOTGEX†*	6.86	3.84	5.61	<0.0001	<0.000 1	-	<0.000 1	-	<0.000 1	-	-	-	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	<0.000 1	Intermediate
LGTGINΩ*	0.89	0.70	0.85	-	-	-	-	-	-	-	17.14	<0.000 1	Intermediate
NBRAYO‡*	29.10	25.27	32.20	-	-	0.05	-	0.23	-	<0.000 1	-	-	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	-	<0.000 1	-	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

Table 4. Results of the "a posteriori" classification of the three species using canonical discriminant analysis of 87 accessions and 34 morphological characters.

		A posteriori group		Percentage correctly	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 1. Geographical and ribotype distribution of the three species under study in east of their distribution range. Dashed lines, diagonal lines and darked-gray regions indicate the distribution of *S. boreale, S. novi-belgii* and *S. anticostense*, respectively. Ellipse, hexagonal and rectangular shapes are ribotype symbols for *S. boreale, S. novi-belgii*, and *S. anticostense*, respectively. Letters inside the shapes correspond to the clades indicated in Figure 4 and Table 1. This map was produced using the Adobe Illustrator V. 24 (Microsoft Co.) created by the first author from sources in the public domain.

Figure 2. Principal Component Analysis (PCA) of the morphological data of 28 accessions of *S. anticostense,* 29 of *S. novi-belgii*, and 30 of *S. boreale*. The inset represents the character vectors, which were scaled to 1 in this analysis. Abbreviations used in the inset graph are provided in Table 2.

Figure 3. Canonical Discriminant Analysis (CDA) of the morphological characters including 28 accessions of *S. anticostense,* 29 of *S. novi-belgii*, and 30 of *S. boreale*. Three "a priori" groups were defined for the analysis. Variation belonging to each function is indicated within parentheses. The symbols are provided in Figure 2.

Figure 4. Phylogenetic tree of the ITS data resulting from a ML analysis of the three species under study, 37 diploid species, and 3 outgroup genera. Clades A, B, C, D, E, F, and G comprise the unresolved ribotypes that are expanded in Figure 6. Numbers above branches are bootstrap support values.

Figure 5. Ribotype information related to the three species under study. The number between the arrows indicates shared ribotypes among two or/and three taxa. The numbers below or above the taxon names are total/unique ribotypes/no. of individuals per taxon.

Figure 6. Simplified ribotype network of the ITS data set using clades A to G of Figure 4. The ellipse, hexagonal and rectangular shapes indicate ribotypes of *S. boreale, S. novi-belgii* and *S. anticostense,* respectively. The three forms are collapsed when the species share a ribotype. Two large circles (clades E and G) indicate ribotype shared between *S. boreale* and the other diploid species. The small circles indicate unsampled ribotypes. The size of symbols is proportional to the number of shared ribotypes. The derivatives of each clade are indicated inside the broken-line rectangles. Sa, *S. anticostense*; Sn, *S. novi-belgii*; Sb, *S. boreale*; Spu, *S. puniceum*; Sde, *S. depauperatum*; Spa, *S. parviceps*; Spo, *S. porteri*; Se, *S. elliottii*; San, *S. anomalum*; Sw, *S. welshii*; Sd, *S. dumosum*; Sna, *S. nahanniense*; Su, *S. urophyllum*; Spl, *S. plumosum*; Sra, *S. racemosum*; Ssh, *S. shortii*; Sf, *S. firmum*.

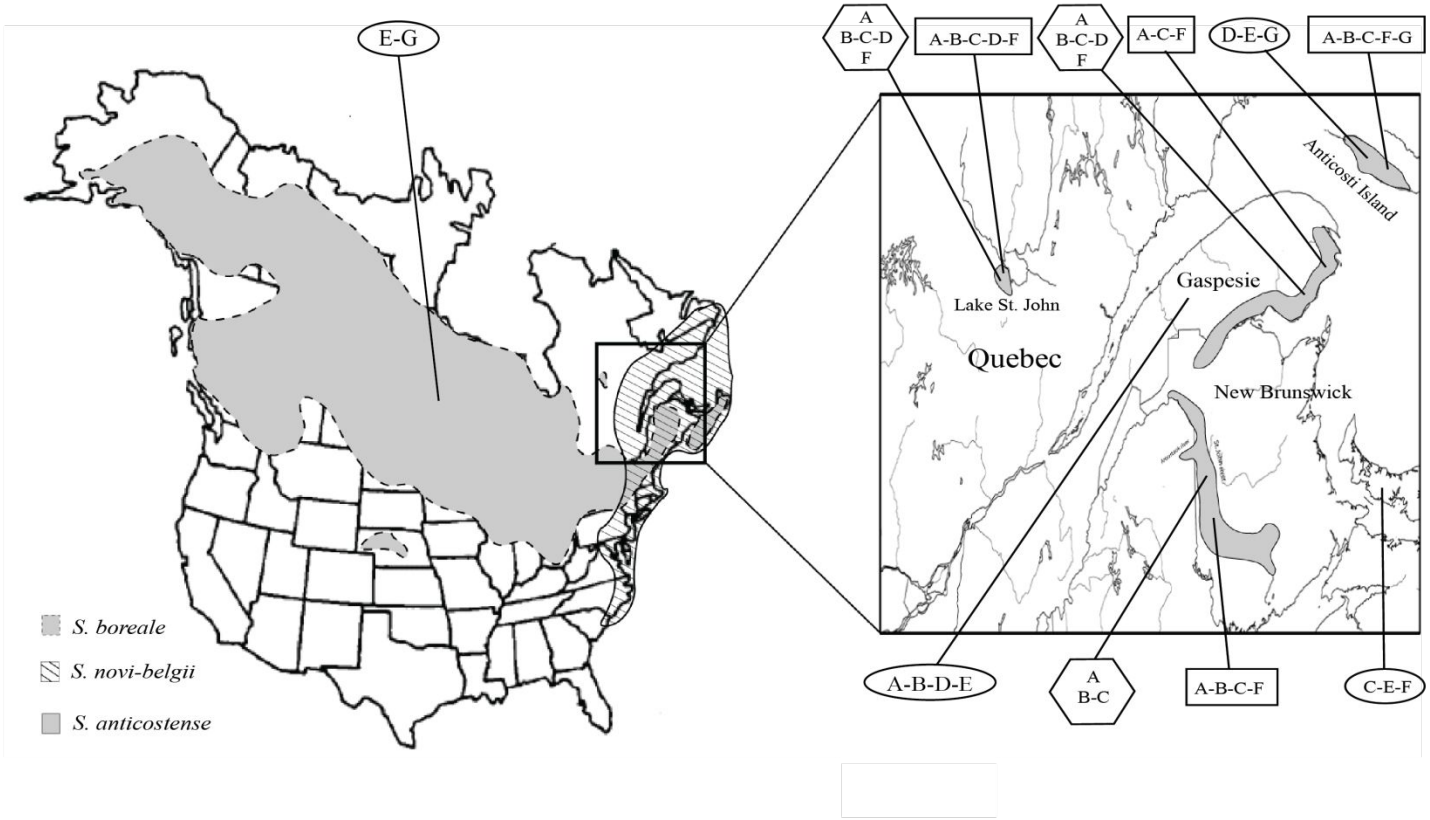

Figure1.

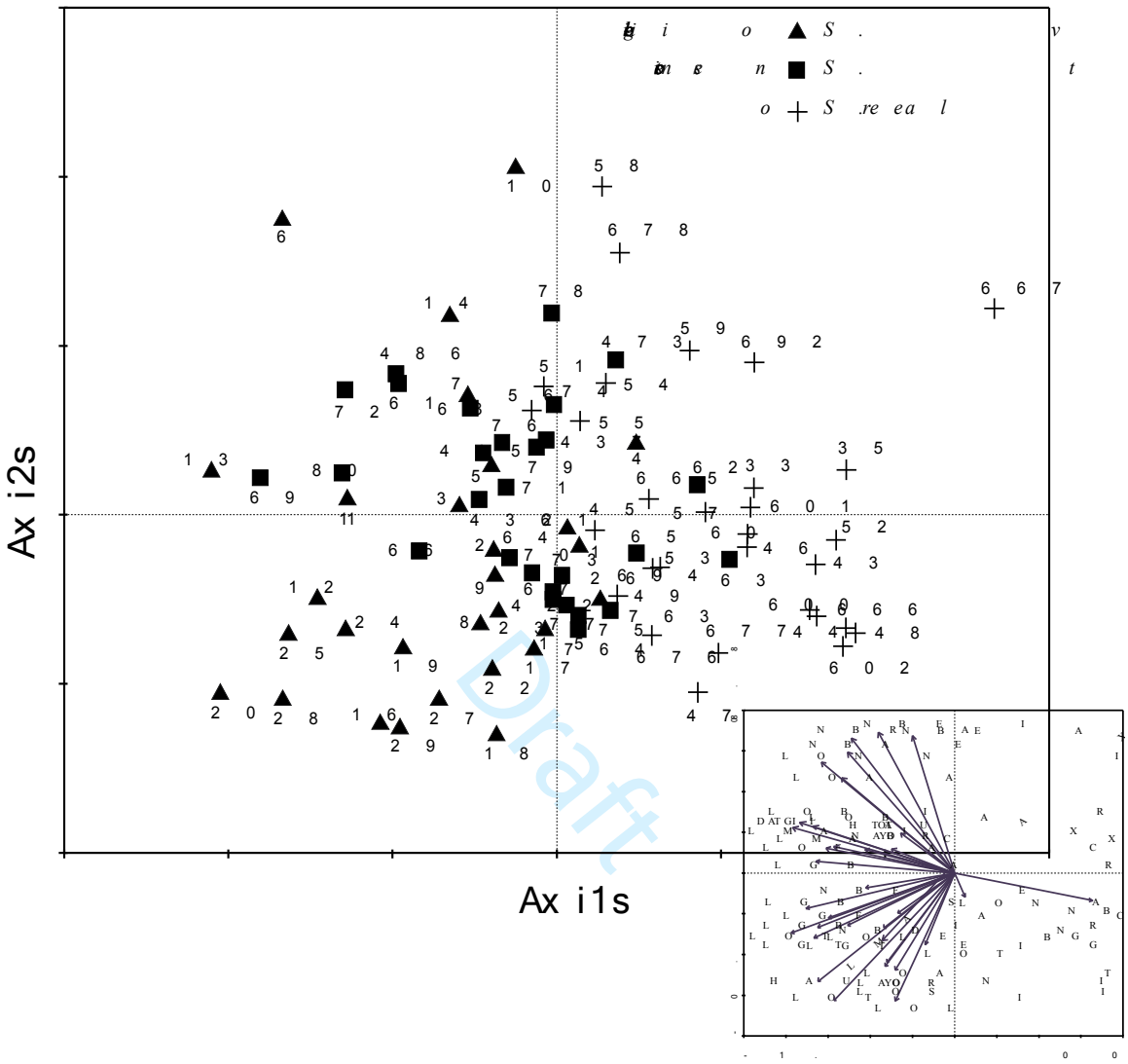

Figure 2.

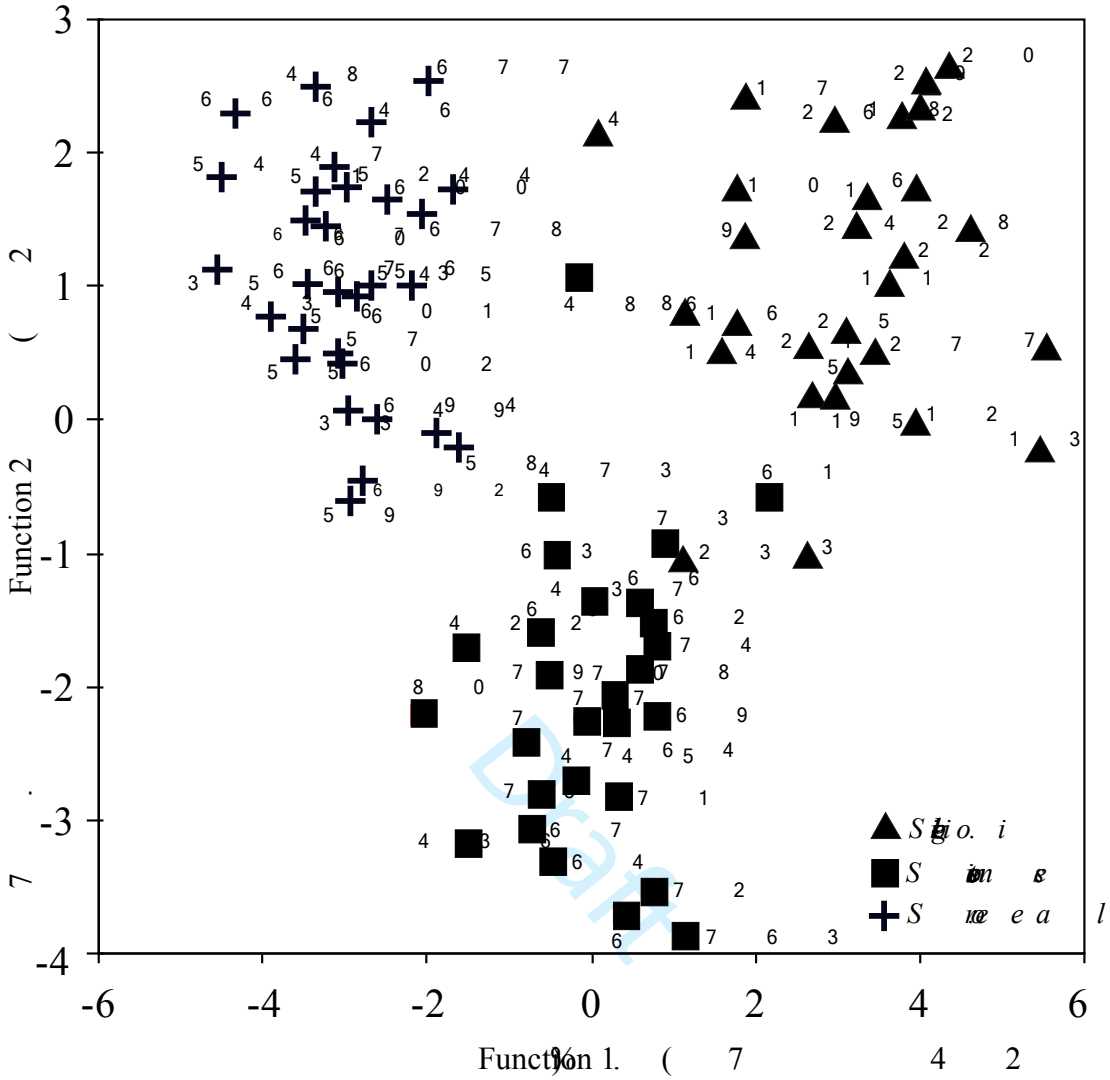

Figure 3.

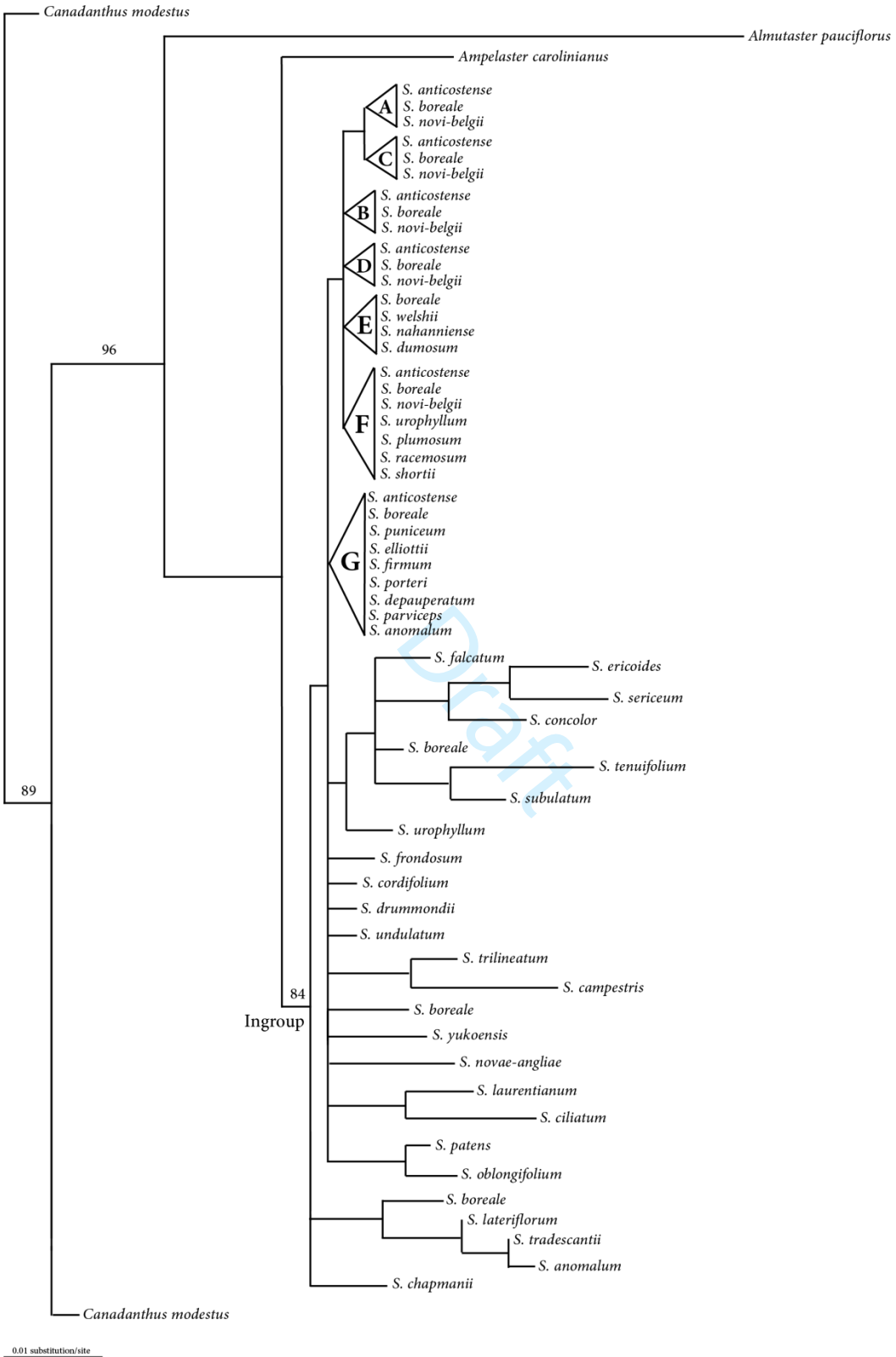

Figure 4.

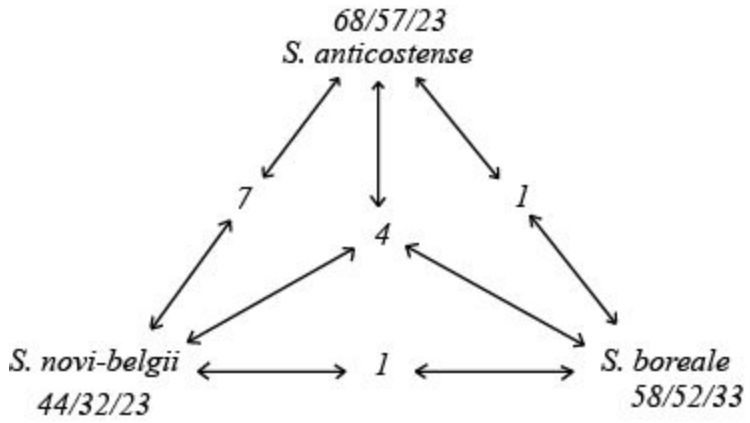

Figure 5.

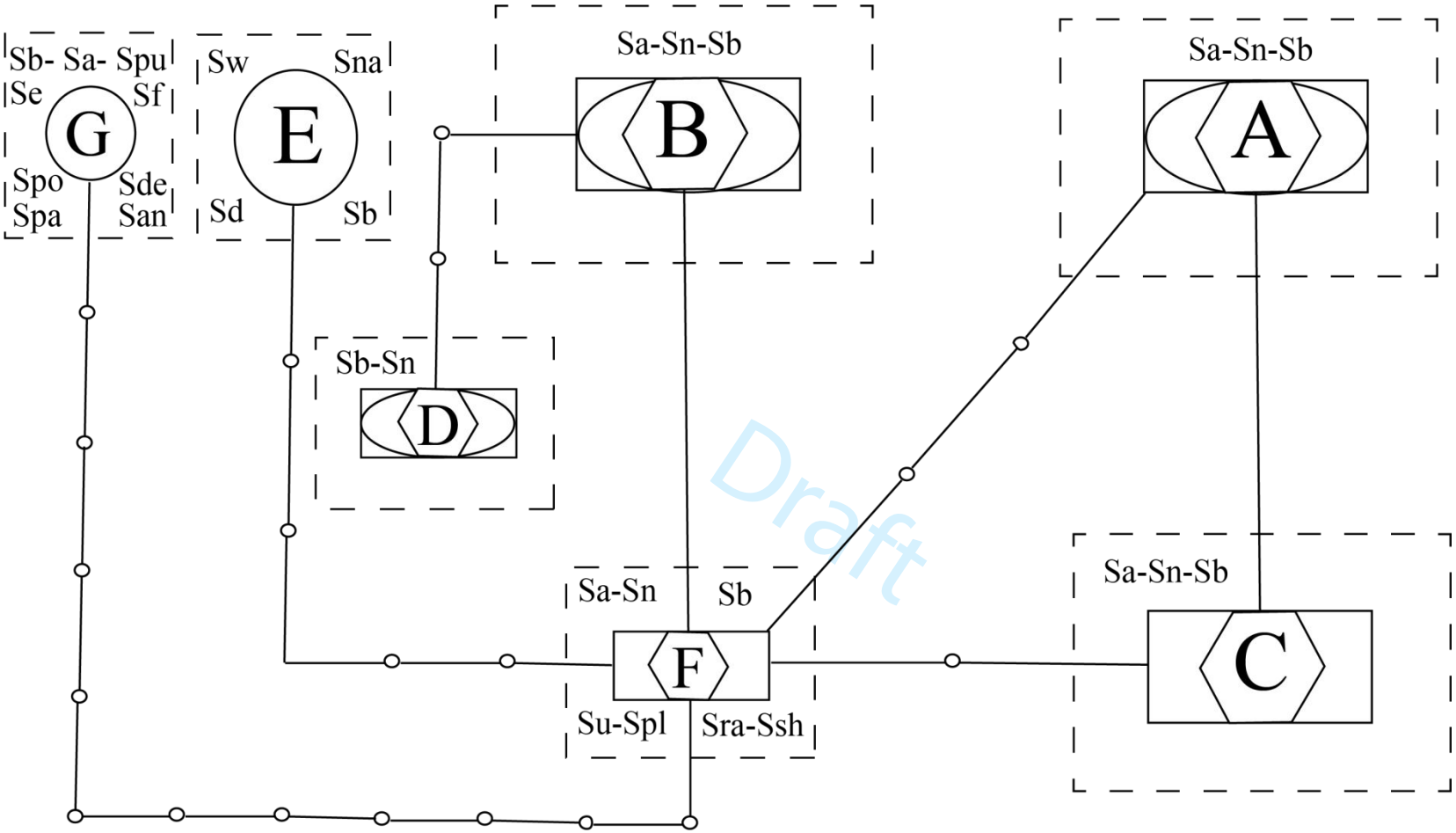

Figure 6.