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Systematics, biogeography, and character evolution of *Sparganium* (Typhaceae): Diversification of a widespread, aquatic lineage[1]

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- *Premise of the study: Sparganium* (Typhaceae) is a genus of aquatic monocots containing ±14 species, with flowers aggregated in unisexual, spherical heads, and habit ranging from floating to emergent. *Sparganium* presents an opportunity to investigate diversification, character evolution, and biogeographical relationships in a widespread temperate genus of aquatic monocots. We present a fossil-calibrated, molecular phylogeny of *Sparganium* based on analysis of two chloroplast and two nuclear markers. Within this framework, we examine character evolution in both habit and stigma number and infer the ancestral area and biogeographic history of the genus.
- *Methods:* Sequence data from two cpDNA and two nDNA markers were analyzed using maximum parsimony, maximum likelihood, and Bayesian inference. We used the program BEAST to simultaneously estimate phylogeny and divergence times, S-DIVA and Lagrange for biogeographical reconstruction, and BayesTraits to examine locule number and habit evolution.
- *Key results:* Two major clades were recovered with strong support: one composed of *S. erectum* and *S. eurycarpum*; and the other containing all remaining *Sparganium*. We realigned the subgenera to conform to these clades. Divergence time analysis suggests a Miocene crown origin but Pliocene diversification. Importantly, the floating-leaved habit has arisen multiple times in the genus, from emergent ancestors—contrary to past hypotheses.
- *Conclusions:* Cooling trends during the Tertiary are correlated with the isolation of temperate Eurasian and North American taxa. Vicariance, long-distance dispersal, and habitat specialization are proposed as mechanisms for *Sparganium* diversification.

Key words: aquatic habit; biogeography; BEAST; Beringia; bur-reed; monocot; Pliocene; Poales; *Sparganium*; Typhaceae.

Sparganium (Typhaceae) is an aquatic genus of ±14 species. Species of *Sparganium* are ecologically important in aquatic communities, providing cover and food for a variety of waterfowl and mammals (Arber, 1920; Fassett, 1940). Bur-reeds provide forage for cattle, especially during dry periods (Aston, 1987), but may become a nuisance, choking waterways (Cook and Nicholls, 1987). Traditionally, bur-reeds have been used as a medicinal herb to improve blood circulation (Tulin et al., 1999) and as a food source (Moerman, 1998). Though of minor economic importance, *Sparganium* are of biogeographical, phylogenetic, and evolutionary interest. We examine here with nuclear (nDNA) and chloroplast (cpDNA) regions the phylogenetic relationships of extant species, and test the current classification of the genus; use a fossil-calibrated chronogram to uncover patterns of biogeographic spread across the Northern Hemisphere under vicariance and dispersal models; and estimate the transitions and evolutionary implications of two key morphological features of this aquatic genus—growth form and ovary/stigma number.

Sparganium occupy aquatic habitats primarily in temperate and cool regions, and several species show wide-ranging, circumboreal distributions. The latter include *S. angustifolium*, *S. emersum*, *S. glomeratum*, *S. natans*, and *S. hyperboreum.* These species have limited range extensions southward at high elevations in Eurasia and North America. The remaining species are limited to either Eastern or Western Hemisphere distributions. The centers of diversity in the genus are eastern North America (9–10 spp.), East Asia (10–13 spp.), and Europe (7 spp.; Cook and Nicholls, 1986; Kaul, 1997; Sun and Simpson, 2010). *Sparganium fallax* and *S. subglobosum* are primarily East Asian, but also appear in the mountains of New Guinea (Cook and Nicholls, 1986). Only two species (*S. subglobosum* and *S. erectum*) reach the Southern Hemisphere in Australia and New Zealand, where *S. erectum* was probably introduced (Cook and Nicholls, 1987). Distributional patterns and morphological similarities have suggested close relationships between North American and Eurasian species pairs, such as *S. fluctuans–S. gramineum* and *S. americanum–S. japonicum* (Cook and Nicholls, 1987), but these relationships have not been evaluated in a molecular context. Though *Sparganium* was until recently unknown from Central and South America (Cook and Nicholls,

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1986), its occurrence has been documented in west-central Mexico (Kaul, 1997; Socorro González Elizondo et al., 2007) and Colombia, and vouchers from both locales were reviewed for this study (see Appendix 1). Without DNA evidence, it is difficult to say whether these represent disjunct populations of *S. americanum* (as identified), *S. emersum*, or undescribed species.

Though *Sparganium* has traditionally been placed in Sparganiaceae, *Sparganium* and *Typha* are now both placed in Typhaceae (APG III, 2009), as was earlier advocated by Müller-Doblies (1970) based on the lack of significant differences in flower and inflorescence morphology. Support for the monophyly of *Typha* + *Sparganium* is well established based on intensive phylogenetic study of monocots in recent years (Bremer, 2000; Chase et al., 2006; Givnish et al., 2007, 2010, 2011; Soltis et al., 2011). *Sparganium* and *Typha* constitute an early-diverging lineage within the Poales (Bremer, 2000; Chase et al., 2006; Givnish et al., 2010, 2011), with phylogenetic analysis of whole plastome sequences across monocots placing the family as the second-diverging lineage after Bromeliaceae (Givnish et al., 2010). However, the position of Typhaceae has the lowest support within the Poales (87–97% bootstrap). The placement of *Sparganium* as an early-diverging lineage in Poales, complemented with a rich fossil record dating to the Paleocene, make this group an intriguing subject for molecular dating of key events.

Despite the distinctiveness of *Sparganium* as a genus and its apparent monophyly based on the inclusion of five *Sparganium* species in a recent phylogenetic study of *Typha* (Kim and Choi, 2011), the phylogenetic relationships within *Sparganium* have not previously received intensive study. Its species and their relationships are still poorly understood, and some species are dubiously differentiated from their presumed Eurasian or North American vicariant counterparts (e.g., *S. eurycarpum*–*S. erectum;* Kaul, 1997). Species identification is made difficult by phenotypic plasticity and confusing taxonomy (Cook and Nicholls, 1986, 1987; Kaul, 1997). Adding to the systematic confusion, hybridization is widely suspected but rarely rigorously verified (Harms, 1973; Cook and Nicholls, 1986, 1987; Les and Philbrick, 1993; Kaul, 1997). Linnaeus (1753) recognized only two species, corresponding broadly to the two growth forms found within the genus: *S. erectum* (plants emergent) and *S. natans* (plants floating). Wladislaw Rothert worked on a monograph of *Sparganium* in the 1910s but died in 1916, and his manuscripts were lost (Cook, 1961). Later, Fernald (1922) published an account of the North American species, based in part on the work of Rothert. Important regional taxonomic treatments of *Sparganium* include Beal (1960) and Thieret (1982) for the southeastern United States, Crow and Hellquist (1981) for New England, Brayshaw (1985) for British Columbia, Kaul (1997) for North America, Cook (1961) for Britain, Yuzepchuk (1968) for Russia, Chen (1981) and Sun and Simpson (2010) for China, and Kadono (1994) for Japan. The most comprehensive treatment to date is the worldwide monograph by Cook and Nicholls (1986, 1987), who recognized 14 species. Species of *Sparganium* have been consistently placed within two subgenera (Holmberg, 1922; Cook, 1961; Cook and Nicholls, 1986, 1987). Subgenus *Xanthosparganium* Holmberg comprised short-statured species with light-colored tepals, whereas subg. *Sparganium* contained tall-statured species with dark-pigmented tepals. No phylogenetic analysis has tested the monophyly of the subgenera.

Aquatic plants have long been intriguing from an evolutionary perspective because of their specialized adaptations to aquatic life and multiple independent origins within angiosperms (Sculthorpe, 1967; Cook, 1996; Barrett and Graham, 1997). Recently, increased attention has been focused on examples of species radiation and character evolution in aquatic groups of angiosperms using phylogenetic methods. Several recent studies have applied character state reconstruction to assess diversification in aquatic growth form, revealing complex patterns of transition between emergent, floating, and submerged habits (Barrett and Graham, 1997; Chen et al., 2004, 2012; Moody and Les, 2007) and between rosulate (rosette) and vittate (caulescent) habits (Les et al., 2008). The genus *Sparganium* presents an opportunity to assess the evolution of divergent growth forms and locule numbers in a widespread, primarily Northern Hemisphere genus of aquatic monocots.

Rooted aquatic plants can be divided into three groups based on growth form: emergent, floating, or submerged (Sculthorpe, 1967; Schuyler, 1984). *Sparganium* species exhibit a striking dichotomy in growth form between emergent and floating habits (Fig. 1), an uncommon occurrence within a single genus of aquatic vascular plants. Whether the ancestral habit in *Sparganium* was emergent or floating has been the subject of controversy. Cook and Nicholls (1986, p. 227) suggested that the floating growth form was the ancestral state and that the "evolutionary trend from small aquatic species to large terrestrial species is clear." Kaul (1972), in comparing leaf anatomy in floating and emergent *Sparganium*, identified features such as stomata on the underside of floating leaves as evidence that the floating form was derived. No rigorous phylogenetic analysis of growth form evolution in *Sparganium* has been done, or evidence of multiple growth form transitions presented.

Sparganium species share a number of morphological adaptations that are common to many unrelated, aquatic angiosperm groups. These include monoecy, wind-pollination, floral reduction, vegetative spread by rhizomes and stolons, and seeds capable of long-distance dispersal (Sculthorpe, 1967; Cook, 1988; Barrett et al., 1993; Santamaria, 2002). Across angiosperms generally, and in aquatic plants specifically, a transition to wind pollination is often accompanied by loss or reduction of the perianth and a reduction in locule and/or ovule number to one (Friedman and Barrett, 2009; Givnish et al., 2010) and is strongly correlated to open habitats in the Poales, including Typhaceae (Givnish et al., 2010). In *Sparganium* the tepals are minute, and the pistillate flowers have a single ovule per locule with either one or two locules per flower. The evolutionary transition in number of locules or ovules per flower has not been examined in a phylogenetic context within *Sparganium*.

In this study, we employ a combined set of nuclear (nDNA) and chloroplast (cpDNA) gene regions, temporally fossil-calibrated, to investigate a series of questions related to phylogenetics, biogeography, and character evolution in *Sparganium*. Our data set is comprised of two cpDNA gene regions (*psbJ-petA*, *trnL-trnF*), and two regions of nDNA (phytochrome C [*phyC*], nuclear ribosomal internal transcribed spacer region [ITS]). We sampled the four gene regions across the broadest taxon sampling within *Sparganium* to date to shed light on the following questions about *Sparganium* evolution: (1) Are the subgenera composed of natural clades? (2) Is there evidence of hybridization among co-occurring species? (3) When and where did divergence events among *Sparganium* species occur, and are these events primarily dispersal or vicariant based? (4) How has *Sparganium* evolution been influenced by changing geographic and climatic conditions? (5) How many transitions between emergent and floating form and between one- and two-loculed ovaries have occurred in *Sparganium*, and what are the ancestral states?

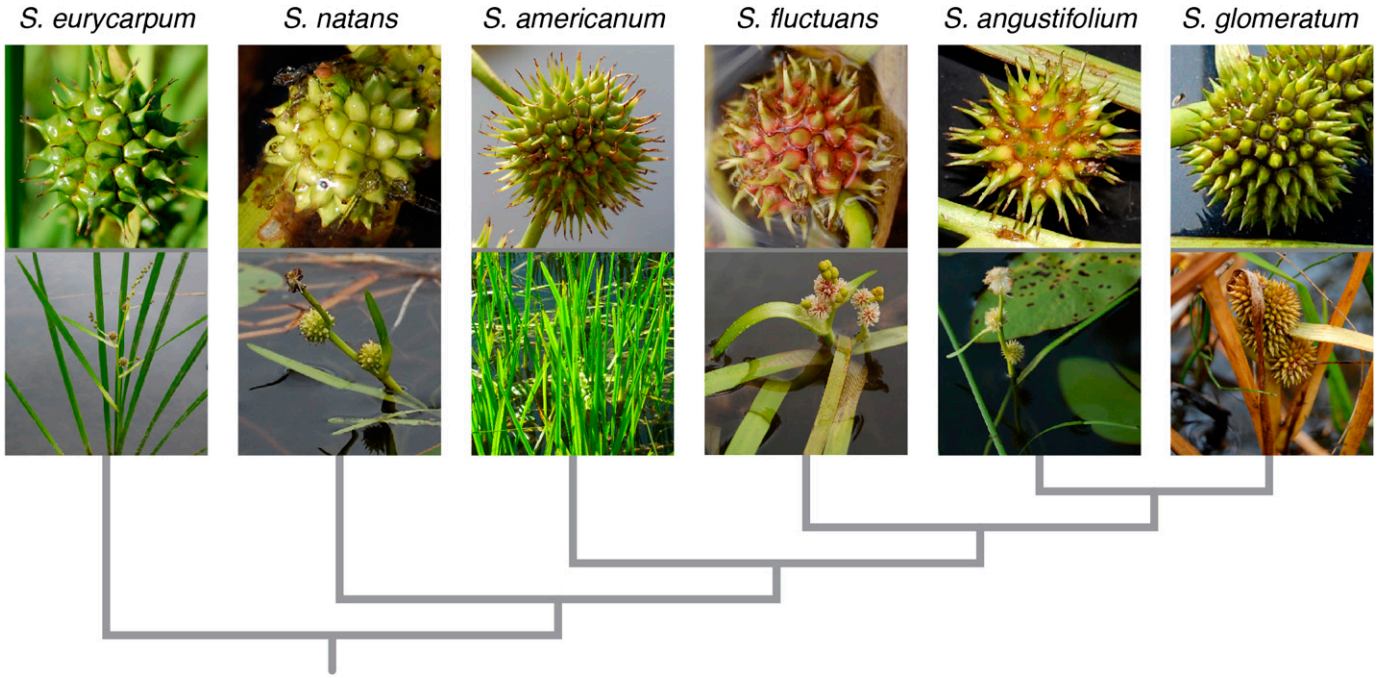

Fig. 1. Representative vegetative and reproductive variation within North American *Sparganium*. Species are arranged based on the results of the combined molecular phylogenetic data. Emergent-leaved species are *S. eurycarpum* and *S. americanum*. Floating-leaved species are *S. natans*, *S. fluctuans*, and *S. angustifolium*. *Sparganium glomeratum* is polymorphic, occurring either as floating or emergent. Previous infrageneric classification placed (of those shown) *S. eurycarpum* and *S. americanum* in subg. *Sparganium*, and the remainder in subg. *Xanthosparganium*. *Sparganium americanum* is now placed in the latter (see text). All photos by J. D. Sulman.

MATERIALS AND METHODS

***Nomenclature and sampling*—**Our species delimitations largely follow those of Cook and Nicholls (1986, 1987) and Kaul (1997), and nomenclature follows Govaerts (2013). We recognize *S. erectum* and *S. eurycarpum* as allopatric, polymorphic species which are only weakly differentiated from each other, largely on the basis of stigma number, though stigma number may vary across populations and even between flowers on a single plant (Kaul, 1997; S. G. Smith, unpublished data). We treat *S. erectum* as a Eurasian species, with flowers mostly unistigmatic, and *S. eurycarpum* as a North American species, with flowers mostly distigmatic. Within *S. erectum,* we recognize five subspecies (see Cook and Nicholls, 1987) and one additional variety, *S. erectum* subsp. *stoloniferum* var. *macrocarpum* (Makino) H. Hara, from Japan [= *S. eurycarpum* subsp. *coreanum* (Léveillé) Cook and Nicholls]. Within *S. eurycarpum*, we recognize var. *greenei* (Morong) Graebn. from the west coast of North America, which was included in *S. erectum* subsp. *stoloniferum* by Cook and Nicholls (1987).

In total, we analyzed 37 accessions, including 30 from 14 species of *Sparganium* (Appendix 2). Outgroup taxa were four species of *Typha* (Typhaceae), one species of *Brocchinia*, and two species of *Puya* (Bromeliaceae); the latter three served as a monophyletic ultimate outgroup for rooting purposes. Of our 37 accessions, all contained at least three of our four sample gene regions (*trnL-trnF*, *psbA-petJ*, ITS, *phyC*) except *Brocchinia prismatica,* which was missing ITS and *phyC*. Gene *phyC* was missing from *Sparganium emersum* (accessions 2–4), *S. erectum* (accessions 3, 4), and *S. subglobosum* (accession 2), and the two *Puya* accessions were missing *psbJ-petA*. To minimize the effects of missing data (and potential hybrids) on our divergence time estimation, a subset of taxa (28) was used for divergence time estimation and subsequent biogeographical reconstruction and character analyses. The six *Sparganium* accessions that lacked *phyC* entirely and *S. hyperboreum 2* with only a partial sequence of *phyC* were removed, though all *Sparganium* species were still represented in the subset.

***DNA extraction, amplification and sequencing*—**Molecular data were obtained from specimens collected by J. D. Sulman, E. Hayasaka, M. Yamazaki, and others and preserved in the field using silica gel; additional material was sampled from herbarium sheets (see Appendix 2). Genomic DNA was extracted from silica-dried plant material and herbarium specimens using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) according to the manufacturer's specifications. A list of accessions and genes sequenced is provided in Appendix 2.

The four gene regions used in our study were chosen based on their relatively high rates of variability and were selected after a broad survey of gene regions. The cpDNA markers sampled were *trnL-trnF* and *psbJ-petA*. We amplified and sequenced *trnL-F* using the Tab C, D, E, and F primers (Taberlet et al., 1991). For the nuclear genes sampled, ITS was amplified and sequenced using the ITS1 and ITS4 primers (Baldwin, 1992), and *phyC* was amplified and sequenced using primers of Jabaily and Sytsma (2010) for *Sparganium* accessions and, for *Typha* accessions, using the newly designed primers bdphyC-f2 (GCDTTGAARTCRTATAARCTYGC) and bdphyC-r1 (AGGRTCRTGTTTY-GCACCACC). Polymerase chain reaction (PCR) amplifications of *psbJ-petA* followed the protocol from Shaw et al. (2007), while the other three DNA regions were amplified following the protocol of Jabaily and Sytsma (2010). Samples that amplified were diluted 30× with distilled water and cycle sequenced in a MJ Research PTC-200 thermal cycler in 10-μL samples: 8 μL of master mix (2.9 μL distilled water, 1.6 μL primer, 1.0 μL DMSO, 2.0 μL sequencing buffer, and 0.50 μL Big Dye [Applied Biosystems, Foster City, California, USA]) and 2 μL diluted PCR product. Cycle sequencing products were cleaned with magnetic beads (Agencourt, Beverly, Massachusetts, USA), and the 10-μL samples were electrophoresed on an Applied Biosystems 3730xl automated DNA sequencing instrument. Data were analyzed using PE-Biosystems version 3.7 of Sequencing Analysis at the University Wisconsin-Madison Biotechnology Center.

***Sequence analyses and divergence time estimation*—**All sequences were edited in the program Sequencher 4.7 (Gene Codes, Ann Arbor, Michigan, USA) prior to being manually aligned in MacClade 4.08 (Maddison and Maddison, 2005). Our nucleotide alignment philosophy followed the procedure of Wheeler (1996). Insertions and deletions were not coded, and gaps were treated as missing data. The alignments used in this study are available in the database TreeBASE (http://treebase.org; study number 13860). Concatenated alignments of cpDNA, nDNA, and the four gene regions combined were compiled and analyzed separately. To assess congruence between the cpDNA and nDNA data

sets (using only ingroup taxa common in all four gene regions; 27 *Sparganium* accessions), we used the incongruence length difference (ILD) test (Farris et al., 1994) as implemented with parsimony in the program PAUP* v4.0b10 (Swofford, 2002). Despite its limitations (Yoder et al., 2001), the ILD test can be useful to identify broad-scale incongruence between data sets (Hipp et al., 2004). Maximum parsimony (MP) analyses were performed in PAUP* by sampling 1000 random addition replicates and using tree-bisection-reconnection (TBR) branch swapping. Bootstrap (Felsenstein, 1985) values were taken from 1000 heuristic searches, with 10 random addition replicates per bootstrap replicate, TBR branch swapping, and no more than 5000 trees saved per replicate. For maximum likelihood (ML) analyses, we used the program GARLI v2.0 (Zwickl, 2006). For Garli analyses, we partitioned our data by gene region and used the models of evolution (detailed below) suggested by the program ModelTest v3.7 (Posada and Crandall, 1998). One hundred bootstrap repetitions were conducted using the same ML settings as the initial search. Bayesian inference (BI) was conducted using the program MrBayes v.3.2.1 (Huelsenbeck and Ronquist, 2001) as implemented in the Cyberinfrastructure for Phylogenetic Research (CIPRES) cluster (http://www.phylo.org/). Analyses were run for 3 million generations using the default settings and GTR + Γ + I as the model of evolution. After confirming that the potential scale reduction factor (Gelman and Rubin, 1992) was ~1.00 and examining the sump files in tracer to confirm adequate mixing, we discarded the first 25% of the trees as burn-in. To assess whether our runs achieved convergence, we checked that the standard deviation of split frequencies fell below 0.01 (achieved after ca. 1.0–1.2 million generations).

BEAST analysis for divergence times—Estimation of divergence times and simultaneous phylogeny estimation was performed on a 28 taxa, four-gene region alignment using the program BEAST v1.7.4 (Drummond and Rambaut, 2007). To account for sequence rate heterogeneity, we partitioned the data into four sections that corresponded to our different gene regions. For each of the four partitions, we used a model of evolution as suggested by the Akaike information criterion (AIC; Akaike, 1974) obtained in ModelTest v3.7. The GTR + Γ + I model was chosen for ITS and *phyC*, the K81uf + Γ model was selected for *psbJ-petA*, while the TVM + Γ model was suggested for the *trnL-trnF* region. These were used as molecular evolution models for each respective partition in BEAST by selecting the "unlink substitution model" option and adjusting the appropriate prior and operator settings (for the TVM and K81uf models). We unlinked our clock models, and we used a Yule tree prior (Yule, 1925). The crown of the Typhaceae was given a minimum age of 70 Myr using a lognormal distribution prior based on fossil evidence (see below). Additionally, the root of the tree (Typhaceae + Bromeliaceae) was calibrated with a uniform distribution from 90–105 Ma (see below). We also explored the effect (especially on the *Sparganium* crown node) of using no fossil calibration and only placing the prior on the root. We conducted two separate BEAST analyses of 20 million generations each, with samples taken every 2000 generations. For each run, the first one million generations were discarded as burn-in, and the trees (19 000) and log files were combined using the program LogCombiner v1.7.4 (http://beast.bio.ed.ac.uk/LogCombiner). The trees were then interpreted by TreeAnnotator v1.7.4 prior to visualization in FigTree v1.4. Log files were analyzed in tracer (Rambaut and Drummond, 2007), and the effective sample size values (ESS) were over 300 for all parameters.

Calibration points for DNA—The root of the tree was secondarily calibrated with a uniform distribution ranging from 90–105 Ma, reflecting the 95% consistency index (CI) variance from Givnish et al. (unpublished manuscript). The crown age of Typhaceae (*Typha* and *Sparganium*) was estimated to be at least 70 Myr. We calibrated the node using a lognormal prior with an offset of 70, a mean of 1.5, and a standard deviation of 0.5. This date is based on the earliest known fossils of *Typha* (seeds *T. ochreacea* Knobloch and Mai and *T. protogaea* Knobloch and Mai, from the Maastrichtian of Eisleben, Germany) giving an approximate age for the stem divergence of *Typha* (and *Sparganium*) (Knobloch and Mai, 1986). Crepet et al. (2004), however, question their (and almost all monocot fossil) identities. Justification for a late Cretaceous dating of the Typhaceae crown includes two arguments. First, the monocot-wide dating studies of Bremer (2000) and Givnish et al. (2011) used this fossil for the crown divergence of Typhaceae. The latter study used six Cretaceous fossils (including *Typha*) across the monocots. Crown divergence of the Typhaceae around 70 Myr was still obtained when only the five other fossils were used (Givnish et al., 2011), providing correlative support for the fossil dating of the node. Second, besides the Cretaceous *Typha* seed fossils, both *Typha* and *Sparganium* possess a fairly abundant and distinctive fossil record (including inflorescences and leaves) that begins in the Paleocene. The stem lineages of both *Typha* and *Sparganium* are thus already differentiated in the Paleocene, supporting a slightly older Cretaceous crown node for the family. The Cretaceous *Typha* fossil predates the oldest *Sparganium* fossil by about 10 Myr. Fossils of *Sparganium* first appear in strata starting in the late Paleocene (ca. 60 Ma). Paleocene records from the northern Great Plains of North America include *S. parvum* Hickey (Hickey, 1977) and *Sparganium* sp. (Postnikoff, 2009). Abundant fossils of *Sparganium* are seen in the Canadian High Arctic at the end of the Paleocene or early Eocene (McIver and Basinger, 1999). Eocene records include *S. antiquum* (Newberry) Berry from Wyoming, USA (Berry, 1924) and *S. fushunense* Geng from northeastern China (Geng, 1999). These macrofossils bear globose heads with or without a peduncle, similar to modern-day *Sparganium* (Brown, 1962). Some Oligocene-age *Sparganium* fossils had ovaries with numerous locules: *S. multiloculare* Reid & Chandler from England had 2–5, and *S. balticum* Dorofeev from Russia had 4–7 (Cook and Nicholls, 1986). Such locule numbers are not seen in extant *Sparganium* and indicate that these fossils are representatives of the *Sparganium* stem lineage.

***Reconstruction of ancestral areas*—**Ancestral area reconstruction (AAR) and estimating geographic patterns of diversification within *Sparganium* were done with two programs, Lagrange (Ree and Smith, 2008) and RASP 2.0 (Reconstruct Ancestral State in Phylogenies, formerly S-DIVA; Yu et al., 2010, 2011). These two programs incorporate quite different assumptions and thus provide contrasting methods to examine biogeography of *Sparganium* across the Northern Hemisphere. All taxa were scored for geographic range, based on their presence in each of five regions: (1) western North America, (2) eastern North America, (3) western Eurasia (including North Africa), (4) eastern Eurasia, and (5) southeast Asia/Australia (including New Zealand). We attempted to use natural boundaries between regions: the Rocky Mountains separating eastern/western North America; the Urals and Caucasus separating eastern/western Eurasia; and the mountains of Yunnan separating eastern Eurasia from southeast Asia. The geological time scale of the Geological Society of America (Walker and Geissman, 2009) was used to determine time splits between epochs within the Tertiary Period.

A subset of 20 accessions of *Sparganium* and one of *Typha* were used for AAR. As we did not have complete representation of accessions for each area for some widespread species, we used a conservative scoring of areas for these accessions and coded each accession with the entire range of the species regardless of their biogeographic source. We did not score *S. erectum* for southeast Asia/Australia, though it occurs there, because it is believed to be a recent introduction (Aston, 1987; Cook and Nicholls, 1987). We explored two alternative biogeographical scorings for the outgroup *Typha* (one accession). First, we allowed the single placeholder for *Typha* to occupy all five areas based on the almost worldwide, present distribution of the genus. Second, we used the recent phylogenetic analysis of *Typha* (Kim and Choi, 2011) to estimate the AAR of the genus, a recommendation of Ronquist (1997) for outgroup scoring. Although not an explicit finding of Kim and Choi (2011), the placement of Eurasian *T. minima* as sister to all other *Typha* species and the placement of several other species from western and eastern Eurasia in several early-diverging clades strongly argue for a widespread Eurasian AAR for the genus. Thus, in a second analysis we allowed the *Typha* placeholder to only occupy western and eastern Eurasia.

DEC analyses—Lagrange uses a likelihood model incorporating assumptions of area connectivity at discrete time intervals and estimates both dispersal and extinction parameters while allowing area vicariance, as part of the dispersal–extinction–cladogenesis (DEC) model (Ree and Smith, 2008). The model of dispersal route possibilities between pairs of areas was based in part on previous DEC models examining plants distributed in the Northern Hemisphere (Clayton et al., 2009; Xie et al., 2009; Drew and Sytsma, 2012; Nie et al., 2012; Sessa et al., 2012) but refined with known geological event dates affecting the Northern Hemisphere (Tiffney, 1985a, b, 2000; Manchester, 1999; Scotese, 2001; Tiffney and Manchester, 2001; Morley, 2003; Graham, 2011). In particular for *Sparganium*, we employed timing of geological events (formation or break-up) involving the Beringian land bridge between Asia and North America, and the North Atlantic connection between North America and Europe. Dispersal probabilities range from 0.1 for well-separated areas to 1.0 for contiguous areas and are provided for three time intervals spanning the Pleistocene, to the mid Miocene (crown divergence of *Sparganium*), and the late Cretaceous (stem divergence of *Sparganium*) (Table 1). The BEAST-generated BI tree (see above) from the combined analysis of *Sparganium* was pruned of nonincluded taxa in the program R 2.15.1 (R Development Core Team, 2012) and entered into Lagrange. We explored AAR under assumptions that (1) all ancestral ranges of three and (2) four combined areas were permitted based on extant

TABLE 1. Dispersal probabilities among areas implemented in Lagrange for *Sparganium*. Dispersal probabilities are given for 0–2.6 Ma, 2.6–8.0 Ma, and 8.0–73 Ma, respectively.

Area	W North America	E North America	W Eurasia	E Eurasia	SE Asia/ Australia
W North America	—	1.0/0.7/0.7	0.1/0.1/0.1	0.5/0.7/1.0	0.2/0.3/0.5
E North America		—	0.5/0.7/1.0	0.1/0.5/0.7	0.1/0.3/0.5
W Eurasia			—	1.0/1.0/0.7	1.0/1.0/0.7
E Eurasia				—	1.0/1.0/1.0
SE Asia/Australia					—

and potentially plausible ranges. For a given node in each tree, the AAR was accepted as significant if it was greater than 2 likelihood units relative to alternative AAR scenarios.

Statistical-DIVA analyses—RASP attempts to overcome some of the limitations inherent in the original DIVA (Ronquist, 1996, 1997; Nylander et al., 2008; Harris and Xiang, 2009; Kodandaramaiah, 2010; Harris et al., 2013). AAR under DIVA strongly models vicariance and minimizes assumptions of dispersal and extinction. RASP allows assessment of phylogenetic uncertainty in AAR by examining multiple trees (e.g., by using a subset of post burn-in BI trees), and thus greatly extends the utility of DIVA. A random sample of 100 BI posterior probability (PP) trees from the combined analysis was pruned of nonincluded taxa in R (R Development Core Team, 2012) and then used to estimate probabilities of ancestral areas at each node using the Statistical Dispersal-Vicariance Analysis (S-DIVA) option in RASP. We explored the impact of restricting the number of unit areas allowed in ancestral distributions by using the maxareas option (4 and 2 areas), as well as using both biogeographical scorings for *Typha*. AAR for all nodes was visualized on the BEAST tree used in the DEC analysis.

***Estimating transitions in habit and ovary evolution*—**We explored the evolutionary transitions of habit (emergent vs. floating growth form) and ovary (one vs. two loculed) in *Sparganium* using BayesTraits (Pagel and Meade, 2007). These analyses in conjunction with the temporal setting provided by the BEAST-derived chronogram permitted estimates of numbers, directionality, and timing of transitions between each pair of character states. We used the same reduced taxa set (20 *Sparganium* and 1 *Typha* accessions) as employed in the biogeographical analyses. Aquatic habit had two states: emergent or floating. Each taxon was scored according to its typical growth form at the time of flowering, as reported in taxonomic treatments and based on review of herbarium material and observations in the field. *Sparganium emersum* and *S. glomeratum* were scored as polymorphic. Locule number in the ovary had two states, 1 or 2. Some taxa in the *S. erectum* group are frequently 1- or 2-locular, and thus scored polymorphic. Locule number and stigma number are effectively the same character, based on the number of carpels. There is evidence suggesting that the fruits of *Typha* and most *Sparganium* are pseudomonomerous, with a single locule comprised of one functional carpel and one highly reduced, vestigial one (Müller-Doblies, 1969, 1970; Cronquist, 1981; Thieret, 1982; see Discussion). We scored species, however, according to the functional locule/stigma number, with the aim of capturing recent evolutionary trends in floral merosity.

We implemented ML optimization of trait evolution (Pagel, 1999) with the BayesMultiState options in BayesTraits v.1.0 (Pagel and Meade, 2007), allowing kappa to be estimated (Friedman and Barrett, 2008), and using a random set of 100 Bayesian PP trees (see RASP analysis above). Probabilities of ancestral states are displayed using the chronogram provided by the BEAST tree used in the biogeographical analyses. In addition, we used maximum parsimony to infer state transitions in habit and ovary condition with the "trace character" option in MacClade (Maddison and Maddison, 2005) and the resolving option of "all most parsimonious states at each node". The resulting ancestral-state reconstructions were visually displayed by color-coding the branches of the BEAST chronogram.

RESULTS

***cpDNA analysis*—**The 37-taxa, aligned cpDNA data matrix was 2040 characters (*psbJ-petA*: 1018; *trnL-trnF*: 1022). The cpDNA ML and BI trees were identical in topology with BI posteriori probabilities (PP) generally higher than ML bootstrap support (BS) (Fig. 2A). The MP bootstrap consensus tree was consistent with the ML/BI tree but with fewer resolved nodes. There was strong support for the monophyly of *Sparganium* (100% ML BS, 100% MP BS, 1.00 PP). The genus is divided into two strongly supported clades that do not correspond to previously accepted subgenera. The first clade consists of *S. eurycarpum* and *S. erectum* (97% ML BS, 98% MP BS, 1.00 PP). Neither species was resolved as monophyletic. The second clade (95% ML BS, 89% MP BS, 0.99 PP) contains all remaining species of *Sparganium*. The MP bootstrap consensus yielded a polytomy at the crown of the second clade, though a strict consensus of the most parsimonious trees recovered a topology congruent with the ML and BI analyses (data not shown). In the ML and BI analyses, a floating-leaved clade of *S. natans* + *S. hyperboreum* (79% ML BS, 89% MP BS, 1.00 PP) was placed as sister to a clade comprised of the remaining 10 species, although there is little support for it. The backbone of this latter clade is poorly resolved, except for the clades of *S. fluctuans* + *S. gramineum* (93% ML BS, 93% MP BS, 1.00 PP) and *S. emersum* + *S. glomeratum* (71% ML BS, 61% MP BS, 0.99 PP).

***Nuclear DNA analysis*—**The 36-taxa (no *Brocchinia*), aligned nDNA data matrix included 1680 characters (ITS: 803; phyC: 877). The nDNA tree (Fig. 2B) resolved *Sparganium* as a well-supported clade (100% ML BS, 100% MP BS, 1.00 PP). Within *Sparganium*, the same two clades as in the cpDNA tree are resolved: the first clade consisting of *S. erectum* +*S. eurycarpum* (95% ML BS, 98% MP BS, 1.00 PP) and the second clade including all remaining species (97% BS, 96% MP BS, >0.99 PP). In the first clade, relationships are better resolved than with cpDNA. *Sparganium eurycarpum* is monophyletic (79% ML BS, 81% MP BS, 0.99 PP) and together with *S. erectum* var. *macrocarpum* is sister to a clade of the other three accessions of *S. erectum.* Although the backbone of the second clade was more resolved with nDNA than with cpDNA, a number of the nodes are weak in the ML tree. A clade of *S. natans* + *S. hyperboreum* was well supported (100% ML BS, 100% MP BS, 1.00 PP). A well-supported clade of *S. gramineum* + *S. fluctuans* (93% ML BS, 97% MP BS, 1.00 PP) together with *S. angustifolium, S. japonicum, S. fallax*, *S. emersum*, and *S. glomeratum*, formed a fairly well-supported clade (88% ML BS, 86% MP BS, 1.00 PP) not seen in the cpDNA tree.

***Identification of putative hybrids*—**Two *Sparganium* accessions were identified as potential hybrids, based on these molecular as well as morphological evidence. Appendix S1 (see Supplemental Data with the online version of this article) gives the ML tree based on the combined cpDNA and nDNA of all 36 taxa. Putative *Sparganium angustifolium* × *S. emersum* was sister to *S. angustifolium* in the cpDNA, nDNA, and combined data trees. The cpDNA sequence for the possible hybrid closely matched *S. angustifolium,* while nDNA character states matched those of *S. angustifolium* or *S. emersum* at different sites. The putative hybrid was morphologically intermediate between the suggested parent species for habit, inflorescence, and leaf shape and was collected in Wyoming, USA, within the range of both species, but well outside of the range of other potential parent species, e.g., *S. glomeratum*. The second potential hybrid, *Sparganium japonicum* × *S. fallax*, was sister to *S. japonicum* in the cpDNA, nDNA, and combined data trees. The nDNA sequences showed polymorphisms at nine base positions in ITS and one in *phyC*, where one variant matched *S. japonicum* and the other *S. fallax.* The cpDNA sequences were identical to those of *S. japonicum.*

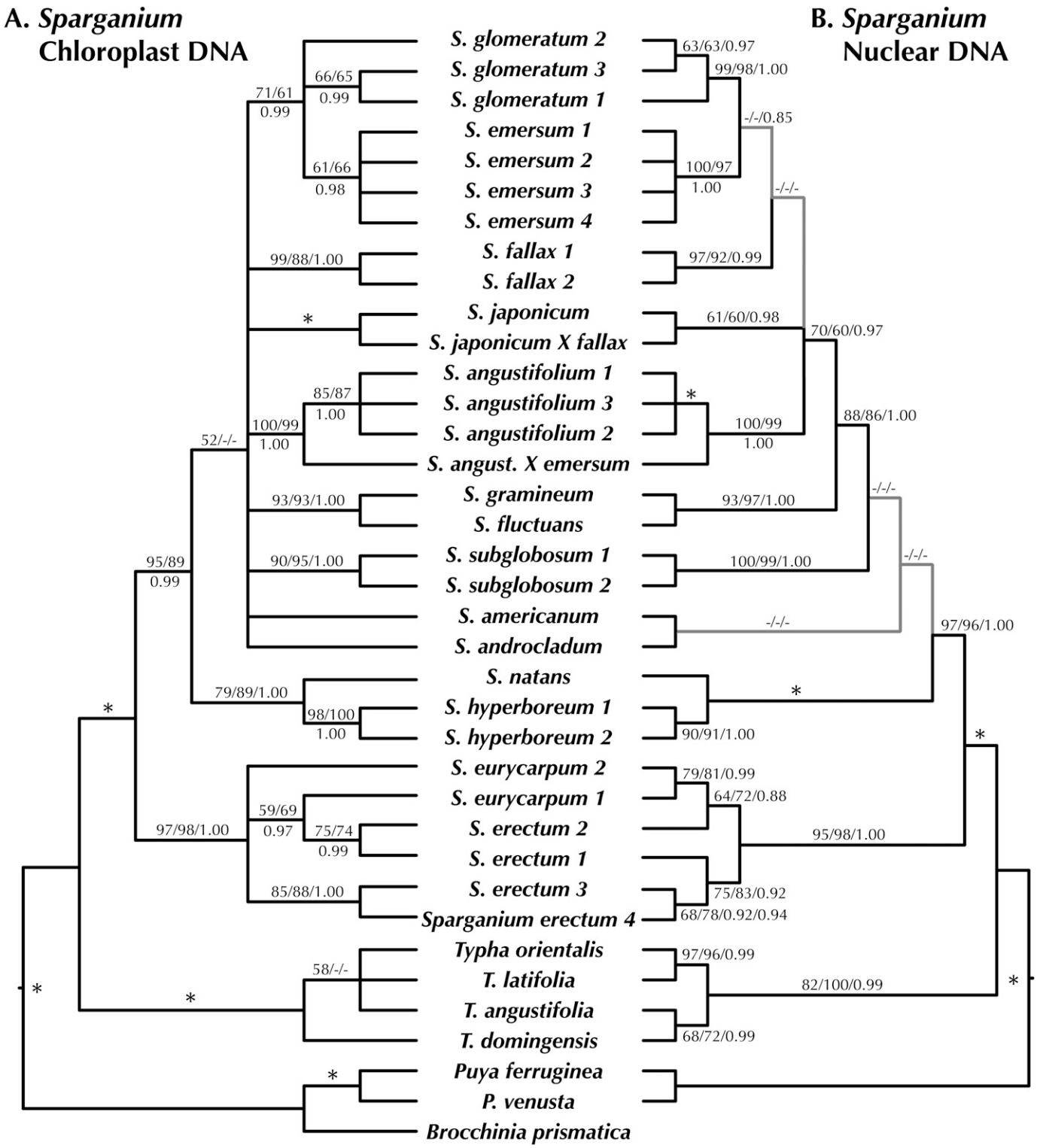

Fig. 2. *Sparganium* ML phylogenetic trees for cpDNA vs. nDNA based on the 37-taxa data set. Trees are shown as ML cladograms for ease in viewing short branches. Support values are shown as follows: ML/MP/PP (for BS > 50%; PP > 0.70). Dashes indicate BS < 50% or PP < 0.70. An asterisk indicates that the node received 100/100/1.00 support. Branches with less than 50% ML BS are shown in gray.

The voucher specimen had poorly developed fruits, suggesting this may be a largely sterile F1 hybrid between maternal *S. japonicum* and paternal *S. fallax*. This hybrid combination has not been previously described. Despite the lack of discordance in the placements of these two putative hybrids among the cpDNA, nDNA, and combined DNA trees, both were removed (along with accessions with missing data) for combined data set congruency tests and BEAST analyses of 28 accessions.

Combined data set analysis—The ILD test of congruence between the cpDNA and nDNA partitions from the reduced 28-taxa data set (including only *Sparganium*) yielded a *p*-value of 0.3, indicating there was not significant incongruence between the two data sets. In the 28-taxon alignment, the cpDNA data matrix was 2011 characters (*psbJ-petA*: 989; *trnL-trnF*: 1022), and the nDNA data matrix included 1678 characters (ITS: 801; phyC: 877) for a total of 3689 characters. The analysis of the combined cpDNA and nDNA 28-taxa data set provides a better-resolved and more strongly supported ML tree than either cpDNA or nDNA does alone (Appendix S2; see Supplemental Data with the online version of this article). This ML tree is identical in topology to the BI BEAST tree (Fig. 3; see below). The combined data set analysis provides strong support for monophyly of *Sparganium* (support values all 100%). Within *Sparganium*, the same two clades as in the cpDNA and nDNA trees are resolved: the first clade, consisting of *S. erectum* +*S. eurycarpum* (support values all 100%), and the second clade, with all remaining species (support values all ≥ 99%). Within the first clade, *S. eurycarpum* including *S. eurycarpum* var. *greenei* is monophyletic (71% ML BS, 78% MP BS, 1.00 PP). The monophyly of *S. erectum* (including var. *macrocarpum*) is not supported by ML or MP and only weakly by BI (0.72 PP). Within the second clade, almost all nodes are now resolved and with increased support values (especially PP). *Sparganium natans* + *S. hyperboreum* form a clade (support values all 100%) that is sister to a clade of 10 remaining species (72% ML BS, 0.95 PP). A clade of *S. fluctuans* + *S. gramineum* (support values all ≥ 99%) is sister to a clade of *S. angustifolium*, *S. japonicum*, *S. fallax*, *S. emersum*, and *S. glomeratum* (69% ML BS, 66% MP BS, 1.00 PP).

Divergence time analyses with BEAST—The BEAST chronogram of the combined 28-taxa data set is shown in Fig. 3. The root age (divergence of Typhaceae and Bromeliaceae; secondarily calibrated at 90–105 Myr) is 100 Myr

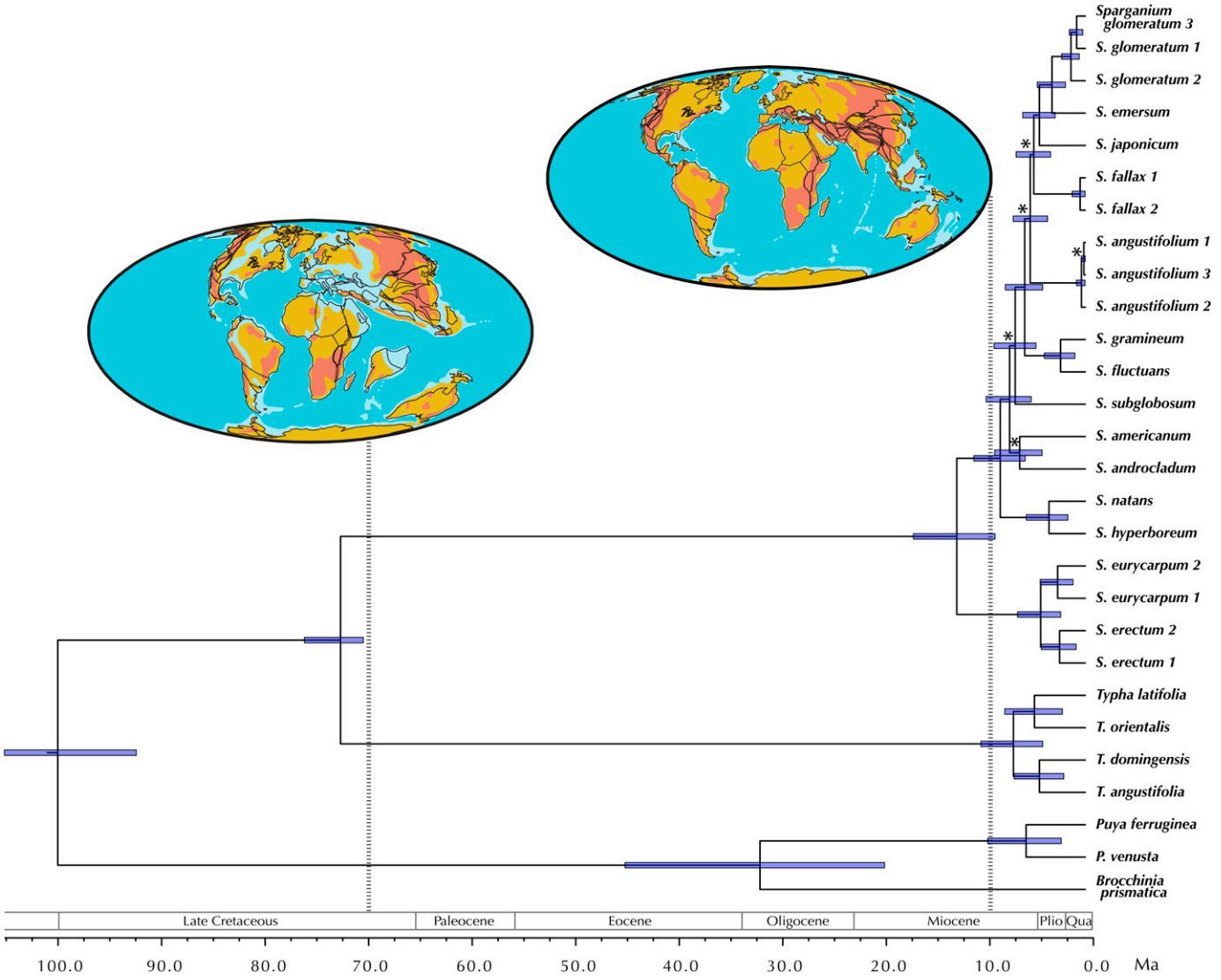

Fig. 3. *Sparganium* BEAST chronogram for the 28-taxa, combined four-gene data set. Only nodes with asterisks have less than 95% PP. Fossil constraint values and secondary node calibration values are given for the *Sparganium* stem and Poales root, respectively. Blue bars represent 95% confidence intervals for the estimated mean dates. Past positions of continents are portrayed (70 Ma: late Cretaceous; 10 Ma: late Miocene; adapted from Scotese, 2001) for near the stem and crown nodes, respectively, of *Sparganium*.

(95% CI = 92–105). The stem age of *Sparganium* (fossil-calibrated at 70 Myr) is estimated to be late Cretaceous (72 Myr; 95% CI = 70–76). The BEAST chronogram (not shown) using no fossil calibration and only dating the root provides a stem age of *Sparganium* at 40 Myr; this date is clearly unrealistic as it is at least 20 Myr younger than the oldest known Paleocene *Sparganium* fossils (Hickey, 1977). The fossil-calibrated BEAST tree (Fig. 3) indicates that the crown diversification of *Sparganium* into two clades (now recognized as subgenera, see Discussion) occurs by the end of the mid-Miocene (13 Myr; 95% CI = 8.9–17), prior to the radiation of our more limited sample of *Typha* (7.1 Myr; 95% CI = 4.1–10). Our results suggest that the main diversification of *Sparganium* (all species lineages) occurred rather recently, during the late Miocene and Pliocene. The earliest speciation events leading to single extant species occur in the late Miocene: *S. subglobosum* (6.9 Myr), *S. americanum* (6.4 Myr), and S. *androcladum* (6.4 Myr). The latest speciation event gave rise to the New World/Old World pair of *S. fluctuans*/*S. gramineum* at 2.4 Ma at the Pliocene–Pleistocene border.

***Ancestral area reconstruction*—**Results from the DEC analysis in Lagrange and RASP are largely congruent for ancestral area reconstruction (AAR) except for several of the most basal nodes (Fig. 4). DEC analyses invoking either a limited *Typha* ancestral range (Eurasia) or a widespread range (all five areas) were very similar and only the results based on a widespread *Typha* and maxarea = 4 are depicted and discussed. Nodes with significant AAR (>2 log likelihood units; Edwards, 1992; Ree and Smith, 2008) are asterisked and are generally confined to more recent events. DEC invoked 18 dispersal events and only two vicariance events, at the crown radiations of *Sparganium* and of subg. *Sparganium*. In contrast to the DEC analyses, considerably more variation in S-DIVA results was evident between the two *Typha* scorings and between using maxarea = 4 vs. 2, especially in basal nodes and in the node giving rise to the North American–Eurasian pair of floating-leaved species, *S. fluctuans* and *S. gramineum*. For ease in direct comparison to DEC results, the AAR results are shown for S-DIVA based on a widespread *Typha* and maxarea = 4 (Fig. 4). AAR results utilizing a Eurasian *Typha* ancestral distribution are indicated only at nodes with significant differences relative to those based on a widespread *Typha* scoring. S-DIVA invoked three vicariance and 37

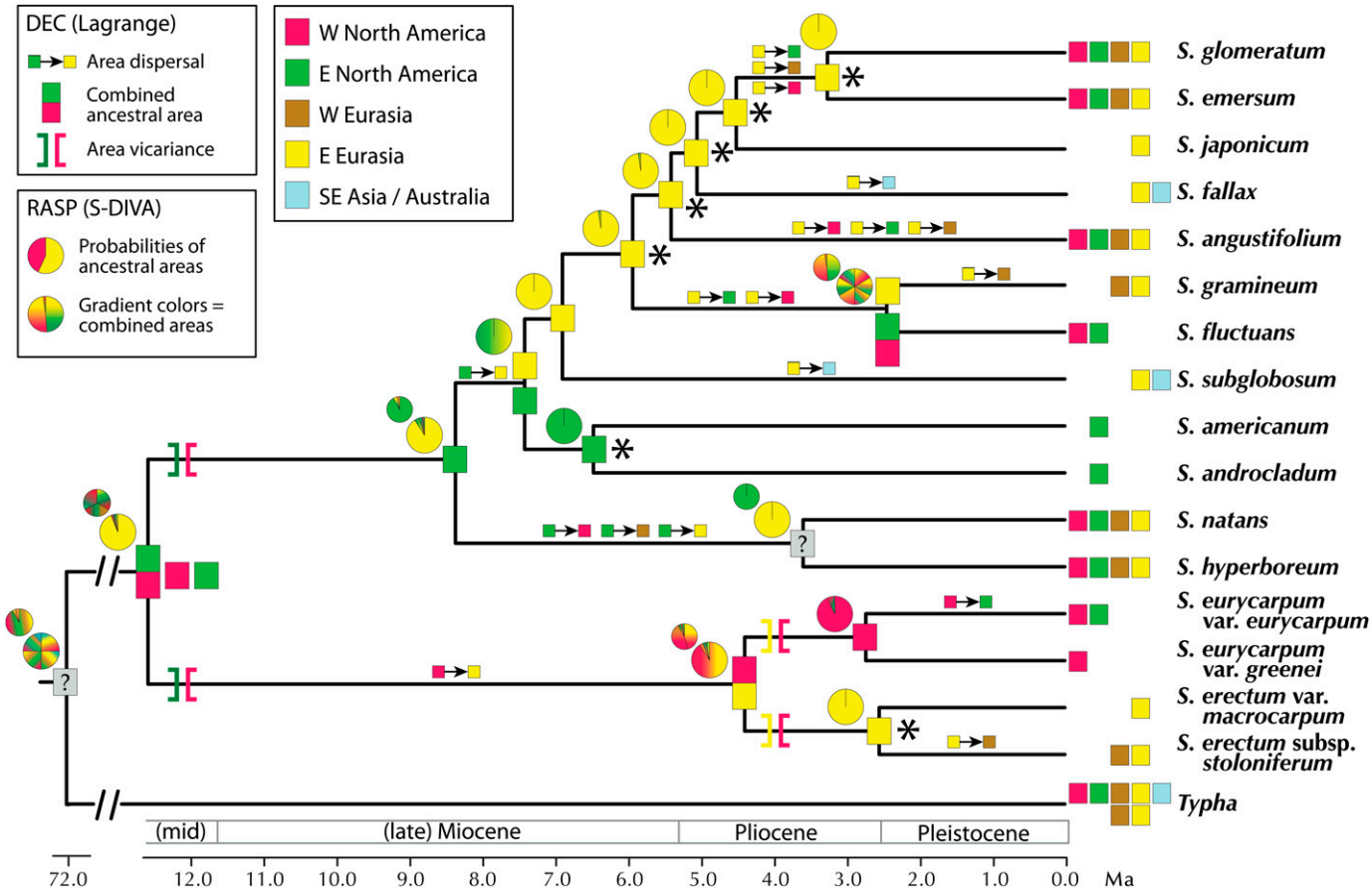

Fig. 4. Dispersal–extinction–cladogenesis (DEC) and S-DIVA models of ancestral area reconstruction (AAR) in *Sparganium* based on a reduced BEAST combined-gene chronogram. Shown is a simplified 17 taxa chronogram from the 21 taxa analyzed; multiple accessions of the widespread *S. glomeratum* and *S. angustifolium* were removed for clarity. DEC ancestral area reconstructions with highest likelihood are shown as colored boxes at each node. Single area boxes indicate an ancestor confined to a single geographic area; combined boxes indicate an ancestor with a distribution encompassing two or more areas; two boxes separated by a space indicate the ancestral ranges inherited by each of the daughter lineages arising from the node. Asterisks indicate nodes where AAR is significant (i.e., next probable reconstruction is 2 likelihood units worst). Three likely AAR are shown for the crown radiation of *Sparganium*. Boxes with "?" mean that many different scenarios are equally probable. Inferred dispersal or vicariance events are indicated. S-DIVA ancestral area reconstructions are shown by large pies at each node assuming *Typha* is widespread across all five areas and MaxArea = 4. Smaller pies at six nodes indicate significantly different AAR when *Typha* is ancestrally restricted to Eurasia. Inferred combined areas (2–5 areas) are indicated by gradient colors.

dispersal events. Generally, the S-DIVA results involving a Eurasian *Typha* ancestral distribution, and not those based on a widespread *Typha* distribution, are more similar to DEC results based on a widespread *Typha* distribution.

The AAR of the stem node of *Sparganium* in the late Cretaceous is ambiguous or comprises a combined area of North America and Eurasia under both DEC and S-DIVA (Fig. 4). The AAR of the crown node of *Sparganium* at the end of the mid Miocene is a combined area of western and eastern North America (or either one alone as alternative likely scenarios) under DEC, but strongly eastern Eurasia under S-DIVA (although North America dominates under S-DIVA with a restricted *Typha* distribution). The first vicariance event invoked by DEC involves western and eastern North America at the crown radiation of *Sparganium*. This separation was followed by a dispersal event from western North America to eastern Eurasia in the *S. eurycarpum/erectum* clade (DEC). The second vicariant event occurs in this clade through the break-up of a combined western North American and eastern Eurasian area, presumably by sundering of the Beringian connection. In the larger clade sister to the *S. eurycarpum/erectum* clade, a dispersal event from eastern North America to eastern Eurasia in the late Miocene sets up a dominant eastern Eurasian biogeographical presence for most of the nodes in this clade.

***Estimating transitions in habit and ovary evolution*—**BayesTraits analysis of stigma/locule number indicates an ancestral state of 1 in both *Sparganium* and *Typha* (Fig. 5). A single transition to two locules occurred in the *Sparganium eurycarpum/erectum* clade. No further transitions occurred in the second clade, which is entirely unistigmatic. Thus, two-loculed ovaries are shared by the *S. eurycarpum/erectum* clade apart from all other species of *Sparganium* and *Typha*. However, the polymorphic retention of the plesiomorphic unilocular condition as well as the derived state in *S. erectum* and *S. eurycarpum* var. *greenei* complicates the interpretation of the evolutionary transition of ovary locule number.

The evolution from the plesiomorphic emergent aquatic habit to the derived floating aquatic habit is more complex (Fig. 5). Character state analysis of the aquatic growth form shows that the ancestral habit or the stem and crown nodes of *Sparganium* was likely emergent (100% and 80%, respectively). The pattern of habit evolution is dependent on the method and assumption of state reconstruction and is complicated by the polymorphic nature of *S. emersum* and *S. glomeratum* (Fig. 5). The ML implementation in BayesMultiState suggests three or four separate origins of floating-leaved aquatics in *Sparganium* involving: (1) the widespread clade of *S. natans* and *S. hyperboreum*, sometime during the late Miocene to Pliocene; (2) the clade comprising the

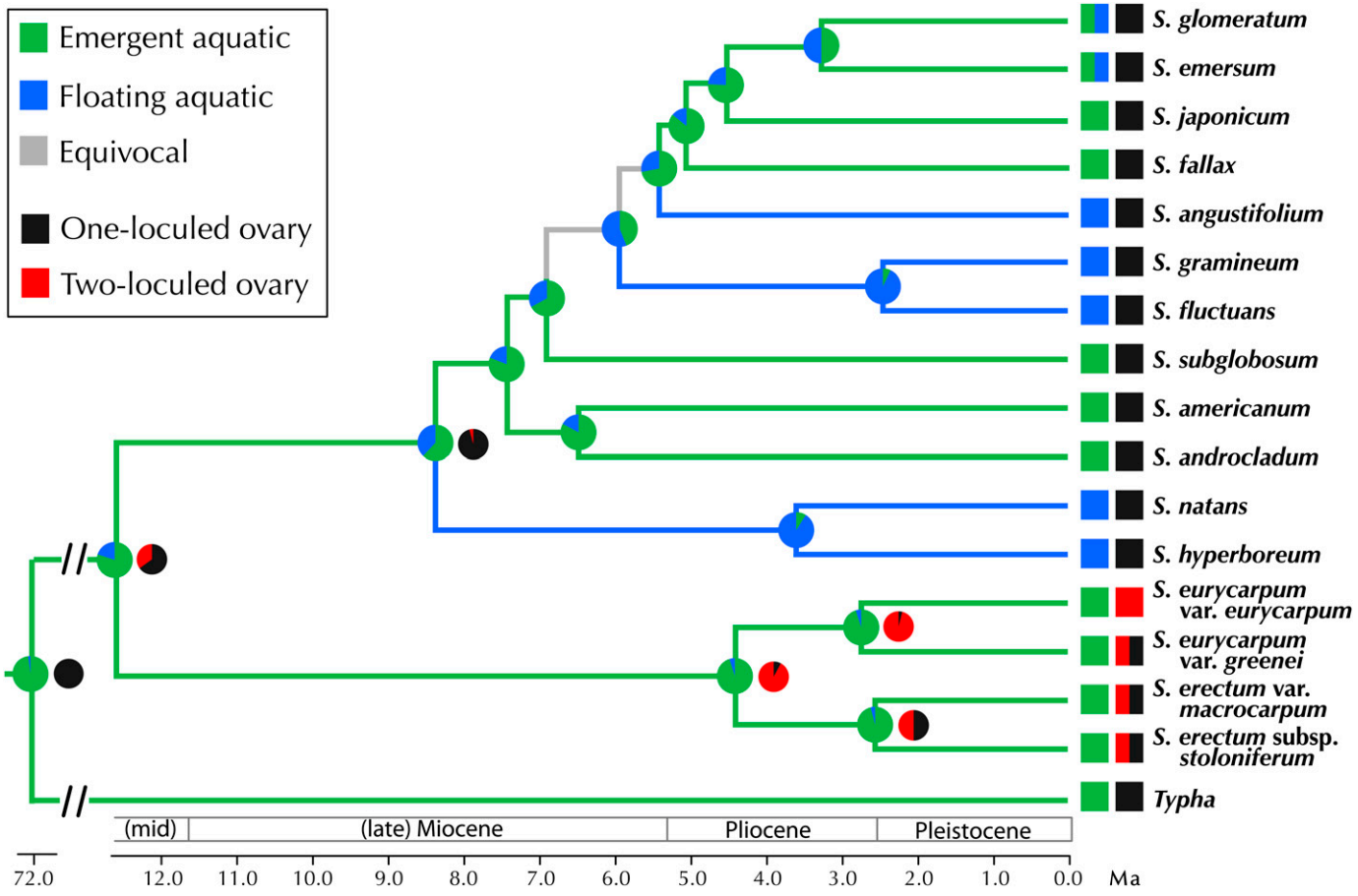

Fig. 5. Habit transitions and ovary evolution within *Sparganium* based on a reduced BEAST combined-gene chronogram. Pies represent probabilities of emergent and floating-leaved habit at each node as inferred under Bayesian framework in BayesMultiStates using 100 random posterior probability (PP) trees. Branch colors indicate conservative MP reconstruction of habit in MacClade. Smaller pies represent probabilities in BayesMultiStates of one vs. two-loculed ovaries. Only values for basal nodes are depicted, as all other nodes are 100% unilocular.

New World and Old World, respectively, *S. fluctuans* and *S. gramineum*, sometime during the Pliocene; (3) the widespread *S. angustifolium*, sometime during the Pliocene to Pleistocene; and (4) the polymorphic *S. emersum* and *S. glomeratum* also during the Pliocene to Pleistocene. The MP reconstruction is likewise equivocal (see branch coloring in Fig. 5), indicating either three or four separate origins as with ML. Assuming the gain of the floating-leaved habit is rare, one transition to the derived state could account for its presence in *S. fluctuans*, *S. gramineum*, and *S. angustifolium*, followed by a reversal to the emergent aquatic condition in the common ancestor of *S. fallax*, *S. japonicum*, *S. glomeratum*, and *S. emersum*.

DISCUSSION

The results of the phylogenetic analyses presented here constitute the most comprehensive molecular analysis of worldwide *Sparganium* diversity to date and provide a well-resolved and supported combined cpDNA and nDNA tree. We employ a fossil-calibrated chronogram, character trait analysis, and ancestral area reconstruction to gain insight into how and when diversification occurred. The results support the monophyly of *Sparganium,* and separate the genus into two clades that do not correspond to previously accepted subgenera. The chronogram dates the divergence of the two clades by the end of the mid-Miocene, and the divergence of extant floating lineages beginning during the late Miocene and Pliocene, a period of global cooling. Within this new phylogenetic framework, we discuss the implications of climate change, growth form, and biogeography on the evolution of a widely distributed group of aquatic monocots.

Occurrence of hybridization in Sparganium—Due to the presumed, but little verified, presence of hybrids within *Sparganium* (Cook and Nicholls, 1986, 1987; Les and Philbrick, 1993; Kaul, 1997), our sampling was directed toward accessions that appeared to have morphologies representative of the species, while also including suspected hybrids to assess the validity of hybridization with molecular data. Despite the lack of overall incongruence between the cpDNA and nDNA ML trees (Fig. 2), the use of sequence data from both the plastid and nuclear genomes allowed for identification of potential hybridization events. We identified polymorphic nrITS sequences that are often indicative of hybridization and used the cpDNA relationships to identify the maternal lineage (Les et al., 2008; Tippery and Les, 2011). Our findings highlight the need for assessing both genomes when hybridization is suspected (e.g., Smith and Sytsma, 1990; Spooner et al., 1991; Rieseberg, 1991, 1997; Wendel et al., 1991; Wendel and Doyle, 1998; Drew and Sytsma, 2013), and perhaps especially with aquatic plants (Les and Philbrick, 1993; Kuehn et al., 1999; Les et al., 2010; Moody and Les, 2010; Kim and Choi, 2011; Kirk et al., 2011). Hybrids may be particularly difficult to detect in *Sparganium* due to phenotypic plasticity and subtle morphological differences among species, and between hybrids and parental species. The nrITS data were effective in distinguishing among species and in identifying a potential hybridization event based on polymorphic characters and should be a useful region for DNA barcoding applications in *Sparganium*. In *S. japonicum* × *S. fallax,* we inferred that *S. japonicum* was the maternal parent based on their identical cpDNA sequences. The collection shows poorly developed fruits, suggesting that this hybrid may be partly sterile. *Sparganium angustifolium* × *S. emersum* is reportedly widespread in western North America (Cook and Nicholls, 1986; Kaul, 1997). The phylogenetic data are inconclusive as to whether the morphologically intermediate plant sampled represents a *Sparganium angustifolium* × *S. emersum* hybrid. Its placement in the tree does not reflect discordance between the chloroplast and nuclear genome, perhaps due to introgression and backcrossing with *S. angustifolium*.

Phylogenetic relationships and subgeneric realignment—
Sparganium is divided into two strongly supported clades. The first consists of two species, *S. erectum* and *S. eurycarpum*, the only species that produce distigmatic flowers and bilocular fruits. Endocarps that bear longitudinal ridges form an additional synapomorphy for the pair (Cook, 1961). Because these ridges are also shared by some extinct *Sparganium* with two or more locules (Cook and Nicholls, 1986), this feature may represent a plesiomorphic state for *Sparganium* retained only in *S. erectum* and *S. eurycarpum* (see below for further discussion on stem lineage features). Typically these two species are tall (to 2 m), with strongly keeled leaves, and branching stems bearing the pistillate and staminate heads. Although both cpDNA and nDNA suggest, usually weakly, paraphyly of one or the other species, the combined data provides limited support for the monophyly of both. The combined data analyses using different numbers of accessions (Fig. 3; online Appendices S1, S2) provide support for the placement of *S. eurycarpum* var. *greenei* within *S. eurycarpum* rather than in *S. erectum.*

The second major clade comprises all the remaining members of *Sparganium* included in our sample. They are a morphologically diverse group of 12 species, which includes all the floating-leaved species. The synapomorphies for this clade are a stigma number of one, unilocular fruits, and endocarps without longitudinal ridges. The monophyly of each of these species, where examined, is supported at least with the combined data set. Although a well-resolved set of relationships based on the combined ML tree is evident (Fig. 3; Appendices S1, S2), bootstrap support is weak along portions of the backbone of this apparently rapidly and recently diverging clade. The five entirely floating-leaved species (two others are polymorphic) represent three different clades (Fig. 5), and not an ancestral lineage as previously proposed (Cook and Nicholls, 1986).

The molecular phylogenetic results (Figs. 2, 3; Appendices S1, S2) are not compatible with the previous infrageneric classification and composition of two subgenera in *Sparganium* (Holmberg, 1922; Cook, 1961; Cook and Nicholls, 1986, 1987). The two subgenera were based on morphological features of tepal color/texture and plant stature. In light of the two well-supported clades (both > 95% BS, PP) at the crown radiation of *Sparganium*, we revise the subgenera to create natural phylogenetic groups. The new description and species composition of the two subgenera are as follows:

Sparganium L. subgenus ***Sparganium***. Stigmas and ovary locules 2 (at least on some flowers on some plants); endocarps with longitudinal ridges; plants emergent.

Included species: S. *erectum*, *S. eurycarpum.*

Excluded species previously placed in subgenus: *S. americanum*, *S. androcladum*, *S. fallax*, *S. japonicum*, *S. subglobosum.*

Sparganium L. subgenus ***Xanthosparganium*** Holmberg. Stigma and ovary locule 1; endocarps without longitudinal ridges; plants floating or emergent.

Included species: *S. americanum*, *S. androcladum*, *S. angustifolium*, *S. emersum*, *S. fallax*, *S. fluctuans*, *S. glomeratum*, *S. gramineum*, *S. hyperboreum*, *S. japonicum*, *S. natans*, *S. subglobosum.*

The two proposed subgenera of extant *Sparganium* are easily separated based on the combined features of stigma/ovary locule number, presence or absence of longitudinal ridges on endocarps, and habit. BayesTraits analyses presented here (Fig. 5) indicate that the two-loculed/stigma condition in subgenus *Sparganium* is derived from the apparent plesiomorphic uniloculed/stigma condition seen in *Typha* and all other *Sparganium*. Likewise, the endocarps with longitudinal ridges possessed only by subgenus *Sparganium* (and not *Typha* and all other *Sparganium*) would suggest that such endocarp sculpting is derived as well.

However, understanding the evolutionary transitions of these two key characters is complicated by features seen in the middle to early Tertiary *Sparganium* fossils. In their examination of Oligocene *Sparganium* fossils, Cook and Nicholls (1986) described extinct species that had longitudinal ridges or even wings on the endocarp and multiple locules, ranging from 2 to 7. Based on their ages (well before the crown radiation of *Sparganium* in the late-Miocene, Fig. 5), these fossils must represent extinct members of the *Sparganium* stem lineage and its offshoots. Both the longitudinal ridges and locule number would suggest an affinity of these fossils to extant subg. *Sparganium*. Inclusion of fossil evidence might thus argue that these two character states seen in subg. *Sparganium* are actually plesiomorphic in *Sparganium*. This apparent reduction in ovary locule/stigma number through the latter half of the Tertiary calls into question the results of the BayesTraits analysis (based on extant taxa only) showing an evolutionary transition from an ancestral unilocular ovary to a derived bilocular condition in subg. *Sparganium* (Fig. 5). These results were predicated on scoring *Typha* as unilocular. The single carpel evident in extant *Typha*, often interpreted to be pseudomonomerous (Müller-Doblies, 1970; Cronquist, 1981; Thieret, 1982), might well have been derived via a similar reduction process. If so, multilocular ovaries would be plesiomorphic for Typhaceae with subsequent reduction to unilocular ovaries occurring in parallel in *Sparganium* and *Typha*. The reduction in carpel number perhaps occurred as an adaptation for anemophily in both *Sparganium* and *Typha* (Friedman and Barrett, 2008, 2009), with other modifications in inflorescence structure and reduction in carpel size occurring as adaptations to anemochory in *Typha* (Müller-Doblies, 1970). The bilocular ovaries in *S. eurycarpum* and *S. erectum* may thus be a case of phylogenetic conservatism, and not of adaptive significance (Givnish et al., 2010).

Evolution of growth form—The two or three separate origins of floating habit from emergent ancestors, plus the partial transition seen in polymorphic *S. emersum* and *S. glomeratum* (Fig. 5), represent a rare example of multiple habit shifts within a single genus of aquatic angiosperms. Several phylogenetic studies have assessed shifts in aquatic habit within families, e.g., Pontederiaceae (Barrett and Graham, 1997), Haloragaceae (Moody and Les, 2007), and Hydrocharitaceae (Chen et al., 2012), or orders, e.g., Alismatales (Alismatidae) (Chen et al., 2004). The only other phylogenetically based examination of aquatic habit transition within a genus, to our knowledge, examined shifts from rosulate (rosette) to vittate (caulescent) growth form in the submerged aquatic *Vallisneria* (Les et al., 2008). Our results support the view of Kaul (1972) that the floating habit was derived and contradict the view of Cook and Nicholls (1986) that the floating habit was ancestral. The multiple transitions to the aquatic habit from emergent ancestors in *Sparganium* parallels the broader pattern of repeated evolution of aquatic plants across the angiosperms, as well as the patterns in Pontederiaceae, Haloragaceae, and Alismatales.

Divergence between the floating and emergent habits in *Sparganium* may be related to specialization of the floating habit to specific climatic and habitat conditions. *Sparganium* species with the floating habit are widely distributed in cold climates, while in temperate climates, they appear to be limited to nutrient-poor habitats or deep water (Sulman, 2010). Theoretically, plants with the floating habit—with lower allocation to unproductive support tissue at a given depth than emergent plants—should be better able to persist in unproductive habitats (i.e., in sites with short growing seasons and/or infertile soils) and invade deeper water than plants with the emergent habit. Conversely, the emergent habit should have a competitive advantage in overtopping floating species in shallow water and in sites where nutrient availability and growing season length are greater, and permit positive yearly carbon balance in emergent plants (Givnish, 1986, 1987, 1990, 1995; T. J. Givnish, University of Wisconsin, personal communication). These predictions largely accord with the observed distribution of floating vs. emergent *Sparganium* species in temperate North America (Nichols, 1999; Sulman, 2010), and at broader scales across the Northern Hemisphere.

In contrast to the *Sparganium* species with a floating-leaved habit that show repeated and rapid morphological evolution, the emergent habit species often exhibit pronounced morphological stasis. Morphological stasis, as one line of argument, has been used to explain the recurring pattern seen in East Asian–eastern North American Tertiary disjuncts in which morphologically similar, suspected vicarid pairs are shown to (1) have relatively old divergences and/or (2) not be even closely related (Wen, 2001; Milne and Abbott, 2002). Cook and Nicholls (1987, p. 10) suggested a close relationship between East Asian *S. japonicum* and eastern North American *S. americanum,* based on morphology, stating that *S. japonicum* "could well be recognized at an infraspecific rank [of *S. americanum*]." Our results, however, indicate that this putatively vicarid pair of emergent species is not closely related, and their most recent common ancestor dates to 7.4 Ma in the Miocene (Fig. 5). *Sparganium americanum* and another North American emergent species, *S. androcladum*, are also morphologically similar and considered closely related (Cook and Nicholls, 1987). CpDNA does not resolve them as sister and nDNA only weakly (Fig. 2); only with the combined data set is there stronger support (although <75% PP) for these two species to be sister to each other (Fig. 3; Appendices S1, S2). The divergence time between these sympatric species is 6.2 Ma in the late Miocene (Fig. 5), the oldest (and most weakly supported) of any sister species pair in *Sparganium*. Whether phylogenetic constraint or stabilizing selection (see Wen, 1999, 2001; Milne and Abbott, 2002) is operating in causing morphological stasis among these three emergent *Sparganium* species is open to question.

Biogeographical patterns of differentiation—*Sparganium*, along with *Typha*, comprise the second-earliest diverging lineage after Bromeliaceae in the order Poales (Givnish et al., 2010), an order with a strong South American (Guayana Highlands) AAR dating to around 100–110 Ma in the mid-Cretaceous (Givnish et al., 2011). However, the long 28 Myr stem lineage of Typhaceae and the even longer 60 Myr stem lineage for *Sparganium* (Fig. 3) all but obscures any biogeographical signal until the Miocene (Fig. 4). *Sparganium* was already widespread in

the Paleocene and Eocene fossil record in both North America and Eurasia, growing in what was then a subtropical climate (Berry, 1924; Hickey, 1977; Tiffney and Manchester, 2001). However, using either the widespread area scoring for *Typha* or the more likely Eurasian ancestral area scoring (Kim and Choi, 2011), no consistent geographical AAR is evident for the 13 Ma crown node of *Sparganium* under DEC and S-DIVA models (Fig. 4). The former suggests a combined area of western and eastern North America (or either alone) as a likely AAR, but S-DIVA (at least under one scoring option for *Typha*) suggests an Asian AAR. A number of factors may contribute to this lack of clarity in AAR at the crown of *Sparganium*: (1) the combination of long stem and crown lineages leading to and from the crown of *Sparganium*, (2) extensive overland connections (see below) across Beringia (intermittently at least) and across the North Atlantic Land Bridge (up to the late Eocene) during the early to mid-Tertiary (Tiffney, 1985a), and (3) the widespread distributions today of five of the extant species.

A striking feature of the temperate flora of the Northern Hemisphere are the disjunct congeners distributed in two or more of the four now isolated areas: eastern Asia, eastern North America, western North America, and Europe (reviewed by Li, 1952; Graham, 1972; Wood, 1972; Tiffney, 1985b; Manchester, 1999; Wen, 1999; Milne and Abbott, 2002; Donoghue and Smith, 2004; Milne, 2006; Harris et al., 2013). Other than long distance dispersal (LDD; discussed later), two land bridges have been invoked for explaining these patterns: the North Atlantic Land Bridge (NALB) via Greenland and the Beringian Land Bridge (BLB). The lifespan of the NALB as an effective route for both plants and animals has been actively debated, especially in regard to both the timing and nature of the northern and southern routes and the possibility of a "stepping stones" route (see reviews by Milne and Abbott, 2002; Milne, 2006). In some form, the NALB probably was effective as a bridge for biota until the Eocene or possibly early Miocene for plants (Manchester, 1999; Tiffney, 2000; Tiffney and Manchester, 2001; Milne and Abbott, 2002; Milne, 2006; Harris et al., 2013). Thus, although the NALB presumably was important in the earlier spread of stem lineage *Sparganium* (and *Typha*), it would have factored little in the geographical spread of crown *Sparganium* in the late Miocene and Pliocene. Thus, our results provide limited support for vicariance in *Sparganium*, possibly in only two instances.

The chronogram (Fig. 4) indicates that radiation of *Sparganium* into its two clades occurs during the late Miocene and Pliocene. DEC analysis indicates a vicariant event during this subgeneric differentiation, in which the combined area of North America separates to form ancestral areas for the two main clades: eastern North America for subg. *Xanthosparganium* and western North America for subg. *Sparganium*. The late Miocene and Pliocene are epochs in which aridification, cooling, and seasonality intensified across the midcontinental regions of North America (and Eurasia), accompanied by the spread of C_4 grasslands (Tiffney, 1985a, b; Edwards et al., 2010). Climate change and the deterioration of *Sparganium* habitat in the Great Plains could be responsible for this vicariant event. However, DEC does not show significant support for AAR at this node, nor does S-DIVA provide a consistent AAR either. Thus, alternative reconstructions and biogeographical interpretations may be feasible at the crown radiation of *Sparganium*.

The second vicariant event suggested by DEC analysis involves Beringia and occurs at the crown radiation of subg. *Sparganium* at 4.4 Ma in the Pliocene (Fig. 4). A combined ancestral area of western North America + eastern Eurasia was sundered by the closing of the BLB, giving rise to the North American *S. eurycarpum* and the Eurasian *S. erectum*. External support for contemporaneous BLB-based vicariance comes from geological evidence for the BLB and molecular dates for divergences of other disjunct plants. The timing of the BLB in terms of opening and closing (often in an intermittent fashion) during the Tertiary has been extensively reviewed (e.g., Tiffney, 1985a; Manchester, 1999; Marincovitch and Gladenov, 1999; Tiffney and Manchester, 2001; Milne and Abbott, 2002; Vila et al., 2011). It is now known with some precision that the final closure of the BLB occurred at 5.5–5.4 Ma (Gladenkov et al., 2002) in the Pliocene, matching closely to our time estimate (4.4 Ma) of the eastern Asian–western North American diversification of *Sparganium* subg. *Sparganium*. As in *Sparganium*, the severing of the BLB in the Pliocene has been implicated as a likely explanation for a cluster of divergence times of around 5 Ma for Tertiary relict disjuncts (reviewed by Milne and Abbott, 2002).

Long distance dispersal and/or overland migration are implicated for the remainder of *Sparganium* (subg. *Xanthosparganium*) based on AAR in both DEC and S-DIVA analyses (Fig. 4): five of the 12 species are widespread across the Northern Hemisphere, showing a propensity in the boreal species to extend their ranges with repeated migrations during the Pliocene and Pleistocene. There is ambiguity in the AAR for the crown radiation of this subgenus (eastern Eurasia vs. eastern North America) between DEC and S-DIVA. However, with both models, there is strong support for an AAR of eastern Eurasia for most of the nodes in subg. *Xanthosparganium*; two notable patterns emerge in this group. First, the majority of dispersal events (see Fig. 4) involve the floating-leaved species. Floating-leaved species, perhaps due to their occurrence in higher-latitude regions with more circumboreal extent (see above), may more readily disperse than emergent species. Second, most of the North American species (possibly with the exception of *S. americanum* and *S. androcladum*) have been derived from eastern Asian ancestors under both models. The majority of the taxa found in North America arrived during the Pliocene by dispersal/migration from eastern Asia. Such dispersal events could have occurred even after direct land connections across Beringia (or NALB) were severed, providing that plants were tolerant of the climate near the BLB and NALB, and had capabilities for dispersal (Milne and Abbott, 2002; Les et al., 2003). During the Quaternary glacial epochs, as sea levels fell and the BLB reappeared (Elias et al., 1996), it could harbor species of arctic or boreal affinity (Hultén, 1937; Murray, 1981; Abbott et al., 2000; Milne and Abbott, 2002).

The importance of LDD in our AAR is supported by evidence of the effective LDD capabilities of *Sparganium*. Seeds of *Sparganium* are eaten by and readily transported long distances via migrating birds (Arber, 1920; Fassett, 1940; Cook and Nicholls, 1986; Pollux et al., 2005, 2006, 2007). LDD may be one of the key adaptations that have allowed *Sparganium* to succeed in the high latitudes during the widely fluctuating climates of the Pliocene and Pleistocene. Dispersal south along mountain belts during periods of glaciation has been an important mechanism of survival for Arctic species (Abbott and Brochmann, 2003), and several *Sparganium* species today show a similar pattern. Rapid colonization of new habitat after retreat of ice sheets in the Pleistocene would have been aided by LDD of seeds by birds.

Pleistocene climate-driven speciation, related to the sundering of contiguous regions of suitable habitat by glacial advance, likely occurred in one clade of floating-leaved *Sparganium*. The divergence between the Eurasian *S. gramineum* and the North American *S. fluctuans* (at 2.4 Ma, Fig. 4) follows a late-Pliocene peak of Northern Hemisphere glaciation at 2.74 Ma (Bartoli et al., 2005) that would have partitioned an ancestral, circumboreal distribution. Today, their ranges are in cool-temperate, forested regions, separated by 4000 km of ocean.

The Miocene to Pliocene diversification in *Sparganium* exhibits the response of an aquatic plant lineage to the dynamic conditions of the Tertiary. Our findings point to a late Miocene–Pliocene diversification, multiple origins for the floating habit from emergent ancestors within the genus, and support the occurrence of hybridization between species. We implicate long distance dispersal for explaining wide species ranges and morphological stasis for explaining similarities between emergent species that had formerly been considered close relatives. Dispersal ability and divergence in growth form could have provided ways for *Sparganium* species to respond to cooling climates during the late Tertiary and to exploit niches in aquatic habitats. Future molecular work should include extensive sampling within species and across species ranges to uncover evidence of additional hybridization and to better understand the timing and nature of dispersal/migration events. Additional work is needed to clarify the relationships within subgenus *Sparganium*, including among the subspecies of *S. erectum,* and to determine the relationships of disjunct *Sparganium* from Mexico and Colombia within subgenus *Xanthosparganium*. Thorough sampling of *Sparganium* across Eurasia, and their inclusion in future, *Sparganium*-wide phylogenetic work will add to a more detailed understanding of the evolution and diversification of this dynamic aquatic lineage.

LITERATURE CITED

ABBOTT, R. J., AND C. BROCHMANN. 2003. History and evolution of the arctic flora: In the footsteps of Eric Hultén. *Molecular Ecology* 12: 299–313.

ABBOTT, R. J., L. C. SMITH, R. I. MILNE, R. M. M. CRAWFORD, K. WOLFF, AND J. BALFOUR. 2000. Molecular analysis of plant migration and refugia in the Arctic. *Science* 289: 1343–1346.

AKAIKE, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.

APG III [ANGIOSPERM PHYLOGENY GROUP III]. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.

ARBER, A. 1920. Water plants: A study of aquatic angiosperms. Cambridge University Press, Cambridge, UK.

ASTON, H. I. 1987. Sparganiaceae. *In* A. F. George [ed.], Flora of Australia, vol. 45. Australian Government Publication Service, Canberra, Australia.

BALDWIN, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3–16.

BARRETT, S. C. H., C. G. ECKERT, AND B. C. HUSBAND. 1993. Evolutionary processes in aquatic plant populations. *Aquatic Botany* 44: 105–145.

BARRETT, S. C. H., AND S. W. GRAHAM. 1997. Adaptive radiation in the aquatic plant family Pontederiaceae: Insights from phylogenetic analysis. *In* T. J. Givnish and K. J. Sytsma [eds.], Molecular evolution and adaptive radiation, 225–258. Cambridge University Press, Cambridge, UK.

BARTOLI, G., M. SARNTHEIN, M. WEINELT, H. ERLENKEUSER, D. GARBE-SCHÖNBERG, AND D. W. LEA. 2005. Final closure of Panama and the onset of Northern Hemisphere glaciation. *Earth and Planetary Science Letters* 237: 33–44.

BEAL, E. O. 1960. *Sparganium* (Sparganiacae) in the southeastern United States. *Brittonia* 12: 176–181.

BERRY, E. W. 1924. A *Sparganium* from the Middle Eocene of Wyoming. *Botanical Gazette (Chicago, Ill.)* 78: 342–348.

BRAYSHAW, T. C. 1985. Pondweeds and bur-reeds, and their relatives, of British Columbia. *Occasional Papers of the British Columbia Provincial Museum* 26: 1–167.

BREMER, K. 2000. Early Cretaceous lineages of monocot flowering plants. *Proceedings of the National Academy of Sciences, USA* 97: 4707–4711.

BROWN, R. W. 1962. Paleocene flora of the Rocky Mountains and Great Plains. Geological Survey Professional Paper 375. U.S. Government Printing Office, Washington D.C., USA.

CHASE, M. W., M. F. FAY, D. S. DEVEY, O. MAURIN, N. RØNSTED, T. J. DAVIES, Y. PILLON, ET AL. 2006. Multigene analyses of monocot relationships: A summary. *Aliso* 22: 63–75.

CHEN, J. M., G. W. ROBERT, AND Q. F. WANG. 2004. Evolution of aquatic life-forms in Alismatidae: Phylogenetic estimation from chloroplast *rbcL* gene sequence data. *Israel Journal of Plant Sciences* 52: 323–329.

CHEN, L. Y., J. M. CHEN, R. W. GITURU, AND Q. F. WANG. 2012. Generic phylogeny, historical biogeography and character evolution of the cosmopolitan aquatic plant family Hydrocharitaceae. *BMC Evolutionary Biology* 12: 30.

CHEN, Y. D. 1981. A study on Chinese *Sparganium. Acta Phytotaxonomica Sinica* 19: 43–56.

CLAYTON, J. W., P. S. SOLTIS, AND D. E. SOLTIS. 2009. Recent long-distance dispersal overshadows ancient biogeographical patterns in a pan-tropical angiosperm family (Simaroubaceae, Sapindales). *Systematic Biology* 58: 395–410.

COOK, C. D. K. 1961. *Sparganium* in Britain. *Watsonia* 5: 1–10.

COOK, C. D. K. 1988. Wind pollination in aquatic angiosperms. *Annals of the Missouri Botanical Garden* 75: 768–777.

COOK, C. D. K. 1996. Aquatic plant book. Academic Publishing, New York, New York, USA.

COOK, C. D. K., AND M. S. NICHOLLS. 1986. A monographic study of the genus *Sparganium*. Part 1: Subgenus *Xanthosparganium. Botanica Helvetica* 96: 213–267.

COOK, C. D. K., AND M. S. NICHOLLS. 1987. A monographic study of the genus *Sparganium*. Part 2: Subgenus *Sparganium. Botanica Helvetica* 97: 1–44.

CREPET, W. L., K. C. NIXON, AND M. A. GANDOLFO. 2004. Fossil evidence and phylogeny: The age of major angiosperm clades based on mesofossil and macrofossil evidence from Cretaceous deposits. *American Journal of Botany* 91: 1666–1682.

CRONQUIST, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, New York, USA.

CROW, G. E., AND C. B. HELLQUIST. 1981. Aquatic vascular plants of New England: Part 2. Typhaceae and Sparganiaceae. *New Hampshire Agricultural Experiment Station Bulletin* 517: 1–21.

DONOGHUE, M. J., AND S. A. SMITH. 2004. Patterns in the assembly of temperate forests around the Northern Hemisphere. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 359: 1633–1644.

DREW, B. T., AND K. J. SYTSMA. 2012. Phylogenetics, biogeography, and staminal evolution in the tribe Mentheae (Lamiaceae). *American Journal of Botany* 99: 933–953.

DREW, B. T., AND K. J. SYTSMA. 2013. The South American radiation of *Lepechinia* (Lamiaceae): phylogenetics, divergence times and evolution of dioecy. *Botanical Journal of the Linnean Society* 171: 171–190.

DRUMMOND, A. J., AND A. RAMBAUT. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.

EDWARDS, A. W. F. 1992. Likelihood. Johns Hopkins University Press, Baltimore, Maryland, USA.

EDWARDS, E. J., C. P. OSBORNE, C. A. E. STRÖMBERG, S. A. SMITH, AND C4 GRASSES CONSORTIUM. 2010. The origins of C4 grasslands: Integrating evolutionary and ecosystem science. *Science* 328: 587–591.

ELIAS, S. A., S. K. SHORT, C. H. NELSON, AND H. H. BIRKS. 1996. Life and times of the Bering Land Bridge. *Nature* 382: 60–63.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance and congruence. *Cladistics* 10: 315–319.

FASSETT, N. C. 1940. A manual of aquatic plants. University of Wisconsin Press, Madison, Wisconsin, USA.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.

FERNALD, M. L. 1922. Notes on *Sparganium. Rhodora* 24: 26–34.

FRIEDMAN, J., AND S. C. H. BARRETT. 2008. A phylogenetic analysis of the evolution of wind pollination in the angiosperms. *International Journal of Plant Sciences* 169: 49–58.

FRIEDMAN, J., AND S. C. H. BARRETT. 2009. Wind of change: New insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany* 103: 1515–1527.

GELMAN, A., AND D. B. RUBIN. 1992. Inference from iterative simulation using multiple sequences. *Statistical Science* 7: 457–472.

GENG, B. Y. 1999. A new species of *Sparganium* from the Eocene of Fushun, Liaoning Province. *Acta Phytotaxonomica Sinica* 37: 204–208.

GIVNISH, T. J. 1986. Economics of support. *In* T. J. Givnish [ed.], On the economy of plant form and function, 413–420. Cambridge University Press, Cambridge, UK.

GIVNISH, T. J. 1987. Comparative studies of leaf form: Assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist* 106 (supplement): 131–160.

GIVNISH, T. J. 1990. Causes of depth zonation of emergent, floating-leaved, and submerged aquatic macrophytes. [Ecological Society of America, Abstracts] *Bulletin of the Ecological Society of America* 71: 166.

GIVNISH, T. J. 1995. Plant stems: Biomechanical adaptations for energy capture and influence on species distributions. *In* B. L. Gartner [ed.], Plant stems: Physiology and functional morphology, 3–49. Chapman and Hall, New York, New York, USA.

GIVNISH, T. J., M. S. AMES, J. R. MCNEAL, M. R. MCKAIN, P. R. STEELE, C. W. DEPAMPHILIS, S. W. GRAHAM, ET AL. 2010. Assembling the tree of the monocotyledons: Plastome sequence phylogeny and evolution of Poales. *Annals of the Missouri Botanical Garden* 97: 584–616.

GIVNISH, T. J., M. H. BARFUSS, B. VAN EE, R. RIINA, K. SCHULTE, R. HORRES, P. A. GONSISKA, ET AL. 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny. *American Journal of Botany* 98: 872–895.

GIVNISH, T. J., K. C. MILLAM, P. E. BERRY, AND K. J. SYTSMA. 2007. Phylogeny, adaptive radiation, and historical biogeography of Bromeliaceae inferred from *ndhF* sequence data. *Aliso* 23: 3–26.

GLADENKOV, A. Y., A. E. OLEINIK, L. MARINCOVICH, AND K. B. BARINOV. 2002. A refined age for the earliest opening of Bering Strait. *Palaeogeography, Palaeoclimatology, Palaeoecology* 183: 321–328.

GOVAERTS, R. 2013. World checklist of Typhaceae. Facilitated by the Royal Botanic Gardens, Kew. Available at http://apps.kew.org/wcsp/ [accessed 18 May 2013].

GRAHAM, A. 1972. Outline of the origin and historical recognition of floristic affinities between Asia and eastern North America. *In* A. Graham [ed.], Floristics and paleofloristics of Asia and eastern North America, 1–18. Elsevier, Amsterdam, Netherlands.

GRAHAM, A. 2011. The age and diversification of terrestrial New World ecosystems through Cretaceous and Cenozoic time. *American Journal of Botany* 98: 336–351.

HARMS, V. L. 1973. Taxonomic studies of North American *Sparganium,* I. *Sparganium hyperboreum* and *S. minimum. Canadian Journal of Botany* 51: 1629–1641.

HARRIS, A. J., AND Q.-Y. XIANG. 2009. Estimating ancestral distributions of lineages with uncertain sister groups: A statistical approach to dispersal–vicariance analysis and a case using *Aesculus* L. (Sapindaceae) including fossils. *Journal of Systematics and Evolution* 47: 349–368.

HARRIS, A. J., J. WEN, AND Q.-Y. XIANG. 2013. Inferring the biogeographic origins of inter-continental disjunct endemics using a Bayes-DIVA approach. *Journal of Systematics and Evolution* 51: 117–133.

HICKEY, L. J. 1977. Stratigraphy and paleobotany of the Golden Valley Formation (Early Tertiary) of western North Dakota. Geological Society of America Memoir 150. Geological Society of America, Boulder, Colorado, USA.

HIPP, A. L., J. C. HALL, AND K. J. SYTSMA. 2004. Phylogenetic accuracy, congruence between data partitions, and performance of the ILD. *Systematic Biology* 53: 81–89.

HOLMBERG, O. R. 1922. Anteckningar till nya Skandinaviska Floran. II. *Botaniska Notiser* 1922: 206–207.

HUELSENBECK, J. P., AND F. R. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.

HULTÉN, E. 1937. Outline of the history of arctic and boreal biota during the Quarternary Period. Cramer, New York, New York, USA.

JABAILY, R. S., AND K. J. SYTSMA. 2010. Phylogenetics of *Puya* (Bromeliaceae): Placement, major lineages, and evolution of Chilean species. *American Journal of Botany* 97: 337–356.

KADONO, Y. 1994. Aquatic plants of Japan. Bun-ichi Sogo Shuppan, Tokyo, Japan.

KAUL, R. B. 1972. Adaptive leaf architecture in emergent and floating *Sparganium. American Journal of Botany* 59: 270–278.

KAUL, R. B. 1997. Sparganiaceae. *In* Flora of North America Editorial Committee, [eds.], Flora of North America North of Mexico, vol. 22. Oxford University Press, New York, New York, USA.

KIM, C., AND H.-K. CHOI. 2011. Molecular systematics and character evolution of *Typha* (Typhaceae) inferred from nuclear and plastid DNA sequence data. *Taxon* 60: 1417–1428.

KIRK, H., C. CONNOLLY, AND J. R. FREELAND. 2011. Molecular genetic data reveal hybridization between *Typha angustifolia* and *Typha latifolia* across a broad spatial scale in eastern North America. *Aquatic Botany* 95: 189–193.

KNOBLOCH, E., AND D. H. MAI. 1986. Monographie der Früchte und Samen in der Kreide von Mitteleuropa. *Rozpravy Ústředního Ústavu Geologického* 47: 1–219.

KODANDARAMAIAH, U. 2010. Use of dispersal–vicariance analysis in biogeography—A critique. *Journal of Biogeography* 37: 3–11.

KUEHN, M. M., J. E. MINOR, AND B. N. WHITE. 1999. An examination of hybridization between the cattail species *Typha latifolia* and *Typha angustifolia* using random amplified polymorphic DNA and chloroplast DNA markers. *Molecular Ecology* 8: 1981–1990.

LES, D. H., D. J. CRAWFORD, R. T. KIMBALL, M. L. MOODY, AND E. LANDOLT. 2003. Biogeography of discontinuously distributed hydrophytes: A molecular appraisal of intercontinental disjunctions. *International Journal of Plant Sciences* 164: 917–932.

LES, D. H., S. W. L. JACOBS, N. P. TIPPERY, L. CHEN, M. L. MOODY, AND M. WILSTERMANN-HILDEBRAND. 2008. Systematics of *Vallisneria* (Hydrocharitaceae). *Systematic Botany* 33: 49–65.

LES, D. H., AND C. T. PHILBRICK. 1993. Studies of hybridization and chromosome number variation in aquatic plants: Evolutionary implications. *Aquatic Botany* 44: 181–228.

LES, D. H., S. P. SHELDON, AND N. P. TIPPERY. 2010. Hybridization in hydrophiles: Natural interspecific hybrids in *Najas* (Hydrocharitaceae). *Systematic Botany* 35: 736–744.

LI, H. 1952. Floristic relationships between eastern Asia and eastern North America. *Transactions of the American Philosophical Society* 42: 371–429.

LINNAEUS, C. 1753. Species plantarum, vol. 1, 971. Laurentius Salvius, Stockholm, Sweden.

MADDISON, D. R., AND W. P. MADDISON. 2005. MacClade 4: Analysis of phylogeny and character evolution, version 4.08. Sinauer, Sunderland, Massachusetts, USA.

MANCHESTER, S. R. 1999. Biogeographical relationships of North American Tertiary floras. *Annals of the Missouri Botanical Garden* 86: 472–522.

MARINCOVICH, L., AND A. GLADENOV. 1999. Evidence for an earlier opening of the Bering Strait. *Nature* 397: 149–151.

MCIVER, E. E., AND J. F. BASINGER. 1999. Early Tertiary floral evolution in the Canadian High Arctic. *Annals of the Missouri Botanical Garden* 86: 523–545.

MILNE, R. I. 2006. Northern Hemisphere plant disjunctions: A window on tertiary land bridges and climate change? *Annals of Botany* 98: 465–472.

MILNE, R. I., AND R. J. ABBOTT. 2002. The origin and evolution of Tertiary relict floras. *Advances in Botanical Research* 38: 281–314.

MOERMAN, D. E. 1998. Native American ethnobotany. Timber Press, Portland, Oregon, USA.

MOODY, M. L., AND D. H. LES. 2007. Phylogenetic systematics and character evolution in the angiosperm family Haloragaceae. *American Journal of Botany* 94: 2005–2025.

MOODY, M. L., AND D. H. LES. 2010. Systematics of the aquatic angiosperm genus *Myriophyllum* (Haloragaceae). *Systematic Botany* 35: 121–139.

MORLEY, R. J. 2003. Interplate dispersal paths for megathermal angiosperms. *Perspectives in Plant Ecology, Evolution and Systematics* 6: 5–20.

MÜLLER-DOBLIES, D. 1970. Über die Verwandtschaft von *Typha* und *Sparganium* im Infloreszenz- und Blütenbau. *Botanische Jahrbücher* 89: 451–562.

MÜLLER-DOBLIES, U. 1969. Über die Blütenstände und Blüten sowie zur Embryologie von *Sparganium. Botanische Jahrbücher* 89: 359–450.

MURRAY, D. E. 1981. The role of arctic refugia in the evolution of the arctic vascular flora: A Beringian perspective. *In* G. E. Scudder and J. L. Reveal [eds.], Evolution today, 11–20. Hunt Institute, Pittsburgh, Pennsylvania, USA.

NICHOLS, S. A. 1999. Distribution and habitat descriptions of Wisconsin lake plants. Wisconsin Geological and Natural History Survey, Bulletin 96, Madison, Wisconsin, USA.

NIE, Z.-L., H. SUN, S. R. MANCHESTER, Y. MENG, Q. LUKE, AND J. WEN. 2012. Evolution of the intercontinental disjunctions in six continents in the *Ampelopsis* clade of the grape family (Vitaceae). *BMC Evolutionary Biology* 12: 17.

NYLANDER, J. A. A., U. OLSSON, P. ALSTRÖM, AND I. SANMARTÍN. 2008. Accounting for phylogenetic uncertainty in biogeography: A Bayesian approach to dispersal-vicariance analysis of the thrushes (Aves: *Turdus*). *Systematic Biology* 57: 257–268.

PAGEL, M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology* 48: 612–622.

PAGEL, M., AND A. MEADE. 2007. BayesTraits, version 1.0. Computer program and documentation distributed by the author, website: http://www.evolution.rdg.ac.uk [accessed 17 October 2012].

POLLUX, B. J. A., M. DE JONG, A. STEEGH, J. M. VAN GROENENDAEL, AND M. KLAASEN. 2006. The effect of seed morphology on the potential dispersal of aquatic macrophytes by the common carp (*Cyprinus carpio*). *Freshwater Biology* 51: 2063–2071.

POLLUX, B. J. A., N. J. OUBORG, J. M. VAN GROENENDAEL, AND M. KLAASEN. 2007. Consequences of intraspecific seed-size variation in *Sparganium emersum* for dispersal by fish. *Functional Ecology* 21: 1084–1091.

POLLUX, B. J. A., L. SANTAMARÍA, AND N. J. OUBORG. 2005. Differences in endozoochorous dispersal between aquatic plant species, with reference to plant population persistence in rivers. *Freshwater Biology* 50: 232–242.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: Testing the model of DNA substitution. *Bioinfornatics* 14: 817–818.

POSTNIKOFF, C. L. 2009. Flora of the Ravenscrag Formation of the Big Muddy Valley, Willow Bunch Lake Map Area (72H), Saskatchewan. Master's thesis, University of Saskatchewan, Saskakatoon, Saskatchewan, Canada.

R DEVELOPMENT CORE TEAM. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org/ [accessed 29 December 2012].

RAMBAUT, A., AND A. J. DRUMMOND. 2007. Tracer, version 1.5. Computer program and documentation distributed by the author, website http://beast.bio.ed.ac.uk/Tracer [accessed 10 October 2012].

REE, R. H., AND S. A. SMITH. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* 57: 4–14.

RIESEBERG, L. H. 1991. Homoploid reticulate evolution in *Helianthus*: Evidence from ribosomal genes. *American Journal of Botany* 78: 1218–1237.

RIESEBERG, L. H. 1997. Hybrid origin of plant species. *Annual Review of Ecology and Systematics* 28: 359–389.

RONQUIST, F. 1996. DIVA, version 1.1. Computer program and manual available by anonymous FTP from Uppsala University, Uppsala, Sweden.

RONQUIST, F. 1997. Dispersal–vicariance analysis: A new approach to the quantification of historical biogeography. *Systematic Biology* 46: 195–203.

SANTAMARIA, L. 2002. Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. *Oecologica* 23: 137–154.

SCHUYLER, A. E. 1984. Classification of life forms and growth forms of aquatic macrophytes. *Bartonia* 50: 8–11.

SCOTESE, C. R. 2001. Atlas of earth history. PALEOMAP project. University of Texas, Arlington, Texas, USA. Website http://www.scotese.com [accessed 15 December 2012].

SCULTHORPE, C. D. 1967. The biology of aquatic vascular plants. Edward Arnold, London, UK.

SESSA, E. B., E. A. ZIMMER, AND T. J. GIVNISH. 2012. Phylogeny, divergence times, and historical biogeography of New World *Dryopteris* (Dryopteridaceae). *American Journal of Botany* 99: 730–750.

SHAW, J., E. B. LICKEY, E. E. SCHILLING, AND R. L. SMALL. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *American Journal of Botany* 94: 275–288.

SMITH, R. L., AND K. J. SYTSMA. 1990. Evolution of *Populus nigra* (sect. *Aigeiros*): Introgressive hybridization and the chloroplast contribution of *Populus alba* (sect. *Populus*). *American Journal of Botany* 77: 1176–1187.

SOCORRO GONZÁLEZ ELIZONDO, M. S., M. GONZÁLEZ ELIZONDO, AND M. A. MÁRQUEZ LINARES. 2007. Vegetación y ecorregiones de Durango. Plaza y Valdes, Mexico D. F., Mexico.

SOLTIS, D. E., S. A. SMITH, N. CELLINESE, K. J. WURDACK, D. C. TANK, S. F. BROCKINGTON, N. F. REFULIO-RODRIGUEZ, ET AL. 2011. Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal of Botany* 98: 704–730.

SPOONER, D. M., K. J. SYTSMA, AND J. F. SMITH. 1991. A molecular reexamination of diploid hybrid speciation of *Solanum raphanifolium* (Solanaceae). *Evolution* 45: 757–764.

SULMAN, J. D. 2010. A systematic study of the genus *Sparganium*. M.S. thesis, University of Wisconsin, Madison, Wisconsin, USA.

SUN, K., AND D. SIMPSON. 2010. Typhaceae. *In* Z. Y. Wu, P. H. Raven, and D. Y. Hong [eds.], Flora of China, vol. 23 (Acoraceae through Cyperaceae). Science Press, Beijing, China; Missouri Botanical Garden Press, St. Louis, Missouri, USA.

SWOFFORD, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer, Sunderland, Massachusetts, USA.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

THIERET, J. W. 1982. The Sparganiaceae in the southeastern United States. *Journal of the Arnold Arboretum* 63: 341–355.

TIFFNEY, B. H. 1985a. Perspectives on the origin of the floristic similarity between eastern Asia and eastern North America. *Journal of the Arnold Arboretum* 66: 73–94.

TIFFNEY, B. H. 1985b. The Eocene North Atlantic land bridge: Its importance in Tertiary and modern phytogeography of the Northern Hemisphere. *Journal of the Arnold Arboretum* 66: 243–273.

TIFFNEY, B. H. 2000. Geographic and climatic influences on the Cretaceous and Tertiary history of Euramerican floristic similarity. *Acta Universitatis Carolinae. Geologica* 44: 5–16.

TIFFNEY, B. H., AND S. R. MANCHESTER. 2001. The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere Tertiary. *International Journal of Plant Sciences* 162 (S6): 3–17.

TIPPERY, N. P., AND D. H. LES. 2011. Evidence for the hybrid origin of *Nymphoides montana* Aston (Menyanthaceae). *Telopea* 13: 285–294.

TULIN, L., Y. DINGJIANG, AND M. CHUNQIN. 1999. Studies on antiplatelet aggregation and antithrombosis action of total flavonoids of common burreed (*Sparganium stoloniferum*). *Chinese Traditional and Herbal Drugs* 6: 17.

VILA, R., C. D. BELL, R. MACNIVEN, B. GOLDMAN-HUERTAS, R. H. REE, C. R. MARSHALL, Z. BÁLINT, ET AL. 2011. Phylogeny and palaeoecology

of *Polyommatus* blue butterflies show Beringia was a climate-regulated gateway to the New World. *Proceedings of the Royal Society, B, Biological Sciences* 278: 2737–2744.

WALKER, J. D., AND J. W. GEISSMAN. 2009. Geological time scale: Geological Society of America. Website http://www.geosociety.org/science/timescale/ [accessed 9 December 2012].

WEN, J. 1999. Evolution of eastern Asian and eastern North American disjunct pattern in flowering plants. *Annual Review of Ecology and Systematics* 30: 421–455.

WEN, J. 2001. Evolution of eastern Asian and eastern North American biogeographic disjunctions: a few additional issues. *International Journal of Plant Sciences* 162: S117–S122.

WENDEL, J. F., AND J. J. DOYLE. 1998. Phylogenetic incongruence: Window into genome history and molecular evolution. *In* P. Soltis, D. Soltis, and J. Doyle [eds.], Molecular systematics of plants II, 265–296. Kluwer, Dordrecht, Netherlands.

WENDEL, J. F., J. MCD. STEWART, AND J. H. RETTIG. 1991. Molecular evidence for homoploid reticulate evolution among Australian species of *Gossypium. Evolution* 45: 694–711.

WHEELER, W. 1996. Optimization alignment: The end of multiple sequence alignment in phylogenetics? *Cladistics* 12: 1–10.

WOOD, C. E. 1972. Morphology and phytogeography: The classical approach to the study of disjunctions. *Annals of the Missouri Botanical Garden* 59: 107–124.

XIE, L., W. L. WAGNER, R. H. REE, P. E. BERRY, AND J. WEN. 2009. Molecular phylogeny, divergence time estimates, and historical biogeography of *Circaea* (Onagraceae) in the Northern Hemisphere. *Molecular Phylogenetics and Evolution* 53: 995–1009.

YODER, A. D., J. A. IRWIN, AND B. A. PAYSEUR. 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. *Systematic Biology* 50: 408–424.

YU, Y., A. J. HARRIS, AND X. J. HE. 2010. S-DIVA (Statistical Dispersal-Vicariance Analysis): A tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution* 56: 848–850.

YU, Y., A. J. HARRIS, AND X. J. HE. 2011. RASP (Reconstruct Ancestral State in Phylogenies) 2.0b. Computer program available at http://mnh.scu.edu.cn/soft/blog/RASP.

YULE, G. U. 1925. A mathematical theory of evolution, based on the conclusions of Dr. JC Willis, FRS. *Philosophical Transactions of the Royal Society of London, B, Containing Papers of a Biological Character* 213: 21–87.

YUZEPCHUK, S. V. 1968. Sparganiaceae. *In* V. L. Komarov [ed.], Flora of the USSR, vol. 1, 230–263. Botanical Institute of the Academy of Sciences of the USSR, Leningrad, USSR [translated in 1968 by Israeli Program for Scientific Translations, Jerusalem, Israel].

ZWICKL, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, University of Texas, Austin, Texas, USA.

APPENDIX 1. Collections of *Sparganium* from Mexico and Colombia reviewed for this study.

***Sparganium americanum* Nutt.** Colombia: Antioquia, Municipio Urrao. Lado del camino a Pávon, 1850 m. *Julio Betancur & Richard W. Pohl 218* Sept. 11 1986 (MO, HUA). Mexico: Durango, El Salto. Streamside along Rio Salto, broad open valley in pine–oak zone, 8200 ft. *Robert W. Dickerman 1005* July 7, 1956 (MIN).

APPENDIX 2. List of taxa, vouchers, and Genbank accession numbers for material examined.

Taxon; *Name in figures*; Collection locale; *Voucher*; GenBank numbers: *trnL-trnF, psbJ-petA,* ITS, phyC. Dash (—) indicates no sequence.

Sparganium americanum Nutt.; *S. americanum*; Allequash Creek, Vilas Co., WI, USA; *J. Sulman 856* (WIS); KF265398, KF265433, KF265365, KF265468. ***Sparganium androcladum*** (Engelm.) Morong; *S. androcladum*; Pershing Wildlife Area, Taylor Co., WI, USA; *J. Sulman 868* (WIS); KF265399, KF265434, KF265366, KF265469. ***Sparganium angustifolium*** Michx.; *S. angustifolium* 1; Camp Lake, Vilas Co., WI, USA; *J. Sulman 861* (WIS); KF265403, KF265438, KF265370, KF265473. ***Sparganium angustifolium*** Michx.; *S. angustifolium* 2; Ribbon Lake, Yellowstone Nat'l Park, WY, USA; *J. Sulman SANG2* (WIS); KF265401, KF265436, KF265368, KF265471. ***Sparganium angustifolium*** Michx.; *S. angustifolium* 3; Meadow Lark Lake, Bighorn Mts., WY, USA; *J. Sulman 920* (WIS); KF265402, KF265437, KF265369, KF265472. ***Sparganium angustifolium*** Michx. × ***S. emersum*** Rehmann; *S. angust.* × *emersum;* Yellowstone Nat'l Park, WY, USA; *J. Sulman 918* (WIS); KF265400, KF265435, KF265367, KF265470. ***Sparganium emersum*** Rehmann; *S. emersum 1*; Nixon Creek, Vilas Co., WI, USA; *J. Sulman 854* (WIS); KF265404, KF265439, KF265371, KF265474. ***Sparganium emersum*** Rehmann; *S. emersum 2*; Allequash Lake, Vilas Co., WI, USA; *J. Sulman & S. Knight 912* (WIS); KF265426, KF265461, KF265391, —. ***Sparganium emersum*** Rehmann; *S. emersum 3*; Bluff Creek, Walworth Co., WI, USA; *J. Sulman & G. Virnig s.n.* [photo]; KF265427, KF265462, KF265392, —. ***Sparganium emersum*** Rehmann; *S. emersum 4*; Lauderdale Lakes, Walworth Co., WI, USA; *J. Sulman & G. Virnig 922* (WIS); KF265428, KF265463, KF265393, —. ***Sparganium erectum*** L. subsp. ***stoloniferum*** (Buch.-Ham. ex Graebn.) H. Hara; *S. erectum 1;* Sapporo, Hokkaido, Japan; *M. Yamazaki s.n.* (SAPS, WIS); KF265406, KF265441, KF265373, KF265476. ***Sparganium erectum*** L. subsp. ***stoloniferum*** (Buch.-Ham. ex Graebn.) H. Hara var. ***macrocarpum*** (Makino) H. Hara; *S. erectum 2;* Hanyu, Higashine-shi, Yamagata Pref., Japan; *E. Hayasaka 4803* (TUS, WIS); KF265407, KF265442, KF265374, KF265477. ***Sparganium erectum*** L. subsp. ***stoloniferum*** (Buch.-Ham. ex Graebn.) H. Hara; *S. erectum 3;* Novosibirsk, Russia; *I. Krasnoborov s.n.* (MO); KF265430, KF265465, KF265395, —. ***Sparganium erectum*** L. ***subsp. microcarpum*** (Neuman) Domin; *S. erectum 4;* Ta. Iitti, Saaranen, Finland; *I. Kukkonen & M. Korhonen 9099* (WIS); KF265429, KF265464, KF265394, —. ***Sparganium eurycarpum*** Engelm.; *S. eurycarpum 1*; Wausau, WI, USA; *J. Sulman 825* (WIS); KF265408, KF265443, KF265375, KF265478. ***Sparganium eurycarpum*** Engelm.var. *greenei* (Morong) Graebn.; *S. eurycarpum 2*; Olema, Marin Co., CA, USA; *J. Sulman & E. McGrath 894* (WIS); KF265415, KF265450, KF265382, KF265485. ***Sparganium fallax*** Graebn.; *S. fallax 1;* Hiraizumi, Iwate Pref., Japan; *N. Numakunai, K. Abe, S. Arakida 650* (TUS, TKPM, WIS); KF265409, KF265444, KF265376, KF265479. ***Sparganium fallax*** Graebn.; *S. fallax 2;* Yuanyang Lake, Taiwan; *Chien-I Huang 1439* (HAST); KF265410, KF265445, KF265377, KF265480. ***Sparganium fluctuans*** (Engelm. ex Morong) B.L.Rob.; *S. fluctuans*; Shearer Lake, Taylor Co., WI, USA; *J. Sulman 867* (WIS); KF265411, KF265446, KF265378, KF265481. ***Sparganium glomeratum*** (Laest. ex Beurl.) Beurl.; *S. glomeratum 1*; Ishikari kanai, Hokkaido, Japan; *M. Yamazaki, s.n.* (SAPS, WIS); KF265412, KF265447, KF265379, KF265482. ***Sparganium glomeratum*** (Laest. ex Beurl.) Beurl.; *S. glomeratum 2*; Sichuan: Maerkang (Barkam) Xian, China; D. E. Boufford, K. Fujikawa, S. L. Kelley, R. H. Ree, B. Xu, J. W. Zhang, T. C. Zhang, W. D. Zhu 39555 (A); KF265419, KF265454, KF265386, KF265489. ***Sparganium glomeratum*** (Laest. ex Beurl.) Beurl.; *S. glomeratum 3*; Superior, WI, USA; *J. Sulman 892* (WIS); KF265413, KF265448, KF265380, KF265483. ***Sparganium gramineum*** Georgi; *S. gramineum*; Uryu numa Mire, Hokkaido, Japan; *M. Yamazaki s.n.* (SAPS, WIS); KF265414, KF265449, KF265381, KF265484. ***Sparganium hyperboreum*** Laest. ex Beurl.; *S. hyperboreum 1*; Noatak Nat'l. Preserve, AK, USA; *C. L. Parker, R. Elven & H. Solstad 15183* (ALA); KF265416, KF265451, KF265383, KF265486. ***Sparganium hyperboreum*** Laest. ex Beurl.; *S. hyperboreum 2*; Denali Nat'l Park, AK, USA; *A. Larsen, C. Roland & A. Batten 01-0973* (ALA); KF265431, KF265466, KF265396, —. ***Sparganium japonicum*** Rothert; *S. japonicum*; Hanyu, Higashine-shi, Yamagata Pref., Japan; *E. Hayasaka 4801* (TUS, WIS); KF265417, KF265452, KF265384, KF265487. ***Sparganium japonicum*** Rothert × ***S. fallax*** Graebn.; *S. japonicum* × *fallax*; Takagi, Japan; *K. Sawa 1* (TUS, WIS); KF265405, KF265440, KF265372, KF265475. ***Sparganium natans*** L.; *S. natans*; Micmac Lake, Lake Co., MN, USA; *J. Sulman & E. McGrath 891* (WIS); KF265418, KF265453, KF265385, KF265488. ***Sparganium subglobosum*** Morong; *S. subglobosum 1*; Futami-cho, Japan; *M. Usuba s.n.* (TUS, WIS); KF265420, KF265455, KF265387, KF265490. ***Sparganium subglobosum*** Morong; *S. subglobosum 2*; Tazawa, Murayama-shi, Yamagata Pref., Japan; *E. Hayasaka 4810* (TUS, WIS); KF265432, KF265467, KF265397, —.

Typha orientalis C.Presl.; *Typha orientalis*; Fukushima Pref., Honshu, Japan; *M. Usuba s.n.* (TUS, WIS); KF265424, KF265459, —, KF265494. ***Typha angustifolia*** L.; *T. angustifolia*; Minocqua, Oneida Co., WI, USA; *J. Sulman 848* (WIS); KF265421, KF265456, KF265388, KF265491. ***Typha latifolia*** L.; *T. latifolia*; Minocqua, Oneida Co., WI, USA; *J. Sulman 847* (WIS); KF265423, KF265458, KF265390, KF265493. ***Typha domingensis*** Pers.; *T. domingensis*; Shizuoka Pref., Honshu, Japan; *K. Kitamura 4* (TUS, WIS); KF265422, KF265457, KF265389, KF265492.

Brocchinia prismatica L.B.Sm.; *Brocchinia prismatica*; Cerro Yapacana, Amazonia, Venezuela; *T. Givnish s.n.* (WIS); KF265425, KF265460, —, —.

Puya ferruginea (Ruiz & Pav.) L.B.Sm.; *Puya ferruginea*; *Chase 23826* (K) & *R. Jabaily 146* (WIS); EU780865 & EU780877, —, KF265363, FJ968250. ***Puya venusta*** (Baker) Phil.; *P. venusta*; *R. Jabaily 166* & *203* (WIS); JF299263, —, KF265364, FJ968261.