SYSTEMATICS, BIOGEOGRAPHY, AND CHARACTER EVOLUTION OF *SPARGANUM* (TYPHACEAE): DIVERSIFICATION OF A WIDESPREAD, AQUATIC LINEAGE

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Objectives: To present a fossil-calibrated, molecular phylogeny of *Sparganium* based on analysis of two chloroplast and two nuclear markers. We examine character evolution in both habit and stigma number and infer the ancestral area and biogeographic history of the genus. We realigned the subgenera to conform to the 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.

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 biogeographic, 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 \textit{S. americanum} (as identified), \textit{S. emersum}, or undescribed species.

Though \textit{Sparganium} has traditionally been placed in Sparganiaceae, \textit{Sparganium} and \textit{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 \textit{Typha + Sparganium} is well established based on intensive phylogenetic study of monocots in recent years (Bremer, 2000; Chase et al., 2004, 2011; Solits et al., 2011). \textit{Sparganium} and \textit{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 \textit{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 \textit{Sparganium} as a genus and its apparent monophyly based on the inclusion of five \textit{Sparganium} species in a recent phylogenetic study of \textit{Typha} (Kim and Choi, 2011), the phylogenetic relationships within \textit{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., \textit{S. eurycarpum—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: \textit{S. erectum} (plants emergent) and \textit{S. natans} (plants floating). Wladislaw Rorth worked on a monograph of \textit{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 Rorth. Important regional taxonomic treatments of \textit{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 \textit{Sparganium} have been consistently placed within two subgenera (Holmberg, 1922; Cook, 1961; Cook and Nicholls, 1986, 1987). Subgenus \textit{Xanthosparganium} Holmberg comprised short-statured species with light-colored tepals, whereas subg. \textit{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 \textit{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). \textit{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 \textit{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 \textit{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 \textit{Sparganium} has been done, or evidence of multiple growth form transitions presented.

\textit{Sparganium} species share a number of morphological adaptations that are common to many unrelated, aquatic angiosperm groups. These include monoeory, 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 \textit{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 \textit{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 \textit{Sparganium}. Our data set is comprised of two cpDNA gene regions (\textit{psbA-trnM}, \textit{trnL-trnF}), and two regions of nDNA (phytochrome C [\textit{phyC}], nuclear rDNA internal transcribed spacer region [ITS]). We sampled the four gene regions across the broadest taxon sampling within \textit{Sparganium} to date to shed light on the following questions about \textit{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 \textit{Sparganium} species occur, and are these events primarily dispersal or vicariant based? (4) How has \textit{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 \textit{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 allotopic, 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. Har, 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 prismitica, 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 psbA-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 psbA-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 Systma (2010) for Sparganium accessions and, for Typha accessions, using the newly designed primers bdphyC-F2 (GCDTTGAAARTCRTATAARCTYGC) and bdphyC-r1 (AAGCRTCRTGTTTTYGCAACCACC). Polymerase chain reaction (PCR) amplifications of psbA-petA followed the protocol from Shaw et al. (2007), while the other three DNA regions were amplified following the protocol of Jabaily and Systma (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 used 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 six times 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 replications were conducted using the same ML settings as the initial search. Bayesian inference (BI) was conducted using the program MrBayes v3.2.1 (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 + I + 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 Akaile information criterion (AIC; Akaile, 1974) obtained in ModelTest v3.7. The GTR + Γ + I model was chosen for ITS and phyC, the GTR+I+Γ model was selected for psbI-petA, while the TVM + Γ + I 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 0.5 to 1.5, and a standard deviation of 0.5. This date is based on the earliest known fossils of *Typha* (seeds of *T. parvum* – The root of the tree was secondarily calibrated as 1.0–1.5, and a standard deviation of 0.5. This date is based on the earliest known fossils of *Sparganium* – (1) the earliest Cretaceous fossil (Postnikoff, 2009) with three and (2) four combined areas were permitted based on extant fossils of *Sparganium* 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–Eocene (Yoder et al., 2001), and the late Cretaceous (Newberry) Brown, 1962). Some Oligocene-age *Sparganium* fossils had ovaries with numerous locules: *S. multiloculare* Reid & Chandler from England had 2–5, and *S. balantium* 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 (Lee 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 northern Africa, (4) eastern/ western 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 (Roe 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 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 (10–5 Myr), to the Miocene (5–2 Myr), and to the Cenozoic (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.

<table>
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<th>Area</th>
<th>W North</th>
<th>E North</th>
<th>SE Asia/Australia</th>
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<td>W North America</td>
<td>1.00/0.70/0.7</td>
<td>0.10/1.0/1.0</td>
<td>0.50/0.7/1.0</td>
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<tr>
<td>E North America</td>
<td>—</td>
<td>0.50/0.7/1.0</td>
<td>0.10/0.5/0.7</td>
</tr>
<tr>
<td>W Eurasia</td>
<td>—</td>
<td>1.0/1.0/0.7</td>
<td>1.0/1.0/0.7</td>
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<tr>
<td>E Eurasia</td>
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<td>SE Asia/Australia</td>
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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; Kodandaramiah, 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 states 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 field.

Nuclear DNA analysis—The 36-taxon (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. angustifoilum*, *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 angustifoilum* × *S. emersum* was sister to *S. angustifoilum* in the cpDNA, nDNA, and combined data trees. The cpDNA sequence for the possible hybrid closely matched *S. angustifoilum*, while nDNA character states matched those of *S. angustifoilum* 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.*
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-taxon data set (including only *Sparganium*) yielded a p-value of 0.3, indicating there was no 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-taxon 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. *macrorhizum*) 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-taxon data set is shown in Fig. 3. The root age (divergence of Typhaceae and Bromeliaceae; secondarily calibrated at 90–105 Myr) is 100 Myr.

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**Fig. 3.** *Sparganium* BEAST chronogram for the 28-taxon, 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*.
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

(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.

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.
Estimating transitions in habit and ovary evolution—Bayes-Traits 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 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 surrendering 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.

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 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 distigmate 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, paralogy 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.

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*. These results were predicated on scoring *Typha* as unilocular. The single carpel evident in extant *Typha*, often interpreted to be pseudomonomeros (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 florescence 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 vicariant 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 vicariant pair of emergent species is not closely related, and their most recent common ancestor dates to 7.4 Ma in the late 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 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 (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. *Xanthosporangium* 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 C₄ 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 was2010). Climate change and the deterioration of for AAR at this node, nor does S-DIVA A provide a consistent ant event. However, DEC does not show signifi cant support for vicariance 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. *Xanthosporangium*) 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. *Xanthosporangium*; 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 (Eläis 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 surrendering 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-Pleistocene peak of Northern Hemisphere glaciation at 2.74 Ma (Bartoli et al., 2005) that would have partitioned an ancestral, circumtropical 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 Xanthosporangium. 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


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.


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.


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

**Taxon:** *Sparganium americanum* Nutt.; *S. americanum* Allequash Creek, Vilas Co., WI, USA; J. Sulman 855 (WIS); KF265398, KF265433, KF265365, KF265468.  
**Sparganium androcladum** (Engelm.) Morong; *S. androcladum* Pershing Wildlife Area, Taylor Co., WI; J. Sulman 868 (WIS); KF265399, KF265434, KF265366, KF265469.  
**Sparganium angustifolium** Michx.; *S. angustifolium* 1; Camp Lake, Vilas Co., WI; 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; J. Sulman 920 (WIS); KF265402, KF265437, KF265369, KF265472.  
**Sparganium angustifolium** Michx.; *S. angustifolium* 4; Bluff Creek, Walworth Co., WI, USA; J. Sulman & G. Vrtnig s.n. [photo]; KF265427, KF265462, KF265392.  
**Sparganium angustifolium** Michx.; *S. angustifolium* 5; Superior, WI, USA; J. Sulman & S. Knight 918 (WIS); KF265426, KF265461, J. Sulman 918 (WIS); KF265400, KF265435, KF265367, KF265470.  
**Sparganium emersum** Rehmann; *S. emersum* 1; Nixon Creek, Vilas Co., WI; 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, J. Sulman 912 (WIS); KF265428, KF265462, 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, KF265411, 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. Krasnovorov s.n. (MO); KF265430, KF265465, KF265395.  
**Sparganium erectum** L. subsp. *microcarpum* (Neuman) Domínguez & E. Domínguez; *S. erectum* 4; Ta. Iitti, Saaranen, Finland; J. Kokkonen & M. Korhonen 9099 (WIS); KF265429, KF265464, KF265394.  
**Sparganium eurycarpum** Michx.; *S. eurycarpum* 1; Wausau, WI; J. Sulman 825 (WIS); KF265408, KF265443, KF265375, KF265478.  
**Sparganium eurycarpum** Michx.; *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, Tazawa, Murayama-shi, Yamagata Pref., Japan; E. Hayasaka 4810 (TUS, WIS); KF265432, KF265467, KF265397.  
**Sparganium fallax** Graebn.; *S. fallax* 2; Yuyang Lake, Taiwan; Chien-I Huang 1439 (HAST); KF265410, KF265445, KF265377, KF265480.  
**Sparganium fallax** Graebn.; *S. fallax* 3; Taoyuan, Taiwan; S. Takagi, Japan; K. Sowa 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; Takawa, Murayama-shi, Yamagata Pref., Japan; E. Hayasaka 4810 (TUS, WIS); KF265432, KF265467, KF265397.  
**Typha orientalis** C.Presl.; *T. orientalis*; Fukushima Pref., Honshu, Japan; M. Usuba s.n. (TUS, WIS); KF265424, KF265459.  
**Typha angustifolia** L.; *T. angustifolia*; Minocqua, Oneida Co., WI, USA; J. Sulman 847 (WIS); KF265431, KF265448, KF265388.  
**Typha latifolia** L.; *T. latifolia*; Minocqua, Oneida Co., WI, USA; J. Sulman 847 (WIS); KF265432, KF265458, KF265390, KF265493.  
**Typha domingensis** Pers.; *T. domingensis*; Shizuoka Pref., Honshu, Japan; K. Kitamura 4 (TUS, WIS); KF265422, KF265457, KF265389, KF265482.  
**Brochocladia prismaticata** L.B.Sm.; *Brochocladia prismaticata*; 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.  
**Puya venusta** (Baker) Phil.; *P. venusta*; R. Jabaily 166 & 203 (WIS); JF299263, —, KF265364, JF968250.  
**Puya yungai** (Ruiz & Pav.) L.B.Sm.; *Puya yungai*; Chase 23826 (K) & R. Jabaily 146 (WIS); EU780865 & EU780877.  

**Note:** Collection numbers not shown.