Molecular systematics and character evolution of *Typha* (Typhaceae) inferred from nuclear and plastid DNA sequence data

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Abstract Species identification and analysis of phylogenetic relationships within the genus *Typha* are difficult because of the high degree of variability among morphological characters and frequent interspecific hybridization. Traditionally, two sections (*T.* sect. *Ebracteolatae* and sect. *Bracteolatae*) have been recognized within the genus based on the presence or absence of bracteoles in the female flowers. The aims of this study were to reconstruct the phylogeny of *Typha* using DNA sequence data from nuclear *LEAFY* and three plastid regions, and to evaluate previous classifications. We sampled nine species from various regions that were each invariant at the molecular level. A parsimony consensus tree recovered three clades in the genus: clade I including *T. angustifolia*, *T. elephantina*, *T. domingensis*, and *T. capensis*; clade II including *T. orientalis* and *T. laxmanni*; and clade III comprising *T. latifolia* and *T. shuttleworthii*. *Typha minima* was found sister to the rest of the *Typha* species with maximal bootstrap support. The results do not support previous classifications of *Typha*. Character analysis showed that bracteole loss, spatulate stigma, lack of a gap between staminate and pistillate inflorescences, and monad pollen are derived characteristics in *Typha*.

Keywords *LEAFY*; plastid DNA; phylogenetic relationship; species delimitation

■ INTRODUCTION

Typha L. (Typhaceae), known as the cattail, is widely distributed in temperate and tropical regions of the Northern and Southern Hemispheres, existing on all continents except Antarctica (Smith, 1987; Kim, 2002). The genus includes 10 to 15 species and comprises highly productive aquatic weeds (Boyd, 1971) and rapid colonizers of disturbed or newly formed wetlands (Steinbachova-Vojtiskova & al., 2006). They grow successfully in many aquatic habitats, including pond and lake margins, freshwater and brackish marshes, ditches, and even industrial waste-contaminated reservoirs (Grace & Wetzel, 1982, 1998).

Palynological, serological, and plastid DNA sequence data firmly associate *Typha* with *Sparganium* L. (Lee & Fairbrothers, 1972; Punt, 1975; Chase, 2004; Janssen & Bremer, 2004), and are in support of traditional taxonomic treatments. The earliest fossil record of either genus is from the Paleocene Fort Union Formation, based on typhaceous pollen (Wilson & Webster, 1946). Based on unequivocal fruits from the Eocene series of Wyoming (Berry, 1924), *Sparganium* has a slightly older fossil record than *Typha* (Daghlian, 1981).

The members of the genus *Typha* share several synapomorphies including dense, elongate, cylindrical spike-like inflorescences, as well as female flowers having numerous capillary bristles (Takhtajan, 2009). Because of the conspicuous morphological synapomorphies of *Typha*, it is not difficult to recognize a plant as being a member of the genus. There are difficulties, however, with the identification of species and the analysis of phylogenetic relationships within the genus. The taxonomic difficulties in *Typha* are due in part to the high degree of variability of vegetative and reproductive characters (Grace & Wetzel, 1982; Kim, 2002). Another important source of taxonomic complexity is the frequent occurrence of interspecific hybridization (Smith, 1967; Krattinger & al., 1979; Kuehn & White, 1999; Selbo & Snow, 2004). For example, *T. glauca* Godr. is considered a hybrid between *T. latifolia* L. and *T. angustifolia* L. based on morphologic, isozyme, and microsatellite-based analyses (Fassett & Calhoun, 1952; Bayly & O'Neill, 1971; Snow & al., 2010). Although *Typha* species from Europe, Russia, India, North America, Australia, and East Asia are reasonably well known within each respective region (Hotchkiss & Dozier, 1949; Cook, 1980; Finlayson & al., 1985; Fedchenko, 1986; Kadono, 1996; Thieret & Luken, 1996; Guha & Mondal, 1998; Kim & al., 2003), species delimitation within *Typha* on a world basis is not yet firmly established.

Typha was first described by Linnaeus (1753) as *Typha angustifolia* L. Kronfeld (1889) proposed the first classification of the species of *Typha* (Table 1), based on the presence/absence of a bracteole in the female flowers, the ratio of staminate inflorescence length to pistillate inflorescence length, and plant height. Kronfeld recognized two sections within the genus, each with two subsections: *Typha* sect. *Ebracteolatae* Kronf. with subsect. *Schuria* Kronf. and subsect. *Engleria* Kronf. and *T.* sect. *Bracteolatae* Kronf. with subsect. *Schnizleinia* Kronf. and subsect. *Rohrbachia* Kronf. Graebner (1900) recognized the two sections but did not accept the subsections distinguished by Kronfeld. Smith (1987) placed 11 species into six groups, without recognition of section or subsection, based on stigma shape, the presence or absence of a bracteole, and pollen cell unit (Table 1). From sect. *Bracteolatae*, *T. minima* Funck ex Hoppe has been placed into the new genus *Rohrbachia* (Kronf.) Mavrodiev on

the basis of its unique morphology (e.g., hairs on stalk of female flowers swelling at tip; Mavrodiev, 2001). Previous classification attempts using morphological data have been hampered by extensive quantitative and overlapping characters, and evolutionary relationships among *Typha* species remain unclear.

The use of molecular techniques has helped in delineating species and understanding phylogenetic relationships in other taxonomically difficult groups (e.g., Devos & al., 2006; Kim & al., 2009, 2010). In this study, four DNA sequence regions (nuclear *LEAFY* and plastid *trnL-F*, *trnC-petN*, and *psbM-trnD*) are used to examine the interspecific relationships within *Typha*. *LEAFY* is a transcription factor known to affect inflorescence development in Poaceae (Bomblies & Doebly, 2005). This gene is distributed in all plants including mosses, ferns, gymnosperms, and angiosperms (Frohlich & Parker, 2000; Himi & al., 2001). Phylogenetic analyses of amino acid sequences of *LEAFY* suggest that the gene duplicated on the stem lineage leading to seed plants, but that one copy was lost in angiosperms, making it a single copy gene in diploid angiosperms (Frohlich & Parker, 2000; Himi & al., 2001). The nucleotide sequence from the second intron of *LEAFY* has been used in many phylogenetic studies with high utility (Oh & Potter, 2005; Kim & al., 2010). Although many plastid DNAs (ptDNA) showed low level of genetic variation compared to *LEAFY*, ptDNA sequences have proven useful for understanding the evolutionary relationships and classification in many plant groups (e.g., Lee & Wen, 2004; Su & al., 2008; Kim & al., 2010).

The objectives of this study were to present a comprehensive phylogenetic reconstruction of *Typha* using nuclear and plastid DNA sequence data and to evaluate the previous classification systems in the light of our findings. Based on the inferred phylogeny, we also provide a discussion on the evolution of morphological characters within the genus.

■ MATERIALS AND METHODS

Plant materials. — A complete list of taxa used in this study, including voucher information, is presented in the Appendix. Nine of eleven previously recognized species of *Typha* (Cook, 1980; Smith, 1987) were sampled: *T. angustifolia* L., *T. capensis* Rohrb., *T. domingensis* Pers., *T. elephantina* Roxb., *T. latifolia* L., *T. laxmanni* Lepech., *T. minima* Funck ex Hoppe, *T. orientalis* C. Presl, and *T. shuttleworthii* W.D.J. Koch & Sond. Two hybrid species, *T. glauca* and *T. subulata* Crespo & Perez-Moreau were not included (Smith, 1987). Our sampling included of all recognized sections and subsections of *Typha* (Kronfeld, 1889; Graebner, 1900). Sixty samples of the nine *Typha* species were sequenced for nuclear *LEAFY* and ptDNA. We used the five *Sparganium* species (*S. hyperboreum* Laest. ex Beurl., *S. emersum* Rehm., *S. fallax* Graebner, *S. erectum* L., *S. eurycarpum* subsp. *coreanum* (H. Lév.) C.D.K. Cook & M.S. Nicholls) as outgroups based on results from previous monocotyledon phylogenies (Duvall & al., 1993; Janssen & Bremer, 2004; Givnish & al., 2006).

DNA isolation, PCR amplification, and sequencing. — Total genomic DNA was isolated from fresh or dried leaves by a rapid DNA minipreparation method (Chen & Ronald,

Table 1. Previous classifications of *Typha*.

Kronfeld (1889)	Graebner (1900)	Smith (1987)	
Sect. *Ebracteolatae*	Sect. *Ebracteolatae*	Group I	*T. latifolia*
Subsect. *Schuria*	*T. latifolia*		*T. shuttleworthii*
T. latifolia	*T. shuttleworthii*	Group II	*T. angustifolia*
T. shuttleworthii	*T. capensis*[b]		*T. domingensis*
T. capensis	*T. orientalis*[c]		*T. subulata*
T. orientalis	*T. laxmanni*	Group III	*T. angustifolia* × *latifolia* (= *T. glauca*)
Subsect. *Engleria*			*T. orientalis*
T. laxmanni			*T. capensis*
Sect. *Bracteolatae*	Sect. *Bracteolatae*	Group IV	*T. laxmanni*
Subsect. *Schnizleinia*	*T. angustifolia* (= *T. angustata*)[a]	Group V	*T. minima*[d]
T. angustifolia (= *T. angustata*)[a]	*T. domingensis*	Group VI	*T. elephantina*
T. domingensis	*T. elephantina*		
T. elephantina	*T. minima* (= *T. gracilis*)[d]		
Subsect. *Rohrbachia*			
T. minima			
Hybrid species	Hybrid species		
T. glauca	*T. glauca*		

[a] Kim (2002) considered that *T. angustata* should be included in *T. angustifolia*.
[b] Graebner (1900) placed *T. capensis* as a subspecies of *T. latifolia*.
[c] Graebner (1900) placed *T. orientalis* as a subspecies of *T. shuttleworthii*.
[d] *Typha minima* comprises three species (*T. lugdunensis*, *T. martinii*, *T. gracilis*) according to Cook (1980).

1999). Two degenerate primers, LFtxr and LFsxl-3, were used for *LEAFY* amplification from *T. latifolia*, *T. angustifolia*, and *Sparganium erectum* (Frohlich & Meyerowitz, 1997). PCR products were excised from agarose gels, purified with a Gel and PCR Cleanup Kit (Solgent, Daejeon City, South Korea), and cloned using the pGEMT-easy Vector System (Promega Biosciences, San Luis Obispo, California, U.S.A.). Plasmid DNA was purified using the plasmid DNA Miniprep System (Qiagen, Valencia, California, U.S.A.) and sequenced with T7 and Sp6 universal primers on an ABI3730 automated sequencer (Applied Biosystems, Foster City, California, U.S.A.). Fifteen clones from the three taxa were sequenced. Specific primers TlfyF-3 (5′-GGCGTTCGGCCCTTTGGGAG) and TlfyR-2 (5′-AGTGTTCCGGTACGCCAAGAA) were then developed based on the cloned sequences and used to amplify *LEAFY* sequences for additional individuals. The ptDNA *trnL-F* region (including the *trnL* gene, *trnL* intron, and *trnL-F* intergenic spacer) was amplified using primers "c" and "f" (Taberlet & al., 1991). A new primers combination for the amplification of ptDNA *trnC-petN* and *psbM-trnD* intergenic spacer region was designed based on *T. latifolia* ptDNA sequence available from GenBank (accession number GU195652; Guisinger & al., 2010). The *trnC-petN* region was amplified using primers TytrnCF (5′-AGCGGTAAGGCAGGGGACTGCAA) and TypetNR (5′-TAAAGCAGCCCAAGCGAGACTT). PCR amplification of the *psbM-trnD* region was performed with primers TypsbMF (5′-ACTAGAATGAACAGTGCAGTAGC) and TytrnDR (5′-TCAAGGCGGAAGCTGCGGGTTCGA).

Genomic DNA (200 ng in 25 µl) was amplified with a PTC-200 thermal cycler (MJ Research, Waltham, Massachusetts, U.S.A.) programmed for 4 min at 94°C; 40 cycles of 1 min at 94°C (denaturation), 1 min at 55°C–58°C (annealing; 55°C, *LEAFY* and *trnL-F*; 58°C, *trnC-petN* and *psbM-trnD*), and 1 min 30 s at 72°C (extension), with a final cycle of 10 min at 72°C.

Amplified DNA samples were purified for sequencing using a PCR purification kit (Qiagen), in accordance with the supplier's specifications, to remove small molecular fragments of primer and dNTPs. All PCR products were directly sequenced in both directions using the amplification primers on an ABI3730 automated sequencer (Applied Biosystems). All DNA sequence data have been deposited in GenBank under the following accession numbers: GU646680–GU646687, GU646689, GU646694–GU646696, GU646698, GU646701, GU646704–GU646709, GU646711, GU646714, GU646717–GU646718, GU646723, GU646727–GU646733, GU646738, GU646743, GU646751, GU646760–GU646767, GU646770–GU646771, JF319445–JF319659 (Appendix).

Phylogenetic analysis. — Complementary DNA sequences were assembled for each individual using Codoncode Aligner v.3.7.1 (http://www.codoncode.com). Multiple-sequence alignment was performed using ClustalX v.1.81 with the default alignment parameters (Thompson & al., 1997) and then manually adjusted by use of the alignment criterion presented by Zurawski & Clegg (1987) in which gaps are considered as characters and the number of evolutionary events is attempted to be minimized. Parsimony-informative gaps were coded as binary characters using simple indel coding (Simmons & Ochoterena, 2000). The incongruence length difference (ILD) test was conducted to assess data congruency (Farris & al., 1995) with one accession per species because all species sampled are each monophyletic (see Results). The ILD test was performed using PAUP* v.4.0b10 (Swofford, 2002) and 1000 heuristic search replications. This test has been used in several phylogenetic studies as a criterion to assess the appropriateness of combining data from different genes (e.g., Dolphin & al., 2000). Most published reports suggest that assessing the significance of incongruence at $P < 0.05$ although this threshold is too conservative for the homogeneity test (Cunningham, 1997).

Maximum parsimony (MP) analyses were performed using PAUP* v.4.0b10. All characters and character states were weighted equally and unordered. Each dataset was analyzed separately and then in combined analyses with ptDNA and then a simultaneous analysis (Kluge, 1989; Nixon & Carpenter, 1996) was performed with all regions. Combined data matrices for this study were deposited in TreeBASE (http://www.treebase.org/; study accession number, SN11267). Searches were conducted using 100 random-taxon-addition replicates with tree bisection-reconnection (TBR) branch swapping, and MulTrees in effect, using maxtrees = 100,000. Bootstrap analyses (BP, 1000 pseudoreplicates) were conducted to examine the relative level of support for clades on the cladograms (Felsenstein, 1985). Phylogenetic analyses of *LEAFY* and combined data were also conducted under Bayesian MCMC inference (BI; Yang & Rannala, 1997) using MrBayes v.3.12 (Ronquist & Huelsenbeck, 2003). Applying the Akaike information criterion (AIC; Akaike, 1974), MODELTEST v.3.1 (Posada & Crandall, 1998) assigned the GTR+G model for the simultaneous-analysis sequence data. For *LEAFY* and combined analyses in which gap coding data was included, the datatype = standard option of MrBayes for the non-nucleotide data partition was used. Four chains of the Markov Chain Monte Carlo (MCMC) were run simultaneously, sampled every 100 generations for a total of two million generations. The first 200,000 generations (i.e., 2000 trees) were deleted as the "burn in" of the chains. We plotted the log-likelihood scores of sample points against generation time using TRACER v.1.5 (Rambaut & Drummond, 2003) to ensure that stationarity was achieved after the first 200,000 generations by checking whether the log-likelihood values of the sample points reached a stable equilibrium (Huelsenbeck & Ronquist, 2001). In addition, we used AWTY (Nylander & al., 2008) to compare split frequencies in the different runs and to plot cumulative split frequencies to ensure that stationarity was reached. Of the remaining 36,000 trees (18,000 from each parallel run), a maximum a posteriori tree was constructed by summarizing the remaining trees from parallel runs in a majority rule consensus tree, yielding the posterior probability (PP) values for each clade.

Morphological character evolution analysis. — *Typha* species exhibit a wide range of morphological characters that have been shown to be taxonomically useful in species recognition (Kim, 2002). Because many traits are quantitative and have overlapping variation ranges, such traits were not selected for character analysis due to difficulties in coding the variations as

discrete character states. In this study, four morphological characters (absence or presence of bracteoles in female flower, shape of stigma, gap between staminate and pistillate inflorescence, number of pollen units) were included in the morphological character evolution analysis. All four traits were selected because of their potential for inferring relationships among species in *Typha* (Kronfeld, 1889; Graebner, 1900; Smith, 1987). The number of pollen units was coded as (0) monad or (1) tetrad; stigma shape as (0) filiform or (1) spatulate; bracteoles in female flower as (0) absent or (1) present; gap between staminate and pistillate inflorescence as (0) present or (1) absent. Each character was scored by examining live material and herbarium specimens from AJOU, BRI, CANB, IBIW, LE, WU.

The most parsimonious topology from the analysis of the combined molecular data (*LEAFY*+three ptDNA loci) was applied to reconstruct histories of character states of key morphological characters. Unweighted and unordered MP reconstructions of characters states were performed in Mesquite v.2.0 (Maddison & Maddison, 2007) using unambiguous optimization.

■ RESULTS

***LEAFY* data analysis. —** The *LEAFY* alignment, comprising 65 accessions from nine *Typha* species and five outgroups, contained 835 characters including 46 simple gap coding characters. There were 293 variable sites, of which 250 (29.9%) were parsimoniously informative (Table 2). The parsimony analysis of *LEAFY* data resulted in >100,000 equally parsimonious trees (tree length = 359, ensemble consistency index [CI; Kluge & Farris, 1969] = 0.920, ensemble retention index [RI; Farris, 1989] = 0.978).

The strict consensus tree is shown in Fig. 1. The BI phylogram was identical in topology to the MP strict consensus tree (BI tree not shown) except two individuals (TC_Sa01, TC_Sa03) of *T. capensis* were sister group to the clade including *T. angustifolia*, *T. elephantina*, *T. domingensis*, one individual (TC_Sa02) of *T. capensis*, *T. orientalis*, and *T. laxmanni* species. All *LEAFY* sequences were grouped according to their taxonomic species, except for *T. shuttleworthii* and *T. capensis* (Fig. 1). The samples of *T. shuttleworthii* and *T. latifolia* were not resolved and *T. capensis* was resolved as paraphyletic (albeit with <50% BP). Several relationships were inferred from the strict consensus tree (Fig. 1): (1) *T. minima* is a sister to the clade consisting of the rest of the *Typha* species with maximal support (BP = 100%, PP = 1.00); (2) *T. angustifolia* and *T. elephantina* are sister groups (BP = 96%); (3) *T. orientalis* and *T. laxmanni* are sister groups (BP = 89%). *Typha domingensis* is sister to *T. angustifolia–T. elephantina* but with low statistical support (BP = 58%, PP = 0.75). Although most of the large clades within *Typha* clade were poorly supported, sections *Ebracteolatae* and *Bracteolatae* were resolved as paraphyletic and polyphyletic groups, respectively (Fig. 1).

Plastid DNA data analysis. — The characteristics and statistics of individual ptDNA region from the MP analyses are presented in Table 2. The *trnC-petN* sequence produced the greatest proportion of variable and parsimony-informative characters. Each individual ptDNA region provided very low resolution among *Typha* species, with most branches unresolved in the three separated MP strict consensus trees (data not shown). Despite this, analyses of ptDNA *trnL-F* and *trnC-petN* region showed that three species, *T. domingensis*, *T. elephantina*, and *T. capensis* formed a monophyletic group.

The ILD test indicated that three ptDNA sequence data partitions were not significantly incongruent ($P = 0.101$). Maximum parsimony analysis performed on combined ptDNA data matrix yielded a strict consensus tree from >100,000 equally parsimonious trees (tree length = 581, CI = 0.880, RI = 0.971). The BI phylogram was identical in topology to the strict consensus tree sampled by the MP analysis, except that *T. orientalis* was sister to a clade consisting of *T. latifolia* and *T. shuttleworthii* (BI phylogram not shown). The strict consensus MP tree clearly indicated the distinct nature of *Typha* species, including *T. shuttleworthii*, *T. capensis* which were not grouped to their taxonomic species in *LEAFY* data (Fig. 2). Also, classification according to sections *Ebracteolatae* and *Bracteolatae* is not supported. Consistent with results from *LEAFY*, *T. minima* is sister to all remaining *Typha* species (BP = 100, PP = 1.00). Within the remaining species, one clade (*T. elephantina*, *T. domingensis*,

Table 2. Tree statistics of the *LEAFY*, *trnL-F*, *trnC-petN*, *psbM-trnD*, and combined datasets from maximum parsimony (MP) analysis.

					Combined	
Parameters	*LEAFY*	*trnL-F*	*trnC-petN*	*psbM-trnD*	3 ptDNA	*LEAFY*+3 ptDNA
Number of sequences (ingroup/outgroup)	65 (60/5)	65 (60/5)	65 (60/5)	65 (60/5)	65 (60/5)	65 (60/5)
Aligned length (bp/indel characters)	835 (789/46)	999 (968/31)	1024 (1003/21)	1150 (1119/31)	3173 (3090/83)	4008 (3829/129)
Variable characters (%)	293 (35.1%)	124 (12.4%)	230 (22.5%)	146 (12.7%)	500 (15.8%)	793 (19.8%)
Parsimony informative characters (%)	250 (29.9%)	110 (11.0%)	145 (14.2%)	138 (12.0%)	393 (12.4%)	643 (16.0%)
Number of trees (MP)	>100,000	>100,000	1	>100,000	>100,000	>100,000
MP tree length	359	150	254	161	581	960
Ensemble consistency index (CI)[a]	0.920	0.853	0.941	0.928	0.880	0.873
Ensemble retention index (RI)	0.978	0.966	0.985	0.985	0.971	0.967
Model selected (AIC)	GTR+G				GTR+G	GTR+G

[a]The ensemble consistency index is calculated excluding uninformative characters.

and *T. capensis*) could be recognized (BP = 86%, PP = 0.99). *Typha latifolia* formed a clade with *T. shuttleworthii* but with only weak support (BP = 53%, PP = 0.75). The individuals of *T. latifolia* were divided into East Asia and North America clades (Fig. 2). In addition, *T. latifolia* from Finland is sister to those of North American *T. latifolia* (BP = 90%, PP = 0.99).

Combined analysis of datasets. — The ILD test indicated that *LEAFY* and ptDNA sequence data partitions were not significantly incongruent ($P = 0.069$). When all molecular datasets were combined, the strict consensus MP tree was better resolved than either individual analysis. Among the 4008 characters of the combined datasets, 643 (16.0%) were parsimoniously

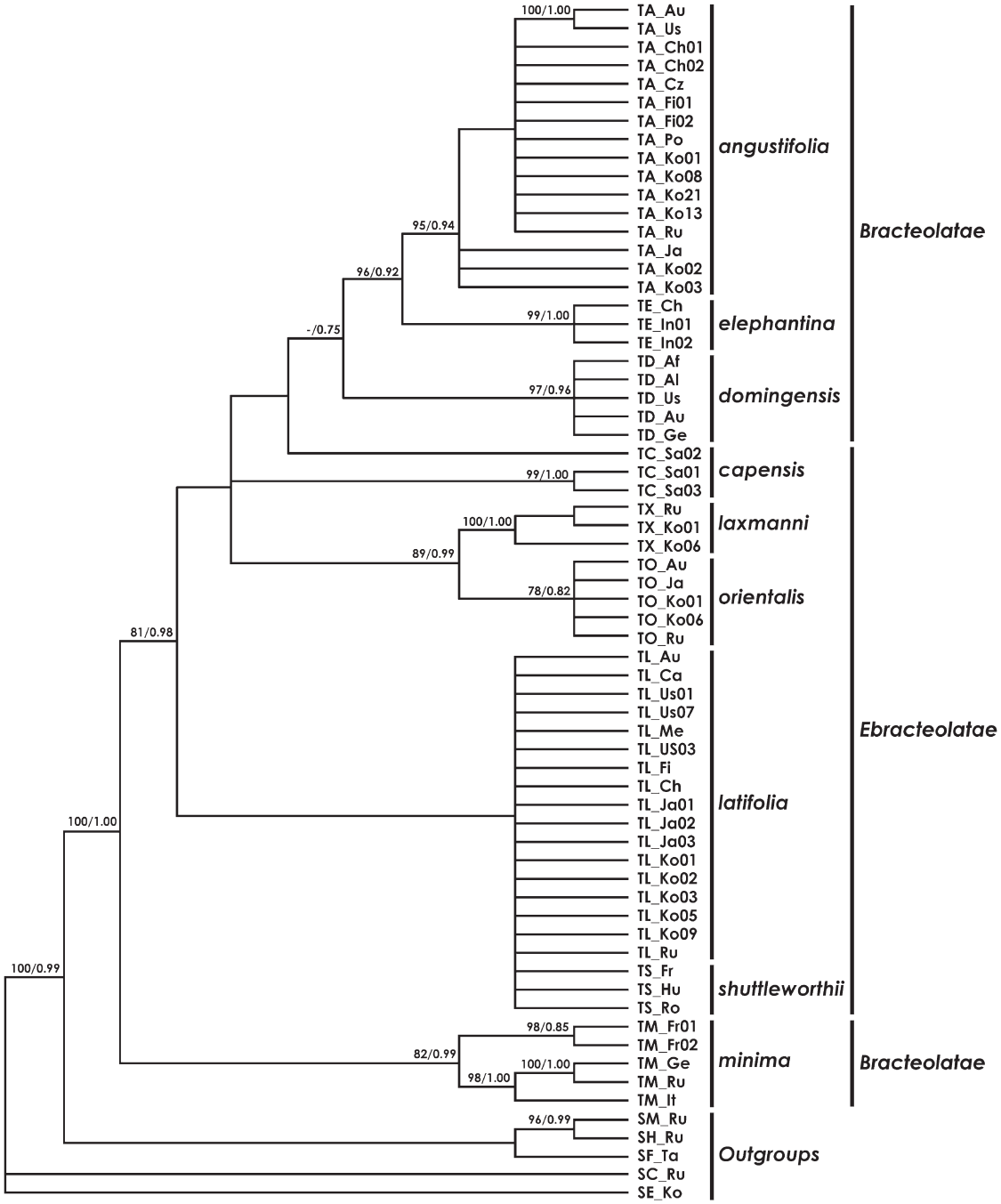

Fig. 1. Strict consensus of 100,000 most parsimonious trees (tree length = 359, CI = 0.920, RI = 0.978) from parsimony analysis of *LEAFY* sequence data of *Typha* and *Sparganium* species. Numbers above branches indicate support values (maximum parsimony bootstrap [BP]/Bayesian posterior probability [PP]); dash (-) indicates that a node did not receive >75% BP by MP analysis. Geographic origin of *Typha* and *Sparganium* specimens indicated by country abbreviations (Af, Afghanistan; Al, Algeria; Au, Australia; Ca, Canada; Ch, China; Cz, Czech Republic; Fi, Finland; Fr, France; Ge, Germany; Hu, Hungary; In, India; It, Italy; Ja, Japan; Ko, South Korea; Me, Mexico; Po, Poland; Ro, Romania; Ru, Russia; Sa, South Africa; Ta, Taiwan; Us, U.S.A.). See Appendix for OTU abbreviations.

informative (Table 2). Phylogenetic analysis of the combined dataset resulted in >100,000 equally parsimonious trees, each of 960 steps (CI = 0.873, RI = 0.967). The BI phylogram was identical in topology to the MP strict consensus tree sampled (BI phylogram not shown). In the strict consensus tree, three main clades (I, II, III) in the genus *Typha* were recognized (Fig. 3). Clade I included *T. angustifolia*, *T. elephantina*, *T. domingensis*, and *T. capensis* (BP = 84%, PP = 0.99). Within this clade, *T. angustifolia* was sister to *T. elephantina* with moderate support (BP = 78%, PP= 0.95). Clade II included *T. orientalis* and *T. laxmanni* (BP = 88%, PP = 1.00) and was sister to clade I with moderate support (BP = 75%, PP = 0.90). Clade III comprised *T. latifolia* and *T. shuttleworthii* (BP = 98%, PP = 0.99). *Typha minima* was sister to the group consisting of the rest of the *Typha* species, with maximal support (BP = 100%, PP = 1.00). Consistent with individual results from *LEAFY* and ptDNA, sections *Ebracteolatae* and *Bracteolatae* were perceived to be paraphyletic and polyphyletic groups, respectively (Fig. 3).

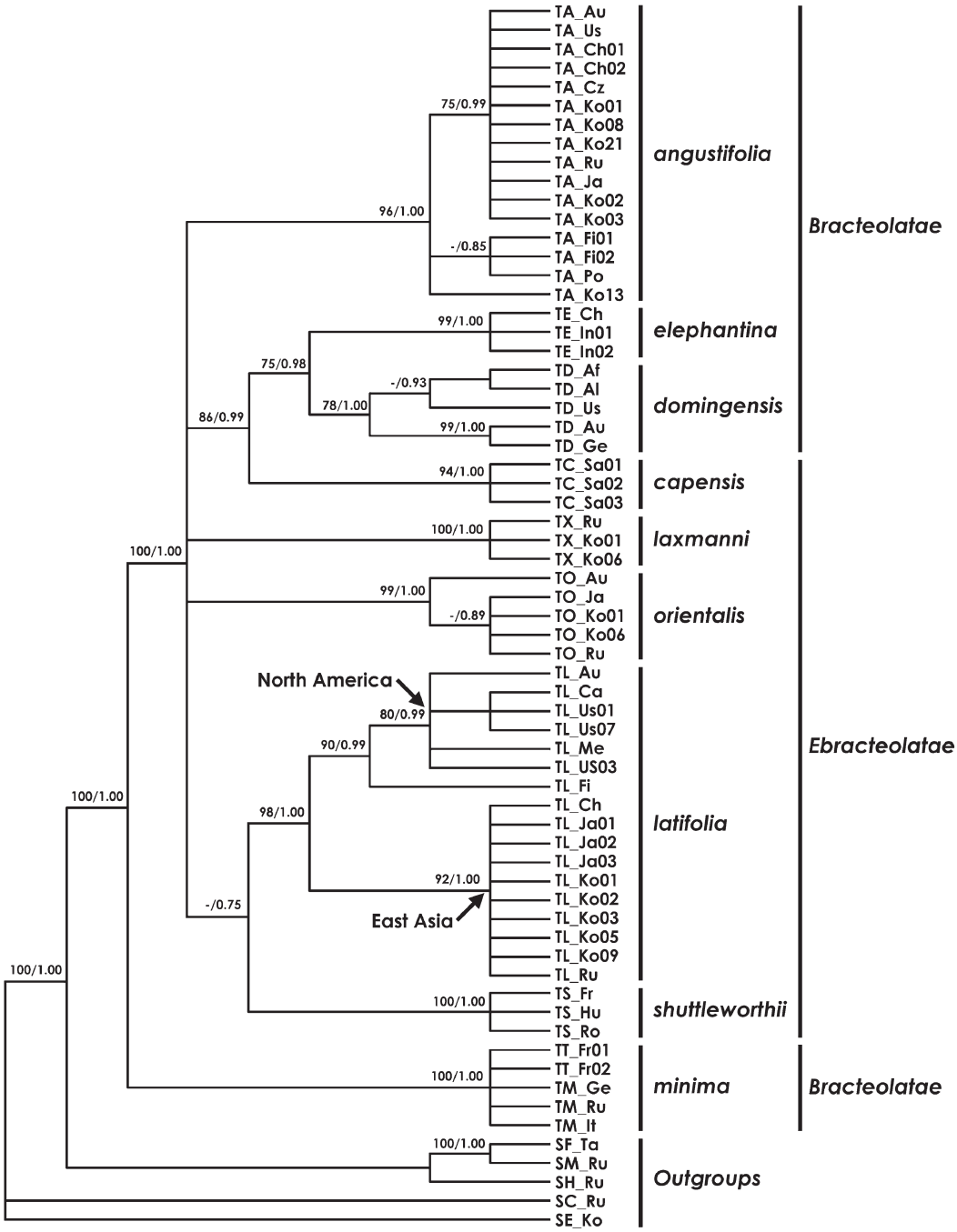

Fig. 2. Strict consensus of 100,000 most parsimonious trees (tree length = 581, CI = 0.880, RI = 0.971) from parsimony analysis of combined plastid DNA sequences data of *Typha* and *Sparganium* species. For further explanation see Fig. 1.

Morphological character evolution analysis. — Using the cladogram constructed from combined *LEAFY* and ptDNA sequence data as a reference (Fig. 4), two species (*T. latifolia*, *T. shuttleworthii*) of clade III have identical states for all four morphological characters although no synapomorphies for this clade were identified. The character states of stigma shape and bracteoles in the female flower appear to have evolved at least two times within *Typha*, whereas the gap between staminate and pistillate inflorescence and the number of pollen units appear to have evolved at least three times (Fig. 4). All ingroup taxa

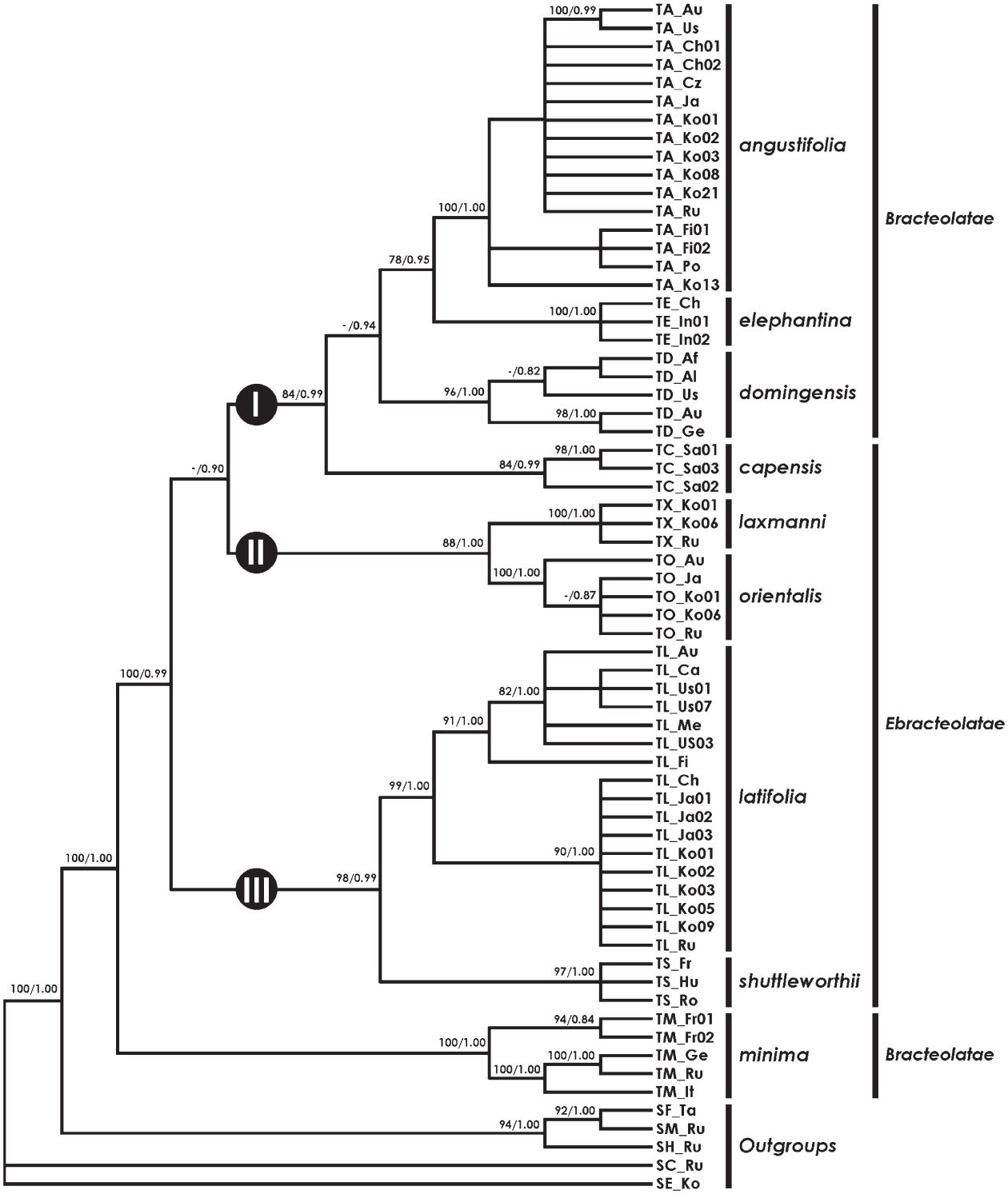

Fig. 3. Strict consensus of 100,000 most parsimonious trees (tree length = 960, CI = 0.873, RI = 0.967) from parsimony analysis of combined *LEAFY* and plastid DNA sequences data of *Typha* and *Sparganium* species. For further explanation see Fig. 1.

possessed alternative states for two characters, stigma shape and bracteoles in female flowers, indicating that they were strongly correlated. Stigma shape is also highly correlated with the character state of gap between staminate and pistillate inflorescence in all taxa except *T. laxmanni* (Fig. 4). However, the pollen unit is not correlated with the stigma shape, bracteoles, and gap between staminate and pistillate inflorescence. *Typha minima* and two species of clade III (*T. latifolia*, *T. shuttleworthii*) have tetrad pollen units whereas species of clade I (except *T. elephantina*) and II have a monad pollen units (Fig. 4).

■ DISCUSSION

Species classification. — Accurate taxon identification is a prerequisite for the evaluation of phylogenetic relationships. Many previous attempts to classify *Typha* species have resulted in the adoption of a loosely defined and intuitive approach to taxonomy, based largely on field observations and incorporating the analysis of some micromorphological characters (Hotchkiss & Dozier, 1949; Finlayson & al., 1985). The delimitation of *Typha* species based on morphology alone is often not possible (Kuehn & White, 1999; Zhang & al., 2008) due to the high degree of plasticity among the morphological characters. Therefore, a number of molecular markers (e.g., randomly amplified polymorphic DNA [RAPD], amplified fragment length polymorphism [AFLP], or sequence information of nuclear and ptDNA) have been used for *Typha* species delineation, at least as a supplemental tool (Kuehn & al., 1999; Zhang & al., 2008; Na & al., 2010).

The molecular-phylogenetic analysis confirmed the traditional delimitation of *Typha* species even when samples were included from different continents, with the exception of *T. capensis* and *T. shuttleworthii* in the *LEAFY* phylogeny (Fig. 1). *Typha capensis* formed an unresolved group with *Typha* species including *T. angustifolia*, *T. elephantina*, *T. domingensis*, *T. laxmanni*, and *T. orientalis* (Fig. 1). According to Smith (1987), *T. capensis* is very similar to North American hybrids (e.g., *T. latifolia* × *T. angustifolia* [= *T. glauca*]) or *T. orientalis* from Australia. Although there is no obvious morphological autapomorphy for *T. capensis* in this study, this species can be distinguished from *T. glauca* and *T. orientalis* by a subulate anther (Kim, 2002). Moreover, ptDNA and combined analyses indicated that *T. capensis* is distinct from *T. orientalis* (Figs. 2–3).

Unexpectedly, *LEAFY* sequences of *T. shuttleworthii* from Europe and *T. latifolia* from Eurasia, North America, and Australia were identical (Fig. 1). According to Krattinger & al. (1979), there seems to be no genetic isolation mechanism preventing hybridization between *T. shuttleworthii* and *T. latifolia* in Switzerland. However, *T. shuttleworthii* is distinguished from *T. latifolia* by two morphological characters: a short staminate inflorescence (<6 cm) and narrow leaves (Kim, 2002). Plastid DNA sequences also showed differences between individuals of *T. shuttleworthii* and *T. latifolia* (Fig. 2). Therefore, we conclude that European *T. shuttleworthii* is genotypically distinct from *T. latifolia*.

Phylogenetic relationships. — Our study recovered *T. minima* as sister to the rest of the species of *Typha* (Fig. 3). Traditionally, *T. minima* has been included in sect. *Bracteolatae* based on the presence of bracteole morphology (Kronfeld, 1889; Graebner, 1900; but see Mavrodiev, 2001). However, this species does not have a close relationship with the other species of sect. *Bracteolatae*. Unlike other *Typha* species, which have a long vegetative shoot (>1 m), *T. minima* has a relatively

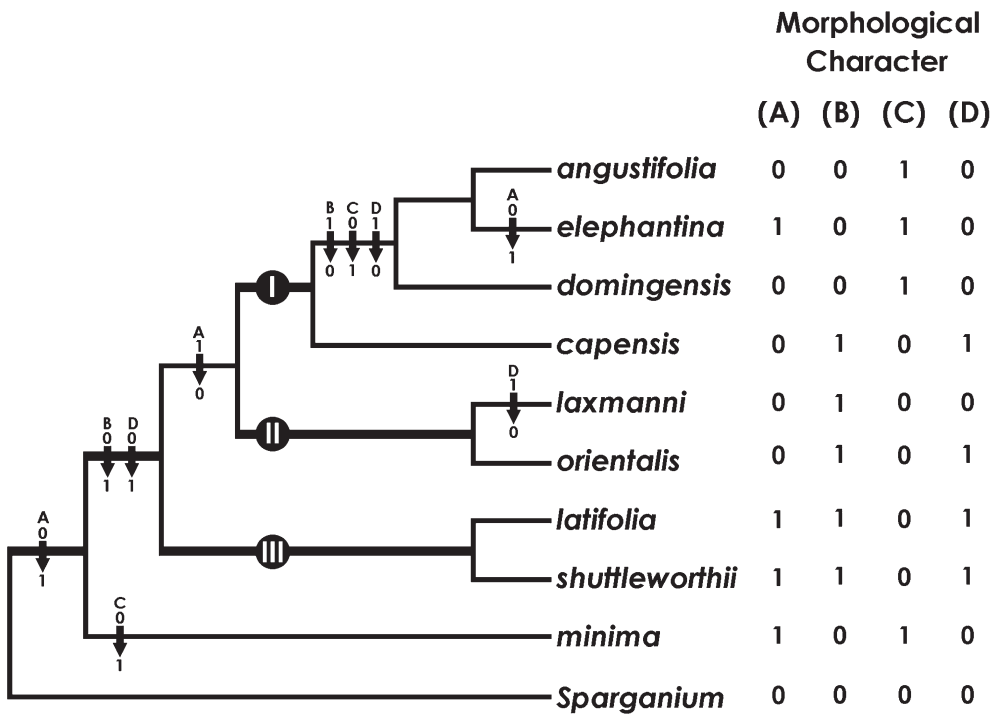

Fig. 4. Distribution of morphological traits on the MP strict consensus tree topology from the combined molecular data. (A) pollen unit, (B) stigma shape, (C) presence or absence of bracteoles in female flowers, (D) gap between staminate and female inflorescence. Character states of *Sparganium* coded as "0". Strong branch support (BP > 80%, PP > 0.99) indicated by bold lines.

short shoot (usually a maximum of 0.6 m) and hairs on the stalk of female flowers swelling at tip (Smith, 1987; Mavrodiev, 2001). Hybridization experiments indicated that the species is genetically distinct (Krattinger & al., 1979). Recently, the transfer of *T. minima* to the new genus *Rohrbachia* was suggested by Mavrodiev (2001). However, additional molecular studies should be pursued to determine the relationships of *T. minima* to the species of *Rohrbachia* (e.g., *R. alekseevii* Mavrodiev).

As to species of clade I (*T. angustifolia*, *T. elephantina*, *T. domingensis*, *T. capensis*), some authors recognized *T. elephantina* as a distinct group based on different morphology (e.g., stiff trigonal leaves and tetrad pollen; Smith & al., 1987; Sharma & Gopal, 1980). However, in our analysis this species is sister to *T. angustifolia* with moderate support. Several morphological features, including the gap between staminate and pistillate inflorescence, filiform stigma, and presence of bracteoles in the female flower were shared in *T. angustifolia*, *T. domingensis*, and *T. elephantina* (although no such synapomorphies were inferred for the clade of *T. angustifolia*+*T. elephantina*). The close relationship among the three species corresponds to sect. *Bracteolatae* subsect. *Schnizleinia* Kronfeld.

A conflict between our results and previous classifications involved *T. capensis*. This species has previously been placed in sect. *Ebracteolatae* (Kronfeld, 1889; Graebner, 1900). *Typha capensis* is morphologically distinct within clade I; it has no gap between staminate and pistillate inflorescences, has spatulated stigmas, and lacks bracteoles in female flowers. Although the phylogenetic placement of *T. capensis* in clade I is surprising from a morphological perspective, our ptDNA data suggested that *T. capensis* is sister to *T. elephantina* and *T. domingensis* (Fig. 2). Although we do not have the morphological characters to support the relationship of the three species in the current study, they can be distinguished from the other *Typha* species by sharing a subulate anther (Kim, 2002). Our results also indicated that the nature of the bracteoles and the gap between staminate and pistillate inflorescence may be reversals in *T. capensis* (Fig. 4).

The two species of clade II (*T. orientalis*, *T. laxmanni*) share morphological characters such as spatulate stigma, absence of bracteoles, and monad pollen, but are different in presence/absence of gap between staminate and pistillate inflorescences (Fig. 4). The two subsections *Schuria* and *Engleria* proposed by Kronfeld (1889) were not supported by our studies. Of the species of subsect. *Schuria* sampled, *T. orientalis* is sister to *T. laxmanni* of subsect. *Engleria* (Fig. 3). Based on pollen morphology and abortive flower shape, Mavrodiev (2002) suggested that *T. orientalis* should be placed in *Engleria*. A close relationship between *T. orientalis* and *T. laxmanni* was also indicated in phenetic analysis of *Typha* species from Korea and far eastern Russia using 25 quantitative morphological characters (Kim & al., 2003). Graebner (1900) treated *T. orientalis* as a subspecies of *T. shuttleworthii*. Indeed, plants of *T. orientalis* are very similar to *T. shuttleworthii* in general appearance. The two taxa share narrow leaves, short staminate inflorescence, and no gap between staminate and pistillate inflorescence (Kim, 2002). However, the present study indicates that *T. orientalis* is sister to *T. laxmanni* instead of *T. shuttleworthii*.

The sister relationship of the two species of clade III, *T. latifolia* and *T. shuttleworthii*, is morphologically supported by absence of a gap between staminate and pistillate inflorescences, spatulate stigma, absence of bracteoles in the female flower, and pollen dehisced in tetrads (Fig. 4). The two species can be distinguished from those of clades I (but not *T. elephantina*) and II by tetrad pollen (Fig. 4). The close relationship between *T. latifolia* and *T. shuttleworthii* is consistent with previous classifications (Kronfeld, 1889; Graebner, 1900; Smith, 1987); both species were placed in subsect. *Schuria* by Kronfeld (1889).

The most wide ranging *Typha* species are *T. latifolia* and *T. angustifolia*. This study offered the opportunity to compare the biogeographic and genetic patterns of these two species in East Asia and North America. Although an intercontinental comparison based on a limited sampling is difficult, our results allow some preliminary conclusions. *Typha latifolia* showed relatively high genetic divergence between the East Asian and North American disjunction, as measured with ptDNA sequence data (Fig. 2). On the other hand, a low sequence divergence (Fig. 2) was found between East Asian and North American *T. angustifolia*. *Typha angustifolia* is considered to be indigenous to North America, but may actually have been introduced during early European settlement (Stuckey & Salamon, 1987). The commonness of the species in North America has rapidly increased since the mid-20th century (Shih & Finkelstein, 2008). A recent introduction of *T. angustifolia* in North America would be supported by the low sequence divergence found in this study.

Evolution of morphological characters. — Although vegetative morphological characters are considered as homoplasious states in *Typha*, the evolution of four reproductive characters (absence or presence of a bracteole in the female flower, stigma shape, the gap between staminate and pistillate inflorescence, and the number of pollen units) can be inferred in clades I, II, and III based on the combined strict consensus tree (Fig. 4). Changes in reproductive structure are of fundamental importance in the early evolution and subsequent diversification of species (Rudall & al., 2005). The four character state distributions are discussed here in view of their support for the major clades of *Typha* and their adaptive significance in terms of breeding system.

In general, a bracteole in the female flower may serve to control seed germination and aid seed dispersal by wind and water (Gustafsson, 1973; Osmond & al., 1980; Ungar & Khan, 2001). Osmond & al. (1980) indicated that bracteoles of *Atriplex* spp. could aid in the wind dispersal of seeds. Bracteoles may also be significant in determining the distribution of species through the dispersal of seeds by water (Gustafsson, 1973). Although some molecular analyses have not been able to provide fine enough resolution to infer the evolution of bracteole shape, bracteoles can be used as a stable character of taxonomic groups of basal monocots (Reznicek, 1990; Remizowa & al., 2006). In the combined consensus tree, *T. minima*, which has a bracteole in the female flower, is a sister to the rest of *Typha* species (Fig. 3). Bracteole loss occurred in species of clades II and III. Therefore, bracteole loss is a derived characteristic (Fig. 4).

Stigmas capture pollen, support hydration and germination, and offer entry points and guidance to pollen tubes en route to the ovaries (Soreng & Davis, 1998; Edlund & al., 2004). Stigma shape is therefore relevant to the breeding system of *Typha* species, which are wind-pollinated. A spatulate stigma is putatively more efficient at trapping airborne pollen than is a filiform stigma (Lee, 1975). In addition, a spatulate stigma is correlated with the lack of a gap between staminate and pistillate inflorescence, with the exception of the stigma of *T. laxmanni* in clade II (Fig. 4). Inflorescence characteristics are also related to the pollination system of *Typha* species (Lee, 1975; Na & al., 2010). In *T. latifolia* absence of a gap between staminate and pistillate inflorescences favors inbreeding, whereas the presence of a gap in *T. angustifolia* favors outbreeding. Interestingly, our findings indicate that spatulate stigma and lack of a gap between staminate and pistillate inflorescence are correlated with the absence of a bracteole in the female flower (Fig. 4). Therefore, we suggest that the spatulate stigma and no gap between staminate and pistillate inflorescence are derived characteristics in *Typha*.

Difference in the number of pollen grains per unit is taxonomically important in *Typha*. According to Edlund & al. (2004), the structure of the stigma generally correlates with pollen units in angiosperms. However, we found no correlation between pollen unit and stigma shape (Fig. 4). We therefore infer that the pollen tetrads arose independently in *Typha*, regardless of stigma shape. Although pollen tetrads have been described as an advanced characteristic over monad grains in angiosperms (Walker & Doyle, 1975), our finding does not support this. Instead, the monad pollen of the species of clades I (except *T. elephantina*) and II is a derived characteristic (Fig. 4). In some instances, e.g., Cyperaceae, monads may have secondarily evolved from tetrads and in such cases tetrad grains represent a primitive rather than an advanced character state (Walker, 1971; Walker and Doyle, 1975).

In conclusion, our molecular evidence suggests that all currently recognized morphological species in *Typha* are exclusive lineages. Our phylogenetic analyses have, for the first time, revealed phylogenetically meaningful clades within *Typha*. Our phylogenetic analyses support the Eurasian *T. minima* as the basal taxon in the genus. The traditional sections *Ebracteolatae* and *Bracteolatae* are paraphyletic and polyphyletic, respectively. Our results provide a clearer concept of phylogenetic relationships in *Typha* and will aid a future taxonomic revision. This study also improves our understanding of character evolution within the genus *Typha*, particularly in terms of the evolution of the presence or absence of a bracteole in female flowers, stigma shape, the gap between staminate and pistillate inflorescence, and the number of pollen units.

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■ LITERATURE CITED

Akaike, H. 1974. A new look at the statistical model identification. *I.E.E.E. Trans. Automatic Control* 19: 716–723.

Bayly, I.L. & O'Neill, T.A. 1971. A study of introgression in *Typha* at Point Pelee Marsh, Ontario. *Canad. Field-Naturalist* 85: 309–314.

Berry, E.W. 1924. A *Sparganium* from the Middle Eocene of Wyoming. *Bot. Gaz.* 78: 324–348.

Bomblies, K. & Doebley, J.F. 2005. Molecular evolution of *FLORICAULA/LEAFY* orthologs in the Andropogoneae (Poaceae). *Molec. Biol. Evol.* 22: 1082–1094.

Boyd, C.E. 1971. Further studies on productivity, nutrient and pigment relationships in *Typha latifolia* populations. *Bull. Torrey Bot. Club* 98: 144–150.

Chase, M.W. 2004. Monocot relationships: An overview. *Amer. J. Bot.* 91: 1645–1655.

Chen, D.-H. & Ronald, P.C. 1999. A rapid DNA minipreparation method suitable for AFLP and other PCR applications. *Pl. Molec. Biol. Reporter* 17: 53–57.

Cook, C.D.K. 1980. Typhaceae. Pp. 275–276 in: Tutin, T.G., Heywood, V.H., Burges, N.A., Valentine, D.A., Walters, S.M. & Webb, D.A. (eds.), *Flora europaea*, vol. 5. Cambridge: Cambridge University Press.

Cunningham, C.W. 1997. Can three incongruence tests predict when data should be combined? *Molec. Biol. Evol.* 14: 733–740.

Daghlian, C.P. 1981. A review of the fossil record of monocotyledons. *Bot. Rev. (Lancaster)* 47: 517–555.

Devos, N., Raspe, O., Oh, S.-H., Tyteca, D. & Jacquemart, A.-L. 2006. The evolution of *Dactylorhiza* (Orchidaceae) allotetraploid complex: Insights from nrDNA sequences and cpDNA PCR-RFLP data. *Molec. Phylog. Evol.* 38: 767–778.

Dolphin, K., Belshaw, R., Orme, C.D.L. & Quicke, D.L.J. 2000. Noise and incongruence: Interpreting results of the incongruence length difference test. *Molec. Phylog. Evol.* 17: 401–406.

Duvall, M.R., Clegg, M.T., Chase, M.W., Clark, W.D., Kress, W.J., Hills, H.G., Eguiarte, L.E., Smith, J.F., Gaut, B.S., Zimmer, E.A. & Learn, G.H. 1993. Phylogenetic hypotheses for the monocotyledons constructed from *rbcL* sequence data. *Ann. Missouri Bot. Gard.* 80: 607–619.

Edlund, A.F., Swanson, R. & Preuss, D. 2004. Pollen and stigma structure and function: The role of diversity in pollination. *Pl. Cell* 16: S84–S97.

Farris, J.S. 1989. The retention index and the rescaled consistency index. *Cladistics* 5: 417–419.

Farris, J.S., Källersjö, M., Kluge, A.G. & Bult, C. 1995. Testing significance in incongruence. *Cladistics* 10: 315–319.

Fassett, N.C & Calhoun, B. 1952. Introgression between *Typha latifolia* and *T. angustifolia*. *Evolution* 6: 367–379.

Fedchenko, B.A. 1986. Family Typhaceae. Pp. 165–170 in: Komarov, V.L. (ed.), *Flora of U.S.S.R.*, vol. 1. Koenigstein: Koeltz Scientific Books.

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

Finlayson, M., Forrester, R.I., Mitchell, D.S. & Chick, A.J. 1985. Identification of native *Typha* species in Australia. *Austral. J. Bot.* 33: 101–107.

Frohlich, M.W & Meyerowitz, E.M. 1997. The search for flower homeotic gene homologos in basal angiosperms and Gnetales: A potential new source of data on the evolutionary origin of flowers. *Int. J. Pl. Sci.* 158: S131–S142.

Frohlich, M.W. & Parker, D.S. 2000. The mostly male theory of flower evolutionary origins: From genes to fossils. *Syst. Bot.* 25: 155–170.

Givnish, T.J., Pires, J.C., Graham, S.W., McPherson, M.A., Prince, L.M., Patterson, T.B., Rai, H.S., Roalson, E.H., Evans, T.M., Hahn, W.J., Millam, K.C., Meerow, A.W., Molvray, M., Kores, P.J., O'Brien, H.E., Hall, J.C., Kress, W.J. & Sytsma, K.J. 2006. Phylogenetic relationships of monocots based on the highly informative plastid gene *ndhF*: Evidence for widespread concerted convergence. *Aliso* 22: 25–51.

Grace, J.B. & Wetzel, R.G. 1982. Variations in growth and reproduction within populations of two rhizomatous plant species: *Typha latifolia* and *Typha angustifolia*. *Oecologia* 53: 258–263.

Grace, J.B. & Wetzel, R.G. 1998. Long-term dynamics of *Typha* populations. *Aquatic Bot.* 61: 137–146.

Graebner, P. 1900. Typhaceae. Pp. 1–18 in: Engler, A. (ed.), *Das Pflanzenreich*, vol. 1 (IV. 8). Berlin: Engelmann.

Guha, R. & Mondal, M.S. 1998. Revision of Indian Typhaceae with special reference to palynotaxonomy. *J. Econ. Taxon. Bot.* 22: 517–526.

Guisinger, M.M., Chunmley, T.W., Kuehl, J.V., Boore, J.L. & Jansen, R.K. 2010. Implifications of the plastid genome sequence of *Typha* (Typhaceae, Poales) for understanding genome evolution in Poaceae. *J. Molec. Evol.* 70: 149–166.

Gustafsson, M. 1973. Evolutionary trends in the *Atriplex triangularis* group of Scandinavia. I. Hybrid sterility and chromosomal differentiation. *Bot. Not.* 126: 345–392.

Himi, S., Sano, R., Nishiyama, T., Tanahashi, T., Kato, M., Ueda, K. & Hasebe, M. 2001. Evolution of MADS-box gene induction by *FLO/LFY* genes. *J. Molec. Evol.* 53: 387–393.

Hotchkiss, N. & Dozier, H.L. 1949. Taxonomy and distribution of North American cattails. *Amer. Midl. Naturalist* 41: 237–254.

Huelsenbeck, J.P. & Ronquist, F. 2001. MR BAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Janssen, T. & Bremer, K. 2004. The age of major monocot groups inferred from 800+ *rbcL* sequences. *Bot. J. Linn. Soc.* 146: 385–398.

Kadono, Y. 1996. *Aquatic plants of Japan*. Tokyo: Bun-ichi Sogo Shuppan. [in Japanese]

Kim, C. 2002. *Morphology and molecular systematic of Typhaceae*. Dissertation, Ajou University, Suwon, Republic of Korea. [in Korean with English abstract]

Kim, C., Na, H.R., Shin, H. & Choi, H.-K. 2009. Systematic evaluation of *Isoëtes asiatica* Makino (Isoëtaceae) based on AFLP, nrITS, and chloroplast DNA sequences. *J. Pl. Biol.* 52: 501–510.

Kim, C., Shin, H. & Choi, H.-K. 2003. A phenetic analysis of *Typha* in Korea and far east Russia. *Aquatic Bot.* 75: 33–43.

Kim, C., Shin, H., Chang, Y.-T. & Choi, H.-K. 2010. Speciation pathway of *Isoëtes* (Isoëtaceae) in East Asia inferred from molecular phylogenetic relationships. *Amer. J. Bot.* 97: 958–969.

Kluge, A.G. & Farris, J.S. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* 18: 1–32.

Kluge, A.G. 1989. A concern for evidence and a phylogenetic hypothesis for relationships among *Epicrates* (Boidae, Serpentes). *Syst. Zool.* 38: 7–25.

Krattinger, K., Rast, D. & Karesch, H. 1979. Analysis of pollen proteins of *Typha* species in relation to identification of hybrids. *Biochem. Syst. Ecol.* 7: 125–128.

Kronfeld, M. 1889. Monographie der Gattung *Typha* Tourn. *Verh. Zool.-Bot. Ges. Wien* 39: 89–192.

Kuehn, M.M. & White, B.N. 1999. Morphological analysis of genetically identified cattails *Typha latifolia*, *Typha angustifolia*, and *Typha* ×*glauca*. *Canad. J. Bot.* 77: 906–912.

Kuehn, M.M., Minor, J.E. & White, B.N. 1999. An examination of hybridization between the cattail species *Typha latifolia* and *Typha angustifolia* using random amplified polymorphic DNA and chloroplast DNA markers. *Molec. Ecol.* 8: 1981–1990.

Lee, C. & Wen, J. 2004. Phylogeny of *Panax* using chloroplast *trnC-trnD* intergenic region and the utility of *trnC-trnD* interspecific studies of plants. *Molec. Phylog. Evol.* 31: 894–903.

Lee, D.W. & Fairbrothers, D.E. 1972. Taxonomic placement of the Typhales within the monocotyledons: Preliminary serological investigation. *Taxon* 21: 39–44.

Lee, D.W. 1975. Population variation and introgression in North American *Typha*. *Taxon* 24: 633–641.

Linnaeus, C. 1753. *Species plantarum*. London: Adlard and Son Bartholomew Press.

Maddison, W.P. & Maddison, D.R. 2007. Mesquite: A modular system for evolutionary analysis, version 2.0. http://mesquiteproject.org/.

Mavrodiev, E.V. 2001. *Rohrbachia*, a new genus of the Typhaceae. *Bot. Zhurn. (Moscow & Leningrad)* 86: 120–124. [in Russian with English abstract]

Mavrodiev, E.V. 2002. Two new species of *Typha* L. (Typhaceae Juss.) from far east of Russia and from Mongolia. *Feddes Repert.* 113: 281–288.

Na, H.R., Kim, C. & Choi, H.-K. 2010. Genetic relationship and genetic diversity among *Typha* taxa from East Asia based on AFLP markers. *Aquatic Bot.* 92: 207–213.

Nixon, K.C. & Carpenter, J.M. 1996. On simultaneous analysis. *Cladistics* 12: 221–242.

Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. 2008. AWTY (Are We There Yet?): A system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* 24: 581–583.

Oh, S.H. & Potter, D. 2005. Molecular phylogenetic systematic and biogeography of tribe Neillieae (Rosaceae) using DNA sequences of cpDNA, rDNA, and *LEAFY*. *Amer. J. Bot.* 92: 179–192.

Osmond, C.B., Bjorkman, O. & Anderson, D.J. 1980. *Physiological processes in plant ecology*. Berlin: Springer.

Posada, D. & Crandall, K.A. 1998. Modeltest: Testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Punt, W. 1975. Sparganiaceae and Typhaceae. *Rev. Palaeobot. Palynol.* 19: 75–88.

Rambaut, A. & Drummond, A.J. 2003. Tracer, version 1.5. http://tree.bio.ed.ac.uk/software/tracer/.

Remizowa, M.V., Sokoloff, D.D. & Rudall, P.J. 2006. Patterns of floral structure and orientation in *Japonolirion*, *Narthecium*, and *Tofieldia*. *Aliso* 22: 159–171.

Reznicek, A.A. 1990. Evolution in sedges (*Carex*, Cyperaceae). *Canad. J. Bot.* 68: 1409–1432.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Rudall, P.J., Stuppy, W., Cunniff, J., Kellogg, E.A. & Briggs, B.G. 2005. Evolution of reproductive structures in grasses (Poaceae) inferred by sister-group comparison with their putative closest living relatives, Ecdeiocoleaceae. *Amer. J. Bot.* 92: 1432–1443.

Selbo, S.M. & Snow, A.A. 2004. The potential for hybridization between *Typha angustifolia* and *Typha latifolia* in a constructed wetland. *Aquatic Bot.* 78: 361–369.

Sharma, K.P. & Gopal, B. 1980. A note on the identity of *Typha elephantina* Roxb. *Aquatic Bot.* 9: 381–387.

Shih, J.G. & Finkelstein, S.A. 2008. Range dynamics and invasive tendencies in *Typha latifolia* and *Typha angustifolia* in eastern North America derived from herbarium and pollen records. *Wetlands* 28: 1–16.

Simmons, M.P. & Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analysis. *Syst. Biol.* 49: 369–381.

Smith, S.G. 1967. Experimental and natural hybrids in North America *Typha* (Typhaceae). *Amer. Midl. Naturalist* 78: 257–287.

Smith, S.G. 1987. *Typha*: Its taxonomy and the ecological significance of hybrids. *Arch. Hydrobiol., Beih. Ergebn. Limnol.* 27: 129–138.

Snow, A.A., Travis, S.E., Wildova, R., Fer, T., Sweeney, T.M.,

Marburger, J.E., Windels, S., Kubatova, B., Goldberg, D.E. & Mutegi, E. 2010. Species-specific SSR alleles for studies of hybrid cattails (*Typha latifolia* × *T. angustifolia*; Typhaceae) in North America. *Amer. J. Bot.* 97: 2061–2067.

Soreng, R.J. & Davis, J.I. 1998. Phylogenetics and character evolution in the grass family (Poaceae): Simultaneous analysis of morphological and chloroplast DNA restriction site characters. *Bot. Rev. (Lancaster)* 64: 1–85.

Steinbachova-Vojtiskova, L., Tylova, E., Soukup, A., Novicka, H., Votrubova, O., Lipavska, H. & Cizkova, H. 2006. Influence of nutrient supply on growth, carbohydrate, and nitrogen metabolic relations in *Typha angustifolia*. *Environm. Exp. Bot.* 57: 246–257.

Stuckey, R.L. & Salamon, D.P. 1987. *Typha angustifolia* in North America: A foreigner masquerading as a native. *Amer. J. Bot.* 74: 757.

Su, Y.C.F., Smith, G.J.D. & Saunders, R.M.K. 2008. Phylogeny of the basal angiosperm genus *Pseuduvaria* (Annonaceae) inferred from five chloroplast DNA regions, with interpretation of morphological character evolution. *Molec. Phylog. Evol.* 48: 188–206.

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

Taberlet, P., Gielly, L., Pauton, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.

Takhtajan, A. 2009. *Flowering plants*, 2nd ed. New York: Springer.

Thieret, J.W. & Luken, J. 1996. The Typhaceae in the southeastern United States. *Harvard Pap. Bot.* 8: 27–56.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. 1997. The Clustal X windows interface: Flexible strategies for multiple sequences alignment aided by quality analysis tools. *Nucl. Acids Res.* 24: 4876–4882.

Ungar, I.A. & Khan, M.A. 2001. Effect of bracteoles on seed germination and dispersal of two species of *Atriplex*. *Ann. Bot.* 87: 233–239.

Walker, J.W. & Doyle, J.A. 1975. The bases of angiosperm phylogeny: Palynology. *Ann. Missouri Bot. Gard.* 62: 664–723.

Walker, J.W. 1971. Pollen morphology, phytogeography, and phylogeny of the Annonaceae. *Contr. Gray Herb.* 202: 1–132.

Wilson, R.L. & Webster, R.M. 1946. Plant microfossils from a Fort Union Coal of Montana. *Amer. J. Bot.* 33: 271–278.

Yang, Z. & Rannala, B. 1997. Bayesian phylogenetic inference using DNA sequences: A Markov Chain Monte Carlo method. *Molec. Biol. Evol.* 14: 717–724.

Zhang, X.-H., Tapia, M., Webb, J.B., Huang, Y.-H. & Miao, S. 2008. Molecular signatures of two cattail species, *Typha domingensis* and *Typha latifolia* (Typhaceae), in South Florida. *Molec. Phylog. Evol.* 49: 368–376.

Zurawski, G. & Clegg, M.T. 1987. Evolution of higher-plant chloroplast DNA-encoded genes: Implications for structure-function and phylogenetic studies. *Annual Rev. Pl. Physiol.* 38: 391–418.

Appendix. Voucher information and GenBank accession numbers for taxa used in this study.

Species name – abbreviation of OTU, voucher (herbarium acronym), country, GenBank accession number for *LEAFY*, *trnL-F*, *trnC-petN*, and *psbM-trnD*

Typha angustifolia L. – TA_Au, *C Kim 2006-11* (AJOU), Australia, JF319515, JF319450, JF319600, JF319535; TA_Ch01, *C Kim 2002-12* (AJOU), China, GU646728, JF319451, JF319601, JF319536; TA_Ch02, *HR Na 80591-1* (AJOU), China, GU646729, JF319452, JF319602, JF319537; TA_Cz, *SJ Wiesbaur 984* (WU), Czech Republic, GU646762, JF319453, JF319603, JF319538; TA_Fi01, *R Alava & I Kukkonen 4488* (LE), Finland, JF319516, JF319454, JF319604/JF319539; TA_Fi02, *I Kause & E Seikkula s.n.* (LE), Finland, JF319517, JF319455, JF319605, JF319540; TA_Ja, *H-K Choi 551* (AJOU), Japan, GU646730, JF319456, JF319606, JF319541; TA_Ko01, *HR Na 80181* (AJOU), Korea, GU646731, JF319457, JF319607, JF319542; TA_Ko02, *H Kim s.n.* (AJOU), Korea, GU646732, JF319458, JF319608, JF319543; TA_Ko03, *C Kim 492* (AJOU), Korea, GU646733, JF319459, JF319609, JF319544; TA_Ko08, *HR Na 40016* (AJOU), Korea, GU646738, JF319460, JF319610, JF319545; TA_Ko13, *H Kim s.n.* (AJOU), Korea, GU646743, JF319461, JF319611, JF319546; TA_Ko21, *HR Na 40017* (AJOU), Korea, GU646751, JF319462, JF319612, JF319547; TA_Po, *T Tacik s.n.* (LE), Poland, JF319518, JF319463, JF319613, JF319548; TA_Ru, *H-K Choi 83* (AJOU), Russia, GU646760, JF319464, JF319614, JF319549; TA_Us, *C Kim 429* (AJOU), USA, GU646761, JF319465, JF319615, JF319550. ***T. capensis*** Rohrb. – TC_Sa01, *H-K Choi s.n.* (AJOU), South Africa, JF319519, JF319466, JF319616, JF319551; TC_Sa02, *H-K Choi s.n.* (AJOU), South Africa, GU646706, JF319467, JF319617, JF319552; TC_Sa03, *C Kim 2007-3* (AJOU), South Africa, GU646707, JF319468, JF319618, JF319553; ***T. domingensis*** Pers. – TD_Af, *D Podlech 12788* (LE), Afghanistan, JF319520, JF319469, JF319619, JF319554; TD_Al, *VP Bochantsev 1218* (LE), Algeria, JF319521, JF319470, JF319620, JF319555; TD_Au, *K Stephens 124* (BRI), Australia, GU646765, JF319471, JF319621, JF319556; TD_Ge, *P Ascherson s.n.* (LE), German, GU646766, JF319472, JF319622, JF319557; TD_Us, *C Kim 441* (AJOU), U.S.A., GU646767, JF319473, JF319623, JF319558; ***T. elephantina*** Roxb. – TE_Ch, *C Kim 2002-11* (AJOU), China, GU646763, JF319474, JF319624, JF319559; TE_In01, *HR Na 10190* (AJOU), India, JF319522, JF319475, JF319625, JF319560; TE_In02, *K Holpobus s.n.* (LE), India, GU646764, JF319476, JF319626, JF319561; ***T. latifolia*** L. – TL_Au, *T Shimizu 39212* (CANB), Australia, GU646680, JF319477, JF319627, JF319562; TL_Ca, *WJ Cody & JM Matte 8904*, Canada, JF319523, JF319478, JF319628, JF319563; TL_Ch, *H-K Choi 771* (AJOU), China, GU646681, JF319479, JF319629, JF319564; TL_Fi, *I Kukkonen 9448* (LE), Finland, JF319524, JF319480, JF319630, JF319565; TL_Ja01, *HR Na 7J095* (AJOU), Japan, GU646682, JF319481, JF319631, JF319566; TL_Ja02, *HR Na 7J125* (AJOU), Japan, GU646683, JF319482, JF319632, JF319567; TL_Ja03, *H-K Choi 550* (AJOU), Japan, GU646684, JF319483, JF319633, JF319568; TL_Ko01, *Y Park s.n.* (AJOU), Korea, GU646685, JF319484, JF319634, JF319569; TL_Ko02, *HR Na 40016* (AJOU), Korea, GU646686, JF319485, JF319635, JF319570; TL_Ko03, *H-K Choi s.n.* (AJOU), Korea, GU646687, JF319486, JF319636, JF319571; TL_Ko05, *C Kim 366* (AJOU), Korea, GU646689, JF319487, JF319637, JF319572; TL_Ko09, *HR Na 80373* (AJOU), Korea, GU646694, JF319488, JF319638, JF319573; TL_Me, *M Rosas 384* (LE), Mexico, JF319525, JF319489, JF319639, JF319574; TL_Ru, *H-K Choi 101* (AJOU), Russia, GU646695, JF319490, JF319640, JF319575; TL_Us01, *H-K Choi s.n.* (AJOU), USA, GU646696, JF319491, JF319641, JF319576; TL_Us03, *C Kim 386* (AJOU), USA, GU646698, JF319492, JF319642, JF319577; TL_Us07, *C Kim 388* (AJOU), USA, GU646701, JF319493, JF319643, JF319578; ***T. laxmanni*** Lepech. – TX_Ko01, *C Kim 55* (AJOU), Korea, GU646718, JF319507, JF319657, JF319592; TX_Ko06, *C Kim 104* (AJOU), Korea, GU646723, JF319508, JF319658, JF319593; TX_Ru, *H-K Choi 304* (AJOU), Russia, GU646727, JF319509, JF319659, JF319594. ***T. minima*** Funck ex Hoppe – TM_Fr01, *C Martin s.n.* (LE), France, JF319526, JF319494, JF319644, JF319579; TM_Fr02, *R Weibel s.n.* (LE), France, JF319527, JF319495, JF319645, JF319580; TM_Ge, *A Zick 2056* (WU), German, GU646770, JF319496, JF319646, JF319581; TM_It, *D Podlech 9140* (LE), Italia, JF319528, JF319497, JF319647, JF319582; TM_Ru, *LN Androssow 2606* (WU), Russia, GU646771, JF319498, JF319648, JF319583. ***T. orientalis*** C. Presl – TO_Au, *R Dowling 220* (BRI), Australia, GU646708, JF319499, JF319649, JF319584; TO_Ja, *M Togashi 3382* (WU), Japan, GU646711, JF319500, JF319650, JF319585; TO_Ko01, *H Kim s.n.* (AJOU), Korea, GU646709, JF319501, JF319651, JF319586; TO_Ko06, *HR Na 80372* (AJOU), Korea, GU646714, JF319502, JF319652, JF319587; TO_Ru, *H-K Choi 280* (AJOU), Russia, GU646717, JF319503, JF319653, JF319588. ***T. shuttleworthii*** W.D.J. Koch & Sond. – TS_Fr, *Seelano & Berne s.n.* (WU), France, GU646704, JF319504, JF319654, JF319589; TS_Hu, *NO Tuberbach 2418* (WU), Hungary, GU646705, JF319505, JF319655, JF319590; TS_Ro, *A Mesterhazy s.n.* (AJOU), Romania, JF319529, JF319506, JF319656, JF319591; ***Sparganium emersum*** Rehm. – SM_Ru, *O Mochapova s.n.* (IBIW), Russia, JF319514, JF319449, JF319599, JF319534. ***S. erectum*** L. – SE_Ko, *C Kim 2010-101* (AJOU), Korea, JF319511, JF319446, JF319596, JF319531; ***S. eurycarpum*** subsp. ***coreanum*** (H. Lév.) C.D.K. Cook & M.S. Nicholls – SC_Ru, *EA Luvarb s.n.* (IBIW), Russia, JF319510, JF319445, JF319595, JF319530; ***S. fallax*** Graebner – SF_Ta, *M Sugiyama 1422* (AJOU), Taiwan, JF319512, JF319447, JF319597, JF319532; ***S. hyperboreum*** Laest. ex Beurl. – SH_Ru, *M Kozhin s.n.* (IBIW), Russia JF319513, JF319448, JF319598, JF319533.