Hydrobiologia (2016) 770:117–128
DOI 10.1007/s10750-015-2574-9

PRIMARY RESEARCH PAPER

Comparison of genetic diversity in four *Typha* species (Poales, Typhaceae) from China

Beibei Zhou · Dan Yu · Zhenjie Ding · Xinwei Xu

Received: 28 March 2015 / Revised: 27 October 2015 / Accepted: 2 November 2015 / Published online: 12 November 2015

Abstract Life history traits play an important role in the level and distribution of genetic diversity, and comparing closely related species with similar life histories can provide insight into the determinants of genetic variation in plant populations. In this study, we used variations of one chloroplast DNA fragment, one nuclear gene, and six microsatellites to compare the levels and distributions of genetic diversity in four widespread *Typha* species from China. Surveys were conducted on 898 individuals from 120 sites. The individuals of all four species formed monophyletic clades and distinct genetic clusters, suggesting no hybridization between *T. angustifolia* and *T. latifolia* in China. The levels of cpDNA nucleotide diversity followed the order *T. latifolia* > *T. laxmannii* > *T. angustifolia* > *T. orientalis*, whereas the genetic diversity in nDNA and nSSR of *T. laxmannii* and *T. angustifolia* was higher than that of *T. latifolia*. In *T. angustifolia*, *T. laxmannii*, and *T. orientalis*, more than half of genetic variation occurred within populations, and in *T. latifolia*, most of genetic variation occurred among populations. The variation in the levels and distributions of genetic diversity among the four species can be attributed to differences in inflorescence characteristics which either limit or enhanced outcrossing rates.

Keywords *Typha* · Genetic diversity · Chloroplast DNA · Nuclear DNA · Hybridization · China

Handling editor: Sidinei Magela Thomaz

Electronic supplementary material The online version of this article (doi:10.1007/s10750-015-2574-9) contains supplementary material, which is available to authorized users.

B. Zhou · D. Yu (✉) · Z. Ding · X. Xu (✉)
National Field Station of Freshwater Ecosystem of Liangzi Lake, College of Life Sciences, Wuhan University, Wuhan, People's Republic of China
e-mail: lakeyd@163.com

X. Xu
e-mail: xuxw@whu.edu.cn

Introduction

Life history traits, such as breeding system, life form, seed dispersal mechanisms, and geographic range, have a profound and significant effect on the level and distribution of genetic diversity in plant species (Hamrick & Godt, 1989, 1996; Nybom, 2004). By comparing species with different combinations of traits, patterns of genetic diversity can be identified. For example, the genetic variability of taxa with outcrossing mating system is greater within populations, whereas the genetic variability of taxa with inbreeding mating system is greater among populations. Evolutionary history also plays an important role in the level and distribution of genetic diversity (Loveless & Hamrick, 1984; Hamrick et al., 1992; Hamrick & Godt, 1996). Such inferences can be effectively verified and confirmed by comparing

closely related species because these taxa often have a similar evolutionary history or some identical life history traits (e.g., Mateu-Andrés & de Paco, 2006; Skrede et al., 2009; Carrió et al., 2010; Robuchon et al., 2014; Martins et al., 2014; Ng et al., 2015).

The genus *Typha* L. (Typhaceae) is aquatic herbs and includes 8–13 species that are distributed nearly worldwide (Smith, 1987; Cook, 1990). *Typha* species have similar life history traits: widespread, perennial, and seed dispersal by wind. Because of their rapid clonal growth, *Typha* species often dominate a variety of wetland habitats in both temperate and tropical regions (Smith, 1987). In recent decades, many studies have focused on the genetic variation of several widespread *Typha* species, e.g., *T. angustifolia* L. and *T. latifolia* L. Various markers have been used to estimate the genetic diversity of *Typha*, including isozymes (Lee & Fairbrothers, 1973; Mashburn et al., 1978; Sharitz et al., 1980), variable number tandem repeats (VNTRs) (Keane et al., 1999), amplified fragment length polymorphisms (AFLPs) (Lamote et al., 2005; Na et al., 2010), microsatellites (Tsyusko et al., 2005), and DNA sequences (Zhang et al., 2008). Despite the different markers, sample sizes, and geographic regions analyzed in these studies, the general conclusion is that *Typha* species have low genetic diversity and that *T. angustifolia* has greater genetic diversity than *T. latifolia*.

Another popular focus for *Typha* studies is interspecific hybridization. A total of 7 hybrids have been reported in Europe, North America, and South America, and five hybrids have been verified by experiments (Smith, 1987). Among these, the hybrid *T. angustifolia* × *T. latifolia* (*T.* × *glauca* Godron) is well known, as it is frequently and broadly found in sympatry with the parental species (Smith, 1987). This hybrid has been identified based on its morphology (Figert, 1890; Alm & Weimarck, 1933; Luther, 1947; Smith, 1967), isozyme variation (Lee & Fairbrothers, 1973; Lee, 1975), random amplified polymorphic DNA (RAPD) (Kuehn et al., 1999, Nowińska et al., 2014), and microsatellites (Snow et al., 2010; Travis et al., 2010; Kirk et al., 2011). However, these investigations were exclusively conducted in North America and Europe. Conversely, this hybrid has not been reported in Asia, where the two parental species, *T. angustifolia* and *T. latifolia*, are also found.

In China, four *Typha* species (*T. angustifolia*, *T. latifolia*, *T. laxmannii* Lepechin, and *T. orientalis* C. Presl) are common and widespread (Sun & Simpson, 2010). Although two of the four species, *T. angustifolia* and *T. latifolia*, can hybrid (Smith, 1987) and mixtures of them are often observed in the same stand in the field, no hybrids have been reported in this region. To date, almost all studies on *Typha* genetic variation have been conducted in North America and Europe, with the exception of one in East Asia (Na et al., 2010), and only a few have examined the population genetic structure (e.g., Tsyusko et al., 2005). In this study, we investigated the four *Typha* species with extensive sampling by using chloroplast DNA (cpDNA) and nuclear DNA (nDNA) sequences and nuclear microsatellite (nSSR) data. Our aims are to (1) explore whether hybridization between *T. angustifolia* and *T. latifolia* has occurred in China and (2) compare the genetic diversity and population genetic structure among the four *Typha* species. Such a comparison of closely related species with similar life histories will provide insight into the determinants of genetic variation in plant populations.

Materials and methods

Plant materials and morphological measurement

A total of 898 individuals were collected from 120 sites throughout China during the summers of 2010, 2011, and 2012 (Fig. 1, Online Resource 1). Multiple *Typha* species were often observed at the same sites, and we classified *Typha* samples as one of the four species based on morphological characteristics (Na et al., 2010; Sun & Simpson, 2010). We also conducted morphological analyses at ten collection sites with multiple species in Northeast China to test species' morphological variations. The plant height, leaf width, and inflorescence length (male inflorescence, female inflorescence, and gap) of 163 individuals were examined. All morphological data used for analyses are provided in Online Resource 2. The morphological variations among the four species were compared by one-way ANOVA followed by a Tukey HSD test using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). For undistinguishable plants (about one-fourth, i.e., those with no flowers or intermediate morphologies), no a priori species assignment was made in the field until plant height and leaf width were measured

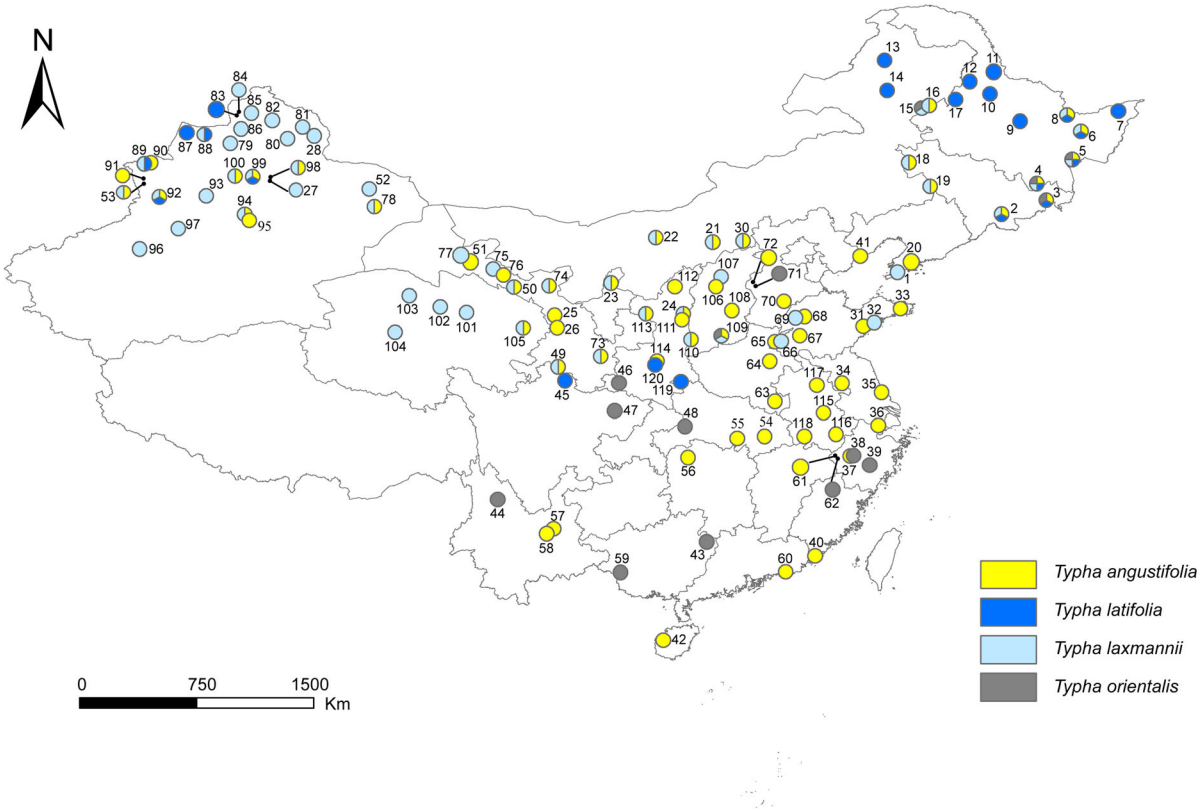


Fig. 1 The 120 sampling sites of the four *Typha* species. The species were identified using both morphology (see Fig. 2) and DNA sequences. Data for different species are shown in *different colors*

and sequencing done. The plants were collected randomly at intervals of at least 10 m to minimize sampling ramets from a single clone. The fresh leaves of the samples were dried in silica gel in the field and frozen at −20°C after being transported to the laboratory for subsequent DNA extraction. Voucher specimens were deposited in the herbarium of Wuhan University (WH).

Amplification, sequencing, cloning, and genotyping

Total genomic DNA was extracted using DNAsecure Plant Kit (Tiangen Biotech, Beijing, China). Randomly selected 36 individuals from the four species were used to screen for polymorphisms in five chloroplast fragments *rps*16, *trn*L-*trn*F, *rpl*32-*trn*L, *trn*H-*psb*A, and *trn*S-*trn*G, (Shaw et al., 2005, 2007). The former three fragments were successfully amplified and sequenced in all individuals, and intraspecific variations were only observed in the *rpl*32-*trn*L fragment, which was chosen for further studies. Polymerase chain reaction (PCR) amplifications were performed using 10–30 ng genomic DNA, 0.1 μM each primer, 0.2 mM each dNTP, 2 mM $MgCl_2$, and 0.6 U ExTaq DNA polymerase (TaKaRa, Otsu, Japan) in a 25 μl volume under the following conditions: 3 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 55°C, 90 s at 72°C, and a final 5 min extension at 72°C. The amplifications were performed in a Veriti 96-well thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR products were purified and sequenced in both directions by Beijing Genomic Institute in Wuhan, China. Three nuclear genes phytochelatin synthase (*PS*), Malate synthase and Metallothionein-like protein, which have been used in studies of *T. latifolia* and *T. domingensis* Pers. (Zhang et al., 2008), were screened in preliminary experiments. Because amplifications were not successful for all four of the *Typha* species, and the sequence of *PS*

gene was long enough to re-design primers, we designed a pair of new primers (PS-TF: 5′-GGTT GGACATTGAGGAC and PS-TR: 5′-GCTTCATG CAAAGATTG) based on the *PS* sequences of *T. latifolia* and *T. domingensis*. The PCR and sequencing methods using these primers were performed under the same conditions as those for *rpl*32-*trn*L. The program Sequencher 4.5 (GeneCodes Corporation, Ann Arbor, Michigan, USA) was used to evaluate chromatograms for base confirmation and to edit contiguous sequences. *Typha* individuals can be either homozygous or heterozygous at the nuclear PS locus. For a heterozygote, however, it may not be possible to directly read the sequences of two alleles in a chromatogram when multi-point mutations or length differences caused by insertions or deletions exist between the two alleles. In these cases, purified PCR products were cloned using the pGM-T vector (Tiangen Biotech), and then the two alleles were determined separately by sequencing multiple clones. We also used nuclear microsatellite (nSSR) data to compare genetic diversity. Eleven loci from Tsyusko-Omeltchenko et al. (2003) were screened and six loci (TA5, TA8, TA15, TA16, TA20, and TA21) were used for further studies due to their successful amplification and genotyping in three species *T. angustifolia*, *T. latifolia,* and *T. laxmannii*. We conducted nSSR data analyses in 23, 6, and 17 populations of *T. angustifolia*, *T. latifolia*, and *T. laxmannii*, respectively, each with more than five individuals. PCR amplification followed Tsyusko-Omeltchenko et al. (2003) and PCR products were run on an ABI 3730XL DNA analyzer and genotyping was performed using GeneMapper 4.0 (Applied Biosystems).

Data analysis

All sequences were aligned with the MAFFT 6.7 software (Katoh et al., 2002) using the L-INS-i algorithm, with "maxiterate" set to 1000. Identical sequences were collapsed into a single haplotype using DNASP 5.10 (Librado & Rozas, 2009), and all sequences with unique haplotypes were deposited in GenBank (Accession Nos. KR265866-KR265896). A phylogenetic tree based on all haplotypes of the four *Typha* species was constructed by the neighbor-joining (NJ) method with a p-distance algorithm and 1000 bootstrap replicates in MEGA 5.05 (Tamura et al., 2011). Our aim is to explore whether the four species were corresponding to distinct clades rather than their phylogenetic relationship; therefore, no outgroup was used to root the tree. For each *Typha* species, a haplotype network was constructed using TCS 1.18 (Clement et al., 2000), which utilizes statistical parsimony to connect haplotypes constrained by 95% confidence intervals. In this analysis, indels were treated as single mutation events. Nucleotide diversity (π) and haplotype diversity (Hd) were calculated using ARLEQUIN 3.0 (Excoffier et al., 2005). Two measures of population differentiation, G_{ST} and N_{ST}, were calculated using the program HAPLONST (Pons & Petit, 1996). G_{ST} considers haplotype frequencies, whereas N_{ST} takes into account both haplotype frequencies and their genetic distances. An N_{ST} value significantly higher than G_{ST} typically indicates the presence of phylogeographic structure in which closely related haplotypes are found in the same area more often than less closely related haplotypes (Pons & Petit, 1996). Regarding nSSR data, three measures of genetic diversity, the mean number of alleles per locus (Na), the observed heterozygosity (Ho), and expected heterozygosity (He), were calculated using FSTAT 2.9.3 (Goudet, 1995). The Bayesian clustering approach implemented in STRUCTURE 2.0 (Falush et al., 2003) was used to detect interspecific hybrids between *T. angustifolia* and *T. latifolia* based on the nSSR data. Ten independent runs were performed at $K = 3$ under the admixture model, with a burn-in period of 20,000 and 100,000 Markov chain Monte Carlo (MCMC) iterations. Genetic variation within and among populations was assessed by analyses of molecular variance (AMOVA), implemented in ARLEQUIN. A Mantel test, performed in IBD 1.52 (Bohonak, 2002), was used to examine the correlation between geographical and genetic distances, as measured by $F_{ST}/(1 - F_{ST})$ (Rousset, 1997).

Results

Species confirmation and haplotype distribution

Morphological data were obtained from a total of 163 plants, including 64 plants of *T. angustifolia*, 22 of *T. latifolia*, 27 of *T. orientalis* and 50 of *T. laxmannii*, respectively (Online Resource 2). Statistical analyses showed significant morphological differences between

species in all six measurements. Each species could be distinguished from other species according to at least one measurement (Fig. 2).

We obtained sequences of the chloroplast *rpl*32-*trn*L fragment from 855 individuals and the nuclear PS gene from 681 individuals. A total of 11 and 20 haplotypes were obtained from chloroplast and nuclear sequences, respectively. In the NJ trees of both the chloroplast and nuclear haplotypes, four distinct clades were resolved with robust support and corresponded to the four *Typha* species (Fig. 3). All samples with chloroplast haplotypes in the clade of each species also possessed nuclear haplotypes exclusively of the clade of the same species. Furthermore, STRUCTURE analysis revealed three genetic clusters corresponding to three species, and all individuals were assigned to each species with a posterior probability higher than 0.97 (Fig. 4). These results suggested that no interspecific hybrid between *T. angustifolia* and *T. latifolia* was found.

Based on both morphology and molecular data, we identified multiple species at 32 collection sites, with 2, 7, and 23 sites having 4, 3, and 2 species, respectively. Both *T. angustifolia* and *T. latifolia* were found at eight sites, and all of the sites contained multiple species distributed in northern China (Fig. 1). *T. angustifolia* was found at 69 sites throughout China; *T. latifolia* exhibited a disjunctive distribution and was found at 24 sites in northeastern China (Heilongjiang, Inner Mongolia and Jinlin), central China (Sichuan

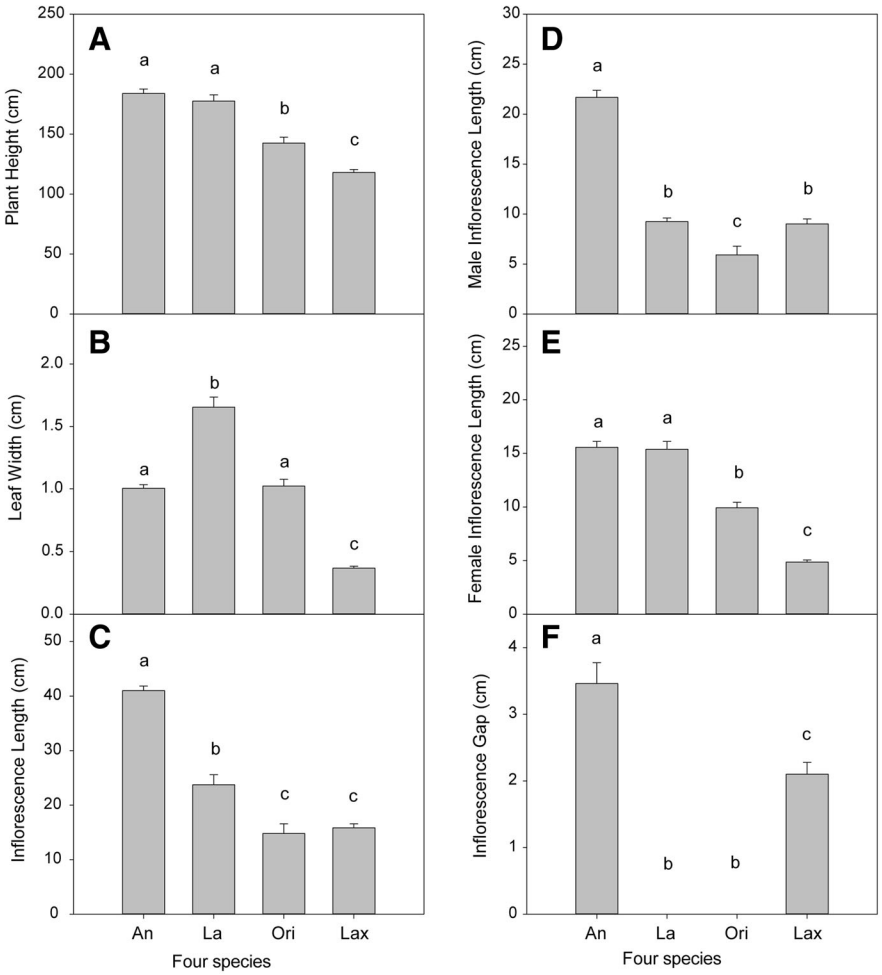


Fig. 2 Comparisons of six morphological characteristics of four *Typha* species. Data indicate means ± SDs. Significant differences among clades are indicated by *different letters*

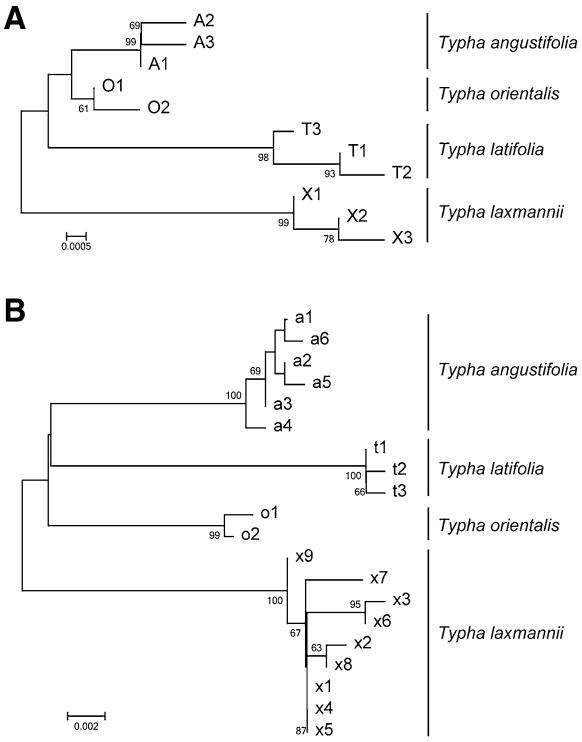


Fig. 3 Neighbor-joining tree of haplotypes in the four *Typha* species. **A** Haplotypes of the chloroplast fragment *rpl*32-*trn*L. **B** Haplotypes of the nuclear phytochelatin synthase (*PS*) gene. Bootstrap values of 60 and above are shown at the nodes (based on 1000 replicates)

and Shaanxi), and northwestern China (Xinjiang). *Typha laxmannii* was widespread throughout northern China and was found at 55 sites. *Typha orientalis* was found at 15 sites, with a northeastern to southwestern distribution (Fig. 1).

No clusters were observed in the haplotype network of each species (Fig. 5). For *T. angustifolia*, three chloroplast haplotypes and six nuclear haplotypes were identified. More chloroplast haplotypes were found in the eastern part of northern China, whereas more nuclear haplotypes were found in eastern China (Fig. 5). With regard to *T. latifolia*, three chloroplast

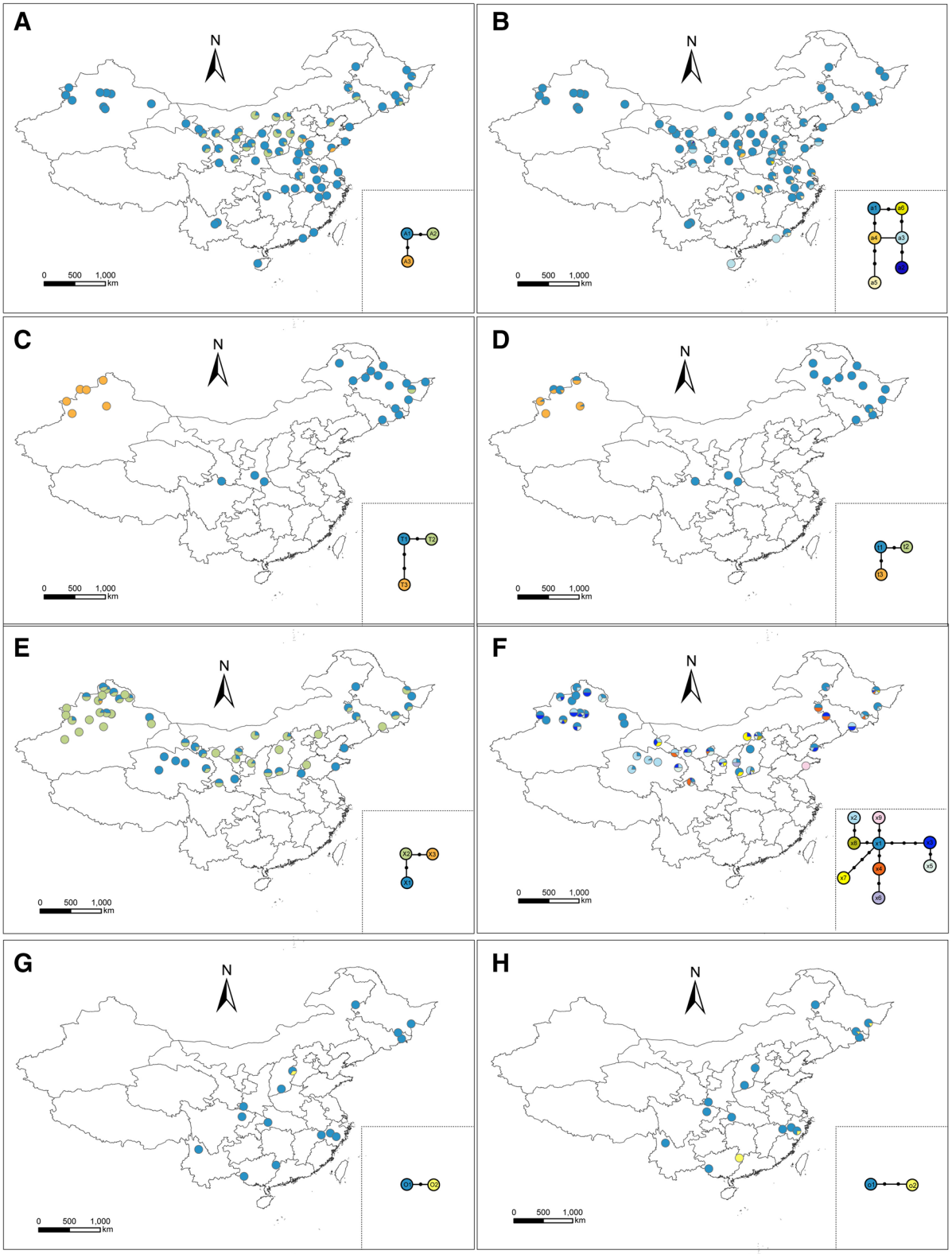
Fig. 5 Geographical distribution and network of haplotypes in ▶ four *Typha* species. **A** cpDNA haplotypes of *T. angustifolia*, **B** *n*DNA haplotypes of *T. angustifolia*, **C** cpDNA haplotypes of *T. latifolia*, **D** *n*DNA haplotypes of *T. latifolia*, **E** cpDNA haplotypes of *T. laxmannii*, **F** nDNA haplotypes of T. *laxmannii*, **G** cpDNA haplotypes of *T. orientalis*, **H** *n*DNA haplotypes of *T. orientalis*

and nuclear haplotypes each were identified. Two common chloroplast haplotypes, T1 and T3, were found in both central and northeastern China and northwestern China, respectively. The nuclear haplotype t1 was present in all populations except one in northwestern China, and t3 was present in all populations from northwestern China (Fig. 5). Three chloroplast haplotypes and nine nuclear haplotypes were observed for *T. laxmannii*, but their geographical distribution showed no distinct patterns (Fig. 5). In *T. orientalis,* two chloroplast haplotypes and two nuclear haplotypes were identified. One chloroplast haplotype occurred in all populations, yet the other haplotype occurred in only one population. Nuclear haplotypes were similarly distributed (Fig. 5).

Genetic diversity

The cpDNA nucleotide diversity ranged from high to low in the four *Typha* species as follows: *T. latifolia* (0.943×10^{-3}), *T. laxmannii* (0.569×10^{-3}), *T. angustifolia* (0.28×10^{-3}), and *T. orientalis* (0.065×10^{-3}). The nDNA nucleotide diversity of the species also ranged from high to low as follows: *T. laxmannii* (2.641×10^{-3}), *T. angustifolia* (0.433×10^{-3}), *T. latifolia* (0.374×10^{-3}), and *T. orientalis* (0.307×10^{-3}). The percentage of heterozygous individuals (from high to low) was *T. laxmannii* (43.8%), *T. angustifolia* (10.1%), *T. orientalis* (5.7%), and *T. latifolia* (4.1%). Regarding nSSR data of three species, genetic diversity (from high to low) was *T. laxmannii* (Ho: 0.544 and He: 0.523), *T. angustifolia* (Ho: 0.167

Fig. 4 The bar plot depicts the STRUCTURE admixture coefficients for all populations when $K = 3$ for 46 populations of three *Typha* species. A *single vertical bar* displays the membership coefficient of each individual, with site names shown on the *bottom*


A
N
0 500 1,000 km
A1
A2
A3
B
N
0 500 1,000 km
B1
B2
B4
B3
B5
C
N
0 500 1,000 km
C1
C2
C3
D
N
0 500 1,000 km
D1
D2
D3
E
N
0 500 1,000 km
E2
E1
E3
F
N
0 500 1,000 km
F2
F9
F8
F1
F3
F5
F4
F7
F6
G
N
0 500 1,000 km
G1
G2
H
N
0 500 1,000 km
H1
H2

and He: 0.205), and *T. latifolia* (Ho: 0.069 and He: 0.101).

In *T. angustifolia*, the nucleotide diversity of polymorphic populations ranged from 0.161×10^{-3} to 1.126×10^{-3} based on cpDNA data and from 0.259×10^{-3} to 1.66×10^{-3} based on nDNA data. Of the 67 populations for which both cpDNA and nDNA data were obtained, 40.3% (27) were monomorphic (Online Resource 1). According to cpDNA and nDNA data, only one polymorphic population and six polymorphic populations, respectively, were present in *T. latifolia*; both datasets identified 14 of 20 populations as monomorphic (Online Resource 1). In *T. laxmannii*, the nucleotide diversity of polymorphic populations ranged from 0.327×10^{-3} to 0.764×10^{-3} based on cpDNA data and from 0.415×10^{-3} to 5.16×10^{-3} based on nDNA data. Of the 43 populations for which both cpDNA and nDNA data were obtained, only 14% (6) were monomorphic (Online Resource 1). In *T. orientalis*, only one polymorphic population and three polymorphic populations were present, as assessed by cpDNA and nDNA data, respectively, and both datasets identified 11 of 15 populations as monomorphic (Online Resource 1).

Genetic structure

AMOVAs of the cpDNA data revealed that most of genetic variation occurred within *T. angustifolia*, *T. laxmannii*, and *T. orientalis* populations, whereas almost all of genetic variation occurred among populations in *T. latifolia* (Table 1). The global F_{ST} values of *T. angustifolia*, *T. laxmannii*, *T. orientalis*, and *T. latifolia* were 0.248, 0.441, 0.147, and 0.965, respectively; a similar trend was found for the nDNA data AMOVAs, with global F_{ST} values of 0.438, 0.250, 0.359, and 0.678, respectively (Table 1). Regarding the nSSR data of three species, the trend is also similar and the global F_{ST} values of *T. angustifolia*, *T. laxmannii*, and *T. latifolia* were 0.413, 0.293, and 0.723, respectively (Table 1). The differences between the N_{ST} and G_{ST} values were not significant ($P > 0.05$) for any of the four species based on either cpDNA data or nDNA data, indicating the absence of phylogeographic structure with regard to haplotype variation. Significant correlations of geographical and genetic distances were only detected for *T. latifolia* ($r^2 = 0.214$, $P < 0.001$) and *T. laxmannii* ($r^2 = 0.009$, $P < 0.05$) based on cpDNA data, *T. latifolia* ($r^2 = 0.057$, $P < 0.001$) based on nDNA

Table 1 Analyses of molecular variance (AMOVA) of cpDNA and nDNA data from four *Typha* species

Species	Source of variation	cpDNA		*n*DNA		*n*SSR	
		Percentage of variation	Fixation index	Percentage of variation	Fixation index	Percentage of variation	Fixation index
T. angustifolia	Among populations	24.80	$F_{ST} = 0.248$*	43.77	$F_{ST} = 0.438$*	41.28	$F_{ST} = 0.413$*
	Within populations	75.20		56.23		58.72	
T. latifolia	Among populations	96.54	$F_{ST} = 0.965$*	67.78	$F_{ST} = 0.678$*	72.25	$F_{ST} = 0.723$*
	Within populations	3.46		32.22		27.52	
T. laxmannii	Among populations	44.05	$F_{ST} = 0.441$*	25.01	$F_{ST} = 0.250$*	29.28	$F_{ST} = 0.293$*
	Within populations	55.95		74.99		70.72	
T. orientalis	Among populations	14.68	$F_{ST} = 0.147^{NS}$	35.88	$F_{ST} = 0.359$*		
	Within populations	85.32		64.12			

NS not significant ($P > 0.05$)

* $P < 0.001$

data, and *T. latifolia* ($r^2 = 0.341$, $P < 0.05$) based on nSSR data.

Discussion

Interspecific hybridization

Natural hybridization between *T. angustifolia* and *T. latifolia* is widely observed in Europe and North America (Smith, 1987), and it was recently confirmed by some molecular studies (e.g., Snow et al., 2010; Travis et al., 2010; Kirk et al., 2011; Nowińska et al., 2014). However, no putative hybrid was found in our study based on DNA sequences and nSSR data, despite the large overlapping areas and the inclusion of eight co-occurring sites. Because substantial overlap in flowering time is found between *T. angustifolia* and *T. latifolia* (Selbo & Snow, 2004; Ball & Freeland, 2013; Nowińska et al., 2014), interspecific pollination is likely. However, as this observation of flowering times was recorded for North American and European populations, detailed investigations on the flowering time of Chinese populations are still required. Because limited individuals with inflorescence were sampled in sites with *T. angustifolia* and *T. latifolia* in our current study, the hybrids were likely missed in the collections. Therefore, to verify the conclusion of no hybrid between *T. angustifolia* and *T. latifolia* in China, it is necessary to conduct further investigations on these sites including samples as many as possible.

Genetic diversity

Different levels of genetic variation in *Typha* have been detected using different markers (e.g., Sharitz et al., 1980; Keane et al., 1999; Tsyusko et al., 2005; Zhang et al., 2008). Although markers can vary in their mutation rates and their polymorphisms cannot be compared directly, *Typha* species tend to have relatively low genetic diversity compared to other species evaluated using the same makers (Mashburn et al., 1978; Sharitz et al., 1980; Keane et al., 1999; Galeuchet et al., 2002; Tsyusko et al., 2005). Similarly, the nucleotide diversity of *Typha* species in the present study was much lower than that based on nuclear *Adh1a* sequences of another perennial and aquatic clonal species (*Zizania latifolia*) from the same area (0.0054; Xu et al., 2008).

Genetic diversity is often compared among *Typha* species, especially between *T. angustifolia* and *T. latifolia*, and previous studies based on AFLP markers or microsatellites have noted that *T. angustifolia* exhibits approximately 1.5 times greater genetic diversity than *T. latifolia* (Tsyusko et al., 2005; Na et al., 2010; Kirk et al., 2011). This difference may be attributed to these species' reproductive (Tsyusko et al., 2005) or inflorescence characteristics (Na et al., 2010). In our study, similar trend was present in nSSR (He: 0.205 vs. 0.101) and nDNA data (π: 0.433×10^{-3} vs. 0.374×10^{-3}), whereas according to the measurements of cpDNA data, *T. latifolia* exhibited greater genetic diversity than *T. angustifolia* (π: 0.28×10^{-3} vs. 0.943×10^{-3}). The relatively high genetic diversity exhibited by *T. latifolia* in this study may be caused by the fact that we have captured the level of genetic diversity from different geographical regions and sampling from a larger geographic area.

Our study based on nuclear *PS* sequences and nSSR data revealed that *T. laxmannii* has the highest genetic diversity (at least 2.5 times greater than the other three *Typha* species). However, a previous study revealed that *T. angustifolia* has the highest genetic diversity and that *T. latifolia* has the lowest genetic diversity of the four *Typha* species based on AFLP markers (Na et al., 2010). The genetic diversity of *T. laxmannii* was likely underestimated due to its limited sampling range (nine sites from Korea and Russia) compared with that of *T. angustifolia* (28 sites from Korea, China, Japan and Russia). Differences in the breeding system may be cited as explanations of genetic diversity patterns. As *T. angustifolia* has lighter pollen and higher-positioned male inflorescences, this species has a higher cross-pollination rate than *T. latifolia* (Krattinger, 1975). Although inbreeding of *T. laxmannii* and *T. orientalis* has not yet been investigated, the cross-pollination rate of *T. laxmannii* should be higher because these species share similar inflorescence characteristics with *T. angustifolia* and *T. latifolia*, respectively (Sun & Simpson, 2010). Inbreeding increases the frequency of homozygous individuals compared to that expected under random mating (Hartl, 2000). Heterozygote frequency in *T. laxmannii* was 4- to 10-fold higher than that in the three other species, suggesting a mixed-mating or even outcrossing breeding system. In addition, there was a much lower percentage of monomorphic populations in *T.*

laxmannii (14%) than in *T. angustifolia* (40.3%), *T. latifolia* (70%), or *T. orientalis* (73.3%); this pattern suggests that sexual recruitment is likely to be important for *T. laxmannii* and that asexual reproduction plays a less extensive role compared to other species, e.g., *T. latifolia* (Keane et al., 1999). Nonetheless, detailed comparative investigations are still needed to quantify differences in breeding system and reproductive strategy.

Genetic structure

The levels of population differentiation measured by maternally inherited chloroplast markers are expected to be higher than that measured by biparentally inherited nuclear markers due to differences in seed and pollen migration parameters (Ennos, 1994; Petit et al., 2005). However, this trend is not exhibited in *T. angustifolia* and *T. orientalis* (Table 1), perhaps due to the low outcrossing rates and different levels of marker polymorphism (Hamrick & Godt, 1989; Nauta & Weissing, 1996). Of the four species, *T. latifolia* showed the highest level of genetic differentiation among its populations ($F_{ST} = 0.965$, 0.678 or 0.723), values that are higher than those previously reported for this species (0.32–0.41, Keane et al., 1999; 0.29, Tsyusko et al., 2005). This finding may be explained by the present study's inclusion of geographically isolated populations, i.e., separated by a distance of at least 1800 km. Except for *T. latifolia*, genetic differentiation among *Typha* populations was lower than that of *Phragmites australis* in China ($F_{ST} = 0.484$ for cpDNA sequences, An et al., 2012), even though this species has similar reproductive, pollination, and seed dispersal strategies and often co-occurs with *Typha*. This pattern may be due to the lighter weights of *Typha* seeds (Ekstam & Forseby, 1999). As discussed above, the low level of *n*DNA and *n*SSR genetic differentiation among *T. laxmannii* populations is likely associated with its high outcrossing rate.

Isolation by distance (IBD) is a common model of gene flow and often serves as an explanation for the positive correlation of genetic and geographic distances (Slatkin, 1987). According to cpDNA, nDNA, and nSSR data, an IBD pattern was observed for *T. latifolia* in this study. Based on the same microsatellite loci, the correlation between genetic and geographic distances, albeit significant, was relatively low for *T. latifolia* populations from Ukraine with a range of approximately 1000 km and was absent in populations from Czech Republic at a spatial scale of 100 km (Tsyusko et al., 2005; Fér, 2008). In the current study, the presence of IBD in *T. latifolia* was associated with the high genetic differentiation between disjunctive populations. As no correlation was detected in separate analyses of northeast and northwest China, the geographic scale appears to be important for the isolation of *Typha* species. With regard to the three other species, only *T. laxmannii* showed a significant, but relatively weak, correlation of genetic and geographic distances based on cpDNA data, which was not supported by the study of Na et al. (2010) based on AFLP data. For aquatic plants, except for life history traits, many factors such as geographical features, colonization processes, and hydrological connectivity played roles in shaping gene flow patterns (Terer et al., 2015). Comprehensive and detailed investigations at finer scale are needed to elucidate their influences on gene flow patterns.

In conclusion, the four widespread *Typha* species studied here formed four highly supported clades and distinct genetic clusters, suggesting no hybridization between *T. angustifolia* and *T. latifolia* in China. Substantial variation in the levels and distributions of genetic diversity among the four *Typha* species was found, which is associated with different breeding system mechanisms.

Acknowledgments This study was supported by grants from the National Natural Science Foundation of China to Xinwei Xu (31070190 and 31270265) and Dan Yu (30930011). We thank the members of Dan Yu's group for field assistance.

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

Alm, C. G. & H. Weimarck, 1933. *Typha angustifolia* L. × *latifolia* L. funnen i Skåne. Botaniska Notiser 1933: 279–284.

An, J. X., Q. Wang, J. Yang & J. Q. Liu, 2012. Phylogeographic analyses of *Phragmites australis* in China: native distribution and habitat preference of the haplotype that invaded North America. Journal of Systematics and Evolution 50: 334–340.

Ball, D. & J. R. Freeland, 2013. Synchronous flowering times and asymmetrical hybridization in *Typha latifolia* and *T.*

angustifolia in northeastern North America. Aquatic Botany 104: 224–227.

Bohonak, A. J., 2002. IBD (isolation by distance): a program for analyses of isolation by distance. Journal of Heredity 93: 153–154.

Carrió, E., A. D. Forrest, J. Güemes & P. Vargas, 2010. Evaluating species nonmonophyly as a trait affecting genetic diversity: a case study of three endangered species of *Antirrhinum* L. (Scrophulariaceae). Plant Systematics and Evolution 288: 43–58.

Clement, M., D. Posada & K. A. Crandall, 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657–1659.

Cook, C. D. K., 1990. Aquatic plant book. SPB Academic Publishing, Hague.

Ekstam, B. & Å. Forseby, 1999. Germination response of *Phragmites australis* and *Typha latifolia* to diurnal fluctuations in temperature. Seed Science Research 9: 157–163.

Ennos, R. A., 1994. Estimating the relative rates of pollen and seed migration among plant populations. Heredity 72: 250–259.

Excoffier, L., G. Laval & S. Schneider, 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1: 47–50.

Falush, D., M. Stephens & J. K. Pritchard, 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164: 1567–1587.

Fér, T., 2008. Study of plant dispersal in river corridors using molecular markers. Ph.D. dissertation, Charles University Prague, Prague.

Figert, E., 1890. Botanische Mitteilungen aus Schlesien. III. *Typha latifolia* × *Typha angustifolia*. Deutsche Botanische Monatsschrift 8: 55–57.

Galeuchet, D. J., R. Husi, C. Perret, M. Fischer & B. Gautschi, 2002. Characterization of microsatellite loci in *Lychnis flos-cuculi* (Caryophyllaceae). Molecular Ecology Notes 2: 491–492.

Goudet, J., 1995. FSTAT: a computer program to calculate statistics, version 1.2. Journal of Heredity 86: 485–486.

Hamrick, J. L. & M. J. W. Godt, 1989. Allozyme diversity in plant species. In Brown, A. H. D., M. T. Clegg, A. L. Kahler & B. S. Weir (eds), Plant population genetics, breeding and genetic resources. Sinauer Associates, Sunderland: 43–63.

Hamrick, J. L. & M. J. W. Godt, 1996. Effects of life history traits on genetic diversity in plant species. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 351: 1291–1298.

Hamrick, J. L., M. Godt & S. Sherman-Broyles, 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests 6: 95–124.

Hartl, D. L., 2000. A primer of population genetics. Sinaue, Sunderland.

Katoh, K., K. Misawa, K. I. Kuma & T. Miyata, 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059–3066.

Keane, B., S. Pelikan, G. P. Toth, M. K. Smith & S. H. Rogstad, 1999. Genetic diversity of *Typha latifolia* (Typhaceae) and the impact of pollutants examined with tandem-repetitive DNA probes. American Journal of Botany 86: 1226–1238.

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

Krattinger, K., 1975. Genetic mobility in *Typha*. Aquatic Botany 1: 57–70.

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

Lamote, V., M. De Loose, E. Van Bockstaele & I. Roldán-Ruiz, 2005. Evaluation of AFLP markers to reveal genetic diversity in *Typha*. Aquatic Botany 83: 296–309.

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

Lee, D. W. & D. E. Fairbrothers, 1973. Enzyme differences between adjacent hybrid and parent populations of *Typha*. Bulletin of the Torrey Botanical Club 100: 3–11.

Librado, P. & J. Rozas, 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452.

Loveless, M. D. & J. L. Hamrick, 1984. Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics 15: 65–95.

Luther, H. E., 1947. *Typha angustifolia* × *latifolia* L. (*T.* × *glauca* Godr.) I Ostfennoskandien. Memoranda Societatis Flora et Fauna Fennica 23: 66–75.

Martins, E., R. Lamont, G. Martinelli, C. Lira-Medeiros, A. Quinet & A. Shapcott, 2014. Genetic diversity and population genetic structure in three threatened *Ocotea* species (Lauraceae) from Brazil's Atlantic Rainforest and implications for their conservation. Conservation Genetics 16: 1–14.

Mashburn, S. J., R. R. Sharitz & M. H. Smith, 1978. Genetic variation among *Typha* populations of the southeastern United States. Evolution 32: 681–685.

Mateu-Andrés, I. & L. De Paco, 2006. Genetic diversity and the reproductive system in related species of *Antirrhinum*. Annals of Botany 98: 1053–1060.

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

Nauta, M. J. & F. J. Weissing, 1996. Constraints on allele size at microsatellite loci: implications for genetic differentiation. Genetics 143: 1021–1032.

Ng, W. L., Y. Onishi, N. Inomata, K. M. Teshima, H. T. Chan, S. Baba, S. Changtragoon, I. Z. Siregar & A. E. Szmidt, 2015. Closely related and sympatric but not all the same: genetic variation of Indo-West Pacific *Rhizophora* mangroves across the Malay Peninsula. Conservation Genetics 16: 137–150.

Nowińska, R., B. Gawrońska, A. Czarna & M. Wyrzykiewicz-Raszewska, 2014. *Typha glauca* Godron and its parental plants in Poland: taxonomic characteristics. Hydrobiologia 737: 163–181.

Nybom, H., 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Molecular Ecology 13: 1143–1155.

Petit, R. J., J. Duminil, S. Fineschi, A. Hampe, D. Salvini & G. G. Vendramin, 2005. Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. Molecular Ecology 14: 689–701.

Pons, O. & R. Petit, 1996. Measuring and testing genetic differentiation with ordered versus unordered alleles. Genetics 144: 1237–1245.

Robuchon, M., L. Le Gall, S. Mauger & M. Valero, 2014. Contrasting genetic diversity patterns in two sister kelp species co-distributed along the coast of Brittany, France. Molecular Ecology 23: 2669–2685.

Rousset, F., 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145: 1219–1228.

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

Sharitz, R. R., S. A. Wineriter, M. H. Smith & E. H. Liu, 1980. Comparison of isozymes among *Typha* species in the eastern United States. American Journal of Botany 67: 1297–1303.

Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling & R. L. Small, 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. American Journal of Botany 92: 142–166.

Shaw, J., E. B. Lickey, E. E. Schilling & R. L. Small, 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. American Journal of Botany 94: 275–288.

Skrede, I., L. Borgen & C. Brochmann, 2009. Genetic structuring in three closely related circumpolar plant species: AFLP versus microsatellite markers and high-arctic versus arctic–alpine distributions. Heredity 102: 293–302.

Slatkin, M., 1987. Gene flow and the geographic structure of natural populations. Science 236: 787–792.

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

Smith, S. G., 1987. *Typha*: its taxonomy and the ecological significance of hybrids. Archiv fü Hydrobiologie 27: 129–138.

Snow, A. A., S. E. Travis, R. Wildová, T. Fér, P. M. Sweeney, J. E. Marburger, S. Windels, B. Kubátová, D. E. Goldberg & E. Mutegi, 2010. Species-specific SSR alleles for studies of hybrid cattails (*Typha latifolia* × *T. angustifolia*; Typhaceae) in North America. American Journal of Botany 97: 2061–2067.

Sun, K. & D. Simpson, 2010. Typhaceae. In Wu, Z. Y. & P. H. Raven (eds), Flora of China. vol. 23. Science Press, Beijing: 158–163.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei & S. Kumar, 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731–2739.

Terer, T., A. M. Muasya & L. Triest, 2015. Strong isolation by distance revealed among *Cyperus papyrus* populations in the Rift Valley lakes, Lake Victoria, and isolated wetlands of Kenya. Aquatic Botany 121: 57–66.

Travis, S. E., J. E. Marburger, S. Windels & B. Kubátová, 2010. Hybridization dynamics of invasive cattail (Typhaceae) stands in the Western Great Lakes Region of North America: a molecular analysis. Journal of Ecology 98: 7–16.

Tsyusko, O. V., M. H. Smith, R. R. Sharitz & T. C. Glenn, 2005. Genetic and clonal diversity of two cattail species, *Typha latifolia* and *T. angustifolia* (Typhaceae), from Ukraine. American Journal of Botany 92: 1161–1169.

Tsyusko-Omeltchenko, O. V., N. A. Schable, M. H. Smith & T. C. Glenn, 2003. Microsatellite loci isolated from narrow-leaved cattail *Typha angustifolia*. Molecular Ecology Notes 3: 535–538.

Xu, X. W., W. D. Ke, X. P. Yu, J. Wen & S. Ge, 2008. A preliminary study on population genetic structure and phylogeography of the wild and cultivated *Zizania latifolia* (Poaceae) based on Adh1a sequences. Theoretical and Applied Genetics 116: 835–843.

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