Development of genomic resources for cattails (*Typha*), a globally important macrophyte genus

Running title: Development of genomic resources for *Typha*

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Abstract

A critical knowledge gap in freshwater plants research is the lack of genetic resources necessary to answer fundamental questions about their demographic histories, adaptation, and taxonomy. One example of this is *Typha*, a macrophyte genus essential to wetlands that is also becoming an increasingly problematic biological invader in numerous regions worldwide; while important insights have been discovered for this genus, currently available genetic markers are insufficient to resolve its phylogenetic relationships, population structure, and hybridization dynamics. We performed a cost and time–accessible library preparation for high–throughput sequencing to develop a suite of genomic resources for *Typha*. Genome–wide nuclear SNPs of 140 *Typha* samples from North America, temperate Eurasia and Africa revealed three independent genetic clusters corresponding to *T. angustifolia*, *T. domingensis* and *T. latifolia*. Data from ~40% of the nuclear genome was obtained, permitting the characterization of 119,324 nuclear diagnostic markers (SNPs that differentiate the three species). A reference–guided workflow to reconstruct whole–chloroplast–genome sequences was implemented, recovering ~60% of the genome per sample. Three genetic lineages were identified from the cpDNA phylogenetic analysis, agreeing with those determined from nrDNA. With a cost below 15 USD per sample and a processing time of two hours for the library preparation, this is a rapid and cost–effective protocol for population genomic studies. The genomic locations of diagnostic markers and the chloroplast sequences produced in this study will be permanent resources that can be incorporated into future studies of *Typha,* a globally important and evolutionarily dynamic genus.

Keywords: Hybridization, diagnostic markers, chloroplast–genome assembly, high–throughput sequencing, *Typha* phylogenomics, introgression.

Introduction

Freshwater plants are essential to aquatic ecosystems, shaping their habitats' structure and ecological functions (Chambers et al., 2008; Christie et al., 2009; Rejmankova, 2011). Although freshwater plants have been increasingly incorporated into applications that include habitat restoration and invasive species management, they remain highly understudied compared to terrestrial plants (Evangelista et al., 2014; Iversen et al., 2022). One key knowledge gap in freshwater plant research is the lack of genetic tools necessary to answer fundamental questions about their demographic histories, adaptation, and phylogenetic relationships (Fay et al., 2019; Maréchal, 2019; O'Hare et al., 2018; Yannelli et al., 2022).

Genomic characterization of freshwater plants has been hampered by biological and technical challenges, plus biases in scientific research (Matheson & McGaughran, 2022; Troudet et al., 2017). Hundreds to thousands of molecular markers are often required for genomic–based research on topics such as gene flow and adaptation of non–model organisms (da Fonseca et al., 2016; Stapley et al., 2010), but the *de novo* development of genomic resources can be both time–consuming and expensive (Hu et al., 2020; Ortega et al., 2020; Prieto et al., 2021). Overcoming these challenges is feasible with novel, rapid and cost–effective methods that capture genome–wide genetic variation, allowing researchers to address questions related to taxonomy, evolution, and conservation (Andrews et al., 2016; Goodwin et al., 2016).

Typha L. (cattails) is a global genus of freshwater plants foundational to wetlands (reviewed in Bansal et al., 2019). Cattails are a valuable ecosystemic and human resource that play a fundamental ecological role by cycling nutrients, preventing erosion, maintaining stable water levels and providing food and shelter for wildlife (Andrews & Pratt, 1978; Bonanno & Cirelli, 2017; Dieye et al., 2017; Kimmerer, 2013; Svedarsky et al., 2019). While

their importance is undisputed, one major challenge in *Typha* research has been taxonomic identification, which cannot be fully accomplished using morphological characters due to their high intraspecific variability and interspecific hybridization. Consequently, the richness of cattail species, their taxonomy, their provenance (e.g., alien versus native lineages), and their phylogenetic relationships remain incompletely resolved (Ciotir & Freeland, 2016; Volkova & Bobrov, 2022; Zhou et al., 2018). A refined *Typha* taxonomy accompanied by diagnostic markers, i.e., those loci that have alternative fixed alleles between two species, is necessary to identify lineages of both introduced and hybrid taxa, which are increasingly documented as invasive, e.g., *T. domingensis* Pers. in Western Europe and Central America, *T. latifolia* L. in Oceania, and *T.* × *glauca* Godr. (*T. angustifolia* L. × *T. latifolia* L.) in North America (Bansal et al., 2019; *GISD*; Govaerts, 2004; Hall, 2009; Maldonado, 2019).

High–throughput sequencing technologies, novel, cost and time–accessible genome library preparations, and the recent assembly of the *T. latifolia* genome (Goodwin et al., 2016; Rowan et al., 2019; Widanagama et al., 2022) collectively present an opportunity to develop a suite of genomic resources for *Typha*. In addition to taxonomic resolution, these resources can facilitate investigations of the genetic variation, local adaptation, population structure, evolutionary history, and hybridization dynamics in this genus. We applied a high–throughput sequencing protocol for enzymatic library preparation and data processing to produce genome–wide resources in *Typha* spp. By optimizing the method from Rowan et al. (2019), we generated a catalogue of nuclear SNPs that differentiate *T. angustifolia*, *T. domingensis*, and *T. latifolia*, plus extensive chloroplast genome sequences in a fast, straightforward, and cost–efficient manner. These permanent resources will be incorporated into future evolutionary and population genetics research in *Typha*.

Materials and methods

Reference genome

We used the reference–based scaffolder Chromosomer 0.1.4a (Tamazian et al., 2016) to align the 1158 *T. latifolia* scaffolds from Widanagama et al. (2022) (Genbank accession: JAIOKV000000000.1) with the *T. latifolia* isolate L0001 (15 chromosomes, GenBank accession: JAAWWQ000000000.1) to produce a local chromosome–level *T. latifolia* genome. Widanagama et al. (2022) had a significantly higher mapping success of unrelated re–sequenced *Typha* spp. compared to the isolate L0001, suggesting it was a more representative *Typha* genome assembly. The scaffolds were aligned as chromosomes using BLAST+ 2.12.0 (Camacho et al., 2009) with the software default settings, and the alignments were anchored in Chromosomer, establishing a gap length = 0 and a ratio threshold = 1.

Sampling, DNA extraction, and sequencing

DNA extractions were obtained either from previous studies (Bhargav et al., 2022; Ciotir et al., 2017; Pieper et al., 2020, 2017; Tangen et al., 2022; Tisshaw et al., 2020) or from samples collected in Eurasia and extracted at Trent University following the published protocols. We obtained DNA from 140 *T. angustifolia*, *T. domingensis*, and *T. latifolia* samples (Figure 1), previously identified using a combination of morphological characteristics (Grace & Harrison, 1986; Smith, 1967) and genetic analyses of microsatellite loci (Bansal et al., 2019; Kirk et al., 2011; Pieper et al., 2020; Snow et al., 2010). Extracted DNA was quantified using a Qubit fluorometer (Thermofisher Scientific) and calculated as the mean of three independent readings from each sample. All samples were either standardized to 2 ng/µL by dilution with nuclease–free water or, if at concentrations less than 2 ng/µL (0.4 – 1.9 ng/µL), left undiluted.

Each DNA sample was prepared for Nextera XT sequencing libraries by enzymatic fragmentation followed by ligation of short adapter sequences with the Illumina Tagment DNA enzyme (TD) and buffer kit (small kit, #20034210). As the ratio of TD enzyme to DNA is crucial for the reaction, we initially followed the recommendations of Rowan et al. (2019) and subsequently optimized the reagent volumes for our library preparation as 5.5 µL of 5× TD buffer, 0.5 µL of 1× TD enzyme, and 4 µL of DNA (standardized or undiluted), keeping all reagents on ice during the preparation. Samples were incubated at 55°C for 10 minutes and left at room temperature for five minutes. 5 µL of each sample were run on an agarose gel to confirm the efficacy of the tagmentation reaction, evidenced by visible smears. The fragmented DNA was then amplified using unique dual indexing based on combinations from a total of 24 N7 (47 bases) and 8 S5 (51 bases) adapters (Alpha DNA, Canada). The PCR cocktail included 0.2 µM of each index, 0.5U of KAPA HiFi HotStart DNA polymerase (Roche), 12.5 µL of 5× KAPA reagent, 5 µL of tagmented DNA, and 6.5 µL of nuclease–free water to a final volume of 25 µL. The PCR cycle comprised 72°C (3 minutes); 95°C (30 seconds); and 14 cycles of 95°C (10 seconds), 55°C (30 seconds), and 72°C (30 seconds). Visible smears confirmed amplification success after running 5 µL of the PCR product on an agarose gel; then, 10 µL from each sample were pooled, and the remaining PCR products were stored at -20°C. The pooled library was purified with a QIAquick PCR purification kit (QIAGEN) following the manufacturer's protocol, with a final elution in 50 µL of buffer. The library was quantified using a D1000 Tapestation assay (Agilent Technologies, USA) and a Qubit fluorometer (Thermo Fisher Scientific). A quality–control paired–end sequencing was executed using a Miseq (151 bp) to ensure the library was compiled successfully. Finally, paired–end sequencing was performed on a Novaseq 6000 (126 bp) at The Centre for Applied Genomics (Toronto, Ontario).

Raw data processing, filtering, and SNP–calling

The quality of the demultiplexed raw sequences was evaluated using FastQC 0.11.9 (Andrews, 2017) and MultiQC 1.14 (Ewels et al., 2016). Read pairing and adapter removal were carried out with trimmomatic 0.39 (Bolger et al., 2014), removing any cleaned reads shorter than 100 bp. Paired and remaining unpaired reads were mapped to our chromosome–level *T. latifolia* nuclear genome and the *T. latifolia* chloroplast reference (Genbank accession: NC_013823.1) using the mem module of BWA 0.7.17 (Li & Durbin, 2009). Mapped reads from Miseq and Novaseq 6000 sequencers were merged, and mapping statistics were evaluated with the flagstat and coverage modules of SAMtools 1.15.1 (Li et al., 2009).

SNP–calling was performed with ANGSD 0.93 (Korneliussen et al., 2014) following the SAMtools model, exclusively retrieving SNPs with a minimum *P*–value of $1e^{-6}$, minimum mapping and sequencing qualities of 20, omitting any triallelic sites, and outputting a variant call format (VCF) file (*-doGeno 4 -gl 1 -skipTriallelic 1 -SNP_pval 1e-6 -minMapQ 20 -minQ 20 -doMajorMinor 1 -domaf 1 -doPost 1 -doCounts 1 -dovcf* 1). For the nuclear analyses, SNPs with > 50% missing data across all samples and sites that mapped to the chloroplast genome were removed with vcftools 0.1.16 (Danecek et al., 2011). We did not apply additional filters to SNP identification: our samples represented broad geographical sampling and thus were not expected to be in Hardy–Weinberg equilibrium; additionally, as allele frequencies were unlikely to be representative of regional allele frequencies we did not apply a minor allele frequency filter. Neither did we filter for linkage equilibrium, as eliminating alleles that are in linkage disequilibrium is likely to decrease the power of analyses to detect hybridization and introgression (Alexander, 2020; Pearman et al., 2022).

Genetic structure and diagnostic markers

We first used nuclear SNPs to assess the most likely number of genetic clusters across all samples and the membership of each plant to these clusters using three complementary approaches. ADMIXTURE 1.3.0 (Alexander & Lange, 2011) was run with K = 1 – 10, and the optimal number of clusters was chosen via the cross–validation procedure. A neighbour–joining tree from the samples' pairwise genetic distance matrix (transformed on the R 4.2.2 package ape (Paradis et al., 2004; R Core Team, 2022)), and a Principal Component Analysis (PCA) without an *a priori* assumption were produced in Plink 1.90 (Purcell et al., 2007). We verified that the assignment of samples to genetic clusters (corresponding to three species, see *Results*) was consistent for each approach.

Potential introgression was tested by running ADMIXTURE (K = 1 – 5) on three datasets, each comprising a unique combination of two genetic clusters, using only those SNPs that remained variable based on the two clusters being compared. We confirmed that the cross–validation procedure for the runs of each cluster pair chose the optimal number of clusters as two and used the admixture proportion (Q score in $K = 2$) as an index of potential introgression of each sample. Applying Senn & Pemberton (2009) and Smith et al. (2018) thresholds, individuals whose Q score was $0.05 \leq Q \leq 0.95$, were considered as introgressed.

To compare the levels of differentiation between clusters, values of Weir and Cockerham's genetic differentiation (F_{ST}; Weir & Cockerham, 1984) for every variable site and genetic divergence (d_{xy}; Nei & Miller, 1990) in 10 Kbp windows between species pairs were computed using pixy 1.2.7 (Korunes & Samuk, 2021), and the means were calculated. Species–specific SNPs for each pair of genetic clusters were identified with DiagnoSNPs 1.0 (Arce-Valdés, 2022). We removed introgressed individuals before estimating genetic differentiation levels and identifying species–specific SNPs.

178 *Chloroplast genome reconstruction and phylogenetic analysis*

179 We implemented a reference–guided workflow to reconstruct whole–chloroplast–
180 genome sequences. Nucleotide calling was performed individually for each of the 140
181 samples in ANGSD using the reads that mapped to the chloroplast genome reference,
182 requiring minimum mapping and base qualities of 20, and using Ns as missing data (*-dofasta*
183 *2 -minMapQ 20 -minQ 20 -doCounts 1*). Sequences were aligned to the chloroplast genomes
184 of *T. przewalskii* Skvortsov, *T. lugdunensis* P. Chabert, *T. orientalis* C. Presl, and
185 *Sparganium natans* (GenBank accessions: NC_061354.1, NC_061353.1, NC_050678.1, and
186 NC_058577.1), following Smith et al. (2021) by applying MAFFT 7.0 default settings (plus
187 the flag *--nwildcard*) (Katoh et al., 2019). Snp–sites 2.5.1 (Page et al., 2016) was used to
188 remove sections of the genome with ambiguous positions, gaps, and missing data, such that if
189 any of those was found in a sequence, that position was removed for all sequences.
190 Nucleotide diversity (π) was calculated in the R package pegas (Paradis, 2010).
191 The phylogenetic relationships of the chloroplast genome sequences were
192 reconstructed through RAxML-NG 1.1 (Kozlov et al., 2019). Model selection was based on
193 jmodeltest 2.1.10 results (Darriba et al., 2012) using the AIC. A transversion model was
194 established, assuming gamma–distributed rates with four substitution categories and
195 estimating the proportion of invariable sites (TVM + G4 + I). RAxML was run with a
196 thorough bootstrap starting from 100 random trees, with *Sparganium natans* as the outgroup,
197 and letting automatic bootstrapping (--autoMRE). The best–scoring ML tree was plotted.

Results

Genome scaffolding, mapping statistics, and genotyping

Approximately 99.81% of the scaffold sequences were aligned to the template genome. The scaffolds were anchored to 15 chromosomes producing a genome of 285.11 Mb (Pending GenBank accession: JAIOKV000000000.2). The total assembled size was comparable to the *T. latifolia* genome sizes of Widanagama et al. (2022) (287.19 Mb) and the isolate L0001 (214.13 Mb). Updating the chromosome–level assembly genome simplified our downstream analyses while keeping the highest mapping success of unrelated re–sequenced *Typha* spp. and facilitating an accurate recombination map for future studies of speciation, hybridization, and the genomic landscape of introgression in *Typha*.

After quality control, 982 M clean paired–end reads were retained, and ~98% mapped to the reference genome. With minimum mapping and sequencing qualities = 20, the average depth and breadth of coverage of the nuclear sequences were 4× and 42%, respectively. Over 60% of the chloroplast genome breadth was covered across all samples (mean depth = 711×), enabling us to use 96,591 bp for the phylogenetic reconstruction. We assembled 12,177,703 bi–allelic nuclear SNPs across the 140 *Typha* samples (7,122,151 with a MAF > 0.05). The total genotyping rate, i.e., the mean proportion of samples with data for each SNP, was 0.68.

Genetic structure and diagnostic markers

The admixture analysis, the PCA, and the neighbour–joining tree each established the most likely number of genetic clusters as three ($K = 3$) (Figure 1): in line with previous taxonomic identifications, 38 samples were identified within the *T. angustifolia* cluster (15 of which had *T. latifolia* introgression and 5 of which had both *T. domingensis* and *T. latifolia* introgression); 25 samples were in the *T. domingensis* cluster (one with *T. angustifolia* introgression, 12 with *T. latifolia* introgression, and 3 with both *T. angustifolia* and *T. latifolia*

introgression); and 77 samples were in the *T. latifolia* cluster (one with both *T. angustifolia* and *T. domingensis* introgression, and one with *T. angustifolia* introgression). Using only the 18 *T. angustifolia*, 9 *T. domingensis*, and 75 *T. latifolia* not introgressed individuals, the mean pairwise interspecific F_{ST} and d_{xy} values ranged from 0.25 to 0.49 and 0.28 to 0.35, respectively, with *T. latifolia* showing the highest differentiation with both *T. angustifolia* and *T. domingensis.* We identified 119,324 nuclear species-specific SNPs (Table 1) based on the comparisons between species pairs.

Chloroplast genome reconstruction and phylogenetic relationships

After removing all ambiguities and missing data from the chloroplast genomes, we were left with an alignment of 96,591 bp across all 140 sequences and the four reference genomes, with 4,916 segregating sites and $\pi = 0.003$. The phylogenetic reconstruction was consistent with the nuclear genetic structure results, grouping the 143 *Typha* samples into three lineages, with *T. angustifolia* in one clade, *T. domingensis* and *T. orientalis* sharing another, and *T. latifolia* and *T. przewalskii* in a third clade (Figure 2). All interspecific nodes were strongly supported (100%, Felsenstein bootstrap proportion).

Discussion

We aimed to produce a suite of genome–wide resources to facilitate investigations into the taxonomy and population genetics of *Typha* and to advance the general understanding of wetland plants. Following a fast, straightforward, and cost–efficient genomic library preparation protocol (Rowan et al., 2019), we sequenced 140 *Typha* samples, obtaining an average breadth of 42% of the nuclear genome, characterizing 119,324 nuclear SNPs that collectively differentiate three *Typha* spp., and producing chloroplast sequences with a breadth of > 60% per sample. With a cost below 15 USD per sample and a processing time of two hours for the library preparation, our workflow is a rapid and cost–effective protocol that

246 can be applied in population genomic studies for investigating levels of genetic diversity and
247 differentiation, identifying conservation units and alien taxa, characterizing hybrid classes,
248 and examining hybridization dynamics, among other purposes.

249 Three genetic clusters were identified from both nuclear and chloroplast genomes,
250 corresponding to *T. angustifolia*, *T. domingensis*, and *T. latifolia*; and potential introgression
251 was detected among the three species. Hybridization of *T. domingensis* with *T. angustifolia*
252 and *T. latifolia* has been historically documented (Govaerts, 2004; Smith, 1967); however,
253 those inferences were based on experimental and taxonomical observations and recently
254 exposed with microsatellite loci by Ciotir et al. (2017). Future investigations should address
255 the extent to which hybridization is shaping the genetic variation among these three species.
256 Furthermore, *T. domingensis* is increasingly invading Costa Rica (Trama et al., 2017), Nigeria
257 (Ringim et al., 2016), and North America, potentially expanding its range across the latter
258 (Spencer & Vincent, 2013; Zhang et al., 2008). However, the taxonomic identity of these
259 plants is unclear –are they hybrids, non–native lineages, or native lineages responding to
260 environmental change? By identifying SNPs that characterize *T. domingensis*, we provide a
261 valuable resource to answer this and other questions across the evolutionary and hybridization
262 history of *Typha*.

263 The markers that differentiate *T. angustifolia* from *T. latifolia* will have important
264 applications in North America, where the two species interbreed across a large area and
265 produce an invasive interspecific hybrid (*T.* × *glauca*) that dominates wetlands, alters nutrient
266 cycling, and reduces biodiversity across the Great Lakes Region (Bansal et al., 2019);
267 additionally, this hybrid is expanding throughout the Prairie Pothole Region, causing native
268 plant diversity to decrease in invaded potholes (Jones et al., 2023), and may impact essential
269 habitat for millions of breeding and migratory waterfowl species (Tangen et al., 2022). Until

now, molecular resources to differentiate *T. angustifolia*, *T. latifolia*, and *T.* × *glauca* were limited to sets of relatively few individual markers that have produced important insights: RAPDs, chloroplast DNA sequences, and codominant SSR loci have contributed to exposing the sexual fertility of first–generation hybrids (F1) (Snow et al., 2010), asymmetric hybridization (with *T. angustifolia* being mainly the maternal parent) (Ball & Freeland, 2013; Kuehn et al., 1999; Pieper et al., 2017), overall comparable levels of sexual and clonal reproduction in parents and F1s (Pieper et al., 2020; Travis et al., 2011), heterosis in F1s (Bunbury-Blanchette et al., 2015; Travis et al., 2010; Zapfe & Freeland, 2015), a high frequency of F1s in natural populations (Kirk et al., 2011; Travis et al., 2010), and partial sterility of F1s coupled with hybrid breakdown of F2s and advanced–generation hybrids (Bhargav et al., 2022; Pieper et al., 2017). However, critical questions remain unresolved because existent markers are insufficient to expose the prevalence of advanced–generation hybrids and backcrosses in wild populations. The expansive suite of SNPs identified in this study will facilitate investigations into the extent of hybridization, hybrid breakdown dynamics, and adaptive introgression across the *T.* × *glauca* hybrid zone, allowing researchers to understand the evolutionary processes shaping this genus speciation and species boundaries, and to inform conservation and management strategies.

Fundamental genetic tools are essential for understanding macrophytes biology, management, and conservation (O'Hare et al., 2018). The protocol described in this paper, the updated chromosome–level assembly genome of *T. latifolia*, the SNPs catalogue, and the chloroplast sequences produced for each sample comprise permanent resources that can be applied to study the genetic composition of *Typha* populations and hybrid zones. Genome–wide sequencing techniques and reference–based chloroplast genome assemblies are promising tools to clarify the demographic histories, dispersal, adaptation, and taxonomy of

294 multiple congeneric macrophyte species (Russello et al., 2015; Straub et al., 2012), and
295 substantial genome–wide research will allow us to tackle these and other knowledge gaps in
296 *Typha* and other taxa.

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Data Accessibility Statement

The updated genome assembly, raw reads supporting this study's findings, SNP information and chloroplast sequences produced in the present study will be deposited into online databases upon publication. Upon publication, related metadata and all the scripts used will be available (https://gitlab.com/WiDGeT_TrentU). We will provide unique sample identifier tags that can be matched to the deposited genetic data, metadata, and scripts.

Benefit–Sharing Statement

Benefits from this research accrue from sharing our data and results on public databases, as described above.

Author Contributions

Joanna R. Freeland, Aaron B. A. Shafer and Marcel E. Dorken conceived this study. Joanna R. Freeland and Polina A. Volkova collected and characterized the plant tissue samples. Tulsi Patel performed the lab work. Alberto Aleman executed the bioinformatics and wrote the manuscript. All authors contributed to the manuscript and approved this version.

Conflict of Interest

The authors declare no conflict of interest.

Supporting Information

Tables and Figures

Table 1. Mean Weir and Cockerham's pairwise genetic differentiation (F_{ST}) for every variable site, mean genetic divergence (d_{xy}) measured in 10 Kbp windows, number of SNPs, and diagnostic markers (SNPs with fixed opposite alleles) between three *Typha* spp.

Pairwise comparison	F_{ST}	d_{xy}	SNPs	Diagnostic SNPs
T. angustifolia – T. domingensis	0.25	0.28	10,358,977	33,436
T. angustifolia – T. latifolia	0.44	0.35	8,786,870	30,113
T. domingensis – T. latifolia	0.49	0.34	9,380,607	55,775

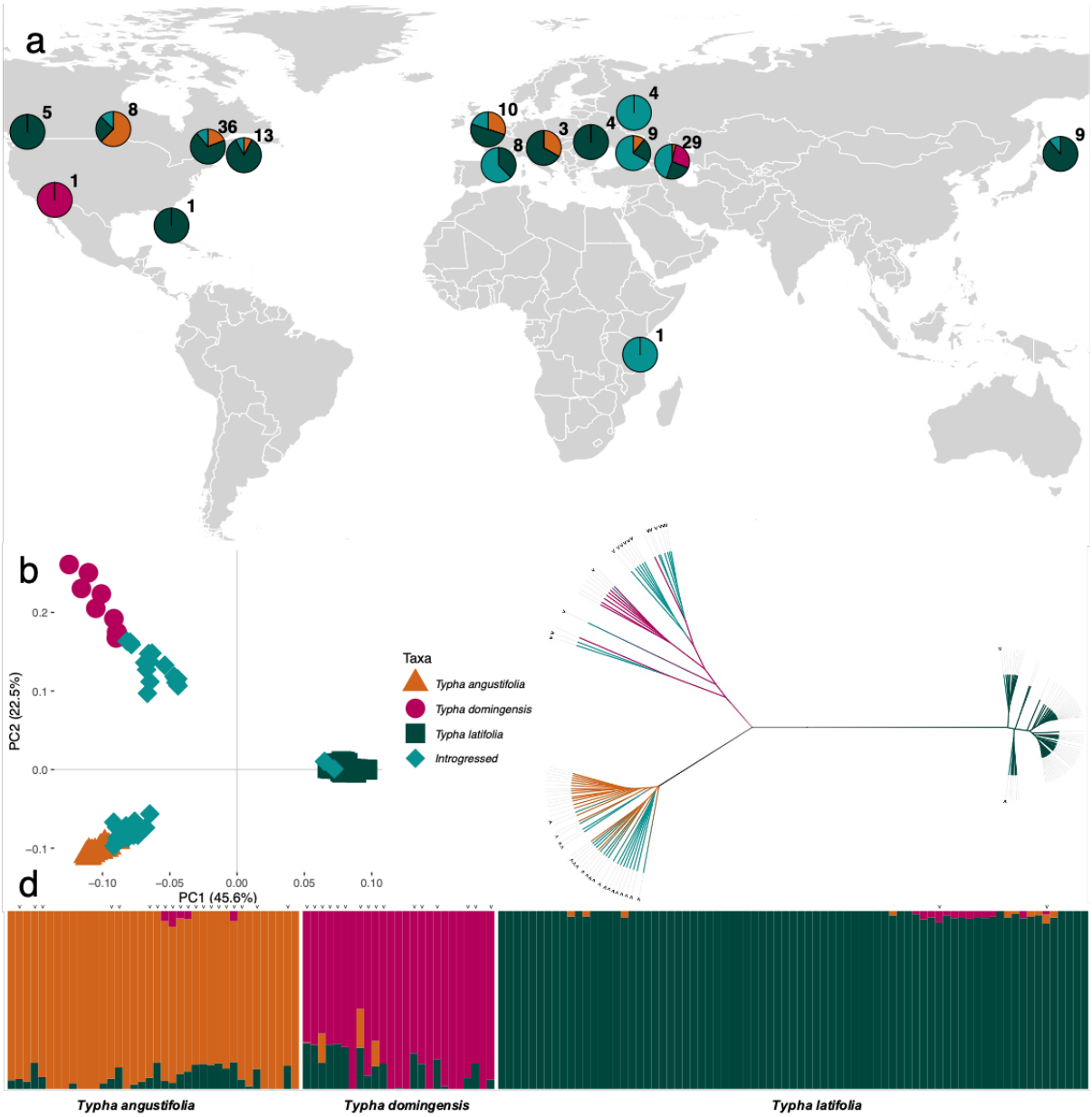

Figure 1. Genetic structure results of 12,177,703 nuclear SNPs obtained for three *Typha* spp. (a) Sampling locations in this study. Coloured areas of the pie charts reflect the proportion of samples from each taxon, and the number of samples is written top–right of each pie chart. (b) Principal Component Analysis for PC1 and 2. Shapes represent individuals, colours represent taxa, (c) Neighbour–joining tree. Tips represent individuals; colours represent clusters as a and b, (d) ADMIXTURE analysis for $K = 3$. Vertical bars represent individuals, and the probability of belonging to different taxa is coded with different colours. Arrows in b and d indicate the location of potentially introgressed individuals. Colour and shape code as the box in b: *T. angustifolia* (orange, triangles), *T. domingensis* (pink, circles), *T. latifolia* (green, squares), and potentially introgressed individuals (cyan, diamonds). a-b plotted in ggplot2 (Wickham, 2016), c plotted with iTOL v6 (Letunic & Bork, 2021), and d plotted in R.

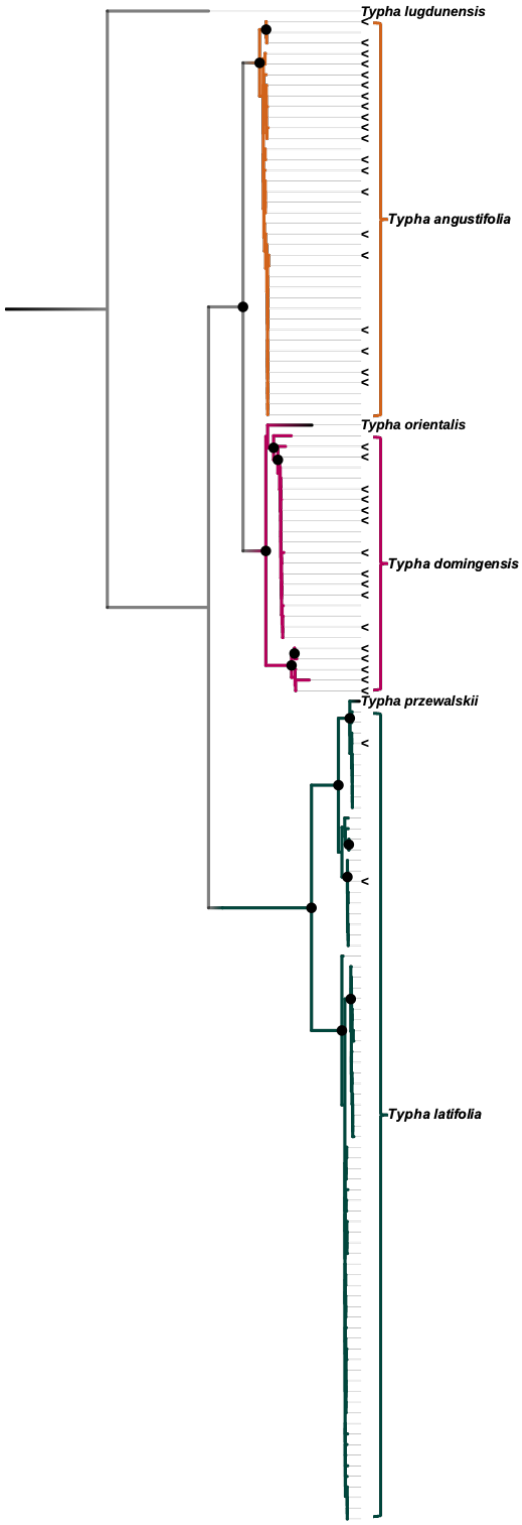

Figure 2. Chloroplast phylogeny of 140 *Typha* samples and four NCBI references, based on 96,591 bp. The tree was produced with *Sparganium natans* as the outgroup and drawn without it. Coloured lines recreate the genetic structure results in Figure 1, i.e., *T. latifolia* (green), *T. angustifolia* (orange), and *T. domingensis* (pink). Black circles indicate branch support = 100% (Felsenstein bootstrap proportion). Arrows indicate the location of potentially introgressed individuals (genetic structure results).