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A phenetic analysis of *Typha* in Korea and far east Russia

Changkyun Kim^a, Hyunchur Shin^b, Hong-Keun Choi^{a,*}

^a Division of Natural Science, Ajou University, Suwon 442-749, South Korea
^b Division of Biological Science, Shoonchunhyang University, Asan 337-880, South Korea

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Abstract

We used principal components analysis (PCA) and UPGMA cluster analysis to determine taxonomically definable limits and to estimate the phenetic relationships among four *Typha* species from Korea and far east Russia using 25 quantitative characters. A scatter plot of the first two principal components resolved four clusters among the 77 specimens of *Typha* examined from Korea and far east Russia. The clusters corresponded to the four currently recognized species. *Typha latifolia* was readily distinguished from other species by having wide leaves and female inflorescences. *Typha angustifolia* was distinguished from *T. orientalis* and *T. laxmanni* by the long male inflorescences and large gap between male and female inflorescences. *Typha laxmanni* was distinguished from *T. orientalis* by a higher ratio of male and female inflorescence lengths than others. UPGMA analysis also showed that individuals of *Typha* species from Korea and far east Russia form discrete clusters corresponding to four species.

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1. Introduction

Typha L., the sole genus of the family Typhaceae, is one of the most common aquatic plants found in marshes and shallow waters throughout the world (Dahlgren et al., 1985). *Typha*, with 9–18 species, is characterized by dense, elongate, cylindrical spikelike inflorescences, and female flowers having numerous capillary bristles (Kronfeld, 1889; Graebner, 1900; Fedchenko, 1934; Takhtajan, 1997).

Typha was described by Linnaeus in 1753. Kronfeld (1889) and Graebner (1900) produced monographs of the genus, but its taxonomy remains confusing (Cook et al., 1974),

^{*} Corresponding author. Tel.: +82-31-219-2618; fax: +82-31-219-1615. *E-mail address:* hkchoi@madang.ajou.ac.krAbstract (H.-K. Choi).

because of the variability in its reproductive and vegetative characters (Smith, 1967; Grace and Wetzel, 1982), and its frequent hybridization (Smith, 1987; Kuehn and White, 1999; Kuehn et al., 1999). Recently, microscopic characters (e.g. stigma width) have been found to be reliable indicators of species identity, due to the absence of environmental selective pressures on these characters (Kuehn and White, 1999). However, these characters cannot be used in the case of immature or senescent plants, so the identification of sterile *Typha* plants can be uncertain (Sharitz et al., 1980). This has necessitated regional taxonomic revisions. Such studies have been produced in India (Saha, 1968; Sharma and Gopal, 1980), Iran and Pakistan (Bokhari, 1983), North America (Hotchkiss and Dozier, 1949; Grace and Harrison, 1986; Kuehn and White, 1999), Argentina (Crespo and Perez-Moreau, 1967), Australia (Finlayson et al., 1985), Europe (Cook, 1980), the U.S.S.R. (Fedchenko, 1934), and eastern Asia (Noda, 1971; Yang, 1978; Ohwi, 1978; Kadono, 1996), as either taxonomic reexaminations or as part of comprehensive floristic studies.

In Korea, *Typha orientalis* Presl was first reported by Komarov (1901). Subsequently, *T. angustata* Bory and Chaubard (Nakai, 1952; Chung, 1957), *T. latifolia* L. (Rim, 1957), *T. angustifolia* L. (Choi, 2000), and *T. laxmanni* Lepechin (Kim and Choi, 2001) have been added to the Korean flora. Most of these reports are floristic in nature. Thus, the taxonomic confusion associated with these species names remains unaddressed. The taxonomic identification of *Typha* species in Korea has remained ambiguous.

The objectives of this study were (1) to depict morphological relationships among Korean *Typha* taxa by analyzing their variational patterns, (2) to clarify their taxonomic identities, and (3) to provide a basis for the delimitation of *Typha* species from Korea and far east Russia.

2. Materials and methods

2.1. Plant material

A total of 374 specimens, collected in Korea and far east Russia from August 1994 to September 1999, was examined to determine morphological variation patterns in the genus *Typha*. Specimens were identified primarily using characters emphasized by previous authors (Kronfeld, 1889; Graebner, 1900; Fedchenko, 1934; Ohwi, 1978). After removal of duplicates from the same populations, 77 plants, including 45 specimens of *T. angustifolia* L., 9 specimens of *T. latifolia* L., 10 specimens of *T. orientalis* Presl and 13 specimens of *T. laxmanni* Lepechin were used for principal components analysis and UPGMA cluster analysis. Disparity in number of specimens was due to the their geographical distributions. *Typha angustifolia* has a wide distribution than *T. latifolia*, *T. orientalis*, and *T. laxmanni* in Korea.

2.2. Morphological measurements

Measurements were made for 25 quantitative characters. Among the characters, 10 were vegetative and 10 reproductive. Five characters were derived from ratios of leaf and inflorescences. Lengths of stigma plus style, ovary and gynophore were measured with

a stereo-microscope, from 10 flowers per female inflorescence. Although the lengths of the stigma and style were distinguished in three species, those of *T. angustifolia* could not be measured distinctly. Consequently, the lengths of the stigma and style were combined and treated as one morphological character. The number of septa and the thickness of the leaf were measured using hand-sectioned preparations with a stereo-microscope. The first leaf below the female inflorescence was chosen for the measurement of leaf characters.

2.3. Data analysis

Statistical analysis and principal components analysis (PCA) were carried out using the SAS computer programs 6.12 (SAS Institute Inc., 1996; Shin and Choi, 1997), to provide non-hierarchical relationships among the individuals. Cluster analysis (UPGMA) was also performed using NTSYS 1.70 (Rohlf, 1992) to create dendrograms depicting similarities among the individuals.

Table 1 Characters used in morphological analysis of Typha in Korea and far east Russia

Morphological characters			
a	Length of vegetative shoot (cm)		
b	Width of stem base (mm)		
c	Length of leaf (cm)		
d	Width of leaf (cm)		
e	Thickness of leaf (mm)		
f	Number of leaf blade septa		
g	Angle of leaf apex (degree)		
h	Angle of leaf sheath (degree)		
i	Number of main veins		
j	Number of lateral veins		
k	Length of male inflorescence (cm)		
1	Length of female inflorescence (cm)		
m	Width of female inflorescence (upper portion) (cm)		
n	Width of female inflorescence (middle portion) (cm)		
0	Width of female inflorescence (lower portion) (cm)		
p	Gap between male and female inflorescences (cm)		
q	Length of stigma plus style (mm)		
Γ	Length of ovary (mm)		
S	Length of gynophore (mm)		
t	Length of female flower $(q + r + s)$ (mm)		
и	Thickness of leaf (cm)/Width of leaf (cm) (e/d)		
v	Length of male inflorescence (cm)/Length of female inflorescence (cm) (k/l)		
w	Width of female inflorescence (upper portion) (cm)/Width of female inflorescence		
	(middle portion) (cm) (m/n)		
X	Width of female inflorescence (middle portion) (cm)/Width of female inflorescence		
	(lower portion) (cm) (n/o)		
у	Gap between male and female inflorescence (cm)/Width of female inflorescence (middle portion) (cm) (p/n)		

See Fig. 1 for further clarification.

3. Results

3.1. Morphological variation

Twenty-five quantitative characters of *Typha* species from Korea and far east Russia were analyzed (Table 1 and Fig. 1), and their means and standard deviations determined (Table 2). In general, the species showed some degree of overlap in characters, with the exception of nine characters which included the vegetative shoot length (character *a*; character codes here and subsequently refer to the character codes in Table 1), width of stem base (character *b*), leaf width (character *d*), angle of leaf apex (character *g*), number of lateral leaf veins (character *f*), male and female inflorescences lengths (characters *k* and *f*), female inflorescence width (character *n*), and gap between male and female inflorescences (character *p*). The individuals of *T. laxmanni* tended to have a shorter length of female inflorescences, narrower leaves and stem bases, and fewer lateral veins than the others. In contrast, those of *T. latifolia* showed a tendency to have wider leaves and female inflorescences than the others. In the cases of *T. angustifolia* and *T. orientalis*, a considerable overlap occurred in more than nine characters, but not in the length of male inflorescence; those of

Table 2 Comparison of 25 morphological characteristics of *Typha* in Korea and far east Russia

Morphological characters	A ($N = 204$)	L ($N = 26$)	O ($N = 53$)	X (N = 91)
a	127 ± 22.5	138 ± 12.2	115 ± 15.4	85 ± 15.4
b	1.8 ± 0.5	2.1 ± 0.4	1.3 ± 0.4	0.7 ± 0.2
c	112.5 ± 26.8	81.3 ± 30.9	94.3 ± 39.2	68.2 ± 21.0
d	0.7 ± 0.2	1.2 ± 0.3	0.6 ± 0.2	0.3 ± 0.1
e	1.6 ± 0.6	1.5 ± 0.5	1.3 ± 0.4	0.9 ± 0.3
f	10.0 ± 1.6	12.6 ± 2.1	8.5 ± 1.4	6.5 ± 1.2
g	5.0 ± 1.0	11.2 ± 2.7	6.3 ± 1.7	2.6 ± 0.6
h	64.0 ± 23.1	64.6 ± 22.9	78.2 ± 20.1	62.1 ± 23.3
i	10.0 ± 1.6	11.6 ± 1.8	8.3 ± 1.4	9.4 ± 2.7
j	3.0 ± 1.1	3.4 ± 1.5	2.9 ± 1.1	0.7 ± 0.7
k	22.8 ± 4.9	10.5 ± 1.5	5.4 ± 1.6	11.0 ± 2.8
1	15.4 ± 5.3	15.4 ± 4.9	10.1 ± 3.3	4.7 ± 1.4
m	1.1 ± 0.5	2.0 ± 0.7	1.2 ± 0.4	1.3 ± 0.4
n	1.4 ± 0.5	2.2 ± 0.6	1.2 ± 0.4	1.5 ± 0.5
o	1.3 ± 0.5	1.7 ± 0.5	1.0 ± 0.3	1.2 ± 0.4
p	4.4 ± 1.5	0.0 ± 0.1	0.1 ± 0.4	2.5 ± 0.8
\overline{q}	1.7 ± 0.5	3.5 ± 1.0	2.0 ± 0.6	2.1 ± 0.6
r ·	0.9 ± 0.3	1.2 ± 0.3	0.8 ± 0.2	1.0 ± 0.3
S	2.6 ± 1.3	4.0 ± 1.6	2.0 ± 1.2	3.0 ± 0.9
t	5.2 ± 1.7	8.7 ± 2.3	4.8 ± 1.8	6.0 ± 1.1
u	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
v	1.6 ± 0.3	0.8 ± 0.2	0.6 ± 0.2	2.2 ± 0.4
w	0.9 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	0.9 ± 0.2
X	0.1 ± 0.2	1.3 ± 0.3	1.2 ± 0.3	1.4 ± 0.3
y	3.5 ± 1.9	0.0 ± 0.1	0.1 ± 0.3	1.9 ± 1.2

Character codes corresponded to those in Table 1. Symbols—A: *T. angustifolia*; O: *T. orientalis*; L: *T. latifolia*; X: *T. laxmanni*; *N*: number of individual of each species.

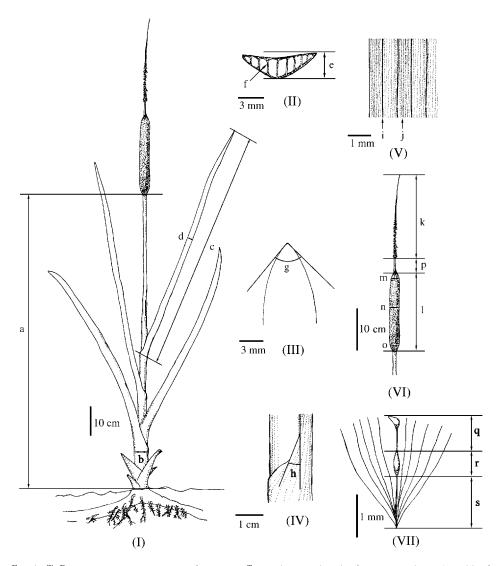


Fig. 1. (I) Diagrammatic representation of a mature *Typha* plant—a: length of vegetative shoot; b: width of base; c: length of leaf; d: width of leaf. (II) Cross-sectional diagram of leaf (middle) of *Typha—e*: thickness of leaf; f: number of septa. (III) Leaf apex of *Typha—g*: angle of leaf apex. (IV) Upper leaf sheath of *Typha—h*: angle of leaf sheath. (V) Veins of *Typha* leaf—i: main vein; j: lateral vein. (VI) Male and female inflorescence of *Typha—k*: length of male inflorescence; t: length of female inflorescence; m: width of female inflorescence (upper); n: width of female inflorescence (middle); o: width of female inflorescence (lower); p: gap between male and female inflorescence. (VII) Fertile flower of *Typha—q*: length of stigma plus style; r: length of ovary; s: length of gynophore.

T. angustifolia had a distinctive gap between male and female inflorescence (over 3 cm) and longer male inflorescences than those of *T. orientalis*.

3.2. Principal components analysis

Three principal components accounted for 28.9, 20.4, and 12.2% of the total variance, respectively (Table 3). The first principal component reflected high positive loadings for characters related to the length of the vegetative shoot, the width of the stem base (characters a and b) and leaf (characters d, f and g), and the length of female inflorescence (character l); high negative loadings were associated with the ratio of the lengths of male and female inflorescences (character v) and ratio of leaf thickness and width (character u). The length of male inflorescence (character k), the gap between male and female inflorescences (character p), and the ratio of the gap between male and female inflorescences and the width of female inflorescence (character y) influenced the separation of groups in the second principal

Table 3
Loading of the first three principal components for 25 characters from the analysis of 77 individuals of *Typha* in Korea and far east Russia

Morphological characters	Components				
	1	2	3		
a	0.305497	0.095227	-0.058313		
b	0.269020	0.156901	-0.096255		
c	0.173888	0.287400	0.042013		
d	0.328238	0.020876	-0.142450		
e	0.171649	0.181926	-0.057335		
f	0.317283	0.091568	-0.044782		
g	0.312324	-0.075814	-0.186137		
$\stackrel{\smile}{h}$	-0.011362	-0.131828	-0.052544		
i	0.212956	0.052446	0.010531		
j	0.229867	0.153050	-0.058969		
k	0.086737	0.326630	0.271918		
I	0.241959	0.246190	-0.020923		
m	0.217514	-0.251233	0.246431		
n	0.192916	-0.214755	0.329921		
o	0.183924	-0.096656	0.383066		
p	-0.042059	0.304316	0.352696		
\overline{q}	0.131640	-0.287069	-0.032093		
Γ	0.061639	-0.154145	0.265051		
S	0.185542	-0.190436	0.268066		
t	0.190755	-0.266642	0.213859		
u	-0.204489	0.008151	0.110775		
v	-0.205320	0.057781	0.369467		
w	0.076447	-0.187434	-0.142551		
X	-0.015118	-0.215771	-0.061988		
y	-0.046333	0.339683	0.205145		
Eigenvalue	7.22	5.12	3.03		
Comulative % of eigenvalue	28.9	49.3	61.5		

Character codes corresponded to those in Table 1.

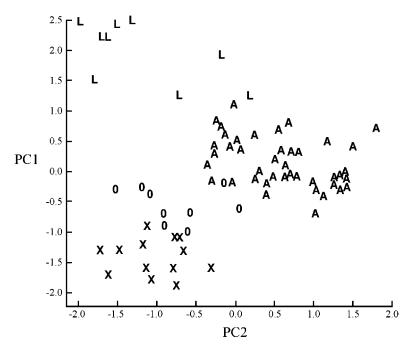


Fig. 2. Scatter diagram between PC 1 and PC 2 from principle component analysis of *Typha* using 25 quantitative characters. Symbols—A: *T. angustifolia*; O: *T. orientalis*; L: *T. latifolia*; X: *T. laxmanni*.

components with positive loadings, and the length of stigma plus style (character q) and the length of a female flower (character t) influenced those with high negative loadings (Table 3).

The scatter plot of the first two principal components from 25 quantitative characters indicated that all individuals clustered into four separate groups, corresponding to the taxa (Fig. 2). However, clusters of individuals of *T. laxmanni* and *T. orientalis* overlapped. The first principal component strongly distinguished the individuals of *T. latifolia* from those of *T. laxmanni* and *T. orientalis*, and weakly differentiated the individuals of *T. laxmanni* and *T. orientalis*, whereas, the second principal component distinguished individuals of *T. angustifolia* from *T. laxmanni*, and little overlapped the those of *T. angustifolia* and *T. orientalis* (Fig. 2).

3.3. Cluster analysis

The UPGMA dendrogram revealed two primary clusters at a taxonomic distance of ca. 1.8 (Fig. 3). The first large cluster (I in Fig. 3) consisted of most individuals representing three species (*T. angustifolia*, *T. orientalis* and *T. laxmanni*) and the second cluster (II in Fig. 3) included those of *T. latifolia* only. In cluster I, there were three subclusters, *T. angustifolia*(A), *T. orientalis* (O), and *T. laxmanni* (X) at a taxonomic distance of ca. 1.3. In general, the individuals of *Typha* species from Korea and far east Russia formed unique

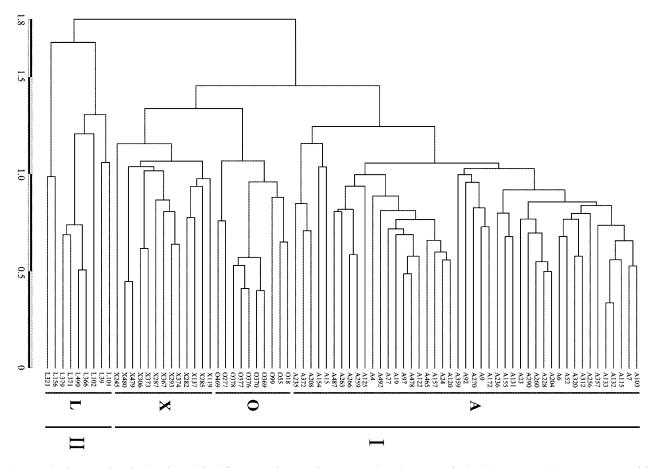


Fig. 3. UPGMA dendrogram of *Typha* showing relationships among four putative taxa based on the 25 quantitative characters. Symbols—A: *T. angustifolia*; O: *T. orientalis*; L: *T. latifolia*; X: *T. laxmanni*. The scale bar represents the taxonomic distances.

clusters according to traditionally recognized species. This dendrogram showed similar grouping patterns to the scatter plot of principal components analysis (Fig. 2).

4. Discussion

4.1. Diagnostic characters of Typha from Korea and far east Russia

Species of the genus *Typha* from Korea and far east Russia can be more readily identified by the length of the gap between male and female inflorescences, length of female inflorescence and width of the leaf (Ohwi, 1978; Kadono, 1996), than by the presence/absence of bracteoles, and the length of bracteole and female flowers (Kronfeld, 1889; Graebner, 1900). The results of principal components analysis support this interpretation revealed (Fig. 2). However, except for leaf width, these characters are useless if plants lack inflorescences. In such cases, the cross-sectional shape of the leaf and presence/absence of spots on the inside of the leaf sheath (qualitative characters not used in the analyses) are useful diagnostic characters. Individuals of *T. orientalis* have a more or less triangular shape whereas the others have a semilunar or flat shape. Plants of *T. angustifolia* have spots, while those of *T. latifolia* have the widest leaf (over 1 cm), and those of *T. laxmanni* have the narrowest leaf (< 0.3 cm).

4.2. Taxonomic identity of Typha species from Korea and far east Russia

Individuals of *T. angustifolia* cluster together in both analyses, and are distinguished by a long gap between male and female inflorescences and a long male inflorescence. In addition, plants of *T. angustifolia* have distinctive bracteoles in the female inflorescence, characters separate used to the genus *Typha* into two sections (Kronfeld, 1889; Graebner, 1900). In absence of reproductive organs, *T. angustifolia* is easily identified by spots on the leaf sheath.

T. orientalis and *T. laxmanni* formed two discrete clusters in the cluster analysis, whereas, they formed a single group in the principal components analysis with relatively weak separation (Figs. 2 and 3). However, these two species differ both in reproductive and vegetative morphological characters; the former has a relatively broad leaf and longer female inflorescence compared to the latter. Plants of *T. orientalis* are similar to *T. angustifolia* in appearance except for the gap between male and female inflorescences. Individuals of *T. orientalis* were distinguished from those of *T. angustifolia* by the triangular shape in the cross-sectional leaf and by ebracteolate flowers.

Typha latifolia forms a cohesive group in cluster analysis and principal components analysis. T. latifolia clearly shows discontinuity for leaf width compared to other Typha taxa, although this species is known to be quite variable (Kuehn et al., 1999). In addition, the female inflorescence is widest in Typha taxa from Korea and far east Russia. Therefore, T. latifolia is a clearly delimited taxon. However, the cluster of T. latifolia in the dendrogram indicates higher morphological variation within this species than other species (Fig. 3). Although extensive morphological variation may occur among some ecotypes, these differences are not associated with fine genetic variation (Mashburn et al., 1978; Sharitz et al., 1980; Kuehn et al., 1999; Keane et al., 1999). Also, leaf surface area (related to leaf length

and width), varies according to water depth (Grace and Wetzel, 1982). Therefore, it is necessary to examine variational patterns according to ecological factors such as water depth, salinity, and geographic isolation.

4.3. Taxonomic position of Typha latifolia

Although the genus *Typha* has been separated into two sections by the bracteoles in female flowers (Kronfeld, 1889; Graebner, 1900), this division is not reflected in the dendrogram (Fig. 3); *T. latifolia* does not form a cluster with *T. laxmanni* or *T. orientalis*, neither of these three taxa have the bracteoles in the female flowers, so they are assigned to Section *Ebracteolatae* Kronfeld (1889). On the other hand, the individuals of *T. latifolia* formed a cohesive group, separated at a taxnomic distance of ca. 1.8. The dendrogram, which of course does not comprise all the taxa of genus *Typha*, provides insight into the classification of the genus *Typha* (Fig. 3).

Within the genus *Typha*, according to Kronfeld (1889), six species, including *T. latifolia* (Section *Ebracteolatae*), and *T. minima* (Section *Bracteolatae*), have tetrad pollen, irrespective of the classification system based on the bracteoles in the female flowers. The phylogenetic significance or the biological function of the bracteoles in the female flowers has not been confirmed yet, and most of the taxonomic literature does not mention it as an important or major character in *Typha* (Cronquist, 1981; Takhtajan, 1997). However, pollen morphology is considered an important character in systematic treatments to separate genera and species phylogenetically (Skvarla and Larson, 1963). Also, pollen tetrads have been described as an advanced character over solitary grains in angiosperms (Walker and Doyle, 1975). Therefore, the classification of genus *Typha* needs to be reexamined using phylogenetically informative characters such as palynological characters.

In conclusion, our study provides definable limits and phenetic relationships by the quantitative morphological characters among four *Typha* species from Korea and far east Russia.

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