



Genetic delimitation and population structure of three *Trapa* taxa from the Yangtze River, China



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ABSTRACT

Water chestnuts (genus *Trapa*) are an annual floating-leaved aquatic plants widely distributed in the Old World. The taxonomy of the genus is extremely confusing worldwide. The plants of *Trapa* are abundant in lakes of mid-to-lower reaches of the Yangtze River, China. The genetic relationship and diversity among three common *Trapa* species in the area were evaluated using amplified fragment length polymorphism (AFLP) markers. A total of 249 unambiguous bands, of which 192 (77.1%) were polymorphic, was produced with four pairs of primers. The genetic relationship estimated by different approaches (NJ tree, STRUCTURE, PCA and UPGMA) consistently indicated that all the three *Trapa* taxa formed genetically distinct groups, which confirmed the taxonomic status of the three separate species (*T. quadrispinosa*, *T. japonica* and *T. bispinosa*). The three *Trapa* taxa appeared to possess low level of gene diversity ($H_E = 0.073\text{--}0.107$) compared with the perennial aquatic macrophytes in the same habitat and range distribution. The main factor responsible for that was the habitat deterioration of recent years combined with the annual life history of the plants. Evident genetic structure was found among populations for each *Trapa* taxon, contributing more than 50% of the total gene diversity. The high genetic differentiation could be due to the restricted gene flow among populations ($N_m = 0.165\text{--}0.243$) and the high degree of inbreeding ($F_{IS} = 0.482\text{--}0.503$) in their reproductive system. These results were important in relation to conservation management of *Trapa* taxa.

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1. Introduction

Trapa L. (Trapaceae), known as water chestnut, is a genus of annual floating-leaved aquatic plants widely distributed in subtropical and temperate regions of Africa, Asia, and Europe and was introduced into Australia and North America (Chen et al., 2007). Its seeds are important food for humans and animals in East and Southeast Asia due to the high content of starch (Suriyagoda et al., 2007; Hoque et al., 2009). However, the taxonomy of the genus is extremely confusing world-wide because of the wide variability in morphological traits of the genus and the limited diagnostic characters (Yu, 1998; Kim et al., 2010). Cook summarized that the genus contained only one polymorphic or up to ca. 20 species in the

world (Cook, 1990). The classification of Chinese *Trapa* is also problematic. Flora Republicae Popularis Sinicae documented 15 species and 11 varieties in the genus (Wan, 2000), while only two species were reported in its revised edition, the Flora of China (Chen et al., 2007). The confused situation has greatly hindered further genetic researches of the genus. As valuable food and natural resources, the genetic diversity of *Trapa* populations should be investigated in order to effectively conserve or manage them. However, previous genetic researches only focused on the classification of the genus (Bao et al., 2004; Jiang and Ding, 2004; Takano and Kadono, 2005; Kim et al., 2010), and few efforts have been made on the genetic diversity and population structure of *Trapa* populations.

Trapa plants are common in northeastern and southern part of China, occurring in freshwater wetlands, sluggish reaches of rivers, lakes, ponds and estuaries (Wan, 2000; Chen et al., 2007). The plants have floating leaves and submersed ones. The floating leaves have spongy and swollen float in petioles, which allows the foliage to form a rosette on the surface of the water. The plants are capable of extensive clonal propagation (Groth et al., 1996). Additionally,

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Table 1
Locations of the three *Trapa* taxa.

Site (code)	Latitude (N)	Longitude (E)	Species (population code)	Vouchers and collector
Changhu Lake (CH)	30.374	112.338	<i>T. quadrispinosa</i> (CHsj)	Chenyuanyuan0013, Chen et al. (2012)
			<i>T. japonica</i> (CHqj)	Chenyuanyuan0011, Chen et al. (2012)
			<i>T. bispinosa</i> (CHl)	Chenyuanyuan009, Chen et al. (2012)
Yuhu Lake (YH)	29.950	112.205	<i>T. quadrispinosa</i> (YHsj)	Chenyuanyuan0016, Chen et al. (2012)
			<i>T. quadrispinosa</i> (HHsj)	Chenyuanyuan0022, Chen et al. (2012)
Honghu Lake (HH)	29.857	113.405	<i>T. japonica</i> (HHqj)	Chenyuanyuan0024, Chen et al. (2012)
			<i>T. bispinosa</i> (HHl)	Chenyuanyuan0020, Chen et al. (2012)
			<i>T. quadrispinosa</i> (XLSj)	Chenyuanyuan0026, Chen et al. (2012)
Xiliang Lake (XL)	29.979	114.127	<i>T. japonica</i> (XLqj)	Chenyuanyuan0025, Chen et al. (2012)
			<i>T. bispinosa</i> (XLI)	Chenyuanyuan0027, Chen et al. (2012)
			<i>T. quadrispinosa</i> (YZsj)	Chenyuanyuan0029, Chen et al. (2012)
Yezhu Lake (YZ)	30.826	114.086	<i>T. bispinosa</i> (YZl)	Chenyuanyuan0030, Chen et al. (2012)
			<i>T. quadrispinosa</i> (DHsj)	Chenyuanyuan0034, Chen et al. (2012)
Donghu Lake (DH)	30.575	114.387	<i>T. japonica</i> (DHqj)	Chenyuanyuan0032, Chen et al. (2012)
			<i>T. bispinosa</i> (DHI)	Chenyuanyuan0033, Chen et al. (2012)
			<i>T. quadrispinosa</i> (LZsj)	Chenyuanyuan0037, Chen et al. (2012)
Liangzi Lake (LZ)	30.425	114.541	<i>T. japonica</i> (LZqj)	Chenyuanyuan0036, Chen et al. (2012)
			<i>T. bispinosa</i> (LZI)	Chenyuanyuan0035, Chen et al. (2012)
			<i>T. quadrispinosa</i> (OHsj)	Chenyuanyuan0049, Chen et al. (2012)
Ouhuasai Lake (OH)	30.685	117.136	<i>T. japonica</i> (LGqj)	Chenyuanyuan0044, Chen et al. (2012)
Longgan Lake (LG)	30.023	116.261	<i>T. bispinosa</i> (LGI)	Chenyuanyuan0041, Chen et al. (2012)
Shengjin Lake (SJ)	30.381	117.059	<i>T. quadrispinosa</i> (Sjsj)	Chenyuanyuan0055, Chen et al. (2012)
			<i>T. japonica</i> (SJqj)	Chenyuanyuan0051, Chen et al. (2012)
Poyang Lake (PY)	29.515	116.172	<i>T. quadrispinosa</i> (PYSj)	Chenyuanyuan0059, Chen et al. (2012)
			<i>T. japonica</i> (PYqj)	Chenyuanyuan0058, Chen et al. (2012)

Trapa plants are considered to be of high degree of autogamy because of the selfing-pollinating behavior before flowering ([Arima et al., 1999](#)). Because of the similar vegetative characters in *Trapa* plants, the morphology of fruits (nuts) offers the best diagnostic criteria for the classification of *Trapa*. Variations of fruit morphology, including fruit size, the number of spines, the shape of lower protuberances and tubercles on the surface of the fruits and so on, have been used for recognition of various taxa ([Staszkiwicz and Wojcicki, 1979, 1981](#); [Chung et al., 1987a, 1987b](#); [Kadono, 1987](#)).

The mid-to-lower reaches of the Yangtze River include the largest floodplain in China. This area contains thousands of shallow lakes and sustains a rich variety of aquatic macrophytes ([Wang and Dou, 1998](#)), which makes the area one of the six biodiversity hotspots in the large river ecoregions of the world ([Olson and Dinerstein, 1998](#)). As one of the main distribution ranges of *Trapa* in China, several *Trapa* taxa grow in mixed populations in lakes of this area during our field investigation (2011). Among them, the most common *Trapa* taxa were *T. japonica* Flerow, *T. bispinosa* Roxb. and *T. quadrispinosa* Roxb (taxonomy follows Flora Republicae Popularis Sinicae; [Wan, 2000](#)). According to morphological features described in Flora Republicae Popularis Sinicae ([Wan, 2000](#)), their fruits show obvious differences among the three species. For *T. bispinosa* and *T. quadrispinosa*, their fruits are nuts with two and four barbed spines, respectively, and the tubercles on the nut hulls are not obvious. *T. japonica* has the two-horned nuts with the pseudo horns replaced by blunt protuberances. Additionally, the snout of *T. japonica* nuts is more obvious than that of *T. bispinosa* and *T. quadrispinosa*. All three taxa were included in the species *T. natans* L in Flora of China ([Chen et al., 2007](#)). The three *Trapa* taxa grow in mixed populations in most lakes. What is the extent of hybridization and gene flow among congeners in these mixed populations?

Molecular markers, due to the high polymorphism and resolution, have been considered a preferred method for identifying species and evaluating the genetic structure of plant populations in the past years ([Nybom, 1994, 2004](#)). In the present study, amplified fragment length polymorphism (AFLP) was employed for the analysis of genetic relationships and genetic diversity among populations of the three commonly-found *Trapa* taxa in the mid-to-lower reaches of the Yangtze River. The specific questions addressed are: (I) Can AFLP markers discriminate among the three *Trapa* taxa? (II)

What is the level of genetic diversity in the *Trapa* populations? (III) Is there any genetic structure within *Trapa* taxa and, if so, are the populations in different lakes completely isolated, or is there any gene flow among populations? The baseline genetic information will be beneficial for the conservation and sustainable utilization of the natural resources in the area.

2. Materials and methods

2.1. Sample collection

Eleven lakes from the mid-to-lower reaches of the Yangtze River were extensively investigated in the autumn of 2011. The foliar rosettes of *Trapa* with matured seeds were randomly collected at intervals of at least 4 m. The morphological characters of fruits and leaves were measured and recorded. The samples were identified according to the description of Flora Republicae Popularis Sinicae ([Wan, 2000](#)). All the voucher specimens have been logged in the Plant Herbarium, Wuhan Botanical Garden, Chinese Academy of Sciences (HIB). For each taxon, samples from the same lake were handled as a population. For most populations, 16–22 individuals were sampled in every population. In all, a total of 482 individuals were collected from the three taxa, including ten (213 individuals), eight (140 individuals) and seven (128 individuals) populations of *T. quadrispinosa*, *T. japonica* and *T. bispinosa*, respectively ([Table 1](#); [Fig. 1](#)). Young leaves from the foliar rosettes were immediately dried in silica gel and brought back to our laboratory for DNA extraction.

2.2. DNA extraction and AFLP analysis

Total genomic DNA was extracted from 0.3 to 0.5 g of dried leaves using the modified CTAB (Cetyltrimethyl Ammonium Bromide) protocol outlined in [Doyle and Doyle \(1987\)](#). The AFLP analysis was performed essentially as described by [Vos et al. \(1995\)](#). Total genomic DNA (200 ng) was digested using 3 units of *EcoRI* and *MseI* endonuclease mixture (New England Biolabs, Ipswich, MA, USA) in a total volume of 20 μ L for 3 h at 37 °C and digests were confirmed by electrophoresis on 1.5% agarose gels. Then, 10 μ L of ligation solution containing 4 pM *EcoRI* adaptor, 40 pM

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