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Comparison of the extent of genetic variation of *Vallisneria natans* and its sympatric congener *V. spinulosa* in lakes of the middle–lower reaches of the Yangtze River

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ABSTRACT

Vallisneria natans and *Vallisneria spinulosa* are two morphologically very similar and sympatrically dominant submerged macrophytes in lakes of the middle–lower reaches of the Yangtze River. Genetic variation was compared based on a total of 196 individuals from six *V. natans* populations and 201 individuals from seven *V. spinulosa* populations. Using eight ISSR primers, a total of 139 and 129 DNA fragments were generated with 121 being polymorphic in *V. natans* and 99 in *V. spinulosa*. The two species maintained higher genetic variation both at the species and population levels in comparison with other aquatic macrophytes. A higher level of genetic diversity among populations was found in *V. natans* than in *V. spinulosa*: the percentage of polymorphic loci (*PPL*) in *V. natans* was 52–62% vs. 38–47% in *V. spinulosa*; gene diversity (*H*) was 0.21 in *V. natans* vs. 0.17 in *V. spinulosa*.

Both an analysis of molecular variance (AMOVA) and *F*-estimation (F_{ST}) showed that most of the total genetic variation resided within populations of both species (AMOVA: 85% and 80%; F_{ST}: 0.132 and 0.202), indicating low genetic differentiation between populations. Principal coordinates analysis (PCA) indicated evident gene flow between populations of both species. The outcrossing reproductive mode and pervasive gene flow might have played important roles in maintaining high genetic diversity and in shaping low population differentiation of the two *Vallisneria* species, while the extent of clonal growth might account for the different levels of population divergence between them.

1. Introduction

Shallow lakes with dense areas of submerged plants often have clear water and low concentrations of nutrients and phytoplankton (e.g. Havens et al., 2004; Caraco et al., 2006), suggesting that submerged plants are important for the ecological recovery of eutrophic water bodies. The middle–lower reaches of the Yangtze River form the largest floodplain in China, with thousands of shallow lakes that are generally less than 3 m deep. Most of these lakes can sustain a rich variety of submerged plants. However, due to recent eutrophication and excessive human disturbance, the population size and distribution of submerged plants such as *Vallisneria* spp. have decreased (Wang et al., 2005). The continued decline of submerged vegetation will further worsen aquatic conditions for fish and other wildlife in these areas. It appears urgent to take action and re-introduce submerged plants to restore environmental conditions, allowing wildlife to subsequently re-colonize naturally.

Since *Vallisneria* spp. are thought to provide food for waterfowl, as a nursery habitat for fish, a substrate for invertebrates, and have a strong influence on water quality, they are used as pioneer plants in freshwater ecosystem restoration and have attracted attention in recent years (e.g. by Korschgen et al., 1997; Li et al., 2005; Ke and Li, 2006; Xie et al., 2006a, 2007; Xiao et al., 2007; Wang et al., 2008; Wu et al., 2009). Three species of the genus *Vallisneria* are recorded in China: *Vallisneria spinulosa* Yan, *Vallisneria denseserrulata* (Makino) Makino, and *Vallisneria natans* (Lour.) Hara. *V. spinulosa* is thought to be endemic to China, only occurring in the middle–lower reaches of the Yangtze River; *V. denseserrulata* is restricted to China and Japan; and *V. natans* is a cosmopolitan species, especially

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in tropical and subtropical zones (Xie et al., 2006a,b, 2007). In China, these can be found in different freshwater locations including lakes, ponds, rivers and paddy fields.

V. natans, *V. spinulosa* and *V. denseserrulata* are very similar morphologically, making it difficult to distinguish them clearly in the field without flowers or fruit (Les et al., 2008). Especially *V. natans* and *V. spinulosa* usually occur sympatrically and form mixed populations in the middle–lower reaches of the Yangtze River. These two species have somewhat different life histories, however. For example, Chen et al. (2008b) found that *V. natans* flowered earlier than *V. spinulosa* and produced fewer vegetable tubers, suggesting that stronger clonal growth occurs in *V. spinulosa*. This would be expected to result in population genetic variation differences, since clonal growth can have a strong influence on population diversity and genetic structure (Ellstrand and Roose, 1987).

The present study was designed to investigate the genetic diversity levels of the two *Vallisneria* species in lakes of the middle–lower reaches of the Yangtze River by DNA molecular markers with careful field sampling during the flowering period. Following an unsuccessful attempt to estimate genetic variations using the SSR markers developed by Chen et al. (2006), this time we used inter-simple sequence repeat (ISSR) assays. The method applies the principle of SSR-anchored polymerase chain reaction (PCR) amplification by designed primers that can randomly amplify DNA fragments of the inter-repeat regions. Since prior DNA sequence information is not required, it is a particularly useful method for studying species whose sequence information is not known (Zietkiewicz et al., 1994; Jones et al., 2009). Although the dominance of the ISSR marker limits the utilization of the method for estimating heterozygosity and mating systems, it is commonly used in studies of population genetics, taxonomy and phylogeny of many plant species (e.g., Meimberg et al., 2006; Angelone et al., 2007).

V. spinulosa has considerable genetic variation and hardly any population genetic differentiation, probably due to extensive hydrologic connectivity among populations by the Yangtze River (Chen et al., 2007), suggesting that in practice any population can be used as stock for re-introduction. In a comparison with the sympatric and phenotypically similar congener *V. spinulosa*, we addressed the following questions: (1) does *V. natans* also hold rather genetic variation and present a similar pattern of population divergence as *V. spinulosa*? (2) can we find evidence of interpopulation gene flow in *V. natans*?

2. Materials and methods

2.1. Sample collection

Of the 26 characters used to classify species in the genus of *Vallisneria* by Les et al. (2008, their Table 2), only two, i.e. the number of locules/anthers and the form of the fruit cross-section, can be used as reliable identifiers of *V. natans* and *V. spinulosa* under field conditions. Thus, the useful time to collect and separate the two species in the field should be during the flowering period. In September to October, 2007, when both species flowered, we sampled plant material from 12 lakes in the middle–lower reaches of the Yangtze River using the above characters for identification.

At each population, if the plants were distributed continuously, one 500 m × 2 m transect was established and one individual was randomly collected at 5 m intervals (Chen et al., 2007). When plants occurred in patches, one individual was collected every 5 m in each patch. A total of 196 samples from 6 populations of *V. natans* and 201 samples from 7 populations of *V. spinulosa* were collected (Table 1). Plant material was stored dry in silica gel and brought back to the laboratory for DNA extraction.

2.2. Total DNA extraction

Total genomic DNA was isolated from 0.5 g of silica-dried leaf tissue using a modification of the hexadecyl trimethyl-ammonium bromide (CTAB) extraction procedure of Doyle and Doyle (1987). Leaf material was powdered with liquid nitrogen, mixed with 2 mL extraction buffer (1.4 M NaCl, 100 mM Tris–HCl (pH 8.0), 20 mM EDTA, 2% (V/V) CTAB and 2% 2-mercaptoethanol) at 65 °C, and incubated at 65 °C for 30 min with gentle shaking every 5 min. Proteins were extracted twice with 2 mL of chloroform:isoamylalcohol (24:1), then centrifuged at 10,000 × *g* for 2 min. RNase (10 μg mL^{-1}) was added to the supernatant and incubated for 2 h at 37 °C. The mixture was centrifuged at 10,000 × *g* for 2 min. The sediment was washed twice in 70% ethanol, air-dried, resuspended in 100 μL 0.1 × TE, and then stored at −20 °C.

Table 1
Sample size and parameters of genetic diversity of *Vallisneria* populations. *G* = number of genotypes detected; *PPL* = percentage of polymorphic loci; *H* = Nei's gene diversity.

Species	Locality	Sample size	*G*	*PPL* (%)	*H* ± sd
Vallisneria natans					
NSVn	Niushan Lake, Hubei Province (E114°31.1′/N31°20.7′)	29	29	55	0.24 ± 0.23
BAVn	Baoan Lake, Hubei Province (E114°43.9′/N30°12.1′)	30	29	52	0.22 ± 0.22
PYVn	Poyang Lake, Jiangxi Province (E115°53.5′/N29°14.2′)	32	32	62	0.28 ± 0.23
LGVn	Longgan Lake, Hubei Province (E116°01.4′/N29°57.1′)	35	35	53	0.24 ± 0.23
DPVn	Dongpu Lake, Anhui Province (E117°12.1′/N31°52.7′)	44	44	58	0.23 ± 0.22
DSVn	Dianshan Lake, Shanghai city (E120°55.1′/N31°06.9′)	26	24	56	0.23 ± 0.24
Mean				56	0.24 ± 0.20
Total		196	193	87	0.32 ± 0.21
V. spinulosa					
HHVs	Honghu Lake, Hubei Province (E113°23.2′/N290°50.7′)	26	24	47	0.21 ± 0.23
DCVs	Diaocha Lake, Hubei Province (E113°43.2′/N30°43.2′)	32	32	44	0.19 ± 0.22
XLVs	Xiliang Lake, Hubei Province (E114°26.5′/N30°19.3′)	28	27	42	0.18 ± 0.22
BAVs	Sympatric with BSVn	36	34	45	0.21 ± 0.23
LGVs	Sympatric with LGVn	26	23	38	0.17 ± 0.22
DPVs	Sympatric with DPVn	25	25	38	0.18 ± 0.23
DSVs	Sympatric with DSVn	28	25	39	0.16 ± 0.21
Mean				42	0.18 ± 0.21
Total		201	190	77	0.28 ± 0.22