

# Divergences in reproductive strategy explain the distribution ranges of *Vallisneria* species in China



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## ABSTRACT

Closely related plant species usually have similar characteristics in life history, morphology and reproduction and thus similar requirements for their environment, resulting in intense interspecific competition that may constrain the co-occurrence of congeneric plants. In this study, the reproductive strategies of *Vallisneria natans*, *Vallisneria spinulosa* and *Vallisneria denseserrulata* in China were compared based on studies on natural populations and common garden experiments. Variability in reproductive density (including flowering ramets, spathes, flowers, fruits and seeds) and phenotypic characteristics were significantly higher for *V. natans* than the other two species. *V. spinulosa* allocated a considerable proportion of biomass (~27%) to vegetative reproduction through the formation of turions. *V. denseserrulata* invested less in both sexual and vegetative reproduction than *V. natans* or *V. spinulosa*. The differences in reproductive strategy and phenotypic variation may explain the distribution patterns of the three *Vallisneria* species in eastern China.

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## 1. Introduction

Closely related species usually possess similar life history, morphological, physiological and reproductive characteristics, resulting in similar requirements for their environment (Losos, 2008). As a consequence, intense interspecific competition is likely to occur between closely related congeneric species, especially when they occupy the same habitat (Lavergne et al., 2004; Garcia-Serrano et al., 2007). Nevertheless, fine divergences in life history and/or reproductive strategy, such as growth form, growth rate, competition ability, resistance to herbivory, phenology, pollination mode and reproductive effort, may facilitate the co-occurrence of congeneric plant species with similar niche requirements (Pitelka, 1977; Schnitzler et al., 1992; Lavergne et al., 2004; Garcia-Serrano et al., 2007). The sexual reproductive structures, in particular, are usually less varied within a genus, but the number of reproductive structures (e.g., inflorescence, flower, fruit and seed), reproductive effort (i.e., the proportion of the total biomass allocated to sexual reproduction), and reproductive mode (e.g., sexual or asexual)

are more likely to vary among plant species (Primack, 1979), or even among populations of the same species (Phillips et al., 1983; Kleunen et al., 2002). Comparative studies among congeners have shown that divergences in reproductive strategy enable them to co-occur; this could therefore lead to preferences for particular niches within habitats (Pitelka, 1977; Brock, 1983) or even segregation of geographic distributions (Kay, 2006). In addition, such studies indicate that species with higher capability of sexual reproduction have wider distribution ranges relative to their congeneric species with lower capability of sexual reproduction (Lavergne et al., 2004; Hoyo and Tsuyuzaki, 2015).

Because of the relative uniformity and connectivity of water environments, aquatic plants usually show limited interspecific morphological variation and relatively wide distribution, and sympatric distribution of congeneric species is therefore common (Barrett et al., 1993; Santamaría, 2002). Even so, high variations of reproductive strategy among congeners may be found in aquatic plants coexisting in the same water body (Santamaría, 2002). For example, the upper-intertidally distributed *Zostera americana* (now known as *Z. japonica* (Short, 2011)) allocates more resources into sexual reproduction than its mid- to low-intertidally distributed congener, *Z. marina* (Harrison, 1979). Other studies also indicate that the reproductive strategies (e.g., flowering phenology,

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**Table 1**  
Differences in morphological characters among the three *Vallisneria* species.

Trait	<i>V. natans</i>	<i>V. spinulosa</i>	<i>V. denseserrulata</i>
stolon	smooth	spinose	smooth
leaf texture	fragile	fragile	tough
leaf apex	acute	obtuse	obtuse
leaf edge	smooth, with sparse teeth	with triangular teeth	smooth, with sparse teeth
anther number	one	two	two
female flower cross-sect.	circular	triangular	triangular
fruit cross-sect.	circular	triangular	circular
seed	fusiform, wingless	ovoid, winged	fusiform, wingless

flowering frequency and reproductive effort) of *Zostera* species are heavily influenced by environmental gradients such as light, temperature, salinity, water depth and drought (Phillips et al., 1983; Harwell and Rhode, 2007). In another case, Brock (1983) investigated three sympatric species of *Ruppia* in Southern Australia and found that species growing in ephemeral habitats allocate more resources to reproduction (both sexual and asexual) than species growing in permanent waters. These studies suggest that divergence in reproductive strategy plays an important role in niche segregation of congeneric aquatic plants, leading to sympatric distribution, as well as differences in distribution range.

*Vallisneria* (Hydrocharitaceae) is a dioecious, submerged genus containing ~15 species and usually forms monodominant communities in many freshwater ecosystems worldwide (Chen et al., 2008; Les et al., 2008). *Vallisneria* species also play important roles in the maintenance and stabilization of freshwater ecosystems, such as providing food for waterfowls, nursery habitats for fishes, and substrates for invertebrates and purifying water quality (Li et al., 2005; Wu et al., 2009), and are therefore used frequently to restore freshwater ecosystem (Korschgen et al., 1997; Xie et al., 2007).

Three species of *Vallisneria* are recorded in China: *V. natans* (Loureiro) H. Hara, *V. spinulosa* S. Z. Yan and *V. denseserrulata* (Makino) Makino (Sun, 2009; Wang et al., 2010b). These three species have relatively different distribution ranges, but they overlap in eastern China (Fig. 1): *V. natans* has a relatively wider geographic range from northeast to southwest China, *V. denseserrulata* is distributed mainly in southern China, and *V. spinulosa* is endemic to the mid-lower reaches of Yangtze River, where the other two species also commonly occur (Chen et al., 2008; Sun, 2009; Wang et al., 2010a,b). A recent phylogenetic study show that *V. spinulosa* and *V. denseserrulata* are sister species, whereas *V. natans* is relatively distinct from the other two species (Les et al., 2008). Given their high morphological homoplasy and similarity in their growth forms, flowering times and pollination modes (Les et al., 2008; Sun, 2009; Wang et al., 2010b), we can expect that the three species have similar niche requirements and, consequently, that intense interspecific competition will constrain their co-occurrence. Thus, the question emerges of what permits the co-occurrence of the three species in the mid-lower reaches of the Yangtze River.

To answer this question, we investigate the populations of each of the three species under natural and common garden conditions. We focus especially on the possible divergence of reproductive strategy between species by detecting (1) the capability for sexual reproduction; and (2) the amplitude of phenotypic variation.

## 2. Materials and methods

### 2.1. Study species

The three species of *Vallisneria* native to China have rosette-like ramets with multiple belt-like leaves. New ramets are produced through extension of stolons. The spathe is located among bases of leaves with hundreds of packed male flowers. The male flow-

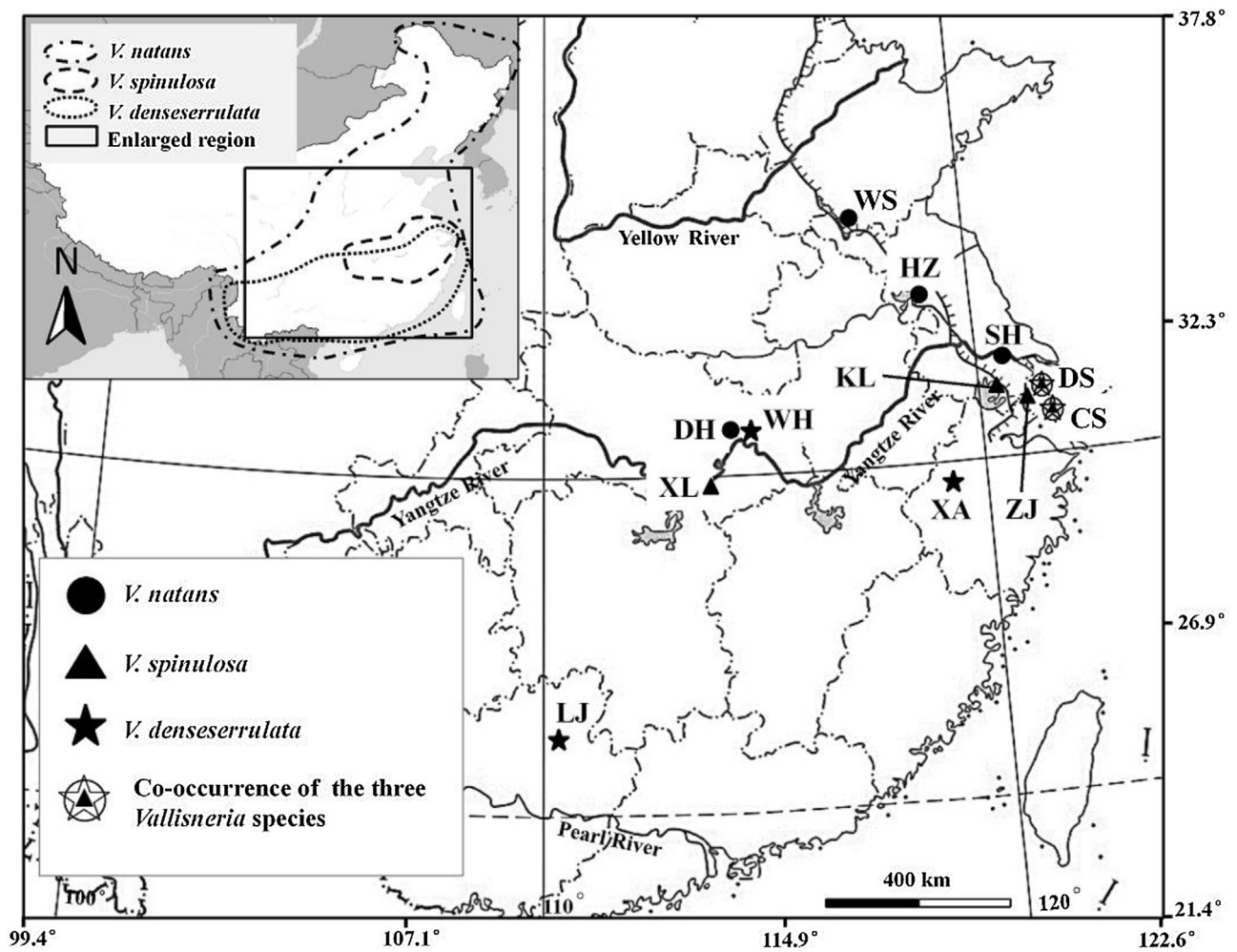
ers are released to the water surface after maturation. A female spathe has only one female flower with a long peduncle reaching the water surface. Pollination occurs on the surface of the water, and the fleshy fruits will be pulled down into the water via a helix of peduncles for further development (Wylie, 1917; Kosuge et al., 2013). The three *Vallisneria* species have overlapping flowering and fruiting periods (from July to November), and their peak flowerings occur in August to September, although *V. denseserrulata* maintains its flowers for a longer period in southern regions (Wang et al., 2010b). Despite the homoplasy of this genus, the three species have several significant differences in morphology based on the descriptions of Les et al. (2008), Wang et al. (2010b), and Sun (2009) and our long-term observations (Table 1), which enable us to distinguish them accurately.

### 2.2. Natural population investigation

In September 2013, when *Vallisneria* species were at peak flowering and fruit-setting stages, we investigated eight lakes/rivers (Fig. 1) in the mid-lower reaches of the Yangtze River in where *Vallisneria* species were the dominant submerged macrophytes. These water bodies are connected and belong to the Yangtze River system, so the environmental factors are relatively homogeneous. Among these water bodies, Chenshan Botanical Garden (CS) and Dianshan Lake (DS) contain all three species, whereas the others are dominated by one of the three species (Table 2). Correspondingly, four populations of each species, totaling 12 natural *Vallisneria* populations, were investigated. We excavated the samples at an interval of ~1 m along a line transect or rectangular quadrat in each population, depending on the density and coverage area of *Vallisneria* plants. One sample was consisted of a clonal shoot (ramet). In total, 62–246 samples were excavated from each of the population according to the available ramets per population (Table 2). Each ramet was identified and recorded as male, female or nonflowering. Between 20–56 male and female ramets of *V. natans* and *V. spinulosa* were randomly selected in the field for posterior detailed trait measurements (see Section 2.4). *V. denseserrulata* had a heavily biased sex ratio, and all flowering shoots were measured (Table 2).

### 2.3. Common garden experiment

To test for species-specific morphology, we constructed a common garden trial involving all three species at Fudan University, Shanghai, East China. The common garden experiment contained four populations per species. For *V. natans* and *V. spinulosa*, 12–15 intact, well-developed fruits were randomly sampled from each natural population in 2013. Because not all natural populations had sufficient fruits, for the common garden experiment, therefore the source populations were not completely consistent with those for the field investigation (Table 2). Fruits of *V. denseserrulata* were very rare at the mid-lower reaches of the Yangtze River (only one fruit was found in the CS population), and >60 vegetative ramets from DS, Wuhan Botanical Garden (WH) and Xin'an River (XA) were used to construct experimental populations. These ramets from natu-



**Fig. 1.** Distribution ranges of the three *Vallisneria* species (Vn: *V. natans*; Vs: *V. spinulosa*; Vd: *denseserrulata*) and locations of the studied populations. Distribution ranges are drawn according to Sun (2009) and Wang et al. (2010b). CS: Chenshan Botanical Garden; DH: Donghu Lake; DS: Dianshan Lake; HZ: Hongze Lake; KL: Kuilei Lake; LJ: Lijiang River; SH: Shanghu Lake; WH: Wuhan Botanical Garden; WS: Weishan Lake; XA: Xin'an River; XL: Xiliang Lake; ZJ: Zhujiyajiao River (see Table 2 for detailed information).

**Table 2**

Information of studied populations and sample numbers of the three *Vallisneria* species.

Population	Abbr.	Geographic location	Species <sup>a</sup>	Water depth (m)	Substrates	Sampling method	Sample number <sup>b</sup>					
							Natural			Experimental		
							M	F	N	M	F	N
Chenshan Botanical Garden, Shanghai	CS	31°04'N, 121°10'E	Vn	0.2–0.5	mud	linear	51(46)	77(56)	0	22(15)	28(15)	0
			Vs	0.4–0.8	mud	linear	48(40)	50(40)	135	6(6)	10(10)	34
			Vd	0.3–0.7	mud	quadrat	7(7)	12(10)	43	–	–	–
Donghu Lake, Hubei	DH	30°33'N, 114°23'E	Vn	– <sup>c</sup>	mud	–	–	–	–	23(15)	26(15)	0
			Vs	0.3–0.8	mud	linear	48(45)	64(55)	3	25(15)	15(15)	0
			Vd	0.5–1.0	mud	linear	46(40)	54(40)	138	9(9)	9(9)	30
Hongze Lake, Jiangsu	HZ	33°16'N, 118°49'E	Vn	0.2–0.5	mud	–	–	–	–	27(15)	22(15)	0
			Vs	0.5–1.8	mud	linear	32(30)	29(23)	62	–	–	–
			Vd	0.8–2.0	gravel	–	–	–	–	16(16)	13(13)	21
Lijiang River, Guangxi	LJ	24°81'N, 110°50'E	Vn	0.1–0.4	mud	quadrat	28(25)	36(25)	0	–	–	–
			Vs	0.4–0.6	mud	quadrat	1(1)	0	63	7(7)	1(1)	42
			Vd	–	–	–	–	–	–	–	–	–
Shanghu Lake, Jiangsu	SH	31°38'N, 120°41'E	Vn	0.1–0.4	mud	quadrat	28(25)	36(25)	0	–	–	–
			Vs	0.4–0.6	mud	quadrat	1(1)	0	63	7(7)	1(1)	42
			Vd	–	–	–	–	–	–	–	–	–
Wuhan Botanical Garden, Hubei	WH	30°33'N, 114°25'E	Vn	0.4–0.6	mud	quadrat	1(1)	0	63	7(7)	1(1)	42
			Vs	–	–	–	–	–	–	–	–	–
			Vd	–	–	–	–	–	–	–	–	–
Weishan Lake, Shandong	WS	34°46'N, 117°08'E	Vn	0.3–0.6	mud	quadrat	27(25)	31(25)	0	–	–	–
			Vs	0.5–2.0	gravel	linear	0	23(20)	87	0	6(6)	43
			Vd	0.7–1.3	mud	–	–	–	–	6(6)	8(8)	33
Xin'an River, Zhejiang	XA	29°29'N, 119°14'E	Vn	0.5–2.0	gravel	linear	0	23(20)	87	0	6(6)	43
			Vs	0.7–1.3	mud	–	–	–	–	6(6)	8(8)	33
			Vd	0.6–1.1	mud	linear	47(40)	43(40)	156	12(12)	9(9)	28
Xiliang Lake, Hubei	XL	29°58'N, 113°58'E	Vn	0.7–1.3	mud	–	–	–	–	6(6)	8(8)	33
			Vs	–	–	–	–	–	–	–	–	–
			Vd	–	–	–	–	–	–	–	–	–
Zhujiyajiao River, Shanghai	ZJ	31°07'N, 121°03'E	Vn	0.6–1.1	mud	linear	47(40)	43(40)	156	12(12)	9(9)	28
			Vs	–	–	–	–	–	–	–	–	–
			Vd	–	–	–	–	–	–	–	–	–

<sup>a</sup> Vn: *V. natans*; Vs: *V. spinulosa*; Vd: *V. denseserrulata*.

<sup>b</sup> Numbers out of the brackets indicate the samples we excavated from natural populations or cultivated from experimental populations, whereas numbers in the brackets indicate the samples we measured. M: male; F: female; N: nonflowering.

<sup>c</sup> Depth of this population is unavailable because we only saw floating fruits, did not ensure where the plants growing in.

**Table 3**F values of ANOVAs for measured traits among the three *Vallisneria* species and among four populations of each species.

Trait	Among species	Among populations		
		Vn	Vs	Vd
Natural populations				
Spathe no. per male flowering ramet	941.54***	22.81***	4.56**	8.68***
Fruit no. per female flowering ramet	399.82***	13.81***	1.70	2.45
Male flower no. per spathe	173.69***	42.05***	31.92***	–
Seed no. per fruit	149.55***	32.97***	12.12***	–
Turion no. per ramet	1477.62***	–	11.43***	–
Experimental populations				
Ramet no. per plant	144.23***	29.17***	9.20***	18.63***
Percentage of flowering ramets	293.18***	9.85***	2.06	2.47
Spathe no. per male flowering ramet	492.38***	1.76	1.52	0.64
Spathe no. per male plant	313.46***	10.98***	0.07	2.79
Fruit no. per female flowering ramet	213.00***	1.69	2.27	1.6
Fruit no. per female plant	254.29***	5.61**	3.60*	4.00*
Male flower no. per spathe	284.05***	1.87	5.99*	23.33***
Seed no. per fruit	241.80***	7.48**	0.15	0.84
%biomass into below-ground structures	1161.42***	11.24***	33.86***	1.93
%biomass into leaves	88.22***	6.28**	5.05**	2.51
%biomass into sexual reproduction	552.80***	2.77*	0.43	2.23
Turion no. per ramet	1605.38***	–	3.73*	–
Turion no. per plant	1624.93***	–	10.64***	–
% biomass into turions	1712.57***	–	6.35**	–

Vn: *V. natans*; Vs: *V. spinulosa*; Vd: *V. denseserrulata*.\*  $p < 0.05$ .\*\*  $p < 0.01$ .\*\*\*  $p < 0.001$ .

ral populations were transplanted separately in pots and reserved under greenhouse conditions for overwintering. In addition, we collected 15 fruits from Lijiang River (LJ) from the Pearl River basin to supply the common garden experiment (Table 2 and Fig. 1).

The flesh and pectin of each fruit were carefully removed, and the seeds from the same population were pooled and then preserved at 4 °C for over 3 months. In March 2014, over 300 seeds of each population were germinated in a plastic jar (10 cm in diameter and 12 cm in height) paved with sterile substrate (mud:sand = 4:1, ~3 cm thick). The jars were filled with water and placed under constant room temperature at 25 °C with a 12 h: 12 h light cycle. In late May 2014, when the *Vallisneria* seedlings produced three to four leaves, we randomly transplanted 50 seedlings of each population into plastic pots (12 cm in upper diameter and 10 cm in height, one seedling per pot) with sterile substrate ~8 cm thick (mud:sand = 4:1). Meanwhile, we randomly collected 50 single ramets from each of the reserved *V. denseserrulata* population (DS, WH and XA; every ramet was collected from a different plant) and transplanted them separately into plastic pots as mentioned above. In total, 600 pots (450 seedlings and 150 ramets) were placed randomly in the experimental pool, the water depth of which was maintained at 0.8 m. In early October 2014, when the fruit setting stage began, the *Vallisneria* plants were harvested for trait measurement (see Section 2.4 for details). Each plant was recorded as male, female or nonflowering. For *V. natans*, all plants flowered, and we randomly selected 15 each of well-developed male and female plants from each population for the trait measurement. All flowering plants of *V. spinulosa* and *V. denseserrulata* were harvested, because only a small proportion of plants produced flowers (see Table S2 for detailed sample numbers).

#### 2.4. Trait measurement

The percentage of flowering ramets of each natural population, the spathe number of each male ramet and the fruit number of each female ramet were measured directly in the field. For experimental populations, we counted both the total and flowering ramet num-

bers to calculate the percentage of flowering ramets of each plant. Spathe number per flowering ramet of experimental populations was measured as the mean of three randomly selected flowering ramets from the same plant, as well as fruit number per flowering ramet. The total number of spathes per male plant and the total number of fruits per female plant were also recorded. In each natural population, a total of 15 male spathes from different samples (for *V. denseserrulata*, all spathes found) were collected to estimate the male flower number of each spathe. For experimental populations, we randomly sampled one mature spathe from each male plant to count male flower number. Seed number per fruit of natural populations was measured based on 15 fruits from different samples (except that only one *V. denseserrulata* fruit was found in the field), and that of experimental populations was measured based on fruits randomly sampled from every female plant.

Biomass (dry weight) measurements were only conducted in experimental populations. Every plant was partitioned into below-ground structures (including roots and stolons), leaves, sexual reproductive structures (including spathes, peduncles and fruits), and vegetative reproductive structures (turions). The plant parts were dried to constant weight at 80 °C, and weighed with Sartorius balances (to the nearest 0.001 g). Considering that some male spathes had withered at the time when the plants were harvested, we randomly sampled five to ten non-dehiscent, mature male spathes of each male plant for biomass weighing (for *V. spinulosa* and *V. denseserrulata*, all mature male spathes were weighed), then divided the value by the number of spathes weighed and multiplied by the total number of spathes to estimate the biomass of sexual reproductive structures of a male plant. Similarly, we randomly selected five to ten intact, mature fruits of each female plant (for *V. spinulosa* and *V. denseserrulata*, all mature fruits were weighed), divided the value by the number of fruits weighed and multiplied by the total number of fruits to estimate the biomass of sexual reproductive structures of a female plant. The biomass of the turions was determined in a similar manner. The percentage of biomass allocated to each part was calculated by the ratio of each part's biomass to the total biomass of a plant.



## 2.5. Data analysis

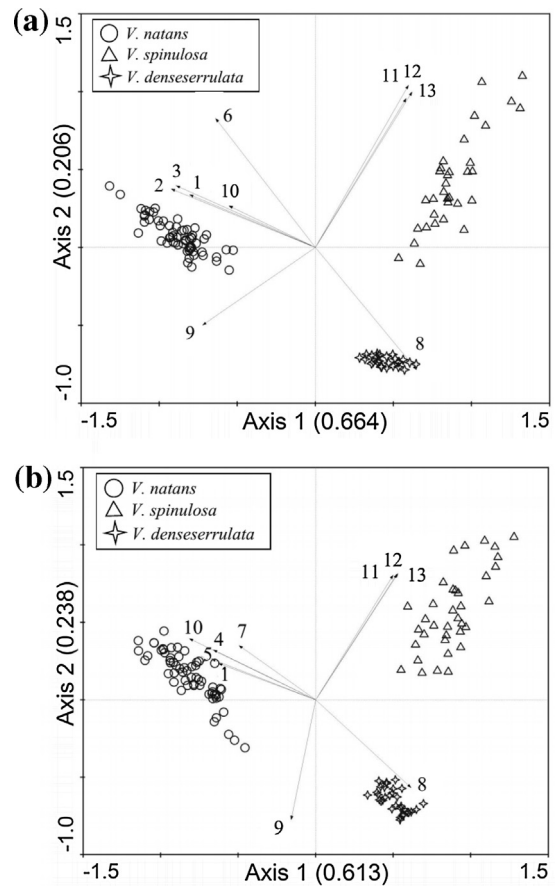
All of the data analyses were conducted using SPSS ver. 19.0 (IBM corporation, Armonk, New York). Data of each measured traits were standardized using min-max method before analyzed. A One-way Analysis of Variance (ANOVA) was applied to determine the effect of population on phenotypic variations, then to test for differences among the three *Vallisneria* species. Due to natural and experimental populations were not entirely consistent and in which different traits were measured respectively, the data of natural and experimental populations were analyzed separately. The amplitude of variation of each trait was estimated by variance-mean-ratio (VMR) for each species separately (Morisita, 1959). We did not use the coefficient of variation (CV) as the indicator because in the cases of *V. spinulosa* and *V. denseserrulata*, the means of some traits (e.g., spathe/fruit number per flowering ramet) were very close to zero, and thus, even a small change (e.g., from 0 to 1) would have led to a great effect on CV (Abdi, 2010). VMR, by contrast, is more suitable in such situations (Cox and Lewis, 1976). In particular, for *V. denseserrulata*, young ramets of three populations instead of seedlings were used for common garden experiment (see 2.3 for detail), so we calculated VMRs of traits from both seedlings of the population LJ and young ramets of the other three populations. The values were not significantly different between the results of experimental populations established with seedlings and young ramets ( $p = 0.447$ ), so we can compare these populations with that of the other *Vallisneria* species. Principal component analysis (PCA) was applied to determine the divergences among the three species. We separated male and female plants to draw PCA diagrams because some traits (e.g., spathe number per male flowering ramet vs. fruit number per female flowering ramet, male flower number per spathe vs. seed number per fruit) were sex-specific and not comparable between the two sexes. All interspecific comparisons of variables were conducted using Duncan's Multiple Range Test with the adjustment for significance level followed by Bonferroni correction ( $p < 0.05/3 = 0.0167$ ).

## 3. Results

All measured traits varied significantly among the three study species under both natural and experimental conditions (Table 3). Flowering frequency varied significantly among the species: *V. natans* having more flowers than the others (Table 4 & Table S1). Five out of 367 ramets from the natural populations of *V. natans* were nonflowering, and all plants at the common garden produced flowers (Table 2). Only 41.4% and 25.3% of total ramets sampled from the natural populations and 35.6% and 30.2% of total plants harvested from the experimental populations produced flowers in *V. spinulosa* and *V. denseserrulata*, respectively.

*V. natans* produced significantly more male spathes and fruits per ramet and per plant than *V. spinulosa* and *V. denseserrulata* (Table 4, Tables S1 & S2). The production of male flowers per spathe and seeds per fruit were also significantly higher for *V. natans* than for the other two species (Table 4, Tables S1 & S2). The differences in the above traits between *V. spinulosa* and *V. denseserrulata* were significant under both natural and experimental conditions. *V. spinulosa* had more sexually reproductive effort (except for spathe number per male plant) and was the only species that produced turions (Table 4, Tables S1 & S2).

All three species invested most of their biomass into leaves, although *V. spinulosa* invested the least, while the other two species had similar investment in leaves (Table 4 & Table S2). *V. denseserrulata* allocated the most to roots and stolons, followed by *V. spinulosa* (turions were not included), and *V. natans* invested only a small part of the total biomass into roots and stolons. *V. spinulosa* allocated



**Fig. 2.** PCA diagram for the measured traits of (a) male and (b) female *Vallisneria* species. Every spot indicates a *Vallisneria* plant in experimental populations. The numbers on the tips of the arrows refer to measured traits: 1. percentage of flowering ramets; 2. spathe number per male flowering ramet; 3. spathe number per male plant; 4. fruit number per female flowering spathe; 5. fruit number per female plant; 6. male flower number per spathe; 7. seed number per fruit; 8. % biomass into below-ground structures; 9. % biomass into leaves; 10. % biomass into sexual reproduction; 11. turion number per ramet; 12. turion number per plant; 13. % biomass into turions.

approximately one-quarter of the total biomass to turions. *V. natans* allocated most biomass to sexual reproduction among the three species; Both *V. spinulosa* and *V. denseserrulata* allocated very little biomass into sexual reproduction, although *V. spinulosa* had a significantly higher sexually reproductive effort than *V. denseserrulata* (Table 4 & Table S2).

The variance-mean-ratios (VMRs) of most traits were higher in *V. natans* than in *V. spinulosa* and *V. denseserrulata* (Table 4), implying that *V. natans* had a relatively higher amplitude of phenotypic variation than the other two species.

Principal component analysis (PCA) showed that the three *Vallisneria* species can be distinctly separated by the traits we measured in this study. The eigenvalues of Axis 1 and Axis 2 for male plants were 0.664 and 0.206, respectively, indicating that ~87% of the total variance could be explained by the two axes. Male *V. natans* were characterized by higher percentage of flowering ramets, spathe number per male flowering ramet/plants, male flower number per spathe and percentage of the total biomass into sexual reproduction (traits 1, 2, 3, 6 and 10 in Fig. 2a). All these traits were associated with higher effort toward sexual reproduction. However, the male plants of *V. denseserrulata* were distinguished by higher percentage of the total biomass into below-ground structures (trait 8 in Fig. 2a), whereas male *V. spinulosa* were distinguished by the production of turions (traits 11, 12 and 13 in Fig. 2a). The results of PCA for female plants were very similar to those for

**Table 4**Means  $\pm$  SE and variance-mean-ratios (VMRs) of measured traits under natural and experimental conditions.

Trait No.	Population	Means $\pm$ SE			VMR (means of four populations $\pm$ SE)		
		<i>V. natans</i>	<i>V. spinulosa</i>	<i>V. denseserrulata</i>	<i>V. natans</i>	<i>V. spinulosa</i>	<i>V. denseserrulata</i>
1	Experimental	0.77 $\pm$ 0.02 <sup>a</sup>	0.16 $\pm$ 0.01 <sup>b</sup>	0.09 $\pm$ 0.01 <sup>c</sup>	0.026 $\pm$ 0.004	0.024 $\pm$ 0.007	0.012 $\pm$ 0.002
2	Natural	23.05 $\pm$ 0.69 <sup>a</sup>	1.93 $\pm$ 0.10 <sup>b</sup>	1.33 $\pm$ 0.17 <sup>b,c</sup>	2.603 $\pm$ 0.243	0.493 $\pm$ 0.109	0.258 $\pm$ 0.182
	Experimental	24.17 $\pm$ 0.86 <sup>a</sup>	1.97 $\pm$ 0.18 <sup>b</sup>	1.64 $\pm$ 0.14 <sup>b,c</sup>	2.144 $\pm$ 0.365	0.523 $\pm$ 0.079	0.265 $\pm$ 0.041
3	Experimental	95.68 $\pm$ 4.66 <sup>a</sup>	3.52 $\pm$ 0.34 <sup>b</sup>	3.87 $\pm$ 0.36 <sup>b</sup>	14.262 $\pm$ 4.303	1.064 $\pm$ 0.161	0.632 $\pm$ 0.094
4	Natural	5.25 $\pm$ 0.20 <sup>a</sup>	0.31 $\pm$ 0.04 <sup>b</sup>	0.01 $\pm$ 0.01 <sup>c</sup>	1.166 $\pm$ 0.233	0.801 $\pm$ 0.065	1.024
	Experimental	6.10 $\pm$ 0.39 <sup>a</sup>	1.11 $\pm$ 0.12 <sup>b</sup>	0.39 $\pm$ 0.08 <sup>c</sup>	0.696 $\pm$ 0.153	0.675 $\pm$ 0.048	0.679 $\pm$ 0.075
5	Experimental	25.33 $\pm$ 1.12 <sup>a</sup>	2.00 $\pm$ 0.25 <sup>b</sup>	0.69 $\pm$ 0.26 <sup>c</sup>	2.431 $\pm$ 0.439	0.276 $\pm$ 0.281	0.729 $\pm$ 0.071
6	Natural	588.42 $\pm$ 13.25 <sup>a</sup>	399.89 $\pm$ 9.77 <sup>b</sup>	231.67 $\pm$ 9.45 <sup>c</sup>	9.082 $\pm$ 0.633	11.589 $\pm$ 1.416	1.307 $\pm$ 1.132
	Experimental	529.25 $\pm$ 14.51 <sup>a</sup>	319.86 $\pm$ 9.58 <sup>b</sup>	163.00 $\pm$ 6.10 <sup>c</sup>	10.211 $\pm$ 2.126	9.621 $\pm$ 0.701	4.686 $\pm$ 0.578
7	Natural	321.09 $\pm$ 8.19 <sup>a</sup>	170.02 $\pm$ 9.97 <sup>b</sup>	24	8.511 $\pm$ 0.856	14.912 $\pm$ 2.208	–
	Experimental	340.68 $\pm$ 8.82 <sup>a</sup>	172.74 $\pm$ 7.90 <sup>b</sup>	15.69 $\pm$ 1.36 <sup>c</sup>	8.806 $\pm$ 0.975	11.672 $\pm$ 2.840	1.474 $\pm$ 0.490
8	Natural	0.00 $\pm$ 0.00 <sup>b</sup>	1.30 $\pm$ 0.13 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	–	0.386 $\pm$ 0.031	–
	Experimental	0.00 $\pm$ 0.00 <sup>b</sup>	1.78 $\pm$ 0.22 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	–	0.363 $\pm$ 0.016	–
9	Experimental	0.00 $\pm$ 0.00 <sup>b</sup>	15.44 $\pm$ 1.76 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	–	1.321 $\pm$ 0.485	–
10	Experimental	0.07 $\pm$ 0.00 <sup>c</sup>	0.27 $\pm$ 0.01 <sup>b</sup>	0.41 $\pm$ 0.01 <sup>a</sup>	0.009 $\pm$ 0.003	0.005 $\pm$ 0.001	0.006 $\pm$ 0.001
11	Experimental	0.60 $\pm$ 0.01 <sup>a</sup>	0.43 $\pm$ 0.01 <sup>b</sup>	0.58 $\pm$ 0.01 <sup>a</sup>	0.015 $\pm$ 0.001	0.011 $\pm$ 0.002	0.004 $\pm$ 0.001
12	Experimental	0.33 $\pm$ 0.01 <sup>a</sup>	0.02 $\pm$ 0.00 <sup>b</sup>	0.01 $\pm$ 0.00 <sup>b,c</sup>	0.032 $\pm$ 0.004	0.030 $\pm$ 0.003	0.009 $\pm$ 0.002
13	Experimental	0.00 $\pm$ 0.00 <sup>b</sup>	0.27 $\pm$ 0.01 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	–	0.012 $\pm$ 0.003	–

Trait number: 1. percentage of flowering ramets; 2. spathe number per male flowering ramet; 3. spathe number per male plant; 4. fruit number per female flowering ramet; 5. fruit number per female plant; 6. male flower number per spathe; 7. seed number per fruit; 8. turion number per ramet; 9. turion number per plant; 10.%biomass into below-ground structures; 11.%biomass into leaves; 12.%biomass into sexual reproduction; 13.%biomass into turions.

Letter superscripts denote a significant difference between trait values among three species based on Duncan's Multiple Range Test adjusted by Bonferroni correction ( $p < 0.05/3 = 0.0167$ ).

male plants: female *V. natans* were distinguished from the other two species by higher percentage of flowering ramets, fruit number per female flowering ramets/plants, seed number per fruit and percentage of the total biomass into sexual reproduction (traits 1, 4, 5, 7 and 10 in Fig. 2b), whereas female *V. spinulosa* and *V. denseserrulata* were distinguished by the production of turions (traits 11, 12 and 13 in Fig. 2b) and a higher percentage of the total biomass into below-ground structures (trait 8 in Fig. 2b), respectively. The eigenvalues of Axis 1 and Axis 2 for female plants were 0.613 and 0.238, respectively, indicating that ~85% of the total variance was explained by the two axes.

#### 4. Discussion

Aquatic plants generally may maintain their populations by vegetative rather than sexual reproduction (Barrett et al., 1993; Philbrick and Les, 1996; Santamaría, 2002). *V. natans* in the common garden experiment allocated approximately one-third of its total biomass to sexual reproduction, which is nearly 18 and 38 times higher than that of *V. spinulosa* and *V. denseserrulata*; and had 47–70 times more male flowers and 23–743 times more seeds than *V. spinulosa* and *V. denseserrulata*, confirming *V. natans* invests more in sexual reproduction than the other two species. This supports the observation of Chen et al. (2008) that divergence in reproductive strategy may have allowed co-occurrence.

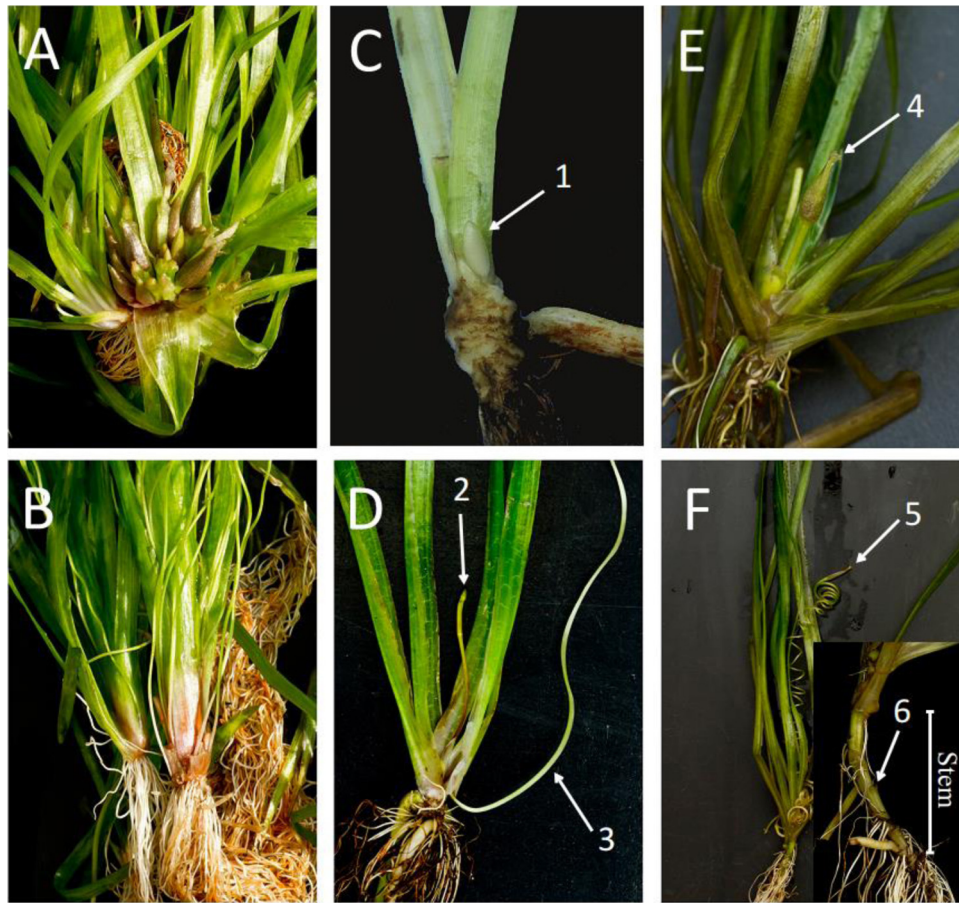
Fruits of *Vallisneria* species will float to nearshore areas after detachment from mother plants, a high abundance of seeds will occur near the bank. Consequently, the seedlings of *V. natans* are undoubtedly more dominant around nearshore areas (Liu et al., 2005). By contrast, *V. spinulosa* and *V. denseserrulata* have relatively stronger clonal growth, which allows them to occur at deeper areas (Table 2). In deeper areas, decreasing light may lower the survival rates of *V. natans* seedlings (Zhou et al., unpublished data), but have less effects on turions and young ramets of *V. spinulosa* and *V. denseserrulata* (Doyle and Smart, 2001; Liu et al., 2005). In addition, nearshore areas usually suffer seasonal drought, which limits the growth of the evergreen *V. denseserrulata*, while the seeds of *V. natans* are more tolerant to such environments.

In addition to reproductive strategy, significant divergences in life history are also found among the three *Vallisneria* species. Dur-

ing our common garden experiment, all of the plants of *V. natans* that we did not harvest for trait measurement died two months later. This supports the opinion that *V. natans* is an annual plant, or at least a semelparous species that ends its life after sexual reproduction (Chen et al., 2008; Les et al., 2008). By contrast, all of the plants of *V. denseserrulata* do not wither even in the coldest season and can revive quickly in the next growing season. We observed an inconspicuous, short stem with leaf scars in most ramets of *V. denseserrulata* (i.e., erect growth form, see Fig. 3), which makes it slightly different from its rosette-like congeners (Les et al., 2008). Conversely, although the above-ground parts of *V. spinulosa* wither in the winter, the turions remain in the substrate and germinate the following year. Therefore, *V. spinulosa* and *V. denseserrulata* are both perennial species with different overwintering strategies. Therefore, it is reasonable to infer that both differences in reproductive strategy and growing season length may allow coexistence of the three species in mid-lower reaches of the Yangtze River.

Among the three species, *V. natans* has the widest range of distribution, which can also be attributed to its higher capability for sexual reproduction (Frankham, 1996; Lowry and Lester, 2006). Seeds have a wider dispersal range and a higher tolerance to stressful environments than vegetative propagules (Oliver et al., 2000; Levin et al., 2003). Sexual reproduction may produce higher genetic diversity due to recombination compared to vegetative reproduction (Barton and Charlesworth, 1998; Rice, 2002). Higher genetic variation is believed to account for higher phenotypic variation (Hughes et al., 2008) and greater potential to adapt to highly variable environments (Lowry and Lester, 2006; Lavergne and Molofsky, 2007), therefore improves the potential for species to occupy larger geographic ranges by conferring a greater niche breadth (Brown, 1984; Gitzendanner and Soltis, 2000; Lowry and Lester, 2006). In the present study, we detected a higher amplitude of phenotypic variation in *V. natans* than that in the other two species. Wang et al. (2010a) also demonstrated that *V. natans* had relatively greater genetic diversity than *V. spinulosa* (Nei's gene diversity  $H = 0.21$  vs. 0.17,  $t$ -test,  $p < 0.001$ ).

Compared with other forms of propagules, seeds are more tolerant to many unfavorable conditions, such as drought, extreme temperature, acid, salt and pathogens (Pammenter and Berjak, 1999; Kaya et al., 2006; Paula and Pausas, 2008). According to life



**Fig. 3.** Morphology of flowering ramet of the three *Vallisneria* species. A, male *V. natans*; B, female *V. natans*; C, male *V. spinulosa*; D, female of *V. spinulosa*; E, male *V. denseserrulata*; F, female *V. denseserrulata*. 1, male spathe; 2, female spathe; 3, peduncle; 4, male spathe; 5, female spathe; 6, the short stem.

history theory, plants with higher levels of sexual reproduction (i.e., higher seed production) are predicted to grow in relatively more fluctuating environments due to the high tolerance of seeds to harsh environments, while plants with higher levels of vegetative growth are adapted to relatively stable environments (Stearns, 1976).

Compared with *V. natans*, the other two species of this genus allocate only a very small part of the total biomass into sexual reproduction but more biomass into below-ground structures (i.e., roots and stolons; see Table 4 and Table S2). In addition to their anchoring and absorbing functions, below-ground structures also play an important role in nutrient and energy storage (Jackson et al., 2007). In unfavorable seasons (e.g., drought, extreme high and/or low temperatures), above-ground plant parts of many herbaceous plants will wither, but surviving below-ground parts may revive in the next growing season (Vegis, 1964). Many perennial aquatic plants also adopt similar strategies to withstand unfavorable conditions (Haag, 1979; Masuda et al., 2006). *V. spinulosa* allocates over half of its total biomass into below-ground structures if the turion is taken into account (~27% total biomass allocated both to turions and to other below-ground structures, Table 4). A turion is a specialized vegetative structure that enables plants to overcome stressful periods in the life span, and one of the main forms of vegetative propagation in aquatic plants (Philbrick and Les, 1996). *V. spinulosa* in the mid-lower zones of the Yangtze River may fall dry in the winter, and survives this period through turions, and it is the most dominant of the three studied species (Chen et al., 2008). *V. denseserrulata*, due to its evergreen life form, is unable to survive in environments with seasonal droughts or freezes. This species can

only persist in more stable and warmer environments such as the southern areas of China.

Our data demonstrate that there are significant differences in reproductive strategy between *V. spinulosa* and *V. denseserrulata*, and that *V. spinulosa* invests obviously more resources into both sexual and vegetative reproduction. The amplitudes of variation of most traits are also higher in *V. spinulosa*, indicating its higher phenotypic plasticity and capability for sexual reproduction. However, the distribution range of *V. spinulosa* is limited to the mid-lower reaches of the Yangtze River, whereas *V. denseserrulata* occupies a wider area of Southern China (Fig. 1). We infer that the more stable environments in warmer areas favor species capable of expanding their populations rapidly and lead to relatively larger distribution ranges. As an evergreen plant, *V. denseserrulata* has a longer growing season than *V. spinulosa*, which is more advantageous in dispersing. In addition, *V. denseserrulata* produces more ramets than *V. spinulosa* within the same time interval, as our common garden experiment shows, implying that *V. denseserrulata* can expand more rapidly than *V. spinulosa* in natural populations. As a consequence, in the areas more favorable to *V. denseserrulata* (e.g., Southern China), it is able to outcompete its congeners and become the dominant species in such freshwater ecosystems.

## 5. Conclusions

The present study shows that reproductive strategies are very different among three *Vallisneria* species native to China: *V. natans* (1) has a significantly higher output of sexual reproductive structures, (2) allocates more biomass into sexual reproduction, and



(3) has higher amplitude of phenotypic variation than its closely related congeners, *V. spinulosa* and *V. denseserrulata*. The latter two species, however, invest more in vegetative growth than into sexual reproduction. These divergences of reproductive strategy among the three species are correlated with their distribution patterns in China. The relatively northern and widespread distribution of *V. natans* is mainly attributed to its higher capability for sexual reproduction. Remarkable divergences in reproductive strategy, and consequent habitat differentiations, exist even in such a small genus (~15 species) with highly conserved morphologies. These findings suggest that we should pay more attention to detailed comparisons among congeneric aquatic plants with different reproductive strategies, which is valuable for ecosystem restoration using aquatic plants.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.aquabot.2016.04.005>.

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