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Local-scale patterns of genetic variation in coexisting floating-leaved *Nymphoides peltata* and submerged *Myriophyllum spicatum* in Donghu Lake*

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Abstract Coexisting floating-leaved and submerged plants experience similar environmental changes but may evolve different patterns of genetic variation. To compare local-scale genetic variation, we collected samples of floating-leaved *Nymphoides peltata* and submerged *Myriophyllum spicatum* coexisting in a disturbed urban lake in China. At the subpopulation level, using microsatellites, *M. spicatum* had higher clonal diversity than *N. peltata*. *M. spicatum* had 28.4% multilocus genotypes (MLGs) shared between subpopulations, but *N. peltata* had only one MLG shared between two adjacent subpopulations. *N. peltata* displayed more genetic variation between subpopulations than within subpopulations, but the reverse was true for *M. spicatum*. Principal components and Bayesian cluster analyses showed that individuals from each subpopulation of *N. peltata* tended to have relatively close genetic relationships. For *M. spicatum*, individuals from each subpopulation were genetically scattered with those from other subpopulations. Our results imply that in unpredictable adverse environments *M. spicatum* may be less subjected to local-deme extinction than *N. peltata* because of genetically diverse clones at the subpopulation level. This characteristic means that following adverse events, *M. spicatum* may rapidly restore subpopulation distributions via re-colonization and intense gene flow among subpopulations.

Keyword: aquatic plants; life forms; microsatellites; clonal diversity; eutrophic lake

1 INTRODUCTION

Floating-leaved plants and submerged plants frequently coexist in aquatic communities (Nikolić et al., 2007; Madgwick et al., 2011), providing heterogeneous habitat for associated organisms (Rejmankova, 2011). Over evolutionary time, the two life forms of plants in common communities experience similar environmental changes but may evolve different patterns of genetic variation because of different biological characteristics (Barrett et al., 1993). Under the increasing influence of global climate change and anthropogenic disturbance, species with different patterns of genetic variation may exhibit differences in resilience (Reusch et al., 2005; Ehlers et al., 2008; Canale and Henry, 2010). Plant species' resilience is correlated with frequencies of subpopulation loss and reoccurrence determined by genetic diversity, clonal structure and propagule dispersal (Hughes and Stachowicz, 2004; Massa et al., 2013). Comparing local-scale genetic variation in floating-leaved and submerged plants can enhance predictions about the development of aquatic communities.

Many aquatic plants have a high capacity for asexual reproduction (clonal propagation), helping them to expand and occupy habitat rapidly in stable environments (Barrat-Segretain, 1996; Li, 2014). However, in unstable environments, subpopulations establishing from limited genets may be subject to maladaptation of some clones and even loss of all clones. In these circumstances sexual reproduction is

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advantageous (Li, 2014). The recovery of subpopulations depends on propagule dispersal and re-colonization within populations. As the major contributor to maintaining populations in stable environments, vegetative reproduction of aquatic plants is achieved via rhizomes, shoot fragments and so on (Sculthorpe, 1967; Philbrick and Les, 1996; Li, 2014). Among these vegetative propagules, fragments are rapidly dispersed by water flow in interconnected spaces throughout growth seasons (Sculthorpe, 1967; Aiken et al., 1979; Philbrick and Les, 1996; Darbyshire and Francis, 2008). In contrast, the dispersal of rhizomes buried in substratum is relatively limited, expanding space only via extension growth (Sherman et al., 2016) and occasional sediment transport (Phan et al., 2017). Differences in propagule dispersal probably affect local-scale genetic structure in aquatic species.

Floating-leaved *Nymphoides peltata* (Gmel.) O. Kuntze and submerged *Myriophyllum spicatum* L. are widely distributed across several continents and frequently co-occur in freshwater plant communities (Zhou and Chen, 1996; Nikolić et al., 2007; Darbyshire and Francis, 2008; Cao et al., 2017). The two species are perennial with over-wintering rhizomes buried in the substratum (Flora of China, 2018). Vegetative spread can occur throughout the growth season via the dispersal of node-bearing fragments (Aiken et al., 1979; Darbyshire and Francis, 2008), though both species can produce seeds (Flora of China, 2018). The pollination modes of *M. spicatum* and *N. peltata* are mainly anemophilous and entomophilous, respectively (Aiken et al., 1979; Wang et al., 2005). *N. peltata*, with its distylous flowers is a self-incompatible species (Wang et al., 2005). Stems of *M. spicatum* up to 2.5 m (Flora of China, 2018), branched near the water surface, can easily produce fragments by mechanical breakage and a self-initiated abscission (Aiken et al., 1979; Smith et al., 2002). *N. peltata* stolons are produced horizontal to the substratum and leaf petioles can be up to 4 m; petiole length depends on water depth (Darbyshire and Francis, 2008; Flora of China, 2018). Mechanical disturbances in the upper layer of water and water surface easily break *N. peltata* leaf blades, but stolons generally remain undamaged because of flexibility and deep submergence (Cao et al., 2016). We studied samples of *N. peltata* and *M. spicatum* in a highly disturbed urban lake to compare local-scale genetic variation. Our specific objectives were to: (1) compare clonal diversity between coexisting floating-leaved *N. peltata* and submerged *M. spicatum* at a local scale; and (2) determine differences in genetic relationships and local-scale genetic structures at a subpopulation level. We expect that our results can enhance our understanding of the development of aquatic communities in eutrophic urban lakes.

2 MATERIAL AND METHOD

2.1 Site description

Donghu Lake (30°31′N–30°36′N, 114°21′E–114°28′ E) is located in Wuhan City in central China (Fig.1) in a subtropical monsoon climate. It is the second largest urban lake in China. The shallow lake has an average depth of 2.2 m (Qiu et al., 2001) and has been segmented into several sublakes by causeways, resulting in lack of interchange and different trophic status among water bodies. We sampled from a sublake, termed Niuchaohu Sublake. Niuchaohu has relatively vigorous plant growth and less pollution (Qiu et al., 2001), though the whole lake has severely polluted in recent decades. When plants sampled, we collected water sample at each sample site and measured the concentration of total nitrogen (1.46±0.215 mg/L) and total phosphorus (0.11±0.009 mg/L) in water of the sublake.

2.2 Plant sample

In the Niuchaohu Sublake, *N. peltata* and *M. spicatum* are dominant in the plant community. In May 2016, we sampled all conspicuous subpopulations of the two species in this sublake and recorded the longitude and latitude of every sampling site by GPS. The distance between sample sites (subpopulations) was within the range of 0.2–2.1 km for *N. peltata* and 0.3–2.4 km for *M. spicatum* (Fig.1). Distances between samples within subpopulations were about 1 m for each species. According to the distribution areas of these plants the sample sizes of the subpopulations were 11–34 for *N. peltata* and 23–39 for *M. spicatum* (Table 1). Leaf samples were desiccated with allochroic silica gel and stored until DNA extraction. The total sample sizes of both species were nearly equal (approximately 210).

2.3 DNA extraction and PCR amplification

Total genomic DNA was extracted from dry leaf samples following a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Saghai-Maroof et al., 1984). We selected 12 and 10 pairs of

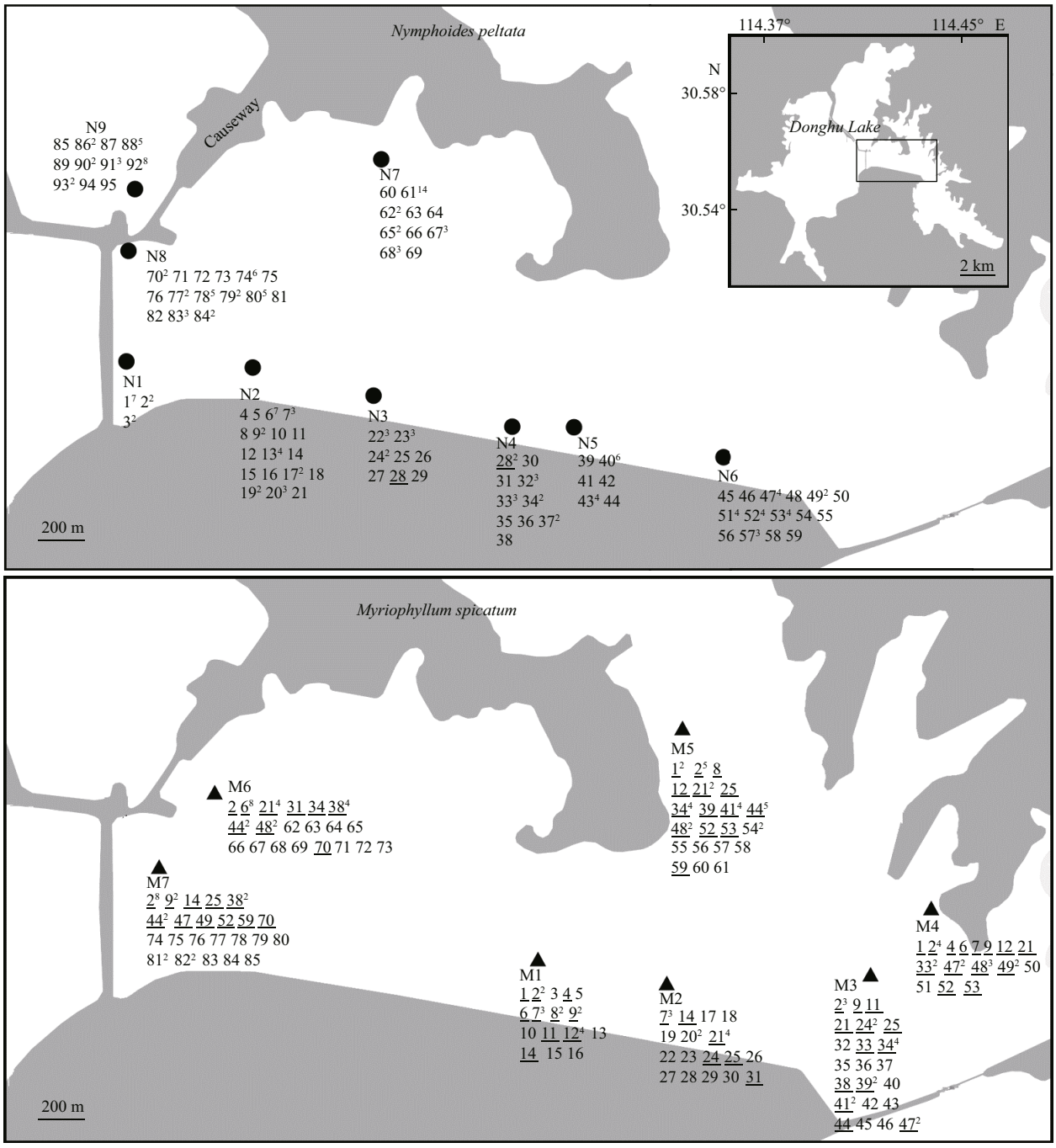

Fig.1 Locations of sampling sites and distribution MLGs of *N. peltata* (subpopulations: N1–N9) and *M. spicatum* (subpopulations: M1–M7) in Donghu Lake

Samples belonging to the same clones are represented by the same normal numbers, among subpopulations of each species. Numbers in superscript are the ramet number of each clone in each subpopulation. Normal numbers underlined denote clones shared between two or more subpopulations.

microsatellite (i.e. simple sequence repeat, SSR) primers amplified polymorphic bands for *N. peltata* and *M. spicatum*, respectively, out of 47 and 20 pairs of primers reported by Uesugi et al. (2005), Yuan et al. (2013) and Wu et al. (2013). Detailed information about primer pairs is provided in the Online Resource.

Amplification of SSR was carried out in 20 μL containing a mix of *Taq* polymerase (0.1 U/μL), dATP, dCTP, dGTP, dTTP (0.4 mmol/L each) and buffer (Beijing ComWin Biotech). PCRs were performed in a C1000 Thermal Cycler (Bio-Rad Laboratories). A denaturation period of 5 min at 94°C was followed by 30 cycles of 30 s at 94°C, 30 s at 58°C and 45 s at 72°C, and then 10 min at 72°C for

Table 1 Genetic diversity and clonal diversity of *N. peltata* and *M. spicatum* in Donghu Lake

Subpopulation	N	A	A_e	H_e	G	G_e	R	N_r
N. peltata								
N1	11	1.8	1.6	0.582	3	2.1	0.20	3.7
N2	34	2.5	1.7	0.936	18	10.9	0.52	1.9
N3	13	2.1	1.7	0.910	8	6.3	0.58	1.6
N4	17	2.2	1.6	0.934	10	8.3	0.56	1.7
N5	14	2.2	1.7	0.769	6	3.5	0.38	2.3
N6	30	2.4	1.7	0.936	15	10.5	0.48	2.0
N7	29	2.6	1.4	0.756	10	3.7	0.32	2.9
N8	34	2.2	1.6	0.925	15	9.8	0.42	2.3
N9	27	2.3	1.8	0.875	11	6.3	0.38	2.5
Mean	23	2.3	1.7	0.847	11	6.8	0.45	2.3
Total population	209	6.1	2.6	0.986	95	52.6	0.45	/
M. spicatum								
M1	24	4.4	3.4	0.957	16	12.0	0.65	1.5
M2	23	4.4	3.3	0.960	17	12.3	0.73	1.4
M3	31	4.8	3.3	0.972	22	16.9	0.70	1.4
M4	24	4.6	3.2	0.957	16	12.0	0.65	1.5
M5	39	4.8	3.0	0.951	21	13.7	0.53	1.9
M6	35	5.0	3.1	0.929	20	10.3	0.56	1.8
M7	35	4.8	3.2	0.945	23	12.1	0.65	1.5
Mean	30	4.7	3.2	0.953	19	12.8	0.62	1.6
Total population	211	5.6	3.3	0.973	85	31.3	0.40	/

N: sample size; A: number of alleles; A_e: effective number of alleles; H_e: Nei's genetic diversity (expected heterozygosity) corrected for sample size; G: number of MLGs; G_e: effective number of MLGs; R: genotypic richness: $R=(G-1)/(N-1)$; N_r: average ramet number of each MLG in each subpopulation ($N_r=N/G$).

final extension. PCR products were analyzed by Tsingke Biological Technology (China), and the length of DNA fragments was determined in GeneMarker v. 1.3 Demo.

2.4 Data analyses

Because *N. peltata* and *M. spicatum* are hexaploid species, comparing the genetic diversity parameters between the species should be reasonable. In each species, the individuals with the same multilocus genotype (MLG) were treated as a clone (genet).

Data used were DNA fragment lengths of amplification products. Analyses of genetic diversity (A, number of alleles; A_e, effective number of alleles; H_e, Nei's genetic diversity corrected for sample size, i.e. expected heterozygosity), clone assignment, clonal diversity and principal components analysis (PCA) were performed in GenoDive 2.0b23 (Meirmans and van Tienderen, 2004) on a Mac. GenoDive is suitable for analysis on asexual organisms with polyploids (Dufresne et al., 2014). Clonal diversity was denoted by the following parameters: G, number of MLGs; G_e, effective number of MLGs; R, genotypic richness, $R=(G-1)/(N-1)$, where N is number of samples (Dorken and Eckert, 2001); and N_r, average ramet number of each MLG in each subpopulation ($N_r=N/G$). Analyses of allele frequencies (A, A_e) and PCAs for individual samples were corrected for the unknown dosage of alleles. Scatter diagrams from the PCAs were drawn in SigmaPlot 10.0 (Systat Software, Chicago, IL, USA).

Bayesian cluster analyses of subpopulation structure were performed using Structure 2.3 (Pritchard et al., 2000) to determine the number of genetic clusters in each species, using the admixture model with independent allele frequencies. We tested K in 10 independent runs from 1 to 9 for *N. peltata* and 1 to 7 for *M. spicatum*, without using sampling location as a prior to assess convergence of Ln P (D) (10 000 burn-in and 100 000 Markov chain Monte Carlo replicates in each run). The value of Ln P (D) is the posterior probability of the data for a given K. ΔK values based on the rate of change in Ln P (D) between successive K values were calculated according to Evanno et al. (2005). Then, based on the distribution of ΔK as a function of K, we identified the correct number of clusters (K) that best explain the data.

Analyses of molecular variance (AMOVA) were conducted for *N. peltata* and *M. spicatum* in software GenAlEx 6.5 (Peakall and Smouse, 2012). In this analysis, binary data were used, because this software cannot analyze DNA fragment length data in hexaploid plants.

3 RESULT

3.1 Genetic diversity and clonal diversity

In Donghu Lake, *N. peltata* (H_e=0.986) and *M. spicatum* (H_e=0.973) had similar values of total genetic diversity based on SSR markers (Table 1). The H_e values of subpopulations of *N. peltata* and *M. spicatum* were in the range of 0.582–0.936 and 0.929–0.972, respectively. Regarding clonal diversity, at the total lake population level the parameter values for *N. peltata* (G=95, G_e=52.6, R=0.45) were greater than those for *M. spicatum* (G=85, G_e=31.3, R=0.40) (Table 1). However, the reverse was true for average values at the subpopulation level (mean G=11, G_e=6.8, R=0.45 in *N. peltata*; mean G=19, G_e=12.8,

R=0.62 in *M. spicatum*). *N. peltata* (N_r=2.3) had a higher average ramet number of each MLG in each subpopulation than *M. spicatum* (N_r=1.6). The greatest ramet number of an individual MLG in each subpopulation of *N. peltata* and *M. spicatum* was 14 (MLG No. 61 in subpopulation N7) and 8 (MLG No. 2 in subpopulation M7 and MLG No. 6 in subpopulation M6), respectively (Fig.1). *M. spicatum* had 28.4% MLGs shared between two or more subpopulations, but *N. peltata* had only one MLG (No. 28) shared between two adjacent subpopulations (N3 and N4). For *M. spicatum*, the greatest number of subpopulations sharing the same MLGs (No. 2) was six out of seven.

3.2 Local-scale genetic structure and genetic relationships between subpopulations

AMOVA analyses showed that genetic variation in *N. peltata* was higher among subpopulations (65%) than within subpopulations (35%) (Table 2). In contrast, genetic variation in *M. spicatum* among subpopulations was only 10% and much smaller than within subpopulations (90%).

At the individual level, PCA and Bayesian cluster analyses found that individuals originating from each subpopulation of *N. peltata* tended to have relatively close genetic relationships, but for *M. spicatum* individuals originating from each subpopulation were genetically scattered with those from other subpopulations (Figs.2 & 3). *N. peltata* individuals from subpopulation N2 were genetically distant from those from the other subpopulations and three genetic groups (N1–N5–N9, N7–N8 and N3–N4) were exhibited (Fig.3).

Table 2 Analysis of molecular variance (AMOVA) of *N. peltata* and *M. spicatum* in Donghu Lake

Source of variation	df	SSD	CV	Total (%)	*P* value
N. peltata					
Among subpopulations	8	1 265.07	6.77	65	<0.001
Within subpopulations	200	732.97	3.67	35	<0.001
Total	208	1 998.04	10.44		
M. spicatum					
Among subpopulations	6	148.57	0.64	10	<0.001
Within subpopulations	204	1 172.03	5.75	90	<0.001
Total	210	1 320.60	6.38		

df: degree of freedom; SSD: sum of squared deviations; CV: variance component estimates; % total: percentage of total variation.

4 DISCUSSION

4.1 Diversity of *N. peltata* and *M. spicatum* in Donghu Lake

Base on SSR markers from the samples in Donghu Lake, high genetic diversity was detected in *N. peltata* (H_e=0.986) and *M. spicatum* (H_e=0.973). Genetic diversity detected in this study is higher than those detected in samples collected extensively in China, e.g. the samples from 21 populations of *N. peltata* (mean H_e in the range of 0.362–0.484, Liao et al., 2013) and ones from 58 populations of *M. spicatum* (mean H_e=0.756, Wu et al., 2016). Our results indicate that these two aquatic species have high local-scale genetic diversity, even when inhabiting a eutrophic urban lake.

Nymphoides peltata is a distylous herb with strong self-incompatibility (Wang et al., 2005) and was not found to have higher genetic diversity than *M. spicatum* at a total lake population level. Generally, genetic diversity is related to the outcrossing rate of plants (Hamrick and Godt, 1996) and self-incompatible *N. peltata* has a high outcrossing rate. Comparably, the characteristics of *M. spicatum* flowers favor cross-pollination by polygamonoecy (having male, female and bisexual flowers on the same plant) and protogyny (stigmas ripen in advance of the stamens) (Aiken et al., 1979; Flora of China, 2018). At the subpopulation level, *N. peltata* had obviously lower genetic diversity (e.g. low allele numbers) than *M. spicatum*. For *N. peltata*, the low genetic diversity of subpopulations might be related to its high ability of vegetative propagation and a low frequency of gene exchange among subpopulations. At a total lake population level *N. peltata* samples (*R*=0.45) from Donghu Lake had a similar genotypic richness to samples (*R*=0.405) collected extensively in China mentioned above (Liao et al., 2013), but *M. spicatum* samples (*R*=0.40) had obvious lower genotypic richness than samples (*R*=0.57) collected by Wu et al. (2016). However, this result showed that clonal diversity in the two species was not low in a eutrophic urban lake. At the subpopulation level the low genotypic richness of *N. peltata* (mean *R*=0.45) relative to *M. spicatum* (mean *R*=0.62) may be related to different types of clonal growth and vegetative propagation. *N. peltata* grows through horizontal extension and branching of stolons (Darbyshire and Francis, 2008) forming large clone sizes (mean N_r=2.3) in each subpopulation. Large *N. peltata* clones may repress establishment of immigrants and yield a relatively low clonal diversity of

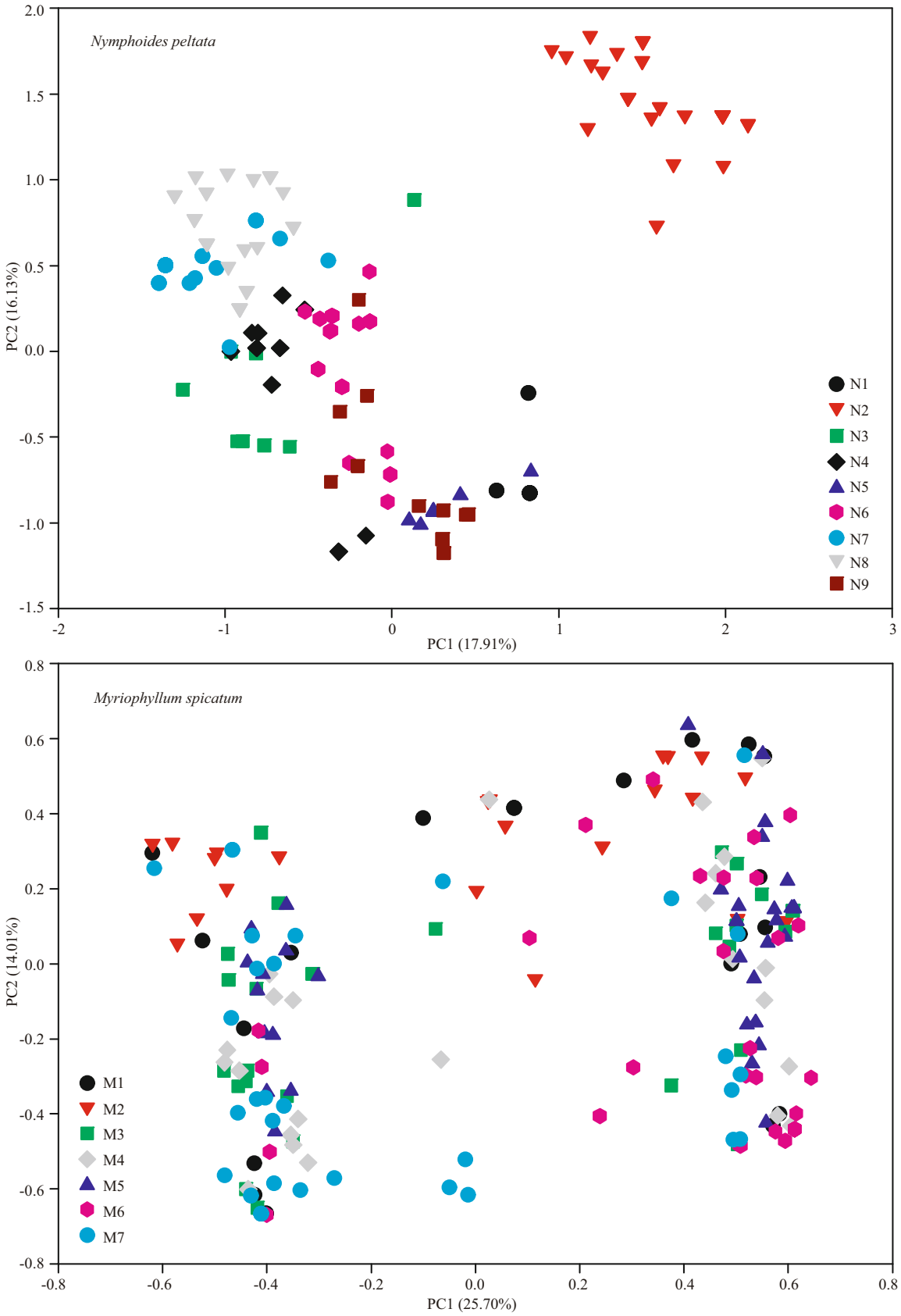

Fig.2 Scatter plots of first and second principle components for analysis of SSR fragment length data for individual *N. peltata* (subpopulation code N1–N9) and *M. spicatum* (M1–M7) in Donghu Lake

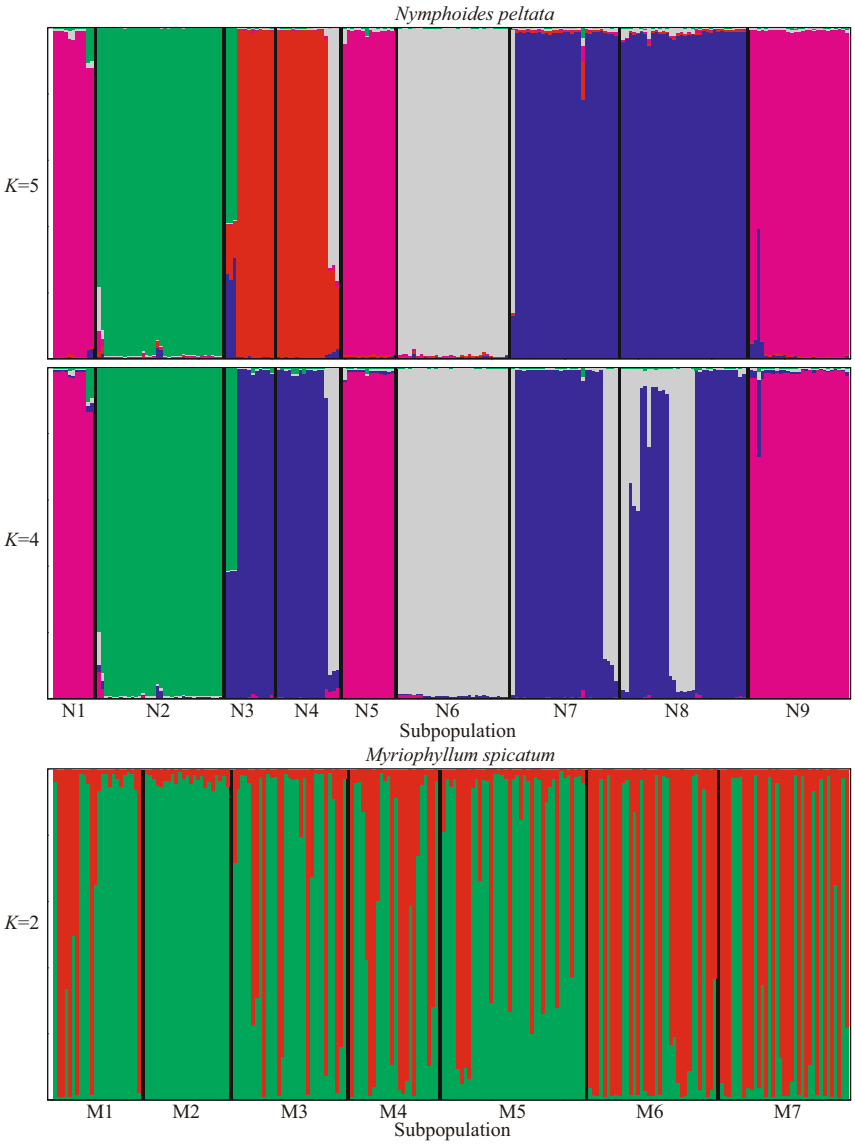

Fig.3 Estimated genetic structure of subpopulations of *N. peltata* and *M. spicatum* in Donghu Lake inferred from Bayesian cluster analyses (Structure 2.3) at an individual level

Black lines indicate different subpopulation origins.

subpopulations. Stems of *M. spicatum* grow upward and branch adjacent to water surface (Aiken et al., 1979), forming small clones (mean N_r=1.6) within subpopulations. Small *M. spicatum* clones may provide a higher chance of immigrant establishment, especially for shoot fragments with the ability to spread rapidly. *M. spicatum* had a relatively high clonal diversity within subpopulations, but a moderate clonal diversity at the population level.

4.2 Local-scale genetic structure at a subpopulation level

High genetic variation (65%) among subpopulations of *N. peltata* and the low variation (10%) among *M. spicatum* subpopulations imply more intense gene flow between subpopulations in *M. spicatum*. The two species can produce seeds, overwinter by rhizomes and propagate and disperse by shoot fragments in growth seasons (Aiken et al., 1979; Darbyshire and Francis, 2008). The production and dispersal of fragments is easier and more frequent in *M. spicatum* than *N. peltata*, which may contribute to differences in gene flow of the two species. This fragment difference may explain the distribution of individuals and clones among subpopulations. Fragments of *M. spicatum* are frequently produced by mechanical breakage such as recreational activity, fishing and self-initiated abscission (Smith et al.,

2002) and dispersed by water flow and animal activity (Aiken et al., 1979; Darbyshire and Francis, 2008). Clones with different genetic backgrounds mix extensively among subpopulations just as the distribution pattern of individuals according to the PCA and Bayesian clustering analyses. We detected 28.4% MLGs shared between *M. spicatum* subpopulations, and one MLG shared in six out of seven subpopulations. In contrast, stolons of *N. peltata* creep along the sediment surface and are immersed deeply in the water column (Darbyshire and Francis, 2008) with little chance of producing fragments by mechanical disturbance in recreational activities, fishing and aquatic plant management. Gene flow between subpopulations of *N. peltata* may benefit little from fragment migration, a proposition supported by the fact that only one MLG was shared (between two neighboring subpopulations). This shared MLG may result from a shoot fragment rarely produced and dispersed by mechanical disturbance or resolution inefficiency of the molecular markers used in this study. Therefore, the migration of seed and pollen could be the major gene movement method for *N. peltata* subpopulations.

There was no significant correlation between genetic and geographical distances for subpopulations in each species, though several adjacent subpopulation pairs had close genetic relationships. The genetic relationships between subpopulations in each species imply that the extent of gene flow was not sensitive to spatial distances at a local scale. Similar results have been reported in other studies on the fine-scale genetic structures of aquatic species (red alga, *Asparagopsis taxiformis*, Andreakis et al., 2009; Australian seagrass, *Zostera muelleri*, Sherman et al., 2016). In particular, the N9 subpopulation of *N. peltata* insulated by a causeway was not genetically distant from other subpopulations, which may be historically connected with the N9 subpopulation. Besides, the causeway between the N9 and other subpopulations may be a barrier only for propagule migration by water flow and not for the dispersion of seed and pollen by animals.

Surprisingly, the N2 subpopulation of *N. peltata* was genetically distant from other subpopulations at the subpopulation and at individual levels. The N2 subpopulation had the highest number of alleles (*A*) and number of MLGs (*G*) among all subpopulations. The N2 subpopulation also had the largest number (11) of single-ramet MLG among all subpopulations, though only five unique alleles at two out of twelve SSR loci were detected in it. Therefore, the distant genetic relationship between the N2 subpopulation and others is difficult to explain. When samples were collected we observed a sandy and stony substratum at the N2 subpopulation site, unlike the silty sediment of other subpopulations. We speculate that the distinct substratum of the N2 subpopulation does not favor clonal growth via rhizome and stolon extension because of nutrient limitation and mechanical hampering, but does favor successful germination and recruitment from seeds with diverse genotypes in canopy gaps. Similar to other studies (Sherman and Ayre, 2008; Sherman et al., 2016), genetic differentiation can occur over local spatial scales under heterogeneous environmental factors. However, explanation for the distinction of the N2 subpopulation remains unclear and requires further work.

4.3 Implication for aquatic communities

Even in a eutrophic urban lake such as Donghu we found high levels of genetic diversity and clonal diversity of floating-leaved *N. peltata* and submerged *M. spicatum*. These species have a high tolerance for eutrophication and human disturbance. If contamination of the lake is alleviated by control of pollutant inputs, the spontaneous recovery of aquatic communities dominated by *N. peltata* and *M. spicatum* is expected via propagule banks. This is consistent with the recovery of submerged vegetation in Donghu Lake according to large-scale enclosure experiments (Qiu et al., 2001).

It is generally regarded that floating-leaved plants are more tolerant to water level changes and eutrophication because of their superior ability to compete for light (Bornette and Puijalon, 2010). However, under increase of human disturbance, climate change and biological invasion, environmental changes tend to be more frequent, more unpredictable and involve more factors (Canale and Henry, 2010; Maclean and Wilson, 2011; Wingfield et al., 2011; Havel et al., 2015). Floating-leaved *N. peltata* grows through stolon extension horizontally, which favors large clones but depresses seedling establishment in closed canopies. Therefore, the clonal diversity of *N. peltata* at the subpopulation level was relatively low and may result in massive loss of plants in a subpopulation or even local extinction when adverse events occur. If local extinction occurs, the subpopulation recovery of *N. peltata* relies on seed dispersal and successful seedling recruitment. Therefore, for *N. peltata*, sexual reproduction is

important in maintaining populations. On the contrary, subpopulations of submerged *M. spicatum*, with clones from diverse genetic backgrounds may survive under unpredictable adverse environments. Frequent migration of shoot fragments of *M. spicatum* can rapidly restore the subpopulation by re-colonization and metapopulation dynamics (Harrison and Hastings, 1996) when local subpopulations go extinct. The recovery of *M. spicatum* may be more likely than *N. peltata* following adverse events because of intense propagule flow among subpopulations, as inferred from differences in local-scale genetic structuring.

5 CONCLUSION

Our results show that *M. spicatum* had higher clonal diversity than *N. peltata* at the subpopulation level. *M. spicatum* had 28.4% MLGs shared between subpopulations, but *N. peltata* had only one MLG shared between two adjacent subpopulations. *N. peltata* displayed more genetic variation between subpopulations than within subpopulations, but the reverse was true for *M. spicatum*. According to principal components and Bayesian cluster analyses, individuals from each subpopulation of *N. peltata* tended to have relatively close genetic relationships. For *M. spicatum*, individuals from each subpopulation were genetically scattered with those from other subpopulations. Our results imply that in unpredictable adverse environments *M. spicatum* may be less subjected to local-deme extinction than *N. peltata* because of genetically diverse clones at the subpopulation level. This property means that following adverse events, *M. spicatum* may rapidly restore subpopulation distributions via re-colonization and intense gene flow among subpopulations.

6 DATA AVAILABILITY STATEMENT

Data of microsatellite (i.e. simple sequence repeat, SSR) primer pairs used for DNA amplification in this study are included in the supplementary information files of this published article. Data of allele information of the two species generated and analyzed during the current study are available from the corresponding author on reasonable request.

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