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Effect of Habitat Modification on the Distribution of the Endangered Aquatic Fern *Ceratopteris pteridoides* (Parkeriaceae) in China

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ABSTRACT.—Sixteen sites in China where *Ceratopteris pteridoides* occurs based on historical records and/or from observations were surveyed during preliminary field surveys. Eight previously recorded populations were found to have been extirpated. Decline in natural populations of *C. pteridoides* has resulted from the destruction or complete loss of its primary habitat. Analysis of 17 parameters of water quality indicated that differences in pH and dissolved oxygen might be principal factors determining the distribution and occurrence of *C. pteridoides*. The sites of the extirpated populations had higher water pH values than those of the sites of the extant populations ($P < 0.05$). The value of dissolved oxygen concentration at the sites of the extirpated populations was lower than at the sites of the extant populations ($P < 0.05$). The degeneration of primary habitats, a decline in the area of wetland coverage and deterioration of water quality caused by human activities are identified as the likely key factors responsible for the reduction in *C. pteridoides* populations. Because the habitat and population characteristics of eleven remaining populations were different, different sites should adopt different conservation methods as appropriate. Some small populations could be conserved by establishing conservation areas; other relatively large populations could be conserved by establishing nature reserves.

KEY WORDS.—*Ceratopteris pteridoides*, endanger, habitat modification, distribution, conservation

Ceratopteris pteridoides (Hook.) Hieron. (Parkeriaceae), an annual diploid (n = 39), is an aquatic, homosporous, floating fern. The species displays clonal growth by means of numerous marginal leaf buds that rapidly develop into plantlets (Hickok *et al.*, 1987). Both spores and the plantlets from marginal leaf buds are dispersed mainly by water flow. *Ceratopteris pteridoides* mainly grows in ponds, lakes, rivers, and ditches (Lloyd, 1974). The species has principally been identified in Central and South America, Southeastern Asia, Eastern India, and China (Diao, 1990; Hickok *et al.*, 1995). In China, *C.*

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pteridoides is mainly distributed in central, eastern, and southern China (Diao, 1990). Although *C. pteridoides* was widely distributed in China, in recent decades the species has declined rapidly in the numbers and sizes of populations, and has even disappeared from many locations (Yu, 1999; Dong *et al.*, 2007). *Ceratopteris pteridoides* is now considered endangered and is listed in the second category of the National Key Protected Wild Plants in China (Yu, 1999). Several factors, including the degeneration of primary habitats, the decline in area of coverage of wetlands, and the deterioration of water quality due to human activities, have been identified as being responsible for the reduction in *C. pteridoides* populations (see Dong *et al.*, 2007). However, no data have been provided to support these assertions.

Earlier studies on *Ceratopteris pteridoides* have mainly focused on taxonomy and morphology (Hickok *et al.*, 1995; Fan and Dai, 1999; Carquist and Schneider, 2000). In recent years, Dong *et al.* (2007, 2010), using RAPD and ISSR data, revealed low levels of genetic diversity (the percentage of polymorphic bands (PPB): RAPD, 33.6%; ISSR, 25.2%) and high levels of gene flow between the remaining *C. pteridoides* populations in China. Tao *et al.* (2008) reported that bensulfuron-methyl inhibits gametophyte growth and sex organ differentiation of *C. pteridoides* at low concentration and may pose a risk to sexual reproduction of *C. pteridoides* in the field. However, the information available on the conservation biology of *C. pteridoides* in China is limited in comparison with that for the more widely studied closely related species *Ceratopteris thalictroides* (L.) Brongn. This latter species is now also considered to be endangered in China (Yu, 1999).

It is probable that additional studies on the various aspects of the conservation biology of *Ceratopteris pteridoides* will provide information that will justify more stringent conservation practices for this rare plant. The important objective in the present study was to report the current distribution of *C. pteridoides* in China, its habitats, and population characteristics. Another aim was to investigate the natural distribution of *C. pteridoides* in China in relation to habitat characteristics, especially wetland and water chemistry parameters, in order to contribute to the available information on the biology of this endangered species.

Materials and Methods

From August of 2003 to October of 2006, sixteen sites throughout the historic geographic distribution of *Ceratopteris pteridoides* in China were investigated (Fig. 1). The sites were identified on the basis of records on labels of herbarium specimens and/or observations during field surveys. The sixteen sites surveyed are located in Hubei, Jiangxi, Anhui, Zhejiang, Jiangsu, Fujian and Shandong provinces in Mainland China. At each sampling station elevation, latitude, and longitude were measured by Global Positioning System (GPS). The habitat characteristics of *C. pteridoides* were recorded (Table 1). The population characteristics of the species including population numbers and

Table 1. Geographic distribution, habitat and populations characteristics of extant and extinct populations of *Ceratopteris pteridoides* in China.

Location	Population code	Extant/ extinct population	Elevation (m)	Latitude(N)/ Longitude(E)	Habitats	Population area (m^2)	Population size
Xiaogang, Hubei province	HB-1	Extant	24	29°55′/113°13′	Ditch, Fishpond	700–800	6000–10000
Jiayu, Hubei province	HB-2	Extant	19	29°54′/114°01′	Lake	40–50	200–500
Liangzihu, Hubei province	HB-3	Extant	15	30°15′/114°34′	Lake	400–500	10000–15000
Yangxin, Hubei province	HB-4	Extant	14	29°55′/113°59′	Fishpond	1800–2000	50000–60000
Jiayu, Hubei province	HB-5	Extant	19	29°55′/113°59′	Pond	30–50	500–1000
Ruichang, Jiangxi province	JX-1	Extant	21	29°48′/115°44′	Lake	200–300	20–50
Huzhou, Zhejiang province	ZJ-1	Extant	-	30°51′/120°04′	Wetland, pond	40–50	500–1000
Wuyishan, Fujian province	FJ-1	Extant	-	27°46′/118°01′	Ditch	3–5	3–5
Nansihu, Shangdong province	SD-1	Extinct	10	34°40′/117°15′	Lake		
Taibaihu, Hubei province	HB-6	Extinct	14	29°58′/115°37′	Lake		
Haikouhu, Hubei province	HB-7	Extinct	18	30°02′/115°17′	Lake		
Honghu, Hubei province	HB-8	Extinct	24	29°55′/113°13′	Lake		
Changhu, Hubei province	HB-9	Extinct	19	30°44′/112°12′	Lake		
Mulanhu, Hubei province	HB-10	Extinct	-	31°06′/112°12′	Reservoir		
Futouhu, Hubei province	HB-11	Extinct	20	30°01′/114°17′	Lake		
Dongliu, Anhui province	AH-1	Extinct	16	30°12′/116°54′	Lake lost		

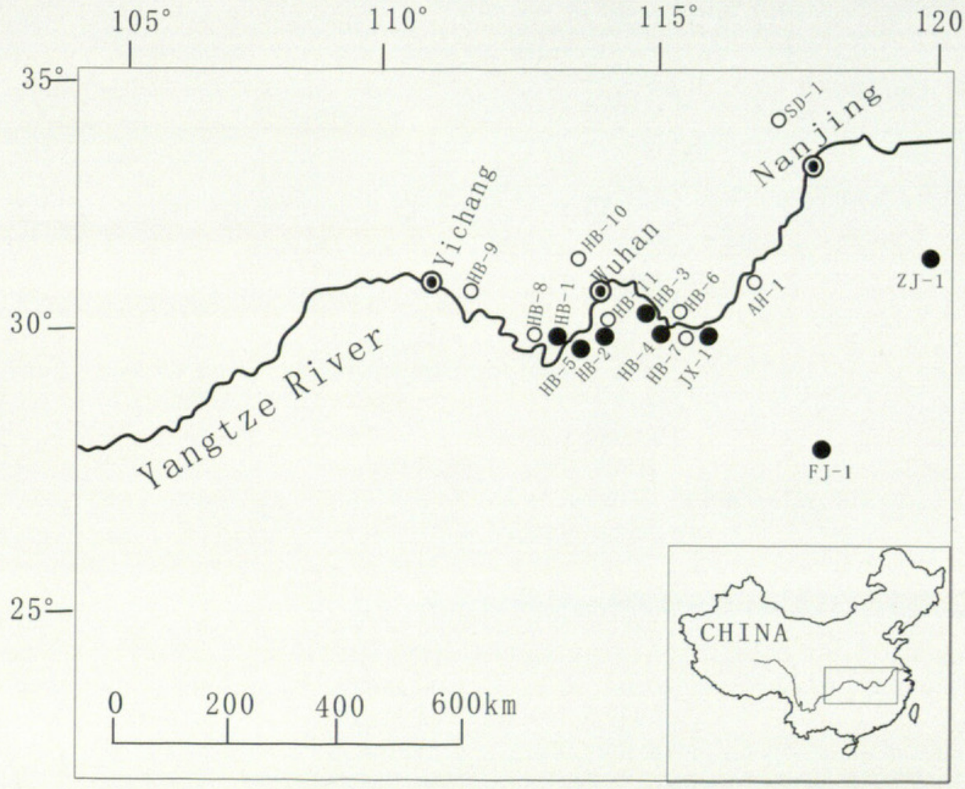

FIG. 1. Distribution map and water sampling sites of *Ceratopteris pteridoides* in China. ● Sites of extant populations. ○ Sites extirpated populations. Eleven water sampling sites including HB-1, HB-2, HB-3, HB-4, HB-6, HB-7, HB-9, HB-10, HB-11, JX-1, and SD-1. Codes and names of populations see Table 1.

population area, population sizes (numbers of individuals), and companion species were investigated (Table 1).

Eleven sites of the sixteen sites wåere investigated in 2003. Thus, in order to decrease statistical error, the water chemistry parameters of only the 11 sites surveyed from August to September of 2003 were analyzed (Fig 1). Five sites which still have populations of *Ceratopteris pteridoides* were designated as type A sites while six sites from which the species has been extirpated were designated as type B sites (Table 2). Seventeen water parameters were measured at seventeen sites (Table 2). The chemical parameters, including NO_3-N, NH_3-N, PO_4^{3-}, total Cl, Ca, Mg, Fe, Cu, Zn, Mo, and Cr^{6+}, were measured from the sample with a multi-parameter ion-specific photometer (C200, Hanna Co., Italy) in the laboratory using a 500 ml water sample collected in a plastic bottle in eleven sites respectively.

pH was measured with a portable meter (HI 98107, Hanna Co., Italy), as was conductivity (HI 983004, Hanna Co., Italy). Dissolved oxygen, dissolved carbon dioxide, alkalinity, and hardness were measured in the field with a portable test kit (HI 3814, Hanna Co., Italy). The water temperature of the water sample sites were about 25–30°C from August to September.

The mean values of the 17 chemistry parameters of water at the type A and type B sites were compared statistically using a one-way ANOVA when the

TABLE 2. Significance tests of water chemical parameters at the sample sites in 2003. Significance level for all comparisons: *$P < 0.05$; Type A: Sites of extant populations; Type B: Sites extirpated populations.

			Statistical Tests	
Parameters	Type A (X ± sd n=5)	Type B (X ± sd n = 6)	One-way ANOVA (F-test)	Rank sum test (U-value)
Cl (mg/L)	0.23 ± 0.03	0.24 ± 0.07	0.02	–
Cu (mg/L)	0.27 ± 0.11	0.45 ± 0.27	1.93	–
Ca (mg/L)	0.17 ± 0.22	0.20 ± 0.20	0.07	–
Mg (mg/L)	0.38 ± 0.16	0.40 ± 0.17	0.03	–
Zn (mg/L)	0.11 ± 0.11	0.20 ± 0.33	0.28	–
Mo (mg/L)	1.28 ± 1.32	1.31 ± 0.95	0.002	–
PO_4^{3-} (mg/L)	0.26 ± 0.25	0.56 ± 0.34	2.68	–
Dissolved carbon dioxide (mg/L)	22.60 ± 9.71	24.83 ± 13.67	0.09	–
pH	7.54 ± 0.22	7.97 ± 0.27	7.90*	–
Dissolved oxygen (mg/L)	7.34 ± 1.11	5.05 ± 1.15	11.57*	–
Alkalinity ($mgCaCO_3$/L)	97.80 ± 28.11	109.50 ± 50.33	0.21	–
Hardness ($mgCaCO_3$/L)	93.00 ± 26.07	136.00 ± 79.41	–	25.00
NH_3-N (mg/L)	0.12 ± 0.04	0.19 ± 0.14	–	24.50
Fe (mg/L)	0.28 ± 0.45	0.06 ± 0.08	–	32.34
Cr^{6+} (ug/L)	2.80 ± 6.26	9.83 ± 23.12	–	28.00
NO_3-N (mg/L)	0.60 ± 0.82	0.19 ± 0.24	–	32.00
Conductivity (ms/cm)	0.22 ± 0.08	0.36 ± 0.27	–	26.00

variance was homoscedastic and using a rank sum test when the variance was heteroscedastic. The homogeneity of variance of all factors was assessed using the Bartlett test (Li, 2002). Any factors that showed significant difference between the type A and type B sites were evaluated further by analysis of multiple comparison using Least Significant Difference. Level of significance was set at $P < 0.05$.

RESULTS

A total of eight extant populations of *Ceratopteris pteridoides* were found in the 16 sites surveyed from August of 2003 to October of 2006 across the natural geographic distribution range of *C. pteridoides* in Mainland China (Fig. 1, Table 1). These extant populations of *C. pteridoides* were found growing in ponds, lakes, rivers, and ditches. These extant populations are located mainly in the middle and lower reaches of the Yangtze River, which is also the site of thousands of shallow lakes most of which are interconnected to the main artery of the Yangtze River. With the exception of five populations (Jx-1, FJ-1, HB-2, ZJ-1 and HB-5) that had fewer than 1000 individuals, the rest of the populations (HB-1, HB-3, and HB-4) had more than 1000 *C. pteridoides* individuals per population. Most of the individuals were floating. *Ceratopteris pteridoides* mainly grows together with *Nelumbo nucifera* Gaertn., *Hydrocharis dubia* (Blume.) Backer., *Phragmites communis* Trin., *Alternanthera philoxeroides* (Mart.) Griseb., *Phalaris arundinacea* Linn., *Trapa bispinosa*

Roxb., and *Potamogeton distinctus* A. Benn. *Ceratopteris pteridoides* was the dominant species in three of the extant populations including (HB-1, HB-4, HB-5). The habitats at the sites of the extinct populations of *C. pteridoides* have been greatly modified. At some stations, including at the Nansi, Taibai, Haikou, Honghu, and Changhu Lakes, water pollution was clearly evident, while some lakes and wetlands including Dongliu Lake (AH-1 population) in Anhui province have vanished (Table 1). Area of coverage of wetland in site of ZJ-1 population was reduced due to uncontrolled real estate development.

Analysis of the 17 parameters of water quality indicated that type B sites had significantly higher mean pH ($P < 0.05$) and lower dissolved oxygen ($P < 0.05$) than type A sites (Table 2). None of the remaining chemical variances differed significantly between type A sites and type B sites.

Discussion

Various kinds of human activities can bring about changes of wetlands and aquatic habitats (Carrier, 1991). Loss of habitat is the single most important cause of extinction of species (Primack, 1993). The accelerated loss of habitat of *Ceratopteris pteridoides* in China, together with a decline in the wetland surface area might have put the species at the risk of becoming extirpated in this expansive region and resulted in the extinction of *C. pteridoides* population at some sites. In recent decades, *C. pteridoides* in Mainland China have declined rapidly in number of individuals and populations, and plants have disappeared from many locations. For instance, loss of Dongliu Lake, which was part of the Yangtze River system in Anhui province undoubtedly led to the disappearance of this species from the site. Excessive aquaculture and water pollution have been identified as the most likely cause for the extinction of *C. pteridoides* at Haikou, Taibai, and Honghu Lakes (Jian *et al.*, 2001; Lu and Jiang, 2003).

Five of the eight extant populations of *Ceratopteris pteridoides* in Hubei province of central China are located in an area which was occupied by a large wetland known as the Yunmeng marshland in ancient times. In 239 B.C. the Yunmeng marshland was reputed to have a surface area spanning more than six million ha and plants of the genus *Ceratopteris* were recorded there (Liu, 1984; Shi *et al.*, 1989; Diao, 1990). Over a period in excess of two thousands years, the surface coverage of the wetland has continued to decline at different rates in different historical periods, mainly due to overexploitation, irrigation and tourism activities. As a result, the number of lakes in Hubei Province, which is known in China as "The province of a thousand lakes", decreased from 305 in the 1950s to 217 currently. Compared with the 1950s, the total area of lakes in Hubei Province was reduced by 66% to 2438.6 km^2 at present (Wang *et al.*, 2009). In addition, due to overexploitation and uncontrolled real estate development (Wan *et al.*, 2004), the number of urban lakes in Wuhan of Hubei Province was reduced from 89 in 1949 to 38, within which *C. pteridoides* only exists in one lake (HB-3 population) in this study (Table 1). Our own field investigations also have indicated that population ZJ-1 is in imminent danger of extirpation due to rapid expansion of Huzhou city. The current body of evidence gathered in several studies, including our present study, shows that the progressive reduction in the numbers and sizes of *C. pteridoides* populations is likely attributable to sedimentation from the upper reaches of the Yangtze River (Shi *et al.*, 1989) and to human activities including farming, excessive aquaculture, overexploitation of water bodies, building of irrigations works, reclamation of land from lakes, tourism activities, uncontrolled real estate development and run-off water pollution (Shi *et al.*, 1989; Jian *et al.*, 2001; Lu and Jiang, 2003; Dong *et al.*, 2007).

Potamic and lacustrine water chemical properties are among the principal factors determining the kind, number, and distribution of aquatic plants (Shi *et al.*, 1989; Yang and Ye, 2001; Liu *et al.*, 2003). Hydrobiological communities are greatly threatened by water pollution (Moyle and leidy, 1992). Analysis of 17 parameters of water quality indicated that type B sites had significantly higher mean pH ($P < 0.05$) than type A sites (Table 2) in 2003. The mean pH of type A was 7.16 ± 0.49 in August of 2011, and lower than that of type B sites, which was consistent with the results in 2003. At the same stations, some sites of extant populations had higher pH values than the recorded primary habitat indicated on the labels of herbarium specimens. For example, the mean water pH value at Haikou Lake site has risen from 6.3 recorded in 2001 to 7.9 (Jian *et al.*, 2001). While the pH value at both type A and Type B sites indicated a progressive rise in the recent past, the proportional increase is markedly higher for the type B sites. Significant variability of pH at different sites might be largely due to different levels of pollution at the sites. Excessive aquaculture and land usage (Ivahanenko *et al.*, 1988; Downey *et al.*, 1994) could have a profound influence on pH variation in wetlands.

Significant changes in water pH may interfere with physiological activities of aquatic plants (Tang *et al.*, 2002). Water pH affects the bioavailability of Ca, Fe, Mn and Zn to rooted aquatic macrophytes (Jackson *et al.*, 1993). Furthermore, variation of pH may upset the subtle ionic balance in the environment; For example, pH variability affects the ionic balance between ammonium and ammonia in water (Körner *et al.*, 2001). pH variation has been shown to be the principal factor affecting the distribution of *Ceratopteris thalictroides*, a closely related species which is also endangered in China (Dong *et al.*, 2005), as well as the endangered aquatic fern *Isoëtes sinensis* Palmer (Wen *et al.*, 2003). Considering that pH may influence the absorption of ions and metabolic activities of aquatic plants including *C. pteridoides*, it could be an important factor leading to extirpation of this endangered species from its habitats. This finding suggests that change in pH may be associated with the disappearance of *C. pteridoides* populations from the type B sites.

The different tests of water chemical parameters of these sample sites indicated that type B sites had significantly lower dissolved oxygen ($P < 0.05$) than type A sites (Table 2). Several studies, including Yang *et al.* (2001) and Shi *et al.* (1989), have demonstrated that dissolved oxygen is a principal factor that affects the distribution of aquatic plants. The absorption of minerals by plant roots is closely related to respiration in plants. Variation in oxygen availability influences absorbability of minerals by the roots. Generally speaking, the higher the oxygen concentration, the more efficient the absorption of minerals by roots. *Ceratopteris pteridoides* is primarily a floating plant in lakes and ponds and absorbs minerals chiefly through its roots. It is probable that the lower concentrations of dissolved oxygen at these sites of extinct populations affects the intake of oxygen and consequently respiration in *C. pteridoides* plants, thus interfering with the physiological activities of the plants. This could be detrimental to the health of the populations and may have contributed to the extirpation of the species from these sites. Therefore, the significant differences in pH and dissolved oxygen between type A and type B sites indicated that distribution and occurrence of *C. pteridoides* are closely correlated with water chemical characteristics.

Biodiversity has declined in freshwater lakes in China in recent times (Jian *et al.*, 2001). The factors leading to biodiversity reduction mainly include water pollution and excessive aquaculture, with the latter having been identified as the single most important factor (Jian *et al.*, 2001). At the sites of the eight extinct populations at Taibai, Haikou, Honghu and Changhu Lakes, observations and interviews with the locals revealed a long history of intensive aquaculture. It is likely that this is a major reason for the decline of *Ceratopteris pteridoides* at these sites and elsewhere in China. Field surveys also showed that fishing activities by local fishermen destroyed *C. pteridoides* in the Yangxin (HB-4) and Jiayu (HB-5) populations in Hubei province, which compromised the self- maintenance and self- renewal abilities of the populations, leading to gradual decline of the populations.

Five populations among the eight extant populations of *Ceratopteris pteridoides* were relatively small with fewer than 1000 individuations (Table 1). Most of the eight extant *C. pteridoides* populations grew in ponds, ditches, shallow lakes, and kaleyards, while others (ZJ-1, HB-3, and HB-5) were located among city regions that have undergone rapid expansion. Such populations can be highly susceptible to the effects of environmental changes and disasters, which heighten the possibility of species extirpation.

We have shown that the distribution and occurrence of *Ceratopteris pteridoides* are correlated with water chemistry, with pH value and dissolved oxygen being the most important factors. Evidence from the present study supports the idea that the observed decline in *C. pteridoides* populations is associated with the destruction and the loss of their primary habitats, especially the reduction in wetland areas and the increase in water pollution. Human activities such as farming, tourism, real estate development, fisheries, and run- off water pollution are the most important reasons. It is of critical importance that measures are taken to establish appropriate conservation strategies to stem and even reverse the decline in populations of *C. pteridoides* observed in mainland China.

Both *in situ* and *ex situ* conservation approaches are important conservation strategies for rare and endangered species. However, the most appropriate conservation strategy is to protect the habitats of the species (Primack 1993). Because habitat and population characteristics of the remaining populations are different, a uniform approach may not apply for all sites, and different sites should adopt different conservation methods as appropriate. Some small populations such as Jx-1, FJ-1, HB-2, ZJ-1, and HB-5 could be better conserved by establishing small, protected areas. Other relatively large populations, such as HB-1, HB-3, HB-4 should be conserved by establishing nature reserves. At the same time, there should be a government policy implementing complete cessation of farming activities in selected areas to allow forest to regenerate.

In recent years, in order to protect natural species and renew lake habitat, some government policies have been implemented at Xilianghu Lake (HB-2), Liangzihu Lake (HB-3) in Hubei province, including a complete cessation of farming activities, purse seine cultures, and fill-up of urban lakes. The HB-3 population, located at Wuhan, Hubei province, could be better conserved by actualizing local projects such as the expansion of lake areas and the connection of the surrounding lakes. These policies and measures could be a good approach towards improving the lacustrine ecosystem and protecting threatened aquatic life forms including *Ceratopteris pteridoides*. At the same time, in order to better protect threatened aquatic species, we advise establishing a list of lakes for conservation in China, and taking effective and long-term population and community characteristic measurements of these lakes, as well as monitoring the changes in water chemical parameters.

Botanical gardens have played an increasingly important role in the *ex situ* conservation of rare and endangered plants (Maunder, 1994). Wuhan Botanical Garden (WBG), is ranked as one of three core research botanical gardens in China. It houses the largest aquatic plant garden in East Asia, and has theme gardens such as Central China relicts, and a rare and endangered plant garden. WBG currently plays an important role in the conservation of aquatic species. The botanical garden has shown preliminary success in the conservation of *Ceratopteris* including *C. pteridoides* and *C. thalictroides*. *Ceratopteris pteridoides* was mainly protected at a pond in a zone of aquatic plants. The species in WBG mainly grows together with *Nelumbo nucifera* Gaertn., *Hydrocleys nymphoides* (Willd.) Buchenau., *Potamogeton lucens* Linn., *Triarrherca sacchariflora* (Maxin.) Nakai., *Pistil stratiotes* L., and *Vallisneria natans* (Lour.) Hara.

A key aim of conservation, in addition to habitat preservation, is to maintain a species' existing level of genetic variation in order to maximize its chances for persistence in the face of changing environments (Keiper and McConchie, 2003). Dong *et al.* (2007, 2010) used ISSR and RAPD data that revealed low levels of genetic diversity at the species level and low levels of genetic variation among populations of *Ceratopteris pteridoides* in China. The studies have also demonstrated a high level of interpopulation gene flow in the extant populations of *C. pteridoides* in China. In light of the genetic information for *C. pteridoides*, we recommend establishing as many *in situ* conservation spots as possible and cross-transplanting plants between populations in order to increase gene flow and preserve to the greatest extent possible the genetic resources of the species.

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