Ecophysiological Traits of Leaves of Three *Marsilea* Species Distributed in Different Geographical Regions

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ABSTRACT: *Marsilea*, an amphibian fern genus (containing ca. 80 species) characterized by their unusual leaves and reproductive structures, is distributed over the five continents. To investigate the adaptation traits of three *Marsilea* species (*M. crenata*, *M. quadrifolia*, and *M. schelpiana*), distributed in different geographic regions, to terrestrial conditions, we compared morphological features, optical properties and photosynthetic performance of leaflets of the three species grown in terrestrial environment. The results showed that leaflets of the three species had significant differences in some of the ecophysiogical traits. Among the three species, *M. quadrifolia* (distributed in temperate region where receiving low precipitation) had the highest trichome density on its leaflet surface and the highest water use efficiency, *M. schelpiana* (mainly in southern Africa where accepting high level of solar irradiance) had the tallest petiole and the highest leaf dissection index, total stomatal pore area index, PSII electron transport rate and photosaturated photosynthetic rate, *M. crenata* (mainly in southeastern Asia region where receiving high precipitation and with high humidity) had the lowest leaf dissection index and water use efficiency. Accordingly, leaf characteristics of the three *Marsilea* species reflect the climate pattern of their habitats. The results also suggest that water availability and light intensity are two of the important factors contributing to the geographic distribution of the three species.

KEY WORDS: *Marsilea*, gas exchange, chlorophyll fluorescence, water use efficiency, leaf morphology, trichome density, stomatal density.

INTRODUCTION

Marsileaceae is a family of rooted amphibious ferns. In addition to have ability to live in both aquatic and terrestrial environments, the family shares many features in common with aquatic angiosperms, such as simplified vascular systems, polymorphic vegetative characters, and highly specialized reproductive systems (Schneider and Pryer, 2002). Due to these important features, the family has long been of interests to researchers studying plant morphology, physiology and evolution. There are three genera in this family, i.e. *Marsilea*, *Regnellidium* and *Pilularia*. *Regnellidium* is a monotypic genus known only from three localities in southern Brazil and adjacent Argentina (Pryer, 1999), *Pilularia* is a genus of six species of wide but disjunctive distribution (Tryon and Tryon, 1982), in contrast, *Marsilea* is a cosmopolitan genus of 50-80 species (Launert, 1968; Lesho, 1994; Pryer, 1999; Nagalingum et al., 2007). The widely distribution pattern and species-rich characteristics make the genus *Marsilea* an important subject for understanding mechanisms of adaptation and evolutionary diversification of the Marsileaceae.

Marsilea is found primarily in seasonally wet habitats, where it grows in shallow water and at the edges of ponds, lakes, or rivers (Johnson, 1986). It is reported that *Marsilea* produces different types of leaves in response to changes in natural environment and culture conditions (Allsopp, 1955; Lin and Yang, 1999; Lin et al., 2007). It is also known that climate plays an important factor affecting the distribution of plant species (Hogan et al., 1994; Lambers et al., 1998; Lambers et al., 2008). Plants distributed in regions with different climate might have evolved different morphological and/or physiological traits. Accordingly, comparing traits of plants distributed in different climate zones might provide clues on the environmental factors affecting the distribution pattern of plant distribution.

As the primary site for photosynthesis, leaves are of fundamental and functional important to green plants. Differences in morphological, anatomical and physiological characteristics of leaves along environmental gradients can be found across species (Givnish, 1987). In this study, we compared ecophysiological traits of leaves of three *Marsilea* species distributed in different geographic regions with different climate pattern. *M. quadrifolia* is mainly distributed in Europe and east America, *M. schelpiana* in southern Africa and *M. crenata* in southeastern Asia (Nagalingum et al., 2007). The three species all have

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ability to develop aerial (emergent) and water leaves in aquatic habitats and terrestrial leaves in terrestrial environment (Wu and Kao personal obs.). The ability to develop heterophyll is proposed as one of the adaptive traits enabling the group of amphibious ferns to survive in contrasting habitats. However, in comparison to plants in aquatic environment, those in terrestrial environment generally experience more variation in environmental factors. Thus it is expected that the selection pressures would exert more on terrestrial leaves than on aquatic leaves of the amphibious plants. The fact that *Marsilea* is distributed worldwide suggests its possible evolution of various adaptation traits for living in different habitats. Accordingly, we compared leaves of terrestrial-grown plants of the three species, identify leaf traits related to their habitats, and tested the hypothesis that leaf characteristics of the species reflect their habitat climates.

MATERIALS AND METHODS

Plant materials

Rhizomes of *M. crenata* were transplanted from a wetland in Shenkeng District (25º0' N, 121º37' E), Taipei, Taiwan. *M. quadrifolia* was cultivated from the submerged sporophyte in aseptic culture, which is obtained from Dr. Bai-Ling Lin (Genomic Research centre, Academic Sinica, Taiwan). Plants of *M. schelpiana* were purchased from a local market and identified according to Edmund Launert's monographic survey (Launert, 1968). Rhizomes of three *Marsilea* species with 2-4 nodes were planted in plastic plots (diameter of 7 inch) filled with a mixture of perlite: vermiculite: peat of 1: 1: 2 by volume. Plants were grown under natural light in a greenhouse, from April to May of 2007, and watered to soil saturation every other day. The microclimates in the greenhouse during the culture period were: mean photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1} (max. 487, min 233 μmol m^{-2} s^{-1}), air temperature 20-23ºC, and ambient humidity 40-50 %. Inorganic fertilizer (N: P: K of 20:20:20, Hyponex 2, Hyponex, Japan) was added once every two weeks. These plants produce leaves with four leaflets expanded perpendicular to the petiole. We conducted following measurements on the new fully expanded leaflets.

Leaf morphological traits

Parameters including leaf dissection index, specific leaf area (SLA, leaf area/ dry mass), petiole height and diameter, trichome and stomatal density on upper and lower leaf surfaces, stomatal pore length, and guard-cell size were measured as following. We took black-and-white images of excised leaves using a page scanner (the resolution of 600 dpi). Images were then analyzed by the image processing program (Photoshop CS3 Extended, Adobe) and the leaf perimeter and area were estimated. We then calculated the leaf dissection index using following equation (Nicotra et al., 2008):

$$\text{Leaf dissection index} = \text{leaf perimeter} / \sqrt{\text{leaf area}}.$$

Following the measurement of the leaf perimeter and area, we dried the leaf in an oven temperature controlled at 60°C for 48 hours then measured its dry weight. Specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry weight.

The morphology of trichomes on leaf surface was observed under a scanning electron microscope (TM 1000, Hitachi). Before making the SEM scanning, we used liquid nitrogen to fix the samples. Some of trichomes were detached from the surface by the treatment. Hence, we estimated trichome density on both surfaces with a dissecting microscope instead of calculating the number of trichomes by SEM scanning. The total trichome density was calculated as the sum of the trichome density on upper and lower surfaces. To measure stomatal density, pore length, and guard-cell size, we peeled epidermal strips from the leaflets and scanned the strips at 600× magnification with a light microscope equipped with a calibrated ocular micrometer. The total stomatal density was calculated as the sum of the stomatal density on upper and lower surfaces. After the epidermal strip was peeled from a leaflet, due to the closure of stomata it was hard to have an accurate measurement of stomatal pore area. Instead of measuring the pore area, we calculated total stomatal pore area index (SPI = total stomatal density × stomatal pore length^2) which has been demonstrated to be a determinant of maximum stomatal conductance (Sack et al., 2003).

Leaf chlorophyll content and optical properties

Leaf chlorophyll content was determined by a portable chlorophyll meter (SPAD-502, Minota). The chlorophyll content was estimated using regression equations previous determined (Lin and Kao, unpublished):

chlorophyll content of *M. crenata* = 1.067 × SPAD value -10.22;

chlorophyll content of *M. quadrifolia* = 0.783 × SPAD value - 4.31;

chlorophyll content of *M. schelpiana* = 1.072 × SPAD value - 14.51.

Leaf optical properties were measured on adaxial surfaces of leaflets using a custom-built dual integrating sphere system(Wu and Kao, 2009). Leaf spectral transmissivity (T(λ)) and reflectivity (R(λ)) were measured from 400 nm to 700 nm in 0.5 nm resolution using a fiber-optic spectrometer (HR2000, Ocean

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Optics) integrated with an integrated sphere, FOIS-1 (for T(λ) measurement; Ocean Optics) or ISP-REF (for R(λ) measurement; Ocean Optics) (Runcie and Durako, 2004). For T(λ) measurement, after a collimated beam of light from a tungsten-halogen light (LS-1, Ocean Optics) passed through a leaflet, it was directed into the entrance port and to an exit port of the integrated sphere, connected to the spectrometer by an optic fiber. For measuring the R(λ), the beam of light was irradiated into the entrance port of the integrated sphere and directed to the opposites side with a leaflet attached. The light reflected by a leaflet sample was collected to the exit port of the integrated sphere, which is connected to the spectrometer, too. Spectral reflectance was calibrated by a 99% reflectance standard (WS-1-SS, Ocean optics). After measurements of T(λ) and R(λ), we then calculated leaf absorptivityA(λ) = 1-T(λ)-R(λ).

Gas exchange and chlorophyll fluorescence measurement

Photosaturated photosynthetic rate (A_{max}), stomatal conductance (g_s), and transpiration rates (E) were measured with an infrared gas exchange system (Li-6400, Li-Cor, Lincoln, Nebraska, USA). Measurement condition within the cuvette were: photosynthetically active radiation (PAR) of 800 μmol $m^{-2}s^{-1}$, cuvette temperature 25°C, leaf-to-air water vapor pressure difference (VPD) 1.1–1.3 kPa, and ambient CO_2 concentration 370 ppm. Photosynthetic water use efficiency (WUE) was calculated as A_{max}/E (Upadhyaya and Cooke, 1988).

To compare the response of the efficiency of excitation capture by open photosystem II (the effective quantum yield of PSII or Y) and the proportion of open PSII (photochemical quenching coefficient of PSII or qP) to different PAR of the three species, we conducted fluorescence induction on leaflets using a portable, pulse amplitude modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany). After plants being transferred into the laboratory, their leaflets were dark adapted for 30 minutes before the fluorescence measurement was taken. The PAR were controlled at 880, 570, 370, 250, 160, 70, 50, 30 μmol m^{-2} s^{-1}, respectively. Leaflets were illuminated with the desired PAR for about 10 minutes before the fluorescence parameters, effective quantum yield (Y = (Fm'-Ft) / Fm', where Fm' is maximum fluorescence and Ft steady-state fluorescence of the illuminated leaf) and photochemical quenching coefficient (qP = (Fm'- Ft) / (Fm'- Fo'), where Fo' is the minimal fluorescence yield of the illuminated leaf), were taken. The electron transport rate of PSII (ETR, μmol $m^{-2}s^{-1}$) was calculated as ETR = PAR × Y × 0.5 × A(λ) (values from optical analysis) (Genty et al., 1989).

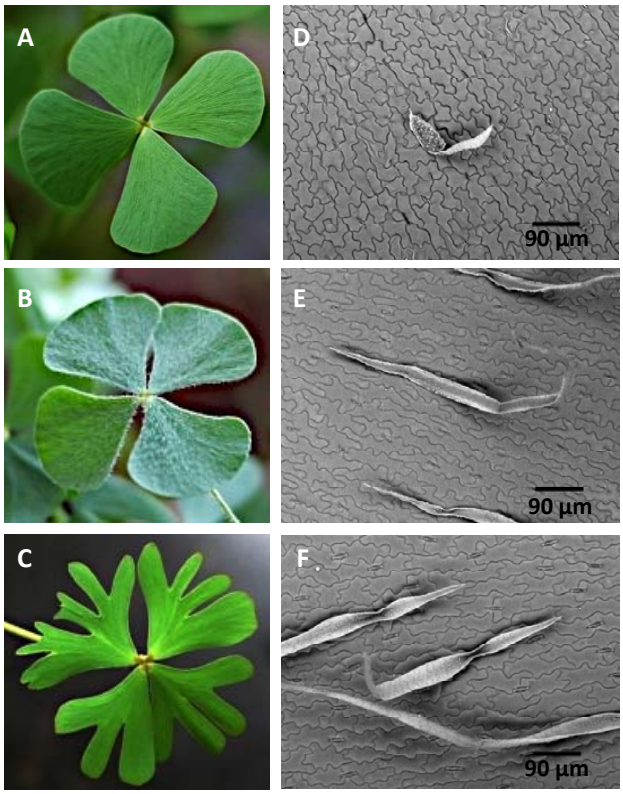


Fig. 1. The morphology of leaves (A, B, C) and SEM pictures of trichomes on leaf lower surfaces of *M. crenata* (A, D), *M. quadrifolia* (B, E), and *M. schelpiana* (C, F).

Statistics

All statistic tests were performed with the computer software Origin (OriginLab, Massachusetts, USA). Data were first analyzed with one-way ANOVA model and then the mean was tested with Tukey honestly significant difference (HSD) comparison. Significant differences are reported as $p < 0.05$.

RESULTS

Leaf morphological traits

Leaflets of the three species looked different, those of *M. crenata* had entire edge (Fig. 1A), and those of *M. schelpiana* formed lobes (Fig. 1C). We quantified this character by calculating the leaf dissection index. The results showed that they did have significant difference in this parameter (Table 1). Among the three species, *M. schelpiana* had the highest dissection index, *M. quadrifolia* the intermediate, and *M. crenata* the least. *M. schelpiana* also had significantly longer petiole with wider diameter than the other two species. In contrast, the three species had similar specific leaf area.

All three species produced multicellular trichomes, with protuberance (Figs. 1D, E, F), on upper and lower

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Table 1. Morphological traits, including leaf dissection index, specific leaf area (SLA), petiole length and diameter, trichome density on upper (TUS) and lower surface (TLS) and total trichome density, stomatal density on upper (SUS) and lower surface (SLS), total stomatal density, guard-cell length, pore length and total stomatal pore area index of leaflets of the three *Marsilea* species (mean ± S.E., n = 6). Value within the same row followed by different superscripts represent significant difference at $p = 0.05$.

	M. crenata	*M. quadrifolia*	*M. schelpiana*
Leaf dissection index	7.28±0.22 ^{c}	8.07±0.08 ^{b}	11.06±0.20 ^{a}
SLA (cm^{-2} g^{-1})	544.70±57.25	503.08±34.33	417.19±49.31
Petiole length (cm)	15.35±1.65 ^{b}	16.24±1.63 ^{b}	26.73±1.94 ^{a}
Petiole diameter (mm)	1.24±0.04 ^{b}	0.62±0.01 ^{c}	1.78±0.04 ^{a}
TUS (cm^{-2})	9.6±2.4 ^{b}	1509.4±172.5 ^{a}	7.0±2.6 ^{b}
TLS (cm^{-2})	324.0±39.5 ^{b}	1517.1±185.6 ^{a}	716.5±63.6 ^{b}
Total trichome density (cm^{-2})	333.6±62.5 ^{b}	3026.4±369.3 ^{a}	723.6±65.6 ^{b}
SUS (mm^{-2})	206.8±11.6 ^{b}	273.9±14.3 ^{a}	182.0±14.8 ^{b}
SLS (mm^{-2})	153.8±10.7 ^{ab}	189.1±9.6 ^{a}	136.7±14.3 ^{b}
Total stomatal density (mm^{-2})	360.6±21.1 ^{b}	463.1±22.2 ^{a}	318.7±28.6 ^{b}
Guard-cell size (μm)	25.85±0.97 ^{b}	24.55±0.58 ^{c}	36.80±0.64 ^{a}
Pore length (μm)	10.57±0.35 ^{b}	11.21±0.45 ^{b}	17.15±0.59 ^{a}
Total stomatal pore area index	0.040±0.002 ^{b}	0.058±0.001 ^{b}	0.096±0.008 ^{a}

leaflet surface (Table 1). However, the size and density of trichomes varied significantly among the three species. In general, trichomes of *M. quadrifolia* (Fig. 1E) and *M. schelpiana* (Fig. 1F) were longer than those of *M. crenata* (Fig. 1D). In *M. crenata* and *M. schelpiana*, almost all the trichomes were distributed on the lower surface of leaflets, while in *M. quadrifolia* trichomes were located evenly on both surfaces. In comparison among the three species, *M. crenata* had the shortest and the least density of trichomes, *M. quadrifolia* had significantly more trichomes than the other two species (Table 1).

All the three species had stomata distributed on both surfaces of their leaflets. In general, more stomata were found in upper surface than in lower surface (the ratio of upper/lower stomatal density ratio was 1.33-1.44), though the difference was not significant. Compared to the other two species, *M. schelpiana* had less total stomatal density, but significantly larger size of guard-cell, pore length, and total stomatal pore area index (Table 1).

Leaf chlorophyll content and optical properties

Leaflets of the three species had significant difference in chlorophyll content (Table 2). Among the three species, *M. schelpiana* had the highest, *M. crenata* the intermediate, and *M .quadrifolia* the least chlorophyll content. In consistent with the result of chlorophyll content, the optical analysis revealed that among the three species, leaflets of *M. schelpiana* had the lowest transmissivity and the highest absorptivity, while *M. quadrifolia* had the lowest absorptivity. There was significantly more light reflected by leaflets of *M. quadrifolia* compared to the other two species (Table 2).

Table 2. The chlorophyll content (μg cm^{-2}) and optical properties of leaflets of three *Marsilea* species (mean ± S.E., n = 6). Value within the same row followed by different superscripts represent significant difference at $p = 0.05$.

	M. crenata	*M. quadrifolia*	*M. schelpiana*
Chlorophyll content	0.252±0.010 ^{b}	0.189±0.004 ^{c}	0.366±0.011 ^{a}
Transmissivity	0.085±0.006 ^{a}	0.065±0.009 ^{a}	0.036±0.003 ^{b}
Reflectivity	0.027±0.004 ^{c}	0.055±0.003 ^{a}	0.038±0.005 ^{b}
Absorptivity	0.898±0.008 ^{a}	0.880±0.010 ^{b}	0.926±0.008 ^{a}

Table 3. The photosaturated photosynthetic rate (A_{max}, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), and water use efficiency (WUE, μmol CO_2 $mmol^{-1}H_2O$) of three *Marsilea* species (mean ± S.E., n = 6). Value within the same row followed by different superscripts represent significant difference at $p = 0.05$.

	M. crenata	*M. quadrifolia*	*M. schelpiana*
A_{max}	11.98±1.13 ^{b}	12.29±1.31 ^{b}	18.34±2.01 ^{a}
g_s	0.135±0.007 ^{b}	0.139±0.004 ^{b}	0.280±0.009 ^{a}
E	2.30±0.32 ^{a}	1.47±0.14 ^{b}	2.61±0.21 ^{a}
WUE	5.20±0.25 ^{b}	8.35±0.46 ^{a}	7.02±0.35 ^{a}

Gas exchange and chlorophyll fluorescence measurement

Table 3 showed similarity and difference in photosynthetic gas exchange parameters of the three species. Leaflets of *M. schelpiana* had the highest photosaturated photosynthetic rate (A_{max}) and stomatal conductance (g_s) among the three species. Though *M. crenata* and *M. quadrifolia* had similar A_{max} and g_s, the former had significantly higher transpiration rate (E) than the later. As a result, leaflets of *M. quadrifolia* had

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significantly higher WUE (=A_{max} / E) than those of *M. crenata*. *M. schelpiana* also had significantly higher E than *M. quadrifolia*, however, the former species also had higher A_{max} than the later. Consequently, both species showed similar WUE (Table 3).

Results from the measurement of chlorophyll fluorescence illustrated that the effective quantum yield (Fig. 2A) and photochemical quenching coefficient of PSII (qP, Fig. 2B) decreased with increasing PAR, while the reduction rate was much higher in *M. crenata* than in the other two species. Compared at the same PAR, no significant difference was found in the effective quantum yield and qP between leaves of *M. quadrifolia* and *M. schelpiana* (Fig. 2C). In contrast to yield and qP, ETR increased with increasing PAR and reached a saturation point. For *M. quadrifolia* and *M. schelpiana*, their ETR saturated at PAR of 600 μmol m^{-2} s^{-1}, while for *M. crenata* that at PAR of 400 μmol m^{-2} s^{-1}. At PAR lower than 160 μmol m^{-2} s^{-1}, there was no significant difference in ETR among the leaves of the three species. While when PAR was higher than 160 μmol m^{-2} s^{-1}, *M. schelpiana* leaves showed significantly higher ETR than the other two species (Fig. 2C). In addition an apparent reduction in ETR was found when leaflets of *M. crenata* were exposed to irradiance higher than 400 μmol m^{-2} s^{-1}. Consequently, under an illumination of PAR higher than 400 μmol m^{-2} s^{-1}, *M. crenata* leaflets had significantly lower ETR than the other two species compared at the same PAR (Fig. 2C).

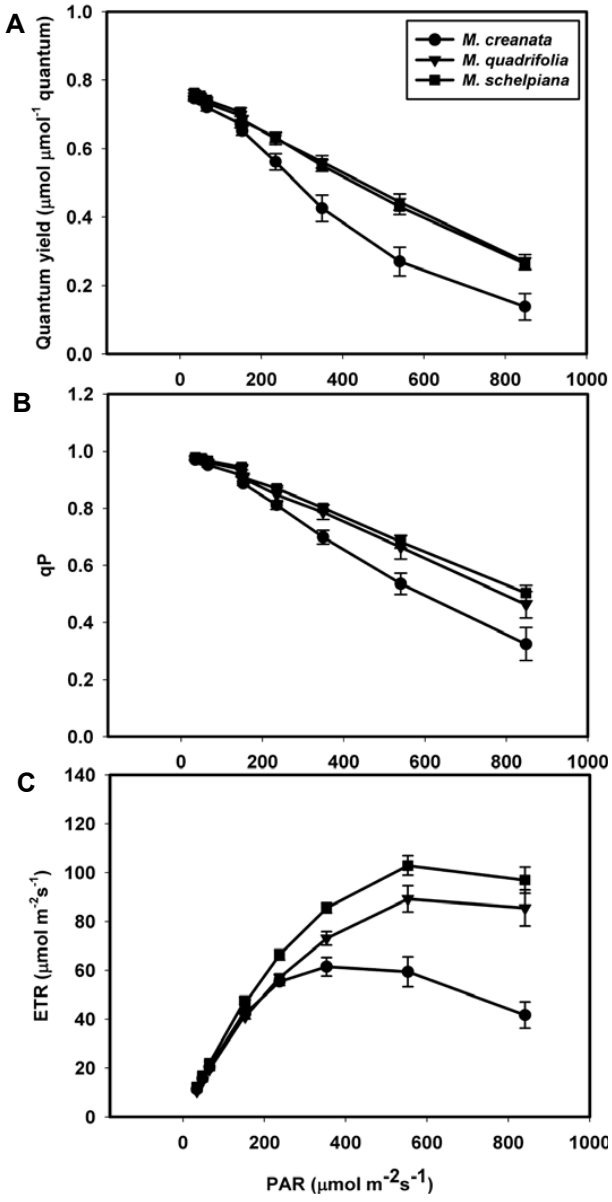


Fig. 2. The effect of photosynthetically active radiation(PAR) on (A) effective quantum yield of photosystem II, (B) photochemical quenching coefficient (qP), and (C) the electron transport rate (ETR) of leaflets of *M. crenata*, *M. quadrifolia* and *M. schelpiana* (mean ± s.e., n=6).

DISCUSSION

Most of *Marsilea* studies have focused on the formation and structural difference of its heterophyll (Allsopp, 1952, 1953, 1954, 1955; Gaudet, 1963; Liu, 1984; Lin and Yang, 1999; Hsu et al., 2001) and the systematics or phylogenetics of the genus (Launert, 1968; Johnson, 1986; Lin and Yang, 1999; Pryer, 1999; Jacono and Johnson, 2006; Nagalingum et al., 2007), in contrast, few on the ecological aspects (Gopal, 1968b; Gopal, 1968a; Gopal, 1969; Kornas, 1988). To our knowledge, comparisons of physiological and morphological traits among *Marsilea* species distributed in habitats with different climates have not been conducted. Results of this study revealed similarity and differences among three species of *Marsilea*, *M. crenata*, *M. quadrifolia* and *M. schelpiana*, distributed in different geographic regions, which not only enhance our understanding of the adaptation traits but also offer clues on possible environmental constrains contributing to the differential distribution of the three species.

Higher trichome density, high reflectivity, and low transpiration rate were the greatest difference between *M. quadrifolia* and the other two species (Tables 1, 2, 3). It has been shown that trichomes on the leaflets reduced significant water loss in *M. quadrifolia* (Wu and Kao, 2009). Thus the lower transpiration rate measured in this species might due to its dense trichomes covering leaf surface. In addition, a higher reflectivity in leaflets of *M. quadrifolia* might result in a significantly lower absorptivity than the other two species, which could reduce light absorption. With a reduction of light absorption, leaflets of this species would have a lower

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leaf temperature, which might also contribute to the reduction in transpirational water loss. In a previous study (Wu and Kao, 2009), we found that increase in trichome density did not increase reflectivity of *M. quadrifolia*. Thus the higher reflectivity found in this species might not be its dense trichome density. Though *M. quadrifolia* and *M. crenata* had similar A_{max}, however, due to a lower transpiration rate, the former had a significantly higher WUE. Plants with high water use efficiency would be favored by natural selection in dry habitat (Ackerly et al., 2000; Picotte et al., 2007). The result is consistent with the distribution of *M. quadrifolia* which mainly in temperate area with less precipitation.

Results of the study also revealed that the morphological traits work in concert with the physiological traits allowing *M. schelpiana* to grow in high light (maybe also high temperature) environment. Morphologically, the most obvious differences between *M. schelpiana* and the other two species were its lobed leaflets, high total stomatal pore area index (SPI), and long petiole with a wide diameter. Physiologically, among the three *Marsilea* species, *M. schelpiana* had the highest chlorophyll content per leaf area, absorptivity, A_{max} (Table 3) and light saturated ETR (Fig. 2). These are characters of sun-adapted leaves (Talbert and Holch, 1957; Héraut-Bron et al., 2001; Sack et al., 2003). When leaves of *M. schelpiana* receive high radiant energy for photosynthesis, they also face rising in leaf temperature. Plants with lobed leaves could partially eliminate the problem because lobed leaves have thinner boundary layers and therefore greater convective cooling than whole-margin leaves (Vogel, 1970; Nicotra et al., 2008). Hence, lobed leaves are considered selectively advantageous in conditions of high irradiance (Semchenko and Zobel, 2007). However, lobed leaves also indicate high transpirational water loss due to the reduction in boundary layer resistance. This is exactly what being found in *M. schelpiana*, which had the highest transpiration rate among the three species (Table 3). With a high transpiration rate the species would need a more efficient way of water transport to compensate for the water loss. Producing petioles with a wider diameter found in this species would help transporting water more efficiently than the other two species. Another morphological feature might also help the species in transporting water. Though *M. schelpiana* had the lowest stomatal density but it had the largest pore length (Table 1, Fig. 1F) among the three species. As a result, the species had significantly the highest stomatal pore area index (SPI) (Table 1). Several studies have demonstrated a positive correlation between the SPI and leaf hydraulic conductance (K_{leaf}) (Meinzer, 2003; Sack et al., 2003). High hydraulic conductance would also increase water transport (Brodribb et al., 2004; Gullo et al., 2010). As long as there is water available in soil, leaflets of the *M. schelpiana* equipped with the morphological features could use high irradiance achieving high photosynthetic rate without suffering water deficit.

The relationship found between the SPI and leaf hydraulic conductance (Meinzer, 2003; Sack et al., 2003) also indicates that leaves with high K_{leaf} also have fast rate for CO_2 diffusion through the stomatal pore. For example, Aasamaa and Sober (2001) found a positive correlation between K_{leaf} and photosynthetic gas exchange related parameters, including photosynthetic rate per leaf area. In consistent with the morphological traits (SPI value), physiological measurement also revealed that among the three *Marsilea* species, *M. schelpiana* had the highest g_s, A_{max} (Table 3), and electron transport rate through PSII under high irradiance (Fig. 2).

Among the three species, *M. crenata* had the lowest WUE, which is consistent with the climate pattern of its distribution range (mainly in Asia receiving high annual rainfall and with high humidity). In addition, a rise in PAR decreased the ETR value of *M. crenata* leaves exposed to irradiance higher than 400 μmol m^{-2} s^{-1}, which implies photoinhibition. *M. crenata* also had significantly lower qP, effective quantum yield and ETR than the other two species when PAR was above 400 μmol m^{-2} s^{-1} (Fig. 2). The results suggested that in comparison to the other two species, the photosynthetic behavior of *M. crenata* is more like that of a shade adapted plant.

Specific leaf area (SLA) calculated as the ratio of leaf area over leaf dry weight has been suggested an important adaptation trait. Leaves of plants adapted to environments of less water availability and/or high light intensity or nutrient deficient generally have low SLA (Wright et al., 2004). To our surprise, the three *Marsilea* species, distributing in habitats receiving different precipitation and irradiance level, when grown in the same condition did not showed significant difference in their SLA (Table 1). It implies that SLA might not be the selected trait for *Marsilea* species growing in habitats with different precipitation and/or irradiance. However, further studies comparing more *Marsilea* species distributing and/or growing under different environments are needed to evaluate the adaptive value of the trait.

Among environmental factors, water, solar irradiance and temperature are the three important factors affecting plant distribution (Lambers et al., 2008). Present study did not allow us to identify the importance of temperature in affecting the distribution

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of the three species. In addition, the combination of the factors might play an equally important role in affecting the distribution of the three species. Hence, further studies on the response of the three species to the variations of air temperature and to the combinations of the environmental factors would provide us a clear clue upon the driving force for the diversification of the *Marsilea* species.

In conclusion, the three *Marsilea* species when grown under the same environmental conditions showed significant differences in some of the morphological and physiological traits. *M. quadrifolia* (which is mainly distributed in temperate region) had the highest water use efficiency, *M. crenata* (mainly distributed in southeastern Asia) had the worst water use efficiency among the three species, and *M. schelpiana* (mainly distributed in southern African) showed high-light adapted characters. Thus, leaf characteristics of the three species reflect their habitat climates. It also indicates that water availability and light intensity are two of the important factors contributing to the geographic distribution of the three species.

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三種不同地理分佈的田字草之葉片生理生態特徵探討

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摘要：田字草（約有八十種）是兩棲性蕨類，具特殊的葉片形態及生殖構造，其分布遍及五大洲。為了探討分布在不同地理區域的三種田字草（*Marsilea crenata*、*M. quadrifolia* 及 *M. schelpiana*）其適應陸生環境的特性，我們將這三種田字草種植在相同環境下、比較陸生植株其小葉形態特徵、光學特性及光合作用表現。結果發現三種田字草小葉在某些生理生態特徵上有顯著的不同：在三者中，*M. quadrifolia*（分布於降雨較少的溫帶）的小葉有最高的表皮毛密度及水分使用效率；*M. schelpiana*（分布於有較高光照量的南非）有最長的葉柄及最大的裂葉指數、總氣孔面積指數、第二光系統電子傳遞鏈速率及光飽和光合作用速率；*M. crenata*（主要分布於有較高降雨量及溼度的東南亞）有最低的裂葉指數及水分使用效率。因此，這三種田字草的葉部特徵反應其棲地之氣候形態，結果也顯示水可利用度及光照量是影響這三種田字草地理分布不同的兩個重要因子。

關鍵詞：田字草、氣體交換、葉綠素螢光、水分使用效率、葉部形態、表皮毛密度、氣孔密度。

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