Ottelia fengshanensis, a new bisexual species of Ottelia (Hydrocharitaceae) from southwestern China

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Abstract

Ottelia fengshanensis, a new species (Hydrocharitaceae) from southwestern China is here described and illustrated. Comparing its morphological features to putative close relatives O. guanyangensis, it has 3–4 flowers (vs. 2–5) each spathe, hexagonal-cylindric fruit, white styles (vs. yellow), green leaves (vs. dark green) and fruit tiny winged (vs. winged obviously). Molecular phylogenetic investigation of four DNA sequences (ITS, rbcL, trnK5’ intron and trnS-trnG) and the Poisson Tree Processes model for species delimitation (PTP) analysis, further resolves O. fengshanensis as a new species that is close to O. guanyangensis with distinct support.

Keywords

karst, bisexual flowers, molecular phylogeny

Introduction

Ottelia Persoon (1805:1) has about 22 species and is widely distributed in the tropical, subtropical and temperate regions. In comparison with other genera within the family Hydrocharitaceae, Ottelia is morphologically complex and variable, e.g. the leaf
type of the genus is exceptionally erratic even within an individual depending on the developmental stage, as well as within the varieties or populations (Li et al. 2018). The flower sexuality varies within species and flowers can be either bisexual or unisexual. Southwestern China possesses complex terrain and various ecosystems and is a center of diversity for *Ottelia* species (Chen et al. 2017, Zhai et al. 2018). To date, six species and three varieties of *O. acuminata* Dandy (1934: 132) have been recorded from the area with narrowly endemic distribution in karst rivers or lakes. Among these, just three species, *O. alismoides* Persoon (1805: 273), *O. balansae* Dandy (1934: 137) and *O. guanyangensis* Z.Z. Li, Q.F. Wang & S. Wu (2018: 294) are bisexual and can only be found in specific karst regions, except for the widespread species *O. alismoides* (Cook et al. 1984, Cook and Urmi-Konig 1984, Li 1981).

In 2017–2018, we found and reported a new bisexual species *O. guanyangensis* in Guilin City, China (Li et al. 2018). We deemed that there are some previously undetected potentially new *Ottelia* species in Guangxi province’s karst streams (Fig. 1). We made further aquatic plant investigations in Guangxi province, China, in 2018. From the Fengshan County, we found once again a species with bisexual flowers which generally appeared to be like *O. balansae*. Based on investigations of herbarium specimens in GXMG, HIB, IBSC, KUN and PE, and literature review, only three bisexual species of *Ottelia* are known from China. These are *O. guanyangensis*, a species described in 2018 (Li et al. 2018), *O. balansae*, and *O. alismoides*, the latter two recorded from “Flora of China”. Compared to the recorded three bisexual species, it was interesting that the population from Fengshan county had some unique flowers (e.g. white styles and over three flowers each spathe) and leaf traits (e.g. triplinerved with obvious cross-

![Figure 1](image_url). Distribution record of *Ottelia fengshanensis* Z.Z. Li, S.Wu & Q.F.Wang (red triangle) from Fengshan county, Guangxi province, China.
Ottelia fengshanensis, a new bisexual species from China

Material and methods

Morphological study

The morphological characteristics of the new species were collected during fieldwork in July 2018. We randomly selected 10 individuals, took pictures of each part and measured the characteristics of flowers, leaves and fruits (Fig. 2). The pollen grains of new species were gold-coated, and photographed using a Hitachi S-800 SEM system at Wuhan Botanical Garden, CAS. Simultaneously, we collected voucher specimens and several fresh leaves were dried using silica gel for DNA extraction. For further detailed morphological analysis, we transplanted five living individuals to a greenhouse at Wuhan Botanical Garden. We also observed the characteristics of flowers, leaves and fruits of these two bisexual species in our greenhouse for further comparative analysis (Table 1).

Table 1. The voucher information and GenBank accession numbers for the sequences of internal transcribed spacer (ITS) and three cp regions (trnS-trnG, rbcL and trnK5’ intron) in the present study.

| Taxon                        | Individual code | Locality | Voucher no. | ITS       | rbcL       | trnK5’ intron | trnS-trnG    |
|------------------------------|-----------------|----------|-------------|-----------|------------|--------------|-------------|
| O. acuminata var. jingxiensis | 01, 10_1, 10_2  | Luguhu, Yunan | HIB-Otte011 | MG751784/ | MH257626/  | MH257644/    | MH257662/    |
| O. acuminata var. crispa     | 02, 10_2        | Heqing, Yunan | HIB-Otte003 | MG751786  | MH257625    | MH257643    | MH257661    |
| O. acuminata var. acuminata  | 15              | Jinchuan, Yunan | HIB-Otte006 | MG751787  | MH257637    | MH257655    | MH257673    |
| O. acuminata var. lananensis | 16              | Shillin, Yunan | HIB-Otte008 | MG751789  | MH257629    | MH257647    | MH257665    |
| O. acuminata var. songmingensis | 21_1, 21_2     | Songming, Yunan | HIB-Otte007 | MG751790/ | MH257631/  | MH257649/    | MH257667/    |
| O. balansae                  | 29              | Huaxi, Guizhou | HIB-Otte005 | MG751792  | MH257634    | MH257652    | MH257670    |
| O. emersa                    | 41              | Guigang, Guangxi | HIB-Otte004 | MG751794  | MH257638    | MH257656    | MH257674    |
| O. cordata                   | 40              | Haikou, Hainan | HIB-Otte001 | MG751795  | MH257639    | MH257657    | MH257675    |
| O. alismoides                | 42              | Changping, Fujian | HIB-Otte002 | MG751796  | MH257640    | MH257658    | MH257676    |
| O. guanyangensis             | 32              | Guanyang, Guangxi | HIB-Otte015 | MG751797  | MH257635    | MH257653    | MH257671    |
| O. fengshanensis             | 35              | Fengshan, Guangxi | HIB-lzz51  | MK31550   | MK31552    | MK31553    | MK31551    |
| B. japonica                  | 36              | Wuyishan, Fujian | HIB-Bly001 | MG751799  | MH257641    | MH257659    | MH257677    |
Figure 2. *Ottelia fengshanensis* Z.Z.Li, S.Wu & Q.F.Wang. **A** Habitat **B** flowering plant **C** bisexual flower with red-green sepals **D** individual and leaf: triplinerved with conspicuous cross veins **E** fruit: Hexagonal-cylindric with tiny wings **F** the character of pollens by SEM **G** spathe **H** seeds.

**Phylogenetic analysis**

Total genomic DNA of one sample, collected from Fengshan county, Hechi city, Guangxi province, was extracted following Li et al. (2018). One nuclear DNA region (ITS) and three chloroplast DNA regions (*trnS-trnG, rbcL* and *trnK5' intron*) were sequenced; the primers and PCR protocols followed Li et al. (2018). The same sequence
regions from other species were downloaded from the NCBI (Table 2). The sequence alignments were made using MAFFT with default settings (Kuraku et al. 2013). The best nucleotide substitution model was detected using jModeltest 2.1.4 (Darriba et al. 2012) with the Akaike Information Criterion (AIC). The Maximum Likelihood (ML) analysis was made using IQtree with 5000 bootstrap replicates (Nguyen et al. 2003). The Bayesian Inference (BI) was analyzed by MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2015), with 20,000,000 generations and four chains run with sampling after every 2000 generations. The first 25% of generations were discarded and a majority rule consensus tree (> 50%) was computed from the remaining trees. In order to test molecular support for species delimitation in Ottelia, the Poisson Tree Processes model for species delimitation (PTP) was applied to the tree with the following parameters: 500,000 generations; thinning: 100; burnin: 0.1 and seed: 123 (Zhang et al. 2013).

Results and discussion

The comparison among three bisexual species, *O. fengshanensis*, *O. guanyangensis* and *O. balansae*, is presented in Table 2. The new species had unique features, including the number of flowers, white styles, trinerved venation with distinct cross veins and longer leaf shape.

Morphological characters distinguish *O. fengshanensis* from the three bisexual species. For *O. alismoides*, there is only one flower in each spathe and it is easy to distinguish from the new species. However, *O. guanyangensis* and *O. balansae*, which are

| Characters                  | Ottelia fengshanensis | Ottelia guanyangensis | Ottelia balansae  |
|-----------------------------|-----------------------|-----------------------|-------------------|
| Flowers                     | bisexual              | bisexual              | bisexual         |
| Sepals                      | 1.0–1.5 cm, red green | 1.0–1.5 cm long, red brown | 2.0–2.5 cm long, green |
| Stamens                     | 3; filaments 3.0–5.0 mm long | 3; filaments 5.0–7.0 mm | 3; filaments 4.0–5.0 mm |
| Ovary                       | 5–10 cm long, hexagonal-cylindrical to cylinder | 4–5 cm long, hexagonal-cylindrical | 3.5–5.0 cm long, trianular-cylindrical |
| Styles                      | 3, bifid to base, white | 3, bifid nearly to base, yellow | 3.5–5.0 cm long, yellow |
| Spathe                      | 3–4 (3) flowed | 2–5 flowed | 3–11 flowed |
| Leaf shape                  | Linear or oblong, 30–70 × 8–14 cm, base rounded, apex acute or obtuse; petiole 8.0–10.0 cm long | Linear, 15–50 × 2.5–4.0 cm, base rounded, apex acute, petiole 8.0–13.0 cm long | Oblong or ovate, 20–40 × 6.0–8.0 cm, base truncate, rounded, or cordate, apex acute or rounded, petiole ca. 20 cm long |
| Texture                     | green, opaque, thick ca. 0.8 mm | dark green, opaque, thick ca. 1.2 mm | green, translucent, thick ca. 0.5 mm |
| Venation                    | trinerved with obvious cross veins, distance 4.0–6.0 cm to base, longitudinal veins 9 | trinerved with obvious cross veins, distance 4.0–6.0 cm to base, longitudinal veins 9 | Basal veins, longitudinal veins 7 |
| Fruit                       | hexagonal-cylindrical, winged unobviously | hexagonal-cylindrical, winged | Narrowly elliptic, unwinged |
| Seed                        | fusiform, ca. 1.0 mm long | fusiform, ca. 1.5 mm long | Cylindric to fusiform, ca. 3.0 mm long |
| Pollen                      | spheroidal, inaperturate, ca. 40 × 40 μm | spheroidal, inaperturate, ca. 35 × 45 μm | Spheroidal, inaperturate, ca. 49 × 53 μm |
| Flowering time              | April to November | April to October | June to November |
distributed in Guangxi province and Guizhou province respectively, are closest to the new species. The critical diagnostic characters of *O. fengshanensis* include having white styles, longer leaf shape and number of flowers in each spathe. Moreover, these three species are also isolated geographically, *O. fengshanensis* was only found in Fengshan county, but *O. guanyangensis* was found in Guilin city. *O. balansae* was only recorded in Guizhou province based on a recent survey. Karst terrain will play an important role in species divergence in this lineage.

Four sequence regions (ITS, *trn*S-*trn*G, *rbc*L and *trn*K5' intron) were aligned and concatenated into a 3623 bp sequence. 605 variable nucleotides were detected. Two clades were displayed with high support (BS = 70, PP = 0.7). PTP analysis further recognized four species with *O. fengshanensis* having the highest support (0.678). Based on phylogenetic analyses, *O. fengshanensis* was resolved as sister to *O. guanyangensis* with high support (BS = 100, PP = 1.0) and only distantly related to *O. balansae*, which clusters together with *O. acuminata* (Fig. 4), and based on PTP analysis, *O. balansae* was not supported as a species, but was more likely to be treated as a bisexual variety of *O. acuminata*. In combination, the morphological and molecular phylogenetic analyses support that *O. fengshanensis* is a distinct species closely related to *O. guanyangensis*, a species also distributed in Guangxi province.

*Ottelia* possesses complex floral traits and may have bisexual and unisexual flowers. Based on the previous studies (He 1991, Chen et al. 2012) bisexual flowers have evolved multiple times in *Ottelia*. Here we report a new bisexual species *O. fengshanensis* and verify that bisexual flower indeed has multiple origins in *Ottelia*. *Ottelia fengshanensis* probably has a common ancestor with the unisexual *O. acuminata* var. *songmingensis*. Besides, we also suggest that *O. balansae* should be treated as a variety of *O. acuminata*. This point has also been put forward by Yu Ito et al. (2019). It will also help us have a better understanding of the diversity and evolution of sex evolution in *Ottelia*.

**Description of the new species**

*Ottelia fengshanensis* Z.Z.Li, S.Wu & Q.F.Wang, sp. nov.

urn:lsid:ipni.org:names:77202741-1

Fig. 3

**Description.** The new species is similar to *Ottelia guanyangensis* in having bisexual flowers, three stamens, but differs through having (3)-4 flowers in each spathe (vs. 2–5), white styles (vs. yellow), green leaves (vs. dark green) and by fruits which are tiny winged (vs. obviously winged).

**Type.** CHINA. Guangxi, Hechi City, Fengshan County, elev. 507 m, 24°34’20"N, 107°10’17"E, 11 September 2018, Z. Z. Li & S. Wu-Otte51 (holotype HIB-lzz51!).

Annual or perennial herb. Rhizome, short. Leaves entirely submerged, dark green and opaque, linear or oblong, 30–70 × 8–14 cm, base rounded, apex acute or obtuse; longitudinal veins 9; midrib conspicuous, stretched to the apex, becoming trinerved with obvious cross-veins at a distance of 5–7 cm from the base; petiole smooth, green, 8.0–10.0 cm long,
Figure 3. Illustration of *Ottelia fengshanensis* Z.Z.Li, S.Wu & Q.F.Wang. Drawn by Shuai-Jie Li.

the base expanded into a sheath. Spathe oblate, ca 3.0 × ca. 3.5 cm, warty along edges or smooth, longitudinally ribbed and winged on the lateral margins, containing 3–4 (3) flowers; flowers bisexual; sepals reddish green, 1.0–1.5 × ca. 0.5 cm, with longitudinal ribs; petals white with yellow base, obovate, ca. 2.0 × ca. 2.0–2.5 cm, with longitudinal pleats;
Figure 4. Phylogenetic tree and PTP analysis of *O. fengshanensis* sp. nov. and *Blyxa japonica* as an outgroup. Posterior probabilities (PP > 0.70) and bootstrap values (BS > 70) based on Bayesian Inference and maximum likelihood (ML) analysis are shown above the branches.
Ottelia fengshanensis, a new bisexual species from China

stamens 3, opposite to sepals, anthers elliptic, connective obscure, filaments 3.0–5.0 mm long; glands 3, 0.5–1.0 × 0.5–1.5 mm, opposite to petals, pale yellow. Ovary hexagonal-cylindric to the cylinder, 5–10 cm long, with 3 carpels; styles 3, white, slender and hairy, 1.2–1.5 cm long, stigma bifid, divided to base; stigmas 6, linear and hairy, ca. 8 mm long. Fruit a hexagonal-cylindric capsule, with 6 inconspicuous wings, dark green, with persistent calyx, 4.0–9.0 cm × ca. 6.5 mm, always longer than spathe. Seeds numerous, fusiform, ca. 1.0 mm long, both ends hairy. Pollen, subglobe, ca. 40μm in diam, with spiny granules.

**Distribution and habitat.** Ottelia fengshanensis is known from a single population in Fengshan County, Guangxi Province, China. The species inhabits a karst river less than 1.5 m in depth. Due to the complex underground river system in the karst region, it is probable that the species occurs in nearby areas as well.

**Conservation status.** Only one population of new species was found at Fengshan County, Guangxi Province, China. Although it might be distributed in adjacent karst rivers. Until now, approximately 50–100 individuals were found in a single population. However, there is not enough information on population size and dynamics. According to the IUCN Red List Categories and Criteria (IUCN 2017), we suggested that the species be evaluated as Data Deficient (DD).

**Phenology.** The new species was found in flower from April to November.

**Etymology.** The epithet is derived from the name of Fengshan County, which is the only known locality of occurrence.

**Other specimens examined (paratypes).** CHINA. Guangxi, Hechi City, Fengshan County, elev. 507 m, 11 September 2018, Z. Z. Li & S. Wu Otte 056 (HIB!)

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