The Genus *Nymphoides* Ségui er (Menyanthaceae) in Taiwan

Sung-Po Li (1), Tsung-Hsin Hsieh (2,3), Chun-Chi Lin (1)

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ABSTRACT: The genus *Nymphoides* Ségui er (Menyanthaceae) in Taiwan is revised based on comparative morphological, palynological, and cytological studies. Five native taxa, *N. aurantiaca* (Dalzell) O. Kuntze, *N. coreana* (H. Leveilie) H. Hara, *N. hydrophylla* (Lour.) O. Kuntze, *N. indica* (L.) Cb Kuntze, and *N. lungtanensis* P, Li, T, H. Hsieh & C. C. Lin, sp. nov., and a cultivated species *N. peltata* (Gmelin) O. Kuntze are recognized in this paper. The ornamentation of seed coat and chromosome number are good characters in separating the Taiwanese taxa. SEM micrographs of pollen grains and seed coat, chromosome number, a key to species, descriptions, and taxonomic notes are provided.

KEY WORDS: *Nymphoides*, Menyanthaceae, Seed, Pollen grain, Chromosome number, Revision, Taiwan.

INTRODUCTION

The genus *Nymphoides* Ségui er (Menyanthaceae), consisting of about 40 species, occurs mainly in wet place of the temperate and tropical regions of both the Old World and the New World (Ho & Ornduff, 1995).

There are some confusing about this genus in Taiwan. Liu and Kuo (1978) treated the yellow flower plant as *N. aurantiaca* in Flora of Taiwan according to the only collection (Shimada 6545), but Ying (1989) and Lu (1999) treated it as *N. peltata* (Gmelin) O. Kuntze and cited the same specimen.

Due to the plants indistinguishable vegetatively, and the flowers dry so poorly that they are usually useless for identification purpose (Ornduff, 1970b). The specimens were usually misidentified in herbaria and confusing for a long time in Taiwan.

Recently, a new species *N. lungtanensis* (Figs. 1A and B) was collected from the pond in Lungmei Road, Lungtan, Taoyuan County by the third author (Lin, 2000). Thus the infrageneric classification in Taiwan is rather confused, therefore a new revision is desirable.

MATERIALS AND METHODS

Fresh materials used in this study were collected from throughout Taiwan. Most specimens were pressed and dried for voucher specimens and deposited in the Herbarium, Department of Science Education, National Tainan Teachers College (NTNTC).

Pollen grains were acetolysed according to the procedures outlined by Erdtman (1952). The acetolysed grains were dehydrated in an ethanol series and dried in air. Dried grains were coated with gold and examined using SEM.

The seeds were kept in desiccator (relative humidity 40-50%) for 24 hours, coated with gold, and examined by SEM.

1. Lung-Hai Elementary School, Taichung 434, Taiwan.
2. Department of Science Education, National Tainan Teachers College, Tainan 700, Taiwan.
3. Corresponding author. E-mail: thhsieh@ipx.ntntc.edu.tw
Root tips for chromosome counts were cut from living plants. After pretreatment with 0.002 M 8-hydroxyquinoline for 3-4 hours at 18-20°C, the materials were fixed in a mixture of absolute alcohol and glacial acetic acid (3:1/v:v), then macerated with 1% pectinase, stained with acetic orcein, squashed, and observed with a Nikon microscope.

RESULTS

External morphology
The leaf blade of Nymphoides is ovate-orbicular, rounded, deeply cordate at base with narrow sinuses. Although the shape is similar, N. indica has the largest leaves (up to 20 cm across); N. lungtanensis has median size leaves (up to 12 cm across); the other species has small leaves (up to 8 cm across).

Although the specimens in herbaria are usually misidentified, the flower morphology can be used to distinguish this genus in Taiwan. The corolla of N. aurantiaca and N. peltata is yellow; the others is white with a yellow throat. In yellow corolla group, N. peltata has large
corolla (2.5-3 cm across); *N. aurantiaca* has small corolla (8-10 mm across). In white corolla group, *N. indica* has the largest flower (2.5 cm across); *N. lungtanensis* has median size flower (1.2-1.5 cm across); the other species has small flower (1.0-1.2 cm across).

**Pollen grains**

The pollen grains of *Nymphoides* are parasyncolpate, isopolar; suboblate to oblate-spheroidal in equatorial view, semi-angular in polar view, and the sexine with tuberculate processes (Fig. 2).

On the other hand, the pollen grains of *N. lungtanensis* are either parasyncolpate (ca. 30%), sexine with smaller tubercules than the other species (Figs. 2G, H) or irregular small ones (ca. 70%).

**Seeds**

It is easy to distinguish the taxa by seeds in Taiwan. The seed of *N. hydrophylla* is globose, ca. 2 mm across and with many short papillose projection (Figs. 3E and F). On the other hand, the seeds of the other species are ellipsoid, ca. 1 mm long. *N. aurantiaca* has densely aculeate surface (Figs. 3A and B); *N. coreana* has sparsely tubercles rounded at the distal ends (Figs. 3C and D); *N. indica* has smooth and shining surface (Figs. 3G and H).

The ornamentation of *N. aurantiaca* and *N. hydrophylla* is consistent with Sivarajan et al. (1989) for Indian *Nymphoides*, except for *N. indica*, which has scattered cluster of tuberculate protuberances in Indian population (Sivarajan et al., 1989). But, Cook (1996) described seed surface of *N. indica* with smooth and shining or with a few small tubercles from India. Therefore, there are maybe two kinds of seeds or populations in this taxon and further study is needed.

**Chromosome number**

The chromosome number of *N. hydrophylla* (Fig. 4B) and *N. indica* (Fig. 4C) is 2n = 18 (diploid); the chromosome number of *N. coreana* (Fig. 4A) is 2n = 36 (tetraploid) and *N. lungtanensis* (Fig. 4D) is 2n = 27 (triploid).

The chromosome number of *N. coreana* was reported to be 2n = 56 for Japanese plants (Shigenobu & Tanaka, 1980), but our result shows that the chromosome number of Taiwanese plant is 2n = 4x = 36.

**TAXONOMICAL TREATMENT**

**Key to species of Taiwan**

1. Flower yellow
   2. Stem branched; leaves opposite at upper stem; flower 2.5-3 cm in diameter .................. 6. *N. peltata*
   2. Stem unbranched; leaves alternate at stem; flower 0.8-1 cm in diameter ...................... 1. *N. aurantiaca*
1. Flower white
   3. Corolla-lobes with a longitudinal crest along the center; seeds globose ...................... 3. *N. hydrophylla*
   3. Corolla-lobes either fimbriate or with hairs; seeds ellipsoid or absent
   4. Corolla-lobes with fimbriate margin ................................................................. 2. *N. coreana*
   4. Corolla-lobes densely covered with long, white hairs
   5. Leaves up to 20 cm across; flower 2.5 cm across; seed ellipsoid, ca. 1 mm across .......... 1. *N. indica*
   5. Leaves up to 12 cm across; flower 1.2-1.5 cm across; seed absent .............................. 5. *N. lungtanensis*
Fig. 2. SEM micrographs of pollen grains of *Nymphoides* in Taiwan. A and B: *N. coreana*; C and D: *N. hydrophylla*; E and F: *N. indica*; G and H: *N. lungtanensis*. Scale bar = 5 μm in A, C, E, and G; in B, D, F, and H scale Bar = 1 μm.
Fig. 3. SEM micrographs of seeds coat of Nymphoides in Taiwan. A and B: N. aurantiaca; C and D: N. coreana; E and F: N. hydrophylla; G and H: N. indica. Scale bar = 300 μm in A, C, D, E, and G, except 30 μm in B, F, and H.


Rhizome short, elongate, bearing leaves and flowers at regular intervals. Petiole-like shoots with several nodes, simple, flexible; nodes occasionally rooting. Petioles 3-9 cm long; leaves on flowering stems alternate; blades floating, orbicular-rounded, 2-5(-8) cm in
diameter, deeply cordate at base with narrow sinuses, purple on abaxial surface. Flowers usually 2 per node. Pedicels 1.5-4.5 cm long. Calyx deeply (4-) 5-lobed; lobes ovate-lanceolate, 3-6 mm long, glandular outside. Corolla yellow. Corolla tube 5-7 mm long, lobes (4) 5, obovate-oblong, 4-7 mm long, apex distinctly 2 lobed, margin with fimbriately toothed wings. Stamens as many as petals, inserted at the sinuses of the corolla, each with a small tufts of glandular hairs. Pistil bottle-shaped. Capsule ovoid or obovoid, 3.5-4.5 mm in diameter, 10-15-seeded. Seed ellipsoid, ca. 1 mm long, surface densely aculeate.

Distributed in S. India and Sri Lanka. Taiwan found in Taoyuan County, but no specimens have been collected recently.

Specimens examined: Taoyuan: S. Sasaki s. n. Jun 1923 (TAI); Kizan, Heicho, Taiko, Y. Shimada 3461(NTUF); en route from Taoyuan to Nankan, Y. Yamamoto s. n. May 1929 (TAI).

Notes: This species is close to *N. peltata* (Gmel.) O. Kuntze, but differs from it by having unbranched stem, solitary leaf at stem, two flowers per node, much smaller flower (8-10 mm in diameter), and aculeate seed surface. On the other hand, *N. peltata* has branched stem, opposite leaves at upper portion of stem, large corolla (2.5-3 cm in diameter), and compressed seed with densely ciliate surface.


Rhzome with branching scars and scale leaves, sending out stolons which develop new plantlets. Fertile branches many, petiole-like, highly variable in length. Petiole variable, 0.5-5 cm long. Leaves blade ovate-oreibicular, rounded, 2-8 cm across, deeply cordate at base, abaxially densely glandular dotted. Flowers ca. 1.0-1.2 cm in diameter, 6-10 clustered at nodes, 4-5-merous. Pedicels 3-5 cm long. Calyx deeply 4-5-lobed to near base; lobes ca. 3 mm long, ovate-lanceolate, apex obtuse, margins hyaline. Corolla white with a yellow throat; lobes 4-5, oblong-lanceolate, up to 5.5 mm long, apex 2-lobed, margin with fimbriate hairs. Stamens as many as corolla lobes, inserted in the throat of the corolla, each with a small tufts of glandular hairs on not. Pistil bottle-shaped; style 2-lobed; hypogynous disc glands as many as stamens, orbicular, ciliolate at tips with papillose hairs. Capsule globose, ca. 3 mm long. Seed ellipsoid, ca. 1 mm long, with sparse tubercles rounded at the distal ends. Chromosome number 2n = 4x = 36.

Distributed in E. Russia, Korea and Japan. Taiwan found throughout the island and Lanyu island, but is very rare now.

Note: This species is distributed both in Taiwan and Lanyu island and has geographical differentiation. The population in Taiwan has bigger leaves (up to 8 cm across), petal with a small tufts of glandular hairs at base and few turbaculæ on seed margin (Fig. 3C), but the population in Lanyu island has smaller leaves (2-5 cm across), petal without a small tufts of glandular hairs at base and more turbaculæ on seed margin (Fig. 3D). The chromosome number of Taiwanese plant is $2n = 36$, but $2n = 56$ in Japanese plant (Shigenobu & Tanaka, 1980). So, a further study is needed for this taxon.


**Menyanthes hydrophylla** Lour., Fl. Cochinch. 1: 105. 1790.
**Menyanthes cristata** Roxb., Pl. Corom. 2: 3. pl. 105. 1798.


Rhizome short, elongate, bearing leaves and flowers at regular intervals. Petiole-like shoots with several nodes, simple, flexible; nodes occasionally rooting. Petioles 1-2.5 cm long; leaves few per node; blades orbicular-rounded, 1-6 cm across, deeply cordate at base with narrow sinuses, purple on abaxial surface. Flowers 2-10 per node, 5-merous. Pedicels 3-6 cm. Calyx deeply 5 lobed; lobes narrowly lanceolate, 3-5 mm long. Corolla white with a yellow throat, campanulate. Corolla tube 7-8 mm long, lobes 5, oblong, with flexuous, membranous wings on the margins and a similar median longitudinal crest within. Stamens as many as petals, inserted at the sinuses of the corolla, each with a small tufts of glandular hairs. Pistil bottle-shaped, with 5 hypogynous disc glands, orbicular, ciliolate at tips with papillose hairs. Capsule oblong, 3 mm in diameter, 1-6-seeded. Seeds globose, ca. 2 mm in diameter, densely covered with many short papillose projections. Chromosome number $2n = 18$.

Distributed in India, Sri Lanka, Malaysia, and South China. Taiwan found in southern part. The only population was found in Chung-Cheng Lake, Meinong Hsiang, Kaohsiung County.

**Specimens examined:** KAOHSIUNG: Meinong, T. H. Hsieh 4805 (TAI, NTNTC).

Notes: The present species has long been named under **Nymphoides cristata** (Roxb.) O. Kuntze. Sivarajan and Joseph (1993), Toyokuni and Yamazaki (1993), and Cook (1996) treated **N. cristata** as a synonym of **N. hydrophylla**, but Ho & Ornduff (1995) and Yang et al. (2001) could distinguish them and **N. cristata** is also distributed in Taiwan. Whether these two entities should represent distinct species are open to debate. Since the Taiwanese materials can fit **N. hydrophylla** by Sivarajan and Joseph’s publication, here we tentatively treat them as the same species.


**Menyanthes indicum** L., Sp. Pl. 145. 1753.
Rhizome 1-5 cm thick with numerous branching scars and scale leaves, sending out stolons which develop new plantlets. Fertile branches many, petiole-like, highly variable in length. Petiole short, to 2 cm long. Leaves blade ovate- orbicular, rounded, deeply cordate at base with narrow sinuses, 8-20 cm across, abaxially densely glandular. Flowers bisexual, distylic, clustered at nodes, 5-merous. Pedicels 5-12 cm long. Calyx deeply 5-lobed to near base; lobes 5-7 mm long, ovate-lanceolate, apex obtuse, margins hyaline. Corolla white with a yellow throat, 2.5 cm across; lobes (4-) 5-7 (-8), oblong-lanceolate, up to 15 mm long, surface and margins densely fimbriate, with multicellular, cotton-like outgrowths. Stamens as many as corolla lobes, dimorphic, inserted in the throat of the corolla, each with a small tufts of glandular hairs. Pistil bottle-shaped; style stout, 2-lobed; hypogynous disc glands as many as stamens, orbicular, ciliolate at tips with papillose hairs. Capsule ellipsoid to obovoid, 4.5-6 mm across. Seeds brownish, ellipsoid, ca. 1 mm long, smooth and shining. Chromosome number 2n = 2x = 18.

Widely distributed in warm temperate to tropical regions in E. and S. Asia, Australia, America, and Africa.

**Specimens examined:**

5. **Nymphoides lungtanensis** S. P. Li, T. H. Hsieh & C. C. Lin, sp. nov. — TYPE: TAIWAN.

Taoyuan Hsien: Lungtan Hsiang, Lungmei Road, C. C. Lin 170 (Holotype: TAIF).

Figs. 1A, B, C, 5

**Affinis speciei N. indica, sed folio minuti, corollae 1.2-1.5 cm. longa, ovaria abortive, et chromosomatum numberus 2n = 3x = 27 differt.**

Rhizome with numerous branching scars and scale leaves, sending out stolons which develop new plantlets. Fertile branches many, petiole-like, highly variable in length. Petiole short, to 2 cm long. Leaves blade ovate- orbicular, rounded, deeply cordate at base with narrow sinuses, to ca. 12 cm across, abaxially densely glandular dotted. Flowers bisexual, 1.2-1.5 cm in diameter, 6-10 clustered at nodes, 4-5-merous. Pedicels 3-5 cm long. Calyx deeply 4-5-lobed to near base; lobes ca. 3 mm long, ovate-lanceolate, apex obtuse, margins hyaline. Corolla white with a yellow throat; lobes 4-5, oblong-lanceolate, up to 7 mm long, surface and margins densely fimbriate, with multicellular, cotton-like outgrowths. Stamens as many as corolla lobes, inserted in the throat of the corolla, each with a small tufts of glandular hairs. Pistil bottle-shaped; style 4-grooved; hypogynous disc glands as many as stamens, orbicular, ciliolate at tips with papillose hairs. Capsules lacking. Chromosome number 2n = 3x = 27.

**Notes:** *Nymphoides lungtanensis* is most closely related to *N. indica* which is also distributed in Taoyuan County. Both have a white corolla with a yellow throat and densely fimbriate petal with multicellular, cotton-like outgrowths. However, this new species can be
distinguished from *N. indica* by its smaller leaves (up to 12 cm across), smaller flower (1.2–1.5 cm across), 4-grooved stigma, and chromosome number being $2n = 3x = 27$. On the other hand, *Nymphoides indica* of the Old World has bigger leaves (up to 20 cm across), bigger flowers (2.5 cm across), bilobed stigma (Fig. 1D), capsules and seeds present, and chromosome number $2n = 2x = 18$ (Ornduff, 1970a; Ornduff and Mosquin, 1970).

*Nymphoides lungtanensis* is sterile and can only reproduce vegetatively, forming large, uniform colonies. Because habitat was destroyed, this new species is very rare now (Lin, 2000).


Notes: Based on our field work and the available herbarium specimens, *N. peltata* does not seem to present in Taiwan, though the binomial had, in the past, been enumerated without cited any specimen (Sasaki, 1928; Masamune, 1936 and 1954). Liu and Kuo (1978) treated the yellow flower plant as *N. aurantiaca* in Flora of Taiwan according to the only collection (Shimada 6545), but Ying (1989) and Lu (1999) treated it as *N. peltata* (Gmelin) O. Kuntze and cited the same specimen. Unfortunately, we can’t find the voucher specimen in herbaria including TAI, TAIF, NTUF, HAST. The plant with yellow flower of *Nymphoides* has only taxon and can be identified as *N. aurantiaca* in Taiwan. Therefore, *Nymphoides peltata* may be introduced and cultivated in Taiwan recently.

Specimens examined: TAICHUNG: Lung-Hai Elementary School, S. P. Li 717 (TNM) (cultivated).

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台灣之苦菜屬（睡菊科）
李松柏(1)、謝宗欣(2, 3)、林春吉(1)

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摘　　要

根據外部形態、花粉、種子和細胞學來訂正台灣苦菜屬(Nymphoides)植物。本研究指出台灣有5個分類群，分別為黃花苦菜(N. aurantiaca (Dalzell) O. Kuntze)、小苦菜(N. coreana (H. Léveillé) H. Hara)、龍骨瓣苦菜(N. hydrophylla (Lour.) O. Kuntze)、印度苦菜(N. indica (L.) O. Kuntze)、龍潭苦菜(N. tungitanensis S. P. Li, T. H. Hsieh & C. C. Lin, sp. nov.)和一個栽培種苦菜(N. peltata (Gmelin) O. Kuntze)。本文並提供花粉、種子、檢索表、分類群描述等資料。

關鍵詞：苦菜屬、睡菜科、種子微細構造、染色體、分類訂正、台灣。

1. 龍潭國民小學，台中縣 434，台灣。
2. 國立台南師範學院自然科學教育系，台南市 700，台灣。
3. 通信作者。