

Exploration of Thai native waterlilies related with *Nymphaea siamensis* Puripany.

V. Puripunyanich, W. La-onsri, K. Boonsirichai and P. Chukiatman



Funded by



Part I

Nymphaea siamensis 'Jongkolnee' the historic water lily in Thailand



**By : Vichai Puripunyanich, Dr. Woranuch La-ongsri,
Dr. Kanokporn Boonsirichai, and Primlarp (Wasuwat) Chukiatman**

Pigat Bua 5 : An ancient Thai medicine – 5 waterlilies

Formulation for healing fevers including Typhoid



One of them is *N. siamensis* ‘Jongkolnee’

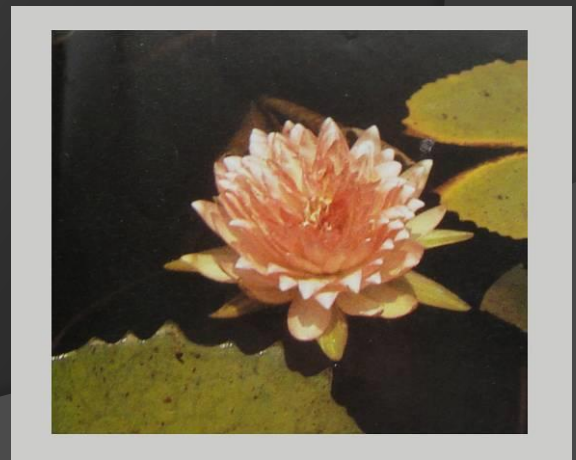
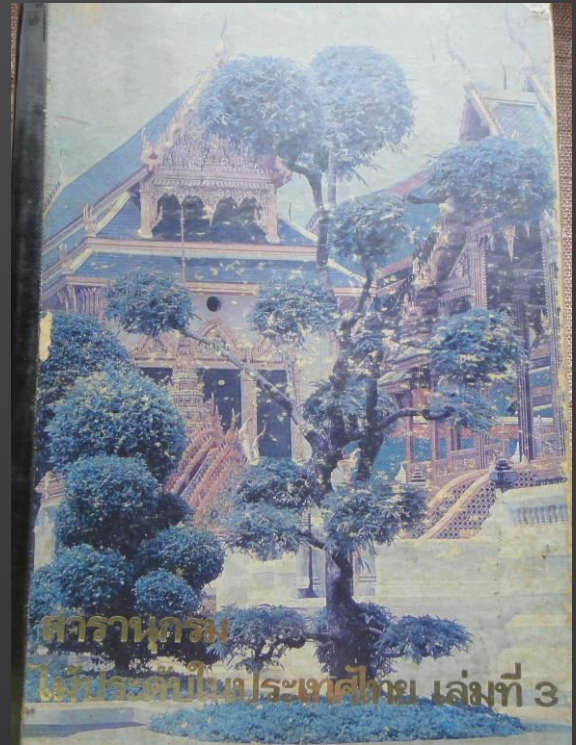


Dr. Slearmlarp Wasuwat, a Thai waterlily guru

Described *Nymphaea siamensis*
'Jongkolnee' characteristics and history in
his books

“Encyclopedia of Ornamental Plants of
Thailand” vol.3 and “Culture of
Ornamental Waterlily”

It was mentioned that *N. siamensis* had
been present in Thailand for a long time.



Genus *Nymphaea* L.

Group Apocarpiae

Apocarpous carpel

Subgenus *Anecphyra*

Australian water lily

Subgenus *Brachyceras*

Tropical day blooming water lily

Subgenus *Confluentes*

Australian water lily

Group Syncarpiae

Syncarpous carpel

Subgenus *Hydrocallis*

Subgenus *Lotos*

Tropical night blooming water lily

Subgenus *Nymphaea*

Sub-tropical water lily

***Nymphaea siamensis* has no sexual reproductive organs.**

Propagation is by bulblets only.

What are bulblets?



Since *N. siamensis* lacks both stamens and pistils, its only means of reproduction is via bulblets which are attached to the vertical rhizome.

Usually, these bulblets will give rise to leaves. However, when separated from the rhizome, they will give rise to a new plant.

Bulblets : the unique characteristic of *Nymphaea siamensis*



Nymphaea siamensis is similar to Thai purple 'Bae' ('Nilubol') in term of bulblets



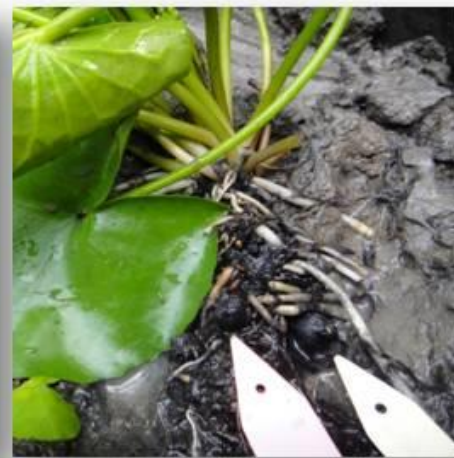
N. 'Nilubon'



N. siamensis



N. colorata



Thai purple 'Bae'

N. siamensis

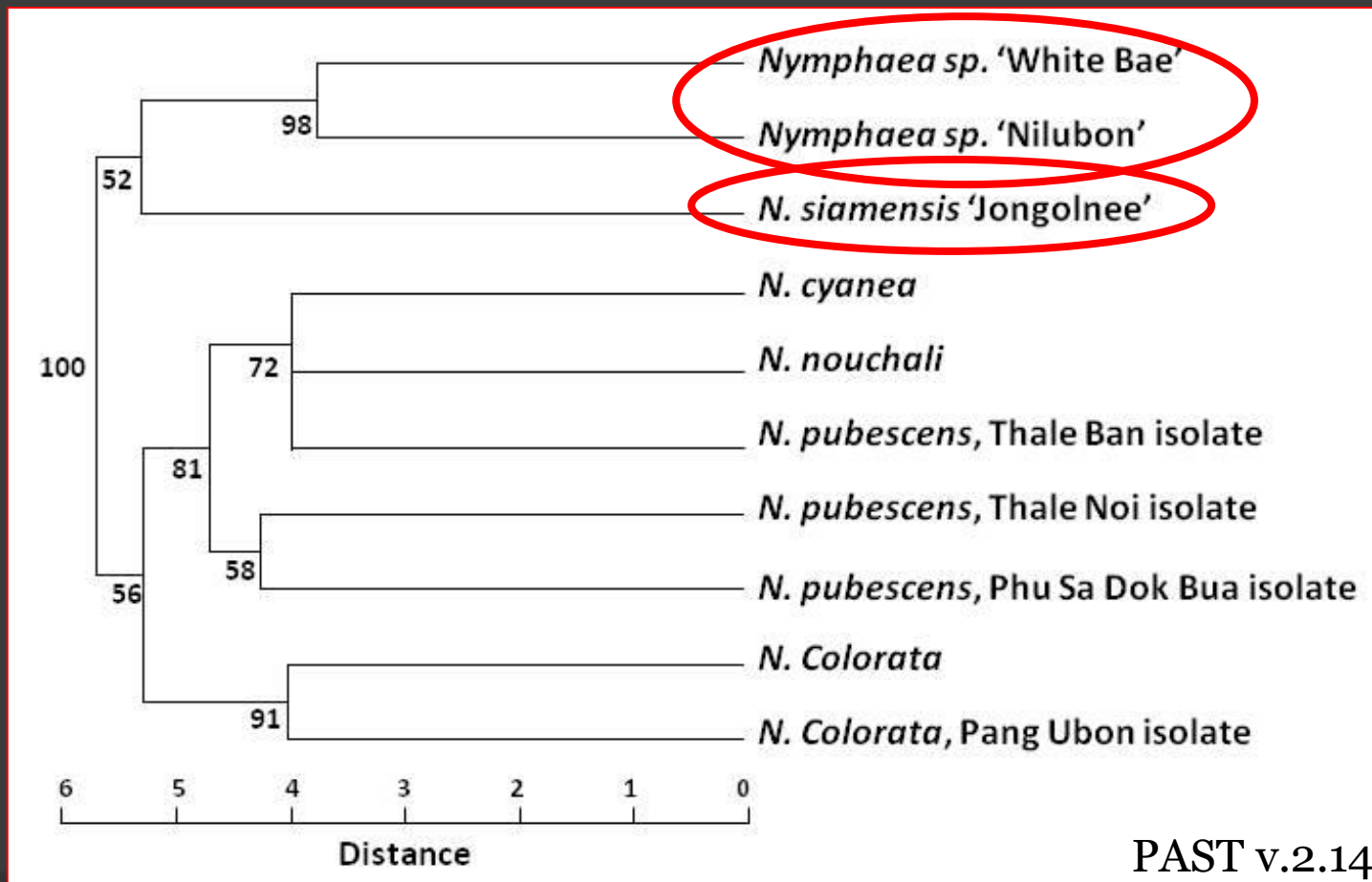
N. colorata

N. cyanea

Cluster analysis:

RAPD data (64 polymorphic loci)

- DNA fingerprint of *N. siamensis* is grouped together with *N.* 'Nilubon' and Thai white 'Bae'



Nymphaea siamensis

Nymphaea siamensis 'Jongkolnee' cannot be classified into apocarpiae or syncarpiae group because

- 1) *N. siamensis* 'Jongkolnee' lacks locules
- 2) *N. siamensis* 'Jongkolnee' propagates via bulblets without sexual reproductive organs. (Unique characteristic waterlily)
- 3) *N. siamensis* 'Jongkolnee' DNA fingerprinting data grouped it separately from other known species from Thailand

Therefore, *Nymphaea* 'Jongkolnee' should be established as a new species and given the name *Nymphaea Siamensis*.

Acta Horticulturae
Number 1035

**Proceedings of the
Sixth International Symposium
on
the Taxonomy of Cultivated Plants**

Editors

**Qixiang Zhang
Xiaobai Jin**



Nymphaea siamensis, the New Species of Waterlily in Thailand

V. Puripunyanich^{1,a}, W. La-onsri², K. Boonsirichai¹ and P. Chukiatman³

¹ Research and Development Division, Thailand Institute of Nuclear Technology, Ongkharak, Nakhon Nayok, Thailand

² Queen Sirikit Botanic Garden, Botanical Garden Organization, Chiang Mai, Thailand

³ Pang U Bon Garden, Nonthaburi, Thailand

Keywords: *Nymphaea siamensis*, new species, nomenclature, morphology, RAPD

Abstract

Morphological and genetic comparison between *Nymphaea siamensis* and other *Nymphaea* species were conducted. *N. siamensis* is a new species of *Nymphaea* found in Thailand. Lacking carpels and anthers, its indeterminate flowers feature multiple whorls of pink petals. The species could be propagated vegetatively via bulblets and shows closest similarities to *Nymphaea* 'Nilubon', an unclassified landrace found in the northeast of Thailand. *N. siamensis* and *N.* 'Nilubon' have similar leaf, stem and root morphology. Like *N. siamensis*, *N.* 'Nilubon' also produces bulblets. However, flowers of *N.* 'Nilubon' have purple petals and are complete with both stamens and pistils. *N. siamensis* differs from the other *Nymphaea* species in its lack of locules, which made it difficult to be classified. Nonetheless, its morphological characters appear closer to species in the subgenus *Brachyceras* than in the subgenus *Lotos*. As a consequence, the species may be classified under the subgenus *Brachyceras* of the genus *Nymphaea*. Cluster and neighbor joining analyses of 34 polymorphic RAPD alleles revealed that *N. siamensis* was most similar to *N.* 'Nilubon' in our study. In addition, parsimony analysis revealed that it might have a separate origin from the other *Nymphaea* species in our studies. We propose that *N. siamensis* be qualified as a new plant species native to Thailand.

INTRODUCTION

Nymphaea is a very diverse genus of the family *Nymphaeaceae* with species distribution in the temperate and tropical regions of the northern as well as the southern hemispheres (Borsch et al., 2011). It comprises six subgenera: *Anecphya*, *Brachyceras*, *Confluentes*, *Hydrocallis*, *Lotos* and *Nymphaea* (Borsch et al., 2007; Jacobs, 2007). In recent years, extensive DNA studies have driven new evolutions for taxon classification

Garden, Nonthaburi, Thailand. Morphological comparisons concerning bulblets, rhizomes, leaves, flowers and floral composition of these *Nymphaea* species against *N. siamensis* were performed. Taxonomic placements were conducted according to the Angiosperm Phylogeny Group (2009).

For cluster analysis, RAPD fingerprinting was performed. Genomic DNAs were extracted from leaves using Genomic DNA Mini Kit (Plant) (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's instruction. RAPD PCR setups included 50-100 ng genomic DNA, 1.33 mM MgCl₂, 0.7 μM primer (Williams et al., 1990), 0.2 mM each dNTP, and 2.5 U *Taq* DNA polymerase (Fermentas, Thermo Scientific, USA) in the manufacturer-supplied buffer with 40 cycles of 30 s at 94°C, 45 s at 35°C and 2 min at 72°C (C1000™ Thermal Cycler, Bio-Rad Laboratories, USA). PCR products were separate in 2% agarose in Tris-borate buffer (TBE). 2-3 samples were analyzed for each cultivar. Cluster analyses were performed using PAST v.2.14 (Hammer et al., 2001).

RESULTS AND DISCUSSION

A new species of *Nymphaea* from Thailand is described in this report. Specimens of this species were previously identified as *Nymphaea* 'Jongkolnee', but its flowers are unmatched by any other *Nymphaea* species (Wasuwat, 1982; Chukiatman, 2006; Knotts, 2010). Considering its long history together with its distinctive characteristics, it is described here as a new species.

Scientific name

Nymphaea siamensis V. Puripanyavanich, sp. nov. (Figs. 1-4)

1. Type, Thailand, Chiang Mai, Queen Sirikit Botanic Garden, Mae Rim District, Alt. 800 m, 20 August 2000, W. La-onsri 204 (HOLOTYPE: QBG). The type is a cultivated specimen in the Queen Sirikit Botanic Garden Herbarium (QBG) grown at the Queen Sirikit Botanic Garden, and received from Pang ubon Garden.

Tuberous rhizomes erect, ovoid, to 15 cm long, to 5 cm in diameter, produces oval bulblets, when fully mature. Leaf simple, leaf blades subcoriaceous, alternate, ovate to broadly elliptic, to 25 cm long, to 23 cm wide, rounded or retuse at apex, peltate 0.5-3 mm from base of sinus, margins crenate and sinuate near sinus, sinus open with

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International Society for Horticultural Science

Acta Horticulturae books 1049 - 1000

Nr	Title	Place	Published
1035	VI International Symposium on the Taxonomy of Cultivated Plants	Beijing, China	May 2014

In Thailand, four native and one introduced species of *Nymphaea* have been recorded. Through herbarium consultations and explorations of wetlands, La-onsri and colleagues (2009) reported encounters of *Nymphaea cyanea*, *Nymphaea nouchali*, *Nymphaea pubescens*, *Nymphaea rubra* and *Nymphaea capensis*, the latter one being introduced in Thailand in 1897 (Chomchalow and Chansilpa, 2009). However, other native waterlilies still exist, which differ markedly from known *Nymphaea* species and have never been given a specific rank (Wasuwat, 1982; Horticultural Science Society of Thailand, 2007).

*vichaipuri@hotmail.com

Proc. 6th IS on the Taxonomy of Cultivated Plants

Eds.: Qixiang Zhang and Xiaobai Jin

Acta Hort. 1035, ISHS 2014

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Nymphaea siamensis

Code

Nymphaea siamensis V. Puripanyavanich, sp. nov.

Type: Thailand, Chiang Mai, Queen Sirikit Botanic Garden,
Mae rim district, alt. 800 m, 20 August 2000, W. La-onsri 204
(HOLOTYPE:QBG)

Part II Exploration of Thai native waterlilies

In Thailand, four native and one introduced species of *Nymphaea* have been recorded.

1. *Nymphaea cyanea*

2. *Nymphaea nouchali*

3. *Nymphaea pubescens*

4. *Nymphaea rubra*

(5) *Nymphaea capensis*

However, other native waterlilies might still exist, which differed markedly from the known *Nymphaea* species and had never been given a specific rank.

Exploration of Thai native waterlilies

◎ Before *N. siamensis* was given a scientific name, a group of researchers had tried to prove that *N. siamensis* had originated or could only be found in Thailand. Explorations were taken around Thailand to investigate native waterlilies which might be related to *N. siamensis*. Some of them were used as evidences for scientific nomenclature of *N. siamensis*, but some data were not previously published. A lot of varieties' details were interesting in many aspects and some varieties emerged as endangers and should be placed under conservation.

Exploration of Thai native waterlilies

- Waterlily samples were collected from multiple regions of Thailand. Samples of leaves, flowers, bulblets, rhizomes and seeds were collected, studied, and photographs were taken. The exploration areas included the followings:
 - - **Phu Sa Dok Bua National Park** in Yasothon and Mukdahan provinces,
 - - **Queen Sirikit Botanic Garden and Saraphi district** in Chiang Mai province,
 - - **Thale Ban National Park** in Satun province,
 - - **Thale-Noi Wildlife Conservation Cvelopment and Extension Station** in Phatthalung province,
 - - **Pang U Bon Waterlily Garden** in Nonthaburi province,
 - - **Nong Han Lake** near Kasetsart University, Sakon Nakhon campus, in Sakon Nakhon province,
 - - **Wetland areas in the northeastern part of Thailand** in Roi Et, Yasothon, Mukdahan, Khon Kaen, Ubon Ratchathani, and other provinces,
 - - **Wetland areas in the central plain** and the lower northern part of Thailand in Ayutthaya, Chai Nat, Phitsanulok, Sukhothai, and other provinces.

Exploration of Thai native waterlilies

The collected samples were propagated by rhizomes or bulblets at Queen Sirikit Botanic Garden in Chiang Mai and at Pang U Bon Waterlily Garden in Nonthaburi.

Morphology and taxonomy were studied at Queen Sirikit Botanic Garden.

Jongkolnee

The scientific nomenclature of 'Jongkolnee' has recently been accepted upon the publication of

"Nymphaea siamensis, the New Species of Waterlily in Thailand" in *Acta Horticulturae* no. 1035 in May, 2014.



‘Bua Khap’ or ‘Bua Khap Pak Klang’

(Nymphaea cyanea)

The name ‘Bua Khap’ might refer to many types of waterlilies. This was because the word “Khap” in Thai means the color of the feathers of a bird called Khap (Nok Khap). Its feathers have many color tones, but all are in the shade of light blue or purple blue. When the local people saw a waterlily with blue petals, they often called it ‘**Bua Khap**’.

'Bua Khap' or 'Bua Khap Pak Klang'

(*Nymphaea cyanea*)



‘Nilubon’ or ‘Bua Bae Muang’ (*Nymphaea cyanea* ?)

Our research group found ‘Bua Bae Muang’ in a natural pond in Roi Et. It is a tropical day blooming waterlily with light blue or purple blue petal shade. **Interestingly, bulblets were found attached to its rhizomes similarly to *N. siamensis*.**

After RAPD DNA fingerprinting was performed, it was determined that ‘Bua Bae Muang’ or ‘Nilubon’ was far different from ‘Bua Khap’

It was also called ‘Bua Khap’ in some old articles too.

‘Nilubon’ or ‘Bua Bae Muang’ (*Nymphaea cyanea* ?)



‘Bua Bae Kao’ waterlily (*Nymphaea sp.*)

Our research group found a white waterlily with very similar morphology to ‘Bua Bae Muang’ growing in Mukdahan. That’s the first time we knew about ‘Bua Bae Kao’. Its petals were white. **Also, it was found to produce bulblets very similar to *N. siamensis* and ‘Nilubon’.** This was the third waterlily variety in our exploration which produced bulblets that we have known of. **RAPD fingerprinting was performed and showed that ‘Bua Bae Kao’ was closed to ‘Nilubon’ or ‘Bua Bae Muang’ and in close relation to *N. siamensis*.**

‘Bua Bae Kao’ is a Thai native waterlily that must be urgently placed under a conservation program.

‘Bua Bae Kao’ waterlily (*Nymphaea sp.*)



‘Bua Phan’ and ‘Bua Phuean’ (*Nymphaea nouchali* Burm. f.)

- ◎ **‘Bua Phan’** is also well known in Thailand but harder to find in natural areas because of the development. ‘Bua Phan’ produces green leaves with faint brownish blotches on top, pink or blue violet underneath. The leaf shape is oval to round, with sinuate margin; the sinus is usually open. The size of leaf varies from 13-15 cm . The petal is pale bluish purple with bluish white sepal. The petal changes its color to pink after the second day of blooming. The flower has stellate shape with the size of 5-13 cm.
- ◎ **‘Bua Phuean’** is a waterlily that is found growing in rice fields, natural ponds and many places in every region Thailand. As many literatures described its characteristics, it has smaller flowers than another common variety called ‘Bua Phan’. The flower has stellate shape with the size of less than 5 cm. The petals are white with pale bluish purple tips .

‘Bua Phan’ and ‘Bua Phuean’ (*Nymphaea nouchali* Burm. f.)



Bua Phan



Bua Phuen

‘Bua Sai’ in Saraphi district, Chiang Mai (*Nymphaea pubescens* Willd.)

- ◎ This waterlily variety is a native waterlily, but no botanists had collected them previously. We found that ‘Bua Sai’ in Saraphi district showed distinctive characteristics. Its petals were not totally white, pink, or red like other ‘Bua Sai’ that are commonly found. The flowers of this particular specimen exhibited small white petals with a pale pink rib.
- ◎ Upon encountering it, our research team reported it to the Plant Varieties Protection Division, Department of Agriculture. Later, the authorities went to explore their origin and discovered that this waterlily variety was not isolated in its habitat. In addition to Saraphi district, Chiang Mai, it was also found in some other areas of Chiang Mai and Lamphun provinces with some variation in its characteristics.

‘Bua Sai’ in Saraphi district, Chiang Mai

(Nymphaea pubescens Willd.)



‘Bua Sai’ in Phu Sa Dok Bua National Park, Yasothon

(*Nymphaea pubescens* Willd.)

- ⦿ This waterlily variety is an isolated, native night blooming waterlily. It looked similar to ‘Bua Sai’ from Saraphi district, Chiang Mai.
- ⦿ The flowers of ‘Bua Sai’ found in Phu Sa Dok Bua national Park showed a varying pink shade on the outer side of their petals, but the inner side was white. It had rarely been found anywhere else and likely was facing extinction.
- ⦿ In the natural pond on a mountain top in Phu Sa Dok Bua National Park. The other commercial ornamental waterlilies were also found growing. This situation could threaten the existence of this particular ‘Bua Sai’ variety in its own habitat. Therefore, we informed the authorities of the situation and recommended that it should be placed under a conservation program.

‘Bua Sai’ in Phu Sa Dok Bua National Park, Yasothon
(Nymphaea pubescens Willd.)



‘Bua Sai’ Thale-Noi Wildlife Conservation Development and Extension Station, Phatthalung (*Nymphaea pubescens* Willd.)

- ◎ ‘Bua Sai’ found in Thale Noi Conservation Station looked similar to ‘Bua Sai’ from Phu Sa Dok Bua National Park and ‘Bua Sai’ from Saraphi district, Chiang Mai.
- ◎ The pink-white ‘Bua Sai’ in Thale-Noi Conservation Station was very highly specific in its habitat. It was found only in a small location which was called “Koh Nangyuan” and nearby area. However, ‘Bua Sai’ in Thale-Noi Conservation Station was still not endangered. During our later exploration trip, we could still find them growing in the same area without interruption from other invading waterlily species.

‘Bua Sai’ Thale-Noi Wildlife Conservation Development and Extension Station, Phatthalung (*Nymphaea pubescens* Willd.)



‘Bua Sai’ Thale Ban National Park, Satun (*Nymphaea pubescens* Willd.)

- ① ‘Bua Sai’ in Thale Ban National Park also belonged to *Nymphaea pubescens* species, but there was something unique.
- ② ‘Bua Sai’ from Thale Ban National Park had a long pink stripe running from the top to the bottom of their petals.
- ③ Similarly to ‘Bua Sai’ in Thale-Noi, this ‘Bua Sai’ was not yet endangered, likely because the population was rarely disturbed as it was growing in a faraway national park close to the border of Thailand and Malaysia where hardly any persons traveled to.

Surprisingly, this variety is easy to find out as a cut flower in ornamental market on Buddhist important days in the present time (2017). We try to ask the resources of this flowers and known that some farmers plant them in their gardens nearby Bangkok but the original natural resources still in doubt.

‘Bua Sai’ Thale Ban National Park, Satun (*Nymphaea pubescens* Willd.)



‘Bua Sai’ (*Nymphaea pubescens* Willd.)

It should be noted that at the present time, Thai native tropical night blooming waterlilies called ‘Bua Sai’ or ‘Bua Kinsai’ (*Nymphaea pubescens*) which have small pink-white flowers can be discovered in the north, northeastern and southern regions of Thailand, but cannot be found in the central plain area (except growing in collectors’ ponds). The reason for this situation because central Thailand is a highly populated and urbanized region with much fewer natural wetland areas, making it harder to find this particular group of waterlilies there.

'Bua Sai' (*Nymphaea rubra*)



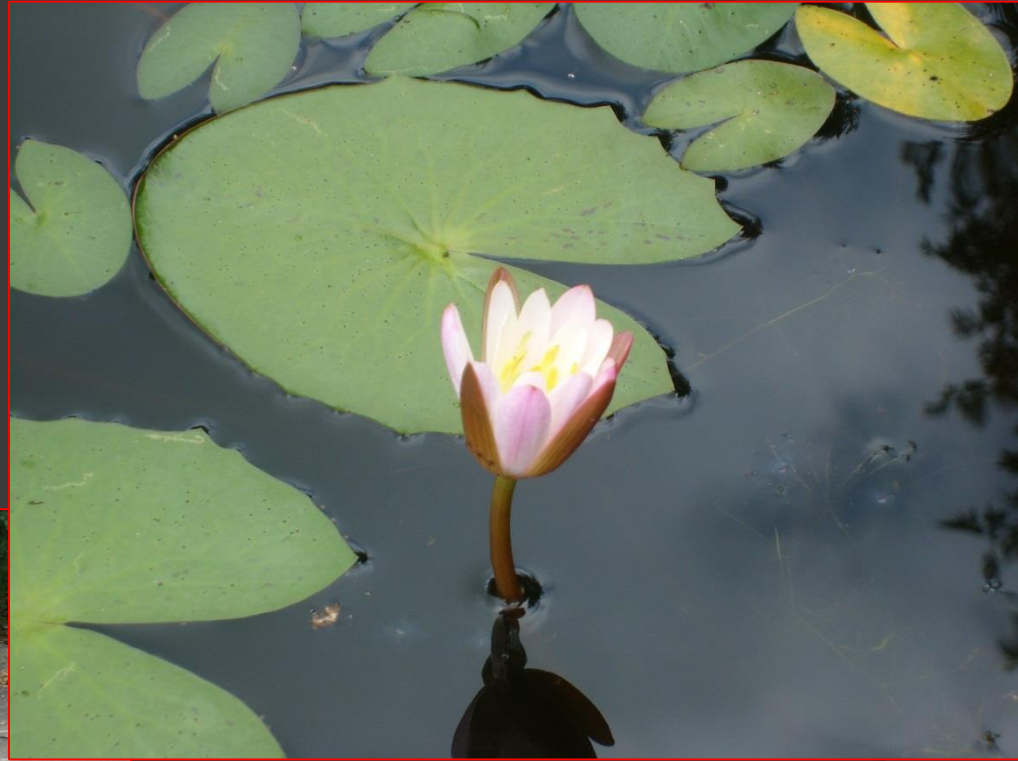
'Bua Sai' (*Nymphaea lotus*)



'Bua Sai' (*Nymphaea sp.*)



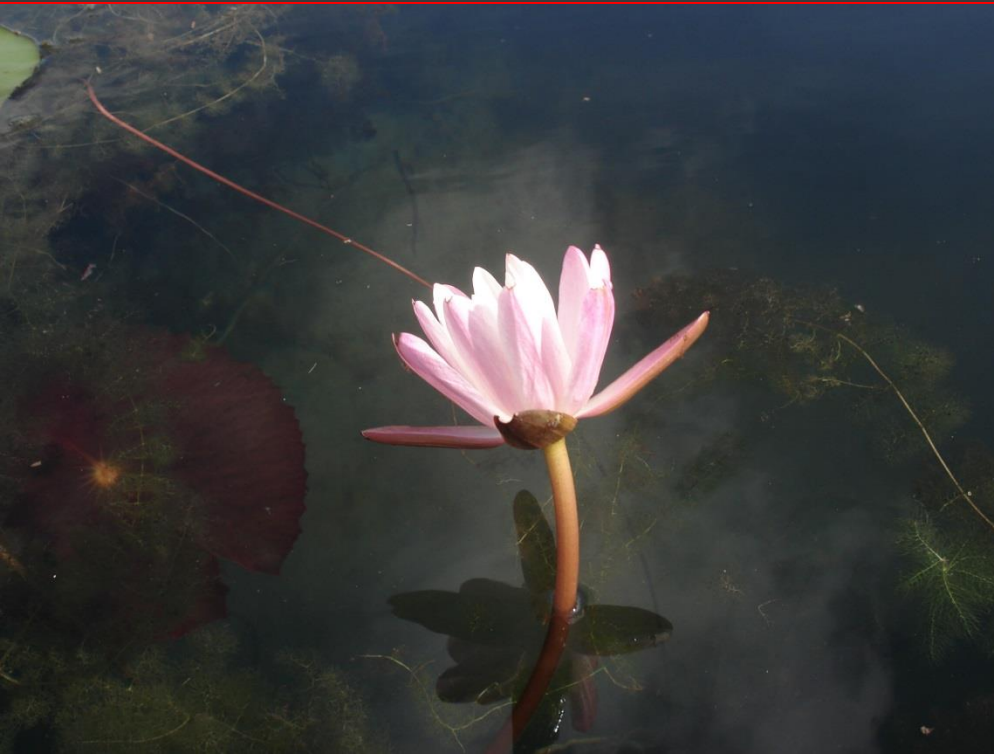
Exploration around Thailand



Exploration around Thailand



Exploration around Thailand



Exploration around Thailand



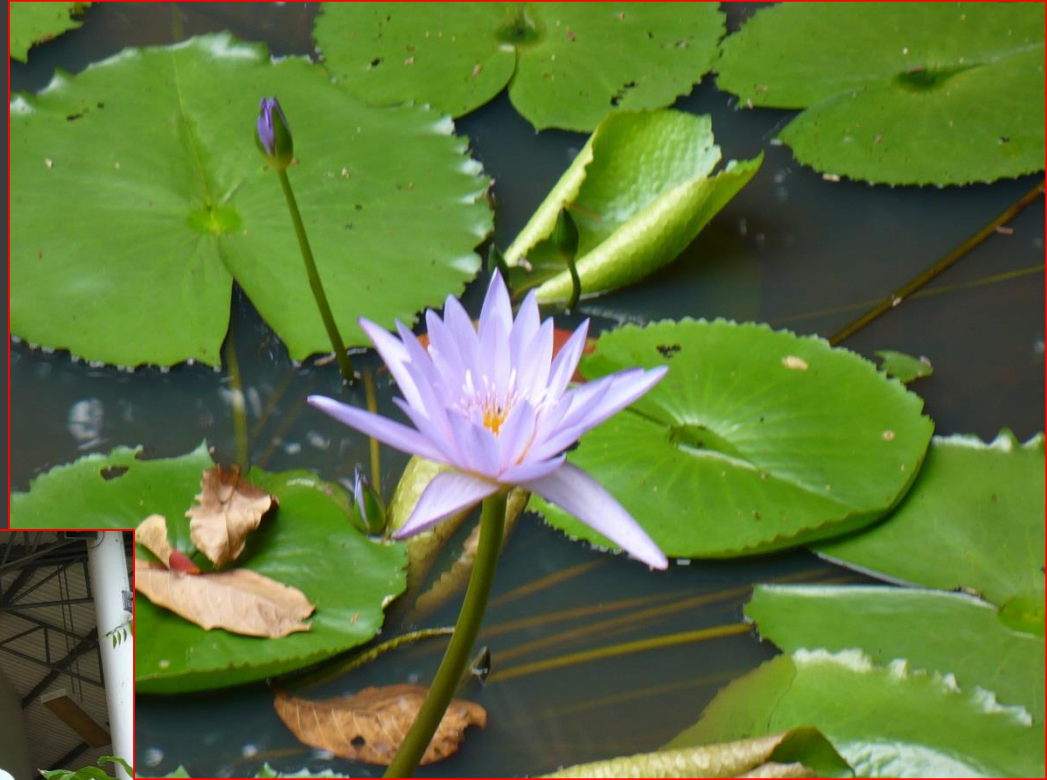
Exploration around Thailand



Exploration around Thailand



Exploration around Thailand



Genus *Nymphaea* L.

Group Apocarpiae

Apocarpous carpel

Subgenus *Anecphyta*

Australian water lily

Subgenus *Brachyceras*

Tropical day blooming water lily

Subgenus *Confluentes*

Australian water lily

Group Syncarpiae

Syncarpous carpel

Subgenus *Hydrocallis*

Subgenus *Lotos*

Tropical night blooming water lily

Subgenus *Nymphaea*

Sub-tropical water lily

Syncarpous and Apocarpous Carpels in Waterlily



Syncarpous ovaries
of subgenus *Nymphaea*

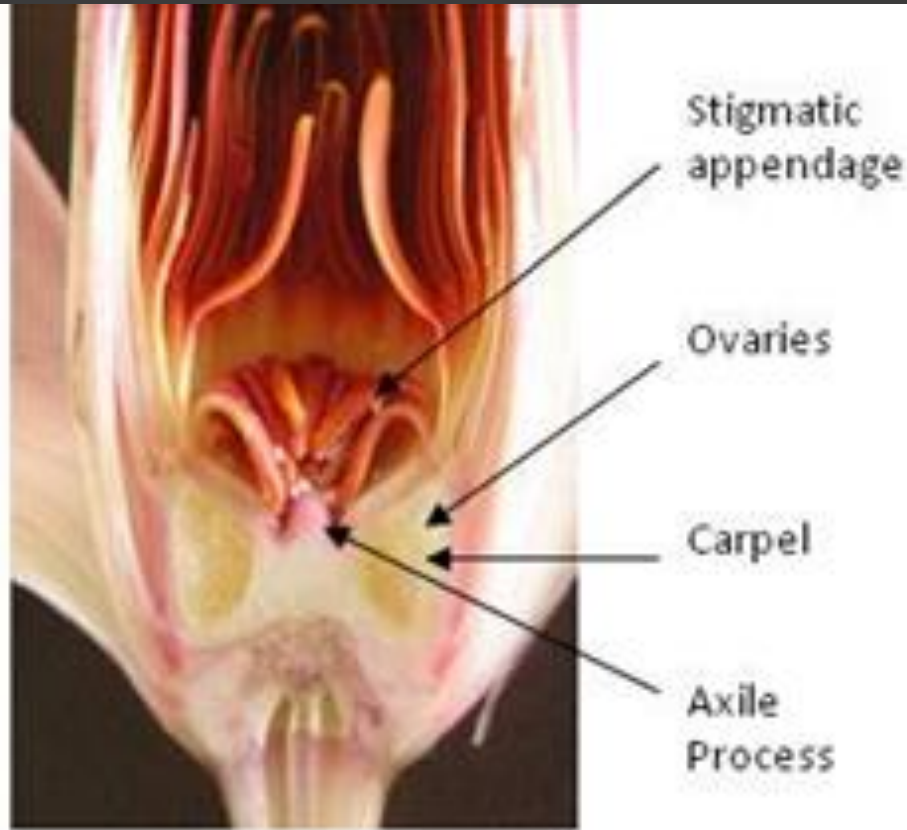


Apocarpous ovaries
of subgenus *Brachyceras*

Nymphaea siamensis 'Jongkolnee' has no locules

Nymphaea lotos

Nymphaea siamensis



Taxonomy of Genus *Nymphaea*.

- ⦿ Genus *Nymphaea* is categorized into two groups with a total of six subgenera, where the two groups are distinguished by **locule characteristics**;
- ⦿ **Apocarpiae group**: apocarpous ovaries consisting of separate carpels. There are three subgenera in this group: *Anecphyra*, *Brachyceras* and *Confluentes*.
- ⦿ **Syncarpiae group**: syncarpous ovaries consisting of united carpels. There are also three subgenera in this group: *Hydrocallis*, *Lotos* and *Nymphaea*.
- ⦿ *N. siamensis* has previously been placed under Apocarpiae group, subgenus *Brachyceras*. Is this correct?
- ⦿ ***N. siamensis* has no ovaries, no locules, no carpels, no pistils and no stamens.** It is only propagated asexually by bulblets. Therefore, *N. siamensis* could not essentially be placed under either Apocarpiae group or Syncarpiae group and could not be placed under any existing subgenera. In fact, ***N. siamensis* should be introduced to a new subgenus in non-carpiae group.**

กิตติกรรมประกาศ

ขอขอบคุณ

- ที่ปรึกษาโครงการ ดร.เสริมลาภ วสุวัต (ถึงแก่กรรม วันที่ 12 เมษายน พ.ศ. 2557)
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