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Systematics of Water Lilies (Genus *Nymphaea* L.) Using 18S rDNA Sequences

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Abstract**—**Water lily (*Nymphaea* L.) is the largest genus of Nymphaeaceae. This family is composed of six genera (*Nuphar*, *Ondinea*, *Euryale*, *Victoria*, *Barclaya*, *Nymphaea*). Its members are nearly worldwide in tropical and temperate regions. The classification of some species in *Nymphaea* is ambiguous due to high variation in leaf and flower parts such as leaf margin, stamen appendage. Therefore, the phylogenetic relationships based on 18S rDNA were constructed to delimit this genus. DNAs of 52 specimens belonging to water lily family were extracted using modified conventional method containing cetyltrimethyl ammonium bromide (CTAB). The results showed that the amplified fragment is about 1600 base pairs in size. After analysis, the aligned sequences presented 9.36% for variable characters comprising 2.66% of parsimonious informative sites and 6.70% of singleton sites. Moreover, there are 6 regions of 1-2 base(s) for insertion/deletion. The phylogenetic trees based on maximum parsimony and maximum likelihood with high bootstrap support indicated that genus *Nymphaea* was a paraphyletic group because of *Ondinea*, *Victoria* and *Euryale* disruption. Within genus *Nymphaea*, subgenus *Nymphaea* is a basal lineage group which cooperated with *Euryale* and *Victoria*. The other four subgenera, namely *Lotos*, *Hydrocallis*, *Brachyceras* and *Anecphya* were included the same large clade which *Ondinea* was placed within *Anecphya* clade due to geographical sharing.

Keywords**—**nrDNA, phylogeny, taxonomy, Waterlily

I. Introduction

NYMPHAEA L. is a large genus of family Nymphaeaceae comprising six genera: *Nuphar*, *Ondinea*, *Euryale*, *Victoria*, *Barclaya* and *Nymphaea*. Genus *Nymphaea* called water lily was described by [1], consisting of 5 subgenera: *Anecphya*, *Brachyceras*, *Hydrocallis*, *Lotos* and *Nymphaea* [2], [3]. The members of these subgenera have unique morphological characters in petal and stamen shape, blooming form and time because of worldwide distribution both in tropical and temperate zones. The water lily in subgenus *Anecphya* is called as Australian water lily distributing only in Australia continent including genus *Ondinea* as monotypic species (*O. purpurea* Hartog) and their flowers will bloom during the day. The members of two subgenera (*Brachyceras* and *Lotos*) are tropical water lily in Asia while members in subgenus *Hydrocallis* grow in tropical America as well. The subgenus *Nymphaea* or hardy water lily is distributed in northern hemisphere of temperate region i.e. America and Europe [4] Since members of this genus are widespread around the world, classification by using only morphological characteristics for this genus remains unclear so data increasing is needed for solving confused taxonomy. In modern time, molecular techniques were applied and extended for classification and identification because DNA molecules give stable and reliable information with accurate results.

Generally, plant classification and systematics were evaluated using DNA sequences or barcodes in regions of nuclear DNA (nrDNA) or chloroplast DNA (cpDNA) because of being unchangeable in various locations and stages of growth and development; moreover, molecular data help understand plant phylogeny and evolution [5]. In general, cpDNA markers for reconstructing phylogenetic relationship are ribulose-bisphosphate carboxylase gene (*rbcL*) and muturase K gene (*matK*). However,maternal inheritance lacks recombination so it is difficult to use for classifying in species level [6]. The other molecule widely used is ribosomal DNA such as Internal Transcribed Spacer (ITS) or 18S rDNA [7].

Ribosomal DNA (rDNA) is one of non-coding regions in nuclear DNA which codes for ribosomal RNA (rRNA). It has been used for studying molecular phylogenetics of any organism. When comparing with conserved coding region, rDNA sequences can be more changeable so they are more suitable for using in classification and genetic variation studies. Even though many copies of rDNA appeared in nuclear genome, each copy is highly similar because of sharing the same way in evolutionary trait. 18S rDNA sequences have been used for phylogeny reconstruction within many groups of eukaryotes [8] such as green algae [9], [10], group of bryophytes [11], yew family [12], conifer family [13], group of gymnosperms [14] and group of angiosperms [15]. Therefore, this paper attempted to investigate 18S rDNA for phylogenetic relationship analyses within genus *Nymphaea* and related genera for clarify classification comparing maximum parsimony, maximum likelihood and neighbor joining methods.

II. Materials and Methods

A. Plant Materials

Fresh leaves of 52 water lilies were collected from natural field around Phitsanulok, Thailand and some obtained as fresh leaf in silica gel from Suan Luang Rama IX botanical garden, Bangkok and Rajamangala University of Technology Tawan-ok, Chonburi, including as dry leaf in herbarium from Virginia Polytechnic Institute and State University in USA. All fresh

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samples in Thailand were collected as voucher specimens and deposited at herbarium of Department of Biology, Faculty of Science, Naresuan University, Photsanulok, Thailand. In addition, another sequencing data of related genera were referred from shared international GenBank database (Table I).

B. DNA Extraction

Genomic DNA solution was extracted from young leaves of all *Nymphaea* samples using two modified conventional CTAB methods depending on the amount of leaf. First method was prepared according to [16] for fresh leaf from field with the following modifications: After the samples were suspended in 6 ml 1X CTAB extraction buffer at 60 °C for 1 hr; 6 ml chloroform:isoamyl alcohol (24:1) was added and the samples were shaken gentle then centrifuged at 6000 rpm for 10 min in room temperature. The supernatant was transferred into 15 ml new tubes and 2/3 volume of isopropanol was added to precipitate DNA and then centrifuged at 13,000 rpm for 10 min in room temperature. The supernatant was discarded; the DNA pellet was washed with 0.5 ml of 70% ethanol and then centrifuged at 13,000 rpm for 5 min at room temperature. The pellet was dried at room temperature and then dissolved in 500 µl RNase buffer and 5 µl RNase A for RNA degradation. Next, 500 µl phenol:chloroform:isoamyl alcohol (25:24:1) was added and the samples were shaken gentle then centrifuged at 10000 rpm for 10 min at 4 °C. The supernatant was transferred to new microcentrifuge tube and 450 µl chloroform:isoamyl alcohol (24:1) was added and the samples were shaken gentle then centrifuged at 10000 rpm for 10 min at 4 °C. The supernatant was transferred to new microcentrifuge tube and 2 volume of absolute ethanol and 1/10 volume 3 M sodium acetate were added to precipitate DNA and then centrifuged at 13,000 rpm for 10 min at 4 °C. The pellet was washed by adding 500 µl of 70% ethanol before dissolving in 50-100 µl TE buffer and stored at −20 °C. Second method was modified from [17] for fresh leaf in silica gel and dry leaf from herbarium with the following modifications: After the samples were suspended in 600 µl 1X CTAB extraction buffer at 60 °C for 1 hr; 600 µl chloroform was added and the samples were shaken gentle then centrifuged at 10000 rpm for 10 min in room temperature. The supernatant was transferred to 1.5 ml new microcentrifuge tube and 10 µl of 10 mg/ml RNase A was added to degrade RNA at 37 °C and then repeated with 500 µl chloroform twice. The last supernatant was added 450 µl absolute ethanol and 45 µl 3 M sodium acetate to precipitate DNA and then centrifuged at 11,000 rpm for 10 min. The pellet was washed by adding 500 µl of 70% ethanol before dissolving in 50-100 µl TE buffer and stored at −20 °C.

All DNA solutions were checked for purity and concentration after 0.8% agarose gel electrophoresis and ethidium bromide staining using the GeneRuler 1 kb Ladder (Fermentas, Lithuania) and with a UV/Vis spectrophotometer (Analytik, Jena, Germany). The value of absorbance at 260 and 280 nm was used for calculating ratio 260/280 and concentration of extracted DNA.

TABLE I
SAMPLES OF GENUS *NYMPHAEA*

No.	Species	Subgenus
1.	*N. stellata* Willd. 1[a]	*Brachyceras*
2.	*N. stellata* Willd. 2[a]	*Brachyceras*
3.	*N. stellata* Willd. 3[a]	*Brachyceras*
4.	*N. stellata* Willd. 4[a]	*Brachyceras*
5.	*N. stellata* Willd. 5[a]	*Brachyceras*
6.	*N. colorata* Peter[c]	*Brachyceras*
7.	*N. thermarum* Eb.Fisch[d]	*Brachyceras*
8.	*N. elegans* Hook.[d]	*Brachyceras*
9.	*N. flavogrisea* Lehm.[d]	*Brachyceras*
10.	*N. capensis* Thunberg 1[c]	*Brachyceras*
11.	*N. capensis* Thunberg 2[c]	*Brachyceras*
12.	*N. capensis* Thunberg 3[c]	*Brachyceras*
13.	*N. gracillis* Zucc.[d]	*Brachyceras*
14.	*N. minuta* Landon[c]	*Brachyceras*
15.	*N. caerulea* Savigny[d]	*Brachyceras*
16.	*N. micrantha* Guill. and Perr[b]	*Brachyceras*
17.	*Nymphaea* 'Khao Thamanoon'[c]	*Brachyceras*
18.	*Nymphaea* 'Mueang Wiboonlak'[c]	*Brachyceras*
19.	*Nymphaea* 'Yellow Nang Kwaug'[a]	*Brachyceras*
20.	*Nymphaea* 'White Nang Kwaug'[a]	*Brachyceras*
21.	*Nymphaea* 'Violet Nang Kwaug'[a]	*Brachyceras*
22.	*Nymphaea* 'Pink Nang Kwaug'[a]	*Brachyceras*
23.	*Nymphaea* 'Jongkolnee'[c]	*Brachyceras*
24.	*Nymphaea* sp.[c]	*Brachyceras*
25.	*N. atrans* S.W.L. Jacobs[b]	*Anecphya*
26.	*N. gigantea* Hook.[c]	*Anecphya*
27.	*N. violacea* Lehm.[b]	*Anecphya*
28.	*N. immutabilis* S.W.L. Jacobs [b]	*Anecphya*
29.	*N. macrosperma* Merr. & L.M.Perry[d]	*Anecphya*
30.	*N. conardii* Wiersema 1[d]	*Hydrocallis*
31.	*N. conardii* Wiersema 2[d]	*Hydrocallis*
32.	*N. lingulata* Wiersema[d]	*Hydrocallis*
33.	*N. prolifera* Wiersema[d]	*Hydrocallis*
34.	*N. jamessoniana* Planch.[d]	*Hydrocallis*
35.	*N. petersiana* Klotzsch[d]	*Lotos*
36.	*N. pubescens* Willd. 1[a]	*Lotos*
37.	*N. pubescens* Willd. 2[a]	*Lotos*
38.	*N. pubescens* Willd. 3[a]	*Lotos*
39.	*N. pubescens* Willd. 4[a]	*Lotos*
40.	*N. pubescens* Willd. 5[c]	*Lotos*
41.	*N. lotus* L. (Chompoolinjon)[c]	*Lotos*
42.	*N. lotus* L. (Khao Suan Luang)[c]	*Lotos*
43.	*N. rubra* Roxb.[c]	*Lotos*
44.	*N. alba* L.[b]	*Nymphaea*
45.	*N. odorata* Aiton[d]	*Nymphaea*
46.	*N. tuberose* Paine 1[d]	*Nymphaea*
47.	*N. tuberose* Paine 2[d]	*Nymphaea*
48.	*N. mexicana* Zucc.[d]	*Nymphaea*
49.	*N. tetragona* Georgi[d]	*Nymphaea*
50.	*Nymphaea* 'Sunrise'[c]	-
51.	*Victoria amazonica* (Poepp.) J.C. Sowerby 1[c]	-
52.	*Victoria amazonica* (Poepp.) J.C. Sowerby 2[d]	-
53.	*Ondinea purpurea* Hartog	-
54.	*Nuphar variegate* Durand.	-
55.	*Barclaya longifolia* Wall.	-
56.	*Euryale ferox* Salisb.	-
57.	*Brasenia schrebeni* J.F.Gmel.	-
58.	*Cabomba caroliniana* A.Gray	-
59.	*Amborella trichopoda*	-

[a]Provinces of Thailand
[b]Rajamangala University of Technology Tawan-ok, Chon Buri, Thailand
[c]Public park and Botanical Garden in Bangkok, Thailand
[d] Virginia Polytechnic Institute and State University in USA

C. DNA Amplification and Sequencing

Amplicons of all samples were amplified using specific

primers designed from 4 sequences alignment of outgroups (no. 53-56 in Table I) in region of 18S rDNA nuclear gene, namely W18S-F1 (5'-AAG ATT AAG CCA TGC ATG GG-3') and W18S-R1 (5'-AGG TTC AAT GAA CTT CTC GC-3'). Amplification was performed in a GeneAmp PCR Systems 9700 thermal cycler (Applied Biosystems, USA) as follows: 5 min at 94 °C; 30 cycles of 1 min at 94 °C, 1.30 min at 54-56 °C depending on samples, 3 min at 72 °C; followed by final extension for 5 min at 72 °C. Size and yield of PCR amplicons were detected by electrophoresis through a 1% agarose gel and ethidium bromide staining using the GeneRuler 1 kb Ladder (Fermentas, Lithuania). PCR products were sequenced directly at Macrogen Inc. in South Korea after purification by HiYield™ Gel/PCR DNA Fragments Extraction Kit (RBCBioscience, Taiwan)

D. Sequence Alignment and Phylogenetic Tree Construction

All sequencing data were compared with GenBank database using BLASTn (ncbi.nlm.nih.gov) for getting right sequences of plants before analyses in next step. Upon completion of DNA sequencing, base calling was verified by testing fluorographs in Chromas version 1.45 [18] and corrections were made as necessary. Sequence alignment was done manually using GeneDoc version 2.6.002 [19] and checked again by ClustalX [20]. Phylogenetic analyses were inferred using maximum parsimony, maximum likelihood and Neighbor joining methods in MEGA 5.2 [21]. To search for the phylogenetic tree, heuristic search with hundred random sequence additions, tree bisection reconnection (TBR) branch swapping were used. Bootstrap was used to obtain a measure of support for each branch. Thousand bootstrap replications were carried out using full heuristic search. Each representative species of genus *Brasenia*, *Cabomba* and *Amborella* sequences were used as outgroups. The results of phylogenetic tree analysis were compared with morphological traits of *Nymphaea*.

III. Results

A. DNA Extraction and Amplification

DNA was extracted from young leaves using two modified CTAB method according to [16] and [17]. Most of extracted DNA solutions were colorless but some samples showed light yellow color. From electrophoresis result, size of DNA strand was larger than 10 kb comparing with 1 kb standard ladder. Some samples especially that are from herbarium specimens showed smear of DNA which caused by fragmentation. On the other hand, extracted DNA using modified method from [17] showed a better quality and more quantity than method [16] using spectrophotometer. All DNA samples were amplified by using PCR technique with specific primer to 18S rDNA that showed specific band (approx. 1650 bp).

B. Phylogenetic Analyses

The sequencing results of all samples have 1,575 bp of *N. alba* to 1578 bp (*N. lotus* (Khao Suan Luang)). The sequence alignment of all samples is 1,582 bp in length. The conserved characters are 1,434 bp (90.64%) and the variable characters are 144 bp (9.36%) composing of 42 bp (2.66%) of phylogenetically informative sites and 102 bp (6.70%) of singleton sites.

C. Maximum Parsimony Tree

The maximum parsimony trees (Fig. 1) showed that all species in genus *Nymphaea* were separated from out groups with high bootstrap support (100%). The result pointed that water lilies in genus *Nymphaea* were divided into two major clades. The first major clade was members of *Victoria*, *Euryale* and subgenus *Nymphaea* whereas the second clade was composed of subgenus *Lotos*, *Hydrocallis*, *Brachyceras* and *Anecphya-Ondinea* which supported with moderate bootstrap (80%). Moreover, *Ondinea purpurea*, monotypic species, was grouped within the same clade of subgenus *Anecphya*.

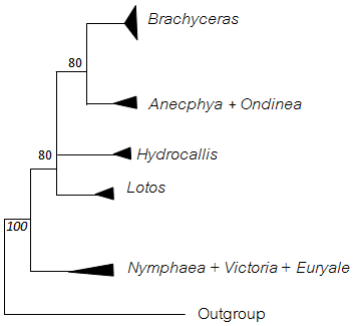

Fig. 1 Maximum parsimonious tree

D. Maximum Likelihood Tree

Comparing maximum parsimony analysis, tree topology from maximum likelihood (Fig. 2) looked similar but it showed very low bootstrap support (<60%). The genetic relationship of genus *Nymphae*a was unclear because it could be divided into many clades although subgenus *Nymphaea* clade was disrupted by *Euryle* and *Victoria*. Meanwhile, other clades were still formed following to four subgenera and *Ondinea* was still placed in subgenus *Anecphya* with low bootstrap.

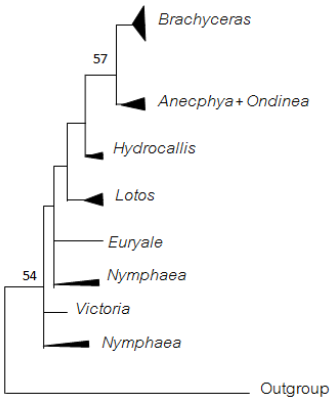

Fig. 2 Maximum likelihood tree

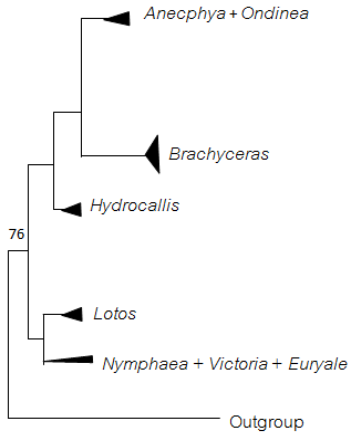

Fig. 3 Neighbor joining tree

E. Neighbor Joining

Comparing maximum parsimony and maximum likelihood, the neighbor joining tree (Fig. 3) was slightly different because two subgenera, namely *Lotos* and *Nymphaea*, were joined in the same clade while other subgenera, particularly *Hydrocallis*, *Barchycera* and *Anecphya*, were formed in the other clade. Both clades were separated with moderate bootstrap support (76%). The former clade, subgenus *Nymphaea* still related closely with genus *Victoria* and genus *Euryale*. The latter clade, subgenus *Anecphya* was grouped with *Ondinea*.

IV. Discussion

Phylogenetic trees performed by three genetic distance analyses showed that the genetic relationship based on 18S rDNA of genus *Nymphaea* was concordant with morphological data [8] and previous works for chloroplast DNA data [3], [24]. Phylogenetic tree from maximum parsimony showed the highest bootstrap value for supporting clades. This is meant that parsimonious tree was more reliable than both trees from maximum likelihood and neighbor joining methods. However, trees from maximum parsimony and neighbor joining pointed to 5 distinct clades, namely *Nymphaea*+ *Victoria*+*Euryale*, *Lotos*, *Brachyceros*, *Anecphya*+*Ondinea* and *Hydrocallis*; whereas, tree from maximum likelihood separated subgenus *Nymphaea* into two clades and both genera, *Victoria* and *Euryale*, as well.

Totally, phylogenetic trees presented 5 clades in genus *Nymphaea* which was paraphyletic group unless it included 3 genera of *Victoria*, *Euryale* and *Ondinea*. Subgenus *Nymphaea* was placed as basal lineage with highly support while other subgenera were grouped together in large clade. Both groups were divided following to the ecological habitat. Members of subgenus *Nymphaea* or hardy water lily distributed in temperate zone of America and Europe continents. The others in large clade were tropical water lilies [9] dispersed around the world. In addition, *Victoria* and *Euryale* were grouped with *Nymphaea* clade because triple of them shared the same distribution. Large clade was composed of three subgroups; *Lotos*, *Hydrocallis* and *Anecphya*-*Brachyceras*. Two subgenera, namely *Anecphya* and *Brachyceras* were sister taxa which shared the apocarpous pistil characteristic while *Lotos* and *Hydrocallis* subgenera shared syncarpous pistil [22]. Subgenus *Anecphya* was separated from *Brachyceras* using stamen shape without appendage. Subgenus *Anecephyta* was round shape while *Brachyceras* was slim shape. Subgenus *Lotos* was isolated from *Hydrocallis* with stamen shape, appendage presence and flower blooming. Members in *Lotos* have straight stamen, no appendage and flower blooming ranged from half-circle to semi-circle*;* whereas, species in *Hydrocallis* have slim stamen with appendage and blooming as bell-shape. Furthermore, *Ondinea purpurea* was clustered with Australian water lilies in subgenus *Anecphya* because of geographical sharing

In this study, members in subgenus *Nymphaea* were consisting of 5 species and 1 cultivar. All are day blooming, syncarpous pistil, no appendage on the top of stamen and entire edge of leaf. All species of this subgenus distributed around northern hemisphere of temperate region. *N. alba* and *N. odorata* are sister taxa because of white petal character sharing while *N. mexicana* presents yellow petal liked as *Nymphaea* 'Sunlight'. *Nymphaea* 'Sunrise' is the hybrid of *N. mexicana* and *N. odorata* [22], [23]. *N. tuberosa* used to be variety of *N. odorata*; however, this molecular study showed that both species appeared distinct position on the tree and did not form sister taxa. In addition, genus *Victoria* and genus *Euryale* were included inside this clade based on maximum parsimony and neighbor joining analyses according to previous research based on combined data among morphological characters, *rbcL*, *matK* and 18S rDNA sequences [23] even though published data based on *trnT-L-F* indicated that *Victoria-Euryale* clade was separated and placed as basal lineage of genus *Nymphaea* [24] similar to maximum likelihood tree in this study.

Members of subgenus *Lotos* were consisting of 9 samples of 4 species, namely *N. lotus*, *N. pubescens*, *N. rubra* and *N. petersiana*. All distributed in tropical region of Asia and Africa and their flowers were blooming at night. The shared characters of this subgenus were petiole, peduncle and beneath leaf was covered with numerous hairs. Syncarpous pistil, serrate leaf margin, appendages appearance at stamen and pistil were found in waterlilies of this subgenus. *N. pubescens* and *N. rubra* were very closely related; it was reported that *N. rubra* was derived from *N. pubescens* [22], [23], [25]. There are a few distinct characters between two species; first is shade of petal color which is pale pink in *N. pubescens*, dark pink or red in *N. rubra* and second is leaf color because leaf surfaces of *N. pubescens* show greenish in upper and reddish in lower whereas *N. rubra* is reddish in both of leaf surfaces. Furthermore, *N. lotus* is greenish in both leaf surfaces as well. Hence, the color of leaf surface may be one efficient character to classify triple species of subgenus *Lotos* in preliminary surveys. Anyways, *N. lotus* is easy to classify and separate from *N. pubescens* since when flower is blooming, floral shape of *N. lotus* acts as reflex angle (more than 180 degree) while floral shape of *N. pubescens* blooms as acute angle (less than 180 degree). Generally, petals of *N. lotus* are white or

pink. Firstly based on morphology, *N. petersiana* is classified within subgenus *Brachyceras*. Much molecular information such as ITS [26] and *trnT-L-F* [3] supported to move *N. petersiana* to subgenus *Lotos*.

Waterlilies in subgenus *Hydrocallis* in this study presented 5 samples in 4 species which were all found in neotropical zone and South-Africa. Their flowers will bloom at night and pistil shows syncarpous. Stamen and pistil also presented appendages. The genetic relationship in this subgenus is unclear because each species was separated and showed polytomy. Moreover, the data of them are still rare except in 2011 Borsch et al. [27] posted that this subgenus migrated to the New World while subgenus *Lotos* bended to the Old World and radiated to South America during the Miocene. Based on *trnT-L-F* sequences, subgenus *Hydrocallis* was sister taxa to subgenus *Lotos* with high bootstrap support (92%).

Subgenus *Anecphya* consists of 5 species distributed only in Australia and New Guinea [24]. Flowers of members in this genus will open at daytime and pistil shows apocarpous. Both male and female reproductive parts have no appendage. The margin of leaf is undulate. The genetic relationship of these 5 species was unsolvable using only 18S rDNA. The combination of many DNA regions, for example ITS for nuclear DNA and *trnT-L-F* for chloroplast DNA, could separate into two clades. Firstly, clade of *N. gigantea* and *N. macrosperma* presented large seed. Secondly, clade of *N. atrans* and *N. immutabilis* shared many similar characteristics [26], [23]. *N. atrans* is only species that can change petal color from white to pink according to ages of flower and light intensity. In subgenus *Anecphya* clade, it was noticeable that *Ondinea purpurea* was added. This relationship was also presented in phylogenetic tree using *trnT-L-F* chloroplast DNA sequences [24]. The result was found that *O. purpurea* was classified to the same group with subgenus *Anecphya* because of shared geographical distribution [22], even though it presented different morphological characteristics. Genus *Ondinea* is monotypic containing only one species; therefore, many reports suggested that *O. purpurea* should be transferred into genus *Nymphaea* and called *Nymphaea ondinea* [3], [24], [26], [27].

Most specimens were belonging to subgenus *Brachyceras* consisting of 24 samples in 10 species, 6 cultivars, 1 expected natural variety (*Nymphaea* 'Jongkolnee') and 1 unknown species. All members of this subgenus are distributed in tropic area. Their flowers will bloom at daytime and their leaf margin is undulate. Both stamen and pistil appear appendages and pistil is apocarpous. Following phylogenetics analysis, it was proposed that the relationship within this subgenus was unclear except *N. stellate* no.4 and *Nymphaea* sp. because they shared character of flower shape; however, the bootstrap support value of this subclade was below 50%.

Five species were proposed as outgroups consisting *Amborella trichopoda*, *Cabomba* spp. and *Brasenia* spp. Two species representative from genus *Nuphar* and *Barclaya* were settled as basal lineages of this family respectively in neighbor joining tree. Genus *Nuphar* showed outer seed coat in hood-shape and cup-shape which were derived from semiannular integument [28]. Furthermore, its micropyle was separated from hilum with narrow testa which was similar to *Cabomba* [29], [30] and its leaf shape was lanceolate as primitive character [31].

V. Conclusion

Phylogenetic relationship analysis from18S rDNA sequences showed that the relationship within genus *Nymphaea* was paraphyletic group. This genus has been disturbed by *Victoria*, *Euryale* and *Ondinea* genus. Genus *Nymphaea* could be divided into 5 clades which were concordant with morphological traits and previous chloroplast DNA identification.

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