

## The phylogenetic position of *Aglaodorum* Schott (Araceae – Aroideae – Aglaonemateae)

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*Abstract:* Analysis of chloroplast DNA sequences (trnL intron and trnL-trnF intergenic spacer) of the helophytic colony-forming *Aglaodorum griffithii* (Schott) Schott and other representative species of Araceae supported its placement within *Aglaonema* Schott. It is therefore proposed that *Aglaodorum* Schott should be recognised as a generic synonym of *Aglaonema*.

*Keywords:* Araceae, *Aglaonema griffithii*, *Aglaodorum griffithii*, trnL, trnL-trnF.

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### Introduction

*Aglaonema griffithii* Schott was first described by Schott (1856) from Peninsular Malaysia. Two years later, he realized that this species have some differences from other species of *Aglaonema* Schott (see below) and so he created a new genus, *Aglaodorum* Schott, and transferred *Aglaonema griffithii* Schott into it (Schott 1858). Schott's view was not always followed, however. For example, Teijsmann & Binnendijk (1863) described *Aglaonema palustre* Teijsm. & Binn., which is a synonym of *Aglaodorum griffithii* (Schott) Schott (Boyce et al. 2012), from Sumatra, and

Hooker (1883: 981) and Ridley (1925: 100) both treated *Aglaodorum* as a synonym of *Aglaonema*. Nevertheless, Engler, the great authority on Aroids after Schott, maintained *Aglaodorum* as a genus allied to but separate from *Aglaonema* (Engler 1915: 34), and since then *Aglaodorum* has been accepted as a monotypic genus the sole species of which is distributed in southern Cambodia, southern Vietnam, south and west through Peninsular Malaysia and Sumatra, and east to northern and western Borneo (Mayo et al. 1997; Boyce et al. 2012).

The morphology of *Aglaodorum griffithii* is known to be extremely similar to that of *Aglaonema*, especially in the structure of spathe, male and female spadix, ovary, stigma, ovule and leaf blade (Nicolson 1969). However, there are some notable differences: *Aglaodorum griffithii* is traditionally distinguished from the species of *Aglaonema* by having one whorl of pistillate flowers (versus several whorls) and peduncles of 40-50 cm in length (versus the much shorter ones). Moreover, *Aglaodorum griffithii* has large green spongy fruits adapted to floating dispersal, spongy rhizomes, peduncles and petioles apparently adapted to growing on soft mud and in water, and is helophytic in open conditions, while *Aglaonema* species are terrestrial on tropical forest floor, almost always in shade and the fruits are red or more rarely pink and bird-dispersed (Schott 1858; Nicolson 1969; Mayo et al. 1997; Boyce et al. 2012). However, these morphological characteristics are known individually to vary greatly within other genera of Araceae. Arrangement of pistillate flowers in only one whorl is known in *Aglaonema costatum* N.E.Br. (Ridley 1925) and thus it is not a distinct characteristic for *Aglaodorum*. We therefore set about investigating whether these genera really can be maintained distinct.

Here, we analyse molecular data of eleven taxa of Araceae and one Acoraceae in order to determine the phylogenetic affinity of *Aglaodorum griffithii*. We also evaluated taxonomic usefulness of morphological characters that have traditionally placed *Aglaodorum griffithii* within *Aglaonema*.

## Material and Methods

Eleven taxa of Araceae and one Acoraceae used in this study were collected from southern regions of Vietnam (Tab. 1), including three species of *Aglaonema*. Each taxon had one sample except for *Aglaodorum griffithii* with three samples from the Mekong Delta provinces of Tien Giang (H. T. Van 103A), Ben Tre (H. T. Van 103B) and Long An (H. T. Van 103C). All voucher specimens are stored at SGN Herbarium. The trnL and trnL-trnF sequences obtained were submitted to GenBank and the assigned accession numbers are listed in Tab. 1.

Total genomic DNA was extracted from fresh leaf tissues using a Genomic DNA Purification Mini Kit (Thermo, USA). The trnL intron and trnL-trnF IGS chloroplast DNA region were amplified by polymerase chain reaction (PCR). List of primers is shown in Tab. 2. The PCR reactions were performed in an Eppendorf Mastercycler Gradient using a volume of 25µl reaction mixture: 12.5 µl go taq green master mix (Promega, USA), 1.25µl of each forward and reverse primers (10 µM), 9.5µl ultrapure water and 0.5 µl DNA template (25ng). PCR cycles consisted of an initial denaturation

**Tab. 1 Specimens of eleven Araceae and one Acoraceae species used in this study.**

Studied specimens (SGN)	Species	Accession numbers ( <i>trnL</i> intron/ <i>trnL-trnF</i> IGS)
H.T.Van 99	<i>Acorus calamus</i> L.	MN844006/MN844022
H.T.Van 103A	<i>Aglaodorum griffithii</i> (Schott) Schott	MN844014/MN844028
H.T.Van 103B	<i>Aglaodorum griffithii</i> (Schott) Schott	MN844015/MN844029
H.T.Van 103C	<i>Aglaodorum griffithii</i> (Schott) Schott	MN844016/MN844030
H.T.Van 23	<i>Aglaonema cochinchinense</i> Engl.	MN844007/MN844023
H.T.Van 91	<i>Aglaonema simplex</i> (Blume) Blume	MN844012/MN844026
H.T.Van 116	<i>Aglaonema costatum</i> form. <i>immaculatum</i> (Ridl.) Nicolson	MN844008/MN844020
H.T.Van 79	<i>Arisaema roxburghii</i> Kunth	MN844011/MN844025
H.T.Van 18	<i>Colocasia esculenta</i> (L.) Schott	MN844013/MN844027
H.T.Van 62	<i>Alocasia macrorrhizos</i> (L.) G.Don	MN844009/MN844024
H.T.Van 03	<i>Amorphophallus paeoniifolius</i> (Dennst.) Nicolson	MN844010/MN844021
H.T.Van 102	<i>Pistia stratiotes</i> L.	MN844018/MN844032
H.T.Van 61	<i>Pothos chinensis</i> (Raf.) Merr.	MN844017/MN844031
H.T.Van 42	<i>Typhonium trilobatum</i> (L.) Schott	MN844019/MN844033

for 5 min at 95°C; 35 cycles of denaturation (1 min at 94°C), annealing (1 min at 50°C) and extension (1:30 min at 72°C); and a final extension at 72°C for 10 min. The PCR products were visualized on 1.5 % agarose gel and sent for purification and direct sequencing at Nam Khoa Biotek Company Ltd (Vietnam) by using ABI 3130 XL Sequencer.

For multiple alignments, the ClustalW (Thompson et al. 1994) was used to recognize the homology between sequences. Phylogenetic analysis was carried out with the software PAUP\* 4.0a146 (Swofford 2002) and MrBayes (Ronquist & Huelsenbeck 2003) using the maximum parsimony and Bayesian methods with *Acorus calamus* L. (Acoraceae) as outgroup, following Cabrera et al. (2008); Cusimano et al. (2011); Nauheimer et al. (2012). The maximum parsimony trees were calculated based on chloroplast sequence data with gaps treated as missing data and heuristic search algorithms (Nei & Kumar 2000) with the following parameters: 1000 random addition sequence replicates, tree bisection reconnection (TBR) branch swapping, 10 parsimonious trees held after each replicate (Yulita et al. 2005; Harrison & Langdale 2006). All characters were equally weighted and treated as unordered (Fitch 1971). The fit of characters to the trees was also tested by calculating the consistency index (CI), the retention index (RI) and the rescaled consistency index (RC) (Kluge & Farris 1969; Farris 1989). The Bayesian Markov-chain Monte Carlo (MCMC) was constructed on the software MrBayes (Ronquist & Huelsenbeck 2003). The algorithm used in this study was a two million-generation MCMC with one cold and three heated chains, which was set to start from random trees and sampled every 100<sup>th</sup> generation. The standard deviation of split frequencies was used as an index to assess the convergence, the values of which

**Tab. 2 Primers used in the present study. (\*) direction of primer F (forward), R (reverse).**

Primers (*)	Regions	Sequence (5'-3')	References
A (F)	<i>trnL</i> intron	CGAAATCGGTAGACGCTACG	Taberlet et al. (1991)
B (R)	<i>trnL</i> intron	GGGGATAGAGGGACTTGAAC	
C (F)	<i>trnL-trnF</i> IGS	GGTTCAAGTCCCTCTATCCC	
D (R)	<i>trnL-trnF</i> IGS	ATTGAACTGGTGACACGAG	

**Tab. 3 Mean pairwise genetic distances between ingroup species based on the combined data set of *trnL* intron and *trnL-trnF* IGS.**

	1	2	3	4	5	6	7	8	9	10	11
1. <i>Aglaonema costatum</i>											
2. <i>Aglaodorum griffithii</i>	0.003										
3. <i>Aglaonema simplex</i>	0.006	0.009									
4. <i>Aglaonema cochinchinense</i>	0.017	0.020	0.020								
5. <i>Pothos chinensis</i>	0.065	0.068	0.068	0.080							
6. <i>Amorphophallus paeoniifolius</i>	0.046	0.049	0.049	0.061	0.087						
7. <i>Alocasia macrorrhizos</i>	0.061	0.065	0.065	0.073	0.105	0.051					
8. <i>Pistia stratiotes</i>	0.078	0.082	0.082	0.087	0.127	0.068	0.053				
9. <i>Arisaema roxburghii</i>	0.072	0.075	0.075	0.075	0.109	0.058	0.035	0.061			
10. <i>Typhonium trilobatum</i>	0.059	0.063	0.062	0.071	0.104	0.046	0.038	0.054	0.046		
11. <i>Colocasia esculenta</i>	0.056	0.060	0.059	0.068	0.096	0.043	0.028	0.046	0.038	0.030	

reaching <0.005 are considered as a good convergence. The initial 10% of trees were discarded as burnin while the remaining of them were used to build up 50% majority-rule consensus trees. The pairwise genetic distances (Kimura 1980) were calculated using the software MEGA6 (Tamura et al. 2013).

## Results and discussion

The length of combined data set (*trnL* intron and *trnL-trnF* IGS) ranged from 760 to 892 bp. The entire aligned length of two regions is 976 bp. The mean pairwise genetic distances between the genera based on the combined data set are shown in Tab. 3 with the lowest value belonging to *Aglaonema* and *Aglaodorum*. Accordingly, the genetic distances between *Aglaodorum griffithii* and *Aglaonema costatum* f. *immaculatum* (Ridl.) Nicolson, *A. simplex* (Blume) Blume and *A. cochinchinense* Engl. were 0.003, 0.009 and 0.020, respectively. The phylogenetic analysis of the combined data set resulted in one of the most-parsimonious tree (length: 471 steps, CI: 0.82, RI: 0.79, RC: 0.71) and the Bayesian tree (Fig. 1 & 2). Both analyses grouped *Aglaodorum* and *Aglaonema* in one clade with maximum bootstrap value (100%). All specimens of *Aglaodorum griffithii* (103A, 103B, 103C), *Aglaonema simplex* and *A. costatum* f. *immaculatum* are grouped together and nested in *Aglaonema* clade with *Aglaonema cochinchinense* as sister.

Our phylogenetic analyses are consistent with previous studies on the generic groups, indicating the very close affinity of *Aglaonema* and *Aglaodorum*, both belonging to the tribe Aglaonemateae (Mayo et al. 1997; Cabrera et al. 2008; Cusimano et al. 2011; Nauheimer et al. 2012). However, these studies only used one species of *Aglaonema* and *Aglaodorum griffithii* to construct the phylogenies while three species of *Aglaonema* were analysed in our study. All individuals of *Aglaodorum griffithii* collected from three different provinces in southern Vietnam are clearly nested within *Aglaonema* in both the maximum parsimony or Bayesian trees. Therefore, we propose that the monotypic genus *Aglaodorum* should be reduced to the synonymy of *Aglaonema* and the name *Aglaonema griffithii* be resurrected.

As an aside, an interesting parallel is found in another Asian aroid group, *Homalomena* Schott, which is an almost entirely forest floor genus with one species being a colony-forming helophyte that grows in open conditions (often with *Aglaodorum*) – the Bornean *H. expedita* A.Hay & Hersh. (Hay & Hershovitch 2002), the position of which within *Homalomena* Schott, despite distinctive reproductive features (Wong et al. 2011) is also supported by molecular studies (Wong et al. 2013).

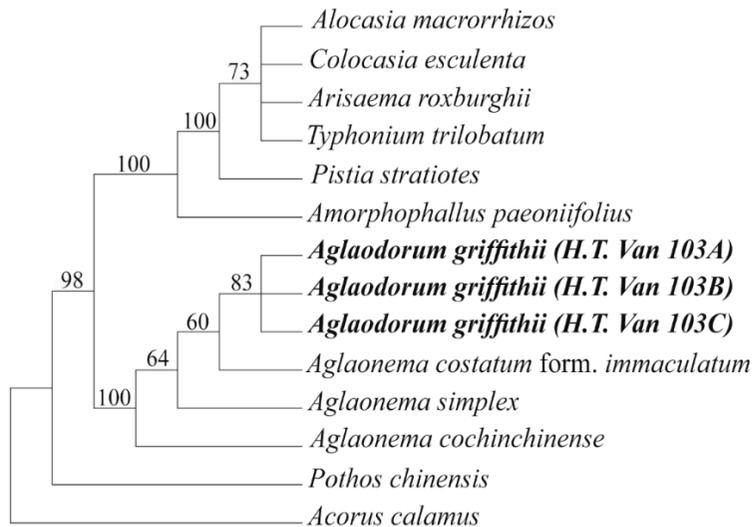
#### **Taxonomic treatment**

*Aglaonema griffithii* Schott, Syn. Aroid. 123, 1856; Hook. f., Fl. Brit. India 6: 528, 1893; Ridl., Mat. Fl. Malay Penins. 19, 1907; Ridl., Fl. Malay Penins. 5: 100, 1925; – *Aglaodorum griffithii* (Schott) Schott, Gen. Aroid. 58: 58, 1858; Schott, Prodr. Syst. Aroid.: 306, 1860; Engl. in A DC. & C DC, Monogr. Phan. 2: 443, 1879; Benth. & Hook. f., Gen. Pl. 3(2): 981, 1883; Engl. in Engl. & Prantl, Nat. Pflanzenfam. 2(3): 135, 1889; Engl. in Engl., Pflanzenr. IV, 23Dc (Heft 64): 34, Fig. 16, 1915; Nicolson, Smiths., Contr. Bot. 1: 36, 1969; Mayo, Bogner & Boyce, Gen. Arac. 225: pl. 78, 123A, 1997; Phamh., Illustr. Fl. Vietn. 3: 351, fig. 9114, 2000; V.D. Nguyen, Arac. Vietn.: 78, 2006; P.C. Boyce et al., Fl. Thail. 11: 110, 2012; – Type: MALAYSIA, Malacca, without date (ca. 1842), W. Griffith 5991 (K, holo & iso, seen on-line).

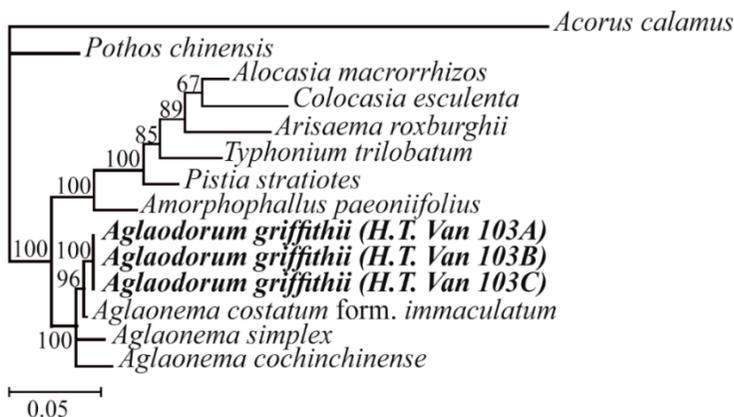
Evergreen, helophytic colony-forming herbs, 40 – 60 cm high. Stem rhizomatous, spongy, 2.0–2.5 cm in diameter. Leaves few, each subtended by cataphylls; petioles terete, spongy, dark green, 30–40 cm long, ca. 1.5 cm in diameter; sheath very short; leaf blade oblanceolate, thick, 20–30 cm long, 12–15 cm wide, dark green above, pale green under side; midrib thick, impressed adaxially and prominent abaxially, lateral veins diverging from the midrib and toward margin. Inflorescence solitary; peduncle terete, spongy, dark green, 30–50 cm long, ca. 1 cm in diameter; spathe oblong, cuspidate, not differentiated into a lower tube and upper limb, green, 7–8 cm long, ca. 1 cm in diameter; spadix stipitate, 6–7 cm long; female part with a single whorl of flowers, loosely arranged; ovaries pale green or pale yellow, 1-locular with 1 basal ovule; style absent; stigma yellow, concave at centre, 4 mm long, 2–2.5 mm wide; male part cylindrical, thecae opening apically by elongate pore. Infructescence

ultimately declinate; fruit obovoid to ellipsoid, pale green, 3 – 4 cm long, 1 – 2 cm wide, floating (Fig. 3).

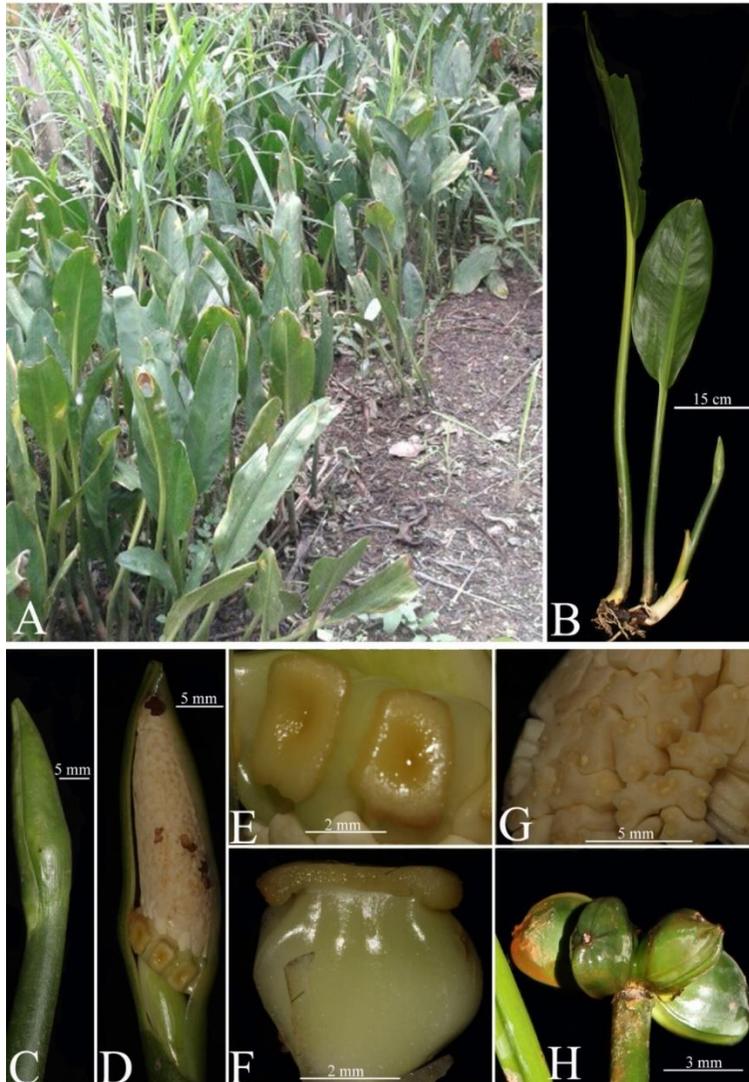
Distribution: Southern Vietnam, Thailand, Cambodia, south and west through Peninsular Malaysia and Sumatra, and East to North Borneo.



**Fig. 1 One of most-parsimonious tree obtained based on the combined data set of trnL intron and trnL-trnF IGS. Gaps treated as missing data. The bootstrap values of 50% or more are shown above the nodes. Tree length = 471 steps, CI = 0.82, RI = 0.79, and RC = 0.71.**



**Fig. 2 Bayesian tree obtained from the combined data set of trnL intron and trnL-trnF IGS. The bootstrap values of 50% or higher are shown above the nodes.**



**Fig. 3** *Aglaonema griffithii* Schott. (A) habit and habitat, (B) petiole, leaf blade and inflorescence, (C) spathe, (D) spadix, (E) stigma, (F) ovary, (G) stamens, (H) fruits.

Studied specimens: Vietnam, Tien Giang Province, Cho Gao District, 10°19'17"N; 106°29'08"E, 10 m elevation, 20 July 2015, collected by Hong Thien Van, H. T. Van 103A (SGN!); Ben Tre Province, Tam Phuoc District, 10°17'700"N; 106°21'16"E, 15 m elevation, 01 August 2015; collected by Hong Thien Van, H. T. Van 103B (SGN!); Long An Province, Ben Luc District, 10°41'124"N; 106°33'972"E, 12 m elevation, 05 August 2015, collected by Hong Thien Van, H. T. Van 103C (SGN!); Saigon (Ho Chi Minh City), 20 May 1921, Poilane 520 (P, seen images); Malaysia, 1856, Griffith 5991 (K, seen images); Sai Gon City, Vietnam, 20 May 1921, Poilane 520 (P, seen images); Malaysia, Malacca, Griffith 5991 (K, seen images).

## References

- Boyce P. C., Sookchaloem D., Hetterscheid W. L. A., Gusman G., Jacobsen N., Idei T. & Nguyen V. D. (2012): Araceae. – *Flora of Thailand* 11: 101–321.
- Cabrera L. I., Salazar G. A., Chase M. W., Mayo S. J., Bogner, J. & Dávila P. (2008): Phylogenetic relationships of aroids and duckweeds (Araceae) inferred from coding and noncoding plastid DNA. – *Am. J. Bot.* 95/9: 1153–1165. doi.org/10.3732/ajb.0800073
- Cusimano N., Bogner J., Mayo S. J., Boyce P. C., Wong S. Y., Hesse M., Hetterscheid W. L. A., Keating R. C. & French J. C. (2011): Relationships within the Araceae: comparison of morphological patterns with molecular phylogenies. – *Am. J. Bot.* 98/4: 654–668. doi.org/10.3732/ajb.1000158
- Engler A. (1915): Anubiadeae, Aglaonemateae, Dieffenbachieae, Zantedeschieae, Typhonodoreae, Peltandreae. – *Das Pflanzenreich IV.23Dc (Heft 64)*: 1–78.
- Farris J. S. (1989): The retention index and the rescaled consistency index. – *Cladistics* 5/4: 417–419. doi.org/10.1111/j.1096-0031.1989.tb00573.x
- Fitch W. M. (1971): Towards defining the course of evolution: minimum change for a species tree topology. – *Syst. Zool.* 20/4: 406–416. DOI: 10.2307/2412116
- Harrison C. J. & Langdale J. A. (2006): A step by step guide to phylogeny reconstruction. – *Plant J.* 45/4: 561–572. DOI: 10.1111/j.1365-313X.2005.02611.x
- Hay A. & Herscovitch C. (2002): Two remarkable new West Malesian *Homalomena* (Araceae) Species. – *Gard. Bull. Singapore* 54: 171–178.
- Hooker J. D. (1883): Aroideae. – In: Bentham G. & Hooker J. D. *Genera Plantarum* 3: 955–1000.
- Kimura M. (1980): A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. – *J. Mol. Evol.* 16: 111–120. doi.org/10.1007/BF01731581
- Kluge A. & Farris J. (1969): Quantitative phyletics and the evolution of anurans. – *Syst. Zool.* 18/1: 1–32. DOI: 10.2307/2412407
- Mayo S. J., Bogner, J. & Boyce, P. C. (1997): *The Genera of Araceae*. – Royal Botanic Gardens, Kew.
- Nauheimer L., Dirk M. & Susanne S. R. (2012): Global history of the ancient monocot family Araceae inferred with models accounting for past continental positions and previous ranges based on fossils. – *New Phytologist* 195/4: 938–950. DOI: 10.1111/j.1469–8137.
- Nei M. & Kumar S. (2000): *Molecular Evolution and Phylogenetics*. – Oxford University Press, New York, 333p.
- Nicolson D. H. (1969): A revision of the genus *Aglaonema* (Araceae). – *Smithsonian Contr. Bot.* 1: 36. https://doi.org/10.5962/bhl.title.123257
- Ridley H. N. (1925): Aroideae. – *The Flora of the Malay Peninsula* 5: 84–131.
- Ronquist F. & Huelsenbeck J. P. (2003): MrBayes 3: bayesian phylogenetic inference under mixed models. – *Bioinformatics* 19/12: 1572–1574. doi.org/10.1093/bioinformatics/btg180
- Schott H. W. (1856): *Synopsis Aroidearum*. – Vindobonae.
- Schott H. W. (1858): *Genera Aroidearum*. – Vindobonae.
- Swofford D. L. (2002): PAUP\*. *Phylogenetic Analysis Using Parsimony (\*and Other Methods)*. Version 4.0 Beta. – Sinauer Associates, Sunderland, Massachusetts. DOI: 10.1111/j.0014-3820.2002.tb00191.x

- Taberlet P., Gielly L., Patou G. & Bouvet J. (1991): Universal primers for amplification of three non-coding regions of chloroplast DNA. – *Plant Mol. Biol.* 17: 1105–1109. doi.org/10.1007/BF00037152
- Tamura K., Stecher G., Peterson D., Filipski A. & Kumar S. (2013): MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. – *Mol. Biol. Evol.* 30/12: 2725–2729. doi: 10.1093/molbev/mst197
- Teijsmann J. E. & Binnendijk S. (1863): *Plantea novae in Horto Bogoriensi cultae.* – *Tijdschrift voor Nederlandsch-Indië* 25: 399–428.
- Thompson D. J., Higgins D. G. & Gibson T. J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. – *Nucleic Acids Res* 22/22: 4673–4680. DOI: 10.1093/nar/22.22.4673
- Yulita K. S., Bayer R. J. & West J. G. (2005): Molecular study of *Hopea* and *Shorea* (Dipterocarpaceae): Evidence from the trnL–trnF and internal transcribed spacer regions. – *Plant Spec. Biol.* 20/3: 167–182. doi.org/10.1111/j.1442-1984.2005.00136.x
- Wong S. Y., Boyce P. C. & Ahmad F. B. (2011): Studies on Homalomeneae (Araceae) of Borneo III: The helophytic *Homalomena* of Sunda. – *Gardens' Bull. Singapore* 62/2: 313–327.
- Wong S. Y., Tan P. J., Ng K. K., Othman A. S., Lee H. B., Ahmad F. B. & Boyce P. C. (2013): Phylogeny of Asian *Homalomena* (Araceae) based on the ITS region combined with morphological and chemical data. – *Systematic Botany* 38/3: 589–599. doi.org/10.1600/036364413X670430

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