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Original Article

Phenetic study of the genus *Monochoria* in Thailand

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Abstract

Monochoria C. Presl is aquatic flowering plants belonging to Pontederiaceae. It consists of 8 species worldwide, occurring in tropical and subtropical regions. Currently, taxonomic status of Thai *Monochoria* remains unclear due to its morphological plasticity and different classification viewpoints. Some authors accept three monotypic species i.e. *M. hastata, M. elata* and *M. vaginalis*, whereas others recognized infraspecific taxa. To clarify this problem, intensive specimen collections were made throughout Thai floristic regions. Then, 22 quantitative and 23 qualitative characters from 550 specimens were analyzed. Cluster analysis indicated that 550 specimens could be divided into five groups. Similarly, canonical discriminant analysis showed that it comprises of five groups distinguished by lamina width of floral-leaf, petiole length of floral-leaf and flowering stem length. To recapitulate, phenetic data support the segregation into five groups: *M. vaginalis* and its closely related species, *M. elata* and its closely related taxon and *M. hastata,* a monotypic species.

Keywords: *Monochoria,* phenetic study, infraspecific taxa, OTUs, Thailand

1. Introduction

Monochoria C. Presl, a morphologically variable genus (Cook, 1989; Guofang & Horn, 2000; Wang *et al.*, 2003; Wang *et al.*, 2004), belongs to an aquatic plant family, Pontederiaceae and comprises 4-8 species worldwide (Mabberley, 2008). They naturally occur in tropical and subtropical regions, especially in Asia. So far, five species are recognized in Asia: *M. hastata* (L.) Solms*, M. korsakowii* Regel & Maack, *M. vaginalis* (Burm.f.) C. Presl ex Kunth var. *vaginalis* and *M. vaginalis* var. *angustifolia* G.X. Wang, *M. elata* Ridl. and *M. valida* G.X.Wang & Nagam (Wang *et al.*, 2004). *M. valida* was an additional species to the world *Monochoria* account and is closely related to *M. elata*, but differed in size of leaf and number of flower per inflorescence (Wang *et al.*, 2004). Some taxonomists, however, have recognized *M. valida* as *M. elata* (Backer, 1951; Chayamarit, 2005; Guofang & Horn, 2000; Ridley, 1924; Yang, 1976), while some recognized *M. elata* and *M. valida* as a variety, i.e. *Monochoria hastata* var. *elata* (Ridl.) Backer (Backer, 1951; The Plantlist, 2013).

According to the 'Flora of Thailand', there are three monotypic species, i.e. *M. elata* Ridl., *M. hastata* and *M. vaginalis* (Chayamarit, 2005) in the Thai *Monochoria*. Wang *et al.* (2004), on the other hand, investigated morphology of the genus *Monochoria* which included some Thai *Monochoria*, and reported that *Monochoria* in Thailand contained three species and two varieties: *M. hastata,*

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M. elata, M. vaginalis var. *vaginalis* and *M. vaginalis* var. *angustifolia,* based solely on quantitative characters without any statistical tests. They proposed a new variety, *M. vaginalis* var. *angustifolia* based on four Thai herbarium specimens (Wang *et al.*, 2003). However, some taxonomists still recognized *M. vaginalis* as a monotypic species (Chayamarit, 2005; Lansdown, 2013; The Plantlist, 2013).

It can be seen that the classifications of *Monochoria* species in Thailand as well as in Asia are different, suggesting the need for further taxonomic assessment. The objectives of this research are as follows: (1) to investigate the morphometric relationship and determine the suitability of the species circumscriptions of each known species and of other related taxa, and (2) to resolve the importance of both vegetative and reproductive characters that can be used to distinguish these taxa. With these objectives in mind, both cluster analysis and canonical discriminant analysis were performed based on both qualitative and quantitative characters.

2. Materials and Methods

2.1 Specimen collection

First of all, all the water-bodies throughout Thailand were located using Google Earth™ as well as data from previous collection sites of herbarium specimens. Then target provinces were selected to cover all floristic regions of the country (Santisuk & Larsen, 2011). The 550 living *Monochoria* specimens from 25 populations were found and collected (Table 1). Each specimen was considered as an operational taxonomic unit (OTU). All the living plant materials were identified using the key-to-species and description in the existing Floras (Backer, 1951; Chayamarit, 2005; Guofang & Horn, 2000; Ridley, 1924; Yang, 1976). Then all the specimens were verified from types and voucher specimens from AAU, BCU, BK, BKF, BM, K, KYO L, M and TNS (herbarium abbreviations according to Thiers, continuously updated).

Table 1. Twenty five populations with the number of OTUs of the genus *Monochoria* used in multivariate analyses.

Population No.	Population Names	Number of OTUs	Floristic Regions	Taxa in Flora of Thailand
1	MH Ratchaburi	20	Central	*M. hastata* (L.) Solms
2	MH Sing Buri	22	Central	*M. hastata* (L.) Solms
3	MH Phatthalung	20	Peninsular	*M. hastata* (L.) Solms
4	MH Nakhon Si Thammarat	20	Peninsular	*M. hastata* (L.) Solms
5	MH Chaiyaphum	17	Eastern	*M. hastata* (L.) Solms
6	MH Nakhon Pathom	20	Central	*M. hastata* (L.) Solms
7	MH Nakhon Sawan	20	Northern	*M. hastata* (L.) Solms
8	MH Phra Nakhon Si Ayuthaya	20	Central	*M. hastata* (L.) Solms
9	MV Kanchanaburi	20	South-Western	*M. vaginalis* (Burm.f.) C. Presl ex Kunth
10	MV Chiang Mai	20	Northern	*M. vaginalis* (Burm.f.) C. Presl ex Kunth
11	MV Ratchaburi	30	Central	*M. vaginalis* (Burm.f.) C. Presl ex Kunth
12	MV Suphan Buri	25	Central	*M. vaginalis* (Burm.f.) C. Presl ex Kunth
13	MV Angthong	19	Central	*M. vaginalis* (Burm.f.) C. Presl ex Kunth
14	MV Nan	20	Northern	*M. vaginalis* (Burm.f.) C. Presl ex Kunth
15	MV Phatthalung	25	Peninsular	*M. vaginalis* (Burm.f.) C. Presl ex Kunth
16	MV Chachoengsao	30	South-Western	*M. vaginalis* (Burm.f.) C. Presl ex Kunth
17	MV Phatthalung	25	Peninsular	*M. vaginalis* (Burm.f.) C. Presl ex Kunth
18	MV Chumphon	30	Peninsular	*M. vaginalis* (Burm.f.) C. Presl ex Kunth
19	ME Chaiyaphum	19	Eastern	*M. elata* Ridl.
20	ME Udon Thani	20	North-Eastern	*M. elata* Ridl.
21	ME Nakhon Ratchasima	16	Eastern	*M. elata* Ridl.
22	ME Chachoengsao	30	South- Eastern	*M. elata* Ridl.
23	ME Phatthalung	30	Peninsular	*M. elata* Ridl.
24	ME Surat Thani	15	Peninsular	*M. elata* Ridl.
25	ME Nakhon Si Thammarat	17	Peninsular	*M. elata* Ridl.
	(25 populations)	(550 OTUs)		(3 Taxa)

Note: The population names come from the candidate species follow the Flora of Thailand and the collected provinces. MH, MV and ME is the candidate species of *M. hastata, M. vaginalis* and *M. elata*, respectively.

2.2 Character measurements

A total of 45 characters (22 quantitative and 23 qualitative characters) of all living specimens from 25 populations were analyzed and selected for multivariate analyses (Table 2 and 3). Both vegetative and reproductive structures were employed to measure their morphological characters. For the macroscopic characters, linear measurements were carried out using standard ruler or digital caliper. The microscopic characters were observed under a compound light microscope equipped with 10X lens attached to a micrometer disc and 10X or 40X objectives.

2.3 Data analyses

For cluster and canonical discriminant analyses, 22 quantitative (Table 2) and 23 qualitative characters (Table 3) were used (data matrices are available from the corresponding author). Cluster analysis was carried out based on quantitative and qualitative characters using the Gower similarity coefficient (Gower, 1971) and UPGMA clustering in the MVSP program (Kovach Computing Services, MVSP Plus, version 3.1). All characters used in these analyses were assumed to be of equal importance, and were not weighed. In cluster analyses, we ensured the accuracy of the results by analyzing only quantitative characters and constructing the dendrogram. We then compared this dendrogram with another dendrogram constructed with both quantitative and qualitative characters. Canonical discriminant analysis was conducted using SPSS for Windows (SPSS, 2007). Stepwise discriminant analysis was used to select a subset of characters that maximized differences among 550 specimens to estimate character weights from correlations between canonical variables and original variables. Apart from the characters selected in this analysis, correct classification rate were also used to determine the division of the specimens into groups. Univariate analysis was performed using SPSS for Windows (SPSS, 2007) in order to summarize the range of variation between and within segregated groups on each character.

Table 2. Twenty two quantitative characters of reproductive and vegetative structures used in multivariate analyses of the genus *Monochoria* with their scoring methods.

Characters	Details of measurements and counts
Leaf	
- length	Length of leaf (cm)
- width	Width of leaf (cm)
Spathe length	Length of spathe (cm)
Floral leaf	
- lamina length	Length of lamina of floral-leaf (cm)
- lamina width	Width of lamina of floral-leaf (cm)
- petiole length	Length of petiole of floral-leaf (cm)
Flowering stem length	Length of flowering stem (cm)
Peduncle length	Length of peduncle (cm)
Number of flowers per inflorescence	Number of flowers per inflorescence
Pedicels length	Length of pedicels (cm)
Outer perianth	
- length	Length of outer perianth (cm)
- width	Width of outer perianth (cm)
Inner perianth	
- length	Length of inner perianth (cm)
- width	Width of inner perianth (cm)
Largest stamen	
- filament length	Filament length of the largest stamen (cm)
- anther length	Anther length of the largest stamen (cm)
- anther width	Anther width of the largest stamen (cm)
Normal stamens	
- filament length	Filament length of normal stamens (cm)
- anther length	Anther length of normal stamens (cm)
Style length	Length of style (cm)
Number of longitudinal ridges per seed	Number of longitudinal ridges per seed
Seed length	Length of seed in (μm)

Table 3. Twenty three qualitative characters of reproductive and vegetative structures used in multivariate analyses of the genus *Monochoria* with their scoring methods.

Characters	Details of measurements and counts
Leaf	
- shape	1. lanceolate-linear, 2. lanceolate, 3. ovate- lanceolate, 4. ovate-cordate, 5. cordate, 6. broadly cordate
- apex	1. narrowly acute, 2. broadly acute, 3. acuminate, 4. broadly acuminate, 5. acute
- base	1. obtuse, 2. cordate
- midrib (on upper surface)	1. present, 2. absent
Stipules	
- appendage	1. present, 2. absent
Longitudinal ridge of petiole	1. present, 2. absent
Floral leaf	
- shape	1. lanceolate, 2. cordate
- lamina apex	1. narrowly acute, 2. broadly acute, 3. acuminate, 4. broadly acuminate, 5. acute
- lamina base	1. obtuse, 2. cordate
- midrib (on upper surface)	1. present, 2. absent
Height of leaf tip comparing with inflorescence tip	1. lower, 2. higher
Height of flora leaf tip comparing with inflorescence tip	1. lower, 2. higher
Longitudinal ridges of flowering stem	1. present, 2. absent
Sequence of blooming	1. starting at the top of inflorescence (determinate inflorescence), 2. starting at the base of inflorescence (indeterminate inflorescence)
Outer perianth	
- shape	1. narrowly elliptic, 2. lanceolate, 3. ovate, 4. oblong, 5. narrowly obovate
- color of costa on abaxial	1. light purple, 2. green, 3. white
Inner perianth	
- shape	1. elliptic, 2. broadly elliptic, 3. narrowly elliptic, 4. elliptic-ovate
- apex	1. broadly acute, 2. obtuse, 3. acute
Color of costa on abaxial of inner perianth	1. light purple, 2. green, 3. white
Style color	1. light purple, 2. white
Fruit with screwy persistent perianth	1. present, 2. absent
Seeds shape	1. oval, 2. barrel
Seed longitudinal ridges	1. deep, 2. shallow

3. Results and Discussion

3.1 Cluster analysis

The first dendrogram deriving from twenty-two quantitative characters showed the segregation of 550 specimens (OTUs) into five groups or probably four species at the 0.76 phenon level of Gower similarity coefficient (Figure 1). Group A contains *M. vaginalis* 1 (population no. 9-14), while Group B contains *M. vaginalis* 2 (population no. 15-18). Group C consists of *M. elata* 1 (population no. 19-21), whereas Group D contains *M. elata* 2 (population no. 22-25). The last group, group E comprises *M. hastata* (population no. 1-8).

Similar clustering result was exhibited when both twenty-two quantitative characters and twenty-three qualitative characters were employed in the second cluster analysis (Figure 2). The 550 OTUs were segregated into five different

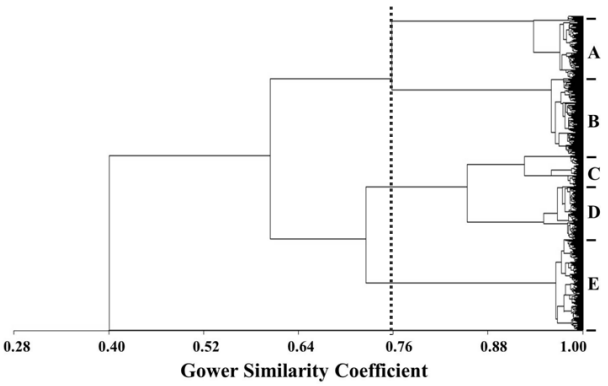

Figure 1. UPGMA phenogram of the 550 specimens based on Gower Similarity Coefficient calculated between means of 22 quantitative characters of the genus *Monochoria* in Thailand. A: *M. vaginalis* 1, B: *M. vaginalis* 2, C: *M. elata* 1, D: *M. elata* 2 and E: *M. hastata*

groups which corresponded with those from the first cluster analysis.

According to the 'Flora of Thailand' (Chayamarit, 2005), the specimens belonging to *M. vaginalis* 1 and 2 are *M. vaginalis* based on leaf shape, pedicel length and inflorescence type. However, our result from cluster analysis indicated that the specimens are clearly classifiable into 2 groups i.e., group A (*M. vaginalis* 1) and B (*M. vaginalis* 2), probably at the species level. This result could, to a certain degree, support the previous study that *M. vaginalis* in Thailand was not a monotypic species (Wang *et al.*, 2004). Wang *et al.* (2004) used both quantitative and qualitative characters, such as leaf size and shape in their determination.

It can be seen that cluster analysis did not support the unification of *M. elata*'s populations (population no. 19-25) in Thailand into a single species as was recognized in the Flora of Thailand. The Flora used inflorescence type, style length and the height of leaf tip comparing with inflorescence tip as important characters. The OTUs can be divided into two groups based on Gower similarity coefficient (Figure 1), viz. Group C (*M. elata* 1) and D (*M. elata* 2). Additionally, we examined the type specimen of *M. valida* and the studied specimen of *M. elata* which were reported in previous work (Wang & Nagamasu, 1994; Wang *et al.*, 2004). We also collected and analyzed the living specimens of *M. elata* from the same localion, Chachoengsao province, Thailand (Table 1). It was found that morphological characters of Group C (*M. elata* 1) and Group D (*M. elata* 2) matched well with the description of *M. valida* and *M. elata*, respectively (Wang *et al.*, 2004; Table 5). The result from this study indicated that Group C and D seem more closely related than Group A and B, and share some common characters as infraspecific taxa. Hence, it may not suitable to separate the so called '*M. elata*' in Thailand as two distinct species as was proposed earlier (Wang & Nagamasu, 1994; Wang *et al.*, 2004).

It can be seen from Figures 2 and 3 that Group E is the most homogeneous group. This group comprises a bulk of *M. hastata*'s specimens (population no. 1-8), collected from four Thai floristic regions (Table 1). This phenetic study strongly indicated that *M. hastata* is a monotypic species which corresponds to the previous classification in the Flora of Thailand (Chayamarit, 2005). The specimens share common characters in inflorescence type, leaf shape, leaf base and height of leaf tip comparing with inflorescence tip. In summary, the result from both cluster analyses supported the recognition of five taxa within the genus *Monochoria* in Thailand: *M. vaginalis* and its related taxon, *M. elata* and its related taxon, and a monotypic species i.e. *M. hastata*.

3.2 Canonical discriminant analysis

In canonical discriminant analysis, twenty-two quantitative characters from 550 specimens were assessed using stepwise discriminant analysis in order to determine which characters are important for dividing the specimens of *Monochoria* into groups. From the twenty-two quantitative characters, only one character i.e. number of longitudinal ridges per seed was not selected as an important character in giving the best separation of the groups (Table 4). The discriminant function classification result showed 100% correct classification of the 550 specimens (OTUs) into the respective groups (A, B, C, D, or E). The classification rates are extremely high considering the existing variations among the five groups. For this reason, the discriminant function (Table 4) can be effectively used to classify the genus *Monochoria* in Thailand.

The canonical correlation of the first canonical discriminant function is 100% with all the variables and the variance explained by it is 62%. This is most highly influenced by flowering stem length (Table 4). The canonical correlation of the second canonical discriminant function is also 100%, and the variance explained by it is 22%; this axis is most highly associated with lamina width of floral-leaf and petiole length of floral-leaf (Table 4). The cumulative percentage from the first three canonical discriminant functions is 99%. So far these functions have been effective in determining the important characters of 550 OTUs in this study.

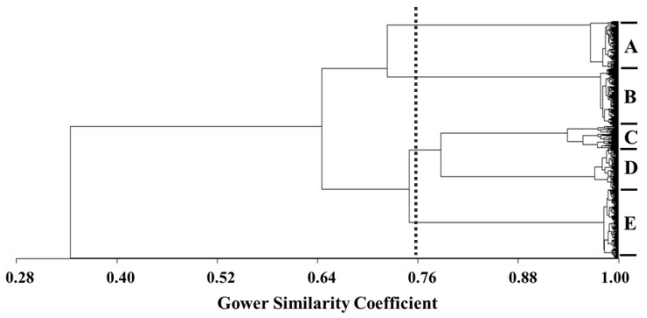

Figure 2. UPGMA phenogram of the 550 specimens based on Gower Similarity Coefficient calculated between means of 23 qualitative and 22 quantitative characters of the genus *Monochoria* in Thailand. A: *M. vaginalis* 1, B: *M. vaginalis* 2, C: *M. elata* 1, D: *M. elata* 2 and E: *M. hastata*

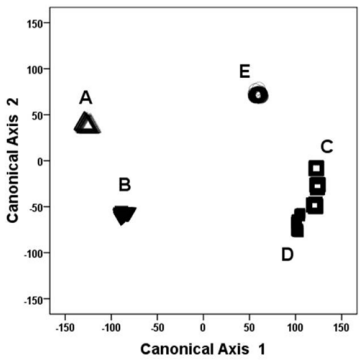

Figure 3. Ordination plot of 550 specimens from 25 populations of Thai *Monochoria* based on 22 quantitative characters. A (△): populations from *M. vaginalis* 1, B (▼): *M. vaginalis* 2, C (□): *M. elata* 1, D (■): *M. elata* 2 and E (○): *M. hastata*

Table 4. Pooled results within canonical structure of 5 group taxa (A, B, C, D and E) according to the results of canonical discriminant analysis; F-value, mean and standard errors of 22 quantitative structures of the 5 groups.

Characters		Axis 1	Axis 2	F-value	Sign.	Group A		Group B		Group C		Group D		Group E	
						mean	± SE	mean	± SE	mean	± SE	mean	± SE	mean	± SE
Leaf	- length	0.14	0.06	5265.00	0.00	6.77	0.037	9.73	0.033	18.15	0.661	22.19	0.288	25.84	0.095
	- width	0.00	0.22	14620.00	0.00	1.71	0.011	6.76	0.056	4.50	0.331	0.68	0.024	14.78	0.049
Spathe length		0.40	-0.15	37170.00	0.00	2.73	0.007	3.53	0.005	12.12	0.039	11.33	0.028	6.08	0.009
Floral leaf	- lamina length	0.07	0.25	7858.00	0.00	6.52	0.014	5.21	0.034	14.30	0.161	3.78	0.134	25.98	0.025
	- lamina width	0.04	0.54*	33190.00	0.00	1.71	0.004	1.43	0.005	3.31	0.332	0.28	0.009	14.12	0.018
	- petiole length	0.16	-0.57*	47090.00	0.00	4.30	0.015	26.66	0.059	26.06	0.009	18.19	0.012	10.17	0.019
Flowering stem length		0.81*	0.13	140000.00	0.00	10.67	0.018	15.55	0.035	160.20	0.140	130.35	0.107	106.41	0.239
Peduncle length		-0.05	-0.25	5852.00	0.00	2.92	0.006	5.05	0.029	4.09	0.009	3.90	0.009	1.86	0.009
Number of flowers per inflorescence		0.06	-0.02	999.90	0.00	4.24	0.125	15.75	0.333	57.53	1.438	96.71	1.453	50.97	0.793
Pedicel length		0.17	0.28	14090.00	0.00	1.31	0.003	0.41	0.003	1.71	0.004	1.58	0.004	2.54	0.005
Outer perianth	- length	0.22	0.14	12480.00	0.00	0.74	0.003	0.52	0.001	1.61	0.002	1.49	0.002	1.55	0.004
	- width	0.10	0.06	2885.00	0.00	0.27	0.0005	0.18	0.0006	0.50	0.0014	0.40	0.0014	0.37	0.0013
Inner perianth	- length	0.14	0.18	7222.00	0.00	0.32	0.001	0.18	0.001	0.73	0.017	0.46	0.003	0.82	0.003
	- width	0.21	0.14	11720.00	0.00	0.80	0.003	0.58	0.002	1.74	0.004	1.56	0.003	1.64	0.005
Largest stamen	- filament length	0.08	-0.01	1819.00	0.00	0.19	0.001	0.14	0.0004	0.43	0.002	0.38	0.002	0.24	0.002
	- anther length	0.08	0.01	1780.00	0.00	0.43	0.002	0.33	0.001	0.81	0.002	0.68	0.003	0.53	0.002
	- anther width	0.01	0.00	29.02	0.00	0.14	0.019	0.07	0.0004	0.14	0.002	0.11	0.001	0.09	0.001
Normal stamens	- filament length	0.11	0.03	8396.00	0.00	0.75	0.003	0.08	0.001	1.80	0.006	1.49	0.008	0.35	0.002
	- anther length	0.08	-0.13	6903.00	0.00	0.44	0.002	0.27	0.001	0.74	0.015	0.93	0.004	0.27	0.001
Style length		0.07	-0.02	1254.00	0.00	0.49	0.002	0.39	0.003	1.19	0.006	0.99	0.010	0.61	0.003
Number of longitudinal ridges per seed [a]		0.00	-0.03	34.76	0.00	8.44	0.109	10.24	0.108	12.35	0.292	7.79	0.077	7.88	0.062
Seed length		0.08	0.00	1479.00	0.00	406.45	1.042	458.47	1.608	581.56	2.218	728.31	1.891	649.92	1.156

* Denotes largest absolute correlation between each character and any discriminant function; a. A variable not selected by stepwise discriminant analysis to be used in further canonical discriminant analysis.

Table 5. Morphological comparison of Thai *M. elata* (Group C and D), Asian species, *M. elata* and *M. valida* KYO (isotype!).

Characters	This study (number of specimens examined)		Wang *et al.* 2004 (number of specimens examined)	
	Group C (55)	Group D (92)	*M. elata* (1)*	*M. valida* (4)*
Leaf				
- shape	sagittate or hastate	hastate, sagittate or linear	narrowly hastate or narrowly sagittate	broadly sagittate
- length (cm)	18.15	22.19	22.5	18.75
- width (cm)	4.50	0.68	1.65	5.50
Floral leaf				
- shape	sagittate, hastate or auriculate	linear	linear or sagittate	sagittate
- lamina length (cm)	14.30	3.78	7.00	23.00
- lamina width (cm)	3.31	0.28	1.40	7.00
Longitudinal ridge of petiole	absent	present	present	absent
Flowering stem length (cm)	160.20	130.35	125.00	155.00
Longitudinal ridges of flowering stem	absent	present	present	absent
Peduncle length (cm)	4.09	3.90	2.00	5.00
Number of flowers per inflorescence	57.53	96.71	27.50	80.00
Outer perianth				
- shape	lanceolate	lanceolate	lanceolate	lanceolate
Inner perianth				
- shape	elliptic or oblong	obovate	obovate	elliptic

*Note: we also investigate the same specimens examined of Wang *et al.* 2004 e.g.; *M. elata* - Thailand: Chachoengsao, Murata *et al.* T-37035 (BKF, KYO); *M. valida* - China: Hainan, G.X. Wang 901001 Isotype! (KYO).

Table 6. First three canonical discriminant functions from 550 OTUs of the genus *Monochoria.*

canonical discriminant functions	canonical correlation	percent of variance	cumulative percentage
Function 1	100%	62%	62%
Function 2	100%	22%	84%
Function 3	100%	15%	99%

The ordination plot of 550 specimens (Figure 3) showed the separation of the genus *Monochoria* in Thailand into five groups (Group A, B, C, D, and E) on canonical axis 1. Specimens of each group correspond with those of the five groups attained from the cluster analyses. It can be seen that Group C and D are rather heterogeneous, while the other three groups (Group A, B and E) are homogeneous.

The result from cluster analyses (Group E from Figure 2 and 3) and canonical discriminant analysis (Group E from Figure 3) showed that only *M. hastata* is a monotypic species. In contrast, *M. elata* formed two groups, viz. C and D in cluster analyses (Figure 1) and canonical discriminant analysis (Figure 3), when only quantitative characters were used. Group C and D should be placed in the same species, probably due to their overlapping quantitative characters. Similarly, when quantitative and qualitative characters were analyzed together, group C and D show the same trend of heterogeneous groups within *M. elata*. The ordination plot (Figure 3) depicted rather close relationship between group C and D which are heterogeneous groups. It is evident from this study that the populations of both groups belong to the same species, and *M. elata* in Thailand should consist of two infraspecific taxa.

Moreover, it is clearly seen from both cluster and canonical discriminant analyses that *M. hastata* (Group E), *M. elata* 1 (Group C) and *M. elata* 2 (Group D) are close to each other more than the other groups based on quantitative and qualitative characters. *M. elata* was previously recognized as a variety of *M. hastata,* i.e. *M. hastata* var. *elata* by Backer (1951) and also in present classification (The Plantlist, 2013). However, additional studies using many specimens by many researchers indicated that it should be

considered as a new species of the genus *Monochoria* (Backer, 1968; Chayamarit, 2005; Guofang & Horn, 2000; Wang *et al.*, 2004). It has been recognized as *M. elata* Ridl. until now. This present study also supports the classification of *M. elata* as a species.

The phenetic analysis separated *M. vaginalis* into two related groups (taxa), probably at the rank of species, since both cluster analyses (Figure 2 and 3) and canonical discriminant analysis (Figure 3) demonstrated a distinct separation of group A and B. Future studies on the phylogenetic relationship of the genus *Monochoria* may produce more evidences for the classification of this plant group.

It is worth noting that this phenetic analysis is the first report on the genus *Monochoria*. It displays an interesting point that quantitative characters are useful for the classification of Thai *Monochoria*. This approach is also useful for the classification of various plant groups. It can also be useful for investigating the populations of *Afgekia sericea* Craib (Boonkerd, 2001), delimiting species within the genus Annonaceae (Chatrou, 1997), determining species boundary of subfamily Polycnemoideae, family Amaranthaceae (Masson & Kadereit, 2013), investigating morphological variations among the species of *Stipa* L. sections *Smirnovia* and *Subsmirnovia* (Gonzalo *et al.*, 2013) and determining *Microsorum punctatum* complex of the family Polypodiaceae (Petchsri *et al.*, 2012).

4. Conclusions

Even though *Monochoria* has been established for more than two centuries (Presl, 1827), the taxonomic status of its species member is still unclear due mainly to different opinions in species concepts. In this study, we focused on numerical taxonomy of 550 specimens, collected from their natural habitats throughout Thailand. Cluster analyses were performed using two sets of data, i.e. both quantitative and qualitative characters set and only quantitative character set; and canonical discriminant analysis was conducted. Both dendrograms from cluster analyses and the ordination plot from canonical discriminant analysis clearly indicate the presence of 5 groups (taxa) within the genus *Monochoria* in Thailand, i.e. *M. hastata*, *M. vaginalis* and its related taxon, *M. elata* and its related taxon.

To summarize, phenetic data may be used effectively along with traditional taxonomic methodology. Nonetheless, these phenetic results do not exhibit the evolutionary relationship among the OTUs. Therefore, the results require careful interpretation. However, these computerized analytical techniques have confirmed to be one of the most effective tools for classification of the species in Thai *Monochoria*.

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