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Numerical taxonomic study of *Ardisia* subgenus *Crispardisia* (Primulaceae) in Thailand

Suparman Suparman[1,2], Pranom Chantaranothai[1,*] & Pimwadee Pornpongrungrueng[1]

[1] Applied Taxonomic Research Center, Khon Kaen University, Khon Kaen 40002, Thailand

[2] Department of Biology Education, Khairun University, Ternate, North Maluku 97719, Indonesia

Abstract. A systematic study of 16 taxa belonging to 14 species of *Ardisia* subgenus *Crispardisia* distributed in Thailand was conducted by means of numerical analysis on 13 morphological characters, including vegetative and floral characters. Based on UPGMA clustering and PCA, calyx length is the most important character and three main groups are recognised by multivariate analysis of morphological characters. The result of this study and literature review recommends that *A. crenata* Sims subsp. *obtusifolia* Chatan & Promprom must be placed under *A. crenata* Sims. *Ardisia crenata* Sims var. *angusta* C. B. Clarke and *A. symplocifolia* (C. Chen) K. Larsen & C. M. Hu are new synonyms of *A. crispa* (Thunb.) A. DC. and *A. maculosa* Mez, respectively.

Keywords: morphometrics, Myrsinaceae, synonym, Thailand

Introduction

The genus *Ardisia* (Swartz, 1788) is one of the largest genera in Primulaceae, comprising approximately 720 species, distributed mainly in the tropics (Julius & Utteridge, 2021). The genus was traditionally placed in the Myrsinaceae, but a new classification in APG III (2009) based on morphology that has free-central placentation and antipetalous stamens, and molecular studies, now places the genus in the Primulaceae subfamily Myrsinoideae. Mez (1902) divided the genus into 14 subgenera then Larsen & Hu (1995) added one subgenus namely *Tetrardisia*. Fletcher (1937) and Larsen & Hu (1996, 2001) contributed enormously to our knowledge of the genus *Ardisia* in Thailand. In Thailand, nine subgenera were recognised with 71 species,

* Corresponding author: pranom@kku.ac.th

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one of which is *Crispardisia* Mez with 14 species including the most common and variable species, *Ardisia crenata* Sims. The *Ardisia* subgenus *Crispardisia* is characterised by having crenate leaves with a gland in each marginal sinus.

Taxonomically *Ardisia crenata* is a large, common and heterogenous, and the problems of classification emerge at the infraspecific level. In the Flora of Thailand, the taxonomic problems relate to the differentiation of three different ranks; a typical taxon, subspecies and variety because only a few morphological characters were traditionally used to classify into these ranks. These are variable within the species, or they conflict with one another and may not support natural groups.

The morphological species concept is still the most important way for separating the species in one genus. The method is still easier and cheaper than the modern method. The molecular marker, for example, must be run more than one marker to recognise the species and intraspecies level. This work aims to use numerical analysis to better understand the phenetic relationships between species within *Ardisia* subgenus *Crispardisia* in Thailand, with respect to their taxonomic treatment.

MATERIALS AND METHODS

Sixteen taxa of *Ardisia* subgenus *Crispardisia* in Thailand *i.e*. *A. bractescens* Ridl., *A. confusa* K. Larsen & C. M. Hu, *A. corymbifera* Mez, *A. crenata* Sims, *A. crenata* subsp. *obtusifolia* Chatan & Promprom, *A. crenata* var. *angusta* C. B. Clarke, *A. longipedicellata* H. R. Fletcher, *A. maculosa* Mez, *A. pilosa* H. R. Fletcher, *A. ridleyi* King & Gamble, *A. rosea* King & Gamble, *A. stipitata* H. R. Fletcher, *A. symplocifolia* (C. Chen) K. Larsen & C. M. Hu, *A. translucida* H. R. Fletcher, *A. villosa* Roxb. and *A. virens* Kurz were managed as the Operational Taxonomic Units (OTUs).

The morphological data were obtained from herbarium specimens at BK, BKF, K, KKU, and PSU; additionally, images of herbarium specimens were examined on virtual herbaria of L and M. All herbarium acronyms cited follow Thiers (2021), see Table 1. Data gathered from fieldwork was also included in this study and all specimens were identified based on Flora of Thailand (Larsen & Hu, 1996), see Table 2. For each species, 1−3 specimens were sampled. A total of 13 important characters that were very significant in key identification were examined, comprising seven qualitative and six quantitative characters. The characters were converted into binary states and multi-states (interval) codes (Table 3). Two types of analyses were performed using Paleontological Statistics Software Package for Education and Data Analysis (PAST) software version 4.03 (Hammer *et al*., 2001). The data matrix was subjected to cluster analysis using UPGMA (Unweighted Pair-Group Method with Arithmetic mean) and a dendrogram was constructed to show the relationship among the species. The factor analysis using a PCA (Principal Component Analysis) as part of the ordination technique was to gain the most important character in every component by reducing dimensions.

Table 1. List of specimens used in the study.

Taxa	Specimens investigated (Herbarium)
A. bractescens	*Kloss 6883* (K)
A. confusa	*Prayad 1457* (BK)
A. corymbifera	*Put 3724* (BK); *Santisuk 1145* (BKF); *Smitinand 10290* (BK)
A. crenata	*Kantachote 126* (KKU); *Kerr 17988* (BK); *Prayad 844* (BK)
A. crenata subsp. *obtusifolia*	*Suparman 09* (KKU); *Suparman 82* (KKU)
A. crenata var. *angusta*	*Hosseus 311A* (M); *Maxwell 96-1046* (L)
A. longipedicellata	*Chayamarit 2020* (BKF); *Larsen 3192* (BKF); *Triboun 7157* (KKU)
A. maculosa	*Chantaranothai 99/2003* (KKU); *Kerr 6059* (BK); *Suparman 85* (KKU)
A. pilosa	*Kerr 20111* (BK); *Prayad 1093* (BK); *Suparman 117* (KKU)
A. ridleyi	*Kerr 7120* (BK); *Niyomdham 6343* (BKF); *Pooma et al. 4417* (BKF)
A. rosea	*Chantaranothai 1266* (KKU); *Santisuk 717* (BKF); *Triboun 42* (BK)
A. stipitata	*Chantaranothai 1162* (KKU); *Kerr 8523A* (BK); *Suparman 79* (KKU)
A. symplocifolia	*Maknoi 1886* (BKF); *Prayad 905* (BK); *Suparman 83* (KKU)
A. translucida	*Kerr 16891* (BK)
A. villosa	*Bunwong 294* (KKU); *Nanakorn 3818* (PSU); *Sakol 4118* (BK)
A. virens	*Maxwell 97-1358* (BKF); *Phengklai 4044* (BKF); *Put 3735* (BK)

Results and Discussion

The data observed from all taxa are compiled in Table 2. The UPGMA dendrogram with a Euclidean distance and scatterplots of PCA of the genus *Ardisia* subgenus *Crispardisia* (Figs. 1−2) clearly discriminated 14 species into three main groups. The first group (I) comprises two species viz. *A. bractescens* and *A. villosa*. The second group (II) comprises three species (five taxa) viz. *A. confusa*, *A. crenata*, *A. crenata* subsp. *obtusifolia*, *A. crenata* var. *angusta* and *A. longipedicellata*. The third group (III) comprises nine species viz. *A. corymbifera*, *A. maculosa*, *A. pilosa*, *A. ridleyi*, *A. rosea*, *A. stipitata*, *A. symplocifolia*, *A. translucida* and *A. virens*.

The plot of 16 OTUs on the three principal components is shown in Figure 2. These components interpret 66.98 % of the total variation and segregation is demonstrated among three groups. The first, second and third components explain 34.88, 19.71 and 12.39 % of the total variation, respectively (Table 4). The main characteristics explaining this separation (characters with a high factor

Table 2. Morphological data observed of the genus *Ardisia* subgenus *Crispardisia*.

Taxa	Branch surface	Leaf arrangement on flowering branch	Leaf length (mm)	Lower surface of leave	Petiole length (mm)	Inflorescence type	Peduncle length (mm)	Peduncle surface	Pedicle length (mm)	Calyx length (mm)	Calyx punctation	Calyx lobe margin	Corolla length (mm)
A. bractescens	ferruginous short hairs when young	scattered	90–180	sparsely scurfy puberulous	6–10	compound	10–15	ferruginous short hairs	7–12	5	scattered, with many black dots	entire	4–5
A. confusa	glabrous	tufted at the branchlet ends	160–270	glabrous	20–30	compound	2–4	glabrous	*ca.* 15	1–1.75	many brown dots	ciliate	6
A. corymbifera	fulvous pubescent when young	scattered	80–150	pubescent	5–8	compound	5–10	ferruginous puberulous	10–15	3	many thick black dots	entire	6–8
A. crenata	glabrous	tufted at the branchlet ends	60–150 (–200)	glabrous	4–10	simple & compound	0–3	glabrous	5–10	1.5	scattered, with many black dots	entire	4–6
A. crenata subsp. *obtusifolia*	glabrous	tufted at the branchlet ends	20–110	glabrous	3–5	simple & compound	0–3	glabrous	7–10	1.8	many black dots	entire	4–6
A. crenata var. *angusta*	glabrous	scattered	60–150	glabrous	4–10	simple	0–3	glabrous	5–27	1.5	scattered, with many black dots	entire	4–6
A. longipedicellata	glabrous	tufted at the branchlet ends	60–190	rusty scale	5–10	simple	none	glabrous	*ca.* 10 (15–30)	1– 1.8	many purple dots	entire	4.5
A. maculosa	glabrous	scattered	100–190	glabrous	10–15	compound	5–10	ferruginous short hairs	10–15	1.5–2	many brown dots	entire	5–6
A. pilosa	densely rusty pilose	tufted at the branchlet ends	25–80	pilose with rusty scale	3–5	simple	none	densely rusty-pilose	15–25	2.5–3	many thick dark dots	entire	5–6
A. ridleyi	ferruginous pubescent	tufted at the branchlet ends	80–100	rusty peltate scale	5–15	simple	none	ferruginous pubescent	17–34	1–1.5	many black dots	entire	*ca.* 3.75
A. rosea	ferruginous pubescent	scattered	50–110	rusty scale	6–10	compound	5–10	ferruginous puberulous	15–20	1–1.5	absent	entire	5–6
A. stipitata	ferruginous scurfy hairs	tufted at the branchlet ends	60–160	few rusty scale	3–10	simple	2–4	ferruginous puberulous	10–15	2–2.5	absent	entire	6

Table 2. Morphological data observed of the genus *Ardisia* subgenus *Crispardisia* (Cont.).

Taxa	Branch surface	Leaf arrangement on flowering branch	Leaf length (mm)	Lower surface of leave	Petiole length (mm)	Inflorescence type	Peduncle length (mm)	Peduncle surface	Pedicle length (mm)	Calyx length (mm)	Calyx punctation	Calyx lobe margin	Corolla length (mm)
A. symplocifolia	glabrous	scattered	70–180	glabrous	5–10	compound	10–30	ferruginous short hairs	10–15	2.5–3	very few	entire	5–6
A. translucida	sparsely puberulous	scattered	150–200	glabrous	1–2	simple	10	ferruginous puberulous	8–10	2	absent	entire	6
A. villosa	densely ferruginous villous, hairs crisped	tufted at the branchlet ends	40–200	densely villous or hirsute	5–10	simple & compound	2–5	villous	5–10	5–7.5	scattered, with many black dots	entire	4–5
A. virens	glabrous	scattered	90–200	glabrous	10–15	compound	2–4	glabrous	15–25	2.5–3.5	many thick black dots	entire	6–8

TABLE 3. Characters and character states used in numerical analysis of the genus *Ardisia* subgenus *Crispardisia*.

Characters	Character states
1. Branch surface	0: glabrous; 1: hairy
2. Leaf arrangement on flowering branch	0: scattered; 1: tufted at the branchlet ends
3. Leaf length (cm)	0: 0–1; 1: 1.01–1.5; 2: more than 1.5
4. Lower surface of leaves	0: glabrous; 1: hairy
5. Petiole length (mm)	0: 0–1; 1: more than 1
6. Inflorescence type	0: simple only; 1: simple or simple & compound
7. Peduncle surface	0: glabrous; 1: hairy
8. Peduncle length (mm)	0: 0–1; 1: more than 1
9. Pedicle length (mm)	0: 0–1.5; 1: more than 1.5
10. Calyx lobe length (mm)	0: 1–2; 1: 2.2–3; 2: 3.1–3.9; 3: 4 or more
11. Calyx punctation	0: very few or absent; 1: many
12. Calyx lobe margin	0: entire; 1: ciliate
13. Corolla lobe length (mm)	0: 3–5; 1: 5.1–6.9; 2: 7 or more

TABLE 4. The latent root for the first three principal components obtained from the *Ardisia* subgenus *Crispardisia*.

Component	Eigenvalue	Percent of variance	Total
1	1.43	34.88	34.88
2	0.81	19.71	54.59
3	0.51	12.39	66.98

TABLE 5. Loading for the first three components of 13 characters used derived from PCA of Thai *Ardisia* subgenus *Crispardisia*.

No	Characters	PC 1	PC 2	PC 3
1	Branch surface	0.27	-0.26	-0.32
2	Leaf distribution	-0.07	-0.32	0.35
3	Leaf length	-0.06	**0.44**	-0.03
4	Leaf surface	0.26	**-0.40**	-0.06
5	Petiole length	-0.03	0.14	0.20
6	Inflorescence type	0.10	0.35	0.16
7	Peduncle length	0.12	0.20	-0.14
8	Peduncle surface	0.27	-0.08	**-0.48**
9	Pedicle length	-0.08	-0.31	0.10
10	Calyx lobe length	**0.86**	0.20	0.28
11	Calyx punctation	0.04	-0.11	**0.59**
12	Calyx lobes margin	-0.06	0.08	0.11
13	Corolla lobe length	-0.12	0.38	-0.12

The bold are the highest values (>±0.40) in every component

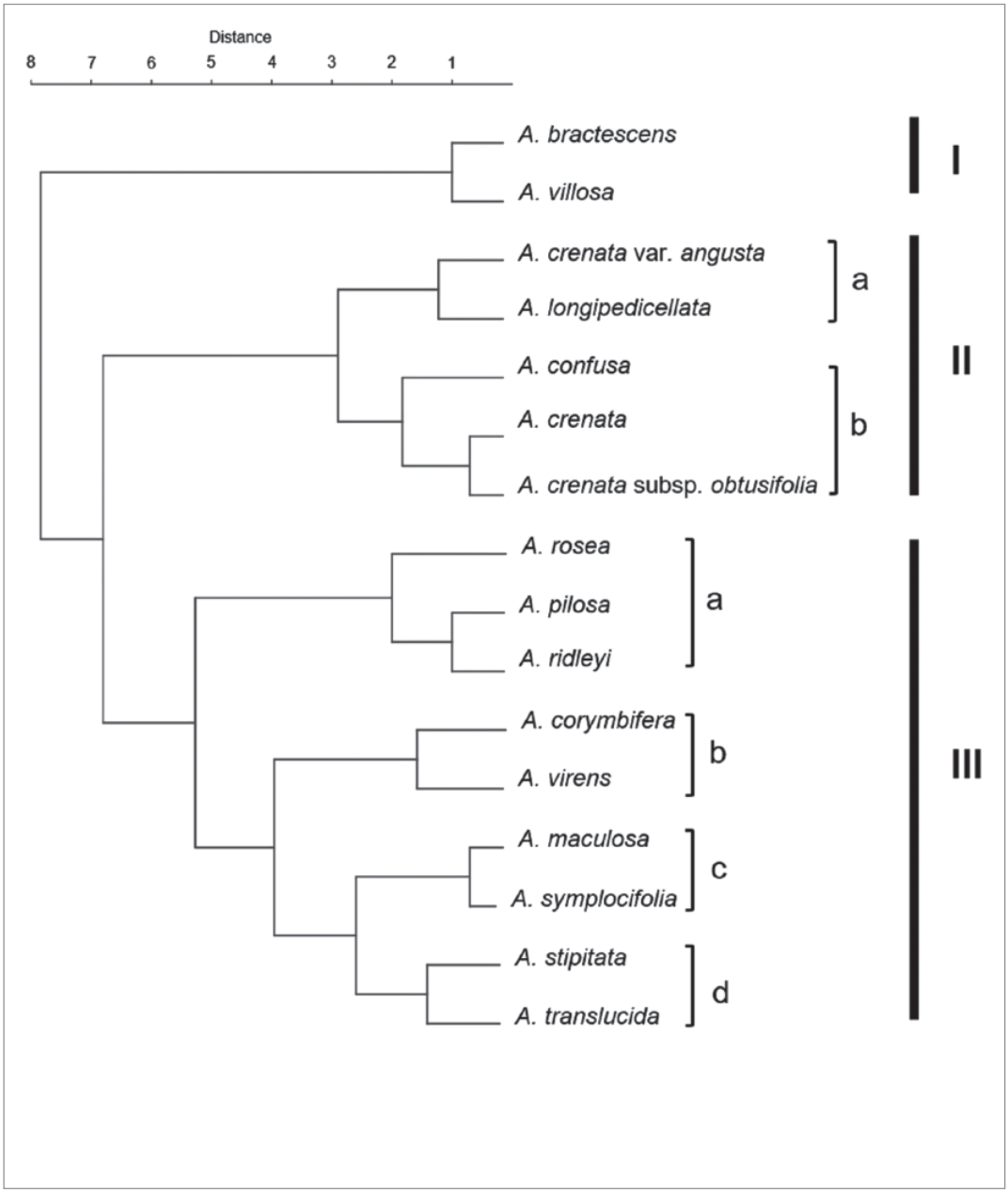

Figure 1. UPGMA dendrogram from 16 taxa of *Ardisia* subgenus *Crispardisia*. Three main groups result from cluster analysis based on morphological characters.

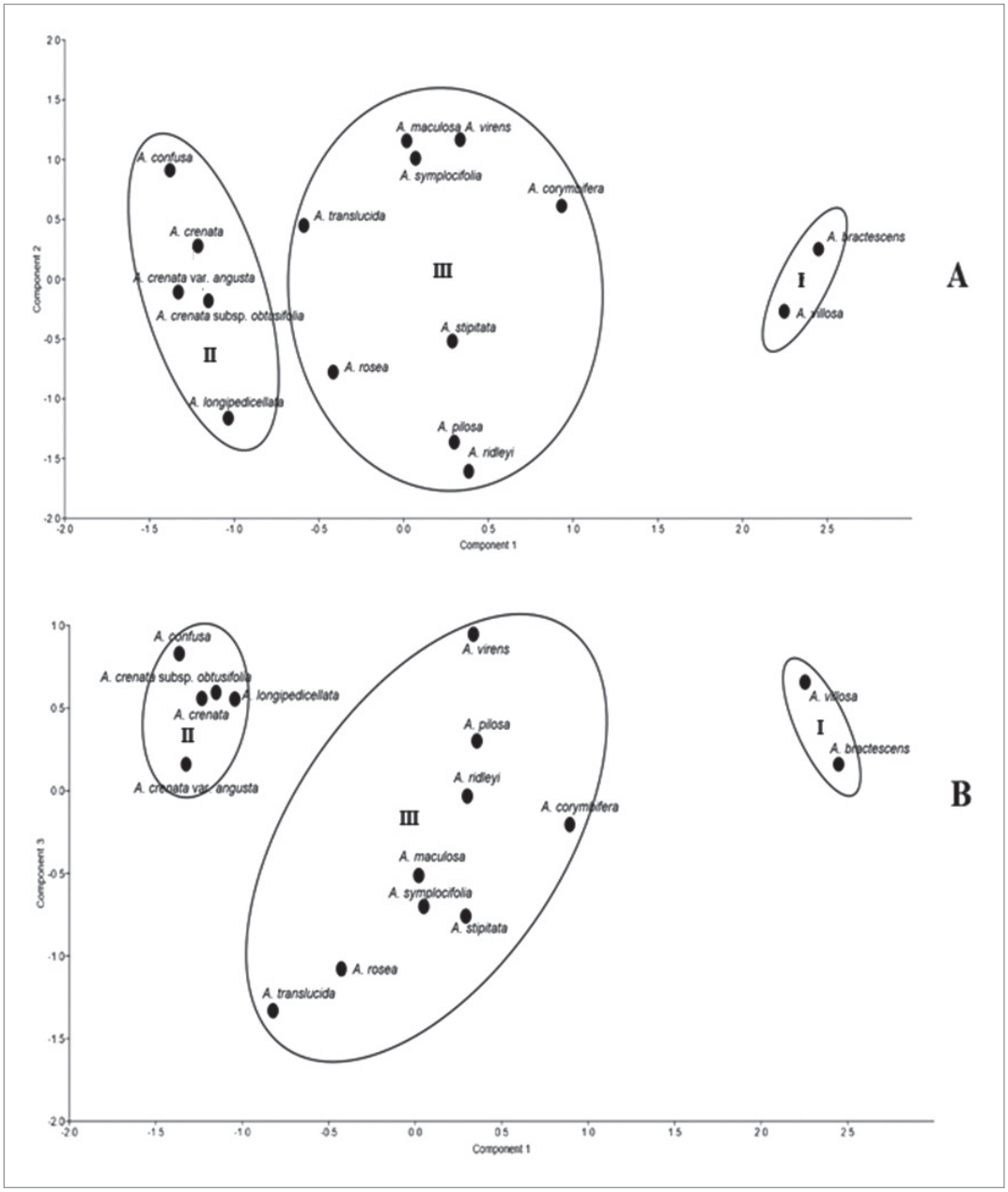

FIGURE 2. Scatterplot of PCA: A. axis 1 against axis 2 (account for 54.59 % of total variance); B. axis 1 against axis 3 (account for 47.27 % of total variance).

loading of 0.4) are calyx lobe length, leaf length and surface, and calyx punctation and peduncle surface for the first, second and third components, respectively (Table 5).

The calyx lobe length separated group I from groups II and III, the first group has a very long calyx lobe, from 5–7.5 mm long, especially *A. villosa,* which is the longest calyx lobe in this subgenus. The other groups have a shorter calyx length (1–3.5 mm long). In group I, *A. bractescens* differs from *A. villosa* by the scattered leaves along the flowering branches (vs tufted at the ends of the branches), the longer peduncle (10–15 vs 2–5 cm long) and glabrous on upper surface but very sparsely scurfy-puberulous or glabrescent on the lower leaf surface (vs villous on lower surface). The long calyx of both species is the most distinct characteristic and Larsen & Hu (1996) use this character to separate them from the others.

Group II consists of five taxa from three different species which is divided into two subgroups (IIa and IIb). Interestingly, three taxa of *A. crenata* are separated and located in different subgroups. *Ardisia crenata* var. *angusta* and *A. longipedicellata* are in the first group (IIa) based on punctate calyx and simple inflorescences. However, both taxa are different, the former has a very long acuminate leaf apex and distinct floral punctation but acuminate or obtuse leaf apex and without punctate flowers in the latter. *Ardisia crenata* var. *angusta* was proposed by Clarke (1882), followed by Larsen & Hu (1996). Although Mez (1902) accepted this taxon as *A. crispa* var. *angusta*. The numerical study and examination of the holotype, *Griffith 3584* (K000756755) at Kew, shows that *A. crenata* var. *angusta* is different from *A. crenata* by having a very long acuminate leaf apex, but actually, *A. crenata* var. *angusta* belongs to *A. crispa*. Morphologically, *A. crispa* has scattered leaf distribution and simple inflorescences and both are shown in *A. crenata* var. *angusta*.

The second subgroup (IIb) comprises *Ardisia confusa*, *A. crenata* and *A. crenata* subsp. *obtusifolia*. *Ardisia confusa* differs from *A. crenata* by the leaves are longer and obscurely crenate margin, with small and inconspicuous marginal glands. Both *A. crenata* and *A. crenata* subsp. *obtusifolia* share many characteristics in common. Chatan & Promprom (2017) described *A. crenata* subsp. *obtusifolia* is a new subspecies that is different from *A. crenata* in many aspects of the shape, apex, margin and texture of the leaves, the primary rachis of inflorescence and the calyx. But in fact, all the characters in *A. crenata* are very variable, especially the size, shape and apex of the leaves. The leaf size varies from 6 by 2 cm to 15(–20) by 4 cm. The leaves are elliptic-lanceolate to oblanceolate and oblong-lanceolate. The leaf apex varies from acute to acuminate, sometimes obtuse to rounded. The primary rachis is generally absent if present it can be 2–4 cm long. The base of the sepals is sometimes overlapped. After careful examination, the apex of some leaves of *A. crenata* subsp. *obtusifolia*, is acuminate which resembles *A. crenata*. The leaf apex is a variable character and from the CA the *A. crenata* subsp. *obtusifolia* is close to

A. crenata. Therefore, *A. crenata* subsp. *obtusifolia* is placed herein as a new synonym of *A. crenata*.

Group III comprises nine different species which is divided into four subgroups (IIIa, IIIb, IIIc and IIId). The first subgroup (IIIa) comprises, namely *A. pilosa, A. ridleyi* and *A. rosea*. These species are hairy on their branches. The first two species have simple inflorescence while *A. rosea* has compound inflorescence. *Ardisia pilosa* differs from *A. ridleyi* by having densely pilose branches and coriaceous leaves. The second subgroup (IIIb) consists of *A. corymbifera* and *A. virens*. They share many similar characteristics viz. compound inflorescences, black punctate on the flowers, and more or less the same length as the calyx and corolla. The lower surface of the leaves of *Ardisia corymbifera* is hairy while glabrous or sparingly lepidote in *A. virens*. The third subgroup (IIIc) comprises two species viz. *A. maculosa* and *A. symplocifolia*. *Ardisia maculosa* was described by Mez (1902) and *A. maculosa* var. *symplocifolia* was proposed by Chen (1978). Later, Larsen & Hu (1996) raised this taxon as a species, *A. symplocifolia*. In addition, Chen & Pipoly (1996) placed *A. maculosa* and *A. maculosa* var. *symplocifolia* as synonyms of *A. virens*. However, we disagree with Chen and Pipoly's view because some morphological characters of *A. maculosa* and *A. virens* are different. The leaves, calyx, and corolla of *A. virens* are very distinct punctate but these characters are absent in *A. maculosa*. Moreover, the leaf apex of *A. virens* is acute whereas on *A. maculosa* is acuminate. From the work of Hu (1999) finally, *A. virens* is now placed under *A. polysticta* Miq. accepted by Hu & Vidal (2004) and followed by Toyama *et al*. (2013).

In our study, we found that the peduncle length of *A. maculosa* and *A. symplocifolia* is 0.5–1 cm and 1–3 cm, respectively and the leaf shape is variable between oblong-elliptic to oblanceolate. These characters showed that the differences between them are so small and unreliable that there are best united by put *A. symplocifolia* under *A. maculosa*. The fourth subgroup (IIId) comprises *A. stipitata* and *A. translucida*. Both species share common characteristics viz. simple inflorescences, epunctate and short calyx (2–2.5 mm long), and hairy branch and peduncle. The floral position of *A. translucida* is lateral with simple inflorescences while umbellate terminal inflorescences on flowering branches are found in *A. stipitata*.

CONCLUSION

The phenetic result from 13 characters brings three dominant characters to separate 16 taxa of *Ardisia* subgenus *Crispardisia*, with calyx length (0.86), calyx punctation (0.59) and peduncle surface (0.48) as the most important characters. The conclusion leads to some important taxonomic impacts in this subgenus. Two taxa *i.e*. *A. crenata* subsp. *obtusifolia* and *A. crenata* var. *angusta* became new synonyms of *A. crenata* and *A. crispa*, respectively while *A. symplocifolia* is a synonym of *A. maculosa*. The study also confirms *A. virens* no longer exists.

In general, the results display congruence between the CA and PCA analyses. Three groups were identified which have been given the names I, II and III. The results obtained from CA and PCA analyses confirmed three well-distinguished groups.

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