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Morphological Characteristics and Taxonomical Positions of Annonaceae in Indonesia

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ABSTRACT

Annonaceae is one of the most important and economically valuable plant families in Malesia region. Taxonomical studies could contribute to further utilization and conservation of the studied taxa. Due to their importance, an update in the morphological characters and taxanomical position of Annonaceae is required. This study aims to characterize the taxonomical position and relationships of 24 species belongs to the family Annonaceae found in the Purwodadi Botanic Gardens. In this work, we reported the taxonomical position and the phenetic clustering of 24 Annonaceae species collected from the Purwodadi Botanic Garden, East Java, Indonesia. The taxonomical study was performed based on morphological and phenetic approach where 81 characters (leaves, stem, and flower) were used in the analysis. A dendogram was produced following the scoring of the character using statistical program PAST ver. 3.0. (Hierarchial Cluster Analysis using Jukes Cantor Similarity approach). The dendogram showed clear separation between members of sub-family Annonoideae and Malmeoideae; however Alphonsea javanica and Sageraea lanceolata were not grouped into their respective tribe or sub-family. Further studies are required to evaluate these taxonomical separations. Overall, from the 24 studied species, we found that they grouped into 2 sub-family (Annonoideae and Malmeoideae), 3 tribes (Annoneae, Miliuseae, and Uvarieae), and 13 genus (Alphonsea, Annona, Desmos, Fissistigma, Miliusa, Mitrephora, Orophea, Polyalthia, Popowia, Pseuduvaria, Sageraea, Stelechocarpus, dan Uvaria).

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INTRODUCTION

Annonaceae, a plant family belonging to the order Magnoliales, is commonly found in Malesia, and holds high importance in the tropical rainforest ecosystem due to its ecological and economical values (Kessler, 1993; Kessler et al., 2002; Singh, 2010). In Indonesia, members of this family can be found in natural forests, conservation areas, and botanic gardens. A previous study reported that 56 spesies of Annonaceae can be conserved in Purwodadi Botanic Gardens, East Java, Indonesia

(Lestari & Azrianingsih, 2019). Members of Annonaceae exhibit diverse habits, including trees, shrubs, and lianas.

They have flowers with a variable structure and shape, usually with floral parts in multiples of three. The flower has 3 sepals and 6 petals, arranged in two whorls. The fruits, called carpids, vary in shape and include forms such as monocarps/apocarps and syncarps/pseudocarps (Rugayah et al., 2011 & Chatrou et al., 2014). There are 4 subfamilies of Annonaceae: Anaxagoreoideae,





Ambavioideae, Annonoideae and Malmeoideae (Chatrou et al., 2012; Chatrou et al., 2014). Annonoideae and Malmeoideae are the two largest subfamilies and members of these subfamilies can be abundantly found at the Purwodadi Botanic Garden. This area a serve as a good habitat model for studying their diversity and classification.

Morphological characters been extensively used by plant taxonomists to analyze phylogeny, evolution, and taxonomical classification of plants. Leaf, stem, and flower characters are the most well-studied and commonly used characters in taxonomical studies, especially leaf and stem characters because they are not seasonal and can be characterized anytime during plant development. Taxonomical relationship and classifications in plants can be analyzed using various methods, one of them being phenetic approach, which classifies plants based on similarities. These phenotypical phenotypical similarities might represent evolutionary relationships, but are also often found to be not related to evolutionary tree; therefore, phenetic analysis is separated from phylogeny analysis (Terry, 2000).

This study aimed to characterize taxonomical position and relationships of 24 species belongs to the family Annonaceae found in the Purwodadi Botanic Gardens. Results from this study clarify the taxonomical relationships within

the family and based onphenetic analysis, and support conservation efforts for the family in Indonesia. Overall, research on the morphological characteristics and taxonomical positions of Annonaceae in Indonesia is fundamental for botanical science. biodiversity conservation. ecological understanding, and potential applications in medicine and economics. It provides crucial insights into the diversity, evolution, and ecological significance of this important plant family in a region of immense botanical richness using automorphy morphological characters.

MATERIALS AND METHODS

Plants were sampled from the Purwodadi Botanic Garden, Pasuruan, East Java Indonesia and taxonomical analysis was performed Biosystematic Laboratory, Faculty of Science and Technology, Airlangga University, Surabaya, East Java, Indonesia. The 24 species of Annonaceae used in this study are: Alphonsea javanica, Annona glabra, A. montana, A. muricata, A. squamosa, Desmos chinensis, D. dumosus, Fissistigma latifolium, Miliusa horsfieldii, M. lineata, Mitrephora javanica, M. polypyrena, Orophea enneandra, O. hexandra, O. polycarpa, Polyalthia celebica, P. lateriflora, P. littoralis, Popowia pisocarpa, Pseuduvaria reticulata, Sageraea lanceolata, Stelechocarpus burahol, Uvaria concava, dan Uvaria schizocalyx (Figure 1).



Figure 1. Morphological characters of vegetative (stem and leaf) and reproductive organs (flower) from 24 Annonaceae species sampled from the Purwodadi Botanic Garden are as follows: a. Alphonsea javanica, b. Annona glabra, c. A. montana, d. A. muricata, e. A. squamosa, f. Desmos chinensis, g. D. dumosus, h. Fissistigma latifolium, i. Miliusa horsfieldii, j. M. lineata, k. Mitrephora javanica, l. M. polypyrene, m. Orophea enneandra, n. O. hexandra, o. O. polycarpa, p. O. Polyalthia celebica, q. P. lateriflora, r. P. littoralis, s. Popowia pisocarpa, t. Pseuduvaria reticulata, u. Sageraea lanceolata, v. Stelechocarpus burahol, w. Uvaria rufa, dan x. U. schizocalyx





The morphological character of vegetative (stem and leaf) and reproductive organs (flower) were characterized from the samples, supported by secondary data published previously by Lestari et al. (2017) and Lestari & Ningrum (2021). The 81 morphological characters used in this study can be found in Table 1. The collected descriptive data were converted to numerical data by assigning scores to each morphological characters so each OTU (*Operational Taxonomy Unit*) or taxon will have certain value based on their character states. The scoring was performed to facilitate the

grouping of OTUs. Each morphological character was scored from 1 to5, depending on the number of variations found in that morphological character. The scoring of the characters can be found in Table 1. Following scoring, the data were analyzed using Hierarchial Cluster Analysis method, performed using the statistical software PAST ver. 3.0, with Jukes-Cantor similarity approach. The analysis produced a dendogram that grouped taxa (OTUs) based on morphological characters, these groupings phenetic relationships between represent the studied members of Annonaceae.

Table 1. Morphological characters of the studied 24 members of Annonaceae

Characters, Character State and Scoring

Stem,

Habitus: 1. tree, 2. shrub, 3. liana

Crown shape; 1. umbrella, 2. cone, 3. cylinder, 4. irregular

Development of main stem; 1. monopodial, 2. Sympodial

Location of branches on main stem; 1. constant, 2. rhythmic

stem growth Direction; 1. perpendicular (erectus), 2. climbing (scandens)

Shape; 1. not rounded, 2. rounded

Length of stem; 1. length 0-10 m, 2. length 10,1-35 m, 3. length >35 m

Stem surface; 1. without trichome, 2. with trichome, 3. with lenticel, 4. with lenticel and trichome

Stem sculpturing; 1. without sculpturing, 2. with sculpturing

branch growth direction; 1. plagiotropic, 2. orthotropic

Stem color; 1. grey, 2. dark grey, 3. brown, 4. dark brown

r young branchesColo; 1. light green, 2. green, 3. dark green, 4. other

outer bark color; 1. light green, 2. green, 3. dark green, 4. Light brown, 5. brown, 6. Dark brown, 7. grey

inner bark color; 1. light green, 2. green, 3. dark green, 4. yellowish green, 5. yellow, 6. brownish yellow, 7. light brown

Leaf

Leaf texture; 1. thin and soft (herbaceous), 2. leathery (perkamentous)

Leaf apex/tip; 1. pointed (acute), 2. tapered (acuminate)

Leaf base; 1. pointed (acute), 2. cordate, 3. rounded

Leaf surface; 1. With trichome (glabrous), 2. with simple trichome (simple hairs), 3. with star-shaped trichome (stellate hairs)

Trichome on the surface of adaxial leaves; 1. absent, 2. Present

mature leaf Color; 1. green, 2. dark green

young leaf Color; 1. light green, 2. reddish yellow, 3. red, 4. purplish red, 5. other

Leaf venation; 1. non-pinnate, 2. pinnate

Leaf-blade venation; 1. submerged, 2. intermediate, 3. raised

leaf blade Shape; 1. lanceolate, 2. elliptical, 3. ovate, 4. obovate, 5. other

leaf margin; 1. entire, 2. Undulate

leaf length; $1. \le 15$ cm, 2. 15, 1 - 19, 5 cm, 3. > 19, 5 cm

Leaf diameter; 1. diameter 0-5 cm, 2. diameter 5,1-9,5 cm

Leaf arrangement; 1. alternate, 2. opposite

Leaf composition; 1. compound, 2. single

leaf stalk indumentum; 1. glabrous, 2. simple hairs, 3. stellate hairs

Length of leaf stalk (petiole); 1. length 0-1 cm, 2. length >1 cm

Lateral leaf stipule; 1. present, 2 absent

leaf Smell; 1. not fragrant, 2. fragrant, 3. very fragrant





Flower

Reproductive organ; 1. unisexual, 2. bisexual

Flower; 1. solitary, 2 compound

Flower arrangement; 1. axillar, 2. internodal, 3. apical, 4. across the leaf 5. At twig and branches, 6. At stem

Number of sepals; 1. has no 3 sepals, 2. with 3 sepals Number of petals; 1. with 6 petals, 2. with 3 petals

Number of whorls; 1. 1 whorl, 2. 2 whorls

Outer petal; 1. present, 2. absent Inner petal; 1. present, 2. absent Perianth; 1. present, 2. absent

Comparison between petal; 1. outer petal is shorter, 2. outer petal is smaller, 3. outer petal is longer, 4. outer petal is bigger, 5. outer petal is smaller and shorter, 6. outer petal is longer and bigger, 7. outer and inner petal have similar size and length

flower stalk Color; 1. light green, 2. green, 3. dark green, 4. yellowish green, 5. reddish green, 6. brownish green, 7. Brown

flower stalk surface; 1. glabrous, 2. simple trichome (simple hairs), 3. star-shaped trichome (stellate hairs)

sepal trichome; 1. absent, 2. present

Sepal type; 1. valvate, 2. reduplicate-valvate, 3. apert, 4. others

sepal fusion; 1. free, 2. connate

Sepal shape; 1. depressed to narrowly ovate, 2. triangular, 3 cup-shaped calyxes

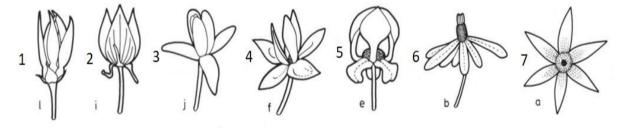
Sepal texture; 1. *not fleshy*, 2. *fleshy* Sepal thickness; 1. thin, 2. thick

Sepal size; 1. short (0-25 mm), 2. long (>25 mm)

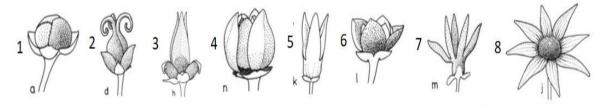
Sepal surface; 1. glabrous, 2. simple trichome (simple hairs), 3. star-shaped trichome (stellate hairs)

Sepal color; 1. light orange, 2. light green, 3. green, 4. dark green, 5. brownish green, 6. green with reddish edges, 7. brown

Expansion of petal (Van Heusden, 1992);



petal aestivation (Van Heusden, 1992);



Petal fusion; 1. free, 2. connate

Outer petal color; 1. white, 2. white with purplish stripes, 3. white to white yellowish, 4. light yellow, 5. yellow, 6. yellowish, 7. pale yellow to yellowish, 8. yellow to peach, 9. peach yellowish to yellow, 10. light green, 11. green, 12. greenish, 13. green yellowish, 14. Orange, 15. Reddish to pinkish, 16. Red

Inner petal color; 1. white to white yellowish, 2. white with purplish stripes, 3. light yellow, 4. yellow, 5. yellow with purple stripes, 6. yellowish, 7. peach yellowish, 8. green yellowish, 9. greenish, 10. yellow to orange, 11. orange, 12. peach to pink, 13. pinkish to yellow, 14. red brownish, 15. red, 16. dark red

Trichome at petal; 1. absent, 2. present

Petal texture; 1. not fleshy, 2. fleshy





Petal thickness; 1. thin, 2. thick

Petal size; 1. small (<0,5 cm), 2. intermediate (0,5-14,5 cm), 3. big (>14,5 cm)

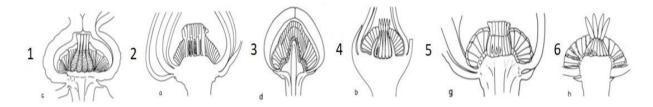
Petal shape; 1. elliptic, 2. ovate, 3. elliptic (outer petal) and mitre-shaped (inner petal), 4. ovate (outer petal) dan mitre-shaped (inner petal)

Inner petal shape; 1. valvate, 2. mitriform

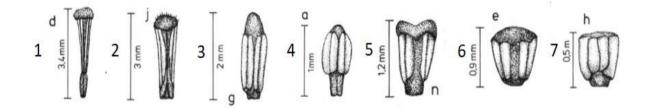
Floral glands; 1. absent, 2. present

Petal surface; 1. Without trichome (glabrous), 2. with simple trichome (simple hairs), 3. with star-shaped trichome (stellate hairs)

Torus of petal (Van Heusden, 1992);



Number of stamens; 1. few (1-6), 2. intermediate (7-12), 3. many (>12) Shape of stamen (Van Heusden, 1992)



Stamen type; 1. uvarioid, 2. miliusoid

Stamen length; 1. short (0,1-7 mm), 2. long (>7 mm)

Stamen Color; 1. white, 2. creamy white, 3. yellowish white, 4. cream, 5. yellowish cream, 6. orange yellow, 7. pale yellow, 8. brownish yellow

Stamen texture; 1. not fleshy, 2. fleshy

Carpel shape; 1. dolabriform, 2. spatula, 3. ellipsoid, 4. cylindrical, 5. ovoid, 6. cordate, 7. other

Number of carpels; 1. Few (<6), 2. intermediate (6-20), 3. many (>20)

Fusion of carpel; 1. apocarpous, 2. syncarpous

Carpel length; 1. short (0,5-10 mm), 2. long (>10 mm)

Trichome on carpel; 1. absent, 2. present

Hook/tendrils; 1. absent, 2. present

RESULTS AND DISCUSSION

The morphological characters of plants, particularly in the Annonaceae family, are highly consistent and are frequently used as taxonomical markers. However, there are instances where collections from certain regions, such as those from East Java conserved in the Purwodadi Botanic Gardens, present challenges due to unclear morphological characteristics. These challenges can obscure the taxonomical position of the species, sometimes leading to plants remaining unidentified at the species level.

Morphological/Phenetic Characters

According to Simpson (2010), there are several characters specific to Annonaceae. This family is known to have single leaf composition, alternate leaf arrangement, and nostipules. Members of Annonaceae are often found as trees, shrubs, or lianas, all with cambium in the stem. The flowers can be found in solitary or compound compositions with bisexual or unisexual reproductive organs. These flower characters differentiate it from Magnoliaceae, as all members of Magnoliaceae have solitary flowers, although both are member of Magnoliales.





Dendrogram of studied Annonaceae species based on morphological character

The dendogram construction grouped the taxa into two subfamilies and three tribes (Figure 2). Sageraea lanceolata (SGL) is separated from the rest of the studied taxa and grouped into the tribe Annoneae,, leading to the assumption that this species belongs to the subfamily Annonoideae. However, Chatrou et al. (2012) mentioned that S. lanceolata is a member of the tribe Miliuseae and subfamily Malmeoideae. The separation of S. lanceolata from subfamily Malmeoideae in our dendogram is probably due to the present of perianth flower in this species (sepal and petal are fused together) (Figure 3). Only a few members of Malmeoideae have this characteristic while in many members of Malmeoideae this character state is absent. We also observed the grouping of Alphonsea javanica (APJ) into Annoneae, while Guo et al. (2017) reported that this species belongs to Miliuseae. This is probably due to morphological similarities between A. javanica and members of Annoneae, such as similarity in sepal and petal thickness, stamen shape, stamen type (uvarioid),

carpel shape (dolabriform), and stamen type. Missplacement of taxa in dendrogram could occur when insufficient fruit and seed characters are used during phenetic analysis. The accuracy of phenetic analysis based on morphological characters can be enhanced by using diverse characters from reproductive organs (flower, fruit, and seed). However, reproductive organs are not always available for phenetic studies since some species rarely produce reproductive organs and some other only produce it at specific and short period of times (Lestari, 2019). Nevertheless, the dendogram showed clear separation between members of Annonoideae and Malmeoideae. Members of these two subfamilies are separated because they have different petal and stamen shapes. Members of Annonoideae have valvate petals, while members of Malmeoideae have *mitriform* petals (Figure 3). These findings are in accordance with report from Lestari et al. (2017). In addition, stamens of Annonoideae are short and broad, while stamens of Malmeoideae have other forms.

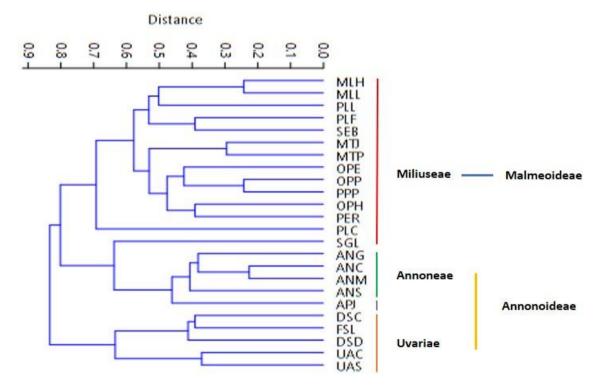


Figure 2. Dendrogram of 24 studied Annonaceae species based on phenetic analysis. Red lines represent members of the tribe Miliuseae, green lines represent members of the tribe Annoneae, and orange lines represent members of tribe Uvarieae. Abbreviations: Alphonsea javanica (APJ), Annona glabra (ANG), Annona montana (ANM), Annona muricata (ANC), Annona squamosa (ANS), Desmos chinensis (DSC), Desmos dumosus (DSD), Fissistigma latifolium (FSL), Miliusa horsfieldii (MLH), Miliusa lineata (MLL), Mitrephora javanica (MTJ), Mitrephora polypyrena (MTP), Orophea enneandra (OPE), Orophea hexandra (OPH), Orophea polycarpa (OPP), Polyalthia celebica (PLC), Polyalthia lateriflora (PLF), Polyalthia littoralis (PLL), Popowia pisocarpa (PPP), Pseuduvaria reticulata (PER), Sageraea lanceolata (SGL), Stelechocarpus burahol (SEB), Uvaria concava (UAC), dan Uvaria schizocalyx (UAS)







Figure 3. Flower characteristics of 24 studied Annonaceae species are as follows; a. Alphonsea javanica, b. Annona glabra, c. A. montana, d. A. muricata, e. A. squamosa, f. Desmos chinensis, g. D. dumosus, h. Fissistigma latifolium, i. Miliusa horsfieldii, j. M. lineata, k. Mitrephora javanica, l. M. polypyrene, m. Orophea enneandra, n. O. hexandra, o. O. polycarpa, p. O. Polyalthia celebica, q. P. lateriflora, r. P. littoralis, s. Popowia pisocarpa, t. Pseuduvaria reticulata, u. Sageraea lanceolata, v. Stelechocarpus burahol, w. Uvaria rufa, and x. U. schizocalyx





Tabel 2. Classification of 24 studied Annonaceae species

Subfamily	Tribe	Genus	Species
			Annona glabra
			Annona montana
	Annoneae	Annona	Annona muricata
Annonoideae			
			Annona squamosa
	Uvarieae	Desmos	Desmos chinensis
			Desmos dumosus
		Fissistigma	Fissistigma latifolium
		Uvaria	Uvaria concava
			Uvaria schizocałyx
Malmeoideae		Alphonsea	Alphonsea javanica
		Miliusa	Miliusa horsfieldii Miliusa lineata
		Mitrephora	Mitrephora javanica Mitrephora polypyrena
		Orophea	Orophea enneandra
			Orophea hexandra
			Orophea polycarpa
	Miliuseae		Polyalthia celebica
		Polyalthia	
			$Polyalthia\ lateriflora$
			Polyalthia littoralis
		Popowia	Popowia pisocarpa
		Pseuduvaria	Pseuduvaria reticulata
		Sageraea	Sageraea lanceolata
		Stelechocarpus	Stelechocarpus burahol

Members of tribe Uvariae are grouped together because all of them are lianas with climbing (scandens) stem growth and trichomes in stem surface and abaxial leaf surface (stellate hairs in Uvaria and simple hairs in Desmos and Fissistigma; Turner (2012)). Members of tribe Miliuseae are clustered together because mainly their habitus is trees and shrubs and the absent of hooks (tendrils). Mols dan Keβler (2003) mentioned that Miliuseae are characterized by their stamen type (miliusoid), valvate petals, and free carpels.

Taxonomical positions of studied Annonaceae species

Annonaceae are divided into four subfamilies: Anaxagoreoideae, Ambavioideae, Annonoideae and Malmeoidea. The subfamily Annonoideae consists of seven tribes: Bocageeae, Xylopieae, Duguetieae, Guatterieae, Annoneae, Monodoreae, and Uvarieae. Similarly, the subfamily Malmeoideae also comprises seven tribes: Piptostigmateae, Malmeeae, Fenerivieae, Maasieae, Dendrokingstonieae Monocarpiae, and Miliuseae.Most species within Annonaceae belong to these \ two subfamilies

(Annonoideae and Malmeoideae) (Chatrou et al., 2012).

From the seven tribes of the subfamily Annonoideae, two tribes, Annoneae and Uvarieae, are included in this study. The members of Annoneae studied here are from the genus Annona, while the members of the tribe Uvarieae assessed in this study belong to genus Desmos, Fissistigma and Uvaria. In this study, Miliuseae are represented by various gene including Miliusa, Orophea, Polyalthia, Pseuduvaria, Sageraea, and Stelechocarpus. Tribes that belong to subfamily Malmeoideae are monophyletic taxa (Chaowasku et al., 2014). The classification of 24 Annonaceae species analyzed in this study is based on reports by Hooker & Thomson (1855), Chatrou et al. (2012), Chaowasku et al. (2014) and Chaowasku et al. (2018) that summarized and presented in Table 2.

Morphological characters in Miliuseae tribes based on similarity (synapomorphy) of the habit of trees, development of the main stem is monopodial, location of branches on the main stem is rhythmic, direction of stem growth is perpendicular (erect), direction of branch growth





Chaowasku et al. (2014) classify each genus in the tribe Miliuseae based on pollen forms, with the genus of Mitrephora and Pseuduvaria having pollen in the form of disulculate; tetrad, the genus Orophea, Meiogyne, Popowia, Miliusa Polyalthia having pollen in the form of disulculate; monads, and the genus of Stelechocarpus having pollen in the form of pollen cryptoaperturate/disulculate; monads. Based on both groupings, distinctions are predominantly made byhabit and generative characteristics, especially in flowers (especially in staminodes, connective apical prolongation, and pollen characters). The results of this study add characters to the grouping by each including the development of the main stem as monopodial, the location of branches on the main stem as rhythmic, the direction of stem growth as perpendicular (erect), and the direction of branch growth as plagiotropic with the smell of bark being fragrant.

The taxonomic relationship between species in plant systematics can be analyzed based on morphology of phenotypiccharacters. In general, morphological characters are used as the basis for phenetic analysis. Taxonomic relationship based on phenetic character represent morphological similarities between group of taxa or species (Rasnovi, 2004). The outcome of phenetic analysis based on morphological characters can be influenced by environmental factors that induce phenotypic variation in a species. Therefore, in phenetic analysis plastic characters need to be avoided (Nikmah et al., 2020).

An example of such plastic character is the variation in glands character in the genus *Orophea* collected from Purwodadi Botanic Garden (Lestari, 2011). The phenetic analysis based on morphological characters clearly separates the 24 studied species into two subfamilies and three tribes. However, we also observed missplacement of *S. lanceolata* and *A. javanica*. These two species should be grouped together with the other members

of the tribe Miliuseae and subfamily Malmeoideae. More accurate grouping can be achieved by involving members of other subfamilies, including Anaxagoreoideae and Ambavioideae.

The grouping and classification of Annonaceae constantly revised and updated because taxonomist continuouslyadd new characters to the analysis than can produce better separation between Classification based on morphological characters is constantly updated by Hooker & Thomson (1855), Van Heusden (1992) and Keßler (1993) using flower and fruit characters, by Koek-Norman & Westra (2012) using stem anatomy, by Doyle & Thomas (2012), Yunyun & Xu (2017), and many other publications. Currently, classification based on morphological characters is also supported by molecular datato resolve and better clarify Annonaceae classification. Recent reports related to Annonaceae classification using molecular markers have been published by Guo et al. (2017) regarding the mega-phylogeny of Annonaceae, Couvreur et al. (2019) regarding the phylogenomics of Annonaceae, Chaowasku (2020) regarding the phylogenetic reclassification of the subfamily Ambavioideae, and many other publications. The findings of this research can be used as a basis for the classification of Annonaceae from Indonesia and further studies related to the taxonomical position of species within Annonaceae.

The primary aim of this study is to address these issues by classifying and identifying the species that have not been clearly identified. By doing so, the taxonomical position of these species can be elucidated. This classification and identification process will involve a comprehensive examination of morphological features, potentially including but not limited to leaf shape, flower structure, fruit characteristics, and other relevant traits specific to the Annonaceae family.

CONCLUSION

In this study, we revealed the taxonomical position of 24 Annonaceae species from Purwodadi Botanic Gardens. They are grouped into the tribes Annoneae and Uvariae, which belongs to the subfamily Annonoideae, and the tribe Miliuseae which belongs to the subfamily Malmeoideae. Compared to other tribes, Annoneae and Uvariae are more closely related. Based on the 81 phenetic characters used in this study, including stem, leaf, and flower characters, each studied species was found to have both shared and specific characters.





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