Variation and clustering analysis of several species of soursop family (Annonaceae) based on vegetative morphology characters

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ABSTRACT

The Annonaceae family exhibits a high level of diversity and is spread over many regions. The designation of the names of several species of the Annonaceae family varies in each region. Therefore, characterization is needed to confirm and classify the taxonomy position of the species. This study aims to characterize the variation and analyze the clustering pattern of several species of the Annonaceae based on vegetative morphology characters. The type of method used in this research is exploratory descriptive, with seventeen specimen species consisting of ten Annonaceae studied in this research. Characters observed include tree age, stem diameter, plant height, stem color, type of sapling, type of branching, leaf shape, leaf tip shape, leaf base shape, leaf width, leaf length, leaf thickness, petiole length, and thickness, leaf hairs, color of young and mature leaves, type of leaf edge, number of leaf veins, color of young branches, leaf hairs on young branches, and number of nodes on the stem per meter. These qualitative and quantitative characters were observed using Descriptors for Cherimoya (Annona cherimola Mill.) from Bioversity International and CHERLA. Data analysis was conducted in the Paleontological Statistics program with cluster analysis method (Bray-Curtis similarity index) to produce a dendrogram topology. Results showed that there were variations in vegetative morphological characteristics among the ten species examined. Dendrogram topology was divided into 3 groups relatively following the tribe division, with a similarity index of 0.64 to 0.96. Xylopia sp. was separated from the others in Group 1 (Xylopiae). Group 2 (Uvariae) consisted of Fissistigma sp., Desmos chinensis, Artabotrys suaveolens, Uvaria purpurea, and Desmos sp. Group 3 (Annoneae) consisted of Annona muricata, Annona montana, Stelechocarpus burahol, and Annona glabra. Vegetative morphological characters have proven useful and effective in differentiating species in the Annonaceae family; it is recommended for the initial identification of species, especially in the Annonoideae subfamily.

Keywords: *annonaceae; dendrogram; diversity; grouping; morphological*

INTRODUCTION

The Annonaceae family has about 109 genera and 2,440 species consisting of several plant habitus, namely trees, shrubs and lianas. They are commonly recognized as the custard apple family or the soursop family (Couvreur et al., 2012). Nowadays, Annonaceae are widely distributed across several countries in tropical to subtropical regions around the world, including Indonesia (Lestari & Ningrum, 2021). Ecologically, the Annonaceae plants also have an important role in regulating temperature, humidity or water reserves, especially in tropical rain forest ecosystems of Kalimantan (Trimanto et al., 2021). The high diversity and distribution of Annonaceae has many potential uses, including as edible fruits (food source), perfume, cosmetics, traditional medicine, biological pesticides, ornamental plants, *etc*. (Akpabio & Akpakpan, 2012; Jun Cheng, 2012; Wang et al., 2012; Aziz et al., 2016).

Several species from the Annonaceae family are widely cultivated and utilized by communities, such as *Annona muricata*, *Annona squamosa*, *Annona montana*, and *Stelechocarpus burahol*. Beside their edible fruits, the leaves of *Annona muricata* and *Stelechocarpus burahol* are used by the people in Yogyakarta to treat high blood pressure and gout (Nahdi et al., 2016). While the roots, bark, fruit and leaves of *Annona muricata* are used to treat malaria, fever by residents in India, Madagascar and Indonesia (Frausin et al., 2014). The fruit of *Stelechocarpus burahol* is used to get rid of body odor (Darusman et al., 2012). It has traditionally been used as a perfume ingredient, especially in ancient times by the palace princesses to scent their sweat, breath and urine (Tisnadjaja et al., 2006).

Specifically in Indonesia, each region has different local names for several species of the Annonaceae: Annonoideae. For an example is the *Annona muricata*, known as nangka sebrang or nangka landa (Java), nangka walanda, sirsak (Sunda), nangka buris, nangkelan (Madura), srikaya jawa (Bali), boh lona (Aceh), durio ulondro (Nias), durio betawi (Minangkabau), jambu landa (Lampung), nangko belando (Palembang), etc. (Inda, 2021). Differences in the local designation of species of the Annonaceae family in each region may affect the diversity data. In addition, the

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taxonomic position of Annonaceae species is still debatable, especially grouping based on morphological characters which needs to be clarified (Lestari et al., 2017). Therefore, characterization is needed to confirm and classify the taxonomy position of the species. One approach to analyzing plant diversity and its grouping is using the morphological character (Gusmiati et al., 2018; Probojati et al., 2023a).

The morphological characteristics of plants have long been used as the basis for identifying plant species and inferred the taxonomic position (Lestari et al., 2017; Santoso & Purnomo, 2021). Moreover, plant morphological characterization is significant for detecting the special traits desired, identifying duplicated accessions, and structuring populations for conservation purposes (Sobir, 2006; Sukartini, 2007; Rahajeng, 2015). However, morphological characterization requires a long time because it is needed to wait for the complete plant life cycle to flowering and fruiting (Gusmiati et al., 2018; Probojati et al., 2023b). Meanwhile, the vegetative morphology is always available throughout the year so it is significant to study, as a key distinguishing character.

Hence, this study aims to characterize the variation and analyze the clustering pattern of several species of the Annonaceae family based on vegetative morphology characters. The result of this research hopefully can be used as a basis and guide for identification, collection, conservation, development and breeding efforts to improve the genetic characteristics of soursops in the future, as well as developing them into products that have better value and utilization.

MATERIALS AND METHODS

This research uses an exploratory descriptive method, the results of this method are used to characterize and analyze the grouping patterns of the Annonacea family. The plant materials examined in this study were 17 specimens from the Annonaceae family. It consists of 9 species from the Annonoideae subfamily and 1 species from the Malmeoideae subfamily (Table 1) (Chatrou et al., 2012). The plant specimens are living collections of Purwodadi Botanic Garden, National Research and Innovation Agency located in Purwodadi, Pasuruan, East Java, Indonesia. They were originally collected from several regions in Indonesia including East Java, Yogyakarta, West Java, East Kalimantan and Sulawesi (Table 1, Figure 1).

Observations were conducted on 22 selected vegetative morphological characters, both qualitative and quantitative based on the Descriptors for Cherimoya (*Annona cherimola* Mill.) from Bioversity International and CHERLA (2008). Characters observed included tree age, stem diameter, plant height, stem color, tiller type, branching type, leaf shape, leaf tip shape, leaf base shape, leaf width, leaf length, leaf thickness, petiole length and thickness, hair on leaves, color of young and mature leaves, type of leaf edge, number of leaf veins, color of young branches, leaf hairs on young branches, and number of nodes on stem per meter. The detailed morphological characters and character states observed were presented in Table 2.

The morphological characterization data of each species from observations were compiled, analyzed and discussed descriptively to determine the distinguishing characters. Furthermore, the characterization data were quantified prior to clustering analysis. Characters with nominal and ordinal data were scored (1, 2, 3, up to the-n). Meanwhile, quantitative characters data were converted into interval scale (1, 2, 3, up to the-n) (Gusmiati et al., 2018). The quantified data were then subjected to clustering analysis using Paleontological Statistics (PAST) version 4.04 software. The dendrogram clustering was reconstructed using multivariate-clustering-classical menu options, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm and Bray-curtis similarity index. The similarity indices among species were also retrieved (Hammer et al., 2001).

RESULTS AND DISCUSSION

The characterization results showed that there were variations in vegetative morphological characteristics among the ten species examined. Leaf shape has become the distinguishing vegetative morphological characters in some species. The leaf shape of the majority of species is ovate, but in *Artabotrys suaveolens* and *Uvaria purpurea* are obovate. Meanwhile, *Xylopia sp.* is lanceloate (Figure 2). Furthermore, the ten species examined are belong to four tribes, i.e. Miliuseae, Annonea, Uvariae and Xylopiae (Chatrou et al., 2012). According to this study, they shared similar characteristics in acuminate leaf apex shape and entire leaf margin (Figure 2).

Stelechocarpus burahol has an ovate leaf shape with acute tip and acuminate base (Figure 2.A.). Group of Annona species varied in leaf characteristics. The leaves of *Annona muricata* are ovate shaped with acute tip and base, similar to *Desmos sp.* but slightly larger in size (Figure 2.B.). Meanwhile, *Annona montana* has an ovate shape, acute tip but acuminate base (Figure 2.C.). There is a difference that is unique to *Annona glabra*, i.e. the shape of the leaf is elliptic with rounded tip and acute base (Figure 2.D.). *Stelechocarpus burahol* has similar leaf characters with the Annoneae group, which influences the grouping pattern of this species. According to Chatrou et al., (2012) based on molecular phylogenetics of eight plastid markers, *Stelechocarpus burahol* belongs to the Miliuseae tribe, Malmeoideae subfamily. However, the result of this study based on vegetative morphological characteristics is in accordance with Lestari et al., (2017) where the tribe of Miliuseae is nested in the Annoneae cluster of the Annonoideae subfamily.

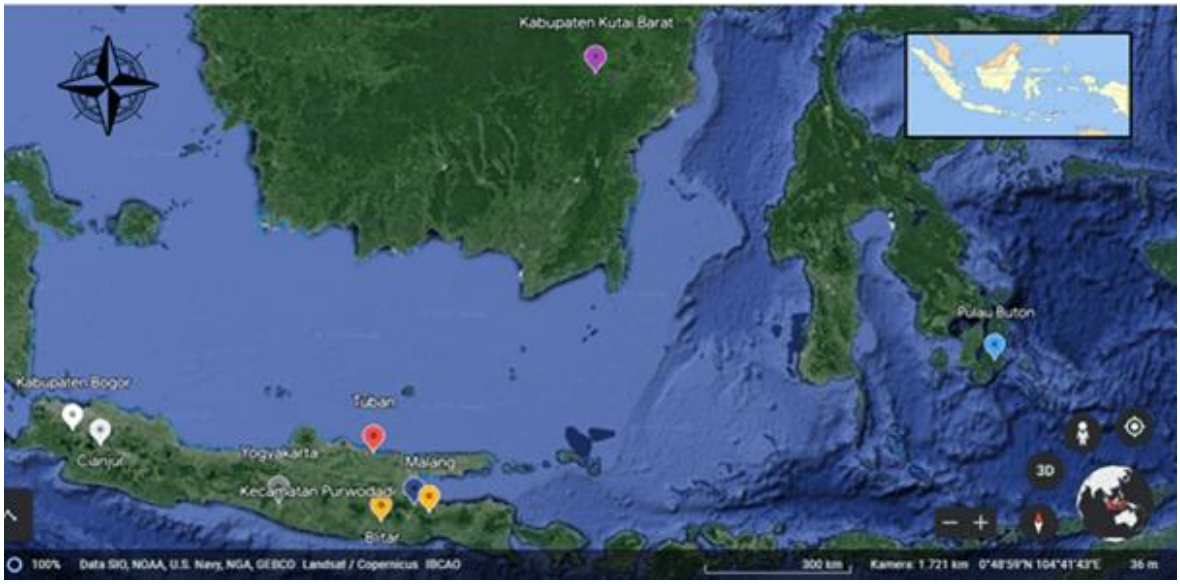

Figure 1. Map of locations where specimens from the Annonaceae family were collected

Table 1. List of plant specimens of the Annonaceae: Annonoideae examined in this study

No	Species name	Tribe	Subfamily	Collection number	Registration number	Locality
1	*Stelechocarphus burahol (Blume) Hook.f. & Thomson*	Miliuseae	Malmeoideae	I.A.48	P1977040130	Pasuruan, East Java
2	*Stelechocarphus burahol (Blume) Hook.f. & Thomson*	Miliuseae	Malmeoideae	XII.G.D.4	P1965010005	Malang, East Java
3	*Stelechocarphus burahol (Blume) Hook.f. & Thomson*	Miliuseae	Malmeoideae	XIV.G.II.8	P1997110065	Pasuruan, East Java
4	*Annona muricata L.*	Annoneae	Annonoideae	XVIII.C.28	P1977090001	Malang, East Java
5	*Annona muricata L.*	Annoneae	Annonoideae	XVIII.C.18	P1977020046	Yogyakarta, D.I Yogyakarta
6	*Annona montana Macfad*	Annoneae	Annonoideae	II.B.13	P1947060002	Bogor, West Java
7	*Annona montana Macfad*	Annoneae	Annonoideae	II.B.13a	P1947060002	Bogor, West Java
8	*Annona montana Macfad*	Annoneae	Annonoideae	XVIII.C.15	P1981040069	Pasuruan, East Java
9	*Annona montana Macfad*	Annoneae	Annonoideae	XVIII.C.15a	P1981040069	Pasuruan, East Java
10	*Annona montana Macfad*	Annoneae	Annonoideae	XVIII.C.15b	P1981040069	Pasuruan, East Java
11	*Annona glabra L.*	Annoneae	Annonoideae	XVIII.C.27	P1980030009	Cianjur, West Java
12	*Fissistigma latifolium (Dunal) Merr.*	Uvariae	Annonoideae	XVIII.C.30	P1982030062	Tuban, East Java
13	*Desmos chinensis Lour.*	Uvariae	Annonoideae	XVIII.C.6	-	Blitar, East Java
14	*Desmos sp.*	Uvariae	Annonoideae	XIX.B.I.3	P2003060019	Kutai Barat, East Kalimantan
15	*Uvaria purpurea Blume.*	Uvariae	Annonoideae	XIX.B.I.7	P2003080074	Kutai Barat, East Kalimantan
16	*Artabotrys suaveolens Blume*	Xylopiae	Annonoideae	XVIII.C.8	P1979070075	Malang, East Java
17	*Xylopia sp.*	Xylopiae	Annonoideae	XIX.B.I.15	P2002060160	Buton, Sulawesi

Data source: DPKI BRIN Purwodadi

Table 2. Morphological characters and character states observed in Annonaceae

No.	Characters	Character state
1	Tree age (years)	1. 1-15, 2. 16-30, 3. 31-45, 4. 36-60, 5. 61-75
2	Crown diameter (cm)	1. 1-28, 2. 29-56, 3. 57-84, 4. 85-112, 5. 113-140
3	Tree height (cm)	1. 1-360, 2. 361-720, 3. 721-1080, 4. 1081-1440, 5. 1440-1800 cm
4	Trunk colour	1. Light grey, 2. Grey, 3. Dark grey,
5	Trunk ramification	1. One branch, 2. Two branches, 3. Three or more branches
6	Suckering tendency	1. Absent , 2. ≤5 Suckers, 3. ≥Suckers
7	Leaf blade shape	1. Ovate, 2. Elliptic, 3. Obovate, 4. Lanceolate
8	Shape of leaf apex	1. Acute, 2. Rounded, 3. Acuminate
9	Shape of leaf base	1. Acute, 2. Rounded, 3. Obtuse, 4. Cordate
10	Leaf length (cm)	1. 1-6, 2. 7-12, 3. 13-18, 4. 19-24, 5. 25-30
11	Leaf width (cm)	1. 1-2, 2. 3-4, 3. 5-6, 6. 7-8, 7. 9-10
12	Leaf thickness (mm)	1. 0.1-0.6, 2. 0.7-0.12, 3. 0.13-0.18, 4. 0.19-0.24, 5. 0.25-0.30
13	Petiole length (cm)	1. 0.1-0.5, 2. 0.6-1.0, 3. 1.1-1.5, 4. 1.6-2.0, 5. 2.1-2.5
14	Petiole thickness (mm)	1. 1-1.78, 1.79-2.56, 2.57-3.34, 3.35-4.12, 4.13-4.9
15	Pubescence of leaf upper surface	1. Absent, 2. Present
16	Colour of mature leaves	1. Light green, 2. Green, 3. Greyish green, 4. Dark green,
17	Colour of young leaves	1. Light green, 2. Green, 3. Dark green,
18	Leaf margin	1. Entire, 2. Undulate
19	Number of primary veins in the leaf blade	1. 1-6, 2. 7-12, 3. 13-18, 4. 19-24, 5. 25-30
20	Colour of young branches	1. Light green, 2. Green, 3. Dark green
21	Pubescence of young branches	1. Absent, 2. Present
22	Number of nodes per meter of branch	1. 1-5, 2. 6-10, 3. 11-15, 4. 16-20, 5. 21-25

The tribe of Uvarieae (Annonoideae subfamily) was divided into two groups based on the vegetative characteristics of the leaves. Group 1 comprises *Desmos sp*. and *Desmos chinensis*; whilst Group 2 consists of *Fissistigma latifolium* and *Uvaria purpurae*. Group 1 was characterized by ovate leaf shape with acute tip and acute base (Figures 2.F. and 2.G). Whereas, group 2 was characterized by obovate leaf shape, with acute tip and cordate base (Figures 2.E. and 2.H.). The Uvaria and Fissistigma genera have similar leaf characteristics. In addition, most of the Uvariae tribe have morphological characteristics: stellate hairs, valvate aestivation of petal and basally connate (Zhou et al., 2010).

Artabotrys suaveolens has an obovate leaf shape with an acute tip and obtuse base (Figure 2.I.), meanwhile, *Xylopia sp.* has a lanceolate leaf shape with acute tip and acute base (Figure 2.J.). Previous studies reported that genus Artabotrys was included in the tribe of Xylopieae (Annonoideae subfamily) (Chatrou et al., 2012; Chen et al., 2020); and considered as a sister group (Johnson & Murray, 2018). However, their vegetative characters observed in this study showed similarities to the Uvariae tribe. A characterization using a combination of both vegetative and generative morphological characters by Lestari et al., (2017) reported that *Artabotrys uncinatus* was clustered in the Uvariae tribe.

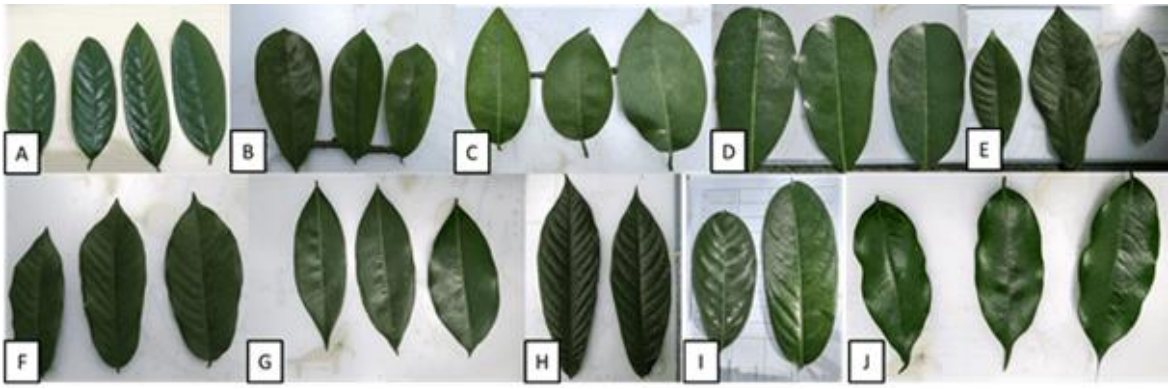

Figure 2. The leaf morphological variation of ten species of Annonaceae: Tribe Miliuseae: A. *Stelechocarpus burahol*; Tribe Annoneae: B. *Annona muricata*, C. *Annona montana*, D. *Annona glabra*; Tribe Uvariae: E. *Fissistigma latifolium*, F. *Desmos chinensis*, G. *Desmos sp*., H. *Uvaria purpurea*; Tribe Xylopiae: I. *Artabotrys suaveolens*, and J. *Xylopia sp.* (Source: research documentation, 2023)

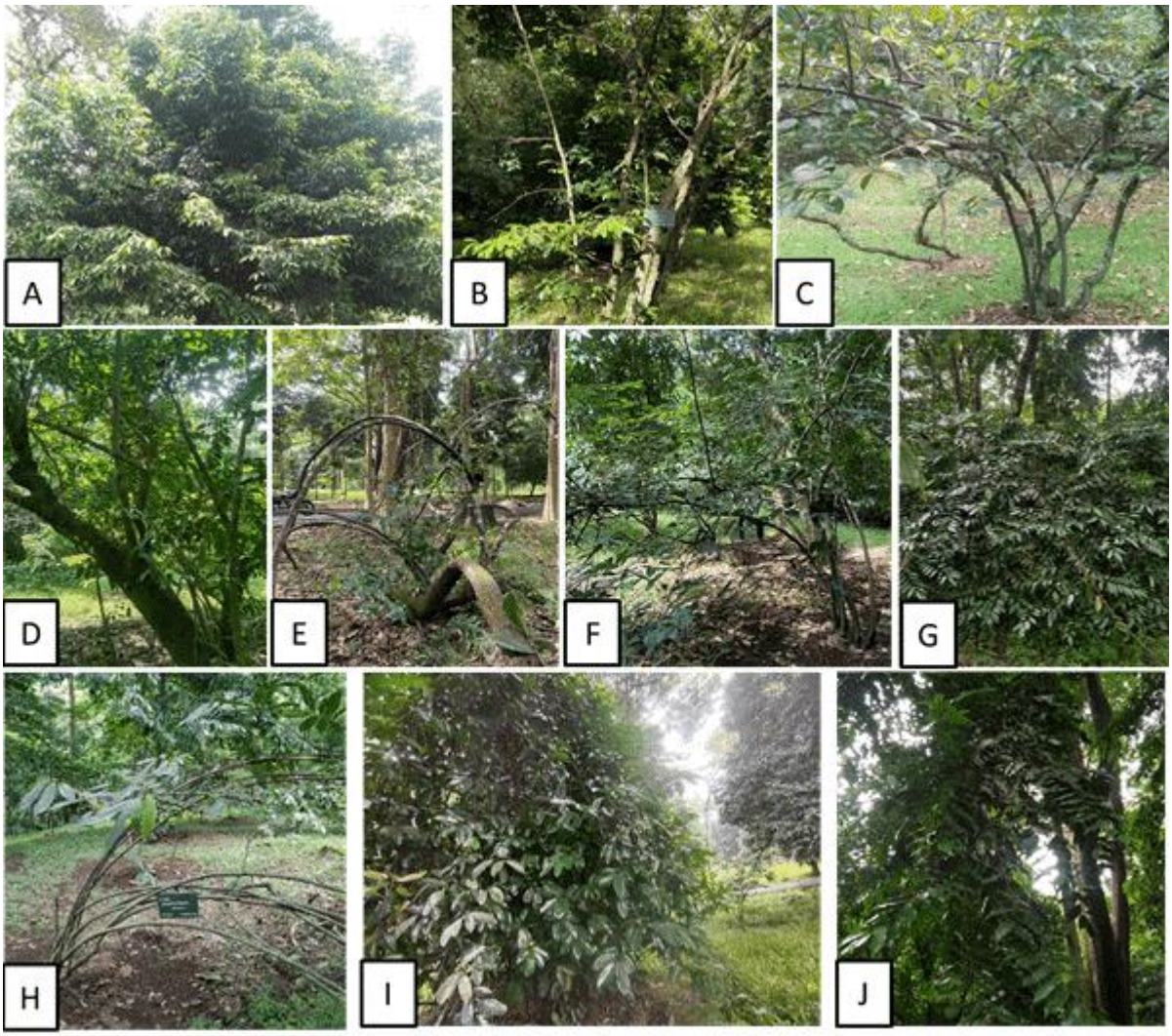

Figure 3. Plant habitus variation of ten species of Annonaceae. Tribe Miliuseae: A. *Stelechocarpus burahol*; Tribe Annoneae: B. *Annona muricata*, C. *Annona montana*, D. *Annona glabra*; Tribe Uvariae: E. *Fissistigma latifolium*, F. *Desmos chinensis*, G. *Desmos* sp., H. *Uvaria purpurea*; Tribe Xylopiae: I. *Artabotrys suaveolens*, and J. *Xylopia* sp.

The plant habit character is also significant in distinguishing at the subfamily level of the Annonaceae family. The subfamily of Malmeoideae has tree habits and sub-family of Annonoideae mostly has a habit of woody climbers (lianas) and some of the trees (Chatrou et al., 2000; Chatrou et al., 2012; Lestari et al., 2017). Furthermore, the plant habit characters are also effectively used as initial identification to differentiate between tribes, in addition to other vegetative and generative characters. The Annoneae tribe comprising *Annona muricata*, *Annona montana*, and *Annona glabra* have tree habits. *Stelechocarpus burahol* from the Miliuseae tribe also has a tree habit like the Annoneae. In detail, *Stelechocarpus burahol* has only one branch type of trunk ramification (Figure 3.A.), while the Annona species has 2-3 branches of type trunk ramification with a suckering tendency of <5 (Figure 3.B-D). Whereas, the Uvariae tribe has a woody climber habit. All species in the Uvariae tribe have in common 2 branches of type trunk ramification and suckering tendency of <5 Suckers (Figure 3.E-H.).

Likewise, *Artabotrys suaveolens* (Xylopiae tribe) has the same habitus character as the Uvariae tribe, i.e. the woody climber (Figure 3.I.). It could support the finding of previous studies by Tan and Wiart (2014) and Lestari et al., (2017) that Artabotrys has a woody climber habit. Chatrou et al., (2012) also described that although there are differences in morphological characters, the two genera of Artabotrys and Xylopia have been recognized in the past as closely related genera, but their synapomorphies have not yet been identified. Whilst, *Xylopia* sp. (*Xylopiae tribee*) has a tree habit (Figure 3.J.), with one branch of type trunk ramification and no suckering tendency.

The similarity index analysis of 17 specimens of Annonaceae resulted in a coefficient range of 0.64 to 0.96 (Table 4). The low similarity index (close to 0) indicates that

the more distant the relationship among specimens and vice versa (Wijayanto et al., 2013). The highest similarity index was observed between *Fissistigma* sp. and *Desmos chinensis*, they shared a high character identity at 0.96 similarities (Table 4). Both species are classified in the same tribe of Uvariae with several common morphological characteristics of leaf shape and plant habit. Whilst, the lowest similarity index was found between *Uvaria purpurea* and *Xylopia* sp. with a coefficient value of 0.64 (Table 4). Both species belong to the Annonoideae sub-family but in different tribes i.e Uvariae and Xylopiae, respectively. Further, Uvariae and Xylopiae are reported distinguished by the absence/presence of hook climbers on the stem and hairs on vegetative and generative organs (Lestari & Ningrum, 2021).

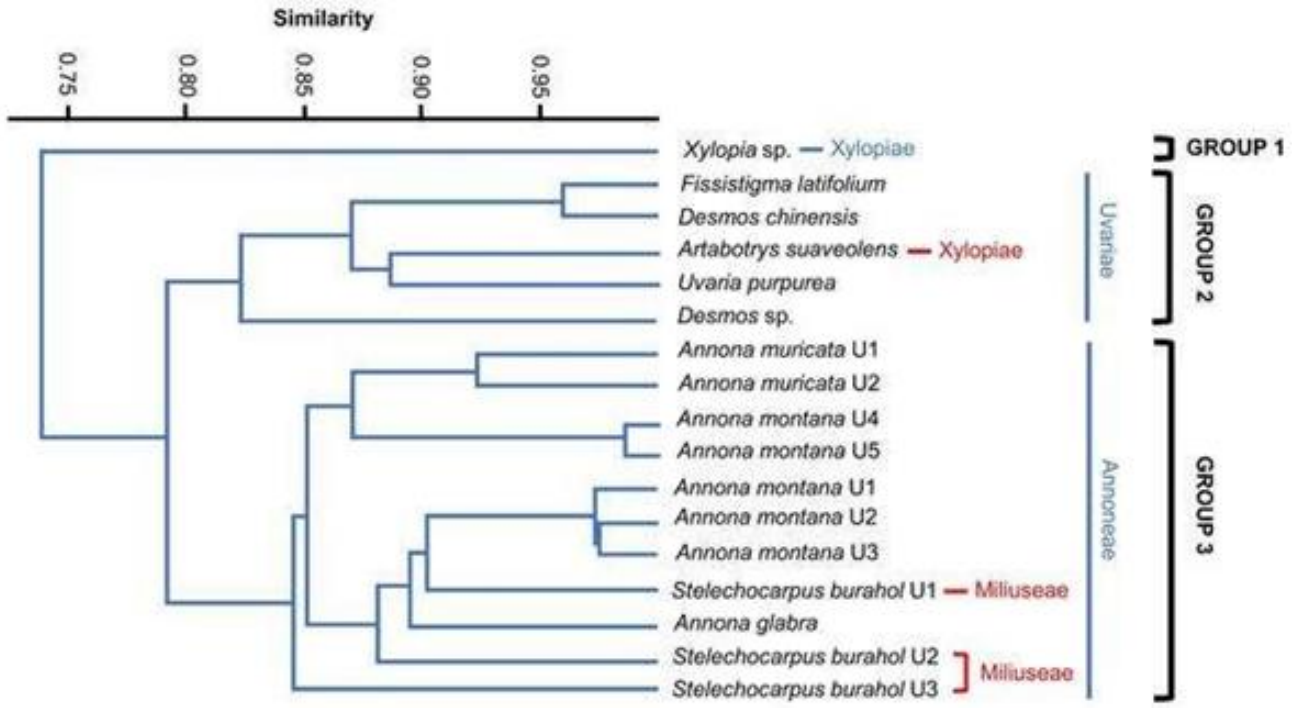

Figure 4. Dendrogram clustering of 17 specimens of Annonaceae based on vegetative morphological characters

Table 3. Similarity index of 17 specimens of the Annonaceae

Specimen	SB1	SB2	SB3	AMu1	AMu2	AMo1	AMo2	AMo3
SB1	1							
SB2	0.90	1						
SB3	0.88	0.88	1					
AMu1	0.84	0.84	0.84	1				
AMu2	0.85	0.85	0.88	0.92	1			
AMo1	0.91	0.88	0.86	0.85	0.88	1		
AMo2	0.89	0.86	0.83	0.85	0.89	0.97	1	
AMo3	0.91	0.89	0.83	0.85	0.89	0.97	0.97	1
AMo4	0.82	0.84	0.84	0.86	0.90	0.88	0.88	0.88
AMo5	0.81	0.83	0.83	0.85	0.88	0.86	0.89	0.87
AG	0.89	0.87	0.79	0.86	0.82	0.88	0.90	0.90
FL	0.85	0.77	0.82	0.81	0.84	0.85	0.86	0.86
AS	0.86	0.76	0.81	0.77	0.77	0.79	0.80	0.82
UO	0.79	0.69	0.78	0.72	0.72	0.74	0.75	0.75
DC	0.83	0.77	0.82	0.81	0.85	0.81	0.81	0.81
DS	0.74	0.74	0.82	0.81	0.81	0.77	0.78	0.78
XS	0.76	0.76	0.75	0.76	0.80	0.79	0.77	0.79

Table 4. Similarity index of 17 specimens of the Annonaceae

Specimen	AMo4	AMo5	AG	FL	AB	UP	DC	DS	XS
AMo4	1								
AMo5	0.99	1							
AG	0.81	0.83	1						
FL	0.84	0.83	0.84	1					
AS	0.78	0.77	0.83	0.90	1				
UO	0.73	0.72	0.76	0.88	0.89	1			
DC	0.85	0.83	0.80	0.96	0.86	0.84	1		
DS	0.81	0.80	0.74	0.85	0.78	0.78	0.89	1	
XS	0.77	0.76	0.71	0.67	0.69	0.64	0.70	0.71	1

Remarks: SB= *Stelechocarpus burahol*; AMu= *Annona muricata*; AMo= *Annona montana*; AG = *Annona glabra*.; FL = *Fissistigma latifolium*.; AB= *Artabotrys suaveolens* ; UP = *Uvaria purpurea*; DC.= *Desmos chinensis*; DS= *Desmos* sp.; and XS = *Xylopia* sp.

The results of clustering analysis based on vegetative morphological characteristics of 17 Annonaceae plant specimens was divided into 3 groups, relatively following the tribe divisions of the Annonoideae, with a few exceptions (Figure 4). Xylopia sp. (Xylopiae tribe) was separated from the others in Group 1, and served as the root. Group 2 occupied by the Uvariae tribe consisted of *Fissistigma* sp., *Desmos chinensis*, *Uvaria purpurea*, and *Desmos* sp., with the exception of *Artabotrys suaveolens* (Xylopiae) nested in this group. Meanwhile, Group 3 consisted of Annoneae tribe including *Annona muricate*, *Annona montana*, and *Annona glabra*, with the exception of *Stelechocarpus burahol* (Malmeoideae: Miliuseae) nested in this group (Figure 4).

This grouping pattern was in line with previous study by Lestari et al. (2017) which characterized using both vegetative and generative organs. As discussed previously, *Artabotrys suaveolens* has a plant habit as a woody climber thus it joins the Uvariae group, not with *Xylopia* sp. which has tree habit. *Stelechocarpus burahol* has a tree habit with some vegetative morphological characteristics similar to Annona species hence it is grouped with the Annoneae tribe. Hence, using only vegetative morphology characters is considered moderately effective in differentiating species in the Annonaceae family, so it is recommended for initial identification of species, especially in the Annonoideae sub-family. Since vegetative morphological characters are always available throughout the year while waiting for plants to flower (generative phase) requires a long time, so that it will be more efficient.

CONCLUSION

The vegetative morphological diversity of the ten species of Annonaceae family showed variation among species. Characters that contribute greatly to the diversity and clustering including leaf shape, leaf tip shape, leaf base shape, tiller type, branching type also plant habit. The dendrogram topology was divided into 3 groups, relatively following the tribe division i.e. Xylopiaeae (*Xylopia* sp.), Uvariaeae (*Fissistigma* sp., *Desmos chinensis*, *Artabotrys blumei*, *Uvaria purpurae*, and *Desmos* sp.) and Annoneae (*Annona muricata*, *Annona montana*, *Stelechocarpus burahol*, and *Annona glabra*). Vegetative morphology characters could be used for initial identification of species in the Annonaceae family for efficiency. Further research using genetic markers or DNA barcodes is necessary to confirm and complement the limitations of morphological characters to provide a more valid result.

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AUTHORS CONTRIBUTIONS

YS completed the field sample collection, which was later assisted by RTP and LH in data analysis and drafting the manuscript. S and EK were involved in interpreting the results and reviewing the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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