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Morphological study and DNA fingerprint pattern of *Goniothalamus tapis* and *G. umbrosus* (Annonaceae) using M13 marker and its taxonomic implication

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Abstract. *Mahadi I, Fathurrahman F, Sofiyanti N, Susatya A. 2023. Morphological study and DNA fingerprint pattern of* Goniothalamus tapis *and* G. umbrosus (*Annonaceae*) *using M13 marker and its taxonomic implication. Biodiversitas 24: 6299-6306.* The species of *Goniothalamus* (Blume) JD Hooker & Thomson (Annonaceae) is widely considered a source of traditional medicine and contains goniothalamin, which is used for anti-cancer and anti-bacterial drugs. *Goniothalamus tapis* and *G. umbrosus* are traditional medicines in Sumatra, Indonesia, and Malaysia Peninsula, respectively. The community uses this plant as a source of traditional medicinal ingredients. Clinically, *Goniothalamus* contains the compound goniothalamin, an anti-cancer and anti-bacterial drug. The existence of this plant is gradually maintained. *G. tapis* is often used as a traditional medicine for the Malay community in Sumatra, and *G. umbrosus* is widely used by the Malay community in the Peninsular Malaysia. These two plants are morphologically similar, but the two species are different. Some researchers consider these plants to be the same. For this reason, it needs to be studied by combining two methods, both molecular and morphological. *G. tapis* and *G. umbrosus* are species of the Goniothalamus distributed in Sumatra and Peninsular Malaysia. Their taxonomic status needs to be clarified due to the morphological character similarity. This study aimed to study the morphological characters of *G. tapis* and *G. umbrosus* as well as their DNA fingerprint pattern using M13 Marker. The morphological examination showed that *G. tapis* and *G. umbrosus* characters are distinguished by leaf, flower, and fruit. This result is supported by a DNA fingerprint pattern using an M13 marker. The results show that these two species are different both in terms of morphology and fingerprints. So, it can be concluded that these two species are different.

Keywords: DNA fingerprinting, genetic relatedness, *Goniothalamus tapis*, *Goniothalamus umbrosus*, molecular identification, M13 marker

INTRODUCTION

Indonesia is a region with very high plant diversity. Many plants contain substances that can be used as natural medicinal ingredients (Nordin et al. 2015). One of them is from the genus *Goniothalamus* (Blume) JD Hooker & Thomson (Aslam et al. 2016). In Riau Province, especially the Sakai tribe and the Malay community in the Peninsular Malaysia, this plant is used as a medicine after giving birth to clean postpartum blood. This plant is also commonly used as a fever medicine, fever-reducing medicine, and ulcer reliever (Suchaichit et al. 2015; Li et al. 2016). Parts of the *Goniothalamus* plant that can be used include stems, roots, leaves, and fruit. This plant can be used as medicine for public health (Mahadi et al. 2021).

The *Goniothalamus* plant contains the goniothalamin compound, a biologically active substance extracted from the *Goniothalamus* plant (Seyed et al. 2014; Suchaichit et al. 2015). Goniothalamin also plays a role in inhibiting the growth and development of cancer cells in body tissues (Khaw-On et al. 2019; de Souza et al. 2020) and inhibits the proliferation of human ovarian cancer cells (Lúcio et al. 2015; Nordin et al. 2015). *Goniothalamus* has styrylpyrone compound is a drug for fighting against herpes simplex virus type 1 (Fouzi et al. 2021) and essential oils (Shakri et al. 2021). In Malay communities in Southeast Asia, the leaves, fruit and roots of this plant are used to treat stomach aches, fever, abortion, and cleanse after childbirth. The bark of this plant can be used as a mosquito repellent (Mat-Salleh 2001).

According to Saunders (2003), the *Goniothalamus* is a shrub with broad leaves with a smooth surface, a single flower with 3 crowns that grow from the trunk or twigs of the tree; in one carpel, it can bear 5-20 fruit. This plant has a habitat in moist lowland forest areas and around river streams (Mahadi et al. 2021). Two species, namely *Goniothalamus tapis* and *Goniothalamus umbrosus*, are widely used as a source of medicine for the community (Sinclair 1955). These plants are morphologically is not similar. They are two different species.

The members of this genus are characterized by having slightly or not spreading outer petals, while inner petals are smaller, clawed, erect and edges adhering (Yang et al. 2020). The occurrence of *Goniothalamus* species in Peninsular Malaysia was reported by Adhya et al. (2020), and smaller numbers of *Goniothalamus* are recorded in this region (18 species). The studies on *Goniothalamus* in Sumatra, Indonesia, have been reported by Saunders

(2002), with 14 *Goniothalamus* species , while Mat-Salleh (2001) recorded a higher number of species from this genus (30 species). The distribution of *Goniothalamus* plants found in almost every topography, especially in Southeast Asia, is numerous and well-known (Guo et al. 2017). In lowland and submontane tropical forests across Southeast Asia, the genus *Goniothalamus* is extensively dispersed (Thomas et al. 2017).

The early revision of *Goniothalamus* was carried out by Saunders (2002). He proposed a new species of *Goniothalamus*, *G. umbrosus* J. Sinclair that showed close morphological characters to *G. tapis* Miq. (Leeratiwong et al. 2021) treated *G. umbrosus* as a distinct species from *G. tapis* based on morphological characteristics, especially leaf texture and shape, as well as length of stipe. Kim et al. (2020) assumed that *G. umbrosus* is the synonym of *G. tapis* due to unclear diagnosis characters used by Sinclair. *G. tapis* is one of *Goniothalamus* species reported from Sumatra (Indonesia) and Malaysia. Our detailed observation of morphological characters of *G. tapis* from Sumatra and Malaysia and *G. umbrosus* from Malaysia showed different features on leaves, flowers, and fruit within the specimen observed. Previously, Sinclair (1955) described that *G. tapis* differed from *G. umbrosus*. Sinclair determined that *G. umbrosus* has different morphological characters from *G. tapis* based on examining the spescimen. This finding supported us in a further study using a molecular method to determine the taxonomic status of those specimens.

Taxonomists have widely used molecular approachto solve taxonomic problems within plant species (Tang et al. 2015). Amplification-directed DNA Minisatellite (DAMD) using universal primer minisatellite M13 is one useful molecular data in plant taxonomy (Selvakumari et al. 2017). M13 primer resulted in a variation of DNA fingerprint pattern on close taxa and showed polymorphism within the species (Abdelaali et al. 2018). M13 primer was an effective and extremely useful tool in producing DNA fingerprints in many plant taxa. Therefore, using DNA fingerprint patterns, we used M13 primer in this study to prove the difference in the taxonomic status of *G. tapis* and *G. umbrosus*s. This research is to complete the molecular data that the species *G. Tapis* and *G. umbrosus* are different. The implication is that people do not make the mistake of choosing *G. umbrosus* which is better used for extracting goniothalamine for medicine compared to *G. tapis.* *G. umbrosus* contains more goniothamine compounds than *G. tapis* (Mahadi et al 2021).

MATERIALS AND METHODS

Sample collection

Moreover, 20 herbarium specimens were collected from Sumatra and Malaysia. They consisted of 10 specimens of *G. tapis* from Riau and Terengganu, Malaysia Peninsula, and 5 specimens of *G. umbrosus* from various places in Malaysia Peninsula. Closely relative species, including *G. malayanus*, *G. montanus*, *G. holttumii*, and *G. woodii*, were singly collected in their respective specimens. A distant sister species, *Polyalthia angustissima* was also collected as its specimen. All specimens were collected from July 2022 to July 2023 (Table 1). All specimens were collected from the field by taking them directly to where the *Goniothalamus* was grown. Accession samples were used as specimens and observed for morphological data. Table 1 shows the specimens examined in this study: the characters utilized in treatments on the *Goniothalamus*, especially of the leaf, floral, and fruit characteristics.

Table 1. List of examined specimens

Species	Voucher	Specimen code
G. tapis 1	Indonesia, Riau, Taman Hutan Raya Sultan Syarif Hasyim, Imam & Mat Salleh G.tapis1*	IMM90
G. tapis 2	Indonesia, Riau, Taman Hutan Raya Sultan Syarif Hasyim..Imam & Mat Salleh G.tapis2*	IMM94
G. tapis 3	Indonesia, Riau, Taman Hutan Raya Sultan Syarif Hasyim. Imam & Mat Salleh G.tapis3*	IMM96
G. tapis 4	Indonesia, Riau, Taman Hutan Raya Sultan Syarif Hasyim. Imam & Mat Salleh G.tapis4*	IMM100
G. tapis 5	Indonesia, Riau, Taman Hutan Raya Sultan Syarif Hasyim. Imam & Mat Salleh G.tapis5*	IMM101
G. tapis 6	Malaysia, Terengganu, Jambu Bongkok forest Reserve. Imam & Mat Salleh. G.tapis6*	KMS5391
G. tapis 7	Malaysia, Terengganu, Jambu Bongkok forest Reserve. Imam & Mat Salleh. G.tapis7*	IMM35
G. tapis 8	Malaysia, Terengganu, Jambu Bongkok forest Reserve. Imam & Mat Salleh. G.tapis8*	IMM36
G. tapis 9	Malaysia, Terengganu, Jambu Bongkok forest Reserve. Imam & Mat Salleh. G.tapis9*	IMM37
G. tapis 10	Malaysia, Terengganu, Jambu Bongkok forest Reserve. Imam & Mat Salleh. G.tapis10*	IMM38
G. umbrosus J. Sinclair 1	Malaysia, Kelantan. Kg. Kubang Batang. Imam & Mat Salleh. G.umbro1.*	IMM40
G. umbrosus 2	Malaysia, Kelantan, Kg. Setul, Imam & Mat Salleh. G.umbro2.*	IMM41
G. umbrosus 3	Malaysia, Terengganu. Kuala Berang. Imam & Mat Salleh. G.umbro3*	KB01
G. umbrosus 4	Malaysia, Perlis, Wang Keluang. Imam & Mat Salleh. G.umbro4*	IMM48
G. umbrosus 5	Malaysia, Penang, Penang Botanical Garden. Imam & Mat Salleh GU5.*	IMM50
G. malayanus	Indonesia, Riau, Taman Hutan Raya Sultan Syarif hasyim. G.aff	IMM 120
G. montanus J. Sinclair	Malaysia, Kelantan, Stong Mountain	IMM63
G. holttumii J. Sinclair	Malaysia, Pahang, Frasers Hill.	KMS4825
G. woodii Merr. Ex. Mat Salleh	Malaysia, Sabah, Sepilok, Feeding Platform & Mat Salleh.GW1.	KMS2475
Polyalthia angustissima Riddl.	Malaysia, Selangor, Bangi Forest Reserve. P.angu	IMM148

Note : *specimens used for morphological observation

Morphological observation on *G. tapis* and *G. umbrosus*

Morphological analysis was conducted by comparing morphological characteristics of fresh leaf, flower, and fruit of *G. tapis* and *G. umbrosus.* Additional species description of these two species from Sinclair (1955) and Saunders (2002, 2003) was also used to support the analysis. Leaf morphology comparison included leaf shape, size, and leaf apex. The flower characteristics were compared to the petal's color, size, and shape. A similar comparison was also conducted on the fruit stall's shape, size, color, and length. Monoblock caliper measured the size of leaf, flower, and fruit. In this study we examined the detailed morphological characters of these parts were described.

DNA isolation

DNA analysis was based on fresh young leaves from all collected specimens, where each of these fresh young leaves was cut by 2 cm^2 size and put into a sealed plastic bag with 5 g silica gel according to the species. All these fresh leaves were then prepared for DNA analysis. The genomic DNA of 20 specimens was isolated using the modified Cetiltrimetil Ammonium Bromide (CTAB) method (Doyle and Doyle 1987). DNA was then purified using phenol-chloroform and purifying of phenol (PCI). The diagnostic fragments were identified from the DNA profiles of sample individuals generated using M13 universal primer. Those diagnostic fragments were extracted from the agarose gel, purified using the QIAquick Gel Extraction Kit, and cloned into the pGem® -T Easy Vector (Promega). Three clones were selected for plasmid extraction using Wizard® Plus SV Minipreps DNA Purification System (Promega) sequenced by an automated ABI 3130xl Genetic Analyzer using a Big Dye (dRhodamine) terminator cycle sequencing-ready reaction kit.

Amplification with Polymerase Chain Reaction (PCR)

PCR was performed in 25 µL. PCR reaction mixture contained 2.5 mL 10X PCR buffer (20mM Tris-HCl pH 8.3, 100 mM KCl) (Promega), 3 mM $MgCl_2$, 200 µM dNTP, 15 pmol primer M13 (5'- TTATGAAACGA CGGCCAGT -3'), 2 U *Taq* polymerase (Promega) and 20 ng of genomic DNA template and distilled water. Amplification was done using the 'Thermal cycle' Gene Amp® PCR system 9700 Perkin Elmer. The first two cycles comprised 2 min pre-denaturation at 94°C and 1 min denaturation at 94°C. These cycles followed subsequent 35 cycles comprising 1 min annealing at 48°C, 2 min elongation at 72°C, with 10 min final extension at 72°C during the last cycle. The PCR products were then separated in TAE buffer using 1.5% agarose gel electrophoresis at 65V for 2.5 h, stained in ethidium bromide. DNA band pattern was visualized and documented using a UV transiluminator following E. Selvakumari et al. (2017). Molecular weight estimation was carried out using Lamda Hind III (Promega).

Data analysis

The amplified M13 bands were scored as present (1) or absent (0) based on Ibrahimi et al. (2023). Unclear and weak because these DNA bands were doubtful of originating from secondary metabolic chemical compounds and not from genomic DNA and did not meet DNA quality standards for analysis. Besides that, DNA bands were excluded because PCR amplification could not conducted, and stable and consistent DNA profile bands could not be used. Pairwise genetic similarity was calculated using the similarity index, Jij = Cij / (ni + nj - Cij). The data was then subjected to cluster analysis by the Unweighted Pair-Group Method with Arithmetical Averages (UPGMA) method as described (Takezaki and Nei 1996). The binary matrix was prepared for clustering. Cluster analysis was then carried out on the Jaccard coefficient with UPGMA (the unweighted pair-group method of averages) using NTSYSpc software (Zheng et al. 2017). An illustration of the dendrogram was obtained through the Tree plot program. The probability (P) of two identical fragments that display genotype is calculated by the formula J^x, where J is the similarity coefficient, and x is the mean of the number of fragments all accession.

RESULTS AND DISCUSSION

Morphological comparison of *G. tapis* and *G. umbrosus*

In this study, we observed the morphology of leaves, flowers, and fruit of *G. tapis* and *G. umbrosus.* Figure 1 and Table 2 present the morphology of these two species' leaves, flowers and fruits. We observed specimens of *G. tapis* collected from Sumatra Island, Indonesia, and Malaysia. Based on morphological comparison, *G. tapis* and *G. umbrosus* are different. *G. tapis* grown in Sumatra and *G. tapis* grown in Malaysian have the same morphological form, except that only a few organ sizes are not significantly different (Table 2) found in the leaves are margins and veins and in the flowers are the length and color of the corolla.

According to Sinclair (1955), *G. tapis* has a 3-10 m high shrub with smooth grey bark. Young twigs glabrous, brownish or pale, striate. Leaves coriaceous, oblong to, abruptly, shortly and bluntly acuminate, base rounded or slightly cuneate, edges recurved when dry, both surfaces dull, glabrous, brown when dry; Midrib sunk above, terete, prominent beneath and slightly rough; Main nerves about 16 pairs, slender and faint but raised on both surfaces, spreading with 1-3 secondary nerves between the main ones; Reticulations faint and lax; Length 15-27 cm; Breadth 6-12 cm; Petiole 5-7 mm, stout, channeled. Flower of *G. tapis* is solitary, occasionally in pairs, axillary, and fragrant. Pedicels with 3-4 mm, basal bracts, stouter than in G. *malayanus*, broadening towards calyx 0,5 cm-1 cm long. Sepals green, sometimes tinged with purple, ovate, acute, free to base, pubescent outside, glabrous inside, 5-6 mm long. Petals coriaceous, pubescent to glabrous, greenish, later creamy-white, often tinged with pink, outer ovate-lanceolate, sharply acuminate. Contracted and thickened at the base, one-veined outside, 3-5 cm long; Inner ovate, acute, clawed, 1-1.7 cm long. Stamens 2.5 mm long with apiculate connectives and large pollen grains. Ovaries are 3 mm long, elongate, tomentose; Style filitem, stigma funnel-shaped with the two lobes of the funnel-mouth split down the inner side. Carpels are ripening red, then purple, and

finally black, ellipsoid, apiculate, glabrous, thin-walled, sessile, 1-2 cm long, 1-seeded.

This difference is clear with *G. umbrosus* having a tree 3 meters maximum high. The young twig is slender, glabrous, white-grey, minute striatie. Folia is 14-18 cm long, 4-5 cm broad, lanceolate, glabrous, membranous, shiny above, dark-green, paler underneath, tip sharp or acuminate, sharp base; Nerve 14-16 pairs slender and dark, the second place between the primary; Petioles are slender, deeply canaliculate is 8 mm long. Flower flavor-virides. Pedicels 6 mm long are provided with three plates at the base: Sepals triangular, acute, glabra, and 3 mm long. Petals are external 4-4.5 cm long, 1-2 cm wide, lanceolate, acute, glabrous, distinctive unguiculate, 1-nervosa; petals interior 1-2 cm long, oblong-lanceolate, acute, unguiculate, marginibe pubescent. Stamen 3 mm long, connetives apiculate terminate. Ovari 3 mm long, tomentosa, *carpells* (vix mature) rose, oblong, leviter apiculate, 1.3-1.5 cm long; Stipites 8 mm-1 cm long. Stamen 1 that this specimen might well be placed in either species, but this is not the case since it has apiculate stamens, which *G. malayanus* does not have.

Both *G. umbrosus* come nearest *G. tapis* have apiculate stamens. However, *G. tapis* has stalked and not sessile carpels, as seen in *G. umbrosus*. Furthermore, *G. tapis* tends to have a rounded base, but the base can be acute less frequently. Meanwhile, *G. umbrosus* has a lanceolate shape that is less conaceous and is more acute at the base of the leaf.

The description carried out by Sinclair (1955) was supported by Meyer (1992) and Mat-Salleh (2001). Both concluded that *G. tapis* is morphologically different from *G. umbrosus*. The similar morphological characteristics between the *G. tapis* from Sumatra and the Peninsular Malaysia indicate they are the same species. Meanwhile, *G. umbrosus* is not found in Sumatra; it only grows in Kelantan, Peninsular Malaysia. Thus, Saunders's (2002) statement does not match the morphological description of these two species.

M13 DNA fingerprint pattern of *Goniothalamus*

PCR amplification using M13 primer gave polymorphism on DNA bands. The length of DNA bands ranged from 150 bp to 1500 bp. Figure 2 shows the gel electrophoresis result (electrophoregram) of 20 examined specimens. Figure 3 presents a dendrogram constructed based on the similarity coefficient of the M15 band scoring.

Based on the dendrogram in Figure 3, we observed that *Goniothalamus* specimens from Sumatra, Indonesia, and Malaysia were clustered in one cluster (I). These specimens were separated from *G. umbrosus* specimens that clustered in cluster II. Occurrences of random genes and mutations can lead to the transfer of an organism to another place and bring the parents' attributes to the next generation to form a new population (İnce and Karaca 2019). Thus, the relationship between *G. tapis* from Sumatra and G. *tapis* from Peninsular Malaysia clearly shows the possibility that they came from the same parent. Observations show that *G. tapis* non-endemic species. Both taxa can be found in Peninsular Malaysia, Sumatra and Borneo. So, it is believed that the two taxa have a close relationship. This opinion is acceptable because according to Metcalfe (2013), the island of Sumatra comes from the same earth plate as Peninsular Malaysia and Borneo before continental drift events occur.

This area is called the Sunda plate; during the Pleistocene events, plants and animals were transferred between islands (Gaffney 2022). It was believed that *G. tapis* Peninsular Malaysia was already scattered in Sumatra. After the events of continental drift occurred, the two taxa and the movement of these islands were separated. According to Metcalfe (2017), the islands in the Sunda complex, including Peninsular Malaysia, Sumatra, Java, Borneo, and the southern Philippines before the Pleistocene events, were only separated by rivers that have now become the sea. Thus, the possibility of gene drift and mutation occurs between accession-accession *G. tapis* from peninsular Malaysia to Sumatra or otherwise before the events of the Greater Sunda.

Table 2. Morphological comparison between *Goniothalamus tapis* and *G. umbrosus*

Characters	*G. tapis*		*G. umbrosus*
	Sumatra	Peninsular Malaysia	
Leaf			
Shape	Corioceous-oblong	Corioceous-oblong	Lanceolate
Size length	15-27 cm	15-30 cm	12-18 cm
Tip	Bluntly acuminate	Bluntly acuminate	Acute-acuminate
Flower			
Color of petal	Creamy white	Creamy white	Brownish yellow
Width of outer petal	1.5-2.2 cm	1.5-2.1 cm	2.5-3.7 cm
Length of outer petal	4.5-7.2 cm	4.3-7.2 cm	5.0-7.0 cm
Tip of petal	Acuminate	Acuminate	Acute
Fruit			
Pedicel	0.8-1.5 cm	0.8-1.5 cm	1.2-1.7 cm
Shape	Somewhat oval	Somewhat oval	Ellipsoid
Base of fruit	Somewhat lanceolate	Somewhat lanceolate	Lanceolate
Tip	Even	Even	Tapering
Color of mature fruit	Bright red	Bright red	Reddish black
Length	0.9-1.3 cm	0.9-1.3 cm	1.2-2.3 cm
Width	0.7-1.0 cm	0.7-1.0 cm	0.8-1.9 cm
Length of stalk	0.5-1.0 cm	0.5-1.0 cm	1.3- 1.7 cm

Additionally, *G. tapis* can also be found in Borneo. According to Selvakumari et al. (2017), the origin of the appearance of the flora of Kinabalu Mountain or Borneo as a whole is related to the range connecting Borneo to the continent of Asia.. Studies on the relationship between each species of *Goniothalamus* found in Borneo and Peninsular Malaysia are needed in the next study to support this hypothesis. However, looking at the probability of the identical accession-accession being quite high (0.39), it is unlikely that clonal accession to identical accession would occur.

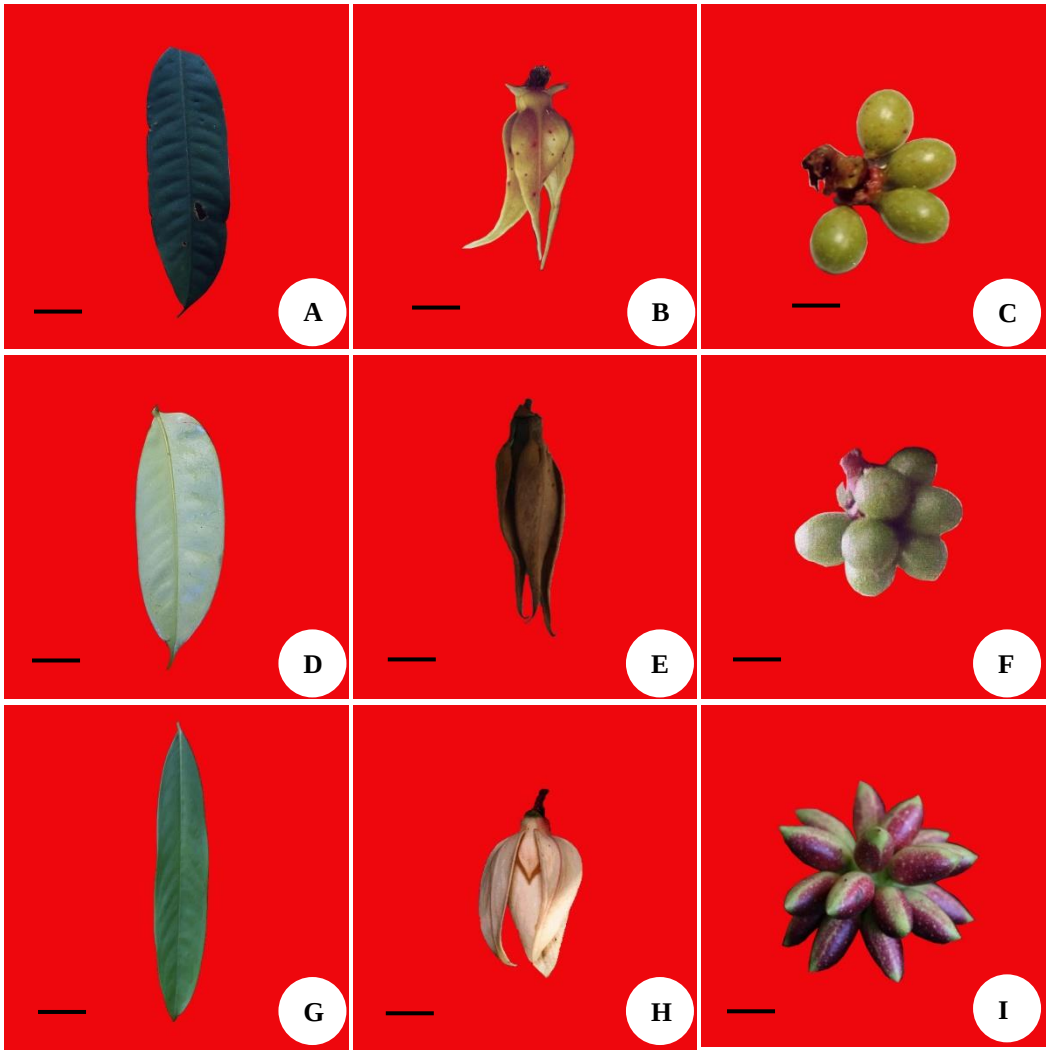

Figure 1. Leaf, flower and fruit morphologies of *Goniothalamus tapis* (A-C. From Sumatra, D-F. From Peninsular Malaysia, and G-I. *G. umbrosus*. (Scale: A-C: 5 cm, D-F: 2 cm, H-I: 1 cm)

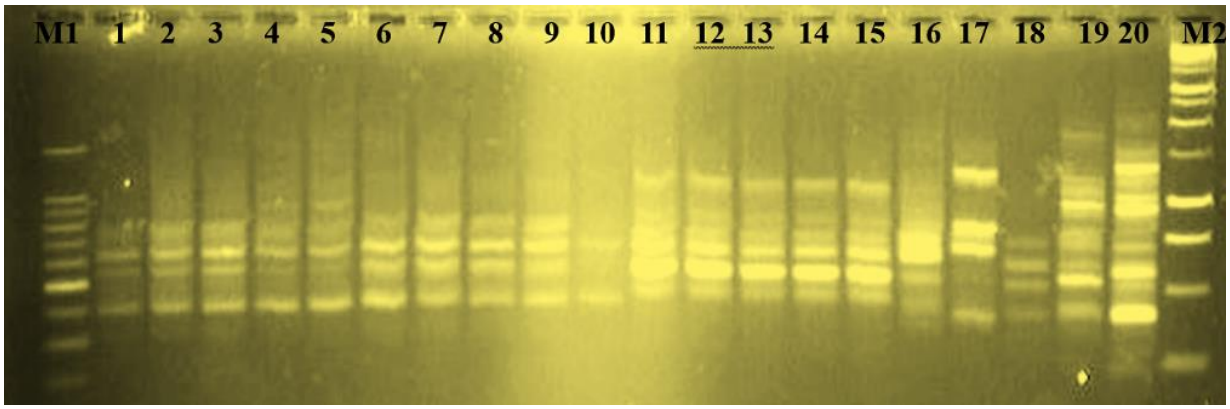

Figure 2. Electrophoregram of *Goniothalamus* PCR product using M13 DNA marker. 1-10. *G. tapis* (G.tapis1-10); 11-15. *G. umbrosus* (G. umbro1-5), 16. *G. Malayanus*, 17. *G. montanus*, 18. *G. holttumii*, 19. *G. woodii*, 20. *Polyalthia angustissima*

Morphologically, the results of our findings showed that the differences between *G. tapis* Sumatra and *G. tapis* Peninsular Malaysia are on the lower leaf surface, where *G. tapis* Sumatra sputter (wavy) like leaves G. *umbrosus* and size length is 15-27cm whereas *G. tapis* Peninsular Malaysia is flat and size lenght is 15-30cm, it may be influenced by the environment of the plant. However, this difference does not affect the genetic variation based on the study of DNA with the high similarity between 0:50 to 1:00.

In cluster III, there is only one species, namely *G. wodii*. Morphologically, *G. wodii* is similar to *G. tapis* but differs in several organs in the flower structure, namely the petals, and pistils; the fruit stalk is longer than *G. tapis* and *G. umbrosus*. Meanwhile, in cluster IV, there are *G. momtanus* and *G. malayanus*. Morphologically, they are not a group with *G. tapis* and *G. umbrosus*. However, these two species are closely related regarding flower morphology and habitat. The fruit's shape and the flowers' size are different from *G. tapis* and *G. umbrosus*.

The relationship between *G. umbrosus* with *G. tapis*

According to Leeratiwong et al. (2021), the distribution of *G. tapis* in Peninsular Malaysia, Sumatra, Borneo, and Sarawak; *G. tapis* is not recorded in Sarawak but found in Sabah. Meanwhile, Kartonegoro et al. (2021) argue *G. tapis* has geographical distribution in Peninsular Malaysia, Sumatra, and southern Thailand. The distribution to southern Thailand has not been recorded. Possibly, the species reviewed by Saunders is *G. umbrosus*; by then, Souders believed that *G. umbrosus* was synonymous with *G. tapis.*

The dendrogram in Table 3, Figure 3 was obtained from cluster *G. umbrosus* forming its own cluster, with 3 subclusters. The first subcluster found that 3 accessions are identical (J=1), namely *G. umbrosus* 1, *G. umbrosus* 2, and *G. umbrosus* 3. Subcluster second forms a variation of *G. umbrosus* 4 (J=0.86), and subcluster third *G.umbrosus* 5 (J=0.71). However, their relationship is close (J=0.71-1.0). The separation of the cluster and the low value of the Jaccard equation (0.39) for both groups in this taxon makes clear that *G. umbrosus* is a different species from *G. tapis.* Accessions from Kelantan and Terengganu showed them identical; clonal accessions can cause this, as G. *umbrosus* 1, *G.umbrosus* 2, and *G. umbrosus* 3 each have a value of J=1.00, the mean number of fragments per genotype is 0.5, then the probability P (two identical genotypes) is 1.00:5.0 or 2% Given the probability P (two identical genotypes) is not high, then most likely the occurrence of clonal accession. In addition, *G. umbrosus* 3 is located on the border between the states of Kelantan and Terengganu, where an environment is much more similar, which can lead to the development of clone accession between the accession from both states.

This species is synonymous with *G. tapis*. He added that Sinclair (1955) did not clarify the conditions of flowers and fruits in more detail. Apart from that, the interesting thing is that *G. tapis* is located in South Peninsular Malaysia, like in Johor, probably not *G. tapis* but the new species *G. endauensis* Inet, which was previously considered *G. tapis* (Mat-Salleh 2001). However, the results of DNA studies have shown that *G. umbrosus* differs from *G. tapis* as described above. We make a morphological check of flowers and fruits for the two species below, which are considered different. On the outside of *G. holttumii* are in one *G. tuberculosis* cluster, but the relationship between adjacent access is far (J=0.38); this species is derived from the highland. *G. umbrosus* is a lowland species that grows on cliffs and lowlands.

The third cluster of *G. woodii* and the fourth cluster of *G. montanus* and *G. malayanus* clearly show that each species is different, supported by a very low inequality value of 0-0.36. Morphologically, these species are easily distinguishable (Mat-Salleh 2001).

Table 3. Triangular matrix of Jaccard similarity coefficient values between the 20 accessions that have been determined

	Gtp1	Gtp2	Gtp3	Gtp4	Gtp5	Gtp6	Gtp7	Gtp8	Gtp9	Gtp10	Gum1	Gum2	Gum3	Gum4	Gum5	Gholt	Gmon	Gwod	GaffU	P.clau
Gtp1	1.00																			
Gtp2	1.00	1.00																		
Gtp3	1.00	1.00	1.00																	
Gtp4	0.75	0.75	0.75	1.00																
Gtp5	0.75	0.75	0.75	1.00	1.00															
Gtp6	1.00	1.00	1.00	0.75	0.75	1.00														
Gtp7	1.00	1.00	1.00	0.75	0.75	1.00	1.00													
Gtp8	1.00	1.00	1.00	0.75	0.75	1.00	1.00	1.00												
Gtp9	1.00	1.00	1.00	0.75	0.75	1.00	1.00	1.00	1.00											
Gtp10	0.75	0.75	0.75	0.50	0.50	0.75	0.75	0.75	0.75	1.00										
Gum1	0.43	0.43	0.43	0.29	0.29	0.43	0.43	0.43	0.43	0.29	1.00									
Gum2	0.43	0.43	0.43	0.29	0.29	0.43	0.43	0.43	0.43	0.29	1.00	1.00								
Gum3	0.43	0.43	0.43	0.29	0.29	0.43	0.43	0.43	0.43	0.29	1.00	1.00	1.00							
Gum4	0.29	0.29	0.29	0.14	0.14	0.29	0.29	0.29	0.29	0.33	0.83	0.83	0.83	1.00						
Gum5	0.38	0.38	0.38	0.25	0.25	0.38	0.38	0.38	0.38	0.25	0.86	0.86	0.86	0.71	1.00					
Ghol	0.33	0.33	0.33	0.17	0.17	0.33	0.33	0.33	0.33	0.17	0.43	0.43	0.43	0.29	0.38	1.00				
Gmon	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.10	0.11	0.09	0.00	1.00			
Gwod	0.14	0.14	0.14	0.00	0.00	0.14	0.14	0.14	0.14	0.16	0.25	0.25	0.25	0.29	0.22	0.14	0.13	1.00		
Gmalay	0.17	0.17	0.17	0.08	0.08	0.17	0.17	0.17	0.17	0.18	0.23	0.23	0.23	0.25	0.31	0.17	0.36	0.27	1.00	
P.ang	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.08	0.08	0.08	0.12	1.00

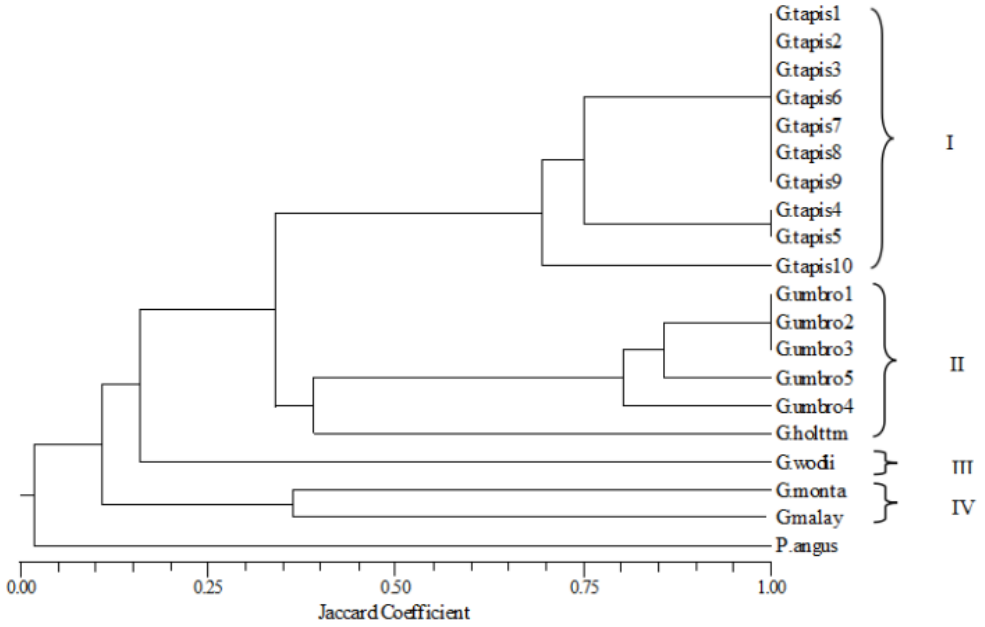

Figure 3. Dendrogram indicates a genetic relationship of 20 accessions studied

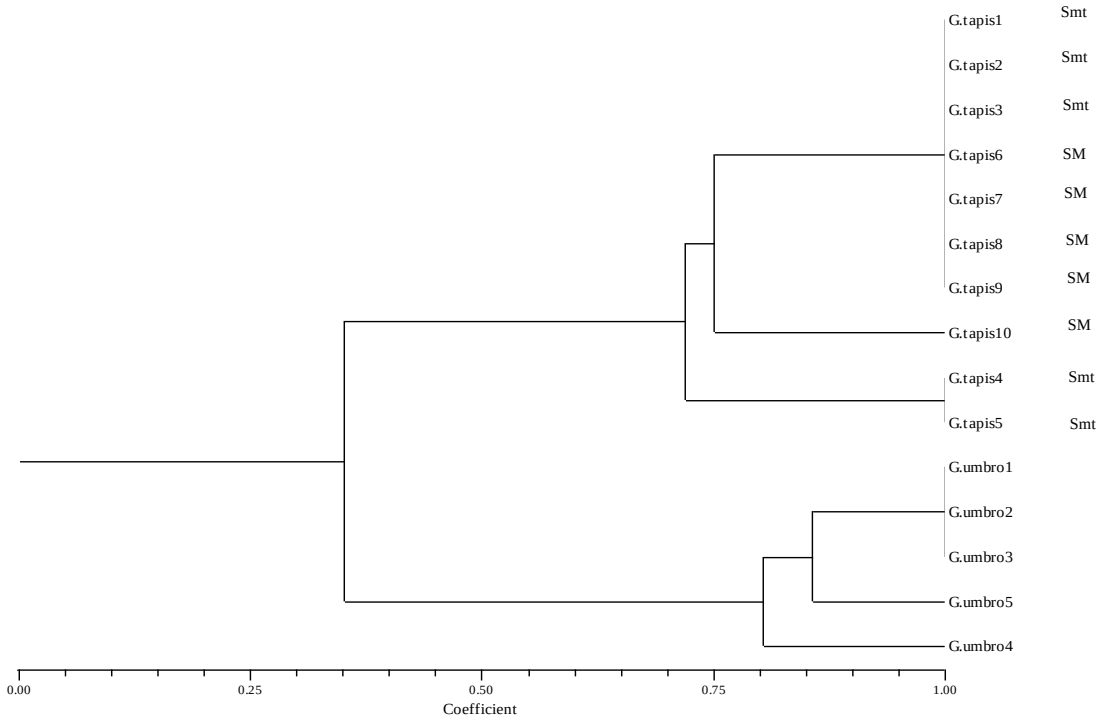

Figure 4. Dendrogram indicate a genetic relationship between *Goniothalamus tapis* and *G. umbrosus*

The genetic relationship between *G. tapis* and *G. umbrosus* forms a separate cluster; this is shown in Figure 4. In the *G. tapis* cluster, Sumatra and Peninsular Malaysia accessions are interconnected in the same cluster. The formation of subclusters in *G. tapis* explains that these accessions were taken from different locations. Even though the locations are separated by islands, namely Sumatra and the Peninsular Malaysia, the *G. tapis* accessions are still in the same cluster. Meanwhile, the *G. umbrosus* cluster also formed a separate cluster (Table 1). All *G. umbrosus* accessions are in one cluster. Furthermore, 3 subclusters show that *G. umbrosus* accessions were taken from different locations on the Peninsular Malaysia; *G. umbrosus* is an endemic species to the Peninsular Malaysia.

In conclusion, the results of research based on morphological and fingerprint studies using M13 primer show that *G. tapis* and *G. umbrosus* are different in terms of morphology and fingerprints of these two species. This research describes two species in taxonomic and molecular analyses with different patterns, and it can be concluded that two species are different.

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