

MORPHOLOGICAL AND CYTOLOGICAL DIFFERENTIATION OF *ENDOSPERMUM DIADENUM* AND *ENDOSPERMUM QUADRILOCULARE*: IMPLICATIONS FOR FOREST PLANTATION SELECTION

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Endospermum diadenum, locally known as sesenduk, is a promising timber species for forest plantation in Peninsular Malaysia. However, it appears as two morphologically distinct entities—differing in leaf, flower, fruit morphology, and germination rates—yet generally referred to as *E. diadenum*. This study employed morphological and karyotype analyses to investigate whether these entities, termed Entity A and Entity B, represent separate species. Samples were collected from three locations in Peninsular Malaysia where both entities coexist. Morphological comparisons using Chi-square tests revealed significant differences in leaf, flower, and fruit characteristics. Cytological analysis confirmed that the somatic chromosome numbers were $2n=46$ for Entity A and $2n=48$ for Entity B, suggesting species-level differences. Consequently, Entity A was identified as *Endospermum diadenum* and Entity B as *Endospermum quadriloculare*. Given its higher germination rate, Entity B is recommended for forest plantations.

Keywords: *Endospermum diadenum*, sesenduk, morphological characteristics, chromosomes

INTRODUCTION

The decline of natural forests in developing countries is driven by human settlement, agriculture expansion, and growing demands from wood and furniture industries. In response, the Malaysian government has promoted sustainable forest plantations to secure continuous log supplies for wood-based industries. The Forest Research Institute of Malaysia (FRIM) has assessed several timber species for plantations, including *Endospermum diadenum*, due to its rapid growth and suitability for open planting environments (Darus et al. 1990, Ang & Mohd Affendi 1991).

The size of natural forests in developing countries is gradually decreasing due to the need for human settlement, agriculture, and the growing demand from wood and furniture manufacturing industries. For decades, the Malaysian government has promoted sustainable

timber forest plantations to ensure a continuous supply of logs for wood-based industries. FRIM has taken the initiative to assess the feasibility of several species, including *Endospermum diadenum*, for forest plantations in Peninsular Malaysia. This species has been recommended for plantations due to its rapid growth rate and suitability for open conditions (Darus et al. 1990, Ang & Mohd Affendi 1991).

Endospermum malaccense M.A., also known as *E. diadenum* or locally as sesenduk, is a timber species belonging to the family Euphorbiaceae, subfamily Crotonoideae, tribe Adenoclineae, and subtribe Endosperminae. Schaeffer (1971) identified 12 to 13 *Endospermum* species, including *E. medullosum* (whitewood), *E. diadenum*, and *E. peltatum*. This genus is distributed from India (Assam) to South China and throughout Southeast Asia and Malesia

to Australia and the West Pacific (Fiji Islands) (Guerrero & van Welzen 2011). Schaeffer (1971) and Corner (1988) confirmed that only one *Endospermum* species, *E. diadenum*, is present in Peninsular Malaysia. It is widely distributed in lowland secondary forests at altitudes of up to 1000 m and is usually found in logged-over forests (Whitmore 1973). It is classified as a light hardwood with a wood density ranging from

400 to 460 kg/m³ (Ashaari et al. 2017). This wood is suitable for pattern making, matchbox manufacturing, splints, toys, blackboards, and drawing boards (Whitmore 1973). Additionally, it is commonly used for making trays, plywood, crates, wooden clogs, and disposable chopsticks (Mohd Shukari 1982).

The infestation of the seed borer (*Dichocrocis punctiferalis* Guenée) has resulted in a high

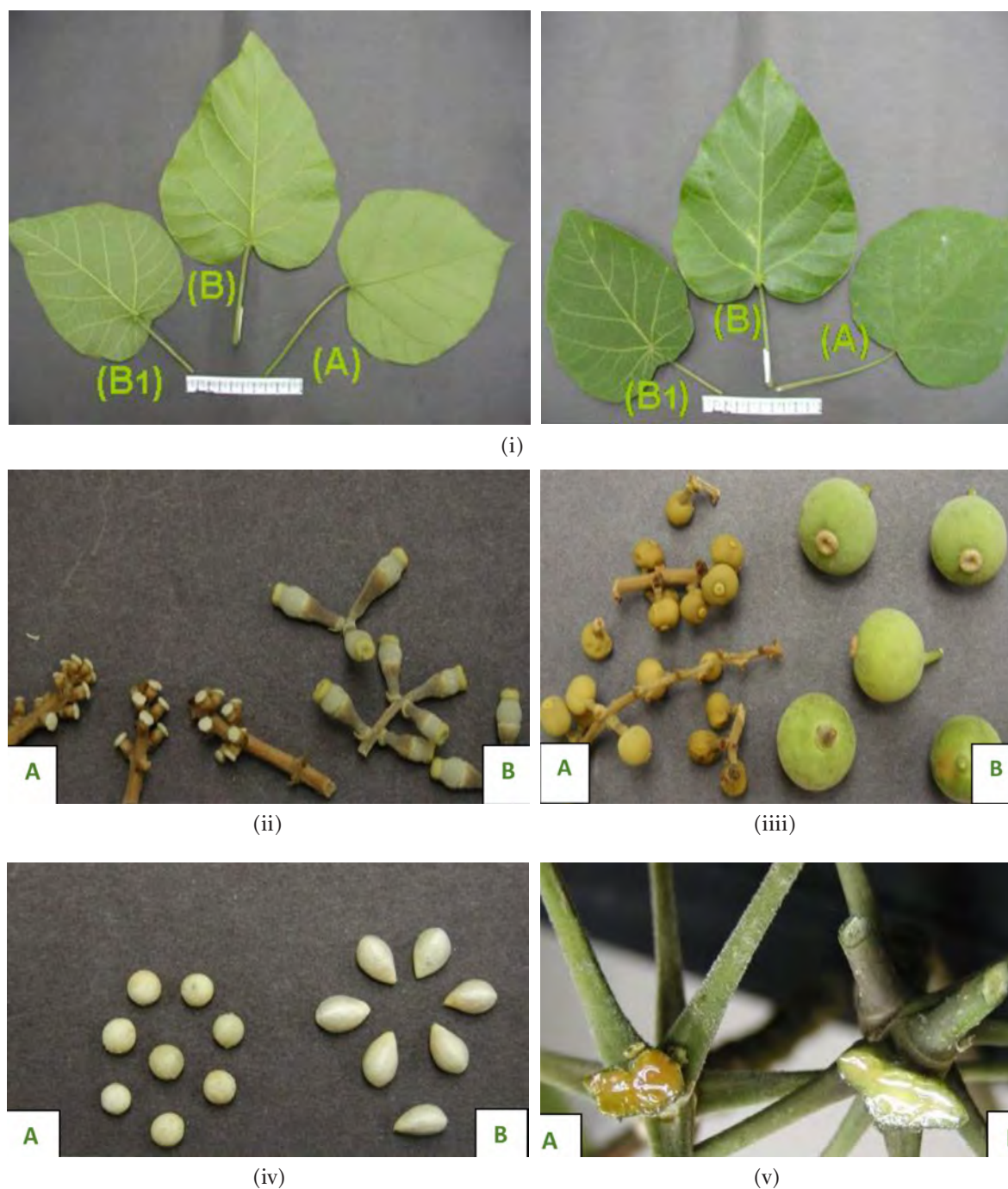


Figure 1 Morphological characteristic of *E. diadenum* Entities A and B. i) leaf ii) Pistillate flower, iii) Fruits, v) Seeds, and vi) Sap colour

proportion of non-viable seeds, affecting the supply of planting materials (Yap & Razali 1980, Darus et al. 1990). In 2007, a technical committee of the Malaysian Timber Industries Board (MTIB) identified eight species suitable for the forest plantation programme, excluding *E. diadenum*. However, several studies have focused on producing planting materials from this species. Other studies have also found variations in its seed germination percentages (Darus et al. 1990, Ang & Affendi 1991), which were closely related to morphological variations in tree structure, leaf shape, flower and fruit size, and sap colour (Figure 1). Such differences can also impact wood yield and quality.

In the review paper *Revision of Malesian Endospermum (Euphorbiaceae) with Notes on Phylogeny and Historical Biogeography* (Arias & Van Welzen 2011), *Endospermum banghamii* and *Endospermum ronaldii* were identified as synonyms of *Endospermum quadriloculare*. Similarly, Siti Salwana (2009) classified Entity B as *E. quadriloculare* based on morphological and

karyotype studies of leaves, flowers, and fruits. Furthermore, Siti Salwana (2009) conducted a comparative study and found that Entity A and Entity B are distinct species, with Entity A identified as *Endospermum diadenum* and Entity B as *Endospermum quadriloculare*. These findings are consistent with the review by Arias & Van Welzen (2011). Therefore, this study will discuss the morphological and chromosomal characteristics as evidence.

MATERIALS AND METHODS

Plant material

The leaves, fruits, and flowers of approximately 90 adult trees of *E. diadenum* Entities A and B were collected for morphological and cytological studies from three population sites in Peninsular Malaysia: Gunung Tampin Forest Reserve, Negeri Sembilan; Lentang Forest Reserve, Pahang; and Ulu Tranum Forest Reserve, Pahang (Figure 2). Both entities were present at all study sites.

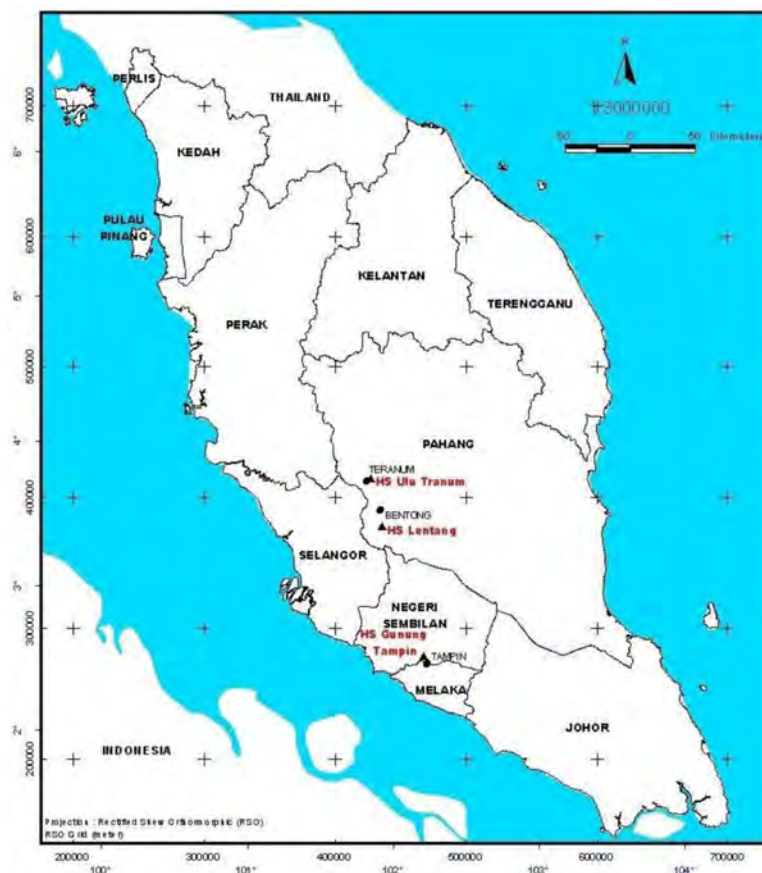


Figure 2 Populations sites of *E. diadenum* in Peninsular Malaysia used in the study
(Source: Google Map edited population site by author)

Morphological study

The morphological study focused on the external form or appearance of the plant. These features have been used longer than anatomical or molecular evidence and were the only source of taxonomic evidence in early plant systematics (Radford et al. 1974, Judd et al. 2002). The classification of trees as Entity A or Entity B was based on consistently observed characteristics within the population.

Entity A produced small fruits measuring 5 to 10 mm in diameter, typically containing two seeds per fruit. The fruits turned golden-yellow when ripe, and the leaves exhibited a velvety texture. Additionally, cut twigs of Entity A exuded a clear orange sap (Figure 1). In contrast, Entity B produced larger fruits, ranging from 15 to 18 mm in diameter, with three to five seeds per fruit. The fruits turned to yellowish-green when ripe, while the leaves were glabrous and papyraceous. Cut twigs of Entity B exude a clear sap (Figure 1)

Entities A and B were distinguished based on the characteristics of their leaves, flowers, fruits, and sap colour. Voucher specimens (UT5, UT18, UT45, L29, PT1, PT10, PT12, & PT14) were prepared following the methods described by Radford et al. (1974) and Khairuddin (1989) and were deposited in the herbarium of FRIM (KEP). Samples were observed under a light microscope to describe leaf characteristics such as shape, base, margin, apex, and the presence of glands and hairs on the midrib and both surfaces of the leaf. Data collected from direct observation and microscopic analysis were statistically analysed. Leaf measurements were taken from three leaves per tree to assess petiole length, leaf length, and leaf width. The data were then analysed using a t-test. Additionally, both staminate and pistillate flowers, as well as fruits, were also examined.

Cytological study

Entities A and B were investigated cytologically. Mature seeds, with the exocarp, mesocarp, and endocarp removed, were sown in germination beds containing 100% river sand. Actively growing root tips were collected after three months of germination. Dyer's conventional rapid squash method (1963) was used to prepare

squash slides of root tips for chromosome counting.

The root tips were pre-treated with 2 mM 8-hydroxyquinoline at 18°C for about 4 to 5 hours. Samples were then fixed in Farmer's fluid (ethanol: propionic acid in a 3:1 ratio) and refrigerated overnight. The fixed root tips were hydrolysed in 1N HCl at 60°C for 10 minutes and stained with Feulgen for 2 hours. Squash preparations were made in diluted 1% acetocarmine after treatment with a 2% cellulase and 20% pectinase enzyme solution for 30 minutes at room temperature. Cells with well-spread metaphases were examined and photographed using a Zeiss Axiophot microscope (ZEISS, Oberkochen, Germany) at 1,000x magnification. Chromosome numbers were determined using five good metaphase spreads for each entity.

RESULTS AND DISCUSSION

Morphological characteristic

Morphological analysis of both vegetative (leaves) and reproductive (flowers and fruits) structures was conducted to verify the visual differences between the two entities. The results showed a significant difference in leaf morphology, particularly in the leaf base and the presence of hairs on the midrib, across the combined data from three populations (Table 1).

Both entities are featured an ovate leaf shape but varies in the leaf base. Entity A had a predominantly cordate leaf base, while Entity B more frequently exhibited a cuneate/round leaf base (Table 1). Entity B also displayed a significant occurrence of peltate leaves (aged between two to ten months) at the sapling stage, whereas Entity A was never exhibited peltate leaf bases.

Previous studies on the botanical description of *E. diadenum* leaves have shown some dissimilarities. Corner (1939) described the leaves of *E. diadenum* as ovate, with an acute apex and a cordate base, measuring 27 cm in length and width for saplings. He also noted an elliptical obovate leaf shape with a cuneate or short cordate base and no peltate leaves. Conversely, Whitmore (1973) described the leaves as ovate, with a round or cordate base, but

Table 1 Leaf morphological characteristics of two *E. diadenum* entities

Morphological characteristics	Character	Entity A (%)	Entity B (%)	X ² _{value}
Shape	Ovate	100	100	0
Base	Cordate	84.8	34.2	21.52*
	Cuneate/round	11.4	44.3	19.43*
	Peltate	0	3.8	-
	Cordate + cuneate/ round	3.8	8.9	2.05
	Cordate + peltate	0	3.8	-
	Cuneate/ round + peltate	0	5	-
Margin	Entire	100	100	0
Apex	Acute	100	100	0
Glands		47	76	6.84
Midrib	Hairy	100	24	46.58*
Upper surface	Hairy	100	0	-
Lower surface	Hairy	100	82.3	1.72

Note: * significant difference

peltate at the sapling stage. Some plant species may experience significant changes in leaf shape in response to environmental conditions, a process known as heterophylly (Zotz et al. 2011). Therefore, the juvenile tree's leaves may differ significantly from those of the adult tree, and the adult tree may have a variety of leaves on the same branch (Corner 1939, Judd et al. 2002). Both entities had entire leaf margins and acute leaf apices (Table 1).

There was a significant difference in the length and width of leaves between Entities A and B, except for petiole length (Table 2). Entity B had significantly longer leaves than Entity A, while Entity A had a significantly greater leaf width than Entity B. However, the petiole length

of Entity A did not differ significantly from that of Entity B. Leaves of Entity A had hairs on both the upper and lower surfaces, while Entity B lacked hairs on the upper surface and instead had a waxy coating. Hairs on the lower surface of Entity B leaves were either absent or very rare (Figure 3).

The floral morphology of both entities confirmed that they are dioecious, with male and female flowers occurring on separate plants. Both entities exhibited indeterminate female inflorescences, where the main axis terminating in a flower. However, the pistillate flowers of Entity A differed significantly from those of Entity B. The results showed that the female inflorescence of Entity A is a spike with sessile

Table 2 Length and width of leaves, and length of petioles of two entities of *E. diadenum*

Characteristics	Entity		t-value	t-critical
	A	B		
Leaf length(cm)	12.7 (±2.7)	13.8 (±2.6)	4.03*	1.96
Leaf width (cm)	11.2 (±2.7)	10.1 (±2.2)	2.6875*	1.96
Petiole length (cm)	9.2 (±3.2)	8.5 (±2.0)	1.6483	1.96

Note: * significant difference at p = 0.05

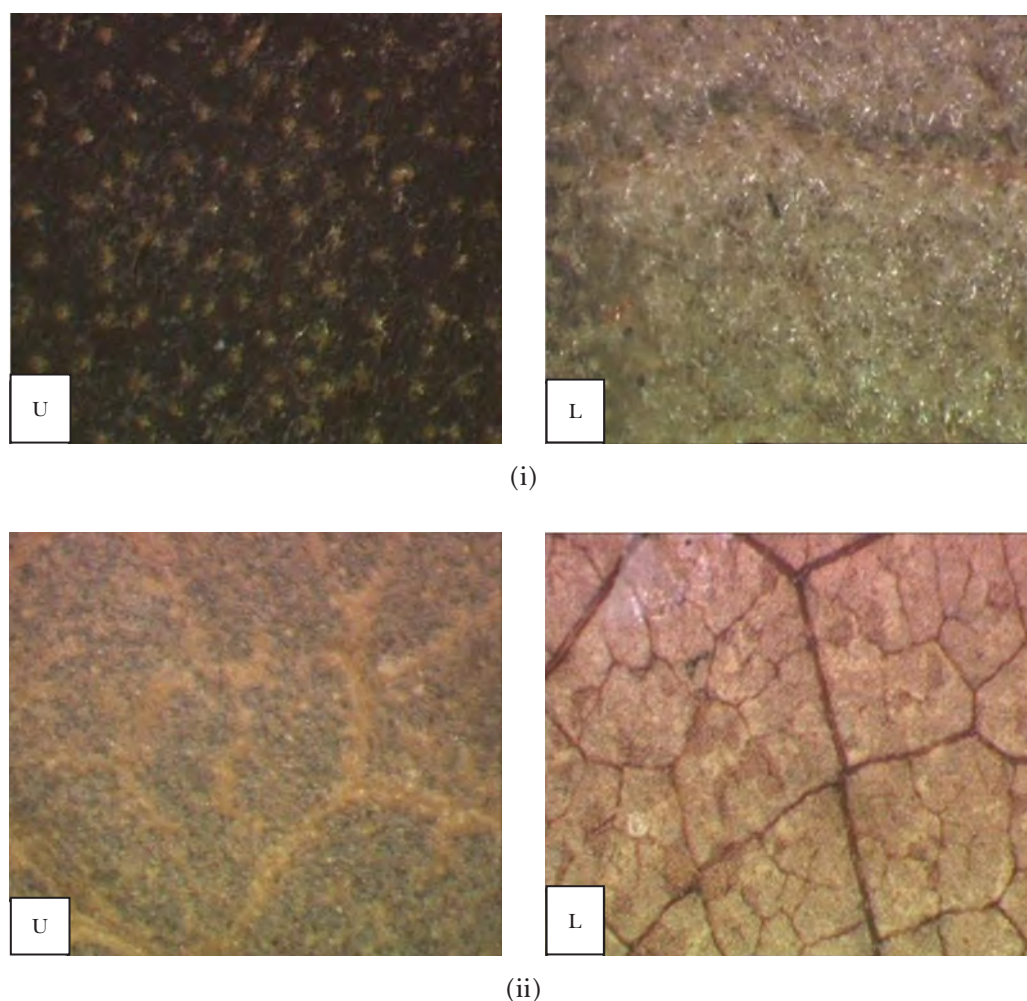


Figure 3 Upper (U) and lower (L) leaf surfaces of *E. diadenum* (i) Entity A and (ii) Entity B. (35× magnification)

flowers (no pedicel or stalk), which are small and vary in size from 2 to 4 mm. In contrast, the female inflorescence of Entity B is a raceme with pedicellate flowers ranging in size from 6 to 8 mm.

The cross-section of the pistillate flower structure revealed that Entity A had an average of 2-locular ovaries, whereas Entity B had 3 or 4-locular ovaries (Figure 4). The staminate flowers of both entities were similar in shape, ranging in size from 3 mm to 4 mm, and contained 8 to 11 stamens. The flowers emitted a sweet fragrance, likely to attract insects as pollinators. However, differences in inflorescence structure and anther shape were observed between the two entities. The male inflorescence of Entity A is a raceme with short branches, and its anthers are 4-lobed. In contrast, Entity B has a panicle inflorescence with 3-lobed anthers (Figure 5).

The fruits of both entities also differed significantly in size, colour, and the number of seeds per fruit. The ripened fruits of Entity A were golden yellow and ranged from 5 to 12 mm in cross-sectional size. Each fruit contained an average of two seeds. Conversely, the ripened fruits of Entity B were yellowish-green, measuring from 15 mm to 18 mm in size. Each fruit contained three to four seeds, and these distinguishing characteristics were consistent across all samples (Figure 6).

Many of Entity A's fruits were infested with insect larvae (Figure 7), leading to a significantly low germination rate (10%). In contrast, Entity B had a much higher germination rate (>80%), likely due to the presence of a thick pericarp that protected the seeds from insect invasion. According to Yap & Razali (1980), a high percentage of *Endospermum* fruits were infested with insects, which significantly reduced seed

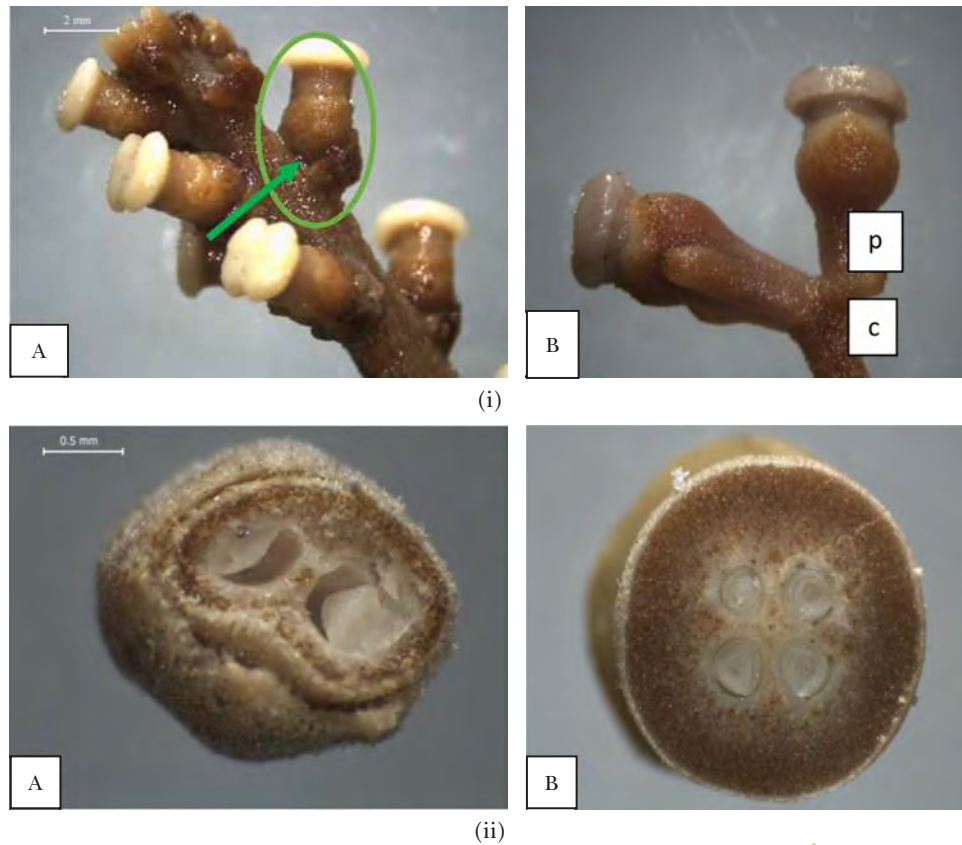


Figure 4 (i) Pistillate flowers of *E. diadenum* for Entity A; no pedicel (➤) (ii) Entity B; p-pedicel; c-bract. (ii) Cross section of a pistillate flower *E. diadenum* Entities A & B

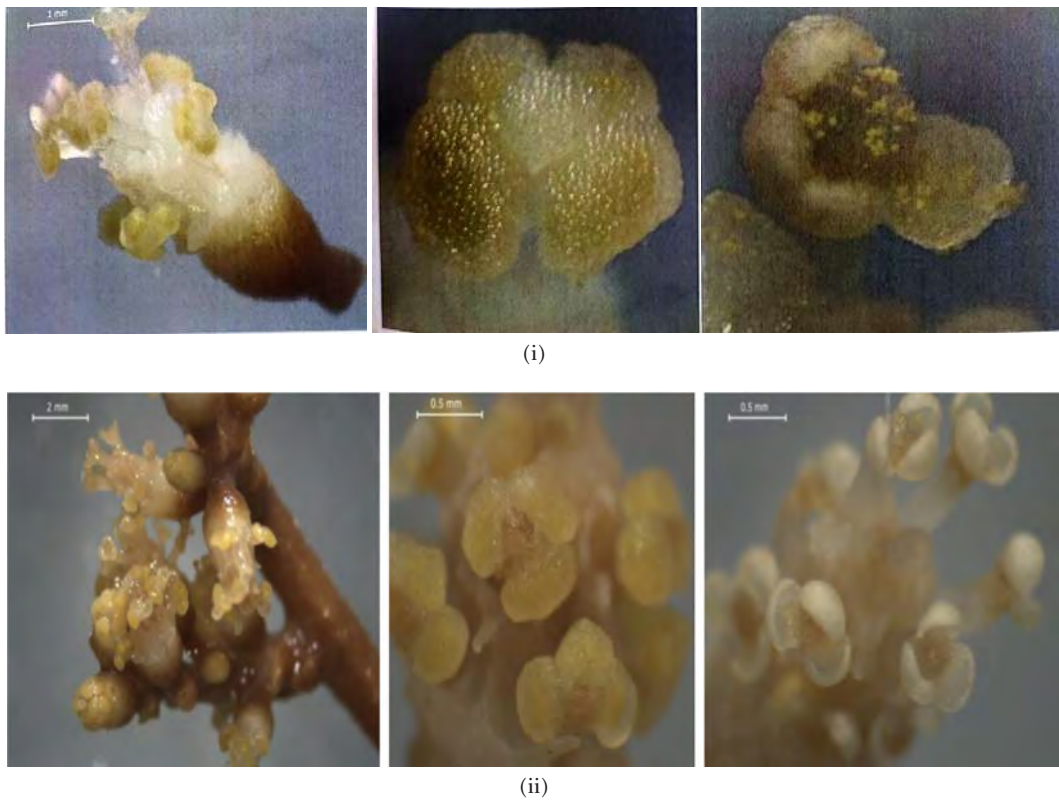


Figure 5 Staminate flowers of *E. diadenum* (i) Entity A & (ii) Entity B

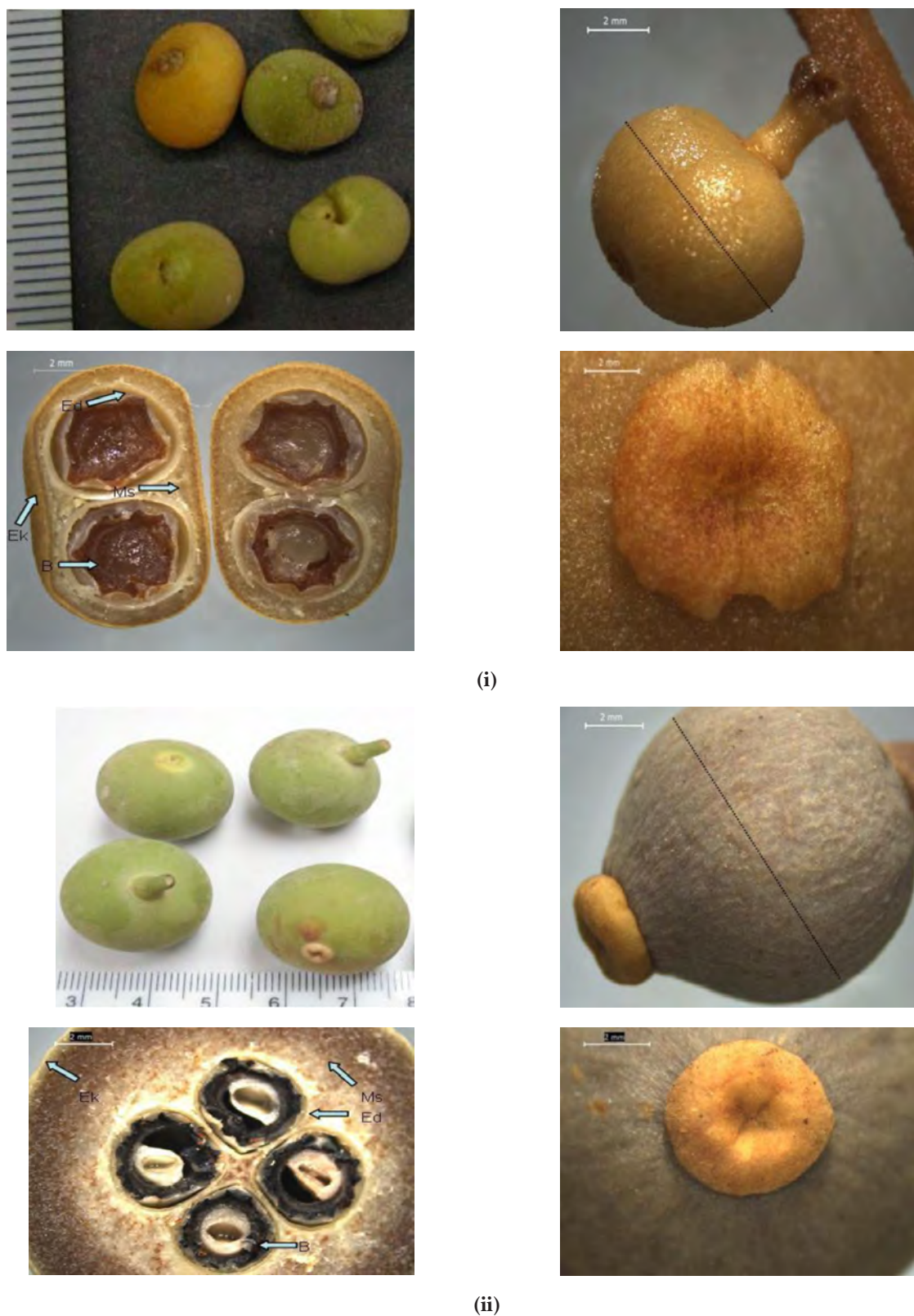


Figure 6 Morphological characteristic of fruits of *E. diadenum* (i) Entity A; (ii) Entity B. Ek- exocarp; Ms-mesocarp; Ed- endocarp; B – seed

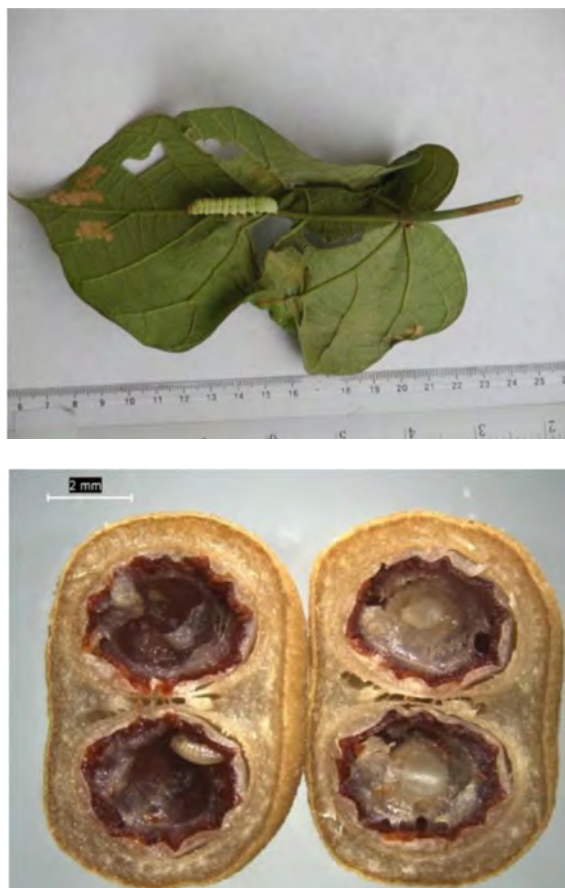


Figure 7 Larvae of *Dichocrocis punctiferalis* Guenée invaded on leaf and seed of *E. diadenum* Entity A

viability. Similarly, Darus et al. (1990) reported that insect larvae attacks led to very low seed germination rates in *Endospermum*. However, the thicker pericarp of Entity B fruits likely prevented insect infestation, contributing to their higher germination success.

Chromosome study

Chromosomal analysis revealed differences between the two entities. The number of chromosomes at metaphase indicated that Entity A and Entity B had distinct chromosome counts. The somatic chromosome number was diploid, with Entity A having $2n = 46$, while Entity B had $2n = 48$ (Figure 8).

The chromosome count for Entity B was similar to that reported for *E. mollucanum* from Papua New Guinea (Oginuma et al. 1999). These findings suggest that Entities A and B are two different species based on their chromosome numbers, further supporting the morphological distinctions described earlier.

Botanical description

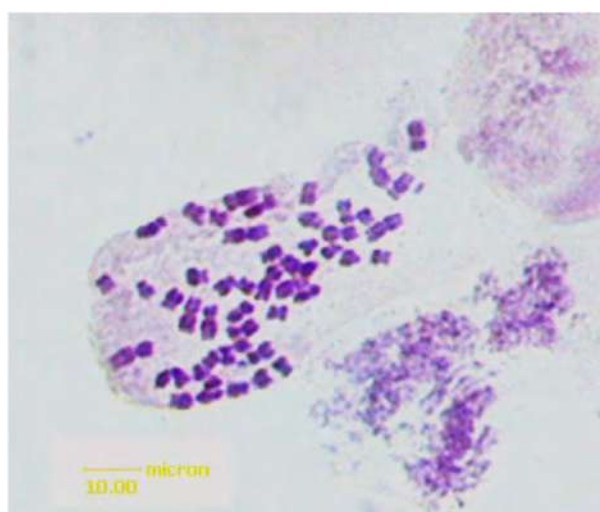
Endospermum diadenum Entity A

Entity A is a medium to large tree with a diameter of up to 100 cm. The twigs, leaf stalks, and the undersides of leaves are finely velvety. When cut, twigs exude clear orange sap. The leaf blade is typically ovate, averaging 11.2 cm in width and 12.7 cm in length. The leaf apex is acute, while the base varies between cordate or cuneate/rounded, but it is never peltate. The leaf stalk is often long, usually with two raised glands at the top and sometimes a gland at nerve junctions below. The upper leaf surface is hairy, and the lower surface is densely to slightly hairy.

Staminate flowers of Entity A have a raceme inflorescence with short branches. Pistillate flowers form a spike with sessile flowers (no pedicel). Staminate flowers occur singly or in groups of up to three in the axils of bracts, with 4-lobed anthers and 8 to 11 stamens that release a



(i)



(ii)

Figure 8 Metaphase chromosomes of *E. diadenum* (i) Entity A (ii) Entity B.

fragrant scent. Pistillate flowers have no pedicel, with 1 to 3-locular ovaries and a small stigma measuring 2 to 4 mm. Fruits are golden yellow when ripe, measuring 4 to 10 mm in diameter. The pericarp is thin, and fruits typically contain 1 to 3 seeds, which are easily attacked by insects. The chromosome number for Entity A is $2n = 46$. Botanical drawings for Entity A are presented in Figures 9 and 10.

Endospermum diadenum Entity B

Entity B is a medium to large tree with a diameter of up to 102 cm. The twigs, leaf stalks, and undersides of leaves are glabrous or papery. When cut, twigs exude clear sap. The leaf blade is typically ovate, averaging 10.1 cm in width and 13.8 cm in length. The apex is acute, while the base is cordate or cuneate and occasionally

peltate at the young stage. The leaf stalk is often long, with two raised glands at the top and sometimes an additional gland at nerve junctions below. The upper leaf surface is glabrous, while the lower surface is slightly hairy.

Staminate flowers of Entity B have a panicle inflorescence. Pistillate flowers form a raceme with pedicellate flowers. Staminate flowers occur singly or in groups of up to three in the axils of bracts, with 3-lobed anthers and 8 to 11 stamens that release a fragrant scent. Pistillate flowers have a pedicel, with 3 to 5-locular ovaries and a diffuse stigma measuring 6 to 8 mm. Fruits are yellowish-green when ripe, measuring 15 to 18 mm in diameter. The pericarp is thick, and fruits typically contain 3 to 5 seeds. The chromosome number for Entity B is $2n = 48$. Botanical drawings for Entity B are presented in Figures 11 and 12.

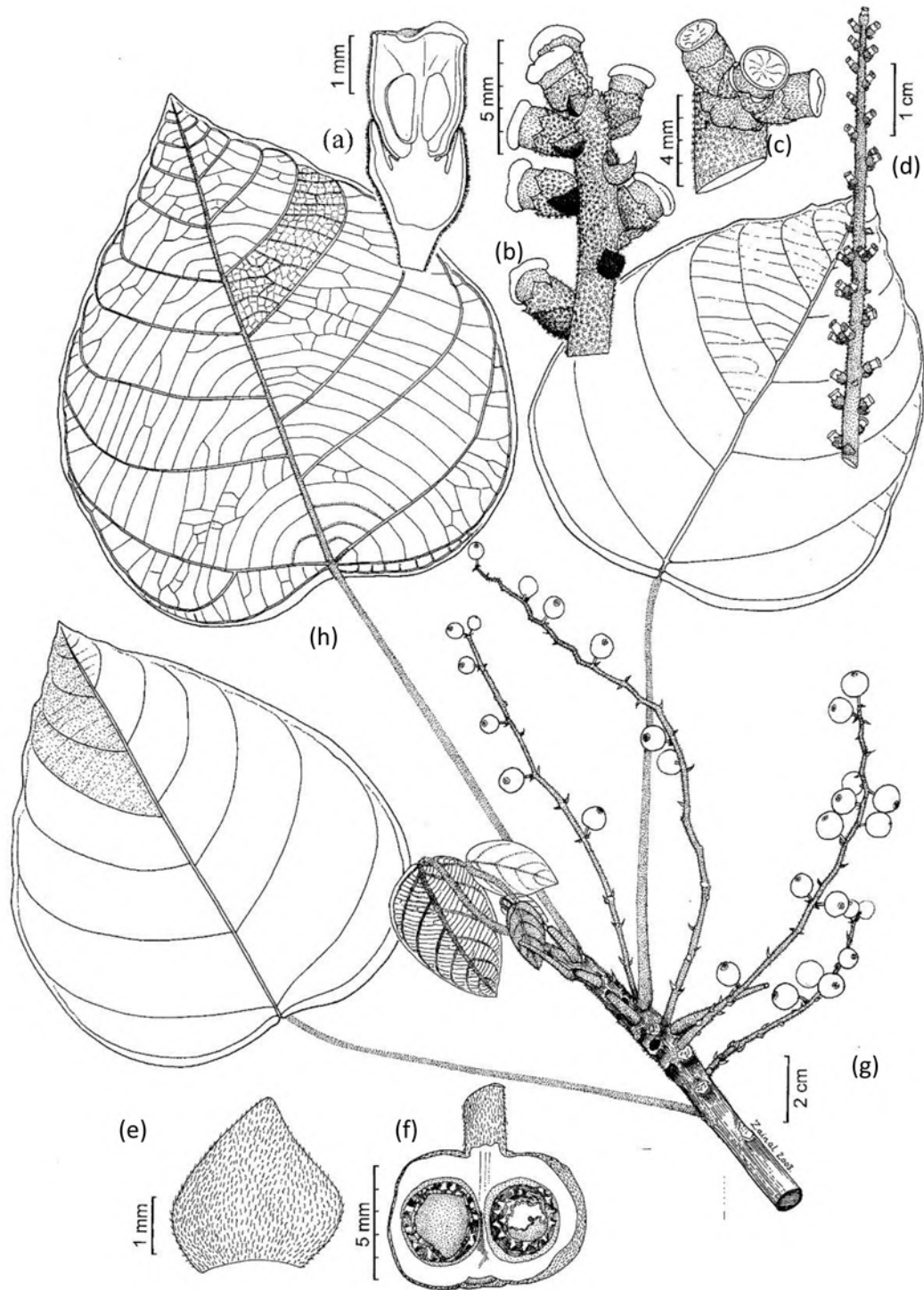


Figure 9 *Endospermum diadenum* Entity A. (a) longitudinal section of a pistillate flower, (b)-(d) inflorescence, (e) bract, (f) fruit in longitudinal section (insect larvae inside the seed), (g) infructescence and (h) leaf

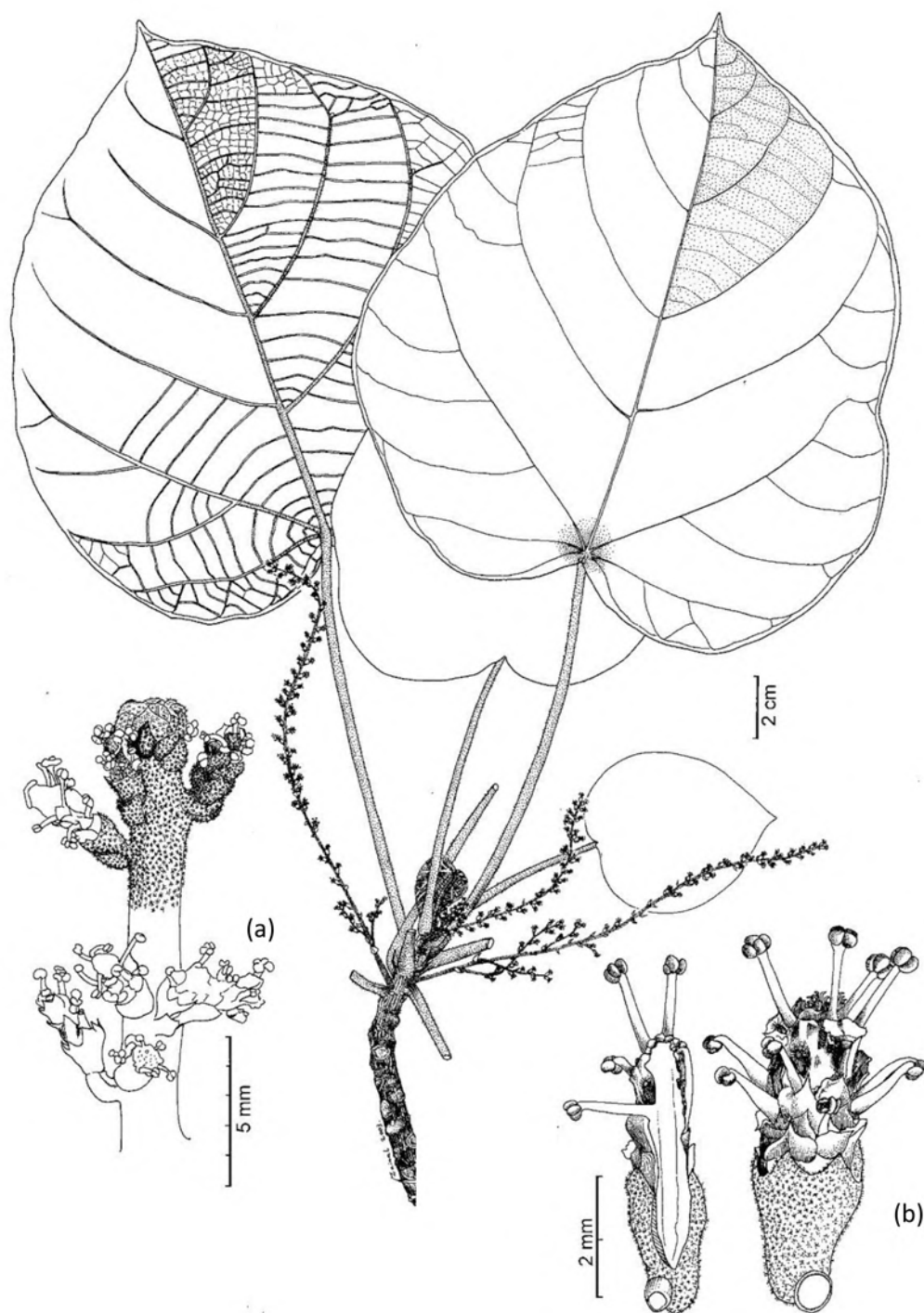


Figure 10 *Endospermum diadenum* Entity A. (a) inflorescence of a staminate flower and (b) structure

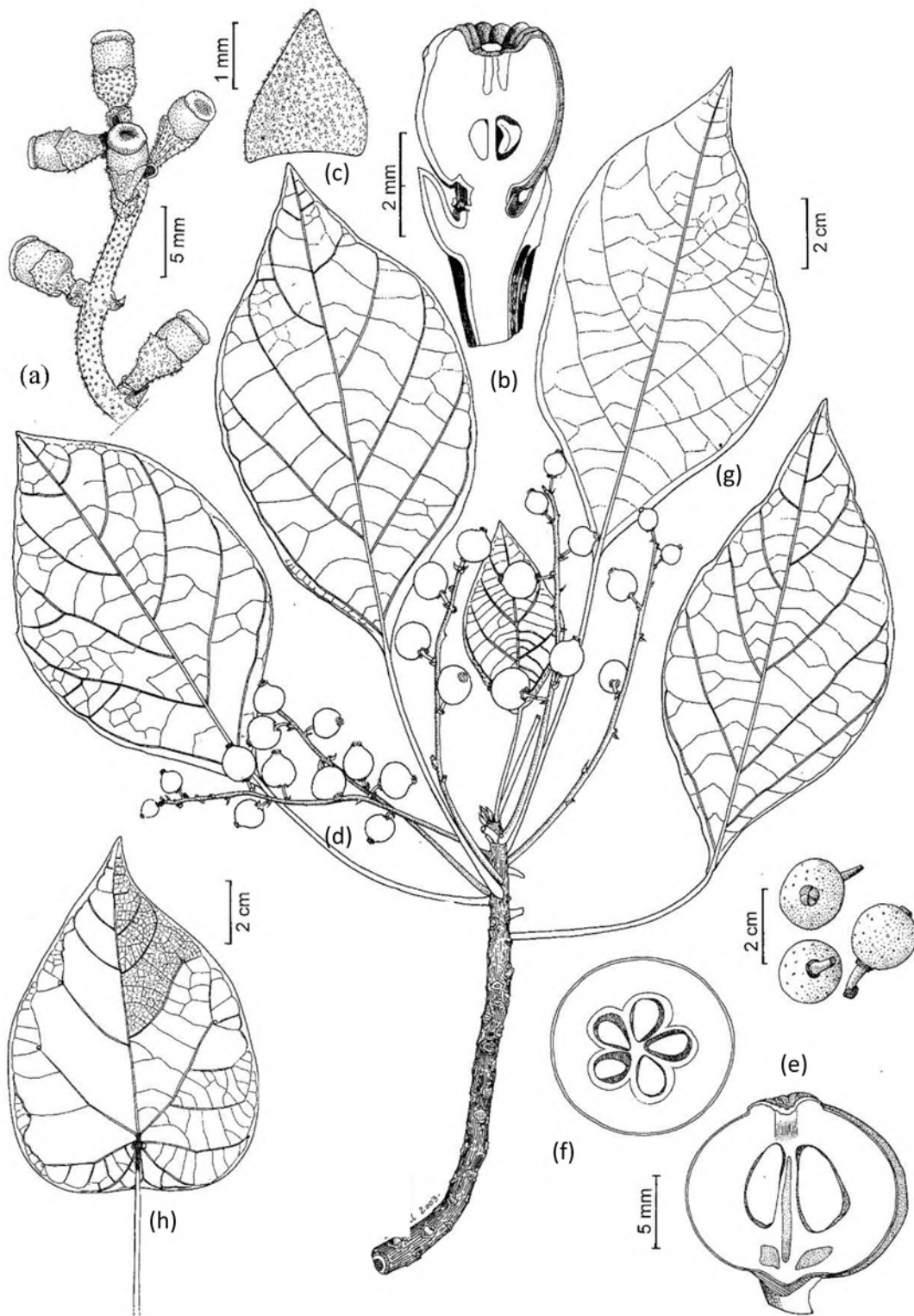


Figure 11 *Endospermum diadenum* Entity B. (a) inflorescence of a pistillate flower, (b) longitudinal section, (c) bract, (d) infructescence, (e) fruit in longitudinal section, (f) fruit in cross section, (g) leaf and (h) peltate leaf

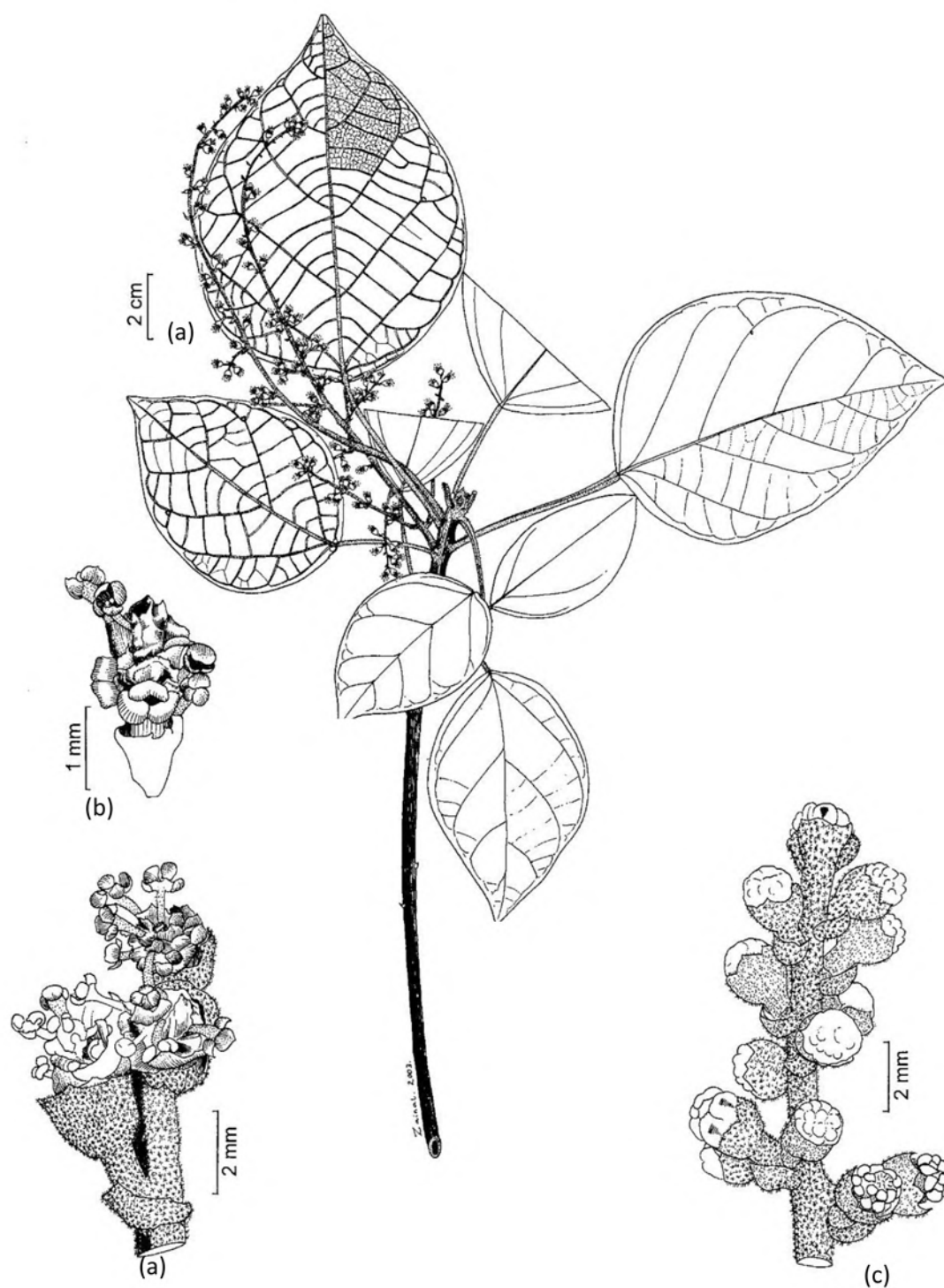


Figure 12 *Endospermum diadenum* Entity B. (a) inflorescence of a staminate flower (b) structure and (c) immature staminate flower

CONCLUSION

This study confirms that Entity A and Entity B are two distinct species, with Entity A identified as *Endospermum diadenum* and Entity B as *Endospermum quadriloculare*, based on clear differences in their morphological and cytological characteristics. The morphological analysis revealed significant differences in leaf base shapes, flower structures, fruit size, and seed characteristics. Additionally, cytological studies showed a difference in somatic chromosome numbers, with Entity A having $2n = 46$ and Entity B having $2n = 48$, further supporting their classification as separate species.

Among the two species, Entity B (*E. quadriloculare*) stands out as a more suitable candidate for forest plantation programs due to its thicker pericarp and significantly higher germination rate (>80%), which help protect its seeds from insect infestation—a known challenge for *Endospermum* species. These features make it a more viable option for sustainable timber production and long-term plantation success. Although *E. diadenum* was previously introduced as a plantation species, this study highlights *E. quadriloculare* as a better alternative for future forest plantation efforts.

To fully realise the potential of *E. quadriloculare* as a plantation species, further research is required. Field trial plots should be established to assess its growth performance, adaptability, and resistance to pests and diseases under various environmental conditions. Comprehensive studies on its wood properties, economic value, and market potential will provide critical data for its successful commercialization and integration into plantation programs.

In conclusion, the findings of this study provide strong evidence to support the differentiation and practical application of these two species in forest plantation selection. Entity B (*E. quadriloculare*) offers new opportunities for enhancing the sustainability the sustainability and productivity of timber plantations in Malaysia. However, careful evaluation through additional research will be essential before its official introduction into large-scale forestry practices.

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