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Highlights

- Two *Amyema* species differ in the color of flowers in which pink for *A. curranii* while red for *A. seriata*.
- The two species differ in haustorial attachment where *A. curranii* has several haustoria as it is creeping, ovary wall has presence of hairs, while *A. seriata* has solitary attachment and ovary wall is smooth.
- This is the first morpho-anatomical study of species in genus *Amyema* in the Philippines, which can be used as a baseline information for further studies on morpho-anatomy of the said genus.

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Comparative Morpho-Anatomical Studies of two Philippine Endemic Species of *Amyema* Tiegh. (Loranthaceae)

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Running head: Comparative Morpho-Anatomical Studies

Abstract. *Amyema* are epiphytic hemiparasitic plants on different types of woody host plants, and are abundant in temperate, subtropical, and tropical regions. In Marilog Forest Reserve, Southern Philippines, two Philippine endemic species of *Amyema* were recorded, viz., *Amyema curranii* (Merr.) Danser and *A. seriata* (Merr.) Barlow. In this study, these two species were compared and examined for their morphology and anatomy. Data revealed that the two *Amyema* species are morphologically distinct, in which *A. curranii* has lanceolate leaves, pink flowers and red-colored fruit, whereas *A. seriata* has obovate leaves, red flowers and fruits. For the morpho-anatomy, *A. curranii* has single-layered epidermis, paracytic stomata, collateral open vascular bundles, eustele type of stele with pith at the center, and inferior free central type with hairy ovary wall. Meanwhile, *A. seriata* has a pinkish and single-layered epidermis, paracytic stomata, collateral open vascular bundles, eustele type of stele with the presence of pith at the center, and inferior free central ovary type. Hence, employing gross morphology and morpho-anatomy of these species could scrutinize in future evaluation and taxonomic placements of these species.

Keywords: Clearing Technique, Free-Hand Technique, Hemiparasitic, Marilog Forest Reserve, Philippine Endemic

INTRODUCTION

Loranthaceae, also known as Showy Mistletoes, are perennial flowering plants which are known for their vivid inflorescence and enigmatic appearance (Devkota 2015). One of the genera of this family is *Amyema*, an epiphytic hemiparasitic plant (partially parasitic) on the xylem tissues of their host (Kuijt 1969; Calder & Bernhardt 1983; Kuijt 2009; Arruda *et al.* 2012), inhabiting a wide range of host plants (Devkota *et al.* 2011; Ogunmefun *et al.* 2015) and acquire water, nutrient and sugar while having the capability of photosynthesis (Norton & Carpenter 1998).

Amyema is the second genus with high diversity in Loranthaceae, second *Psittacanthus* which is the most specious (Kuijt 2009). The Philippine archipelago has a total

number of 24 *Amyema* species (Pelser *et al.* 2011 onwards), of which 19 are endemic to the country (Danser 1934; Barlow 1974, 1984, 1992; Pelser & Barcelona 2013). This genus is widely distributed from Southeast Asia to the mainland Australia and the southwest Pacific (Danser 1934; Barlow 1974, 1984, 1992, Pelser & Barcelona 2013). Recently, three *Amyema* species have been described, *viz.*, *A. nickrentii* Barcelona & Pelser in the Philippines (Pelser & Barcelona 2013), *A. lisae* Pelser & Barcelona also in the Philippines (Pelser *et al.* 2018), and *A. xiphophylla* Wege & Start in western Australia (Wege & Start 2020). Regardless of their detrimental effects on the host plant, they are still important in determining the floral diversity in forest ecosystem worldwide (Kujit 1964; Hawksworth 1983; Calder 1983; Polhill & Wiens 1998; Devkota *et al.* 2011).

Marilog Forest Reserve in Marilog District, Davao City has a total land area of 63,000 ha. This area has a rich diversity of flora which makes it as one of the priority areas for biodiversity studies by Conservation International. It is home to different parasitic plants, such as *Balanophora papuana* Schltr. (Balanophoraceae), *Amyema curranii* (Merr.) Danser and *A. seriata* (Merr.) Barlow (Loranthaceae), *Mitrastemon yamamotoi* Makino (Mitrastemonaceae) and *Christisonia scortechinii* Prain (Orobanchaceae) documented by Acma *et al.* (2021). Of these, the two *Amyema* species were studied and examined for their gross morphology and anatomy.

MATERIAL AND METHODS

Botanical Fieldworks

Botanical samplings were conducted from August to November 2019. Necessary permits were obtained from the local people and Gratuitous Permit (GP) from the Department of Environment and Natural Resources (DENR). The specimens of *A. curranii* were collected from Busay Garden Resort near the base of Mt. Malambo (minor peak), while *A. seriata* was collected in Mt. Antayapan, Sitio Tribal Village, both in Brgy. Datu Salumay, Marilog District, Davao City, Philippines (Fig. 1). Repeated transect walks and opportunistic samplings were employed to survey and collect specimens. Moreover, characteristics of habitat, vegetation type, and documentation of host plants and anthropogenic disturbances in the areas were recorded.

Identification of the Specimens

Specimens available at the Journal Storage (JSTOR) were used for the comparison, identification and classification of *Amyema* species. Books and journals (e.g., Pelser & Barcelona 2013) and online databases (The Global Plant List and Co's Digital Flora of the Philippines by Pelser *et al.* 2011 onwards) were used for the verification of the morphological and anatomical features of the plants.

Plant Measurements and Descriptions

Morphological characters of *A. curranii* and *A. seriata* were examined, documented, and described. Five plants were treated for each species. Lengths of the vegetative parts of living specimens were measured using a tape measure. Measurable features, such as plant height, stem, length and width of leaf, length and diameter of petiole, length and diameter of

haustorium, length of flowers and fruits, and detailed floral parts were described and documented. Terminology of Wilson and Calvin (2006) was used in this study.

Anatomical Analysis

Anatomy was done at the laboratory of the Department of Biology, Central Mindanao University, Musuan, Bukidnon. The procedure of Johansen (1940) for anatomical studies was followed. Free-hand technique was done through cutting small pieces (about 1 mm transverse sections) of the different parts of the plants. Clearing technique was done on the young leaves to study the venation pattern, stomatal type and epidermal composition. Close up views of the anatomy were taken using light and stereo microscopes (Table 2).

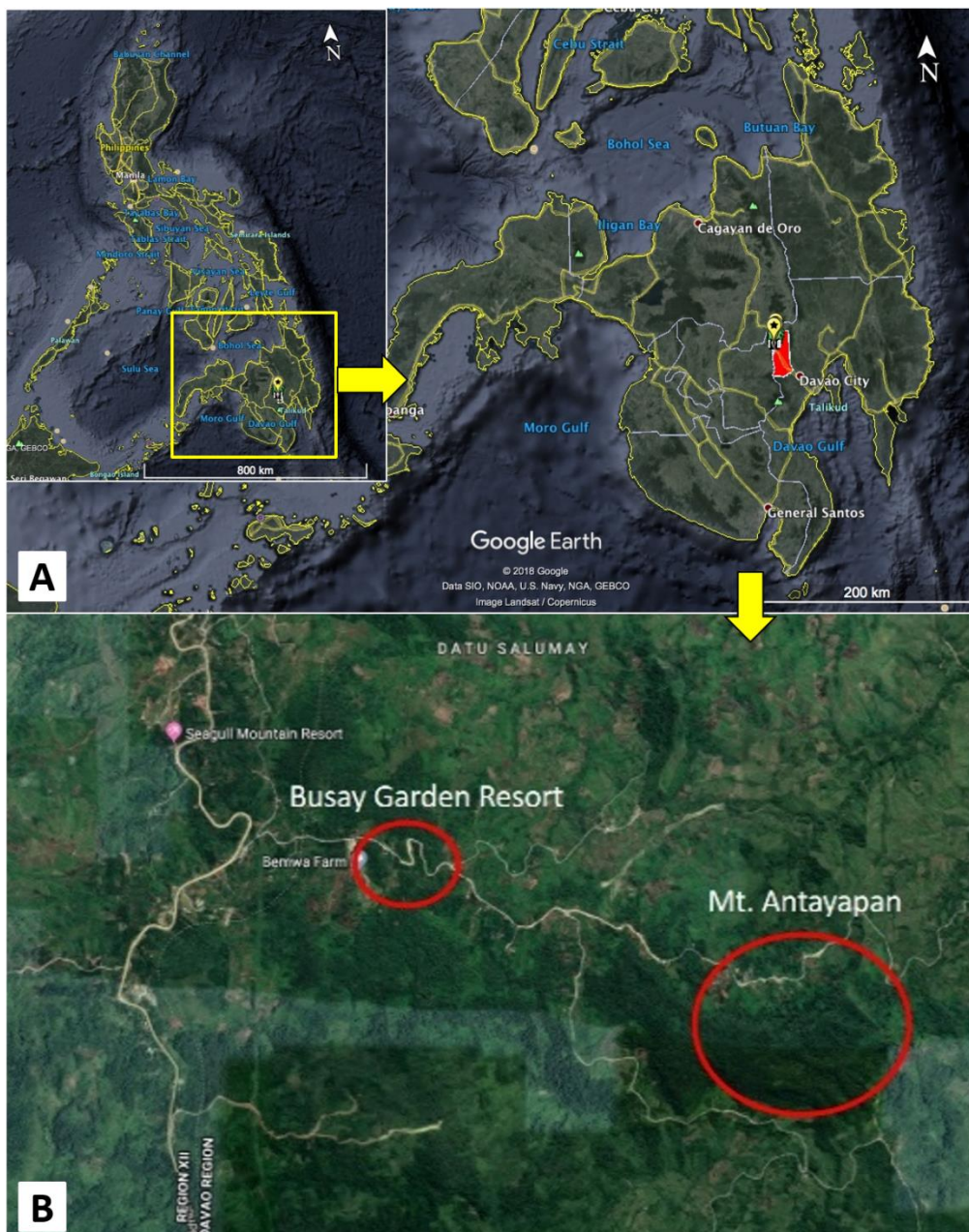


Figure 1. Study sites. (A) Map of Mindanao Island (inset Philippine map), (B) Marilog District, Davao City showing Mt. Antayapan and Busay Garden Resort.

RESULTS AND DISCUSSION

A. Gross Morphology

a. *Amyema curranii* (Fig. 2)

Amyema curranii stems creeping around the host plant; leaves lanceolate to oblanceolate, 10–18 cm long. Inflorescence pink, umbel, pedunculate inflorescence with a yellowish corolla tip. Flowers 10–15 with at least 5–5.5 cm long; fruits ovate, berry, aggregate, green when young, red when mature. It has a very close resemblance to *A. incarnatiflora* (Elmer) Danser which has a pinkish, umbel, pedunculate inflorescence. They differ in the tip of corolla, where *A. incarnatiflora* has a pinkish coloration from base to the tip, while *A. curranii* has a yellowish corolla tip. Further, *A. curranii* has dimorphic leaves (Table 1).

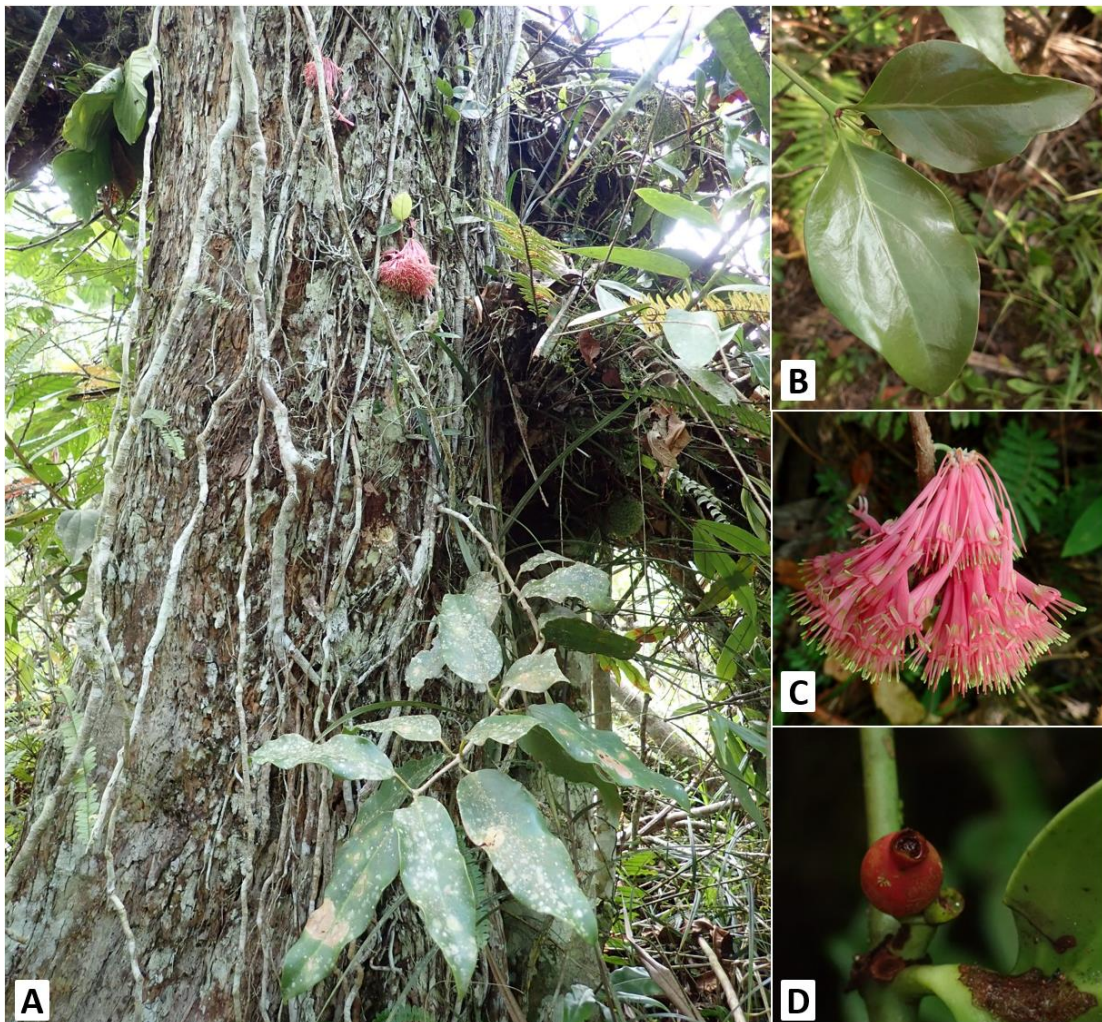


Figure 2. Morphology of *Amyema curranii* (Merr.) Danser. A) Habit, B) Leaves, C) Inflorescence, D) Fruit.

b. *Amyema seriata* (Fig. 3)

Amyema seriata stems erect and mostly attached to the haustorium; leaves obovate to oblanceolate, 10–13 cm long. Inflorescence pedunculate, umbel and red inflorescence and dark red at the tip of the corolla with at least 6–8 flowers attached, ca. 2.5–3 cm long; fruits 2 cm long, berry, ovate, aggregate, green when young and red upon maturity. *A. seriata* closely resembles *A. celebica* (Tiegh.) Danser, except for the latter having a red, umbel, pedunculate inflorescence and obovate leaves. They also differ at the tip of the corolla where *A. celebica* has a yellowish tip and outward infolded when fully bloom. It has also a pedicel attached to each flower (Table 1).

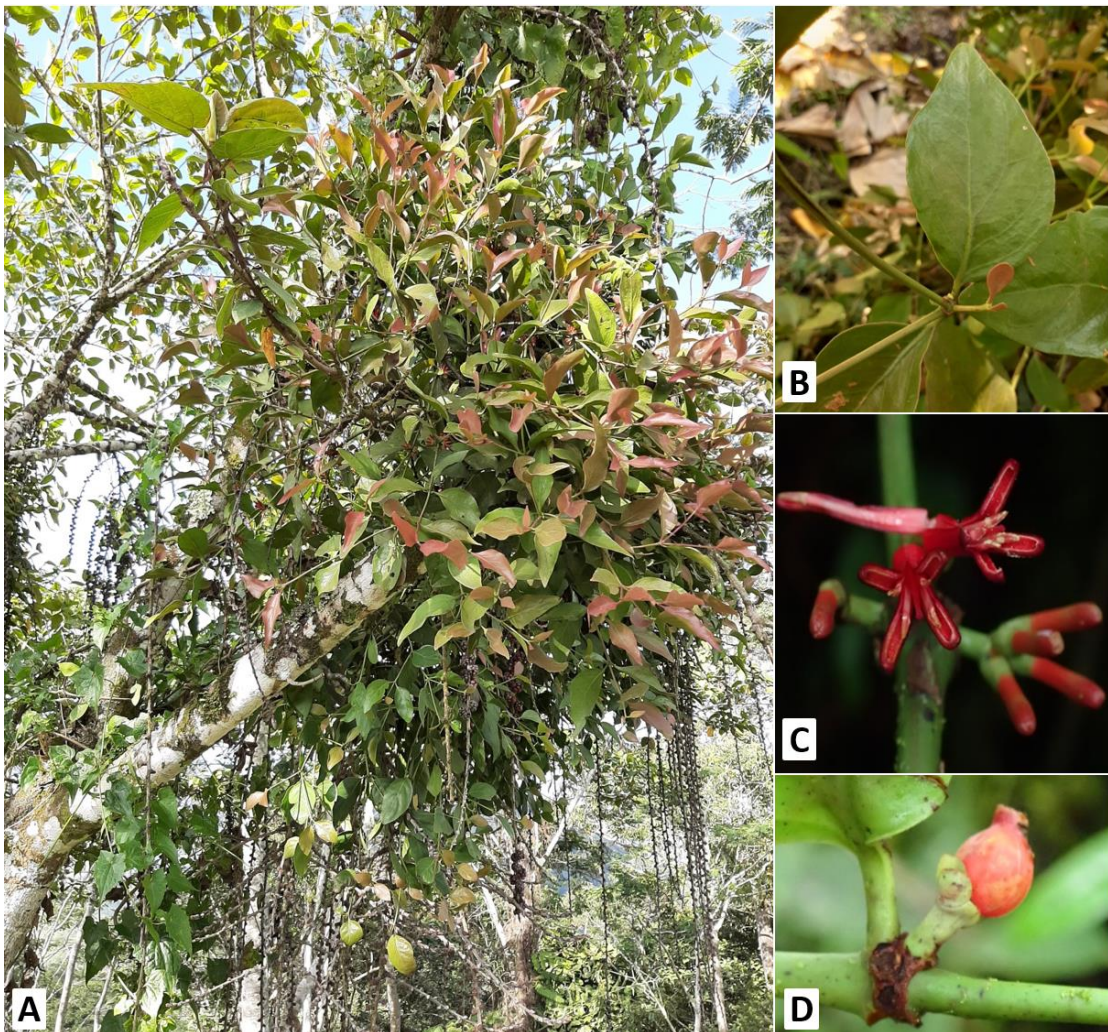


Figure 3. Morphology of *Amyema seriata* (Merr.) Barlow. **A)** Habit, **B)** Leaves, **C)** Inflorescence, **D)** Fruit.

Table 1. Comparative morphological characters of the two *Amyema* species.

Gross Morphology	<i>A. curranii</i>	<i>A. seriata</i>
Leaves		
Arrangement	Opposite	Opposite
Shape	Oblanceolate/lanceolate	Oblanceolate to obovate; 4–5 in long
Color	Brownish green (Young), Green (Mature)	Brownish green (Young), Green (Mature)
Texture	Smooth	Smooth
Venation pattern	Netted	Netted
Base	Obtuse	Obtuse
Apex	Acute	Acute
Margin	Simple	Simple
Length	10–18 cm	10–12 cm
Width	5–10 cm	5–8 cm
Stem	Creeping around the host plant	Erect mostly attached to haustorium
Flower		
Inflorescence	Umbel	Umbel
Corolla (petal)	Pink with yellowish color on the apex	Red with deep reddish coloration at the apex
Length	5–5.3 cm	2–2.5 cm
Peduncle	Present	Present
Pedicel	Present	Present
Fruit		
Type	Berry	Berry
Placentation	Aggregate	Aggregate
Texture	Smooth	Smooth
Color	Green when young, Red when ripe	Green when young, Red when ripe
Peduncle	Present	Present
Pedicel	Present	Present
Shape	Obovate	Obovate
Length	1–1.5 cm	0.8–1.2 cm

B. Anatomy

Amyema curranii leaf epidermis single layer, pinkish, stomata paracytic. Palisade mesophyll 5–6 layers, spongy mesophyll 3–5 layers, vascular bundle collateral open; petiole epidermis single layer, cortex 12–14 layers and collateral open vascular bundles. Stem epidermis single layer, 10–11 layered cortex, eustele with pith at the center; several haustoria attached to the secondary xylem or the wood. Ovary inferior, free central, hairy (Fig. 4; Table 2).

A. seriata leaf epidermis single layer, pinkish color, stomata paracytic. Palisade mesophyll 4–5 layers, spongy mesophyll 5–6 layers, vascular bundle collateral open. Petiole epidermis single layer, pinkish color, cortex 10–11 layers,

vascular bundle collateral open. Stem epidermis single layer, cortex 8–10 layers, eustele type of stele with pith at the center; haustorium solitary, attached to the secondary xylem. Ovary inferior, free central, smooth (Fig. 5; Table 2).

According to study Costa and Ceccantini (2015), the host plants should have a distinct thick-walled latewood as compared to that of the haustoria of parasitic plants. Haustorium evolution was complex in the aerial Loranthaceae with multiple origins of each basic haustorial type (Wilson & Calvin 2006). They also tend to accumulate much of the woods' water reservoir in which it could harm or either a way to kill the host plants (Hawksworth 1983). Some host plants also tend to resist the haustorium formation of *Amyema* by forming wound periderm or by means of change in host plants tissue (Yan, 1993). This statement can be shown in Fig. 2 (E1 and E2) where both *Amyema* species have not fully penetrated the entire latewood or secondary xylem of the host plant.

Table 2. Anatomy of vegetative and reproductive parts of the two *Amyema* species.

Anatomy	<i>A. curranii</i>	<i>A. seriata</i>
Leaf		
Epidermis	Single layer, pinkish color	Single layer, pinkish color
Stomata	Paracytic	Paracytic
Palisade Mesophyll	5–6 layers	4–5 layers
Spongy Mesophyll	3–5 layers	5–6 layers
Vascular Bundles	Collateral open	Collateral open
Petiole		
Epidermis	Single layer	Single layer
Cortex	12–14 layers	10–11 layers
Vascular Bundle	Collateral open	Collateral open
Stem		
Epidermis	Single layer	Single layer
Cortex	10–11 layers	8–11 layers
Stele	Eustele	Eustele
Pith	Present	Present
Haustorium		
Type	Several, attached to the secondary xylem or wood	Solitary, attached to the secondary xylem or wood
Ovary		
Type	Hairy, inferior, free central	Smooth, inferior, free central

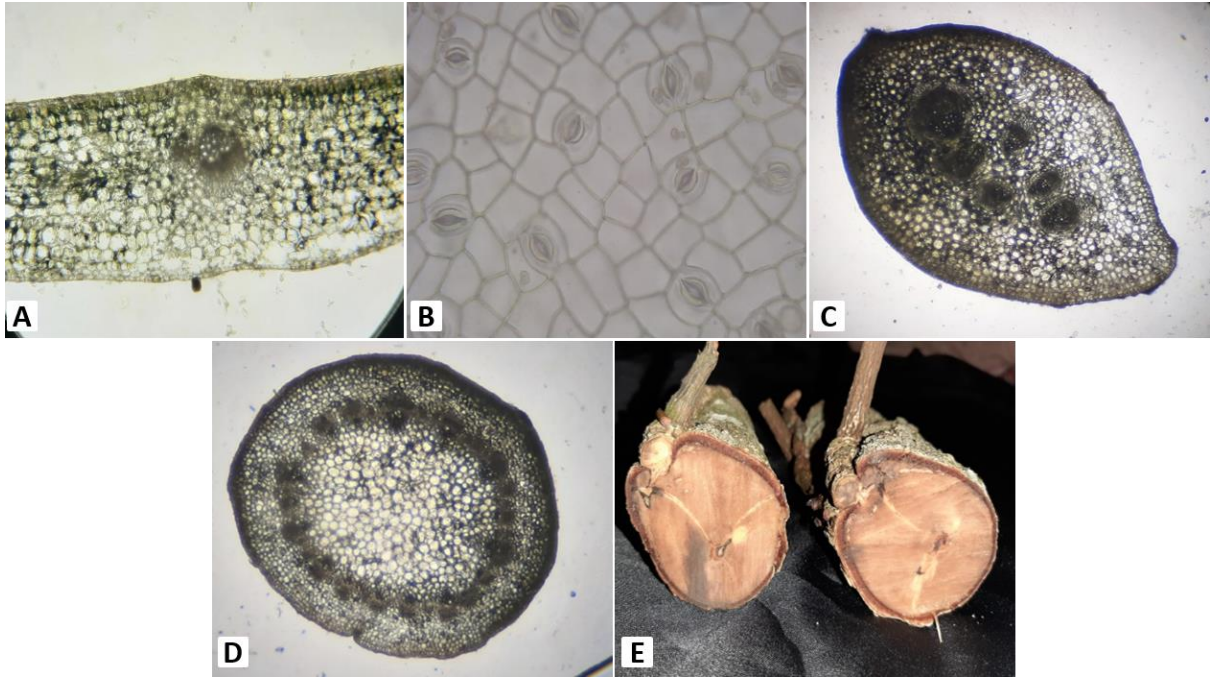


Figure 4. Anatomy of the vegetative parts *A. curranii*. A) Leaf cross section, B) Cleared leaf epidermis, C) Cross section of petiole, D) Stem cross section, E) Transverse section of haustorium.

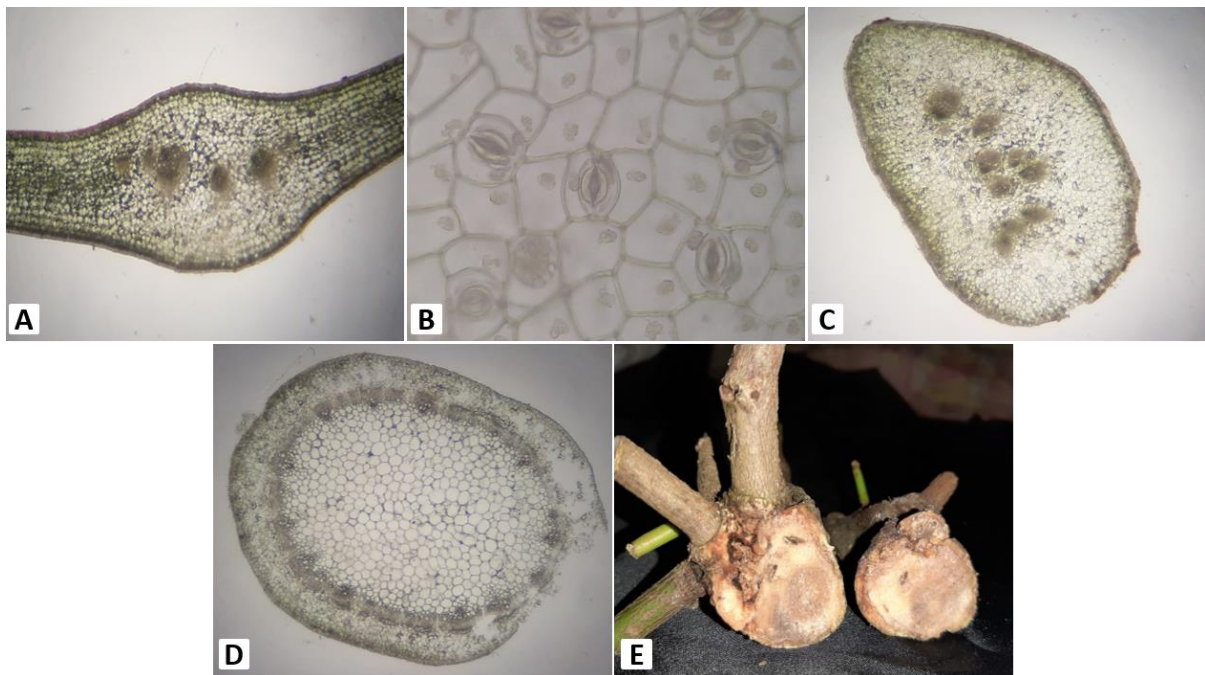


Figure 5. Anatomy of the vegetative parts *A. seriata*. A) Leaf cross section, B) Cleared leaf epidermis, C) Cross section of petiole, D) Stem cross section, E) Transverse section of haustorium.

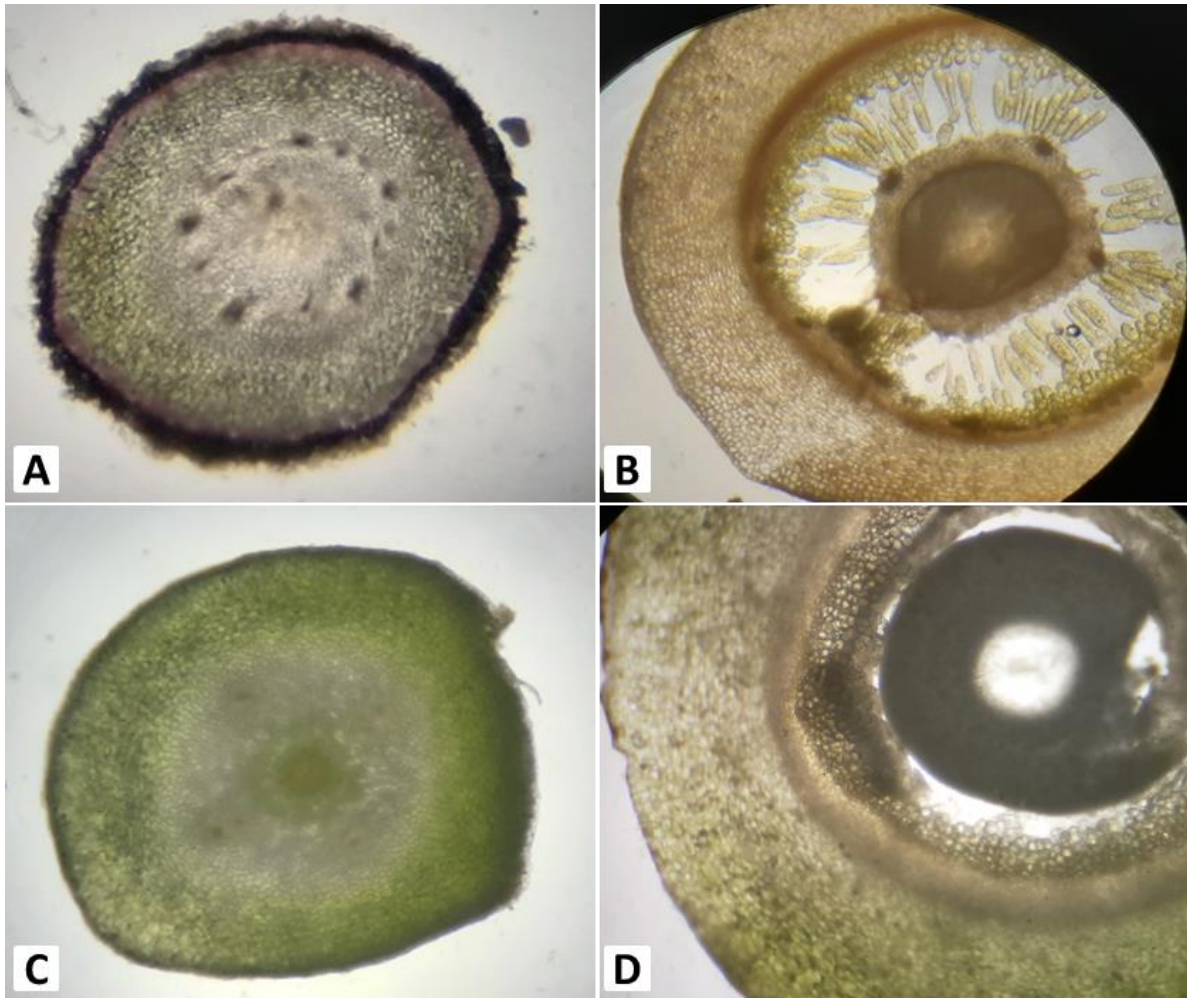


Figure 6. Anatomy of the ovary. A) Transverse section of the young ovary of *A. curranii*, B) Transverse section of mature ovary of *A. curranii*, C) Transverse section of the young ovary of *A. seriata*, D) Transverse section of mature ovary of *A. seriata*.

CONCLUSIONS

The two *Amyema* species differ in the color of flowers in which pink for *A. curranii* while red for *A. seriata*. They also differ in haustorial attachment where *A. curranii* has several haustoria as it is creeping, while *A. seriata* has solitary attachment, and ovary wall in which *A. curranii* has presence of hairs, whereas *A. seriata* is smooth.

FUTURE SCOPE

Conservation and protection of the Marilog Forest Reserve should be implemented due to the increasing forest and habitat disturbances caused by several anthropogenic activities placing these plants to vulnerability or depletion. Anatomical studies using other techniques are also suggested to carefully record the differences in their parts.

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