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Taxonomic Significance of Leaf Anatomy of the Genera *Canthium* Lam., *Canthiumera* K.M.Wong & Mahyuni, *Meyna* Roxb. ex Link, and *Psydrax* Gaertn. (Rubiaceae: Vanguerieae) in Thailand

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ABSTRACT. – The taxonomic significance of leaf anatomy within the genera *Canthium*, *Canthiumera*, *Meyna*, and *Psydrax* (Rubiaceae: Vanguerieae) in Thailand were investigated. Leaf and petiole anatomy of eight species of *Canthium*, one species of *Canthiumera* K.M.Wong & Mahyuni, three species of *Meyna* Roxb. ex Link, and three species of *Psydrax* Gaertn. were analyzed using the transverse sections, epidermal peels, and scanning electron microscopy (SEM). Anatomical characters were evaluated using Principal Component Analysis (PCA) and Unweighted Pair Group Method Algorithm (UPGMA). The anatomical analysis indicates that the genera can be distinguished based on the number of palisade mesophyll layers, the presence or absence of stomata on the leaf surface, and the presence or absence of an adaxial parenchymatous hypodermis. Anatomical features support the recognition of *Canthium* in a narrow sense: *Canthium*, *Canthiumera*, *Meyna*, and *Psydrax*. These findings emphasize the potential of leaf anatomical characters as a reliable tool for taxonomic classification.

KEYWORDS: Taxonomy, leaf anatomy, Ixoroideae, PCA, UPGMA

INTRODUCTION

The Rubiaceae family, comprising approximately 13,150 species about 600 genera, ranks as the fourth largest family of flowering plants. The family is characterized by opposite simple leaves with interpetiolar stipules, fused petals forming a corolla tube, and an inferior ovary. Molecular investigations revealed that Rubiaceae includes three subfamilies: Cinchonoideae, Ixoroideae, and Rubioideae (Bremer and Eriksson, 2009).

The genus *Canthium* Lam. continues to undergo various taxonomic revisions, primarily based on morphological data. *Canthium* was named by Jean-Baptiste Lamarck in 1785 in the *Encyclopédie Méthodique*. Later, *Psydrax* was first established by Joseph Gaertner (1788) in his treatise on plant fruits, seeds, and pyrene morphology, followed by the establishment of *Meyna* by Johann Heinrich Friedrich Link in 1820 based on the presence of spines, and fruits. Hooker (1875) studied *Canthium* and classified species with two locules under *Canthium*, including the genus *Psydrax* Gaertn. Bridson (1992) conducted a comprehensive study of *Canthium* in South Africa. Bridson merged with *Meyna* Roxb. ex Link, *Psydrax*, and *Pyrostria* Roxb into *Canthium* based on the presence of two or more locules and spines. The phylogenetic relationships of the Vanguerieae were analyzed using morphological and molecular data, revealing a close genetic relationship between *Meyna* and *Canthium*, which led to the merging of *Meyna* into *Canthium* (Wong, 1989; Lantz and Bremer 2004; Chen et al., 2011). In 2018, Wong and colleagues proposed the new genus *Canthiumera* K.M.Wong & Mahyuni, reclassifying four species previously assigned to *Canthium*. This reclassification was based on leaf subcoriaceous when fresh but may dry with a chartaceous texture, inflorescences with short peduncles and branches, style as long as the corolla tube, and the pyrene featuring a prominent keel-like apical-dorsal crest and lateral shoulders. The genus was based on *Canthiumera glabra* (Blume) K.M.Wong & Mahyuni (*Canthium glabrum* Blume), the type species. In Thailand, Craib (1932) reported 26 species of *Canthium* and 3 species of *Meyna* based on morphological characteristics, and this view was followed by Sangrattanaprasert (2013). In 2005, Puff and colleagues listed approximately 20 species of *Canthium* in a broad sense, around 3 species of *Meyna*, and about 10 species of *Psydrax* based on morphological characteristics and noted that another genus within the *Canthium*-*Psydrax* alliance is *Meyna*. Species previously included in Craib's broader concept of *Canthium*, have been narrowed down to 16 species under the narrow sense of the genus *Canthium*. Among these 16 species, one has been renamed, and three have been described as new species (De Wilde & Duyfjes, 2022). Utteridge and Davis (2009) and Duyfjes et al. (2023) reported two species of *Pyrostria* in Thailand, resulting from the transfer of *Canthium brunnescens* Craib and *Canthium cochinchinense* Pierre ex Pit. Duyfjes et al. (2023) also reported two species of *Canthiumera* in Thailand by transferring *Canthium siamense* (K.Schum.) Pit. and *Canthium carinatum* Pierre ex Pit. *Psydrax* in Thailand is classified into four species, according to data from Plants of the World Online (POWO, 2024). *Canthium* is characterized by paired supra-axillary spines, brachyblasts in some species, solitary flowers or few-flowered fascicles, and with a 2 celled ovary (De Wilde and Duyfjes, 2022). *Meyna* deviates by having a 4-5 celled ovary (Puff et al., 2005). *Canthiumera* is prominent with thornless plants with panicle-like inflorescences and corolla tubes with downward-directed moniliform hairs (Wong et al., 2018). *Psydrax* is recognized by its thornless habit, cyme-like or umbellate inflorescences, and long slender styles (Wong et al., 2018; De Wilde and Duyfjes, 2022). Many species of *Canthium* are used as medicinal plants. Components of *Canthium coromandelicum* (Burm.f.) Alston. are used for their antimicrobial activity, antioxidant activity, hepatoprotective activity, antimalarial activity, anti-diabetic activity, anti-asthmatic and antibacterial activity (Patro et al., 2014). *Meyna spinosa* Roxb. is traditionally used to treat skin infection, fever, diabetes, liver disease, dysentery, indigestion, intestinal worm, and frequent urination (Sen et al., 2013; Rudrapaul et al., 2014). *Psydrax subcordatus* (DC.) Bridson is used for antimicrobial and antioxidant activity (Anokwah et al., 2016).

Anatomical analyses have long been valuable for taxonomy and have been applied efficiently. The use of anatomy to sort species and resolve taxonomical problems in angiosperms is customary in Rubiaceae and anatomical studies span multiple taxonomic levels (Martínez-Cabrera et al., 2009; Moraes et al., 2011; Romero et al., 2019; Judkevich et al., 2020). In The subfamily Rubioideae is recognized by the presence of raphides crystals, while Ixoroideae and Cinchonoideae reveal druse crystals (Martínez-Cabrera et al., 2009; Moraes et al., 2011; Judkevich et al., 2020). The tribe Hamelieae, Gardenieae, and Psychotrieae can be distinguished using specific vascular tissue types in the midrib and petiole (Martínez-Cabrera et al., 2009). The leaf anatomical features, including the presence of cuticle striations on the leaf, trichome type, the shape of the anticlinal cell wall, the presence of subepidermal parenchymatous hypodermis, a layer of palisade cells, thickness of spongy parenchyma, shape of the main vascular bundles, and the contour of the petiole are beneficial in genera circumscription such as *Canthium s.l.*, *Cephalanthus* L., *Psychotria* L., *Randia* L., *Oldenlandia* L., *Ixora* L. and *Mitragyna* L. (Tilney et al., 1990; Moraes et al., 2011; Romero et al., 2019; Judkevich et al., 2020; Patil and Patil, 2020; Ekeke et al., 2021; Essiett and Umoh, 2014; Ghazalli et al., 2021). In the study of *Hedyotis corymbosa* L. complex, anatomical characteristics that can differentiate this complex include the shape of the anticlinal cell wall (Panda, 2014). Tilney et al. (1990) examined 14 species of *Canthium s.l.* in southern Africa (nine species of *Canthium s.s.*, four species of *Psydrax*, and one species of *Keetia* Phill.), while Sangrattanaprasert (2013) analyzed nine species of *Canthium s.l.* in Thailand (four species of *Canthium s.s.*, four species of *Psydrax*, and one species of *Canthiumera*). Both studies identified leaf anatomical features valuable for classification, such as hair structure, lamina thickness, midrib shape, cuticle thickness, epidermal cell shape, and mesophyll composition. Tilney et al. (1990) classified three groups based on leaf anatomy, corresponding to the genera *Canthium s.s.*, *Psydrax*, and *Keetia*, noting anatomical consistency within groups and distinct traits for individual species. However, classification of *Canthium*, remains unclear within this group, while anatomical data have been studied for only 16.25% of the species in the genus in Africa and Asia and 25% of the species in the genus in Thailand. The significance of anatomical data for the classification of Thai *Canthium s.l*, has not been elucidated. Therefore, the current interpretations expressed in this report aim to examine the leaf and petiole anatomical characteristics of four genera including *Canthium*, *Canthiumera*, *Meyna*, and *Psydrax* in Thailand and to construct anatomical keys for genera and provide additional data to classify *Canthium*, *Canthiumera*, *Meyna*, and *Psydrax* in Thailand and to analyze anatomical data using Principal Component Analysis (PCA) and cluster similarity analysis for use in the clarification of generic limitation.

MATERIALS AND METHODS

Plant materials

The specimens of *Canthium* Lam., *Canthiumera* K.M.Wong & Mahyuni, *Meyna* Roxb. ex Link and *Psydrax* Gaertn. were collected throughout Thailand. The leaf and petiole anatomy of 15 species belong to four genera were examined, consisting of eight species of *Canthium*, one species of *Canthiumera*, three species of *Meyna*, and three species of *Psydrax*. The plant was identified by the first author using the following literature: Robyns (1928), Wong et al. (2018), De Wilde and Duyfjes (2022), and Duyfjes et al. (2023). The number of species examined for each genus represents more than or equal to 50% of the species recognized in Thailand. Voucher specimens were deposited at the Queen Sirikit Botanic Garden Herbarium (QBG) and Forest Herbarium (BKF) (Table 1).

Light microscopy

Mature leaves were stored in 70% ethanol for leaf and petiole examinations. Mature leaves and petiole were selected from the 3rd to 5th nodes from the shoot

TABLE 1. List of specimens examined for leaf anatomical features.

Species	Voucher specimens	Locality
Canthium berberidifolium E.T.Geddes	*P. Poosongsee 032* (BKF)	Khon Kaen
Canthium calvum Craib	*P. Poosongsee 064* (BKF)	Sakon Nakhon
Canthium coffeoides Pierre ex Pit.	*P. Poosongsee 078* (BKF)	Chiang Rai
Canthium ferrugineum Craib	*P. Poosongsee 070* (QBG)	Chumphon
Canthium horridum Blume	*P. Poosongsee 043* (QBG)	Surat Thani
Canthium horridulum Craib	*P. Poosongsee 052* (QBG)	Trang
Canthium parvifolium Roxb.	*P. Poosongsee 075* (QBG)	Chiang Mai
Canthium quadratum Craib	*P. Poosongsee 072* (QBG)	Chumphon
Canthiumera siamensis (K.Schum.) K.M.Wong	*P. Poosongsee 076* (QBG) *P. Poosongsee 105* (QBG)	Chiang Mai Bueng Kan
Meyna grisea (King & Gamble) Robyns	*P. Poosongsee 074* (QBG)	Mae Hong son
Meyna spinosa Roxb. ex Link	*K. Wangwasit 200619-4* (QBG) *P. Poosongsee 047* (QBG) *P. Poosongsee 055* (QBG) *P. Poosongsee 080* (QBG)	Chiang Mai Surat Thani Udon Thani Sakon Nakhon
Meyna velutina Robyns	*P. Poosongsee 023* (BKF) *P. Poosongsee 061* (BKF)	Loei Sakon Nakhon
Psydrax dicoccos Gaertn.	*P. Poosongsee 024* (BKF) *P. Poosongsee 077* (BKF)	Loei Prachuap Khiri Khan
Psydrax nitidus (Craib) K.M.Wong	*P. Poosongsee 058* (BKF)	Sakon Nakhon
Psydrax umbellatus (Wight) Bridson	*P. Poosongsee 031* (QBG) *P. Poosongsee 066* (BKF)	Khon Kaen Bueng Kan

apex. Leaves and petiole were cut at the middle part. The midrib, leaf margin, the area between the midrib and leaf margin and petiole were prepared by paraffin methods (applied from Johanson, 1940). All samples were transversely sectioned with a thickness of 8−12 µm, using a rotary microtome. Samples were stained with safranin O and fast green, then permanently mounted in DePeX. Adaxial and abaxial leaf surfaces were prepared via epidermal peeling method. The unbesirable tissues at the centre between the base and the apex of the lamina were removed using razor blade. The cleared surfaces were stained with safranin O. Afterward, they were treated in a sequential series of alcohol concentrations for 10–15 minutes at each concentration. Finally, the prepared epidermal samples were mounted in DePeX medium. Permanent slides were examined, with images captured using a Leica DM 750 light microscope.

Scanning electron microscopy

Leaf samples were collected from the central area between the base and apex of the leaf lamina. The dried leaf was affixed to aluminum stubs with double sided adhesive carbon taps. They were coated with gold using a SPIMODULE at 11.5 mA for 5 minutes. Examination and documentation of image were conducted using a scanning electron microscope, JEOL JSM6460LV.

Data analysis

A binary code was created from the leaf and petiole anatomical and micromorphological data, with each character assigned a score of either present (1) or absent (0). The presence or absence of 19 anatomical characters was evaluated for the 15 species in four genera (Supplementary material 1). Unweighted Pair Group Method Algorithm (UPGMA) cluster dendrograms and principal component analysis (PCA) were generated using of R software version 3.4.2 and R Studio (R Core Team, 2024).

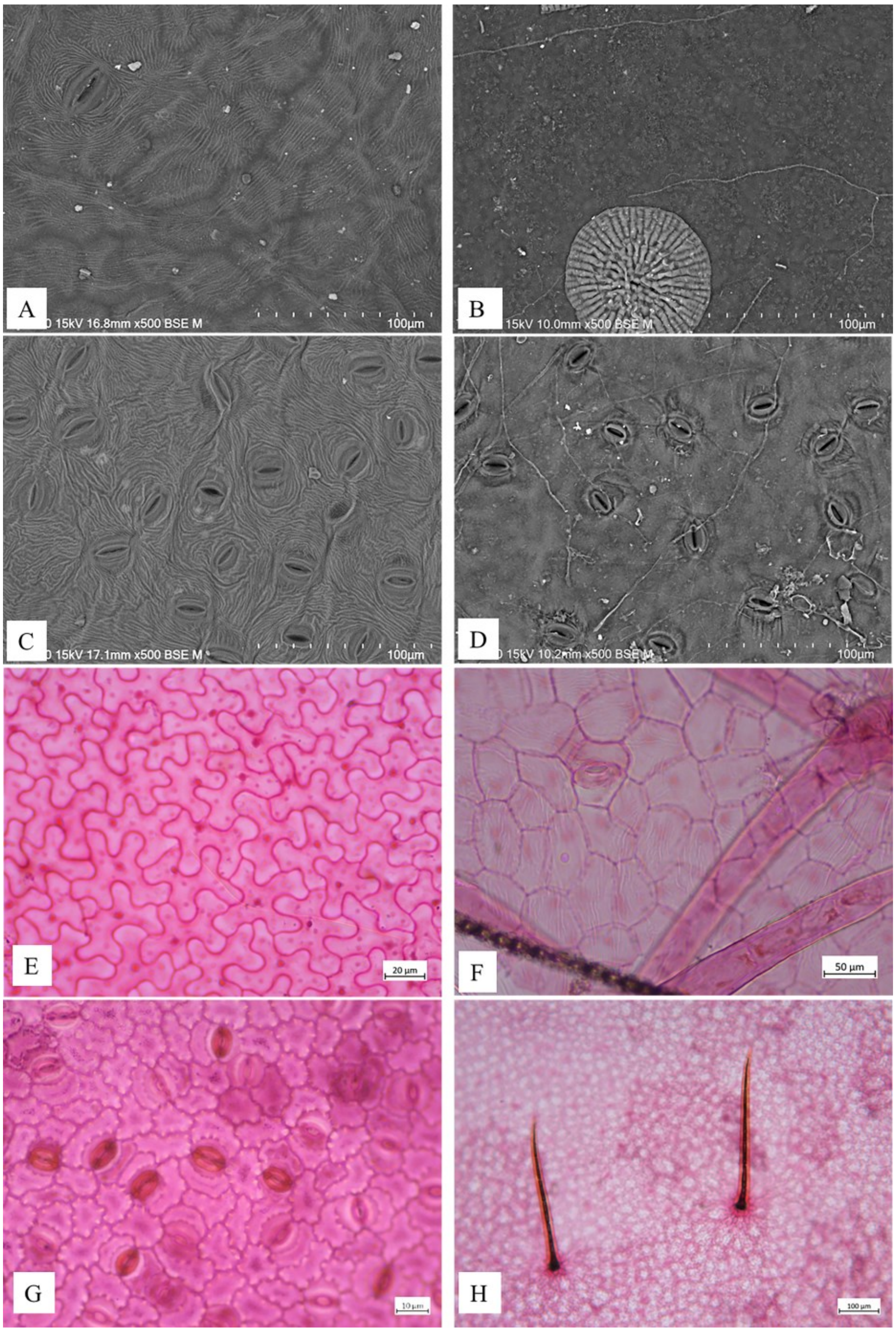

FIGURE 1. Cuticular ornamentation on the upper leaf surface (A-B); A. *Meyna velutina*; striated ornamentation with stomata. B. *Psydrax nitidus*; absent ornamentation with peltate trichome. Cuticular ornamentation on lower leaf surface (C-D); C. *Meyna velutina*: striated ornamentation D. *Canthium coffeoides*: absent ornamentation. Upper leaf surface (E-F); E. *Canthium parvifolium*: jigsaw shape and anticlinal walls undulate. F. *Meyna grisea*: polygonal shape and anticlinal walls straight. Lower leaf surface (G-H); G. *Psydrax dicoccos*: irregular shape. H. *Canthium ferrugineum*: multicellular non-glandular trichomes.

RESULTS

The anatomical study of 15 species from four genera (*Canthium*, *Canthiumera*, *Meyna*, and *Psydrax*) in Thailand examined epidermal peels and transverse sections of leaves and petioles. PCA and UPGMA were used to cluster the genera and support their taxonomic classification.

Anatomical characteristics of *Canthium, Canthiumera, Meyna and Psydrax*

Lamina surface

Striated cuticular ornamentation on the adaxial surface was present mainly (Fig. 1A), but it was absent in *Canthiumera siamensis* and *Psydrax nitidus* (Fig. 1B). Most species lacked ornamentation on the abaxial surface (Fig. 1D), except for *Meyna velutina* (Fig. 1C) which had a striated pattern. Epidermal cells of the

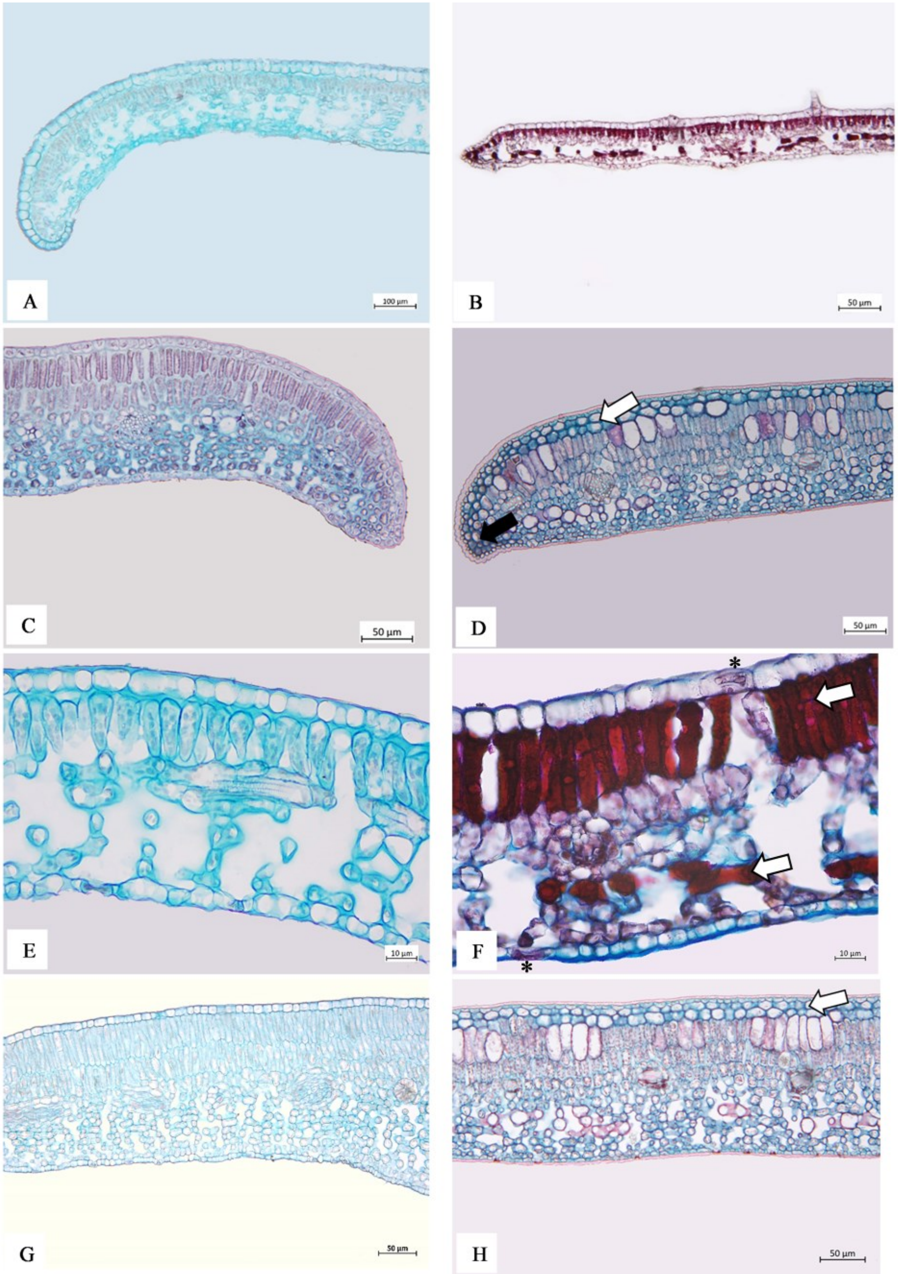

FIGURE 2. Leaf margins in transverse sections (A-D); A. *Canthium coffeoides*: obtuse and downward margin. B. *Meyna velutina*: acute and straight margin. C. *Canthiumera siamensis*: obtuse and downward margin. D. *Psydrax dicoccos*: obtuse and downward margin, subepidermal parenchymatous hypodermis (white arrow) and angular collenchyma (black arrow). The region between leaf margin and midrib (E-H); E. *Canthium ferrugineum*: with single layer palisade parenchyma and spongy parenchyma thickness more than 50% of lamina thickness. F. *Meyna grisea*: amphistomatic leave (asterisk) and dark staining deposits (white arrow). G. *Canthiumera siamensis*: with multiple layer palisade parenchyma and spongy parenchyma thickness less than 50% of lamina thickness. H. *Psydrax dicoccos*: subepidermal parenchymatous hypodermis (white arrow).

upper and lower epidermis were primarily similar with a jigsaw shape (Fig. 1.E) or irregular (Fig. 1G), but *Meyna grisea* had polygonal upper epidermal cells (Fig. 1F). Unicellular non-glandular trichomes and multicellular non-glandular trichomes were normally exposed on both leaf surfaces (Fig. 1H), whereas *Canthium coffeoides*, *Canthiumera siamensis*, and *Psydrax nitidus* (Fig. 1B) revealed peltate trichomes on the upper epidermis. The genera examined commonly had hypostomatic leaves with paracytic stomata, except for the genus *Meyna* which is remarkable with amphistomatic leaves (Fig. 2F).

Lamina transverse section

The leaves demonstrated a bifacial type. Typical stomata were generally studied in all species. The outline of the leaf margin is mostly obtuse and downward (Fig. 2A, C−D), while genus *Meyna* has an acute and straight margin (Fig. 2B). Unicellular non-glandular trichomes and multicellular non-glandular trichomes were normally exposed on the leaf margin,

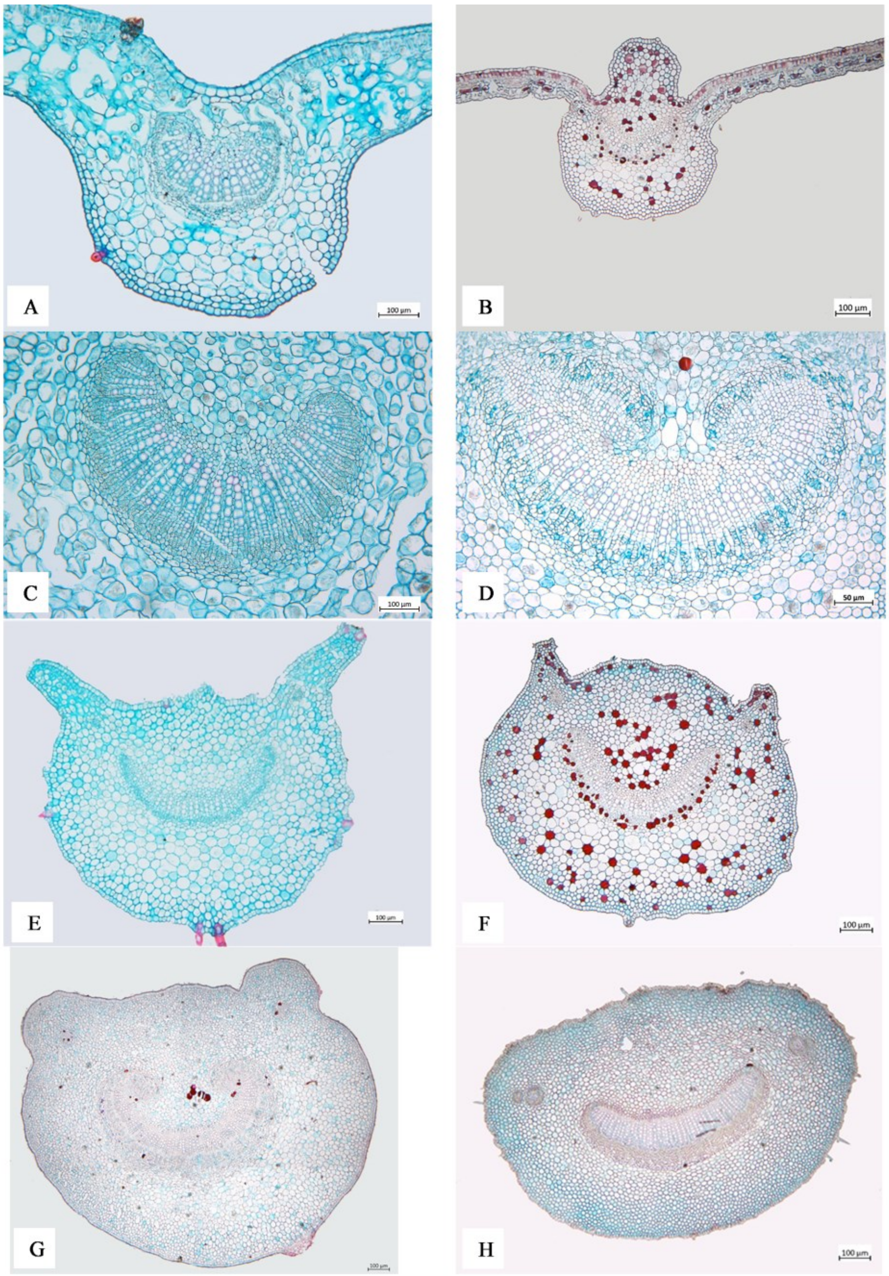

FIGURE 3. Midribs in transverse sections (A-D); A. *Canthium coffeoides*: concave shape in the adaxial surface. B. *Meyna velutina*: biconvex outline. C. *Canthium coffeoides*: vascular bundles arrange in arc-shaped without invaginated ends. D. *Canthiumera siamensis*: vascular bundles arrange in arc-shaped with invaginated ends. Petioles in transverse sections (E-H); E. *Canthium parvifolium*: semicircular shape without lateral vascular bundles. F. *Meyna velutina*: circular shape with lateral vascular bundles. G. *Canthiumera siamensis*: semicircular shape with lateral vascular bundles. H. *Psydrax umbellatus* semicircular shape with lateral vascular bundles.

except *Canthium berberidifolium*, *C. calvum*, *C. coffeoides*, *C. quadratum*, *Canthiumera siamensis, Psydrax dicoccos, P. nitidus and P. umbellatus* in which trichomes are absent. Adaxial parenchymatous hypodermis is lacking in most species examined (Fig. 2E−G), except in the genus *Psydrax* in which several layers of adaxial parenchymatous hypodermis were observed both in the leaf margin and midrib (Fig. 2D, H). The mesophyll mainly consists of a single-layered palisade mesophyll (Fig. 2E–F), whereas *Psydrax* and *Canthiumera* show a multilayered palisade mesophyll (Fig. 2G–H). The spongy parenchyma thickness was more than 50% of the lamina thickness (Fig. 2E−F), while *Psydrax and Canthiumera* were less than 50% of the lamina thickness (Fig. 2G−H). The shape of the midrib was typically biconvex (Fig. 3B). The adaxial side was concave in *Canthium coffeoides* (Fig. 3A), *Canthium horridum* and *Canthium parvifolium*. The vascular bundle was usually of collateral type with a parenchymatous bundle sheath. The vascular bundle in the midrib was generally arc-shaped without invaginated ends (Fig. 3C), whereas in the genus

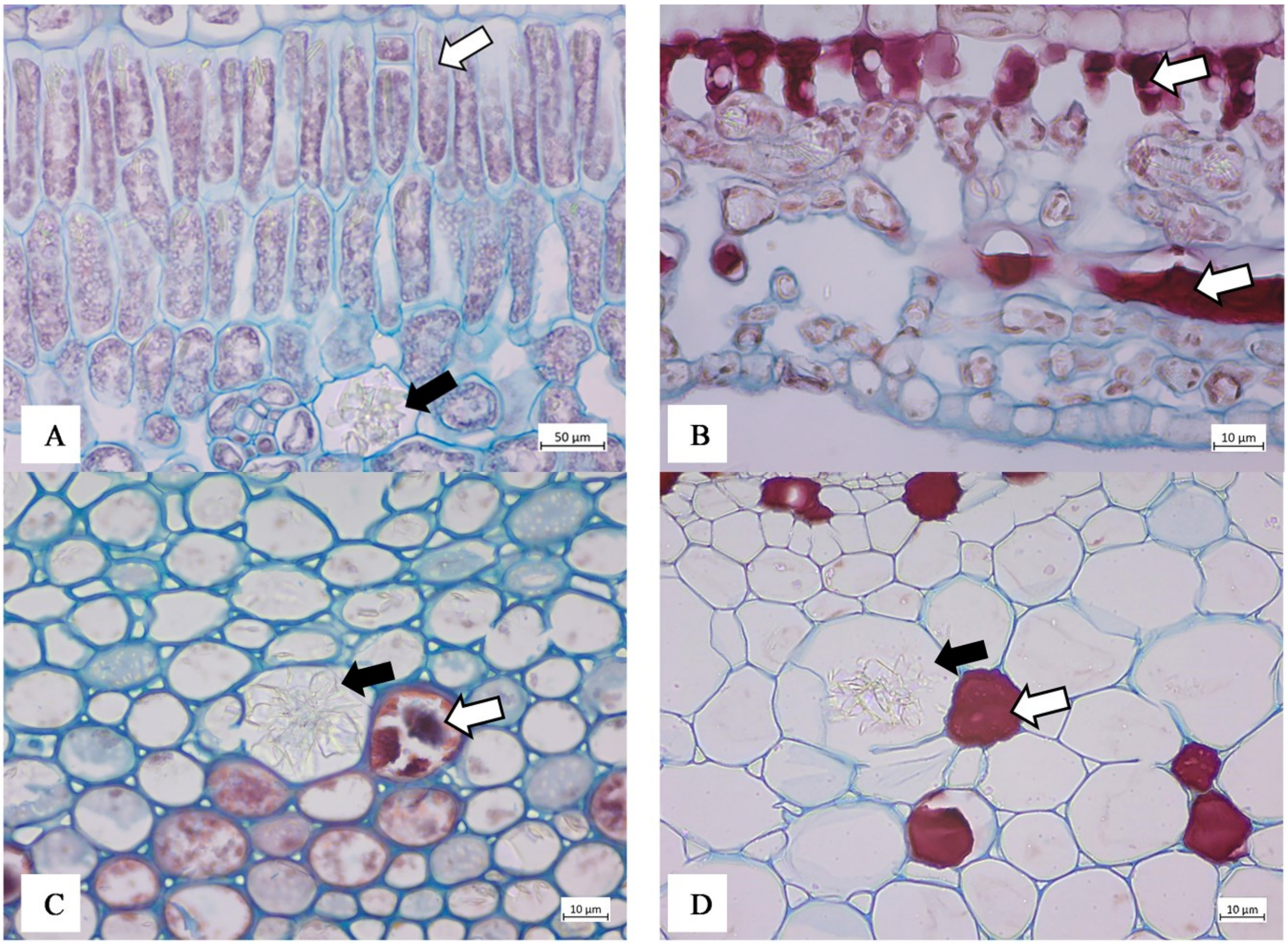

FIGURE 4. Deposition in mesophyll (A-B); A. *Canthiumera siamensis*: styloids in palisade parenchyma (white arrow) and druses in spongy parenchyma (black arrow). B. *Meyna spinosa*: dark staining deposits in palisade and spongy parenchyma (white arrow). Deposition in petioles (C-D); C. *Psydrax dicoccos*: druses (black arrow) and dark staining deposits (white arrow). D. *Meyna velutina*: druses (black arrow) and dark staining deposits (white arrow).

Canthiumera, it was exclusively arc-shaped with invaginated ends (Fig. 3D). The ground tissue of most species was composed of parenchyma and angular collenchyma. The angular collenchyma occurs underneath the epidermis and is restricted to the upper side in *Meyna grisea*, while in *Canthium horridulum*, *Meyna spinosa* and *M. velutina,* they were absent. Druses were present in *Psydrax* and *Canthiumera* (Fig. 4A). Styloids were present in *Canthiumera* (Fig. 4A). Dark-staining deposits were commonly found in mesophyll (Fig. 4B), except in *Canthium calvum*, *C. coffeoides*, *C. ferrugineum*, *C. parvifolium* and *C. quadratum*.

Petiole transverse section

The petiole is mostly semicircular (Fig. 3E, G−H), except in genus *Meyna* and *Canthium quadratum* which are circular (Fig. 3F). Unicellular non-glandular trichomes and multicellular non-glandular trichomes were typically found (Fig. 3H), except in the genus *Canthiumera siamensis* (Fig. 3G) and *Psydrax dicoccos*. The ground tissue of most species is comprised of parenchyma and angular collenchyma. The angular collenchyma occurs underneath the epidermis. The vascular bundles were of the type and shape as those of the midrib. The lateral vascular bundles were remarkable in *Canthium coffeoides*, *Canthiumera siamensis* (Fig. 3G), genus *Meyna* and genus *Psydrax* (Fig. 3F, H). Dark-staining deposits and druses were noted in *Canthium berberidifolium*, *C. horridum*, *C. horridulum*, *Canthiumera siamensis*, genus *Meyna* and genus *Psydrax* (Fig. 4C−D).

Anatomical character analysis

The relationships among members of *Canthium*, *Canthiumera*, *Meyna*, and *Psydrax* are investigated using twenty-two accessions from fifteen species in Thailand. Based on the binary data matrix, nineteen leaf and petiole anatomical characters are analyzed using a Principal Component Analysis (PCA) and Unweighted Pair Group Method Algorithm (UPGMA) cluster dendrograms. The PCA results are presented in Figure 5, while the UPGMA cluster dendrograms are illustrated in Figure 6.

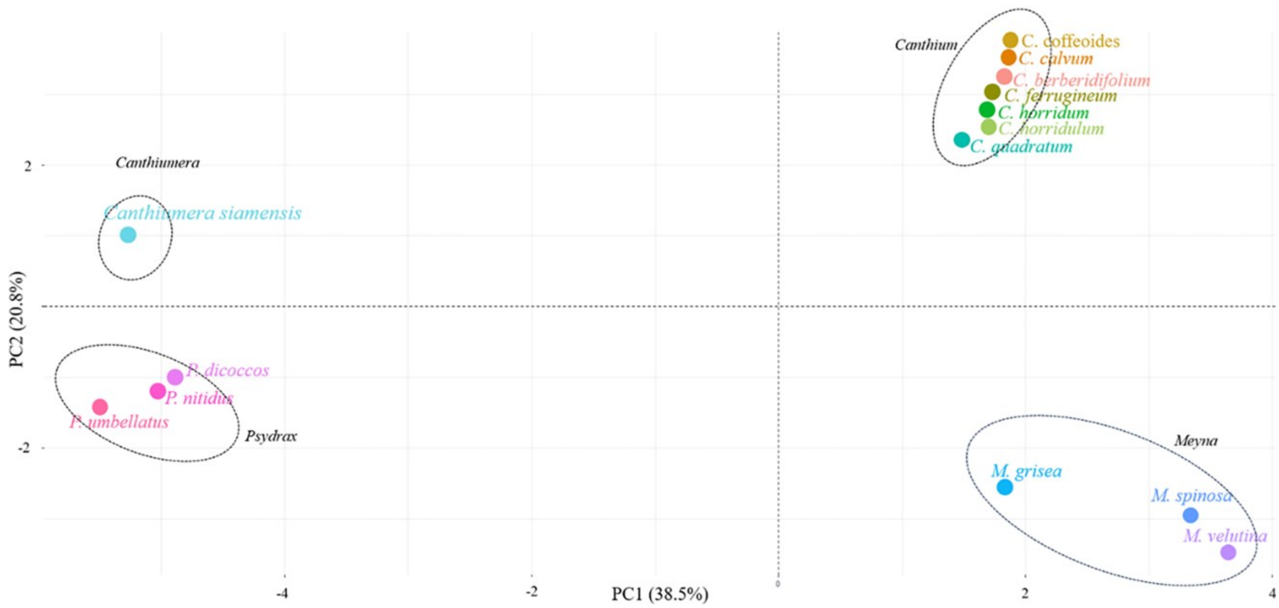

FIGURE 5. Principal Component Analysis (PCA) of anatomical data showing their distinct clustering.

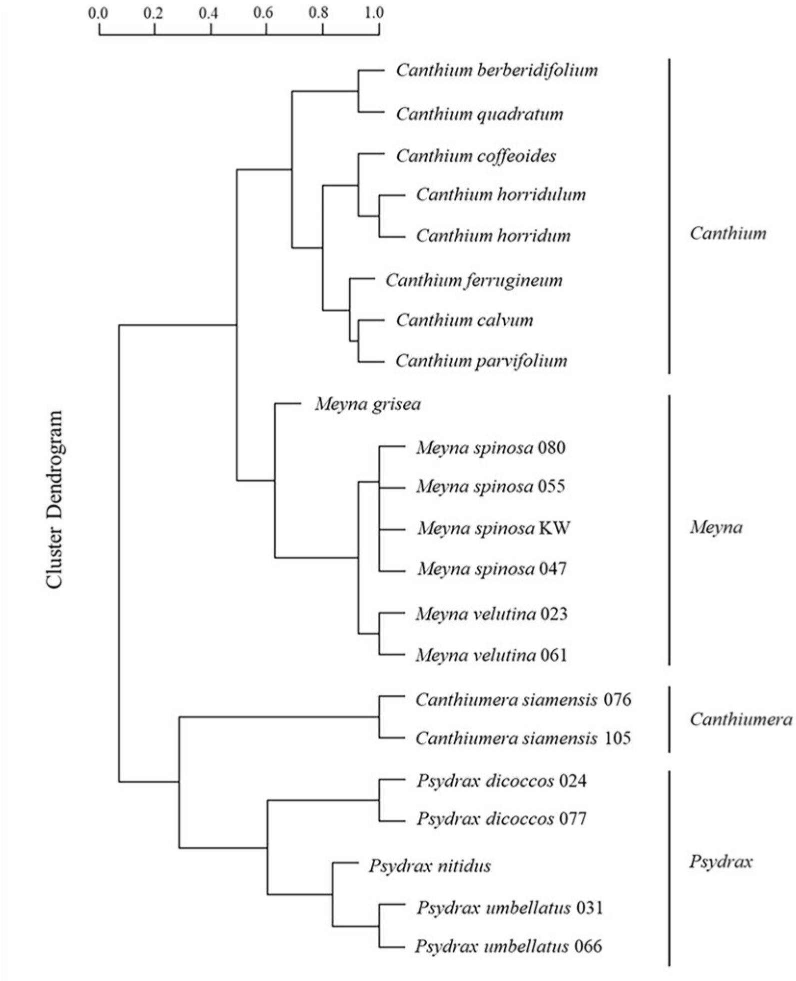

FIGURE 6. Cluster similarity analysis of *Canthium*, *Canthiumera*, *Meyna*, and *Psydrax* species studied based on leaf anatomical data.

The Principal Component Analysis (PCA) provides a complementary perspective to the UPGMA cluster analysis. PC1 explains 38.5% of the variation between the species based on the anatomical features. PC2 adds an additional 20.8% to the explanation of the variation. Together, PC1 and PC2 account for a significant portion (about 59.3%) of the overall variation. *Canthium* clusters tightly together, showing less variation within the genus. The *Meyna* cluster is more spread out along PC1, indicating a greater degree of variation within this genus. *Psydrax* and *Canthiumera* are both clearly distinct from the other two genera, with less internal variation.

The analysis indicates two distinct main groups of genera based on the number of palisade mesophyll layers. The first group, positioned on the right side of the PCA plot, includes the genera *Canthium* and *Meyna*, which share a key feature: a single-layered palisade mesophyll. The groups show moderate similarity (~50%), as indicated in the UPGMA cluster dendrogram. Within this group, division is based on the presence of stomata on leaf surface: *Canthium* possesses hypostomatic leaves with a high similarity value of about 70%, while *Meyna* reveals amphistomatic leaves with a moderate similarity value of about 60%. The second group is located on the left side of the PCA plot, comprises the genera *Canthiumera* and *Psydrax*. Both are characterized by a multi-layered palisade mesophyll and exhibit low overall similarity (~30%) in the UPGMA cluster dendrogram. These genera are further distinguished by the presence of an adaxial parenchymatous hypodermis in *Psydrax*, with a similarity value of about 60%, whereas its absence in *Canthiumera*, exhibiting a remarkably high similarity value of about 98%.

Based on anatomical character analysis the key for investigated identification is presented.

Key to the genera of *Canthium*, *Canthiumera*, *Meyna*, and *Psydrax* using leaf anatomical features

1. Mesophyll compose of single layered palisade mesophyll
    2. Stomata are present on abaxial leaf surface; hypostomatic leaf…………………….…….....…*Canthium*
    2. Stomata are present on adaxial and abaxial leaf surface; amphistomatic leaf….………………....*Meyna*
1. Mesophyll compose of multilayered palisade mesophyll
    3. Adaxial parenchymatous hypodermis present……… ……………………………………………...*Psydrax*
    3. Adaxial parenchymatous hypodermis absent……… …………………………………..….*Canthiumera*

DISCUSSION

Striated ornamentation on the adaxial surface was observed in most species of *Canthium* and *Psydrax*, aligning with the findings of Sangrattanaprasert (2013). However, Sangrattanaprasert noted the absence of ornamentation in *C. berberidifolium* and *C. coffeoides*, which contrasts with the observations in this study. The jigsaw and irregular shape of epidermal cells of *Canthium* and *Psydrax* are consistent with the investigations of Sangrattanaprasert (2013). Non-glandular trichomes were commonly observed in the examined species of *Canthium*, consistent with the results of Tilney et al. (1990) and Sangrattanaprasert (2013). Paracytic stomata are dominant in recent research as previously described for several members of the Rubiaceae (Moraes et al., 2011; Romero et al., 2019; Judkevich et al., 2020). The hypostomatic leaves appear to be the most common case among the Rubiaceae (Moraes et al., 2011). In the present studies, hypostomatic leaves are previewed in *Canthium* and *Psydrax*, which corresponds with Tilney et al. (1990) and Sangrattanaprasert (2013), except in *Meyna*, in which amphistomatic leaves are prominent; this is the first report of amphistomatic leaves in Rubiaceae.

The occurrence of a bifacial leaf, typical stomata, subepidermal parenchymatous hypodermis, and druse crystals in *Canthium* and *Psydrax* agree with Tilney et al. (1990) and Sangrattanaprasert (2013). The single layered palisade mesophyll is common in the species *Canthium*, and multilayered palisade mesophyll occurs in *Psydrax*, which corresponds to Tilney et al. (1990) and Sangrattanaprasert (2013). Martínez-Cabrera et al. (2009) and Judkevich et al. (2020) reported that druse crystals are typically present in the subfamily Ixoroideae, in which *Canthium* and *Psydrax* have traditionally been classified (Lantz and Bremer, 2004).

The petioles in *Canthium* and *Psydrax* exhibit a semicircular shape in transverse section, angular collenchyma, and an arc-shaped vascular bundle without invaginated ends, which corresponds to the findings of Tilney et al. (1990) and Sangrattanaprasert (2013). However, the angular collenchyma of *C. berberidifolium* and *C. coffeoides* was not observed by Sangrattanaprasert (2013). The central vascular patterns in *Canthium*, *Meyna*, and *Psydrax* form an open arc without invaginated ends and accompany with two pairs of lateral bundles, whereas in *Canthiumera*, the central trace forms an open arc with invaginated ends and attached to a single pair of lateral bundles. These central vascular patterns data are consistent with Martínez-Cabrera et al. (2009).

Significance of leaf anatomical features for delimitation of *Canthium*, *Canthiumera, Meyna*, and *Psydrax* in Thailand

The UPGMA and PCA analyses based on anatomical characteristics correspond with the classification derived from morphological features. *Canthium*, *Mayna*, *Canthiumera* and *Psydrax* are accepted in the narrow sense by Wong et al. (2018), De Wilde and Duyfjes (2022), and Duyfjes et al. (2023) with main characteristics including: (i) growth form (shrub or tree with or without spines), (ii) type of inflorescence, and (iii) type of hairs in flowers. The anatomical analysis categorizes the examined plants into two groups based on the number of palisade mesophyll layers. The first group, characterized by a single-layered palisade mesophyll, correlates with the thorny plants: *Canthium* and *Meyna*. The group is further divided into two subgroups based on the presence of stomata on the leaf surface: hypostomatic leaves, recognized in *Canthium*, and amphistomatic leaves, observed in *Meyna*. The second group, distinct with a multilayered palisade mesophyll, corresponds to the thornless plants: *Canthiumera* and *Psydrax*. The adaxial parenchymatous hypodermis is present in *Psydrax* but absent in *Canthiumera*. The leaf anatomical features observed in this research provide substantial taxonomic support for the delimitation of the genera *Canthium*, *Canthiumera*, *Meyna*, and *Psydrax* in Thailand and support the treatment by Wong et al. (2018), De Wilde and Duyfjes (2022), and Duyfjes et al. (2023) in the narrow sense.

Tilney et al. (1990) proposed the separation of *Canthium s.l.* in Southern African into *Canthium s.s.* and *Psydrax* based on the differentiation of mesophyll into palisade and spongy parenchyma. In the present study, UPGMA and PCA analyses segregate *Canthium* and *Psydrax* based on the number of palisade mesophyll layers. The results indicate that *Canthium* exhibits a high level of internal similarity, characterized by a single layered palisade mesophyll. *Psydrax* shows a high level of internal similarity, distinguished by multilayered palisade mesophyll. This analysis provides strong support for the taxonomic distinction between *Canthium* and *Psydrax* based on robust anatomical characteristics and validated statistical data, thereby confirming their recognition as divided genera, in agreement with Tilney et al. (1990).

In conclusion, the anatomical, UPGMA and PCA analyses based on anatomical character can be used to distinguish the four studied genera: *Canthium*, *Canthiumera*, *Meyna*, and *Psydrax*. *Canthium* and *Meyna* are characterized by a single layered palisade mesophyll, while *Canthiumera* and *Psydrax* are grouped by multilayered palisade mesophyll. *Canthium* and *Meyna* are separated by hypostomatic leaves, which are present in *Canthium*, and amphistomatic leaves, which are outstanding in *Meyna*. *Canthiumera* and *Psydrax* are divided by the presence of adaxial parenchymatous hypodermis, which is present in *Psydrax*, but absent in *Canthiumera*. These anatomical characters strongly support the separation of the genera and validate the narrow concept of *Canthium*. However, *Canthiumera* in Thailand comprises two species, but this research examines only one, while *Pyrostria*, which also includes two species in Thailand, is not included in this interpretation due to the inability to collect plant samples. Therefore, anatomical data of *Canthiumera* and *Pyrostria* are still essential to further clarify and support taxonomic studies of the *Canthium* group.

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