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Leaf Anatomy of *Vatica* L. (Dipterocarpaceae) in Thailand

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ABSTRACT.– The leaf anatomical characters of all species of the genus *Vatica* L. in Thailand were investigated by leaf epidermal clearing, transverse sectioning of the lamina and petiole, and examination by scanning electron microscopy (SEM). The generalized anatomical characteristics in all species are as follows: 1) one-layered epidermal cells; 2) hypostomatic leaves; 3) the presence of bundle sheath extensions to both epidermises or at least on the adaxial side; 4) the presence of stellate trichomes on the petioles; 5) the presence of resin canals; and 6) druse crystals in the mesophyll, midribs and petioles. Moreover, the present investigation indicates that anatomical characters which are significant for species delimitation are 1) cuticular ornamentation; 2) the level and types of stomata; 3) the distribution and types of trichomes; 4) the occurrence of a palisade cell layer in the laminas; 5) the number of resin canals in the petioles; 6) the presence/absence of sclereid cells in the midribs; 7) the presence/absence of fiber cells in the mesophyll and leaf margins; 8) the crystal types; and 9) the starch grains in ground tissue. In addition, the genus can be divided into two distinct groups based on the petiole anatomy by having the different numbers of resin canals, which supports the grouping based on the morphological data.

KEY WORDS: Leaf anatomy, *Vatica* L., Thailand.

INTRODUCTION

The genus *Vatica* L. (Dipterocarpaceae) contains around 71 species distributed from India, Sri Lanka to Myanmar, Indochina and Malaysia. In Thailand, nine species have been recorded that occur in evergreen dipterocarp forests and peat swamps in the lowlands, and in dry areas or in rugged limestone (Pooma and Newman, 2001; Pooma, 2002). These plants are distinguished from other genera of the Dipterocarpaceae by the absence of looped intramarginal nerves on the leaves, the winged fruits enclose less than half of the nut, anthers are glabrous and the style is stout (Ashton, 1982; Pooma and Newman, 2001). Systematic studies of the genus *Vatica* have been carried out by many authors, e.g. Smitinand et al. (1980), Ashton (1982) and Maury-Lechon and Curtet (1998). Many species of the families are doubtfully identified. Previously, phylo-

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TABLE **1.** Species and specimens examined anatomically.

Species	Locality	Habitat	Collector Number
V. bella	Sirindhorn Waterfall, Waeng District, Narathiwat	Evergreen forest	A. Srinual 152
V. diospyroides	Takua Pa District, Phangnga	Evergreen forest	A. Srinual 114
V. harmandiana	Mu Ko Surin, Phangnga	Dry areas on open rocky	A. Srinual 121
V. mangachapoi ssp. *obtusifolius*	Tham Suea, Krabi	On limestone ridges	A. Srinual 120
V. odorata	Tha Takiap District, Chachoengsao	Evergreen forest	A. Srinual 106
V. pauciflora	To Daeng, Narathiwat	Peat swamp forest	A. Srinual 132
V. philastreana	Dong Fa Huan, Ubon Ratchathani	Dipterocarp forest	A. Srinual 164
V. stapfiana	Waeng District, Narathiwat	Evergreen forest	A. Srinual 131
V. umbonata	Waeng District, Narathiwat	Hillside in evergreen forest	A. Srinual 153

genetic relationships within the dipterocarps have been addressed using morphological data, fossil records, pollen analysis and anatomy (Desch, 1941; Ashton, 1982). In particular, anatomical data has provided valuable taxonomic information at the subfamily, tribe, generic and species levels for different groups of plants (Stuessy, 1990), including the delimitation of species in the Dipterocarpaceae.

Solereder (1908) first highlighted the anatomical features of the leaves in this family in terms of paracytic stomata, the presence of large crystals and resin canals in the mesophyll. Metcalfe and Chalk (1957) then reported the importance of the stomatal types, the distribution and types of trichomes, the resin canals, and the crystals in the mesophyll as informative anatomical characteristics. Tewary and Sarkar (1985a) studied the leaf epidermis of Dipterocarpaceae in Indian forests and recognized two stomatal types in *Vatica*: cyclocytic and tetracytic stomata. Recently, Rojo (1987) pointed out that the petiole vascular patterns can be used as a taxonomic implication of the Philippine dipterocarps when associated with other anatomical features, such as, the number of resin canals and the presence/absence of sclerenchymatous tissue surrounding the vascular bundle. Although considerable work has been done on the family, Thai species are completely untouched hitherto. This is, to the best of our knowledge, the first attempt to use leaf anatomical characters for the identification of Thai *Vatica* species. The aims of this study are to compare the leaf anatomy of the genus *Vatica* in Thailand and to provide additional evidence for the delimitation of species occurring in Thailand and supporting the identification based on morphological data.

MATERIALS AND METHODS

The leaf anatomical characters of all species of the genus *Vatica* in Thailand were investigated by leaf epidermal clearing, transverse sectioning of the lamina and petiole, and examination by scanning electron microscopy (SEM). All specimens used in this study were collected from the field in Thailand (Table 1). The leaf specimens were taken from midway between

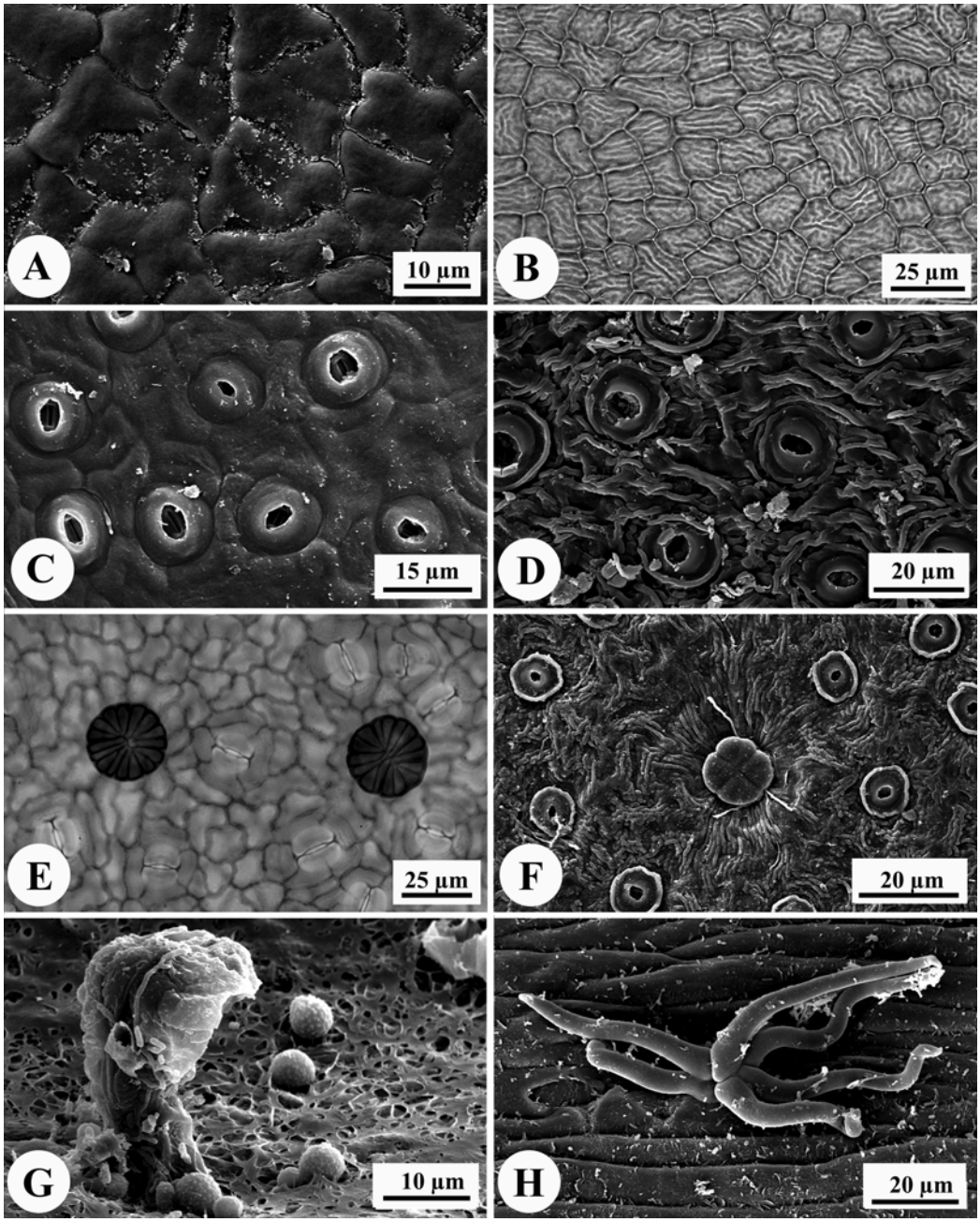

FIGURE 1. The leaf epidermis of Thai *Vatica*. **A.** Smooth ornamentation (*V. stapfiana*) and **B.** striate periclinal wall (*V. pauciflora*) on the adaxial surface. **C.** Cyclocytic stomata (*V. mangachapoi* ssp. *obtusifolius*). **D.** Randomly orientated striae on the abaxial surface and prominent peristomatal rims of the cuticle (*V. stapfiana*). **E.** Peltate trichomes (*V. harmandiana*). **F.** Peltate trichome; randomly orientated striae on abaxial surface and prominent peristomatal rims of cuticle (*V. pauciflora*). **G.** Stalked glandular (*V. diospyroides*) and **H.** stellate (*V. umbonata*) trichome.

the apex and the base of a mature lamina. For epidermal studies; the pieces of lamina were cleared in 5% (w/v) KOH and stained with 1% (w/v) Safranin (applied from Lersten and Curtis, 2001). Transverse sections of the leaf margins, midribs, the middle parts between the midrib and margin of lamina, and petioles were dehydrated, embedded in paraffin, sectioned on a sliding microtome with 8-14 μm thickness and stained with Safranin and Fast green (Thammathaworn, 1995). Permanent slides were mounted with DePeX artificial mounting medium. Photographs were taken under Normaski illumination using an Olympus BX51 microscope fitted with an Olympus DP11 digital camera.

For SEM studies; the lamina were dehydrated with alcohol series (70, 95 and 100% (v/v)), dried with CO_2 in a critical point dryer, then affixed on aluminum stub with a double-sided carbon tape, coated with gold particles and photographed through LEO 1450VP SEM. Ten to fifteen samples were used for each species, depending on the extent of its geographical distribution. All specimens examined and slide collections are kept at the KKU (Khon Kaen University) Herbarium.

RESULTS

General anatomical characters of Thai *Vatica*

1. Lamina

1.1 Leaf surface

The cuticular ornamentation, deposited on the outer wall of the epidermal cells of the adaxial and abaxial surfaces, vary among species. Most species have a smooth ornamentation on both surfaces, except *V. stapfiana* (King) Slooten and *V. umbonata* (Hook.f.) Burck which are smooth on the adaxial surface but with randomly orientated striae on the abaxial surface, and *V. pauciflora* (Korth.) Blume with randomly orientated striae on both surfaces (Fig. 1A, B F).

The epidermal cells on both surfaces are polygonal to irregular in shape with straight to slightly undulate anticlinal walls. However, *V. pauciflora* and *V. stapfiana* have striate periclinal walls. The epidermal cells on the midrib are smaller than those on the blade. Stomata are confined to only the abaxial surface. Most species have cyclocytic stomata, except *V. bella* Slooten, *V. harmandiana* Pierre and *V. mangachapoi* ssp. *obtusifolius* (Elmer) P.S.Ashton which present both cyclocytic and actinocytic stomata (Fig. 1C). The peristomatal rims of the cuticle are prominent in *V. pauciflora* and *V. stapfiana* (Fig. 1D, F). Trichomes are usually present on both surfaces, except in *V. harmandiana* and *V. mangachapoi* ssp. *obtusifolius* which have trichomes only on the abaxial surface. Five types of trichomes were recorded here: (i) peltate trichomes, which were present in all species studied (Fig. 1E, F), except *V. mangachapoi* ssp. *obtusifolius*; (ii) subsessile glandular trichomes, and found to occur in *V. bella, V. diospyroides* Symington, *V. harmandiana, V. odorata* (Griff.) Symington, *V. pauciflora* and *V. stapfiana* (Fig. 2A, B); (iii) stalked glandular trichomes, which occur only in *V. diospyroides* (Fig. 1G); (iv) unicellular trichomes, found only in *V. bella;* and (v) stellate trichomes, which are present in *V. bella, V. diospyroides, V. mangachapoi* ssp. *obtusifolius, V. pauciflora, V. philastreana* Pierre, *V. stapfiana* and *V. umbonata* (Fig. 1H).

TABLE 2. Anatomical characters of the lamina of Thai *Vatica*. (A = actinocytic stomata, BI = bifacial leaf, C = cyclocytic stomata, CO = collenchyma cell, CV = convex, D = druse crystal, F = fiber cell, P = peltate trichome, PA = parenchyma cell, PR = prism crystal, R = raise stomata, RA = randomly orientated striae, S = stellate trichome, SC = sclereid cell, SF = slightly flat, SG = stalked glandular trichome, SM = smooth, ST = starch grains, SU = subsessile glandular trichome, T = typical stomata, U = unicellular trichome, UN = unifacial leaf, - = absent)

Species	Epidermis						Mesophyll	
	Cuticle		Stomata		Trichome		Palisade layers	Types of leaf
	Adaxial	Abaxial	Type	Level	Adaxial	Abaxial		
V. bella	SM	SM	A, C	T	P, S, U	SU, P, S	1-3	UN
V. diospyroides	SM	SM	C	T	P, S, SG, SU	SU	1-2	UN
V. harmandiana	SM	SM	A, C	T	-	SU, P	1	UN
V. mangachapoi ssp. *obtusifolius*	SM	SM	A, C	T	-	S	1-2	UN
V. odorata	SM	SM	C	T	P	SU, P	1-2	UN
V. pauciflora	RA	RA	C	T	SU, P	SU, S	1	BI
V. philastreana	SM	SM	C	T	P	P, S	1-2	UN
V. stapfiana	SM	RA	C	R	SU, P, S	SU, S	1-2	UN
V. umbonata	SM	RA	C	T	P	P, S	1-2	UN

1.2 Lamina TS

The cuticular thickness is equally thick on both the adaxial and abaxial surfaces, except in *V. mangachapoi* where the outer wall of the adaxial surface is thicker than that of the abaxial surface (Fig. 2F). The epidermis is one-layered, with round to polygonal epidermal cells and with the adaxial cells being slightly larger than the abaxial cells. The epidermal cells of *V. bella* contain reddish-brown contents which stained with both Safranin and Fast green (Fig. 2C).

The stomata are markedly typical in most species while they are slightly raised in *V. stapfiana*. The mesophyll has a well differentiated palisade and spongy regions in all species. The majority of the mesophyll is composed of palisade cells with 1-3 cell layers on both surfaces (unifacial leaves), except in *V. pauciflora* which has a palisade cell layer on the adaxial side and spongy cells on the abaxial side (bifacial leaves) (Fig. 2D, E). In *V. diospyroides* and *V. harmandiana,* fiber cells are also present in the mesophyll. The vascular bundle is collateral. The bundle sheath is comprised of both parenchyma and sclerenchyma cells. The parenchyma cells are around the vascular bundle whereas sclerenchyma cells present as caps above and below the vascular bundle. The bundle sheath extensions to both epidermises, or at least on the adaxial side, are frequently present on the largest vein whilst absent on the small veins (Fig. 2F). Resin canals are usually present in the mesophyll between and embedded within the vascular bundles (Fig. 2G, H), whilst druse crystals are common in the mesophyll of all species (Fig. 2C).

1.3 Midrib TS

The outline of the midrib in transverse section at the adaxial surface is convex (Fig. 3A), except in *V. mangachapoi* ssp. *obtusifolius* and *V. umbonata* where it is slightly flat (Fig. 3B, C). The abaxial

Midrib							Cells of the margin
Outline		Ground tissue	Resin canals	Crystal types	Inclusions	Idioblast	
Adaxial	Abaxial						
CV	CV	PA, CO	5	D	-	-	F, PA
CV	CV	PA, SC	4	D	ST	SC	F, CO
CV	CV	PA	1	D	ST	-	CO
SF	CV	PA, SC, F	1	D	-	-	F, CO
CV	CV	PA, SC	1	D	ST	SC	F, PA
CV	CV	PA, CO	4	D, PR	ST	SC	F, CO
CV	CV	CO	2	D	ST	SC	F, PA, CO
CV	CV	PA	9	D	ST	SC	CO
SF	CV	PA, CO	1	D	ST	SC	CO

surface is convex in all species studied. Parenchyma predominates in ground tissue, and in some species (Table 2) collenchyma, fiber and sclereid cells are present. The vascular bundles exhibit a very complex structure with the distal end as a closed or very slightly open ring, surrounding a central medullary region. The vascular bundles are surrounded by sclerenchyma cells (Fig. 3D, E). Resin canals are associated with the outer part of the xylem on the midrib. The number of resin canals varies between species from 1 to 9 canals on the midrib (Fig. 3E). Druse crystals occur in the parenchyma cells above and below the vascular bundle of all species as well as in the phloem tissue. In *V. pauciflora,* druse and prism crystals are present in the vascular tissue. Starch grains present in the ground tissue of the midrib in all species (Fig. 3F), except *V. bella* and *V. mangachapoi* ssp. *obtusifolius*.

1.4 Leaf margin TS

The outline of the leaf margin varies from straight, slightly downwards or distinctly downwards (Fig. 3G, H). Collenchyma and fiber cells are observed in the leaf margins of some species (Table 2). Inclusions are found in *V. diospyroides* and *V. mangachapoi* ssp. *obtusifolius*. Only in *V. mangachapoi* ssp. *obtusifolius* was druse crystals observed on the leaf margin.

2. Petiole TS

The outline of the petioles in transverse section is mostly circular shaped, however; in some species it is concave on adaxial side *e.g. V. mangachapoi* ssp. *obtusifolius, V. pauciflora, V. philastreana* and *V. umbonata* (Fig. 4A, B). The epidermis in transverse section of all species studied is circular or semicircular, and cells are usually smaller than cells in the ground tissue. Trichomes: (i) Stellate trichomes were present in all species (Fig. 4C). (ii) Subsessile glandular trichomes were only found in *V. bella, V. diospyroides, V. pauciflora, V. philastreana* and *V. stapfiana,* and (iii) stalked glandular

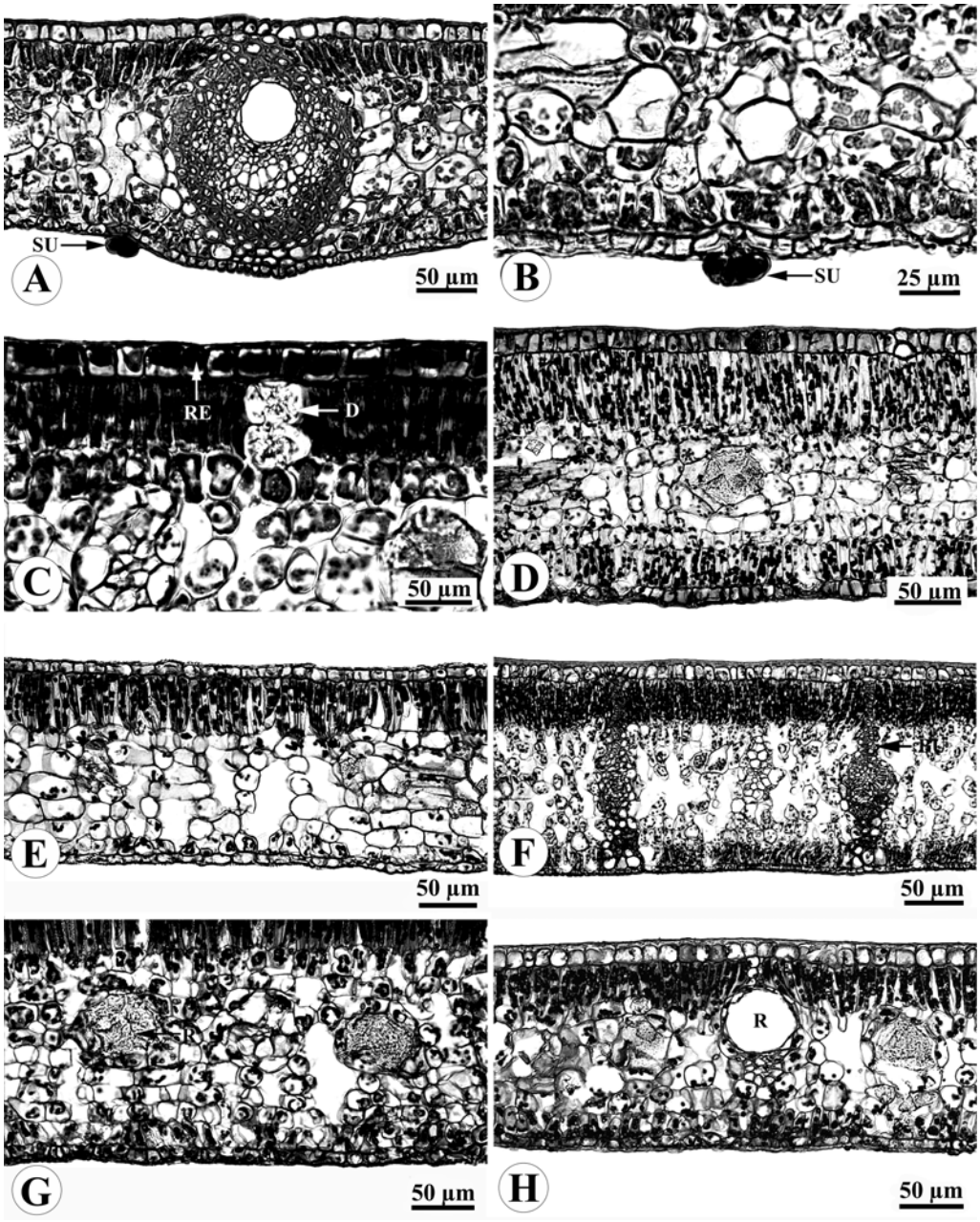

FIGURE 2. Transverse sections of lamina. **A and B.** Subsessile glandular trichomes (*V.diospyroides* (A) and *V. odorata* (B)). **C.** Reddish-brown content in the mesophyll (*V. bella*). **D.** Unifacial leaves (*V. philastreana)*. **E.** Bifacial leaves (*V. pauciflora*). **F.** Bundle sheath extension to both epidermises (*V. mangachapoi* ssp. *obtusifolius*). **G and H.** Resin canals in the mesophyll (*V. umbonata* (G) and *V. odorata* (H)). (BU = bundle sheath extension, D = druse crystal, R = resin canal, RE = reddish-brown content, SU = subsessile glandular trichome).

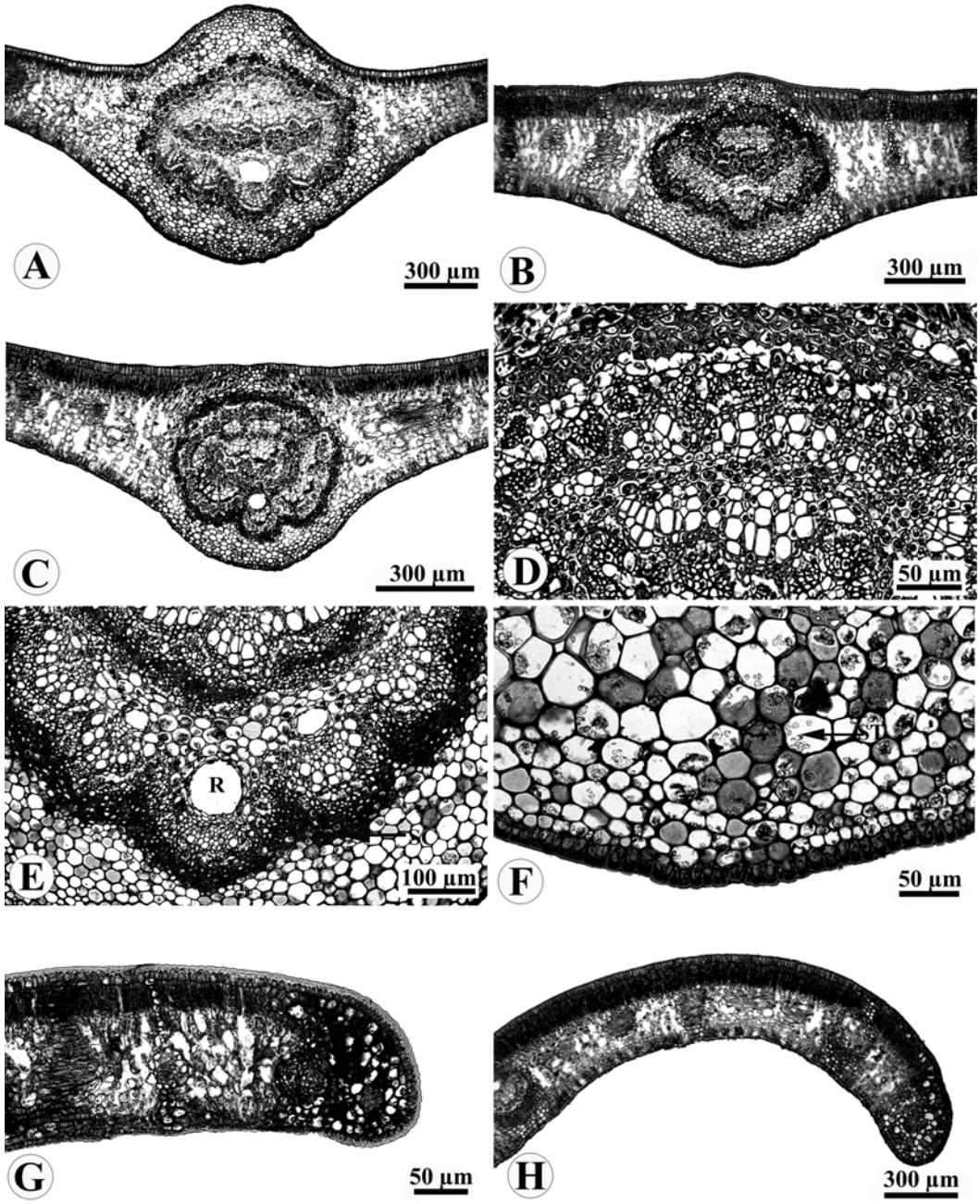

FIGURE 3. Transverse sections of midribs (A-F) and leaf margins (G and H). **A-C.** The outline of midribs (*V. harmandiana* (A), *V. mangachapoi* ssp. *obtusifolius* (B) and *V. umbonata* (C)). **D.** The vascular tissue (*V. umbonata*). **E.** The sclerenchyma cells surrounding the vascular bundles and resin canals in the midrib (*V. diospyroides*). **F.** Starch grains in the ground tissue on the midrib. **G and H.** Leaf margins (*V. mangachapoi* ssp. *obtusifolius* (G) and *V. stapfiana* (H)). (R = resin canal, SC = sclerenchyma cells, ST = starch grains).

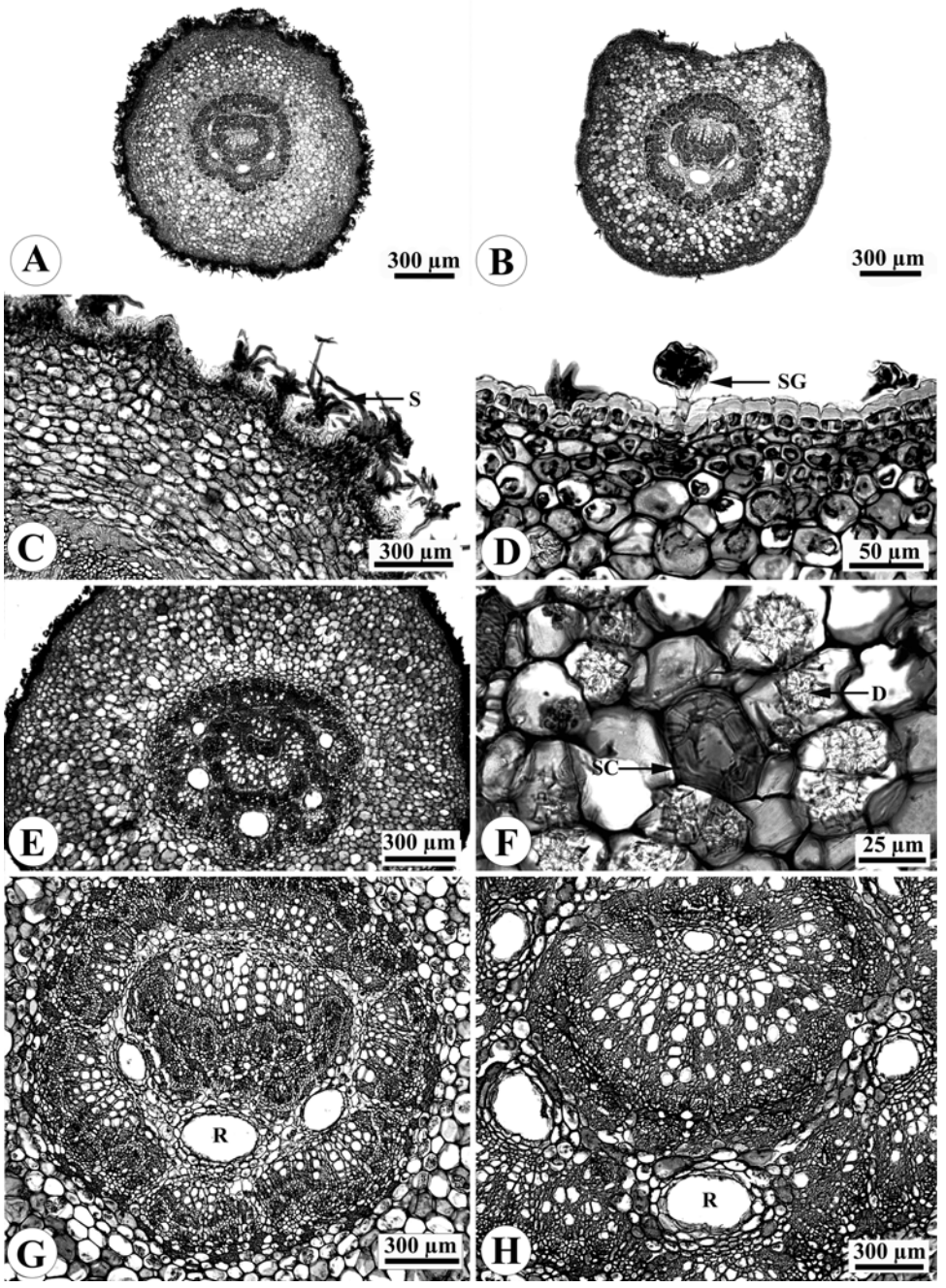

FIGURE 4. Transverse sections of petiole. **A.** Circular shaped outline (*V. bella*). **B.** Concave shaped outline on adaxial (*V. mangachapoi*). **C.** Stellate trichome (*V. odorata*). **D.** Stalked glandular trichome (*V. mangachapoi*). **E and F.** Sclereid cells (*V. umbonata* (E) and *V. philastreana* (F)). **G and H.** Vascular bundles associated with resin canals (*V. mangachapoi* (G) and *V. pauciflora* (H)). (D = druse crystal, R = resin canal, S = stellate trichomes, SC = sclereid cell, SG = stalked glandular trichome).

trichomes occurred only in *V. mangachapoi* ssp. *obtusifolius* (Fig. 4D). Ground tissue: The parenchyma cells were mainly present in the ground tissue, with sclereid cells in all species studied (Fig. 4E, F). The vascular bundles varied in shape and exhibit a very complex structure with the distal end as a closed or very slightly open ring, surrounding a central medullary region with the accessory bundles embedded in it (Fig. 4G, H). Resin canals occurred in all species and were associated with the xylem. The number of resin canals varied among species from 3 to 13 canals (Fig. 4G, H). Druse crystals were present in all species (Fig. 4F). In *V. pauciflora,* both druse and prism crystals were found. Starch grains were present in ground tissue of all species, except *V. harmandiana* (Table 3).

Key to Thai Species of *Vatica* Based on Anatomy of Lamina and Petiole.

1a. Trichomes present only on the abaxial surface of the lamina..... 2
1b. Trichomes present on both surfaces.. 3
2a. Stalked glandular trichomes present on the petiole..... *V. mangachapoi* ssp. *obtusifolius*
2b. Stalked glandular trichomes absent..... *V. harmandiana*
3a. Raised stomata position, the number of resin canals of the petiole more than 10 canals.....*V. stapfiana*
3b. Typical stomata position, the number of resin canals of the petiole up to 10 canals..... 4
4a. Leaves bifacial, druse and prism crystals present.....*V. pauciflora*
4b. Leaves unifacial, only druse crystals present..... 5
5a. Stalked glandular trichomes present on lamina..... *V. diospyroides*
5b. Stalked glandular trichomes absent.... 6
6a. Unicellular trichomes present on lamina, actinocytic stomata present on abaxial surface..... *V. bella*
6b. Unicellular trichomes and actinocytic stomata absent..... 7
7a. Subsessile glandular trichomes present on lamina..... *V. odorata*
7b. Subsessile glandular trichomes absent..... 8
8a. Subsessile glandular trichomes present on petiole, lamina cuticle smooth on both surfaces..... *V. philastreana*
8b. Subsessile glandular trichomes absent, lamina cuticle with randomly orientated striae on abaxial surface... *V. umbonata*

DISCUSSION AND CONCLUSIONS

Summaries of the anatomical characters of the lamina and petiole of Thai *Vatica* are shown in table 2 and 3, respectively. The present investigation reveals that leaf anatomical characters of the genus are typical of the Dipterocarpaceae that have been reported by previous studies *e.g.* Solereder, 1908, Metcalfe and Chalk, 1957.

The present study shows that in *Vatica,* some characters are useful for delimitation of species in the genus and these characters might be useful for species identification in other genera of the Dipterocarpaceae. The anatomical characters of the genus *Vatica* are constant for the genus whereas some characters are different and useful for distinguishing between species.

The generalized anatomical characteristics of all species of *Vatica* are as follows: 1) the one-layered epidermal cells; 2) the hypostomatic leaves; 3) the presence of

TABLE 3. Anatomical characters of the petiole of Thai *Vatica*. (CC = concave, CI = circular, D = druse crystals, SU = subsessile glandular trichome, PR = prism crystals, S = stellate trichome, SG = stalked glandular trichome, - = absent)

Species	Outline		Trichomes	Resin canals	Crystal types	Inclusions
	Adaxial	Abaxial				
V. bella	CI	CI	S, SU	5	D	Starch
V. diospyroides	CI	CI	S, SU	7	D	Starch
V. harmandiana	CI	CI	S	3	D	-
V. mangachapoi ssp. *obtusifolius*	CC	CI	S, SG	3	D	Starch
V. odorata	CI	CI	S	3	D	Starch
V. pauciflora	CC	CI	S, SU	6	D, PR	Starch
V. philastreana	CC	CI	S, SU	4	D	Starch
V. stapfiana	CI	CI	S, SU	13	D	Starch
V. umbonata	CC	CI	S	6	D	Starch

bundle sheath which extends to both epidermises or at least to the adaxial side; 4) the presence of stellate trichomes on the petioles; 5) the presence of resin canals; and 6) the presence of druse crystals in the mesophyll, midribs and petioles. On the other hand, there are other characters that may be considered invaluable for species delimitation; these are discussed in detail below.

The petiole anatomical features in Thai *Vatica* are not recommended as useful characters alone for species delimitation. These characters, however; may be of taxonomic utility when combined with the lamina features. Nevertheless, the number of resin canals in the petiole is helpful for division of the genus. According to Pooma (2002), nine species in two sections have been recorded in Thailand; six species in *section Vatica* (*V. bella*, *V. diospyroides*, *V. pauciflora*, *V. philastreana*, *V. stapfiana*, and *V. umbonata*), and three species in *section Sunaptea* (*V. harmandiana*, *V. mangachapoi* ssp. *obtusifolius* and *V. odorata*). The present study indicates that the genus can be divided into two distinct groups based on the petiole anatomy, by having the different numbers of resin canals associated with the vascular bundles. The first group is a group with three resin canals, *i.e. V. harmandiana*, *V. mangachapoi* ssp. *obtusifolius* and *V. odorata*. In contrast to the first group, the second group has more than three resin canals, such as *V. bella*, *V. diospyroides*, *V. pauciflora*, *V. philastreana*, *V. stapfiana*, and *V. umbonata*. The grouping based on the anatomical characters was supported by the grouping based on the morphological data.

Tewary and Sarkar (1985b) suggested that the cuticular ornamentation pattern was one of the important and informative characters which proved to be of diagnostic taxonomic value for Indian *Vatica*. In Thai *Vatica,* the cuticular ornamentation on both the adaxial and abaxial surfaces is smooth or randomly orientated striae, equally thick on both surfaces, or thicker on the adaxial surface than that of the abaxial one. In surface view, the epidermal cells on both surfaces are polygonal to irregular in shape with straight to slightly undulate anticlinal walls, and in some cases striate periclinal walls.

Stomata are restricted to the abaxial surface only, with cyclocytic and actinocytic stomata. This is in contrast to the works of Solereder (1908) and Metcalfe and Chalk (1957) which only recognized the paracytic stomata in Dipterocarpaceae, whilst Tewary and Sarkar (1985a) recorded two stomatal types in *Vatica*: cyclocytic and tetracytic stomata. Indeed, the stomatal position is markedly typical and slightly raised only in *V. stapfiana*. Some species have a prominent peristomatal rim of cuticle.

The trichomes are usually present on both surfaces except in some species where the leaves are glabrous. Five trichome types are observed in this genus: 1) peltate trichomes; 2) multicellular, subsessile glandular trichomes; 3) multicellular, stalked glandular trichomes; 4) unicellular, uniseriate trichomes; and 5) stellate trichomes. The latter type has never been recorded for the genus *Vatica* in the previous works by Solereder (1908), Metcalfe and Chalk (1957) and Tewary and Sarkar (1985a, b). Therefore; the stellate trichomes are reported here for the first time in the genus, but it waits to be resolved if this is unique to these Thai species isolates of *Vatica* or is more common but was previously overlooked.

The mesophyll has a well differentiated palisade and spongy regions; all species have unifacial leaves except *V. pauciflora* which has bifacial leaves. This species is found in a different habitat from the other species, as it grows in peat swamp forests. Therefore, it likely has palisade tissue only on the adaxial surface as an adaptation to its environment. In some species, fiber cells are found in the mesophyll. Metcalfe and Chalk (1957) noted that this character is diagnostic for a specific level. The vascular bundles in midrib and petiole exhibit a very complex structure with the distal end as a closed or very slightly open ring, surrounding a central medullary region. The bundle sheath extensions to both epidermises were present in all species studied. The resin canals are distinguished in all species and are present in the mesophyll between the vascular bundle in the lamina and are associated with the outer part of the xylem on the midribs and petioles. The number of resin canals varied amongst the different species. This is in agreement with the studies by Metcalfe and Chalk (1957) who reported that the variation in vascular structure, and the number and distribution of resin canals are apparently highly distinctive in different genera and species. In addition, druse crystals were commonly found in all species, whilst prism crystals were present only in some species. Starch grains were present in the ground tissue of the midrib and petiole of some species.

The results revealed a number of interesting features that may be useful for identification of some closely related species which are as follows:

Vatica harmandiana and *V. odorata*: these two species have very similar morphological characters and current identification between them is based upon the number of leaf pairs. Here the leaf anatomical characters, such as the cuticular ornamentation types, trichome types, and the number of resin canals on the petiole, also supported the differentiation between these two species and thus appear as additional informative characters.

Vatica pauciflora and *V. stapfiana*: *V. pauciflora* can be differentiated from *V. stapfiana* by having a typical stomatal position, the presence of stellate trichomes

on the adaxial surface, the bifacial leaves and the presence of both druse and prism crystals in the midrib and petiole. Whereas, *V. stapfiana* has a raised stomatal position, unifacial leaves and only druse crystals present in the midrib and petiole.

In conclusion, the leaf anatomical data of Thai *Vatica* has apparently valuable (informative) characters for solving the species delimitation problems and is useful for the identification of morphologically closely related species because these characters appear to be consistent within species and distinguishable between species, although of course further evaluation on geographically disparate accessions is required to confirm this trend seen here in Thai accessions. Nevertheless, the present investigation indicates that the anatomical characters which are apparently significant for species delimitation are 1) cuticular ornamentation; 2) the level of and types of stomata; 3) the distribution and types of trichomes; 4) the occurrence of the palisade cell layer in the laminas; 5) the number of resin canals in the petioles; 6) the presence/absence of sclereid cells in the midrib; 7) the presence/absence of fiber cells in the mesophyll and leaf margins; 8) the crystal types; and 9) the starch grains in ground tissue. Furthermore, the number of resin canals in the petiole is helpful in separation of the genus into two distinct groups based upon having three or more than resin canals, respectively. The grouping based on the anatomical characters is congruent with the group based on morphological characters.

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LITERATURE CITED

Ashton, P. S. 1982. Flora Malesiana. Series I-Spermatophyta. Martinum Nijhoff Pub, The Netherlands,

Desch, H. E. 1941. Manual of Malayan timbers. Malayan Forest Records 15: vol. I, vol. II.

Lersten, M. R. and Curtis, J. D. 2001. Idioblast and other unusual internal foliar secretory structures in Scrophulariaceae. Plant Systematics and Evolution, 227: 63-73.

Maury-Lechon, G. and Curtet, L. 1998. Biogeography and evolutionary systematics of Dipterocarpaceae. In: Appanah S. and Turnbull J. M. (eds.). A review of Dipterocarps: Taxonomy, Ecology and Silviculture. Centre for International Forestry Research (CIFOR), Indonesia, pp. 5-44.

Metcalfe, C. R. and Chalk, L. 1957. Anatomy of the Dicotyledons. Vol. I. Oxford University Press, London. 220 pp.

Pooma, R. 2002. Futher notes on Thai Dipterocarpaceae. Thai Forest Bulletin (Botany) 30: 7-27.

Pooma, R. and Newman, M. 2001. Checklist of Dipterocarpaceae in Thailand. Thai Forest Bulletin (Botany) 29: 110-187.

Rojo, J. P. 1987. Petiole anatomy and infrageneric interspecific relationship of Philippine *Shorea* (Dipterocarpaceae). In: Kostermans A. J. G. H. (ed.). Proceedings of the Third Round Table Conference on Dipterocarps. Papers presented at an International Conference Held at the Mulawarman University, Indonesia. pp. 573-598.

Smitinand, T., Santisuk, T. and Phengklai, C. 1980. The manual of Dipterocarpaceae of Mainland

South-East Asia. The Forest Herbarium, Royal Forest Department, Bangkok. 133 pp.

Solereder, H. 1908. Systematic anatomy of the Dicotyledons. The Clarendon Press, Oxford.

Stuessy, T. F. 1990. Plant taxonomy: the systematic evaluation of comparative data. Columbia University Press, U.S.A. pp. 233-250.

Tewary, P. K. and Sarkar, A. K. 1985a. Leaf epidermal studies in Dipterocarpaceae-The genera *Vatica* L. and *Vateria* L. Journal of Plant Anatomy and Morphology, 2: 67-72.

Tewary, P. K. and Sarkar, A. K. 1985b. Taxonomical, ecological and foliar epidermal studies in Indian Dipterocarpaceae. In: Kostermans A.J.G.H. (ed.). Proceedings of the Third Round Table Conference on Dipterocarpaceae, Regional Office for Science and Technology for Southeast Asia, Indonesia. pp. 537-567.

Thammathaworn, A. 1995. Handbook for microtechnique in Biology. Department of Biology, Faculty of Science, Khon Kaen University, Thailand. pp. 1-50. (in Thai).

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