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Petiole Vascular Bundles and its Taxonomic Value in the Tribe Dipterocarpeae (Dipterocarpaceae)

(Berkas Vaskular Petiol dan Nilai Taksonominya dalam Tribus Dipterocarpeae (Dipterocarpaceae))

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ABSTRACT

*A comprehensive survey and descriptions on the petiole anatomy was undertaken on 16 species belonging to four selected genera (*Anisoptera, Cotylelobium, Vatica *and* Upuna*) in the tribe Dipterocarpeae to investigate variations in vascular bundle structure. Methods used in this study were petiole sectioning using a sliding microtome, staining, mounting and observation under light microscope. Findings had shown that all species studied have complex vascular bundle structures, consisting of outer and medullary vascular bundles. Four different types of vascular bundle arrangements were found and described as Types 1, 2, 3 and 4. The vascular bundle types were determined based on the arrangement and system of vascular bundle strands present in the petiole transverse sections. These vascular bundle types were determined and suggested for easy reference. The results showed that vascular bundles can be used for identification of certain species and can definitely be used for classification of different genera. The presence of sclerenchyma cells is useful in differentiating genera. As a conclusion, the petiole vascular bundle anatomical characteristics have taxonomic value, especially in the identification of some species and possibly applicable in the classification of the tribe Dipterocarpeae.*

Keywords: Anisoptera; Cotylelobium; *Dipterocarpaceae; Dipterocarpeae; petiole anatomy; vascular bundles;* Vatica

ABSTRAK

*Kajian dan huraian komprehensif telah dijalankan ke atas anatomi petiol 16 spesies daripada empat genus terpilih (*Anisoptera, Cotylelobium, Vatica *dan* Upuna*) dalam tribus Dipterocarpeae untuk mengkaji variasi dalam struktur berkas vaskular. Kaedah yang digunakan dalam kajian ini ialah kaedah hirisan petiol menggunakan mikrotom gelongsor, pewarnaan, pelekapan dan pencerapan di bawah mikroskop cahaya. Hasil kajian menunjukkan semua spesies mempunyai berkas vaskular kompleks yang terdiri daripada berkas vaskular luar dan medulari. Empat jenis susunan berkas vaskular telah ditemui dan dihuraikan sebagai Jenis 1, 2, 3 dan 4. Jenis berkas vaskular ditentukan berdasarkan susunan dan sistem berkas vaskular dalam keratan rentas petiol. Jenis berkas vaskular ini ditentukan dan dicadangkan untuk memudahkan rujukan. Hasil kajian menunjukkan berkas vaskular boleh digunakan untuk pengecaman spesies dan terbukti boleh digunakan untuk pembezaan genus. Kehadiran kelompok sel sklerenkima juga berguna untuk membezakan genus. Kesimpulannya ciri anatomi berkas vaskular mempunyai nilai taksonomi terutamanya untuk pengecaman spesies dan berkemungkinan boleh diaplikasikan dalam pengelasan tribus Dipterocarpeae.*

Kata kunci: Anatomy petiol; Anisoptera; *berkas vaskular;* Cotylelobium; *Dipterocarpaceae; Dipterocarpeae ;* Vatica

INTRODUCTION

Dipterocarpaceae is an important timber family with 17 genera and approximately 510 to 680 species worldwide (Ashton 1982; Maury-Lechon & Curtet 1998; Symington 2004). Their distribution is pantropical from northern South America to Africa, Seychelles, Sri Lanka, the Philippines, India, China, Thailand, Indonesia and Malaysia, with the greatest diversity and abundance in western Malaysia (Cronquist 1981; Ashton 1998; Gamage et al. 2006). There are 14 genera with approximately 160 species in Peninsular Malaysia and 13 genera and 269 species in Borneo (Symington 2004).

The Dipterocarpaceae is subdivided into three subfamilies, namely Dipterocarpoideae, Monotoideae and Pakaraimodeae. Monotoideae is represented in Africa, Madagascar and South America by three genera. Ashton (1979) classified the Asian Dipterocarpaceae into two tribes, namely Dipterocarpeae and Shoreae. The two tribes are recognizable by a few characteristics. Tribe Dipterocarpeae has valvate fruit calyx lobes, scattered resin canals and a basal chromosome number of $\times$=11. Members of this tribe are *Vateria, Vateriopsis, Stemonoporus, Vatica*, *Cotylelobium, Upuna, Anisoptera* and *Dipterocarpus*. The other tribe is Shoreae, which has imbricate fruit calyx lobes, resin canals in tangential bands and a basal chromosome number of $\times$=7. The members of this tribe are *Dryobalanops, Parashorea, Hopea*, *Neobalanocarpus* and *Shorea*.

Dipterocarpaceae species are unique in their flowering characteristics. Flowering in this family is generally considered to be irregular (Burgess 1972), with a few species having specific flowering patterns (Sasaki et al.

1979; Sakai 2002). This general way of flowering is a phenomenon unique to lowland dipterocarp forests (Sakai 2002). During this mass flowering season, nearly all dipterocarp species, together with species of other families, come heavily into flower (Sakai 2002). Therefore, because of this phenomenon, one of the problems in identification of species is the difficulty in obtaining specimens in flower. Difficulties in identification of sterile specimens without either flowers or fruits may result in wrong identification of species.

Candolle (1879) made the first comprehensive survey of petiole anatomy and he described several fundamental concepts of vascular bundles. He distinguished between 'opened system' and 'closed system' in the vascular bundles. There are very few studies on the petiole anatomy of Dipterocarpaceae. Rojo (1987), who conducted a study on the petiole anatomy of some Philippines *Shorea* species, stated that the petiole vascular patterns can be used in taxonomic distinction of certain taxa. A study on some Malaysian Dipterocarpaceae species by Munawirah et al. (1991) showed that certain characters are useful in identification of species. Those characters are present in the petiole transverse sections such as the pattern of vascular tissues, the presence or absence of sclerenchyma cells around the vascular bundles, the presence of medullary vascular bundles and the types of trichomes. A few studies showed evidence for grouping genera by petiole anatomy and the petiole anatomy is useful for identification of species (Dehgan 1982; Kamel & Loutfy 2001; Kocsis & Borhidi 2003; Agbagwa & Ndukwa 2004; Noraini & Cutler 2009). A previous study on 17 *Dipterocarpus* species undertaken by Ruzi et al. (2009) had shown that petiole anatomy can be used to form groups in the genus *Dipterocarpus*. Therefore, the main objective of this study was to investigate the taxonomic value of petiole vascular bundle anatomical characteristics in the tribe Dipterocarpeae.

MATERIALS AND METHODS

This study was conducted on 16 species belonging to three genera, namely *Vatica* (10 species), *Anisoptera* (4 species),

TABLE 1. List of species studied

Species and specimen code	Locality	Date of collection, Collectors
Anisoptera costata Korth. AHH7	Arboretum FRIM, Kepong Selangor	20.01.2010 Abu Husin Harun (FRIM)
Anisoptera curtisii Dyer *ex* King AHH4	Bukit Lagong, Selangor	05.05.2003 Abu Husin Harun (FRIM)
Anisoptera laevis Ridley AHH6	Arboretum FRIM, Kepong Selangor	20.01.2010 Abu Husin Harun (FRIM)
Anisoptera scaphula (Roxb.) Kurz AHH5	Arboretum FRIM, Kepong Selangor	20.01.2010 Abu Husin Harun (FRIM)
Vatica badifolia P.S. Ashton AHH17	Arboretum FRIM, Kepong Selangor	20.01.2010 Abu Husin Harun (FRIM)
Vatica bella Slooten AHH11	Arboretum FRIM Kepong, Selangor	20.01.2010 Abu Husin Harun (FRIM)
Vatica cinerea King AHH16	Arboretum FRIM, Kepong Selangor	20.01.2010 Abu Husin Harun (FRIM)
Vatica cuspidata (Ridl.) Symington AHH2	Kledang Sayong, Selangor	06.08.2003 Abu Husin Harun (FRIM)
Vatica hullettii (Ridl.) P.S. Ashton AHH10	Gunung Berlumut, Kluang, Johor	15.06.2009 Abu Husin Harun (FRIM)
Vatica lowii King AHH1	Bukit Lagong, Selangor	05.05.2003 Abu Husin Harun (FRIM)
Vatica maingayi Dyer AHH3	Hutan Simpan Pasoh, Negeri Sembilan	10.08.2003 Abu Husin Harun (FRIM)
Vatica nitens King AHH14	Arboretum FRIM Kepong, Selangor	20.01.2010 Abu Husin Harun (FRIM)
Vatica odorata (Griff.) Symington AHH12	Arboretum FRIM Kepong, Selangor	20.01.2010 Abu Husin Harun (FRIM)
Vatica pauciflora (Korth.) Blume AHH13	Arboretum FRIM Kepong, Selangor	20.01.2010 Abu Husin Harun (FRIM)
Vatica umbonata (Hook. *f*.) Burck AHH15	Arboretum FRIM, Kepong Selangor	20.01.2010 Abu Husin Harun (FRIM)
Cotylelobium lanceolatum Craib AHH8	Arboretum FRIM, Kepong Selangor	20.01.2010 Abu Husin Harun (FRIM)
Upuna borneensis Symington AHH9	Arboretum FRIM Kepong, Selangor	20.1.2010 Abu Husin Harun (FRIM)

Cotylelobium (1 species) and *Upuna* (1 species) (Table 1). Fresh specimens were collected from the Bukit Lagong Forest Reserve in Selangor and the FRIM Arboretum in Kepong, Selangor. Voucher specimens were deposited at the Universiti Kebangsaan Malaysia Herbarium (UKMB). Specimens were fixed in a 3:1 AA solution (70% alcohol: 30% acetic acid), sectioned using a sliding microtome through the middle part of the petiole and stained in Safranin and Alcian Green. Following dehydration in a series of ethanol solutions, the sections were mounted in Euparal. Anatomical images of the sections were taken using video camera DP 25 Olympus attached to a Olympus microscope and images were processed using Cell^B Software. Fixation and embedding followed the method by Johansen (1940) and Sass (1958) with suitable modifications.

RESULTS

Figure 1 shows the illustrations of petioles with vascular bundles and the classification of genus based on types of vascular bundle arrangements in the petioles. The four types of vascular bundle arrangements found in this study are listed in Table 2. Vascular bundles: Type 1, present in *Vatica badifolia*, *V. bella, V. cinerea, V. cuspidata, V. hullettii,*

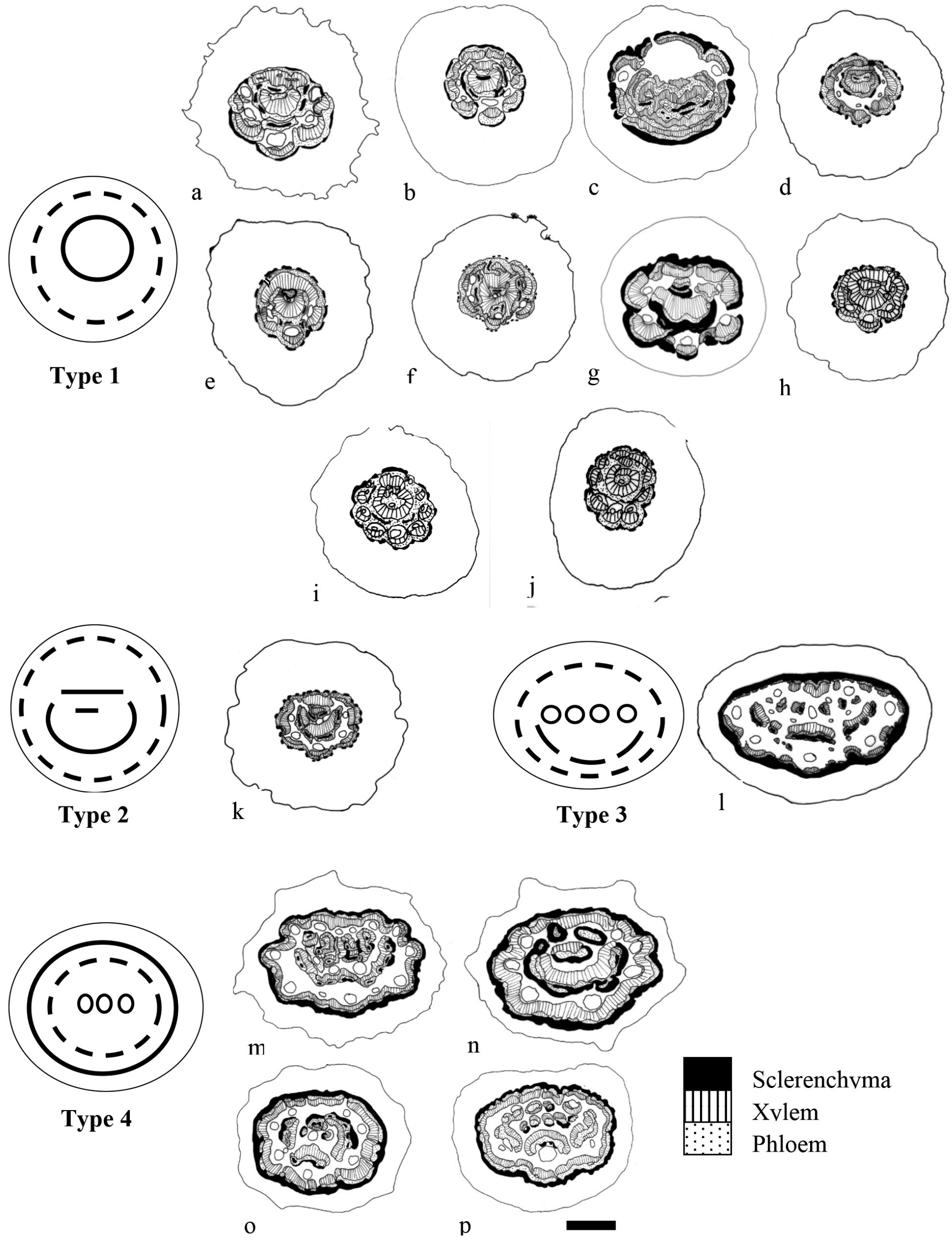


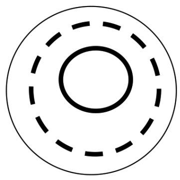
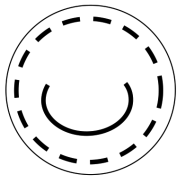
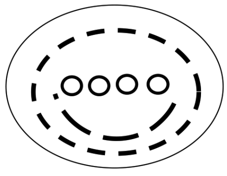
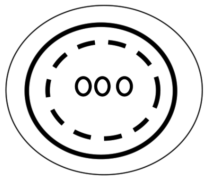
FIGURE 1. Illustrations of petiole tranverse sections. Type 1. a) *V. badifolia*, b) *V. bella*, c) *V. cinerea*, d) *V. cuspidata*, e) *V. lowii*, f) *V. maingayi*, g) *V. nitens*, h) *V. odonata*, i) *V. pauciflora* and j) *V. umbonata*; Type 2. k) *Cotylelobium lanceolatum*; Type 3. l) *Upuna borneensis*; Type 4. m) *A. curtisii*, n) *A. scaphula*, o) *A. laevis*, p) *A. costata* Scale bar: 200 μm

V. lowii, V. maingayi, V. nitens, V. odorata, V. pauciflora and *V. umbonata*. Type 2, present in *Cotylelobium lanceolatum*. Type 3, present in *Upuna borneensis*. Type 4, present in *Anisoptera costata, A. curtisii, A. laevis* and *A. scaphula*. Sclerenchyma cells: Sparsely scattered fibres around peripheral vascular bundles were present in *Cotylelobium lanceolatum*. Continuous layers of sclerenchyma cells around peripheral vascular bundles were found in *Upuna borneensis, Anisoptera costata, A. curtisii, A. laevis* and *A. scaphula*. Discontinuous layers of sclerenchyma cells around peripheral vascular bundles were present in *Vatica badifolia*, *V. bella, V. cinerea, V. cuspidata, V. hullettii, V. lowii, V. maingayi, V. nitens, V. odorata, V. pauciflora* and *V. umbonata*. Phloem sclerenchyma was present between phloem in central vascular bundles in all the species studied.

Discussion

Solereder (1908) stated that the cortical vascular bundles in the branch, the structural details of the vascular system in the petiole and the position as well as the number of the resin canals may be useful for the diagnosis of the genera in the Dipterocarpaceae. Hare (1942) described the various arrangements of vascular bundles in the petiole and stated that the petiole anatomical characters are diagnostic and possibly of value for the purpose of classification. Howard (1974, 1962) considered the vascular structure of the petiole to be of value as a taxonomic character and most useful at the generic level and in a few cases, at the family level. He also suggested that the systematic level of the taxonomic value may vary from one group to another. In some cases, families can be recognised, in other cases genera, species or varieties can be distinguished on the basis of petiole vascular pattern. Many previous studies on the petiole anatomy have proved that this character has systematic significance in many plant families such as in genus *Anaxagorea* in Annonaceae (Scharaschkin & Doyle 1997). Stephan and Haron (2002) studied the midrib and petiole vascular patterns of Icacinaceae and their findings can be used in dividing Icacinaceae into four groups. This is a good example of the usefulness of vascular bundle patterns for species classification and identification.

TABLE 2. Four types of vascular bundle arrangement found in this study

Types	Descriptions	Illustrations
1	• Consists of outer and medullary vascular bundles • Outer vascular bundles closed system, an interrupted O-shaped ring of several vascular bundles • Medullary vascular bundles closed system, a circular vascular bundle	
2	• Consists of outer and medullary vascular bundles • Outer vascular bundles closed system, an interrupted O-shaped ring of several vascular bundles • Medullary vascular bundles opened system, U-shaped from several vascular bundles on abaxial side of petiole	
3	• Consists of outer and medullary vascular bundles • Outer vascular bundles closed system, an interrupted O-elliptical shaped ring of several vascular bundles • Medullary vascular bundles closed system, consists of several vascular bundles with U-shaped interrupted vascular bundles on abaxial side and several separated vascular bundles on adaxial side	
4	• Consists of outer and medullary vascular bundles • Outer vascular bundles closed system, continuos O-shaped ring of several vascular bundles • Medullary vascular bundles closed system, consists of an interrupted O-shaped ring of several vascular bundles and also stack/s of vascular bundle/s	

Metcalfe and Chalk (1979) pointed out that the petiolar vascular structure in Dipterocarpaceae is always complex and exhibits a range of structure that might prove to be of considerable diagnostic value but needs further investigation. He reported that although three separate vascular bundles enter the base of the petiole, transverse sections through the distal end exhibit a closed or very slightly open ring of vascular bundles surrounding a central medullary region with accessory bundles embedded in it. He also reported that the vascular system of the petiole always exhibits a very complex structure in transverse sections through the distal end, including a number of resin canals accompanying the vascular strands. He suggested that a thorough reinvestigation of the petiolar structure of the Dipterocarpaceae based on accurately named materials should provide data of the highest diagnostic value.

Rojo (1987) stated that the petiole vascular patterns can be used in taxonomic distinction of certain taxa. The degree of usefulness varies from generic to specific level of taxa. The vascular patterns in Philippines *Shorea* can only be used in delimiting subgeneric taxa when associated with other anatomical features of the petiole. In *Dipterocarpus*, Rojo (1987) differentiated six species to three groups based on the secretory canal associated with the first xylem ring. Pardi et al. (1991) in their studies reported that all of the *Dipterocarpus* species have a complex vascular structure and closed ring of vascular bundles. A previous study by Noraini and Cutler (2009) stated that the variability in vascular structure and the combination of petiole outline with a type of vascular bundle arrangement could be very useful in the classification of genus *Parashorea* of Dipterocarpaceae.

The results have shown that all the species and genera studied in the tribe Dipterocarpeae have a complex vascular structure with a closed or an opened ring system and consist of outer and medullary bundles that supported the findings by Metcalfe and Chalk (1979), Rojo (1987), Ruzi et al. (2009) and Noraini and Cutler (2009). Ashton (1982) also reported that the complex petiolar vascular supply, characteristic of many genera in the family, reaches its greatest elaboration in *Dipterocarpus*, *Vateria* and the type sections of *Shorea* and *Hopea*. The supply is reduced to three peripheral bundles in many species of *Hopea* section *Dryobalanoides* and is relatively simple in the other sections of *Shorea,* but also in the putatively primitive *Vatica*. He reported that the large-leaved *Dipterocarpus* and *Shorea* (including species of section *Pachycarpae*) have very much more complex systems than small-leaved ones and genera with slender petioles such as *Dryobalanops* and *Anisoptera* have simpler systems than *Dipterocarpus,* though more complex than most *Hopea* and *Shorea* species. The complex vascular structure present in all the species studied consists of outer and medullary vascular bundles with closed or opened system.

The results showed that Type 1 was present in all the *Vatica* species studied (*V. badifolia, V. bella, V, cinerea, V. cuspidata, V. lowii, V. maingayi, V. nitens, V. odorata, V. pauciflora* and *V. umonata*) while Type 2 was present in only one species, namely *Cotylelobium lanceolatum*. Type 3 was observed in *Upuna borneensis,* while Type 4 was present in all the *Anisoptera* species studied, namely *A. curtisii, A. scaphula, A. laevis* and *A. costata*. Based on the vascular bundles arrangement in the petiole, it is clearly shown that this character can be really useful in genus differentiation, especially for the four studied genera. It is also clearly shown that types of vascular bundle systems can be used for species grouping in the tribe Dipterocarpeae. These findings are similar to those of Ruzi et al. (2009), who have stated that the types of vascular bundle based on the arrangement and system in the vascular bundle strands can be used for grouping of species in *Dipterocarpus* and also help in species identification.

As Metcalfe (1942) stated, the anatomy of both leaf and stem gives numerous characteristics, which in combination are reliable for diagnostic purposes, some species can be identified with certainty based on the vascular bundles arrangement. The findings in this study support the statement by Heim (1892) and Maury (1978), who reported that in Dipterocarpaceae petiolar anatomy and particularly the arrangement of the vascular bundles as seen in transverse section at the distal end is a guide to classification and species determination. For example *Upuna borneensis* and *Cotylelobium lanceolatum* can be identified with certainty based on the petiole vascular bundles arrangement.

Other outstanding characteristic in the petiole vascular bundles is the presence of sclerenchyma cells. The presence of sclerenchyma cells or fibres sparsely scattered around peripheral vascular bundles in *Cotylelobium lanceolatum* is a good characteristic to differentiate this species from the others. For the other species, continuous layers of sclerenchyma cells were present around the peripheral vascular bundles in *Upuna borneensis, Anisoptera costata, A. curtisii, A. laevis* and *A. scaphula*. Discontinuous layers of sclerenchyma cells around peripheral vascular bundles were present in all the *Vatica* species studied (*V. badifolia*, *V. bella, V. cinerea, V. cuspidata, V. hullettii, V. lowii, V. maingayi, V. nitens, V. odorata, V. pauciflora* and *V. umbonata*). A previous study by Noraini (2006) also reported that the presence of fibres or sclerenchyma cells accompanying peripheral or outer vascular bundles in species and genera belonging to the Shoreae has taxonomic value.

Conclusion

In conclusion, this study indicates that by using different types of vascular tissue arrangement, four different genera can be distinguished and authentication of some species can be done. Therefore, the vascular bundles arrangement can be used in addition to other characters for genus identification and classification in the tribe Dipterocarpeae. This study confirms that petiole vascular bundles arrangement of the Malaysian Dipterocarpeae has taxonomic value.

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