Studies on Leaf Venation in Selected Taxa of the Genus Ficus L. (Moraceae) in Peninsular Malaysia

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INTRODUCTION

Ficus (figs) belongs to the Moraceae family and is one of the largest angiosperm genera, with approximately 1000 species worldwide (Berg 1990) distributed throughout tropical and subtropical areas of Asia, Australia, Africa and America.
Frodin (2004) has ranked this genus as one of the 21 largest genera of seed plants. The Malaysian forest is extremely rich in *Ficus* species, with approximately 16% (101) of known species (Ng 1978). With regard to growth habit, Harrison (2005) considers this genus as one of the most diverse. *Ficus* includes a large number of indoor ornamental plants and garden and roadside trees, such as *F. religiosa*, *F. elastica* and *F. microcarpa*. The genus is easy to identify by the highly characteristic fruits, or syconia, and also by the milky juice, the prominent stipules that leave a scar after abscission and the minute unisexual flowers often arranged on variously shaped receptacles (Hutchinson & Dalziel 1958). However, some species do not bear syconia, and their morphological characteristics are similar, contributing to difficulty in species identification, especially in the field.

Anatomical studies of leaf venation have shown that ornamentation of the veins and the course of traces in the lamina are useful additional characters for the identification of species of *Euphorbia* (Sehgal & Paliwal 2008). Dede (1962) also described the foliar venation patterns in Rutaceae, proving them to be useful for the identification of various species. Hickey (1973) stated that leaf venation is correlated with plant evolution and has systematic significance in plant identification and classification. Indeed, leaf venation plays a very important role in the identification of incomplete plants, e.g., sterile specimens, archaeological remains and fragmentary fossils of non-reproductive organs. The objective of this study was to determine the patterns of variation in leaf venation that may have taxonomic value for group identification of this taxon.

**MATERIALS AND METHODS**

A total of 21 taxa of the genus *Ficus* were used in this study. Details of the species studied are presented in Table 1. The fresh specimens used in this study were obtained from many locations in Malaysia, including several forest reserves in Selangor, Terengganu and Perak. Dried specimens were obtained from the Herbarium of Universiti Kebangsaan Malaysia (UKM). The fresh leaf specimens collected were fixed in AA (70% ethanol:30% acetic acid in ratio of 1:3); the dried herbarium samples were boiled. A 1 x 1 cm sample of the leaf lamina and margin area were cleared using Basic Fuchsin solution (10% Basic Fuchsin and 10% KOH, Bendosen Laboratory, Selangor, Malaysia) in an oven at 60°C for 1 to 2 days, depending on the thickness of the leaf specimen. The cleared leaf specimens were then dehydrated in an alcohol series, cleared in xylene and mounted on slides using Canada Balsam (R&M Chemical, Essex, UK); the samples were then placed in an oven at 60°C for nearly 2 weeks. The slides were photographed using a digital camera (Olympus BX43F, Tokyo) mounted on an Olympus microscope (Olympus Soft Imaging Solutions GmbH, Münster, Germany). The leaf venation patterns were observed using Cell B Software (Olympus Soft Imaging Solutions GmbH, Münster, Germany) under 10x, 20x, 40x and 100x magnifications. Details of the analysis and descriptions of the leaf venation types followed the classification of Hickey (1973).
Table 1: List of *Ficus* specimens and taxa studied.

<table>
<thead>
<tr>
<th>Species</th>
<th>Collector, collection number</th>
<th>Locality</th>
<th>Date of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. Zainuddin, AZ 04390</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. Zainuddin, AZ 6794</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>F. aurantiacea</em> Griff. var. <em>aurantiacea</em></td>
<td>B. Ummu-Hani, UHB 05</td>
<td>Recreational Forest of Sungai Salu, Perak Batu Berangkai, Kampar, Perak Km 87, road to Dungun from Kuantan</td>
<td>10.11.2011</td>
</tr>
<tr>
<td></td>
<td>B. Ummu-Hani, UHB 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. Zainuddin, AZ 4665</td>
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<td></td>
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<tr>
<td><em>F. aurata</em> Miq.</td>
<td>B. Ummu-Hani, UHB 35</td>
<td>Bukit Bauk, Terengganu</td>
<td>06.03.2012</td>
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<tr>
<td></td>
<td>B. Ummu-Hani, UHB 64</td>
<td>Bukit Chini, Pahang</td>
<td>31.10.2012</td>
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<tr>
<td><em>F. benjamina</em> L.</td>
<td>A. Hussin, AH 01</td>
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<tr>
<td></td>
<td>B. Ummu-Hani, UHB 02</td>
<td>Section 15, Bangi, Selangor</td>
<td>20.10.2011</td>
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<tr>
<td></td>
<td>A. Hussin, AH 07</td>
<td></td>
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<tr>
<td><em>F. deltoidea</em> Jack var. <em>kunstleri</em> (King) Corner</td>
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<td>A. Latiff, ALM 1899</td>
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<td><em>F. depressa</em> Blume</td>
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<td>M. Kassim, MK 315</td>
<td>Batu 20, Kuala Selangor Selangor</td>
<td>05.10.1972</td>
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<tr>
<td></td>
<td>B. Ummu-Hani, UHB 71</td>
<td>Recreational Forest, Hulu Bendul, Negeri Sembilan</td>
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<td></td>
<td>A. Zainuddin, AZ 5466</td>
<td>Pulau Tioman, Rompin</td>
<td>28.04.1995</td>
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<td>Species</td>
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<td>Locality</td>
<td>Date of collection</td>
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<tr>
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<tr>
<td>F. heteropleura Blume</td>
<td>A. Hussin, AH 26</td>
<td>Road of Balau, FRIM, Kepong, Selangor</td>
<td>04.04.2012</td>
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<td>A. Zainuddin, AZ 4709</td>
<td>Bukit Wang, Jitra, Kedah</td>
<td>23.10.1993</td>
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<td></td>
<td>A. Zainuddin, AZ 4709</td>
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<td>04.04.2012</td>
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<td>Chini, Pahang</td>
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<td>A. Zainuddin, AZ 4709</td>
<td>Bukit Wang, Jitra, Kedah</td>
<td>23.10.1993</td>
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F. hispida L.f.

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<th>Date of collection</th>
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F. microcarpa L.f.

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F. religiosa L.

<table>
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<td>04.04.2012</td>
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<td>B. Ummu-Hani, UHB 38</td>
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F. sagittata Vahl

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F. schwarzii Koord.

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F. superba (Miq.) Miq.,

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<td>A. Latiff, ALM 2658</td>
<td>Pulau Aur, Mersing, Johor</td>
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F. tinctoria G. Forst

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F. ucinata (King) Becc.

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F. vasculosa Wall. ex Miq.

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<td>A. Zainuddin, AZ 5480</td>
<td>Pulau Tioman, Pahang</td>
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<td>A. Zainuddin, AZ 3886</td>
<td>Bukit Bauk, Terengganu</td>
<td>20.10.1991</td>
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RESULTS

The leaf venation patterns are described below with illustrations. The comparison and identification of the studied taxa based on their leaf venation patterns are shown in Tables 2 and 3.

Table 2: Comparison of leaf venation patterns in selected taxa of the genus Ficus.

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of ultimate marginal venation</th>
<th>Type of areolar venation</th>
<th>Types of veinlets</th>
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<tbody>
<tr>
<td></td>
<td>Incomplete</td>
<td>Complete</td>
<td>Incomplete</td>
</tr>
<tr>
<td>F. annulata</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>F. aurantiacea</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>var. aurantiacea</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F. aurata</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. benghalensis</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. benjamina</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. deltoidea</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>var. angustifolia</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>F. deltoidea</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>var. kunstleri</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. depressa</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. elastica</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. fulva</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>F. heteropleura</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>F. hispida</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>F. lepicarpa</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>F. microcarpa</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. religiosa</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. sagittata</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>F. schwarzi</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>F. superba</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. tinctoria</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>F. ucinata</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>F. vasculososa</td>
<td>+</td>
<td>–</td>
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Notes: + present; – absent
Table 3: Identification of taxa studied based on leaf venation.

<table>
<thead>
<tr>
<th>Patterns of leaf venation</th>
<th>Description</th>
<th>Species</th>
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<tbody>
<tr>
<td>1</td>
<td>Complete ultimate marginal venation</td>
<td>F. sagittata</td>
</tr>
<tr>
<td></td>
<td>Closed areolar venation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No veinlets</td>
<td></td>
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<tr>
<td>2</td>
<td>Incomplete ultimate marginal venation</td>
<td>F. aurantiacea var. aurantiacea</td>
</tr>
<tr>
<td></td>
<td>Closed areolar venation</td>
<td>F. fulva</td>
</tr>
<tr>
<td></td>
<td>Simple veinlets</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Incomplete ultimate marginal venation</td>
<td>F. schwarzii</td>
</tr>
<tr>
<td></td>
<td>Incomplete areolar venation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uni-veinlets</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Complete ultimate marginal venation</td>
<td>F. superba</td>
</tr>
<tr>
<td></td>
<td>Closed areolar venation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uni-veinlets</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Incomplete ultimate marginal venation</td>
<td>F. lepicarpa</td>
</tr>
<tr>
<td></td>
<td>Closed areolar venation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uni-veinlets</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Complete ultimate marginal venation</td>
<td>F. aurata</td>
</tr>
<tr>
<td></td>
<td>Incomplete areolar venation</td>
<td>F. heteropleura</td>
</tr>
<tr>
<td></td>
<td>Bi-veinlets</td>
<td></td>
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<tr>
<td>7</td>
<td>Incomplete ultimate marginal venation</td>
<td>F. deltoidea var. angustifolia</td>
</tr>
<tr>
<td></td>
<td>Incomplete areolar venation</td>
<td>F. hispida</td>
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<tr>
<td></td>
<td>Tri-veinlets</td>
<td>F. tinctoria</td>
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<td></td>
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<td>F. ucinata</td>
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<tr>
<td></td>
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<td>F. vasculosa</td>
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<td>Complete ultimate marginal venation</td>
<td>F. benghalensis</td>
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<tr>
<td></td>
<td>Incomplete areolar venation</td>
<td>F. benjamina</td>
</tr>
<tr>
<td></td>
<td>Tri-veinlets</td>
<td>F. deltoidea var. kunstleri</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F. depressa</td>
</tr>
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<td></td>
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<td>F. elastica</td>
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<td>F. microcarpa</td>
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<tr>
<td></td>
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<td>F. religiosa</td>
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</table>

Ultimate marginal venation: Two types of ultimate marginal venation were observed. Type 1: Incomplete venation was present in F. annulata, F. aurantiacea var. aurantiacea, F. deltoidea var. angustifolia, F. fulva, F. hispida, F. lepicarpa, F. schwarzii, F. tinctoria, F. ucinata and F. vasculosa [Fig. 1(a–j)]. Type 2: complete venation was observed in F. aurata, F. benghalensis, F. benjamina, F. deltoidea var. kunstleri, F. depressa, F. elastica, F. heteropleura, F. microcarpa, F. religiosa, F. sagittata and F. superba, [Fig. 1(k) and (l), Fig. 2(a–i)].
Figure 1: Ultimate marginal venation. In-complete venation; a) *F. annulata*; b) *F. aurantiacea*; c) *F. deltoidea* var. *angustifolia*; d) *F. fulva*; e) *F. hispida*; f) *F. lepicarpa*; g) *F. schwarzi*; h) *F. tinctoria*; i) *F. ucinata*; j) *F. vasculosa*. Complete venation; k) *F. aurata*; l) *F. benghalensis*. 
Figure 2: Ultimate marginal venation. Complete venation; a) F. benjamina; b) F. deltoidea var. kunstleri; c) F. depressa; d) F. elastic; e) F. heteropleura; f) F. microcarpa; g) F. religiosa; h) F. sagittata; i) F. superba.

Areolar venation: Two types of areolar venation were present. For Type 1, the majority of species showed incomplete-ending veinlets, including F. annulata, F. aurata, F. benghalensis, F. benjamina, F. deltoidea var. angustifolia, F. deltoidea var. kunstleri, F. depressa, F. elastica, F. heteropleura, F. hispida, F. microcarpa, F. religiosa, F. schwarzii, F. superba, F. tinctoria, F. ucinata and F. vasculosa [Fig. 3(a–i), Fig. 4(a–h)]. For Type 2, the majority presented closed venation, such as in F. aurantiacea var. aurantiacea, F. fulva, F. lepicarpa and F. sagittata [Fig. 4(i–l)].
Veinlets: Five types of veinlet ending were observed. Type 1: no veinlets were observed in *F. sagittata* [Fig. 5(a)]. Type 2: Simple veinlets consisting of linear to curved endings, as observed in *F. aurantiacea* var. *aurantiacea* and *F. fulva* [Fig. 5(b) and (c)]. Type 3 comprises uni-veinlets, as in *F. lepicarpa, F. schwarzii* and *F. superba* [Fig. 5(d)]. Type 4 presents bi-veinlets or dichotomous, which was present in *F. aurata* and *F. heteropleura* [Fig. 5(e)]. Type 5, tri-veinlets, was present in *F. annulata, F. benghalensis, F. benjamina, F. deltoidea var. angustifolia, F. deltoidea var. kunstleri F. depressa, F. elastica, F. hispida, F. microcarpa, F. religiosa, F. tinctoria, F. ucinata and F. vasculosa* [Fig. 5(f)].
Figure 4: Areolar venation. Incomplete venation; a) F. benjamina; b) F. microcarpa; c) F. religiosa; d) F. schwarzi; e) F. tinctoria; f) F. ucinata; g) F. superba; h) F. vasculosa. Closed venation; i) F. aurantiaceae; j) F. fulva; k) F. lepicarpa; l) F. sagittata.
Figure 5: Areolar venation. Veinlet endings; a) nil or no veinlet; b and c) simple veinlets, linear to curve endings; d) Uni-veinlets; e) bi-veinlets; f) tri-veinlets.

Swollen-ending veinlets: Swollen veinlet ending was present in some Ficus species, such as F. annulata, F. benghalensis, F. benjamina, F. depressa, F. elastica, F. heteropleura, F. microcarpa, F. sagittata and F. superba [Fig. 6(c)]. Cystolith cell: The majority of the species contain a cystolith cell, except for F. aurata and F. fulva [Fig. 6(a) and (b)]. Trichomes: The trichomes are simple and unicellular in some taxa, such as F. aurantiacea var. aurantiacea, F. aurata, F. benghalensis, F. fulva, F. hispida, F. lepicarpa, F. sagittata and F. superba [Fig. 6(d)].

DISCUSSION

Plant leaves are determinate structures responsible for primary productivity and arise as swellings on the flanks of the shoot apex in accordance with a specific phyllotactic pattern (Fosket 1994). All the main functions of the leaf (light harvesting, gas exchange, water transport and distribution of photosynthate) depend upon the architecture, which is defined as the position and form of all the elements that constitute the outward-expressed structure of the organ (Hickey 1988). One such architectural element is the arrangement of the veins of the lamina, which is referred to as the venation pattern (for a recent review, see Nelson & Dengler 1997). Although there are numerous studies on the leaf vasculature of higher plants, very little is known about venation pattern formation. In fact, there is a rich diversity of venation patterns in both monocotyledonous (Inamdar et al. 1983) and dicotyledonous plants (Hickey 1973).
Leaf venation can be classified into some characters or patterns that may have taxonomic value for the identification and classification of species, including veinlets, ultimate marginal venation, areolar venation and areolation shape (Hickey 1973; Sehgal & Paliwal 2008). In this study, two types of ultimate marginal venation were observed. Type 1 is incomplete ultimate marginal venation, consisting of freely ending veinlets directly adjacent to the margin (Fig. 1). Type 2 is complete ultimate marginal venation, which refers to higher vein orders fused into a vein running just inside the margin (Figs. 1 and 2).

Areolar venation is the smallest area of the leaf tissue surrounded by veins, which taken together forms a contiguous field over most of the area of the leaf. Findings have shown that there are two types of areolar venation: Type 1, for which the majority of the leaf venation consists of incomplete ending veinlets (Figs. 3 and 4); and Type 2, largely showing closed venation (Fig. 4).

Veinlets are the freely ending ultimate veins of the leaf and veins of the same order that occasionally cross the aeroles to become connected distally. Hickey (1973) stated that the main characteristics of veinlets are divided into three types, namely, no veinlets, simple without branches and branches giving rise to ramifications by dichotomising, i.e., uni-veinlets, bi-veinlets and tri-veinlets. Based on Hickey’s (1973) classification, all 21 taxa of Ficus studied can be classified into 8 leaf venation patterns (see Tables 2 and 3). Pattern 1 consists of species with no veinlets, closed areolar venation and complete ultimate marginal venation (F. sagittata). Pattern 2 has simple veinlets that are either linear or curved veinlet and closed areolar and incomplete ultimate marginal venation.
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(F. aurantiacea var. aurantiacea and F. fulva). Patterns 3, 4 and 5 consist of uni-veinlets that are different in areolar and ultimate marginal venation and incomplete in both areolar and ultimate marginal venation, as in F. schwarzii (Pattern 3), and incomplete areolar and complete ultimate marginal venation, as in F. superba (Pattern 4); Pattern 5 is with closed areolar and incomplete ultimate marginal venation, as in F. lepicarpa. Pattern 6 is branched-ending veinlets in two or bi-veinlets and incomplete areolar and complete ultimate marginal venation, as present in F. aurata and F. heteropleura. Patterns 7 and 8 comprise tri-veinlets and incomplete areolar venation, but both are different in ultimate marginal venation, which is incomplete in F. annulata, F. deltoidea var. angustifolia, F. hispida, F. tinctoria, F. ucinata and F. vasculosa (Pattern 7) while complete in F. benghalensis, F. benjamina, F. deltoidea var. kunstleri, F. depressa, F. elastica, F. microcarpa and F. religiosa (Pattern 8) (Tables 2 and 3).

Dilcher (1974) stated that a study on the nature and structure of leaf venation has significant implications for the relationship between taxonomy and phylogeny. Levin (1929) also explained that leaf venation patterns have high taxonomic value and suggested that a species has a constant number of veins that can be used for species identification. Studies on leaf texture and secondary venation have taxonomic value and can be used for the identification of some species in the genera Corchorus and Grewia (Sharma 1991). Therefore, the variations in the patterns of leaf venation represented in this study is not only significant for group identification but can also be used directly to identify some Ficus species, such as F. lepicarpa, F. sagittata, F. schwarzii, and F. superba. The presence of swollen veinlets or swollen tracheids, cystolith cells and trichomes can also be used as an additional tool for the differentiation of species among groups [Figs. 6(a) and (b)]. For example, swollen veinlets are only present in F. heteropleura (Pattern 6) and F. annulata (Pattern 7). Pattern 8 swollen veinlets are present in F. benghalensis, F. depressa, F. elastica and F. microcarpa.

The cystolith (consisting of calcium carbonate) is located in lithocysts; they occur in the form of papillate or hair-like lithocysts, mostly in the epidermis of leaves (Mauseth 1988). Metcalfe and Chalk (1950) noted that "true cystoliths" are known to occur in some genera of Moraceae, such as Broussonetia, Chlorophora, Conocephalus, Ficus and Morus. Therefore, the presence of cystoliths in this study is a common characteristic of the genus Ficus, except for F. aurata and F. fulva only. Klimko and Truchan (2006) stated that various trichomes, such as straight and long and short and peltate can be found on leaves in Ficus taxa. In this study, simple and unicellular trichomes were observed in the leaf venation of some species, such as in F. aurantiacea var. aurantiacea, F. aurata, F. benghalensis, F. fulva, F. hispida, F. lepicarpa, F. sagittata and F. superba.
CONCLUSION

The presence of variable patterns of leaf venation is significant because it can be used as an additional piece of data, especially in the group identification of species and also to directly differentiate some Ficus species, such as F. lepicarpa, F. sagittata, F. schwarzii and F. superba. However, other species may require more anatomical characters for differentiation.

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