

## Vegetative Anatomy of Subtribe Ischaeminae (Poaceae) in Thailand

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**ABSTRACT.**— The structure of the epidermis, and the anatomy of leaves and culms, as seen in transverse sections, were studied in 10 species representing five genera of the subtribe Ischaeminae in Thailand. A number of characters, such as the leaf blade outline, ribs and furrows on both surfaces, bulliform cells, midribs and keels, number of vascular bundles in the keels, shape of sclerenchyma at blade margins, intercostal long cells, intercostal short cells, papillae, prickles, macro-hairs, silica bodies, costal short cells, culm outline and central cavity in ground tissue of culm, are regarded as particularly distinct characters among the studied species and genera. It seems likely that vegetative anatomical characters are taxonomically useful in classification of the genera (at the generic level) of these grass groups.

**KEY WORDS:** Culm, grass, Graminae, leaf blade, light microscopy, taxonomy

### INTRODUCTION

The present paper is part of an ongoing study of the anatomy of the Poaceae, and attempts to determine whether five genera of the subtribe Ischaeminae can be distinguished by their leaf and culm anatomy.

The anatomy of this tribe was investigated by Renvoize (1981), which indicated that anatomical information can be very useful in delimiting the tribe. However, some of the genera failed to conform completely to the general pattern; namely the nodular silica bodies in the genus *Apluda*, stomata being present in more than 10 rows across the intercostal zone of the lower epidermis and more than seven rows across the intercostal zone of the upper epidermis in the genus *Thelepogon*.

The limited studies on the vegetative anatomy of Ischaeminae are distributed across the tribe in such a way that most

species found in Thailand have not been included. Metcalfe (1960) described the anatomy of the leaves and culms of *Apluda mutica* var. *aristata* (L.) Pilger.; *Ischaemum australe* R.Br.; *I. brachyatherum* Frnzl ex Hack.; *I. rugosum* Salisb. and *I. triticeum* R. Br.; *I. laxum* R.Br. (now known as *Sehima nervosum* (Rottl. ex Willd) Stapf, plus the culm only in *I. commutatum* Hack. and *I. santapau* Bor.

Watson and Dallwitz (1992) also published a detailed description of the leaf structure in the genera *Sehima* and *Thelepogon*, and described on the basis of these observations the diagnostic generic characters. In most studies the anatomical characters have proved to be an important tool in grass identification and classification.

Accordingly our revision of the subtribe Ischaeminae in Thailand is based upon anatomical investigations, and our goal is to

**TABLE 1.** List of species examined, their collection localities and herbarium reference voucher identity.

Genus	Species	Localities	Collectors
<i>Apluda</i> L.	<i>A. mutica</i> L.	Sattahip, Chon Buri Khun Tan, Lampang	P. Traiperm 152 P. Traiperm 321
<i>Ischaemum</i> L.	<i>I. hirtum</i> Hack.	Nam Nao, Phetchabun	P. Traiperm 171
	<i>I. hubbardii</i> Bor	Doi Inthanon, Chiang Mai	P. Traiperm 308
	<i>I. muticum</i> L.	Muang, Songkhla Tarutao, Satun	P. Traiperm 138 P. Traiperm 199
	<i>I. rugosum</i> Salisb.	Muang, Khon Kaen Rayong	P. Traiperm 127 P. Traiperm 293
	<i>I. tenuifolium</i> A.Camus	Ubon Ratchathani Makham, Chanthaburi	P. Traiperm 233 P. Traiperm 287
	<i>I. thomasi</i> Traiperm and Boonkerd (ined.)	Khao Soi Dao, Chanthaburi	P. Traiperm 327
<i>Kerriochloa</i> C.E. Hubbard	<i>K. siamensis</i> C.E. Hubbard	Ubon Ratchathani	P. Traiperm 235
<i>Sehima</i> Forssk.	<i>S. nervosum</i> (Rottler) Stapf	Nam Nao, Phetchabun	P. Traiperm 170
		Khun Tan, Lampang	P. Traiperm 323
<i>Thelepogon</i> Roem. and Schult.	<i>T. elegans</i> Roem. and Schult.	Muang, Saraburi	P. Traiperm 276

support the systematics of this subtribe. The results of this long-term project will be embodied in a published Flora of Thailand.

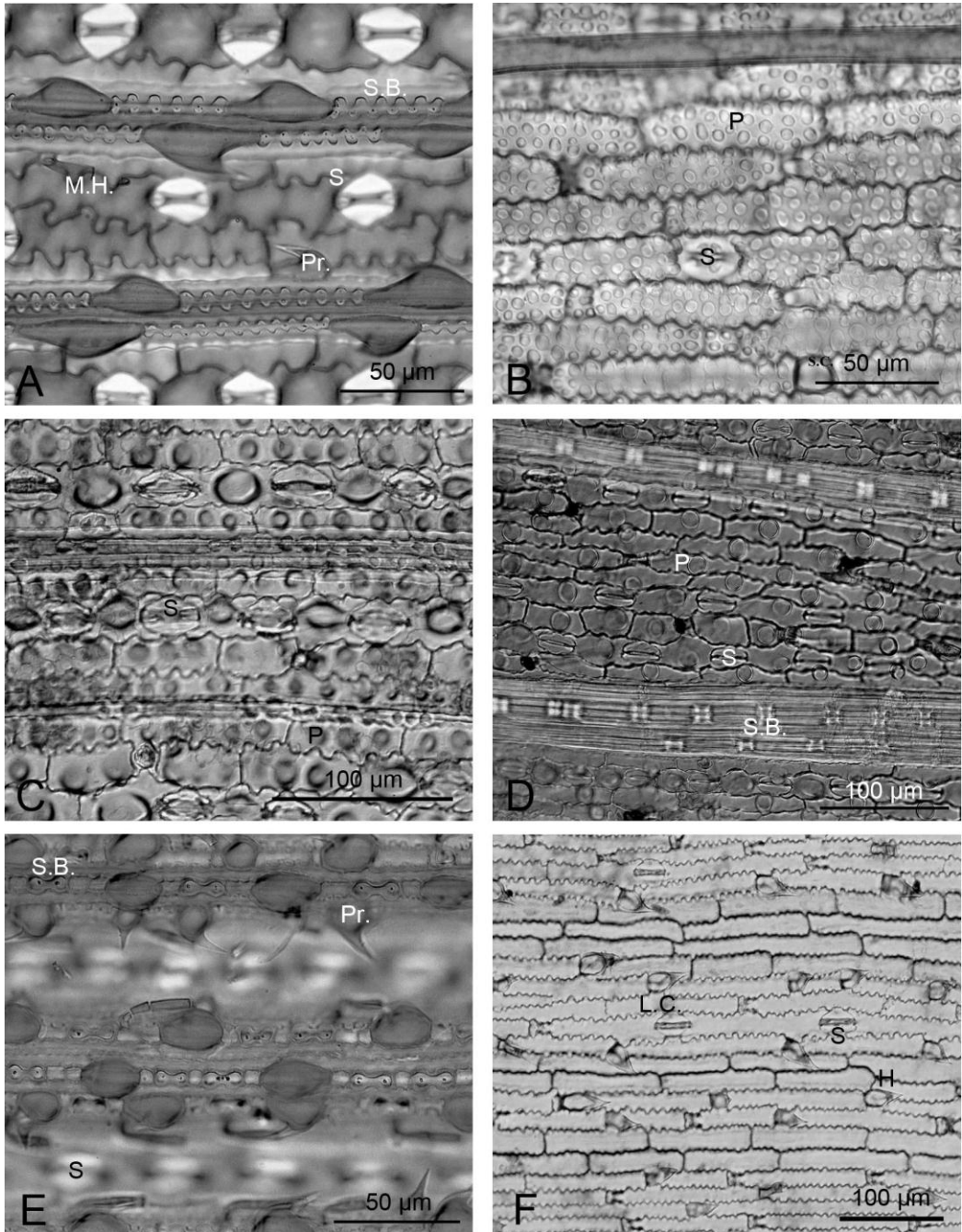
## MATERIALS AND METHODS

**Types of samples.**— Vegetative anatomy of 10 species of the subtribe Ischaeminae were examined in the present study, with the specimens listed in Table 1.

**Preparation of samples for anatomical observation.**— Living materials were collected in the field throughout Thailand. Herbarium voucher specimens were prepared for identification purposes. The permanent slides were prepared using the paraffin methods. The specimens were fixed in 70% (v/v) FAA (90 parts 70% (v/v) EtOH; 5 parts glacial acetic acid; 5 parts 40% (v/v) formaldehyde) or in 70% (v/v) ethanol. Leaves from the mid-culm were used, excluding the lowermost leaf and the leaf directly subtending the inflorescence. Transverse sections of culms and the midribs

at median level, as well as transverse sections of the leaf-blades in the intercostal region and in the margin were obtained. The samples were dehydrated in an increasing ethanol concentration series (up to 100% (v/v)), embedded in paraffin, sectioned with a microtome at 8 - 16  $\mu$ m thickness, stained in safranin and fast green, cleared with xylene and mounted in DePeX. Epidermal preparations were made by scraping pieces of softened leaves with a safety razor blade, dehydrated in an increasing ethanol series (as above), stained in safranin, cleared with xylene and mounted in DePeX. The anatomy characters were observed and recorded photographically with an Olympus BX 51 microscope and Olympus DP11 camera, respectively.

The leaf anatomy of each group was further evaluated by examination of all the anatomical characters of the leaf blade in transverse section, and the epidermis, using the details in accord with Ellis (1976, 1979) and Metcalfe (1960).



**FIGURE 1.** Abaxial epidermal structure as seen in surface view. (A) *Apluda mutica*: M.H.: micro-hairs; Pr.: prickles; S: stomata; S.B.: silica bodies, (B) *Ischaemum hirtum*: P: papillae; S.C.: short cell, (C) *Ischaemum hubbardii*, (D) *Kerriochloa siamensis*, (E) *Sehima nervosum* and (F) *Thelepogon elegans*.

## SYSTEMATICS

### Genus *Apluda* L.

#### *Apluda mutica* L. (Figures 1A, 2A and 3A)

#### Leaf surface (Fig. 1A)

ABAXIAL EPIDERMIS.— *Intercostal long cells*: elongated cells, length 3x or more than wide; cell rectangular, square or trapezoidal; moderately undulating. *Stomata*: subsidiary cells dome-shaped, one row of stomata in each intercostal zone. *Intercostal short cells*: absent. *Papillae*: absent. *Prickle hairs*: common in costal zones, overlying any vascular bundle-order, large prickles, one to three rows along whole length between all vascular bundle. *Micro-hairs*: bicellular, two-celled, basal and distal cells approximately equal in length, apex of distal cell sharply pointed, base or attachment of basal cell: parallel-side, point of attachment small. *Macro-hairs*: absent. *Silica bodies*: nodular. *Costal short cells*: absent.

ADAXIAL EPIDERMIS.— Basically similar to the abaxial epidermis. *Stomata* present, very rare, in regular form, usually found at the leaf margins.

#### Leaf in transverse section (Fig. 2A)

*Outline*: blade expanded, slightly undulating or nearly straight; no ribs or furrows present on either surface. *Epidermis*: bulliform cells present in simple fans; the central cell is not much larger than bundle sheath parenchyma. *Midrib outline*: present, keel conspicuous and developed, triangular-shaped; one vascular bundle comprising the keel. *Vascular bundle arrangement in the lamina*: one median bundle, 10 or more third-order bundles

between one second-order bundle; all bundles situated in the center of the blade. *Primary-order vascular bundles*: circular or round to slightly square-shaped in outline. *Intercostal sclerenchyma*: crescent-shaped cap; sclerenchyma extends shortly along both abaxial and adaxial side of the leaf margins.

#### Culm in transverse section (Fig. 3A)

*Outline*: circular, culm examined 1.2-1.5 mm in diameter. *Epidermis* subtended by about 9-12 layers of cells with thickened, lignified walls, with small non-circular assimilatory tissue. *Inner ground tissue* without a central cavity. *Vascular bundles* of outermost circle are embedded in the layers of thickened cells and are smaller than the remainder.

### Genus *Ischaemum* L.

#### *Ischaemum hirtum* Hack. (Figures 1B, 2B and 3B)

#### Leaf surface (Fig. 1B)

ABAXIAL EPIDERMIS.— *Intercostal long cells*: elongated cells, length 3x or more longer than wide; cell rectangular, square or trapezoidal, sometimes shape varies: angled outwards, cells hexagonal; moderately undulating. *Stomata*: subsidiary cells dome- or low-dome-shaped, one to five rows of stomata in each intercostal zone. *Intercostal short cells*: solitary short cells, tall and narrow or rectangular in shape, smooth outline. *Papillae*: circular or rounded, more than one papillus per cell. *Prickle hairs*: absent. *Hooks*: present but very rare on the intercostals zone. *Micro-hairs*: bicellular, two-celled, basal cell only slightly shorter than the distal cell; apex of distal cell slightly tapered, tapering to a round apex, base or attachment of basal cell:

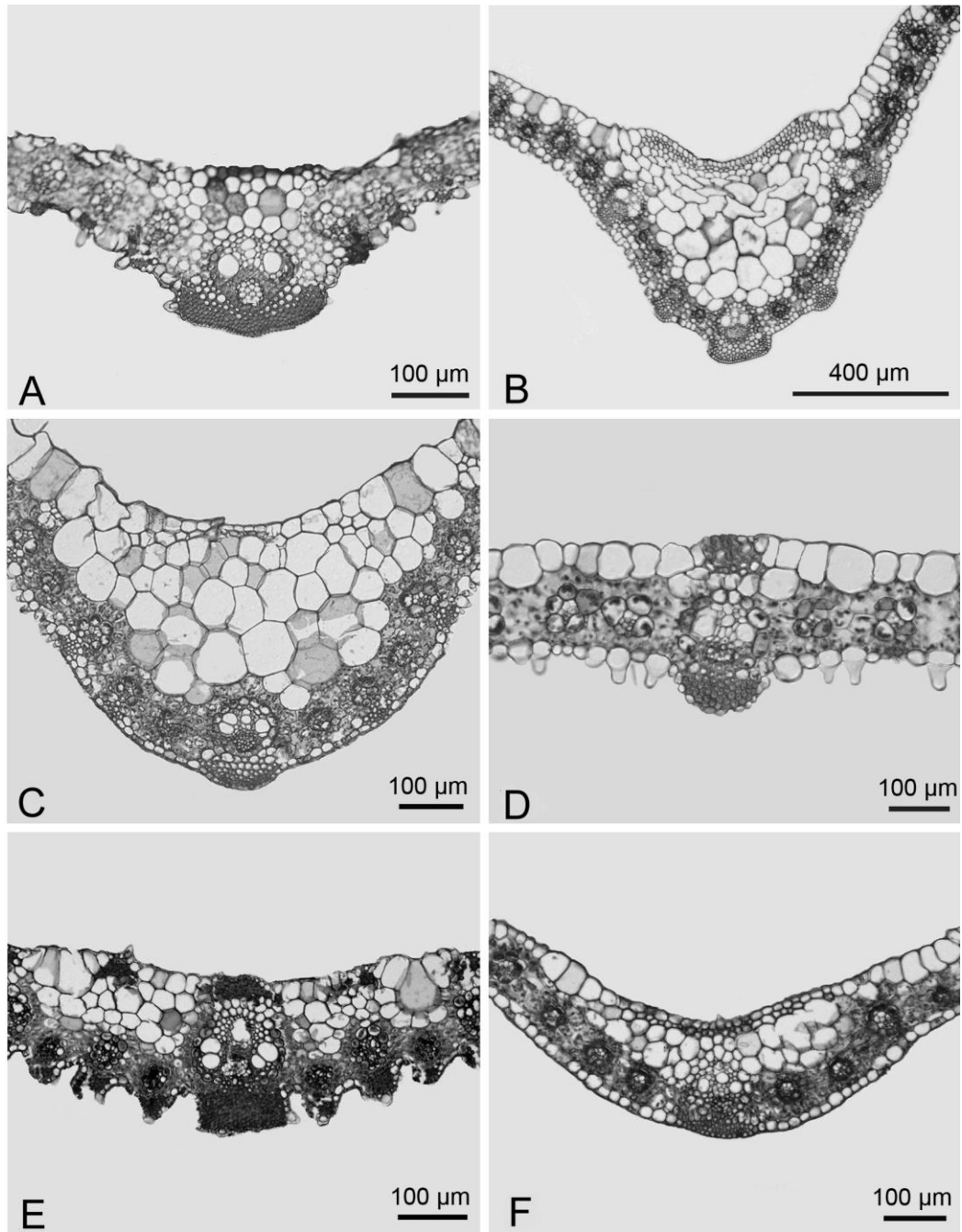


FIGURE 2. Leaf blade anatomy as seen in transverse section through the midribs. (A) *Apluda mutica*, (B) *Ischaemum hirtum*, (C) *Ischaemum hubbardii*, (D) *Kerriochloa siamensis*, (E) *Sehima nervosum* and (F) *Thelepogon elegans*.

parallel-side, point of attachment small. *Macro-hairs*: unicellular, many hard, long hairs, usually smaller, specialized epidermal cell accompanying base of hair. *Silica bodies*: intermediate between cross and dumb-bell-shaped, *Costal short cells*: square or rectangular in shape, smooth outline, present very rare.

**ADAXIAL EPIDERMIS.**— *Intercostal long cells*: variable shape, hexagonal cells, or bowed outwards, inflated or rectangular cells, moderately to deeply undulating.

#### **Leaf in transverse section (Fig. 2B)**

*Outline*: two halves of lamina curved upwards on either side of the midrib; no ribs or furrows are present on either surface. *Epidermis*: bulliform cells are present but not arranged in regular groups. *Midrib outline*: present, keel conspicuous and developed, triangular-shaped; many vascular bundles are present in the keel, all vascular bundles abaxially arranged: three first-order bundles and smaller bundles comprise keel. *Vascular bundle arrangement in the lamina*: 2-4 third-order bundles between consecutive larger bundles; all bundles situated in abaxial of the blade. *Primary-order vascular bundles*: round or circular in outline. *Intercostal sclerenchyma*: point-cap shaped of sclerenchyma at the margin, contact with the lateral bundle.

#### **Culm in transverse section (Fig. 3B)**

*Outline*: circular or oval, culm examined 1-2 mm in diameter. *Epidermis* subtended by a sclerenchyma ring consisting of some 9-12 layers of fibres and assimilatory tissue bounded on the inner side, alternating with the outermost vascular bundle. *Inner ground tissue* consisting of many layers of large, thin-walled cells, extending to the hollow centre of the culm, which has a somewhat irregular cavity. *Vascular bundles*:

outermost vascular bundles are embedded in the inner sclerenchyma ring.

### ***Ischaemum hubbardii* Bor (Figures 1C, 2C and 3C)**

#### **Leaf surface (Fig. 1C)**

**ABAXIAL EPIDERMIS.**— *Intercostal long cells*: shortened cells, length less than 3x longer than wide; cell is rectangular, square or trapezoidal; moderately undulating. *Stomata*: subsidiary cells dome-shaped, one to four rows of stomata in each intercostal zone. *Intercostal short cells*: tall and narrow in shape, smooth in outline. *Papillae*: circular or rounded, more than one papillus per cell. *Prickle hairs*: absent. *Hooks*: absent. *Micro-hairs*: not detected. *Macro-hairs*: unicellular, hard, long hairs, many smaller specialized epidermal cell accompanying base of hair. *Silica bodies*: dumb-bell-shaped, constricted, narrow central portion. *Costal long cells*: narrower than intercostal long cells.

**ADAXIAL EPIDERMIS.**— *Intercostal long cells*: short and inflated cells.

#### **Leaf in transverse section (Fig. 2C)**

*Outline*: two halves of lamina curved upwards on either side of the midrib, sometimes V-shaped; no ribs or furrows are present on either surface. *Epidermis*: bulliform cells are present on adaxial surface, not arranged in regular groups. *Midrib outline*: present, keel conspicuous and develop, triangular-shaped to slightly round; many vascular bundles are present in the keel, all vascular bundles abaxially arranged: first-order bundle and smaller bundles comprise keel. *Vascular bundle arrangement in the lamina*: 10 or more third-order bundles between consecutive larger bundles; all bundles situated in

abaxial of the blade. *Primary-order vascular bundles*: round or circular in outline. *Intercostal sclerenchyma*: cap-shaped of sclerenchyma at the margin, cap equal or less than the width of a third-order bundle, contact with the lateral bundle.

### Culm in transverse section (Fig. 3C)

*Outline*: somewhat circular or oval, culm examined 1.5-2.5 mm in diameter, intercellular space present about three cells below the epidermis around the periphery of the culm, the outermost vascular bundle alternating with the spaces. Intercellular spaces followed on the inner side by a somewhat sinuous, continuous ring of fibres about 2-3 cells wide, the fibrous ring being bounded on the inner side by a zone of thin-walled ground tissue, extending to the hollow centre of the culm, with a somewhat circular cavity.

### *Ischaemum muticum* L.

#### Leaf surface

ABAXIAL EPIDERMIS.— *Intercostal long cells*: elongated cells, length 3x or more longer than wide; cell rectangular, square or trapezoidal; moderately undulating. *Stomata*: subsidiary cells dome-shaped, three to six rows of stomata in each intercostal zone. *Intercostal short cells*: solitary cells, tall and narrow in shape, crenate in outline. *Papillae*: circular or rounded, more than one papillus per cell. *Prickle hairs*: absent. *Hooks*: present but very rare in intercostal zone. *Micro-hairs*: bicellular, two-celled, basal cell only slightly shorter than the distal cell; apex of distal cell slightly tapered, tapering to a round apex, base or attachment of basal cell: parallel-side, point of attachment small. *Macro-hairs*: absent. *Silica bodies*: dumb-bell shaped. *Costal long cells*: narrower than

intercostals long cells. *Costal short cells*: square or rectangular in shape.

ADAXIAL EPIDERMIS.— *Intercostal long cells*: variable shape, hexagonal cells, or bowed outwards, inflated or rectangular cells, moderately undulating.

#### Leaf in transverse section

*Outline*: blade expanded, straight; no ribs or furrows are present on either surface. *Epidermis*: bulliform cells are present in fan-shaped. *Midrib outline*: present, keel conspicuous and developed, triangular-shaped; many vascular bundles are present in the keel, all vascular bundles abaxially arranged: first-order bundle and smaller bundles comprise keel. *Vascular bundle arrangement in the lamina*: one first-order bundle, many third-order bundles between one second-order bundles; all bundles situated in abaxial of the blade. *Primary-order vascular bundles*: round to elliptical in outline. *Intercostal sclerenchyma*: point-cap-shaped of sclerenchyma at the margin, contact with the lateral bundle.

#### Culm in transverse section

*Outline*: somewhat circular in outline, culm examined 1-2 mm in diameter. *Epidermis* subtended by a zone of 1-2 layers of fibres of small diameter with thick walls, followed by a zone of thin-walled tissue, about 4-5 cells wide, bounded internally by a sclerenchyma ring of about 5-6 cells wide. *Inner ground tissue* consisting of many layers of large, thin-walled cells, extending to the hollow centre of the culm. Center of the culm has a somewhat irregular cavity. *Vascular bundles* of the outermost circle are situated at the outer or center boundary, and the next circle at the inner boundary, of the sclerenchyma ring.

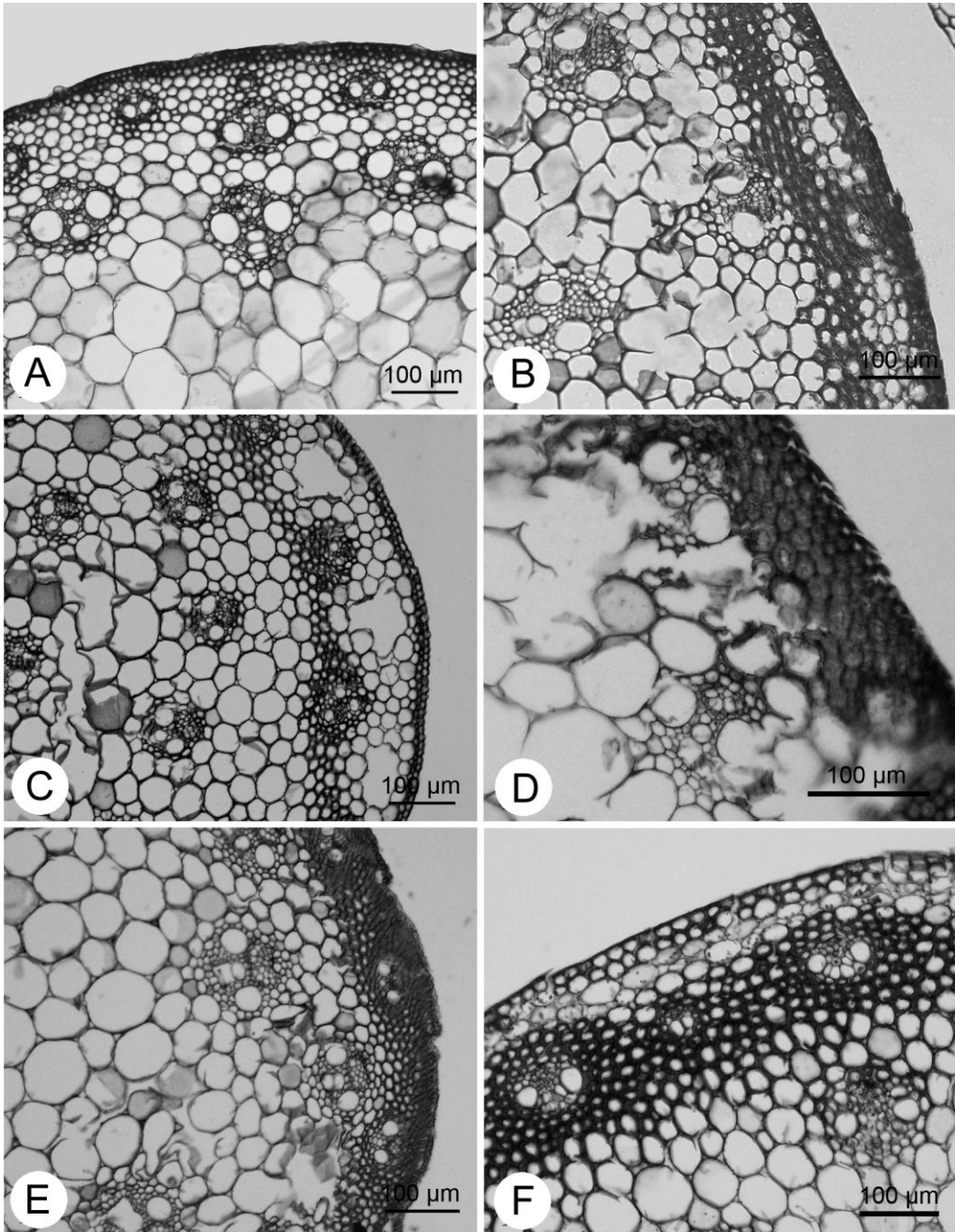


FIGURE 3. Culm anatomy as seen in transverse section. (A) *Apluda mutica*, (B) *Ischaemum hirtum*, (C) *Ischaemum hubbardii*, (D) *Kerriochloa siamensis*, (E) *Sehima nervosum* and (F) *Thelepogon elegans*.

***Ischaemum rugosum* Salisb.**

**Leaf surface**

ABAXIAL EPIDERMIS.— *Intercostal long cells*: elongated cells, length 3x or more longer than wide; cell rectangular, square or trapezoidal; deeply undulating. *Stomata*: subsidiary cells dome- or low-dome shaped, one to four rows of stomata in each intercostal zone. *Intercostal short cells*: solitary cells, tall and narrow in shape, crenate in outline, *Papillae*: absent. *Prickle hairs*: absent. *Hooks*: absent. *Micro-hairs*: bicellular, two-celled, basal cell only slightly shorter than the distal cell; apex of distal cell slightly tapered, tapering to a round apex, base or attachment of basal cell: parallel-side, point of attachment small. *Macro-hairs*: unicellular, hard, short, stiff hairs, two, or sometimes one specialized epidermal cell accompanying base of hair. *Silica bodies*: dumb-bell shaped. *Costal long cells*: narrower than intercostals long cells. *Costal short cells*: tall and narrow in shape, crenate in outline.

ADAXIAL EPIDERMIS.— *Papillae*: circular or rounded, more than one papillus per cell. *Prickle hairs*: common in costal zones, medium prickles. *Hooks*: present very rare on the intercostals zone. *Macro-hairs*: unicellular, hard and long hairs, many smaller specialized epidermal cell accompanying base of hair. *Silica bodies*: dumb-bell shaped, constricted, narrow central portion, or sometimes nodular silica bodies.

**Leaf in transverse section**

*Outline*: lamina rolled inwards towards the adaxial surface, inrolled from one margin only; margins wrapped around each other; ribs and furrows present on both surface; on adaxial surface: medium

furrows, a quarter to one half the leaf thickness, furrows obtuse angle, furrows between all vascular bundles; triangular ribs, apex pointed, one vascular bundle in each rib; on abaxial surface shallow and wider than the adaxial ribs, present opposite all vascular bundles. *Epidermis*: bulliform cells present on adaxial surface or sometimes in abaxial surface, associated with colourless cells in fan-shaped groups penetrating deeply into the mesophyll. *Midrib outline*: present, inconspicuous keel; one vascular bundle comprising the keel. *Vascular bundle arrangement in the lamina*: 1-4 third-order bundles between consecutive larger bundles; all bundles situated in center of the blade. *Primary-order vascular bundles*: elliptical in outline. *Intercostal sclerenchyma*: point-cap shaped of sclerenchyma at the margin, contact with the lateral bundle.

**Culm in transverse section**

*Outline*: circular, culm examined 1.5-2 mm in diameter. *Epidermis* subtended by a zone of 3-4 layers of sclerenchyma. *Inner ground tissue* consisting of 7-8 layers of large, thin-walled cells, extending to the hollow centre of the culm, which has a somewhat circular cavity. *Vascular bundles* of outermost embedded in the center sclerenchyma ring.

***Ischaemum tenuifolium* A. Camus**

**Leaf surface**

ABAXIAL EPIDERMIS.—*Intercostal long cells*: elongated cells, length 3x or more longer than wide; cell rectangular, square or trapezoidal; moderately undulating. *Stomata*: subsidiary cells low-dome shaped, one or rarely two, rows of stomata in each intercostal zone., *Intercostal short cells*: solitary short cells, tall and

narrow in shape, crenate in outline, *Papillae*: absent. *Prickle hairs*: absent. *Hooks*: absent. *Micro-hairs*: only basal cell remains. *Macro-hairs*: absent. *Silica bodies*: dumb-bell shaped elongated with rounded ends. *Costal long cells*: narrower than intercostal long cells.

**ADAXIAL EPIDERMIS.**— *Intercostal long cells*: variable shape, hexagonal cells, or bowed outwards, inflated or rectangular cells, slightly undulating. *Prickle hairs*: common in intercostal zones, very large prickles, base at least twice as long as the stomata; long barb.

#### **Leaf in transverse section**

*Outline*: heart-shaped in outline or sometimes two halves of lamina curved upwards on either side of the midrib and inrolled in one side; ribs and furrows present on adaxial surface: slight, shallow furrows, between all vascular bundles; triangular ribs, one vascular bundle in each rib; sometimes no ribs or furrows on either side of the midrib. *Epidermis*: bulliform cells present on adaxial surface, associated with colourless cells in fan-shaped groups penetrating deeply into the mesophyll and abaxial side. *Midrib outline*: present, keel conspicuous and developed, triangular to round-shaped, three to six vascular bundles present in the keel, all vascular bundles abaxially arranged: first-order bundle and smaller bundles comprise keel. *Vascular bundle arrangement in the lamina*: 3-8 third-order bundles between consecutive larger bundles; all bundles situated in center of the blade. *Primary-order vascular bundles*: round or slightly elliptical in outline. *Intercostal sclerenchyma*: point-cap shaped of sclerenchyma at the margin, not contact with the lateral bundle.

#### **Culm in transverse section**

*Outline*: oval, culm examined 1.5-2 mm in diameter. *Epidermis* subtended by a zone of 4-5 layers of sclerenchyma. *Inner ground tissue* consisting without a central cavity. *Vascular bundles* of outermost embedded in the inner sclerenchyma ring.

#### ***Ischaemum thomasi* Traiperm and Boonkerd (ined.)**

#### **Leaf surface**

**ABAXIAL EPIDERMIS.**— *Intercostal long cells*: shortened cells, length less than 3x longer than wide, or varies in shape; cell rectangular, square or trapezoidal; moderately undulating. *Stomata*: subsidiary cells dome-shaped, one to four rows of stomata in each intercostal zone. *Intercostal short cells*: absent. *Papillae*: circular or rounded, more than one papillus per cell. *Prickle hairs*: absent. *Hooks*: absent. *Micro-hairs*: none seen. *Macro-hairs*: densely, unicellular, long and slender hairs, many smaller specialized epidermal cells accompanying base of hair. *Silica bodies*: dumb-bell shaped. *Costal long cells*: absent.

**ADAXIAL EPIDERMIS.**— *Intercostal long cells*: short and inflated cells.

#### **Leaf in transverse section**

*Outline*: two halves of lamina curved upwards on either side of the midrib, or U-shaped; no ribs or furrows present on both surface. *Epidermis*: bulliform cells present on adaxial surface not arranged in regular groups. *Midrib outline*: present, keel conspicuous and develop, triangular, sometimes inconspicuous; five vascular bundles present in the keel, all vascular bundles abaxially arranged: first-order bundle and smaller bundles comprise keel. *Vascular bundle arrangement in the lamina*:

TABLE 2. The main anatomical differences separating the generic taxa in the subtribe Ischaeminae

Character	<i>Apluda</i>	<i>Ischaemum</i>	<i>Kerriochloa</i>	<i>Sehima</i>	<i>Thelepogon</i>
<b>Leaf anatomy</b>					
Leaf blade outline	Blade expanded, slightly undulating or nearly straight	Blade expanded or straight or two halves of lamina curved upwards on either side of the midrib or rolled	Blade expanded, undulating gently or nearly straight	Blade broad wide, horizontally elongated	Two halves of lamina curved upwards on either side of the midrib
Adaxial ribs and furrows	Absent	Present or Absent	Absent	Present	Absent
Abaxial ribs and furrows	Absent	Present or Absent	Absent	Present	Absent
Bulliform cells on abaxial surface	Simple fans	Fans-shaped or arranged in irregular groups	Arranged in irregular groups	Fans-shaped	Arranged in irregular groups
Midrib and Keel	Keel conspicuous, triangular-shaped	Keel conspicuous, triangular-shaped or inconspicuous keel (rare)	Inconspicuous keel	Inconspicuous keel	Keel conspicuous, rounded or semicircular-shaped
No. of vbs in keel	One	One to many	One	One	Many
Sclerenchyma at margins	Crescent-shaped cap	Point-cap or cap shaped	Absent	Crescent-shaped cap	Point-cap shaped
Abaxial intercostal long cells	Elongated cells, moderately undulating	Elongated cells or variable in shape, deeply or moderately undulating	Variable shape, moderately undulating	Elongated cells, deeply undulating	Elongated cells, moderately undulating
Abaxial intercostal short cells	Absent	Solitary	Solitary	Absent	Solitary
Papillae	Absent	More than one papillus per cell on abaxial or adaxial surface, absent in <i>I. tenuifolium</i>	One papillus per cell on abaxial surface	Absent	Absent
Abaxial prickles	Present (common)	Absent	Absent	Present (common)	Absent
Abaxial macro-hairs	Absent	Present or absent	Present	Absent	Present
Abaxial silica bodies	Nodular	Dumb-bell or intermediate between cross and dumb-bell shaped	Cross-shaped	Dumb-bell shaped	Cross, dumb-bell or intermediate between cross and dumb-bell shaped
Abaxial costal short cells	Absent	Present	Absent	Present	Present
<b>Culm anatomy</b>					
Culm outline and width	Circular, 1.2 - 1.5 mm in diameter	Circular or oval, 1 - 2 mm in diameter	Somewhat circular, 1 - 1.5 mm in diameter	Circular, 1.2 - 1.5 mm in diameter	Circular or somewhat oval, 2 - 3 mm in diameter
Central cavity in ground tissue	Absent	Present, except <i>I. tenuifolium</i>	Absent	Absent	Absent

10 or more third-order bundles between consecutive larger bundles; all bundles situated in abaxial of the blade. *Primary-order vascular bundles*: round or circular in outline. *Intercostal sclerenchyma*: cap shaped of sclerenchyma at the margin, cap more than the width of a third-order bundle, contact with the lateral bundle.

**Genus *Kerriochloa* C.E. Hubbard**

***Kerriochloa siamensis* C.E. Hubbard  
(Figures 1D, 2D, 3D)**

**Leaf surface (Fig. 1D)**

ABAXIAL EPIDERMIS.— *Intercostal long cells*: variable shape; intercostal zone, hexagonal cells centrally and rectangular

cells laterally; moderately undulating. *Stomata*: subsidiary cells low dome-shaped, ovoid, one to five rows of stomata in each intercostal zone. *Intercostal short cells*: solitary cells, square or rectangular in shape, smooth walls, present at only one end of each long cell, in 20% of all long cells. *Papillae*: circular or rounded papillae as seen in surface view, large: diameter more than or equal to  $\frac{1}{2}$  the vertical width of the long cells, one papillus per cell. *Prickle hairs*: absent. *Hooks*: absent. *Micro-hairs*: not detected. *Macro-hairs*: unicellular, hard, short, stiff hairs, one specialized hemispherical epidermal cell accompanying base of hair, swollen in relation to hair thickness. *Silica bodies*: cross-shaped, equidimensional, vertical and horizontal dimensions approximately equal, acutely angled; sometimes found transverse silica bodies tall and narrow dumb-bell shape on intercostal long cells. *Costal long cells*: bowed outwards, cells inflated, thin and smooth wall, separated by silica bodies. *Costal short cells*: absent.

**ADAXIAL EPIDERMIS.**— *Intercostal long cells*: variable shape, hexagonal cells, or bowed outwards, inflated or rectangular cells, slightly undulating. *Intercostal short cells*: solitary or paired, tall and narrow, crenate in outline, present at near the leaf margins of intercostal zones only. *Hooks*: present at near the leaf margins of intercostal zones only. *Micro-hairs*: bicellular, two-celled, basal and distal cells approximately equal in length, apex slightly tapered, tapering to rounded apex, base or attachment of basal cell: parallel-side, point of attachment small.

#### **Leaf in transverse section (Fig. 2D)**

*Outline*: blade expanded, undulating gently or nearly straight; no ribs or furrows present on either surface. *Epidermis*:

bulliform cells present, not arranged in regular groups. *Midrib outline*: present, inconspicuous keel; one vascular bundle comprising the keel. *Vascular bundle arrangement in the lamina*: one median bundle, 10 or more third-order bundles between one second-order bundles; all bundles situated in the center of the blade. *Primary-order vascular bundles*: circular or round in outline. *Intercostal sclerenchyma*: no sclerenchyma developed in association with the margin.

#### **Culm in transverse section (Fig. 3D)**

*Outline*: somewhat circular, culm examined 1-1.5 mm in diameter. *Epidermis* subtended by about 4-5 layers of cells with thickened, lignified walls, with a circular of small, oval, columns of assimilatory tissue embedded in the thickened ground tissue. *Inner ground tissue* without a central cavity. *Vascular bundles* of outermost circle embedded in the layers of thickened cells and being smaller than the remainder.

#### **Genus *Sehima* Roem. and Schult.**

##### ***Sehima nervosum* (Rottler) Stapf (Figures 1E, 2E and 3E)**

#### **Leaf surface (Fig. 1E)**

**ABAXIAL EPIDERMIS.**— *Intercostal long cells*: elongated cells, length 3x or longer than wide; cell rectangular, square or trapezoidal; deeply undulating. *Stomata*: subsidiary cells dome-shaped, always one to two rows of stomata in each intercostal zone, if two: rows adjacent to one another. *Intercostal short cells*: absent. *Papillae*: absent. *Prickle hairs*: common in costal zones, medium prickles, more than three rows along whole length between all vascular bundles. *Hooks*: present in margins of costal zones only. *Micro-hairs*: bicellular,

two-celled, slender, basal and distal cells approximately equal in length; distal cell very slender look like long caudate, angle base emerges in cells at margins of costal zones. *Macro-hairs*: absent. *Silica bodies*: dumb-bell shaped, elongated with rounded ends. *Costal short cells*: alternating silica cells and costal short cells, short to square short or cork cells.

**ADAXIAL EPIDERMIS.**— *Intercostal long cells*: variable shape, hexagonal cells; sometimes adjacent with single short cells. *Intercostal short cells*: solitary, silicified silica cells containing distinct silica body or phytolith, silica body and silica cell of same or similar shape.

#### **Leaf in transverse section (Fig. 2E)**

*Outline*: blade broad wide, horizontally elongated; ribs and furrows present on both surfaces; on adaxial surface: slight, shallow furrow less than a quarter of the leaf thickness, furrow wide and open, furrows between first-order and second-order vascular bundles, present over third-order bundles; ribs slides rounded with flat top; on abaxial surface taller than the adaxial ribs, present opposite all vascular bundles; composed of sclerenchyma in form of rounded caps on second and third-order bundles; composed of girder or strand of sclerenchyma in contact with epidermis on first-order bundles. *Epidermis*: bulliform cells present in fan-shaped present over third-order bundles. *Midrib outline*: present, inconspicuous keel; one vascular bundle comprising the keel. *Vascular bundle arrangement in the lamina*: 4-6 first-order bundles in half lamina, 1-3 second-order bundles between first-order bundle, 1-4 third-order bundles between second-order bundle, first and second-order bundles central and third-order bundle displaced abaxially in ribs. *Primary-order vascular*

*bundles*: circular or round in outline. *Intercostal sclerenchyma*: crescent-shaped cap; sclerenchyma extends shortly along both abaxial and adaxial side of the leaf margins.

#### **Culm in transverse section (Fig. 3E)**

*Outline*: circular, culm examined 1.2-1.5 mm in diameter. *Epidermis* subtended by about 9-12 layers of cells with thickened, lignified walls, small non-circular assimilatory tissue. *Inner ground tissue* without a central cavity. *Vascular bundles* of outermost circle embedded in the layers of thickened cells and smaller than the remainder.

#### **Genus *Thelepogon* Roem. and Schult.**

##### ***Thelepogon elegans* Roem. and Schult. (Figures 1F, 2F and 3F)**

#### **Leaf surface (Fig. 1F)**

**ABAXIAL EPIDERMIS.**— *Intercostal long cells*: elongated cells, length 3x or more longer than wide, sometimes with inflated cells separated from the narrower cells; cell rectangular, square or trapezoidal; moderately undulating. *Stomata*: subsidiary cells low-dome to dome shaped, many or upto 18 rows of stomata in each intercostal zone. *Intercostal short cells*: solitary short cells situated between long cells, tall and narrow in shape, smooth in outline, usually connect to transverse silica bodies. *Papillae*: absent. *Prickle hairs*: absent. *Hooks*: present very rare in intercostal zones. *Micro-hairs*: bicellular, two-celled, basal cells shorter than distal cells, basal cells less than ½ distal cell length; slightly tapered, tapering to a rounded apex, base or attachment of basal cell: expanded base, constriction above bulbous base. *Macro-hairs*: unicellular, hard, short, stiff hairs, one

TABLE 3. The main anatomical differences separating the species in the genus *Ischaemum*

	<i>I. hirtum</i>	<i>I. hubbardii</i>	<i>I. muticum</i>	<i>I. rugosum</i>	<i>I. tenuifolium</i>	<i>I. thomasi</i>
<b>Leaf anatomy</b>						
Midrib and Keel	Keel conspicuous, triangular-shaped	Keel conspicuous, triangular-shaped to slightly round	Keel conspicuous, triangular-shaped	Keel inconspicuous	Keel conspicuous, triangular to round-shaped	Keel conspicuous or inconspicuous, triangular-shaped
Ribs and furrows	Absent	Absent	Absent	Present on both surfaces	Present on adaxial surface	Absent
Number of vascular bundles in the keel	Many	Many	Many	One	Three to six	Five
Papillae	Present on abaxial surface	Present on abaxial surface	Present on abaxial surface	Present on adaxial surface	Absent	Present on abaxial surface
Macro-hairs	Present	Present	Absent	Present	Absent	Present
<b>Culm anatomy</b>						
Central cavity in ground tissue	Present, somewhat irregular cavity	Present, somewhat circular cavity	Present, somewhat irregular cavity	Present, somewhat circular cavity	Absent	-

specialized hemispherical epidermal cell accompanying base of hair. *Silica bodies*: transverse cross-shaped with four rounded apices in intercostal zones; costal zones with dumb-bell shaped, relatively short, not elongate, sometimes intermediate between cross and dumb-bell shaped. *Costal short cells*: solitary of short cells situated between long cells, tall and narrow in shape, smooth in outline, connect to silica bodies.

**ADAXIAL EPIDERMIS.**— *Intercostal long cells*: variable shape, hexagonal cells, or bowed outwards, inflated or rectangular cells, moderately undulating. *Hooks*: densely with hooks around the intercostal zones.

#### Leaf in transverse section (Fig. 2F)

*Outline*: two halves of lamina curved upwards on either side of the midrib; no ribs or furrows present on either surface. *Epidermis*: bulliform cells present, not

arranged in regular groups. *Midrib outline*: present, keel conspicuous and develop, rounded or semicircular-shaped; many vascular bundles present in the keel, all vascular bundles abaxially arranged: first-order bundle and smaller bundles comprise keel. *Vascular bundle arrangement in the lamina*: one first-order bundle, 10 or more third-order bundles between one second-order bundle; all bundles situated in the center of the blade except median bundle. *Primary-order vascular bundles*: round to slightly elliptical in outline. *Intercostal sclerenchyma*: point-cap shaped of sclerenchyma at the margin, not in contact with the lateral bundle.

#### Culm in transverse section (Fig. 3F)

*Outline*: circular or somewhat oval, culm examined 2-3 mm in diameter. *Epidermis* subtended by about 1-3 layers of thin-walled cells; this zone being bounded on its inner

side by a sclerenchyma ring consisting of some 6-8 layers of fibres. *Inner ground tissue* without a central cavity. *Vascular bundles* of outermost circle embedded in sclerenchyma ring, smaller than next circle.

#### DISCUSSION AND CONCLUSION

In this study, the anatomy of the leaf blades and culms of 10 species representing five genera of subtribe Ischaeminae were comparatively examined with light microscopy.

The results of these anatomical observations suggested that anatomical data could have a considerable taxonomic significance for separating the related genera in the subtribe Ischaeminae of Thai grasses, and in particular the leaf blade outline, ribs and furrows on both surfaces, bulliform cells, midrib and keel, number of vascular bundles in the keels, shape of sclerenchyma at margins, intercostal long cells, intercostal short cells, papillae, prickles, macro-hairs, silica bodies, costal short cells, culm outline and central cavity in ground tissue of culm. Table 2 shows that in many species certain specific characters have been found useful in identifying this group of plants.

Previously, systematists have studied the gross morphology of this group of plants and also the internal morphological patterns of leaves and stem. Metcalfe (1960), Renvoize (1981) and Watson and Dallwitz (1992) studied the leaf blades by light microscopy and concluded that the anatomical characters of leaf blades were of significant value in grass identification. This paper shows more apparent variations between the genera and describes some potentially useful characters of the culms which have not been reported before.

According to the investigation of Renvoize (1981), the aberrant features of some genera in Andropogoneae could not be combined into a single generalized description. This study also supports that conclusion. For example, the silica bodies in all genera are cross-shaped, dumb-bell-shaped or in between cross and dumb-bell-shaped, except in the genus *Apluda* in which they are nodular.

Prickles are common in the genera *Apluda* and *Sehima*, with the later showing distinctive ribs and furrows on both surfaces. This character also appears in *I. rugosum* but it is completely different from *Sehima* in the degree and shape of the ribs and furrows and the masses of sclerenchymatous cells below the epidermis. The ribs slides were rounded with a flat top and wide and open furrow in the genus *Sehima* while triangular ribs and obtuse furrows are present in *I. rugosum*.

The midrib of *Thelepogon* is usually composed of a few vascular bundles, with a conspicuous keel that is rounded or semicircular-shaped because of the sclerenchymatous cell mass below the upper epidermis and above the lower epidermis. There are one to a few rows of colorless parenchyma cells below the upper epidermis and chlorenchyma surround the small vascular bundles. In contrast to this, the midrib of *Kerriochloa* and *Sehima* are arranged in a straight or somewhat uneven row, in which a large bundle is situated in the center and a few small bundles are placed on both sides of it. The keel is inconspicuous, not associated with any developed parenchyma and the projection due to position or size of bundle and sclerenchyma is on abaxial surface.

The occurrence of papillae is a good diagnostic character for the genera, whilst within the genera *Apluda*, *Sehima* and

*Thelepogon* they are absent, but rather are present only in the genera *Kerriochloa* and *Ischaemum*. The papillae are comprised of more than one papillus per cell in the *Ischaemum* and solitary in the *Kerriochloa*. However, some species fail to conform completely to this general pattern, but have the papillae as absent and the culm with no central cavity in *Ischaemum tenuifolium* (Table 3). The absence of papillae appears to be associated with morphological trends within the genera, and it is possible to separate the other groups below the generic level.

Although anatomical features alone are insufficient for the delineation of some generic groups, in general, we consider that the features have considerable systematic values in providing additional support to the distinctiveness of the species. However, like all other taxonomic evidences, leaf anatomical characters must be interpreted with caution.

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#### LITERATURE CITED

- Ellis, R.P. 1976. A procedure for standardizing comparative leaf anatomy in the Poaceae I: the leaf blade as viewed in transversal section. *Bothalia*, 12: 65-109.
- Ellis, R.P. 1979. A procedure for standardizing comparative leaf anatomy in the Poaceae II: the epidermis as seen in surface view. *Bothalia*, 12: 641-671.
- Metcalf, C. R. 1960. *Anatomy of the Monocotyledons I. Poaceae*. U.K.: Oxford University Press.
- Renvoize, S. A. 1981. A survey of leaf-blade anatomy in Grasses. I Andropogoneae. *Kew Bulletin*, 37: 315-321.
- Watson, E. and Dallwitz, M. J. 1992. *The grasses genera of the world*. Cambridge U.K.: University Press.
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