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A Comparative Anatomy of *Salomonia* Lour. (Polygalaceae) in Thailand

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ABSTRACT.– The anatomy of five species of *Salomonia* in Thailand was studied. Capsule and flower characters along with roots were not discriminatory at species level within the genus but two sets of leaf characters were found to allow discrimination between all five species of *Salmonia* endemic to Thailand. The following characters were found to be discriminatory and distinctive at the generic level. The leaf characters are dorsiventral leaves with anomocytic stomata, unicellular hairs present, collateral vascular bundles, unilacunar with one trace node, siphonostele and presence of fibre strands around vascular bundles and inside the ribs on stems, presence of idioblasts which contain yellowish substances and schizogenous cavities in root cortex. The leaf characters; cuticular appearance and thickness, presence of spine-like hairs or papillae on leaf margins, presence of hairs and density and characters of epidermal cells on capsule faces are significant for species identification.

KEY WORDS: anatomy, *Salomonia*, Thailand

INTRODUCTION

Salomonia Lour. is one of approximately 17 genera within Polygalaceae with around 12 species in the world (Hutchinson, 1967). The genus, which is mostly similar to *Polygala,* but distinguished from the latter by the absence of alae, comprises five species found from India to Japan, throughout Malaysia and North Australia. These five species are also recorded in Thailand with 2 endemics; *S. kradungensis* and *S. thailandica* (Pendry, 2001) and two of them; *S. cantoniensis* and *S. longiciliata*, have been used by natives of northeastern Thailand as medicinal plants (Mahidol University Foundation, 2000). All of them have a unique odor in their roots, the same odor being found in some *Polygala* species. Because of the similarity of their flowers, leaf and especially capsule morphology, they are recognised as identification characters and are used in as such in the keys presented by Koyama (1995) and Pendry (2001). Most attention within the genus has been paid to relationships within the family with only a few species included. For example; Rao (1964) revealed the embryology of *S. cantoniensis*; Eriksen (1993a,b) compared floral morphology and anatomy between 15 genera which were then used for cladistic

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analysis of Polygalaceae; and Persson (2001) studied phylogenetic relationships within the family by using plastid DNA sequences. For anatomical studies, Metcalfe (1950) reported the general characters of another 13 genera, and some characters are reported for certain groups such as Moutabeae (Styer 1977) and *Xanthophyllum* (Dickison 1973), but no attention was given to *Salomonia* itself in these studies. The present study was undertaken for two purposes: (i) to reveal the anatomical features of the genus and (ii) to provide an identification key based on anatomical features.

MATERIALS AND METHODS

Plant materials used in this study were taken from field collections and deposited at Khon Kaen University (KKU) as follows: *Salomonia cantoniensis* Lour., Narumol 26; *S. ciliata* (L.) DC., Narumol 56; *S. kradungensis* H. Koyama, Narumol 88; *S. longiciliata* Kurz, arumol 22; *S. thailandica* H. Koyama, Narumol 80.

Leaves, nodes, roots, flowers and capsules from spirit specimens were dehydrated and paraffin embedded. Transverse sections, 8-12 μm thickness, were cut on a sliding microtome and the sections stained with Safranin and Fast Green (Thammathaworn, 1995). Leaves were cleared in 2% (w/v) NaOH and stained with 1% (w/v) Chlorazol Black E as described by Lersten and Curtis (2001). Anatomical characters were observed under light microscopy and photographed through Normaski Olympus BX51 fitted with Olympus DP11 digital camera. Leaves and capsules were dehydrated with ethanol series; 50%, 60%, 70%, 80%, 90%, 95%, 100% (v/v), critical-point dried, sputtered with gold particles, and cuticular ornamentations were examined and photograped through LEO 1450VP SEM Generally, five to ten pieces of each organ were examined.

RESULTS

Root Anatomy

The secondary root of all species is characterized by a single-layered epidermis covered with a thin cuticle. Epidermal cells are ellipsoid or rounded in transectional outline. Some epidermal cells of *S. cantoniensis* contain oil droplets which then disappeared between slide preparations. The cortex, which is composed of parenchyma cells, is arranged into four zones: (i) the outermost cells of cortex are arranged in one to two layers adjacent to epidermis; (ii) subjacent to the outermost is a zone composed of cells arranged in a radial row alternated with schizogenous cavities; (iii) this is followed by two to three layers of enlarged cells filled with a yellowish substance; and (iv) the innermost layer is an endodermis which is distinguished by cells which are two to three times smaller than in other zones and without a Casparian strip. The unique type of root stele is a protostele with radially arranged xylem (Fig. 1A).

Stem and Nodal Anatomy

The stem is ellipsoid or rounded with four to six prominent ribs in transectional outline. The ribs are long and narrow in *S. cantoniensis* (Fig. 1B), dome-shaped in *S. ciliata*, *S. kradungensis* and *S. thailandica*

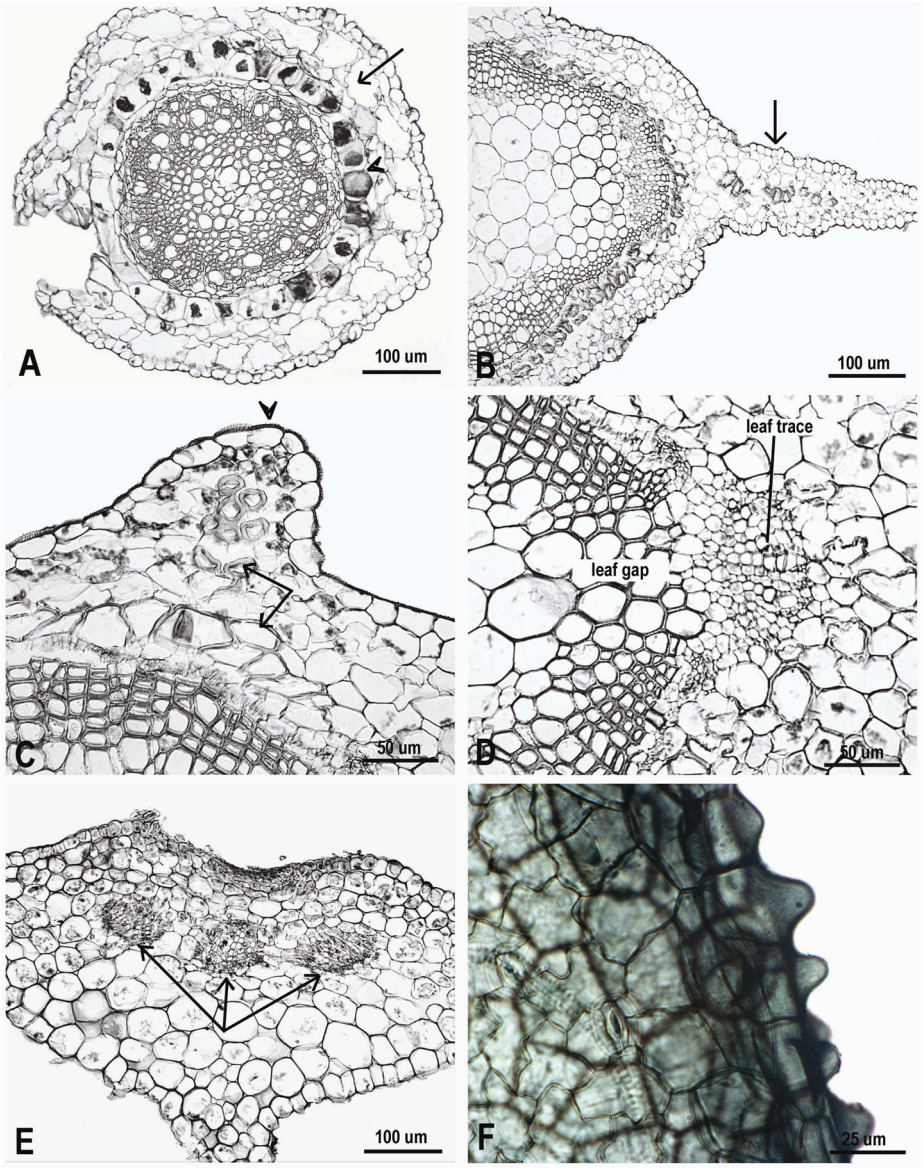

FIGURE 1. A. *S. kradungensis*; root with schizogenous cavities (arrow) and cells containing yellowish substance (arrowhead) in cortex and protostele with radially arranged xylem. **B.** *S. cantoniensis*; long and narrow rib (arrow) on stem. **C-D.** *S. thailandica;* **C.** dome-shaped rib (arrowhead) on stem, cortex composed of chlorenchyma cells and unlignified fibres (arrows) with large lumen surrounding vascular cylinder and in rib; **D.** nodal anatomy with one leaf trace and leaf gap. **E.** *S. longiciliata*; petiole in transectional outline and vascular bundle divided into three to five bundles (arrows). **F.** *S. cantoniensis*; papillae on leaf margin.

(Fig. 1C), and are a more prominent dome-shape in *S. longiciliata*. The epidermis is composed of single layer of rectangular cells covered with a long-paralleled striate cuticle. Unicellular hairs are present in all species. The cortex is composed of

chlorenchyma cells loosely arranged into spongy-liked layers and fibre bundles inserted in ribs (Fig. 1C).

The nodal anatomy of this genus is unicellular with one trace type (Fig. 1D). The leaf trace shows variability in shape from rounded to arc-shaped and is divided into three to five bundles in the petiole. The vascular cylinder of the stem forms a distinct siphonostele which is surrounded by unlignified fibre strands. The fibre cells are rectangular in transectional outline with a distinctly large lumen (Fig. 1C). Pith cells are isodiametric parenchymatous with triangular intercellular spaces. There are no crystals found in stems of all specimens of all five species examined.

Petiole Anatomy

The petiole is heart-shaped to broadly heart-shaped in transectional outline and comprised of a single layer of epidermal cells covered with long-paralleled striae. Unicellular hairs are commonly present in all species. Isodiametric parenchyma cells of cortex are arranged tightly with or without very small intercellular spaces. The vascular bundle within the petiole is a single bundle soon after diverging from the cauline vascular cylinder and typically subdivides into three to five separate bundles which are arranged in horizontal lines before entering into the leaf base. No sclerenchyma is found in any part of the petiole (Fig. 1E).

Leaf Blade Anatomy

Unicellular hairs with warty cuticular ornamentation are commonly present in both adaxial and abaxial surfaces but are confined at the base and midrib on the adaxial side in both *S. ciliata* and *S. thailandica*. In *S. cantoniensis*, papillae are present on the leaf margins (Fig. 1F). Multicellular spine-like shaped hairs are commonly present on the leaf margins of *S. longiciliata* (Fig. 2A). The cuticular thickness is equally thick on both surfaces and varies among species: it is thin, less than 2.5 μm, and unprominent in *S. cantoniensis*; 2.5-7.5 μm in *S. ciliata* and *S. kradungensis*; and 5-25 μm in *S. longiciliata* and *S. thailandica*. Cuticular ornamentation on both surfaces is smooth with grooves on anticlinal walls in *S. cantoniensis* (Fig. 2B). In contrast, in *S. kradungensi* it is smooth on the adaxial surfaces but with an undulate striate on the abaxial surface which is raised above the general cuticular level anticlinal walls on both surfaces (Fig. 2C). In *S. ciliata*, *S. longiciliata* and *S. thailandica* the ornamentation on the leaf surfaces is densely undulate striae and is continuous over several cells (Fig. 2D).

In surface view, both adaxial and abaxial epidermal cells are polygonal to irregular shaped with sinuous anticlinal walls in *S. cantoniensis*, *S. kradungensis* and *S. longiciliata* but slightly undulated in *S. ciliata* and *S. thailandica*. Stomatal type is typically anomocytic and confined to both surfaces. Stomatal position is markedly typical in *S. cantoniensis* while it is slightly raised in others. Guard cells are 6-11 μm wide and 23-32 μm long. Outer stomatal rims are slightly raised but not prominent.

In transverse section, the single layer of epidermal cells are polygonal to rounded with the adaxial cells slightly larger than the abaxial cells. The mesophyll is unifacial with well differentiated palisade and spongy regions in *S. cantoniensis* (Fig.

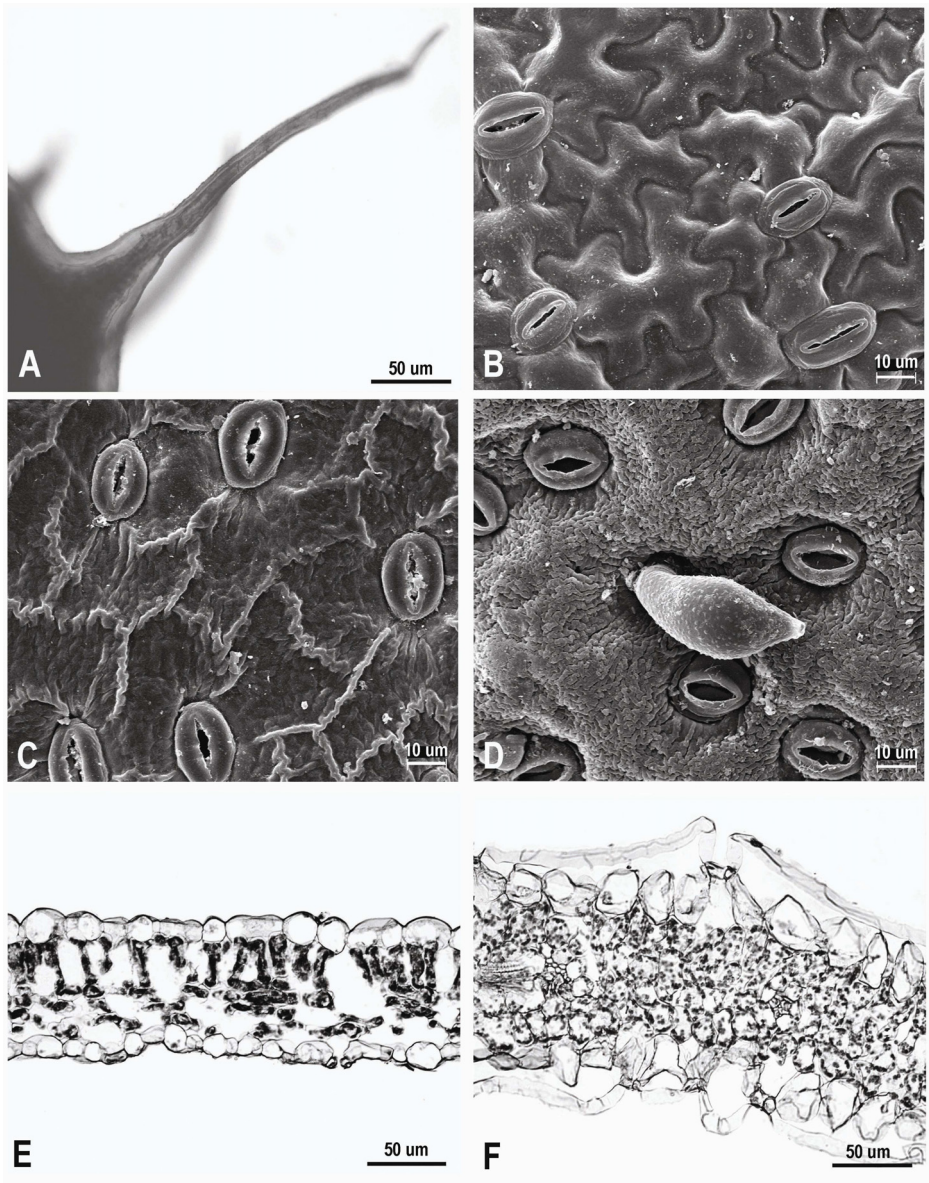

FIGURE 2. A. *S. longiciliata*; spine-like hair on leaf margin. B. *S. cantoniensis*; leaf cuticle with smooth periclinal wall and groove on anticlinal walls. **C.** *S. kradungensis*; leaf cuticle with undulate striae and raised anticlinal walls. **D.** *S. longiciliata*; leaf cuticle with densely undulate striae and unicellular hairs with warty surface. **E.** *S. cantoniensis*; mesophyll with well differentiated palisade and spongy regions. **F.** *S. ciliata*; mesophyll with poorly differentiated palisade and spongy regions.

2E), *S. kradungensis* and *S. longiciliata*, with the palisade length equal to about half the mesophyll width. In both *S. ciliata* and *S. thailandica* (Fig. 2F), the palisade and spongy regions are poorly differentiated with short palisade cells and densely packed spongy cells.

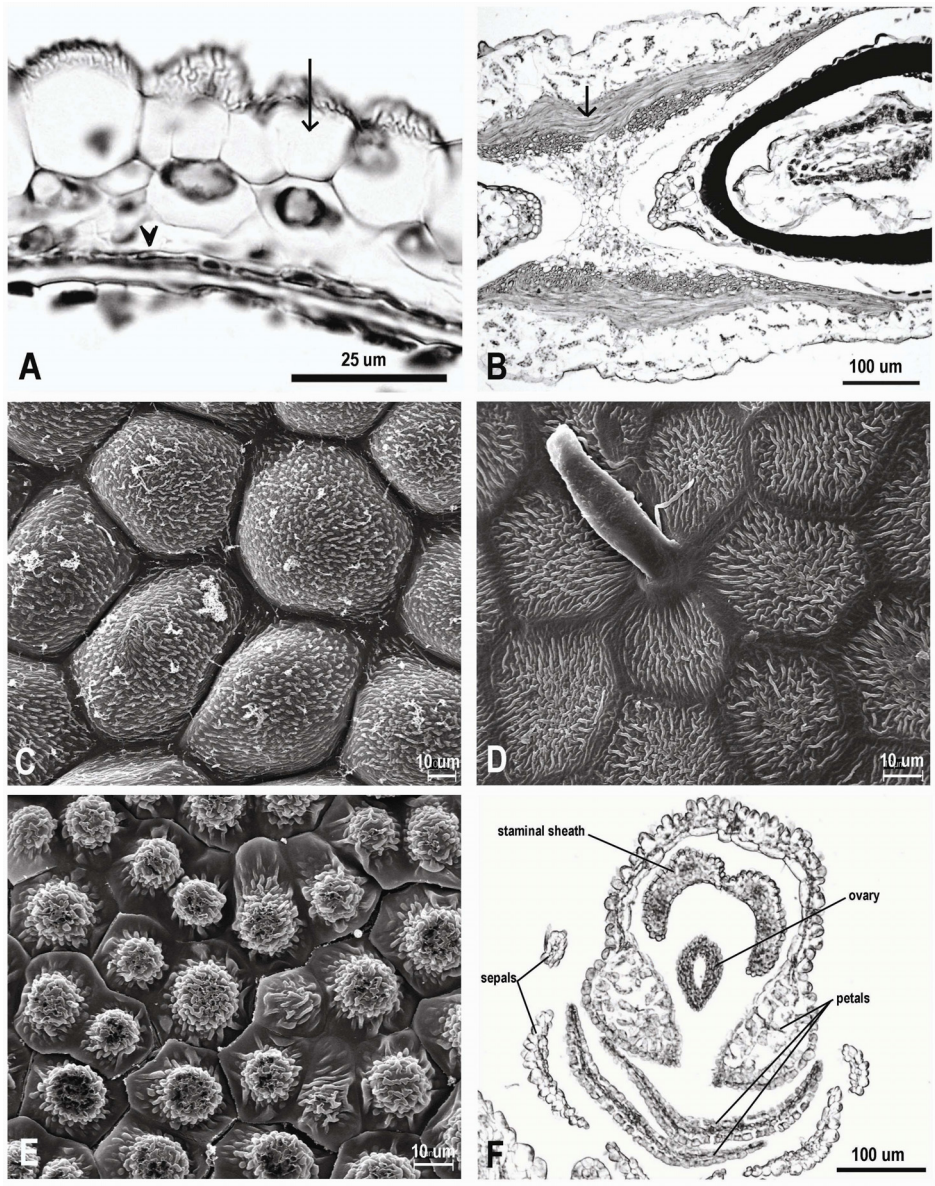

FIGURE 3. A. *S. kradungensis*; outer (arrow) and inner (arrowhead) epidermal cells of capsule in transverse section. **B.** *S. longiciliata*; fibres (arrow) in the middle of capsule cortex. **(C)**. *S. thailandica*; epidermal cells on capsule face. **D.** *S. ciliata*; epidermal cells on capsule face. **E-F.** *S. cantoniensis*: **E.** periclinal walls of cells on capsule face with densely undulate striae on papillae; **F.** flower in transverse section under stamens and stigma level shows parenchymatous component of perianth parts.

All veins are formed from a single collateral bundle which is variable in size from the largest in the midrib to the smallest with phloem only at the vein endings. Parenchymatous sheath is uniformly single cell layered without extension and is recognised in all foliar bundles (Fig. 2E-F).

Capsule Anatomy

In transverse section, the epidermal cells are one-layered in both the inner and outer epidermis. The inner epidermis is composed of small cells with a rectangular to ellipsoid shape in transectional outline and a polygonal to irregular shape with sinuous anticlinal walls in surface view. These inner epidermal cells are characterized by their lignified walls. In contrast, the outer epidermis is composed of much larger cells than the inner one (Fig. 3A). Epidermal cell shapes are ellipsoid to rectangular in transectional outline and polygonal with straight anticlinal walls in surface view. Unlike the inner cells, these outer epidermal cells contain unlignified walls. In *S. cantoniensis*, the outer epidermal cells are remarkably different from others and are characterized by the protruding papillae on their outer periclinal walls especially for cells in the capsule face regions. The cortex is composed of two distinct types of cells, fibre and parenchyma. The groups of fibres are located in the middle of capsule margins, middle parts and base of spines while parenchyma cells fill in the whole of cortex (Fig. 3B).

When looking at the cuticular ornamentation of cells which cover the capsule faces, in surface view, there are short undulate striae randomly arranged on periclinal walls of each cell but which are smooth on anticlinal walls in all species (Fig. 3C-D) except *S. cantoniensis*, in which the striae densely cover papillae but are smooth around papillae base and anticlinal walls (Fig. 3E).

Petal and Sepal Anatomy

Perianth parts of *Salomonia* species are composed of a single layer of epidermis, an undifferentiated parenchymatous cortex and vascular bundles with phloem only. No difference in tissue components was observed among the five species (Fig. 3F).

Key to species

1a. leaf mesophyll with well-differentiated palisade and spongy regions, in surface view, epidermal cells shapes are polygonal with markedly sinuous anticlinal walls.....**2**

b. leaf mesophyll with poorly differentiated palisade and spongy regions, in surface view, epidermal cells shapes are polygonal with slightly undulate anticlinal walls.....**4**

2a. spine-like hair present on leaf margins.....***S. longiciliata***

b. spine-like hair absent on leaf margins... .....**3**

3a. leaf cuticle with undulate striae on abaxial epidermal cells, cuticular thickness 2.5-5 μm.....***S. kradungensis***

b. leaf cuticle without undulate striae on both adaxial and abaxial epidermal cells, cuticular thickness less than 2.5 μm.....***S. cantoniensis***

4a. epidermal cells on capsule faces with convex periclinal walls, cuticle on leaf surfaces with deeply undulate striae.....***S. thailandica***

b. epidermal cells on capsule faces with flattened periclinal walls, cuticle on leaf surface with shallowly undulate striae.....***S. ciliata***

DISCUSSION

The following five features are constant and very similar in all five species of *Salomonia* examined; (i) stems with ribs and siphonostele surrounded by unlignified fibre strands; (ii) amphistomatic leaves with anomocytic stomata; (iii) collateral foliar bundles with parenchymatous sheath; (iv) some cells in root cortex contain yellowish substances and (v) schizogenous cavities are present. Thus these traits, along with capsule and flower characters are not diagnostic at the species level. Therefore, the variable leaf characters are the most useful and those suitable for species recognition are (i) cuticular ornamentation, (ii) epidermal cells shapes, (iii) types of hairs and (iv) the degree of differentiation of mesophyll cells; as shown in the key. According to this study, the poorly differentiated mesophyll of both *S. ciliata* and *S. thailandica* is significantly different from *S. kradungensis* although these three species are found in a similar habitat, that is an open rocky floor with shallow soil which becomes swampy in the rainy season. Therefore, this feature should be constant among the studied species. If not, we should find both mesophyll characters in the same species examined.

Comparing the data of this study with that reported by Metcalfe (1950), it appears that *Salomonia* shares some typical characters with most polygalaceous species, especially in tribe Polygaleae, in the following features: presence of unicellular hair; anomocytic stomata; hypodermis absent; vascular bundles of leaf veins not accompanied by sclerenchyma cells; midrib with a single band vascular bundle; and the stem bundle appearing as a closed ring. However not all traits are in agremmenet with all other members of Polygaleae. The smooth and striate cuticle shows little difference within these five species, in contrast to the rarely striated cuticle reported by Metcalfe (1950). In addition, in all five plants studied, granular thickening was absent and warty thickening found just on the hair cuticles but not on epidermal cells surfaces. The petiole bundle is divided into three to five bundles after diverging from the stem bundle rather than a single bundle or completely enclosed ring through the distal end as in other genera reported. It should be explained that the vascular bundles at the distal end of the petiole seem to be divided to support the primary veins which are arranged in parallel in leaf blades.

This can be compared with *Epirixanthes,* which was included in *Salomonia* in Hutchinson's (1967) system because of the similarity of flowers and inflorescences but, nowadays is reclassified as a saprophytic genus with scale leaves (Pendry, 2001). A vascular cylinder surrounded by fibres is the common character shared between the two genera but the most different feature is the absence of stomata in scale leaves of *Epirixanthes*. The presence of fibres in ribs on the stems and the presence of cortical cells which contain yellowish substance in roots, especially fibrous roots, are similar to some *Polygala* species. But cells containing yellowish substance(s) are confined to the young roots of some *Polygala*, as present in *P. senega*, and disappears when the secondary structure develops (Metcalfe, 1950), whereas it is still present in the fibrous roots of *Salomonia*.

Considering perianth parts, as in other genera, tissue components are all paren-

chymatous with a lack of crystals in common with some other genera such as *Bredemeyera*, *Nylandtia*, *Polygala*, *Securidaca*, *Xanthophyllum* (Eriksen, 1993a).

In addition, following the works of Metcalfe (1950), Dickison (1973) and Styer (1977), the significant characters of plants in Moutabeae and Xanthophylleae which are markedly different from *Salomonia* can be listed as follows: presence of hypodermis in some species of *Moutabea* and *Xanthophyllum*; presence of long sclerosed cells in palisade tissue of *Moutabea* species; hypostomatic leaves with paracytic stomata; presence of fibre bundle sheath or sclerenchymatous cells in vascular bundles; sclereids present in stem cortex and presence of prismatic crystals in both tribes; and the presence of tracheoid idioblasts at the end of veinlets in *Xanthophyllum*. Though most leaf characters are very different, the nodes of both *Xanthophyllum*, the tree genus, and *Salomonia*, the small herb genus, are typically common with unilacunar type. Thus, the results support the data from morphology and DNA sequences that *Salomonia* is more closely related to Polygaleae than Moutabeae and Xanthophylleae (Hutchinson, 1967; Eriksen, 1993b; Persson, 2001).

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