VI. SUMMARY AND CONCLUSION
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The "milk-worts" comprise the wide spread but predominantly tropical family Polygalaceae, having a peculiar design of zygomorphy and there seems to be no intermediate forms connecting them with less particularized groups. Apart from their scientific significance, some of the members of the family are also economically important due to medicinal value. An attempt has been made to consolidate the taxonomic data of the family.

This investigation purports to deal with the leaf histomorphology, pollen morphology, seed micro-morphology and embryology of the Polygalacean members available in South India. The work was carried out to utilize the variations of different features in the delimitation of taxa at different taxonomic hierarchy and to analyse the evolutionary trends within the family. The foliar epidermal features, histochemistry, pollen morphology, seed micro-morphology and embryology are investigated by conventional
methods coupled with light and electron microscopy. The parameters under investigation are:

(1) Stomatal studies - stomatal index and stomatal frequency

(2) Palisade ratio

(3) Histochemistry of foliar epidermis

(4) Pollen morphology

(5) Seed morphology

(6) Embryology

Twenty species belonging to three genera, *Salomonia* DC., *Polygala* Linn. and *Xanthophyllum* Roxb. were gathered for this work from Kerala, Tamil Nadu, Karnataka and Maharashtra. *S. oblongifolia*, DC., available only in South India, is a very delicate herbaceous annual. All species of *Polygala*, Linn. are small or large herbs except *P. arillata*, Ham. which is a shrub and *X. flavescens*, Roxb. a small tree, the sole representative in South India. The roots of all these plants when plucked fresh from soil possess a characteristic odour - the sure mark of Polygalacean identity. The floral characters, fruits, seeds and pollen are almost alike in all the members and contribute to the uniqueness of the family among the angiosperms. Some of the plants have high medicinal value and are of ethnobotanical significance.
The leaves are amphistomatic in majority of the taxa except *P. arillata*, Ham. and *X. flavescens*, Roxb. where they are hypostomatic. The stomata are distributed all over the leaf surface without any definite pattern of orientation. The distribution of stomata on both surfaces of leaves is highly variable, but the pattern of variation is taxon specific. The stomatal indices and stomatal frequencies are high on the lower surface. Stomatal index is almost constant within species which are collected from even different ecological conditions. Stomatal frequency values are highly variable and proved to be of little taxonomic value. The stomata encountered in the members of the family are of the anomocytic type. In most of the plants, stomatal abnormalities like contiguous stomata, guard cells with cytoplasmic connection, degenerating guard cells, unequally sized guard cells, stomata with single guard cell and giant stomata are observed.

The reliability of palisade ratio as a taxonomic character is emphasised by the present study. This criterion is more useful in delimiting the taxa at intra-specific levels than at generic levels. The palisade ratio was constant and specific for each and every taxon, even when they are collected from extreme environmental conditions.

Histochemical localization of plant metabolites like starch, polyphenols, insoluble polysaccharides, sulphated and carboxylated
polysaccharides, proteins and lipids in the foliar epidermis of eighteen taxa has been carried out. The guard cells of all the taxa contain prominent starch grains and their number, size and orientation vary in the different species. The number of starch grains ranged from 4 to 14 and the colour from brown to black. The maximum number of starch grains was recorded in *P. arvensis*, Willd. and the minimum in *S. oblongifolia*, DC. In the degenerating guard cells, the starch grains are present at the polar region.

Insoluble polysaccharides and polyphenols are localized in epidermal cells and guard cells with toluidine blue O dye. This dye gives a differential staining reaction to these two compounds. Phenolic compounds when present, take up a turquoise blue colour and were frequently observed in the epidermal, subsidiary and guard cells. Their concentration in the cells of the different taxa shows remarkable differences which can be due to the influence of different environmental factors. Higher concentration of phenolics was observed in woody plants like *P. javana*, DC.; *P. arillata*, Ham. and *X. flavescens*, Roxb.

With PAS reaction for sulphated and carboxylated polysaccharides, the subsidiary cells and epidermal cells stained almost equally well but the guard cells developed a deeper pinkish-red colour. This indicated more concentration of these
polysaccharides in the guard cells. Proteins are present in the cytoplasm of epidermal, subsidiary and guard cells of all the taxa in an amorphous form. The plastids of the guard cells also stained well indicating the presence of proteins. The nucleus of the epidermal cells shows positive staining reaction for proteins. Black lipid granules are observed in the cytoplasm of all the cells. More number of granules was noted in the subsidiary and epidermal cells.

Pollen morphology has been studied with the help of light microscopy and scanning electron microscopy on eighteen species of the three genera. Morphological analysis was based on aperture characters, exine ornamentation, exine strata and the size and shape of pollen grains. Polygalaceae is a stenopalous family, showing only one basic type of pollen grain but with variations in aperture number, size and shape of the grain and exine thickness. The number of apertures varies from 9 as seen in X. flavescens, Roxb. to 22 in P. chinensis, Linn. P. crotalariodes, Ham., P. elongata, Klein and P. japonica, Houtt. and their position is always zonal. In these multicolpate grains, the colpi from the upper and lower sides fuse in the middle to form the ora. Here the ora is a compound one, formed by the fusion of several os and so the grains are described as stephanocolporate. All the furrows meet at the equatorial region, fuse with collars of the upper and lower halves to form an operculum or an equatorial ring or girdle. This
is a characteristic feature of the pollen grains of the members of this family and also a sure mark of identification. The size of the pollen grains varies from 112 μm to 159 μm and they belong to two groups - small and medium. The different pollen shapes encountered are prolate, subprolate and prolate spheroidal.

Both light microscopic and scanning electron microscopic studies were carried out on the seed surface morphology of twenty species belonging to the three genera. Certain standards are proposed for the study of morphological features of seeds. The length and breadth of seeds are calculated as main axis (MA) and cross axis (CA) respectively. The size, shape and testa microsculpture represent characters of taxonomic and phylogenetic significance. The position of hilum was considered as the primary, the presence or absence of a strophiole as secondary and the nature and distribution of surface hairs as tertiary characters for the basis of evolving standards for the description of seeds. Of the three genera selected for the present study, only Polygala, Linn. had strophiolate seeds. The estrophiolate seed of X. flavescens, Roxb., protected inside the single indehiscent fruit, was very large compared to the seeds of the other two genera.

In Polygala, Linn. and Salomonia, DC. the fruits are laterally compressed, dehiscent, bearing two seeds, one in each locule. Seeds of S. oblongifolia, DC. are very small. In all the
species of *Polygala*, Linn. except *P. arillata*, Ham., the seed surface was covered with acicular hairs. Based on the distribution pattern of hairs and the nature of the trilobed caruncle, the seeds exhibited great diversity. Considering the above mentioned characters, the following conclusions are made with regard to the seed morphology of Polygalaceae:

1) The indehiscent single seeded fruits and seeds with glabrous testa surface are primitive characters (eg. *X. flavescens*, Roxb.).

2) Laterally compressed seeds with prominent surface ornamentations have the next order of importance (eg. *Polygala*, Linn.).

3) Globose seeds with large strophiolar lobes almost entirely covering the seed but lacking surface hairs differentiate *P. arillata*, Ham. from other species of *Polygala*, Linn.

4) The nature and form of strophiole varied greatly among the different species of *Polygala*, Linn.

5) Based on distribution of hairs, three major types of seeds could be recognized – (a) surface densely hairy (b) surface moderately hairy except at the basal region where the hairs are longer and denser and (c) surface only radially hairy, exposing the seed outline.
6) Small dehiscent two seeded fruits and seeds with rugulate testa surface of *Salomonia*, DC. form an intermediate genus between *Xanthophyllum*, Roxb. and *Polygala*, Linn.

It may be concluded that the presence or absence of caruncle and surface hairs on seeds are remarkable features and have taxonomic and phylogenetic significance.

The embryological studies of the three taxa, *S. oblongifolia*, DC.; *P. elongata*, Klein ex Willd. and *X. flavescent*, Roxb. include features like development of anther, wall layers, microsporogenesis, structure of pollen grains, ovule ontogeny, megasporogenesis and structure and development of seed coat and caruncle. In *S. oblongifolia*, DC., there are four monadelphous stamens with 1 mm long filaments basally united into a split staminal tube, that is also adnate to the corolla. The anthers open by apical pores. *P. elongata*, Klein ex Willd. has eight monadelphous stamens with filaments connate to form a tube, split on one side and adnate to corolla. The anthers dehisce by apical pores as in *S. oblongifolia*, DC. There are eight stamens in *X. flavescent*, Roxb. which are almost free and the anthers are oblong, erect and dehisce longitudinally by double slits. The development of anther in all the three plants is of the typical dicotyledonous type. The sporogenous tissue is enclosed by a four-layered anther wall, comprising an epidermis, an endothecial layer, a middle layer and the inner most
tapetum. The tapetum is of the secretory type and its cells become binucleate at the beginning of microspore formation. In a cross section of a young anther lobe, six to eight sporogenous cells are seen in *S. oblongifolia*, DC. and *P. elongata*, Klein ex Willd. while eight to ten cells are seen in *X. flavescens*, Roxb. The tetrads of uninculeate haploid microspores when released assume a spherical form and increase in size. The pollen grains are stephanocolporate, prolate to prolate-spheroidal and provided with a thick, psilate exine.

In *S. oblongifolia*, DC. and *P. elongata*, Klein ex Willd., the ovary is bilocular, laterally compressed, with one ovule in each chamber. The ovary in *X. flavescens*, Roxb. is globose, sessile, unilocular with two to four developing ovules, but when mature there exists only one surviving ovule. The ovules in all the three taxa are anatropous, pendulous from apex in *S. oblongifolia*, DC. and *P. elongata*, Klein ex Willd. and obliquely parietal in *X. flavescens*, Roxb. The ovules are bitegmic with each integument being two cell layers thick. The endosperm present in *S. oblongifolia*, DC. and *P. elongata*, Klein ex Willd. is copious while it is absent in *X. flavescens*, Roxb. In *S. oblongifolia*, DC. and *P. elongata*, Klein ex Willd. the cotyledons are moderately thick while in *X. flavescens*, Roxb. they are fleshy, extremely thick and planoconvex.

In *P. elongata*, Klein ex Willd. cells of the upper region
of outer integument divide to form a massive exostome which later produces three lobes that grow downwards along the seed so as to cover the hilum or even below. The aril lobes are glabrous but on the upper side they bear a loose tuft of hairs. Colour of the caruncle is white to pale yellow and very prominent in the mature seed. In the remaining portion of the seed, the outermost layer of the outer integument differentiates to form an epidermis with a thick outer wall. From these epidermal cells protrude one-celled, long acicular hairs which ensheath the entire seed surface.

The flowers of Xanthophyllum, Roxb. are different from those of Polygala, Linn. and Salomonia, DC. when fully grown. In Xanthophyllum, Roxb., the fusion among petal lobes and among stamens is lacking, and the adnation of stamens to petals is only at the base. The nature of dehiscence of the anthers in Xanthophyllum, Roxb. differs from that of Polygala, Linn. and Salomonia, DC. The genus Xanthophyllum, Roxb. has a thick walled, indehiscent fruit with limited number of ovules.

Any extra tissue or appendage found on the seed surface contributes to its primitiveness. Greater the protection, more the primitiveness. In the present study Salomonia, DC. has estrophiolate seeds in dry dehiscent bilocular fruits while Polygala, Linn. has strophiolate seeds in bilocular dehiscent fruits and Xanthophyllum, Roxb. bears estrophiolate seeds in thick walled unilocular
indehiscent fruits. Hence it is proper to consider the seeds of *Salomonia*, DC. advanced when compared with the highly ornamented seeds of *Polygala*, Linn. and the estrophiolate but well protected seeds of *Xanthophyllum*, Roxb.

The chapters so far discussed reveal the immense scope for the present day taxonomists to further enhance the understanding on the systematics and evolutionary trends of the world's valuable flora. Though the family Polygalaceae does not have much of commercial significance so far, the morphological, palynological, embryological and seed studies have revealed its unique characteristics which will be helpful in the further analysis of their role in nature and evolution of plant kingdom. It is highly essential to preserve the rare species of Polygalaceae which contributes a great deal to biodiversity. Eventhough there is enough literature on the medicinal utility of certain Polygalaceous members, it has not been optimally and commercially utilized. This may be due to the insufficiency of biochemical and biotechnological studies which may help in its commercial application. Therefore it requires further research by employing modern techniques for a better and deeper comprehension about the family.