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Abstract

Pollen morphology of one species of *Mitracarpus*, *Richardia* and four species of *Spermacoce* were studied in the present paper. The specimens were collected from Pyin Oo Lwin Township and pollen recorded by using light microscope and photomicrographs. All of the grains were observed one type of aperture (colpate), exine sculpture (distinctly reticulate) and the shape in oblate type. The number of aperture (colpi) are varied in the studied species. The size of the pollen were small, medium or large. The classification of pollen morphology has been described on the basis of shape, size, apertures, sculpture patterns and pollen wall stratification.

Introduction

Rubiaceae family is widely distributed in the tropic and subtropic with some species represented in the temperate regions. Most tropical species are trees or shrubs, while all temperate ones are herbaceous.

Palynology was coined by Hyde and Williams in 1944, to denote pollen and spore science; they chose this term based on the Greek words *paluno* meaning "to sprinkle" and pale meaning "dust" (Latin for pollen). The word pollen, originally meaning "fine flour", deals chiefly with the walls of pollen grains and spores, not with their live interior (Erdtman 1952).

Pollen analysis can be tool for the reconstruction of past vegetation and environments, and its also be applied concerning climate change, archaeology, geology, honey analysis and forensis science (Moore, Webb and Collinson 1991). Pollen in the atmosphere causes an allergic reaction in many peoples and the study of the nature of this sensitivity involves an understanding both of the chemical nature, the periodicity of pollen release and its mode of transport.

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Pollen have been used for a wide variety of basic as well as applied studies, eg., plant taxonomy and breeding, genetics, palynology, large scale production of haploid and homozygous diploids, control of fertilization, parthenocarpy, chemotropism, incompatibility reactions, the nutritive value for insects and human beings, allergic responses in man, cell wall formation, and role of nucleic acids in growth and development (Vasil 1987).

Walker and Doyle (1975) stated that, angiosperms have two basic pollen grains, the monosulcate and tricolpate. Monosulcate types are boat - shaped with one long germinal furrow and aperture. It is characteristics of primitive dicotyledons, the majority of monocotyledons, cycads and pteridosperms. Palynologists agree that the first flowering plants probably had monosulcate grains.

The living pollen grain of an angiosperm has a wall that is made up of two layers. The outer layer is called the exine and is composed of a very unusual substance, sporopollenin (Zetzsche 1932), together with smaller quantities of polysaccharides (Rowley *et al.*, 1981), sometimes referred to as the glycocalyx. The inner layer, or intine, is of cellulose and very similar in construction to an ordinary plant cell wall. The main aim of the present study to evaluate the value of pollen features in identification of the taxa of Rubiaceae.

Materials and Methods

1. Plant Collection

The plants were collected from Pyin Oo Lwin Township. All the collected species are recorded by photographs while flowing times. Describing and classifying of the species are made by fresh specimens.

Pollen collection

Pollen samples were freshly collected from the anthers of blooming flowers. For each species pollen was stored in glass vials with 1cc of glacial acetic acid and the specimen was labelled with the scientific names.

Methods

Acetolysis of pollen grains (Erdtman 1952)

The specimen in a glass vial was crushed with a glass rod and 1cc of acetic acid was added to it. The above mixture was transferred into a test tube and 5-9 drops (depending on the quantity of the specimen) of concentrated sulphuric acid was added. The test tube was put in a water-bath (in a beaker of water) for 15-30 minutes (depending on the size, sculpturing and structure of the pollen grain). On cooling, the material was diluted with distilled water and transferred to a centrifuge tube and centrifuged for 20-30 minutes. This process was repeated at least twice, decanting the distilled water each time. Glycerine jelly with saffranin was added to the residue. This was then slightly warmed to evaporate the remaing water and finally cooled and stored in air-tight vials.

Preparation of glycerine jelly

The glycerine jelly was prepared according to Kisser's formula (Erdtman 1952). 50 gms gelatin, 150cc of glycerine and 7gms of hpenol crystals were mixed with 175 cc of distilled water in a beaker and stirred with a glass rod. This was heated for about 3 hours in a water-bath till homogeneous and 0.05 gm of safranin was finally added before removal and storage. Only the amount of material needed should be removed from the storage bottle. A water bath was used for warming the glycerine jelly as it should not be directly heated to prevent overheating.

Slide Preparation

The sample bottle was warmed in a water bath and stirred with a glass rod. A drop of jelly was taken out and placed on the glass slide, and then covered with a glass cover slip. The glass slide well mounted with pollen sample were examined under light microscope with (X 400) and photomicrograph by camera (DSC-W 300) SONY. The size of the pollen were measured by polar axis and equatorial diameter when observed from the equatorial view, and 20 pollen from each species were measured and recorded.

Results

The present research consists of 6 herbaceous species of Rubiaceae.

A. Pollen key to the species

1. Colpi more than 15 ---------- *Richarsia brasiliensis*
1. Colpi less than 14 ---------- 2
   2. Colpi longicolpate ---------- *Mitracorpus hirtus*
   2. Colpi brevicolpate ---------- 3
3. Grain more than 45 μ length ---------- 4
3. Grain less than 40 μ length ---------- 5
   4. Exine more than 3.8 μ thick ---------- *Spermacoce lattifolia*
   4. Exine less than 2.8 μ thick ---------- *S. articularis*
5. The lumina more than 1.5 μ in width ---------- *S. assurgens*
5. The lamina less than 0.8 μ in width ---------- *S. ramanii*

B. Pollen Description of Each Taxa

1. *Mitracarpus hirtus* (L.) DC., Prod. 4:527. 1830. (Fig. 1. A, B, C)

Myanmar name : Unknown

English name : Unknown

Flowering period : September to December

Penta-hexacolpate, suboblate, small, 20-25 × 23-28μ in length and breadth; amb 5- to 6-lobed; colpi longicolpate, 16-21 × 1.25-2.50μ in length and breadth; exine 2.5-3.0μ thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 0.5-1.0μ in width, the muri simplibaculate, about 0.25μ wide.

2. *Richardia brasiliensis* Gomes. Men. Ipecae 31. t. 2. 1801. (Fig. 1. D, E, F)

Myanmar name : Unknown

English name : Unknown

Flowering period : June to August

Polycolpate (colpi 16-21), zonocolpate, oblate, large, 55-60 × 70-85μ in length and breadth; amb circular; colpi brevicolpate, 18-20 × 1.25-2.00μ in length and breadth; exine 2.5-3.0μ thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 0.75-1.25μ in width, the muri simplibaculate, about 0.5μ wide.

3. *Spermacoce articularis* L. f., Suppl. Pl. 119. 1782. (Fig. 2. A, B, C)

Myanmar name : Unknown

English name : Unknown

Flowering period : September to December

Polycolpate (colpi 10-13), zonocolpate, oblate, large, 50-60 × 57-67μ in length and breadth; amb circular; colpi brevicolpate, 5-7 × 2.5-4.0μ in length and breadth; exine 1.75-2.50μ thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 1-2μ in width, the muri simplibaculate, about 1μ wide.

4. *Spermacoce assurgens* Ruiz & Pavon, Fl. Perce 1:60, t. 92. 1798.

(Fig. 2. D, E, F)

Myanmar name : Unknown

English name : Unknown

Flowering period : July to October

Polycolpate (colpi 8-10), zonocolpate, suboblate, medium, 28-33 × 32-36μ in length and breadth; amb circular; colpi brevicolpate, 3.75-4.20 × 1.00-1.25μ in length and breadth; exine 2.50-3.75μ thick, sexine thicker than nexine; scupturing distinctly reticulate, the lumina heterobrochate, 1.5-2.0μ in width, the muri simplibaculate, about 0.75μ wide.

5. *Spermacoce lattifolia* Aubl., Pl. Guiane Fr. 1:55, t. 19/1. 1755.

(Fig. 3. A, B, C)

Myanmar name : Unknown

English name : Unknown

Flowering period : July to September

Polycolpate (colpi 10-13), zonocolpate, oblate, large, 50-53 × 58-63μ in length and breadth; amb circular; colpi brevicolpate, 7.5-10.0 × 2.5-3.0μ in length and breadth; exine 4.0-4.5μ thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 1.00-1.25μ in width, the muri simplibaculate, about 0.75μ wide.

6. *Spermacoce ramanii* Sivarajan & Nair, Taxon 35:367. 1986.

(Fig. 3. D, E, F)

Myanmar name : Unknown

English name : Unknown

Flowering period : July to September

Polycolpate (colpi 8-10), zonocolpate, oblate, medium, 30-35 × 35-40μ in length and breadth; amb circular; colpi brevicolpate, 3.5-4.2 × 1.00-1.25μ in length and breadth; exine 2.5-3.0μ thick, sexine thicker than nexine; sculpturing distinctly reticulate, the lumina heterobrochate, 0.50-0.75μ in width, the muri simplibaculate, about 0.75μ wide.

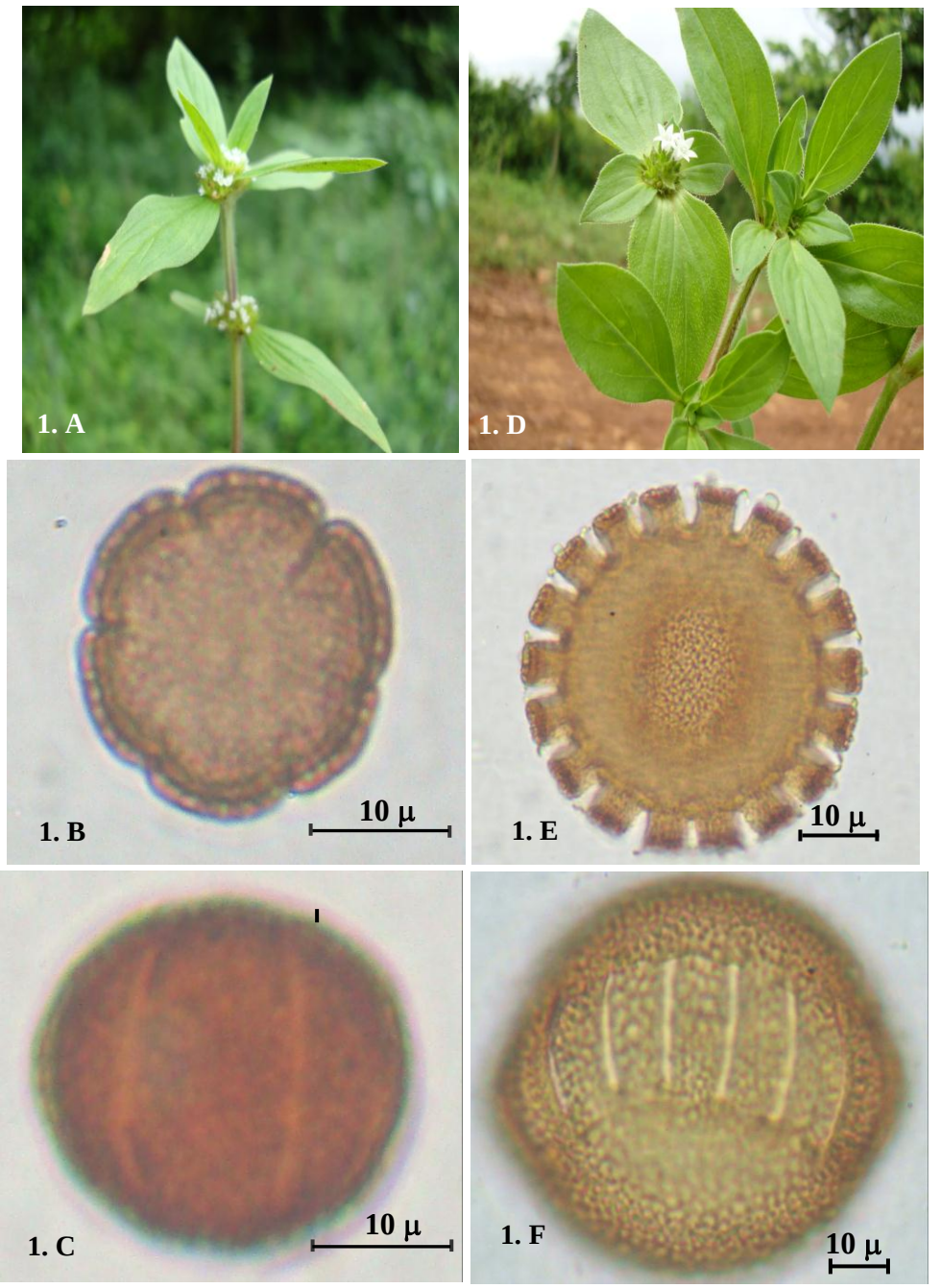

Figure 1. **A. Inflorescences, B. Polar view, C. Equatorial view of *Mitracarpus hirtus* (L.) DC.**
D. Inflorescences, E. Polar view, F. Equatorial view of *Richardia brasiliensis* Gomes

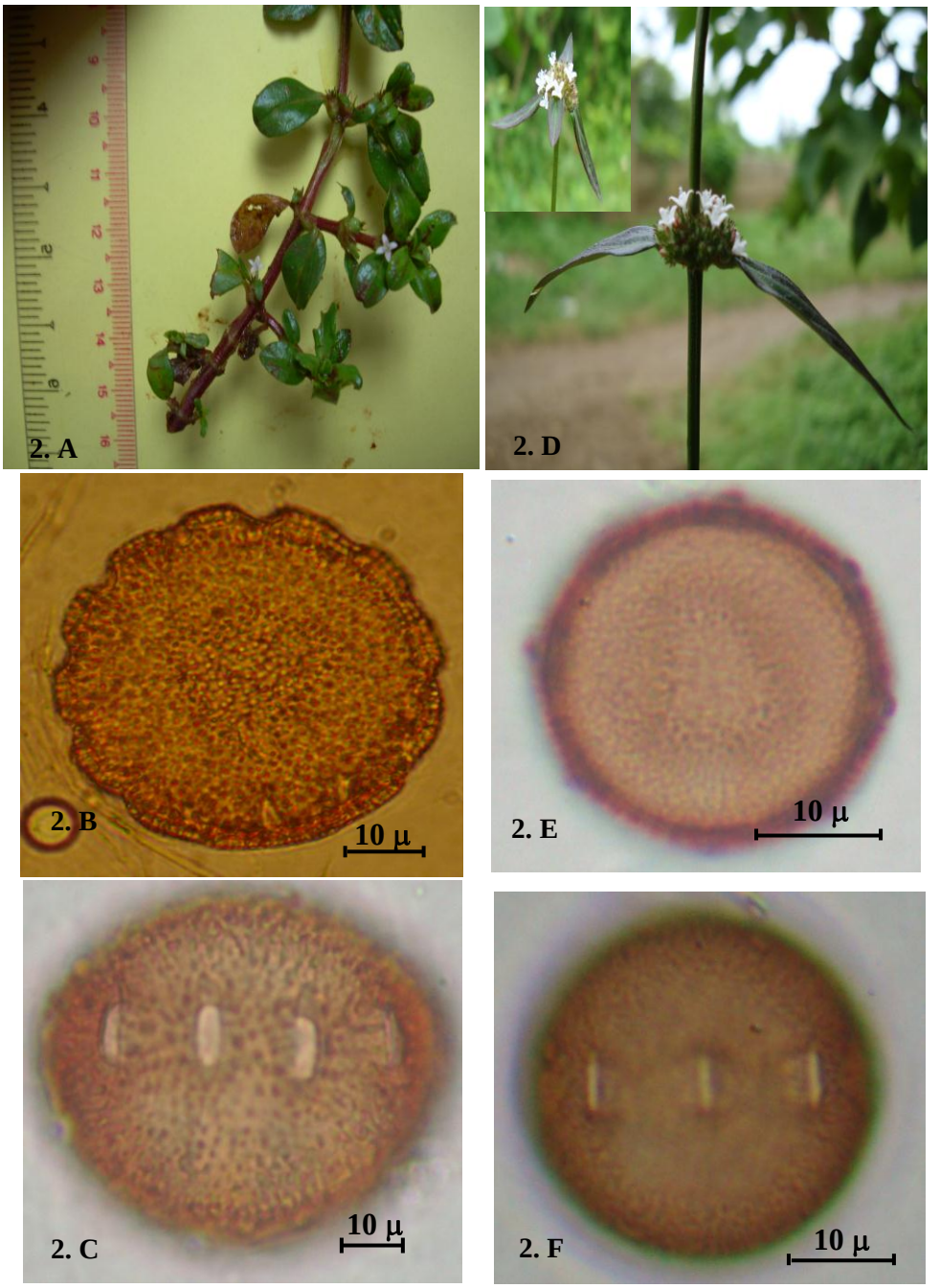

Figure 2. A. Inflorescences, B. Polar view, C. Equatorial view of *Spermacoce articularis* L.f.
D. Inflorescences, E. Polar view, F. Equatorial view of *Spermacoce assurgens* Ruiz & Pavon.

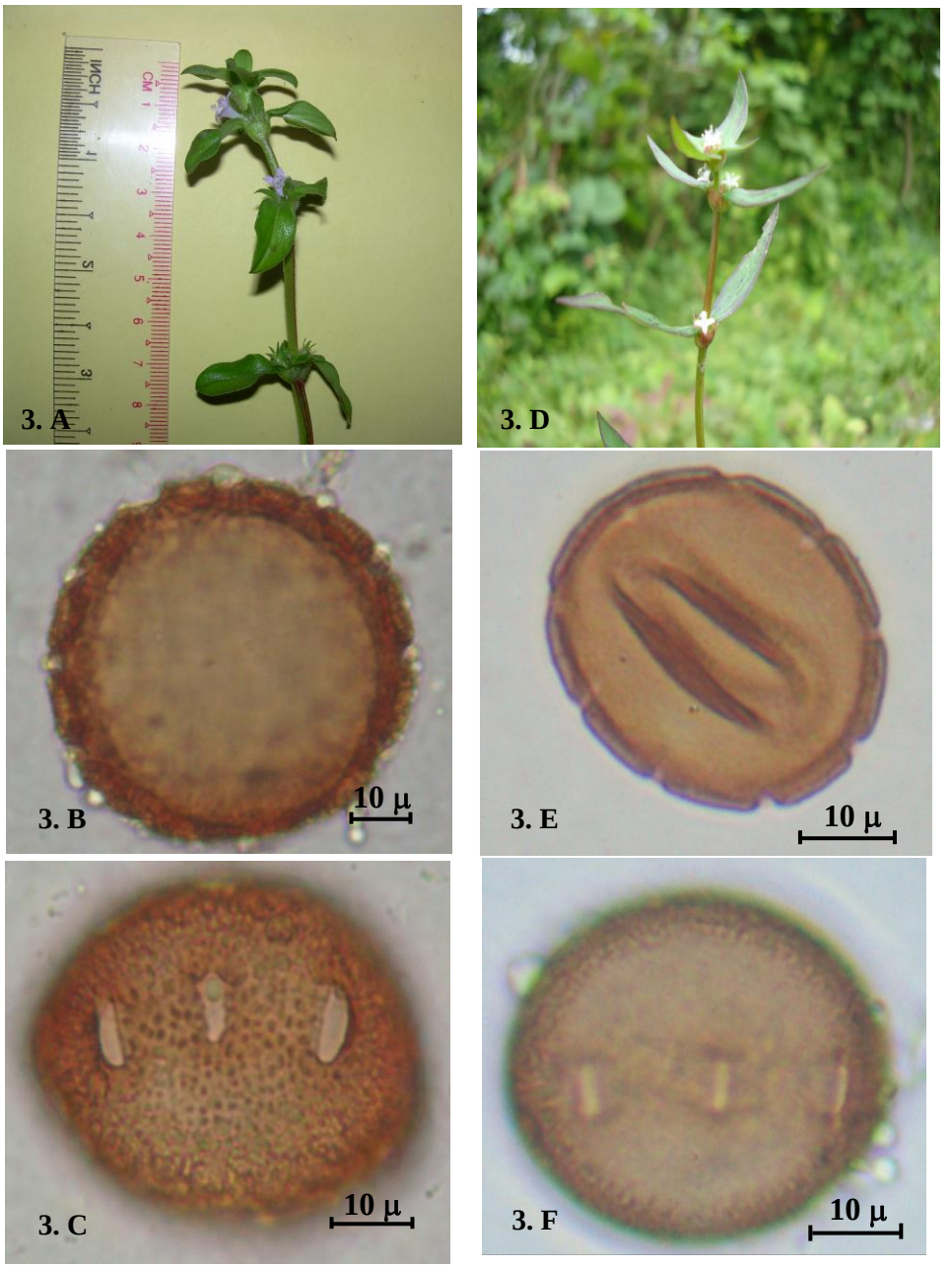

Figure 3. **A. Inflorescences, B. Polar view, C. Equatorial view of *Spermacoce lattifolia* Aubl.**
D. Inflorescences, E. Polar view, F. Equatorial view of *Spermacoce ramanii* Sivarajan & Nair

Discussion and Conclusion

The most important diagnostic features of the pollen are its shape, size, number of apertures and sculpture of exine ornamentation. These characteristics can be studied in fresh and fossilized pollen. In the present study six herbaceous species of Rubiaceae were observed from the freshly collected pollen grains.

The shape in equatorial view, pollen is described by the P/E ratio (P, polar axis; E, equatorial diameter). In the studied species, all of the grains were observed oblate or suboblate shape. The size of the pollen grains varies with the species on measurement of length. They are found small, medium or large size. The smallest axis is 20-25 μ in *Mitracarpus hirtus* and the largest axis is 55-60 μ in *Richardia brasiliensis*.

Apertures of Rubiaceae is colpate, porate and colporate grains. In the present study the grains were found only one of colpate type. The number of colpi is variable, the colpi (5-6) in *Mitracarpus hirtus*, (16-21) in *Richardia brasiliensis*, (8-10) in two species of *Spermacoce assurgens*, *S. ramanii* and (10-13) in two species of *S. articularis*, *S. lattifolia*. The colpi length are found longicolpate and brevicolpate. The longest axis (longicolpate) is 16-21 μ in *Mitracarpus hirtus* and the shorted axis (brevicolpate) is 3.5-4.2 μ in *Spermacoce ramanii*. Dessein *et al*., (2005 b) stated that, the length of the ectocolpus is very variable in the Rubiaceae.

In this study, the sculpturing pattern of pollen are occurred in one type of distinctly reticulate. It has been observed that the thickness of the exine also ranged from 1.75-2.50 μ in *Spermacoce articularis* and 4.0-4.5 μ in *S. lattifolia*. This shows that the thickness of exine also varies from one to another species.

All these extremely interesting pollen features are undoubtedly important for the future taxonomic studies. Thus, it is concluded that the pollen morphological data may be used for further identification of the taxa.

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