

## ORIGINAL RESEARCH ARTICLE

# Pollen morphology of selected species of the family menispermaceae in kerala

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## Abstract

The pollen grains of 10 species from 9 genera of the family Menispermaceae occurring in the state of Kerala were analysed using light microscopy (LM) and scanning electron microscopy (SEM): *Anamirta cocculus* (L.) Wight & Arn., *Cissampelos pareira* L. var. *hirsuta* (Buch.-Ham. ex DC.) Forman, *Cocculus hirsutus* (L.) W.Theob., *Cyclea peltata* (Lam.) Hook.f. & Thomson, *Diploclisia glaucescens* (Blume) Diels., *Stephania wightii* (Arn. ex Wight) Dunn., *Tiliacora acuminata* (Lam.) Hook.f. & Thomson, *Tinospora cordifolia* (Willd.) Miers, *Tinospora crispa* (L.) Miers ex Hook. f. & Thomson., *Fibraurea darshani* Udayan & Ravikumar. Pollen grains of menispermaceae family are usually radially symmetric, isopolar and tricolporate. The results revealed five types of pollen based on tectum ornamentation (finely reticulate, coarsely reticulate, reticulate-perforate, reticulate and coarsely perforate). Pollen grains also differ in their shape, aperture features and exine thickness. The study also suggested that each genus has its own distinguishing pollen features.

**Keywords:** Aperture, Exine, Menispermaceae, Pollen, Shape.

## Introduction

The Menispermaceae comprises 71 genera with 450 species (Kessler, 1993). In Kerala the family comprises 11 genera and 19 species (Nayar, 2014). Menispermaceae are mostly dioecious climbling plants and majority of species can be found in countries with tropical climate. The family had curved seed, hence the common name moonseed family. The family contains plants that are mostly lianas, sometimes small trees or shrubs and occasionally perennial herbs. Menispermaceae contains many medicinal plants with systematically recognized vital pharmacological activities (Troupin 1956, Botha 1975).

Pollen morphology characters of Menispermaceae were revised by Thanikaimoni (1968). The palynological study of the family Menispermaceae and their associations with the tribes Coscinieae, Fibraureae, Menispermeae, and Triclisieae were analysed (Ferguson 1975, 1978; Harley & Ferguson 1982; Harley 1985). The present study is to investigate the pollen morphological characters of Menispermaceae species in Western Ghats. The study helps to identify the diagnostic characters at the genus or species level and provide an understanding of the taxonomic interrelationship amongst the members of this family.

## Materials and methods

Pollens were excised from fresh samples and were dried naturally. Different samples were prepared for each species to confirm the strength of pollen characters within a species. The pollen grains were prepared and analysed using light microscopy and scanning electron microscopy (SEM) (Erdtman 1952). For light microscopy, pollen grains were prepared and mounted on glycerine jelly and observed under microscope. For Scanning electron microscopy, pollen grains were dusted onto specific Aluminium stubs coated with silver. The stubs were coated with gold and observed and photographed in Carl Zeiss EVO 18 Research. Qualitative and quantitative characters such as polar length, equatorial length and the length of the colpi were studied. The

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**How to cite this article:** Prabha MA, Kumar TR, Kumar NS. (2024). Pollen morphology of selected species of the family menispermaceae in kerala. *J. Indian bot. Soc.*, 104(4):222-228 Doi: 10.61289/jibs2024.04.20.1123

**Source of support:** Nil

**Conflict of interest:** None.

pollen shape was adopted: oblate-spheroidal, spheroidal, prolate-spheroidal, subprolate and prolate, according to the classification proposed by Erdtman (1952, 1960):

## Results

The plant species includes *Anamirta cocculus* (L.) Wight & Arn., *Cissampelos pareira* L. var. *hirsuta* (Buch.-Ham. ex DC.) Forman, *Cocculus hirsutus* (L.) W.Theob., *Cyclea peltata* (Lam.) Hook.f. & Thomson, *Diploclisia glaucescens* (Blume) Diels., *Stephania wightii* (Arn. ex Wight) Dunn., *Tiliacora acuminata* (Lam.) Hook.f. & Thomson, *Tinospora cordifolia* (Willd.) Miers, *Tinospora crispa* (L.) Miers ex Hook. f. & Thomson., *Fibraurea darshani* Udayan & Ravikumar.

### General pollen characteristics

Pollen grains were usually radially symmetric, isopolar and tricolporate. The average size of the pollen was usually small (10–25  $\mu\text{m}$ ). The shape of the pollen grain is considered by the relation between the length of the polar axis (P) and the equatorial axis (E). The shape of varied from oblate-spheroidal to prolate. The colpus length varied from species to species. In some species length of the colpus was same as that of the length of polar axis or three fourth of that of polar axis. Generally the colpus is covered by a sheath of membranes. As an extension of the membrane of the colpus a small circular outgrowth called operculum was present in some species.

The thickness of exine wall varied from 1-22  $\mu\text{m}$ . sexine was generally thicker than nexine. Tectum orientation is one of the most identifying characters among different species were shown in Table 1. Orientation varies from coarsely reticulate to mega reticulate. Lumina are the space inside the tectum. It was generally irregular in shape, compressed or free. Pictures of pollen in equatorial view, polar view and exine ornamentation under light microscope and scanning electron microscopy were shown in Plate 1, 2 and 3.

#### *Anamirta cocculus*

Pollen grains were small in size. The average length of the polar axis was 17.36 (15.44-18.82)  $\mu\text{m}$  and the average length of the equatorial axis 16.79 (15.90-17.26)  $\mu\text{m}$ . The pollen grains were prolate - spheroidal in shape. The average length of colpus was 15.90 (14.30-16.99)  $\mu\text{m}$  and tricolporate. The colpus was long, narrow and sunken and ends of colpus were acute and colpus membrane is granular-papillose. The endoapertures was elliptical in shape. The total exine thickness was 1.60 (1.04-2.11)  $\mu\text{m}$  and the sexine was thicker than nexine. The tectum ornamentation was finely reticulate. Lumina were irregular and isodiametric.

#### *Cissampelos pareira* var. *hirsuta*

Pollen grains were small in size. The average length of the polar axis was 16 (14.74-17.36)  $\mu\text{m}$  and the average length of the equatorial axis 16.28 (14.81-18.66)  $\mu\text{m}$ . The pollen grain were oblate spheroidal in shape. The average length of colpus was 14.64 (12.05-15.26)  $\mu\text{m}$  and tricolporate. The colpus was

short, narrow, ends of colpus are acute and length of colpus membrane is two third of the polar axis with a smooth circular operculum. The endoapertures was circular in shape. The total exine thickness was 1.28 (1.00 -1.50)  $\mu\text{m}$  and sexine was thicker than nexine. The tectum ornamentation was coarsely reticulate. Lumina were variable in size and isodiametric.

#### *Cocculus hirsutus*

Pollen grains were small in size. The average length of the polar axis was 15.14 (13.93 -17.16)  $\mu\text{m}$  and the average length of the equatorial axis 13.13 (12.15-17.48)  $\mu\text{m}$ . The pollen grains were sub-prolate in shape. The average length of colpus was 14.56 (12.60-17.23)  $\mu\text{m}$  and tricolporate. The colpus was long, narrow, ends of colpus are acute and the colpus membrane was granular and thickened at the equator. The endoapertures was circular in shape. The total exine thickness was 1.30 (1.00 -1.50)  $\mu\text{m}$  and sexine was thicker than nexine. The tectum ornamentation was reticulate perforate. Lumina were variable in size and isodiametric.

#### *Cyclea peltata*

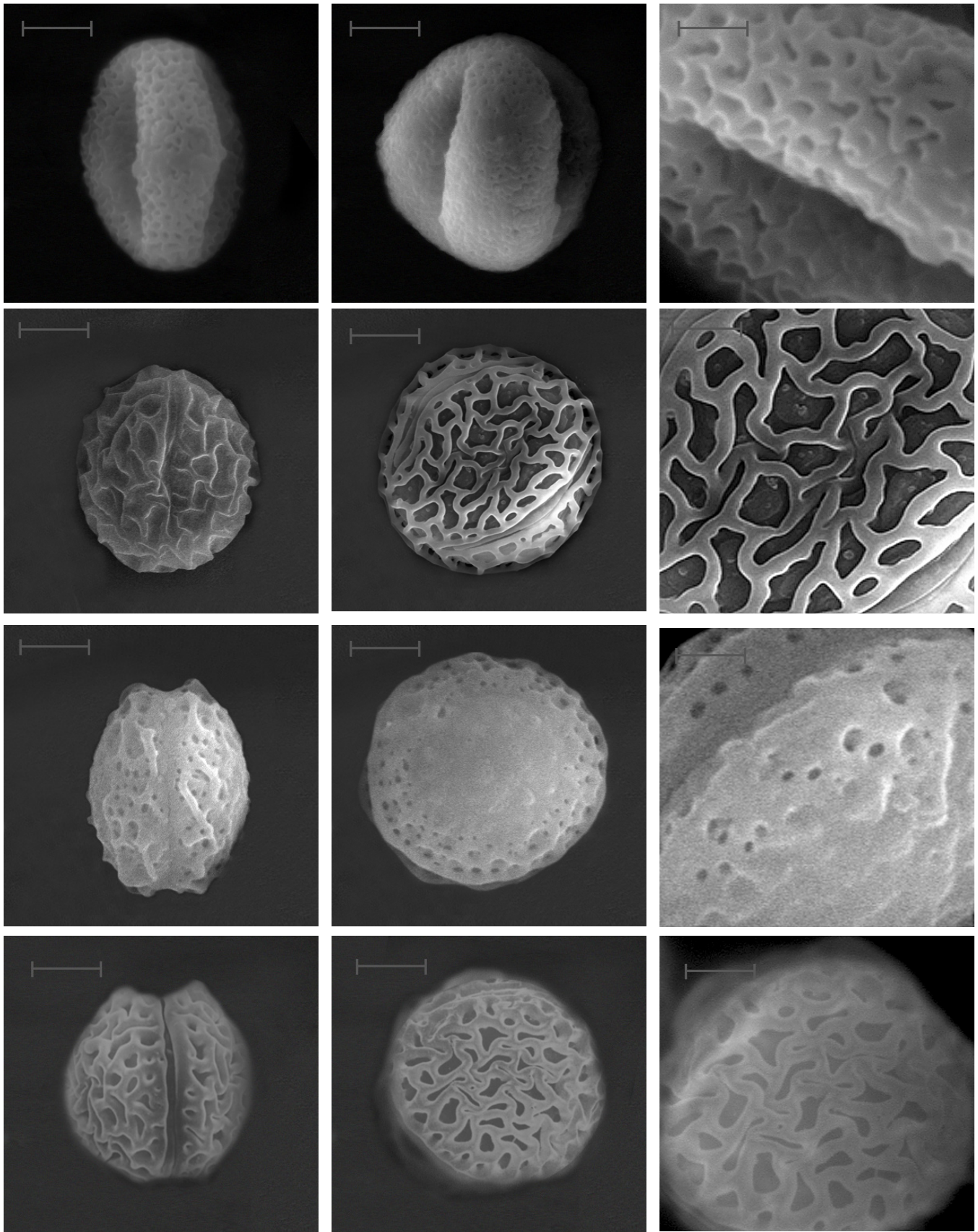
Pollen grains were small in size. The average length of the polar axis was 14.85 (12.26 -17.80)  $\mu\text{m}$  and the average length of the equatorial axis 14.22 (12.74-17.73)  $\mu\text{m}$ . The pollen grains were prolate-spheroidal in shape. The average length of colpus was 14.26 (12.00-16.49)  $\mu\text{m}$  and tricolporate. The colpus was long, narrow, ends of colpus are acute and the colpus membrane was three-fourth of the polar axis with a smooth circular operculum granular. The endoapertures was circular in shape. The total exine thickness was 1.79 (1.20 -2.55)  $\mu\text{m}$  and sexine was thicker than nexine. The tectum ornamentation was coarsely reticulate. Lumina were variable in size and isodiametric.

#### *Diploclisia glaucescens*

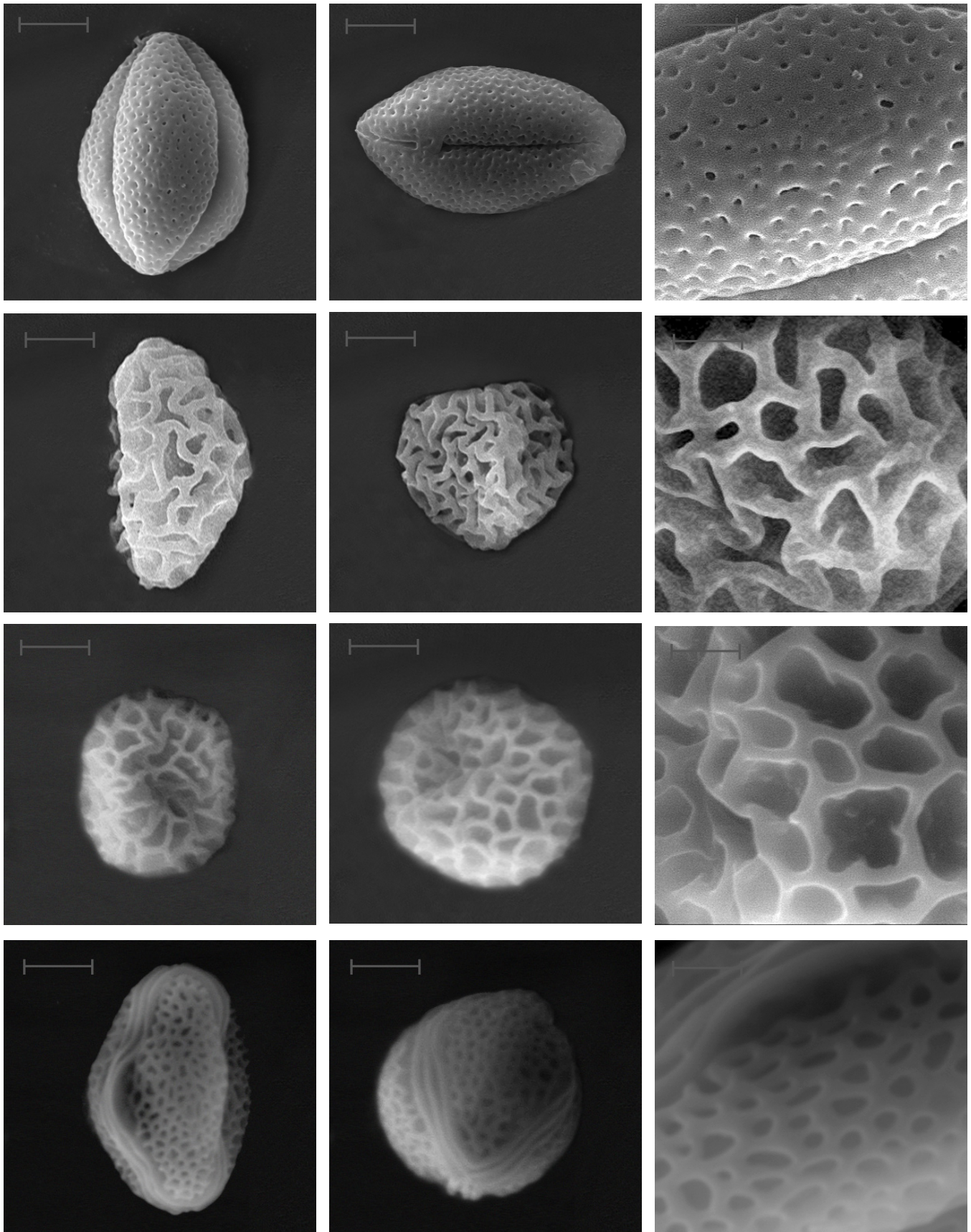
Pollen grains were small in size. The average length of the polar axis was 16.97 (14.49 -18.16)  $\mu\text{m}$  and the average length of the equatorial axis 14.76 (12.52-17.33)  $\mu\text{m}$ . The pollen grains were prolate-spheroidal in shape. The average length of colpus was 13.24 (12.45-16.25)  $\mu\text{m}$  and tricolporate. The colpus was long, narrow, ends of colpus are acute and the colpus membrane was three-fourth of the polar axis and the colpus membrane was granular. The endoapertures was circular in shape. The total exine thickness was 1.38 (1.10 -2.25)  $\mu\text{m}$  and sexine was thicker than nexine. The tectum ornamentation was reticulate-perforate. Lumina were variable in size and shape.

#### *Stephania wightii*

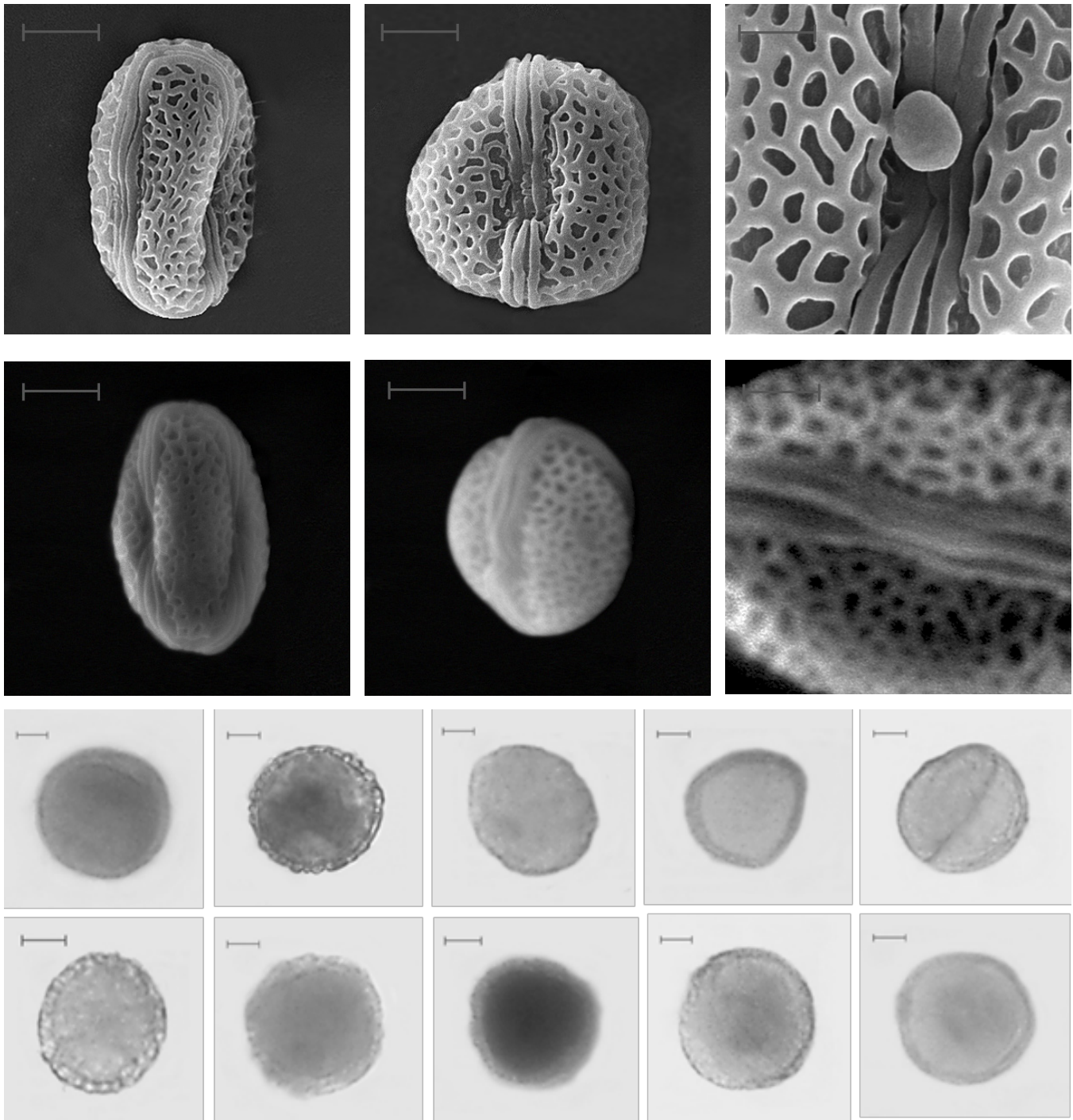
Pollen grains were small in size. The average length of the polar axis was 13.55 (11.24 -16.43)  $\mu\text{m}$  and the average length of the equatorial axis 14.91 (12.64-17.72)  $\mu\text{m}$ . The pollen grains were oblate-spheroidal in shape. The average length of colpus was 13.74 (11.75-16.35)  $\mu\text{m}$  and triporate. Pores were of irregular in shape and are small in size with pore membrane. The total exine thickness was 1.38 (1.10



**Plate 1:** Pollen grains of Menispermaceae. Equatorial view, Polar view and exine ornamentation under scanning electron microscopy 1-3 *Anamirta cocculus*, 4-6 *Cissampelos pareira* var. *hirsuta*, 7-9 *Cocculus hirsutus*, 10-12 *Cyclea peltata*.



**Plate 2:** Pollen grains of Menispermaceae. Equatorial view, Polar view and exine ornamentation under scanning electron microscopy 13-15 *Diploclisia glaucescens*, 16-18-*Stephania wightii*, 19-21- *Tiliacora acuminata*, 22-24 *Tinospora cordifolia*.



**Plate 3:** Pollen grains of Menispermaceae. Equatorial view, Polar view and exine ornamentation under scanning electron microscopy 25-27 *Tinospora crispa*, 28-30 –*Fibraurea darshani*.

Pollen grains of Menispermaceae using light microscope. 1-10 *Anamirta cocculus*, *Cissampelos pariera* var *hirsuta*, *Cocculus hirsutus*, *Cyclea peltata*, *Diploclisia glaucescens*, *Stephania wightii*, *Tiliacora acuminata*, *Tinospora cordifolia*, *Tinospora crispa*, *Fibraurea darshani*.

-2.25)  $\mu\text{m}$  and sexine was thicker than nexine. The tectum ornamentation was coarsely reticulate. Lumina were variable in size and isodiametric in shape.

***Tiliacora acuminata***

Pollen grains were small in size. The average length of the

polar axis was 20.59 (18.93-22.83)  $\mu\text{m}$  and the average length of the equatorial axis was 15.84 (14.76-17.59)  $\mu\text{m}$ . The pollen grains were sub-prolate in shape. The average length of colpus was 16.73 (14.59-17.25)  $\mu\text{m}$  and inaperatuate. The total exine thickness was 2.18 (1.50-2.25)  $\mu\text{m}$  and sexine was thicker than nexine. The tectum ornamentation is reticulate. Lumina

**Table 1:** Pollen types of the 10 species and their genera of the family Menispermaceae based on their tectum ornamentation.

Sl No	Pollen types	Tectum ornamentation	Genera	Species
1	Type 1	Finely reticulate	Anamirta	Anamirta cocculus
2	Type 2	Coarsely reticulate	Cissampelos	Cissampelos pariera var hirsuta
			Cyclea	Cyclea peltata
			Stephania	Stephania wightii
			Tinospora	Tinospora cordifolia Tinospora crispa
3	Type 3	Reticulate-perforate	Cocculus	Cocculus hirsutus
			Diploclisia	Diploclisia glaucescens
4	Type 4	Reticulate	Tiliacora	Tiliacora acuminata
5	Type 5	Coarsely perforate	Fibraurea	Fibraurea darshani

were large and variable in size and isodiametric in shape.

#### ***Tinospora cordifolia***

Pollen grains were small in size. The average length of the polar axis was 19.52 (17.87-21.72)  $\mu\text{m}$  and the average length of the equatorial axis 14.41 (12.35-16.46)  $\mu\text{m}$ . The pollen grains were prolate in shape. The average length of colpus was 16.80 (14.57-18.78)  $\mu\text{m}$  and tricolporate. The colpus was long, narrow, ends of colpus were acute and the colpus membrane was three-fourth of the polar axis and the colpus membrane was granular. The endoapertures was circular in shape. The total exine thickness was 1.84 (1.25-2.00)  $\mu\text{m}$  and sexine was thicker than nexine. The tectum ornamentation was coarsely reticulate. Lumina were variable in size and polygonal in shape.

#### ***Tinospora crispa***

Pollen grains were small in size. The average length of the polar axis was 24.48 (22.64-25.62)  $\mu\text{m}$  and the average length of the equatorial axis 17.50 (15.82-18.74)  $\mu\text{m}$ . The pollen grains were prolate in shape. The average length of colpus was 20.83 (18.97-21.74)  $\mu\text{m}$  and tricolporate. The colpus was long, narrow, ends of colpus are acute and the colpus membrane was three-fourth of the polar axis and the colpus membrane was granular. The endoapertures was circular in shape. The total exine thickness was 1.25 (1.12-2.00)  $\mu\text{m}$  and sexine was thicker than nexine. The tectum ornamentation was coarsely reticulate. Lumina were variable in size and polygonal in shape.

#### ***Fibraurea darshani***

Pollen grains were small in size. The average length of the polar axis was 15.47 (16.25-18.20)  $\mu\text{m}$  and the average length of the equatorial axis 13.79 (11.59-16.71)  $\mu\text{m}$ . The pollen grains were prolate-spheroidal in shape. The average length of colpus was 14.55 (12.38-17.82)  $\mu\text{m}$  and tricolporate. The colpus was long, narrow, ends of colpus are acute and the colpus membrane was three-fourth of the polar axis and

the colpus membrane was granular. The endoapertures was lalongate in shape. The total exine thickness was 1.09 (1.00-1.75)  $\mu\text{m}$  and sexine was thicker than nexine. The tectum ornamentation was coarsely perforate. Lumina were variable in size and polygonal in shape.

Based on the pollen features, all the studied taxa can be keyed out as below:

- 1a Pollen grains aperturate
- 2a Pollen grains tricolporate
- 3a Tectum ornamentation coarsely reticulate
  - 4a Pollen grain oblate spheroid ----- *Cissampelos pariera* var *hirsuta*
  - 4b Pollen grain prolate or prolate spheroid
    - 5a Pollen grain prolate
    - 6a Exine thickness <math>\leq 1.5 \mu\text{m}</math> ----- *Tinospora crispa*
    - 6b Exine thickness >math>1.5 \mu\text{m}</math> ----- *Tinospora cordifolia*
    - 5b Pollen grain prolate spheroid
      - 7a Pollen grains operculate ----- *Fibraurea darshani*
      - 7b Pollen grains not operculate ----- *Cyclea peltata*
  - 3b Tectum ornamentation reticulate perforate
    - 8a Pollen grain subprolate ----- *Cocculus hirsutus*
    - 8b Pollen grain prolate spheroid ----- *Diploclisia glaucescens*
  - 2b Pollen grains triporate ----- *Stephania wightii*
  - 1b Pollen grains inaperturate ----- *Tiliacora acuminata*

### **Discussion and Conclusion**

Nine genera of the family Menispermaceae in Kerala were studied. Each genus was palynologically distinct and could be identified by their pollen characters. The pollen grains studied are mostly dispersed in monads and are isopolar. The pollen shape of the genus *Anamirta*, *Cissampelos*, *Diploclisia* and *Fibraurea* were found to be prolate-spheroidal where as the *Cocculus* and *Tiliacora* was sub-prolate. Both the species of genus *Tinospora* showed prolate pollen grains. Five pollen types were identified based on their tectum ornamentation; finely reticulate, coarsely reticulate, reticulate-perforate, reticulate and coarsely perforate. Coarsely reticulate tectum were observed in the genus *Cissampelos*, *Cyclea*, *Stephania*, *Tinospora*.

The pollen grains were predominantly tricolporate; porate and inaperturate pollen grains were also observed and they were found to be derived pollen characters (Thanikaimoni 1986). The thickness of sexine and nexine pollen wall varied among different genus and mostly sexine was thicker than nexine. The length of colpi were three fourth of the length of the polar axis and in most of the genera colpus is long, wide and sucken.

Anjum and Mohammad (2003) reported morphological character of pollen of the family Menispermaceae from Pakistan. Based on the study two distant pollen types were recognized *Cocculus pendulus* type and *Tinospora malabarica* type. The former type had sub-prolate pollen with reticulate or reticulate-rugulate tectum whereas latter coarsely reticulate tectum. Twelve species of Menispermaceae in Brazil were analyzed by Teixeira et al (2013).

Prabhakar and Ramakrishna (2013) studied the palynodiversity of important climbers from Andhra Pradesh. These climbers are having variety in ethnomedicinal importance. *Tinospora cordifolia* is one of the climber they examined and reported that pollen grains were prolate and tricolporate. Colpi are linear and operculate. Sexine are thicken than nexine with microreticulate tectum. Hoot (2009) defined the apomorphic character of colporate pollen and the presence of an operculum were homoplastic condition. From the present study it was concluded that each genera have distinct pollen characteristics which can be used as a distinguishable character in identifying and classifying each genera and also useful for further taxonomic studies.

### Acknowledgement

The authors sincerely acknowledge Kerala State Council for Science, Technology & Environment (KSCSTE), and Government of Kerala for providing the financial assistance in terms of KSCSTE Research fellowship.

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