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Research Article

Pollen morphology of selected taxa of Ehretiaceae from Western Ghats, India.

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Abstract: Pollen Morphology is an important tool in the identification of a genera. Data on pollen morphology is used as a reference in other fields of palynology like allergic studies, melissopalynology, tracing the history of vegetation, genetic and evolutionary studies, climate change studies etc. Pollen morphology of two genera of Ehretiaceae family is studied using Scanning electron microscope. Palynological contributions are still fragmentary in the family Ehretiaceae of southern India. *Ehretia pubescens* Benth is a small tree belonging to Ehretiaceae family located at the foothill of Chamundi hill reserve forest which is a part of Western ghat near Mysuru and *Cormona retusa* (Vahl) Masam is a shrub found 3400ft above on top of Chamundi Hill. Pollen grains were acetolyzed and Scanning Electron Microscopic studies conducted to obtain data on pollen morphology. The study is conducted to assess the taxonomic significance of pollen morphology in relation to their phylogenetic origin of the two genera of Ehretiaceae in the region. The palynological evidence shows *Ehretia pubescens* with tri-zonocolporate and heterocolpate pollen grains and *Cormona retusa* with tricolpate pollen grain without the pseudocolpi. Both genera have foveolate tectum. Palynological data indicate that these two naturalized taxa ie. *Ehretia pubescens* and *Cormona retusa* belong to two different lineage of Ehretia and can be identified by their pollen morphology. Diversity in the pollen characters points towards the eurypalynous nature in Ehretiaceae.

Keywords: Pollen morphology; Palynology, Ehretiaceae, *Ehretia pubescens* Benth, *Cormona retusa* (Vahl) Masam, Colporate, Colpate, Foveolate.

Introduction

Apertures and sculpturing of exine wall make pollen grain a highly recognized object by which a genera and even a species can be recognized. (Scheel. R 1996; Perveen. A, 2000). The pollen grains of the family Boraginaceae showed great diversity in exine wall pattern and aperture structure which had been studied in different parts of the world (Ahn & Lee, 1986; Ghazaly,1995; Scheel, 1996; Perveen, 2000; Khahtamsaz, 2001; Retief & Wyk, 2001).

Traditionally Boraginaceae was divided into five subfamilies namely Boraginoideae, Cordioideae, Ehretioideae, Heliotropioideae and Wellstedioideae which was accepted by many earlier botanist. But based on molecular data and phylogenetic analyses (Gottschiling *et al.,* 2014) a consensus classification was proposed which suggest recognition of 11 families under Boraginales namely Boraginaceae s. str., Codanaceae, Coldeniaceae, Cordiaceae, Ehretiaceae, Heliotropiaceae, Hoplestigmataceae, Hydrophyllaceae, Lennoaceae, Namaceae and Wellstediaceae. (Luebert *et al.,* Boraginales Working group, 2016).

Ehretiaceae is identified as a major lineage of the Boraginales (Gottschling *et al.,* 2014, Luebert *et al.,* Boraginales Working Groups). Ehretiaceae family members are shrubs and trees, pantropical in distribution and existing in central America, Africa, East Asia and comprise of 150 species (Miller, 2003). Molecular data analysis indicated two clades of Ehretia. Ehretia I species with four parted endocarp and Ehretia II species with two parted endocarp with *Carmona retusa* as its closest relative (Gottschling *et al.,* 2014). Enough morphological data are not available to resolve the relationship within the Ehretiaceae.

The objective of the study is to obtain data on pollen morphology of *Ehretia pubescens* Benth and *Cormona retusa* (Vahl) Masam (Gamble, J.S., 1921), both being the natural component of Chamundi hill reserve forest located near Mysuru which is the part of Western Ghats and to assess the taxonomic relationship between the taxa and to evaluate interspecific and interfamilial relationship.

Ehretia pubescens Benth, a tropical dry deciduous tree, belonging to Ehretiaceae is present at Chamundi Hill reserve forest (Saldana,1996), Mysuru. Around eight trees are seen in the study area. *E. pubescens* is a small deciduous tree with a height of 7-10feet. Stem arise from the base, grey in color, simple pubescent leaves, elliptical-oblong with the entire margin. Inflorescences are in the terminal, dichotomously branched, biparous cyme.Flowers are white. fruit a

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drupe, globose and red in colour at maturity. Flowering is from June to July. (Fig.1A)

Sepals are five, valvate aestivation, green, pubescent and persist in the fruit. Petals are white and cream, five in number, imbricate aestivation. Stamens are five in number, epipetalous. Anthers are white, transparent succulent at anthesis and turn to brown at post anthesis stage. Bifid style with two stigmatic branches is prominent. Stigma and style are persistent after fertilization. (Fig.1A & B)

Figure 1: A & B- Ehretia pubescens
C & D Cormona retusa (Not scaled)

Cormona retusa is an evergreen shrub of 4-5 feet in height. The stem is brown and rough. The leaves appear in the cluster of three. Leaves are obovate. They are narrow near the petiole and broadens towards the apex. The apex of the leaf is toothed. Sepals are 5 and united with valvate aestivation. Sepals have unequal lobes. Corolla is white, gamopetalous and with imbricate aestivation. Andrecium is five, attached to the petal at the base. The position of the stamens is below the bifid style. Gynoecium is with bifid style and branched stigma which is persistent in the fruit. Bifid Style split till near the base. Fruit is a drupe and orange in color but dispersed as a single endocarp. (Fig.1C & D)

Materials and Methods

Study area- Chamundi Hills:

Chamundi hills are located 13 km in the southwest direction of Mysuru city. The average elevation is 1000 m (3300 ft.). The hillock has a periphery of c14 km and surface area of c.17 km^2. Scanty rainfall supports the tropical deciduous thorn-scrub type of vegetation in this reserve forest(Karnataka state Gazetteer,1988).

Collection of sample

There were around eight small trees belonging to *E.pubescens*. Flowers were collected randomly from all the eight plants. *Cormona retusa* was collected from the top of Chamundi hill located at 3200 ft. There were around ten shrubs in the region. The anthers at anthesis were removed and pollens collected from them.

Processing the pollen for SEM scan

Pollens were processed by acetolysis as per (Erdtman, 1943) and Scanning electron microscopic studies was carried out. The acetolyzed pollen grains were dried naturally at room temperature and then was transferred onto a stub covered with double-stick tape, sputter coated with gold in a polaran sputtering apparatus and photographed on a Leo 435 UP Scanning Microscope from Leo electron microscopy Ltd Cambridge at CISF unit of CFTRI. The sputter coating system was from Watford England model no E5100. The pollen grains are described as per Webb, Moore &Collinson (1991).

Results

Pollen Morphology

Ehretia pubescens: The pollen grains are shed as monad. The pollen grains are isopolar and radially symmetrical. The pollen grain diameter ranges from 11.35µm to19.03µm. The average diameter is 15.03 µm in the equatorial view. The grains are small (10-24µm). Pollen is subprolate to prolate (P/E1.18-1.739). It is circular to triangular at a polar view and elliptical in equatorial view. Apertures are trizonocolporate, three parallel-sided colpi with pores arranged on the equator. They are long and narrow extending between both the poles. The length of the colpus ranges from15.88µm to 21.19 µm and diameter from 1.273 µm to 1.817 µm at the equator.

The three porate colpi are arranged on the equator alternating with non-porate colpi which is situated exactly between the porate colpi. The distance between colpus to pseudocolpus is 3.99 µm.Apocolpus is convex. Non-porate colpi are narrower and longer than porate colpi. The pseudocolpi varies in from 22.19 µm to 25.86 µm. Endoapertures are large. They are 5.113 µm long and 1.32 wide µm and meridionally elongated (lolangate). Aperture membranes are granular. Exine tectum is foveolate. Mesocolpia is convexed. Mesocolpium distance is 6.34µm. There is a considerable variation in the size of the reticulum lumina or foveolae. Muri is wider than the lumina but of irregular width. Lumina diameter ranges from 0.035 µm to 0.153µm. (Fig 2).

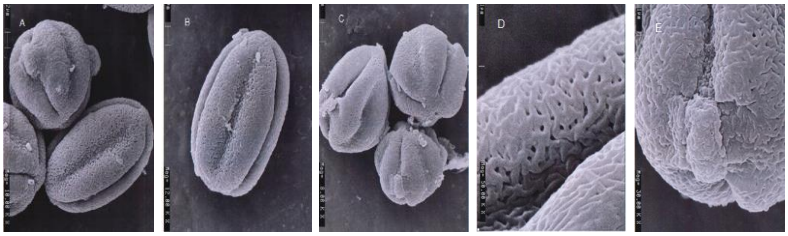

Figure 2 A-E: Pollen morphology of *Ehretia pubescens*. A & C: Pollen in polar and equatorial view showing porate colpus alternating with non-porate colpus; B: Single pollen in equatorial view showing longer and narrow pseudocolpi : D: foveolate tectum; E; Granular aperture membrane. Scale bar-A:2µm,10kx; B:2µm, 12kx; C:3µm, 8kx; D:1µm, 50kx; E:1µm, 30kx.

Cormona retusa: The pollen is shed as a monad. The pollen is radially symmetrical, isopolar, 3-colpate grain. The shape is elliptical-rhomboidal in the equatorial view and triangular in polar view (Fig.3A). In the equatorial view, the polar axis measures 18.70µm and equatorial axis is 27.21µm and in polar view ,the polar axis measures 22.22µm and the equatorial axis is 20.19µm. TheP/E ratio ranges from 1.455µm to 1.729µm. Apertures are 3 in number and colpate (endoaperture is seen not clearly in SEM picture), Colpus is narrow, slit-like and long (29.17µm), arranged equatorially (Zonocolpate). Mesocolpium measures 14.52µm and concaved. The presence of endoaperture was not evident. The lumina is wider in this region. The pseudocolpus is not present in the pollen grain. The tectum is foveolate and lumina dimension ranges between 0.262 µm-0.156 µm.(Fig.3)

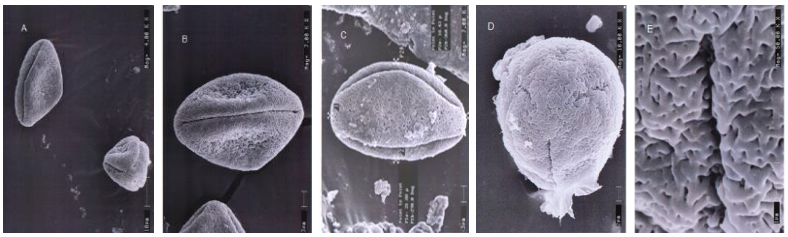

Figure 3 A-E: *Cormona retusa*. A:Pollen shape in polar and equatorial view, B&C: pollen showing colpus and mesocolpium . D: pollen in polar view. E: pollen showing tectum and aperture membrane. Scale bar-A:10µm,4kx, B&C:3µm, 7.00kx, D:2µm, 10.00kx, E:1µm,10.00kx.

Discussion

The findings of this study indicates that pollen grains of *Ehretia pubescens* and *Cormona retusa* are triaperturate, shed as monad, isopolar, small sized grain (below24 µm), with foveolate tectum, but *E. pubescens* has colporate aperture alternating with pseudocolpi (fig.2 A & B) whereas the *C. retusa* is a colpate grain and lacking pseudocolpi (fig.3A & B). *E.pubescens* and *C.retusa* can be identified by their respective pollen morphology. The *E.pubescens* shows pollen grains with pseudocolpi which are absent in *C.retusa*. The pollen grains of *E.pubescens* are trizonocolporate and *C.retusa* shows pollen grain with trizonocolpate. The results of my study show that palynological evidence does support the molecular studies conducted which support two different lineages of *E. pubescens* and *C.retusa*. (Gottsching *et al.,* in 2014).

Pollen morphology of species belonging to Ehretia from different parts of the world showed diversity in pollen aperture and exine sculpturing. (Table 1). Retief and Van Wyk (2001) studied the pollen grains of Ehretia belonging to southern Africa and found that two types of pollen exist in the genus. Hexagonal (equatorial view)pollen grains with broad apertures and forrow like mesocolpium centers eg pollen of *E.rigida* subsp. rigida. The second type of pollen grains were elliptical or rectangular (equatorial view) pollen grains with narrow apertures and furrow like mesocolpium centers occur. The pollen morphology of *E.pubescens* resembels *E.rigida* in having furrow like mesocolpium center but differed from it in having foveolate tectum.

Table 1: Comparative pollen characters in different species of Ehretia from a different part of the world

→ Pollen character↓	*Ehretia pubescens*	*Cormona retusa*	*Ehretia ovalifolia*[2]	*Ehretia laevis*[1]	*Ehretia buxifolia(india)*	*Ehretia* [1]*obtusifolia*
Dispersal unit	Monad	Monad	Monad	Monad	Monad	Monad
Polar axis and equatorial axis	18.7µm 15.8 µm	27.21 µm 18.70 µm	20.76µm, 19.04µm	17 µm	25.0 µm 22.5 µm	15.6-20µm 15.6-19.5µm

Shape	Subprolate-prolate	Prolate	Oblate spheroidal-prolate	Prolate spheroidal	Prolate spheroidal -spheroidal	Spheroidal
Symmetry	Isopolar	Isopolar	Isopolar	Isopolar	Isopolar	Isopolar
No. of aperture	3	3	3	3	3-4	3-6
Position of aperture	Equatorial	Equatorial	Equatorial	Equatorial	Equatorial	Equatorial
Aperture structure	Heterocolpate	Isocolpate	Isocolpate	Isocolpate	Isocolpate	Isocolpate
Pseudo aperture	Yes	No	No	No	No	No
Tectum	Foveolate	Foveolate	Foveolate	Reticulate - rugulate	Reticulate-reticulate rugulate	Foveolate but coarsely regulate in the center.
Mesocolpium	Convex	Concave	Slightly concave	Concave at the center of mesocolpia	-	Slightly concave

1–Description by Anjum Parveen, 1995(from Pakistan); 2–Described by Ahn and Lee, 1986 (from Korea)

Anjum. P *et al.,* (1995) studied pollen grains of 20 genera from Pakistan belonging to the Boraginaceae family which included *Ehretia obtusifolia* and *Ehretia laevis* from Ehretiaceae family. The pollen grains of *E.obtusifolia* is characterized by 6-colporate, zonoaperturate, striate-regulate tectum with lalongate endopore. *E.laevis* differed from *E.obtusifolia* in having reticulate-rugulate tectum. *E.obtusifolia* from Iran showed tricolporate, foveolate tectum. (Khahtamsaz, 2001). The pollen grain of *E.pubescens* differed from *E.obtusifolia* and *E.laevis* in containing pseudocolpi, lolongate endoaperture and foveolate tectum. *C.retusa* differed from *E.obtusifolia* and *E.laevis* in having tricolpate aperture and foveolate tectum.

Ahn & Lee (1986) studied 13 genera belonging to Boraginaceae from Korea. He described pollen grain of *E.ovalifolia* Hassk. as tricolporate with a foveolate tectum which is different from *E.pubescens* in lacking psedocolpi. *C.retusa* lacks colporate grain. Thus species of Ehretiaceae shows eurypalynous nature and can be identified by its pollen morphology. Phylogenetically among the two taxa studied the tricolpate pollen of *C.retusa* is considered as primitive and tricolporate with pseudocolpus is derived from tricolpate grain. (Walker,1974:Ahn &Lee,1986). All the species of Boraginaceae from santa catarina state (southern Brazil) have an isopolar, radially symmetrical, zonoaperturate, tricolporate pollen grains(Scheel *et al.,* 1996) which is also observed in the Ehretiaceae members studied in India.

The *C.retusa* resemble Borageineae, lithospermae tribes of Boraginaceae in having isocolpate pollen grains. *E.pubescens* resembles cynoglosseae, eritrichieae and Heliotropiaceae in having pseudocolpi alternating with poratecolpi but lack constriction at the equatorand the equatorial band found in Eritrichieae (Hargrove and Simpson, 2003). Palynological data indicates the strong relationship the taxa share with the Boraginaceae and Heliotropiaceae under Boraginales.

Conclusion

Ehretia pubescens Benth shows tricolporate, foveolate pollen grains but have a unique feature of lolongate endopore with an endocingulam nature. It shows pseudocolpus aperture alternating with true colporate apertures whereas *Cormona retusa* differ in pollen morphology. It had colpate aperture and no pseudocolpus was found. Individual species of Ehretia can be identified by their pollen morphology due to eurypalynous nature of the family. The *E.pubescens* and *C.retusa* resemble Boraginaceae family in their pollen morphology indicating an affinity with the family from which it had been separated to a family level. Phylogenetically the pollen of both the species studied indicates two different line of development.

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