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RESEARCH ARTICLE

POLLEN MORPHOLOGICAL STUDIES IN FIVE SPECIES OF *LEEA* D. ROYEN EX L. FROM KERALA.

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Abstract

The pollen morphology of ten accessions representing five species of *Leea* D. Royen ex L. collected from different parts of Kerala State, South India, were studied. The major palynological features appear to be highly conserved with the pollen grains being consistently 3-colporate, prolate-spheroidal to subprolate and medium-sized with microreticulate exine ornamentation. SEM analysis revealed large broad colpi with slightly winged margins, tall muri with warted upper surfaces and irregularly heterobrochate narrow lumina. The luminal floors were not clearly visible due to luminal depth and heavy wax deposition, more frequently at the mesocolpium. The lumina tend to elongate and narrow at the mesocolpium assuming a rugulate nature. Earlier reports of similar pollen traits from the close kin Vitaceae reflect the palynological affinity between the two groups, justifying the recent inclusion of the genus as a subfamily under the Vitaceae.

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Introduction:-

Leea D. Royen ex L., the monophyletic member of the tropical family Leeaceae, comprises 70 species world over, and has 34 species reported from Asia and Africa, of which six species have so far been recorded from Kerala (Sasidharan, 2004). The members are mostly shrubs, trees or herbs with simple or pinnate leaves, many flowered corymbose cymes, reflexed petals, variously lobed stamina tubes, basal ovules in 2-4 carpellary pistils and indehiscent berries (Risidale, 1974, 1975). The genus was originally considered under the economically important grape family, the Vitaceae, from which it was later separated out as a distinct family - the Leeaceae owing to the absence of tendrils, shrubby habit, presence of floral disc, presence of staminoidal tube, variations in ovule and carpel number etc. (Molina, 2009). However, the genus is presently being considered as a subfamily (Leeoideae) under the Vitaceae (APG I 1998 to APG IV, 2016).

Phylogenetic studies and taxonomic categorizations have at present assumed a multidimensional aura, demanding the collection of data from variant sources for systematic elucidations. Pollen microstructural analysis employing Scanning Electron microscopy unveils the pollen surface architecture yielding a vast array of details pertaining to the exine ornamentation and aperture, which along with other traits such as exine strata, pollen size, pollen shape etc. have become very valuable as 'palynological markers' at all taxonomic hierarchies. A perusal of the literature on the pollen of the genus *Leea* yielded scanty results, as such studies have focused largely on the palynology of the larger group, the Vitaceae (Erdtman, 1952; Tarnavski and Petria, 1968; Moore et al. 1991; Inceoglu et al. 2000;

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Punt et al. 2003; Perveen and Qaiser, 2008; Najmaddin et al. 2011, Najmaddin, 2014). Consequently, *Leea* pollen have received meagre attention, and the aim of the present study is to gather data on the pollen morphology of the genus. The study includes detailed comparative interspecific analysis of the pollen from five species of *Leea* from different parts of Kerala, South India, using LM and SEM studies.

Materials and Methods:-

The details regarding the five species of *Leea* collected from various locations in the State of Kerala for the present study are furnished in Table 1. Fresh polliniferous materials from the collected specimen were fixed directly in glacial acetic acid. Pollen preparations were made by the acetolysis method standardized by Erdtman (1952) and modified by Nair (1970).

Table 1:- List of five species of *Leea* collected from different parts of Kerala with locality

Sl.No	Plant Name	Locality	District	North	East
1	<i>Leea asiatica</i> (L.) Ridsdale	Nilambur	Malappuram	11°16'43.788"	76°14'21.1452"
2	<i>Leea asiatica</i> (L.) Ridsdale	Muthappanpuzha	Kozhikode	11°26'40.6032"	76°5'14.7984"
3	<i>Leea guineensis</i> G. Don	Palaruvi	Kollam	8°57'3.5136"	77°9'24.4332"
4	<i>Leea guineensis</i> G. Don	Pamba	Pathanamthitta	9°24'26.9604 "	77°4'9.39"
5	<i>Leea indica</i> (Burm. f.) Merr.	Venjaramoodu	Thiruvananthapuram	8°40'57.7056"	76°54'34.3476"
6	<i>Leea indica</i> (Burm. f.) Merr.	Sulthan Bathery	Wayanad	11° 39'53.6256"	76°15'46.6272"
7	<i>Leea macrophylla</i> Roxb. ex Hornem.	Nilambur	Malappuram	11°16'28.0704"	76°13'5.2176"
8	<i>Leea macrophylla</i> Roxb. ex Hornem.	Venjaramoodu	Thiruvananthapuram	8°40'57.7056"	76°54'34.347 "
9	<i>Leea rubra</i> Blume ex Spreng.	Mundakkal	Kollam	8°52'41.0196"	76°36'3.8268"
10	<i>Leea rubra</i> Blume ex Spreng.	Venjaramoodu	Thiruvananthapuram	8°40'57.7056"	76°54'34.3476"

The acetolysed pollen were stored in 1:1 glycerine and distilled water and permanent slides for light microscopic studies were prepared by mounting the acetolysed pollen in glycerine jelly and sealing with paraffin wax. LM photomicrographs of the pollen preparations were taken using an Olympus CH - 20 Research Microscope with Digital camera attachment facility. The slides of pollen preparation are deposited in the Department of Botany. SEM pictures were taken at the NIIST, Pappanamcode, Trivandrum using a JEOL – JSM – 5600LV scanning electron microscope. Morphological features of pollen grains including those relating to the aperture, exine ornamentation, pollen size and shape were studied from LM and SEM observations. The value of P/E ×100 was found in the case for ascertaining the shape class, following the pollen shape classification suggested by Erdtman (1966) and pollen size classes were ascertained following Walker & Doyle (1975). The terminologies suggested by Punt *et al.* (1994) have been used for describing the aperture types and exine ornamentation pattern. The quantitative pollen traits (polar and equatorial pollen diameter, P/E ratio, exine thickness and aperture number were measured using an ocular micrometer, and in each case, the mean values of the measurements were taken from a random sample of 30 pollen grains. Qualitative palynological characters studied include pollen shape, type, size class, exine ornamentation, mural nature (shape, wall, width and height), luminal nature (depth, floor, shape and wax plugging) and aperture shape.

Results and Discussion;-

Details of pollen morphological characters observed from 10 accessions representing five species of *Leea* D. Royen ex L., collected from different parts of Kerala State are represented in Tables 2 and 3. The pollen are in general medium-sized, 3-colporate and spheroidal with the polar and equatorial diameters ranging from $46.5 \pm 4.24 \mu\text{m}$ and $46.8 \pm 3.1 \mu\text{m}$ to $42.6 \pm 3.8 \mu\text{m}$ and $39.9 \pm 4.5 \mu\text{m}$ respectively (Table 2). Exine $3.15 \pm 0.71 \mu\text{m}$ to $3.9 \pm 0.63 \mu\text{m}$ thick, thinner towards the colpi margins and microreticulate. The aperture number was consistently 3 in all the ten taxa studied.

Table 2:- Quantitative pollen morphological characters in five species of *Leea* from Kerala

Characters	<i>L. asiatica</i>	<i>L. guineensis</i>	<i>L. indica</i>	<i>L. macrophylla</i>	<i>L. rubra</i>
Polar diameter –P (μm)	45 ± 4.9	43.8 ± 3.1	46.5 ± 4.24	42.6 ± 3.8	45.6 ± 4.73
Equatorial diam.- E (μm)	40.8 ± 3.1	46.8 ± 3.1	40.5 ± 4.24	39.9 ± 4.5	41.4 ± 7.9
P/E	1.09 ± 0.06	0.94 ± 0.01	1.15 ± 0.02	1.07 ± 0.05	1.11 ± 0.17
Exine thickness (μm)	3.48 ± 0.90	3.21 ± 0.7	3.9 ± 0.63	3.15 ± 0.71	3.36 ± 0.55
Aperture number	3	3	3	3	3

The major qualitative palynological features were also conserved within the genus with the members having mostly prolate-spheroidal, 3-colporate, medium-sized pollen with microreticulate exine ornamentation (Table 3). The pollen in *Leea guineensis* and *Leea indica* are oblate-spheroidal and subprolate respectively. Tricolporate pollen have been observed consistently in *Leea* (Tarnavski and Petria 1968; Najmaddin, 2014), while several studies have shown that they are characteristic of the closely related Vitaceae also, being mostly sub-prolate or prolate to prolate-spheroidal (Inceoglu et al., 2000; Marasali et al., 2005; Perveen and Qaiser, 2008).

Table 3:- Qualitative pollen morphological characters in five species of *Leea* from Kerala

Characters	<i>L. asiatica</i>	<i>L. guineensis</i>	<i>L. indica</i>	<i>L. macrophylla</i>	<i>L. rubra</i>
Pollen shape	Prolate-spheroidal	Oblate-spheroidal	Subprolate	Prolate-spheroidal	Prolate-spheroidal
Pollen type	3-colporate	3-colporate	3-colporate	3-colporate	3-colporate
Pollen size-class	Medium	Medium	Medium	Medium	Medium
Exine ornamentation	Microreticulate	Microreticulate	Microreticulate	Microreticulate	Microreticulate
Muri shape	Heterobrochate	Heterobrochate	Heterobrochate	Heterobrochate	Heterobrochate
Muri wall nature	Warted moderately	Warted highly	Warted moderately	Warted slightly	Warted highly
Muri width	Moderately broad	Moderately broad	Moderately broad	Moderately broad	Moderately broad
Muri height	Tall	Tall	Tall	Tall	Tall
Lumina depth	Deep	Deep	Deep	Deep	Deep
Lumina floor nature	Not visible	Not visible	Not visible	Not visible	Not visible
Lumina shape	Irregular	Irregular	Irregular	Irregular	Irregular
Wax plugging in lumina	Present more towards the mesocolpium	Present more towards the mesocolpium	Present more towards the mesocolpium	Present more towards the mesocolpium	Present more towards the mesocolpium
Aperture shape	Colporate	Colporate	Colporate	Colporate	Colporate
Colpi margin	Slightly winged	Slightly winged	Slightly winged	Slightly winged	Slightly winged

The lumina were heterobrochate and irregular in shape, bounded by tall, moderately broad muri with small granular warts on their upper surfaces. The mural surfaces were heavily warted in *Leea guineensis* and *Leea rubra*, while those of *Leea macrophylla* showed the least incidence of warts. The narrow lumina limited by tall muri appeared

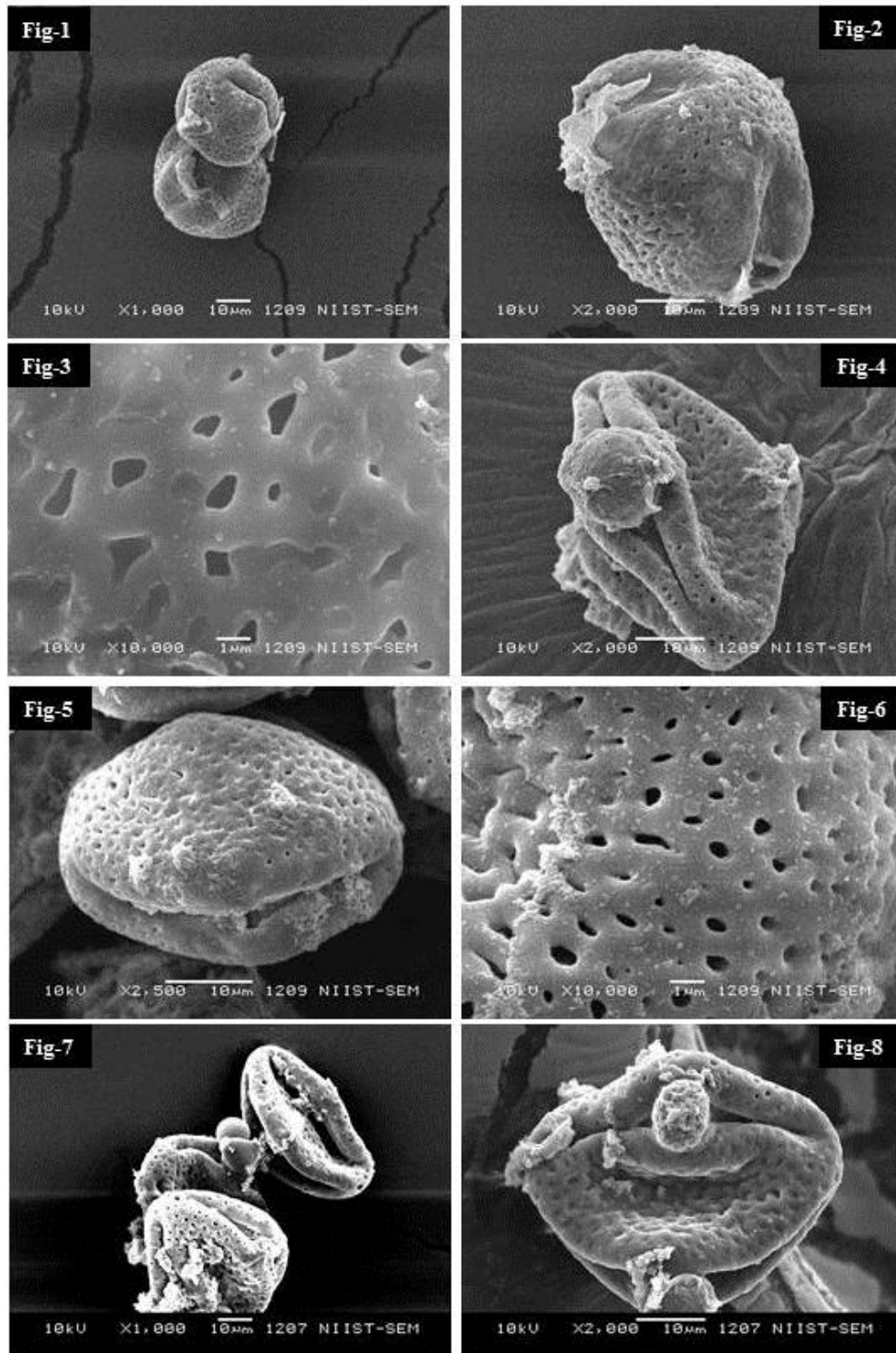
deep, making the pollen microreticulate rather than reticulate. The very deep luminal floors were not clearly visible due to the height of the mural walls. The lumina appeared to be variously shaped assuming irregularly oval, ellipsoidal or round appearances. In all cases the lumina appeared to be very often plugged with waxy deposits, more frequently at the mesocolpium. Roytchev et al. (1994) noted that the colpi are usually wide and deep in the equatorial zone, narrowing towards the poles. In addition, the present study revealed that the colpi margins are slightly winged in *Leea*.

Both Leeaceae and its close kin the Vitaceae have been reported to be stenopalynous, being characterised by tricolporate, reticulate pollen. Perveen and Qaiser (2008) grouped the pollen of Vitaceae into three types based on exine ornamentation, among which the *Ampelopsis vitifolia* subsp. *hazaraganjiensis*-type alone had reticulate exine. Although the Leeaceae pollen have been repeatedly reported as reticulate, the narrow deep lumina bounded by tall muri give them a porous appearance and consequently the exine ornamentation may be more aptly considered to be microreticulate. The reticulum is more clear towards the apocolpia, while the lumina tend to elongate and narrow at the mesocolpium assuming a rugulate nature. This was observed in all the five species and appears to be more effected by the waxy embedding at the mesocolpium. Reduction in luminal width towards the equatorial region was observed earlier by Faegri and Iversen (1989) in the closely related genus *Vitis*, and this was later recorded as foveolate-rugulate or rugulate-striate exine ornamentation at the mesocolpium (Inceoglu et al. 2000; Najmaddin et al 2011) based on the SEM studies on the pollen of *Vitis* species. However, the authors have not come across such observations on the pollen of *Leea*.

The systematic hierarchy of *Leea* in relation to its closely related family the Vitaceae has been flooded with much controversy, with taxonomists either separating it out as a distinct family - the Leeaceae owing to its unique erect shrubby habit, absence of tendrils, terminal inflorescences, large stipules, complex floral disc, staminoidal tube, secondary septa in ovary, variations in ovule and carpel number etc. (Dumortier, 1829; Risdale, 1974, 1976; Wen, 2007; Molina, 2009), or including the genus as a separate subfamily Leeoideae along with the larger subfamily Vitioidae under the Vitaceae (APG I 1998 to APG IV, 2016), due to the common possession of pearl glands, barbed raphides, phloem-plastids, antipetalous stamen, ruminant seeds with oily endosperm etc. and similar floral development. The present study unveiled much similarity between the pollen morphological traits of the two subfamilies of the Vitaceae, the details of which have already been discussed in the paragraphs above. The stenopalynous nature of the two subfamilies along with the conservation of the major pollen characters in them support the inclusion of the genus *Leea* as a subfamily (Leeoideae) under the family Vitaceae.

Conclusions:-

The monotypic genus *Leea* of the stenopalynous family Leeaceae is characterized by mostly prolate-spheroidal to subprolate, 3-colporate, medium-sized pollen with microreticulate exine ornamentation. The colpi demarcated by slightly winged margins, are wider and deeper towards the equatorial zone. The tall muri with warty upper surfaces enclose deep irregularly heterobrochate narrow lumina, very often plugged with waxy deposits, more frequently at the mesocolpium. The reticulum is more clear towards the apocolpia, while the lumina tend to elongate and narrow at the mesocolpium assuming a rugulate nature. These major pollen traits appear to be heavily conserved not only within the genus, but also within the closely related family Vitaceae as well, justifying the inclusion of the genus as a separate subfamily Leeoideae along with the larger subfamily Vitioidae under the Vitaceae.



Figs. 1-3:-*Leea asiatica* (L.) Ridsdale, pollen- 1000x, 2000x, 10000x; **Figs. 4-6:** *Leea guineensis* G. Don, pollen- 2000x, 2500x, 10000x; **Figs. 7-8:** *Leea indica* (Burm. f.) Merr., pollen- 1000x, 2000x

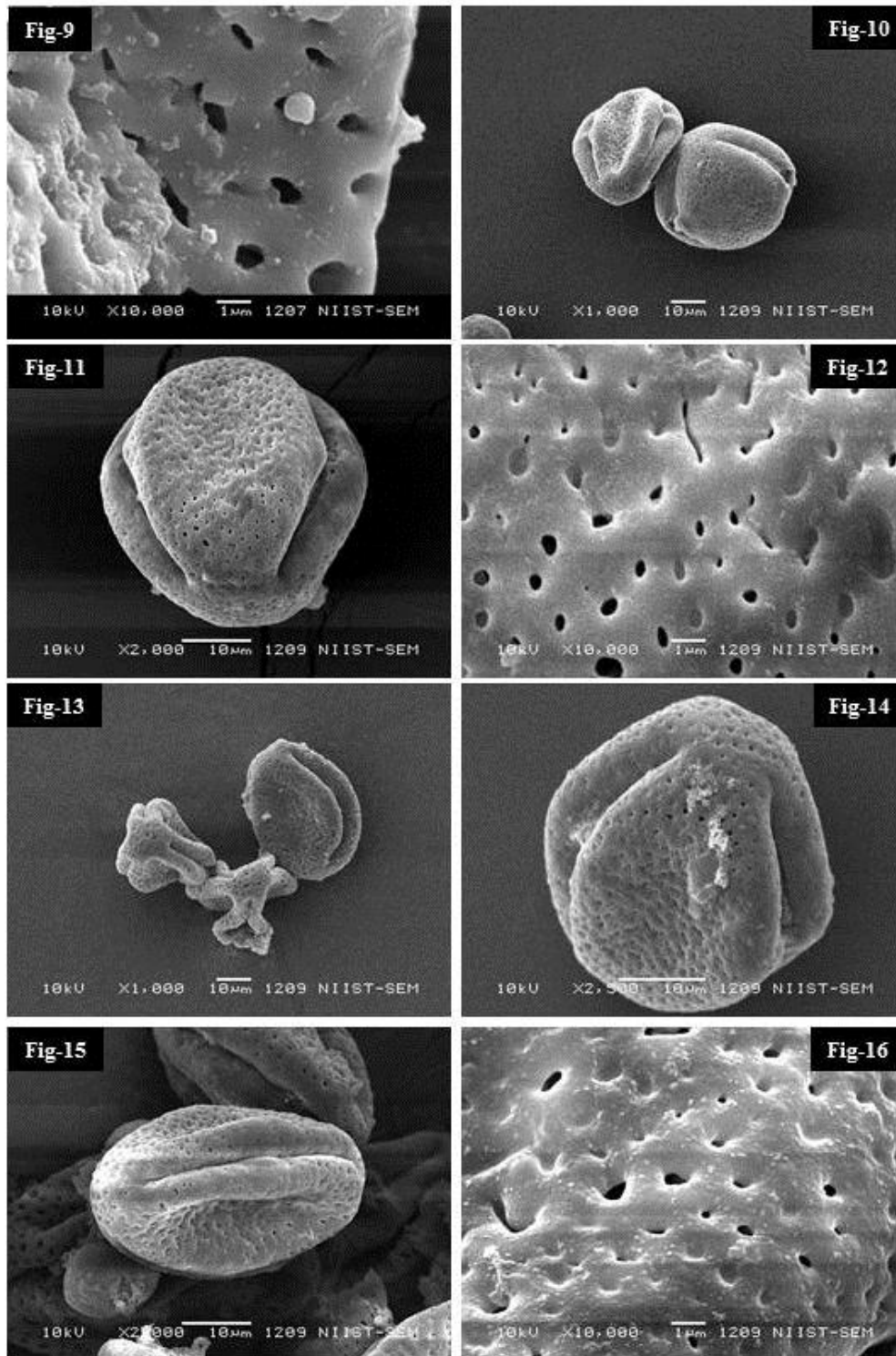


Fig. 9:-*Leea indica* (Burm. f.) Merr., pollen- 10000x; **Figs. 10-12:** *Leea macrophylla* Roxb. ex Hornem., pollen- 1000x, 2000x, 10000x; **Figs. 13-16:** *Leea rubra* Blume ex Spreng., pollen- 1000x, 2500x, 2000x, 10000x

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