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Pollen diversity in *Phyllanthus* species (Phyllanthaceae) Andhra Pradesh, India

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ABSTRACT

The present study deals with the pollen morphological diversity and taxonomic importance among the *Phyllanthus* species belonging to the Phyllanthaceae family from combined Andhra Pradesh. Sixteen species belonging to genus *Phyllanthus* analyzed the pollen morphology using SEM. The size, shape, symmetry, aperture and ornamentation were recorded in all test species, among them ten were tricolporate, three tetracolporate while the remaining indicates the pantosyncolpate. The reticulate ornamentation is common and predominant in all taxa. The evaluation of taxonomic diversity among *Phyllanthus* species hitherto not known from Andhra Pradesh, hence the present study is the first report and gains importance. It is clear that the pollen characteristic features are significant in the identification of endemic species (*P.scabrifolius* and *P.narayanaswamii*) and confirmation of potential medicinal plants (*P. amarus* and its allied species) with the help of micromorphological pollen studies.

Key words: Endemic species, *Phyllanthus*, Pollen morphology, Taxonomic diversity.

INTRODUCTION

Phyllanthus L. (*s.l.*) often divided into six subgenera, namely, Cicca, Eriococcus, Isoclades, Kirgenelia, *Phyllanthus* (*s.s*) and Xylophylla. The subgenus *Phyllanthus* (*s.s.*) is characterized by herbs or semi-woody shrubs bearing colporate pollen grains, tricarpellary capsular fruits with six striate and/or foveolate seeds. The Genus *Phyllanthus* L. is represented by 51 species from India which grows in forests, wastelands and open fields (Chaudhary and Rao 2002).

The genus *Phyllanthus* widely distributed in Sri Lanka, India, Burma, Indonesia, Pacific Islands and West Indies (Mariappan *et al*., 2015). The erstwhile *P.niruri* non L. is divided into three species *viz*., *P. amarus* (s.s), *P.fraternus and P.debilis.* (Theerakulpisut 2008). Many publications from India, on *P.niruri* non L. were found to be referring to any of the three species and not on the true *P.niruri* L. which is endemic to Central America. The true *P.niruri* L. is native species of Central America which does not occur in India. The group of herbs, *P.amarus, P.fraternus, P.debilis* and *P.urinaria* has been referred to as 'Bhumyamalaki', in Indian literature which is also known as *P.niruri* complex. They are similar in morphology but difficult to separate into distinct species on exomorphological features. Specific morphological features that aid the identification of *P.amarus, P.debilis, P.maderaspatensis and P.virgatus* have been reported. The literature revealed about 20 species that occur in Andhra Pradesh (Pullaiah *et al.,* 2018). *P.acidus, P.indofisheri, P.fraternus and P. leschenaultia* were not relocated. The distinction among the herbaceous *Phyllanthus* like *P.scabrifolius, P.rheedei, P.debilis, P.airy-shawii* and *P.kozhikodianus* is not well demarcated since most of the morphological features are identical, however, *P.kozhikodianus* Sivaranjan and Manilal is reported as a synonym of *P.rheedei* Gangopadhyay *et al.,* (2004). *P.debilis* Hook.f is different from *P.debilis* Willd. and the later was replaced by *P.airyshawii* Brun. and Roux (Mitra and Nayar 2017).

Recently a new species was reported from Tamilnadu (Parthipan *et al.,* 2017) earlier it was named as *P.urinaria* var. hookeri Mull.Arg. Raju *et al.,* (2011) *P.narayanaswamii* Gamble, an endemic to the forests of Visakhapatnam and East Godavari districts and reported as endangered due to mining practices in their region. *P.scabrifolius* endemic to south India but recorded as rare with restricted populations. Ambivalent exomorphological features of *P.narayanaswamii* and *P.scabrifolius* are resembled with *P.virgatus and P.rheedei* respectively (Lalithamba, 2014).

The confusion is rooted mostly due to a lack of methodological rigor of taxonomy. It may due to the reason

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that there is no specific distinctiveness among the species in the form of a taxonomic key. At any rate, even rather visually different *P.urinaria* L. is often used interchangeably with these species known earlier as *P.niruri non*.L (*P.amarus, P.fraternus, P.debilis*) in traditional/ indigenous medicine systems (Kirtikar and Basu 1935) Practitioners did not differentiate properly due to the lack of taxonomic understanding. A similar situation exists in Malaysia, where the same name applies to both *P.niruri* and *P.urinaria.*

The present attempt is mainly to focus on avoiding ambiguity in morphological characters of *Phyllanthus* species, in which the microscopic and morphological attributes of the pollen grain is provided. Pollen morphological data is particularly valuable in strengthening the taxonomic diversity among the members of *Phyllanthus* which include palynological characters *viz*., pollen size, shape, polarity, symmetry, apertural structure, ornamentation and other characters can be used to highlight taxonomic relationship (Erdtman 1952), (Bhattacharya 2014). *Phyllanthus* is the largest genus of the Phyllanthaceae (Euphorbiaceae), contains about 830 species (Govaert *et al.*, 2000). Various morphologists and taxonomists have pointed out the great and complex diversity of pollen in Euphorbiaceae (including Phyllanthaceae). The subfamily Phyllanthoideae under Euphorbiaceae later elevated to a separate family i.e. Phyllanthaceae in general, particularly the genus *Phyllanthus*, have received much attention in recent times (Punt, 1962: Punt and Rentrop, 1974).

Studies on pollen morphology can also provide important information in the forensic investigation in allergens, pollinator biology and honey analysis (Ganga Kailas *et al.,* 2016): Devender *et al.,* 2016). The pollen description of *P.columnaris* and *P.debilis* from the Andaman Islands was provided (Ganga Kailas *et al.,* 2016).

Punt (1987) reviewed on the pollen profile of the subgenera of *Phyllanthus* which indicates that the pollen morphological variation using SEM. The pollen morphology of 21 Philippine *Phyllanthus* species belonging to five subgenera and eleven sections were studied under the genus *Phyllanthus*.

MATERIALS AND METHODS

Study area

The study area is the harbors primarily tropical deciduous vegetation found in Orissa, Telangana andhra Pradesh, Tamilnadu and some parts of the Karnataka States of isolated hill ranges of the Eastern Ghats in peninsular India. The Eastern Ghats of Andhra Pradesh and Telangana (the link between 13°30′19.07"N; 77°28′84.45" E) covers the hilly terrain of coastal Andhra with nine districts and three districts in the Telangana States. The intensive field studies were conducted in the forests of the Eastern Ghats in Andhra Pradesh in between 2017 to 2018 yields 16 *Phyllanthus* spp. Among them, 12 are herbaceous, 2 shrubs and 2 are trees (Fig 1).

Plant collection

Plant samples were collected from the original localities from

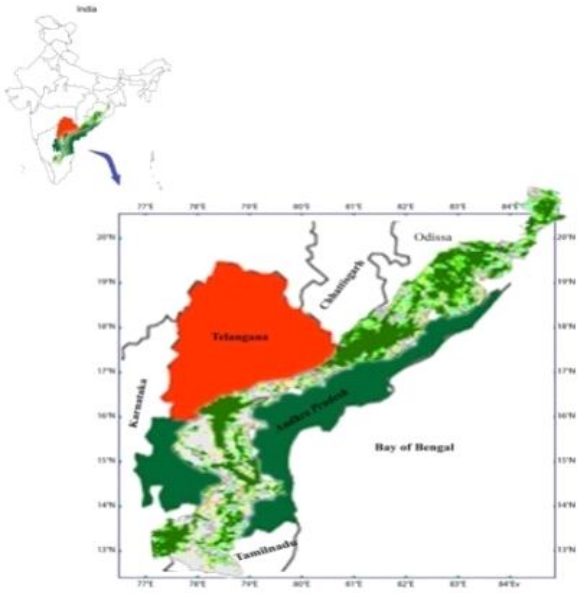

Fig 1: Location of the Andhra Pradesh and Eastern Ghats, India.

which the specimens were collected, dried and poisoned with saturated mercuric chloride with ethyl alcohol (Jain and Rao 1977). Collected specimens were identified with the help of floras and monographs and voucher specimens were prepared and confirmed by comparing with authentic specimens housed at Sri Krishnadevaraya University Herbarium (SKU), Anantapuramu. The Madras Herbarium (MH), Coimbatore. The voucher specimens were deposited at SKU, Sri Krishnadevaraya University, Anantapuramu (Table 1).

Preservation

Male flowers of all the sixteen tested species were separated and preserved in FAA solution (Formaldehyde 5%, Glacial acetic acid 5% and 90% Ethyl alcohol). The Pollen grains were separated from preserved specimens and subjected to SEM analysis.

SEM analysis

The dried/dehydrated method (Pathan, *et al.,* 2008) was followed for pollen analysis. The Sirion resolution SEM equipped with a Schottky field emission source with a high voltage variable between 200 V and 300 Kv. Detectors include Everhart-Thorley and solid-state backscatter detectors and an in-lens detector for high-resolution imaging resolution: 1.5nm at >10 kV, 2.5nm at 1 kV, 3.5nm at 500V. The pollen grains were mounted onto a metallic stub with double-sided adhesive tape. The Gold coating of few nanometers was applied using a sputter coating machine to avoid charging Capture high-quality images and recorded the surface morphological features of the test species.

RESULTS AND DISCUSSION

1. *Phyllanthus airy-shawii* Brun. and Roux. (Plate 1, Fig 1, 2)

Symmetry: Bilateral.
Shape: Subprolate.

Polarity: Isopolar.
Size: PA 16.5-18 µm. EA 13.5-15 µm.
Polar view (amb): Circular, 15-17 µm.
Aperture: Tricolporate.
Sporoderm stratification: Exine 1.5 µm thick, sexine as thick as nexine.
Ornamentation: Psilate.

2. *Phyllanthus amarus* Schum. andThonn. (Plate 1, Fig 3, 4)

Symmetry: Bilateral.
Shape: Subprolate.
Polarity: Isopolar.
Size: PA 17-19 µm. EA 14-16 µm.
Polar view (amb): Triangular, 12-14 µm.
Aperture: Tricolporate.
Sporoderm stratification: Exine 1 µm thick, sexine as thick as nexine.
Ornamentation: Granular.

3. *Phyllanthus debilis* Kl. ex Willd. (Plate 1, Fig 5, 6).

Symmetry: Radial.
Shape: Spheroidal.
Polarity: Isopolar.
Size: PA 20-25 µm. EA 20-25 µm.
Polar view (amb): Circular, 21-25 µm.
Aperture: Tricolporate, colpi long, tips acute.
Sporoderm stratification: Exine 1.5 µm thick, sexine as thick as nexine.
Ornamentation: Reticulate.

4. *Phyllanthus emblica* L.
(Plate 1, Fig 7, 8)
Symmetry: Radial.
Shape: Spheroidal.
Polarity: Isopolar.
Size: PA 22-28 µm. EA 22-28 µm.
Polar view (amb): Circular, 22-27 µm.
Aperture: Tetracolporate.
Sporoderm stratification: Exine 1.5 µm thick, sexine as thick as nexine.
Ornamentation: Reticulate.

5. *Phyllanthus leschenaultia* Mull.Arg.
(Plate 1, Fig 9, 10)
Symmetry: Bilateral.
Shape: Subprolate.
Polarity: Isopolar.
Size: PA 30-35µm. EA 29-32µm.
Polar view (amb): Triangular, 25-27 µm.

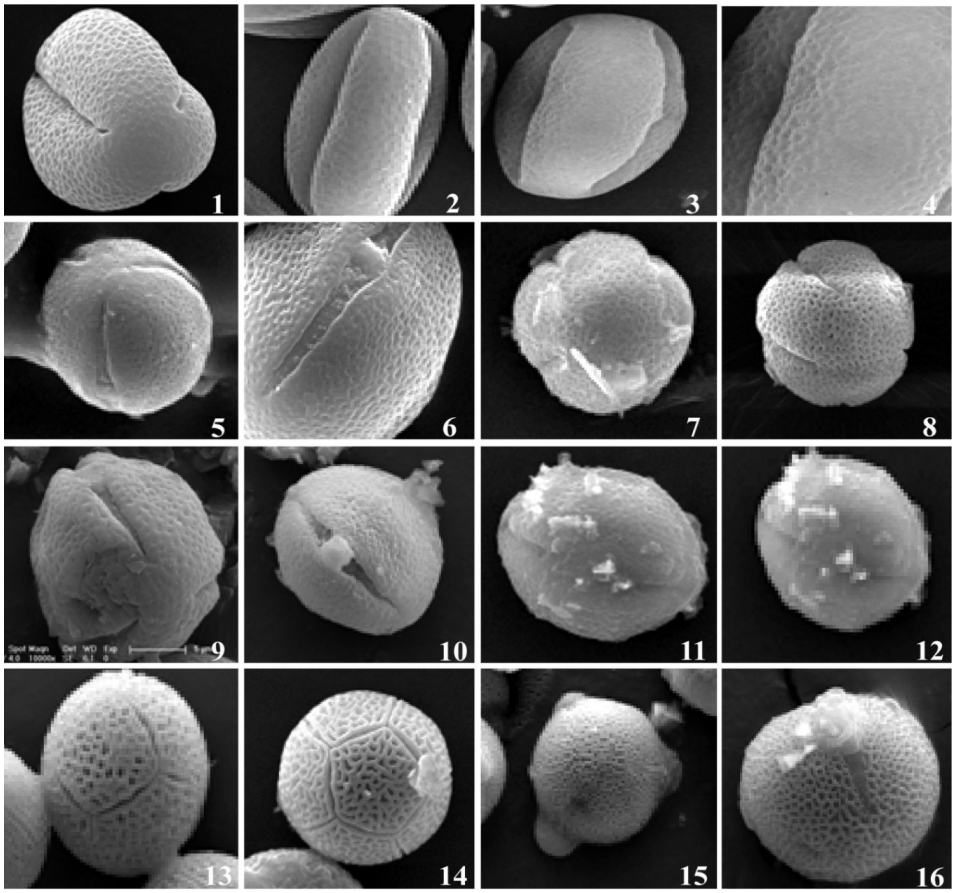

Plate 1: 1, 2. *P. airy-shawii* Brun. and Roux.; **3, 4.** *P. amarus* Schum. and Thonn.; **5, 6.** *P. debilis* Kl. ex Willd.; **7, 8.** *P. emblica* L.; **9, 10.** *Phyllanthus leschenaultia* Mull. Arg; **11, 12.** *P. maderaspatensis* L.; **13, 14.** *P. narayanaswamii* Gamble and **15, 16.** *P. pinnatus* (Wt.) Webster. scalebar 5µm.

Aperture: Tricolporate.
Sporoderm stratification: Exine 1.5 µm thick, sexine as thick as nexine.
Ornamentation: Granular.

6. *Phyllanthus maderaspatensis* L. (Plate 1, Fig 11, 12)

Symmetry: Bilateral.
Shape: Prolate.
Polarity: Isopolar.
Size: PA 21-23 µm. EA 15-17 µm.
Polar view (amb): Triangular, 16.5-18 µm.
Aperture: Tricolporate.
Sporoderm stratification: Exine 1 µm thick, sexine as thick as nexine.
Ornamentation: Reticulate.

7. *Phyllanthus narayanaswamii* Gamble (Plate 1, Fig 13, 14)
Symmetry: Radial.
Shape: Spheroidal.
Polarity: Isopolar.
Size: PA 19.5-21µm. EA 19.5-21 µm.
Polar view (amb): Circular, 19.5-22 µm.
Aperture: Pantosyncolpate.
Sporoderm stratification: Exine 2µm thick, sexine thicker than nexine.
Ornamentation: Reticulate.

8. *Phyllanthus pinnatus* (Wt.) Webster (Plate 1, Fig 15, 16)

Symmetry: Radial.
Shape: Spheroidal.
Polarity: Isopolar.
Size: PA 15-17 µm. EA 15-17 µm.
Polar view (amb): Triangular, 15-16 µm.
Aperture: Tricolporate.
Sporoderm stratification: Exine 1µm thick, sexine as thick as nexine.
Ornamentation: Reticulate.

9. *Phyllanthus polyphyllus* Willd. (Plate 2, Fig 17, 18)

Symmetry: Radial.
Shape: Spheroidal.
Polarity: Isopolar.
Size: PA 12-14 µm. EA 12-14 µm.
Polar view (amb): Circular, 12-14 µm.
Aperture: Tetracolporate.
Sporoderm stratification: Exine 1.5µm thick, sexine thicker than nexine.
Ornamentation: Reticulate.

10. *Phyllanthus reticulatus* Poir. (Plate 2, Fig 19, 20)

Symmetry: Radial.
Shape: Spheroidal.
Polarity: Isopolar.
Size: PA 23-28 µm. EA 23-28µm.
Polar view (amb): Circular, 23-28µm.
Aperture: Tricolporate.
Sporoderm stratification: Exine 2 µm thick, sexine thicker than nexine.
Ornamentation: Coarsely reticulate.

11. *Phyllanthus rheedei* Wt. (Plate 2, Fig 21, 22)

Symmetry: Bilateral.
Shape: Subprolate.
Polarity: Isopolar.
Size: PA 25.5-27µm. EA 19.5-21 µm.
Polar view (amb): Circular, 18-20 µm.
Aperture: Tricolporate, oralolongate.
Sporoderm stratification: Exine 1.5 µm thick, sexine thicker than nexine.
Ornamentation: Psilate.

12. *Phyllanthus rotundifolius* Kl. ex Willd. (Plate 2, Fig 23, 24)

Symmetry: Bilateral.
Shape: Prolatespheroidal.
Polarity: Isopolar.
Size: PA 30-34µm. EA 26-30 µm.
Polar view (amb): 28-31 µm.
Aperture: Tricolporate.
Sporoderm stratification: Exine 2 µm thick, sexine thicker than nexine.
Ornamentation: Reticulate.

13. *Phyllanthus scabrifolius* Hook.f. (Plate 2, Fig 25, 26)

Symmetry: Bilateral.
Shape: Prolate.
Polarity: Isopolar.
Size: PA 22.5-24 µm. EA 15-17 µm.
Polar view (amb): Circular, 16.5-18 µm.
Aperture: Tricolporate, ora circular
Sporoderm stratification: Exine 2 µm thick, sexine as thick as nexine.
Ornamentation: Reticulate.

14. *Phyllanthus tenellus* Roxb. (Plate 2, Fig 27, 28)
Symmetry: Radial.
Shape: Spheroidal.
Polarity: Isopolar.
Size: PA 28-32µm. EA 28-32µm.
Polar view (amb): Circular, 27-32 µm.
Aperture: Tetracolporate.
Sporoderm stratification: Exine 1.5 µm thick, sexine as thick as nexine.
Ornamentation: Reticulate.

15. *Phyllanthus urinaria* L. var. *najarana* (Plate 2, Fig 29, 30)

Symmetry: Bilateral.
Shape: Subprolate.
Polarity: Isopolar.
Size: PA 18-20 µm. EA 15-17µm.
Polar view (amb): Circular, 13.5-15 µm.
Aperture: Tetracolporate, colpi long, oralalongate.
Sporoderm stratification: Exine 1.5 µm thick, sexine as thick as nexine.
Ornamentation: Reticulate.

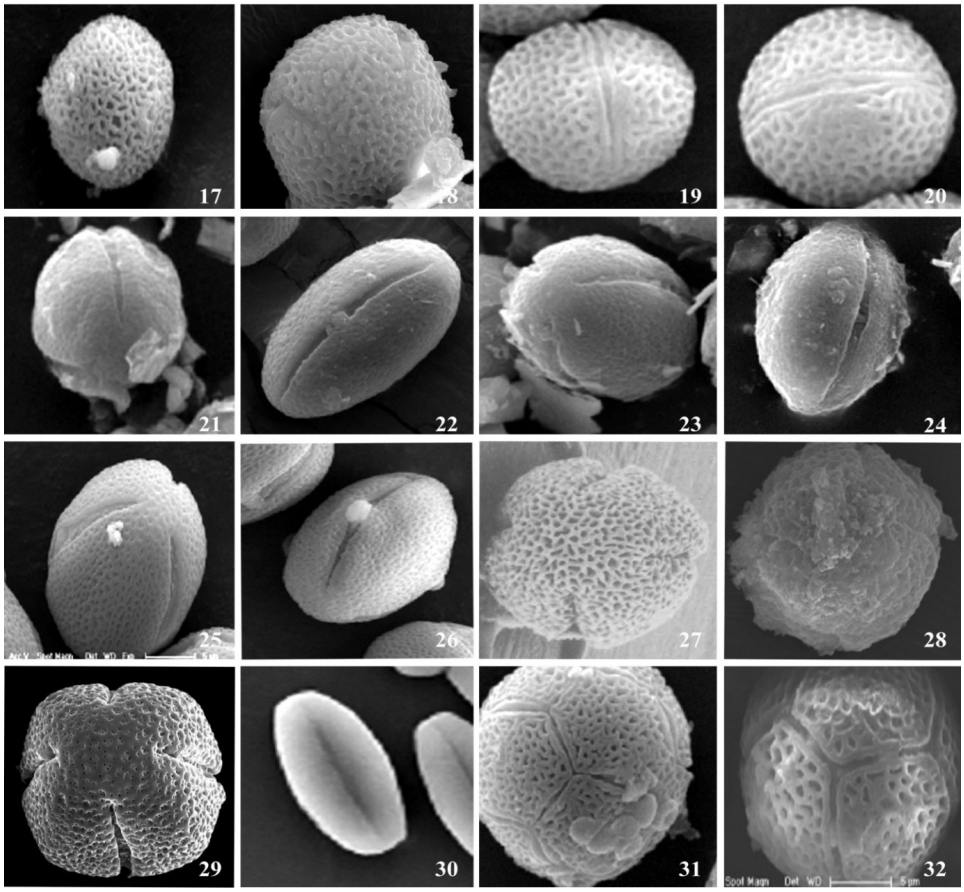

Plate 2: 17, 18. *P. polyphyllus* Willd. **19, 20**; *P. reticulatus* Poir.; **21, 22.** *P. rheedei* Wt.; **23, 24.** *P. rotundifolius* Kl. ex Willd.; **25, 26.** *P. scabrifolius* Hook.f.; **27, 28.** *P. tenellus* Roxb.; **29, 30.** *P. urinaria* L.var. *najarana.* **31, 32.** and *P. virgatus* G.Forst. scalebar 5µm.

16. *Phyllanthus virgatus* G.Forst. (Plate 2, Fig 31, 32)
Symmetry: Radial.
Shape: Spheroidal.
Polarity: Isopolar.
Size: PA 18-20 µm. EA 18-20 µm.
Polar view (amb): Circular, 18-20 µm.
Aperture: Pantosyncolpate.
Sporoderm stratification: Exine 2 µm thick, sexine as thick as nexine.
Ornamentation: Reticulate.

The pollen samples of 16 *Phyllanthus* species collected from the Eastern Ghats of Andhra Pradesh were analyzed (Table 1). Half of the test species (8 spp) exhibit radial symmetry while the other half indicates bilateral symmetry but all taxa possess isopolar condition. The pollen size and shape were recorded based on the P/E×100 (P= Polar axis diameter, E= equatorial diameter) and enumerated (Table 2), spheroidal shape revealed in majority species (9 spp) followed by sub-prolate (4), prolate (2 spp.) and prolate-spheroidal found in 1 species only, However, ornamentation of pollen wall plays an important role in taxonomic identificationof the taxa Bhattacharya *et al.,* (2009).

The sixteen taxa of *Phyllanthus* in this present study reveal that a significant diversity in pollen morphology. Among these taxa, 3 types of apertures *viz*., tricolporate, tetracolporate and pantosyncolpate, whereas 3 types of ornamentations *viz*., psilate, granular and reticulate were recorded. Reticulate ornamentation is the common feature of Phyllanthaceae Punt and Rentrop (1973). *Phyllanthus airy-shawii and P.rheedei* has tricolporate with psilate ornamentation whereas, *P.leschenaultii* has tricolporate with granular; while *P.amarus P.debilis, P.maderaspatensis, P.pinnatus, P.reticulatus and P.rotundifolius* have tricolporate with reticulate ornamentation. Earlier Punt and Rentrop (1973) Bor (1979), Punt (1980), Long and Yu (1984), Chen and Wu (1997 and 2009) have reported prolate spheroidal to subprolate, tricolporate with reticulate ornamentation in *P. amarus*. Tetracolporate with reticulate ornamentation is found in P.*urinaria, P. emblica, P.polyphyllus and P.tenellus.* Earlier Punt and Rentrop (1973), Bor (1979), Long and Yu (1987), Rossignol *et al.,* (1987), Lobreau-callen *et al.,* (1988) and Chen and Wu (1997 and 2009) were recorded prolate in shape, tetracolporate, narrow colpi with reticulate ornamentation in *P.urinara.*

The pollen of *P.virgatus* and *P.narayanaswamii* has pantosyncolpate which is rare associated character with reticulate ornamentation. These grains were also studied by Punt (1980 and 1987) recorded spheroidal, pantosyncolpate condition.

Table 1: The *Phyllanthus* species collected from Eastern Ghats of Andhra Pradesh.

Name of Test species	Voucher specimen	Habit	Latitude and Longitude	Location
P.airy-shawii Brun. and Roux.	SKU50214	Herb	15°24′25.61"N; 78°41′30.24"E	Chelama (Kurnool Dist.)
P.amarus Schum. and Thonn.	SKU50217	Herb	14°28′32.32"N; 78°43′08.32"E	Yvu garden (Kadapa Dist.)
P.debilis Kl. ex Willd.	SKU50216	Herb	17°37′47"N; 81°37′01"E	Maredumelli (East Godavari Dist.)
P.emblica L.	SKU50219	Tree	14°28′26.75"N; 78°43′06.56"E	Yvu garden (Kadapa Dist.)
P. leshnaultii Mull.Arg.	SKU50220	Herb	14°25′44.82"N; 78°59′15.22"E	Palakondalu (Kadapa)
P.maderaspatensis L.	SKU50221	Herb	14°36′43"N; 77°38′42"E	S.K.U Campus (Ananthapuramu Dist.)
P.narayanswamii Gamble	SKU50206	Herb	18°16′27"N; 82°19′26"E	Araku (Visakhapatnam Dist.)
P.pinnatus (Wt.) Webster	SKU50201	Shrub	15°00′38.82"N; 78°01′25.53"E	Bhugga (Ananthapuramu Dist.)
P. polyphyllus Willd.	SKU50222	Tree	13°41′57"N; 79°20′21"E	Tirumala (Chittoor Dist.)
P.reticulatus Poir.	SKU50223	Shrub	14°12′21.72"N; 78°07′43.35"E	Kalasamudram (Ananthapuramu Dist.)
P. rheedei Wt.	SKU50204	Herb	15°53′07.39"N; 78°49′34.22"E	Rollapenta (Kurnool Dist.)
P.rotundifolius Kl. ex Willd.	SKU50224	Herb	17°43′21"N; 83°19′29"E	A.U. Campus (Visakhapatnam Dist.)
P. scabrifolius Hook.f.	SKU50215	Herb	15°24′25.61"N; 78°41′28.58"E	Chelama (Kurnool Dist.)
P.tenellus Roxb.	SKU50225	Herb	13°42′46"N; 79°20′31"E	Tirumala (Chittoor Dist.)
P.urinaria L. var. *najarana*	SKU50207	Herb	15°07′46.70"N; 78°40′41.63"E	Ahobilum (Kurnool Dist.)
P.virgatus G. Forst	SKU50202	Herb	14°14′39.70"N; 78°09′48.36"E	Kalasamudram (Ananthapuramu Dist.)

Table 2: Pollen morphology of *Phyllanthus* species (Phyllanthaceae).

Name of The species	P	E	P/E	Shape	Ornamentation	Aperture
P. airy-shawii Brun. and Roux.	16.5-18 µm	14-16 µm	113	Subprolate	Psilate	Triccolporate
P. amarus Schum. and Thonn.	17-19 µm	20-25 µm	85	Subprolate	Granular	Tricolporate
P.debilis Kl. ex Willd.	20-25 µm	20-25 µm	100	Spheroidal	Reticulate	Tricolporate
P. emblica L.	22-28 µm	22-28 µm	100	Spheroidal	Reticulate	Tetracolporate
P. lawii J.Grah.	30-35 µm	29-32 µm	103	Subprolate	Granular	Tricolporate
P. maderaspatensis L.	21-23 µm	15-17 µm	137	Prolate	Reticulate	Tricolporate
P. narayanaswamii Gamble	19.5-21 µm	19.5-21 µm	100	Spheroidal	Reticulate	Pantosyncolpate
P. pinnatus (Wt.) Webster	15-17 µm	15-17 µm	100	Spheroidal	Reticulate	Tricolporate
P. polyphyllus Willd.	12-14 µm	12-14 µm	100	Spheroidal	Reticulate	Tetracolporate
P. reticulatus Poir.	23-28 µm	23-28 µm	100	Spheroidal	Coarsely reticulate	Tricolporate
P. rheedei Wt.	25.5-27 µm	25.5-27 µm	100	Spheroidal	Psilate	Tricolporate
P. rotundifolius Kl. ex Willd.	30-34 µm	26-30 µm	114	Prolatespheroidal	Reticulate	Tricolporate
P.scabrifolius Hook.f.	22.5-24 µm	15-17 µm	162	Prolate	Reticulate	Tricolporate
P. tenellus Roxb.	28-32 µm	28-32 µm	100	Spheroidal	Reticulate	Tetracolporate
P. urinaria L. Var. najarana	18-20 µm	15-17 µm	118	Subprolate	Reticulate	Tetraolporate
P. virgatus G.Forst.	18-20 µm	18-20 µm	100	Spheroidal	Reticulate	Pantosyncolpate

P=Polar axis, E= Equatorial axis.

CONCLUSION

Since time immemorial, *Phyllanthus* species have been used for the treatment of several human and veterinary diseases all over the world. Most of the medicinally important *Phyllanthus* species are often adulterated with other ingredients due to their close similarities with their related species. It is evident that the pollen morphological characters provide helpful attributes in distinguishing *Phyllanthus* species within the genus and the characteristic features of aperture are significant in confirmation of taxonomic identity. The taxonomic discrimination among the members of *Phyllanthus* species depends on pollen morphology along with other microscopic characters as a majority of them are identical in exo- morphological features. Hence the present study supports classical taxonomic diversity in strengthening of key characters to enable easy identification.

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