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Pollen morphology of poaceae with special emphasis on the occurrence of diporate apertures

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Abstract
Poaceae family is considered to be stenopalynous as all the member exhibit similar pollen morphology being monoporate and psilate (Sometimes microreticulate). However, the occurrence of diporate pollen grains which is a rare phenomenon in the family, has been reported by various authors in recent times from different parts of the world, occurring in few species. Palynomorphological studies of Poaceae in India are quite meagre and the report of diporate pollen grains in Indian Poaceae is scanty.
The present study deals with 19 species of Indian Poaceae to investigate the presence of diporate pollen grains. Freshly collected pollen grains were acetolysed and studied under bright field light microscopy and their respective microphotographs were taken.
The study reveals the existence of diporate pollen grains in one of the members i.e. *Paspalidium flavidum*, which is not reported in earlier studies. *Paspalidium flavidum* exhibits both the type of pollen morphs, i.e. monoporate as well as diporate but monoporate pollen grains are dominant. It can serve as a taxonomic tool for identification of the species and it can be assumed that the increase in the number of apertures in Poaceae members could be an evolutionary phenomenon with an effect of environmental condition acting on it.

Keywords: diporate, *Paspalidium flavidum*, poaceae, pollen

Introduction
The family Poaceae is one of the largest family of Angiosperms belonging to the monocotyledons, having 12000 species distributed among 780 genera (Christenhusz and Byng, 2016) [3]. The members are mostly grasses and bamboos being abundantly distributed all over the world, providing staple foods and fodder. In India the family is represented by 266 genera and 1506 species (Kellogg *et al*., 2020) [11].
Pollen morphological analysis of this family has been carried out by many workers from time to time, all around the world. However, considering the vast number of species, palynological studies carried out in this family is quite less and in India it is quite meagre. Works of Erdtman (1952) [7] , Siddiqui and Qaiser (1988) [21], Chaturvedi *et al*. (1998) [2], Skvarla *et al*. (2003) [22], Liu *et al*. (2004)[12], Perveen (2006) [17], Perveen and Qaiser (2012) [16], Nazir *et al*. (2013) [15], Morgado *et al*. (2015) [13], Radaeski (2016), Radaeski *et al*. (2017) [20], Ghosh and Karmakar (2017) [9], Guimaraes *et al*. (2018) [10] and Mursyidin *et al*. (2018) [14] are worth to be mentioned.
Poaceae has been considered to be a stenopalynous family from a very early time due to the presence of more or less uniform type of pollen grains, distributed among its members. The pollen grains are basically spheroidal and monoporate having more or less similar exinous pattern. However, with the passage of time, as more palynological researches on Poaceae are being carried out in different parts of the world, the existence of diporate pollen grains in the family was reported in various members, eg. *Pharus lappulaceus, Digitaria ciliares, Papalum pauciciliatum* (Radaeski *et al*. 2016) [19] from Southern Brazil, *Enneapogon persicus, Aristida adscensionis, Melanocenchris abyssinica* (Perveen, 2006) [17] from Pakistan are few to be mentioned. However, from India very scanty report is available regarding the pollen morphology of Poaceae. So, the present study has been undertaken to investigate the presence of diporate pollen grains in the family.

Materials and Methods
The polleniferous materials of 19 species of Poaceae were freshly collected from their natural habitats, growing in different regions of West Bengal, India. The pollen grains were acetolysed following the acetolysis method of Erdtman (1952) [7] and were thoroughly studied using bright field light microscopy**,** under a Leitz laborlux S (Germany) microscope with Leica DFC 295 digital camera attachment.
The measurements of the pollen size, exine thickness, aperture etc. were taken using a stage and ocular micrometer under 100x oil immersion lens of the microscope. Diluted Safranin stain has been used in some grains. The pollen grains were counted using the dilution method as per the methodologies of Cruden (1977) [4].
The basic terminologies of Punt (2007) [18] were followed in describing the pollen grains.
The scientific names of the studied species have been consulted with the website: *The Plant List* (http://www.theplantlist.org/).

Results and Discussion

The palynomorphological details of the selected taxa are as follows.

***Arundinella pumila* (Hochst.) Steud. (Fig. 1: A-B)**

Pollen grains are radially symmetrical, heteropolar, spheroidal, 35.6 µm in diameter, amb circular, monoporate, pore circular, 5.1 µm in diameter, annulate, crassimarginate; exine tegillate, 1 µm in thickness, crassisexinous; surface microreticulate.

***Arundo donax* L. (Fig. 1: C-D)**

Pollen grains are radially symmetrical, heteropolar, spheroidal, 39.4 µm in diameter, amb circular, monoporate, pore circular, 3.5 µm in diameter, annulate, crassimarginate; exine tegillate, 1 µm in thickness, crassisexinous; surface microreticulate.

***Chrysopogon aciculatus* (Retz.) Trin. (Fig. 1: E-F)**

Pollen grains are radially symmetrical, heteropolar, spheroidal, 45.9 µm in diameter, amb circular, monoporate, pore circular, 4.4 µm in diameter, operculate, a circular patch of exine is present at the centre of the pore, annulate, crassimarginate; exine tegillate, 1.5 µm in thickness, crassisexinous; surface microreticulate.

***Coix lacryma jobi* L. (Fig. 1: G-H)**

Pollen grains are radially symmetrical, heteropolar, spheroidal to slightly prolate spheroidal, slightly urn-shaped, 96.6 µm in diameter, amb circular, monoporate, pore circular, 5 µm in diameter, annulate with a centrally located circular operculum, crassimarginate; exine tegillate, 1.7 µm in thickness, crassisexinous; surface psilate.

***Cynodon dactylon* (L.) Pers. (Fig. 1: I-J)**

Pollen grains are radially symmetrical, heteropolar, spheroidal, 54.7 µm in diameter, amb circular, monoporate, pore circular, 3.2 µm in diameter, annulate, crassimarginate; exine tegillate, 2.4 µm in thickness, crassisexinous; surface microreticulate.

***Dactyloctenium aegyptium* (L.) Willd. (Fig. 1: K-L)**

Pollen grains are radially symmetrical, heteropolar, P X E 43.4x48.3 µm, oblate-spheroidal, amb circular, monoporate, pore circular, 4.5 µm in diameter, annulate, crassimarginate; exine tegillate, 1.8 µm in thickness, crassisexinous; surface microreticulate.

***Desmostachya bipinnata* (L.) Stapf (Fig. 1: M)**

Pollen grains are radially symmetrical, heteropolar, 38.6 µm in diameter, spheroidal, amb circular, monoporate, pore circular, 2.5 µm in diameter, annulate, crassimarginate; exine tegillate, 2 µm in thickness, crassisexinous; surface psilate.

***Eleusine indica* (L.) Gaertn. (Fig. 1: N)**

Pollen grains are radially symmetrical, heteropolar, spheroidal, 33.8 µm in diameter, amb circular, monoporate, pore circular, 2.8 µm in diameter, annulate, crassimarginate; exine tegillate, 0.8 µm in thickness, crassisexinous; surface psilate.

***Oplismenus burmanni* (Retz.) P.Beauv. (Fig. 1: O-P)**

Pollen grains are radially symmetrical, heteropolar, spheroidal, 38.6 µm in diameter, amb circular, monoporate, pore circular, 3.1 µm in diameter, annulate, crassimarginate; exine tegillate, 1.4 µm in thickness, crassisexinous; surface microreticulate.

***Oplismenus compositus* (L.) P.Beauv. (Fig. 1: Q-R)**

Pollen grains are radially symmetrical, heteropolar, spheroidal, 44 µm in diameter, amb circular, monoporate, pore circular, 3.8 µm in diameter, annulate, crassimarginate; exine tegillate, 1.9 µm in thickness, crassisexinous; surface microreticulate.

***Oryza sativa* L. (Fig. 1:S-T)**

Pollen grains are radially symmetrical, heteropolar, spheroidal, 36 µm in diameter, amb circular, monoporate, pore small, annulate, circular, 3.5 µm in thickness, crassimarginate; exine tegillate, crassisexinous; surface psilate.

***Paspalidium flavidum* (Retz.) A.Camus (Fig. 1: U-V)**

Pollen grains are radially symmetrical, heteropolar, P X E 37x40.2 µm, oblate- spheroidal, amb circular, monoporate, pore circular, 3 µm in diameter, annulate, crassimarginate; exine tegillate, 1.6 µm in thickness, crassisexinous; surface microreticulate.

Some di-porate grains are also observed where the two pores are present close to each other.

***Polypogon monspeliensis* (L.) Desf. (Fig. 1:W-X)**
Pollen grains are radially symmetrical, heteropolar, P X E 30.5 X 33.8 µm, oblate-spheroidal, amb circular, monoporate, pore circular, 4.8 µm in diameter, annulate, crassimarginate; exine tegillate, 0.9 µm in thickness, crassisexinous; surface microreticulate.

***Saccharum spontaneum* L. (Fig. 1:Y-Z)**
Pollen grains are radially symmetrical, heteropolar, P X E 61.3 X 57.4 µm, prolate-spheroidal, slightly urn shaped, amb circular, monoporate, pore circular, 3.4 µm in diameter, annulate, crassimarginate; exine tegillate, 1.6 µm in thickness, crassisexinous; surface microreticulate.

***Setaria pumila* (Poir.) Roem. & Schult. (Fig. 1:A1-A2)**
Pollen grains are radially symmetrical, heteropolar, spheroidal, 59.5 µm in diameter, amb circular, monoporate, pore circular, 4.5 µm in diameter, annulate, crassimarginate; exine tegillate, 1.7 µm in thickness, crassisexinous; surface psilate.

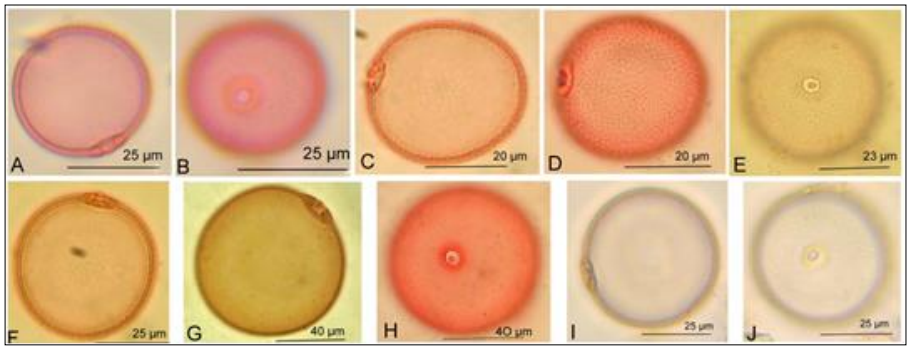
***Setaria verticillata* (L.) P.Beauv. (Fig. 1:A3-A4)**
Pollen grains are radially symmetrical, heteropolar, spheroidal, 49.8 µm in diameter, amb circular, monoporate, pore circular, 2.5 µm in diameter, annulate, crassimarginate; exine tegillate, 1.4 µm in thickness, crassisexinous; surface microreticulate.

***Setaria viridis* (L.) P.Beauv. (Fig. 1:A5-A6)**
Pollen grains are radially symmetrical, heteropolar, spheroidal, slightly urn shaped, 54 µm in diameter, amb circular, monoporate, pore circular, 5.1 µm in diameter, annulate, crassimarginate; exine tegillate, 0.8 µm in thickness, crassisexinous; surface microreticulate.

***Vetiveria zizanioides* (L.) Nash (Fig. 1:A7-A8)**
Pollen grains are radially symmetrical, heteropolar, spheroidal, 51.5 µm in diameter, slightly urn shaped, amb circular, monoporate, pore circular, 4.3 µm in diameter, annulate, crassimarginate; exine tegillate, 1.6 µm in thickness, crassisexinous; surface microreticulate.

***Zea mays* L. (Fig. 1:A9-A10)**
Pollen grains are radially symmetrical, heteropolar, P X E 161 X 130.2 µm, sub-prolate, slightly urn-shaped, amb circular, monoporate, pore circular, annulate with a centrally located circular operculum, 7.6 µm in diameter, crassimarginate; exine tegillate, 2.1 µm in thickness, crassisexinous; surface psilate. (P: polar axis; E: equatorial axis of the pollen grain) It has been observed that majority of the pollen grains are spheroidal in shape while few are oblate-spheroidal and the size ranges from 35.6 to 61.3 µm, thus fitting into the *mediae* and *magnae* size (Erdtman, 1969) [8]. The pollen grains of *Zea mays* and *Coix lacryma jobi* are exceptionally larger in size being 130 µm and 96.6 µm in diameter. The apertural pattern in all the pollen grains is monoporate (except for one of the species i.e. *Paspalidium flavidum*). Pores are annulated, circular, provided with operculum in few species, i.e. *Zea mays*, *Coix lacryma jobi* and *Chrysopogon aciculatus*. The surface is microreticulate in majority of the species while few exhibits psilate exine, they are *Zea mays*, *Setaria pumila*, *Oryza sativa*, *Eleusine indica*, *Desmostachya bipinnata* and *Coix lacryma jobi*. The morphology of the studied pollen grains are quite similar to that of the previous authors mentioned earlier in this work. The pollen grain of *P. monspeliensis* has been studied earlier by Nazir *et al*. (2013) [15] where the surface pattern was mentioned to be of verrucate type, whereas in our present study it is of microreticulate type. Siddiqui and Qaiser (1988) [21] mentioned about the occurrence of diporate pollen grains in this species. Di-porate pollen grains are observed only in *Paspalidium flavidum* which is not a common phenomenon in Poaceae. Monoporate pollen grains are the dominant pollen morph in *P. flavidum* and the proportion of diporate pollen grains in the species is quite less. The diporate pollen morph constitutes only 22.4 % of the total pollen count of the species. The pollen grains of *P. flavidum* has been studied earlier by Ghosh and Karmakar (2017) [9] but there was no mention of presence of diporate pollen grains.

Figures



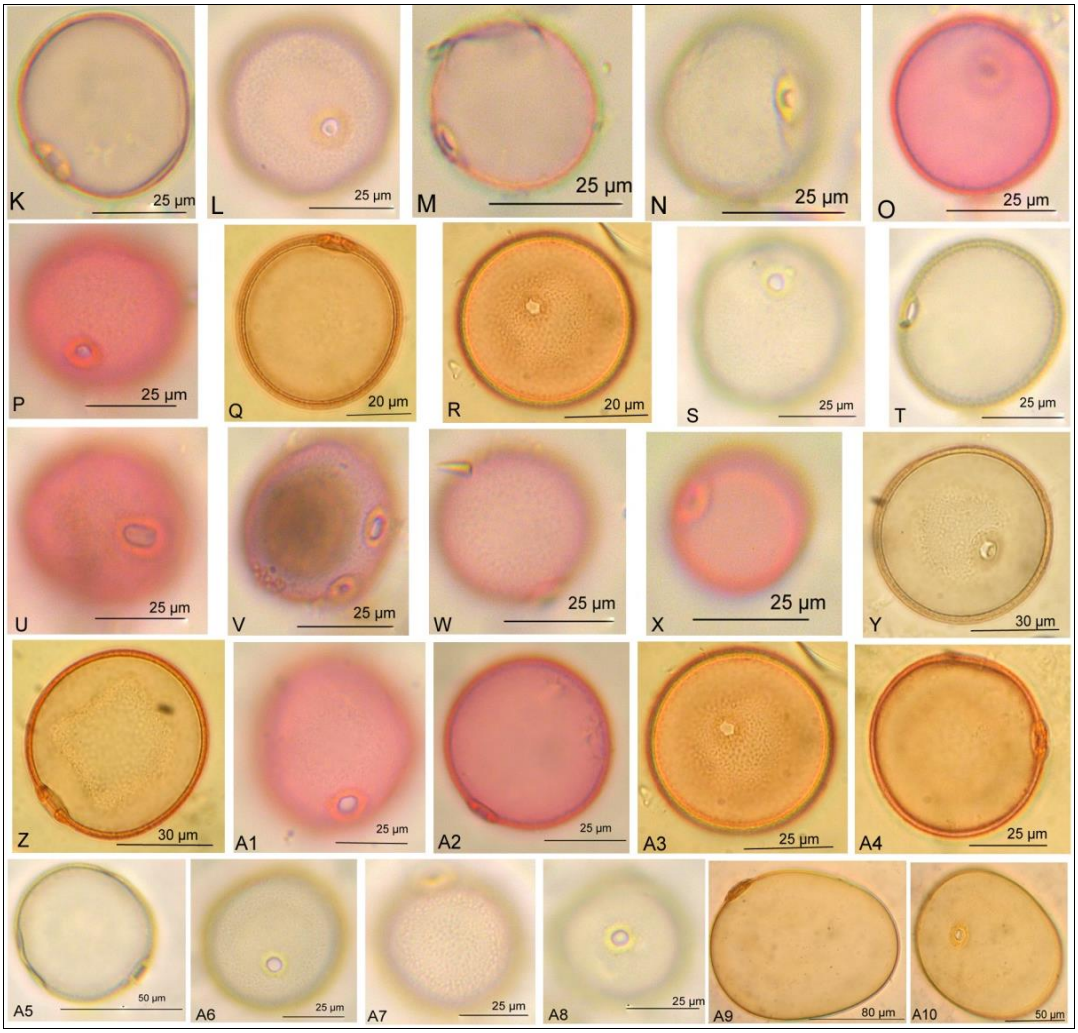


Fig 1: Pollen grains of the respective species of Poaceae. A–B. *Arundinella pumila*. C–D. *Arundo donax*. E–F. *Chrysopogon aciculatus*. G–H. *Coix lacryma jobi.* I–J. *Cynodon dactylon.* K–L. *Dactyloctenium aegyptium.* M. *Desmostachya bipinnata.* N. *Eleusine indica*. O–P. *Oplismenus burmanni*. Q–R. *Oplismenus compositus.* S–T. *Oryza sativa.* U–V. *Paspalidium flavidum.* W–X. *Polypogon monspeliensis*. Y–Z. *Saccharum spontaneum.* A1–A2. *Setaria pumila.* A3–A4. *Setaria verticillata.* A5–A6. *Setaria viridis.* A7–A8. *Vetiveria zizanioides.* A9-A10. *Zea mays.*

Conclusions

The presence of diporate pollen grains in the species *Paspalidium flavidum*, has been reported for the first time in the present work although the species has been investigated earlier by Ghosh and Karmakar (2017) [9]. The presence of diporate pollen grains in the family Poaceae is quite rare but has been reported earlier in species like *Digitaria violascens, Tragus roxburgii, Polypogon monspeliensis* (Siddiqui and Qaiser, 1988) [21], *Pharus lappulaceus, Digitaria ciliares, Papalum pauciciliatum* (Radaeski *et al*. 2016) [19], *Enneapogon persicus, Aristida adscensionis, Melanocenchris abyssinica* (Perveen, 2006) [17], *Agrostis gigantea, A. munroana, A. viridus, Festuca rubra, Hyalopoa nutans, Hyparrhenia hirta, Koeleria macrantha, Lolium temulentum* (Perveen and Qaiser, 2012)[16] and *Dicanthelium sabulorum, Echinochloa polystachya, Zizaniopsis bonariensis* (Radaeski *et al*. 2017)[20]. So, the co-existence of diporate pollen grains along with the monoporate ones in few species of Poaceae along with variations in size and exine ornamentations questions the stenopalynous nature of the family. Number of aperture of pollen grains is said to have increased over the geological time (Chaloner, 1976; Van Campo, 1976; Doyle and Hotton, 1991)[1, 23, 6], so it could be assumed that an increase in the number of pores in the pollen grains of Poaceae, as witnessed in many species in recent times, could have been an evolutionary phenomenon. Increase in aperture number leads to faster germination of pollen tube but on the other hand lowers life expectancy (Dajoz *et al*. 1991) [5]. So, further studies are required to throw light in this regard which could be done by taking into account the germination rate and life expectancy of both the pollen morphs of the species, i.e. the monoporate and diporate pollen grains of *Paspalidium flavidum*. The diporate pollen grain in Poaceae serves as a taxonomic tool in identifying of the species.

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