Pollen Morphology of Indian Species of *Saraca* L. (Leguminosae)-A Threatened and Legendary Medicinal Tree

Sujit Sil[1, 2], Tanmoy Mallick[2], Tuhin Pal[1], Animesh Mondal[1], Kalyan Kumar De[1] and Asok Ghosh[2,*]

[1]Postgraduate Department of Botany, Hooghly Muhammad Mohsin College (Estd.1836), Chinsurah, 712101, Hooghly, West Bengal, India.

[2]UGC-CAS Department of Botany, Taxonomy of Angiosperms and Biosystematics Laboratory, University of Burdwan, Golapbag, Burdwan, 713104, West Bengal, India.

[*]Corresponding Author: Asok Ghosh. Email: asokcarex@gmail.com; asokcarex@rediffmail.com.

Abstract: The genus *Saraca* L. (Leguminosae) is a universal panacea in herbal medicine. The present study investigates the comparative pollen morphology of four species of *Saraca viz*. *S. asoca* (Roxb.) de Wilde, *S. declinata* (Jack) Miq., *S. indica* L., and *S. thaipingensis* Cantley ex Prain growing in India to reveal differences of their pollen structures to aid taxonomic and evolutionary values. The detailed morphology and surface structure of pollen grains were studied and described using light microscopy and scanning electron microscopy. The pollen grains of *Saraca* showed isopolar, para-syncolporate, tricolporate, with radially symmetric, prolate and prolate-spheroidal structure. The surface of pollen of *S. indica* is rugulate with large lirae but in *S. declinata,* the surface is micro-rugulate to vermiculate with relatively thin lirae and that of *S. thaipingensis* is indistinct as the psilate surface with a frequent protuberance and fewer perforations were observed along with the gemmae like structure. Exine ornamentation helped to separate *S. indica* and *S. asoca*. Exine thickness varies from 3-4 µm. Presence of protuberance and exine thickness varies among individuals of the species spread over different locations. Present work also provides a unique palynological identity and interrelationship of these four species based on cluster analysis taking 23 pollen characters with the help of statistical method like the plotting of ternary graph. Ternary plots also helped to calculate the level of plasticity of each character in the intra- and inter-specific level.

Keywords: Cluster analysis; palynology; SEM study; surface ornamentation; ternary graph

1 Introduction

The genus *Saraca* L. of the family- Leguminosae, subfamily- Detarioideae [1] and the tribe- Detarieae [2], is a small to medium-sized evergreen rainforest trees growing to a maximum height of nine meters. 11 species are distributed in tropical and subtropical rain forests of southeastern and southern Asia [3]. Four species of *Saraca* are distributed in India; i.e., *S. asoca* (Roxb.) de Wilde (Fig. 1(A)) is a small tree distributed in different places of India. *S. declinata* (Jack) Miq. is distributed in India and other countries like Burma, Malaysia, Myanmar, Sri Lanka and commonly known as Red Ashok (Fig. 1(D)). *S.indica* L. is a medium-sized tree and commonly known as Sita Ashok (Fig. 1(G)), frequently planted in association with Buddhist temples [2]. *S. thaipingensis* Cantley ex Prain is present in India under captivating and frequent in Myanmar, Thailand, Malaysia and Indonesia having yellow flowers (Fig. 1(J)) and thus commonly known as Yellow *Saraca* or Yellow Ashok. *S. asoca* having close morphological resemblance with *S. indica* and co-exists in India with *S. indica* [3]. Central and eastern Himalayan foothills, northern plains and western coasts are reported to have a rich distribution of *S.indica* [4]. All of these plants are considered to contain medicinal properties. The crude extract of flowers of *S.asoca* is considered the best uterine tonic [5,6]. Due to the decline in population *S. asoca* is listed as 'globally vulnerable' in the Red List of Threatened Species of International Union for Conservation of Nature and Natural Resources [7] (http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T34623A9879360.en).

Little is known about the pollen morphology of the species of *Saraca* [8-10] and it is important to know the detailed structural evaluation of pollen as it can help the research on gene flow, aerobiology, reproductive biology, and systematics. Morphology of the pollen grain is often a pertinent taxonomic marker in the identification of higher plants [11] and is not influenced by environmental conditions. Palynology is unique to provide a tremendous amount of information and the variation of pollen morphological characters have importance for phylogenetic structuring [12] and having phylogenetic significance [13] it was applied to evaluate intergeneric relationships [14] and classify the eudicots [15].

Ternary plots were used to evaluate the correlation of floral characters related to pollination which helped to establish the evolutionary relationships among pollinators [16]. Selection of non-variable characters among the large set of morphological features of *Saraca* was also done with the help of ternary plots [10].

The present study was undertaken to specify the pollen morphology of four species of *Saraca* and the phenetic relationship among them, evaluating the stability and plasticity of characters with the help of ternary plots.

Figure1: Photographs and micrographs of four species of *Saraca*. A: Flowering twig of *S. asoca*. B: Equatorial view of pollen grain of *S. asoca*. C: Polar view of pollen grain of *S. asoca*. D: Flowering twig of *S. declinata*. E: Equatorial view of pollen grain of *S. declinata*. F: Polar view of pollen grain of *S. declinata*. G: Flowering twig of *S. indica*. H: Equatorial view of pollen grain of *S. indica*. I: Polar view of pollen grain of *S. indica*. J: Flowering twig of *S. thaipingensis*. K: Eqatorial view of pollen of *S. thaipingensis*. L: Polar view of pollen *S. thaipingensis*

2 Materials and Methods

Partially opened matured fresh flowers of four species of *Saraca viz. S. asoca*, *S. declinata*, *S. indica*, and, *S. thaipingensis* were collected at 9 AM from the month of April 2015 to March 2018 for palynological studies from the plants grown in Acharya Jagadish Chandra Bose Indian Botanic Garden, Shibpur, West Bengal, India and other localities mentioned in Tab. 1. Polliniferous samples were collected from different places of India and all the voucher specimens of the studied species deposited at Herbarium of the University of Burdwan (Acronym BURD [17]).

Table 1: Details of the collection of all plants under consideration

Sl No.	Name of species	Location	Global position	Date of Collection	Voucher	Altitude
1	*S. asoca* (Roxb.) de Wilde (AS-1)	Ramanathpur, West Bengal	88°21′93″E 22°68′50″N	26/04/15	BURD12008	7 M
2	*S. asoca* (Roxb.) de Wilde (AS-2)	Tarkeswar, West Bengal	88°01′42″E 22°87′86″N	13/04/15	BURD12049	14 M
3	*S. declinata* (Jack) Miq. (DE-1)	Shibpur, West Bengal	88°31′17″E 22°57′21″N	13/01/16	BURD12005	7 M
4	*S. declinata* (Jack) Miq. (DE-2)	Chinsurah, West Bengal	88°39′19″E 22°88′60″N	08/01/18	BURD12009	17 M
5	*S. declinata* (Jack) Miq. (DE-3)	Long island, Andaman	92°93′51″E 12°39′60″N	02/03/17	BURD12012	68 M
6	*S. indica* L. (IN-1)	Shibpur, West Bengal	88°31′17″E 22°57′25″N	17/06/17	BURD12004	7 M
7	*S. indica* L. (IN-2)	Serampore, West Bengal	88°33′86″E 22°74′80″N	27/03/16	BURD12010	11 M
8	*S. thaipingensis* Cantley ex Prain (TH-1)	Shibpur, West Bengal	88°31′17″E 22°57′20″N	26/01/16	BURD12006	7 M
9	*S. thaipingensis* Cantley ex Prain (TH-2)	Bengaluru, Karnataka	75°95′79″E 11°95′50″N	28/03/18	BURD12050	1467 M

2.1 Sample Preparation and Light Microscopy

Pollen grains were isolated from the anther of each species separately and acetolysis was done following the method of Erdtman [18,19]. For the light microscopic study, the acetolysed pollen grains were centrifuged to decant for 3-5 minutes. The pollen material was mounted on the slide with glycerin jelly without any staining. The pollen grains were examined under oil immersion objective using a 10 × eyepiece of a compound microscope (Olympus CH20i microscope) fitted with CMOS Camera (IS 500, 5.0 MP) and its attachment with a computer. Both the polar axis and the equatorial axis of pollen grains (5 samples for each voucher), aperture size, and exine thickness were measured. Quantitative measurements (Tab. 2) of pollen grains were done with the aid of VIEW 7 image analysis software. LM images of pollen grains are shown in Figs. 1(B), 1(C), 1(E), 1(F), 1(H), 1(I), 1(K), 1(L) and Supplementary Figs. 1(B), 1(C), 1(F), 1(G), 1(J), 1(K), 1(N), 1(O), 1(R), 1(S).

2.2 SEM Study

For scanning electron microscopic (SEM) study, the previously acetolysed pollen material was mounted on a metallic stub, coated with gold, in a sputtering chamber (Ion-sputter JFC-1100). The coating was restricted to 150Å and SEM examination was carried out with a Carl Zeiss EVO 40 (EDX Oxford Instrument No-7636) system and its relevant software, Carl Zeiss Smart SEM 0.5.03. The terminology for the description of pollen grains is used after different literatures [18,20,21]. SEM images of pollens are represented in Fig. 2 and Supplementary Figs. 1(D), 1(H), 1(L), 1(P), 1(T) and pollen morphological data are represented in Tab. 2.

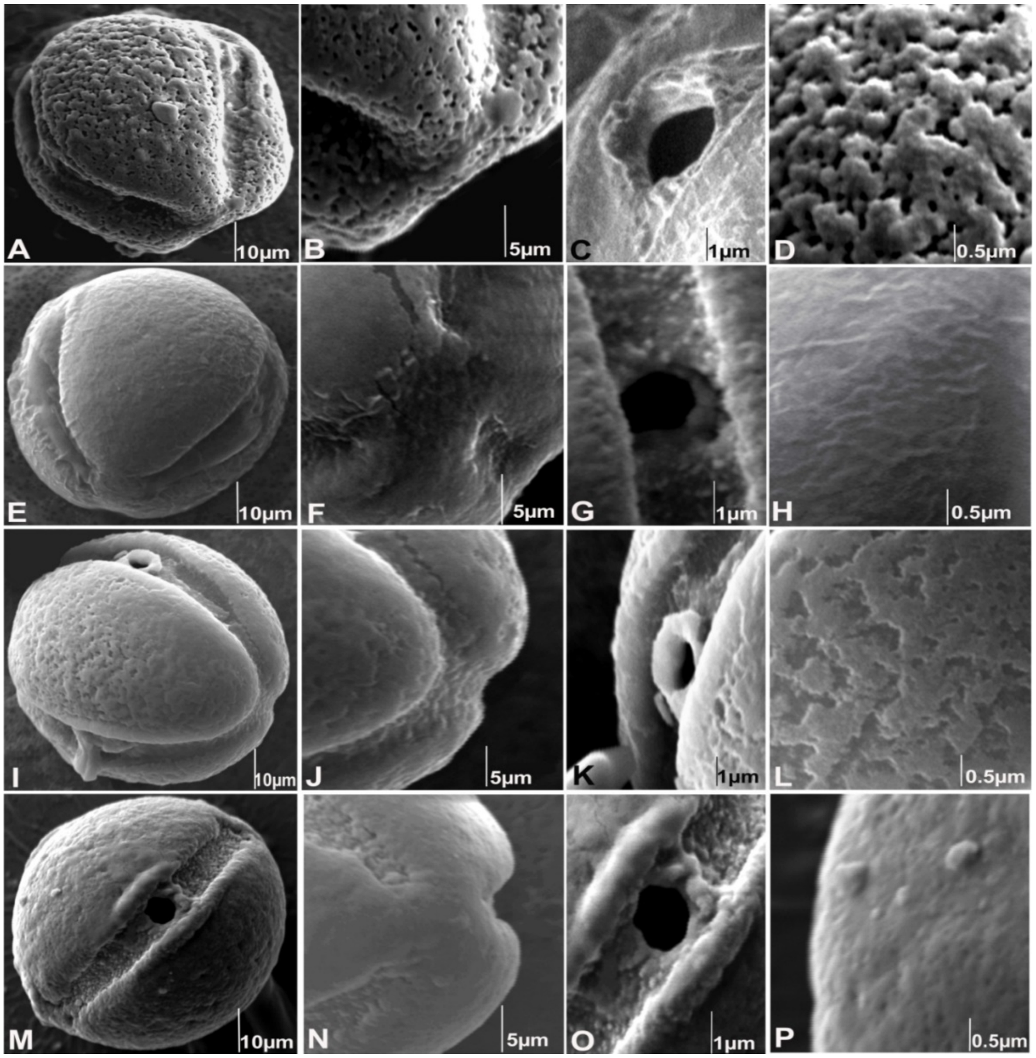

Figure 2: SEM micrographs of pollen of four species of *Saraca*. A: Pollen grain of *S. asoca*. B: Apocolpium region of *S. asoca*. C: Aperture of *S. asoca*. D: Surface ornamentation of pollen grains of *S. asoca*. E: Pollen grains of *S. declinata*. F: Apocolpium region of *S. declinata*. G: Aperture of *S. declinata*. H: Surface ornamentation of pollen grains of *S. declinata*. I: Pollen grain of *S. indica*. J: Apocolpium region of *S. indica*. K: Aperture of *S. indica*. L: Surface ornamentation of pollen of *S. indica*. M: Pollen grains of *S. thaipingensis*. N: Apocolpium region of *S. thaipingensis*, O: Aperture of *S. thaipingensis*. P: Surface ornamentation of pollen of *S. thaipingensis*

Table 2: Comparative quantitative data of pollen morphology of three species of *Saraca* (Data expressed as a mean of 10 replicates ± SD)

Ch. No.	Characters of pollen grains	*S. asoca* (AS-1)	*S. asoca* (AS-2)	*S. declinata* (DE-1)	*S. declinata* (DE-2)	*S. declinata* (DE-3)
1	*PA (µm)	68.65 ± 2.86	59.85 ± 3.2	48.49 ± 1.96	57.73 ± 1.92	61.4 ± 1.78
2	ǂED (µm)	53.2 ± 2.13	54.17 ± 0.98	39.83 ± 1.13	56.38 ± 1.1	50.74 ± 1.2
3	P/E Ratio	1.2 ± 0.43	1.1 ± 0.31	1.22 ± 0.27	1.02 ± 0.29	1.21 ± 0.28
4	Length of colpus (µm)	52.6 ± 4.63	52.34 ± 3.17	38.22 ± 3.96	54.65 ± 4.08	46.65 ± 4.1
5	Breadth of colpus (µm)	6.6 ± 1.14	4.93 ± 0.69	5.31 ± 0.69	4.06 ± 0.34	7.8 ± 0.31
6	Depth of colpus (µm)	4.9 ± 0.39	3.36 ± 0.41	2.79 ± 0.39	5.34 ± 0.42	2.04 ± 0.37
7	§L/B ratio of colpus	7.97 ± 0.69	10.62 ± 0.82	7.20 ± 0.79	13.46 ± 0.41	5.98 ± 0.37
8	Area of colpus (μm^2)	129.47 ± 41.7	207.66 ± 20.3	205.97 ± 23.14	204.7 ± 17.5	198.36 ± 26.4

9	Perimeter of colpus	67.2 ± 5.89	79.22 ± 6.23	88.94 ± 5.32	86.22 ± 5.11	84.32 ± 4.07
10	[ʃ]CL/PA ratio	98.87 ± 6.63	96.62 ± 5.77	78.82 ± 7.11	96.93 ± 5.35	91.94 ± 7.51
11	Pore size (µm)	3.62 ± 0.32	5.95 ± 0.49	3.63 ± 0.49	4.85 ± 0.47	4.96 ± 0.52
12	Pore area (µm^2)	54.26 ± 6.75	39.72 ± 6.32	24.93 ± 1.26	24.21 ± 3.79	26.17 ± 2.73
13	Perimeter pore (µm)	29.33 ± 0.38	14.21 ± 1.12	18.02 ± 0.87	17.23 ± 0.98	18.51 ± 1.06
14	Height of annulus (µm)	1.71 ± 0.73	0.31 ± 0.04	0.67 ± 0.1	0.62 ± 0.07	0.63 ± 0.08
15	Amb value	1230.13 ± 179.62	1010.27 ± 211.5	1422.14 ± 198.65	1526.17 ± 301.39	1453.21 ± 213.54
16	Apocolpium index	11.97 ± 1.06	21.71 ± 3.45	26.17 ± 2.41	24.27 ± 5.11	25.31 ± 4.36
17	Area of apocolpium space (µm^2)	12.06 ± 1.8	24.17 ± 3.52	69.74 ± 6.79	67.25 ± 8.59	70.11 ± 7.03
18	Angle of apocolpium space	60.02 ± 0.04	60.01 ± 0.08	60.04 ± 0.08	60.02 ± 0.05	60.05 ± 0.07
19	Perimeter of apocolpium(µm)	21.31 ± 4.74	19.16 ± 1.06	38.22 ± 2.13	36.28 ± 2.87	39.31 ± 3.01
20	No of gemmae like structure (per µm^2)	0	0	0	1 ± 1	0
21	Sexine thickness(µm)	1.48 ± 0.41	1.81 ± 0.16	0.87 ± 0.12	0.91 ± 0.12	0.82 ± 0.09
22	Nexine thickness(µm)	0.71 ± 0.16	1.29 ± 0.12	0.78 ± 0.06	0.78 ± 0.09	0.67 ± 0.05
23	Perimeter of the pollen grain (µm)	127.9 ± 20.08	121.5 ± 9.21	139.47 ± 17.59	117.3 ± 17.2	110.5 ± 9.89

Ch. No.	Characters of pollen grains	*Saraca indica* (IN-1)	*S. indica* (IN-2)	*S. thaipingensis* (TH-1)	*S. thaipingensis* (TH-2)
1	[*]PA (µm)	46.23 ± 2.9	59.2 ± 2.7	44.98 ± 1.91	62.16 ± 1.86
2	[ǂ]ED (µm)	34.41 ± 1.52	54.98 ± 1.39	40.57 ± 1.32	55.86 ± 1.36
3	P/E Ratio	1.34 ± 0.25	1.08 ± 0.23	1.11 ± 0.34	1.11 ± 0.35
4	Length of colpus (µm)	43.36 ± 3.15	42.48 ± 3.07	37.41 ± 4.21	46.62 ± 4.51
5	Breadth of colpus (µm)	6.83 ± 0.76	7.07 ± 0.75	8.06 ± 1.63	5.48 ± 1.43
6	Depth of colpus (µm)	3.67 ± 0.41	3.09 ± 0.43	3.99 ± 0.41	5.67 ± 0.63
7	[§]L/B ratio of colpus	6.85 ± 0.76	6.01 ± 0.68	4.64 ± 0.43	8.51 ± 0.49
8	Area of colpus (µm^2)	117.21 ± 17.55	132.42 ± 19.5	209.17 ± 26.41	201.41 ± 31.6
9	Perimeter of colpus	73.36 ± 5.59	76.75 ± 4.77	85.92 ± 5.27	86.4 ± 7.12
10	[ʃ]CL/PA ratio	93.79 ± 6.32	77.26 ± 6.79	83.17 ± 8.05	83.46 ± 9.11
11	Pore size (µm)	3.84 ± 0.51	3.96 ± 0.17	4.71 ± 0.53	4.97 ± 0.59
12	Pore area (µm^2)	56.17 ± 3.91	57.67 ± 3.97	18.32 ± 0.79	20.12 ± 1.81
13	Perimeter pore (µm)	27.39 ± 1.33	28.62 ± 1.23	14.95 ± 0.69	16.72 ± 1.14
14	Height of annulus(µm)	1.89 ± 0.17	1.43 ± 0.07	0.28 ± 0.03	0.27 ± 0.02
15	Amb value	1249.97 ± 76.41	1170.92 ± 157.67	1028.67 ± 147.42	1092.26 ± 197.49
16	Apocolpium index	12.47 ± 2.16	13.18 ± 2.19	20.28 ± 2.01	20.65 ± 2.64
17	Area of apocolpium space (µm^2)	13.21 ± 1.17	12.97 ± 2.45	23.67 ± 1.89	23.63 ± 3.17
18	Angle of apocolpium space	60.05 ± 0.09	60.17 ± 0.16	60.17 ± 1.33	60.09±0.12
19	Perimeter of apocolpium (µm)	15.99 ± 1.28	21.97 ± 2.33	22.86 ± 1.32	22.67±2.93
20	No of gemmae like structure (per µm^2)	0	3 ± 1	1 ± 1	3 ± 2
21	Sexine thickness (µm)	0.64 ± 0.07	1.78 ± 0.25	1.31 ± 0.43	1.57 ± 0.42
22	Nexine thickness (µm)	0.67 ± 0.06	1.19 ± 0.23	0.37 ± 0.04	0.96 ± 0.13
23	Perimeter of the pollen grain (µm)	131.26 ± 14.71	137.6 ± 19.4	118.85 ± 18.21	133.6 ± 14.7

[*]PA = polar axis, [ǂ]ED = equatorial diameter, [§]L/B ratio = ratio between length and breadth of colpus, [†]R = range, [ʃ]CL/PA = Ratio between the length of the colpus and polar axis and A = average

2.3 Cluster Analysis and Preparation of Dendrogram

A dendrogram (Fig. 3) was obtained using PAST3 software to conduct multivariate cluster analysis (Correlation outgroup) applying 23 character states of pollen morphological features and data matrix presented in Supplementary Tabs. 2 and 3. Four species of *Saraca* and their different individuals were considered as IN-1 to TH-2 (Tab. 1) and character states were oriented with four character codes i.e., 0, 1, 2 and 3.

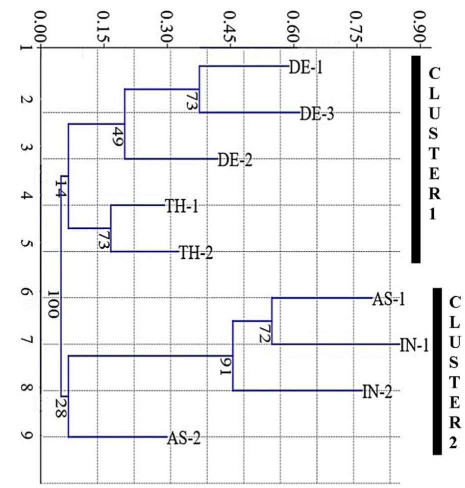

Figure 3: Dendrogram representing nine different specimens of *Saraca*

2.4 Measuring the Mean Proportion Percentage and Preparation of Ternary Plots

Mean of Proportion Percentage $= \frac{\text{sum of the value of characters of three species}}{3} \times 100\%$ Values of character states were converted to proportion percentage with the formula obtained (Supplementary Tab. 4-10). Then the data were plotted in the datasheet of PAST 3 software and using the toolkit, transposition of arrangement was done. Then entering into the plot option and applying Ternary plot option, specific plot-diagramme was obtained.

Application of ternary plots done here in two ways; firstly, the combination of four species of *Saraca* in four ternary plots (Supplementary Fig. 2), one species at a time helped to select identifying characters of pollen and secondly, combination of each species with their closely related individuals in the ternary plot (Supplementary Fig. 3) helped to characterize plasticity and stability of characters of pollen. Colored space within the ternary shows the range of characters. The affinity of dots towards the epicenter of the triangle shows the stability of characters whereas the increase of the distance of a dot from the epicenter represents the increase of plasticity of the character represented by the dot.

3 Results

3.1 General Morphology of the Pollen

Pollen grains of four species of *Saraca* are usually prolate to prolate-spheroidal. Pollen grains of *S. asoca* (Figs. 1(B), 1(C), Supplementary Figs. 1(B), 1(C), 1(D)) are usually prolate-spheroidal and that of *S. declinata* is sub-prolate (Figs. 1(E), 1(F), Supplementary Figs. 1(F), 1(G), 1(H), 1(J), 1(K), 1(L)) and pollen grains of *S. indica* (Figs. 1(H), (I), Supplementary Figs. 1(N), 1(O), 1(P)) and *S. thaipingensis* (Figs. 1(K), 1(L), Supplementary Figs. 1(R), 1(S), 1(T)) also vary from prolate to prolate-spheroidal. The amb is circular or sub-triangular with zonoaperturates. The diameter of pollen grains varies from 39 µm to 57 µm.

Pollen grains of *Saraca* spp. are usually medium to large in size (Medium = 25-50 µm, Large ≥ 50 µm), Isopolar (In *S. declinata*, Voucher BURD12005; both isopolar and heteropolar pollen grains were observed), parasyncolporate, parasyncolpate, sometimes syntricolpate, occasionally parasyncolporate, radially symmetric, prolate to subprolate (Figs. 2(A), 2(B), 2(C), 2(E), 2(F), 2(G), 2(I), 2(J), 2(K), 2(M), 2(N), 2(O)).

The variability of pollen aperture is higher in different species of *Saraca*. as well as the shape and size. The surface ornamentation also varies considerably like reticulate, macro-rugulate, micro-rugulate, psilate. The presence of gemmae like structure is also a very important character.

3.2 Apertures

The number and type apertures are similar among four species of *Saraca*. Most of the species have angulaperture. The compound type of aperture is present in all cases as endoaperture is present as pore at the equator of each of the three colpi (Figs. 2(C), 2(G), 2(K), 2(O)). The length and breadth ratio of the aperture (colpi) is greater than 2 in all of the four species. Each endoaperture contain distinct ring-like thickening or annulus and the height of it varies in four species. The annulus of *S. indica* has the maximum height (Fig. 2(K)) and that of *S. thaipingensis* (Fig. 2(O)) is apparently very minute and the annulus is medium in height in *S. declinata* (Fig. 2(G)). The height of the annulus of *S. asoca* is also remarkably high (Fig. 2(C)). The amb (ambitus or contour or circumference of the pollen grains in polar view) is peritreme to angulaperturate. Three colpi fuse or anastomose with each other at each pole producing apocolpial region and that area varies in four species (Figs. 1(C), 1(F), 1(I), 1(L), Figs. 2(B), 2(F), 2(J), 2(N)). However, in *S. thaipingensis* the area and nature of the apocolpial field itself vary in different samples as the colpa becomes anastomose (Supplementary Fig. 1(R), (T)) in some and remains separated in others (Fig. 2(N)).

3.3 Exine Morphology and Surface Ornamentation

The surface ornamentation varies as rugulate with large lirae in *S.indica* (Fig. 2(L)), micro-rugulate with relatively thin lirae but in *S. declinata* (Fig. 2(H)) and in *S. thaipingensis* pollen surface is indistinct but psilate with gemmae like structure (Fig. 2(P)). Pollen grains of *S. asoca* is relatively large in size in respect to other species but the surface ornamentation (Fig. 2(D)); i.e., reticulate type, produces the remarkable difference with other pollen grains. Exine thickness varies from 3-4 µm. *S. asoca* has thicker exine than *S. indica.* Tectum ornamentation is present but somewhat indistinct. Detailed qualitative features of four species and their other individuals are presented in Tab. 3. Qualitative characters of each type of pollen grains are represented in Supplementary Tab. 1.

3.4 Pollen Morphology of Different Species Examined

3.4.1 S. asoca

Pollen grains (Voucher BURD12008) are large, tricolporate with angulaperture and sub-triangular amb (Figs. 1(B), 1(C), Fig. 2(A)). Colpus is approximately 52.6 µm long and uniformly deep (4.9 µm) and wider at the equatorial region where an endoaperture, i.e., the pore (Fig. 2(C)) is situated. The area of the pore is relatively larger (54.26 µm) and the annulus is 1.71 µm high. The exine is with reticulate ornamentation (Fig. 2(D)). The sexine is thicker than nexine.

Pollen grains of another sample of the same species (Voucher BURD12049) showed similar characters inrespect to shape and size (Supplementary Figs. 1(B), 1(C), 1(D)). The apocolpium has a larger area (24.17 µm^2). The thickness of sexine and nexine is relatively broader than others.

3.4.2 S. declinata

Pollen grains of *S. declinata* (Voucher BURD12005) are medium in size (48.49 µm), tricolporate, angulaperturate with circular amb (Figs. 1(E), 1(F), Fig. 2(E)). Length of colpi is 38.22 µm and depth is 2.79

μm. Area of the pore (Fig. 2(G)) is 39.72 $μm^2$. The height of the annulus is considerably low (0.31 μm). The exine is micro-rugulate (Fig. 2(H)) and the thickness of sexine and nexine are relatively less thick.

Pollen grains from the voucher BURD12009 (Supplementary Figs. 1(F), 1(G), 1(H)) and voucher BURD12012 (Supplementary Figs. 1(J), 1(K), 1(L)) have a larger size (57.73 μm and 61.4 μm respectively). The length of colpi is longer in them and the breadth of it is surprisingly high in the pollen grains of voucher BURD12012 (7.8 μm). The area of endoaperture is less variable. The apocolpium space is relatively greater. The surface ornamentation is also non-variable.

3.4.3 S. indica

The size of the pollen grains of *S. indica* also varies considerably. Pollen grains are tricolporate with sub-triangular amb (Figs. 1(H), 1(I), Fig. 2(I)). Length of colpi is longer but the depth is relatively shallow. The thickness of the sexine and nexine also varies considerably. Pores (Fig. 2(K)) are relatively larger and the height of the annulus is relatively higher. Amb is sub-triangular in shape. The exine is observed to have rugulate surface (Fig. 2(L)).

The thickness of pollen grains is variable. Pollen grains of voucher BURD12004 has thin exine but that of voucher BURD12010 is with thick exine (Supplementary Figs. 1(N), 1(O), 1(P)). Pollen grains contain larger pores and the apocolpium index is relatively small. The surface is macro-rugulate like other samples.

3.4.4 S. thaipingensis

The size of the pollen varies from medium to large but the basic morphology is very similar among all of the samples (Figs. 1(K), 1(L), Fig. 2(M) and Supplementary Figs. 2(R), 2(S), 2(T)). Tricolporate pollen grain of voucher BURD12006 with circular amb has the 37.41 μm of colpi with 3.99 μm of depth. Endoaperturates are relatively small (18.32 $μm^2$) and the annulus is also low in height (Fig. 2(O)). Apocolpium index is also relatively high. Thickness of sexine is greater than that of nexine. The surface of the pollen grains is psilate but the presence of gemmae like structure (Fig. 2(P)) is specific to the species.

The variability of morphological features is low. The L/B ratio and thickness of nexine vary considerably in the pollen grains collected from another sample, voucher BURD12050 (Supplementary Figs. 1(R), 1(S), 1(T)).

3.5 Description of Dendrogram

Qualitative characters of each type of pollen grains are represented in Supplementary Tab. 1. The dendrogram (Fig. 3) obtained from the cluster analysis applying the data matrix (Supplementary Tab. 3) showed two distinct clusters, where cluster 1 contains relatively higher similarity among DE-1, DE-3 and DE-2, and TH-1 and TH-2 showed a relative difference. In cluster 2 AS-1, IN-1 and IN-2 showed higher similarity but AS-2 is separated. Three separate ternary plots were prepared including DE-1, DE-2, and DE-3 in the first, AS-1, IN-1 and IN-2 in the second and TH-1, TH-2 and AS-2 in the third ternary plot.

3.6 Selection of Characters Using Ternary Plots

Triangular or ternary diagram obtained from proportion percentage of each character for three of these four species of *Saraca* shows that 100% of each character is represented by the apex of the angles. Lateral sides of the triangle indicate the scale of species-specific character in percentage. From the ternary plot in the Supplementary Fig. 2(A), it is obtained that the height of annulus character (Tab. 2 character no. 14, Yellow color in the ternary plot) value in the ternary diagram is highest (68%) in case of *S. indica* representing its unique pollen characters. Similarly, the area of apocolpium space (Tab. 2 character no. 17, dark green color in the ternary plot) is the unique pollen character for *S. declinata* representing nearly 66% of correlation. In case of *S.thaipingensis* exine ornamentation, containing psilate surface with gemmae like structure and few perforations (Tab. 2. character no. 20, red coloring in the ternary plot) is unique and the value is maximum, representing 100%. Supplementary Fig. 2(B) represents the combination of *S. indica*, *S. declinata*, and *S. asoca* and only the characters represented by the character no. 17 (Tab. 2); i.e., area of apocolpium space is unique for *S. declinata*. Supplementary Fig. 2(C) represents the combination of *S. indica*, *S. thaipingensis* and *S. asoca* and the character of the surface of pollen, i.e. presence of exine protuberance represented by the character no. 20 (Tab. 2), is unique for *S. thaipingensis* and characters like the area of the pore (Tab. 2 character no. 12) and height of annulus (Tab. 2 character no. 14) are equally important for the identification of *S. indica* and *S. asoca*. Ternary plot with the combination of characters of *S. declinata*, *S. thaipingensis* and *S. asoca* (Supplementary Fig. 2(D)) represent the presence of protuberance on the surface of pollen is important for *S. thaipingensis*, large area of apocolpium space (Tab. 2 character no. 17) is important identifying character of pollen of *S. declinata* and the greater height of annulus (Tab. 2 character no. 14) is definitive for pollen of *S. asoca*. From this analysis, it is represented that the selection of definite key character for the identification of pollen of *S. declinata* and *S. thaipingensis* from the set of quantitive characters is possible but that is difficult to separate *S. indica* and *S. asoca*.

Ternary plots like supplementary Figs. 3(A) to 3(D) are the combination of pollen characters of three specimens from each cluster of the dendrogram (Fig. 3). Supplementary Fig. 3(B) showed that pollen of *S. indica* (voucher BURD12010) has exine protuberance (Tab. 2 character no. 20) unlike any other representative of *S. indica* and perimeter of the pollen is much higher than *S. asoca* (voucher BURD12008). Supplementary Fig. 3, C showed that *S. declinata* (voucher BURD12012) has the pollen with the protuberance in its surface but its abundance is rare. Supplementary Fig. 3(D) showed that both *S. thaipingensis* (voucher BURD12005 and voucher BURD12050) has protuberance on the surface of their pollen but the abundance is more in the pollen grains of voucher BURD12050. Presence of protuberance showed inter-specific variations. From supplementary Figs. 3(A) to 3(D) it is observed that the presence of exine protuberance (Tab. 2 character no. 20), the thickness of sexine (Tab. 2 character no. 21) and nexine (Tab. 2 character no. 23) are with the higher level of plasticity. Principal differences among these four species are given in the Tab. 2.

Table 3: Principal differences of pollen morphology of four species of *Saraca*

Ch. No.	Characters	*S. asoca*	*S. declinata*	*S. indica*	*S. thaipingensis*
1	Polarity	Isopolar	both isopolar and heteropolar	Isopolar	Isopolar
2	Endoaperture	Medium	Small	large	Small
3	Annulus Height	Large	Small	large	Small
4	Presence of gemmae	Absent	Absent	absent	Present
5	Surface ornamentation	Reticulate	macro-rugulate	microrugulate	Nudate

4 Discussion

The shape of pollen, apertural types and exine ornamentation were found to be more significant pollen characters in *Onobrychis* of Fabaceae [22]. Size of the pollen plays a greater role in the sub-generic grouping of Polygonaceae [23]. The present investigation revealed that the shape of the pollen grains of the studied species of *Saraca* varies from prolate to prolate-spheroidal. The prolate-spheroidal shape of the pollen grain is found in *S. indica,* but in *S. declinata* pollen grains are sub-prolate and in *S. thaipingensis* the shape varies from prolate to prolate spheroidal. It was reported that P/E values and morphological features in polar view are relatively stable in each group in polygonate [24]. Pollen grains of different samples collected from different localities of *Saraca* also showed relative stability of P/E ratio but morphological features obtained from polar view varies considerably. According to Matamoro-Vidal et al. [25], exine thickness is an important character for the taxon delimination. Exine thickness is most variable character shown in the analysis in both intra- and inter-specific taxa of *Saraca*.

Pollen grains are usually categorized largely on the basis of their shape, size, aperture types, symmetry, polarity and exine sculpturing [14,26]. The type of aperture varies from tricolporate to syntricolporate and the size varies from medium (25-50 μm) to large (above 50 μm). Present study reflects that the variation in pollen size and the thickness of exine are less effective for the specification of Indian *Saraca*. Pollen grains were categorized into four groups based on the surface ornamentation as i) reticulate ii) macro-rugulate iii) micro-rugulate and iv) psilate. Present study also revealed that some of the pollen characters like annulus height (comparatively higher), narrowest apocolpium space, macro-rugulate surface ornamentation, etc. are unique in *S. indica*; annulus is relatively lower in height, higher apocolpium space and micro-rugulate surface ornamentation are in *S. declinata;* and annulus lowest in height, narrow apocolpium space, psilate ornamentation and gemmae like protuberance and few perforations are present in *S. thaipingensis*. The ternary plot (Supplementary Fig. 2) revealed that character of the annulus in *S. indica*, apocolpium space in *S. declinata* and surface ornamentation in *S. thaipingensis* and *S. asoca* are potent marker characters turned to be useful traits for species identification.

Palynological characters provide very important information for the phylogenetic reconstructions [12]. Moreover, from a phylogenetic as well as evolutionary point of views, the polarity, symmetry, aperture types and exine sculpturing are the most important pollen characters [13]. It was concluded that the latitudinal gradient including moisture and temperature is important in the evolution of size and shape of pollens due to harmomegathy [27]. The diversity of shape and size of pollen of *Saraca* may also carry such influence. Harmomegathic stress produced due to loss of water caused the decrease in size and change of shape of pollen to avoid dehydration [28]. Pollen of *S. asoca* has relatively greater size as the abundance of this species can be observed near the forests having higher rainfall. The shape of the pollen often causes reproductive barriers among related sub-species [29]. The difference in the size of pollen of *S. indica* and *S. asoca* is not very much pronounced but the reproductive barrier may be caused due to the difference of exine sculpturing. Pollen grains with greater apocolpium space is an advanced feature [30-32]. Pollen grains of *S. thaipingensis* with higher apocolpium space is advanced though that of *S. declinata* is higher because *S. declinata* produce heteropolar pollen grains. Ornamentation pattern of pollen surface is important in the study of plant evolution and systematic taxonomy [33]. According to the concept of evolution of pollen grains stated by Yang et al. [34], it can be found that the surface morphology of pollen of *S. asoca* having the reticulate type of exine is primitive among all. Both coarse rugulate (psilate) and rugulate-perforate exine sculpture were evolved independently from (micro) reticulate sculpturing of exine [35]. Walker [13] Concluded that evolution of pollen depends upon the gradual reduction of exine sculpture. From above concept, it can be concluded that the macro-rugulate surface of pollen of *S. indica* evolved from reticulate (Fig. 2(D)) type of *S. asoca* and micro-rugulate (Fig. 2(H)) type of surface of pollen of *S. declinata* is derived from macro-rugulate type (Fig. 2(L)). Most advanced type of pollen surface is psilate (Fig. 2(P)) with perforated tectum and gemmae like structure which is found in the pollen of *S. thaipingensis* which is derived from the micro-rugulate type of surface. The proposed evolutionary trends of pollen of four species of *Saraca* are given in Fig. 4.

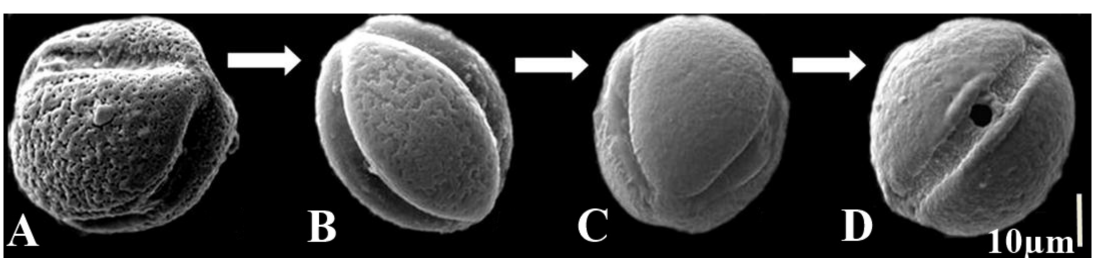

Figure 4: Sequence of Scanning Electron Microscopic images showing evolutionary lineage of pollen grains of *Saraca* based on their surface ornamentation. A: Pollen of *S. asoca* (AS-1). B: Pollen of *S. indica* (IN-1). C: Pollen of *S. declinata* (DE-1). D: Pollen of *S. thaipingenssis* (TH-1)

Palynology *vs*. Systematics of *S. indica* and *S. asoca*

In the present palynological investigation, pollen size, shape, aperture of both the species (i.e., *S. asoca* and *S. indica*) are of the same type as described for two other species of *Saraca* [10]. Though, two distinctly different types of exine ornamentation were observed; *S. asoca* showed perforate to reticulate surface (Fig. 2(D)) and *S. indica* showed micro rugulate surface ornamentation (Fig. 2(L)). Therefore, the palynological study confirms the existence of two different sets of taxa comparable to two different species as treated by Zuijderhoudt [36] and Ding et al. [3].

Variation of quantitative data of pollen grains makes difficulty for morphology-based taxonomic identification of different species of *Saraca*. Samples collected from different geographical locations for the same species showed a considerable degree of character variation which leads to the conclusion that most of the considered characters of *Saraca* are plastic in nature. Though, the presence of protuberance in all the studied individuals is a strong identifying character for the species *S. thaipingensis*. Plasticity of shape, size and surface ornamentation may be regulated by harmomegathic stress produced due to changing humidity. Pollen of *S. asoca* is primitive due to the presence of reticulate exine ornamentation and from which pollens of *S. indica* evolved which has a rugulate type of ornamentation. Gemmae like exine ornamentation present in the pollen of *S. tahipingensis* is the most advanced type. Exine ornamentation of *S. indica*, i.e., macro-rugulate, differs from *S. asoca* which has the reticulate type of ornamentation indicates the existence of two different taxa. It can also be concluded that the qualitative characters of pollen (Tab. 3) are more applicable than quantitative characters (Tab. 2) to distinguish different species of *Saraca*, taxonomically.

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References

1. The Legume Phylogeny Working Group (2017). A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. *Taxon, 66(1),* 44-77.
2. Tucker, S. C. (2000). Floral development and Homeosis in *Saraca* (Leguminosae: Caesalpinioideae: Detarieae). *International Journal of Plant Sciences, 161,* 537-549.
3. Ding, H., Larsen, K., Larsen, S. S. (1996). Cesalpiniaceae. In: Kalkman, C. et al. (eds.), Flora Malesiana, vol. 12, pp. 660-673. Rijksherbarium/Hortus Botanicus, Leiden, Netherlands.
4. Preeti, B., Bharti, A., Sharma, A. N., Singh, V. (2012). A review on *Saraca indica* plant. *International Research Journal of Pharmacy, 3,* 80-84.
5. Pradhan, P., Joseph, L., Gupta, V., Chulet, R., Arya, H. et al. (2009). *Saraca asoca*(Ashoka): a review. *Journal of Chemical and Pharmaceutical Research, 1,* 62-71.
6. Mishra, S. B., Vijayakumar, M. (2015). Floral morphology and HPTLC fingerprint profile of *Saraca asoca* (Roxb.) De Wilde (Caesalpiniaceae): a consecrated tree of India. *International Letter Natural Sciences, 43,* 54-62.
7. CAMP Workshops on Medicinal Plants, India, 1998. *Saraca asoca*. The IUCN Red List of Threatened Species 1998: e.T34623A9879360.
8. Australasian Pollen and Spore Atlas (2007). http://apsa.anu.edu.au/family/FABACEAE/genus/Saraca.

9. Halbritter, H., Weis B. (2017). *Saraca declinata*. *PalDat* (a palynological database) https://www.paldat.org/pub/*Saracadeclinata*/302810.
10. Sil, S., Mallick, T., De, K. K., Pramanik, A., Ghosh, A. (2018). Comparative morphological study of three species of *Saraca* L. (Fabaceae) by the statistical approach to find out the logic of potent morphological markers. *Beni-Suef University Journal of Basic and Applied Science, 7,* 612-619.
11. Aytug, B. (1959). Polinolojinin tavsif ve sınıflandırmaya hizmeti. *İstanbul Üniversitesi Orman Fakültesi Dergisi,* 118-125.
12. Ozler, H., Pehlivan, S., Celep, F., Dogan, M., Kahraman, A. et al. (2013). Pollen morphology of *Hymenosphace* and *Aethiopis* sections of the genus *Salvia* (Lamiaceae) in Turkey. *Turkish Journal of Botany, 37,* 1070-1084.
13. Walker, J. W. (1976). Comparative pollen morphology and phylogeny of the ranalean complex. In: Beck, C.B. (ed.), *Origin and early evolution of Angiosperms*, pp. 241-299. Columbia University Press, New York.
14. Zhao, Z., Skvarla, J. J., Jansen, R. K., Devore, M. L. (2000). Phylogenetic implications of pollen morphology and ultrastructure in the Barnadesioideae (Asteraceae). *Lundellia, 3,* 26-40.
15. Doyle, J. A., Hotton, C. (1991). Diversification of early angiosperm pollen in a cladistic context. In: Blackmore, S., S. H., Barnes (eds.), *Pollen and spores, pattern of diversification*, vol. 44, pp. 169-195. The Systeatics Association, Clarendon Press, Oxford.
16. Ackermann, M., Weigend, M. (2006). Nectar, floral morphology and pollination syndrome in Loasaceae subfam. Loasoideae (Cornales). *Annals of Botany, 98,* 503-514.
17. Thiers, B. (2016). Index Herbarium: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/.
18. Erdtman, G. (1952). *Pollen morphology and plant taxonomy. Angiosperms. an introduction to palynology I.* Almqvist & Wiksell, Stockholm, Sweden.
19. Erdtman, G. (1960). The acetolysis method, a revised description. *Svensk Botanisk Tidskrift, 54,* 561-564.
20. Punt, W., Hoen, P. P., Blackmore, S., Nilson, S., Thomas, A. L. (2007). Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology, 143,* 1-81.
21. Hesse, M., Halbritter, H., Weber, M., Buchner, R., Frosch-Radivo, A. et al. (2009). *Pollen terminology: an illustrated handbook.* Springer, Wein, NewYork.
22. Amirabadizadeh, H., Jafari, A., Mahmoodzadeh, A. (2015). Comparative morphology, anatomy and palynological studies of perennial species of *Onobrychis* (Fabaceae) in northeast Iran. *Nordic Journal of Botany, 33,* 159-169.
23. Ayodele, A. E. (2005). The morphology and taxonomic significance of pollen in the west African Polygonaceae. *Thaiszia Journal Botany, 15,* 143-153.
24. Wang, L., Gu, L., Zhao, C., Liu, J. (2018). Pollen morphology of Polygonatae and its systematic significance. *Palynology, 42,* 255-272.
25. Matamoro-Vidal, A., Raquin, C., Brisset, F., Colas, H., Izac, B. et al. (2016). Links between morphology and the function of the pollen wall: an experimental approach. *Botanical Journal of Linnean Society, 180,* 478-490.
26. Luz, C. F., Maki, E. S., Horak-Terra, I., Vidal-Torrado, P., Mendonca-Filho, C. V. (2013). Pollen grain morphology of Fabaceae in the special protection area (SPA) Pau-de-Fruta, Diamentina, Minas Gerais, Brazil. *Annals Academia Brasileira Ciencias, 85,* 1329-1344.
27. Kriebel, R., Khabbazian, M., Sytsma, K. J. (2017). A continuous morphological approach to study the evolution of pollen in a phylogenetic context: an example with the order Myrtales. *PloS One*. https://dx.doi.org/10.1371/journal.pone.0187228.
28. Blackmore, S., Barnes, S. H. (1984). Harmomegathic mechanisms in pollen grains. In: Blackmore, S. & Ferguson, I. K. (eds.), *Pollen and spores: form and function*, pp. 137-149. Academic Press, London, UK.
29. Cruzan, M.B. (1990). Variation in pollen size, fertilization ability, and post-fertilization siring ability in *Erythronium grandiflorum*. *Evolution, 44,* 843-856.
30. Donoghue, M. J. (1985). Pollen diversity and exine evolution in *Viburnum* and the Caprifoliaceae *sensu lato*. *Journal of Arnold Arboretum, 66,* 421-469.

31. Perveen, A., Qaiser, M. (2001). Pollen Flora of Pakistan-XXVI. Balsaminaceae. *Turkish Journal of Botany, 25,* 35-38.

32. Ressayre, A., Godelle, B., Raquin, C., Gouyon, P. H. (2002). Aperture pattern ontogeny in angiosperms. *Journal of Experimental Zoology, 294,* 122-135

33. Zhang, W. X., Zhao, M. M., Fan, J. J., Zhou, T., Chen, Y. X. et al. (2017). Study on relationship between pollen exine ornamentation pattern and germplasm evolution in crabapple. *Scientific Reports, 7,* 1-11.

34. Yang, M., Zhang, D., Zheng, J., Liu, J. (2001). Pollen morphology and its systematic and ecological significance in *Rheum* (Polygonaceae) from China. *Nordic Journal of Botany, 21,* 411-418.

35. Sarwar, A. K. M. G., Hoshino, Y., Araki, H. (2015). Pollen morphology and its taxonomy significance in the genus *Bomarea*Mirb. (Alstroemeriaceae)-I. Subgenera *Baccata*, *Spharine* and *Wichuraea*. *Acta Botanica Brasilica, 29,* 425-432.

36. Zuijderhoudt, G. F. P. (1967). A revision of the genus *Saraca* L. (Legum.-Caes.). *Blumea, 15,* 413-425.

Appendix A: Supplementary Tables 1-10

Supplementary Table 1: Qualitative characters of individuals of *Saraca* under study

Ch. No.	Name of the species	Shape of the pollen	Aperture type	Surface ornamentation
1	***S. asoca*** **(AS-1)**	Prolate-spheroidal	Syn- tricolporate	Reticulate pattern present; Exine protuberance absent
2	***S. asoca*** **(AS-2)**	Prolate-spheroidal	Para, syn- tricolporate	Rugulate with fine lirae; Exine protuberance absent
3	***S. declinata*** **(DE-1)**	Sub-prolate	Para, syn- tricolporate	Micro-rugulate
4	***S. declinata*** **(DE-2)**	Sub-prolate	Syn- tricolporate	Micro-rugulate to Vermiculate
5	***S. declinata*** **(DE-3)**	Prolate-spheroidal.	Para, syn- tricolporate	Micro-rugulate to Vermiculate
6	***Saraca indica*** **(IN-1)**	Prolate	Syn- tricolporate	Macro-rugulate with large lirae
7	***S. indica*** **(IN-2)**	Prolate	Syn- tricolporate	Rugulate with large lirae
8	***S. thiapingensis*** **(TH-1)**	Prolate-spheroidal	Para, syn- tricolporate	Psilate with verrucate sculpturing
9	***S. thaipingensis*** **(TH2)**	Prolate-spheroidal	Para, syn- tricolporate	Psilate with verrucate sculpturing

Supplementary Table 2: Characters and character states of pollen considered for cluster analysis and coding of character states

Ch. No.	Character of pollen grains	0	1	2	3
1	PA (μm)	Below 20	20 t0 40	40 to 60	Above 60
2	ED (μm)	Below 20	20 to 40	40 to 60	Above 60
3	P/E Ratio	Below 0.6	0.6 to 1.2	1.2 to 1.8	Above 1.8
4	Length of colpus(μm)	Below 20	20 to 40	40 to 60	Above 60
5	Breadth of colpus (μm)	Below 4	4 to 8	8 to 12	Above 12
6	Depth of colpus (μm)	Below 3	3 to 6	6 to 9	Above 9
7	L/B ratio of colpus	Below 6	6 to 8.5	8.5 to 11	Above 11
8	Area of colpus (μm^2)	Below 100	100 to 150	150 to 200	Above 200
9	Perimeter of colpus (μm)	Below 40	40 to 60	60 to 80	Above 80
10	CL/PA ratio	Below 30	30 to 60	60 to 90	Above 90
11	Pore size (μm)	Below 2	2 to 4	4 to 6	Above 6
12	Pore area (μm^2)	Below 21	21 to 42	42 to 63	Above 63
13	Perimeter of pore (μm)	14 to 17	17 to 20	20 to 23	Above 23
14	Height of annulus (μm)	Below 0.4	0.4 to 0.8	0.8 to 1.2	Above 1.2
15	Amb value	400 to 800	800 to 1200	1200 to 1500	Above 1500
16	Apocolpium index	Below 8	8 to 16	16 to 24	Above 24
17	Area of apocolpium space (μm^2)	Below 20	20 to 40	40 to 60	Above 60
18	Angle of apocolpium space	Below 50	50 to 100	100 to 150	Above 150
19	Perimeter of apocolpium (μm)	Below 8	8 to 16	16 to 24	Above 24
20	No of gemmae like structure (per μm^2)	0	1	2	3
21	Sexine thickness (μm)	Below 0.6	0.6 to 1.2	1.2 to 1.8	Above 1.8
22	Nexine thickness (μm)	Below 0.4	0.4 to 0.8	0.8 to 1.2	Above 1.2
23	Perimeter of the pollen (μm)	Below 60	60 to 120	120 to 180	Above 120

*PA = polar axis, [ǂ]ED = equatorial diameter, [§]L/B ratio = ratio between length and breadth of pollen, [†]R = range, [ʃ]CL/PA = Ratio between length of the colpus and polar axis and A = average

Supplementary Table 3: Character state and data matrix

Ch. No.	Characters	AS-1	AS-2	DE-1	DE-2	DE-3	IN-1	IN-2	TH-1	TH-2
1	[*]PA (µm)	2	2	2	3	2	2	2	3	3
2	[ǂ]ED (µm)	1	2	1	2	2	1	2	2	2
3	P/E Ratio	2	1	2	2	1	2	1	2	1
4	Length of colpus(µm)	1	2	1	2	2	2	2	2	2
5	Breadth of colpus (µm)	2	1	1	2	1	1	2	2	1
6	Depth of colpus (µm)	1	1	0	0	1	1	1	1	1
7	[§]L/B ratio of colpus	0	2	1	0	3	1	1	1	2
8	Area of colpus(µm^{2})	3	3	3	2	3	1	1	1	3
9	Perimeter of colpus	3	2	3	3	3	2	2	2	3
10	[ʃ]CL/PA ratio	2	3	2	3	3	2	2	3	2
11	Pore size (µm)	2	2	1	2	2	1	1	1	2
12	Pore area (µm^{2})	0	0	1	1	1	2	2	2	0
13	Perimeter pore(µm)	0	0	1	1	1	3	3	3	0
14	Height of annulus(µm)	0	0	1	1	1	3	3	3	0
15	Amb value	1	1	2	2	3	2	1	2	1
16	Apocolpium index	2	2	3	3	3	1	1	1	2
17	Area of apocolpium space (µm^{2})	1	1	3	3	3	0	0	0	1
18	Angle of apocolpium space	1	1	1	1	1	1	1	1	1
19	Perimeter of apocolpium(µm)	2	2	3	3	3	2	2	2	2
20	No of gemmae like structure (per µm^{2})	0	0	0	0	1	0	3	1	3
21	Sexine thickness(µm)	2	3	1	1	1	1	2	2	2
22	Nexine thickness(µm)	0	3	1	1	1	1	2	2	2
23	Perimeter of the pollen(µm)	1	2	2	2	1	2	2	2	2

[*]PA = polar axis, [ǂ]ED = equatorial diameter, [§]L/B ratio = ratio between length and breadth of pollen, [†]R = range, [ʃ]CL/PA = Ratio between length of the colpus and polar axis and A = average

Supplementary Table 4: Mean proportion percent of each of the character compared among *S. declinata* (DE-1), *S. indica* (IN-1) and *S. thaipingensis* (TH-1)

Ch. No.	*S. declinata* (DE-1)	*S. indica* (IN-1)	*S. thaipingensis* (TH-1)
1	34.71	33.09	32.19
2	34.69	29.97	35.34
3	33.24	36.51	30.25
4	32.12	36.44	31.44
5	26.29	33.81	39.9
6	26.69	35.12	38.18
7	38.52	36.65	24.83
8	38.69	22.02	39.29
9	35.83	29.55	34.61
10	30.81	36.67	32.52
11	29.8	31.53	38.67
12	25.07	56.49	18.43
13	29.85	45.38	24.77
14	23.59	66.55	9.86

15	38.43	33.78	27.79
16	44.42	21.16	34.42
17	65.41	12.39	22.2
18	33.31	33.31	33.38
19	49.59	20.75	29.66
20	0	0	100
21	30.85	22.69	46.45
22	42.86	36.81	20.33
23	35.8	33.69	30.51

Supplementary Table 5: Mean proportion percent of each of the character compared among *S. asoca* (AS-1), *S. declinata* (DE-1) and *S. indica* (IN-1)

Ch. No.	*S. asoca* (AS-1)	*S. declinata* (DE-1)	*S. indica* (IN-1)
1	42.02	29.68	28.29
2	41.75	31.25	27
3	31.92	32.45	35.64
4	39.2	28.48	32.32
5	35.22	28.34	36.45
6	43.13	24.56	32.31
7	36.19	32.69	31.11
8	28.6	45.5	25.89
9	29.28	38.75	31.96
10	36.42	29.03	34.55
11	32.64	32.73	34.63
12	40.09	18.42	41.49
13	39.24	24.11	36.65
14	40.05	15.69	44.26
15	31.52	36.44	32.03
16	23.65	51.71	24.64
17	12.69	73.4	13.9
18	33.32	33.34	33.34
19	28.22	50.61	21.17
20	0	0	0
21	49.49	29.09	21.4
22	32.87	36.11	31.02
23	32.08	34.99	32.93

Supplementary Table 6: Mean proportion percent of each of the character compared among *S. asoca* (AS-1), *S. indica* (IN-1) and *S. thaipingensis* (TH-1)

Ch. No.	*S. asoca* (AS-1)	*S. indica* (IN-1)	*S. thaipingensis* (TH-1)
1	42.94	28.92	28.14
2	41.5	26.85	31.65
3	32.88	36.71	30.41
4	39.44	32.51	28.05
5	30.71	31.78	37.51
6	39.01	29.22	31.77
7	40.96	35.2	23.84

8	28.4	25.71	45.89
9	29.67	32.39	37.94
10	35.85	34	30.15
11	29.75	31.55	38.7
12	42.14	43.63	14.23
13	40.92	38.22	20.86
14	44.07	48.71	7.22
15	35.06	35.62	29.32
16	26.77	27.88	45.35
17	24.64	26.99	48.37
18	33.3	33.32	33.383
19	35.42	26.58	37.99
20	0	0	100
21	43.15	18.66	38.19
22	40.57	38.25	21.14
23	33.84	34.72	31.44

Supplementary Table 7: Mean proportion percent of each of the character compared among *S. asoca* (AS-1), *S. declinata* (DE-1), and *S. thaipingensis* (TH-1)

Ch. No.	*S. asoca* (AS-1)	*S. declinata* (DE-1)	*S. thaipingensis* (TH-1)
1	42.35	29.91	27.74
2	39.82	29.81	30.37
3	33.99	34.56	31.45
4	41.02	29.8	29.17
5	33.05	26.59	40.36
6	41.95	23.89	34.16
7	40.23	36.35	23.42
8	23.77	37.82	38.41
9	27.76	36.74	35.49
10	37.9	30.22	31.88
11	30.27	30.35	39.38
12	55.65	25.57	18.79
13	47.08	28.92	23.99
14	64.29	25.19	10.53
15	33.42	38.64	27.95
16	20.49	44.79	34.71
17	11.44	66.12	22.44
18	33.3	33.31	33.39
19	25.86	46.39	27.75
20	0	0	100
21	40.44	23.77	35.79
22	38.17	41.94	19.89
23	33.12	36.11	30.77

Supplementary Table 8: Mean proportion percent of each of the character compared among *S. asoca* (AS-2), *S. indica* (IN-1), and *S. indica* (IN-2)

Ch. No.	*S. indica* (IN-1)	*S. indica* (IN-2)	*S. asoca* (AS-1)
1	26.56	34.01	39.44
2	24.13	38.56	37.31
3	37.02	29.83	33.15
4	31.32	30.69	37.99
5	33.32	34.49	32.19
6	31.48	26.5	42.02
7	32.89	28.85	38.26
8	30.92	34.93	34.15
9	33.76	35.32	30.92
10	34.75	28.62	36.63
11	33.63	34.68	31.69
12	33.41	34.31	32.28
13	32.09	33.54	34.37
14	37.57	28.43	33.99
15	34.24	32.07	33.69
16	33.15	35.03	31.82
17	34.55	33.92	31.54
18	33.32	33.38	33.3
19	26.98	37.068	35.95
20	0	100	0
21	16.41	45.64	37.95
22	26.07	46.3	27.63
23	33.08	34.68	32.24

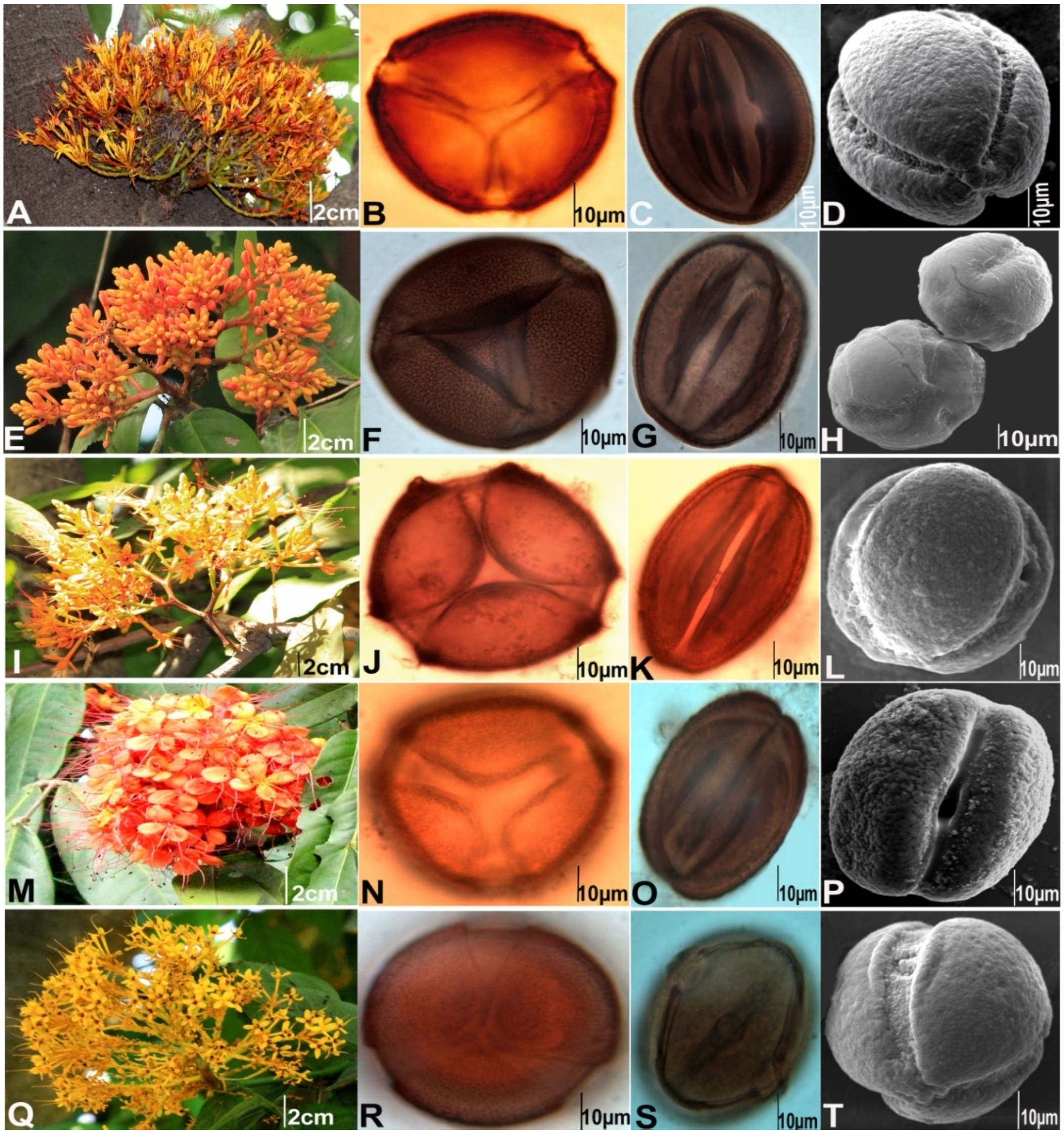
Supplementary Table 9: Mean proportion percent of each of the character compared among *S. declinata* (DE-1) and *S. declinata* (DE-2) and *S. declinata* (DE-3)

Ch. No.	*S. declinata* (DE-1)	*S. declinata* (DE-2)	*S. declinata* (DE-3)
1	28.93	36.63	34.44
2	27.1	34.53	38.37
3	36.1	34.67	29.23
4	27.39	33.44	39.17
5	30.93	45.43	23.65
6	27.43	20.06	52.51
7	26.72	22.54	50.74
8	33.82	32.57	33.61
9	34.28	32.49	33.23
10	29.35	34.39	36.26
11	27.01	36.9	36.09
12	33.1	34.75	32.15
13	33.52	34.43	32.05
14	34.89	32.81	32.29
15	32.31	33.02	34.67
16	34.55	33.41	32.04
17	33.67	33.85	32.47
18	33.34	33.34	33.32
19	33.58	34.54	31.88
20	0	0	100
21	33.46	31.54	35
22	34.98	30.05	34.98
23	37.97	30.09	31.94

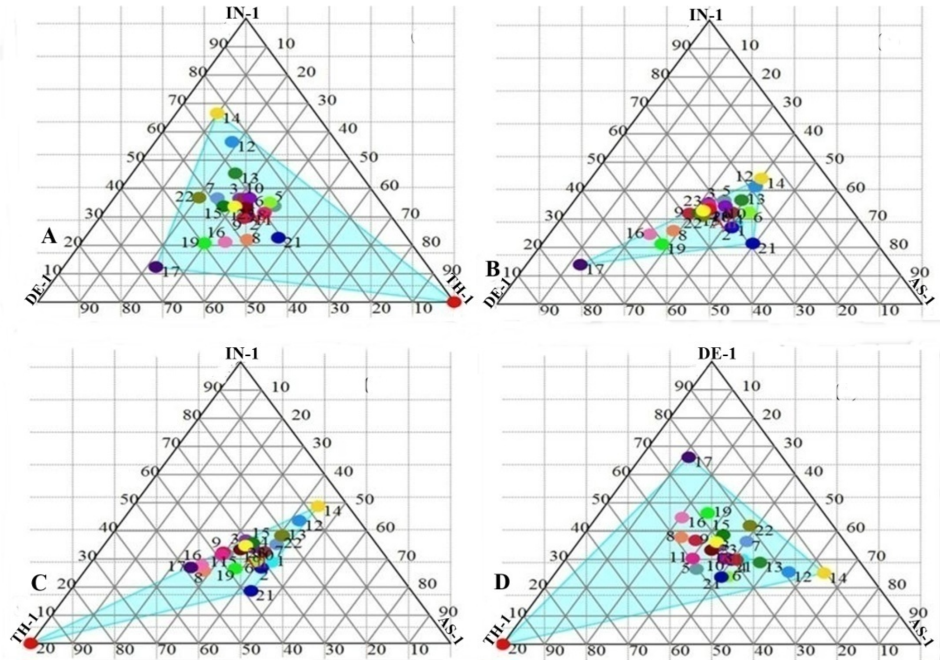
Supplementary Table 10: Mean proportion percent of each of the character compared among *S. asoca* (AS-2), *S. thaipingensis* (TH-1) and *S. thaipingensis* (TH-2)

Ch. No.	*S. asoca* (AS-2)	*S. thaipingensis* (TH-1)	*S. thaipingensis* (TH-2)
1	35.84	26.94	37.22
2	35.97	26.94	37.09
3	33.13	33.43	33.43
4	38.38	27.43	34.19
5	26.69	43.64	29.67
6	25.81	30.65	43.55
7	44.68	19.52	35.8
8	33.59	33.83	32.58
9	31.49	34.16	34.35
10	36.7	31.59	31.7
11	38.07	30.13	31.79
12	33.9	31.5	58.16
13	30.97	32.59	36.44
14	36.05	32.56	31.39
15	32.26	32.85	34.88
16	34.66	32.38	32.97
17	33.82	33.12	33.06
18	33.29	33.38	33.33
19	29.62	35.38	35.04
20	0	25	75
21	38.59	27.93	33.48
22	49.24	14.12	36.64
23	32.49	31.78	35.73

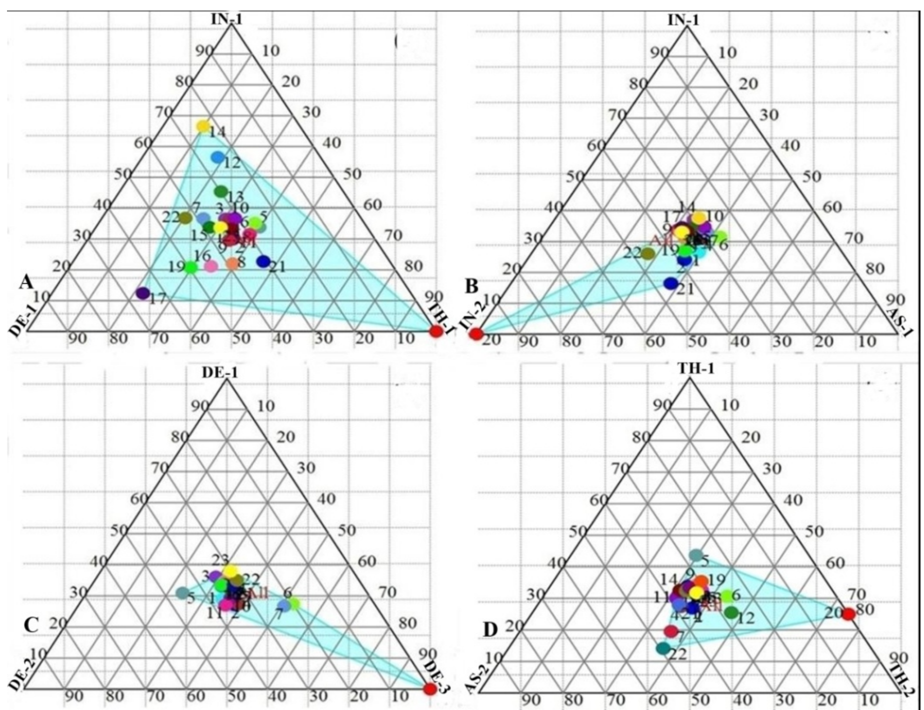
Appendix B: Supplementary Figures 1-3



Supplementary Figure 1: Photographs and micrographs of different specimen of *Saraca* spp. A: Flowering twig of AS-2. B: Pollen of AS-2 at polar view. C: Pollen of AS-2 at equatorial view. D: SEM image of pollen of AS-2. E: Flowering twig of DE-2. F: Pollen of DE-2 at polar view. G: Pollen of DE-2 at equatorial view. H: SEM image of pollen of DE-2. I: Flowering twig of DE-3. J: Pollen of DE-3 at polar view. K: pollen of DE-3 at equatorial view. L: SEM image of pollen of DE-3. M: Flowering twig of IN-2. N: Pollen of IN-2 at polar view. O: Pollen of IN-2 at equatorial view. P: SEM images of pollen of IN-2. Q: Flowering twig of TH-2. R: Pollen of TH-2 at polar view. S: Pollen of TH-2 at equatorial view. T: SEM image of pollen of TH-2



Supplementary Figure 2: Ternary plots of permutation and combination of data from pollen grains of four species of *Saraca*. A: comparative ternary plot including IN-1, DE-1 and TH-1. B: Comparative ternary plot including IN-1, DE-1 and AS-1. C: Comparative ternary plot including IN-1, TH-1 and AS-1. D: Comparative ternary plot including DE-1, TH-1 and AS-1



Supplementary Figure 3: Ternary plots of combination of four species of *Saraca* with their different samples. A: Comparative ternary plot including IN-1, DE-1 and TH-1. B: Comparative ternary plot including IN-1, IN-2 and AS-1. C: Comparative ternary plot including DE-1, DE-2 and DE-3. D: Comparative ternary plot including TH-1, AS-2 and TH-2