Pollen morphology of some species of *Buddleja* L. (Buddlejaceae) from North-east India and its taxonomic significance

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Abstract

Pollen grains of five species of *Buddleja* L. (Buddlejaceae), collected from North-east India have been examined thoroughly both by Light Microscopy (LM) and Scanning Electron Microscopy (SEM). On the basis of number of pollen apertures, the species are divided into two basic types, viz., Type I: 4-5-colporate grains and Type II: 3-colporate grains. Differentiation of the individual species has been done on the basis of critical differences of micromorphological characters of pollen aperture and exine stratification/ornamentation. The interspecies heteromorphism of pollen features are used to recognize the studied species. The taxonomic significance of these characters is discussed. Finally, an artificial key has been constructed for easy and correct identification of the taxa studied.

Key words: *Buddleja* L., North-east India, Pollen characters, Taxonomic significance

INTRODUCTION

Buddleja L. (Buddlejaceae), commonly known as the butterfly bush, is a genus comprising of about 90 species in the world, endemic to tropics and subtropics of America, Africa, and Asia, especially in South-east Asia (Mabberley 2017). In India, the genus is represented by 11 species (Santapau & Henry 1973). Rich concentration of the wild inhabitants (ca. 8 species) is found in the North-eastern states of the country, viz, Arunachal Pradesh, Meghalaya, Mizoram, Nagaland, Sikkim and Darjeeling Hills of West Bengal (C. B. Clarke 1883; Das &

Table 1. Distribution of *Buddleja* species in NE India

Name of Species	Distribution in India	Places of collection	Collection number
<i>Buddleja asiatica</i> Lour.	Almost throughout India	West Bengal (Darjeeling), Sikkim	<i>Siddhanta & Maity</i> 303, 305, <i>Siddhanta</i> 338
<i>Buddleja colvilei</i> Hook.f. & Thomson	NE India (including Darjeeling)	Sikkim	<i>Maity</i> 20231
<i>Buddleja crispa</i> Benth.	NE India (cultivated)	Arunachal Pradesh	<i>Siddhanta & Bera</i> 312
<i>Buddleja macrostachya</i> Wall. ex Benth.	NE India (including Darjeeling)	Arunachal Pradesh, Meghalaya, Sikkim	<i>Siddhanta & Bera</i> 315, 316
<i>Buddleja paniculata</i> Wall. ex Roxb.	NE India (including Darjeeling)	Arunachal Pradesh, Manipur	<i>Siddhanta & Bera</i> 349, <i>Bera</i> 126

Chanda 1987; Chowdhery 2008; Murmu 2016; Dash & Singh 2017; Maity *et al.* 2018). Present investigation deals with five species of the genus occurring in North-east India of which only *B. crispa* is a common garden plant and others are wild (Table 1). All these four wild species are also recorded from Darjeeling and Terai regions of West Bengal (Murmu 2016). Das & Chanda (1987) recorded *B. asiatica*, *B. davidii*, *B. paniculata* from the temperate hills of Darjeeling.

The species of *Buddleja* are morphologically well characterized, however, only few fragmentary works on pollen morphology of the genus is available globally (Erdtman 1952; Punt & Leenhouts, 1967; Mosyakin & Tsybalyuk 2013). Long back, Wilhelm (1910) used pollen characters of Buddlejaceae for separating the genus from rest of the members of the family. Notably, in India pollen characterization or a suitable key to the species for classifying the genus on the basis of pollen features is not yet being reported inspite of its abundance in the North eastern states. Furthermore, *B. asiatica* is abundant in Darjeeling hills and Terai-Duars and *B. paniculata* is quite common in Darjeeling hills and is frequent in Duars.

Present study deals with detailed investigation of the palynomorphs of the five species of North-east Indian *Buddleja* out of eight species known to occur in this region (Clarke 1883; Das & Chanda 1987; Li & Leeuwenberg 1996; Chowdhery 2008; Murmu 2016; Dash & Singh 2017; Maity *et al.* 2018). Pollen grains were studied through evaluation of overall shape and size, nature and number of apertures, detail aperture characteristics and exine wall stratification. All these characters are used in detail characterization as well in grouping of the taxa. Furthermore, Pollen load count of individual anther and pollen viability percentage of each species are also provided. An artificial key is prepared for easy and correct identification of the species.

MATERIALS AND METHODS

Mature flower buds and immature flowers of the five studied species of *Buddleja* were collected from different parts of Arunachal Pradesh, Meghalaya, Manipur, West Bengal (Darjeeling) and Sikkim. All the species were properly identified with the help of relevant literature (Clarke 1883; Li & Leeuwenberg 1996; Rae 2001; Chowdhery 2008; Murmu 2016; Dash & Singh 2017). The pollen grains were properly acetolyzed following standard methodology (Erdtman 1960) and prepared for light microscopy (LM) and scanning electron microscopy (SEM). Pollen Load Count (PLC) from the anthers was also recorded using haemocytometer (Kearns & Inouye 1993). Pollen viability percentage (PV) of each species was calculated following Norton (1966).

RESULT

Buddleja asiatica Lour., Fl. Cochinch. 1: 72. 1790. [Figure 1A,B,C]

Pollen grains monads, homomorphic, tricolporate, prolate-spheroidal to sub-prolate, 15.6-18 µm x 12-15 µm in equatorial view, 12-18 µm x 9-15 µm in polar view; colpi 12-15 µm long, homomorphic, membranes psilate, margins rugose; endocolpi psilate; apocolpium present; ora lalongate about 3.2 µm diam; amb angular, goniotreme, about 3 µm diam; exine psilate with microporations; exine thicker than intine, exine and intine 4.4 µm and 0.6 µm thick respectively; tectate, tectum perforated, baculae indistinct.

<i>NPC number</i>	:	345
<i>PLC</i>	:	3,666 (approx.)
<i>PV</i>	:	72.80 %

Figure 1: Details of pollen grains of studied *Buddleja* species: A - C. *B. asiatica*; D & E. *B. colvillei*; F. *B. crispa*.

***Buddleja colvillei* Hook.f., Ill. Himal. Pl. t. 18. 1855. [Figure 1D,E]**

Pollen grains monads, heteromorphic, pentacolporate, prolate-spheroidal, 27.9-30 μ m x 26.7-27 μ m in equatorial view, 27-36 μ m x 24-36 μ m in polar view; colpi heteromorphic, central colpus about 15 μ m long, laterals 18-21 μ m long respectively; colpi membranes psilate with microperforations, margins rugose; endocolpi rugose; apocolpium present; ora lalongate, heteromorphic, ora of all laterals about 5 μ m diam and central colpus about 5.9 μ m diam; amb semiangular to circular, goniotreme to peritreme, about 2-3 μ m diam; exine foveolate;

exine thicker than intine; exine and intine 4.4 μm and 0.6 μm thick respectively; tectate, tectum perforated, baculae indistinct.

<i>NPC number</i>	:	545
<i>PLC</i>	:	4,733 (approx.)
<i>PV</i>	:	93.10%

***Buddleja crispa* Benth., Scroph. Ind. 43. 1835. [Figure 1F, 2A]**

Pollen grains monads, homomorphic, tricolporate, sub-prolate, 15.9-18 μm x 12-15 μm in equatorial view, 12-15 μm x 9-12 μm in polar view; colpi about 13.5 μm long, homomorphic, membranes rugulate, margins rugose; endocolpi psilate; apocolpium present; ora lalongate, about 3.2 μm diam; amb angular, goniotreme, about 3 μm diam; exine rugulate; exine thicker than intine, exine and intine about 4.4 μm and 0.6 μm thick respectively; tectate, tectum perforated, baculae indistinct.

<i>NPC number</i>	:	345
<i>PLC</i>	:	2,830 (approx.)
<i>PV</i>	:	67.41%

***Buddleja macrostachya* Benth., Scroph. Ind. 42. 1835. [Figure 2B,C]**

Pollen grains monads, heteromorphic, tetracolporate, rarely pentacolporate, prolate-spheroidal to sub-prolate, 18-24 μm x 15-21 μm in equatorial view, 15-18.9 μm x 12-16.2 μm in polar view; colpi heteromorphic, lateral colpi 12-13.5 μm long, central colpus 12.7-15 μm long; all colpi membranes rugose with microperforations, margins rugose; endocolpi psilate; apocolpium present; ora lalongate, about 3.2 μm diam; amb angular to rarely circular, goniotreme to rarely peritreme, about 3 μm diam, rarely 2 μm diam; exine psilate with microperforations; exine thicker than intine, exine and intine 4.7 μm and 1.5 μm thick respectively; tectate, tectum perforated, baculae indistinct.

<i>NPC number</i>	:	445, rarely 545
<i>PLC</i>	:	6,100 (approx.)
<i>PV</i>	:	75.98%

***Buddleja paniculata* Wall. in Roxb., Fl. Ind. 1: 412. 1820. [Figure 2D,E,F]**

Pollen grains monads, homomorphic, tricolporate, sub-prolate, 15-18 μm x 12-15.7 μm in equatorial view, 12-15 μm x 9-12 μm in polar view; colpi 9-14.7 μm long, homomorphic, membranes psilate, margins rugose; endocolpi rugose; apocolpium present; ora lalongate, about 2.9 μm diam; amb angular, goniotreme, about 3 μm diam; exine psilate with microperforations; exine thicker than intine, exine and intine about 4.4 μm and 0.6 μm thick respectively; tectate, tectum perforated, baculae indistinct.

<i>NPC number</i>	:	345
<i>PLC</i>	:	4,605 (approx.)
<i>PV</i>	:	86.77%

A comparison of major pollen features of the studied species of *Buddleja* has been represented in Table 2.

Figure 2: Details of pollen grains of studied *Buddleja* species: A. *B. crispa*; B & C. *B. macrostachya*; D – F. *B. paniculata*

DISCUSSION

The studied species of *Buddleja* exhibits a wide range of variation in pollen characters. The smallest pollen grains are recorded in *B. asiatica* (15 – 18 μ m $\times$ 12 – 15 μ m), whereas, the largest one found in *B. colvilei* (27.9 – 30 μ m $\times$ 26.7 – 27 μ m). Among all the studied taxa of the genus, *B. asiatica*, *B. crispa* and *B. paniculata* are homomorphic and *B. colvilei* and *B. macrostachya* are with heteromorphic pollen grains. Moreover, the pollen grains of *B. colvilei* have heteromorphic colpi. Central colpus of pollen in this species

Table 2. Comparison of major pollen features of the studied species of *Buddleja* L.

Name of species	Grains			Colpi		Oras	Pollen Sculptures
	Size (µm)	Shape	NPC No.	Nature	Size (µm)	Nature	Exine ornamentation
	Polar Axis (µm) × Equatorial Diameter(µm) (Equatorial view; Polar view)						
<i>Buddleja colvilei</i>	27.9-30 × 26.7-27; 27-36×24-33	Prolate-Spheroidal	545	Hetero-morphic	Central colpus about 15, laterals 18-21	Lalongate	Foveolate
<i>Buddleja macrostachya</i>	18-24 ×15-21; 15-18.9×12-16.2	Prolate – Spheroidal to Sub-prolate	445, rarely 545	Hetero-morphic	12.7-15 in laterals from in central colpus about 12-13.5	Lalongate	Psilate with microperforations
<i>Buddleja paniculata</i>	15.6-18 × 12-15.7; 12-15×9-12	Sub – Prolate	345	Homo-morphic	12-15	Lalongate	Psilate with microperforations
<i>Buddleja crispa</i>	15.9-18 × 12-15; 12-15×9-12	Sub - Prolate	345	Homo-morphic	13.5	Lalongate	Rugulate
<i>Buddleja asiatica</i>	15-18 × 12-15; 12-18×9-15	Prolate – Spheroidal to Sub-prolate	345	Homo-morphic	9-14.7	Lalongate	Psilate with microperforations

µm

Table 3. Tabular representation for distinguishing the pollen types and subtypes among the species of *Buddleja* L. (Buddlejaceae)

<i>Buddleja</i> species	Pollen type	Pollen subtype
<i>Buddleja colvilei</i>	I	-
<i>Buddleja macrostachya</i>	I	-
<i>Buddleja crispa</i>	II	II.I
<i>Buddleja asiatica</i>	II	II.II
<i>Buddleja paniculata</i>	II	II.II

measures about 15 µm, laterals varies from 18 – 21 µm. *B. macrostachya* exhibits heteromorphism regarding its grain aperture number and size of colpi. It has dimorphic pollen. Both 4-aperturate and 5-aperturate grains are recorded in this species. Moreover, the central colpus, measuring 12-13.5 µm, is somehow smaller and wider than the other three laterals (12.7 – 15 µm).

The number of aperture varies significantly in the studied members. Pollen grains in *B. colvilei* are strictly 5-aperturate. *B. macrostachya* is with dimorphic grains with both 4- and 5-apertures. In contrast, *B. asiatica*, *B. crispa* and *B. paniculata* are having strictly 3-aperturate grains.

The shape of the pollen grains is variable in all five species. Prolate-spheroidal grains are found in *B. colvilei* whereas in *B. crispa* and *B. asiatica* pollen grains are sub-prolate. Significantly, in other two species, viz. *B. macrostachya* and *B. paniculata* prolate-spheroidal to sub-prolate grains are recorded.

The colpi membranes exhibit wide range of variation in these species. It is smooth in *B. asiatica*, smooth with microperforations in *B. colvilei*, rugulate in *B. crispa*, rugose with microperforations in *B. macrostachya* and smooth in *B. paniculata*. Colpi margins are without projections (costae) in all the species. The size of colpi in the species with homomorphic grains varies from 12 – 15 μm . Whereas, the heteromorphic grain bearing species like *B. colvilei*, the second colpus is shortest and widest (15 μm) than the other four (laterals 18 – 21 μm). The other species with heteromorphic pollen grains *B. macrostachya*, the central colpus is a bit shorter and wider (12 – 13.5 μm) than the rest three laterals (12.7 – 15 μm). Ora is alonate in all the studied species.

The exine stratification spectrum in the studied species is impressive. Exine ornamentation varies from foveolate (*B. colvilei*), rugulate (*B. crispa*) to psilate with microperforations (*B. asiatica*, *B. macrostachya* and *B. paniculata*) and these features are important towards the correct identification of species as well as in grouping of the species. This heteromorphism in pollen structure, both qualitative as well as quantitative, have great taxonomic significance towards correct identity of the species.

Based on this observation, the pollen grains of the studied taxa have been divided into two basic types (Table 3). Type I is characterized by 4 – 5-aperturate grains (*B. colvilei* and

Key to the studied species of *Buddleja* L.

1a. Grains 3-aperturate.....	2
1b. Grains 4-5-aperturate.....	4
2a. Exine rugulate.....	<i>B. crispa</i>
2b. Exine psilate.....	3
3a. Length of colpi 9-14.7 μm	<i>B. asiatica</i>
3b. Length of colpi 12-15 μm	<i>B. paniculata</i>
4a. Grains 4-aperturate, exine psilate.....	<i>B. macrostachya</i>
4b. Grains 5-aperturate, exine foveolate.....	<i>B. colvilei</i>

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