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Pollen morphological characters variation among species of *Strobilanthes* s.l. from Sumatra, Indonesia and its taxonomic implications

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Abstract. *Suratman, Suranto, Muzzazinah, Purnomo. 2022. Pollen morphological characters variation among species of* Strobilanthes *s.l. from Sumatra, Indonesia and its taxonomic implications. Biodiversitas 23: 4636-4648.* The objective of this study was to evaluate pollen morphological characters variation and its taxonomic value among species of *Strobilanthes* s.l. from Sumatra. A total of 32 species from four genera previously recognized within the subtribe *Strobilanthineae* were used for pollen morphology observations using Light Microscope (LM) and Scanning Electron Microscope (SEM). A total of ten pollen morphological characters (both quantitative and qualitative) were observed in this study. In general, a great variation occurs in the majority of investigated pollen morphological characters. A similarity dendrogram among species of *Strobilanthes* s.l. based on pollen morphological character data revealed two major clusters. Cluster I consist of 25 species that differed from species in Cluster II in having pollen type I, pollen type II and pollen type III with a similarity coefficient of 0.61. Cluster II consists of seven species and has pollen type IV, pollen type V, pollen type VI and pollen type VII with a similarity coefficient of 0.46. An identification key to the species of *Strobilanthes* s.l. based on pollen morphology is also established for easy discrimination of the investigated taxa. This study revealed that pollen morphology was taxonomically useful character for identification and taxa delimitation of *Strobilanthes* s.l., especially at the species level. The results of this study also support earlier works through molecular studies, statistical analysis, gross morphology observations and leaf anatomy studies that all segregate genera of the subtribe *Strobilanthinae* should be merged under a single and well-defined genus as *Strobilanthes* s.l.

Keywords: Pollen morphological characters, *Strobilanthes* s.l., Sumatra, taxonomic problems, variation

INTRODUCTION

Strobilanthes Blume s.l. (sensu lato) is the second largest and much diverse genus of the family Acanthaceae (House 2016; Deng 2019). *Strobilanthes* s.l. can be distinguished from other members of Acanthaceae in having floral characteristics such as membraneous sheath formed by united filament, bifid stigma with a rudimentary posterior lobe, and two rows of hairs retaining the style against the posterior wall of the corolla throat (Bremekamp 1944; Carine and Scotland 2000; Manktelow 2000; Moylan et al. 2004). *Strobilanthes* s.l. contains *Strobilanthes* and its allied genera in the subtribe *Strobilanthineae* (sensu Bremekamp 1944) such as *Aechmanthera, Baphicacanthus, Clarkeasia, Hemigraphis, Stenosiphonium* (Scotland and Vollesen 2000) and numerous additional genera had been proposed previously to accommodate the morphological variation (Tripp et al. 2013).

Strobilanthes s.l. comprises approximately more than 400 species. The genus is native and mainly distributed in the subtropical and tropical regions in Asia with some species extending to Northern Australia and Pacific Islands (Mabberley 2008; Chen et al. 2019; Deng 2019; POWO 2022). *Strobilanthes* s.l. is also introduced into Western Africa (Benin, Cameroon, Chad) and Middle America (Belize, Cuba, Dominican Republic, El Salvador, Haiti, Honduras, Jamaica, Nicaragua, Panamá, Trinidad-Tobago) (POWO 2022).

Some species of *Strobilanthes* s.l. have been used for economic purposes, such as ornamental plants, natural dyes producers and medicinal plants (Baby and Raphael 2018; Li et al. 2019; Sreekumar et al. 2021). They have many important properties, such as anti-inflammatory, antimicrobial, antidiabetic, anticancer, antioxidant (Ismail et al. 2012; Nurraihana and Norfarizan-Hanoon 2013; Preethi and Suseem 2014; Nilanthi 2019), antiviral (Gu et al. 2015; Zhou et al. 2017; Tsai et al. 2020), antipyretic (Yu et al. 2021), antifungal, anthelmintic (Baby and Raphael 2018), indigo dyes (Liu et al. 2014; Fan et al. 2018).

Classification of *Strobilanthes* s.l. has long been controversial and problematic (Moylan 2004). No taxonomic account has satisfactorily dealt with the variation within *Strobilanthes*. Therefore, species delimitation and generic circumscription of this group remain ambiguous (Carine and Scotland 1998). There were three alternative classifications of the group proposed by Anderson (1867), Bremekamp (1944) and Terao (1983). Anderson (1867) recognized four genera in this group based on their ovule number. Three genera comprising species with more than four ovules were retained, namely *Hemigraphis*, *Aechmanthera* and *Stenosiphonium* whereas a large genus of *Strobilanthes* can be distinguished from the other three genera in having 2 or 4 ovules in their ovary (Carine and Scotland 2002; Deng 2019). In his monograph, Bremekamp (1944) divided the subtribe *Strobilanthinae* into 54 genera (with maintained a large genus *Strobilanthes* and three allied genera such as *Hemigraphis*, *Aechmanthera* and *Stenosiphonium*, the others consist of smaller genera) and arranged into 27 informal groups to account for 356 species primarily based on pollen and seed morphological characters (Carine and Scotland 2002; Bennet and Scotland 2003; Deng 2019). Terao (1983) then proposed a broad concept of *Strobilanthes* and merged all species and genera of the subtribe *Strobilanthinae* (sensu Bremekamp 1944) into a single large genus. In this classification, the group then divided into nine sections, eighteen subsections, and two series (Terao 1983; Carine and Scotland 2002).

The pollen morphological characters have been widely used in various plant groups of Angiosperms (Telleria et al. 2013; Al-Hakimi et al. 2015; Zhao et al. 2016; Mander 2016; Kriebel et al. 2017; Silva et al. 2017; Xiong et al. 2019; Raza et al. 2020). They can be used to solve the taxonomic problems of plant groups, such as identification, taxa delimitation, classification and relationship assessment (Yao and Zhang 2016; Al-Hakimi et al. 2017; Tripathi et al. 2017; Ruiz-Domínguez et al. 2019; Chen et al. 2019; de Souza et al. 2019; Ragho 2020). The pollen morphological characters such as polar axis length (P), equatorial diameter (E), P/E ratio, pollen sculpture or exine ornamentation (Via do Pico and Dematteis 2013; Ferrauto and Pavone 2014; Sarwar et al. 2015; Al-Hakimi et al. 2018), pollen size, pollen shape, exine thickness (Akhila and Beevy 2015; Krachai and Pornpongrungrueng 2015; Noedoost et al. 2021) are valuable for distinguishing and identification of many plant groups.

The members of the Acanthaceae have highly variable pollen morphological features. Therefore, it makes pollen morphology an attractive characteristic to investigate for its taxonomic significance. Pollen morphology is an important character that has been used extensively in formulating classification systems in the Acanthaceae (House 2016). Therefore, the studies of pollen grains of Acanthaceae seem to have become mandatory for the description of new species (Silva et al. 2017).

The potential utility of pollen morphology variability of *Strobilanthes* for generic delimitation was firstly recognized by Radlkofer (1883) and then followed by Lindau (1895). Afterwards, pollen morphology of *Strobilanthes* s.l. has been studied extensively. Many earlier workers have investigated the pollen morphology of the genus in specific geographical regions in order to develop a classification (House 2016), e.g. in Bhutan (Wood 1994), southern India and Sri Lanka (Carine and Scotland 1998), China (Wang and Blackmore 2003), Java (Bennett and Scotland 2003), and Myanmar (Wood et al. 2022). However, pollen morphology of *Strobilanthes* s.l. in Sumatra (Indonesia) is still poorly understood. Therefore, investigation in detail of pollen morphology of *Strobilanthes* s.l. from Sumatra is needed to increase their contribution to taxonomic problem solving for this area. For this reason, this study aims to evaluate pollen morphological characters variation and its taxonomic value among species of *Strobilanthes* s.l. from Sumatra.

MATERIALS AND METHODS

Plant material

This study was based on observations of obtained pollen samples from herbarium specimens of *Strobilanthes* s.l. were collected from various collection sites in Sumatra and kept in Herbarium Bogoriense (BO) (Table 1, Figure 1). A total of 32 species from four genera previously accepted within the subtribe *Strobilanthineae* (*Strobilanthes, Hemigraphis, Sericocalyx, Semnostachya*) were used for pollen morphology observation.

Procedures

The pollen grains of 32 species were examined using Light Microscope (LM) and Scanning Electron Microscope (SEM). Pollen samples were acetolysed following the procedures of Erdtman (1960) and separated into two parts, one for LM and the other for SEM. The glass slides were prepared for LM observations by mounting pollen samples in glycerine jelly (Wang and Blackmore 2003). The transparent nail polish was also deposited along the margins of glass slides with cover glass to make it semi-permanent for LM observation (Raza et al. 2020). For LM observations, light microscopic (Model: Nikon Eclipse E100, magnification of 10 x for ocular and 40 x for objective) were used (Suratman et al. 2022). Random observations of the glass slides were conducted based on ten pollen grains (Rueangsawang et al. 2013). For the SEM analysis, prepared pollen samples were dehydrated in an ethanol series, and mounted on glass coverslips, then air dried from 95% ethanol. The coverslips were then attached to aluminum stubs with double-sided cellophane tape and dried, then sputter coated with a gold-palladium mixture (Wang and Blackmore 2003; Rueangsawang et al. 2013). Observations were made using a Scanning Electron Microscope Hitachi SU 3500 at accelerating voltage 5KV and magnification of 1.500 x.

A total of ten pollen morphological characters (both quantitative and qualitative) were observed in this study. The measurement of quantitative characters, include five characters i.e. polar axis length (P), equatorial diameter (E), polar axis length (P): equatorial diameter (E) ratio (P/E), exine thickness and number of longitudinal ribs. Averages of the quantitative characters for each species were then used in further analyses (Suratman et al. 2016; Pitoyo et al. 2018). There were five observed qualitative pollen morphological characters, included pollen shape, pollen size, exine ornamentation, presence of spines, and presence of septate ribs. The terminology of pollen morphology used in this study then follows Carine and Scotland (1998), Punt et al. (2007), and Halbritter et al. (2018).

Table 1. Voucher specimens of *Strobilanthes* s.l. from Sumatra which pollen was sampled

No.	Species	Voucher specimens	Collection sites	Altitude (m a.s.l.)
1.	*Strobilanthes alboviridis* J. B. Imlay	Lorzing 11502	Simalungun, North Sumatra	350
2.	*Strobilanthes atropurpurea* Nees	Steenis 9349	Gayo, Aceh	1.600
3.	*Strobilanthes axilliflora* Clarke ex Moore	de Wilde 12219	Gunung Leuser, Aceh	200-400
4.	*Strobilanthes backeri* (Bremek.) J. R. Benn.	Poulsen et al. 11	Gunung Singgalang, West Sumatra	1.800
5.	*Strobilanthes barisanensis* (Bremek.) J. R. I. Wood	Steenis 8436	Gayo, Aceh	2.100-2.250
6.	*Strobilanthes bibracteata* Blume	Afriastini 2390	South Tapanuli, North Sumatra	1.300
7.	*Strobilanthes bunnemeyeri* J. R. I. Wood	Bunnemeijer 9874	Gunung Kerinci, Jambi	2.200
8.	*Strobilanthes capillipes* C. B. Clarke ex Ridl.	Jacobson 302	Suban Ayam, Bengkulu	1.200
9.	*Strobilanthes cernua* Blume	Yates 1295	East Coast, Riau	-*
10.	*Strobilanthes cusia* (Nees) Kuntze	Djarwaningsih TD 2573	North Tapanuli, North Sumatra	-*
11.	*Strobilanthes echinata* Nees	Bunnemeijer 3190	Agam, West Sumatra	850
12.	*Strobilanthes hamiltoniana* (Steud.) Bosser & Heine	Wyssling 104	Gunung Kerinci, Jambi	1.300
13.	*Strobilanthes hossei* C. B. Clarke	Steenis 6030	Takengon, Aceh	1.290
14.	*Strobilanthes inflata* T. Anderson	Steenis 8363	Gayo, Aceh	2.500
15.	*Strobilanthes involucrata* Blume	Lorzing 15645	Toba, North Sumatra	1.550
16.	*Strobilanthes multiflora* Ridl.	Lorzing 16771	Pematangsiantar, North Sumatra	800
17.	*Strobilanthes ovatifolia* (Bremek.) J. R I. Wood	Bunnemeijer 4156	Gunung Malintang, West Sumatra	1.150
18.	*Strobilanthes palawanensis* Elmer	Afriastini 773	Bukit Pelalawan, Riau	1.450
19.	*Strobilanthes parabolica* Nees	Bunnemeijer 9159	Gunung Kerinci, Jambi	2.000
20.	*Strobilanthes pateriformis* Lindau	de Voogd 10	Danau Ranau, South Sumatra	-*
21.	*Strobilanthes pedunculosa* Miq.	Rappard K-19	Gunung Kaba, Bengkulu	900
22.	*Strobilanthes polybotrya* Miq.	Hotta & Okada 1640	Kelok Sembilan, West Sumatra	700
23.	*Strobilanthes pubescens* (Bremek.) J. R. I. Wood	de Voogd 1437	Bukit Daun, Bengkulu	1.600
24.	*Strobilanthes rufopauper* C.B.Clarke	Lorzing 15500	Habinsaran, North Sumatra	1.200
25.	*Strobilanthes speciosa* Blume	Steenis 9799	Gunung Kemiri, Aceh	1.100
26.	*Strobilanthes sumatrana* Miq.	Bunnemeijer 8043	Gunung Kerinci, Jambi	1.100
27.	*Strobilanthes violascens* Ridl.	Bunnemeijer 408	Padang, West Sumatra	400
28.	*Sericocalyx crispa* (L.) Bremek.	de Voogd 574	Balai, Bengkulu	500
29.	*Hemigraphis alternata* (Burm. f.) T. Anderson	Lorzing 14014	Medan, North Sumatra	20
30.	*Hemigraphis blumeana* (Nees) Boerl.	Teysmann 4484	Lampung	-*
31.	*Hemigraphis serpens* (Nees) Boerl.	Steenis 3357	Palembang, South Sumatra	500
32.	*Semnostachya benculensis* Bremek.	Ajoeb 349	Suban Ayam, Bengkulu	1.200

Note: * = No information about altitude from specimens label

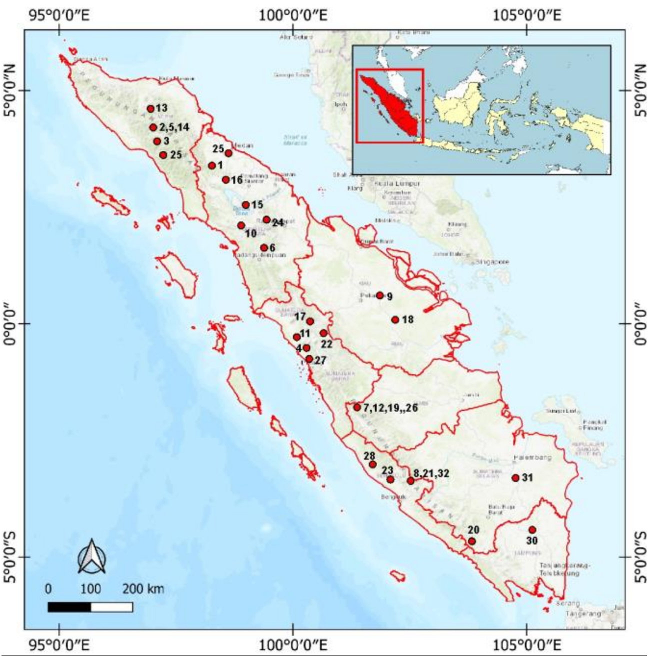

Figure 1. Distribution map of Sumatran *Strobilanthes.* S.No. refer to Table 1.

Data analysis

The measured quantitative pollen morphological characters were analyzed with Duncan's multiple range test ($p < 0.05$) using SPSS 20.0 version software to test the significance of variation among all of the tested species through analysis of variance (Suratman et al. 2016; Pitoyo et al. 2018; Restykania et al. 2019). All of the tested pollen morphological characters (both quantitative and qualitative) then subjected to principal component analysis using software of PAST 4.03 version in order to determine the relative contribution of each character to the total variation and the importance of the tested characters for discrimination of all of the tested taxa (Muzzazinah et al. 2021). A dendrogram was generated by the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) procedure through the cluster analysis method in order to group all of the tested species based on pollen morphological characters similarity. A computer programme, NTSYS ver. 2.00, then used to perform this analysis (Suratman et al. 2016; Pitoyo et al. 2018; Restykania et al. 2019).

RESULTS AND DISCUSSION

Results

A total of ten pollen morphological characters consisting of five quantitative and five qualitative characters were selected for pollen morphological comparison among species of *Strobilanthes* s.l. The quantitative pollen morphological characters of *Strobilanthes* s.l. from Sumatra are summarized in Table 2 and Table 3.

A highly significant difference among species of *Strobilanthes* s.l. for all tested quantitative pollen morphological characters was revealed by the analysis of variance in this study. Polar axis length varied highly significant and showed wide variation and ranging from 41.31 µm (*H. blumeana*) to 102.51 µm (*S. pedunculosa*) with an average 62.65 µm. Equatorial diameter values exhibited highly significant variation and displayed a range from 28.33 µm (*S. pubescens*) to 58.56 µm (*S. pateriformis*) with an average 41.98 µm. The ratio of polar axis length and equatorial diameter (P/E) then reveals highly significant variation among all tested species. *Strobilanthes axilliflora* displayed the lowest value of ratio P/E (0.96) whereas the highest value (2.3) can be found in *S. multiflora* with an average 1.53. Exine thickness also showed highly significant variability with range from 2.22 µm (*H. alternata)* to 6.39 µm (*S. parabolica)* and an average of 4.00 µm for this character. The highly significant variation can be also found in a number of longitudinal ribs and ranged from 0 (*S. pateriformis*) to 21 (*S. capillipes* and *S. pubescens)* with an average 17.04. The number of longitudinal ribs had the highest coefficient of variance (23.40 %) then followed by exine thickness (22.87%), polar axis length (21.84%), ratio of polar axis length and equatorial diameter (19.80%) and equatorial diameter (18.91%).

Qualitative pollen morphology characters among species of *Strobilanthes* s.l. are summarized in Table 4. Pollen grain's shape can be classified based on the ratio of polar axis length and equatorial diameter (P/E) values. There are nine classes of pollen shape i.e. peroblate (P/E ≤ 0.50), euoblate (P/E 0.51-0.75), suboblate (P/E 0.76-0.87), oblate spherodial (P/E 0.88-0.99), spherical (P/E 1.00), prolate spheroidal (P/E 1.01-1.14), subprolate (P/E 1.15-1.33), euprolate (P/E 1.34-1.99) and perprolate (P/E ≥ 2.00) (Walker and Doyle 1975). Based on pollen grains shape, majority species of *Strobilanthes* s.l. have euprolate pollen such as *S. alboviridis*, *S. atropurpurea*, *S. barisanensis*, *S. bibracteata*, *S. bunnemeyeri*, *S. capillipes*, *S. cusia*, *S. echinata*, *S. hamiltoniana*, *S. hossei*, *S. inflata*, *S. involucrata*, *S. multiflora*, *S. ovatifolia*, *S. palawanensis*, *S. pedunculosa*, *S. polybotrya*, *S. pubescens*, *S. rufopauper*, *S. speciosa*, *S. sumatrana*, *S. violascens*, *Se. crispa*, *H. serpens,* and *Sm. benculensis.* Only two species of *Strobilanthes* s.l. have perprolate pollen grains namely, *S. pubescens* and *S. multiflora*. Perprolate and euprolate pollen grains usually can be considered as ellipsoidal pollen grains shape. The subprolate pollen grains are distributed in three species of *Strobilanthes* s.l. i.e. *S. backeri*, *H. alternata* and *H. blumeana.* A few species have prolate spheroidal pollen grains, such as *S. cernua*, *S. parabolica* and *S. pateriformis* whereas the oblate spherodial pollen grains only was found in *S. axilliflora.* The subprolate, prolate spheroidal, and oblate spheroidal pollen grains may be grouped together under the category of subspheroidal pollen (Walker and Doyle 1975).

The size of pollen grains can be classified into six classes i.e. minute (<10 µm), small (10-24 µm), medium (25-49 µm), large (50-99 µm), very large (100-199 µm) and gigantic (≥200 µm) (Walker and Doyle 1975). Based on pollen size, most of the studied species have large-sized pollen grains such as *S. alboviridis*, *S. atropurpurea*, *S. axilliflora*, *S. backeri*, *S. barisanensis*, *S. bibracteata*, *S. capillipes*, *S. cernua*, *S. cusia*, *S. echinata*, *S. hamiltoniana*, *S. hossei*, *S. inflata*, *S. involucrata*, *S. multiflora*, *S. ovatifolia*, *S. palawanensis*, *S. parabolica*, *S. pateriformis*, *S. polybotrya*, *S. pubescens*, *S. rufopauper*, *S. speciosa*, *S. sumatrana*, *S. violascens*, and *Sm. benculensis.* A few species of *Strobilanthes* s.l. have medium-sized pollen grains such as *S. bunnemeyeri*, *Se. crispa*, *H. alternata*, *H. blumeana*, *H. serpens*, whereas the very large-sized pollen grains only found in one species, namely as *S. pedunculosa.*

Majority species of *Strobilanthes* s.l. have pseudocolpate pollen grains, except for *S. pateriformis*. The pseudocolpi then divide the exine into longitudinal ribs or flanges. Most of the studied species have longitudinal ribs with septa forming crossbars between the ribs such as *S. alboviridis*, *S. atropurpurea*, *S. axilliflora*, *S. barisanensis*, *S. bibracteata*, *S. bunnemeyeri*, *S. capillipes*, *S. cusia*, *S. echinata*, *S. hamiltoniana*, *S. hossei*, *S. inflata*, *S. involucrata*, *S. multiflora*, *S. ovatifolia*, *S. palawanensis*, *S. pedunculosa*, *S. polybotrya*, *S. pubescens*, *S. speciosa*, *S. sumatrana*, *S. violascens*, *Se. crispa*, *H. alternata*, *H. serpens* and *Sm. benculensis.* Only a few species have no septa forming crossbars between the ribs, such as *S. backeri*, *S. cernua*, *S. parabolica*, *S. rufopauper*, and *H. blumeana.*

Table 2. Estimates of the variability of pollen morphological characters among species of *Strobilanthes* s.l. from Sumatra

Pollen morphological characters	Mean	Min	Max	Standard Deviation	Coefficient of Variance (%)	P*(F-test)
Polar axis length (P) (µm)	62.65	41.31	102.51	13.68	21.84	0.00**
Equatorial diameter (E) (µm)	41.98	28.33	58.56	7.94	18.91	0.00**
Ratio of polar axis length and equatorial diameter (P/E)	1.53	0.96	2.3	0.30	19.80	0.00**
Exine thickness (µm)	4.00	2.22	6.39	0.91	22.87	0.00**
Number of longitudinal ribs/ flanges	17.04	0	21	3.98	23.40	0.00**

Note : **P < 0.01= highly significant

Table 3. Quantitative pollen morphological character variation among species of *Strobilanthes* s.l. from Sumatra

Species	Polar axis length (P) (µm)	Equatorial diameter (µm)	Ratio of polar axis length/equatorial diameter (P/E)	Exine thickness (µm)	Number of longitudinal ribs/flanges
S. alboviridis	59.13±3.28ghijk	41.23±3.61ijklm	1.44±0.12defgh	3.99±0.76cdefghij	15.4±0.51de
S. atropurpurea	72.55±7.35mn	39.12±3.00ghijkl	1.86±0.12ijkl	3.29±0.60abcdef	14.5±0.52cd
S. axilliflora	51.20±6.57def	53.06±5.06qr	0.96±0.06a	3.15±0.67abcdef	20.6±0.51j
S. backeri	65.46±22.8jkl	50.67±7.14pqr	1.32±0.48cdef	5.14±1.22jkl	12.4±0.84b
S. barisanensis	73.26±2.21mno	48.76±2.16nopq	1.51±0.07efgh	2.94±0.88abcd	17.4±0.96fg
S. bibracteata	79.75±7.11nop	50.89±7.35pqr	1.60±0.24fghi	3.55±0.63cdefgh	15±0de
S. bunnemeyeri	48.75±5.46cd	33.57±3.45bcdefg	1.47±0.25efgh	2.84±0.77abc	20.4±1.26
S. capillipes	51.94±3.36defg	32.67±4.87bcde	1.62±0.24ghi	3.99±0.66cdefghij	21±0j
S. cernua	50.65±5.75de	49.65±6.19opqr	1.03±0.10ab	4.55±1.08ghijkl	17.2±1.03fg
S. cusia	63.49±8.57ijkl	44.63±5.14lmno	1.44±0.27defgh	5.19±0.73kl	20.7±0.48j
S. echinata	80.32±4.08op	51.31±2.73pqr	1.57±0.13fgh	4.70±1.02hijkl	15.2±1.03de
S. hamiltoniana	58.65±7.17fghijk	38.75±6.13ghijk	1.57±0.41fgh	3.66±0.61cdefgh	17.6±0.84fg
S. hossei	61.71±10.62hijkl	40.30±5.55hijkl	1.57±0.41fgh	4.33±1.40fghijk	17.8±0.63g
S. inflata	67.21±5.68lm	43.87±4.48klmn	1.54±0.17efgh	3.91±0.75cdefghi	18.8±1.03hi
S. involucrata	73.40±7.04mno	46.58±4.74mnop	1.59±0.24fghi	4.12±0.56defghijk	15.3±0.48de
S. multiflora	92.94±7.07q	41.12±6.26ijklm	2.30±0.29m	3.15±0.30abcdef	20.8±0.42j
S. ovatifolia	60.23±7.18hijkl	40.02±5.91hijkl	1.52±0.17efgh	3.49±0.62bcdefg	20.7±0.48j
S. palawanensis	57.62±4.31efghij	36.69±2.60defghi	1.58±0.14fgh	3.44±0.90bcdefg	20.5±0.52j
S. parabolica	55.29±6.17defgh	52.85±3.65qr	1.05±0.12abc	6.39±1.57m	13.8±0.63c
S. pateriformis	61.56±7.69hijkl	58.56±8.84s	1.08±0.25abc	5.21±1.36kl	0±0a
S. pedunculosa	102.51±14.29r	55.16±14.46rs	1.93±0.35kl	5.08±1.18ijkl	15±1.05de
S. polybotrya	63.12±3.52ijkl	38.52±4.26fghijk	1.66±0.25ghijk	4.20±0.84efghijk	15±0de
S. pubescens	54.35±3.61defgh	28.33±8.12b	2.07±0.62lm	4.10±1.13defghijk	21±0j
S. rufopauper	72.93±7.78mn	37.32±4.32efghij	1.99±0.39l	3.66±0.52cdefgh	14.9±0.56de
S. speciosa	64.00±5.09ijkl	42.65±3.65jklm	1.52±0.22efgh	5.51±2.26lm	18.9±1.85i
S. sumatrana	61.03±6.55hijkl	36.57±4.08defghi	1.69±0.27hijkl	3.42±1.88bcdefg	15.5±0.52e
S. violascens	61.70±6.93hijkl	44.82±7.99lmno	1.42±0.33defgh	4.05±1.08cdefghijk	18±0gh
Se. crispa	42.91±3.69bc	31.36±3.56bcd	1.38±0.11defg	3.23±0.75abcdef	16.8±1.03f
alternata	41.69±7.36ab	36.73±7.04defghi	1.18±0.34abcd	2.22±0.31a	20.7±0.48j
H. blumeana	41.31±4.18ab	32.89±2.45bcdef	1.26±0.13bcde	3.01±0.55abcde	18±0gh
H. serpens	49.58±2.91cd	30.15±1.71bc	1.65±0.10ghij	5.01±2.36ijkl	17.4±0.96fg
Sm. benculensis	64.83±6.82jkl	34.59±4.97bcdefgh	1.90±0.30jkl	3.60±1.26cdefgh	19.2±2.89i
Average	62.65	41.98	1.53	4.00	17.04

Note: * Values are means ± standard deviation. Values followed by the different letters in the same column indicate significant differences in Duncan Multiple Range Test ($p < 0.05$)

Exine of all studied pollen grains has various ornamentation such as bireticulate, reticulate, micoreticulate, echinulate and caruncuIate. Most of species *Strobilanthes* s.l. have bireticulate ornamentation such as *S. alboviridis, S. atropurpurea, S. barisanensis, S. bibracteata, S. bunnemeyeri, S. capillipes, S. cusia, S. echinata, S. hamiltoniana, S. hossei, S. inflata, S. involucrata, S. multiflora, S. ovatifolia, S. palawanensis, S. pedunculosa, S. polybotrya, S. pubescens, S. rufopauper, S. speciosa, S. sumatrana,* and *S. violascens.* The microreticulate tectal ornamentation can be found in some species, such as *S. axilliflora, S. backeri, S. cernua* and *S. parabolica* whereas a few species have reticulate ornamentation such as *Se. crispa, H. alternata, H. serpens* and *Sm. benculensis.*The echinulate ornamentation was only distributed in *S. pateriformis* whereas the caruncuIate ornamentation can be found in *H. blumeana.* Majority of species have no spines in their exine, except for *S. pateriformis* and *H. blumeana. Strobilanthes pateriformis* has pointed spines whereas *H. blumeana* has rounded ones.

Based on their morphological character, the pollen of *Strobilanthes* s.l. from Sumatra can be classified into seven types (Figure 2). Type I has ellipsoid, bireticulate ornamentation, septate ribs pollen grains, consist of 21 species such as *S. alboviridis, S. atropurpurea, S. barisanensis, S. bibracteata, S. bunnemeyeri, S. capillipes, S. cusia, S. echinata, S. hamiltoniana, S. hossei, S. inflata, S. involucrata, S. multiflora, S. ovatifolia, S. pedunculosa, S. polybotrya, S. pubescens, S. speciosa, S. sumatrana,* and *S. violascens*. Type II has ellipsoid, reticulate ornamentation, septate ribs pollen grains, consisting of four species, such as *S. palawanensis, Se. crispa, H. serpens* and *Sm. benculensis.* Type III has ellipsoid, reticulate ornamentation, no septate ribs pollen grains and consist of only one species, namely *S. rufopauper.* Type IV has subspheroidal, multireticulate ornamentation, no septate ribs pollen grains, consist of three species, such as *S. backeri, S. cernua* and *S. parabolica.* Type V has subspheroidal, bireticulate or reticulate ornamentation, septate ribs pollen grains and consist of two species, namely *S. axilliflora* and *H. alternata.* Type VI has subspheroidal, caruncuIate ornamentation, no septate ribs pollen grains and consists of one species, i.e. *H. blumeana.* Type VII has subspheroidal, echinulate ornamentation, no septate ribs pollen grains and consist of only one species namely as *S. pateriformis.* An identification key to the species of *Strobilanthes* s.l. based on pollen morphology is provided below for easy discrimination of the investigated taxa.

The quantitative and qualitative pollen morphological characters data set then subjected to PC analysis and revealed the first three principal components with eigenvalues greater than 1 and accounted for about 79.75 % of the total variation (Table 5). The first principal component (PC1) exhibited 41.62 % of the total variation with an eigenvalue of 4.16 was dominated by polar axis length, ratio P/E, number of longitudinal ribs, pollen shape, pollen size and presence of septate ribs, but some characters such as equatorial diameter, exine thickness, exine ornamentation, presence of spines showed negative values. The second PC (PC2) with an eigenvalue of 2.74 and 27.37 % variation was positively associated with polar axis length, equatorial diameter, ratio P/E, exine thickness, pollen shape and pollen size but negatively associated with a number of longitudinal ribs, tectal ornamentation, presence of spines, and presence of septate ribs. The third PC (PC3) with 10.76 % variation and eigenvalue of 1.08 showed polar axis length, ratio P/E, pollen shape, tectal ornamentation, and presence of spines as highly loaded characters but equatorial diameter, exine thickness, number of longitudinal ribs, pollen size, and presence of septate ribs as the negatively loaded characters.

Key to the species of *Strobilanthes* s.l. from Sumatra based on pollen morphology

1a. Pollen grains subspheroidal ……………………………… 2
1b. Pollen grains ellipsoid …………………………………… 8
2a. Exine ornamentation bireticulate or reticulate, septate longitudinal ribs present …………………………………… 3
2b. Exine ornamentation micoreticulate or caruncculate or echinulate, no septate longitudinal ribs …………………… 4
3a. Pollen grains oblate spheroidal, bireticulate, polar axis 51.20 µm, diameter equatorial 53.06 µm, P/E 0.96 …… *S. axilliflora*
3b. Pollen grains subprolate, reticulate, polar axis 41.69 µm, diameter equatorial 36.73 µm, P/E 1.18 ……….... *H. alternata*
4a. Exine ornamentation microreticulate, spines absent, large-sized pollen grains …………………………………………. 5
4b. Exine ornamentation caruncculate or echinulate, spines present, medium or large-sized pollen grains ……………………… 7
5a. Pollen grains subprolate, P/E 1.15-1.33 ………… *S. backeri*
5b. Pollen grains prolate spheroidal, P/E 1.01-1.14 …………… 6
6a. Polar axis 50.65 µm, diameter equatorial 49.65 µm, P/E 1.03, 16-18 longitudinal ribs …………………………… *S. cernua*
6b. Polar axis 55.29 µm, diameter equatorial 52.85 µm, P/E 1.05, 12-14 longitudinal ribs ……………………… *S. parabolica*
7a. Medium-sized pollen grains, polar axis 41.31 µm, diameter equatorial 32.89 µm, P/E 1.26, caruncculate …… *H. blumeana*
7b. Large-sized pollen grains, polar axis 61.56 µm, diameter equatorial 58.56 µm, P/E 1.08, echinulate …... *S. pateriformis*
8a. Septate longitudinal ribs absent, exine ornamentation reticulate ………………………………………. *S. rufopauper*
8b. Septate longitudinal ribs present, exine ornamentation bireticulate or reticulate ………………………………….. 9
9a. Exine ornamentation reticulate …………………………… 10
9b. Exine ornamentation bireticulate ………………………… 13
10a.Large-sized pollen grains, 50-99 µm long ………………. 11
10b.Medium-sized pollen grains, 25-49 µm long ……………. 12
11a. Polar axis 57.62 µm, diameter equatorial 36.69 µm, P/E 1.58 ………………………………………….. *S. palawanensis*
11b. Polar axis 64.83 µm, diameter equatorial 34.59 µm, P/E 1.90 ………………………………………….. *Sm. benculensis*
12a. Polar axis 42.91 µm, diameter equatorial 31.36 µm, P/E 1.38 ………………………………………………… *Se. crispa*
12b. Polar axis 44.58 µm, diameter equatorial 30.15 µm, P/E 1.65 ……………………………………………….. *H. serpens*
13a. Pollen grains perprolate, P/E ≥ 2.00 …………………….. 14
13b. Pollen grains euprolate, P/E 1.34-1.99 ………………….. 15
14a. Polar axis 92.94 µm, diameter equatorial 41.12 µm, P/E 2.30 ……………………………………………… *S. multiflora*
14b. Polar axis 54.35 µm, diameter equatorial 28.33 µm, P/E 2.07 ……………………………………………….. *S. pubescens*
15a. Very large-sized pollen grains, 100-199 µm long ………… ………………………………………….. *S. pedunculosa*
15b. Large-sized (50-99 µm long) or medium-sized (25-49 µm long) pollen grains ……………………………………… 16
16a. Medium-sized pollen grains, 25-49 µm long…*S. bunnemeyeri*
16b. Large-sized pollen grains, 50-99 µm long ……………… 17
17a. Longitudinal ribs ≥ 20 …………………………………… 18
17b. Longitudinal ribs < 20 …………………………………… 20
18a. Longitudinal ribs 20-2 ………………………… *S. ovatifolia*
18b. Longitudinal ribs 21 ……………………………………… 19
19a. Polar axis 51.94 µm, diameter equatorial 32.67 µm, P/E 1.62 ……………………………………………… *S. capillipes*
19b. Polar axis 63.49 µm, diameter equatorial 44.63 µm, P/E 1.44 ………………………………………………….. *S. cusia*
20a. Polar axis < 60 µm ……………………………………… 21
20b. Polar axis > 60 µm ……………………………………… 22
21a. Polar axis 59.13 µm, diameter equatorial 41.23 µm, P/E 1.44 ……………………………………………… *S. alboviridis*
21b. Polar axis 58.65 µm, diameter equatorial 38.75 µm, P/E 1.57 ……………………………………..…….. *S. hamiltoniana*
22a. Polar axis 60-70 µm ……………………………………… 27
22b. Polar axis 71-81 µm ……………………………………… 23
23a. Longitudinal ribs < 18 …………………………………… 24
23b. Longitudinal ribs 18 ………………………… *S. barisanensis*
24a. P/E 1.86 …………………………………… *S. atropurpurea*
24b. P/E < 1.86 ……………………………………………… 25
25a. P/E 1.60 …………………………………… *S. bibracteata*
25b. P/E > 1.60 ……………………………………………. 26
26a. Polar axis 80.32 µm, diameter equatorial 51.31 µm, P/E 1.57, exine thickness 4.70 µm ……………………… *S. echinata*
26b. Polar axis 73.40 µm, diameter equatorial 46.58 µm, P/E 1.59, exine thickness 4.12 µm ………………… *S. involucrata*
27a. Longitudinal ribs < 18 …………………………………… 28
27b. Longitudinal ribs ≥ 18 …………………………………… 29
28a. Longitudinal ribs 16, diameter equatorial 36.57 µm, P/E 1.69, exine thickness 3.42 µm ………………… *S. sumatrana*
28b. Longitudinal ribs 15, diameter equatorial 38.52 µm, P/E 1.66, exine thickness 4.20 µm ………………… *S. polybotrya*
29a. Exine thickness 5.51 µm ……………………… *S. speciosa*
29b. Exine thickness < 5.51 µm ……………………………… 30
30a. Exine thickness 3.91 µm, longitudinal ribs 18-20 ….*S. inflata*
30b. Exine thickness > 3.91 µm, longitudinal ribs 18 ………… 31
31a. Exine thickness 4.33 µm, diameter equatorial 40.3 µm, P/E 1.57 ………………………………………………… *S. hossei*
31b. Exine thickness 4.05 µm, diameter equatorial 44.82 µm, P/E 1.42 ……………………………………………. *S. violascens*
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Table 4. Qualitative pollen morphological character variation among species of *Strobilanthes* s.l. from Sumatra

Species	Pollen shape*	Pollen size *	Exine ornamentation	Presence of spines	Septate ribs/flanges
S. alboviridis	Euprolate	Large	Bireticulate	Absent	Present
S. atropurpurea	Euprolate	Large	Bireticulate,	Absent	Present
S. axilliflora	Oblate spherodial	Large	Bireticulate	Absent	Present
S. backeri	Subprolate	Large	Microreticulate	Absent	Absent
S. barisanensis	Euprolate	Large	Bireticulate	Absent	Present
S. bibracteata	Euprolate	Large	Bireticulate	Absent	Present
S. bunnemeyeri	Euprolate	Medium	Bireticulate	Absent	Present
S. capillipes	Euprolate	Large	Bireticulate	Absent	Present
S. cernua	Prolate spheroidal	Large	Microreticulate	Absent	Absent
S. cusia	Euprolate	Large	Bireticulate	Absent	Present
S. echinata	Euprolate	Large	Bireticulate	Absent	Present
S. hamiltoniana	Euprolate	Large	Bireticulate	Absent	Present
S. hossei	Euprolate	Large	Bireticulate	Absent	Present
S. inflata	Euprolate	Large	Bireticulate	Absent	Present
S. involucrata	Euprolate	Large	Bireticulate	Absent	Present
S. multiflora	Perprolate	Large	Bireticulate	Absent	Present
S. ovatifolia	Euprolate	Large	Bireticulate	Absent	Present
S. palawanensis	Euprolate	Large	Reticulate	Absent	Present
S. parabolica	Prolate spheroidal	Large	Micoreticulate	Absent	Absent
S. pateriformis	Prolate spheroidal	Large	Echinulate	Pointed spines	Absent
S. pedunculosa	Euprolate	Very large	Bireticulate	Absent	Present
S. polybotrya	Euprolate	Large	Bireticulate	Absent	Present
S. pubescens	Perprolate	Large	Bireticulate	Absent	Present
S. rufopauper	Euprolate	Large	Bireticulate	Absent	Absent
S. speciosa	Euprolate	Large	Bireticulate	Absent	Present
S. sumatrana	Euprolate	Large	Bireticulate	Absent	Present
S. violascens	Euprolate	Large	Bireticulate	Absent	Present
Se. crispa	Euprolate	Medium	Reticulate	Absent	Present
H. alternata	Subprolate	Medium	Reticulate	Absent	Present
H. blumeana	Subprolate	Medium	Caruncülate	Rounded spines	Absent
H. serpens	Euprolate	Medium	Reticulate	Absent	Present
Sm. benculensis	Euprolate	Large	Reticulate	Absent	Present

Note : * Classification based on Walker and Doyle (1975)

Table 5. Eigenvectors coefficient of ten selected pollen morphological characters among species of *Strobilanthes* s.l. from Sumatra for the first three principal components with eigenvalues, individual and cumulative percentage of the total variation

Pollen morphological characters	Principal Components (PC)		
	PC 1	**PC 2**	**PC3**
Polar axis length (P) (µm)	**0.197**	**0.488**	**0.249**
Equatorial diameter (E) (µm)	-0.189	**0.471**	-0.317
Ratio of polar axis length and equatorial diameter (P/E)	**0.360**	**0.100**	**0.565**
Exine thickness (µm)	-0.151	**0.366**	-0.177
Number of longitudinal ribs/ flanges	**0.292**	-0.317	-0.304
Pollen shape	**0.429**	**0.043**	**0.239**
Pollen size	**0.118**	**0.522**	-0.074
Exine ornamentation	-0.442	-0.105	**0.226**
Presence of spines	-0.358	-0.042	**0.507**
Septate ribs/flanges	**0.408**	-0.090	-0.155
Eigenvalue	**4.16**	**2.74**	**1.08**
Individual percentage	**41.62**	**27.37**	**10.76**
Cumulative percentage	**41.62**	**68.99**	**79.75**

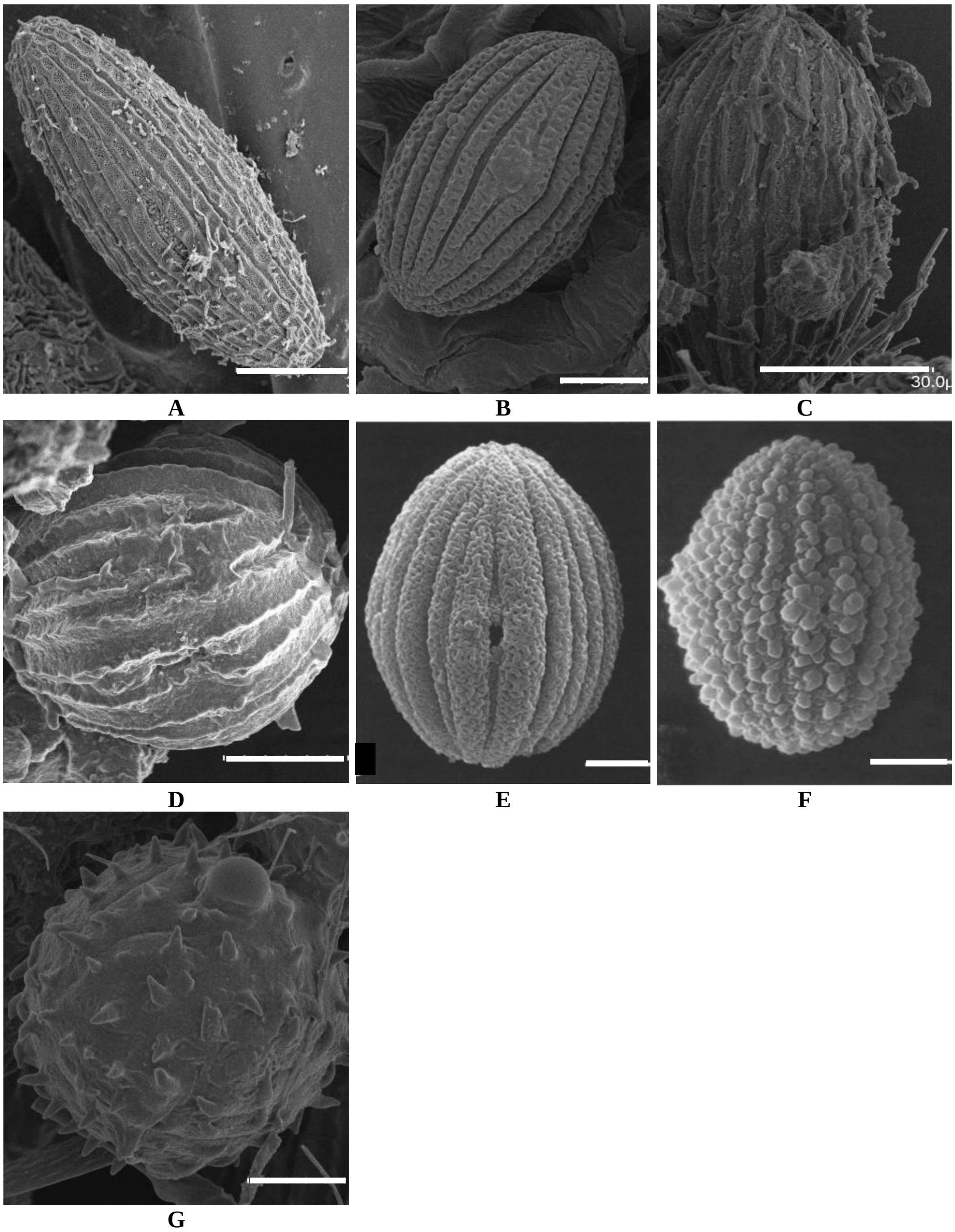

Figure 2. Scanning electron micrographs of pollen grains group of *Strobilanthes* s.l. from Sumatra A. Type I: ellipsoid, bireticulate, septate ribs (*S. involucrata*); B. Type II: ellipsoid, reticulate, septate ribs (*H. serpens*); C. Type III: ellipsoid, reticulate, no septate ribs (*S. rufopauper*); D. Type IV: subspheroidal, multireticulate, no septate ribs (*S. parabolica*); E. Type V: subspheroidal, reticulate, septate ribs (*H. alternata*); F. Type VI: subspheroidal, carunculate, no septate ribs (*H. blumeana*); G. Type VII: subspheroidal, echinulate, no septate ribs (*S. pateriformis*). Scale bars A, C = 30 μm; B, D, G = 15 μm; E = 5 μm, F = 10 μm. E and F from Moylan et al. (2002)

Plotting of the species of *Strobilanthes* s.l. based on pollen morphological characters across the first two PCs (PC1 and PC2) through PCA analysis showed clearly separation of groups across the PC1 axis (Figure 3). Species with higher values for PC1 (such as *S. multiflora, S. pubescens, S. pedunculosa, S. capillipes*) had higher polar axis length, ratio P/E and number of longitudinal ribs than species with lower PC1 values, such as *S. pateriformis, S. parabolica, S. backeri, S. cernua, H. blumeana*. Species with higher values for PC2 (*S. pedunculosa, S. pateriformis, S. echinata, S. backeri, S. parabolica)* had higher polar axis length, equatorial diameter, exine thickness and pollen size than species with lower PC2 values such as *S. alternata, S. blumeana, S. bunnemeyeri, S. crispa, H. serpens.* The results of PC analysis also showed that the species of *Strobilanthes* s.l. clearly grouped into six groups (A-F) based on pollen morphological characters.

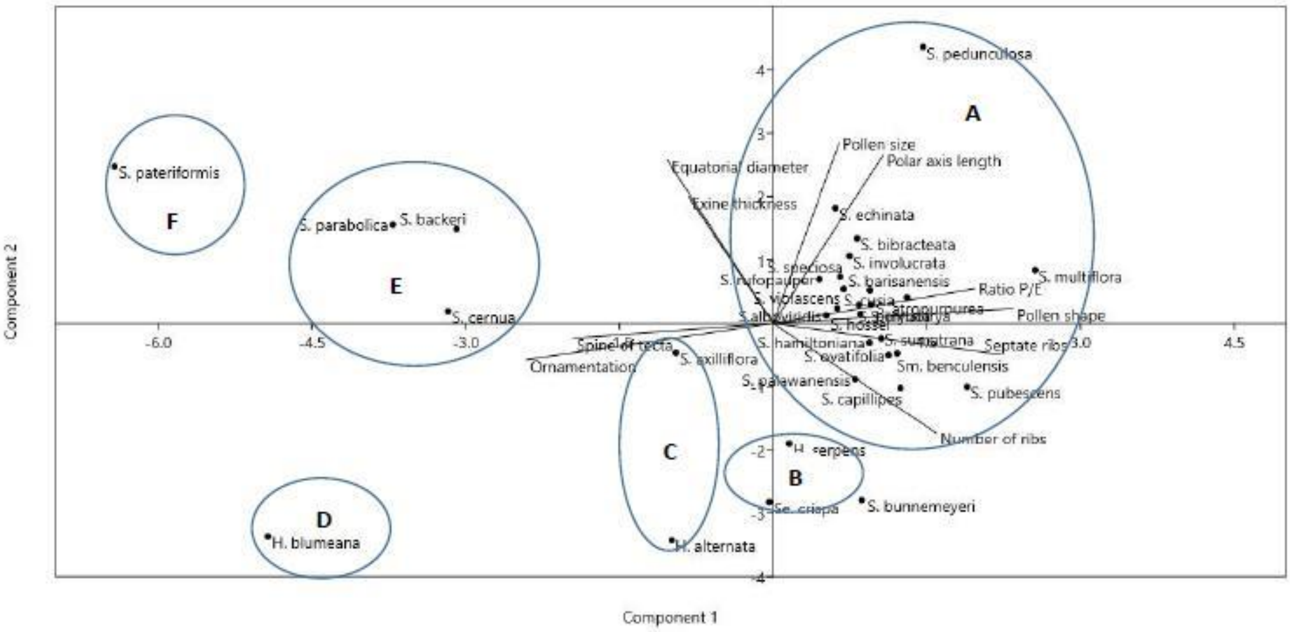

Figure 3. Scatter diagram of 32 species of *Strobilanthes* s.l. from Sumatra and ten selected pollen morphological characteristics when plotted against the first two principal components (explaining 68.99 % of the total variation). Groups A-F indicate the grouping of species based on pollen morphology

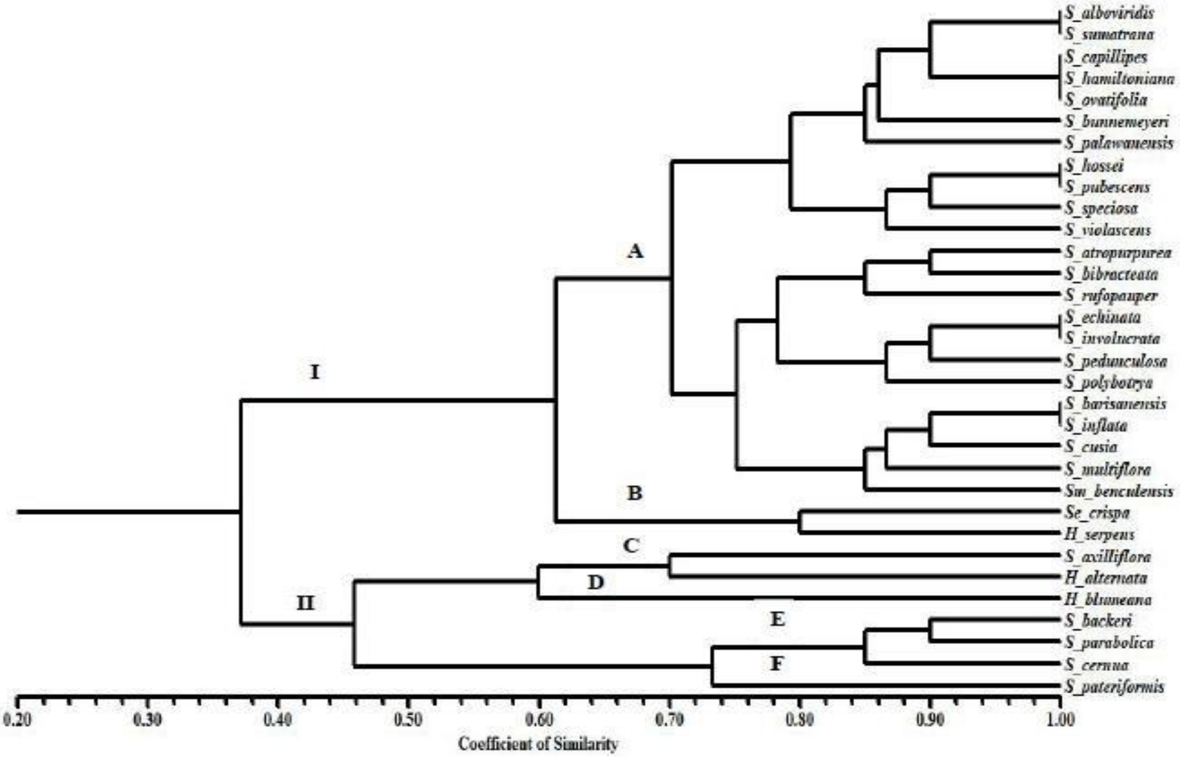

Figure 4. A dendrogram of similarity among species of *Strobilanthes* s.l. from Sumatra based on pollen morphological characters data

A dendrogram of similarity among species of *Strobilanthes* s.l. from Sumatra derived by UPGMA from the similarity matrix of the pollen morphological characters data is presented in Figure 4. The result of UPGMA cluster analysis showed that the species of *Strobilanthes* s.l. from Sumatra can be divided into two main clusters. Cluster I consist of 25 species which differed from species in Cluster II in having pollen type I (ellipsoid, bireticulate, septate ribs pollen grains), pollen type II (ellipsoid, reticulate, septate ribs pollen grains) and pollen type III (ellipsoid, reticulate, no septate ribs pollen grains) with a similarity coefficient of 0.61. This cluster then can be separated into two sub-clusters i.e. sub-cluster A and sub-cluster B. A total of 23 species supported sub-cluster A consist of 22 species have pollen type I (such as *S. alboviridis, S. atropurpurea, S. barisanensis, S. bibracteata, S. bunnemeyeri, S. capillipes, S. cusia, S. echinata, S. hamiltoniana, S. hossei, S. inflata, S. involucrata, S. multiflora, S. ovatifolia, S. palawanensis, S. pedunculosa, S. polybotrya, S. pubescens, S. speciosa, S. sumatrana, S. violascens* and *Sm. benculensis*) and one species has pollen type III (*S. rufopauper*) with a similarity coefficient of 0.70. Sub-cluster B only consists of two species that have pollen type II (*Se. crispa* and *H. serpens*) and are closely related to sub-cluster A with a similarity coefficient of 0.80.

Cluster II consists of seven species and can be distinguished from species in Cluster I in having pollen type IV (subspheroidal, multireticulate, no septate ribs pollen grains), pollen type V (subspheroidal, bireticulate or reticulate, septate ribs pollen grains), pollen type VI (subspheroidal, caruncula te, no septate ribs pollen grains) and pollen type VII (subspheroidal, echinulate, no septate ribs pollen grains) with a similarity coefficient of 0.46. This cluster then can be divided into four sub-clusters i.e. sub-cluster C, D, E and F. Sub-cluster C consist of two species have pollen type V (*S. axilliflora* and *H. alternata*) with a similarity coefficient of 0.70. Sub-cluster D only consists of one species that has pollen type VI namely *H. blumeana* and is closely related to sub-cluster C with a similarity coefficient of 0.60. A total of three species supported sub-cluster E have pollen type IV (*S. backeri, S. cernua* and *S. parabolica*) with a similarity coefficient of 0.85. Sub-cluster F only supported by one species has pollen type VII (*S. pateriformis*) and is closely related to sub-cluster E with a similarity coefficient of 0.73.

Discussion

Pollen morphological characters variation

In the present study, the pollen morphological characters (both qualitative and quantitative) of 32 species of *Strobilanthes* s.l. from Sumatra were observed. This study determines various quantitative pollen morphological characters such as polar axis length (P), equatorial diameter (E), polar axis length (P): equatorial diameter (E) ratio (P/E), exine thickness and a number of longitudinal ribs. Then, the observed qualitative pollen morphological characters, including pollen shape, pollen size, exine ornamentation, presence of spines, and presence of septate ribs/flanges.

In general, there was a great variation in the majority of pollen morphological characters (both qualitative and quantitative) among species of *Strobilanthes* s.l. The analysis of variance results showed highly significant differences among species of *Strobilanthes* s.l. for all of the tested quantitative characters. Similarly, observation of the qualitative pollen morphological characters also showed a great variability occurs among species of *Strobilanthes* s.l. These findings also indicate that these characters could be employed to distinguish all of the tested taxa (Osawaru et al. 2013).

The presence of pollen morphological characters variation among species of *Strobilanthes* s.l. in this study was also confirmed by PC analysis. PC analysis was used to determine the highly loaded pollen morphological characters that can be considered to delimit all of the investigated taxa. PC analysis showed that the first three PC accounted for about 79.75 % of the total variation which indicated a very strong correlation among the characters being studied (Tuhina-Khatun et al. 2015). The PC1 was dominated by highly loaded characters such as polar axis length, ratio P/E, number of longitudinal ribs, pollen shape, pollen size and presence of septate ribs. In the PC2, some characters showed highly loaded characters such as polar axis length, equatorial diameter, ratio P/E, exine thickness, pollen shape and pollen size. The PC3 showed polar axis length, ratio P/E, pollen shape, tectal ornamentation, and the presence of spines as highly loaded characters. These first components are defined as the important components, since it accounts for the greatest part of the total variation found in the original data (Arriel 2007). Thus, this method can be used to estimate the level of characters variation within and among species. Therefore, through PC analysis, it becomes clear which characters cause the discrimination among taxa and can be useful to distinguish species (Al-Ghamadi et al. 2013).

In this study, PC analysis revealed that pollen morphological characters are important for differentiating taxa, especially at the species level, although they are not working well yet at the genera level. Pollen morphological characters such as polar axis length, equatorial diameter, ratio P/E, exine thickness and the number of longitudinal ribs are important descriptors that can be used for distinguishing and identification of species in this study as reported by earlier workers in the genus *Strobilanthes* (Carine and Scotland 1998; Wang and Blackmore 2003) or other members of Acanthaceae such as genus *Justicia* L. (Rueangsawang et al. 2013), *Barleria* L. (Al-Hakimi et al. 2018), *Rhinacanthus* Nees and *Hypoestes* Sol. (Al-Hakimi et al. 2015).

Pollen size and pollen shape also can further give information concerning species identification (Krachai and Pornpongrungrueng 2015; House 2016; Noedoost et al. 2021). The study noticed that pollen size and shape were the characters that helped to distinguish the taxa at the species level (Akhila and Beevy 2015). Based on pollen shape, most species of *Strobilanthes* s.l. have euprolate pollen (P/E 1.34-1.99), only a few species of *Strobilanthes* s.l. have perprolate (P/E $\geq$ 2.00), subprolate (P/E 1.15-1.33), prolate spheroidal (P/E 1.01-1.14), and oblate spherodial (P/E 0.88-0.99) pollen grains. Perprolate and euprolate pollen usually can be considered the ellipsoidal pollen grains whereas the subprolate, prolate spheroidal, and oblate spheroidal pollen grains are usually classified as the subspheroidal pollen grains (Walker and Doyle 1975). Thus, the species of *Strobilanthes* s.l. can be divided into two major groups based on their pollen shape, namely the ellipsoid and subspheroidal pollen groups. The pollen size can also be used to distinguish species of *Strobilanthes* s.l. Based on pollen size, most of the investigated species have large-sized pollen grains (50-99 µm long), only a few species have medium-sized pollen grains (25-49 µm long) or very large-sized pollen grains (100-199 µm).

Pollen morphological characters correlated with pollen sculpture, such as exine ornamentation, presence of spines, and presence of septate ribs can be used as good diagnostic features for taxonomic studies (Wang and Blackmore 2003; Rueangsawang et al. 2013; House 2016). Pollen grains are identified by prominent morphological features represented in their exine, and make them as important criteria for characters comparisons in the flowering plants (Tripathi et al. 2017). Exine ornamentation is an important taxonomical feature for determining species. However, this feature is often highly characteristic, consistent within taxa (Raza et al. 2020) and helps to distinguish the taxa at the species level (Akhila and Beevy 2015). The observation of pollen sculpture (especially exine ornamentation) using SEM made it possible to determine which types of apertural membrane were found in the pollen grains that would otherwise be difficult to identify using LM. Therefore, the exine ornamentation is one of the most powerful features for evaluating the diversity of pollen morphology (Akhila and Beevy 2015; Silva et al. 2017). In this study, exine ornamentation can be used for discrimination features among species of *Strobilanthes* s.l. The majority species of *Strobilanthes* s.l. have bireticulate ornamentation, only a few species have reticulate, echinulate and caruncülate ornamentation.

The results of PC analysis also confirmed the findings of cluster analysis. PC analysis based on pollen morphological characters clearly separated 32 species of *Strobilanthes* s.l. into six groups (Figure 3). Plotting of the species of *Strobilanthes* s.l. based PC analysis showed that some pollen morphological characters such as pollen shape, ratio P/E, polar axis length, number of longitudinal ribs, and presence of septate ribs grouped some species of *Strobilanthes* s.l. into Group A and B. On the other hand, the others pollen morphological features such as equatorial diameter, exine thickness, exine ornamentation, and presence of spines grouped the other species of *Strobilanthes* s.l. into Groups C-F. A dendrogram of similarity was generated by the UPGMA procedure through cluster analysis also revealed a similar topology and cluster membership with PC analysis results (Figure 4). Hence, UPGMA gives insight into the degree of similarity among all of the tested species and whether they clustered and gives an indication of the level of variation within and among species (Al-Ghamadi et al. 2013). Thus, PC analysis helps us to understand how the taxa of similar categories group together compared to dissimilar ones. Therefore, the results of PC analysis in this study can be used to verify the result of cluster analysis. PC analysis thereby confirmed the accuracy of the constructed dendrogram obtained from cluster analysis. These results also indicate that the data on pollen morphology obtained from this study were accurate, precise, and reliable (Tuhina-Khatun et al. 2015; Muzzazinah et al. 2021).

Taxonomic implications

In the present study, the pollen types do not always correspond with the infrageneric classification of *Strobilanthes* s.l. Members of a different genus may have the same pollen type. For example, pollen type II has ellipsoid, reticulate, septate ribs pollen grains, consisting of four species, such as *S. palawanensis, Se. crispa, H. serpens* and *Sm. benculensis*. In this case, these four genera have the same type of pollen. Similarly, species belonging to pollen type V are distributed in two different genus, i.e. *S. axilliflora* and *H. alternata*. On the contrary, pollen type I has ellipsoid, bireticulate ornamentation, septate ribs pollen grains, consisting of 21 species only distributed in genus *Strobilanthes* such as *S. alboviridis, S. atropurpurea, S. barisanensis, S. bibracteata, S. bunnemeyeri, S. capillipes, S. cusia, S. echinata, S. hamiltoniana, S. hossei, S. inflata, S. involucrata, S. multiflora, S. ovatifolia, S. pedunculosa, S. polybotrya, S. pubescens, S. speciosa, S. sumatrana*, and *S. violascens*. This result showed that pollen morphology could not be used consistently to differentiate at the genus level. However, to some extent, species of the same genus, if present within the same pollen type, can still be distinguished based on pollen morphological characters (Qaiser and Perveen 2019). Therefore, pollen morphology has been widely used in species delimitation of *Strobilanthes* (Chen et al. 2019).

The relationship dendrogram of *Strobilanthes* s.l. based on pollen morphology in this study also showed that *Strobilanthes* nested together with the allied genera in the tribe subtribe *Strobilanthineae* (*Hemigraphis, Sericocalyx* and *Semnostachya*). This finding indicates that these genera are closely related and not easily distinguishable. The results of this study also support earlier works through molecular studies (Moylan et al. 2004), statistical analysis (Carine and Scotland 2002), gross morphology observations (Terao 1983; Wood 1994) and leaf anatomy studies (Suratman et al. 2022) that the majority of groups into which the subtribe *Strobilanthinae* was divided by Bremekamp (1944) could not be easily split into smaller component genera (Deng 2019). Thus, the separation of this subtribe into segregate genera (eg *Hemigraphis, Sericocalyx, Strobilanthes*, etc) however should be neglected (Suratman et al. 2022). As a consequence of these findings, the only practical solution to this taxonomy problem seems to be to place all segregate genera of the subtribe *Strobilanthinae* under an expanded, single and well-defined genus as *Strobilanthes* s.l. within the tribe Ruelliinae as proposed by Terao (1983) then followed by many authors (Scotland and Vollesen 2000; Tripp et al. 2013; Souladeth et al. 2017; Wood 2014; Augustine 2018; Deng 2019; Chen et al. 2020; Wood and Scotland 2021; Wood et al. 2022).

In conclusion, this study revealed that pollen morphology was taxonomically useful character for identification and taxa delimitation of *Strobilanthes* s.l., especially at the species level. However, pollen morphological characters are not working well yet at the genera level because of the separation of *Strobilanthes* s.l. into smaller component genera can not easily conduct in this taxa (Deng 2019). Observation of pollen morphology of *Strobilanthes* s.l. based on LM and SEM in this study could make significant contributions toward a better understanding of the relationships and systematics of

Acanthaceae (Rueangsawang et al. 2013; Al-Hakimi et al. 2017).

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