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Variations in the Indonesian *Sauromatum horsfieldii* Miq. (Araceae) Based on Characteristics of Morphology, Anatomy and Cytology

(Variasi *Sauromatum horsfieldii* Miq. (Araceae) dari Indonesia Berdasarkan Ciri Morfologi, Anatomi dan Sitologi)

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ABSTRACT

Sauromatum horfieldii Miq. (Araceae) is distributed from India, China to South East Asia. In Indonesia, the species only occurs in Sumatera, Jawa, and Bali. This study investigated the morphological, anatomical, and cytological diversity of three populations of the species, one from each of these Indonesian islands. The results showed that there was variation in morphology of the petiole, number of leaflets and leaf margin. The stomatal complex consisted of elliptical pores with reniform guard cells and two or three subsidiary cells. The Indonesian plants of *S. horsfieldii* possessed two stomatal types, namely anomocytic and anisocytic, both with abaxial distribution (hypostomatic). Measurements on each population showed average stomatal indices (SI) of 10.01, 10.02 and 14.55, average stomatal lengths of 34.6, 33.8 and 29.50 µm, and average stomatal widths of 20.8, 21.1 and 16.50 µm for Sumatera, Jawa, and Bali, respectively. The epidermal cells are mostly irregular and somewhat undulate. The chromosome number for all the accessions was 2n = 26.

Keywords: Anatomy; cytology; Indonesia; morphology; *Sauromatum horsfieldii*

ABSTRAK

Taburan *Sauromatum horfieldii* Miq. (Araceae) meliputi India, China hingga ke Asia Tenggara. Di Indonesia, spesies ini hanya dijumpai di Sumatera, Jawa dan Bali. Penyelidikan ini mengkaji variasi morfologi, anatomi dan sitologi spesies ini daripada tiga populasi di Indonesia (Sumatera, Jawa dan Bali). Hasil kajian menunjukkan terdapat variasi morfologi pada petiol, bilangan anak daun dan tepi daun bervariasi. Kompleks stomata terdiri daripada liang elips dengan sel penjaga berbentuk ginjal dan dua atau tiga sel tetangga. *S. horsfieldii* dari Indonesia mempunyai dua jenis stomata, iaitu anomosit dan anisosit yang tersebar di permukaan bawah daun (hipostoma). Ukuran pada setiap populasi menunjukkan indeks stomata purata bernilai 10.01, 10.02 dan 14.55, panjang stomata purata bersaiz 34.6, 33.8 dan 29.50 µm, lebar stomata purata bersaiz 20.8, 21.1 dan 16.50 µm masing-masing bagi sampel Sumatera, Jawa dan Bali. Sebahagian besar sel epidermis didapati tidak teratur dan agak beralun. Bilangan kromosom bagi semua aksesi adalah 2n = 26.

Kata kunci: Anatomi; Indonesia; morfologi; *Sauromatum horsfieldii*; sitologi

INTRODUCTION

The genus *Sauromatum* (Araceae) is recognised as a distinct genus from *Typhonium* (Cusimano et al. 2010), consisting of nine or ten species (Boyce & Croat 2020; Odyuo et al. 2015; WFO 2021). Two species of Sauromatum are found in Indonesia, i.e., *S. brevipilosum* (Hett. & Sizemore) Cusimano & Hett. and *S. horsfieldii* Miq. The former is endemic to Sumatera, whereas the latter is a widespread species that is native to China (South-Central and Southeast China), Myanmar, Laos, Vietnam, Cambodia, Thailand, and Indonesia (Cusimano et al. 2010; Haigh et al. 2011; Hetterscheid & Boyce 2000). Within the Indonesian archipelago, the species is only found in Sumatera, Jawa and Bali.

Sauromatum horsfieldii was first published by Miquel in 1856, based on materials collected by Thomas

Horsfield from Oengaran (old spelling of Ungaran) in Jawa Tengah in 1802. This holotype (Figure 1) is lodged at the Herbarium of the Royal Botanic Gardens, Kew (K). *Sauromatum horsfieldii* is rare in Indonesia, although sometimes found locally abundant (Backer & van den Brink 1968; as *Typhonium horsfieldii*). This rarity means that previous attempts to locate populations in the field have been largely unsuccessful. Recent fieldworks conducted in Lampung Province of Sumatera (in 2017), Jawa Barat (2018) and Bali (2019) found some individuals of *S. horsfieldii* mostly in highland areas. The aim of this study was to evaluate the morphological, anatomical, and cytological characters of these Indonesian populations of *S. horsfieldii* to clarify the circumscription of the species.

Source: http://plants.jstor.org/stable/10.5555/al.ap.specimen.k000099894

FIGURE 1. Holotype of *S. horfieldii* lodged at the Royal Botanic Gardens, Kew (K), K000099894

Materials and Methods

Plant materials

Plant materials were collected from Lampung Barat Regency in Sumatera (Regist 48B Palakiah Protected Forest and Regist 9B Lumbok Seminung Protected Forest), Cianjur Regency in Jawa Barat (Pasir Sarongge Village, Pacet District), and Bedugul in Bali. The first two was collected from the natural habitats, whereas the last was sampled from cultivated plants at Kebun Raya Eka Karya Bali (KREK). *Sauromatum horsfieldii* specimens at KREK were originally collected from Bukit Tapak Nature Reserve which is located adjacent to the garden. All the specimens were collected from highland regions, at an elevation of 850-1,500 m. All plants used in this study were subsequently cultivated at Kebun Raya Bogor (KRB) in Jawa Barat, Indonesia, and each population contained ten specimens.

LEAF PARADERMAL ANATOMY

STOMATA

An impression method was employed to observe stomata using clear nail polish. Leaf samples were collected between 0800 and 0900 h. A thin layer of nail polish was spread on both leaf surfaces between the primary veins and left to dry. A clear adhesive tape was applied and pressed down firmly over the dry nail polish. The tape was then gently peeled from the leaf surface, together with the layer of nail polish with the adhering leaf impression. Subsequently, the tape with leaf impression was examined under a trinocular microscope (Olympus CX31), at 40×10 magnification. The type and number of stomata were recorded, and stomatal index was calculated. The definition of stomata types followed van Cotthem (1970). Stomatal index (SI) was calculated based on Salisbury (1928), as follows:

$$SI = \frac{S}{S + E} \times 100$$

where SI is the Stomatal Index; S is the number of stomata per unit area, and E is the number of epidermal cells in the same area.

Stomatal Density (SD) was calculated based on Stace (1965), as follows:

$$SD = \frac{\Sigma \text{ Stomata}}{\text{Unit Area (mm}^2\text{)}}$$

LEAF CROSS-SECTION

Preparation of samples was conducted following Sass (1951). Leaf sections were fixed in FAA, and dehydrated by alcohol at different concentrations from 70% to 96%. The dehydrated samples were then dipped into solutions of mixed alcohol-xylol and pure xylol. The samples were cut using rotary microtome Leica RM 125RT. Infiltration was done using liquid paraffin in an incubator at 65 ºC, and dyes used were safranin 1% and fast green 2%.

CYTOLOGY

Squash method (Manton 1950) modified by Darnaedi (1991) was applied for somatic chromosome observation. In this study, chromosome number was observed from root tips. The roots were collected in the morning from 0900 to 1000 h, then washed and cleaned. The root tips were cut at 1 cm long, put in small bottles containing 0.002 M 8-hydroxyquinolin, and then stored at 20 ºC for 24 h. Subsequently, 45% acetate acid was used for fixation (for 10 min). The roots were then removed from the acetate acid liquid, and put in substrate of 1N hydrochloric acid HCl: 45% acetic acid CH_3COOH (3:1), at 60 ºC for 2-2.5 min. After the incubation, the roots were stained with 2% acetic orcein to intensify the staining of mitotic cells. Subsequently, sections of the root tip (1-2 mm long) were stained by a drop of 2% orcein, then covered with a cover glass and gently squashed. The glass slide was then heated slightly over a Bunsen burner. The chromosome number was counted under an Olympus CX31 microscope at 100×10 magnification.

RESULTS AND DISCUSSION

Inflorescence features are useful taxonomic character for defining species in Araceae. The habit of *S. horsfieldii* was somewhat different among specimens from Sumatera, Jawa and Bali (Figure 2) in their life forms, leaf blade features and petioles. The inflorescences, however, were not significantly different among the three populations. A comparison of the inflorescences of this species from the three different Indonesian localities proved to be relatively invariant (Figure 3). Although this information confirmed the species identification of these specimens, they differ slightly in the length of the male zone, staminodes direction and the tip of the appendix.

The habit of the examined *S. hosfieldii* showed morphological variation among specimens from the three different islands, which may mislead the identification of the plants. Specimens from Sumatera formed clusters consisting of more than three petioles which were short, rather tough and green. Specimens from Jawa did not form clusters, instead these plants consisted of 1 or 2 petioles which were long, flexible and reddish. Interestingly, specimens from Bali were somewhat intermediate, having relatively long, tough petiole, and green with red basally.

Differentiation was also found in some characters of the leaf blade. Specimens from Sumatera had a smooth, shiny leaf surface with margin crenate, whereas the leaves of those from Jawa were somewhat velvety with margin entire, and specimens from Bali had leaves that were densely velvet with margin either entire or crenate. These differences of morphological variation were probably caused by genetic flexibility which is often found in the family. Liu, Zheng and Qi (2020) reported that plants show phenotypic and anatomical plasticity strategies under various environmental conditions to optimize resource utilization. Moreover, Guo et al. (2017) stated that most of plants, within species, react to climate changes by modifying their leaf traits such as leaf thickness in shrubs.

FIGURE 2. The habit of cultivated plants of *S. horsfieldii*. A. specimen from Sumatera (bar = 5 cm), B. specimen from Jawa (bar = 5 cm) and C. specimen from Bali (bar = 3 cm)

LEAF PARADERMAL ANATOMY

STOMATA

The Balinese specimens differed from the other two populations in stomatal characteristics, that is, number, size, SI and SD. The average number of stomata from Bali specimens was higher compared to that of Sumatera and Jawa specimens, but smaller in the average length and width (Table 1). The SI and SD were significantly greater for Balinese specimens than for those from Sumatera and Jawa (Table 2). However, the epidermal and palisade cells were not significantly different (Table 3 & Figure 4).

In addition, variations in leaf anatomy among the three Indonesian populations of *S. horsfieldii* included the number and type of stomata and epidermal cells. The stomata of *S. horsfieldii* were hypostomatic, occurring only on the abaxial surface. Based on the form of stomata, this species showed anomocytic and anisocytic types (Figure 4). Anomocytic type was characterized by stoma surrounded by a limited number of cells that were of the same size or form, whereas anisocytic type was classified as stoma surrounded by three cells, one of which is distinctly smaller in size than the others (van Cotthem 1970).

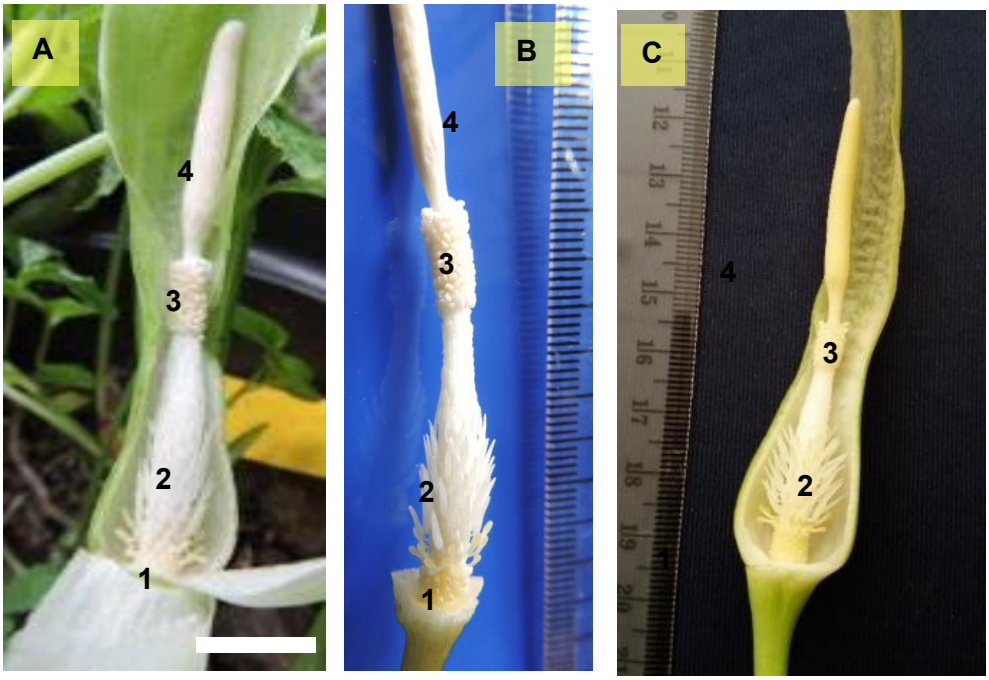

FIGURE 3. Inflorescences of Indonesian S. horsfieldii. A. specimen form Sumatera (bar = 5 cm), B. specimen from Jawa and C. specimen from Bali. Spathe cut/removed showing the female (1), staminodes (2), male (3) and appendix (4) zonations

It is interesting to note that anisocytic stomata type was found in both specimens from Jawa and Bali but not from Sumatera. Moreover, more anisocytic stomata were found in Bali specimens than Jawa specimens (Figure 5). This phenomenon was probably influenced by environmental factors, such as temperature, light intensity and humidity. Liu, Zheng and Qi (2020) stated that leaf anatomy characters are key roles in plant functions in adaptive evolutionary to the circumstance of environment changes.

The Bali specimens showed the highest number of stomata (14.93), but smaller stomata size (length = 29.50 µm, width = 16.50 µm), compared to the other two specimens (Table 1). According to Izza and Laily (2015), the total number, density and type of stomata, and the numbers of open and closed stomata were influenced by several factors, such as altitude, temperature, humidity, light intensity and CO_2 availability. Among the three localities, specimens from Bali grew at the highest elevations (1250–1500 m asl), whereas the other two were found at below 1200 m asl. Almost all specimens were found growing in shaded areas. Consequently, the stomata responded to the limitations of existing environmental factors that were increased air humidity, lower air temperature and decreased CO_2 availability.

Field observations found that most of the plants received low light intensity in shaded habitats. Haryanti (2010) stated that shade-tolerant plants show a higher photosynthesis rate when grown in places with low-intensity light. They are adaptable to use low light to maximize photosynthesis. In addition, Hetherington and Woordward (2003) reported that photosynthesis has a close relation to stomata in that CO_2 diffusion into leaf lamina during the photosynthesis process is controlled by the opening and closing of stomata. They also reported that process of opening and closure of stomata is influenced by light intensity.

Higher sunlight intensity results in a greater number of stomata but lower size, whereas lower sunlight intensity results in a smaller number of stomata but bigger size (Ruban 2009). Lower sunlight intensity also causes slower photosynthesis process as an effect of slow CO_2 diffusion that causes stomata to close (Leopold & Kriedemann 1975; Mahanani et al. 2020; Ruban 2009). The process of formation and division of prospective stomata cells is probably inhibited in such shaded habitats so that a smaller number of stomata is

formed. Furthermore, Listia et al. (2019) stated that the sunlight intensity, air temperature and pressure in highlands are lower compared to those of the lowlands. This causes plants to respond by reducing the size of stomata.

The Bali specimens showed the highest SI and SD (Table 2). The SI indicates the level of SD. Sundari and Atmajaya (2011) stated that SD is influenced by environmental variables, such as water availability, light intensity, temperature and CO_2 concentration. The exchange of CO_2 and O_2 generally occurs in the stomata. Meanwhile, SD controls the exchange of CO_2 and water vapour between the leaf and the atmosphere (Ogaya, Llorens & Peñuelas 2011). When CO_2 is limited and light intensity is low, plants will increase the SD to fulfill the requirement of CO_2 to increase the photosynthesis rate, as appeared to happen in the Balinese plants of *S. horfieldii*. According to Izza and Laily (2015), SD is closely related to the number of stomata, in which a larger number of stomata will correspond with a higher density.

TABLE 1. Stomatal numbers and sizes of Indonesian *S. horsfieldii* on abaxial epidermis

Specimen	Number of stomata		Stomata size (length)		Stomata size (width)	
	Range/ unit area	Average/ unit area	Range (µm)	Average (µm)	Range (µm)	Average (µm)
Sumatera	3-9	6.07	23.88-47.22	34.80	11.31-38.94	20.57
Jawa	4-10	7.10	19.66-50.89	33.80	12.48-29.45	21.10
Bali	12-20	14.93	15.55-43.48	29.50	7.70-25.75	16.50

TABLE 2. Stomatal index and density of Indonesian *S. horsfieldii* on abaxial epidermis

Specimen	Stomatal Index (SI)		Stomata Density (SD)	
	Range SI	Average SI	Range SD (mm^2)	Average (mm^2)
Sumatera	4.55-13.24	10.01	39.06-117.19	78.99
Jawa	5.80-12.70	10.02	65.10-130.21	92.45
Bali	10.91-18.87	14.55	166.25-247.40	199.44

TABLE 3. Epidermal cells and palisade of Indonesian *S. horsfieldii*

Specimen	Adaxial epidermal cell length (µm)		Adaxial epidermal cell width (µm)		Palisade length (µm)	
	Range	Average	Range	Average	Range	Average
Sumatera	5.86-16.11	11.03	4.17-8.72	5.63	6.11-19.72	12.58
Jawa	6.95-14.17	9.75	2.50-7.14	3.78	6.44-14.81	11.78
Bali	10.29-16.54	13.03	6.07-10.50	8.36	7.29-14.01	11,09

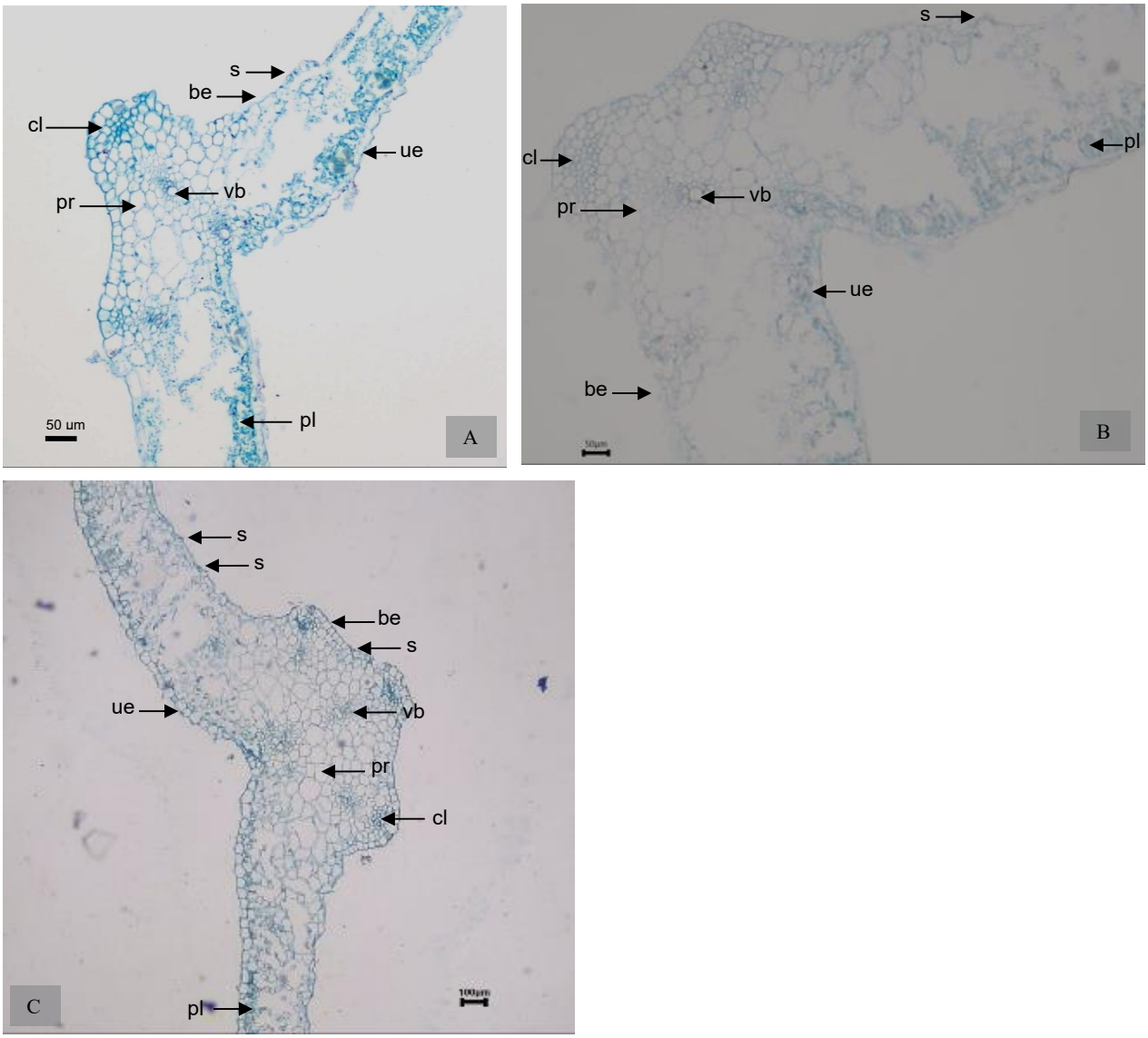

FIGURE 4. Cross sections of Indonesian *S. horsfieldii* leaf. A. Sumateran specimen (20× magnification), B. Jawa specimen (40× magification) and C. Bali specimen (4× magnification). be = below (abaxial) epidermis, ue = upper (adaxial) epidermis, vb = vascular bundle, pl = palisade arrangement, pr = parenchyma, cl = collenchyma, s = stomata

LEAF CROSS-SECTION

Leaf epidermal characters, including stomata and epidermal cells, are of taxonomic significance in the Araceae family (Zade 2016). Epidermal cells of Indonesian *S. horsfieldii* were rounded to elongated, irregularly elongated, or elongated sinuous (Figures 4 & 5). However, Zade (2016) reported other forms of epidermal cells from another species *S. venosum* (Dryand. ex Ait.) Kunth [as *S. pedatum* (Link & Otto) Schott] which have isodiametric and polygonal epidermal cells with irregularly wavy or slightly undulate cell walls. Nevertheless, what induces this shape during development is unknown because the explanation given by the existing hypothesis is regarded as insufficient (von Sengbusch 2019). Furthermore, von Sengbusch (2019) mentioned that elongated epidermal cells can be found on leaf veins or lamina of monocotyledons. Panawala (2017) stated that epidermal cells are tightly

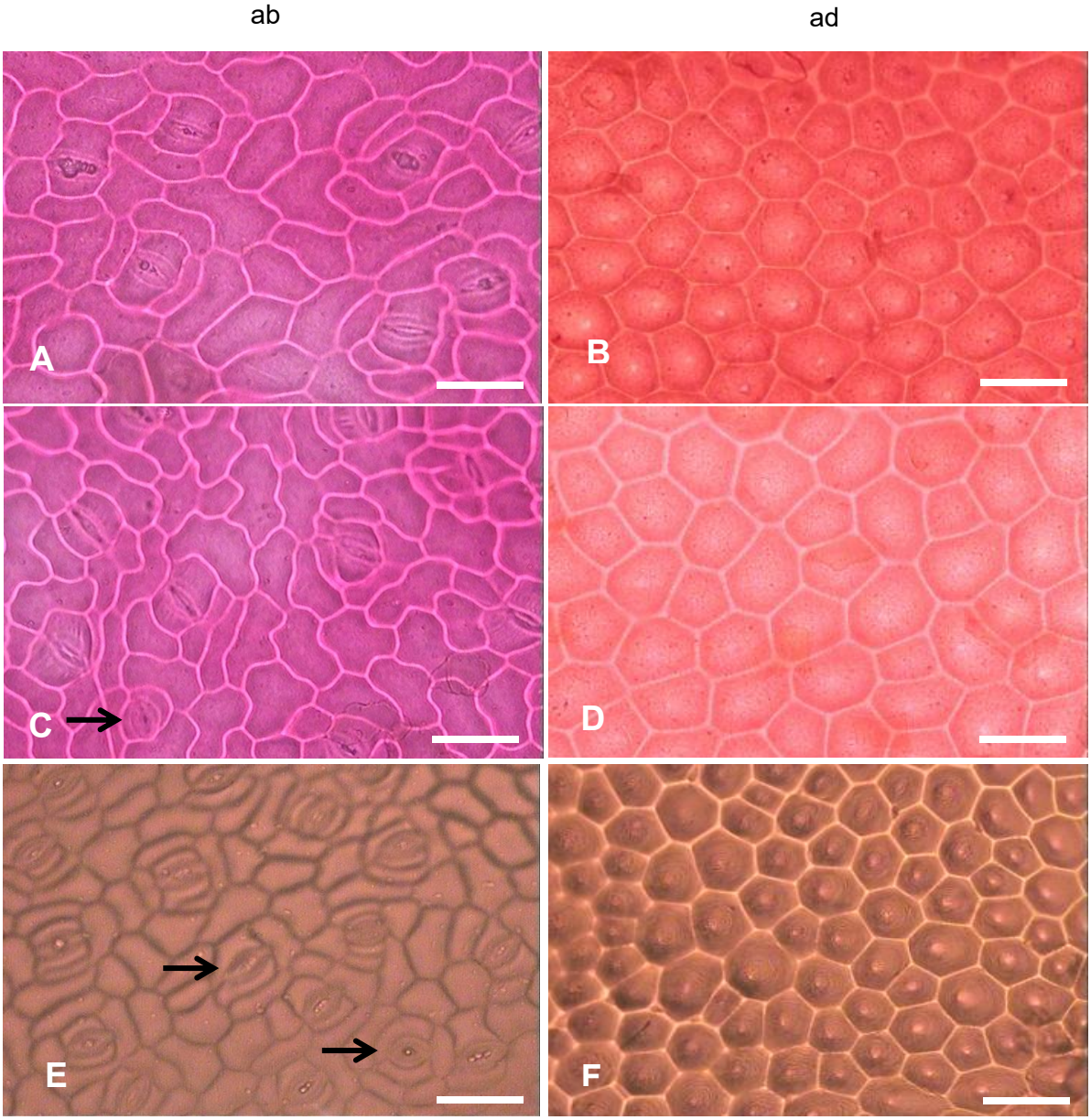

FIGURE 5. *Sauromatum horsfieldii* produced hypostomatic leaf where stomata occurs only abaxially (left/ab) and none adaxially (right/ad). Sumateran specimen (A-B): anomocytic stomata, rounded to elongated epidermal cells. Jawa specimen (C-D): anomocytic and anisocytic stomata (arrows), irregularly elongated epidermal cells. Bali specimen (E-F): anomocytic and anisocytic stomata (arrows), elongated sinouos epidermal cells. Bars = 50 µm with 400× magnification

bound to each other and provide mechanical support for the plant. This study demonstrated that the length and width of the epidermal cells and the length of the palisade cells of *S. horsfieldii* from the three different localities were not significantly different (Table 3).

CYTOLOGY

The chromosome number of all specimens was consistently 2n = 26 (Figure 6), confirming the conclusion of Cusimano et al. (2010). The chromosomes of the Jawanese specimens were the longest, but this

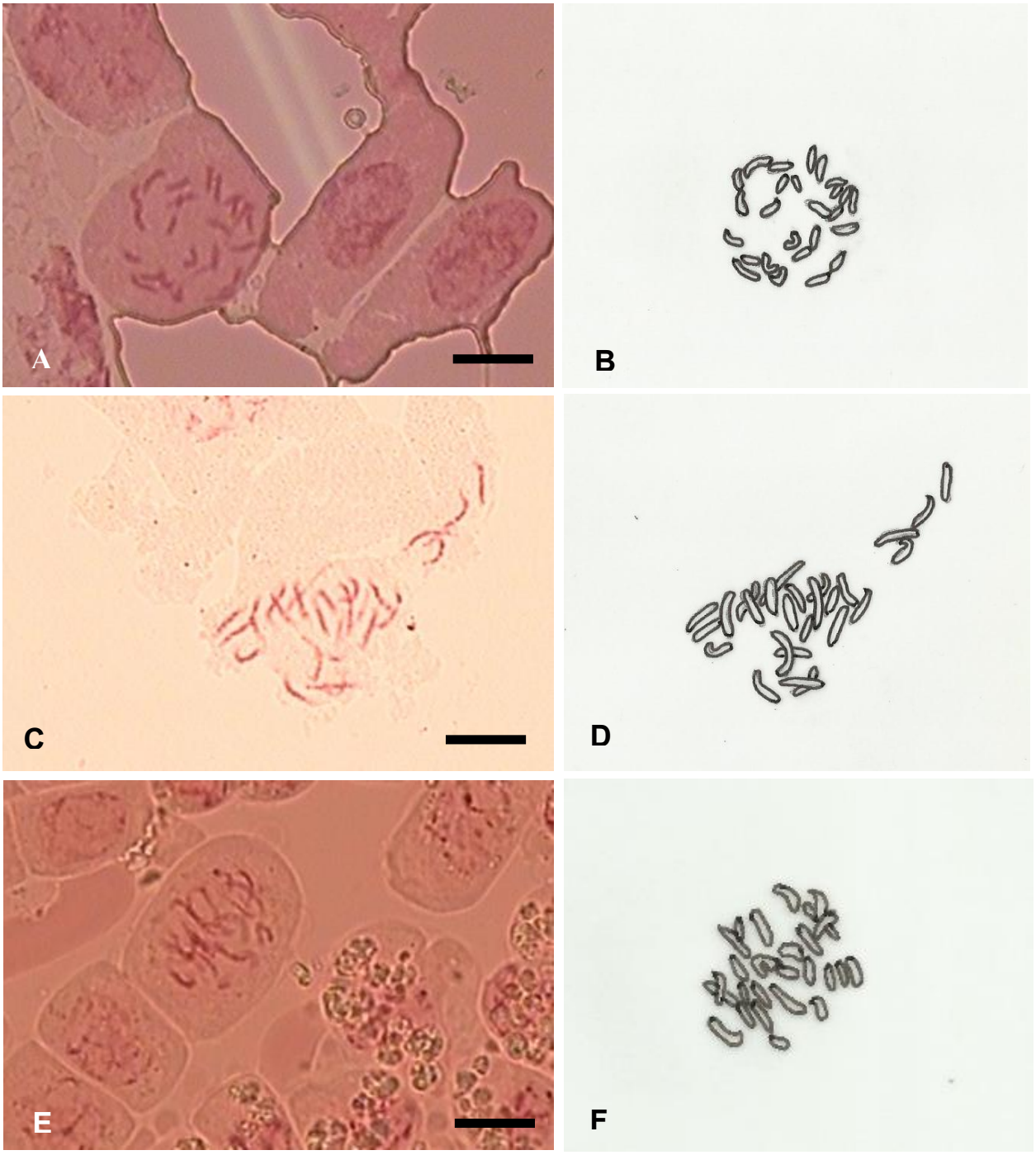

FIGURE 6. Chromosomes of *S. horsfieldii* with 100× magnification. Left images (A, E and E) are the chromosomes of Sumatera, Jawa and Bali specimens, respectively; Right images (B, D and F) are line drawings of the chromosomes of Sumatera, Jawa and Bali specimens, respectively. All samples showed the same number of chromosomes, 2n = 26. Bars = 0.01 mm

was probably an artifact caused by different fixation periods, in which longer fixation results in shorter chromosomes (Dr. Titien Ng. Praptosuwiryo pers. comm.).

DESCRIPTION OF INDONESIAN *Sauromatum horsfieldii* MIQ

Synonyms:
Heterostalis pedate (Schott) Schott, Ann. Mus. Bot. Lugduno-Batavum 1 (1864) 278; *Typhonium fallax* N.E.Br, J. Linn. Soc. Bot. 18 (1880) 260; *Typhonium horsfieldii* (Miq.) Steenis, Bull. Jard. Bot. Buitenzorg III, 17 (1948) 403.

Herb, with depressed globose, sometimes oblong tuber. *Petiole* pale green to reddish brown, or greenish to dark green, flexing to erect, 7-33.5 cm long. *Leaves* compound, pedately 3-11-foliolate. Leaflets arranged in half circle, elliptic to narrowly elliptic, yellowish green to dark green adaxially, pale green abaxially, leaflets with surface smooth, shiny, sparsely to densely velvet, 3-12 cm long, 1-3.3 cm wide, margin entire, slightly undulate to crenate, with apex acute to acuminate. *Rachis* 0.5-1.5 cm long, middle leaflet sessile. *Midrib* sunken adaxially, prominent abaxially; *primary veins* 3-10 on each side, sunken adaxially, slightly above or flush with lamina abaxially; *inter-marginal veins* conspicuous, 0.3-0.5 cm from margin. *Inflorescence* solitary. *Peduncle* as petiole in colour, 6-10 cm long. *Spathe* 9-11 cm long, slightly constricted at the sterile zone. *Lower spathe* ovate, 3-3.5 cm long, yellowish-green or mid-green with or without several prominent stripes outside, whitish green inside. *Limb* boat-shaped with apex acuminate, paper-like, 6-7.5 cm long, abaxial greenish, adaxial pale green to whitish. *Spadix* sessile, shorter than spathe, 6-7 cm long. *Female zone* cylindrical, 0.2-0.4 cm long, 0.3-0.5 cm in diameter. *Ovary* ovoid, pale yellow; stigma sessile, button-like. *Sterile zone* narrowly conic, 2.2-2.4 cm long, 0.4-0.6 cm in diameter at base, entirely covered by staminodes, proximal half clavate, bend upward, sub-basal staminodes linear, pointed upwards, white, c. 0.4 cm long, abruptly shorter, and then naked toward male zone. *Male zone* cylindrical, 0.8-1 cm long, c. 0.3 cm in diameter. *Male flowers* creamy, sub-clavate to spathulate. *Appendix* sub-sessile or stipitate, creamy, somewhat clavate or slightly fusiform, 2.2-3.5 cm long, slightly tapering to obtuse apex, smooth.

CONCLUSION

The number of chromosomes of *S. horsfieldii* was 2n = 26. The stomatal distribution of this species was hypostomatic and consisted of both anomocytic stomata (found in all specimens) and anisocytic (only seen in plants from Jawa and Bali). Based on observations, the specimens from the three localities of Sumatera, Jawa and Bali differed in the number, length and width of stomata. Specimens from Bali showed the highest SI and SD. The stomatal variation in Indonesian *S. horsfieldii* is presumed as having arisen from environmental factors such as altitude, temperature, humidity, light intensity and CO_2 availability.

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