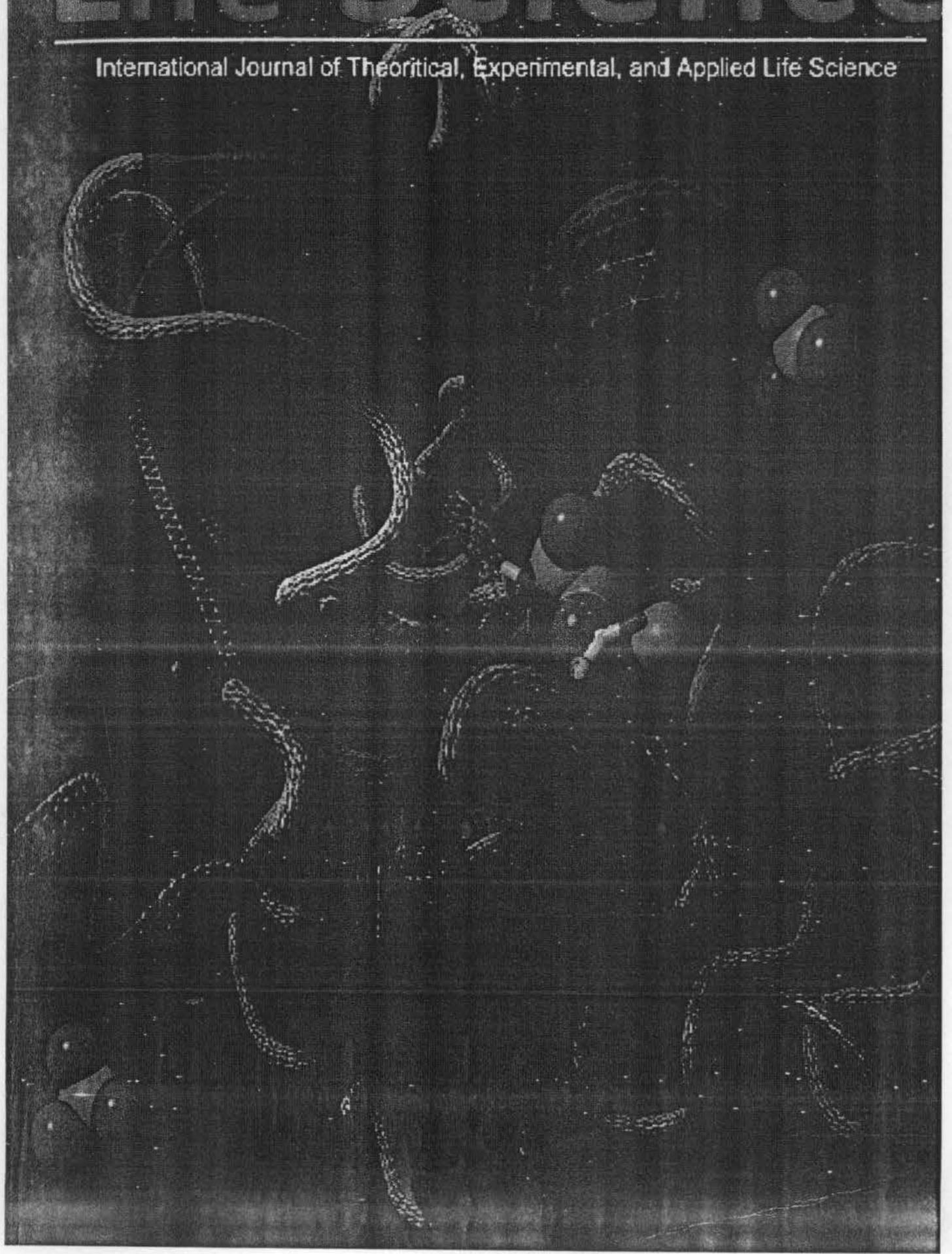


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## Anatomical and Cytological Features of *Spathoglottis plicata* from Java Island

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### ABSTRACT

*Spathoglottis plicata* is a ground orchid that displays large variation in flower features. In spite of large variation on morphological characters, its anatomical and cytological characters are not well known. This paper described the leaf anatomical and chromosomal features of *S. plicata*. Leaf anatomy was observed from both transverse and paradermal sections of leaves. Chromosome numbers were observed from root tip cells. Epidermis of all *S. plicata* variants comprised of two cell shapes, the rectangle cells located above veins, while the polygonal one located in between veins. Stomatal type was brachitetracyclic, which were only found on lower epidermis. Leaf transversal section comprised of one layer of upper and lower epidermis, spongy mesophyll, and vascular tissue. The number of leaf hair varied depended on the original habitat and altitude. All variants of *S. plicata* had same chromosome number ( $2n=40$ ).

**Keywords:** anatomical, chromosome, *Spathoglottis plicata* variation

### INTRODUCTION

*Spathoglottis plicata* is an exotic ground orchid that belongs to the family Orchidaceae. This orchid is characterized by several plicate leaves arose from each pseudobulb, and tall inflorescences arose from the axil of the basal leaves. Flower has free tepals which spread widely and a trilobed lip, which is not saccate or spurred, but with two knobs near its base [1].

*S. plicata* is popular as ornamental plant because of its nice leaves and beautiful flowers. In addition, it has ability to adapt to unfavorable environment making it easy to grow and care it. This ground orchid is distributed in a wide environment, from area with hot temperature such as tropical area to cold temperature such as temperate area. In nature, it is found from 125 to 1650 m above sea level. The wider geographical distribution of plant species was correlated to the higher diversity of plant [2]. It is not surprising that *S. plicata* displays high variation in morphological characters especially its flower structure and color.

Since anatomical characters and chromosome number are valuable as well as morphological characters for plant systematic study, it is interesting to know whether the high variation in morphological character of *S. plicata* followed by high variation in anatomical and cytological characters. Previous study showed that many anatomical and cytological characters are good to be used for species determination or solving taxonomical problem. For example, the character of leaf epidermis layer could be used to distinguish among Javanese *Pandanus* species [3]. This paper described the leaf anatomical character and chromosome number of *S. plicata* from Java island.

### MATERIALS AND METHODS

Plant materials consisted of 40 populations of *S. plicata* collected from 18 locations in Java island and 2 population of *S. aurea*. Leaf anatomy were observed from both paradermal and transverse sections. The leaf paradermal section were made by soaking the leave in FAA solution for 1 to 3 months, followed by soaking it in 1 N hydroxide solution for 1 hour, boiling it in a solution of 1 N chlonic acid: 45 % acetic acid (3:1) for 15 to 20 minutes, and then skinning (scraped) it using tweezers, and put the epidermis on a slide. The section were observed under light microscope. The observed characters were mid-

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vein, protrusion of the midvein, midvein shape, the ratio of protrusion of the midvein and midvein length, midvein tip, stomatal position and shape, the arrangement of metaxylem, upper and lower epidermis cell shape, and mesophyll cell shape. Leaf transverse section were obtained by soaking the mature leave in FAA solution (Formalin: acetic acid: 70 % ethanol = 3: 2: 95) for 2 to 12 weeks, then cutting them using frozen microtome, and making a slide of each slice. Both paradermal and transverse sections were stained in 1 %, safranin and then observed under microscope using glycerin as a medium.

Chromosome number observation was done by examining the chromosome number at metaphase of mitosis of root tips using squash method modified. Several (7-10) root tips were collected. One cm length of each root tip was fixed in 0.002 M 8-hydroxyquinolin and incubated at 20 °C for 24 hour. The root tips were then moved into 45% acetic acid for 10-15 minutes, followed by soaking in a solution of 1 N chloric acid : 45 % acetic acid (3:1) at temperature 60 °C for 2-2.5 minutes. The root tip was then cut into 1-2 mm pieces, then put one piece on a slide, dropped 2 % acetoorcein on it and cover it. The glas slide containing the root tip was then tapped using pencil. Chromosome numbers were observed under light microscope [4].

## RESULTS AND DISCUSSION

### Paradermal Section

Epidermis layer of *S. plicata* leave was generally consisted of two cell types, which were 1. Rectangle cells located under and above veins with ratio length to width was 5: 1.2. Polygonal cells located in between veins with 4 to 6 angles, and the ratio length to width was 1-3: 1 (Figure 1A and 1B). Polygonal cells were arranged in good order. Stomata were found only in lower epidermis layer and distributed among polygonal cells. Stomata shape was brachitetracyclic, with 4 subsidiary cells. The length of the guard cells was parallel to the leaf length. Small hairs were found sparsely in both epidermis layers. Hairs were attached to small epidermis cells. Hair was straight, consisted of 1 to 3 cells. The cell number of hair was correlated to the hair length. The higher the cell number the longer the hair length.

### Transverse sections of leaves

The characteristic of leaf transverse section were midvein very prominent, raphide cell very sparse, lobe of epidermis cells shallow, guard cells parallel to the epidermis cells, vascular shape of midveins ovate, vascular tip of midveins

obtuse, the distribution of meta-xylem cells ditributed into Y pattem. *S. plicata* leaves were transversally differentiated into one layer of upper epidermis cells, mesophyll cells, and one layer of lower epidermis cells. The leaf surface was protected by thin cuticle with  $\pm 0.6 \mu\text{m}$  thick (Figure 1C and 1D). The diameter of epidermis cells was smaller than mesophyll cells. The lower epidermis cells was smaller than that of upper cells. Epidermis cells on the veins were also smaller that that of the other parts. The epidermis cell shape was elliptic. The mesophyll was arranged of spongy parenchyma without palisade parenchyma, and vascular bundle was distributed in it. Mesophyll cell shape was elliptic. Rhapid were sometimes found among spongy parenchyma.

All *S. plicata* variants had the similar anatomical characters, and there was no diagnostic anatomical characters that could differentiate among variants. Therefore, anatomical characters of *S. plicata* can not be used to judge whether the variant can be classified under species such as variety or subspecies. The similar result was also found in *Artemisia* [5]. They found no significant differences in the anatomy of the leaves between variety of *Artemisia absinthium* var. *absinthium* and its Polish endemic variety *A. absinthium* var. *calcigena* that have differences in color leaves. However, several previous results showed that anatomical characters were powerful for taxonomical purposed when it was used in the higher categories such as genus. Another research [6] was able to distinguish *Cedrus atlantica*, *Cedrus libani*, and *Cedrus brevifolia* using the anatomical characteristics of the needles. When we compared the anatomical character of *S. plicata* to another *Spathoglottis* such as *S. aurea*, we were able to easily distinguish between both species, especially from the characters of leaf transverse section, i.e. protrusion of the midvein, midvein shape, the ratio between protrusion of the midvein and midvein length, midveid tip, stomatal position, the arrangement of meta-xylem, the number of raphid cells (Table 1, Figure 1C, and 1D).

In orchids, leaf anatomy is often typical of the genus rather than the species and there are rather large groups of genera that share quite similar leaf structure. However, there was found a useful range of character variation, such as silica bodies. In our case, we found the number of raphid cells in *S. plicata* is less than that of *S. aurea*. Leaf surfaces may also provide information about the normal habitat in which the plants occur. Plants that grow in dry, or periodically dry conditions, such as epiphytic orchids, often have very thick cuticles. Conversely, plants that grow in moist or

humid conditions, the cuticle is often thin and smooth. Within *S. plicata* collected from the natural habitat, hair size was influenced by plant environment. Short hairs were found in plant that grew in open environment, while long hairs were found in plant that grew among crowd

bushes. Hair density varied and was influenced most by plant environment and also altitude. On the open areas, hair density was higher than that of the crowd bushes. The hair density decreased in the higher areas.

Table 1. Anatomical characters of *S. plicata* and *S. aurea*

No	Characters	<i>S. plicata</i>	<i>S. aurea</i>
1	Protrusion of the midvein	Very prominent	Less prominent
2	Vascular bundle shape of midveins	Ovate	Five angles
3	Epidermis lobes	Shallow	Deep
4	The position of stomatal guard cells to epidermis cells	Parallel	Prominent
5	Vascular bundle tip of midveins	Obtuse	Rounded
6	The widest part of midveins	Under middle	Above middle
7	Meta-xilem arrangement	Y pattern	) ( Pattern
8	Raphids cells	Sparse	Many
9	Lamina lobes	Beringgitan	Berpicisan

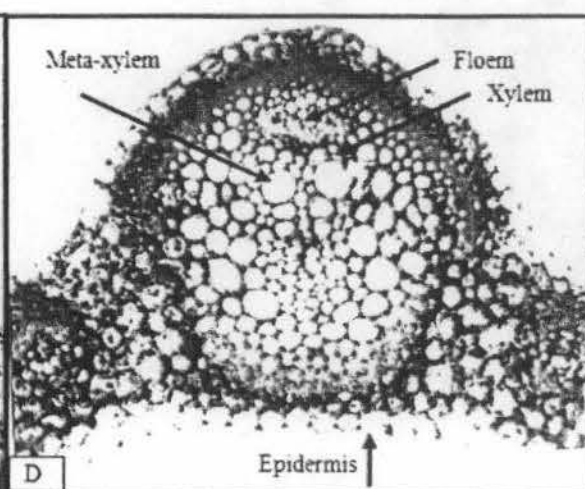
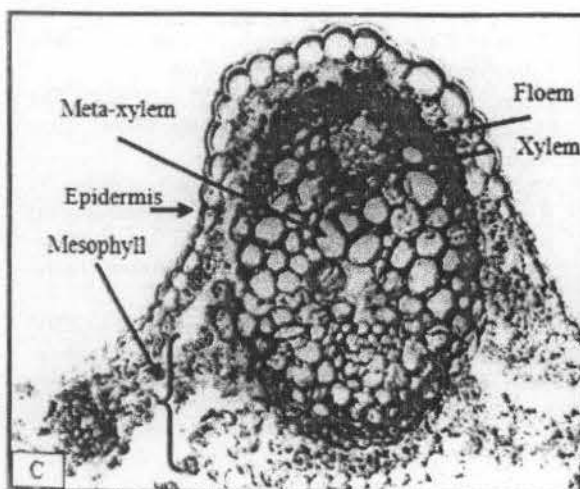
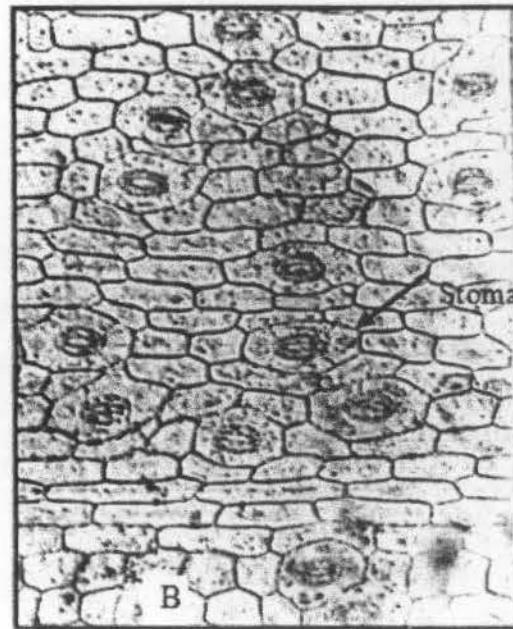
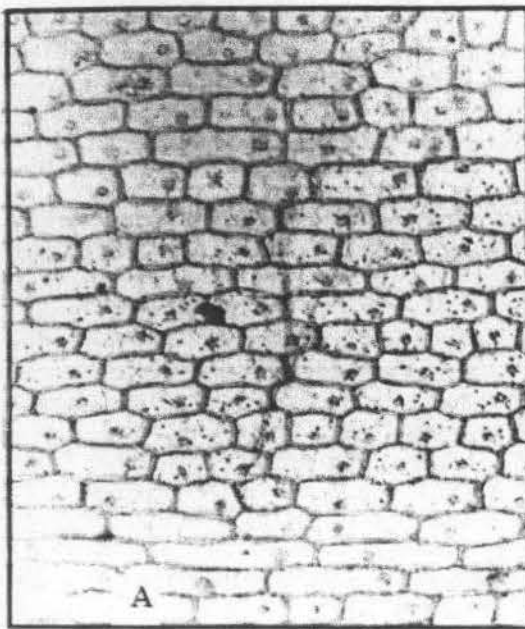


Figure 1. Leaf paradermal section upper (A) and lower (B) epidermis of *S. plicata* (40x); leaf transversal section of *S. plicata* (C) and *S. aurea* (D) showing the shape of midveins (10x)

### Chromosome number

Chromosome number is of particular interest because closely related species and genera often have the same number and hence a similar amount of genetic material in their tissues. All *S. plicata* variants have the same chromosome number which are  $2n=40$ . This same result reported by Tanaka dan Kamemoto (1974) [7]. Previous studies also showed that most orchid genera have constant chromosome number, therefore, the most prevalent form of speciation in orchids appears to be occur without any change in the number of chromosomes. The immensely vast *Dendrobium* (1500 species) has very diverse morphological variations in the vegetative and floral characters, however it has only two chromosome numbers, 38 and 40 (or their multiples) [8]. Since changes in number of chromosomes between species are not so prevalent in orchids, it is to be assumed that changes within the complement are responsible for directing the course of evolution in the family. It could be brought about by hybridisation, cryptic structural changes in the chromosomes and gene mutations [10].

In spite of the tendency towards constancy of chromosome numbers in orchids, another research recorded three species of the *Cymbidium* have distinct inter-specific variation in the arm ratio of few homologous pairs in the complements [9]. The karyotypes of the species were more or less symmetrical. Therefore, it is interesting to know the karyotype of all *S. plicata* variants in the future. We hope that karyotype variation may be found among *S. plicata* variants.



Figure 2. Chromosome of *S. plicata* ( $2n=40$ ) (100 $\times$ )

### CONCLUSION

All *S. plicata* variants had the similar anatomical characters. *S. plicata* leaves are transversally arranged into one layer of upper epidermis cells, mesophyll cells, and one layer of lower epidermis cells. The mesophyll consisted of spongy tissue, and vascular bundle was distributed in it. Epidermis layer of *S. plicata* leave is generally arranged of two cell types, which are rectangular and polygonal cells. Stomata were found in the lower epidermis. All *S. plicata* variants have same chromosome number.

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