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MORPHOLOGY AND PALYNOLOGY OF THE GENUS *KAEMPFERIA* L. (KENCUR) FROM WEST SUMATRA

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ABSTRAK

Atiqa Zhafira Syahputri Rinaldi, Nurainas, Syamsuardi 2024. Morfologi dan Palinologi Genus Kaempferia L. (Kencur) dari Sumatra Barat. Floribunda 7(5): 202 – 210 — Marga *Kaempferia* yang dikenal di Indonesia dengan "kencur" merupakan salah satu marga dari Zingiberaceae yang sangat banyak dimanfaatkan masyarakat Indonesia sebagai obat tradisional, rempah, dan tanaman hias. Penelitian ini bertujuan untuk mengobservasi jenis *Kaempferia* di Sumatra Barat dan menganalisis karakter morfologi serbuk sari serta kontribusinya sebagai karakter pembeda pada marga *Kaempferia*. Hasil penelitian didapatkan lima jenis *Kaempferia* di Sumatra Barat yaitu *Kaempferia angustifolia, K. elegans, K. galanga, K. pulchra*, dan *K. rotunda.* Morfologi polen dari lima jenis *Kaempferia* tersebut telah diamati dengan menggunakan mikroskop cahaya (LM) dan mikroskop elektron (SEM). Karakter butiran serbuk sari yang ditemukan semuanya *monolete, isopolar*, ukuran termasuk kategori *large* dan *very large*, berbentuk *subspheroidal, inaperture* dengan ornamentasi *reticulate* dan *rugulate*. Karakter morfologi polen *Kaempferia* memiliki kemiripan dengan genus *Boesenbergia*, *Cornukaempferia*, dan *Curcuma*, namun struktur tectumnya berkontribusi untuk membedakan genus *Kaempferia* dari genus lain dalam famili Zingiberaceae.

Kata kunci: *Kaempferia*, Kencur, Pollen, Sumatra, Taxonomic, Zingiberaceae.

Atiqa Zhafira Syahputri Rinaldi, Nurainas, Syamsuardi 2024. Morphology and Palynology of the Genus *Kaempferia* L. (Kencur) from West Sumatra. Floribunda 7(5): 202 – 210 — The *Kaempferia*, commonly known as "kencur" in Indonesia, is one of the genus of Zingiberaceae which is widely used by Indonesian people as traditional medicine, spices, and ornamental plants. In this study, we observed *Kaempferia* species in West Sumatra and aimed to analyze the morphological characteristic including pollen morphology and its contribution as a distinguishing character in the *Kaempferia* genus. Pollen morphology observations were done using a light microscope (LM) and scanning electron microscope (SEM). The results revealed that there were five species of *Kaempferia* in West Sumatra, namely *K. angustifolia*, *K. elegans*, *K. galanga*, *K. pulchra*, and *K. rotunda*. Vegetative and generative morphological characters that play an important role in distinguishing the five *Kaempferia* species in West Sumatra are the shape of rhizome, leaf, *lateral staminode*, *labellum*, stigma type, and apex of anther crest. The pollen grain characters found were all *monolete, isopolar*, large to very large in size, *sub-spheroidal, inaperture*, and also *reticulate* and *rugulate* in tectum character. The pollen morphology character of Kaempferia resembles with the genera *Boesenbergia, Cornukaempferia*, and *Curcuma* but its tectum has contributed to distinguishing the *Kaempferia* genus from other genus in the Zingiberaceae family.

Keywords: *Kaempferia*, Kencur, Pollen, Sumatra, Taxonomic, Zingiberaceae.

In Indonesia, it is reported that there are six species of the *Kaempferia* genus, namely *K. vittata* N.E.Br., *K. elegans* Wall., *K. galanga* L., *K. angustifolia* Roscoe, *K. rotunda* L., and *K. pandurata* Roxb. (syn. *Boesenbergia rotunda* (L.) Mansf.) (Newman, *et al*., 2004; Suhono & LIPI Team, 2010). Three of the six species of which *K. angustifolia*, *K. rotunda*, and *K. vittata* are found in Sumatra (Miquel 1862; Newman *et al* ., 2004). However, in West Sumatera, there has been no definite report on the *Kaempferia* distribution, so a new report on the distribution of species of this genus is needed.

The character similarity to other genus makes *Kaempferia* an unstable genus. Some species are now simply synonyms of other species in different genus. For example, *K. pandurata* Roxb. is a synonym of *Boesenbergia rotunda* (L.) Mansf. Roxb (Newman *et al* ., 2004). In addition, *K. rotunda* is often misunderstood by the general public as white turmeric (*Curcuma*) (Ochse & Bakhuizen, 1977). Misidentification in plants will have an impact on the information delivery in the utilization of these plants. Therefore, to increase the characters' stability in the *Kaempferia* genus, it is necessary to add more characters from various aspects.

The character of pollen is one of the characters that play an important role in taxonomic and phylogenetic studies of the Zingiberales order (Kress *et al*., 1978; Saensouk *et al*., 2015). Many studies have used pollen characters as additional evidence in establishing new taxa such as at the species and even subspecies level and their application in the classification of certain taxa. This can be seen especially in monocot plants, one of which is in the Zingiberaceae family (Saensouk *et al*., 2015; Acma & Mendez, 2018; Ardiyani *et al*., 2021; Saensouk & Saensouk, 2022). Zingiberaceae has a unique pollen character in each genus (Mangaly & Nayar, 1990). In the *Curcuma*, the morphological character of pollen can be a distinguishing character between several *Curcuma* species (Saensouk *et al*., 2015). Pollen character information is important for understanding its relation to morphological characters, elemental composition, and future studies on in vitro germination of selected species (Acma & Mendez, 2018). In this study, we observed *Kaempferia* from various locations in West Sumatra. The study aimed to analyze the morphological characters and pollen morphology of *Kaempferia* in West Sumatra and to explore its implications in taxonomy.

MATERIALS AND METHODS

The sampling method used is a survey method that involves direct observation and collection in the field. The technique for collecting and preparing specimens follows the guidelines of Smith (1981) and Sirirugsa (1992). All collected samples were identified using the Zingiberaceae Herbarium specimen, which had been identified at the Andalas University Herbarium (ANDA) and referenced in the literature by Holttum (1950), Ridley (1924), Sirirugsa (1992), Smith (1981), and Larsen *et al*. (1999). The terminology used follows the guidelines of Harris & Harris (1994).

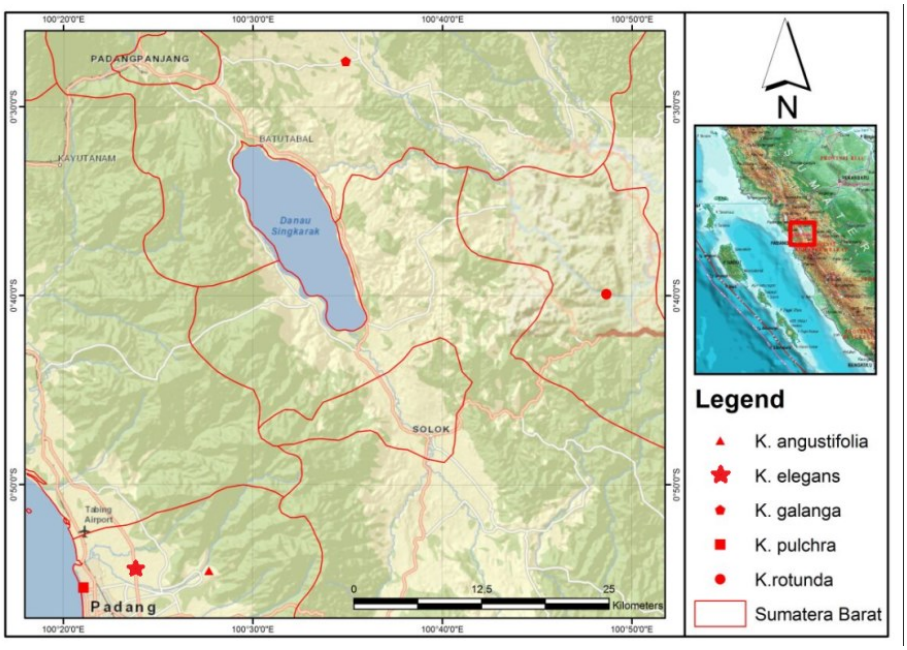

Figure 1. The distribution of *Kaempferia* sample collection locations in West Sumatra (Source: Indomap.shp).

Materials for pollen observations were obtained from fresh flowers of *Kaempferia* which were collected from several locations in West Sumatra (Figure 1). To avoid mixing with pollen from other plants, we choose and collect the unopened anthers or anthers on newly bloomed flowers. The collected anthers are then put into a 70% alcohol solution.

Pollen was observed using a Light Microscope (LM) and Scanning electron microscope (SEM). The unit, shape, and size of pollen were observed with LM 400x magnification which was carried out in the Laboratory of Plant Structure and Development, Department of Biology, Andalas University, West Sumatra. The preparation of samples for capturing using SEM consists of the specimen preparation, mounting, coating, and capturing processes. Ornamental characters were observed using SEM (JSM-5000LV Scanning Microscope) at the Zoology Division SEM Laboratory, The National Research and Innovation Agency (BRIN), Cibinong. The use of terminology for pollen refers to Erdtman (1952), Punt *et al*. (2006), and Hesse *et al*. (2009).

RESULT AND DISCUSSION

A. Species and Morphological characters of Kaempferia in West Sumatra

In this study, we found five species of *Kaempferia* in West Sumatera. The five species were *K. angustifolia, K. elegans, K. galanga, K. pulchra*, and *K. rotunda* (Fig.2). Previously, three species of *Kaempferia* in Sumatra had been reported by Miquel (1862) and Newman *et al* . (2004). The three species namely *K. angustifolia, K. galanga*, and *K. rotunda*, while some others such as *K. vittata* and *K. pandurata* Roxb. (syn. *Boesenbergia rotunda* (L.) Mansf.) have been included in the taxa *Boesenbergia*. From our current study, novel information obtained is the presence of *K. elegans* and *K. pulchra* species in West Sumatra. Where it was previously known that *K. elegans* is distributed in Malaysia Peninsular, Borneo (Newman *et al* ., 2004), and *K. pulchra* is distributed in the southern and southwestern parts of Thailand, Myanmar, Singapore, Malaysia, and Indonesia (Holttum, 1950; Sirirugsa, 1992; Picheansoonthon & Koonterm, 2008). This is because both types of *Kaempferia* are known and cultivated as ornamental plants (Valeton, 1918; Hashiguchi *et al* ., 2022), thus making their distribution quickly to various regions, especially in West Sumatra.

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The people of West Sumatra cultivate several species of *Kaempferia*, including *K. galanga*, *K. elegans*, *K. pulchra*, and *K. rotunda*. These species are cultivated for their various uses in society, such as being used as a spice in cooking, in traditional medicines, and as ornamental plants (Valeton, 1918; Pham *et al*., 2021; Hashiguchi *et al*., 2022). One commonly used type of *Kaempferia* in West Sumatra is *K. galanga*, known locally as "cakua". It is used as a spice in the preparation of a dish called "lotek" and is also used as a traditional medicinal herb to treat coughs, bloating, nausea, swelling, and ulcers (Arbain *et al*., 2014).

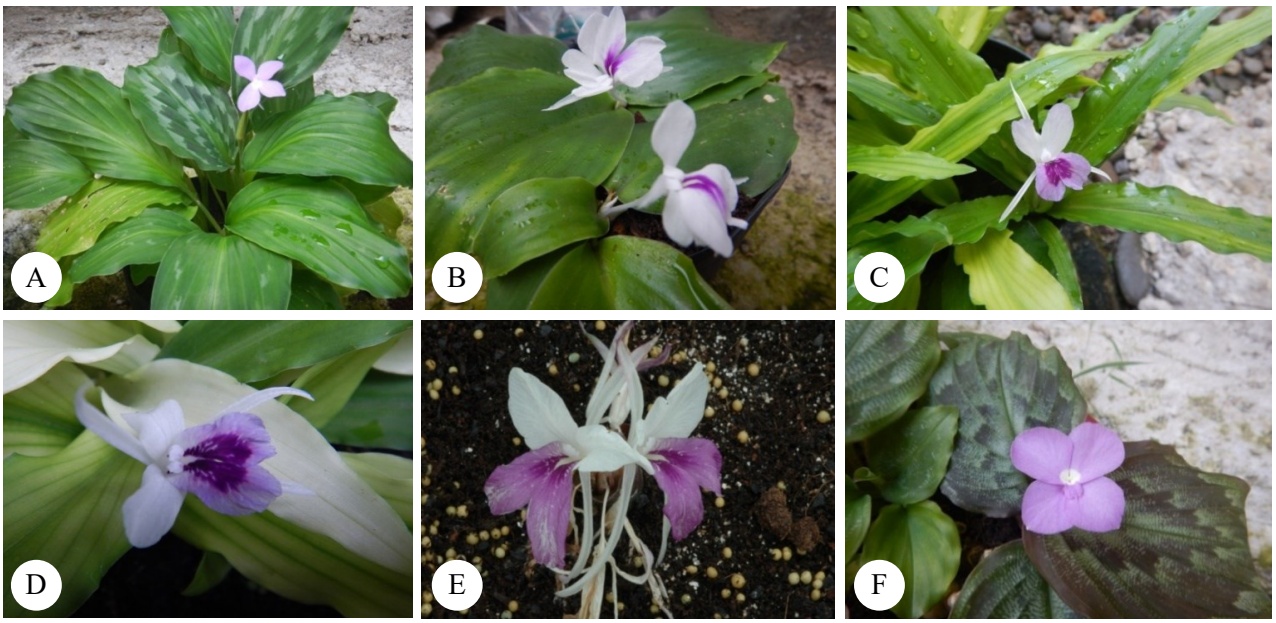


Figure 2. The plant habit and flower of *Kaempferia* from West Sumatra. A. *Kaempferia elegans*; B. *K. galanga;* C and D. *K. angustifolia*; E. *K. rotunda (source : http://www.eol.org)*; F. *K. pulchra.*

The morphological characteristics of five *Kaempferia* species in West Sumatra exhibit variations in rhizome shape (Figure 3A-B), leaf shape, *lateral staminode* shape, *labellum shape* (Figure 3C-D), stigma type (Figure 3E-F), and apex anther crest (Figure 4). Based on the *labellum* shape, the five *Kaempferia* species can be categorized into two groups. The first group, characterized by a *bilobed labellum* shape (Figure 3C) with sinus < ½ to > ½, includes *K. angustifolia*, *K. galanga*, and *K. rotunda*. The second group, defined by a divided *labellum* shape (Figure 3D), consists of *K. elegans* and *K. pulchra*.

Among the five *Kaempferia* species, *K. rotunda* stands out with distinct morphological characteristics, such as a *globose-ovoid* rhizome shape (Figure 3A), a longer *pseudostem*, inflorescence appearing before the vegetative organs, and an upright position of the anther crest (Figure 4C).

Kaempferia pulchra and *K. elegans* have almost similar morphological characteristics that are difficult to identify, but these two species can be distinguished on the character of the leaf growth direction, *pseudostem* length, leaf surface color, and variation of apex anther crest (Table 1).

Tabel 1. Morphological differences between *Kaempferia elegans* and *K. pulchra*.

No	Morphological character	*Kaempferia elegans*	*Kaempferia pulchra*
1.	Leaf growth direction	erect leaves	leaves appressed to the ground
2.	Pseudostem length	1-2 cm	3-5cm
3.	Leave shape	*Obovate*	*Oblong-Obovate*
4.	Leaf surface color	green with silver streaks	dark brownish-green with silver patterns
5.	Variation of apex anther crest	*acutus, trilobed*, and *entire*	*Acuminate* (like a claw)

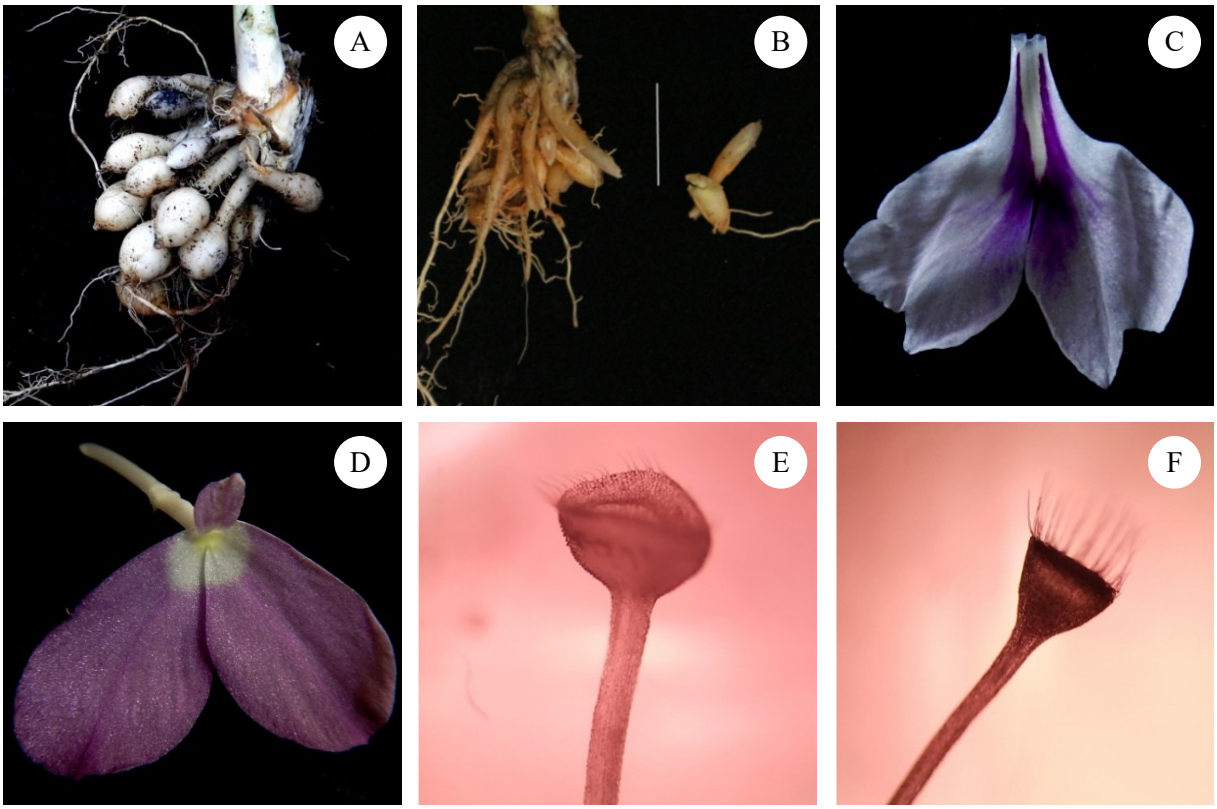


Figure 3. Morphological characteristics of rhizome, labellum, and stigma of *Kaempferia* from West Sumatra: A. Rhizome globose-ovoid on *K. rotunda;* B. Rhizome torulose with cylindrical tubers on *K.angustifolia*; C. Labellum bilobed on *K. galangal*; D. Labellum divided on *K. elegans*; E. Stigma cup with ellipse margins on *K. galanga*; F. Stigma cup with rounded margin on *K. pulchra*. Scale bar = 2 cm

The vegetative and generative morphological characteristics play an important role in distinguishing the five species of *Kaempferia* in West Sumatra. The growth direction and the appearance position of inflorescence (inflorescence appearing before the vegetative parts or inflorescence appearing between the two innermost leaves) also serve as distinguishing characters in the five species of *Kaempferia* in West Sumatra.

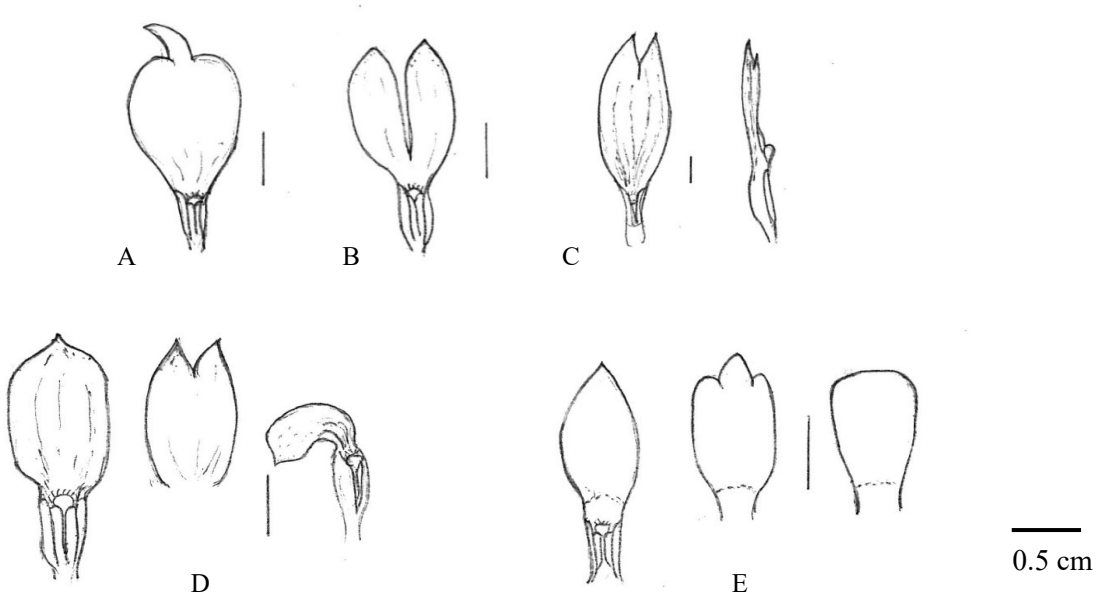


Figure 4. Variations of anther crest of *Kaempferia* in West Sumatra: A. *K. pulchra*; B. *K. galanga*; C. *K. rotunda*; D. *K. angustifolia* ; E. *K. elegans*. Scale bar = 0.5 cm

B. Pollen morphology of Kaempferia in West Sumatra

Pollen units in the *Kaempferia* genus have a *monad* or *monolete* type, which means the unit consists of only one whole pollen grain. Pollen grains in *Kaempferia* separate freely from one another. The polarity of *Kaempferia* pollen found is isopolar, where the shape of the proximal and distal poles is the same (Figure. 5). The size and shape of the pollen in the five observed species of *Kaempferia* did not vary much. The *Kaempferia* pollen is classified as large to very large, with an average diameter of 87.26 to 113.57 µm in the polar area and 85.81 to 109.98 µm in the equatorial area. The shape of the pollen is sub-spheroidal, determined based on the polar to equatorial (P/E) comparison index (Table 2).

Tabel 2. Pollen morphology characteristics in *Kaempferia* genus in West Sumatra

No	Spesies	Average E (µm) ± SD	Average P (µm) ± SD	P/E ratio (µm)	Shape	Size chategory	Tectum
1	*K. angustifolia*	87,78 ± 7,9	95,80 ± 8,5	1,09	Subspheroidal	Large	Rugulate
2	*K. elegans*	85,81 ± 3,4	87,26 ± 3,7	1,01	Subspheroidal	Large	Rugulate
3	*K. galanga*	109,98 ±13,6	113,57 ± 13,7	1,03	Subspheroidal	Very Large	Rugulate
4	*K. pulchra*	91,59 ± 5,8	96,89 ± 7,2	1,05	Subspheroidal	Large	Rugulate
5	*K. rotunda*	105,13 ±15,3	113,34 ± 15,5	1,07	Subspheroidal	Very Large	Reticulate

The tectum is the outermost layer of pollen, which has a variety of morphological structures, making it a distinguishing characteristic for identification. In our current study, we identified two types of tectum in *Kaempferia*: *reticulate* and *rugulate*. *Reticulate tectum* is characterized by a net-like engraving on the pollen surface with protruding engravings (lumina). Meanwhile, *rugulate tectum* is in the form of several broad protrusions on the pollen surface. *Reticulate tectum*

was only found on the pollen surface of *K. rotunda*, while *rugulate tectum* was found on *K. angustifolia*, *K. elegans*, K. *galanga*, and *K. pulchra* (Figure. 6-10). These results demonstrate the relationship between the morphological and pollen micromorphological characters of these five types of *Kaempferia*.

The morphological characteristics of *K. rotunda* set it apart from the other four types of *Kaempferia*. These characteristics include inflorescences appearing on rhizomes without leafy vegetative organs, erect anther crests, oblongated, and hairy ovaries (Sirirugsa, 1992). Previous research on Curcuma also supports classification of species based on variations in pollen forms and morphological characters (Saensouk *et al*., 2015).

Comparing the tectum characteristics of the *Kaempferia* genus with its closest genus, such as *Boesenbergia*, *Cornukaempferia*, *Curcuma,* and *Zingiber* (Kress *et al*., 2002; Smith, 1981), shows distinct differences. The tectum variations in the closest genus of *Kaempferia* are *cereboid*, *spiro-striate*, and *psilate* (Theilade *et al*., 1993; Saensouk *et al*., 2009; Chen & Xia, 2011), whereas, for the *Kaempferia* genus, the tectum is rugulate and reticulate. Therefore, the tectum character can serve as a distinguishing factor between the genus of the Zingiberaceae family. This is supported by Syamsuardi *et al*. (2010) and Nurainas *et al*. (2011), who stated that the morphological character of pollen in Zingiberaceae at the genus level, especially the tectum shape, can be a stable distinguishing factor. This has also been demonstrated in *Amomum* and *Curcuma*, where the morphological characteristics of pollen exhibit variations in size, shape, and tectum (Kaewsri & Paisooksantivans, 2007; Saensouk *et al*., 2015).

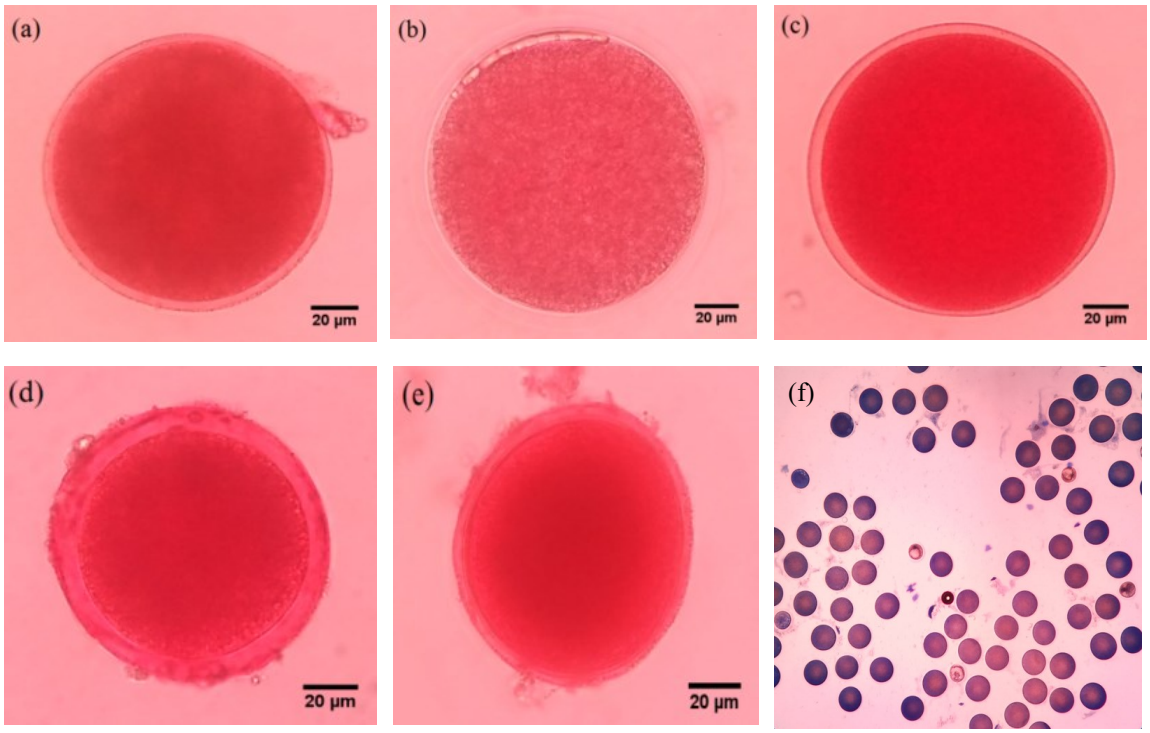
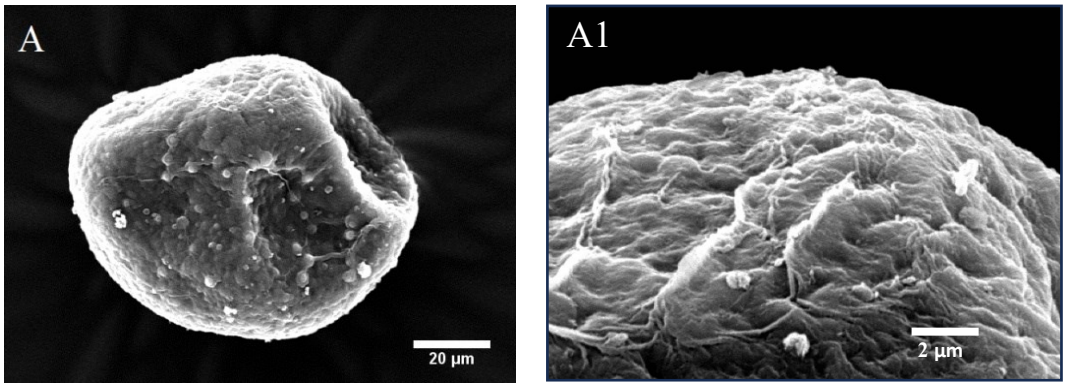


Figure 5. LM micrographs of pollen grains in *Kaempferia* from West Sumatra. *Kaempferia angustifolia* (a), *K. elegans* (b), *K. galanga* (c), *K. pulchra* (d) and *K. rotunda* (e and f). Scale bars: 20 µm



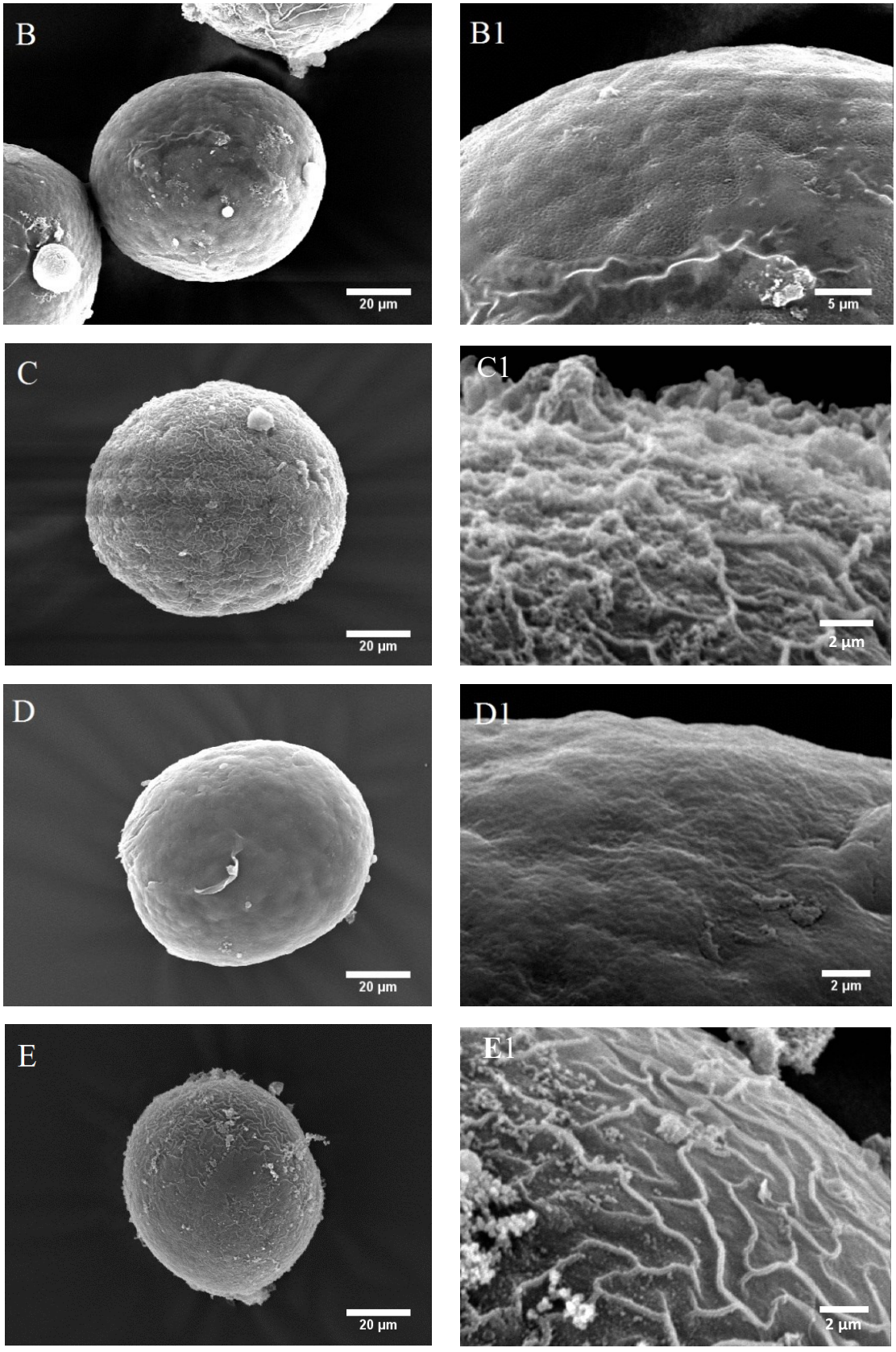


Figure 6. SEM micrographs of pollen grains in *Kaempferia* from West Sumatra. A and A1: *Kaempferia Angustifolia,* B and B1: *Kaempferia elegans,* C and C1: *Kaempferia galanga,* D and D1: *Kaempferia pulchra.* E and E1: *Kaempferia rotunda.* Scale bars: 20 µm

CONCLUSION

In this study, we found five species of *Kaempferia* distributed in West Sumatra, namely *K. angustifolia, K. elegans, K. galanga, K. pulchra,* and *K. rotunda.* The vegetative and generative morphological characteristics, shape of rhizome, leaf, *lateral staminode*, *labellum*, stigma type, and apex anther crest are useful for identification of the five species of *Kaempferia* in West Sumatera. The *reticulate* and *rugulate* pollen tectum in *Kaempferia* can contribute to one of the distinguishing characters in certain species within the *Kaempferia* and significant as a distinguishing character at the genus level on Zingiberaceae family.

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