



## PRELIMINARY STUDY OF THE POLLEN MORPHOLOGY OF MALAYSIAN ZINGIBERACEAE (TRIBE ALPINIEAE) AND THE TAXONOMIC RELATIONSHIP

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### ABSTRACT

MOHAMAD, S., MEEKIONG, K & SEDEK, A. S. 2023. Preliminary study of the pollen morphology of Malaysian Zingiberaceae (tribe Alpinieae) and the taxonomic relationship. *Reinwardtia* 22(2): 91–102. — The family Zingiberaceae forms an important herbaceous layer in the tropical forests of Malaysia. From a phylogenetic point of view, certain genera of the problematic tribe Alpinieae are non-monophyletic and in need of more taxonomic evidence to support the classification. This study demonstrates how the palynological data could correlate with the proposed phylogenetic data, using representatives from the Malaysian species. The pollen morphology of 21 species from the tribe Alpinieae was investigated. Parameters including polarity, symmetry, shape, size, apertures, exine ornamentation, size of spine, type of spine apex, spine density, and distance between spine were analysed. The results demonstrated that the studied species were conveniently divided into two major groups based on the exine sculpturing of the spheroidal pollens, either psilate as in *Etilingera* and *Hornstedtia*, or echinate as in *Alpinia*, *Conamomum*, *Meistera*, *Plagiostachys*, *Sundamomum*, and *Sulettaria*. Hence, as far as the study is concerned, the main sculpturing is considered useful to generally distinguish the genera in the tribe.

**Key words:** Diversity, Johor, systematics, Sarawak, taxonomy, wild gingers.

### ABSTRAK

MOHAMAD, S., MEEKIONG, K & SEDEK, A. S. 2023. Penelitian awal morfologi serbuk sari Zingiberaceae Malaysia (puak Alpinieae) dan hubungan taksonominya. *Reinwardtia* 22(2): 91–102. — Suku Zingiberaceae membentuk lapisan herba penting di hutan tropis Malaysia. Dari sudut pandang filogenetik, marga tertentu dari puak Alpinieae yang bermasalah adalah non-monofiletik dan membutuhkan lebih banyak bukti taksonomi untuk mendukung klasifikasi. Studi ini menunjukkan data palinologi dapat berkorelasi dengan data filogenetik yang diusulkan, menggunakan perwakilan dari jenis Malaysia. Morfologi polen dari 21 jenis dari puak Alpinieae telah diamati. Parameter yang dianalisis termasuk polaritas, simetri, bentuk, ukuran, bukaan, perhiasan eksin, ukuran duri, tipe ujung duri, kerapatan duri, dan jarak antar duri. Hasilnya menunjukkan bahwa jenis yang diteliti dengan mudah dibagi menjadi dua kelompok besar berdasarkan perhiasan eksin serbuk sari yang membulat, baik *psilate* seperti pada *Etilingera* dan *Hornstedtia*, atau *echinate* seperti pada marga *Alpinia*, *Canamomum*, *Meistera*, *Plagiostachys*, *Sundamomum*, dan *Sulettaria*. Oleh karena itu, perhiasan utama dianggap berguna untuk membedakan marga dalam puak secara umum.

**Kata kunci:** Jahe liar, Johor, keragaman, Sarawak, sistematika, taksonomi.

### INTRODUCTION

Zingiberaceae is the largest family among the eight families in the monophyletic order, Zingiberales and has at least 60 genera and 1,900 species (POWO, 2022; Banaticla-Hilario & Altamirano, 2023). The species occur in the tropics and subtropics, of which the Asian tropics hold the highest

diversity and number of taxa (Lamb *et al.*, 2013; POWO, 2022). Most Zingiberaceae are evergreen plants that form the important herbaceous layer of the tropical rainforest, especially in forest gaps where light is not scarce. Within Zingiberaceae, diverse genera such as *Alpinia* and *Amomum* are often dominant in humid tropical lowlands, while *Etilingera*, *Hornstedtia*, and *Plagiostachys* can pre-

dominate in habitats that provide more sunlight, such as disturbed areas or secondary forests (Lamb *et al.*, 2013). In Malaysian Borneo, as botanical exploration progresses, more than 250 named taxa in Zingiberaceae have been reported and subfamily Alpinioideae encompassed the highest number of species (De Boer *et al.*, 2018; Mohamad & Meekiong, 2019). On top of that, several Alpinieae genera are highly distributed or endemic to Sarawak, in particular *Epiamomum* (all six species, 100%), *Sundamomum* (10 species out of 16.63%), and *Plagiostachys* (13 taxa out of 33.38%) (POWO, 2020; WCVP, 2020; Mohamad *et al.*, 2020; Mohamad & Meekiong, 2020). Of *Etilingera* in Borneo, 32 taxa out of the known 42 (80%) occur in Sarawak, 30 taxa in Sabah (70%), 25 taxa (65%) in Kalimantan, and 13 taxa (33%) in Brunei (Poulsen, 2006). It is difficult to compare the species richness of Borneo with that of other islands, as only a relatively small number of detailed studies have been carried out (Poulsen, 2006). By 1999, Peninsular Malaysia had documented a lower count of Zingiberaceae species, with a total of 160 species across 18 genera, as reported by Larsen *et al.* (1999). In 2001, Khaw conducted a revision of *Etilingera* in Peninsular Malaysia, which initially comprised 12 species (Khaw, 2001). Subsequently, in the same year, Lim's report added two more species to the list, totaling 14 species (Lim, 2001).

A new classification of Zingiberaceae was proposed by Kress *et al.* (2002) based on the DNA sequence data who recognised four subfamilies and six tribes, namely Siphonochiloideae (tribe Siphonochileae), Tamijioideae (tribe Tamijieae), Alpinioideae which comprises most of the former tribes Alpinieae (tribe Alpinieae and tribe Riedelieae), and Zingiberoideae which includes the former tribes Hedychieae and Globbeae (tribe Zingibereae and tribe Globbeae). The subfamily Alpinioideae is diagnosed by the plane of distichous leaves perpendicular to the rhizome and the absence or reduction of the two lateral staminodes. The taxonomic status of the tribe Alpinieae still needs further clarification, especially for the non-monophyletic genera, including *Alpinia* which consists of different clades (Kress *et al.*, 2005), *Plagiostachys* (nested deep within *Zerumbet* clade IV in *Alpinia*) (Kress *et al.*, 2005; Julius *et al.*, 2008), and *Hornstedtia* (two species are nested within the *Amomum* clade and *H. leonurus* is nested within the *Etilingera* clade) (Pedersen, 2004; De Boer *et al.*, 2018). In the current development, species that were formerly placed under *Elettaria* have been moved to a new genus, *Sulettaria* based on works by Poulsen *et al.* (2019). In the long-term, the species in these genera may have to be transferred to another genus, but for the time being, they are ideally classified based on morphological characters.

The pollen morphology in Zingiberaceae have been investigated in certain genera and several characteristics, including shape, size, symmetry, and polar aperture of the pollen grains, were discovered as beneficial in identifying the species of the particular genus (Larsen *et al.*, 1998; Saensouk *et al.*, 2009; Chen & Chia, 2011; Saensouk *et al.*, 2015) and for classification at the generic and sectional levels (Theilade *et al.*, 1993). In 1990, Mangaly & Nayar, who studied the South Indian Zingiberaceae, reported that the shape of pollen grains ranged from spheroidal, subspheroidal, ovoid, and ellipsoid, whilst the sculpturing of the exine can be echinate, regulate, scabrate, striate, psilate, or verrucate. In addition, they proposed that the thin layer of exine does not provide mechanical strength for the pollen grain, thus most of the taxa have low resistance to acetolysis treatment (Mangaly & Nayar, 1990). Previously, the pollen of Zingiberaceae was regarded as exineless and inaperturate (Dahlgren *et al.*, 1985), but this was proved untrue by Mangaly & Nayar (1990) who discovered that among the 21 taxa studied, an exine was only absent in *Kaempferia galanga*. All other taxa portrayed a distinct exine layer covering the pollen grain, as found in other genera in the Zingiberales order: *Heliconia* (Heliconiaceae), *Strelitzia* (Strelitziaceae) and *Tapeinochilos* (Costaceae). In contrast, the intine consisted of different layers that are well developed and lamellated (Mangaly & Nayar, 1990).

Of the tribe Alpinieae, Mangaly & Nayar (1990) recorded that the pollen shapes of the seven studied species were either spheroidal or subspheroidal, while the exine sculpture was spinulose in most of the tribe, *viz.* *Alpinia calcarata*, *A. galanga*, *A. zerumbet*, *Amomum hypoleucum*, *A. pterocarpum*, and verrucate as in *Elettaria cardamomum*. In contrast, the pollen shapes of *Zingiber* (tribe Zingibereae) were either spheroidal with rugulate ornamentation or ellipsoidal and striate, which closely followed the subgeneric boundaries. Among the different taxa studied, the well developed exine only occurred in *Zingiber*. The presence or absence of ornamentation, however, clarified that there is no correlation to the thickness of the exine layer (Mangaly & Nayar, 1990). Variation in pollen morphology was shown as following the taxonomic borders, even though the tribes encompassed more than one pollen type.

Theilade *et al.* (1993), who explored the pollen structure and morphology of 18 *Zingiber* species from Peninsular Malaysia, Thailand, and Myanmar reported two groups of pollen structure, particularly spherical with cerebriform or reticulate sculpturing in section *Zingiber* and section *Dymerizewiczia*, and ellipsoidal with a spiro-striate surface for section *Cryptanthium*. The pollen morphology of *Zingiber* offered a more useful criterion than the ambiguous inflorescence habit for the classification into sec-