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Scanning Electron Microscopy (SEM) Leaf Anatomy and Micromorphology for New Zingiberaceae Species in Borneo

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ABSTRACT

This study aims to explore and document the micromorphological characteristics of four newly identified species of the Zingiberaceae family in Singai Bau, Sarawak: *Sulettaria meekiongii*, *Alpinia songet*, *Zingiber singaiensis,* and *Amomum jackliamii*, using Scanning Electron Microscopy (SEM). Conducted in the biodiverse region of Mount Sijanjang Singai, the research focuses on the anatomical features of leaves, including trichomes, stomata, epidermal cells, subsidiary cells, epicuticular wax, and glands. Collaborating with botanists and utilizing herbarium records and MyBIS for classification, the study meticulously prepared leaf samples for high-resolution SEM imaging. Findings revealed distinct anatomical features: *Sulettaria meekiongii* exhibited long non-glandular trichomes and rectangular wax patterns; *Alpinia songet* displayed diverse trichome distributions and protective wax layers*; Zingiber singaiensis* showed smooth resinous layers and stunted trichomes; and *Amomum jackliamii* featured dense stomatal arrangements and fissured wax. Additionally, the study assessed the traditional uses and ecological significance of these species within the Bidayuh community, highlighting the integration of traditional knowledge and scientific techniques. This research provides a foundational understanding of micromorphological diversity within the Zingiberaceae family, demonstrating the value of combining traditional knowledge with modern scientific approaches for holistic biodiversity conservation.

Key Words: Zingiberaceae, Micromorphology, Scanning Electron Microscopy (SEM), Traditional Knowledge and Biodiversity Conservation

Abbreviations: SEM: Scanning Electron Microscopy, Formalin-Acetic Acid-Ethanol fixative: FAA, MyBIS: Malaysia Biodiversity Information System

INTRODUCTION

The Zingiberaceae family, commonly known as the ginger family, is a vital group of monocotyledonous flowering plants with significant ecological, economic, and cultural importance. Comprising over 50 genera and approximately 1,300 species, this family is predominantly found in tropical and subtropical regions, with

Southeast Asia recognized as its center of diversity (Kumar et al., 2020; Tan et al., 2020). This study focuses on the micromorphological analysis of four newly identified Zingiberaceae species in Singai Bau, Sarawak: *Sulettaria meekiongii, Alpinia songet, Zingiber singaiensis,* and *Amomum jackliamii.*

Mount Sijanjang Singai, located about 40 kilometers from Kuching city, is home to the Bidayuh community, one of the indigenous groups in Sarawak. The Bidayuh community has long utilized local flora, particularly Zingiberaceae plants, for medicinal purposes. This traditional knowledge is invaluable for sustainable resource use and conservation efforts (Sayok et al., 2019; Kumar et al., 2021).

Micromorphological studies provide critical insights into the structural and functional adaptations of plants. These studies involve examining fine details of plant surfaces, such as trichomes, stomata, and epicuticular waxes, which are essential for understanding plant physiology and ecology (Barthlott et al., 2019). Scanning Electron Microscopy (SEM) is crucial for taxonomic classification, ecological studies, and developing new applications in agriculture and pharmacology (Zhang et al., 2019).

The aim of this study is to document and analyze the micromorphological characteristics of the leaves of *Sulettaria meekiongii, Alpinia songet, Zingiber singaiensis,* and *Amomum jackliamii* using SEM. Additionally, this study aims to highlight the traditional uses of these plants by the Bidayuh community, emphasizing the need to conserve both biological and cultural heritage (Chen et al., 2019; Kopustinskiene et al., 2020).

The study's methodological approach involves several key steps. Initially, plant specimens were collected from Mount Sijanjang Singai. Identification of these species was conducted in collaboration with botanists, utilizing herbarium records and online databases such as MyBIS (Kress et al., 2021). The leaf samples were prepared for SEM analysis through a series of steps, including fixation in FAA fixative, dehydration in an ethanol series, and critical point drying (Goldstein et al., 2019). Once prepared, the samples were coated with platinum to enhance the quality of SEM imaging. The SEM analysis focused on both the abaxial and adaxial surfaces of the leaves, providing high-resolution images that revealed the detailed structures of trichomes, stomata, and epicuticular waxes. These images were analyzed to identify and classify the unique micromorphological features of each species (Bozzola & Russell, 2020). The findings provide a comprehensive understanding of the micromorphological characteristics of the four new Zingiberaceae species. Each species exhibited unique features: *Sulettaria meekiongii* showed long simple non-glandular trichomes and rectangular epicuticular wax patterns; *Alpinia songet* had diverse trichome distributions and protective wax layers; *Zingiber singaiensis* displayed smooth resinous layers and stunted trichomes; and *Amomum jackliamii* featured dense stomatal arrangements and fissured epicuticular wax (Chakraborty et al., 2023). The study also highlighted the traditional uses of these plants by the Bidayuh community. For instance, *Sulettaria meekiongii* is used to make strings from mature leaf sheaths, while *Alpinia songet's* rhizomes are employed in treating skin diseases. These practices underscore the cultural significance of these species and the need to preserve this knowledge for future generations (Lakshmi et al., 2021; Alkandahri et al., 2021).

The significance of this study extends beyond science to ecological preservation and cultural heritage. By documenting and analyzing micromorphological characteristics and traditional uses of four new Zingiberaceae species, it offers essential insights for biodiversity conservation, cultural preservation, and scientific advancement. This holistic approach is essential for effective conservation strategies, ensuring the preservation of both biological and cultural heritage (Aswati et al., 2021; Chaidir et al., 2021). This study advances existing knowledge by combining cutting-edge SEM technology with traditional ecological knowledge to provide a comprehensive understanding of new Zingiberaceae species. The detailed micromorphological analysis offers new data that can be used to refine taxonomic classifications and understand plant adaptations in tropical environments. Furthermore, by documenting the cultural uses of these plants, the study highlights the importance of integrating traditional knowledge into scientific research. This approach not only enriches the scientific understanding of biodiversity but also promotes the conservation of both biological and cultural diversity in Borneo. This work sets a precedent for future studies in ethnobotany and plant taxonomy, advocating for interdisciplinary research that bridges the gap between modern science and indigenous knowledge systems.

Objectives of the Study

- To explore and document the micromorphological characteristics of four new Zingiberaceae species in Singai Bau, Sarawak.
- To identify and classify the unique anatomical features of the new Zingiberaceae species.
- To assess the ecological and cultural significance of the newly identified Zingiberaceae species.

METHODOLOGY

1. **Study site**

The study was carried with the Bidayuh in Singai community in Sarawak.

Figure 1: Map of Sarawak (Source: http://www.malaysia-maps.com)

The study involved with Bidayuh community in Singai Bau Sarawak, and the study site namely Mount Sijanjang, Singai (figure 1). This area was selected for the study due to the historically established Bidayuh settlements (Sayok et al., 2014). Currently, the ethnic community in this region continues to practice traditional medicine, with Zingiberaceae plants playing a crucial role (Sayok et al., 2014). This area is not only houses rich biodiversity but also supplies clean water to the villages. The majority of ginger species grow in the moist, shaded undergrowth, with their green stems emerging through the humid forest floor. Some of the species were found growing on cliffs beneath trees.

2. **Plant Collection**

The Zingiberaceae species were identified in the field, and notes on the habitat and GPS coordinates were recorded. Mature and healthy specimens representing the typical characteristics of the species were collected for herbarium samples. Fresh, healthy leaves were also collected for SEM analysis, with multiple samples taken from different parts of the plant for comparison

3. **Herbarium Preparation**

The specimens were pressed as soon as possible after collection to preserve their form, using a plant press and regularly changing the blotting paper to prevent mold. Each specimen was labeled with detailed information, including genera name, collection date, location, and other relevant data such as altitude and usage. The specimens were then dried in a well-ventilated area, with a drying oven set at a controlled temperature not exceeding 60°C.

4. **Identification of Plant Species**

To identify plant species at a herbarium, identification keys, botanical literature, and comparisons with identified specimens were used. Collaboration with botanists, online databases, cross-referencing SAR of the Sarawak Forest Department herbarium records, and expert verification confirmed identifications.

5. **SEM Sample Preparation**

The leaf samples were carefully cleaned using distilled water to remove any dirt or debris without damaging the leaf surface. The leaf samples from both the abaxial and adaxial parts of each species were then cut into small pieces

6. **Fixation of Leaf Samples**

The samples were immersed in FAA fixative (3.7% v/v formaldehyde, 50% ethanol, 5% acetic acid) and subjected to a light vacuum until they sank. They were then fixed overnight (approximately 18 hours) at 4°C. After fixation, the samples were rinsed three times in 25 mM sodium phosphate buffer (pH 7) before being dehydrated in an ethanol series (30%, 50%, 70%, 95%, and 100% dry, 30 minutes each step). The 100% dry ethanol was changed twice, and the tissue was stored overnight at 4°C before being processed with the Critical Point Drier (CPD) the next day.

7. **Sample Preparation using Critical Point Drier (CPD K850)**

The blue valve was turned clockwise to pre-cool the chamber to 5°C. After loading the specimens, the chamber insert was secured by ensuring the thumbscrew was correctly tightened. The green valve was then turned clockwise to fill the chamber with liquid CO2. The specimens were soaked and stirred for 3 minutes while maintaining the temperature below 10°C. This process was repeated three times. The specimens were then heated for 35 minutes to reach 35°C and 1250 psi pressure. The samples were depressurized for 20 minutes.

8. **Mounting and Sputter Coating**

The dried specimens were mounted on aluminum stubs using carbon tape to ensure stability and proper positioning for imaging. The samples were then coated with a thin layer of platinum using a sputter coater to prevent charging under the electron beam, preparing them for SEM imaging.

9. **SEM imaging**

The SEM analysis was conducted, focusing on the micromorphological features relevant to the study, including trichomes, stomata, epidermal cells, subsidiary cells, epicuticular wax, and glands. High-resolution images were obtained, providing crucial insights into the morphological characteristics of the leaf surfaces, which in turn offered understanding into their physiological functions and adaptations. The characteristics of trichomes, stomata, epidermal cells, subsidiary cells, epicuticular wax, and glands on both the abaxial and adaxial sides of the leaves were then described

10. Naming the New Species

Following the identification and verification processes, the new species was named based on its unique morphological characteristics and ecological niche. The naming followed the International Code of Botanical Nomenclature guidelines, ensuring the name was unique and descriptive. The chosen name reflected both the plant's distinguishing features and its habitat, honoring the traditional knowledge of the local communities involved in the study. The newly named species was then documented in the herbarium records and published in relevant botanical journals for recognition and further research.

RESULT

The study compiled the four new species were encountered in Singai. Two species each are belonging to *Zingiber* and *Amomum,* and one species each belonged to *Alpinia and Sulettaria* genera. These new species were described.

1. New species

A total of four species were identified as new species. One of the species is cultivated by the local community of Bidayuh in Singai for-medicinal purposes and six other species are originally grown in Mount Sijanjang, Singai. All of these species are used by the local community for various uses.

1. *Sulettaria meekiongii* Ripen & S.P. Teo sp. nov.

This plant has up to 10 pseudostems, 50-70cm tall, and light green leaves. It has a rhizome with 6-8 leafless sheaths and a leaf margin. The plant has alternate leaf arrangements and a petiole. Inflorescences are erect, conical, and have a rachis 3-5cm long. Bracteoles are tubular, and the calyx is tubular. The flowers are tubiform, with 2-3 open at a time. The ovary is glabrous, and the labellum is pale yellow.

Traditional use: The sheaths of matured leaf are splits into strips and use as string.

Figure 2 illustrates various parts of *Sulettaria meekiongii* Ripen & S.P. Teo in detail. Panel A displays the entire inflorescence with its flowers, highlighting the arrangement and distribution. Panel B provides a close-up view of an individual flower, emphasizing its morphological characteristics. Panel C focuses on the labellum and stamen, showing their intricate structure and positioning. Panel D depicts the plant with immature fruit, illustrating this stage of its lifecycle. Panel E presents a close-up of the ovary with the calyx, and Panel F showcases the oval-shaped and reddish bracts, emphasizing their unique coloration and form.

Figure 2: *Sulettaria meekiongii* Ripen & S.P. Teo. A. Inflorescence with flowers. B. Close-up flower C. Close-up of labellum and stamen D. Plant with immature fruit. E. Ovary with calyx. F. Oval -shaped, reddish bracts.

Figure 3 provides a comprehensive view of *Sulettaria meekiongii* Ripen & S.P. Teo by showcasing various parts of the plant. Panel G displays the whole plant, giving an overview of its general structure and growth habit. Panel H focuses on the rhizome, the underground stem from which roots and shoots emerge. Panel I highlight the short stilt-rooted aspect of the plant, showing its root structure. Panel J illustrates the inflorescence with flowers, emphasizing the floral arrangement. Panel K shows the short ligule, a small outgrowth at the junction of the leaf and leafstalk. Lastly, Panel L presents the acuminate apex, detailing the pointed tip of the leaf.

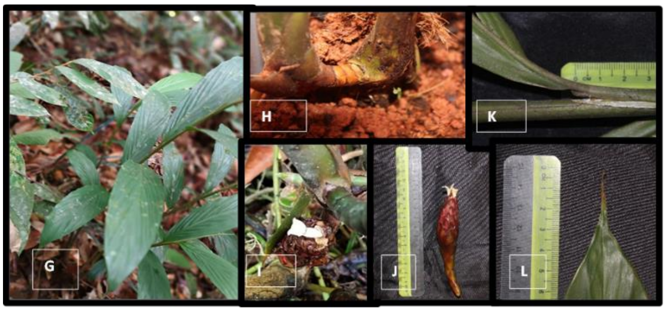

Figure 3: *Sulettaria meekiongii* Ripen & S.P. Teo. G. Whole plant. H. Rhizome. I. short stilt-rooted J. Inflorescence with flowers. K. Ligule short. L. Apex acuminate.

Scanning Electron Micrographs of *Sulettaria meekiongii*

Figure 4 presents Scanning Electron Micrographs of *Sulettaria meekiongii*, focusing on the plant's microstructural details. Panel A shows the distribution of trichomes and stomata on the adaxial (upper) surface of the lamina, while Panel B also emphasizes this distribution. Panel C details long, simple, non-glandular trichomes, highlighting their pyramidal and unicellular structure. Panel D presents short, cone-shaped trichomes. Panel E illustrates the distribution of trichomes and stomata on the abaxial (lower) lamina. Panel F captures the rectangular epicuticular wax pattern. Panel G shows trichomes without segmentation, and Panel H compares short and long trichomes on the adaxial leaf surface, providing a comprehensive understanding of the leaf's microanatomy.

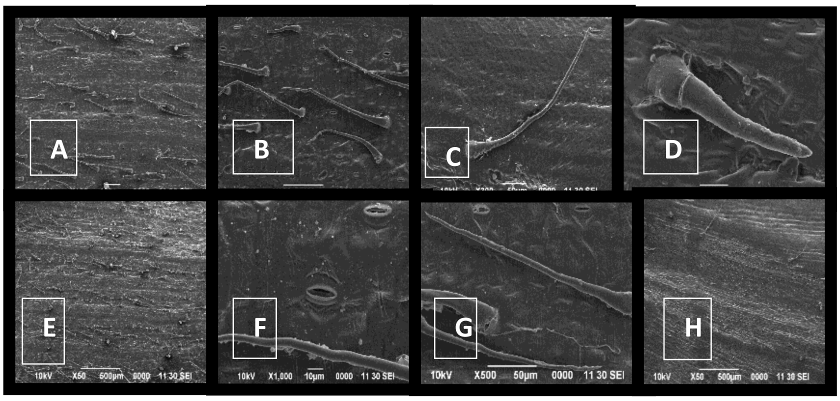

Figure 4: Scanning Electron Micrographs of *Sulettaria meekiongii* A. Distribution of trichomes and stomata on adaxial lamina. B. Distribution of trichomes and stomata on adaxial lamina. C. Long, simple, non-glandular trichomes, pyramidal, and unicellular trichome of leaf adaxial. D. Short and cone shape trichome of adaxial lamina. E. Distribution of trichomes and stomata on abaxial lamina F. Rectangular of epicuticular wax on leaf abaxial. G. trichomes without segment. H. Short and long trichomes of leaf adaxial.

2. ***Alpinia songet* Ripen & Meekiong sp. nov.**

This plant has up to 10 pseudostems, 1.4-1.7 m tall, and pale green with stout rhizomes. It has 6-8 pairs of alternate leaf blades, elongate blades, and apex acuminate. The inflorescence is terminal, erect, with 20-25 cincinni flowers per cincinni. The flower is c. 4 cm long, with 2-5 flowers open at a time. The calyx is tubular, and the ovary is glabrous and greenish whit. The fruit is globose or rounded, with a persistent calyx that turns black when ripe. The mesocarp is thick and has three locules with many seeds.

Traditional use: The ripen fruit are eaten by bird and wild boar and the rhizome is used to treat skin diseases.

Figure 5 depicts various aspects of *Alpinia songet.* Panel A shows the inflorescence with its flowers, illustrating the floral arrangement. Panel B focuses on a green flower, highlighting its unique coloration. Panel C provides a close-up of the labellum and stamen, detailing their structure and arrangement. Panel D presents the plant with immature fruit, showing this developmental stage. Panel E captures an immature fruit with a semipersistent calyx, emphasizing its transitional stage. Finally, Panel F displays a black mature fruit, indicating the plant's fruit at full maturity.

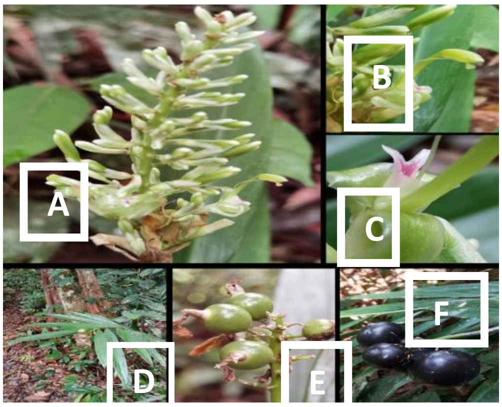

Figure 5: *Alpinia songet* A. Inflorescence with flowers. B. Green flower C. Close up of labellum and stamen D. Plant with unmatured fruit. E. Unmatured fruit with semipersistent calyx, F. Black matured fruit.

Figure 6 provides an overview of Alpinia songet by highlighting different parts of the plant. Panel A shows the whole plant, giving a complete view of its structure and growth habit. Panel B focuses on the rhizome and roots, illustrating the underground components that support the plant. Panel C displays the terminal inflorescence, highlighting the flowering part at the end of the stem. Panel D presents the fruit with an open seed, showing the internal structure of the fruit. Finally, Panel E shows the seeds with white flesh, providing a close-up view of the seed's texture and appearance.

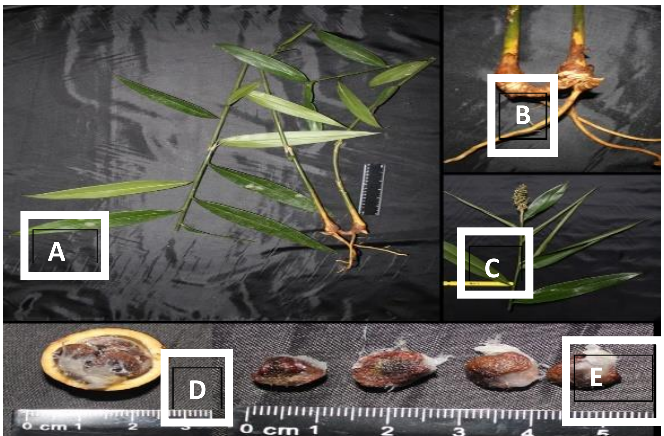

Figure 6: *Alpinia songet* A. Whole plant. B. Rhizome and roots. C. Terminal inflorescence. D. Fruit with open seed. E. Seeds with white flesh

Scanning Electron Micrographs of *Alpinia songet*

Figure 7 showcases Scanning Electron Micrographs of *Alpinia songet*, focusing on the microstructural details of the plant. Panel A displays the distribution of trichomes and stomata on the abaxial (lower) surface of the lamina, while Panel B highlights the distribution of trichomes and stomata on the adaxial (upper) lamina. Panel C illustrates the distribution of wax on the trichomes of the adaxial leaf surface, emphasizing the wax's pattern and coverage. Finally, Panel D presents the short, cone-shaped trichomes on the abaxial lamina, detailing their shape and structure.

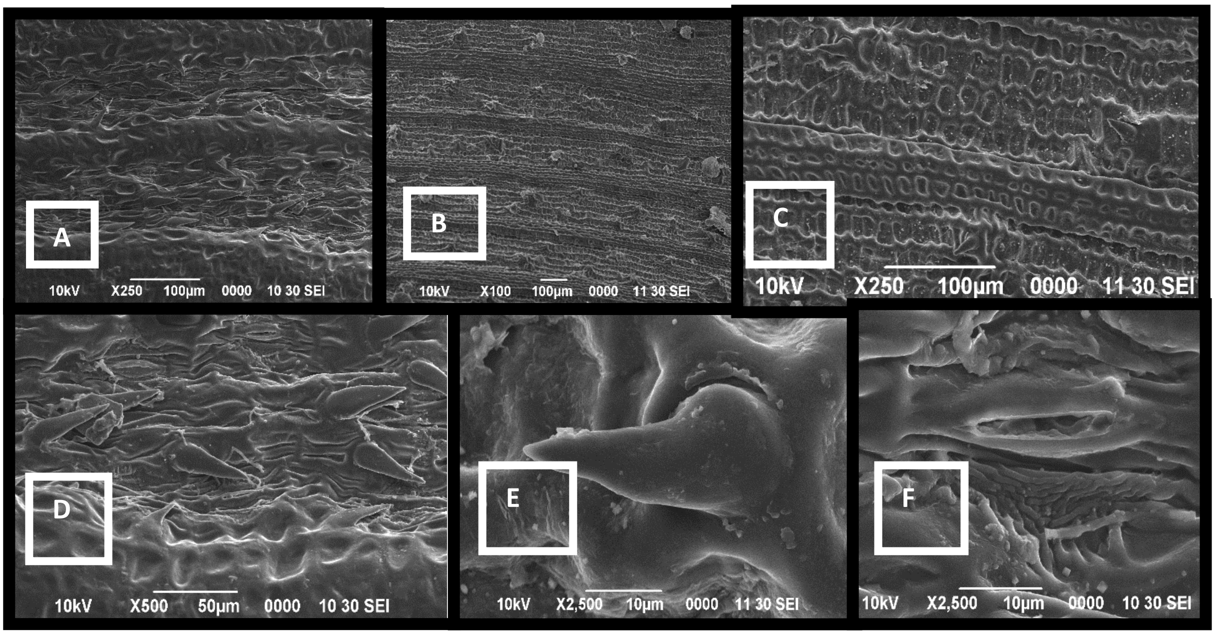

Figure 7: Scanning Electron Micrographs of *Alpinia songet*. A. Distribution of trichomes and stomata on abaxial lamina. B. Distribution of trichomes and stomata on adaxial lamina. C. Distribution of wax on trichomes of leaf adaxial. D. Short and cone shape trichome of abaxial lamina

Figure 8 presents Scanning Electron Micrographs of ***Alpinia songet***, specifically focusing on the epicuticular wax on the abaxial (lower) leaf surface. Panel G shows the distribution of rectangular crystalloids of epicuticular wax on the trichomes, highlighting their unique shape and arrangement. Panel H illustrates the distribution of these rectangular crystalloids on the stomata, emphasizing how the wax interacts with the stomatal structures.

Panel I provide a close-up view of the rectangular form of the wax crystalloids, detailing their specific shape and texture.

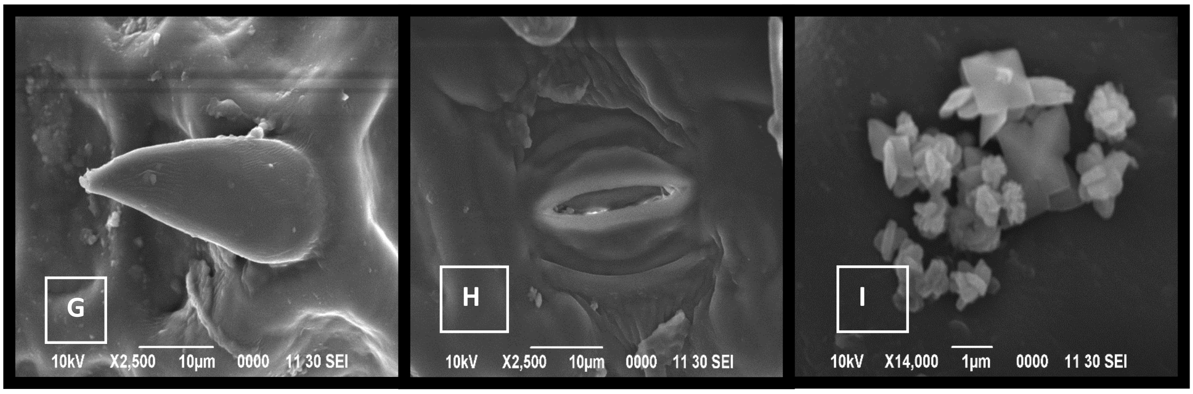

Figure 8: Scanning Electron Micrographs of *Alpinia songet* epicuticular wax on abaxial leaf. G. Distribution of rectangular crystalloid of epicuticular wax on trichome. H. Distribution of rectangular crystalloid of epicuticular wax on stomata I. Wax crystalloid rectangular form.

3. *Zingiber singaiensis* Ripen & Meekiong sp. nov

This short ginger plant has stout rhizomes and leaves with a pale green basal part and yellow rhizomes. The leaves are thick and alternate, with elongate blades and a rounded apex. The inflorescence forms an open pouch when the flower produces, with closely overlapping bracts. The flower is 4 cm long and produces one open at a time. The plant has a cream bracteole, a creamy white calyx, and a glabrous, green ovary. The plant has staminodes and an anther that is greenish and crested.

Traditional use: The inflorescence is eaten by animal such as wild boar and bird. It is potentially to be use as ornamental as the inflorescence has orange and pink colour.

Figure 9 illustrates various aspects of *Zingiber singaiensis*, showcasing different parts and features of the plant. Panel A presents the entire Zingiber singaiensis plant, giving an overview of its general structure and growth habit. Panel B displays the inflorescence with its characteristic orange color, highlighting the vibrant floral arrangement. Panel C focuses on the inflorescence with flowers, showing the white flowers in detail. Panel D provides a close-up view of the labellum and stamen, detailing their intricate structure and positioning. Panel E presents the cone-like flowering head, emphasizing its unique shape. Finally, Panel F depicts the inflorescence with an opened pink bract and black seed, illustrating the plant's reproductive structures and stages.

Figure 9: *Zingiber singaiensis* A. *Zingiber singaiensis* plant. B. Inflorescence with orange color. C. Inflorescence with flowers. C. White flower D. Close up of labellum and stamen E. Cone-like flowering head. F. Inflorescence with opened pink bract and black seed

Figure 10 provides detailed views and comparisons of the flower and its dissected parts of *Zingiber singaiensis*. Panel I shows a close-up of the entire flower, highlighting its overall structure and coloration. Panel J presents the labellum, dorsal corolla lobes, and stamen, illustrating these key floral components together. Panel K focuses on the three lobes of the labellum, detailing their shape and arrangement. Panel L provides a view of the dorsal corolla lobes, emphasizing their structure. Panel M shows the long, creamy white stamen, highlighting its length and color. Panel N presents a close-up of the anther of the flower, detailing the pollen-producing part. Finally, Panel O provides a close-up view of the ovary, illustrating its structure and position within the flower.

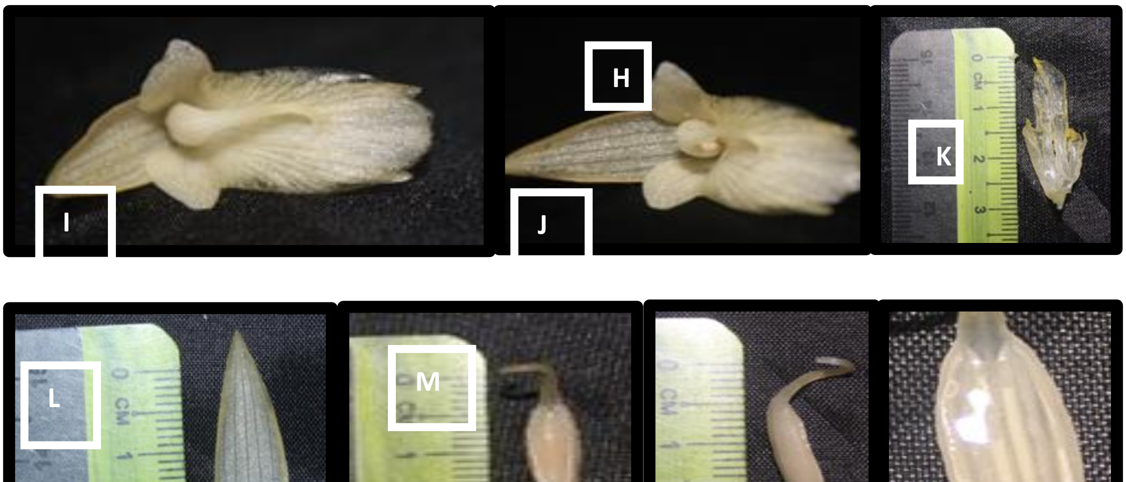

Figure 10: *Zingiber singaiensis* Flower and Its Dissected Parts in Comparison I. Close up of flower. J. Labellum, dorsal corolla lobes and stamen. K. Three lobes of labellum. L. Dorsal corolla lobes. M. Long, creamy white stamen. N. Anther of flower. O. Close up of ovary

Scanning Electron Micrographs of *Zingiber singaiensis*

Figure 11 features Scanning Electron Micrographs of *Zingiber singaiensis*, focusing on the detailed microstructural aspects of the plant's leaves. Panel A shows the abaxial (lower) surface of the leaf, highlighting its texture and features. Panel B displays the adaxial (upper) surface of the leaf, providing a comparative view. Panel C illustrates the distribution of stomata on the abaxial lamina, emphasizing the pattern and density of these openings. Panel D presents the smooth coverings described as 'resinous layers' and 'soft waxes,' showing the protective coatings on the leaf surface. Panel E focuses on the short and stunted trichomes on the abaxial lamina, detailing their structure and appearance. Finally, Panel F provides a close-up view of the stomata with bean-shaped guard cells, highlighting the morphology of these important structures for gas exchange.

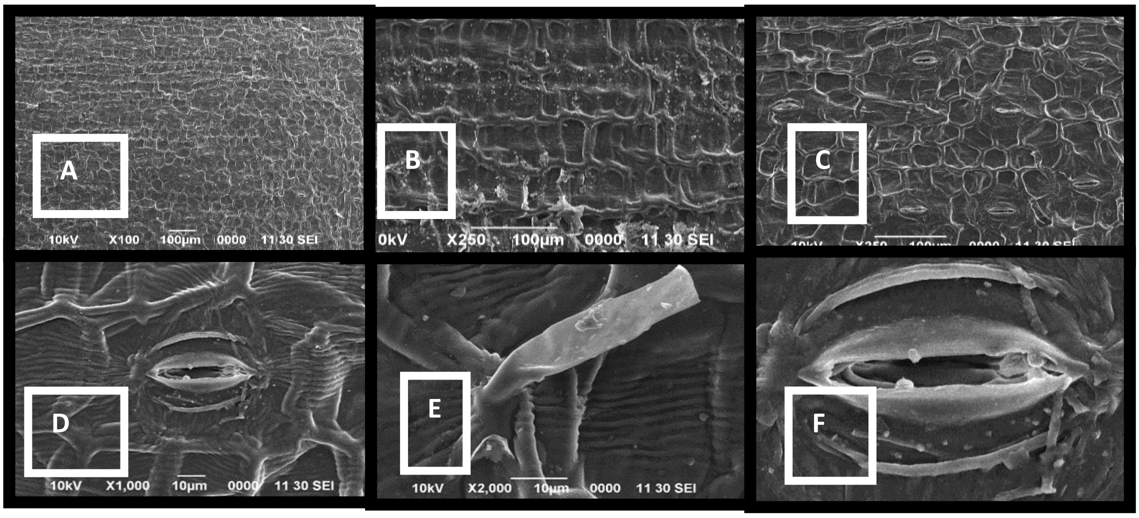

Figure 11: Scanning Electron Micrographs of *Zingiber singaiensis* A. Leaf abaxial B. Leaf adaxial. C. Distribution of stomata on abaxial lamina. D. Smooth coverings like ‘resinous layers’ and ‘soft waxes’. E. Short and stunted trichome of abaxial lamina. F. Stomata with bean-shaped guard cells

4. *Amomum jackliamii* Ripen & Meekiong sp. nov.

Amomum jackliamii is a perennial herbaceous species with a creeping rhizome, up to 60 cm tall, and 5-7 leaves. It produces lemony and strong smells when crushed. The leaves have a glabrous margin and white prickly hairs.

Inflorescences grow on the ground, with white-cream flowers. The plant has a tubular calyx, corolla, and labellum. The fruit is not seen, but it is fast-growing, ornamental, and scented, and grows in humid areas.

Traditional use: Locally known as Dowen poras due to its leaves that produce a slightly lemon grass smell. The leaves are crushed and rubbed on forehead for headaches.

Figure 12 illustrates various aspects of *Amomum jackliamii*, highlighting its key features and structures. Panel A shows a herbarium sample of the Amomum jackliamii plant, providing a preserved view of its overall form. Panel B presents the erect inflorescence, demonstrating its upright floral arrangement. Panel C focuses on the white prickly hairs towards the leaf tip, detailing their unique texture and appearance. Panel D depicts the inflorescence with flowers prostrate on the surface soil, showing this distinctive growth pattern. Panel E offers a close-up view of the inflorescence, emphasizing the arrangement and detail of the flowers. Finally, Panel F provides a close-up of the labellum and stamen, highlighting their intricate structure and positioning.

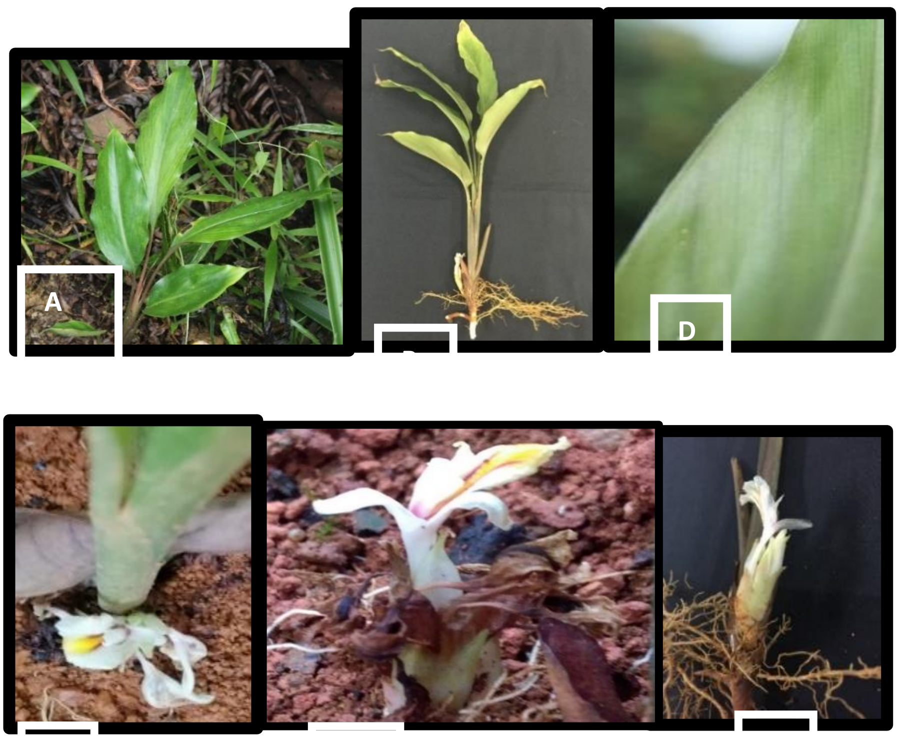

Figure 12. *Amomum jackliamii* A. Herbarium sample of *Amomum jackliamii* plant. B. Erect inflorescence C. White prickly hairs towards the leaf tip D. Inflorescence with flowers prostrate in the surface soil E. Close up of inflorescence F. Close up of labellum, stamen

Figure 13 provides a detailed examination of the flower and its dissected parts of *Amomum jackliamii*. Panel H shows a close-up of the labellum and stamen, highlighting their intricate structures. Panel I presents the front view of the flower, offering a complete perspective of its form. Panel J displays the labellum, dorsal corolla lobes, and stamen together, emphasizing the key floral components. Panel K provides a close-up side view of the flower, showcasing its lateral structure, while Panel L illustrates the flower with its protective calyx.

Figure 13: *Amomum jackliamii* Flower and Its Dissected Parts in Comparison. H. Close up of labellum and stamen. I Front view of flower J. Labellum, dorsal corolla lobes and stamen. K. Close up of flower (side view) L. Flower with calyx

Scanning Electron Micrographs of *Amomum jackliamii*

Figure 14 presents Scanning Electron Micrographs of *Amomum jackliamii*, focusing on the microstructural details of its leaves. Panel A shows the distribution of stomata and trichomes on the abaxial (lower) surface of the leaf, highlighting their arrangement and density. Panel B illustrates the distribution of wax around the stomata on the abaxial leaf surface, emphasizing the protective wax layer. Panel C provides a view of the short and stunted trichomes on the abaxial lamina, detailing their structure. Panel D depicts the distribution of stomata and trichomes on the adaxial (upper) leaf surface, offering a comparative view. Panel E shows a half-opened stomata on the adaxial leaf surface, capturing the stomatal opening mechanism. Finally, Panel F highlights the fissured layer of epicuticular wax on the abaxial leaf surface, showcasing the detailed texture and pattern of the wax layer.

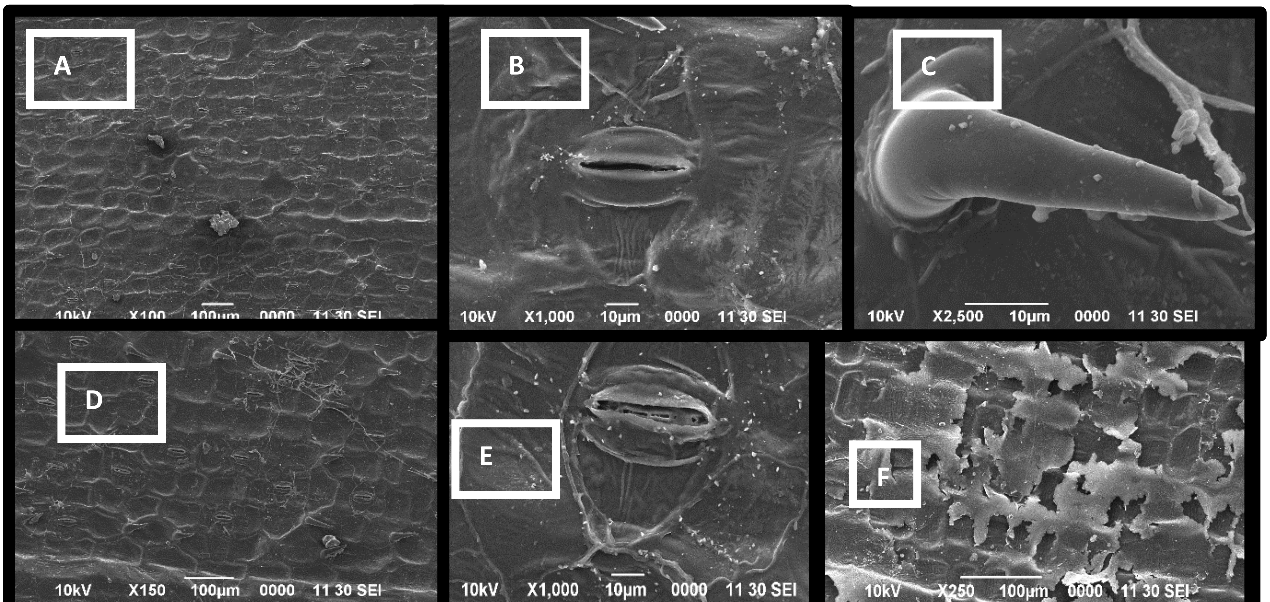

Figure 14: Scanning Electron Micrographs of *Amomum jackliamii* A. Distribution of stomata and trichome of leaf abaxial. B. Distribution of wax around stomata of leaf abaxial C. Short and stunted trichome of abaxial lamina. D. Distribution of stomata and trichome of leaf adaxial E. Half opened stomata of leaf adaxial F. Fissured layer of epicuticular wax on leaf abaxial.

DISCUSSION

Characterization of Four New Zingiberaceae Species in Singai: Morphological Descriptions and Traditional Uses

The study identified four new species in the Singai area, comprising two species from the genera *Zingiber* and *Amomum* and one species each from *Alpinia* and *Sulettaria*. These species are integral to the local community's traditional practices and are described in detail based on their morphological characteristics and uses. For instance, *Sulettaria meekiongii* Ripen & S.P. Teo is characterized by its light green leaves, erect conical inflorescences, and pale yellow labellum. Traditionally, its mature leaf sheaths are split into strips and used as string. The scanning electron micrographs further reveal the intricate distribution of trichomes and stomata on the leaf surfaces, providing a comprehensive understanding of the plant's microanatomy.

The objective to explore and document the micromorphological characteristics of four new Zingiberaceae species in Singai Bau, Sarawak, was achieved through the use of Scanning Electron Microscopy (SEM) to capture detailed images of their microstructural features. The high-resolution images provided a comprehensive view of the trichomes, stomata, epidermal cells, and epicuticular waxes, revealing unique morphological characteristics that distinguish these species from others. Documenting these micromorphological traits is crucial as it enhances our understanding of how these plants adapt to their environment, which is vital for their conservation. This study not only introduces new species but also contributes significantly to the broader body of knowledge regarding plant micromorphology in tropical environments. These findings have important implications for taxonomy, ecology, and conservation biology, providing a foundation for future research on plant adaptations and their ecological roles.

The study on the micromorphological characteristics of four new Zingiberaceae species in Singai Bau, Sarawak, represents a significant advancement in botanical research techniques, particularly through the innovative use of SEM. This technology enabled researchers to capture high-resolution images that reveal intricate details of plant structures, such as trichomes, stomata, epidermal cells, and epicuticular waxes, which are not visible through traditional microscopy. This advanced approach has allowed for a deeper understanding of plant anatomy and adaptations, contributing to the broader field of plant morphology by providing detailed insights into the structural complexities of these newly identified species.

In addition to advancing research techniques, the detailed micromorphological data obtained through SEM has enhanced the precision of plant taxonomy. By identifying unique morphological traits specific to these new Zingiberaceae species, the study aids in the accurate classification of these plants, which is crucial for understanding and preserving biodiversity. This precision is particularly important for conservation efforts, as it ensures that species are correctly identified and categorized, allowing for the development of targeted strategies to protect them. The study's findings are instrumental in refining taxonomic frameworks and enhancing our understanding of species diversity, particularly in tropical environments where biodiversity is rich yet vulnerable.

Beyond its contributions to taxonomy and conservation, the study has broader implications for ecological and technological innovation. The detailed documentation of micromorphological traits provides valuable insights into how these plants adapt to their environment, shedding light on their ecological roles and evolutionary processes. Moreover, the knowledge gained from this research could inspire technological innovations, especially in biomimetics, where biological structures are emulated in the development of new materials and technologies. The unique surface structures observed, such as the arrangement of trichomes and epicuticular waxes, have potential applications in designing materials with water-repellent properties or specific optical characteristics. Thus, the study not only enriches scientific knowledge but also paves the way for future technological advancements.

To identify and classify the unique anatomical features of the new Zingiberaceae species.

The identification and classification of these new species were achieved by comparing their anatomical features with existing botanical records. The study employed a systematic approach involving herbarium specimens, taxonomic keys, and expert verification to ensure accurate identification. The classification of these species into the appropriate genera of *Zingiber*, *Amomum*, *Alpinia*, and *Sulettaria* demonstrates the diversity within the

Zingiberaceae family. The discovery of unique anatomical features, such as the distinctive arrangement of stomata and trichomes, not only differentiates these species from known ones but also provides insights into their evolutionary adaptations. This classification contributes to the growing database of plant biodiversity in Borneo, underscoring the region's role as a hotspot for new species discovery.

Alpinia songet Ripen & Meekiong is noted for its tall pseudostems, terminal inflorescences with vibrant flowers, and thick, globose fruits that turn black when ripe. Its rhizomes are traditionally used to treat skin diseases, while the ripe fruits are consumed by birds and wild boars. *Zingiber singaiensis* Ripen & Meekiong features a stout rhizome, elongate leaves, and an inflorescence with closely overlapping bracts. Its flowers, which open one at a time, have potential ornamental uses due to their orange and pink colors. *Amomum jackliamii* Ripen & Meekiong is distinguished by its creeping rhizome, white-cream flowers, and lemony-smelling leaves, which are used to alleviate headaches. The detailed morphological descriptions and traditional uses highlight the ecological and cultural significance of these new species.

Scanning Electron Micrographs of Four New Zingiberaceae Species in Singai

The identification and characterization of four new species of *Sulettaria meekiongii, Alpinia songet, Zingiber singaiensis*, and *Amomum jackliamii* represent significant additions to the botanical knowledge of the Singai region. The document offers an in-depth examination of the microstructural aspects of various plant species through scanning electron micrographs, showcasing the intricate details of their leaf surfaces. For *Alpinia songet*, the micrographs reveal a diverse distribution of trichomes and stomata on both the abaxial (lower) and adaxial (upper) surfaces of the leaves. Panel A highlights the lower surface, illustrating how these tiny structures are spaced and oriented, while Panel B shifts focus to the upper surface, providing a comparative view. Panel C emphasizes the presence and pattern of wax on the trichomes of the adaxial surface, detailing its protective role. Panel D concludes with a close-up of the short cone-shaped trichomes on the abaxial lamina, emphasizing their unique shape and structure.

The micrographs of *Sulettaria meekiongii* offer a detailed look at the various structural features across different parts of the leaf. Panels A and B both emphasize the trichomes and stomata on the adaxial surface, but from slightly different perspectives, providing a comprehensive view of this upper layer. Panel C focuses on the long simple non-glandular trichomes, highlighting their distinctive pyramidal and unicellular structures. Subsequent panels, such as D and E, illustrate the distribution patterns of these features on both the upper and lower surfaces of the leaf. Additionally, Panels F and G explore the epicuticular wax patterns and the non-segmented trichomes, offering insights into the protective mechanisms of the leaf. Panel H compares short and long trichomes, showcasing the leaf's diverse microanatomy.

In the case of *Zingiber singaiensis,* the micrographs delve into the detailed texture and features of both the abaxial and adaxial leaf surfaces. Panel A offers an overview of the lower surface, while Panel B provides a comparative look at the upper surface. Panel C highlights the distribution and pattern of stomata on the abaxial lamina, crucial for understanding gas exchange processes. Panel D reveals the presence of smooth coverings, described as resinous layers and soft waxes, that protect the leaf surface. The short and stunted trichomes on the abaxial lamina are showcased in Panel E, and Panel F provides a close-up view of the stomata, emphasizing the bean-shaped guard cells that are vital for regulating gas exchange.

Lastly, the micrographs of *Amomum jackliamii* offer a comprehensive view of the microstructural details of its leaves. Panel A highlights the arrangement and density of stomata and trichomes on the abaxial surface, while Panel B emphasizes the protective wax layer surrounding the stomata. Panel C focuses on the short and stunted trichomes, detailing their structure and function. The adaxial surface is examined in Panel D, showcasing the comparative distribution of stomata and trichomes. A detailed look at a half-opened stomata is provided in Panel E, capturing the mechanics of stomatal opening. Finally, Panel F highlights the fissured layer of epicuticular wax on the abaxial surface, detailing its texture and protective role. These comprehensive micrographs provide valuable insights into the structural adaptations of these plant species, highlighting their ecological and physiological significance.

The scanning electron micrographs reveal distinct microstructural adaptations in *Alpinia songet, Sulettaria meekiongii, Zingiber singaiensis,* and *Amomum jackliamii. Alpinia songet* shows a notable distribution of trichomes and stomata on both leaf surfaces, with wax patterns providing additional protection. *Sulettaria meekiongii* presents a complex array of trichomes, including long, simple non-glandular and short cone-shaped varieties, along with distinct epicuticular wax patterns enhancing leaf protection. *Zingiber singaiensis* features smooth resinous layers and soft waxes on the abaxial surface, short stunted trichomes, and detailed stomatal structures, emphasizing gas exchange and defense mechanisms. In contrast, *Amomum jackliamii* displays a dense arrangement of stomata and trichomes, with a protective wax layer and fissured epicuticular wax, showcasing its adaptive strategies for environmental stress. Each species demonstrates unique structural features, such as varying trichome types, stomatal distributions, and protective waxes, highlighting their specialized adaptations to thrive in different ecological niches. These micrographs collectively illustrate the diverse evolutionary strategies employed by these plants to optimize their survival and function.

To assess the ecological and cultural significance of the newly identified Zingiberaceae species

The assessment of the ecological and cultural significance of the newly identified Zingiberaceae species is critical for understanding their role within both the natural environment and the local communities that interact with them. These species not only contribute to the rich biodiversity of the Singai Bau region but also play an integral part in the traditional practices of the Bidayuh community. The ecological significance of these plants lies in their adaptation to the unique environmental conditions of the area, as evidenced by their specialized anatomical features such as trichomes, stomata, and epicuticular waxes. These adaptations help the plants thrive in their specific habitats, contributing to the overall health and stability of the ecosystem.

Culturally, these Zingiberaceae species are deeply embedded in the traditional knowledge and practices of the Bidayuh people. Each species has its unique use, whether in traditional medicine, as materials for craft, or as food sources for both humans and wildlife. For instance, *Alpinia songet* is traditionally used to treat skin diseases, while *Amomum jackliamii* is known for its lemony-scented leaves used to alleviate headaches. These uses highlight the importance of preserving both the plants and the knowledge associated with them, as they represent a living heritage that contributes to the community's identity and well-being.

Understanding the ecological and cultural significance of these species is essential for developing conservation strategies that not only protect the plants but also support the communities that rely on them. This holistic approach ensures that conservation efforts are sustainable and culturally sensitive, fostering a deeper connection between people and their environment.

CONCLUSION

The conclusion of this research highlights the significant impact of integrating modern scientific techniques with traditional ecological knowledge in the study of new Zingiberaceae species. By documenting the micromorphological characteristics and traditional uses of these species, the research enhances our understanding of plant biodiversity in tropical environments and underscores the importance of preserving cultural heritage. The use of SEM provided detailed insights into the unique anatomical features of these plants, contributing to more accurate taxonomic classifications and a deeper understanding of their ecological adaptations.

The study underscores the crucial role that traditional knowledge plays in biodiversity conservation. By recognizing and documenting the cultural significance of these species within the Bidayuh community, the research advocates for conservation strategies that are both scientifically rigorous and culturally sensitive. This interdisciplinary approach demonstrates that the preservation of biological and cultural diversity can be achieved through collaborative efforts that bridge the gap between modern science and indigenous knowledge systems.

The study provides significant insights into the micromorphological characteristics of four newly identified species within the Zingiberaceae family. Conducted in Mount Sijanjang Singai, a region renowned for its biodiversity and historical significance, the research documented intricate surface structures of these plants' leaves, including trichomes, stomata, epidermal cells, subsidiary cells, epicuticular wax, and glands through high-resolution SEM imaging. This meticulous analysis revealed unique anatomical features of each species, such as long, simple, non-glandular trichomes and rectangular epicuticular wax patterns in *Sulettaria meekiongii*, diverse trichome distributions and protective wax layers in *Alpinia songet*, smooth resinous layers and stunted trichomes in *Zingiber singaiensis*, and dense stomatal arrangements with fissured epicuticular wax in *Amomum jackliamii*. These findings lay the foundation for further studies and underscore the ecological and cultural importance of these species within the Bidayuh community.

The traditional uses of these Zingiberaceae plants by the Bidayuh community were also documented, emphasizing their cultural significance. For example, *Sulettaria meekiongii* is used to make strings from mature leaf sheaths, *Alpinia songet's* rhizomes are employed in treating skin diseases, *Zingiber singaiensis* is considered for ornamental purposes due to its vibrant orange and pink inflorescences, and *Amomum jackliamii* is known for its lemony-smelling leaves that are used to alleviate headaches. By integrating traditional knowledge with scientific techniques, the study highlights the necessity of preserving both the biological and cultural heritage of the region. This documentation is crucial for ensuring that the valuable traditional practices and knowledge of the Bidayuh community are recognized, respected, and incorporated into conservation strategies.

The ecological significance of the study is profound, as it supports biodiversity conservation efforts by providing detailed documentation of the micromorphological features of these plants. Accurate taxonomic classification is essential for the conservation of biodiversity, and the high-resolution SEM images obtained in this study aid in this classification by revealing intricate details of plant surface structures. Understanding these characteristics not only contributes to taxonomy but also enhances ecological studies and the development of new applications in agriculture and pharmacology. Furthermore, the study's findings emphasize the importance of the Singai Bau region as a biodiversity hotspot, advocating for the protection and sustainable management of its habitats.

Ultimately, this research contributes valuable knowledge that will aid in the ongoing efforts to protect and conserve the rich biodiversity of Borneo, ensuring that both the natural environment and the cultural practices of local communities are preserved for future generations.

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