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Original Article

Diversity, traditional uses, chromosome number, pollen, and leaf anatomy of the genus *Hellenia* in Ubon Ratchathani Province, Thailand

Piyaporn Saensouk[1], Sombat Appamaraka[2], Thawatphong Boonma[2], Pimtida Niamngon[3], Tawanon Niamngon[4], Areerat Ragsasilp[5], Sarayut Rakarcha[6], Kamonwan Koompoot[2], Ahmad Dwi Setyawan[7,8], Arunrat Chaveerach[9], Surapon Saensouk[2]*

[1]Diversity of Family Zingiberaceae and Vascular Plant for Its Applications Research Unit, Department of Biology, Faculty of Science, Mahasarakham University, Kantarawichai, Maha Sarakham, 44150, Thailand
[2]Diversity of Family Zingiberaceae and Vascular Plant for Its Applications Research Unit, Biodiversity Program, Walai Rukhavej Botanical Research Institute, Mahasarakham University, Kantarawichai, Maha Sarakham, 44150, Thailand
[3]Diversity of Family Zingiberaceae and Vascular Plant for Its Applications Research Unit, MSU Science Park, Mahasarakham University, Kantarawichai, Maha Sarakham, 44150, Thailand
[4]Diversity of Family Zingiberaceae and Vascular Plant for Its Applications Research Unit, Faculty of Environment and Resource Studies, Mahasarakham University, Maha Sarakham 44150, Thailand
[5]Diversity of Family Zingiberaceae and Vascular Plant for Its Applications Research Unit, Program of Environmental and Resource Management, Faculty of Environment and Resource Studies, Mahasarakham University, Maha Sarakham 44150, Thailand
[6]Queen Sirikit Botanic Garden, The Botanical Garden Organization, Chiang Mai 50180, Thailand
[7]Department of Environmental Science, Faculty of Mathematics and Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia
[8]Biodiversity Research Group, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia
[9]Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

**Corresponding author's email:* surapon.s@msu.ac.th
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Abstract

The genus *Hellenia* (Costaceae) is noted for its taxonomic complexity and ecological significance. This study addresses the limited understanding of the genus in Northeastern Thailand by investigating species diversity, conservation status, traditional uses, chromosome number, pollen characteristics, and unique peeling leaf morphology in Ubon Ratchathani Province. Fieldwork and laboratory analyses conducted in 2023 identified two species, *Hellenia lacera* (Gagnep.) Govaerts and *H. speciosa Hellenia lacera* (J.Koenig) Govaerts, which differ in their growth patterns. Conservation assessments proposed to placed *H. speciosa* in the Least Concern (LC) category according to the International Union for Conservation of Nature (IUCN) criteria. The consistent diploid chromosome number (2n = 18) and unique pollen morphology offer important taxonomic insights. Additionally, the leaf morphology, marked by epidermal detachment, presents a new characteristic within the genus and may indicate ecological adaptations or evolutionary significance. These findings contribute to the understanding *Hellenia*'s biology and highlight its ecological and taxonomic relevance within the Costaceae family.

Keywords: Chromosomal structure, Conservation status, Costaceae family, *Hellenia*, Leaf anatomy, Pollen morphology, Species diversity, Taxonomy, Thailand, Traditional Uses, Ubon Ratchathani

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Introduction

The Costaceae family, a relative of the Zingiberaceae, encompasses tropical and subtropical plants often known as 'spiral gingers' due to their distinctive spiral leaf arrangement and unique flower structures (Larsen, 2008). This family includes eight recognized genera and 162 species that primarily grow in humid, shaded environments, such as forest understories and stream banks, with a distribution across Central and South America, Africa, and Southeast Asia (Govaerts, 2004; Specht and Stevenson, 2006; Dutta, 2010; POWO, 2024). Traditionally, Costaceae species have been appreciated for their medicinal, culinary, and ornamental uses, often valued for their spiral foliage and eye-catching flowers (Specht and Stevenson, 2006; POWO, 2024). However, several species within this family face significant conservation challenges, with some being threatened in their natural habitats (IUCN, 2023). As habitat loss and environmental changes continue to accelerate, there is an increasing need for conservation efforts, particularly for species like *Hellenia*, which are becoming more vulnerable due to their specialized habitats requirements and restricted distribution.

The genus *Hellenia* Retz., which was previously placed under the genus *Costus* L. within the Costaceae family, consists of eight well-known species found across regions including the Indian subcontinent, Southern China, Southeast Asia, Queensland, and New Guinea (Larsen, 2008; APG IV, 2016; IUCN, 2023; POWO, 2024). These plants are significant locally, as they are collected from natural habitats for traditional medicinal, culinary uses and are also popular ornamental plants in tropical regions (Niamngon et al., 2023). In Thailand, five species of Costaceae are recorded native to the country, with three belonging to *Hellenia* (Larsen, 2008; POWO, 2024). One notable species, *Hellenia speciosa* (crêpe ginger), occurs both in the wild and in cultivation for its decorative value (Natungmol et al., 2019). *Hellenia speciosa*, native to tropical and subtropical Asia, is also found in northeastern Queensland where it thrives in wet tropical conditions (Larsen, 2008).

The genus *Hellenia* plays a crucial role in enhancing the ecological diversity of Ubon Ratchathani, supporting local biodiversity by providing food and habitat for various species. Its presence in both natural and cultivated environments help sustain ecosystem balance. However, the local *Hellenia* populations may be at risk from environmental challenges such as habitat loss and climate change. This study aims to document the species diversity and conservation status of *Hellenia* in Ubon Ratchathani, Eastern Thailand, it investigates the plant's distribution, ecological significance, and traditional uses. Additionally, the study examines chromosome numbers, pollen morphology, and leaf morphology, underscoring the genus's vital role in supporting biodiversity and maintaining ecosystem functions in this unique region.

Material and Methods

Study area

Ubon Ratchathani Province located in northeastern Thailand, was chosen as the study area due to its remarkable biodiversity and the presence of numerous rare and endemic plant species (Brodie et al., 2016; Suksawang, 2018; Saensouk et al., 2024a). The province hosts a variety of habitats, including mixed deciduous forests, dry dipterocarp forests, evergreen forests, and wetlands, all of which play a vital role in plant conservation (Duangchantrasiri et al., 2016; Suksawang, 2018; Iqbal, 2019). Additionally, this region serves as a transitional zone where species from multiple biogeographical regions converge, highlighting its importance for ecological and conservation research (Suksawang, 2018).

Plant material and diversity study

Fresh specimens of *Hellenia lacera* and *H. speciosa* were collected during fieldwork conducted from January to December 2023 and have been deposited at the Mahasarakham University Herbarium (MSU) [*Hellenia lacera*: Ubon Ratchathani, *Saensouk HLN2301* (MSU!). *Hellenia speciosa*: Ubon Ratchathani, *Saensouk HLN2302* (MSU!)]. Field observations were carried out to record ecological information, phenological traits, distribution patterns, and vernacular names of the species. Identification was based on a comprehensive review of botanical literature, examination of herbarium specimens from various herbaria (e.g. BK!, BKF!, KKU!, QBG!, PSU!), and comparison with taxonomic references and online photographs (Govaerts, 2004; Specht and Stevenson, 2006; Larsen, 2008; Bongcheewin et al., 2015; Esser and Saw, 2015; Paton et al., 2016; Ye and Xia, 2016; Ahmad et al., 2017; Bongcheewin et al., 2017; Leeratiwong et al., 2017; Chen et al., 2018; Tokan et al., 2018; Budiyanti et al., 2019; Rozaini et al., 2019; Chantaranothai, 2021; Esser, 2021; Bongcheewin et al., 2022; Ngernsaengsaruay et al., 2022; Ragsasilp et al., 2022; Rakarcha et al., 2022; Singh, 2022; Tagane et al., 2022; Zhang et al., 2022; Wali et al., 2022; Inta et al., 2023; Boonma et al., 2023, 2024; POWO, 2024).

Traditional uses study

Semi-structured interviews were conducted with 20 individuals, including local experts in traditional medicine and plant usage, to investigate the traditional applications of *Hellenia*. Participants were selected for their expertise in the local flora and their knowledge of cultural practices. The interviews focused on documenting the plant's roles as food, spices, ornamental plants, and in rituals. A standardized questionnaire guided discussions on usage types, preparation methods, and cultural significance. The collected information was analyzed qualitatively to identify shared patterns and preserve local knowledge about these species (Trisonthi, 2018; Numpulsuksant et al., 2021; Phatlamphu et al., 2021; Wali et al., 2022; Boonma et al., 2023, 2024; Niamngon et al., 2023; Inta et al., 2023; Phengmala et al., 2023; Sudchaleaw et al., 2023).

Conservation assessment

The conservation status of *Hellenia lacera* and *H. speciosa* was evaluated following the IUCN Red List criteria (IUCN, 2023). Key factors considered included population size, distribution range, habitat threats, and ecological characteristics specific to each species. The classification of their conservation status drew upon data from field observations, consultations with experts, and an examination of relevant local conservation policies. In the case of *Hellenia lacera*, which has been less extensively documented, efforts were prioritized to gather additional information, ensuring a more comprehensive and accurate assessment.

Chromosome numbers observation

Determination of chromosome numbers was conducted using the squash technique as described by Saenprom et al. (2018), Senavongse et al. (2018, 2020), Saensouk et al. (2019, 2023, 2024b), and Sengthong et al. (2023). Root tips of were pretreated with paradichlorobenzene (PDB) at 4°C for six hours and subsequently fixed in 3:1(v/v) solution of ethanol and acetic acid at room temperature for 30 minutes. The fixed samples were then stored at 4°C for future analysis. Subsequently, the root tips were washed in distilled water, hydrolyzed in 1M HCl at 60 degrees Celsius for five minutes, and washed again in distilled water. They were then stained in 2% aceto-orcein and analyzed using the squash technique. Observations were conducted under a light microscope (Zeiss Axiostar Plus) at a magnification of 400x. For data analysis, chromosome numbers were determined from the metaphase chromosomes visible in photomicrographs, with 10 metaphase plates examined for each species. The chromosome numbers for description were based on the terminology by Levan et al. (1964), and corroborated by earlier studies (Senavongse et al., 2018, 2020; Saensouk et al., 2019, 2023, 2024b; Sengthong et al., 2023).

Pollen observation

The anthers of two *Hellenia* species were preserved in 70% ethanol. Pollen grains from the *Hellenia* genus in Ubon Ratchathani Province, Thailand, were examined using scanning electron microscopy (SEM). The samples were dehydrated through a graded alcohol series of 70%, 80%, 95%, and 100%. For LM studies, pollen grains were mounted in silicone oil and sealed with paraffin. A minimum of 30 pollen grains per sample were measured to determine the diameter (µm) along the polar and equatorial axes. Data analyses were conducted using the mean and standard error. The shapes were described based on the P/E ratio (the ratio of the polar axis length to the equatorial axis length). The classification of pollen shape and size followed Erdtman's system. For SEM studies, pollen grains in absolute alcohol were dried on aluminum stubs using double-sided cellophane tape. Samples were then sputter-coated with gold-palladium and examined with a JEOL JSM 8460LV SEM to analyze the exine sculpturing and aperture. The terminology used to describe the palynological characteristics followed Punt's guidelines (Rakarcha et al., 2018; Saensouk and Saensouk, 2022, 2023; Hanchana et al., 2023).

Peeling leaf anatomy

Specimens were prepared by mechanically scraping the epidermal peelings between the midrib and margin of the leaf lamina. The upper and lower epidermis of the center regions of fully developed leaves were removed from fresh leaves. Next, the specimens underwent dehydration using a series of graded alcohol concentrations, specifically 30%, 50%, and 70%. Finally, they were stained with safranin. After staining, the specimens underwent two washes in 70% alcohol and were then dehydrated using a series of alcohol concentrations: 80%, 95%, and 100%. This was followed by a mixture of absolute alcohol and xylene in a 1:1 ratio, and finally xylene alone. The specimens were subsequently embedded in DePeX and captured at magnifications of 100× and 400× using a ZEISS Axio Lab.A1 light microscope. Analyzed were over 15 photos per leaf for each species, with an emphasis on the types of epidermal cells, the shapes and quantities of subsidiary cells surrounding pairs of guard cells, and the density of stomata per leaf area (Rakarcha et al., 2018; Hanchana et al., 2023). The Mahasarakham University Herbarium houses and maintains all specimens and slide collections.

Statistical analysis

To ensure reproducibility and accuracy, statistical analyses were conducted using descriptive statistics (mean, standard deviation) for phenological and morphological data. Chromosome number data were analyzed qualitatively through photomicrographs, and differences between species were assessed using standard comparative methods. Data from the traditional uses study were analyzed using thematic analysis to identify common themes and usage patterns. The findings were then integrated into the overall study to inform conservation recommendations.

Results

1. Diversity of *Hellenia* genus from Ubon Ratchathani Province, Thailand

Hellenia lacera (Gagnep.) Govaerts (Figure 1A) and *H. speciosa* (J. Koenig) Govaerts (Figure 1B), have been identified in Ubon Ratchathani Province, Thailand. This finding is consistent with the work of Larsen (2008) and the data from POWO (2024). *Hellenia* was initially treated as part of the genus *Costus*. However, based on differences in morphological characteristics, particularly in stem structure and floral anatomy, taxonomists have recognized *Hellenia* as a distinct genus. The reclassification was supported by detailed studies, including those by Larsen (2008), which highlighted the differences between *Hellenia* and other closely related genera within the Costaceae family.

1.1. Morphology of *Hellenia lacera* (Gagnep.) Govaerts

The plant has roots that branch out from the rhizome. The rhizome has a segmented appearance, while the aerial stem is straight and does not twist when mature, reaching a height of 1-2 m. The stem is upright, with leaves arranged alternately in a spiral pattern. Therefore, *Hellenia lacera* (Gagnep.) Govaerts is characterized by a vertical stem that remains straight and does not spiral as it matures. The leaves are simple, elliptical, 20-40 cm long, and 6-15 cm width. Leaves are smooth adaxially and dark green, while the abaxial side is covered with fine hairs and pale green, apex pointed, base rounded. The petioles are short, and the ligules are also short, with the leaf sheaths clasping around the stem. The flowers are borne in inflorescences at the stem tips, with the inflorescence measuring about 10 cm long and 5-7 cm width. The flower stalk is approximately 3 cm long and 1 cm wide. The bracts are lanceolate, about 9 cm long and 7 cm wide, and they split into fibers when mature. The smaller bracts are around 6 cm long and 3.5 cm wide. The flowers have green calyx tubes that turn red when mature, measuring about 3 cm long and 1.8 cm wide, and they split into three lobes. The corolla tube is shorter than the calyx, white with a pink tint, measuring 3-5 cm long, and 2.5-3 cm width. The labellum c. 4 cm long, and 2.5 cm wide, with a hairy surface and wavy margins. The stamens on either side of the labellum are usually sterile, while the fertile stamen is yellow, with a filament c. 5 cm long, and c. 3 cm wide. The anther has two lobes. The pistil has a triangular ovary, a single, slender style, and a semicircular stigma with nectar glands. The fruit is angular, and the seeds are black. (Figure 1A)

The primary distinguishing feature of *Hellenia lacera* (Gagnep.) Govaerts is its vertical stem, which remains straight throughout its development, lacking the spiraling growth pattern seen in some other species within the genus. This straight, non-twisting growth habit serves as a key characteristic that differentiates *Hellenia lacera* from related species. The consistency

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of this feature with previous observations, such as those reported by Larsen (2008), further supports its significance as a defining trait of the species.

1.2. Morphology of *Hellenia speciosa* (J.Koenig) Govaerts

The plant has roots that branch out from the rhizome. The rhizome is segmented and about 1-2 m long. The aerial stem twists into a spiral as it matures, reaching a height of 1-2 m, and the upper part of the stem branches out when mature. Therefore, the stem of *Hellenia speciosa* (J. Koenig) Govaerts begins as straight but gradually curves into a spiral shape as it matures. The leaves are simple and arranged alternately in a spiral pattern, resembling the shape of a snail's shell. The leaf blade is lanceolate, measuring about 23 cm long, and 6 cm width. Leaves are smooth adaxially and dark green, while the abaxial side is covered with fine hairs and pale green, apex pointed, and rounded base. The petioles are short, and the ligules are also short, with the leaf sheaths clasping around the stem. The flowers are borne in inflorescences at the stem tips, with the inflorescence measuring about 12 cm in length and 7 cm in width. The flower stalk is approximately 4 cm long and 2 cm wide. The bracts are lanceolate, about 4 cm long and 2 cm wide. The smaller bracts are around 2.5 cm long and 1.5 cm wide, with pointed tips. The flowers have green calyx tubes that turn red when mature, measuring about 4.5 cm long and 1.5 cm wide, and they split into three lobes. The corolla tube is shorter than the calyx, white in color, measuring 3-5 cm in length and 2.5-3 cm in width. The labellum is white, about 4.5 cm long and 2 cm wide, with a hairy surface and wavy margins. The stamens on either side of the labellum are usually sterile, while the fertile stamen is yellow, with a filament of about 3 cm long and 1.5 cm wide. The anther has two lobes. The pistil has a triangular ovary, a single, slender style, and a semicircular stigma with nectar glands. The fruit is angular, and the seeds are black. (Figure 1B).

The dominant characteristic of *H. speciosa* (J. Koenig) Govaerts is its distinctive spiraling stem, which initially grows straight but gradually curves into a spiral as the plant matures. This spiraling growth pattern serves as a key distinguishing feature of the species. These findings are consistent with those reported by Larsen (2008).

Figure-1. Two species of the *Hellenia* genus from Ubon Ratchathani Province, Thailand: A. *Hellenia lacera* (Gagnep.) Govaerts, and B. *H. speciosa* (J. Koenig) Govaerts.

1.3 The ecology information

Ecological data for both *Hellenia* species were gathered from various forest types, building on earlier studies by Larsen (2008) and POWO (2024). These observations shed light on how environmental factors, such as rainfall patterns and temperature fluctuations, influence the phenological behavior of these species.

Hellenia lacera (Gagnep.) Govaerts is typically found in mixed deciduous forests, which are characterized by a diverse assemblage of tree species that seasonally shed their leaves. Unlike pure deciduous forests, these ecosystems exhibit a more complex canopy structure, resulting in variable light conditions and microclimates. The phenology of *H. lacera* appears to be closely linked to these environmental dynamics, with flowering and fruiting periods timed to seasonal shifts in rainfall and temperature. For example, flowering typically coincides with the onset of the rainy season, providing optimal conditions for seedling establishment. Variations in soil properties, light availability, and plant associations within these forests suggest that *H. lacera* is well-adapted to environments with fluctuating moisture and light levels throughout the year.

Hellenia speciosa (J. Koenig) Govaerts is predominantly found in deciduous dipterocarp forests, which undergo extended dry periods due to seasonal rainfall patterns. During the dry season, dipterocarp trees shed their leaves, leading to marked fluctuations in soil moisture and temperature. The phenological behavior of *H. speciosa* appears closely tied to these seasonal shifts, with its flowering period often occurring at the end of the dry season, just before the rainy season begins. This timing coincides with more favorable moisture and temperature conditions, facilitating growth and reproduction. The species' resilience to dry periods is evident in its ability to thrive in habitats with limited moisture availability during the dry season. The collected ecological data further confirm that *H. speciosa* is well-adapted to environments characterized by pronounced seasonal variations in rainfall and temperature.

These ecological patterns align with previous studies, reinforcing the reliability of the findings. The close correlation between the phenology of both *Hellenia lacera* and *H. speciosa* and environmental factors such as rainfall and temperature highlight the critical role these climate variables play in shaping their growth, distribution, and ecological functions.

The ecological data for both species corroborates the foundational work of botanists and ecologists, particularly Larsen's (2008) research on Southeast Asian flora, which provides key insights into habitat preferences and phenological traits of *Hellenia* species. Additionally, Govaerts (2004) contributions offer further evidence regarding the ecological distribution and habitat conditions favorable to these species. The comprehensive data from POWO (2024) further validate the observed ecological characteristics by presenting an updated global perspective on the distribution of *Hellenia*. These combined findings emphasize the intricate relationship between the phenology of *Hellenia* species and the environmental factors of their habitats. This relationship underscores the ecological adaptations of these species and enhances our understanding of their role within diverse ecosystems.

1.4 The phenology

The phenology of *Hellenia lacera* (Gagnep.) Govaerts and *H. speciosa* (J. Koenig) Govaerts was observed from May to October, a period critical for understanding their seasonal growth and reproductive behaviors. During this time, key life cycle stages such as flowering, fruiting, and vegetative growth were documented. Both species typically flower within this period, with variations in bloom frequency, duration, and timing influenced by environmental conditions. The fruiting phase, including seed development and maturation, was also closely monitored to gain insight into reproductive success and dispersal mechanisms. Additionally, vegetative growth patterns, such as leaf emergence and stem elongation, were tracked to assess responses to seasonal changes in temperature and moisture. The observed phenological events aligned with variations in environmental factors, such as rainfall, temperature, and humidity, which were recorded to understand their role in shaping the species' growth and reproductive cycles. These findings were consistent with previous studies by Larsen (2008), Govaerts (2004), and the POWO database (2024), though some discrepancies were noted when compared to the research of Smith (1987) and Wu and Raven (2000), potentially due to regional differences or variations in environmental conditions across study areas.

2. The conservation status of *Hellenia* genus from Ubon Ratchathani Province, Thailand

The conservation status of the *Hellenia* genus, including *H. lacera* (Gagnep.) Govaerts and *H.*

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speciosa (J. Koenig) Govaerts), is summarized based on the latest available data and relevant conservation resources. Both species are not endemic, meaning they are found across multiple regions rather than being restricted to a specific geographical area, a status confirmed by the Plant of the World Online (POWO, 2024) database. Regarding *H. lacera*, as of 2024, the species is not listed on the IUCN Red List, suggesting that it has either not been assessed or is excluded from the list. This lack of formal evaluation points to a potential gap in knowledge, indicating the need for further studies to assess the species' conservation status and potential risks. In contrast, *H. speciosa* is currently classified as Least Concern (LC) by the IUCN, signifying that the species is not at immediate risk of extinction. While the classification indicates a stable population, it does not rule out the possibility of threats to tis long-term survival.

Potential Threats and Challenges

Despite *Hellenia speciosa* being classified as Least Concern, it is important to acknowledge that even species with stable populations may still encounter threats that could undermine their long-term viability. Habitat loss, particularly through deforestation, is a significant concern. The mixed deciduous and dipterocarp forests where these species are found are increasingly vulnerable to human activities such as logging, agricultural expansion, and urbanization. These factors contribute to the fragmentation of suitable habitats, threatening the integrity of ecosystems vital for these species. Additionally, climate change represents an emerging challenge, as shifts in temperature and rainfall patterns may disrupt the timing of key phenological events, such as flowering and fruiting, which are essential for reproductive success. The heightened frequency and intensity of droughts in regions where *H. speciosa* occurs could further alter soil moisture levels, potentially affecting its growth and reproductive capacity. Similarly, *H. lacera*, which currently lacks a formal conservation status, may face comparable risks, particularly given its reliance on the specific conditions provided by mixed deciduous forests, which are also increasingly threatened by human-driven activities.

Implications and Conservation Efforts

Given the potential threats facing both *Hellenia speciosa* and *H. lacera*, it is essential to maintain ongoing monitoring of their populations. The IUCN Red List classification should be periodically reviewed to incorporate new data on population trends and habitat degradation. To mitigate some of these threats, habitat protection initiatives, such as establishing conservation areas or creating buffer zones around critical habitats, should be considered. Additionally, increasing public awareness about the ecological significance of these species and their habitats will play a key role in their long-term conservation.

Further ecological research is recommended to better understand the specific environmental needs of these species and identify regions at risk of habitat loss or degradation. Such studies will inform targeted conservation efforts, ensuring that appropriate actions are taken to maintain stable populations of *Hellenia* species despite ongoing environmental changes.

General Considerations

Regular assessments and updates are critical for effective conservation management. The conservation status of species can shift due to various environmental influences, including climate change, habitat destruction, and human-driven pressures. To ensure comprehensive evaluations of the conservation needs of *Hellenia* species, it is important to monitor updates from key databases such as IUCN (2023) and POWO (2024), as well as review recent studies, including those by Hussain et al. (2023). Research by Govaerts (2004), Larsen (2008), and the IUCN (2023) identifies potential risks to *Hellenia* species, particularly from habitat loss and environmental alterations. However, studies such as Smith et al. (2005) suggest that while there are concerns, the urgency of conservation measures may not be as critical. This underscores the need for a balanced and thorough evaluation of conservation priorities for *Hellenia* species.

3. The traditional uses of the genus *Hellenia* in Ubon Ratchathani, Thailand

The *Hellenia* species in Ubon Ratchathani, hold multiple roles in traditional practices, serving as source of food, ornamental display, religious rituals, and medicine.

3.1. The traditional uses of *Hellenia speciosa* (J.Koenig) S.R. Dutta

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3.1.1. Edible Vegetation

During the rainy season, when the plants are at their tenderest and most abundant, various parts of *Hellenia speciosa* are harvested for consumption. To make the plant more palatable, particularly its leaves and stems, they are boiled to remove any strong odor. This step is important for enhancing the flavor and making the plant more suitable for culinary applications.

3.1.2. Culinary Uses

The boiled or blanched plant parts are typically used as a condiment, often paired with a variety of vegetables and a popular staple in Thai cuisine: chili paste. This preparation process softens the plant tissues, allowing them to complement the spicy and savory taste of the paste. The young, fresh leaves of *Hellenia speciosa* are especially valued for their mild flavor and tender texture. They are often consumed raw as an accompaniment to chili pastes such as bamboo shoot chili paste or mushroom chili paste, creating a flavorful dish that is both nutritious and traditional.

3.1.3. Animal Fodder

Beyond human use, *Hellenia speciosa* is also utilized as animal feed. Its leaves and stems are consumed by livestock, including cattle and buffalo, providing a nutritious food source, particularly in rural areas where access to other forage might be limited.

3.1.4. Medicinal proposes

The plant has various medicinal applications. It is commonly used to treat respiratory issues like asthma, coughs, and bronchitis due to its ability to clear mucus and improve airflow. Additionally, a paste made from the rhizome is applied topically to treat skin conditions, such as infections and boils. The plant’s beneficial properties extend to cosmetics, where it is used to improve skin texture and tone.

3.1.5. Ornamental proposes

Hellenia speciosa is a favored plant in tropical and subtropical gardens, prized for its attractive foliage and striking flowers. The plant’s tall, spiral stems and large, green leaves make it an eye-catching feature in garden beds and tropical-themed landscapes. It is often used as a backdrop in mixed borders, and its flowers and bracts are frequently cut for floral arrangements, adding a touch of exotic beauty to tropical flower displays.

3.1.6. Cultural and Nutritional Importance

Cultural and nutritional value are significant aspects of *Hellenia speciosa*. It plays a vital role in local customs and food practices, particularly in the rainy season when it provides important nutrients. The plant offers essential vitamins, minerals, and fiber, contributing to a well-balanced diet during times when other food sources may be scarce. The connection between local communities and the plant highlights its enduring role in cultural traditions and daily sustenance.

3.2. The traditional uses of *Hellenia lacera* (Gagnep.) Govaerts

3.2.1. Medicinal Uses

The rhizomes and leaves of *Hellenia lacera* have been used for centuries in traditional medicine, particularly to alleviate stomach discomfort. The plant is commonly believed to aid in digestion and provide relief from gastrointestinal issues. Additionally, both the rhizomes and leaves are used in preparing remedies for respiratory ailments, such as coughs, with the plant thought to help clear mucus and soothe the throat.

3.2.2. Ornamental Uses

Hellenia lacera is prized for its ornamental value, with its attractive foliage and vibrant flowers making it a favored choice for enhancing gardens. Its distinctive spiral growth and striking flowers add a unique visual element, making it an ideal addition to a variety of landscape designs.

3.2.3. Cultural and Ritual Uses

In certain cultural traditions, particularly in areas where *Hellenia lacera* is native, the plant may be used in ceremonial practices. It is sometimes believed to possess protective or purifying properties, which could contribute to its role in rituals or religious observances. The traditional uses of *Hellenia* species in Ubon Ratchathani, as documented by researchers like Govaerts (2004) and Larsen (2008), highlight the plant’s importance in food, decoration, and medicinal practices. However, it is important to note that other studies, such as those by Smith (1987) and Wu and Raven (2000), might not fully reflect the specific cultural and regional contexts of these uses in Thailand.

4. Chromosome numbers

The chromosome numbers for *Hellenia lacera* (Gagnep.) Govaerts (Figure 2A, B) and *H. speciosa* (Figure 2C, D) have been studied to some extent, and here are the known details:

Hellenia lacera **(Gagnep.) Govaerts: Chromosome Number:** 2*n* = 18 (Figure 2A, B).

Similarly, *Hellenia lacera* (Gagnep.) Govaerts has also been reported to have a diploid chromosome number of 18, aligning with other species within the Costaceae family. This finding represents the first documented study of the chromosome number for *H. lacera*.

Hellenia speciosa **(J. Koenig) Govaerts: Chromosome Number:** 2*n* = 18 (Figure 2C, D).

Hellenia speciosa (J. Koenig) Govaerts is known to have a diploid chromosome number of 18. This chromosome number is consistent across various studies, making it a typical feature of the species.

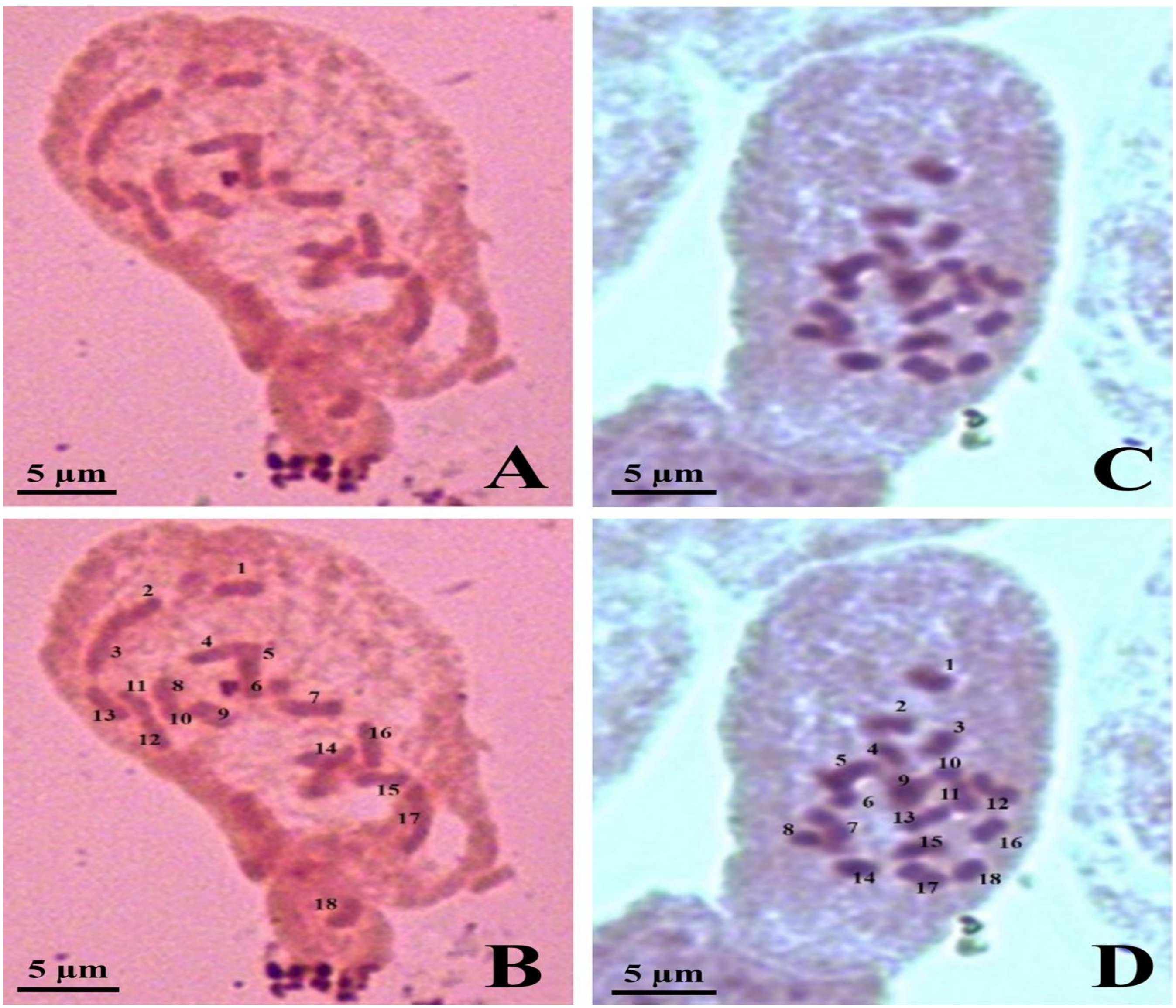

Figure-2. The chromosome numbers of two species of the *Hellenia* genus from Ubon Ratchathani Province, Thailand: **A.** and **B.** *H. lacera* (Gagnep.) Govaerts (2n = 18); **C.** and **D.** *H. speciosa* (J. Koenig) Govaerts (2n = 18) (Scale bars = 5 µm).

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5. The pollen of *Hellenia lacera* (Gagnep.) Govaerts and *H. speciosa* (J. Koenig) Govaerts

The pollen of *Hellenia lacera* and *H. speciosa* displays characteristics typical of the Costaceae family. Below is an overview of the pollen morphology for these two species.

Pollen morphology of genus *Hellenia*

The pollen grains of *Hellenia* species are characterized by being single, radially symmetrical, with an isopolar axis and a monocolpate aperture. The surface of the pollen can either be smooth or feature blunt spines, with the grains being notably large in size. The shape of the pollen grains typically ranges from prolate spheroidal to subprolate, and the wall pattern is spinuate and regulated.

5.1. Pollen Morphology of *Hellenia lacera* (Gagnep.) Govaerts

The pollen grains of *Hellenia lacera* are large, single, and radially symmetrical, exhibiting an isopolar axis and a monocolpate aperture. The average length of the aperture measures 754.57 ± 27.86 µm, with a width of 32.62 ± 8.68 µm. The pollen grains have no spines, and their shape is prolate spheroidal, with an average polar axis length of 105.49 ± 7.24 µm and an average equatorial axis length of 101.26 ± 6.85 µm. The wall pattern is rugulate (Figure 3A).

5.2. Pollen morphology of *Hellenia speciosa* (J. Koenig) Govaerts

The pollen grains are single, and radially symmetrical, exhibiting an isopolar axis and a monocolpate aperture. The average length of the aperture is 653.39 ± 62.44 (544.42-700.09) µm, and the average width is 52.83 ± 5.92 (42.44-57.38) µm. The surface features blunt spines. The pollen grains are very large, with a prolate spheroidal shape. The average length of the polar axis is 99.15 ± 3.93 (92.31-105.79) µm, and the average length of the equatorial axis is 95.58 ± 2.93 (90.91-100.61) µm. The wall pattern is spinulate, with an average spine height of 5.89 ± 1.35 (2.91-7.27) µm, an average spine width of 7.64 ± 1.10 (5.82-9.45) µm, and an average spine density of 42.77 ± 10.67 (25.92-51.84) spines per square millimeter (Figure 3B).

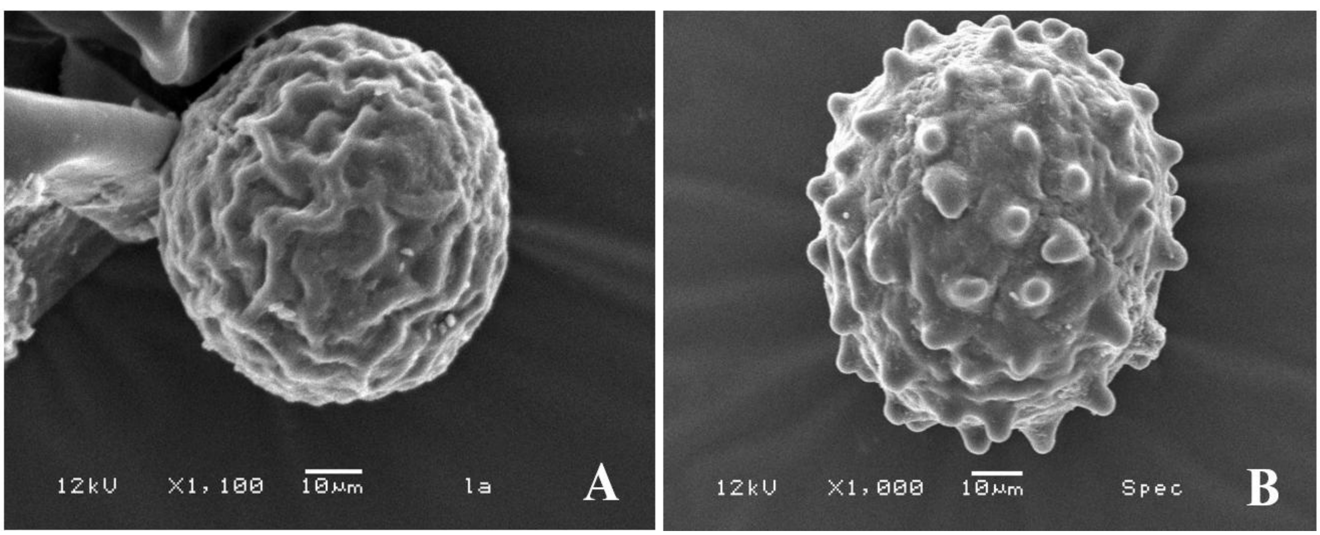

Figure-3. The pollen of two species of the *Hellenia* genus from Ubon Ratchathani Province, Thailand: **A.** *H. lacera* (Gagnep.) Govaerts; **B.** *H. speciosa* (J. Koenig) Govaerts (Scale bars = 10 µm).

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6. Peeling leaf surfaces anatomy of *Hellenia* species

The peeling leaf surfaces of *Hellenia* species (*H. lacera* (Gagnep.) Govaerts and *H. speciosa* (J. Koenig) Govaerts) exhibit several notable anatomical features:

6.1. The peeling leaf surface anatomy of the genus *Hellenia* in Ubon Ratchathani Province.

On the upper leaf surface, the cells in the epidermal tissue are polygonal with 4-7 sides and have smooth walls. The stomata are of the paracytic and tetracytic types, with two subsidiary cells adjacent to each stoma. The guard cells are bean-shaped, and prismatic crystals are present.

The epidermal cells on the lower surface of the leaf are polygonal, typically with 4 to 7 sides, and feature smooth walls. Stomata are found in both paracytic and tetracytic arrangements, with two adjacent subsidiary cells for each stoma. The guard cells are bean-shaped, and prismatic crystals can be observed within the tissue. Additionally, multicellular trichomes are present.

6.2. The peeling leaf surface anatomy of *Hellenia lacera* (Gagnep.) Govaerts in Ubon Ratchathani Province.

The epidermal cells on the upper leaf surface are polygonal, typically ranging from 4 to 7 sides, and have smooth walls. Stomata are of the paracytic type, with two subsidiary cells positioned next to each stoma. The guard cells are bean-shaped, with an average length of 25.97 ± 2.34 µm and an average width of 8.64 ± 1.40 µm. The average stomatal density is 20.83 ± 8.62, with a range from 0.00 to 36.75 stomata per square millimeter. Prismatic crystals are present (Figure 4A).

On the lower leaf surface, the cells in the epidermal tissue are similarly polygonal with 4-7 sides and have smooth walls. The stomata are of the paracytic type, with two subsidiary cells adjacent to each stoma. The guard cells are bean-shaped, with an average length of 43.40 ± 3.37 (38.37-48.79) µm and an average width of 8.90 ± 1.11 (6.50-10.13) µm. The average stomatal density is 158.03 ± 27.97 (122.50-196.01) stomata per square millimeter. Prismatic crystals and multicellular trichomes are present, with an average trichome length of 80.91 ± 12.12 (68.55-107.92) µm and an average trichome density of 319.735 ± 94.088 (232.757-526.766) trichomes per square millimeter (Figure 4B).

6.3. The peeling leaf surface anatomy of *Hellenia speciosa* (J. Koenig) Govaerts in Ubon Ratchathani Province:

On the upper leaf surface, the cells in the epidermal tissue are polygonal with 4-7 sides and have smooth walls. The stomata are of the paracytic and tetracytic types, with two subsidiary cells adjacent to each stoma. The guard cells are bean-shaped, with an average length of 48.68 ± 3.53 (43.63-53.92) µm and an average width of 13.09 ± 2.14 (9.89-16.67) µm. The average stomatal density is 14.70 ± 4.13 (0.00-24.50) stomata per square millimeter. Prismatic crystals are present (Figure 4C).

On the lower leaf surface, the cells in the epidermal tissue are polygonal with 5-7 sides and have smooth walls. The stomata are of the paracytic and tetracytic types, with two subsidiary cells adjacent to each stoma. The guard cells are bean-shaped, with an average length of 41.50 ± 1.85 (38.73-44.30) µm and an average width of 13.28 ± 2.35 (9.87-17.11) µm. The average stomatal density is 51.45 ± 12.65 (36.75-73.50) stomata per square millimeter. Prismatic crystals and multicellular trichomes are present, with an average trichome length of 277.99 ± 32.67 (238.04-329.67) µm and an average trichome density of 64.93 ± 8.27 (49.00-73.50) trichomes per square millimeter (Figure 4D).

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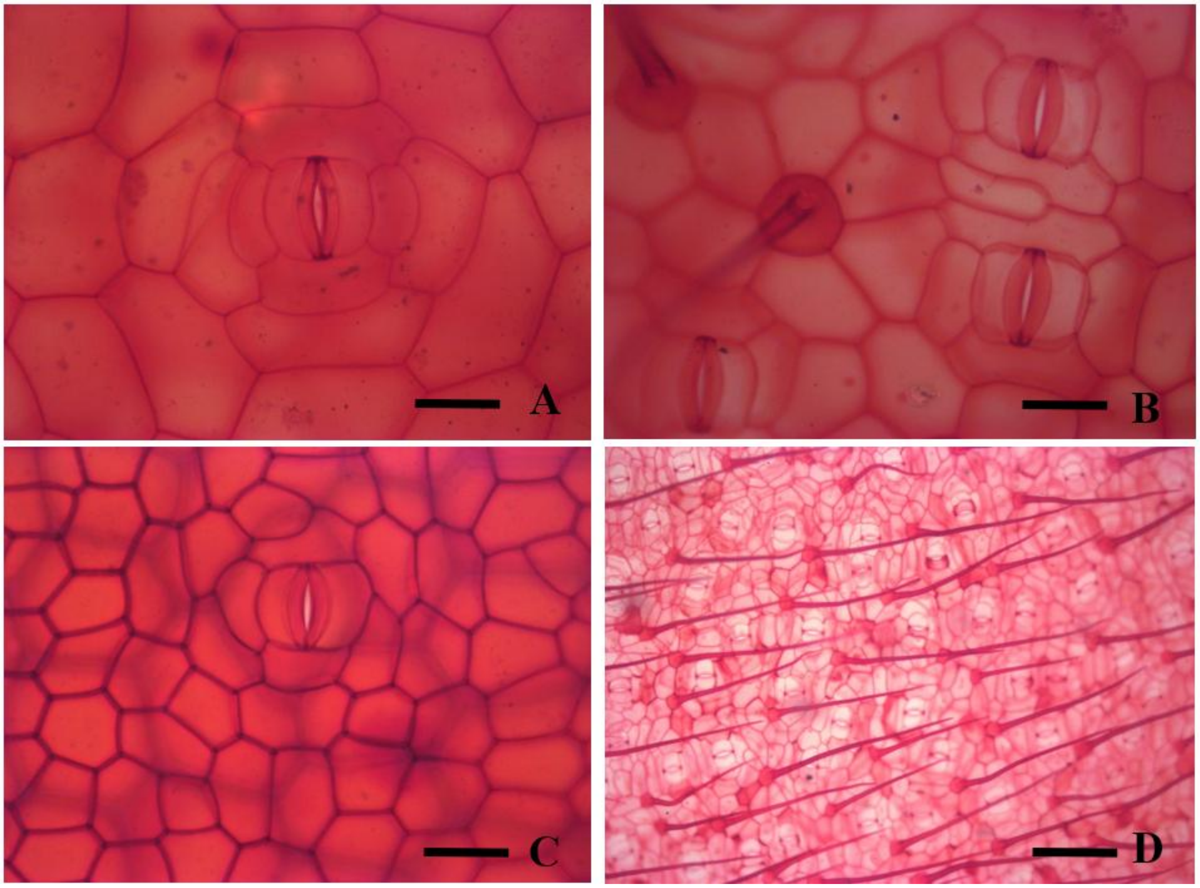

Figure-4. The peeling leaf surface anatomy of the genus *Hellenia* in Ubon Ratchathani Province, Thailand: **A.** Upper leaf surface of *H. lacera* (Gagnep.) Govaerts; **B.** Lower leaf surface of *H. lacera* (Gagnep.) Govaerts; **C.** Upper leaf surface of *H. speciosa* (J. Koenig) Govaerts; **D.** Lower leaf surface of *H. speciosa* (J. Koenig) Govaerts (Scale bars = 10 µm).

Discussion

In Ubon Ratchathani Province, Northeastern Thailand, two species of the genus *Hellenia* have been identified, each displaying distinctive anatomical and morphological traits that may influence their ecological adaptability. *Hellenia lacera* (Gagnep.) Govaerts, with its straight, non-spiraling stem, may represent an adaptive strategy for maximizing vertical growth in more stable environments, where mechanical support against environmental forces is not critical. In contrast, the gradual spiral growth pattern of *H. speciosa* (J. Koenig) Govaerts could reflect an adaptation to dynamic environments, where increased flexibility and resilience against wind or herbivory confer an ecological advantage. The differences in stem growth may reflect adaptive strategies that have evolved in response to environmental pressures.

The pollen morphology of *Hellenia lacera* and *H. speciosa* reveals key distinctions that underscore their uniqueness within the Costaceae family. While both species share large, radially symmetrical pollen grains with a monocolpate aperture, *H. lacera* has a smooth surface and a rugulate wall pattern, whereas *H. speciosa* is distinguished by blunt spines and a spinulate wall pattern. Additionally, the average aperture length of *H. lacera* (754.57 µm) is greater than that of *H. speciosa* (653.39 µm), which may suggest an adaptive advantage in pollination. These morphological differences not only aid in the identification of the two species but also imply that they have evolved distinct ecological roles and adaptations to their environments.

The findings of this study align with earlier reports on the chromosome number of the species, consistently identifying a diploid count of 2n = 18. This observation is supported by research conducted by Chattopadhyay and Sharma (1983); Sharma and Chattopadhyay (1983); Chen et al. (1986); Subrahmanyam and Khoshoo (1986); Tyagi and Gupta (1987) and Omanakumari and Mathew (1988), all of whom reported similar results. However, discrepancies are noted in studies by Mukhopadhyay and Sharma (1990) and Sarkar (1990), both of which found 2n = 36, as well as Kamble (1993), who recorded 2n = 27.

Several factors may account for these inconsistencies. The use of different sample populations, each potentially exhibiting chromosomal variations, could explain the conflicting results. Distinctions in stem growth among populations might be linked to underlying chromosomal differences. Additionally, methodological differences in cytogenetic techniques, such as slide preparation and staining, may influence the accuracy of chromosome counts. Taxonomic misidentification, possibly arising from confusion between closely related species or varieties, could also contribute to the observed variations. Environmental conditions, including cultivation practices, may further impact chromosomal stability, leading to aberrations in specific populations. Lastly, inaccuracies in data interpretation or reporting cannot be ruled out. These observations emphasize the necessity of employing standardized methodologies, precise taxonomic verification, and careful consideration of environmental factors when assessing chromosome numbers in plant species.

Although the diploid chromosome number of 2n = 18 is well-documented, this study sheds light on its distribution among *Hellenia* species in Ubon Ratchathani. The uniformity of this chromosome number across species in the region suggests a level of genetic consistency that may be indicative of ecological and environmental stability. This finding provides a basis for future investigations into chromosomal evolution within *Hellenia*, with particular attention to its geographic range and environmental adaptations. By documenting these chromosome numbers in local populations, the study contributes valuable insights into the genetic diversity of *Hellenia* species. Such information is critical for conservation efforts, particularly in areas where habitat loss and environmental changes threaten these species.

The peeling leaf surfaces observed in *Hellenia* species, marked by epidermal detachment and changes in the cuticle, suggest a functional adaptation to environmental pressures. This peeling may assist in regulating water retention or transpiration, offering resilience in the fluctuating moisture conditions typical of deciduous dipterocarp and mixed deciduous forests. When comparing the peeling leaf surface anatomy of *Hellenia lacera* and *H. speciosa*, distinct differences in stomatal types, guard cell dimensions, trichome density, and crystalline presence highlight specific adaptations for each species. *H. lacera* exhibits a higher stomatal density on the lower leaf surface (158.03 stomata per mm^2) and smaller guard cells on the upper surface (25.97 µm in length), indicating enhanced gas exchange and better adaptation to drier conditions. While *H. speciosa* has larger guard cells (48.68 µm) and a combination of paracytic and tetracytic stomata on the upper surface, traits that may help with water regulation in more humid environments. Furthermore, the two species differ in trichome morphology, *H. lacera* has more numerous but shorter trichomes (319.735 per mm^2, 80.91 µm in length), while *H. speciosa* has fewer, but longer trichomes (64.93 per mm^2, 277.99 µm). This difference may reflect varied protective strategies, with *H. lacera* being better suited to more exposed environments. Both species contain prismatic crystals, though their function may vary based on habitat, potentially aiding in defense against herbivores or helping with light modulation. These anatomical differences underline the ecological importance of leaf surface traits in *Hellenia* species, showing how environmental factors influence structural variations.

The adaptive features in *Hellenia* species, such as stem morphology and stomatal adaptations, echo ecological adjustments observed in other plant species, like Sorghum, across different altitudes (Ahmad et al., 2022). These modifications in traits such as trichome density and stomatal structure help plants cope with specific environmental challenges, whether it's moisture variability in tropical forests or cold stress at high altitudes. This comparative perspective sheds light on how plants in diverse environments leverage structural adaptations to thrive in their habitats.

A similar study by Ahmad et al. (2018) on plant adaptations to environmental gradients demonstrates how species from various altitudinal zones develop unique morphological and physiological traits, such as changes in leaf area and stomatal features, to optimize their performance in specific environmental conditions. The anatomical adaptations observed in *Hellenia* species in Ubon Ratchathani are consistent with these findings, including variations in trichome density and leaf structure. Both studies underscore the critical role of structural changes in helping plant species manage abiotic stresses and resource fluctuations, illustrating the broader ecological strategies employed by plants in different habitats and at various elevations.

The leaf-peeling trait observed in *Hellenia* species plays a crucial role in their ecological adaptability and provides important insights into their evolutionary biology. This trait likely evolved as an adaptation to environmental pressures, such as water scarcity, herbivory, or microbial threats. By shedding outer leaf layers, the plants may improve water-use efficiency, protect inner tissues from herbivores, or limit pathogen access, especially in humid or tropical environments where these challenges are common. These adaptive features enable *Hellenia* species to thrive in diverse and harsh habitats, demonstrating their ecological resilience.

Leaf-peeling also highlights the phenotypic plasticity of *Hellenia* species. Variations in this morphology across populations or environments indicate their ability to adapt to different ecological conditions. This flexibility not only helps the species survive in varying habitats but also allows them to colonize new ecological niches. Such plasticity is essential for their evolutionary success, enabling *Hellenia* species to respond to environmental changes and potentially expand their range.

From a phylogenetic perspective, the evolution of leaf-peeling morphology may offer insights into the lineage's divergence and adaptive history. Comparative analyses with closely related species in the same family or genus could reveal ancestral traits and the evolutionary pathways that led to this adaptation. By examining the distribution of this trait across species, researchers can gain a better understanding of the selective pressures that influenced the evolution of *Hellenia* and its ecological specializations.

The leaf-peeling trait appears to offer ecological benefits that could contribute to niche differentiation and the process of speciation within the genus. Variations in this characteristic among populations may influence reproductive isolation, potentially leading to the emergence of new species over time. This highlights the role of environmental pressures and morphological adaptations in promoting diversification within Hellenia. To gain a deeper understanding of the importance of this trait, and to assess how these species might adapt to future environmental changes, further studies combining ecological, morphological, and phylogenetic approaches are essential.

These anatomical features enhance our understanding of how these species are structurally adapted to their environments and how they maintain functionality in response to varying environmental stresses, contributing to their ecological success (Ahmad et al., 2016; Ahmad et al., 2023). Extending this comparative approach, studying other species in the Costaceae family would provide additional context for understanding the adaptive mechanisms of *Hellenia*. Species within the family that exhibit different stem structures or non-spiral growth patterns could reflect distinct evolutionary responses to similar environmental challenges. Investigating how *Hellenia*'s adaptations compare to related species will deepen our understanding of evolutionary biology within the Costaceae, highlighting how structural traits like stem morphology and leaf anatomy may represent convergent or divergent adaptive strategies.

The spiral growth patterns in *Hellenia* species likely reflect adaptive strategies shaped by their specific environmental conditions. Species with spiral growth, often found in shaded or densely vegetated habitats, may use this pattern to optimize light capture. In areas where light is limited due to competition, the helical arrangement of leaves allows them to be spaced evenly, reducing self-shading and improving photosynthetic efficiency. This adaptation is particularly useful in forest understories or regions with restricted sunlight. Additionally, the spiral growth form may provide structural advantages, helping plants maintain a compact, resilient shape capable of withstanding environmental stresses such as wind or herbivory. Conversely, non-spiral growth, seen in species from open, sunlit habitats, may help maximize sunlight absorption by spreading leaves horizontally. In environments with less light competition, this arrangement allows for broader light capture. Moreover, this growth form could reduce water loss in dry conditions by minimizing leaf overlap, thus aiding water retention. These contrasting growth patterns reflect the species' different ecological needs and emphasize the importance of morphological traits in their evolutionary success. Ultimately, spiral growth in *Hellenia* species highlights how plants adapt to varying environmental pressures, shaping their ecological interactions, resource use, and potential for speciation.

Conclusion

This research offers valuable insights into the taxonomy, morphology, and conservation status of two *Hellenia* species found in Ubon Ratchathani Province, Northeastern Thailand: *H. lacera*, which is characterized by its straight stem, and *H. speciosa*, known for its spiral stem. Both species share a diploid chromosome number of 2n = 18 and display distinct pollen morphology, reinforcing their taxonomic differentiation. The peeling leaf trait adds another layer of morphological distinction to the genus *Hellenia*. Conservation assessments have classified *H. speciosa* as Least Concern, yet *H. lacera* requires more immediate attention, given the limited knowledge about its ecology and distribution.

For the long-term conservation of *Hellenia lacera*, it is recommended to focus on habitat protection, potentially through the creation of protected areas or community-led conservation initiatives. Regular population monitoring should be implemented to track species dynamics and identify potential threats. Raising awareness in local communities about the ecological role of *Hellenia* could also promote community-based conservation efforts. Further research is essential to address gaps in understanding the reproductive biology and pollination mechanisms of *H. lacera*, which are critical for assessing its population sustainability. Exploring the genetic diversity of both species across different populations could provide valuable information on their adaptability and help inform potential ex-situ conservation strategies. Furthermore, studies on habitat preferences and responses to environmental changes, including climate fluctuations, would be instrumental in guiding habitat management practices. By addressing these research gaps and implementing robust conservation strategies, this study contributes significantly to our understanding of *Hellenia* and underscores the need for proactive conservation efforts to ensure the survival of these ecologically important species within the Costaceae family.

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Contribution of Authors

Saensouk P, Appamaraka S, Niamngon P, Niamngon T, Ragsasilp A, Rakarcha S, Koompoot K, Setyawan AD & Chaveerach A: Conceptualization, methodology, validation, formal analysis, investigation, writing-review and editing.

Boonma T: Conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing–review and editing and visualization.

Saensouk S: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing–original draft preparation, writing–review and editing, visualization, project administration and funding acquisition.

Saensouk P & Saensouk S: Managed resources and supervised the research.

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