



RESEARCH ARTICLE

Anatomical adaptations of *Launaea sarmentosa* (Willd.) Kuntze (Asteraceae) from the Andaman coast, Thailand

Pornsawan Sutthithon¹, Phuripong Meksuwan² & Yurachat Meksuwan^{3*}

¹Department of Botany, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

²Science and Mathematics Program (Biology), Faculty of Science and Technology, Phuket Rajabhat University, Phuket 83000, Thailand

³Faculty of Technology and Environment, Prince of Songkla University, Phuket Campus, Phuket 83120, Thailand

*Correspondence email - yurachat.y@phuket.psu.ac.th

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Abstract

Launaea sarmentosa (Willd.) Kuntze, a threatened coastal species endemic to the Andaman coastline of Thailand, is undergoing significant population decline due to habitat degradation. Despite its ecological and economic importance, limited knowledge exists regarding its anatomical adaptations to coastal environments, knowledge that is essential for effective conservation and restoration strategies. This study aimed to comprehensively investigate the vegetative anatomy of *L. sarmentosa* and to identify taxonomically significant features that reflect its adaptation to environmental stressors. Samples of leaves, stems, stolons and roots were collected from Mai Khao Beach, Phuket and examined using paraffin embedding and light microscopy techniques. The results revealed several adaptive features, including amphistomatic leaves, thick cuticle layers, homobaric leaf structure and the formation of periderm in stems and roots. Stomatal density was significantly higher on the adaxial surface compared to the abaxial surface, indicating differential regulation of gas exchange. The presence of a thick cuticle and epidermis further highlights its adaptations to the harsh coastal environment, characterized by high light intensity and drought conditions. Stolons exhibited chlorenchyma and stomata, suggesting their role in photosynthesis and water regulation. These anatomical traits reflect the ability of *L. sarmentosa* to thrive in dune habitats and provide essential information for future conservation and habitat restoration initiatives. Understanding these adaptations is crucial for the effective preservation and rehabilitation of this species.

Keywords: amphistomatic; homobaric leaves; linhar; Mai Khao Beach; Phuket

Introduction

Launaea sarmentosa (Willd.) Kuntze (Asteraceae), locally known as linhar in Thai, is a threatened coastal plant species found along the Andaman coast of Thailand (1). Historically, this species was abundantly distributed along the sandy shorelines of Prachuap Khiri Khan, Ranong, Phangnga, Krabi and Phuket provinces; however, it is now rare and occurs only in scattered populations. The leaves are considered as a delicacy and are sold for approximately 300 Thai baht (about 10 USD) per kg in Phuket and Phangnga. Several inhabitants of Mai Khao Beach, a major tourist destination in Phuket, cultivate this plant for additional income. *L. sarmentosa* is an important medicinal herb, commonly used in traditional Vietnamese remedies for the treatment of various diseases, particularly inflammatory conditions. Different parts of the plant have been traditionally used to treat a range of human diseases. The aerial parts are considered to be effective against hypertension and diabetes, while the white milky latex is widely applied to treat skin injuries caused by fish spines during fishing activities (2). Phytochemical analysis has revealed a high concentration of polyphenols in the leaves (3). The roots are reputed for treating jaundice. In Vietnam, they are used as a component in treatments for various disorders,

mainly inflammatory diseases. Extracts of *L. sarmentosa* have demonstrated pharmacological properties, including antipyretic and anti-inflammatory characteristics (4, 5).

Despite its ecological, medicinal and economic importance, recent studies have reported alarming declines in population due to habitat destruction, pushing this once-prevalent species toward local extinction along the Andaman coastline. A suitable medium for the tissue culture of *L. sarmentosa* has been developed to support both commercial propagation and conservation efforts (6). However, the anatomical mechanisms underlying its survival in such constrained and harsh environments remain largely unexplored.

Coastal sand habitats are typically characterized by high salinity, high temperatures, limited water availability, intense sunlight, strong winds and nutrient-poor soil (7). To survive under such stress conditions, coastal plant species develop specialized structural adaptations. Understanding these anatomical traits is vital for predicting plant responses to environmental stress and for devising effective conservation strategies (8). Plant anatomy offers valuable insights into how species adapt to their environments and life cycles (9). For

example, anatomical traits such as the shape of epidermal cells and the presence of a hypodermal layer have proven useful in the taxonomical identification of *Mangifera* species in Thailand (10). Vegetative anatomical studies have also been conducted on coastal sand plants, such as *Zygophyllum album* L., a salt-tolerant species from the Balouza dunes of Egypt (11). That study found adaptations including isolateral mesophyll, a thick cuticle and protective periderm tissue in the roots that help withstand drought and mechanical injuries. Glandular trichomes were also observed, contributing to the plants' defence against temperature stress and water loss (12).

The Asteraceae family, to which *L. sarmentosa* belongs, is predominantly distributed in tropical regions, particularly in grasslands. A total of 34 species within this family have demonstrated a broad range of adaptive anatomical traits, such as a thickened cuticle, reinforced epidermal cell walls, epicuticular wax and amphistomatic leaves (13). These characteristics are commonly associated with environmental resilience and are therefore anticipated in *L. sarmentosa* as well. However, to date, no detailed anatomical study has been conducted on this species to validate the presence of such features. Consequently, understanding the anatomical and physiological adaptations of *L. sarmentosa* is critical for informing conservation strategies.

This study aims to investigate the vegetative morphology of *L. sarmentosa* and to identify taxonomically relevant features that reflect its adaptation to environmental stressors. Understanding its structural adaptations can provide insights into its survival mechanisms of the species and aid conservation efforts aimed at protecting and restoring this threatened plant species from the Adaman coastal ecosystem.

Materials and Methods

Mature rosettes of *L. sarmentosa*, each comprising 12-15 leaves, were collected from Mai Khao Beach in Phuket, Thailand, in October 2023 (Fig. 1). A voucher specimen was deposited at the PSU Herbarium, Division of Biological Science, Faculty of Science, Prince of Songkla University, Thailand, under accession number 20077. For anatomical analysis, the eighth leaf from the apex, stems, stolons, main roots (20-25 mm from the root base) and lateral roots (25 mm from the root tip) were excised from ten rosettes using a sharp blade. The plant materials were preserved for 48 hr in FAAL (a solution of formaldehyde, glacial acetic acid and 70 % ethyl alcohol in a

5:5:90 v/v/v ratio) (14). Following fixation, the samples were washed three times with 70 % ethanol, with each wash lasting three hr.

Dehydration was carried out using a graded ethanol-tertiary butyl alcohol series (70 %, 85 %, 95 % and 100 %), after which the samples were infiltrated with liquid paraffin and embedded in paraffin wax (15). Paraffin-embedded materials were sectioned into 10-15 μm thick slices with a rotary microtome (Leica HistoCore BIOCUT). The sections were mounted on microscope slides, stained with Safranin and Fast Green and sealed with mounting media (14). Photographs were taken with an Olympus BX-51 microscope equipped with a DP28 digital camera (Olympus Corporation, Tokyo, Japan). The samples were examined under 400X magnification to study epidermal structures. Quantitative anatomical measurements were conducted using ImageJ software, with each anatomical feature replicated six times per sample (six measurements per micrograph).

For stomatal analysis, the central sections of the lamina near the leaf margin, on both the adaxial and abaxial sides, was peeled and mounted on glass slides. These samples were washed with distilled water, stained with Safranin O and mounted in 10 % glycerin (16). The types and morphology of epidermal cells, as well as stomatal density (number of stomata per mm^2), were examined using a light microscope (ZEISS Axiocam 208 Color) and ZEISS ZEN 3.8 software. Stomatal length, density and stomatal index (SI) were measured at six positions on each leaf. A total of twenty leaves were sampled from 20 individual plants. The SI was calculated using the following equation (17):

$$\text{SI (\%)} = \frac{\text{SD}}{\text{SD} + \text{E}} \times 100 \quad (\text{Eqn. 1})$$

where SI is the stomatal index, SD is the stomatal density (number of stomata per mm^2) and E is the number of epidermal cells per mm^2 . To compare stomatal features between the adaxial and abaxial leaf surfaces, a paired sample t-test was performed using SPSS software (version 20.0), with significance determined at ($p < 0.05$).

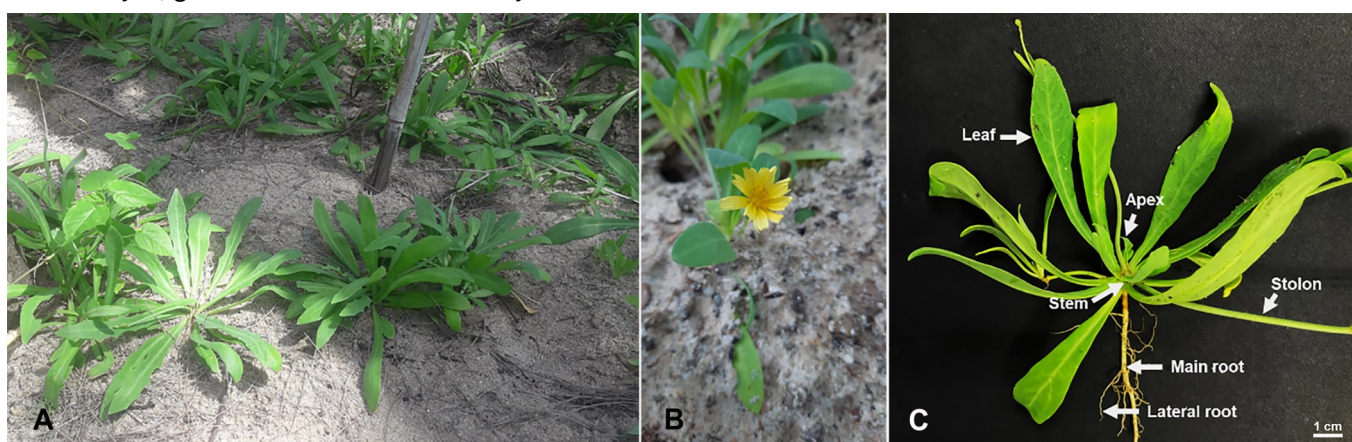


Fig. 1. *L. sarmentosa* from Mai Khao Beach, Phuket, Thailand. A) Rosettes in natural habitat; B) Flower; C) Morphology of a mature rosette.

Results

Leaf

The leaves of *L. sarmentosa* were amphistomatic, possessing stomata on both surfaces. Both the adaxial and abaxial surfaces exhibited an anomocytic stomatal apparatus, characterized by the absence of distinct subsidiary cells (Fig. 2A and 2B). The stomatal density (SD) on the abaxial epidermis ($128.96 \pm 4.07 \text{ N.mm}^{-2}$) was significantly higher than that on the adaxial surface ($111.04 \pm 3.61 \text{ N.mm}^{-2}$) (t-test, $p < 0.001$). However, the average stomatal length ($31.52 \pm 3.72 \mu\text{m}$ on the adaxial and $32.61 \pm 4.21 \mu\text{m}$ on the abaxial surface) and the SI (15.68 ± 0.91 and $17.07 \pm 0.77 \%$, respectively) did not show a statistically significant difference between the two surfaces.

Transverse sections of the leaves revealed that the epidermis was uniseriate and composed of polygonal cells with a thick cuticle layer measuring approximately ($8.57 \pm 0.54 \mu\text{m}$). The midrib was biconvex in shape (Fig. 3A). The adaxial and abaxial epidermal thickness were approximately $40.65 \pm 1.44 \mu\text{m}$ and $33.98 \pm 1.29 \mu\text{m}$, respectively. The thickness of mesophyll was $339.78 \pm 10.84 \mu\text{m}$ and was dorsiventral in structure, consisting of distinct palisade and spongy parenchyma layers. The palisade parenchyma was 1-2 cell layers thick, composed of vertically elongated, tightly packed parenchymatous cells (Fig. 3B). In contrast, the spongy parenchyma consisted of loosely arranged, irregularly shaped parenchyma cells with large intercellular air spaces (Fig. 3B).

Collateral, open vascular bundles with visible cambium (Fig. 3C), along with lignified vessels, were predominantly observed in the midrib region (Fig. 3C). Additionally, small vascular bundles aligned in the median portion of the mesophyll along the leaf blade. The absence of bundle sheath extensions indicated that the leaves were homobaric (Fig. 3D), a trait that may enhance lateral diffusion of gases within the leaf, promoting uniform photosynthetic activity.

Stem

The stem *L. sarmentosa* exhibited a circular outline with a eustelic stele organization (Fig. 4A). In transverse section, the stem displayed a uniseriate epidermis covered by a cuticle approximately $5.5 \mu\text{m}$ thick. Collateral vascular bundles, typically numbering between 20-22 bundles, were arranged in a concentric ring surrounding the central pith (Fig. 4B). During secondary growth, the differentiation of cortical parenchyma cells into cork cambium was observed, giving rise to periderm development (Fig. 4C). Lenticel structures (Fig. 4C, arrowhead),

along with remnants of epidermal cells that had been replaced by periderm, were also present (Fig. 4D).

Next to the periderm, the cortex, measuring approximately $411.56 \pm 49.85 \mu\text{m}$ in thickness, was composed of 15-18 layers of parenchyma cells, which were either oval or rounded. The vascular cambium, situated between the secondary xylem and secondary phloem, consisted of 2-3 layers of small, thin-walled, rectangular meristematic cells (Fig. 4E). The xylem region contained prominently lignified vessels of considerable size (Fig. 4E). The pith was composed of thin-walled, small oval or rectangular parenchyma cells.

Stolon

The stolon appeared circular in transverse section (Fig. 4F). A uniseriate epidermis containing stomata was observed (Fig. 4G & H). Immediately beneath the epidermis was a hypodermal layer composed of parenchyma cells. The cortex, composed of 3-4 layers of chloroplast-containing parenchyma (chlorenchyma), was clearly evident (Fig. 4G). The vascular bundles, typically numbering between 20 and 22, were oval in shape, collateral in arrangement and surrounded the central pith in a concentric ring. Lignified vessel walls were visible within the vascular bundles (Fig. 4F). Adjacent to the vascular ring, 1-3 layers of sclerenchyma were present. The central pith consisted of parenchymatous cells.

Root

The young roots exhibited a circular outline with both concave and convex surfaces (Fig. 5A). Root hairs covered with a thick cuticle were observed (Fig. 5B). The root cortex consisted of approximately 9-10 layers of thin-walled parenchyma cells. These parenchymatous cells were generally polygonal in shape and interspersed with intercellular spaces. Numerous secretory ducts were dispersed throughout the cortical parenchyma (Fig. 5C). The secretory epithelium lining the ducts comprised cuboidal or columnar cells and the lumens were schizogenous in nature (Fig. 5C). Idioblasts containing druse crystals were also present throughout the cortical parenchyma (Fig. 5D). In transverse sections, an endodermis with distinct casparian strips was not observed (Fig. 5E). The vascular cylinder, located in the central region of the root, was arranged radially (Fig. 5E). The xylem exhibited an exarch configuration. Xylem and phloem were separated by conjunctive tissue composed of parenchymatous cells. Several vessels with secondary cell wall thickening were clearly visible (Fig. 5F). The pith was absent.

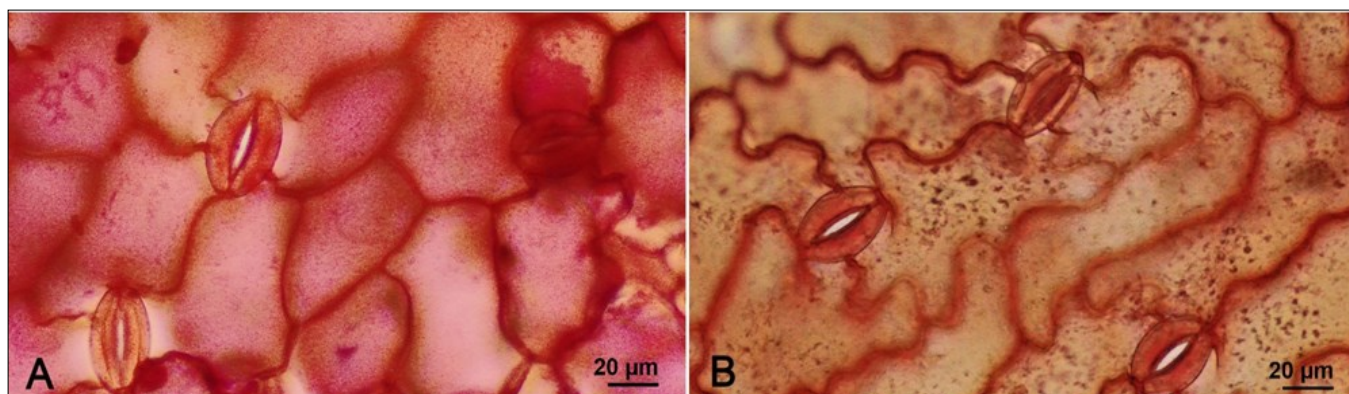


Fig. 2. Epidermis in frontal view of *L. sarmentosa*. A) Anomocytic stomata on adaxial surface of the epidermis; B) Anomocytic stomata on abaxial surface of the epidermis.

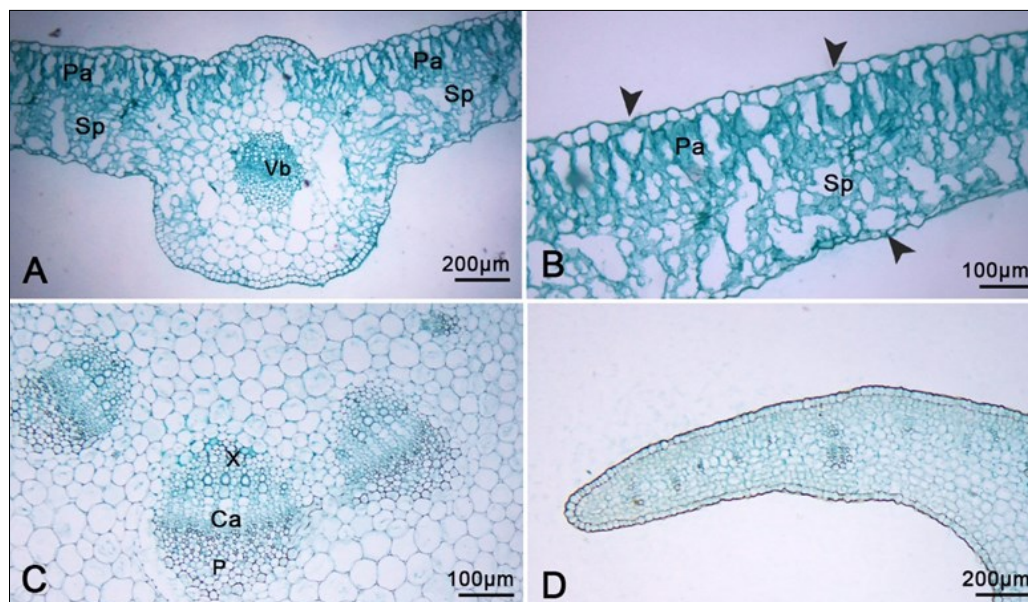


Fig. 3. Cross-sections of *L. sarmentosa* leaves. A) Transverse section of a biconvex midrib presenting collateral vascular bundle; B) Transverse sections of the lamina of the eighth leaves showing stomata on both sides (arrowheads); C) Magnification of collateral vascular bundles with visible cambium and lignified vessels; D) Downward curling of the leaf margin with large epidermal cell and no bundle sheath extensions (homobaric leaves) (Pa: Palisade mesophyll; Sp: Spongy mesophyll; X: Xylem; P: Phloem; Vb: Vascular bundle; Ca: Vascular cambium).

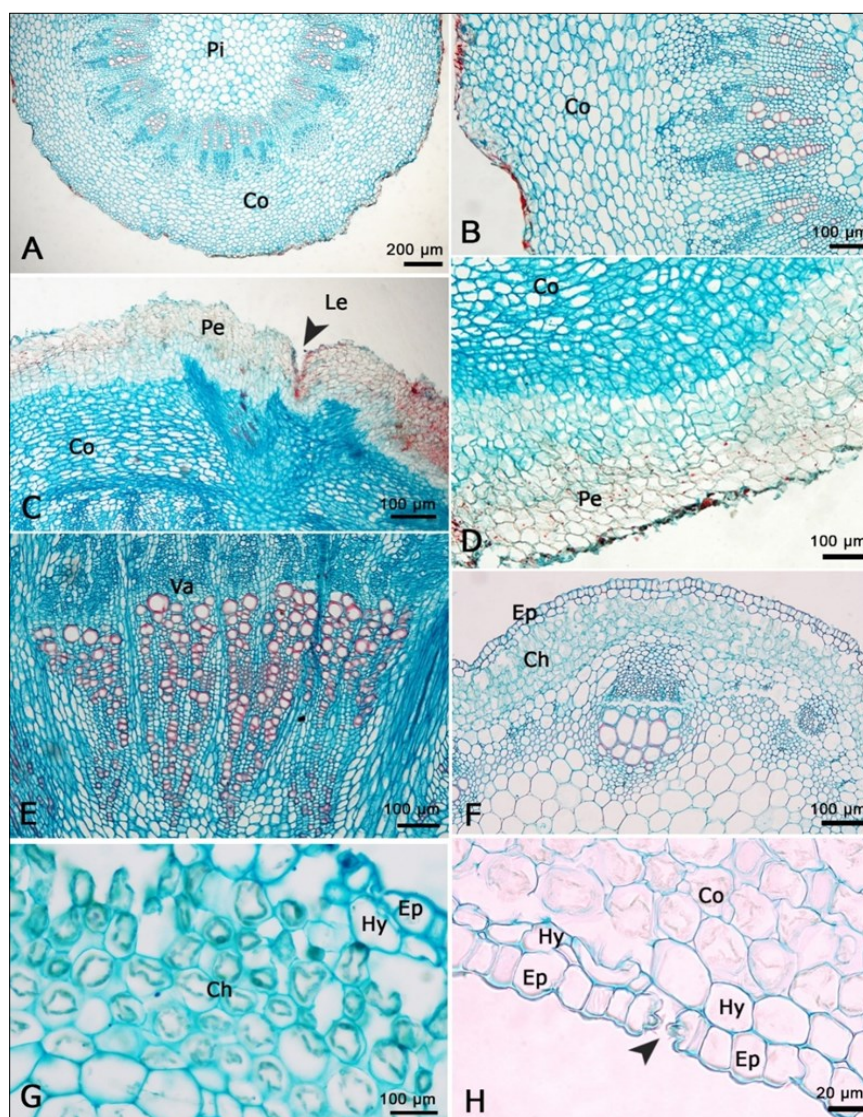


Fig. 4. Stem anatomy of *L. sarmentosa*. A) Transverse section of the aerial stem showing a ring of vascular bundles; B) Magnified view of cortex in aerial stem; C) Lenticel in rhizome bark (arrowhead); D) Magnified view of periderm; E) Vascular cambium lignin between xylem and phloem; F) Transverse section of running stolon; G) Chlorenchyma and hypodermis in stolon; H) Magnified view of stomata in stolon (arrowhead) (Ch: Chlorenchyma; Co: Cortex; Ep: Epidermis; Hy: Hypodermis; Le: Lenticel; Pe: Periderm; Pi: Pith; St: Stomata, Va: Vascular cambium).

Additionally, roots undergoing secondary growth displayed 5-6 layers of periderm and a prominent cortex (Fig. 5G & H). The cortex consisted of 16-18 layers of thin-walled parenchyma cells varying in size from large to small and shaped from circular to oval. These cells surrounded triangular or square-shaped intercellular spaces (Fig. 5G). Both endodermis and pericycle were indistinct. The vascular bundles maintained a radial arrangement and the xylem retained an exarch pattern. The pith remained absent.

Discussion

The vegetative anatomy of *L. sarmentosa* exhibits notable differences compared to other species within the Asteraceae family. Moreover, the anatomical features observed in this study indicate specific adaptations to environmental conditions. The leaves of *L. sarmentosa* were amphistomatic and exhibited anomocytic stomata. Anomocytic stomata are common among members of the Asteraceae, e.g., *Aspilia africana* (Pers.) C.D. Adams, *Ageratum conyzoides* L., *Solidago chilensis* Meyen and

Solidago chilensis Meyen (18, 19). However, a range of stomata, such as tetracytic, anisocytic, brachyparacytic and diacytic have been recorded within Asteraceae, (20, 21). Stomatal characteristics have proven to be taxonomically significant. Given that fossil evidence suggests anomocytic stomata are an ancestral trait in early-diverging angiosperm (21, 22), *L. sarmentosa* may represent a more primitive lineage within the Asteraceae family (23).

Although the present study did not identify other stomatal traits typically linked to arid environments such as sunken stomata and trichome-covered stomata, amphistomatic leaves have been associated with different stress conditions.

For instance, amphistomatic characteristics are observed in aquatic plants (e.g., *Potamogeton* spp.), crop plants such as *Zea mays*, herbaceous weeds like *Xanthium strumarium* L. and CAM plants such as *Opuntia ficus-indica* and *Kalanchoë daigremontiana* (24). Amphibious species such as *Veronica anagallis-aquatica* L. (Plantaginaceae) have also developed amphistomatic leaves, enabling them to thrive in both

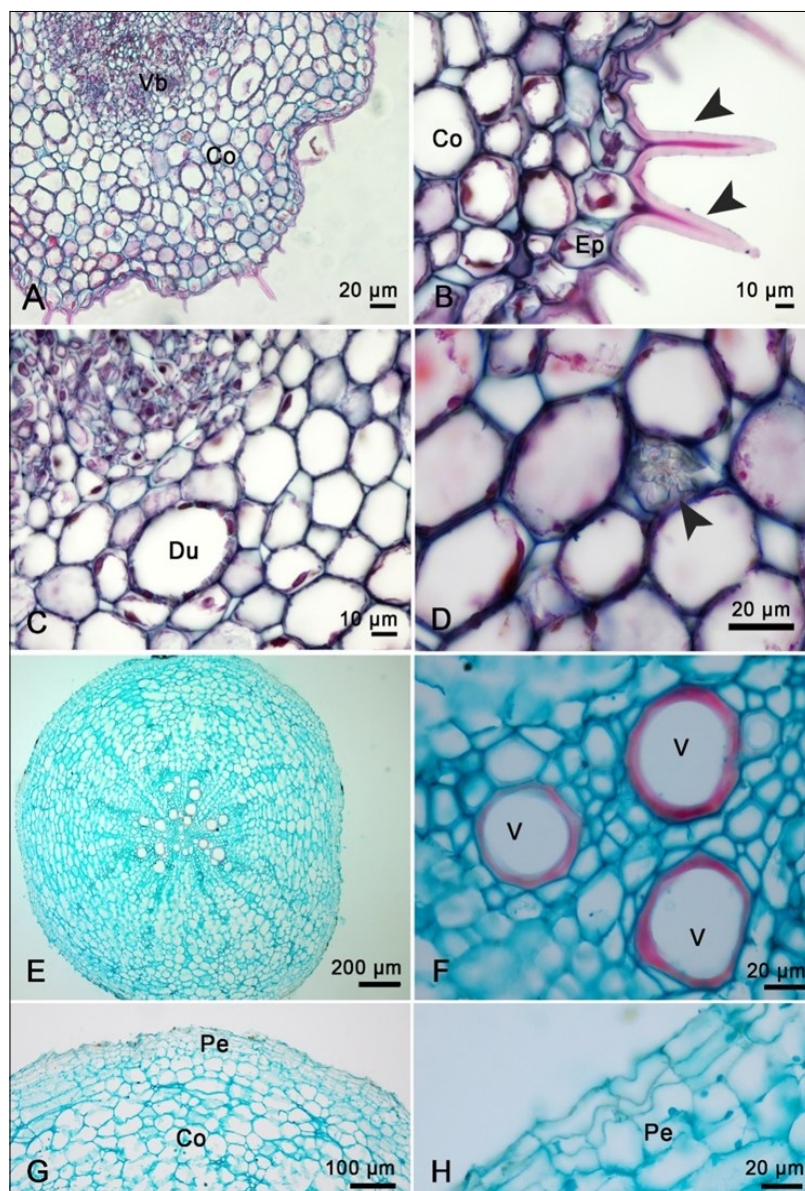


Fig. 5. Transverse sections of *L. sarmentosa* roots. A) The young root section presenting both concave and convex surfaces; B) Root hair in young root (arrowheads); C) Secretory duct in the cortex of root; D) Druse calcium oxalate in young root (arrowhead); E) Round shape of mature root; F) The old root section showing lignified vessel; G) Periderm and cortex in mature root; H) Magnified view of periderm (Co: Cortex; Du: Secretory duct; Ep: Epidermis; Vb: Vascular cylinder; V: Vessel; Pe: Periderm; Vb: Vascular bundle).

submerged and emergent aquatic environments. Typically, leaves exposed to water-limited conditions and intense light exhibit increased thickness and an amphistomatic nature (25). Amphistomatic leaves are considered an adaptive feature that enhances stomatal conductance and supports a high photosynthetic rate, both of which are crucial for plant development in high-light environments (26).

Habitat humidity has been suggested to impact stomatal type in *Pogostemon* Desf. (Lamiaceae) species (27). Consequently, a notable adaptive characteristic exhibited by *L. sarmentosa* is its capacity for amphistomy, a trait that may contribute to the species occurrence across different microhabitats within the coastal region, including both littoral zones and inland terrestrial habitats. Amphistomatic leaves may have evolved in response to increasing aridity during the tertiary period, as they increase leaf conductance to CO₂ (28). Therefore, amphistomatic leaves, which promote high maximum leaf conductance, are commonly found in species growing in arid environments (29). It is seen especially among herbs and shrubs from different environments such as early successional forests, deserts and swamps (26). In this study, 60 % of the observed species had amphistomatic leaves, consistent with findings in *Ambrosia cordifolia*, where most species grow in full sunlight during the short rainy season (26).

The average stomatal length and SI did not differ significantly between the adaxial and abaxial epidermis. However, the abaxial epidermis exhibited a significantly higher stomatal density (SD) than the adaxial epidermis. This finding is consistent with previous observations on *Pogostemon* spp. and on *Eucalyptus globulus* Labill. (Myrtaceae), where the stomatal density on abaxial surfaces was greater than on adaxial surfaces (27, 30). This pattern is commonly observed across many species because the abaxial surface is typically less exposed to direct sunlight, reducing water loss through transpiration. Moreover, this phenomenon is related to the differential regulation of gas exchange between the surfaces. In wheat, the abaxial surface exhibits a higher potential for gas exchange in comparison to the adaxial surface (31). The current study found that both the cuticle and epidermis were notably thick, a characteristic also previously reported (32). The presence of thickness in both the cuticle and the epidermis may be involved in a protective mechanism against dryness and sunlight. It also performs functions against the action and entry of pathogens and herbivores and prevents the overheating of internal tissues and the loss of water into the atmosphere.

The homobaric nature of leaves in *L. sarmentosa* is interesting as it deviates from the typical heterobaric condition observed in most Asteraceae species (33). This deviation may reflect ecological specialization or the retention of an ancestral trait. Generally, the leaves being homobaric is a notable feature. Homobaric leaves are characterized by uniform pressure and gas exchange throughout the leaf, as they lack internal compartments like veins or special barriers that would separate different regions of the leaf (8). This uniformity allows for more efficient gas movement and photosynthesis across the leaf surface. Most species in the Asteraceae family, however, tend to have heterobaric leaves, which feature prominent veins and internal barriers that compartmentalize the leaf into different zones with varying gas exchange capabilities. Heterobaric leaves

are thought to confer advantages in terms of water-use efficiency and stress tolerance, under environmental conditions, such as drought or high light intensity, by controlling gas exchange more precisely in different regions of the leaf. Therefore, the homobaric nature of *L. sarmentosa* leaves could indicate either a primitive condition or an adaptation to specific environmental conditions. It may suggest that the species evolved in environments where uniform gas exchange across the leaf surface was advantageous, perhaps in stable or humid habitats. On the other hand, the more common heterobaric structure in Asteraceae species reflects an adaptation to diverse environmental pressures, allowing for better regulation of water loss and photosynthetic efficiency under varying conditions.

L. sarmentosa possesses aerial stems, rhizomes and stolons. Rhizomes generally function as storage organs and serve to anchor the plant within the soil (8). In our observations, rhizomes exhibited the presence of a periderm, consistent with findings in *S. chilensis* Meyen (19). Additionally, lenticels-specialized structures in woody plants that facilitate gas and water exchange between internal tissues and the external environment through the bark-were observed on the aerial stem (8, 34). The presence of lenticels in the stem suggests an adaptive strategy for balancing water retention with gas exchange required for photosynthesis. This is supported by the structure of the stolon, where stomata, hypodermis and chlorenchyma were present. Within Asteraceae, chlorenchyma has been identified in the stolon cortex (19). Furthermore, the occurrence of a hypodermis as a feature that helps protect plants from desiccation (35). Stomata observed on the stolon surface may contribute to gas exchange regulation (8). In strawberries, stomatal activity and water potential in stolons are known to be influenced by environmental factors such as drought and flooding (36). Drought stress particularly impacts stolons more than leaves, given that stolons are responsible for transporting water and nutrients to developing daughter plants. Thus, stolons are a vital organ for *L. sarmentosa*, supporting survival in dune habitats.

In *L. sarmentosa*, the formation of abundant new rosettes was observed. The development of rosettes allows the plant to anchor itself more effectively and explore the surrounding soil. Root systems play a pivotal role in dune plant survival, as deep root penetration not only prevents soil erosion but also facilitates access to water sources (37). Extensive root systems are crucial for accessing deep moisture reserves (35). Accordingly, the abundance of lateral roots in *L. sarmentosa* is likely associated with structural support and water uptake. However, the roots of *L. sarmentosa* did not display a visibly distinct casparian strip. While the casparian strip typically regulates water movement and enhances selective permeability, its absence in this species may represent an adaptation for survival under arid conditions by permitting greater water influx. Additionally, the presence of periderm in the main root was observed, consistent with its recognized dual role in gas exchange and defense. The periderm functions as a primary protective barrier against biotic and abiotic stresses (11). In *L. sarmentosa*, the formation of periderm likely contributes to resilience against environmental challenges such as windstorms, water erosion and flooding.

Conclusion

The anatomical adaptations of *L. sarmentosa* reveal its survival mechanisms in the harsh coastal environments of Thailand's Andaman coast. Key features such as amphistomatic leaves, thick cuticles, homobaric leaf structure and periderm development in both stems and roots highlight the plant's ability to withstand drought, high light intensity and environmental stress. These traits, combined with its robust root system and specialized stolons, enable *L. sarmentosa* to thrive in coastal dune habitats despite ongoing habitat degradation. Future research should focus on its physiological responses to varying stressors, the genetic basis of its adaptations and methods for large-scale propagation. Such studies will inform comprehensive conservation strategies to protect and rehabilitate this threatened species, including habitat restoration, establishment of seed banks and *in situ* conservation efforts.

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Authors' contributions

YM originated the research framework. YM & PM conducted the fieldworks. PS performed anatomical procedures. YM & PS discussed the results and findings. All authors observed and described the anatomical features as well as read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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