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Anatomy of vegetative organs of *Lycopodiella cernua* (L.) Pic. Serm. and *Selaginella biformis* A. Braun ex Kuhn

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Abstract. This study examines the anatomical structures of vegetative organs in two Lycophyte species—*Lycopodiella cernua* (L.) Pic. Serm. and *Selaginella biformis* A. Braun ex Kuhn—based on six specimens collected from Cat Ba National Park and Phong Dien Nature Reserve (Hue). Using established morphological methods and double-staining techniques, we analyzed root, stem, and leaf anatomy to explore evolutionary adaptations in early diverging vascular plants. *L. cernua* exhibited primitive features, including a mixed protostele in both roots and stems, undifferentiated endodermis, and simple leaves lacking clear mesophyll differentiation. In contrast, *S. biformis* showed more derived traits: a well-defined endodermis with Casparian strips, exarch protostele in roots, and polystelic stems with trabeculae. Its leaves displayed a dorsiventral structure, chloroplasts confined to the upper epidermis, and stomata on the lower surface, along with a flattened lamina and large intercellular spaces. These anatomical differences suggest that *S. biformis* has evolved structural specializations that enhance water conduction, photosynthesis, and mechanical support, compared to the more ancestral organization in *L. cernua*. The findings contribute valuable data to the anatomical database of Lycophytes and offer insights into the evolutionary transitions of vascular tissues in seedless plants. Moreover, the study provides additional evidence for species-level anatomical adaptations within Lycophytes, highlighting their evolutionary strategies for survival in both xeric and mesic environments. The comparative data between genera also serves as a useful reference for future taxonomic, ecological, and evolutionary research in Lycophyta.

Keywords: anatomy of lycophytes, *Lycopodiell*a, *Selaginella*

1 Introduction

Lycophytes are one of the most ancient lineages of vascular plants, comprising three main families: Lycopodiaceae, Selaginellaceae and Isoetaceae. Lycophytes retain many primitive characteristics, such as having small leaves (microphylls) and reproducing via spores [1, 2]. *Lycopodium*, *Lycopodiella* (Lycopodiaceae) possesses a primitive protostele [3, 4], reflecting a simple and ancestral anatomical structure. In contrast, *Selaginella* (Selaginellaceae) shows a more advanced condition with a plectostele or polystele, a stele fragmented into multiple meristeles, each surrounded by its own endodermis, indicating structural specialization and adaptation.

This study contributes to building a database on the anatomical features of *Lycopodium* and *Selaginella*, which retain many primitive traits.

However, anatomical studies focusing on internal structures of vegetative organs such as roots, stems, and leaves in the genera *Lycopodium* and *Selaginella* are still very limited. In Vietnam, there is almost no published material on the anatomy of these genera. Therefore, developing detailed anatomical illustrations and descriptions of them is essential. Lycophytes—seedless

vascular plants—are essential for research, education, and plant taxonomy in Vietnam. The image dataset obtained from this research serves as a foundation for future studies on the evolutionary trends of vascular bundle development in seedless vascular plants.

2 Materials and methods

A total of six specimens of 2 species, included: *Lycopodiella cernua* (CB2024-001, CB2024-002, PD2023-0501) and *Selaginella biformis* (PD2023-0913, PD2023-0929, CB20024-003). They are widely distributed throughout Vietnam, from the South to the North [5]. Specimens were collected in Cat Ba National Park (Hai Phong) and Phong Dien Nature Reserve (Hue).

Species identification was conducted using the expert-based approach. The scientific names of two species were confirmed by Prof. Dr. Tran The Bach, Department of Botany, Institute of Biology, Vietnam Academy of Science and Technology.

This study applied the morphological analysis methods of Nguyen Ba [6], Bendre and Kumar [7]; anatomical sectioning and double-staining procedures were carried out following the protocols of Hoang Thi San and Nguyen Phuong Nga [8], as well as Bendre and Kumar [7]. The samples were bleached with 12% sodium hypochlorite (NaOCl) for 3 minutes, neutralized with 3% HCl for 2 minutes, stained with methylene blue for 30 seconds, and finally stained with carmine for 10 minutes, with thorough rinsing in distilled water between each step.

Sections' positions of vegetative organs were illustrated in Fig. 1. For each position of stems, roots and leaves, 7-10 continuous thin sections were made. Transverse sections of the specimens were captured at an appropriate magnification using Olympus CX23 microscope. Cell dimensions were measured by micrometry and PortableCapture Pro software.

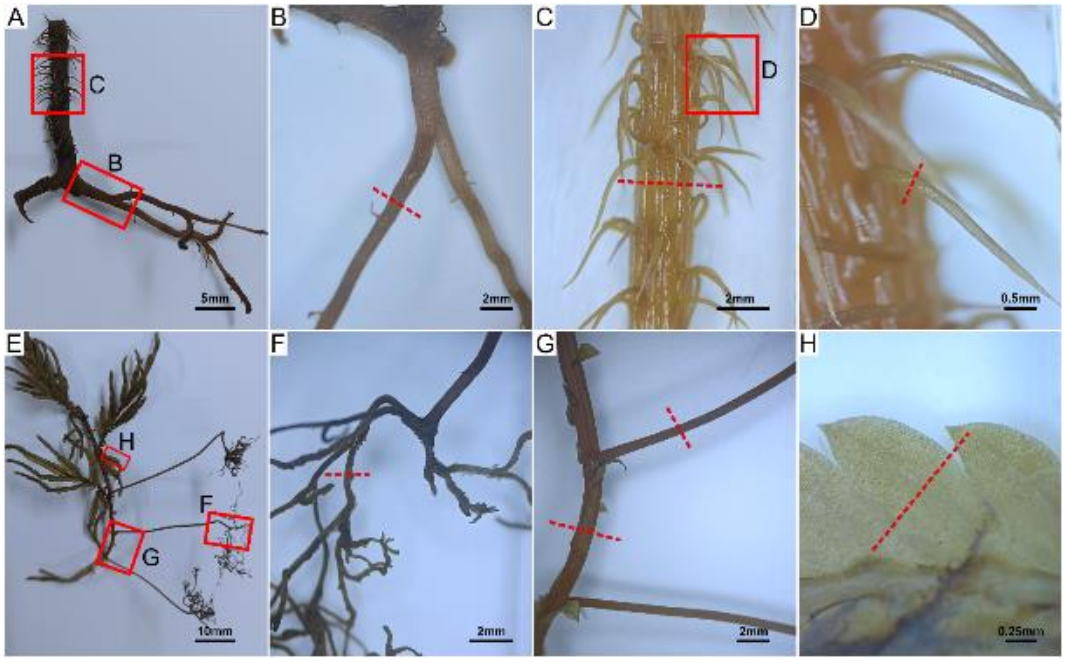

Fig. 1. Illustration of sections' positions on each specimen. A, B, C, D: *Lycopodiella cernua* (L.) Pic. Serm.; E, F, G, H : *Selaginella biformis* A.Braun ex Kuhn

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3 Results and discussion

3.1 Root anatomy of *L. cernua* (L.) Pic. Serm. and *S. biformis* A.Braun ex Kuhn

L. cernua: The outermost layer, the epidermis, consists of thin-walled cells (Fig. 2B) without root hairs and has an average thickness of 39.32 ± 7.86 μm. Directly beneath the epidermis lies a sclerenchymatous layer (Fig. 2B), which originates from parenchymatous cells that have undergone lignification, providing enhanced mechanical support. The inner cork layers are made up of thin-walled parenchymatous cells lacking intercellular spaces (Fig. 2C). The innermost layer could not be identified as an endodermis, since it shows no clear structural distinction from the neighboring cells. The stele is mixed or dissected protostele, where xylem strands are distributed throughout the xylem mass, instead of being exclusively in the center or at the periphery, with several protoxylem pole leading to exarch maturation of xylem (Fig. 2C). Xylem is composed exclusively of tracheary elements, lacking any parenchyma (Fig. 2C). The transverse section of xylem vessel measured 44.31 ± 8.34 μm in length and 33.26 ± 5.88 μm in width. Phloem is dissected by xylem strands into several solid masses distributed throughout the stele (Fig. 2A, 2C). Phloem consists solely of conducting elements without companion cells (Fig. 2C).

S. biformis: Roots arise from rhizophore, which is a simple leafless cylinder that lacks a root cap and root hairs, root-bearing organ arising from the stem, only involved in Lycophytes [9]. Although morphologically distinct from both roots and stems, it typically gives rise to adventitious roots at its tip. The rhizophore is a specialized structure arising from the stem and growing downward, it also serves a mechanical support function, enhancing the plant's stability and resistance to external physical effects. Cross sections of rhizophore and root clearly show the structures consisted of mainly 2 parts: cortex and stele, which can be easily distinguished by endodermis between them (Fig. 2D, 2G). Epidermis, 12.94 ± 1.74 μm in thickness, and 3-4 layers beneath, which is called hypodermis, are thick walled and sclerified leading to improved mechanical support (Fig. 2E). Many inner layers of cortex are formed by parenchymatous cells without intercellular space (Fig. 2F). The innermost layer of cortex is endodermis (Fig. 2F), which is a layer of suberized parenchymatous cells. Endodermal cells with Casparian strips can be easily recognised (Fig. 2F), which function as apoplastic barriers, regulating the selective uptake of water and solutes into the vascular cylinder. Pericycle consisted of 1-2 cell layers (Fig. 2F), located just inside the endodermis, playing a crucial role in the initiation of lateral roots. Vascular bundle is amphicribral with a protoxylem pole at the outermost of the xylem core (Fig. 2F). The stele is typically protostele and exarch condition with a xylem mass in the center surrounded by phloem, except opposite the protoxylem pole (Fig. 2F). Xylem and phloem are consisted only of conducting elements (Fig. 2F). Xylem vessel cross-section: 23.29 ± 7.40 × 18.51 ± 5.49 μm.

The anatomy of the root is almost similar to rhizophore, except the thickness of hypodermis while epidermis's thickness is approximately equal, 13.64 ± 1.93 μm. Hypodermis in root is thinner than in rhizophore (Fig. 2E, 2H) due to function of root, which facilitates the transport of ions and water through cortex region. Endodermis in root is much thicker walled and fully suberized walled (Fig. 2F, 2I) to ensure selective uptake of water and ions, minimize water loss, and protect vascular tissues from soil-borne stress. In contrast, rhizophores are non-absorptive structures, and their thinner endodermis facilitates the emergence of adventitious roots.

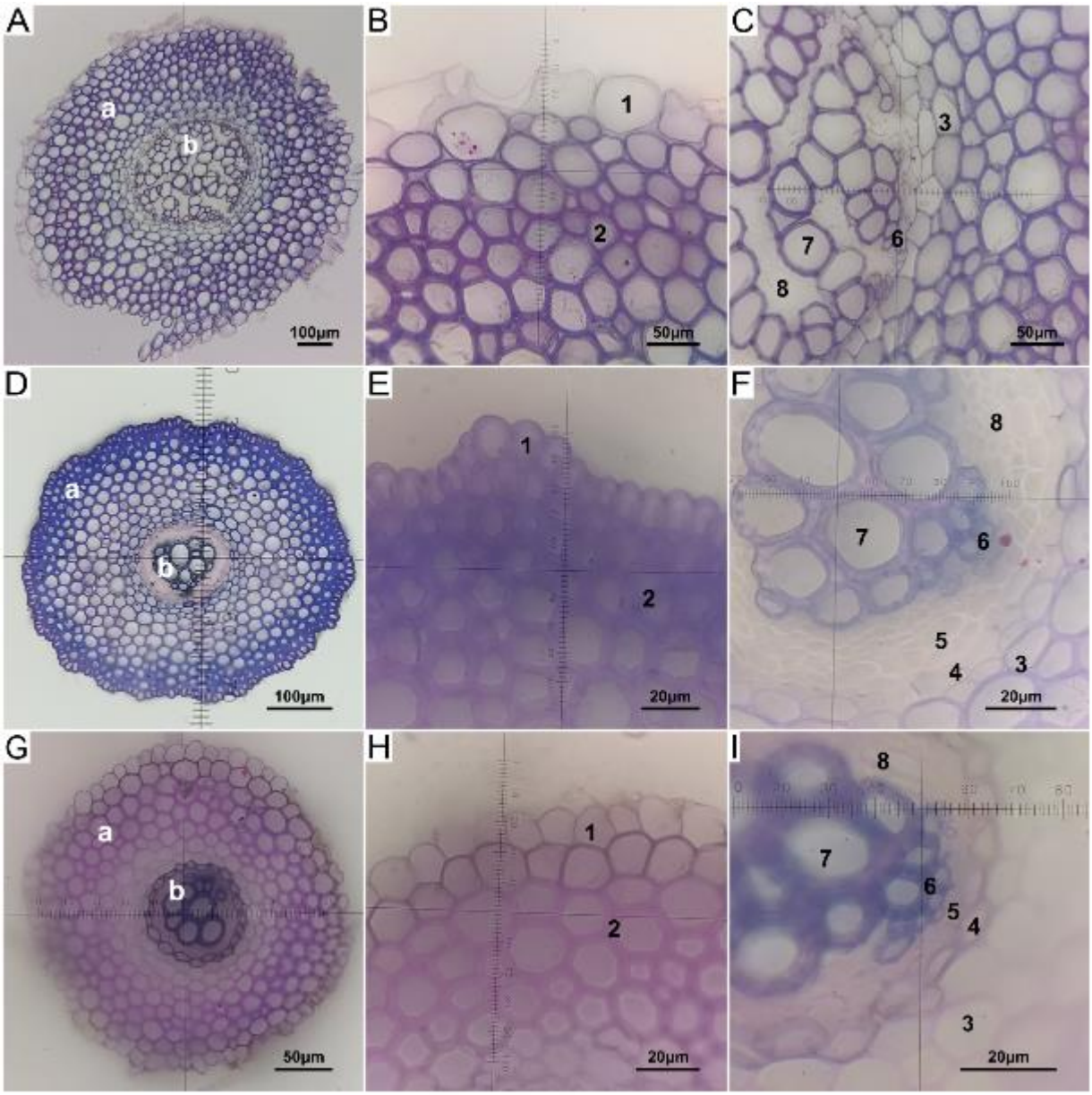

Fig. 2. Tranverse microsections of roots and rhizophore. A, B, C: root of *Lycopodiella cernua* (L.) Pic. Serm.; D, E, F: rhizophore of *Selaginella biformis* A.Braun ex Kuhn; G, H, I: root of *Selaginella biformis* A.Braun ex Kuhn; a: cortex; b: stele; 1: epidermis; 2: sclerenchyma; 3: parenchyma; 4: endodermis; 5: pericycle; 6: protoxylem; 7: metaxylem; 8: phloem. (Photos: Cao Hoang Tuan)

3.2 Stem anatomy of *L. cernua* (L.) Pic. Serm. and *S. biformis* A.Braun ex Kuhn

L. cernua: Transverse microsections of the stem shows 2 parts: cortex and stele (Fig. 3A). Cortex consists of 3 distinct components. Outer cortex is composed of the outermost layer of epidermis, 24.20 ± 5.03 µm in thickness, and several layers of parenchyma beneath (Fig. 3B). Located inside the outer cortex is middle cortex (Fig. 3B, 3C), consists of 4-5 layers of cells which are thick walled and undergoing lignification. It functions as an external supporting framework for the plant while simultaneously protecting the inner ground tissue and vascular cylinder. The inner cortex is formed by parenchymatous tissue without any intercellular space (Fig. 3D). The innermost layer

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of cortex is constructed from thin walled and smaller cells (Fig. 3D), and cannot be clearly defined as endodermis. Distributed throughout the cortex region are leaf traces formed by the first 1-2 layers of stele – pericycle (Fig. 3C, 3D), which are located right under the last cortex parenchyma layer. The stele in stem is organized into a mixed protostele similar to root in which the xylem strands lie scattered in phloem (Fig. 3D). The xylem is exarch with protoxylem facing towards the periphery and metaxylem towards the centre (Fig. 3D). Cross-section of a xylem vessel: 92.25 ± 18.93 × 63.25 ± 19.78 μm. The absence of vascular transition between the stem and the root suggests that the stele structure in this group remains primitive [3].

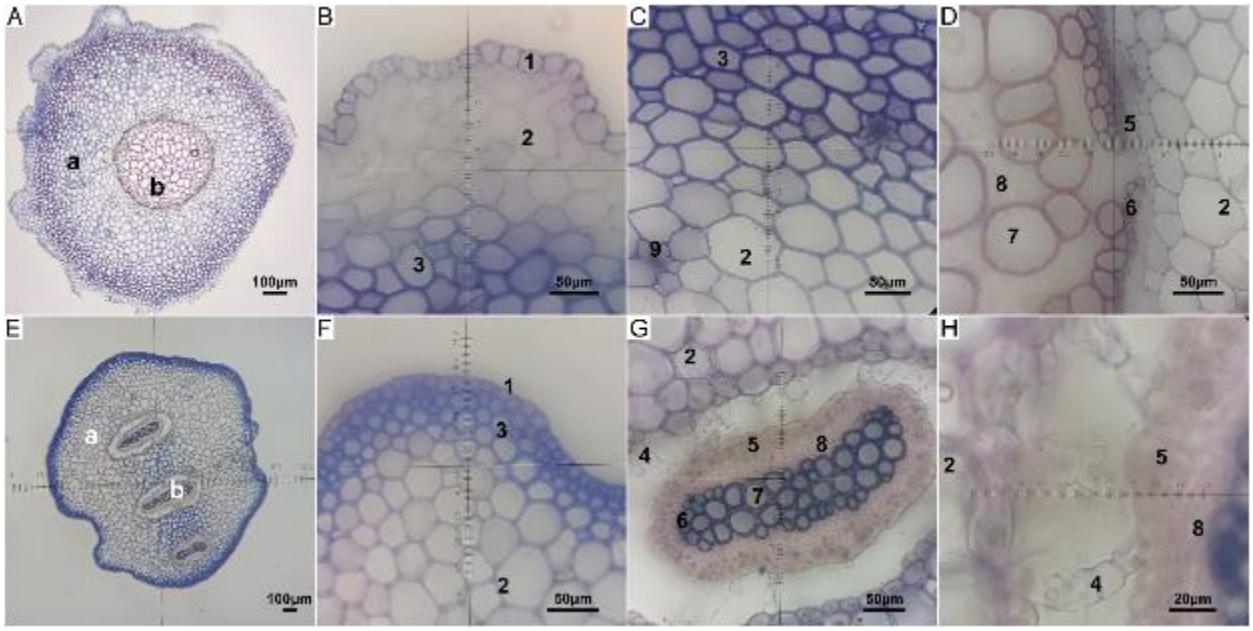

Fig. 3. Transverse microsections of stems. A, B, C, D: *Lycopodiella cernua* (L.) Pic. Serm.; E, F, G, H: *Selaginella biformis* A.Braun ex Kuhn; a: cortex; b: stele; 1: epidermis; 2: parenchyma; 3: sclerenchyma; 4: trabeculae; 5: pericycle; 6: protoxylem; 7: metaxylem; 8: phloem; 9: leaf trace. (Photos: Cao Hoang Tuan)

S. biformis: Transverse microsection of the stem shows a special structure including cortex and 3 steles, which is called polystele (Fig. 3E). Epidermis, 13.09 ± 1.90 μm in thickness, and several outer layers of cortex are composed of thick walled cells (Fig. 3F), meanwhile, ground tissues which located between 2 steles also undergo lignification (Fig. 3G), these 2 structures provide the stem a stable framework which give mechanical support so that plant can adapt to arid condition. The still region of cortex is made of thin walled parenchymatous cells without any intercellular space (Fig. 3E, 3F). The endodermis separates steles from the cortex region, by radially elongated endodermis cell, called trabeculae (Fig. 3H), with conspicuous intercellular spaces between 2 trabeculae. First 1-2 layers of each stele are pericycle surrounding xylem and phloem and follows endodermis (Fig. 3G). Each stele is generally protoxylem with xylem core fully surrounded by phloem (Fig. 3G). Xylem in cross-section appears in oval or elongated shape (Fig. 3G). Xylem vessel cross-section: 24.76 ± 6.23 × 21.89 ± 6.01 μm. Phloem consists of smaller cells with dense protoplasm. Polystele allows for greater internal flexibility and redundancy (damage to one stele may not affect others), the distribution of vascular tissues throughout the cortex may reduce the overall mechanical rigidity of the axis. It may offer adaptive advantages by

distributing mechanical loads more evenly across the organ. The transition from a protostele in roots to a polystele in stems reflects a functional and evolutionary adaptation [3]. While the compact protostele provides mechanical strength and efficiency for water uptake underground, the polystele in stems enhances transport capacity, structural flexibility, and supports lateral organ formation—marking a step toward more advanced vascular architectures in plant evolution.

3.3 Leaf anatomy of *L. cernua* (L.) Pic. Serm. and *S. biformis* A.Braun ex Kuhn

L. cernua: Transverse section of leaf shows the typical structure of a primitive plant's leaf. The outermost layer is epidermis with no differentiation into upper and lower parts. Epidermal cell dimensions were 238.69 ± 57.18 μm in length, 27.16 ± 5.12 μm in width and 20.46 ± 2.84 μm in thickness. This is a single layer of compactly arranged cells without any intercellular space (Fig. 4A) and likely with a cuticle. It functions as a protective barrier, may participate in gas exchange and prevent water loss. Chloroplasts can be easily recognised in epidermal cells. Some epidermal cells differentiated into guard cells forming stomata (Fig. 5B, 5C), which increases the gas exchange through air pore between each 2 guard cells. Beneath the epidermis is mesophyll (Fig. 4B) - a region of loosely arranged parenchyma cells with visible intercellular spaces and some chloroplasts, greenish with several pyrenoid-like bodies, facilitating active photosynthesis. However, it does not show clear differentiation into palisade and spongy mesophyll – a more evolved trait found in angiosperm leaves for optimized photosynthesis and gas exchange. This is an indication that the tissue organization is not highly evolved, making it suitable primarily for moist or semi-aquatic environments [9]. Located centrally is a vascular bundle (Fig. 4C) with highly lignified cells, supports conduction of water and mechanical support. Cross-section of a xylem vessel: 4.68 ± 1.42 × 3.21 ± 0.92 μm. The venation is only composed of a longitudinal bundle bounded by bundle sheath without branching.

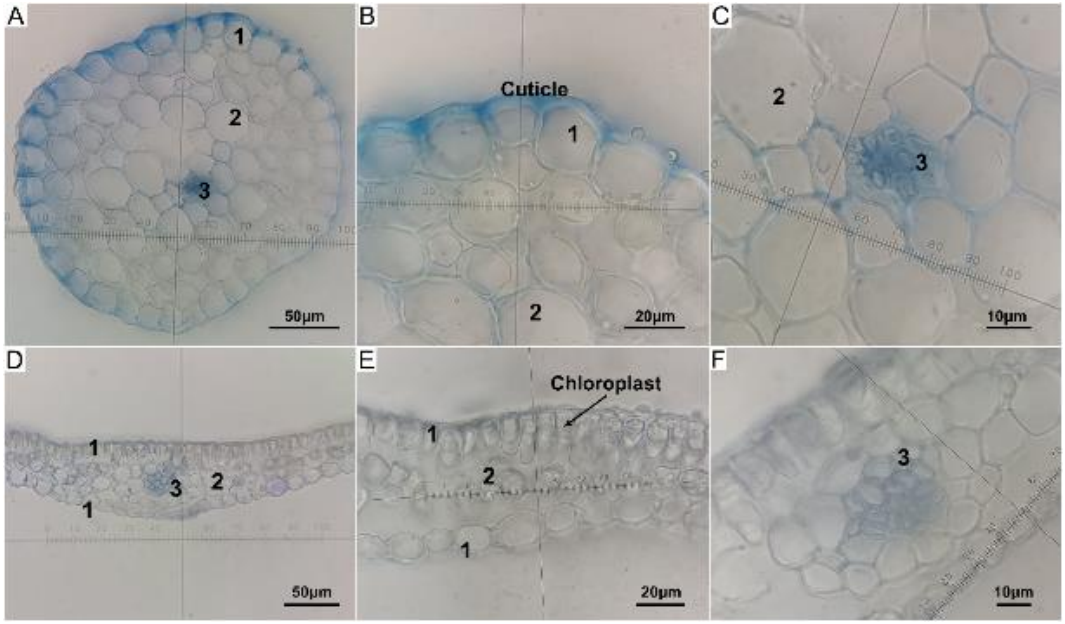

Fig. 4. Tranverse microsections of leaves. A, B, C: *Lycopodiella cernua* (L.) Pic. Serm.; D, E, F: *Selaginella biformis* A.Braun ex Kuhn; 1: epidermis; 2: parenchyma; 3: vascular bundle. (Photos: Cao Hoang Tuan)

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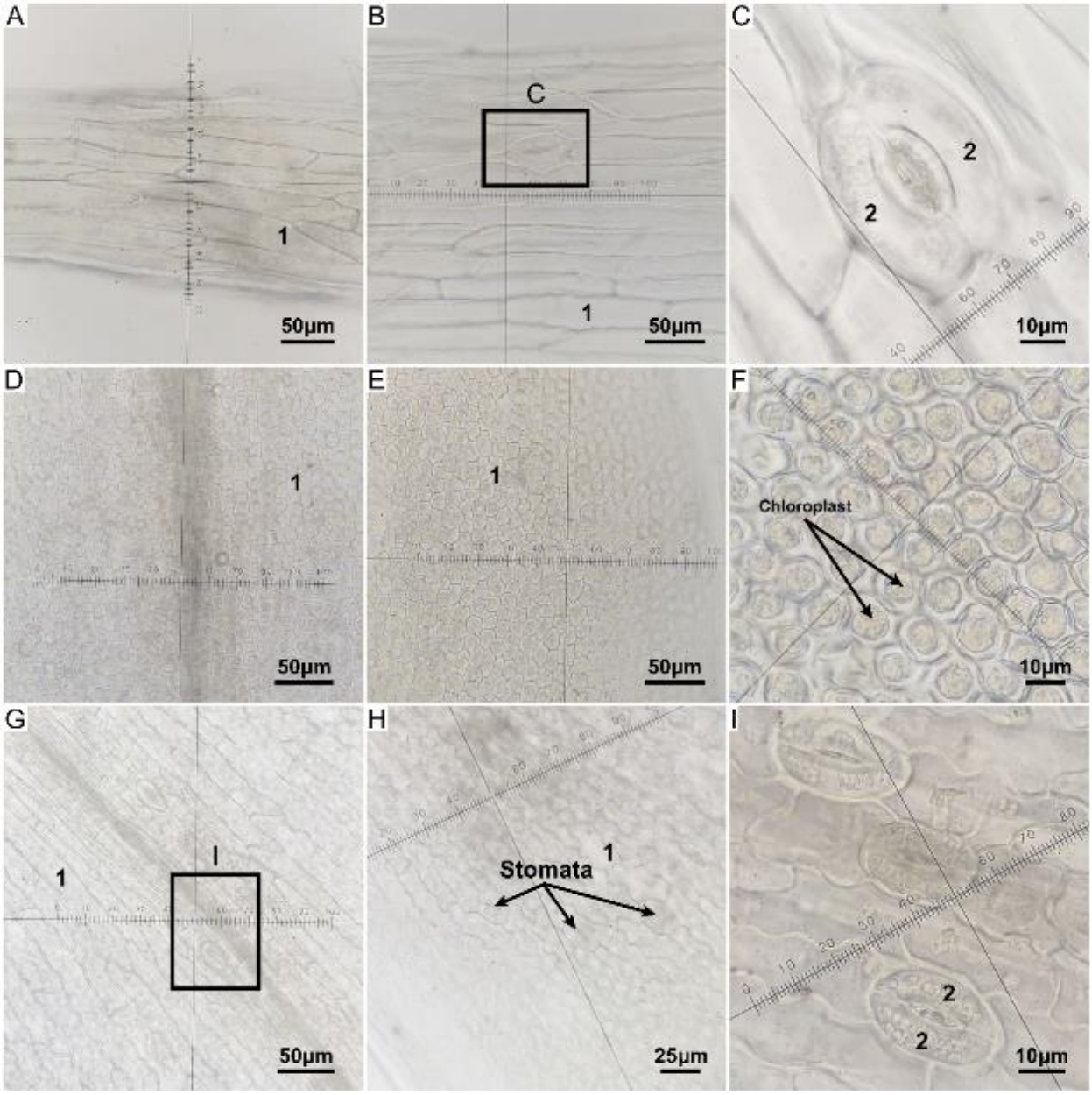

Fig. 5. Epidermis. A, B, C: *Lycopodiella cernua*; D-I : *Selaginella biformis* (D, E, F: upper epidermis; G, H, I: lower epidermis). 1: epidermal cell; 2: guard cell. (Photos: Cao Hoang Tuan)

S. biformis: Transverse microsections (Fig. 4) also show a primitive structure of leaf. The epidermis forms a continuous outermost layer of compact cells, serving as a protective covering (Fig. 4D). The epidermis of the leaf is differentiated into an upper and a lower layer. The upper epidermal cells are slightly elongated in shape, while the lower epidermal cells are more spherical. The upper epidermis measured 33.51 ± 3.63 µm in length and 28.43 ± 2.66 µm in width, with a thickness of 20.75 ± 1.78 µm, while the lower epidermis measured 187.47 ± 31.68 µm in length and 27.26 ± 4.01 µm in width, with a thickness of 13.37 ± 2.14 µm. Chloroplasts are localized exclusively in the cells of the upper epidermis. Notably, stomata are absent from the upper epidermal surface (Fig. 4E, 5E, 5F). Some epidermal cells on the lower epidermis differentiate into guard cells forming stomata

(Fig. 4D), which increases the gas exchange. Stomata are predominantly concentrated along both sides of the main vein, with a more scattered distribution across the lamina ((Fig. 5G, 5H). The leaf has a flattened laminar structure (Fig. 4D), which contrasts with the more three-dimensional leaf morphology of *L. cernua*. This flattened form increases the surface area of the leaf, thereby enhancing its capacity to capture light for photosynthesis. Just beneath it lies the mesophyll without being differentiated into palisade and spongy mesophyll (Fig. 4D). It shows many conspicuous intercellular spaces, which allows for the efficient diffusion of CO_2 into photosynthetic cells and the release of O_2 and water vapor as byproducts of photosynthesis and transpiration [11]. In primitive plants or those adapted to humid environments, large intercellular spaces help maintain internal aeration, preventing hypoxia in tissues. The vascular bundle appears as a continuous strand running along the length of the leaf (Fig. 4D).

Table 1. Anatomical measurements of root, stem, and leaf in L. cernua (L.) Pic. Serm

Parameter		Root			Stem			Leaf		
		$\bar{x}$	S	CV(%)	$\bar{x}$	S	CV(%)	$\bar{x}$	S	CV(%)
Epidermis thickness (μm)		39.32	7.86	19.99	24.20	5.03	20.79	20.46	2.84	13.90
Xylem vessel cross-section dimensions (μm)	**Length**	44.31	8.34	18.82	92.25	18.93	20.52	31.57	4.68	30.40
	Width	33.26	5.88	17.69	63.25	19.78	31.27	3.21	0.92	28.56
Epidermal cell dimensions (μm)	**Length**							238.69	57.18	23.96
	Width							27.16	5.12	18.85

Table 2. Anatomical measurements of root, rhizophore, stem, and leaf in S. biformis A.Braun ex Kuhn

Parameter		Root			Rhizophore			Stem			Leaf		
		$\bar{X}$	S	CV(%)	$\bar{X}$	S	CV(%)	$\bar{X}$	S	CV(%)	$\bar{X}$	S	CV(%)
Epidermis thickness (μm)		13.64	1.93	14.13	12.94	1.74	13.42	13.09	1.90	14.49	20.75	1.78	8.56
Upper epidermis thickness (μm)											13.37	2.14	15.99
Lower epidermis thickness (μm)											6.73	1.16	17.25
Xylem vessel cross-section dimensions (μm)	**Length**	23.29	7.40	31.76	18.17	4.07	22.40	24.76	6.23	25.14	35.36	4.12	11.66
	Width	18.51	5.49	29.65	14.02	4.06	28.94	21.89	6.01	27.45	5.79	0.96	16.53
Upper epidermal cell dimensions (μm)	**Length**										33.51	3.63	10.83
	Width										28.43	2.66	9.36
Lower epidermal cell dimensions (μm)	**Length**										187.47	31.68	16.90
	Width										27.26	4.01	14.72

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Vascular bundle is simple and unbranched, with no lateral veins —features commonly seen in more advanced vascular plants. The structure likely represents a protostele, where xylem and phloem are arranged in a central, solid core without a pith. Xylem vessel cross-section: 6.73 ± 1.16 × 5.79 ± 0.96 μm. This arrangement is characteristic of primitive vascular plants [10], where water and nutrient conduction is handled by a central, narrow stele without complex vascular architecture, which is suitable for small organs in plants that do not require extensive structural support or long-distance transport like those seen in larger, more evolved vascular plants [12].

4 Conclusion

This study highlights key anatomical differences in the vegetative organs of *Lycopodiella cernua* and *Selaginella biformis*. While both exhibit primitive vascular traits, *S. biformis* shows more specialized structures such as defined endodermis, polystele in stem, and flattened leaves—suggesting adaptive evolution. In contrast, *L. cernua* retains more ancestral features like mixed protostele and undifferentiated tissues. These findings contribute to the anatomical database of early-diverging vascular plants and provide insights into the evolutionary adaptations of lycophytes. The anatomical features of the two studied species are consistent with adaptations to either mesic environments (*L. cernua*: thin cuticle; mixed protostele in roots and stems; undifferentiated endodermis; simple leaf structure without mesophyll differentiation) or xeric habitats (*S. biformis*: exarch protostele in roots, polystele in stems, distinct endodermis with casparian strips; conspicuous intercellular spaces and stomata only on the lower surface).

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