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MORPHOLOGICAL AND ANATOMICAL ADAPTATIONS OF THE TUBER FOR PARASITISM IN THREE SPECIES OF *Balanophora* J.R. Forst. et G. Forst. FROM MU CANG CHAI, YEN BAI

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Abstract. The genus *Balanophora* J.R. Forst. et G. Forst. (Balanophoraceae) includes obligate holoparasitic plants that lack chlorophyll and are completely dependent on their hosts for water and nutrients. This study investigates the morphological and anatomical characteristics of the tuber in three *Balanophora* species (*B. fungosa, B. laxiflora, and B. harlandii*) collected from Mu Cang Chai, Yen Bai, Vietnam. Detailed observations were conducted to describe the structural adaptations related to parasitism, focusing on the organization of the tuber and the development of composite bundles that form the host–parasite interface. The results reveal that the tuber functions as a highly specialized parasitic organ, consisting of an outer cortex, matrix parenchyma, parasite vascular bundles, and composite bundles. The composite bundles play a pivotal role in resource exchange between the host and the parasite, with transfer cells facilitating efficient nutrient absorption. These findings provide new insights into the structural and functional adaptations of *Balanophora*, contributing to a better understanding of the evolutionary strategies of holoparasitic plants.

Keywords: *Balanophora*, tuber anatomy, holoparasitic plants, composite bundle, parasitic adaptation, Vietnam**.**

1. Introduction

The genus *Balanophora* (Balanophoraceae) comprises approximately 15 - 20 species worldwide, with five species also well known as medicinal plants in Vietnam [1], [2].

Members of this genus are obligate holoparasitic plants, entirely lacking chlorophyll and incapable of photosynthesis, and thus depend completely on their host plants for nutrients [3].

Parasitic strategies in plants display remarkable diversity and complexity, largely determined by the structure and function of the haustorium, a specialized organ that enables attachment to the host, establishes vascular connections, and mediates the bidirectional transfer of water, minerals, and organic compounds between the parasite and the host. Depending on the species, haustoria may develop from stem tissues, root tissues, or arise exclusively from the apical meristem of the root tip [4].

Species of *Balanophora* typically parasitize the roots of various host families, including Fabaceae, Ericaceae, and Urticaceae. At the site of host–parasite contact, the tissues of the parasite differentiate into a cylindrical or nearly spherical tuberous structure, which may or may not be branched. This tuber serves as the critical interface for resource exchange between the parasite and its host [5], [6].

In Vietnam, research on parasitic plants, particularly on species within *Balanophora*, remains limited. Previous studies have primarily focused on the morphological characteristics of pollen grains and the phytochemical characterization of lignans isolated from *B. fungosa* subsp. *indica* [3]. Additionally, a few taxonomic reports have documented newly recorded *Balanophora* species within the Vietnamese flora. However, comprehensive investigations into the haustorial anatomy and parasitic mechanisms of these species are still lacking. Understanding the specialized structures involved in parasitism, particularly haustorial cells and transfer cells, is critical to elucidating how these plants efficiently transport nutrients across the host–parasite boundary. Such knowledge provides insight into the evolutionary adaptations and ecological strategies of holoparasitic plants [7]-[9].

The present study aims to (1) describe in detail the anatomical features of haustorial structures in three *Balanophora* species distributed in Mu Cang Chai, Yen Bai Province, Vietnam, and (2) illustrate and explain the vascular connections between the xylem and phloem of the host and the parasite. These findings will contribute to a better understanding of parasitic strategies within the Balanophoraceae and serve as a foundation for future taxonomic, anatomical, and ecological studies of obligate holoparasites.

2. Content

2.1. Materials and methods

2.1.1. Sampling and preliminary processing

A total of 19 specimens representing three species (*B. fungosa*, *B. laxiflora*, *B. harlandii*) were collected in Mu Cang Chai, Yen Bai, Vietnam, in November and December 2024 and then stored in Hanoi Herbarium (HN), Vietnam Academy of Science and Technology, and Department of Botany, Faculty of Biology.

Specimens were collected, processed, and preserved following the procedure of Nguyen Nghia Thin [10]. Species identification was conducted based on expert consultation. The scientific names of the three species were confirmed by Prof. Dr. Tran The Bach, Department of Botany, Institute of Biology, Vietnam Academy of Science and Technology.

2.1.2. Morphological examination

The morphological characteristics of the specimens were analyzed following the method of Nguyen NT [10] and Bendre and Kumar [11].

The principal morphological characteristics were examined, documented, and compared with other *Balanophora* specimens as well as relevant taxonomic references [7]-[9].

2.1.3. Double-staining technique and anatomical observation

Anatomical sectioning and double-staining procedures were performed following the protocols of HT San and NP Nga [12], as well as those of Bendre and Kumar [11]. The samples were bleached in 12% NaClO bleach (Javel water) until discoloration was complete, then neutralized in 3% HCl for 2 minutes, stained with methylene blue for 30 seconds, and counterstained with carmine for 10 minutes, with thorough rinsing in distilled water between each step.

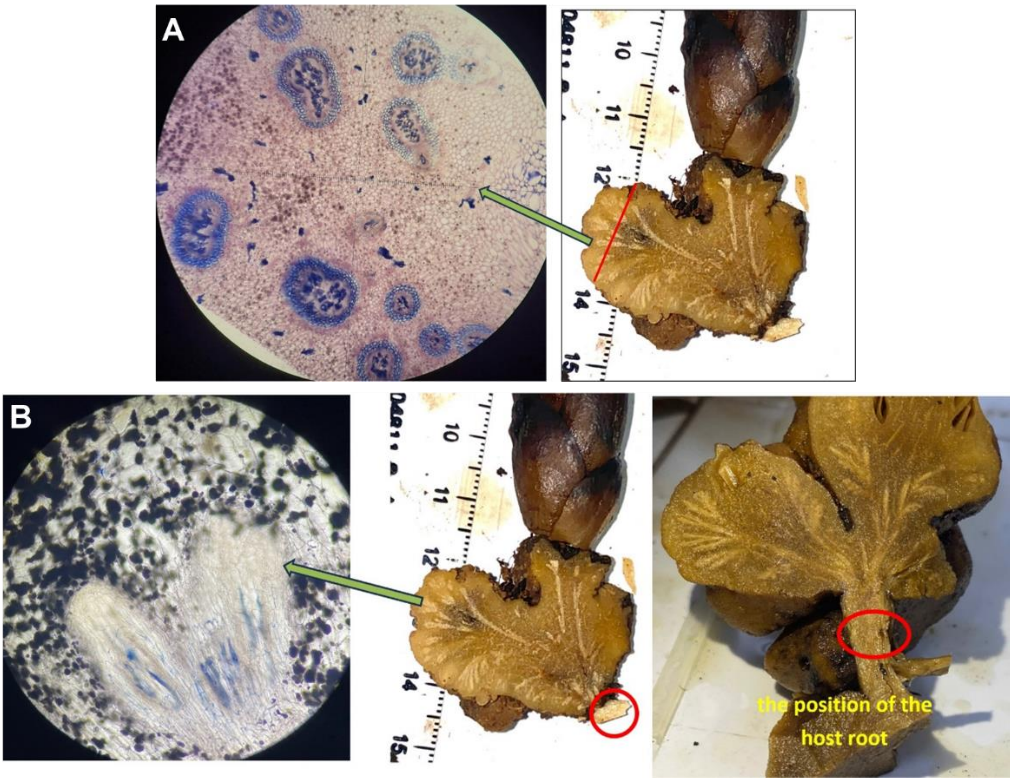

Figure 1. Transverse sections (A) and longitudinal sections (B) of B. laxiflora tuber

The sectioning positions of each specimen are illustrated in Figure 1. For each position, 10 - 15 continuous thin transverse and longitudinal sections were prepared. Micrographs of the specimens were captured at appropriate magnifications using an Olympus CX23 microscope.

Statistical data, including the mean (x̄), standard deviation (s), and coefficient of variation (CV), were calculated and analyzed using Microsoft Excel Office 2022.

2.2. Results

2.2.1. Morphological and anatomical descriptions of tubers

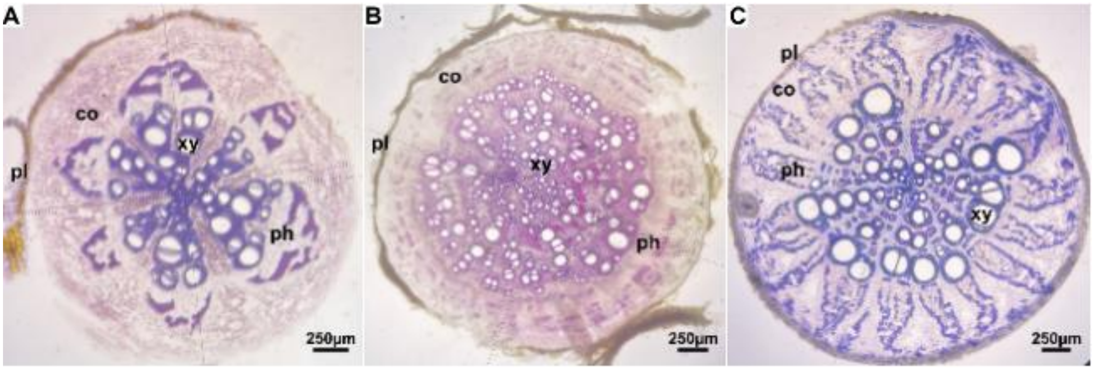

Figure 2. Anatomy of three host roots without parasitism
A. B. fugosa; B. B. harlandii; C. B. laxiflora. pl: phellem; co: cortex; ph: phloem; xy: xylem

Species of the genus *Balanophora* are obligate holoparasitic plants (Gonzalez & Mauseth, 2010). They lack true stems and roots, bear scale-like leaves devoid of chlorophyll, and are dioecious. Previous studies have reported that *Balanophora* species typically parasitize the roots of host plants belonging to families such as Fabaceae, Sapindaceae, or Rosaceae [13].

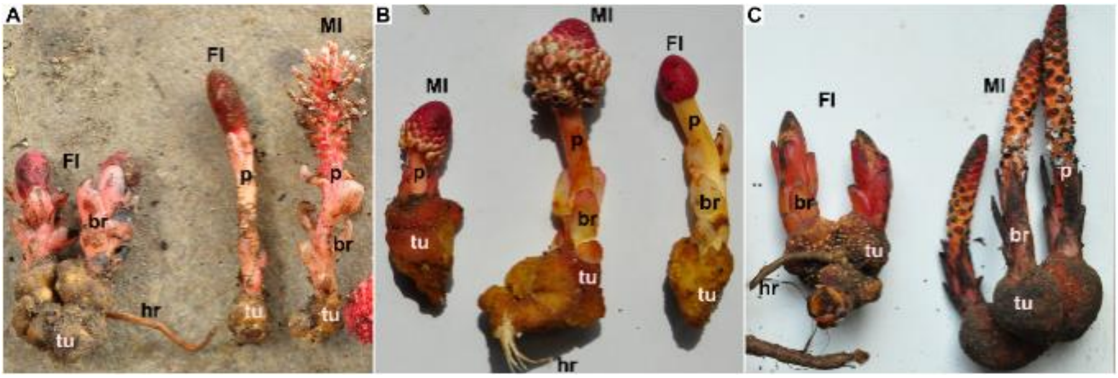

Figure 3. Morphological characteristics of the tubers in Balanophora species. (photo: NQ Hung) A. B. fungosa; B. B. harlandii; C. B. laxiflora; FI: female inflorescence; MI: male inflorescence; hr: host root; p: peduncle; tu: tuber; br: bract

In the present study, although the host plants could not be identified to the family level, anatomical observations of transverse sections of the host roots (Figure 2) indicate that they belong to dicotyledonous plants. This identification is based on diagnostic root characters, including collateral vascular bundles featuring visible secondary xylem, alternately arranged medullary rays, and the absence or occasional presence of a small pith, well-defined activation of vascular cambium and cork cambium, which confirm that these are secondary roots of dicotyledonous plants. Morphologically, the tuber constitutes the main vegetative organ of *Balanophora*, functioning as a substitute for true roots. It is firmly attached to the host root system and develops into an irregularly shaped mass, varying in color from yellow to brown or black, with a generally rough surface. Reproductive (inflorescence) shoots arise directly from the tuber (Figure 3), highlighting its central role in both vegetative support and reproductive development.

Host roots parasitized by *Balanophora* exhibit pronounced anatomical modifications, particularly in the vascular tissues and the surrounding parenchyma. These changes are likely associated with the formation and development of composite bundles and haustorial tissues. Comparable anatomical changes, including distortion of xylem and phloem elements, increased proliferation of parenchymatous tissue, and localized thickening of cell walls, have been documented in various other holoparasitic plant systems and are interpreted as structural responses of the host root that facilitate vascular integration between the host and the parasite. The anatomical observations shown in Figure 4 reveal a distinctive feature of the parasite tissue: the presence of small, dark brown to black spherical bodies within the parasite cell mass, referred to as balanophin, described by Hsiao (1995). In contrast, such structures are absent from the host cell mass, providing a clear anatomical criterion for distinguishing parasite-derived tissues from those of the host.

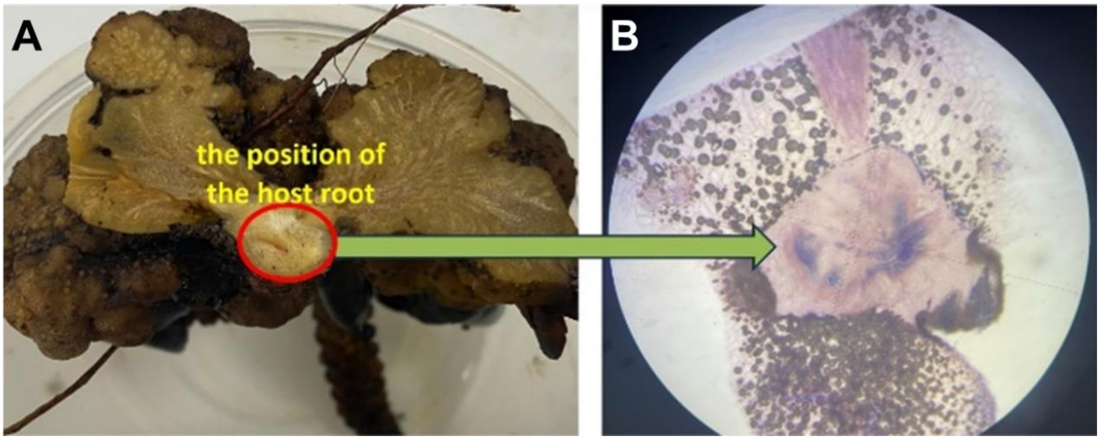

Figure 4. Longitudinal (A) and transverse (B) sections of B. laxiflora tubers

Overall, the tuber can be divided into four main regions as follows:

(i) Outer cortex (peripheral region): the outermost protective and mechanical layer.

The outermost layer of the tuber consists of an epidermis or cork-like protective tissue, with cells often thick-walled and sometimes lignified or suberized; sclereids (stone cells) are scattered within this layer, providing rigidity and mechanical strength.

In some species, a periderm-like tissue forms, acting as a protective barrier similar to the cork layer in roots. The outer cortex (peripheral region) of the tuber serves as the primary protective layer and consists of one to several layers of compactly arranged cells with relatively thickened walls, compared to the inner parenchymatous matrix.

(ii) Parenchymatous matrix: the ground tissue forming the bulk of the tuber.

Tuber tissues of *Balanophora* show clear functional differentiation related to holoparasitism. The outer protective layers provide mechanical protection and reduce water loss despite the absence of a true periderm, whereas the parenchymatous matrix, which lacks conspicuous sclereids, is primarily involved in storage and metabolic functions. This tissue organization is consistent across the three species and reflects specialization of the tuber for a parasitic lifestyle. The cell walls of these parenchyma cells range from thin to moderately thick, relative to those of the outer cortex, and this pattern is generally consistent across the three studied species.

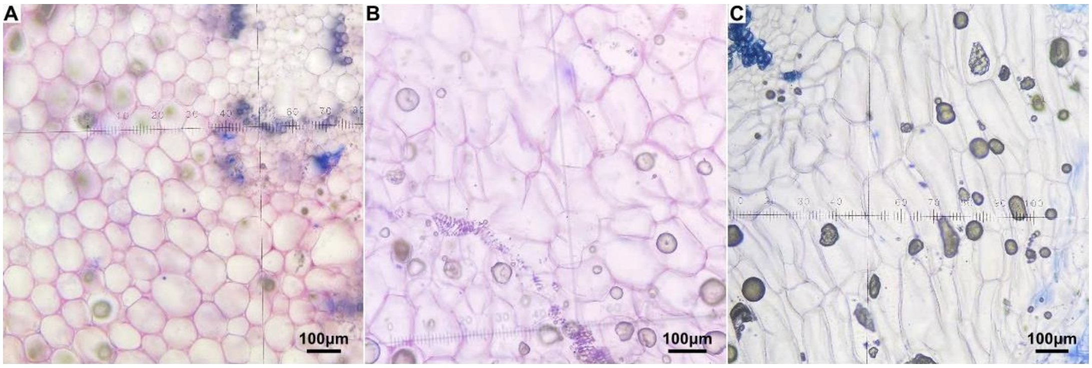

Figure 5. Parenchymatous matrix of three Balanophora species
A. B. fungosa; B. B. harlandii; C. B. laxiflora

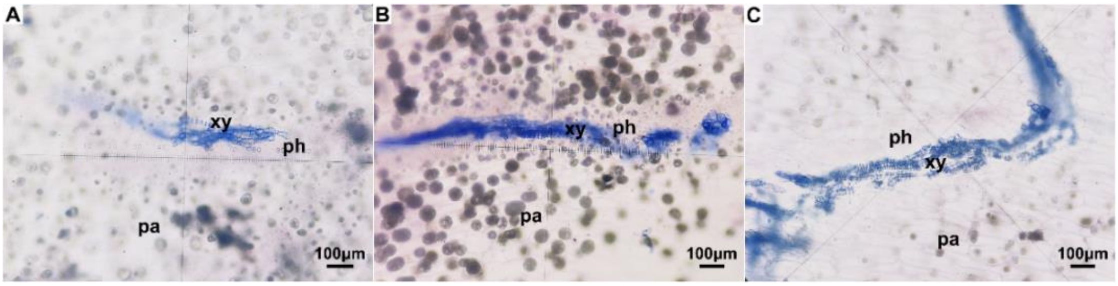

Figure 6. Vascular bundles of three Balanophora species
A. B. fungosa; B. B.; C. B. laxiflora; pa: parenchyma; xy: xylem; ph: phloem

(iii) Vascular bundles/parasite internal conducting system: vascular bundles are located within the matrix parenchyma but consist exclusively of parasite cells, without any direct host tissue involvement, and include.

Phloem: well-developed and dominant, composed of sieve tube members and companion cells, responsible for the transport of organic nutrients within the parasite.

Xylem: reduced and often represented by simple tracheids or a few primitive vessel elements.

Parenchyma cells: associated with the vascular tissue, functioning in storage and lateral transport.

Transfer cells: present in small numbers, facilitating efficient short-distance nutrient transfer within the parasite.

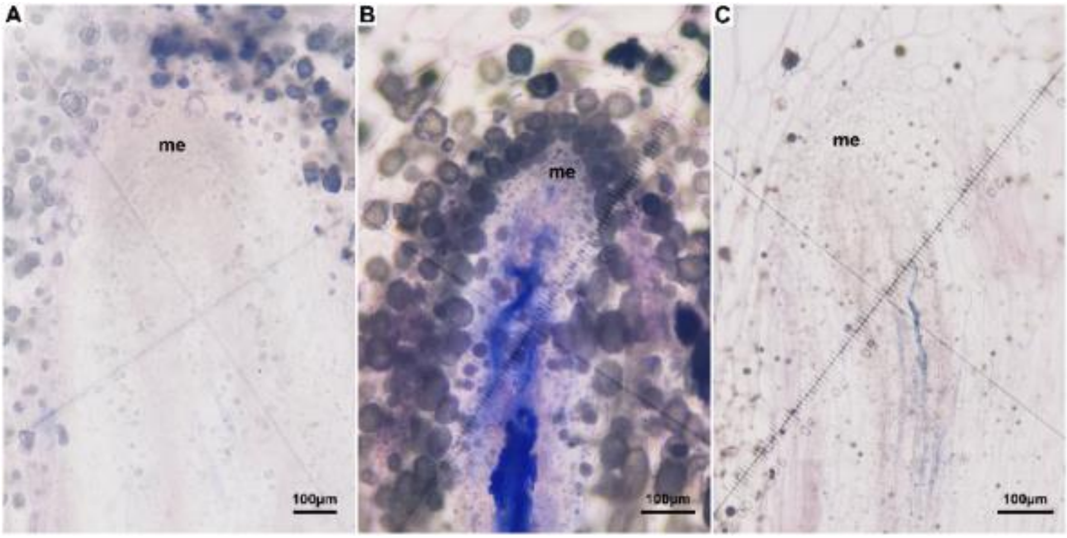

Figure 7. Composite strand meristem in three Balanopha species
A. B. fungosa; B. B. laxiflora; C. B. harlandii; me: meristem.

(iv) Composite bundles: specialized vascular systems, including parasite vascular bundles and composite bundles, which form the direct host-parasite interface (Figure 8B, C, D; Figure 9B, C, D; Figure 10B, C, D).

Sheath region (parasite side): located on the outer side, adjacent to the matrix parenchyma.

Composed exclusively of parasite tissue, specifically sheath parenchyma consisting of thin-walled parenchyma cells; sheath vascular bundles containing both phloem and xylem elements of the parasite, as well as numerous transfer cells. These sheath vascular bundles connect directly to the internal vascular bundles of the parasite.

Vascular core (host side): located at the center of the composite bundle, in direct contact with the host tissue.

Composed primarily of host vascular tissue, including both xylem and phloem.

Transfer cells of the parasite are interspersed among the host vascular elements; these cells have wall ingrowths, dramatically increasing surface area for efficient nutrient absorption; they are the primary site of nutrient uptake from the host.

A pericycle-like parenchyma layer of host origin separates the vascular core from the sheath region.

(v) Apical development of composite bundles.

The apical development of composite bundles describes the formation and growth of these specialized host–parasite interface structures in *Balanophora* from their apical (growing) region within the tuber (Hsiao, Mauseth & Peng, 1995).

At the very tip of the composite bundle, the tissue is composed of undifferentiated, meristematic-like parenchyma cells (Figure 7). As these cells mature, they gradually differentiate into two distinct and highly specialized regions (Figure 7).

Sheath region (parasite tissue): the outer layer, derived entirely from parasite cells, forming the sheath parenchyma and sheath vascular bundles, which later connect with the parasite's internal vascular system.

Vascular core (host tissue): the inner central region, composed of host vascular elements (xylem and phloem) intermingled with parasite transfer cells, which are essential for the absorption of water and nutrients.

The growth is basipetal, meaning that new cells are continuously added at the apex, pushing older, fully differentiated portions downward. This allows the composite bundles to elongate and branch, creating multiple active contact points between the parasite and the host root system. In essence, the apical development process explains how initially simple, undifferentiated tissue at the tip progressively develops into the complex, mature composite bundles that function as the physiological bridge for nutrient exchange between the host and the parasite.

2.2.2. Anatomical characteristics of*B. fungosa* tuber

The tuber surface is covered by thick epidermal layers, with some irregularly shaped, protruding cells that provide mechanical protection and reduce water loss (Figure 8A). The stem-like structure of the tuber in *B. fungosa* is dominated by parenchymatous tissue, interspersed with scattered vascular bundles (Figure 8B). The composite vascular bundles are well developed as large strands, radiating from the host root and branching dichotomously (Figure 8E, F).

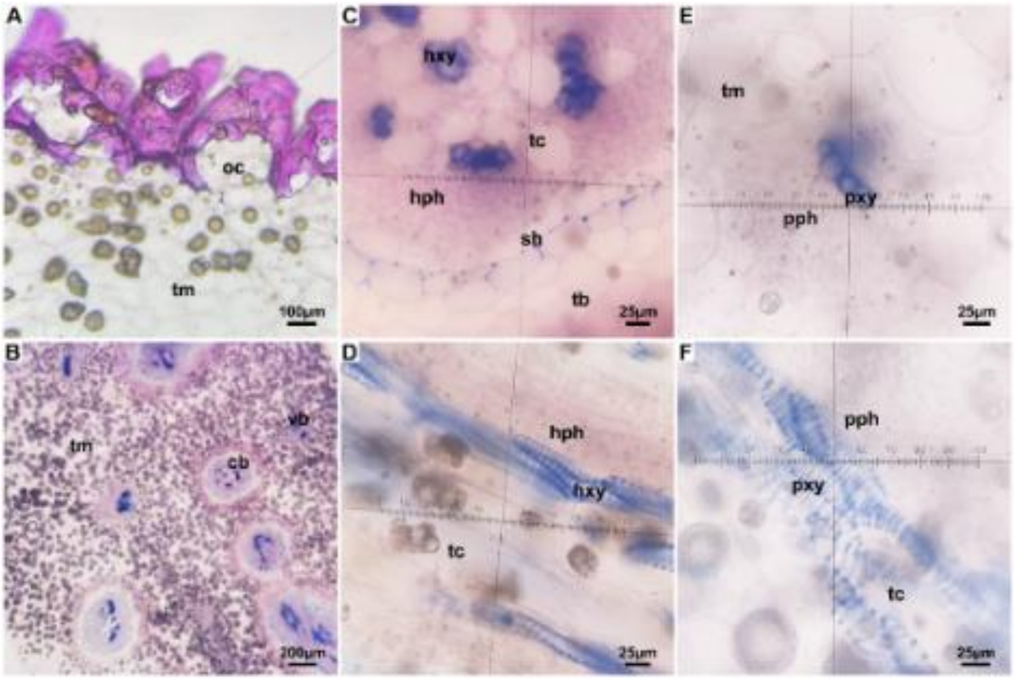

Figure 8. Anatomical features of B. fungosa tuber;oc: outer cortex; tm: tuber matrix; cb: composite bundle; vb: vascular bundle; hph: host phloem; hxy: host xylem; tc: transfer cell; sh: sheath cell; pph: parasite phloem; pxy: parasite xylem

2.2.3. Anatomical characteristics of *B. laxiflora* tuber

The outer epidermis consists of a single layer of polygonal cells arranged closely together, functioning to provide mechanical protection and prevent water loss. The ground tissue is predominant and primarily composed of storage tissue that serves as a nutrient reserve for the species. The mixed-vascular bundles are well-developed, radiating from the host plant's roots and branching dichotomously. These vascular bundles are scattered within the ground tissue, clearly showing the alternation between transfer cells and the host vascular tissue.

The outer epidermis consists of multiple layers of polygonal cells arranged compactly (Figure 9A), while the vascular bundles are well-developed and branch dichotomously. Composite vascular bundles extend longitudinally in parallel arrays and are scattered within the tissue; large parenchyma cells serve as storage units for essential nutrients (Figure 9B, C).

The parasite-derived transfer cells are large, closely packed, and thick-walled, with an average diameter of 31.16 ± 1.35 µm, and are surrounded by host xylem elements (Figure 9C, D). Host xylem elements, averaging 20.90 ± 0.70 µm in diameter, not only surround the transfer cells but are also intermingled with them, thereby optimizing nutrient transport from the host. In *B. laxiflora*, the xylem elements within the vascular bundles have a diameter of 19.17 ± 1.25 µm (Figure 9E, F).

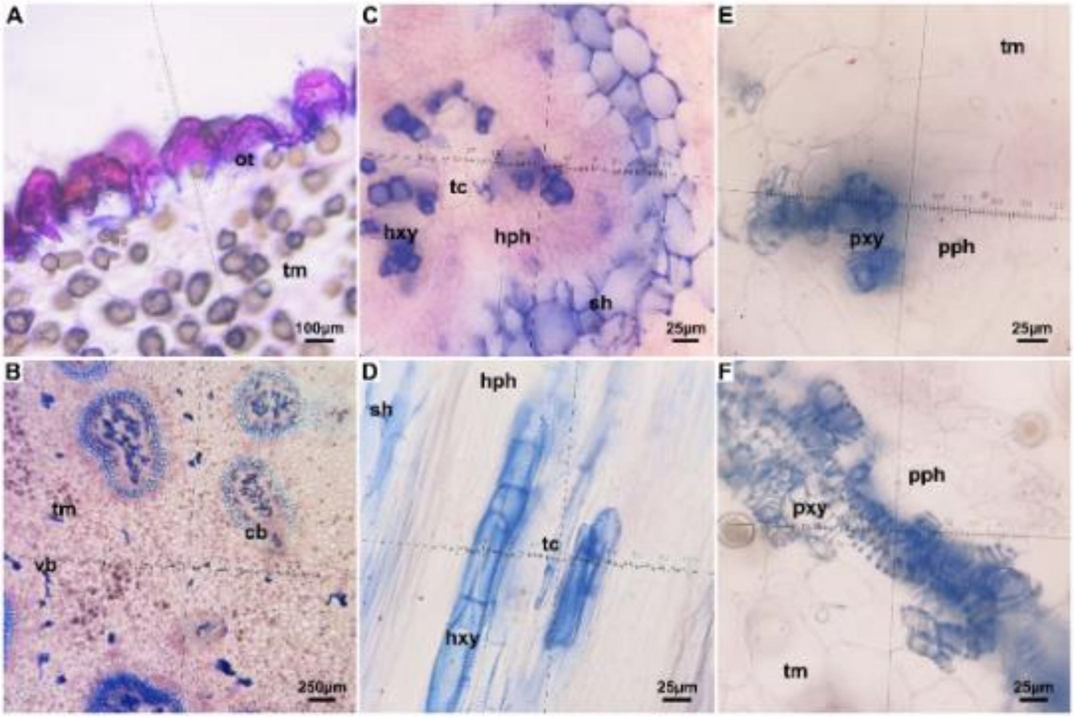

Figure 9. Anatomical features of B. laxiflora tuber;
oc: outer cortex; tm: tuber matrix; cb: composite bundle; vb: vascular bundle;
hph: host phloem; hxy: host xylem; tc: transfer cell; sh: sheath cell;
pph: parasite phloem; pxy: parasite xylem

2.2.4. Anatomical characteristics of *B. harlandii* tuber

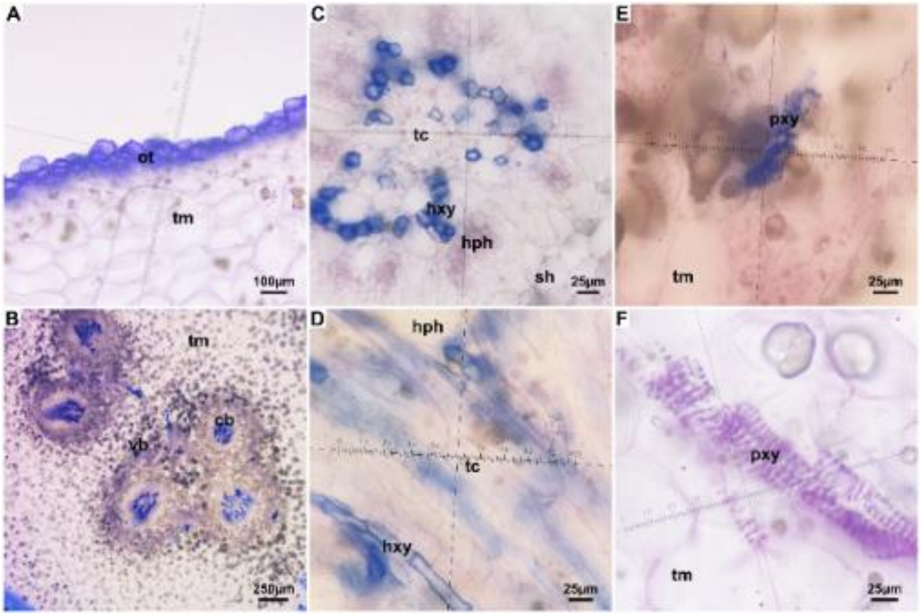

Figure 10. Anatomical features of B. harlandii tuber; oc: outer cortex; tm: tuber matrix; cb: composite bundle; vb: vascular bundle; hph: host phloem; hxy: host xylem; tc: transfer cell; sh: sheath cell; pph: parasite phloem; pxy: parasite xylem

The anatomical features of this species follow the same pattern as *B. laxiflora*, including the multilayered epidermis (Figure 10A) and the distribution of scattered vascular bundles and storage parenchyma (Figure 10B, C).

However, specific dimensions vary: the parasite-derived transfer cells average 34.37 ± 1.49 µm in diameter (Figure 10C, D). These are surrounded by host xylem elements (mean diameter of 17.00 ± 0.51 µm) to optimize nutrient transport. For *B. harlandii*, the xylem elements within the bundles have a diameter of 12.76 ± 0.60 µm (Figure 10E, F).

3. Conclusions

The tuber of *Balanophora* represents a highly specialized structure that ensures the complete dependence of the parasite on its host. The outer cortex and parenchymatous matrix provide mechanical protection, structural stability, and nutrient storage. Parasite vascular bundles form an internal conducting network, while the composite bundles serve as the sole physiological interface between host and parasite. Transfer cells, characterized by extensive wall ingrowths, are strategically positioned within the composite bundles to maximize nutrient absorption and translocation. This structural complexity enables *Balanophora* to thrive as a holoparasitic organism, entirely lacking chlorophyll and photosynthetic capacity, while efficiently exploiting host resources. These findings highlight the remarkable anatomical adaptations that underlie the survival and ecological success of *Balanophora* species.

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REFERENCES

[1] Nguyen TB (editor) et al., (2005). *Checklist of Plant Species of Vietnam*, Agricultural Publishing House, 3, 9-10 (in Vietnamese).

[2] Tran TB, Bui TH & Nguyen VQ, (2019). A study on the diversity of taxon ranks of Magnoliophyta distributed in both Vietnam and Thailand. *HNUE Journal of Science, Natural Sciences,* 64(10A), 128-132.

[3] Kirschner GK, Xiao TT, Jamil M, Salim AB, Lube1 V & Blilou I, (2023). A roadmap of haustorium morphogenesis in parasitic plants. *Journal of Experimental Botany*, 74(22), 7034-7044, (doi.org/10.1093/jxb/erad284).

[4] Yoshida S, Cui S, Ichihashi Y and Shirasu K, (2016). The haustorium is a specialized invasive organ in parasitic plants. *Annual Review of Plant Biology,* 67, 643-667, (doi.org/10.1146/annurev-arplant-043015-111702.

[5] Hsiao SC, Mauseth JD & Peng CI, (1995). Composite bundles, the host/parasite interface in the holoparasitic angiosperms *Langsdorffia* and *Balanophora*. *American Journal of Botany*, 82(1), 81-91.

[6] Gonzalez AM & Mauseth JD, (2010). Morphogenesis is highly aberrant in the vegetative body of the holoparasite *Lophophytum leandrii* (Balanophoraceae): All typical vegetative organs are absent and many tissues are highly modified. *International Journal of Plant Sciences*, 171, 499-508.

[7] Nguyen TT, Ho TT, Nguyen QH, Nguyen QB & Nguyen VT, (2019). Comparative study of plant morphology and pollen morphology of some species of the genus *Balanophora* JR & G Forst in Vietnam. *Journal of Pharmacy,* 524, (59), 44-49 (12/2019, ISSN 0866 - 786) (in Vietnamese).

[8] Nguyen TT, Nguyen VT & Nguyen QH, (2017). *Balanophora subcupularis* PC Tam (Balanophoraceae): New record species for the Flora of Vietnam. *Journal of Pharmacognosy & Natural Products,* 3(2), Doi: 10.4172/2472-0992.1000142).

[9] Nguyen TT, Nguyen VT, Nguyen QH, (2018). First record of *Balanophora tobiracola* Makino (Balanophoraceae) from Vietnam. *Bioscience Discovery*, 9(2), 297-301, (4/2018. ISSN: 2231-024X online).

[10] Nguyen NT, (2007). *Methods of Plant Research*. Vietnam National University Press, Hanoi, 25-31 (in Vietnamese).

[11] Bendre AM, Kumar A, (2009-2010). *A textbook of practical botany 1*. Revised ed. Meerut (India), Rastogi Publications, 4-15.

[12] Hoang TS & Nguyen PN, (2003). *Plant Morphology - Anatomy*. University of Education Publisher, 18-20; 278-285 (in Vietnamese).

[13] Joel DM, Gressel J, Musselman LJ, (2013). *Parasitic Orobanchaceae*. Berlin, Heidelberg: Springer Berlin Heidelberg.