

# Comparative Morpho-Anatomical and Ecological Study of *Ophioglossum raphaelianum* and *Ophioglossum rubellum* in Menal Forest, Rajasthan, India

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**Abstract:** The present study reports the first occurrence of *Ophioglossum rubellum* Welw. ex A. Braun and *Ophioglossum raphaelianum* in the Menal Forest of Rajasthan, India, significantly extending their known geographical distribution. Detailed morphological, palynological, and ecological analyses were conducted to differentiate these species and understand their adaptations to semi-arid conditions. *O. rubellum* exhibits lophate spores, a globose-fusiform rhizome, and copper-tinged tropophylls, while *O. raphaelianum* is characterized by foveolate spores, a globose-ellipsoid rhizome, and bluish-green foliage. Both species were found in bryophyte-rich microhabitats, indicating moisture-dependent survival strategies in an otherwise arid region. This discovery underscores the ecological uniqueness of Menal Forest and highlights the need for targeted conservation efforts to protect these ferns and their fragile habitats. The study contributes to the global understanding of *Ophioglossum* diversity and emphasizes the importance of further floristic explorations in understudied regions.

**Keywords:** *Ophioglossum*, adder's-tongue ferns, spore morphology, SEM, microhabitat, pteridophyte conservation, semi-arid ecosystems

## 1. Introduction

The genus *Ophioglossum* L. (Ophioglossaceae), commonly known as adder's-tongue ferns, represents one of the most ancient lineages of vascular plants, with a fossil record dating back to the Paleozoic era (Clausen, 1938; Goswami, 2008). These ferns are characterized by their simple morphology, consisting of a single sterile tropophyll and a fertile spike bearing embedded sporangia. Despite their evolutionary significance, *Ophioglossum* species remain understudied due to their cryptic growth habits and small size (Burrows & Jones, 2001).

Globally, the genus comprises approximately 50 species, with India emerging as a major diversity hotspot, hosting around 17 species (Mahabale, 1962; Fraser-Jenkins et al., 2017). Most Indian *Ophioglossum* species are restricted to tropical and subtropical regions, particularly the Western Ghats and Eastern Himalayas (Yadav & Shingade, 2006; Anto et al., 2016). Their occurrence in arid or semi-arid regions like Rajasthan is exceptionally rare, primarily due to the prevailing xeric conditions.

This study documents the first records of *O. rubellum* and *O. raphaelianum* in Rajasthan's Menal Forest, a region characterized by a semi-arid climate with distinct monsoon-driven microhabitats. *O. rubellum* was previously known from tropical Africa and southern India (Kerala), while *O. raphaelianum* was reported only from Kerala (Anto et al., 2016). Their discovery in Rajasthan not only expands their distributional range but also provides insights into their ecological plasticity.

## 2. Objectives

- 1) To provide detailed morphological descriptions of *O. rubellum* and *O. raphaelianum* from their new locality.
- 2) To compare spore morphology using Light Microscopy (LM) and Scanning Electron Microscopy (SEM).
- 3) To analyze habitat preferences and ecological adaptations enabling their survival in semi-arid conditions.
- 4) To discuss conservation implications for these species in Rajasthan.

## 3. Materials and Methods

### 3.1 Study Area

Menal Forest, located in southern Rajasthan, presents a mosaic of seasonal streams, shaded ravines, and grassy plains within a semi-arid landscape. Elevations range between 500 to 650 meters above sea level, with monsoonal rainfall creating localized moist niches conducive to fern growth.

### 3.2 Field Collection and Preservation

Systematic surveys were conducted during the monsoon season of 2023. Fern specimens were photographed in situ and collected for herbarium preparation. Standard protocols (FAA preservation, pressing, drying) were followed to maintain morphological integrity.

### 3.3 Morphological and Anatomical Analysis

Morphological characters were examined under a stereomicroscope. Transverse sections of lamina and petiole

were taken for anatomical studies, stained with safranin-fast green, and observed under compound light microscopy.

### 3.4 Palynological Studies

Spore surface structures were examined using both LM and SEM. Measurements were made using image analysis software, with attention to ornamentation patterns, polar and equatorial axes, and perine layer characteristics.

## 4. Results and Observations

### 4.1 General Morphology

*Ophioglossum raphaelianum* is characterized by a smaller stature (1.4 – 6.0 cm), single ovate-orbicular trophophyll, and bluish-green coloration. Rhizomes are globose-ellipsoid. The fertile spike is shorter (1 – 3 cm) with 6–7 pairs of sporangia.

In contrast, *O. rubellum* reaches 2.1 – 7.8 cm in height, has multiple leaves (2–4) that are elliptic, spatulate, or sub-orbicular, and displays a copper-tinged hue. Rhizomes are globose-fusiform, and fertile spikes extend to 7 cm with 6–10 sporangial pairs.

### 4.1 Morphological Comparisons

Table 1 provides a comparative analysis of the morphological characteristics of *Ophioglossum rubellum* and *Ophioglossum raphaelianum*. *O. rubellum* exhibits a taller growth habit, with plant height ranging from 2.1 to 7.8 cm, whereas *O. raphaelianum* is comparatively shorter, measuring between 1.4 and 6.0 cm. The rhizome of *O. rubellum* is globose-fusiform with a diameter of 0.5–1 cm, in contrast to the globose-ellipsoid rhizome of *O. raphaelianum*, which has a consistent diameter of 0.8 cm. The trophophyll shape also differs, being ovate to spatulate-obovate in *O. rubellum*, while *O. raphaelianum* displays a widely ovate-orbicular form. Fertile spikes in *O. rubellum* are longer, ranging from 1.5 to 7.0 cm, compared to the 1.0 to 3.0 cm length observed in *O. raphaelianum*. Additionally, *O. rubellum* typically bears 6 to 10 sporangia, whereas *O. raphaelianum* has slightly fewer, ranging from 6 to 7. The spore type is also diagnostic, with *O. rubellum* producing lophate spores characterized by muri with ridges, in contrast to the foveolate and granulose spores of *O. raphaelianum*. These distinct morphological features are key in differentiating the two species.

**Table 1:** Comparison between characteristics of *O. rubellum* and *O. raphaelianum*

Characteristic	<i>O. rubellum</i>	<i>O. raphaelianum</i>
Plant Height (cm)	2.1–7.8	1.4–6.0
Rhizome	Globose-fusiform (0.5–1 cm diam.)	Globose-ellipsoid (0.8 cm diam.)
Trophophyll Shape	Ovate/spatulate-obovate	Widely ovate-orbicular
Fertile Spike (cm)	1.5–7.0	1.0–3.0
Sporangia Count	6–10	6–7
Spore Type	Lophate, muri with ridges	Foveolate, granulose

### 4.2 Anatomical Features

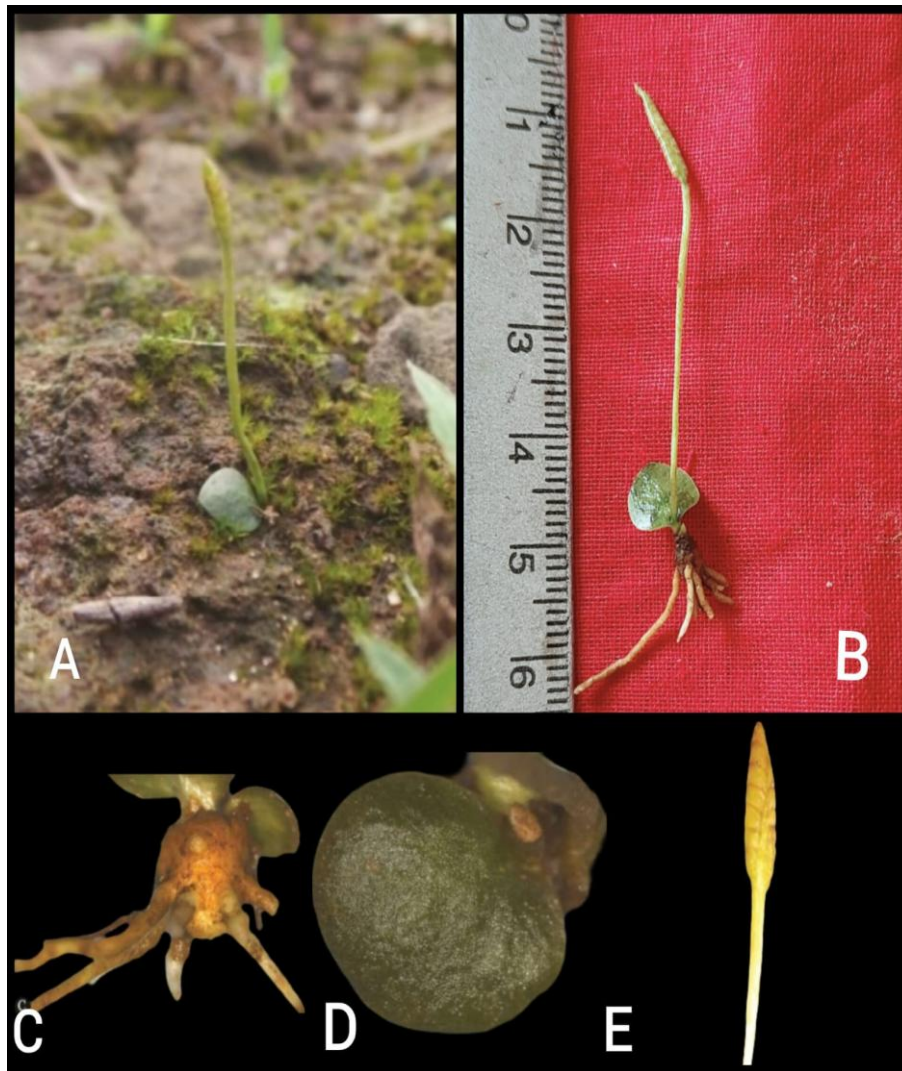
Both species possess a thick cuticle, indicating xeromorphic adaptations. Mesophyll cells are tightly packed. Vascular bundles are concentric, and stomata are sunken. *O. raphaelianum* shows elongated stomatal apparatus with foveolate spores, whereas *O. rubellum* has polygonal stomata with lophate spores.

### 4.3 Spore Morphology and SEM Observations

Figure 3 presents detailed images of the spores of *Ophioglossum raphaelianum* captured under both light microscopy (LM) and scanning electron microscopy (SEM), offering insights into their morphological features. Image A

shows the distal surface of the spore under LM, revealing a nearly spheroidal shape with a reticulate exine pattern.

Image B illustrates the proximal surface under LM, where the trilete mark is visible at the center, surrounded by a similar textured exine. SEM images in C and D provide enhanced structural details. Image C displays the distal surface, emphasizing the prominently reticulate ornamentation of the exine, with a coarse, net-like structure. Image D shows the proximal surface, clearly exhibiting the trilete scar and the comparatively smoother central area. The SEM images confirm the overall trilete, subglobose form of the spores and highlight the distinct surface ornamentation, contributing to species-level identification and palynological characterization.

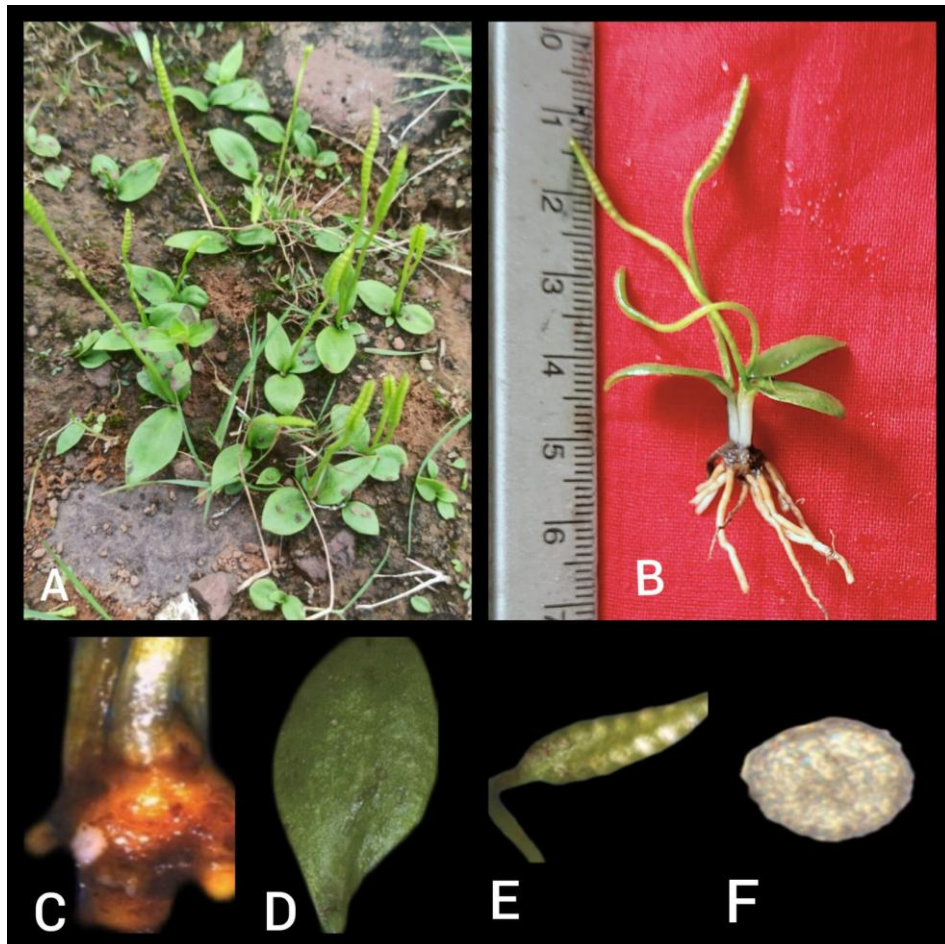


**Figure 1:** A) *Ophioglossum raphaelianum* in Habitat, B) Close-up View of *Ophioglossum raphaelianum*, C) View of *Ophioglossum raphaelianum* root, D) Close up view of *Ophioglossum raphaelianum* leaf E) Detailed View of Spike.

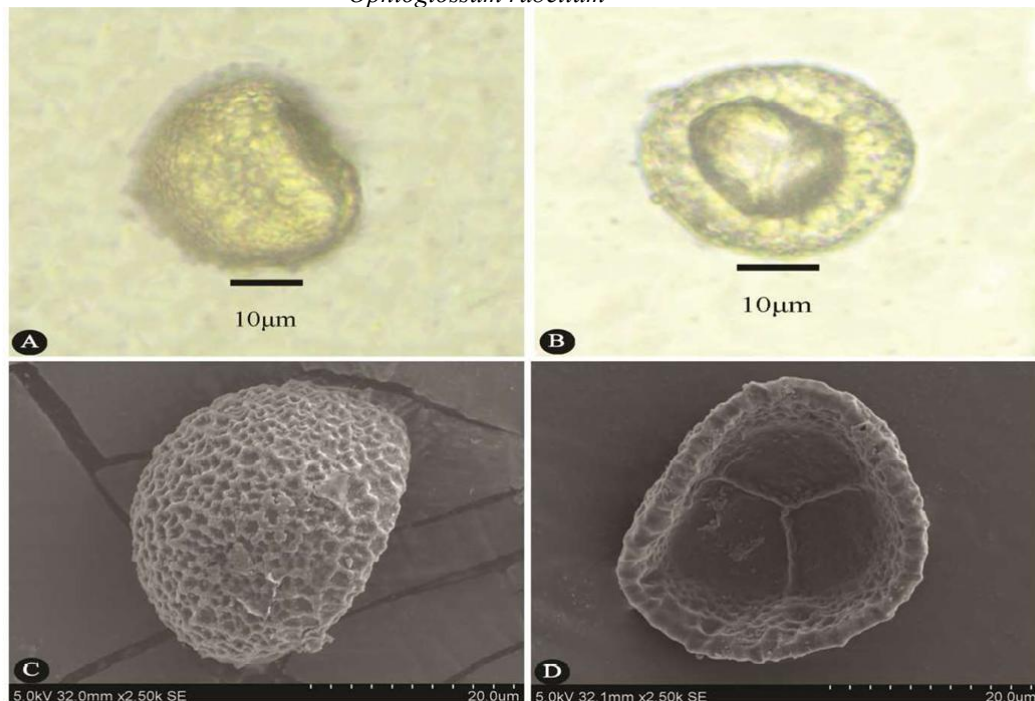
Figure 3 (*O. raphaelianum*) shows globose-ellipsoid, trilete spores with foveolate ornamentation. The distal face is granulose, and muri are narrow with no pointed tips.

- Distal face size:  $26.24 \times 20.44 \mu\text{m}$
- Polar axis:  $14.27 - 18.96 \mu\text{m}$
- Equatorial axis:  $30.53 - 37.4 \mu\text{m}$
- P/E ratio:  $0.53 \pm 0.04$

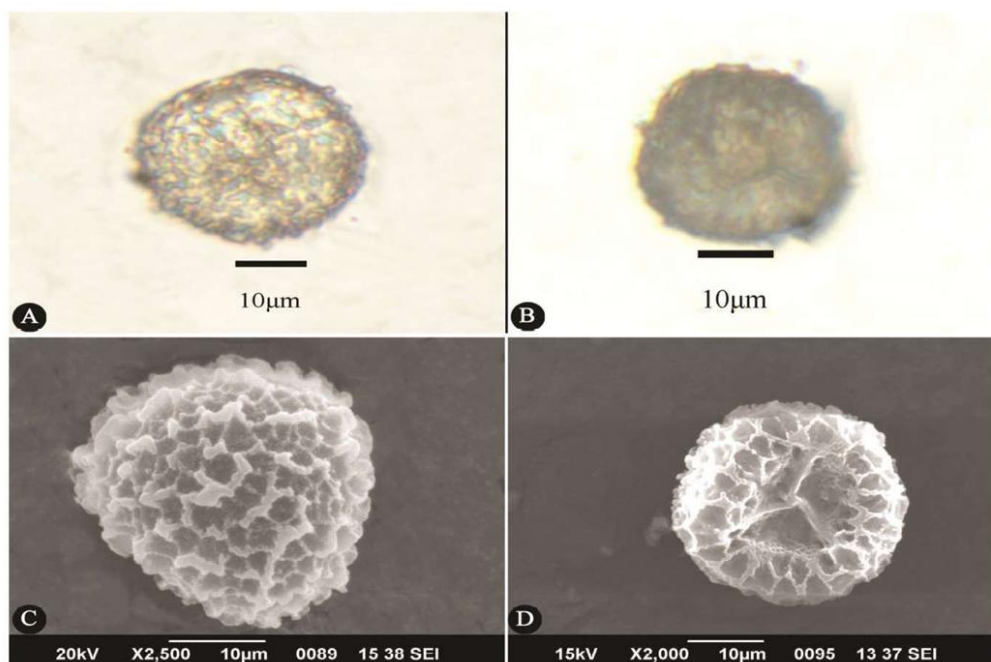




**Figure 2:** A) *Ophioglossum rubellum* in Habitat, B) Close-up View of *Ophioglossum rubellum* Leaf and Roots, C) View of *Ophioglossum rubellum* Sporophyte, D) View of *Ophioglossum rubellum* root, E) Detailed View of Spike. F) spore of *Ophioglossum rubellum*



**Figure 3:** Spores of *Ophioglossum raphaelianum*: A) Distal surface under LM. B) Proximal surface under LM. C) Distal surface under SEM. D) Proximal surface under SEM



**Figure 4:** Spores of *Ophioglossum rubellum*: A) Distal surface under LM. B) Proximal surface under LM. C) Distal surface under SEM. D) Proximal surface under SEM

Figure 4 illustrates the spore morphology of *Ophioglossum rubellum* as observed through light microscopy (LM) and scanning electron microscopy (SEM), showcasing both distal and proximal surfaces. Image A displays the distal surface under LM, where the spore appears circular to slightly subglobose with a granular or faintly reticulate texture. Image B reveals the proximal surface under LM, presenting a similarly rounded appearance with the trilete mark faintly visible at the center. In Image C, the SEM view of the distal surface shows a distinct and densely reticulate to verrucate exine pattern, indicating a rough, uneven surface. Image D provides the SEM image of the proximal surface, clearly exposing the trilete mark with the surrounding exine maintaining its coarsely reticulate ornamentation. These features underline the diagnostic characteristics of *O. rubellum* spores, which are crucial for species identification and comparative palynological studies within the genus. Fig. 4 (*O. rubellum*) displays globose, trilete or alete spores with lophate or smooth ornamentation. The exospore forms ridges, and lumina are polygonal. Distal face size is:  $22 - 33 \times 25.69 - 22.29 \mu\text{m}$ .

#### 4.4 Ecological Parameters

*O. raphaelianum* was found in open grassy plains with loam-clay soil and partial shade. *O. rubellum* was restricted to shaded, moss-covered zones with loamy soil and high humidity. Their occurrence during July–August suggests monsoon dependency for sporogenesis.

#### 5. Discussion

The morpho-anatomical and ecological distinctions observed between the two species suggest divergent adaptive strategies to exploit niche habitats within a semi-arid biome. While *O. raphaelianum* demonstrates greater ecological amplitude by surviving in open grassy terrains, *O. rubellum* appears to be more stenotopic, restricted to bryophyte-laden, shaded

locales. Spore surface differences further validate taxonomic distinctness and reflect evolutionary divergence.

#### 6. Conservation Implications

The discoveries of *O. raphaelianum* and *O. rubellum* in Menal Forest highlight the ecological significance of microhabitats in semi-arid landscapes. Conservation efforts should include:

- Protection of microrefugia (moss-covered and shaded niches)
- Prevention of habitat degradation due to grazing and tourism
- Ex-situ conservation (spore banking and cultivation trials)
- Awareness campaigns among local communities

#### 7. Conclusion

This comparative study reveals that *O. raphaelianum* and *O. rubellum*, despite sharing a genus, differ significantly in morphology, ecology, and spore architecture. Their co-occurrence in Menal Forest underscores the area's hidden biodiversity and emphasizes the need for continued botanical exploration in Rajasthan. These findings contribute to fern taxonomy, biogeography, and conservation biology.

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