



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>
Journal DOI: [10.21474/IJAR01](https://doi.org/10.21474/IJAR01)

INTERNATIONAL JOURNAL
OF ADVANCED RESEARCH

RESEARCH ARTICLE

OPHIOGLOSSUM RAPHAELIANUM (OPHIOGLOSSACEAE) - A NEW SPECIES FROM SOUTH INDIA.

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Manuscript Info

Manuscript History:

Received: 18 March 2016
Final Accepted: 22 April 2016
Published Online: May 2016

Key words:

Heterobrochate, Ophioglossum raphaelianum, Trophophore.

Abstract

A new species of Ophioglossum with small size, bluish green colour is described and illustrated here. It collected from Mangadu, Thrissur District, Kerala. This small Ophioglossum named *Ophioglossum raphaelianum*, become distinct from all other reported species in having subglobose rhizome, orbicular, obtuse, bluish green and appressed trophophyll and sheathing trophophore. The spore lumina shallow, muri reticulate, virugose, heterobrochate and rhizome with large amount of VAM association.

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Introduction:-

The species concept of Ophioglossum is greatly misunderstood and the species are consequently poorly delimited, largely because of the simple morphology of the genus and resulting lack of characters up on which to base species (Burrows, Edwards, 1995). When the species become tinier the problem becomes great complex.

The genus Ophioglossum L. commonly known as Adder's tongue or snake tongue fern of eusporangiate belonging to the family Ophioglossaceae, the world wide Ophioglossum includes about 45 or more species along with varieties (Banwari L. Yadav, Hit K. Goswami, 2010; Pichi sermolli, 1958). In India the fern is represented by 13 species (Clausen, 1938; Khandelwal and Goswami, 1984; Khandelwal, 1987, 1989; Vasudeva and Bir, 1993; Yadav and Tripathi, 2002; Goswami *et al*, 2008; Banwari L. Yadav, Hit K. Goswami, 2010). Manickam and Irudayaraj (1992) reported 5 Ophioglossum species from south Western Ghats and Bhuskute (1999) reported 6 species from Maharashtra. Since there has been no authenticated study on Ophioglossum, while investigated study of Ophioglossum of Thrissur district as a part of M. Sc project we find one of the taxa become new to the genus Ophioglossum.

Reported small species in the world include *Ophioglossum indicum*, *O. gramineum*, *O. gomezianum*, *O. crotalophoroides*, *O. nudicaule*, *O. parviflora*, *O. polyphyllum* etc. Spore morphology and the vegetative characters of the genus become characters of the taxa limitation. This new taxa has bluish green colour, small size, and spore patterns which is distinct from other small species of Ophioglossum. It differs from *O. indicum* in the Trophophyll which is subterranean, circular, bluish green, with sheathing Trophophore and it is arranged in appressed on the soil. It is similar to that of *O. crotalophoroides* in general appearance. But it is differ from this species with trophophyll arrangement, round shape and downward recurved margins.

It differs from *O. nudicaule* in size of trophophyll, shape of rhizome and solitary appearance in habitat and not propagated through running rhizome. It differs from *O. gramineum* in the position and shape of trophophyll and

rhizome. All the small size species are almost similar in size but very much differ in morphology especially trophophyll and spore (Table1).

Materials and methods:-

The present study is based on fresh *Ophioglossum* plants collected from different localities of Thrissur district, from hill valley areas of Mangadu during 2015 June-July. Photographs of the plants were taken using Canon EOS 1200 D, camera from the field showing their habit and habitat. The plants were taken to the laboratory for morphological as well as microscopic analysis. Morphological characters of Rhizome, Rhizoid, Trophophore, Trophophyll, and the anatomical observations were made using a stereomicroscope (LABOMED Lx 300). Characters of the spores of different species were studied by Scanning Electron Microscopy (SEM) analysis and their photographs (SEM images) were taken from National Institute of Technology (NIT) Calicut and Sophisticated Analytical Instruments Facility (SAIF), department of Cochin University. The observations were made from populations of living plants as well as the herbarium specimens and identification of the plants were done with the help of available literatures, floras, monographs and also consulting the experts in the field.

Description of the new species:-

Ophioglossum raphaelianum Anto P.V., Afsana Khan, Franklin Francis and Ignatius Antony, sp. nov:-
Figs 1, 2

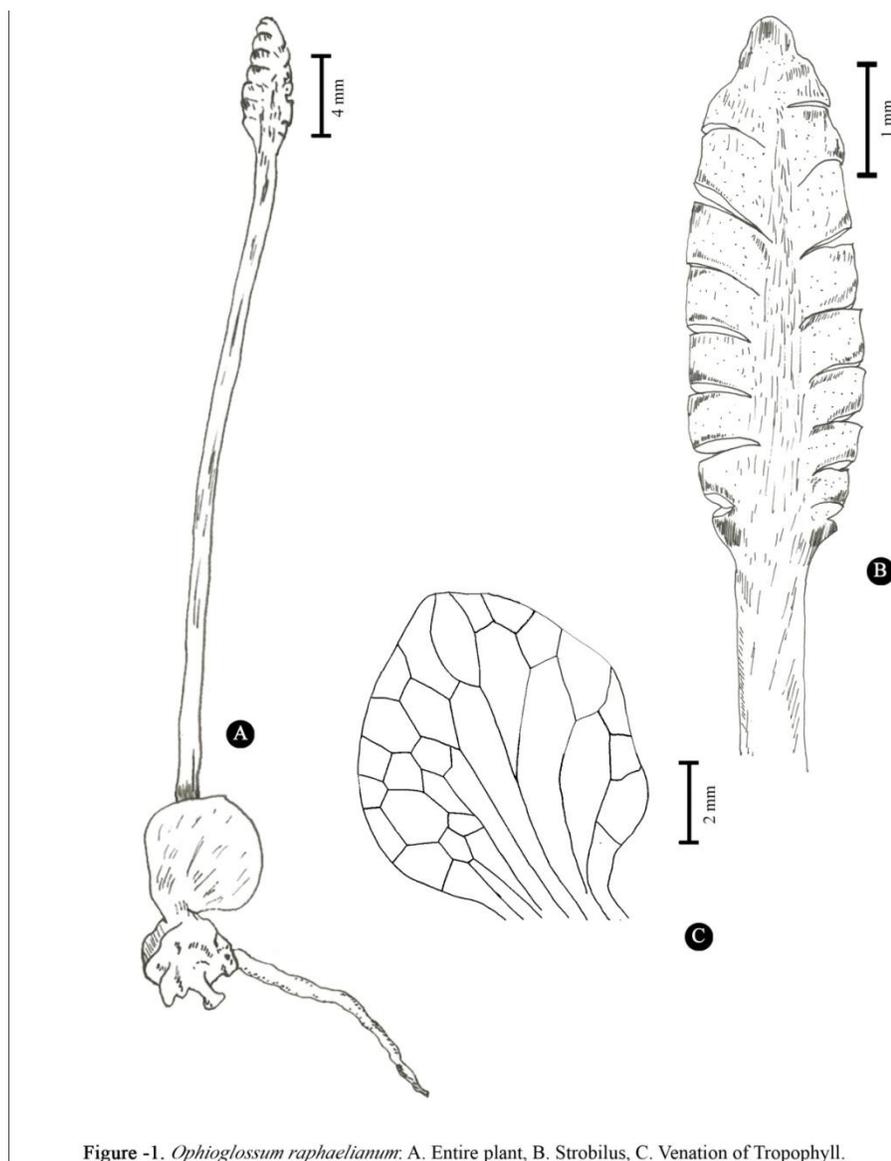


Figure -1. *Ophioglossum raphaelianum*: A. Entire plant, B. Strobilus, C. Venation of Trophophyll.

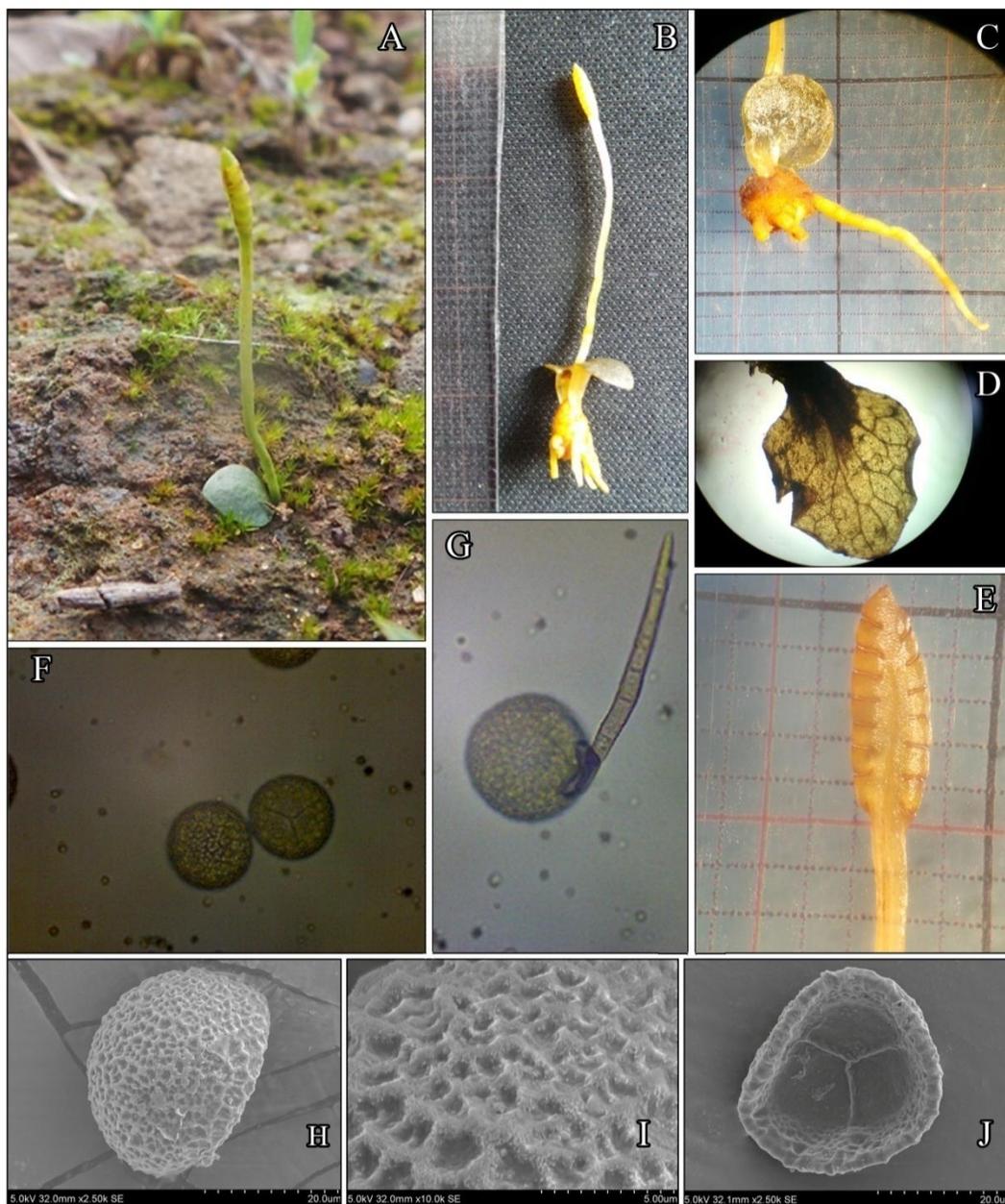


Figure 2. *Ophioglossum raphaelianum* : A. Habit; B. Entire plant; C. Rhizome with tropophyll; D. venation of tropophyll; E. strobilus; F. Spores - Dorsal, Ventral views; G. Germinated spore; (H,I,J - SEM Images of Spore) H. Dorsal view 2500 times enlarged; I. Dorsal view 10000 times enlarged; J. Ventral view 2500 times enlarged.

Diagnosis:- The new species is distinguished from *O. crotalophoroides* and *O.indicum* with subglobose rhizome, orbicular, obtuse, bluish green and appressed tropophyll and sheathing trophophore. The spore lumina shallow, muri reticulate, virugose, heterobrochate and rhizome with large amount of VAM association.

Type. India, Kerala, Thrissur, Hill valleys of Mangadu. 10° 42' 30" N, 76° 11' 43" E, alt. 100 m. Anto, P.V., 112, 26/6/2015. (Holotype CALI!.; Isotype CAL!, MH!, BLAT!, India).

Description:- Whole plant minute, 1-3 cm in length. Rhizome small, subglobose, tuberous, 2-6 mm long, 9 mm to 1.2 cm wide, outside of rhizome mostly with a brown coating; several short roots arises from the mid part of the rhizome, white, 4-7 in number, c. 1cm long, up to 2 mm wide; the rhizome bears 1-2 fronds at its apex, tropophylls subterranean in origin, olivaceous or bluish green in colour, glabrous, orbicular, minute, 1-5 mm long, 3-4 mm wide, their bases continuous to the fertile stalk, borne close to and lying \pm flat on the ground, or slightly upward-directed, margin entire, slightly recurved or convex, apex rounded to very slightly, but obtusely narrowed; venation showing one mid-vein split into to 2 in the slightly widened, pale leaf-sheath and divided into 5 veins in each lamina, each one anastomosing to form 3 or 4 large hexagonal areoles with 4 or 5 small areoles at the periphery.

The strobilus arises directly from the apex of the rhizome, peduncle pale green, 2-3 cm long, 2-3 mm wide; fertile apex very short (c. 5-6 mm long, 1-3 mm wide), cylindrical with a short, sterile pointed apex, sporangia oppositely arranged, 6-7 in number on either side, small, yellow (Figure 1 and 2).

Anatomical preparations of the rhizome show a heavy symbiotic association of VAM mycorrhizal microorganisms in the rhizome and root.

Spore sizes below 22×33 micrometer, In Light microscope (LM), it is globose and trilete with a small triradiate mark which is not extending to the margins, the laesalar arms are more or less wavy and jointed, up to the middle of the proximal cavity. In Scanning Electron Microscope (SEM) the laesalar arms are pointing the wall in between trilobed proximal end. In LM the distal face is granulose to verrucate. In SEM depressed lumina are shallowly reticulate, hetrobrochate, which are more or less square shaped, ranges 2 to 4 micrometer, the muri with numerous warts like processes or verrucate (Fig 2: I.).

Spore germination starts from the proximal cavity. The germinating stalk grows perpendicular to polar axis. The proximal cavity plugged with thick tissue. The germination of spore starts with in one or two days after the rupture of sporangia with favourable environment and media (Fig 2: G.).

Phenology:-

June-July.

Etymology:-

The specific epithet of this species is given for honouring our manager Mar. Raphael Thattil, Who gave great contribution to education in Thrissur District, Kerala, India.

Distribution and Ecology:-

It is found in hill base regions of isolated hills in Thrissur district. It is very rare and has solitary appearance. It is an ephemeral which seen during June to July. It has symbiotic association with microorganism, which localized in the rhizome and root.

Discussion:-

Ophioglossum raphaelianum is a new species find during our investigation from mangadu and Perumala. It is characterized by minute size, subglobose, tuberous rhizome, olivaceous or bluish green subterranean, orbicular tropophyll. This species not agree with any reported species of *Ophioglossum* when we search for the species in many literatures. We compare this species with other reported small species including *O. indicum*, *O. crotalophoroides* and *O. nudicaule* and arrived at a conclusion that it will be a new species of *Ophioglossum*.

O. crotalophoroides Is very much similar to that of *O. raphaelianum*, but its habitat is 4300 m. altitude where as *O. raphaelianum* below 200 m. Other morphological features of *O. crotalophoroides* disagree with the illustrated new species which are rhizome globosely corm like, no sheathing leaf base, the distance between rhizome and tropophyll $2/3^{\text{rd}}$ of its total length, sterile strobilus tip narrowly elongated, curved and pointed and shape of leaf deltate-cordate or ovate lanceolate. Kew database herbarium of *O. crotalophoroides* specimen No: K000589309, collected by Bridges with collection No: 341 were also be referred here.

Ophioglossum indicum is pink brown in colour and shows many differences in spore characters. Spore sizes below 22×33 micrometer, In Light microscope (LM), it is globose and trilete with a small triradiate mark which is not

extending to the margins, this is similar to those of *O. indicum*. But there are some marked differences between *O. raphaelianum* and *O. indicum*. In *O. raphaelianum* the laesalar arms are more or less wavy and jointed, up to the middle of the proximal cavity. In Scanning Electron Microscope (SEM) the laesalar arms are pointing the wall in between trilobed proximal end. In LM the distal face is granulose to verrucate. In SEM depressed lumina are shallowly reticulate, heterobrochate, which are more or less square shaped, ranges 2 to 4 micrometer, the muri with numerous warts like processes or verrucate. But in *O. indicum* the laesalar arms are straight and extending up to the rim of proximal end. Distal end contains many minute depressions, which are more or less cone shaped, negatively reticulate.

So the illustrated new species is distinct from all other small species of *Ophioglossum*.

The comparison with other species is described in table 1.

Table 1:- Comparison of morphological characters of *O. raphaelianum* with *O. indicum*, *O. crotalophoroides* and *O. nudicaule*.

Character	<i>O.indicum</i>	<i>O.raphaelianum</i>	<i>O.crotalophoroides</i>	<i>O.nudicaule</i>
Plant Colour	Pink	Bluish-green	Palegreen	Green
Size	2.5-3.6cm	1.5-3.8 cm	3-10	3-5 cm
Rhizome shape	Knobe shaped	Subglobose,tuberous	Corm like	Creeping cylindrical
Rhizome length	0.4-0.6cm	0.2-0.6cm	1cm	1-2 cm
No.of tropophylls	One	one	Two	One
Tropophyll shape	Elliptic-lanceolate	Orbicular	Deltate-cordate	Elliptical ovate
Tropophyll apex	Acute	Obtuse	With apiculum	Obtuse
Tropophyll colour	Pink	Bluish-green	Palegreen	Green
Tropophyll position	Afew cm above from ground, parallel to strobilus	Llying on ground, appressed, subterrestrial	Afew cm above from ground, margins upwardly curved	Laying above the ground
Strobilus	0.4-0.6mm	5-6mm	Less than 1cm	4-5 mm
Fertile stalk	1-1.2cm	2-3cm	0.6cm	1.2-1.8 cm
Sporangia-number	7-10 pairs	6-7 pairs	3- 8 pairs	5-6 pairs
Trophophore	no sheathing	sheath present	No sheathing	No sheathing

Acknowledgements:-

The authors are grateful to Dr. Jenson P. O., Principal, Jacob Abraham Pulikal, Head of the Department of Botany St. Thomas' College, Thrissur, Authorities of Herbarium Database of Royal Botanical Garden, KEW and the financial assistant as M.Sc. student project of K.S.C.S.T.E. for providing funds and all necessary facilities to carry out this work.

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