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## Research Article

### Reshuffle Within the Genome of *Goswamia costata* Zhang & Zhang Accelerates the Origin of a Species “*Goswamia glossii*” sp. nov.

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#### Abstract

During ongoing studies since 1970, a rare trait of development of spores on the margins of trophophylls (leaves) was observed in a few species of *Ophioglossum*. Recently, a plant population exhibiting similar rare features along with development of primitive sporangia on the margins and veins of trophophylls have been observed on plants resembling *Goswamia costata* Li Bing Zhang & Liang Zhang (= *Ophioglossum costatum* RBr L). Field and laboratory studies based on light microscopy and SEM investigations indicate that plants under study are different from *Goswamia costata*. Possession of very thick elevated or swollen mid vein bearing “pockets containing developing spores and small anucleate cells like nurse cells; (primitive sporangium;), well developed spores also on veins; origin of parallel veins forming linear closed areas to enclose globular spore-like structures of variable sizes on other veins, and cross linking fine veins on mid vein shaping like “stitched midvein” are regular traits on many trophophylls of the same plant. Such traits may be called “profern traits”. Some traits on trophophylls also resemble a few leaf genera of the *Glossopteris* flora of the Palaeozoic era. Hence these plants are being named as *Goswamia glossii* sp. nov.

**Keywords:** *Goswamia glossii* sp. nov, Intragenomic reshuffle, Ophioglossaceae, Primitive sporangia on trophophylls, Spores and nurse cells, Relic traits, Evolutionary genetics, Speciation and genomics

#### Introduction

Search for variations in natural populations of *Isoetes* L and *Ophioglossum* L etc. has been in progress since 1966, covering several localities and areas in different parts of India by one of us (HKG). According to recent data, out of nearly 50 species of *Ophioglossum* L in the world flora [1], India has more than twenty-five species reported from various parts of India [2-5]. This communication is based on a series of rare variants observed on the species, *Goswamia costata* Zhang & Zhang, [6] for several years. Hereunder, we report a stable population of a species evolved within the same area, with traits which are hitherto unknown in the literature of a leaf of any living plant. This paper also has a background of early

reports of observing external and internal variations (plants even with albino sporophores) within the shape, size and venation pattern of the trophophylls in natural populations for about two decades which until now, were considered to be some rare expressions of the supposedly heterozygous populations of *Ophioglossum costatum* L which is now renamed as *Goswamia costata* Zhang & Zhang. According to them [6] the plants with bulbous or cylindrical larger rhizome with many or proportionately swollen roots possess totally different phylogenetic status and have been renamed as *Goswamia* Zhang & Zhang and those with the linear rhizome with few thin roots are known as *Ophioglossum* L. So as they

mention there are now following genera within the family Ophioglossaceae which include, *Botrychium* Sw; *Botrypus* Michx; *Japanobotrychium* Masam; *Sceptridium* Lyon; *Ophioglossum* L, *Goswamia* Zhang and Zhang, *Haukia* Zhang and Zhang and *Whittieria* Zhang and Zhang which according to molecular phylogenetics are all deeply diverged from the rest of *Ophioglossum* (*Ophioglossum* s.s.) as demonstrated by the long branches or numerous molecular synapomorphies of each of them [6].

Furthermore, the intra-generic molecular divergence among the genera, *Goswamia*, *Haukia*, *Ophioglossum* s.s., and *Whittieria* have been faster than the other genera. Molecular dating by Testo and Sundue [7] estimated the genera to have diverged from their sisters ca. 134, 127, and 179, respectively, million years ago (MA). Obviously the segregation of different genera was evolutionarily installed millions of years ago and the genome of the genus *Goswamia* may be rich and diversified. Our detailed follow up studies on different ophioglossaceous plants have revealed the present population of plants to be different from *Goswamia costata* except in gross morphology hence these plants are being named as *Goswamia glossii* sp. nov.

## Material and Methods

Among collections of various species of the genus *Ophioglossum* L two variant plants partly resembling *Goswamia costata* (Fig.1A) were collected bearing white/albino sporophores, large lanceolate and small round trophophylls with bulbous rhizomes and bunch of roots [8]. Follow-up studies carried out on the spots of collections from the locality 50 kms away from Bhopal to Budni ((Lat- 22°50' 38.9" N- Long 77° 39' 32.8" E) further revealed three plants with the same gross morphology bearing albino sporophores (Fig. 1B) along with a large number of plants only with long lanceolate trophophylls possessing parallel veins (Fig. 2 B) arising from the base and bearing normal long sporophores. Since then, yearly collection schedules have recorded many plants to possess larger 4 to 10 lanceolate trophophylls possessing very thick and raised up (swollen) nodulated midvein. Interestingly, many spores were found to be developed on the margins of trophophylls during earlier collections [8-11] and these new variant plants were labelled as "evolving" species related to *Goswamia costata*. We have been following under mentioned approach to evaluate the status of this unique species (Fig.2 A):

(I) At the outset we ascertained that blackish –brown spots on sporophores and margins and veins of trophophylls [9-10] are not any fungal or bacterial infections. Slides prepared after teasing with the help of needles and forceps and examining under the light microscope we had confirmed that actually clusters of spores were produced by the vegetative tissue (Fig. 2 C--F). No traces of any hyphae were observed. (II) After digging out 6-8 plants from the soil, only tropho-

phylls were cut from other plants so as to keep rhizomes with roots to generate fresh plants in the next rainy season. Trophophylls were fixed in 70% ethanol. Next day, these trophophylls were made feebly translucent by passing through alcohol- xylene series; for examining under stereo-microscope and also under light microscope (Fig. 3 A, B).

(III). Spores observed from the sporangia in sporophore (Fig.3 C, D) as well as on margins and on parallel veins and midvein were photographed.

(IV). Thick mid veins of many trophophylls showing elevated points were scratched and mounted revealing a large number of small spores (Figs. 3 F, 4 A-D, 5).

(V). Spores from sporophore--sporangia and midvein pockets were also screened under the scanning electron microscope (SEM; Figs. 5 E, 6) at Junagadh facility in Gujarat.

## Results

### Taxonomic treatment

*Goswamia glossii* Hit Kishore Goswami, Sachin Kumar, Shivani Vadhiya and R. Raviya; sp.nov.

**Type:-** India, Madhya Pradesh; Bhopal hilly plateau on Hoshangabad road; 50-55 Km away from Bhopal (latitude (Lat- 22°50' 38.9" N- Long 77° 39' 32.8" E)

Holotype: Cal;

**Isotypes:** Cal; K; Herbarium Junagadh (BKNM UNIVERSITY, Department of Life Sciences); Herbarium Society of Biologists, 24 Kaushalnagar, Misrod, Bhopal, India

**Diagnosis** *Goswamia glossii* Hit Kishore Goswami, Sachin Kumar, Shivani Vadhiya, and R. Raviya (Figures 2-4,6).

**Plants** are large in size, up to 16 cm with 4 to 10 lanceolate **trophophyll:** (leaves), measuring 3 to 6 cm in length, in some plants trophophyll reach 10 cm in length; 1.2 to 1.5 cm in breadth in the middle of the trophophyll; some of the trophophyll of each plant have very much thickened and nodules all over mid vein; **midvein:** always very thick, elevated or swollen with two or more parallel veins; some trophophyll of the same plant have additional thickened vein like structures on margins appearing as pseudo-veins; midvein of some (not all) trophophyll has crossing fine thread like "stitches" appearing all over the midvein. **Sporophore:** arises as usual from the adaxial base of the trophophyll and the stalk bearing fertile sporophore measures from 6 to 10 cm. Sporophore (fertile spike) measures 2 to 3 cm bearing almost opposite about 40 to 62; sporangia: on the mature sporophore. **Spores:** trilete round but also some proportion of oblong spores, fully mature spores possess outer perine, measure 20-26  $\mu$ m in diameter, develop in the sporangia of sporophores; **spores developing** on the midvein and other veins all across the trophophyll are smaller (10-12  $\mu$ m) but with very thick spiny or warty exine. A few globular or elongated pockets develop to enclose very small cells (2-3  $\mu$ m) as well as large and small spores. These cells are too small and unlike spores have very thin outer layer. Such structures

appearing as “nurse” cells are also seen in some sporangia (Fig. 3 D) on sporophores.

**Etymology:** The specific epithet is in the recognition of an excellent hitherto unknown trait of possessing nodules throughout the parallel running two or more veins with cross linkers of fine veinlets and development of reproductive structures on the mid vein; which are comparable to a few glossopterid leaves broadly related to the composite genus *Glossopteris* Brongniart.

**Distribution and ecology:** Randomly distributed in open forest on the slopes and plain fields around the road leading to Budni, Hoshangabad after 50 km away from Bhopal MP. Plants also have, at some places the intermixed growth of some angiosperms (grasses; *Scilla* sp etc) and *Goswamia costata*, Zhang & Zhang; *G. malvae* Patel & Reddy; *G. Indica* Zhang & Zhang and *O. gramineum* Willd.

**Conservation status:-** At present plants are widely distributed on both sides of the road leading to Budni but ongoing construction and widening of the roads has started squeezing the distribution area. However, due to the properly fenced area of the Ratapani reserved forest, several smaller areas should remain protected.

## Discussion

### Comparative Traits

These plants with many [4-10] long lanceolate trophophyll are expressing a large number of non-comparable features which are exclusively confined to the species. Table 1 presents a comparative account of some very relevant species including *Goswamia costata* and *Goswamia bispora* Vadhiya, S., Raviya, R., & Goswami H.K. Interestingly *G. bispora* plants are too small having different morphometry but are worthy of a noteworthy resembling trait. A few plants in each collection possess spores on the margins of trophophyll (Fig. 5 A-D) although sometimes also on the veins on abaxial face of the trophophyll. Another significant resemblance is the development of two different shapes of the spores within the sporangia in sporophore (Figs. 5 D; 6 B) of both the species *G. glossii* sp. nov. and *G. bispora*. Some morphological features which are comparable with some of the glossopterid leaves [12] of these plants have been published elsewhere [10,11,13]. Such a comparison has not been a matter of stretched imagination but appears to be a hard fact as also opined by M. Kato [14,15]. Such instances of appearance of altogether new traits, some of these directly comparable to the species and genera flourishing in the past geological era, are not rare [2. 16-21] the evolutionary strategies of biological evolution.

As recently pointed out by Munoz Mora et al. [22] the genetic load on polyploids also can exert threats and this implies exactly so on the genera referable to the genera *Ophioglossum* L and *Goswamia* Zhang & Zhang in particular, because of very high paleopolyploidy [16-18]. Sudden appearance

of several unknown features encountered in a population of plants also indicates the role of stem cells present in the meristematic tissue of the lamina [10,23]. We will have to study these plants by multidisciplinary approach.

### Phylogenetic considerations

The genomic constitution of the genus *Ophioglossum* is evolutionary rich and geologically too ancient [24-26] many new and abnormal features have been observed in various populations; many consistent variants have also been described as new species within past two decades [16,27-30]. Each of them has exhibited certain morphological features exclusively confined to the species itself (see, reviews), [1,2,4,5,9,10,13,27,31].

Kato [14] had pointed out a resemblance between ophioglossoid fertile leaves and glossopterid fructification, which some workers have suggested as a possible archetype for the angiosperm carpel [15]. Kato has repeatedly considered that the genus *Ophioglossum* among other plants of ophioglossaceae has the most plausible resemblances with glossopterid leaves and progymnosperms. The range of variation of ophioglossoid leaves in the position of sporophore insertion and the number of sporophores per leaf is apparently comparable with a few glossopterids (see [(9,10,12–15,32,33]. Kato considers the glossopterid fructification as fundamentally the same structure as ophioglossoid leaves, inserted on the adaxial side of a trophophyll. Since presently described variant plants have tuberoses and large rhizome bearing proliferous roots, these are assignable to the genus *Goswamia* as per revised nomenclature proposed by multidisciplinary phylogenetic studies based on anatomical and plastome studies [3,6]. Since the trophophylls of the plants also express some glossopterid traits [13] we name this species as *Goswamia glossii* sp. nov.

### Spores on trophophylls

Repeatable observations on development of spores on the margins and veins of the trophophylls' lamina of *Goswamia bispora* and *Goswamia glossii* sp. nov. not only resemble in many ways but also confirm earlier observations to support hypothesis that the genes for the development of sporangia on leaves (a feature of ferns) are deeply conserved within the genome of ophioglossaceous genera, particularly within the *Goswamia costata* Zhang and Zhang and *G. eliminata* Zhang and Zhang genomes [8]. In some species the conserved genes appear to be triggered by some epigenetic mechanism [18,19,21,34]. As a matter of fact, theoretically, the development of sporangia requires activation of different set of genes inherent within the stem cells in order to steer up simultaneous origin of sporangial wall, differentiation of sporogenous tissue and series of mitotic divisions for the development of spores. Since mesophyll cells cannot produce spores, obviously “*new spore mother cells*” are generated within the mesophyll and spores so produced might be ex-

pressing some genes which might have been silenced during ongoing-evolutionary strategies within the species. Stem cells located on margins of trophophyll (leaves) [11,35,37] must have exerted some epigenetic mechanism. Since the spores on margins and veins of trophophyll develop in clusters and also seen as octads (6 to 8 interconnected spores; Fig. 4 C-D) quite possibly the spore mother cells undergo continuous divisions producing clusters of spores. Also possibly, these spores may be produced due to amitotic divisions of spore mother cells and may even be “diploid”. The spores in chains, however confirm amitotic divisions.

Another extremely unusual feature is that spores developed within the mesophyll tissue differ in exine ornamentation with the spores produced by the reproductive tissue in the sporophore of the same plant. Exactly as in *Goswamia bispora* [9,10] spores on mesophyll do not resemble with spores produced in the sporangia of sporophore (see Fig.3 C D, E) and even are totally new ones not resembling any of the species of ophioglossaceous genera. Also never before, any ophioglossaceous species nor even any true fern, has ever shown presence of simple, small and anucleate cells (Fig. 3 D) appearing as nurse cells. Such empty small cells cannot be aborting spores because spores abort after attaining some size with any wall layer formation.

#### Significance of formation of “primitive sporangia”

Prantl [38] and earlier) had emphatically mentioned that the leaf is the part of the pteridophyte to which spore formation is confined and the growing points in the leaf margin are the original loci of soral inception [39,40]. According to Mettenius

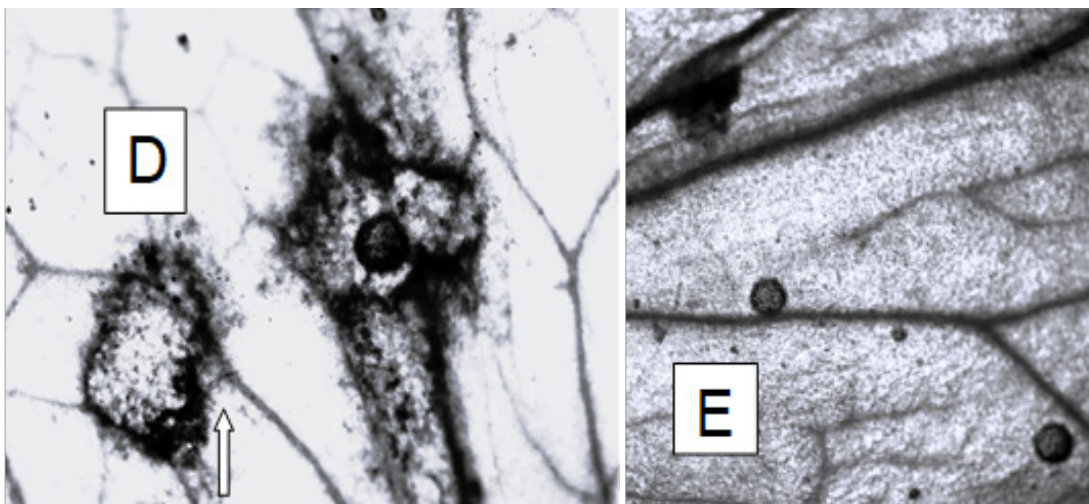
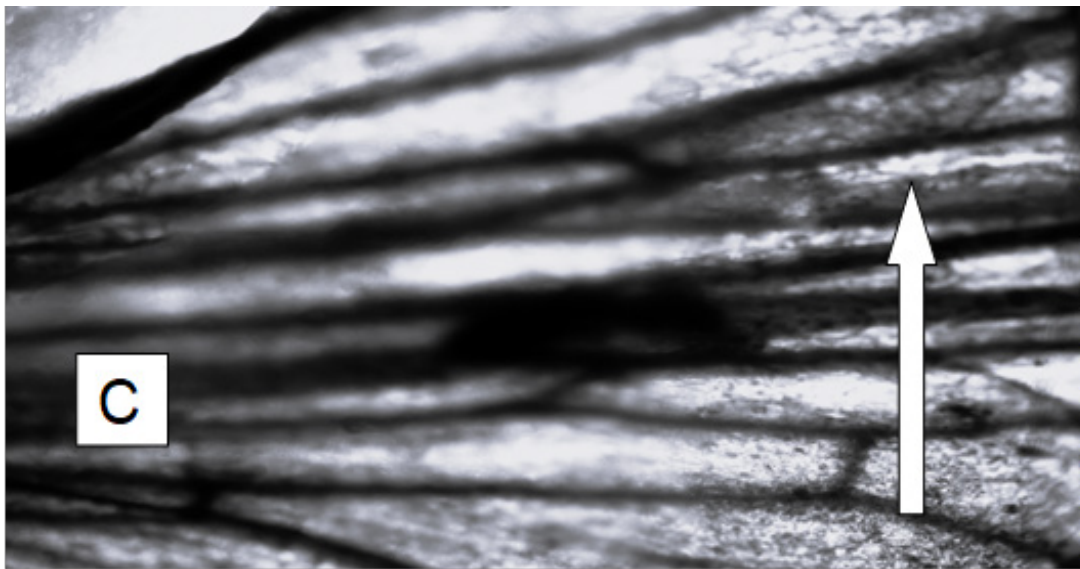
[41] neither the venation of the sterile leaves nor the formation of the sori in itself are sufficient criteria for delimiting the species or the pteridophytic genera. But the most important criterion is dependent on “fertile veins” and relationships of sori with veins. Mettenius also classified different sori on the basis of sori position on veins. From an evolutionary point of view Prantl considered development of marginal sorus to be a primitive state of organisation in the soral evolution. Shifting of the sorus from the leaf margin onto the abaxial face has to be dependent on phylogenetic strategies. Prantl also distinguished and named sori; the sorus with many sporangia was termed *aspolysporangial* sorus and a sorus with a single sporangium as *monosporangial* sorus. From finding clusters of spore formation on the margins of trophophylls in species viz. *Goswamia eliminata* and more frequently in *Goswamia bispora* [10] now we have *Goswamia glossii* plants which actually demonstrate the situation of shifting reproductive structures (spores) from margins to the mid vein and other veins (Figs. 2-4).

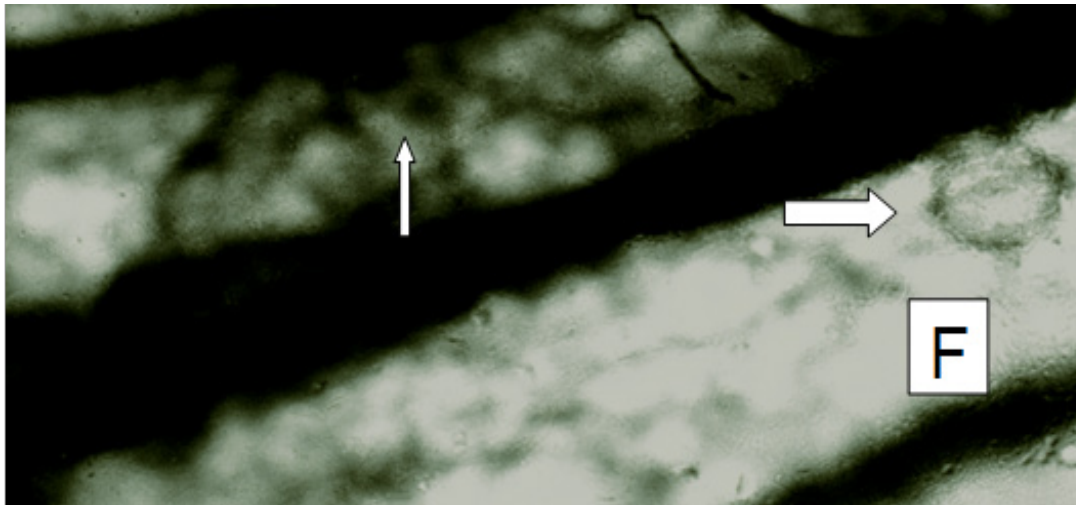
There are no structures like sporangia but some enclosed areas (pockets) within the network of veins (arrowed in Figs. 2 C-E, 3 A) but not on trophophyll margin. Spores on margin are in clusters but strangely develop even trilete mark as if, these have been formed after a transverse and longitudinal divisions of a spore mother cell. Pocket like enclosures on veins can be considered as “primitive sporangia” because these contain developing, spores along with many anucleate too small cells (Fig. 3 D) which may be “nurse cells”. We will follow up this species for further investigations.

### Figures and Tables

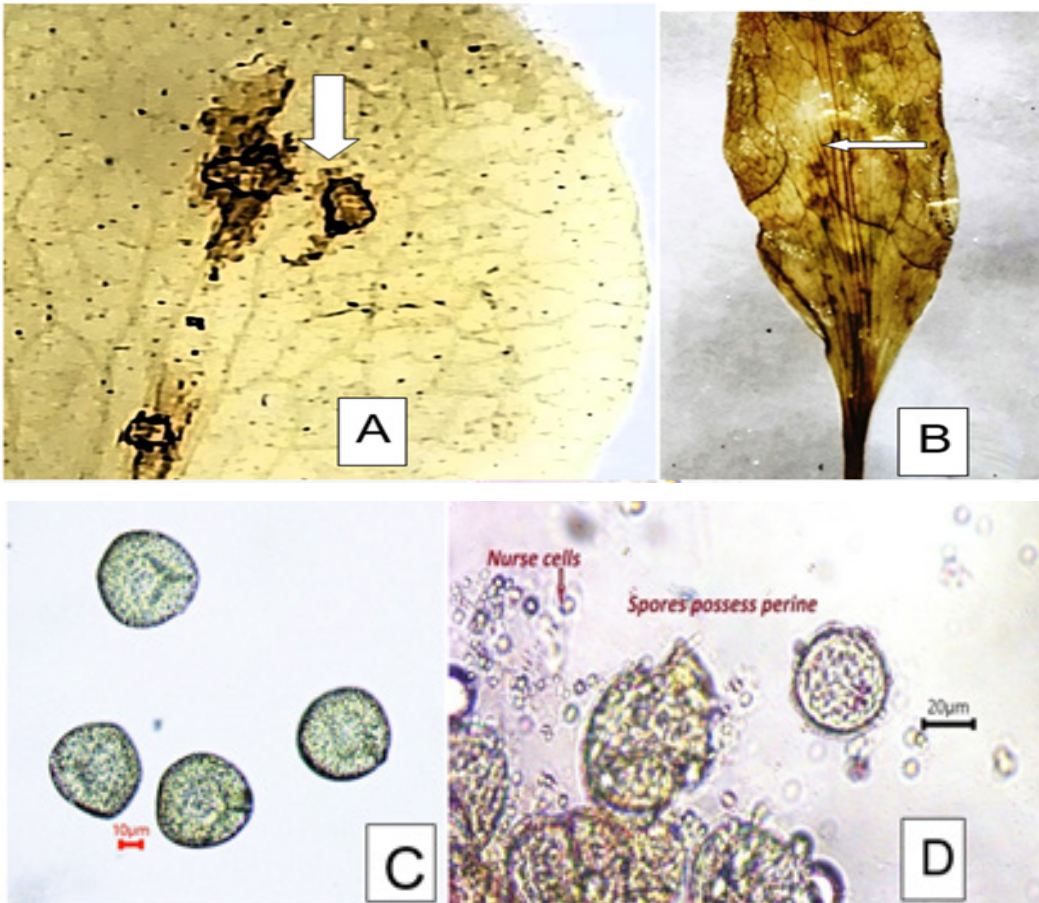


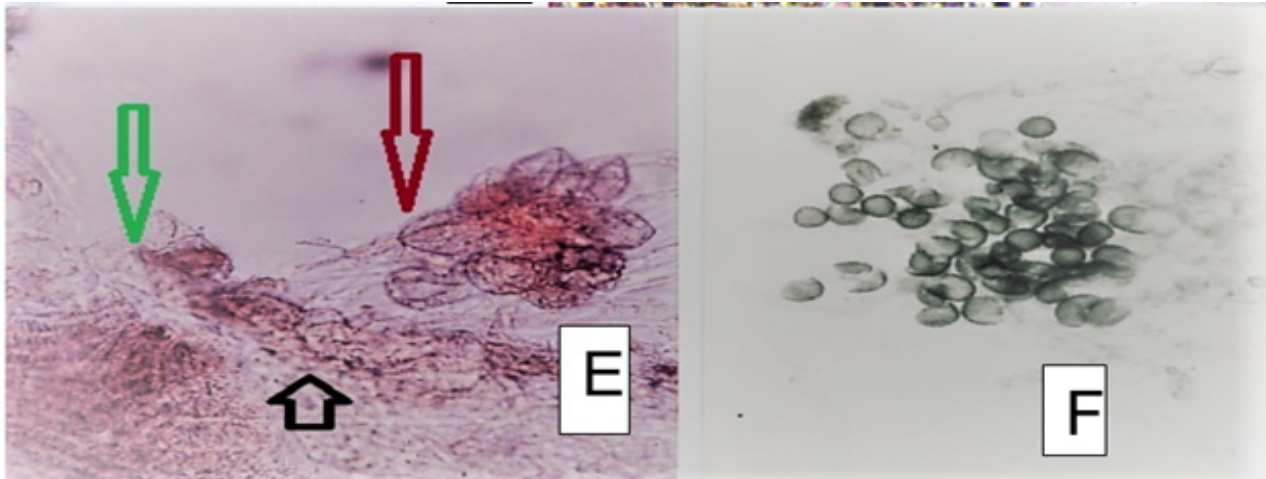
**Figure 1: A** Normal plant of *Goswamia costata* Zhang & Zhang (= *Ophioglossum costatum*) (x 1) **B**. Trophophyll of new species observed in 2025 showing albino sporophore and origin of parallel veins (x 2). The albino sporophores (spikes) in a few plants were also recorded in 1987, 2007 in a few plants exhibiting variants.



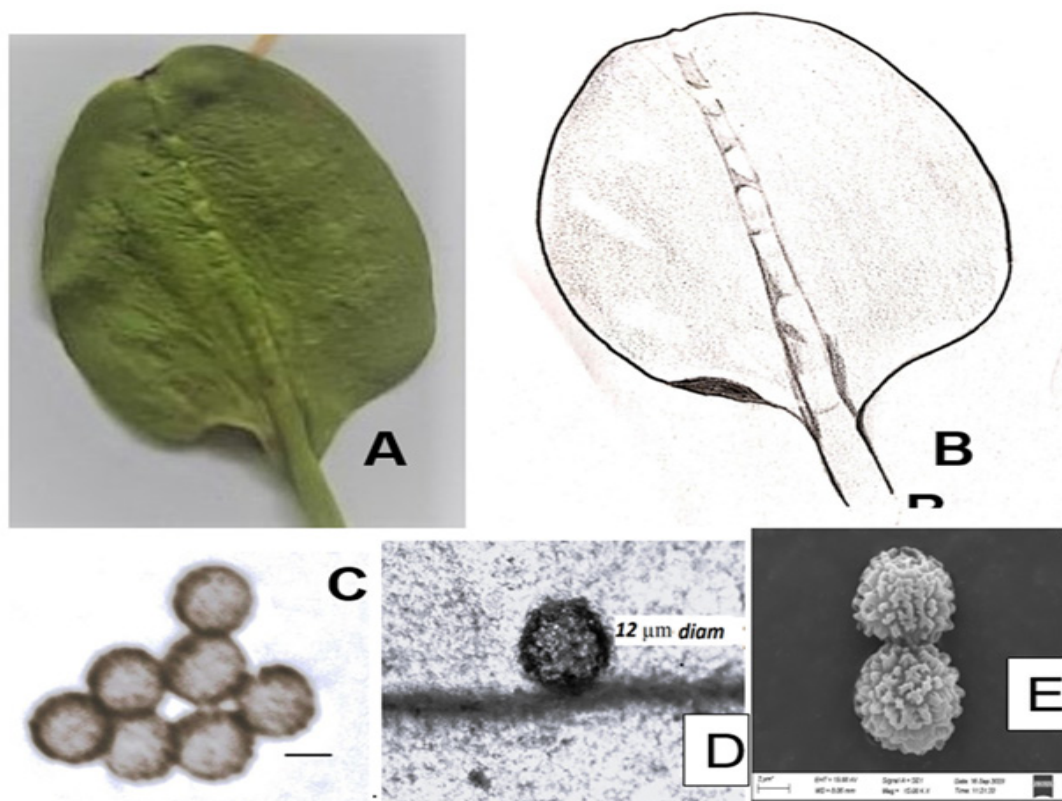


**Figure 2:** *Goswamia glossii* sp.nov. A The holotype of the plant *Goswamia glosii* sp. nov. of the new species on Herbarium sheet trophophylls (x 1) B, Enlarged dried trophophyll to show many parallel veins and cross-linking veinlets passing over the entire length of the thick elevated midvein (arrowed ; x 2); C, Many parallel veins arise to fill up the lamina and strangely, many of them form linear “pockets” containing globular balls (arrowed) may be an early stage of spore development; D, other two round pockets surrounded by thick cell wall, small containing very fine and small cells and the bigger pocket possesses one large spore with small cells (? nurse cells in the primitive sporangium); E, Mature spores on the mid vein and other vein, note the thick walled warty exine of the spores; a few small developing spores also seen on the veins; F, Enlarged part of midvein area showing cluster of developing spores (upper arrow) and a developed spore (arrowed; all photographed enlarged after under 10 x 40 magnification).

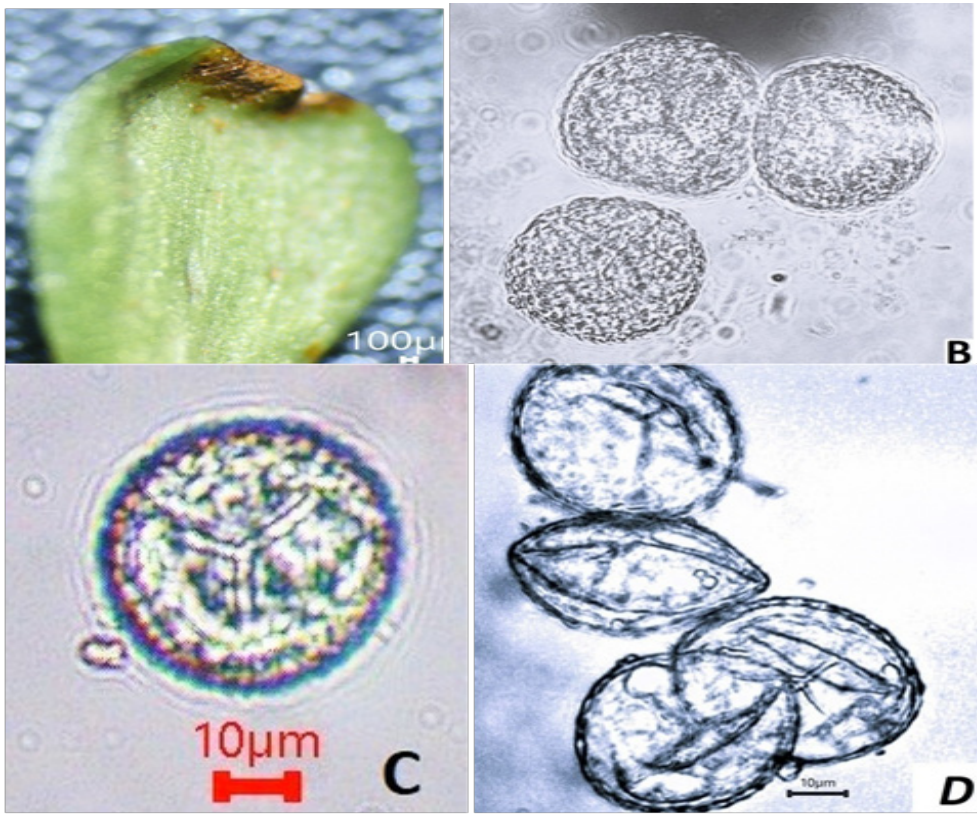




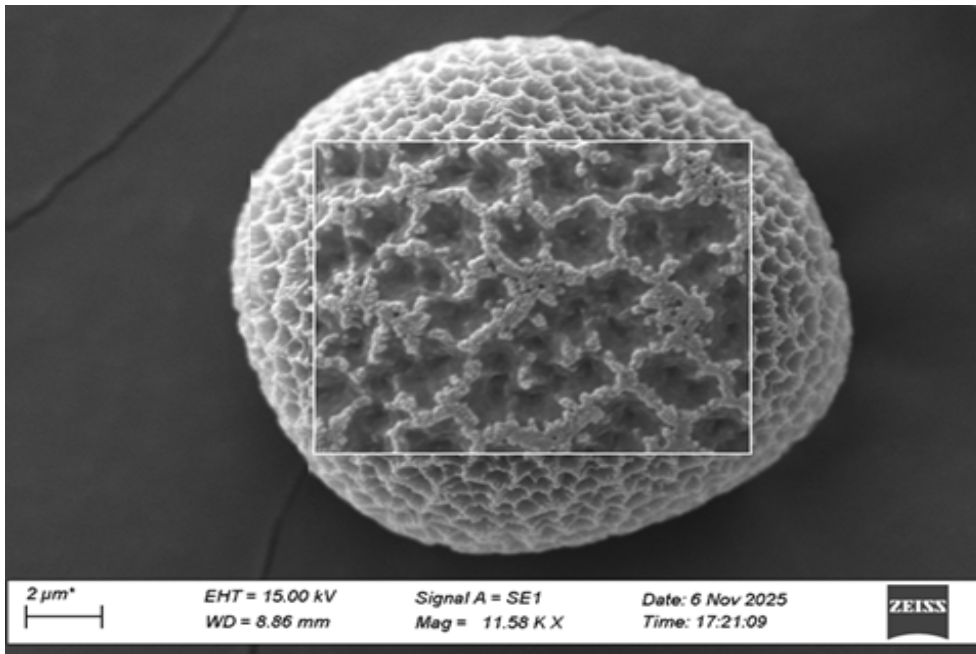
**Figure 3:** *Goswamia glossii*. A. A young translucent trophophore showing “primitive sporangia” on the midvein and other veins; the inner globular contents are exactly same as shown in Figure 2 E (x 3); . B. Mature trophophore showing many parallel and irregular veins; (x 1 ) C. Trilete spores released from young sporophore .Both proximal and distal faces of mature spores possess fine granulate deposition of sporopollenin on exine; D. Mature spores released from the sporangia of sporophore, mature spores (25  $\mu\text{m}$ ) possess outer perine (perispore) layer and also typically show presence of large number of too small (less than 3  $\mu\text{m}$ ) globular anucleate “ nurse cells”. E. Monolete spores on the marginal tissue of the trophophore also some tissue filled up with small structures (arrowed), another trophophore possesses younger stages of spore development, (pictures photographed under 10 x 40 magnification; spores measure 10-12  $\mu\text{m}$ ).



**Figure 4:** A An usual trophophore showing nodulated and broad midvein (x1), B. Line diagram showing strips of the same trophophore (x1), C. Spores from scratched from the id vein (the line bar = 10 $\mu\text{m}$ ), D. Only one spore on the mid vein (Fig. 2 E ) enlarged to show; warty exine ; E. SEM picture of the similar type of spores from the trophophore, the sporopollenin forms totally different elevated short nodulations all around spores not found on the spores from sporophore.



**Figure 5:** A. *Goswamia bispora* : A trophophyll showing cluster of spores (Vadhiya et al, 2024) ;B. spores released from a sporophore presenting younger phase while C, a fully mature spore showing perispore (outermost layer) which does not appear in developing spores; D. Two shapes of spores within one and the same sporangium of sporophore (never observed in any species of *Ophioglossum*). In fact the spores on the trophophylls are also totally different from the spores in sporophore (Vadhiya et al, 2024).



**Figure 6A:** *Goswamia glossii*. The distal face of a triradiate spore showing hexagonal foci with sporopollenin deposition in beads. The presence of pits on both sides of the spore resembles *Goswamia costata* but extra deposition of loosely spread beads offer difference.



**Figure 6B:** *Goswamia glossii*. A low power SEM picture reveals two shapes of the spores; oblong spores are seen among mainly round spores (see also Fig.5 D, *G bispora* spores)

Organ	<i>Goswamia costata</i>	<i>G. glossii</i> sp nov	<i>G. bispora</i>	Remark
<b>Sporophore</b> (spike) 1.Length- 2.Length with the Stalk of the sporophore  3.Number of sporangia per sporophore	2 to 4 cm  Up to 6 cm  20 to 40	3 to 6 cm  4-to 12cm  30 to 50	0.5 to1 cm  1.5 cm  6-13	<i>Goswamia bispora</i> plants are very small ; sporophores and trophophylls have brown patches (Vadhiya et al 2024) Fig. 5A
Spores in Sporophore 1.Shape and size of spores  2.Wall layers (under light microscope)  3. Exine (SEM) ornamentation	Trilete round; 20 to 36 µm  Only three, perine or perispore is absent  Webbed, Equal sized pits on both faces of elevated exine layers	Trilete round measuring 16-20 µm,; spores are round, few oblong  Only three, perine or perispore is present on mature spores  Webbed fovea forming pits resembles <i>G.costata</i> but loose beads on margins are different (Fig 6 A)	Trilete round 12- 24 µm, but some spores may have different shapes (boat /bilateral shaped) of two Only three, perine or perispore is seen in maturing spores (see Fig. 5)  (Vadhiya,et al, 2024)	<i>Both the species G. bispora</i> and <i>G.glossii</i> possess two shapes of spores (Fig 5 D 6 B)
<i>Two shapes of spores in the sporangia in sporophore</i>	Not known	Small proportion of spores have oblong and irregular shape	Present round and bilateral /boat shaped spores	<i>G. bispora</i> Fig.4 D

Rhizome	Globose , large	Large globose	Globose and proportionately large	G bispora Plants are too small
Roots	Prolific roots	Prolific roots	Very thick roots	

**Table 1:** Comparative observations on morphological traits with some resembling species

## Declarations

**Conflict of Interests:-** None

**Funding:-** None

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**Special remarks:-** *Goswamia eliminata* Zhang & Zhang rarely exhibited two shapes of spores and also spores on trophophyll margin (2,27–29,32) while *Goswamia bispora* Vadhiya et al. plants, which are too small (less than 3 cm) possess both features as almost normal traits. Now, we have *Goswamia glossii* sp.nov. plants which possess spores on trophophyll margin and also on laminar veins along with formation of primitive sporangia. Spores in sporophores are different than those on vegetative parts and also have two different shapes. These three species of the genus *Goswamia* have common features but are too different from each other in shape and size of plants and exine wall sporopollenin depositions on spores as evidenced by SEM studies.

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