



REVIEW ARTICLE

Changing trends in pteridology: I. A reference to ophioglossum L

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Abstract

This paper intends to emphasize the importance of Poly-trait diagnostic approach (Ptda) for the diagnosis of any species. The genus *Ophioglossum* in particular which is known to possess $2n=1450 + 10$ microchromosomes as the highest chromosome number among all living organisms, has been discovered to exhibit interspecific and intraspecific chromosomal variations thereby declaring chromosome number of very little importance for species diagnosis. Also new natural hybrids are being discovered from India and other countries which have been exhibiting too many variations in gross morphology of lot many species. Thus, this is becoming almost mandatory to observe all possible morphological features of leaf, corm and spike and must also observations be made on some biochemical parameters. Additional features which offer reliable diagnosis are comparative molecular phylogenetic studies on *rbcl* and other chloroplast genes along with SEM studies on spore morphology. Based on such comprehensive studies over several years some highly exciting inferences have been obtained. The family ophioglossaceae known to comprise of *Ophioglossum*, *Botrychium*, *Helminthostachys*, *Mankuya* now also has phylogenetically distinct three genera raised by Zhang and Zhang (viz.: *Goswamia*, *Haukia*, and *Whittieria*) raising the total to seven (07) genera. According to molecular phylogenetics, all are deeply diverged from the rest as demonstrated by the long branches or numerous molecular synapomorphies of each of them..

Our poly-trait diagnosis approach (Ptda) investigating on all above parameters have not only authenticated diagnosis of species described from India but also assert that the most reliable morphological features are SEM of spores to exhibit exine ornamentation and shape and size of corms and number of roots. This revised study indicates that conventional *Ophioglossum* plants with bulbous corm / rhizome with bulk of roots have to be identified as *Goswamia* Zhang and plants with small, linear corm with a few roots as *Ophioglossum* L.

Keywords: Ophioglossaceae, Evolutionary significance of *Ophioglossum* and *Goswamia*; Multidiagnostic approach for diagnosis; Comparative Phylogenetic analyses, Morphological and genetic diversity.

Introduction

There have been series of studies on members of Ophioglossaceae ever since the discovery of *Ophioglossum* by Bauhin (1620). Since then the identification of species of *Ophioglossum* L and their distribution in forests hills and plains in India and elsewhere have been based on gross morphological characteristics and sometimes on chromosome counts. In past fifty years we have reports of interspecific as well as intraspecific variations within many species of the genus and therefore chromosome count

did not always offer any definite clue to identification of a species. Also, many biochemical studies including paper chromatic separation of aminoacids, enzyme variations by disc and gel electrophoresis have been helping but lately, the genomic extractions and assessing comparative traits of chloroplast genes (*rbcl* in particular) have been found to be of universal importance.. So, comparative phylogenetic studies assisted with SEM studies on spores and other gross morphological features have become almost mandatory for exact diagnosis of species. Natural hybrids have been discovered from India and also from other countries; no one character can obviously be a final one unless too significant and consistently present.

A few of them need special mention because altogether new approaches involving morphology, taxonomy, cytology, biochemical studies and phytogeography were investigated by various authors which paved the way for recognition of several new species (Beddome, 1883; Campbell 1911; Clausen 1938; Bower, 1935; Ninan 1956; Mahabale, 1962; Khandelwal and Goswami, 1976,1977a,b, 1984; Khandelwal et al., 1980; Dixit 1984, 2000; Chakravarti, 1951; Balkrishnan et al., 1960; Pant and Khare 1969; 1971; Pant et al., 1995; Bir

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and Vasudev, 1971; Goswami and Khandelwal, 1973 a,b, 1976, 1980; 1984; Goswami, 1987, 1998, 1999, 2007; Manickam and Irudharaj 1992; Manickam, 2007; Bir and Verma 2010 and many more). Recently, Mukhopadhyay (2019) has reported a brief account of number and distribution of genera in India.

Lately, we have many new genera and species described, some of them as natural hybrids from different parts of the globe which have further enriched the germplasm of this group of pteridophytes. Among most workers, the genus *Ophioglossum* has attracted the maximum attention by most workers more particularly since the beginning of this century (Burrows and Jones 2001; Muller 2000; Munster et al 2002; Goswami, 2007, 2008, 2010, 2012, 2013; Singh et al., 2009; Peruzzi et al., 2015; Yadav and Goswami, 2010; Olejnik, et al 2018; Anto et al., 2016 ; Patel and Reddy, 2018, 2019 ; Patel et al., 2018; Goswami and Patel 2019; Goswami et al., 2020).

Evolutionary Background

Bower (1935) had related Ophioglossaceae with Rhyniaceae and Coenopteridaceae. Campbell in 1907 (cited from Smith, 1955) mentioned that the gametophytes of *Ophioglossum*, Psilotaceae and *Lycopodium* resembled each other in bearing subterranean structure destitute of chlorophyll. Banks (1968) had probably rightly suggested that Ophioglossales, Coenopteridales and Filicales might have originated separately from Rhyniopsida. Our observations on periderm (Khandelwal and Goswami, 1977a) in three genera, *Ophioglossum*, *Helminthostachys* and *Botrychium* affirm a distinctive anatomical feature from ferns. Population studies have revealed that the production of periderm could be a genetic trait prevalent in some and absent in other plants at the same time in the same population.

Dating evolution in terms of millions of years Pryer et al (2001, 2004) have estimated that the Psilotalean and Ophioglossoid lineages might have diverged from one another in the late Carboniferous (360myrs). This and other evidences reemphasizing that *Ophioglossum*, so pretty – fragile possesses the genome having survived for more than three billion years without any anatomical strength (no sclerenchyma in Ophioglossaceae), and even without reproductive production measures (protective sporangial cover). A serious question emerges as to what are inherent genomic strength; are these high chromosome numbers (ranging from 100 to 1400 chromosomes per cell and very high grades of C - values ?..Yet another feature, which is also found in ferns and a large number of plants having bulbous subterranean rhizomes is the presence of highly aromatic oil and rich contents of proteins with sulphur containing amino acids (methionine, ornithine etc). These compounds help in prolific vegetative reproduction and maintenance of plants in populations (Fowden 1965; Khandelwal and Goswami, 1976; Khandelwal et al., 1980) and as a matter of fact, due to the treasures of most valuable compounds pteridophytes are considered to be “evolutionary boon as medicinal plants” Goswami et al., 2016).

Recent Studies

Zhang et al., (2020) Zheng et al., (2021) and Zhang & Zhang (2022) have carried out very exhaustive studies and have reconstructed new phylogenetic trees. Zhang and Zhang have emphatically concluded, on the basis of investigating on nearly 100 ophioglossaceous species (of all genera) on four datasets: Sanger sequences of eight plastid markers of 184 accessions, 22 plastomes (12 are new), 29 morphological characters, and combined Sanger and morphological data that:

- The relationships among the four subfamilies are well resolved and strongly supported in Bayesian and parsimony analyses based on plastomes: *Mankyua* is sister to the rest, followed by Ophioglossoidae which are sister to Helminthostachys + Botrychioideae;
- Sanger data, plastomes, and combined Sanger and morphological data recovered and strongly supported the monophyly of *Ophioglossum* in its current circumscription (sensu lato; s.l.) in Bayesian and/or parsimony analyses;
- within *Ophioglossum* s.l., four deeply diverged clades are identified and the relationships among the four clades are well resolved;
- Evolution of 34 morphological characters has been analyzed in the context of the new phylogeny, among which shape of rhizomes, germination time of spores, shape of early gametophytes, and a number of other characters are found to contain interesting phylogenetic signals.
- Based on the new phylogeny and character evolution, Zhang and Zhang, now include seven (07) genera within the family Ophioglossaceae which include, *Botrychium*, *Botrypus*, *Japanobotrychium*, *Sceptridium*, *Ophioglossum*, *Goswamia*, *Haukia*, and *Whittieria* considering their molecular, morphological, ecological, and biogeographical distinctiveness.

Discussion

Morphological Significance.

Goswamia, *Haukia*, and *Whittieria* can not be accommodated in one genus, because they are paraphyletic with respect to *Ophioglossum*. Species of *Goswamia* have tuberose or bulbous/cylindrical (Plate 1) and often with proliferous roots, clearly different from those of *Ophioglossum* spp which have linear cylindrical rhizomes. Based on the phylogenetic separations following changes based on morphological identifications have been indicated as under :

Goswamia Zhang & Zhang

Plants terrestrial; mature rhizomes tuberose or subglobose; roots 2–5 (–25) per rhizome, up to 1.5 mm diam., often strongly proliferous; trophophores erect to spreading and often forming an angle of 0–20° from horizontal; venation reticulate; sporangial clusters up to 10 cm. Approximately

15 species distributed in Africa, Southeast & South Asia, and Australasia.

Goswamia costata (R. Br.) Li Bing Zhang & Liang Zhang, comb. nov. Basionym: *Ophioglossum costatum* R. Br., Prodr. Fl. Nov. Holland. 163. 1810. In its current definition, *Goswamia costata* occurs from Australia to India and Africa.

Goswamia eliminata (S. Khandelwal & H.K. Goswami) Li Bing Zhang & Liang Zhang, comb. nov. Basionym: *Ophioglossum eliminatum* S. Khandelwal & H.K. Goswami, Fern Gaz. 12(6): 330. 1984. This species is endemic to India.

Goswamia gomeziana (Welw. ex A. Braun) Li Bing Zhang & Liang Zhang, comb. nov. Basionym: *Ophioglossum gomezianum* Welw. ex A. Braun, Fil. Afr. 176. 1868 & Prantl 315 t. 7f. 13. NPfl. 467. 1868. This species occurs in Africa.

Goswamia gujaratensis (S.M. Patil, R.N. Kachhiyapatel, R.S. Patel & K.S. Rajput) Li Bing Zhang & Liang Zhang, comb. nov. Basionym: *Ophioglossum gujaratense* S.M. Patil, R.N. Kachhiyapatel, R.S. Patel & K.S. Rajput, Phytotaxa 251(4): 274, f. 1. 2018. This species is endemic to India.

Goswamia hitkishorei (M. Patel & M.N. Reddy) Li Bing Zhang & Liang Zhang, comb. nov. Basionym: *Ophioglossum hitkishorei* M. Patel & M.N. Reddy, Bot. Lett. 166(4): 426. 2019.

Goswamia indica (B.I. Yadav & H.K. Goswami) Li Bing Zhang & Liang Zhang, comb. nov. Basionym: *Ophioglossum indicum* B.I. Yadav & H.K. Goswami, Bull. Natl. Mus. Nat. Sci., Tokyo, B. 36(4): 155–159; figs. 2010. This species is endemic to India.

Goswamia isanensis (S. Petchsri, Li Bing Zhang & T. Jaruwattanaphan) Li Bing Zhang & Liang Zhang, comb. nov. Basionym: *Ophioglossum isanense* S. Petchsri, Li Bing Zhang & T. Jaruwattanaphan, Phytotaxa 533(3): 158–164. 2022. This species is endemic to Thailand.

Goswamia malviae (M. Patel & R.M. Reddy) Li Bing Zhang & Liang Zhang, comb. nov. Basionym: *Ophioglossum malviae* S.M. Patel & R.M. Reddy, Sci. Rep.: 8(art. 5911): 1, f.1–4. 2018. This species is endemic to India.

Goswamia raphaeliana (Anto, Afs. Khan, F. Francis & I. Antony) Li Bing Zhang & Liang Zhang, comb. nov. Basionym: *Ophioglossum raphaelianum* Anto, Afs. Khan, F. Francis & I. Antony, Int. J. Advanced Res. 4 (5): 1269, f.1–2. 2016. This species is endemic to India.

Goswamia trilokinathii (B.I. Yadav, M.K. Meghvansi, K. Meena & C. B. Gena) Li Bing Zhang & Liang Zhang, comb. nov. Basionym: *Ophioglossum trilokinathii* B.I. Yadav, M.K. Meghvansi, K. Meena & C.B. Gena, Sci. Rep. 11:24396. 2021. This species is endemic to India.

***Ophioglossum* L**

Typical morphology of *Ophioglossum* plants known for centuries, but the plants must have slightly thick or swollen linear rhizome (but not bulbous or as a big knot) with not many roots: see (Tables 1 & 2; Plate 1). Several classical species of *Ophioglossum* like *O. reticulatum*, *O. vulgatum*, *O. gramineum*, *O. chalonerii*, *O. thermale* etc remain within the

genus because all collections of past several years exhibiting both young and mature plants possess thicker linear rhizome with about 6-10 roots emerging from the respective corm /rhizomes.

(C & D) *Haukeria* Zhang & Zhang and *Whitteria* Zhang & Zhang

Plants exactly assignable to both these genera have not been segregated among our collections. Needless to mention, we have to plan for extensive field work, hopefully we would be able to find these rare specimens in nature.



Diagnostic features among spores

As per large number of publications and revised diagnostic criteria this has been unequivocally established that besides comparative assessments on the basis of chloroplast genes (plastomes, Figure 1; Pryer *et al.*, 2001;2004; Zhang and Zhang, 2022 and several others) the exact diagnosis would also need spore wall layers both under light as well as SEM studies on exine ornamentations (Goswami, 1987, 2010, 2013, 2021; Peruzi *et al.*, 2015; Olejnik *et al.* 2018; Goswami and Patel, 2019; Goswami *et al.*, 2020, Goswami and Patel, 2021 ; Stensvold and Farrar, 2017; Patel and Reddy, 2019) because sporopollenin deposition on pattern of exine layer constitution needs controlled and directed deposition of many organic compounds and all this mechanism must involve many genes. Briefly, SEM study of spores (Plate 2) also offers valuable tool for diagnosis. This exactly holds true for all kinds of spores and pollens of other groups of plants.

Table 1: Registered Sequences of rbcL genes of different species

<i>Species name</i>	<i>Chloroplast gene name</i>	<i>NCBI Accession number New Name of</i>
<i>O. chalonerii</i>	rbcL gene	MH605181
<i>O. chalonerii</i>	psbA-trnH	MH605182
<i>O. chalonerii</i>	trnL-trnF	MH605183
<i>O. malviae</i>	rbcL gene	MF184998
<i>O. malviae</i>	psbA-trnH	MG875321
<i>O. malviae</i>	trnL-trnF	MG875324
<i>O. parvifolium</i>	rbcL gene	MG875326
<i>O. parvifolium</i>	psbA-trnH	MG875322
<i>O. parvifolium</i>	trnL-trnF	MG875323
	rbcL gene	MK120496
<i>O. aletum</i>	rbcL gene	MK120497
<i>O. hitkishorei</i>	rbcL gene	MK360156
<i>O. hitkishorei</i>	psbA-trnH	MK358464
<i>O. hitkishorei</i>	trnL-trnF	MK358465

Plate 1: new genera among revised classification of ophioglossaceae

	Main Features	Figure/ Remark
<i>Ophioglossum</i>	Typical simple conventional outline a leaf with adnate spike often arising from the base of the tropophyll (tropophore/ leaf); corm / rhizome small, linear or cylindrical with not many roots (nonproliferous roots)	 <i>Ophioglossum chaloneri</i>
<i>Goswamia</i>	Typical simple conventional outline a leaf with adnate spike often arising from the base of the tropophyll (leaf); stout, cylindrical or round often large with lot many proliferous roots; roots are almost thicker than stalk of the leaf	 <i>Goswamia costata</i>
<i>Haukeria</i>	similar to <i>Ophioglossum</i> s.s. in having similar aerial morphology, but it has tuberous rhizomes, non-proliferous roots, sporophores arising from the rhizome top	Not yet identified among samples of various collections
<i>Whittieria</i>	Similar to <i>Ophioglossum</i> s.s. in having similar aerial morphology, but it has double venation with secondary veins within primary larger and thicker veins,	Not yet identified among samples of various collections

Palingenetic significance

Precisely, *Ophioglossum* genus has three additional sister genera *Goswamia*, *Haukia*, and *Whittieria* which according to molecular phylogenetics are all deeply diverged from the rest of *Ophioglossum* ss (Table 2 & Fig.1) : as demonstrated by the long branches or numerous molecular synapomorphies of each of them (See Zhang & Zhang 2022). These authors find that infrageneric molecular divergence in Ophioglossaceae is generally much lower, as observed in Botrychioideae than the one among *Goswamia*, *Haukia*, *Ophioglossum* s.s., and *Whittieria*. Molecular dating conducted in 2016 by Testo and Sundue (cited from Zhang and Zhang 2022) had estimated the three genera to have diverged from their sisters ca. 134, 127, and 179, million years ago (MA) respectively,. In comparison, the other four genera in the same family, *Botrychium*, *Botrypus*, *Japanobotrychium*, and *Sceptridium*, which have been recognized by Hauk *et al.* (2003, 2012), Shinohara *et al.* (2013), PPG I (2016), and Zhang

Table 2: Revised Nomenclature

<i>Ophioglossum costatum</i> R.Br.	<i>Goswamia costata</i> [Rhizome bulbous with many roots]
<i>Ophioglossum eliminatum</i> Khandelwal & Goswami	<i>Goswamia eliminata</i> [Rhizome bulbous with many roots]
<i>Ophioglossum aletum</i> Patel, Reddy & Goswami	<i>Goswamia aleta</i> [Rhizome bulbous with many roots]
<i>Ophioglossum hitkishorei</i> Patel and Reddy	<i>Goswamia hitkishorei</i> [Rhizome bulbous with many roots]
<i>Ophioglossum malviae</i> Patel & Reddy	<i>Goswamia malviae</i> [Rhizome bulbous with many roots]
<i>Ophioglossum gramineum</i> Willd	<i>Ophioglossum gramineum</i> [Rhizome erect, linear; few roots]
<i>Ophioglossum chaloneri</i>	<i>Ophioglossum chaloneri</i> [Rhizome erect, linear; few roots]
<i>Ophioglossum reticulatum</i>	<i>Ophioglossum reticulatum</i> [Rhizome erect, linear; few roots]
<i>Ophioglossum vulgatum</i>	<i>Ophioglossum vulgatum</i> [Rhizome erect, linear; few roots]



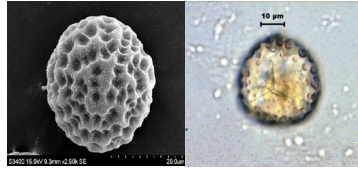
Figure 1: Comparative phylogenetics of different species of the family Ophioglossaceae (to be updated after revising studies on all specie). This phylogenetic tree recognizes again that even when several overlap in gross morphology most conventionally known and newly discovered species as well are quite distinct phylogenetically (eg. *O.aletum*, *O. chaloneri*, *O. reticulatum*, *Goswamia hitkishorei*, and also *Goswamia. costata*).

Plate 2: Comparative observations on exine ornamentation on spores of some *Ophioglossum* and *Goswamia* species under SEM
Type (s) of Exine ornamentation Example (s)

1. Exine uniformly deposited on distal face with several deeper pits

The exine deposition is uniform with larger pits on both faces of spores. These are quite clear under SEM as well as on light microscopic observation

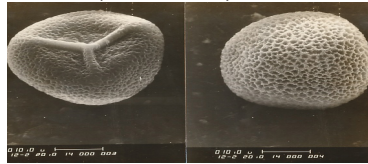
Proximal face : Triradiate round *Ophioglossum gramineum*



2. Exine broad- flat ridges forming deep pits

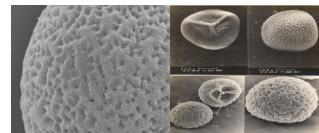
Both Surfaces (Proximal and Distal) uniformly covered with of variable shapes and sizes.

Triradiate round spores; *Goswamia costatata* (= *O. costatum*)



3. Exine broad zigzag plates often mixed up forming smaller gaps ridges and furrows; such broad zigzag plates are seen on spores of *O. vulgatum* and *O. polyphyllum*. But then shape of leaf helps in distinction.

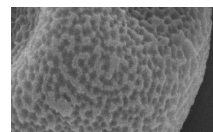
Exine on distal face of spores of ; *O. polyphyllum* and *O. vulgatum*



4. Elevated ribbon shaped stripes/ ridges of exine ;

Small globular dots on proximal face appearing as pseudo-tubercles. (Elevated strips have never been observed on distal face of any species of *Ophioglossum*)

Triradiate round spores with zigzag elevated strips *Ophioglossum chaloneri*



5. Exine with small and larger pits

many depressed areoles united in to negative reticulation; covered with fly perispore (a rare feature)

Triradiate round spors with perisporal cover on exine



Goswamia indica

6. Exine with mesh of cavities/foveae margins covered with grains

filled up all around (Such grains on margins of foveae have never been reported as essential part of exine ornamentaion

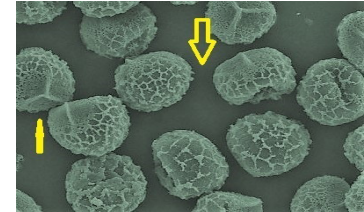
Triradiate round spores sometimes mixed up with a few spores which are triangular in *Goswamia eliminata* (= *O. eliminatum*).



7. Exine forming beaded strings

tied and spread all over the distal face; proximal face with grains forming grannular mesh around triradiate mark Pointers in the figure indicate proximal part as simple granulate while distal face shows webbed strings of exine ornamentation

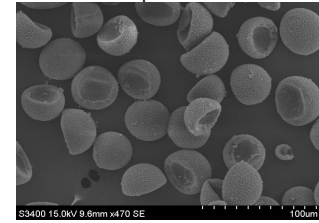
Triradiate round spores of *Goswamia hitkishorei*



8. Alete spores

During developmental stages these alete spores are seen in chains demonstration amitotic divisions (budding phenomenon)

Alete round spores



Goswamia aleta (= *Ophioglossum aletum*)

et al. (2020a), were dated to have diverged ca. 18, 99, 40, and 21 MA, respectively.

Due to above studies, one very important question becomes partly answerable, ie, the problem realetd to palaeoploidisation among *Ophioglossum* ss (Wagner and Wagner 1980, 1987 and 1990 ;see review Goswami, 2013). This was rightly hypothesized several years ago that since almost all species possess intraspecific polyploidy (Goswami and Khandelwal, 1980, Goswami, 1987, 2007; Khandelwal, 1990) the intragenomic variability must be loaded with interspecific hybridization contents including chromosomal aberrations (Goswami and Khandelwal 1980; Goswami 1998, 1999, 2013). Quite possibly, this may be one of the reasons that homologous chromosomes rarely form trivalents and multivalents during early meiotic stages, despite the fact that palaeo-autoploidy must have resulted in copying homologous chromosomes (Goswami and Patel 2019). In recent years, repeated phylogenetic analysis of chloroplast *rbcl* genes have revealed that many species in India appear to be offshoots of a " hybrid clade" which is likely to be the hybridization products involving *Ophioglossum costatum* (now *Goswamia costata*) *O. vulgatum*, and or also *Ophioglossum reticulatum*, including other possible interspecific hybridizations. For example, based on morphological and paper chromatographic evaluation of aminoacids variation at the time of spike initiation and development (Goswami and Khandelwal, 1976; Khandelwal, et al., 1980), as well as other repeated experimental trials strongly suggested that only *O. gramineum* contains iminoacid proline as found in *O. eliminatum* but in no other specis. *Ophioglossum eliminatum* (now, *Goswamia eliminata*) has been understood to be a hybrid in between *O. costatum* x *O. gramineum*.

This is most remarkable that some of the new taxa published by us, viz. *O. eliminatum* (now, *Goswamia eliminata*) *O. indicum* (now, *Goswamia indica*) *O. aletum* and *O. chalonerii* (both these and many other classical species viz. *O. reticulatum*, *O. vulgatum*, *O. gramineum* etc retain *Ophioglossum* genus identity on account of linear and thick with a few roots; but not bulbous rhizomes with too many roots), explicitly show distinct phylogenetic relationship maintaining independent genomic identity (Plate 2 & Figure 1).

Furthermore, each new species shows some rare uniquely confined traits (on Itropophylls / leaves, spikes and exine morphology on spores not possessed by any other species of the genus (Goswami, 1987, 2008, 2013) Recently the presence of intracellular blue green algae to be a symbiont has been confirmed (Goswami and Patel 2021) in *Goswamia costata*, *Goswamia eliminata*, *Goswamia indica*, *Ophioglossum chalonerii* and *Goswamia aleta* (*Ophioglossum aletum*). I am sure, many such biochemical associations will be known soon in several species. Such an adaptive achievement appears to be of significant biochemical importance. Several new observations on morphological traits (Goswami et al., 2020) suggest that the plants may be expressing extremely rare genic-combinations which might have been brought forth due to autopolyploidy and/or allopolyploidy (hybridizations) during past several decades or more. Obviously, we need an exhaustive multidisciplinary approach (morphological, anatomical, biochemical and phylogenetic assessments using many more genes) in order to identify different species of *Ophioglossum*, *Goswamia*, *Whitteria*, *Haukeria* and related genera with new and unusual genomic identity and also to understand possible evolutionary mechanisms undergoing within the geologically too old genome of ophioglossaceae. Similar situations where polygenic genetic factors have been offering both morphological as well as genetic changes have been elaborated in detail by Stensvold and Farrar (2017) in *Botrychium lunaria* complex. Morphological and genetic variations are also related to geographical distribution of this unique species in the world flora. Additionally, I have found too many variables among *Helminthostachys* (Goswami and Khandelwal, 1980; Goswami, 1987, 2014) plants collected from different areas in UP, Bengal and Kerala. So many recurrent variations in natural populations must involve (Haldane 1932; Goswami, 2008; Fournier and Schacherer, 2017) genetic mechanisms on way of knitting some stable changes.

Ecological significance

There appears to be no direct influence of any ecological or environmental factor(s) to be positively correlated with bulbous rhizomes with a large number of roots (eg: *Goswamia costata*) and plants with linear simple rhizome with a few roots (eg. *Ophioglossum gramineum*). Several species assignable to *Goswamia* as well as *Ophioglossum* are found

within the radius of 100 cm, (at many places where too many species are found in a particular area/spot of collection). However as argued earlier by me, palaeoloidisation in *Ophioglossum*s (particularly as of now *Goswamia* and *Ophioglossum*) has been mainly (*prima facie*) responsible (Goswami, 2013) for very many adaptive measures for their survival. This is intriguing that the plants of the same species occurring at different localities do possess intracellular blue green alga (Goswami and Patel, 2021), but also do not possess intracellular alga at some places (unpublished).

Conclusion

Based on published background over six decades on various aspects of Biology of *Ophioglossum* and *Helminthostachys* some important conclusions must be mentioned so as to ignite basic interests in evolutionary biology of these wonderful genera..

- The days of field identifications on the shape and size of leaf, better "gross morphology," are gone; because as I often claim, "there are many a men who look like apes but they are not apes" (Goswami, 2010).
- Natural hybrids are being discovered from India and other countries which have been exhibiting too many variations in gross morphology of many species. Thus, this is becoming almost essential to observe all possible morphological features of leaf, corm and spike and must also observations be made on some biochemical parameters. Comparative phylogenetic studies on *rbcl* and other chloroplast genes along with SEM studies on spore morphology are becoming more reliable tools.
- Changing ecological, climatic conditions world over as well as natural hybrids are resulting in too many variables and we often come across many fundamental events and structures of evolutionary significance; which are liable to be ignored.
- These abnormal variants if maintained in a population for several years/ generations then, we can look for all such segregates carefully as if, altered variations are genic combinations selected under interplay of ecological genetics.. We already have discovered many structures never seen before.
- Poly-disciplinary approach (detailed internal morphology of leaves; shape and size of corms and roots, spore morphology with analysis of wall layers and exine ornamentations must be assisted with some biochemical investigations on enzymes and if possible with molecular phylogenetic studies with genomic DNA samples of variants in particular.
- What we know today is the surface flora of India; there are several valleys, hills and remote forest areas whose enormous wealth has not been searched meticulously. Search for more genera and species of *Ophioglossaceae* will be honourable efforts.

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References

- Anto P.V, A Khan, F Francis and I Antony (2016). *Ophioglossum raphaelianum* – A New Species from South India. *International Journal of Advanced Research.*, **4(5)** : 1268-1273
- Balakrishnan N P, K Thothathriand and A N Henry (1960). Some Indian *Ophioglossums*-Taxonomy and Distribution. *Bulletin Botanical Survey of India* 2(3&4): 335-339.
- Banks H P (1968). The Early History of Land plants. In, Evolution and Environment a symposium Presentation- Museum of Natural History of Yale University, E Drake (Ed). New Haven and London, pp 73-107.
- Bauhin C (1620). *Ophioglossum and Lunaria*. In: *Prodromus TheatriBotanici*, Frankfurt, Germany, pp. 354-355.
- Beddome R.H. (1883). Handbook to the ferns of British India. Ceylon and Malay Peninsula Thacker Spink & Co., Calcutta, pp. 500.
- Bir S S and Vasudeva S M (1971). Pteridophytic flora of Kodaikanal (South India). *Journal of the Bombay Natural History Society* **68** 169-195.
- Bir, S S and S C Verma (2010). Chromosome Atlas of the Indian Pteridophytes (1951-2009). Bishen Singh Mahendra Pal Singh, Dehradun, pp: xx, 346 [ISBN : 978-81-211-0772-3] 2010
- Bower FO (1935). Primitive Land plants. MacMillan & Co. Ltd London.(Reprint 1959)
- Burrows J E and Jones RJ (2001). Ophioglossaceae. (eds Beentje, H.J.), *Flora of Tropical East Africa: Ophioglossaceae* pp. 1-19.
- Cambell D H (1911). The Eusporangiatae. *Carnegi Inst Publ* 140. Washington
- Chakravarty H L (1951). Indian *Ophioglossum*. *The Botanical Society Bengal* **5**: 1-10.
- Clausen RT (1938). A monograph of the Ophioglossaceae. *Memoirs of the Torrey Botanical Club.* **19:5**, 1-177.
- Dixit RD (1984). A Census of Indian Pteridophytes. *Botanical Survey of India, Calcutta, India*, pp. 1-177.
- Dixit RD (2000). Conspectus of Pteridophytic Diversity in India. *Indian Fern Journal* **17**:77-91.
- Doyle J J and J L Doyle (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* **19**: 11–15.
- Fourneir T and J Schacherer (2017). Genetic backgrounds and hidden trait complexity in natural populations. *Current opinion in Genetics and Development.* **47**: 48- 53.
- Fowden L (1965). Origins of the amino acids. In: Bonner J and Varner JE (eds) *Plant Biochemistry*. New York: Academic Press pp. 361–390.
- Goswami, H. K. and S Khandelwal, (1973a). Abnormalities in *Ophioglossum* L. *Brit. Fern. Gaz.*:10 (6): 311-314.
- Goswami, H K and S Khandelwal (1973b). Wall layers in normal abnormal spores of *Ophioglossum* L. *J. Ind. bot. soc.*: **52**: 206-213.
- Goswami H K and S Khandelwal (1976). Free aminoacids in *Ophioglossum* leaves at the time of spike initiation. *Israel J. bot.* **25**: 211-213
- Goswami H K and S Khandelwal (1980). Chromosomal elimination in natural populations of *Ophioglossum*. *Cytologia*; **45**: 77-86
- Goswami H K and S Khandelwal (1980). Observations on *Helminthostachya* Kaulfus. *Acta Botanica Neerlandica* **29**: 192-202
- Goswami HK (1987). Ophioglossales:I. An Overview. *Bionature* **7**: 47-89.
- Goswami H K. (1998). Ecological Genetics of a natural hybrid. In, Perspectives in Environment. Ed. Agrawal *et al.* New Delhi (ISBN 81-7024-972-4).
- Goswami H K (1999). Ophioglossales II. Population Biology of *phioglossum* L. In : Progress in Cytogenetics. Ed. P. Kachroo Dehradun (ISBN B1-211-0163-8).
- Goswami HK (2007). Biology of *Ophioglossum* L. *Bionature*. Pp **27**: 1-73.
- Goswami HK (2008). Recurrent variations in natural populations may indicate genomic involvements: I. *Ophioglossum* L & *Psilotum nudum* (L) P Beav. In: SC Verma, SP Khullar HK Cheema (editors): Perspectives in Pteridophytes, pp. 465-474, Bishen Singh Mahendra Pal Singh, Dehradun, India;
- Goswami HK (2010). Teaching of Botany and Genetics through pteridophytes: Reminiscences from my Journey as a Biologist. *Indian Fern J.* 2010. **27**: 5-28.
- Goswami HK (2012). Observations on *Ophioglossum eliminatum* (pteridophyta : Ophioglossaceae) with comments on the lowest chromosome number in the genus. *Acta Botanica Hungarica.* **54(3-4)**: 283–300
- Goswami HK (2013). Palaeoploidization and adaptation: An Evolutionary strategy among pteridophytes with a reference to *Ophioglossum* L. *Nucleus* **56 (2)**: 69-80.
- Goswami HK (2014). *Helminthostachys zeylanica* populations need serious attention: Disclosures from unpublished observations. *Ind Fern J.* 31: 136-139
- Goswami HK, Sen K and Mukhopadhyay R (2016). Pteridophytes: evolutionary boon as medicinal plants. *Plant Genetic Resources.* (published on line) <http://dx.doi.org/10.1017/S1479262116000290>. Pp: **14(4)** 328-355
- Goswami H K and M Patel (2019). Comparative phylogenetics of some species of *Ophioglossum* L. (Ophioglossaceae: Pteridophyta) in India with comments on evolutionary significance of high palaeoploidy and rare morphological traits. *Nucleus* <https://doi.org/10.1007/s13237-019-00287-y>
- Goswami HK, M Patel M and K K Nag (2020). *Ophioglossum chaloneri*: A new species of *Ophioglossum* (Ophioglossaceae: Pteridophyta) from India. *Phytotaxa* :**468 (1)** 101-111. <https://doi.org/10.11646/Phytotaxa.468.1.6>.
- Goswami HK (2020). The Genus *Ophioglossum* L in India: Brief Review on Field work. In, *New Vistas in Indian Flora* : Edts, L J Singh & V Ranjan; 187-198 ISBN: 978-00-000-0000-0; M/s Bishen Singh Mahendra Pal Singh, Dehra Dun, India
- Goswami HK (2021). Comparative SEM assessment of exine ornamentations on spores is reliable for diagnosis of specie:

- A Reference to *Isoetes* L. *Indian Fern J.* **38** : 72-80.
- Goswami HK and Patel M (2021). Cyanobacteria (Blue Green Algae) revisited in leaves of *Ophioglossum* L. *Glob Imaging Insights*, 2021 doi: 10.15761/GII.1000217 **6**: 1-2
- Hauk WD, Parks CR and Chase MW (2003). Phylogenetic studies of Ophioglossaceae: evidence from rbcL and trnL-F plastid DNA sequences and morphology. *Mol. Phylogenet. Evol.* **28** (1), 131–151.
- Hauk WD, Kennedy L and Hawke H M (2012). A Phylogenetic Investigation of *Botrychium* s.s. (Ophioglossaceae): evidence from three plastid DNA sequence datasets. *Syst. Bot.* **37**, 320–330.
- Haldane JBS (1957). The cost of natural selection *J Genetics.* **55**: 511-524
- Khandelwal S and Goswami H K (1976). Amino acid differentiation in *Ophioglossum* L. by paper Chromatography. *Curr. Sci.* **45** (2): 62-63.
- Khandelwal S and Goswami H K (1977a). Periderm in ophioglossaceae. *Acta Soc. bot. Polomae.* 46: 641-646
- Khandelwal S and Goswami. H K (1977b). Stomata in *Ophioglossum palmatum* L. *Curr.Sci.* 1977: 45: 51-54
- Khandelwal S and Goswami H K (1984). A new *Ophioglossum* from India. *Fern Gaz.* 1984.12: 330.
- Khandelwal S, H K Goswami and A K Chatterji (1980). Nitrogen and oil contents in *Ophioglossum* L. *J. Indian bot. Soc.* **59**: 78-80
- Mahabale T S (1962). Species of *Ophioglossum* in India: their taxonomy and phylogeny. *Bulletin of Botanical Survey of India.* **4**:71-74
- Manickam V S (2007). Checklist of Pteridophyte species. A database available at <http://www.tnenvi.nic.in>
- Muller S (2000). Assessing occurrence and habitat of *Ophioglossum vulgatum* L. and other Ophioglossaceae in European forests. Significance for nature conservation. *Biodiversity and Conservation* **9**: 673–681,
- Mukhopadhyay R (2019). Ophioglossales : its Evolutionary trends- A Review. *J Botan Soc Bengal* **73** (1) 1-15
- Munster T, Faigl W, Saedler W and G Theissen (2002). Evolutionary aspects of MADS-box genes in the Eusporangiate fern *Ophioglossum*. *Plant Biol.* **4**: 474-483
- Ninan CA (1956). Cytology of *Ophioglossum*. *Curr. Sci.* **25**:161-162.
- Olejnik N, Celka Z, Szkudiarz P and Shevera M V 2018 Taxonomic significance of morphological characters of spores in the family ophioglossaceae (psilotopsida). *Rev of Palaeobotany and Palynology.* <https://doi.org/10.1016>
- Panigrahi G and Dixit R D (1969). Studies in Indian Pteridophytes IV. The family Ophioglossaceae. *Proceedings of National Academy of Science in India.* **35(B)**:230-266.
- Pant D D and Khare P K (1969). Epidermal structure and stomatal ontogeny in some eusporangiate ferns. *Ann. Bot.* **33**: 795-805
- Pant DD and Khare P K (1971). Notes on the spore morphology of Ophioglossaceae and the occurrence of *Ophioglossum* and its gametophytes in the Gangetic valley. *Geophytology.* **1**: 48-53
- Pant D D, Nautiyal D D and Misra D R (1984). *Gametophytes of Ophioglossaceae. Phytograph monograph No.1*, 1-111.
- Pant D D, Nautiyal D D, Misra D and Shukla AK (1995). Comparative study of exine ornamentation in some ophioglossaceous spores under LM and SEM. *Birbal Sahni Centenary Volume.*, Allahabad, India
- Patil, S and Dongare M (2014). *Ophioglossum* from Western Ghats of India; *Indian Fern J.* **31**: 17-24.
- Patel M and Reddy M N (2019). Revealing a new species of *Ophioglossum* Ophioglossaceae-Pteridophyta) from India with palynological and phylogenetic implications. *Botany Letters* **166**: 425–433. DOI: 10.1080/23818107.2019.1636405
- Peruzzi L, Pietini B, Magrini S, Andreucci A, Marchetti D and Viane R. (2015). Three new hybrids of *Ophioglossum* (Ophioglossaceae) from Monte Pisano, Tuscany (Central Italy) *Plant Biosystems*, <http://dx.doi.org/10.1080/11263504.2015.1057264>
- Puneet., Goswami HK, Indira and Srivastava GK (1989). New epidermal features on leaves of *Ophioglossum eliminatum*. *Bionature.* **9**: 39-42.
- Patel M and M N Reddy (2018). Discovery of the world's smallest terrestrial Pteridophyte. *Scientific Reports*, 2018
- Pryer KM, H Schneider A R Smith R Cranfill, Wolf P G, Hunt J S and Sipes S D (2001). Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. *Nature* **409**: 618–622.
- Pryer KM, E Schuettpelz P G Wolf H Schneider A R Smith and R Cranfill (2004). Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. *Amer J Bot.* **91**:1582–98
- Rajput K S, Kachhiyapatel R N, Patel S K and Raole V M (2016). Study on pteridophyte diversity of Gujarat state, Western India. *Plant Science Today.* **3(4)**:327-338
- Singh A P, Mishra S, Gupta S, Behera S K and Khare P B (2009). Studies on the genus *Ophioglossum* L. in Pachmari Biosphere Reserve, Madhya Pradesh, India. *Taiwania.* ; **54(4)**:353-364.
- Shinohara W, Nakato N, Yatabe-Kakugawa Y, Oka T, Kun Kim J, Murakami N, Noda H and Sahashi N (2013). The use of matK in Ophioglossaceae phylogeny and the determination of Mankyua chromosome number shed light on chromosome number evolution in Ophioglossaceae. *Syst. Bot.* **38**: 564–570.
- Smith GM (1955). *Cryptogamic botany Vol II. Bryophytes & Pteridophytes.* McGraw Hill Book Co. Ny.
- Smith AR, Pryer KM, Schuettpelz E, Korall P, Schneider H and Wolf PG (2006). A Classification of extant ferns. *Taxon* **55**: 705-731
- Stebbins GL (1971). *Chromosomal Evolution in Higher Plants* Edward Arnold, London
- Stensvold M C and Farar D R (2017). Genetic Diversity in the Worldwide *Botrychium lunaria* (Ophioglossaceae) complex, with New species and New combinations. *Brittonia* 69 (2) 148-175
- Wagner DH (1987). Meiotic pairing success affected by environment in a *Polystichum* hybrid. *Amer.J. Bot* **74**: 762-763
- Wagner W H Jr and Wagner F S (1980). Polyploidy in pteridophytes. In, "Polyploidy : Biological relevance (Ed. W.H.Lewis). Plenum New York.Pp: 199-214.
- Wagner WH Jr (1990). Ophioglossaceae. In: Kramer, K.U., Green, P.S. (Eds.), *The Families and Genera of Vascular Plants. Vol. 1. Pteridophytes and Gymnosperms.* Springer-Verlag, Berlin, pp. 193–197.
- Yadav BL and Goswami HK (2010). A New, Pink Brown *Ophioglossum* (Ophioglossaceae) from India. *Bulletin of the Science Museum Series B* 36: 155-159.
- Yadav BL and Tripathi. M K (2002). *Ophioglossum* Linn. In:P.C. Trivedi Rajasthan–Taxonomy and Distribution. In *Advances in Pteridology.* Pointer Publisher, Jaipur, pp. 248-267.
- Zhang L, X-M Zhou Z-L Liang X-P Fan N T Lu M-S Song R Knap X-F, Gao, H Sun and L-B Zhang (2020). Phylogeny and classification of the tribe Lepisoreae (Polypodiaceae; pteridophyta)

with the description of a new genus, *Ellipinema* gen. nov., segregated from *Lepisorus*. *Mol. Phylogenet. Evol.* 148, 106803. doi: <https://doi.org/10.1016/j.ympev.2020.106803>.

Zhang L and Li-Bing Zhang (2022). Phylogeny, character evolution, and systematics of the fern family Ophioglossaceae based on Sanger sequence data, plastomes, and morphology.

Molecular Phylogenetics & Evolution.**173**: 107512 <https://doi.org/10.1016/j.ympev.2022.107512>

Zheng S, He J, Lin Z, Zhu Y, Sun JL and Li (2022). Two MADS-box genes regulate vascular cambium activity and secondary growth by modulating auxin homeostasis in *Populus*. *Plant Comm.* 2, 100134