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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 ther 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 ophioglossacea 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 G*oswamia* 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 (*rbc*L 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 Irudaraj 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 Ophioglossoideae 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 Ohioglossaceae 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.l.Yadav & H.K.Goswami) Li Bing Zhang & Liang Zhang, comb. nov. Basionym: Ophioglossum indicum B.l.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.l. Yadav, M.K. Meghvansi, K. Meena & C. B. Gena) Li Bing Zhang & Liang Zhang, comb. nov. Basionym: Ophioglossum trilokinathii B.l. 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 posses 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 sporopolenin 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

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

Plate 1: new genera among revised classification of ophioglossaceae

	Main Features	*Figure/ Remark*
Ophioglossum	Typical simple conventional outline a leaf with adnate spike often arising from the base of the tropophyll (tropophore/ leaf); corm / rhizome small, linear orcylindrical *with not many roots* (nonproliferous roots)	 *Ophioglossum chaloneri*
Goswamia	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	 *Goswamia costata*
Haukeria	similar to *Ophioglossum* 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
Whitteria	Similar to *Ophioglossum* 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

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

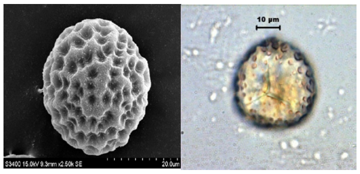
Figure. 1: Comparative phylogenetics of different species of the family Ophioglossaceae (to be updated after revising studies on all specie). This phylogenetic tree recognizes again that even when several overlap in gross morphology most conventionally known and newly discovered species as well are quite 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

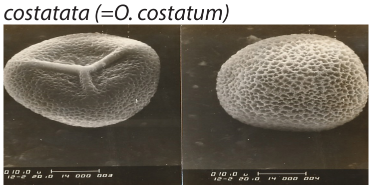
Proximal face : Triradiate round *Ophioglossum gramineum*



2. ***Exine broad- flat ridges forming deep pits***

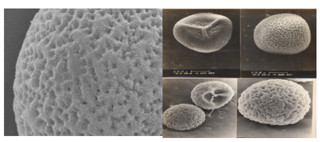
Both Surfaces (Proximal and Distal) uniformly covered with of variable shapes and sizes.

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



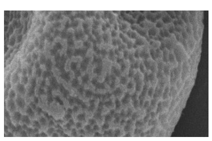
3. Exinne broad ziz zag plates often mixed up forming smaller gaps ridges and furrows;' such broad zigzag plates are seen on spores of *O. vulgatum* and *O.polyphyllum*. But then shape of leaf helps in distinction.

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



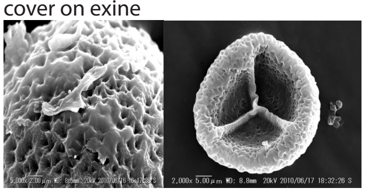
4. ***Elevated ribbon shaped stripes/ ridges of exine ;***
Small globular dots on proximal face appearing as pseudo-tubercles. (Elevated strips have never been observed on distal face of any species of *Ophioglossum)*

Triradiate round spores with zigzag elrvated strips
Ophioglossum chaloneri



5. Exine with small and larger pits
many depressed areoles united in to negative reticulation; covered with fily perispore (a rare feature)

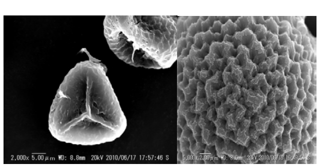
Triradiate round spors with perisporal cover on exine



Goswamia indica

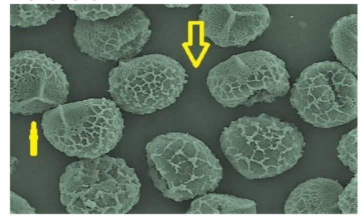
6. ***Exine with mesh of cavities/foveae margins covered with grains***
filled up all around (Such grains on margins of foveae have never been reported as essential part of exine ornamentaion

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



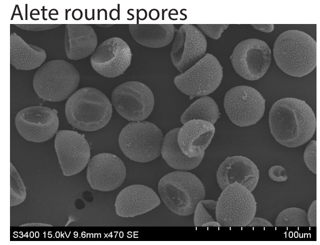
7. Exine forming beaded strings
tied and spread all over the distal face; proximal face with grains forming grannular mesh around triradiate mark Pointers in the figure indicate proximal part as simple granulate while distal face shows webbed strings of exine oornamentation

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 palaeoplodisation 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 intraspecifc polyploidy (Goswami and Khandelwal, 1980, Goswami, 1987, 2007; Khandelwal, 1990) the intragenomic variability must be loaded with interspecifc 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 interspecifc 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.gramineuum*.

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 confned traits (on ltropophylls / 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 smybiont 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 diferent 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 word 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; Fourneir 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 Ophioglossums (particularly as of now *Goswamia* and *Ophioglossum)* has been mainly (*prima faci)*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 Biologu 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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