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ISOETES PANTII GOSWAMI & ARYA IS "*PAR EXCELLENCE"* IN PLANT MORPHOLOGY : A REAPPRAISAL

HIT KISHORE GOSWAMI

Retired Professor of Genetics, 24, Kaushalnagar, P.O. Misrod, Bhopal (MP) India
Email: hitkishoreg@gmai.com

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As far as known, *Isoetes pantii* Goswami & Arya is the only species in the world flora to have exhibited the evolution and continued transmission of some traits that have never been known in the genus so far. These traits, well published within past five decades are:(i) development of heterosporangia (microsporangia containing thousands of alete, monolete and trilete microspores along with megaspores within one and the same sporangium; recently collected plants of *I. pantii* from Gujarat also possess the same features);(ii) megaspores in heterosporangia are sometimes too abnormal, resembling some of the fossil lycopods; (iii) the megaspores and microspores germinate within the heterosporangium, *in situ* germination and photographs of gametophytes have been the first ever reports; (iv) many plants show series of chromosomal evolution based on a new basic number x/ n=12 (2n= 36, 48 and 60 chromosomes); (v) Plants with 2n 36 and 48 chromosomes exhibit by and large, normal meiosis and possess variable counts of X, Y and B chromosomes (the first ever sex chromosomal mechanism in any pteridophyte). This paper offers repeated recognition of these significant discoveries. Certain plants, with 2n=60 chromosomes and also on the basis of detailed spore morphological and SEM studies, have been named as new species (described as *Isoetes fuchsii* Bhu, Goswami, Sharma & Bajpai, and *I. narsinghgarensis* Goswami & Mazumdar). Actually, mitotic and meiotic divisions and behaviour of chromosomes among certain plants have had suggested 50 years ago that the genetic make up of *Isoetes pantii* is on the track of evolution of sex chromosomes. Followup population cytogenetic studies since then, from the same and different localities have aptly demonstrated all these evolutionary mechanisms thereby verifying that *Isoetes pantii* is typically characetized by exhibiting presence of X, Y and two B chromosomes. Molecular genetic experiments (Southern blot, probing with human Y chromosome sequence & DNA blasting) have also confirmed older hypothesis that *I coromandelina* L and *I. sampathkumaranii* Rao might have crossed in the natural aquatic habitat and must have donated chromosomes along with causing genomic reshuffle (11+1 & 33+1 chromosomes respectively). We have always recorded some plants with chromosome fragmentations and translocations. This is also the reason that we have encountered, over these fifty years plants with variable 2n=36, 38, 39, 48 and 60 chromosomes. While plants show many small breaks, laggards and translocations plants with 2n=48 and 2n=60 chromosomes appear to be stable and both mitosis and meiotic divisions are more or less as expected with regards to pairing and lagging of chromosomes during anaphasic movements.. Apart from chromosomal survey, based on the most reliable differences in exine ornamentation in combination with phylogenetic assessments (vide rbcL- chloroplast genes), the segregating plant populations over these years have appeared constituting "*pantii complex"* with four species, viz. *I pantii* Goswami & Arya (2n=48); *I. fuchsii* Bhu *et al.* (2n=60), *I. narsinghgarensis* Goswami & Mazumdar (2n=60) and *I.pantii* var *jaideepii* Goswami & Patel. Exhaustive follow-up travels for collections and laboratory studies from a large number of localities in many states of India have also lead to record many other fundamental observations which are like *rediscoveries* for the genus *Isoetes.* One of the most significant observation has been about the ligule which develops below the labium; in other words what we see on the adaxial face on the sporangium is the labium, not ligule as was labelled in earlier textbooks and research publications before my publication. Further, a unique relationship with ligule and labium was discovered that on maturity, either the ligule or the labium may shrivel down, depending upon, obviously as per genetic make up of the plants. Similarly, development of dimorphic or polymorphic spores in a sporangium has been explained due to the genic differences among spore mother cells. Obviously, the rare assemblage of many unique and hitherto unknown features, the taxon, *Isoetes pantii* is being designated as "*par excellence"* in plant morphology.

Key Words: Heterosporangium in *Isoetes pantii ,* Natural hybridization and speciation, New basic chromosome number 12 ; Sex chromosome evolution within the genome; Some megaspores inside heterosporangia resemble fossil lycopods ; Interrelationships in between ligule and labium

Isoetes L., also known as Merlin's grass is an aquatic weed belonging to the group of heterosporous lycopsids possessing a large number of ever increasing count of species with the cosmopolitan distribution of the genus (Taylor *et al*, 1985, Srivastava *et al.* 1993, Liu *et al.* 2004, Shukla *et al.* 2005, Singh *et al.* 2018, Kim *et al.* 2010 a, b, Troia *et al.* 2016. Shukla *et al.* 2017) in the world flora. As is well known, *Isoetes coromandelina* was the first species described from India by C.A. Linne. Fi. In 1782 from the Coromandel coast of India. This had paved the way to search *Isoetes* L. plants in the vast land of Indian sub continent with extreme climatic and highly diversified environmental conditions in different regions and by now, we have more than fifteen species described from various

parts of India (Rao 1944, Shende 1945, Pant and Srivastava 196, Goswami and Arya 1970, Bharadwaj 1984, Srivastava *et al.* 1992, 1993, 1996, Srivastava 1995, Sharma 2005, 2013, Bir & Verma 2010 and others). Indisputably, *I. coromandelina* has been found widely adapted and has been reported from almost all states in India, also from many Asian countries and as well as from Australia (Marsden 1976) . On account of some minor differences a few varietal forms of *coromandelina* have also been described. Identification of species has been principally based on exine ornamentation of spores. In her monograph, Pfeiffer (1922) recognized the exine (outer surface of the spore) to be a real distinguishing feature of a species on the basis of the greater reliability of these features (Punt *et al.* 2007; Takamiya 1999, 2001, Goswami, 2010) as, gross morphological (colour, shape and size etc) characteristics might be easily affected by environmental factors. In order to be more accurate Pfeiffer had recognized four sections of megaspore surface ornamentations, viz. *Tuberculatae, Echinatae, Cristatae* and *Reticulate* and subsequently, Fuchs-Eckert added a fifth section *Laevis* (for smooth surfaced megaspores; see also, Fuchs-Eckert, 1992; personal discussions). By and large, almost all species described so far in the world flora have been described on megaspore and microspore ornamentation besides adding features of ligule, labium, corm and leaf characteristics also to be cumulatively more helpful in delimiting species of the genus (Pant and Srivastava 1962, Goswami and Arya 1970, Sharma 2005, 2013, 2015, Sharma and Bohra, 1978, 2001,2002, 2011, Goswami, 1996; Hickey 1984, Small and Hickey 2001, Prada and Rolleri 2005; Shukla *et al.* 2005, Troia & Greuter 2014, Takamiya 1999, 2001, Sharma and Purohit 2019). Lately however, this is becoming absolutely essential that biochemical, chromosomal and molecular characteristics need more serious attention in order to add authenticity to the species diagnosis because neither gross morphology nor chromosome number offer definitive inferences on species diagnosis. The modern trend demands that many pteridophytic genera including *Isoetes* should not be identified exclusively on gross morphological structures (for example, the ligule-labium association and presence/ absence of velum (Scott and Hill 1900, Pant & Srivastava 1962, Goswami 1976, 2005, Bhu 1990, Pant *et al.* 2000, Budke *et al.* 2005 Sharma & Purohit 2001) because dozens of plants within the same niche show same and variable shapes and sizes. So practically, identification of species has to be based, besides on general morphology, also on details of exine ornamentation of spores, as well as on taxonomically distinct and important structures which may differ among other taxa; for example: chromosome number and behaviour of chromosomes during mitosis and meiosis (Ninan 1958, Goswami 1975, Bhu and Goswami 1990, Takamiya 1994, 1996, Liu *et al.* 2004, Zican 2004). Lately, modern molecular genetic studies assessing phylogenetic comparisons have become almost indispensible. Unfortunately, certain seemingly over ambitious publications have been revising a large number of species of pteridophytes including *Isoetes* found in India almost blindly on gross morphology alone (mainly size of the plant) and adding revised names after some of these important discoveries. This has been pointed out elsewhere (Goswami 2010, Goswami and Arya 2012, Sharma 2011, 2013, Verma 2018) that this freedom, exercised by the classical morpho-taxonomists has to be based on identifications of conventional and Internationally acknowledged detailed methodologies rather than simple size of the plants and "general appearance" in the field or on the herbarium sheets. Population biology of studies for several years on the same taxa have exposed enormous variables among existing flora, therefore multidisciplinary approaches can only offer reliable information.

Phylogenetic considerations along with spore morphological descriptions on exine ornamentations are becoming more reliable with rbcL genic comparative approaches (Rydin and Wikstrom 2002, Goswami and Patel 2019, Hemsley 1997Troia *et al.* 2016).

Five decades ago, through the pages of this journal, Goswami & Arya (1970) discovered a new species of this aquatic weed and was named as *Isoetes pantii.* The study initiated in 1966 was based on exhaustive study of *Isoetes* species in India by Pant and Srivastava (1962, 1965; Dixit, 1984)) and search on morphological, chromosomal, anatomical and biochemical studies is still going on for more than fifty years now and as many as two dozen species of *Isoetes* have been collected and or examined from various localities in India and European countries. Various herbaria have been visited for comparative assessments and also Isotypes have been deposited as and when required. This paper mainly focuses on those vibrant rare features observed consistently in plants of *Isoetes pantii* Goswami & Arya not so far encountered in any species of the genus in the world flora of the genus. Obviously, *Isoetes pantii* , as already indicated (Goswami and Arya 1968, 1970, 2012, Goswami 2012, 2014) exhibits extremely rare features on account of genomic reshuffle most likely brought about by natural hybridization in *Isoetes coromandelina* L and *I. sampathkumaranii* with some additional evolutionary mechanisms . Now these rare features are the integral part of the genome of *I. pantii*, viz, speciation of some species constituting pantii-complex (Goswami & Mazumdar, 2019) on the basis of chromosome numbers, n=12, (Goswami and Goswami, 1986; Bhu and Goswami 1990, Jermy 1991; Liu *et al.* 2004) instead of speciation path of n=11, which is the basic number encountered among all species of *Isoetes* in the world flora reported from different countries (Hickey 1984, Small and Hickey 2001, Takamiya *et al.* 1994, 1996; Musselman *et al.* 1997, Shukla *et al.* 2005; Zican *et al.* 2004, Tro`ıa 2001, Tro`ıa, *et al.* 2016, Singh *et al.* 2018). The second unique character is the presence of heterosporangium (microsporangium with both microspores and megaspores). This paper chiefly concerns with the evolutionary significance of extremely rare features discovered during followup studies since 1966 until today on *Isoetes* populations discovered in and around Narsinghgarh and also from very remote areas in Central India as well as in several other states. Observations on *Isoetes pantii* over these decades have been so significant that no other species of the genus has ever lead to so many discovery and rediscoveries. Morphological, chromosomal , anatomical, histochemical and molecular genetic studies have compelled to designate *Isoetes pantii* as "*par excellence in plant morphology.* . Additionally, this paper also offers a brief account of seven species of the genus studied over fifty years and also ventures revising morphological, cytological molecular genetic studies along with emphasis on those features which are typically diagnostic features of respective species.

MATERIALAND METHODS

Plant Collections and basic approaches

Unbelievable though, the author has been collecting *Isoetes* populations mostly from forests and hilltop- small ponds located in more than two dozen localities in Central India for over six decades and several species of *Isoetes* particularly, *I. sampathkumaranii,*Rao *I..panchananni* ,Pant & Srivastava *I. indica,* Pant & Srivastava; *I. coromandelina,*L. *I.dixitei,* Shende *I. pantii,* Goswami & Arya, *I. fuschii* Bhu *et al.* and, *Isoetes narsinghgarensis* have been studied by various morphological, chromosomal, biochemical, embryological and anatomical approaches. Many reviews have appeared on important observations and specific approaches and their pertinent methodologies

have been described (Goswami and Arya 1968, 1970, 2012; Goswami and Bhu, 2000; Goswami and Kumar 2006, Goswami 1975a b,1996, 2001a,b,c, 2004a,b, 2010, 2014; Bir and Verma 2010).

Though, this may be only for theoretical satisfaction but is of immense importance that exhaustive collections from remote areas of Indian State, Chhatisgarh (particularly South Bastar, viz. Jagdalpur, Narainpur forest ponds and remote interiors of so called Abhujmand) have many unique species distributed which are totally distinct. Many species are still unpublished as these collections could not be repeated for either chromosomal or DNA studies during 1990s and thereafter. We have enormous wealth of *Isoetes* germplasm and this is a totally invalid concept that India can not have many species.

DNA extraction, amplification and sequencing

In order to offer comparative phylogenetic relationships among various Indian species of the genus, experimental efforts have been made by suitable genomic DNA extractions (Doyle and Doyle 1987). The detailed aspects of methodology regarding amplification of *rbcL* chloroplast gene /DNA sequences (cpDNA) using a protocol with published primer details (Levin *et al.* 2003, Kress and Erickson 2007, Taberlet *et al*. 1991, Kress *et al.* 2009) have been published by us (Patel *et al.* 2018, Goswami and Patel 2019). Successful amplifications were purified, and commercially sequenced. Purification of amplified PCR product was done using a GenElute™ PCR clean-up kit. Eurrofins Genomics India Pvt Ltd., Bangalore, sequenced the purified PCR product. The nucleotide sequences were aligned with ClustalW (Thompson and Gibson, 2002) embedded in MEGA 7.0 (Tamura *et al*. 2003). *RbcL* gene molecular phylogenetic analyses (using newly generated sequences of *Isoetes* species and *Selaginella simplex* as an out group) were performed using Maximum likelihood (ML) and method. The *rbcL* gene dataset was analyzed in Partition Finder (Lanfear *et al*. 2012) to select the best partitioning scheme. The same partition scheme was selected for ML analyses. ML analysis was employed to infer the phylogenetic relationships in RAxML . An ML analysis was run for 1000 bootstrap replicates under the GTR + I model to assess clade support. Strict protocols (Hall 1999, Silvestro and Michalak 2012; Goswami and Patel, 2019) were followed as also published earlier and suitable statistical approaches were used finally to construct phylogenetic tree. Brief results are presented here (Fig. 6).

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OBSERVATIONS AND COMMENTS

The central theme of this presentation fundamentally revolves around discovery of a few new species of *Isoetes*, the most remarkable features present in these taxa, trying to understand their mode of origin (which conform to incidence of natural hybridization), their distribution along with comparative assessments of genetic variations and similarities among species particularly reported from India. A brief description of species reported from Narsinghgarh and Maksoodangarh (districts Rajgarh & Guna respectively: M P) is presented hereunder.

Brief description of species

I. *Isoetes pantii* Goswami & Arya, J. . Ind. Bot. Soc. 49: 30. 1970. ; R. D. Dixit, Census 18.1984.*Isoetes coromandelina* sensu auctt. Fraser-Jenkins no nL; Revalidated as *Isoetes pantii* Goswami & Arya 2012

Plants measure 24 - 60 cm (Fig.1, A; B-D) mature leaves are 22-58cm long, leaves are unequal, no four leaves are of equal length among 20 to 30 leaves per plant; **corm:** three lobed, one lobe smaller than others making corm often tilted; **ligule:** cordate and spongy (1-3 x 2-5 mm) under labium, with long attenuating middle flap with serrate margins, shrivels down on mature sporangia; **labium**:

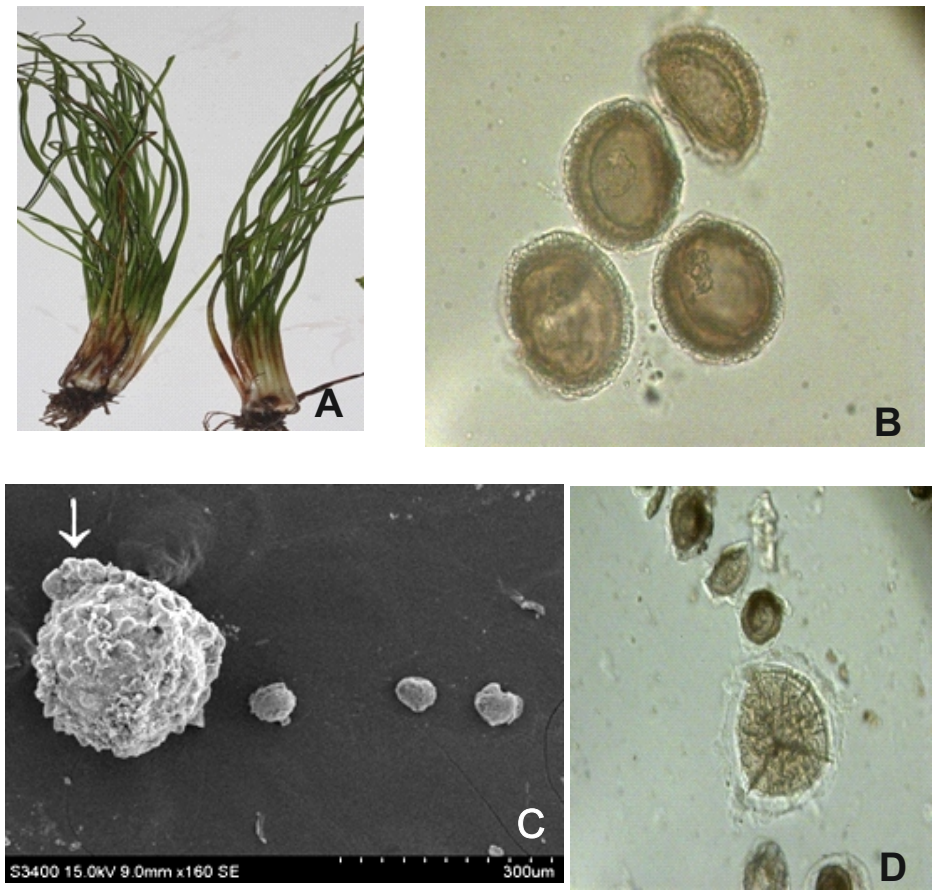


Figure 1 (A-D): A.Plants of *Isoetes pantii* collected from Gujarat (plants measure 18-21cm in length); **B.** A glycerine mount showing round alete and and bilateral monolete microspores (24-28mu) from the heterosporangium (heterosporous microsporangium) **C.** SEM of spores from the same sporangium showing large megaspore and a few microspores, some are attached at the upper face (arrowed); **D.** Glycerine mount of spores showing bilateral microspores and a trilete megaspore under light microscope

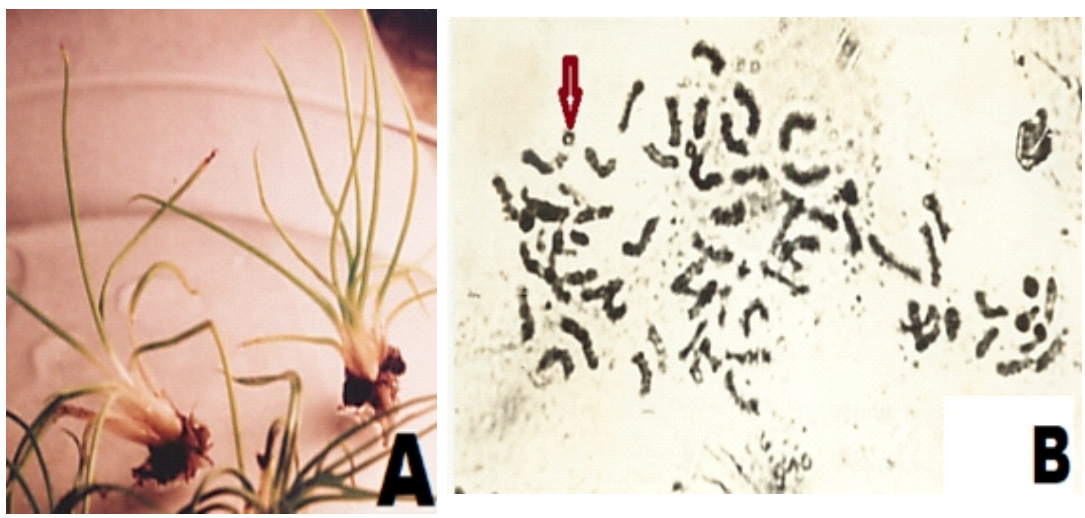


Figure 2 (A-B): A. Plants of *I . narsinghgarensis* (Plants measure less than 6 cm) **B** 2n=60 chromosomes in root tip mitosis stained primarily by Feulgen's method and counterstained with 2% acetic-orcein; presence of a B chromosome (arrowed) and two small fragments are seen (Photographed under 1000 magnification)

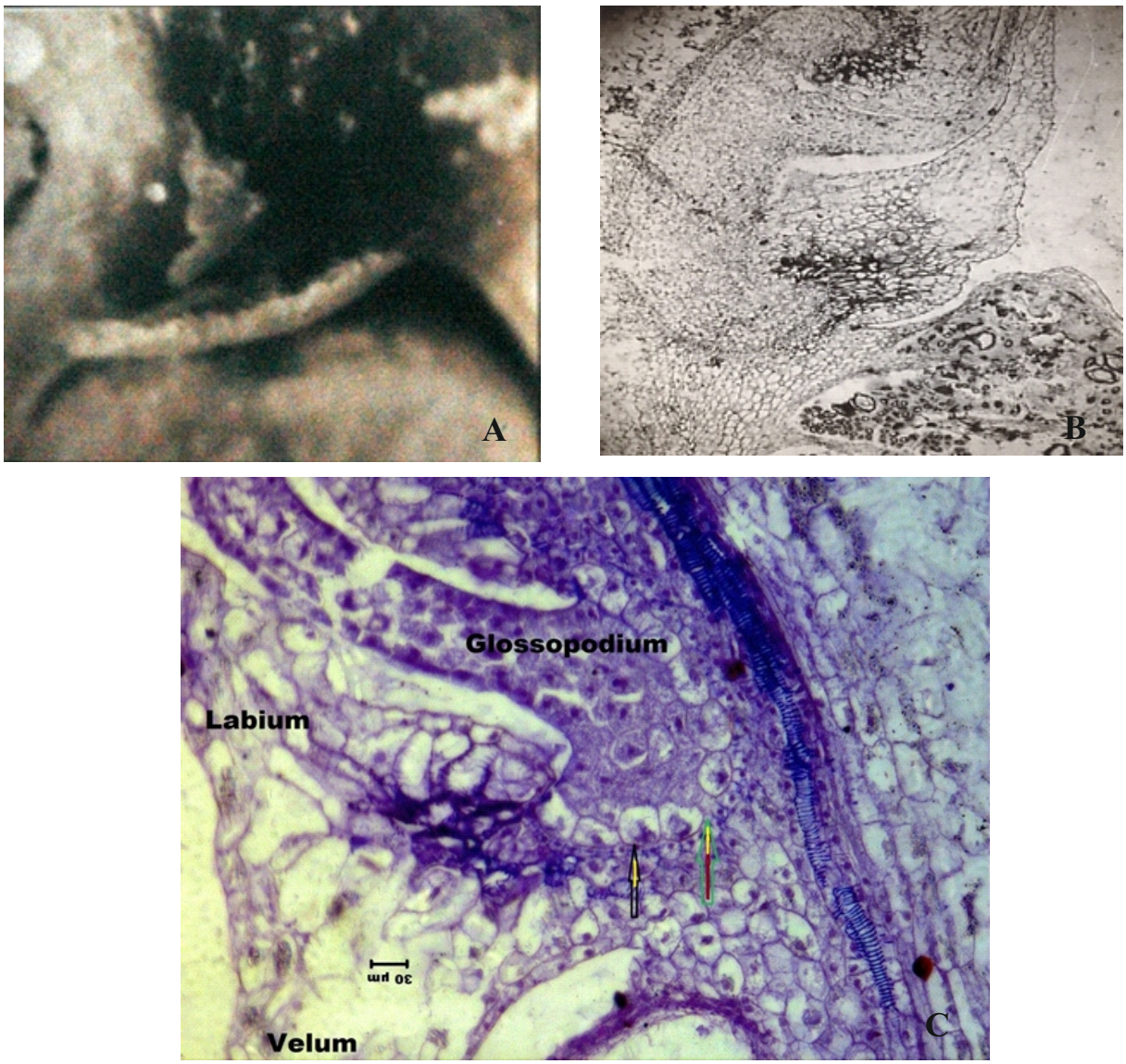


Figure 3 (A-C) : A. *Isoetes pantii* showing ligule and labium; ligule is in between sporophyll and flap of labium; **B.** L S of the heterosporangium showing that ligule is covered and protected by over arching labium; **C.** L S of young sporangium showing globose glossopodium and upper notch of labium partly covering ligule and lower bending flap indicating labium. Arrows indicate the haustorial function of basal cells , few basal cells are vanished creating gap so as to allow entering of water and solutes from adjoin cells of labium (see Goswami, 1976, 2005, 2010).

large, much broader (4-7mm) than long (3-4 mm)remains as a thick flap on mature sporangia; **velum** : absent, but seen as rudimentary in very young sporangia; **Sporangia, megasporangia:** round to oval 4-16 mm long and 2-5 mm broad at the upper part, containing 180- 500 trimorphic, round tuberculate megaspores, large megaspores, 370-560 mu), medium (240-360 mu) and small megaspores measure 120 – 200 mu in diam

Heterosporangia : 0-4 per plant ; contain more than 70,000(seventy thousand) microspores (12-46 mu) microspores trimorphic, round alete, bilateral monolete, and triangular to globular trilete along with, several large megaspores with reticulate exine of variable shapes and sizes ranging 165-180mu in diam. **Chromosome number:** sex chromosomes present, 2n=48 and some plants with 2n=36; B-chromosome (1-2), always

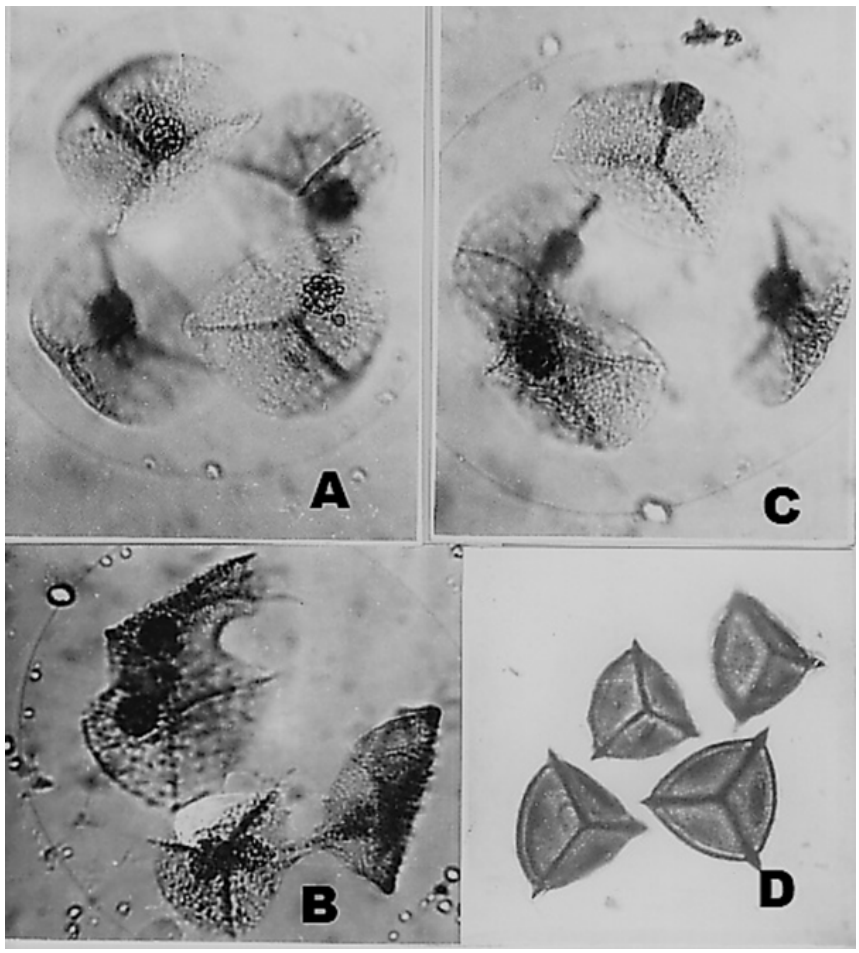


Figure 4 (A-D): A. *Isoetes pantii :* A megaspore mother cell divides to produce four normal spores**; B.** Another megaspore mother cell divides to produce joint spores, siamese spores, and tubular joints ; **C.** Yet another megaspore mother cell may produce normal and slightly deshaped spores; **D. *Isoetes fuchsii*:** Four small megaspores perfectly normal with one tubercle in each pyramidal area of each spore (The definite ratio of normal, Joint and abortive spores in the ratio of 5:2:1 had suggested that spore mother cells are not exact clones but differ in genetic make up and each spore mother ell on completing meiosis produces spores which may have different expression for certain traits) This also explains that spores produced by some SMCs may have different exine ornamentation and would differ with spores produced by other SMCs within the same sporangium.

present.

Distribution : Narsinghgarh and Rampura in Madhya Pradesh, -some forest ponds in South Gujarat, India

Voucher specimens: Plant collections in herbarium- Bionature –HKG/ 1990-2004; HKG/-2010 2018.

II. *Isoetes x fuchsii* I Bhu, HK Goswami, US Sharma and AK Bajpai,

. *Isoetes fuchsii:* Bhu, et al. (2001: 12) Revised: Goswami HK, Mazumdar J (2019) *Isoetes pantii* Complex is Evolving with New Basic Chromosome Number. J Adv Plant Sci 2: 107 (P1-7)

Description: Plants measure 20-30cm (Figure 1A), rhizomorph 3 or 4 lobed; leaves measure 20-26cm, almost of equal in length; velum absent; megaspores with round tubercles; possesses trimorphic megaspores with round tubercles and belong to Section Palustres (=Tuberculatae). About 15–20 % megaspores have large spines, a feature never reported so far in any species of the genus *Isoetes* in the world flora. Among Small megaspores, some have pits on the middle of round tubercle and some have nipple like protuberance as if the

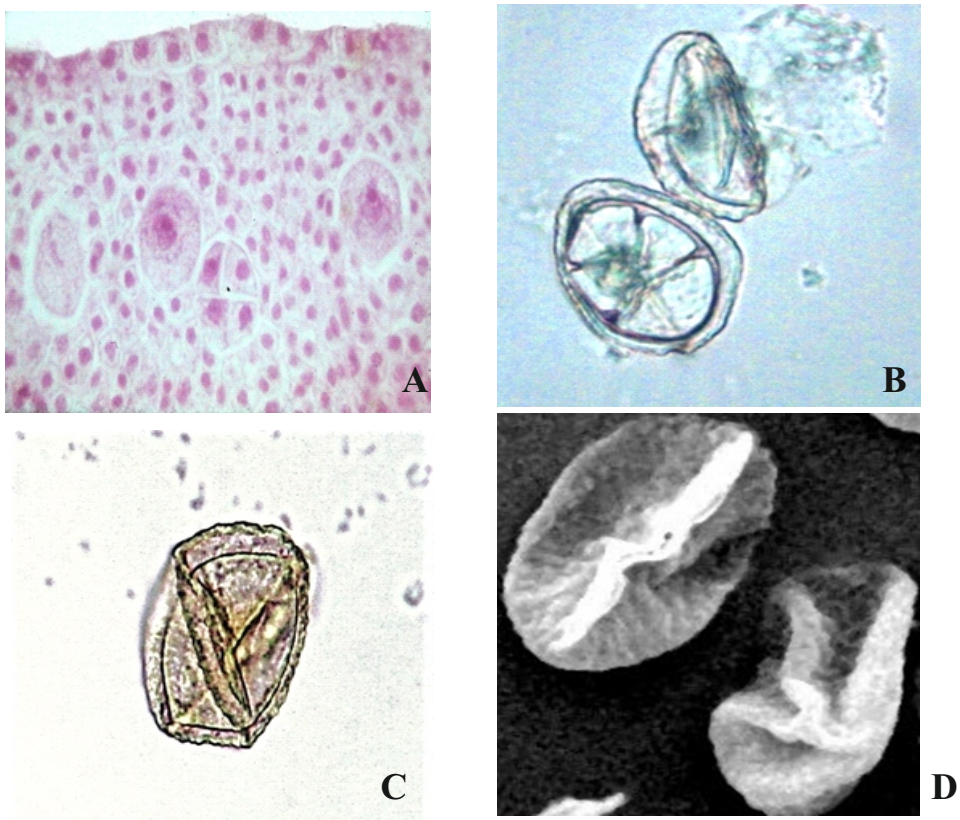


Figure 5(A-D): Microspores of *Isoetes pantii*: A. A part of young microsporangium showing early activity of megaspore mother cells ; microspore mother cells have not yet entered meiosis. **B.** Dil glycerine mount of microspores showing irregular and monolete ridge**; C.** Another microspore showing irregular fold of perispore/perine; **D.** SEM of microspore showing striations and granular sporopollenin deposition on exine. Such monolete bilateral spores measure 20-26 micron in length and nearly half in width.

two had attached earlier during sporogenesis. Large megaspores white when dry, ash coloured when wet, diameter range from 350-400µm, 374µm (average of 50 megaspores). Microsporangia are rare, not on all plants; microspores dimorphic, only monolete and trilete spores have been observed, microspores measure 20-29µm with wide perispore; no megaspores have been observed in microsporangia. Root tip mitosis shows 2n=60 chromosomes

This new species has been named in honour of Swiss botanist Prof. Hans Peter Fuchs-Eckert (1928-1999) for his noteworthy contributions to the genus *Isoetes* and also approving *I. fuchsii* as new species. Chromosome number: Root tip mitosis shows 2n=60 chromosomes ; meiotic studies did not reveal excellent preparations, but no laggards were observed at any stage. Diagnosis: I. fuchsii possesses trimorphic megaspores with round tubercles and belong to Section Palustres (=Tuberculatae). About 15–20 % megaspores have large spines, a feature never reported so far in any species of the genus *Isoetes* in the world flora. Among Small megaspores, some have pits on the middle of round tubercle and some have nipple like protuberance as if the two had attached earlier during sporogenesis. Many a times, one tubercle of the pyramidal area instead of possessing spine shows dismantled tubercles exhibiting fibrous mass of sporopollenin (exiine deposition; Hemsley, 1997; see Fig. 1 C).

III *Isoetes narsinghgarensis* Goswami HK, Mazumdar J (2019) *Isoetes pantii* Complex is Evolving with New Basic Chromosome Number. J Adv Plant Sci 2: 107 (P1-7)

Ponds in Narsinghgarh (Pachtalai pond in particular) and in adjoining villages, plant

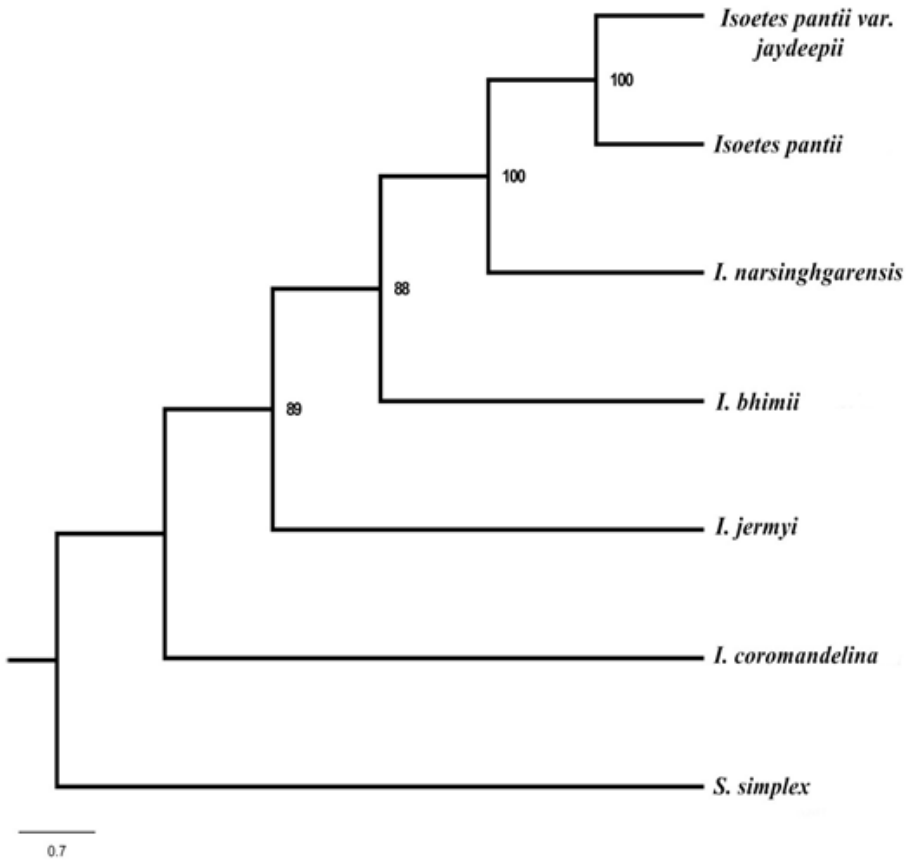


Figure 6: Phylogenetic analysis of rbcL chloroplast DNA sequences of some variant collections of *Isoetes* from different localities including *Isoetes narsinghgarensis* and *Isoetes pantii*. Both the species are retrieve as a distinct lineage and found to diverge from one of the parents, *I. coromandeliana.* Since *I. coromandelina* resembles in general morphology and possesses tuberculate megaspores the differences in phylogenetic distance is of great significance. This phylogenetic distance validates the detailed morphological enumeration of exine ornamentation of spores of both microspores and megaspores and reconfirms that *I. pantii* genome has inherent genetic makeup to produce heterospory within one and the same microsporangium (now named as heterosporangium). *I. pantii* plants collected from Narsinghgarh (MP) and forest near forest area in South of Surat (Gujarat), both possess polymorphic megaspores inside megasporangia and polymorphic microspores and megaspores inside the heterosporangia. Minor differences in exine ornamentation of megaspores (see text and Tables 1 &2) matter the difference hence named as *I. pantii var jaideepii.* Additionally, also near Surat in South Gujarat, a species possessing megaspores within the megasporangia with hyaline and smooth walled exine (plain without any markings of exine ornamentation) have been observed. Isoetes species with such megaspores has been named as Isoetes jermyi (after late A Clive Jermy). Detailed paper describing two species . Viz. I. bhimii & I.jermyi are being published elsewhere.

collections had initially identified three species*; I. coromandelina, I sampathkumaranii* and *I. pantii* as well as certain variants, one of which was erroneously identified as *Isoetes muricata* (Bhu & Goswami 1990). Our follow-up studies had then recorded the growth of small plants confined to the periphery of the same pond at Narsinghgarh which were smaller but typically characterized by 2n=60 chromosomes (Fig.2 A & B) and very strangely, megaspores exhibited both reticulate exine as well as presence of round tubercles. Such a feature has never been observed in any species so far. Assuming this to be a clear hybrid, this was published as *I. pantii* var.*hybrida* . Now, on the basis of repeated observations and ascertaining about the permanency of all specific traits, we have renamed these plants as *I. narsinghgarensis* (Goswami & Mazumdar 2019).

Table 1: Common features in the genome of *Isoetes pantii* but rare among others species as recorded during 1966-2019

MORPHOLOGICAL relating other species with *Isoetes pantii*	**Typical features**	**Remarks**
1. Height and appearance of the plant	Plants always more than 18 cm, one corm smaller than other two	Many species in India (*Isoetes coromandelina, I.indica, I tuberculata, I.rajasthanesnsis* and others are large plants but - Plants of any species do not give "tilted " shape to the plant
2.Habitat : submerged or on amphibious habitat due to receding water in the waterbody	Not many plants grow on amphibious soil	Many species are found partly or totally submerged; do not grow on dried margins of the pond exactly like *I. sampathkumaranii* and *I. coromandelina*. But , *Isoetes narsinghgarensis* always found around drying margins.
3.Labium	Broad tongue shaped thick parenchymatous flap survives until maturity and mostly covers Ligule beneath its flap (**Fig 3 A)**	What appears on the adaxial face of the sporangium is the Labium not ligule ; this applies to ALL species of the genus worldwide. **(Fig 3)**
4.Ligule	Spongy, tongue shaped; gradually shrinking as the sporangium matures, finally dries out, but sometimes haustoira like structure and *dissolution* of endodermis like marginal cells of glossopodium are observed **(Fig.3 C , arrowed)**	Common shape of the ligule in many species; but no such structures or mechanisms to are known in any species
5.Megasporangia	Outer whorl sporangia are often round but inner whorls possess long elongated /oval sporangia; contain 500 to 800 megaspores	Basic principle same, in many species of the genus, but number of spores may depend on the size of the megasporangium; *Isoetes narsinghgarensis* plants have very small sporangia containing 12-20 spores
6.Megaspores in the megasporangium		
I.(Types, shapes and sizes)	Triradiate, round to triangular ; exine with round big and small tubercles three types, differing in size range, : Large (300 to 650 micron); medium (200- 320 mu) small (150 to 180 mu) . All megaspores have round tubercles	Similar situation in most species of *Isoetes* with round tubercles ; some may have only two, large and small megaspores *Isoetes pantii var jaideepii shows a major difference that one type of megaspores possess pointed or triangular tubercles, instead of round tubercles, a feature partly comparable to spores of Isoetes indica* Pant & Srivastava . *However in I indica, all three sizes of megaspores show pointed tubercles.*
II. Abortive , irregular & Joint megaspores	Very often observed in those plants whose root tip counts showed variations and meiosis showed laggards ; (Goswami, 1975a,b) Typical *Isoetes pantii* plants always show 5: 2: 1 ratio to display normal, joint, abortive spores; obviously involving 50% ratio among spore mother cells (see Text, Fig 4)	Irregular spores are recorded in many species in India; Joint spores seen in many other specis but not with a definite ratio as in *I. pantii* (see, Pant & Srivastava, 1962; Goswami & Arya, 1970; 2012) Joint spores are present but the exact ratio is not observed in the megasporangia of *Isoetes pantii* var *jaideepii* (collected from a pond in South Gujarat) (**Fig**. 4C)
7. Microsporangia	Often elongated but Round or oval , often in inner whorl; some large plants had shown microsporangium 2cm long	Even among larger plants such as *Isoetes coromandelina, I indica*, and others microsporangia are not as large as in *I. pantii; but in I pantii* var *jaideepii microsporangia* are small; thus indicating that size of the sporangia has to be proportionate to the size of the plant.
8. Microspores	Polymorphic microspores (round alete, bilateral monolete and small round trilete spores (22micron to 28 micron in diam) (Fig. 5 A-C	No species has polymorphic spores ; recently also recorded in *I.pantii* var *jaideepii* collected from South Gujarat (Fig. 1 A-D)

9. Scanning Electron microscopy of spores A. Megaspores	*I pantii* megaspores always have round tubercles on the both proximal and distal faces; large , medium and small size of spores only differ in the sizes and counts of tubercles in respective pyramidal areas (Goswami & Arya, 1970,2012; Goswami, 1975a)	Many species of the genus have typical round tubercles (eg. *I.coromandelina; I tuberculata* etc) pointed tubercles (eg. *I. indica*) or reticulate exine ornamentation (eg. *I. sampathkumaranii*) **but no species** possesses spores with different exine ornamentations as is a consistent feature in *I. narsinghgarensis* , *I. pantii var jaideepii,* and *I. fuchsii,* . (Goswami & Mazumdar, 2019) (Table 2)
B.Microspores		Microspores of many species viz. *I.indica, I.coromandelina, I. divyadarshanii* etc are spiny all around/or echinate (Srivastava, 1995; Shukla, et al,2017; Singh et al 2018) while *I.pantii* spores are (trimorphic) seen with sporopollenin grains (triradiate spores) /granulate ; plain or minor striations of exine layer on round and bilateral monolete microspores. (Figs 5 D)

Description of Isoetes narsinghgarensis

Plants attain height up to 10cm , rhizome bi-lobed; rarely 3 lobes; sporangia do not show velum; mega sporangia oval to round containing 47 & 233 megaspores in outer and middle sporangia respectively; dimorphic, tri-radiate, large megaspores 100-250µm in diameter, proximal face possesses 3-8 small round tubercles; on distal face exine typically reticulate or wavy; few spores may possess spiny outgrowth; small megaspores tri-radiate 50-95µm in diameter, both proximal face and distal face have small round tubercle like structures; each sporangium consists of 5 to 6% anucleate and or abortive deshaped spores; presence of both tubercles on proximal face and wavy, reticulate exine on the distal face of large spores along with presence of abortive spores as a regular feature decidedly indicates this to be a natural segregate; microsporangia have not been found so far.

General considerations

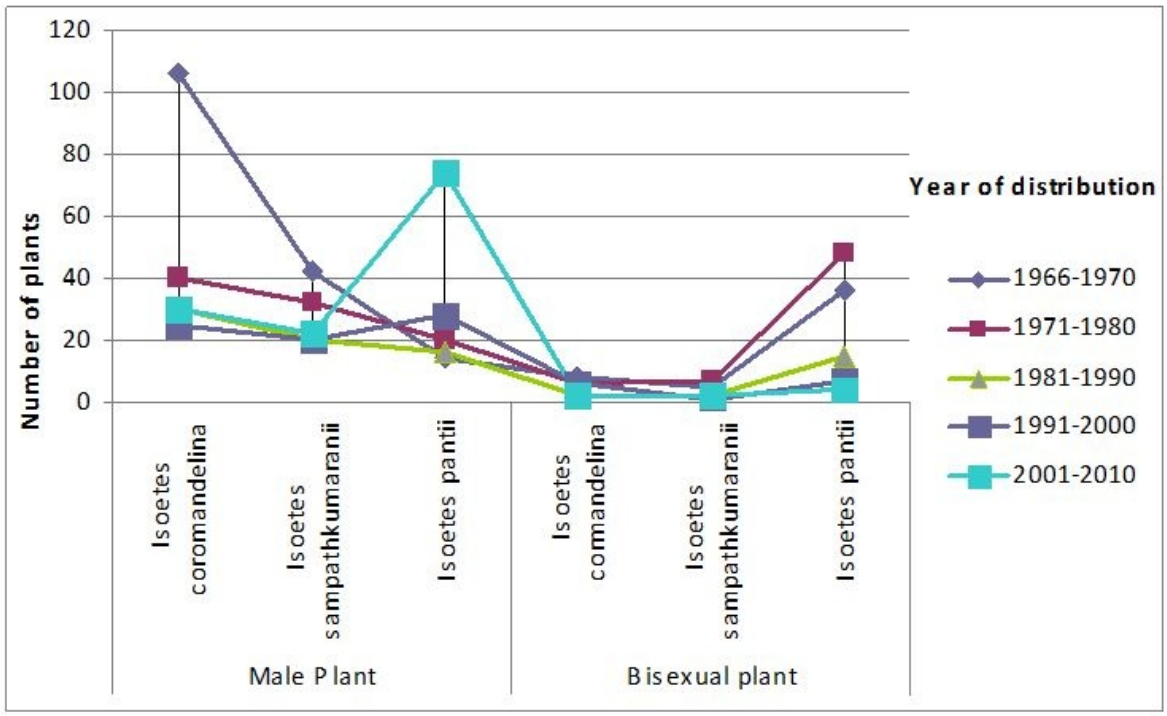
(I): *Rediscovery of ligule and labium association*

Despite the fact that Scott & Hill (1900) had clearly demonstrated the exact developmental stages of labium and ligule, and the hard fact that ligule develops below the labium, the presence of labium was ignored by almost all workers (including Pitot in 1959, see Hall 1971, Bhu 1992) did recognize the presence but called it as "pseudoligule". None of the textbooks and other notable publications (Bhambie 1963; (Bower,1935; Eames, 1936; Sporne, 1970) did realize the truth and always mentioned that what is apparently present at the adaxial face above the sporangium is ligule. Contradicting these all descriptions, Goswami (1976) pointed out that what we see above the sporangium on the adaxial face of the sporophyll is 'labium" and the ligule is just behind the labium. In other, words if we wish to see ligule then we will have to turn down the thicker flap (labium) and locate the spongy exactly tongue like ligule (Goswami 1976, 2005, 2010, Bhu 1992). We have also demonstrated that the ligule is secretory, nutritive as well as haustorial (Goswami 1976, 2005, Sharma and Bohra 2002,; Sharma and Purohit 2001) organ and also serves, to retain enough water in order to pass it on to the developing sporangium thereby offering nutritive support for the development of sporocytes, through network of tracheides. The base of the ligule, known as glossopodium even helps in flow of water and nutrients sometimes even by dissolving a few marginal, endodermis like cells (arrowed in Fig. 3C) probably to facilitate uninterrupted flow of solutes as well.

Labium, lying or covering the ligule on the adaxial face of the sporangium (see Figs 3 A-C) protects the ligule. (Goswami 1976 2005; Bhu 1992, Pant *et al.* 2000, Budke *et al.* 2005). Goswami did further discover that there exists interspecific variation regarding life span of ligule or labium. In other words, in some species (*I. pantii, I coromandelina* ;

Table 2: Extremely rare traits exclusively within the genome of *Isoetes pantii* Goswami & Arya (Never reported in any other species of the genus)

Traits	Comparative remarks
(A) Morphological	
1.*Permanent traits:*	Microsporangia examined from *Isoetes pantii* plants have always shown large megaspores; sometimes, a few megaspores have morphologically resembled fossil lycopod megaspores and above all these megaspores are fertile and germinate on original soil medium to produce gametophytes (Goswami & Arya,1968, Goswami & Goswami, 1986; Bhu & Goswami 1990; Goswami & Sharma, 1995, 1997; Goswami & Bhu, 1998; Goswami & Kumar, 2006Goswami 2001, 2014)
I.Heterosporangium [Microsporangia possessing mixed occurrence of fertile microspores and megaspores have been now designated as heterosporangia].	***Heterosporangium also present in Isoetes pantii var jaideepii*** **.**
II. Tilted rhizomorph due to one smaller lobe of the corm	(Goswami & Arya 1970, 2012); also present in *I.jaideepii*
1. *Extremely Rare Traits* **i.***in situ* germination of microspores and megaspores observed ; megagametophyte with archegonium photographed and described	***Not observed in I.pantii var jaideepii*** Observed in 1998 and 2004 (Goswami & Bhu 1998, Goswami 2004, see also Goswami 2014).
ii. Biflagellate antherozoids	Observed only once (Belajf in 1898; Goswami 2004a,b; Goswami & Kumar 2006)
iii -Megagametophyte produced on soil culture bore young embryo possessing long single celled rhizoid with pointed tubules	Observed only when fossil resembling megaspores from the heterosporangium were attempted for germinationGoswami & Sharma, 1997; Goswami, 2004,a,b)
iv. -Linear Tetrads	Spore mother cells sometimes have shown meiotically produced spores in linear fashion (Bhu & Goswami, 1992)
v Rare :-Sterile cells in the sporangia	Bhu & Goswami (1992) have described sterile cells with regular occurrence of microspores and megaspores in the sporangium
(B) Chromosomal Features	***Not yet successful in studying meiosis in I. pantii var jaideepii***
I 2n=36, 38-39; 48 chromosomes in root tips of many plants with B chromosome	All species in the world flora have chromosomes in multiples of n=11 (22,44, 55, 66, ------etc) hence as often expected triploid plants are often sterile. But the triploid plants of *Isoetes pantii* possess 2n=36 chromosomes, often show paired bivalents with one or two trivalents and univalents; some plants possess 2 or 3 extra chromosomes which are seen as laggards. The megaspores and microspores from the heterosporangium are fertile. We have species with 2n=60 chromosomes (Fig 2 A B)
II Presence of Sex chromosomes And B chromosomes	B chromosome is known in many species of *Isoetes* in India but more than two laggards caught attention; (Fig.2 B) Pairing behaviour of chromosomes and anaphasic movements of segregating chromosomes in *anaphase I* revealed a long submetacentric and one or two small chromosomes as laggards along with univalents, (Goswami 1975a,b); staining with Feulgen and C banding confirmed sex chromosomes (Goswami & Goswami, 1986; Bhu & Goswami, 1990) Presence of long submetacentric **X** chromosome and small triangular shaped **Y** chromosome have been repeatedly confirmed in followup studies (Goswami & Arya, 2012; Goswami, 2014, Verma, 2018**)**



	1966-1970	1971-1980	1981-1990	1991-2000	2001-2010
Isoetes coromandelina	106	40	30	25	30
Isoetes sampathkumaranii	42	32	20	20	22
Isoetes pantii	14	20	16	28	74
Isoetes cormandelina	8	6	2	6	2
Isoetes sampathkumaranii	5	7	2	1	2
Isoetes pantii	36	48	15	7	4

Figure 7: Decline in *Isoetes* plants among ponds in and around, Narsinghgarh (MP) India

most velumless species) the labium survives and the ligule shrivels down (even dried and detached) and in other species (eg. *I.sampathkumaranii, I.panchananii*; most species with velum) the labium dies out as and when the sporangium matures. In those species when ligule shrivels down, the bean shaped glossopodium appears lonely mass of tissue with traces of ligular detachment (Goswami, 1976, 2010). Since the mature sporangium may have only one structure on the adaxial face above the sporangium, most workers have had ignored and thought that whatever present on the face must be ligule. Both structures can be visualised only when sporangium is at the mid-phase of maturity (Fig. 3C).

(ii) *Chromosome evolution:*

Population cytogenetic studies have shown the evolution of new basic chromosome number ie, n=12 and we have discovered plants with 2n 36 and 48 chromosomes; 2n=48 with X, Y and B chromosomes appears to be a stable chromosome number (Goswami 1975a,b; 1996, 2010, 2011; Bhu and Goswami, 1990; Goswami and Goswami, 1986; Liu *et al.* 2004, Goswami and Arya 2012, Verma 2018). Additionally, new species with 2n=60 + 1 or 2 B chromosomes have also been described (Goswami and Mazumdar 2019) constituting "panti complex" as these species, viz. *Isoetes narsinghgarensis* Bhu *et al.* and *Isoetes*

fuchsii Goswami & Mazumdar, also possess partial resemblance with *Isoetes pantii* Goswami & Arya . This is remarkable that world over all species which have been chromosomally investigated, either from India or from any other part of the globe, except *Isoetes hystrix* (which has n=10; 2n=20 chromosomes) possess chromosomes based on X=11/n=11 (Abraham and Ninan, 1958; Ninan, 1958; Hickey, 1984; Troia 2001, Troia *et al.* 2016; Goswami and Bhu, 2000; Kim et al 2010a, b; Goswami, 2011; Zican *et al.* 2004; Singh *et al.* 2018; Shukla *et al.* 2017. Apart from this, there is no species in the world flora among pteridophytes as a whole to possess sex chromosomal mechanism.

(iii) ***Heterosporangium:***

Origin and evolution of heterospory has been the greatest testimony to the ongoing evolutionary strategies in the plant kingdom. We are aware of the fact that the endless origin and diversity of the flora over the geological period has been mainly on account of the evolutionary phases inherently operative in different ecosystems (Bateman and Di Michele 1994; Seward, 1933; Pigg 1992, 2001, Kar and Dilcher, 2002)

Since *Isoetes* is a bisexual genus, plants of most species often show development of microsporangia (male sporangia) and megasporangia (female sporangia) which release microspores and megaspores respectively and do germinate to produce respective gametophytes. As well established for more than a hundred years, species are distinguished form one another by scoring differences on various morphological parameters mainly depending also on exine ornamentations of microspores and megaspores. The microsporangia of *Isoetes pantii* plants have had presented extremely rare features ever since we first described them (Goswami and Arya, 1968, 1970, 2012) and which are the established diagnostic features of the species, these microsporangia have been designated as heterosporangia (Goswami, 2014). Brief description would be essential hereunder.

Three kinds of microspores (alete, monolete and trilete) developing inside one and the same microsporangium were reported in 1968 (Goswami and Arya, 1968) a feature which is a regular inherent feature within the genome of *Isoetes pantii* (Fig. A- D). For the first time in the botanical literature the microspores have been found to germinate within the heterosporangia (microsporangia) to produce microgametophytes and developmental stages have been clearly photographed and published (Goswami 2004). The heterosporangium also possesses large trilete megaspores which have reticulate exine (not tuberculate, as are the megaspores in the megasporangium of the same plant). Furthermore, as an extremely rare event, we have seen these megaspores germinating into female gametophyte, also *in situ* (within the heterosporangium); archegonium has been seen (Goswami and Bhu 1998; Goswami 2004,2014) photographed and published for the first time. As far as we know, we have conceived the archegonium only through excellent diagrams of earlier pteridologists like La Motte, (1933,1937; cited from Smith 1955, Goswami and Bhu, 1998; Goswami 2004, 2014). Some of these megaspores possess abnormal or very large perispore flanges within which fine tubules and or spines extend to provide variable shapes and sizes. These abnormal spores are directly comparable to spores of fossil lycopds (Goswami, 1975; Goswami and Goswami, 1986; Goswami and Sharma, 1995, 1997; Goswami and Bhu, 1998, 2000; Goswami, 2004b). Both apparently normal, as well as megaspores resembling fossil lycopods germinate within the hetersporangium and megagametophyte shows extended archegonium coming out of the gametophytic tissue (exoscopic; Goswami 2004).

(iv) ***Some extremely rare features***

Many other extremely rare features hither to

unknown, (Goswami and Kumar, 2006) include: linear tetrads (Bhu and Goswami, 1992). Sperms observed to be released from the male gametophyte in the heterosporous sporangia of *I. pantii* were multiflagellate but in longitudinal sections of such sporangia the sperms were also seen biflagellate (Goswami 2004) with bifid tips. Also, emergence of some gametophytic tissue beyond the confines of the spore wall (breaking the distinction between endosporic and ectosporic gametophytes; formation of central vacuole in the female gametophyte) were unusual embryological features.

DISCUSSION

Species diagnosis

Pfeiffer (1922) laid the foundation of species diagnosis on the basis of ornamentation of outer coat of the megaspores (exine ornamentation). Accordingly, now we know five categories of spore ornamentation,Viz. Tuberculatae, Cristateae, Echinatae, Reticulatae and Laevis. In most of the species microsporangia are not commonly found but each and every fertile *Isoetes* plant has a megasporangium hence megaspore morphology including colour shape and size, tritadiate mark and nature of pyramidal areas on proximal face etc have been most reliable and widely used diagnostic features for species differentiation (Pant and Srivastava 1962, Goswami and Arya 1970, Goswami 2010,2014, Srivastava *et al.* 1993; Small and Hickey, 2001; Shukla *et al.* 2005). In the entire *Isoetes* flora, *I. pantii* Goswami & Arya has been the only species, as far as known , which has consistently shown, unique features.

Polymorphism of megaspores

The development of two or three kinds of megaspores within one and the same megasporangium of *Isoetes* has been known for more than a century and has been well published from various parts of the world (Pfeiffer 1922, Pant and Srivastava 1962, Goswami and Arya 1968, 1970, 2012, Hall, 1971, Marsden 1976, Goswami and Arya, 1968, Jermy1991, Goswami 1996, Kim *et al.* 2008, 2010a,b; Musselman *et al.* 1995, 1996, 1997, Takamiya 1999; Shukla *et al.* 2005; Singh *et al,.*2018).There has been uncertainty regarding explanation of the prevalence of dimorphism and polymorphism of megaspores, sometimes even blaming chromosomal nondisjunction during meiosis, a feature quite prevalent among many species of *Isoetes* in India (Abraham and Ninan, 1958; Verma 1961, Pant and Srivastava 1965, Goswami, 1975). Marsden (1976) had even opined that polyploidy and apomixis may be the possible causes of polymorphism of megaspores in *I. muelleri* A. Br.

None of the explanations offered so far have been exactly accurate but the perfect explanation for the production of two or three kinds of megaspores have been guided by the discovery of strict heritable variation of joint and free megaspores in the ratio of 5:2:1 (free, joint and abnormal spores) in the megasporangia of *Isoetes pantii* (Goswami and Arya 1970, 2012; Bhu and Goswami,1998, 2001, Goswami !975, 1996, 2010, 2014). This has been ascertained that spore mother cells have different genetic make up (not all spore mother cells have exactly same genes so that meiotic products: the spore could be identical with regard to size and exine ornamentation) This is best proved by following developmental evidences:

(i) First evidence is by the consistent inheritance of fixed ratio of free, joint and abnormal spores in the ratio of 5:2:1 (indicating that one spore mother cell produces all four normal megaspore while the other megasporemother cell produces one joint (2 spores) one normal and one abnormal/abortive spore (Fig.4 A–D). Several thousand spores from more than 100 megasporangia of *Isoetes pantii* have been examined during past 50 years and the joining of spores has been

validated as a heritable trait.

(ii) The second evidence comes from megaspores developing inside the megasporangia of hybrid species, viz. *I fuchsii, I narsinghgarensis* (Goswami and Goswami, 1986, Goswami and Bhu 1998, 2000; Bhu *et al.* 2000) which show both tuberculate as well as different expressions of exine ornamentations.

(iii) The most important evidence is offered by the megaspores developing inside the (microsporangium) heterosporangium of *Isoetes pantii* ; these megaspores always show reticulate and webbed exine but never tubercles as expected because megaspores inside the megasporangium of the same plant are tuberculate. These megaspores with reticulate exine have obviously expressed genes of *I. sampahtkumarini*, one of the putative parent (Goswami and Arya, 1968; Goswami and Bhu 1998,2000; Bhu *et al.* 2001, Goswami & 2011, 2014).

(iv) Another evidence that spore mother cells may differ in genetic make up from among crowd of megasporemother cells within the same sporangium comes from a rare phenomenon of " linear tetrads" sometimes encountered within the heterosporangia of *I. pantii* (Bhu and Goswami, 1992; Goswami and Kumar 2006; Goswami 2001c; Goswami, 2010).

Cradle of heterospory: :the Heterosporangium

This has been hypothesized on the basis of consistent observations that the heterospory to begin with, might have originated within a sporangium and both microsporogenesis as well as megasporogenesis must have exclusively dependent on genetic make up of spore mother cells so as to produce different microspores and megaspores (Fig. 5 A-D) and even, onset of respective divisions also might (Fig 5A) differ. Quite likely, thereafter, separate sporangia should have evolved in due course of evolution (Goswami and Goswami, 1986; Goswami, 2014). This new category of a sporangium, is hereby named as "Heterosporangium", conceived to have been the cradle sporangium for the origin of heterospory in most of the cases. Such a sporangium, may have possessed three types of spores, viz. microspores, megaspores and morphologically distinct spores possessing resemblances with spores of past lineages/ fossils. Such a classic structure has been exemplified by a fundamental discovery of " Intasporangial heterospory" in 1968 by Goswami and Arya, in the living taxon, *Isoetes pantii*.. This appears that this evolutionary phase should have followed the path of sequential evolution of *Isosprangium –anisosporangium –heterosporangium* and finally, leading to Intersporangial heterospory in the form of independent *microsporangium* and *megasporangium (*Goswami 2014). Then on, independent sex differentiated sporangia must have had evolved by selective degeneration (Bell, 1996; Goswami, 2014) of spore mother cells in a differentiating young heterosporangium. In other words, a microsporangium should have evolved by the degeneration of megasporemother cells and megasporangium by early degeneration of microspore mother cells. This selective degeneration must have been operative on account of some genetic mechanism as suggested by Bell in 1996 (Goswami, 2001a, b, 2011,2012) and later, demonstrated by our observations (Goswami and Lee, 2001, Goswami 2014) on epigenetic mechanisms suspecting mainly abrupt hypomethylation of DNA, to be responsible event as indicated in heterosporangia of *Isoetes pantii.* Origin of heterospory is one of the most significant landmark (Seward 1933, Bateman and DiMichele 1994, Kar and Dilcher 2002; Pig, 1992, 2001, Goswami, 2014) in the evolutionary history of plant kingdom.

Phylogenetic considerations

Comparative profile to exhibit differences in lineage among some species of *Isoetes* related to *Isoetes pantii* on the basis of sequences of chloroplast rbcL (chloroplast) genes is

presented in Fig 6. This attempt reveals three major points; One that *I.coromandelina* appears to be far distinct despite the fact identical plant size or round tubercles are recorded in many species; and second and important information is that *I pantii* and the closely related species, which possess hybrid features of exine ornamentation of megaspores and microspores, and also possess a different path of chromosome evolution are also possess individual identity on the basis of rbcL assay. Last and most significant information is that *I. pantii* collected from Narsinghgarh and now also obtained from Gujarat are similar yet show differences. The fundamental resemblance is the possession of "heterospsorangium" (heterosporous microsporangium; Goswami 2014) meaning thereby that production of heterosporangium is genetically inherent in the species (See Figs. 1 & 6). Molecular genetic studies are obviously gaining wider recognition to resolve such problems (Bagella *et al.* 2011; Rydin, and Wikstrom. 2002 ; Kim *et al.* 2010 a,b; Goswami and Patel 2019).

Important Concluding Remarks

Repeated observations on development of gametophytes (microgametophytes and megagametophytes developing within the heterosporangia (germination of spores to produce gametophytes *in situ* is not known in the genus; see Goswami 2004 for detailed description and figures) not only confirm that the species *Isoetes pantii* is a fertile hybrid but also suggest that the evolution of heterosporangia offers definite pathway for the origin of heterospory (Goswami, 2014). Some other important observations affirm this taxon being a unique in the plant kingdom. These are :

(1) *I. coromandelina* plants (2n=22+1) and *I. sampathkumaranii* plants (2n=66+1) possess expected chromosomes in respective plants but plant populations of *Isoetes pantii* show mixture of plants with chromosome variables (possessing 2n= 24, 36, 38-39,or 48 chromosomes) within the same pond. Meiotic studies have shown irregular segregation, too many fragments of chromosomes, perfectly moving bivalents, and also laggards;

(2) Plants with 2n=36-39 chromosomes have consistently shown 8 -12 megaspores exhibiting unusual morphology inside the heterosporangia which have been compared with many fossil lycopods (Goswami, 1975a, Goswami and Goswami, 1986; Bhu and Goswami 1990);

(3) In some plants with 2n=36 chromosomes almost regular meiotic segregations have been observed; obviously a triploid plant has more or less identical loci on chromosomes which can facilitate partial pairing of homologues.

(4) Perfectly mature and fertile plants of *Isoetes pantii* possess 2n=48 chromosomes with perfect demonstration of C bands verifying the presence of X, Y and B chromosomes. In meiosis, unpaired movements and irregular segregation of sex and B chromosomes confirm their classical nature (Goswami, 1975b, 2011, 2014; Bhu and Goswami 1990) ;

(4) Plants at a distant locality (Maksoodangarh, District Guna) were found to possess 2n=60 chromosomes and were named as *Isoetes fuchsii* Bhu *et al.* 2001). Another segregate (small plants measuring 4-8 cms) found in the peripheral areas of Narsinghgarh (Central India) ponds, recently named as *Isoetes narsinghgarensis* Goswami & Mazumdar also possesses 2n=60 chromosomes. All the above categories of plants possess basic homologies of tuberculate megaspore exine-ornamentation but yet, show alarming differences in some morphological details. For example, megasporangia in *I. narsinghgarensis* possess megaspore showing both tuberculate and reticulate exine ornamentation obviously showing expression of genes from *I coromandelina* (tuberculate) and *I sampathkumaranii* (reticulate) respectively.

(5) This paper opines that natural

hybridization wherein each putative parent might have contributed one B chromosome along with expected gametic number of chromosomes and also is accompanied with chromosomal aberrations (particularly fission) might have actually triggered genomic reshuffle to evolve with new basic chromosome number (n=12 / X=12) . Obviously, apparent genomic reshuffle (Bajpai *et al.* 2004) has resulted in expression of highly unusual traits of great evolutionary significance. This is becoming almost essential to attempt for molecular genetic studies to (Bajpai *et al.* 2004, Bagella *et al.* 2011; Troia2001, Rydin and Wikstrom 2002, Leitch *et al.* 2005, Troia & Greuter 2015, Troia 2016) decipher interrelationships among species and also try to understand underlying genetic mechanisms. Simple phenotypic resemblances or differences can not be held as final verdicts (Goswami 2001a,b,c; Goswami and Lee 2001; Goswami and Bajpai 200) as so clear;ly evident by rbcL chloroplast gene assay (Fig.6). Most larger plants often measuring 16- 20 cm resemble *I coromandelina* but the most widely distributed *I coromandelina* is far away in the phylogenetic tree. Infact this species should be considered as a "parent" species for a variety of larger *Isoetes* plants which possess tubeculate megaspores.

(6) Conservation : Fig 7 with attached table has indicated the gradual loss of the bisexual plants of Isoetes in original ponds. Despite my best efforts I could not prevent agricultural and public usage of these ponds in and around Narsinghgarh (MP: India) by making all round approaches with important public men and forest personnel during 1978-2000. Being aware of increasing public load on these waterbodies and encroachment of domestic animals I have been fearing since the beginning in late 1970s that this evolutionary genuine taxon will be lost or reduced to a very limited distribution. I am still trying to conserve helplessly, though !!.

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