**Isoetes Pantii Complex is Evolving with New Basic Chromosome Number**

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**Abstract**

A new species, *Isoetes pantii* HK Goswami and BS Arya was described more than fifty years ago from Narsinghgarh in Central India. Many of the rare features of this species, like heterosporous microsporangia (now known as heterosporangia), three types of microspores (alete, monolette, trilete) and several plants possessing 2n=48 chromosomes (basic n=12) have been repeatedly confirmed since then. Continued search for *Isoetes* plants from several localities further yielded two new species. Plants of both these species resembled *I. pantii* in having round tuberculation megaspores but exhibited n=12 series and showed 2n=60 chromosomes in roots. However both these species have exhibited extremely rare features. *I. fuchsii* sp Nov possessed 25% megaspores with spines on the proximal face in only one pyramidal area, a feature never observed so far. Plants of another species published earlier as *I. pantii var. hybridra* (hereunder being revised and named as *Isoetes narsinghgarhensis* sp Nov) were very small (less than 10cms) and exhibited a very strange mixed/hybrid trait on exine ornamentation of megaspores; megaspores possess both reticulate exine as well as clearly demarcated round tubercles. Such mixed exine ornamentation has never been described in the botanical literature. Since both these species were not properly published we wish to describe them again and confirm the validity of *I. fuchsii* and also propose a new name *Isoetes narsinghgarhensis* sp Nov for *I. pantii var. hybridra*. We also assert that the *Isoetes pantii* complex is essentially characterized by round tuberculation megaspores but with chromosome counts based on n=12 chromosome series. As far as known, no species of *Isoetes* in the world flora possess multiples of n=12 chromosomes; infact all other species possess n=11 chromosome series. While all essential evidences already published, *Isoetes pantii* is a confirmed sexual species (male and female gametophytes and developing sporophytes have been well described earlier) we have so far not found any sexual stage either in *I. fuchsii* or in newly named *I. narsinghgarhensis* sp Nov hence we presume these plants to be sterile hybrid segregates constituting *I. pantii* complex.

**Keywords:** Cytology; India; Isoetaceae; *Isoetes pantii* Complex; New Species

**Introduction**

The genus *Isoetes* Linnaeus (1753: 1100; Linneus filius C.) [1,2] (Isoetaceae) is represented by about 250 species in the World (PPG I 2016). [3]. In a recent revision Fraser-Jenkins, et al. [4] published that only four species occur in India. Certainly such a statement is oversimplification of the fact and as such cannot be accepted. This treatment has not been accepted by many workers; however denial of genuine facts ignoring excellently published papers. In the meantime, Troia, et al. [5] observed that the taxonomy of *Isoetes* in India is unclear. The hard fact on the contrary is that there are many more species validly reported from India [6-10] established on the basis of spore morphology (as strongly confirmed by Turland, et al., Art. 39.1; Turland, et al. Art. 40.1) and chromosome numbers [11]. The criticism of recognition of *Isoetes pantii* HK Goswami and BS Arya in particular was once advocated by those workers who do not give any cognizance to chromosome constitutional variation (position of centromere on the chromosomes) and chromosome number. *I. pantii* has been shown to possess sex chromosomal variation in meiosis and mitotic studies observed since early days of 1970 and excellent demonstration by shape, size, meiotic behaviour and molecular genetic studies have been well documented [7,12-17]. This has been proved by repeated studies since then that *I. pantii* has evolved with basic chromosomes n=12 and several variations have been described with stable count of 2n=48 chromosomes among many plants [15,18,19]. Another relevant feature with great morphological impact worth emphasizing regarding *Isoetes pantii* Goswami & Arya (1970: 30) [20] (Figure 1A), has been the development of three kinds of microspores (alete, monolette and trilete) in the microsporangia along with highly unusual large megaspores inside within the same microsporangia [15,18-21]. These all features have been repeatedly observed since 1966 onwards and are excellent acquisition within the genome of *Isoetes pantii*. No species in
the world flora of the genus *Isoetes* has ever shown a combination of these traits which decidedly indicate strong genetic divergence from other species. Elsewhere, molecular genetic studies have been published to opine as to why very unusual megaspores resembling fossil lycopod megaspores develop inside the microsporangium of *I. pantii* and what mechanism might be responsible for triggering female “genes” inside the male sporangium (?). This switching on mechanism might be due to one of the putative parental genes (*I. sampathkumaranii* LN Rao) becoming active because megaspores inside the microsporangia possess reticulate exine while megaspores inside the mega sporangium are round tuberculation. These large megaspores are fertile and produce female gametophyte [15,18,22-26]. The presence of sex chromosomes reported in *I. pantii* [7,13,15,16,26-29] have been well cited thereby establishing an altogether different path of chromosomal evolution (Slides of chromosome preparations; spore morphologies and microtome sections along with plant materials were shared with Late Professors DD Pant, Fuchs-Eckert during their visits to Bhopal and excellent photographs have been published over these years). Follow-up cytogenetic studies on *Isoetes* populations have had further yielded two new species. Plants of both these species resemble *I. pantii* in having round tuberculation megaspores, possess (n=12 series) 2n=60 chromosomes in roots and have also exhibited extremely rare features. *I. fuchsii* sp Nov possessed 25% megaspores with spines on the proximal face a feature never observed so far. Plants of another species described earlier as *I. pantii var hybridia* (hereunder being revised and named as *Isoetes narsinghgarhensis* sp Nov) were very small (less than 10cms) and exhibited a very strange mixed/hybrid trait on exine ornamentation of megaspores; megaspores possess both reticulate exine as well as clearly demarcated round tubercles. Since both these species were not properly published earlier, we wish to describe them again and confirm the validity of *I. fuchsii* and also propose a new name *Isoetes narsinghgarhensis* sp Nov for *I. pantii var hybridia*. We also assert that the *Isoetes pantii* complex is essentially characterized by round tuberculation megaspores but with chromosome counts based on n=12 chromosome series. All other species of the genus possess n=11 chromosome series. While all essential evidences already published, *Isoetes pantii* is a confirmed sexual species (male and female gametophytes and developing sporophytes have been well described earlier) we have so far not found any sexual stage either in *I. fuchsii* or in newly named *I. narsinghgarhensis* sp Nov hence we presume these plants to be sterile hybrid segregates constituting *I pantii* complex.

### Materials and Methods

While the major localities and ponds for collections were at Narsinghgarh (MP) India, extensive collections (since 1966 onwards) and population based comparative morphological, anatomical, biochemical, chromosomal, and molecular genetic studies have been carried out (Table 1) (Figure 1, 2) from various parts of India and series of papers have been published (some of them among present references) dealing with many species Viz. *Isoetes coromandelina, I pantii, I indica, I. sampathkumaranii, I. dixitei, I. panchananii,* and a few more which are under repeated scrutiny [7,8]. All related methodologies have already been published. Briefly, plants were collected from respective localities and young roots were put in 8-hydroxyquinolene for four hours and then transferred to 45% acetic acid. After a few hours roots were squashed and stained with 1.5% acetic-orcein and immediately examined under a light microscope attached with camera. Good and well stained metaphases were photographed.

<table>
<thead>
<tr>
<th>Features</th>
<th><em>I. pantii</em></th>
<th><em>I. fuchsii</em></th>
<th><em>I. narsinghgarhensis</em></th>
<th><em>I. coromandelina</em></th>
<th><em>I. sampathkumaranii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizome</td>
<td>3 lobed, one lobe always smaller than the other two</td>
<td>3-4 lobed</td>
<td>2 lobed</td>
<td>3-4 lobed</td>
<td>2 lobed</td>
</tr>
<tr>
<td>Velum</td>
<td>Young sporangia may have small velum, but absent on maturity</td>
<td>absent</td>
<td>covering ¼ of sporangium</td>
<td>absent</td>
<td>covering ½ of sporangium</td>
</tr>
<tr>
<td>Megaspore</td>
<td>trimorphic</td>
<td>trimorphic</td>
<td>dimorphic</td>
<td>dimorphic</td>
<td>dimorphic</td>
</tr>
<tr>
<td>Megaspore exine ornamentation</td>
<td>tuberulate</td>
<td>tuberculate</td>
<td>Mixed tuberculate -- Reticulate (Figure 1F)</td>
<td>tuberculate</td>
<td>reticulate</td>
</tr>
<tr>
<td>Spine in megaspore</td>
<td>absent</td>
<td>Present on proximal face at one pyramidal area (Figure 1E)</td>
<td>Rarely, small spine present on abortive spores (Figure 1G)</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Chromosomes</td>
<td>2n= 24, 36, 48 (including sex chromosomes and two B chromosomes)</td>
<td>2n=60 with 1 or 2 B chromosomes</td>
<td>2n=60 with 1 or 2 B chromosomes</td>
<td>2n=33, 44 + 1B</td>
<td>2n=66 + 1 B</td>
</tr>
</tbody>
</table>

Table 1: Comparative morphology of *I. pantii* complex with related species
Figure 1: (A) Habit of plants: *Isoetes fuchsii* (left), *I. narsinghgharensis* (middle), *I. pantii* (right); (B, C) Megaspores of *I. fuchsii* from mega sporangium stained with safranin showing joint spores with small spiny outgrowths all around periphery. Some spores also exhibit large spine emerging from the round tubercle; (D) A megaspore of *I. fuchsii* showing spine emerging from feebly developed tubercle in one pyramidal area; (E) Plants of *I. narsinghgharensis*; (F) Megaspores of *I. narsinghgharensis* from mega sporangium showing mixed occurrence of reticulate exine with sporadic round tubercles, a combination never so far known on any exine ornamentation; (G) An ill developed young megaspore of *I. narsinghgharensis* with prominent spine under SEM.

Figure 2: Mitotic chromosomes of *Isoetes fuchsii* 2n=60; the lower black arrow indicates an acrocentric chromosome in association with a chromosome (telocentric one) and the upper one solid black arrow is in the vicinity of a B chromosome (detailed significance with differential staining is still unpublished).
The inherent intention of this paper is to recognize the taxonomic status of *I. pantii* complex suspected earlier [12,13] and recognize proposed these two species, *Isoetes x fuchsii* I Bhu, HK Goswami, US Sharma and AK Bajpai, sp. Nov and *I. narsinghgarhensis* H K Goswami and J Mazumdar, sp Nov as the major constituent.

**Observations and Comments**

**Taxonomic account**

*Isoetes pantii*: HK Goswami and BS Arya (1971: 30) (Figure 1A)

**Type:** INDIA. Madhya Pradesh: Rajigarh, Narsinghgarh, HK Goswami GA: IA (holotype BM!; isotypes DD!, Herbarium of Botany Department Allahabad University, Allahabad! Herbarium of Botany Department Government College, Narsinghgarh! Herbarium of Botany Department Govt. Science College, Gwalior!; plants and slides showing mixed occurrence of micro and megaspores have also been deposited at Kew and BME, England.

**Description:** Plants 24-60cm (Figure 1A); details of diagnosis of the species have already been described [7,14,19-21]. Pertinent points worth mentioning are that plants possess 2n=48 chromosomes including sex chromosomes and 02 B (dot) chromosomes [7,12-14,18,19] are fertile and possess hybrid features of exine ornamentation on megaspores. Peculiarly, megaspores developing inside heterosporangia [21,26] show reticulate exine like megaspores of *I. sampathkumaranii* and megaspores in megasporangia are round tuberculate resembling *I. coromandelina*. Since both *I. coromandelina* and *I. sampathkumaranii* grow intermixed in the ponds at Narsinghgarh (MP, India) several morphological, biochemical and molecular genetic studies on genomic DNA procedures including Southern blot experiments on many related species were conducted. The hypothesis that both the above species should have crossed in nature [13,23,30,31] and the variant plants in ponds being observed since 1966 and thereafter, might be actually natural segregates of the natural hybridization. Furthermore, as germination of megaspores to produce female gametophytes and young embryos have already been published earlier (23, 24,25, 26) we strongly believe that *I. pantii* is a fertile hybrid.

**Etymology:** Named in honour of renowned Indian botanist Prof. Divya Darshan Pant (1919-2001).

**Chromosome number:** Root tip mitosis shows 2n=48 including sex chromosomes and two B chromosomes; A few plants with 2n=36 including B-chromosome (1-2). These plants also show typical features of *I. pantii*.

**Ecology:** Plants are submerged in water body.

**Fertility:** August - September–October.

**Distribution:** So far only known from India (Narsinghgarh and Rampura in Madhya Pradesh).

*Isoetes x fuchsii*

*Isoetes x fuchsii* I Bhu, HK Goswami, US Sharma and AK Bajpai, sp Nov (Figure 1A).

**Isoetes x fuchsii**

*Isoetes x fuchsii* I Bhu, HK Goswami, US Sharma and AK Bajpai, sp Nov (Figure 1A).

**Type:** INDIA. Madhya Pradesh: Bhu, Maksoodangarh, H.K. Goswami HKG-2000 (holotype Herbarium of Society of Botanists, Bhopal!; Isotypes K!, Herbarium of Bioscience Department (pteridophyte section) South Gujarat University, Surat!).

**Description:** Plants measure 20-30cm (Figure 1A), rizomorph 3 or 4 lobed; leaves measure 20-26cm, almost of equal in length; velum absent; megaspores with round tubercles; mega sporangia many, containing about 400 to 490 megaspores; megaspores trimorphic with round tubercles, large megaspores white when dry, ash coloured when wet, diameter range from 350-400µm, 374µm (average of 50 megaspores), globose to elongated in shape; tri-radiate ridge often straight, rarely sinuous; proximal face with 3-4 round tubercles, distal face many small size tubercles; medium size spores, tubercles measure 200 to 300µm in diameter; proximal face with 1-3 uneven tubercles, distal face possesses 20 or more tubercles (Figure 1B and C) about 20% spores show unusually significant spine emerging from the pyramidal area (Figure 1E) small megaspores not many in number even less than 5% among spores measure less than 100µm in diameter; proximal face with one large tubercle having a nipple like protuberance and the corresponding small spores may show depression/ pit in the middle of tubercle [32]; microsporangia not present in all plants; microspores dimorphic, only monolet and trilet 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 (Figure 2).

**Etymology:** This new species is 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 (Figure 2); 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.
 Isoetes narsinghgarhensis

**Description:** Plants attain height up to 10cm (Figure 1A, D), 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 (Figure 1G); 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 (Figure 1F) decidedly indicates this to be a natural segregate; microsporangia have not been found so far.

**Type:** INDIA. Madhya Pradesh: Rajgarh, Narsinghgarh, H.K. Goswami HKG/ IS-2004 (holotype Herbarium of Society of Bionaturalists, Bhopal; isotypes Herbarium of Bioscience Department (pteridophyte section), South Gujrat University, Surat!, Bapalal Vaidya Botanical Research Centre, Surat!; Isotypes K!).

**Isoetes pantii:** Goswami and Arya (1971: 30) var *hybrida* Goswami (2004: 152) nom. inval.

**Isoetes narsinghgarhensis** HK Goswami and Mazumdar sp Nov (Figure 1A,D,F and G)

**Diagnosis:** Unique combination of features: bi-lobed rhizome and tuberculation and reticulate megaspores in same mega sporangium (Figure 1F) distinguish it from other species of *I. pantii* complex.

**Etymology:** This species is named after the locality Narsinghgarh (Madhya Pradesh, India) where it was first discovered. Earlier epithet "hybrida" was derived from the belief that this new taxon was a natural hybrid between *I. coromandelina* and *I. sampathkumaranii* Rao (1944: 287)[33].

**Distribution:** So far only known from in India (Narsinghgarh and Bhopal in Madhya Pradesh).

**Ecology:** Plants grow as amphibious in periphery of water body.

**Fertility:** October –November- December

**Discussion**

**Taxonomic relevance**

Unfortunately *I. fuchsii* was published without a latin diagnosis [11] and *I. pantii* var *hybrida* without a holotype citation [11] and thus invalid as pointed out by Fraser-Jenkins, et al. [4]. These names are republished here for inclusion in the World flora of *Isoetes*, along with synopsis of *I. pantii* complex. So also the publication of *I. pantii* var. *Hybrida* (Goswami, 2004) [8] was pointed out to be invalid as per taxonomic practices. Hence we republish these two species along with a brief background of evolutionary relevance.

*I x fuchsii* Bhu, et al.

A new species named after Professor Fuchs-Eckert was published [32] which showed very peculiar features of possessing nearly 20-25% megaspores showing large spines and root tips showing 2n=60 chromosomes, corresponding to the chromosomal path of evolution based on n=12 chromosomes. Since the latin diagnosis was not added, which was essential at that time of publication, the validity of species was obviously questioned. We therefore revise and offer this text so as to properly validate *I. fuchsii* Bhu, et al. As already mentioned plants have been deposited at various herbaria. This species is a natural sterile hybrid

**Isoetes x narsinghgarhensis** Goswami and Mazumdar

Ponds in Narsinghgarh (pachtalai pond in particular) and in adjoining villages plant collections had initially identified three species; *I. coromandelina, I sampathkumaranii* and *I. pantii* as well as certain variants [12,18]. Our follow-up studies also 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 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* [8]. Now, on the basis of repeated observations and ascertaining about the permanency of all specific traits, we have renamed these plants as *I. narsinghgarhensis* (Figure 1D and F).
Evolutionary significance of “pantii complex”

Since August 1966 more than a dozen papers on morphological, anatomical, population cytogenetic screening and genomic assays have been published [6,11-13,19,20,24,27]. The discovery of *I. pantii* has lead to establish a new series of chromosomal evolution [15-17,34] and three taxa with basic chromosome number n=12, have been observed to constitute the assemblage of three species *I. pantii* (2n=48), *I. fuchsi* (2n=60) and *I. narsinghgarhensis* with 2n=60 chromosomes. Additionally, these species have genes for exine ornamentation from *I. coromandelina* as well as from *I. sampathkumaranii*; megaspores developing inside the microsporangia of *I. pantii* possess reticulate and webbed exine (like *sampathkumaranii*) while the megaspores inside the mega sporangia of the same plant possess round tubercles (like, *coromandelina*). This is relevant to record here that *I. narsinghgarhensis* megaspores possess mixed occurrence of reticulate exine and round tubercles on the distal as well as on proximal face; which is an extremely rare morphological combination of exine ornamentation.

Needless to mention, these species can not be considered to be cytotypes because each one has distinct features of taxonomic importance. These species appear to have evolved on a chromosomal path totally different and have assemblage of such DNA sequences which are endowed with rare sequences comparable to many other organisms (details are out of present context; see Goswami & Chandorkar, Goswami & Bhu, Bajpai & Goswami [22,24,3126]). Obviously, then “panti complex” members have got to have chromosomes based on, n =12 series.

Summary

Besides observing and ascertaining a new series of chromosomal evolution based on n=12 [15-17], we have also encountered evolution of new traits over these years. Prominent among these are, large spines on the proximal face of some megaspores (Figure 1) on one pyramidal area of *I. fuchsii* (Figure 1B and C) and mixed occurrence of reticulate exine as well as tubercles on the distal and proximal faces of *I. narsinghgarhensis* (Figure ID and F).

Conservation status

Ever increasing human interference on lakes and ponds and invasion of weeds like *Ipomea, Lantana camara* etc on marshy area around water bodies are the commonest incidences noticed at all places thereby reducing the populations of these small quillworts [25]. Furthermore, villagers have started ploughing on these sites therefore survival of flora particularly growing on catchment marshy areas are getting terribly reduced. Until some institutional conservation strategies are not opted, it would be difficult to conserve these evolutionary important species [26]. HKG has been trying in vain to convince local villagers, local teachers and students of botany for protection of small lakes?

Acknowledgements

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