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Comparative studies of six populations of *Isoetes panchganiensis* from India

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Abstract: Six populations of *Isoetes panchganiensis* G.K.Srivast., D.D.Pant & P.K.Shukla growing in different environmental conditions of the Western Ghats have been studied to determine the range of variation within the species. The present investigation does not show any variation in habitat (aquatic), rhizomorph (trilobed), velum (fenestra), and presence or absence of peripheral strands; however, there is a subtle variation in the length of plants, number of leaves, size of megasporangia, and size and ornamentation of the megaspores. The basic organisation of the megaspores of all the populations is almost same, but the finer details of the ornamentation of the megaspores exhibit subtle differences. They may be retate to reticulate. The muri may be complete or incomplete. Megaspores of the Kolahapur population are the largest in diameter, whereas the megaspores of the Khinger population are the smallest in diameter.

Key words: Spore, environment, population, Western Ghats, India

Introduction

The Western Ghats is one of the hot spots of Indian biodiversity. It is the abode of a number of rare and precious species. The quillworts are an important component of the Western Ghats flora. They are widely distributed in Maharashtra and Karnataka states (Figure 1). *Isoetes sahyadriensis* Mahab. was the first species of the genus described by Mahabale (1938) from the Panchgani tableland of Maharashtra and the Sahyadri hills of Karnataka. Subsequently, Shende (1945) described the second species, *Isoetes dixitei* Shende, from the Panchgani tableland. After a gap of 48 years, Srivastava et al. (1993) described the third species, *I. panchganiensis* G.K.Srivast., D.D.Pant & P.K.Shukla, from the same locality. Thus, the Panchgani tableland is the type locality of the 3 Indian species of *Isoetes* L. However, our search for *I. sahyadriensis* was unsuccessful. We could not collect even a single plant of this species from Maharashtra and the Sahyadri hills. There is no report of this species after 1938. It may have become extinct. *I. dixitei* and *I. panchganiensis* grow together in all localities observed by us.

Isoetes panchganiensis is an aquatic species found growing in shallow water, while the plants of *I. dixitei* occur along the periphery of the pool. *I. dixitei* is robust, tall, and more resistant than *I. panchganiensis*, which is delicate, small, and less resistant. Both species are important parts of the Western Ghats flora. Srivastava et al. (2001) made preliminary studies of the 2 populations of *I. panchganiensis*. Wagai et al. (2008) studied the megaspores of *I.*

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Figure 1. Distribution of *Isoetes panchganiensis*.

dixitei to understand the diversity, evolution, and intraspecific variation in the megaspores of different populations. In the present study, 6 populations of *I. panchganiensis* have been examined to determine the range of morphological variation in different populations.

Materials and methods

The fully mature plants of *Isoetes panchganiensis* for the present study were collected between September and November of 2008-2010 from an open area of tropical evergreen forest of the Western Ghats. The populations that were explored and studied were the tablelands of Panchgani, Dhandighar, Khinger, and Kolahapur (Maharashtra); and Bababuddangiri and Kemmangundi (Karnataka). Habitat and morphometric details were observed in the field. Fresh materials were fixed in formalin–acetic acid–alcohol for further examination in the laboratory. The herbarium vouchers were prepared from each locality and deposited in the Botanical Survey of India (BSA), Central Circle, Allahabad.

Ten randomly selected plants per population were used to record the range of variation in a population. Morphometric observations of megaspore features were observed under light microscope and scanning electron microscope (SEM). Thirty megaspores of 10 individuals per population were measured in the equatorial plane and statistical analysis was done using

STATISTICA software. For SEM studies, spores were stereoscanned for microstructures under suitable magnification at an accelerating potential of 15 kV using a LEO 430 scanning electron microscope at the Birbal Sahni Institute of Palaeobotany, Lucknow. The descriptive terms used in the present paper follow the terminology of Jackson (1928), Kremp (1965), Hickey (1986), and Lellinger and Taylor (1997).

Results

The present studies of 6 different populations of *I. panchganiensis*, growing in the Western Ghats, exhibit some subtle differences in the length of plants, number of leaves per plant, and their characters (Table 1). The ornamentation of the megaspore is reticulate in the plants of the type locality, but it is variable in other populations (Table 1).

Table 1. Comparative morphological observation of 6 populations of *Isoetes panchganiensis*.

Locality	Length of plant leaves (cm)	No. of leaves	Size of megasporangium		Megaspore size		Velum	Ornamentation of megaspores
			Length (mm)	Width (mm)	Large (µm)	Small (µm)		
Panchgani	10–14 Av. = 12 S.d. = 1.58 C.V. = 13.17 S.E. = 0.70	15–21 Av. = 17.65 S.d. = 2.58 C.V. = 14.54 S.E. = 1.15	3.5–5 Av. = 4.2 S.d. = 0.67 C.V. = 15.97 S.E. = 0.30	2.5–3.5 Av. = 3.08 S.d. = 0.37	357–386 Av. = 370 S.d. = 11.5 C.V. = 3.1 S.E. = 2.98	214–257 Av. = 235 S.d. = 14.2 C.V. = 6.04 S.E. = 3.67	Fenestra	Reticulate with prominent muri and regular network
Dhandighar	5–14 Av. = 10.4 S.d. = 3.57 C.V. = 18.63 S.E. = 1.60	9–22 Av. = 15 S.d. = 5.83 C.V. = 38.87 S.E. = 2.60	3.5–5.5 Av. = 4.6 S.d. = 0.82 C.V. = 17.86 S.E. = 0.37	3.0–3.50 Av. = 3.33 S.d. = 0.26	308–420 Av. = 365.8 S.d. = 34 C.V. = 9.4 S.E. = 8.94	168–280 Av. = 229 S.d. = 31.1 C.V. = 13.2 S.E. = 8.05	Fenestra	Reticulate with prominent muri and regular network
Khinger	9–15 Av. = 12 S.d. = 2.23 C.V. = 21.32 S.E. = 1.00	15–25 Av. = 21 S.d. = 3.96 C.V. = 18.69 S.E. = 1.77	4–5.50 Av. = 4.60 S.d. = 0.65 C.V. = 14.17 S.E. = 0.29	3–3.50 Av. = 352 S.d. = 27.36	312–411 Av. = 352 S.d. = 27 C.V. = 7.7 S.E. = 7.06	156–284 Av. =222 S.d. = 32.1 C.V. = 15 S.E. = 8.52	Fenestra	Reticulate with prominent muri and regular network
Kolahapur	9–15 Av. = 11 S.d. = 2.34 C.V. = 21.32 S.E. = 1.04	11–20 Av. = 14.6 S.d. = 3.91 C.V. = 26.79 S.E. = 1.74	3–4.50 Av. = 4.0 S.d. = 0.61 C.V. = 15.30 S.E. = 0.27	3.50–4 Av. = 3.8 S.d. = 0.26	360–420 Av. = 394 S.d. = 18 C.V. = 4.6 S.E. = 4.73	225–285 Av. = 254 S.d. = 21.5 C.V. = 8.5 S.E. = 5.56	Fenestra	Reticulate with less prominent muri and larger areoles
Kemmangundi	10–16 Av. = 14.5 S.d. = 2.70 C.V. = 18.4 S.E. = 1.20	12–35 Av. = 20 S.d. = 9.26 C.V. = 44.96 S.E. = 4.14	3.5–5 Av. = 4.2 S.d. = 0.67 C.V. = 15.97 S.E. = 0.30	3–4 Av. = 3.58 S.d. = 0.49	340–411 Av. = 368 S.d. = 23 C.V. = 6.3 S.E. = 6	213–284 Av. = 246 S.d. = 25 C.V. = 9.8 S.E. = 6.20	Fenestra	Retate with prominent muri and incomplete areoles
Bababuddangiri	10–17 Av. = 14.40 S.d. = 2.70 C.V. = 18.74 S.E. = 1.21	12–35 Av. = 20 S.d. = 9.26 C.V. = 44.96 S.E. = 4.14	4–5.50 Av. = 4.9 S.d. = 0.67 C.V. = 13.30 S.E. = 0.29	3–4 Av. = 3.58 S.d. = 0.49	330–450 Av. = 384 S.d. = 34 C.V. = 9.07 S.E. = 9	210–300 Av. = 262 S.d. = 25 C.V. = 9.1 S.E. = 6.35	Fenestra	Retate with prominent muri and incomplete areoles

Abbreviations: Av. = Average, S.d. = standard deviation, C.V. = coefficient of variance, S.E. = standard error.

The megaspores of all of the populations are dimorphic, and their colour is grey (wet) and white (dry). On the basis of the ornamentation of the megaspores, length of plants, number of leaves per plant (Table 1), altitude, latitude, longitude, soil colour, and geographical distribution (Table 2), plants of different populations of *I. panchganiensis* were grouped into 3 categories: Panchgani, Kolahapur, and Bababuddangiri (Table 2).

Panchgani: It includes Panchgani, Dhandighar, and Khinger in nearby localities.

Larger megaspores are pyramidal globose, reticulate with prominent muri that form a regular network on the distal side, proximal area with flattened muri, 357–386 µm in diameter (average [Av.] = 370, standard deviation [S.d.] = 11.56, coefficient of variance [C.V.] = 3.12), triradiate ridges 30–47 µm high, equatorial ridge slightly undulating and 10–15 µm high, girdle distinct with vague ornamentation. Smaller megaspores, somewhat flattened, 214–257 µm in diameter (Av. = 235 µm, S.d. = 14.2, C.V. = 6.02) (Figure 2, Table 1).

Kolahapur: Larger megaspores are pyramidal globose, reticulate, muri less prominent, forming larger areoles on both distal and proximal sides, 360–420 µm in diameter (Av. = 394, S.d. = 18, C.V. = 4.65); triradiate ridge thin and straight, 25–38 µm high; equatorial ridge indistinct, girdle area very prominent. Smaller megaspores 225–300 µm in diameter (Av. = 254, S.d. = 21.2, C.V. = 8.4) (Figure 2, Table 1).

Bababuddangiri: It includes Bababuddangiri and Kemmangundi. Larger megaspores triangular to subglobose, retate, muri prominent and generally forms incomplete areoles on distal sides, 330–450 µm in diameter (Av. = 384, S.d. = 34.84, C.V. = 5.63), triradiate ridge (28–45 µm) distinct but height is greater than width. Equatorial ridge slightly undulating and 12–18 µm high, girdle area may or may not be present (Figure 3). Smaller megaspores 210–300 in diameter (Av. = 262, S.d. = 25, C.V. = 9.1) (Figure 2, Table 1).

SEM microphotographs of all 3 categories are almost the same, except for the density of siliceous fibres. The entire surface of the perispore is covered by siliceous gel fibres that are interconnected with one another. The ends of the siliceous fibres are generally broken and form an irregular net-like pattern (Figure 3). This appears to be the most conservative and stable character to keep the plants of different populations in one species.

Discussion

Srivastava et al. (1993) provided a key for the identification of the Indian representatives of *Isoetes* on the basis of the ornamentation of megaspores. The plants of *Isoetes* were kept in 2 sections, Tuberculatae and Reticulatae, by Pfeiffer (1922). Later on, Srivastava (1998) divided the Indian species of *Isoetes* into 3 complexes:

I. coromandeliana complex,

I. dixitei complex,

I. panchananii complex.

I. panchganiensis belongs to the *I. panchananii* complex. It is a unique species of the Western Ghats. It belongs to the section Reticulatae (Terrestres) and differs from all other Indian species of the section Reticulatae (Table 3) in the following characters:

A. Rhizomorph (corm): Rhizomorph of *I. panchganiensis* is trilobed, while it is bilobed in other species belonging to the section Reticulatae (Terrestres).

Table 2. Habitat-based data of *Isoetes panchganiensis*.

Category	Altitude	Latitude	Longitude	Soil
Panchgani (Maharashtra)	1372 m	17°50′N	73°49′E	Brown red
Kolahapur (Maharashtra)	563 m	16°42′N	74°16′E	Reddish black
Bababuddangiri (Karnataka)	1900 m	13°53′N	76°21′E	Dusky red

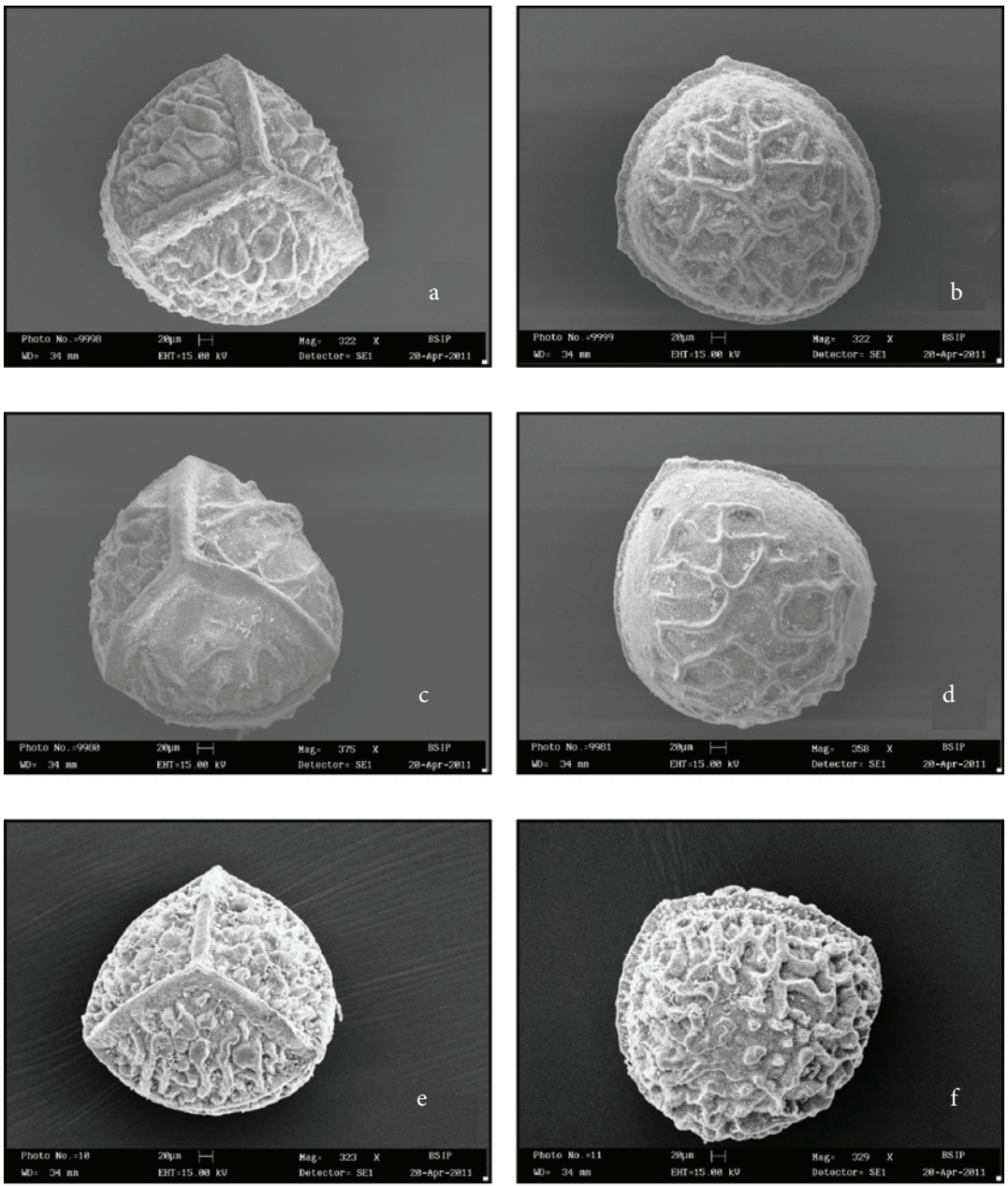

Figure 2. SEM micrographs of megaspores of *Isoetes panchganiensis*. A) Proximal side of Panchgani population, B) distal side of Panchgani population, C) proximal side of Kolahapur population, D) distal side of Kolahapur population, E) proximal side of Bababuddangiri population, and F) distal side of Bababuddangiri population.

B. Velum: Velum is complete (fenestra). It is present in all the populations of *I. panchganiensis*. This is a unique feature of this species. In other species of this section, it is variable, ranging from 1/4 to 2/3 and rarely complete. Thus, in this character, the species stands apart from other Indian species.

C. Megaspore: Megaspores of the Panchgani population are reticulate, generally, and muri form complete areoles. In this character, it comes closer to *I. panchananii*, but it differs from it in the size of areoles and from other species of the section Reticulatae in the thickness of siliceous gel fibres on the surface of the perispore wall of megaspores.

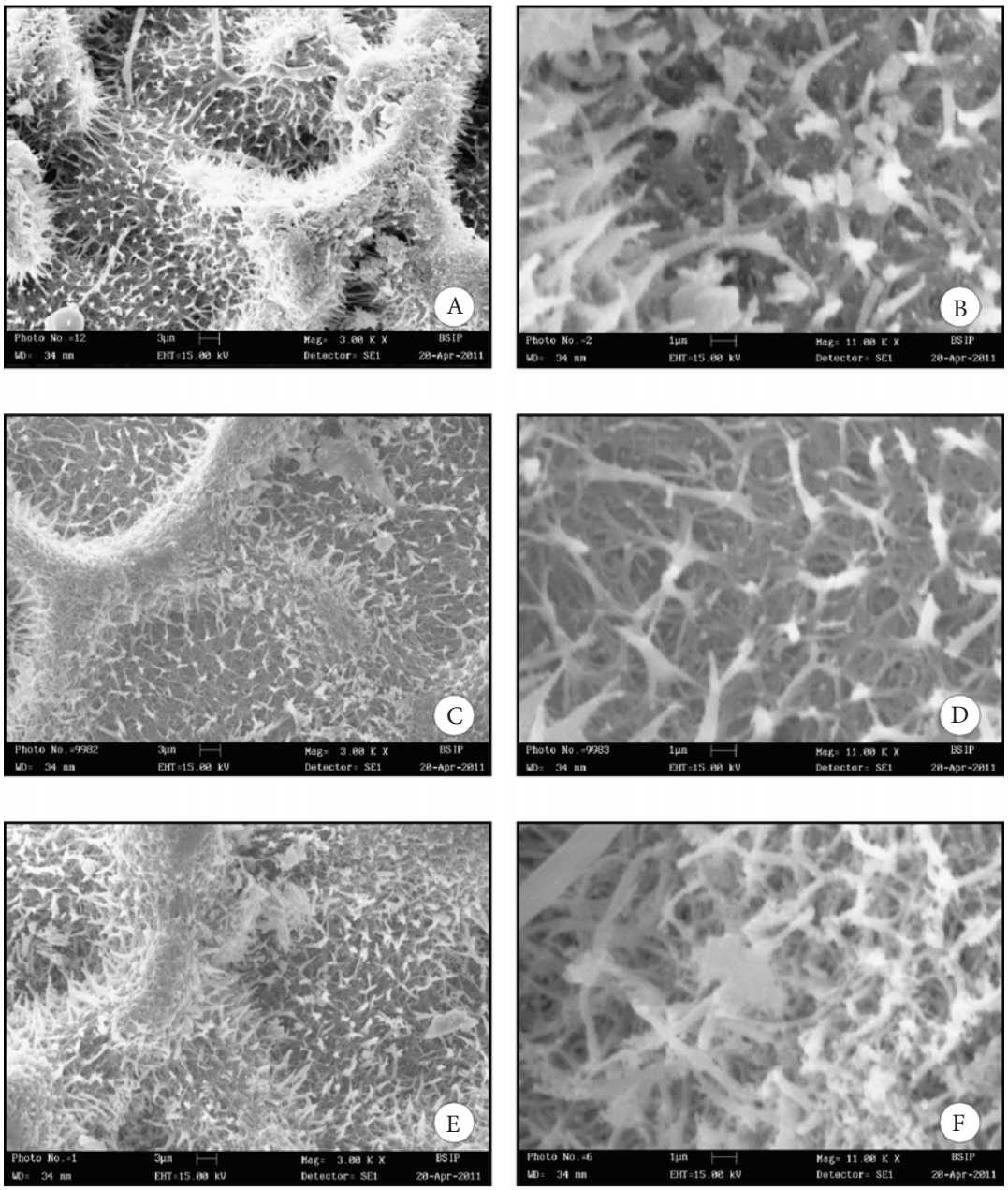

Figure 3. SEM micrographs showing infrastructural details of megaspores of *Isoetes panchganiensis*. A and B) Panchgani population, C and D) Kolahapur population, and E and F) Bababuddangiri population.

The megaspores of all of the populations of *I. panchganiensis* are dimorphic (larger and smaller) and reticulate, but the degree of reticulation varies; areoles may be complete or incomplete (Figure 2). The larger megaspores of the Kolahapur population are the largest in diameter, i.e. 360–420 µm (Av. = 394), while those of the Khinger population are the smallest, i.e. 312–411 µm (Av. = 352). However, the Bababuddangiri and Dhandighar populations show the highest degree of spread of distribution of megaspore size (S.d. = 34, C.V. = 9.4) (Table 1).

Table 3. Comparisons between *Isoetes panchganiensis* and closely related taxa.

Characters	***I. sampathkumaranii***	***I. rajasthanensis***	***I. mahadevensis***	***I. panchananii***	***I. reticulata***	***I. panchganiensis***
Plant habit[α]	Amphibious, terrestrial	Amphibious, terrestrial	Amphibious, terrestrial	Amphibious, lacustrine	Amphibious, terrestrial	Lacustrine
Rhizomorph[β]	2-lobed	2-lobed	2-lobed, rarely 3	2-lobed, rarely 3	2-lobed, rarely 3	3-lobed
Velum[λ]	1/2 to 3/4	3/4	Rudimentary	1/2, 2/3, 3/4 to rarely complete	1/2, 2/3, 3/4 to almost complete	Complete (fenestra)
Megaspores[©]	256–458 µm, cristate, muri incomplete, never form areoles	330–350 µm, cristate-reticulate, muri never form areoles	215–524 µm, retate	185–548 µm, reticulate, muri rarely form areoles	160–245 µm, reticulate, forming areoles	149–456 µm, reticulate, generally muri forming areoles, equatorial girdle prominent
Chromosomes[€]	2n = 66	2n = 44	2n = 44 + 1	2n = 44 + 1, 66, 110 + 1	2n = 33 + 1, 55 + 1	2n = 33 + 1 (unpublished)

Data sources: α, β, λ, © = Rao (1944), Gena and Bhardwaja (1984), and Shukla et al. (2007); € = Srivastava (1998), Takamiya (1999), and Shukla et al. (2007).

The smaller megaspores of the Bababuddangiri populations are the largest in diameter at 210-300 µm (Av. = 262) and those of the Khinger population are the smallest at 156-284 µm (Av. = 222). However, the Khinger population shows a higher degree of spread of distribution of smaller megaspore size (S.d. = 32.2, C.V. = 15) (Table 1).

D. Equatorial girdle: The presence of the girdle is another unique character of the megaspores of this species. However, it is absent in all other Indian species, including section Reticulatae. The megaspore surfaces of the Panchgani and Kolahapur populations show prominent equatorial girdles on the distal sides of the megaspores (Figure 2). It may be present or absent in the Kemmangundi and Bababuddangiri populations (Figure 2). Bagella et al. (2011) studied the different populations of *Isoetes histrix* Bory in Sardinia and reported that the presence or absence of the equatorial girdle and megaspore ornamentation are the most discriminant features usually used in species identification and diagnosis.

Our present work supports the earlier creation of the new variety of *I. panchganiensis*. The plants of Kemmangundi and Bababuddangiri were assigned to *I. panchganiensis* var. *kemangundiensis* G.K.Srivast., D.D.Pant & P.K.Shukla by Srivastava et al. (1993). It differs from the Panchgani population in the length of plants, number of leaves, and ornamentation of megaspores (retate). The areoles are incomplete and the equatorial girdle may or may not be present (Figure 2).

ITS sequences were done in collaboration with Dr W.C. Taylor and his associates. Tree topology shows that *I. panchganiensis*, *I. mahadevensis* G.K.Srivast., D.D.Pant & P.K.Shukla, *I. reticulata* Gena & Bhardwaja, and *I. panchananii* are in an Australasian clade with *I. brevicula* from western Australia and *I. taiwanensis* De Vol from Taiwan (unpublished). These 4 Indian species are very distinct from each another morphologically as well as in terms of molecular phylogeny. Bolin et al. (2008) studied the preliminary molecular phylogeny and morphology of *Isoetes* occurring in south-western Asia. Thus, the molecular analysis is necessary to know the relationship between closely associated species. All 4 Indian species belong to the Australasian clade. This indicates that these species are not of Indian origin but of Australian origin.

Srivastava and Srivastava (2001) described how the impacts of soil and other environmental factors play an important role in determining the morphological variation within the species. Soil nutrients like copper, potassium, phosphorous, nitrogen, and soil pH exhibit a negative correlation with plant length. The amount of copper is at its maximum at Panchgani and the length of plants is at its minimum, while at Kemmangundi, the amount of copper is at its minimum and length of plants is at its maximum. Manganese, however, shows a positive correlation with plant length, i.e. the amount of manganese is at maximum at Kemmangundi and at minimum at Panchgani, in proportion to the length of the plants.

It appears that the nutrient status of the soil is important, but alone it may not be the cause of the morphological variation in the genus *Isoetes*. Other factors such as climate, altitude of region, biotic interference, and competition with associated plants are equally important in determining the morphological variation in characters. The higher altitude and low biotic interference with high rainfall increases the acidity of soil (basaltic rock rich in iron) due to excessive rainfall, which leads to leaching and cation exchange in which hydrogen replaces the bases. Thus, the pH decrease at higher altitude favours maximum growth at Kemmangundi and shorter lengths of plants at lower altitudes due to low acidity (high pH), high biotic interference, and low rainfall. Thus, the ecological factor may be among the important factors creating variation.

Reproductive biology

Isoetes panchganiensis is confined only to the Western Ghats of India. It has not been reported from any other parts of the subcontinent. Sexual reproduction and microspores are unknown. Srivastava et al. (1996) described how the plants propagate through rhizomorphs or apogamy. All plants collected from the Western Ghats are megasporangiate. We could not collect even a single microsporengiate plant after repeated collections. Thus, the main mode of reproduction in this species may be apogamous.

Conservation status

Shukla et al. (2002), for the first time, discussed in detail the various aspects of conservation of Indian species. Later on, Srivastava (2005) reported that Panchgani tableland is very precious because it is the type locality of 3 species of *Isoetes*, *I. sahyadriensis*, *I. dixitei*, and *I. panchganiensis*. Because of disturbance, the place is unsafe for the further survival of these species of *Isoetes*.

I. panchganiensis is found growing in the natural ponds of the Panchgani tablelands and nearby tablelands of Maharashtra. Due to construction and decoration on the Panchgani tablelands to attract tourists and school children, the natural ponds are being destroyed. The same is the case for Panhala Fort at Kolahapur (Maharashtra), and Kemmangundi (Karnataka), due to the iron ore mines and construction of hotels. Melia et al. (2012) stated that abnormalities taking place at different stages of sexual reproduction are responsible for the low capacity for seed formation. This is comparable with the present species, in which sexual reproduction is unknown so far. The absence of sexual reproduction may be responsible for the formation of sexually reproducing megaspores. There is no immediate danger to the survival of the species, but if precautions are not taken to conserve these plants, the future is unsafe. *I. panchganiensis* plays an important role in understanding the evolution and diversification of the genus *Isoetes*. Therefore, it is necessary to conserve this species both ex situ and in situ.

Conclusion

The present investigation of 6 populations of *I. panchganiensis* growing in different geographical distributions, altitudes, soil profiles, etc. has revealed that there is no change in the key characters of the species (e.g., rhizomorph, ligule, velum, peripheral strands), but other characters like growth, habitat, size and number of leaves, and surface ornamentation of megaspores are likely to be influenced by these factors. Our study supports the earlier observations of Hickey (1986), who studied 3 localities of Costa Rica and found that the 3 populations varied in elevation/ecological cline. He also observed that geography and elevations are common clines for the change in the spore ornamentation and show intergradations in spore morphology. Therefore, a species should be recognised only after determining the range of variation within different populations of a species. Thus, the population study is very important in understanding the species as a whole.

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