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A new subspecies of *Isoetes coromandelina* (Isoetaceae) from Gujarat, India

Santosh Kumar SHUKLA[1], Sarvesh Kumar SINGH[2,*], Pradeep Kumar SHUKLA[3], Nawal Kishor DUBEY[2], Husnara KHANAM[4] and Gopal Krishna SRIVASTAVA[4]

1. Department of Botany, Kamala Nehru Institute of Physical and Social Sciences, Sultanpur-228118, UP., India.
2. Department of Botany, Institute of Science, Banaras Hindu University, Varanasi–221005, UP., India.
3. Department of Botany, Brahmanand Post Graduate College, Kanpur-208002, UP., India.
4. Department of Botany, University of Allahabad, Allahabad–211002, UP., India.
[*] *Corresponding author's email: pteridologicalexpress@gmail.com*

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ABSTRACT: In the present study *Isoetes coromandelina* ssp. *thanensis* S.K. Shukla, S.K. Singh, P.K. Shukla, N.K. Dubey, H. Khanam & G.K. Srivastava a micro endemic new subspecies is described from the Than in Rajkot District of Gujarat state in coastal zone of India. Morphologically, the new subspecies is most similar to *I. coromandelina* ssp. *coromandelina* but differs in its microspore with levigate surface ornamentation and chromosome number 2n = 22 + 1. Different morphological features of *I. coromandelina* ssp. *thanensis* are discussed. Images and a distributional map are also provided. In the present study, we made a correlation between microspore ornamentation and ploidal status at the infra-specific level which helpful in taxonomy of closely related taxa.

KEY WORDS: Diploid, *Isoetes*, India, Microspore, Subspecies, Taxonomy.

INTRODUCTION

The lycophyte family Isoetaceae contains only one extant cosmopolitan, heterosporous (having both megaspores and microspores) and ligulate genus *Isoetes* L. that are found in the ditches, ponds, rivers, lakes, wetlands and terrestrial habitats (Taylor and Hickey, 1992). The genus comprises about 350 species distributed in different geographical locations around the world (Hickey *et al*., 2003), out of which 192 taxa accepted, 112 synonyms, 14 hybrids and 26 unresolved names (Troia *et al*., 2016). In India, 16 species have been reported from different geographical regions, and of these, 15 species are endemic (Srivastava *et al*., 1993; Shukla *et al*., 2002, 2007; Yadav *et al*., 2012, 2015). The endemism of *Isoetes* species has commonly been seen in Asian species (Merill and Perry, 1940; Choi *et al*., 2008; Kim *et al*., 2010; Jung *et al*., 2014). In India, first species of the genus, *I. coromandelina* L. f. was described by the son of Linnaeus in 1781 from the Cormandel Coast, Tamilnadu (Pant and Srivastava, 1962). *Isoetes coromandelina* is the most wide spread species in the Indian subcontinent (Bangladesh, India, Nepal, Pakistan and Sri Lanka) and also reported from the Northern Territory of Australia and Cambodia as well (Shukla *et al*., 2002, 2007; Chandra *et al*., 2008; Marsden, 1976; Jung *et al*., 2014).

At infra-specific level, the composition of the *I. coromandelina* includes two subspecies, namely, *I. coromandelina* ssp. *coromandelina* and *I. coromandelina* ssp. *macrotuberculata* Marsden and a variety, *viz*., *Isoetes coromandelina* var. *raipurensis* Unni (Unni, 1967; Marsden, 1976; Srivastava, 1998; Srivastava *et al*., 1993; Troia *et al*., 2016). Recently, Troia *et al*. (2016) have published a provisional checklist of the genus *Isoetes* in which both subspecies are kept under accepted taxa and a variety as unresolved category.

In part of our ongoing regular plant collection visits, the authors came across to a new population of *Isoetes* in Than, Rajkot, Gujarat which resemble *I. coromandelina* ssp. *coromandelina* in their gross morphology. A comparative and critical review of the literatures clearly shows that there is only one species of *Isoetes*, i.e. *I. coromandelina*, known from the Gujarat State of India. After detailed study, the authors found that some of the plants in this population show subtle differences in the microspores surface morphology and cytology as well. In the present investigation an effort has been made to assign to a new subspecies to *I. coromandelina* based on marked differences in microspore morphology and cytology.

MATERIAL AND METHODS

Sample collection and herbarium

Investigations were carried out on the specimens collected during field trips in Gujarat, in October 2012. The first hand initial observation on megamorphic features such as habitat, leaf shape and colour, plant length, soil colour etc. have been recorded in the field. Living plants along with soil (for cytological study) and another samples without soil (for morphological studies) brought to the laboratory for further examination. Plant samples procured for morphological studies were preserved in FAA (formalin: acetic acid: alcohol). This was followed by herbarium preparations including that

of plants found growing closely associated with *Isoetes* species and voucher specimens were deposited at Botanical Survey of India, Kolkata (CAL), India.

Morphological and Scanning electron microscopy

Randomly five plants from a single locality (type) for each cytotype were selected for documenting organo-graphical morphology. Fifty megaspores (dry) from five megasporangium (one from each plant) not subjected to acetolysis were measured in equatorial plane using light microscope (LM). Similarly, 50 microspores from five microsporangium (one from each plant) mounted in safranin-glycerin jelly, without subjected to any treatment, were measured in equatorial plane using LM.

For Scanning electron microscope (SEM) studies, both megasporangia and microsporangia were dehydrated in an alcohol series and ruptured on the glass slide, after drying in a critical point dryer, they were placed on double-sided adhesive tape affixed to an aluminum stub and coated with gold-palladium in a sputtering chamber (Polaron sputter coater, SC 7640). Both megaspores and microspores were examined for the surface microstructure under suitable magnification at an accelerating potential of 15 kV and a working distance of 22–35 mm using an SEM LEO 430 at Birbal Sahni Institute of Palaeoscience, Lucknow, India.

Cytology and terminology

For the cytological studies, randomly young and healthy root tips were selected and pre-treated with aqueous solution of Para Dichloro Benzene (PDB) for 3 hours at 4°C in the dark, after thoroughly washed with distilled water, they were transferred for fixation in freshly prepared solution of absolute ethanol : glacial acetic acid (3 : 1) and kept for 48 hour penetration. Thereafter, root tips were stored in 70% ethanol for 24 hours. The stored root tips were hydrolyzed with 1N HCl at 60°C in a thermostat for 30 minutes and then they were allowed to stain in 2% acetic-carmine for nearly 30 minutes. Then, terminal 0.5mm portions of meristematic tissue of root tips were cut, macerated and squashed. Several slides were observed in order to confirm the results under Leica DMLB DC300 micro-photographic research microscope. The selected voucher slides were in Department of Botany, University of Allahabad, Allahabad, UP., India.

The sporoderm surface terminology and descriptive terms follow Jackson (1928), Kremp (1956), Hickey (1986b) and Punt *et al*. (2007).

TAXONOMIC TREATMENTS

Isoetes coromandelina* ssp. *thanensis S.K. Shukla, S.K. Singh, P.K. Shukla, N.K. Dubey, H. Khanam & G.K. Srivastava ***ssp. nov.*** **Figs. 1 A, B & 2**

Type: India. Gujarat, Rajkot district, Than, 30 October 2012, *S.K. Shukla 8401* (Holotype: S.K. Shukla 8401, CAL).

Isoetes coromandelina ssp. *thanesis* is allied to *I. coromandelina* ssp. *coromandelina* in gross morphology but it differs from the other Indian species and *I. coromandelina* ssp. *coromandelina* by monomorphic microspores that are levigate and chromosome number 2n = 22 + 1, as well as by monomorphic microsporangium.

Description. Plants herbaceous, tufted, amphibious, deciduous. Rootstock corm globose, dark brown, 10–20 mm wide, 5–12 mm high, deeply embedded in the habitat soil, perennial, tri-lobed, each lobe 5–11 mm wide. Roots synchronously arising along the central grooves, facing downward, unequal dichotomies from each of main roots, follows in branches and branchlets, subsequently become wiry, branching one to five times, older roots dark blackish while younger ones creamy white, up to 70 in number, 60–90 mm long, 2.0–2.4 mm thick at the bases. Scale membranous, scalerified, trianguloid, dark blackish, 7–10 mm long, 3–5 mm wide and 4–8 in number. Phyllopodia absent. Leaves up to 86 in number, nearly all sporophyllous, spirally arranged and twisted in basal portion, simple, linear, apices attenuate, edges undulating, spreading on ground, occasionally erect, yellowish-green, 30–70 cm long and 6–10 mm wide at base, 3.5–6 mm wide above the sporangium, 1.5–2.5 mm wide at mid-length, bases broadened, gradually tapering towards apex, apex attenuate; leaves generally megasporangiate rarely microsporangiate, alae yellow-green extending upward for ½ of the total leaf length, margin undulating, 1.5–3 mm wide above the sporangium, 0.25–0.4 mm wide at mid-length, subula trigonal; interstellar canal 3; 4 longitudinal transversally septet air chamber with internal hair; stomata present in upper ¾ of leaf. Peripheral fibrous bundles 5–10 minor, strongly developed. Ligule triangular, cordate, serrate, delicate, 3.5–5 mm long and 2–3.5 mm wide at base. Labium persistent, firmly attached with glossopodium, 2–3.5 mm long and 4–8 mm wide at base, margin serrate. Velum is absent. Sporophylls are mostly megasporophyllous, 20–60 in number; rarely microsporophylls 4–7 in number. Sporangia single, sessile, adaxial, basal, embedded in basal cavity of leaf base, positioned 0.6–0.8 mm above base, elongated dark-brown (microsporangia) to light-brown (megasporangia); microsporangia 6–7 mm long, 4–5 mm wide, with numerous microspores; megasporangia 6–9 mm long, 5–7 mm wide, contains 350-410 megaspores per sporangium; sporangial wall composed of thick-walled, elongated, brown, irregularly shaped cells, with no internal pigmentation. Megaspores trilete, pyramidal-globose, dimorphic, grey when wet, white when dry, larger megaspore 457–585 × 429–517 µm, smaller megaspores 214–400 × 214–285 µm, both proximal and distal surface have pustulate

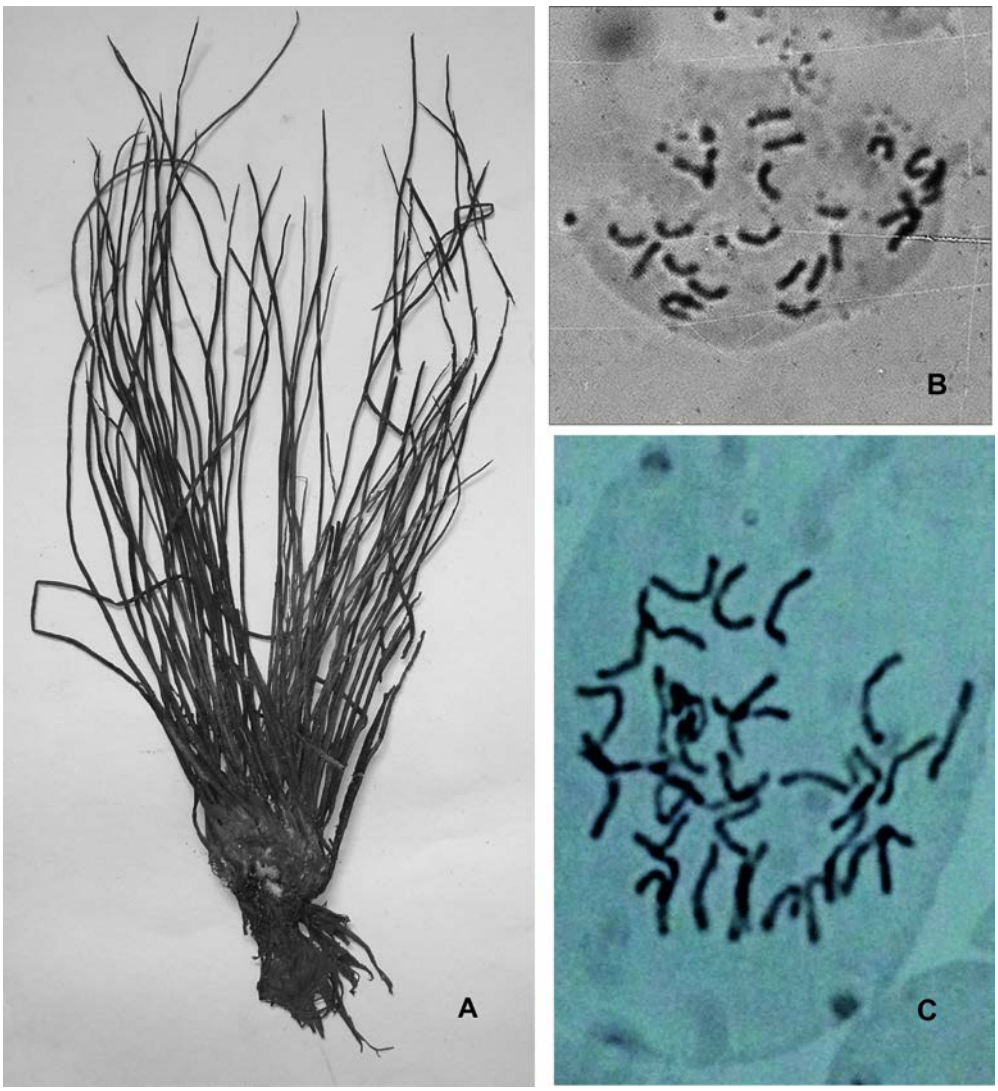

Fig. 1. A. Holotype of ***Isoetes coromandelina*** ssp. ***thanensis*** [India. Gujarat, Rajkot district, Than, 30 October 2012, *S.K. Shukla 8401* (Holotype: S.K. Shukla 8401, CAL)] and scale bar 30 mm, B. Chromosome number of diploid plant (2n = 22 +1), C. Chromosome number of triploid plant (2n = 33 +1).

ornamentation, pustules 8–12 in proximal face while 20–28 in distal face, length and width of pustules 43–71µm, infrastructural details show entire surface of perispore covered by siliceous gel-fibers and interconnected with each other by finger-like thick network forming a regular networking. Microspores monomorphic, monolete, ovate rarely rounded, equatorial diameter 42–71 µm, both proximal and distal surfaces have levigate ornamentation. Ploidal status diploid, 2n = 22 + 1.

Etymology. The epithet refers to the type locality, where it was originally discovered. The word 'than' has no meaning in indigenous language.

Phytogeography. *Isoetes coromandelina* ssp. *thanesis* grows as an amphibious in very small seasonal rainy drainage pools near the Than railway station in Rajkot District of Gujarat State which is a lowland population (about 10 m). It shares its understory habitat with *Marsilea minuta* L. and many angiospermic plants. Huge numbers of tiny earthworms were found in the cluster of basal portions of sporophylls.

Phenology. Late June to October (spore mature in late September).

Endemism. It has been collected from a single locality of Gujarat. Therefore, it is a micro endemic to this geographical region.

Conservation status. Following the IUCN guideline ver. 12 (IUCN 2016), *Isoetes coromandelina* subsp. *thanensis* is assessed as 'Data Deficient' (DD), as only few individuals were located during the floristic survey. Further explorations in the adjacent area are necessary to ascertain the status of the subspecies. Presently, the area is not well protected and habitat is prone to the anthropogenic activity and to grazing.

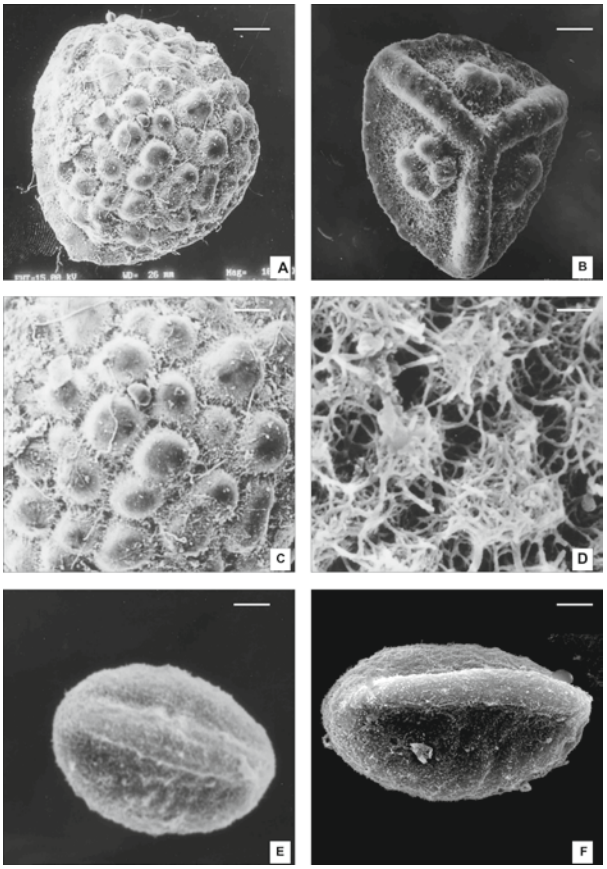

Fig. 2. Scanning electron photomicrographs of ***Isoetes coromandelina*** ssp. ***thanensis*** spores, **A–D**. Megaspores with pustulate ornamentation, **A**. Distal surface and scale bar 45 µm, B. Proximal surface and scale bar 90 µm, **C–D**. A portion of magnified to show infrastructural details of surface ornamentation and scale bar 250 µm, **E–F**. Monolete microspores with levigate ornamentation and scale bar 40 µm.

DISCUSSION

The establishment of the present subspecies now raises the number of subspecies of *Isoetes coromandelina* from two to three. All the previously described infra-specific taxon of *I. coromandelina* show almost similar organo-graphical characters, such as, presence of scale, prominent ligule and labium, dominant megasporangiate plants, dimorphic megaspores, globose with pustulate ornamentation, dimorphic microspores, ovate to reniform with echinate ornamentation and their cytological analysis shows diploidy to hexaploidy (Abraham and Ninan, 1958; Verma, 1961; Pant and Srivastava, 1965; Srivastava and Shukla, 2000; Srivastava *et al*., 1997, 2003; Shukla *et al*., 2007). Some Indian authors have established new infra-specific taxa on the basis of a few characters which are undoubtly plastic in their nature (Unni, 1967; Panigarhi, 1981). In contrast, *I. coromandelina* ssp. *thanensis* differs principally from other infra-specific taxon in following basic characters such as 1. Microspores have levigate ornamentation and 2. Chromosome number 2n = 22 + 1. Remarkably, the microspores of other subspecies of *I. coromandelina* and other Indian species are described as echinate ornamentation (Srivastava *et al*., 1996). Thus, these characters are enough to justify its assignment to a new subspecies (Tables 1, 2).

Table 1. Comparative assessment of morphological characters in the Than population of ***I. coromandelina*** based on ploidal status.

Characters	Diploid (22 + 1)	Triploid (33 + 1)
Plant	Robust and smaller	Weaker and larger
Root stock	3–lobed	3–lobed
Root		
Length (mm)	60–90 (x̄ 72)	89–137 (x̄ 107)
Number	Up to 70	Up to 53
Branching	Up to 5 times	Up to 3 times
Scale	Trianguloid	Trianguloid
Phyllopodia	Absent	Absent
Leaves		
Length (cm)	30–70 (x̄ 54)	42–102 (x̄ 83)
Number	Up to 86	Up to 53
x-sectional shape	Trigonal	Trigonal
Peripheral fibrous strands	5–10 minor	5–10 minor
Ligule		
Size (l x b) (mm)	3.5–5 × 2–3.5	3.5–5 × 2–3.5
Shape	Cordate	Cordate
Labium		
Size (l x b) (mm)	2–3.5 × 4.0–8	2–3.5 × 4.0–8
Velum coverage	Absent	Absent
Megasporangia		
Size (l x b)(mm)	6–9 (x̄ 7.5) × 5–7 (x̄ 6)	6–11(x̄ 8.5) × 5–7 (x̄ 6)
Shape	Elongated	Elongated
Nature	Dimorphic	Dimorphic
No. of spores/ sporangium	350–410 (x̄ 380)	550–710 (x̄ 637)
Megaspores		
Shape	Pyramidal–globose	Pyramidal–globose
Size (µm)	457–585 (x̄ 542)	476–680 (x̄ 592)
Perispore	Pustulate	Pustulate
Microsporangia		
Size (mm)	6–7 (x̄ 6.5) × 4–5 (x̄ 4.6)	6–7 (x̄ 6.5) × 4–5 (x̄ 4.6)
Shape	Elongated	Elongated
Nature	Monomorphic	Dimorphic
Microspore		
Shape	Ovate rarely rounded	Ovate to reniform
Size (µm)	43–71 (x̄ 54)	38–98 (x̄ 63)
Perispore	Levigate	Echinate
Frequency	Rare	Frequent

Megaspore morphology

Fuchs (1962) assigned the smooth walled megaspores to section *Laevis* Fuchs. Hickey (1986b) has recognized seven discriminate types of megaspore surface ornamentation such as pustulate, tuberculate, levigate, saccate, clavate, verrucate and baculate within the previously classed tuberculate type of Pfeiffer (1922), Fuchs (1962) and Fuchs-Eckert (1981a, 1981b). Thus, on the basis of megaspore surface morphology *I. coromandelina* belongs to tuberculate/pustulate type and assignable to the section *Palustres* (A. Braun ex Grenier in Grenier and Godron (1855–1856), emend., Fuchs-Eckert (1981a, 1981b) [= section *Tuberculatae*

Table 2. Comparison between the subspecies of ***Isoetes coromandelina.***

	ssp. *coromandelina*	ssp. *macrotuberculata*	ssp. *thanensis*
Larger megaspores			
Diameter (µm)	470–660	420–530	457–585
Ornamentation	Tuberculate, both proximal and distal surface have short blunt tubercles	Tuberculate, both proximal and distal surface have large globular tubercles	Tuberculate/pustulate, both proximal and distal surface have globular pustules
Ridges	Triradiate and commissual ridge almost smooth	Triradiate and commissual ridge irregularly corrugate	Triradiate and commissual ridge almost smooth
Smaller megaspores			
Diameter (µm)	350–460	330–410	214–400
Ornamentation	Tuberculate, tubercles are borader than hight	Tuberculate, tubercles are higher than broad	Tuberculate/pustulate, pustules are borader than hight
Ridges	Triradiate and commissual ridge almost smooth	Triradiate and commissual ridge irregularly corrugate	Triradiate and commissual ridge almost smooth
Microspores			
Diameter (µm)	40–92	–	43–71
Ornamentation	Rugose to papillate or echinate	–	Levigate
Chromosome number			
	2n = 33 + 1or 2 or 3, 2n = 44 + 1 or 2, 2n = 66 + 1	–	2n = 22 + 1

Data source: Pfeiffer (1922), Knox (1950), Abraham and Ninan (1958), Goswami (1975), Marsden (1976), Srivastava (1995, 1998), Srivastava *et al.* (1996), Srivastava *et al.* (1997, 2003), Jung *et al.* (2014).

Pfeiffer (1922)]. At infra-specific level of classification this subspecies comes under *I. coromandelina* (Taylor and Hickey, 1992). Previously described all the subspecies of *I. coromandelina* have megaspores with tuberculate/pustulate surface ornamentation and their ultrastructure was found to be similar (Marsden, 1976; Panigrahi, 1981; Jung *et al*., 2014), although there is considerable overlap in their size range (Table 2).

Microspore morphology and cytological data

The delineation of taxa at species level in the genus *Isoetes* as a whole or even at infra-specific level including *I. coromandelina* has primarily been based upon the megaspore surface ornamentation (Pferiffer, 1922; Marsden, 1976; Hickey, 1986b; Jung *et al.*, 2014) and its morphology along with other associated vegetative characters (such as leaf morphology, presence or absence of velum and its coverage over sporangia, corm lobe, plant length etc.) or in some cases the cytological data in terms of ploidy levels (Hickey, 1984, 1986a; Srivastava, 1998, 2005; Shukla *et al*., 2005; Yadav *et al*., 2012). Remarkably, almost none of the taxa in the genus were delineated exclusively on the basis of microspore morphology and have largely been neglected in taxonomic treatments (Musselman, 2003). The probable reasons may be their rare occurrence or sometimes complete absence in some of the species and in some of the earlier studies the poor resolution of surface details may be the cause of its negligence (Pant and Srivastava, 1962; Marsden, 1976; Jung *et al*., 2014). In addition, the descriptive terms for surface sculpturing of these micro bodies have also not been standardized (Musselman, 2003). The ornamentation of microspores in *I. coromandelina* has been described previously as rugose to papillate or echinate either at individual level or at the level of population (Pfeiffer, 1922; Knox, 1950; Marsden, 1976; Srivastava, 1995; Srivastava *et al*., 1996), but in none of the case it has been used as the key character in delineation of taxa at infra-specific level. Thus, this is the first attempt where the establishment of taxa is based exclusively upon the differences in ornamentation of microspore associated with ploidal status at the level of individual and populations. As a consequence this has enhanced the importance of microspores in dealing taxonomy, evolution and also helps in distinguishing the closely related taxa.

The microspores in the genus *Isoetes* are described as greyish or brownish in colour, bilaterally symmetrical, sharply angled at the proximal ridge, with two distal surfaces or single curved distal surface, the proximal ridge may be with a groove, in some cases with a semispherical swelling, reniform in shape, mostly falls within the diameter range of 20–50 µm, and with a single 'monolete' aperture. The ornamentation of the microspores are described as smooth or textured with spines, tubercles or ridges (Taylor *et al*., 1993), echinate, aculeate, cristate, psilate and laevigate (Musselman, 2003), echinate, rugulate and tuberculate (Macluf *et al*., 2006). The microspore surface ornamentation is also strongly convergent (Hickey, 1986b; Musselman, 2003; Macluf *et al*., 2006) as in many other pteridophytes (Tryon and Lugardon, 1991). The polymorphism is also common in these reproductive micro-bodies. The taxa exhibiting the levigate microspores (smooth walled or psilate) either as sole feature or with the mixture of other ornamentation types in the genus *Isoetes* have been reported from almost all parts of the world (Table 3). Remarkably in similarity with the Laurasian origin, the taxa of *Isoetes* exhibiting the similar ornamentation in the microspores of Gondawana origin is also relatively equal (Table 3).

Table 3. List of ***Isoetes*** species exhibiting smooth pattern in their perispores of microspore along with ploidal status

Taxon of Gondwana Origin			Taxon of Lauracian Origin		
Species	Microspore ornamentation	Ploidal status	Species	Microspore ornamentation	Ploidal status
I. brsiliensis	Smooth or rugulate	Not known	*I. brittonii*	Smooth with scattered tubercles	2n = 33
I. ekmanii	Smooth or rugulate	Not known	*I. engelmanii*	Psilate, smooth–papillose	2n = 22
I. favulata	Smooth or rugulate	Not known	*I. hyemalis*	Psillate–laevigate	2n = 44
I. fusco-marginata	Smooth or rugulate	Not known	*I. tuckermanii*	Smooth	Not known
I. gardnerina	Smooth or rugulate	Not known	*I. hieroglyhica*	Smooth	2n = 44
I. itaboensis	Smooth or rugulate	Not known	*I. eatonii*	Smooth	Not known
I. panamensis	Smooth or rugulate	2n = 44	*I. echinospora*	Bacillate, smooth–spinulose	2n = 22
I. ramboi	Smooth or rugulate	Not known	*I. tennesseensis*	Levigate	2n = 88
I. sehnemii	Smooth or rugulate	Not known	*I. japonica*	Levigate, echinate or tuberculate	2n = 66, 67, 77, 87, 88
I. smithii	Smooth or rugulate	Not known	*I. hopei*	Levigate	Not known
I. spannagellii	Smooth or rugulate	Not known	*I. melanospora*	Smooth–papillose	2n= 22
I. araucaniana	Levigate or minutely regulate–granulate	Not known	*I. orcuttii*	Smooth–papillose	2n= 22
I. anatolica	Psilate, occasionally spinules	Not known	*I. asiatica*	Levigate	2n= 22
I. pusilla	Smooth or with slight projection	Not known	*I. pringlei*	Smooth	2n= 22
I. brevicula	Smooth	Not known	*I. tuerekheimii*	Smooth	Not known
I. caroli	Smooth or spinose	Not known	*I. unguiensis*	Lavigate–granulate	2n = 22
I. mongerensis	Almost smooth	Not known	*I. macrospora*	Smooth	2n = 44
I. coromandelina	Smooth or textured with spine, tubercles or ridges	2n = 22 + 1or 2, 33 + 1, or 2, 44 + 1or 2, 66 + 1			
I. rajasthanensis	Smooth	2n = 44 + 1			
I. toximontana	Aculeate	Not known			

Data source:The data set are based upon Pfeiffer (1922), Marsden (1976), Croft (1980), Johnson (1984), Brunton and Taylor (1990), Kott and Britton (1983), Taylor *et al*. (1993), Watanabe *et al*. (1996), Chinnock (1998), Takamiya (1999), Wang *et al*. (2002), Luebke and Badke (2003), Musselman and Roux (2002), Musselman (2003), Prada and Rolleri (2005), Macluf and Hickey (2007).

The ornamentation of microspore in *I. coromandelina* was described as smooth, rugose to papillate or echinate by various authors (Pfeiffer, 1922; Knox, 1950; Srivastava, 1995; Srivastava *et al*., 1996). Its ploidal status ranges from diploidy to hexaploidy (Abraham and Ninan, 1958; Verma, 1961; Pant and Srivastava, 1965; Srivastava *et al*., 1997, 2003; Troia, 2001; Shukla *et al*., 2007; Jung *et al*., 2014). Earlier a direct correlation was found between ploidal status of species and the size of megaspores and microspores (Kott and Britton, 1983; Cox and Hickey, 1984; Hickey, 1986a; Toria, 2001; Luebke and Budke, 2003; Macluf *et al*., 2006) up to an extant, however, no clear cut relationship in this regard was established in the genus (Musselman, 2003). Therefore, the present study for thefirst time, establish a direct correlation between ploidal status and patterns of microspore ornamentation at infra-specific level in the subspecies of *I. coromandelina*, i.e. smooth pattern of microspore is associated with diploidy while triploidy with echinate pattern in this population (Table 1). An analysis of the data presented in table 1 clearly point out that triploid plants are larger in root length, leaf length, sporangial length, number of megaspores within the sporangium, size of megaspores and microspores than their diploid counterparts. In contrast, they exhibit reduction in number of roots and branching pattern and number of leaves per plant than the diploid individuals. Similar trend in variation with respect to length of plant (leaves) and number of leaves between triploid and tetraploid plants in the same species, i.e. *I. coromandelina* was also recorded earlier (Srivastava, 1998). Therefore, under such a concrete background it can now be postulated with high degree of certainty that during the subsequent course of evolution in the geological past, the variable degree of echinate patterns should have been evolved from the basic diploid individuals having levigate pattern of microspore ornamentation due to auto-polyploidyzation. This hypothesis also get support from the present day reflection of cytological, morphological and distribution data of this very species, i.e. the echinate microspore pattern and a series of auto-polyploids in almost all the populations (Abrahm and Ninan, 1958; Ninan, 1958; Pant and Srivastava, 1965; Srivastava, Goswami, 1975; Bhardwaja and Gena, 1992; Srivastava, 1998) as stated earlier is most frequent

and abundant in the nature and undoubtedly is an adoptive success story of this species in the modern time.

Molecular phylogeny has confirmed a close relationship among several species including *I. coromandelina* on continents that originated from Gondwana (Hoot *et al*., 2006; Jung *et al*., 2014). Since species belonging to Gondwana land origin including *I. coromandelina* are phylogenetically primitive than Laurasian (Hickey, 1986b, 1990; Taylor and Hickey, 1992; Rydin and Wickstrom, 2002; Shukla *et al*., 2005) therefore, the results of present investigation may be hopefully throw considerable light in understanding of basal and sub basal level relationships among the species of the genus and helpful in resolving the intricate evolutionary patterns.

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