

Status of *Isoetes coromandeliana* L.f. and *Equisetum debile* Roxb. ex Voucher in Gujarat State, Western India

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Abstract

Occurrence of *Isoetes coromandeliana* L.f. in natural ponds of Harni, Savali and Tuwa (India) is known since 1956 by earlier workers. *Equisetum debile* Roxb. ex Voucher was also reported in 1962 growing as wild at Savali. Available literature indicates that *I. coromandeliana* falls under the category of 'near threatened' in Asian continents and as an 'endangered species' at national (India) level. In the current field work study, the authors could not locate the investigated species from the locations earlier documented by researchers. Few saplings of *I. coromandeliana* were observed at Talod and Vaktapur near Gandhinagar, a new location for the species. In contrast, *E. debile* appeared to be lost in wild from Gujarat. Their extinction from earlier reported locations is associated with anthropogenic pressure and thus legal action for their protection is needed. The present paper suggests further survey and habitat based studies and recommends conservation and management action plans based upon the ecology of the habitat.

Keywords: conservation, diversity, extinction, habitat loss, pteridophytes

Introduction

Pteridophytes are the early land dwellers and most primitive group of vascular plants that appeared on this planet in the mid-Palaeozoic era (e.g. around 438 million years ago) during the Silurian period (Dudani *et al.*, 2011, 2014). Their adaptation to terrestrial conditions by evolving specialized tissues for the translocation of water and food is responsible for their greater colonization in terrestrial ecosystems. Development of vascular tissue not only contributed in water and food translocation but also played crucial role in mechanical support. Therefore, many of them such as *Cyathea* and *Wilsonia* (tree ferns) could attain great heights like trees. This interesting group of plants is considered as an important component of forest ecosystems and forms the link between the non-vascular cryptogams and the seed plants. They occupy various niches on the land, in marshes, swamps and in water bodies (Dudani *et al.*, 2011). They flourish in moist tropical and temperate forests while they also occur in different eco-geographical regions of the world where conditions are not conducive for growth (Dixit, 2000). The majority of them thrive well in shady and moist places but a few survive in rock crevices and dry places while some of them such as *Salvinia*, *Marselia* and *Azolla* grow in aquatic habitats (Bower, 1923, 1963).

Extensive field work was carried out in 2012 to survey the diversity of pteridophytes of Gujarat state. During the hereby study, all locations named from earlier reports were visited

(Phatak *et al.*, 1953; Chavan and Mehta, 1956; Gaekwad and Deshmukh, 1956; Chavan and Sabnis, 1961; Chavan and Padate, 1962, 1963; Shah and Vaidya, 1964; Padate, 1969; Inamdar and Shah, 1967; Patel *et al.*, 2010; Dabgar, 2012; Modi and Dudani, 2013) and were documented for the diversity of pteridophytes in Gujarat. During this survey, it was noticed that *Isoetes coromandeliana* and *Equisetum debile* lost their original habitat. Therefore, the main aim of the present study was to investigate their status in the Gujarat state, Western part of India.

Materials and Methods

During the survey work, field work was carried out at all the locations, in different parts of the Gujarat, such as Harni Lake, Savali, Tuwa, Timba and water bodies around Vadodara city, where earlier researchers studied the occurrence of *Isoetes coromandeliana* and *Equisetum debile*. Beside these locations, different water bodies were also investigated in central and Northern Gujarat including Pilo, Talod, Vaktapur, Polo forest, Jessore and Balamram sanctuary, during 2012-2015. From these different locations, the authors could locate *Isoetes* only at Talod and Vaktapur. Since, numbers of individual were decreased significantly; only five plants of *Isoetes coromandeliana* were collected for the preparation of herbarium and laboratory

work, for confirmation of their identity. Field photographs were taken in their natural habitat with digital camera (Cannon SLR 1200D). The morphological characteristic, diagnostic features and relevant field notes of the specimens were recorded in the field using fresh plant materials. Collected specimens were pressed in blotting paper and after drying specimens were poisoned by using 0.5% mercuric chloride dissolved in ethanol. Subsequently, the specimens were air dried under fan and the herbariums have been stored in the BARO herbarium, Department of Botany, Faculty of Science, the Maharaja Sayajirao University of Baroda, Vadodara, Gujarat (India). Identification of collected specimens was carried out by referring to Pteridophyte flora (Pant and Srivastava, 1962). Further confirmation of their identity was done by Dr. Sachin Patil from Department of Botany, Shivaji University Kolhapur (Maharashtra state).

Molecular identification

For molecular identification, genomic DNA was extracted using fresh samples. Extraction has been done by using Plant/Fungi DNA isolation kit (Sigma Cat#E5038) and manually by CTAB method as described by Doyle and Doyle (1990). PCR was carried out using 1X final concentration of Ready Mix™ Taq PCR Reaction Mix (Sigma) and template DNA (50 ng/μl). Amplification of the DNA was performed by using Thermal cycler (Applied Biosystems Veriti®). The genomic DNA was amplified by PCR machine with the primers rbcLaF and rbcLaR as described by Levin *et al.* (2003) and Kress and Erickson (2007). The amplified products were purified using Purelink™ Quick PCR purification kit (Cat#K310001). Successful PCR purified products were sent for sequencing to Eurofins Genomics India Pvt Ltd, Bangalore.

Sequence data obtained after sequencing was subjected to sequence match analysis using Basic Local Alignment Search Tool (BLAST) on NCBI for identification of pteridophytes. Identification was done by 99% base-pair match of the sequence obtained to the closest available reference sequences. After the preliminary analysis, the sequence was submitted to NCBI by using BankIt tool and also submitted to Bold Systems according to their guidelines.

Results and Discussion

Studies on pteridophytes of Gujarat started about a century ago and the first report on *Ceratopteris thalictroides* comes from the work of Saxton and Sedgwick (1918) while working on flora of North Gujarat. Subsequently, further studies on this group were taken up by Phatak *et al.* (1953), Chavan and Mehta (1956), Gaekwad and Deshmukh (1956), Chavan and Sabnis (1961), Chavan and Padate (1962, 1963), Mahabale (1948, 1963), Shah and Vaidya (1964), Padate (1969), Inamdar and Shah (1967). However, studies on pteridophytes were neglected thereafter. Therefore, intensive fieldwork was carried out throughout the Gujarat to study the diversity and status of pteridophytes occurring in the state.

Isoetes coromandeliana is an endemic species in India, particularly Coromandel Coast, from where it dispersed to other states of India and Asian subcontinent (Shukla *et al.*, 2002). It is the most common species in different parts of the country except Himalayas (Srivastava *et al.*, 1993) and from Western Ghat (Shukla *et al.*, 2002). Other than Indian subcontinent, it is also

recorded from different parts of Australia by earlier researchers (Marsden, 1976; Clifford and Constantine, 1980; Chinnock, 1998). Therefore, the species has a twin continental endemism, India and Australia respectively (Shukla *et al.*, 2002). Occurrence of *I. coromandeliana* was documented by Gaekwad and Deshmukh (1956) and Chavan and Sabnis (1961) from the Harni Lake near Vadodara city and at Tuwa village (GEC 1996). Similarly, Chavan and Padate (1962) and Padate (1969) reported *Equisetum debile* from Savli taluka. Beside these studies, no published information is available on the occurrence of *Isoetes* and *Equisetum* from Gujarat. The authors visited most of the lakes and natural ponds in different months in Savli taluka at Tuwa village, but it was not possible to relocate both species. Increasing boundaries of the suburban areas and newly developing housing societies invaded the lake margins and interference of human population on Harni Lake Ecosystem resulted in habitat loss of the *I. coromandeliana*. Due to discussions with local residents of Harni village, it was noted that population of this species declined gradually from 1996, whereas few individuals were seen until 2003. In contrast, lakes around the Tuwa village are deepened down for rainwater harvesting which consequently lost the habitat from Tuwa.

Nevertheless, *I. coromandeliana* was observed in a new location at Talod and Vaktapur, near Gandhinagar. Unfortunately, few individuals were observed at this location, since major part of the lake is also deepened for rainwater harvesting.

During the survey, *E. debile* could not located as reported in earlier floristic studies by Chavan and Padate (1962) and Padate

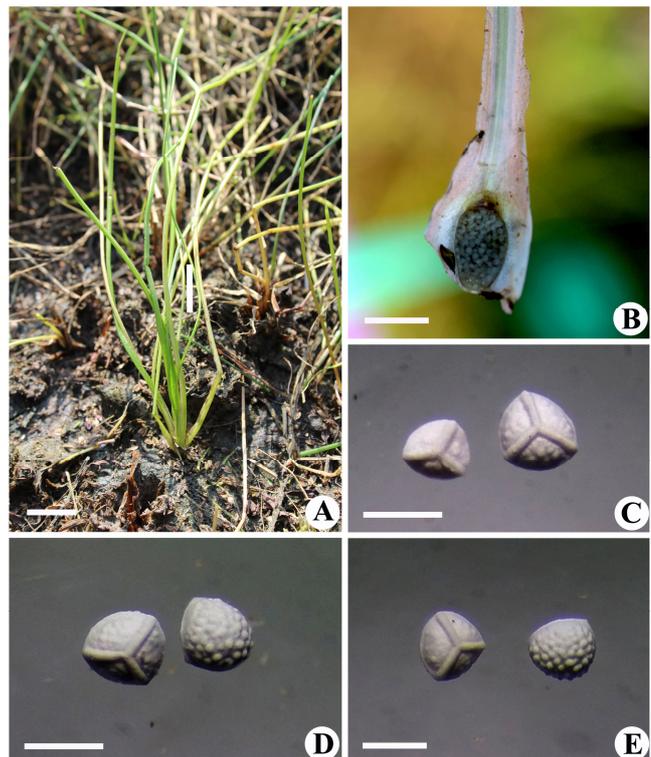


Fig. 1. *Isoetes coromandeliana* L.f.

A: Mature plant of *Isoetes coromandeliana* in the field.
 B: Basal part of sporophyll of mature plant with sporangium.
 C: Proximal view of dry (small and large) megaspores.
 D: Proximal and distal of large (dry) megaspore.
 E: Proximal and distal view of small (dry) megaspore.
 Scale bar: A = 2 cm, B = 1 cm, C, D = 500 μm, E = 350 μm

(1969). According to these authors, it is abundantly available in Savali taluka. After these studies, no documented evidence is available on its occurrence at different location in Gujarat. Even after discussions with local residents the authors could not get any information about this species. Therefore, it appears that *E. debile* has lost its natural habitat from Gujarat and only few saplings are under cultivation in the botanical garden of the Maharaja Sayajirao University of Baroda, Vadodara.

Conclusions

Isoetes coromandeliana is critically endangered in Gujarat and needs legal protection to save the ponds from biotic pressure and human activities. Moreover, there is an urgent need to formulate necessary action plan to conserve this species. Unavailability of *E. debile* on its reported location indicate that it might have been lost from Gujarat. The paper suggests further extensive field studies to search for new locations (if any) and recommends the development of conservation and management based action plans to protect this species based upon ecology and habitat.

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