Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Irudayaraj, V., Benniamin, A., & Arokia Raj, S. (2023). Apogamous *Isoetes coromandelina* L.f. (Isoetaceae) with asynaptic meiosis. *Caryologia* 76(4): 51-57. doi: 10.36253/caryologia-2222

Received: July 02, 2023

Accepted: January 24, 2024

Published: March 14, 2024

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Apogamous *Isoetes coromandelina* L.f. (Isoetaceae) with asynaptic meiosis

V. Irudayaraj[1,2], A. Benniamin[3,*], S. Arokia Raj[1]

[1] *Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu – 627002*
[2] *Present Address: 23/116, Darlingnagar, Maharajanagar Post, Tamil Nadu, India-627 011*
[3] *Botanical Survey of India, Western Regional Centre, Pune, India- 411 001*
*Corresponding author. E-mail: fernsbenni@gmail.com

Abstract. This cytological study on *Isoetes coromandelina* L.f (Isoetaceae) from Tirunelveli District, Tamil Nadu, South India shows the presence of twenty two individual chromosomes along with a fragment (n=2n=22+1) during meiosis in megaspore mother cells without the presence of any bivalent. Cytomixis between two megaspores is very common, resulting in the formation of a high percentage of abortive spores. The present material is of an apogamous taxon with asynaptic Meiosis as reported by Abraham and Ninan from Kerala and Karnataka.

Keywords: *Isoetes coromandelina*, asynaptic meiosis, apogamy.

INTRODUCTION

Lycophytes and Pteridophytes form the dominant vegetation on the earth next to flowering plants. Fern allies and ferns are a monophyletic group and the closest living relatives to seed plants (Pryer *et al*., 2001). This group of plants, with homosporous clubmosses, heterosporous spikemosses and quillworts, is the ancestor for Lycopodiopsida and Monilophytes Smith *et al*. (2006). Quillworts are considered by some to be the last remnant of the fossil tree lycopsid with which they share some unusual features including the development of wood and bark, a modified shoot system acting as roots, bipolar growth, and an upright stance (Retallack, 1997). *Isoetes,* one of the extant members of Lycopodiopsida, is a heterosporous fern. They are small aquatic herbs and they are commonly called quillworts or Merlin grass. Many aquatic plant populations including quillworts are under severe threat in the Western Ghats, particularly in Kerala and Maharashtra. For example, the endangered *Isoetes panchganiensis* has been reported from temporary ponds and pools on the high altitude plateaus of Panchgani table lands in Maharashtra and Kemmangundi Hills in Karnataka (Molur *et al*., 2011). Some aquatic species, often found in mixed populations containing taxa of different ploidy, appear to have evolved abruptly via interspecific hybridiza-

tion and chromosome doubling. Evidence from distribution patterns, megaspore morphology and viability, chromosome numbers, and electrophoretic profiles of leaf enzymes supports a hypothesis of allopolyploid speciation (Taylor & Hickey, 1992).

Isoetes is not only a very complicated genus but also a remarkable genus with lot of things yet to be studied and understood. From India, large number of species under *Isoetes* has been reported as new species. After Fraser-Jenkins *et al*. (2017) it is clear that there are only four different species (*I. coromandelina* L.f., *I. dixitii* Shende, *I. sahyadrii* Magab. ex L. N. Rao, *I. udipiensis* P. K. Shukla, G. K. Srivast., S. K. Shukla and P. K. Rajagopal) of *Isoetes* in India. As the species of *Isoetes* are mostly aquatic and Tamil Nadu can have low rainfall for several years with rare occurrence of normal rainfall, the occurrence of *Isoetes* species is rare and seasonal. But once there is enough rainfall, they flourish immediately and cover large area in several acres as mono-dominant species which is the characteristic features of several related fossil lycophytes like *Pleuromeia rossica,* which grew in wet shore side habitats and formed mono-dominant communities. Such ecological type of a mono-dominant plant community is quite typical of such hygrophilous vegetation in both the geological past and the present-day world, but it is inhabited by taxonomically different plants. The occurrence of mono-dominat *Isoetes coromandelina* L. f. has been reported from Kallidaikurichy, Tirunelveli District by Manickam and Irudayaraj (1992) and from a lake near Bharathidasan University, Tiruchirapalli, Tamil Nadu on 1999. They have mentioned that although the species is common throughout India, it is a rare species in Tamil Nadu. With the presence of rainfall above the average amount during the year 2014, this aquatic weed started to grow in and around several ponds and water pits in several localities, including the same locality Kallidaikurichy of Manickam and Irudayaraj (1992). Thanks to the availability of this seasonal aquatic weed, we aimed to make detailed cytological studies on the quillwort *Isoetes coromonandelina* L. f.

MATERIALS AND METHODS

Materials for the present study were collected from Kallidaikurichy, Tirunelveli District, Tamil Nadu, India during 2014 (Figure 1 A,B). Sporangia (Figure 1 C, D) were fixed in the mixture of Absolute alcohol, Chloroform and Acetic acid (6:3:1). Acetocarmine squash technique was followed to study the chromosomes behaviour in megaspore mother cells. Voucher has been deposited in St. Xavier's College Herbarium, Palayamkottai.

RESULTS AND DISCUSSION

Meiosis was observed in megaspore mother cells. Thin, network chromosomes, without the identity of individual chromsosme were observed in the leptotene stage and long, thick, rod like overlapping chromosomes were observed in zygotene stage (Figure 2, A-C). Pachytene and diplotene stages were not observed. After zygotene stage, each and every chromosome remains as such without pairing of homologous chromosomes. Instead of bivalents formation, they all remain as univalents. Thus all the chromosomes were like that of mitotic ones. There are 22 stout and thick chromosomes of more or less uniform size along with a very short chromosome. The chromosomes are of variable in length and type (Figure 2, D-E). The meiosis is more or less regular from I metaphase to II telophase except the irregular behavior of the extra chromosome (22 + 1) which either forms Chromosome Bridge or lies little away from others during first anaphase and telophase (Figure 2 F-I). There is no indication for the presence of this extra chromosome, in the form of micronucleus, in tetrads or young spores (Figure 2 L-Q). The mature megaspores are in different size and the surface of the megaspores is with prominent tubercles (Figure 1, E-H).

The careful observation on number of chromosomes in each group of Anaphase I, clearly shows the unreduction of chromosome number during first meiosis (Figure 2, F, G, H). From the present study on meiosis in megaspore mother cells of *Isoetes coromandelina* L.f from Kallidaikurichy, Tirunelveli District, Tamil Nadu, it is concluded that the chromosome number is n=22 +1. This is the first count for *I. coromandelina* L. f from Tamil Nadu and it is in agreement with Abraham and Ninan (1958), who have observed the presence of complete asynapsis in this species in populations from Kerala and Karnataka. They have concluded that this species from South India is of asynaptic apogamous form.

Cytological reports on Indian Quillworts are available. Plants of *I. panchananii, I. indica* and Varanasi plants of *I. coromandelina* are with forty-four chromosomes and a fragment at mitosis in root tip smears but the plants of *I. coromandelina* growing in Lohgarha and Konark are with only thirty-three chromosomes and a fragment (Pant & Srivastava, 1965). As a rule, the first meiotic division shows almost complete asynapsis, most of the chromosomes being univalents although a few are bivalents and multivalents. The association of bivalent and multivalent chromosomes takes place in various ways. About 50% of the spores are nucleate and the remaining 50% are enucleate, which again suggests that a second nuclear division is normally absent in the spore mother cells of

Figure 1. A. Large colony of *Isoetes coromandelina* L.f. in Kallidaidurichi, Tirunelveli District, Tamil Nadu, India, B. Habit, C-D. Sporophylls, E-H. Megaspores under light microscope (E, G) and Electron Microscope (F,H).

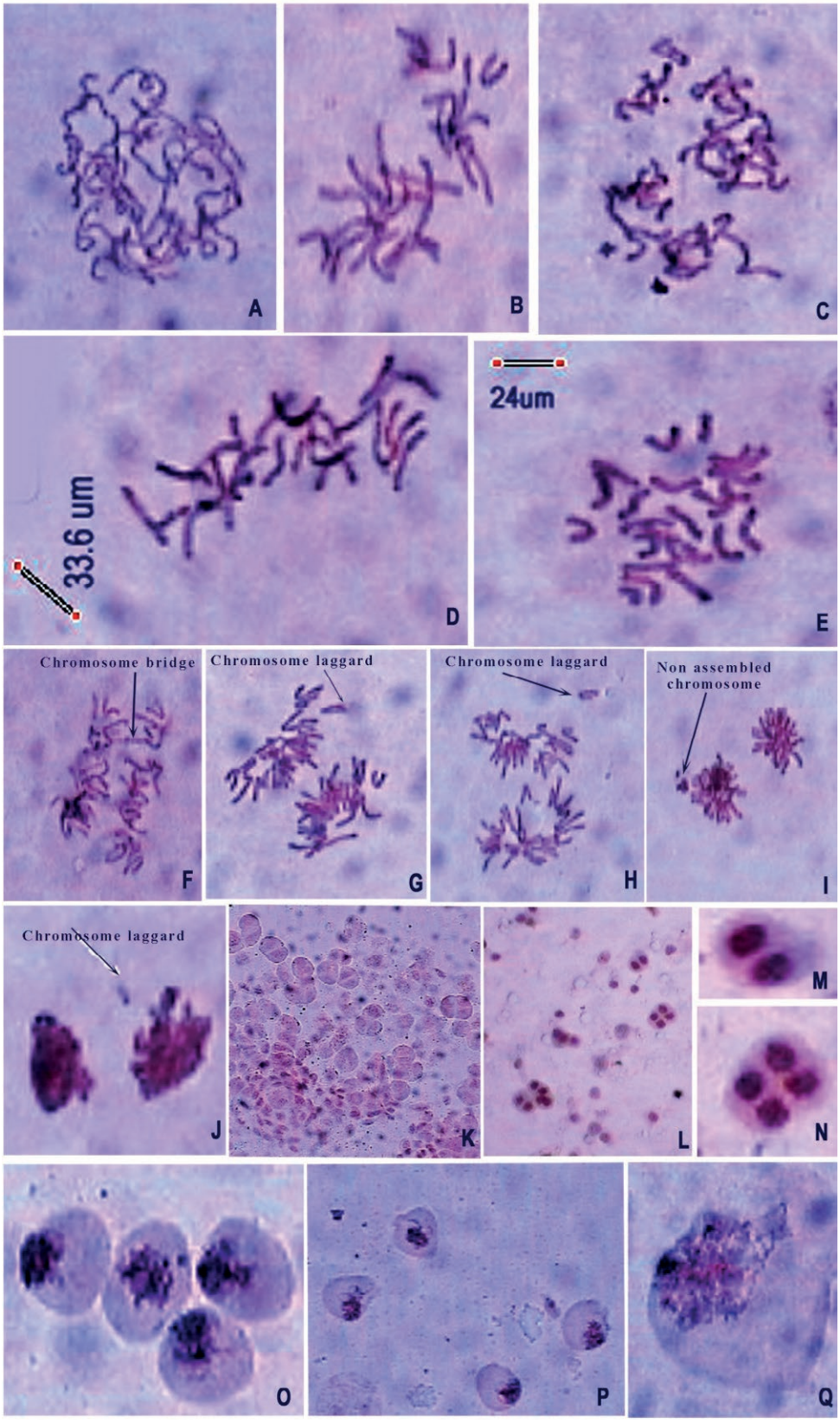

Figure 2. Meiosis in megaspore mother cells of *Isoetes coromandelina* L. f. A-J Meiosis I. K-N. Diads and tetrads; O,P,Q- Young spores.

Indian species of *Isoetes* and that their tetrads are formed by cytokinesis of the two dyads. The young larger megaspores of *I. coromandelina, I. indica* and *I. panchananii* usually show one nucleus and one "centrosomic plastid" while the smaller ones in the same species are enucleate with only one "centrosomic plastid". Karyomorphology on triploid *I. coromandelina* from South India shows Trisomy and translocations (Kuriachan and Ninan, 1974a, b). In general, *I. coromandelina* L.f. in India is a larger species complex with varied rhizome morphology, ornamentation of megaspore and cytology with the presence of diploid apomictic, triploid apomictic and triploid hybrid, tetraploid apomictic, pentaploid hybrid and hexaploid. *I. udipiensis* P.K.Shukla, G.K.Srivast., S.K.Shukla & P.K.Rajagopal is diploid sexual (Fraser-Jenkins *et al.* 2017). *I. dixitii* Shende is with 4x sexual and 3x, 6x sterile hybrids (Bir and Verma 2010).

In the present study, considerable percentage of microspores and megaspores are abortive ones with high reduction in size and irregular in shape. Another kind of abnormality in both microspores and megaspores is the presence of cytomixis. Mixing of chromatin materials of two spores through a tubular connection of the spore wall is a common phenomenon in the presently studied quillwort. The tubular connection is of different size. It is very thin and narrow, or very thick and short. Sometimes two spores are compactly and closely joined together without any tubular connection (Figure 3 A-S). Chromosomal abnormalities including cytomixis occur in meiotic process of pollen mother cells of many angiosperm species at high frequency, but similar phenomenon was rarely reported in lower vascular plants. Irregular chromosomal behaviour, especially cytomixis, is present in *Isoetes sinensis* Palmer (Heng-Chang *et al.,* 2007). It appeared in each stage of meiotic division from prophase I to telophase II. The cytoplasmic channels between microspore mother cells are narrow and stretched. The genetic material connection is maintained mainly by visible chromatin fiber. Cytomixis occurs between two adjacent microspore mother cells of same stage. A variety of other abnormal chromosomal behaviors, such as chromosomal stickiness, fragmentation, bridge, laggards, micronuclei are also present but with low frequency. Indeed, the cause of cytomixis formation and the evolutionary significance of it are still source of huge controversy. Bobak and Herich (1978) and Morisset (1978) believed it was just a pathological phenomenon by pharmaceuticals or mechanical pressure. Ghanima and Talaat (2003) stressed the impacts of extreme environmental factors. Nirmala and Rao (1996) thought cell fusion and chromatin degeneration were probably caused by both environmental and genetic factors.

Isoetes species are cytologically much variable with the presence of diploid, triploid, tetraploid and hexaploid species/cytotypes based on the base number x=11. Majority of them are sexual with few apogamous taxa. In *Isoetes* reticulate evolution by complex allopolyploid speciation has well established. So it is necessary to increase the sampling of *Isoëtes* looking for undescribed, missing species and additional intraspecific variation (Hoot *et al.,* 2004). Apogamy is not common among fern allies. In the present case of the apogamous *Isoetes coromandelina* L.f., it is peculiar in forming diplospores by avoiding normal reduction division during first meiosis in spore mother cells. Usually, the first meiosis is heterotypic division by the reduction of chromosome number and the second meiosis is homotypic division with normal mitosis. In the present case, both first and second meiosis are homotypic division without the reduction of chromosome number resulting in the formation of diplospores. Formation of diplospores with total asynapsis has been reported in several cases and the genetic control of total asynapsis in apogamous taxa has also been explained. The level and mode of diploid megaspore formation has been studied in full-sib diploid potato clones with either normal or desynaptic meiosis (Jongedijk *et al.,* 1991). The present observation of diplospory in the apogamous *I. coromandelina* L.f is similar one of pseudohomotypic division during first meiosis.

Apomixis has been repeatedly observed in fern lineages that experienced frequent reticulate evolution combined with polyploidization. The apomictic lineages showed no increase in speciation rate. Instead, all apomictic lineages appeared to be short lived despite some evidence for post origin diversification. In general, apomictic ferns are evolutionary dead ends in the long term but maintain the short-term potential to be highly successful in particular ecological conditions such as climates with strong seasonality (Liu *et al*., 2012).

There are several reports of asynaptic meiosis both in ferns and angiosperms. Manton and Sledge (1954) reported complete absence of chromosome pairing at meiosis in two separate wild collections of filmy fern *Hymenophyllum javanicum* Sprengel from Ceylon. In another filmy fern *Trichomanes insigne* v.d.B forma α is with normal meiosis, while forma β is with asynaptic meiosis (Mehra & Singh, 1957). The filmy fern, *Trichomanes proliferum* Bl. from Sarawak, is with asynaptic diakinesis with 108 univalents in spore mother cell and 32 spores in sporangia (Bell, 1960). Braithwaite (1964) described a new type of apogamy in *Asplenium aethiopicum* complex from Africa with complete asynapsis at diakinesis resulting in restitution nucleus which further mitotically divide to form 16 diplospores in each spo-

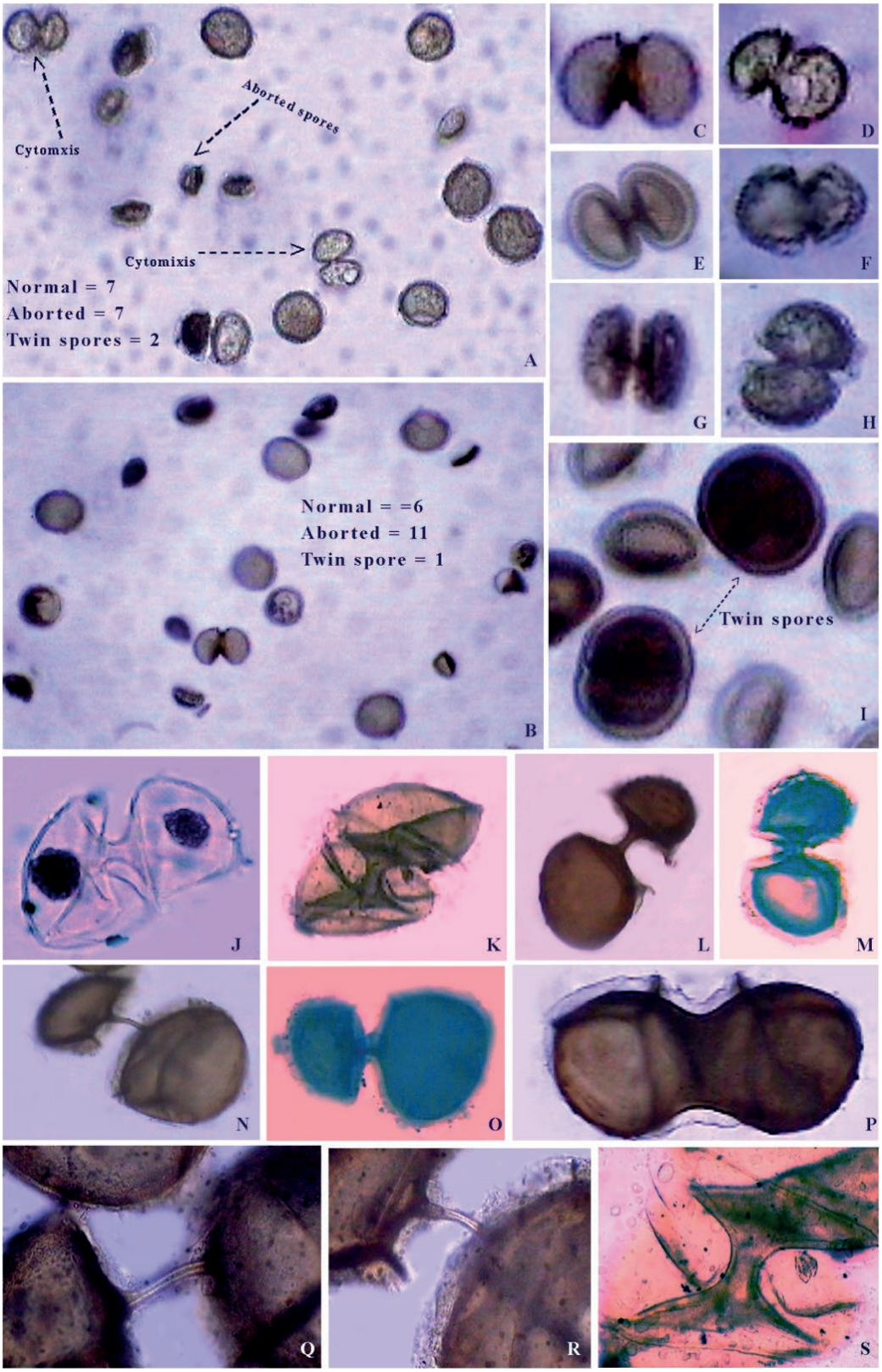

Figure 3. *Isoetes coromandelina* L.f. Cytomixis in microspores and megaspores. A,B- Mixture of spores with normal, aborted and twin spores; C-H- Cytomixis in microspores. I-Completely mingled twin microspores, J-S- Cytomixis in megaspores.

rangium. Asynaptic meiosis with the formation of unreduced gamete has been reported in interspecific hybrid of *Trifolium* (Ansari *et al.* 2022).

REFERENCES

Abraham A, Ninan, CA. 1958. Cytology of *Isoetes*. Curr. Sci. 27(2): 60-61.

Ansari, H. A., Ellison, N. W., Verry, I. M. & Williams, W. M. 2022. Asynapsis and unreduced gamete formation in a *Trifolium* interspecific hybrid. BMC Plant Biology, 22: 14.

Bell, P. R. 1960. The morphology and cytology of sporogenesis of *Trichomanes proliferum* Bl. *New Phytol.* 59: 53.

Bobak M, Herich R. 1978. Cytomixis as a manifestation of pathological changes after the application of trifluraline. Nucleus India, 21: 22-26.

Braithwaite, A. F. 1964. A new type of apogamy in ferns. New Phytologist, 63(3): 293-305.

Fraser-Jenkins CR, Gandhi KN, Kholia, BS, Benniamin A. 2017. An Annotated Checklist of Indian Pteridophytes Part 1. Lycopodiaceae to Thelypteridaceae. Bishen Singh & Mahendra Pal Singh, Dehra Dun.

Ghanima AM, Talaat AA. 2003. Cytomixis and its possible evolutionary role in a Kuwaiti population of *Diplotaxis harra* (Brassicaceae). Bot. J. Linn. Soc. 143: 169-175.

Heng-Chang W, Li JQ, He ZC. 2007. Irregular meiotic behavior in *Isoetes sinensis* (Isoetaceae), a rare and endangered fern in China. Caryologia, 60(4): 358-363.

Hoot SB, Napier NS, Taylor WC. 2004. Revealing unknown or extinct lineages within *Isoëtes* (Isoëtaceae) using DNA sequences from hybrids. American J. Bot. 91(6): 899-904.

Jongedijk E, Ramanna MS, Sawor Z, Hermsen JG. 1991. Formation of first division restitution (FDR) 2n-megaspores through pseudohomotypic division in ds-1 (desynapsis) mutants of diploid potato: routine production of tetraploid progeny from 2xFDR × 2xFDR crosses. Theor Appl Genet. 82(5): 645-56.

Kuriachan PI, Ninan CA. 1974 a. Karyomorphology of a triploid *Isoetes coromandelina* with a note on the karyotype evolution in the species. New Bot. 1: 160-169.

Kuriachan PI, Ninan CA. 1974 b. Trisomy and translocations in *Isoetes coromandelina* L. pp. 307-317. In P. Kachroo (ed.), Advancing Frontiers in Cytogenetics. Hindustan Publ. Co., Dehli.

Liu HM, Dyer RJ, Guo ZH, Meng Z, Li JJ, Schneider H. 2012. The eveolutionary dynamics of apomixes in ferns: A case study from polystichoid ferns. J. Bot. Article ID 510478.

Manickam VS, Irudayaraj V. 1992. *Pteridophyte Flora of the Western Ghats, South India*. BI Publications, Pvt. Ltd. New Delhi.

Manton, I & Sledge, WA. 1954. Observations on the cytology and taxonomy of the pteridophyte flora of Ceylon. Phi. Trans. B238: 127.

Mehra, PN & Singh, G. 1957. Cytology of Hymenophyllaceae. J. Genet. 55: 379.

Molur S, Smith KG, Daniel BA, Darwall WRT. (Compilers). 2011. The status and distribution of freshwater biodiversity in the Western Ghats, India. IUCN, Cambridge, UK and Gland, Switzerland.

Morisset P. 1978. Cytomixis in pollen mother cell of *Ononis* (Leguminosae). Can. J. Genet. Cytol. 20: 383-388.

Nirmala A, Rao PN. 1996. Genesis of chromosomal numerical mosaicism in higher plants. Nucleus, 39: 151-175.

Pant DD, Srivastava GK, 1965. Cytology and reproduction of some Indian species of *Isoetes*. Cytologia, 30: 239-251.

Pryer KM, Schneider H, Smith AR, Cranfil LR, Wolf PG, Hunt JS, Sipes SD. 2001. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. Nature, 409: 618-622.

Pryer KM, Schuettpelz E, Wolf PG, Schneider, H, Smith AR, Cranfill R. 2004. Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. Amer. J. Bot. 91(10): 1562-1598.

Smith AR, Pryer KM, Schuettpelz E, Korall P, Schneider H, Wolf PG. 2006. A classification for extant ferns. Taxon 55(3): 705-731.

Retallack GJ. 1997. "Earliest Triassic Origin of *Isoetes* and Quillwort Evolutionary Radiation." Journal of Paleontology, 71(3): 500-521.

Taylor WC, Hickey RJ. 1992. Habitat, evolution, and speciation in *Isoetes*. Ann. Miss. Bot. Gard. 79(3): 613-622.