

CYTOLOGY OF SEVEN APOGAMOUS FERN TAXA FROM KERALA

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SUMMARY

Cytological study was carried out in seven apogamous fern taxa *Doryopteris ludens* (Wall) J. Sm., *Hemionitis arifolia* (Burm) Moore (2 cytotypes), *Adiantum caudatum* L., *A. latifolium* Lam., *A. philippens* L. and *A. hispidulum* Sw. from Kerala. Except two diploids (*A. caudatum* and *A. latifolium*), all the others are polyploids at different ploidy levels, such as triploid, tetraploid (on $x = 30$), and hexaploid (on $x = 29$). Apogamy in ferns is a type of asexual reproduction in which the gametophytes arising from diplospores produce sporophytes without fertilization. In the present taxa, premeiotic endomitosis in sporangial archesporium resulted in half the usual number of spore mother cells (8) in sporangia, in which diploid number of regular bivalents were formed at meiosis, which together yield 32 diplospores. Recent studies using conventional and modern tools and techniques have highlighted phylogenetic and evolutionary significance of apogamy in ferns, which offer prospects for future studies of apomixis in ferns, and its role in fern evolution. The instance of two of the present species complexes (*H. arifolia* and *A. caudatum*), is pointed out to warrant detailed study aimed at probing into the role of apogamy in evolution of ferns of this region.

Keywords: Apogamous ferns, cytology, Kerala.

INTRODUCTION

Apogamy in ferns, in the broad sense, is a type of asexual reproduction in which diplospores are produced in sporangia, and the resultant gametophytes produce sporophytes of the next generation without fertilization (Manton 1950). The phenomenon usually occurs in polyploids in ferns as an alternative mechanism for circumventing the bottleneck of sterility imposed by polyploidy (Manton 1950), and hence, construed essentially as an escape from sterility, but also an escape into a 'blind ally' of evolution (Darlington 1939). Sporogenesis in ferns takes place within the sporangium, where spores are generated through meiosis. In sexual lineages, there would be 16 spore mother cells in which meiosis leads to formation of 64 haploid cells (spores), each containing half of the parental number of chromosomes. The apogamous ferns, by contrast, follow one of the two alternative spore-generating pathways to yield chromosomally unreduced diplospores following the restitution nucleus formation, either before or during meiosis such as (1) by premeiotic endomitosis (PE), also referred to as 'Dopp-Manton sporogenesis' (Dopp 1932, Manton 1950) or (2) by meiotic first division restitution (MFDR),

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also known as 'Braithwaite sporogenesis' (Braithwaite 1964, Walker 1985). The 32 diplospores in either case, each having one full chromosome complement germinate, and the resulting prothalli are capable of generating new sporophytes from the somatic cells, which are usually located near the apical notch of the prothallus, without fusion of sperm and egg.

Apogamy is more common in ferns than in any other group of vascular plants. Globally, around 10% of the fern species are apogamous (Lovis 1977), and the frequency varies in different fern floras - 15% in the Japanese flora (Takamiya 1996), 20% in Sri Lankan flora (Manton & Sledge 1954), 8% in South Indian flora (Abraham et al. 1962) and 10% in the sub-Himalayan flora (Mehra 1961). Among the North Indian ferns, the phenomenon was detected in species of 15 genera (Mehra 1961), and from South India in species of 5 genera which include *Pteris* Linn., *Dryopteris* Adanson, *Cyrtomium* Presl. and *Adiantum* Linn. In either Indian flora, apogamous species predominated in polyploids, especially triploids. Both the above studies had not however, furnished cytological events associated with the phenomenon. Apogamy in ferns is characterized by the invariable occurrence of the same gametic (meiotic) and somatic chromosome numbers, accompanied by incidence of half the usual number of spore mother cells (SMCs) (8) and spores (32) in the sporangia as against the normal 16 and 64 respectively in the sexual species. Since the first discovery of the phenomenon in ferns by Farlov (1874), there have been much strides in the study of apogamy in ferns. Grusz (2016) has recently reviewed the progress of the study of apogamy in ferns accomplished using both conventional and modern tools and techniques including DNA sequencing, and highlighted the phylogenetic, ecological and evolutionary significance and implications of the phenomenon. The present authors have screened species of three genera of Pteridaceae (sensu Copeland 1947) such as *Doryopteris* J. Sm., *Hemionitis* Linn., and *Adiantum* Linn., from Kerala for apogamy, and this paper is aimed at presenting the details of cytological events associated with apogamy in seven taxa such as *D. ludens* (Wall) J.Sm, *H. arifolia* (Burm) Moore (2 cytotypes), and *A. caudatum* L., *A. latifolium* Lam., *A. philippens* L. and *A. hispidulum* Sw.

MATERIALS AND METHODS

The materials for the study were procured from plants established in garden conditions which were originally collected from different low, medium and high altitude regions (viz. Bonacaud, Peermade and Munnar) in the Kerala sector of the Western Ghats, and two species of *Adiantum* were collected locally from Thiruvananthapuram, where they grow as weeds in moist shady places. The ferns are difficult materials for cytological study, especially meiotic study because of the small size of SMCs and hard texture of the sporangial wall (Abraham et al. 1962). For meiotic study and for screening of the SMCs and spore contents, young sporophylls were fixed in Carnoy's fluid for 2 or 3 d which were smeared and stained in 2% acetocarmine. For the sporangial content determination, the fixed sporangia were pressed under a cover slip for discharging the contents, and which were also stained in acetocarmine. Somatic chromosomes were studied from squashes of young root tips, also fixed in Carnoy's fluid and stained in acetocarmine. Pretreatment of root tips by keeping in ice chamber for 3–4 h prior to fixation was found useful for chromosome condensation and good spreading. The cytological preparations were photomicrographed using a Leica DM 100 digital camera attached to Leica DM trinocular microscope.

OBSERVATIONS

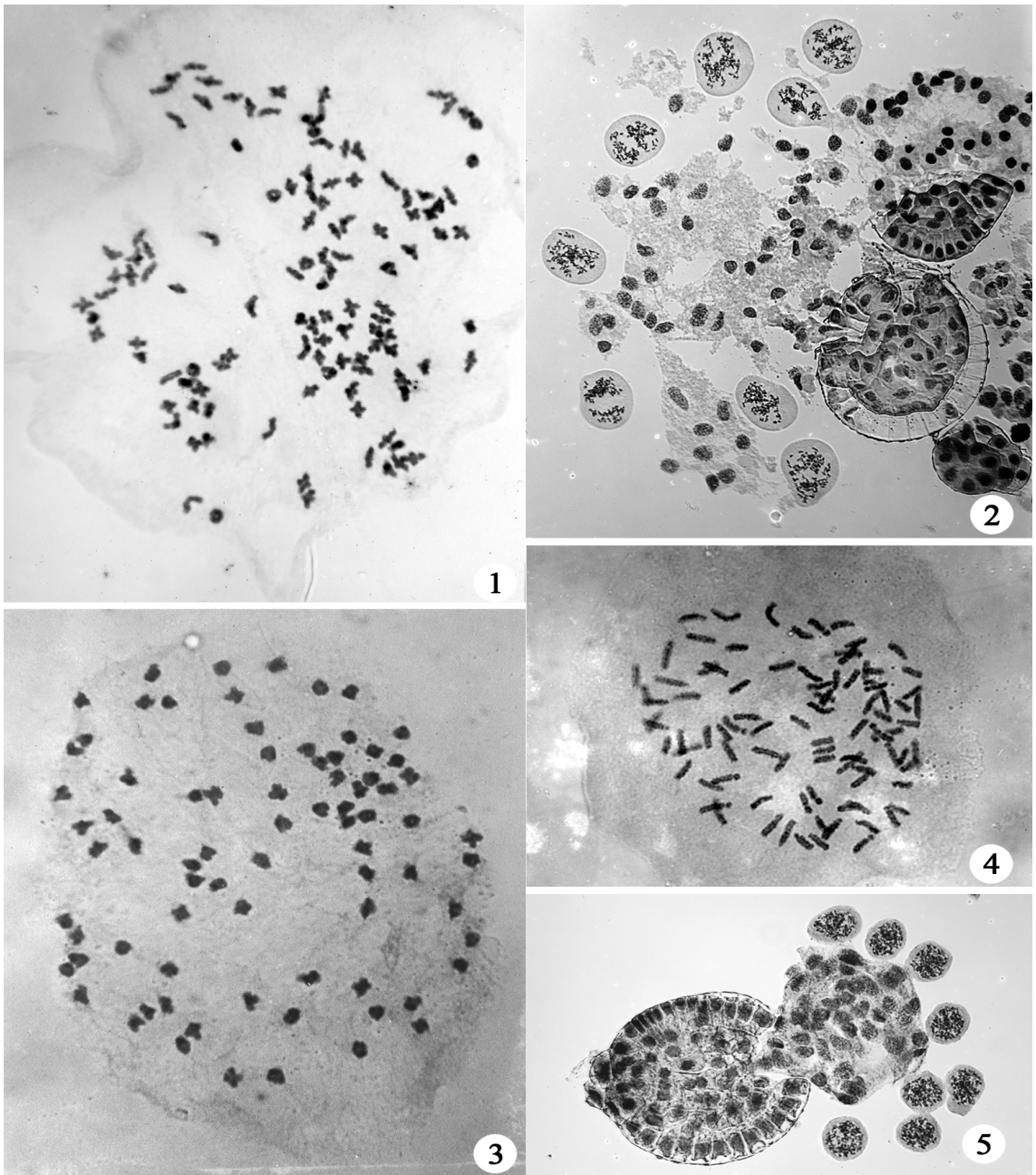
In all the fern species studied here, each sporangium has been found to have eight SMCs which undergo meiosis producing 32 unreduced diplospores, which germinate and produce diploid gametophytes (prothalli), from which the sporophytes are formed asexually without fertilization. The diplosporous nature of the taxa has been confirmed by determining the somatic chromosome number from root tip cells. The details of the cytological data of the seven apogamous taxa are given in Table 1 and depicted in Figs 1–21.

TABLE 1: Cytological data of seven apogamous fern taxa.

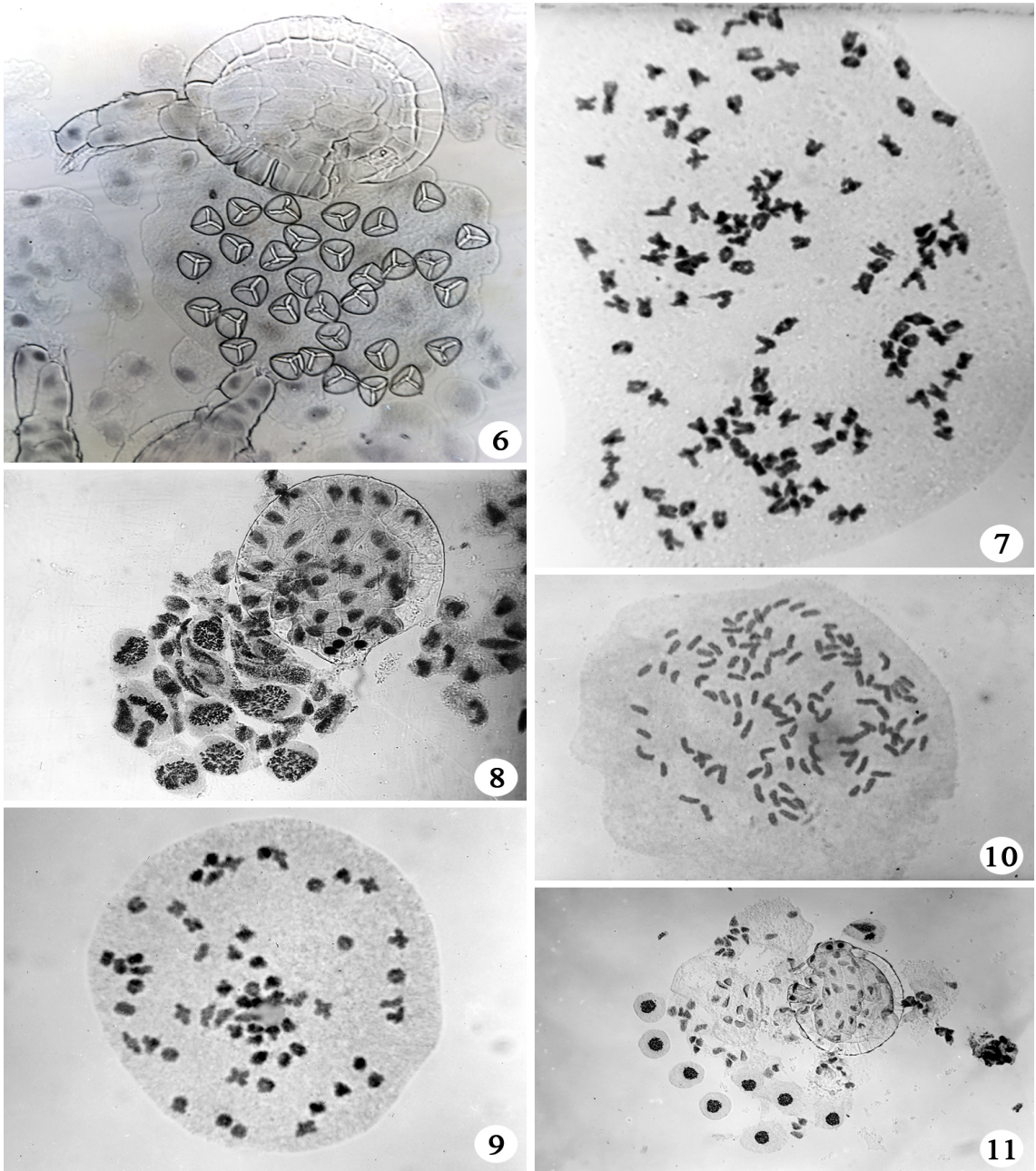
Taxon	Chromosome number		SMCs/ sporangium	Spores/ sporangium	Basic chrom. No.	Ploidy	Figure Nos.
	n	2n					
<i>D. ludens</i>	120	120	8	32	30	4x	1, 2
<i>H. arifolia</i> (cytotype I)	90	90	8	32	30	3x	...
<i>H. arifolia</i> (cytotype II)	120	120	8	32	30	4x	7, 8
<i>A. caudatum</i>	60	60	8	32	30	2x	9–11
<i>A. latifolium</i>	60	60	8	32	30	2x	12–15
<i>A. philippens</i>	90	90	8	32	30	3x	16–18
<i>A. hispidulum</i>	174	174	8	32	29	6x	19–21

DISCUSSION

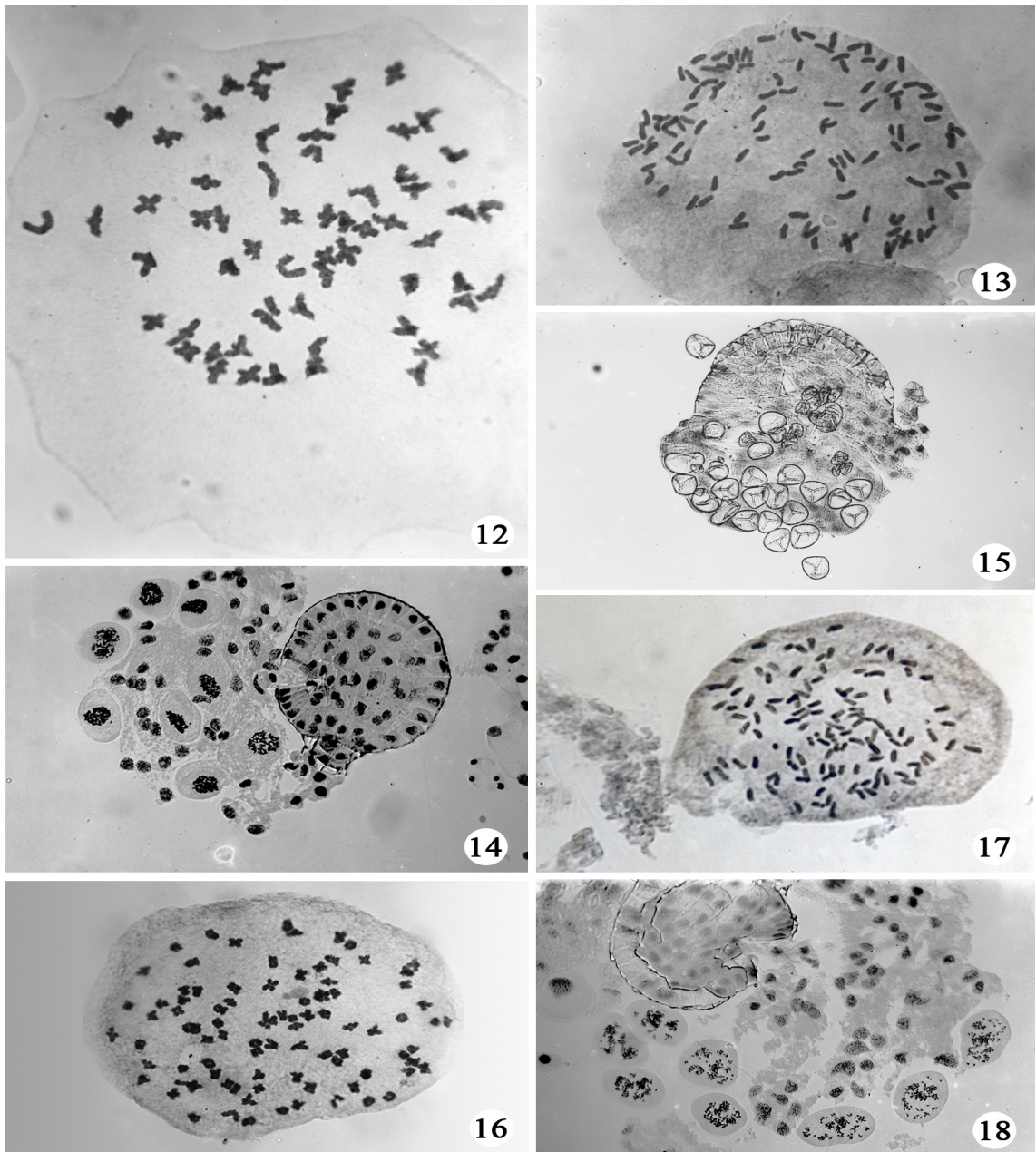
All the taxa of ferns studied here were screened for apogamy, and the pertinent features of the phenomenon such as the same gametic (meiotic) and somatic chromosome numbers, the reduced number of SMCs (8) and of spores (32) in sporangia were noticed. The somatic chromosome numbers in the different taxa show that, except two diploid taxa (*A. caudatum*, *A. latifolium*), all the others are polyploids at different levels such as triploid (cytotype I of *H. arifolia* and *A. philippens*), tetraploid (*D. ludens* and cytotype II of *H. arifolia*) and hexaploid (*A. hispidulum*). All the taxa, except *A. hispidulum*, are $x = 30$ basic. *A. hispidulum* with $2n = 174$ is an aneuploid derivative at the hexaploid level on $x = 29$, which in turn a descending aneuploid derivative of the deep rooted and stable basic number of $x = 30$ of the genus *Adiantum* (Abraham et al. 1962, Mathew & Cinthya Christopher 2018, Ninan & Mathew 2016). It may be noted that all the present apogamous taxa invariably possess only 8 SMCs at meiosis and 32 spores in each sporangium as against the normal 16 and 64 respectively in sexual species.



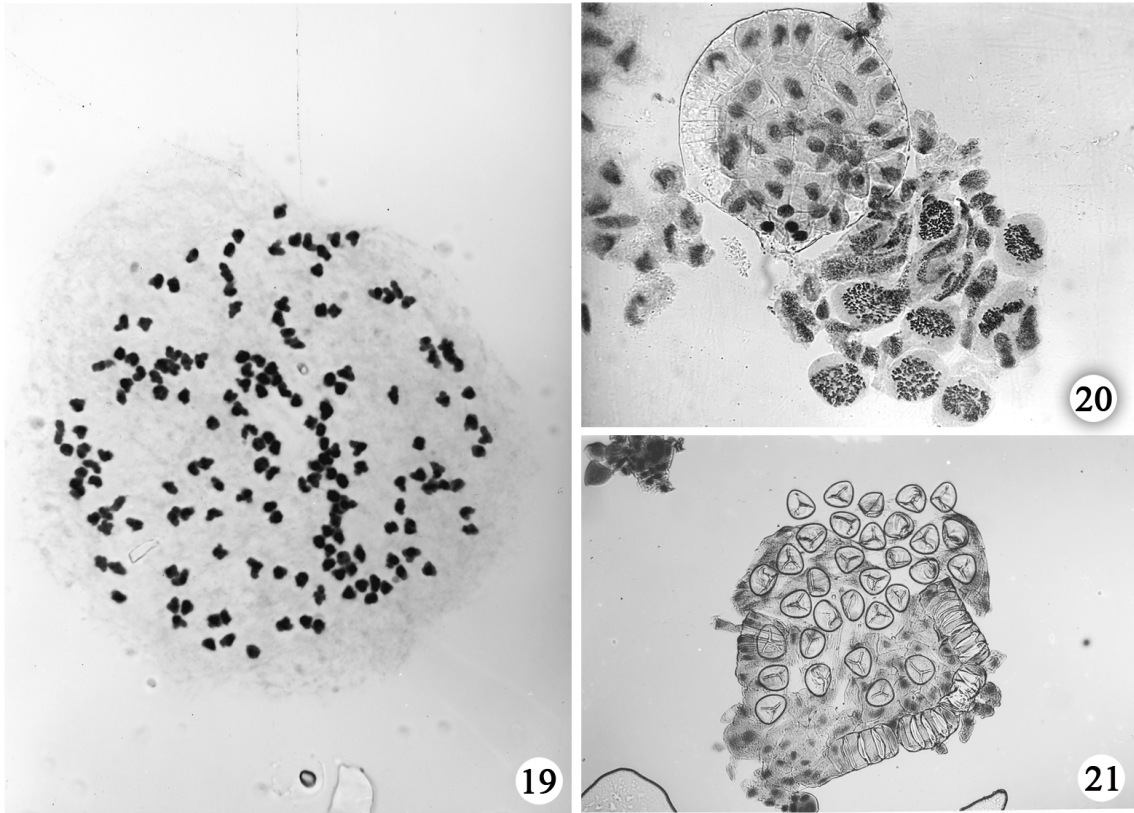
Figs 1–5: 1. *D. ludens*. SMC showing 120 bivalents. 2. Sporangium with 8 SMCs. 3–5. *H. arifolia* (cytotype I). 3. SMC showing 90 bivalents. 4. Root tip cell showing 60 chromosomes. 5. Sporangium with 8 SMCs at meiosis. (Figs. 1, 3, 4 x1000, Figs 2, 5 x250)



Figs 6–11: 6. *H. arifolia* (cytotype I). Sporangium with 32 spores. 7, 8. *H. arifolia* (cytotype II). 7. SMC showing 120 bivalents. 8. Sporangium with 8 SMCs. 9–11. *A. caudatum*. 9. SMC showing 60 bivalents. 10. Root tip cell showing 60 chromosomes. 11. Sporangium with 8 SMCs at meiosis. (Figs 7, 9, 10 x 1000, Figs 6, 8, 11 x 250)



Figs 12–18: 12–15. *A. latifolium*. 12. SMC showing $n = 60$ bivalents. 13. Root tip cell showing 60 chromosomes. 14. Sporangium with 8 SMCs. 15. Sporangium with 32 spores. 16–18. *A. philippens*. 16. SMC showing 90 bivalents. 17. Root tip cell showing 90 chromosomes. 18. Sporangium with 8 SMCs. (Fig. 12 $\times 1200$, Figs 13, 16, 17 $\times 900$, 14, 15, 18 $\times 250$)



Figs 19–21: *A. hispidulum*. 19. SMC showing 174 bivalents. 20. Sporangium with 8 SMCs. 21. Sporangium with 32 spores. (Fig. 19 x900, 20, 21 x250)

The premeiotic endomitosis (PE) and the meiotic first division restitution (MFDR) involve different modifications to the standard sporogenesis. Both PE and MFDR types usually begin with three successive mitotic divisions originating from the archesporial cell. The apomictics, undergoing the PE type, proceed to a fourth and final mitotic division, which is endomitotic, and produce eight SMCs, each with twice the parental chromosome complement. These mother cells undergo normal meiosis forming twice the number of ($2n$) bivalents, ultimately yielding 32 diplospores per sporangium as against the 64 in the sexual counterparts. Unlike the PE type, the MFDR pathway undergoes a normal cell division in the final mitosis also preceding meiosis, producing 16 spore mother cells. The first cellular division of meiosis in them is incomplete, the chromosomes fail to pair resulting in univalents, and a restitution nucleus formed prior to the onset of meiosis (Braithwaite 1964). The abbreviated meiosis ends in diads. In both the PE and MFDR types, the final meiotic outcome is 32 diplospores per sporangium, each containing $2n$ number of chromosomes, and thus the inherent requirement of apogamous ferns for an unreduced chromosome complement to compensate for the absence of sexual reproduction is satisfied

(Braithwaite 1964). Unlike with the MFDR type, the genome duplication that precedes meiosis in the PE type apomicts, can facilitate the pairing of and recombination between nonidentical hom(eo)ologous chromosomes during meiosis (Ootsuki et al. 2011). Although both the PE and MFDR pathways occur in ferns, the former is far more common (Walker 1985). The higher incidence of the PE type is attributed to genome stability imparted by high fidelity in bivalent chromosome pairing (Gastony & Windham 1989). The normal pairing phenomenon forming bivalents takes place because of the presence of two entirely homologous sets of chromosomes. In all the present apogamous taxa, irrespective of ploidy difference, regular diploid number of bivalent formation was evident in the spore mother cells.

Apogamy is one of the potential aspects of fern reproduction. The review of the progress of the study of apogamy in the past decades reveal that nearly half of the fern families contain apomictic species (Abraham et al. 1962, Braithwaite 1964, Gastony & Windham 1989, Grusz 2016, Hand & Koltunow 2014, Manton 1950, Mehra 1961, Ootsuki et al. 2011 and Walker 1985), most of which appearing in the larger families. Recent analyses reported that, in ferns as a whole, incidence of apogamy is correlated with species diversification and evolution (Liu et al. 2012, Hori et al. 2014 and Huang et al. 2011). The results also were in agreement with the concept of apomictic lineages themselves being apparently young, placing the ages of extant apomictic ferns in relatively recent evolutionary times (Grusz 2016). Nearly 10% of the ferns, for which reproductive modes have been determined, exhibit apomixis. The elevated frequency and distinctive meiotic characteristics of apogamy in ferns suggest that this form of asexuality may play dynamic role in fern evolution (Grusz 2016), and the evidences produced since 1950 in the study of apogamous ferns have laid strong foundation highlighting the significance of the phenomenon in fern evolution. In the apogamous ferns, the offspring from a sporophyte is expected to be clonal and genetically the same, and hence the amount of genetic variation within apogamous species would be low (Ootsuki et al. 2011). Despite the clonal nature of reproduction, many apogamous species of ferns show extensive morphological and genetic variation often forming species complexes in which the morphological species are difficult to distinguish because of their continuous variation (Hori et al. 2014). This is considered to be due to the apogamous species of ferns readily hybridizing with other sexual species producing apogamously reproducible taxa.

The recent literature on apogamous ferns indicates that substantial progress has been achieved in understanding of the phenomenon in ferns. Diverse contributions using both conventional and modern tools and techniques have highlighted the phylogenetic and evolutionary significance of the phenomenon of apogamy in ferns (Grusz 2016). Even so, the reproductive mode still remains undetermined fully for most fern taxa, and much remains to be explored as regards the origin of apomictic taxa, and the implication of apogamy in fern population dynamics, biogeography, colonization and even evolution. Traditional morphological and cytological techniques, combined with new technologies like DNA sequencing for assessing parentage in apomictic polyploids based on ISSR markers or whole genome sequencing for exploring DNA methylation in apomictic lineages offer

exciting prospects for future studies of apomixis and its role in fern evolution (Grusz 2016, Schneller & Krattinger 2010). The instance of two of the present species complexes such as *H. arifolia* and *A. caudatum* assumes importance. One of the cytotypes of *H. arifolia* is an apogamous triploid with $n = 2n = 90$, and the other apogamous tetraploid with $n = 2n = 120$; while both the cytotypes of *A. caudatum* are diploid, of which one sexual with $n = 30$ and $2n = 60$, and the other apogamous with $n = 2n = 60$. In either case of species complexes, there is recognizable degree of plant morphological difference apparent between the cytotypes, especially as regards the size of fronds and sporophylls; and the matter merits further detailed study, which is underway aimed at probing into the role of apogamy in species diversification and evolution of ferns of this region.

REFERENCES

- ABRAHAM A, NINAN C A & MATHEW P M 1962 Studies on the cytology and phylogeny of the Pteridophytes VII Observations on one hundred species of South Indian ferns *J Indian Bot Soc* **41** 339–421
- BRAITHWAITE A F 1964 A new type of apogamy in ferns *New Phytologist* **63** 293–305
- COPELAND E B 1947 *Genera Filicum* Waltham Mass Chronica Botanica Co London
- DARLINGTON C D 1939 *The evolution of genetic systems* Cambridge University Press London
- DOPP W 1932 Die apogamic *Aspidium* AL Br *Planta* **17** 87–152
- FARLOW W G 1874 An asexual growth from the prothallus of *Pteris cretica* *J Cell Sci* **14** 266–272
- GASTONY G J & WINDHAM M D 1989 Species concepts in Pteridophytes The treatment and definition of apogamosporous species *Am Fern Jour* **79** 65–77
- GRUSZ A L 2016 A current perspective on apomixis in ferns *Inst Bot Chinese Acad Sci* **54** 656–665
- HAND M L & KOLTUNOWA M G 2014 The genetic control of apomixis Asexual seed formation *Genetics* **197** 441–450
- HORI K, AKITAKA T, FUJIMOTO K, KATO J, EBIHARA A, WATANO Y & MURAKAMI N 2014 Reticulate evolution in the apogamous *Dryopteris varia* complex (Dryopteridaceae: subgenus *Erythrovariae* sect *Variae*) and its related sexual species in Japan *J Pl Res* **127** 661–684
- HUANG Y M, HSU S Y, HSIEH T H, CHOU H M & CHIOU W L 2011 Three *Pteris* species (Pteridaceae: Pteridophyta) reproduce by apogamy *Bot Stud* **52** 79–87
- LIU H M, DYER R J, GUO Z Y, MENG Z & SCHNEIDER H 2012 The evolutionary dynamics of apomixis in ferns: A case study from Polystichoid ferns *J Bot* **10** 57–68
- LOVIS J D 1977 Evolutionary patterns and processes in ferns *Adv Bot Res* **4** 229–415
- MANTON I 1950 *Problems of cytology and evolution in the Pteridophytes* Cambridge University Press Cambridge
- MANTON I & SLEDGE W A 1954 Observations on the cytology and taxonomy of the Pteridophyte flora of Ceylon *Phil Trans* **238 B** 127–185
- MATHEW P M & CINTHYA CHRISTOPHER 2018 Cytology in the systematics phylogeny and evolution in plants I Pteridophytes *J Cytol Genet* **19** (NS) 47–53
- MEHRA P N 1961 Chromosome numbers in the Himalayan ferns *Res Bull Punjab Univ Sci (NS)* **12** 139–164

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- NINAN C A & MATHEW P M 2016 *Ferns and Lycophytes of Kerala Taxonomy Cytology and Evolution* Jawaharlal Nehru Tropical Botanic Garden and Research Institute Thiruvananthapuram
- OOTSUKI R, SHINOHARA W, SUSUKI N & MURAKAMI N 2011 Genetic variation in the apogamous fern *Cyrtomium fortunei* (Dryopteridaceae) *Acta Phytotax* **62** 1–14
- SCHNELLER J & KRATTINGER L 2010 Genetic composition of Swiss and Austrian members of the apogamous *Dryopteris affinis* complex (Dryopteridaceae Polypodiopsida) based on ISSR markers *Plant Syst Evol* **286** 1–6
- TAKAMIYA M 1996 *Index to chromosomes of Japanese Pteridophyta (1910–1916)* Japan Pteridological Society Tokyo
- WALKER T G 1985 Some aspects of agamospory in ferns – The Braithwaite system *Proc Roy Soc B Biological Sciences* **86** 59–68