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Chromosome numbers of some species of *Pteris* (Pteridaceae) in Java, Indonesia

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Abstract. *Praptosuwiryo TNg, Mumpuni M. 2018. Chromosome numbers of some species of Pteris (Pteridaceae) in Java, Indonesia. Biodiversitas 19: 2118-2126. Pteris* L. (Pteridaceae) is a large fern genus consisting of about 250 species which distributed predominantly in tropical and subtropical countries. The genus grows in a diversity of ecosystems, either terrestrially or lithophytically, although most species occur in the forest. A study of the cytology of *Pteris* in Java is being undertaken for the conceptual understanding of the interrelationships between various fern species in the Malesian region. This aims of our study are: (1) to observe somatic chromosome number of some species of *Pteris* in Java; (2) to determine the reproduction types of the observed species by counting the spore number per sporangium; (3) to discuss polyploidy in the species in relation to plant morphological variation across their geographical distribution. Chromosome counts for eight species are reported. *Pteris biaurita* is an apogamous diploid species (2n = 58). *Pteris ensiformis* var. *ensiformis* has chromosome numbers of 2n = 87 (apogamous triploid) and 2n = 116 (sexual tetraploid), while *P. ensiformis* var. *victoriae* has a chromosome number 2n = 58 and is a sexual diploid. *Pteris fauriei* is an apogamous triploid species (2n = 87). *Pteris longipinnula* has a chromosome number of 2n = 116 (tetraploid). *Pteris multifida* and *P. vittata* are sexual tetraploids (2n=116). *Pteris tripartita* has two ploidy levels (sexual diploid and tetraploid). Another currently unplaced Javanese *Pteris* sp. has 2n = 87 (apogamous triploid). Diploid *P. biaurita*, tetraploid *P. ensiformis*, triploid *P. fauriei*, diploid *P. tripartita*, and teraploid *P. vittata* are new record cytotypes for Central Java. Polyploidy in the genus in relation to plant morphological variation across its geographical range is discussed.

Keywords: Chromosome, Java, *Pteris*

INTRODUCTION

Chromosome number is the most basic feature concerning the genome of a species (Peruzzi et al. 2012). Analyses of chromosome numbers are very important as they represent a fundamental step in the study of any group of organisms. Chromosome counts provide indispensable information on genetic discontinuities within and among species, and they contribute for the understanding of phylogenetic relationships at all taxonomic levels (Windham and Yatskievych 2003). Cytological studies have resolved various taxonomic problems and have revealed details of evolutionary patterns and speciation processes in Pteridophytes (Lovis 1977). Because it is considered that ferns have been developed in evolution by hybridization and doubling (Wagner 1954).

As pointed out by Ivanova & Pie˛kos-Mirkowa (2003) polyploidy seems to be the most important processes among the processes that have played significant roles in pteridophyte differentiation and speciation in ferns. Cytological and cytogenetic investigations have revealed that polyploidy is very common in Pteridophyta. It was estimated that polyploidy in ferns as high as 95% (Walker 1979; Grant 1981). The majority of polyploid ferns appear to be allopolyploids, and the success of polyploidy seems closely associated with hybridization (Bir 1988, Cubas 1989).

Pteris is a fairly large fern genus which consisting of about 250 species (Holttum 1954; Tryon et al. 1990). Morphologically, *Pteris* species are recognized by their marginal sori supported by a collecting vein and protected by an indusium formed by the reflexed lamina margin, and by trilete spores which are usually with an equatorial cingulum (Holttum 1954; Martínez 2016). *Pteris* contains herbaceous perennials with short-creeping to erect, usually compact rhizomes, stalked fronds with laminae divided into a simply pinnate to bipinnate arrangement, or sometimes tripartite, usually with the basal pinnae bearing an enlarged basiscopic pinnule (Holttum 1966; Prado and Windisch 2000; Martínez 2011). It is a cosmopolitan fern genus which distributed in temperate, subtropical, and tropical regions of the world, extending to the south in China, New Zealand, and Tasmania, west to South Africa, north to Korea and Japan, across the United States, as well as the Mediterranean region of Europe (Copeland 1947; Tryon and Tryon 1982). This genus grows either terrestrially or lithophytically (on rocks) on a diverse ecology although most species occur in forest, frequently secondary forests in opening or along rocky stream banks, coastal areas and xeric niches (Copeland 1947; Chao et al. 2014).

Many species of *Pteris* have several ploidy levels (Walker 1966; Wang 1989; Praptosuwiryo 2003; Praptosuwiryo and Darnaedi 2008; Chao et al. 2012a, 2012b; Jaruwattanaphan et al. 2013; Nakato and Ebihara 2016) and are found in several geographical areas, such as *P. cretica* and *P. vittata* (Manton 1950; Khare and Kaur 1983; Wang 1989) which likely reflect the ecological differentiation within species. The first extensive cytological study of the genus *Pteris* was carried out by Walker (1966). This study included 82 species and reported that the base number of chromosomes in *Pteris* is 29. Recently data on the cytological of 106 *Pteris* species were reviewed by Chao et al. (2012b). It is presumed that about 60% of *Pteris* species are polyploids, including triploids, tetraploids, pentaploids, hexaploids, and octoploids (Walker 1956, Kuriachan and Ninan 1976; Wang 1989; Chao et al. 2012b).

It is estimated that Sout East Asia has about 80 species of *Pteris* (Schneider and Rusea 2003). Malesian region has more than 40 species of *Pteris*. In Java, Backer and Posthumus (1939) recorded 19 species of *Pteris*. Praptosuwiryo (2013) reported two new species record of *Pteris* for Java, namely *P. insignis* and *P. walichiana*, from Mount Slamet. Nevertheless recently reports on the cytological study of this genus in Malesian region are only a few. The first cytological record on *Pteris* of Jawa was reported by Walker (1962), *viz*. diploid type of *P. biaurita*, but he did not mention the specific locality of the voucher specimen. Darnaedi (1992) reported three species from Gede-Pangrango National Park, West Java, namely: *P. biaurita, P. pellucida,* and *P. longipinnula*. Zubaidah (1998) studied cytology of *P. biaurita* in East Java. Praptosuwiryo (2003) reported one species from Lombok Island, *P. venulosa* Bl. (2n = 3x). Praptosuwiryo and Darnaedi (2008) reported chromosomes account on six wild species of *Pteris* from Bogor Botanic Gardens, viz.: *P. biaurita* (2n = 58), *P. ensiformis* (2n = 116), *P. fauriei* (2n = 87), *P. multifida* (2n = 116), *P. tripartita* (2n = 58 and 2n = 116) , and *P. vittata* (2n = 116). Hastuti et al. (2011) reported two ploidy level of *P. multifida,* apogamous triploid (2n=3x) and sexual tetraploid (2n=4x). Mumpuni et al. (2015) studied on the intraspecific polyploid of *P. vittata* from Java and found apogamous pentaploid (2n=5x) and sexual tetraploid (2n=4x). Efendi et al. (2014) reported chromosome account of *Pteris ensiformis* var. *victoriae* (2n=58 and 2n=87) and var. *ensiformis* (2n=116).

Here we continue cytological study on *Pteris* of the Malesian fern flora by choosing some species of *Pteris* and collecting plants material from some localities of Java. This study contributes to the interrelationships concepts of the various fern species in Malesian region, especially to support data that may be helpful to phylogenetic studies of the genus *Pteris*. The aims of this study are: (i) to observe somatic chromosome number of some species of *Pteris* in Java; (ii) to determine the reproduction types of the observed species by counting the spore number per sporangium; (iii) to discuss polyploidy in the species in relation to plant morphological variation across their geographical distribution.

MATERIALS AND METHODS

Plant specimen

A number of living plants specimen for the cytological studies were collected from the natural population of some locations in Java (Table 1.). Collected specimens were identified by consulting Backer and Posthumus 1939, Holttum (1966), and Edie (1978). Voucher specimens were deposited at BOHB (Herbarium of Bogor Botanical Gardens).

Chromosome observation

Preparation of chromosome samples was carried out by using squash method as this method can be used as an alternative to ensure good physical separation of chromosomes (Fukui 1996). The preparation procedure followed the procedures developed by Manton (1950) and modified by Praptosuwiryo and Darnaedi (2008). Root tips were pretreatment in 0.002 M 8-hydroxyquinoline solution for 24 hours at 3-4º C. They were fixed in 45% acetic acid for 10 minutes and macerated in a mixture of CH3COOH 45 %: I N HCl = 1 : 3 at 60º C for 3-4 minutes. The fixed roots were stained and squashed in aceto-orcein solution. Chromosome observation was carried out under the microscope by using 1000x magnification.

Reproduction types observation

Reproduction mode was determined by spore counting for each sporangium by following procedures described by Manton (1950), Knobloch (1966), Lovis 1977 and Walker (1979, 1984). Each individual plant was taken 10-15 spores for counting under a binocular microscope. The spore number was recorded as “64 s/s” if approximately 64 spores each were counted at least in two sporangia, and as ‘32 s/s” if approximately 32 spores each were counted (Ebihara et al. 2014). The spores number per sporangium is related to the reproduction mode. Plants with 64 spores generally have sexual reproduction, whereas those with 32 or 16 spores usually have apogamous reproduction (Knobloch 1966; Walker 1984).

RESULTS AND DISCUSSION

New data on chromosome numbers and reproduction types of eight species of 33 individuals plants collected from five localities in Java are reported (Table 1 and Figure 1.). There is only one ploidy level on *P. biaurita*, apogamous diploid. *Pteris ensiformis* var *ensiformis* shows the chromosome number of 2n = 87 (apogamous triploid) and 2n = 116 (sexual tetraploid), while *P. ensiformis* var. *victoria* reveals 2n = 58 (sexual diploid). *Pteris fauriei* is apogamous triploid (2n = 87). *Pteris longipinnula* shows chromosome number 2n = 116 (tetraploid). *Pteris multifida* and *P. vittata* are sexual tetraploids (2n=116). *Pteris tripartita* has two ploidy level (sexual diploid and tetraploid). *Pteris* sp. has 2n = 87 (apogamous triploid). New record cytotypes of *Pteris* for Central Java are reported, *viz.* diploid *P. biaurita*, tetraploid *P. ensiformis*, triploid *P. fauriei*, diploid *P. tripartita* and teraploid *P. vittata*. Discussion for each species is presented.

Table 1. Chromosome Number and Reproduction Types of *Pteris* in Java, Indonesia

Species	Voucher	Chromosome number (2n)/ ploidy level	Types of reproduction	Locality
Pteris biaurita L.	TNgP 3219	58/diploid	-	Petak 41a, Northern Slope of Mt. Slamet, Desa Karang Mangu, Kec. Pulosari, Kab. Pemalang (RPH Karang Sari, BKPH Moga, KPH Pekalongan Barat). 1300 m asl., Central Java.
	MM 144	58/diploid	Apogamous	Bogor Botanical Gardens, West Java. Ca. 250 m asl.
	TNgP & YF 25	58/diploid	Apogamous	ibid.
	TNgP & YF 17	58/diploid	Apogamous	Ibid.
	TNgP & NI 03	58/diploid	Apogamous	Among *Acalypa siamensis,* Ngadirejo country, Mojosongo sub-district, Boyolali District, Central Java. Ca. 295 m asl.
	TNgP & NI 06	58/diploid	Apogamous	Near ravine and bridge, between Masahan-Wates Country, Mojosongo Sub-district, Boyolali District, Central Java. Ca. 300 m asl.
P. fauriei Hieron	MM 156	87/triploid	Apogamous	Bogor Botanical Gardens, West Java. Ca. 250 m asl.
	TNgP & YF 11	87/triploid	Apogamous	Ibid.
	TNgP & NI 05	87/triploid	Apogamous	Near ravine and bridge, between Masahan-Wates Country, Mojosongo Sub-district, Boyolali District, Central Java. Ca. 300 m asl.
	TNgP & NI 07	87/triploid	Apogamous	ibid.
	TNgP & NI 08	87/triploid	Apogamous	Ibid.
	TNgP & NI 10	87/triploid	Apogamous	Ibid.
P. ensiformis var. *ensiformis* Burm. f.	TNgP 2719 c	87/triploid	Apogamous	Bringin, Semarang, Central Java.
	TNgP 2719	116/tetraploid	-	Ibid.
	DD s.n. 1 May 2000	116/tetraploid	-	Bogor, West Java.
	MM 157	116/tetraploid	Sexual	Bogor Botanical Gardens, West Java. Ca. 250 m asl.
	MM 152	116/tetraploid	-	Ibid.
	TNgP & NI 08	116/tetraploid	-	Near ravine and bridge, between Masahan-Wates Country, Mojosongo Sub-district, Boyolali District, Central Java. Ca. 300 m asl.
P. ensiformis var. victoriae Baker	TNgP s.n.	58/diploid	Sexual	Bogor, West Java.
P. longipinnula Wall. ex J. Agardh	MM 161	116/tetraploid	-	Cibodas Botanical Gardens, West Java. Ca. 1270 m asl.
	MM 163	116/tetraploid	-	Ibid.
P. multifida Poir.	MM 145	116/tetraploid	Sexual	Paledang, Bogor, West Java. 250 m asl.
P. tripartita Sw.	MM 158	58/diploid	Sexual	Bogor Botanical Gardens, West Java. Ca. 250 m asl.
	MM 142	116/tetraploid	-	ibid.
	MM 148	116/tetraploid	-	Ibid.
	TNgP & YF 04	58/diploid	Sexual	Bogor Botanical Gardens, West Java . Ca. 250 m asl.
	TNgP & YF 26	58/diploid	Sexual	ibid.
	TNgP s.n. (20 Feb. 2015)	58/diploid	Sexual	Ibid.
	TNgP s.n. (13 Arpil 2015)	58/diploid	Sexual	Bogor Botanical Gardens, West Java.
	TNgP & NI 04	58/diploid	-	Among *Acalypa siamensis* plants, Ngadirejo country, Mojosongo sub-district, Boyolali District, Central Java. Ca. 295m asl.
P. vittata L.	MM 149	116/tetraploid	Sexual	Paledang, Bogor, West Java Province. Ca. 250 m asl.
	TNgP & NI 02	116/tetraploid	Sexual	Ngadirejo Country, Mojosongo Sub-district, Boyolali District, Central Java. Ca. 285 m asl.
Pteris sp.	MM 185	87/triploid	Apogamous	Curug Nangka, Bogor , West Java.

Note: DD = Dedy Darnaedi; MM = Mugi Mumpuni; NI = Niko Islamika; TNgP = Titien Ng. Praptosuwiryo; YF = Yogi Febriyanto.

***Pteris biaurita* L.**

Pteris biaurita is regarded as a pantropic species and occurs both in lowland and mountains, in moist places. It is differentiated from other species of *Pteris* by characters as follows: Basal pinnae branched but not longer than the others; the lamina on the basiscopic side of the midrib of a pinna is nearly wider than that on the acroscopic side; veins forming a regular series of narrow areoles on the side of the costa (Holttum 1966). The thin leaf brake fern *P. biaurita* L. in Java usually is found in semi opened and or shady places with humid condition on humus-rich soil as well as on semi-sandy soil (Backer and Posthumus 1939).

The beginning chromosome account of *P. biaurita* was reported by Walker (in Manton and Sledge 1954), Abraham et al. (1962), Walker (1962, 1966) and Roy and Sakya (in Fabbri 1963). They found the diploid type of *P. biaurita*. Apogamous diploid of *P. biaurita* was first reported from Jamaica (1966). Praptosuwiryo and Darnaedi (2008) reported apogamous diploid of *P. biaurita* growing spontaneously in the Bogor Botanic Gardens in moist and fairly shady place.

In the present study, two individuals of *P. biaurita* from West Java (MM 144) and Central Java were found to be a diploid apogamous with 32 spores per sporangium. Punetha and Sen (1989) reported *P. biaurita* from Kumaon (northwest Himalaya) as apogamous triploid, chromosome number n = 87 with 32 spores per sporangium. In Malang, East Java, Zubaidah (1998) found apogamous diploid and apogamous tetraploid *P. biaurita*. Interestingly, we recognize three cytotypes of *P. biaurita* in its distribution area, namely apogamous diploid, apogamous triploid and apogamous tetraploid, but we have not found its sexual types, yet. Further cytotaxonomical studies are needed to unravel this species complex.

***Pteris ensiformis* Burm. f.**

Pteris ensifromis is distributed in Ceylon and South India, North India and China, through Malesia to Australia and Polynesia (Holttum 1966). This species is conspicuously common fern in Java. In Java, this species consists of two varieties, namely *P. ensiformis* var. e*nsiformis* and *P. ensiformis* var. v*ictoriae*. The first variety is widely distributed in Java, whereas the second variety is commonly found as ornamental cultivation and the locality of origin is not reported. The two varieties are distinguished by the coloration of the lamina. *Pteris ensiformis* var. *ensiformis* has full green color of lamina whereas *P. ensiformis* var. *victoriae* shows white and green coloration of lamina.

It was observed that the two varieties of *P. ensiformis* also show differences in chromosome number or ploidy level. *Pteris ensiformis* var. *ensiformis* has two ploidy levels, viz. sexual tetraploid (2n = 116) and apogamous triploid (2n = 87), whereas *P. ensiformis* var. *victoriae* shows only sexual diploid (Table 1.) Cytological information on *P. ensiformis* is more distributed than other species of *Pteris*. Brownlie (1965) recorded n = 58 from Kalabere, New Caledonia, without information of its spore number per sporangium or reproduction type. Wang (1989) reported sexual tetraploids with n=58 from Chine without information on its variety. Tetraploid sexual was also found in Jamaica (Walker 1966). Efendi et al. (2014) reported new cytotypes of *Pteris ensiformis* var. *victoriae* and one cytotype of var. *ensiformis* from Indonesia; *Pteris* var. *victoriae* with 2n=58 (sexual diploid) was from Gorontalo, North Sulawesi, and 2n=87 (triploid) from Lombok Island and Bogor, West Java, and P. *ensiformis* var. *ensiformis* with 2n=116 (sexual tetraploid).

***Pteris fauriei* Hieron.** In comparison with the closely related species of *P. fauriei, P. latipinna* Y.S.Chao & W.L.Chiou, Chao et al. (2017) described the morphological diagnostic characteristics of *P. fauriei* var. *fauriei* as follows. Lamina 15-40 cm long, 10-35 cm wide; length/width ratio 1.2-1.5. Lateral pinnae of sterile fronds 2-7 pairs, straight, sessile or short petiolate, lanceolate, not narrowed at base, 2-3.5 cm width; basal pinna-segments free to the rachis. Terminal pinnae are smaller than lateral pinnae. *Pteris fauriei* is distributed in Japan, China, the Ryukyu Islands, Taiwan and Vietnam (Shieh 1975, 1994).

Roy and Holttum (1965) reported diploid *P. fauriei* from Southern China. Kato et al. (1992) reported apomixes triploid of *P. fauriei* of Yunnan, Southwestern China. In Taiwan, two varieties of *P. fauriei* have been confirmed, and both varieties have different cryptic characteristics and prefer different niches (Chao et al. 2017). *Pteris fauriei* Hieron. var. *fauriei*, apomictic and triploid (2n = 87), usually has herbaceous laminae and prefers cooler sites; *P. fauriei* var. *minor* Hieron., sexual and diploid (2n = 58), usually has coriaceous laminae and is found in warmer sites (Huang et al. 2006, Huang et al. 2007). The two varieties can also be differentiated by their gametophyte characteristics. *Pteris fauriei* var. *minor* produces sporophytes by sexual mode, whereas *P. fauriei* var. *fauriei* produces sporophytes by apogamy (Huang et al. 2006). In Taiwan, in general, triploids are found at higher elevations than the diploids (Huang et al. 2007). Roy and Holttum (1965) stated that *P. fauriei* of China and Japan belongs to the complex of *P. quadriaurita* Ret.

***Pteris longipinnula* Wall. Ex J. Agardh.**

The first record on chromosome account of *P. longipinnula* was reported by Walker (1962). Walker (1962) found three cytotypes of *P . longipinnula*, *viz*. diploid (2n = 58), triploid (2n = 87), and tetraploid (2n = 116). Present study only reported two individuals of tetraploid collected from Mt. Gede, Gede-Pangrango National Park, West Java. Darnaedi (1992) also reported a tetraploid type (2*n*=116) from Mt. Gede. Diploid and triploid type of *P. longipinnula* have not been recorded from Java.

Holttum (1966) stated that *P. longipinnula* is one of the species of the group of *P. quadriaurita,* which is so polymorphic in the tropics of both Old and New Worlds. Holttum (1966) explained that typically *P. longipinnula* has green stipe and rachis, broad pinnae narrowed at the base and caudate at the apex, the lobes broad, with sori almost from base to apex, the veins only once forked, the costal spines short and costular spines few. Based on specimens collected from Malay Peninsula, Holttum (1966) recognized three groups of specimens which are out of the

typical forms: (i) with narrower pinnae, the lowest branched; (ii) with somewhat narrower pinnae which are often broadly cuneate at the base and only slightly caudate at the apex, narrower and closer lobes, the basal pinnae branched, stipe and rachis more or less flushed with purple. This group comes somewhat between *P. longipinnula* and *P. asperula* J. Sm.; (iii) Pinnae narrow (lowest 2,5-3,5 cm wide), with 10-24 pairs of veins, rather shortly caudate; veins forked well above their bases; lobes about 5 mm wide above the base; basal pinnae forked; old stipes and rachis a little flushed with purple. These plants are intermediate between *P. biaurita* and *P. longipinnula*. Further studies for these complex species from their geographical range should be done to unravel their status. As stated by Holttum (1966) that many specimen collections that cover all stages of development, from young to fully mature plants must be studies before we can judge their status. The morphological variation occurs owing to environmental differences should be found out to judge their status. Therefore further study on the cytotaxonomy of *P. longipinnula* and its closely related species need to be done.

***Pteris multifida* Poir.**

The spider brake fern, *P. multifida,* is recognized by morphological characters as follow: Lamina simple pinnate; terminal pinnae linear-oblanceolate; lateral pinnae 1-4 pairs; basal pinnae 1-3 branched; upper portion of rachis winged (Edie 1978). Morphologically, *P. multifida* is similar to *P. cretica*. However, the differentiation between two species can be recognized by the following characteristics. The mature lower pinnae of *P. multifida* regularly show both acroscopic and basiscopic lobes. In *P. cretica,* lobes are formed on the basiscopic sides only. The decurrent part of terminal pinnae of *P. multifida* are longer and run parallel to the rachis, whereas the decurrent part of the terminal pinnae of *P. cretica* are shorter. *Pteris multifida* is widely distributed in China and Japan, and also widely cultivated in other countries (Roy and Holttum 1965).

Cytological observations of the spider brake fern have been carried out by several researchers, such as in South China (Roy and Holttum 1965),) Japan (Mitui 1980), Ceylon, Hongkong, Himalaya (Walker 1962), and Java (Praptosuwiryo and Darnaedi 2008). Results of these studies revealed that *P. multifida* has infraspecific variation. Three ploidal levels were found, namely diploid type (Walker 1962), triploid type (Praptosuwiryo and Darnaedi 2008), and tetraploid type (Walker 1962; Roy and Holttum 1965; Mitui 1980; Praptosuwiryo and Darnaedi, 2008).

We report only one individual cytological record for *P. multifida*, viz. MM 145 with tetraploid sexual (Fig)**.** Recently a cytological study on *P. multifida* from Bogor, West Java, also showed two ploidy level, namely apogamous triploid (2n = 87) and sexual tetraploid (2n = 116) (Hastuti et al. 2011). The two cytotypes are very hard to be differentiated morphologically, but anatomically the two cytotypes can be differentiated by their epidermal cell number. The sexual tetraploid type has a greater number of epidermal cell than those apogamous triploid type.

***Pteris tripartita* Sw.**

Pteris tripartita, in abroad sense, is very widely distributed in the tropics of the Old World (including Africa), Australia and Polynesia (Holttum and Roy 1965, Holttum 1966). This species can be found in the lowlands and at moderate elevations on the mountains, in open ground, well-drained but moist places. *P. tripartita* can be recognized by diagnostic characters as follow: Frond tripartite, the middle branch deeply bipinnatifid. The lateral branches as long as the middle branch, each with large secondary bipinnatifid branch on the lower side towards the base, the lowest basal pinna on this branch usually bearing several pinnatifid leaflets on its lower side, the lamina on its upper side lobed as in the other pinnae. The texture of lamina thin, herbaceous; veins forming a series of narrow costa areoles, each the series of costa areoles continuous from one costule to the next, a series of shorter areoles on either side of the costule almost to its apex (Holttum 1966).

All plants so named hitherto examined cytologically before Holttum and Roy (1965) have been tetraploid. Now, in this study, we report two cytological types of *P. tripartita*, diploid and tetraploid. Recently finding of tetraploid plants of Jawa was reported by Praptosuwiryo and Darnaedi (2008). The first diploid of *P. tripartita* was reported by Holttum and Roy (1965) from New Guinea, East Malesia. Diploid type of *P. tripartita* from West Malesia (West Java: wild plants of Bogor Botanical Gardens) was reported by Praptosuwiryo and Darnaedi (2008). The diploid type of *P. tripartita* from New Guinea has venation typical of *P. tripartita*, but lobes of pinnae are closer together than in the lowland form of the species which is common in Malaya (Holttum and Roy 1965). However, Praptosuwiryo and Darnaedi (2008) did not find the morphological differences between the diploid and tetraploid type of *P. tripartita* in West Java. Further studies dealing with cytomorphology of *P. tripartita* in its areas distribution range are needed.

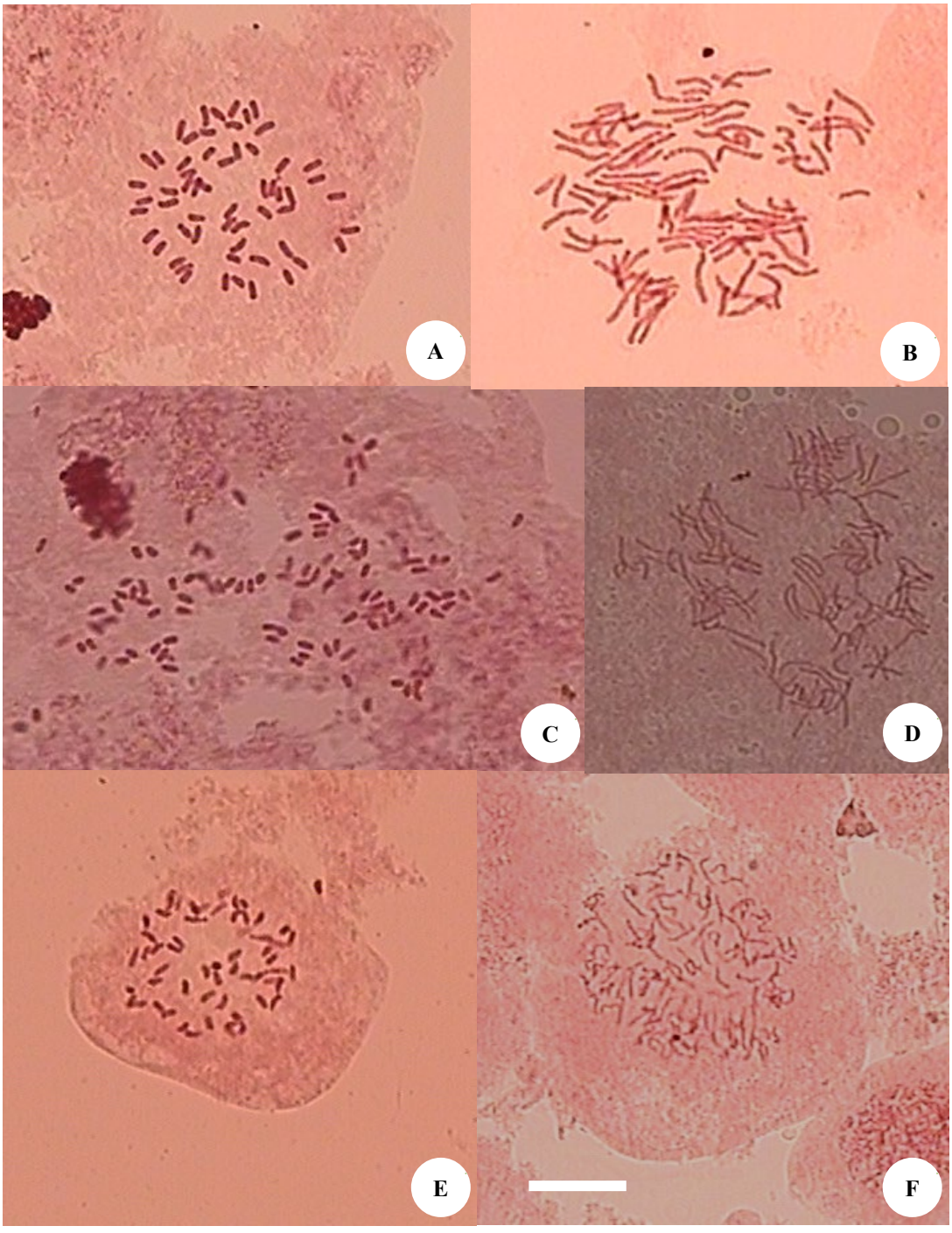
***Pteris vittata* L.**

Pteris vittata is very widely distributed in the tropics and subtropics of the Old World. This plant is also one of the common species in Java. In Java**,** it can be found from the lowlands to the higher coverage until approximately 2000 m, in the open, sunny to the slightly shaded area, steep earth, and rock wading, on old walls, especially on limestone rocks, also occurring elsewhere, often in great numbers together (Backer and Posthumus 1939).

This species is very easy to be recognized in the field by diagnostic characters as follow: Frond simply pinnate with a terminal pinna like the lateral ones; pinnae numerous, 1-4 cm apart, middle ones longest, basal ones gradually reduced and often very short, upper ones only slightly reduced and much shorter than the terminal pinna except in larger fronds, all pinnae sessile and oblique. Veins free, nearly at right angle to the costa, usually forked near the costa. Sori continuous from near the base to near the apex of each pinna (Holttum 1966). This species is highly valued as an ornamental plant (Srivastava et al.

2007). It has recently been reported as phytoremediator; this species can accumulate a large concentration of arsenic in its aboveground biomass (up to 23,000 mg per kg) (Ma et al. 2001).

Cytological study on the ladder brake fern *P. vittata* is very interesting as this species is widely distributed in the world with multi-level habitats. Cytological study on *P. vittata* from Chine by Wang (1989) showed that this species is a species complex which includes several cytotypes. Wang (1989) reported a sexual tetraploid from south Guangdong and south Yunnan. He also added that there were a sterile triploid with the chromosome number of n=201 Ⅰ +26 Ⅱ +5 Ⅲ and a sterile tetraploid hybrid with the chromosome number of n=9 Ⅰ +45 Ⅱ +3 Ⅲ +21 Ⅴ from south Yunnan and south Guangdong respectively. As stated by Wang (1989) the distribution pattern of different cytotypes in *P. vittata* complex may indicate that the tropics is more favorable to the formation and surviving of polyploid than the subtropics. However, Wang (1989) also stated that its natural ancestral diploid had been widely found in the subtropical regions of China, such as Sichuan, Guizhou, Yunnan and Hubei Provinces.



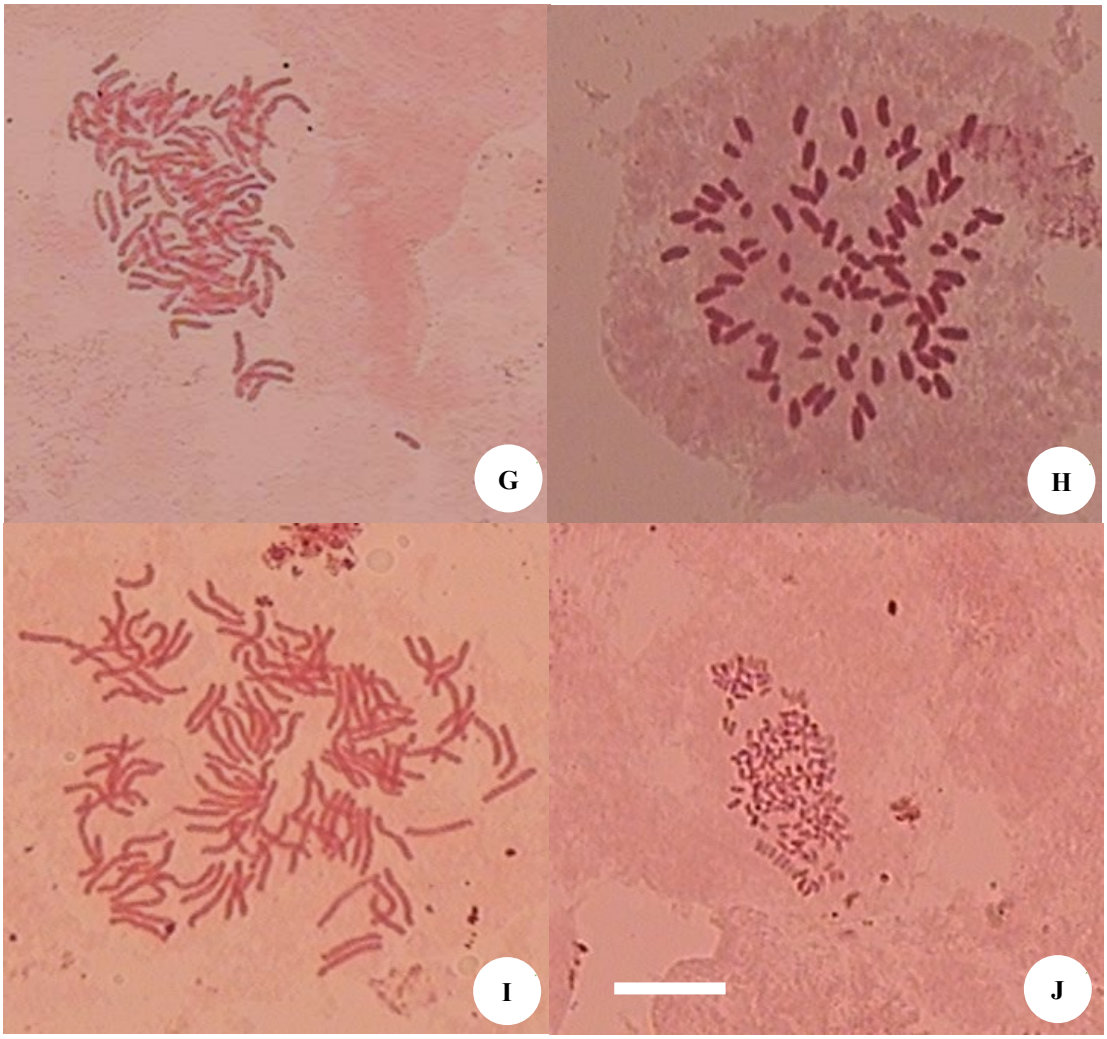


Figure 1. Somatic chromosome numbers of *Pteris*. A. *Pteris biaurita* (TNgP & YF 25, 2n = 58); B. *Pteris fauriei* (MM 156, 2n = 87); C. *Pteris ensiformis var ensiformis* (TNgP & NI 08, 2n = 116); D. *Pteris tripartita* (MM 142, 2n = 116); E. *Pteris tripartita* (TNgP & YF 26, 2n = 58); F. *Pteris multifida* (MM 145, 2n = 116); G. *Pteris sp.* (MM 185, 2n = 87); H. *Pteris fauriei* (TNgP & NI, 2n = 87); I. *Pteris longipinnula* (MM 163, 2n = 116); J. *Pteris vittata* (TNgP & NI 02, 2n=116). Scale bar = 10 µm for all.

As reviewed by Chao et al. (2012), the Chinese ladder brake *P. vittata* shows considerable morphological variations and wide geographical distribution throughout the world. Diverse ploidy levels and reproductive modes have been recorded, including sexual diploids, triploids, sexual and apogamous tetraploids, pentaploids, and hexaploids (Grant 1981; Walker 1962; Khare and Kaur 1983a; Wang 1989; Tsai and Shieh 1984; Roy et al. 1971; Kato et al 1992; Mitui 1976; Srivastava et al. 2007). The variability of the ploidy in *P. vittata* revealed that we are dealing with an apogamic species-complex (Brullo et al. 1982), as has already been verified in other species of this genus (Walker 1962; Verma and Khullar 1965). Among these polyploids, tetraploid is the most common whereas pentaploid and triploid are rare (Khare and Kaur 1982b). A similar situation was also reported from Java, pentaploid *P. vittata* were rarer among the most common cytotype, tetraploid type (Mumpuni et al. 2015).

Diploid type of *P. vittata* is also rare in the world. Sexual diploid was found in Yunnan, Southwestern China (Kato et al. 1992). Bronwlie (1965) reported *P. vittata* with chromosome number n = 58 from Kalabere, New Caledonia, but there was no information on its type reproduction of this record so that we do not recognize whether this record was apogamous diploid or sexual tetraploid. Cytological surveys on *P. vittata* conducted by Srivastava et al. (2007) in India indicated that at least four cytotypes, namely diploid, triploid, pentaploid and hexaploid, are threatened in their native habitats. The diploid type of *P. vittata* from the Malesian region has not been reported yet (Praptosuwiryo and Darnaedi 2008; Mumpuni et al. 2015). Therefore most of the cytotypes of *P. vittata* reported from many locations in the world were tetraploid.

In this study, *P. vittata* is found to be tetraploid sexual (2n = 116). This plant was collected from lowland areas,

ca. 250-300 m asl. Cytological observation of *P. vittata* of Himalaya (Bowali, Kumaon, Nortwest Himalaya) which was growing at 1800 m asl. revealed sexual diploid (2n = 58) (Punetha and Sen 1989). It is clearly proved that the ploidy level in *P. vittata* is not affected by altitude. Recently the cytological report on *P. vittata* in Java (Mumpuni et al. 2015) showed also that the ploidy level of this species was not affected by altitude. The tetraploid and pentaploid *P. vittata* were found in both lowland and mountainous area.

The cytological data of *Pteris* in Java has given an evident that Indonesia is one the centers speciation of *Pteris* in the world. Walker (1962) stressed that *Pteris* had many centers of speciation, mainly the West Indies and Central America, Madagascar, Indonesia, New Guinea, the Philippines, and the Sino-Japanese area. Table 1. also indicates several patterns of speciation on the fern genus *Pteris* in Java have been occurred, namely polyploidy and breeding structure. Of eight species of *Pteris* investigated from Java, seven species have polyploid types. The dynamic aspect of ploidal evolution was pointed out by Haufler (1987). Speciation is the formation of a species. Polyploidy is now widely recognized as one of the principal methods for the formation of new species among the higher plants (Stebbins 1950). Two breeding structure are occurred in Javan *Pteris*, namely sexual and apogamous.

In conclusions, all species of *Pteris* have basic chromosome number x = 29. *Pteris biaurita* is apogamous diploid (2n = 58). *Pteris ensiformis* var *ensiformis* shows the chromosome number of 2n = 87 (apogamous triploid) and 2n = 116 (sexual tetraploid), while *P. ensiformis* var. *victoriae* reveals 2n = 58 (sexual diploid). *Pteris fauriei* is apogamus triploid (2n = 87). *Pteris longipinnula* shows chromosome number 2n = 116 (tetraploid). *Pteris multifida* and *P. vittata* are sexual tetraploids (2n=116). *Pteris tripartita* has two ploidy level, viz sexual diploid (2n = 58) and sexual tetraploid (2n=116). *Pteris* sp. has 2n = 87 (apogamous triploid). Another currently unplaced Javanese *Pteris* sp. has 2n = 87 (apogamous triploid). New record cytotypes of *Pteris* for Central Java are reported, *viz.* diploid *P. biaurita*, tetraploid *P. ensiformis*, triploid *P. fauriei*, diploid *P. tripartita* and teraploid *P. vittata*. The cytological data of *Pteris* in Java has given an evident that Indonesia is one of the speciation centers of *Pteris* in the world. Discussing ploidy in the species of *Pteris* in relation to plant morphological variation across their geographical distribution revealed that *Pteris* has many centers of speciation in the world, notably the West Indies and Central America, Madagascar, Indonesia, New Guinea, the Philippines, and the Sino-Japanese area. The distribution pattern of different cytotypes in *Pteris* may indicate that the tropics is more favourable to the formation and surviving of polyploid than the subtropics.

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