



NOTE

Chromosome numbers and reproductive modes of some species of the fern genus *Tectaria* from the Malesian region

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ABSTRACT: Chromosome numbers and reproductive mode for nine species of Tectarioid ferns of Indonesia are reported here to add new cytological accounts for the genus *Tectaria* (Tectariaceae) of the Malesian region. This study aimed to (1) observe the somatic chromosome number of some species of *Tectaria* in the Malesian region; and (2) determine the reproduction types of the observed species by counting the spore number per sporangium. The results verified the formerly reported basic chromosome numbers $x = 40$ for the genus *Tectaria*. The present study has revealed the presence of 5 species of sexual diploids, one species of sterile triploid, and two species of sexual tetraploids. This study also reported new records of chromosome numbers for Tectarioid ferns of the Malesian region from sexual diploid ($2n = 80$) of *T. aurita* (Sw.) S.Chandra, *T. melanocaulos* (Blume) Copel., *T. palmata* (Mett.) Copel., and *T. zollingeri* (Kurz.) Holttum, and sterile triploid ($2n = 120$) *T. menyanthidis* (C.Presl) Copel. The chromosome numbers of sexual diploid ($2n = 80$) *T. angulata* (Willd.) Copel. and tetraploid ($2n = 160$) *T. barberi* (Hook.) Copel., sexual tetraploid ($2n = 160$) *T. polymorpha* (Wall. ex Hook.) Copel., sexual diploid ($2n = 80$) and sexual tetraploid ($2n = 160$) *T. semipinnata* (Roxb.) C.V.Morton are reported for the first time collected from Central Kalimantan, Java and Sumatra, respectively.

KEY WORDS: chromosome number, Indonesia, Malesian region, reproduction mode, *Tectaria*.

INTRODUCTION

Chromosome data is a fundamental character in plant systematics and evolution. The cytotaxonomist accepts that chromosomes are valuable characters for understanding species relationships (Britton, 1974). Chromosome number and homology largely determine pairing behavior and fertility, and hence the breeding behavior and patterns of variation, knowledge of which is fundamental to achieving a classification that reflects evolution (Stace, 2000). Cytological studies have resolved various taxonomic problems and have revealed details of evolutionary patterns and speciation processes in Pteridophytes (Lovis, 1977).

Chromosome numbers are a valuable indicator of biodiversity (Stace, 2000) and help in understanding of issues of genetic diversity in the conservation of rare and endangered species (Murray and Young, 2001; Severns and Liston, 2008). Change in chromosome number and structure raises questions about the origin, extent, and evolutionary relationships of the chromosome variants (Murray and Young, 2001). Information on cytological variation is also crucial when conservation strategies are planned for the restoration of depleted populations or in establishing new ones, because mixing cytotypes can lead to hybrid dysgenesis (Dobzhansky, 1951).

The genus *Tectaria* was first described by Cavanilles (1799). It is a great genus with 263 accepted species (POWO, 2023). This genus is well distributed in tropical and subtropical regions (Holttum, 1986, 1991), primarily tropical species in Africa, the Americas, Asia, and the Indian Ocean and Pacific Ocean islands (Zhang and

Zhang, 2018). *Tectaria* is a genus of terrestrial ferns in the rainforest that are common components of lowland forests, with most species occurring from the sea level to 1500 m a.s.l. (Dong *et al.*, 2020).

Holttum (1991) stated that a common feature of all species of *Tectaria* except a few which have entire frond or unlobed basal pinnae is that the basal pinnae are larger than those next above them and have elongate basal basisopic lobes or pinnules. The shape and size of the basal pinnae of fronds of mature plants provide some of the most distinctive characteristics for distinguishing between species. The rhizome and stipe covered with lanceolate scales, the adaxially non-grooved frond-axes, and the ctenoid hairs on the adaxial surface of frond-axes are also important characters in distinguish the member of *Tectaria* (Holttum, 1991; Dong *et al.*, 2020). Ching (1931) recognized the majority of *Tectaria* with additional diagnostic characteristics, including the less dissected and herbaceous lamina, anastomosing veins, and round sori.

Tectaria is morphologically diverse and complex in terms of recognizing species, therefore, it causes wide disagreements on its circumscription (Ding *et al.*, 2014, 2018). It has dryopteroid morphology but has been placed in a separate family of Tectariaceae based on molecular taxonomic studies (Smith *et al.*, 2008). Phylogenetic analyses of selected molecular markers (*atpB*, *ndhF*, *ndhF-trnL*, *rbcL*, *rps16-matK*, *matK*, and *trnL-F*) have been used to infer the relationship among taxa of *Tectaria* or to test the systematic positions of some species with unique morphological characters (Dong *et al.*, 2018). These works have greatly enhanced the understanding of the generic



circumscription and infrageneric phylogeny for *Tectaria* (Ding *et al.*, 2014, 2018; Moran *et al.*, 2014; Wang *et al.*, 2014; Zhang *et al.*, 2016, 2017).

Tectaria remains a poorly studied genus amongst ferns for its chromosome numbers. According to Löve *et al.* (1977), Rice *et al.* (2015), and Nakato and Ebihara (2018), it is estimated that not more than 24% of species of *Tectaria* of the world were recognized their chromosome numbers. In Japan, only three species out of eight native species were reported (Mitui, 1976; Takamiya, 1996; Nakato and Ebihara, 2018). From India, 14 out of 25 species were reported (Irudayaraj and Manickam, 1987; Sankari and Bhavanandan, 1993; Vijayakanth *et al.*, 2018; Sundari *et al.*, 2020). For the Malesian region, only 23 out of 105 species (Holtum, 1991) reported their chromosome numbers (Manton, 1954; Manton and Sledge, 1954; Bidin and Go, 1995).

The present study is projected to contribute more knowledge about the cytology of *Tectaria* ferns in the Malesian region, providing a basis for future studies on the structure, diversity and evolution of the genomes of these vascular plants. This study aims to: (1) observe the somatic chromosome number of some species of *Tectaria*; and (2) determine the reproduction modes of the observed species by counting the spore number per sporangium.

MATERIALS AND METHODS

Materials

Living specimens for the cytological studies were planted in the Fernery Green House and Nursery of the Bogor Botanic Gardens, Indonesia. These plants were collected from the natural populations of some locations in Indonesia, Malesian region (Table 1.). Collected specimens were identified by consulting Holtum (1991) and the validated specimens were deposited in the Herbarium Bogoriense (BO). All voucher specimens will be deposited in BO.

Chromosome observation

The preparation of chromosome samples was carried out using the squash method, as this method can be used as an alternative to ensure good physical separation of chromosomes (Fukui, 1996). The preparation procedure followed Praptosuwiryo and Darnaedi (2008). Root tips ca. 1 – 2 mm in length were excised in the morning and then pretreated in 0.002 M 8-hydroxyquinoline solution for 24 hours at 3 – 4 °C. After a brief wash, the roots were fixed in 45% acetic acid for 10 minutes and macerated in a mixture of 45% acetic acid : 1 N HCl = 1 : 3 at 60 °C for 2.5 – 3.5 minutes. The fixed roots were stained and squashed in a 2% aceto-orcein solution.

Chromosome observation was carried out under the microscope by using 1000x magnification. Chromosome counts were carried out from metaphase plates of mitotic

division cells in which individual chromosomes were clearly distinguishable. An Olympus microscope U-TV0, 5XC-3 5H12344, connected to a computer was used to document the pictures.

Reproduction mode observation

The reproductive mode was determined by counting spore number per sporangium and observing spore shape regularity in the voucher specimens or cultivated living collections. According to Walker (1979), there were two reproductive types in ferns, i.e., the sexual reproductive type with 64 spores per sporangium and the apogamous one with 32 spores.

Data Analysis

The chromosome number of each specimen was analyzed from metaphase chromosomes based on the collected photographs. All data obtained is analyzed descriptively.

RESULTS

New chromosome counts of nine species of *Tectaria* from the Malesian region are presented in Table 1. The chromosome photographs of mitotic materials from nine species of *Tectaria* are provided in Fig. 1 – 2. The present study reported the presence of 5 sexual diploids, one sterile triploid, and two sexual tetraploids of the genus *Tectaria* from the Malesian region.

Sexual diploid ($2n = 80$) plants were found in *T. angulata*, *T. aurita*, *T. melanocaulos*, *T. semipinnata*, and *T. zollingeri*. A specimen with sterile fronds of *Tectaria palmata* from West Kalimantan was confirmed to be diploid. The sterile triploid ($2n = 120$) was found in *T. menyanthidis*. A specimen of *T. barberi* with only sterile fronds found is tested to be tetraploid. Two individuals with sexual tetraploid ($2n = 160$) were found in *T. semipinnata* and *T. polymorpha*. Fig. 3. Presents photographs of the evidences of sexual reproductive type with 64 spores per sporangium and the apogamous one with 32 spores.

DISCUSSION

Tectaria angulata (Willd.) Copel. --- $2n = 80$ (2x, sexual)

Fig. 1B

The sexual diploid is the first cytological record for *T. angulata* in Central Kalimantan, Indonesia (Fig. 3.A-B). The first account of *T. angulata* from the Malesian region was reported by Bidin and Go (1995) from Bukit Takon, Selangor, Malay Peninsula, but without any chromosome illustration.

Tectaria angulata is widely distributed in Peninsular Thailand, Malesia, and the Solomon Islands. This species is usually found on rocks or among rocks in lowland forests near streams (Holtum, 1991).

**Table 1.** New chromosome counts of *Tectaria* from the Malesian region.

No. Species	Chromosome No. (2n)/Ploidy Level	Reproductive Mode (Spore No.)	Voucher	Locality
1. <i>T. angulata</i> (Willd.) Copel.	80/diploid	Sexual (64)	JRW 323A	Tanjung Puting National Park, Central Kalimantan, Borneo
	80/diploid	NA	JRW 232C	Tanjung Puting National Park, Central Kalimantan, Borneo
2. <i>T. aurita</i> (Sw.) S.Chandra	80/diploid	Sexual (64)	PW 496A	Mt. Ciremai, West Java
	80/diploid	NA	PW 496B	Mt. Ciremai, West Java
	80/diploid	Sexual (64)	PW 496C	Mt. Ciremai, West Java
	80/diploid	NA	PW 496D	Mt. Ciremai, West Java
	80/diploid	NA	TT1125	Central Sulawesi
3. <i>T. barberi</i> (Hook.) Copel.	160/tetraploid	NA	TNgP 3471A	Sri Maram Forest, Sabung vill., Subah sub district, Sambas district, West Kalimantan, Borneo
	160/tetraploid	NA	TNgP 3471B	Sri Maram Forest, Sabung vill., Subah sub-district, Sambas district, West Kalimantan, Borneo
	160/tetraploid	NA	TNgP 4316B	Mt. Bentarang, Aruk vill. , Sajingan Besar sub-district, Sambas district, West Kalimantan, Borneo
	160/tetraploid	NA	TNgP 4316C	Mt. Bentarang, Aruk vill., Sajingan Besar sub-district, Sambas district, West Kalimantan, Borneo
	160/tetraploid	NA	XIXCXII85	Bogor Botanic Gardens
4. <i>T. melanocaulos</i> (Blume) Copel.	80/diploid	NA	TNgP 2505	Kerinci Seblat National Park, Resort Bukit Tapan, Km 25-28. S. Kunyi. Air Hangat Timur subdistrict, Jambi Province, Sumatra
	80/diploid	Sexual (64)	DM 2083A	Baturraden, Mt. Slamet, Central Java. 715 m a.s.l.
	80/diploid	NA	YN 149	Bali: Riverside, 644 m a.s.l., Hutan Pilan Kerta vill., Banjar Dinas Pilan,. Payangan subdistrict, Gianyar, Bali
5. <i>T. menyanthidis</i> (C.Presl) Copel.	120/triploid	Sterile (32)	DW 1001	West Papua
6. <i>T. palmata</i> (Mett.) Copel.	80/diploid	NA	TNgP 4280	Sungai Marimi, secondary forest, Taman Wisata Asuansang, Sungai Bening vill., Sajingan Besar subdistrict, Sambas district, West Kalimantan province. 81 m a.s.l.
7. <i>T. polymorpha</i> (Wall. ex Hook.) Copel.	160/tetraploid	NA	TPBBG 14	Bogor Botanic Gardens, West Java
	160/tetraploid	NA	TPBBG 15	Bogor Botanic Gardens, West Java
	160/tetraploid	NA	TPBBG 16	Bogor Botanic Gardens, West Java
	160/tetraploid	NA	TPBBG 19	Bogor Botanic Gardens, West Java
	160/tetraploid	Sexual (64)	TPBBG 20	Bogor Botanic Gardens, West Java
	160/tetraploid	Sexual (64)	TPBBG 21	Bogor Botanic Gardens, West Java
	160/tetraploid	Sexual (64)	TPBBG 22	Bogor Botanic Gardens, West Java
	160/tetraploid	Sexual (64)	TPBBG 24	Bogor Botanic Gardens, West Java
	160/tetraploid	NA	TPBBG 25	Bogor Botanic Gardens, West Java
	160/tetraploid	NA	TNgP 3730B	Teak production forest, Petak 8, Singget vill., Tangen subdistrict, Sragen District, Central Java. 141 m a.s.l.
	160/tetraploid	NA	TNgP 3730C	Production forest, Petak 8, Singget vill., Tangen subdistrict, Sragen district, Central Java. 141 m a.s.l.
160/tetraploid	NA	TNgP 3730D	Production forest, Petak 8, Singget vill., Tangen subdistrict, Sragen district, Central Java. 141 m s.l.	
8. <i>T. semipinnata</i> (Roxb.)C.V.Morton	80/diploid	NA	DM 1598A	West Sumatra
	160/tetraploid	Sexual (64)	TNgP 2670	Tebing Bukit Tengkorak, Air Koprass vill., Lebong Utara subdistrict, Kab. Lebong district, Bengkulu Province, Sumatra. 750-790 m a.s.l.
	80/diploid	Sexual (64)	TT 1279A	Riau, Sumatra
9. <i>T. zollingeri</i> (Kurz) Holttum	80/diploid	Sexual (64)	TT 1279B	Riau, Sumatra
	80/diploid	Sexual (64)	TPBBG 043	Bogor Botanic Gardens, West Java
	80/diploid	Sexual (64)	TPBBG 044	Bogor Botanic Gardens, West Java
	80/diploid	Sexual (64)	TPBBG 045	Bogor Botanic Gardens, West Java
	80/diploid	NA	TPBBG 046	Bogor Botanic Gardens, West Java

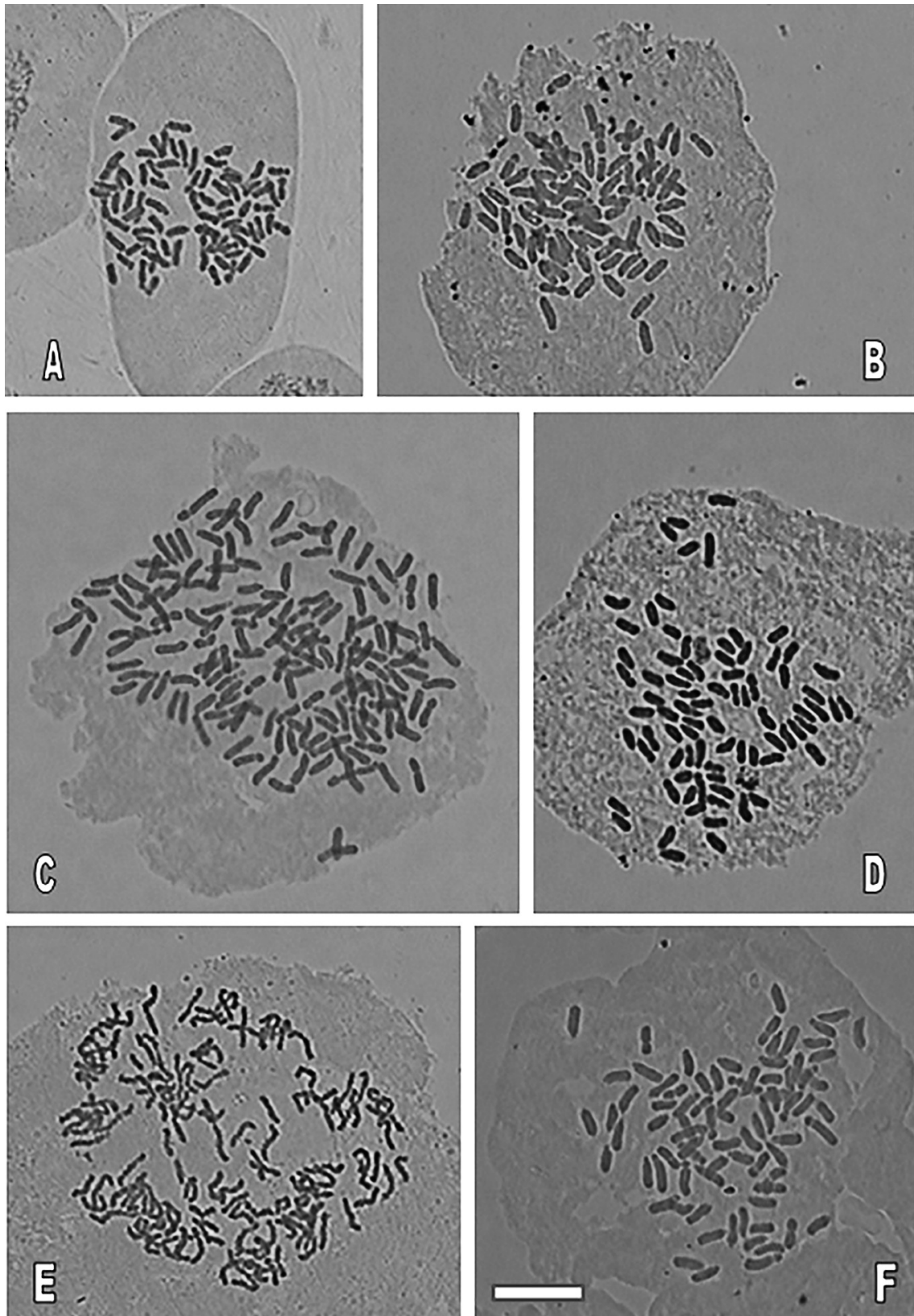


Fig.1. Photographs of somatic chromosome numbers of *Tectaria* from Malesian region. **A.** *T. aurita* (Java: PW 496A, $2n = 80$); **B.** *T. angulata* (Borneo: JRW 323A, $2n = 80$); **C.** *T. barberi* (Borneo: TNGP 4316C, $2n = 160$); **D.** *T. melanocaulos* (Java: TNGP 2505, $2n = 80$); **E.** *T. menyanthidis* (Papua: DW 1001, $2n = 120$); **F.** *T. palmata* (Borneo: TNGP 4280, $2n = 80$). Scale bar = 10 μm for all.

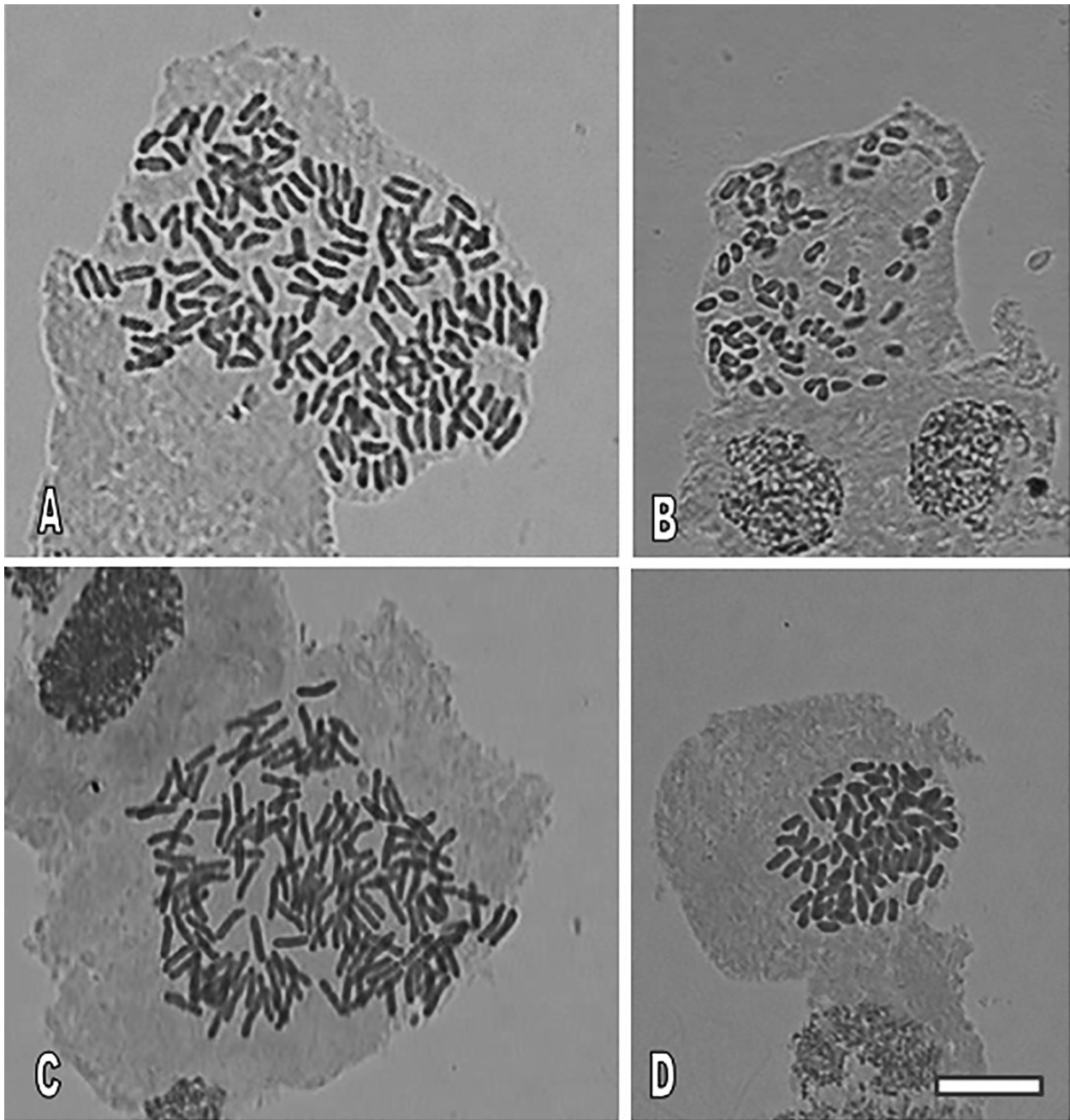


Fig. 2. Photographs of somatic chromosome numbers of *Tectaria* from Malesian region; **A.** *T. polymorpha* (Java: TPBBG 20, $2n = 160$); **B.** *T. semipinnata* (Sumatra: DM 1598A, $2n = 80$); **C.** *T. semipinnata* (Sumatra: TNpP 2670, $2n = 160$); **D.** *T. zollingeri* (Java, TPBBG-043, $2n = 80$). Scale bar = 10 μm for all.

***Tectaria aurita* (Sw.) S.Chandra ---- $2n = 80$ (2x, sexual)**

Fig. 1A

Diploid *T. aurita* of Java (Fig. 4C–D) and Sulawesi are new record cytotypes. *Tectaria aurita* has been reported by Friebel (1933) and Walker (1973) from New Ireland under *Stenosemia aurita* (Sw.) Presl.

Tectaria aurita is closely related to *T. nayari* Mazumdar (Dong *et al.*, 2018), the later was previously known as *Heterogonium pinnatum* (Copel.) Holttum in the Flora Malesiana (Holttum, 1991). The two species are

shared in having morphological characteristics as follows: fronds strongly dimorphous, veins forming aroles along costae of pinnae, and all parts of fertile frond narrow (Holttum, 1991).

Tectaria aurita is distributed throughout Malesia except for the Malay Peninsula. This species was reported as growing on rocky stream banks in the forest, or sometimes on limestone, and also terrestrial in the forest (Holttum, 1991).

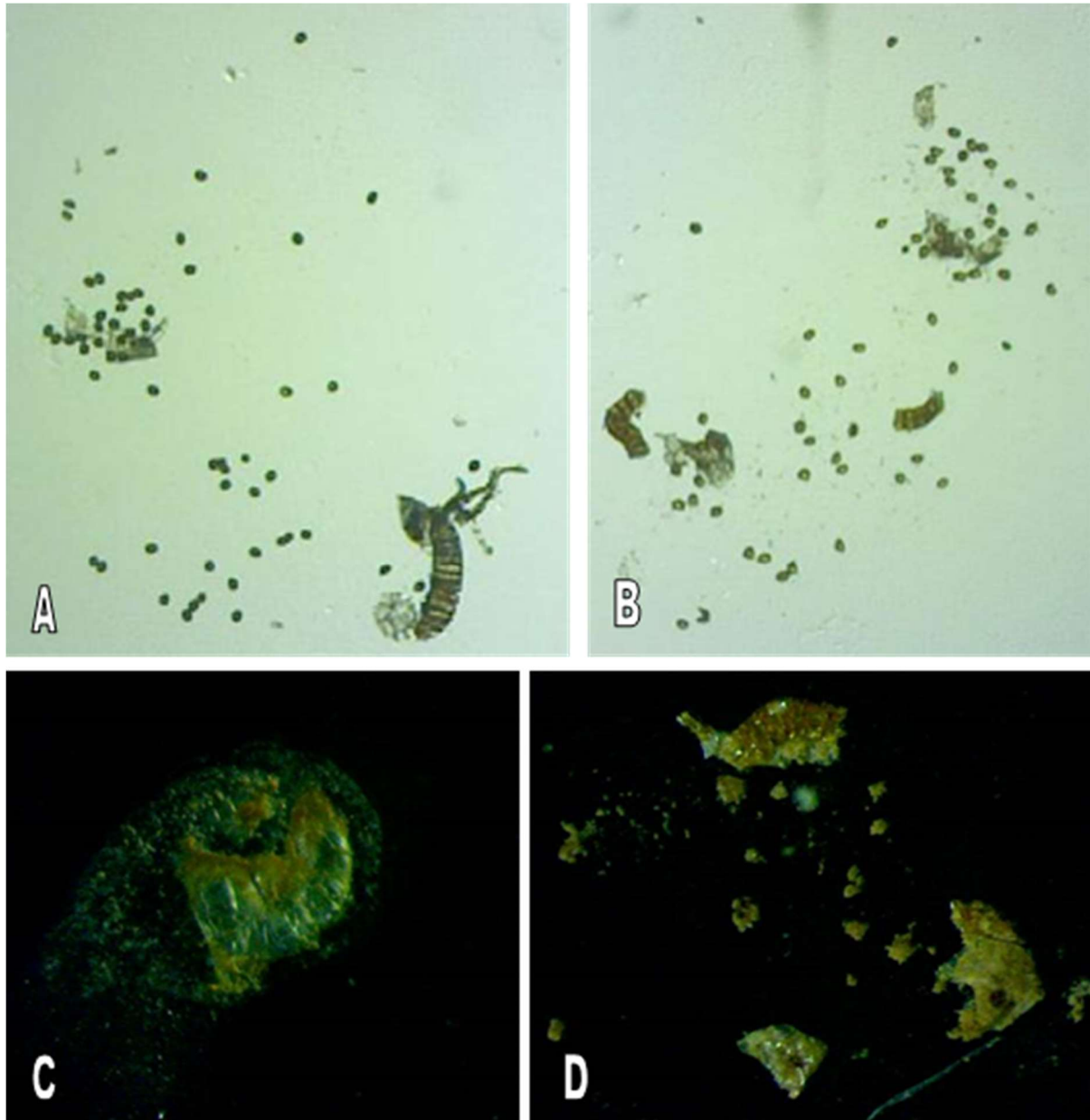


Fig. 3. Photographs of sexual and apogamy sporangia of *Tectaria*. **A.** *T. melanocaulos* (Java: DM 2083A, sexual, S/S = 64); **B.** *T. zollingeri* (Java: TPBBG 043, sexual, S/S = 64; **C–D.** *T. menyanthidis* (Papua: DW 1001, sterile); **C.** Abortive sporangium (S/S = empty); **D.** S/S = ca. 32

***Tectaria barberi* (Hook.) Copel. ---- $2n = 160$ (4x, unknown) Fig. 1C**

The chromosome number of the Malesian region for *T. barberi* was first reported by Manton (1954) from Singapore. It was tetraploid ($n = 78–80$). Bidin and Go (1995) reported a haploid chromosome number $n = 40$ for this species from Perak, Malaysia, without any chromosome illustration. Our present result ($2n = 160$, tetraploid) is the first formal chromosome record for this species for West Kalimantan, Indonesia (Fig. 4E–F). Therefore this finding confirms the existence of tetraploid *T. barberi* in the Malesia region.

Tectaria barberi usually grows in lowland forests and is locally abundant in wet, shady places (Piggott and Piggott, 1988; Holttum, 1991). This species is distributed in Southern Myanmar and Thailand. In Malesia, this species is found in Sumatra, the Malay Peninsula, Borneo, and the Sulu Archipelago (Holttum, 1991).

***Tectaria melanocaulos* (Blume) Copel. ---- $2n = 80$ (2x, sexual) Fig. 1D**

The sexual diploid is the first cytological record for *T. melanocaulos* (Fig. 4G–H) of *Tectaria* ferns of the Old World. The native range of *T. melanocaulos* is the



Andaman Islands, Peninsular Thailand to Vanuatu. In Malesia, this species is recognized to be distributed in Sumatra, Malay Peninsula, Java, Philippines (Luzon, Negros, Sulu Archipelago), Sulawesi, and New Guinea (Holttum, 1991).

Holttum (1991) wrote the name of this species as *Tectaria melanocaula* (Blume) Copel. We follow Fraser-Jenkins *et al.* (2018) and POWO (2023) in accepting this species name.

***Tectaria menyanthidis* (C.Presl) Copel. ---- $2n = 120$ (3x, sterile)**

Fig. 1E

The sterile triploid is the first cytological record for *T. menyanthidis* (Fig. 4I–J) in the Old World *Tectaria*. In Malesia, this species is distributed in the Philippines and New Guinea; its wider distribution is Solomon Island, Banks Island, and Fiji (Holttum, 1991). *Tectaria menyanthidis* is usually found in lowland forests, frequently occurring near stream banks and occasionally on limestone (Holttum 1991; Dong *et al.* 2018).

Based on phylogenetic analyses using sequences of five plastid regions (*atpB*, *ndhF*, *ndhF-trnL*, *rbcl*, *rps16-matK*, *matK*, and *trnL-F*), *T. menyanthidis* (in the southeastern Philippines to Solomon Islands and Fiji) is closely related to *T. ternata* (in Borneo), and *T. variabilis* (in Vietnam) and the three species are not true members of *Tectaria* but represent a distinct lineage, being sister to *Pteridrys* (Dong *et al.*, 2018). The three species are morphologically very similar; they are common in having long-creeping rhizomes, imparipinnate fronds with entire pinnae, fully anastomosing veins, and sori being in two irregular rows between lateral veins. Supported by combining these characteristics, Dong *et al.* (2018) proposed that the *T. menyanthidis* should be recognized as a separate genus *Polydictyum*.

***Tectaria palmata* (Mett.) Copel. var. *palmata* ---- $2n = 80$ (2x, unknown)**

Fig. 1F

The diploid is the first cytological record for *T. palmata* (Fig. 4K–L) in the Old World *Tectaria*. Holttum (1991) recognized four varieties of *T. palmata*, namely *T. palmata* var. *dimorpha*, *T. palmata* var. *palmata*, *T. palmata* var. *platanifolia*, and *T. palmata* var. *sumatrana*. The morphology of our present material corresponds to the form of var. *palmata*. *Tectaria palmata* var. *palmata* is distributed in Borneo (Central and South East Kalimantan), and this variety can be differentiated from the others variety by characteristics combination as follows: fertile fronds isomorphous with sterile ones; lamina on mature plants deeply 5-lobed, up to c. 20 cm long and wide; costae densely short-hairy on upper surface; sori very distinct on all sori (Holttum, 1991).

Holttum (1991) recognized *Tectaria palmata* var. *palmata* to be distributed in Central and South East

Kalimantan (Borneo). The present result shows that this taxon has a broader distribution in Borneo, including West, Central, South, and East Kalimantan.

***Tectaria polymorpha* (Wall. ex Hook.) Copel. ---- $2n = 160$ (4x, sexual)**

Fig. 2A

The sexual tetraploid is the first cytological record for *T. polymorpha* (Fig. 4M–N) in Java, Indonesia. The tetraploid cytotypes of *T. polymorpha* var. *cuneifolia* have been reported in the Malay Peninsula by Manton and Sledge (1954) from Fraser's Hill but without any chromosome illustration. In India, the diploid cytotypes were reported by Ammal and Bhavanandan (1993), whereas the tetraploid cytotypes were reported by Abraham *et al.* (1962).

Tectaria polymorpha has a wide distribution, from Nepal to Yunnan and North Thailand to Tonkin, Taiwan, South India and Sri Lanka; In Malesia, it is found in East Java, Lesser Sunda Islands (Timor), Philippines (Luzon), and Sulawesi (Holttum, 1991). The present study records that this species is also distributed in Central and West Java. In Java's teak forest, it is usually found along the banks of streams, on humus-rich soil or sandy soils, at 100 – 250 m a.s.l. In West Java, this species is widely growing on rock crevices or cutting edges at 250 – 275 m a.s.l. Xing *et al.* (2013) also reported that this species grows on rocks and wet soils in China.

***Tectaria semipinnata* (Roxb.) C.V.Morton ---- $2n = 80$ (2x, sexual) and $2n = 160$ (4x, sexual)**

Fig 2B & C

The first chromosome account of *T. semipinnata* from the Malay Peninsula, was reported by Manton (1954), *viz.* diploid with $n = 40$ – 41 , as *T. maingayi*. The sexual diploid and tetraploid are the first cytological record for *T. semipinnata* (Fig. 4O–P) in Sumatra, Indonesia.

The distribution of this species is in Peninsular Thailand and the Malesia region. In Malesia, it is distributed in the Malay Peninsula and North Sumatra (Holttum, 1991).

***Tectaria zollingeri* (Kurz.) Holttum ---- $2n = 80$ (2x, sexual)**

Fig. 2D

All the wild plants of *T. zollingeri* (Fig. 4Q) in the Bogor Botanic Gardens (BBG) showed chromosome numbers with $2n = 80$, diploid. This is the first report of chromosome number for the species in Malesian region.

In Bogor Botanic Gardens, *T. zollingeri* grows naturally in a somewhat shady place on sloping ground. In the forest, this species is growing on steep earth banks in shady places near streams at low altitudes (Holttum, 1991). In the present days, this species is found in Malesian region only and distributed in Java, South West

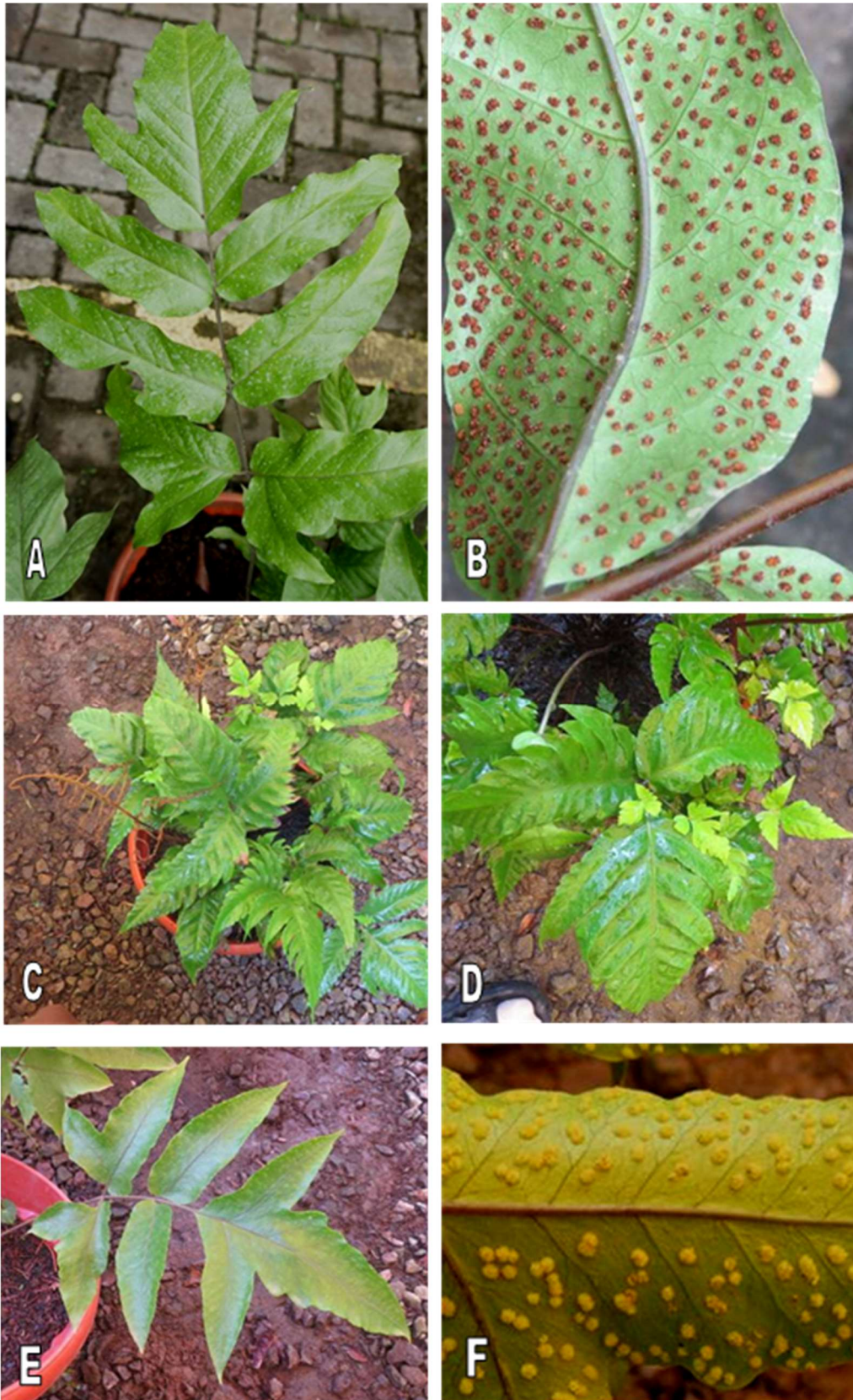


Fig. 4. Photographs of living materials of *Tectaria* from Malesian region. **A–B.** *T. angulata* (Borneo: JRW 323A, $2n = 80$); **C–D.** *T. aurita* (Java: PW 496A, $2n = 80$); **E–F.** *T. barberi* (Borneo: TNgP 4316C, $2n = 160$).

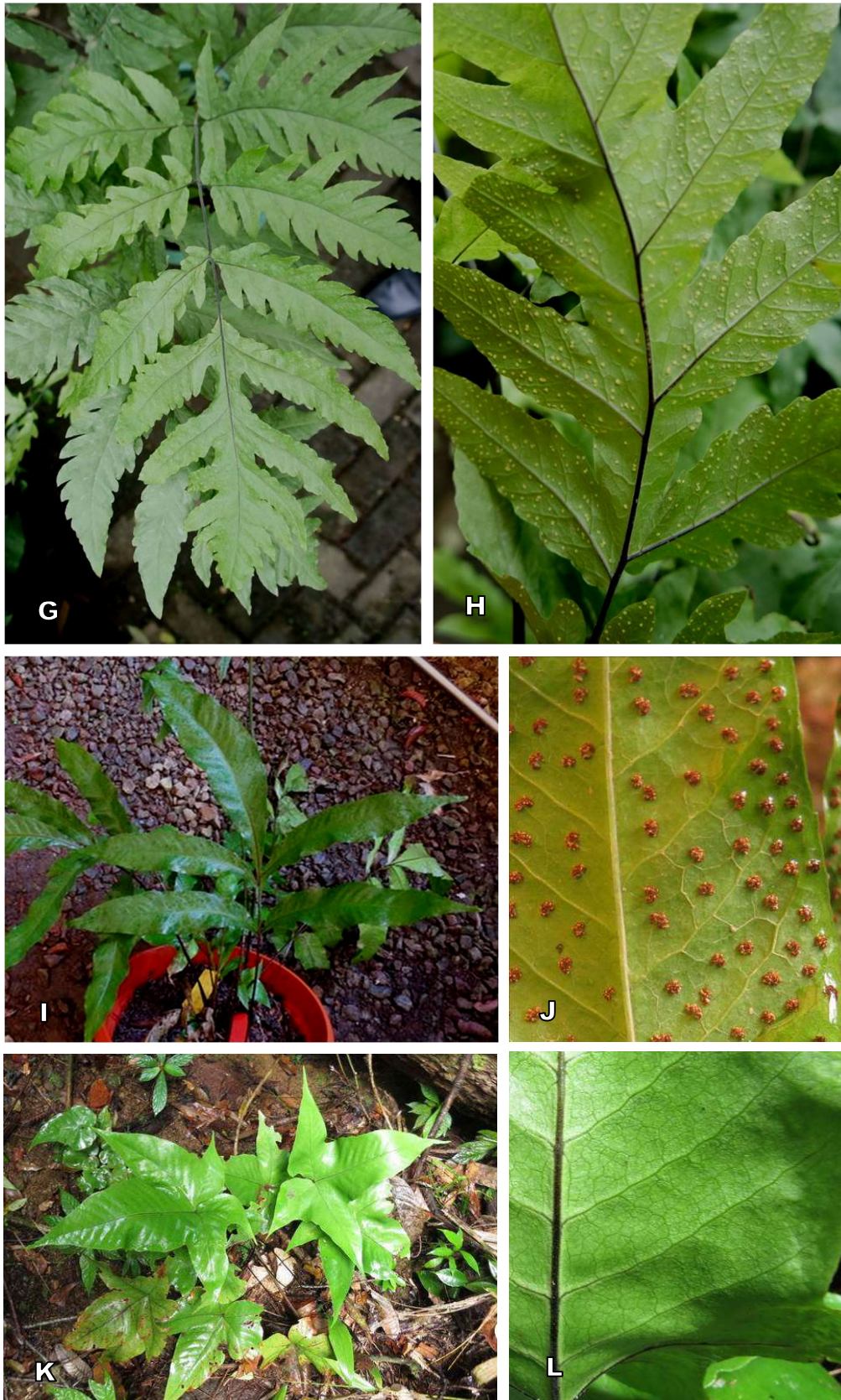


Fig. 4. Continued. **G–H.** *Tectaria melanocaulos* (Java: DM 2083, $2n = 80$); **I – J.** *T. menyanthidis* (Papua: DW 1001, $2n = 120$); **K–L.** *T. palmata* (Borneo: TNgP 4280, $2n = 80$).

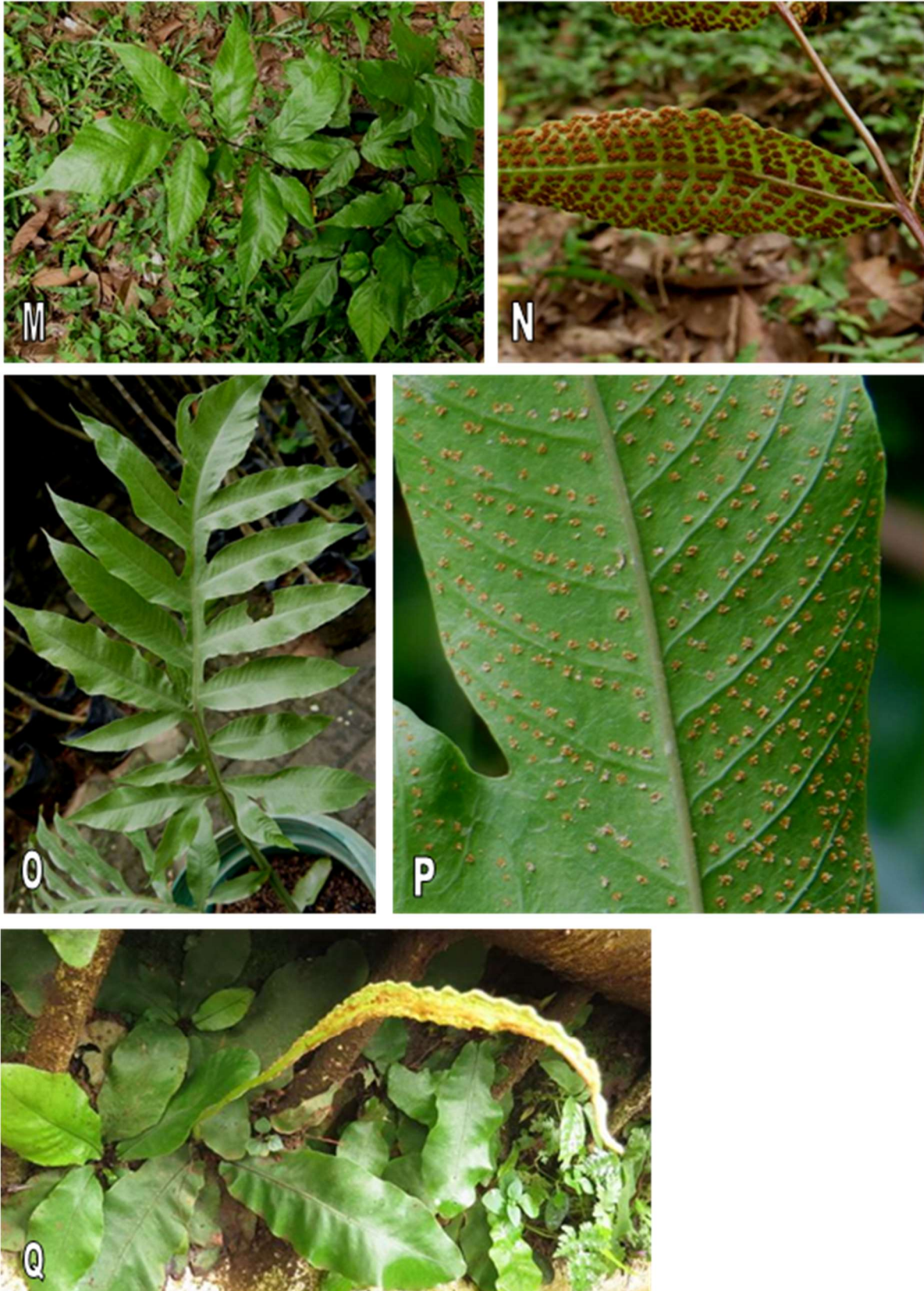


Fig. 4. Continued. M–N. *T. polymorpha* (Java: TPBBG 20, 2n = 160); O–P. *T. semipinnata* (Sumatra: TT 1279A, 2n = 80); Q. *T. zollingeri* (Java, 2n = 80).



Sulawesi, and Moluccas (Ambon and Tanimbar Island) (Holttum, 1991). Praptosuwiryo (2013) reported *T. zollingeri* in Mt. Slamet, Central Java, as a rare species growing on rocky soils.

Basic chromosome numbers and taxonomic implication

The present study verifies most of the formerly reported basic chromosome numbers of the genus *Tectaria*. In general, the basic chromosome number of *Tectaria* is determined as $x = 40$ (Abraham *et al.*, 1962; Love *et al.*, 1977; Takamiya, 1996; Cheng and Zhang, 2010). Recent cytological observations of *Tectaria* species also support the basic chromosome number $x = 40$ (Vijayakanth *et al.*, 2018; Nakato and Ebihara, 2018). Our results are in line with the previous reports on the chromosome account of *Tectaria* from the Old World with basic chromosome number $x = 40$. Cytological study of ferns from South India reported somatic chromosome number of *Tectaria* with $x = 40$ ($2n = 80$ and $2n = 200$) (Bhavanandan, 1981; Sundari and Iyudarayaj, 2020). Cytotaxonomic study of ferns from Yunnan (China) reported sexual diploid *T. fuscipes* ($2n = 80$) (Kato *et al.*, 1992). Zhao and Dong (2016) also supported the basic chromosome number $x = 40$ based on their cytological observation on the apogamous triploid ($2n = 120$) of *Tectaria* × *hongkongensis* from southern China. However, Bidin and Go (1995) reported the basic chromosome number $x = 39$ in *T. brachiata*, *T. grandidentata*, *T. semibipinnata*, *T. siifolia*, *T. simonsii*, *T. singaporeana*, *T. translucens*, and *T. vasta*, but they did not provide any chromosome illustration, except for *T. semibipinnata*. Our question is whether the base number $x = 39$ represents a dysploidy of the base number $x = 40$. Chromosome number reduction through dysploidy or chromosome ‘fusion’, combined with polyploidy has been suggested as an important evolutionary process in several genera of ferns (Lovis, 1977; Windham and Yatskievych, 2003; Bellefroid *et al.*, 2010). Further study is needed. Integrating the known chromosome counts and molecular phylogenetic trees to obtain certain answers to this question is possible: which is more ancestral, $x = 39$ or $x = 40$?

A phylogenetic analysis for the pantropical fern genus *Tectaria*, with emphasis on the Old World species, based on sequences of five plastid regions (*atpB*, *ndhF plus ndhF-trnL*, *rbcL*, *rps16-matK plus matK*, and *trnL-F*), that included ca. 56 species of *Tectaria* s.l. and 36 species of ten related genera supported the monophyly of *Tectaria* in a broad sense (Ding *et al.*, 2014). The base chromosome number $x = 40$ supported the broadly circumscribed *Tectaria*.

Spore number and sexuality

The present study has revealed the presence of 5 species of sexual diploids, one species of sterile triploid, and two species of sexual tetraploids (Table 1.). All species of the sexual diploid and sexual tetraploid

produced fertile fronds with 64 spores per sporangium. There was only one species with the fertile fronds having 32 spores per sporangium, viz. triploid *T. menyanthidis*, but the spores are irregular in size and shape. Therefore, this plant should be interpreted as sterile. Sixty-four-spored plants are considered sexual, whereas 32-spored plants are agamosporous (Lovis, 1977; Walker, 1979, 1984).

Haufler (1987) stressed the dynamic aspect of ploidal evolution in ferns. It is proposed that there is a correlation between ploidy and reproductive mode (Lovis, 1977; Walker, 1979, 1984). Further study is required to determine the relation between ploidy level, sporogenesis, and sexuality of *Tectaria*.

In conclusion, a study on chromosome numbers and reproductive biology of nine species of *Tectaria* of Indonesia has added new cytological accounts for the genus *Tectaria* (Tectariaceae) of the Malesian region. This study supported the genus *Tectaria*'s basic chromosome number $x = 40$. The present study added new cytological data of five species for the genus *Tectaria*, namely: (1) Sexual diploid *T. aurita* ($2n = 80$); (2) Sexual diploid *T. melanocaulos*; (3) Apogamous triploid *T. menyanthidis*; (4) Diploid *T. palmata*; (5) Sexual diploid *T. zollingeri*. Sexual diploid ($2n = 80$) *T. angulata* and tetraploid ($2n = 160$) *T. barberi* are new record cytotypes for Central Kalimantan. Sexual tetraploid ($2n = 160$) *T. polymorpha* is a new cytological record of Java. Sexual diploid ($2n = 80$) and sexual tetraploid ($2n = 160$) *T. semipinnata* (Roxb.) are new records for Sumatra.

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LITERATURE CITED

- Abraham, A., Ninan, C.A., Mathew, P.M. 1962 Studies on the cytology and phylogeny of the pteridophytes: VII. Observation on one hundred species of South Indian ferns. *J. Indian. Bot. Soc.* **41**(3): 339–421.
- Ammal, L.S., Bhavanandan K.V. 1993 Karyomorphological studies on three species of *Tectaria* Cav. *Indian Fern J.* **10**: 35–39.
- Bhavanandan, K.V. 1981 Studies on the cytology of South Indian Aspidiaceae. *Cytologia* **46**(1/2): 195–207.
- Bidin, A.A., Go, R. 1995 Chromosome base numbers for *Tectaria* and allied genera in Peninsular Malaysia. *Nat. Hist. Res.* **3**(2): 115–122.



- Bellefroid, E., Khadijah Rambe, S., Leroux, O., Viane, R.L.L.** 2010 The base number of 'loxoscapoid' *Asplenium* species and its implication for cytoevolution in Aspleniaceae. *Ann. Bot.* **106(1)**: 157–171.
- Britton, D.M.** 1974 The significance of chromosome numbers in ferns. *Ann. Missouri Bot. Gard.* **61(2)**: 310–317.
- Cavanilles, A.J.** 1799 Nuevos caracteres genericos de los Helechos por Smith. *Anal. Historia Nat.* 108–115.
- Cheng, X., Zhang, S.Z.** 2010 Index to chromosome numbers of Chinese Pteridophyta (1969–2009). *J. Fairylake Bot. Gard.* **9(1)**: 1–58.
- Ching, R.-C.** 1931 The studies of Chinese ferns 7, a revision of the genus *Tectaria* from China and Sikkim-Himalaya. *Sinensia* **2**: 9–36.
- Ding, H.-H., Chao, Y.-S., Callado, J.R., Dong, S.-Y.** 2014 Phylogeny and character evolution of the fern genus *Tectaria* (Tectariaceae) in the Old World inferred from chloroplast DNA sequences. *Mol. Phylogenet. Evol.* **80**: 66–78.
- Dobzhansky, T.** 1951 *Genetics and the Origin of Species*. Columbia University Press, New York.
- Dong, S.-Y., Chen, C.-W., Tan, S.-S., Zhao, H.-G., Zuo, Z.-Y., Chao, Y.-S., Chang, Y.-H.** 2018 New insights on the phylogeny of *Tectaria* (Tectariaceae), with special reference to *Polydictyum* as a distinct lineage. *J. Syst. Evol.* **56(2)**: 139–147.
- Dong, S.-Y., Haque, A.K.M. K., Rahman, M.S., Rahim, M.A., Khan, S.A.** 2020 A taxonomic revision of the fern genus *Tectaria* (Tectariaceae) from Bangladesh. *Taiwania* **65(4)**: 567–574.
- Fraser-Jenkins, C.R., Gandhi, K.N., Kholia, B.S.** 2018 An Annotated Checklist of Indian Pteridophytes 2. BSMPS, Dehra Dun.
- Friebel, H.** 1933 Untersuchungen zur Cytologie de Farne. *Bietr. Biol. Pflanzen* **21**: 167–731.
- Fukui, K.** 1996 Plant chromosome at mitosis. In: **Fukui, K., Nakayama, S.** (eds.) *Plant Chromosome: Laboratory Methods*, CRC Press, Boca Raton.
- Hauffler, C.H.** 1987 Electrophoresis is modifying our concepts of evolution in homosporous pteridophytes. *Am. J. Bot.* **74(6)**: 953–966.
- Holtum, R.E.** 1986 Studies in the Fern-Genera Allied to *Tectaria* Cav. VI: A conspectus of genera in the Old World regarded as related to *Tectaria*, with descriptions of two genera. *Gard. Bull. Sing.* **39(2)**: 153–167.
- Holtum, R.E.** 1991 *Flora Malesiana, series II, Pteridophyta, vol. 2, part 1, Tectaria group*. Rijksherbarium / Hortus Botanicus, Leiden, 132 pp.
- Irudayaraj, V., Manickam, V.S.** 1987 SOCGI plant chromosome number reports. *VJ. Cytol. Genet.* **22**: 156–163.
- Kato, M., Nakato, N., Cheng, X., Iwatsuki, K.** 1992 Cytotaxonomic study of ferns of Yunnan, southwestern China. *Bot. Mag. Tokyo* **105(1)**: 105–124.
- Lovis, J.D.** 1977 Evolutionary patterns and processes in ferns. *Adv. Bot. Res.* **4**: 229–415.
- Löve, A., Löve, D., Pichi-Sermolli, R.E.G.** 1977 *Cytotaxonomical Atlas of the Pteridophyta*. A.R. Ganner Verlag, Vaduz.
- Manton, I.** 1954 Cytological notes on one hundred species of Malayan ferns. In: **Holtum R.E.** *A Revised Flora of Malaya*. II. Appendix: 623–627.
- Manton, I., Sledge, W.A.** 1954 Observations on the Cytology and Taxonomy of the Pteridophyte Flora of Ceylon. *Phil. Trans. Royal Soc. London. Ser. B, Biol. Sci.* **238(654)**: 127–185.
- Moran, R.C., Labiak, P.H., Hanks, J.G., Prado, J.** 2014 The phylogenetic relationship of *Tectaria brauniana* and *Tectaria nicotianifolia*, and the recognition of *Hypoderris* (Tectariaceae). *Sys. Bot.* **39(2)**: 384–395.
- Murray, B.G., Young, A.G.** 2001 Widespread chromosome variation in the endangered grassland forb *Rutidosia leptorrhynchoides* F. Muell. (Asteraceae: Gnaphalieae). *Ann. Bot.* **87(1)**: 83–90.
- Nakato, N., Ebihara, A.** 2018 Chromosome numbers of eleven ferns in Japan (Athyraceae, Dryopteridaceae and Tectariaceae). *Bull. Natl. Mus. Nat. Sci. Ser. B* **44(1)**: 23–30.
- Piggott, A.G., Piggott, C.J.** 1988 *Fern of Malaysia in Colour*. Tropical Press Sdn. Bhd., Kuala Lumpur.
- POWO** 2023. *Plants of the World Online*. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; <http://www.plantsoftheworldonline.org/>. Retrieved 28 January 2023.
- Praptosuwiryo, T.Ng., Darnaedi, D.** 2008 Cytological observation on fern genus *Pteris* in the Bogor Botanic Gardens. *Bul. Kebun Raya Indonesia* **11(2)**: 15–23.
- Praptosuwiryo, T.Ng.** 2013 The rare Pteridophytes of Mt. Slamet with three species new records for Java. *Floribunda* **4(6)**: 138–146.
- Rice, A., Lior, G., Abadi, S., Einhorn, M., Kopelman, N.M., Salman-Minkov, A., Mayzel, J., Chay, O., Mayrose, I.** 2015 The Chromosome Counts Database (CCDB) - a community resource of plant chromosome numbers. *New Phytol.* **206(1)**: 19–26.
- Sankari, A.L., Bhavanandan, K.V.** 1993 Karyomorphological Studies on three species of *Tectaria* Cav. *Indian Fern J.* **10**: 35–39.
- Severns, P.M., Liston, A.** 2008 Intraspecific chromosome number variation: A neglected threat to the conservation of rare plants. *Conserv. Biol.* **22(6)**: 1641–1647.
- Smith, A.R., Pryer, K.M., Schuettpelz, E., Korall, P., Schneider, H., Wolf, P.G.** 2008 *Ferns and Lycophytes*. Cambridge University Press, Cambridge.
- Stace, C.A.** 2000 Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21st centuries. *Taxon* **49(3)** Golden Jubilee Part 1: 451–477.
- Sundari, M.S., Benniamin, A., Irudayaraj, V.** 2020 Chromosome study on an endangered oak leaf fern *Tectaria zeilanica* (Houtt) Sledge (Dryopteridaceae) from South India. *Int. J. Plant Biol. Res.* **8(1)**: 1115.
- Takamiya, M.** 1996 *Index to chromosomes of Japanese Pteridophyta (1910–1996)*. Japan Pteridological Society, Tokyo.
- Vijayakanth, P., Sahaya Sathish, S., Dominic Rajkumar, S., Irudayaraj, V., Kavitha, R., Mazumdar, J.** 2018 Studies on the chromosome numbers of ferns from Kolli Hills, Eastern Ghats, Tamil Nadu, India. *Caryologia* **71(4)**: 380–396.
- Wang, F.-G., Barratt, S., Falcon, W., Fay, M.F., Lehtonen, S., Tuomisto, H., Xingand, F.-W., Christenhusz, M.J.M.** 2014 On the monophyly of subfamily Tectarioideae (Polypodiaceae) and the phylogenetic placement of some associated fern genera. *Phytotaxa* **164(1)**: 1–16.



- Walker, T.G.** 1973 Evidence from cytology in the classification of ferns. In: **Jermy A.C., Crabble J.A., Thomas B.A.** (eds.). The phylogeny and classification of the ferns. Bot. J. Linn. Soc. **67**, Suppl.1: 91–110.
- Walker, T.G.** 1979 The Cytogenetics of Ferns. In: **Dyer, A.F.** (ed). The Experimental Biology of Ferns. Academic Press, London.
- Walker, T.G.** 1984 Chromosomes and evolution in pteridophytes. In: **Sharma, A.K., Sharma, A.** (eds.). Chromosomes in Evolution of Eukaryotic Groups. Vol. 2, pp. 103–141. CRC Press, Boca Raton.
- Windham, M.D., Yatskievych, G.** 2003 Chromosome studies of cheilanthoid ferns (Pteridaceae: Cheilantheoideae) from the western United States and Mexico. Am. J. Bot. **90**(12): 1788–1800.
- Xing, F.W., Yan, Y.H., Dong, S.Y., Wang, F.G., Christenhusz, M.J.M., Hovenkamp, P.H.** 2013 Tectariaceae. Pp. 730–746. In: **Wu, Z.Y., Raven, P.H. Hong, D.Y.** (eds.). Flora of China, Vol. 2–3 (Pteridophytes). Science Press, Beijing; Missouri Botanical Garden Press, St. Louis.
- Zhao, H.-G., Dong, S.-Y.** 2016 A new hybrid of *Tectaria* (Tectariaceae) from southern China. Phytotaxa **266**(3): 213–218.
- Zhang, L., Zhang, L.-B.** 2018 A classification of the fern genus *Tectaria* (Tectariaceae: Polypodiales) based on molecular and morphological evidence. Ann. Missouri Bot. Gard. **103**(2): 188–199.
- Zhang, L., Schuettpelz, E., Rothfels, C., Zhou, X.-M., Gao, X.-F., Zhang, L.B.** 2016 Circumscription and phylogeny of the fern family Tectariaceae based on plastid and nuclear markers, with the description of two new genera: *Draconopteris* and *Malajifilix* (Tectariaceae). Taxon **65**(4): 723–738.
- Zhang, L., Zhou, X.-M., Chen, D.-K., Schuettpelz, E., Knapp, R., Thi Lu, N., Tam Luong, T., Tri Dang, M., Duan, Y.-F., He, H., Gao, X.-F., Zhang, L.-B.** 2017 A global phylogeny of the fern genus *Tectaria* (Tectariaceae: Polypodiales) based on plastid and nuclear markers identifies major evolutionary lineages and suggests repeated evolution of free venation from anastomosing venation. Mol. Phylogenet. Evol. **114**: 295–333.