

## Cytotaxonomic Studies on Thirteen Ferns of Taiwan

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**Abstract** Mitotic chromosome numbers of 13 fern taxa collected in Taiwan were counted, and their reproductive modes were estimated by counting the number of spores per sporangium. Four taxa (*Pteris angustipinna*, *P. longipinna*, *Thelypteris angustifrons* × *T. glanduligera* and *Dryopteris enneaphylla* var. *enneaphylla*) were not studied in previous cytological studies. New cytotypes were recorded for four others (*Coniogramme japonica*, *Pteris dactylina*, *Blechnum hancockii* and *Dryopteris varia*), and for three taxa (*Cyrtomium devexiscapulae*, *Dryopteris integriloba* and *D. labordei* var. *labordei*), the present results are the first cytological records of Taiwanese plants of the taxa. The chromosome counts for the remaining two taxa (*Stegnogramma tottooides* and *Acystopteris tenuisecta*) matched those previously reported from Taiwan.

**Key words** : chromosome number, ferns, Taiwan.

Studies on the fern and lycophyte flora of Taiwan have made remarkable progress in recent years, and no less than 747 taxa are recorded in the latest flora (Knapp, 2013). To infer their origins or relationships with conspecifics/closely-related species in adjacent regions, information on ploidy levels and reproductive modes is necessary, even when DNA markers are used. However, ploidy level information is available only for 359 taxa (48.1%) of ferns and lycophytes of Taiwan (= 322 species enumerated in Tsai [1992] plus subsequently published records at least for 37 taxa [Kuo and Chao, data not shown]), and both the taxon coverage and the coverage of infraspecific cytotype variations of the taxa are insufficient. Although flow cytometry has been frequently used for rapid determination of relative genome sizes in recent studies, we made chromosome counts for collections of reliable cytological records in this study.

### Materials and Methods

Root tips for mitotic chromosome counts were collected from 19 cultivated stocks (Table 1). They were pretreated with 2 mM 8-hydroxyquinoline solution for 4–5 h, fixed in Carnoy's solution for 1–24 h, macerated in 1 N HCl at 60°C for 1 min, and then squashed in aceto-orcein. Spore number per sporangium was counted (and spore size was measured for *P. dactylina*) under a light microscope using fresh material and/or the voucher specimens when fertile fronds with mature spores were available. 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. The former is generally a feature representing sexual reproduction, and the latter is one representing apogamous reproduction (Walker, 1984). The voucher specimens were deposited in TNS (Table 1).

Table 1. Plant materials used in this study with their results

Species	Chromosome number	Ploidy	Reproductive mode (spore count)	Coll. No.	Voucher	Locality	Figure
<i>Coniogramme japonica</i>	2n = 60	2x	N/A	K017026*	TNS VS-1181102	Nantou Co., Mt. Wu-ling	1
<i>Pteris angustipinna</i>	2n = ca.87	3x	Apo. (32 s/s)	K017247*	TNS VS-1181090; TAIF	Nantou Co., Patungkuan to Lele	2
<i>Pteris dactylina</i>	2n = 58	2x	Apo. (32 s/s)	AE3096	TNS VS-1170720	Nantou Co., Ren-ai, Mt. Bilu Trail	3
<i>Pteris longipinna</i>	2n = 58	2x	Sex. (64 s/s)	K013585*	TNS VS-1181096	Taitung Co., Mt. Hsinkang	4
<i>Pteris longipinna</i>	2n = 58	2x	Sex. (64 s/s)	K017629*	TNS VS-1181094	Taitung Co., Tienlung Suspension Bridge	—
<i>Stegogramma tottoides</i>	2n = 72	2x	Sex. (64 s/s)	AE3101	TNS VS-1170714	Nantou Co., Ren-ai	5
<i>Thelypteris angustifrons</i> × <i>T. glanduligera</i>	2n = 108	4x	[irregular]	K018566*	TNS VS-1181100	Taipei City, Mt. Yangming, Erhtzuping	6
<i>Acystopteris tenuisecta</i>	2n = 84	2x	Sex. (64 s/s)	AE3124	TNS VS-1170767	Ilan Co., Datong, near Ming-Chih	7
<i>Blechnum hancockii</i>	2n = 62	2x	N/A	K030809*	TNS VS-1181101	Taoyuan Co., Mt. Peichatien	8
<i>Blechnum hancockii</i>	2n = 62	2x	N/A	K030810*	TNS VS-1181066	Taoyuan Co., Mt. Peichatien	—
<i>Cyrtomium devexiscapulae</i>	2n = 164	4x	Sex. (64 s/s)	K030840*	TNS VS-1181084	Miaoli Co., Tai-an, Mt. Hu	9
<i>Dryopteris enneaphylla</i> var. <i>enneaphylla</i>	2n = 82	2x	Sex. (64 s/s)	AE3108	TNS VS-1170791	Taichung City, Wuling Rd.	10
<i>Dryopteris integriloba</i>	2n = 123	3x	N/A	K017561*	TNS VS-1181104	Nantou Co., Lienhuachih	11
<i>Dryopteris labordei</i> var. <i>labordei</i>	2n = ca.123	3x	Apo. (32 s/s)	K019873*	TNS VS-1181089	Ilan Co., Yuanyang Lake	12
<i>Dryopteris varia</i>	2n = 82	2x	Sex. (64 s/s)	K013434*	TNS VS-1181085	New Taipei City, Pinglin	13
<i>Dryopteris varia</i>	2n = 82	2x	Sex. (64 s/s)	SM TW03–209	TNS VS-1116777	New Taipei City, Wulai, Hungzhou Valley	—
<i>Dryopteris varia</i>	2n = 82	2x	Sex. (64 s/s)	SM TW03–228	TNS VS-1116778	ibid.	—
<i>Dryopteris varia</i>	2n = 82	2x	Sex. (64 s/s)	SM TW03–229	TNS VS-1116779	ibid.	—
<i>Dryopteris varia</i>	2n = ca.123	3x	Apo. (32 s/s)	K012647*	TNS VS-1181086	New Taipei City, Wulai, Tunghou logging trail	14

\* Cultivated plants in Dr. Cecilia Koo Botanic Conservation Center collected in January 2013.

## Results and Discussion

The results of chromosome counts and the spore numbers are summarized in Table 1.

### Pteridaceae

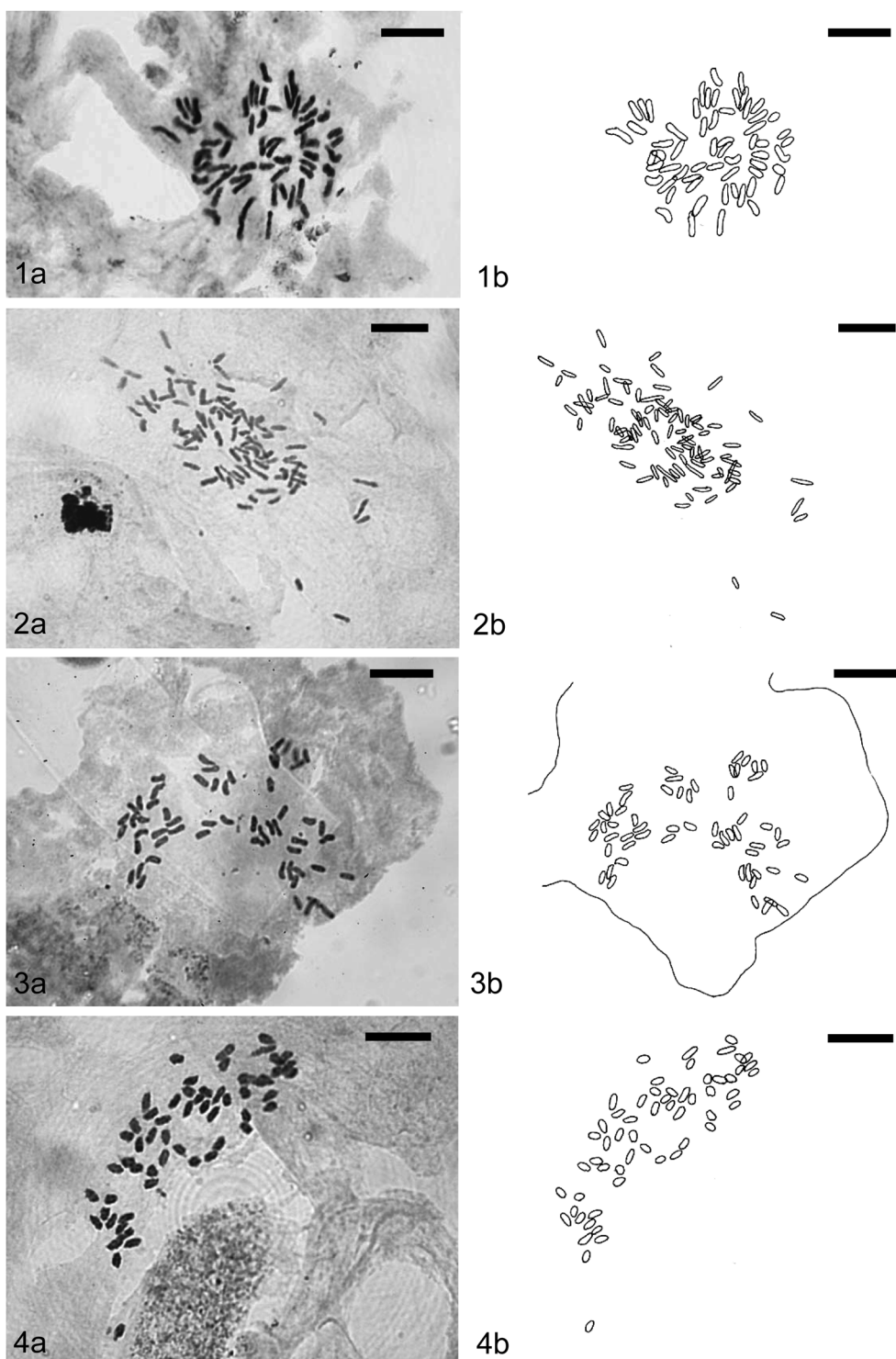
*Coniogramme japonica* (Thunb.) Diels  
2n = 60 [diploid, x = 30] (Fig. 1).

Note: This is a new cytotype and the first cytological record from Taiwan, although its reproductive mode was not examined due to the lack

of fertile frond. All the previous records are tetraploid (n = 60, Kurita, 1963, Japan; Weng and Qiu, 1988, China; n = ca. 60, Chiu 1981, China; n = 60, locality unknown, Manton and Sledge, 1954). Compared with the plants in Japan, the type locality, Taiwanese plants tend to have less anastomosing veins of lamina.

*Pteris angustipinna* Tagawa

2n = ca. 87 [triploid, x = 29] (Fig. 2), 32s/s [apogamous].



Figs. 1–4. Somatic chromosomes of Taiwanese ferns. (a) microphotographs and (b) explanatory illustrations of somatic chromosomes. All scale bars = 10 μm. 1. *Coniogramme japonica* ( $2n=60$ ). 2. *Pteris angustipinna* ( $2n=ca. 87$ ). 3. *Pteris dactylina* ( $2n=58$ ). 4. *Pteris longipinna* ( $2n=58$ ).

Note: This is the first cytological record for this species endemic to Taiwan. The species is difficult to distinguish from *P. actinopteroides* H.Christ and *P. henryi* H.Christ in China; the latter two were reported as triploid (Walker, 1962) and diploid (Kato *et al.*, 1992), respectively. More evidence is needed to clarify the relationship among those species.

*Pteris dactylina* Hook.

$2n = 58$  [diploid,  $x = 29$ ] (Fig. 3), 32 s/s [apogamous].

Note: This is a Sino-Himalayan species and apogamous triploids have been reported from Yunnan Prov., China ( $n' = 87$  and  $88$ ; Lin *et al.*, 1996), India ( $n' = 87$ ; Verma in Mehra, 1961, Khullar and Mehra, 1972, Punetha, 1989, Punetha and Sen, 1989; earlier records  $n = 33, 55, 57, 2n = 66, 78$  [Sharma and Majumder, 1956] are possible miscounts), and Nepal ( $2n = 87$ ; Walker, 1962). The present result, which is the first record for the Taiwanese material, is the discovery of a new cytotype for the species. Spore sizes of the apogamous diploid (38–45  $\mu\text{m}$  in diameter) are slightly smaller than those of the specimens from the Himalayan region (larger than 50  $\mu\text{m}$ ) which are probably apogamous triploids. Since we also observed spore sizes of ca. 40–45  $\mu\text{m}$  with spore counts of 32 spores per sporangium in a specimen collected in Nantou Co., Taiwan (TNS-VS-808247), apogamous diploids may be widely distributed in Taiwan. Possible taxonomic issue is existed in this disjunctive distribution species, Himalayan and Taiwan. In China, *P. dactylina* is morphological similar to *P. gallinopes* Ching, but which is reported as tetraploid (Wang, 1989). Based on our field study around the Himalayan region, triploid plants in this group have not been found.

*Pteris longipinna* Hayata

$2n = 58$  [diploid,  $x = 29$ ] (Fig. 4), 64 s/s [sexual].

Note: This is the first cytological record for this endemic species to Taiwan. In Taiwan, the most morphologically similar species is *P. venusta* Kunze. However, according to the *Pteris* phylog-

eny study until now (Chao *et al.*, 2012), the two species are belonged to two distinctly different and independent lineages. The origin of this endemic species is curiously.

**Thelypteridaceae**

*Stegnogramma tottooides* (Hayata ex H.Itô) K. Iwats.

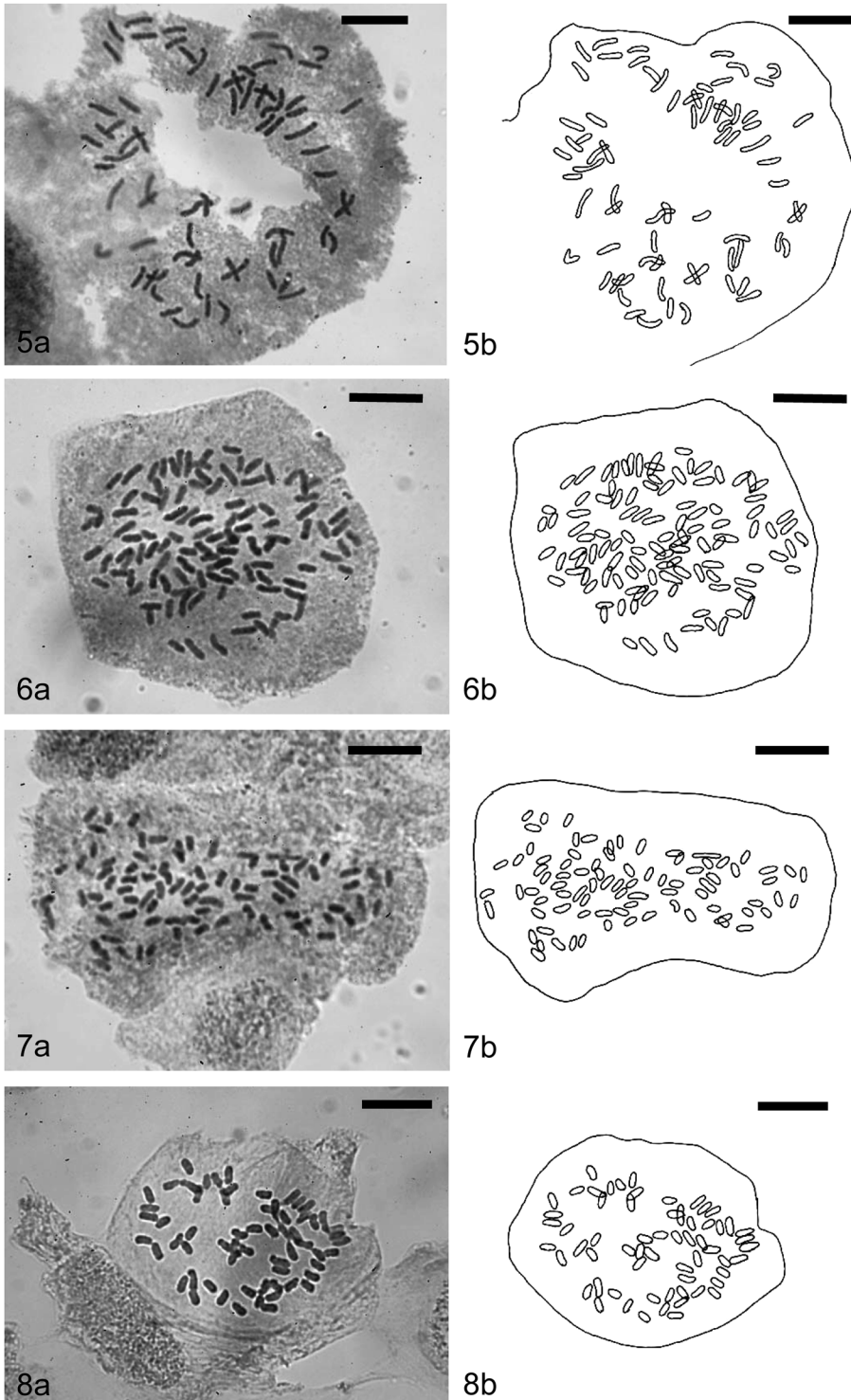
$2n = 72$  [diploid,  $x = 36$ ] (Fig. 5), 64 s/s [sexual].

Note: Our result matches the previous report,  $n = 36$ , diploid, 64 s/s based on a Taiwanese plant (Nantou Co.) by Tsai and Shieh (1985). The Asian species of *Stegnogramma* sect. *Leptogramma*, especially *S. pozoi* and its allied species including *S. tottooides* seem to form a species complex considering their wide range of morphological, cytological and molecular variation so far reported (Yatabe *et al.*, 1998; Bir and Irudayaraj, 2001; He and Zhang, 2012).

*Thelypteris angustifrons* (Miq.) Ching  $\times$  *T. glanduligera* (Kunze) Ching

$2n = 108$  [tetraploid,  $x = 27$ ] (Fig. 6), spores abortive (Fig. 15).

Note: This plant is morphologically intermediate between *T. angustifrons* and *T. glanduligera* (i.e. the upper basal pinnule of each pinna is a little larger than the others, nearly independent but not cleft to the base in lower pinnae). A hybrid of this combination has not yet been published, but we have already noticed several herbarium specimens collected in Japan (e.g. *T. Hosokura* s.n. [TNS-VS-1162522], Numazu-shi, Shizuoka Pref.), and this is the first report from Taiwan. Cytological variation is known in *T. angustifrons* in Japan — triploid, tetraploid and hexaploid are present, but only the hexaploid is widely found throughout the country (Nakato *et al.*, 2002), while *T. glanduligera* is a tetraploid (Nakato *et al.*, 2002). Our present count is best explained as a hybrid between tetraploid *T. angustifrons* and tetraploid *T. glanduligera*, though only diploids were recorded in both the parental species in Taiwan (Tsai and Shieh, 1977, 1983, 1985).



Figs. 5–8. Somatic chromosomes of Taiwanese ferns. (a) microphotographs and (b) explanatory illustrations of somatic chromosomes. All scale bars = 10  $\mu\text{m}$ . 5. *Stegnogramma tottoides* ( $2n=72$ ). 6. *Thelypteris angustifrons* × *T. glanduligera* ( $2n=108$ ). 7. *Acystopteris tenuisecta* ( $2n=84$ ). 8. *Blechnum hancockii* ( $2n=62$ ).



### Cystopteridaceae

*Acystopteris tenuisecta* (Blume) Tagawa

$2n = 84$  [diploid,  $x = 42$ ] (Fig. 7), 64 s/s [sexual].

Note: Previous studies reported both sexual diploid ( $n = 42$ ; Tsai and Shieh, 1975, 1985) and tetraploid ( $n = 84$ ; Tsai and Shieh, 1978, 1983, 1985) from Taiwan, and our record matched the former. The chromosome number  $n = 41$  cited by Cheng and Zhang (2010) is probably an error. Both sexual tetraploid ( $n = 84$ ) and sterile triploid ( $2n = 126$  with irregular meiosis) were recorded in India (Bir in Mehra, 1961, Bir, 1971).

### Blechnaceae

*Blechnum hancockii* Hance

$2n = 62$  [diploid,  $x = 31$ ] (Fig. 8).

Note: Due to the lack of fertile frond in the stocks we examined, the reproductive mode was unknown. A previous study reported this species as sexual tetraploid ( $n = 62$ , 64 s/s; Tsai, 1973) based on a Taiwanese material, thus, diploid is a new cytotype for this species. This quasi-endemic species to Taiwan is closely related to *B. niponicum* (Kunze) Makino endemic to Japan as demonstrated by their completely matching chloroplast *rbcl* sequences (Ebihara, 2011), and the present chromosome count matches those of *B. niponicum* ( $n = 31$ ,  $2n = 62$ , sexual diploid, see references in Takamiya, 1996). Although *B. hancockii* is said to be distinguishable from *B. niponicum* by shorter fertile fronds, almost the same in length as the sterile ones, we should still consider the possibility of *B. hancockii* being an infraspecific variation of *B. niponicum* in its southernmost distribution range.

### Dryopteridaceae

*Cyrtomium devexiscapulae* (Koidz.) Ching

$2n = 164$  [tetraploid,  $x = 41$ ] (Fig. 9), 64 s/s [sexual].

Note: This is an amphidiploid species derived from hybridization between *C. falcatum* (L.f.) C.Presl subsp. *australe* nom. nud. (diploid) and an unknown diploid species (Matsumoto, 2003; Ebihara and Matsumoto, unpublished data) distributed in Japan, Taiwan, China, South Korea and Vietnam (Zhang *et al.*, 2013). Although dis-

tinguishable from the other members of the *C. falcatum* complex (diploid and triploid) by several characters such as inland distribution, larger fronds and microscales on the upper surface of the lamina (Matsumoto, 2003), it is still often misidentified as *C. falcatum*. The occurrence of this species in Taiwan was already confirmed by Matsumoto *et al.* (2006) based on morphological characters including the counts of 64 spores per sporangium, and this is the first record of its ploidy level for Taiwanese plants.

*Dryopteris enneaphylla* (Baker) C.Chr. var. *enneaphylla*

$2n = 82$  [diploid,  $x = 41$ ], 64 s/s [sexual] (Fig. 10).

Note: This is the first cytological record for this taxon distributed in Taiwan and continental China (Hubei and Zhejiang Prov. [Zhang *et al.*, 2013]). *Dryopteris sieboldii* (van Houtte ex Mett.) Kuntze, a morphologically closely related species distributed in continental China and Japan is known as a sexual tetraploid based on material from Japan (Kurita, 1962; Mitui, 1968; Hirabayashi, 1969). The chromosome count  $n = 82$  reported from Taiwan as "*D. sieboldii* var. *sieboldii*" (Tsai and Shieh, 1985) is perhaps a misidentification of *D. enneaphylla* var. *enneaphylla*, but their result (tetraploid) does not match the present result.

*Dryopteris integriloba* C.Chr.

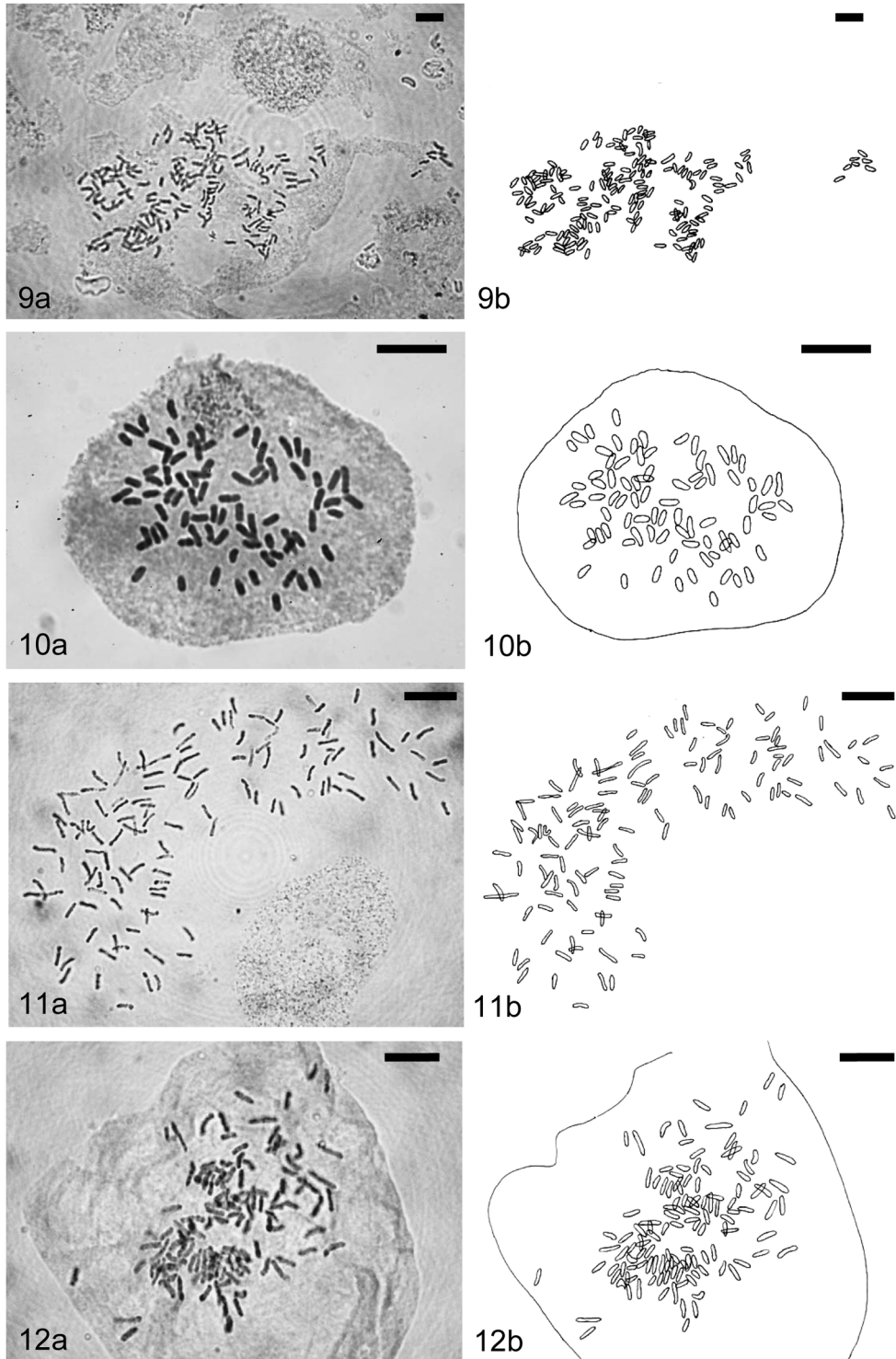
$2n = 123$  [triploid,  $x = 41$ ] (Fig. 11).

Note: Although its reproductive mode has not been clarified due to the lack of fertile frond in the stocks examined, this is the first cytological record from Taiwan. Both sexual tetraploid ( $n = 82$ , Weng, 1989a) and apogamous triploid ( $2n =$  ca. 120 and ca. 123, Gibby, 1985) have been reported from continental China, and our plant from Taiwan seems to match the latter.

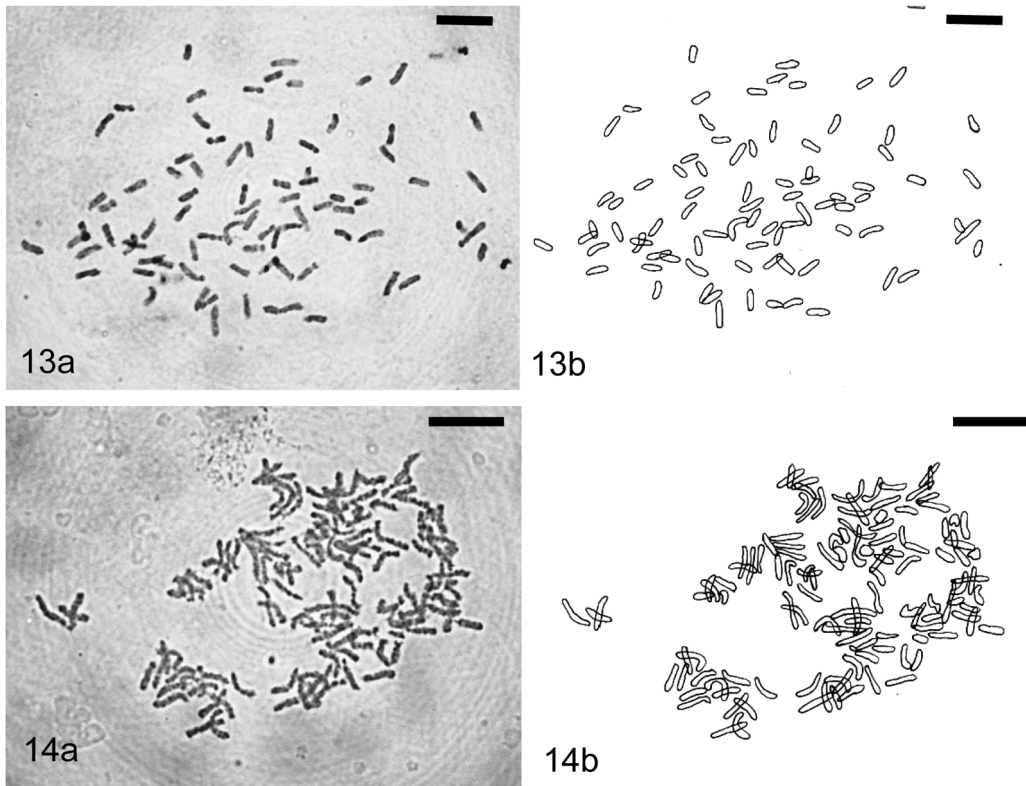
*Dryopteris labordei* (H.Christ) C.Chr. var. *labordei*

$2n =$  ca. 123 [triploid,  $x = 41$ ] (Fig. 12), 32 s/s [apogamous].

Note: This is the first cytological record from Taiwan for this taxon distributed in Taiwan, continental China and Japan (Zhang *et al.*, 2013),



Figs. 9–12. Somatic chromosomes of Taiwanese ferns. (a) microphotographs and (b) explanatory illustrations of somatic chromosomes. All scale bars = 10 μm. 9. *Cyrtomium devexiscapulae* ( $2n = 164$ ). 10. *Dryopteris enneaphylla* var. *enneaphylla* ( $2n = 82$ ). 11. *Dryopteris integriloba* ( $2n = 123$ ). 12. *Dryopteris labordei* var. *labordei* ( $2n = \text{ca.}123$ ).



Figs. 13–14. Somatic chromosomes of *Dryopteris varia*. (a) microphotographs and (b) explanatory illustrations of somatic chromosomes. All scale bars = 10  $\mu$ m. 13. A diploid ( $2n = 82$ ). 14. A triploid ( $2n = \text{ca. } 123$ ).

and it matched the previous record from continental China ( $n' = 123$ , apogamous triploid [Weng, 1989b]). An apogamous diploid with the chromosome number " $n = 41$ " was reported by Weng (1989a), but it needs confirmation because the 41 bivalents in the figure are unlike chromosomes of apogamous species.

*Dryopteris varia* (L.) Kuntze

$2n = 82$  [diploid,  $x = 41$ ] (Fig. 13), 64 s/s [sexual];  $2n = \text{ca. } 123$  [triploid] (Fig. 14), 32 s/s [apogamous].

Note: The sexual diploid is the first reliable record for this species widely distributed in Asia — as Tsai and Shieh (1985) listed diploid *D. varia* in Table 1 but not in Table 3, their record of diploid is likely an error. Previously known cytotypes and reproductive modes are as follows: apogamous diploid ( $n = 82$ , Hirabayashi, 1966, 1967, 1974, Japan), apogamous triploid ( $n' = 123$ ,

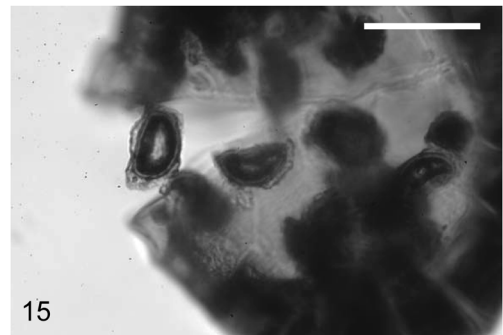


Fig. 15. Irregular-shaped spores of *Thelypteris angustifrons*  $\times$  *T. glanduligera*. Scale bar = 50  $\mu$ m.

Mitui, 1966, 1968, Hirabayashi, 1970, Japan;  $2n = 123$ , Lin *et al.*, 2003, Japan;  $2n = 123$ , Gibby, 1985, China and Hong Kong;  $2n = \text{ca. } 123$ , Nakato *et al.*, 1995, China;  $n' = 123$ , Weng, 1989a, China;  $2n = 123$ , Lin *et al.*, 2002, China;  $n = 41\text{III}$ , Tsai and Shieh, 1985, Taiwan), sexual tetraploid ( $n = 82$ , Tsai and Shieh, 1975, 1985,



Taiwan). In consequence, sexually reproducing individuals are so far known only from Taiwan.

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