

Chromosome Numbers of 18 Ferns in Japan: Toward Completion of Chromosome Information in Japanese Ferns

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Abstract Mitotic chromosome numbers of 18 fern species (Plagiogyriaceae, Dennstaedtiaceae, Pteridaceae, Thelypteridaceae and Polypodiaceae) in Japan were counted and their reproductive modes were estimated by spore number per sporangium. Chromosome numbers were recorded for the first time for nine taxa: *Plagiogyria koidzumii*, *Microlepia obtusiloba* var. *obtusiloba*, *M. ×muroyae*, *Pteris satsumana*, *P. yakuinsularis*, *Thelypteris hattorii*, *T. castanea*, *Lepisorus uchiyamae* and *Colysis decurrens* × *C. pteropus*. New chromosome numbers or new cytotypes were recorded for three taxa: *Pteris oshimensis*, *Thelypteris uraiensis* and *Goniophlebium someyae*.

Key words: chromosome number, *Colysis*, *Goniophlebium*, *Lepisorus*, *Microlepia*, *Plagiogyria*, *Pteris*, *Thelypteris*.

The fern and lycophyte flora of Japan comprising ca. 1,100 taxa (including species, subspecies, varieties and interspecific hybrids) are accompanied with rich cytotaxonomic information covering 596 taxa (more than 54% of the taxa) (Takamiya, 1996). After publication of Takamiya's Chromosome Index in 1996, several studies focusing on certain groups provided new chromosome counts for the species without previous information (Ebihara *et al.*, 2005, 2009, 2014; Hori *et al.*, 2015; Lin *et al.*, 2003; Masuyama and Watano, 2005; Matsumoto, 2003; Nakato, 1998a; Nakato and Ebihara, 2011; Nakato and Kato, 2002; Nakato and Yamaoka, 2003; Nakato *et al.*, 2002, 2004; Ohta and Takamiya, 1999; Takamiya, 2006; Takamiya *et al.*, 1997, 1999, 2000, 2001; Takamiya and Ohta, 2001; Terada and Takamiya, 2006) for a total of approximately 90 additional taxa, including some published before 1996 but not included in the Takamiya (1996), and raised the coverage to 61% (683 taxa). If hybrids are excluded, the coverage is

approximately 74% (539/726 taxa). We are making further efforts to fill the lacking cytological information of the taxa as information on ploidies and reproductive modes is quite important in trying to clarify the biological entity.

Materials and Methods

Materials used for chromosome counting are listed in Table 1. All the voucher specimens are deposited in TNS. Methods for mitotic chromosome counting in root tips of living stocks followed those used in Ebihara *et al.* (2014). Reproductive modes were determined by counting spore number per sporangium and by spore shape regularity in the voucher specimens or in cultivated stocks except for *Plagiogyria koidzumii*. As fertile frond was lacking in our material of *P. koidzumii*, we observed the spores in two sheets of herbarium specimen collected in Iriomote Island, Japan (*Shimabuku 1808* [TNS VS-401427]; *Kurata & Nakaïke 479* [TNS

Table 1. Plant materials used in this study with their chromosome counts

Taxon	Chromosome number and ploidy	Reproductive mode	Voucher	Locality	Chromosome figure
Plagiogyriaceae					
<i>Plagiogyria koidzumii</i> Tagawa	2n = 130 (2x, x = 65)	sexual (64 s/s)*1	AE 3526 [TNS VS-1232394]	Okinawa Pref., Yaeyama-gun, Taketomi-cho (Iriomote Isl.)	Fig. 1
Dennstaedtiaceae					
<i>Microlepia obtusiloba</i> Hayata var. <i>obtusiloba</i>	2n = 168 (4x, x = 42)	sexual (64 s/s)	AE 3525 [TNS VS-1232391]	Okinawa Pref., Yaeyama-gun, Taketomi-cho, Urauchigawa River (Iriomote Isl.)	Fig. 2
<i>Microlepia sinostrigosa</i> Ching	2n = 168 (4x, x = 42)	sexual (64 s/s)	Nakato 2588 [TNS VS-1247769]	Shizuoka Pref., Shimoda-shi, Rendaiji	Fig. 3
	2n = 168 (4x, x = 42)	sexual (64 s/s)	Nakato 2589 [TNS VS-1247771]	ibid.	
	2n = 168 (4x, x = 42)	sexual (64 s/s)	Nakato 2600 [TNS VS-1247766]	Shizuoka Pref., Kamo-gun, Kawazu-cho, Ohnabe	
	2n = 168 (4x, x = 42)	sexual (64 s/s)	Nakato 2601 [TNS VS-1247768]	ibid.	
<i>Microlepia</i> × <i>muroyae</i> Sa. Kurata, nom. nud.	2n = 126 (3x, x = 42)	sterile (spore irregular)	AE 3382 [TNS VS-1209694]	Miyazaki Pref., Minaminaka- gun, Kitago-cho, Inohae (cultivated by T. Minamitani)	Fig. 4
<i>Microlepia speluncae</i> (L.) T. Moore	2n = 172 (4x, x = 43)	sexual (64 s/s)	Nakato 3194 [TNS VS-1249716]	Okinawa Pref., Yaeyama-gun, Taketomi-cho, Funaura (Iriomote Isl.)	Fig. 5
Pteridaceae					
<i>Pteris oshimensis</i> Hieron.	2n = 58 (2x, x = 29)	sexual (64 s/s)	AE 3379 [TNS VS-1200898]	Kagoshima Pref., Ohshima- gun, Sumiyoh-son, Sumiyoh River (Amami-oshima Isl.)	Fig. 6
	2n = 58 (2x, x = 29)	apogamous (32 s/s)	AE 3457 [TNS VS-1219665]	Saga Pref., Karatsu-shi, Ouchi-cho, Hirayamashita	
	2n = 58 (2x, x = 29)	apogamous (32 s/s)	Nakato 3151 [TNS VS-1247849]	Kumamoto Pref., Amakusa- shi, Futaura-machi	
	2n = 58 (2x, x = 29)	apogamous (32 s/s)	AE 3234 [TNS VS-1176826]	Miyazaki Pref., Saito-shi, Minoh	
	2n = 58 (2x, x = 29)	not examined	AE 3233 [TNS VS-1176825]	ibid.	
	2n = 58 (2x, x = 29)	not examined	AE 3236 [TNS VS-1176828]	ibid.	
	2n = 58 (2x, x = 29)	not examined	AE 3237 [TNS VS-1176829]	ibid.	
<i>Pteris natiensis</i> Tagawa	2n = 58 (2x, x = 29)	apogamous (32 s/s)	AE 3460 [TNS VS-1225898]	Kagoshima Pref., Satsuma- sendai-shi, Odake (Shimo- koshiki Isl.)	Fig. 14
<i>Pteris satsumana</i> Sa. Kurata	2n = 58 (2x, x = 29)	apogamous (32 s/s)	AE 3445 [TNS VS-1226039]	Kagoshima Pref., Izumi-shi, Takaono-cho, Takaono Dam	Fig. 15
<i>Pteris yakuinsularis</i> Sa. Kurata	2n = 87 (3x, x = 29)	apogamous (32 s/s)	AE 3246 [TNS VS-1176838]	Miyazaki Pref., Saito-shi, Minoh	Fig. 16
	2n = 87 (3x, x = 29)	apogamous (32 s/s)	AE 3240 [TNS VS-1176832]	ibid.	
	2n = 87 (3x, x = 29)	apogamous (32 s/s)	AE 3241 [TNS VS-1176833]	ibid.	
	2n = ca. 87 (3x, x = ca. 29)	not examined	AE 3237 [TNS VS-1176829]	ibid.	
	2n = 87 (3x, x = 29)	not examined	AE 3238 [TNS VS-1176830]	ibid.	
<i>Pteris laurilsilvicola</i> Sa. Kurata	2n = 58 (2x, x = 29)	apogamous (32 s/s)	AE 3239 [TNS VS-1176831]	ibid.	Fig. 17
	2n = 58 (2x, x = 29)	not examined	AE 3235 [TNS VS-1176827]	ibid.	
Thelypteridaceae					
<i>Thelypteris hattorii</i> (H. Itô) Tagawa f. <i>nemoralis</i> (Ching) Ebihara et Nakato	2n = 136 (4x, x = 34)	sexual (64 s/s)	Nakato 3096 [TNS VS-1248154]	Ehime Pref., Kamiukena-gun, Kumakogen-cho, Kamihata- nokawa	Fig. 18

Table 1. Continued

Taxon	Chromosome number and ploidy	Reproductive mode	Voucher	Locality	Chromosome figure		
<i>Thelypteris quepaertensis</i> (Christ) Ching	2n = 68 (2x, x = 34)	sexual (64 s/s)	<i>Nakato 2878</i> [TNS VS-1247754]	Gunma Pref., Tone-gun, Minakami-machi, Yubiso	Fig. 19		
	2n = 68 (2x, x = 34)	sexual (64 s/s)	<i>Nakato 2907</i> [TNS VS-1247755]	Nagano Pref., Saku-shi, Tateshina-machi			
<i>Thelypteris esquirolii</i> (Christ) Ching	2n = 70 (2x, x = 35)	sexual (64 s/s)	<i>Nakato 2037</i> [TNS VS-1247763]	Shizuoka Pref., Tagata-gun, Amagi-yugashima-cho, Joren-no-taki Fall	Fig. 20		
	2n = 70 (2x, x = 35)	sexual (64 s/s)	<i>Nakato 2379</i> [TNS VS-1247759]	ibid.			
	2n = 70 (2x, x = 35)	sexual (64 s/s)	<i>Nakato 2380</i> [TNS VS-1247760]	ibid.			
	2n = 70 (2x, x = 35)	sexual (64 s/s)	<i>Nakato 2381</i> [TNS VS-1247761]	ibid.			
	2n = 70 (2x, x = 35)	sexual (64 s/s)	<i>Nakato 2556</i> [TNS VS-1247756]	Kochi Pref., Aki-shi, Harikawa River			
	2n = 70 (2x, x = 35)	sexual (64 s/s)	<i>Nakato 2559</i> [TNS VS-1247765]	Kochi Pref., Susaki-shi, Nagatake			
	2n = 70 (2x, x = 35)	sexual (64 s/s)	<i>Nakato 2562</i> [TNS VS-1247764]	ibid.			
	2n = 70 (2x, x = 35)	sexual (64 s/s)	<i>Nakato 2327</i> [TNS VS-1247762]	Kagoshima Pref., Oshima-gun, Yamato-son, Materia Fall (Amami-oshima Isl.)			
	2n = 70 (2x, x = 35)	sexual (64 s/s)	<i>Nakato 2408</i> [TNS VS-1247757]	Okinawa Pref., Kunigami-gun, Kunigami-son, Benoki (Okinawa Isl.)			
	<i>Thelypteris castanea</i> (Tagawa) Ching	2n = 62 (2x, x = 31)	sexual (64 s/s)	<i>AE 3176</i> [TNS VS-1176779]		Okinawa Pref., Yaeyama-gun, Taketomi-cho, Urauchigawa River (Iriomote Isl.)	Fig. 21
		2n = 62 (2x, x = 31)	sexual (64 s/s)	<i>AE 3177</i> [TNS VS-1176780]		ibid.	
2n = 62 (2x, x = 31)		sexual (64 s/s)	<i>AE 3178</i> [TNS VS-1176781]	ibid.			
2n = 62 (2x, x = 31)		sexual (64 s/s)	<i>AE 3179</i> [TNS VS-1176782]	ibid.			
<i>Thelypteris uraiensis</i> (Rosenst.) Ching	2n = 140 (4x, x = 35)	sexual (64 s/s)	<i>Nakato 2343</i> [TNS VS-1197316]	Kagoshima Pref., Mt. Yuwan (Amami-oshima Isl.)	Fig. 22		
	2n = 140 (4x, x = 35)	sexual (64 s/s)	<i>Nakato 2344</i> [TNS VS-1197317]	ibid.			
Polypodiaceae							
<i>Goniophlebium someyae</i> (Yatabe) Ebihara	2n = 72 (2x, x = 36)	sexual (64 s/s)	<i>Nakato 2610</i> [TNS VS-1197321]	Kochi Pref., Kami-gun, Monobe-son	Fig. 23		
<i>Lepisorus uchiyamae</i> (Makino) H.Itô	2n = 70 (2x, x = 35)	sexual (64 s/s)	<i>AE 3359</i> [TNS VS-1200880]	Kagoshima Pref., Amami-shi (Amami-oshima Isl.)	Fig. 24		
<i>Colysis decurrens</i> (Wall. ex Hook. et Grev.) Nakaïke × <i>C. pteropus</i> (Blume) Bosman	2n = 108 (3x, x = 36)	sterile (sporangium not developed)	<i>AE 3192</i> [TNS VS-1176790]	Okinawa Pref. Yaeyama-gun, Taketomi-cho, Yutsun River (Iriomote Isl.)	Fig. 25		

*1 Reproductive mode was examined in two herbarium specimens as the stock did not produce fertile frond.

VS-368807]). For *Pteris oshimensis*, we examined reproductive modes based on spore number per sporangium in additional herbarium specimens in TNS (Table 2: ten from Amami-oshima Isl, seven from Honshu, one from Shikoku and five from Kyushu).

Results and Discussion

Plagiogyriaceae

Plagiogyria koidzumii Tagawa—2n = 130 (2x, sexual) [Fig. 1]

First chromosome count for the species. This species is distributed across Japan (Iriomote Island) and Taiwan (Nantou Co.), but rare in both

Table 2. Examined specimens of *Pteris oshimensis* for spore number per sporangium

Specimen No. [TNS VS-]	Collection number	Coll. date	Locality
Sexual form with 64 spores per sporangium			
311030	<i>R. Yoroï 4426</i>	1972-12-30	Kagoshima Pref., Ohshima-gun, Sumiyoh-son, Nishinakama (Amami-oshima Isl.)
311047	<i>R. Yoroï 4394</i>	1972-12-30	ibid.
295840	<i>M. Sakakibara s.n.</i>	1972-12-30	ibid.
365275	<i>M. Sakakibara s.n.</i>	1972-12-30	ibid.
366360	<i>A. Yamamoto, T. Nakaike & M. Ishizuka 55</i>	1977-11-25	Kagoshima Pref., Ohshima-gun, Sumiyoh-son, Yanma (Amami-oshima Isl.)
808623	<i>F. Miyamoto 8602</i>	1983-01-31	Kagoshima Pref., Ohshima-gun, Setouchi-cho, Yakugachi River (Amami-oshima Isl.)
965217	<i>J. Haginiwa 15217</i>	1989-11-12	Kagoshima Pref., Ohshima-gun, Sumiyoh-son (Amami-oshima Isl.)
1103520	<i>G. Kokubugata, M. Yokota & H. Yamashita 12801</i>	2010-07-02	Kagoshima Pref., Amami-shi, Kamiya, Sumiyoh-gawa (Amami-oshima Isl.)
1023664	<i>M. Ono s.n.</i>	1988-01-15	Kagoshima Pref., Ohshima-gun, Amagi-cho, Mikyo (Tokunoshima Isl.)
1023715	<i>M. Ono s.n.</i>	1988-01-17	ibid.
Apogamous form with 32 spores per sporangium			
1165877	<i>T. Yamamoto s.n.</i>	1976-10-02	Shizuoka Pref., Kamo-gun, Kamo-mura, Ugusu
9515970	<i>K. Satake s.n.</i>	1962-08-12	Shizuoka Pref., Kamo-gun, Kawazu-cho, Numanokawa
332547	<i>K. Satake s.n.</i>	1960-12-25	ibid.
344406	<i>S. Ohtani s.n.</i>	1959-08-12	ibid.
1033423	<i>H. Itô s.n.</i>	1959-10-24	Mie Pref., Owase-shi, Kata
808615	<i>T. Iwasaki 1179</i>	1970-08-22	Wakayama Pref., Nishimuro-gun, Shirahama-cho, Shagawa
808616	<i>T. Iwasaki 1180</i>	1970-08-22	ibid.
364784	<i>K. Yamaoka s.n.</i>	1977-02-13	Kochi Pref., Aki-gun, Toyo-cho, Kannoura
364685	<i>S. Kurata s.n.</i>	1961-08-11	Saga Pref., Kurokami-yama
364694	<i>Y. Tanno s.n.</i>	1975-08-20	Saga Pref., Imari-shi
364874	<i>S. Kurata s.n.</i>	1975-07-29	Saga Pref., Higashimatsuura-gun, Kitahatamura, Kishidake
344676	<i>Y. Kuranari s.n.</i>	1975-07-29	ibid.
364848	<i>B. Matsubayashi s.n.</i>	1975-11-03	Nagasaki Pref., Nagasaki-shi, Iwaya-yama

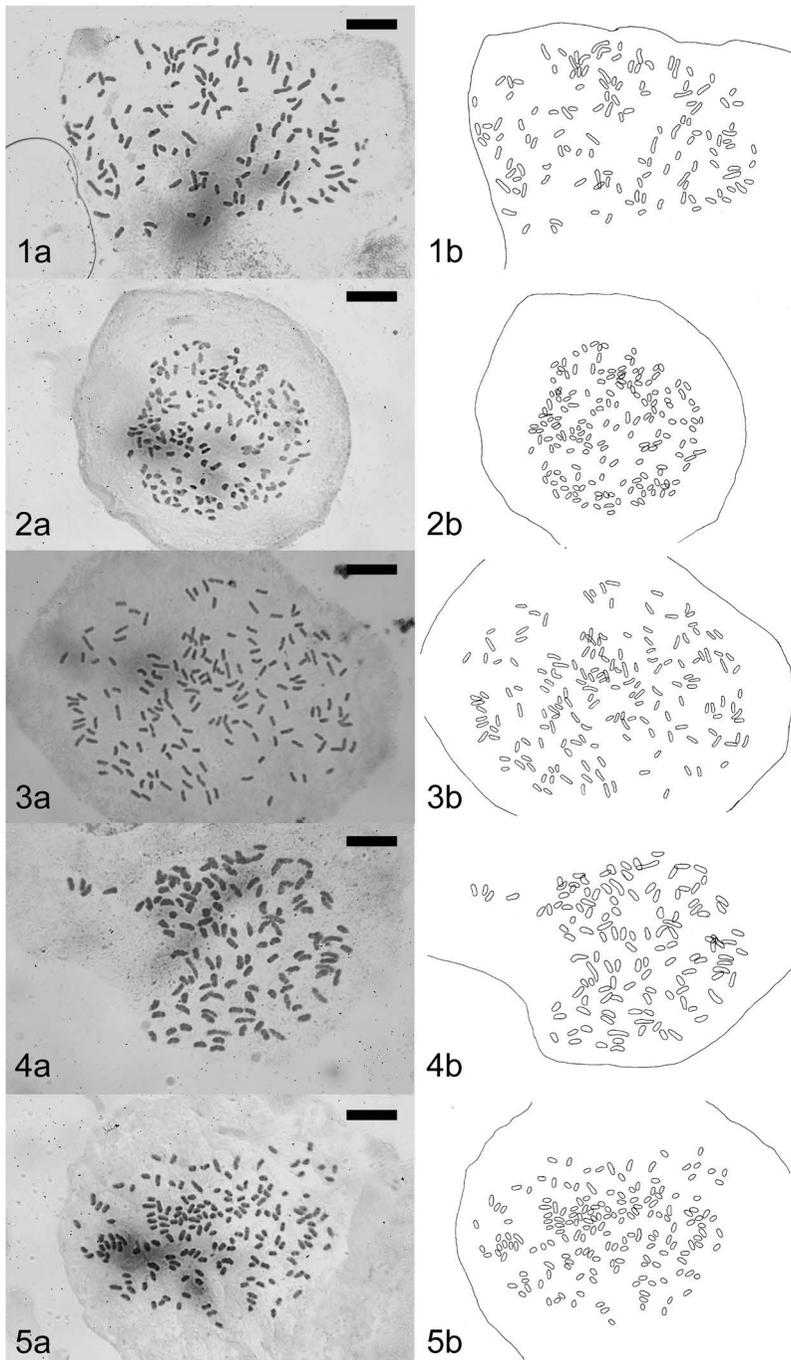
areas. The circum-Pacific genus *Plagiogyria* comprising ca. 15 species shows chromosome base number $x=65$ and 66 (Nakato and Mitui 1983; Walker, 1966), and almost half of the species with known chromosome numbers are polyploids—tetraploid, pentaploid and hexaploid, e.g. Nakato and Mitui (1983), Nakato (1988), Nakato and Kato (2002). This is the fourth diploid species for the genus. The taxonomic treatment by Zhang and Nooteboom (1998) which synony-

mized this species under *P. euphlebia* (Kunze) Mett. is not supported due to difference in the ploidy level (diploid for *P. koidzumii* versus tetraploid for *P. euphlebia* [Kurita, 1963; Nakato and Mitui, 1983]).

Dennstaedtiaceae

Microlepia obtusiloba Hayata var. *obtusiloba*— $2n = 168$ ($4x$, sexual) [Fig. 2]

First chromosome count for the variety. Two



Figs. 1–5. Mitotic metaphase chromosomes. (a) microphotographs and (b) explanatory illustrations. Scale bars = 10 μ m. 1. *Plagiogyria koidzumii* ($2n = 130$). 2. *Microlepidia obtusiloba* var. *obtusiloba* ($2n = 168$). 3. *Microlepidia sinostrigosa* ($2n = 168$). 4. *Microlepidia* \times *muroyae* ($2n = 126$). 5. *Microlepidia speluncae* ($2n = 172$).

varieties are recognized in *M. obtusiloba* based on lamina shapes (Serizawa, 1972): var. *obtusiloba* distributed in Taiwan (type locality), China, Japan and Indochina and var. *angustata* Seriz. recorded only in Yakushima Island, Japan. Tetraploidy ($2n = 168$) was reported in var. *angustata* (Nakato, 1987 as "*Microlepidia obtusiloba*"), and we made a first cytological record for var. *obtusiloba* in the present study (tetraploid). There seems no cytological difference between the two varieties.

Microlepidia sinostrigosa Ching— $2n = 168$ (4x, sexual) [Fig. 3]

Previous studies clarified that it is tetraploid (Kurita, 1967; Sasamoto, 1970; Mitui, 1975), but the base number remained ambiguous ($x = 42$ or 43). In the present study, we confirmed the base number $x = 42$ based on four individuals from two localities. Although the Japanese populations of *M. sinostrigosa* are sometimes treated as an independent species *M. izupeninsulae* Sa.Kurata, we followed the treatment of Serizawa (1972) who synonymized *M. izupeninsulae* under *M. sinostrigosa*. In contrast, Yan *et al.* (2013) synonymized *M. sinostrigosa* under *M. pseudostrigosa* Makino, but we do not accept this treatment on the grounds that the former is clearly different from the latter in that it has glabrous adaxial rachis and more deeply dissected (bipinnate) lamina.

Microlepidia × *muroyae* Sa.Kurata, nom. nud.— $2n = 126$ (3x, sterile) [Fig. 4]

First chromosome count for the hybrid. This hybrid was originally recognized as an interspecific hybrid between *M. izupeninsulae* (= *M. sinostrigosa*) and *M. substrigosa* (Kurata and Nakaike, 1994), but there is another opinion that it is a hybrid between *M. obtusiloba* and *M. substrigosa* (S. Serizawa, personal communication). Our present result suggesting its sterile triploidy does not support either hypothesis because the presumable parent species, *M. sinostrigosa*, *M. obtusiloba* and *M. substrigosa*, are all tetraploid, not diploid (present study; Mitui, 1968; Nakato,

1987). Besides, strangely enough, none of the putative parent species has been found around any single site of the hybrid (T. Minamitani, personal communication). Further study to identify the parent species is needed.

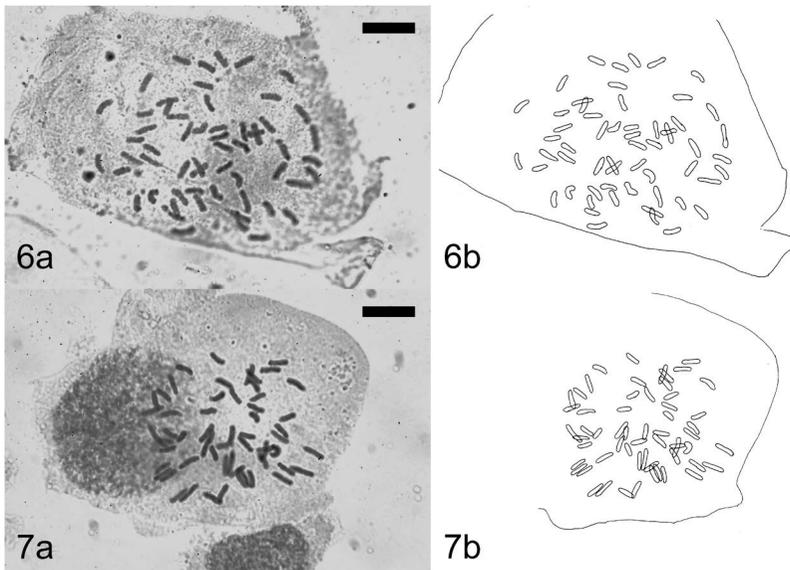
Microlepidia spelunca (L.) T. Moore— $2n = 172$ (4x, sexual) [Fig. 5]

This species is widely distributed throughout the tropics, and sometimes infraspecific taxa are recognized (Holttum, 1954). The morphology of our present material corresponds to the form of "var. *hancei* (Prantl) C.Chr. et Tardieu" often occurs in open *Ananas* fields which is with smaller fronds (less than 80 cm long), and its chromosome number matches the sole previous chromosome record from Japan based on a material from Hahajima, Bonin Islands (Mitui, 1973) which is probably a typical understory form. Correlation between morphological/habitat variations and cytotypes (diploid, tetraploid and hexaploid have been known outside of Japan [Manton and Sledge, 1954; Manton, 1959; Bir and Verma, 2010]) needs further study.

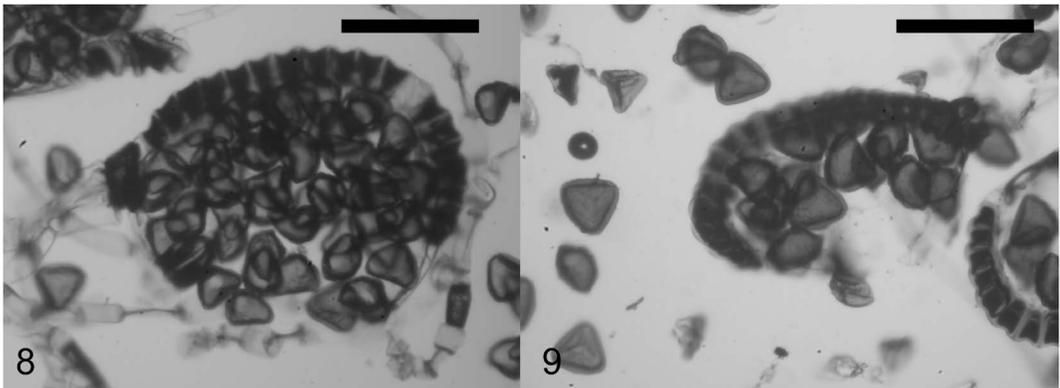
Pteridaceae

Pteris oshimensis Hieron.— $2n = 58$ (2x, sexual and 2x, apogamous) [Figs. 6–12]

Apogamous diploid (Nakato, 1996, four localities) and apogamous triploid (Mitui, 1967, 1968, based on a single observation on a plant collected in Amami-oshima Isl.) have been known for this species. In the present study, sexual diploid was newly discovered in Amami-oshima Isl. and Tokunoshima Isl. in the Ryukyu Islands (Figs. 6, 8), and all the remaining stocks from three distant localities in Kyushu are apogamous diploids (Figs. 7, 9). As far as we examined the specimens of *P. oshimensis* deposited in TNS (a total of 10 sheets), those from Amami-oshima and Tokunoshima Islands had 64 spores per sporangium without exception, suggesting their reproduction mode is sexual. We also confirmed that the voucher specimen for which chromosome count $2n = 87$ (apogamous triploid) was given by Mitui (1967, 1968) is actually a misidentification of *P.*



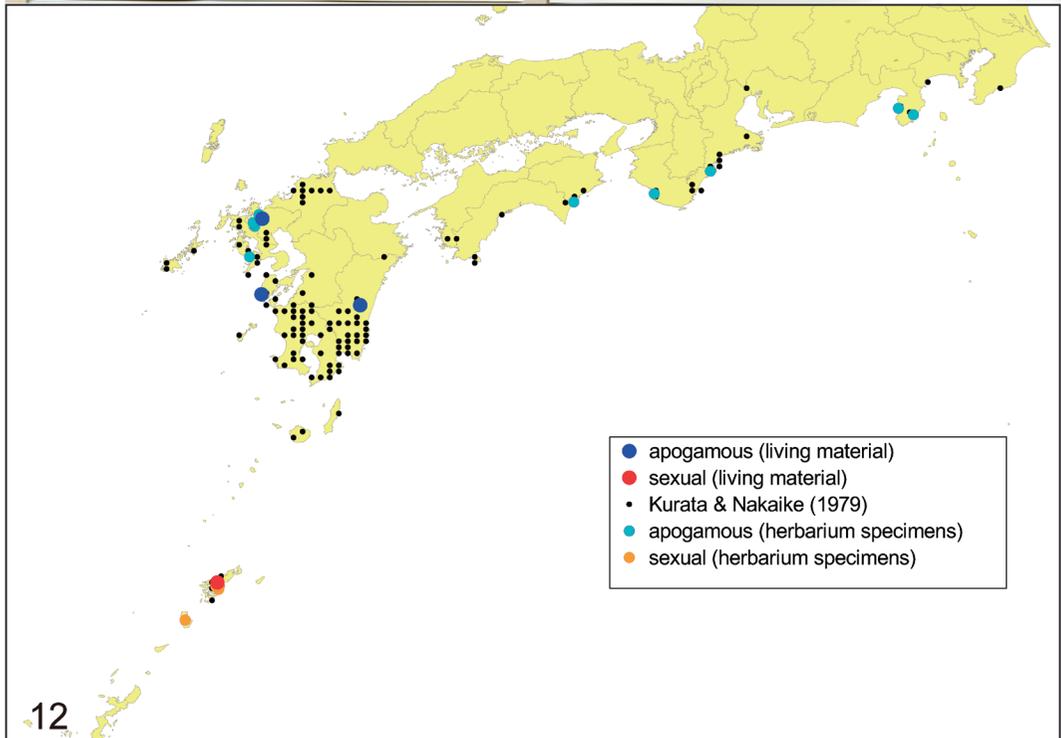
Figs. 6–7. Mitotic metaphase chromosomes of *Pteris oshimensis*. (a) microphotographs and (b) explanatory illustrations. Scale bars = 10 μm . 6. AE3379 (Kagoshima Pref., Amami-oshima Isl.), ($2n = 58$). 7. AE3234 (Miyazaki Pref., Saito-shi, Minoh), ($2n = 58$).



Figs. 8–9. Spores of *Pteris oshimensis*. Scale bars = 100 μm . 8. AE3379 (Kagoshima Pref., Amami-oshima Isl.), 64 spores per sporangium, sexual. 9. Nakato 3151 (Kumamoto Pref., Amakusa-shi, Futaura-machi), 32 spores per sporangium, apogamous.

fauriei Hieron. (Fig. 13). Consequently, there are two allopatric cytotypes in *P. oshimensis* (sexual diploid and apogamous diploid)—the former is confined to the Amami Islands while the latter is widespread but not in the Amami Islands (Fig. 12). The two forms are also distinguishable by frond morphology such as number of pinna pairs (often more than 8 pairs in the sexual form)

(Figs. 10–11); the holotype specimen of *P. oshimensis* (*Faurie 4013*, P!) collected in “Insula Oshima” (= Amami-oshima Isl.) well matches the morphology of the sexual form. The update of their taxonomic treatments is pending until the genomic compositions of both forms become clear.



Pteris natiensis Tagawa— $2n = 58$ ($2x$, apogamous) [Fig. 14]

Pteris satsumana Sa.Kurata— $2n = 58$ ($2x$, apogamous) [Fig. 15]

Pteris yakuinsularis Sa.Kurata— $2n = 87$ ($3x$, apogamous) [Fig. 16]

First chromosome counts for *P. satsumana* and *P. yakuinsularis*. These three species are occasionally lumped into one polymorphic species, *P. natiensis* (e.g. Iwatsuki *et al.*, 1995), but a recent Red Data Book of Japan (Japanese Ministry of Environment, 2015) recognized these three as separate endangered species. In general, they are distinguishable by the following morphological characteristics: *P. natiensis* s.str. has 2 to 4 pairs of lateral pinna with shorter lamina (less than 30 cm); *P. satsumana* has longer lamina than *P. natiensis* s.str., and the pinna and segments are more spaced; *P. yakuinsularis* has thicker lamina than the others and with distinct scales at the stipe base. *Pteris natiensis* is already known as an apogamous diploid (Kurita, 1962), and ploidy levels and reproductive modes of *P. satsumana* and *P. yakuinsularis* are reported here for the first time. Our observation suggested that *P. satsumana* is an apogamous diploid based on a sample collected in the same area as the type locality (Mt. Toya-dake, Kagoshima Pref., Japan). It also suggested that *P. yakuinsularis* is an apogamous triploid based on multiple individuals collected from mixed populations with *P. laurisilvicola* and *P. oshimensis* in Minoh, Saito-shi, Miyazaki Prefecture. These results confirmed that *P. natiensis* s.lat. is an apogamous species complex including cytologically heterogeneous lineages.

Pteris laurisilvicola Sa.Kurata— $2n = 58$ ($2x$, apogamous) [Fig. 17]

Two cytotypes have been reported for this species: apogamous diploid (Shimura, 1980;

Nakato, 1996) and triploid with unknown reproductive mode (Nakato, 1996). Our present result matched the former.

Thelypteridaceae

Thelypteris hattorii (H.Itô) Tagawa f. *nemoralis* (Ching) Ebihara et Nakato, **stat. nov.** — $2n = 136$ ($4x$, sexual) [Fig. 18]

≡ *T. nemoralis* Ching, Bull. Fan Mem. Inst. Biol. 6: 338 (1936) ≡ *T. hattorii* (H.Itô) Tagawa var. *nemoralis* (Ching) Sa.Kurata, Enum. Jap. Pterid. 317, 343 (1961) ≡ *Metathelypteris hattorii* (H.Itô) Ching var. *nemoralis* (Ching) Nakaike, New Fl. Jap. Pterid. (Rev. & Enl.) 817, 842 (1992).

The forma *nemoralis* differs from the forma *hattorii* by glabrous stipes, rachises and costae in the abaxial side. According to Iwatsuki *et al.* (1995), stipe color is “pale green to stramineous with faint purplish tint” in var. (f.) *hattorii* while “without purplish tint” in var. (f.) *nemoralis*, but the “faint purplish tint” is not a stable characteristic of f. *hattorii* based on observation on the specimens in TNS. Thus, these taxa are better to be distinguished at forma rank.

This is the first cytological record for the whole species, and the number ($2n = 136$, tetraploid) matched that recorded in a closely related species *T. laxa* (Franch. et Sav.) Ching (Nakato, 1998b, but aneuploids $2n = 134$ were also reported).

Thelypteris quelpaertensis (Christ) Ching— $2n = 68$ ($2x$, sexual) [Fig. 19]

One record each of $n = 34$ (Mitui, 1970, material from Nagano Pref.) and $n = 36$ (Hirabayashi, 1970, material from Hokkaido Pref.) were made by previous studies, and our present result from two localities (Gunma and Nagano Prefectures) matched the former.

Figs. 10–12. *Pteris oshimensis*. 10. A specimen of the sexual form with 64 spores per sporangium (TNS VS-311030, Kagoshima Pref., Amami-oshima Isl.). 11. A specimen of the apogamous form with 32 spores per sporangium (AE3457, TNS VS-1219665, Saga Pref., Karatsu-shi). 12. A distribution map of the species in Japan (black dots: records by Kurata and Nakaïke (1979), red/orange dots: the sexual form identified by the present study and blue/light blue dots: the apogamous form identified by the present study).



Fig. 13. The voucher specimen of chromosome count, $n = 87$ (apogamous triploid) by Mitui (1967, 1968) as *Pteris oshimensis* (TNS VS-321860, Kagoshima Pref., Amami-oshima Island), but it is probably an immature frond of *P. fauriei*.

Thelypteris esquirolii (Christ) Ching— $2n = 70$ ($2x$, sexual) [Fig. 20]

Two varieties are often distinguished within the species in Japan (Iwatsuki *et al.*, 1995): i.e. var. *esquirolii* with relatively large fronds mostly in the subtropics and var. *glabrata* (Christ) K. Iwats. with smaller fronds in the temperate areas. Although two of the nine present materials fall into the range of var. *esquirolii*, there were no variation in chromosome numbers and reproductive mode among the nine materials collected from five distant localities as well as previous counts (Kurita, 1961; Mitui, 1968; Hirabayashi, 1969). Considering their continuous morphological variation as well as homogeneous cytological nature, we suspend recognizing the varieties within the species.

Thelypteris castanea (Tagawa) Ching— $2n = 62$ ($2x$, sexual) [Fig. 21]

First chromosome count for the species distributed in Taiwan and southern Japan (Iriomote Isl.). Although this species is occasionally synonymized under *T. japonica* (e.g. Knapp, 2011), cytologically it does not correspond to *T. japonica* ($2n = 124$, tetraploid [Nakato *et al.*, 2004], but shows the same number as *T. musashiensis* (Hiyama) Nakato, Sahashi et M.Kato (Nakato *et al.*, 2004). In addition, spores with echinate ornamentation are shared by *T. castanea* and *T. musashiensis*, but those with membraneous ornamentation in *T. japonica*.

Thelypteris uraiensis (Rosenst.) Ching— $2n = 140$ ($4x$, sexual) [Fig. 22]

Our present count, $2n = 140$ (tetraploid of $x = 35$) based on materials from Amami-oshima Island is inconsistent with the previous record $n = 62$ (tetraploid of $x = 31$) from Yakushima Island (Hirabayashi, 1969).

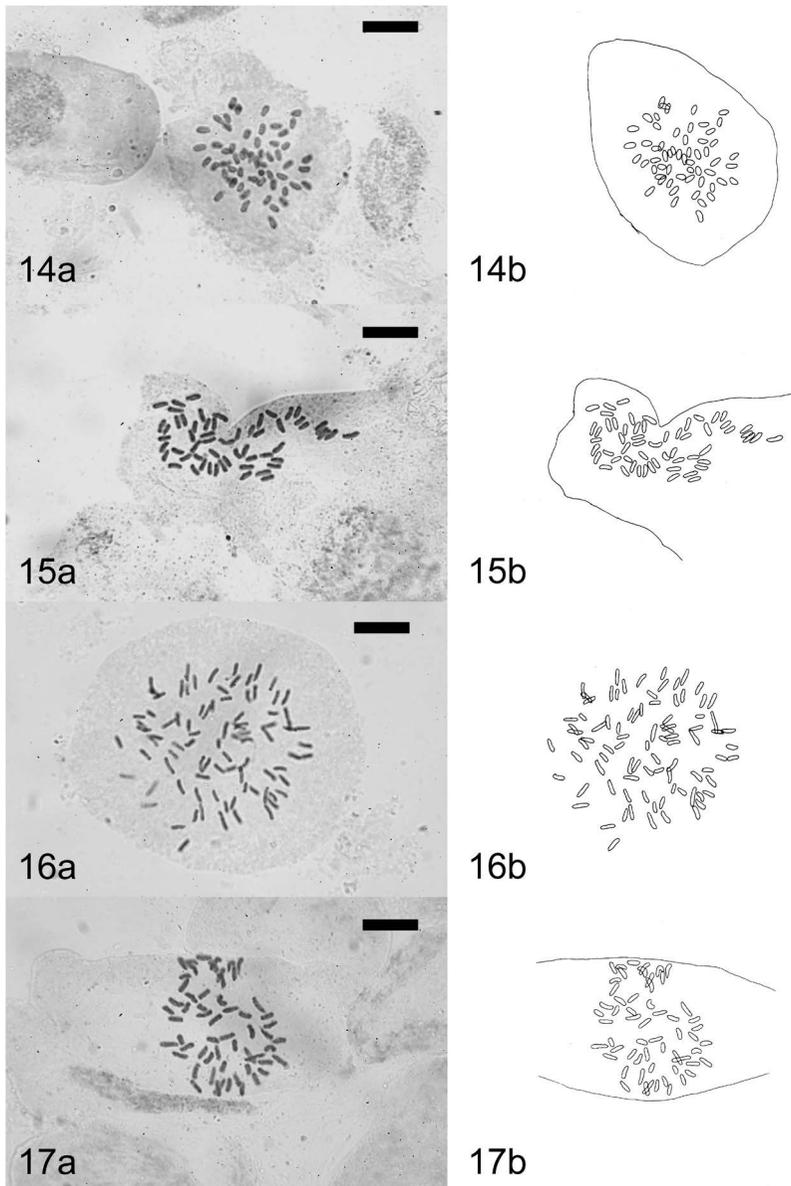
Polyodiaceae

Goniophlebium someyae (Yatabe) Ebihara— $2n = 72$ ($2x$, sexual) [Fig. 23]

The geographically isolated populations of *G. someyae* in Shikoku are sometimes separated as “var. *awaense* (Tagawa) Nakaïke”, characterized by a larger number of lateral lobes (10–20 pairs) with shallow sinus between adjacent main lateral veins (Iwatsuki *et al.*, 1995). Chromosome base number of present count using a Shikoku material ($2n = 72$, diploid of $x = 36$) is different from that of a Honshu population, $n = 37$ (Shimura *et al.*, 1980, material from Shizuoka Pref.). Further sampling is necessary to judge the effectiveness of chromosome base number to distinguish the two varieties.

Lepisorus uchiyamae (Makino) H.Itô— $2n = 70$ ($2x$, sexual) [Fig. 24]

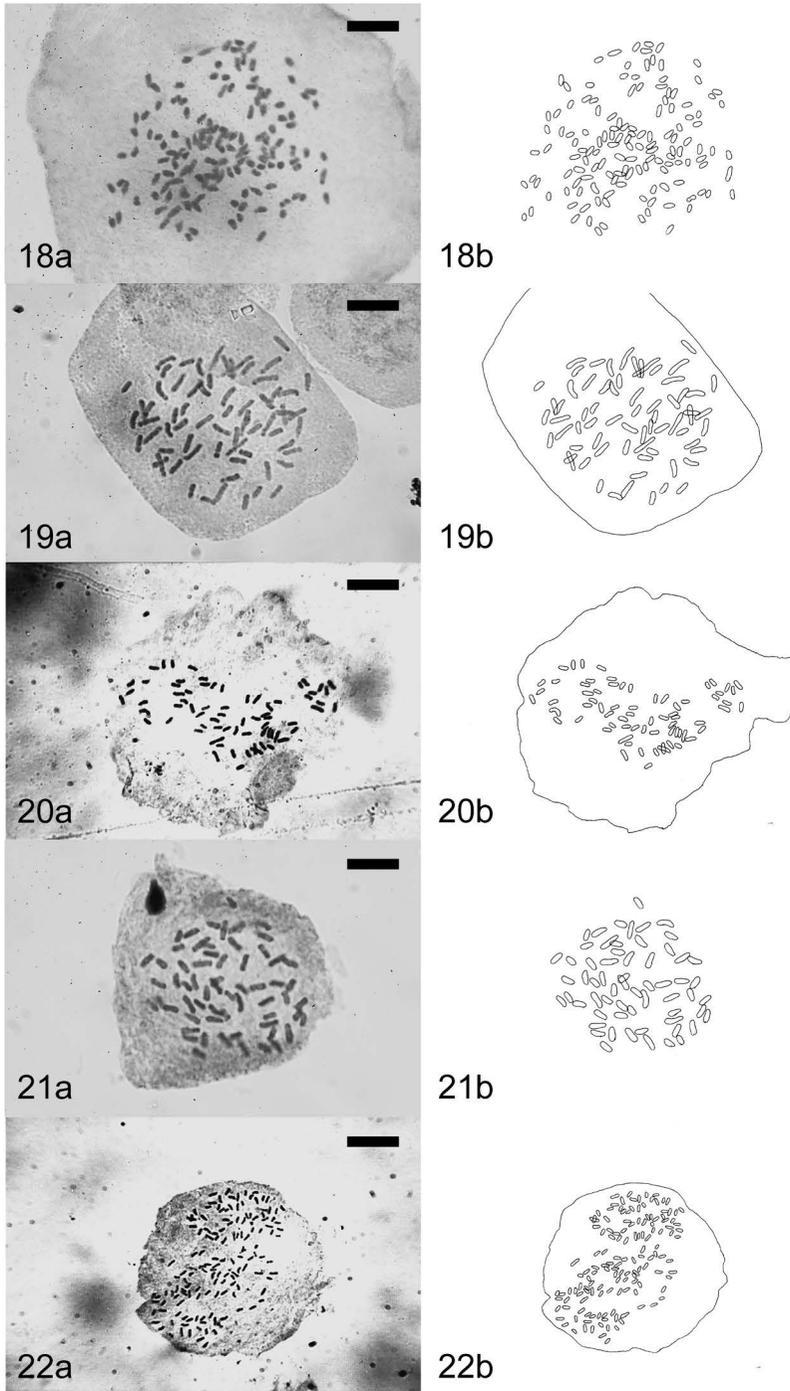
Serizawa (2015) split *L. uchiyamae* into two species based on their morphology and habitat, i.e. *L. uchiyamae* grows on coastal rocks in western Japan (Kii Peninsula of Honshu, Shikoku,



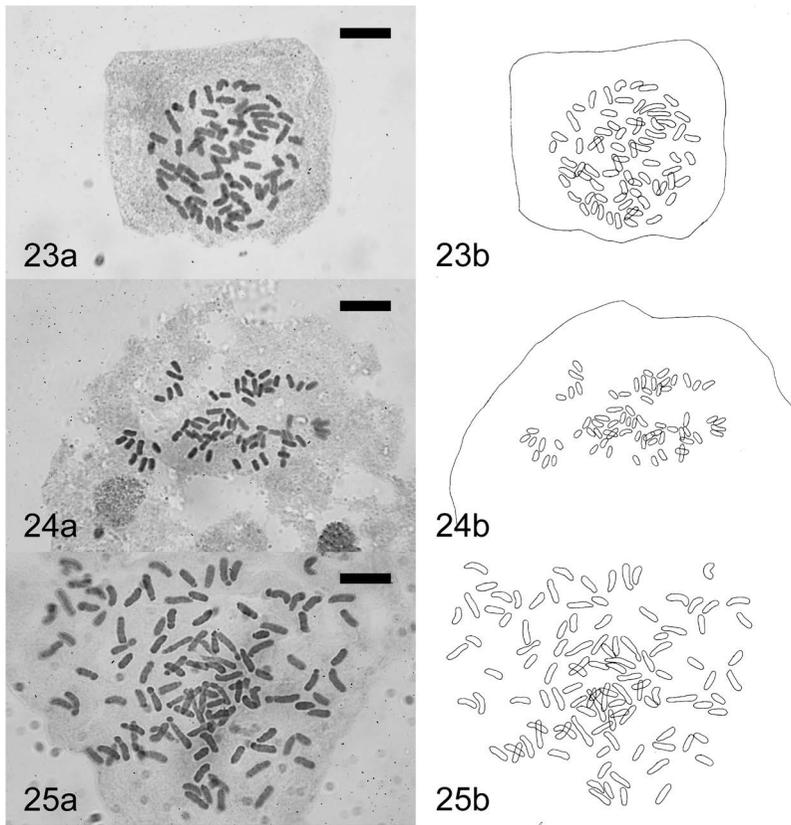
Figs. 14–17. Mitotic metaphase chromosomes. (a) microphotographs and (b) explanatory illustrations. Scale bars = $10\mu\text{m}$. 14. *Pteris natiensis* ($2n = 58$). 15. *Pteris satsumana* ($2n = 58$). 16. *Pteris yakuinsularis* ($2n = 87$). 17. *Pteris laurisilvicola* ($2n = 58$).

Kyushu and Ryukyu) and *L. yamaokae* Seriz. usually grows on artificial stonewalls in Honshu (Izu Peninsula westward), Shikoku and Kyushu (Oita Pref.). As the previous chromosome count as “*L. uchiyamae*” (Mitui, 1971, $n = 70$, tetraploid of $x = 35$) is actually a count for *L. yamao-*

kae, present count $2n = 70$ (diploid of $x = 35$) is a first cytological record for *L. uchiyamae* sensu Serizawa (2015). Thus, the taxonomic treatment by Serizawa (2015) is supported by cytological evidences.



Figs. 18–22. Mitotic metaphase chromosomes. (a) microphotographs and (b) explanatory illustrations. Scale bars = 10 μ m. 18. *Thelypteris hattorii* f. *nemoralis* ($2n = 136$). 19. *Thelypteris quelpaertensis* ($2n = 68$). 20. *Thelypteris esquirolii* ($2n = 70$). 21. *Thelypteris castanea* ($2n = 62$). 22. *Thelypteris uraiensis* ($2n = 140$).



Figs. 23–25. Mitotic metaphase chromosomes. (a) microphotographs and (b) explanatory illustrations. Scale bars = 10 μ m. 23. *Goniophlebium someyae* ($2n = 72$). 24. *Lepisorus uchiyamae* ($2n = 70$). 25. *Colysis decurrens* \times *C. pteropus* ($2n = 108$).



Figs. 26–27. *Colysis decurrens* \times *C. pteropus* on streamside rocks in Yutsun, Iriomote Island, Okinawa Pref. 26. Habitat. 27. Sorus.

Colysis decurrens (Wall. ex Hook. et Grev.) Nakaike \times *C. pteropus* (Blume) Bosman— $2n = 108$ (3x, sterile) [Figs. 25–27]

Jap. Name: Urano-shida

First chromosome count for the hybrid. Although this hybrid was originally recognized as an “intergeneric hybrid” between *Colysis* and *Microsorium*, the generic classification of the microsorioid ferns is still awaiting a global taxonomic revision and *Microsorium* is a polyphyletic genus (Kreier *et al.*, 2008). We tentatively transferred *M. pteropus* to *Colysis*, and placed it closer in position to *Colysis* species than typical *Microsorium* (Ebihara, 2011), though *Colysis* is likely to be merged into *Leptochilus*, a closely related and supersede name by future taxonomic revisions. The hybrid growing on stream-side rocks in Iriomote Island, Ryukyu showed triploidy with irregular spores, which is well explained by hybridization between diploid *C. decurrens* (Kurita, 1968; Mitui, 1966; Takei, 1982, all as “*C. pothifolia*”) and tetraploid *C. pteropus* (Kurita, 1976).

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