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Chromosome Numbers of Eleven Ferns in Japan (Athyriaceae, Dryopteridaceae and Tectariaceae)

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Abstract Mitotic chromosome numbers of eleven leptosporangiate fern taxa (Athyriaceae, Dryopteridaceae and Tectariaceae) in Japan were counted and their reproductive modes were estimated by observing spore number per sporangium. Chromosome numbers were recorded for the first time accompanied by chromosome illustrations for five species (*Deparia coreana*, *D. henryi*, *D. kiusiana*, *D. minamitanii* and *Tectaria phaeocaulis*), two infraspecific taxa (*Dryopteris sparsa* var. *ryukyuensis* and *Arachniodes yasu-inouei* var. *angustipinnula*) and three putative interspecific hybrids (*Anisocampium* × *saitoanum*, *Athyrium* × *bicolor* and *Arachniodes* × *mirabilis*).

Key words: *Anisocampium*, *Arachniodes*, *Athyrium*, chromosome number, *Deparia*, *Tectaria.*

Japan is one of the best-studied areas for chromosome numbers of ferns and lycophytes (i.e. pteridophytes) in the world, with data available for 667 taxa (ca. 60% of the total pteridophyte flora; Nakato and Ebihara, 2016). This coverage increases to ca. 74% (538 taxa) if hybrid taxa are excluded. Furthermore, coverage reaches nearly 80% (368/463 taxa) for eupolypods, a large clade comprising ca. 6000 species including Aspleniaceae, Athyriaceae, Dryopteridaceae, Polypodiaceae and Thelypteridaceae (Schneider *et al.*, 2004). Although base chromosome numbers of eupolypods are not highly variable and mostly range from $x=31$ to 41 (Smith *et al.*, 2006), information drawn from chromosome observations such as ploidy, spore fertility and reproductive mode can be powerful clues for identifying biological entities in species complexes that have resulted from reticulate evolution (e.g. Ebihara *et al.*, 2014). With the goal of contributing to the complete taxon sampling of chromosome observations for Japanese pteridophytes, we observed mitotic chromosomes and spores in eight species plus three interspecific hybrids of Japanese ferns that previously lacked published chromosome information.

Materials and Methods

Materials used for chromosome counting are listed in Table 1. All voucher specimens are deposited in TNS. Methods for counting mitotic chromosomes in root tips of living stocks followed those of Ebihara *et al.* (2014). Reproductive mode was determined by counting spore number per sporangium and by spore shape regularity in the voucher specimens or in cultivated stocks.

Results and Discussion

Athyriaceae

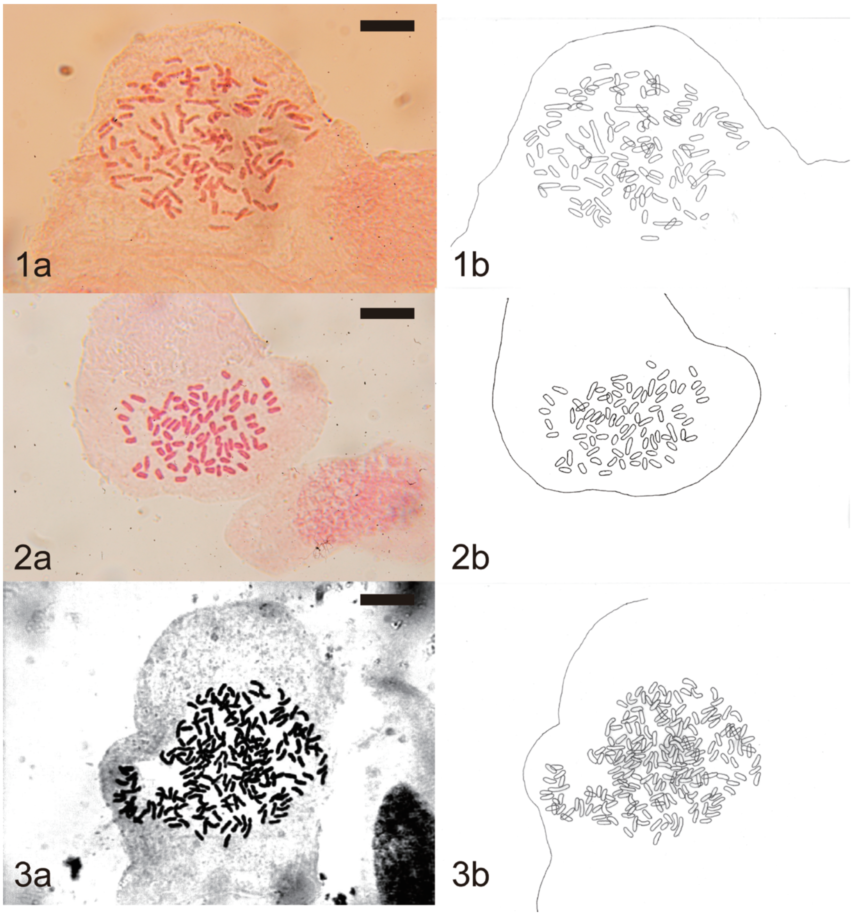
Anisocampium × *saitoanum* (Sugim.) M.Kato — $2n=120$ ($3x$, sterile) [Fig. 1]

A putative hybrid taxon between *Anisocampium niponicum* (Mett.) Y.C.Liu, W.L.Chiou et

Table 1. Plant material used in this study with chromosome counts

Taxon	Chromosome number	Reproductive mode	Voucher	Locality	Chromosome figure
Athyriaceae					
Anisocampium × *saitoanum* (Sugim.) M.Kato	$2n=120$ ($3x$, $x=40$)	sterile (spore irregular)	Nakato 2804 [TNS VS-1268662]	Fukuoka Pref., Kasuya-gun, Sasaguri-machi, Mt. Wakasugi	Fig. 1
Athyrium rupestre Kodama	$2n=80$ ($2x$, $x=40$)	sexual (64 s/s)	*Nakato & Ebihara 3420* [TNS VS-1286239]	Aomori Pref., Mutsu-shi, Mt. Kamafuse	
	$2n=80$ ($2x$, $x=40$)	sexual (64 s/s)	*Nakato & Ebihara 3422* [TNS VS-1286241]	ibid.	Fig. 2
	$2n=80$ ($2x$, $x=40$)	sexual (64 s/s)	*Nakato & Ebihara 3424* [TNS VS-1286242]	ibid.	
Athyrium × *bicolor* Seriz.	$2n=200$ ($5x$, $x=40$)	sterile (spore irregular)	*Nakato 1675* [TNS VS-1277150]	Fukushima Pref., Yama-gun, Atsushiokano-mura	Fig. 3
Deparia coreana (H.Christ) M.Kato	$2n=160$ ($4x$, $x=40$)	sexual (64 s/s)	*AE 3547* (= *Nakato 3178*) [TNS VS-1265113]	Aomori Pref., Hachinohe-shi, Shirahama	Fig. 4
Deparia kiusiana (Koidz.) M.Kato	$2n=$ ca. 240 ($6x$, $x=40$)	sexual (64 s/s)	*Nakato 2870* [TNS VS-1280185]	Aichi Pref., Kitashitara-gun, Shitara-cho (cultivated in Tsukuba Botanical Garden)	Fig. 5
Deparia minamitanii Seriz.	$2n=240$ ($6x$, $x=40$)	sexual (64 s/s)	*Nakato 2871 (KS2007-285)* [TNS VS-774852]	Miyazaki Pref., Kobayashi-shi (cultivated in Tsukuba Botanical Garden)	Fig. 6
Deparia henryi (Baker) M.Kato	$2n=120$ ($3x$, $x=40$)	apogamous (32 s/s)	*AE 3533* [TNS VS-1268624]	Osaka Pref., Takatsuki-shi, Kawakubo	Figs. 7, 8
Dryopteridaceae					
Dryopteris sparsa (Buch.-Ham. ex D.Don) Kuntze var. *ryukyuensis* Seriz.	$2n=164$ ($4x$, $x=41$)	sexual (64 s/s)	*A. Ebihara, T. Oka & T. Minamitani 3247* [TNS VS-1176839]	Miyazaki Pref., Miyazaki-shi, Tano, Miyazaki University Experimental Forest	Fig. 9
Arachniodes yasu-inouei Sa.Kurata var. *angustipinnula* Seriz.	$2n=164$ ($4x$, $x=41$)	sexual (64 s/s *1)	*Nakato 3002* [TNS VS-1244729]	Miyazaki Pref., Kobayashi-shi, Nagakuino (cultivated by T. Minamitani)	Fig. 10
Arachniodes × *mirabilis* Sa.Kurata	$2n=123$ ($3x$, $x=41$)	sterile (sporangium empty)	*Nakato 3000* [TNS VS-1247772]	Miyazaki Pref., Ebino-shi, Iino (cultivated by T. Minamitani)	Fig. 11
Tectariaceae					
Tectaria phaeocaulis (Rosenst.) C.Chr.	$2n=80$ ($2x$, $x=40$)	sexual (64 s/s)	*AE 3563* [TNS VS-1265102]	Okinawa Pref., Kunigami-gun, Kunigami-son, Iyudake	Fig. 12

*1 Spore number counted on a herbarium specimen (TNS VS-1261195).

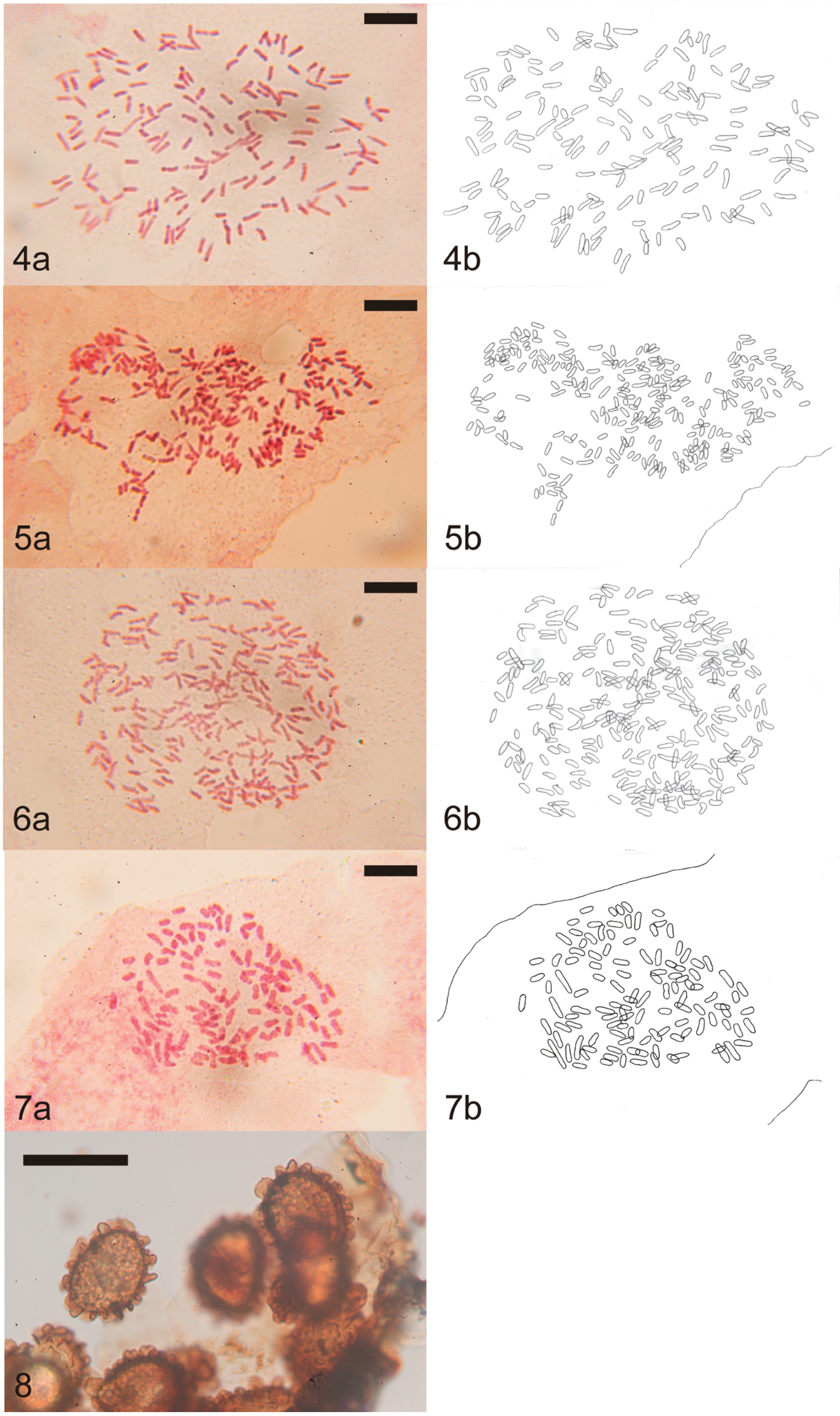


Figs. 1–3. Mitotic metaphase chromosomes. (a) microphotographs and (b) explanatory illustrations. Scale bars = 10 μm. 1. *Anisocampium* × *saitoanum* (2*n* = 120). 2. *Athyrium rupestre* (2*n* = 80, *Nakato & Ebihara 3422*). 3. *Athyrium* × *bicolor* (2*n* = 200).

M.Kato and *A. sheareri* (Baker) Ching. The present result (triploid) can be explained by combining the ploidies of the putative parent species: diploid *A. niponicum* (*n* = 40 [Kurita, 1960; Mitui, 1968; Hirabayashi, 1970], 2*n* = 80 [Tatuno and Okada, 1970]) and tetraploid *A. sheareri* (*n* = 80 [Kurita, 1976]). Although this is the first formal chromosome record for this taxon, the triploid individual with irregular meiosis that Kurita (1976) reported as *Athyrium* (*Anisocampium*) *sheareri* was almost certainly this hybrid (S. Kurita, personal communication). This hybrid sometimes occurs where *A. sheareri* is not found, but the individual we examined produced fully irregular spores and is not suggestive of apogamous reproduction. Further sampling is necessary to determine the rate of fertility of spores for this hybrid.

Athyirum rupestre Kodama — 2*n* = 80 (2*x*, sexual) [Fig. 2]

Although the base chromosome number of *Athyrium* had been considered to be *x* = 40 (Kramer *et al.*, 1990), Nakato (1988) observed *x* = 39 in *A. nikkoense* Makino, a member of the *A. yokoscense* group, and a subsequent careful study (Tsuji, 2006) confirmed that *A. yokoscense* (Franch. et Sav.) H.Christ and its close relatives (*A. kirishimaense* Tagawa and *A. tashiroi* Tagawa) have the base number *x* = 39 without



Figs. 4–8. Mitotic metaphase chromosomes. (a) microphotographs and (b) explanatory illustrations. Scale bars = 10 μm. 4. *Deparia coreana* (2*n* = 160). 5. *Deparia kiusiana* (2*n* = ca. 240). 6. *Deparia minamitanii* (2*n* = 240). 7. *Deparia henryi* (2*n* = 120). 8. Spores of *Deparia henryi* (AE3533). Scale bars = 100 μm.

exception. *Athyrium rupestre* is a closely allied species to *A. yokoscense* in appearance, but its inclusion in the *A. yokoscense* clade has not yet been supported by any molecular phylogenetic analyses (Adjie *et al.*, 2008; Ebihara, 2011). Our present count confirms the base number $x = 40$ ($n = 40$ and $2n = 80$) by Mitui (1970, 1980), and it suggests that *A. rupestre* retains the plesiomorphic state of the base number.

Athyrium × *bicolor* Seriz. — $2n = 200$ (5*x*, sterile) [Fig. 3]

We here report the first chromosome count for the taxon. It is a putative hybrid between *A. vidalii* (Franch. et Sav.) Nakai and *A. neglectum* Seriz. subsp. *neglectum*, often collected in sympatry. Considering the present result (pentaploid) and the tetraploidy of *Athyrium vidalii* ($n = 80$ [Kurita, 1960; Mitui, 1968]), we propose that the other parental species *A. neglectum* subsp. *neglectum* is likely hexaploid.

Deparia coreana (H.Christ) M.Kato — $2n = 160$ (4*x*, sexual) [Fig. 4]

Shimura (1984) reported a haploid chromosome number $n = 80$ without any chromosome illustration or citation of a voucher specimen for this species. Our present result ($2n = 160$, tetraploid) is the first formal chromosome record for this species, and is congruent with Shimura's report. Among the six species belonging to the *Dryoatyhrium* (DR) clade (Kuo *et al.*, 2016) in Japan, this is the sole tetraploid species — the remaining members are include two diploid sexual species (*D. pterorachis* (H.Christ) M.Kato and *D. viridifrons* (Makino) M.Kato) and three triploid apogamous species (*D. okuboana* (Makino) M.Kato, *D. unifurcata* (Baker) M.Kato and *D. henryi* (Baker) M.Kato, see below) (Takamiya, 1996).

Deparia kiusiana (Koidz.) M.Kato — $2n =$ ca. 240 (6*x*, sexual) [Fig. 5]

This is the first chromosome count for this species. Closely related *Deparia dimorphophylla* (Koidz.) M.Kato, formerly treated at the varietal rank under *D. kiusiana*, is also known as a hexaploid (Nakato, 1996). The *Athyriopsis* (AT) clade (Kuo *et al.*, 2016) shows high species diversity in Japan (seven species), but its constituent taxa are exclusively polyploid (tetraploid and hexaploid [Takamiya, 1996; Aman and Serizawa, 2011]).

Deparia minamitanii Seriz. — $2n = 240$ (6*x*, sexual) [Fig. 6]

This is the first chromosome count for this species. This is a critically endangered species confined to just a few localities in Kyushu, and it has closely related species with unknown ploidy distributed in China (Kuo *et al.*, 2016).

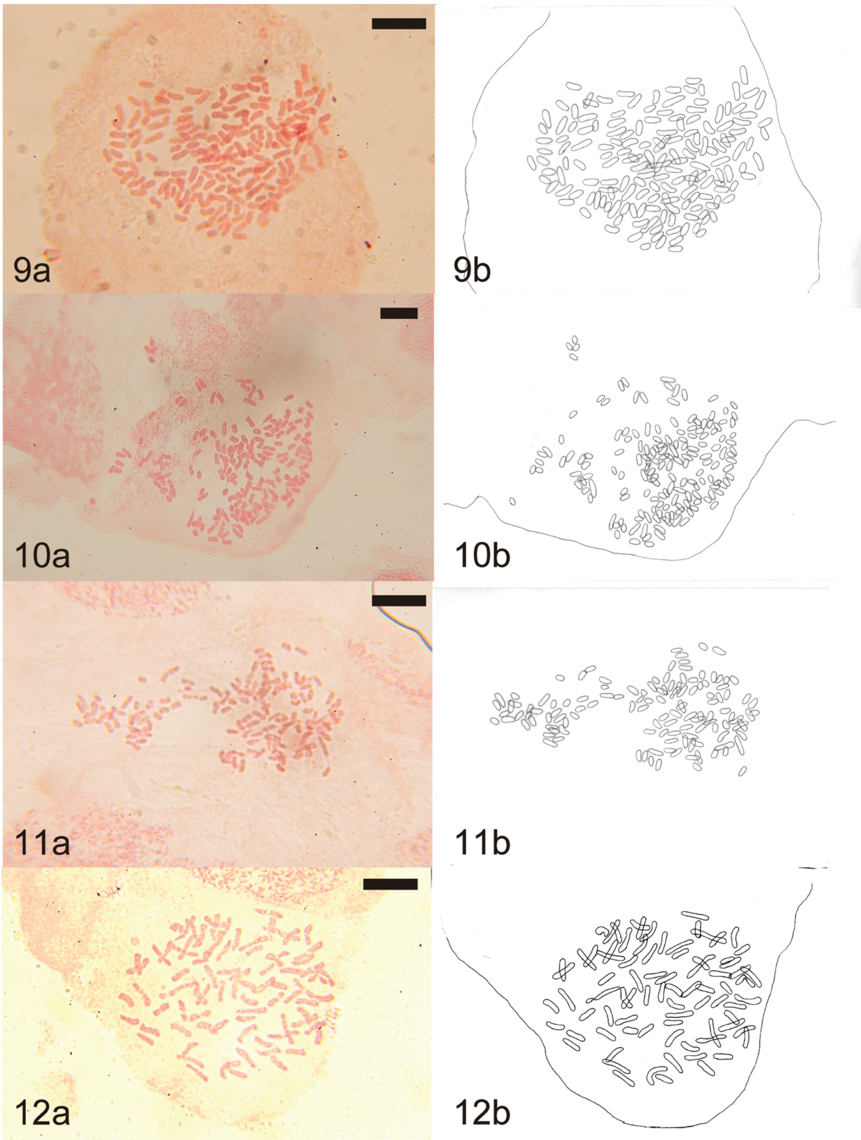
Deparia henryi (Baker) M.Kato — $2n = 120$ (3*x*, apogamous) [Figs. 7, 8]

This species was unrecognized in most of the florae of Japan (e.g. Iwatsuki *et al.*, 1995) except for misapplication of the name to *D. okuboana* (Makino) M.Kato (e.g. Kurita, 1960). Mitsuta (2003) first noticed its occurrences in western Japan. Its laminar shape is similar to *D. coreana*, but the round-reniform soral shape is more similar to *D. okuboana*. Our present result suggests *D. henryi* is also distinct in its apogamous reproduction from sexually reproducing *D. coreana*. On the other hand, *D. okuboana* and *D. henryi* likely form a species complex largely composed of apogamous triploid individuals, and are awaiting global and comprehensive revision.

Dryopteridaceae

Dryopteris sparsa (Buch.-Ham. ex D.Don) Kuntze var. *ryukyuensis* Seriz. — $2n = 164$ (4*x*, sexual) [Fig. 9]

The present result is the first chromosome count for this taxon. It was recognized by Serizawa (1971) as distinguishable from var. *sparsa* in having smaller fronds and growing on cliffs. Except for having cleft indusia at the mature stage, *D. hayatae* Tagawa is morphologically similar to this taxon, but is cytologically distinct, being a sexual diploid (Mitui, 1966, 1968, 1976; Kurita, 1967; Hirabayashi, 1969). It is notable that var. *sparsa* is cytologically variable (Dar-



Figs. 9–12. Mitotic metaphase chromosomes. (a) microphotographs and (b) explanatory illustrations. Scale bars = 10 μm. 9. *Dryopteris sparsa* var. *ryukyuensis* (2*n* = 164). 10. *Arachniodes yasu-inouei* var. *angustipinnula* (2*n* = 164). 11. *Arachniodes* × *mirabilis* (2*n* = 123). 12. *Tectaria phaeocaulis* (2*n* = 80).

naedi *et al.*, 1989) amongst the populations in Japan, which include sexual tetraploid (Darnaedi and Iwatsuki, 1987; Darnaedi *et al.*, 1989; Kurita, 1966; Hirabayashi, 1966, 1974), apogamous diploid (Darnaedi *et al.*, 1989) and apogamous triploid (Darnaedi and Iwatsuki, 1987; Darnaedi *et al.*, 1989; Hirabayashi, 1974) cytotypes.

Arachniodes yasu-inouei Sa.Kurata var. *angustipinnula* Seriz. — 2*n* = 164 (4*x*, sexual) [Fig. 10]

This is the first chromosome count for this taxon. It was initially collected in 1976, but thought to be extinct due to logging of the forest at the original site. Later it was described at the varietal rank under *A. yasu-inouei* (Serizawa, 2009), followed by rediscovery in November, 2013 nearby the type locality. We observed chromosome numbers of the rediscovered stock collected and cultivated by T. Minamitani, and counted 64 spores per sporangium on a herbarium specimen. These results confirmed that its ploidy level and chromosome number are the same as those of *A*. *yasu-inouei* var. *yasu-inouei* ($n = 82$ [Shimura *et al.*, 1982]).

Arachniodes × *mirabilis* Sa.Kurata — $2n = 123$ (3*x*, sterile) [Fig. 11]

This is a putative hybrid between *A*. *amabilis* (Blume) Tindale var. *fimbriata* K.Iwats. (tetraploid, $n = 82$ [Mitui, 1966, 1968]) and *A*. *yoshinagae* (Makino) Ching (tetraploid, $n = 82$ [Kurita, 1966]; $n =$ ca. 80 [Mitui, 1968]). Shimura (1983) reported chromosome number $2n =$ ca. 164, $n =$ ca. 50II + ca. 64I without providing any chromosome illustration. Our count is the first formal chromosome record for this hybrid, but the ploidy level (triploid) is inconsistent with former record (tetraploid) by Shimura (1983), and suggests one of the parents is not tetraploid *A*. *amabilis* var. *fimbriata*, but rather another diploid taxon. One candidate diploid taxon is *A*. *amabilis* var. *amabilis*, which is reported as a sexual diploid (Shimura *et al.*, 1982), but is so far undiscovered in Miyazaki Prefecture (Ebihara, 2017; T. Minamitani, pers. comm.).

Tectariaceae

Tectaria phaeocaulis (Rosenst.) C.Chr. — $2n = 80$ (2x, sexual) [Fig. 12]

This is the first chromosome count for this species. *Tectaria* remains a poorly studied genus amongst Japanese ferns for chromosome numbers, that is, previously counts have been reported for only two of eight native species (i.e. *T. harlandii* (Hook.) C.M.Kuo [$n =$ ca. 80, Mitui, 1976] and *T. subtriphylla* (Hook. et Arn.) Copel. [$n = 40$, Mitui, 1976]).

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