BIODIVERSITAS
Volume 23, Number 6, June 2022
Pages: 3285-3296

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230658

Chromosome number and reproductive mode of some *Pteris* species (Pteridaceae) in Thailand

PONPIPAT LIMPANASITTICHAI♥, TASSANAI JARUWATTANAPHAN♥♥
Department of Horticulture, Faculty of Agriculture, Kasetsart University. Chatuchak, Bangkok 10900, Thailand.
Tel./Fax.: +66-2-579-0113, ♥email: ponpipat.lim@gmail.com, ♥♥tassanai.j@ku.th

Manuscript received: 28 April 2022. Revision accepted: 30 May 2022.

Abstract. *Limpanasittichai P, Jaruwattanaphan T. 2022. Chromosome number and reproductive mode of some* Pteris *species (Pteridaceae) in Thailand. Biodiversitas 23: 3285-3296.* The fern genus *Pteris* L. (Pteridaceae) consists of 329 species. This genus is one of the largest fern genera and is quite diverse with many different ploidy levels and reproductive modes resulting from complicated hybrid speciation events in their evolution. However, there have been very few observations of Pteridophytes in Thailand. This study aims to investigate chromosome number and reproductive mode of *Pteris* in Thailand. Mitotic chromosome numbers of 9 species collected from natural habitats were observed by hematoxylin squash method and their reproductive modes were estimated by counting the number of spores per sporangium. Three cytotypes were found, namely diploid, triploid and tetraploid. Nevertheless, only sexual individuals were recognized. First reports are provided for 3 species, namely *P. dalhousieae* ($2n = 2x = 58$, sexual), *P. decrescens* ($2n = 2x = 58$, sexual), and *P. heteromorpha* ($2n = 2x = 58$; $2n = 3x = 87$ and $2n = 4x = 116$, sexual) and the chromosome numbers from the remaining taxa match those reported from other countries. Two species, namely *P. heteromorpha* and *P. vittata* exhibited diverse cytotypes.

Keywords: Cytotype, polyploid, *Pteris*, reproductive mode, speciation

INTRODUCTION

Pteris L. (Pteridaceae) is one of the most diverse fern genera, comprising about 329 species distributed in all tropical and subtropical regions (POWO 2022). This genus is characterized by submarginal sori that are covered by the reflexed leaf margin. Zhang and Zhang (2018) classified *Pteris* into 3 subgenera and 15 sections by phylogeny, morphology, and geographic distribution. Although there are many publications, taxonomic confusions among *Pteris* species are still found (Fraser-Jenkins et al. 2017). The morphological variations are caused by polyploidization and hybridization, primary phenomena of evolution and speciation in ferns (Haufler 2008; Chao et al. 2012a; Jaruwattanaphan et al. 2013; Uday and Bhakat 2021). To better understand the origin, speciation, and evolutionary relationships of *Pteris*, more information on their chromosomes and reproductive modes is very important, even as DNA studies are becoming more prevalent.

Hybrid speciation events via polyploidization are common events in pteridophytes, and it is estimated that about 31% of pteridophyte speciation events are accompanied by a ploidy increase, which is a higher rate than for angiosperms (Wood et al. 2009). The basic chromosome number of *Pteris* is 29 (Walker 1962), whereas 60% of *Pteris* species exhibit polyploidy, including triploids, tetraploids, pentaploids, hexaploids, and octoploids, and 63% of these species resulted from polyploid speciation (Chao et al. 2012b). Ferns reproduce asexually through spores using a system called apogamy. Apogamy is a mechanism that can stabilize the reproduction of polyploid ferns (Jaruwattanaphan et al. 2013). For homosporous leptosporangiate ferns, counting the number of spores in each sporangium is a way of identifying which reproductive mode that fern uses. Generally, if there are 64 spores in each sporangium, then it is a sexual fern and if there are 32 spores it is an apogamous fern. Chao et al. (2012b) reported that sexual reproduction was the most commonly found reproductive mode in this genus (45%), followed by apogamous (31%) and both types of reproduction (12%). Some taxa, such as *P. terminalis* var. *terminalis* (sexual form) and *P. terminalis* var. *fauriei* (apogamous form) have been divided at varietal rank by reproductive mode (Ebihara et al. 2017).

According to Tagawa and Iwatsuki (1985), 29 *Pteris* species were recorded in Thailand, but only a few Thai species have been characterized cytologically. Thirteen of those species were found to have the sexual mode of reproduction, based on studies of the spore count per sporangium in herbarium specimens (Yingchutrakul 2016). A few cytotypes were reported from cultivated plants in Thailand, *P. semipinnata* ($2n = 116$), *P. multifida* 'Crestata' ($2n = 116$), *P. quadriaurita* var. *argyraea* ($2n = 58$), and *P. cretica* 'Albolineata' ($2n = 87$) (Thumdee 1996; Rattamanee 2006). Only one cytotype was reported from a wild plant (tetraploid *P. ensiformis*) (Paitoonyakul 2018).

Pteris are largely diverse with many different cytotypes in consequence of complicated hybrid speciation events. However, evidence about the detailed characteristics of different *Pteris* species is still lacking, especially those found in Thailand. Chromosome number and reproductive mode studies should help determine whether species have uniform chromosome numbers and which have cyto-reproductive variation, as well as shed light on whether one or more species should be recognized in some debatable taxa, and questions about parental lineage (Ivanova and Piekos-Mirkowa 2003). Although the flow cytometry technique has been widely employed in recent research to quickly determine relative genome sizes, we made chromosomal counts for collections of trustworthy cytological data in this work. Therefore, this study aims to investigate chromosome number and reproductive mode of some *Pteris* species in Thailand to provide more basic information available to fill the lacking cytological information of ferns taxa and information on ploidy level and reproductive modes for further systematic and evolutionary studies of this genus.

MATERIALS AND METHODS

Plant materials

The 9 species and 6 sections of *Pteris* used for this study are listed in Table 1. Each individual was collected from natural habitats in several provinces and identified with Flora of Thailand (Tagawa and Iwatsuki 1985), China (Zhang et al. 2013), and Malaya (Holttum 1968). Their rhizomes were grown in a moist chamber, with growing media (peat moss: pumice: perlite: sand = 2: 2: 2: 1) to promote new roots at the Plant Diversity Laboratory, Department of Horticulture, Kasetsart University, Thailand. The voucher specimens were deposited in the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation (BKF), Thailand.

Table 1. Plant materials used in this study and the results

Section	Species	Chromosome number	Reproductive mode	Voucher	Phytogeographical areas/ Locality	Fig.
Cadierii	*P. decrescens**	$2n = 2x = 58$	Sexual	181220-3	N/Chom Thong Dis., Chiang Mai Prov.	1A
		$2n = 2x = 58$	Sexual	201212-1	N/Khun Nan NP, Nan Prov.	1B
		$2n = 2x = 58$	Sexual	181220-4	N/Chom Thong Dis., Chiang Mai Prov.	
		$2n = 2x = 58$	Sexual	201211-1	N/Khun Nan NP, Nan Prov.	
		$2n = 2x = 58$	Sexual	201211-2	N/Khun Nan NP, Nan Prov.	
	*P. heteromorpha**	$2n = 2x = 58$	n/a	190704-1	N/Mueang Chiang Mai Dis., Chiang Mai Prov.	1C
		$2n = 3x = 87$	n/a	190703-14	N/Mae Tha Dis., Lamphun Prov.	1D
		$2n = 3x = 87$	n/a	190703-1	N/Mae Tha Dis., Lamphun Prov.	
		$2n = 4x = 116$	Sexual	181220-21	N/Chom Thong Dis., Chiang Mai Prov.	1E
		$2n = 4x = 116$	Sexual	190703-3	N/Mae Tha Dis., Lamphun Prov.	
		$2n = 4x = 116$	n/a	181117-9	N/Mueang Mae Hong Son Dis., Mae Hong Son Prov.	
		$2n = 4x = 116$	n/a	181220-6	N/Chom Thong Dis., Chiang Mai Prov.	
	P. venusta	$2n = 2x = 58$	n/a	190703-6	N/Mae Tha Dis., Lamphun Prov.	1F
		$2n = 2x = 58$	n/a	190922-13	N/Umphang Dis., Tak Prov.	1G
		$2n = 2x = 58$	n/a	190922-11	N/Umphang Dis., Tak Prov.	
		$2n = 2x = 58$	n/a	181117-2	N/Mueang Mae Hong Son Dis., Mae Hong Son Prov.	
		$2n = 2x = 58$	n/a	181123-10	SE/Soi Dao Dis., Chanthaburi Prov.	
		$2n = 2x = 58$	n/a	181125-1	SE/Soi Dao Dis., Chanthaburi Prov.	
		$2n = 2x = 58$	n/a	190702-1	N/Thoen Dis., Lampang Prov.	
		n/a	Sexual	181217-2	N/Tha Song Yang Dis., Tak Prov.	
Campteria	*P. blumeana*	$2n = 2x = 58$	n/a	181125-3	SE/Soi Dao Dis., Chanthaburi Prov.	1H
		$2n = 2x = 58$	n/a	190716-2	P/Phanom Dis., Surat Thani Prov.	
		n/a	Sexual	201211-3	N/Khun Nan NP, Nan Prov.	
Excelsae	*P. ensiformis*	$2n = 4x = 116$	Sexual	201219-2	SW/Kaeng Krachan NP, Phetchaburi Prov.	1I
		$2n = 4x = 116$	Sexual	200915-1	P/Lang Suan Dis., Chumphon Prov.	
		$2n = 4x = 116$	n/a	181211-2	NE/Nong Hin Dis., Loei Prov.	
		$2n = 4x = 116$	n/a	190909-1	P/Phanom Dis., Surat Thani Prov.	
Hypsopodium	*P. amoena*	$2n = 4x = 116$	Sexual	200902-3	NE/Lom Kao Dis., Phetchabun Prov.	1J
	P. bella	$2n = 2x = 58$	Sexual	200902-7	NE/Lom Kao Dis., Phetchabun Prov.	1K
		$2n = 2x = 58$	Sexual	200902-8	NE/Lom Kao Dis., Phetchabun Prov.	
Pteris	*P. vittata*	$2n = 2x = 58$	Sexual	191222-3	N/Umphang Dis., Tak Prov.	1L
		$2n = 3x = 87$	n/a	191223-7	N/Umphang Dis., Tak Prov.	1M
		$2n = 4x = 116$	n/a	181219-4	N/Mae Sariang Dis., Mae Hong Son Prov.	1N
Semipinnatae	*P. dalhousieae**	$2n = 2x = 58$	Sexual	210103-1	P/Ron Phibun Dis., Nakhon Si Thammarat Prov.	1O

Note: *: First chromosome count, NP: National Park; Dis.: district, Prov.: province, Phytogeographical areas of Thailand: N: Northern; NE: North-eastern; E: Eastern; SW: South-western; SE: South-eastern; P: Peninsular (van Welzen et al. 2011)

Procedures

Chromosome number observation

Mitotic chromosome numbers were counted by hematoxylin squash method described by Jaruwattanaphan et al. (2013). Growing root tips or circinate fronds were pretreated with 2 mM 8-hydroxyquinoline for 6-8 h at 16-18°C. After fixation in freshly mixed Carnoy's fixative solution (absolute ethanol: glacial acetic acid = 3: 1) for 12-24 h, samples were hydrolyzed with 1 M HCl for 5 min at 60°C and then squashed in 1% hematoxylin. Chromosomes were observed and photographed under a compound microscope at 1000x magnification with a digital camera.

Reproductive mode observation

Reproductive modes were identified based on the number of spores per sporangium (Huang et al. 2011). Fresh and mature fertile fronds were collected and 20 sporangia from each individual were examined. Each sporangium was transferred to a drop of 50% glycerol and the number of spores was counted under a light microscope.

Data analysis

Chromosome number of each specimen was analyzed from metaphase chromosomes based on the collected photographs. These numbers were also compared with Walker (1962) for their ploidy levels. For reproductive mode, individuals with 64 spores per sporangium were identified as sexual plants, and those with up to 32 spores were identified as apogamous plants (Ebihara et al. 2017). In addition, distributed continuity of each cytotype was observed by correlated with preceding records from adjacent regions (Walker 1962; Takamiya 1996; Bir and Verma 2010; Cheng and Zhang 2010; Chao et al. 2012b; Praptosuwiryo and Mumpuni 2018).

RESULTS AND DISCUSSION

The results of chromosome number and reproductive mode observation are summarized in Table 1. A total of 34 specimens belonging to 9 species and 6 sections were examined. The concept of taxa here follows Zhang and Zhang (2018). In all of the specimens for which reproductive mode was examined, 64 spores in each sporangium and no irregular-shaped spores were found.

Pteris **section** ***Cadierii*** **(Shieh) Liang Zhang & Li Bing Zhang**

Pteris decrescens Christ

$2n = 2x = 58$, [diploid sexual]

This is the first chromosome count for the species, which is distributed in China, Thailand, Laos, Cambodia, and Vietnam (Hwang et al. 2015; Chao et al. 2017). *P. decrescens* is a bipinnatifid species that has ciliate pseudoindusia, bristles on red to dark-brown stipes and basal pinnae without a basiscopic branch. It is similar to *P. parviloba* Christ, which was once treated as a variety of *P. decrescens*, but can be distinguished by spore morphology (Chao et al. 2017). *P. decrescens* is placed in section *Cadierii* (Zhang and Zhang 2018). Unlike *P. cadieri* complex, *P. decrescens* has variations in morphological characters but in this study only sexual diploids ($2n = 2x = 58$) were found (Figure 1A, 1B). These morphological variations are probably the consequence of the adaptation to the habitats of this species. Moreover, this species was distributed in many phytogeographical areas of Thailand (N, NE, E, SE, and P), so collecting additional samples to cover the distribution area is needed.

Pteris heteromorpha Fée

$2n = 2x = 58$, [diploid]; $2n = 3x = 87$, [triploid]; $2n = 4x = 116$, [tetraploid sexual]

This is the first cytological record for the species although its reproductive mode has not been clarified in diploid ($2n = 2x = 58$) (Figure 1C) and triploid plants ($2n = 2x = 97$) (Figure 1D) due to the lack of fertile fronds in the specimens collected. Furthermore, tetraploid sexual plants ($2n = 2x = 116$) were found (Figure 1E). Despite being widely distributed in Southeast Asia, India, and China (Fraser-Jenkins et al. 2017), a taxonomic study of this plant is still lacking. *P. heteromorpha* have unstable frond construction from simply pinnate to pectinate. The irregular frond morphologies found in some species complexes resulted from their hybrid origins, such as *P. quadriaurita* complex (Walker 1958) and *P. cadieri* complex (Chao et al. 2012a). In this study, the diverse variations in frond morphology mirrored the variations in cytotypes that were observed. The specimens that were simply pinnate with no segments were found to be triploid, while those with a small number of segments were tetraploid and those with a large number of segments were diploid. From frond morphologies and diverse ploidy levels of *P. heteromorpha*, we can surmise it probably has a hybrid origin, perhaps descending from the hybridization of a simply pinnate species and a pectinate pinnate species (Walker 1958; Ebihara et al. 2017). Since *P. venusta* is a simply pinnate species and closely related to *P. heteromorpha* with very low generic variation (Zhang and Zhang 2018), this species seems likely to be the ancestor of this complex. It is recommended that there should be further phylogenetic analysis studies of *P. heteromorpha* species complex to try to elucidate and affirm the history of polyploidization in this group of ferns.

Pteris venusta Kunze

$2n = 2x = 58$, [diploid]

All plant material of *P. venusta* collected from five places proved to be diploid, with $2n = 2x = 58$ chromosomes (Figure 1F, 1G). This species has continuous variation in frond from simple to pinnate, and the lowest pinna of some specimens has branches, which has often caused it to be confounded with a Philippine species, *P. pellucida* C. Presl (Fraser-Jenkins et al. 2017). Since some authors treated *P. venusta* as a synonym of *P. pellucida*, cytological data was also confounded. Although it is distributed in Southeast Asia, India, Bhutan, Nepal, and China (Zhang et al. 2013; Dong and Haque 2021), cytotypes of true *P. venusta* have only been reported from India, including sexual diploids and sexual tetraploids

(Fraser-Jenkins et al. 2017). In Thailand, this species is a common fern, especially in the northern region. Despite various types of fronds being examined in this research, such as wide segments, narrow segments, or white-striped segments, only diploid cytotypes were found (Figure 1F, 1G). Some individuals had very narrow segments, making them look very similar to *P. heteromorpha*. To resolve taxonomic confusion, further study on the speciation or evolution of these species is suggested.

Pteris sect. *Campteria* (C. Presl) Ching

Pteris blumeana J. Agardh

$2n = 2x = 58$, [diploid]

This species is widely distributed from China to tropical Asia (Dong and Haque 2021; POWO 2022), and only one cytotype (apogamous diploid; $2n = 58$) was reported from India (Bir and Verma 2010). This present study also found a diploid plant (Figure 1H) and some sexual individuals, as reported in Yingchutrakul (2016). Due to *P. blumeana* having morphological variations and being quite well distributed throughout, it is one of the species complexes in Thailand. The report by Tagawa and Iwatsuki (1985) of *P. asperula* and *Pteris* sp. *aff. longipinnula* was in error for *P. blumeana*. The key character of this species has short spines on costules but they often fall out during herbarium specimen preparation, especially on the older ones. However, only diploid cytotypes were found in this study. More evidence is needed to clarify the variations of this complex.

Pteris sect. *Excelsae* (Shieh) Liang Zhang & Li Bing Zhang

Pteris ensiformis Burm.f.

$2n = 4x = 116$, [tetraploid sexual]

Pteris ensiformis is distributed in tropical and subtropical Asia to Pacific (Praptosuwiryo and Mumpuni 2018; Dong and Haque 2021; POWO 2022). Although some publications observed cytological records on the ornamental plant *P. ensiformis* var. *victoriae*, $2n = 87$ (Chao et al. 2012b; Praptosuwiryo and Mumpuni 2018), only the wild type of *P. ensiformis* var. *ensiformis* was focused on in the current study. Previous studies mostly recorded sexual tetraploids from China (Cheng and Zhang 2010), Japan (Takamiya 1996), Taiwan, Sri Lanka, Malaysia (Walker 1962), India (Bir and Verma 2010), Indonesia (Praptosuwiryo and Mumpuni 2018), and Thailand (reproductive mode was unknown) (Paitoonyakul 2018). Diploid cytotype was reported from China and Taiwan (Cheng and Zhang 2010) while apogamous triploid was reported from Indonesia (Praptosuwiryo and Mumpuni 2018). In Thailand, *P. ensiformis* is one of the ferns in the genus *Pteris* that have variations in morphology but only sexual tetraploids are found (Figure 1I) even if samples were collected in different phytogeographical areas of Thailand (NE, SW, and P) (Table 1). This species was placed into section *Excelsae* by poorly resolved relationships among subclades (Zhang and Zhang 2018) and seemed to be a cryptic species originating from multiple hybridizations (Chao et al. 2012b).

Pteris sect. *Hypsopodium* Shieh

Pteris amoena Blume

$2n = 4x = 116$, [tetraploid sexual]

Pteris amoena is distributed from China to New Guinea (Fraser-Jenkins et al. 2017) and is considered a critically endangered species in India (Fraser-Jenkins 2012). It is discontinuously found in Thailand, in lower montane forests at 1,200-1,500 m above sea level (Tagawa and Iwatsuki 1985). In a plant of *P. amoena* collected from a Northeastern area, Lom Kao District, Phetchabun Province, we observed $2n = 4x = 116$ with 64 spores (sexual) (Figure 1J). *P. amoena* is placed in section *Hypsopodium* (Zhang and Zhang 2018) and is considered to be autopolyploid by having 29 tetravalent chromosomes (Chao et al. 2012b). Previous studies reported diploid specimens ($2n = 58$) from Taiwan (Cheng and Zhang 2010) and a sexual tetraploid ($2n = 116$) from Japan (Takamiya 1996) under the name *P. tokioi* Masam., which is now considered a synonym with *P. amoena* (Fraser-Jenkins et al. 2017). The morphological character of this species is often confused by having costular areoles (Fraser-Jenkins et al. 2017). Now, it is understood that *P. amoena* comprises specimens both with and without costular areoles. Although sexual tetraploids derived from an individual that had some costular areoles were found in the present result, variation in each cytotype remains unclear due to the lack of plant materials, especially samples that lack the costular areole.

Pteris bella Tagawa

$2n = 2x = 58$, [diploid sexual]

This species is distributed from China to Indo-China and Taiwan (Lindsay and Middleton 2012; POWO 2022). Previous studies reported diploids, apogamous triploids, and sexual tetraploids from Taiwan only (Chao et al. 2012b). A diploid sexual plant ($2n = 2x = 58$) was also found in the same area of *P. amoena* (Figure 1K). The same as *P. amoena*, *P. bella* belongs to the section *Hypsopodium*. Although intraspecific polyploids and monophyly were found in each subclade (Zhang and Zhang 2018), morphological characters would still amaze. Color of stipes, the margin of segments, and architecture of fronds are the key characters to identify at species rank but species exceptions are located at section rank. To clarify taxonomic confusions, the investigation of speciation or evolution of each species complex is proposed.

Pteris sect. *Pteris*

Pteris vittata L.

$2n = 2x = 58$, [diploid sexual]; $2n = 3x = 87$, [triploid]; $2n = 4x = 116$, [tetraploid]

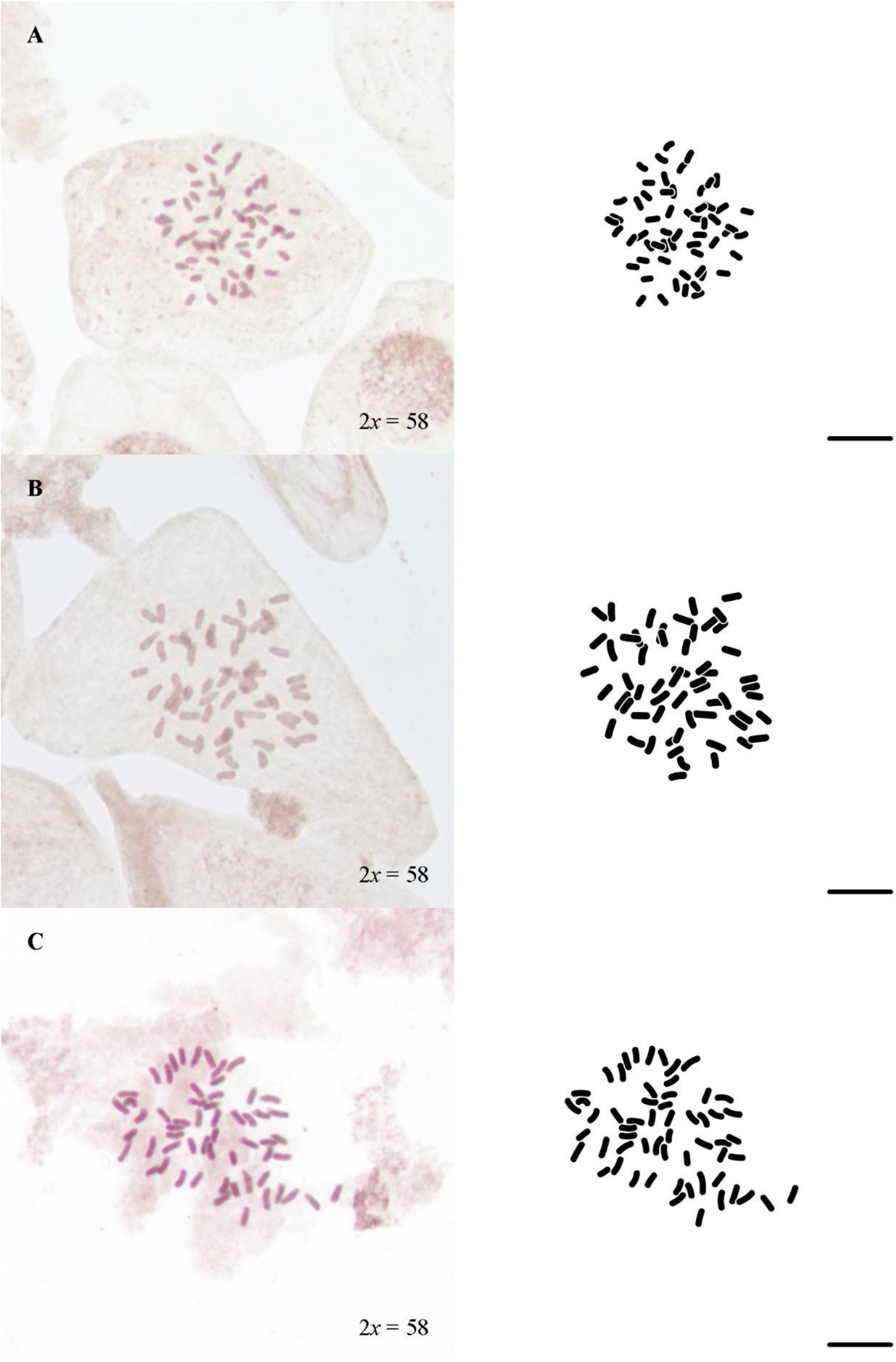
This species is widely distributed in the tropics and subtropics of the Old World (Dong and Haque 2021; Morajkar and Hegde 2021). Previous studies clarified that it is a species complex with diverse cytotypes, including diploid, triploid, tetraploid, pentaploid, and hexaploid, while most cytotypes reported from many countries were tetraploid (Walker 1962; Takamiya 1996; Bir and Verma 2010; Cheng and Zhang 2010; Praptosuwiryo and Mumpuni 2018) and diploid is a rare cytotype (Kato et al. 1992). Because cases of irregular meiosis were recorded, this complex likely originated through hybridization (Khare and Kaur 1983; Wang 1989). In Thailand, this species has a vast distribution and was recorded in all phytogeographical areas. We examined three plants that were collected from two populations in Northern Thailand (Table 1) and obtained chromosome counts of 2*n* = 58 (diploid) (Figure 1L), 2*n* = 87 (triploid) (Figure 1M), and 2*n* = 116 (tetraploid) (Figure 1N) in mitotic metaphase. These chromosome numbers are identical to those reported in China (Wang 1989). Praptosuwiryo and Mumpuni (2018) reported tetraploid sexual specimens from Indonesia and did not find other cytotypes, especially diploid type, which has not been previously reported in the Malesian region at all. Diploid types are only found in the Indochina region, and the type of speciation events that have occurred with *P. vittata* could be specific to each different region of distribution (Praptosuwiryo and Mumpuni 2018).

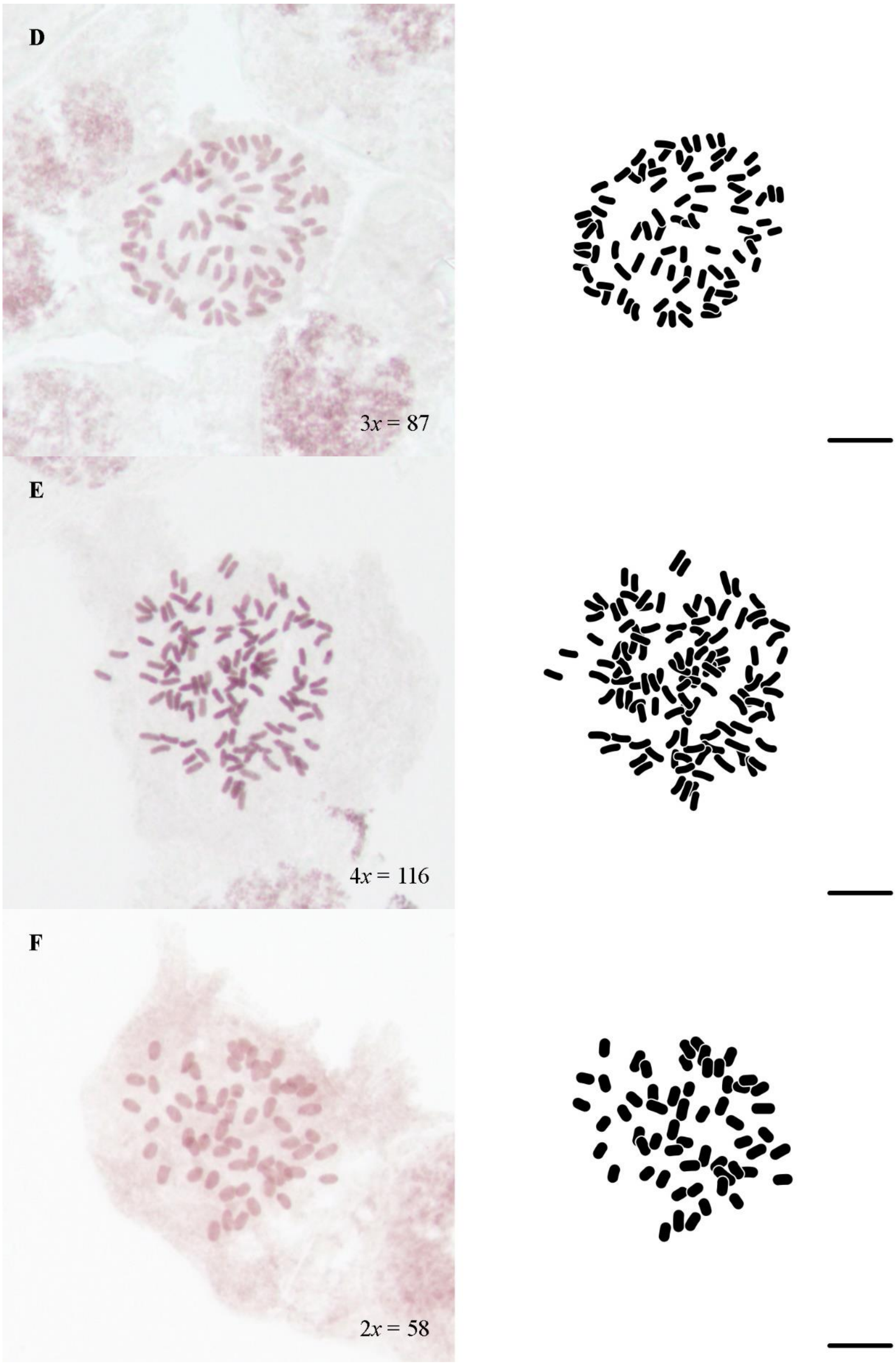
All of the different cytotypes were found in India (Bir and Verma 2010), so it seems to be the center of speciation. A recent cytological survey on *P. vittata* in India was published by Srivastava et al. (2007), and only tetraploids were found in natural habitats. Diploid, triploid, pentaploid, and hexaploid were considered extinct in their habitats. Recently, a new variant of *P. vittata*, dubbed *P. vittata* “nano” was recorded based on ecotype and morphological characters. This variant was found only on concrete surfaces and the spore number per sporangium was less than 64 spores, also with spore ornamentation, but they could not study the ploidy level (Morajkar and Hegde 2021). This variant of concrete buildings in urban areas of Thailand was also observed. The collection of additional specimens from urban areas might help us reach a better understanding of the relationship between ploidy levels and ecotypes found in different environments, especially in Thailand, which is a hybrid zone and is home to cytotypes with different ploidy levels (Hanušová et al. 2019). Although this species is widely distributed and some intraspecific classification was published, most discussions focused on Indian plants. To resolve the confusion, further taxonomical study on this species complex is needed. Works on morphology, cytology, and molecular phylogeny of this complex from other regions are proposed.

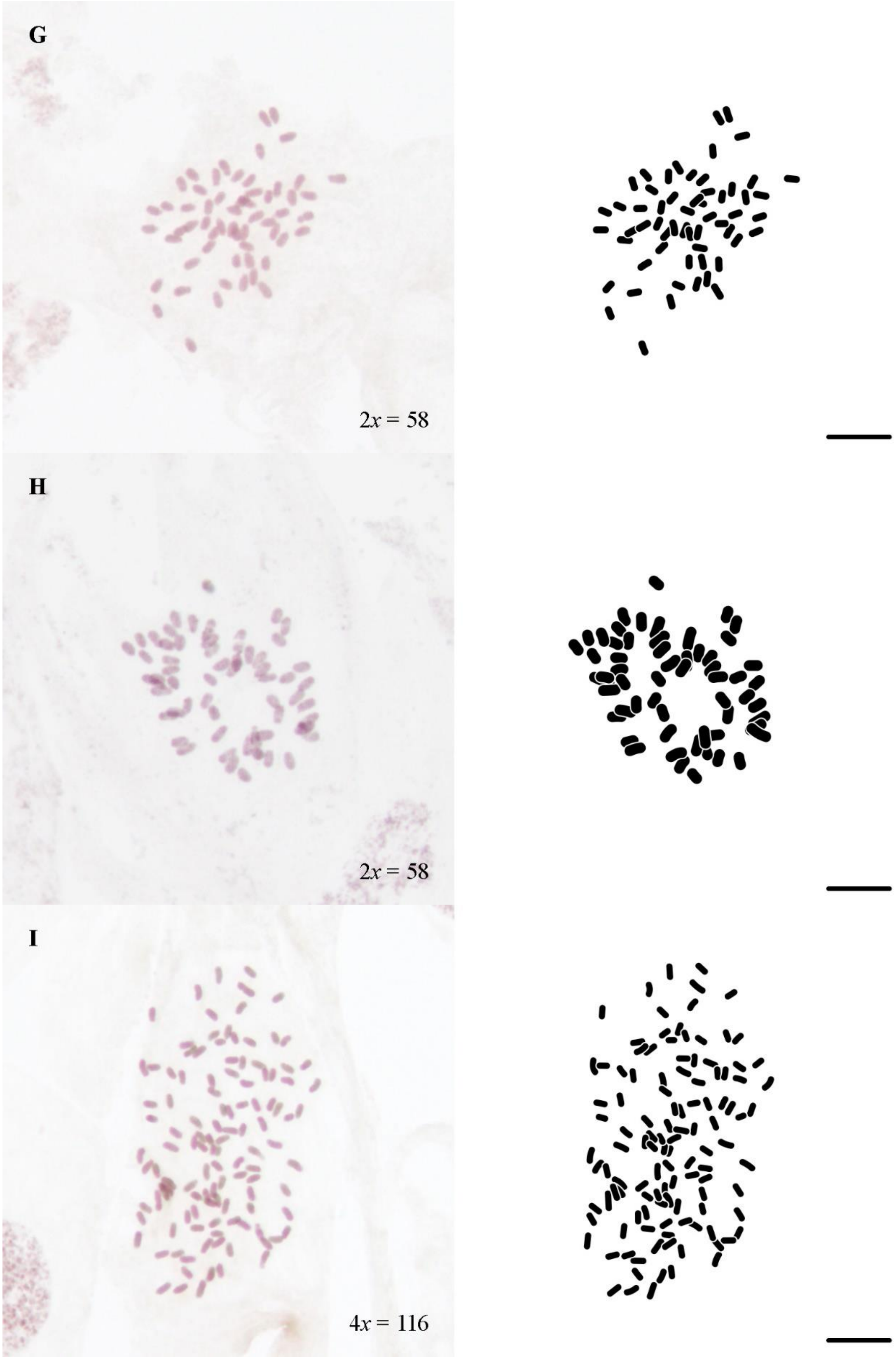
***Pteris* sect. *Semipinnatae* Liang Zhang & Li Bing Zhang**
Pteris dalhousieae Hook.
2*n* = 2*x* = 58, [diploid sexual]

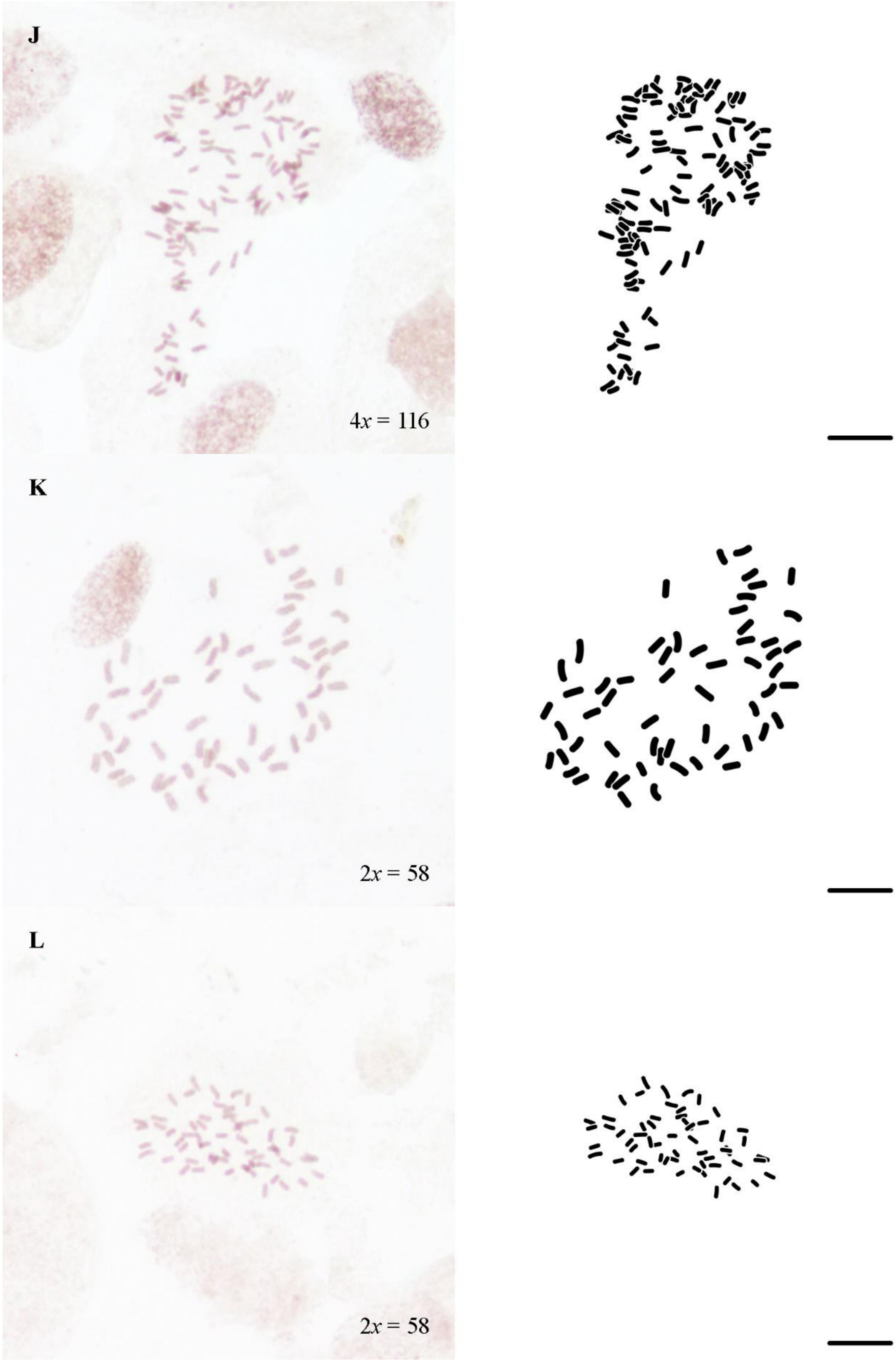
This is the first cytological record for this species, specifically a diploid sexual (2*n* = 2*x* = 58) (Figure 1O). *P. dalhousieae* is characterized by the segments that are restricted to the basiscopic side, while the acroscopic side is found with fewer segments or entire. They are distributed from South Indo-China to Indonesia (Lindsay and Middleton 2012; POWO 2022) and are closely related to *P. semipinnata*. It might be a case of hybridization; some individuals are intermediate between them (Holttum 1968) and may also be misidentified with *P. dissitifolia* Baker (Yang et al. 2011). Although *P. dalhousieae* was never included in a molecular study before (Chao et al. 2014; Zhang et al. 2015; Zhang and Zhang 2018), it should belong to section *Semipinnatae* of its morphological characteristics.

Based on chromosome observation of 9 *Pteris* species, 4 species with polyploid cytotypes were found and we did not detect any with evidence of using the apogamous reproductive system. This might be some specimens studied had not yet formed fertile fronds. Additional studies should be done because many genera of ferns with triploid plants are functionally sterile and use apogamous reproductive mode (Chao et al. 2012b; Nakato and Ebihara 2016; Nakato et al. 2020). Triploid apogamous plants have intermediate forms of morphological characteristics, which might arise from interspecific hybridization or intraspecific hybridization. The diverse cytotypes of *P. heteromorpha*, comprising 2*x*, 3*x*, and 4*x*, were characterized by similarly diverse morphology of fronds, so these different characters could be used for more detailed infraspecific classification schemes, as has been done in the case of *P. terminalis* (Ebihara et al. 2017). Nuclear DNA marker studies are needed to try to see if the different cytotypes have certain alleles that might have come from other species. If they do not, then they can be segregated at a varietal rank (Jaruwattanaphan et al. 2013; Ebihara et al. 2017). Based on molecular, morphological, and ecological evidence, an infrageneric classification of this genus was proposed and it was classified into 3 subgenera and 12 sections (Zhang and Zhang 2018). The number of chromosomes or the ploidy level cannot be used to classify at the infrageneric level, especially for ferns in the genus *Pteris*. Many of them utilize multiple cyto-reproductive types, and this can bring about reticulate evolution within and between species. In each section of *Pteris*, a multitude of diverse section cytotypes could be seen (Walker 1958; Chao et al. 2012b; Jaruwattanaphan et al. 2013).


A
2x = 58
B
2x = 58
C
2x = 58


D
3x = 87
E
4x = 116
F
2x = 58


G
2x = 58
H
2x = 58
I
4x = 116


J
4x = 116
K
2x = 58
L
2x = 58

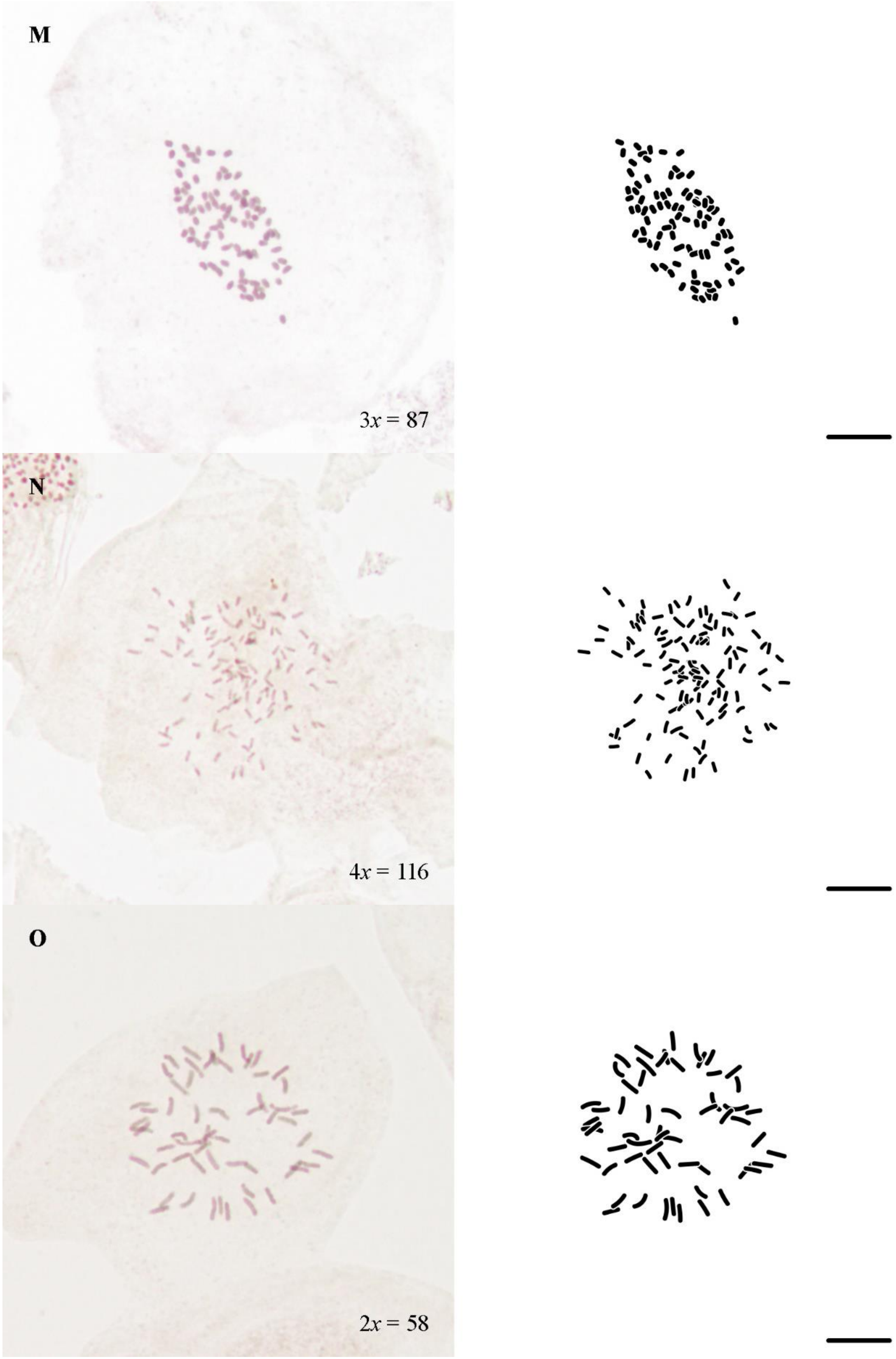


Figure 1. Mitotic chromosome numbers of ferns in genus *Pteris*. Microphotographs (left) and explanatory illustrations (right). A-B. P. *decrescens*; C-E. *P. heteromorpha*; F-G. *P. venusta*; H. *P. blumeana*; I. *P. ensiformis*; J. *P. amoena*; K. *P. bella*; L-N. *P. vittata*; O. *P. dalhousieae*. Scale bar = 10 µm

In conclusion, *Pteris* specimens from Thailand investigated in this study had the basic chromosome number $x = 29$. This was the first published account of the chromosome numbers of *P. decrescens*, *P. heteromorpha*, and *P. dalhousieae*. Diverse cytotypes in *P. vittata* and *P. heteromorpha*, including diploid, triploid and tetraploid were observed. This information on the cytotypes and reproductive biology of ferns in genus *Pteris* will be essential in phylogenetic and evolutionary studies of genus *Pteris*, especially for species with distribution contiguous with Thailand and adjacent regions.

ACKNOWLEDGEMENTS

We thank the editors and anonymous reviewers for providing helpful comments. This research is supported in part by the Graduate Program Scholarship from the Graduate School, Kasetsart University, Thailand.

REFERENCES

Bir SS, Verma SC. 2010. Chromosome Atlas of the Indian Pteridophytes (1951-2009). Bishen Singh Mahendra Pal Singh, Dehra Dun.

Chao YS, Dong SY, Chiang YC, Liu HY, Chiou WL. 2012a. Extreme multiple reticulate origins of the *Pteris cadieri* complex (Pteridaceae). Intl J Mol Sci 13 (4): 4523-4544. DOI: 10.3390/ijms13044523.

Chao YS, Liu HY, Chiang YC, Chiou WL. 2012b. Polyploidy and speciation in *Pteris* (Pteridaceae). J Bot 2012: 1-7. DOI: 10.1155/2012/817920.

Chao YS, Rouhan G, Amoroso VB, Chiou WL. 2014. Molecular phylogeny and biogeography of the fern genus *Pteris* (Pteridaceae). Ann Bot 114: 109-124. DOI:10.1093/aob/mcu086.

Chao YS, Mustapeng AMA, Chen CW, Chiou WL. 2017. *Pteris borneensis* (Pteridaceae), a new species from Borneo, with re-circumscription of *Pteris decrescens* and *Pteris parviloba*. Syst Bot 42 (4): 724-732. DOI:10.1600/036364417X696609.

Cheng X, Zhang SZ. 2010. Index to chromosome numbers of Chinese pteridophyta (1969-2009). J Fairylake Bot Gard 9 (1): 1-58.

Dong SY, Haque AKMK. 2021. A taxonomic study on *Pteris* L. (Pteridaceae) of Bangladesh. Bangladesh J Pl Taxon 28 (1): 131-140. DOI: 10.3329/bjpt.v28i1.54213.

Ebihara A, Nakato N, Jaruwattanaphan T. 2017. A new taxonomic treatment for the apogamous counterpart of *Pteris terminalis* (Pteridaceae). Phytotaxa 314 (1): 73-80. DOI:10.11646/phytotaxa.314.1.5.

Fraser-Jenkins CR. 2012. Rare and threatened pteridophytes of Asia (2) endangered species of India: the higher IUCN categories. Bull Natl Mus Nat Sci B 38 (4): 153-181.

Fraser-Jenkins CR, Gandhi KN, Kholia BS, Benniamin A. 2017. An Annotated Checklist of Indian Pteridophytes Part-1 (Lycopodiaceae to Thelypteridaceae). Bishen Singh Mahendra Pal Singh, Dehra Dun.

Hanušová K, Čertner M, Urfus T, Koutecký P, Košnar J, Rothfels CJ, Jarolímová V, Ptáček J, Ekrt L. 2019. Widespread co-occurrence of multiple ploidy levels in fragile ferns (*Cystopteris fragilis* complex; Cystopteridaceae) probably stems from similar ecology of cytotypes, their efficient dispersal and inter-ploidy hybridization. Ann Bot 123: 845-855. DOI: 10.1093/aob/mcy219.

Haufler CH. 2008. Species and Speciation. In: Ranker TA, Haufler CH (eds). Biology and Evolution of Ferns and Lycophytes. Cambridge University Press, New York.

Holttum RE. 1968. A Revised Flora of Malaya II. Ferns of Malaya. Government Printing Office, Singapore.

Huang YM, Hsu SY, Hsieh TH, Chou HM, Chiou WL. 2011. Three *Pteris* species (Pteridaceae: Pteridophyta) reproduce by apogamy. Bot Stud 52: 79-87.

Hwang IC, Moon MO, Bounphanmy S, Yoon N, Sun BY. 2015. New records of ferns in the flora of Laos (1). Korean J Pl Taxon 45 (2): 109-113. DOI: 10.11110/kjpt.2015.45.2.109.

Ivanova D, Piekos-Mirkowa H. 2003. Chromosome numbers of Polish ferns. Acta Biol Cracov Ser Bot 45 (2): 93-99.

Jaruwattanaphan T, Matsumoto S, Watano Y. 2013. Reconstructing hybrid speciation events in the *Pteris cretica* group (Pteridaceae) in Japan and adjacent regions. Syst Bot 38 (1): 15-27. DOI: 10.1600/036364413X661980.

Kato M, Nakato N, Cheng X, Iwatsuki K. 1992. Cytotaxonomic study of ferns of Yunnan, southwestern China. Bot Mag Tokyo 105: 105-124. DOI: 10.1007/BF02489407.

Khare PB, Kaur S. 1983. Intraspecific polyploidy in *Pteris vittata* Linn. Cytologia 48: 21-25. DOI: 10.1508/cytologia.48.21.

Lindsay S, Middleton DJ. 2012. Ferns of Thailand, Laos and Cambodia. www.rbg-web2.rbge.org.uk/thaiferns

Morajkar S, Hegde S. 2021. DNA barcoding identifies a potential new ecotype of Chinese brake fern, *Pteris vittata* L. nano. Proc Natl Acad Sci India B 91 (2): 335-341. DOI: 10.1007/s40011-021-01231-4.

Nakato N, Ebihara A. 2016. Chromosome numbers of 18 ferns in Japan: toward completion of chromosome information in Japanese ferns. Bull Natl Mus Nat Sci B 42 (1): 25-40.

Nakato N, Ebihara A, Watanabe M, Tsutsumi C. 2020. New cytotaxonomic records on threatened fern species in Japan. Bull Natl Mus Nat Sci B 46 (1): 17-27.

Paitoonyakul S. 2018. Diversity and Chromosome Numbers of Monilophytes at Khao Laem, Mu Ko Chang National Park, Trat Province. [Thesis]. Kasetsart University, Bangkok. [Thai]

POWO. 2022. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew.
www.plantsoftheworldonline.org

Praptosuwiryo TN, Mumpuni M. 2018. Chromosome numbers of some species of *Pteris* (Pteridaceae) in Java, Indonesia. Biodiversitas 19 (6): 2118-2126. DOI: 10.13057/biodiv/d190618.

Rattamanee C. 2006. Inheritance of Crested Frond and Chromosome Number in Some *Asplenium*, *Pteris* and *Nephrolepis*. [Thesis]. Kasetsart University, Bangkok. [Thai]

Srivastava J, Ranade SA, Khare PB. 2007. Distribution and threat status of the cytotypes of *Pteris vittata* L. (Pteridaceae) species complex in India. Curr Sci 93 (1): 81-85.

Takamiya M. 1996. Index to Chromosomes of Japanese Pteridophyta (1910-1996). Japan Pteridological Society, Tokyo.

Tagawa M, Iwatsuki K. 1985. Pteridophytes. In: Smitinand T, Larsen K (eds). Flora of Thailand. Vol. 3 Part 2. Phonphan Printing Co., Ltd., Bangkok.

Thumdee S. 1996. Studies on Time to Collect Circinate Fronds and Root Tips for Chromosome Counting of Four Species of Fern in Genus *Pteris*. [Special Problems]. Kasetsart University, Bangkok. [Thai]

Uday UKS, Bhakat RK. 2021. Short Communication: Assessment of Pteridophytes' composition and conservation status in sacred groves of Jhargram District, South West Bengal, India. Biodiversitas 22 (5): 3171-3178. DOI: 10.13057/biodiv/d220620.

van Welzen PC, Madern A, Raes N, Parnell JAN, Simpson DA, Byrne C, Curtis T, Macklin J, Trias-Blasi A, Prajaksood A, Bygrave P, Dransfield S, Kirkup DW, Moat J, Wilkin P, Couch C, Boyce PC, Chayamarit K, Chantaranothai P, Esser HJ, Jebb MHP, Larsen K, Larsen SS, Nielsen I, Meade C, Middleton DJ, Pendry CA, Muasya AM, Pattharahirantricin N, Pooma R, Suddee S, Staples GW, Sungkaew S, Teerawatananon A. 2011. The current and future status of floristic provinces in Thailand. In: Trisurat Y, Shrestha RP, Alkemade R (eds). Land Use, Climate Change and Biodiversity Modeling: Perspectives and Applications. Information Science Reference, Hershey.

Walker TG. 1958. Hybridization in some species of *Pteris* L. Evolution 12 (1): 82-92. DOI: 10.2307/2405906.

Walker TG. 1962. Cytology and evolution in the fern genus *Pteris* L. Evolution 16 (1): 27-43. DOI: 10.2307/2406264.

Wang ZR. 1989. A preliminary study on cytology of Chinese *Pteris*. Acta Phytotax Sin 27 (6): 421-438. [Chinese]

Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH. 2009. The frequency of polyploid speciation in vascular plants. Proc Natl Acad Sci USA 16 (33): 13875-13879. DOI:10.1073/pnas.0811575106.

Yang DM, Xing FW, Wang FG. 2011. Lectotypification of three species in the fern genus *Pteris* (Pteridaceae) from China. Novon 21 (4): 515-516. DOI: 10.3417/2010094.

Yingchutrakul M. 2016. Reproductive Mode Studies of *Pteris* Fern in Thailand. [Special Problems]. Kasetsart University, Bangkok. [Thai]

Zhang GM, Liao WB, Ding MY, Lin YX, Wu ZH, Zhang XC, Dong SY, Prado J, Gilbert MG, Yatskievych G, Ranker TA, Hooper EA, Alverson ER, Metzgar JS, Funston AM, Masuyama S, Kato M. 2013. Pteridaceae. In: Wu ZY, Raven PH, Hong DY (eds). Flora of China. Vol. 2-3. Science Press, Beijing.

Zhang L, Rothfels CJ, Ebihara A, Schuettpelz E, Péchon TL, Kamau P, He H, Zhou XM, Prado J, Field A, Yatskievych G, Gao XF, Zhang LB. 2015. A global plastid phylogeny of the brake fern genus *Pteris* (Pteridaceae) and related genera in the Pteridoideae. Cladistics 31: 406-423. DOI: 10.1111/cla.12094.

Zhang L, Zhang LB. 2018. Phylogeny and systematics of the brake fern genus *Pteris* (Pteridaceae) based on molecular (plastid and nuclear) and morphological evidence. Mol Phylogenet Evol 118: 265-285. DOI: 10.1016/j.ympev.2017.09.011.