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Cytogenetic study of five species of medicinal plants from Maha Sarakham Province, Thailand

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Abstract. *Saensouk P, Saen-in N, Saensouk S. 2022. Cytogenetic study of five species of medicinal plants from Maha Sarakham Province, Thailand. Biodiversitas 23: 3593-3603.* The cytogenetic study of five species of medicinal plants, namely *Butomopsis latifolia* (D. Don) Kunth, *Iris domestica* (L.) Goldblatt & Mabb., *Limnocharis flava* (L.) Buchenau, *Murdannia loriformis* (Hassk.) R.S. Rao & Kammathy, and *Pontederia hastata* L. from Maha Sarakham Province, northeastern Thailand was conducted. This study aimed to investigate the chromosome numbers, fundamental number (NF), karyotype forms, and perform ideogram analysis of the five species. The cytogenetic characteristics, including the chromosome number, karyology (fundamental number (NF), karyotype formula), symmetrical karyotype, chromosome size, relative length (RL), centromeric indexes (CI), and ideograms of *B. latifolia* were studied for the first time. The chromosome numbers of five medicinal plant species were reported as 2*n* = 14 (*B. latifolia*) - 80 (*I. domestica*). Moreover, the NF had 28 (*B. latifolia*) - 80 (*I. domestica*). Two species (*M. loriformis* and *P. hastata*) were recognized as having symmetrical karyotypes. Three species, namely *B. latifolia, I. domestica*, and *L. flava* were reported as having asymmetrical karyotypes. Chromosomes of large size (L), chromosomes of medium size (M), and chromosomes of small size (S) were observed. Ideograms of five species were provided in this study. The NF, RL, CI, chromosome size, and ideograms of all species were reported for the first time in this study. The karyotype formulae of the five species, except the karyotype formula of *P. hastata*, differed from those previously studied. The cytogenetic data of the five species in this study can be used for identification.

Keywords: Chromosome, chromosome number, ideogram, karyotype

INTRODUCTION

Thailand has an abundance of forests. These forests consist of plants that are used as food, as herbs, in rituals, as equipment, and in construction, etc. People in different regions of Thailand are increasingly taking advantage of plants from these natural resources. Therefore, the local plants in each community in different regions of Thailand are important, and the awareness of the benefits of local plants have been receiving increasing attention due to people in different regions increasingly making use of native plants from nature (Inta et al. 2013; Khuankaew et al. 2014; Cruz-Garcia et al. 2016; Panyadee et al. 2016; Saensouk et al. 2016; Pholhiamhan et al. 2018; Phumthum et al. 2018; Junsongduang et al. 2017, 2020; Punchay et al. 2020; Saensouk and Saensouk 2021d; Numpulsuksant et al. 2021; Phatlamphu et al. 2021; Saisor et al. 2021; Ragsasilp et al. 2022).

Maha Sarakham Province is located in Northeastern Thailand with the lowest proportional forest area of any province. Deciduous dipterocarp forests have been found to be the most common forest type in Maha Sarakham Province. They are an important source of food and medicinal plants, and as a place for livestock. The villagers in Maha Sarakham Province depend on the natural plant resources of neighboring forests for food, medicine, firewood, building houses, and making home appliances, etc. (Numpulsuksant et al. 2021; Saisor et al. 2021; Saensouk and Saensouk 2022). Therefore, the forest in this province is important to the lives of the nearby villagers from the past to the present.

Medicinal plants refer to a group of plants that are commonly used for medicinal purposes, to nourish the body, for detoxification, and local wisdom, including uses in traditional and modern medicine. Some medicinal plants are popular as ingredients in daily food as well (such as banana, basil leaf, cucumber, galangal, garlic, ginger, lemon grass, rice, shallot, tamarind, and tomato, etc.), used as a health food supplement (such as finger root, garlic, ginger, galangal, tamarind, tomato, and turmeric, etc.), or to produce cosmetics (such as cucumber, tamarind, tomato, and turmeric). Medicinal plants are very important to human daily life. Therefore, villagers from Thailand cultivate medicinal plants in their home gardens, i.e., banana, basil, black pepper, cucumber, finger root, garlic, ginger, galangal, gooseberry, kaffir lime, lime leaves, lemon grass, mango, mint, pandan, pepper, sesame, shallot, tamarind, tomato, and turmeric, etc. (Pholhiamhan et al. 2018; Saensouk and Saensouk 2021a; Numpulsuksant et al. 2021; Phatlamphu et al. 2021; Saisor et al. 2021; Ragsasilp et al. 2022). Several authors have studied the cytology of some species of medicinal plants, i.e., Banerji and Haldar (1942), Harada (1943), Majumdar (1953), Banerjee (1974), Bhattacharya and Ghosh (1979), Patwary et al. (1989),

Wang and Wang (1989), Uchiyama (1989), Forni-Martins and Calligaris (2002), Kundu (2005), Wang et al. (2007), Feitoza et al. (2010), Saensouk and Saensouk (2020). Villagers from communities in Maha Sarakham Province, northeastern Thailand usually use five species - namely *Butomopsis latifolia* (D. Don) Kunth, *Iris domestica* (L.) Goldblatt & Mabb., *Limnocharis flava* (L.) Buchenau, *Murdannia loriformis* (Hassk.) R.S. Rao & Kammathy, and *Pontederia hastata* L. as medicinal plants, which are cultivated in home gardens. Therefore, the chromosome structure, variations in chromosome number, and karyotypes of those medicinal plants might be different from previous studies (Saensouk and Saensouk 2020; Saensouk and Saensouk 2021a). Moreover, the information from this research will support future cytological studies to be more comprehensive. In this paper, we aimed to study the chromosome numbers, fundamental number (NF), karyotype forms, and ideogram analysis of five species in medicinal plants from Maha Sarakham Province, Northeast Thailand.

MATERIALS AND METHODS

Sample collection

Five species of medicinal plants - namely *Butomopsis latifolia* (D. Don) Kunth, *Iris domestica* (L.) Goldblatt & Mabb., *Limnocharis flava* (L.) Buchenau, *Murdannia loriformis* (Hassk.) R.S. Rao & Kammathy, and *Pontederia hastata* L. were collected from home gardens in Maha Sarakham Province, northeastern Thailand. All medicinal plant species were grown in a nursery at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, Maha Sarakham Province, Thailand. Voucher specimens of the five species were deposited at Mahasarakham University, Thailand.

Mitotic, karyotype, and ideogram analysis

The chromosome numbers were studied from root tips according to the methods of Saensouk et al. (2019) and Senavongse et al. (2018, 2020). The root tips were pre-treated in 2mM 8-hydroxyquinoline for 8 h at 4°C, fixed for 30 min in ethanol: acetic acid (3:1, v:v) at room temperature, and stored at 4°C. The root tips were hydrolyzed in 1 M HCl for 5 min at 60°C. The root tips were stained and squashed in 2% aceto-orcein on slides, and then they were sealed with a transparent color nail polish before observation under a microscope. Photographs were taken using a light microscope (Zeiss: Axiostar plus) at 100× magnification. Karyomorphological observations were performed on chromosomes at the mitotic metaphase, and karyotype formulas were derived from measurements of the photomicrographs of metaphase chromosomes. The nomenclature of the chromosome morphology was based on Levan et al. (1964). The chromosome number, chromosome length range, haploid chromosome length, arm ratio, relative length, and karyotype formula were determined from 10 metaphase cells in each species. For the arrangement of the chromosomes in the karyotypes, the parameters for the average length of the short arm (Ls), the average length of the long arm (Ll), length of each chromosome (LT), average relative length (RL), chromosome index (CI), and standard deviations (SD) of RL and CI from the metaphase chromosomes were calculated according to Senavongse et al. (2018, 2020), Saensouk et al. (2019), and Saensouk and Saensouk (2020, 2021b, c).

RESULTS AND DISCUSSION

During the authors' survey of medicinal plants from Maha Sarakham Province, northeastern Thailand, many medicinal plant species were found in home gardens, such as *B. latifolia*, *I. domestica*, *L. flava*, *M. loriformis*, and *P. hastata* Villagers from communities in Maha Sarakham Province used *B. latifolia* as a medicinal plant and local vegetable. *I. domestica* was also used as a tonic and ornamental plant in home gardens and schools. *L. flava* was used as a medicinal plant (as a tonic and as an antipyretic) and local vegetable. Moreover, villagers used *M. loriformis* as a medicinal plant to be used as a heart tonic. *P. hastata* was used as a medicinal plant (as a tonic), vegetable, and ornamental plant.

The chromosome numbers from the root tips of *B. latifolia*, *I. domestica*, *L. flava*, *M. loriformis*, and *P. hastata* from Maha Sarakham Province, northeastern Thailand are shown in Table 1. In addition, the karyology (fundamental number (NF), karyotype formula), ideograms, and location in this study and those studied previously, of five species in this study are presented in Table 1. Moreover, the chromosome size, the relative length (RL), and the centromeric indexes (CI) are shown in Tables 2-6.

Family Alismataceae

Two species, *B. latifolia* and *L. flava*, belonging to family Alismataceae, were studied cytogenetically.

***Butomopsis latifolia* (D. Don) Kunth -** The somatic chromosome number of *B. latifolia* was presented as 14 (Figure 1.A). The NF was found to be 28. The karyotype formula of this species was asymmetrical, karyotype 4m+4sm+6st, including two pairs of metacentric (m)-type, two pairs of submetacentric (sm)-type, and three pairs of subtelocentric (st)-type (Figures 1.B, 1.C and Table 2). The chromosome size of this species was recognized as three pairs of large size, three pairs of medium size, and one pair of small size. The short arm length (Ls) ranged from 1.71±0.84 to 5.52±0.65 μm, the long arm length (Ll) ranged from 3.86±0.47 to 6.87±0.47 μm, and the total chromosome length (Lt) ranged from 5.65±0.84 to 12.40±0.91 μm. The relative length (RL) of the karyotype was between 9.13 to 20.08% (Table 2). The centromeric indexes (CI) were 0.56-0.75 (Table 2 and Figures 1.B, 1.C). The created ideogram was based on the lengths of the chromosome arms and presented the point of the centromere (Figure 1.C). The NF, karyotype, chromosome size, RL, CI, and ideogram of *B. latifolia* were reported for the first time (Tables 1-2).

Table 1. Chromosome number, karyological study, and ideogram of *Butomopsis latifolia*, *Iris domestica*, *Limnocharis flava*, *Murdannia loriformis*, and *Pontederia hastata* investigated in this study and those studied previously

Family	Species	Chromosome numbers (2*n*)	NF	Karyotype formula	Ideogram	Symmetrical karyotype	Location	Previous studied
Alismataceae	*Butomopsis latifolia*	14*	28*	4m+4sm+6st*	✓*	Asymmetry	Thailand	*Present study*
	Limnocharis flava	20	40*	4m+14st+2a	✓*	Asymmetry	Thailand	*Present study*
		20	-	4m+16a	-		Brazil	Forni-Martins and Calligaris (2002)
		20	-	4m+16a	-		Australia	Feitoza et al. (2010)
		20	-	-	-		Japan	Harada (1943)
		26, 39	-	-	-		India	Bhattacharya and Ghosh (1979)
		20	-	-	-		Japan	Uchiyama (1989)
Commelinaceae	*Murdannia loriformis*	20	40*	6m+14sm	✓*	Symmetry	Thailand	*Present study*
		20	-	8m+12sm	-		Thailand	Saensouk and Saensouk (2020)
Iridaceae	*Iris domestica*	40	80*	14m+22sm+4st	✓*	Asymmetry	Thailand	*Present study*
		32	-	- 20m+10sm+2st - 20m+8sm+4st	-		China	Wang et al. (2007)
Pontederiaceae	*Pontederia hastata* (syn.*Monochoria hastata*)	28	56*	2m+26sm	✓*	Symmetry	Thailand	*Present study*
		28, 80	-	-	-		Bangladesh	Patwary et al. (1989)
		28	-	-	-		China	Wang and Wang (1989)
		28	-	-	-		India	Banerji and Haldar (1942)
		28	-	-	-		India	Majumdar (1953)
		28	-	-	-		India	Kundu (2005)
		28,	-	2m+26sm	-		India	Banerjee (1974)
		40, 70, 76, 80, 82	-	-	-		India	Banerjee (1974)

Note: *first time report, a: acrocentric chromosome, m: metacentric chromosome, NF: fundamental number, sm: submetacentric chromosome, st: subtelocentric chromosome, and −: not available

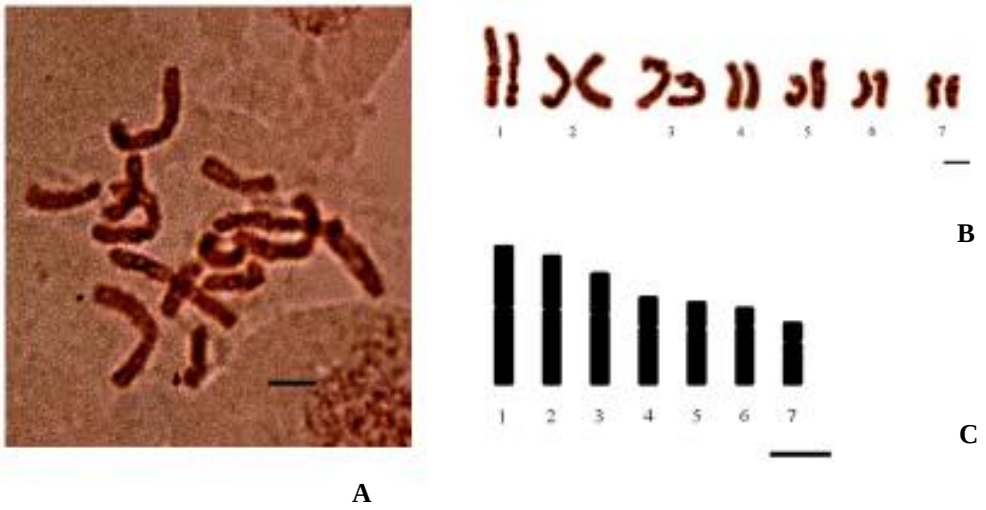

Figure 1. Chromosomes of *Butomopsis latifolia*. A. Somatic metaphase chromosome number showing 2*n* = 14, B. karyotype showing 4m+4sm+6st, C. Ideogram, scale bars = 5 μm

Table 2. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL and CI from 20 metaphases, and chromosome size of *Butomopsis latifolia* (2*n* = 14)

Chromosome pair	Ls (μm) ± SD	Ll (μm) ± SD	LT (μm) ±SD	RL (%)	Chromosome size	CI	Chromosome type
1	5.52±0.65	6.87±0.47	12.40±0.91	20.08	L	0.56	Metracentric
2	4.83±1.09	6.71±0.83	11.54±1.78	18.69	L	0.58	Metracentric
3	3.75±0.55	6.22±0.35	9.97±0.75	16.14	L	0.63	Submetracentric
4	2.76±0.57	5.13±0.44	7.90±0.95	12.76	M	0.66	Submetracentric
5	2.46±0.71	4.97±0.69	7.43±1.32	12.02	M	0.70	Subtelocentric
6	1.71±0.84	5.19±0.36	6.90±1.17	11.18	M	0.75	Subtelocentric
7	1.79±0.53	3.86±0.47	5.65±0.84	9.13	S	0.70	Subtelocentric

Note: L: chromosome large size, M: chromosome medium size, and S: chromosome small size

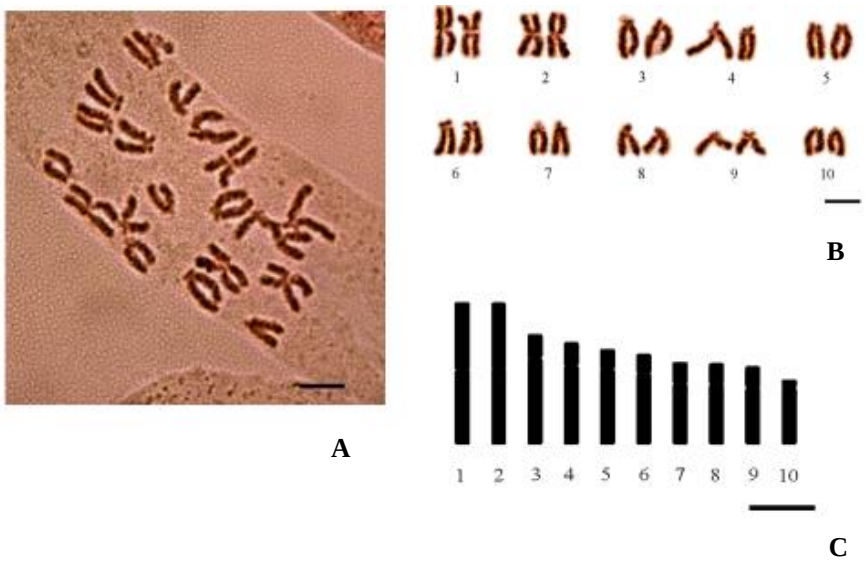

Figure 2. Chromosome of *Limnocharis flava*. A. Somatic metaphase chromosome number showing 2*n* = 20, B. Karyotype showing 4m+14st+2a, C. Ideogram, scale bars: 5 μm

Table 3. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL and CI from 20 metaphases, and chromosome size of *Limnocharis flava* (2*n* = 20)

Chromosome pair	Ls (μm) ± SD	Ll (μm) ± SD	LT (μm) ±SD	RL (%)	Chromosome size	CI	Chromosome type
1	3.38±1.01	3.71±0.36	7.09±0.81	13.72	L	0.59	Metracentric
2	3.28±0.06	3.78±0.39	7.06±0.39	12.81	L	0.57	Metracentric
3	1.18±0.69	4.29±0.41	5.48±0.62	11.60	L	0.78	Subtelocentric
4	1.14±0.13	3.94±0.21	5.08±0.23	10.75	M	0.78	Subtelocentric
5	1.03±0.97	3.73±0.40	4.76±0.84	10.07	M	0.78	Subtelocentric
6	0.97±0.26	3.53±0.26	4.50±0.42	9.52	M	0.79	Subtelocentric
7	1.09±0.77	3.03±0.36	4.12±0.60	8.73	M	0.73	Subtelocentric
8	1.03±0.40	3.01±0.39	4.05±0.35	8.56	M	0.75	Subtelocentric
9	1.07±0.98	2.83±0.58	3.89±0.71	8.23	M	0.72	Subtelocentric
10	0.38±0.23	2.84±0.43	3.22±0.34	7.68	S	0.84	Acrocentric

Note: L: chromosome large size, M: chromosome medium size, and S: chromosome small size

***Limnocharis flava* (L.) Buchenau –** The somatic chromosome number of *L. flava* was observed to be 20 (Figure 2.A). The NF was found to be 40. The karyotype formula of this species was asymmetrical, karyotype 4m+14st+2a, including two pairs of metacentric (m)-type, seven pairs of subtelocentric (st)-type, and one pair of acrocentric (a)-type (Figures 2.B, 2.C and Table 3). The chromosome size of this species can be divided into three pairs of large size, six pairs of medium size, and one pair of small size. The short arm length (Ls) ranged from 0.38±0.23 to 3.38±1.01 μm, the long arm length (Ll) ranged from 2.83±0.58 to 4.29±0.41 μm, and the total chromosome length (Lt) ranged from 3.22±0.34 to 7.09±0.81 μm. The relative length (RL) of the karyotype had a value between 7.68 and 13.72 % (Table 3). The centromeric indexes (CI) were 0.57-0.84 (Table 3 and Figures 2.B, 2.C). The created ideogram was based on the lengths of the chromosome arms and presented the point of the centromere (Figure 2.C). The NF, chromosome size, RL, CI, and ideogram of *L. flava* were reported for the first time (Tables 3). This study found the same as several other scientists who studied the chromosome numbers of *L. flava*, as show in Table 1, such as Forni-Martins and Calligaris (2002), Feitoza et al. (2010), Harada (1943), and Uchiyama (1989), while it differed from that of Bhattacharya and Ghosh (1979) of 2*n* = 26, 39 (Table 1). The karyotype formula in this study differed from previous reports due to the effects of environmental factors, i.e., Forni-Martins and Calligaris (2002) and Feitoza et al. (2010), who reported 4m+16a without chromosome satellites (Table 1).

Family Commelinaceae

One species, *M. loriformis*, in the family Alismataceae was studied cytogenetically.

***Murdannia loriformis* (Hassk.) R.S. Rao & Kammathy –** The diploid chromosome number of *M. loriformis* was found to be 20 (Figure 3.A). The NF was found to be 40. The karyotype formula of this species was symmetrical, karyotype 6m+14sm, including three pairs of metacentric (m)-type and seven pairs of submetacentric (sm)-type (Figures 3.B, 3.C and Table 4). The chromosome size of this species can be divided into four pairs of large size and six pairs of medium size. The short arm length (Ls) ranged from 1.00±0.17 to 2.00±0.21 μm, the long arm length (Ll) ranged from 1.50±0.12 to 2.87±0.28 μm, and the total chromosome length (Lt) ranged from 2.55±0.40 to 4.87±0.36 μm. The relative length (RL) of the karyotype was a value between 7.27 to 13.60 % (Table 4). The centromeric indexes (CI) were 0.52-0.65 (Table 4 and Figures 3.B, 3.C). The created ideogram was based on the lengths of the chromosome arms and presented the point of the centromere (Figure 3.C). The NF, chromosome size, RL, CI, and ideogram of *M. loriformis* were reported for the first time (Table 4). This study showed the same as Saensouk and Saensouk (2020) who studied the chromosome numbers of *M. loriformis* as shown in Table 4. The karyotype formula in this study differed from previous reports due to the effects of environmental factors, i.e., Saensouk and Saensouk (2020), who reported 8m+12sm without chromosome satellites (Table 4).

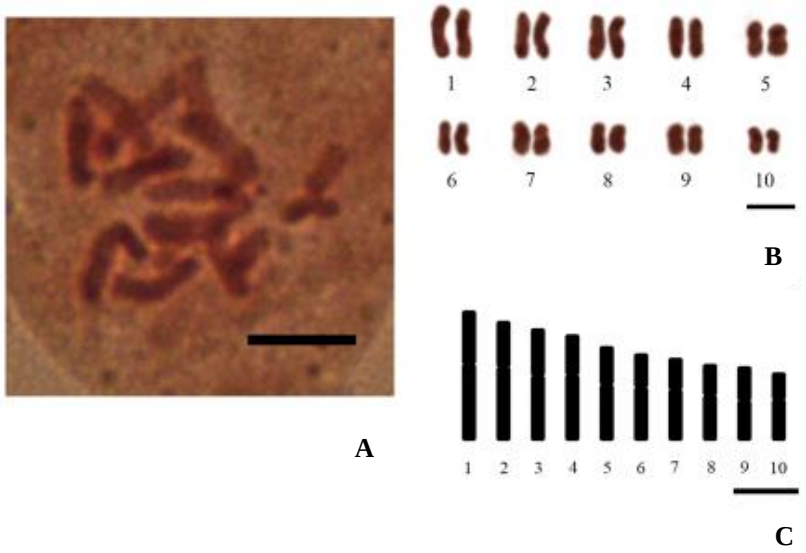

Figure 3. Chromosomes of *Murdannia loriformis*. A. Somatic metaphase chromosome number showing 2n = 20, B. Karyotype showing 6m+14sm, C. ideogram, scale bars: 5 μm

Table 4. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL and CI from 20 metaphases, and chromosome size of *Murdannia loriformis* (2*n* = 20)

Chromosome pair	Ls (μm) ± SD	Ll (μm) ± SD	LT (μm) ±SD	RL (%)	Chromosome size	CI	Chromosome type
1	2.00±0.21	2.87±0.28	4.87±0.36	13.60	L	0.62	Submetracentric
2	1.71±0.37	2.77±0.61	4.48±0.89	12.35	L	0.63	Submetracentric
3	1.79±0.15	2.43±0.20	4.22±0.33	11.73	L	0.61	Submetracentric
4	1.53±0.49	2.46±0.80	3.99±1.22	11.11	L	0.62	Submetracentric
5	1.45±0.48	2.10±0.73	3.55±1.20	9.34	M	0.54	Metracentric
6	1.27±0.20	2.00±0.40	3.27±0.59	9.08	M	0.58	Metracentric
7	1.20±0.25	1.91±0.38	3.11±0.59	8.88	M	0.65	Submetracentric
8	1.18±0.16	1.70±0.42	2.88±0.58	8.38	M	0.61	Submetracentric
9	1.30±0.09	1.50±0.12	2.79±0.16	8.27	M	0.52	Metracentric
10	1.00±0.17	1.54±0.27	2.55±0.40	7.27	M	0.64	Submetracentric

Note: SD: standard deviation, L: chromosome large size, M: chromosome medium size, S: chromosome small size

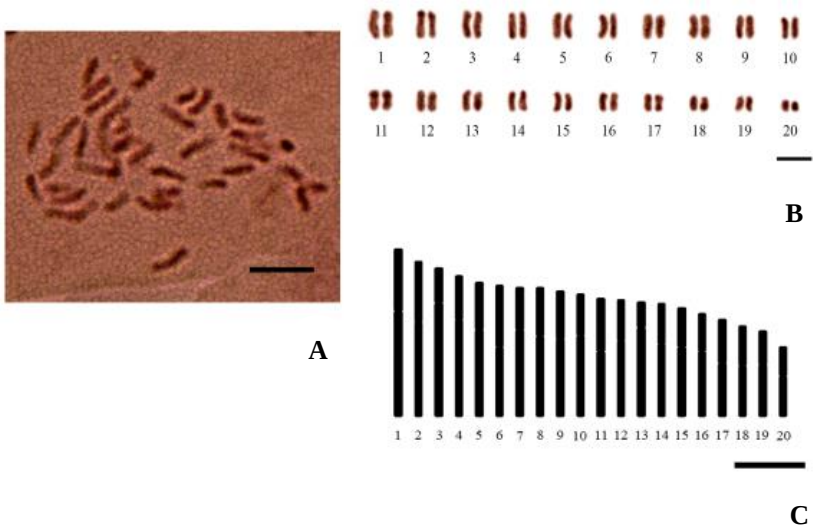

Figure 4. Chromosome of *Iris domestica.* A. Somatic metaphase chromosome number showing 2*n* = 40, B. Karyotype showing 14m+22sm+4st, C. Ideogram, scale bars: 5 μm

Table 5. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL and CI from 20 metaphases, and chromosome size of *Iris domestica* (2*n* = 40)

Chromosome pair	Ls (μm) ± SD	Ll (μm) ± SD	LT (μm) ±SD	RL (%)	Chromosome size	CI	Chromosome type
1	1.32±0.24	2.23±0.33	3.55±0.46	6.98	L	0.63	Submetracentric
2	1.29±0.13	2.00±0.18	3.29±0.07	6.47	L	0.61	Submetracentric
3	0.76±0.09	2.40±0.10	3.16±0.10	6.20	L	0.70	Subtelocentric
4	0.94±0.06	2.06±0.15	2.99±0.21	5.86	L	0.69	Submetracentric
5	1.00±0.21	1.84±0.19	2.84±0.20	5.56	L	0.65	Submetracentric
6	1.32±0.13	1.47±0.08	2.79±0.07	5.46	L	0.59	Metracentric
7	0.91±0.15	1.84±0.14	2.75±0.08	5.38	L	0.67	Submetracentric
8	1.05±0.05	1.70±0.23	2.75±0.26	5.38	L	0.58	Metracentric
9	1.03±0.17	1.64±0.27	2.67±0.43	5.23	L	0.57	Metracentric
10	0.88±0.23	1.72±0.19	2.60±0.10	5.10	L	0.66	Submetracentric
11	1.15±0.10	1.37±0.19	2.52±0.10	4.94	L	0.59	Metracentric
12	0.87±0.15	1.60±0.27	2.48±0.39	4.86	M	0.65	Submetracentric
13	0.56±0.24	1.88±0.20	2.44±0.21	4.78	M	0.71	Subtelocentric
14	0.87±0.09	1.53±0.09	2.40±0.02	4.70	M	0.64	Submetracentric
15	0.85±0.26	1.47±0.23	2.31±0.06	4.53	M	0.64	Submetracentric
16	0.81±0.06	1.38±0.06	2.19±0.04	4.28	M	0.63	Submetracentric
17	0.93±0.37	1.13±0.32	2.06±0.14	4.03	M	0.59	Metracentric
18	0.86±0.19	1.07±0.10	1.91±0.14	3.75	M	0.57	Metracentric
19	0.71±0.13	1.11±0.15	1.82±0.07	3.57	M	0.61	Submetracentric
20	0.63±0.14	0.86±0.33	1.49±0.45	2.94	S	0.57	Metracentric

Note: L: chromosome large size, M: chromosome medium size, and S: chromosome small size

Family Iridaceae

Only one species, *I. domestica*, in the family Iridaceae was studied cytogenetically.

***Iris domestica* (L.) Goldblatt & Mabb. –** The diploid chromosome number of was found to be 40 (Figure 4.A). The NF was found to be 80. The karyotype formula of this species was an asymmetrical karyotype 14m+22sm+4st, including seven pairs of metacentric (m)-type, eleven pairs of submetacentric (sm)-type, and two pairs of subtelocentric (st)-type (Figures 4.B, 4.C and Table 5). The chromosome size of this species can be divided into eleven pairs of large size, eight pairs of medium size, and one pair of small size. The short arm length (Ls) ranged from 0.63±0.14 to 1.32±0.24 μm, the long arm length (Ll) ranged from 0.86±0.33 to 2.40±0.10 μm, and the total chromosome length (Lt) ranged from 1.49±0.45 to 3.55±0.46 μm. The relative length (RL) of the karyotype was a value between 2.94 to 6.98 % (Table 5). The centromeric indexes (CI) were 0.57-0.71 (Table 5 and Figures 4.B, 4.C). The created ideogram was based on the lengths of the chromosome arms and presented the point of the centromere (Figure 4.C). The NF, chromosome size, RL, CI, and ideogram of *I. domestica* were reported for the first time (Tables 5). The chromosome number 2*n*=40 of *I. domestica* was inconsistent with a previous study by Wang et al. (2007) that had a chromosome number of 2*n*=32, as shown in Table 5. The karyotype formula of *I. domestica* in this study differed from previous reports from China due to the effects of environmental factors and varies by geography, i.e., Wang et al. (2007) who reported 20m+10sm+2st and 20m+8sm+4st without chromosome satellites (Table 5).

Family Pontederiaceae

Pontederia hastata in this family was studied cytogenetically.

***Pontederia hastata* L. –** The cytogenetics of *P. hastata* was studied and it showed that the diploid chromosome number was 28 (Figure 4.A). The NF was found to be 56. The karyotype formula of this species was a symmetrical karyotype 2m+26sm, including one pair of metacentric (m)-type and thirteen pairs of submetacentric (sm)-type (Figures 5.B, 5.C and Table 6). The chromosome size of this species can be divided into seven pairs of large size, six pairs of medium size, and one pair of small size (Table 6). The short arm length (Ls) ranged from 0.63±0.13 to 1.29±0.16 μm, the long arm length (Ll) ranged from 0.84±0.09 to 2.31±0.13 μm, and the total chromosome length (Lt) ranged from 1.47±0.19 to 3.59±0.37 μm. The relative length (RL) of the karyotype was a value between 4.10 to 10.02 % (Table 6). The centromeric indexes (CI) were 0.57-0.68 (Table 6 and Figures 5.B, 5.C). The created ideogram was based on the lengths of the chromosome arms and presented the point of the centromere (Figure 5.C). The NF, chromosome size, RL, CI, and ideogram of *P. hastata* were reported for the first time (Table 6). *P. hastata* had the chromosome number 2*n*=28, consistent with most previous studies, such as Wang and Wang (1989), Banerji and Haldar (1942), Majumdar (1953), and Kundu (2005), but the difference was reported as 2*n*=28, 80 by Patwary et al. (1989). Moreover, Banerjee (1974) also reported 2*n*=28, 40, 70, 76, 80, and 82 as shown in Table 5, due to environmental effects such as evaluation, regions, air, and soil. The karyotype formula of this species in this study was the same as in a previous report by Banerjee (1974) (Table 6).

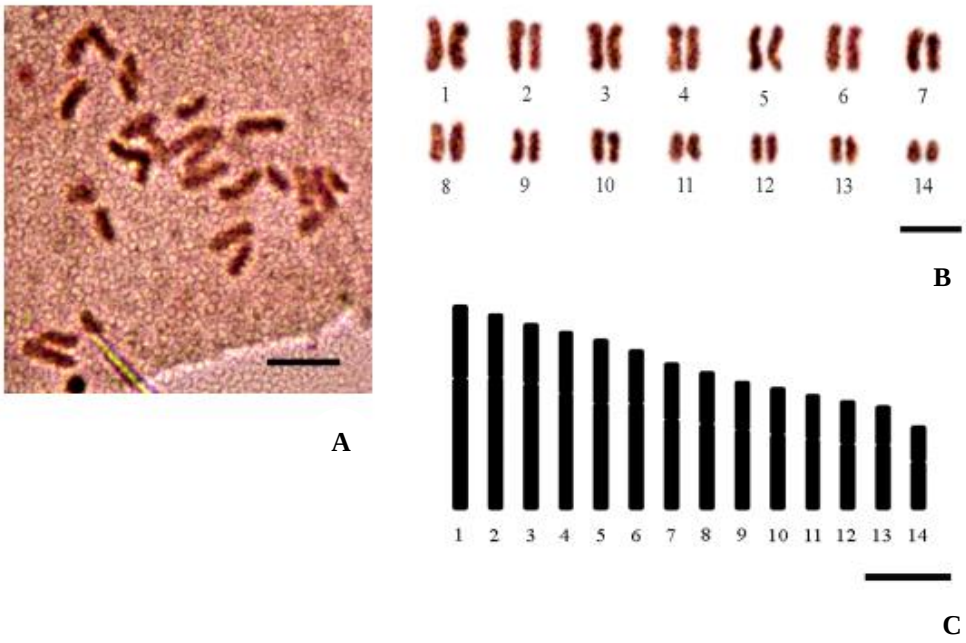

Figure 5. Chromosomes of *Pontederia hastata* A. Somatic metaphase chromosome number showing 2*n* = 28, B. Karyotype showing 2m+26sm, C. Ideogram, scale bars: 5 μm

Table 6. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL and CI from 20 metaphases, and chromosome size of *Pontederia hastata* (2*n* = 28)

Chromosome pair	Ls (μm) ± SD	Ll (μm) ± SD	LT (μm) ±SD	RL (%)	Chromosome size	CI	Chromosome type
1	1.29±0.16	2.30±0.32	3.59±0.37	10.02	L	0.64	Submetracentric
2	1.12±0.08	2.31±0.13	3.43±0.20	9.56	L	0.67	Submetracentric
3	1.05±0.25	2.21±0.64	3.26±0.89	9.09	L	0.68	Submetracentric
4	1.09±0.13	2.03±0.17	3.12±0.09	8.70	L	0.65	Submetracentric
5	1.14±0.11	1.85±0.28	2.99±0.36	8.32	L	0.62	Submetracentric
6	0.95±0.35	1.85±0.50	2.81±0.84	7.80	L	0.65	Submetracentric
7	0.99±0.08	1.58±0.23	2.57±0.29	7.19	L	0.61	Submetracentric
8	0.91±0.03	1.51±0.19	2.42±0.21	6.78	M	0.62	Submetracentric
9	0.86±0.29	1.40±0.66	2.25±0.90	6.32	M	0.62	Submetracentric
10	0.83±0.11	1.32±0.15	2.15±0.07	6.02	M	0.62	Submetracentric
11	0.78±0.08	1.24±0.24	2.02±0.29	5.64	M	0.61	Submetracentric
12	0.76±0.35	1.15±0.79	1.92±1.09	5.36	M	0.60	Submetracentric
13	0.68±0.20	1.14±0.49	1.82±0.61	5.09	M	0.63	Submetracentric
14	0.63±0.13	0.84±0.09	1.47±0.19	4.10	S	0.57	Metracentric

Note: L: chromosome large size, M: chromosome medium size, and S: chromosome small size

In conclusion, the cytogenetic study of the five medicinal plant species, namely *B. latifolia*, *I. domestica*, *L. flava*, *M. loriformis*, and *P. hastata* from Maha Sarakham Province, northeastern Thailand was conducted. The study of *B. latifolia* included the chromosome number, karyology (fundamental number (NF), karyotype formula), symmetrical karyotype, chromosome size, relative length (RL), centromeric indexes (CI), and ideogram. The chromosome numbers of medicinal plant five species were reported as 2n = 14 (*B. latifolia*), 20 (*L. flava*), 20 (*M. loriformis*), 40 (*I. domestica*), and 28 (*P. hastata*). Moreover, the NF had 28 (*B. latifolia*), 40 (*L. flava)*, 40 (*M. loriformis)*, 80 (*I. domestica)*, and 56 (*P. hastata*). The NF was 28 (*B. latifolia*) – 80 (*I. domestica*). The karyotype formulae of these species were symmetrical (two species) – *M. loriformis* and *P. hastata*, or asymmetrical in three species, namely *B. latifolia*, *I. domestica*, and *L. flava*. Three chromosome size types, chromosomes of large size (L), chromosomes of medium size (M), and chromosomes of small size (S), were observed. The chromosomes of large size and medium size were the most diversely reported, while chromosomes of small size were reported less. The ideograms of the five species in this study were provided based on the lengths of the chromosome arms and they presented the point of the centromere. The NF, RL, CI, chromosome size, and ideogram of all species were

reported for the first time in this study. For the karyotype formulae of four species, namely, *B. latifolia*, *I. domestica*, *L. flava*, and *M. loriformis*, differed from previous studies (Saensouk and Saensouk 2020). Whereas, the karyotype formula of *P. hastata* was the same as previously reported in table 1. The cytogenetic data of five species in this study can be used for identification in each species by the chromosome number, karyology (NF, karyotype formula), symmetrical karyotype, chromosome size, RL, and CI, which were determined from the result in this study and could be used to provide more accurate and complete taxonomic data, and to apply this information to the cytogenetics for further research in various fields, such as being able to be developed further in plant breeding for economic potential and for the conservation of these medicinal plants in Maha Sarakham Province to maintain them sustainably for the future.

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