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Original Research Article

Cytogenetics Study of Medicinal plants of Genus *Alpinia* two Rare Species from Thailand

Piyaporn Saensouk[1], Surapon Saensouk[2]*, Santi Watthana[3], Phetlasy Souladeth[4]

[1]*Diversity of Family Zingiberaceae and Vascular Plant for Its Applications Research Cluster, Department of Biology, Faculty of Science, Mahasarakham University. Kantharawichai, Maha Sarakham, 44150, Thailand.*
[2]*Diversity of Family Zingiberaceae and Vascular Plant for Its Applications Research Cluster, Biodiversity Program, Walairukhavej Botanical Research Institute, Mahasarakham University. Kantarawichai, Maha Sarakham, 44150, Thailand*
[3]*School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand*
[4] *Faculty of Forest Science, National University of Laos, Vientiane Capital, Laos PDR*

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ABSTRACT

With more than 250 species, the genus *Alpinia* Roxb. is the largest in the tribe Alpinieae, subfamily Alpinoideae, and family Zingiberaceae. *A. macrostaminodia* Chaveer. and Sudmoon, and *A. latilabris* Ridl, two rare species, were identified. These two unusual species' cytogenetics have never been documented. This study's objective was to examine the chromosomal counts, fundamental numbers, and karyotype structure of two endangered *Alpinia* species from Thailand. Thus, using the squash technique, two rare species, *A. macrostaminodia* and *A. latilabris*, were cytologically examined. Two rare species are known to have the somatic chromosomal counts and karyotypes 2*n* = 48 = 46 sm + 2 st (*A. macrostaminodia*) and 2*n* = 48 = 8m+18sm+22st (*A. latilabris*). Both rare species' NFs were discovered to be 96. Both rare species' ideograms were given. *A. macrostaminodia* and *A. latilabris*' somatic chromosomal counts, NFs, karyotype formulae, and ideograms were the first to be documented. The data of cytology, including NFs, karyotype formulas, karyotype structures, and ideograms in both rare *Alpinia* species, can be identified in each species.

Keywords: *Alpinia*, Chromosome number, Karyotype, Thailand, Zingiberaceae

Introduction

Zingiberaceae, a monocotyledon aromatic and medicinal family with about 52 genera and 1,300 species that can be found in the tropics from India to Malaysia, is a member of the order Zingiberales.[1] The Zingiberaceae family has the most diversity in Southeast Asia.[2,3] It was discovered that the *Alpinia* Roxb. genus, which contains more than 250 species, is the largest genus in the Alpinieae tribe, Alpinoideae subfamily, and Zingiberaceae family. *Alpinia* was found in tropical Asia, Australia, the Solomon Islands, the New Hebrides, New Caledonia, Fiji, and Samoa, as well as other Pacific island nations.[1,4,5] There are over 160 species in the genus, most of which are found in the Malesian region. Larsen examined the genus Alpinia in Thailand in 1996 with 13 species, Saensouk et al. followed in 2003 with 14 species, and Chumroenphat and Saensouk in 2022 with 15 species.[6,7] Furthermore, according to research by Ragsasilp et al. in 2022 and Chumroenphat and Saensouk in 2022, the genus *Alpinia* species were traditionally used as medicinal, food, ornamental, cosmetics, and ritual plants. In particular, *A. macrostaminodia* Chaveer, Sudmoon, and *A. latilabris* Ridl were used as local medicinal and food plants.[3, 5, 8] While the conservation status of *Alpinia* species was determined to be *A. macrostaminodia* Chaveer and Sudmoon (rare and endemic species) and *A. latilabris* Ridl (Least Concern, or LC, and rare species) based on many databases and the literature that was available.[5,9,10,11]

*Corresponding author. E mail: surapon.s@msu.ac.th
Tel: +660880293958

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Many researchers have discussed the cytology of a few species of the *Alpinia* genus, including Darlington and Wylie in 1955, Mahanty in 1970, Moore in 1974, Goldblatt in 1988, Chen and Huang in 1996, Larsen *et al.* in 1998, Eksomtramage *et al.* in 2002, and Augsonkitt *et al.* in 2004. These researchers reported the chromosome numbers 2n = 24, 28, 30, 36, 48, and 50.[1,12-18] However, the genus *Alpinia*'s karyotype has never been investigated. Prior research has never been done on the karyotypes and ideograms of the *Alpinia*, which includes *A. macrostaminodia* Chaveer, Sudmoon, and *A. latilabris* Ridl. So, this investigation will reveal fresh cytogenetics data. This study's conventional goal was to examine the chromosomal counts, fundamental numbers (NF), and karyotype variations of medicinal plants of the genus *Alpinia*, including two endangered species from Thailand. This study will offer valuable information for the primary uses of plants, including plant breeding for traditional medicines, food products, and economic plants.

Materials and Methods

Sample collection

Alpinia macrostaminodia Chaveer and Sudmoon (coll. no. S. Saensouk 3001; Ban Phaeng District, Nakhon Phanom Province; the coordinates of location at 17°58′5″N 104°12′57″E) and *A. latilabris* Ridl. (coll. no. S. Saensouk 3040; Na Kae District, Nakhon Phanom Province; the coordinates of location at 1656′48″NN 104°30′3″E), two medicinal plants with native Thai species, were found in several locations throughout northeastern Thailand in April and May 2021 (Table 1 and Figure 1). The Mahasarakham University Herbarium received these voucher specimens for deposit. The Mahasarakham University nursery (Walai Rukhavej Botanical Research Institute), Maha Sarakham Province, Thailand, provided the rhizomes used to cultivate the roots of each species.

Mitotic, karyotype and ideogram analyses

Both rare species' 1-2 cm long root tips were taken from living plants in order to analyze the mitotic chromosomes using the squash approach.[19] All root tips underwent a 6-hour paradichlorobenzene (PDB) pretreatment at 4°C, followed by a 30-minute room-temperature fixation in ethanol-acetic acid 3:1 (v/v), which was then stored at 4 °C or used right away. Additionally, root tips were washed in distilled water and then hydrolyzed in 1N HCl for 5 minutes at 60°C. The chromosomes of all species' root tips were dyed and damaged with 2% aceto-orcein before being studied using a light microscope (Zeiss: Axiostar Plus), which produced the micrographs depicted in Figures 2, 3, and which were measured using Adobe Photoshop CS3 Extended (Figures 2-3). The chromosomal number investigation adhered to the procedures that various scientists had previously described.[19, 20, 21] The chromosome morphology nomenclature was in accordance with procedures that numerous researchers had previously described.[23, 24, 25] Acrocentric (a), subtelocentric (st), metacentric (m), and subsubmetacentric (sm) were used to categorize all chromosomes. The chromosomes of each species were identified from the 20 visible metaphase cells. The chromosome index (CI = Ll/LT), average length of the short arm (Ls), average length of the long arm (Ll), total length of each chromosome (LT), and standard deviations (SD) of RL and CI from clearly metaphase chromosomes were calculated for the karyotype structure.[19,20,21,23-25] The ideograms that emerged near the centromere were made using the lengths of the chromosomal arms.[24]

Figure 1: The flower morphology of two rare species in their natural habitat from Thailand. A. *A. macrostaminodia*, B. *A. latilabris* (scale bars = 1 cm)

Table 1: A summary of reports in somatic chromosome numbers, karyotype, ideogram of two rare species from Thailand

***Alpinia* species**	**2*n***	***n***	**NF**	**Karyotype formula**	**Ideogram**	**Location in Thailand**	**Previous studied**
A. macrostaminodia	48*	-	96*	46sm+2st*	✓*	Nakhon Phanom Province	The first time reported
A. latilabris	48*	-	96*	8m+18sm+22st*	✓*	Sakon Nakhon Province	The first time reported

* The first time reported

Results and Discussion

The *Alpinia macrostaminodia* Chaveer. and Sudmoon (Figure 1A) was collected from Nakhon Phanom Province, consistent with previously reported by several botanists[26] who collected this species only in Nakhon Phanom Province few years ago but differ from previously reported by several botanists[9, 8] who collected this species only in Bueng Kan Province, Thailand. And *A. latilabris* Ridl. (Figure 1B) was collected as rare species from Nakhon Phanom Province, Thailand, differ from Chumroenphat and Saensouk (2022)[5] who collected this species only in Sakon Nakhon Province, Thailand. Moreover, based on the conservation status of *Alpinia* species in the research area, it was found that *A. macrostaminodia* Chaveer. and Sudmoon were recorded as rare plants, consistent with previous reports by several scientists[9,10,11] who reported this species as a rare and endemic species, and *A. latilabris* Ridl. was recorded as a rare plant, consistent with previous reports by several workers[5, 10, 11] who reported the conservation status of this species to be Least Concern (LC) and rare species. The conservation status of *A. macrostaminodia* Chaveer. and Sudmoon and *A. latilabris* Ridl. was reported as a rare species, which is consistent with previous reports by several scientists[8, 5]

The dominant characteristics of both rare plants and endemic species are provided. *Alpinia macrostaminodia* Chaveer. and Sudmoon have the dominant characteristics of labellum yellow with a plane and all parts of the pseudostem glabrous, which are consistent with what has previously been reported. 8 While, *A. latilabris* Ridl. has the dominant characteristics—labellum yellow with concave and all parts of pseudostem pubescence which are consistent with previously reported.[5] From the available literature and the people who live in the plant area, traditional utilization data of both rare plants and endemic species are provided here. *Alpinia macrostamino*dia Chaveer and Sudmoon's rhizomes, young pseudostems, and young inflorescences were as a food, vegetables, spices, and medicinal plants (tonic and stomachache), which is consistent with previous reported uses.[8] Young *A. latilabris* Ridl. inflorescences and rhizome were as a food, including vegetables, and medicinal plants (stomachache and skin disease), which is consistent with previous studies.[5]

The root tips of *Alpinia macrostami*nodia Chaveer.and Sudmoon, and *A. latilabris* Ridl from the northeastern region of Thailand were analysed for somatic chromosomal counts, which are shown in Table 1. The NF and karyotype formula, ideogram, location, and previously investigated information for the two species are included in the karyology data listed in Table 1. Additionally, Tables 2-3 provided data on the chromosome size, relative length (RL), and centromeric indexes (CI).

Figure 2A shows that the somatic chromosome number of *A. macrostaminodia* Chaveer and Sudmoon is 2*n* = 48, which is in agreement with earlier research by Thai researchers who reported the chromosome number of the genus *Alpinia* as 2*n* = 48.17, 27 NF was discovered to be 96 for this species. The karyotype formula, which was discovered to be 46sm + 2st, was identified as an asymmetrical karyotype. *A. macrostaminodia* Chaveer and Sudmoon's karyological analysis revealed 23 pairs of submetacentric-type chromosomes and 1 pair of subtelocentric-type chromosomes (Table 2 and Figure 2B). The length of the long arm was 0.480.01 to 1.000.02 m, the short arm was 0.320.01 to 0.630.02 m, and the total length was 0.800.01 to 1.570.02 m. In this investigation, the chromosome centromeric indices (CI) ranged from 0.60 to 0.72, while the relative lengths (RL%) ranged from 3.00 to 6.00 (Table 2). Figure 2C has all of the ideograms. The findings showed that the somatic chromosomal counts, NF, karyotype, and ideogram of *A. macrostaminodia* Chaveer and Sudmoon were analyzed for the first time.

With a somatic chromosome number of 2*n* = 48, it was recognized as *A. latilabris* Ridl., which is consistent with prior research by Thai botanists who recorded the chromosomal number of the genus *Alpinia* and discovered it was 2*n* = 48.17 The NF for this species was given as 96. The karyological data of *A. latilabris* Ridl revealed four pairs of chromosomes with metacentric types, nine pairs with submetacentric

types, and eleven pairs with subtelocentric types. The karyotype formula was found to be 8m+18sm+22st, and it was revealed that these karyotypes were asymmetrical (Table 3 and Figure 3B). The length of the long arm ranged from 1.36 to 3.68 m, whereas the short arm's length ranged from 0.75 to 2.07 m. The overall chromosomal length ranged from 2.18 to 4.75 m. The relative lengths (RL%) of the chromosomes in this investigation ranged from 3.000.01 to 6.000.02 according to the centromeric index (CI) (Table 3). It can be found in Figure 3C. First recorded were the somatic chromosomal numbers, nuclear factor, karyotype, and ideogram of *A. latilabris* Ridl.

The findings corroborated those of Santhosh in 1999, who identified plants based on their chromosome morphological characteristics. The results showed that the chromosome structure and karyotype formula of two rare *Alpinia* species, *A. macrostaminodia* Chaveer and Sudmoon and *A. latilabris* Ridl, from Thailand, can be used to identify each species. [27] Because the somatic chromosome number of the genus *Alpinia* was difficult to identify clearly in metaphase, similar with previously investigated species, the cytogenetic study of this genus was hardly documented, particularly karyotype and ideogram. [24, 25] Furthermore, Stebbins proposed in 1971 that the karyotype of organisms incorporates chromosome size and chromosome kinds. These cytological data may be useful in the classification of plant species, but more research is necessary. [28] In addition, Chaiyasut recommended a study of plant karyotypes in 1989 because it could aid in determining the reason for morphological changes that resulted from environmental factors like the plant's location, soil, water, or air, or from a change involving chromosomes, either structurally or numerically. [29, 30, 31]

Conclusion

The somatic chromosome number and karyotypes of two rare *Alpinia* species are 2*n* = 48 with the karyotype formula as 46sm+2st (*A. macrostaminodia* Chaveer. and Sudmoon) and 2*n* = 48 with the karyotype formula as 8m+18sm+22st (*A. latilabris* Ridl.). The NF of both rare species was found to be 96. The ideogram of both rare species was provided based on the lengths of the chromosome arms and the point of the centromere. Therefore, the somatic chromosome numbers, NF, karyotype, and ideogram of *A. macrostaminodia* Chaveer. and Sudmoon and *A. latilabris* Ridl. were studied for the first time. The data on NF, karyotype formula, karyotype structure, and ideogram in this study can be used for the identification of each species.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 2: Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), relative length (RL) and centromere index (CL) RL, CI of *A. macrostaminodia* 2*n* = 48, obtained from 10 metaphase plates

Chromosome pair	Ls ± SD (*μ*m)	Ll ± SD (*μ*m)	LT ± SD (*μ*m)	RL (%)	CI	Chromosome type
1	0.63 ± 0.02	0.95 ± 0.02	1.57 ± 0.02	6.00 ± 0.01	0.60 ± 0.02	Submetacentric
2	0.52 ± 0.01	1.00 ± 0.02	1.52 ± 0.01	5.00 ± 0.02	0.66 ± 0.01	Submetacentric
3	0.50 ± 0.05	0.88 ± 0.03	1.38 ± 0.03	5.00 ± 0.01	0.70 ± 0.02	Submetacentric
4	0.50 ± 0.02	0.82 ± 0.01	1.32 ± 0.02	5.00 ± 0.02	0.71 ± 0.01	Submetacentric
5	0.37 ± 0.01	0.93 ± 0.02	1.30 ± 0.02	5.00 ± 0.01	0.72 ± 0.01	Subtelocentric
6	0.48 ± 0.03	0.82 ± 0.05	1.29 ± 0.05	5.00 ± 0.01	0.63 ± 0.02	Submetacentric
7	0.49 ± 0.03	0.79 ± 0.03	1.28 ± 0.03	5.00 ± 0.02	0.62 ± 0.01	Submetacentric
8	0.42 ± 0.02	0.86 ± 0.01	1.27 ± 0.01	5.00 ± 0.03	0.67 ± 0.02	Submetacentric
9	0.47 ± 0.02	0.80 ± 0.01	1.26 ± 0.01	4.00 ± 0.02	0.63 ± 0.03	Submetacentric
10	0.43 ± 0.01	0.80 ± 0.02	1.23 ± 0.01	4.00 ± 0.02	0.65 ± 0.05	Submetacentric
11	0.50 ± 0.01	0.72 ± 0.02	1.22 ± 0.01	4.00 ± 0.02	0.60 ± 0.01	Submetacentric
12	0.43 ± 0.03	0.76 ± 0.05	1.19 ± 0.04	4.00 ± 0.02	0.64 ± 0.02	Submetacentric
13	0.39 ± 0.01	0.77 ± 0.04	1.16 ± 0.03	4.00 ± 0.02	0.66 ± 0.02	Submetacentric
14	0.40 ± 0.01	0.74 ± 0.02	1.14 ± 0.01	4.00 ± 0.02	0.65 ± 0.03	Submetacentric
15	0.36 ± 0.02	0.78 ± 0.01	1.13 ± 0.01	4.00 ± 0.02	0.69 ± 0.01	Submetacentric
16	0.44 ± 0.01	0.69 ± 0.01	1.12 ± 0.01	4.00 ± 0.02	0.61 ± 0.02	Submetacentric
17	0.38 ± 0.02	0.74 ± 0.01	1.12 ± 0.01	4.00 ± 0.02	0.66 ± 0.05	Submetacentric
18	0.37 ± 0.05	0.70 ± 0.02	1.07 ± 0.03	4.00 ± 0.02	0.65 ± 0.04	Submetacentric
19	0.37 ± 0.05	0.67 ± 0.02	1.04 ± 0.03	4.00 ± 0.02	0.65 ± 0.01	Submetacentric
20	0.36 ± 0.04	0.65 ± 0.01	1.02 ± 0.02	3.00 ± 0.01	0.64 ± 0.02	Submetacentric
21	0.37 ± 0.02	0.58 ± 0.01	0.95 ± 0.01	3.00 ± 0.01	0.61 ± 0.02	Submetacentric
22	0.32 ± 0.01	0.60 ± 0.02	0.92 ± 0.01	3.00 ± 0.01	0.66 ± 0.03	Submetacentric
23	0.33 ± 0.02	0.54 ± 0.01	0.88 ± 0.01	3.00 ± 0.01	0.62 ± 0.01	Submetacentric
24	0.32 ± 0.01	0.48 ± 0.01	0.80 ± 0.01	3.00 ± 0.01	0.61 ± 0.01	Submetacentric

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Table 3: Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), relative length (RL) and centromere index (CL) RL, CI of *A. latilabris* 2*n* = 48, obtained from 10 metaphase plates

Chromosome pair	Ls ± SD (*μ*m)	Ll ± SD (*μ*m)	LT ± SD (*μ*m)	RL (%)	CI	Chromosome type
1	1.06 ± 0.01	3.68 ± 0.02	4.75 ± 0.03	6.00 ± 0.02	0.78 ± 0.02	Subtalocentric
2	1.12 ± 0.01	3.18 ± 0.01	4.30 ± 0.02	5.00 ± 0.02	0.74 ± 0.02	Subtalocentric
3	2.07 ± 0.01	2.17 ± 0.01	4.23 ± 0.01	5.00 ± 0.01	0.51 ± 0.01	Metacentric
4	1.11 ± 0.01	2.97 ± 0.01	4.07 ± 0.02	5.00 ± 0.01	0.73 ± 0.02	Subtalocentric
5	1.97 ± 0.02	2.02 ± 0.01	3.99 ± 0.03	5.00 ± 0.02	0.51 ± 0.03	Metacentric
6	1.06 ± 0.01	2.74 ± 0.02	3.80 ± 0.03	5.00 ± 0.01	0.72 ± 0.03	Subtalocentric
7	1.35 ± 0.01	2.41 ± 0.01	3.76 ± 0.02	5.00 ± 0.02	0.64 ± 0.02	Submetacentric
8	1.35 ± 0.01	2.39 ± 0.01	3.74 ± 0.02	4.00 ± 0.02	0.64 ± 0.02	Submetacentric
9	1.16 ± 0.01	2.57 ± 0.01	3.72 ± 0.02	4.00 ± 0.01	0.69 ± 0.01	Submetacentric
10	1.04 ± 0.01	2.68 ± 0.01	3.72 ± 0.02	4.00 ± 0.01	0.72 ± 0.01	Subtalocentric
11	1.03 ± 0.01	2.69 ± 0.01	3.71 ± 0.02	4.00 ± 0.03	0.72 ± 0.03	Subtalocentric
12	1.16 ± 0.01	2.53 ± 0.01	3.68 ± 0.01	4.00 ± 0.02	0.69 ± 0.02	Submetacentric
13	0.93 ± 0.01	2.68 ± 0.01	3.61 ± 0.02	4.00 ± 0.02	0.74 ± 0.01	Subtalocentric
14	0.86 ± 0.01	2.67 ± 0.01	3.53 ± 0.02	4.00 ± 0.01	0.76 ± 0.02	Subtalocentric
15	0.87 ± 0.01	2.53 ± 0.01	3.40 ± 0.02	4.00 ± 0.01	0.74 ± 0.01	Subtalocentric
16	1.52 ± 0.01	1.87 ± 0.01	3.39 ± 0.01	4.00 ± 0.02	0.55 ± 0.02	Metacentric
17	1.23 ± 0.01	2.07 ± 0.01	3.30 ± 0.01	4.00 ± 0.02	0.63 ± 0.01	Subtalocentric
18	0.86 ± 0.01	2.46 ± 0.01	3.33 ± 0.02	4.00 ± 0.01	0.74 ± 0.02	Submetacentric
19	0.75 ± 0.01	2.42 ± 0.01	3.18 ± 0.02	4.00 ± 0.02	0.76 ± 0.02	Submetacentric
20	1.03 ± 0.02	2.12 ± 0.01	3.15 ± 0.03	4.00 ± 0.01	0.67 ± 0.01	Subtalocentric
21	0.99 ± 0.01	1.76 ± 0.01	2.75 ± 0.01	3.00 ± 0.01	0.64 ± 0.01	Submetacentric
22	0.79 ± 0.01	1.74 ± 0.01	2.52 ± 0.01	3.00 ± 0.01	0.69 ± 0.02	Submetacentric
23	0.98 ± 0.01	1.43 ± 0.01	2.42 ± 0.01	3.00 ± 0.01	0.59 ± 0.02	Metacentric
24	0.82 ± 0.01	1.36 ± 0.01	2.18 ± 0.01	3.00 ± 0.01	0.62 ± 0.01	Submetacentric

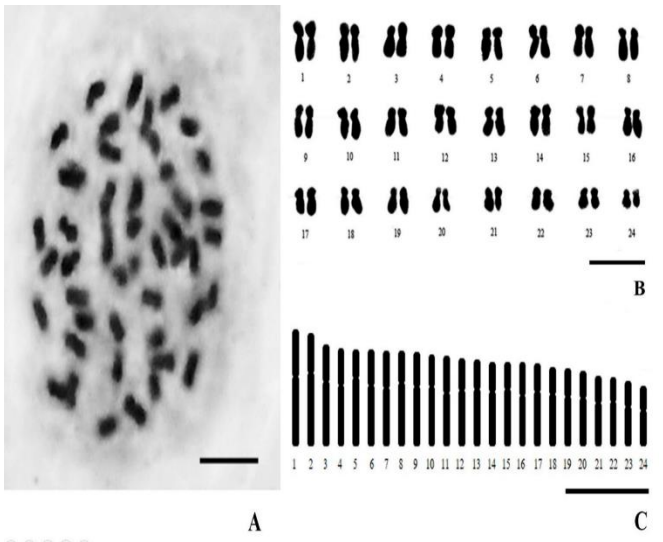

Figure 2: The chromosome of *A. macrostaminodia*. A. Somatic metaphase chromosome number showing 2*n* = 48, B. karyotype showing 46sm+2st, C. Ideogram, scale bars = 5 *μ*m

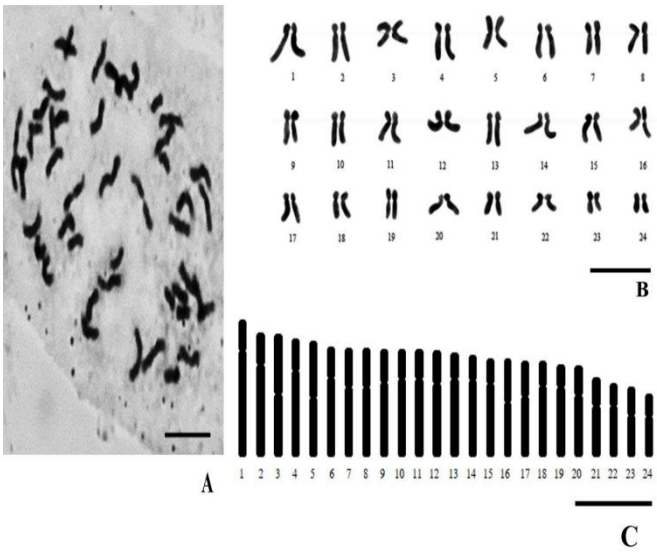

Figure 3: The chromosome of *A. latilabris*. A. Somatic metaphase chromosome number showing 2*n* = 48, B. karyotype showing 8m+18sm+22st, C. Ideogram, scale bars = 5 *μ*m

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