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RESEARCH ARTICLE

First Morphoanatomical and Cytogenetic Characterization of *Picria fel-terrae* Lour.: A Local Accession from Dairi Regency, Indonesia

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Abstract: *Poguntano* (*Picria fel-terrae* Lour.) plant has been widely recognized for its medicinal properties due to its bioactive compounds. However, fundamental biological studies, particularly on its phenological and cytogenetic characteristics, remain limited. This study aimed to characterize the vegetative morphology and karyotypic features of *P. fel-terrae* from Dairi Regency, North Sumatra, to provide a comprehensive cytogenetic database for future study and its potential applications. Morphological observations revealed that *P. fel-terrae* has ovate leaves with serrated margins (*serratus*) and anomocytic-type stomata. Chromosome analysis determined the somatic chromosome number of *P. fel-terrae* as 2n = 20. The ten chromosomal pairs varied in total length (CL), Relative Length (RL), and centromere position, resulting in diverse Centromeric Index (CI) values. Karyotype classification identified four chromosome types: metacentric, submetacentric, subtelocentric, and telocentric. The longest chromosome was 12.61 µm, while the shortest was 6.96 µm. The karyotype asymmetry index (AsK%) was 67.25%, indicating a predominantly asymmetric karyotype with chromosomes exhibiting varying centromere indices. The mean centromere index (XCI) of 0.28 suggests that *P. fel-terrae* chromosomes have relatively shorter short arms compared to their long arms. These findings provide the first cytogenetic report on *P. fel-terrae* from North Sumatra, serving as a valuable reference for further genetic, evolutionary, and conservation studies, as well as potential applications in plant breeding and medicinal research. This research showed that the morphoanatomical characteristic is similar to those in Linderniaceae: However, the cytogenetic characteristics of *Poguntano* (*Picria fel-terrae* Lour.) differ from the Linderniaceae in both the number and the types.

Keywords: Botanical Description, Image Analysis, Karyotype, *Poguntano,* Vegetative Part

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Introduction

Picria fel-terrae Lour. [syn. Curanga fel-terrae (Lour.) Merr.] is an annual herbaceous plant classified under the family Linderniaceae within the order Lamiales. This species is unique as it is the only recognized member of its genus [1]. The plant exhibits an upright growth habit typical of annual herbs and thrives in wet tropical biomes, particularly in regions with high

humidity and ample rainfall [2]. Based on its adaptability, *P. fel-terrae* is widely distributed across various geographical regions, originating from Assam and extending into parts of South China. Its natural habitat spans several countries in South and Southeast Asia, including Bangladesh, the Caroline Islands., (Kosrae), southern and southeastern China, the Eastern Himalayas, and Indonesia [3]. This extensive distribution suggests that the species possesses ecological versatility, allowing it to establish itself in diverse environmental conditions.

The plant is locally referred to as daun kukurang or pogun-/pugun-tano in Indonesia, where it has been traditionally utilized for its medicinal properties [4]. Indigenous knowledge has long recognized the therapeutic potential of *P. fel-terrae*, and in recent years, scientific research has provided empirical support for its pharmacological applications. The growing interest in this plant is evident in the increasing number of studies examining its bioactive compounds and medicinal properties, particularly those derived from accessions found in North Sumatra. Various studies have reported a broad spectrum of pharmacological activities associated with this species, including antibacterial [5], antidiuretic [6], antidiabetic [7], anthelmintic [8], antioxidant [9], cholesterol-lowering [10], cytotoxic [11, 12], hepatoprotective, immunomodulatory [13], and teratogenic effects [14]. These findings indicate that *P. fel-terrae* has a promising future for development as a medicinal plant, further increasing the demand for its sustainable cultivation and large-scale propagation [15].

The utilization of *P. fel-terrae* as traditional medicine is particularly centered in North Sumatra, where the plant has become an integral component of local ethnomedicine [16]. Among the various accessions studied, those originating from Dairi Regency have attracted considerable attention from researchers and practitioners. Dairi Regency is an ecologically and administratively important region that shares borders with Karo, Samosir, Pakpak Barat, and Southeast Aceh. These surrounding areas may contribute to the genetic diversity of local accessions of puguntano, making them an essential focus for conservation and further research. Understanding the botanical characteristics of this species is crucial for elucidating its biological properties and optimizing its pharmacological applications [17]. As a monotypic genus, *P. fel-terrae* holds a unique taxonomic status, presenting an opportunity for cytogenetic studies that could provide deeper insights into its chromosomal characteristics. Until now, there is no report of the chromosomal characteristics of *P. fel-terrae*. Despite its medicinal importance, its comprehensive morphological, anatomical and the cytogenetical descriptions of the species remain limited.

This study aimed to provide the first botanical description of the morpho-anatomical characteristics of *P. fel-terrae* leaves and stems, which constitute the primary plant part used in herbal medicine in the Dairi Regency. By documenting the leaf structure, venation patterns, and microscopic features, this research will contribute to the standardization of identification criteria for the species. Furthermore, karyotypic analysis will be conducted to determine the chromosomal attributes of the local Dairi accession, facilitating a more thorough understanding of its genetic identity.

Materials and Methods

Sample Collection and Cultivation

Thirty samples of *Picria fel-terrae* Lour. were collected from 6 districts, each of 5 of seedling within Dairi Regency, North Sumatra, Indonesia. The seedlings were transplanted into 20×20 cm polybags and cultivated four-weeks then the leaves and stem samplings were observed.

The Morphological Characterization

Morphological characterization was conducted on the vegetative organs of *P. fel-terrae*. Descriptive analysis was performed on the parent plants, assessing stem characteristics (shape, surface texture, growth direction, and leaf arrangement), leaf morphology (apex shape, base shape, margin type, venation pattern, color, surface texture, and thickness), as well as floral and fruit structures. Floral characteristics, including the calyx, corolla, stamens, carpels, floral formula, and floral diagram, were described based on the standard method for botanical description [18].

Anatomical Characterization

Anatomical observations were conducted on the stems and leaves of *P. fel-terrae*. Stem samples were prepared using the freehand sectioning method. Leaf anatomical analysis was performed using histological and micro technique with the paraffin embedding method [19]. The collected plant material was fixed in 50% FAA (Formalin-Acetic Acid-Alcohol) solution. To remove air bubbles from the tissues, an aspirator was used before proceeding with dehydration in a graded ethanol series, followed by infiltration in Tertiary Butyl Alcohol (TBA) solution. The samples were then embedded in paraffin blocks and

sectioned using a rotary microtome at a thickness of 6-8 µm. The sections were stained with safranin and observed under a light microscope.

Cytogenetics Analysis

The chromosomes of *P. fel-terrae* were conducted quantitively and analyses descriptively and the variations were statistically analyzed by standard deviation. The root tip cells of *P. fel-terrae* were used for cytological analysis. Actively growing root tips (1-2 cm in length) were excised and pretreated with 0.01 and 0.005 colchicine for 12 hours at room temperature to arrest mitotic division at metaphase. The root tips were then washed thoroughly with distilled water and fixed in Carnoy's solution (3:1 ethanol: glacial acetic acid) for 24 hours at 4°C. After fixation, the root tips were hydrolyzed in 1N hydrochloric acid (HCl) at 60°C for 5-10 minutes to soften the tissue. Chromosome preparations were carried out using the squash technique, in which the meristematic region of the root tips was carefully excised, macerated, and flattened onto microscope slide. The samples were stained with 5% Giemsa solution for approximately 15 minutes to enhance chromosomal contrast. Excess stain was removed by rinsing with phosphate buffer (pH 7.0), and the slides were air-dried before microscopic examination [20]. Microscopic examination was carried out using light and ARM microscopes, with karyotype visualization enhanced using IdeoKar 1.3 software and Photoshop CS3. At least 10 well-spread metaphase plates were analyzed to determine chromosome number, morphology, and karyotypic features. Karyotype analysis, including the determination of chromosome arm ratios and classification of chromosome types (metacentric, submetacentric, acrocentric, or telocentric), was conducted using IdeoKar 1.3 software [21]. Digital image processing and chromosome arrangement were refined using Adobe Photoshop CS3 to ensure clear visualization of karyotypic features.

Results and Discussion

Picria fel-terrae Lour. is a small herbaceous plant with an upright and spreading growth habit. As an annual species belonging to the Linderniaceae family, it exhibits thin, branching stems with opposite phyllotaxy (Fig. 1). The leaves are simple, consisting of a lamina and petiole, classified as an incomplete leaf (Flos incompletus) (Fig. 2). The leaf shape is ovate to elliptical (Ovatus or Ellipticus), with an acuminate apex (Acutus), a rounded or obtuse base (Obtusus), and a pinnate venation pattern (Penninervis), where the primary vein runs along the midrib with secondary veins branching towards the margins. The margin is crenate (Crenatus), characterized by shallow, rounded teeth along the edges.

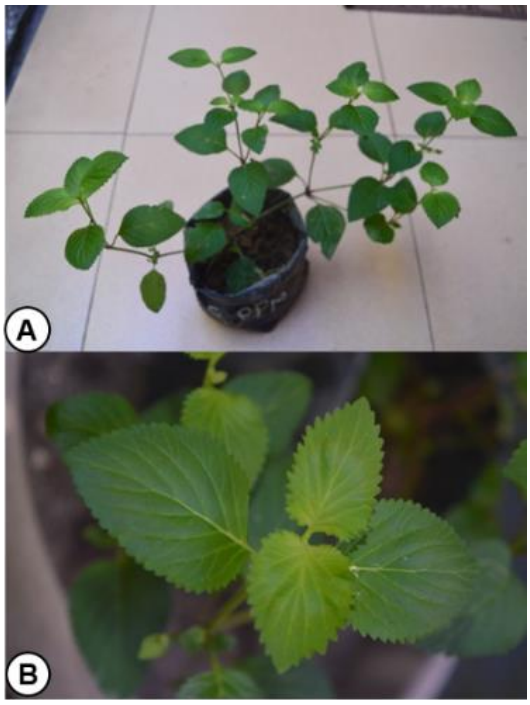

Fig. 1: Plant material of *Picria fel-terrae* Lour. from Dairi Regency cultivated at Laboratory Plat Physiology, Universitas Sumatera Utara. (A), Close-up view of growth habits and phyllotaxy

Fig. 2: Leaf characteristics of *Picria fel-terrae* Lour. from Dairi Regency, North Sumatra, through upper and lower view

The leaf texture is papery (Papyraceus or Chartaceus), and the surface is pubescent (Pilosus), covered with fine hairs. The leaves are arranged in an opposite phyllotaxy (Folio Opposita or Decussata), emerging in pairs at each node, and display a green coloration. The stem is angular (Angularis), with a quadrangular cross-section, and has a pubescent surface (Pilosus), covered with fine hairs. It grows in an ascending orientation (Ascendens), slanting upward, and exhibits a sympodial branching pattern, where lateral branches continue the plant's growth rather than a dominant central stem. The plant bears numerous flowers, making it a multiflora species (Planta multiflora), with scattered flowers (Flores sparsi). The flowers are complete or perfect (Flos completes), possessing both male and female reproductive structures. The calyx is spur-shaped (Calcaratus), forming a tubular extension at the base, while the corolla is bilabiate (Labiatus), with a distinct two-lipped structure. The flower contains two stamens, attached within the corolla tube.

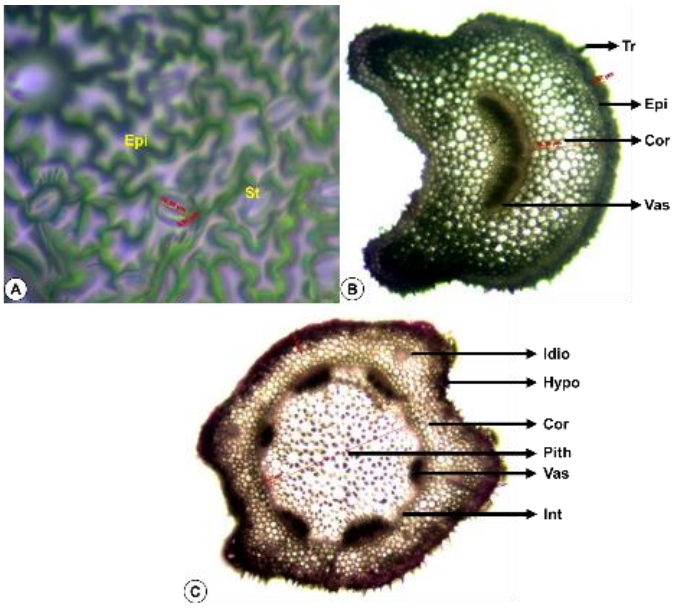

Fig. 3: Anatomical feature of *Picria fel-terrae* showing (A) stomata at 400× magnification, (B) transversal section of petiole, and (C) transversal section of stem at 100× magnification. Note: Epi = Epidermis, Idio = Idioblast, St = Stomata, Tr = Trichome, Cor = Cortex, Vas = Vascular bundle, Int = Intravascular

The fruit is a capsule, a dry, dehiscent structure, which splits open when mature. It has an ovate and flattened shape, measuring approximately 3-4 mm in length. Each capsule contains numerous round seeds, approximately 0.6 mm in diameter, which exhibit eight oval depressions, giving them a pitted appearance. The plant typically produces short, axillary, and terminal racemes, where flowers and fruits develop in clusters along the stems. The anatomical analysis of *Picria fel-terrae* reveals distinctive features in the stomatal structure, petiole, and stem cross-sections (Fig. 3).

The epidermal tissue, observed under a light microscope, displays an undulating pattern of epidermal cells with stomata distributed among them. The stomatal type is classified as anisocytic, characterized by three surrounding subsidiary cells, one of which is distinctly smaller than the others. Anisocytic stomata, commonly observed in certain dicotyledonous plants and succulents, are characterized by guard cells surrounded by three subsidiary cells of unequal size [22]. Their developmental ontogeny differs from anomocytic stomata, which follow an agenous pathway, whereas anisocytic stomata develop through a hemimesogenous process [23]. This stomatal arrangement plays a crucial role in regulating gaseous exchange and transpiration.

The transverse section of the petiole exhibits a concave-convex structure, with a well-developed epidermis forming the outermost layer. Below the epidermis, collenchyma cells provide mechanical support, particularly in the ridges.Collenchyma provides flexibility, allowing plant stems to bend without breaking, especially when exposed to wind or physical pressure. The vascular bundles are arranged in an arc-like pattern, embedded within the fundamental parenchymatous tissue. The vascular system consists of xylem and phloem, with the xylem positioned centrally and the phloem towards the periphery. The petiole also contains trichomes on its outer surface, which may contribute to reducing water loss and deterring herbivory.

The transverse section of the stem reveals a quadrangular outline with prominent ridges.The transport vessels (xylem and phloem) in a rectangular stem tend to be organized along the corners, allowing for more efficient distribution of water and nutrients to the leaves and other organs. The outermost layer comprises a single-layered epidermis covered with trichomes. Beneath the epidermis, a well-differentiated hypodermis, composed of collenchyma cells, provides structural reinforcement. The cortex consists of parenchymatous cells that serve as a storage tissue. The vascular bundles are arranged in a ring, with well-developed xylem and phloem. The xylem occupies the inner portion of the vascular bundles, facilitating water conduction, while the phloem lies towards the outer region, functioning in nutrient translocation. The central portion of the stem is occupied by a prominent pith composed of loosely packed parenchymatous cells, which assist in storage and contribute to the overall lightweight nature of the stem.

The cytogenetic analysis was conducted using colchicine as an inducer and Giemsa as a staining agent, employing two different concentrations (Fig. 4). Observations revealed that at a higher colchicine concentration (0.10%), chromosome aggregation was more pronounced, whereas a lower concentration (0.05%) resulted in better chromosome dispersion. Based on these findings, images from lower colchicine concentration were preferred due to clearer chromosome dispersion (Fig. 4).

These chromosome pairs exhibit variations in total Chromosome Length (CL), Relative Length (RL), and chromosome type. Additionally, the centromere sizes differ, leading to variations in the Centromere Index (CI) among chromosomes (Table 1). The chromosomal measurements revealed a distinct size range within the complement, in which the longest chromosome was measured at 12.61 µm and the shortest was recorded at 6.96 µm. Chromosome 7 has the greatest total Length (L), whereas chromosome 6 is the shortest. However, chromosome 3 has the longest short arm (S), while chromosomes 9 and 10 are the shortest, exhibiting a Centromere Index (CI) of zero and lacking measurable values for short arm length (S), Length Ratio (AR), and r-value.

Chromosome size variation may result from differences in mitotic stages. During mitosis, chromosome size and volume vary due to chromatin condensation and structural modifications [21]. The length ratio index serves as a valuable tool for identifying chromosome variation among individuals and species, contributing to inheritance pattern analysis and the detection of chromosomal abnormalities.

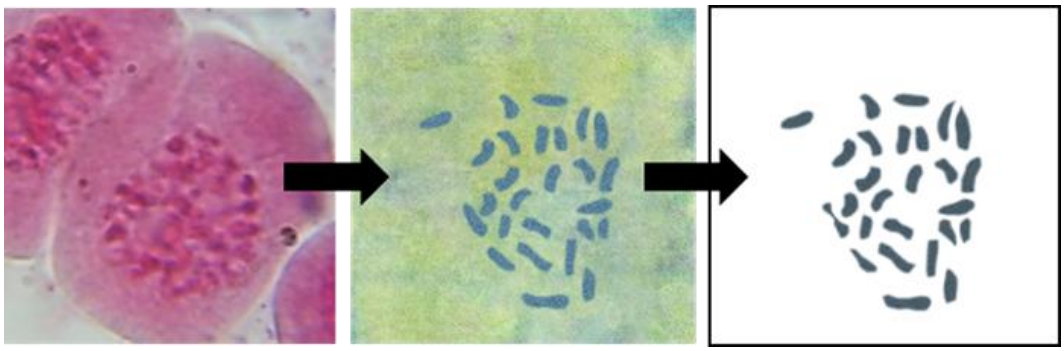

Fig. 4: Image analysis and improved resolution of bulk chromosome of *Picria fel-terrae* into disperse unit(s). Magnification at 4,000× with enhanced image using Ideokar 1.3

Table 1: Karyotypic characteristics of *Picria fel-terrae* chromosomes from Dairi accession. All chromosome length measurements (L, S, and CL) are presented in micrometers (µm)

Chromosome	L	S	CL	AR	*r*-value	%RL	%F	CI	Shape
1	5.12±0.12	3.54±0.14	8.67±0.17	1.46±0.10	0.68±0.08	10.49±0.18	4.29±0.14	0.41±0.05	Metacentric
2	4.86±0.16	1.18±0.08	6.04±0.14	4.5±0.15	0.22±0.04	7.31±0.16	1.43±0.10	0.19±0.05	Subtelocentric
3	6.83±0.23	5.78±0.18	12.61±0.21	1.18±0.08	0.84±0.10	15.26±0.21	6.99±0.14	0.45±0.05	Metacentric
4	4.99±0.14	3.67±0.17	8.67±0.17	1.35±0.10	0.73±0.08	10.49±0.18	4.45±0.15	0.42±0.05	Metacentric
5	6.6±0.15	4.33±0.13	10.38±0.18	1.43±0.10	0.69±0.08	12.55±0.20	5.24±0.14	0.42±0.05	Metacentric
6	4.73±0.13	1.57±0.12	6.3±0.20	3±0.15	0.33±0.05	7.63±0.15	1.9±0.10	0.25±0.05	Submetacentric
7	6.96±0.16	5.25±0.15	12.21±0.21	1.3±0.10	0.76±0.04	14.78±0.20	6.35±0.15	0.42±0.05	Metacentric
8	6.04±0.14	1.7±0.10	7.75±0.15	3.83±0.15	0.26±0.04	9.37±0.15	2.06±0.10	0.21±0.05	Metacentric
9	4.73±0.13	0	4.73±0.13	0	0	5.72±0.12	0	0	Telocentric
10	5.25±0.15	0	5.25±0.15	0	0	6.35±0.15	0	0	Telocentric

Note: L = long arm length (µm); S = short arm length (µm); CL = total chromosome length (µm); AR = arm ratio (L/S); r-value = ratio of short arm to total chromosome length (S/CL); %RL = relative length percentage calculated from (CL/ total haploid complement length) × 100; %F = percentage of the short arm relative to the total chromosome length; CI = centromeric index calculated as S / (L + S)

Not all karyotypes conform to an asymmetric classification, particularly those exhibiting tiny holocentric chromosomes, often measuring less than 1 µm. A karyotype is categorized as symmetric when the Long arm (L) and Short arm (S) show minimal disparity or when the centromere is positioned centrally, resulting in S = 0 [24].

The centromere index is a key parameter for determining centromere position relative to total chromosome length. It is defined as the ratio of the chromosome fragment length from the centromere to the telomere to the total chromosome length, multiplied by 100%. Based on the centromere index, chromosomes are classified as telocentric (d-value = 10, r value = ∞), subtelocentric (d value = 5-10, r value = 3-∞), metacentric (d value 0, r value 1), or submetacentric (d value 0-5, r value 1-3). The chromosomes shapes were gained directly from software Ideokar 1.3. The findings of this study indicate that the karyotype of *P. fel-terrae* is predominantly metacentric. According to [25], the centromere index is calculated by dividing the longest chromosome arm by the total chromosome length, with metacentric chromosomes yielding a ratio of 0.5 and telocentric chromosomes reaching a maximum of 1.0.

Figure 5 presents the karyotypic characteristics of *Picria fel-terrae*, detailing chromosome morphology and organization. Fig. 5A displays chromosomes classified into different categories based on centromeric position: Metacentric (M), Submetacentric (SM), Subtelocentric (ST), and Telocentric (T). These classifications are essential for understanding chromosomal structure and variations in karyotypic evolution. Fig. 5B illustrates the karyogram, where chromosomes are arranged in homologous pairs, numbered in descending order of size. This visualization aids in cytogenetic studies by facilitating the identification of chromosomal features and structural variations in different accessions of *Picria fel-terrae* in the future.

Karyotype asymmetry was analyzed using IdeoKar 1.3 software, revealing a TF% (Total Form Percentage) value of 32.75%, an AsK% (Asymmetry Index) of 67.24%, an XCI (X-chromosome centromere index) of 0.28, and an HCL (Highest Chromosome Length) of 82.64. The TF% represents the asymmetry index of the karyotype and is calculated by dividing the total Short arm length (S) by the total chromosome length (L + S), multiplied by 100% [26]. In this study, a TF% of 32.75% confirms the asymmetric nature of the *P. fel-terrae* karyotype. The HCL value (82.64) further supports this asymmetry, indicating that the Long chromosome arms (L) are dominant compared to the Short arms (S).

The AsK% index measures the extent of karyotype asymmetry by calculating the proportion of long-arm chromosome length relative to total chromosome length [20]. A higher AsK% value signifies a more asymmetric karyotype. The *P. fel-terrae*

karyotype exhibited an AsK% of 67.24%, indicating significant asymmetry, with dominant long-arm chromosomes. The XCI index, which represents the average centromere index across haploid chromosomes [27], was found to be 0.28, indicating a lower proportion of short arms relative to long arms in *P. fel-terrae*. Karyotype analysis and its components serve as valuable tools for understanding karyoevolution and species relationships within a taxonomic group. Centromere position and relative chromosome size play crucial roles in determining karyotypic symmetry and asymmetry. Although the TF% value provides insight into chromosome arm length distribution, it does not account for variations in chromosome arm length ratios or individual chromosome characteristics [26]. Here, in species-level karyotype studies, these characteristics remain essential for assessing chromosome affinity and evolutionary relationships.

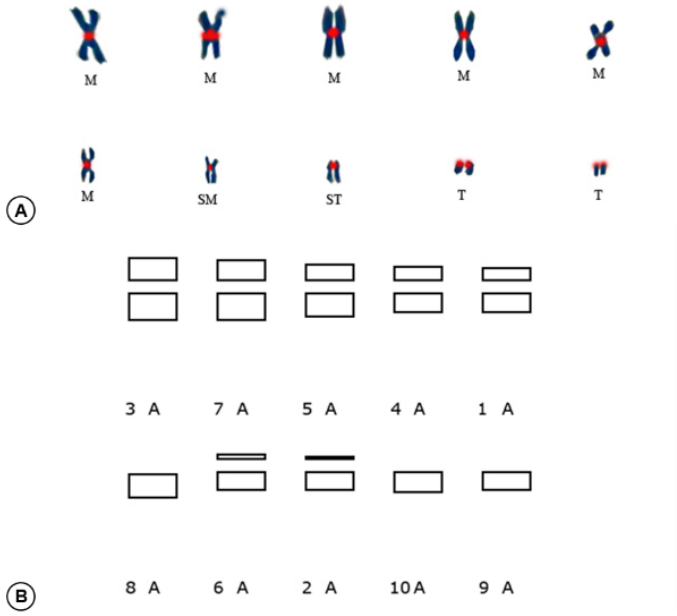

Fig. 5: Karyotypic analysis of *Picria fel-terrae* chromosomes. (A) Representation of chromosome morphology, categorized as metacentric (M), submetacentric (SM), subtelocentric (ST), and telocentric (T) based on centromere position. (B) Schematic karyogram illustrating chromosome pairs, numbered according to decreasing chromosome length, with homologous pairs aligned

IdeoKar has been increasingly applied across a range of plant cytogenetic studies to generate accurate karyometric datasets and standardized ideograms [28]. Its use has been documented in several species, demonstrating its utility for interpreting chromosomal structure and supporting taxonomic assessments. In Hippeastrum papilio, IdeoKar was employed to quantify chromosome length variation and centromere position when comparing diploid and autotetraploid cytotypes, enabling the detection of genome duplication effects on chromosomal morphology [29]. In Iranian Allium species, particularly those belonging to section Acanthoprason, IdeoKar facilitated the extraction of chromosomal measurements and visualization of karyotype patterns, contributing to refined species delimitation within the group [30]. Similarly, in cytogenetic analyses of Beta vulgaris varietal lines, IdeoKar was used to evaluate karyotype symmetry indices, relative chromosome length, and to support the classification of varietal cytotypes [31]. Although the software has been applied in various plant taxa, its use remains more widespread in animal cytogenetic studies. The knowledge and observation of the chromosomes showed differences in number as well as the type compared to those member of Linderniaceae family.

Conclusion

The *Picria fel-terrae* accession from Dairi Regency is reported here for the first time based on an integrated examination of its vegetative morpho-anatomy and karyotype features, providing a clear baseline for its cytogenetic characteristics as a local North Sumatran accession. The dataset established through this work offers a reliable biological reference that can support future studies requiring precise accession identification, including investigations of genetic diversity, population structure, and conservation status. The detailed chromosomal measurements and karyotype composition further create opportunities for subsequent cytogenomic studies, especially those exploring genome organization or informing efforts to

improve propagation and domestication. While this study does not extend into breeding or pharmacological development, the morphological and cytogenetic information presented here forms a critical starting point for future research related to breeding, conservation, and genomic exploration of *P. fel-terrae*.

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Author's Contributions

Elimasni: Conceptualization, data curation, formal analysis, funding acquisition, investigation, supervision.
Deny Supriharti: Resources, software, validation, writing original draft.
Haryati: Project administration, writing reviewing and edited.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript, and no ethical issue is involved.

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