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Genetic Diversity of the Endemic Species *Phanera sirindhorniae* in the Mekong Basin of Thailand Based on ISSR Markers

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Abstract

Phanera sirindhorniae, an endemic species to the Mekong Basin's upper northeastern region in Thailand, is notable for its therapeutic properties in alleviating pain and discomfort. The objective of this study was to assess the genetic diversity of *P. sirindhorniae* derived from four natural sources, specifically Sakon Nakhon, Nakhon Phanom, Bueng Kan and Mukdahan provinces, utilizing Inter-Simple Sequence Repeat (ISSR) markers. A total of 51 samples were analyzed, and 10 primers exhibited distinct, robust amplified fragments and polymorphism. The amplified fragment sizes ranged from 170 to 1,300 base pairs, identifying a total of 113 amplified fragments, of which 107 (94.69 %) displayed polymorphism. Polymorphic information content (PIC) values spanned between 0.112 and 0.429, while Nei's gene diversity ranged from 0.2088 ± 0.2184 to 0.2994 ± 0.1912. Assessment of genetic diversity within and among populations revealed the total genetic diversity (Ht = 0.3443 ± 0.0225) and the genetic diversity within populations (Hs = 0.2617 ± 0.0161). The variability among populations (Gst = 0.2401) indicated a significant degree of gene flow (Nm = 1.580). The Analysis of Molecular Variance (AMOVA) results supported this, with 74 % of genetic differentiation occurring within populations and 26 % among populations. Using the Neighbor-Joining (NJ) generated from Nei's genetic distance-based clustering analysis, Dendrogram construction successfully classified 2 distinct groups. The analysis revealed that *P. sirindhorniae* from Nakhon Phanom, and Bueng Kan constituted 1 group, whereas *P. sirindhorniae* from Mukdahan, and Sakon Nakhon formed another. These findings provide essential data for developing genetic conservation initiatives for this valuable genetic resource.

Keywords: Genetic diversity, ISSR, Mekong Basin of Thailand, *Phanera sirindhorniae*

Introduction

Phanera sirindhorniae K. & S. S. Larsen, Nord. J.Bot (Synonym: *Bauhinia sirindhorniae*), a dicotyledonous plant of the Fabaceae family is an endemic tendril-bearing liana native to the Mekong Basin's upper northeastern region of Thailand. Typically, this plant thrives in direct sunlight and open-air conditions, growing along the edges of dry evergreen or mixed forests [1]. *P. sirindhorniae* flowers have been designated as the official provincial flower by the authorities of Bueng Kan Province. Beyond its ornamental value, *P. sirindhorniae* possesses medicinal properties; Thai practitioners have traditionally used infusions made from boiled leaves, stems and roots to alleviate muscular pain, maintain the nervous system and prevent hypertension and diabetes [2,3].

P. sirindhorniae contains 18 significant constituents in its stems and roots, including various types of cyanoglucosides, flavans, flavanones, flavanonols, flavones, chalcones, chromones, chromone glucosides, triterpenoids, steroid glucosides, phenolic compounds and lignan glycosides. These glycosides exhibit strong antioxidant activity [4]. Additionally, the crude extract from *P. sirindhorniae* roots, stems, leaves and flowers demonstrate substantial antimicrobial activity against pathogens such as *Aeromonas hydrophila* [5], *Bacillus subtilis* [1], *Staphylococcus aureus* [1] and *Streptococcus agalactiae* [6].

A primary conservation goal is to preserve species' evolutionary potential in the face of environmental changes, which necessitates accurate genetic structure analysis and understanding of genetic diversity distribution patterns [7-11]. DNA markers have become an indispensable tool for investigating genetic relationships among plants through the analysis of DNA sequences and allele compositions. Inter-simple Sequence Repeat (ISSR) is a PCR-based DNA marker technique that uses a single primer containing core sequences of simple sequence repeats (SSR), with selective nucleotides located at the 3' or 5' end into the

non-repeat adjacent regions. This design enables the amplification of DNA fragments between closely proximate SSRs, facilitating the examination of polymorphisms at multiple locations rapidly, easily and affordably [12-14]. ISSR techniques have been applied to studies of various endemic plant species within the Fabaceae family, such as *Astragalus cyclophyllon* [15], *Hedysarum theinum* [16] and *Humboldtia brunonis* [17], as well as other endemic plant species like *Hertia cheirifolia* [18], *Rhabdosciadium aucheri* [19] and *Rhynchostylis retusa* [20].

In 2015, based on an assessment by the Herbarium Office using the 2001 IUCN criteria, *P. sirindhorniae* was categorized as an endangered plant [21,22]. This classification highlights the potential decline in the plant's genetic diversity and underscores the importance of its preservation. Molecular markers have thus been employed to examine genetic diversity among *P. sirindhorniae* populations, aiming to support conservation efforts and inform the management of *P. sirindhorniae*'s diversity for sustained viability.

Materials and methods

Plant assessment and collection

Young leaves of *P. sirindhorniae* were collected from wild populations, ensuring a minimum distance of 0.5 km between sampled plants in 4 distinct areas. A total of 51 samples were obtained, comprising 13 samples from Sakon Nakhon Province's Phu Phan National Park, 10 samples from Mukdahan Province's Dong Bang Yi Arboretum, 13 samples from Phu Langka National Park in Nakhon Phanom Province and 15 samples from Bueng Kan Province's Phu Wua Wildlife Sanctuary (**Table 1** and **Figure 1**).

Table 1 The locations of the *P. sirindhorniae* genotypes that were collected in the Mekong Basin of Thailand.

Area	Latitude	Longitude	Altitude (m)	Mean annual rainfall (mm)	Mean annual temperature (℃)
Sakon Nakhon (Phuphan)	16°50'42.23"N	103°54'58.16"E	200 - 567	1,213.0	25.25
Mukdahan (Dongbangaee)	16°24'15.25"N	104°34'35.40" E	160	1,587.0	26.90
Nakhon Phanom (Phu Langka)	17°59'5.28"N	104°08'24.15"E	563	2,178.1	25.50
Bueng Kan (Phuwua)	18°14'47.26"N	103°57'41.99"E	160 - 448	1,544.6	26.00

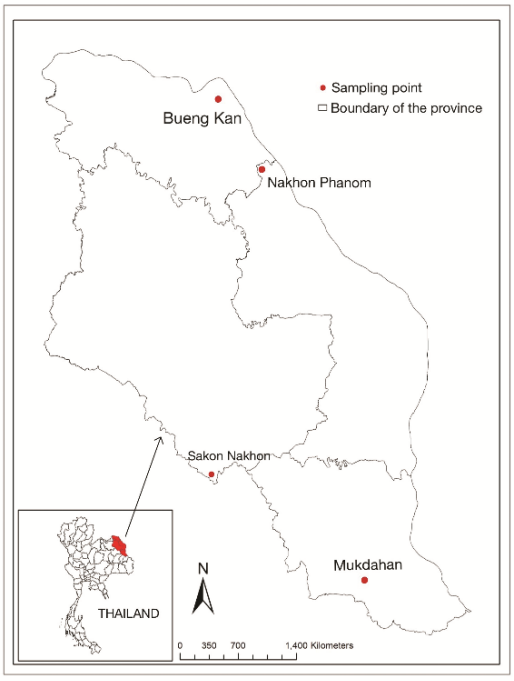

Figure 1 The collecting locations for the *P. sirindhorniae* L. accessions.

DNA isolation

Following Doyle and Doyle [23] methodology, DNA was extracted from *P. Sirindhorniae*'s young leaves using the cetyltrimethylammonium bromide (CTAB) method. Notably, no liquid nitrogen was used when the leaves were crushed into a buffer. A spectrophotometer (Genova nano 737-501, Jenway, United Kingdom) was then used to measure the concentration and purity of the extracted DNA. Using a 1X TBE buffer concentration and an agarose gel with a 1 % concentration, the isolated DNA was then electrophoresed and stained with ethidium bromide. A 100 bp DNA ladder was used as a standard marker to view the electrophoresed gel under ultraviolet light after electrophoresis (**Figure 2**).

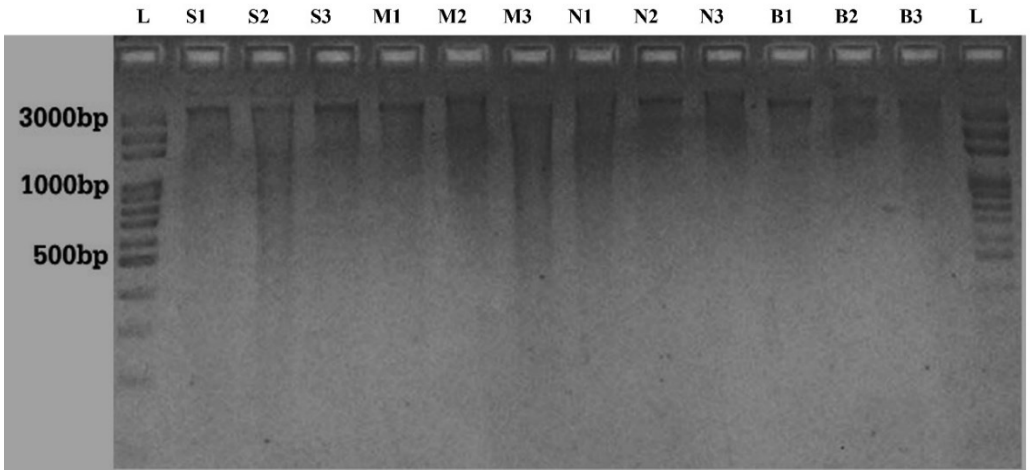

Figure 2 Evaluation of DNA quality utilizing 1 % (w/v) agarose gels in a 1 % Tris-Borate-EDTA (TBE) buffer. Lane L = standard DNA ladder, S = Sakon Nakorn, M = Mukdahan, N = Nakhon Phanom and B = Bueng Kan.

ISSR amplification

In this study, cross-genera transferability primers were identified, derived from the Bauhinia 13 ISSR [24] **(Table 2)**. The PCR reaction involved mixing 10 ng of pooled DNA (10 ng/mL DNA (20 µL) from all samples used as the template for the PCR reaction. The reaction mixture was made up of 0.5 ng of ISSR Primer, 1X HOT FIREPol® Blend Master Mix (Solis Biodyne, Estonia), and 10 ng of pooled DNA. The reaction mixture's final volume was adjusted to 10 µL with DI water. The solution was subjected to PCR (G-storm, GS482, England) under the following PCR conditions: Step 1: Pre-Denature at 95 °C for 15 min for 1 cycle, Step 2: 40 cycles of denature step at 95 °C for 20 s. The annealing step can be selected at 50 - 60 °C for 30 s and the extension step at 72 °C for 30 s. The final extension step was performed at 72 °C for 5 min for 1 cycle. By electrophoresis on a 2 % agarose gel with a 1x concentration of TBE buffer and ethidium bromide dye, the resultant DNA products were examined. The gel was then inspected with a common 100 bp DNA ladder under ultraviolet light. For further examination, the primers that could amplify DNA and produce a clear, robust band were chosen.

Table 2 Primers employed to evaluate genetic variation among 4 *P. sirindhorniae* populations including Polymorphism information content (PIC), Resolving power (RP).

Primer	Sequence	Annealing Temp (°C)	Size of PCR band (bp)	Number of DNA bands			PIC	RP
				Total	Monomorphic	Polymorphic		
1ISPH	$(AG)_8T$	50	170 - 900	10	0	10	0.429	10.706
2ISPH	$(AG)_8G$	60	210 - 550	9	4	5	0.112	16.549
3ISPH	$(GA)_8T$	50	300 - 800	9	0	9	0.359	12.784
4ISPH	$(CA)_8A$	55	200 - 900	11	1	10	0.397	14.000
5ISPH	$(CA)_8G$	55	200 - 950	13	1	12	0.439	14.392
6ISPH	$(TC)_8C$	50	370 - 600	4	0	4	0.245	5.647

Primer	Sequence	Annealing Temp (℃)	Size of PCR band (bp)	Number of DNA bands			PIC	RP
				Total	Monomorphic	Polymorphic		
7ISPH	$(AG)_8YT$	50	220 - 1,300	17	0	17	0.403	16.039
8ISPH	$(AG)_8YC$	50	180 - 790	14	0	14	0.445	16.392
9ISPH	$(AG)_8YA$	55	210 - 900	15	0	15	0.437	14.235
11ISPH	$(CT)_8RC$	55	220 - 700	11	0	11	0.397	14.745

Data analysis

A binary system was employed to represent the collected data comprehensively, assigning a value of 1 for band presence and 0 for band absence. The Polymorphism Information Content (PIC) was calculated to evaluate the efficacy of each ISSR marker using the formula: $PIC = 2fi\ (1 - fi)$, where fi represents the frequency of the amplified allele for a locus, and $1 - fi$ represents the null allele's frequency [25]. Additionally, the resolving power (Rp) of each marker was assessed using the formula $Rp = \sum Ib$, where $Ib = (1-2*(0.5-p))$, and p refers to the proportion of individuals exhibiting a band, indicating the ISSR markers' ability to differentiate among the population [26]. To evaluate the effectiveness of each allele position, the Observed number of alleles (Na), effective number of alleles (Ne), genetic diversity (Nei's gene diversity, h) and Shannon's information index (I) were calculated using Popgene version 1.32 [27]. Using Analysis of Molecular Variation (AMOVA), the ISSR patterns were examined to find variation between and within the 4 studied groups. While using the GEN ALEX version 6.5 program, Principal Coordinate Analysis (PCoA) was used to evaluate the relationships between groups [28]. Utilizing MEGA 11, a Neighbor-Joining (NJ) dendrogram was created utilizing the matrix of Nei's genetic distance.

Results and discussion

ISSR polymorphism

The sustenance of genetic variation is a prerequisite for both the survival of populations and the evolutionary progression of species [29,30]. Although conventional phenotypic traits have been utilized to evaluate plant diversity, their inconsistent expression across varying environmental conditions may constrain their effectiveness. To address this limitation, DNA markers have been employed to investigate genetic diversity in the Bauhinia species, including RAPD [29], ISSR [31] and SSR [32], among others. This study represents the inaugural investigation into the natural population relationships of *P. sirindhorniae* in Thailand. By examining ISSR microsatellites, which disclose information regarding repetitive base sequences, the genetic relationships among *P. sirindhorniae* populations were elucidated, offering insights into the conservation management of this rare endemic plant species in the Mekong River Basin.

In the present research, 13 transferability primers were assessed to ascertain their capacity to generate distinct and robust bands. The findings revealed that 10 primers, specifically primers 1, 2, 3, 4, 5, 6, 7, 8, 9 and 11, yielded 113 amplified fragments with sizes ranging from 170 to 1,300 base pairs. Among these, 107 fragments were identified as polymorphic, constituting 94.69 % of the total. Primer 7 exhibited the highest polymorphism percentage at 94.69 %, generating approximately 17 amplified fragments ranging from 220 to 1,300 base pairs in size. Conversely, Primer 6 displayed the lowest polymorphism percentage at 3.54 %, producing a mere 4 amplified fragments within the range of 300 to 800 base pairs in size. The polymorphic information content (PIC) values of the primers spanned from 0.112 to 0.445, with an average value of 0.366. The agarose gel electrophoresis images depicting the PCR fragments were displayed (**Figure 3**). Additionally, the resolving power fluctuated from 5.647 for Primer 6 to 16.549 for Primer 2, with an average resolving power of 13.55 (**Table 2**).

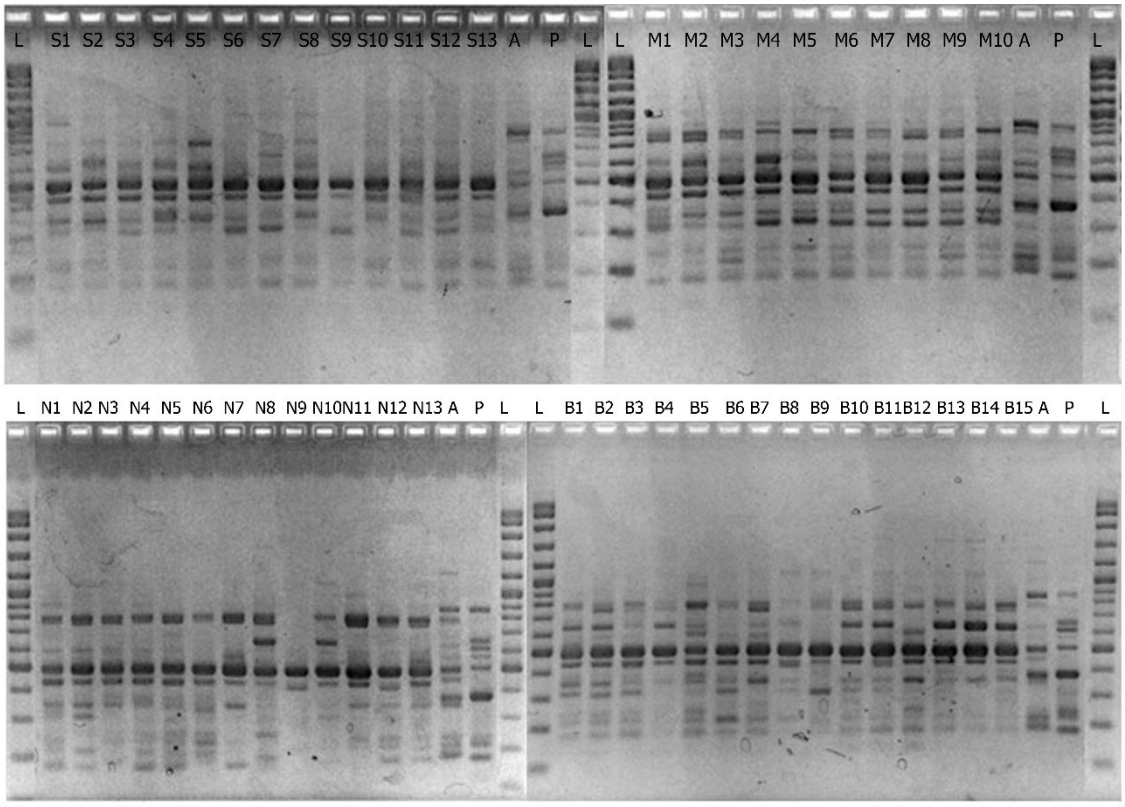

Figure 3 51 samples of *P. sirindhorniae* were amplified by PCR using the 8ISPH [(AG)8YC] Primer**.** Lane L = Standard DNA ladder, S = Sakon Nakorn, M = Mukdahan, N = Nakhon Phanom, B = Bueng Kan and A = *Phanera aureifolia* and *Phanera penicilliloba*.

Generally, endemic plant species exhibit less genetic variation than their more widespread counterparts. The geographical distribution of endemic plants is restricted to specific regions, allowing them to adapt to the unique characteristics of their respective habitats [34,35]. In a study exploring the diversity of *P. sirindhorniae*, Inter-Simple Sequence Repeats (ISSR) were employed as the dominant marker. The Polymorphic Information Content (PIC) values ranged between 0 and 0.5 [36]. This investigation reported a mean PIC value of 0.366, signifying a moderate to high degree of polymorphism. A higher PIC value implies an increased likelihood that 2 randomly selected samples will exhibit substantial differences [37].

The Rp value was utilized to assess the capacity of the ISSR marker to differentiate between organisms [26,38]. For *P. sirindhorniae*, the mean Rp value was 13.55, demonstrating consistency between the PIC and Rp values. Comparable genetic diversity was observed for *P. sirindhorniae* at the species level, with moderate genetic diversity (I = 0.513, h = 0.346). This is analogous to other species, such as *Satureja rechingeri* (I = 0.52, h = 0.35) [39], *Bauhinia variegata* (I = 0.5611) [32] and *Dalbergia nigra* (I = 0.53, h = 0.35) [40].

Genetic diversity examination *of P. sirindhorniae*

This investigation sought to explore the genetic diversity of *P. sirindhorniae* populations across 4 distinct regions, namely Mukdahan, Sakon Nakhon, Nakhon Phanom and Bueng Kan. The percentage of alleles present in polymorphic loci differed among populations, with the lowest observed in Mukdahan at 53.98 % and the highest in Nakhon Phanom at 80.53 %. The observed number of alleles (Na) ranged from 1.540 to 1.805, with an average of 1.708, while the effective number of alleles (Ne) varied from 1.377 to 1.529, with an average of 1.462. The Shannon's information index (I) ranged from 0.304 to 0.441 and Nei's gene diversity (h) ranged from 0.209 to 0.300. The Mukdahan population exhibited the lowest values for these indices (I = 0.304 and h = 0.209), whereas Nakhon Phanom demonstrated the highest genetic diversity (I = 0.441 and h = 0.300). Furthermore, increased genetic diversity was observed at the species level (I = 0.531 and h = 0.346) (**Table 3**).

Table 3 Genetic diversity comparison among 4 of *P. sirindhorniae* accessions based on ISSR markers, including number of accessions of alleles (Na), number of effective alleles (Ne), Nei's genetic diversity (h) and Shannon's information of polymorphic loci index (I).

Population name	Na	Ne	h	I
Sakon Nakhon	1.6991 ± 0.4607	1.4184 ± 0.3894	0.2405 ± 0.2025	0.3587 ± 0.2826
Mukdahan	1.5398 ± 0.5006	1.3766 ± 0.4151	0.2088 ± 0.2184	0.3044 ± 0.3079
Nakhon Phanom	1.8053 ± 0.3977	1.5286 ± 0.3789	0.2994 ± 0.1912	0.4413 ± 0.2622
Bueng Kan	1.7876 ± 0.4108	1.5235 ± 0.3717	0.2979 ± 0.1920	0.4378 ± 0.2669

The cumulative genetic diversity of *P. sirindhorniae* populations was assessed to be 0.344 ± 0.023 (Ht) at the population level and 0.262 ± 0.016 (Hs) within the population. The variability among populations (Gst) was determined to be 0.240 (**Table 4**), in line with the AMOVA analysis, indicating that 26 % of the variance was attributable to genetic differences between populations (PhiPt = 0.2568, $p < 0.001$), while the remaining 74 % was ascribed to intra-population variance (**Table 5**). The gene transfer (Nm) across populations was 1.580 (**Table 4**). The ISSR analysis outcomes revealed significant genetic divergence among the populations with a Gst value of 0.5, signifying an equal distribution of genetic diversity both among and within populations [41,42]. A moderate degree of genetic differentiation was observed among the 4 *P. sirindhorniae*, as evidenced by a Gst of 0.24. Nm and Gst serve as crucial parameters for determining the genetic structure of a single species' population. Furthermore, an Nm value exceeding 1 implies that populations maintain genetic connections over time, while an Nm value below 1 signifies genetic divergence among populations [43,44]. An Nm value greater than 1 indicates the persistence of genetic connections between populations over time, while a value below 1 denotes genetic divergence among populations [35]. An Nm of 1.580 signifies considerable genetic exchange among the populations of this specific species, which mitigates the effects of genetic drift and minimizes genetic differentiation among populations on their structure. Gene flow is generally attributed to pollen dispersal and geographical factors [45,46]. *P. sirindhorniae*, a climbing vine species can freely grow along the treetops at the edge of the Sakon Nakhon basin, situated on the Khorat Plateau and separated by the Phu Phan Uplift [47]. Consequently, *P. sirindhorniae* may be distributed along the mountain ranges of Phu Phan (Sakon Nakhon), Phu Langka (Nakhon Phanom) and Phu Wua (Bueng Kan). Alternatively, human-mediated gene transfer could contribute to its distribution, as *P. sirindhorniae* is a medicinal plant with analgesic properties, commonly cultivated and propagated during migration. Moreover, a comprehensive examination of the relationships among populations was conducted through principal coordinate analysis (PCoA), accounting for 46.47 % of the total variation. The initial principal coordinate (PCo1) was taken into account for 19.20 % of the total variance, while the subsequent principal coordinates, PCo2 and PCo3, contributed 18.11 % and 8.96 % to the variance, respectively (**Figure 4**). The Principal Coordinate Analysis (PCoA) effectively depicted the interrelationships among populations, producing results that aligned with the Neighbor-Joining (NJ) dendrogram generated from Nei's genetic distance-based clustering analysis (**Figure 5**). Both of these analyses exhibited similar distribution patterns, suggesting that *P. sirindhorniae* populations from Nakhon Phanom and Bueng Kan provinces formed a discrete group, distinct from the populations in Mukdahan and Sakon Nakhon provinces. At the regional scale, the highest degree of similarity (0.9098) was observed between Nakhon Phanom and Bueng Kan, whereas the lowest degree of similarity (0.8141) was detected between Mukdahan and Nakhon Phanom. This was determined through the analysis of Nei's unbiased genetic identity in the pairwise population matrix (**Table 6**), with values spanned from 0.8 to 0.9. Indices exceeding 0.8 suggest a limited genetic base [48].

Table 4 Nei's analysis of genetic diversity of 4 populations.

Ht	Hs	Gst	Nm
0.3443 ± 0.0225[a]	0.2617 ± 0.0161[b]	0.2401	1.580

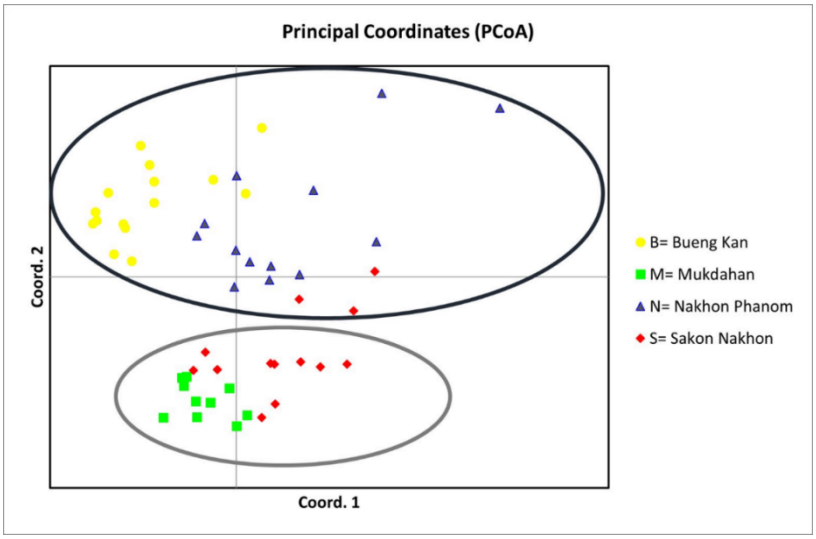

Figure 4 Principal Coordinate Analysis of *P. sirindhorniae* populations.

Table 5 AMOVA data for *P. sirindhorniae* accessions, including sum of squares (SS), mean squares (MS), estimated variations (Est. Var), percentage of genetic variation (%).

Source	df	SS	MS	Est. Var.	%	PhiPT	P (rand ≥ data)
Among pops	3	240.857	80.286	5.159	26		
Within pops	47	701.849	14.933	14.933	74		
Total	50	942.706		20.092	100	0.2568	0.001

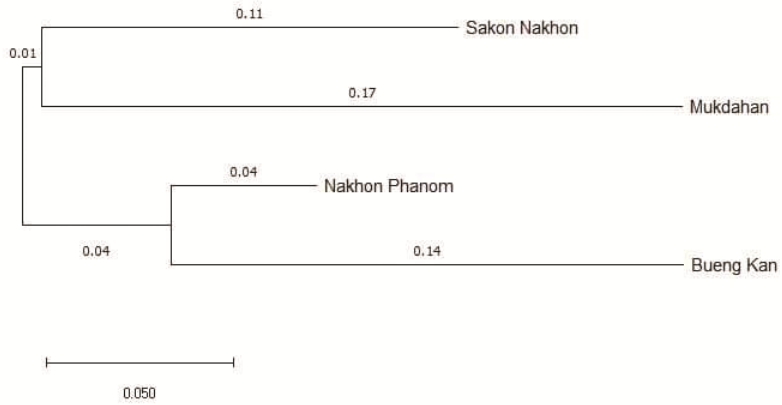

Figure 5 Neighbor-joining (NJ) dendrogram based on Nei's genetic distance.

Table 6 Nei's (1978) unbiased measures of genetic distance (below diagonal) and identity (above diagonal) between four populations of *P. sirindhorniae*.

Population	Sakon Nakhon	Mukdahan	Nakhon Phanom	Bueng Kan
Sakon Nakhon		0.8654	0.9043	0.8711
Mukdahan	0.1445		0.8141	0.8230
Nakhon Phanom	0.1006	0.2056	-	0.9098
Bueng Kan	0.1380	0.1948	0.0944	-

Conclusions

An examination of the genetic diversity of *P. sirindhorniae* across 4 populations in the upper northeastern region of Thailand, employing Inter Simple Sequence Repeat (ISSR) analysis. The population structure analysis identified 2 distinct groups: Group 1, encompassing populations from, Nakhon Phanom, and Bueng Kan provinces, and Group 2, comprising populations from Mukdahan, and Sakon Nakhon province. The populations from Bueng Kan and Nakhon Phanom were determined to be the most genetically similar. To further elucidate *P. Sirindhorniae*'s genetic diversity, it is recommended that DNA barcode sequencing be conducted in future studies. This approach would yield more comprehensive information, which could prove advantageous for conservation management initiatives.

Acknowledgements

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References

[1] S Athikomkulchai, N Sriubolmas and N Ruangrungsi. Antibacterial activity of flavonoids from *Bauhinia sirindhorniae. Thai J. Health Res*. 2005; **19**, 13-9.

[2] K Larsen and SS Larsen. *Bauhinia sirindhorniae* sp. nov. *(Leguminosa Caesalpinioideae)* a remarkable new species from Thailand. *Nord. J. Bot.* 1997; **17**, 113-8.

[3] P Chaisri and N Laoprom. Antioxidant properties and total phenolic content of selected traditional Thai medicinal plants. *Thai Pharmaceut. Health Sci. J.* 2017; **12**, 10-8.

[4] A Sirivan, R Nijsiri and S Toshikazu. Chemical constituents of *Bauhinia sirindhorniae. Herb. Med. J.* 2003; **57**, 150-3.

[5] N Nithikulworawong. Antibacterial activity of *Bauhinia sirindhorniae* extract against *Aeromonas hydrophila* isolated from hybrid catfish. *Walailak J. Sci. Tech.* 2012; **9**, 195-9.

[6] N Nithikulworawong. Efficacy of *Bauhinia sirindhorniae* on resistance to against *Streptococcus agalactiae* in Nile tilapia *(Oreochromis niloticus). Asia Pac. J. Sci. Tech.* 2012; **17**, 715-24.

[7] B Milligan, J Leebens-Mack and A Strand. Conservation genetics: Beyond the maintenance of marker diversity. *Mol. Ecol*. 1994; **3**, 423-35.

[8] MH Mills and MW Schwartz. Rare plants at the extremes of distribution*:* broadly and narrowly distributed rare species. *Biodiversity Conservat.* 2005; **14**, 1401-20.

[9] S Tomasello, I Álvarez, P Vargas and C Oberprieler. Is the extremely rare Iberian endemic plant species Castrilanthemum debeauxii (Compositae, Anthemideae) a 'living fossil'? Evidence from a multi-locus species tree reconstruction. *Mol. Phylogenet. Evol.* 2015; **82**, 118-30.

[10] N Chachai, B Pensuriya, T Pinsuntiae, P Pratubkong, J Mungngam, P Nitmee, P Kaewsri, S Wongsatchanan, R Jindajia, P Triboun and J Sreesaeng. Variability of morphological and agronomical characteristics of *Centella asiatica* in Thailand. *Trends Sci.* 2021; **18**, 502.

[11] M Kamali, D Samsampour,A Bagheri, A Mehrafarin and A Homaei. Association analysis and evaluation of genetic diversity of *Teucrium stocksianum* Boiss. populations using ISSR markers. *Genet. Resour. Crop Evol.* 2023; **70**, 691-709.

[12] O Pakhrou, L Medraoui, C Yatrib, M Alami, A Filali-Maltouf and B Belkadi. Assessment of genetic diversity and population structure of an endemic Moroccan tree (*Argania spinosa* L.) based in IRAP and ISSR markers and implications for conservation. *Physiol. Mol. Biol. Plant*. 2017; **23**, 651-61.

[13] A El-Moneim, EIS ELsarag, S Aloufi, AM El-Azraq, SM ALshamrani, FAA Safhi and AA Ibrahim. Quinoa (*Chenopodium quinoa* Willd.): Genetic diversity according to ISSR and SCoT markers, relative gene expression, and morpho-physiological variation under salinity stress. *Plants* 2021; **10**, 2802.

[14] M Yaman. Determination of genetic diversity in european cranberrybush (*Viburnum opulus* L.) genotypes based on morphological, phytochemical and ISSR markers. *Genet. Resour. Crop Evol.* 2022; **69**, 1889-99

[15] F Aliabadi, A Bagheri, S Abbasi, H Saeidi and FR Blattner. High genetic diversity in an endemic and vulnerable species: Evidence from *Astragalus cyclophyllon* (Fabaceae) in Iran. *Genet. Resour. Crop Evol.* 2023; **70**, 1999-2008.
[16] NS Zvyagina and OV Dorogina. Genetic differentiation of Altai-Sayan endemic *Hedysarum theinum* Krasnob. (Fabaceae) evaluated by inter-simple sequence repeat analysis. *Russ. J. Genet*. 2013; **49**, 1030-5.
[17] SA Dev, M Shenoy and RM Borges. Genetic and clonal diversity of the endemic ant-plant *Humboldtia brunonis* (Fabaceae) in the Western Ghats of India. *J. Biosci.* 2010; **35**, 267-79.
[18] N Rahali, I Yangui, M Boussaid and C Messaoud. Assessment of genetic diversity and population structure of the endemic *Hertia cheirifolia* (L.) Kuntze based on ISSR and SRAP molecular markers. *Biologia* 2022; **77**, 3429-39.
[19] F Kazemeini, Y Asri G Mostafavi, R Kalvandi and I Mehregan. Assessment of genetic diversity, population structure and morphological analyses in an Iranian endemic species *Rhabdosciadium aucheri* Boiss. (Apiaceae) using ISSR markers. *Biologia* 2021; **76**, 441-51.
[20] A Lal, M Pant, G Palni, LMS Kumar, A Kumar and G Kumar. ISSR marker assisted genetic diversity assessment in natural populations of two endemic orchids *Aerides multiflora* and *Rhynchostylis retusa* from Uttarakhand, India. *S. Afr. J. Bot*. 2023; **157**, 151-8.
[21] W Jamjumroon, N Tesna and S Sangrit. *Threatened plants of Phu Wua-Phu Langka forest.* Omega Printing, Bangkok, Thailand, 2015, p. 52.
[22] N Supuntee, W Jamjumroon and N Tesna. Threatened plants of Phu Wua-Phu Langka forest. *In*: Proceedings of the Academic Conference and Academic Presentations, Thailand Forest Ecology Research Network: Ecology Knowledge for Sustainable Management, Phitsanulok, Thailand. 2015, p. 270.
[23] JJ Doyle and JL Doyle. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull*. 1987; **19**, 11-5
[24] CP Lau, L Ramsden and RM Saunders. Hybrid origin of "*Bauhinia blakeana*" (Leguminosae: Caesalpinioideae*)*, inferred using morphological, reproductive, and molecular data. *Am. J. Bot.* 2005; **92**, 525-33.
[25] D Botstein, RL White, M Skolnick and RW Davis. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 1980; **32,** 314-31.
[26] A Prevost and MJ Wilkinson. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet.* 1999; **98**, 107-12.
[27] FC Yen. Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgian J. Bot*. 1997; **129**, 157-63.
[28] R Peakall and PE Smouse. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 2012; **28**, 2537-9.
[29] R Frankham, JD Ballou and DA Briscoe. *Introduction to conservation genetics*. Cambridge University Press, Cambridge, United Kingdom, 2002.
[30] FV Rossum and OJ Hardy. Guidelines for genetic monitoring of translocated plant populations. *Conservat. Biol.* 2022; **36**, e13670.
[31] Y Chen, C Tang, H Li, L Qian, S Luo and X Zhou. Analysis of affinity of *Bauhinia variegata* germplasm resources based on ISSR markers. *Southwest Chin. J. Agr. Sci.* 2020; **33**, 26-31.
[32] S Sraphet, P Sukawutthiya, N Srisawad, DR Smith and K Triwitayakorn. Evaluation of the genetic diversity of *Bauhinia winitii*, an endemic plant of Thailand, using microsatellite markers. *Philippine J. Sci*. 2021; **150**, 557-69.
[33] SCH Barrett and JR Kohn. *Genetic and evolutionary consequences of small population size in plant: Implications for conservation. In*: DA Falk and KE Holsinger (Eds.). Genetic and conservation of rare plants. Oxford University Press, Oxford, United Kingdom, 1991, p. 3-30.
[34] TM Faske, AC Agneray, JP Jahner, LM Sheta, EA Leger and TL Parchman. Genomic and common garden approaches yield complementary results for quantifying environmental drivers of local adaptation in rubber rabbitbrush, a foundational Great Basin shrub. *Evol. Appl*. 2021; **14**, 2881-900.
[35] M Cai, Y Wen, K Uchiyama, Y Onuma and Y Tsumura. Population genetic diversity and structure of ancient tree populations of *Cryptomeria japonica* var. sinensis based on RAD-seq data. *Forests* 2020; **11**, 1192.
[36] CML Serrote, LRS Reiniger, KB Silva, SMDS Rabaiolli and CM Stefanel. Determining the polymorphism information content of a molecular marker. *Gene* 2020; **726**, 144175.
[37] W Powell, GC Machray and J Provan. Polymorphism revealed by simple sequence repeats. *Trends Plant Sci.* 1996; **1**, 215-22.

[38] RRA Giachino. Investigation of the genetic variation of anise (*Pimpinella anisum* L.) using RAPD and ISSR markers. *Genet. Resour. Crop Evol.* 2020; **67**, 763-80.
[39] J Hadian, A Karami, A Azizi and A Khadivi-Khub. Ubiquitous genetic diversity among and within wild populations of *Satureja rechingeri* assessed with ISSR markers. *Plant Syst. Evol.* 2015; **301**, 923-30.
[40] ALD Silva Júnior, RLR Cabral, L Sartori, FDD Miranda, MVW Caldeira, SO Moreira, TD Oliveira Godinho and FSD Oliveira. Molecular markers applied to the genetic characterization of *Dalbergia nigra*: Implications for conservation and management. *Trees* 2022; **36**, 1539-57
[41] L Liao, QS Guo, ZY Wang, L Liu and ZB Zhu. Genetic diversity analysis of Prunella vulgaris in China using ISSR and SRAP markers. *Biochem. Syst. Ecol.* 2012; **45**, 209-17.
[42] S Saboori, Z Noormohammadi, M Sheidai and S Marashi. SCoT molecular markers and genetic fingerprinting of date palm (*Phoenix dactylifera* L.) cultivars. *Genet. Resour. Crop Evol.* 2020; **67**, 73-82.
[43] SG Jian, T Tang, Y Zhong and SH Shi. Conservation genetics of *Heritiera littoralis* (Sterculiaceae), a threatened mangrove in China, based on AFLP and ISSR markers. *Biochem. Syst. Ecol.* 2010; **38**, 924-30.
[44] BR Subedee, GR Tripathi, NN Munankarmi and RP Chaudhary. Genetic diversity in populations of *Girardinia diversifolia* from Nepal Himalaya using ISSR markers. *Ecol. Genet. Genom.* 2022; **23**, e100120.
[45] E Pons, A Navarro, P Ollitrault and L Pena. Pollen competition as a reproductive isolation barrier represses transgene flow between compatible and co-flowering citrus genotypes. *PLoS One* 2022; **6**, e25810
[46] FM Sereshkeh, A Azizi and A Noroozisharaf. Structure of genetic diversity among and within populations of the endemic Iranian plant *Dracocephalum kotschyi*. *Horticult. Environ. Biotechnol.* 2019; **60**, 767-77.
[47] S Li-jian and S Nuchit. The characteristics, formation and exploration progress of the potash deposits on the Khorat Plateau, Thailand and Laos, Southeast Asia. *Chin. Geol*. 2020; **3**, 67-82
[48] AK Bishoyi, VV Pillai, KA Geetha and S Maiti. Assessment of genetic diversity in *Clitoria ternatea* populations from different parts of India by RAPD and ISSR markers. *Genet. Resour. Crop Evol.* 2014; **61**, 1597-609.