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Inter-primer binding site (iPBS) markers reveal the population genetic diversity and structure of tropical climbing *Cissampelopsis* (Asteraceae) in Thailand

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Abstract. *Vanijajiva O, Pornpongrungrueng P. 2020. Inter-primer binding site (iPBS) markers reveal the population genetic diversity and structure of tropical climbing* Cissampelopsis *(Asteraceae) in Thailand. Biodiversitas 21: 3919-3928. Cissampelopsis* is a small climbing tropical Asian genus of Asteraceae-Senecioneae. In Thailand, the genus is represented by two species, *C. corifolia* and *C. Volubilis*, distributed through the mountain evergreen forest. Study on the genetic diversity and structure of populations of both *Cissampelopsis* species provide better understanding of the biology and pattern of species diversification in the genus. To identify the genetic diversity, we used the inter-primer binding site (iPBS) retrotransposon system, in 96 accessions of *Cissampelopsis* species collected from different regions in Thailand. A total of 120 iPBS bands were scored as presence⁄ absence characters. Results from UPGMA and PCoA analyses indicated that *C. corifolia* and *C. volubilis* are different species. Genetic diversity and genetic differentiation among and within populations of *C. volubilis* is higher than *C. corifolia*. Molecular Variance (AMOVA) analysis of both species indicated that the genetic variance value within populations is higher than among populations of each species. Bayesian model-based STRUCTURE analysis detected two gene pools for both *Cissampelopsis* and showed admixture within individuals. Differences among the two *Cissampelopsis* species, in total diversities and levels of population differentiation, indicated that the genetic structure of *Cissampelopsis* populations are congruent with long-lived perennial habit with regional distribution, even for congeneric species, may vary considerably. This study suggests the effectiveness of the iPBS marker system to estimate the population genetic diversity and structure of *Cissampelopsis* genotypes.

Keywords: *Cissampelopsis*, iPBS, genetic diversity, population structure, Thailand

INTRODUCTION

Climbing plants are a significant component of the forest vegetation in tropical areas, and illustrates a notable capability to inhabit and continue in an extensive kind of habitats (Gallagher and Leishman 2012; Estrada-Villegas et al. 2020). The abundance of these plants in a certain habitat depends on several factors, such as light, soil moisture, and nutrients. In tropical forest ecosystems, climbing species have the ability to rapidly colonize treefall openings, compete with trees and suppress tree succession for many years in these gaps and these taxa are habitually highly species-rich over time (Odell et al. 2019; Addo-Fordjour et al. 2020). Although the abundance of climber plants in some ecosystems, their ecology was regularly unobserved in community studies. Probably due to the difficulty of habitat assessment to obtaining the number of individuals, making the measurement uncertain and disfavoring analysis of population structure. According to the different growth strategies of climbing species, the maintaining populations strategies may be different from other habitual plants which can rely more on vegetative propagation (Lau et al. 2009)

Cissampelopsis (DC.) Lem. ex Lindl. is a small scandent perennial genus of Asteraceae-Senecioneae. It comprises about 10 species distributed predominantly in tropical Asia from South Asia eastwards through East Asia and Southeast Asia (Koyama et al. 2016; Li and Ren 2018). Its member is usually distinguished from other Asian genera in Senecioneae by its scandent habit, climbing by means of prehensile petioles. Additional characters aiding to describe the genus include: numerous ovate or triangulate, and unlobed leaves, distributed evenly along branches, commonly large axillary and terminal corymbose synflorescences, composed of abundant discoid or radiate capitula, and caudate anthers with long tails (Li and Ren 2018). The species are naturally found climbing on shrubs or small trees on the margin of mixed deciduous and evergreen montane forests at altitudes up to ca 2,470 m (Koyama et al. 2016).

In Thailand, genus *Cissampelopsis* contains two extant species, *C. corifolia* C. Jeffrey & Y. L. Chen and *C. volubilis* Miq. (Koyama et al. 2016), both of which have a narrow distribution confined to mountain evergreen forest from 1,000 to 2,500 altitudinal meters (Table 1). The species *C. corifolia*, is restricted to Northern in Chiang Mai province, where it is presently known only from two natural populations, i.e., one in the Doi Chiang Dao mountain (CD), and another population in Doi Intanon mountain. Whereas, *C. volubilis* is currently found from

two wild populations limited to Eastern Thailand in Nakhon Ratchasima province at Khao Yai National Park (KY) and South-western Thailand in Phetchaburi province at Pa Noen Tung mountain of Kaeng Krachan National Park (KK). Studies about genetic diversity, reproduction system, and ecological adaptations in *Cissampelopsis* are still unknown. Based on latest revision (Vanijajiva and Kadereit 2008), the provision of conservation assessments of *Cissampelopsis* is necessary. Therefore, study the genetic structure and diversity of *Cissampelopsis* taxa is an important step for conservation of the species, pointing out the variability within and among populations, levels of differentiation, and interrelations. Furthermore, obtaining knowledge about population genetic diversity and structure of both *Cissampelopsis* species in Thailand possibly benefit us to understand better the biology and pattern of species diversification in the genus.

The population genetic variation and structure estimation at the molecular level is available (Minn et al. 2015; Grover and Sharma 2016; Allendorf 2017; Amom and Nongdam 2017; Comes et al. 2017; Nadeem et al. 2018), for several different markers, but only a few dominant markers are most regularly used, such as Randomly Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR), and Amplified Fragment Length Polymorphism (AFLP) (Suratman et al. 2015; Grover and Sharma 2016; Al-Naggar et al. 2017; Bidyaleima et al. 2019; Amom et al. 2020). Despite that, retrotransposon marker method is one of excellent sources of efficient genetic markers (Kalendar et al. 2019; Ghonaim et al. 2020). This marker is reproducible, easy to apply, cheap, and requires basic molecular laboratory facilities. Retrotransposons are one of the most fluid genomic components, instable enormously in copy numbers over relatively short evolutionary timescale, and represent a major constituent of the structural evolution of organism genomes (Kalendar 2011; Schulman et al. 2012). In plants, Long Terminal Repeat (LTR) retrotransposons tend to be more abundant than non-LTR (Macas et al. 2011). Most of retrotransposons are nested, diverse, inverted or truncated in chromosomal sequences. Fragments of LTR with retrotransposons internal fragment are located near other retrotransposons, which permits the use of LTR sequences for PCR amplification. Locations of genome with high density of retrotransposons can be used to perceive their chance link with other retrotransposons (Kalendar et al. 2019).

Kalendar et al. (2010) established inter primer-binding sites (iPBS) retrotransposon indicator systems for eukaryotic organisms, particularly in plants. Due to this, the iPBS technique is an easy-to-use technique that requires no sequence data, cost-effective, not age or tissue-specific, highly informative, and no affected by environmental influences (Nemli et al. 2015; Amom et al. 2020). Thus, the iPBS retrotransposon technique has been selected as a marker to examine population genetic diversity and structure in many plant genera such as *Cicer* (Andeden et al. 2013), *Vitis* (Guo et al. 2014), *Pisum* (Baloch et al. 2015), *Phaseolus* (Nemli et al. 2015), *Psidium* (Mehmood et al. 2017), *Castanea*, *Fagus* and *Quercus* (Coutinho et al. 2018), *Chenopodium* (Hossein-Pour et al. 2019), *Laurus* (Karık et al. 2019), *Hordeum* (Bonchev et al. 2019), *Origanum* (Karagoz et al. 2020). Moreover, in Asteraceae iPBS method has been proved to be a reliable marker for the evaluation of genetic diversity at infraspecific level, as well as the intergeneric hybrid in several genera (Gailite and Rungis 2012; Ali et al. 2019; Bonchev and Vassilevska-Ivanova 2020). To simplify the application of molecular tools and offer a better understanding of the genetic diversity of *Cissampelopsis*, we utilized iPBS molecular markers in these species for the first time. Thus, our main objectives are to evaluate the efficiency of iPBS markers for *Cissampelopsis* species and access the genetic diversity and structure among and within four populations of *C. corifolia* and *C. volubilis* in Thailand. The results of this study can be used to facilitate the sustainable management of *Cissampelopsis*, and this methodology can be extended to other *Cissampelopsis* species.

MATERIALS AND METHODS

Plant materials and DNA extraction

In the current study, a total of 96 individuals from four known natural populations of *Cissampelopsis* in Thailand were collected in silica gel to dried the leaves (Table 1). Voucher specimens representative of all the populations sampled is stored at the Khon Kaen University Herbarium (KKU). Details on these vouchers are given in Table 1. The genomic DNA was extracted using 200 mg of dried leaves from the ground tissue following CTAB procedures (Doyle and Doyle 1990) with minor modifications (Vanijajiva 2020). The DNA was stored at -20 °C, for further use as templates for PCR amplification.

Table 1. Information on sample locations for all populations of *Cissampelopsis* from Thailand

Population code	Locality (Province)	Number	Longitude (N)	Latitude (E)	Altitude (m)	Voucher
C. corifolia						
CD	Doi Chiang Dao (Chiang Mai)	27	19°23'46"	98°53'49"	1,500-2,200	OP005-033
IN	Doi Inthanon (Chiang Mai)	25	18°35'28"	98°29'14"	2,000-2,500	OP034-060
C. volubilis						
KY	Khao Yai (Nakhon Ratchasima)	19	14°26'19"	101°24'42"	1,000-1,300	OP062-082
KK	Kaeng Krachan (Phetchaburi)	25	12°52'05"	99°22'20"	1,000-1,200	OP085-108

Table 2. Characteristics of twenty iPBS primers used in the present study

Primer	Sequence (5'-3')	Optimal annealing, Ta (°C)	Total band number	Scored band sizes (bp)	Polymorphic band number	Polymorphism percentage	Polymorphism information content value (PIC)
2081	GCAACGGCGCCA	65.0	5	250-750	5	100.00	0.462
2272	GGCTCAGATGCCA	55.0	6	200-800	6	100.00	0.392
2076	GCTCCGATGCCA	59.2	7	150-500	6	85.71	0.296
2077	CTCACGATGCCA	55.1	4	350-1,000	4	100.00	0.472
2079	AGGTGGGCGCCA	65.2	5	400-900	5	100.00	0.445
2080	CAGACGGCGCCA	63.3	6	200-750	6	100.00	0.448
2083	CTTCTAGCGCCA	54.6	5	300-1,000	5	100.00	0.379
2085	ATGCCGATACCA	52.8	7	200-2,000	7	85.71	0.484
2374	CCCAGCAAACCA	53.5	5	200-2,500	5	100.00	0.398
2378	GGTCCTCATCCA	53.0	10	200-3,000	9	90.00	0.400
2380	CAACCTGATCCA	50.5	6	200,3,000	6	100.00	0.458
2392	TAGATGGTGCCA	52.2	5	100-2,500	5	100.00	0.442
2393	TACGGTACGCCA	51.0	7	100-1,000	7	100.00	0.372
2394	GAGCCTAGGCCA	56.5	5	200-600	5	100.00	0.462
2273	GCTCATCATGCCA	56.5	6	200-900	6	100.00	0.468
2277	GGCGATGATACCA	52.0	5	200-1,600	5	100.00	0.314
2279	AATGAAAGCACCA	52.0	6	250-2,000	6	100.00	0.375
2382	TGTTGGCTTCCA	50.5	7	250-2,000	5	71.42	0.449
2389	ACATCCTTCCCA	50.0	5	250-800	5	100.00	0.395
2391	ATCTGTCAGCCA	52.6	8	150-800	6	75.00	0.398
	Total		120	1500-3,000	114	95.00	0.415

iPBS-PCR amplification

Initially, 20 iPBS primers designed by Kalendar et al. (2010) were tested on DNA samples (Table 2) and all primers were selected with high clarity and repeatability for polymorphic assessment in studied *Cissampelopsis* accessions (Table 2). DNA amplification was approved by using a modified procedure of Kalendar et al. (2010). To determine iPBS profiles, the size of each DNA band was inferred by evaluation with a 100 bp DNA ladder (Promega), used as a molecular weight marker (M). Data scoring and PCR analysis were performed three different times for each primer to approve band pattern uniformity.

Data analysis

Statistical analysis of iPBS patterns was based on the following assumptions: iPBS fragments in *Cissampelopsis* accessions perform as diploid, dominant markers with presence (amplified) or absence (non-amplified) of alleles, co-migrating fragments representing putatively homologous loci, DNA from nuclear source and biparental inheritance. Only reproducible DNA bands were designated for data analysis. The polymorphic information content (PIC) values were assessed employing the method suggested by Li et al. (2020). The agroupment analysis was run in PAST 3.14 software (Hammer et al. 2001), using Unweighted Pair-group Method with Arithmetic Average (UPGMA) and Principal Coordinate Analysis (PCoA), grouping based on the resulting similarity values of all *Cissampelopsis* accessions.

POPGENE software, version 1.32 (Yeh et al. 1999) was used to analyze the genetic diversity parameters under Hardy-Weinberg equilibrium, percentage of polymorphic bands (PPB), observed number of alleles (N_A), effective number of alleles (N_E), expected heterozygosity (H_E) and observed heterozygosity (H_O). Genetic diversity measures (H_S, average gene diversity within population; H_T, total gene diversity; G_{ST}, coefficient of gene differentiation; N_m, evaluate of gene flow) were tested using Nei's (1987) gene diversity statistics for individual population. The Analysis of molecular variance (AMOVA) was also employed to estimate the hierarchical apportionment of variation. Additionally, parameter *F*-statistic (Φ_{ST}) for describing genetic differentiation of intra-population and inter-population (Holsinger and Weir 2009) was calculated using ARLERQUIN program (Excoffier and Lischer 2010). The genetic structure was determined using model-based cluster analysis (STRUCTURE v. 2.3.4) (Hubisz et al. 2009). The number of populations (K) was estimated every 10 runs for every population, which varied from 2 to 10, characterized by a set of distinctive allele frequencies at each locus, and the individuals were sited in K clusters. Using this method, Markov chain Monte Carlo (MCMC) posterior probabilities were estimated. The MCMC chains were run with a 10,000-iteration burn-in period, followed by 100,000 iterations using a model allowing for admixture and correlated allele frequencies. The most anticipated value for K was predicted with Evanno's ΔK method (Evanno et al. 2005) using STRUCTURE HARVESTER (Earl and van Holdt 2012).

RESULTS AND DISCUSSION

iPBS polymorphisms and relationships analysis

Twenty iPBS primers were initially screened for polymorphism using one DNA accession and all 20 primers generated a PCR product with a varied number of bands. The sizes of reproducible and scorable bands ranged from 150 to 3,500 bp. The 20 iPBS primers produced 120 scorable bands and among them, 114 bands were polymorphic (Table 2). The number of scored bands per primer ranged from 5 to 10. The polymorphism percentage per primer ranged from 71.42% to 100.0% (Table 2). The PIC values ranged from 0.296 (iPBS 2076) to 0.484 (iPBS 2085), with a total mean of 0.415. These results indicate that the iPBS markers can represent a good discriminatory capacity and reveal a wide range of genomic DNA diversity in *Cissampelopsis*.

The agroupment analysis for all 96 samples of *Cissampelopsis* was clearly separated according to the species in two main clusters (Figure 1). The first cluster contained 52 accessions of *C. corifolia* and the second cluster comprised 44 taxa of *C. volubilis*. This indicates that there was a respectable differentiation between both *Cissampelopsis* species in Thailand. The PCoA results corroborated with the dendrogram, similarly indicating two distinct groups (Figure 2). However, at the population level of both species, the clusters are not well delimited. The results showed that the UPGMA and PCoA are most likely reflect a continuous genetic combination between population of each species.

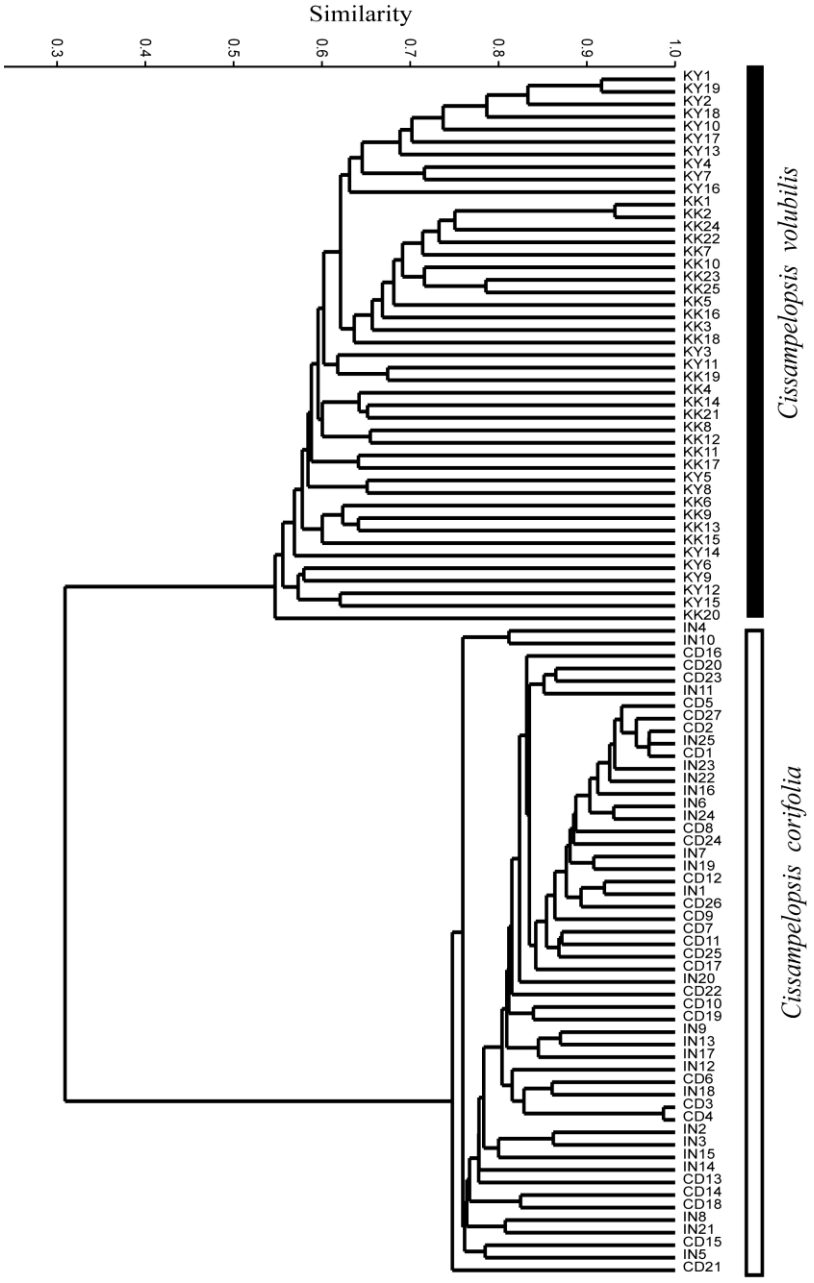

Figure 1. UPGMA cluster diagram of genetic relationships among *Cissampelopsis* accessions based on Jaccard similarity coefficients measures estimated from iPBS data of 96 *Cissampelopsis* accessions

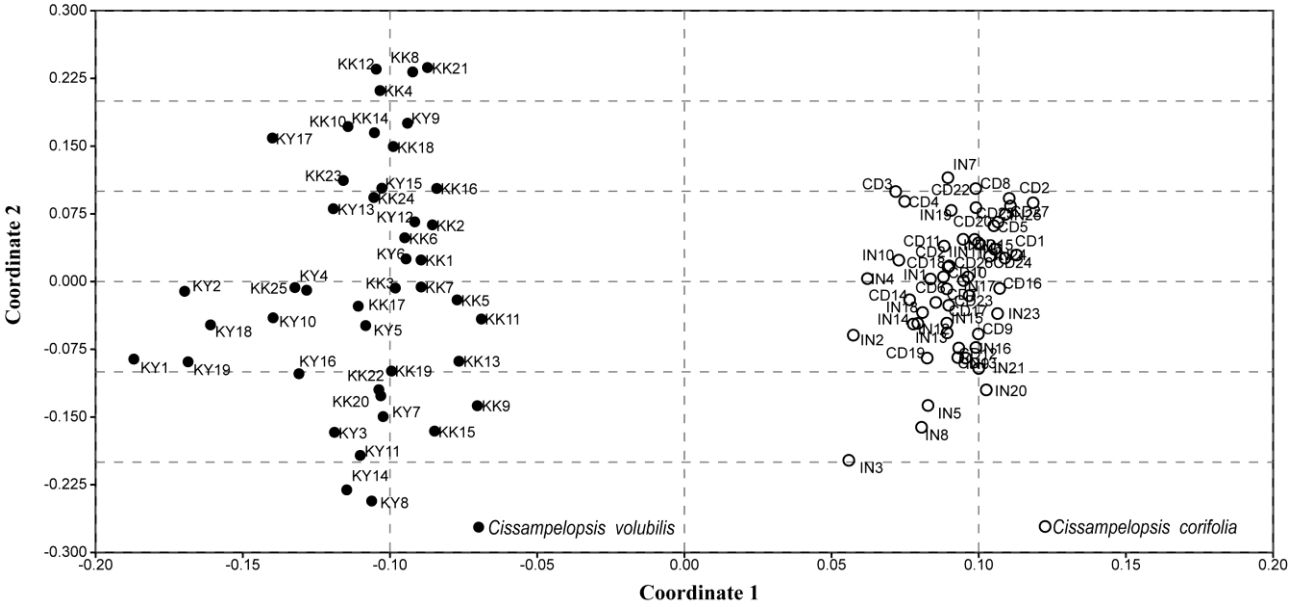

Figure 2. PCoA analysis of genetic relationships among *Cissampelopsis* accessions based on Jaccard similarity coefficients measures estimated from iPBS data of 96 *Cissampelopsis* accessions

Population genetic diversity and structure analysis

At species level, 52 accessions from the two populations of *Cissampelopsis corifolia* gave totally 120 iPBS bands from 20 primers. About 53 (44.17%) polymorphic fragments were recorded. At population level, samples of population CD generated the number of 50 (41.67%) polymorphic bands whereas IN population produced number of 47 (39.17%) polymorphic bands. Under Hardy-Weinberg equilibrium, the mean observed number of alleles (N_A) of population in CD, IN and total was 1.417, 1.392, and 1.442, respectively. The mean effective number of alleles (N_E) of CD, IN and total population was 1.164, 1.186 and 1.183, respectively. The mean genetic diversity (expected heterozygosity, H_E) values of the CD, IN and total population was 0.104, 0.115, and 0.116, respectively. The mean observed heterozygosity (H_O) values of the CD, IN and total population was 0.167, 0.179 and 0.184, respectively. The average gene diversity within populations (H_S) value of *C. corifolia* was 0.109. The total gene diversity (H_T) value of *C. corifolia* population was 0.116. The coefficient of gene differentiation (G_{ST}) value was 0.057. The estimate of gene flow (N_m) was 8.221 (Table 3).

In the STRUCTURE analysis, log probabilities of the data [lnP (D)] showed the highest likelihood at K=2. The result showed that all populations from Thailand (CD and IN) were mixed for cluster I ('red' in Figure 3) and cluster II ('green'). These populations most likely reflect a continuous genetic gradation or admixture of these neighboring groups. The clustering results by UPGMA, PCoA, and STRUCTURE at both the inter- and intraspecific levels, were highly concordant.

We further explored genetic variations within the species *C. volubilis*, a total of 120 alleles were detected with the mean polymorphic bands in KY, KK and total were 86 (71.67%), 90 (75.00%) and 91 (75.83%), respectively. The mean observed number of alleles (N_A) of population in KY, KK, and total was 1.717, 1.750 and 1.758, respectively. The mean effective number of alleles (N_E) of KY, KK, and total population was 1.432, 1.516 and 1.515. The mean genetic diversity (expected heterozygosity, H_E) values of the KY, KK, and total population was 0.248, 0.288, and 0.288, respectively. The mean observed heterozygosity (H_O) values of the KY, KK and total population was 0.371, 0.423, and 0.424, respectively. The average gene diversity within populations (H_S) value of *C. volubilis* population was 0.268. The total gene diversity (H_T) value of *C. volubilis* population was 0.286. The coefficient of gene differentiation (G_{ST}) value was 0.063. The estimate of gene flow (N_m) was 7.464 (Table 4).

A population substructure was found in the STRUCTURE analysis, log probabilities of the data [lnP (D)] clearly showed the highest likelihood at K=2. The result showed that all populations from the Eastern Thailand population (KY) were fixed for cluster I ('Yellow' in Figure 4), while cluster II ('blue') was predominant in the Southern Thailand population (KK).

The analysis of molecular variance among and within-population tested by AMOVA for both species indicated different variations based on combined iPBS markers. A lower variation among populations than within populations was similarly found for *C. corifolia* (Table 5) and *C. volubilis* (Table 6). The Φ_{ST} value was 0.071 (P <0.001) for *C. corifolia* and 0.101 (P <0.001) for *C. volubilis*, respectively. These results suggesting lower differentiation among populations within species. As only few populations from each species were studied, the relationship between genetic distances and geographical distances among populations could not be investigated.

Table 3. Summary of iPBS variation for two populations (52 individuals) of *Cissampelopsis corifolia* from Thailand

Populations	Sample size	Percentage of polymorphic bands (PPB)	Observed number of alleles (N_A)	Effective number of alleles (N_E)	Expected heterozygosity (H_E)	Observed heterozygosity (H_O)
CD	27	41.67	1.417 ± 0.495	1.164 ± 0.279	0.104 ± 0.155	0.167 ± 0.229
IN	25	39.17	1.392 ± 0.490	1.186 ± 0.304	0.115 ± 0.168	0.179 ± 0.246
Total	52	44.17	1.442 ± 0.499	1.183 ± 0.292	0.116 ± 0.162	0.184 ± 0.238

Average gene diversity within populations (H_S)	Total gene diversity (H_T)	Coefficient of gene differentiation (G_{ST})	Estimate of gene flow (N_m)
0.109 ± 0.024	0.116 ± 0.026	0.057	8.221

Table 4. Summary of iPBS variation for two populations (44 individuals) of *Cissampelopsis volubilis* from Thailand

Population	Sample size	Percentage of polymorphic bands (PPB)	Observed number of alleles (N_A)	Effective number of alleles (N_E)	Expected heterozygosity (H_E)	Observed heterozygosity (H_O)
KY	19	86 (71.67)	1.717 ± 0.452	1.432 ± 0.399	0.248 ± 0.198	0.371 ± 0.274
KJ	25	90 (75.00)	1.750 ± 0.435	1.516 ± 0.400	0.288 ± 0.201	0.423 ± 0.278
Total	44	91 (75.83)	1.758 ± 0.430	1.515 ± 0.401	0.288 ± 0.199	0.424 ± 0.274

Average gene diversity within populations (H_S)	Total gene diversity (H_T)	Coefficient of gene differentiation (G_{ST})	Estimate of gene flow (N_m)
0.268 ± 0.037	0.286 ± 0.039	0.063	7.464

Table 5. Molecular variance (AMOVAs) for iPBS variation based on two populations of *Cissampelopsis corifolia* populations sampled from Thailand

Source of variation	df	Sum of squares	Mean squares	Variance components	Percentage of variance	Fixation indices	*P*-value*
Among populations	1	22.445	22.445	0.574	7.07	Φ_{ST} = 0.071	p<0.001
Within populations	50	377.093	7.542	7.542	92.93		p<0.001
Total	51	399.538		8.116	100		

Note: df: Degree of freedom; P-value: probability of null hypothesis. *Significance tests after 1000 permutations

Discussion

Evaluation of iPBS marker

Since the development of applicable iPBS procedures, this marker has been widely used for population genetic diversity and structures analysis (Baloch et al. 2015; Nemli et al. 2015; Hossein-Pour et al. 2019; Karık et al. 2019; Karagoz et al. 2020). This is of advantage because polymorphisms detected by iPBS are expected to be less biased estimators of genetic difference than variation at the level of gene products (Kalendar et al. 2019). To our knowledge, this is the first information describing molecular diversity of *Cissampelopsis* accessions using iPBS markers. The present result shows that iPBS markers are reliable molecular tools for examining genetic variation and structure of populations in *Cissampelopsis*, as well as indicating noticeable genetic differentiation among two *Cissampelopsis* species in Thailand. A total of 120 fragments were obtained, 144 of which showed polymorphism (95%) in generic level. The mean PIC value was calculated as 0.415 ranging from 0.296 (iPBS 2076) to 0.484 (iPBS 2085) (Table 2). Usually, in dominant indicators, the highest value of PIC should be 0.5 (Chesnokov and Artemyeva 2015).

Additionally, genetic agroupment among the 96 *Cissampelopsis* individuals obtained using the UPGMA and PCoA analyses showed that populations of *C. corifolia* and *C. volubilis* tend to be genetically differentiated from each other as indicated by two major clusters which revealed a clear separation of all the *C. corifolia* accessions from *C. volubilis*. The result supports the morphological classification of *C. corifolia* and *C. volubilis* as distinct species. The UPGMA and PCoA analyses were in full agreement with those further obtained by STRUCTURE analysis, regarding the relatedness of the accessions at the intraspecific level. The genetic differentiation between the two species may have resulted from the adaptive selection to varied environmental factors, such as *C. corifolia* preferably occur in habitats with high moisture in evergreen forest whereas *C. volubilis* mostly found in the edge of mixed forests (Vanijajiva and Kadereit 2008), and geographic isolation. A similar successful phenomenon of iPBS technique was observed in several recent plant genetic studies (Kalendar et al. 2019; Amom et al. 2020). The information generated by the iPBS marker system suggests that this system can be used effectively for genetic diversity and structure studies in *Cissampelopsis* species.

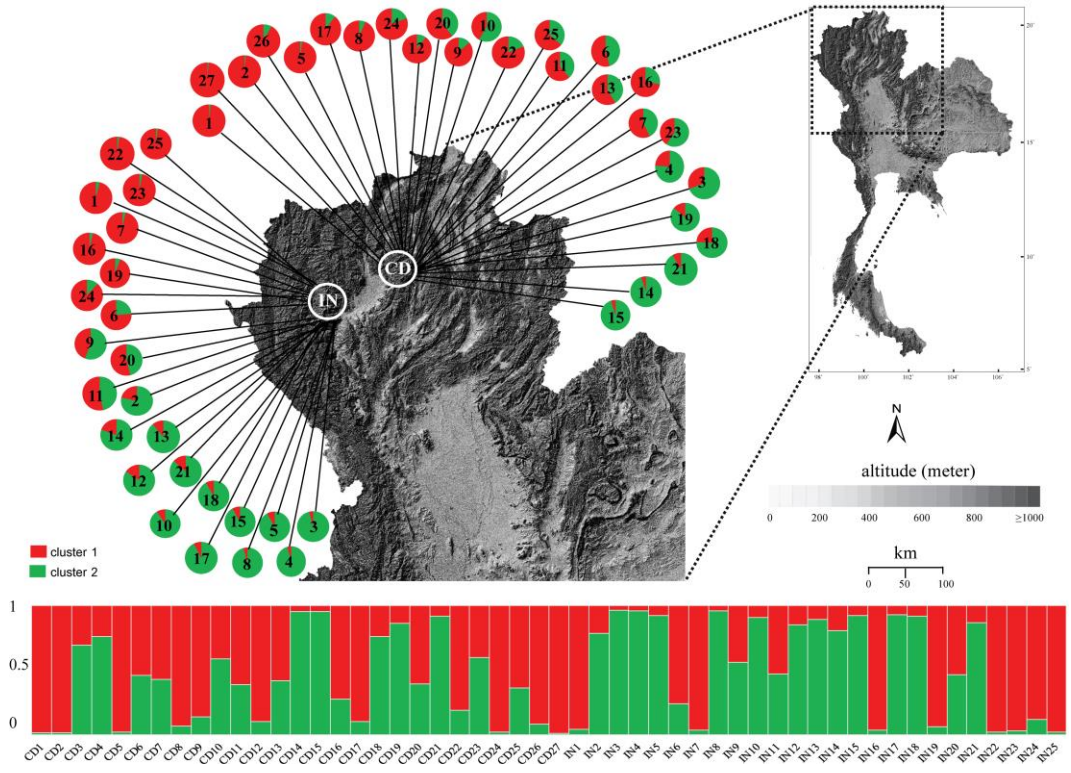

Figure 3. Distribution of the two major iPBS gene pool clusters (I and II) within and among two populations (52 individuals) of *Cissampelopsis corifolia* from Thailand as identified by STRUCTURE based on the ad hoc statistic ΔK. The bar-plot displays the assignment of individuals to the two clusters. The y-axis presents the estimated membership coefficient (Q) for each individual in the two clusters. The x-axis corresponds to population codes as identified in Table 1. The pie charts at the map represent the average proportion of cluster membership computed across individuals per site.

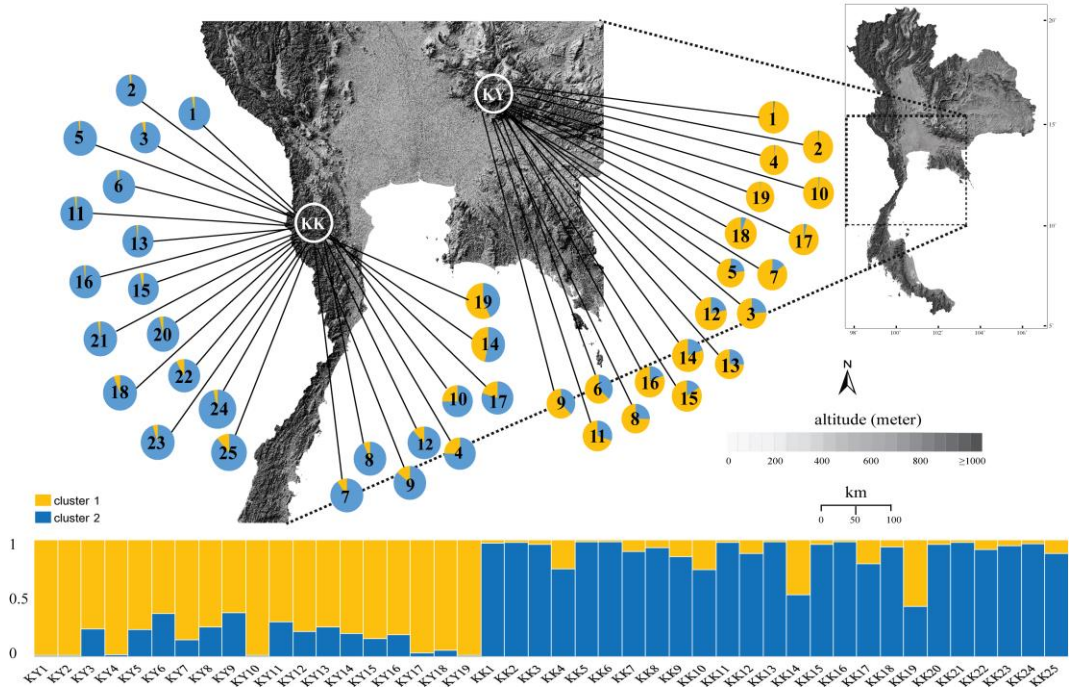

Figure 4. Distribution of the two major iPBS gene pool clusters (I and II) within and among two populations (44 individuals) of *Cissampelopsis volubilis* from Thailand as identified by STRUCTURE based on the ad hoc statistic ΔK. The bar-plot displays the assignment of individuals to the two clusters. The y-axis presents the estimated membership coefficient (Q) for each individual in the two clusters. The x-axis corresponds to population codes as identified in Table 1. The pie charts at the map represent the average proportion of cluster membership computed across individuals per site.

Table 6. Molecular variance (AMOVAs) for iPBS variation based on two populations of *Cissampelopsis volubilis* populations sampled from Thailand

Source of variation	df	Sum of squares	Mean squares	Variance components	Percentage of variance	Fixation indices	*P*-value*
Among Pops	1	59.213	59.213	1.940	10.07	Φ_{ST} = 0.101	p<0.001
Within Pops	42	727.878	17.330	17.330	89.93		p<0.001
Total	43	787.091		19.270	100		

Note: df: Degree of freedom; *P*-value: probability of null hypothesis. *Significance tests after 1000 permutations

Genetic diversity and structure within and among populations

The amount of genetic diversity within a species and its distribution within and among populations provide evidence for the maintenance of variation and gene flow (Jaros et al. 2016; Hamrick et al. 2019; Vasilyeva et al. 2020). These factors are valuable for significant functional populations such as those with members that exchange genes, for identifying potential selection areas and assessing chances for speciation, and in measurements of genetic diversity in plants. The present study is also the first DNA-level study within and among populations of *Cissampelopsis*, and establishes a baseline by which comparisons with other *Cissampelopsis* species may be made. Comparison of the level of genetic variation using iPBS markers based on PPB, N_A, and N_E indicate that *C. corifolia* (Table 3), exhibited low levels of relative genetic diversity in comparison to *C. volubilis* (Table 4). Similarly, combined value of heterozygosity on H_E, H_O, and H_T within species, presented low genetic diversity value on these heterozygosity assessments in *C. corifolia* (Table 3) in comparison to *C. volubilis* (Table 4). Within-population diversity, one of the most usually employed values to estimate its diversity is average gene diversity within populations (H_S). This study found that the mean H_S value of both species has moderate within-population. However, *C. corifolia* (Table 3) revealed lower than *C. volubilis* (Table 4). These probably due to *C. corifolia* has a narrow geographic distribution (from the Himalayas through Myanmar, China to Thailand). The species is characterized by a very few highly scattered small populations. Whereas *C. volubilis* has a broader geographic distribution (from the Himalayas through Myanmar, China, Vietnam, Thailand, Malaysia to Indonesia) but is considered by a regional distributed with small populations. As might be expected, genetic diversity of *C. Corifolia* is lower than *C. volubilis.*

Among-population diversity, genetic differentiation is frequently estimated with G_{ST} according to Nei (1987) who suggested that G_{ST} > 0.25 reflects strong genetic differentiation, whereas G_{ST} between 0.05 and 0.25 among populations indicates moderate genetic differentiation (Nei 1987; Hamrick et al. 2019). Notably, this result is insensitive to assumptions about Hardy-Weinberg equilibrium. Most of the pairwise G_{ST} values among the populations of *C. corifolia* and *C. volubilis* in this study were slightly higher than 0.05 (*C. corifolia* G_{ST} = 0.057 and *C. volubilis* G_{ST} = 0.062). Therefore, "moderate" genetic differentiation occurred among the populations of both *Cissampelopsis* species in Thailand. Likewise, AMOVA revealed an overall Φ_{ST} value is generally considered to indicate 'moderate' genetic differentiation (*C. corifolia* Φ_{ST} = 0.071 and *C. corifolia* Φ_{ST} = 0.109). The result is congruent with the outcomes of most species with outcrossing breeding systems and wind pollination (Tong et al. 2020). Moreover, the estimated number of migrants among populations of both *Cissampelopsis* species (*C. corifolia* N_m = 8.221 and *C. corifolia* N_m = 7.463) are in accordance with values presented for perennial tropical plants. Reis (1996) indicated that N_m values above 1.0 are corporate among populations of long-lived perennial tropical plants that are possibly associated with climbing species with the canopy occupation and the capacity of dispersal and propagules spread (Hamrick and Godt 1996). Furthermore, AMOVA results of all the two *Cissampelopsis* species showed that the major portion, 92.93% in *C. corifolia* and 89.83% in *C. volubilis*, of genetic variance, is residing within populations while only 7.07% in *C. corifolia* and 10.07% in *C. volubilis* of genetic variance is residing among populations. This result indicates higher genetic variability within populations as among populations of each species.

Additionally, the STRUCTURE study (Figure 3-4) inferred that both *Cissampelopsis* species do cluster into two major population groups of each species. In *C. corifolia* populations most possible more reflect a continuous genetic gradation or admixture of these neighboring groups than *C. volubilis* that correspond to geographic regions. The STRUCTURE analyses were performed to confirm the results of previous clustering analyses. The outcomes from all analyses were in agreement concerning the relatedness of the accessions at both the intraspecific and interspecific levels. These results seem to suggest that gene flow and inbreeding are likely to be the major driving force in shaping current population genetic structure of *Cissampelopsis* in Thailand. The population structures of both *Cissampelopsis* are probably mainly determined by colonization dynamics, possibly as a result of the heterogeneous landscape. The mediate genetic differentiation and high level of gene flow between both *Cissampelopsis* populations can result from a combination of factors such as a perennial life form of the plant, self-incompatibility, strong dispersal, and anthropogenic displacement as also concluded by Yan et al. (2016) for *Miscanthus lutarioriparius*. Moreover, continuous gene flow across geographically distant populations might be

caused by long-distance gene dispersal either by pollen or seed. As Rogalski et al. (2016) also found that distribution of genetic variation in the populations can be formed by bidirectional gene flow via pollen and seed. Long-range seed dispersal has been implicated in maintaining links between populations (Hemrová et al. 2017). In many tropical plants, animal-dispersed seeds and insect pollination may also have contributed to gene flow over distance (Ghazoul and Sheil 2010). However, seed or pollen flow seems unlikely between the current, isolated populations. Then, gene flow between *Cissampelopsis* at the two existing sites of each species via seeds and/or pollen was probably extensive and relatively unhindered in the past, before the populations became isolated as also suggested in *Nouelia insignis* a narrowly distributed and endemic species in China (Luan et al. 2006).

In conclusion, this study first revealed the population genetic diversity and structure of two *Cissampelopsis* species, *C. corifolia*, and *C. volubilis*, distributed in Thailand by using iPBS markers. Based on our results, iPBS-retrotransposons should be regarded as a reliable and polymorphic marker method, allowing discrimination among and within *Cissampelopsis* species. The results also support that *C. corifolia* and *C. volubilis* are well separated, confirming the taxonomical treatment of these climbing as separate species. Long-distance gene flow is important for maintaining genetic connectivity as evidenced by the high migration rates of *Cissampelopsis* species. Both *Cissampelopsis* species have moderate genetic variability. Thus, in situ conservation is a priority to protect genetic variation in the two *Cissampelopsis* species. Decreasing local disturbance is also necessary to allow the regeneration of wild populations. We expect this study will be helpful for inspiring biodiversity conservation and further studies of *Cissampelopsis* from other related areas that are valued to understand better the biology and pattern of species diversification in the genus.

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