รายงานวิจัยฉบับสมบูรณ์

โครงการ

ความสัมพันธ์เชิงวิวัฒนาการและประวัติการกระจายพันธุ์ของพืชเผ่า Senecioneae ในประเทศไทย

Phylogeny and diversification of Senecioneae (Asteraceae) in Thailand

โดย

รองศาสตราจารย์ ดร. โองการ วณิชาชีวะ

มิถุนายน 2563

สัญญาเลขที่ RSA5880024

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ผู้วิจัย

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สังกัด

มหาวิทยาลัยราชภัฏพระนคร

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัยและมหาวิทยาลัยราชภัฏพระนคร

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Ongkarn Vanijajiva

บทคัดย่อ

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ชื่อนักวิจัย: รองศาสตราจารย์ ดร. โองการ วณิชาชีวะ มหาวิทยาลัยราชภัฏพระนคร

E-mail address: vanijajiva@pnru.ac.th

ระยะเวลาโครงการ: 1 กรกฎาคม 2558 - 1 มิถุนายน 2563

พืชในเผ่า Senecioneae เป็นพืชเผ่าที่ใหญ่ที่สุดของพืชวงศ์ทานตะวัน (Asteraceae) ประกอบด้วยสมาชิกราว 150 สกุล และ 3,000 ชนิด อย่างไรก็ตามแม้จะมีความพยายามอย่างมากในการจำแนกและเข้าใจความหลากหลายทางสัณฐานวิทยาในเผ่า Senecioneae แต่ยังก็ไม่มีรายงานความสัมพันธ์ของพืชกลุ่มนี้ในประเทศไทย สำหรับประเทศไทยพบว่าพืชเผ่า Senecioneae ประกอบด้วย 9 สกุล ได้แก่ *Cissampelopsis* (DC.) Miq., *Crassocephalum* Moench., *Emilia* (Cass.) Cass., *Erechtites* Raf., *Gynura* Cass., *Kleinia* Mill., *Senecio* L. , *Sinosenecio* B. Nord และ *Synotis* (C. B. Clarke) Jeffrey & Chen ปัจจุบันยังไม่มีข้อมูลการความสัมพันธ์เชิงวิวัฒนาการและการกระจายพันธ์ของพืชเผ่า Senecioneae ของประเทศไทย เพื่อกำหนดรูปแบบการกระจายของ Senecioneae ในภูมิหลังทางชีวประวัติในประเทศไทยยังคงต้องเชื่อมโยงความหลากหลายทางพันธุกรรมของ Senecioneae กับการเปลี่ยนแปลงทางธรณีวิทยาและภูมิอากาศและพยายามที่จะครอบคลุมการเปลี่ยนแปลงทางสัณฐานวิทยาดังนั้นจำเป็นต้องมีการวิเคราะห์รวมถึงสัณฐานวิทยาโมเลกุล ลักษณะนิเวศวิทยา และการกระจายตัวของ Senecioneae ในประเทศไทย พบว่าการจำแนกลักษณะทางโมเลกุลเป็นข้อมูลที่สำคัญสำหรับการวิเคราะห์สายวิวัฒนาการและการกระจายพันธุ์ การศึกษาครั้งนี้ได้นำมาใช้ในการประเมินความสัมพันธ์ของสายวิวัฒนาการของกลุ่มตัวอย่าง Senecioneae จากภูมิภาคทางภูมิศาสตร์ที่แตกต่างกันทั่วประเทศไทย ผลการวิจัยชี้ให้เห็นว่าความสัมพันธ์ระหว่างเก้าสกุลของพืชเผ่า Senecioneae ในประเทศไทย การศึกษานี้ได้ประยุกต์ใช้เทคนิค inter-primer binding site (iPBS) มาช่วยในการศึกษาความสัมพันธ์เชิงวิวัฒนาการของพืชเผ่า Senecioneae ผลจากการทดลองพบว่าสอดคล้องการศึกษาก่อนหน้านี้และกระบวนการส่วนใหญ่ของการกระจายพันธ์ของพืชในเผ่านี้อาจเกิดขึ้นอย่างน้อยตั้งแต่ Miocene ยุคแรกและชัดเจนมากขึ้นในยุค Plio-Pleistocene โดยฤดูกาล อีกทั้งผลของอุณหภูมิ และปริมาณฝนเป็นปัจจัยสำคัญของการกระจาย Senecioneae ในประเทศไทย นำไปสู่การเปลี่ยนแปลงส่งผลให้เกิดสายพันธุ์เฉพาะถิ่นของ Senecioneae ในประเทศไทยหลายชนิด สันนิษฐานว่าบริเวณภูเขาทางภาคเหนือนับเป็นเส้นทางการอพยพที่สำคัญของสมาชิกในเผ่า Senecioneae ในประเทศไทย ดังนั้นประเทศไทยถือได้ว่าเป็นพื้นที่สำคัญสำหรับการกระจายความเสี่ยงของ Senecioneae ในภูมิภาคเอเชียตะวันออกเฉียงใต้

คำหลัก: พืชเผ่า Senecioneae, ความสัมพันธ์เชิงวิวัฒนาการ , ประวัติการกระจายพันธุ์, ประเทศไทย

Abstract

Project Code: RSA5880024

Project Title: Phylogeny and diversification of Senecioneae (Asteraceae) in Thailand

Investigator: Associate Professor Dr. Ongkarn Vanijajiva

E-mail Address: vanijajiva@pnru.ac.th

Project Period: 1 July 2015 – 1 June 2020

Senecioneae is the largest tribe of Asteraceae, comprised of ca. 150 genera and 3,000 species. Despite considerable efforts to classify and understand the striking morphological diversity in Senecioneae, little is known about its intergeneric relationships. In Thailand, Senecioneae is represented by nine genera. These are *Cissampelopsis* (DC.) Miq., *Crassocephalum* Moench., *Emilia* (Cass.) Cass., *Erechtites* Raf., *Gynura* Cass., *Kleinia* Mill., *Senecio* L., *Sinosenecio* B. Nord. and *Synotis* (C. B. Clarke) Jeffrey & Chen. At present, no phylogeny and diversification data of Senecioneae of Thailand. To place patterns of distribution for Senecioneae in a historical biogeographical background in Thailand still need to linked diversifications of Senecioneae lineages to geological and climatological changes and attempting to cover the considerable morphological variation in the group. Additional combined analysis of morphology, molecular, ecology and dispersal of Senecioneae in Thailand is necessary. The molecular characterization is a valuable information for phylogeny and diversification analysis. This present study, inter-primer binding site (iPBS) markers were used to assess the phylogenetic relationship of 984 samples of Senecioneae from different geographical regions across Thailand. The results suggested that the relationships between the nine genera of Senecioneae in Thailand identified in this study from iPBS are congruent between the molecular phylogenies from earlier studies and most of the process of diversification within Senecioneae clades probably occurred since at least the early Miocene and more obvious in the Plio-Pleistocene. The seasonality of both temperature and precipitation is a major determinant of Senecioneae distribution and richness in Thailand and its variation led to a modification of the relative many endemic species of Senecioneae where the Northern Highland is the major immigration route of Senecioneae in Thailand. Thus, Thailand could be considered as a significant area for Senecioneae diversification in Southeast Asian region.

Keywords: Senecioneae, Phylogeny, Diversification, Thailand

Executive summary

A fundamental objective of the study of biological diversity is to understand why divergent regions with similar environments contain divergent numbers of species. Determining the causes of high biodiversity in some regions is of primary importance in biology and a principal aim of biogeographic research. Molecular phylogenetic reconstructions of evolutionary relationships between living organisms are increasingly used to infer these putative causes of diversification within an historic and geographic context. Many current studies show that high numbers of plant species within regions might be due in part to bursts of speciation that occurred during the last few million years triggered by major geophysical and/ or climate change, and that a significant proportion of plant diversity originated during the late Tertiary. However, the number of studies conducted on species rich floras remains low with most centered on groups in Thailand. Thailand is located in a humid climatic region which supports a variety of tropical ecosystems. Unlike those in temperate zone, tropical ecosystems provide wider niches for organism's survival. The county is able to support a much larger variety of plant, animal and microbe species. For plant, Thailand has approximately 15,000 species of plant which account for 10% of estimated total number of plant species found globally. Among the plant families, Asteraceae (Compositae; Sunflower family) is one of the diverse plant families. In Thailand, Asteraceae makes up ca. 10% of total flowering plant specific diversity. Senecioneae is the largest tribe of Asteraceae with about 150 genera and 3,500 species. The tribe has been the subject of much debate with regard to its phylogenetic composition, especially in Thailand relationship of Senecioneae remain poorly known and most genera whose phylogenetic position is unresolved. In Thailand, Senecioneae is represented by nine genera. These are *Cissampelopsis*, *Crassocephalum*, *Emilia*, *Erechtites*, *Gynura*, *Kleinia, Senecio*, *Sinosenecio* and *Synotis*. Recently, a number of molecular marker methods based on retrotransposons sequences, both species-specific and non-specific, have been developed and used for determining genetic structure of several groups of eukaryotic organisms. In this study, inter-primer binding site (iPBS) markers based genome fingerprinting technology used. Based on the results of this study, this study suggested that the inter-primer binding site (iPBS) is a powerful tool to study phylogeny and diversification as well as allows to determine genetic diversity and population structure of Senecioneae in Thailand.

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1. INTRODUCTION

Biogeography and conservation are associated certainly by the relationships between habitat area, primary productivity, earth history, and species richness. This connection is particularly strong in Thailand where the areal extent of the country has repeatedly fluctuated twofold in the last few million years. At present, geography is therefore highly atypical and it will become even more so as the region loses another 10% of its land area this century, and more in the next. At present, Thailand is among the world richest in terms of its biodiversity. The country currently contains more than 15,000 species of vascular plants, equivalent to about 10% of the flora of the world. The country is positioned in a tropical region, between 5°-21° N and 97°-106° E, covering an area about 513,115 sq. km within two major biogeographical regions, the Indochinese region in the North and the Sundaic region in the South and positioned at a unique crossroad of tree main floristic regions. This rich biodiversity is attributed, in part, to the region's geographic position at the transition between the well-differentiated Indo-Burmese, Indo-Chinese and Malesian biogeographic regions, its position in the humid tropics, its history of dramatic changes in land area, and its habitat fragmentation. The diverse communities within the region share a common biogeographic history and many genera and families of plants (Woodruff, 2010).

Among the plant families, Asteraceae (Compositae; Sunflower family) is one of the diverse plant families. It is the largest family or a close second to orchid family (Orchidaceae) in number of species of flowering plants with c. 25,000 species and 1,600 genera. In Thailand, Asteraceae makes up ca. 10% of total flowering plant specific diversity. The family apparently originated already in the Cretaceous, and started to become abundant in the Miocene (Bremer, 1994). Species of Asteraceae today are found throughout the world from the Arctic to alpine meadows and from arid regions to rainforest. Asteraceae are mostly herbaceous plants, but some shrubs, trees and climbers do exist. Asteraceae are generally easy to distinguish from other plants, mainly because of their characteristic inflorescence and other shared characteristics. Systematically, Asteraceae can be divided into two monophyletic groups of markedly unequal size, the small monotribal South American subfamily Barnadesioideae and non-barnadesioid Compositae; it consists of the tribes Inuleae (including Plucheeae), Heliantheae s.l. (including Eupatorieae), Gnaphalieae, Astereae, Anthemideae, Calenduleae, Corymbieae and Senecioneae (Anderberg et al., 2007).

Senecioneae is the largest tribe of Asteraceae with about 150 genera and 3,500 species (Pelser et al., 2010). The number of isolates from the core genus *Senecio* has increased dramatically in the last decade. *Senecio* itself (with over 3,000 binomials) will eventually be defined as a monophyletic but still very large genus by continued removal of discordant elements including several sections. On the tribal level, the phylogeny and relationships within subfamily Asteroideae are not sufficiently known, and it is difficult to discern the closest affinities of Senecioneae among the tribes Astereae, Anthemideae, Calenduleae, Inuleae and Gnaphalieae. The member of Senecioneae show a wide range of growth forms by including annuals, minute creeping alpines and perennial herbs, shrubs, climbers, leaf, stem and root succulents, trees and semi-aquatic herbs. Its species can be easily recognized primarily by the involucre which usually consists of one series of involucral bracts. Frequently the single-rowed involucre is subtended by a smaller outer calyculus (Nordenstam, 1978; Bremer, 1994). Phytochemically, the tribe is rather well characterized by the presence of pyrrolizidine alkaloids in many, but not all genera, and groups of sesquiterpene lactones known as eremophilanes and furanoeremophilanes, and by the absence of polyacetylenes in most genera. Although the evolutionary success of the tribe is striking, as measured by its tremendous number of species and its incredible morphological diversity, the reasons of its success remain largely unknown and unexplored. Bremer (1994) recommended that the success of Senecioneae might be due to their poisonous pyrrolizidine alkaloids. This suggestion has, however, not been further examined, apparently because of the previous lack of a robust phylogeny for Senecioneae and the limited availability of comparative pyrrolizidine alkaloid data for its species. Others have linked diversifications of Senecioneae lineages to geological and climatological changes. At present, Senecioneae diversification have been of continued interests in evolutionary biology. Previous studies revealed that adaptive divergence, polyploidization, hybridization and geohistorical factors contributed to the diversification of Senecioneae as in other groups of flowering plants. Among them, some recent molecular phylogenetic studies suggested that geohistorical factors might have driven rapid speciation in many plant groups during the late Tertiary. In addition, phylogeny and diversification of Senecioneae is poorly known in Thailand. Therefore, phylogeny and diversification of Senecioneae study in Thailand is needed to test this suggestion, further molecular phylogeographic studies in species rich areas are required.

The molecular characterization is a valuable information for phylogeny and diversification analysis. The genetic data estimation at the molecular level is currently available, but only few dominant nuclear DNA markers such as Randomly Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR), Amplified Fragment Length Polymorphism (AFLP) are most regularly used. 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. Due to retrotransposons are one of the most fluid genomic components, instable enormously in copy number 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. (2010) established inter primer-binding sites (iPBS) retrotransposon indicator system for eukaryotic organisms, particular in plants. Due to, the iPBS technique is an easy-to-use technique that requires no sequence data, cost-effective, not age or tissue-specific, highly informative and are not 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. Since no information is available about the genetic information of Senecioneae in Thailand. Assessing the limits of this tribe is a problem that can only be tackled with a broad phylogenetic analysis of entire the tribe.

The objectives of this study are:

1) to evaluate the potential of molecular method for monitoring Thai Senecioneae genetic diversity as a basis for phylogeny-based biogeographic issues
2) to resolve its major lineages of Thai Senecioneae species

2 LITERATURE REVIEWS

2.1 Asteraceae in Thailand

Asteraceae is the most species-rich family in flowering plants, and thus the mechanisms behind its diversification have been of continued interests in evolutionary biology. The family was first described in 1792 by the German botanist Paul Dietrich Giseke. Fundamental work of this family was done by Cassini (1817, 1818, 1826-1834), and Bentham (1873) and Bentham & Hooker (1873). Bentham's tribal classification has stood the test of time, and some modifications were introduced by Hoffmann (1890-1894), Dalla Tore and Harms (1907) and Melchior (1964). Although the 13 tribes recognized by Bentham & Hoffman have been largely accepted up to the present, they are obviously in need of modification considering recent discoveries in biochemistry, palynology analysis, micromorphology, anatomy, cytology, biochemistry especially molecular analysis. At present, a large volume of literature has been published describing, classifying, and discussing the morphological and ecological diversity in Asteraceae (e.g., De Candolle, 1838; Bentham, 1873; Hoffmann, 1890; Cabrera, 1939, 1949, 1985; Nordenstam, 1977, 1978, 2007; Jeffrey & Chen, 1984; Jeffrey, 1986, 1992; Bremer, 1994). Cladistic method has been widely applied, subsequent more precise generic, and tribal concepts have been developed. It has become clear that not only quite a number of genera have been misplaced, but others require a transfer to other tribes (Kadereit & Jeffrey, 2007).

Asteraceae have a cosmopolitan dispersal, and are found everywhere except Antarctica and the extreme Arctic. They are particularly numerous in tropical and subtropical regions notably Central America, eastern Brazil, the Andes, the Mediterranean, southern Africa, central Asia, and southwestern China. In Thailand, the first initial survey of the flora began with the publication of Craib in 1932 which 67 genera and 196 species were recorded. It was followed by several authors e.g. Kerr (1936) in a treatment of Thai Compositae who totally described 16 genera and 196 species. But after Kerr there has been no attempt yet to bring together the vascular flora of Thailand. Smitinand (1980) listed only of the Asteraceae in 70 genera and 139 species. The latest partial enumeration of the Asteraceae of Thailand was made by Koyama, who elaborated in his series of paper since 1981 (Koyama 1981, 1983, 1984a, 1984b, 1985a, 1985b, 1986, 1988, 1989, 1993, 1997, 1998, 2001, 2002, 2003, 2004 and 2005). These work intended to list up the species of Asteraceae known from Thailand with critical notes and identification key. At present, approximately 240 species are enumerated in Thailand (Koyama et al., 2016).

2.2 Phylogeny and diversification of Senecioneae

The Senecioneae are one of the largest tribes in the Asteraceae, with more than 3,000 species in ca. 150 genera, and it exhibits remarkable morphological and ecological diversity (Pelser et al., 2010). The tribe exhibits enormous variation in life-history strategies and morphology. Many species are annual or perennial herbs and include annuals, minute creeping alpines, perennial herbs, shrubs, climbers, succulents, trees and semi-aquatic plants. The remarkable morphological variation is especially apparent in leaf shape, indument, inflorescence type, and flower color (Barkley, 1978). Most taxa of Senecioneae can be identified relatively easily by the possession of capitula with usually uniseriate involucres. Other characteristics of this tribe are the presence of the presence of the macrocyclic senecionine type (Pelser et al., 2007). Systematically, tribe Senecioneae was placed as sister to tribe Calenduleae. This opinion was supported by molecular analysis. However, other datasets provided different hypotheses for the phylogenetic position of Senecioneae within subfamily Asteroideae (Figure 2.1). The short branches that connect Senecioneae with its putative sister clades suggest that the difficulty of placing Senecioneae may be due to rapid diversification early in the evolutionary history of Asteroideae

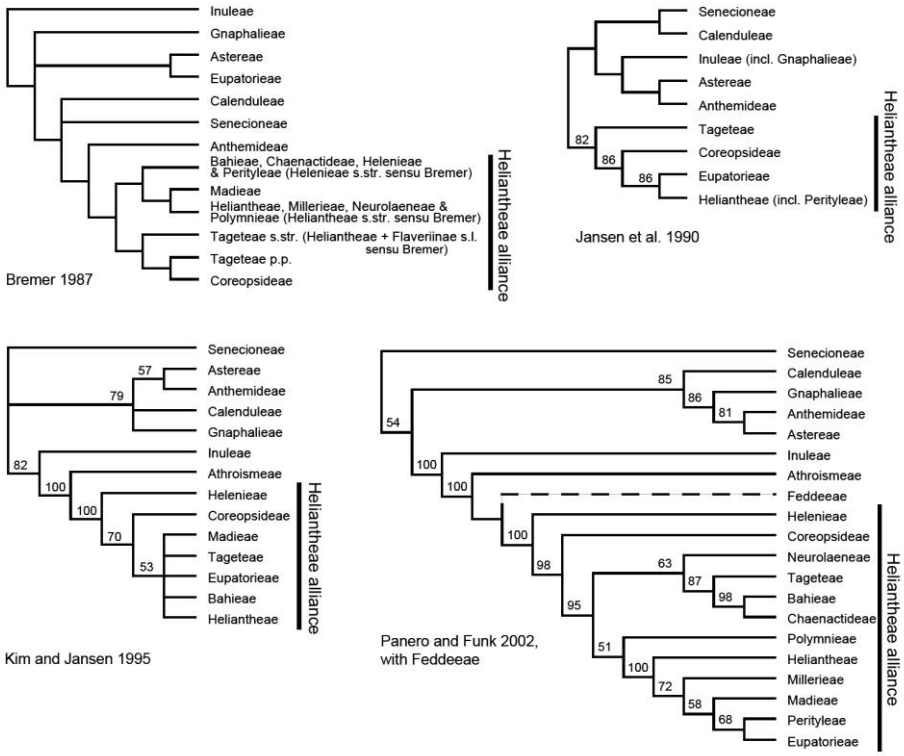

Figure 2.1 Comparison of hypotheses of intertribal relationships of Senecioneae in Asteroideae.

Senecioneae occur in almost all environments. Its members exhibit probably the widest possible range of form to be found anywhere in the entire plant kingdom**.** Even though the tribe has a worldwide distribution, there are some marked centers of generic diversity and speciation. These areas include temperate and subtropical arid or montane regions, continental as well as insular (Figure 2.2). Africa is possibly the continent where Senecioneae alliance had their origin. From there, other continents were colonized, resulting in an almost cosmopolitan distribution. Diversifications of Senecioneae lineages have connected to geological and climatological changes, such as the uplift of the Cordilleran mountain regions in North America, glaciations during the Pleistocene, and a drying trend throughout the Tertiary.

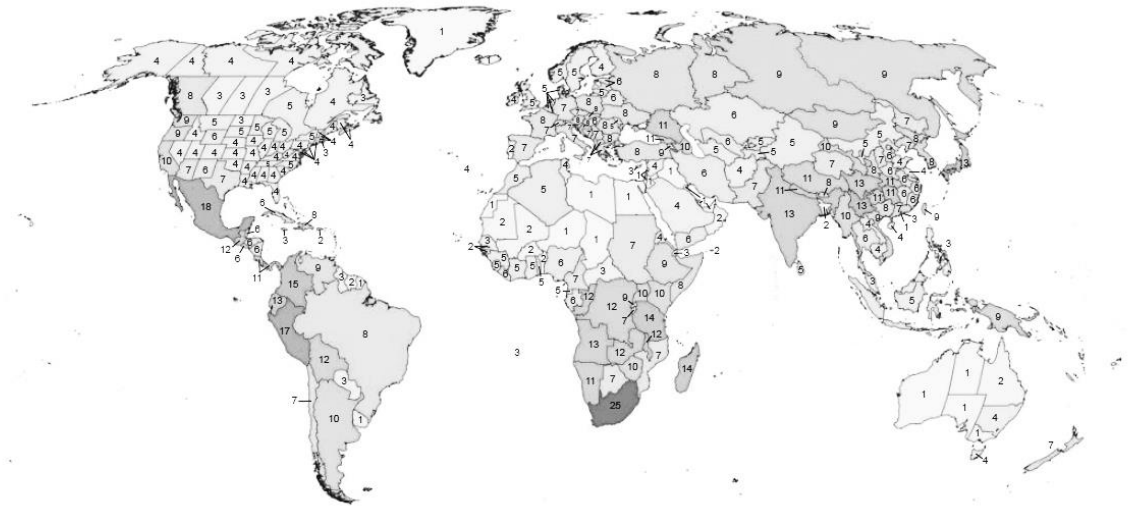

Figure 2.2 Numbers indicate the number of genera in each of the outlined geographic regions.

In Thailand, Senecioneae is represented by nine genera (Figure 2.3). These are *Cissampelopsis* (DC.) Miq., *Crassocephalum* Moench., *Emilia* (Cass.) Cass., *Erechtites* Raf., *Gynura* Cass., *Kleinia* Mill*., Senecio* L., *Sinosenecio* B. Nord. and *Synotis* (C. B. Clarke) Jeffrey & Chen (Koyama et al., 2016). At present, no phylogeny and diversification data of Senecioneae of Thailand. To place patterns of distribution for Senecioneae in a historical biogeographical background in Thailand still need to linked diversifications of Senecioneae lineages to geological and climatological changes and attempting to cover the considerable morphological variation in the group. Therefore, further combined analysis of morphology, molecular, ecology and dispersal of Senecioneae in Thailand is necessary.

Figure 2.3 Morphological character of Senecioneae in Thailand. A. *Cissampelopsis corifolia* B *Crassocephalum crepidioides* C *Emilia sonchifolia* D *Erechtites hieracifolia* E *Gynura pseudochina* F *Kleinia grandiflora* G *Senecio namnaoensis* E. *Sinosenecio oldhamianus* F *Synotis nagensium*

2.3 iPBS marker

Molecular markers are essential in biodiversity application. Entirely eukaryotic genomes contain DNA sequences named repetitive elements that are existing in multiple copies throughout the genome. These repetitive sequences can be displayed in tandem. Otherwise, repetitive elements, such as mobile elements and processed pseudogenes, can be interspersed throughout the genome. Transposable elements (TEs) are highly abundant mobile genetic elements that have multiple classes and constitute a large fraction of most eukaryotic genomes. TEs can be subdivided on the basis of their size, with short interspersed elements being less than 1000 bp long and the rest considered to be long interspersed elements. The class known as retrotransposons, for example, comprises 10–90% of eukaryote genomes. Retrotransposons and related elements are highly abundant in eukaryotic genomes. TEs, particularly long terminal repeat (LTR) retrotransposons, are also predominantly located in heterochromatic regions of the genome. In plants, LTR retrotransposons tend to be more abundant than non-LTR retrotransposons. In many crop plants, between 40% and 70% of the total DNA comprises LTR retrotransposons. TEs are among the most fluid genomic components, fluctuating immensely in copy number over a relatively short evolutionary timescale, and represent a major component of the structural evolution of plant genomes. The movement and accumulation of TEs has been a major force in shaping the genes and genomes of almost all organisms. TEs mostly appear static and nonfunctional. However, some TEs are capable of replicating and mobilizing to new positions in the genome, and even immobile TE copies can be expressed TEs are classified into two main groups in eukaryotic genomes and defined according to their mechanism of transposition. They are classified as Class I TEs transposing through an RNA intermediary, and other transposons (Class II), which do not have an RNA intermediary (Kalendar et al. 2018).

Most of the retrotransposon PCR techniques are anonymous, producing fingerprints from multiple sites of retrotransposon insertion in the genome. However, when one analyses closely related species, it is possible to predict the part of the common PCR amplicons expected in the sample via a phylogenetic approach. All of the techniques use the combination of a known retrotransposon sequence and a variety of adjacent sequences. The targets for PCR primers are generally designed for LTRs close to the joint in domains that are conserved within families but vary between families. Retrotransposon marker systems differ according to the second primer used in the amplification reactions. This primer can be any feature in the genome that is dispersed and conserved (Figure 2.4) (Kalendar et al., 2018).

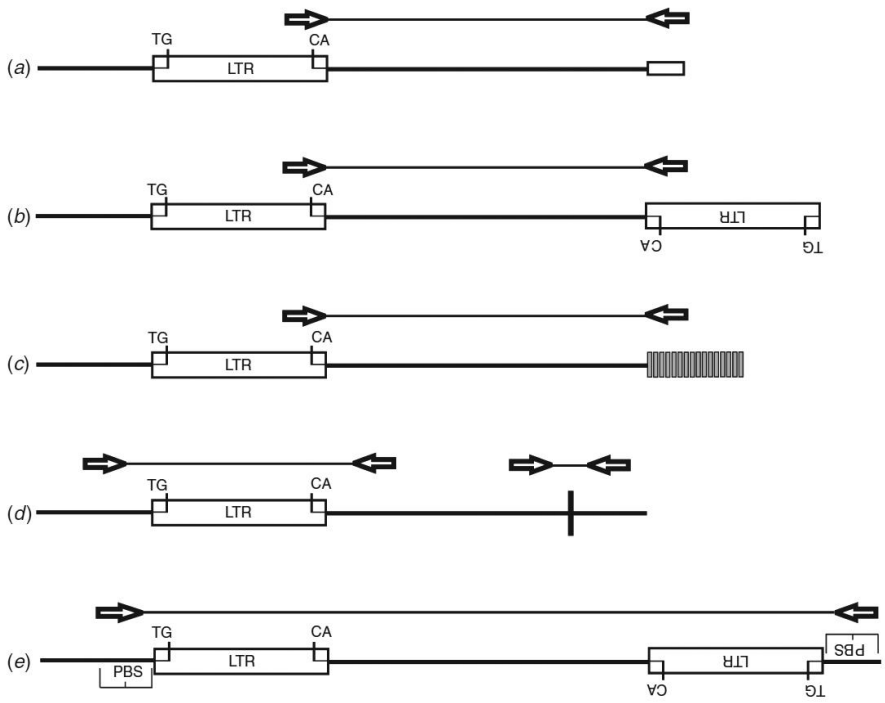

Figure 2.4 Retrotransposon-based molecular marker methods

Multiplex products of various lengths from different loci are indicated by the bars above or beneath the diagrams for each reaction. Primers are indicated by arrows. (*a*) The sequence-specific amplified polymorphism method. The primers used for amplification match the adaptor (empty box) and retrotransposon (the long-terminal repeat (LTR) box). (*b*) The inter-retrotransposon amplified polymorphism method. Amplification takes place between retrotransposons (left and right LTR boxes) near each other in the genome (open bar), using retrotransposon primers. The elements are shown oriented head-to-head, using a single primer. (*c*) The retrotransposon microsatellite amplification polymorphisms method. Amplification takes place between a microsatellite domain (vertical bars) and a retrotransposon, using a primer anchored to the proximal side of the microsatellite and a retrotransposon primer. (*d*) Retrotransposon-based insertion polymorphism. Full sites, depicted on the left, are scored by amplification between a primer in the flanking genomic DNA and a retrotransposon primer. The single product is shown as one bar beneath the diagram. The alternative reaction between the primers for the left and right flanks is inhibited in the full site by the length of the retrotransposon. The product that is not amplified is indicated by a grey bar beneath the diagram. The flanking primers are able to amplify the empty site, on the right, depicted as a bar beneath the diagram. (*e*) The inter-primer-binding site amplification (iPBS) scheme and LTR retrotransposon structure. Two nested LTR retrotransposons in inverted orientations are amplified from a single primer or two different primers from primer binding sites. The PCR product contains both LTRs and PBS sequences as PCR primers in the termini. In the figure, the general structure for PBS and LTR sequences and the several-nucleotide-long spacer between the 50 LTR and PBS are schematically shown (Kalendar et al., 2018).

The inter-PBS amplification (iPBS) method has led to the development of a virtually universal and very efficient technique, which employs the conserved parts of PBS sequences, both for direct visualization of polymorphism between individuals, polymorphism in transcription profiles, and fast cloning of LTR segments from genomic DNA, as well as for database searches of LTR retrotransposons (Figure 2.5). Many retrotransposons are nested, recombined, inverted, or truncated, yet can be easily amplified using conserved PBS primers in all plant species tested. Fragments of retrotransposons containing a 5′ LTR and part of the internal domain are often located near other entire or similarly truncated retrotransposons. Therefore, PBS sequences are very often located sufficiently near to each other to allow amplification (Kalendar and Schulman, 2014). Recently, iPBS markers were developed as an alternative method to explore genetic diversity and relationships and diversity in plants. As a dominant marker system, the iPBS requires no previous knowledge of the genome. Due to the limited genomic information available for Senecioneae in Thailand, this study adopted the iPBS marker system in this study.

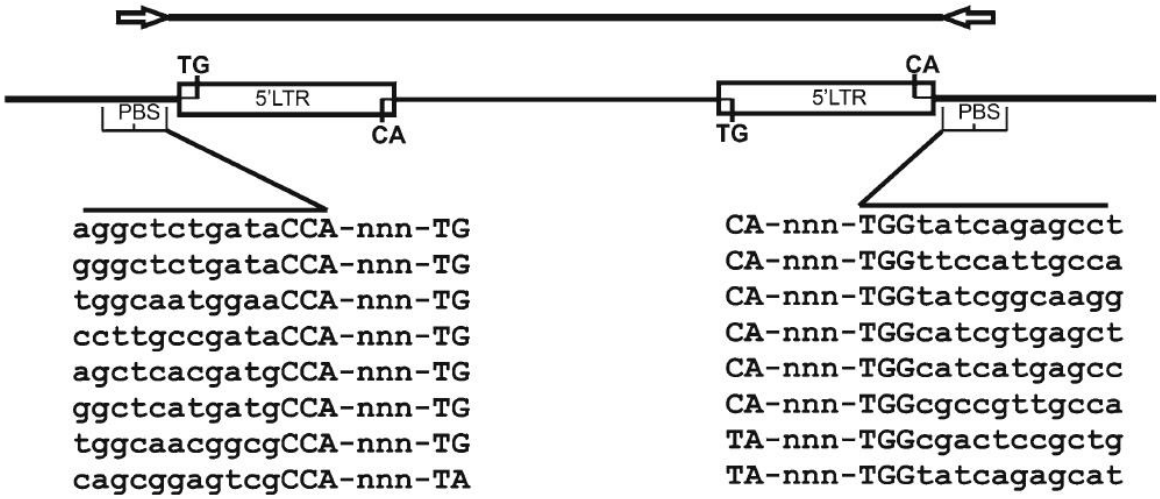

Figure 2.5 The inter-PBS amplification (iPBS) scheme and LTR retrotransposon structure. Two nested LTR retrotransposons in inverted orientation amplified from single primer or two different primers from primer binding sites. PCR product contains both LTRs and PBS sequences as PCR primers in the termini. In the figure, general structure for PBS and LTR sequences and several nucleotides long spacer between 5′LTR (5′-CA) and PBS (5′-TGG3′) are schematically shown (Kalendar and Schulman, 2014).

3. MATERIALS AND METHODS

3.1 Plant materials

Young leaf material of totally about 984 samples of Senecioneae in Thailand were collected in silica gel for DNA extractions. The plants were identified taxonomically based on their habitats and morphological characters according to the treatment of Koyama et al. (2016).

3.2 DNA extraction

The genomic DNA was extracted using 200 mg of dried leaves from the ground tissue following CTAB procedures with minor modifications. The leaves (200 mg) were ground in a mortar with a pestle. Extraction buffer [(1% (w/v) CTAB, 50 mM Tris–HCl (pH 8), 0.7 M NaCl, 0.1% ß-mercaptoethanol)] 500 µl was added and the solution was incubated at 60 C^{o} for 30 min. The homogenate was mixed with 25:24:1 phenol: chloroform: isoamyl alcohol (v/v/v) by gentle inversion. After centrifugation at 13,000 rpm for 15 min, the upper aqueous layer was transferred to a new tube. RNA was removed by treating with 2.5 µl of the RNase (10 µg/µl) for 30 min at 37^{o}C. The extraction of DNA with phenol/chloroform/isoamyl alcohol was repeated one more time. DNA in the solution was precipitated with 0.6 volumes of ice-cold isopropanol and washed with 70% ethanol. Subsequent, the DNA was removed using CTAB DNA extraction protocol without RNase. The procedure was repeated until the DNA pellet was free of color (two to three times) and the final pellet was dissolved in sterile deionized water. DNA quality was using Nanodrop Spectrophotometer (Thermo scientific Nanodrop 1000, USA) at the absorbance ratio of 260 and 280 nm providing a value of 1.7-1.8 which determines pure DNA preparation. Quality of DNA fragment was electrophoretically analyzed through 0.8% agarose gel using 1X TAE buffer. A 100 bp DNA ladder (Promega) was loaded into the gel as molecular size marker. The gels were stained with RedSafe™ Nucleic Acid Staining Solution and photographed under UV light by using gel documentation system alpha imager hp (Innotech, USA). The DNA was stored at -20 C^{o}, for further use as templates for PCR amplification.

3.3 iPBS-PCR amplification

Initially, 20 iPBS primers designed by Kalendar et al. (2010) were tested on DNA samples and all primers were selected with high clarity and repeatability for polymorphic assessment in studied *Senecio namnaoensis* accessions. DNA amplification was approved by

using a modified procedure of Kalendar et al. (2010). PCR was performed using a Thermohybaid Px2 (Roche Molecular Systems, Inc., USA). PCR was optimized for testing the SCoT method. The PCR was performed in a 25 µl reaction mixture containing 5 µl DNA (30-50 ng), 2.5 mM of 10X PCR buffer, 1 µM of primer, 1.2 mM dNTPs, 0.5 unit Taq DNA polymerase, 2 mM $MgCl_2$. The PCR program had an initial hot start at 94 °C for 3 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 43-60°C for 30 seconds and extension at 72°C for 2 minutes. Next, this was a final extension at 72°C for 5 minutes and then the program was finished by holding at 4°C. The reaction was performed in a Bio-Rad T100TM Thermal Cycler with 0.2 mL tubes or 96-well plates. A 10 µl sample of each PCR product was electrophoresed at at 150 A for 30 minutes in 0.04 M TAE (Tris-acetate 0.001 M-EDTA) buffer pH 8. The gels were stained with RedSafe™ Nucleic Acid Staining Solution and photographed under UV light by using gel documentation system alpha imager hp (Innotech, USA). To determine SCoT 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). Identification at all loci were confirmed by three repeating exams for each primer at different times. Data scoring and analysis PCR was performed three times for each primer to approve band pattern uniformity.

3.4 Error rate

To examine the error rate, the 50 replicates were analyzed. For each marker, the number of mismatches between the replicate and the reference sample was calculated. A mismatch was counted if the reference had a presence (1) but the replicate had instead an absent (0) and vice versa at the same marker/loci. The error rate was calculated as the number of mismatches over the number of all loci divided by the total number of loci (Bonin et al., 2007)

3.5 Data analysis

Statistical studies of iPBS patterns were based on the assumptions that iPBS fragments in Senecioneae accessions perform as diploid, dominant markers with alleles being either present (amplified) or absent (non-amplified), co-migrating fragments represent putatively homologous loci and fragments are of nuclear source and inherited bi-parentally. 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). Unweighted Pair-group Method Using Arithmetic Average (UPGMA) dendrogram and Principal Coordinate Analysis (PCoA) grouping were performed for the whole individual-based dataset of each genus and for the subgroups, identified by STRUCTURE analyses. UPGMA and PCoA were calculated in the software PAST version 3 (Paleontological Statistics, (Hammer et al., 2001)) with Dice similarity coefficient (Dice, 1945).

The Bayesian clustering method in STRUCTURE version 2.3.4 (Pritchard et al., 2000) was applied for the whole dataset of each genus STRUCTURE is a mixture model-based Bayesian clustering method that groups individuals into K populations or species and assigns admixture proportions to each individual of these groups (Meudt et al., 2009). For this, the Markov Chain Monte Carlo (MCMC) algorithm is used. It uses the Hardy-Weinberg and linkage equilibrium assumption within genetically homogenous populations. The number of clusters based on posterior probability and numbers of K can be self-specified by the user. The admixture model with correlation of allele frequencies allows the individuals to have mixture ancestries in more than one cluster and the recessive allele model was selected (Falush et al., 2003; Falush et al., 2007). The results from this cluster analysis were subsequently used to guide interpretation of analyses at regional levels (Breinholt et al., 2009). The first run was an exploratory run with K=20 and 5 interations for each K. After this, each following run was K=10 with 10 interations per K. To detect the most likely number of genetic groups (K), the posterior probability of the replicate runs lnP(D) for the particular K and the occurrence of empty groups are helpful to find them (Falush et al., 2007). The R Script STRUCTURSUM was used to create some useful plots where the lnP (D) of the replicate runs for each K were calculated and plotted against the K value. With the function “Structure.table” a plot of lnP (D) against the values of K was calculated. “Structure.simil” is a function which can calculate an average similarity coefficient with standard deviation between the replicate runs against K (Nordborg et al., 2005). Then with the function “Structure-deltaK” four plots were calculated. The first plot with the mean of the logarithmic probability (ln(P)) against the number of groups (K), second the mean of the first order rate of the ln(P) against groups, third the mean of the second order rate of ln(P) against groups and the fourth plot with Delta K = ΔK plotted against the number of groups (K) (Evanno et al., 2005). Delta K calculation is based on the second order rate of change of the likelihood. Delta K shows a clear peak at the meaningful value of K. The run with the highest similarity coefficient and lnP (D) of each K was shown as barplots to visualize the affinity of each individual to the inferred genetic clusters.

4. RESULTS AND DISCUSSION

4.1 Phylogeny and diversification of *Cissampelopsis*

4.1.1 iPBS polymorphisms in *Cissampelopsis*

Overall 96 individuals from 4 populations of *Cissampelopsis* were genotyped with iPBS, successfully. 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 in *Cissampelopsis* (Table 4.1). 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 4.1). The information from these twenty primers, including band polymorphism and mean PIC values is included in Table 4.1. The mean PIC values of iPBS primers varied from 0.296 (2076) to 0.484 (2085). The mean PIC value for these twenty primers was 0.415. These results indicate that the iPBS marker system can representative a good discriminatory capacity and reveal a wide range of genomic DNA diversity in *Cissampelopsis*.

Table 4.1 Characteristics of twenty iPBS primers used in the *Cissampelopsis* 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)
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
2081	GCAACGGCGCCA	65.0	5	250-750	5	100.00	0.462
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
2272	GGCTCAGATGCCA	55.0	6	200-800	6	100.00	0.392
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
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
2382	TGTTGGCTTCCA	50.5	7	250-2,000	5	100.00	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
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
	Total		120	1500-3,000	114	95.00	0.415

4.1.2 Phylogeography of *Cissampelopsis*

To elucidate the genetic relationships among *Cissampelopsis* accessions, a dendrogram and grouping were constructed using Dice similarity coefficients based on the unweighted pair group analysis with the arithmetic mean (UPGMA) dendrogram and the principal component analysis (PCoA) in Past program. According to the results of the UPGMA dendrogram including all 96 samples of *Cissampelopsis* in Thailand were obviously separate under two main clusters (Figure 4.1). 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* genotypes species in Thailand. The results of PCoA similarly indicated two distinct groups in the taxonomic representation of the relative genetic similarity among all *Cissampelopsis* samples (Figure 4.2) , supporting the results presented in the UPGMA dendrogram. The results of our study verified that *C. corifolia* and *C. volubilis* are in the different species.

4.1.3 Diversification of *Cissampelopsis*

The STRUCTURE software assigns individuals to different populations based on allele frequencies of the genotypes. Genetic structure of the population was inferred through allele frequencies (tested for K = 2 to K = 10). K was estimated with the aid of posterior probability of the data for a given. In the STRUCTURE analysis, log probabilities of the data [lnP(D)] showed the highest likelihood at K=2. Therefore, STRUCTURE analysis was conducted for K = 2 and suggesting four clusters for 96 *Cissampelopsis* genotypes as shown in Figure 4.3. At K = 2, Cissampelopsis samples were separated into two clusters. Among *Cissampelopsis* populations in Thailand, *C. corifolia* individuals were largely assigned to one cluster, whereas *C. volubilis* individuals were largely assigned to the second cluster. Based on the latter model, all populations from Thailand were mixed for cluster I (‘red’) and cluster II (‘green’) was in Thailand populations. 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 (Figure 4.3). It was concluded based on present findings that iPBS markers could reliably be used in phylogenetic and diversification analysis of *Cissampelopsis* genotypes.

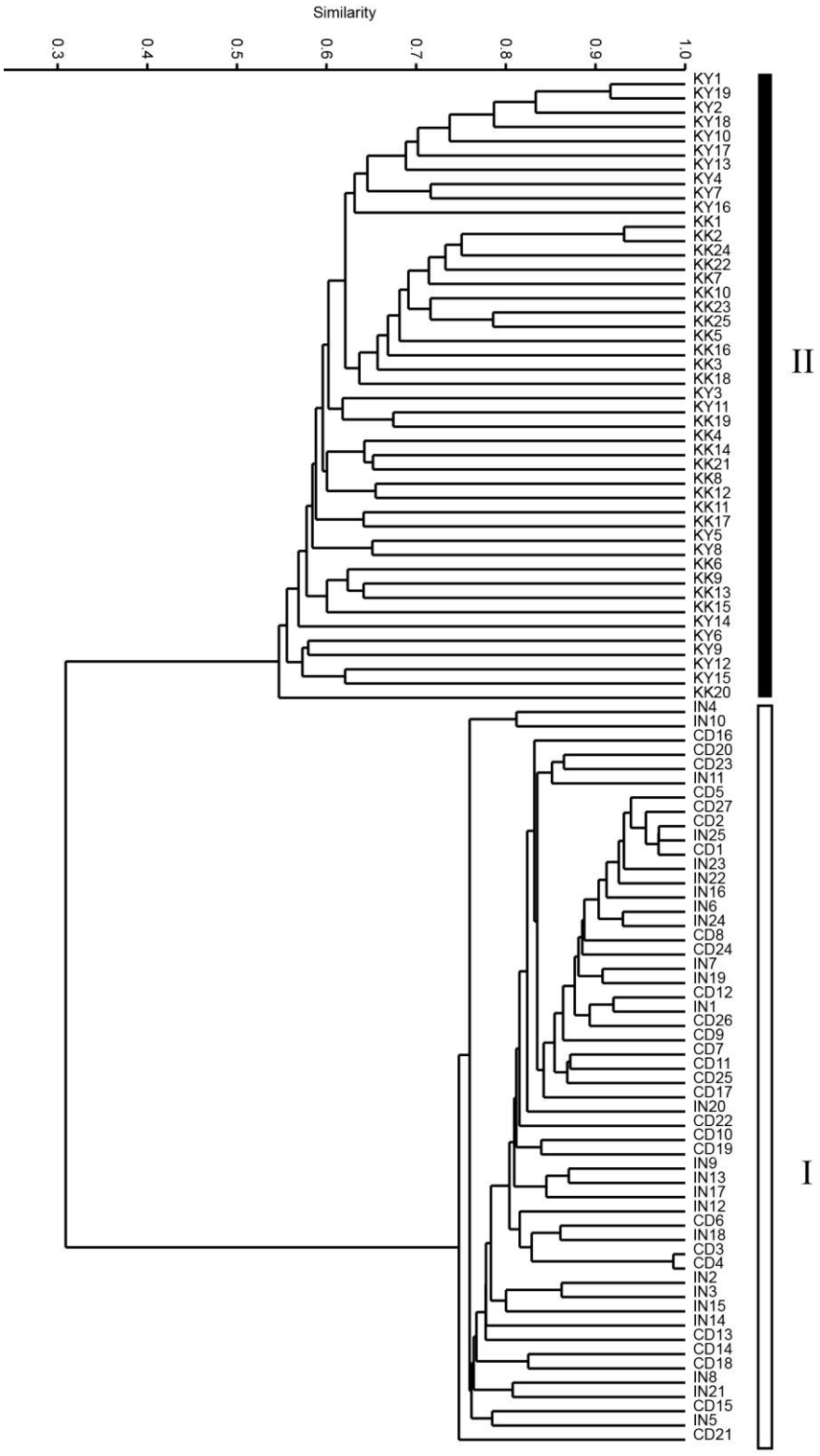

Figure 4.1 Dendrogram of 96 *Cissampelopsis* accessions based on iPBS markers according to UPGMA with the Dice similarity index.

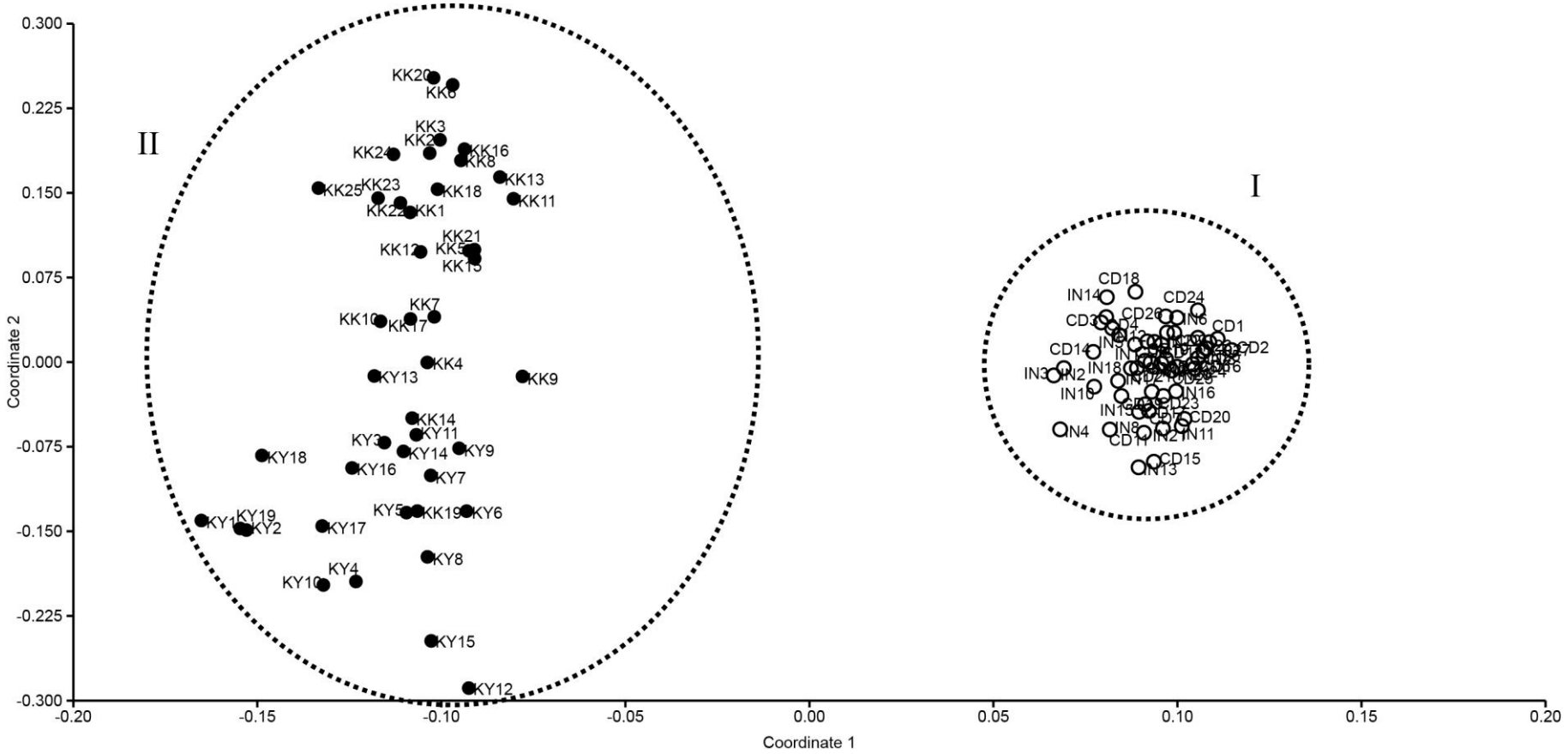

Figure 4.2 PCoA of 96 *Cissampelopsis* accessions based on 20 iPBS markers.

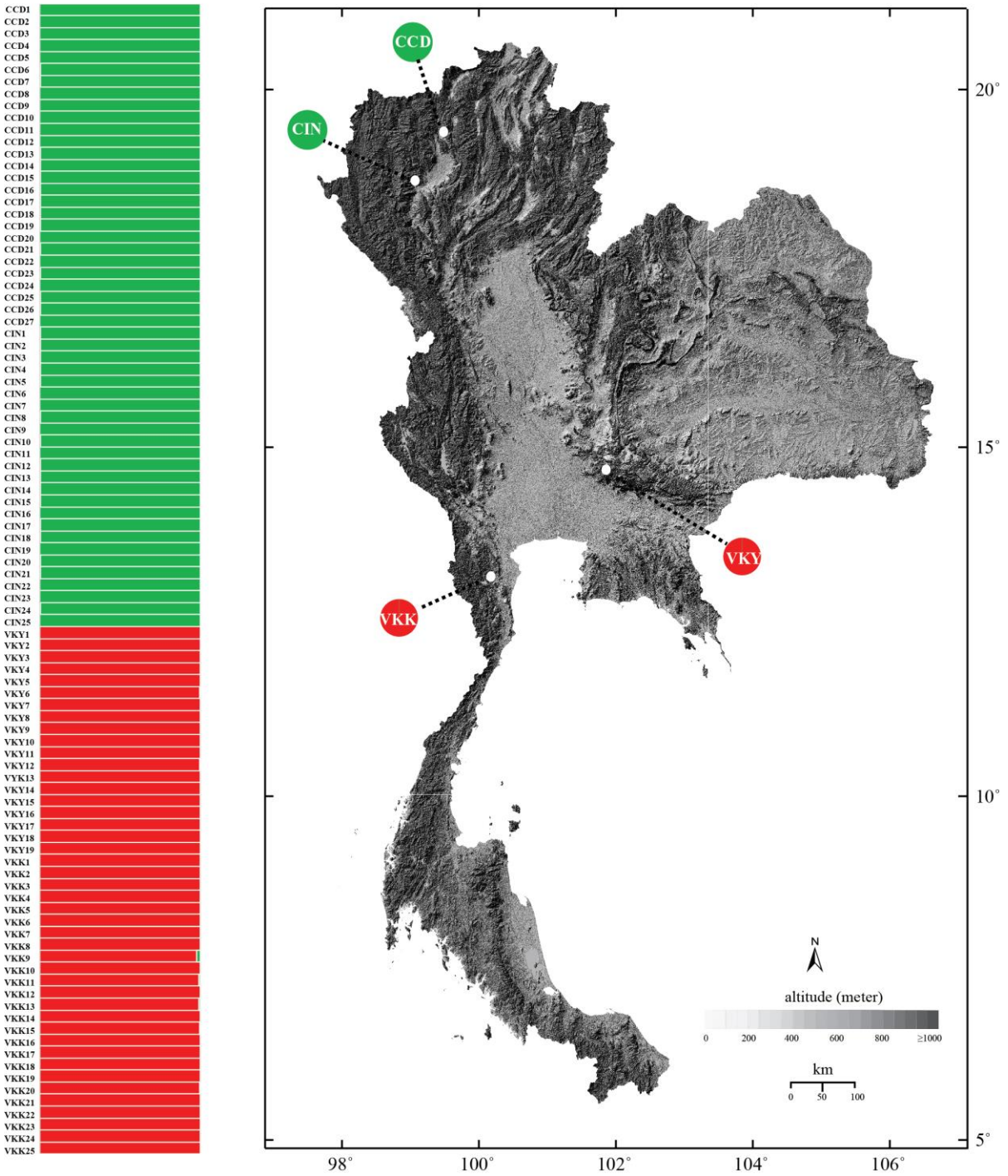

Figure 4.3 Genetic structure of 96 *Cissampelopsis* accessions inferred by STRUCTURE software with 20 iPBS marker data sets. Single vertical line represents an individual accession, and different colors represent genetic stocks/gene pools. Segments of each vertical line show extent of admixture in an individual.

4.2 Phylogeny and diversification of *Crassocephalum*

4.2.1 iPBS polymorphisms in *Crassocephalum*

Overall 116 individuals from 8 populations of *Crassocephalum* were genotyped with iPBS, successfully. All twenty iPBS primers produced 120 scorable bands and among them 43 bands were polymorphic indicating a low degree of genetic variability in *Crassocephalum* (Table 4.2). The sizes of reproducible and scorable bands ranged from 150 to 3,500 bp. The information from all primers, including total band number, band sizes, polymorphic band number, polymorphism percentage and mean PIC values are included in Table 4.2. Primer 2083 produced the highest number of polymorphism percentage bands (80.00%), whereas primer 2081 created the lowest number of polymorphism percentage bands (0%). Primer 2277 had the highest PIC value (0.281), while primer 2081 showed the lowest PIC value (0). The mean PIC value for these twenty primers was 0.176. These results indicate that the iPBS marker system can representative a good discriminatory capacity and reveal a wide range of genomic DNA diversity in *Crassocephalum*

Table 4.2 Characteristics of twenty iPBS primers used in the *Crassocephalum* 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)
2076	GCTCCGATGCCA	59.2	7	150-500	4	57.14	0.239
2077	CTCACGATGCCA	55.1	4	350-1,000	1	25.00	0.109
2079	AGGTGGGCGCCA	65.2	5	400-900	2	40.00	0.187
2080	CAGACGGCGCCA	63.3	6	200-750	3	50.00	0.169
2081	GCAACGGCGCCA	65.0	5	250-750	0	0.00	0.000
2083	CTTCTAGCGCCA	54.6	7	300-1,000	4	80.00	0.235
2085	ATGCCGATACCA	52.8	7	200-2,000	3	42.85	0.190
2272	GGCTCAGATGCCA	55.0	6	200-800	4	66.67	0.255
2273	GCTCATCATGCCA	56.5	6	200-900	2	33.33	0.135
2277	GGCGATGATACCA	52.0	5	200-1,600	3	60.00	0.281
2279	AATGAAAGCACCA	52.0	6	250-2,000	2	33.33	0.119
2374	CCCAGCAAACCA	53.5	5	200-2,500	3	60.00	0.242
2378	GGTCCTCATCCA	53.0	10	200-3,000	4	40.00	0.155
2380	CAACCTGATCCA	50.5	6	200,3,000	3	50.00	0.200
2382	TGTTGGCTTCCA	50.5	5	250-2,000	2	40.00	0.172
2389	ACATCCTTCCCA	50.0	5	250-800	3	60.00	0.254
2391	ATCTGTCAGCCA	52.6	8	150-800	3	37.50	0.147
2392	TAGATGGTGCCA	52.2	5	100-2,500	2	40.00	0.125
2393	TACGGTACGCCA	51.0	7	100-1,000	3	42.86	0.142
2394	GAGCCTAGGCCA	56.5	5	200-600	2	40.00	0.164
	Total		120	1500-3,000	43	35.83	0.176

4.2.2 Phylogeography of *Crassocephalum*

To elucidate the genetic relationships among *Crassocephalum* accessions, a dendrogram and grouping were constructed using Dice similarity coefficients based on the unweighted pair group analysis with the arithmetic mean (UPGMA) dendrogram and the principal component analysis (PCoA) in Past program. According to the results of the UPGMA dendrogram including all 116 samples of *Crassocephalum* in Thailand were obviously separate under three main clusters (Figure 4.4). The first cluster contained 79 accessions of *C. crepidioides*, the second cluster comprised 23 taxa of *C. rubens* and the third cluster included 14 *C. crepidioides* x *rubens*. This indicates that there was a respectable differentiation between all *Crassocephalum* genotypes species in Thailand. The results of PCoA similarly indicated three distinct groups in the taxonomic representation of the relative genetic similarity among all *Crassocephalum* samples (Figure 4.4) , supporting the results presented in the UPGMA dendrogram. The results of our study verified that *C. crepidioides* and *C. rubens* are in the different species.

4.2.3 Diversification of *Crassocephalum*

The STRUCTURE software assigns individuals to different populations based on allele frequencies of the genotypes. Genetic structure of the population was inferred through allele frequencies (tested for K = 2 to K = 10). K was estimated with the aid of posterior probability of the data for a given. In the STRUCTURE analysis, log probabilities of the data [lnP(D)] showed the highest likelihood at K=3. Therefore, STRUCTURE analysis was conducted for K = 3 and suggesting four clusters for 116 *Crassocephalum* genotypes as shown in Figure 4.3. At K = 3, *Crassocephalum* samples were separated into three clusters. Among Crassocephalum populations in Thailand, *C. crepidioides* individuals were largely assigned to one cluster, *C. rubens* were largely assigned to the second cluster, and *C. crepidioides* x *rubens* showed evidence of extensive admixture (Figure 4.6). Based on the latter model, all populations from Thailand were mixed for cluster I (‘green and red’), cluster II (‘blue’) and cluster III (‘green, red and blue’) was in Thailand populations. The clustering results by UPGMA, PCoA and STRUCTURE at both the inter- and intraspecific levels, were highly concordant (Figure 4.6). It was concluded based on present findings that iPBS markers could reliably be used in phylogenetic and diversification analysis of *Crassocephalum* genotypes.

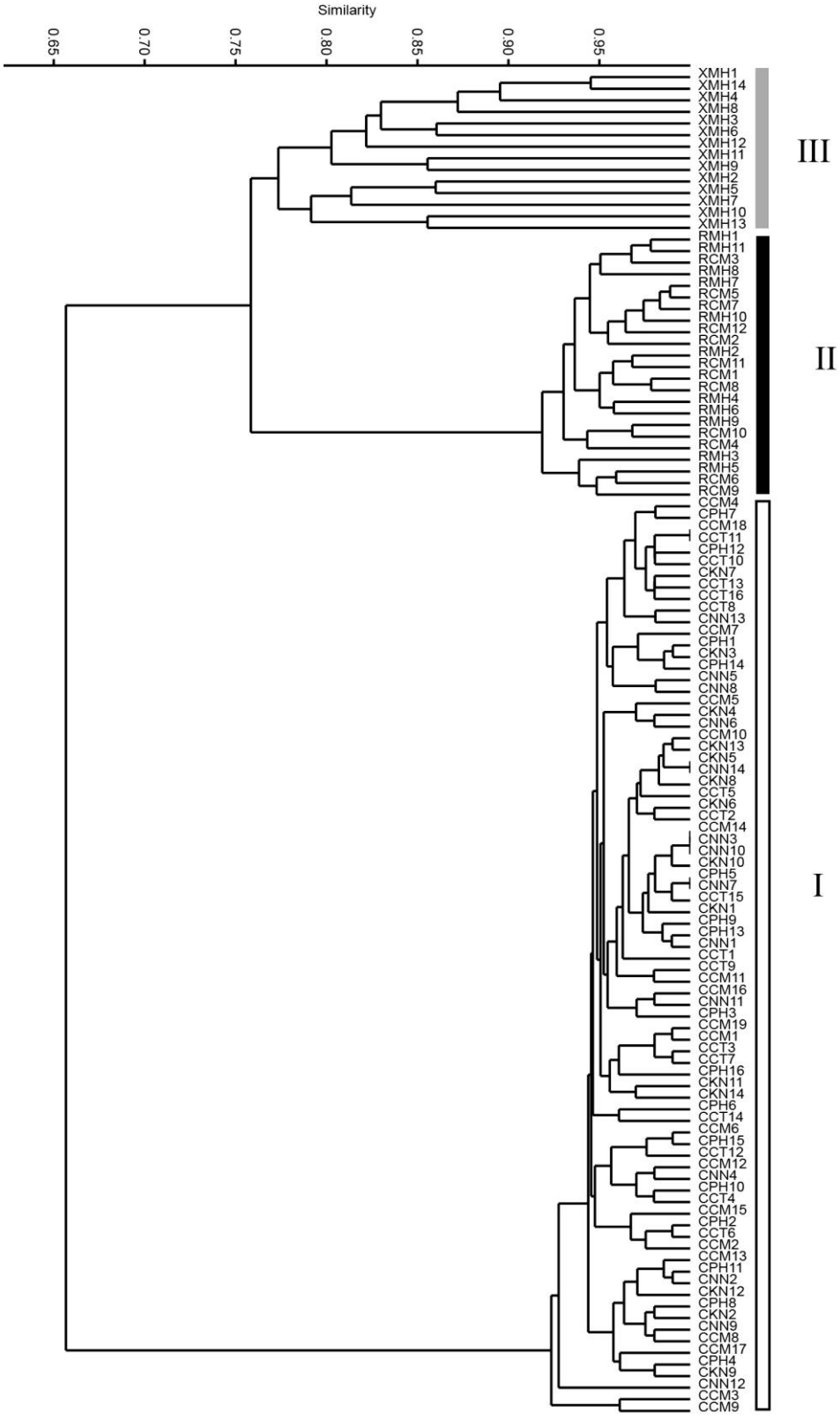

Figure 4.4 Dendrogram of 116 *Crassocephalum* accessions based on iPBS markers according to UPGMA with the Dice similarity index.

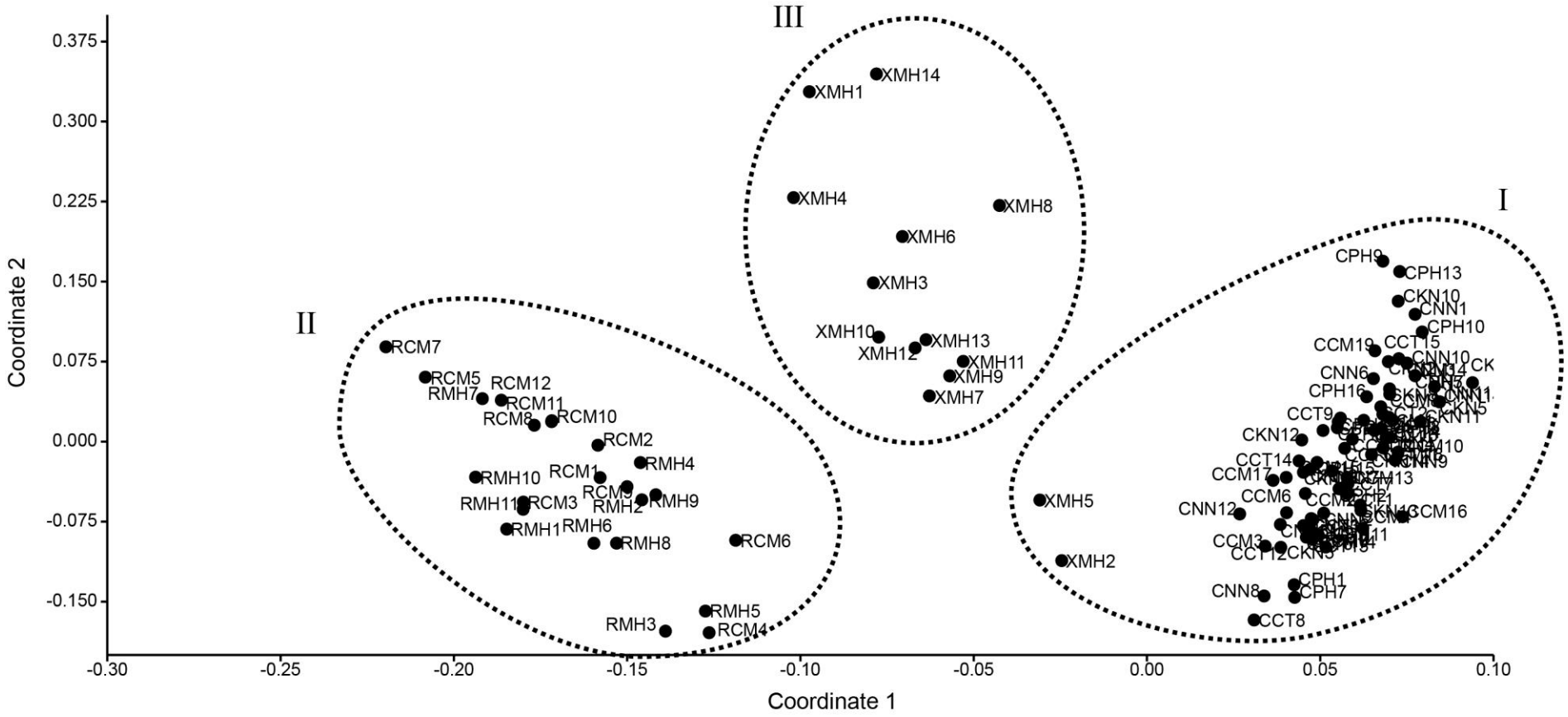

Figure 4.5 PCoA of 116 *Crassocephalum* accessions based on 20 iPBS markers.

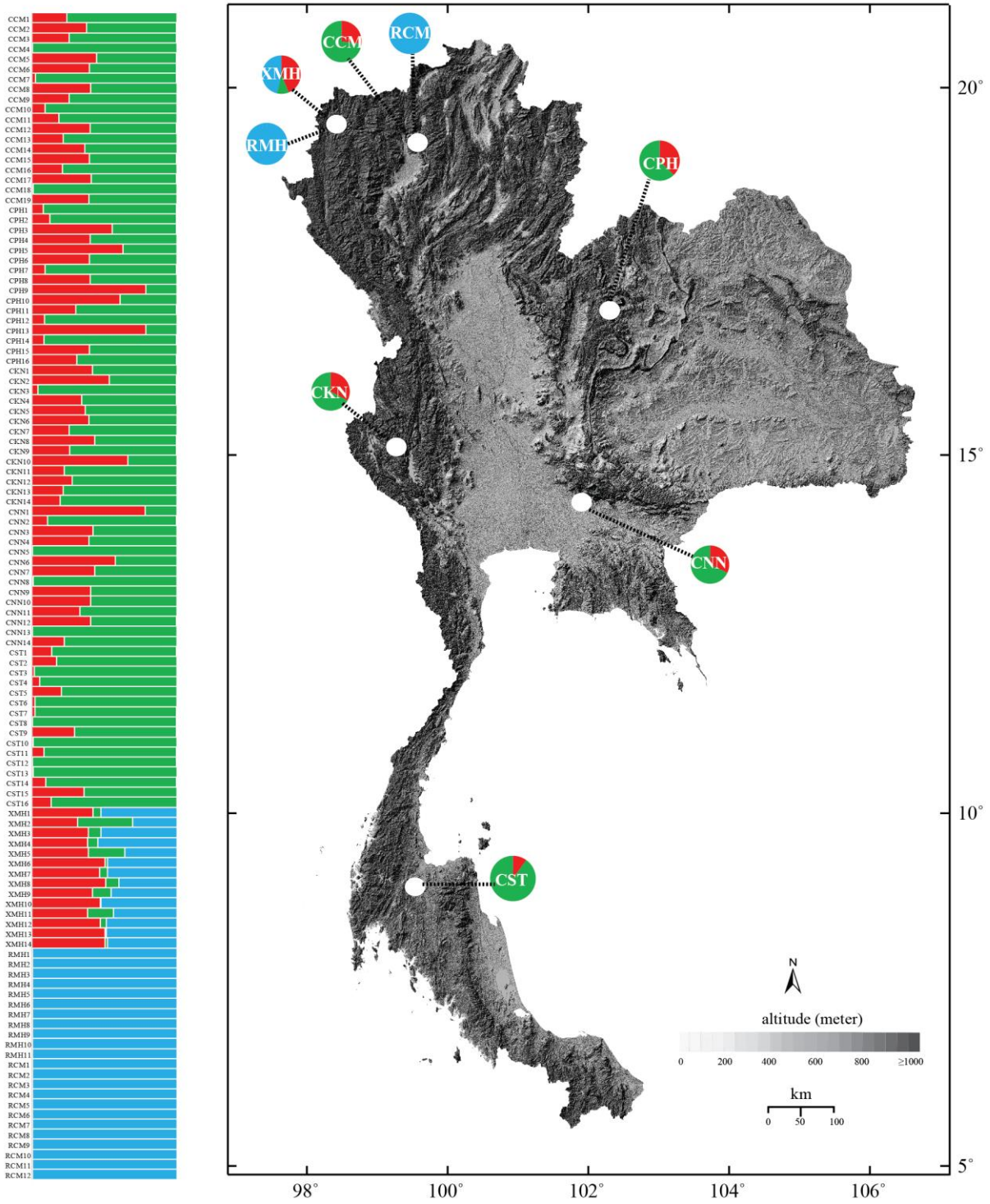

Figure 4.6 Genetic structure of 116 *Crassocephalum* accessions inferred by STRUCTURE software with 20 iPBS marker data sets. Single vertical line represents an individual accession, and different colors represent genetic stocks/gene pools. Segments of each vertical line show extent of admixture in an individual.

4.3 Phylogeny and diversification of *Emilia*

4.3.1 iPBS polymorphisms in *Emilia*

Total 205 individuals from 12 populations of *Emilia* were genotyped with iPBS, successfully. All twenty iPBS primers produced 120 scorable bands and among them 81 bands were polymorphic indicating a high degree of genetic variability in *Emilia* (Table 4.3). The sizes of reproducible and scorable bands ranged from 150 to 3,500 bp. The information from all primers, including total band number, band sizes, polymorphic band number, polymorphism percentage and mean PIC values are included in Table 4.3. Primer 2080 produced the highest number of polymorphism percentage bands (83.33%), whereas primer 2394 created the lowest number of polymorphism percentage bands (40%). Primer 2 076 had the highest PIC value (0.341), while primer 2394 showed the lowest PIC value (0.153). The mean PIC value for these twenty primers was 0. 226. These results indicate that the iPBS marker system can representative a good discriminatory capacity and reveal a wide range of genomic DNA diversity in *Emilia.*

Table 4.3 Characteristics of twenty iPBS primers used in the *Emilia* 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)
2076	GCTCCGATGCCA	59.2	7	150-500	6	85.71	0.341
2077	CTCACGATGCCA	55.1	4	350-1,000	3	75.00	0.221
2079	AGGTGGGCGCCA	65.2	5	400-900	4	80.00	0.310
2080	CAGACGGCGCCA	63.3	6	200-750	5	83.33	0.279
2081	GCAACGGCGCCA	65.0	5	250-750	4	80.00	0.253
2083	CTTCTAGCGCCA	54.6	7	300-1,000	5	71.42	0.257
2085	ATGCCGATACCA	52.8	7	200-2,000	4	57.14	0.168
2272	GGCTCAGATGCCA	55.0	6	200-800	4	66.67	0.237
2273	GCTCATCATGCCA	56.5	6	200-900	4	66.67	0.243
2277	GGCGATGATACCA	52.0	5	200-1,600	4	80.00	0.232
2279	AATGAAAGCACCA	52.0	6	250-2,000	3	50.00	0.160
2374	CCCAGCAAACCA	53.5	5	200-2,500	4	80.00	0.256
2378	GGTCCTCATCCA	53.0	10	200-3,000	7	70.00	0.224
2380	CAACCTGATCCA	50.5	6	200,3,000	3	50.00	0.160
2382	TGTTGGCTTCCA	50.5	5	250-2,000	4	80.00	0.256
2389	ACATCCTTCCCA	50.0	5	250-800	3	60.00	0.192
2391	ATCTGTCAGCCA	52.6	8	150-800	5	62.50	0.200
2392	TAGATGGTGCCA	52.2	5	100-2,500	3	60.00	0.192
2393	TACGGTACGCCA	51.0	7	100-1,000	4	57.14	0.183
2394	GAGCCTAGGCCA	56.5	5	200-600	2	40.00	0.153
	Total		120	1500-3,000	81	67.50	0.226

4.3.2 Phylogeography of *Emilia*

To elucidate the genetic relationships among *Emilia* accessions, a dendrogram and grouping were constructed using Dice similarity coefficients based on the unweighted pair group analysis with the arithmetic mean (UPGMA) dendrogram and the principal component analysis (PCoA) in Past program. According to the results of the UPGMA dendrogram including all 205 samples of *Emilia* in Thailand were obviously separate under two main clusters (Figure 4.7). The first cluster contained 41 taxa of *E. prenanthoidea*, the second cluster comprised 146 accessions of *E. sonchifolia* and 18 accessions *E. khaopawtaensis*. This indicates that there was a respectable differentiation between all *Emilia* genotypes species in Thailand. The results of PCoA similarly indicated two distinct groups in the taxonomic representation of the relative genetic similarity among all *Emilia* samples (Figure 4.8), supporting the results presented in the UPGMA dendrogram. The results of our study verified that *E. prenanthoidea* and *E. sonchifolia* are in the different species. Nevertheless, endemic *E. prenanthoidea* is related to commonly distributed *E. sonchifolia*.

4.3.3 Diversification of *Emilia*

The STRUCTURE software assigns individuals to different populations based on allele frequencies of the genotypes. Genetic structure of the population was inferred through allele frequencies (tested for K = 2 to K = 10). K was estimated with the aid of posterior probability of the data for a given. In the STRUCTURE analysis, log probabilities of the data [lnP(D)] showed the highest likelihood at K=3. Therefore, STRUCTURE analysis was conducted for K = 3 and suggesting four clusters for 205 *Emilia* genotypes as shown in Figure 4.9. At K = 3, *Emilia* samples were separated into three clusters. Among *Emilia* populations in Thailand, *E. khaopawtaensis* individuals were assigned together with *E. sonchifolia* showed largely evidence of extensive admixture to one cluster, *E. prenanthoidea* were assigned to the second cluster (Figure 4.9). Based on the latter model, all populations from Thailand were mixed for cluster I (‘red’), cluster II (‘blue and red’) and cluster III (‘green’) was in Thailand populations. The clustering results by UPGMA, PCoA and STRUCTURE at both the inter- and intraspecific levels, were highly concordant (Figure 4.9). It was concluded based on present findings that iPBS markers could reliably be used in phylogenetic and diversification analysis of *Emilia* genotypes.

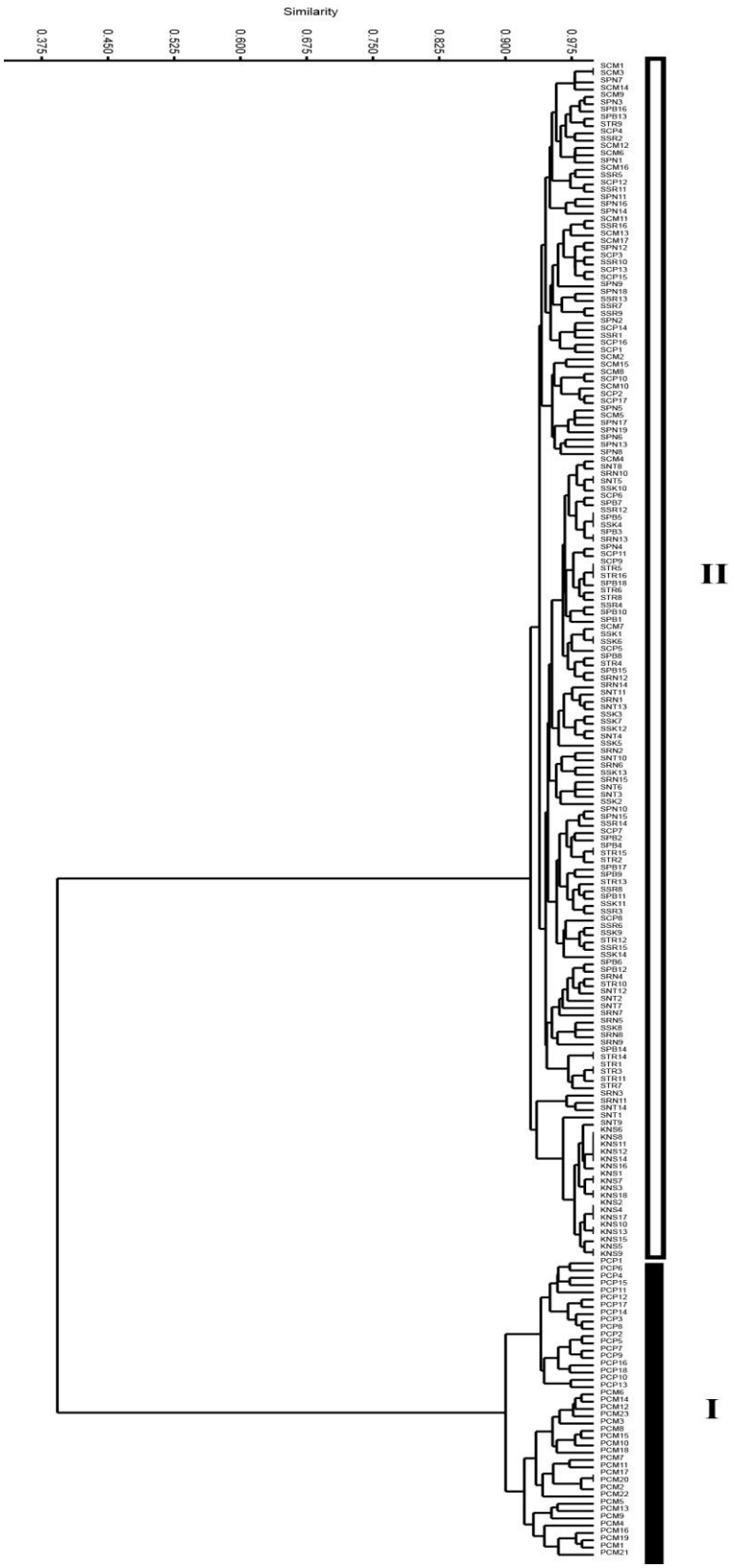

Figure 4.7 Dendrogram of 205 *Emilia* accessions based on iPBS markers according to UPGMA with the Dice similarity index

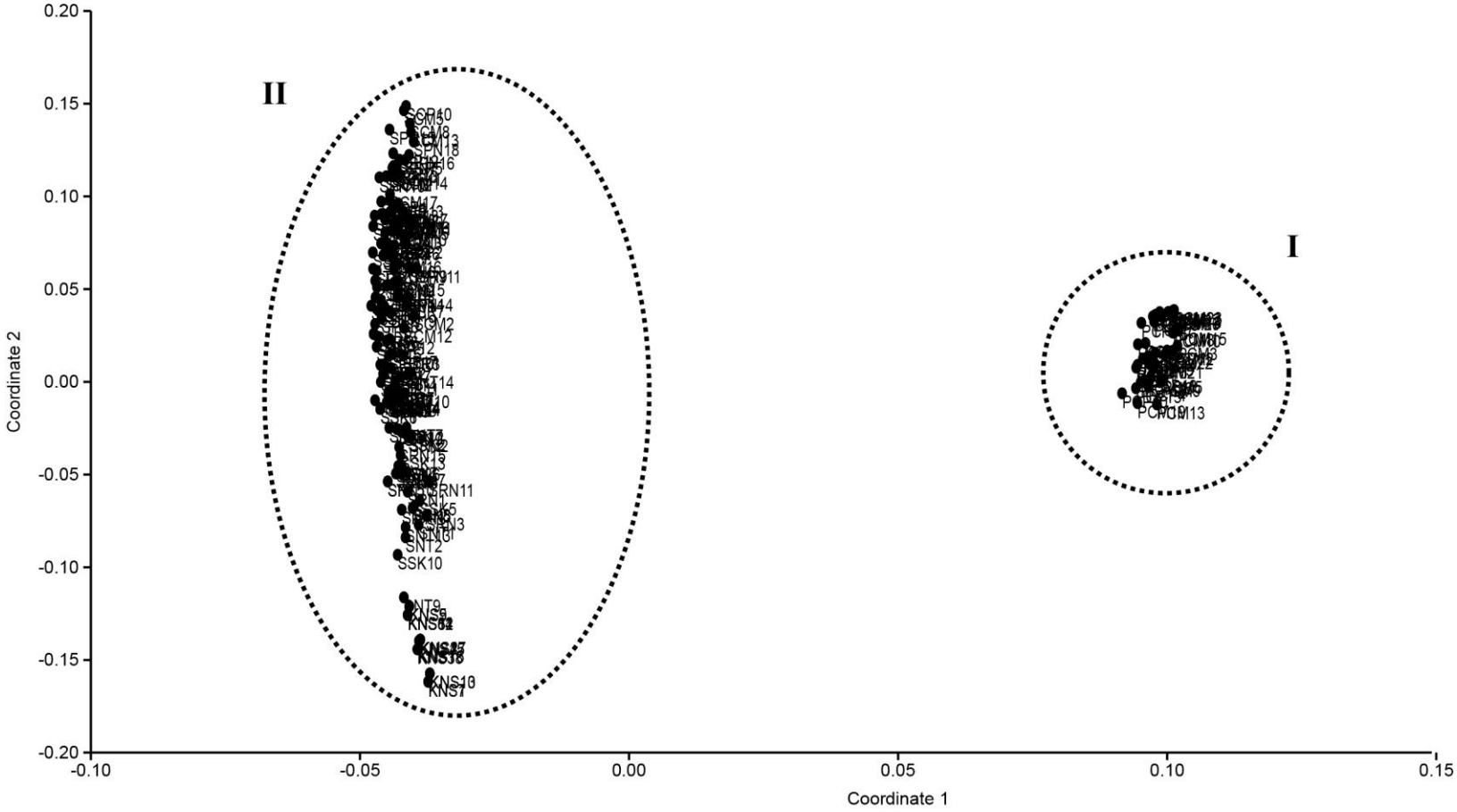

Figure 4.8 PCoA of 205 *Emilia* accessions based on 20 iPBS markers

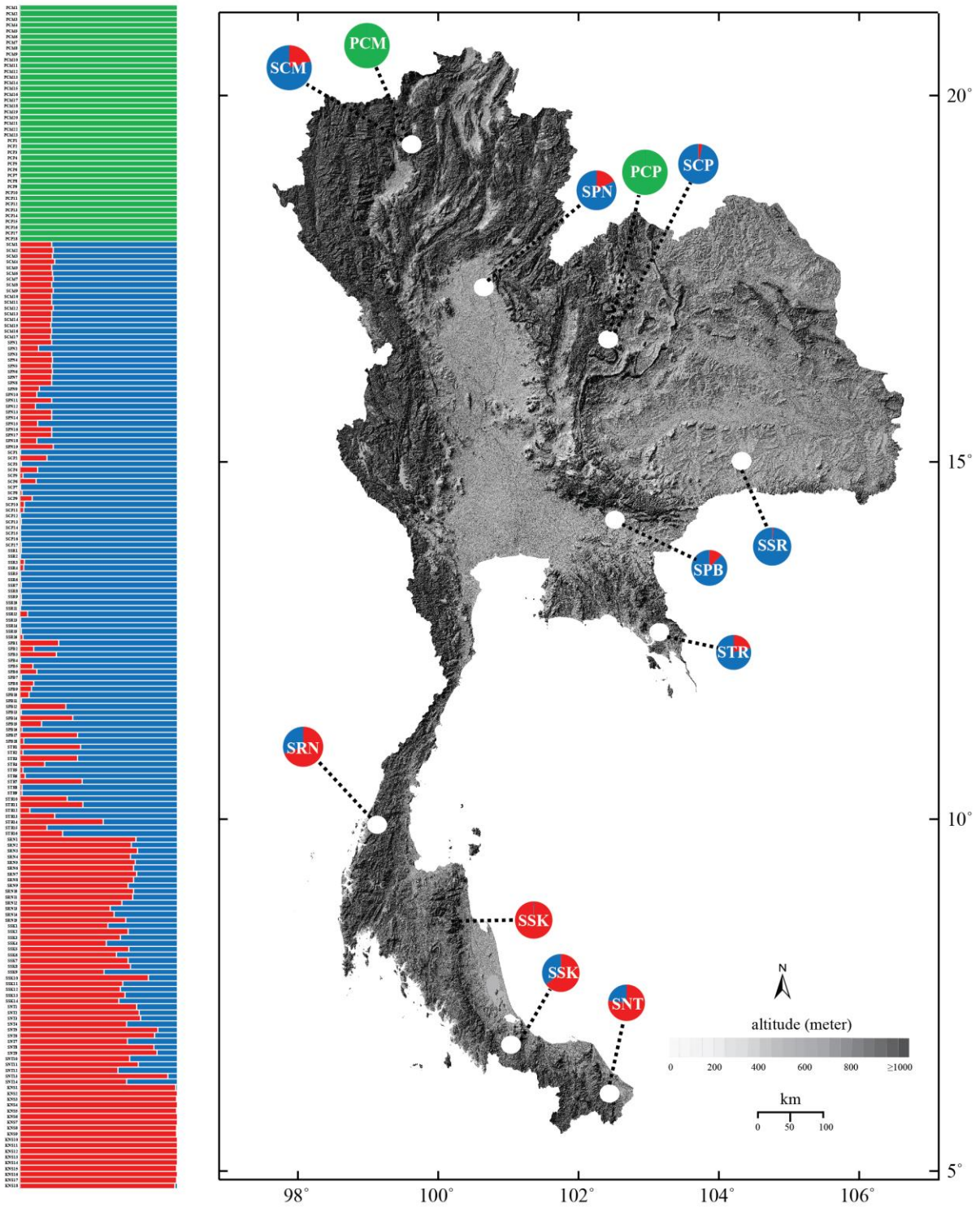

Figure 4.9 Genetic structure of 205 *Emilia* accessions inferred by STRUCTURE software with 20 iPBS marker data sets. Single vertical line represents an individual accession, and different colors represent genetic stocks/gene pools. Segments of each vertical line show extent of admixture in an individual.

4.4 Phylogeny and diversification of *Erechtites*

4.4.1 iPBS polymorphisms in *Erechtites*

Overall 128 individuals from 6 populations of *Erechtites* were genotyped with iPBS, successfully. All twenty iPBS primers produced 120 scorable bands and among them 83 bands were polymorphic indicating a high degree of genetic variability in *Erechtites* (Table 4.4). The sizes of reproducible and scorable bands ranged from 150 to 3,500 bp. The information from all primers, including total band number, band sizes, polymorphic band number, polymorphism percentage and mean PIC values are included in Table 4.3. Primer 2083 and 2085 produced the highest number of polymorphism percentage bands (85.71%), whereas primer 2392 created the lowest number of polymorphism percentage bands (40.00%). Primer 2076 had the highest PIC value (0 .296), while primer 2380 showed the lowest PIC value (0.147). The mean PIC value for these twenty primers was 0.225. These results indicate that the iPBS marker system can representative a good discriminatory capacity and reveal a wide range of genomic DNA diversity in *Erechtites*.

Table 4.4 Characteristics of twenty iPBS primers used in the *Erechtites* 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)
2076	GCTCCGATGCCA	59.2	7	150-500	5	71.43	0.296
2077	CTCACGATGCCA	55.1	4	350-1,000	3	75.00	0.270
2079	AGGTGGGCGCCA	65.2	5	400-900	3	60.00	0.216
2080	CAGACGGCGCCA	63.3	6	200-750	5	83.33	0.279
2081	GCAACGGCGCCA	65.0	5	250-750	4	80.00	0.236
2083	CTTCTAGCGCCA	54.6	7	300-1,000	6	85.71	0.253
2085	ATGCCGATACCA	52.8	7	200-2,000	6	85.71	0.253
2272	GGCTCAGATGCCA	55.0	6	200-800	5	83.33	0.246
2273	GCTCATCATGCCA	56.5	6	200-900	4	66.66	0.197
2277	GGCGATGATACCA	52.0	5	200-1,600	4	80.00	0.236
2279	AATGAAAGCACCA	52.0	6	250-2,000	4	66.67	0.197
2374	CCCAGCAAACCA	53.5	5	200-2,500	4	80.00	0.236
2378	GGTCCTCATCCA	53.0	10	200-3,000	6	60.00	0.177
2380	CAACCTGATCCA	50.5	6	200,3,000	3	50.00	0.147
2382	TGTTGGCTTCCA	50.5	5	250-2,000	3	50.00	0.177
2389	ACATCCTTCCCA	50.0	5	250-800	3	60.00	0.177
2391	ATCTGTCAGCCA	52.6	8	150-800	5	62.50	0.225
2392	TAGATGGTGCCA	52.2	5	100-2,500	2	40.00	0.155
2393	TACGGTACGCCA	51.0	7	100-1,000	5	71.43	0.269
2394	GAGCCTAGGCCA	56.5	5	200-600	3	60.00	0.253
	Total		120	1500-3,000	83	69.17	0.225

4.4.2 Phylogeography of *Erechtites*

To elucidate the genetic relationships among *Erechtites* accessions, a dendrogram and grouping were constructed using Dice similarity coefficients based on the unweighted pair group analysis with the arithmetic mean (UPGMA) dendrogram and the principal component analysis (PCoA) in Past program. According to the results of the UPGMA dendrogram including all 128 samples of *Erechtites* in Thailand were obviously separate under two main clusters (Figure 4.10). The first cluster contained 105 taxa of *E. hieraciifolius*, the second cluster comprised 23 accessions of *E. valerianifolius*. This indicates that there was a respectable differentiation between all *Erechtites* genotypes species in Thailand. The results of PCoA similarly indicated two distinct groups in the taxonomic representation of the relative genetic similarity among all *Erec**htites*** samples (Figure 4.11), supporting the results presented in the UPGMA dendrogram. The results of our study verified that *E. hieraciifolius* and *E. valerianifolius* are in the different species.

4.4.3 Diversification of *Erechtites*

The STRUCTURE software assigns individuals to different populations based on allele frequencies of the genotypes. Genetic structure of the population was inferred through allele frequencies (tested for K = 2 to K = 10). K was estimated with the aid of posterior probability of the data for a given. In the STRUCTURE analysis, log probabilities of the data [lnP(D)] showed the highest likelihood at K=2. Therefore, STRUCTURE analysis was conducted for K = 2 and suggesting two clusters for 128 *Erechtites* genotypes as shown in Figure 4.12. At K = 2, *Erechtites* samples were separated into two clusters. Among *Erechtites* populations in Thailand, *E. hieraciifolius* individuals were assigned showed largely evidence to one cluster, *E. valerianifolius* were assigned to the second cluster (Figure 4.12). Based on the latter model, all populations from Thailand were mixed for cluster I (‘blue’) and cluster II (‘red’) was in Thailand populations. The clustering results by UPGMA, PCoA and STRUCTURE at both the inter- and intraspecific levels, were highly concordant (Figure 4.9). It was concluded based on present findings that iPBS markers could reliably be used in phylogenetic and diversification analysis of *Erechtites* genotypes.

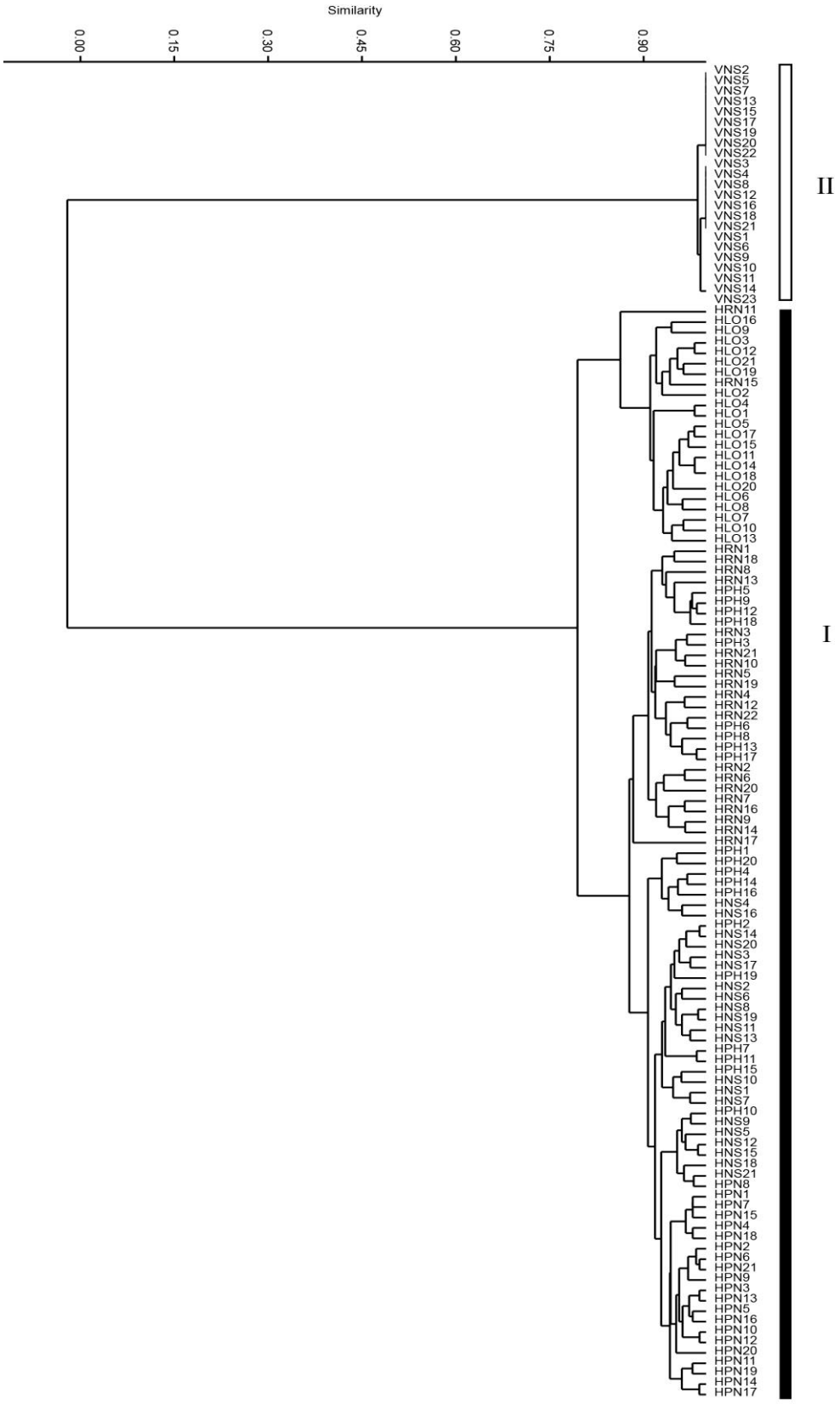

Figure 4.10 Dendrogram of 128 *Erechtites* accessions based on iPBS markers according to UPGMA with the Dice similarity index

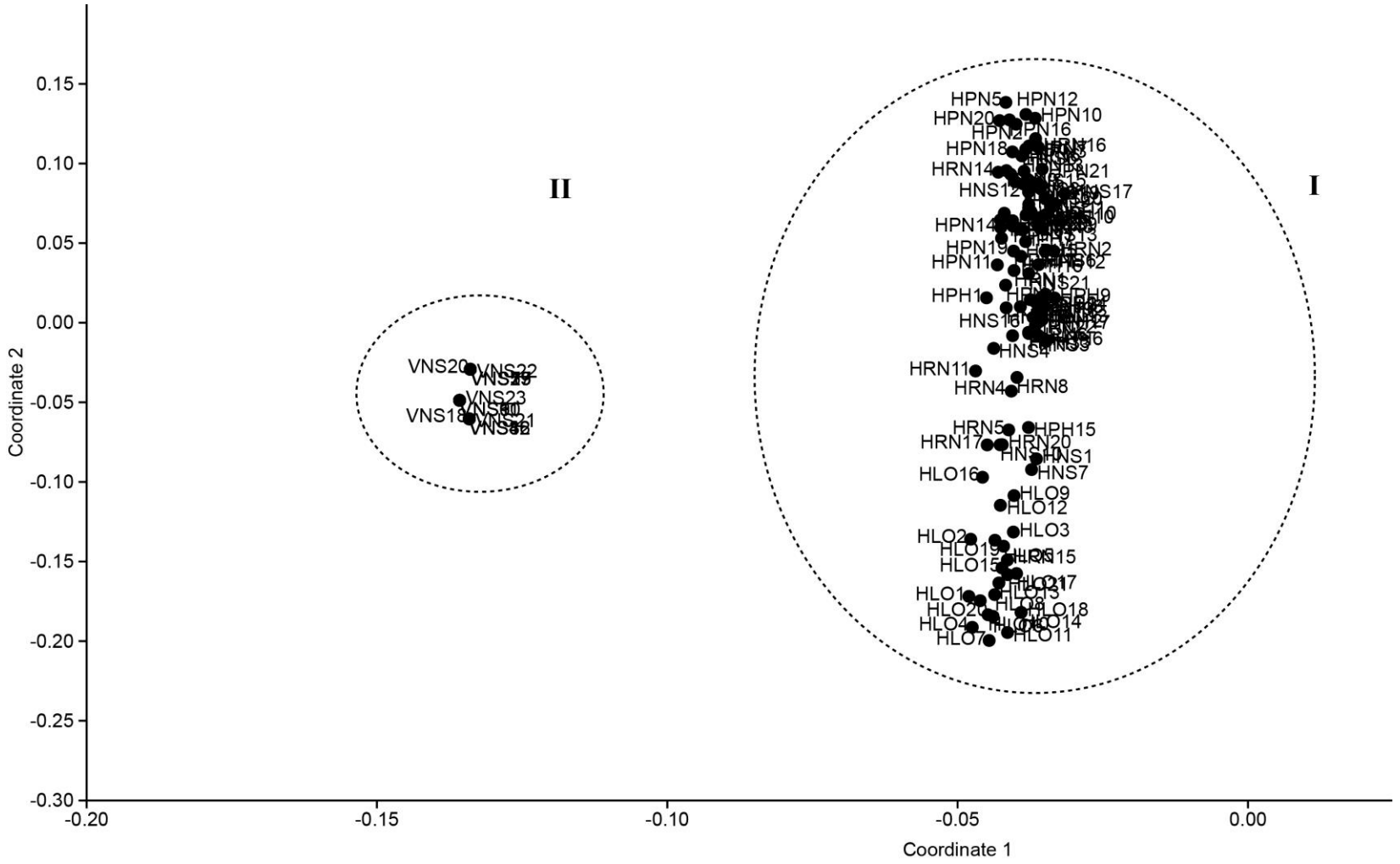

Figure 4.11 PCoA of 128 *Erechtites* accessions based on 20 iPBS markers

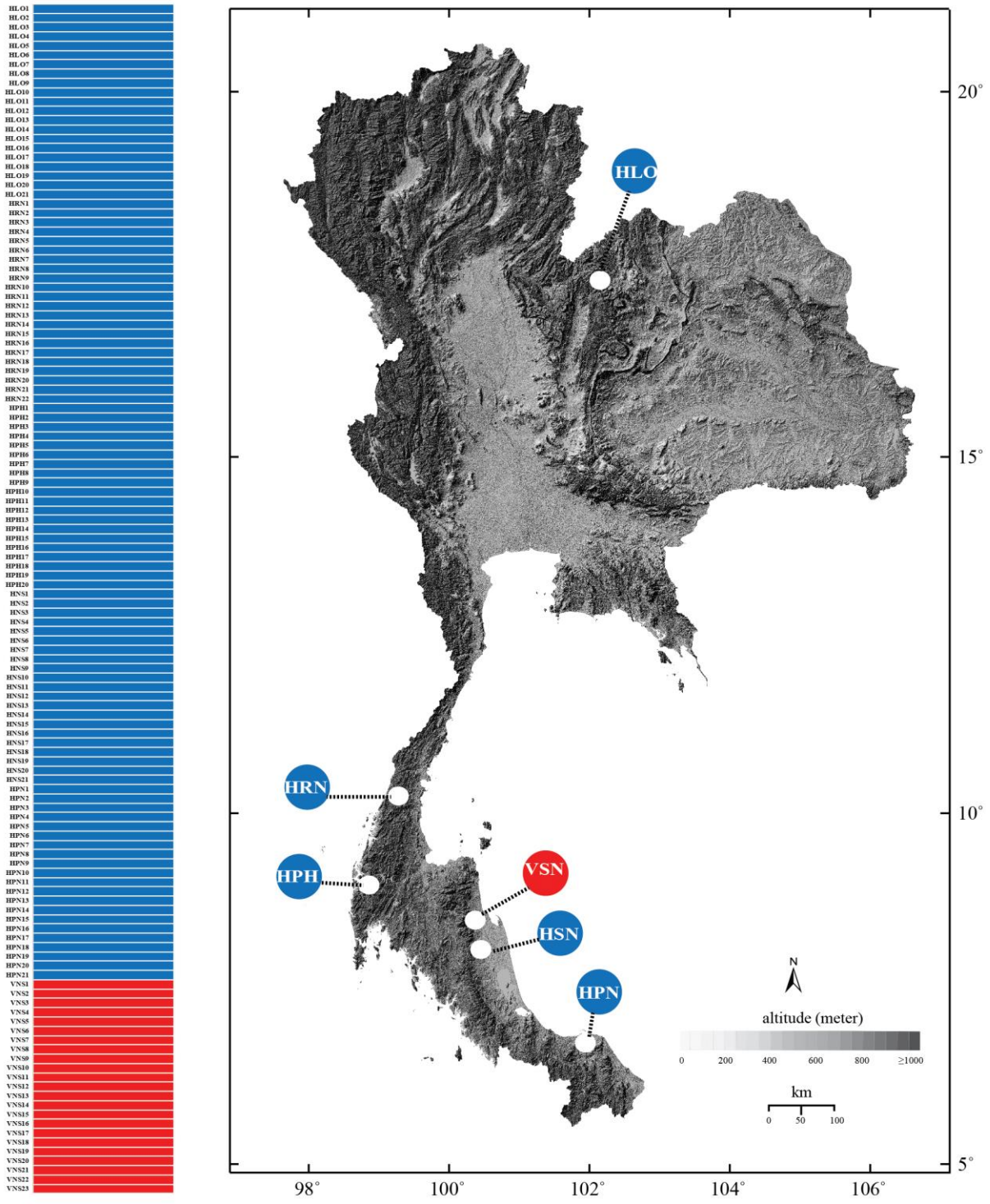

Figure 4.12 Genetic structure of 128 *Erechtites* accessions inferred by STRUCTURE software with 20 iPBS marker data sets. Single vertical line represents an individual accession, and different colors represent genetic stocks/gene pools. Segments of each vertical line show extent of admixture in an individual.

4.5 Phylogeny and diversification of *Gynura*

4.5.1 iPBS polymorphisms in *Gynura*

Overall 141 individuals from 8 populations of *Gynura* were genotyped with iPBS, successfully. All twenty iPBS primers produced 120 scorable bands and among them 113 bands were polymorphic indicating a high degree of genetic variability in *Gynura* (Table 4.5). The sizes of reproducible and scorable bands ranged from 150 to 3,500 bp. The information from all primers, including total band number, band sizes, polymorphic band number, polymorphism percentage and mean PIC values are included in Table 4.5. About 15 primers produced the highest number of polymorphism percentage bands (100%), whereas primer 2279 created the lowest number of polymorphism percentage bands (66.57%). Primer 2 378 and 2380 had the highest PIC value (0 .463), while primer 2279 showed the lowest PIC value (0.301). The mean PIC value for these twenty primers was 0.407. These results indicate that the iPBS marker system can representative a good discriminatory capacity and reveal a wide range of genomic DNA diversity in *Gynura*.

Table 4.5 Characteristics of twenty iPBS primers used in the *Gynura* 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)
2076	GCTCCGATGCCA	59.2	7	150-500	7	100.00	0.437
2077	CTCACGATGCCA	55.1	4	350-1,000	4	100.00	0.414
2079	AGGTGGGCGCCA	65.2	5	400-900	5	100.00	0.386
2080	CAGACGGCGCCA	63.3	6	200-750	5	83.33	0.365
2081	GCAACGGCGCCA	65.0	5	250-750	5	100.00	0.360
2083	CTTCTAGCGCCA	54.6	7	300-1,000	7	100.00	0.423
2085	ATGCCGATACCA	52.8	7	200-2,000	5	71.42	0.328
2272	GGCTCAGATGCCA	55.0	6	200-800	6	100.00	0.426
2273	GCTCATCATGCCA	56.5	6	200-900	6	100.00	0.383
2277	GGCGATGATACCA	52.0	5	200-1,600	4	80.00	0.397
2279	AATGAAAGCACCA	52.0	6	250-2,000	4	66.67	0.301
2374	CCCAGCAAACCA	53.5	5	200-2,500	5	100.00	0.444
2378	GGTCCTCATCCA	53.0	10	200-3,000	10	100.00	0.463
2380	CAACCTGATCCA	50.5	6	200,3,000	6	100.00	0.463
2382	TGTTGGCTTCCA	50.5	5	250-2,000	5	100.00	0.435
2389	ACATCCTTCCCA	50.0	5	250-800	5	100.00	0.454
2391	ATCTGTCAGCCA	52.6	8	150-800	8	100.00	0.423
2392	TAGATGGTGCCA	52.2	5	100-2,500	5	100.00	0.427
2393	TACGGTACGCCA	51.0	7	100-1,000	6	85.71	0.387
2394	GAGCCTAGGCCA	56.5	5	200-600	5	100.00	0.433
	Total		120	1500-3,000	113	94.17	0.407

4.5.2 Phylogeography of *Gynura*

To elucidate the genetic relationships among *Gynura* accessions, a dendrogram and grouping were constructed using Dice similarity coefficients based on the unweighted pair group analysis with the arithmetic mean (UPGMA) dendrogram and the principal component analysis (PCoA) in Past program. According to the results of the UPGMA dendrogram including all 141 samples of *Gynura* in Thailand were obviously separate under eight main clusters (Figure 4.13). Cluster I comprised 21 accessions of *G. calciphila*. Cluster II contained about 13 samples of *G. pseudochina*. Cluster III included 16 accessions of *G. siamensis*. Cluster IV contained 16. G. intergrifolia. Cluster V comprised 20 of *G. divaricate*. Cluster VI included 20 accessions of *G. cusimbua*. Cluster VII comprised 18 samples of *G. procumbens*. Cluster VIII contained 17 accessions of *G. longifolia*. This indicates that there was a respectable differentiation between all *Gynura* genotypes species in Thailand. The results of PCoA similarly indicated eight distinct groups in the taxonomic representation of the relative genetic similarity among all *Gynura* samples (Figure 4.14), supporting the results presented in the UPGMA dendrogram. The results of our study verified that *Gynura* are in the different eight species.

4.4.3 Diversification of *Gynura*

The STRUCTURE software assigns individuals to different populations based on allele frequencies of the genotypes. Genetic structure of the population was inferred through allele frequencies (tested for K = 2 to K = 10). K was estimated with the aid of posterior probability of the data for a given. In the STRUCTURE analysis, log probabilities of the data [lnP(D)] showed the highest likelihood at K=4. Therefore, STRUCTURE analysis was conducted for K = 4 and suggesting four clusters for 128 *Gynura* genotypes as shown in Figure 4.15. At K = 4, *Gynura* samples were separated into four clusters. Among *Gynura* populations in Thailand. Based on the latter model, all populations from Thailand were mixed for cluster I (‘purple’), cluster II (‘green’), cluster III (‘red’) and cluster VI (‘blur’) was in Thailand populations. The clustering results by UPGMA, PCoA and STRUCTURE at both the inter- and intraspecific levels, were highly concordant (Figure 4.15). It was concluded based on present findings that iPBS markers could reliably be used in phylogenetic and diversification analysis of *Gynura* genotypes.

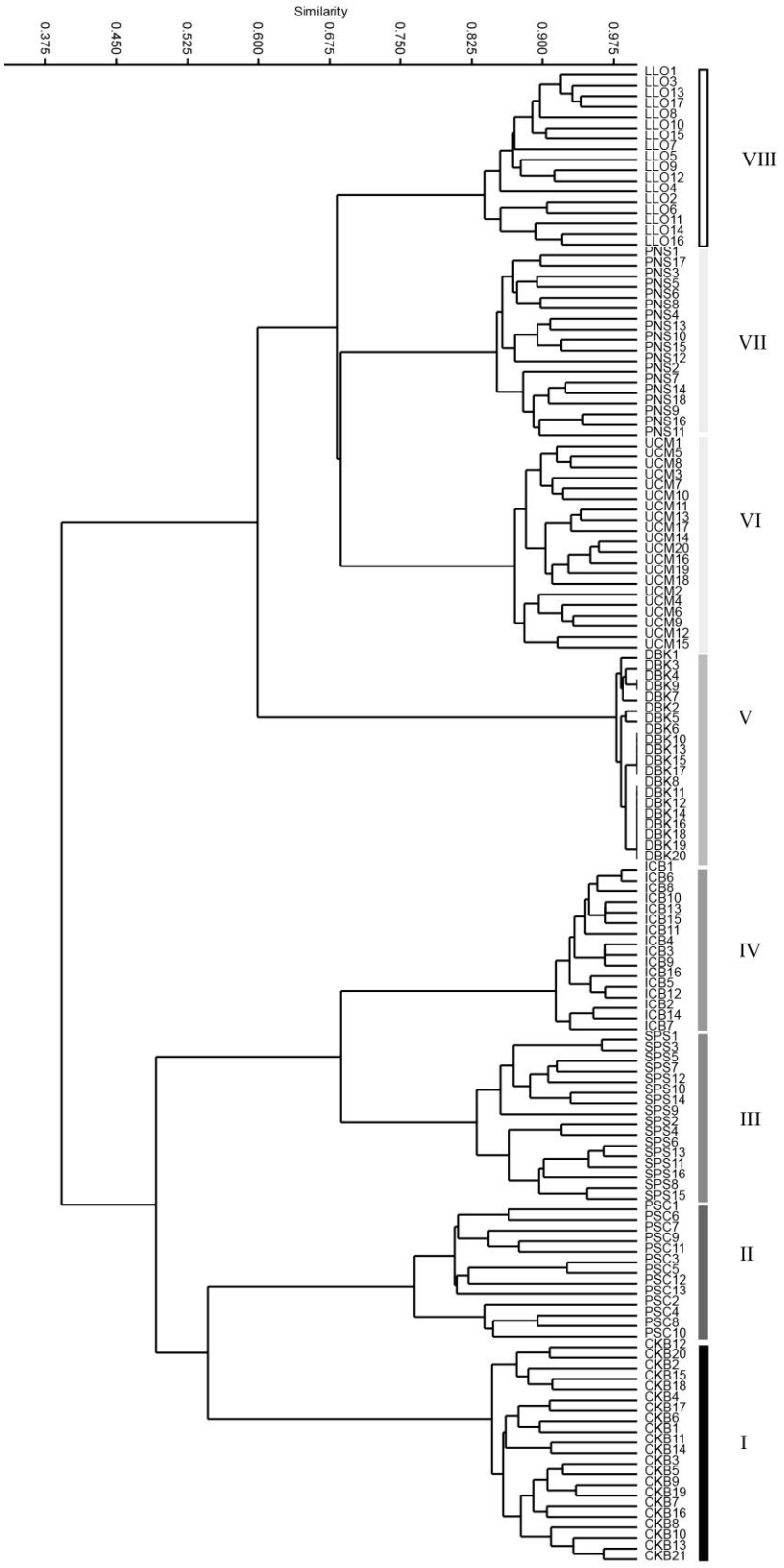

Figure 4.13 Dendrogram of 141 *Gynura* accessions based on iPBS markers according to UPGMA with the Dice similarity index

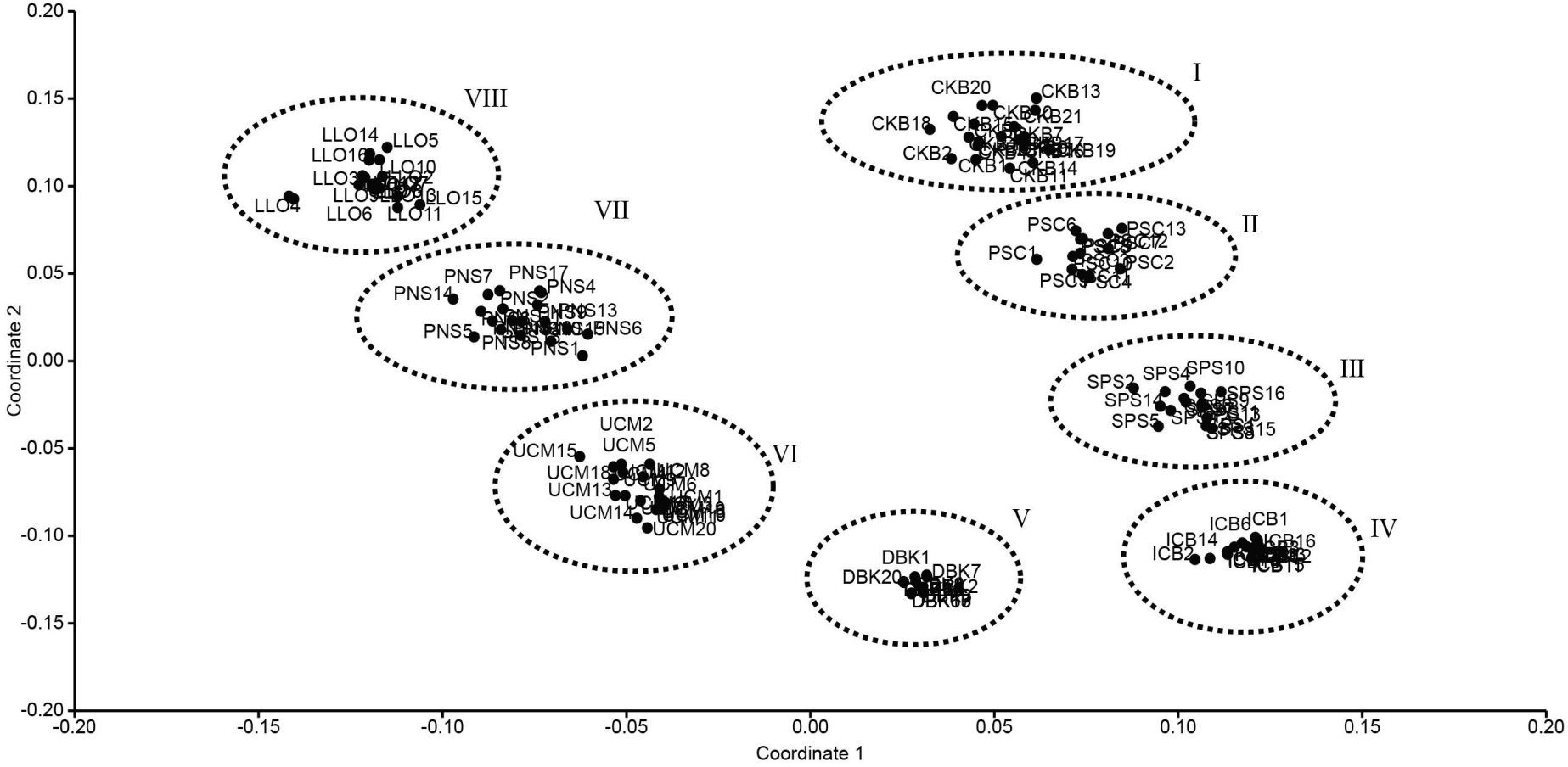

Figure 4.14 PCoA of 141 *Gynura* accessions based on 20 iPBS markers

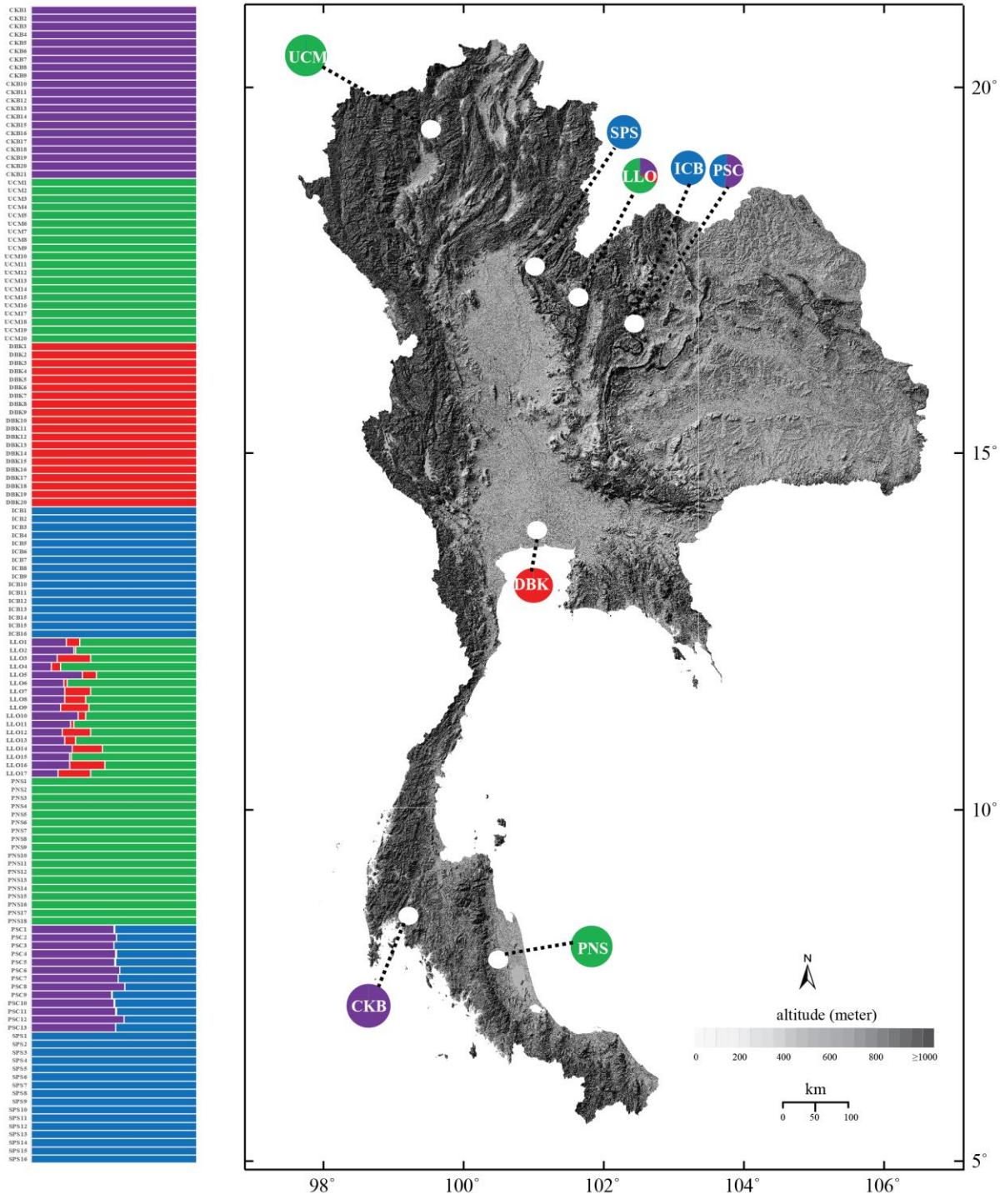

Figure 4.15 Genetic structure of 141 *Gynura* accessions inferred by STRUCTURE software with 20 iPBS marker data sets. Single vertical line represents an individual accession, and different colors represent genetic stocks/gene pools. Segments of each vertical line show extent of admixture in an individual.

4.6 Phylogeny and diversification of *Kleinia*

4.6.1 iPBS polymorphisms in *Kleinia*

Overall 61 individuals from 6 populations of *Kleinia* were genotyped with iPBS, effectively. All twenty iPBS primers produced 120 scorable bands and among them 41 bands were polymorphic indicating a low degree of genetic variability in *Kleinia* (Table 4.6). The sizes of reproducible and scorable bands ranged from 150 to 3,500 bp. The information from all primers, including total band number, band sizes, polymorphic band number, polymorphism percentage and mean PIC values are included in Table 4.6. Primer 2076 produced the highest number of polymorphism percentage bands (57.14%), whereas primer 2081, 2277, 2374, 2382 and 2389 formed the lowest number of polymorphism percentage bands (20%). Primer 2076 had the highest PIC value (0.244), while primer 2077 showed the lowest PIC value (0.088). The mean PIC value for these twenty primers was 0.149. These results indicate that the iPBS marker system can representative a good discriminatory capacity and reveal a wide range of genomic DNA diversity in *Kleinia*.

Table 4.6 Characteristics of twenty iPBS primers used in the *Kleinia* 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)
2076	GCTCCGATGCCA	59.2	7	150-500	4	57.14	0.244
2077	CTCACGATGCCA	55.1	4	350-1,000	1	25.00	0.088
2079	AGGTGGGCGCCA	65.2	5	400-900	2	40.00	0.189
2080	CAGACGGCGCCA	63.3	6	200-750	2	40.00	0.163
2081	GCAACGGCGCCA	65.0	5	250-750	1	20.00	0.099
2083	CTTCTAGCGCCA	54.6	7	300-1,000	3	42.86	0.185
2085	ATGCCGATACCA	52.8	7	200-2,000	2	28.57	0.122
2272	GGCTCAGATGCCA	55.0	6	200-800	3	50.00	0.220
2273	GCTCATCATGCCA	56.5	6	200-900	2	33.33	0.157
2277	GGCGATGATACCA	52.0	5	200-1,600	1	20.00	0.094
2279	AATGAAAGCACCA	52.0	6	250-2,000	2	33.33	0.155
2374	CCCAGCAAACCA	53.5	5	200-2,500	1	20.00	0.088
2378	GGTCCTCATCCA	53.0	10	200-3,000	3	30.00	0.138
2380	CAACCTGATCCA	50.5	6	200,3,000	3	50.00	0.207
2382	TGTTGGCTTCCA	50.5	5	250-2,000	1	20.00	0.092
2389	ACATCCTTCCCA	50.0	5	250-800	1	20.00	0.094
2391	ATCTGTCAGCCA	52.6	8	150-800	3	37.50	0.168
2392	TAGATGGTGCCA	52.2	5	100-2,500	2	40.00	0.147
2393	TACGGTACGCCA	51.0	7	100-1,000	2	28.57	0.134
2394	GAGCCTAGGCCA	56.5	5	200-600	2	40.00	0.191
	Total		120	1500-3,000	41	34.17	0.149

4.6.2 Phylogeography of *Kleinia*

To elucidate the genetic relationships among *Kleinia* accessions, a dendrogram and grouping were constructed using Dice similarity coefficients based on the unweighted pair group analysis with the arithmetic mean (UPGMA) dendrogram and the principal component analysis (PCoA) in Past program. According to the results of the UPGMA dendrogram including all 61 samples of *Kleinia* in Thailand were clearly indicated affinity and relationship between different *Kleinia* accessions (Figure 4.16). About 61 taxa of *K. grandiflora*, within each cluster showed a close relationship. The study thus shows a variability for genetic among different accessions. This indicates that there was a respectable differentiation between all *Kleinia* genotypes species in Thailand. The results of PCoA similarly indicated the relative genetic similarity among all samples (Figure 4.17), supporting the results presented in the UPGMA dendrogram.

4.6.3 Diversification of *Kleinia*

The STRUCTURE software assigns individuals to different populations based on allele frequencies of the genotypes. Genetic structure of the population was inferred through allele frequencies (tested for K = 2 to K = 10). K was estimated with the aid of posterior probability of the data for a given. In the STRUCTURE analysis, log probabilities of the data [lnP(D)] showed the highest likelihood at K=3. Therefore, STRUCTURE analysis was conducted for K = 3 and suggesting two clusters for 61 *Kleinia* genotypes as shown in Figure 4.18. At K = 3, *Kleinia* samples were extensive admixture to one cluster. Among *Kleinia* populations in Thailand, *E. hieraciifolius* individuals were assigned showed largely evidence to one cluster (Figure 4.18). Based on the latter model, all populations from Thailand were mixed for cluster I (‘red’) and cluster II (‘green’) and cluster III (‘blue’) was in Thailand populations. The clustering results by UPGMA, PCoA and STRUCTURE at both the inter- and intraspecific levels, were highly concordant (Figure 4.18). It was concluded based on present findings that iPBS markers could reliably be used in phylogenetic and diversification analysis of *Kleinia* genotypes.

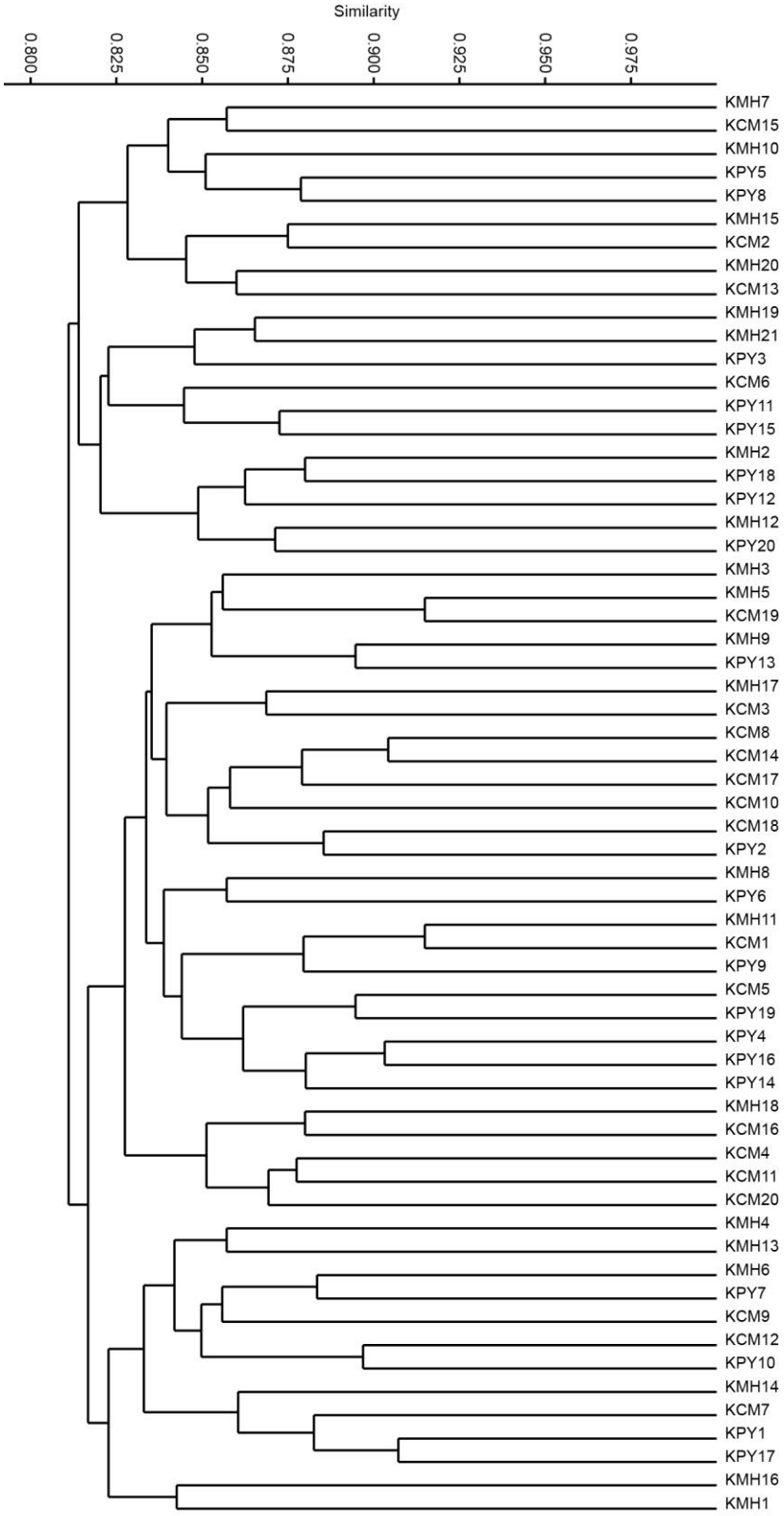

Figure 4.16 Dendrogram of 61 *Kleinia* accessions based on iPBS markers according to UPGMA with the Dice similarity index

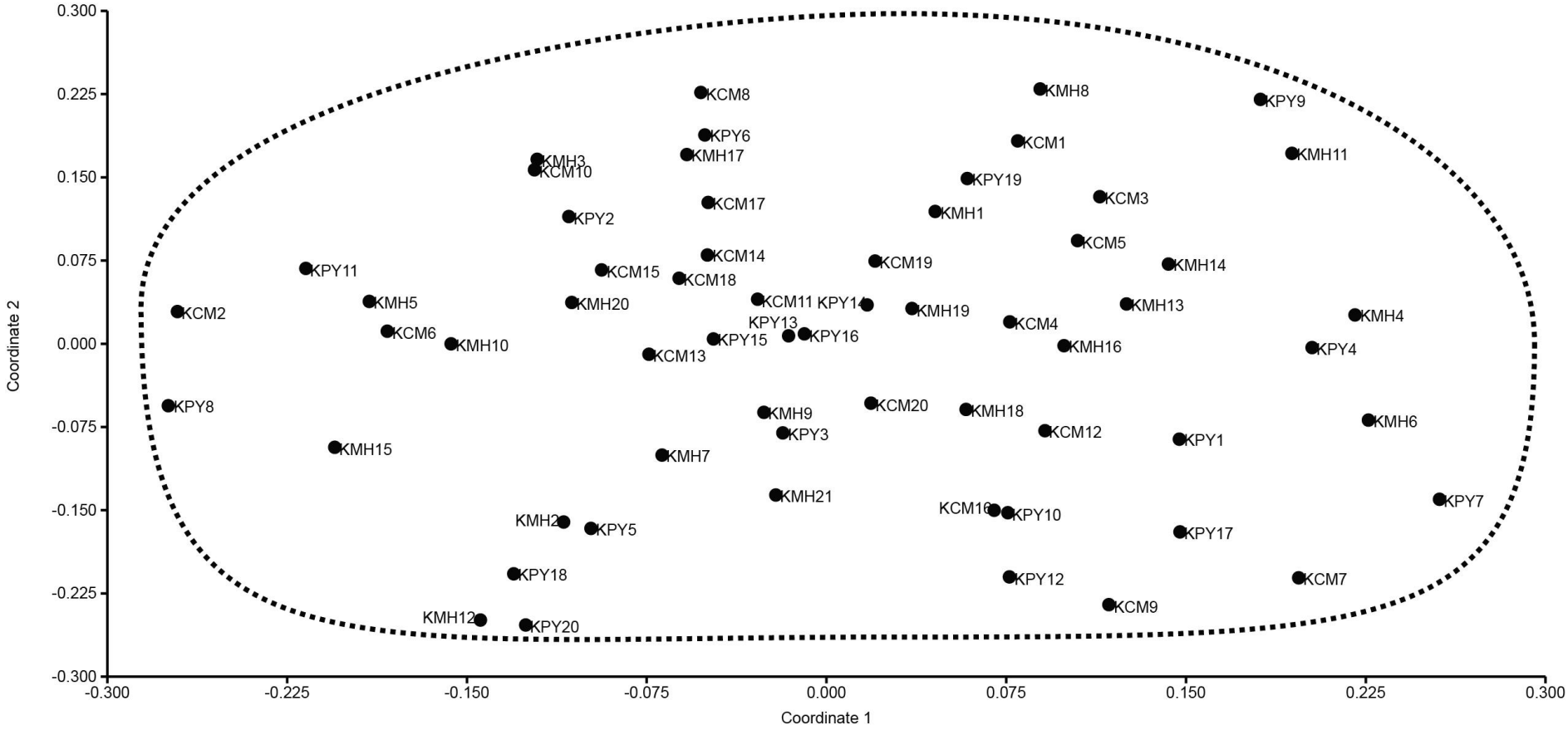

Figure 4.17 PCoA of 61 *Kleinia* accessions based on 20 iPBS markers

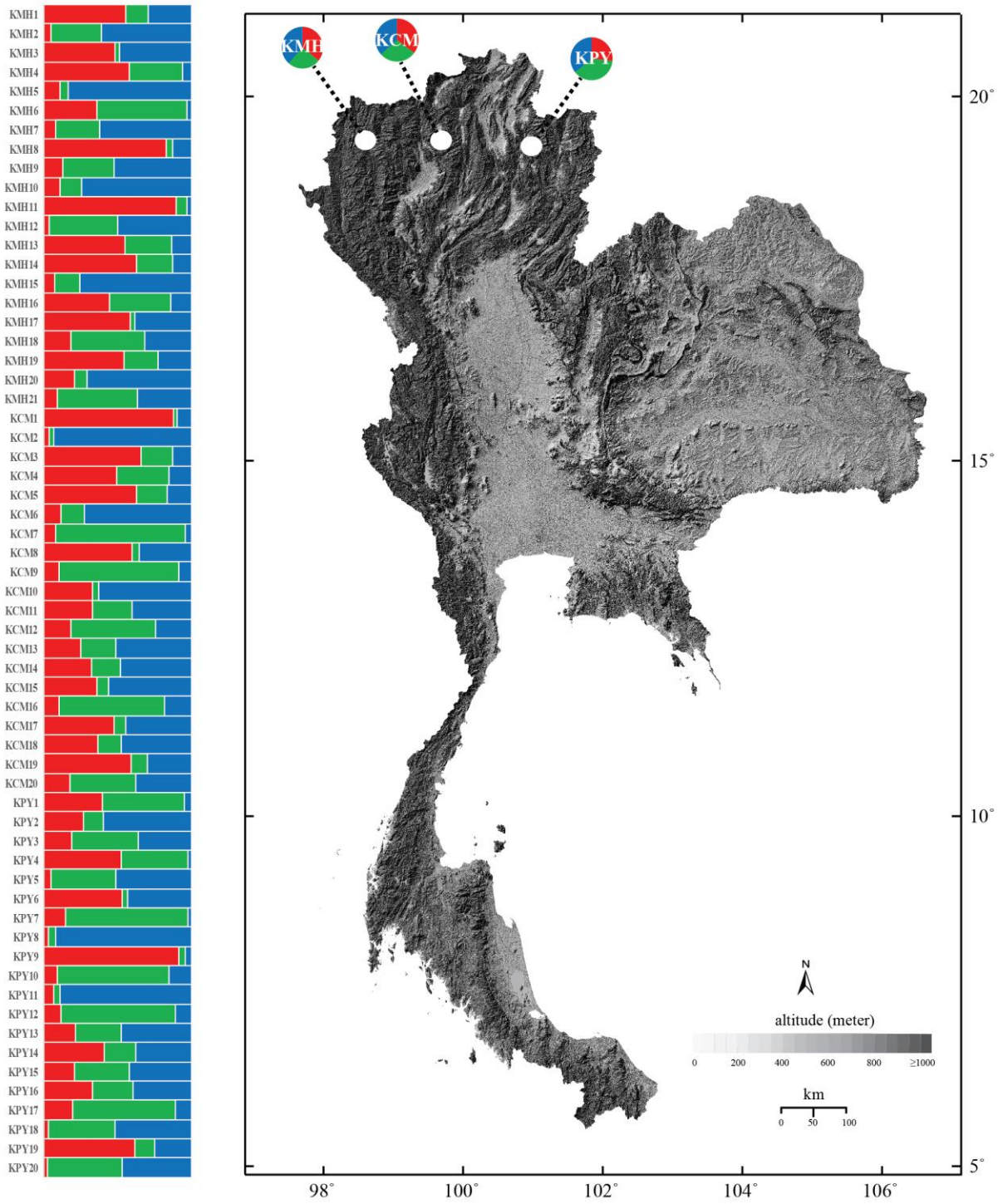

Figure 4.18 Genetic structure of 61 *Kleinia* accessions inferred by STRUCTURE software with 20 iPBS marker data sets. Single vertical line represents an individual accession, and different colors represent genetic stocks/gene pools. Segments of each vertical line show extent of admixture in an individual.

4.7 Phylogeny and diversification of *Senecio*

4.7.1 iPBS polymorphisms in *Senecio*

Overall 100 individuals from 5 populations of *Senecio* were genotyped with iPBS, successfully. All twenty iPBS primers produced 120 scorable bands and among them 81 bands were polymorphic indicating a high degree of genetic variability in *Senecio* (Table 4.7). The sizes of reproducible and scorable bands ranged from 150 to 3,500 bp. The information from all primers, including total band number, band sizes, polymorphic band number, polymorphism percentage and mean PIC values are included in Table 4.7. Primer 2076, 2077 and 2083 produced the highest number of polymorphism percentage bands (100%), whereas primer 2392 created the lowest number of polymorphism percentage bands (20%). Primer 2076 had the highest PIC value (0.453), while primer 2382 showed the lowest PIC value (0.165). The mean PIC value for these twenty primers was 0.284. These results indicate that the iPBS marker system can representative a good discriminatory capacity and reveal a wide range of genomic DNA diversity in *Senecio*.

Table 4.7 Characteristics of twenty iPBS primers used in the *Senecio* 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)
2076	GCTCCGATGCCA	59.2	7	150-500	7	100.00	0.453
2077	CTCACGATGCCA	55.1	4	350-1,000	4	100.00	0.354
2079	AGGTGGGCGCCA	65.2	5	400-900	4	80.00	0.238
2080	CAGACGGCGCCA	63.3	6	200-750	5	83.33	0.404
2081	GCAACGGCGCCA	65.0	5	250-750	3	83.33	0.282
2083	CTTCTAGCGCCA	54.6	7	300-1,000	7	100.00	0.463
2085	ATGCCGATACCA	52.8	7	200-2,000	5	71.42	0.317
2272	GGCTCAGATGCCA	55.0	6	200-800	6	100.00	0.441
2273	GCTCATCATGCCA	56.5	6	200-900	3	50.00	0.202
2277	GGCGATGATACCA	52.0	5	200-1,600	3	83.33	0.254
2279	AATGAAAGCACCA	52.0	6	250-2,000	3	50.00	0.218
2374	CCCAGCAAACCA	53.5	5	200-2,500	3	83.33	0.252
2378	GGTCCTCATCCA	53.0	10	200-3,000	7	70.00	0.327
2380	CAACCTGATCCA	50.5	6	200,3,000	3	50.00	0.237
2382	TGTTGGCTTCCA	50.5	5	250-2,000	2	40.00	0.165
2389	ACATCCTTCCCA	50.0	5	250-800	2	40.00	0.170
2391	ATCTGTCAGCCA	52.6	8	150-800	7	87.50	0.399
2392	TAGATGGTGCCA	52.2	5	100-2,500	1	20.00	0.090
2393	TACGGTACGCCA	51.0	7	100-1,000	4	57.14	0.238
2394	GAGCCTAGGCCA	56.5	5	200-600	2	40.00	0.182
	Total		120	1500-3,000	81	67.50	0.284

4.7.2 Phylogeography of *Senecio*

To construct the genetic relationships among *Senecio* accessions, a dendrogram and grouping were created using Dice similarity coefficients based on the unweighted pair group analysis with the arithmetic mean (UPGMA) dendrogram and the principal component analysis (PCoA) in Past program. According to the results of the UPGMA dendrogram including all 100 samples of *Senecio* in Thailand were obviously separate under three main clusters (Figure 4.19). The first cluster contained 39 accessions of *S. craibianus*, the second cluster comprised 39 taxa of *S scandens* and the third cluster included 22 *S*. *namnaoensis*. This indicates that there was a respectable differentiation between all *Senecio* genotypes species in Thailand. The results of PCoA similarly indicated three distinct groups in the taxonomic representation of the relative genetic similarity among all *Senecio* samples (Figure 4.20) , supporting the results presented in the UPGMA dendrogram. The results of our study verified that *S. craibianus*, *S scandens* and *S. namnaoensis* are in the different species.

4.7.3 Diversification of *Senecio*

The STRUCTURE software assigns individuals to different populations based on allele frequencies of the genotypes. Genetic structure of the population was inferred through allele frequencies (tested for K = 2 to K = 10). K was estimated with the aid of posterior probability of the data for a given. In the STRUCTURE analysis, log probabilities of the data [lnP(D)] showed the highest likelihood at K= 3. Therefore, STRUCTURE analysis was conducted for K = 3 and suggesting four clusters for all 100 *Senecio* genotypes as shown in Figure 4.21. At K = 3, *Senecio* samples were separated into three clusters. Among *Senecio* populations in Thailand, *S. craibianus* individuals were mainly assigned to one cluster, *S scandens* were basically assigned to the second cluster, and *S scandens* showed evidence of extensive admixture (Figure 4.21). Based on the latter model, all populations from Thailand were mixed for cluster I (‘blue and green’), cluster II (‘green and red’) and cluster III (‘red’) was in Thailand populations. The clustering results by UPGMA, PCoA and STRUCTURE at both the inter- and intraspecific levels, were highly concordant (Figure 4.21). It was concluded based on present findings that iPBS markers could reliably be used in phylogenetic and diversification analysis of *Senecio* genotypes.

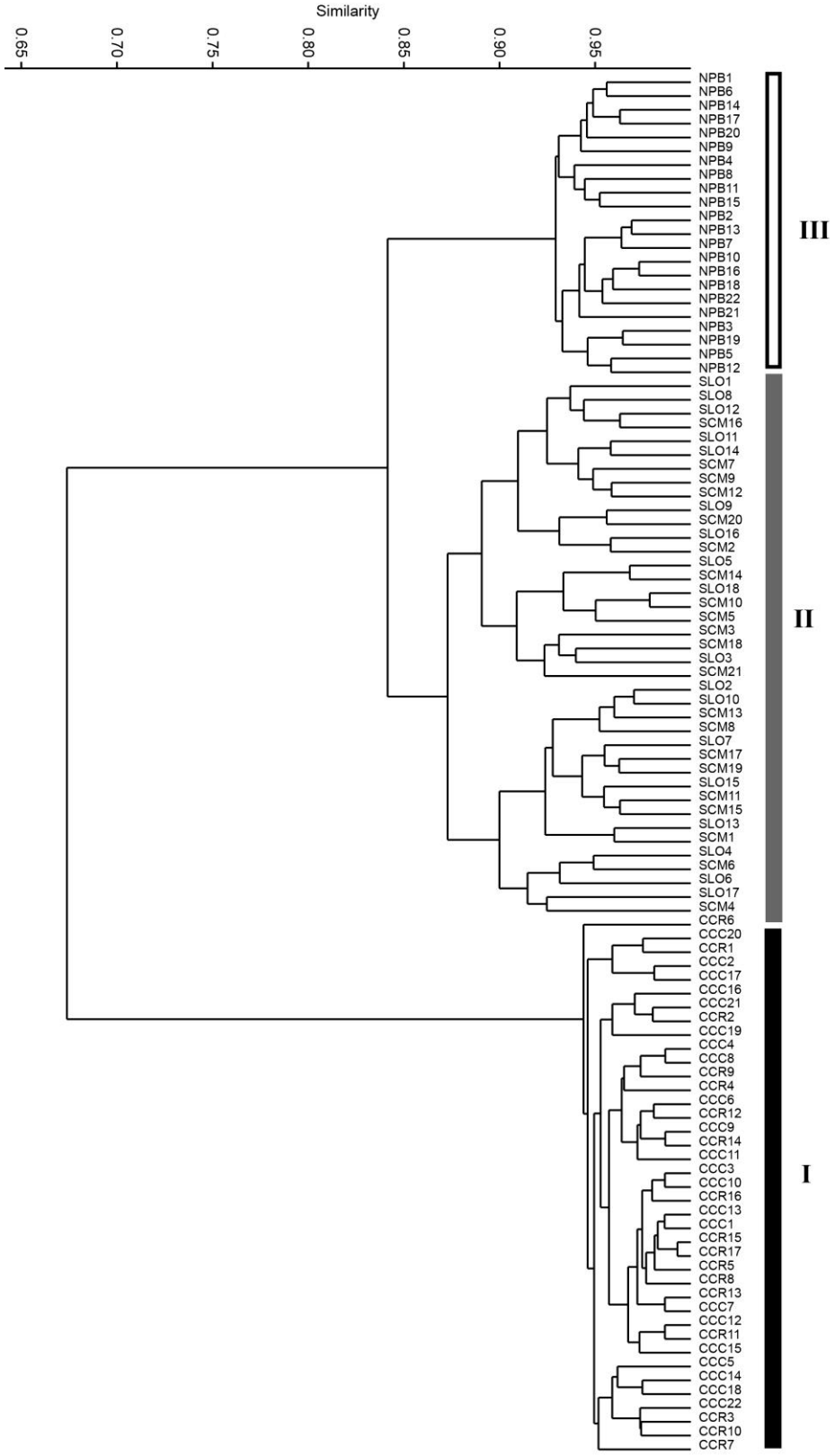

Figure 4.19 Dendrogram of 100 *Senecio* accessions based on iPBS markers according to UPGMA with the Dice similarity index

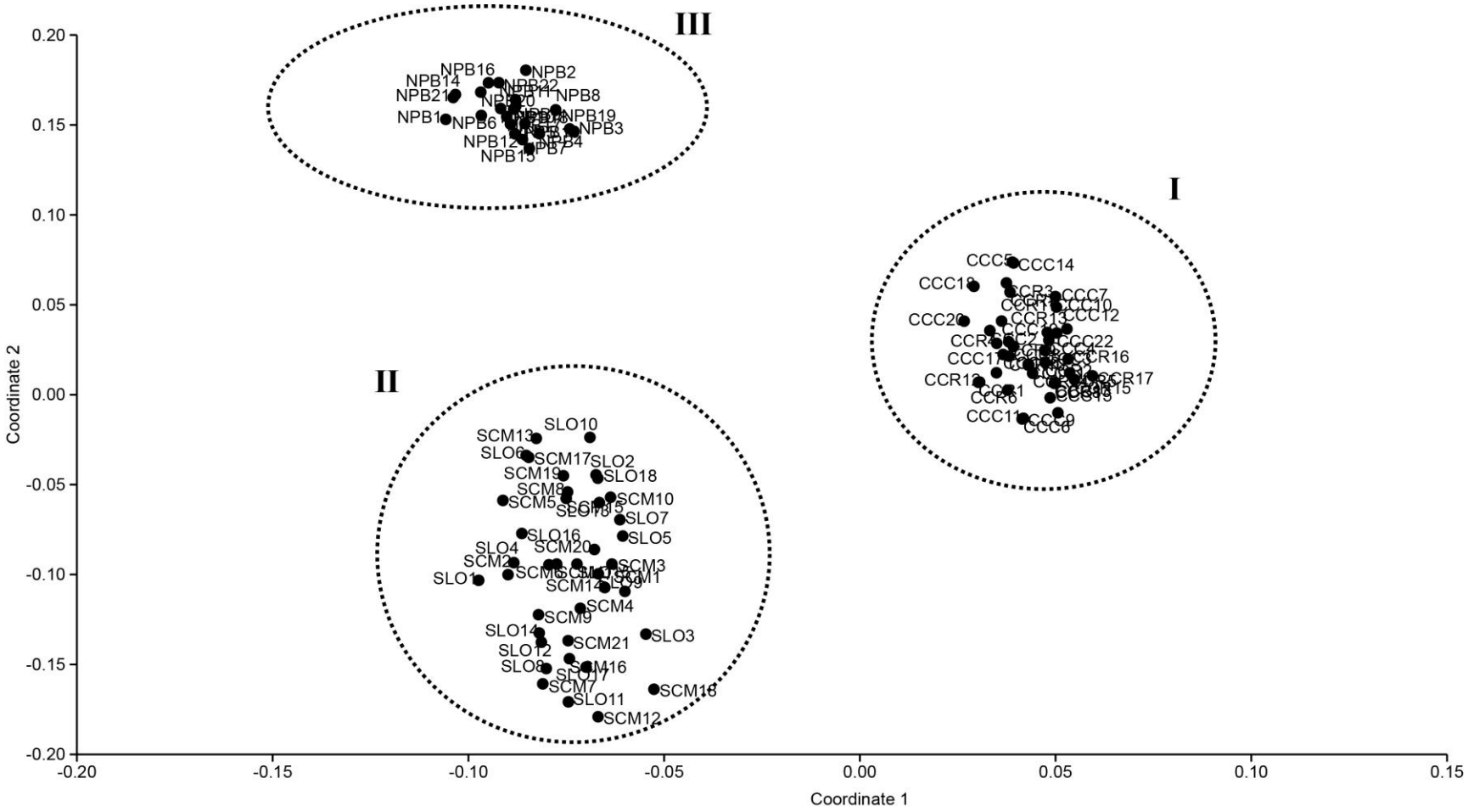

Figure 4.20 PCoA of 100 *Senecio* accessions based on 20 iPBS marker

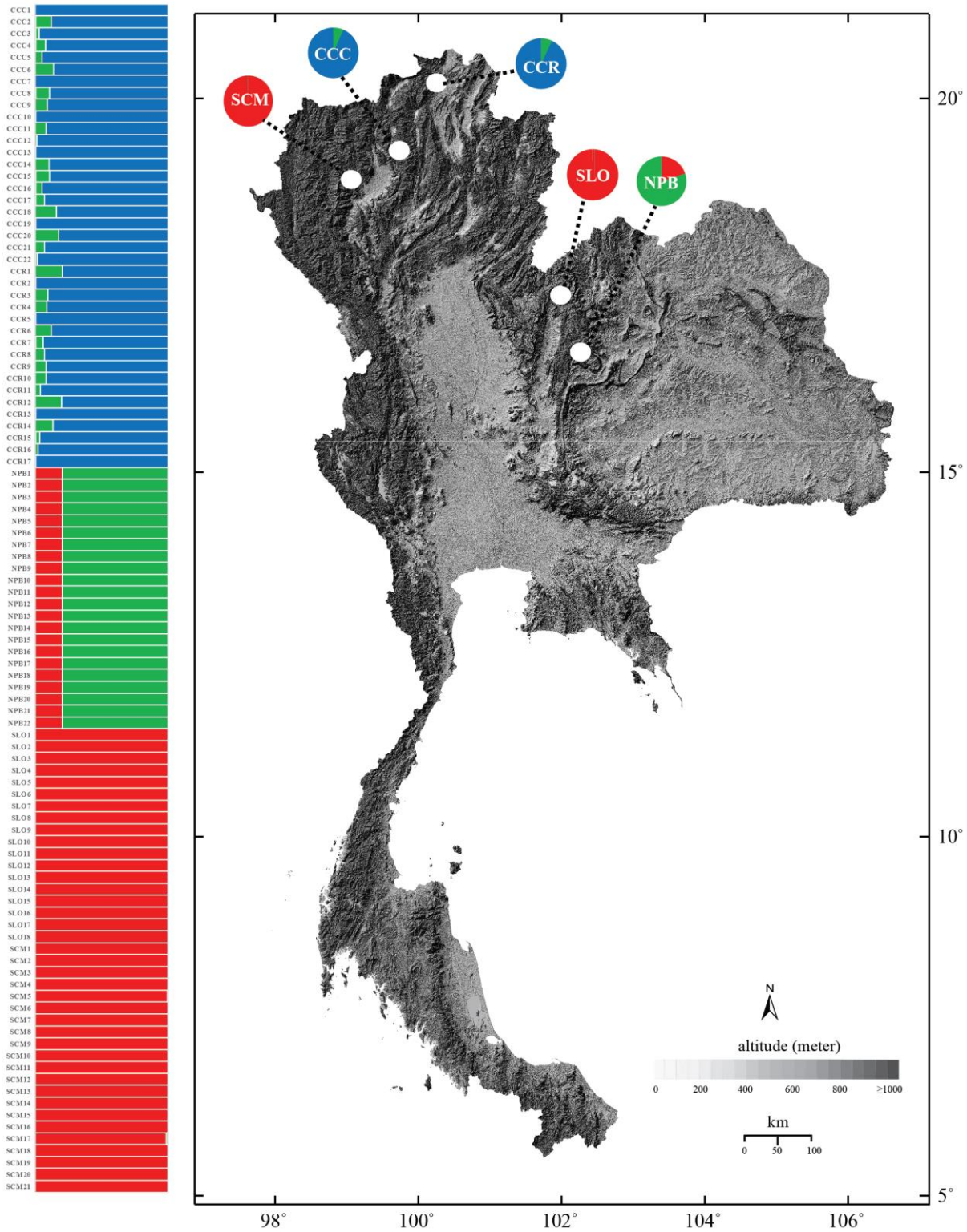

Figure 4.21 Genetic structure of 100 *Senecio* accessions inferred by STRUCTURE software with 20 iPBS marker data sets. Single vertical line represents an individual accession, and different colors represent genetic stocks/gene pools. Segments of each vertical line show extent of admixture in an individual.

4.8 Phylogeny and diversification of *Sinosenecio*

4.8.1 iPBS polymorphisms in *Sinosenecio*

Overall 39 individuals of *Sinosenecio* were genotyped with iPBS, successfully. All twenty iPBS primers produced 120 scorable bands and among them 36 bands were polymorphic indicating a low degree of genetic variability in *Kleinia* (Table 4.8). The sizes of reproducible and scorable bands ranged from 150 to 3,500 bp. The information from all primers, including total band number, band sizes, polymorphic band number, polymorphism percentage and mean PIC values are included in Table 4.8 Primer 2083 produced the highest number of polymorphism percentage bands (71.43%), whereas primer 2081, 2380, 2382 and 2389 formed no polymorphism percentage bands. Primer 2083 had the highest PIC value (0 .207), while primer 2081, 2380, 2382 and 2389 showed no PIC value. The mean PIC value for these twenty primers was 0.126. These results indicate that the iPBS marker system can representative a good discriminatory capacity and reveal a wide range of genomic DNA diversity in *Sinosenecio*.

Table 4.8 Characteristics of twenty iPBS primers used in the *Sinosenecio* 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)
2076	GCTCCGATGCCA	59.2	7	150-500	3	42.86	0.109
2077	CTCACGATGCCA	55.1	4	350-1,000	2	50.00	0.121
2079	AGGTGGGCGCCA	65.2	5	400-900	1	20.00	0.037
2080	CAGACGGCGCCA	63.3	6	200-750	3	50.00	0.150
2081	GCAACGGCGCCA	65.0	5	250-750	-	-	-
2083	CTTCTAGCGCCA	54.6	7	300-1,000	5	71.43	0.244
2085	ATGCCGATACCA	52.8	7	200-2,000	3	42.86	0.181
2272	GGCTCAGATGCCA	55.0	6	200-800	3	50.00	0.187
2273	GCTCATCATGCCA	56.5	6	200-900	2	33.33	0.098
2277	GGCGATGATACCA	52.0	5	200-1,600	2	40.00	0.166
2279	AATGAAAGCACCA	52.0	6	250-2,000	2	33.33	0.148
2374	CCCAGCAAACCA	53.5	5	200-2,500	3	60.00	0.207
2378	GGTCCTCATCCA	53.0	10	200-3,000	2	20.00	0.084
2380	CAACCTGATCCA	50.5	6	200,3,000	-	-	-
2382	TGTTGGCTTCCA	50.5	5	250-2,000	-	-	-
2389	ACATCCTTCCCA	50.0	5	250-800	-	-	-
2391	ATCTGTCAGCCA	52.6	8	150-800	1	12.50	0.041
2392	TAGATGGTGCCA	52.2	5	100-2,500	1	20.00	0.045
2393	TACGGTACGCCA	51.0	7	100-1,000	3	42.86	0.144
2394	GAGCCTAGGCCA	56.5	5	200-600	2	40.00	0.089
	Total		120	1500-3,000	38	31.67	0.128

4.8.2 Phylogeography of *Sinosenecio*

To elucidate the genetic relationships among *Sinosenecio* accessions, a dendrogram and grouping were constructed using Dice similarity coefficients based on the unweighted pair group analysis with the arithmetic mean (UPGMA) dendrogram and the principal component analysis (PCoA) in Past program. According to the results of the UPGMA dendrogram including all 61 samples of *Kleinia* in Thailand were clearly indicated affinity and relationship between different *Kleinia* accessions (Figure 4.22). About 39 taxa of *S. oldhamianus*, within each cluster showed a close relationship. The study thus shows a variability for genetic among different accessions. This indicates that there was a respectable differentiation between all *Kleinia* genotypes species in Thailand. The results of PCoA similarly indicated the relative genetic similarity among all samples (Figure 4.23), supporting the results presented in the UPGMA dendrogram.

4.8.3 Diversification of *Sinosenecio*

The STRUCTURE software assigns individuals to different populations based on allele frequencies of the genotypes. Genetic structure of the population was inferred through allele frequencies (tested for K = 2 to K = 10). K was estimated with the aid of posterior probability of the data for a given. In the STRUCTURE analysis, log probabilities of the data [lnP(D)] showed the highest likelihood at K=3. Therefore, STRUCTURE analysis was conducted for K = 2 and suggesting two clusters for 39 *Kleinia* genotypes as shown in Figure 4.24. At K = 2, *Sinosenecio* samples were extensive admixture to one cluster. Among *Sinosenecio* populations in Thailand, *S. oldhamianus* individuals were assigned showed largely evidence to one cluster (Figure 4.24). Based on the latter model, all populations from Thailand were mixed for cluster I (‘blue’) and cluster II (‘green’) was in Thailand populations. The clustering results by UPGMA, PCoA and STRUCTURE at both the inter- and intraspecific levels, were highly concordant (Figure 4.24). It was concluded based on present findings that iPBS markers could reliably be used in phylogenetic and diversification analysis of *Sinosenecio* genotypes.

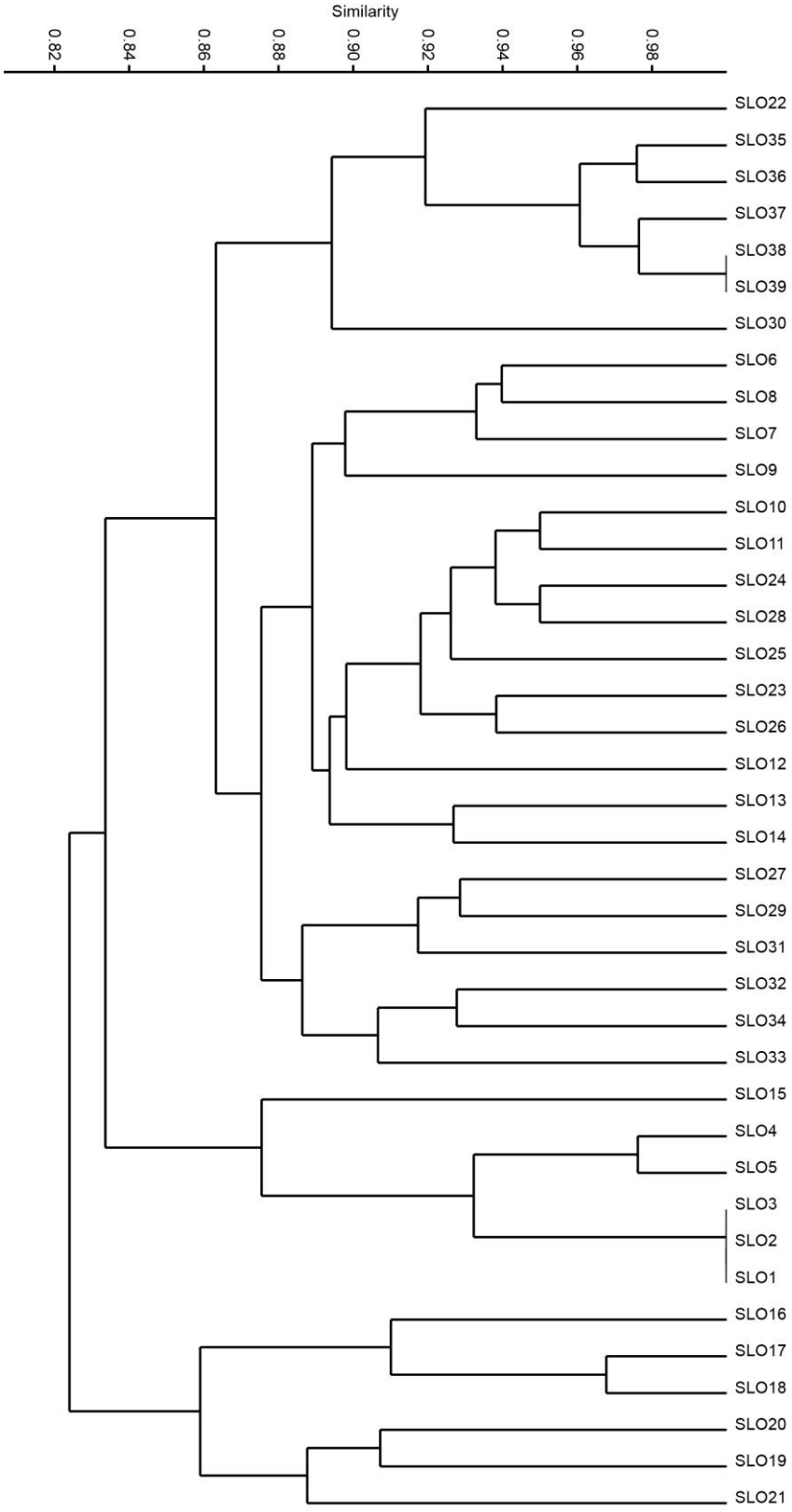

Figure 4.22 Dendrogram of 39 Sino*senecio* accessions based on iPBS markers according to UPGMA with the Dice similarity index

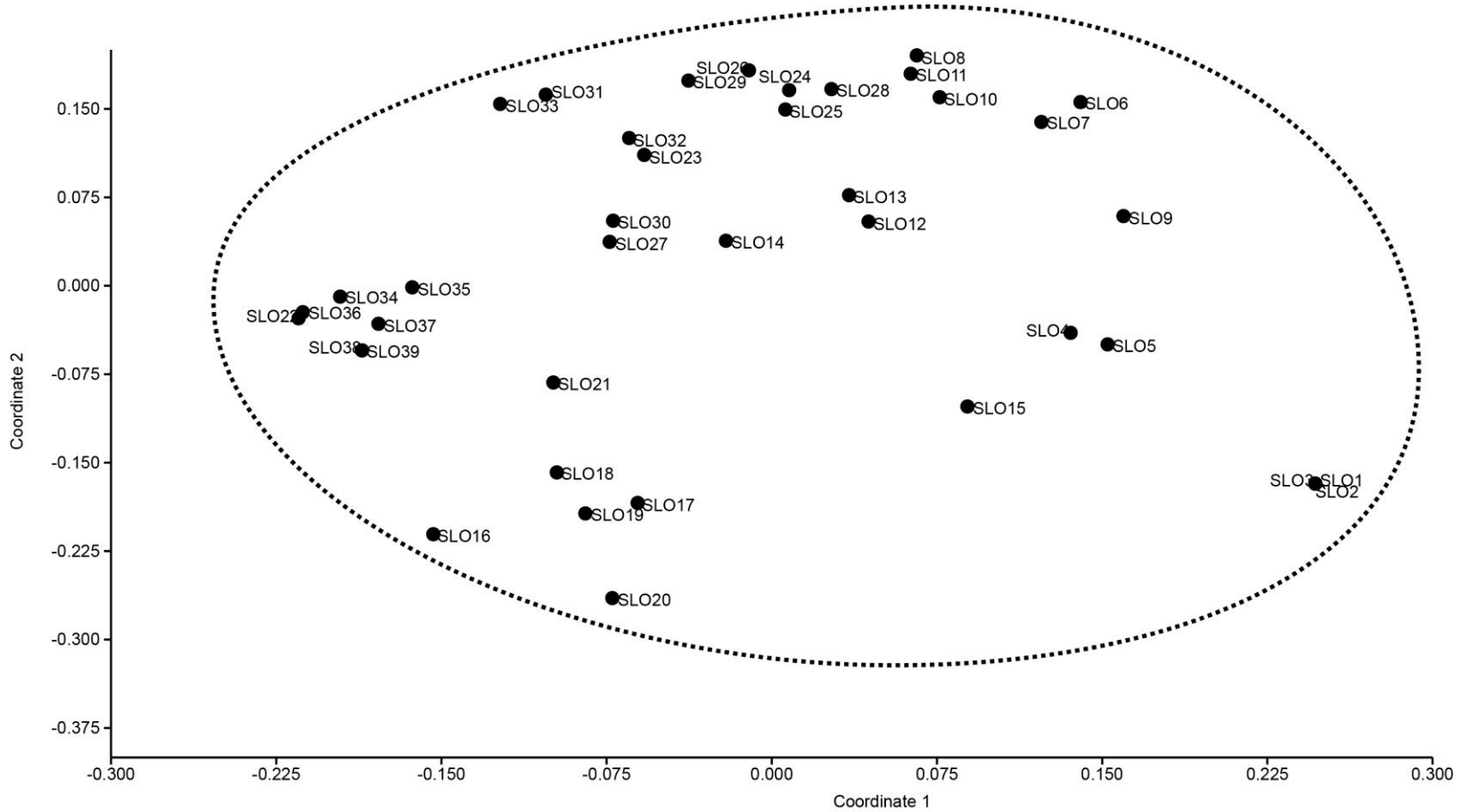

Figure 4.23 PCoA of 39 *Sinosenecio* accessions based on 20 iPBS markers

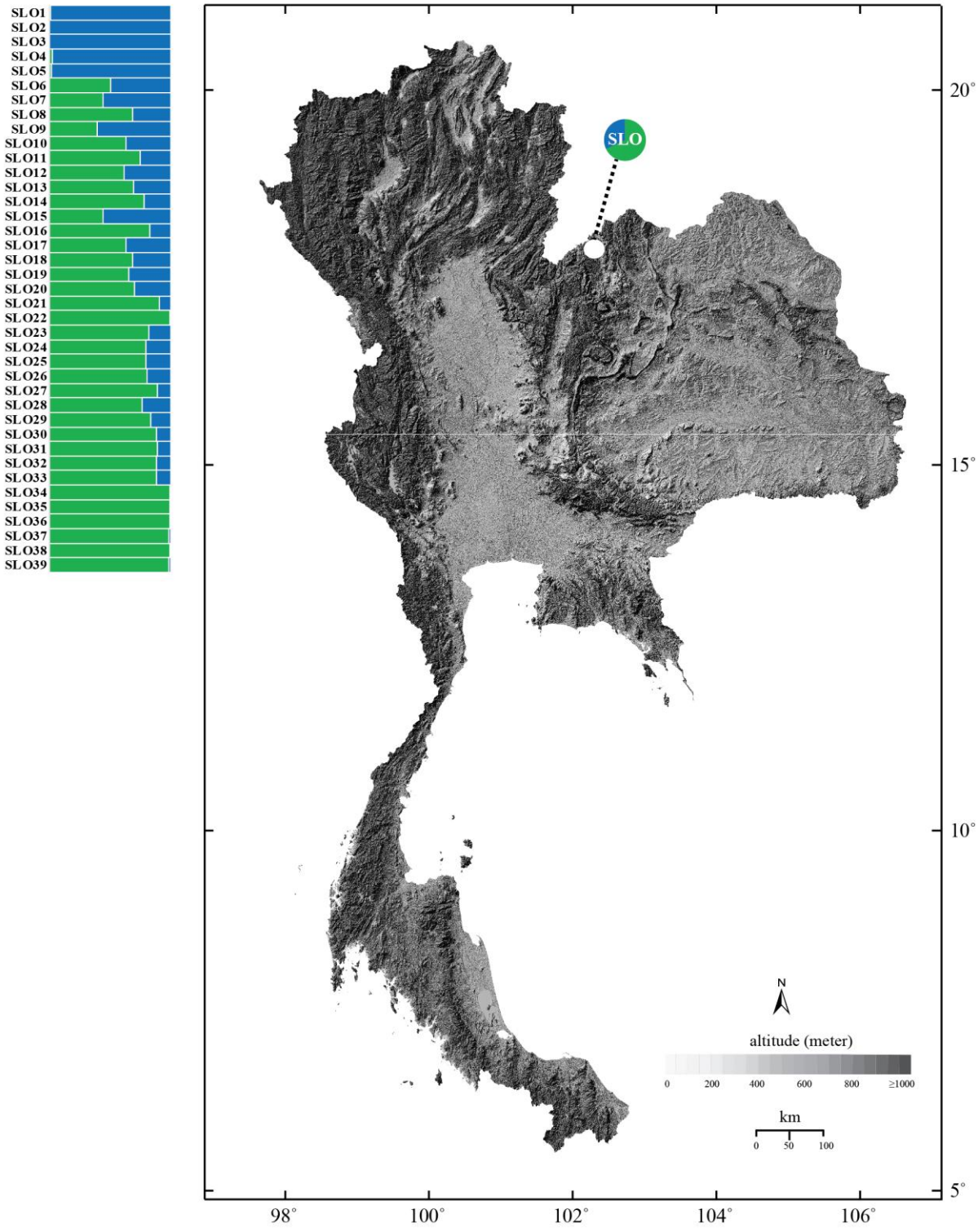

Figure 4.24 Genetic structure of 39 *Sinosenecio* accessions inferred by STRUCTURE software with 20 iPBS marker data sets. Single vertical line represents an individual accession, and different colors represent genetic stocks/gene pools. Segments of each vertical line show extent of admixture in an individual.

4.9 Phylogeny and diversification *Synotis*

4.9.1 iPBS polymorphisms in *Synotis*

Overall 90 individuals from 5 populations of *Synotis* were genotyped with iPBS, successfully. All twenty iPBS primers produced 120 scorable bands and among them 102 bands were polymorphic indicating a high degree of genetic variability in *Synotis* (Table 4.9). The sizes of reproducible and scorable bands ranged from 150 to 3,500 bp. The information from all primers, including total band number, band sizes, polymorphic band number, polymorphism percentage and mean PIC values are included in Table 4.9. Primer 2077, 2081, 2085, 2272, 2273, 2374 and 2380 produced the highest number of polymorphism percentage bands (100%), whereas primer 2394 created the lowest number of polymorphism percentage bands (60%). Primer 2077 had the highest PIC value (0.431), while primer 2382 showed the lowest PIC value (0.211). The mean PIC value for these twenty primers was 0.311. These results indicate that the iPBS marker system can representative a good discriminatory capacity and reveal a wide range of genomic DNA diversity in *Synotis*.

Table 4.9 Characteristics of twenty iPBS primers used in the *Synotis* 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)
2076	GCTCCGATGCCA	59.2	7	150-500	5	71.43	0.271
2077	CTCACGATGCCA	55.1	4	350-1,000	4	100.00	0.431
2079	AGGTGGGCGCCA	65.2	5	400-900	4	80.00	0.320
2080	CAGACGGCGCCA	63.3	6	200-750	5	83.33	0.361
2081	GCAACGGCGCCA	65.0	5	250-750	5	100.00	0.408
2083	CTTCTAGCGCCA	54.6	5	300-1,000	4	80.00	0.251
2085	ATGCCGATACCA	52.8	7	200-2,000	7	100.00	0.383
2272	GGCTCAGATGCCA	55.0	6	200-800	6	100.00	0.392
2273	GCTCATCATGCCA	56.5	6	200-900	6	100.00	0.304
2277	GGCGATGATACCA	52.0	5	200-1,600	4	80.00	0.263
2279	AATGAAAGCACCA	52.0	6	250-2,000	4	66.67	0.212
2374	CCCAGCAAACCA	53.5	5	200-2,500	5	100.00	0.320
2378	GGTCCTCATCCA	53.0	10	200-3,000	9	90.00	0.350
2380	CAACCTGATCCA	50.5	6	200,3,000	6	100.00	0.333
2382	TGTTGGCTTCCA	50.5	7	250-2,000	5	71.43	0.211
2389	ACATCCTTCCCA	50.0	5	250-800	4	80.00	0.328
2391	ATCTGTCAGCCA	52.6	8	150-800	6	75.00	0.291
2392	TAGATGGTGCCA	52.2	5	100-2,500	4	80.00	0.338
2393	TACGGTACGCCA	51.0	7	100-1,000	6	85.71	0.216
2394	GAGCCTAGGCCA	56.5	5	200-600	3	60.00	0.235
	Total		120	1500-3,000	102	85.00	0.311

4.9.2 Phylogeography of *Synotis*

To construct the genetic relationships among *Synotis* accessions, a dendrogram and grouping were created using Dice similarity coefficients based on the unweighted pair group analysis with the arithmetic mean (UPGMA) dendrogram and the principal component analysis (PCoA) in Past program. According to the results of the UPGMA dendrogram including all 90 samples of *Synotis* in Thailand were obviously separate under three main clusters (Figure 4.25). The first cluster contained 22 accessions of *S. cappa*, the second cluster comprised 50 taxa of *S. nagensium* and the third cluster included 18 *S. phupeakensis*. This indicates that there was a respectable differentiation between all *Synotis* genotypes species in Thailand. The results of PCoA similarly indicated three distinct groups in the taxonomic representation of the relative genetic similarity among all *Synotis* samples (Figure 4.26), supporting the results presented in the UPGMA dendrogram. The results of our study verified that *S. cappa*, *S. nagensium* and *S. phupeakensis* are in the different species.

4.9.3 Diversification of *Synotis*

The STRUCTURE software assigns individuals to different populations based on allele frequencies of the genotypes. Genetic structure of the population was inferred through allele frequencies (tested for K = 2 to K = 10). K was estimated with the aid of posterior probability of the data for a given. In the STRUCTURE analysis, log probabilities of the data [lnP(D)] showed the highest likelihood at K=3. Therefore, STRUCTURE analysis was conducted for K = 3 and suggesting four clusters for 90 *Synotis* genotypes as shown in Figure 4.27. At K = 3, *Synotis* samples were separated into three clusters. Among *Synotis* populations in Thailand, *S. cappa* individuals were mainly assigned to one cluster, *S. nagensium* showed evidence of extensive admixture to the second cluster, and *S. phupeakensis* were basically assigned to the third cluster (Figure 4.27). Based on the latter model, all populations from Thailand were mixed for cluster I ('purple'), cluster II ('green and purple') and cluster III ('red') was in Thailand populations. The clustering results by UPGMA, PCoA and STRUCTURE at both the inter- and intraspecific levels, were highly concordant (Figure 4.27). It was concluded based on present findings that iPBS markers could reliably be used in phylogenetic and diversification analysis of *Synotis* genotypes.

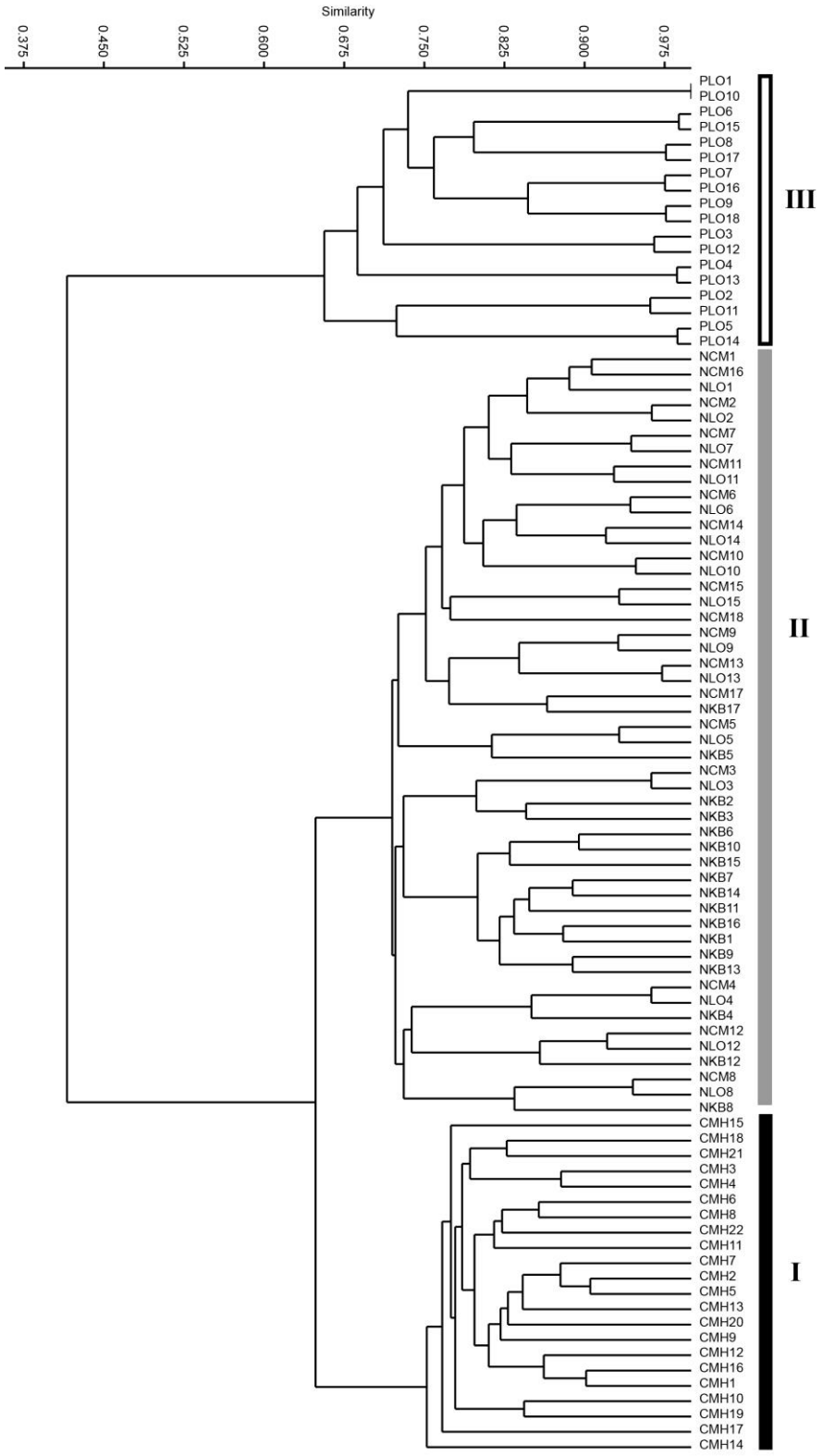

Figure 4.25 Dendrogram of 90 Synotis accessions based on iPBS markers according to UPGMA with the Dice similarity index

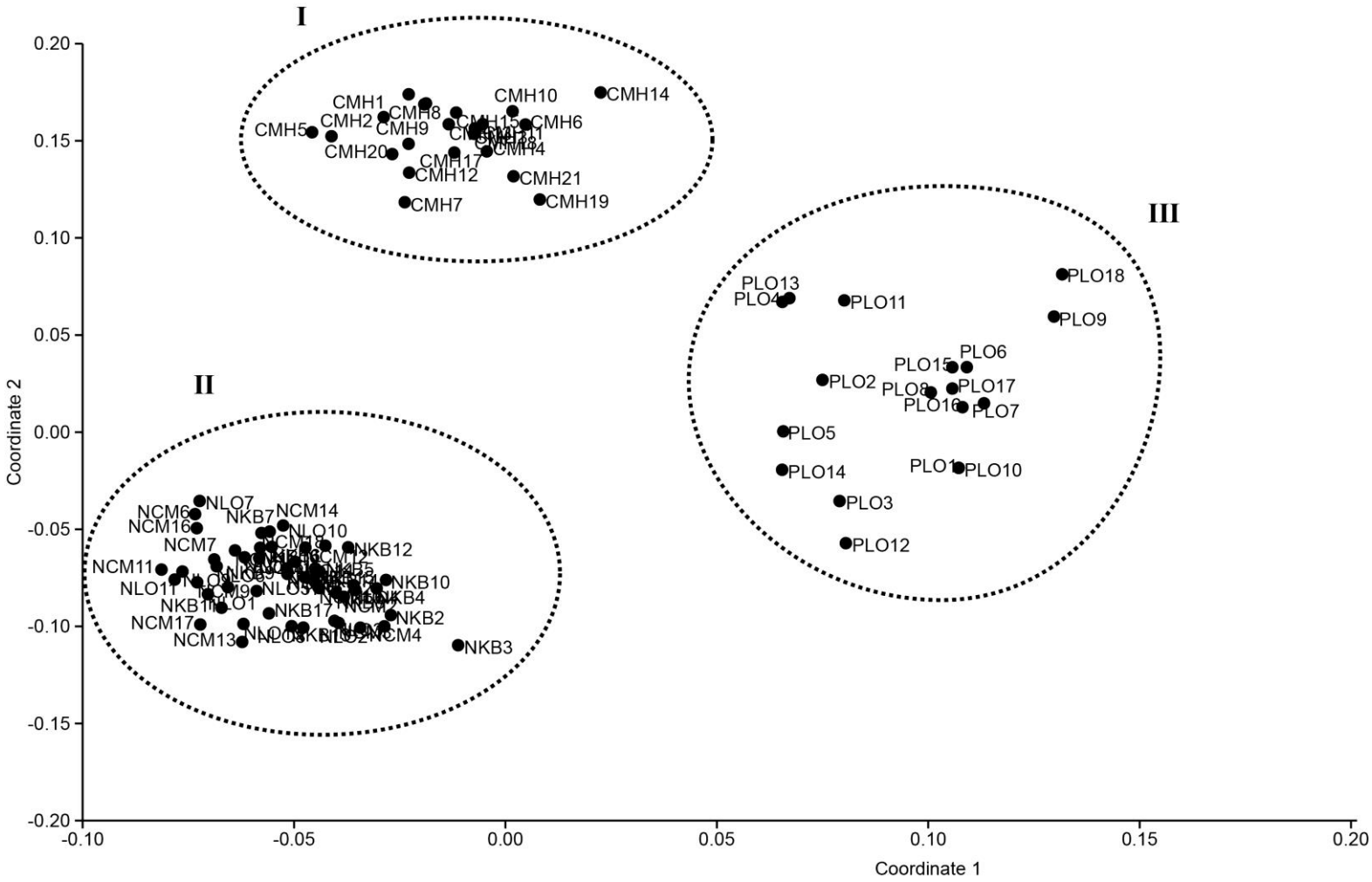

Figure 4.26 PCoA of 90 *Synotis* accessions based on 20 iPBS markers

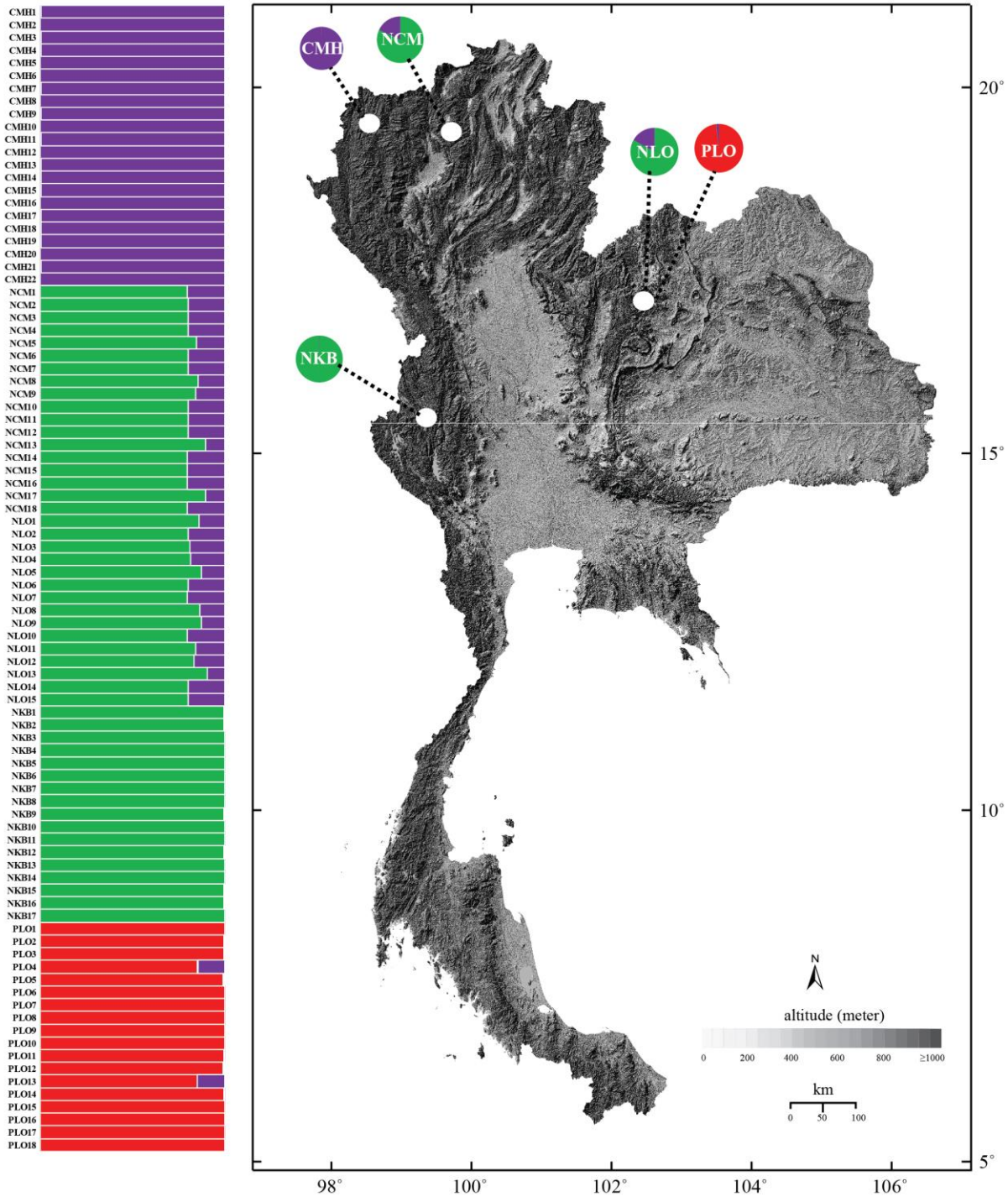

Figure 4.27 Genetic structure of 90 *Synotis* accessions inferred by STRUCTURE software with 20 iPBS marker data sets. Single vertical line represents an individual accession, and different colors represent genetic stocks/gene pools. Segments of each vertical line show extent of admixture in an individual.

4.10 Phylogeny and diversification *Senecioneae*

4.10.1 Phylogeny of *Senecioneae* in Thailand

Molecular phylogenies are powerful tools for developing infrageneric classifications. They allow us to test if traditional morphology-based infrageneric taxa establish evolutionary lineages that merit taxonomic recognition. It is significant that these infrageneric classifications reflect evolutionary relationships, so that they can be used to inform taxon sampling in biological studies that are outside the field of systematics. A well-resolved Senecioneae phylogeny in Thailand is therefore not only important for understanding the evolutionary history and processes that led to its incredible biological diversity, but also to facilitate such studies. The complex patterns of incongruence between Senecioneae phylogenies have thus far prevented a genus-wide phylogenetic hypothesis of the relationships between its species and species groups. For example, molecular phylogenetic studies resulted in a new, monophyletic delimitation of *Senecio*, the identification of lineages and patterns of phylogenetic incongruence in *Senecio* and Senecioneae (Pelser et al., 2010), greater phylogenetic resolution within several *Senecio* lineages, and a better understanding of its biogeographic history and diversification. The present study aims to contribute further to this process by providing phylogeny of *Senecioneae* in Thailand.

The relationships between the nine genera of Senecioneae in Thailand identified in this study from iPBS are congruent between the molecular phylogenies from earlier studies (Pelser et al., 2007) This study indicated that Senecioneae in Thailand is well separated into two clades. The first clade, is composed only *Emilia* species which is considered characteristic elements of the *Pericallis-Emilia* clade (Figure 4.28). The second clade is composed of eight genera of which *Gynura* and *Kleinia* are considered characteristic elements of subtribe Adenostyllinae in *Gynuroid* clade. It also includes the six genera of *Cissampelopsis*, *Synotis, Sinosenecio, Senecio*, *Erechtites* and *Crassocephalum* that are reflected distinguishing features of *Cissampelopsis-Crassocephalum* clade (Figure 4.28). However, in second clade, the relationships among these subclades are uncertain, due to conflicting results in parsimony and Bayesian analyses. In conclusion, the patterns of relationship suggested by the iPBS markers are congruence with ITS data but the results of this study are in conflict with patterns found in the plastid data, and therefore complicate conclusions regarding evolutionary relationships.

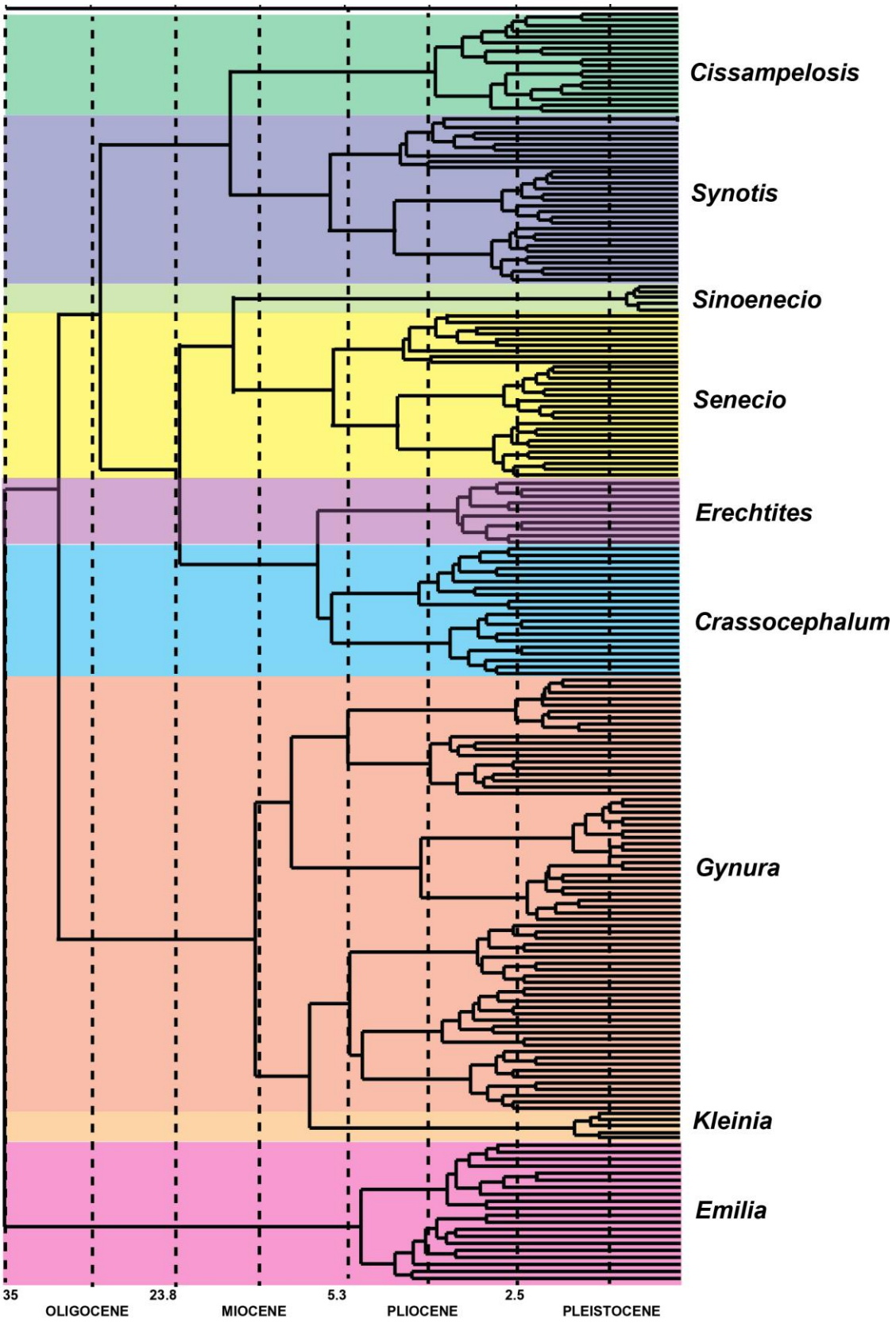

Figure 4.28 Phylogenetic relationships and approximate temporal spreading calibrated according to divergence times derived from Pelser et al. (2010) within Senecioneae in Thailand.

4.10.2 Diversification of *Senecioneae* in Thailand

Tribe Senecioneae is one of the largest tribes in Compositae. Although the evolutionary success of the tribe is striking, as measured by its tremendous number of species and its incredible morphological diversity, the cause(s) of its success remain largely unknown and unexplored. Bremer (1994) suggested that the prosperity of Senecioneae might be due to their poisonous pyrrolizidine alkaloids. This hypothesis has, however, not been further examined, presumably because of the previous lack of a robust phylogeny for Senecioneae and the limited availability of comparative pyrrolizidine alkaloid data for its species. Others have linked diversifications of Senecioneae lineages to geological and climatological changes. Currently, molecular dating analysis suggests that the divergence of Senecioneae clade is remarkably ancient considering that the diversification of the existing members of the whole Asteraceae would have occurred during the last 35 Myr. Africa is probably the continent where Senecioneae had their origin. From there, other continents were colonized, resulting in an almost cosmopolitan distribution for Senecioneae. Assuming a date of the crown age of Asteroideae to be ca. 30-35 Myr. The Senecioneae outside of it are 17 Myr or older and the result of a family-wide rapid Oligocene-Early Miocene diversication. These results are roughly in line with other molecular dating studies in Asteraceae. Therefore, from Africa, other continents seem to have been colonized multiple times independently.

Climatic variables such as temperature and precipitation are major determinants of vegetation types, and major changes in vegetation cover in Thailand occurred during the Pleistocene as a result of climatic oscillations. These vegetation changes may have played a major role in shaping the phylogeographical structure of Senecioneae in Thailand. However, there is no firm consensus on the Quaternary distribution of vegetation types in this region. During the Pleistocene glacial periods, it has been suggested that Thai forests retracted with only a few existing refugia, and that they were replaced by seasonal forests and savanna. In Thailand most of the process of diversification within Senecioneae clades probably occurred since at least the early Miocene and more pronounced in the Plio-Pleistocene. The seasonality of both temperature and precipitation is a major determinant of Senecioneae distribution (Figure 4.29-4.37) and richness in Thailand and its variation led to a modification of the relative abundance of Senecioneae during the Pleistocene where the Northern Highland is the major immigration route of Senecioneae in Thailand (Figure 4.38). Thus, Thailand could be considered as a significant area for Senecioneae diversification in Southeast Asia region.

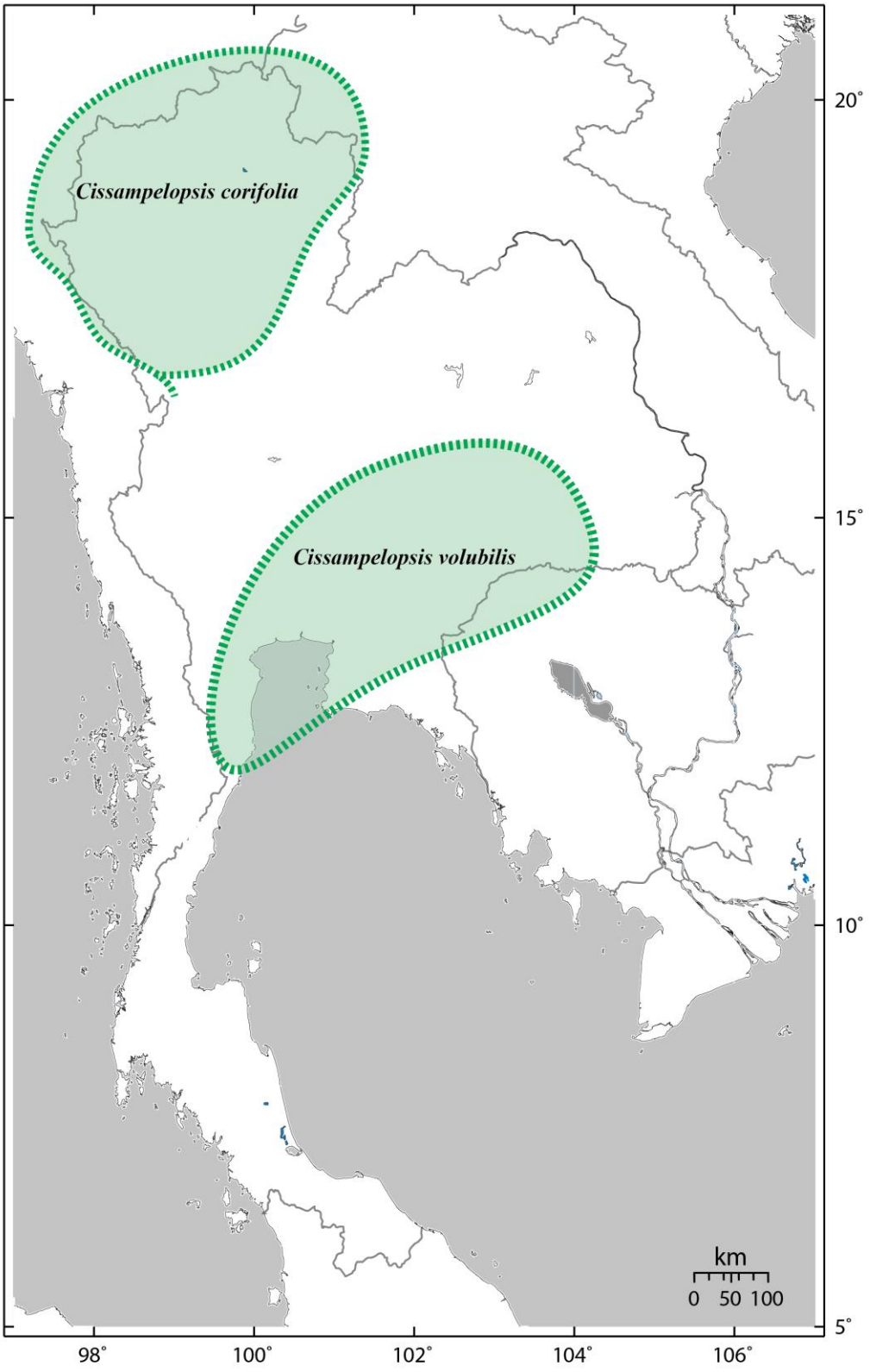

Figure 4.29 Diversification of *Cissampelopsis* in Thailand

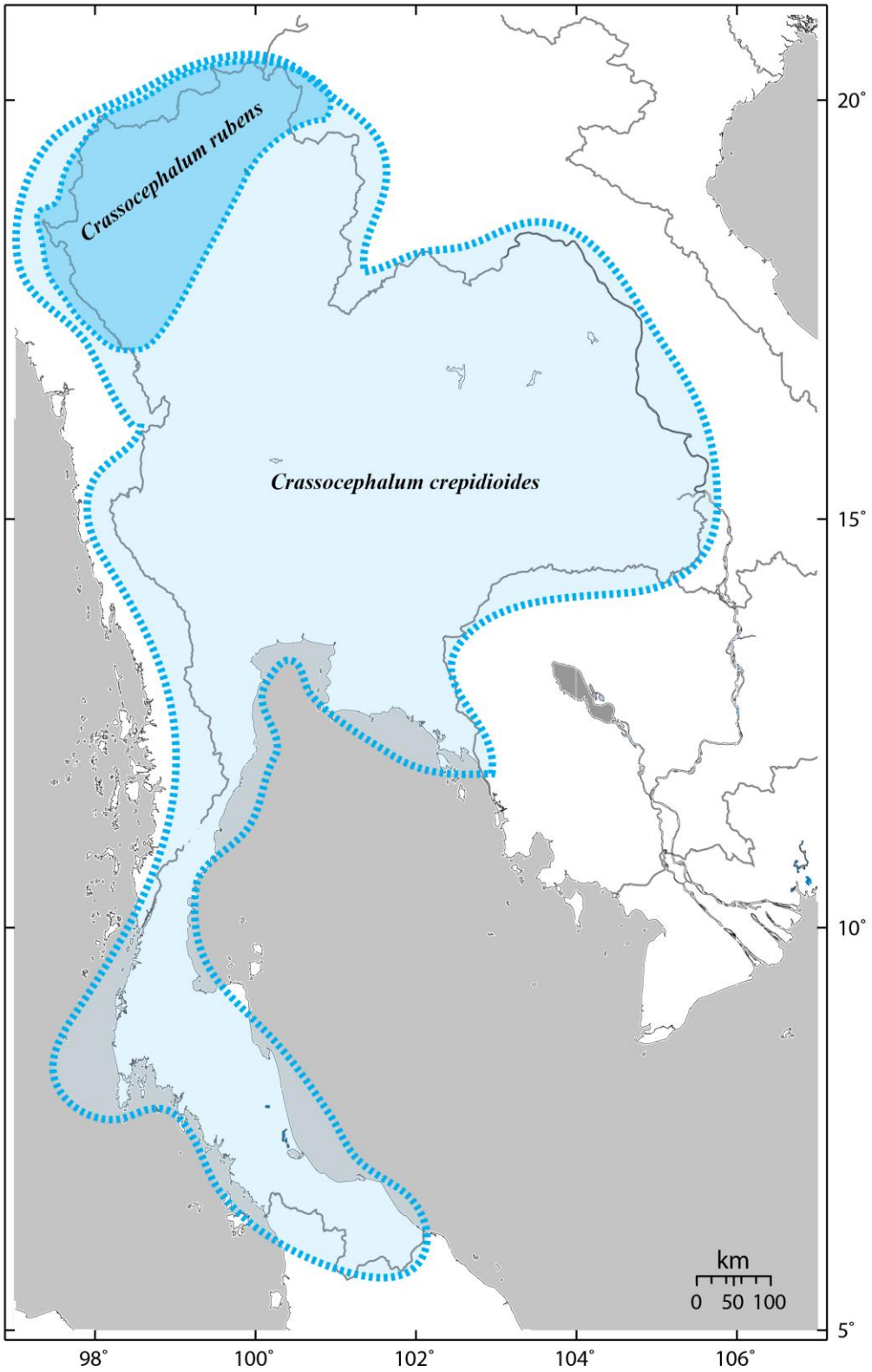

Figure 4.30 Diversification of *Crassocephalum* in Thailand

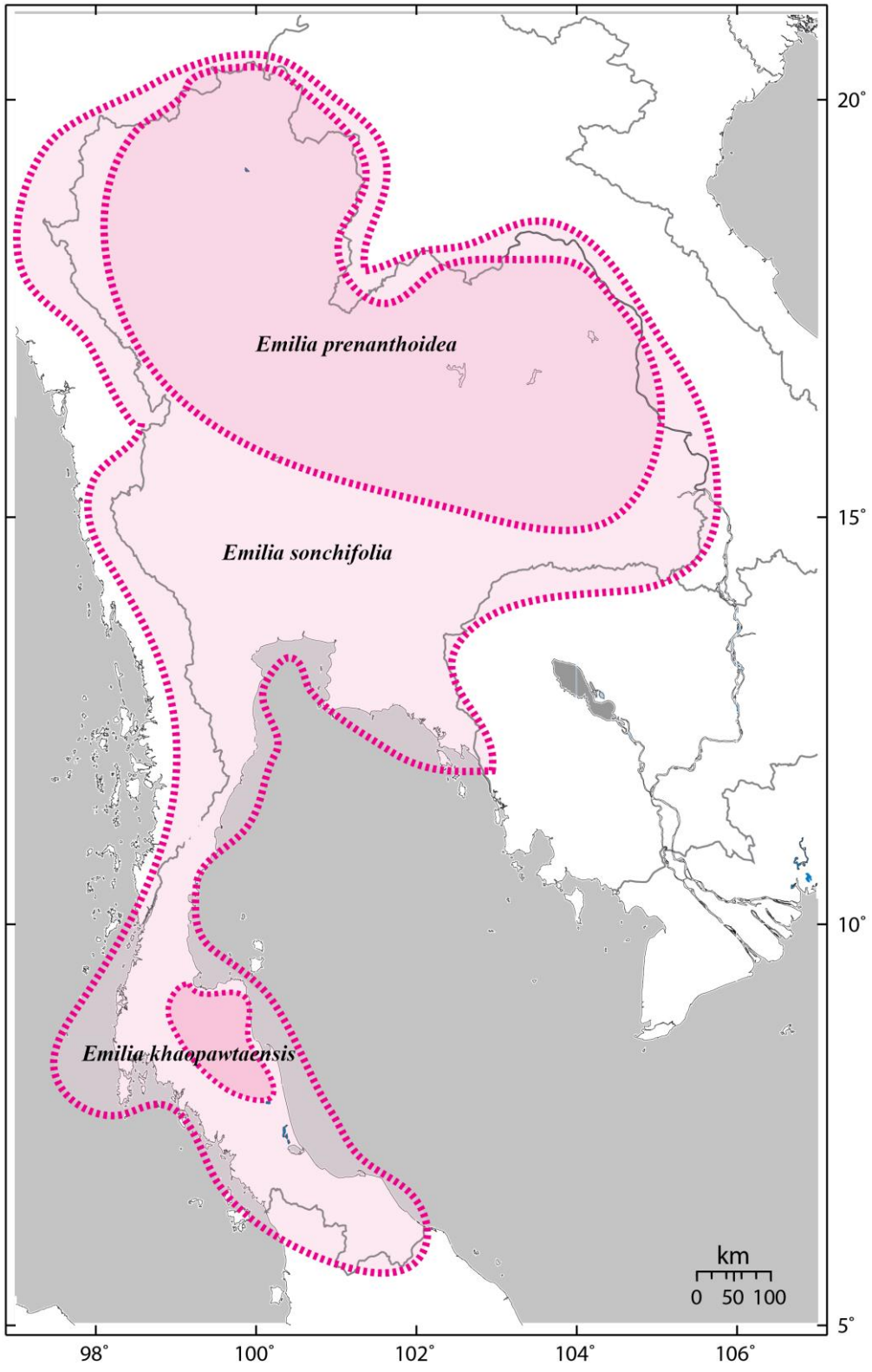

Figure 4.31 Diversification of *Emilia* in Thailand

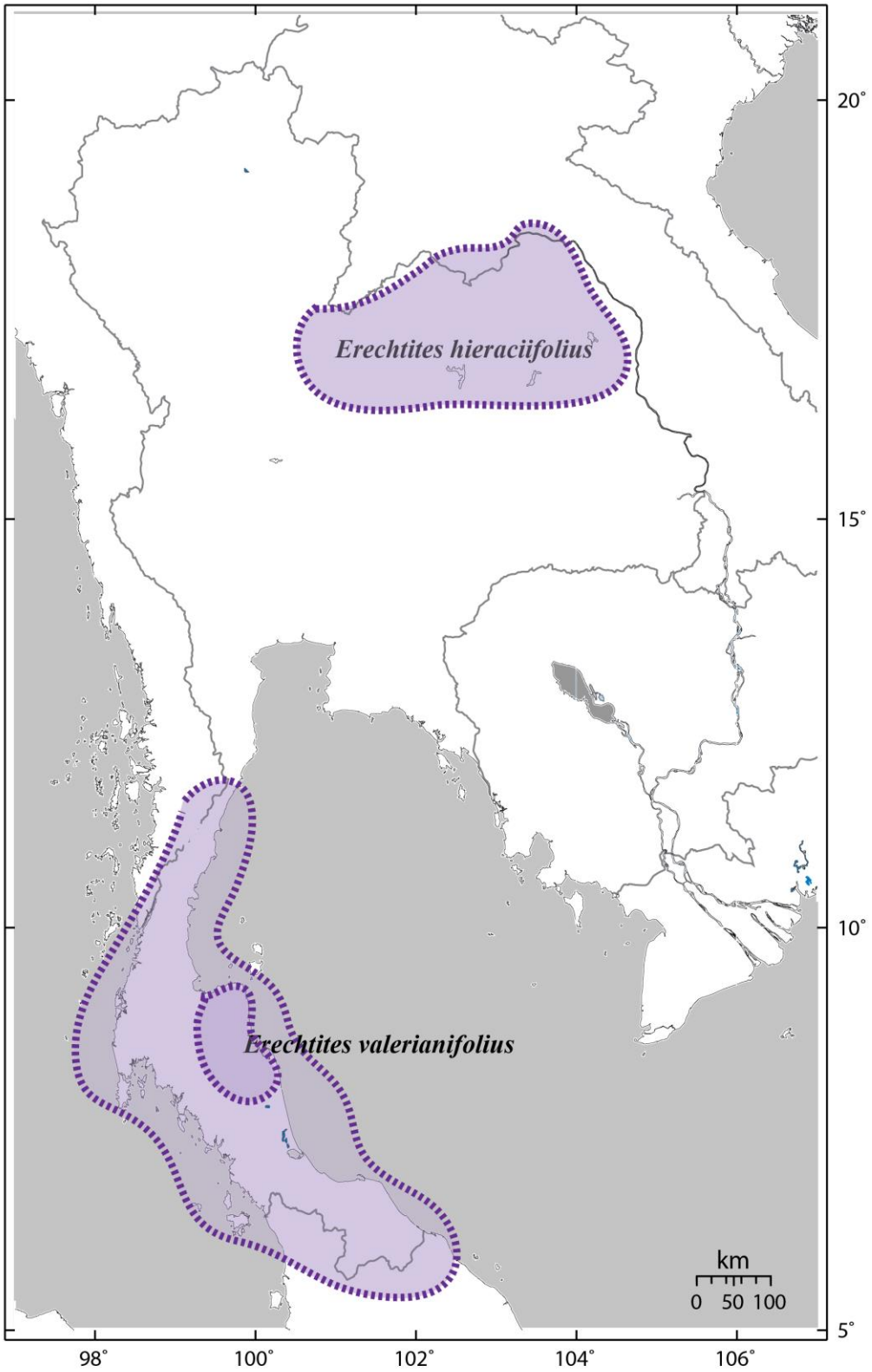

Figure 4.32 Diversification of *Erechtites* in Thailand

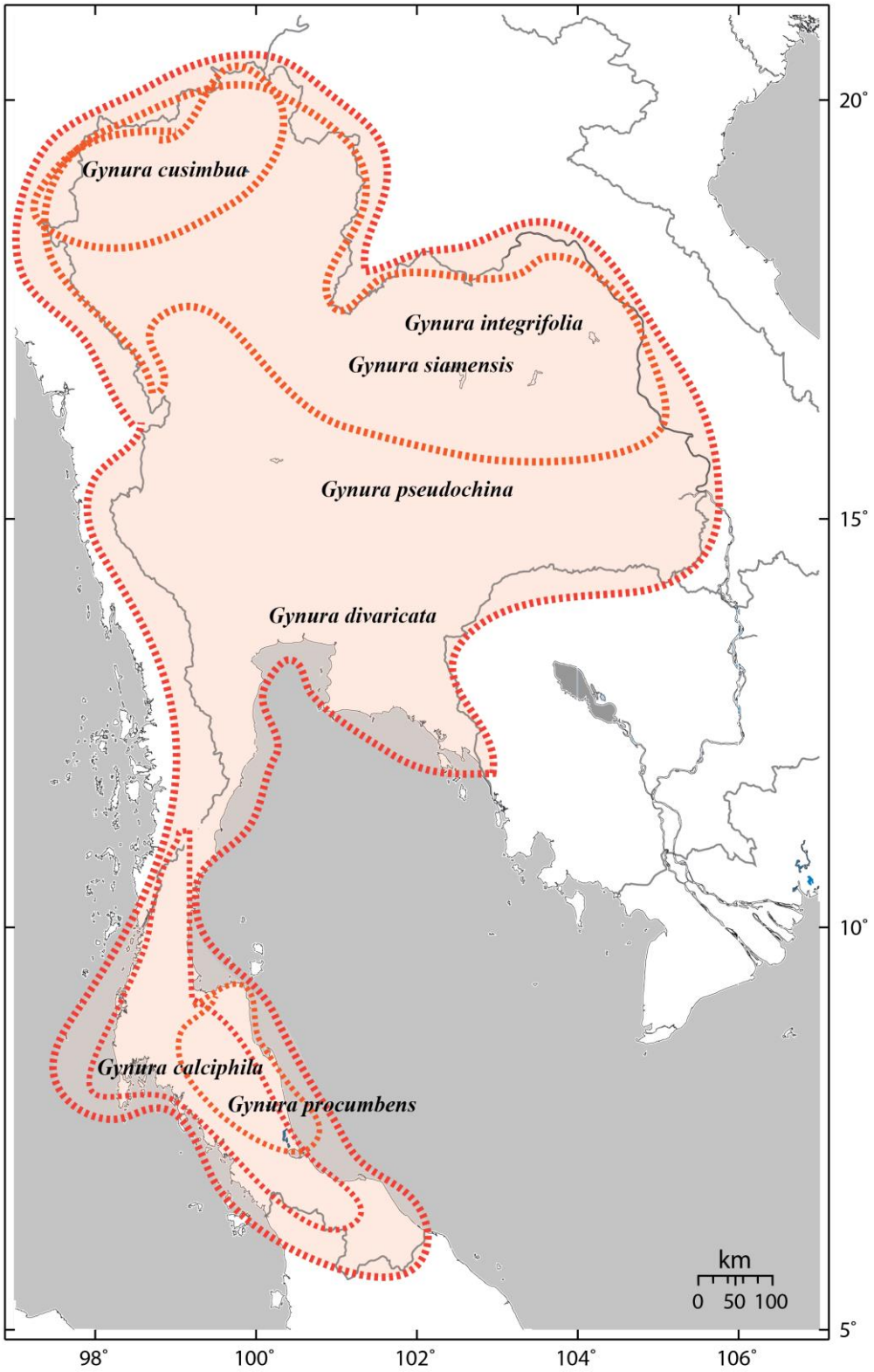

Figure 4.33 Diversification of *Gynura* in Thailand

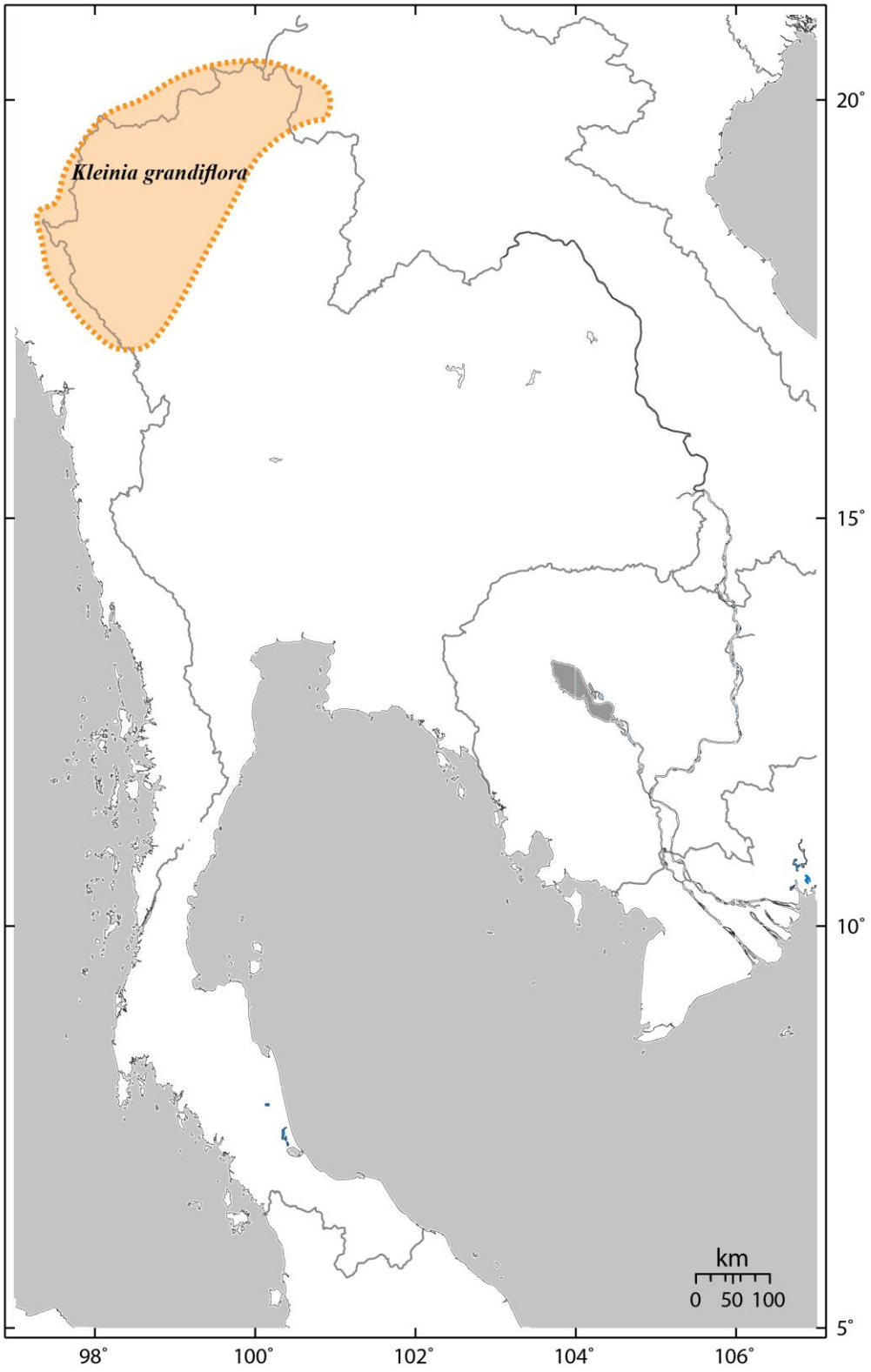

Figure 4.34 Diversification of *Kleinia* in Thailand

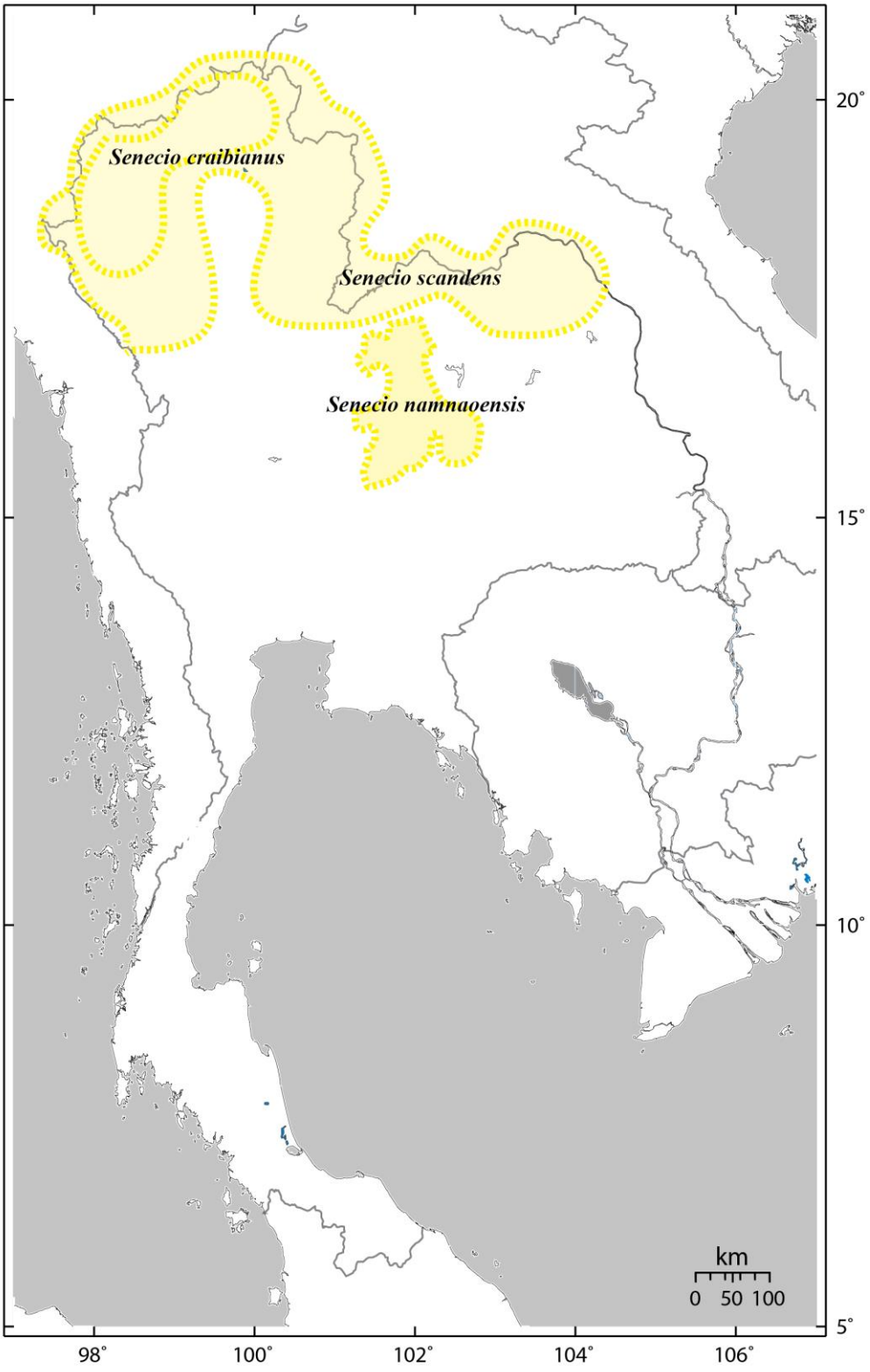

Figure 4.35 Diversification of *Senecio* in Thailand

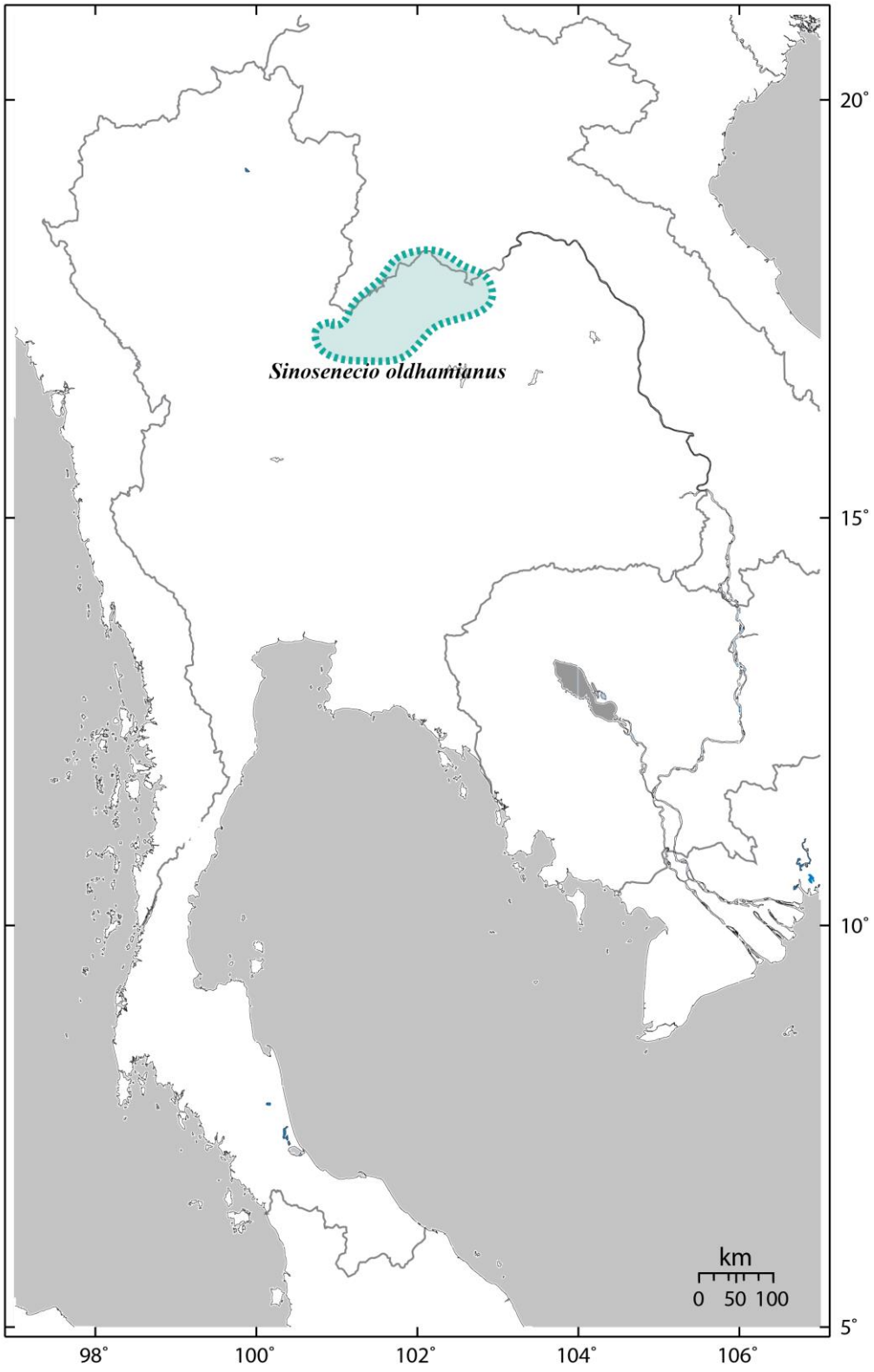

Figure 4.36 Diversification of *Sinosenecio* in Thailand

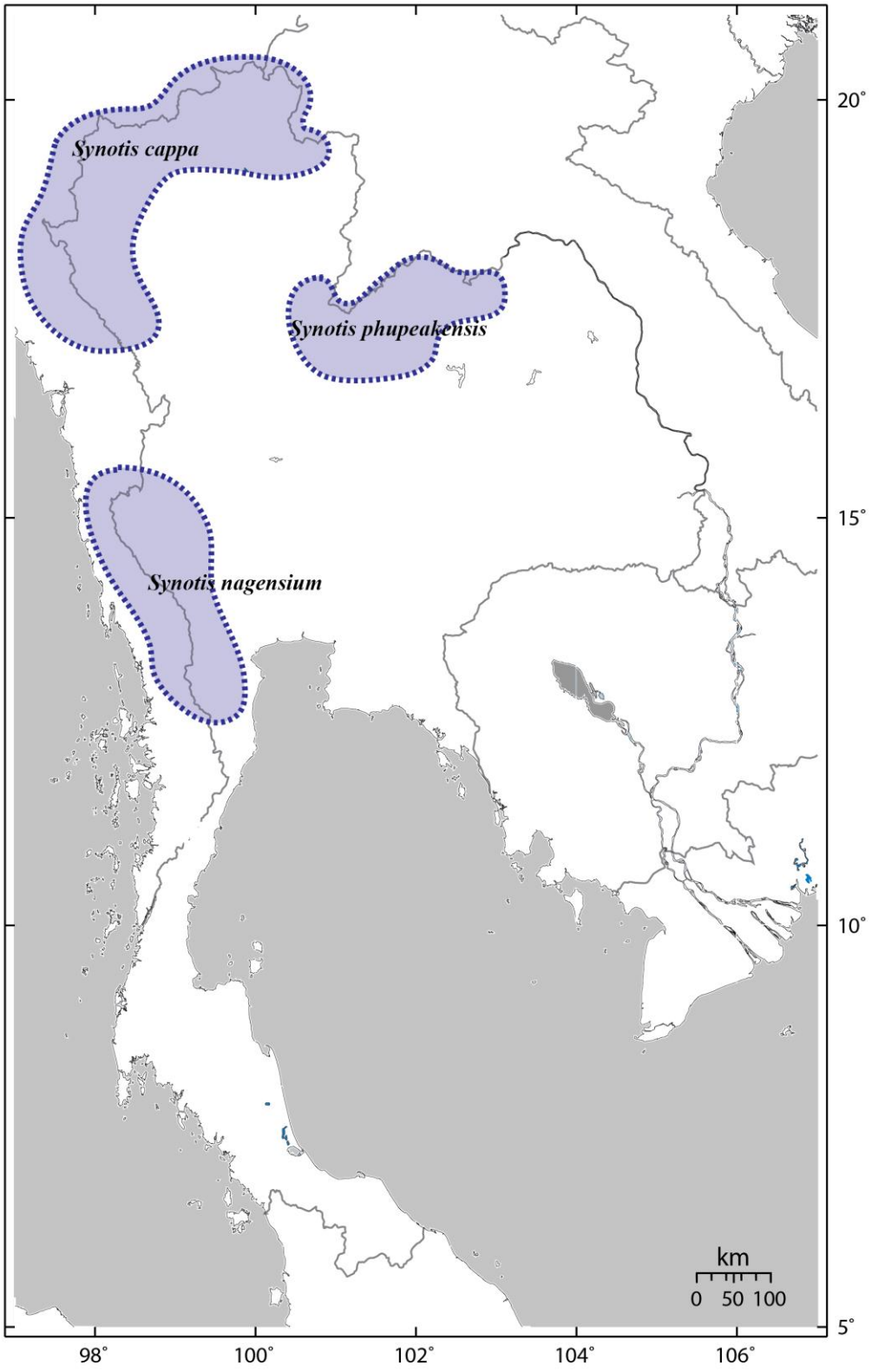

Figure 4.37 Diversification of *Synotis* in Thailand

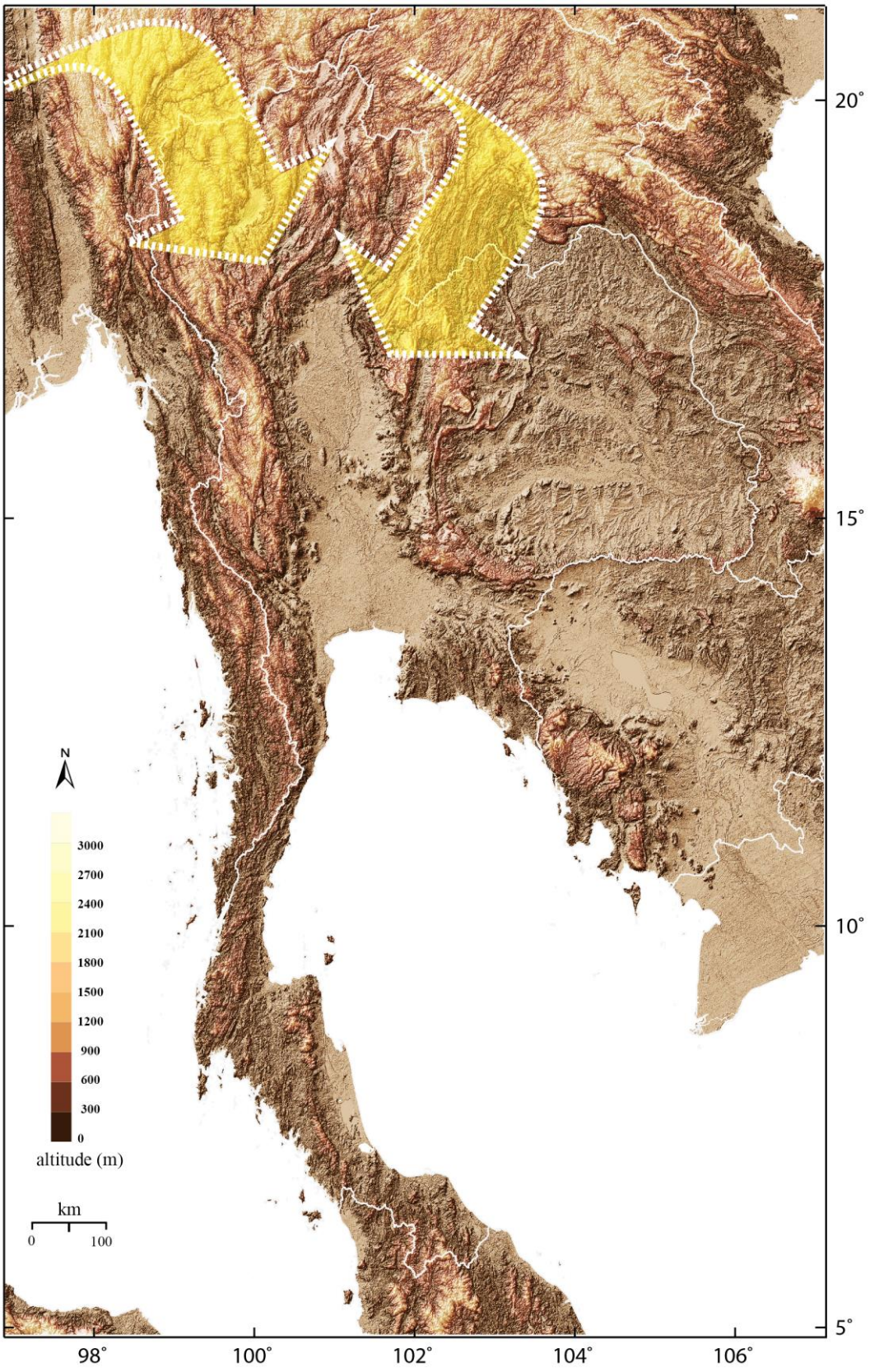

Figure 4.38 The main migration route of Senecioneae in Thailand

5. CONCLUSION

The last decade has witnessed an increased interest in the systematics of Senecioneae, and a wealth of new data has been accumulated. A phylogenetic outline of the tribe has been achieved by analysis of molecular data. However, much work remains before the evolutionary history of the tribe can be described in full detail with accuracy and confidence. Phylogenetic analyses indicated that of Senecioneae in Thailand are closely related with the Old World species. Lineage through time analysis clearly suggests an increase in the diversification rate of the Senecioneae group from the early Miocene and more pronounced in the Pleistocene. The seasonality of both temperature and precipitation is a major determinant of Senecioneae distribution and richness in Thailand and its variation led to a modification of the relative abundance of Senecioneae during the Pleistocene where the Northern Highland is the major immigration route of Senecioneae in Thailand. Thus, Thailand could be considered as a significant area for Senecioneae diversification in Southeast Asia region. Based on the results of this study, this study suggested that the inter-primer binding site (iPBS) is a powerful tool to study phylogeny and diversification as well as allows to determine genetic diversity and population structure of Senecioneae in Thailand.

6. LITERATURE CITED

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7. OUTPUT

Publications in

1. Vanijajiva O. (submitted). Inter-primer binding site (iPBS) markers reveal the population genetic diversity and structure of tropical climbing *Cissampelopsis* (Asteraceae) in Thailand. Biodiversitas. (Q3: IF = 0.880)

2. Vanijajiva O. (submitted). Spatial genetic structure of *Senecio namnaoensis* (Asteraceae), a narrow endemic from Thailand, with implication for conservation. Tropical Natural History (Q2: IF= 0.830)

3. Vanijajiva O. (in prep). Genetic diversity and structure of endemic *Gynura calciphila* in Thailand based on inter-primer binding site (iPBS) markers.

4 Vanijajiva O. (in prep). Population Genetic Variation and Structure of the invasive weed in *Erechtites hieraciifolius* Thailand

Academic applications

The knowledge of this study is applied for development teaching and learning in many subjects such as Taxonomy, Biodiversity and Plant Morphology.