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PHYLOGENETIC ANALYSIS OF WILD GAMBIR (*UNCARIA* SPP.) AND DIVERSIFICATION OF ITS CULTIVATED SPECIES (*UNCARIA GAMBIR* [W. HUNTER] ROXB.)

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SUMMARY

The genus *Uncaria* (Rubiaceae) is a group of widespread woody plants with optimum diversity. Indonesia has over 103 cultivars of gambir and its wild relatives; however, research on wild *Uncaria*, particularly in West Sumatra, Indonesia, remains limited. The related study aimed to analyze the phylogenetic relationship of *Uncaria* spp. in West Sumatra, Indonesia, and recognize the speciation processes in *U. gambir* as an important germplasm. In this study, the collection of 16 gambir samples came from Pesisir Selatan (Siguntur and Taratak Tempatih) and Lima Puluh Kota (Simpang Kapuak and Ampalu), with 12 samples sequenced using data from the GenBank. Extracting DNA used the CTAB method, amplified with ITS markers, and sequenced. Carrying out the analysis engaged the Bioedit application for alignment and MEGA for phylogenetic reconstruction and genetic distance. The ITS sequence was 623 bp long with a GC content of 61.5%. *U. gambir* forms a monophyletic clade, found closely associated with the species *U. yunnanensis* and *U. lanosa*. The genetic distance (0.099–0.102) between *U. gambir* and other species was greater than between its own species (0.000–0.003). These results will support the domestication and selection of *U. gambir* for sustainable breeding and future conservation.

Keywords: *Uncaria* spp., wild relatives, cultivated species, domestication, ITS, phylogenetic analysis, genetic distance, germplasm conservation, West Sumatra

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Key findings: *Uncaria gambir* constitutes a remarkably homogeneous genetic group characterized by the least intraspecific variation. Its close relationship with the species *U. yunnanensis* and *U. lanosa* revealed an evolutionary connection and possible ancestral origin in East Asia. The results support sustainable breeding efforts and future conservation of *Uncaria* germplasm.

INTRODUCTION

The flowering plant genus *Uncaria*, also known as Rubiaceae, is a polytypic genus comprising 34 species. Twenty-nine species existed in Asia and Australia, with three species observed in Africa and Madagascar and two species domesticated in the Americas (Ridsdale, 1978; Turner, 2018). Around 103 gambir cultivars and their wild relatives succeeded in their recognition in Indonesia. Each genotype has specific morphological characteristics, such as leaf shape, fruit shape, seed shape, taste, and fruit aroma (Ruwaida *et al.,* 2009). With numerous *Uncaria* species, Indonesia has emerged as the center of biodiversity along with its considerable distribution worldwide.

Sumatra is the main center of distribution, with seven species of *Uncaria* spp. found in the Sumatra Island (Nurainas *et al.,* 2020). Ridley (1922) reported five species of *Uncaria* spp. existing in West Sumatra, spread across almost all the districts, such as Padang, Agam, Tanah Datar, Sawah Lunto Sijunjung, Lima Puluh Kota, Pesisir Selatan, and Solok, Indonesia. It is a well-known fact that *Uncaria* spp. contain various complex and diverse secondary metabolites (Liang *et al.,* 2020; Wardi *et al.,* 2024), with the most common being the alkaloids and tannins (Munggari *et al.,* 2022). The said species has extensive uses in traditional medicine. However, to date, the community is only aware of the cultivated gambir (*Uncaria gambir*), while knowledge of wild gambir is rare and unexplored for its potential utilization and future conservation.

On *Uncaria* species, little research has taken place in West Sumatra. However, the current research on gambir has mostly focused on the use of cultivated gambir (*Uncaria gambir*) in West Sumatra (Wardi *et al.,* 2024), with less research being conducted on its wild relatives. The wild gambir species have the potential for secondary metabolites; however, their exploration has not fully progressed. Exploration of wild gambir (*Uncaria* spp.) and its relatives is lacking; therefore, it necessitates sustainable conservation and utilization in traditional medicine.

At present, human activities, such as illegal logging, forest conversion, agricultural and plantation activities, and urbanization, have increased pressure on the tropical forests of Sumatra, Indonesia (Tsujino *et al.,* 2016; Eddy *et al.,* 2021), which seemed to be the habitat of wild gambir (Pinto *et al.,* 2022). In the long term, these conditions could threaten various plant species, including wild relatives of gambir, which could lead to the extinction of various wild *Uncaria* species, even before knowing their potential. This underscores the need for significant efforts to conserve germplasm for their sustainable utilization. Phylogenetic analysis of *Uncaria* species has grave consequences for sustainable use of these valuable plant genetic resources and future conservation in West Sumatra.

By understanding the evolutionary relationships among the *Uncaria* species, it becomes possible to identify centers of diversity and prioritize their conservation efforts in areas with high phylogenetic endemism. Additionally, phylogenetic data can help choose the right germplasm for growing and breeding purposes, which will help keep *Uncaria* resources available in the future. Molecular techniques using DNA barcoding approaches, such as ITS, *Mat*K, and *trnL*-F, appeared to be crucial for phylogenetic studies of crop plants in West Sumatra, especially the wild gambir and its relatives (Vijayan and Tsou, 2010; Li *et al.,* 2015; Tnah *et al.,* 2019). Several molecular markers have been applicable to examine the phylogenetic relationship of the selected taxa in West Sumatra (Syamsuardi *et al.,* 2018; Huda *et al.,* 2019; Nuratika *et al.,* 2020; Audina *et al.,* 2025).

Therefore, the study based on exploration of the overall diversity of gambir will be helpful in effective conservation and sustainable utilization of these plant genetic resources. Moreover, phylogenetic analysis can help resolve taxonomic uncertainties and enable accurate identification, thereby facilitating the implementation of targeted bioprospecting strategies for plant diversity, particularly wild gambir. To this end, the following study aimed to determine the phylogenetic relationship of *Uncaria* spp. and analyze the diversification process of cultivated gambir indigenous to West Sumatra, Indonesia.

MATERIALS AND METHODS

Plant material

The collected *Uncaria* spp. totaled 16 from several locations in West Sumatra, Indonesia, such as Pesisir Selatan Regency (Taratak Tempatih and Siguntur) and Lima Puluh Kota Regency (Ampalu). The samples consisted of *U. homomalla*, *U. longiflora, U. lanosa,* and *U. cordata* and three samples each of the *U. gambir* landraces Udang, Cubadak, Riau Besar, and Riau Kecil. Furthermore, 12 sequences came from the GenBank, namely, *U. macrophylla, U. yunnanensis, U. lancifolia, U. laevigata, U. rhynchophylla, U. scandens, U. homomalla, U. hirsuta, U. sessilifructus, U. sinensis, U. lanosa, and Morinda citrifolia* (outgroup) (Table 1). The detailed view of sample collection locations appears in Figure 1. The germplasm collection comprised wild and cultivated gambir from West Sumatra. The detailed information on the gambir germplasm used in this study is available in Table 2.

DNA extraction, amplification, and sequencing

DNA extraction, as carried out, used the CTAB method following the protocol of Doyle and Doyle (1987). The performance of DNA amplification utilized ITS primers (ITS4, 5'TCCTCCGCTTATTGATATGC3' and ITS5, 5'GGAAGTAAAAGTCGTAACAAGG3'), as described by White *et al.* (1990). The preparation of PCR reactions consisted of Bioline MyTaq Red Mix, which underwent the following thermal conditions: an initial pre-denaturation at 95 °C for one minute, followed by 15 s of denaturation at 95 °C, 15 s of annealing at 48.8 °C, and 10 s of extension at 72 °C. The amplification's completion had a final extension step at 72 °C for 10 min. The PCR products' analysis employed electrophoresis before visualization using a Gel Doc system. The conducted sequencing of PCR products used a single-pass DNA sequencing method at Apical Scientific Laboratory, Malaysia.

Data analysis

Data analysis succeeded in processing the forward and reverse sequences obtained from the sequencing results. Initially, both sequences entailed merging into contigs to produce consensus sequences. Performing the alignment process then utilized BioEdit to ensure the accuracy of nucleotide comparisons for subsequent analyses (Ohied and Al-Badran, 2020). The use of MEGA later calculated genetic distances and constructed phylogenetic trees (Audina *et al.,* 2025). Based on molecular data, phylogenetic analysis is an important approach in understanding the relationship among species. This study utilized ITS markers obtained from nuclear DNA for analysis. The said method provided a more profound understanding of the evolutionary relationship and genetic diversity among the studied *Uncaria* spp. populations.

RESULTS AND DISCUSSION

Gambir speciation and phylogenetic analysis

After alignment, the length of the *Uncaria* spp. sequence was 623 bp. Based on the alignment results, the G-C content ranged from 61% to 62.8%, with an average of 61.5% (Tables 1 and 2). The G-C makes the DNA structure

Table 1. Genebank data used in data analysis.

Sample	Accession	Definition	G-C Content (%)	Description
Uncaria macrophylla	MF0333303	*U. macrophylla* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence.	62.8	Wild Gambir
Uncaria yunnanensis	KF881281.1	*U. yunnanensis* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	62.5	Wild Gambir
Uncaria lancifolia	KF881263.1	*U. lancifolia* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	62.2	Wild Gambir
Uncaria laevigata	KF881270.1	*U. laevigata* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	62.4	Wild Gambir
Uncaria rhynchophylla	KP092803.1	*U. rhynchophylla* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	61.8	Wild Gambir
Uncaria scandens	KF881275.1	*U. scandens* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	61.8	Wild Gambir
Uncaria homomalla	KF881251.1	*U. homomalla* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	61.5	Wild Gambir
Uncaria hirsuta	KM057050.1	*U. hirsuta* internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.	61.7	Wild Gambir
Uncaria sessilifructus	MT263757.1	*U. sessilifructus* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence.	62.2	Wild Gambir
Uncaria sinensis	FJ980386.1	*U. sinensis* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	61.0	Wild Gambir
Uncaria lanosa	KC737635.1	*U. lanosa* internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.	62.5	Wild Gambir
Morinda citrifolia	FJ907061.1	*Morinda citrifolia* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	62.9	Outgroup

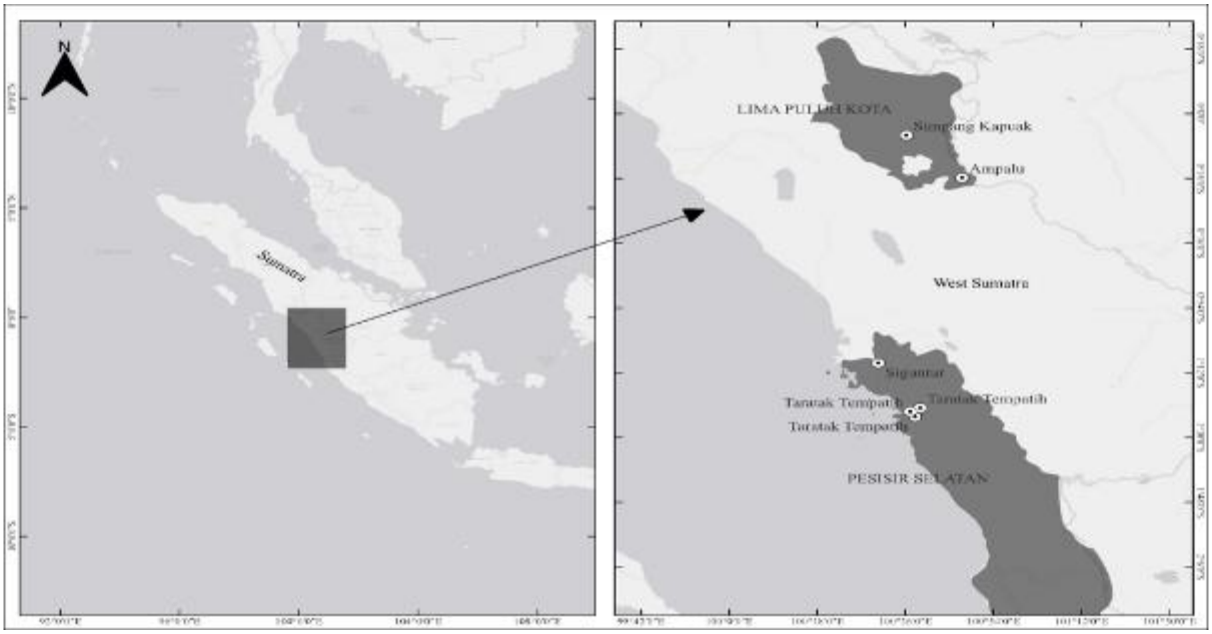

Figure 1. Map of gambir (*Uncaria* spp.) sampling locations in West Sumatra, Indonesia.

Table 2. Distribution of gambir samples used in this study.

Sample	Accession Numbers	G-C Content (%)	Location	Description
Uncaria gambir (Landrace of Riau Kecil_1)	PZ274730	62.5	Siguntur (Pesisir Selatan)	Cultivated Gambir
Uncaria gambir (Landrace of Riau Kecil_2)	PZ274731	62.5		
Uncaria gambir (Landrace of Riau Kecil_4)	PZ274732	62.5		
Uncaria gambir (Landrace of Udang_1)	PZ274733	62.7		
Uncaria gambir (Landrace of Udang_3)	PZ274734	62.7		
Uncaria gambir (Landrace of Udang_4)	PZ274735	62.7		
Uncaria gambir (Landrace of Cubadak_1)	PZ274724	62.5		
Uncaria gambir (Landrace of Cubadak_2)	PZ274725	62.6		
Uncaria gambir (Landrace of Riau Besar_2)	PZ274727	62.7		
Uncaria gambir (Landrace of Riau Besar_3)	PZ274728	62.6		
Uncaria gambir (Landrace of Riau Besar_4)	PZ274729	62.6		
Uncaria gambir (Landrace of Cubadak_5)	PZ274726	62.5	Simpang Kapuak (Lima Puluh Kota)	Cultivated Gambir
Uncaria homomalla	PZ274736	62.2	Taratak Tempatih (Pesisir Selatan)	Wild Gambir
Uncaria longiflora	PZ274722	62.4		
Uncaria lanosa	PZ274723	62.6		
Uncaria cordata	PZ274737	62.0	Ampalu (Lima Puluh Kota)	Wild Gambir

more thermodynamically stable because it has three hydrogen atoms, while A-T (adenine-thymine) only has two (Smarda *et al.*, 2014).

In past studies, the frequent employment of ITS markers prevailed in plant taxonomy and phylogenetic research because of their relatively rapid evolution and high resolution at the species level (Baldwin *et al.,* 1995; Alvarez and Wendel, 2003). By examining the ITS region, the determination of phylogenetic relationships among the *Uncaria* spp. species can succeed through the similarities and differences in nucleotide sequences among the analyzed germplasm. By using the neighbor-joining (NJ) method, the phylogenetic analysis constructs a phylogenetic tree based on genetic distances formulated from nucleotide differences (Saitou and Nei, 1987).

Based on the phylogenetic analysis, the obtained cladogram showed the kinship among the germplasm of *Uncaria gambir* and several wild relatives. The cladogram revealed an informative evolutionary pattern regarding the origin and divergence pattern of the different species. The *Uncaria gambir* samples originating from numerous locations in West Sumatra, Indonesia (landrace of Udang, Cubadak, Riau Kecil, and Riau Besar), form a monophyletic clade and become separated from other *Uncaria* species. Past studies also revealed the monophyletic structure enunciated that *U. gambir* is a lineage sharing a common ancestor and has undergone differentiation and separation from its closest relatives (Ridsdale, 1978; Wardi *et al.,* 2023).

The proximity of the *U. gambir* clade to *U. yunnanensis* illustrated an obvious evolutionary relationship. The gambir species *U. yunnanensis* location was typically in China, whereas *U. gambir* is prevalent in the Sundaland Region, especially in Western Sumatra, Indonesia. This phylogenetic relationship indicated that the ancestor of *U. gambir* likely originated in mainland Southeast Asia, China. The divergence observed in the branches within the *U. gambir* clade revealed intraspecific genetic differentiation. The species *U. gambir* is one of the wild plants that have undergone domestication due to extensive cultivation by the community. The population selection with the highest catechin content and the main bioactive compounds in gambir plant extracts has led to artificial selection for certain morphological and biochemical traits (Munggari *et al.,* 2022). As a result, some locally cultivated cultivars of *U. gambir* showed phenotypic differences for leaf size as compared with wild populations. Although the said process has not formed a new species in the biological sense, this type of domestication reinforces practical reproductive isolation because the cultivated populations were maintained vegetatively and the gene flow to wild populations was rare (Dempewolf *et al.,* 2012; Denham *et al.,* 2020).

Phylogenetic analysis based on the ITS marker yields high bootstrap values (91%–96%). If the bootstrap value is between 70 and 100, the possibility of branch changes decreases, indicating that the resulting clade structure has a high level of consistency. Conversely, if the bootstrap value is less than 70, the likelihood of changes in the clade structure increases (Rosidiani *et al.,* 2013). The phylogenetic tree successfully grouped all the *Uncaria* samples into one main group, separating them from the species *Morinda citrifolia* as an outgroup. The species *M. citrifolia* was an option for outgroup depending on its taxonomic proximity, as both belong to the family Rubiaceae but belong to different genera. The position of the species *M. citrifolia* at the bottom of the tree revealed this species was genetically distant from the genus *Uncaria*, proving it effective for rooting the phylogenetic tree (Figure 2) (Baldwin *et al.,* 1995).

The phylogenetic tree showed that several diverse *Uncaria* species formed a few separate groups. In particular, the species *U. gambir* group (obtained from Riau Kecil, Riau Besar, Udang, and Cubadak) created a single group, revealing these genotypes as genetically very close to each other and sharing a common ancestor. The cladogram places the species *U. gambir* separately from other gambir species in the genus *Uncaria.* These are *U. macrophylla, U. cordata, U. laevigata, U. senssilifructus, U. lancifolia, U. rhynchophylla, U. hirsuta, U. scandens, U. homomalla, U. sinensis, U. longiflora*, and *U. lanosa. U. gambir* proved closely associated with *U. yunnanensis* and *U. lanosa*, indicating that the ancestor of *U. gambir* originated in mainland Southeast Asia, China.

The species *U. macrophylla, U. cordata, U. laevigata, U. senssilifructus, U. lancifolia, U. rhynchophylla, U. hirsuta, U. scandens, U. homomalla,* and *U. sinensis* formed a single group. It indicates these species have relatively smaller genetic distances, ranging from 0.010 to 0.094, because of their close genetic relationship. Several other species' pairings in one clade,

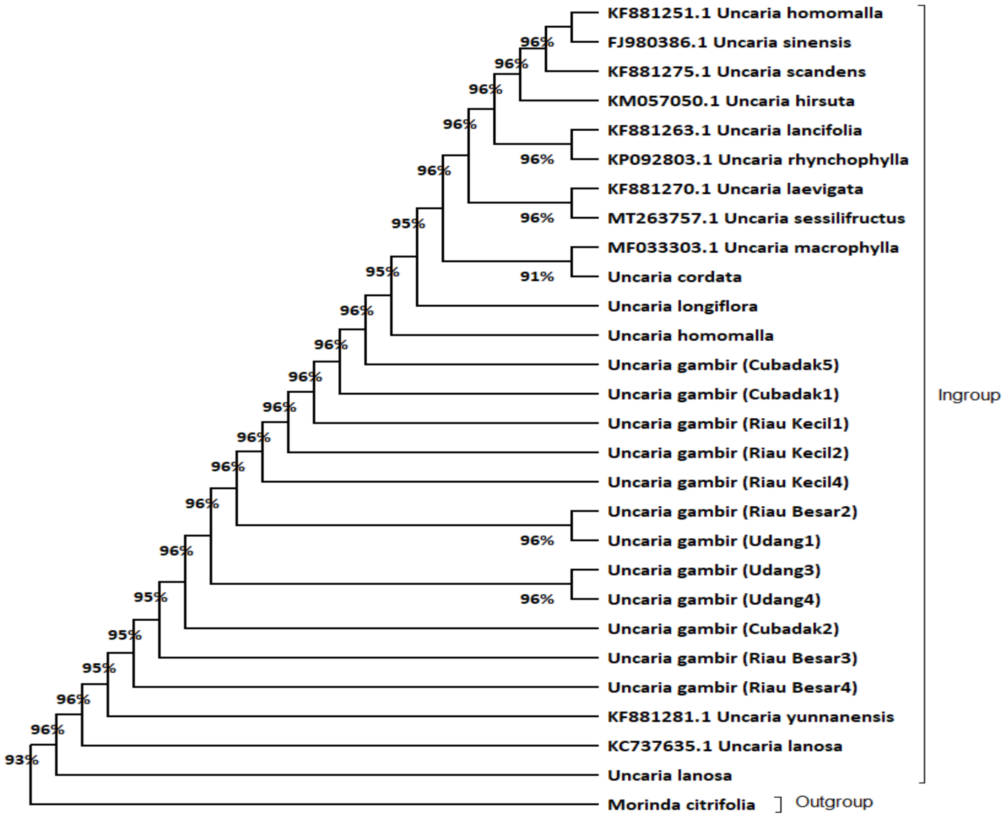

Figure 2. Phylogenetic tree of *Uncaria* spp. based on ITS markers using the neighbor-joining method.

i.e., *U. cordata* and *U. macrophylla, U. laevigata* and *U. senssilifructus*, and *U. lancifolia,* and *U. rhynchophylla*, showed similarities in their nucleotide base composition. Based on the tree created using the neighbor-joining method, one concludes that species *U. cordata* has the longest branching compared with other species (Figure 3). Thus, it became known that this species has a longer evolutionary journey than the other species. Conversely, a short branch reflects a relatively shorter evolutionary journey, as observed in the species *U. gambir*, which has the shortest branch length. In the phylogenetic tree, the branch length provides the evolutionary journey of the different species. The length of the branch indicates the duration of the species' evolutionary journey; a longer branch signifies a more extended evolutionary process, while a shorter branch denotes a more concise evolutionary trajectory (Tabatabaee *et al.,* 2023). This tendency occurred to be associated with a smaller number of nucleotide base genetic variations (Liu *et al.,* 2022). Additionally, the *U. homomalla* sequence obtained from GenBank differs from the sequence generated from field samples. This difference is likely due to intraspecific genetic variation, whereby individuals of the same species but from different geographic locations may undergo genetic divergence as a result of population isolation, local selection, and genetic drift. This is consistent with the findings of Coissac *et al.* (2016), who stated that intraspecific variation in plants can lead to a decrease in sequence similarity, particularly in high-resolution markers.

In this classification, *U. gambir* belonged in group III along with the species *U. longiflora*, while the species *U. cordata* emerged in group I, and the species *U. lanosa* was in group IV. The phylogenetic analysis based on the cladogram showed that some of

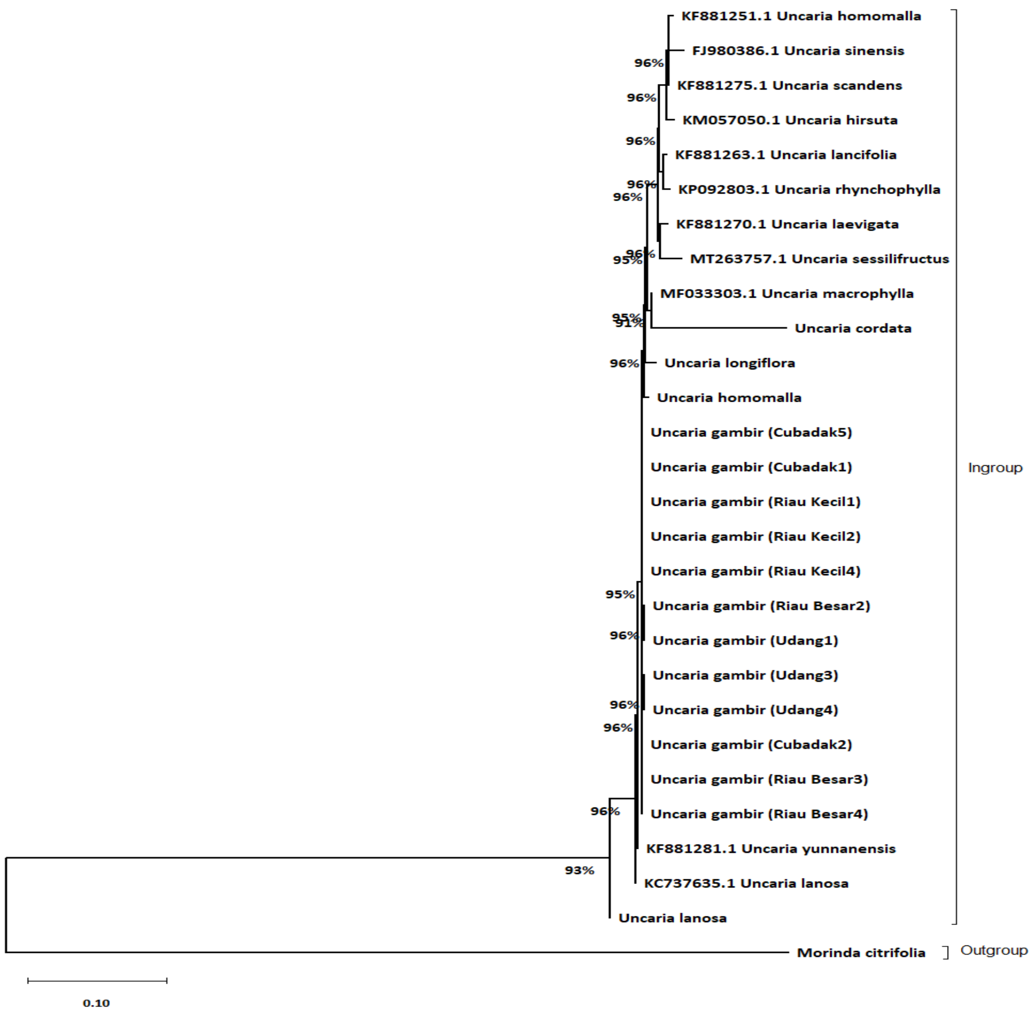

Figure 3. Phylogenetic tree of *Uncaria* spp. using the neighbor-joining method.

these groups form consistent clusters; however, others revealed significant differences. In the phylogenetic tree, the species *U. cordata* appeared separate from the species *U. gambir* group, while close to the *U. macrophylla* species. This information was consistent with the taxonomic classification that places the species *U. cordata* and *U. macrophylla* in group I. In the taxonomic list, the traditional classification of the genus *Uncaria* showed seven major groups (Groups I-VII) (Turner, 2018).

In morphological classification, species *U. lanosa* was in group IV, while in the phylogenetic tree, it appeared in a separate cluster from the species *U. gambir*. This evidence supports that species *U. lanosa* was genetically different. Genetic evidence thus supports the traditional morphological grouping. Nonetheless, certain sections require revisions in accordance with the genetic data (Liu *et al.,* 2022). This comparison implied that morphology-based classification alone may not fully reflect the true evolutionary relationship between the *Uncaria* species. For example, the species *U. gambir* and *U. longiflora* were separated in phylogenetic grouping, whereas in morphological grouping, these two species belonged to group III (Turner, 2018). Therefore, a study highly recommended the integration of morphological, genetic, and phylogenetic data to obtain an accurate and valid evolutionary classification (Villalobos-Cid *et al.,* 2020).

Table 3. Genetic distance of *Uncaria* spp.

Speci	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	2
1																												
2	0.01																											
3	0.01	0.02																										
4	0.01	0.02	0.01																									
5	0.02	0.02	0.00	0.01																								
6	0.01	0.02	0.01	0.01	0.01																							
7	0.02	0.02	0.01	0.01	0.01	0.00																						
8	0.02	0.02	0.01	0.01	0.02	0.00	0.01																					
9	0.02	0.03	0.02	0.02	0.02	0.02	0.03	0.03																				
10	0.03	0.03	0.01	0.02	0.02	0.01	0.01	0.01	0.03																			
11	0.01	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03																		
12	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.01																	
13	0.00	0.00	0.01	0.02	0.01	0.02	0.02	0.02	0.03	0.03	0.00	0.00																
14	0.00	0.00	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.03	0.00	0.01	0.00															
15	0.00	0.00	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.03	0.00	0.01	0.00	0.00														
16	0.00	0.00	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.03	0.00	0.01	0.00	0.00	0.00													
17	0.00	0.00	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.00	0.01	0.00	0.00	0.00	0.00												
18	0.00	0.00	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00											
19	0.00	0.00	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00										
20	0.00	0.00	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00									
21	0.00	0.00	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00								
22	0.00	0.00	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00							
23	0.00	0.00	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00						
24	0.00	0.00	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00					
25	0.00	0.00	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
26	0.00	0.00	0.01	0.02	0.02	0.01	0.02	0.02	0.03	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
27	0.09	0.10	0.10	0.11	0.11	0.11	0.11	0.11	0.12	0.12	0.10	0.10	0.09	0.09	0.09	0.09	0.10	0.09	0.09	0.09	0.09	0.09	0.10	0.10	0.10	0.10		
28	1.02	1.00	1.02	1.02	1.01	1.03	1.05	1.01	1.03	1.05	1.00	1.01	0.97	1.01	1.00	1.01	1.01	1.00	1.00	1.01	1.01	1.01	1.01	1.01	1.01	1.03	1.33	

Description: 1= MF033303.1 *U. macrophylla*, 2= KF881281.1 *U. yunnanensis*, 3= KF881263.1 *U. lancifolia*, 4= KF881270.1 *U. laevigata*, 5= KP092803.1 *U. rhynchophylla*, 6= KF881275.1 *U. scandens*, 7= KF881251.1 *U. homomalla*, 8= KM057050.1 *U. hirsuta*, 9= MT263757.1 *U. sessilifructus*, 10= FJ980386.1 *U. sinensis*, 11= KC737635.1 *U. lanosa*, 12= *U. longiflora*, 13= *U. lanosa,* 14= *U. gambir* (Cubadak1), 15= *U. gambir* (Cubadak2), 16= *U. gambir* (Cubadak5), 17= *U. gambir* (Riau Besar2), 18= *U. gambir* (Riau Besar3), 19= *U. gambir* (Riau Besar4), 20= *U. gambir* (Riau Kecil1), 21= *U. gambir* (Riau Kecil2), 22= *U. gambir* (Riau Kecil4), 23= *U. gambir* (Udang1), 24= *U. gambir* (Udang3), 25= *U. gambir* (Udang4), 26= *U. homomalla* , 27= *U. cordata*, and 28= *Morinda citrifolia* (Outgroup).

Genetic distance analysis

Genetic distance analysis served to evaluate the degree of kinship and genetic differences among the studied individuals. Based on the genetic distance, the values ranged from 0.000 to 0.003, which indicates the highest genetic relationship between *U. gambir* populations (Table 3). The smaller the genetic distance, the closer the kinship of the species; conversely, the greater the genetic distance, the more distant the species kinship (Resida *et al.,* 2023). These results were greatly analogous to the neighbor-joining phylogenetic tree, indicating all the *U. gambir* species form a monophyletic clade, confirming all populations originate from the same ancestor without significant genetic divergence (Nei and Kumar, 2000).

Conversely, the genetic distance between *U. gambir* and other species in the genus *Uncaria* was greater than that between *U. gambir* and other *U. gambir* species, such as *U. sinensis* (0.030), *U. hirsuta* (0.023), and *U. rhynchophylla* (0.021). These values revealed a fairly high level of interspecies genetic divergence, consistent with their morphological and geographical distribution differences. The species *U. sinensis* and *U. hirsuta* existed in East Asia (China and Vietnam), while *U. gambir* was evident in the Western Southeast Asia (Sumatra and the Malay Peninsula). These considerable differences reinforce the hypothesis that geographic isolation played a vital role in this evolution (Coyne and Orr, 2004). Thus, the combination of NJ cladogram results and ITS genetic distances confirmed that *U. gambir* was distinctly a separate taxon from other *Uncaria* species but also remarkable with low intraspecific variation.

CONCLUSIONS

The results showed species *U. gambir* forms a separate monophyletic clade with low genetic distance (0.000–0.003), confirming robust genetic uniformity and obvious separation from other *Uncaria* species. The greater genetic distance between *U. gambir* and other species, such as *U. sinensis, U. hirsuta,* and *U. rhynchophylla* (0.007–0.032), revealed significant interspecific divergence. Furthermore, the close phylogenetic relationship between the species *U. gambir, U. yunnanensis*, and *U. lanosa* (0.003–0.005) suggested a common evolutionary origin. Overall, this study found that the generated ITS-based molecular evidence provides a basis for refining the taxonomy of *Uncaria*.

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