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Molecular phylogenetics uncovers previously unrecognized Mexican diversity within the pantropical Cassytha filiformis complex

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Abstract

Cassytha filiformis is a hemiparasitic species of Lauraceae with a pantropical distribution and subtle morphological variation across its range. In Mexico, populations traditionally assigned to *C. filiformis* occur disjunctly in coastal dune systems of the Yucatan Peninsula and in inland habitats of western and southern Mexico. To investigate their evolutionary relationships within a global context, we sequenced four complete plastomes from Chiapas, Jalisco, Quintana Roo, and Yucatan (one accession per state) and generated nuclear ITS sequences for 40 individuals representing eight populations. Phylogenomic analyses recovered *C. filiformis sensu lato* as monophyletic assemblage comprising three major evolutionary lineages: an Asian lineage, a Tropical widespread lineage spanning Asia, Africa, and the Americas, and a deeply divergent lineage endemic to western Mexico. Mexican populations were not recovered as a single monophyletic group; instead, populations from the Yucatan Peninsula (Quintana Roo, and Yucatan) and Chiapas clustered with Caribbean accessions, whereas western Mexican populations formed an early-diverging lineage. Divergence-time analyses indicate that the western Mexican lineage has remained evolutionarily isolated since the early Miocene (ca. 23.75 Ma). The congruence between plastome and nuclear datasets, together with consistent morphological and ecological differences, supports the recognition of this lineage as the reinstated species *Cassytha brachystachya* (Meisn.) Ruiz-Sanchez & L. Perez. Our results demonstrate that *C. filiformis*, as traditionally circumscribed, represents a geographically structured species complex and illustrate how integrative phylogenomics can uncover overlooked diversity within widespread tropical plants.

Introduction

Cassytha L. is the only genus of parasitic vines within the family Lauraceae (subfamily Cassythoideae, tribe Cassytheae) and comprises approximately 20–23 species distributed mainly in Australia, Southeast Asia, and Africa (Awang et al. 2018; Li et al. 2025; Tian et al. 2021; Yang et al. 2026; Yu et al. 2023). Australia represents the center of diversification of the genus, harboring 19 described species (Liu et al. 2024; Weber 1981; Yu et al. 2023). Among them, *Cassytha filiformis* L. is unique in exhibiting a pantropical distribution, occurring throughout tropical and subtropical regions, particularly in coastal habitats. Its fruits remain buoyant and viable for extended periods, suggesting an important role of oceanic dispersal, whereas birds are considered the main agents of local seed dispersal (Heide-Jørgensen 2008; Mahadevan and Jayasuriya 2013; Nickrent 2020; Zhang et al. 2022). Morphologically, the species is characterized by slender green to orange stems bearing haustoria, reduced triangular leaves, and white flowers arranged in racemose inflorescences (Teixeira-Costa and Davis 2021; Yu et al. 2023). Although *Cassytha* has convergently evolved a vine-like parasitic habit similar to that of *Cuscuta* (Convolvulaceae), both genera belong to distantly related angiosperm families (Nelson 2008; Westwood et al. 2010).

Owing to its hemiparasitic lifestyle, *Cassytha filiformis* has attracted attention because of its ecological impacts on host plants and its evolutionary distinctiveness within Lauraceae. Phylogenetic studies based on mitochondrial, plastid, and nuclear genomes consistently recover *Cassytha* as a well-supported monophyletic lineage that is highly divergent from the woody members of Lauraceae (Li et al. 2025; Song et al. 2020; Song et al. 2024; Yang et al. 2023). This evolutionary divergence is accompanied by extensive plastome reduction. Complete plastomes of *C. filiformis*, *C. melantha* R. Br., and *C. pubescens* R. Br. lack one copy of the inverted repeat, exhibit structural rearrangements, accelerated substitution rates, and have lost several genes associated with photosynthesis (Song et al. 2017; Wu et al. 2017; Yang et al. 2023; Yu et al. 2023). *C. filiformis* possesses one of the smallest plastomes known in Lauraceae (114–116 kb), a pattern consistent with genome reduction observed across multiple parasitic plant lineages (Chen et al. 2024; Claude et al. 2025).

Recent phylogenomic studies have also challenged the traditional circumscription of *Cassytha filiformis*. Analyses of plastid and nuclear genomes have recovered two major evolutionary lineages associated with differences in fruit morphology and habitat preferences, suggesting that the species may represent a complex of cryptic taxa (Liu et al. 2024). Species delimitation within the genus remains problematic because diagnostic morphological characters are inconsistent, and specimens are frequently misidentified as *C. capillaris* Meisn. or *C. larsenii* Kosterm. (Liu et al. 2024; Song et al. 2017; Yu et al. 2023). These studies further suggest that the evolutionary history of *C. filiformis* involved repeated long-distance dispersal events followed by geographic isolation. Despite recent advances, sampling remains heavily biased toward Australia and Southeast Asia. Populations from the Americas, particularly Mexico and Central America, are poorly represented in current phylogenomic datasets, limiting our understanding of the origin and diversification of Neotropical populations. Consequently, whether geographically isolated Mexican populations represent a single evolutionary lineage or multiple independent colonization events remains unresolved.

In Mexico, *Cassytha filiformis* ("fideo de monte") exhibits a disjunct distribution, occurring primarily along the coastal systems of the Yucatan Peninsula (Campeche, Quintana Roo and Yucatan) and in isolated inland localities of western and southern Mexico, including Chiapas, Guerrero and Jalisco (Lorea-Hernández 2002; Villaseñor 2016; Fig. 1). This unusual geographic pattern provides an exceptional opportunity to test whether Caribbean and Pacific populations share a common evolutionary origin or instead represent independently evolving lineages. Moreover, recent ecological studies have documented an expansion of the species in northern Yucatan and demonstrated its negative effects on the reproduction and survival of native coastal shrubs (Anchevida et al. 2024; Parra-Tabla et al. 2023), highlighting the importance of understanding its evolutionary history.

Recent advances in sequencing techniques like genome skimming have greatly improved phylogenetic resolution and enabled the reconstruction of detailed evolutionary and biogeographic histories (Li et al. 2025; Song et al. 2017; Wu et al. 2017; Yang et al. 2026). Here, we present the first complete plastome sequences from Mexican populations traditionally assigned to *Cassytha filiformis* and integrate them with nuclear ITS data in a global phylogenetic framework. We aimed to (1) reconstruct the evolutionary relationships of Mexican populations, (2) estimate the timing of lineage diversification, and (3) evaluate whether the deeply divergent western Mexican lineage warrants taxonomic recognition. By integrating plastome phylogenomics, nuclear ITS sequences, and divergence-time analyses, this study provides the first comprehensive evolutionary framework for Mexican populations of *Cassytha* and offers new insights into the diversification of one of the few pantropical parasitic plant species.

Materials and methods

Plant material

Fresh stem samples of *Cassytha filiformis* were collected in eight locations across four Mexican states (Chiapas, Jalisco, Quintana Roo, and Yucatan) (Fig. 1). Between 11 and 22 individuals were sampled per location, of which, 40 individuals were successfully amplified for nuclear ITS region (Table 1). One representative individual from each state was selected for complete plastome sequencing. Voucher specimens were deposited in the Herbarium of the Instituto de Botánica, Universidad de Guadalajara (IBUG).

Table 1
Sampling localities and geographic information for Mexican populations of *Cassytha filiformis*

Population	Locality	State	Latitude (N)	Length (W)	Elevation (m)	Individuals (ITS)	Plastome sequence
CS	Cañon del Sumidero	Chiapas	16.794015	-93.0889	832	2	✓
P	Palenque	Chiapas	17.563322	-92.01082	63	1	–
PV	Puerto Vallarta	Jalisco	20.51888	-105.2047	1200	10	✓
IB	Isla Blanca	Quintana Roo	21.314143	-86.80968	6	4	–
M	Mayakoba	Quintana Roo	20.691139	-87.01877	0	7	–
PM	Puerto Morelos	Quintana Roo	20.8266	-86.89902	2	4	✓
X	Xcacel	Quintana Roo	20.341528	-87.34362	11	7	–
S	Sisal	Yucatan	21.159	-90.012	5	5	✓

DNA extraction

Total genomic DNA was extracted at the Laboratorio de Identificación y Caracterización Vegetal (LaniVeg), Universidad de Guadalajara, using a modified CTAB protocol (Doyle and Doyle 1987; Garg et al. 2025). For each extraction, approximately 10–20 mg of silica–dried stem tissue was ground to a fine powder, and the resulting DNA was resuspended in 100 µL of TE buffer. DNA concentration and purity were assessed using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), and DNA integrity was verified by electrophoresis on 1% agarose gels.

Plastome sequencing, assembly and annotation

High-quality DNA was used for complete plastome sequencing. Genomic DNA was fragmented by sonication, and sequencing libraries were prepared using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, Ipswich, MA, USA). Paired-end sequencing (2 × 150 bp) was performed on an Illumina NovaSeq 6000 platform at CD Genomics (Shirley, NY, USA). Raw reads were filtered to remove adapters and low-quality bases before plastome assembly. Assembly was performed using GetOrganell v. 1.7.7.1 (Jin et al. 2020) with k-mer values set to 21, 45, 65, 85 and 105. The published plastome of *Cassytha filiformis* (Genbank: NC_036001) was used as a reference to verify assembly completeness (Wu et al. 2017). Plastomes were annotated using the GeSeq (Tillich et al. 2017) and manually inspected. The plastome maps were drawn in OGDRAW (Greiner et al. 2019). The annotated chloroplast genome was submitted to the GenBank database of the National

Center for Biotechnology Information (NCBI) with the accession number (Genbank: PZ835085 (CS2), PZ835086 (PM2), PZ835087 (S3), PZ835088 (PV9).

ITS amplification and sequencing

Nuclear ribosomal Internal Transcribed Spacer (ITS) sequences were generated using Sanger sequencing with the primers ITS-1u and ITS-4u (Cheng et al. 2016). The polymerase chain reaction (PCR) was performed in an AERIS™ thermal cycler (Esco Healthcare, Singapore) under the following conditions: initial denaturation at 94 ºC for 3 min, 30 denaturation cycles, initiation step at 94 ºC for 1 min, hybridization at 50 ºC for 1.30 min, and extension at 72 ºC for 1 min, followed by a final extension at 72 ºC for 10 min. The PCR products were assessed on a 1% agarose gel using SYBR safe DNA Gel Stain (Thermo Fisher Scientific), and visualized with a UV Transilluminator (UVP). The sequencing reaction was purified using the BigDye X Terminator Purification Kit (Thermo Fisher Scientific). Finally, the ITS region was sequenced using a SeqStudio Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the LaniVeg.

Phylogenetic analyses

Complete plastome phylogenetic analysis

Complete plastome sequences available for *Cassytha* species were downloaded from GenBank, including *C. filiformis*, *C. larsenii*, *C. melantha*, and *C. pubescens*. We also included Mexican samples collected in the states of Chiapas, Jalisco, Quintana Roo, and Yucatán (Table 1). The plastome dataset included 69 samples in total (Table S1). Three species, *Cinnamomum camphora* (L.) J. Presl (NC035882, 152,570 bp), *Neocinnamomum delavayi* (MT621607, 150,751 bp), and *Neocinnamomum caudatum* (Nees) Merr. (MT621576, 150,842 bp), were selected as outgroups because *Neocinnamomum* and *Cinnamomum* are among the genera most closely related to *Cassytha* in recent phylogenetic analyses of Lauraceae (Li et al. 2025). Sequences were aligned using MAFFT with default parameters (Katoh et al. 2019). Phylogenetic relationships were inferred using Maximum Likelihood (ML) in raxmlGUI 2.0 (Edler et al. 2021). The best-fit nucleotide substitution model, GTR + I + G, was selected using raxmlGUI. Nodal support was assessed with 1,000 bootstrap replicates.

ITS phylogenetic analysis

For the nuclear phylogenetic analysis, ITS sequences available for *Cassytha* species were downloaded from GenBank, including *C. melantha* and *C. pubescens*. The dataset also included Mexican samples collected in the states of Chiapas, Jalisco, Quintana Roo, and Yucatán. A total of 85 samples were included in the ITS dataset, 40 of which originated from Mexico (Table S2). *Neocinnamomum delavayi* (Lecomte) H. Liu (MT628603) and *Neocinnamomum mekongense* (Hand.-Mazz.) Kosterm. (MT628637) were used as outgroups based on their close phylogenetic relationship with *Cassytha* within Lauraceae (Li et al. 2025). ITS sequences were aligned using MAFFT with default parameters (Katoh et al. 2019). Phylogenetic relationships were inferred using Maximum Likelihood (ML) in raxmlGUI 2.0 (Edler et al. 2021). The best-fit nucleotide substitution model, GTR + I + G, was selected using raxmlGUI and nodal support was evaluated with 1,000 bootstrap replicates.

Divergence time estimation

Divergence times were estimated using the RelTime method (Tao et al. 2020) implemented in MEGA 11 (Tamura et al. 2021). Secondary calibration points were obtained from the plastome-based time-calibrated phylogeny of Lauraceae published by Liu et al. (2024). Three calibration points were applied: the stem age of *Neocinnamomum* (83.43 Ma), the crown age of *Cassytha* (37.04 Ma), and the stem age of the *Cassytha filiformis* complex (23.94 Ma). Divergence time analyses were conducted using the maximum-likelihood phylogeny inferred from complete chloroplast genome sequences in RAxML. Branch lengths were converted into divergence time estimates using the specified calibration constraints.

Results

Plastome variation of Mexican lineages

The plastome length of the Mexican accessions of *Cassytha filiformis* ranged from 114,262 bp (S3, Yucatan) to 114,987 (CS2, Chiapas), and *Cassytha brachystachya* 116,175 bp (PV9, Puerto Vallarta, previously identified as *C. filiformis*), representing approximately 1.9 kb of variation among individuals (Table S3). All plastomes displayed the characteristic non-quadripartite organization previously described for *Cassytha*, in which one of the two inverted repeat regions is absent. Overall GC content varied only slightly among samples (36.7–37%), indicating a highly conserved nucleotide composition. A total of 110–111 genes were annotated in each plastome, including 95–97 protein-coding genes, 32–33 tRNAs, and 4–5 rRNAs. Several photosynthesis-related *ndh* genes were absent or pseudogenized from all Mexican plastomes, whereas gene

content was otherwise highly conserved among accessions. Despite the overall structural conservation, plastome size differences resulted primarily from insertions and deletions in intergenic regions. The Puerto Vallarta plastome exhibited the greatest divergence from all other Mexican samples.

Phylogenetic relationships

The complete plastome alignment comprised 170,776 bp, of which 22,043 sites (12.9%) were variable, and 12,294 (7.2%) were parsimony informative. The nuclear ITS alignment consisted of 784 bp, including 371 variable sites (47.3%) and 263 parsimony-informative sites (33.5%). Maximum likelihood analyses recovered well-resolved phylogenies, with the major clades receiving moderate to strong bootstrap support (BS ≥ 70%) in both datasets. Phylogenetic analyses based on complete plastome sequences recovered the accessions traditionally assigned to *Cassytha filiformis* support, BS ≥ 95%; Fig. 2). Notably, accessions identified as *C. larsenii* from GenBank were nested within *C. filiformis* and did not form an independent lineage, supporting previous suggestions that these taxa are not genetically distinct. Within *C. filiformis*, three major evolutionary lineages were consistently recovered. The Asian lineage (BS = 100%) comprised samples from China, Laos, Thailand, and Indonesia, exhibiting additional phylogeographic structure among Southeast Asian populations. The Tropical widespread lineage (BS = 100%) included accessions from eastern Asia (China and Japan), Africa (Kenya), and the Americas (Caribbean and Mexico), indicating a broad intercontinental distribution. In contrast, a third lineage was represented exclusively by the Puerto Vallarta accession (PV9), which occupied the earliest-diverging position within *C. filiformis* and was recovered as sister to all remaining accessions. Within the Tropical widespread lineage, the Mexican samples from the Yucatan Peninsula (Quintana Roo and Yucatan) and Chiapas formed a strongly supported subclade (BS = 99%) that was sister to Caribbean accessions from the Cayman Islands and Puerto Rico (BS = 100%), indicating a close evolutionary relationship between Mesoamerican and Caribbean populations. Phylogenetic reconstruction based on nuclear ITS sequences recovered a largely congruent topology with plastome phylogenetic tree (Fig. 3). The Asian lineage was again strongly supported (BS = 97%), whereas the Tropical widespread lineage (BS = 93%) included samples from China, Japan, and Mexico. Although ITS resolution was lower than that obtained from complete plastomes, both datasets consistently recovered the Puerto Vallarta lineage as a deeply divergent evolutionary lineage within *C. filiformis*.

Divergence time estimation

The time-calibrated phylogeny estimated the stem age of *Cassytha* at 91.86 Ma, indicating a Late Cretaceous origin for the genus (Fig. 4). The crown of *Cassytha* occurred during the Oligocene 30.85 Ma (95% CI = 30.84–35.76), giving rise to the major extant lineages. Within *C. filiformis*, the Puerto Vallarta lineage diverged first at approximately 23.75 Ma (95% CI = 18.95–31.88), followed by the split between the two principal clades at 19.8 Ma (95% CI = 18.95–24.39). The predominantly Asian lineage originated around 13.47 Ma (95% CI = 12.76–18.84), whereas diversification within the Tropical widespread lineage gave rise to the lineage containing the Mexican, Caribbean, Japanese, and Kenyan accessions at approximately 7.47 Ma (95% CI = 7.24–13.99).

Taxonomic treatment

The plastome and nuclear phylogenetics analyses consistently recovered the Mexican montane (Puerto Vallarta) lineage as a well-supported, evolutionarily independent clade distinct from *Cassytha filiformis sensu stricto*. This lineage also differs morphologically by its shorter inflorescences, fewer flowers per spike, ferruginous pubescence on young stems and peduncles, and its restriction to montane Pine-oak forests of western Mexico. These lines of evidence support recognition of this lineage at the species level. Accordingly, we here elevate *Cassytha americana* var. *brachystachya* Meisn. to species rank and reinstate the name *Cassytha brachystachya* (Meisn.) Ruiz-Sanchez & L. Perez.

Cassytha brachystachya (Meisn.) Ruiz-Sanchez & L. Perez, stat. nov.

Basionym: *Cassytha americana* var. *brachystachya* Meisn. Prodromus Systematis Naturalis Regni Vegetabilis 15(1): 256. 1864.

Revised morphological description of Cassytha brachystachya

Herbaceous hemiparasitic twining vine, stems reaching up to 1 m long. Stems slender, 0.8–1.0 mm in diameter when dry, light green to orange-yellow, apparently becoming orange with age; glabrous on mature portions, young branches sparsely covered with ferruginous pubescence. Leaves reduced to minute scales, lanceolate-triangular, 1.2–1.5 × 0.5–0.7 mm. Inflorescences axillary, spicate, 1–2 cm long, bearing 3–7 flowers; peduncle densely ferruginous-pubescent. Bracts broadly triangular, obtuse, ca. 0.7–1.1 × 0.9 mm, glabrous with ciliate margins, pale green. Flowers sessile, oblong, 2.2–2.4 mm long. Hypanthium inconspicuous at anthesis. Sepals 3, triangular, acute, ca. 0.7 × 0.9 mm, white, glabrous, margins ciliate. Petals 3, ovate to oblong-ovate, 2.0–2.1 × 1.5–1.6 mm, bright white, glabrous, becoming greenish toward the base. Stamens arw arranged in four whorls of three; outer two whorls fertile and introrse; third whorl fertile and extrorse; innermost whorl consisting of obtrullate staminodes. Filaments widening distally into obtrullate anthers, glabrous, white. Ovary ovoid, ca. 1 mm long, unilocular with a single ovule; style ca. 0.4 mm long; stigma slightly capitate. Fruit ellipsoid to ovoid, 5–6 × 4 mm, glabrous, yellow when immature, becoming dark brown to blackish at maturity (Fig. 5).

Habitat and distribution

Cassytha brachystachya is endemic to western Mexico and is currently known only from the municipality of Puerto Vallarta, Jalisco. It grows in open Pine-oak forest at elevations between 1,200 and 1,250 m, parasitizing primarily *Quercus elliptica*, *Clethra sp.*, and *Bejaria mexicana*.

Phenology

Flowering has been recorded from January to May. Fruiting has been observed in May.

Additional specimens examined of Cassytha brachystachya

1) MEXICO. Jalisco: Municipality of Puerto Vallarta, Ojo de Agua, south-facing slope, 7 km southwest of Llanitos, 20°31’13.2”N, 105°11’46.3”W, 1224 m, open Pinus-Quercus Forest, 29 January 2013, *A. Flores-Argüelles and A.R. Romero-Guzmán 624* (IBUG); 2) Ojo de Agua, south-facing slope, 7 km southwest of Llanitos, 20°31’10.23”N, 105°11’47.41”W, 1202 m, open Pinus-Quercus Forest, 7 May 2013, A. *Flores-Argüelles and A.R. Romero-Guzmán 695* (IBUG). 3) Ojo de Agua, south-facing slope, 7 km southwest of Llanitos, 20° 31 '08.0’N, 105° 12 '16.9”W, open Pinus-Quercus Forest, 12 January 2025, 1200 m, *E. Ruiz-Sanchez et al. 873*, *875* (IBUG).

Taxonomic notes

Cassytha brachystachya was originally described as *Cassytha americana* var. *brachystachya* by Meisner (1864), based on its comparatively short inflorescences. Subsequent authors treated this taxon as a synonym of *C. filiformis*, likely owing to the considerable morphological variation exhibited by the latter across its pantropical distribution. However, our plastome and nuclear phylogenies consistently recover the Mexican montane populations as a distinct and well-supported lineage. This phylogenetic evidence, together with their unique ecological niche and stable morphological differences, supports recognition of *C. brachystachya* as a distinct species (Table S4).

Discussion

Despite the pantropical distribution and morphological uniformity traditionally attributed to *Cassytha filiformis*, our analyses of complete plastomes and nuclear ITS sequences reveal that Mexican populations do not represent a single evolutionary lineage. Instead, they are distributed among three deeply divergent clades, with the western Mexican lineage occupying the earliest-diverging position within *C. filiformis*. These findings indicate that Mexican populations likely originated through multiple colonization events rather than diversification from a single ancestral population and provide evidence for previously unrecognized evolutionary diversity within this widespread hemiparasitic species.

Phylogenetic position of the Mexican lineages

The plastomes of the Mexican *Cassytha filiformis* accessions fall within the size range previously reported for the species and retain the characteristic non-quadripartite organization resulting from the loss of one inverted repeat (IR), a genomic feature that appears to be conserved throughout the genus (Liu et al. 2024; Wu et al. 2017; Yu et al. 2023). This structural organization, together with the loss of several *ndh* genes involved in photosynthesis, resembles patterns reported in other hemiparasitic lineages, particularly species of *Cuscuta*, where plastome reduction has accompanied the transition toward a parasitic lifestyle (Claude et al. 2025). Although plastome architecture is highly conserved among sampled populations, phylogenetic analyses based on both complete plastomes and nuclear ITS sequences recovered strong geographic structure within *C. filiformis*. Mexican populations do not form a monophyletic group. Instead, the accession from Puerto Vallarta, Jalisco, occupies the earliest-diverging position within *C. filiformis*, whereas samples from the Yucatan Peninsula and Chiapas cluster with accessions from the Caribbean, Japan, Kenya, and China (Fig. 2, 3). The congruence between plastid and nuclear datasets indicates that these lineages represent independent evolutionary histories rather than incomplete lineage sorting or marker-specific variation. The phylogenetic placement of the Puerto Vallarta lineage suggests that western Mexico harbors an evolutionarily distinct lineage that has remained isolated for a considerable period. Similar levels of genetic divergence have recently been interpreted as evidence for cryptic diversity within Southeast Asian populations of *C. filiformis* (Liu et al. 2024). Likewise, the congruence between plastome and nuclear phylogenies, together with consistent morphological and ecological differences, supports recognition of the western Mexican lineage as the distinct species *Cassytha brachystachya* (Meisn.) Ruiz-Sanchez & L. Perez. Comparable phylogeographic patterns have been documented in several pantropical plant groups, where repeated long-distance dispersal followed by regional isolation generated deeply divergent geographic lineages. Similar evolutionary histories have been proposed for mangroves, tropical lianas, and several pantropical lineages of Annonaceae and Lauraceae (Huang et al. 2025; Li et al. 2024; Lo et al. 2014; Nie et al. 2013), suggesting that recurrent transoceanic dispersal has played an important role in shaping present-day pantropical distributions.

Historical biogeography and divergence of Mexican lineages

Molecular dating indicates that the crown lineage of *Cassytha* originated during the Late Cretaceous, consistent with previous estimates for Lauraceae (Li et al. 2025). Within the genus, *C. filiformis* represents an ancient lineage that has undergone successive diversification events

since the Oligocene. The earliest diversification within *Cassytha* dates to the Eocene–Oligocene transition (~ 30.85 Ma), whereas diversification within *C. filiformis* was concentrated during the Miocene (~ 19.8–7.47 Ma). A period marked by pronounced global cooling, Antarctic glaciation, and extensive reorganization of tropical forests that profoundly influenced diversification across many angiosperm lineages (Morley 2000; Zachos et al. 2001, 2008;). Most diversification events within *C. filiformis* occurred during the middle Miocene to Pliocene (~ 13–3 Ma), coinciding with major climatic fluctuations, repeated expansion and contraction of tropical forests. These climatic oscillations likely promoted repeated fragmentation and reconnection of tropical forests while simultaneously altering coastal environments and migratory routes, thereby increasing opportunities for long-distance dispersal among tropical regions (Herold et al. 2012; Pound et al. 2012; Pound and Salzmann 2017). Similar temporal patterns have been reported in several pantropical plant groups, including Lauraceae, and the genus *Paederia* (Rubiaceae) and *Uncaria* (Pedaliaceae) where Miocene divergence has been interpreted as evidence of repeated transoceanic colonization rather than recent regional diversification (Huang et al. 2025; Li et al. 2025; Nie et al. 2013). Despite the recovery of geographically structured subclades, the broader phylogeny does not follow a strictly continental pattern, as closely related lineages occur across Asia, Africa, the Caribbean, and Mexico. Given the fleshy fruits of the species and its broad coastal distribution, dispersal mediated by migratory birds and ocean currents represents plausible mechanisms explaining the present-day pantropical distribution (Nelson 2008). Both mechanisms are biologically plausible because the fleshy drupes of *C. filiformis* remain buoyant and viable after prolonged exposure to seawater, while frugivorous birds are known to consume and disperse the fruits over long distances (Heide-Jørgensen 2008; Mahadevan and Jayasuriya 2013; Nickrent 2020). Taken together, the phylogenetic relationships, divergence-time estimates, and present-day distribution support a scenario in which Mexico was colonized independently by at least two evolutionary lineages of *C. filiformis*. One lineage gave rise to the populations currently distributed in the Yucatan Peninsula and Chiapas, closely related to Caribbean accessions, whereas the Pacific lineage (Puerto Vallarta) represents an older and evolutionarily independent colonization.

Plastome evolution in Cassytha

The plastomes of Mexican *Cassytha* provide additional evidence of the progressive structural reduction associated with the evolution of parasitism in angiosperms. Despite retaining photosynthetic capability, *Cassytha* exhibits the loss of one inverted repeat and multiple photosynthesis-related genes, indicating that plastome degradation began early during the evolution of the lineage. This evolutionary trajectory resembles patterns observed in *Cuscuta* (Claude et al. 2025), although the degree of plastome reduction is considerably less extensive than that reported for fully parasitic families such as Balanophoraceae and Mystropetalaceae, where plastomes have experienced massive gene loss or have even been completely lost (Ceriotti et al. 2025; Yu et al. 2025). Consequently, *Cassytha* appears to represent an intermediate stage in plastome evolution of the parasitic plants, providing an informative model for understanding the genomic consequences of the transition from autotrophy to parasitism (Liu et al. 2026; Wu et al. 2017).

Morphological differentiation and cryptic diversity

Despite their deep genetic divergence, the Mexican lineages of *C. filiformis* remain morphologically difficult to distinguish. Species delimitation within the genus has long been challenging because most traditional diagnostic characters—including stem pubescence, inflorescence length, flower size, and fruit morphology—show extensive overlap among populations (Kokubugata et al. 2024; Liu et al. 2024; Yu et al. 2023). Recent genomic studies based on genome-wide SNPs have demonstrated that the Japanese taxa *C. filiformis* var. *filiformis* and *C. filiformis* var. *duripraticola* represent genetically distinct lineages despite relatively subtle morphological differences, whereas individuals with intermediate phenotypes appear to be hybrids (Kokubugata et al. 2024). These findings suggest that morphological conservatism, together with occasional hybridization, may have obscured species boundaries throughout the genus. The extensive synonymy currently associated with *C. filiformis*, including nearly thirty published names recognized in historical classifications, likely reflects this taxonomic complexity (World Flora Online, WFO, https://wfoplantlist.org/taxon/wfo-0000589782-2025-06). Consequently, cryptic genetic diversity within the species may ocurr across its pantropical range.

Taxonomic and evolutionary implications

Among the Mexican populations analyzed, the Puerto Vallarta lineage is particularly remarkable because it was consistently recovered as the earliest-diverging lineage of *C. filiformis* in both plastome and nuclear phylogenies. Divergence-time analyses estimate that this lineage separated from all other sampled populations approximately 21 Ma, suggesting prolonged evolutionary isolation. The congruence between plastome and nuclear phylogenies, together with consistent morphological and ecological differences, supports recognition of the western Mexican lineage as *Cassytha brachystachya*. The reinstatement of this species resolves the taxonomic identity of the montane populations from Jalisco and further demonstrates that *C. filiformis*, as traditionally circumscribed, comprises multiple geographically structured evolutionary lineages.

Conclusions

Our phylogenomic analyses reveal that the Mexican populations of *Cassytha filiformis* do not constitute a single evolutionary lineage but instead represent at least two deeply divergent lineages with distinct evolutionary histories. These findings demonstrate that the current circumscription of *C. filiformis* likely encompasses multiple independently evolving lineages that have remained obscured by morphological conservatism. By incorporating the first complete plastome sequences from Mexico into a global phylogenetic framework, this study substantially expands our understanding of the evolutionary history and biogeography of the only pantropical species of *Cassytha*. Our study provides the first phylogenomic evidence supporting the recognition of *Cassytha brachystachya* as a distinct species endemic to western Mexico. More broadly, our results demonstrate how dense geographic sampling combined with plastome phylogenomics, nuclear markers, and morphology can reveal previously overlooked taxonomic diversity within widespread tropical species.

Declarations

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Author Contributions Luz Perez-Garcia: writing – original draft, field collection, laboratory work, data analysis. **Eduardo Ruiz-Sanchez:** Field collection, data analysis, writing – review & editing. **Jessica Perez-Alquicira:** methodology, data analysis, writing – review & editing. **Gabriela Mendoza-González:** field collection, writing – review & editing of the manuscript. All authors reviewed and edited the final version.

Conflicts of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary information The online version contains supplementary material available

Data Availability Statement Plastome sequences (accession numbers PZ835085 to PZ835088) and ITS sequences (accession numbers PZ500133 to PZ500172) are available from GenBank.

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Figures

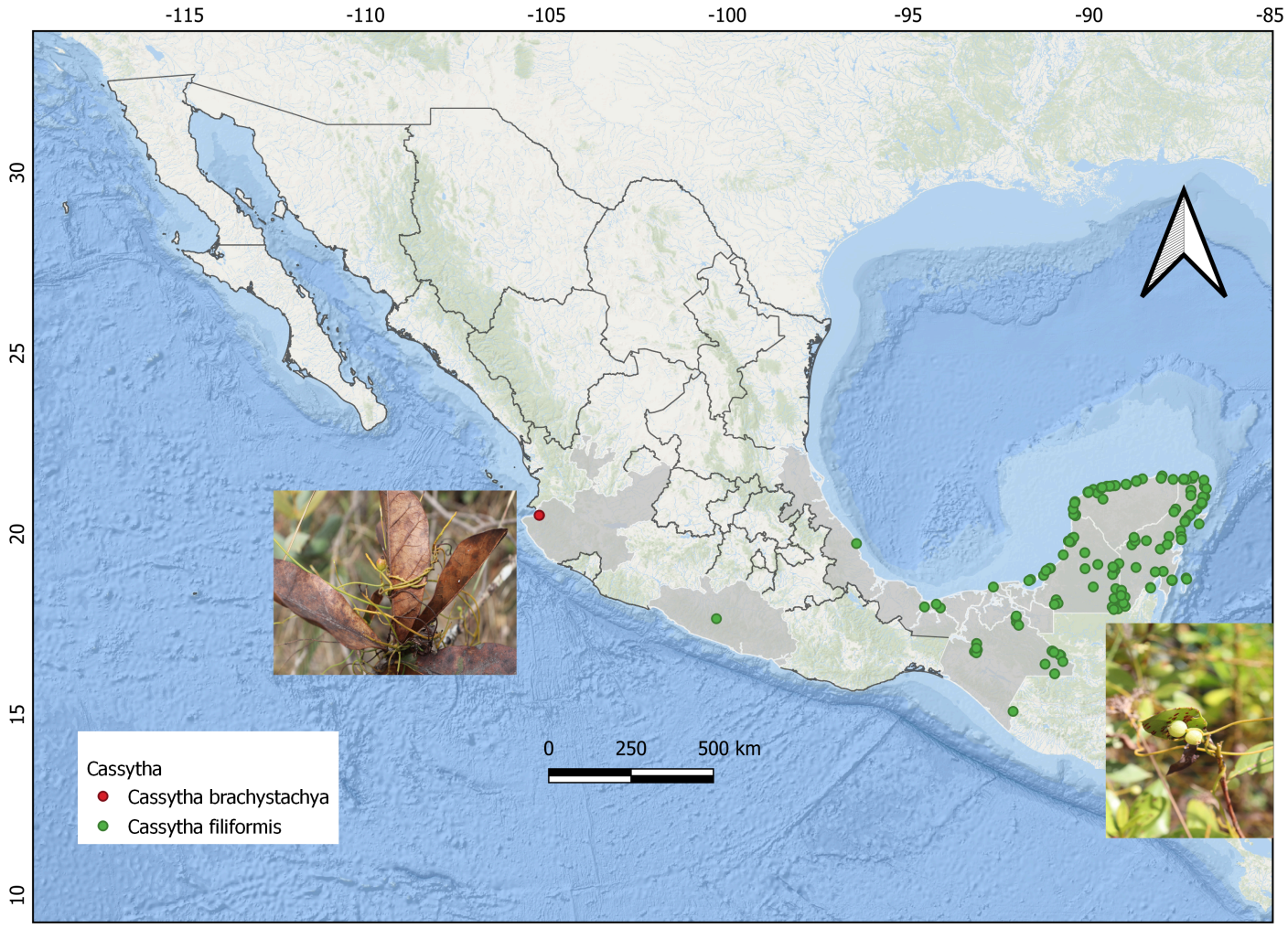


Figure 1

Distribution of in Mexico, based on herbarium and field collections. States in which samples were collected are shown in dark gray

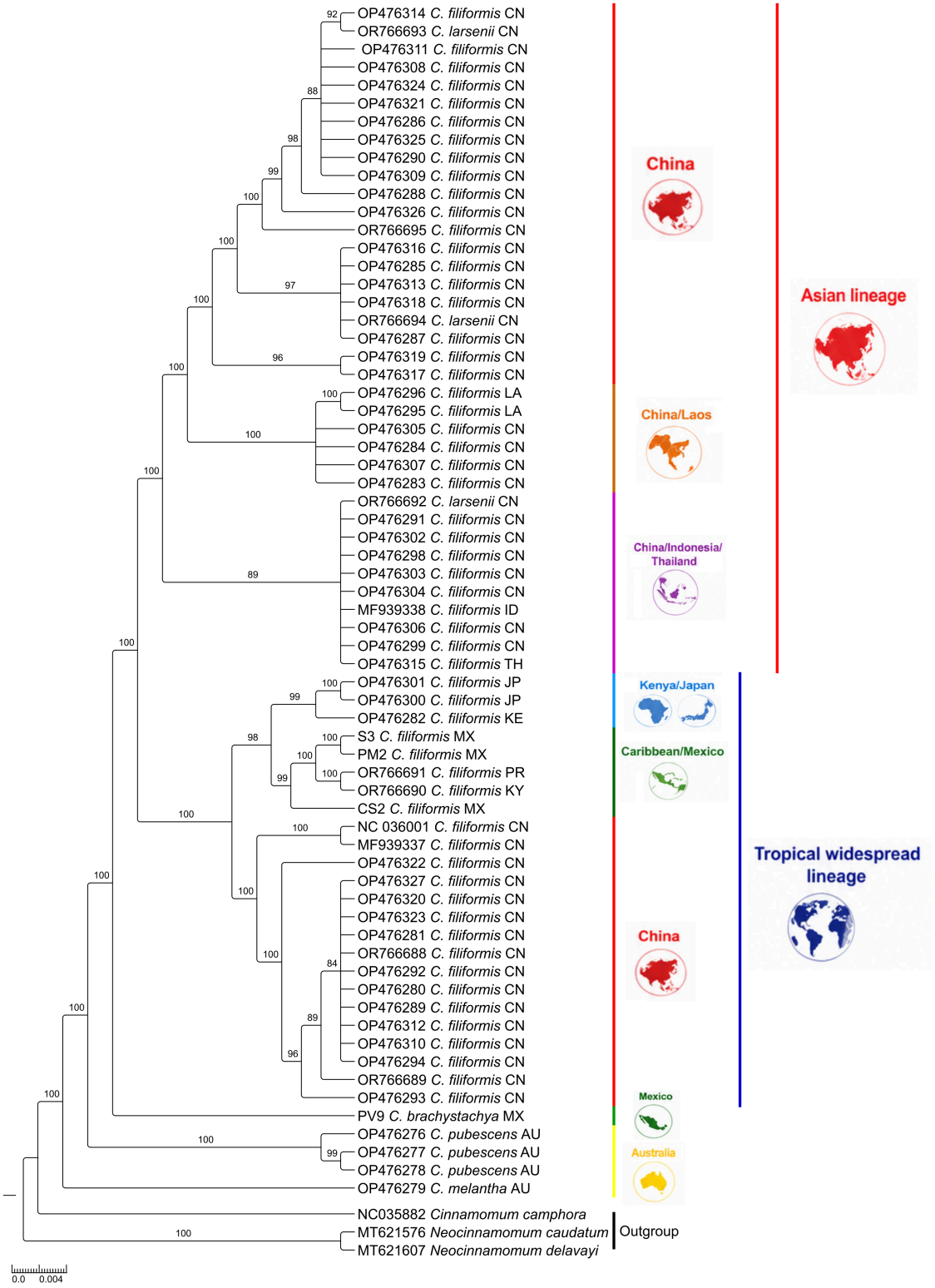


Figure 2

Maximum-likelihood phylogeny based on complete plastome sequences illustrating the evolutionary relationships within genus. Bootstrap support values (BS ≥ 80%) are indicated above branches

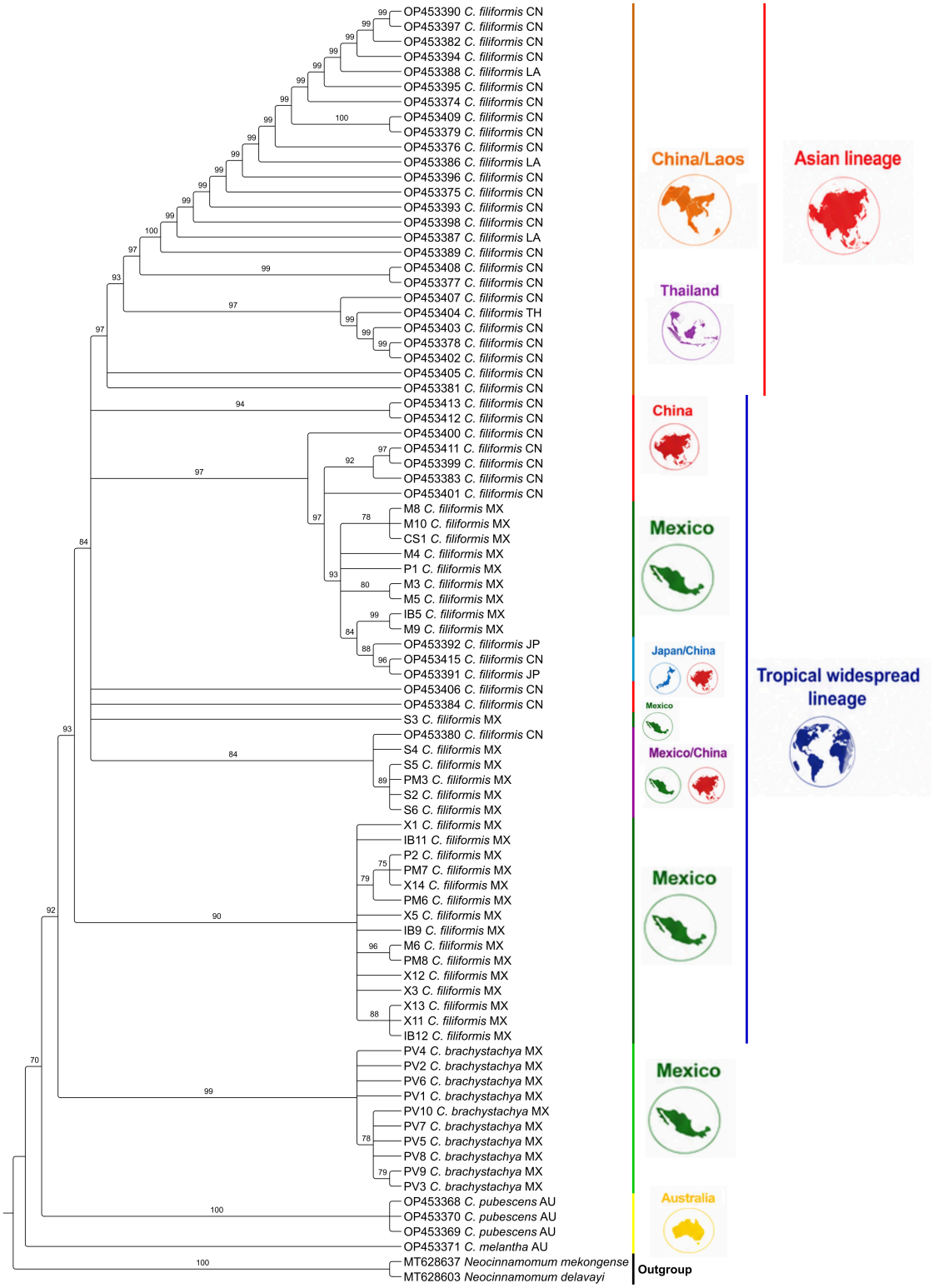


Figure 3

Maximum-likelihood phylogeny inferred from nuclear ribosomal ITS sequences of species. Numbers above branches indicate bootstrap support values (BS); only values ≥ 70% are shown

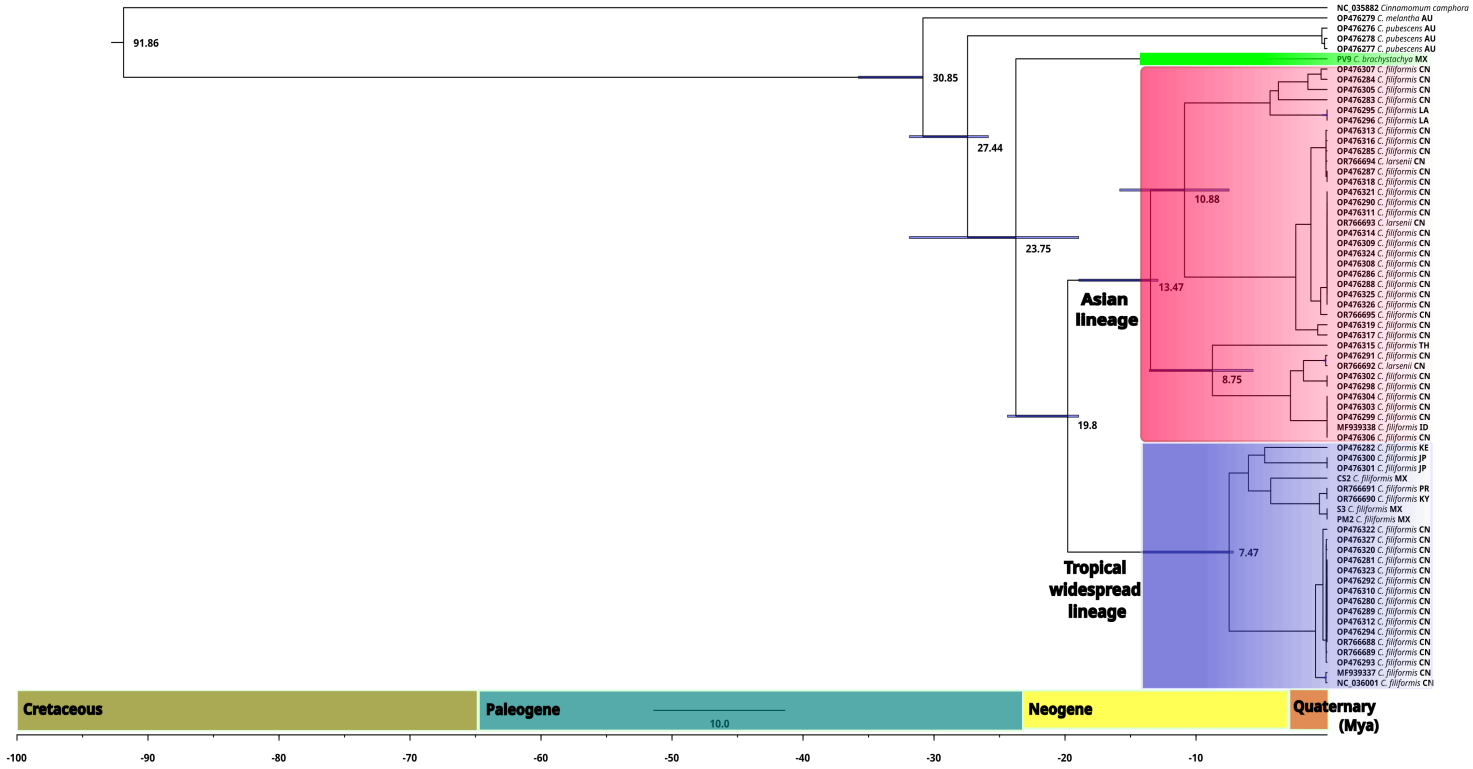


Figure 4

Time-calibrated phylogeny of inferred from complete chloroplast genome sequences. Divergence times (Ma) are shown at the principal nodes and geological periods are indicated along the time scale. Node bars represent the 95% highest posterior density (HPD) interval

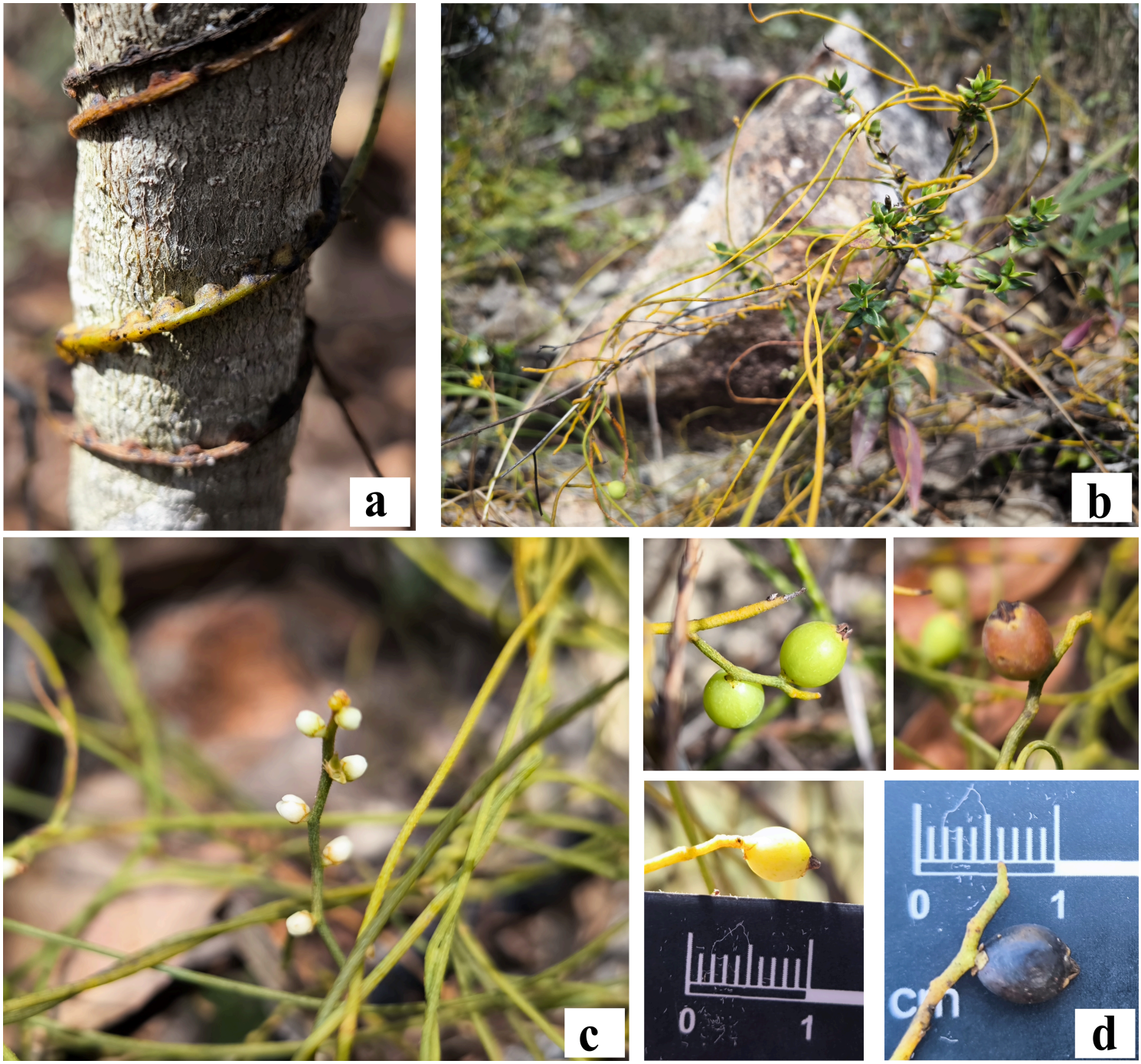


Figure 5

Fig. 5 Diagnostic morphological features of *Cassytha brachystachya* (Meisn.) Ruiz-Sanchez & L. Perez. (a) Twining stem attached to the host. (b) Habit of the hemiparasitic vine in montane Pine-oak forest. (c) Short spicate inflorescence. (d) Fruits at successive stages of development, from immature (green and yellow) to mature (dark brown to black)

Supplementary Files

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