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RESEARCH

Unravelling *Aganope* (*Fabaceae*) in Yunnan and Southeast Asia: insights into species delimitation and generic placement from genomic and ecological evidence

Mareike Roeder[1,2] · **Shook Ling Low**[3,4]

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Abstract

We aimed to identify a sterile *Aganope* specimen collected in Yunnan using genomic and limited morphological evidence. Although *A. thyrsiflora* is the only *Aganope* species currently recorded from Yunnan, our specimen did not match this taxon. To clarify its identity, we sequenced and assembled chloroplast genomes (plastomes) from five Asian *Aganope* species and reconstructed phylogenies using whole plastome data, nuclear ITS, and chloroplast *trnK* sequences. Notably, we recovered complete plastomes from 19th-century herbarium material of *A. polystachya*. Phylogenomic analyses placed *A. polystachya* within *Derris*, suggesting that its current placement in *Aganope* may be inappropriate and consistent with its original description as *D. polystachya*. For the Yunnan specimen, two hypotheses were assessed: (1) *A. balansae* extends beyond Southeast Asia into Yunnan and includes both our unidentified specimen and *A. dinghuensis*, rendering the latter a synonym of *A. balansae*; or (2) *A. balansae*, *A. dinghuensis*, and the Yunnan specimen represents distinct species. Phylogenomic results, climatic niche similarities, and published morphological descriptions support the first hypothesis, indicating that *A. balansae* could be more widely distributed than currently recognized, while *A. dinghuensis* likely represents a local variant. With chloroplast genomes data now available for five out of the six Asian *Aganope* species, this study lays the groundwork for future taxonomic clarification. Given the limited sampling, reliance on partly sterile material, and lack of comprehensive morphological comparisons, future research integrating expanded field surveys, fertile morphological material assessments, and broader molecular sampling will be essential to resolve the taxonomy of *Aganope*, particularly the status of *A. dinghuensis* and its relationship to *A. balansae*.

Keywords Climbers · *Fabaceae* · Historical specimen · Plastome · Yunnan

Introduction

The *Derris*-like species represent a group of genera within the *Fabaceae* family that exhibit striking morphological similarities, often complicating taxonomic delimitation (Bentham 1860; Geesink 1984; Sirichamorn et al. 2012). Over time, these taxa have undergone numerous taxonomic revisions, with genera being split and later recombined (Polhill 1971; Thothathri and Das 1992). One such genus within this group is *Aganope*, which currently comprising 11 accepted species (POWO 2025). The genus exhibits a disjunct distribution, with six species native to Asia and five to Africa. Most *Aganope* species are climbers, including lianas and scrambling shrubs. In Asia, *Aganope* species typically inhabit along mountain streams, in low-elevation forests, or in disturbed habitats (Wu et al. 2008; Sirichamorn et al. 2012).

Among the six Asian *Aganope* species, only two have been recorded in China. *Aganope thyrsiflora* (Benth.) Polhill, a widespread species that now also includes the synonym *A. latifolia* (Prain) T.C.Chen & Pedley, and *A. dinghuensis* (P.Y.Chen) T.C.Chen & Pedley (formerly *Derris dinghuensis*

✉ Mareike Roeder
mareike.roeder2@kit.edu

[1] Karlsruher Institut für Technologie, Josefstr.1, Rastatt 76437, Germany

[2] Centre for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun 666303, Yunnan, China

[3] Institute of Botany, Czech Academy of Sciences, Průhonice 25243, Czech Republic

[4] Forest Research Institute Malaysia (FRIM), Kepong, Selangor, Malaysia

P.Y.Chen), an endemic species previously known only from two populations in Dinghu Mountain (鼎湖山), Guangdong, South China (Wang et al. 2021). The remaining Asian *Aganope* species exhibit varied distributions: *A. heptaphylla* (L.) Polhill occurs broadly across tropical Asia; *A. polystachya* (Benth.) Thoth. & D.N.Das is reported from the eastern Himalayas to Assam; *A. balansae* (Gagnep.) L.K.Phan is distributed in Indochina and *A. agastyamalayana* M.B.Viswan., Manik. & Tangav. is restricted to the southern Western Ghats (Viswanathan et al. 2003; POWO 2025). The taxonomy of *Aganope* remains unsettled, with several species historically transferred between genera. For instance, *A. dinghuensis* originally described in the genus *Derris* (Chen 1984) was later reassigned to *Aganope* (Wu et al., 2008). Likewise, the placement of *A. polystachya* (former *Derris polystachya*) has long been debated and transferred to *Aganope* (Thothathri and Das, 1992), its generic position remains uncertain possibly because such taxa have been excluded from previous phylogenetic analyses (Sirichamorn et al. 2012, 2014; Boonprajan et al. 2024). Such changes highlight the challenges in delineating species boundaries within this group and suggest that additional taxa may require re-evaluation.

In this study, we address the taxonomic identity of a sterile *Aganope* specimen collected during a liana survey in Yunnan. Initially misidentified as *Callerya* (Roeder et al. 2015), the specimen (voucher 75–9-1) was later flagged as problematic in a broader phylogenetic study of *Fabaceae* (pers. comm. E. Asley and M. Wojciechowski 2016). BLAST searches of its DNA barcode years later (*matK*, *rbcL*, ITS, and *trnH-psbA*; from Roeder et al., 2015) subsequently indicated a 100% match with the plastome of *A. dinghuensis* (Wang et al. 2021). This raised a key question involving species known in China: Does the Yunnan specimen represent a disjunct population of the endemic *A. dinghuensis*, or does it belong to another *Aganope* species in Asia, e.g. where no or few genetic data was available?

To address this question, we combined plastome sequencing, nuclear markers, and voucher information to reassess species boundaries and resolve the identity of the Yunnan *Aganope* specimen. We tested two hypotheses: (1) *A. dinghuensis*, *A. balansae*, and the unidentified Yunnan specimen represent a single species, implying that their current taxonomic distinction is unwarranted; or (2) they represent separate species, each with independent evolutionary and biogeographical identities.

Employing multiple lines of evidences is essential, since reliance on morphology alone, or on a single DNA barcode, often leads to misidentification in morphologically conserved lineages. The inclusion of *A. polystachya* further strengthens this study by providing a broader comparative context. By integrating genomic and climatic evidence, as well as literature species description, this study not only clarifies the taxonomic status of the Yunnan *Aganope*, but also re-examines the generic placement of *A. polystachya*. This study will also contribute to establishing a more stable systematic framework for this taxonomically challenging group.

Material and methods

Studied sites and specimen collection

Between November 2011 and March 2012, a liana community survey was conducted in 22 forest plots in Menglun Township, Yunnan Province. In total, more than 3000 liana individuals were recorded. For each species encountered, herbarium vouchers (mainly sterile), tissue samples for DNA extraction, and, when possible, photographs of fresh material were collected. When leaves or diagnostic stem features were inaccessible, stem tissues were sampled for molecular identification.

DNA isolation, Illumina sequencing, and plastome assembly

We obtained plastome data from the unidentified voucher collected in Yunnan in 2011/12, along with six herbarium vouchers representing four Asian *Aganope* species (*A. hetaphylla, A thyrsiflora, A. balansae, A. polystachya*) (voucher ID see Table 1). The only Asian species not included was *A. agastyamalayana*, for which no herbarium material or genomic data were available. However, as it is very distinct from all other species in its published morphological description and illustration (Viswanathan et al. 2003), this taxon was excluded from phylogenetic analysis.

Genomic DNA was extracted using Tiangen DNAsecure Plant Kit (DP320) at the Kunming Institute of Botany (Yunnan, China), following the protocols in Zeng & al. (2018). Due to the fragmented nature of herbarium-derived DNA, blunt-end DNA libraries were prepared using NEBNext Ultra II DNA library Prep kit for Illumina (New England Biolabs Indexed libraries were pooled in equimolar ratio and sequenced on the Illumina XTen platform (Illumina Inc.).

In addition, we downloaded sequencing read archive (SRA) data for *A. dinghuensis* (Wang et al. 2021), and an already published plastome of *A. balansae* (Zhang et al. 2021). Plastome and nuclear assemblies from raw reads were performed using GetOrganelle ver.1.7.6.1 (Jin et al. 2020) on the Metacentrum computational infrastructure (CESNET, Czech Republic). Annotation of assembled plastome sequences were conducted with GeSeq (Tillich et al. 2017). All annotated sequences were deposited to NCBI (see Table 1 for accession numbers).

Table 1 Species, voucher specimen information and accessions numbers in Genbank of all *Aganope* samples used in the study

Species	Geographic origin	Collector's name & number	Herbar-ium	Herbarium number	Plastome	ITS	*trnK*
Aganope balansae (Gagnep.) L.K.Phan	Vietnam	*Poilane 13,016*	(P)	P02770319	PX138910*	PZ212881*	from cp
Aganope balansae	Vietnam	*Poilane 26,751*	(P)	P00736082	n.a	JX506433	n.a
Aganope balansae (Derris alborubra on herbarium label)	China, Yunnan	*(*税玉民*) Y.M. Shui 000681*	(PE)	PE00499877	MW446467	n.a	from cp
Aganope dinghuensis (P.Y.Chen) T.C.Chen & Pedley	China, Guangdong	n.a., isolate: SCBGP293_1	(IBSC)	not found	n.a	KP092717	n.a
Aganope dinghuensis	China, Guangdong	n.a., isolate: SCBGP293_2	(IBSC)	not found	n.a	KP092718	n.a
Aganope dinghuensis	China, Guangdong	n.a	(IBSC)	IBSC0858303	SRR13179903	n.a	from cp
Aganope gabonica (Baill.) Polhill	Gabon, Franceville	*Karmann s.n*	(L)	not found	n.a	JX506438	JX506605
Aganope heptaphylla (L.) Polhill	Thailand, Ranong	*Sidiyasa, K; Ambriansyah; Adriansyah 2598*	(L)	L.1991510	PX138911*	PZ212879*	from cp
Aganope heptaphylla	Indonesia, Kalimantan	*Forman & Blewett 1127*	(L)	L.1991476	n.a	AF467017	n.a
Aganope heptaphylla	Brunei, Belait	*Santisuk 688*	(L)	not found	n.a	JX506432	JX506600
Aganope heptaphylla	Papua New Guinea	*B. Katik P. Verdcourt 4963*	(L)	L.1991433	n.a	MN076212	n.a
Aganope impressa (Dunn) Polhill	Congo, Luki	*Dubois s.n*	(L)	L.1991387	n.a	JX506436	JX506604
Aganope leucobotrya (Dunn) Polhill	n.a	*Versteegh* et al. *150*	(L)	not found	n.a	JX506437	n.a
Aganope polystachya (Benth.) Thoth. & D.N.Das	India, Khasia	*Hooker, JD; Thomson, T s.n*	(L)	L.1941779	PX138913*	PZ212883*	from cp
Aganope polystachya	India, Khasia	*Hooker, J.D. Thomson, T s.n*	(P)	P0075298	PX138909 *	PZ212884*	from cp
Aganope stuhlmannii (Taub) Adema	Ivory Coast, Korhogo	*Versteegh* et al. *456*	(L)	not found	n.a	JX506435	JX506603
Aganope stuhlmannii	Zimbabwe	*Corby 2162*	(K)	not found	n.a	AF467485	AF142708
Aganope stuhlmannii	n.a	n.a	no info	n.a	MN966650	no	from cp
Aganope thyrsiflora (Benth.) Polhill	Cambodia, Phnom Penh	*Cheng, David, Leti, CL 645*	(P)	P00631619	PX138912*	PZ212882*	from cp
Aganope thyrsiflora	Indonesia, Kalimantan	*Sidiyasa, K; Ambriansyah; Adriansyah 2572*	(L)	L.1991511	failed	PZ212880*	Failed
Aganope thyrsiflora	Thailand, Songkhla	*Sirichamorn YSM 2009–22*	(L)	not found	n.a	JX506434	JX506602
Aganope thyrsiflora	Indonesia, Kalimantan	*Sidiyasa 640*	(L)	not found	n.a	AF467018	n.a
Aganope M	China, Yunnan	*Mengsong 75_9_1*	(HITBC)	HITBC0140926	SRR15458559*	HG004878	from cp

For species of other genera and more specific locations see Appendix Table S1. *SRR* raw data, *P* Paris, *L* Leiden, *PE* Beijing, *IBSC* South China Botanical Garden Herbarium, *HITBC* Xishuangbanna Tropical Botanical Garden Herbarium, from cp = extracted from plastome, * newly generated sequences. For some vouchers no herbarium number could be found. n.a. = not available

Plastome alignment and phylogenomic analyses

To determine the phylogenomic placement of the Yunnan specimen, three datasets were compiled: (1) single partition of complete plastome sequences, (2) the nuclear ITS, and (3) the chloroplast *trnK* region. The plastome dataset included seven newly assembled *Aganope* plastomes, the published plastome sequences of African *A. stuhlmanni*, and an unverified *A. balansae* from GenBank, along with five sequences from closely related *Derris* s.l. species. For the ITS and *trnK* datasets, we downloaded publicly available sequences for *Aganope* species and related taxa (Table 1, Appendix Table S1), and also extracted corresponding regions from our plastomes and nuclear assemblies. In total, the *trnK* dataset included eight of the 11 accepted *Aganope* species, whiles the ITS dataset included nine species (Table 1; Appendix Table S1). These single-locus phylogenies (ITS and *trnK*) were used to confirm the identity of herbarium vouchers used for plastome sequencing by comparison with additional conspecific accessions.

Sequences alignments were performed using MAFFT-7.453 (Katoh and Standley 2013). Pairwise sequence identity and GC content (%) was obtained from Geneious prime 2022.0.1 software (https://www.geneious.com). Maximum likelihood (ML) were reconstructed using IQtree2 (Minh et al. 2020), and branch support was evaluated using ultrafast bootstrap replicates. We considered 95% as the threshold for strong ultrafast bootstrap support (Minh et al. 2013). *Dalbergia assamica* and *Dalbergia balansae* were used as outgroups in all analyses.

Climatic data collection and principle component analysis (PCA)

Occurrence records for all six Asian *Aganope* species were compiled from the Global Biodiversity Information Facility (GBIF). For specimens lacking GPS coordinates, geographic positions were estimated from herbarium label information. Spatial outliers such as their occurrence in other continents, oceans, and several thousand km away from core distribution were manually deleted. This resulted in 463 useful records for analysis (Fig. 1). Climatic data at a spatial resolution of 10 min degree were obtained from WorldClim version 2.1 (Fick and Hijmans 2017) for the period 1970–2000. After testing for multicollinearity among the 19 bioclimatic variables through autocorrelation analysis, we selected a subset of seven variables for principal component analysis (PCA), which had a correlation $R < 0.7$, giving priority to seasonality variables wherever possible: mean annual temperature (bio1), mean diurnal range (mean of monthly (max temp – min temp); bio2), temperature seasonality (bio4), annual precipitation (bio12), precipitation seasonality (bio15), precipitation of wettest quarter (bio16), precipitation of warmest quarter (bio18). The PCA was used to explore climatic differentiation among species and assess potential ecological niche divergence. Because species occurrence data often exhibit spatial sampling bias – typically resulting from concentrated research effort near accessible sites or urban areas – we spatially thinned the records to retain only one occurrence per species per grid cell. We used a grid cell size of 0.25°, closely matching the spatial resolution of the climatic variables (≈0.17°). After a first check, 12 more *Aganope* observations had to be excluded, as their climate values were extreme outliers, likely due to strong elevation differences and connected climate data extremes in the Himalaya and unsure coordinates. 218 observations entered the PCA.

Results

Phylogenetic relationships of *Aganope* species

The plastome assemblies for six *Aganope* samples successfully generated > 150 000 bases in length, except one sample,

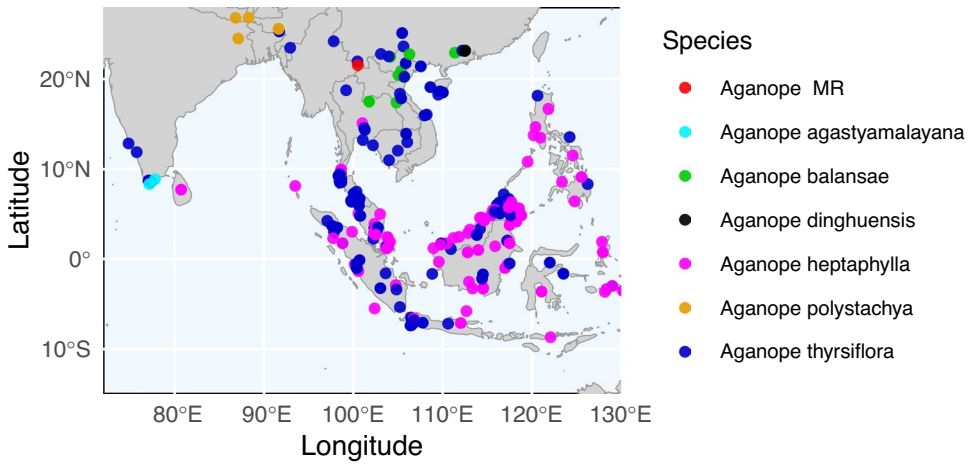

Fig. 1 Occurrence of *Aganope* species in Asia. Observations were extracted from GIBF, some coordinates were estimated by local names

A. polystachya (voucher P00757298) which yielded only approximately 85 000 bases due to partial recovery. The entire plastome dataset comprised 16 accessions, and 178,171 aligned sites (Fig. 3). Pairwise sequence identity between *A. dinghuensis*, *A. balansae* and the unidentified Yunnan specimen was 93.4%, with 88.4% of sites identical. Across all accessions, overall pairwise identity was 81.8%, with 43.0% identical sites. The GC contents across all accessions averaged for 35.3%, meanwhile between *A. dinghuensis*, *A. balansae* and the unidentified Yunnan specimen was 35.4%. The ITS dataset comprised 31 taxa including outgroups, with 691 aligned bp, a pairwise identity of 82.4%, 44.4% identical sites, and a GC content of 55.5%. The *trnK* dataset included 29 taxa, with 2800 aligned bp, a pairwise identity of 90.6%, 64.3% identical sites, and a GC content of 29.7%.

Maximum likelihood (ML) analyses on the plastome dataset produced a strongly supported topology, with high bootstrap support across most nodes (BS > 95; Fig. 2A). In all analyses, *A. dinghuensis* and *A. balansae* consistently formed a well-supported clade (Clade I; BS plastome/*trnK*/ITS: 67/93/97; Fig. 2). While the description of *A. dinghuensis* is considerably shorter and vague than that of *A. balansae* across several morphological traits (e.g. flowers, twigs, leaves), we found no significant morphological contradictions in the diagnostic characters available for both species (Table 2, S2).

The unidentified Yunnan voucher formed a distinct lineage adjacent to the *A. dinghuensis/A. balansae* cluster in the plastome tree (Fig. 2A). In contrast, ITS data placed it as sister to the *A. heptaphylla/A. thyrsiflora* clade (Clade I; Fig. 2C), while *trnK*-based phylogeny nested it within the *A. dinghuensis/A. balansae* cluster (Clade I; Fig. 2B).

No genetic data were previously available for *A. polystachya*. We successfully recovered its complete plastome from one herbarium voucher collected over 150 years ago

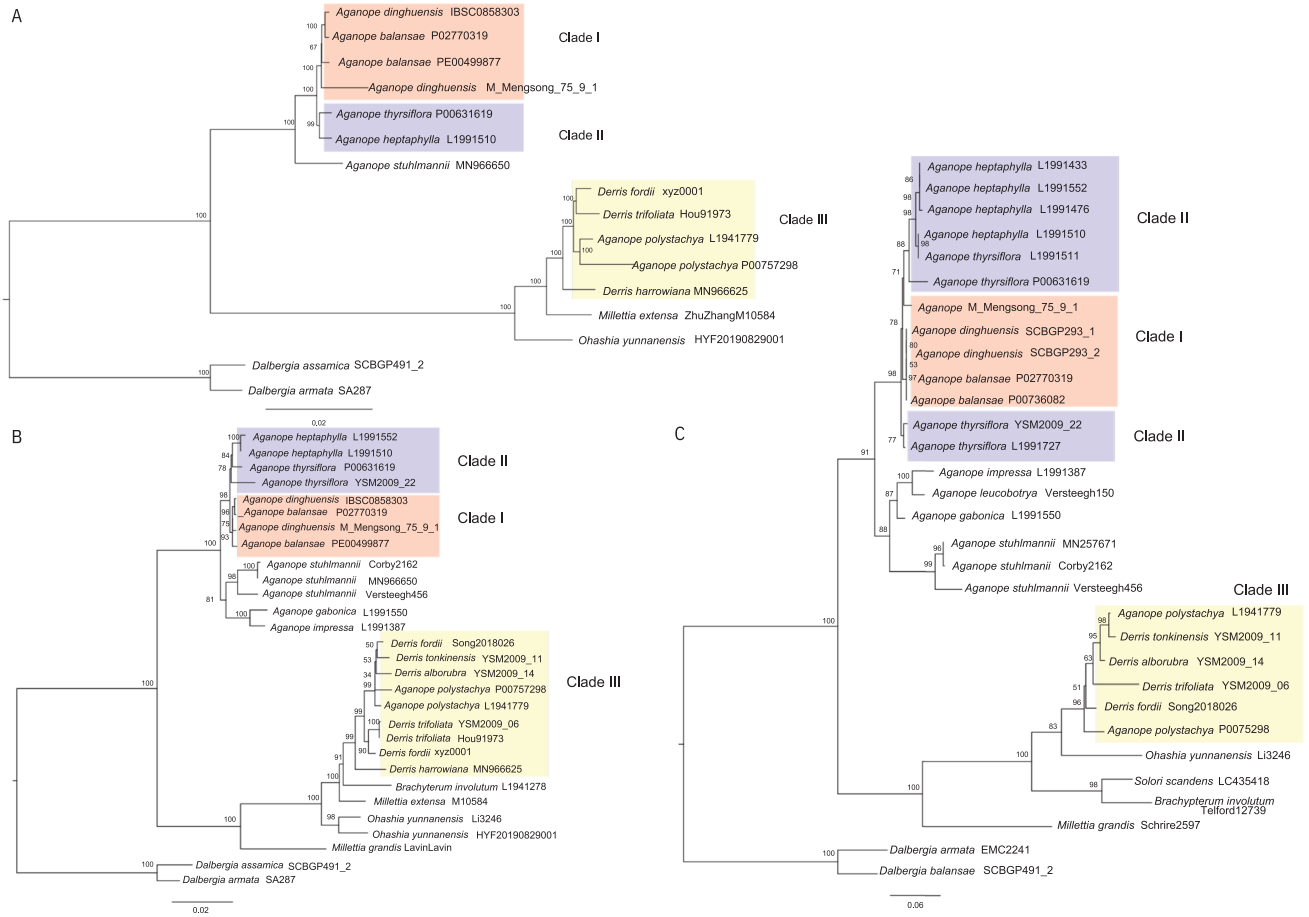

Fig. 2 ML tree reconstructed from (**A**) plastome datasets, including five of the six Asian *Aganope* species; **B** *trnK* sequences including the *mat*K gene, representing eight of eleven global *Aganope* species and additional related taxa; **C** nuclear ITS sequences, encompassing all recognized *Aganope* species except *A. agastyamalayana* and *A. lucida*. Bootstrap support values are indicated at the nodes. Herbarium voucher numbers are provided for each specimen where available, otherwise, collector numbers or GenBank accession number are shown. Clades are color-coded as follows: Red indicates Clade I comprising of *A. dinghuensis*, *A. balansae*, and the unidentified Yunnan sample; blue indicated Clade II comprising of *A. thyrsiflora*, *A. heptaphylla*; and yellow indicates Clade III comprising of *A. polystachya* and *Derris* species

Table 2 Condensed morphological comparison of five Asian *Aganope* species (sources: eFlora Thailand, IPlant.cn, Flora of China, India Flora Online, Flora of Bhutan)

Character	*A. thyrsiflora*	*A. heptaphylla*	*A. balansae*	*A. dinghuensis*	*A. polystachya*
Growth form	Woody climber	Woody climber	Tree or treelet, 4–15 m tall (climber acc. to herbarium notes of collectors)*	Climbing shrub	Climbing shrub
Twigs	Greyish, smooth to pubescent, lenticellate or smooth	Lenticellate, striate	Lenticellate, pubescent	Brown, hollow, pilose. lenticels	Shortly brown puberulous
Stipules	Caducous	Caducous	Caducous	n.a	Persistent
Leaves	17–49 cm; subcoriaceous; petiole 3.5–10.5 cm	22–40 cm; subcoriaceous; petiole 6.5–10.5 cm	45–56 cm; chartaceous to subcoriaceous; petiole 10–17 cm	Pinnate, 35–50 cm	20–35 cm
Leaflets	5–9, elliptic, 6–17.5 cm×3.5–7 cm, 5–7 pairs of lateral veins	5–7, elliptic, 9.5–17.5 cm×6–9.5 cm 8–10 pairs of lateral veins	5–9, elliptic/obovate, 9.5–18 cm×5.3–10.5 cm 6–10 pairs of lateral veins	9, oblong, 10–18 × 7–11 cm 6–8 pairs of lateral veins	7–9, elliptic, not strongly reticulate
Inflorescence	Panicles, up to 35 cm	Panicles, up to 30 cm	Panicles 17–35 cm	Axillary panicle, ~ 20 cm	Branched panicles
Flowers—Corolla	White/cream, **4.5–6 mm standard**	Whitish/green, 13–15 mm standard	Whitish, 13–15 mm standard	White, ~ 15–17 mm	**Red/pink**, ~ 6–8 mm
Flowers—Calyx	Reddish/pinkish green/yellowish, 3.7–4.2 mm, outside sericeous	greenish, cup-shaped, 6–6.5 mm outside sericeous	yellow brown pubescence*, 4–5 mm long, outside sericeous	outside densely yellow–brown pilose, ca. 6 mm	calyx cup finely silky
Pods	Strap-shaped, 5–15 × 2.5–3.8 cm, winged on both sutures, 3–8 mm	Strap-shaped, 4.5–19.5 × 2.5–3.8 cm, **sinuate**, **winged on upper suture**, 2–4 mm	Strap-shaped, 9.5–21 × 3.2–4 cm, winged on both sutures, 5–10 mm	Ligulate-oblong, 10–15 × 3–3.5 cm winged on both sutures, 5–8 mm, bisecting	Thin, oblong, reticulate, ~ 11 × 3 cm, winged on both sutures 2–5 mm
Seeds	1–4, bean-shaped, 11–22 × 9–13 mm	1–2, bean-shaped, 21–22 × 8–9 mm	1–3, bean-shaped, 23–25 × 10–12 mm	1, reniform, ~ 25 × 13 mm	1–2
Habitat	Dry evergreen, disturbed areas, ≤ 100 m	Rainforest, riversides, mangroves, ≤ 120 m	Evergreen forest edges, riversides, ≤ 600 m	Subtropical regions	—
Phenology	Fl. Mar–Nov; Fr. Jun–Dec	Fl. May–Oct; Fr. Jan–Feb	Fl. Mar–Aug; Fr. Jul–Oct	—	—

Leaves size when not given by literature was sum of petiole, rachis, petiolules, terminal leaflet. * additional observations by authors. For complete character list see Appendix Table S2. Outstanding characters are bold

(Fig. 3). Phylogenetic analyses placed *A. polystachya* as sister to *Derris* rather than to other *Aganop*e species (Clade III; BS plastome/*trnK*/ITS: 100/99/96, Fig. 2), raising question on its current generic placement. Both vouchers (including an isotype; Table 1), were collected on the same date and locality by the same collector, but lacked a collector number, suggesting they may derive from the same individual or population.

The placement of *A. thyrsiflora* remains unresolved, and the species appears to be paraphyletic. In plastome- and *trnK*-based phylogenies, it consistently clustered with *A. heptaphylla* (Clade II; BS plastome/*trnK*: 100/78; Fig. 2A, B). However, ITS phylogeny showed incongruence (Clade II, Fig. 2C): two taxa *A. thyrsiflora* (voucher L1991727 and YSM2009_22) formed a basal lineage relative to the clade containing the remaining *A. thyrsiflora* samples, *A. heptaphylla,* the *A. balansae/A. dinghuensis* cluster, and the unidentified Yunnan specimen. Only one *A. thyrsiflora* voucher (P00631619) yielded a complete plastome (Table 1), limiting plastome-based resolution for this species. In contrast, *A. heptaphylla* vouchers consistently formed well-supported clusters in both *trnK* or *ITS* trees (Fig. 2B, C), confirming their species-level identifications.

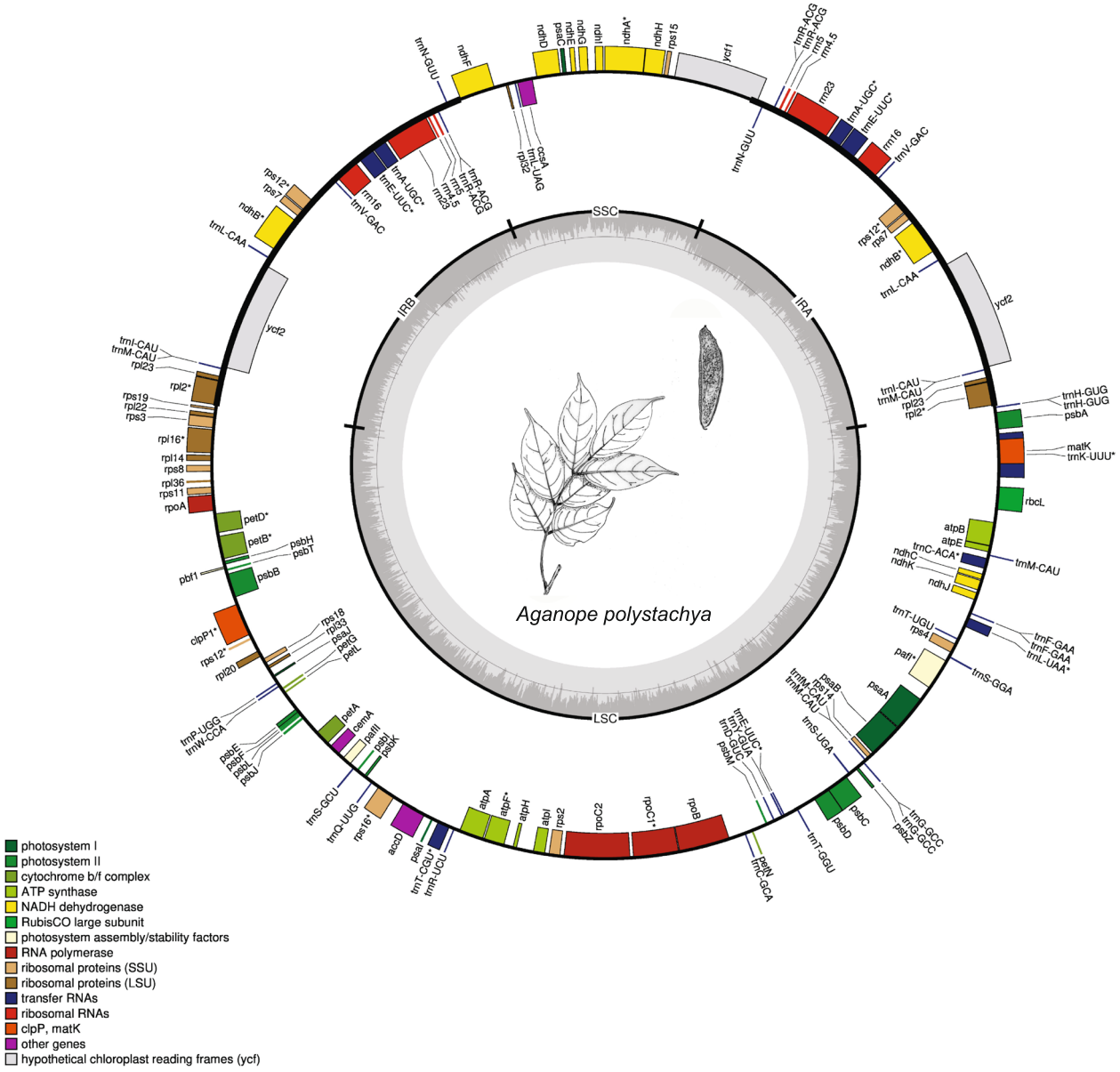

Fig. 3 Chloroplast genome of *Aganope polystachya* (voucher Hooker, JD; Thomson, T s.n., herbarium number L.1941779). Genes positioned outside the outer ring are transcribed in the clockwise direction, while those inside are transcribed counter-clockwise. Gene functions are distinguished by different colours. The dashed region within the inner circle represents the GC content of the chloroplast genome

Climatic niche overlap and PCA of Aganope species

PCA ordination of climatic variables showed that the niche of *A. balansae* encompasses the two recorded localities of *A. dinghuensis* (after thinning just one location) and the unidentified Yunnan record in Mengsong was close by just outside this niche (ellipse containing 95% of data observations). *A. heptaphylla* was more concentrated towards warmer and less seasonal, therefore more tropical climate. *A. thyrsiflora* had the widest climate niche based on the GIBF records, covering most of *A. heptaphylla* niche but with numerous records in more seasonal climates. The first principal component (PC1) explained 36% of the data variance and was primarily associated with annual temperature (bio_1) as well as seasonality of temperature (bio_4) and seasonality of rain (bio_15). The second axis (PC2) accounted for 26% of the variance, with more monsoon related variables like precipitation of wettest quarter (bio_16) and of warmest quarter (bio_18) being the main contributors (Fig. 4).

Discussion

Species identification and taxonomic uncertainty

Our phylogenomic analyses highlight the ongoing uncertainty in delimiting Asian *Aganope* species. The exact number of Asian *Aganope* species remains unresolved – there may be more than the six currently accepted species, or fewer if some represent synonyms. While we generated the first nearly complete plastome framework for Asian *Aganope*, limitations in using old herbarium vouchers, and incomplete sampling constrain definitive conclusions. Nevertheless, these data establish a foundation for future systematic studies of the group.

Based on the two introduced hypotheses, the evidence most strongly supports the interpretation that *A. dinghuensis*, *A. balansae*, and the unidentified sterile specimen represent a single taxon (Hypothesis 1). Our phylogenetic analyses clearly separate this group from *A. thyrsiflora*, *A. heptaphylla*, and the African species. In both plastome and *trnK* phylogenies, these three lineages consistently form a well-supported clade, despite that the ITS phylogeny places them to a slightly lesser extent as sister lineage to *A. heptaphylla* and *A. thyrsiflora*.

Morphological comparison also failed to reveal contradictions for *A. balansae* and *A. dinghuensis* (Table 2, S2). The description as "twany (=yellow brown) pubescent" in various parts (twigs, branches of inflorescence, calyx) in *A. dinghuensis* and "sericeous" in *A. balansae* are functionally similar descriptions of short hairs (Table 2, S2; Cámara-Leret 2015; iPlant 2009). Personal observation on scanned herbarium samples of *A. balansae* also confirmed brown pubescens in inflorescence and calyx. Moreover, the original description of

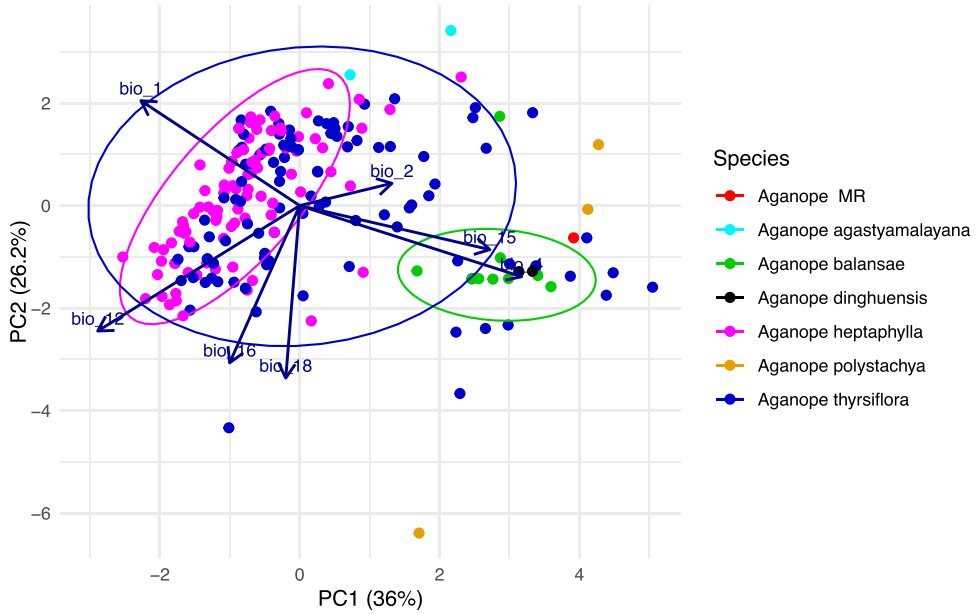

Fig. 4 Ordination of climate variables from occurrence data for each Asian *Aganope* species (GBIF & herbarium notes). Ellipses indicate 95% confidence interval, and could only be calculated for *A. thyrsiflora, A. heptaphylla* and *A. balansae*. Due to autocorrelation, a selection 7 of 19 available bioclimatic variables were used (Fick and Hijmans 2017): bio_1: Mean Annual Temperature, bio_2: Mean Diurnal Range (Mean of monthly (max temp—min temp)), bio_4: Temperature seasonality, bio_12: Annual precipitation, bio_15: Precipitation seasonality, bio16: Precipitation of wettest quarter, bio_18: Precipitation of warmest quarter. PCA Biplot (Bioclim Variables)

A. dinghuensis (Chen 1984) compared it only to *A. thyrsiflora*, the sole closely related species recorded in China at that time. Besides, the Beijing *A. balansae* voucher (PE00499877, genbank accession: MW446467) is closely placed with the other two *A. dinghuensis*/*A. balansae* specimens in the plastome phylogeny (Fig. 2A). Our examination onto the scanned herbaria specimen and *trnK* phylogeny (Fig. 2B) also support its identification by Zhang et al. 2021 as *A. balansae* (instead of *Derris alborubra* as stated in the herbarium). Despite *A. balansae* not being officially listed in China, there are records in China, such as a specimen collected as *A. thyrsiflora* in Ding Hu Mountain (Guangdong, China) that was renamed later to *A. balansae* (Leiden voucher L.2108549), as well as the previously mentioned re-identified voucher used in our plastome phylogeny (PE00499877).

Furthermore, the climatic niche analysis indicates that *A. balansae*, *A. dinghuensis* and the unidentified *Aganope* occupy ecologically similar habitats and climatic envelope along a band of 21°–23° north over South East Asia and China. These evidences suggest that the separation of *A. dinghuensis* from *A. balansae* may not be taxonomically justified.

Taken together, *A. balansae* may be more widely distributed than currently known, and may extend into South China, while *A. dinghuensis* could represent a localized form or synonym of this species.

The second hypothesis is that *A. dinghuensis*, *A. balansae*, and the Yunnan specimen represent distinct species. From this perspective, DNA data alone may not be sufficient for confident taxonomic conclusions. A more comprehensive morphological reassessment across a broader range of specimens, ideally using fertile material, would be necessary to evaluate diagnostic traits and confirm whether these names should remain separate. Under this scenario, *A. dinghuensis* would remain as a narrowly endemic Chinese species restricted to Ding Hu Mountain, *A. balansae* would remain confined to Southeast Asia, and the Yunnan voucher could represent an undescribed species. In this case, the Yunnan region may host any combination of two or three *Aganope* species.

Despite the weight of evidences currently favours the first hypothesis, definitive support for either hypothesis will require extensive field surveys, additional herbarium specimens, comprehensive morphological comparisons of herbarium specimens and broader genomic sampling. To date, *A. balansae* is represented by very few herbarium records, *A. dinghuensis* is known from only two sites, and the sole Yunnan (Mengsong) specimen (Fig. 5) was one of over 3000 liana stems sampled across 22 plots – underscoring the rarity of these taxa and the challenge of comprehensive population sampling. Especially for the Mengsong voucher, where the plot is now likely deforested and located near a border area.

A key additional outcome of this study was the recovery of the complete plastome of *A. polystachya* from a voucher collected in 1859 (Fig. 5). Given that these specimens are over 150 years old, have travelled across continents, and endured varied storage conditions, the quality and utility of the genetic data remain remarkably robust and reliable. Our analyses consistently placed this species within *Derris*, not *Aganope*. Historically, *A. polystachya* was described as *Derris polystachya* by Bentham (Grierson and Long 2003) and was reassigned to *Aganope* based on Polhill's (1971) floral and seed criteria by Thothathri and Das (1992). Polhill identified three key differences separating *Aganope* from *Derris*, including non-adherent wing petals to the keels in the lower half in *Aganope* versus the adherent condition in *Derris,* a markedly eccentric seed hilum with undeveloped radicular lobes (vs submedial hilum in *Derris*), and the spreading radicles in mature seeds (vs incurved radicle in *Derris).* Polhill's key included two Asian (*A. thyrsiflora, A. heptaphylla*) and four African *Aganope* species. He also noted that *Derris* typically has contracted inflorescences, whereas *D. polystachya* had well-spaced flowers, supporting its later reassignment to *Aganope.* Thothathri & Das later re-examined all Indian species of *Derris* section *Aganope*, including *D. polystachya*, and placed them into *Aganope* based on Polhill's criteria (Thothathri and Das 1992). However, they did not provide additional morphological evidence to justify these reassignments. Our plastome data suggest that these characters may be homoplastic. Even so the isotype voucher (P0075298) produced only a partial plastome, the genomic evidence strongly supports reevaluating the status of *A. polystachya*, potentially reinstating it in *Derris*. The here published plastome should be identical for the isotype, since the vouchers are likely from one individual. Interestingly, the two historical herbarium vouchers – despite likely originating from the same individual or population – show slight differences in phylogenetic relationship (Fig. 2A vs. Fig. 2C), indicating hybridization may have occurred.

Phylogenetic analyses yielded conflicting placements of *A. thyrsiflora*. While plastome- and *trnK*-based phylogenies consistently paired it with *A. heptaphylla*, ITS analyses revealed incongruence, with accessions splitting into separate lineages. This pattern may reflect hybridization. Limited plastome recovery from available vouchers further constrains interpretation.

Our phylogeny focuses specifically on *Aganope* and the clade into which *A. polystachya* falls based on plastome data – *Derris*. We acknowledge that this tree is not intended to represent the full phylogenetic structure of the millettioids. Previous studies (Zhao et al. 2021) place *Derris* in the core Millettieae clade, while *Aganope* is a sister group and has been used as an outgroup in recent phylogenies of the Asian core Millettieae (Song et al. 2025). Therefore, our phylogeny is partial and purposefully disjunct, reflecting targeted sampling for species-level resolution rather than broader tribal relationships.

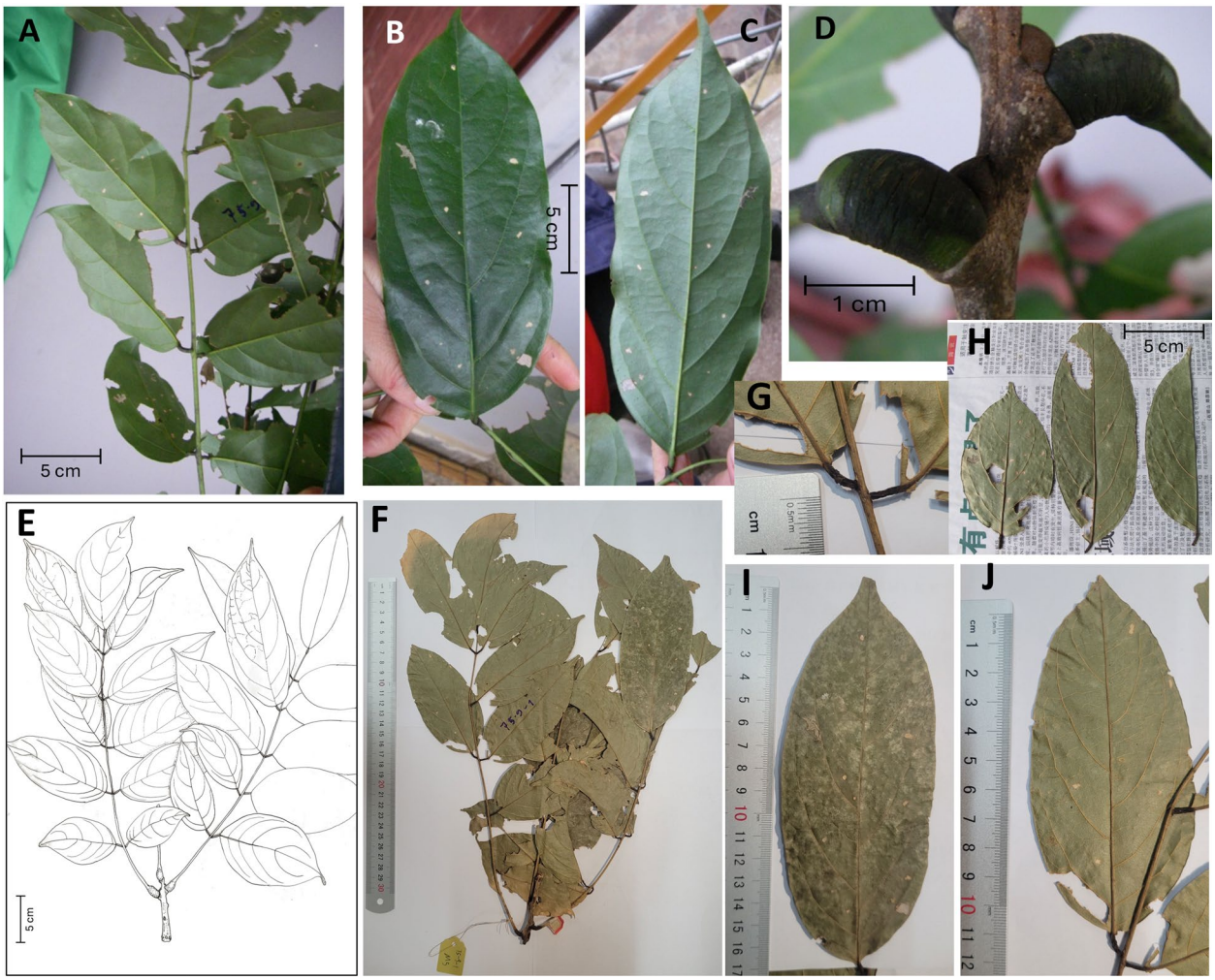

Fig. 5 Fresh (**A**-**D**), dried (**F**-**J**) and illustrated (**E**) sterile herbarium material of the liana individual 75–9-1, collected in Mengsong Forest (Xishuangbanna, Yunnan, CN), now identified as *Aganope*, voucher HITBC0140926. Leaves of this voucher were sequenced as barcodes (2012) and chloroplast genome data (2021). A distinguishing sterile morphological characteristic of this species is its notably swollen pulvinus, which may aid future identification. (Picture credits: MR)

In this study, we address inconsistencies in herbarium records on growth form within *Aganope.* Apparently, liana, shrub and scandent shrub are often interchangeable descriptions, depending on the age and developmental stage at the time of collection, as many liana species begin as free-standing shrubs. Trees should be distinct from the other growth forms. During morphological extraction, various literatures describe *A. latifolia* or *A. balansae* as tree (Wu et al. 2008; Sirichamorn et al. 2012), however herbarium vouchers for *A. balansae* are consistently noted as climbers. The Chinese vernacular name for *A. latifolia* is 大叶鱼藤 (dà yè yú téng, literally "big leaf fish vine") includes the last character meaning "liana", and is by now generally considered a synonym of *A. thyrsiflora*, further supports its interpretation as a climber. We therefore postulate that climbing habitus to be the sole growth form of Asian *Aganope*.

Habitat and climate

The present-day climatic niches of *Aganope* species reflect both their ecological tolerances and historical factors, such as dispersal constraints, that have shaped their distribution and vary substantially between *Aganope* species. *Aganope thyrsiflora* is the most widespread taxon, occurring across a broad range of climatic zones and occupying a broad niche that overlaps all other studied species. In contrast, *A. heptaphylla* is confined to more tropical environments characterized by higher mean temperatures and low seasonality, showing no overlap with *A. balansae/dinghuensis* cluster. The *A. balansae/dinghuensis* cluster is associated with regions experiencing greater daily and annual temperature fluctuations. The larger number of occurrence records available for *A. thyrsiflora* and *A. heptaphylla* – roughly 10 times more

after cleaning for spatial bias than for *A. dinghuensis/A. balansae* cluster – provides a useful framework for assessing climatic tolerances among *Aganope* species.

Despite their geographic separation, the habitats of *A. balansae*, *dinghuensis*, and unidentified Yunnan specimen in Ding Hu (China), in Mengsong (China), and across South East Asia share significant environmental similarities. These areas experience a summer monsoon climate marked by strong rainfall seasonality and temperature fluctuations. While temperature ranges and annual means are comparable across the study sites, annual precipitation varies considerably. *Aganope balansae* occurs across a broad precipitation gradient in Southeast Asia, from approximately 1200 to 2200 mm per year. The Mengsong site lies at the lower end of this range with about 1200 mm of rainfall annually, whereas Dinghu is near the upper margin with roughly 2000 mm. Elevation ranges also differ among sites and species. The *A. balansae* specimens were collected between 18 and 975 m a.s.l., *A. dinghuensis* occurs between 150 and 400 m a.s.l. in the Dinghu Mountain area, and specimen from Mengsong was collected at 1160 m a.s.l. The bedrock types are mainly sandstone and granite, with additional limestone sites in Vietnam, while the soil types are acrisols (ISRIC; Fromaget 1971; Pignatti et al. 1990; Zhu et al. 2015). The vegetation in Ding Hu supporting subtropical evergreen broadleaved forest (Shen et al. 2013), and Mengsong is classify as montane evergreen broad-leaved forest (Zhu et al. 2005). Both forest types nevertheless share substantial floristic overlap with over 60 genera and 16 species in common (Shen et al. 2013, Paudel pers. comm). This combination of floristic and climatic similarity suggests that a single *Aganope* species could feasibly occur across these areas, consistent with the hypothesis that *A. dinghuensis* represents a localized expression of the more widespread *A. balansae*.

Implications

This study began with the coincidental discovery of a barcode from a sterile voucher, underscoring the value of integrating genetic data and other technologies (e.g., near-infrared sensing) into community surveys. Identifying lianas is particularly challenging because diagnostic characters are often inaccessible in the canopy, leaving stems and sterile vouchers as the primary resources in large-scale community surveys.

Our results also highlight the enduring value of historic herbarium specimens in the genomic era. Although degraded DNA can pose technical challenges, these collections provide irreplaceable windows into past biodiversity and biogeography. While plastome data from 19th-century vouchers provide deeper insights into the species boundaries in *Aganope*, traditional barcodes served as a crucial entry point for discovery, covering the broadest range of species until more genomic datasets become available. We may never know if the original individual or its forest still exists, but this case serves as a reminder that significant taxonomic discoveries can be made—even at the kitchen table.

This study shows that even within a relatively small genus, species boundaries remain taxonomically ambiguous. Thus, with the publicly genomic databases continue to expand (example in this case, *A. heptaphylla, A. balansae,* and *A. polystachya*), will continue to refine phylogenetic frameworks and clarify relationships in *Aganope* and related genera. For conservation and floristic studies, these refinements on whether *A. dinghuensis* is a narrow endemic or part of a more widespread species would largely help the assessments of rarity, vulnerability, and management priorities.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11756-026-02290-6.

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Author's contributions MR designed the research, collected the specimens, organized herbarium tissue. SLL analyzed the genomic data. MR and SLL wrote the manuscript. MR https://orcid.org/0000-0002-8312-4118; SLL, https://orcid.org/0000-0002-1821-9655.

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Data availability Accession numbers in GenBank of the newly sequenced plastomes and ITS sequences are in Table 1 and S1. The alignments are available as S3, S4, S5. Plastome raw data will be provided upon request by SLL. For the voucher Mengsong 75–9-1 following data are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/ from earlier submissions: MZ817089 (plastome), associated BioProject, SRA, and Bio-Sample numbers for reads are PRJNA754479, SRR15458559 and SAMN20776253 respectively. In 2014 four barcodes of the same individual were submitted to GenBank, and are available under following accession numbers: rbcL—KF181578; matK—HG005006, ITS1&2—HG004878. For trnH-psbA- (HG005124) the begin and the end of the sequence should be ignored, as the chromatogram had bad resolution. All other used genetic sequences are listed as GenBank accession numbers in table S1.

Declarations

Competing interests The authors declare no competing interests.

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