Molecular and morphological analyses of plants with ethnomedicinal uses in northeastern Peru

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Research

Abstract

Background: In Peru, ethnomedicinal plants have not been extensively assessed in the current context of DNA-based techniques. In the Amazonas region, medicinal plants use for diarrhea and fever treatment are mainly known by local or traditional names, while their phenotypic plasticity limits their proper morphological identification.

Methods: In this regard, selected plants with ethnomedicinal uses in the Amazonas region were confirmed and characterized using morphology and multilocus phylogenies based on three molecular markers (ITS, *mat*K, and *rbc*L).

Results: This study reported four species with ethnomedicinal uses [*Disciphania ernstii* (Menispermaceae), *Psidium fulvum* (Myrtaceae), *Styloceras penninervium* (Buxaceae), *Ugni myricoides* (Myrtaceae)] distributed in humid forest, at 1,000–3,800 masl in the Amazonas region. The genetic markers that showed better resolution to distinguish species of the genera were ITS (*Disciphania*) and *mat*K (*Psidium*, *Ugni*, and *Styloceras*).

Conclusion: An initial screening regarding the diversity of plants with ethnomedicinal uses in the Amazonas region was needed and should include DNA-based techniques using these molecular markers to correctly identify them. This approach will facilitate further evaluation of the ancestral knowledge on the use of medicinal plants in Peru.

Keywords: Amazonas, Buxaceae, DNA barcoding, ethnomedicine, Myrtaceae, Ranunculaceae

Background

Plants have been used as a source of natural medicine for thousands of years by many cultures (Zhang *et al.* 2021). Many medicinal plants have gained popularity as sources of phytochemical compounds that play a key role in the prevention and treatment of various diseases (Hao *et al.* 2017; Zhou *et al.* 2021, Machmudah *et al.* 2022). Most medicinal plants are based on the traditional knowledge of natural resources and are widely used by developed and developing countries due to their accessibility (Barrera & Kindelán 2014, Bailon *et al.* 2015, de Oliveira 2018). Spending on pharmacological drugs in 2017 reached $455.9 million in the United States (Schumock *et al.* 2018) and in 2019 was $1.25 billion worldwide (Mikulic 2020). About thirty percent of these therapeutic drugs were isolated from plants and microorganisms (Newman & Cragg 2012), confirming their medicinal importance due to their bioactive phytochemical content (Ricardo *et al.* 2015, Machmudah *et al.* 2022).

The ethnobotanical use of plant-based substances has proven to be efficient in the prevention and treatment of multiple diseases in the Andean region (Barrera & Kindelán 2014, Bailon *et al.* 2015) since the access to conventional medicine is limited (Bailon *et al.* 2015, Gonzales & Valerio 2006, Irl *et al.* 2015, Tuaza 2020). This ancestral knowledge is recognized and supported by the World Health Organization (WHO) to treat various diseases (WHO 2019). Around 17000 taxa of spermatophytes have been described in the Andean forests of Peru (Brako & Zarucchi 1993, WCVP 2022), of which only 60% were studied and at least 1 400 species were confirmed with medicinal properties (Brack Egg 2004, Bussmann 2013, WCVP 2022). However, many of these are poorly known species, and their ancestral knowledge of traditional medicine is undervalued (Kor *et al.* 2021).

In recent years, exploring biodiversity using DNA-based techniques have proven to be valuable tools, especially in northern Peru, where DNA-barcoding and high-throughput sequencing have confirmed new reports and new species with economic and ecological importance from Amazonas region (Bustamante *et al.* 2019–2020, Tineo *et al.* 2020). In addition, computational advances have facilitated the proper classification of species (Kriebel *et al.* 2017), using ideal loci for a wide range of taxa that can be informative on different evolutionary time scales (Hilu *et al.* 2008), and helping to uncover the species diversity (Carrive *et al.* 2020, Maurin *et al.* 2021). For instance, the markers *mat*K, *atp*B-*rbc*L spacer, and nuclear ITS regions have been traditionally used to reclassify taxa that are not well defined morphologically since these markers allow the establishment of species boundaries based on specific genetic distances (Hilu *et al.* 2008, Hoot *et al.* 2012, Zhai *et al.* 2019).

Some of the best-known medicinal plants from Amazonas region, northeastern Peru, are locally known as "verbena" (*Verbena litoralis* Kunth, Verbenaceae), "achiote" (*Bixa orellana* L., Bixaceae), "molle" (*Minthostachys mollis* (Benth.) Griseb., Lamiaceae), and "llanten" (*Plantago major* L., Plantaginaceae). These plants are used against stomach cramps, kidney disorders and diuretics, intestinal parasites, and prostate and menstruation disorders (Ramírez *et al.* 2020, Corroto *et al.* 2021). Other species such as "granadilla" (*Passiflora ligularis* Juss., Passifloraceae), "elderberry" (*Sambucus peruvianus* L., Adoxaceae), "guasú" (*Desmodium uncinatum* (Jacq.) DC, Fabaceae), "nettle" (*Urtica dioica* L., Urticaceae), "mallow" (*Malva sylvestris* L., Malvaceae), and "chishca" (*Lomatia ligularis* Juss., Proteaceae) are used in the treatment of various ailments and urinary infections for their antibacterial activity (Bussmann & Sharon 2016).

In this region, other four plants with ethnomedicinal potential have not been properly identified with a scientific name due to their phenotypic plasticity. The leaves of these plants are used in the preparation of extracts as a hypoglycemic agent and to combat diarrhea and fever, since they possess antispasmodic and antimicrobial properties (Gutiérrez et al. 2008). The fruits of these plants are also used to make necklaces for traditional festivities in the Amazonas region (Gutiérrez et al. 2008). Therefore, it is difficult to determine whether the knowledge of the use of these medicinal plants is being lost in the Amazon region (Corroto & Macia 2021), based on the low appreciation of this heritage by the new generations (Gupta *et al.* 2021). WHO has already highlighted the dire consequences of the loss of this traditional knowledge of medicinal plants worldwide (WHO, 2002).

Accordingly, these four species, locally used in the Andean communities of the Amazonas region for their pharmacological value, were identified using morphological observations, DNA-barcodes genetic divergences [i.e., maturase K gene (matK), internal transcribed spacer (ITS), and ribulose-bisphosphate carboxylase gene (*rbc*L)], and phylogenetic analyses. This study aims to properly identify theses four species on the basis of DNA-based techniques in order to make their taxonomy available to the scientific community and facilitate further evaluation of the ancestral knowledge on the use of medicinal plants in the Amazonas region.

Materials and Methods

Specimen collection

Four specimens were collected from remote villagers' plots placed in the provinces of Chachapoyas, Luya, and Rodríguez de Mendoza in the Amazonas region (Figure 1). A permit for scientific research on wild flora (RDG N° D000394-2020-MIDAGRI-SERFOR-DGGSPFFS, with authorization code N° AUT-IFL-2020-061) was provided by Servicio Nacional Forestal y de Fauna Silvestre (SERFOR). Tissue samples of approximately 50 mm^2 were taken from leaf tips for molecular analyses and placed in prelabeled 1.5 ml Safelock Eppendorf tubes. Date, time, and GPS coordinates were recorded for each location. Photographs were taken to document sampling locations and site features. In addition, inflorescences, leaves, and fruits were sampled for morphological characterization. Samples were morphologically evaluated according to McVaugh (1958), Köhler (2007) Brako and Zarucchi (1993), and Ulloa-Ulloa *et al.* (2004), and were deposited in the herbarium of Universidad Nacional Toribio Rodríguez de Mendoza (KUELAP), Peru (Table 1) (Thiers 2016).

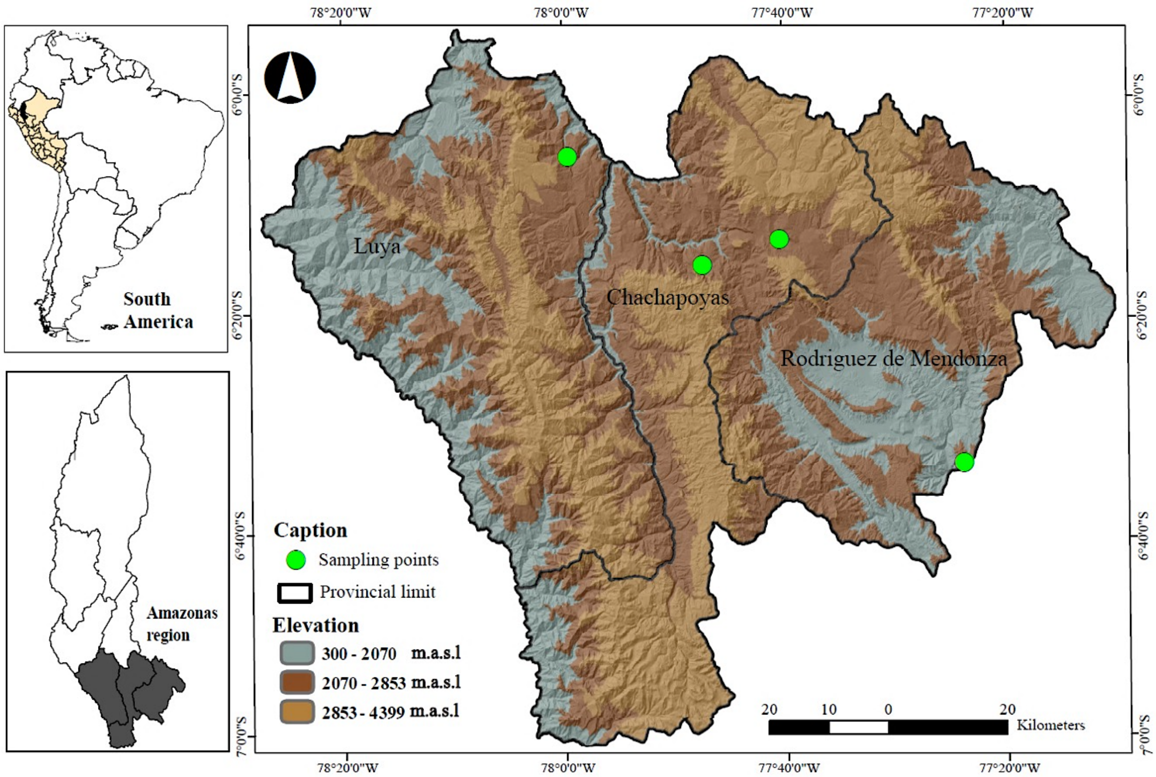

Figure 1. Map showing the sampling of specimens from Region Amazonas, northern Peru.

Table 1. List of samples of ethnomedicinal plants collected in northern Peru.

Species	Voucher; collection code	Collection place	Altitude	UTM	Coordinates	
Disciphania ernstii	KUELAP–314; IARAN053	Nuevo Chirimoto, Rodríguez de Mendoza, Amazonas, Peru	1914	18	6°35'33"	77°16'10"
Psidium fulvum	KUELAP–294; IARAN033	Chachapoyas, Amazonas, Peru	2618	18	6°15'26.56"	77°47'50.47"
Styloceras penninervium	KUELAP–2551; PIC01	San Jerónimo, Luya, Amazonas, Peru	2583	18	6° 5'40.86"	78° 0'6.01"
Ugni myricoides	KUELAP–276; IARAN015	Molinopampa, Chachapoyas, Amazonas, Peru	2538	18	6°13'07.52"	77°40'51.89"

DNA sequencing and alignment preparation

Genomic DNA was extracted from leaf samples using the NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. DNA concentration was quantified by a Quantus™ Fluorometer (Promega, Madison, USA), and quality was measured by 1% agarose gel electrophoresis and visualized on a photodocumenter (SmartView Pro UVCI-1000, Major Science, Saratoga, USA). Two chloroplast markers (*mat*K, maturase K; *rbc*L, ribulose 1,5-biphosphate carboxylase) and one nuclear marker (*nr*ITS, Internal transcribed spacer) were sequenced. Each marker was amplified using polymerase chain reaction (PCR) with MasterMix (Promega, Wisconsin, USA) in the following reaction mixture: 10 ng of DNA and 0.25–0.5 pmol of forward and reverse primers for a total volume of 10 µL. The PCR protocols and primer combinations are summarized in Table 2. Amplicons were purified using the NucleoSpin™ Gel and PCR Clean-up Kit protocol (Macherey-Nagel™, Düren, Germany). The sequences of the forward and reverse strands were determined commercially by Macrogen Inc. (Macrogen, Seoul, Korea). The sequences were manually edited with Chromas V.2.6.6 software. The newly generated sequences (DNA-barcodes) from the four markers (*mat*K, *rbc*L, *nr*ITS) were deposited in GenBank. The newly generated sequences and others obtained from GenBank, after using blast tool and having as far as 98% of genetic similarity (Table 3), were initially aligned with Muscle algorithms (Thompson *et al.* 1994) and were adjusted manually with MEGA10 software (Kumar *et al.* 2018).

Table 2. Sets of primer combinations for *mat*K, *rbc*L and nrITS markers used for specimens from Buxales, Myrtales and Ranunculales (listed 5′→ 3′).

Gene or spacer region	Amplified length (bp)	Primers sequence (5′–3′)	References
ITS	650	F: 5'–GGAAGTAAAAGTCGTAACAAGG–3' R: 5'–TCCTCCGCTATATGATATGC–3'	White et al., 1990 White et al., 1991
*rbc*L	1600	F: 5'–ATGTCACCACAAACAGAAACTAAAGC–3' R: 5'– CTTTTAGTAAAAGATTGGGCCGAG–3'	Chase et al. (1993) Chase et al. (1993)
*mat*K	1500	F: 5'–CTATATCCACTTATCTTTCAGGAGT–3' R: 5'–AAAGTTCTAGCACAAGAAAGTCGA–3'	Ooi et al. (1995) Ooi et al. (1995)

Phylogenetic analysis of concatenated sequence data

Single marker phylogenies were analyzed; however, conclusive phylogenies were based on concatenated data of the three molecular markers (Table 3). For this, each marker was aligned independently, then this alignment was trimmed and finally the three independent alignments were concatenated. Separate phylogenies for Buxaceae (including 26 species and having *Didymeles integrifolia* and *D. perrieri* as outgroup), Myrtaceae (including 22 species and having *Syzygium lateriflorum* and *S. laxeracemosum* as outgroup), and Ranunculaceae (including 19 species and having *Sargentodoxa cuneata* and *Kingdonia uniflora* as outgroup) were evaluated. The best-fitting nucleotide substitution model was selected for each lineage with the three partitions *mat*K, *rbc*L, and *nr*ITS using PartitionFinder (Lanfear *et al.* 2012) (Table 4). The best partition strategy and model of sequence evolution were selected based on the Bayesian information criterion (BIC) for each phylogeny (Table 4). Maximum likelihood (ML) analyses were conducted using the RAxML HPC-AVX program (Stamatakis 2014), implemented in the raxmlGUI 1.3.1 interface (Silvestro & Michalak 2012) using Table 4 models with 1000 bootstrap replications. Bayesian inference (BI) was performed with MrBayes v.3.2.6 software (Ronquist *et al.* 2012) using Metropolis-coupled MCMC and the Table 4 models. Two runs, each with four chains (three hot and one cold) were conducted for 10000000 generations, sampling trees every 1000 generations.3. Finally, because of the limited available accessions in Genbank for the studied genera, the identification was completed by comparing the records and morphologies of our samples to databases and collections such as the Global Biodiversity Information Facility (https://www.gbif.org/), Tropicos from Missouri Botanical Garden (http://www.tropicos.org), the New York Botanical Garden Steere herbarium (http://sweetgum.nybg.org/science), and JSTOR Global Plants (https://plants.jstor.org).

Results and Discussion

A total of eight DNA-barcodes were newly generated for the three molecular markers (1 for ITS, 4 for *mat*K and 3 for *rbc*L) that allowed the construction of multilocus phylogenies (Table 3). The analyzed data matrix included a total of 3 879 base pairs (bp) (2 511 bp for *mat*K, 695 bp for *rbc*L and 673 bp for ITS) from 72 individualsThe exploratory phylogenetic tree obtained from the ML and BI analyses showed four monophyletic lineages belonging to Buxaceae [*Styloceras* Kunth ex A. Juss], Myrtaceae [*Ugni* Turcz. & *Psidium* L.] and one belonging to Ranunculaceae (*Disciphania* Eichle).The single locus phylogenies that better resolved species relationship were based on ITS for Ranunculaceae (Figure S1, S3) and *mat*K for the Myrtaceae and Buxaceae (Figures S2, S4; Figure 2).

Buxaceae

The phylogeny of Buxaceae included 2511 bp for *mat*K containing 26 species. Based on the integration of morphological and molecular analyses,the specimen KUELAP-2551 was recognized as *Styloceras penninervium* A.H. Gentry & Aymard. The genetic divergence of this species with *Styloceras laurifolium* (Willd.) Kunth were 0.08% for *mat*K (Figure 2). *S. penninervium* was founded around 1 700–1800 m.a.s.l. and characterized by having berries with 2 styles separated by 1 cm (Figure 3D–E, Table 5).

Remarks: The genus *Styloceras* is composed of six species distributed in Bolivia, Ecuador and Peru (Torrez & Jørgensen 2010, Ulloa-Ulloa *et al.* 2004, WCVP 2022). In Peru, four species have been recorded *S. Brokawii, S. columnare, S. laurifolium* and *S. penninervium* (Brako & Zarucchi 1993, Ulloa-Ulloa *et al.* 2004). These species have been reported from Amazonas, San Martin, Cusco and Madre de Dios regions (León 2006, Torrez & Jørgensen 2010). This study confirms the presence of *S. penninervium* (KUELAP-2551) in humid forest habitats in the Amazonas region. *S. penninervium* was already recorded from central (Junín and Pasco) and northern Peru (San Martin) at 1000–3800 m.a.s.l, forming sympatric populations with *S. laurifolium* (Gentry & Aymard 1993). *S. penninervium* fruits are mainly consumed to improve digestion, as well as to treat ulcerative lymphangitis (Tamiru *et al.* 2013).

Table 3. List of taxa used in molecular analyses along with voucher numbers followed by GenBank accession numbers. Sequences generated in the present study are in bold.

Species	Voucher, collection code, collection place	ITS	*mat*K	*rbc*L
Buxus arborea	Braimbridge sn, Jamaica		LN877445	
Buxus balearica	B#13477, Spain		LN877446	
Buxus bissei	HFC 77565–A, Cuba		LN877404	
Buxus brevipes	HFC 87054, Cuba		LN877486	
Buxus cacuminis	HFC 75299, Cuba		LN877466	
Buxus crassifolia	1001, Cuba		LN877479	
Buxus gonoclada	HFC 86133, Cuba		LN877437	
Buxus hildebrandtii	YP 2144, Yemen		LN877463	
Buxus jaucoensis	HFC 72333, Cuba		LN877409	
Buxus marginalis	925, Cuba		LN877477	
Buxus mexicana	Koehler sn (B), Mexico		LN877442	
Buxus microphylla	Ackermann & Gonzalez sn, Japan		LN877448	
Buxus pilosula	HFC 78358, Cuba		LN877416	
Buxus rotundifolia	HFC 63382, Cuba		LN877470	
Buxus sclerophylla	HFC 72282, Cuba		LN877418	
Pachysandra axillaris	CPG03495, China		KX526614	
Pachysandra procumbens	VPI:Hinkle 399, China		GU266592	
Pachysandra terminalis	74825, China		AF542581	
Styloceras laurifolium	J. L. Clark 7721, Ecuador		LN877480	
Styloceras penninervium	**KUELAP–2551, PIC1, Peru**		**OP153823**	
Sarcococca confusa	Ra 280, China		LN877482	
Sarcococca conzattii	9759, Mexico		LN877481	
Sarcococca hookeriana	22670, China		LN877488	
Sarcococca saligna	21283 B, China		LN877483	
Didymeles integrifolia	Rabenantoandro et al. 916, (Outgroup)		AM396505	
Didymeles perrieri	Andrianantoanina 387, (Outgroup)		DQ401354	
Disciphania lobata	Ortiz 266, Peru	–	KX384070	–

Disciphania calocarpa	Ortiz et al. 374, Peru	–	KX384068	–
Disciphania domingensis	Ortiz & Pruski 354, Peru	–	KX384069	–
Disciphania ernstii	**KUELAP–314, Peru**	**ON854131**	**OP153819**	**OP153818**
Disciphania killipii	Ortiz & Zarate 310, Peru	KY365645	JN051826	HQ260779
Fibraurea tinctoria	461588	FJ603110	JN051828	FJ026485
Paratinospora sagittata	648882	KY365668	KY365687	KY365715
Burasaia madagascariensis	R.1262	KY365641	JN051813	HQ260767
Penianthus longifolius	461618	KY365654	KC494046	FJ026499
Sphenocentrum jollyanum	Daramota 30, Peru	KY365656	–	JN051687
Aspidocarya uvifera	Hong YP 99190, Peru	KY365639	EF143853	HQ260765
Borismene japurensis	461557	KY365640	KC494024	JN051675
Arcangelisia flava	461553	MG832411	JN051810	LC461723
Anamirta cocculus	KK–AC–08	LC506378	LC506379	LC506380
Tinospora smilacina	461643	KY365675	JN051865	KF496604
Coscinium blumeanum	F Jacques 27	–	JN051822	JN051679
Odontocarya tamoides	1504190/1961252	–	KX384075	KJ594378
Sargentodoxa cuneata	Hong YP 99238, (Outgroup)	–	FJ626515	FJ626605
Kingdonia uniflora	39325, (Outgroup)	–	FJ626519	MN185268
Ugni myricoides	**KUELAP–276, Peru**		**OP153822**	**OP153817**
Psidium fulvum	**KUELAP–294, Peru**		**OP153821**	**OP153816**
Ugni molinae	Conti 110, WIS	–	AY525142	–
Pimenta racemosa	260139	–	DQ088554	–
Calyptranthes pallens	178121		AF368201	
Psidium cattleyanum	375274	–	AB354959	GU135194
Psidium guajava	120290	–	AB354958	KY988321
Psidium environmental	DNAS–EB–121614	–	–	KU887742
Psidium appendiculatum	HUEFS108073	–	–	MT304290
Psidium robustum	ALCB129059	–	–	MT304281
Psidium guineense	260140	–	–	MT708806
Psidium sartorianum	ESA:109980	–	–	MG718504

Plinia pseudodichasiantha	ESA:109393	–	–	MG718205
Archirhodomyrtus beckleri	UNSW23517	–	AF368197	–
Eugenia reinwardtiana	262459	–	KU945995	KM895822
Eugenia myrcianthes	260132	–	AY525131	MG718303
Eugenia supra–axillaris	ESA:109396	–	–	MG718114
Pimenta dioica	Lucas 212 (K)	–	AM490011	–
Siphoneugena reitzii	ESA:119328	–	–	MG833636
Uromyrtus australis	Conti s.n., WIS	–	AY527230	–
Eugenia platysema	ESA:109827	–	–	MG718110
Pimenta guatemalensis	BioBot01807	–	–	JQ592971
Myrcia venulosa	ESA:91792	–	MG718858	MG718335
Myrcianthes fragrans	Conti 108, WIS	–	–	U26328
Myrciaria vexator	260137	–	AY521544	–
Syzygium laxeracemosum	334486, (Outgroup)	–	DQ088586	–
Syzygium lateriflorum	334485, (Outgroup)	–	DQ088585	–

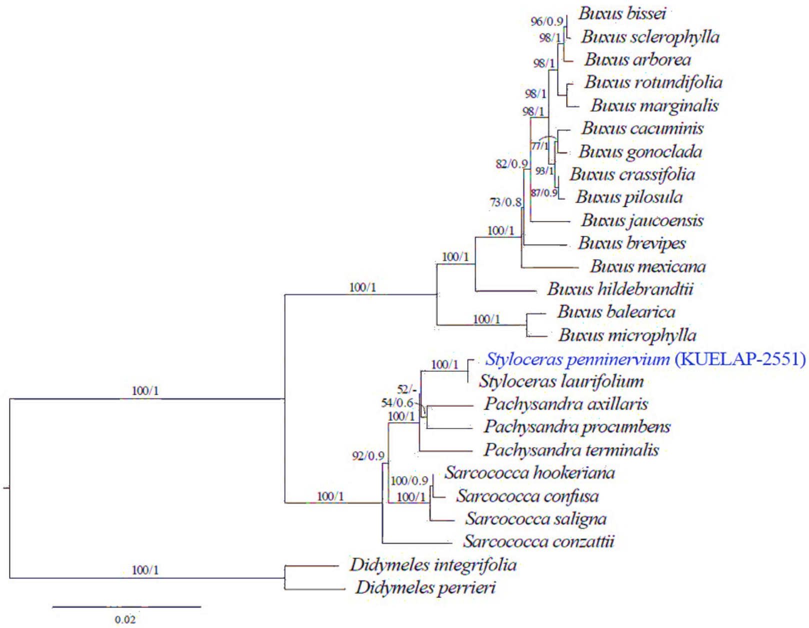

Figure 2. Phylogenetic tree of the Buxaceae lineage based on maximum likelihood inference of *mat*K data. Maximum likelihood bootstrap values (BS; ≥ 50%)/Bayesian posterior probabilities (BPP; ≥ 0.9) are indicated above branches. Values lower than 50% (BS) or 0.90 (BPP) are indicated by hyphens (-). The scale bar indicates the number of nucleotide substitutions per site.

Table 4. Evolutionary models for phylogenetic analyses of specimens from Ericales and Rosales.

Group	Bayesian inferences	Maximum likelihood
Buxaceae	GTR	GTR + G
	GTR + G	K80 +G
	K80+I+G	K80+I+G
Myrtaceae	GTR+G	GTR+G
	GTR	GTR
Ranunculaceae	GTR+G	GTR+G

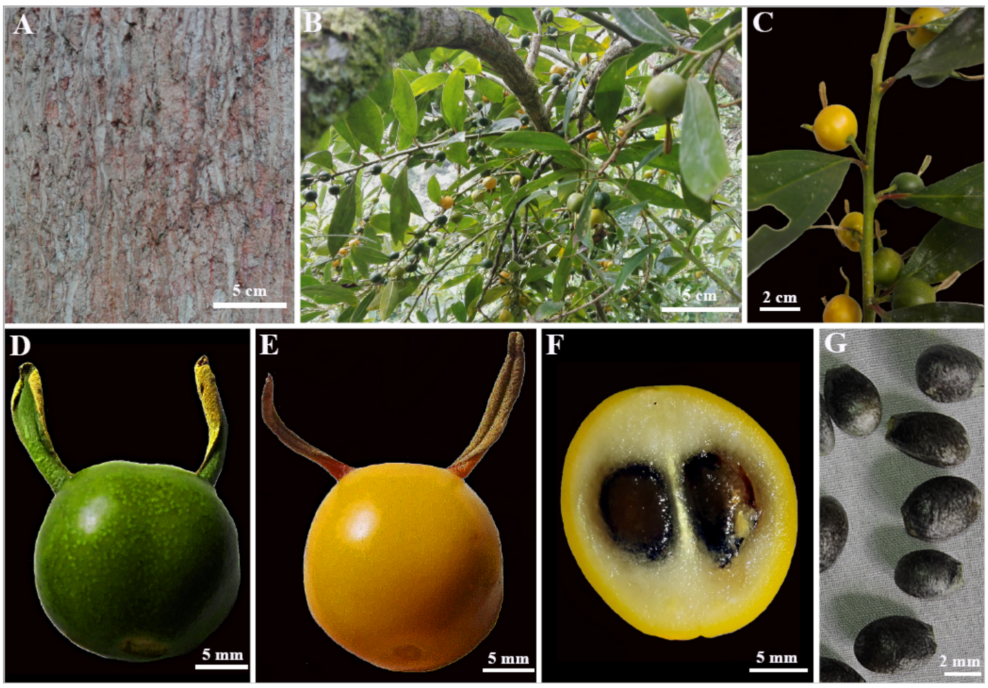

Figure 3. Morphology of *Styloceras penninervium*. Stem (A); Branch (B); Fruitful branch (C); Inmature fruit (D); Mature fruit (E); Longitudinal cut of the fruit (F); Seed (G).

Table 5. Morphological comparisons among species of the genus *Styloceras*.

Species	Habit	Altitude (masl)	Height (m)	Leaves	Fruit diameter	Number seeds/Fruit	Styles	Flowers	anteras (mm)	Localization	References
S. brokawii	subshrub	350–800	2.5–4	Pariraceae to membranaceae	–	–	2 styles separated from the base by up to 7 mm	Yellow with green receptacle	3	Peru/Ecuador/Bolivia	Gentry & Aymard, 1993; Torrez & Jørgensen, 2010
S. columnare	shrub	2533 – 3800	10 – 11	–	1.5– 3	–	2 fused styles, departing from the same point	Glabrous	2	Bolivia/Peru	Gentry & Aymard, 1993; Torrez & Jørgensen, 2010
S. connatum	subshrub	1532	3	semicoriaceus	–	–	2 styles fused from the base 0.7–1 mm	–	1.5–2.6	Bolivia	Gentry & Aymard, 1993; Torrez & Jørgensen, 2010
S. kunthianum	shrub – single monoecious	3208	–	–	–	–	–	Monoecious inflorescence, apical female flowers (botryoides)	3	Ecuador	Gentry & Aymard, 1993; Torrez & Jørgensen, 2010
S. laurifolium	shrub	2200 – 3800	4–16	coriaceous, strongly 3–veined from near base	1.5–2	4	2 styles separated from the base more than 3 mm apart	–	1.5–3	Peru/Bolivia/Ecuador/ Colombia/Venezuela	Gentry & Aymard, 1993; Torrez & Jørgensen, 2010
S. penninervium	shrub	1700–1800	4–5	oblong–lanceolate, coriaceous	–	2–4	2 styles separated by 1 cm	–	2–2.6	Peru	Gentry & Aymard, 1993; Torrez & Jørgensen, 2010; **this study**

Myrtaceae

The phylogeny of Myrtaceae included concatenated data (1152 bp for *mat*K and 695 for *rbc*L) from 27 species. Two specimens were recognized as *Ugni myricoides* (Kunth) O. Berg, (KUELAP-276) and *Psidium fulvum* Mc Vaugh (KUELAP-294). The former species is sister to *Ugni molinae* Turcz (BS/BPP= 88/1.0) and is characterized by black dots on the back of the leaf, white flowers and purple filaments (Figure 5A, Table 6). The genetic divergence between *U. myricoides* and *U. molinae* were 0.1% for *mat*K. Additionally, *P. fulvum* (KUELAP-294) was characterized by elliptic leaves and petiole puberulent and pubescent (Figure 5b; Table 7), and it was sister to *P. robustum* Berg (BS/BPP= 56/0.6) (Figure 4).

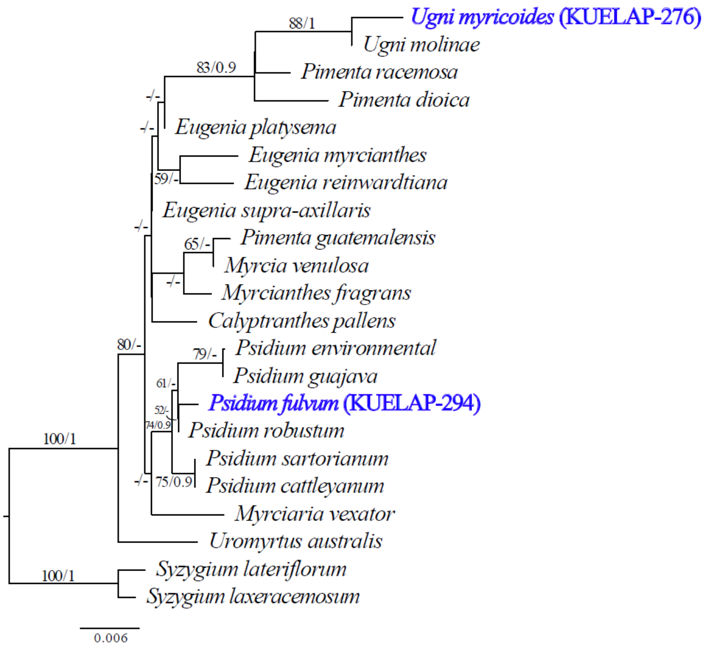

Figure 4. Phylogenetic tree of the Myrtaceae lineage based on maximum likelihood inference of combined *mat*K and *rbc*L data. Maximum likelihood bootstrap values (BS; ≥ 50%)/Bayesian posterior probabilities (BPP; ≥ 0.9) are indicated above branches. Values lower than 50% (BS) or 0.90 (BPP). are indicated by hyphens (-). The scale bar indicates the number of nucleotide substitutions per site.

Figure 5. Morphology of *Ugni myricoides* (A) and *Psidium fulvum* (B).

Table 6. Morphological comparisons among species of the genus *Ugni*.

Species	Habit	Altitude (m above sea level)	Height (m)	Calix	Filaments	Anther	Fruit mature: color	Leaves	Flowers	Corolla	Distribution	References
U. molinae	subshrub	100–400	2–3	–	Reddish	Introrse, whitish	Red	–	Pink to pink–purple	white to rose	Chile	Landrum, 1998
U. candollei	shrub	200 – 1000	1–3	Red	White	White	Red	Small and coriaceous	Pure white, campanulate	White	Peru	Baxter et al., 1998
U. myricoides	subshrub	1000–3000	0.5 – 5	Green	Purple	–	Black	Black spots on the underside	White	White	Peru (Amazonas)	Landrum, 2011; **this study**
U. selkirkii	Tree	200–500	60	–	–	–	Yellowish	Papery to thinly	Buds, brown, white	White	Chile	Breteler, 1999

Table 7. Morphological comparisons among species of the genus *Psidium*.

Species	Altitude (masl)	Height (m)	Number sepals	Leaves	Calix	Petiole	Flowers	Presence of stipulations	Distribution	References
P. acidum	200–500	10–18	4 – 5	Elíptica	closed	channeled	Pyriforme	Caedizas	Peru (Amazonas/Pasco)	Landrum, 2016; Rivero et al., 2012
P. fulvum	1000–1900	12	5	Elíptica	Green	puberulent, pubescent	Solitaire	–	Peru (Amazonas)	Kawasaki & Holst, 2006; **this study**
P. guajava	2000–3100	–	4	Oval or elliptic with dense pubescence	Brown	without pubescence	Solitaire	Caedizas	Peru (San Martin)	Rivero et al., 2012
P. guineense	1000 – 2400	15 m	5	Oval or elliptical	splitting into irregular	–	–	Persistent	Peru (Amazonas/Cusco)	Lim, 2012; Rivero et al., 2013
P. huanucoense	200–1250	1.5–6 m	6	Elliptic/large medium pubescence		puberulento escasamente apresado	Buds pyriform	Caedizas	Peru (Pasco)	Landrum, 2005
P. robustum	~1000	–	–	coriaceous, with rounded apices	Short lobed	Short and robustus	White	White	Bolivia	Soares & Proença, 2008

Remarks: The genus *Ugni* is composed of four species [*U. candollei* (Barnéoud) Berg, *U. molinae* Turcz, *U. myricoides* (Kunth) O. Berg and *U. selkirkii* (Hook. & Arn.) O. Berg] distributed in South and Central America (WCVP 2022). *U. candollei* shares habitats in South America (Chile) and North America (USA) (WCVP 2022). In Peru, two species have been reported *U. molinae* and *U. myricoides* (Brako & Zarucchi 1993, Ulloa-Ulloa *et al.* 2004) and with this study the presence of both species have been confirmed using molecular data (Wilson *et al.* 2005).

These species were previously recorded in the Amazonas region (northern Peru) in montane climates in tropical and subtropical at 2150 m.a.s.l (Landrum & Donoso 1990). This study confirms the distribution of these species in similar habitats (i.e., temperate to humid tropical environments, (Table 6). In the Amazonas region, the fruit of *U. myricoides* is mainly used to improve vision, although the essential oil rich in α-Pineno has also been shown to have analgesic and anti-inflammatory effects (Weston-Green *et al.* 2021).

The genus *Psidium* is composed of 150 species of small trees and shrubs but only 20 species produce edible fruits while the rest are considered wild with inferior quality fruits (Mani *et al.* 2011, Landrum 2016, WCVP 2022). Ten species have been reported in Peru (Brako & Zarucchi 1993, Ulloa-Ulloa *et al.* 2004, Kawasaki & Holst 2006), and three of them have been recorded in the Amazonas region: *P. acidum* (Mart. ex DC.) Landrum, *P. guineense* Sw. and *P. fulvum* Mc Vaugh (Brako & Zarucchi 1993, Ulloa-Ulloa *et al.* 2004, Kawasaki & Holst 2006, WCVP 2022).

Psidium fulvum was found in cold to humid tropical environments at 1000–1900 m.a.s.l (Table 7). In the Amazonas region, leaves of *P. fulvum* are consumed for their anti-inflammatory effect and their high polyphenolic and isoflavonoids (Hussain *et al.* 2021). In contrast, extracts are efficacious for the prevention of tumor development (Sato *et al.* 2010).

Ranunculaceae

The phylogeny of Ranunculaceae included concatenated data (1236 bp for *mat*K, 695 for *rbc*L and 673 bp for ITS) from 19 species. One specimen was recognized as *Disciphania ernstii* Eichler (KUELAP-314). *D. ernstii* characterizes by their greenish-cream flowers and red fruits (Figure 7C, Table 8). Genetically, *D. killipii* and *D. ernstii* were sister species (BS/BPP= 91/0.9), differing by 0.3% for *mat*K, 12.8% for *rbc*L and 6% for ITS. These two species were closely related to *D. calocarpa* Standl (Figure 6).

Remarks: The genus *Disciphania* is composed of 26 species distributed from central Mexico to northern Argentina with a notable concentration of diversity in the upper Amazon basin (Kessler 1993, WCVP 2022). Four out of the 10 species distributed in Peru have been recorded in the Amazonas region (*D. convolvulacea* Barneby, *D. dioscoreoides* Barneby, *D. ernstii* Eichler and *D. heterophylla* Barneby) (Brako & Zarucchi 1993, Ulloa-Ulloa *et al.* 2004, Ortiz-Gentry 2006). This study extends the distribution of *D. ernstii* from Madre de Dios region (southeastern Peru) to Amazonas region (northeastern Peru) (Ortiz-Gentry 2006). *D. ernstii* is a false grape and was found in the cold to humid tropical environments at 400–1914 m.a.s.l (Table 8).

In the Amazonas region, the fruits of *D. ernstii* are consumed for their anti-inflammatory and anti-wrinkle properties; however, more scientific evidence is needed to support these information.

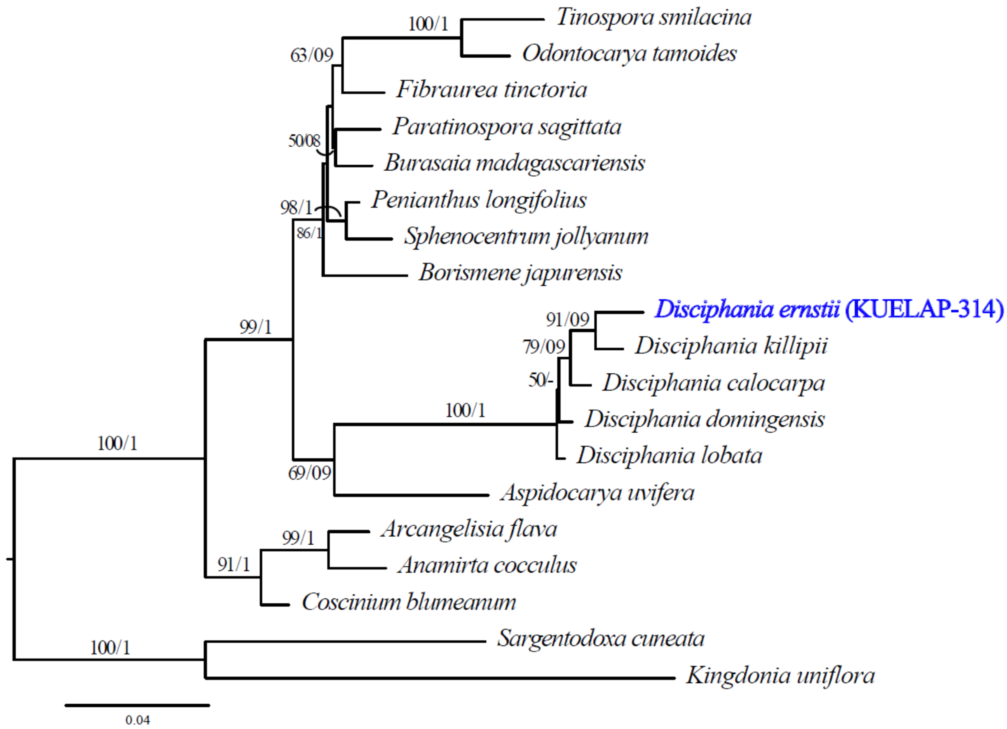

Figure 6. Phylogenetic tree of the Ranunculaceae lineage based on maximum likelihood inference of combined *mat*K, *rbc*L and ITS data. Maximum likelihood bootstrap values (BS; ≥ 50%)/Bayesian posterior probabilities (BPP; ≥ 0.9) are indicated above branches. Values lower than 50% (BS) or 0.90 (BPP). are indicated by hyphens (-). The scale bar indicates the number of nucleotide substitutions per site.

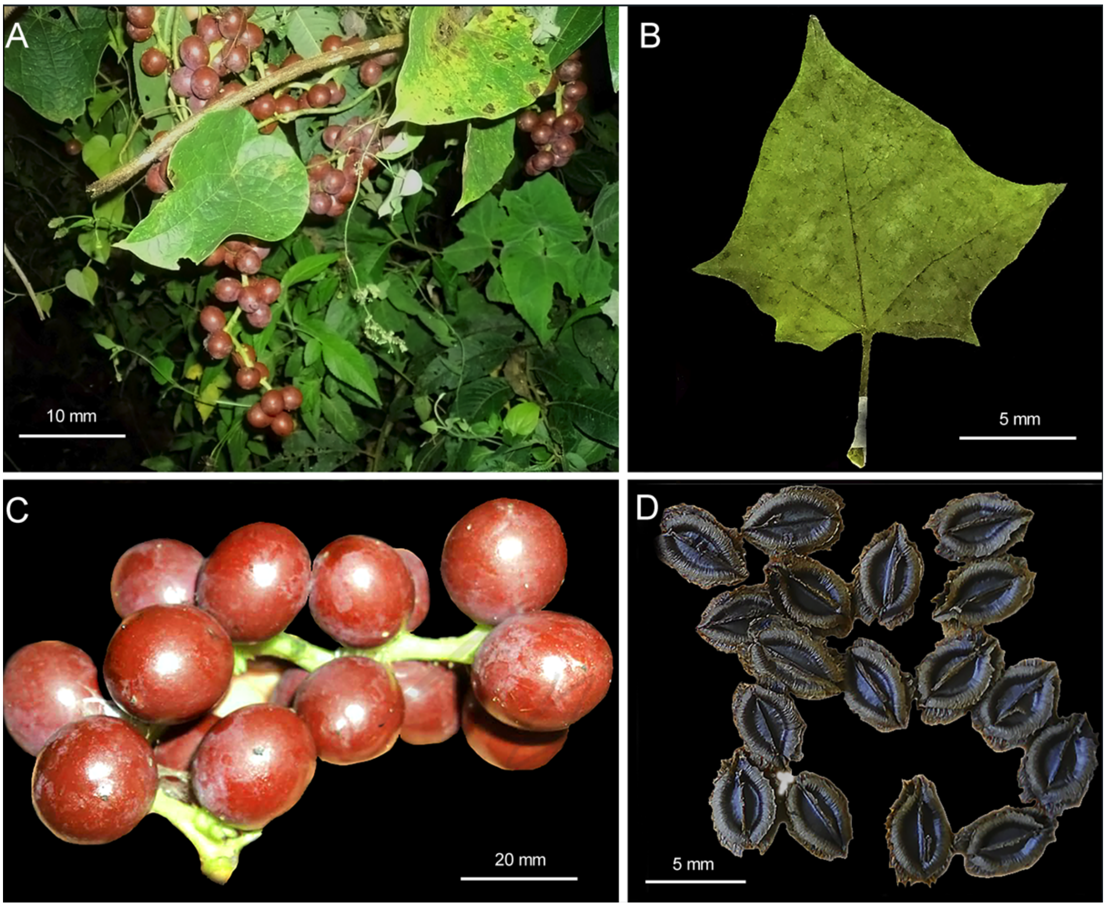

Figure 7. Morphology of *Disciphania ernstii*. Habit (A); Morphology leaf (B); Mature fruit (C); morphology seeds (D).

Table 8. Morphological comparisons among species of the genus *Disciphania*.

Species	**Habit**	**Altitude (m above sea level)**	**Height (m)**	**Leaves**	**Fruit mature: color**	**Flowers**	**Sepals**	**Corolla**	**Distribution in Peru**	**References**
D. convolvulacea	–	100–1000	20	–	–	Sessile, yellow	Introrsun inflexa	–	Amazonas, Cusco	Ortiz–Gentr, 2006; WCVP, 2022
D. cubijensis	Lianas	~260	1		Green	Small green			Madre de Dios	WCVP, 2022
D. dioscoreoides	climber	400 –700	6	Green darck	–	green, reddish powder	Ligth yellow	–	Amazonas/Cusco/San Martin	WCVP, 2022
D. ernstii	variable, long creeping	400–1914		–	Black	Cream grenish	–	–	Madre de Dios	WCVP, 2022; **this study**
D. heterophylla	vine or slender Lianas	1700–2000	4	–	shiny black–purple	Pale green	Green	Orange	Madre de Dios	WCVP, 2022
D. killipii	Lianas	160–270	–	Fleshy	–	Greenish red	–	Greenish red, anthers brownish	Loreto	Pilger, 1933
D. lobata	Lianas	~200	2	Bullate	Black	Red–brown or pink	–	Rose–orange	Loreto	WCVP, 2022
D. remota	Herbaceous	100–250	–	Leathery	–	Flowers removed, glabrous	Elliptical–ovata	6 petals crassiuscula	Loreto	Pilger, 1933
D. tessmannii	Herbaceous climber	100 – 200	–	Glabrous	–	Glabrous sessile flowers	Yellow–green	6 petals, narrow decorated reed	Ucayali	Sleumer, 1967; León, 2006

Conclusions

Using morphological, DNA-barcodes genetic divergences and phylogenetic analyses based on three molecular markers (i.e., ITS, *mat*K, *rbc*L); four species with ethnomedicinal uses from humid forest (at 1000–3800 m.a.s.l) in the Amazonas region were properly identified (i.e., *Disciphania ernstii*, *Psidium fulvum*, *Styloceras penninervium*, *Ugni myricoides*). The genetic markers that showed better resolution to distinguish species of the genera were ITS (*Disciphania*) and *mat*K (*Psidium*, *Ugni*, and *Styloceras*). Accordingly, an initial screening regarding the diversity of plants with ethnomedicinal uses in the Amazonas region is needed and should include DNA-based techniques using these molecular markers. Further studies regarding morphological and molecular analyses of plants with ethnomedicinal uses should be performed in different regions in Peru in order to make their taxonomy available. This approach will facilitate further evaluation of the ancestral knowledge on the use of medicinal plants in Peru.

Declarations

List of abbreviations: Not applicable.

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: Materials are deposited at Herbarium Universidad Nacional Toribio Rodriguez de Mendoza (KUELAP) (http://sweetgum.nybg.org/science/ih/herbarium-details/?irn=259051) which is indexed in the Index Herbariorum of the New York Botanical Garden. The voucher numbers: KUELAP-276, KUELAP-294, KUELAP-310, KUELAP-313, KUELAP-314 and KUELAP-2551 (PIC01). Images of these materials are included in the main manuscript. All Genbank accession numbers are available from https://www.ncbi.nlm.nih.gov/genbank/ under the following accession numbers: OP153816-OP153819, OP153821-OP153823 and ON854131.

Competing interests: The authors declare no conflict of interest.

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Authors' contributions: DT, MSC, DEB: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, writing – original draft, writing – review & editing. DT: Data curation, Project administration; MO: Conceptualization review & editing, Project administration.

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Literature Cited

Bailon N, Romero-Benavides JC, Tinitana-Imaicela F, Ostrosky-Wegman P. 2015. Medicinal plants of Ecuador: A review of plants with anticancer potential and their chemical composition. Medicinal Chemistry Research 246:2283-2296.

Barrera R, Kindelán R. 2014. Utilización de la Medicina Natural y Tradicional en pacientes tratados por Ortodoncia con afecciones de la mucosa oral. Revista Habanera de Ciencias Médicas 133: 466-474.

Brack Egg, A. Ed. 2004. Peru: Biodiversidad, Pobreza y Bionegocios, first ed. Lima, Peru.

Brako LJ, Zarucchi 1993. Catalogue of the Flowering Plants and Gymnosperms in Peru. Mongr. Missouri Botanical Garden 45.

Bussmann RW. 2013. The globalization of traditional medicine in Northern Perú: from shamanism to molecules. Journal of Evidence-Based Complementary Alternative Medicine 2013:291903.

Bussmann RW, Sharon D. 2016. Medicinal plants of the Andes and the Amazon-The magic and medicinal flora of Northern Peru. Ethnobotany Research and Applications 15:1-295.

Bustamante DE, Calderon MS, Leiva S, Mendoza JE, Arce M, Oliva M. 2021. Three new species of Trichoderma in the Harzianum and Longibrachiatum lineages from Peruvian cacao crop soils based on an integrative approach. Mycologia 1135:1056-1072.

Bustamante DE, Oliva M, Leiva S, Mendoza JE, Bobadilla L, Angulo G, Calderon MS. 2019. Phylogeny and species delimitations in the entomopathogenic genus Beauveria Hypocreales, Ascomycota, including the description of B. peruviensis sp. nov. MycoKeys 58:47.

Carrive L, Domenech B, Sauquet H, Jabbour F, Damerval C, Nadot S. 2020. Insights into the ancestral flowers of Ranunculales. Botanical Journal of the Linnean Society 1941:23-46.

Corroto F, Rascón J, Barboza E, Macía MJ. 2021. Medicinal plants for rich people vs. Medicinal plants for poor people: a case study from the Peruvian andes. Plants 108:1634.

Corroto F, Macia MJ. 2021. What is the most efficient methodology for gathering ethnobotanical data and for participant selection? Medicinal plants as a case study in the Peruvian Andes. Economic Botany 751:63-75.

de Oliveira DR. 2018. A consolidação das práticas integrativas e complementares no século 21. VITTALLE-Revista de Ciências da Saúde 301:7-8.

Gentry AH, Aymard G. 1993. A new species of Styloceras Buxaceae from Peru. Novon, 142-144.

Gonzales GF, Valerio LG. 2006. Medicinal plants from Peru: a review of plants as potential agents against cancer. Anti-Cancer Agents in Medicinal Chemistry Formerly Current Medicinal Chemistry-Anti-Cancer Agents 65:429-444.

Gupta A, Raj P, Lama PK, Kumar U, Lalmuansangi C, Dolo Y. 2021. Case study: Awareness of indigenous knowledge associated with traditional herbs for health and sustainable development.

Gutiérrez RMP, Mitchell S. Solis RV. 2008. Psidium guajava: a review of its traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology 117 1-27.

Hussain SZ, Naseer B, Qadri T, Fatima T, Bhat TA. 2021. Guava Psidium Guajava-Morphology, Taxonomy, Composition and Health Benefits. In Fruits Grown in Highland Regions of the Himalayas pp. 257-267. Springer, Cham.

Hao DC, He CN, Shen J, Xiao PG. 2017. Anticancer chemodiversity of Ranunculaceae medicinal plants: molecular mechanisms and functions. Current Genomics 181:39-59.

Hao DC. 2018. Ranunculales medicinal plants: biodiversity, chemodiversity and pharmacotherapy. Academic Press.

Hoot SB, Meyer KM, Manning JC. 2012. Phylogeny and reclassification of Anemone Ranunculaceae, with an emphasis on austral species. Systematic Botany 371:139-152.

Kawasaki L, Holst BK. 2006. Myrtaceae endémicas del Perú. Revista Peruana de Biología 132:463-468.

Kessler PJA. 1993. Menispermaceae. In Flowering Plants. Dicotyledons pp. 402-418. Springer, Berlin, Heidelberg.

Köhler E. 2007. Buxaceae. In Flowering Plants· Eudicots pp. 40-47. Springer, Berlin, Heidelberg.

Kor L, Homewood K, Dawson TP, Diazgranados M. 2021. Sustainability of wild plant use in the Andean Community of South America. Ambio 509:1681-1697.

Kriebel R, Khabbazian M, Sytsma KJ. 2017. A continuous morphological approach to study the evolution of pollen in a phylogenetic context: An example with the order Myrtales PLoS One 1212: e0187228.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 356:1547.

Landrum LR, Donoso C. 1990. Ugni molinae Myrtaceae, a potential fruit crop for regions of Mediterranean, maritime, and subtropical climates. Economic Botany, 536-539.

Landrum LR. 2016. Re-evaluation of Psidium acutangulum Myrtaceae and a new combination in Psidium. Brittonia 684:409-417.

Lanfear R, Calcott B, Ho SY, Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 296: 1695-1701.

León B. 2006. Buxaceae endémicas del Perú. Revista Peruana de Biología, 132:192-192.

Mani A, Mishra R, Thomas G. 2011. Elucidation of diversity among Psidium species using morphological and SPAR methods. Journal of Phytology, 38.

Machmudah S, Fitriana MW, Fatbamayani N, Kanda H, Winardi S, Goto M. 2022. Phytochemical compounds extraction from medicinal plants by subcritical water and its encapsulation via electrospraying. Alexandria Engineering Journal 613:2116-2128.

McVaugh R. 1958. Flora of Peru. Field Museum of Natural History, Chicago

Maurin O, Anest A, Bellot S, Biffin E, Brewer G, Charles-Dominique T, Cowan RS, Dodsworth S, Epitawalage N, Gallego B, Giaretta A, Goldenberg R, Gonçalves DJP, Graham S, Hoch P, Mazine F, Low YW, McGinnie C, Michelangeli FA, Morris S, Penneys DS, Pérez-Escobar OA, Pillon Y, Pokorny L, Shimizu G, Staggemeier VG, Thornhill AH, Tomlinson KW, Turner IM, Vasconcelos T, Wilson PG, Zuntini AR, Baker WJ, Forest F, Lucas E. 2021. A nuclear phylogenomic study of the angiosperm order Myrtales, exploring the potential and limitations of the universal Angiosperms353 probe set. American Journal of Botany 1087:1087-1111.

Mikulic M. 2020. Global spending on medicines, 2020-2024.

https://www.statista.com/statistics/280572/medicine-spending-worldwide/

Newman DJ, Cragg GM. 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of natural products 753:311-335.

Ortiz-Gentry R. 2006. Menispermaceae endémicas del Perú. Revista Peruana de Biología 132:454-454.

Ramírez Viena L, Mostacero León J, López Medina E, De La Cruz Castillo AJ, Gil Rivero AE. 2020. Aspectos etnobotánicos de Cuspón, Perú: Una comunidad campesina que utiliza 57 especies de plantas en sus diversas necesidades. Scientia Agropecuaria 111:7-14.

Ricardo LM, Goulart EMA, Brandão MGL. 2015. Plantas medicinais da Bacia do Rio das Velhas: avaliação das condições para produção e uso em saúde pública. Revista Brasileira de Plantas Medicinais 17:398-406.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu Liang, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic biology 613:539-542.

Sato R, Dang KM, McPherson BG, Brown AC. 2010. Anticancer activity of guava Psidium guajava extracts. Journal of Complementary and Integrative Medicine 71.

Schumock GT, Stubbings J, Wiest MD, Li, EC, Suda KJ, Matusiak LM, Wiest MD, Li EC, Suda KJ, Matusiak ML, Hunkler RJ, Vermeulen LC. 2018. National trends in prescription drug expenditures and projections for 2018. The Bulletin of the American Society of Hospital Pharmacists 7514:1023-1038.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 309:1312-1313.

Silvestro D, Michalak I. 2012. raxmlGUI: a graphical front-end for RAxML. Organisms Diversity Evolution 124:335-337.

Tamiru F, Terfa W, Kebede E, Dabessa G, Roy RK, Sorsa M. 2013. Ethnoknowledge of plants used in veterinary practices in Dabo Hana District, West Ethiopia. Journal of Medicinal PlantsResearch 740:2960-2971.

Torrez V, Jørgensen PM. 2010. Styloceras connatum Buxaceae, una nueva especie de Bolivia. Novon: A Journal for Botanical Nomenclature 203:363-366.

Thiers 2016 B. Thiers Index Herbariorum. A Global Directory of Public Herbaria and Associated Staff New York Botanical Garden's Virtual Herbarium 2016. Available from: http://sweetgum.nybg.org/science/ih accessed 2022

Tineo D, Bustamante DE, Calderon MS, Mendoza JE, Huaman E, Oliva M. 2020. An integrative approach reveals five new species of highland papayas Caricaceae, Vasconcellea from northern Peru. Plos One 1512: e0242469.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 2222:4673-4680.

Tuaza Castro LA. 2020. El COVID-19 en las comunidades indígenas de Chimborazo, Ecuador. Latin American and Caribbean Ethnic Studies 154:413-424.

Ulloa-Ulloa CU, Zarucchi JL, León B. 2004. Diez años de adiciones a la flora del Perú: 1993-2003. Arnaldoa, Ed. Especial 7-242.

Weston-Green K, Clunas H, Jimenez Naranjo C. 2021. A review of the potential use of pinene and linalool as terpene-based medicines for brain health: Discovering novel therapeutics in the flavours and fragrances of cannabis. Frontiers in Psychiatry 12:583211.

Wilson PG, O'brien MM, Heslewood MM, Quinn CJ. 2005. Relationships within Myrtaceae sensu lato based on a matK phylogeny. Plant Systematics and Evolution 2511:3-19.

World Health Organization. 2019. Traditional, complementary and integrative medicine. Available at https://www.who.int/traditional-complementary-integrative-medicine/about/en/

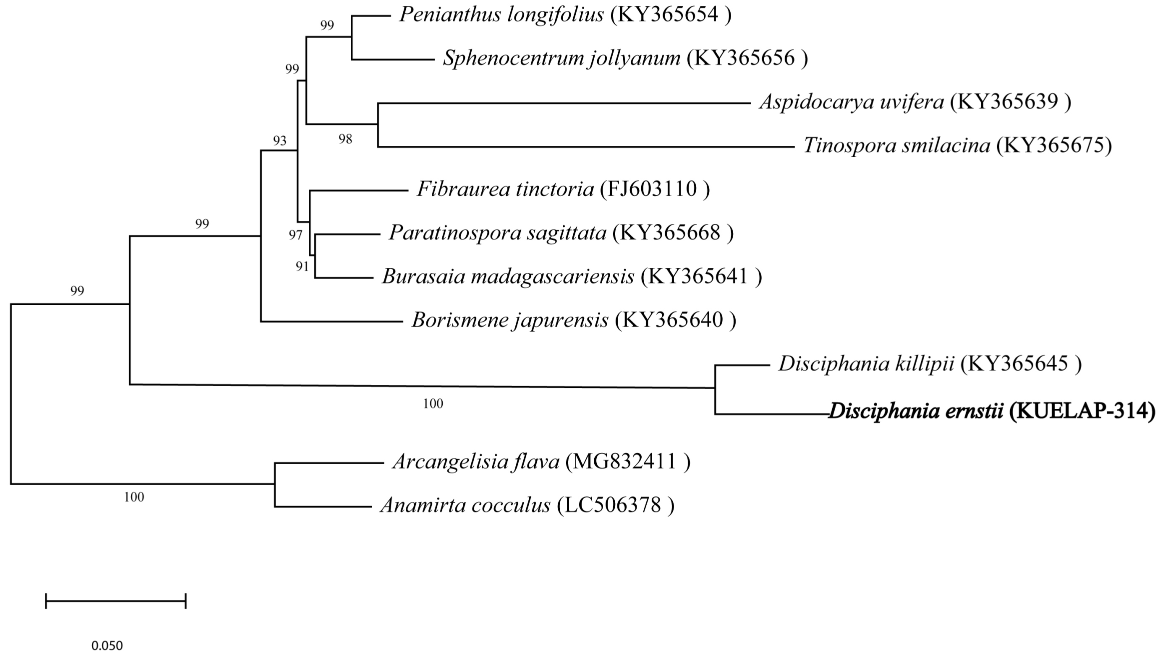
WCVP World Checklist of Vascular Plants**.** Facilitated by the Royal Botanic Gardens, Kew 2022. Published on the internet: http://wcvp.science.kew.org Retrieved 09 July 2022.

Zhai W, Duan X, Zhang R, Guo C, Li L, Xu G, Shan H, Kong H, Ren Y. 2019. Chloroplast genomic data provide new and robust insights into the phylogeny and evolution of the Ranunculaceae. Molecular Phylogenetics and Evolution 135:12-21.

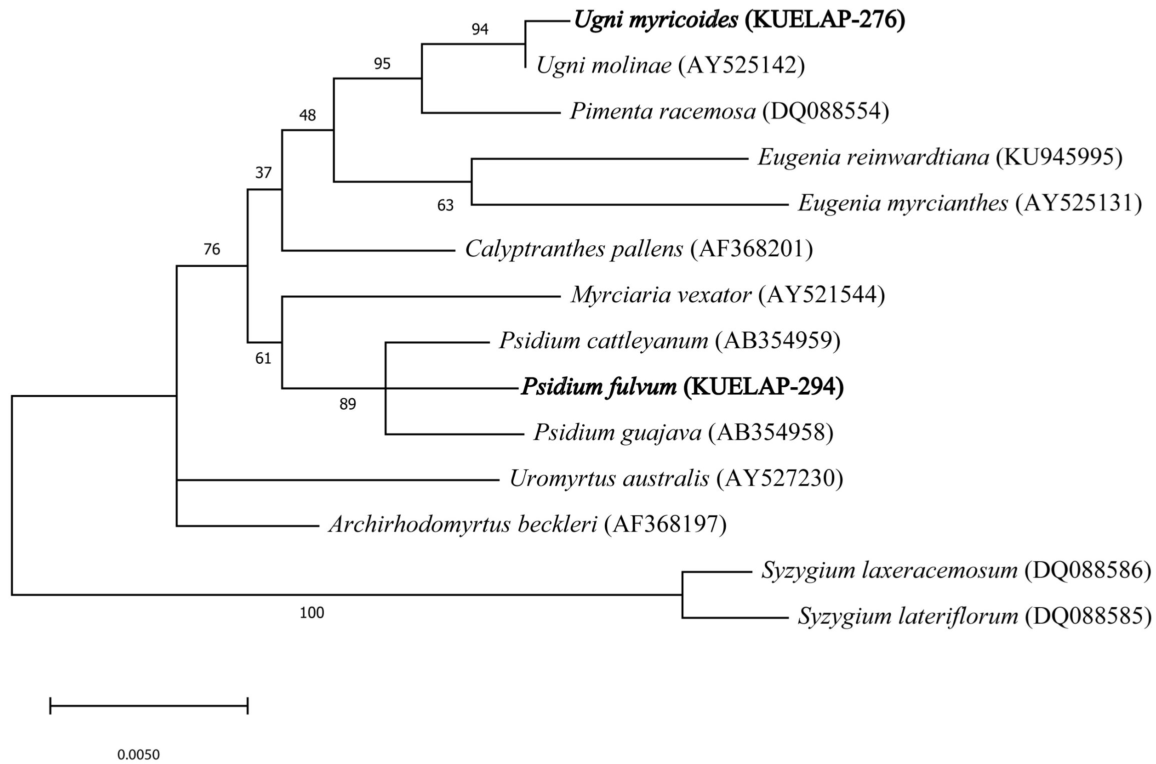
Zhang J, Hu K, Di L, Wang P, Liu Z, Zhang J, Yue P, Song W, Zhang J, Chen T, Wang Z, Zhang Y, Wang X, Zhan C, Cheng YC, Li X, Li Q, Fan JY, Shen Y, Han JY, Qiao H. 2021. Traditional herbal medicine and nanomedicine: Converging disciplines to improve therapeutic efficacy and human health. Advanced Drug Delivery Reviews 178:113964.

Zhou GR, Liao BS, Li QS, Xu J, Chen SL. 2021. Establishing a genomic database for the medicinal plants in the Brazilian Pharmacopoeia. Chinese Medicine 161:1-10.

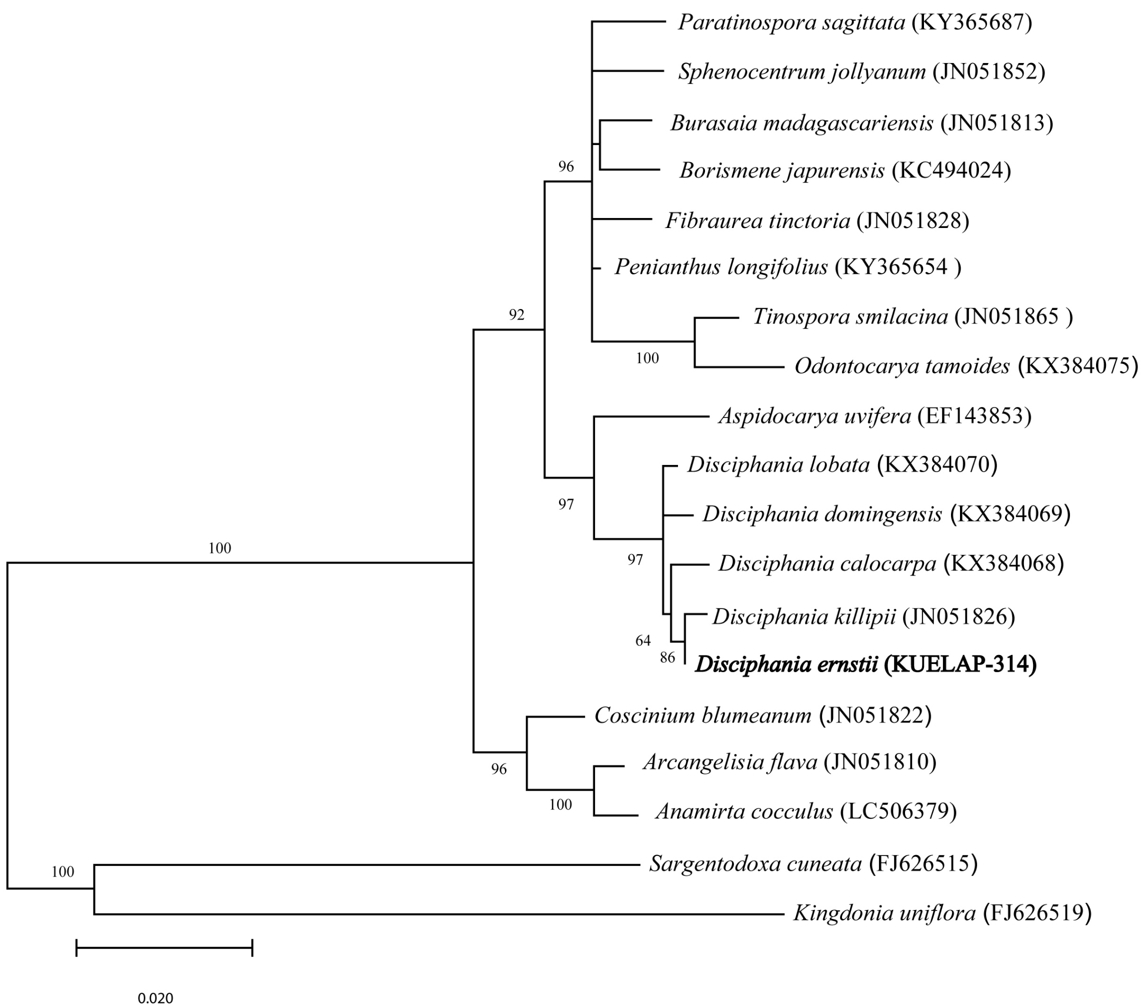
Supplementary material



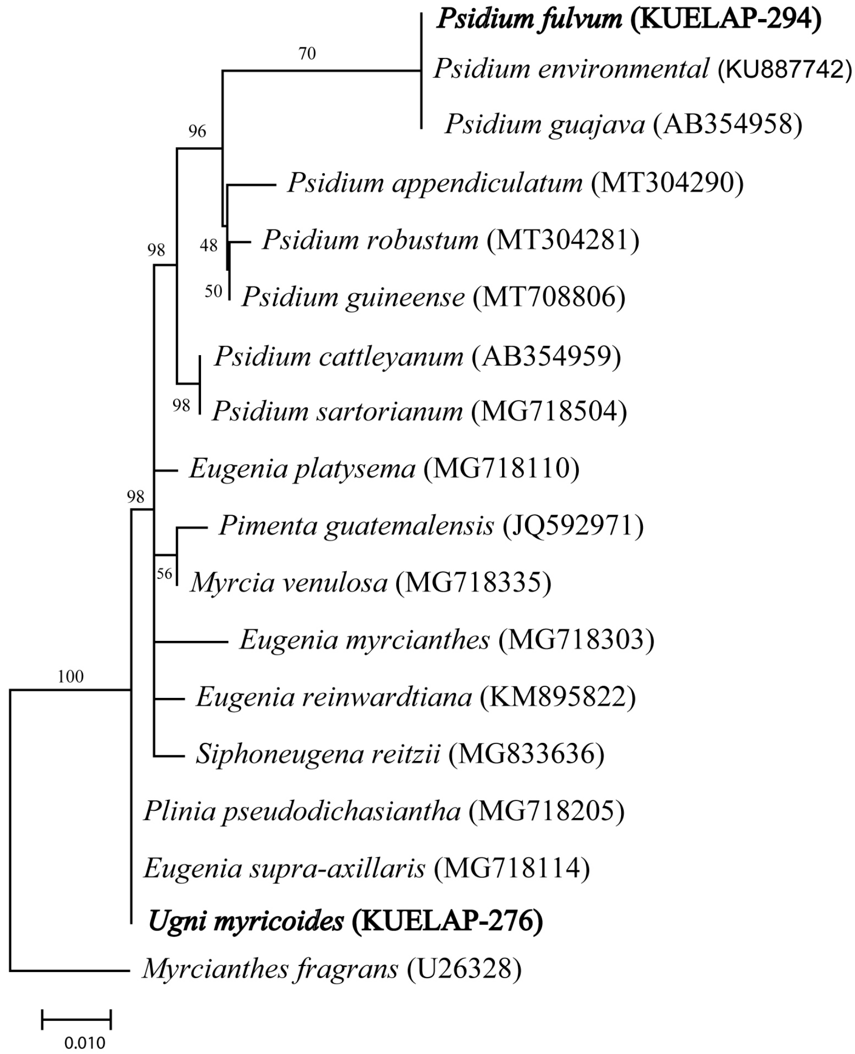
S1. Phylogenetic tree of the Ranunculaceae lineage based on maximum likelihood inference from ITS data. Bootstrap values are indicated below branches.



S2. Phylogenetic tree of the Myrtaceae lineage based on maximum likelihood inference from *mat*K data. Bootstrap values are indicated below branches.



S3. Phylogenetic tree of the Ranunculaceae lineage based on maximum likelihood inference from *mat*K data. Bootstrap values are indicated below branches.



S4. Phylogenetic tree of the Myrtaceae lineage based on maximum likelihood inference from *rbc*L data. Bootstrap values are indicated below branches.