

Phylogenetic relationships of the species of *Abuta* in Peru with emphasis in the affinities of *Abuta grandifolia* s.l. (Menispermaceae)

Relaciones filogenéticas de las especies de *Abuta* en Perú, con énfasis en las afinidades de *Abuta grandifolia* s.l. (Menispermaceae)

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Abstract

Abuta (Anomospermeae, Menispermaceae), comprising 32 species, is the second most diverse genus of Neotropical moonseeds. Several species are known as sources of the arrow poison curare and are used in traditional medicine; among them, *Abuta grandifolia* is the best known for the treatment of various ailments. This species exhibits marked morphological variation, which previous studies have arranged into four informally recognized morphological groups (Agstsl, Agvar, Agll, and Aggu). Despite its widespread use, the phylogenetic relationships of *A. grandifolia* within *Abuta* remain poorly understood. A recent family-wide molecular study recovered *A. grandifolia* as sister to the remaining species of the genus, although taxon sampling was limited. In this study, we expand taxon sampling within *Abuta* to investigate species-level phylogenetic relationships, with focus on the affinities of *A. grandifolia*. Phylogenetic analyses based on combined ITS and two plastid DNA regions support the monophyly of *Abuta* and provide new insights into interspecific relationships. Accessions representing individual species, including the widespread *A. grandifolia*, were recovered as monophyletic. Within *A. grandifolia*, accessions form four well-supported clades that are congruent with the previously recognized morphological groups. Pending resolution of taxonomic limits and associated nomenclature, the species is treated here in a broad sense and referred to as *A. grandifolia* s.l. Notably, *Abuta mycetandra*, a poorly known taxon, was recovered as the sister lineage to *A. grandifolia* s.l.

Resumen

Abuta (Anomospermeae, Menispermaceae), con 32 especies, es el segundo género más diverso de las "moonseed" Neotropicales. Se conocen varias especies que son fuentes del curare, el veneno de flecha, y también se utilizan en la medicina tradicional; entre ellas, *Abuta grandifolia* es la más utilizada en el tratamiento de diversas dolencias y morfológicamente muy variable. Estudios anteriores clasificaron esta variación en cuatro grupos morfológicos reconocidos informalmente (Agstsl, Agvar, Agll y Aggu). A pesar de su uso generalizado, las relaciones filogenéticas de *A. grandifolia* es incierta, recalando la necesidad de un estudio integral del grupo. Un reciente estudio molecular ubicó a *A. grandifolia* como hermana del resto de las especies del género, aunque con un muestreo taxonómico limitado. En este estudio, se amplía el muestreo taxonómico dentro de *Abuta* con el fin de investigar las relaciones filogenéticas interespecíficas, con especial énfasis en las afinidades de *A. grandifolia*. Nuestro análisis basado en la combinación de la región ITS y dos regiones de ADN plastidial respaldan la monofilia de *Abuta* y aportan nueva información sobre las relaciones interespecíficas. Las accesiones representado especies individuales, incluida la ampliamente distribuida *A. grandifolia*, se recuperaron como monofiléticos. Dentro de *A. grandifolia*, las accesiones forman cuatro clados bien soportados, congruentes con los grupos morfológicos reconocidos previamente. Hasta que se resuelvan los límites taxonómicos y la nomenclatura asociada, la especie se trata aquí en un sentido amplio y se denomina *A. grandifolia* s.l. De manera destacable, *Abuta mycetandra*, un taxón poco conocido, fue recuperado como la linaje hermano de *A. grandifolia* s.l.

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Keywords:

Anomospermeae, curare, moonseed, morphological variation, phylogeny.

Palabras clave:

Anomospermeae, curare, filogenia, "moonseed", variación morfológica.

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Introduction

Menispermaceae (moonseed family) comprises dioecious plants that are predominantly climbers, although herbs, shrubs, and small trees also occur within the family (Barneby & White 2004; Ortiz et al. 2016; Ortiz 2025). Several genera are characterized by the presence of diverse bioactive compounds, particularly alkaloids (Barbosa-Filho et al. 2000; Ortiz et al. 2016; Thornber 1970). *Abuta* Aubl., with approximately 32 species, is one of the most diverse genera of Menispermaceae in the Neotropics, occurring mainly in the Amazon Basin and the Guianas (Barneby 2001; Gentry 1991; Ortiz et al. 2016).

Species delimitation within *Abuta*, as in many other genera of the family, is notoriously difficult, largely due to the scarcity of readily comparable morphological characters. As noted by Krukoff and Barneby (1970), this difficulty is almost inevitable in dioecious plants. In addition, the predominantly climbing habit of many species contributes to their underrepresentation in herbarium collections, owing to the practical challenges associated with collecting fertile material.

Various species of *Abuta* were reported as used in the preparation of the arrow poison curare and in traditional medicine (Ayala 1984; Duke and Vásquez 1994; Krukoff & Moldenke 1938). Of these, *Abuta grandifolia* (Mart.) Sandwith, is the most widely reported to treat multiple diseases (Krukoff & Moldenke 1938). Previous molecular-based studies in the Menispermaceae were focused on relationships mostly at the suprageneric level and sampling within individual genera was limited, and in the case of *Abuta* only four species were included (Lian et al. 2019; Ortiz et al. 2016). These studies recovered *A. grandifolia* as the sister lineage to the remaining species of *Abuta*. However, the species as currently delimited is widespread in the Amazon basin and occurs in extra-Amazonian regions, and throughout its distribution, the species exhibits considerable morphological variation (Krukoff & Moldenke 1938). Moreover, ongoing morphological and molecular studies suggest the presence of distinct groups within *A. grandifolia*, although their taxonomic limits are

still unresolved, and therefore uncertainties remain regarding its phylogenetic affinities within *Abuta*.

Several species of *Abuta* have been reported as components of curare arrow poisons and as treatments in traditional medicine (Ayala 1984; Duke & Vásquez 1994; Krukoff & Moldenke 1938). Among these, *Abuta grandifolia* (Mart.) Sandwith is the most widely cited species for the treatment of broad spectrum of morbidities (Krukoff & Moldenke 1938). Previous molecular phylogenetic studies of Menispermaceae have focused primarily on suprageneric relationships, with limited sampling at the intrageneric level; in the case of *Abuta*, only four species were included (Lian et al. 2019; Ortiz et al. 2016). These studies recovered *A. grandifolia* as sister lineage to the remaining species of *Abuta*.

However, as currently delimited, *Abuta grandifolia* is widely distributed throughout the Amazon Basin and extends into extra-Amazonian regions, and across this broad range it exhibits considerable morphological variation (Krukoff & Moldenke 1938). This extensive distribution, together with the observed morphological variability, suggests that *A. grandifolia* may comprise more than one morphologically coherent group, a hypothesis that can be tested through a phylogenetic framework incorporating molecular data, in order to clarify its taxonomic circumscription and phylogenetic placement within *Abuta*.

In the present study, *Abuta grandifolia* is treated in a broad sense (*A. grandifolia* s.l.). The morphological groups recognized within this complex are designated following an informal nomenclature that one of us (Ortiz et al. unpublished results) identified based on the comparative study of 368 herbarium specimens from across the species' distribution, complemented by extensive field observations. These groups are: (1) Agtstl, trees at least six meters tall with small leaves; (2) Agvar, shrubs or lianas with small leaves, the informal name reflecting the variable growth habit of individuals assigned to this group; (3) Agll, shrubs or lianas with large leaves; and (4) Aggu, exclusively climbing plants.

With expanded taxonomic sampling, with particular emphasis on Peruvian species, the objectives of this study were to: (1) re-evaluate the monophyly of the genus *Abuta*; (2) explore phylogenetic relationships among species of *Abuta*; and (3) investigate the placement of *Abuta grandifolia* s.l., a morphologically variable species with multiple documented ethnobotanical uses across its geographic range.

Material and methods

Species concept. In this study, we follow the general lineage species concept (de Queiroz 1998) that consider species as segments of population level lineages that evolve independently. And lineage separation may be inferred by a single or multiple lines of evidence (de Queiroz 2007).

Descriptive morphology. When comparing/contrasting morphological characters for relevant taxa we follow terminology used by Ortiz et al. (2025).

Plant material and taxon sampling. Forty-seven accessions of 23 taxa, including 14 species of *Abuta* (Table 1), were sampled from herbarium material and from samples preserved in silica gel. Sampling for *Abuta grandifolia* s.l. included accessions representing the four recognized morphological clusters, for these we follow the nomenclature as indicated above: 1) Agtstl; 2) Agvar; 3) Agll, and 4) Aggu. The morphological groups are each represented by four accessions. Based on the results of Lian et al. (2019), *Rupertiella boliviana* (Krukoff & Moldenke) Wei Wang & R. Ortiz, *Elissarrhena longipes* Miers, *Elissarrhena solimoesana* (Moldenke) Wei Wang & R. Ortiz, *Telitoxicum krukovii* Moldenke, *Telitoxicum peruvianum* Moldenke, *Elephantomene eburnea* Barneby & Krukoff, *Caryomene grandifolia* Barneby & Krukoff, *Anomospermum chloranthum* Diels, *Anomospermum reticulatum* (Mart.) Eichler, and *Sarcopetalum harveyanum* F. Muell. were included as outgroups. The outgroup taxa are represented by one accession each except for *R. boliviana* which has two accessions.

DNA extraction, PCR amplification and sequence alignment. Total gDNA was extracted from silica gel-dried and herbarium material. We used either innuPREP Plant DNA Extraction Kit (Analytik-Jena) following the protocol provided by the manufacturer of the kit or the modified CTAB protocol of Doyle and Doyle (1990). The *trnL-F* and *rpS16* intron regions, *trnH-psbA* spacer, and *ITS* were chosen based on their utility in resolving relationships in the Menispermaceae in previous studies (Wang et al. 2007; Ortiz et al. 2016; Lian et al. 2019; 2021). The markers were amplified using the primers and protocols described in Table 2. The PCR amplification was performed in a 20 µL reaction mixture with ~2-100 ng template gDNA, 4 µL of 5× PCR buffer (Promega), 0.8 µL dNTP mix (10 mM), 1.5 U of GoTaq G2 Flexi DNA Polymerase, and 0.4 µL of 10 µM forward and reverse primers. Laboratory work was carried out initially at the Jean Christophe-Pintaud Applied Botany Laboratory (LBA-JCP) of the Instituto de Investigaciones de la Ama-

zonía Peruana (IIAP), Tarapoto, Peru; later work was performed at the Ecobiotech Lab in Trujillo, Peru.

PCR products were sent for sequencing at Macrogen Inc. (Seoul, Republic of Korea). For each sample, bidirectional DNA strands were sequenced following gel purification. Contigs were analyzed, edited, and assembled in Geneious Prime® 2025.1.2 (<https://www.geneious.com/features/prime>). All sequences were aligned using the MAFFT v7.490 function (Katoh and Standley 2013) in Geneious and adjusted manually.

Most of the sequences used in this study were newly generated. Exceptions include the *trnL-F* region for *Abuta panamensis* (Standl.) Krukoff & Barneby, *Abuta rufescens* Aubl. (voucher Ortiz 226), and *Abuta sandwithiana* (voucher Ortiz 305), which were retrieved from GenBank. Additionally, accessions for *Abuta grandifolia*-Agvar (voucher Ortiz 221) and *Rupertiella boliviana* (voucher Ortiz 294), originally used by Lian et al. (2019) were downloaded from GenBank for all markers.

Accessions for all outgroups were obtained from GenBank, with the exception of the *ITS* sequences of *Telitoxicum krukovii* and *Elissarrhena longipes*, which were generated for this study. GenBank accession numbers are given in Table 1.

Congruence test and phylogenetic analyses. To examine data compatibility between *ITS* and plastid DNA regions, congruence analyses were assessed by *ILD* test (Farris et al. 1995) and tree-based comparisons (Wiens 1998). The *ILD* test was performed in PAUP v4 (Swofford 2003) via heuristic searches of 100 replicates, each with 10 replicates with random sequence addition and TBR branch swapping. For tree-based comparisons, the thresholds *BS* ≥ 70% and *PP* ≥ 0.95 were used as indicators of significant inconsistency (Lian et al. 2021; Wang et al. 2012). If not, strongly conflicting nodes were recovered, the *ITS* and plastid datasets were combined into a single dataset and used in further analyses.

Phylogenetic analyses were performed using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches. Maximum Likelihood analysis was performed in RAxML v8.2.11 (Stamatakis et al. 2014) with the substitution model for each region and rapid bootstrapping with 1000 replicates. Bayesian Inference analysis was conducted in Mr. Bayes v3.2.5 (Ronquist et al. 2012). Each DNA region was independently assigned the best-fit model of nucleotide substitution, which was determined using jModeltest 2.1.4 (Posada 2008) based on the Akaike Information Criterion (AIC). The analysis involved running two independent sets of Markov Chain Monte Carlo (MCMC) chains, each containing four chains. The chains were run for 50 million generations, with a sampling frequency of every 1000 generations, and initiated with a random tree. The stationarity of the runs was assessed using Tracer 1.7 (Rambaut et al. 2018). The 50% majority-rule consensus tree was obtained after discarding the first 25% of posterior trees as burn-in.

Table 1. Taxa, vouchers and GenBank accession numbers for the sequences used in this study. Herbarium acronyms follow Index Herbariorum at <<http://sciweb.nybg.org/science2/IndexHerbariorum.asp>>. “—” indicates missing sequence. Accession numbers in bold indicate sequences newly generated in this study.

Taxon sampling information		GenBank accession numbers		
Taxon	Voucher	trnL-F	trnH-psbA	ITS
Ingroup				
<i>Abuta fluminum</i>	R. Ortiz et al. 590 (USM, MO)	PZ028744	PZ028710	PZ020011
<i>Abuta imene</i>	R. Ortiz et al. 223 (MO)	PZ028757	PZ028726	PZ020027
<i>Abuta imene</i>	R. Ortiz et al. 340 (MO)	PZ028758	PZ028728	PZ020029
<i>Abuta imene</i>	R. Ortiz et al. 257 (MO)	—	PZ028727	PZ020028
<i>Abuta mycetandra</i>	R. Ortiz et al. 550 (USM, MO)	PZ028760	PZ028730	PZ020031
<i>Abuta mycetandra</i>	R. Ortiz et al. 546 (USM, MO)	PZ028759	PZ028729	PZ020030
<i>Abuta mycetandra</i>	R. Ortiz et al. 558 (USM, MO)	—	PZ028731	PZ020032
<i>Abuta obovata</i>	R. Ortiz et al. 570 (USM, MO)	PZ028761	PZ028732	PZ020033
<i>Abuta pahnii</i>	R. Ortiz et al. 595 (USM, MO)	—	—	PZ020035
<i>Abuta pahnii</i>	R. Ortiz et al. 276 (MO)	PZ028762	PZ028733	PZ020034
<i>Abuta panamensis</i>	R. Kriebel et al. 3496 (MO)	KX384108	PZ028734	PZ020036
<i>Abuta rufescens</i>	R. Ortiz et al. 226 (MO)	JN051738	PZ028735	PZ020037
<i>Abuta rufescens</i>	R. Zañartu 44 (USM)	—	PZ028736	PZ020038
<i>Abuta sandwithiana</i>	R. Ortiz et al. 305 (MO)	KX384109	PZ028737	PZ020039
<i>Abuta solimoesensis</i>	C. Grandez 244 (MO)	—	PZ028738	PZ020040
<i>Abuta</i> sp. 1	R. Ortiz et al. 279 (HOXA, MO)	PZ028764	PZ028740	PZ020042
<i>Abuta</i> sp. 1	R. Ortiz et al. 278 (HOXA, MO)	—	PZ028739	PZ020041
<i>Abuta</i> sp. 7	R. Ortiz et al. 597 (USM, MO)	PZ028765	PZ028742	PZ020045
<i>Abuta</i> sp.2	R. Ortiz et al. 277 (HOXA, MO)	—	PZ028741	PZ020043
<i>Abuta</i> sp.6	R. Ortiz et al. 571 (USM, MO)	—	—	PZ020044
<i>Abuta grandifolia</i> s.l.				
<i>Abuta grandifolia</i> -Agvar	R. Ortiz et al. 541 (USM, MO)	PZ028756	PZ028725	PZ020026
<i>Abuta grandifolia</i> -Agvar	R. Ortiz et al. 221 (MO)	KX384107	MK288751	MK288764
<i>Abuta grandifolia</i> -Agvar	R. Ortiz et al. 537 (USM, MO)	—	PZ028724	PZ020025
<i>Abuta grandifolia</i> -Agvar	R. Ortiz et al. 510 (AMAZ, MO)	PZ028755	PZ028723	PZ020024
<i>Abuta grandifolia</i> -Agltsl	R. Ortiz et al. 538 (USM, MO)	PZ028752	PZ028720	PZ020021
<i>Abuta grandifolia</i> -Agltsl	R. Ortiz et al. 539 (USM, MO)	PZ028753	PZ028721	PZ020022
<i>Abuta grandifolia</i> -Agltsl	R. Ortiz et al. 215 (AMAZ)	—	PZ028719	PZ020020
<i>Abuta grandifolia</i> -Agltsl	R. Ortiz et al. 574 (USM, MO)	PZ028754	PZ028722	PZ020023
<i>Abuta grandifolia</i> -Agll	R. Ortiz et al. 543 (USM, MO)	PZ028750	PZ028717	PZ020018
<i>Abuta grandifolia</i> -Agll	R. Ortiz et al. 545 (USM, MO)	PZ028751	PZ028718	PZ020019
<i>Abuta grandifolia</i> -Agll	R. Ortiz et al. 236 (MO)	PZ028748	PZ028715	PZ020016
<i>Abuta grandifolia</i> -Agll	R. Ortiz et al. 238 (MO)	PZ028749	PZ028716	PZ020017
<i>Abuta grandifolia</i> -Aggu	R. Ortiz et al. 572 (USM, MO)	PZ028747	PZ028714	PZ020015
<i>Abuta grandifolia</i> -Aggu	R. Ortiz et al. 563 (USM, MO)	—	PZ028713	PZ020014
<i>Abuta grandifolia</i> -Aggu	R. Ortiz et al. 224 (AMAZ, MO)	PZ028745	PZ028711	PZ020012
<i>Abuta grandifolia</i> -Aggu	R. Ortiz et al. 289 (MO)	PZ028746	PZ028712	PZ020013
Outgroups				
<i>Rupertiella boliviana</i>	R. Ortiz et al. 294 (MO)	KX384111	MK288750	MK288768
<i>Rupertiella boliviana</i>	R. Ortiz et al. 288 (MO)	PZ028766	PZ028743	PZ020047
<i>Telitoxicum peruvianum</i>	R. Ortiz et al. 218 (MO)	JN051795	—	MK288769
<i>Telitoxicum krukovi</i>	R. Ortiz et al. 261 (MO)	KX384145	—	PZ020048
<i>Elissarrhena longipes</i>	R. Ortiz et al. 243 (MO)	JN051741	—	PZ020046
<i>Elissarrhena solimoesana</i>	R. Ortiz et al. 198 (MO)	JN051743	MK288752	—
<i>Elephantomene eburnea</i>	R. Ortiz et al. 237 (MO)	JN051761	MK288748	MK288770
<i>Caryomene grandifolia</i>	R. Zárate et. al 2136 (MO)	JN051750	MK288749	MK288771
<i>Anomospermum reticulatum</i>	R. Kriebel 3501 (MO)	JN051742	MK288747	MK288766
<i>Anomospermum chloranthum</i>	R. Ortiz et al. 324 (MO)	JN051740	—	MK288765
<i>Sarcopetalum harveyanum</i>	van der Werff et al. 17058 (MO)	JN051785	KM895107	MK288772

Table 2. Statistics from the analyses of datasets.

	No. taxa	Total length	No. informative characters	Model
trnL-F	36	903	28	GTR + I
trnH-psbA	40	531	38	GTR + Γ
ITS	45	581	101	GTR + I + Γ
cpDNA (trnL-F + trnH-psbA)	44	1434	66	-
cpDNA + ITS	46	2015	167	-

Results and discussion

DNA regions, alignments and congruence test. The plastid dataset comprised 1434 aligned nucleotides, as follows: *trnL-F* (903 bp), and *trnH-psbA* (531 bp). We excluded *rpS16* marker due to limited nucleotide variation, and therefore uninformative regarding phylogenetic relationships (not shown here). The ITS dataset comprised 581 base pairs (bp). Table 3 summarizes the number of variable and informative sites, and the best-fit model estimated by jModeltest for each marker dataset.

Phylogenetic relationships. Relationships within *Abuta* recovered by ML and BI analyses of the ITS dataset (Fig. S1) do not differ markedly from those obtained by the plastid dataset (Fig. S2) as indicated by tree-based comparisons, except for the inconsistent placement of *Abuta solimoesensis* Krukoff & Barneby, whose affinities recovered by the ITS dataset differed from the one recovered by the plastid dataset. After this taxon was removed the trees recovered by ML and BI analyses of ITS and plastid datasets were rather congruent. This was confirmed by the ILD test, which had a value of $P = 0.25$. As such, these datasets were combined into a single dataset, which comprised 2015 bp. The results reported here are based on the ML tree resulting from the analysis of the combined data set.

Maximum likelihood and Bayesian Inference recovered similar trees (Fig. 1) *Abuta* is monophyletic with strong support (BS = 99%; PP = 1.0). The long branch of *Sarcopetalum* recovered in the study of Lian et al. (2019) is also observed in our study. As analyses removing the

taxon (not shown) had no influence on phylogenetic relationships among the ingroup, the taxon was kept in further analyses. To simplify representation, the long branch was collapsed in all figures included here. Within *Abuta*, two clades, clade I and clade II, are found. Clade I (BS = 59%; PP = 0.96), consists of *A. panamensis* as sister to a clade formed by a sister relationship between *A. rufescens* and *A. pahni* (Mart.) Krukoff & Barneby (BS = 70%; PP = 1.0) which is in turn sister to a strongly supported (BS = 100%; PP = 1.0) clade that includes *Abuta fluminum* Krukoff & Barneby and various unidentified *Abuta* taxa. These latter taxa are here labelled as *Abuta* sp. 1, *Abuta* sp. 2, *Abuta* sp. 6, and *Abuta* sp. 7, respectively. Clade II, (no BS support; PP = 0.96), comprises *A. mycetandra* Krukoff & Barneby as sister to a larger clade formed by accessions of *A. grandifolia* s.l. (Fig. 1), this latter clade is moderately to strongly supported (BS = 72%; PP = 1.0). Within the *A. grandifolia* s.l. clade, the *A. grandifolia*-Aggu morphological group is sister to the clade formed by *A. grandifolia*-Agll, *A. grandifolia*-Agltsl, and *A. grandifolia*-Agvar morphological groups (MGs) (BS = 70%; PP = 0.99). The sister relationship of *A. grandifolia*-Agltsl and *A. grandifolia*-Agvar MGs is moderately to strongly supported (BS = 70%; PP = 1.0). Of the four MGs, *A. grandifolia*-Aggu and *A. grandifolia*-Agll are independently strongly supported in all analyses (BS = 91%; PP = 1.0 and BS = 98%; PP = 1.0, respectively). Support for *Abuta grandifolia*-Aglts MG is moderate to strong (BS = 63%; PP = 0.99), although a similar moderate BS support (BS = 73%) is found for the *A. grandifolia*-Agvar MG, but it lacks PP support (Fig. 1).

Table 3. Primers used for amplification and sequencing in this study.

Region	Primer	Sequence (5'-3')	Protocol	Reference
ITS	ITS-5A	CCTTATCATTAGAGGAAGGAG	94 °C 3 min; 94 °C 40 s, 52 °C 1 min, 72 °C 1 min, 37 cycles; 72 °C 10 min	Stanford et al. (2000) White et al. (1990) Lian et al. (2019)
	ITS-4	TCCTCCGCTATTGATATGC		
trnH-psbA	psbAF	GTTATGCATGAACGTAATGCTC	94 °C 3 min; 94 °C 40 s, 57 °C 40 s, 72 °C 1 min, 30 cycles; 72 °C 10 min	Shaw et al. (2005) Lian et al. (2019)
	trnHf-05	CGCGCATGGTGGATTACAATCC		
trnL-F	F	CGAAATCGGTAGACGCTACG	94 °C 3 min; 94 °C 40 s, 50 °C 40 s, 72 °C 1 min, 30 cycles; 72 °C 10 min	Taberlet et al. (1991) Lian et al. (2019)
	R	ATTGAACTGGTGACACGAG		
rpS16	rpsF	GTGGTAGAAAGCAACGTGCGACTT	94 °C 3 min; 94 °C 40 s, 57 °C 40 s, 72 °C 1 min, 30 cycles; 72 °C 10 min	Oxelman et al. (1997) Lian et al. (2021)
	rpsR2	TCGGGATCGAACATCAATTGCAAC		

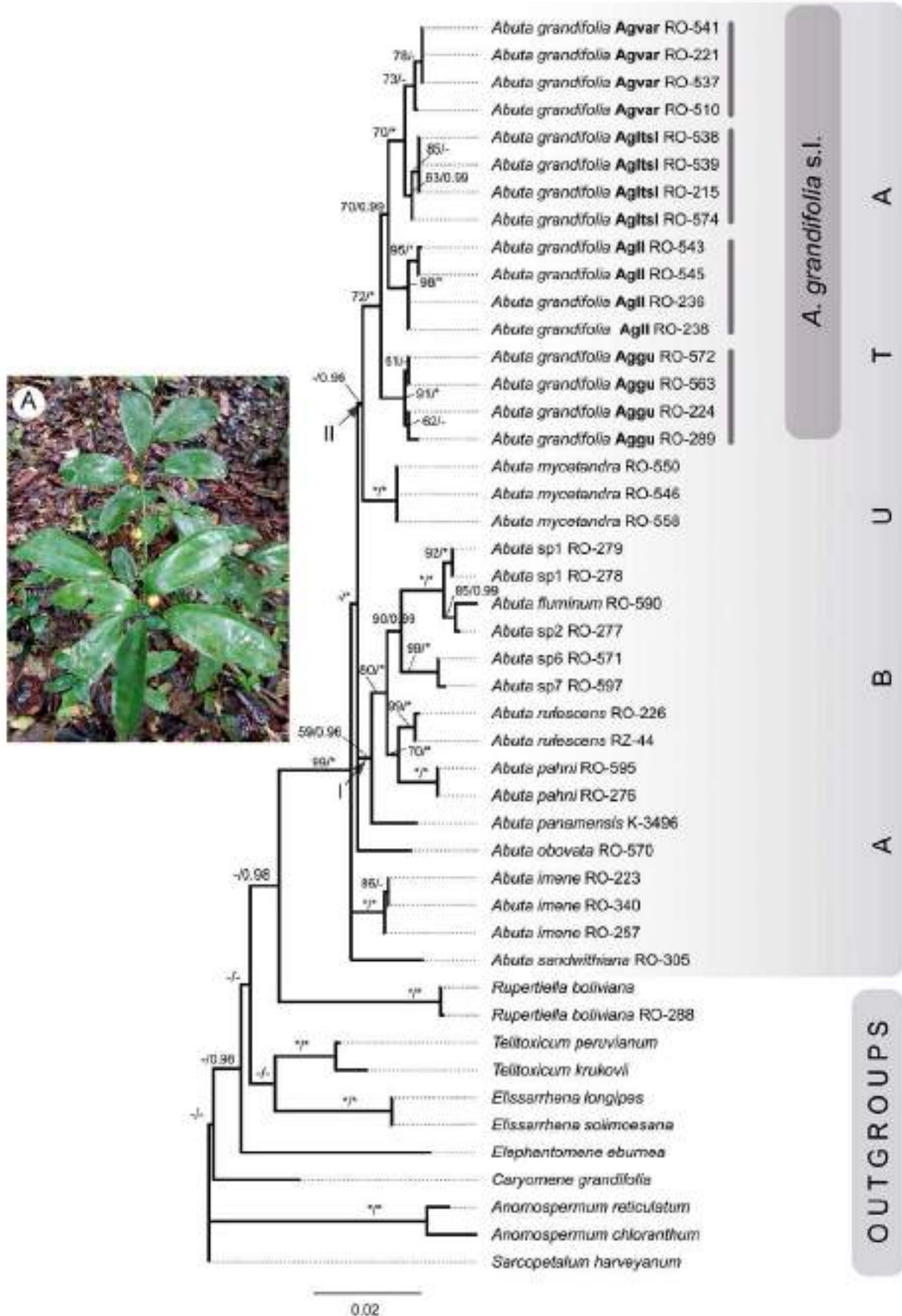


Figure 1. Maximum likelihood tree of the clade *Abuta* inferred from the combined ITS and plastid datasets. Numbers above branches correspond to bootstrap values (BS $\geq 50\%$) and posterior probabilities (PP ≥ 0.95). An asterisk (*) indicates BS = 100% or PP = 1.0). **A.** The picture to the left of the phylogram corresponds to *Abuta grandifolia*-Agvar, which is the most commonly encountered morphological group.

In this study, we used three molecular markers (*trnL-F*, *trnH-psbA*, and *ITS*) to investigate phylogenetic relationships among the *Abuta* species in Peru, although we also included *A. panamensis* from Central America, in order to evaluate the affinities of unidentified species of *Abuta* in Peru, that morphologically resemble the Central American taxon.

Our phylogenetic analyses with and expanded taxon sampling recovered a monophyletic *Abuta* in agreement with Lian et al. (2019) and Ortiz et al. (2016). Within *Abuta*, the two main clades recovered are moderately to strongly supported, albeit with unresolved relationships, similarly unresolved are *A. obovata* Diels, *A. sandwichiana* Krukoff & Barneby and the clade formed by the three accessions of *A. imene* (Mart.) Eichler (Fig. 1). In addition to *A. imene*, within both main clades, the conspecific accessions representing individual species do cluster together, including the various accessions representing individual morphological groups of the widespread *A. grandifolia* s.l. (Fig. 1).

Within clade I, our study supports the sister relationship of the Central American *A. panamensis* to the clade containing *A. rufescens* and *A. pahni*, also *A. fluminum* and four still unidentified species of *Abuta*. A close relationship of the Central American taxon to *A. rufescens* was also recovered in a previous study (Lian et al. 2019), although with a limited taxonomic sampling. Of the species in clade I, *A. rufescens* and *A. pahni* share a moderately to densely pubescent indument and understory leaves of similar shape, although easily to tell them apart when fertile, they are difficult to discriminate when in sterile condition. When fruiting, *A. rufescens* is distinguished by the mostly subglobose fruits that are covered by a dense light brown to cream indument on the surface, whereas *A. pahni* is characterized by the narrowly oblongoid fruits with a dense to relatively sparse rufescent indument (Fig. S3A and Fig. S3B, respectively; Ortiz et al. 2025). In Peru, both species are distributed from low elevations in the Amazon lowlands to mid-elevations in the eastern Andean foothills. The placement of *A. fluminum* is within a clade containing still unidentified *Abuta* taxa (Fig. 1), unfortunately, the specimens associated with those samples are either with immature fruits or in sterile condition.

The two accessions associated with *Abuta* sp. 1 (Fig. 1), superficially resemble *A. fluminum*. In both, *A. fluminum* (Fig. S4A) and *Abuta* sp. 2 (Fig. S4B) the primary veins are shortly plinerved and appear to have a pair of saccate domatia on the angles at the base, those taxa also share a similar minute, appressed, and scarce indument on the abaxial surface of the leaf blade (Fig. S4C, Fig. S4D). *Abuta* sp. 2, the sister taxon of *A. fluminum*, however, is morphologically distinct, the main veins are not plinerved, and no saccate domatia is present (Fig. S4E), additionally, the abaxial surface of the leaf blade is densely pubescent (Fig. S4F). The taxa in this clade (*Abuta* sp. 1 and *Abuta* sp. 2) occur up to about 2200 m a.s.l. whereas *A. fluminum* occurs up to 850 m a.s.l. The remaining two other sister taxa in this clade (*Abuta* sp. 6 and *Abuta*

sp. 7) are morphologically different from each other and from the other remaining taxa, especially in the type of indument on the abaxial surface of the leaf blade, which is long and sericeous, although still scarce (Fig. S4G, Fig. S4H, respectively). Their affinities might be with *A. solimoesensis* or with *A. velutina* Gleason; the latter taxon has not yet been included in molecular based studies. Further studies that include taxa that are still missing in phylogenetic studies are needed to be able to clarify these postulated affinities.

Although our results recover *A. grandifolia* s.l. as monophyletic, four distinct and strongly supported clades are also evident within this widespread taxon. These clades likely represent independent taxonomic units. However, several accessions associated with the Aggu morphological group are either sterile or bear immature fruits; consequently, it remains unclear whether they should be assigned to the Aggu group or instead represent a distinct taxon. Therefore, any formal recognition of these clades as independent taxonomic units remains contingent upon addressing existing research gaps related to the geographic variation and morphological delimitation within *Abuta grandifolia* s.l.

Based on an expanded taxon sampling, our results provide, for the first time, insights into phylogenetic relationships within *Abuta*. In this context, *Abuta mycetandra*, a poorly known taxon, emerges as sister to the clade comprising all accessions of *A. grandifolia* s.l. (Fig. 1).

Although our findings suggest a close relationship between *A. grandifolia* s.l. and *A. mycetandra*, a more comprehensive understanding of relationships within *Abuta* would benefit from the incorporation of genomic data. The potential utility of the Angiosperms353 probe set (Johnson et al. 2019; McDonnell et al. 2021) for resolving phylogenetic affinities within *A. grandifolia* s.l., as well as for assessing possible associations with local environmental conditions, is currently being explored. As *A. grandifolia* represents a Neotropical lineage with multiple reported ethnobotanical uses, including applications in traditional medicine across its geographic range, clarifying its phylogenetic relationships may facilitate the identification of other lineages with potential medicinal value. Notably, several species within the *Abuta* clade have been reported as having traditional medicinal uses (Krukoff & Moldenke 1938). Accordingly, broader interdisciplinary research integrating taxonomy and phytochemical variation would not only enhance our understanding of diversity within the group but also contribute to the recognition and valuation of these natural resources.

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RAZF: Methodology, Research, Data Curation, Writing- Original Draft Preparation.

WW: Writing: Review and Editing.

AMRD-C: Project Administration.

JCR-S: Writing: Review and Editing.

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Aspectos éticos / legales; Ethics / legal:

Authors declare that they did not violate or omit ethical or legal norms in this research.

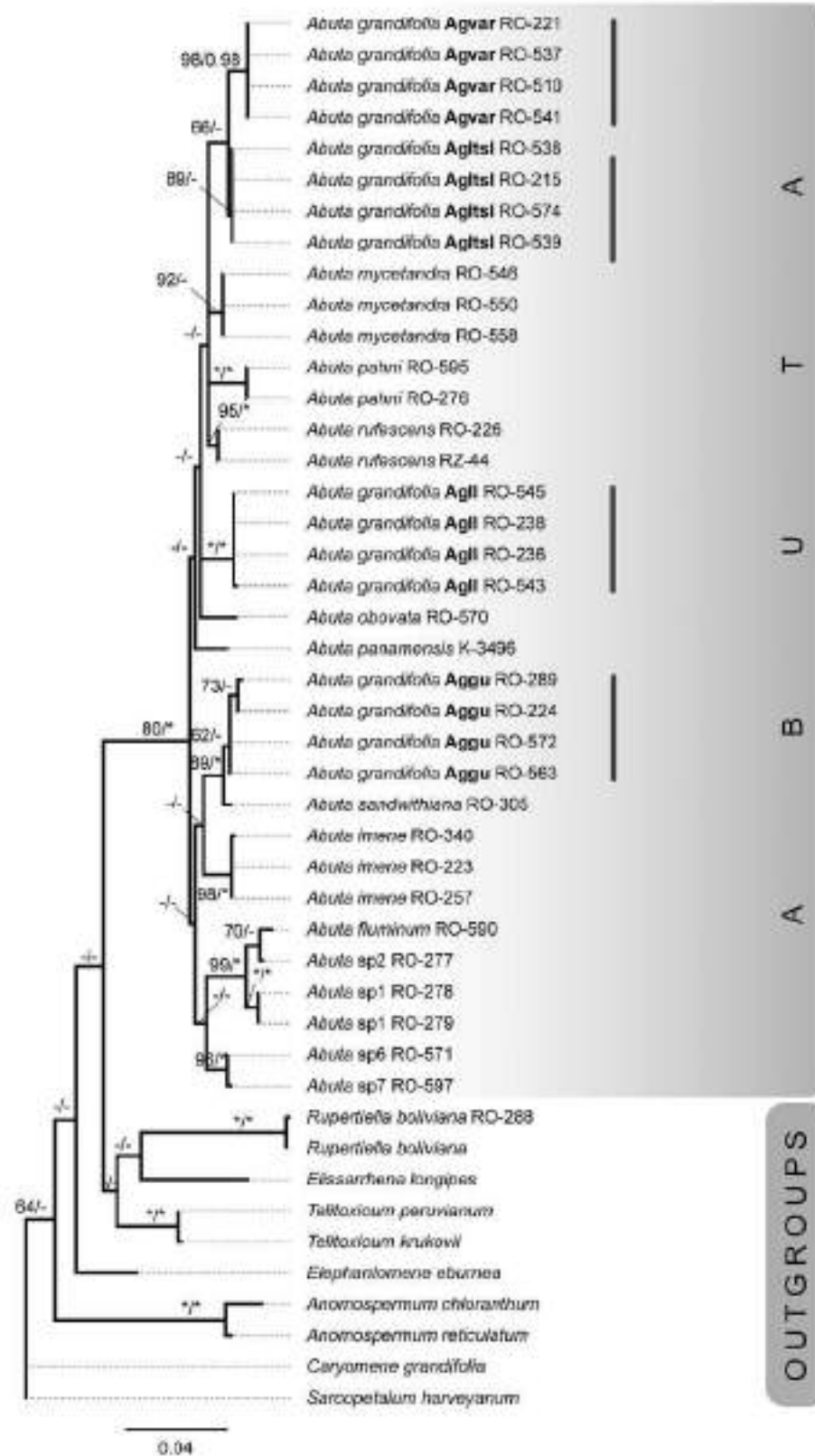


Figure S1. Maximum likelihood tree of the *Abuta* clade inferred from the ITS dataset. Numbers above branches correspond to bootstrap values (BS $\geq$ 50%) and posterior probabilities (PP $\geq$ 0.95). An asterisk (*) indicates BS = 100% or PP = 1.0).

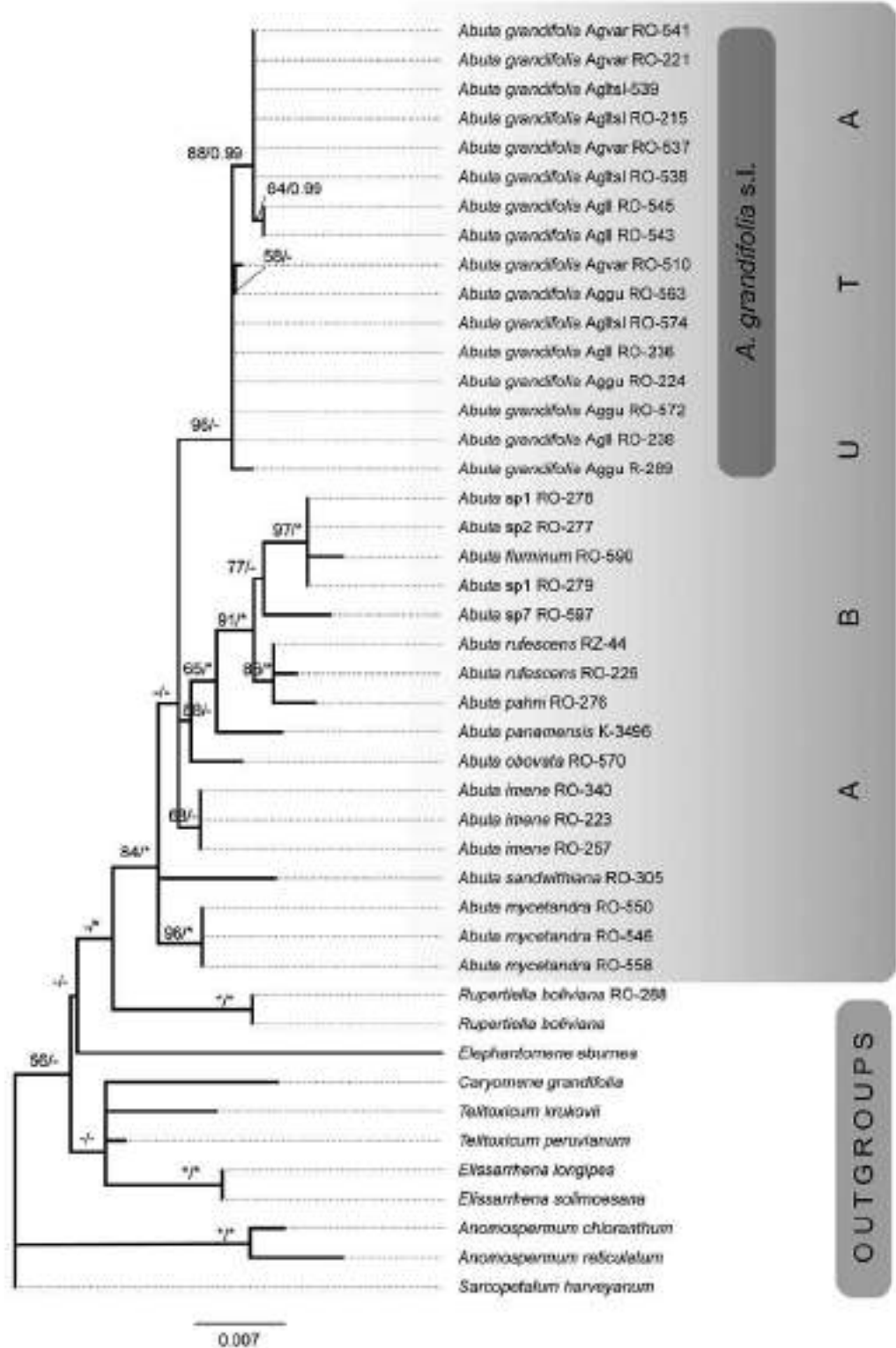


Figure S2. Maximum likelihood tree of the *Abuta* clade inferred from of the combined chloroplast dataset. Numbers above branches correspond to bootstrap values (BS $\geq$ 50%) and posterior probabilities (PP $\geq$ 0.95). An asterisk (*) indicates BS = 100% or PP = 1.0).

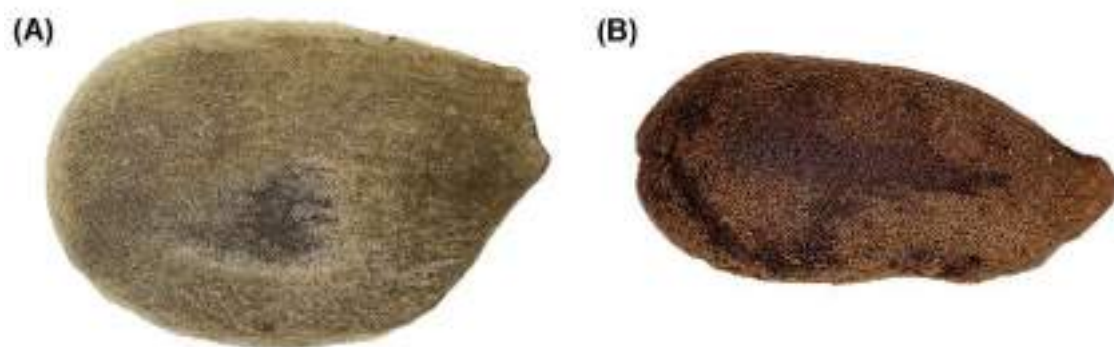


Figure S3. Fruit shapes of *Abuta* Aubl. **A.** Subglobose fruits of *Abuta rufescens* Aubl. (based on Ortiz *et al.* 19). **B.** Narrowly oblongoid fruit of *A. pahni* Mart. (based on Schunke 11771). Images not scaled, photographs taken by R. Zañartu. Both specimens at MO.

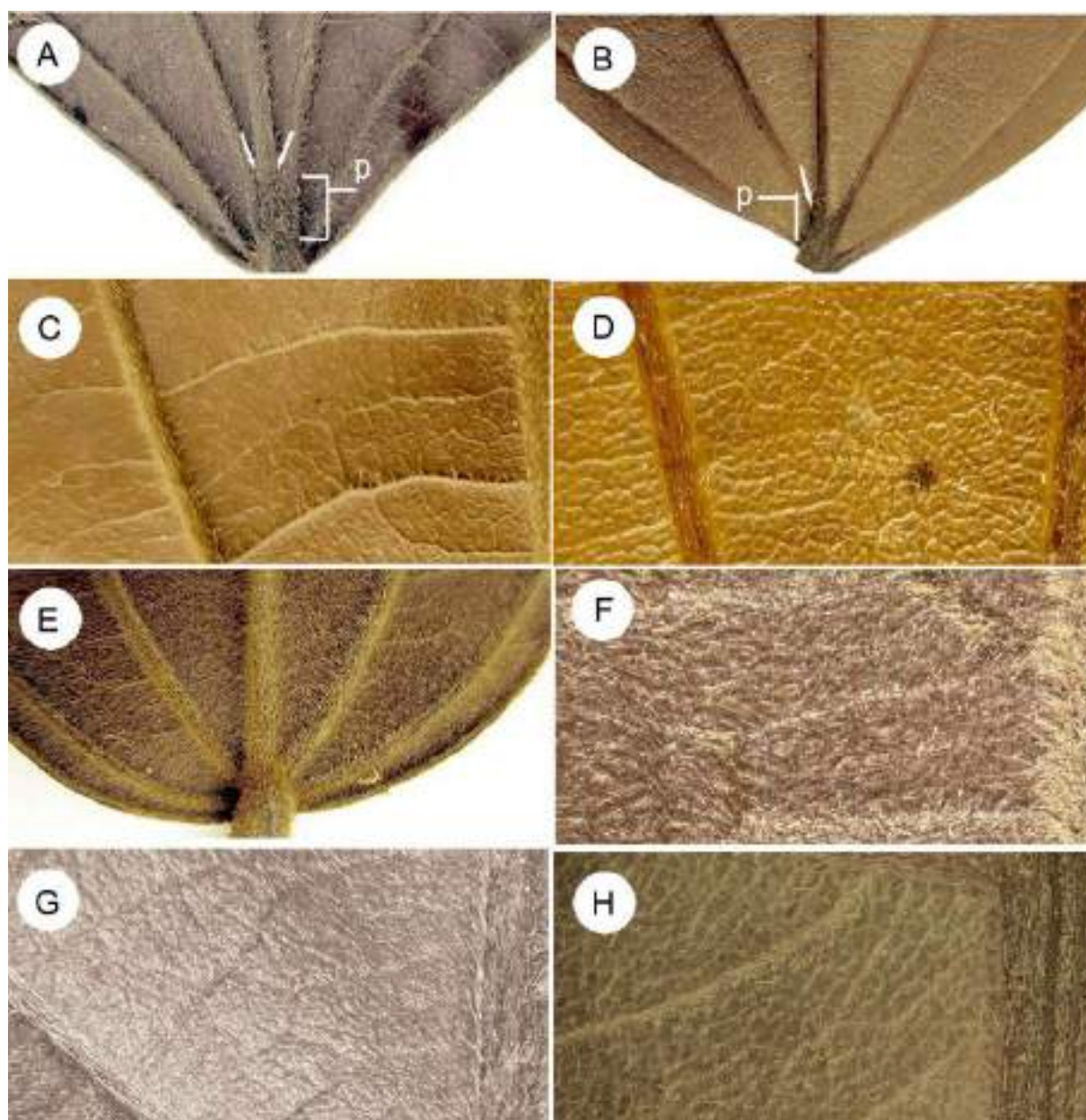


Figure S4. Abaxial leaf surface in selected *Abuta* taxa. **A-B.** Shortly plinerved primary veins, arrows indicate location of saccate domatia, p = plinerved. **A.** *Abuta fluminum* Krukoff & Barneby. **B.** *Abuta* sp 1. **C.** Primary veins conspicuously palmate of *Abuta* sp 2, no domatia present. **D-H.** Indument on abaxial surface of *A. fluminum*, *Abuta* sp 1, *Abuta* sp 2, *Abuta* sp 6 and *Abuta* sp 7. Images not scaled, photographs taken by R. Ortiz. **A, C.** Based on Ortiz *et al.* 590. **B, D.** Based on Ortiz *et al.* 278. **E-F.** Based on Ortiz *et al.* 277. **G.** Based on Ortiz *et al.* 571. **H.** Based on Ortiz *et al.* 597. All vouchers at MO.