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Short Communication: Leaf architectural analysis of taxonomically confusing coffee species: *Coffea liberica* and *Coffea liberica* var. *dewevrei*

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Abstract. *Baltazar AMP, Buot JrIE. 2019. Short Communication: Leaf architectural analysis of taxonomically confusing coffee species:* Coffea liberica and Coffea liberica *var*. dewevrei. *Biodiversitas 20: 1560-1567***.** Coffee is considered as one of the most important crops. The Philippines is known to produce four coffee varieties namely: Arabica (*Coffea arabica*), Robusta (*Coffea canephora*), Excelsa (*Coffea liberica* var. *dewevrei*) and Liberica (*Coffea liberica*). Further, the taxonomy of *C. liberica* and *C. liberica* var. *dewevrei* is still unclear. Since its earliest publication, the relationship of the two taxa have been confusing many taxonomists and also farmers. The status of the two confusing taxa are still under discussions due to contradicting evidence inferred from morphological and molecular data. The two taxa have been claimed as separated species but the markers are considered inconsistent in the field. This study has tried to examine the leaf architectural characters of both species as these have been known to be genetically fixed. Leaf samples of each species including *C. canephora* (outgroup) were collected and examined. Results showed that the two taxa were found to be different in laminar size, domatia distribution, major secondary attachment, and intercostal tertiary veins. Cluster analysis revealed that they are grouped separately. However, the rank of *C. liberica* var. *dewevrei* cannot be decided yet until further taxonomic study, particularly on the anatomy and distribution pattern of domatia, is completed.

Keywords: *Coffea*, Excelsa, leaf architecture, leaf venation, Liberica

INTRODUCTION

The taxonomic work on genus *Coffea* L. has been actively progressing since the late 1980's resulting in a total of 103 species and seven infraspecific taxa to date (Davis et al. 2006). The placement of species under this genus was defined by morphological, biochemical, molecular analyses, etc. (Orozco-Castillo et al. 1996; Roos et al. 1997; Chinnappa and Warner 2008; Davis and Rakotonasolo 2008). However, with these evidence, some infraspecific taxa are still facing confusion up to this day. For example, is the case of *Coffea liberica* W. Bull ex Hiern and *Coffea liberica* var. *dewevrei* (De Wild. & T.Durand) Lebrun which has been designated as two separate species.

Coffea liberica, the currently accepted name, was validly published by William Philip Hiern in 1876 and has six synonyms (IPNI 2005; The Plant List 2013). This species is classified under family Rubiaceae and has one infraspecific taxon in the name of *C. liberica* var. *dewevrei*. This infraspecific taxon was first described and published by Émile Auguste Joseph De Wildeman and Théophile Alexis Durand as *C. dewevrei* in 1899. It was later published and accepted as *C. liberica* var. *dewevrei* by Jean-Paul Antoine Lebrun in 1941 along with *C. liberica* var. *liberica* which is now a synonym of *C. liberica*.

Since its publication in 1941, there are several confusions regarding the identity of the two taxa. In the study of Bridson (1988) and unpublished work of Noirot and Maurin, morphological and molecular evidence were found to support the two as separate varieties (Davis et al. 2006). However, N'Diaye et al. (2005) stated that they are more distinguished than botanical varieties as supported by their differences in morphological traits, molecular markers, and hybrid fertility. The genes involved in this separation are not yet elucidated and in the study by Cao et al. (2014), *rbc*L and *mat*K genes were found to provide clustered data and cannot be used to delineate the two taxa. However, they have found out that leaf morphometric characters were effective in defining the two taxa.

Coffea liberica and *C. liberica* var. *dewevrei* are known in the Philippines as "Barako" and "Excelsa" coffees respectively (Philippine Coffee Board 2018). After the coffee rust infection of coffee farms in Batangas, Philippines in 1889, unidentified seedlings were transferred to Cavite. This initiated the local coffee farmers to devise their own way of identifying the coffee varieties through observance of fruit density and leaf shapes. However, farmers are still confused with Excelsa and Liberica as both possess inconsistent morphological markers and fruit density is highly affected by environment. Also, fruits and flowers are not always available in the field. With this

problem left unresolved, the taxonomic confusion will remain as a threat to the quality of pure coffee beans and coffee industry.

In the absence of reproductive structures, leaf characters can be used in resolving taxonomic confusion. Each plant species has their own leaf fingerprint that may provide morphological, anatomical and biochemical evidence to support the identity of the species. One of this evidence is found in leaf venation pattern which is considered as a taxonomic tool because its primary characteristics are genetically fixed (Roth-Nebelsick et al. 2001).

In this study, leaf architectural analysis was used to differentiate *C. liberica* and *C. liberica* var. *dewevrei*. It was also aimed to provide an alternative way of determining the identity of the unclear coffee variety through difference in leaf architectural characters and hopefully address the confusion between the two taxa.

MATERIALS AND METHODS

Sampling and preparation of plant materials

The plant materials were obtained from the living coffee accessions of Cavite State University-National Coffee Research, Development & Extension Center (CvSU-NCRDEC) in Cavite, Southern Luzon, Philippines. Only one tree for *C. liberica* was sampled because it was the only Liberica tree validated and approved by the National Seed Industry Council (NSIC) in the Philippines. The tree was 72 months old as of the date of registration and was tagged as NSIC 2007 Cf-L 01. However, for the case of *C. liberica* var. *dewevrei*, none has been registered yet at NSIC so instead, leaf samples were gathered from the identified tree by researchers in National Coffee Research Development and Extension Center (NCRDEC). To ensure that the leaf samples are not from unverified coffee species, sampling was only limited to the aforesaid identified trees (Figure 1).

A total of 20 leaf samples from each tree were obtained. To represent the outgroup, leaf samples from *C. canephora* were also acquired. The first fully expanded leaves from the terminal part of the branch were chosen. The leaves were soaked in 5% NaOH and then boiled until the mesophyll tissues of the leaves were soft (Mishra et al. 2010). The soft leaf tissues were carefully brushed to reveal the veins underneath. The leaf skeletons were soaked in hypochlorite to eliminate the pigments. Finally, the leaf skeletons were pressed until completely dried (Figure 2). However, *C. canephora* leaves were not skeletonized because its mesophyll tissues were covered with a very thick cuticle. Leaf skeletons were deposited in Plant Systematics Laboratory Herbarium, Institute of Biological Sciences, University of the Philippines Los Baños, College, Laguna with accession numbers 6878 (*C. liberica*), 6879 (*C. liberica* var. *dewevrei*) and 6880 (*C. canephora*).

Leaf measurement and characterization of leaf venation patterns

The leaf width and length were measured using a ruler and a protractor was used for measuring angles. The leaves were observed under a dissecting microscope (Euromex Edu Blue Series) for the analysis of smaller vein patterns. The leaf characters were divided into two categories: laminal and venation characters. The laminal characters included leaf attachment, leaf arrangement, leaf organization, laminar width, laminar length, laminar area, laminar size, laminar shape, medial symmetry, margin type, special margin features, apex angle, apex shape, base angle, base shape, and domatia distribution. On the other hand, venation characters included primary vein framework, major secondary vein framework, major secondary spacing, major secondary angle, major secondary attachment, intercostal tertiary vein fabric, epimedial tertiaries, exterior tertiary course, quarternary vein fabric, quarternary vein fabric, areolation and freely ending veinlets. The terminologies used to describe and classify leaf characters were based on the Manual of Leaf Architecture by Ellis et al. (2009).

Statistical analysis

To investigate the venation patterns and dissimilarities of *C. liberica*, *C. liberica* var. *dewevrei*, and *C. canephora* (outgroup), a total of 28 leaf characters were observed for each of the 20 leaf samples per individual species. This examination resulted in a total of 1,680 data sets. Since the terminologies from the Manual of Leaf Architecture were qualitative, each leaf character was designated with a numerical value. These values were entered in statistical software to do cluster and ordination analyses. Cluster analysis was done for grouping the species into clusters of similar leaf architectural traits and to illustrate their relationships. Two algorithms were used in this analysis namely, unweighted paired group average (UPGMA) and single linkage (nearest neighbor) both computed with Euclidean similarity index. All computations had bootstrap value of 1000. In addition, ordination analysis using Principal Coordinates Analysis (PCoA) was also done to demonstrate the individual differences of the *Coffea* species. PAST (Paleontological Statistical Software) software by Hammer et al. 2001 was used for the statistical analysis.

RESULTS AND DISCUSSION

The leaves of *C. liberica* and *C. liberica* var. *dewevrei* shared a lot of similar laminal features (Table 1) which further contributes to their taxonomic confusion. *Coffea* spp. are known for their shrub or small tree habit, with simple, petiolate and opposite leaves which were observed in all leaf samples including the outgroup. All leaf samples of *C. liberica* and *C. liberica* var. *dewevrei* were elliptic while *C. canephora* samples had elliptic-obovate shape. Some leaf samples of *C. liberica* and *C. liberica* var. *dewevrei* were observed to exhibit asymmetrical leaves which were said to be influenced by correct auxin transport under a specific environment condition (Scarpella et al. 2010). *Coffea liberica* and *C. liberica* var. *dewevrei* both had entire and undulate margin, acute and convex apex and, acute and straight base. *Coffea canephora* leaves also exhibited the same features except for its acuminate apex.

However, the laminar size of *C. liberica* differ from *C. liberica* var. *dewevrei* and *C. canephora* because of few larger leaf samples that included under microphyll category (18,225-164, 025 mm^2). Another dissimilar laminal feature between the two *Coffea* leaves was the distribution of domatia which harbor small insects in its hollow structure. The domatia of *C. liberica* was consistently found to be distributed along the midrib and the secondary veins while that of *C. liberica* var. *dewevrei* was along the midrib only (Figure 3). Further, domatia in four different *Coffea* species were reported to have varying trichome structures and this can have taxonomic implications (Balinado 2018, pers. com.). However, the formation and distribution of domatia in *Coffea* species could not be considered taxonomic character. As of now, more studies on domatia in other localities and its association with mites or other insects would be urgent. The production of domatia can be influenced by several factors including the presence of beneficial mites (O'Connell et al. 2015).

On the other hand, the venation patterns of *C. liberica*, *C. liberica* var. *dewevrei* leaves also exhibited some similarities. Both have pinnate primary vein; simple brochidodromous, regularly spaced and uniformly angled secondary veins; alternate percurrent epimedial tertiary veins; looped exterior tertiary veins; irregularly reticulate quarternary veins; freely ramifying quarternary veins; moderately developed areolation and dendritic freely ending veinlets (Figure 4 and Table 2). This number of similar features even in higher vein orders makes the two leaves indistinguishable from each other. Conversely, *C. canephora* displayed many dissimilar venation patterns from the confusing species. It had opposite percurrent intercostal and epimedial tertiaries, irregularly reticulate quarternary veins and good areole development.

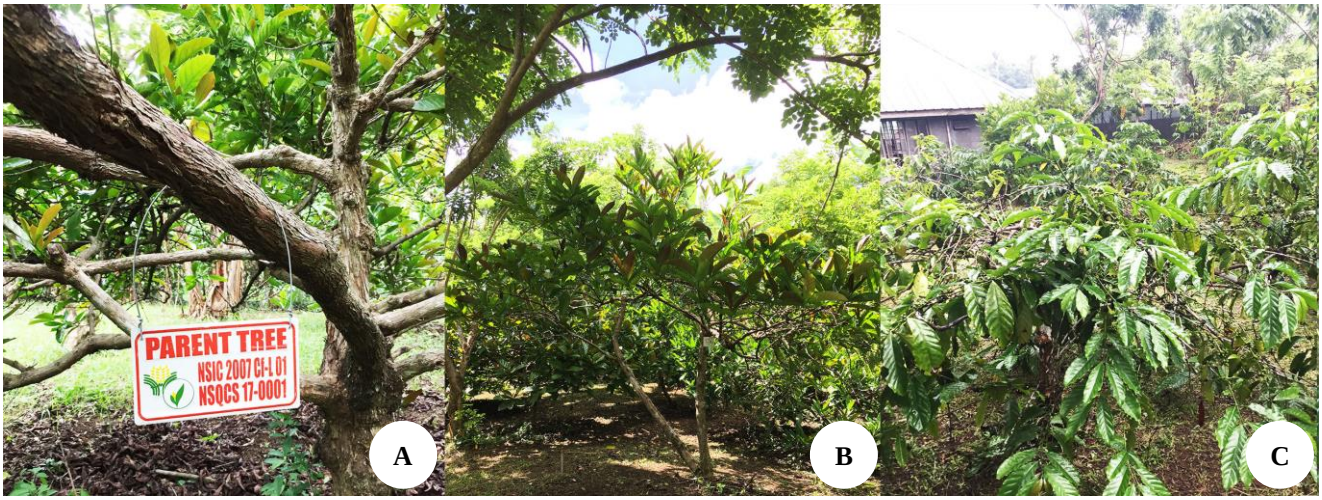

Figure 1. Representative trees of A. *C. liberica*, B. *C. liberica* var. *dewevrei*, C. *C. canephora*. (Photograph: A.M.P. Baltazar)

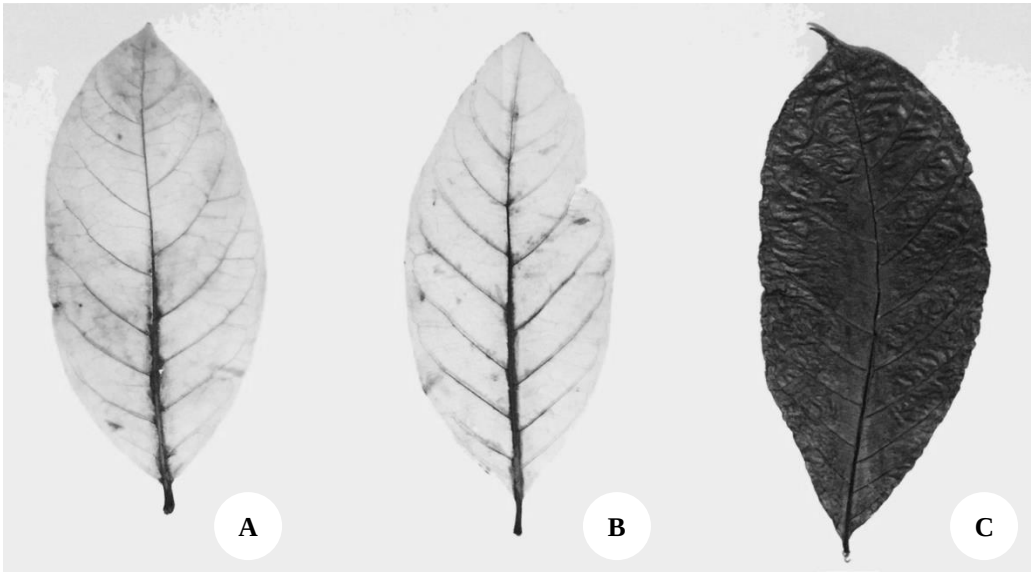

Figure 2. Leaf samples of A. *C. liberica*, B. *C. liberica* var. *dewevrei* and C. *C. canephora* (Photograph: A.M.P. Baltazar)

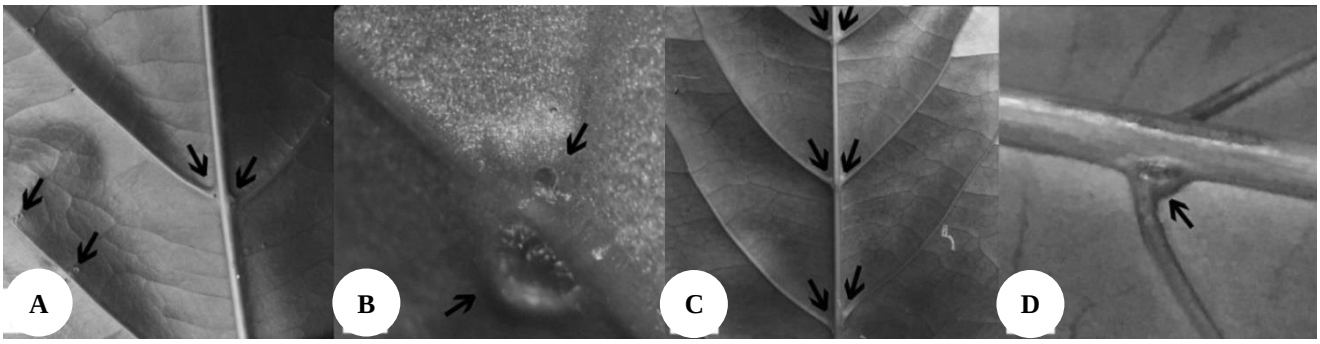

Figure 3. Domatia distribution on the blade of A-B. *C. liberica* and C-D) *C. liberica* var. *dewevrei*. (Photograph: A.M.P. Baltazar)

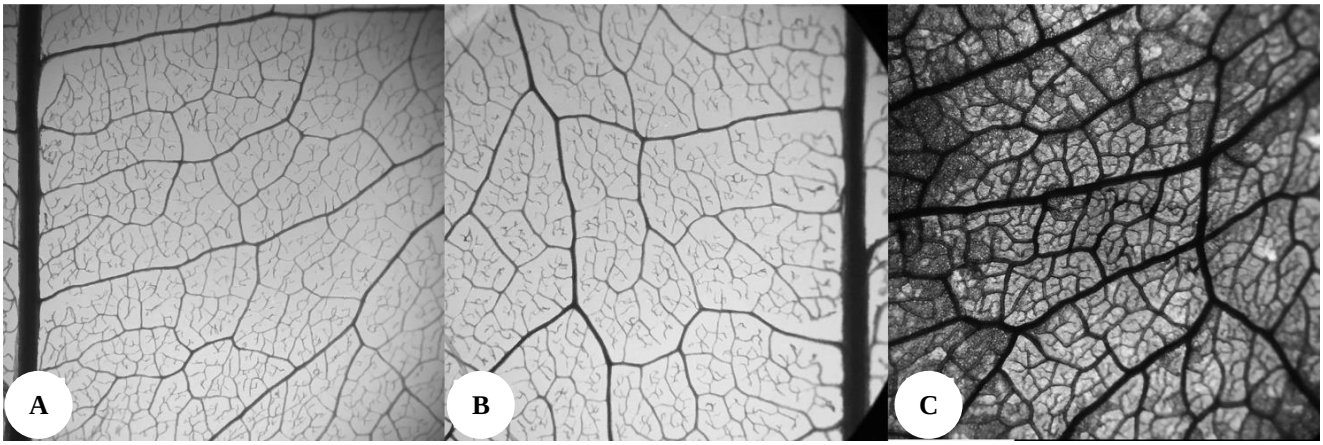

Figure 4. Higher vein orders of A. *C. liberica*, B. *C. liberica* var. *dewevrei*, and C. *C. canephora*. (Photograph: A.M.P. Baltazar)

Table 1. General laminal characters of *C. liberica* and *C. liberica* var. *dewevrei*

Laminal characters	***C. liberica***	***C. liberica* var. *dewevrei***	***C. canephora***
Leaf attachment	Petiolate	Petiolate	Petiolate
Leaf arrangement	Opposite	Opposite	Opposite
Leaf organization	Simple	Simple	Simple
Laminar width	71.5-120 mm	63.5-92.5 mm	60.5-97 mm
Laminar length	164-267 mm	168-212 mm	160-239 mm
Laminar area	8794.5-24,030 mm^2	8001-14,707.5 mm^2	7260-17837.25 mm^2
Laminar size	Mesophyll-microphyll	Mesophyll	Mesophyll
Laminar shape	Elliptic	Elliptic	Elliptic-Obovate
Medial symmetry	Symmetrical-asymmetrical	Symmetrical-asymmetrical	Symmetrical
Margin type	Entire	Entire	Entire
Special margin features	Undulate	Undulate	Undulate
Apex angle	Acute	Acute	Acute
Apex shape	Convex	Convex	Acuminate
Base angle	Acute	Acute	Acute
Base shape	Straight	Straight	Straight
Domatia distribution	Along midrib and secondary veins	Along midrib	Along midrib

Table 2. General leaf venation patterns of *C. liberica* and *C. liberica* var. *dewevrei*

Venation characters	***C. liberica***	***C. liberica* var. *dewevrei***	***C. canephora***
Primary vein framework	Pinnate	Pinnate	Pinnate
Major secondary vein framework	Simple brochidodromous	Simple brochidodromous	Simple brochidodromous
Major secondary spacing	Regular	Regular	Regular
Major secondary angle	Uniform	Uniform	Uniform
Major secondary attachment	Decurrent-proximal secondaries decurrent	Decurrent	Proximal secondaries decurrent
Intercostal tertiary vein fabric	Irregular reticulate	Mixed percurrent	Opposite percurrent
Epimedial tertiaries	Alternate percurrent	Alternate percurrent	Opposite percurrent
Exterior tertiary course	Looped	Looped	Looped
Quarternary vein fabric	Irregular reticulate	Irregular reticulate	Irregular reticulate
Quarternary vein fabric	Freely ramifying	Freely ramifying	Irregular reticulate
Areolation	Moderate development	Moderate development	Good development
FEVs	Dendritic	Dendritic	Dendritic

Table 3. Opposing leaf architectural characters of *C. liberica* and *C. liberica* var. *dewevrei*

Leaf characters	***C. liberica***	***C. liberica* var. *dewevrei***	***C. canephora***
Laminar size	Mesophyll-macrophyll	Mesophyll	Mesophyll
Domatia distribution	Along midrib and secondary veins	Along midrib	Along midrib
Major secondary attachment	Decurrent-proximal secondaries decurrent	Decurrent	Proximal secondaries decurrent
Intercostal tertiary vein fabric	Irregular reticulate	Mixed percurrent	Opposite

However, two venation patterns were found to be different between *C. liberica* and *C. liberica* var. *dewevrei* upon close examination. The major secondary veins of some *C. liberica* leaf samples were observed to be decurrently exhibited by asymptotically attached secondaries to the primary vein. Some leaf samples also had proximally decurrent secondaries which were characterized by both having decurrent proximal secondaries and excurrent distal secondaries. On the other hand, the secondary veins of *C. liberica* var. *dewevrei* were decurrent all throughout. Another difference was the irregularly reticulate arrangement of *C. liberica* tertiary veins exhibited by the formation of irregular polygons shaped by its variously angled tertiary veins. Conversely, *C. liberica* var. *dewevrei* has mixed percurrent tertiary veins demonstrated by the presence of both branched and unbranched tertiary veins crossing two adjacent secondaries. The leaf architectural characters that separated *C. liberica* and *C. liberica* var. *dewevrei* were summarized in Table 3.

A generated dendrogram from UPGMA algorithm illustrated that *C. liberica*, *C. liberica* var. *dewevrei* and *C. canephora* (outgroup) were distinct from each other even with slightly dissimilar leaf characters included in the analysis (Figure 5). *C. liberica* and *C. liberica* var. *dewevrei* were separated from *C. canephora* (outgroup) at distance 2.9 indicating their strong dissimilarities in terms of laminar shape, medial symmetry, apex shape, intercostal and epimedial tertiaries, quarternary veins and areoles. At distance 1.75, *C. liberica* and *C. liberica* var. *dewevrei* were split into two separate groups, also suggesting dissimilarity between the two taxa in terms of laminar size, domatia distribution, major secondary vein attachment, and intercostal tertiary veins. All 20 leaf samples of *C. liberica* clustered together although the dendrogram showed several branching within the group. On the other hand, *C. liberica* var. *dewevrei* leaf samples showed higher degree of similarity among leaf samples exhibited by less branched clades. Moreover, single linkage clustering approach revealed the same trend as UPGMA dendrogram with minor difference in distance (Figure 6). Meanwhile, Figure 7 showed that *C. liberica*, *C. liberica* var. *dewevrei* and *C. canephora* landed on different axes suggesting that they are dissimilar from each other.

However, both analyses on the dissimilarity between *C. liberica* and *C. liberica* var. *dewevrei* in terms of leaf architecture are not enough to decide on the rank that should be employed on the latter. Imprecision in the use of infraspecific ranks such as subspecies and variety has been observed for the past periods but generally, apart from morphological evidence, evolutionary and ecogeographical confirmations should be taken into the picture to understand the true relationship of the two taxa (Hamilton and Reichard 1992). It is suggested that molecular markers

for leaf architecture especially on leaf venation of *C. liberica* and *C. liberica* var. *dewevrei* must be studied to reveal the alignment of morphological and molecular evidence. Furthermore, the consistency of domatia distribution on the leaves of *C. liberica* and *C. liberica* var. *dewevrei* must be studied in larger number of samples under different environmental conditions. If the stability of this character is confirmed, it can be used practically by farmers to rapidly identify the species.

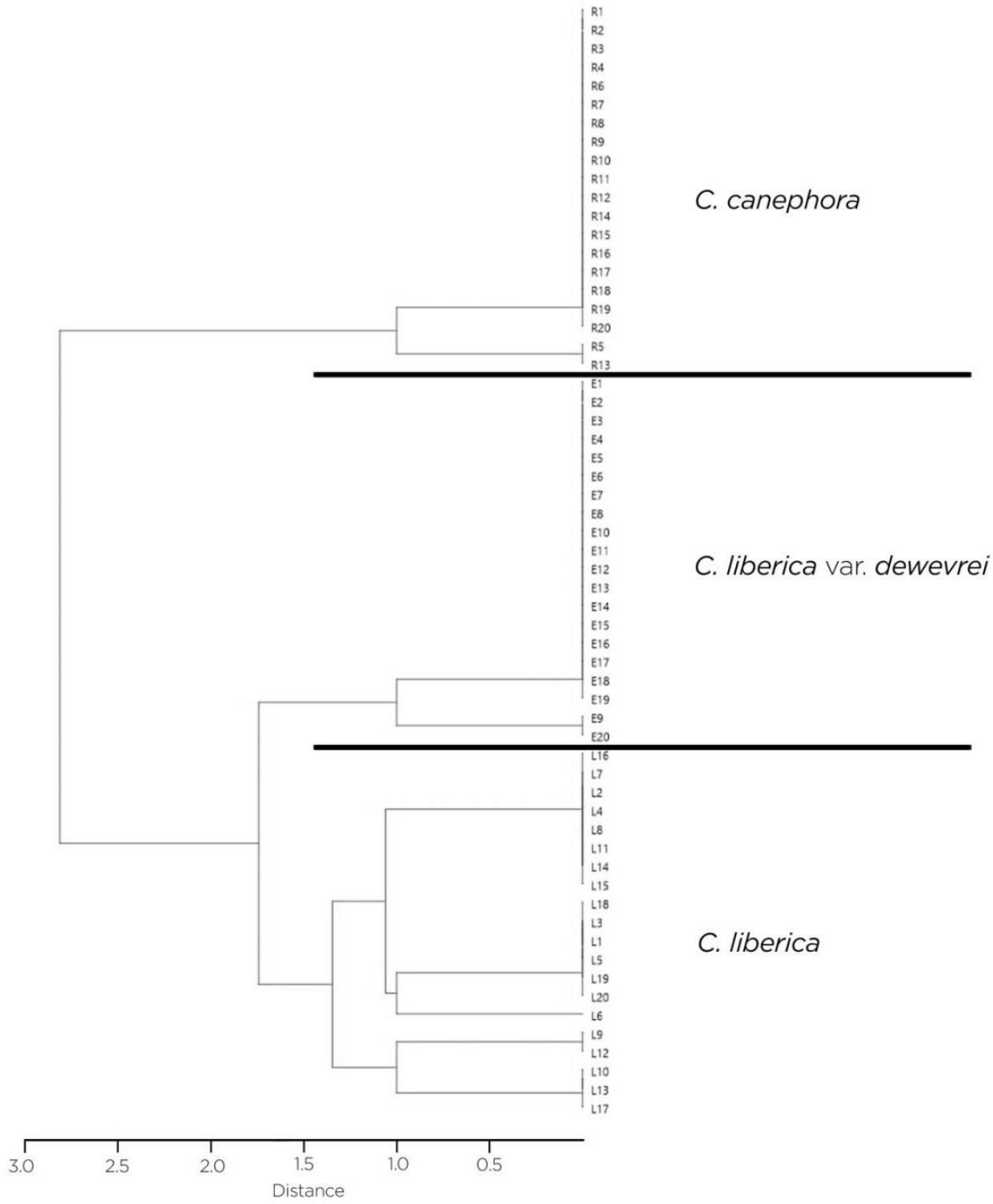

Figure 5. Dendrogram using UPGMA clustering approach using Eucledian as the distance measure. Bootstrap value: 1000

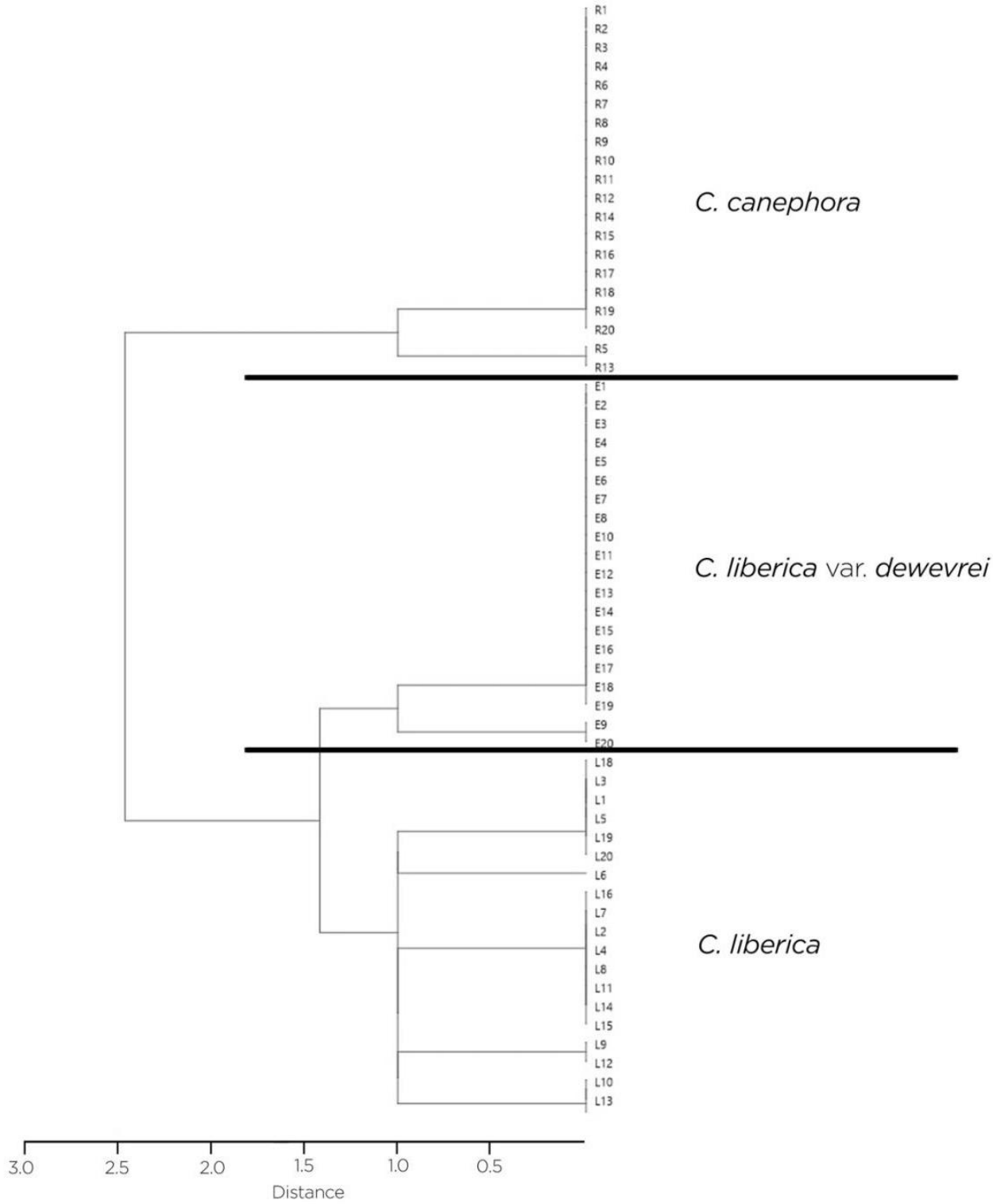

Figure 6. Dendrogram using single linkage clustering approach using Eucledian as the distance measure. Bootstrap value: 1000

In conclusion, the two confusing taxa, of *C. liberica* and *C. liberica* var. *dewevrei* subjected to leaf architectural analysis were found to be different in terms of laminar size, domatia distribution, secondary vein attachment, and intercostal tertiary vein category. Cluster and ordination analyses revealed the dissimilarity of the two taxa. While results showed the difference between *C. liberica* and *C. liberica* var. *dewevrei*, further studies should be taken into consideration to confirm the rank of *C. liberica* var. *dewevrei*. It is also recommended to confirm the consistency and stability of these leaf architectural traits under different environmental conditions. This is the first time that leaf architectural evidence has been used to assess the difference between *C. liberica* and *C. liberica* var. *dewevrei* and their taxonomic significance.

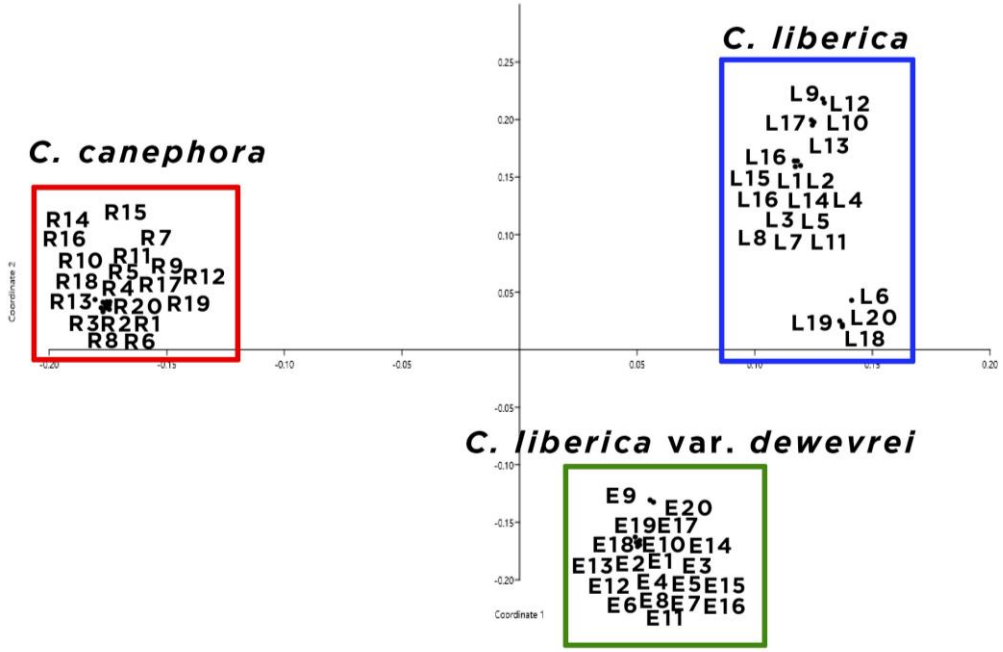

Figure 7. Principal Component Analysis (PCoA) on *C. liberica, C. liberica* var. *dewevrei* and *C. canephora* (outgroup)

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