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Short Communication: Leaf architectural analysis of confusing *Syzygium* species: *Syzygium aqueum* (Burm.f.) Alston and *Syzygium samarangense* (Blume) Merr. & L.M.Perry (Myrtaceae)

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Abstract. *Viacrucis III JDL, Buot Jr. IE. 2021. Short Communication: Leaf architectural analysis of confusing* Syzygium *species:* Syzygium aqueum *(Burm.f.) Alston and* Syzygium samarangense *(Blume) Merr. & L.M.Perry (Myrtaceae). Biodiversitas 22: 3341-3348.* Among species of Myrtaceae family which has economic and ecological importance are the two *Syzygium* species, *Syzygium aqueum* and *Syzygium samarangense*. Both species are cultivated throughout the tropics because of their edible fruits and medicinal properties of various plant parts. However, despite their wide utilization, the two species are often confusing due to their morphological similarity. In this study, leaf architecture of *S. aqueum* and *S. samarangense* have been evaluated to delineate the two confusing taxa. Selected laminal and venation characters were compared. Results showed many similar characters shared between the two species, however, laminar size, apex and base shape, areolation, and freely ending veinlets (FEVs) were found to be distinct. Multivariate analyses, cluster analysis employing Gower similarity index, using unweighted pair-group average (UPGMA) and single linkage (nearest neighbor) algorithm, and ordination using Principal Coordinates Analysis (PCoA), illustrated that they are grouped separately. The variations in laminal and venation characters are additional set of information that will allow accurate differentiation of the two species. However, it is recommended that additional taxonomical analysis using various methods, such as molecular techniques, be employed and characters, such as floral morphology, be studied to contribute in the resolution of the taxonomic complexity of this group and other taxonomic controversies

Keywords: Leaf architecture, *Syzygium*, venation, water apple

INTRODUCTION

The family Myrtaceae has attained relevance worldwide because it comprises several genera of great economic and ecological importance. It is found commonly in many biodiversity hotspots such as South America, Australia, and tropical Asia, with observed occurrences in Europe and Africa (Grattapaglia et al. 2012). However, despite its relevance and diverse representation in wide range of biomes, Myrtaceae is still considered as a taxonomically complex family (McVaugh 1968; Oliveira et al. 2017). Speciation brought about by hybridization and polyploidy, and lack of anatomical and morphological studies were attributed to the difficulty of identification and delimitation of species (Oliveira et al. 2017)

Among generic groups in Myrtaceae, the genus *Syzygium* is the largest with an estimated species count in the world of up to 1,200 (Parnell et al. 2007; Govaerts et al. 2008). Its taxonomy has been long disputed with the genus *Eugenia*. Although morphological, anatomical, and molecular evidences have brought consensus among taxonomists to delineate the genera from one another, conceptual problem within generic and species delimitation within *Syzygium* still remains a problem (Soh 2017).

Several *Syzygium* species are considered as important food resource for insects, birds, and small and large mammals (Parnell et al. 2007). Among them are *S. aqeuem* (Burm. f.) Alstom, and *S. samarengense* (Blume) Merr. & L.M. Perry. *Syzygium aqueum,* known as water apple and watery rose apple, is an economic plant that grows and is cultivated throughout the tropics. In the Philippines, it is locally known as tambis (Panggabean 1992). Traditionally, various parts of the plant have been used as medicine, in particular, as an antibiotic (Manaharan et al. 2011). The powdered dried leaves are used as treatments for cracked tongue and the roots have been used to treat itching and swelling (Osman et al. 2009). Moreover, the leaf extracts were reported to have cosmeceutical properties, such as antioxidant, lipolytic, antityrosinase, and anticellulite activies (Palanisamy et al. 2011). On the other hand, *S. samarangense* is another species of *Syzygium* which bear edible fruits. It is commonly known as wax apple and is the most popular in Southeast Asia because of its economic relevance (Moneruzzaman et al. 2012). In the Philippines, it is most widely known as macopa or makopa (Morton 1987). However, due to their similarity in appearance, *S. aqueum* and *S. samarangense* are often confused with one another (Janick and Paull 2008).

Taxonomic references, such as on morphological characterization of leaves, especially for widely used economic plants as the aforementioned *Syzygium* species, are important for authentication and ultimate identification. These references allow verification and accurate identification of plant species, eliminating confusion and improper use of a particular species, particularly as medicine or cosmetics, which could have adverse effects to health, ecology, and sustainability of plant resource (Oliveira et al. 2017). The aim of this paper is to differentiate the two *Syzygium* species, *S. aqueum* and *S. samarangense*, using leaf characters to contribute to the knowledge of *Syzygium* taxonomy and aid in the resolution of morphological confusion.

In this study, leaf architectural features were investigated to differentiate representative species, *S. aqueum* and *S. samarangense*. It was aimed to provide an alternative in differentiating the two confusing taxa and hopefully, contribute to the differentiation of problematic taxonomic groups in the genus *Syzygium.*

MATERIALS AND METHODS

Sample collection and preparation

Twenty-five (25) mature, fully expanded leaves of *S. aquem* and *S. Samarangense* were collected. To represent the outgroup, *Psidium guajava* leaf samples were also procured. Identification of samples was assisted by locals in Palompon, Leyte and verified by consulting available related publications. A total of 75 leaves were collected. The collected leaves were orderly placed in a tabloid size newspaper, placed in plant press, and sun-dried for two weeks. The representative samples were herborized following standard herbarium procedures and deposited in the Plant Biology Division Herbarium (PBDH) located at Biological Sciences Building, University of the Philippines Los Baños.

Analysis of leaf architecture

Analyses of leaf architecture were done on the leaf samples of *S. aqueum, S. samarangense,* and *P. guajava* (outgroup). Characters and terminologies of Ellis (2009) were used. Laminal characters evaluated include leaf attachment, leaf arrangement, leaf width, leaf length, leaf area, leaf size, leaf shape, leaf symmetry, margin type, apex angle, apex shape, base angle, and base shape. Venation characters were primary vein framework, major secondary vein framework, major secondary spacing, third vein category, third vein course, quarternary vein category, areolation, and freely ending veinlets.

Parameters such as length and width were done using a transparent ruler, while angles of divergence were measured using a protractor. Analysis of smaller vein patterns were done using a crafted light box. A light box was made using a 16 in x 7.5 in box covered with plastic sheet measured 17 in x 12 in. in dimension. A light source was placed inside. The leaves were then pressed in the plastic sheet hanging on the top of the light box. The light passing through the dried leaf specimens allowed easy visualization of the higher vein orders.

Statistical analysis

A total of 25 leaf characters were observed for each of the leaf samples of *S. aquem, S. samarangense, and P. guajava* to investigate their character differences. Qualitative data were assigned with numerical value. To illustrate the relationship of similar leaf architectural traits, cluster analysis was done using two algorithms, unweighted paired group average (UPGMA) and single linkage (nearest neighbor) computed with Gower similarity index. Computations had bootstrap value of 1000. Moreover, to illustrate the individual differences of *Syzygium* species, ordination analysis using Principal Components Analysis (PCoA) was also done. Statistical analyses were done using PAST (Paleontological Statistical Software) software by Hammer et al. 2001.

RESULTS AND DISCUSSION

Leaf characteristics of *S. aqueum, S. samarangense*, and *P. guajava*

Leaf samples of *S. aqueum*, *S. samarangense*, and *P. guajava* (outgroup), shown in Figure 1, were evaluated. General laminar characters evaluated were listed in Table 1. As observed, both species shared many similar laminar features. All leaf samples, including the outgroup, were observed to have pinnately compound, opposite leaves. These characters are commonly observed in most species under Myrtaceae family. Symmetrical leaves with elliptic shape, entire margin, and acute apices were also observed across the species analyzed. The same features were exhibited by *P. guajava* leaves except for its obtuse apex.

Dissimilar laminar characters of *S. aqueum* and *S. samarangense* include the laminar size, apex shape and base shape. Leaf samples of *S. samarangense* have distinct larger laminar areas and were categorized as megaphylls (> 164, 025 mm^2), while *S. aqueum* samples were smaller and were categorized as mesophylls (4,500 – 18,225 mm^2). The outgroup, *P. guajava*, were distinctly smaller and some samples were categorized as notophylls (2,025 – 4,500 mm^2). Similar acute base angles were exhibited by both species. However, difference in base shapes was observed. *S. aqueum* has rounded base shape, while *S. samarangense* has a decurrent shape.

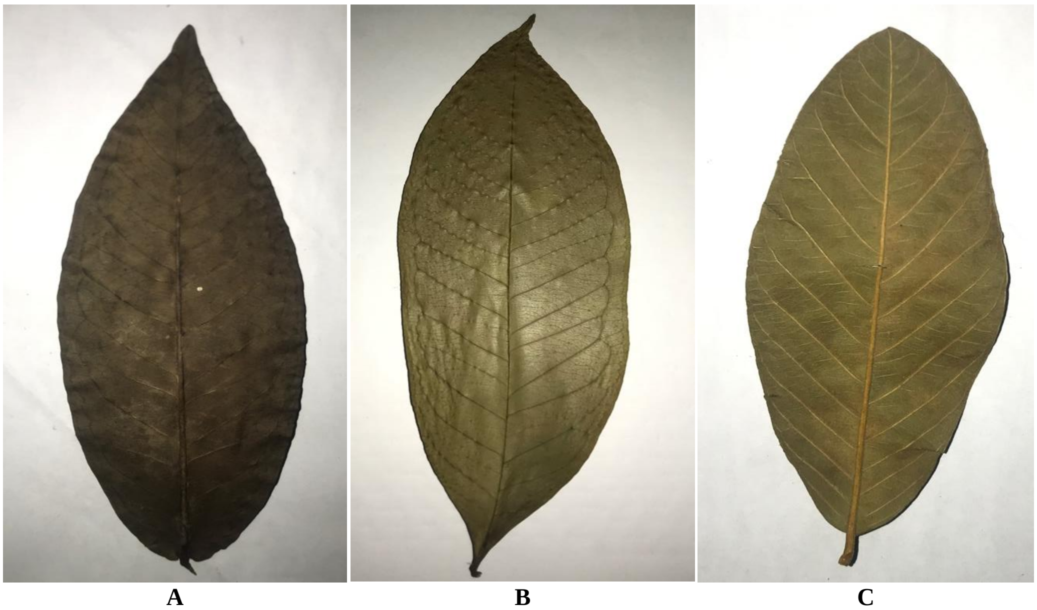

Figure 1. Leaf samples of *Syzygium aqueum* (A), *S. samarangense* (B), and the outgroup *Psidium guajava* (C) Exsicc. Viacrucis 7237, 7238, 7239 (PBDH)

Table 1. Laminar characters of *Syzygium aqueum* and *S. samarangense*

Characters	*S. aqueum*	*S. samarangense*	*P. guajava*
Leaf attachment	Opposite	Opposite	Opposite
Leaf arrangement	Simple	Simple	Simple
Laminar width	6.2 – 8.0	6.2 – 10.3	3.3 – 5.7
Laminar length	14.8 – 19.5	21.0 – 29.3	9.2 – 11.5
Laminar area	97.02 – 156.04	130.2 – 265.05	37.95-58.71
Laminar size	Mesophyll	Mesophyll-megaphyll	Notophyll-mesophyll
Laminar shape	Elliptic	Elliptic	Elliptic
Laminar symmetry	Symmetrical	Symmetrical	Symmetrical
Margin type	Entire	Entire	Entire
Apex angle	Acute	Acute	Obtuse
Apex shape	Convex	Acuminate	Convex
Base angle	Acute	Acute	Acute
Base shape	Rounded	Decurrent	Rounded

Venation characters of *S. aqueum, S. samarangense,* and *P. guajava*

Venation characters of S. *aqueum* and S. *samarangense* leaf samples were also observed and compared as shown in Figure 2. The summary of the observed characters was listed in Table 2. *Syzygium aqueum* and *S. samarangense* exhibited many similar venation characters. Both exhibited pinnate primary vein framework, festooned brochidodromous secondary vein framework, and irregular secondary spacing. The secondary angles in both species were smoothly decreased towards base. Similar vein category and third vein course were exhibited by both species, having alternate percurrent and sinuous characters, respectively. Random reticulate quarternary vein category was also observed in *S. aqueum* and *S. samarangense*. According to Oliveira et al. (2011), camptodromous-brochidodromous secondary venation pattern, ultimate marginal venation in arches, and reticulate tertiary venation were common character occurrences in most of the species under Myrtaceae family. Ultimate marginal venation in arches was observed in all species analyzed, although more prominent in *S. aqueum* and *S. samarangense*. However, reticulate tertiary venation was only observed in the outgroup, *P. guajava*.

Although many similar venation characters were observed in *S. aqueum* and *S. samarangense* species, differences in their areolation and freely ending veinlets (FEVs) are evident. Areoles are considered the smallest areas of the leaf tissue, which are surrounded by quaternary and quinary vein orders. A well-developed areolation was observed in *S. aqueum* leaf samples, while moderately developed areolation was exhibited by *S. samarangense*. Furthermore, FEVs are branched in *S. samarangense*, and absent in *S. aqueum*. The outgroup exhibited a lot of different venation characters, such as the major secondary vein frame-work, major secondary angle, and the third vein course.

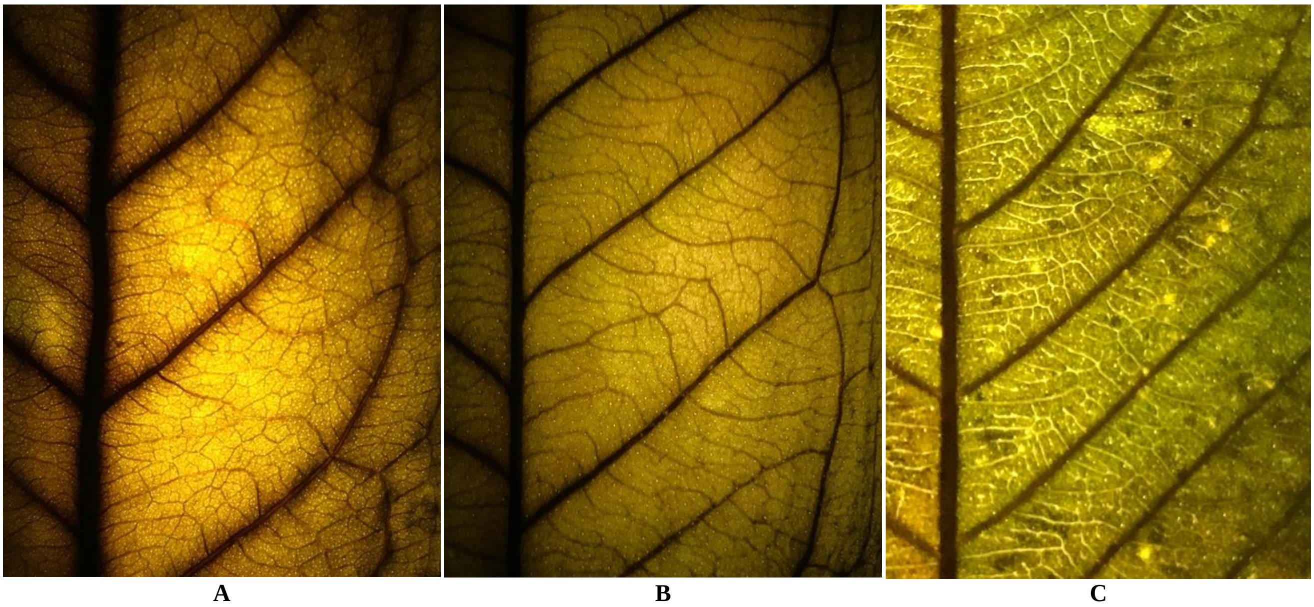

Figure 2. Venation characters of the leaves of *Syzygium aqueum* (A), *S. samarangense* (B), and the outgroup *Psidium guajava* (C), showing their higher vein orders.

Table 2. Venation characteristics of *Syzygium aqueum, S. Samarangense,* and *Psidium guajava*

Characters	*S. aqueum*	*S. samarangense*	*P. guajava*
Primary vein framework	Pinnate	Pinnate	Pinnate
Major secondary vein framework	Festooned brochidodromous	Festooned brochidodromous	Weak brochidodromous
Major secondary spacing	Irregular	Irregular	Irregular
Major secondary angle	Smoothly decreasing towards base	Smoothly decreasing towards base	Uniform
Third vein category	Alternate percurrent	Alternate percurrent	Random reticulate
Third vein course	Sinuous	Sinuous	Ramified
Quarternary vein category	Regular polygonal reticulate	Regular polygonal reticulate	Regular polygonal reticulate
Areolation	Well developed	Moderately developed	Moderately developed
Freely-ending veinlets (FEVs)	Absent	Branched	Branched

Dissimilar laminar and venation characters that could be used to distinguish *S. aqueum* and *S. samarangense* from each other are summarized in Table 3. Distinctive characters of *S. aqueum* and *S. samarangense* are the laminar size, apex, base, areolation, and FEVs. Reports have showed that these distinctive characters can be used as taxonomic characters at species level. For instance, distinct areolation among species have been reported to be one of the most useful leaf characters used as taxonomic tool for delimitation, as seen in the study of *Khaya* species by Oyedapo et al. (2018) and Philippine *Dipterocarpus* species by Hernandez et al. (2020). Moreover, taxonomic significance of leaf apex shape and size have also been elucidated by studies of *Shorea* species by Khan et al. (2016) and Pulan and Buot (2014).

Statistical analysis of leaf architectural characteristics of *S. aqueum, S. samarangense,* and *P. guajava*

Similarities and dissimilarities of the laminar and venation characters of plant species analyzed were visually shown in output patterns using multivariate analysis data exploration. Dendograms using unweighted pair-group average (UPGMA) and single linkage (nearest neighbor) algorithm evidently illustrate the distinction between *S. aqueum, S. samarangense*, and *P. guajava* (outgroup) (Figures 3 and 4). Despite showing several branching within the group, all 25 leaf samples of *S. aqueum*, *S. samarangense*, and *P. guajava* were clustered together. Even with limited dissimilar characters, the analysis showed that all species exhibit variability in the laminal and venation characters, and thus are distinct from one another. The study agreed with the findings of Roth-Nebelsick et al. (2001) and many others (Buot 2020; Paguntalan and Buot 2019; Baltazar and Buot 2019a; Baltazar and Buot 2019b; Baltazar and Buot 2019c) that leaf architectural characters are genetically fixed and hence, can be used favorably in taxonomic studies, especially when dealing with controversial taxa as *S. aqueum* and *S. samarangense.*

Moreover, PCOa data ordination (Figure 5) showed variation among characters of the leaf samples studied . As observed, the species clustered distinctly in groups indicating relevant variabilities between laminal and venation characters of *S. aqueum* and *S. samarangense*. The outgroup, *P. guajava*, has the most distinct cluster between the two indicating strong variability among the two *Syzygium* species.

Table 3. Opposing laminar and venation characters of *Syzygium aqueum* and *S. samarangense*

Characters	***S. aqueum***	***S. samarangense***	***P. guajava***
Laminar size	Mesophyll	Mesophyll-megaphyll	Notophyll-mesophyll
Apex shape	Convex	Acuminate	Convex
Base shape	Rounded	Decurrent	Rounded
Areolation	Well developed	Moderately developed	Moderately developed
Freely-ending veinlets (FEVs)	Absent	Branched	Branched

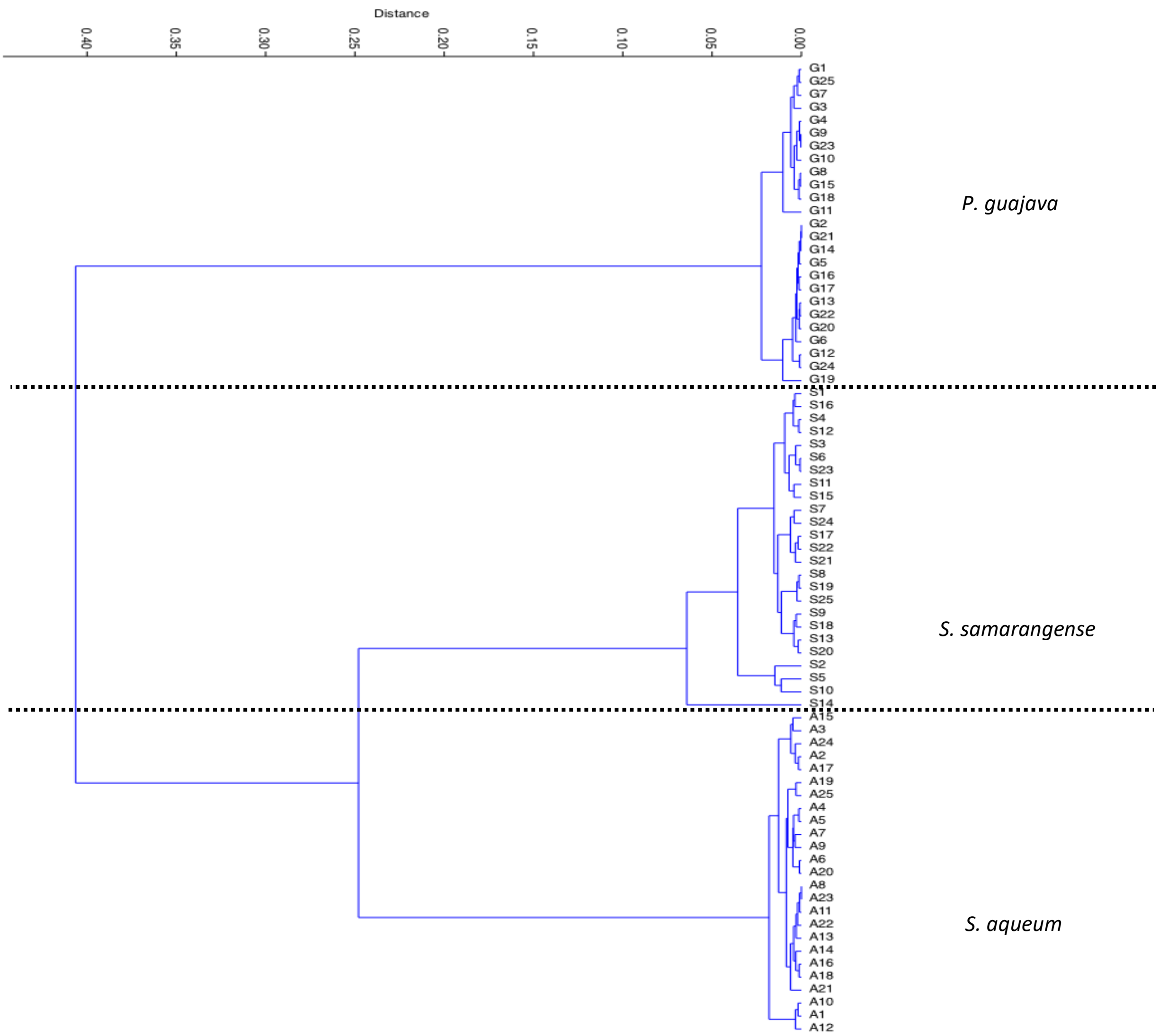

Figure 3. Dendrogram generated using unweighted pair-group average (UPGMA) clustering approach for *Syzygium aqueum*, *S. samarangense*, and *Psidium guajava* (outgroup), showing distinct clusters, thus exhibiting variability in the laminal and venation characters

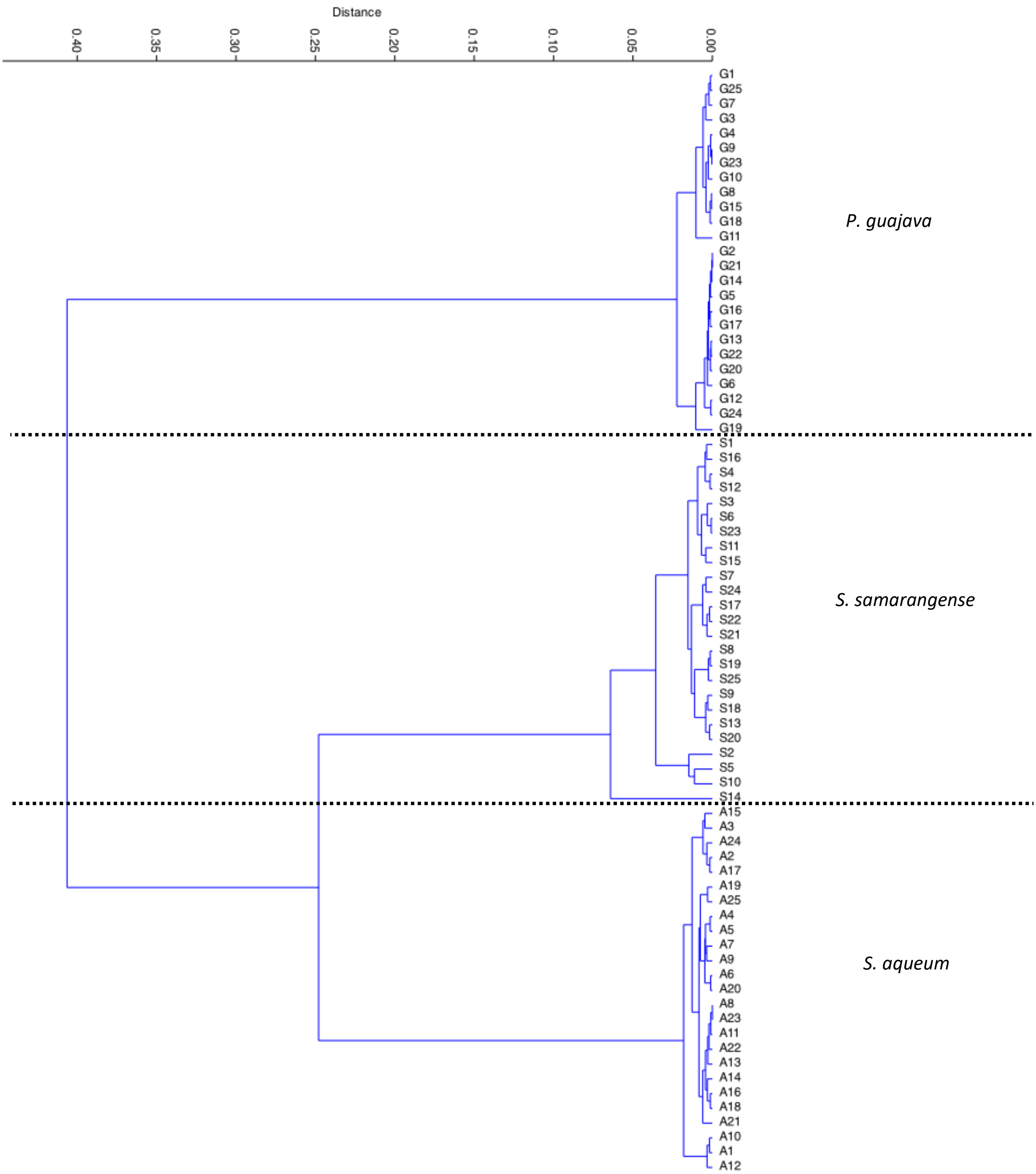

Figure 4. Dendogram generated using single-linkage clustering approach for *Syzygium aqueum*, *S. samarangense*, and *Psidium guajava* (outgroup), showing distinct clusters, expressing variability on laminar and venation characters

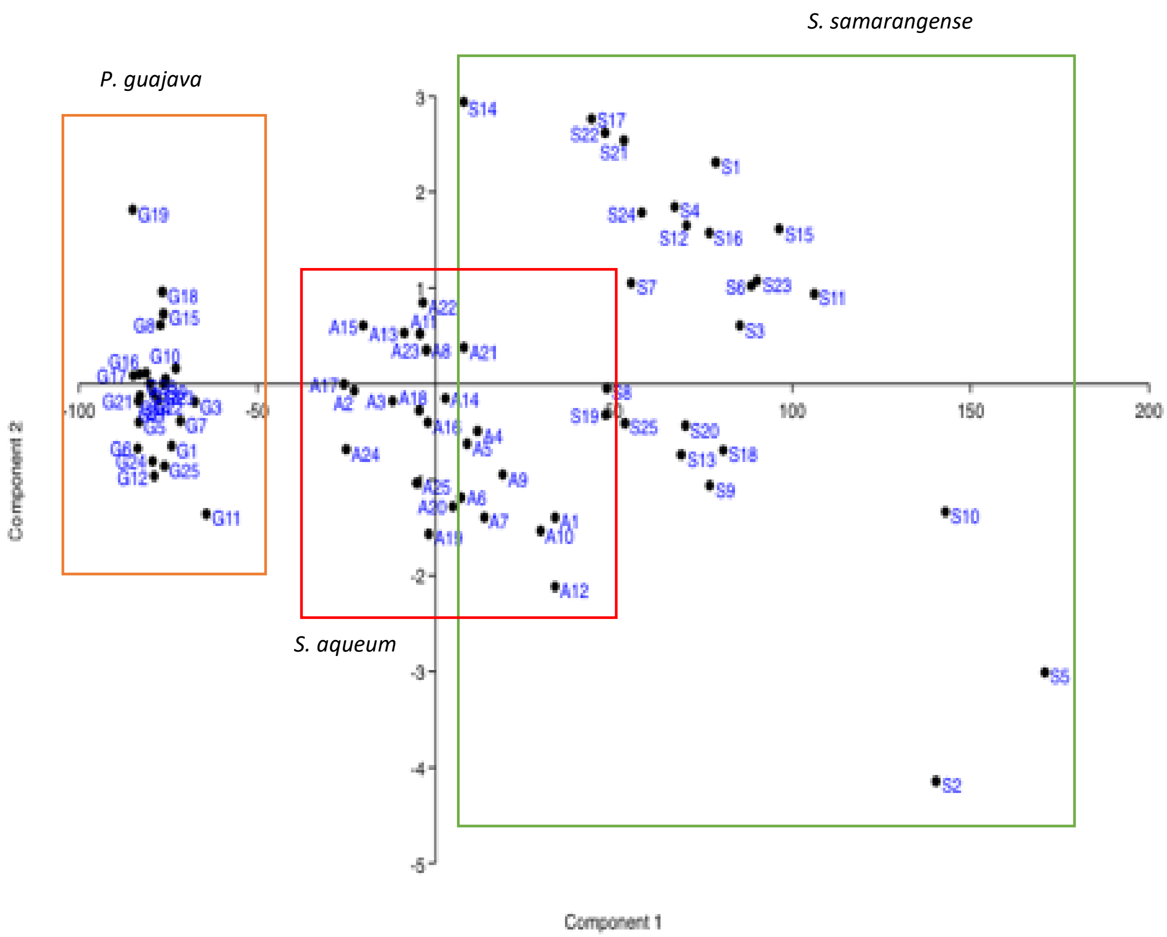

Figure 5. Principal Component Analysis (PCoA) for *Syzygium aqueum*, *S. samarangense*, and *Psidium guajava* (outgroup), showing distinct groups, further validating the distinction of laminar and venation characters.

Discussion

With respect to the biodiversity reported for the *Syzygium* and the described taxonomic complexity of this genus by Oliveira et al. (2017), there is an evident need for comprehensive taxonomic studies on species under this group. Morphological studies of this family, and especially of genus *Syzygium*, could contribute to future phytosociological and ecological studies and provide further evidences to validate taxonomic classification of the group and new species recognition (Oliveira et al. 2017).

In conclusion, laminar and venation characters of *S. aqueum*, *S. samarangense*, and *P. guajava* (outgroup) were evaluated to delineate the two taxonomically confusing species. In this study, *S. aqueum* and *S. samarangense* shared many similar characters which adds to the confusion of the two species, however, they were found to be different in terms of laminar size, apex shape, base shape, areolation, and FEVs. Cluster analyses, using unweighted pair-group average (UPGMA) and single linkage (nearest neighbor) algorithms, and ordination multivariate analyses evidently illustrated the distinction of the two taxa. The variations in laminar and venation characters are additional set of information that will allow accurate delineation of the two species.

Leaf architecture is shown to be a good taxonomic marker and is a useful tool to confirm and identify distinct species using physical characters, such as general laminar and venation characters. With respect to this study, it is suggested that floral characters of *S. aqueum* and *S. samarangense* should be delineated to highlight distinction between species. It is also recommended that taxonomical analysis using various methods, such as molecular techniques, be employed in more *Syzygium* species to contribute in the resolution of the taxonomic complexity of this group and other taxonomic controversies.

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