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Anatomical Relationships among Three Species of Curcubitaceae

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Authors' contributions

This work was carried out in collaboration among all authors. Author JKE designed, supervised and wrote the final manuscript. Author AA collected data and managed the experiment while author AAE performed the statistical analysis and did the literature search. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Anatomical relationship among *Citrullus lanatus* (Thunb.) Matsum. & Nakai*, Cucumeropsis mannii* Naudin and *Lagenaria siceraria* (Molina) Standl planted in controlled conditions in the Botanical garden of the University of Calabar were determined. Standards anatomical (transverse) sections of the leaves were prepared and photo micro graphed. Generalized transverse sections of the leaf, stomata and trichome were studied. The result showed a single layer of epidermal cells, a thick cuticle on both leaf surfaces, presence of hairs and the occurrence of collenchymatous tissues along the median line of the upper surface of each leaf midrib. The palisade parenchyma consists of 2 to 3 layers of elongated, cylindrical cells which are closely packed together in all the species while the spongy parenchyma are loosely arrange with numerous intercellular spaces and are irregular in shape in all species. The vascular bundles in all the species are bicollateral but are differentially arranged depending on species. Anatomy of the leaf section reveals the presence of

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anomocytic stomata in all three species. The stoma was observed as completely surrounded by four to six variable shapes and sizes of subsidiary cells. The cells surrounding the stoma are quite alike to the remaining epidermal cells except among the subsidiary cells surrounding the stoma. The subsidiary cells are four (4) in *L. siceraria*, five (5) in *C. mannii* and variable (4-6) in *C. lanatus.* The trichome of all three species is multicellular and unbranched (uniseriate). However the shape is conical in *L. siceraria* and *C. mannii* but clavate in *C.lanatus.* The number of cells in the trichome varies from 2-3 in *C. lanatus* and 3-4 in *L. siceraria* and *C. C. mannii.* While there are ridges on the trichome of *L. siceraria* the terminal cell in all three species tapers. When these data were converted to numerical taxonomy using Euclidean distance*, L. siceraria* was observed as the out group with *C. mannii* and *C.lanatus* exhibiting about 83.3% and 66.3% shared apomorphies. The differences proved statistically significant at 0.05 confidence limit. Thus the use of anatomical evidence disagrees on the lumping of these species into one genus.

Keywords: Cucumeropsis mannii; dendrogram; numerical taxonomy; anatomy; transverse section.

1. INTRODUCTION

The family Curcubitaceae also commonly called the gourd family has 95 genera and 1000 species [1]. Its members are of high economic importance and widely distributed in the tropics [2]. It consists of mainly herbaceous vines and woody lianas and it is represented in Nigeria by 21 genera and 41 species [3,4].

In Nigeria and some other developing countries where members of this family are ubiquitous, the use of leaf and stem in addressing morphological distinction has proved very challenging. For instance, *Lagenaria siceraria (Molina) Standl. Citrullus lanatus (Thunb) Matsum. & Nakai* and *Cucumeropsis mannii* Naudin. though nested in three different genera are characterized by large, simple, alternate or spirally arranged leaves on long petioles and creeping stems usually with simple tendrils [5]. Morphological affinities has also been shown not only in the leaf base, leaf apex, leaf venation, leaf margin and leaf surface but also across various stem characters [5,6].

Close morphological similarities among *Citrullus lanatus, Cucumeropsis mannii* and *Lageneria siceraria* had prompted calls from several authors to nesting them in a single genus. This is an ongoing debate.

Several taxonomic markers are employed in delimiting taxa with close morphological affinities. One of such is the use of anatomical characters. Anatomical structure of plants has been a critical tool in the hand of taxonomists in the classification and separation of taxa [7]. [8,9] listed variations in pith, cortex, vascular bundles, tendrils and root hairs as anatomical characters of interest to taxonomists. The employment of some of these characters had proved useful in the delimitation of *Discorea, Vernonia, Pinus, Combretum, Ficus and Vitex* members [9-13].

Regrettably, the anatomical profile of these three species has curiously and inexplicably not been studied.

It is in light of these lacunas, that this study aims at determining the anatomical profiles of the leaf of *Lagenaria siceraria, Citrullus lanatus* and *Cucumeropsis mannii* with a view to establishing degree of relationship.

2. MATERIALS AND METHODS

Seeds for the study were obtained from National Centre for Genetic Resources and Biotechnology and International Institute of Tropical Agriculture (NACGRAB).The details for each seed is shown in Table 1.

The seeds were sown in a screen house using a spacing of 1.0 × 1.0 m and a depth of 3.0 cm. Poultry manure was applied to the plots after two weeks to enhance fruiting and mulching of the soil surface.

After 4 weeks, leaf cuts of about 4 cm long were prepared.

The plant material were fixed in formalin, FAA (5 ml 40% formaldehyde, 5 ml glacial acetic acid and 90 ml ethyl alcohol 70%) for 24 hours, washed in several changes of distilled water, dehydrated through alcohol series (30, 50, 70%) for 2hrs in each solution and finally kept in absolute ethanol at room temperature until required. When required, the specimen were collected from the absolute ethanol and sectioned according to [14]. Leaves were then immersed in a mixture of ethanol: Xylene for 30

minutes each in a ratio 3:1, 1:1, 1:3 and 100% Xylene. Sections were rehydrated first by passing through a series of different concentrations of ethanol (absolute, 90%, 70% and 50%) and then in water. Sections were stained in 1% Safranin red for two minutes followed by dehydration through ethanol series. Thin sections were mounted on a slide with glycerin and covered with a cover slip. Photomicrographs were taken with AmScope MA1000 camera using X 100 magnification. The anatomical profiling of these species was obtained using thin sections made from the adaxial surface of the leaf mesophyll. The referenced method used was [14].

3. RESULTS AND DISCUSSION

3.1 Results of Leaf Anatomy

Results obtained from the leaf anatomy are shown in Fig. 1

The result showed that a single layer of epidermal cells, a thick cuticle on both surfaces, presence of hairs and the occurrence of collenchymatous tissues along the median line of the upper surface of each leaf midrib. The palisade parenchyma consists of 2 to 3 layers of elongated, cylindrical cells which are closely packed together in all the species while the spongy parenchyma are loosely arrange with numerous intercellular spaces and are irregular in shape in all species.

The vascular bundles in all species are bicollateral but are differentially arranged depending on species. The species *L. siceraria* and *C. mannii have* three bundles with the largest occurring on the undermost surface and the two smaller bundles lying above. *C. lanatus* on the other hand have four vascular bundles with the largest being at the centre.

3.2 Stomata

Fig. 2 reveals the presence of anomocytic stomata in all three species. The stoma was observed as completely surrounded by same type of four (tetracytic) to six (hexacytic) subsidiary cells with variable shapes and sizes.

The cells surrounding the stoma are quite similar to the remaining epidermal cells except among the subsidiary cells surrounding the stoma. The subsidiary cells are four (4) in *L. siceraria*, five (5) in *C. mannii and* variable [4-6] in *C. lanatus.* These amoeboid cells with variable sizes in *C. lanatus* are generally larger than those observed for the other two species.

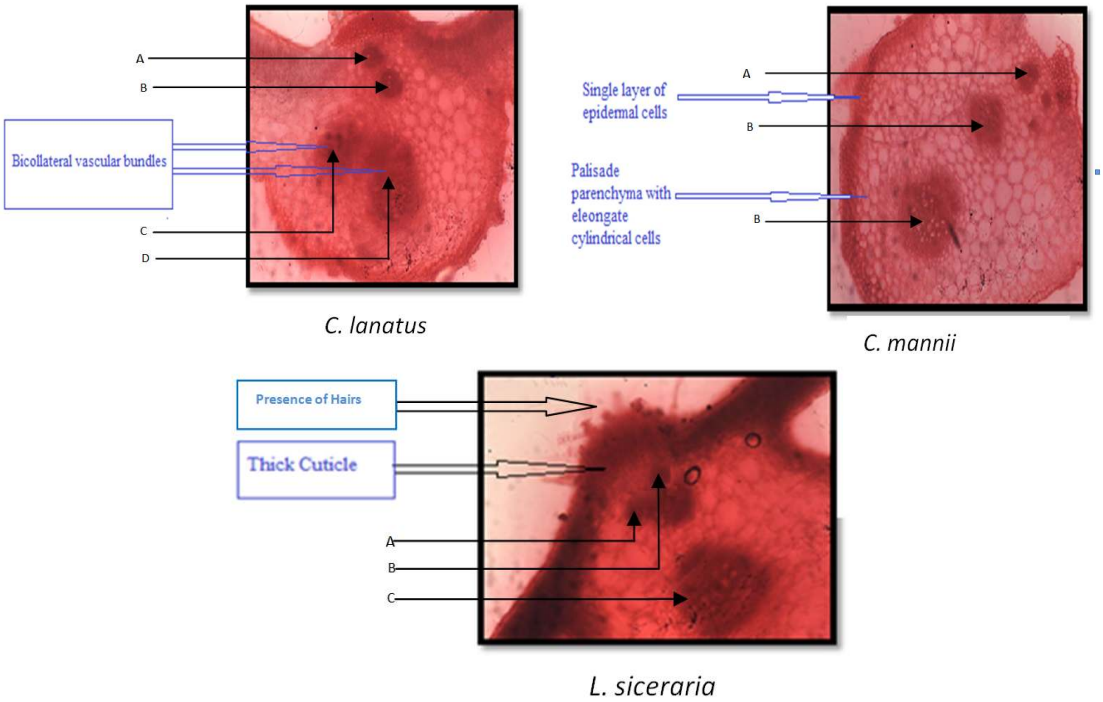

Fig. 1. Cross section of leaf anatomy

3.3 Trichome

The trichomes of all three species are multicellular and unbranched (uniseriate). However the shape is conical in *L. siceraria* and *M.sphaerocarpa* but clavate in *C. lanatus* (Fig. 3).

The number of cells in the trichome also varies. It ranges from 2-3 in *C. lanatus* and 3-4 in *L. siceraria* and *M.sphaerocarpa.* While there are ridges on the trichome of *L. siceraria* the terminal cell in all three species tapers. This result is in agreement with [15,16].

3.4 Taxonomic Relationship

Tables 2, 3 and 4 summarized results for the three species using leaf tissue types (epidermis, palisade mesophyll, spongy mesophyll and leaf bundle), stomata (type, subsidiary cells and size of subsidiary cells) and trichome (Multicellular, uniseriate, Conical, acute apex and Ridge).

The three species were observed to exhibit similar anatomical characters in nine of the twelve characters under investigation. Minor morphological differences were observed in the number of leaf bundle in *C. mannii* the number of trichome in *Citrullus* [2-3], ridge pattern in *Lagenaria* and the extra subsidiary cells in *Citrullus*. When these data were converted to numerical taxonomy using binary notation, the similarities and differences are shown in Table 5.

Table 5 showed that *Lagenaria siceraria* possesses all the 12 anatomical OTU attributes. *Cucumeropsis mannii* shared ten of the twelve attributes as against eight of the attributes shared by *Citrullus lanatus.* Using L. siceraria as the out group to establish relationships, a dendrogram depicting obvious gaps among the studied taxa was obtained as shown in Fig. 4.

At 0.05% confidence limit, using WINKS software, a statistical significant relationship ($P < 0.001$) was obtained between the out group and each of *C. mannii and C. lanatus*. This position justifies the current classification.

In the recent past, a number of research findings employing various taxonomic markers have tended to suggest the merging of these three species into one genus.

For instance, [17] employing phytoliths marker showed strong affinities among the species. The study reported elliptical, cuneiform, clavate, elongate and scrutiform. The resultant dendrogram using nearest neighbor showed a high similarity index among the species.

Close phytochemical affinities also exist as they share a number of secondary metabolites in common. Various reports showed the presence of same or similar species of alkaloid, proteins, steroids, phenols, alkaloids, flavonoids, carbohydrate, saponin, terpenoids and essential oils [18-21].

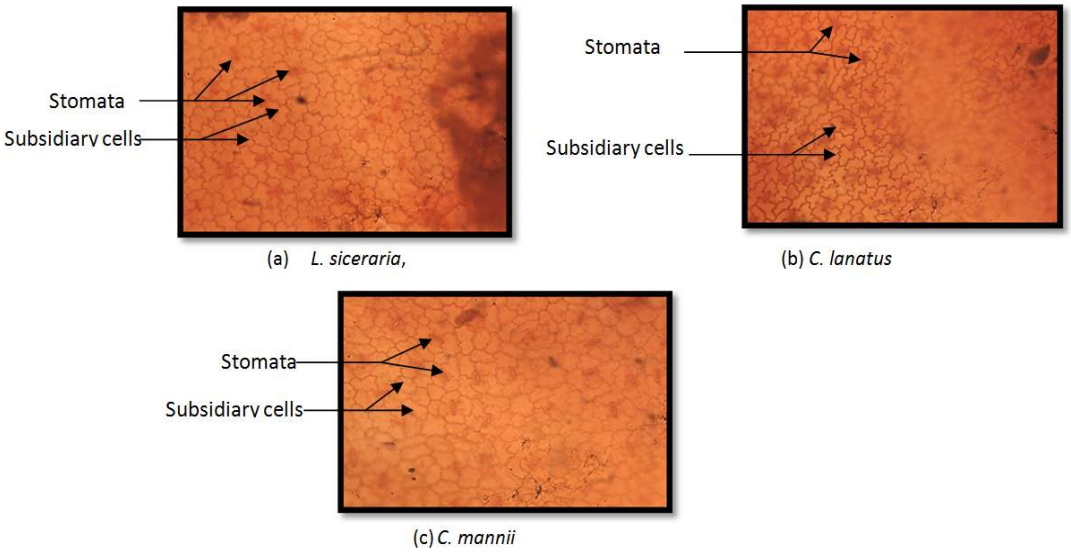

Fig. 2. Transverse Section of Leaf anatomy showing stomata

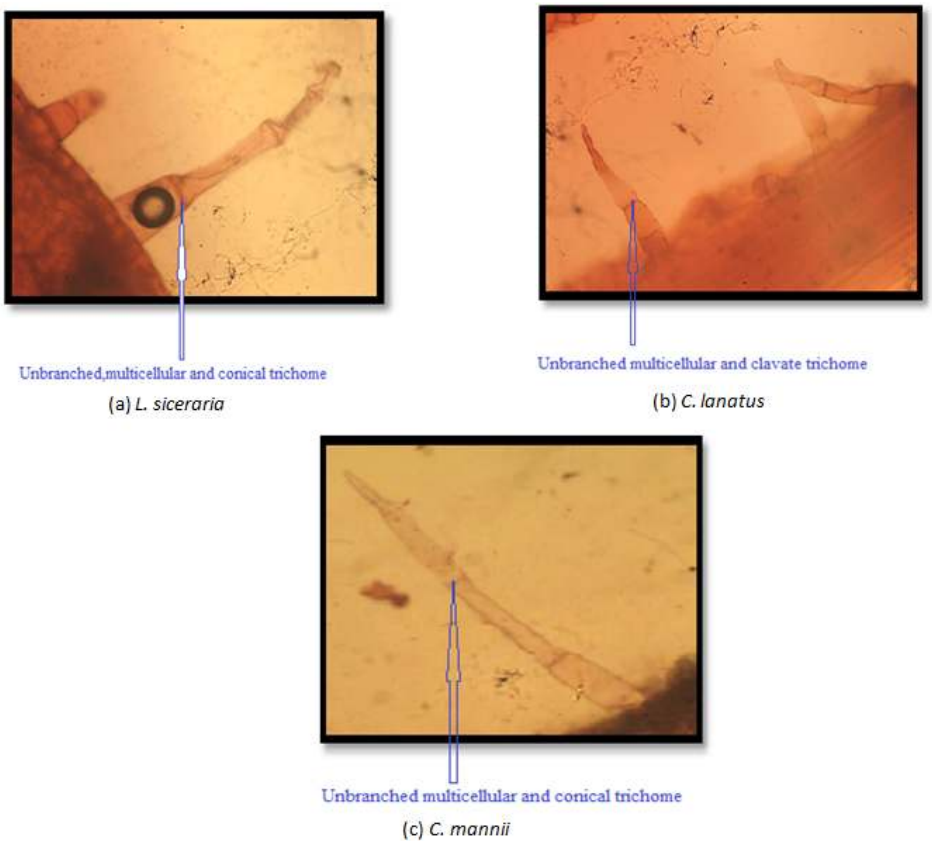

Fig. 3. Leaf anatomy of trichome (X 100)

Cytotaxonomy of *L. siceraria* and *C. lanatus* revealed same number of chromosomes; 2n =22 [22,23] with [22] reporting a statistical insignificant ($p \leq 0.05$) range of 18 to 24 chromosome counts in *C. lanatus*. Osuji et al. 2006 reported that the chromosome count in *C. mannii* (Syn *M. sphaerocarpa*) is 2n=24 which is well within the range of *L. siceraria* and *C. lanatus.*

Information from pollen studies reveals same tricolporate pollen morphology for the three species and a common exine sculpturing pattern of reticulate [24]. [25] reported the grain arrangements as monad across the three species while the pollen shape ranges from oblate-spheroidal to spheroidal prolate.

Morphology evidence obtained from root system, stem, leaf, flower, fruit and seed of the three species under investigation was also reviewed. The three species possess simple, alternate, ovate and pubescent leaves. Flowers are monoecious with axillary inflorescence. Sepals and petals are all five in number. Stamens and carpels are three. Fruits are all pepo. Seeds are all numerous and flat. They all possess herbaceous, pubescent, angular stems. Also they all possess fibrous root system [5,26].

The taxonomic distances obtained in this research do not justify these calls for lumping. For instance, studies revealing lower distances than 16.7% (taxonomic distance between *L. siceraria* and *C. mannii*) and 33.3% (taxonomic distance between *L. siceraria* and *C. lanatus)* were treated as non sisters and subsequently not nested in same genera. [27] showed a taxonomic distance of about 10.3 - 12.5% *for Empidonax. traillii* and *Empidonax. virescens* and argued for their separation into separate genera. Hebert et al 2003 variously showed taxonomic distances among species of same genus to be within 0.7 to 4.6%. [28] variously implied a boundary of not more than 3% among species.

An evaluation of the calls for nesting was shown to be borne out of shared features and not on taxonometric considerations.

Table 1. Source and accession number for seeds used in research

Serial no.	Accession number	Source	Species
1	NG/06/MAR/09/015	NACGRAB	*Lagenaria siceraria (Molina) Standl*
2	NHGB/09/166	NACGRAB	*Citrullus lanatus* (Thunb.) Matsum & Nakai
3	NG/TO/02/12/149	NACGRAB	*Cucumeropsis mannii* Naudin

Table 2. Summary of leaf anatomical characters

Taxa	Epidermis	Palisade mesophyll	Spongy mesophyll	Leaf bundle
L. siceraria	Single layered	2-3 layers cells, elongated and cylindrical	Irregular in shaped	3
C. lanatus	Single layered	2-3 layers cells, elongated and cylindrical	Irregular in shaped	**4**
C. mannii	Single layered	2-3 layers cells, elongated and cylindrical	Irregular in shaped	3

Table 3. Summary of leaf stomata characters

Taxa	Type	Subsidiary cells	Length of subsidiary cells (µ)
L. siceraria	Anomocytic	4-5 cells	33
C. lanatus	Anomocytic	4-6 cells	27
C. mannii	Anomocytic	4-5 cells	35

Table 4. Summary of leaf trichome characters

Taxa	Multicellular	Uniseriate	Conical	Acute apex	Ridge
L. siceraria	3.4 cells	+	+	+	+
C. lanatus	2-3 cells	+	+	+	-
C. mannii	3-4 cells	+	+	+	-

***** *+ = Presence; - = Absence*

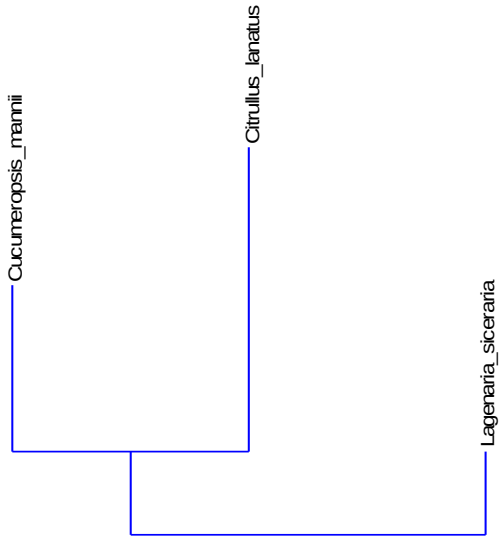

Fig. 4. UPGMA neighbour joining analysis (Past software version 2) of the studied taxa based on anatomical characters using Euclidean distance as measure of similarity

Table 5. Binary matrix

Anatomical characters / Species	Epidermis	Palisade mesophyll	Spongy mesophyll	Leaf bundle	Type	Subsidiary cells	Size of subsidiary cells	Multicellular	Uniseriate	Conical	Acute apex	Ridge
Lagenaria siceraria	1	1	1	1	1	1	1	1	1	1	1	1
Citrullus lanatus	1	1	1	1	0	0	1	0	1	1	1	0
Cucumeropsis mannii	1	1	1	0	1	1	1	1	1	1	1	0

4. CONCLUSION

However, It is imperative that more anatomical details (stomata size, density and frequency) in addition to other taxonomic evidences be conducted to determine if the differences could narrow down taxonomically that would result in them being lumped in same genus or be retained in separate genera as currently been practiced.

5. RECOMMENDATION

Researches utilizing genetic, palynological and other taxonomic lines of evidence should be conducted to further determine degree of differences and/or homologies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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