

Variation Of Phytolith Morphotypes Of Some Members Of Cucurbitaceae Juss.

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Abstract: Phylogenic investigations of eleven members of cucurbitaceae using phytolith morphotypes was examined. The eleven members are *Cucurbita pepo*, *Cucumis sativus*, *Cucumis dipsaceus*, *Citrullus lanatus*, *Melothria sphaerocarpa*, *Cucurbita maxima*, *Luffa cylindrica*, *Telfairia occidentalis*, *Lagenaria siceraria*, *Lagenaria breviflora* and *Telfairia occidentalis*. The specimens were subjected to standard methods of phytolith preparartions. The result indicated forty various morphotypes. Some of them are acuminate, arrow, bilobate, chloridoid, clavate, cordate, crenate, cross thick shank, cuneiform, dumb bell, elongate, fusiform, lanceolate, oblong, orbicular, ovate, rectangular, saddle, scrobiculate, tabular, trapeziform, and several unidentified morphotypes. *Lagenaria siceraria* and *Telfairia occidentalis* had the highest and lowest numbers of morphotypes with eighteen and five respectively. There were some shapes that are characteristics of some species. For instance, forked shape was observed only in *Luffa cylindrica*, Y and pentagular shapes were recorded in *Cucurbita pepo* only, hexagonal and parallelepipedal in *Lagenaria siceraria* while square and carinate were observed in *Cucurbita maxima* only. One unidentified morphotype was found to be common to all the investigated species. When these qualitative data obtained was converted to numerical taxonomy using dissimilarity matrix, a dendrogram was produced using nearest neighbour. Four inferences could be deduce from the study. First, *Citrullus lanatus* is the out group taxon. Second, *Lagenaria breviflora*, *Lagenaria siceraria* and *Cucumis sativus* share closer apomorphies than they do with other taxa. Third, *Cucurbita pepo*, *Trichosanthes cucumerina*, and *Luffa cylindrica* share close affinities among themselves and also with *Cucurbita maxima* and *Melothria sphaerocarpa*. Fourth, *Cucumis dipsaceus* and *Telfairia occidentalis* share close affinities but are distantly related to *Citrullus lanatus*, *Cucumis sativus* and the other taxon under investigation. The study concludes by recommending transfer of *Cucumis dipsaceus* to *Telfairia* and to be nested in the tribe Jolliffieae. Also, the species *Cucurbita pepo* and *Cucurbita maxima* should be re examined with a view to nesting it among *Melothria* in Benincaseae instead of the present Tribe of Cucurbitaceae where it is nested.

Keywords: Cucurbitoid, Dendrogram, Phytolith, Benincaseae, and *Lagenaria*, Tribe.

I. Introduction

Cucurbitaceae, a family of succulent climbing vines whose leaves are simple, palmate and exstipulate (Daniel, 2009) and consisting of about several members in Nigeria are used for a variety of indigenous purposes. These purposes include medicinal, nutritive, fodders, cover crops, musical instruments, spices and other sundry products (Steiner Asiedu et, et. al 2014, Odugbemi, 2006, Warncke 2007, Mabberly, 1997, Gill 1992 and Okoli 1984,). For instance, Odugbemi 2006 and Gills 1992 reported the usage of *Coccinia barteri*, *Coccinia grandis*, *Cucumis melo*, *Cucurbita maxima*, *Cucurbita pepo*, *Lagenaria breviflora*, *Lagenaria siceraria*, *Luffa cylindrica*, *Momordica angustisepala*, *Momordica charantia*, *Momordica foetida*, *Mukia maderaspatana*, *Telfairia occidentalis*, *Trichosanthes cucumerina*, *Cucumis prophetarum*, *Cucumeropsis mannii*, in the treatment of diabetes convulsion, piles, fungal diseases, chest pains, chicken pox, and cough. Okoye, 2013 reported the nutritive value of *Trichosanthes cucumerina*, *Cucumis sativus*, *Cucurbita moschata* and *Cucurbita pepo*. Gills 1992, reported the use of *Cucurbita*, *Luffa*, *Lagenaria* and *Raphanus* species as vegetable crops. Ruminants and other herbivorous fauna species depend on the leaves of most cucurbits for roughages and as fodders. The use of the dried fruits of *Lagenaria* and *Luffa* for flute and as drinking cup for palm wine is a common sight in most Nigerian indigenous communities. The utilization of members of this family as non timber forest products has far reaching positive implications on the lives of the individual and that of the society at large. For instance, in most rural Nigerian communities whose inhabitants live on a less than a dollar per day (FAOSTAT 2015) the utilization of the members of this family for the aforementioned various uses serve as invaluable revenue earner, provide a local alternative to processed products and above all, serve to expose the improvisation ingenuity of

the indigenous craft men. Export of some members of this family had contributed significantly to the Gross Domestic Product of China, Turkey, Iran and Brazil (FAOSTAT 2015). Processing of various species of *Cucumis*, *Melothria*, *Cucurbita* and *Citrullus* is currently driving the vegetable processing industries in Malaysia, Indonesia, Singapore, Thailand, Uzbekistan and Russia (Burieve et. al 2000 and Oripov and Bozorov 2002).

In spite of these obvious economic benefits to the individual and society at large, the taxonomy of members of this family has been challenging. This limitation is better expressed in international and local markets when one species is confused with another. For example, one species of the "melon" brand name(represented in Nigeria by four genera- *Cucumis*, *Melothria*, *Citrullus* and *Cucurbita*) with less attractive nutritive values, hence with lower price regimes is sold to unsuspecting members of the public under the disguise of the other with higher nutritive content and higher price regime. Recent revised classification system of Schaefer & Renner 2011 did not address this taxonomic challenge faced by the melon group as the four genera were still not nested in a single genus as suggested earlier by Pangalo (1950).

Worst still, all the classification systems for this family had utilized all other taxonomic markers except anatomical and phytolith tools. It is based on this deficiency that this study had to utilize phytolith marker for this study.

Phytoliths are siliceous organic matter found in some plant taxa with the ability of occluding absorbed atmospheric carbon (Beilei et al., 2014). Phytoliths have been observed in such plant parts as leaves, stems, roots and fruits (Fuller & Madella, 2001). Several morphotypes of phytoliths exist (Piperno, 1988), however, studies has shown the occurrence of specific and unique morphotypes in poaceae, cyperaceae (Zuo and Lu 2011) and in most plant taxa. Piperno (2006) reported that plant taxa having disparate interval structures and in some cases exhibiting preferential modes of silica deposition can have specific morphotypes for these deposits, meaning that phytolith can be used to identify different plant types at various taxonomic levels. This character state formed the basis for recent delimitation of some *Bambusa* species in Parr et. al (2010). Cucurbitaceae family is one of the family producing high amounts of phytolith (Kealhofer and Piperno, 1994). This marker has been applied in archeological and paleoecological studies of some fossilized members of this family and those of others (Thomasson, 1990; Fredlund and Tieszen, 1997a; Borboni et al., 1999).

II. Materials and Methods

Sample Collection: Fresh species of *Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Cucumis sativus* L., *Cucumis dipsaceus* C.G. Ehrenb. ex Spach., *Cucurbita maxima*, *Cucurbita pepo* Duchesne, *Lageneria siceraria*, (Molina) Standl, *Lageneria breviflora* (Benth.) Roberty, *Luffa cylindrica*, *Melothria sphaerocarpa* (Cogn.) H. Schaeef. & S.S. Renner, **comb. nov.** and *Telfairia occidentalis* were obtained within the precincts of the University of Calabar including the botanical garden. Table 1 shows the botanical names, common names and vernacular names of the plant species in Efik while fig 1 shows the images of the various plant species.

| Species | Common names | Vernacular name in Efik |
|--|------------------|-------------------------|
| <i>Lageneria siceraria</i> , (Molina) Standl | Calabash gourd | Okpon |
| <i>Cucurbita pepo</i> Duchesne | tropical pumpkin | Nnani |
| <i>Cucurbita maxima</i> Duchesne ex Lam. | Giant Pumpkin | Ndise, Nfri |
| <i>Citrullus lanatus</i> (Thunb.) Matsumura. & Nakai | Water melon | Ikon, Ikpan |
| <i>Lageneria breviflora</i> (Benth.)Roberty | Wild colocynth | Ndise ikot |
| <i>Cucumis sativus</i> L | Cucumber | Kokumba |
| <i>Cucumis dipsaceus</i> C.G. Ehrenb. ex Spach | Hedgehog gourd | |
| <i>Luffa cylindrica</i> (L.) Roem. | Sponge gourd | Kusa |
| <i>Melothria sphaerocarpa</i> (Naud.) comb. ined. | Melon | |
| <i>Telfairia occidentalis</i> Hook, | Fluted pumpkin | Ikon ubon |
| <i>Trichosanthes cucumerina</i> L. | Snake gourd | Ikim |

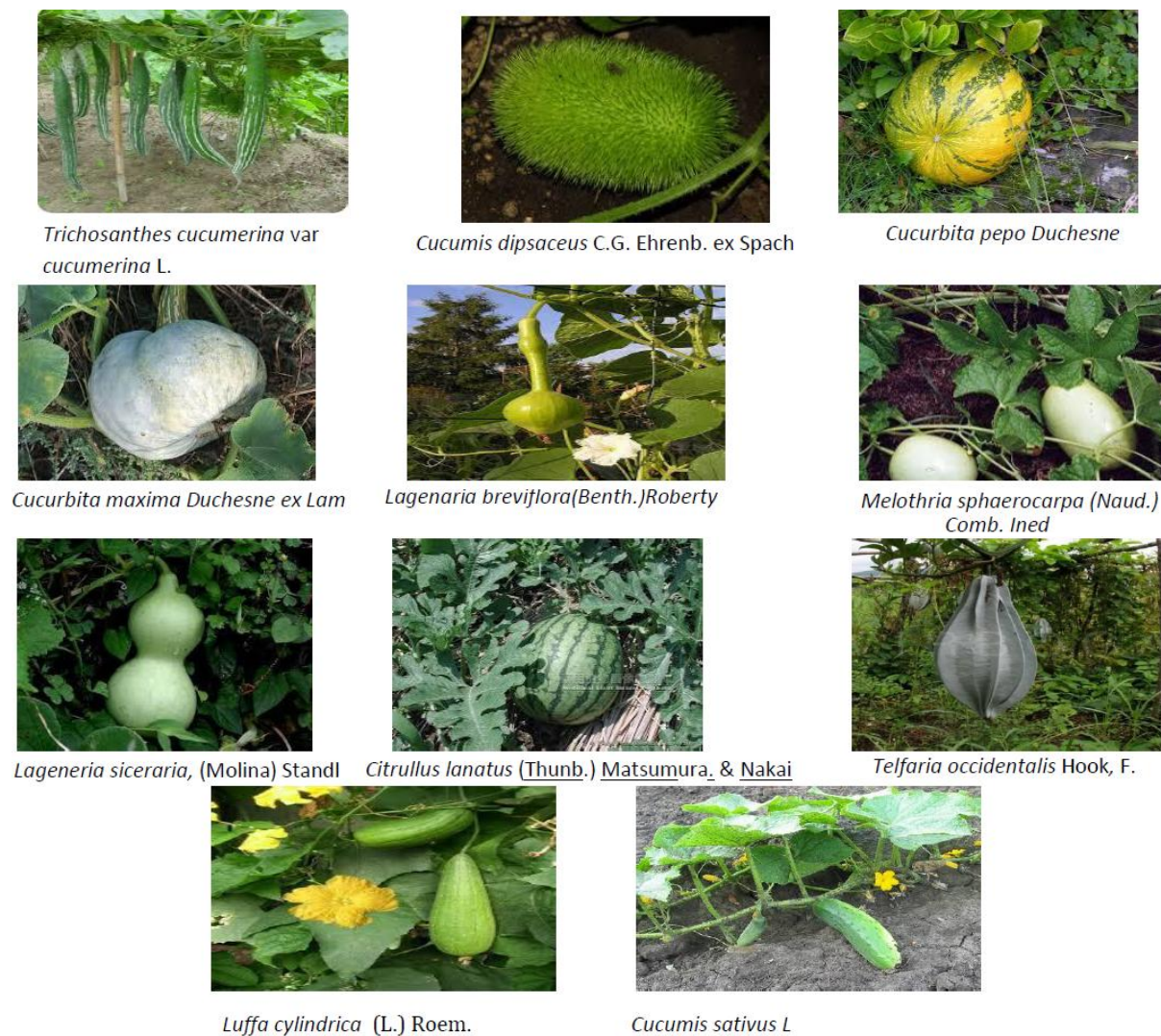


Fig 1: Characteristic representatives of members of Cucurbitaceae studied.

III. Methodology

The plant parts were dismembered into roots, leaves and fruits. Each dismembered part of each were placed in a beaker for further treatment. All of the plant samples were rinsed twice in distilled water and placed in an ultrasonic bath for 20 min, and then dried at 70°C for 24 h. Three parallel samples were selected from each species of plant sample. The method for phytolith extraction was described in detail in [Parr et al 2001]. In this study, a revision to the wet oxidation method was made that aimed to digest the organic matter more completely. The detailed steps are as follows: (1) Weigh about 1 g dry sample into a tube (to the nearest 0.01 mg); (2) add 5 mL HNO₃ into the tube and heat in a water bath at 80°C until reaction stops, then centrifuge 2 times at 3000 r/min for 5 min and decant supernatant; (3) add 10% HCl into a tube and heat in a water bath at 80°C for 30 min, then centrifuge at 3000 r/min for 5 min and decant; (4) add 5 mL HNO₃ into the tube and heat to ensure removal of all organic material, then centrifuge and decant; (5) add 5 mL H₂SO₄ into the tube and heat in a water bath for at least 1 h; (6) cool to room temperature, and reheat in a water bath, and add 30% H₂O₂ slowly until the liquid clears; and (7) centrifuge 4 times at 3000 r/min for 5 min, then dry phytoliths in the tube at 70°C for 24 h. Weigh the phytoliths using an analytical balance and check the samples under an optical microscope at 400× magnification to ensure no organic material exists.

Photomicrography & Sample weighing: The weighed phytoliths were prepared on glass slide viewed and identified with a light microscope at x100 (oil immersion) magnifications. After drying the extract were weighed.

Phytoliths identification: Identification of the various phytolith morphotypes was done using the handbook of phytolith nomenclature (ICPN, 2005). The abundance of each phytolith morphotypes was also determined.

Conversion to Numerical Taxonomy: A Dendrogram was constructed based on the similarity index established among the plant species using the formula

$$\text{Similarity Index (S)} = \frac{N_s}{N_s + N_d}$$

where S = Numerical index; N_s = the number of positive features shared by any OTU (Operational Taxonomic Unit) and N_d = the number of positive features in one OTU and number of negative features in the other OTU

IV. Result

The phytolith morphotypes and their abundance observed for each species is shown in Plate 1.

Result of Phytolith Morphotypes for *Luffa cylindrica*

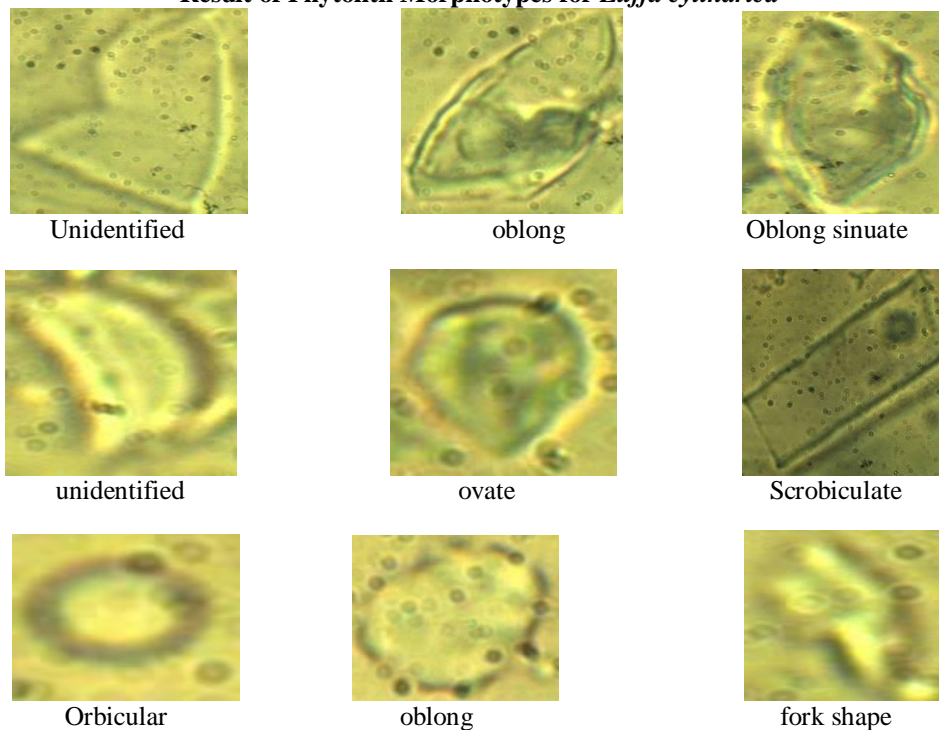
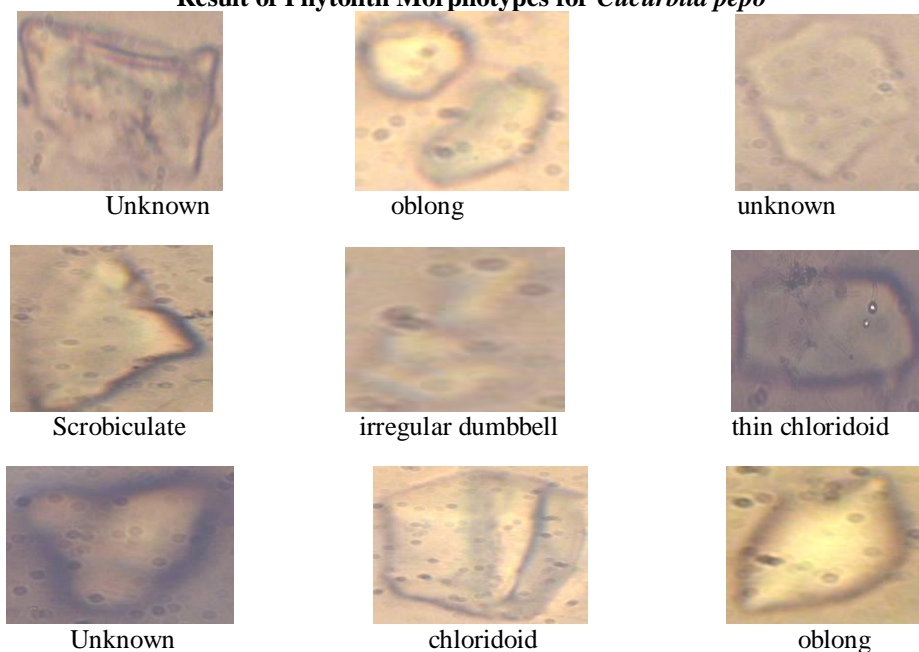


PLATE 1a: Phytolith Morphotypes of *Luffa cylindrica*

The observed phytolith morphotypes for *Luffa cylindrical* include various shapes of oblong, oblong sinuate, ovate, scrobiculate, orbicular and forked shape. Two shapes could not be identified.

Result of Phytolith Morphotypes for *Cucurbita pepo*



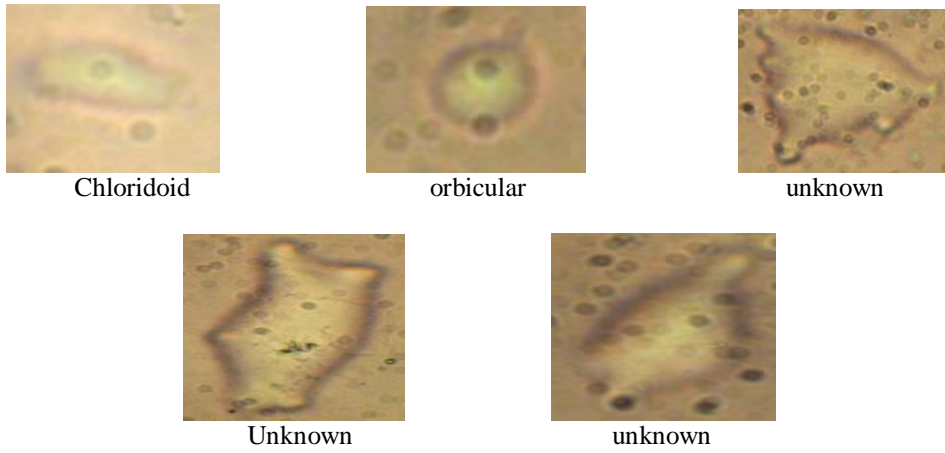
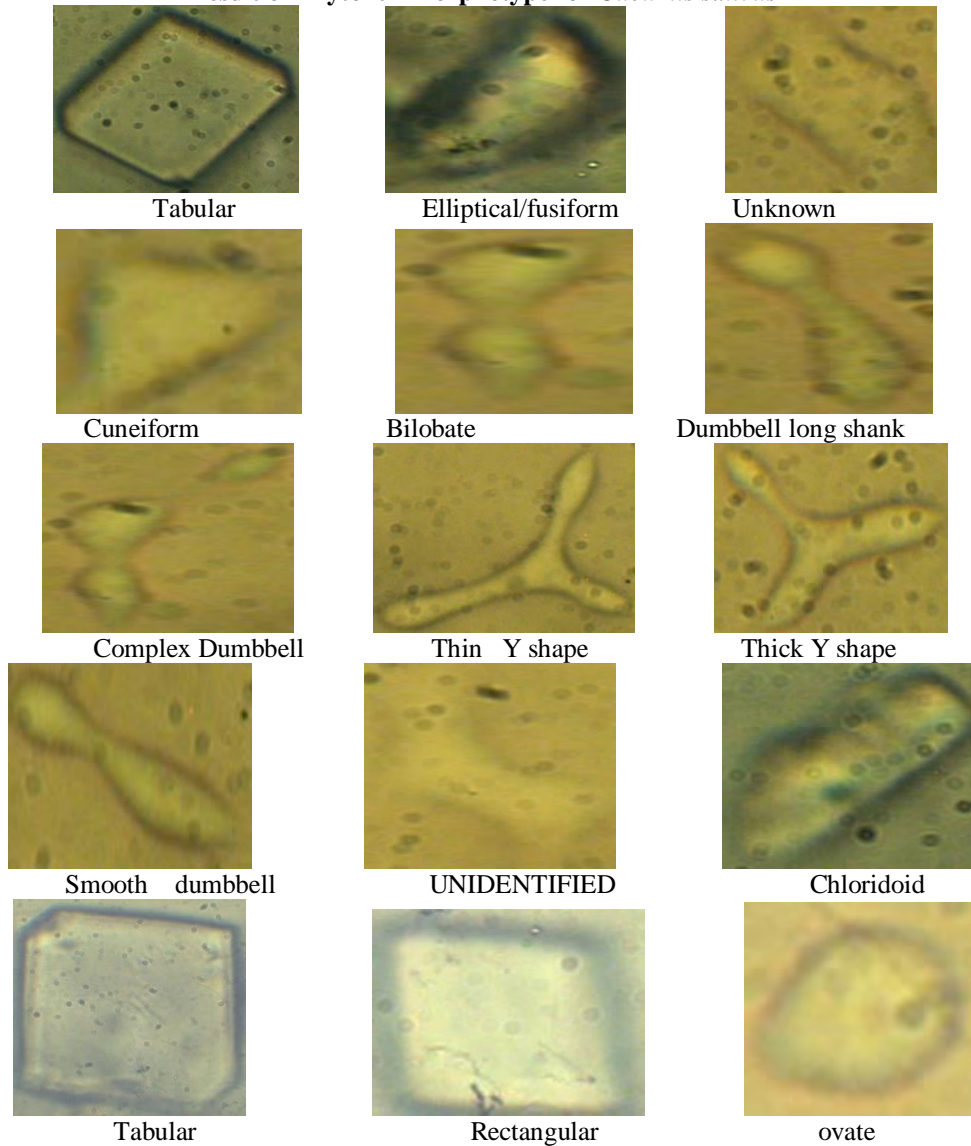
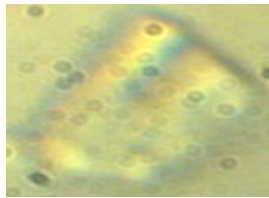


PLATE 1b: Phytolith Morphotypes of *Cucurbita pepo*

The morphotypes of *Cucurbita pepo* included three morpho-shapes of chloridoid, two of oblongs, one each of orbicular, irregular dumb bell , scrobiculate and four unidentified morphotypes .

Result of Phytolith Morphotype for *Cucumis sativus* L





Unknown



Oblong



Unknown



Ovate



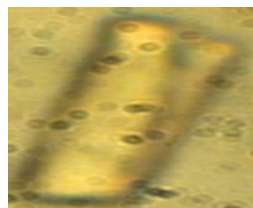
unidentified



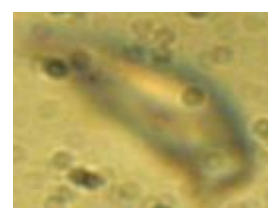
unidentified



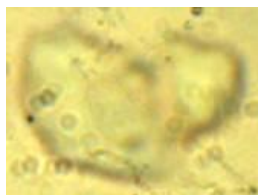
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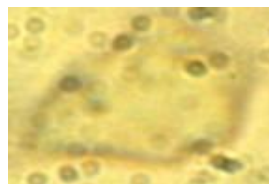
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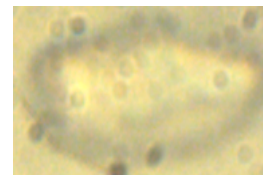
unidentified



Unknown



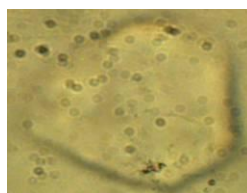
Unknown



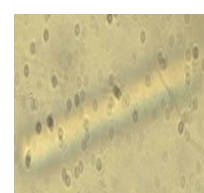
oblong



Unknown



Pentagonal



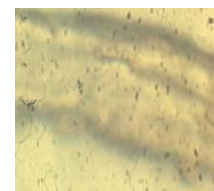
Elongate smooth



Unknown



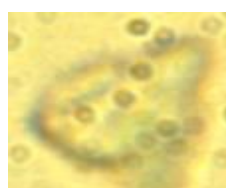
Unknown



Elongate with rough edge



Unknown



Ovate



Oblong

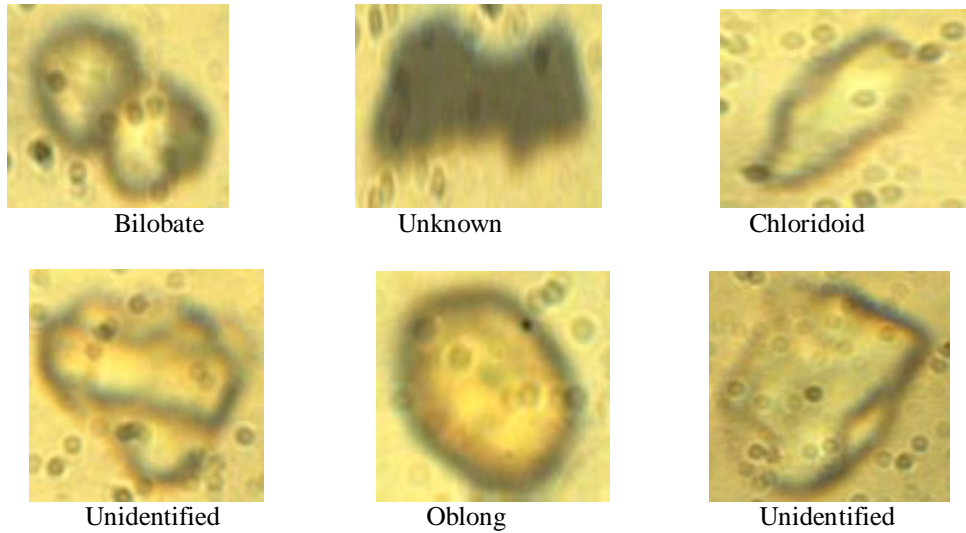


PLATE IC : Phytolith Morphotypes for *Cucumis sativus* L.

The observed phytolith morphotypes for *Cucumis sativus* include three morpho shapes each of dumb-bell, ovate, oblong; two morpho shapes each of Y- shape, bilobate, elongate, tabular; one each of chloridoid, cuneiform, rectangular and pentagonal and eighteen unidentified morphotypes.

Result of Phytolith Morphotypes for *Cucumis dipsaceus* C.G. Ehrenb. ex Spach

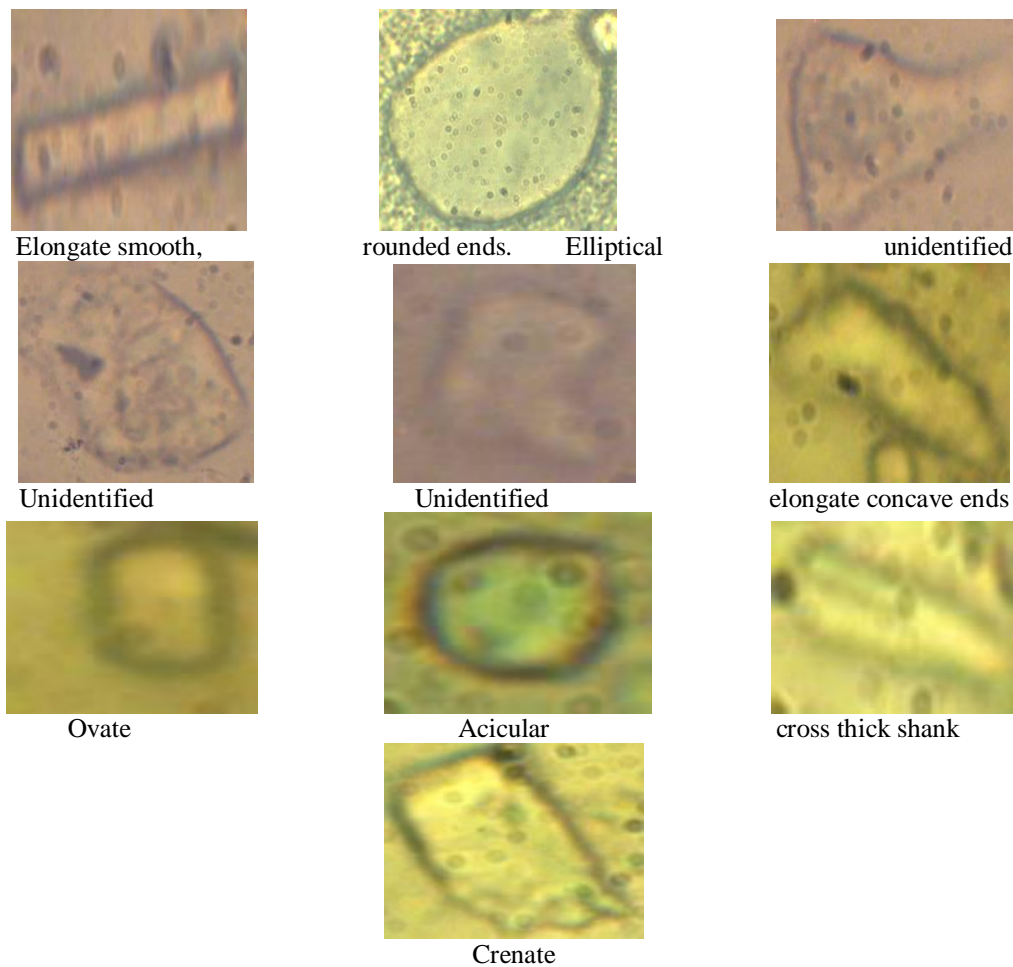
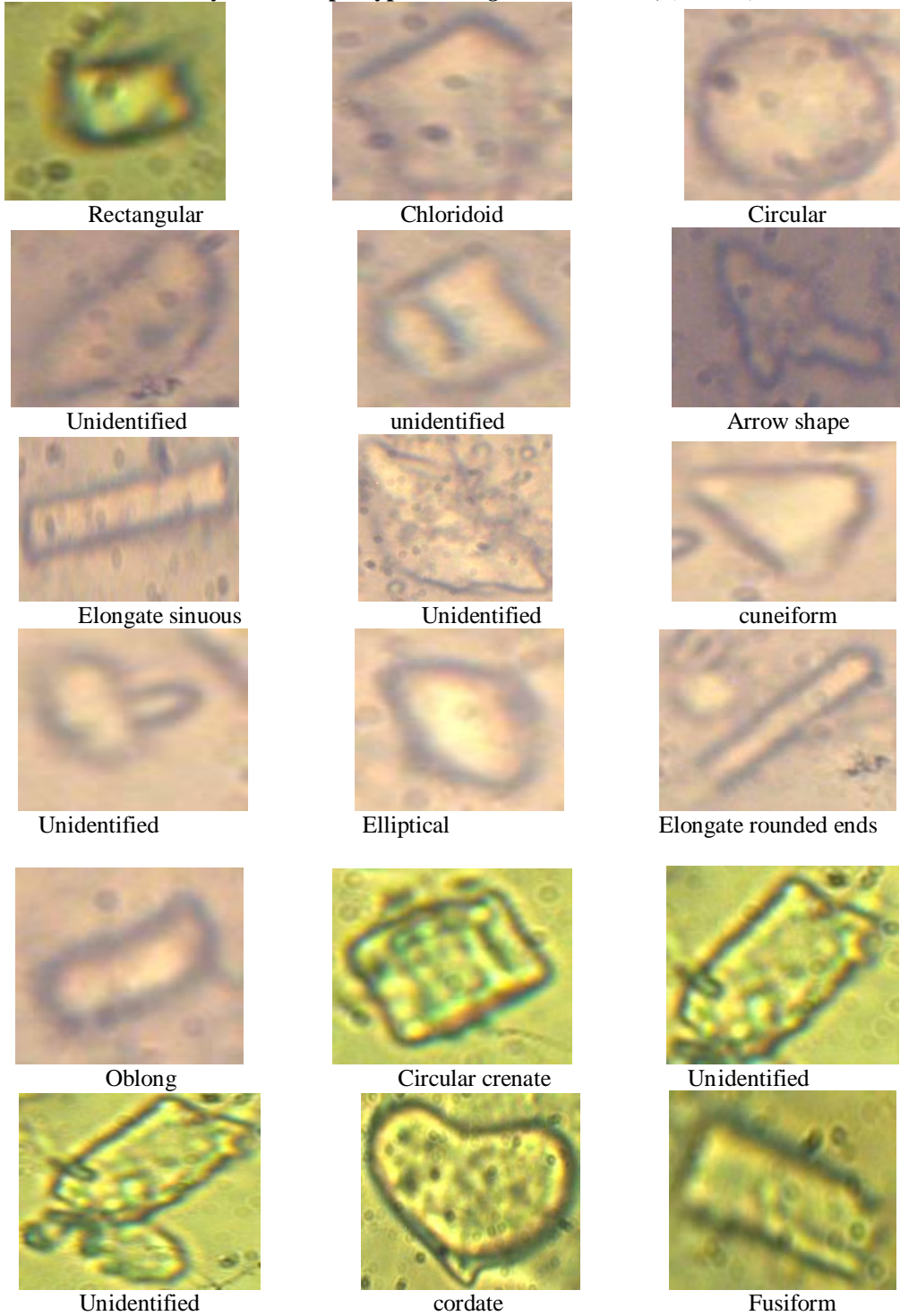
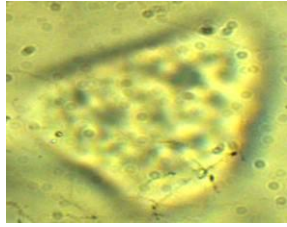


PLATE 1d: Phytolith Morphotypes for *Cucumis dipsaceus* C.G. Ehrenb. ex Spach

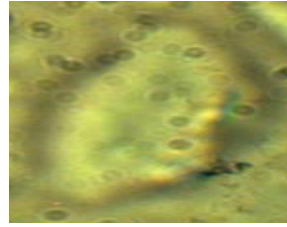
The observed phytolith morphotypes for *Cucumis dipsaceus* are elongate, concave and elongate rounded ends and one morpho shape each of acicular, cross thick shank, crenate, elliptical and three unidentified morphotypes.

Result of Phytolith Morphotypes for *Lageneria siceraria*, (Molina) Standl





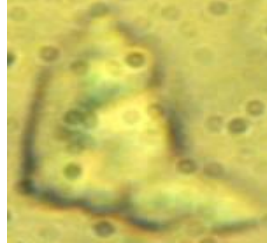
Unidentified



Elliptical



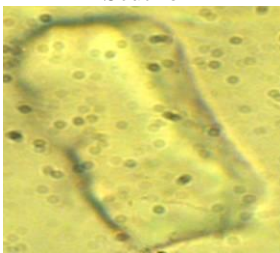
Scutiform



Unidentified



Cuneiform



Parallelepipedal



Elliptical



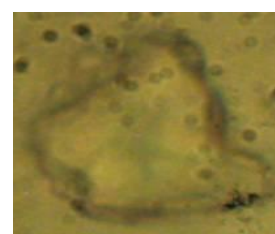
Acicular



Elliptical



Oblong



Cross thick shank



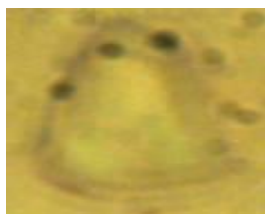
Hexane



Scutiform



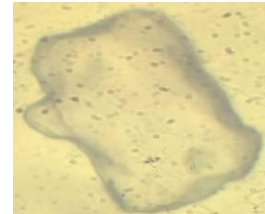
Clavate



Cuneiform



Oblong sinuous



Unidentified



Regular complex dumbbell



Bilobate



Clavate

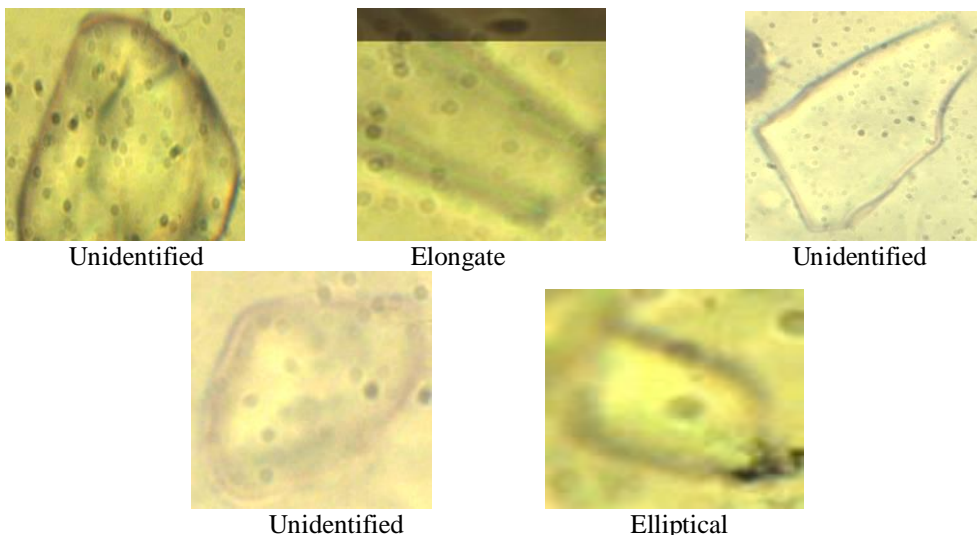


PLATE 1e: Phytolith Morphotypes of *Lageneria siceraria* (Molina) Standl.

The observed morphotypes of *Lageneria siceraria* include five morphoshapes of elliptical morphotypes, three morpho shapes of cuneiform ,oblong, two morpho shapes of the clavate, elongate, scutiform, and one morphotypes each of bilobate , chloridoid, circular , arrow, circular crenate, cordate, fusiform, Parallelepipedal, acircular, cross thick shank, dumb-bell, hexane, rectangular, and twelve various morphotypes that could not be identified.

Result of Phytolith Morphotypes of *Melothria sphaerocarpa* (Cogn.) H. Schaeef. & S.S. Renner,comb. nov

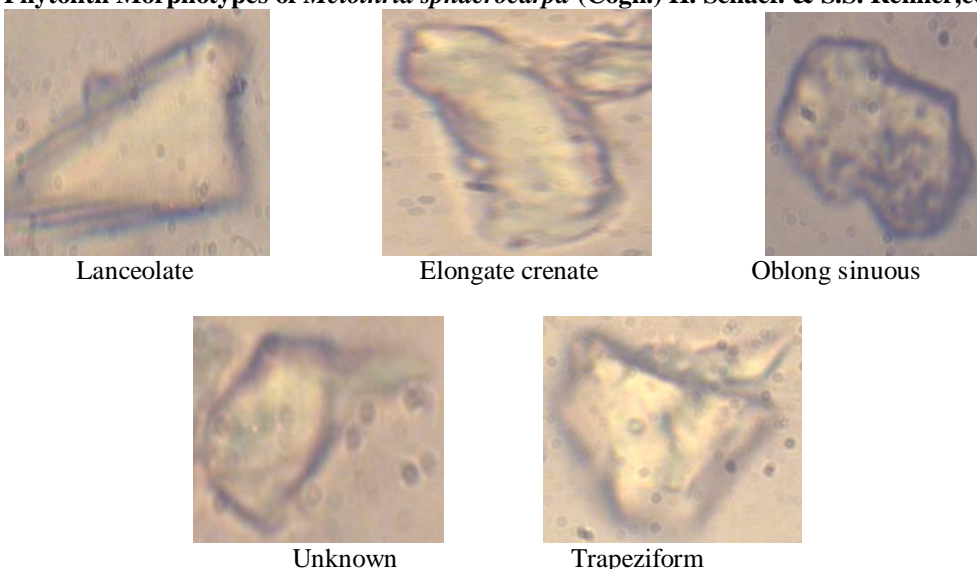
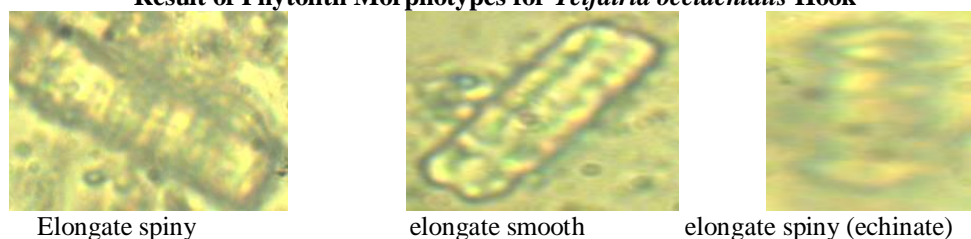


PLATE 1f: Phytolith Morphology of *Melothria sphaerocarpa*(Cogn.) H. Schaeef. & S.S. Renner,comb. nov

The observed phytolith morphotypes for *Melothria sphaerocarpa* are one each of Elongate crenate, Lanceolate, Oblong sinuous , Trapeziform and one that could not be identified .

Result of Phytolith Morphotypes for *Telfairia occidentalis* Hook



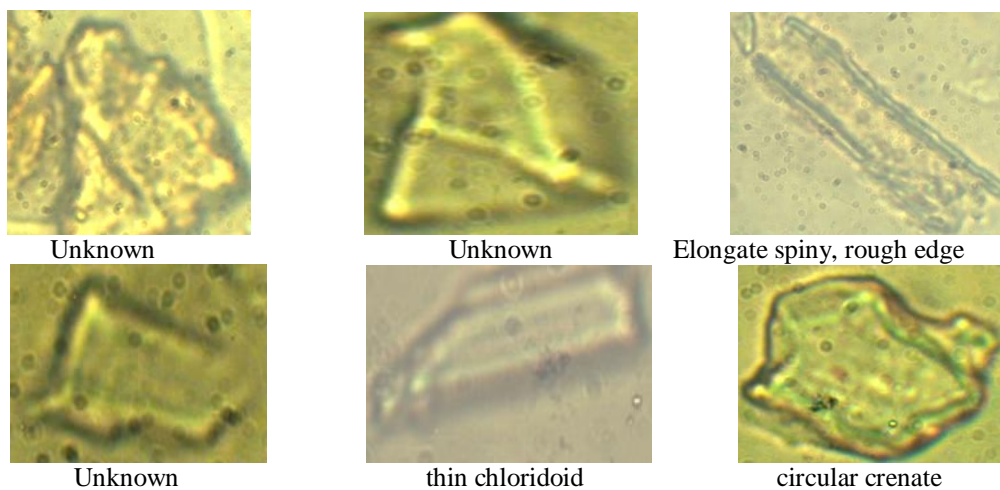
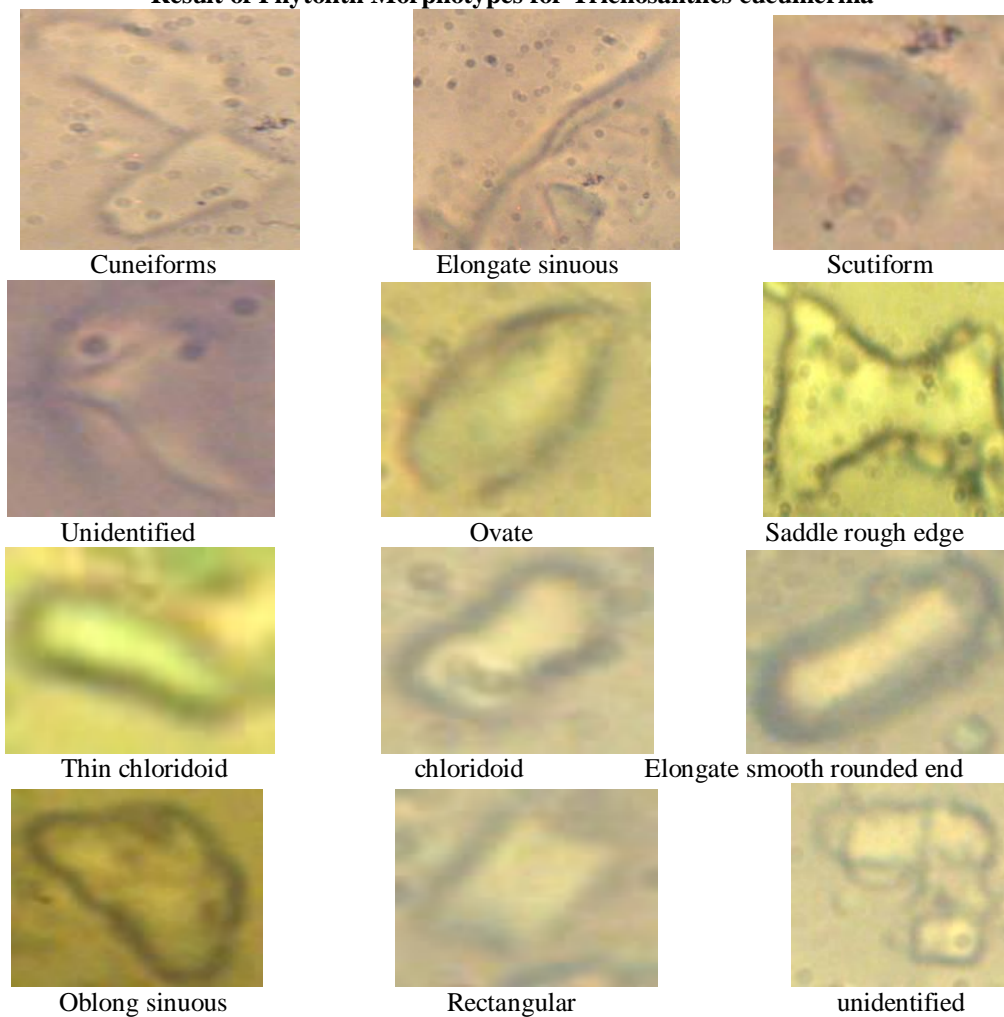


PLATE 1g: Phytolith Morphotypes of *Telfairia occidentalis* Hook.

The observed phytolith morphotypes of *Telfairia occidentalis* included four morpho shapes of elongate, one each of thin chloridoid, circular, crenate and two morphotypes that could not be identified.

Result of Phytolith Morphotypes for *Trichosanthes cucumerina*



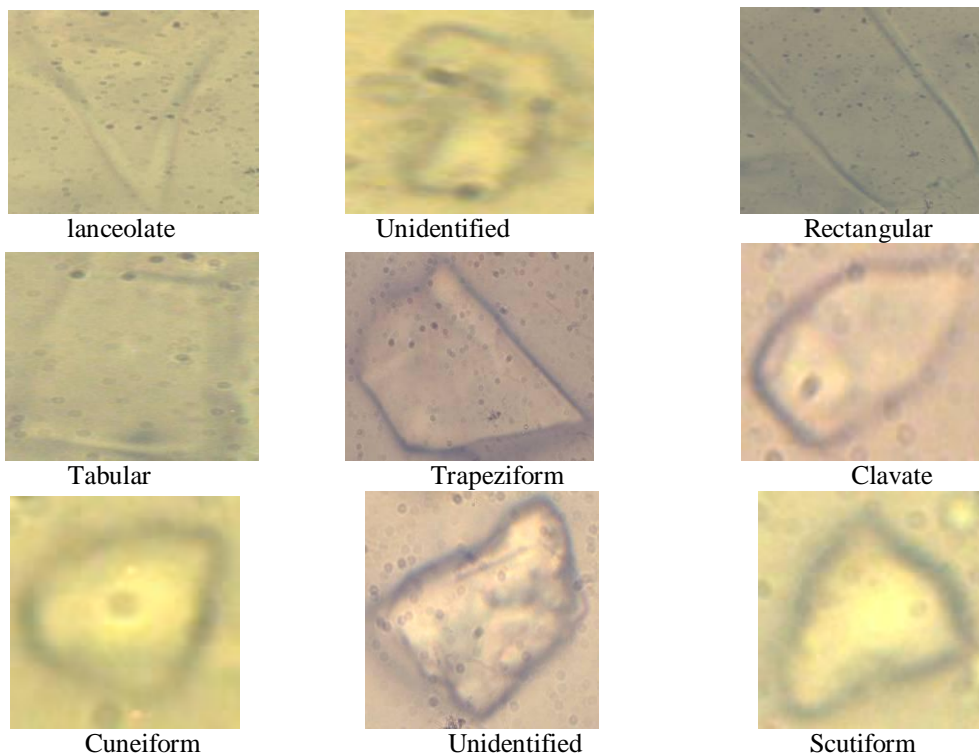
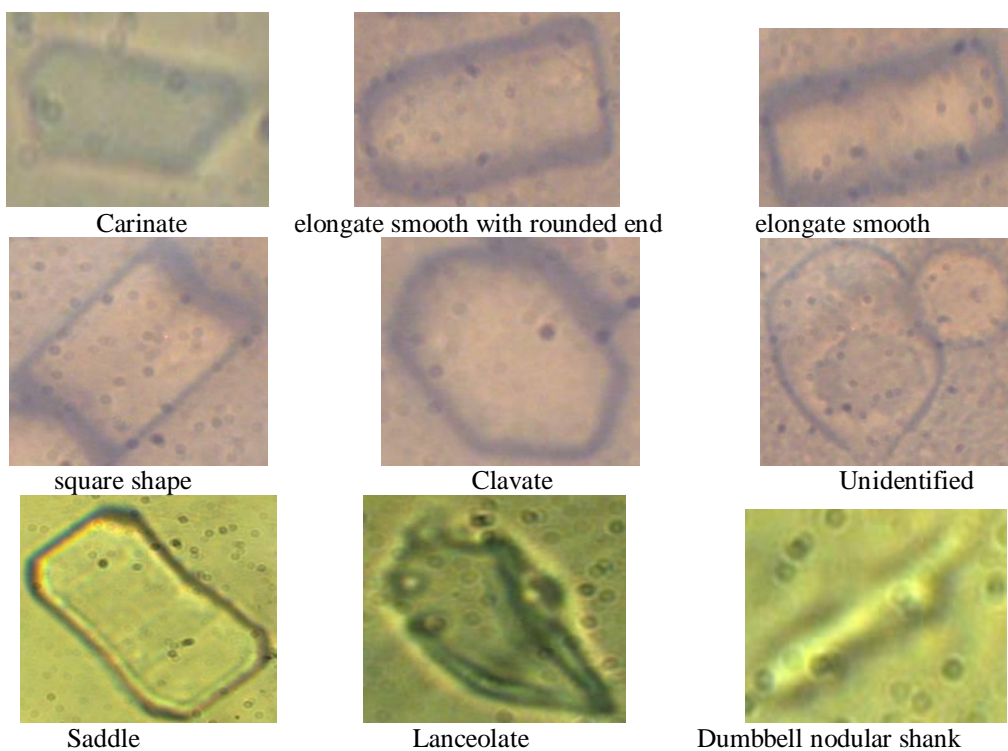


PLATE 1h: Phytolith Morphotypes of *Trichosanthes cucumerina*

The observed phytolith morphotypes of *Trichosanthes cucumerina* include two morpho shapes of chloridoid, cuneiform, elongate, rectangular, scutiform, and one morpho- shape each for Clavate, lanceolate, oblong , ovate , saddle, tabular , Trapeziform and four unidentified morphotypes.

Result of Phytolith Morphotypes for *Cucurbita maxima* Duchesne ex Lam.



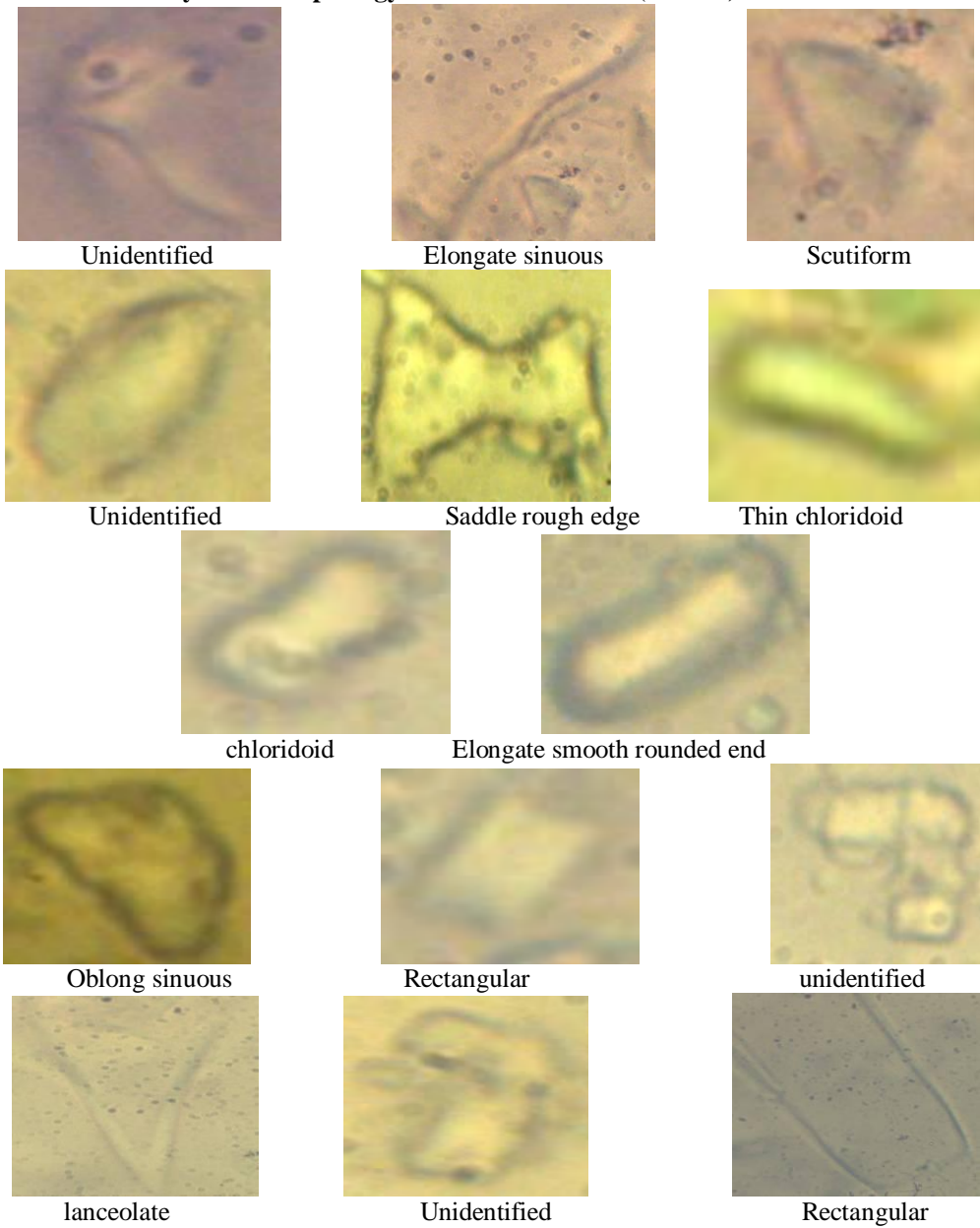


Acuminate

PLATE 1i: Phytolith Morphology of *Cucurbita maxima* Duchesne ex Lam.

The observed phytolith morphotype of *Cucurbita maxima* include two morpho shapes of elongate ,and one each of acuminate, clavate, square, carinate, dumbbell nodular shank, lanceolate, saddle, and one unidentified morphotype.

Result of Phytolith Morphology for *Citrullus lanatus*(Thunb.) Matsum. & Nakai



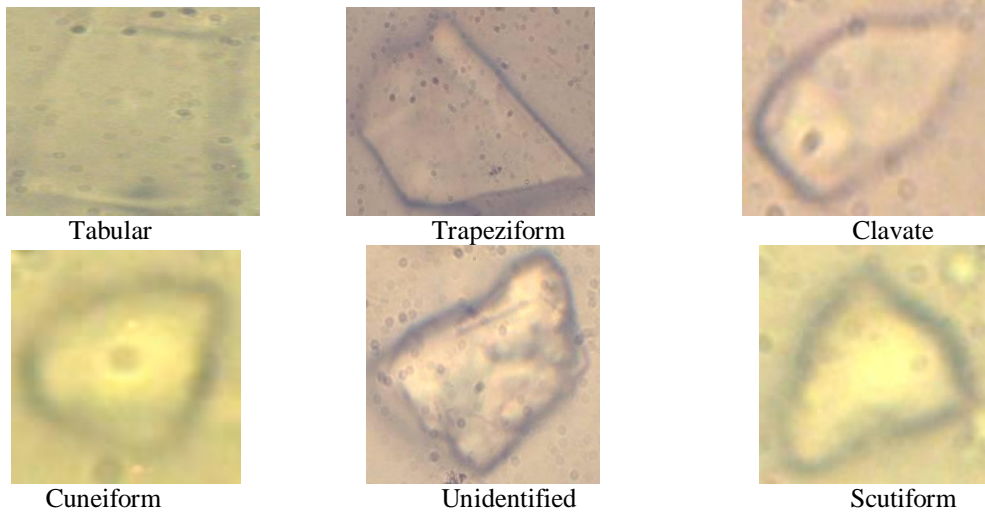


PLATE 1j : Phytolith Morphology of *Citrullus lanatus*(Thunb.) Matsum. & Nakai

The observed phytolith morpho shapes of *Citrullus lanatus* include two morpho shapes each of elongate, chloridoid, cuneiform ,rectangular, and scutiform, one each of clavate, unidentified,, lanceolate, oblong sinus, ovate, Tabular, Trapeziform, and three unidentified morphotypes

Result of Phytolith Morphology of *lagenaria brevilflora*.

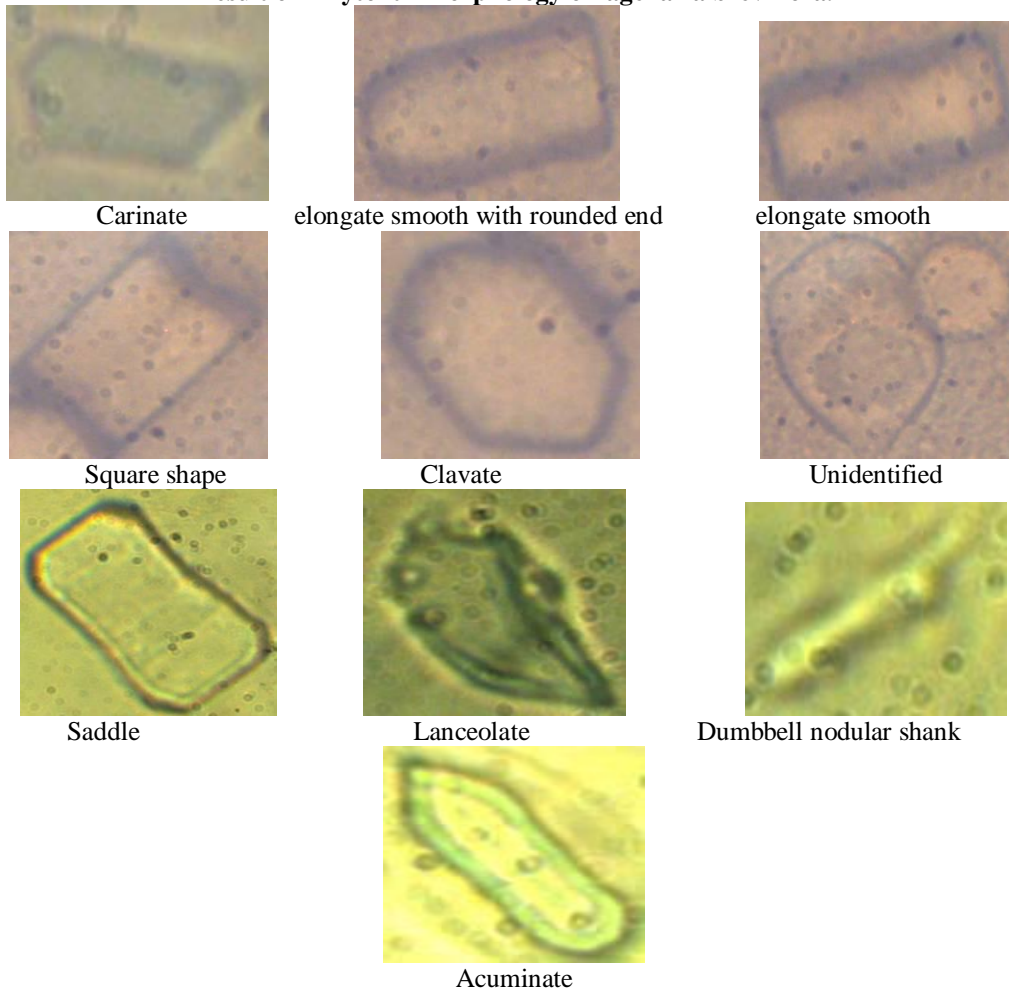


PLATE 1k: Phytolith Morphology of *Lagenaria breviflora*.

The observed morphotypes for *Lagenaria breviflora* include two morpho shapes of elongate and one morpho shape each of carinate, clavate, saddle, lanceolate, dumb bell, acuminate and one unidentified morphotype.

V. Discussion

The dissimilarity matrix as shown in table 1 was used to compute phytolith affinities among the investigating species.

Table 2: Dissimilarity matrix of phytolith morphotypes obtained from eleven cucurbitaceae species

| Species | <i>Citrullus lanatus</i> | <i>Cucumis sativus</i> | <i>Cucumis dipsaceus</i> | <i>Cucurbita pepo</i> | <i>Cucurbita maxima</i> | <i>Trichosanthes cucumerina</i> | <i>Lagenaria seceraria</i> | <i>Luffa cylindrica</i> | <i>Melothria sphaerocarpa</i> | <i>Telfairia occidentalis</i> | <i>Lagenaria breviflora</i> |
|---------------------------------|--------------------------|------------------------|--------------------------|-----------------------|-------------------------|---------------------------------|----------------------------|-------------------------|-------------------------------|-------------------------------|-----------------------------|
| <i>Citrullus lanatus</i> | 0.00 | 0.45 | 0.27 | 0.50 | 0.27 | 0.20 | 0.40 | 0.33 | 0.50 | 0.25 | 0.36 |
| <i>Cucumis sativus</i> | 0.45 | 0.00 | 0.17 | 0.50 | 0.09 | 0.48 | 0.98 | 0.18 | 0.10 | 0.15 | 0.07 |
| <i>Cucumis dipsaceus</i> | 0.27 | 0.17 | 0.00 | 0.31 | 0.14 | 0.27 | 0.27 | 0.09 | 0.33 | 0.57 | 0.15 |
| <i>Cucurbita pepo</i> | 0.50 | 0.50 | 0.31 | 0.00 | 0.21 | 0.83 | 0.33 | 0.63 | 0.27 | 0.44 | 0.23 |
| <i>Cucurbita maxima</i> | 0.27 | 0.09 | 0.14 | 0.21 | 0.00 | 0.43 | 0.29 | 0.33 | 0.44 | 0.18 | 0.12 |
| <i>Trichosanthes cucumerina</i> | 0.20 | 0.48 | 0.27 | 0.83 | 0.43 | 0.00 | 0.30 | 0.38 | 0.42 | 0.40 | 0.29 |
| <i>Lagenaria seceraria</i> | 0.40 | 0.98 | 0.27 | 0.33 | 0.29 | 0.30 | 0.00 | 0.40 | 0.11 | 0.24 | 0.06 |
| <i>Luffa cylindrica</i> | 0.33 | 0.18 | 0.09 | 0.63 | 0.33 | 0.38 | 0.40 | 0.00 | 0.20 | 0.27 | 0.20 |
| <i>Melothria sphaerocarpa</i> | 0.50 | 0.10 | 0.33 | 0.27 | 0.44 | 0.42 | 0.11 | 0.20 | 0.00 | 0.50 | 0.37 |
| <i>Telfairia occidentalis</i> | 0.25 | 0.15 | 0.57 | 0.44 | 0.18 | 0.40 | 0.24 | 0.27 | 0.50 | 0.00 | 0.20 |
| <i>Lagenaria breviflora</i> | 0.36 | 0.07 | 0.15 | 0.23 | 0.12 | 0.29 | 0.06 | 0.20 | 0.37 | 0.20 | 0.00 |

The closest maximum dissimilarity distance of 0.06 was observed between *Lagenaria breviflora* and *Lagenaria seceraria*, followed by a 0.07 distance between *Lagenaria breviflora* and *Cucumis sativus*, *Luffa cylindrica* and *Cucumis dipsaceus* (0.09), *Melothria sphaerocarpa* and *Cucumis sativus*(0.10), followed by *Melothria sphaerocarpa* and *Lagenaria seceraria*(0.11). However, the greatest maximum dissimilarity distance of 0.83 was observed between *Trichosanthes cucumerina* and *Cucurbita pepo*, followed by a 0.63 distance between *Luffa cylindrica* and *Cucurbita pepo* followed by a 0.57 distance shared between *Telfairia occidentalis* and *Cucumis dipsaceus*. It is interesting to note that only these three pairs of shared dissimilarity indices above the threshold distance of 0.50. This findings re affirm the strong affinities shared among the other pairs. A dendrogram showing these relationships is shown in fig 2.

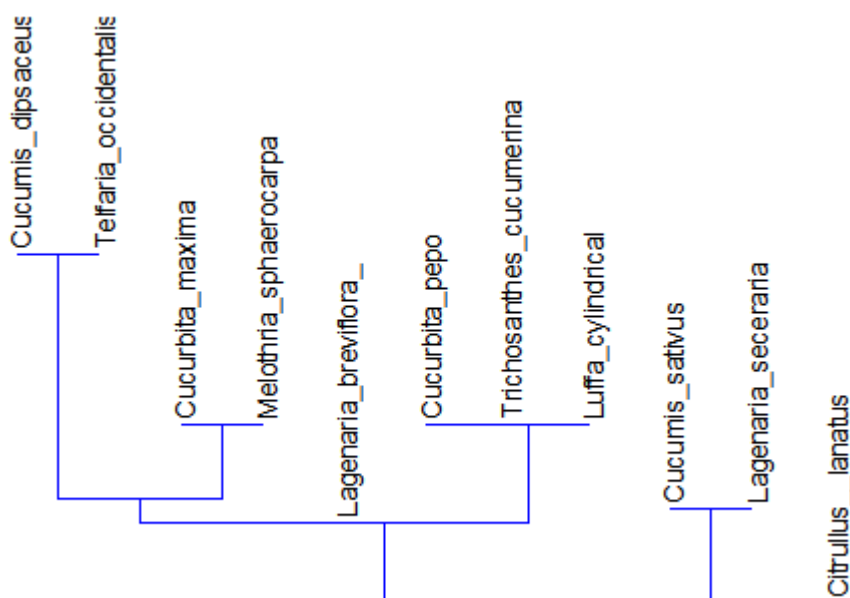


Fig 2 : Neighbor Joining Dendrogram showing phytolith morphotypes relationship among some cucurbitaceae taxa

From the dendrogram, four inferences could be deduce. First, *Citrullus lanatus* is the out group taxon. Second, *Lagenaria breviflora*, *Lagenaria siceraria* and *Cucumis sativus* share closer apomorphies than they do with other taxa. Third, *Cucurbita pepo*, *Trichosanthes cucumerina*, and *Luffa cylindrica* share close affinities among themselves and also with *Curcubita maxima* and *Melothria sphaerocarpa*. Fourth, *Cucumis dipsaceus* and *Telfairia occidentalis* share close affinities but are distantly related to *Citrullus lanatus*, *Cucumis sativus* and the other taxon under investigation.

Other evidences do support *Citrullus sativus* as the out group taxon among the cucurbitaceae (Kocyan et al 2007). However, recent classification by Schaefer and Renner 2011 nesting these species under investigation into four tribes of Jolliffieae (*Telfairia*), Sicyoeae (*Trichosanthes* and *Luffa*), Benincaseae (*Citrullus*, *Lagenaria*, *Cucumis* and *Melothria*), and Cucurbiteae (*Cucurbita*) need re-examination.

VI. Recommendation

Based on this study and other taxonomic evidences from molecular, cytological, morphologically, serological, palynological and incomplete studies on anatomical markers, the generic epithet *Cucumis* in *Cucumis dipsaceus* need be re-examined with a view to transferring it to *Telfairia* and nested in the tribe jolliffieae. Also, the species *Cucurbita pepo* and *Cucurbita maxima* should be re-examined with a view to nesting it among *Melothria* in Benincaseae instead of the present Tribe of Cucurbiteae where it is nested.

VII. Summary and Conclusion

A Taxonomic introspection of the classification system of Schaefer and Renner 2011 need re-examination since all available taxonomic markers were not used in arriving at the present classification.

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