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POLLEN QUANTITATIVE CHARACTERS OF FOUR MEMBERS OF ASTERACEAE IN CRUTECH-CALABAR CAMPUS, NIGERIA

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ABSTRACT

The utilization of quantitative characters of pollen morphology from four species of Asteraceae and its taxonomic relevance was examined. Erdtman acetolysis method was adopted for analyzing fresh pollen samples from the four species. Light Microscope (LM) of X100 objective lens was used to take quantitative micromorphological features such as pollen size, wall thickness, number of pores, length of spines and number of colpi was done respectively. Single Linkage Cluster and ANOVA analysis were carried out to determine similarities and differences among the four species. Results showed insignificant differences in the variations that occurred for each parameter across the four species investigated at $p>0.05$. The similarities observed in the studied characters revealed inter-relationship existing among the four species, and thus lend credence to their present classification.

Key Words: Taxonomy, Palynology, Pollen, Morphology, Delimitation, Taxa

INTRODUCTION

Pollen characters are useful features in resolving conflicting issues of interrelationships between and among taxa ranging from generic to specific level, and up to higher levels. It is a very significant tool for modern Systematist for the delimitation of species, and has become a collaborative approach in plant systematics and evolution.

Ekeke *et al.* (2016) and Agwu and Uwakwe (1992) documented that the main characters of taxonomic value in pollen grains are number and position of furrows, size and spine length, number, position of complexity of apertures, form of sculpturing of the exine and types of pollen. These characters have served as a comparative and very distinctive features employed by systematists for delimiting taxa, and determining phylogenetic relationships.

Mbagwu and Edeoga, (2006) used distinct pollen characters to establish probable evidence of relationships among certain groups of flowering plants in Nigeria. They concluded that pollen features of taxonomic importance are pollen grains sizes, number and position of furrows, spine length, pollen wall morphology, symmetry and shapes. Edeoga and Ikem (2002) in a related study, were able to show that *Boerhavia coccinea* possessed tricolpate pollen grains while *B. ejecta* and *B. diffusa* showed alcopate pollen grains, thus distinguishing *B. coccinea* from other species of *Boerhavia* found in Nigeria. Other authors who have used pollen characters in resolving complicated problems bordering on identification of various taxa

includes; Moona and Ashwaq (2022); Ragho (2020); Agwu and Uwakwe (1992); Khan *et al.* (2018).

Asteraceae is a very large family plant occurring in almost everywhere with highly advanced members. It is the largest family in the plant kingdom with an estimated 1,620 genera and over 25,000 – 35,000 recognized species (Jennifer *et al.*, 2019. Although, some important members of the Asteraceae family are made of shrubs or even trees, the majority of its species are herbaceous weeds, ornamentals, medicinal and green vegetables (Olorode, 1984). Pollen characteristics have been used considerably in the taxonomy of Angiosperms and they have been used in establishing phylogenetic relationships in the family Asteraceae (Ragho, 2020; Moore and Webb, 1978). Pollen grains of fossil and living plants have provided enough clues on the floristic and climatic characters of geological formations (Erdtman, 1943).

The first successful attempt in Nigeria on the use pollen characters in the classification of plants was made by Prasad (Prasad, 1963). Sowunmi, (1973) and Prasad, (1963) were some of the authors who contributed immensely to the understanding of pollen grains morphology and its importance in establishing relationship among plant taxa and its origin. Pollen grains of some members of the family Asteraceae have been studied by several authors in an attempt to understand similarities and dissimilarities among species in order to delimit and established inter-relationship among taxa (Perveen, 1999; Adedeji, 2005; Mbagwu *et al.*, 2009; Mbagwu and Edeoga, 2006).

Although, several studies have dwelled so much on both quantitative and qualitative characters of pollen morphology, this study therefore seeks to rely only on quantitative characters of pollen morphology to delimit and establish inter-relationship among four members of Astereceae.

MATERIALS AND METHODS

Plant species used for the study were collected by the second author (Table 1), authenticated in the CRUTECH Calabar Campus Herbarium, and voucher specimens were deposited in the same herbarium.

Pollen grains from fresh anthers of four members of Asteraceae were collected for the purpose of studying their morphological characters. Pollen grains from 4-5 different flowers of each of the species were collected. These were acetolyzed by treatment with Acetic Anhydride and Concentrated Sulphuric Acid following the procedure of (Erdtman, 1966). The acetolyzed pollen grains were mounted in glycerine. Measurements of very clear and large equatorial and polar diameter, numbers of pores recorded, spine length and wall thickness of pollen grains were taken. Microphotographs of the pollen grains from each plant were taken using Olympus microscope of x100 objective oil immersion lens with an attached camera.

Quantitative data from pollen characters of the studied taxa was subjected to Single Linkage Cluster analysis and ANOVA test to establish relationship, and check for differences.

Table 1: Plant materials and collection information

Scientific name	Common name	Name of collector	Collection date
Aspilia africana (Pers.) C.D. Adams.	Haemorrhage plant	Iso, Obazi Anthony	11/02/2022
Tridax procumbens Linn.	Tridax, Coat buttons		11/02/2022
Chromolaena odorata (L.) R.M. Kings & Robinson	Siam weed, Awolowo leaf		15/02/2022
Ageratum conyzoides Linn.	Goat weed		15/02/2022

RESULTS

Pollen grains of the various species investigated were of the spinate type and in the group media and minuta size, and have spines that are short with thin walls and densely situated pores. The pollen characters measured showed variations in their size, length of spines, thickness of pollen wall and numbers of pores across species (Table 2 and Figure 1).

The values from the pollen parameters of the four species measured were subjected to Single-Linkage Cluster Analysis using the pollen parameters. The result of the cluster analysis is presented in Figure 2. There are two major clusters. The first cluster comprises of *C. odorata and T. procumbens* which were linked at similarity coefficient level of 2.550; while the second cluster comprises of *A. conyzoides* and *A. africana* which were linked with a similarity coefficient level of 2.589.

Table 2: Pollen quantitative characteristics

Scientific name	Pollen size (µm)		P/E Ratio	Spine length (µm)	Pollen wall thickness (µm)	Number of pores
	Polar diameter	Equatorial diameter				
A. conyzoides	19.7 (15.0-20.5)	19.0 (16.5-20.0)	1.04	2.30 (2.00-2.50)	2.90 (2.50-3.50)	11.0
A. africana	14.3 (12.2-15.7)	12.70 (8.7-14.0)	1.13	7.70 (5.45-9.14)	2.90 (2.50-3.30)	9.0
C. odorata	15.5 (12.2-19.2)	11.20 (8.7-14.0)	1.38	1.96 (1.36-2.73)	3.60 (3.20-3.80)	9.0
T. procumbens	18.7 (15.7-21.0)	17.2 (15.7-19.2)	1.09	5.32 (4.09-6.82)	3.40 (3.00-3.60)	3.0

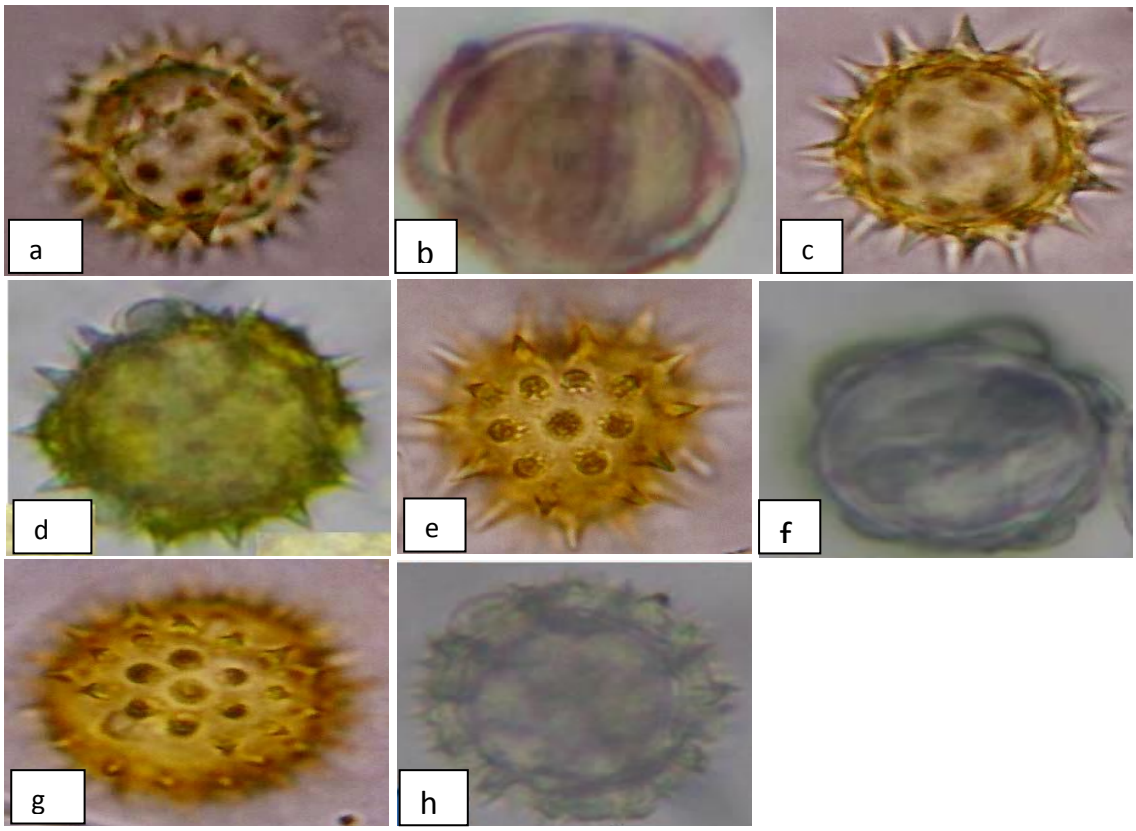

Figure 1: ***Ageratum conyzoides (a-b); Aspilia africana (c-d); Chromolaena odorata (e-f) and Tridax procumbense (g-h)***

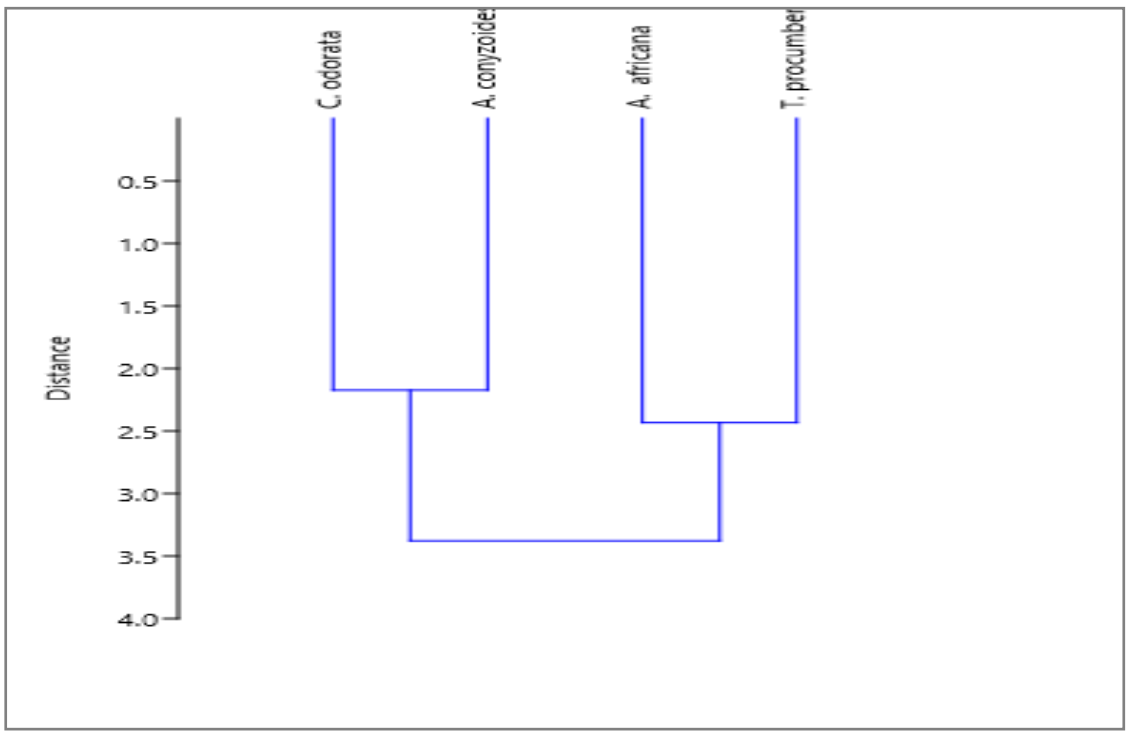

Figure 2: Single Linkage Cluster Analysis of four members of Asteraceae investigated using quantitative characters of pollen morphology.

DISCUSSION

The morphology of pollen grains has shown great significance in the classification and identification of angiosperms as well as in revealing relationship between taxa and among groups of taxa. According to Moore and Webb (1978) application of palynology has not only been tested in the field of taxonomy but has also been found useful in other fields of endeavors such as genetic and evolutionary studies, forensic science, allergy studies, tracing generational history of individual species and taxa, paleo-climatic studies and the study of the past human impact on vegetation.

Pollen diameter of species in Asteraceae has been confirmed to be small (Ekeke *et al.,* 2016; Akinnubi *et al*., 2014). Erdtman, (1952), however, classified pollen grains according to size into six groups: perminuta (diameter less than 10µm), minuta (diameter 10-25µm), media (diameter 25-50µm), magna (diameter 50-100µm), permagna (diameter 100-200µm), giganta (diameter greater than 200µm). Looking at the above classification by Erdtman, it can be deduced that the four members of Asteraceae investigated in this study belong to the group minuta (*A. conyzoides*) and media (*A. africana, C. odorata and T. procumbense*). This therefore, suggests that using pollen size alone, the study has been able to delimit *A. conyzoides* from the other three species studied. This report concurred with the study of Akinnubi *et al*. (2014).

Results from the measurement of spines length as found in the pollen walls of the four members of Asteraceae investigated showed that *A. conyzoides and C. odorata* has short spines (Table 1) while *A. africana*, and *T. procumbens has* long spines in their pollen walls. General, pollen walls of the studied species were observed to be thin, even though it puts *A. conyzoides* and *A. africana* into one group, while *C. odorata* and *T. procumbens* were puts into another group. This outcome as recorded in this study has been documented by (Akinnubi *et al*., 2014; Perveen, 1999) for selected members of Asteraceae. In terms of the numbers of pores, *A. conyzoides* have more pores than other three species studied, even though the difference was not significant when subjected to ANOVA test. According to Adedeji, O. (2005) and Akinnubi *et al*. (2014) number of colpi on pollen grains has showed much relevance in tracing evolutionary relationship among the species.

Furthermore, several studies on pollen morphology of members of Asteraceae have shown that it is difficult to distinguish members from one another under Light Microscope (LM) since they appear very similar. This was however confirmed as ANOVA test showed no significant difference for each pollen parameters measured across the four species at $p>0.05$.

Results from all the parameters studied thus showed shown similar characteristics and close affinity which uphold their present grouping as members of Asteraceae, as well as other useful distinctive features for delimiting one taxon from another.

CONCLUSION

Plant identification and delimitation of taxa is a major setback in conservation study, as well as related field of study such as agriculture, and in ethno-medicine. The importance of pollen studies cannot in providing database for accurate and reliable means of comparison plant character cannot be over emphasized. This study will thus be important for researchers, graduate and post graduate students seeking to gain knowledge and means of plant identification on the basis of shared similarities and dissimilarities of pollen characters. It will also play a very relevant and reliable in forensic study for detection of crime, and adulteration of honey. Pollen morphology is therefore regarded as a very high and significant taxonomic

character especially when used in combination with other taxonomic characters for circumscribing taxa.

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