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Cytomorphological Studies on the *Echinops* L. in the Nigerian Asteraceae (Compositae)

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ABSTRACT: Members of the genus *Echinops* are now widely used in treating brain disorders, ophthalmic problems, chronic fever, pains in the joints and inflammations. They are aphrodisiac and are used for treating pregnant women. The Nigeria species have not been discovered in this way. The plants are therefore being suggested to our scientists, chemists and agriculturists for possible screening for drug development. Cytomorphological studies on 4 taxa distributed in 3 species of the *Echinops* have been studied. Pollen fertility test of the species studied revealed that meiosis was normal with regular bivalent pairing. Thus the plants were normal and hybridization was not responsible for their differences. Chromosome counts for the 3 species are new reports (n=8 for *E. giganteus var lelyi, E. gracilis* and *E. longifolius*) and they are reported for the first time for the African *Echinops*. The base number 8 is suggested as the ancestral base number for the genus. The achene morphology of all the taxa studied are presented for the first time. Distribution maps of all the taxa studied are presented for the first time. The stomatal characteristics and achene morphology have been found to be a reliable taxonomic tool that would help to delimit the genus. The probable pathway of evolution has been traced for the genus for the first time

Keywords: Chromosome number, Base number, Stomata, Achene

Introduction

Echinops L. belongs to the tribe Cardueae Cass and has traditionally been considered the basis for the subtribe Echinopsidinae O. Hoffm, one of the subtribes into which the tribe has been divided. Usually species of *Echinops* are anthropophilous or ruderal plants that are found in fields, on the margins of roads in wastes places or less frequently in conserved habitats usually in mountainous areas (Mozaffarian and Ghahereman, 2002).
The genus consists of approximately 120 species distributed mostly in tropical Africa, Mediterranean basin and temperate regions in Eurasia (Jager, 1987; Bobrov, 1997). In Turkey, the genus is composed of 25 species which are endemic or endangered (Hedge 1975; Gemici and Leblebic, 1992; Vural *et al.,* 2010; Vural 2012; Vural and Sapcl, 2012) while in Iran there are 39 endemic species (Rechinger 1979). In Nigeria the genus is represented by 3 species. (Hutchinson and Dalziel, 1963).
Due to their strong morphological uniformity, their taxonomic classification is very difficult to determine. In most cases the diagnostic characters used for infrageneric delimitation are limited to the bracts of the uniflowered capitula or the number or degree of connation of the inner bracts (Hedge, 1975; Kozuharov, *et al*., 1976; Rechinger, 1979; Bobrov, 1997).
Phytochemical screening of some of the species revealed the presence of triterpenoids, isoflavones, glycosides, phenolic compounds and alkaloids (Maurya *et al.,* 2015). Various parts of the plants are used e.g the roots and seeds of *E. echinatus* have aphrodisiac properties (Kumari and Charantimath, 2011; Patel *et al.,* 2011). Many of their species are economically important, due to the alkaloids and other components that are used as fungicides

and insecticides (Singh *et al.,* 1988; Chang *et al.,* 1990). They are also visited by honeybees, producing good quality honey and used as forage plant for sheep and carmels (Sheidai *et al.,* 2000).

According to Sachez - Jimenez *et al.,* (2012) cytological and karyological data would greatly contribute to the deciphering of a taxon history. Nevertheless, karyological knowledge of *Echinops* is still unknown with less than 50% unaccounted for (Watanabe 2002, 2004). Cytogenetic studies of *Echinops* taxa are in general very limited (Goldblatt and Johnson, 1983; Strid and Frazen, 1981; Moore 1982) and for example there is only one report from Iran (Ghaffari, 1989) and Africa sections have not been represented at all (Garnatje and Martin, 2007).

So far all studies on *Echinops* species are diploids (or rather diploidized ancient poiyploids) however their basic chromosome number varies from X =14, 15, 16 or 17 (Sheidai *et al.,* 2000; Garnatje *et al.,* 2004a,b) which to some extent can be used to verify the systematic position of a species. This variation indicates that there is a centric fusion/fission during species diversification although it is not known which chromosome number is the primitive one (Sheidai *et al.,* 2000).

In addition to morphological studies, molecular techniques have been used to address the taxonomic problems of this genus (Garnatje *et al.,* 2007; Sanchez Jimenez *et al.,* 2009).

Morphological characters are often employed in distinguishing interrelationships between plant groups. Some anatomists believe that several anatomical data found in various parts of a plant species or genus are more reliable and dependable than the commonly employed exomorphological characters.

Metcalfe and Chalk (1950), for example identified the importance of stomatal types as a taxonomic character. Since then, attention has been given to the taxonomic importance of stomatal types and their phylogeny in different angiospermic families

According to Van-Cothen (1973), the morphology of stomatal types are the only valuable diagnostic characters and any conclusion regarding affinities of the various taxa should be confirmed by ontogenetic evidence.

In addition to their importance in systematics and phylogeny, epidermal characters of plants have been helpful in recognizing leaf impression in paleobotany and for recognizing plants employed in traditional medicine. (Johnson, 1975).

The seed morphology of the family Asteraceae had been investigated by Kapil and Bela Sethi (1963), Dittrich (1968) and Vaughan (1970). The nature of ergastic substances in the seeds of Asteraceae was investigated by Vaughan. (1970). Except for the works of Omoigui and Gill (1988), Gill *et al.,* (1991) Idu and Gill (1998) and Omoigui and Aromose (2012) not much work has been done on the morphology and nature of ergastic substances in the seeds *of Echinops* and the family as a whole.

The aim of this paper is to place on record the representatives of this genus in our country and to expose these plants to our scientists for possible screening phytochemically since many of the other members of the genus are very important in other parts of the world.

Materials and Methods

Plant collection and identification: The plants were collected from different parts of the country. The matured plants were critically examined and compared with the voucher specimens deposited in the herbarium of Forestry Research Institute of Nigeria, University of Ibadan, Ahmadu Bello University, Zaria, School of Forestry Jos, Usmanu Danfodio University Sokoto, Bayero University Kano, Obafemi Awolowo University Ile-Ife, Information from literature, herbarium and field surveys were compiled and used to plot the distribution maps.

Cytological studies: Flower buds of different sizes were fixed in 1:3:6 acetic acid, chloroform, ethyl alcohol between 9.00 a.m. – l.00 p.m. for 12 h and subsequently squashed in 2% acetocarmine. Slides were made permanent following the techniques outlined by Gill (1969).

Achene morphology: The seed outlines were drawn by using wild Heerburg Type 256575 microscope fitted with drawing tube and the final details of the seed coat were filled in by examining the seed under a dissecting stereoscopic, microscope and a magnifying lamp each equipped with a fluorescent light. The terminology used for describing the seed morphology is that of Martin and Barkley (1961). Seeds were put in petri-dishes fitted with moist filter paper and left at room temperature for 12 hours. This treatment would softened the seeds rapidly.

Chemical Analysis:

- For starch: This sections of the seeds were made with sharp razor blade and this was placed on a slide. Few drops of Lugol's iodine solution was added. Starch if present reacted with iodine to give a blue black colour.
- For protein: Seeds were macerated and 2ml of 70% nitric acid was added. Presence of protein is indicated by a white precipitate followed by a yellow solution on boiling.

- For fats and oils: Seeds were macerated and 2 drops of alcoholic Sudan III was added. Presence of fats and oils is indicated if on evaporation the colour of the Sudan III (Which is red) is retained.

Phytodermological study: The method of Gill and Karatela (1982) was followed. Fresh material were preserved in FAA (formalin, alcohol, glacial acetic acid mixtures). Phytodermological study was done with matured leaf peeling of abaxial and adaxial surfaces. Peeled surfaces were bleached with 5% sodium hypochloride and stained with 1% safranin. After several changes of distilled water. These epidermal surfaces were examined under light microscope and drain with camera lucida drawings were made.

Results

The taxa studies along with their sources, voucher numbers, chromosome number, base number, ploidy levels and pollen fertility are summarized in Table 1. The epidermal structure and stomatal characteristics of the species studied are summarized in Table 2.

Table 1: Summary of locality and cytological data of the Nigerian *Echinops* (Cardueae – Asteraceae)

Taxon	Locality	Voucher Number	Chromosome Number n	Base Number 2n	Ploidy Level		% Pollen Fertility	Pollen Size
Echinops giganteus A. Rich. var. *lelyi* (C.D. Adams) C.D. Admas	Lekitaba Gembu Road, Gembu Mambilla Plateau, Taraba State	35	8	-	8	Diploid	89	22
[1]*E gracillis* O. Hoffm.	Bauchi Ring Road, Jos, Plateau State	39	8	-	8	Diploid	92	28
[1]*E. longifolius* A. Rich.	Gangere Road, Kaurra Falls, Plateau State	38	8	-	8	Diploid	90	28

Table 2: Summary of the life form, epidermal structure and stomatal characteristics on some Nigeria *Echinops (*Cardueae – Asteraceae)

Taxon	Habit	Voucher Number Nature Material Used	Leaf Surface	Wall Pattern	Distribution of Stomata on Leaf Surface	Morphological Type of Stomata	Ontogenetic Type of Stomata Development	Type of Trichome	Length (µM)	Breadth (µM)	Pore Length (µM)	Pore Breadth (µM)	Frequency (MM^2)
Echinops giganteus A. Rich. *var. lelyi* (C.D. Adams) C D Adams	H	036F	U	Straight	Sunken	Anomocytic	Agenous	Eglandular	30	20	18	8	100
			L	Wavy				Multillular					
E. gracilis O. Hoffm	H	035F	U	Wavy	Hypostomatic	Anomocytic	Agenous		28	22	18	10	40
			L	Wavy									
E. longifolius A. Rich	H	039F	U	Wavy	Amphistomatic	Anomocytic	Agenous	Eglandular	28	26	20	8	10
			L	Wavy				Unicellular	38	25	26	4	10

E. giganteus A. Rich, *var lelyi* (C.D Adams) C.D. Adam (Distribution map 1)
= *E, velutinus* O. Hoffm
= *E. velutinus var lelyi* C.D. Adams

An erect branched herb up to 60 cm high found in open and cultivated land; white florets in spherical heads; stem and midrib of leaves serulose.
A haploid count of 8 was determined at metaphase stage (Fig. 2a), Meiosis and pollen fertility were normal with 89% pollen fertility with an average, pollen size of 22 µm.

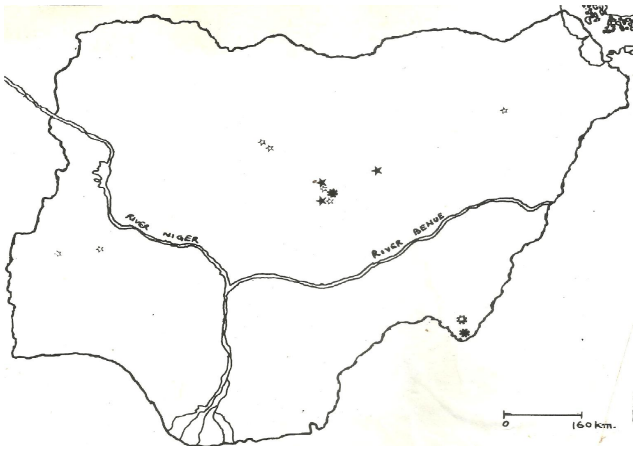

Fig. 1: Distribution map of *Echinops giganteus* var. *lelyi* (*) *E. gracilis* (*) and *E. longifolius* (*) in Nigeria.

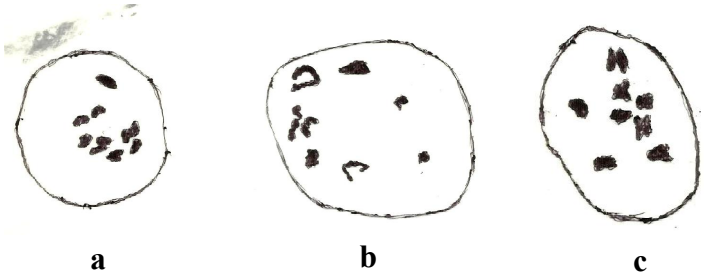

Fig. 2: Chromosome counts of **(a)** *Echinops giganteus var. lelyi* (n = 8) **(b)** *E. gracilis* (n = 8) **(c)** *E. longifolius* (n = 8).

Distribution and Ecology: It is restricted in distribution to lithosols regions of the country with annual rainfall of 1016-1270 mm. Outside Nigeria, it extends eastwards up to Cameroon,
Achene Morphology: Light brown; 1-1.5cm long; (Fig. 3a) oblong-cylindric; body lightly grooved by lengthwise ridges, narrowed to the base; covered by hairs (pappus).

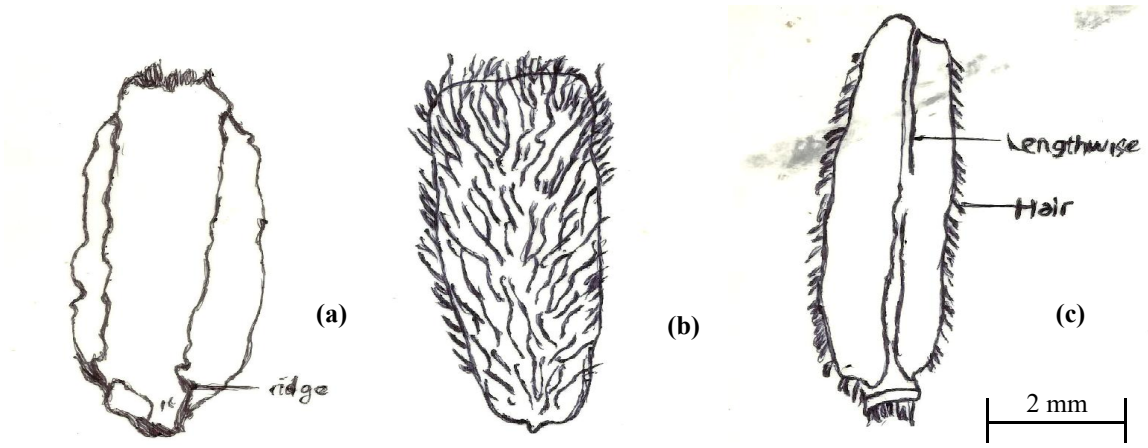

Fig 3: Camera lucida drawing of the achene of **(a)** *Echinops giganteus* var. lely, **(b)** *E. gracilis* **(c)** *E. longifolius*

Ergastic Substances: Achenes tested positive for fats and oils.
Epidermal Morphology: Both surface has straight walled cells, Sunken, anomocytic stomata were observed (Fig. 4a). Eglandular multicellular trichome present. A meristemoid enlarges and is transformed into the guard cell mother cell without giving rise to a subsidiary cell. The guard cell is thus surrounded by a protodermal cell which then divides to form 2 guard cells with a pore between them (Fig. 4b).

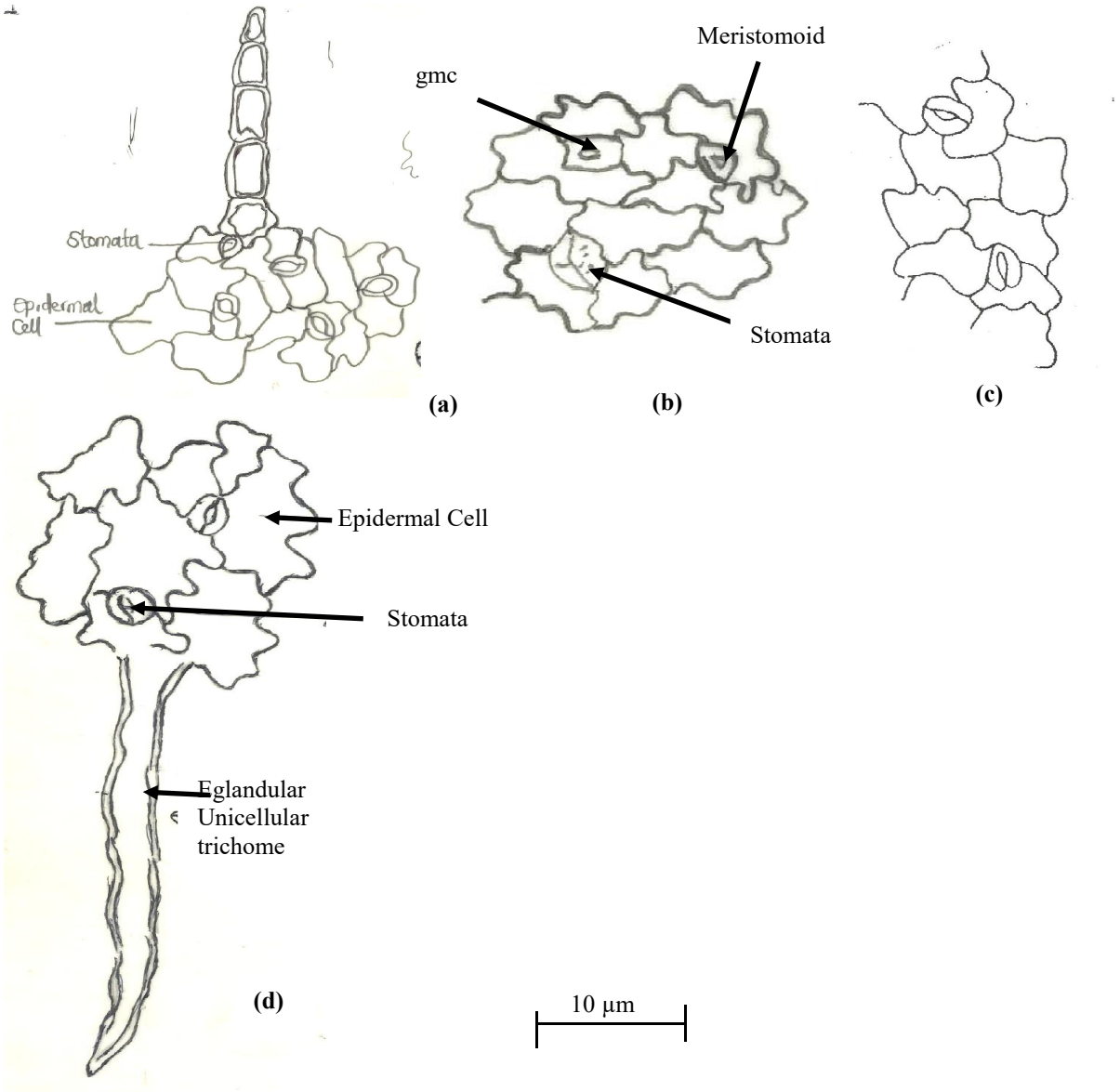

Fig 4: Camera lucida drawings of the lower epidermis of **(a)** *E. giganteus varlelyi* **(b)** *Agenous ontogeny, (gmc) guard cell mother cell* M. meristenoid **(c)** *E. longifolius* **(d)** *E. gracilis*

Exsiccata: FHI 4015 Jos; FHI 41913 Riyom; FHI 57369 Bukuru; FHI 50591; FHI 50591 Cameroon; FHI 30365, 43207 Bamenda; IFE 1433 Riyom; IFE2080 Zaranda (Bauchi mountain), Omoigui 036 Kourra Falls.

Echinops gracilis O. **Hoffm:**
A bushy perennial herb up to 2 m high with the spherical heads sessile; florets white, tap rooted; leaves spinose. A haploid count of 8 was determined at anaphase stage (Fig. 2b). Meiosis and pollen fertility were normal with 92% filled pollen with an average pollen grain size of 28 um.
Distribution and Ecology: This plant is restricted in distribution to lithosols and euthrophic brown soils of the country with annual rainfall 1270 – 1524 mm. It extends eastwards up to Congo and Uganda.
Achene Morphology: Light brown; 1.5 cm long; flat; elliptic; body grooved by lengthwise ridges, body hairs minutely present; pappus dense and short; with the entire achene forming a brush (Fig. 3b).
Eargastic Substances: Achenes tested positive for fats and oils.

Epidermal Morphology: Both surfaces were sinuously walled; (Fig. 4c). Leaves are hypostomatic; the ontogenetic pathway of the anomocyctic stomata is agenous.
Exsiccata: FHI 50592, 10317, Cameroon; FHI 88034, 24729, 61517, 65545, 65545, 88034 Gembu; FHI 31084, 97427, 77234, 92749, 31083 Mambilla; FHI 34841, FHI 34841, 93167, 57355, 29380, 22169, 29379, 40621, Bamenda; FHI 47323 Jos; FHI 59232 Pankshin; FHI 39026 Vom; 41914 Riyom; UIH 17855 Bali (Cameroon), UIH 7143 Vom; UIH 1821, 927 Mambilla, UIH 1091, 316 Vogel Peak; UIH 3624 Cameroon; UIH 2001 Dutsin Pedong; Omoigui 035 Gembu.

***Echinops longifolius* A. Rich (map 1)**
= *E. otarus* Mattf
= *E. bathrophyllus* Mattf.

A glabrous shrubby perennial herb with short spiny leaves and spherical compound heads with tap rooted; leaves lanceolate, whitish under, the top bright green. A haploid count of 8 was determined at metaphase stage (Fig 2c). Meiosis and pollen formation were normal with 90% filled pollen grain with an average grain size of 27.82 µm.
Distribution and Ecology: The plant grow widely in lithosols, ferrasols and ferruginous tropical soils of the country with annual rainfall of 762 -1016 mm (Fig. 1). Outside Nigerian, it extends eastwards up to Tanzania.
Achene Morphology: Achene mnvSmm long; (Fig. 3c) cylindric; deeply grooved by lengthwise ridges; rounded at base; brown; 8mm long; pappus stout; light in colour, body hairs present.
Epidermal Morphology: The epidermal cells of both surfaces are sinuously walled; (Fig. 4d). Eglandular, unicellular trichomes forms a mass on both surfaces. Leaves are amphistomatic. The ontogentic pathway of the mature anomocytic stomata is agenous.
Exsiccata: FHI 50571, 92453, 56963, Jos; FHI 46667, 5399 Shika; FHI 21021 Sho Mountain FHI 45597 Borgu; FHI 45494, Igabi; FHI 24862 Kwaya (Borno); FHI 47841 Kaurra; FHI 101006 Pankshin; FHI 50581 Cameroon; FHI 67387 Bauchi; FHI 67256 Borgu (Kwara); FHI 54814, 26515 Zaria; FHI 13970 Ivory Coast; FHI 59858 Igbetti; IFE 1380 Borgu Game reserve; IFE 6571 Mangu (Plateau); IFE 6315 Keffi Kaduna; IFE 2000 Dutsin Pedong; IFE 523 Kishi; Omoigui 038 Kaurra Falls; Omoigui 039 Jos.

Discussion

The report of n = 8 (2n = 16) for the *Echinops giganteus* A Rich var. *lelyi* (CD. Adams) C.D. Adams, *E. gracilis* O. Hoffm and *E. longifolius* A. Rich are first reports for the African *Echinops.* Chromosome numbers so far reported in *Echinops* are from 2n = 26 (*E.gmelini* Turcz*;* Sanchez-Jimenez *et al.,* 2009) with 2m = 28, 30 and 32 more frequent (Sheidai *et al*., 2000; Garnatje *et al.,* 2004b). The only exception is *E. acantholepis* (= *Acantholepis orientalis* Less) with 2n = 14 (Garnatje *et al*., 2004a). With the physical report of x = 8 from Africa (Nigeria) one can now trace the probable pathway of evolution for the genus (Fig. 5).
According to Sanchez – Jimenez *et al.* (2009) the probable pathway of evolution of this genus have been suggested to be as a result of polyploidy followed by descending dysploidy that is change in chromosome number through chromosomeal rearrangements without considerable alteration (Garcia – Jacas *et al.,* 1996; Garnatje *et al.,* 2004, 2007, 2011; Lavia and Fernandez, 2008).

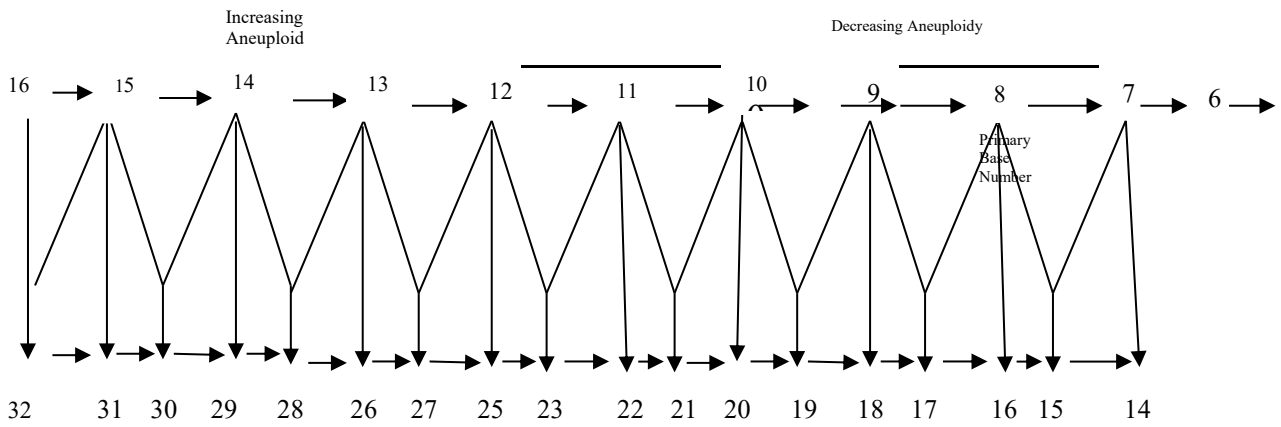

Fig. 5: Evolution of basic chromosome numbers in *Echinops*

According to Pollunin (1961). Plasticity and habitat requirements of a taxon are best judged by the extent of its distribution. From the distribution maps presented the *Echinops* are restricted to high altitudes in the country with pH 7.

Prior to this work, information was not readily available regarding the soil preference in plants in wild habitats. The only previous information of some Nigerian plants is by Husaini (1984). From the results presented it is apparent that majority of the investigated taxa can grow luxuriantly in acidic soils. With pH as low as 4.0. The information presented would be useful to horticulturists and weed scientists

Achene Morphology: The report of achene morphology of the *Echinops* is the first report in the genus. It would be interesting to see the other achenes of the other members of the genus. The result of this study on the shape and structure of the achene agreed with the report of Ayodele (1992) that the pappus hairs showed projections of various shapes and sizes on superficially smooth looking surfaces.

Heywood *et al.* (1977) noted a correlation between the pappus structure and growth habit of some members of the family. They observed that the herbaceous members of the family produced large fruits and the entire length of the pappus had more projections than the shrubby species with their scanty of smooth pappus. The result of this study did not support this correlation between growth habit and the pappus structure. Instead the authors believe that the variation of the pappus structure could be one of an efficient means of dispersal of the achenes by wind.

Ergastic substances: According to Erdtman (1962), ergastic substances are secondary products of plant metabolosim which were formed at certain stage of their metabolic process and were retained when the taxon in question undergoes further development while Gill and Ayodele (1984) are of the opinion that they were infact waste products resulting from cellular activities which are usually simpler in structure than protoplasmic bodies.

The importance of ergastic substances in plant taxonomy has been stressed by various authors (Badenhuzen 1958; Calvin 1983; Earle and Jones, 1962) while Gill *et al*. (1991) believe that the presence or absence of ergastic substances and their nature are of considerable importance in the evolutionary history of the taxon. The result of the ergastic substance have revealed that fats and oils have been found to be present in all the species studied. The nature and quality of the fats and oils is not known. More work is suggested in this area to know if any of these plants can be commercially exploited to provide an alternative source of working oil in the country.

Stomatal Distribution: The distribution of stomata is of considerable taxonomic value although occasionally it is influenced by ecological factors. In the amphistomatic taxa, the number of stomata is greater on the lower surface than their upper surface (Ahmad, 1979) in the family Acanthaceae. Karatela and Gill (1984) observed that within certain limits stomatal distribution appears to be quite constant and to be a reliable taxonomic character. In this genus the three species studied had different patterns. This is the first record of this kind of study in the genus. *Eahinops giganteus var. lelyi* had sunken stomata while *E. gracilis* was hypostomatic, *E. longifolius* was amphistomatic. The use of this character in the delimitation of the genus is limited. More members of the genus need to be studied for the value of this classicization tool to be appreciated.

Two types of trichomes have been found in the species. Eglandular multicellular trichomes in *E. giganteus* var. *lelyi* and Eglandular unicellular in *E. longifolius*. All the species studied had anomcytic type of stomata. Anomocytic stomatal report for the genus agrees with all workers of Asteraceae reports (Metealfe and Chalk, 1950; Kemka and Nwachukwu, 2011).

Anomocytic stomata is more primitive and is expected to be found in woody trees and shrubs. According to Hutchinson (1969) trees are more primitive than shrubs and herbs and woody habit is more primitive than shrubs and the herbs. The result from this study does not agree with this. The anomocytic type of stomata was present in all the 3 species and they all were herbs.

Stomatal Frequency: In the present study, *Echinops giganteus var. lelyi* had as higher stomatal frequency 100 mm^2 followed by *E. gracilis* 40mm^2 and the least was in *E. longifolius.* Stace (1965) states that the frequency of stomata though much is frequently misused. He used this character in the separation of the species in the genus *Luminitxera* (Combretaceae). Other workers have also found it a good taxonomic tool for the delimitation of species in the family Acanthaceae (Ahmad, 1979; Nyawuame, 1988).

Conclusion

More work is suggested for the other members of the genus since this area of study seems promising in resolving the taxonomic difficulty of the *Echinops*.

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