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SYSTEMATIC STUDIES IN SOME *IPOMOEA* LINN. SPECIES USING POLLEN AND FLOWER MORPHOLOGY

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ABSTRACT
The current study was conducted in search of constant morpho-anatomical characters to aid the identification and classification of commonly encountered Ipomoea species in south west Nigeria. Flower and pollen morphology as important repository of constant characters formed the focus of the investigation. Whole flowers were dissected to expose the carpel and stamens for study. For pollen study, after soaking in de-ionized water, the anthers were collected and crushed, stained and placed on a glass slide by needle for observation under light microscope. The lengths of styles and filament all varied in the seven species, highest length in styles was recorded in Ipomoea hederifolia (37.0-38.5mm) while the minimum was recorded in Ipomoea vagans (16.5-19.0mm). United bract into a boat-shaped, doubly acuminate involucre distinguished Ipomoea involucrata from the remaining six species with free bracts. Pollen grains were found to be radially symmetrical; circular in outline, sculpture were echinate, circular aperture, pores equidistantly distributed, oblate, speriodal and oblate-spheriodal. Largest pollen size was recorded in Ipomoea aquatica (60.2-62.5µm), suggesting a less derived position whereas the minimum size (30.7-31.4µm) was observed in Ipomoea hederifolia. The maximum spine length was recorded in Ipomoea involucrate (8.3-9.6µm) and minimum was recorded in Ipomoea hederifolia (3.3-4.0µm). The phylogenetic tree resulting from cluster analysis suggests that the alien species I. aserifolia and I. carnea are sisters to the other native Ipomoea species. As the number of species increases, synapomorphies decrease whereas increased synapomorphies are associated with decreasing number of species.While both I. aserifolia and I. carnea may have naturalized, they represent new records for the Flora of West Tropical Africa.
KEY WORDS: *Ipomoea, morpho-anatomy, pollen, synapomorphy, phylogeny*

INTRODUCTION

The genus *Ipomoea* comprises the largest number of species within the family Convolvulaceae (Morning glory) which are widely cultivated as ornamentals because of their showy and beautiful flowers. Throughout the world; *Ipomoea* is usually estimated to contain more than 600 species in which over half of them are concentrated in the Americans and Asian countries (Judd *et al.*, 2002). According to Hutchinson and Dalziel (1968), *Ipomoea* is represented by 15 species in Nigeria. However, recent study shows that *Ipomoea* is represented by 34 species which are widely distributed among the savanna-north, forest-south, forest-savanna, forest and the savanna climatic zones of Nigeria (Ogunwenmo, 2003). Hallier (1893) recognised the usefulness of pollen characters as being palynologically and taxonomically important and divided the family into subfamily 'Echinoconiae' on the basis of distinct spiny pollen and put *Ipomoea* as echinate pollen (Echinoconiae). The pollen of 170 species in 30 genera was studied by Sengupta (1972) and described four pollen types, each with several subtypes. Van Campo (1976) included Convolvulaceae in the broad group of Angiospermic families with successive form pollen evolutionary pattern from tricolpate-pantoporate. Telleria and Daners (2003) also studied the pollen of 75 species of eleven genera, and three main pollen types were described; some subtypes were recognized in two of these. Osman and Abdel Khalik (2005) studied the pollen morphology of Convolvulaceae in Egypt and observed three main pollen types. Later in 2007 they investigated the seed morphology of 31 taxa belonging to 6 genera of Convolvulaceae from Egypt, tree types of basic anticlinal cell wall boundaries and four different shapes of the outer periclinal cell wall were described.

Japanese workers in the early 1930's produced one of the first plant genetic maps using flower colour variants of *Ipomoea nil* (Imai, 1934) and recent work has positioned *Ipomoea* as a model genus for understanding the genes involved in the floral colour pathway (Durbin et al, 2000; Zufall and Rausher, 2004; Streisfeld and Rausher, 2009). Current work with *Ipomoea* species addresses a range of evolutionary questions that are applicable to many organisms and biological processes, from the evolution of the mating system to plant-herbivore and plant-parasite interaction (Chang and Rausher, 1999; Kniskern and Rausher, 2006a; Simonsen and Stinchcombe, 2007). Some members of *Ipomoea* are invasive; they exhibit a range of mating systems from selfing to obligately outcrossing both within and among species, and vary in form from vines to shrubs to trees.

The main objective of this research is to study the diversity of characters in commonly found *Ipomoea* species in south western region of Nigeria, using both floral and pollen characters with a view to enhancing their identication and classification.

MATERIALS AND METHODS

Seven species of commonly found *Ipomoea* in diverse habitats in south western Nigeria were collected from areas located within GPS Coordinates 6.4166667-7.470706 Latitude and 2.883333-5.194999 Longitude.

These plants were sun-dried, pressed using newspaper sheets and plant press for 6 days until they become dried. All pollen samples were obtained from mature buds of *Ipomoea* plants, the pollen was extracted from ripe anthers in mature buds to avoid contamination, open flowers were used in the absence of mature buds and many flowers were collected in abundance for the purpose of floral study. The specimens of *Ipomoea* were identified at the Forestry Research Institute of Nigeria (FRIN) Herbarium.

All pollen samples were acetolysed as described by Erdtman (1952) with slight modifications. Anthers from the mature flowers were collected and soaked in de-ionized water; this is done to allow absorption of de-ionized water by the anthers for 2-3 hours and was later transferred into 50% alcohol. The collected material was crushed in a Petri dish. The crushed materials were filtered through fine meshes to isolate pollen grains. The pollen grains were prepared for light microscopy by the standard method described by Erdtman (1952). Observations were made with Trinocular Fluorescence Microscope. Measurements and morphological observation were made with the microscope using a ×40 a calibrated eyepiece micrometer. All measurements were based on at least ten pollen grains of each species. Where measurements were made, the range corresponded to two major levels of discontinuity. It is not possible to give the exact values for variation and measuring errors, but the following are thought to influence the results: instrumental error, optical interference, natural variation, distance measures that are not parallel to the plane of the eyepiece micrometer, small deviation in the depth of field resulting in large deviations in measurements, and the theoretical packing effect whereby spines and pores cannot be equidistantly spaced on the pollen surface (Mc Andrew and Swanson, 1967), since it is only possible to place four or twelve equidistant points on a sphere to form regular polyhedrons consisting of triangles (Kristensen and Rindung 1964). Cluster analysis, correlation analysis and ANOVA were conducted on the data obtained. All names of plants are according to the *Flora of West Tropical Africa* (Hutchinson and Dalziel, 1968).

RESULTS AND DISCUSSION

Description of pollen types

The photomicrographs of the pollen types in the species studied are shown in Figs. 1-14 while Table1 contains their quantitative characters and Table 2 comprise qualitative characters, as seen under the light microscope. The pollen characteristics of *Ipomoea* species studied are described as follows.

Ipomoea carnea: Pollen grains are oblate (having a polar axis shorter than the equitorial diameter), radially symmetrical, outline circular, the diameter is 36.6-38.7µm, pores are equidistantly distributed, pantoporate, polytreme, tetragonal area formed by the spine and the ridges of bacula around each extrapolar region (2 divisions in one plane), sculpturing is echinate, spines are 3.6 - 5.4µm long, distance between spine is 3.2-3.6µm, spines are slender with blunt end, pores are 2.0-2.8µm in diameter [Fig. 1-2(LM)].

Ipomoea involucrate: Pollen grains are oblate spheroidal, radially symmetrical, outline circular, with a diameter of 32.5-35.1µm, the pores are equidistantly distributed, pantoporate, polytreme, tetragonal area formed by the spines and the ridges of bacula around each extrapolar region, sculpturing is echinate, spines are 8.3 – 9.6µm long, distance between spines is 3.3–4.0µm, spines are slender, long with pointed end, pores are 4.2–5.2µm in diameter. [Fig. 3-4 (LM)].

Ipomoea vagans: Pollen grains are oblate, radially symmetrical, outline circular, pollen is 31.2–34.4µm in diameter, pores are equidistantly distributed, pantoporate, polytreme, tetragonal area formed by the spines and the ridges of bacula around each extrapolar region, sculpturing echinate, spines are 6.1 - 7.3µm long, distance between spines is 5.7–6.3µm, spines are slender, long and blunt, pores are 2.0 – 2.7µm in diameter. [Figs. 5-6 (LM)].

Ipomoea triloba: Pollen grains are 37.8–39.3µm in diameter, oblate, radially symmetrical, the outline circular, pantoporate, polytreme, pores are equidistantly distributed, tetragonal area formed by the spines and the ridges of bacula around each extra polar region, sculpturing is echinate, spines are 4.1–5.4µm long, distance between spines is 2.2–2.7µm, spines are slender, long and blunt at the ends, pores are 3.0–4.1µm in diameter. [Fig. 7-8 (LM)].

Ipomoea asarifolia: Pollen grains are oblate spheroidal, radially symmetrical, outline circular, 32.3 – 34.2µm in diameter, pantoporate, polytreme, pores are equidistantly distributed, tetragonal area formed by the spines and the ridges of bacula around each extra polar region, sculpturing echinate, spines are 4.0–5.2µm long, distance between spines are 1.7–2.6µm, spines are slender, long, with pointed end, pores are 3.0–3.7µm in diameter. [Table 5, Fig. 9-10 (LM)].

Ipomoea hederifolia: Pollen grains are oblate spheroidal, radially symmetrical, outline circular, 30.7–31.4µm in diameter, pantoporate, polyteme, pores equidistantly distributed, tetragonal area formed by the spines and the ridges of bacula around each extra polar region, sculpturing echinate, spine 3.3–4.0µm long, distance between spines is 1.7–2.4µm, spines are slender, long, with pointed end, pores are 2.0-2.7µm in diameter. [Fig. 11-12 (LM)].

Ipomoea aquatica: Pollen grains are oblate spheriodal, radially symmetrical, outline circular, 60.2-62.5µm in diameter, pantoporate, polytreme, pores are equidistantly distributed, tetragonal area formed by the spine and the ridges of bacula around each extrapolar region, sculpturing is echinate, spine is 4.4-5.9µm long, distance between spines are 5.0-5.6µm, spines are slender, long, with pointed end, pores are 2.8-3.2 in diameter. [Fig. 13-14 (LM)]

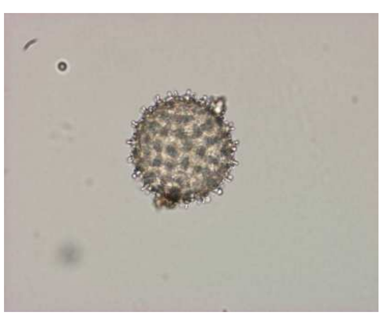


Fig. 1. The pollen grain of *I. carnea* (20X)

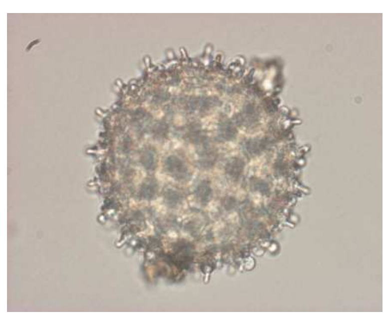


Fig. 2. The pollen grain of *I. carnea* (40X)

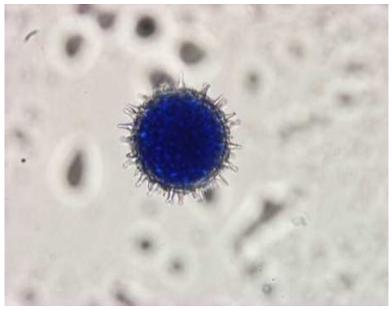
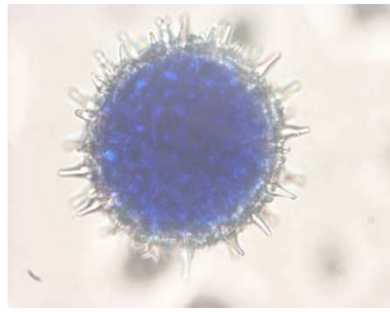



Fig. 3. The pollen grain of *I.involucrata* (20X) **Fig. 4. The pollen grain of *I.involucrata* (40X)**

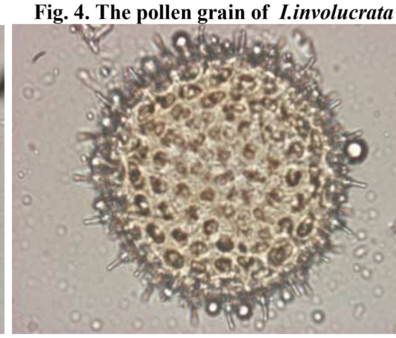



Fig. 5. The pollen grain of *I.vagans* (20X) **Fig. 6. The pollen grain of *I.vagans* (40X)**

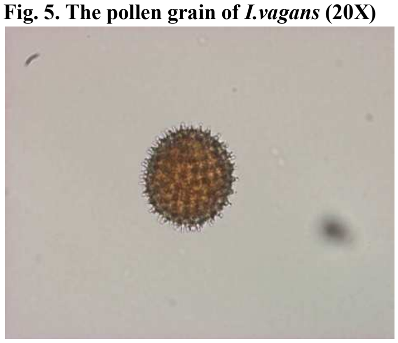
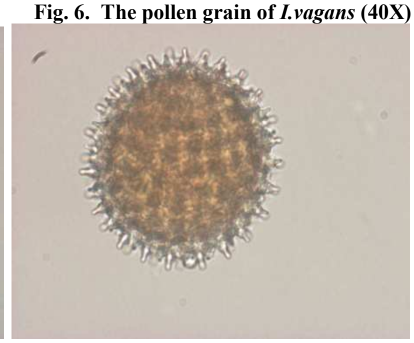



Fig. 7. The pollen grain of *I.triloba* (20X) **Fig. 8.The pollen grain of *I.triloba* (40X)**

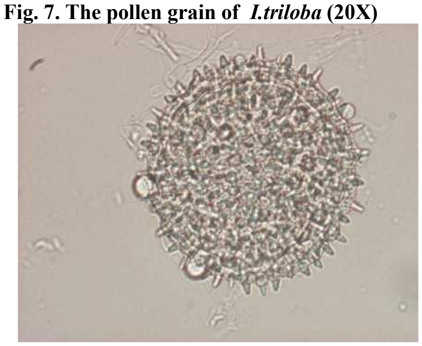
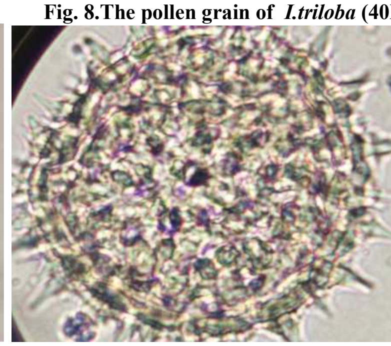
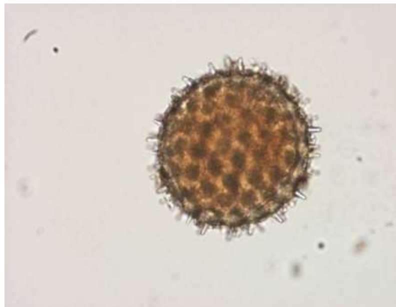



Fig. 9. The pollen grain of *I.asarifolia* (20X) **Fig. 10. The pollen grain of *I.asarifolia* (40X)**



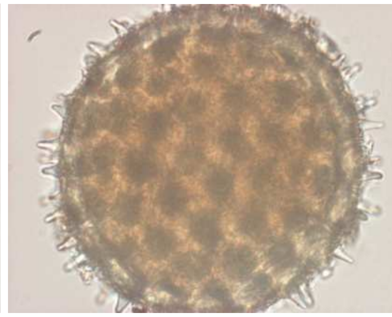


Fig. 11. The pollen grain of *I.hederifolia* (20X) **Fig. 12. The pollen grain of *I.hederifolia* (40X)**

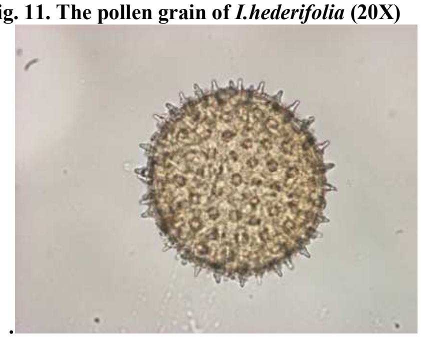


Fig. 13. The pollen grain of *I.aquatica* (20X) **Fig. 14. The pollen grain of *I.aquatica* (40X)**

Description of the flower morphology

Ipomoea carnea: The colour of the flower is pink, pistil and stamens are present (a complete flower), the number of filaments and anthers in each flower is 5, one style and stigma per flower, the lengths of filaments range from 29.0-31.0(mm), the lengths of styles 32-34(mm), sepals are five and hairy, and ovary superior.

Ipomoea involucrate: The flower colour is pink, a complete flower (pistils and stamens present), filaments and anthers are each five in number, one style is present per flower, the filaments range from 26.0-27.0(mm) in length, while the style lengths range from 27.5-28.0(mm), sepals are absent, instead hairy bracts spath-like in shape are present and the ovary is superior.

Ipomoea vagans: Flowers are pink in colour, staminate and pistilate (complete flowers), filaments and anthers are each five in number, one style is present per flower, the lengths of filaments range from 16.0-18.0 (mm), and lengths of styles from 16.5-19.0 (mm), sepals are hairy and five in number. The ovary is superior.

Ipomoea asarifolia: Flowers are pinkish-purple in colour, staminate and pistilate flowers (perfect or complete flowers), numbers of both filaments and anthers are five in each flower, with their lengths ranging from 18.0-29 (mm), and the number of styles is one in each flower with their lengths ranging from 18.5-31.0 (mm), bearing superior ovaries, sepals are glabrous and five in each flower.

Ipomoea triloba: Flower is pink, funnel-shaped with pentagonal edges, both stamens and pistils (complete flowers), filaments five, anthers five in number in each flower, the lengths of filaments range from 19.0-20.0 (mm), one style is present per flower with their lengths rangeing from 20.5-21.0 (mm), pubescent sepals are five in number. Their ovary is superior.

Ipomoea aquatica: Flower is pink in colour, funnel-shaped, a complete flower (stamens and pistils present), filaments five and anthers five in number with the length of filaments ranging from 24.0-25.5 (mm), the flower has one style each and their lengths vary 25.0-25.5 (mm), the ovary is superior and the sepals are not hairy.

Table 1. Quantitative characters of the pollen and styles of the *Ipomoea* species studied

Name of Species	Quantitative characters of the seven Ipomoea species used in the study					
	Pollen characters (μm)				Reproductive characters (mm)	
	Pollen grain diameter	Spine length	Interspinal distance	Aperture diameter	Length of style	Length of filament
I.carnea	36.6- 38.7	3.6-5.4	3.2-3.6	2.0- 2.8	32.0- 34.0	29.0- 31.0
I.involucrata	32.5- 35.1	8.3- 9.6	3.3-4.0	4.2- 5.2	27.5- 28.0	26.0-27.0
I.vagans	31.2- 34.4	6.1- 7.3	5.7- 6.3	2.0- 2.7	16.5- 29.0	16.0- 18.0
I.asarifolia	37.8-39.3	4.1- 5.4	2.2- 2.7	3.0- 4.1	18.5- 31.0	18.0-29.0
I.triloba	32.3- 34.2	4.0- 5.2	1.7- 2.6	3.0-3.7	21.0-20.5	19.0-20.0
I.hederifolia	30.7-31.4	3.3-4.0	1.7-2.4	2.0-2.7	37.0-38.5	35.0-36.0
I.aquatica	60.2-62.5	4.4-5.9	5.0- 5.6	2.8-3.2	25.0-25.5	24.0-25.0

Table 2. Anatomical and morphological characters of the species of *Ipomoea* extracted from micrographs

Species	Location	Character codes													
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
I.carnea	Ile-Ife	+	-	-	+	-	+	-	+	-	+	-	+	-	+
I.involucrata	Ibadan	-	+	+	-	+	-	-	+	-	+	+	+	-	+
I.vagans	Akure	-	+	+	-	+	-	-	+	-	+	+	+	+	-
I.asarifolia	Badagry	+	-	-	+	-	+	-	-	+	-	-	+	-	+
I.triloba	Ondo	-	+	+	-	-	+	-	+	-	+	-	+	+	-
I.hederifolia	Ilesha	-	+	+	-	-	+	+	-	-	+	-	+	-	+
I.aquatica	Ibadan	-	+	+	-	-	+	-	-	-	-	-	+	-	+

Legends: 1.Pollen shape oblate, 2. Pollen shape oblate shperiodal, 3. Pointed end spine, 4. Blunt end spine, 5. Bract shape spathe, 6. Polysepaloid calyx, 7. Red flower colour, 8. Pink flower colour, 9. Pinkish-purple flower colour, 10. Hairy sepals, 11. Radially symmetrical calyx, 12. Funnel-shape flower, 13. Flower deeply lobed, 14. Flower shallowly lobed - = absent; + = present.

The dendrogram obtained from cluster analysis is shown in Fig.15, as a summary of the relationships among the commonly found *Ipomoea* species of south western Nigeria. Two groups of species emerged from the study of the of the clustering pattern, first group consisting of *I.involucrata* and *I.vagans* and *I.triloba,* *I.hederifolia* and *I.aquatica* and a second group comprising *I. carnea* and *I. asarifolia*. appear to be sisters to all to other species in this study as they separate out at the lowest level of similarity while a clade somewhat paraphyletic comprising also emerged. The taxa included within these two clades are not consistent with previous infrageneric and sectional circumscriptions.

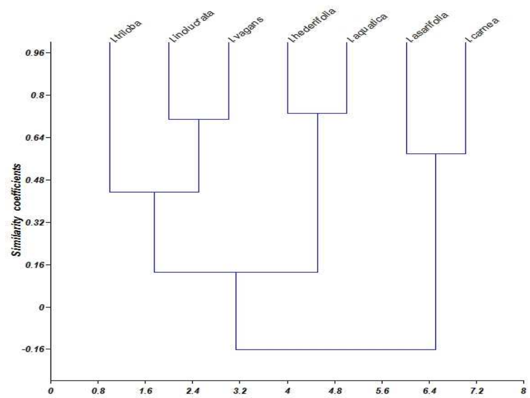


Fig. 15. Dendrogram showing the hierarchy of groups in seven *Ipomoea* species based on coefficients of similarity based on pollen and flower characters.

A close study of Table 1 reveals that characters 7 (red flower colour) and 9 (pinkish-purple flower colour) are unique derived characters, occurring in only one taxon each, as observed here in *I. hederifolia* and I. asarifolia respectively, while character 12 (funnel-shaped flower) is shared derived character or derived synapomorphic character occurring in all the species studied. Characters 2,3,4 and 12 are synapomorphic for *I.triloba*, *I. aquatica* and *I. hederifolia*; characters 2 and 3 are synapomorphic for *I. triloba*, *I. aquatica*, *I. hederifolia*, *I. involucrata* and *I. vegans*; characters 1, 4, 6, 12 and 14 are synapomorphic for *I. carne* and *I. asarifolia*. Correlation analysis between pollen diameter and style length revealed a significant positive correlation (r=0.64). The characters of taxonomic importance are, pointed end spine, blunt end spine, oblate, oblate-spheroid, deeply lobed petals and shallow lobed petals, hairy sepals and flower colours. There are important characters such as blunt end spines found in Ipomoea carnea, and *Ipomoea asarifolia* and pointed end spines observed in Ipomoea involucrata, *Ipomoea hederifolia*, *Ipomoea aquatic*, Ipomoea triloba and *Ipomoea vagans*.

Among the commonly found species of *Ipomoea* of South Western Nigeria, same number of pistils and stamens were observed. It was also observed that all the species studied except *Ipomoea involucrata* had a boat-shaped involucres have 5 sepals (calyx). All the pollen showed polytreme, radially symmetrical, outline circular and pantoporate aperture. Measurements of the lengths of the seven species of *Ipomoea* show variations from species to species and their differences in their spine length, diameter, filament lengths, style lengths, hairy to non-hairy sepals, and differences in their flower colours. Rajurkar, *et al*. (2011) reported the pollen diameter of *I. trilobata* to be 68.93-78.9 μm in diameter, about half that diameter was observed in this study. Hsiao and Kuoh (1995) had divided eighteen *Ipomoea* spp. into two groups based on spines and the ridges of bacula around the extrapolar region, similar observation was made in *Ipomoea triloba* in this study.

Walker and Doyle (1975), assigned standards to pollen grain sizes viz: minute grain <10μm; small grain 10-24μm; medium grain 25-49μm; large grain 50-99 μm; very large grain 100-199μm and gigantic grain < or=200μm. In this study, all pollen grain sizes lie in the medium size group except *I. aquatica* which falls in the large size category. Schols (2005) observed that in *Dioscorea*, pollen size decreases in the more derived clades. It would appear that *I. aquatica* is less derived than other members of the studied species. This is consistent with an ealier work of Manos *et al*., (2001) which categorized the species of *Ipomoea* into phylogenetic groups based on flavonoid chemistry, listing *I. triloba* with other highly advanced species whereas *I. aquatica* and *I. hederifolia* were grouped with the less derived species.

All the pollen grains studied were oblate to oblate-spheroid, the present study also noted echinate charaters in all seven species of *Ipomoea*. These findings are similar exactly to that of Erdtman (1971) and Rao and Lee (1970) observations. All the studied *Ipomoea* species showed metareticulate exine pattern which was previously noted by Borsch and Barthlott (1998) and Telleria and Daners (2003). According to previous studies, generally, the pollen morphology of the Convolvulaceae is known to be highly diverse and of taxonomic importance (Telleria and Daners, 2003). Taxonomy largely relies on morphological characters to define taxa in Convolvulaceae and rarely is pollen character employed. Problems in classification arise when taxa display a large number of variability, due to phenotypic plasticity (van den Berg and Groendijk-Wilders, 1999; Jayeola, 2012). These studies were based on a few morphological characters using pollen grains and flowers to elucidate their relationships among genera within the family. Torres (2000) observed a positive linear correlation between pollen volume and pistil length in Asteraceae, similar to the strong positive correlation between style length and pollen diameter observed in this study, may reflect a functional rather than a phyletic relationship.

The two clades observed in this study are difficult to reconcile with particular patterns of mvariation in flower morphology and pollen characters. Perhaps, it is likely that each is derived from an ancestral complex. *Ipomoea carne* and *Ipomoea asarifolia* both are alien species which may account for their rather isolated position in the dendrogram of phenetic relationships. The *Ipomoea* species placed within Clade 1 do not conform to the sectional treatments but rather are interspersed among other sections. For instance, *I. triloba*, *I. aquatic* and *I. hederifolia* which show strong similarity, being joined at high phenon level, are classified in the Sections Eriospermum, Erpipomoea and Mina respectively.

It appears I. *carnea* and *I. asarifolia* are sisters to other species in this study as they separate out at the lowest level of similarity. There is a strong suggestion of the need of sampling of more African *Ipomoea* (e.g., Van Ooststroom, 1953;Vercourt 1963; Goncalves, 1987) to enable us to refine our understanding of the relationships among taxa previously placed within subgenus *Eriospermum* (section Eriospermum of Van Ooststroom,1943).

The occurrence of a synapomorphic character strongly support the monophyly of Ipomoea, but the relationships of the member species is not yet fully understood. As the number of species increases, synapomorphies decrease whereas increased synapomorphies are associated with decreasing number of species.

CONCLUSION

This study has provided additional characters to those already known about the Ipomoea species in Nigeria. It can be concluded that, pollen characters could be important in *Ipomoea* taxonomy. The similarities in their structure showed interspecies relationships and the reason for them to be in the same genus, while the differences in their flower and pollen structures tell about their existence as a distinct species. Also, pollen characters could provide additional information to improve the identification of this rather poorly known but taxonomically and environmentally important angiosperm family.

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