



POLLEN STUDIES OF MEMBERS OF THE GENUS *BASELLA* LINN. FROM SOUTH WEST, NIGERIA

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<https://dx.doi.org/10.4314/coast.v7i1.2>

Abstract

The pollen studies of members the genus *Basella* L. in South west Nigeria namely, *Basella rubra* Rougx., *Basella alba* Linn. and *Basella cordifolia* Lamk were carried to find if pollen characteristics could be used to explain the taxonomy of the genus. Erdtman (1952) method of acetolysis was used, observed under light microscope; photomicrographs of the acetolysed pollen were made using ACCU-scope trinocular microscope (ACCUscope 33001 led trinocular microscope with 3.2 MP CMOS digital camera); both qualitative and quantitative parameters were taken. Pollen was pantoporate, cuboidal, exine wall reticulate, medium sized. Variations observed classified the species into two groups: *B. rubra* consists of oblate spheroidal pollen, thick walled, longest stipule length while *B. alba* and *B.cordifolia* consist of prolate spheroidal pollen, thin walled exine. *B. alba* is made of large grains, sexinous rods 4.5-5.0 µm long; stipule length is intermediate (6.0-9.0 µm). *B. cordifolia* has pores that are thin and short (5.0 µm long and 1 µm wide). The pollen study showed that *B. rubra* is different from the other two species. The genus *Basella* consists of two species namely: *B.rubra* and *B.alba* while *B.cordifolia* is likely a variant of *B. alba*.

Keywords: *Basella*, cuboidal, pollen, pantoporate, spheroidal

Introduction

Pollen studies or palynology have been very useful in taxonomic studies and their roles at the taxonomic categories are well documented. Palynological evidence may be used to place taxa of uncertain affinities, to suggest rearrangements, withdrawals and separations, as well as corroborate other lines of evidence (Davis and Heywood, 1963). Pollen morphology provides indications concerning the groups to which uncertain elements belong and has also been used to support the amalgamation of families (Erdtman, 1969). Akinwusi and

Illoh (1996) showed that pollen morphology of the *Hibiscus* provided useful data for the taxonomy of the genus. Adedeji (2005) reported that the number of pores on the pollen grains is an attribute effectively and reliably used to separate, delimit and classify the species of *Emilia*.

Comparative pollen morphology of superficially similar pollen provides means of identification (Orijemie, 2018). The characters of taxonomic value in pollen grains are the number and position of aperture (colpi and pori), pollen wall morphology, surface ornamentation, polarity,

symmetry, shape, and size (Walker, 1976; Taia, 1996, 2004; Ugbabe *et al.*, 2013; Ibrahim and Ayodele, 2017). Arogundade and Lawal (2018) noted that the number of pollen units, pollen size and shape were diagnostic for the species, varieties and hybrid of the genus *Talinum*. Olutayo and Arogundade (2022) reported the use of quantitative characters of the pollen such as pollen diameter and distance between colpi as diagnostic characters for the species of *Bryophyllum* and *Kalanchoe*. Ugbabe *et al.* (2013) noted that the careful use of pollen morphology has been a valuable tool in generic delimitation and in understanding relationships within the family and to determine variation within a species and below the species level (Ibrahim and Ayodele, 2017). According to Ahkush (2023) pollen morphology is one of the characteristics which have been used extensively to fine tune most existing classification of the family at the genus level.

The family Basellaceae has four genera and twenty five species distributed in tropical regions of Africa, including Madagascar, America and Asia (Airy Shaw, 1985). In West Tropical Africa the family Basellaceae is monotypic genus consisting of *Basella alba* syn. *B. rubra* (Hutchinson and Dalziel, 1958). Three different taxa of *Basella* exist with descriptions different from that of Hutchinson and Dalziel (1958). These are *Basella rubra* Roux; *Basella alba* Linn.; *Basella cordifolia* Lamk (Adenegan-Alakinde, 2014). *B. alba* and *B. rubra* were considered as two distinct species based on differences in the pigmentation on the leaves and stems (Bittrich and Sperling, 1993; Palada and Chang, 2003; Busuoi and Ifrim, 2004; Larkcom, 2007; Oladele and Aborisade, 2009). Some authors described the species as being synonymous (Hutchinson and Dalziel, 1958). One taxon

is described as a variety of the other (Henry *et al.*; 1987; Sawani, 2005; Greuter and Raus, 2006; Adeyemi, 2007; Roy *et al.*, 2010). Adeyemi (2007) reported that three main cultivars have been distinguished in Nigeria based on leaf shape and colour viz: *B. alba*; *B. rubra* and *B. cordifolia*. *B. cordifolia* is rarely mentioned in literatures especially FWTA but exist in the study area. Adenegan-Alakinde and Akinloye, (2015) and Adenegan-Alakinde and Akinnubi, (2015) reported that the petiole anatomy and epidermal cells and structures of members of the genus indicated that three species occur in Nigeria. Despite the marked differences in petiole anatomy and epidermal structures, the species have features that are common to them. Bolaji *et al.* (2023) reported that there are significant differences in some morphological parameters of the leaves, petioles, fruits and seeds of the green stemmed *B. alba* and the purple stemmed *B. rubra*. But the two plants are very close with respect to their DNA profiling, genetic variation and genetic distance (Bolaji *et al.*, 2022) which suggests the need for more enquiries into the genus. Farooqi *et al.* (2019) worked on the pollen of an extinct species of *Basella* dugged from sediments in India. It was reported that the species *B. keralensis* shared pollen features of *B. alba* and *B. paniculata* by having perfectly cuboidal pollen shape, median pollen size (29-33), exine thin walled and sexine thick.

The taxonomy of the genus *Basella* is not clear and the literatures on the genus in this part of the world are limited. However the search for more evidences from other systematic lines is required to enable us to arrive at an acceptable taxonomic conclusion. Oyelakin *et al.* (2020) emphasized the importance of using pollen characters with other lines of systematic evidences in plant classification. According to them this eliminates the bias associated with using only

plant morphology because pollen characters are genetic and less susceptible to environmental modifications. Hitherto, there is no information on the pollen morphology of the Nigerian species of *Basella*. This study will therefore provide information on the pollen of members of the genus in Nigeria and the possibility of delimiting the species based on pollen features.

Materials and Methods

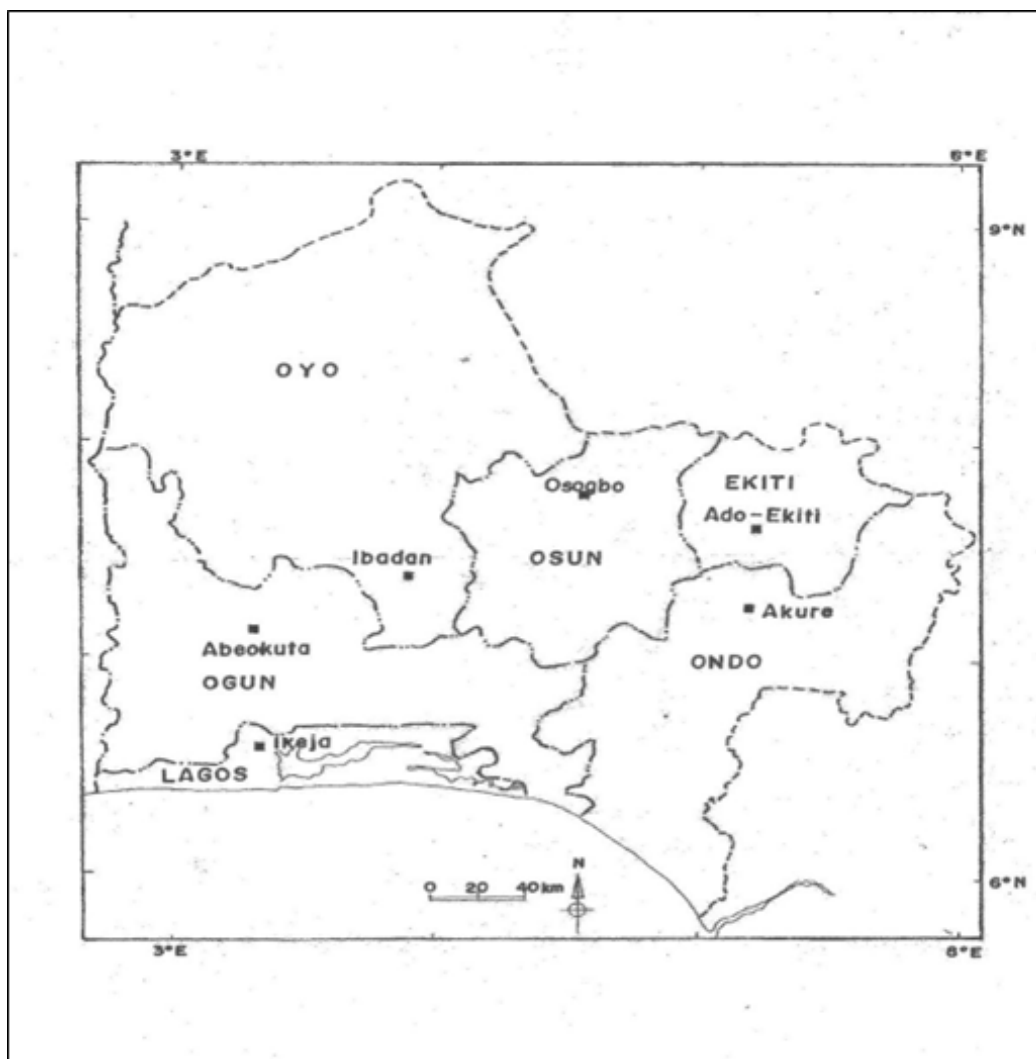
Collection of Plant Materials

Plant materials were collected from Ekiti,

Lagos, Ogun, Ondo, Osun and Oyo States that make up the South - western Nigeria which lies on Latitude 6° - 9°N and Longitude 3° - 9°E (Figure 1). Seeds, seedlings were collected and were sown on the field. The seeds that were not planted immediately were sun dried to prevent spoilage and mould attack.

Study Site

Plants were established inside buckets and on a research field in Adeyemi Federal University of Education, Ondo, Ondo State, Nigeria. years.



Source: Dada *et al.* (2006). Macmillan Nigeria Secondary Atlas, Pp 19.

Figure 1: Map of south western states of Nigeria showing states where plant collections were made.

Pollen acetolysis

Pollen grains from fresh flowers of the species were collected for studying the pollen features. Pollen grains were collected from at least 10 stands of plants per form. These were acetolysed using the method of Erdtman (1960) with modifications. The fresh anthers were placed in a centrifuge tube and macerated in 70 % ethyl alcohol with a stirring rod to dehydrate the specimens. The specimens were centrifuged at 5000 revolutions per minute for fifteen minutes. The alcohol was poured off, glacial acetic acid was added and the mixture was centrifuged again at the same speed. Acetolysis mixture (sulphuric acid and acetic anhydride i.e. 9 part acetic anhydride and 1 part concentrated sulphuric acid) was added. The tubes containing the mixture were kept in a water bath and heated from 70°C to boiling point. The mixture was stirred with a rod and centrifuged again at the same speed as above, after which the acetolysis mixture was decanted and glacial acetic acid added. The mixture was centrifuged again, distilled water was added and the mixture was centrifuged again. The water was decanted and the acetolysed pollen grains were mounted in glycerine jelly.

Photomicrographs of the acetolysed pollen grains were taken using ACCU-scope trinocular microscope (ACCUscope 33001 led trinocular microscope with 3.2 MP CMOS digital camera). Qualitative characters such as exine pattern, type of aperture, type of pollen and shape of the pollen grains were observed. Quantitative characters measured were pollen diameter, equatorial diameter, pollen wall thickness, pore length and width and length of sexinous rods of the pollen grains.

Results

Summarised pollen characters are presented in Table 1 and light photomicrographs of the pollen grains are shown in Figures 1- 4.

Basella rubra

Pollen has six pores (pantoporate). Length of polar axis (P) is 32 and equatorial diameter (E) is 35, P/E is 91.4. Pollen shape on face value is like a dice or cube but palynologically is oblate spheroidal. Pores are 10-12µm long and 4-5µm wide. The exine is thick (1.3µm). Spinule length is 10.5-13.5µm. There are crowded bacules on the exine. Bacules are connected to one another to form a reticulate mesh, i.e. exine pattern is reticulate.

Basella alba

Pollen has six pores i.e. it is pantoporate. Length of polar axis (P) of the pollen in polar view is 40-40.1µm and equatorial diameter (E) is 36µm; P/E is 111.3. Pollen shape, on face value is like a dice or cube but palynologically is prolate spheroidal. Pores are 9µm long and 4µm wide and surrounded by tiny and numerous bacules on the margins. Exine is 1.0µm thick; very difficult to separate into sexine and nexine. Exine has a reticulate pattern; there are sexinous rods (4.5-5.0µm long) arranged in reticuloid pattern on the exine but sexine (outermost part of the exine) seems finely reticulate. Spinule length is 6.0-9.0µm.

Basella cordifolia

Pollen has six pores (pantoporate). Length of polar axis (P) is 27.0µm and equatorial diameter (E) is 25.2µm; P/E is 105.9, pollen shape on face value is like a dice or cube but palynologically is prolate spheroidal. Exine is 1.0µm thick and sexinous rods are 3.0µm long. Pores are very thin (1.0µm in width) and short (5µm in length). Sexinous rod is 3.0 µm; the spinule length is 4.5-6.0 µm.

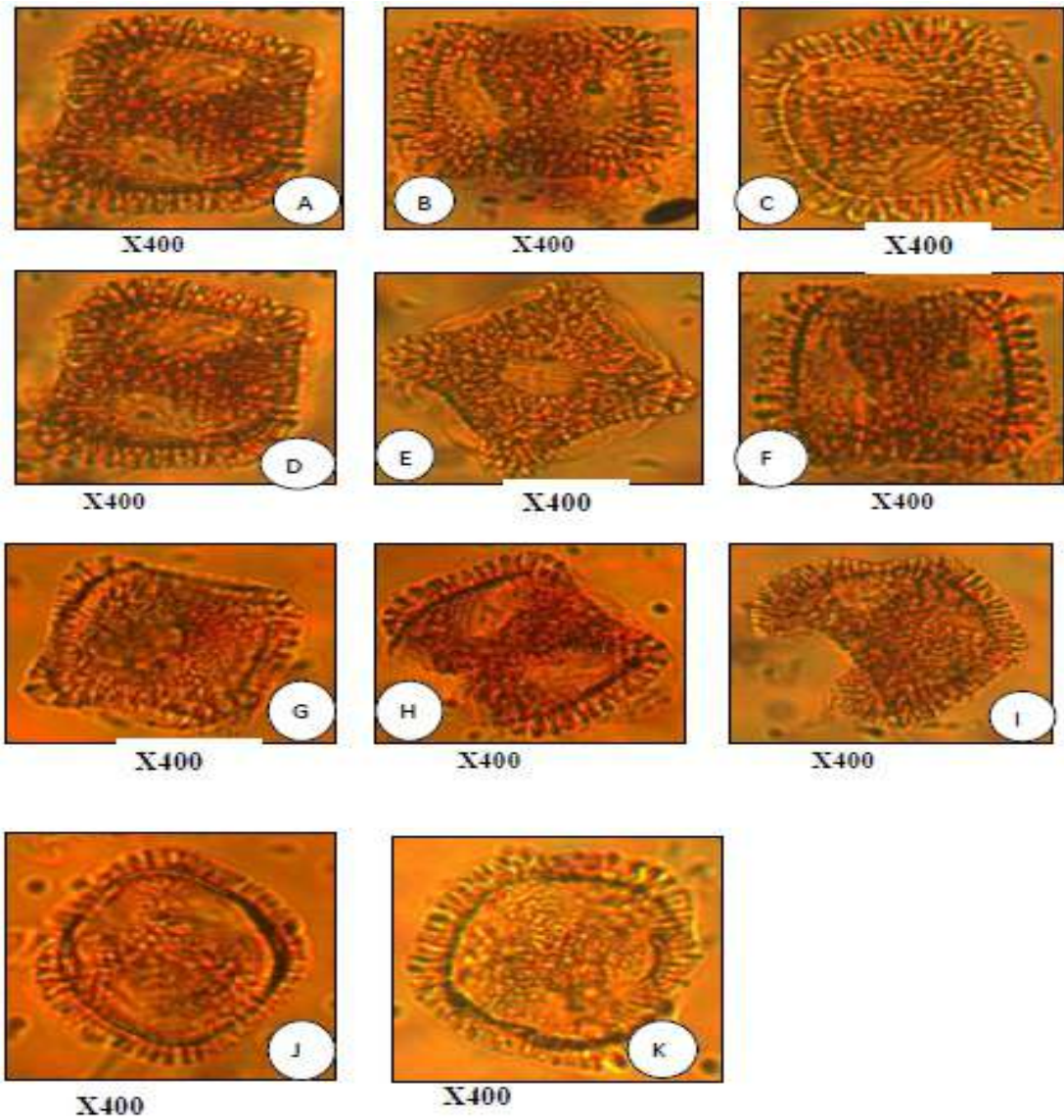


Figure 1: Pollen morphology of *B. rubra*

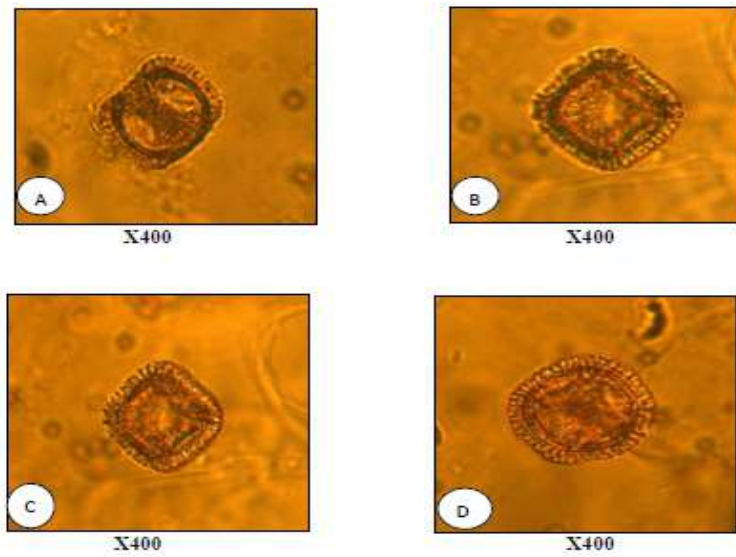


Figure 2: Pollen morphology of *B. alba*

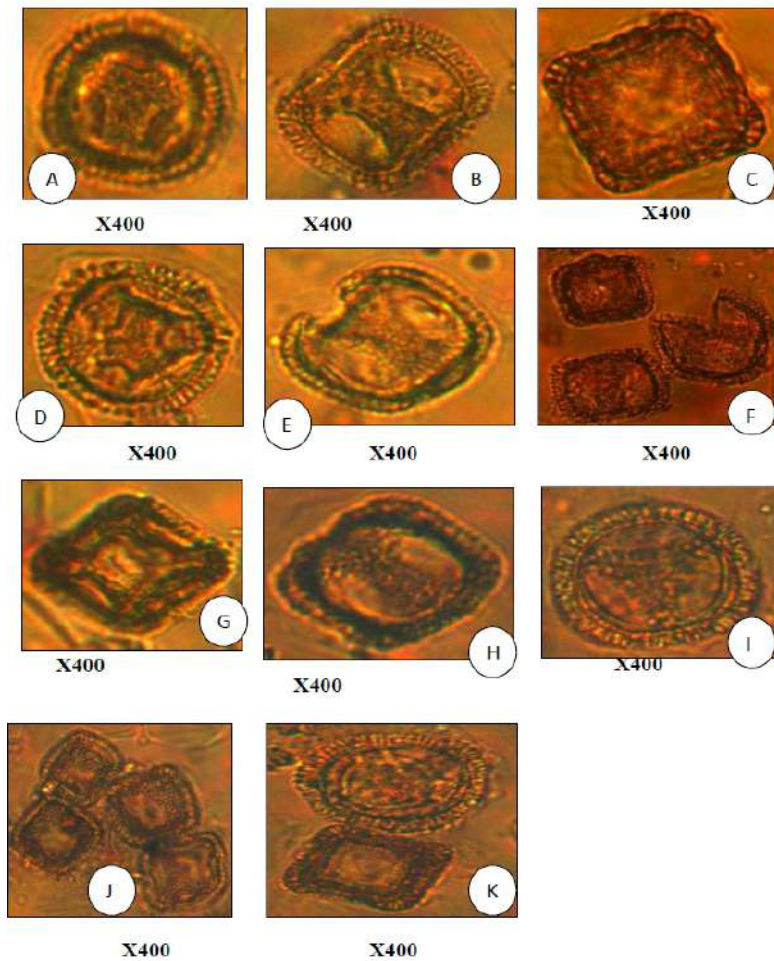


Figure 3: Pollen morphology of *B. cordifolia*

Table 1: Pollen characteristics of the Genus *Basella*

Species/ pollen features	Shape	Size	Pollen class	Exine thickness μm	Exine pattern	Pore length μm	Pore width μm	Sexinous rod length μm	PA μm	ED μm	P/ED	Spinule length μm
<i>B. rubra</i>	Cuboidal	Medium	Oblate spheroidal	1.3	Reticulate	10-12	4-5	3-4	32	35	91.4	10.5- 13.5
<i>B. alba</i>	Cuboidal	Large	Prolate spheroidal	1.0	Reticulate	9.0	4.0	4.5-5.0	40- 40.1	36	111.3	6.0-9.0
<i>B. cordifolia</i>	Cuboidal	Small	Prolate spheroidal	1.0	Reticulate	5.0	1.0	3.0	27.0	25.2	105.9	4.5-6.0

Discussion

The pollen of *Basella* species investigated has similar features. They are pantoporate (6 pores), radially symmetrical, cuboidal in shape from the polar view and the shapes of the sides is concave base on the ratio of the polar diameter to the equatorial diameter; The apertures are 6-pantocolpate; possess sexinous rods and their exine pattern is reticulate. All the pollen grains fall into the medium-sized grain category (25 - 50 μm) based on Erdtman (1952) pollen size classes. The pollen of the species is spheroidal but with variations. The above report is in agreement with those of Nowicke (1975, 1996); Erdtman (1986) and Bittrich and Sperling (1993) and Farooqi *et al.* (2019) for members of the genus occurring outside Nigeria. The similarity in some key features among the species of *Basella* studied indicated close affinity among the members and being members of the same genus.

This study revealed three *informal* pollen size classes: large (P x E $\geq 40 \times 36$), medium (P x E, 32 x 35) and small (P x E, 25-30 μm and ED is 25-30 μm). The large grains occur in *B. alba* and the medium grains occur in *B. rubra*, and the small grains are found in *B. cordifolia*.

Basella rubra is palynologically different from the others because it has thicker exine wall (1.3 μm), medium sized; sub oblate to oblate spheroidal; has longest spinule length, sexinous rod is thick and short (3-4 μm). The green stemmed types are prolate

spheroidal which show closeness between *B. alba* and *B. cordifolia*. The effective use of quantitative pollen morphological characteristics was reported by Olutayo and Arogundade (2022). Members of the genus *Basella* show close affinity and differences in some quantitative pollen morphological characteristics and some are of taxonomic value.

B. alba is made of large grains, sexinous rods 4.5-5.0 μm long; spinule length is intermediate (6.0-9.0 μm). *B. cordifolia* has a sexinous rod (3 μm); spinule length (4.5-6.0 μm) being the shortest with pores which are thin and short (5.0 μm and 1 μm wide). *B. cordifolia*, though quite similar to *B. alba*, is likely a different species of *Basella*; because it has the thinnest pore. Arogundade and Lawal (2018) noted that shape and size of pores in *Talinum* species and F1 hybrids are diagnostic. The thinness of the pores in *B. alba* and *B. cordifolia* is diagnostic. Tomsovic (1997) and Sahreem *et al.* (2008) observed that the presence of thin exine is a characteristic feature of primitiveness in Angiosperms. *B. rubra* is the most advanced due to its thick exine wall while the green stemmed species are primitive.

Some deductions can be made from the above:

1. The pollen of *B. rubra* is different from all others in that it is the only grain that is oblate spheroidal in shape and has the thickest exine (1.3 μm) and more advanced. These factors

suggest that it is palynologically different from the three *Basella alba* species.

2. It appears that the species of *B. cordifolia* is a variant of *B. alba* although the Flora of West Tropical Africa (FWTA) is silent on this.

Although Desmukh and Gaikwad (2020) described *B. alba* and *B. rubra* as forms of the same species due to resemblance in their pollen structure and sculpture, the variations observed in the pollen morphology in this study are diagnostic and separate the genus into two major groups namely:

- i. the red stemmed species, *B. rubra* with oblate spheroidal pollen with thick exine.
- ii. the green stemmed species consisting of *B. alba* and *B. cordifolia* with prolate spheroidal pollens with thin exine.

Conclusion

The study has provided a new line of evidence of the diversity within the genus *Basella*. The study showed that *B. rubra* and *B. alba* are distinct species while *B. cordifolia* is likely a variant of *B. alba*. The taxonomy of the genus needs to be reviewed to accommodate *B. rubra* as a distinct species and not a synonym to *B. alba* as documented in the FWTA. More work need to be done such as molecular studies on the genus.

Authors' contribution

1. Taiwo Adenegan-Alakinde collected plant materials, established them, harvested the pollen and carried out experimental procedures.
2. Olubukola Adedeji carried pollen studies and interpretation of data. The two of us read through the manuscript and made contributions.

Acknowledgement

We appreciate the assistance of Dr Orijemie, E.A. of the University of Ibadan with respect to the photomicrographs of the pollen.

Conflict of interest: None

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