



## Research article

## Comparative studies of *Heterotis rotundifolia* (Sm.) Jacq.-Fel. and *Dissotis erecta* (Guill. & Perr.) Dandy (Melastomataceae) based on macromorphology, foliar epidemis and pollen features in Southeastern Nigeria

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**Abstract:** *Heterotis rotundifolia* and *Dissotis erecta* were comparatively studied to evaluate their macro-morphological, foliar epidermal and palynological features. Standard materials and methods were employed to carry out the studies. The leaf shapes, surfaces, margin, apex and base of the two species are elliptic, glabrous, entire, acute, acute and oblique respectively. The mean values of the leaf length of *Heterotis rotundifolia* is 3.23 cm and *Dissotis erecta* is 3.57 cm while the mean values of the leaf width are 1.96 cm in *Heterotis rotundifolia* and 1.31 cm in *Dissotis erecta*. Anisocytic stomata are recorded only on the abaxial surface of *Heterotis rotundifolia* while tetracytic stomata are common on the abaxial and adaxial surfaces of *Dissotis erecta*. Stomata are not recorded on the adaxial surface of *Heterotis rotundifolia*. Cell shapes of the two species studied are mainly pentagonal in all the surfaces except on the abaxial surface of *Heterotis rotundifolia* where it is isodiametric. Trichomes are absent on the epidermal surfaces. The anticlinal wall patterns are straight and undulate on the surfaces. The pollen grains observed in the two species were tricolpate. The two species which are common in Southeastern Nigeria can easily be differentiated macro-morphologically by their habits and colour of their standard petals. Foliar epidermal features are also of great importance for their differentiations. The palynological report here may be novel since there is no earlier report of pollen features found on the two species studied.

**Keywords:** *Heterotis* - *Dissotis* - Macromorphology - Foliar epidermis - Tricolpate - Stomata.

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### INTRODUCTION

*Heterotis* and *Dissotis* are genera of plant species in the family Melastomataceae which their species are distributed in the tropical and subtropical regions of the world (Edeoga & Eboka 2000). There are 36 species of *Heterotis* and about 140 species of *Dissotis* with medicinal properties (Loigier 1994, The Plant List 2020). According to Willis (1985) *Heterotis* species varied in habit as some were herbs, shrubs and trees with climbing or erect stems. Hutchinson & Dalziel (1966) in their Flora of West Tropical Africa reported 42 species in which five species were not well known. Macroscopically, Abere *et al.* (2009) described *Heterotis rotundifolia* (Sm.) Jacq.-Fel. to have linear leaf shape with glabrous texture, a short petiole, margin entire, apex and leaf base acute with pinnate venation. Microscopically, stomata was anomocytic, epidermal cells were straight and polygonal with uniseriate and multiseriate covering trichomes. Much research has been carried out on *Heterotis*

*rotundifolia*. Four species of *Heterotis* were common in Southern Nigeria. Paracytic stomata were common on abaxial and adaxial surfaces of the four species studied except in *Heterotis rotundifolia* where the stomata were anomocytic. Trichomes were also recorded on the epidermal surfaces of the taxa studied. The anticlinal wall pattern varied from pentagonal to polygonal in both surfaces of the leaf except in *Heterotis rotundifolia* where they were sinuous and irregular in shape (Edeoga & Eboka 2000).

The studies were carried out to evaluate the macro-morphological, foliar epidermal and palynological features of *Heterotis rotundifolia* (Sm.) Jacq.-Fel. and *Dissotis erecta* (Guill. & Perr.) Dandy for their easy identification.

## MATERIALS AND METHODS

Fresh specimens of *Heterotis rotundifolia* and *Dissotis erecta* were collected from different locations of Southeastern Nigeria and identified at Ebonyi State University Herbarium (Fig. 1). The voucher specimens were deposited in the Ebonyi State University Herbarium with the numbers EBSU-H- 0234 and EBSU-H-0235.



**Figure 1.** A, *Heterotis rotundifolia* (Sm.) Jacq.-Fel.; B, *Dissotis erecta* (Guill. & Perr.) Dandy.

### Macromorphological study

Leaf length and width, were measured following the method of Nwankwo & Ayodele (2017). From each specimen, a total of 20 leaves were randomly selected and measured.

### Foliar epidermal studies

Epidermal preparation methods also followed Nwankwo & Ayodele (2017). The standard median portions of the leaves obtained by cutting with razor blade were soaked in concentrated trioxonitrate (v) acid for about 10 to 15 minutes to soften the mesophyll layers for separation. The appearance of air bubbles on the surfaces of the leaves indicated their readiness for separation. They were transferred into some water in the petri dish with a pair of forceps. Both epidermises were carefully separated by tearing them apart and pulling the epidermis back on itself using camel hair brush. The camel hair brush was also used to remove the adhering tissue debris. The separated surfaces were rinsed in distilled water and then transferred into 50% ethanol for about two to three minutes to harden. They were rinsed again in distilled water and stained with safranin for about five minutes and excess stains were washed off in the water. They were mounted in 25% glycerol on slides with the edge of the coverslips sealed with nail varnish to prevent dehydration. The slides were labelled appropriately and examined under the light microscope while photomicrographs of each slide was taken at a magnification (x400), using Canon digital camera fixed to a light microscope and connected to the personal computer.

### Palynology

Preparation of the pollen grains was done following the method of Erdtman (1969). The pollen grains were crushed in plastic centrifuge tubes using glass rods and about 5ml of glacial acetic acid depending on the

quantity of the samples, was added for dehydration. After this, the samples were centrifuged at 5000 rpm for 15 minutes. Acetolysis mixture of acetic anhydride and H<sub>2</sub>SO<sub>4</sub> in the ratio 9:1 respectively was added to the samples in the tubes for oxidation reaction (bleaching). The samples and acetolysis mixture were thoroughly mixed with Vortex Mixer. These were warmed for 13 minutes and centrifuged for 15 minutes at 5000 rpm to remove the supernatants. They were rinsed three times with distilled water to remove the effect of the Acetolysis and then sieved with 120 µm Sieve to remove the plant debris. The acetolysed samples were transferred into vial bottles with a graded series of glycerol (50% and 100%). Temporal slides of the prepared samples in 100% glycerol were mounted on glass slides with cover-slips, sealed with nail varnish and viewed under the (the name is important) light microscope. The slides were properly labelled and proper care was taken to avoid contamination which may introduce error into the result.

## RESULTS

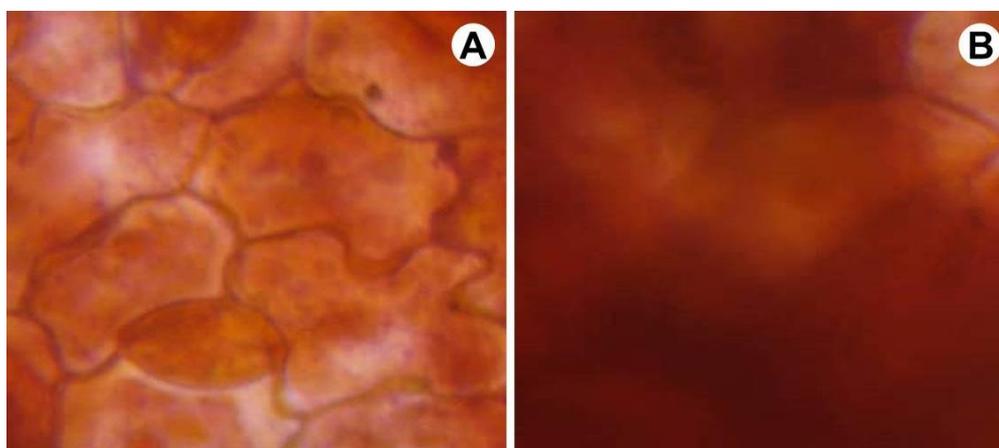
The leaf shapes, surfaces, margin, apex and base of *Heterotis rotundifolia* and *Dissotis erecta* elliptic, glabrous, entire, acute and oblique. The mean values of the leaf length and width show slight variation between the two species (Table 1). Anisocytic stomata are recorded only on the abaxial surface of *Heterotis rotundifolia* while tetracytic stomata are common on the abaxial and adaxial surfaces of *Dissotis erecta* (Table 2). Stomata are not recorded on the adaxial surface of *Heterotis rotundifolia*. Cell shapes of the two species studied are mainly pentagonal in all the surfaces except on the abaxial surface of *Heterotis rotundifolia* where it is isodiametric. Trichomes are absent on the epidermal surfaces. The anticlinal wall patterns are straight and undulate on the surfaces as shown in figure 2 and 3. The results of pollen morphology of *Heterotis rotundifolia* and *Dissotis erecta* were presented in figure 4. The pollen grains observed in the two species of *Dissotis* was tricolpate.

**Table 1.** The qualitative and quantitative macro-morphological features of *Heterotis* and *Dissotis* species studied.

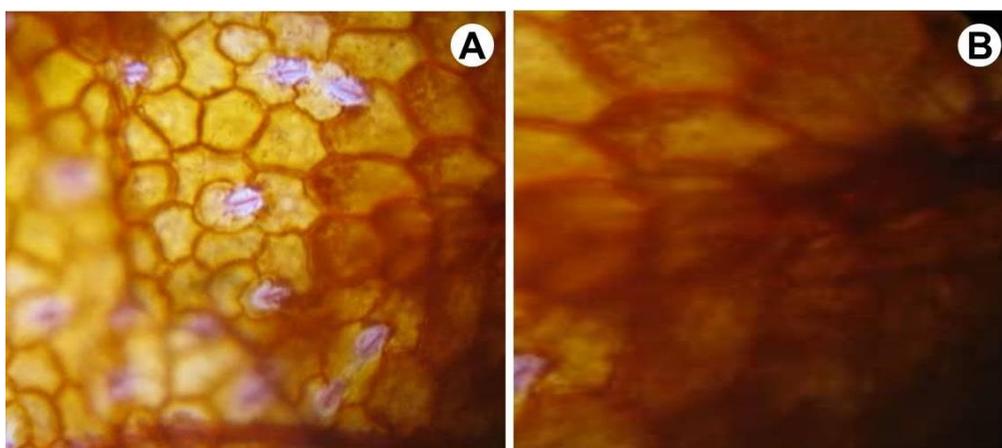
Features of the species	<i>Heterotis rotundifolia</i>	<i>Dissotis erecta</i>
Leaf shapes	Elliptic	Elliptic
Leaf surfaces	Glabrous	Glabrous
Leaf margin	Entire	Entire
Leaf apex	Acute	Acute
Leaf base	Acute	oblique
Mean of leaf length (cm)	3.23	3.57
Mean of leaf width (cm)	1.96	1.31

**Table 2.** Foliar epidermal features of the two *Heterotis* and *Dissotis* species studied.

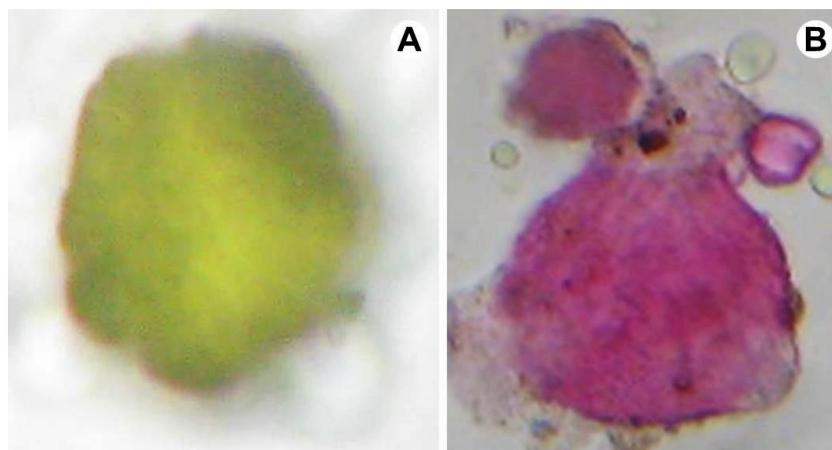
Epidermal feature	<i>Heterotis rotundifolia</i>	<i>Dissotis erecta</i>
Stomata type on abaxial surfaces	Tetracytic stomata	Tetracytic stomata
Stomata type on adaxial surfaces	Anisocytic	absent
Cell shape on adaxial surface	Pentagonal	Isodiametric
Trichome on adaxial surface	absent	absent
Trichome on abaxial surface	absent	absent
Anticlinal wall pattern on adaxial surface	undulate	straight
Anticlinal wall pattern on abaxial surface	Straight	Straight



**Figure 2.** Photomicrographs of the foliar epidermal surfaces of *Heterotis rotundifolia* (Sm.) Jacq.-Fel: **A**, Abaxial surface showing anisocytic stomata; **B**, Adaxial surface.



**Figure 3.** Photomicrographs of the foliar epidermal surfaces of *Dissotis erecta* (Guill. & Perr.) Dandy: **A**, Abaxial surface showing tetracytic stomata; **B**, Adaxial surface showing tetracytic stomata.



**Figure 4.** Photomicrographs of pollen grains: **A**, *Heterotis rotundifolia* (Sm.) Jacq.-Fel.; **B**, *Dissotis erecta* (Guill. & Perr.) Dandy.

## DISCUSSION

The qualitative features of the two taxa studied differ partially from the report of Willis (1985). The mean values of the leaf length of *Dissotis erecta* (3.57 cm) was slightly higher than the leaf length of *Heterotis rotundifolia* (3.23 cm). The result of the macro-morphological features shows that the species are related and this observation agrees with earlier work of Edeoga & Eboka (2000) that used comparative morphology of different species in establishing relationships among various taxa. The report of epidermal features here is totally in disagreement with Edeoga & Eboka (2000) who reported different types of stomata with amphistomatic features and trichomes on the taxa. The disagreement in the reports may be as a result of the environment where the taxa were obtained as Stace (1965) stated that the environment affects epidermal features of plant species.

The pollen grains observed in the species of *Heterotis* and *Dissotis* were tricolporate. The grains of *Heterotis rotundifolia* was separated by triradiate channels extending between its endogerminals while the grains of *Dissotis erecta* is T-tetra with tricolpate apertures. The earlier work of Chantaranonthai (1997), reported two colpi at polar view in another species of *Dissotis* (*Dissotis pelegriana*). The two species studied are related more in their macro-morphological and palynological features than on their foliar epidermal features.

## CONCLUSION

The two species which are common in Southeastern Nigeria can easily be differentiated macro-morphologically by their habits and colour of their standard petals. Foliar epidermal features are also of great importance for their differentiation. The palynological report here may be novel since there is no earlier report of pollen features found on the two species studied.

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