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RESEARCH PAPER

COMPARATIVE FOLIAR ANATOMICAL AND MORPHOLOGICAL STUDIES OF *NEPHROLEPIS BISERRATA* (SWARTZ) SCOTT AND N. *UNDULATA* (SWARTZ) J.SM. IN NIGERIA

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ABSTRACT
The foliar anatomy and morphology of Nephrolepis biserrata and Nephrolepis undulata were investigated. The aim of which is to elucidate their taxonomic knowledge with the use of both foliar anatomical and morphological characteristics that exist between them. The anatomical studies carried out include shape and size of the epidermal cell, venation patterns, stomata type and distribution. One way analysis of variance was used to show whether the two taxa are significantly different. The results of anatomical similarities in the adaxial surfaces of their leaflets were sinuous, anticlinal walls, absence of stomata and trichome, epidermal cells are irregular in shape and variable in sizes. On their abaxial surfaces, epidermal cells are irregular in shapes and variable in sizes, stomata present, predominantly diacytic and anomocytic types with elliptic shapes, thin and wavy anticlinal wall. Anatomical differences include length and width of epidermal cells, absence or presence and distribution of crystal sands, thickness of anticlinal walls on the adaxial surfaces, stomata Index and frequency, length and breadth of guard cell and guard cell area. The venation patterns showed that the mid-rib is sheathed with parenchyma cells and trichome types were observed in N. biserrata but absent in N. undulata. The distinguishing characters of the two taxa studied are of taxonomic value and can be used to identify and delimit each species and thus widen the scope of their taxonomic knowledge.

Keywords: *Foliar anatomy, Nephrolepis, taxonomic value, venation.*

INTRODUCTION
The genus *Nephrolepis* (Swartz) belongs to the family Nephrolepidaceae and order Filicales (David, 1987). There are 40 species *of Nephrolepis* wordwide (Friedrich 2005), out of which six species occur in Nigeria (Alston, 1959). Members are flowerless plants that require water at least during the sexual reproduction (Sporne, 1975). *Nephrolepis biserrata* (Swartz*)* Schott has scaly, short, erect rhizome; rarnenta and compound unipinnate leaf with sessile leaflets having serrated margins, acute to acuminate apices; stipe is polished brown and fertile fronds. It is used as ornamental plant. In Papua New Guinea, the croziers are cooked and eaten as pot herbs while in Micronesia the fronds are used to repel cockroaches (David, 1987). Christensen (1997) reported that

it is used for treating blister, boils, abscesses and sores of the skin in Sarawak whereas in India, the rhizome is used to cure respiratory diseases. It is used as fodder to feed African dwarf goats (Babayemi *et al.*, 2006) due to high nutrient values (Oloyede *et al*., 2008).

Nephrolepis undulata (Swartz) J. Sm. is an epiphytic plant growing mainly on *Elaeis guinensis* as well as decaying wood and inselbergs (Opapeju, 1983). It has a short, erect, tuberous rhizome (Fig. 1 (a) and (b))which persists during the dry season; the fronds are usually with pendent growth form (Dutta, 2005). The leaf is compound unipinnate; leaflet is sessile with serrated margin, acute to acuminate apex, fertile with auriculate base but smaller in size than *N. biserrata*. Stipe is polished brown without ramenta. The bushy form of this plant on *Elaeis guinensis* could harbour some dangerous animals like snakes thereby making local and manual harvesting difficult. *Nephrolepis biserrata* and *N. undulata* look morphologically and anatomically similar such that they pose taxonomic problems especially in their identification. Much more studies therefore, need to be carried out to provide taxonomic features that will delimit the species. This study will widen the scope of taxonomic knowledge with the use of foliar anatomical and morphological characteristics.

MATERIALS AND METHODS

Nephrolepis undulata was collected from the trunk of *Azardiracta indica* opposite staff club and *Elaeis guinensis* growing in the Department of Botany Obafemi Awolowo University, Ile-Ife (Fig. 1(c) and (d)). *biserrata* was collected from Botany Department at Obafemi Awolowo University, Ile-Ife. They were identified using (Alston 1959; Agnew 1974) and Ife Herbarium (IFE) specimens (Fig. 2 (b)) .The morphological features studied were growth habit, rhizome and leaf type. Leaflet shape, arrangements, margin, base and apex; stipe; ramenta and sori arrangements while anatomical studies carried out include the size and shape of the epidermal cell, stomata type and distribution, venation patterns and trichome type.

Anatomical Studies

The anatomical studies carried out included the shape and size of the epidermal cell, stomata type and distribution, as well as venation patterns. Sizeable portions of fresh matured leaflets were cut from the standard median positions (i.e. midway between the base and the apex of the leaflet of each species) and cleared. The leaflets were decolourized by boiling in 70% ethanol at 60ºC for about 10 minutes. The partially decolourized leaflet portions were washed carefully with water to remove all the traces of alcohol. The leaflets were boiled in 2% sodium hydroxide solution for about 5 minutes. The partially cleared leaflets were further cleared by soaking them in Petri dishes containing 2% of domestic bleach (Potassium hypochlorite). The completely cleared leaflet materials were rinsed with water to get rid of the bleach since prolonged stay could cause damage to the cells of the leaflets. The specimens were kept in the specimen bottles containing Formalin Acetic Alcohol (FAA). For microscope examination, the specimens were washed with water, stained with safranin O, placed on a clean glass slide in 25% glycerol, covered with a clean cover slip and mounted on a light microscope for examination, first at low power objective lens followed by high power. Photographs of the venation patterns were taken. For the epidermal studies, scrape method of Metcalfe (1960) was used. The epidermal peels were placed on a clean slide, stained with safrain O and covered with a cover slip. 25% glycerol was added and mounted on the light microscope at high power. Photomicrographs of internal structures of both the adaxial and abaxial surfaces were made. Length and width of the guard cells and the epidermal cells were measured at high power magnification using ocular micrometer. The guard cell area was calculated using the following equation (Franco, 1939):

Guard cell area = (length x width x k) μm^2.

Where k (Franco's constant)= 0.78524.

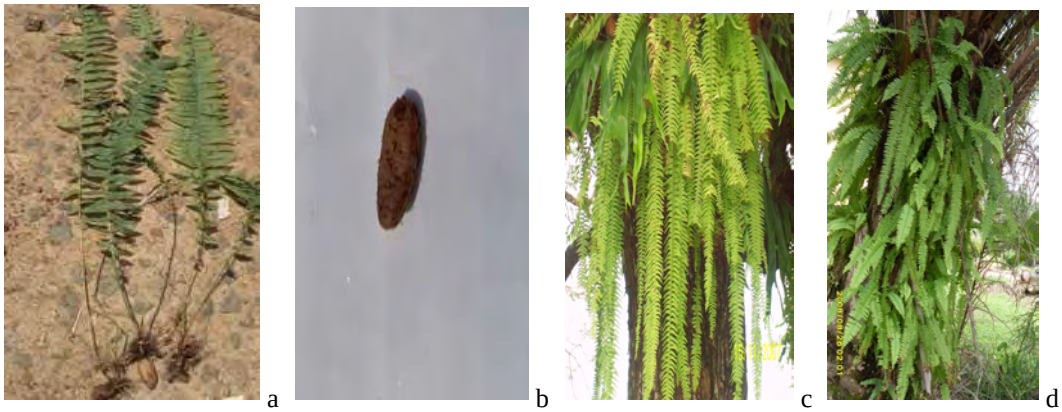


Fig. 1 Plant forms and habits (a): *Nephrolepis undulata* showing tuber on the rhizome. (b): *N. undulata* showing its tuber. (c): *N. undulata* on the (*Azardiracta indica*) tree trunk opposite Staff Club, Obafemi Awolowo University, Ile-Ife. (d): *N. undulata* as epiphytic fern (plant) on *Elaeis guniensis* near the Department of Botany car park, Obafemi Awolowo University, Ile-Ife.

Fig. 2. Plant forms and habits. (a): *Nephrolepis biserrata* showing sori arrangement and crozier covered with whitish substance. (b): Natural habitat and aesthetic value of *N. biserrata* at the Department of Botany, Obafemi Awolowo University, Ile-Ife

The stomata index was obtained by expressing the number of stomata per unit field as a percentage of the total number of epidermal and subsidiary cells in the same unit area, as in the following equation:

Where I = Stomata index, S = Number of stomata per unit area and E = Number of ordinary epidermal cells plus the subsidiary cells in the same unit area.

$$I = \frac{S}{E + S} \times 100\%$$

Statistical Analysis

The results of the quantitative morphometric and anatomical data generated were subjected to statistical analyses using one way analysis of variance (ANOVA) with Duncan multiple range test to show if there exist significant difference in the two species studied.

RESULTS

The summary of the leaflet morphological study of the two species investigated is presented in Table 1. The result shows that the two taxa possessed striking resemblances in their foliar morphological characters such as leaflet apex, indusium, colour of stipe, position and arrangement of sori on the leaflet margin of the abaxial surface as well as the drooping fronds. However, there is variation in their leaflet margin. In *N. biserrata*, it is serrated while in *N. undulata* the serration is not as deep as in *N. biserrata*. Frond number varies in the two species. In *N. biserrata*, it is 34-38 and 2-5 in *N. undulata*. Leaflet number is 86-94 in *N. biserrata* while in *N. undulata* it is 54-63. Table 2 shows the summary of the anatomical study in *N. biserrata* and *N. undulata*.

Table 1: Leaf morphology: qualitative characters of *Nephrolepis biserrata* and *N. undulata*

Characters	***Nephrolepis biserrata***	***Nephrolepis undulata***
Leaflet apex	Acuminate	Acute/acuminate
Leaflet margin	Serrated	Serrated
Leaflet base	Oblong	Auriculate
Ramenta	Seen at the base	Not seen
Indusium	Present, round to reniform	Present, round
Stipe	Polished brown	Polished brown
Sori	Present on the leaflet margin	Present on the leaflet margins
Frond	Drooping, erect, fertile	Drooping, pendent, fertile
Rhizome	Erect, long, perennial	Erect, short, perennial, tuberous

Table 2: Quantitative characteristics of *Nephrolepis biserrata* and *N. undulate*

Plant Species	**Fr. No.**	**Lf. No.**	**Fl. (cm) ± S. E., N = 5**	**Fd. (mm) ± S. E., N = 5**	**Lfl. (cm) ± S. E., N = 5**	**Lfb. (cm) ± S. E., N = 5**
N. biserrata	34-38	86-94	113-280	1.70-3.88	10.31-13.64	1.80-2.74
N. undulata	2-5	54-63	85-170	1.22-1.90	10.00-28.10	2.00-2.66

Fr.- No — Frond Number, Lf. No. —Leaflet Number, Fl. — Frond length, Fd. — Frond diameter, Lfl. — Leaflet length, Lfb. — Leaflet breadth, S .E. — Standard error, N— Number.

Adaxial Surface

***N. biseratta* (Fig. 3 (a))**

Epidermal cells are largely irregular, anticlinal wall is thick, straight and wavy to sinuous, 5.88 -12.04 µm long and 2.50-5.60 µm wide, number of epidermal cells per field is 64-68 (Table 4), crystal sand present and numerous (Table 3).

***N. undulata* (Fig. 3 (b)).**

Epidermal cell generally irregular in shape, anticlinal wall thin, wavy to sinuous (Table 3), 35-37 per field, 8.40-18.20 µm long and 4.76-8.68 µm wide (Table 4). Trichome, crystal sand and stomata are generally absent in the adaxial surface of *N. undulata*.

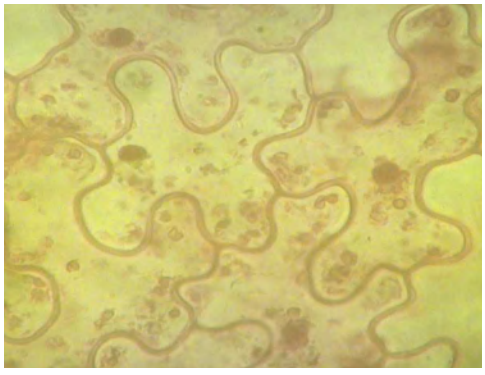
Table 3: Leaf anatomy (adaxial surface only)

Features	***Nephrolepis biserrata***	***Nephrolepis undulata***
Epidermal cell	Irregular in shape	Irregular in shape
Anticlinal wall	Thick, straight and wavy to sinuous	Thin and wavy to sinuous
Stomata	Not seen	Not seen
Trichome	Not seen	Not Seen
Crystal sand	Seen, numerous	Not seen

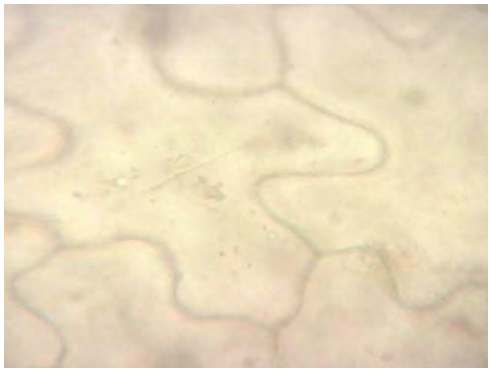
Table 4: Leaf anatomy (epidermal surface)

Plant species	**Leaflet surface**	**Shape of EC.**	**L. of EC. (µm) ± S. E. N = 5**	**Br. of EC. (µm) ± S. E. N = 5**	**Numbers per field**
Nephrolepis biserrata	Adaxial	Irregular	5.88-12.04	2.50-5.60	64-68
	Abaxial	Irregular	5.88-12.04	2.52-5.60	47-54
Nephrolepis undulata	Adaxial	Irregular	8.40-18.20	4.76-8.68	35-37
	Abaxial	Irregular	6.16-18.76	2.80-8.96	24-34

EC—Epidermal cell, S. E—Standard error, N—Number, L—-length, Br—Breadth



a



b

Fig. 3: Anatomical features showing the epidermal cells on the: (a):adaxial surface N. *biserrata* (b) : adaxial surface of *N. undulata*

Abaxial Surface

***N. biserrata* (Fig. 4 (a))**

Epidermal cell irregular with thick and straight to wavy anticlinal wall (Table 5), 47-54 per field, 5.88-12.04 μm long and 2.52-5.60 μm wide (Table 4). Stomata diacytic and anomocytic with elliptical guard cell (Table 5). Non glandular multicellullar, uniseriate trichomes present and numerous, 26.30-57.60 μm long (Table 6 and Fig.7). The guard cell is (4.70-5.80 μm) long and (2.50-3.60 μm) wide (table 7).

***N. undulata* (Fig. 4 (b))**

Epidermal cells irregular, anticlinal wall thin and wavy to sinuous (Table 5): between twenty -four and thirty-four per field, 16.16-18.76 μm long to 2.80-8.96 μm wide. Stomata diacytic and anomocytic, guard cell elliptic (Tables 5 and 7). Trichome absent, crystal sand present and numerous (Table 5). The guard cell is (3.92 -5.88 μm) and (1.96-3.08μm) wide (Table 7)

Venation Pattern (Fig. 5-7)

Venation pattern in the two taxa is dichotomous, areoles not well formed or absent. However, in *N. biserrata*, the midrib has parenchymatous sheath which is absent in *N. undulata* (Table 8). Generally, leaflets in the two species are hypostomatic.

DISCUSSION

Both the foliar anatomical and morphological characters of *Nephrolepis biserrata* (Swartz) Schott and *N. undulata* (Swartz) J. Sm revealed some areas of evolutionary relationship between them although there are characters that separate them. The results of this work agree with the observation of Carlquist (1961) that the leaves of plants can provide variety of anatomical features that can be of taxonomic importance. The commonly used characters like epidermal cell structure, types of stomata, trichomes and crystals, venation patterns and morphological structures were largely employed in this study and the data obtained can be used in the taxonomic separation of the two taxa. Each species showed marked consistency for the anatomical and morphological characters examined.

Exomorphologically, both of them have similar leaf type (compound unipinnate), leaflet apex (acute to acuminate), arrangements (alternate/ opposite) and serrated margins, arrangement of sori, shape of indusia and colour of the stipe. However, variations occur in some morphological character such as growth form, rementa, leaflets (base, numbers, length and width), frond number, length and diameter. Rhizome in

Table 5: Leaf anatomy (abaxial surface only)

Characters	*Nephrolepis biserrata*	*Nephrolepis undulata*
Epidermal cell	Irregular in shape	Irregular in shape
Anticlinal wall	Thick and straight to wavy	Thin wavy to sinuous
Stomata	Present, diacytic, anomocytic type and elliptic in shape	Present, diacytic, anomocytic type and elliptic in shape
Trichome	Seen	Not seen
Crystal sand	Seen, numerous	Seen, numerous

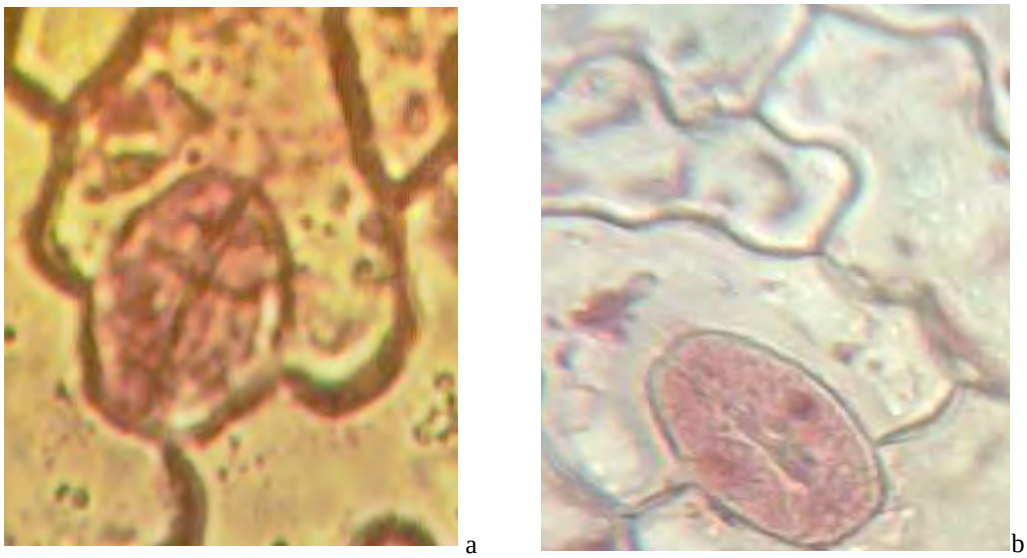
Table 6: Non-grandular uniserrate multicellular trichome on the abaxial surface only

Plant species	Availability	Length (μm)	Quantity
Nephrolepis biserrata	Available	26.30-57.60	Numerous
Nephrolepis undulata	Not available	None	None

Table 7: Leaf anatomy (guard cell on the abaxial surface only)

Plant species	GC. Length (µm)	GC. Width (µm)	GC. Area (µm^2)	Stomata index%	Stomata frequency per field
Nephrolepis biserrata	4.70-5.80	2.50-3.60	9.23-12.29	13.45	7-9
Nephrolepis undulata	3.92-5.88	1.96-3.08	6.89-13.54	13.11	4-5

GC— Guard cell



a b

Fig. 4: Anatomical features showing guard cells and stomata on the: (a): abaxial surface of *N. biserrata*. (b): abaxial surface of *N. undulata*.

Table 8: Summary of the venation patterns in the two species of *Nephrolepis*

Description	*Nephrolepis biserrata*	*Nephrolepis undulata*
Venation type	Dichotomous	Dichotomous
Veinlets	Terminate with each sorus	Terminate with each sorus
Areoles	Not seen	Not seen
Mid-rib	Sheathed	Not sheathed at all
Sheath type	Parenchymatous	Not available

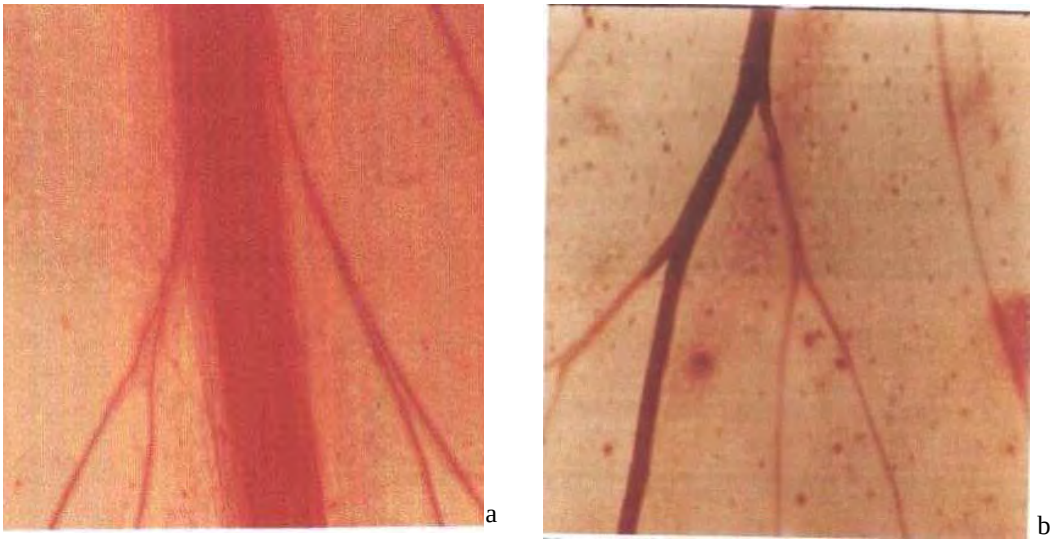


Fig. 5: Anatomical features showing venation pattern in (a): *N.biserrata*. (b): *N. undulata*.

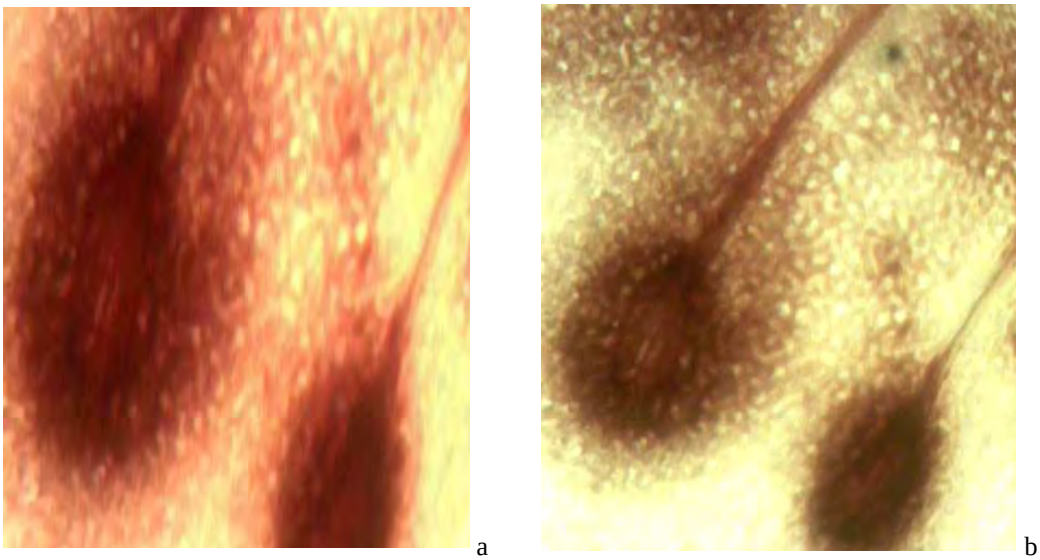


Fig. 6: Anatomical features showing veinlet ending in sori in: (a): *N.biserrata*. (b): *N. undulata*.

N. biserrata is erect and non-tuberous whereas in *N. undulata* it is short, sub-erect and tuberous (Fig. 2 (a) and (b)). Here, it serves as preservative as it is a perennial found under the substrata and able to sprout out new fronds at the onset of rainy seasons. Anatomy of the leaflets revealed some variations in both their adaxial and abaxial surfaces. The anticlinal wall on the adaxial surface of *N. biserrata* is thick while that of *N. undulata* is thin. The presence of numerous multicellular uniserriate trichomes on the abaxial surface of *N. biserrata* delimits it from *N. undulata* which does not have any at all. Similarly, the distributions of crystals in the

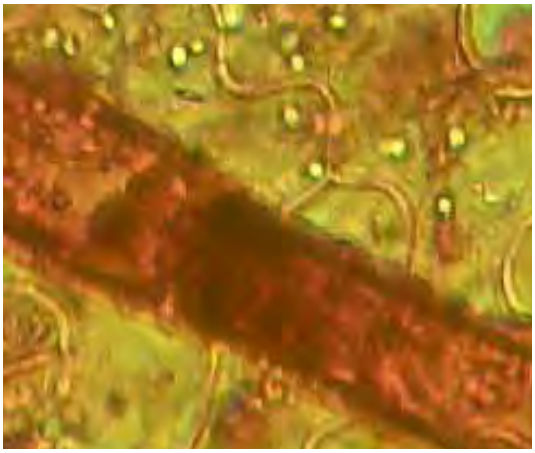


Fig. 7: Anatomical features showing trichome in N. biserrata

two species appear to be taxonomically important in separating them. While numerous crystal sands are present on both the adaxial and abaxial surfaces of *N. biserrata*, they are only present on the abaxial surface of *N. undulata*. According to Cutter (1978), the value of stomata index (I) is reasonably constant for any particular species. Thus, the stomata index can be used as a taxonomic tool to separate them since the two taxa investigated have different values of stomata index. Both of them are hypostomatic (i.e. stomata present on the abaxial surface only) and their stomata type are predominantly diacytic and anomocytic. In comparison, the guard cell is longer and wider with large area in *N. biserrata* than *N. undulata* (Table 7), the range of stomata frequency in *N. biserrata* is 7-9 per field and 4-5 per field in *N. undulata*. This shows that *N. biserrata* has more quantity of stomata than *N. undulata*. Thus, the rate of transpiration is expected to be higher in *N. biserrata* than *N. undulata*. This is an advantage to *N. undulata* since as an epiphyte, it has less access to water and therefore the smaller number of stomata enables it to conserve more water than *N. biserrata*. This might be the reason why *N. biserrata* is restricted to moist or damp ecological areas. Also in contrast, the midrib of *N. biserrata* is parenchymatous sheathed while that of *N. undulata* is not sheathed at all.

CONCLUSION

In conclusion, the two species studied shows close inter-relationships in their anatomical and morphological structures which can be used to classify and delimit them. Thus, the anatomical and morphological similarities exhibited by both of them can be one of the reasons for grouping them in the same genus ***'Nephrolepis'*** Schott (Alston, 1959; Agnew, 1974). The differences in their leaflets anatomy and morphology are taxonomically important for separating them into two different species as *Nephrolepis biserrata* (Swartz) Scott and *N. undulata* (Swartz) J. Sm.

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