

Full Length Research Paper

Morpho-anatomical characters of *Zehneria capillacea* (Schumach) C. Jeffrey and *Zehneria scabra* (L.F.) Sond Cucurbitaceae

Josephine Agogbua, Chimezie Ekeke* and Bosa Ebenezer Okoli

Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Port Harcourt, P.M.B. 5323, Choba, Rivers State, Nigeria.

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Comparative studies on macro-morphology, foliar epidermis, stem and petiole anatomy of two indigenous wild cucurbits (*Zehneria capillacea* and *Zehneria scabra*) in Nigeria were carried out in order to improve the delimitation of the species based on these parameters. The morphological features of significance observed include variations in leaf and stem pubescence density, leaf shape, fruit shape and color, flower color, seed shape and coat texture. Similarities were observed in their growth habit, shape of foliar epidermal cells, stomatal features and stem anatomy. The two species evaluated have a creeping growth habit, uniseriate eglandular trichomes, anomocytic and tetracytic stomata, pentagonal shaped stem with 8 bicollateral vascular bundles. Glandular 4-celled head trichome and isotricytic stoma were only found in *Z. capillacea*. The leaves of both species are amphistomatic having adaxial and abaxial stomatal densities of 2.47 ± 0.058 and 13.68 ± 0.021 for *Z. capillacea* and 3.24 ± 0.125 and 19.72 ± 0.199 for *Z. scabra*. However, the observed petiolar vascular bundle was 5 in *Z. capillacea* and 7 in *Z. scabra*. This distinct number in the petiole provides additional distinguishing information for maintaining them as different species.

Key words: Anatomy, bicollateral vascular bundles, stomata, trichome.

INTRODUCTION

The genus *Zehneria* Endl. belongs to sub-tribe Cucumerinae of the Benincaseae together with eleven other genera (Jeffrey, 2005) and is presently restricted to those minor cucurbits with small, white or yellow, mostly monoecious flowers. There are about 35 species in the Old World tropics, extending from South Africa and Madagascar through tropical Africa and Asia to Japan,

Malaysia, Australia and Polynesia (Jeffrey, 1990; De Wilde and Duyfjes, 2004). Ethno-botanical surveys showed species such as *Zehneria capillacea*, *Zehneria cordifolia*, *Zehneria minutiflora* and *Zehneria mucronata* in the Niger Delta region of Nigeria (Edwin-Wosu and Ndukwu, 2008).

In African countries, villagers generally consume leaves,

*Corresponding author. E-mail: ekeke.uc@gmail.com.

fruits and flowers of cultivated cucurbits and also harvest leaves and fruits of some wild cucurbits for consumption and medicinal use (Jansen van Rensburg et al., 2004). *Zehneria* species have enormous ethno-botanical value and are used by different tribes for food as wild edible plants and treatment of various ailments. In India, the leaves of wild cucurbits such as *Z. maysorensis* are powdered and taken with honey to kill stomach worms (Ayyanar and Ignacimuthu, 2005). The root extract of *Z. scabra* is used with milk to treat fever and diarrhea while the leaf extract is used to treat skin rashes (Anand and Jeyachandran, 2003) and has anti-bacterial and anti-inflammatory properties. Leaves of *Z. scabra* and bark and leaf of *Rumex nervosus* are pounded and rolled in cloth, and tied on swelling to reduce the effect (Amenu, 2007).

In Nigeria, roots of *Z. cordifolia* are used by herbalists to induce abortion (Chike et al., 2006), while the powdered leaves of *Z. hallii* is used for the treatment of tapeworm and as sedatives (Burkill, 1985). The leaves of *Z. capillacea* and *Z. cordifolia* are used as vegetable in soup (Edwin-Wosu and Ndukwu, 2008; Omara-Achong et al., 2012). Despite the immense economic potentials of cucurbits, information on their morphology and anatomy is either scanty or completely lacking (Okoli, 2013).

According to Stace (1980), the anatomical characterization of plants is not affected by environmental changes and the knowledge has been utilized to delimit species, genera and families in plant, this study is widely used in systematic identification, placing anomalous groups in a satisfactory position in classification and explaining patterns of relationship that may have not been clearly expressed in morphological features. Among Nigerian species of cucurbits, the uses of morphological and anatomical characters in their delimitation have been reported (Okoli 1984; Aguoru and Okoli, 2012; Agbagwa and Ndukwu, 2004; Ndukwu, 1988; Ndukwu and Okoli, 1992; Ajuru and Okoli, 2013). Also, taxonomic, cytogenetic and ethno-botanical investigations have been conducted on some of these indigenous cucurbits by researchers in the Universities and national research institutes (Okoli, 1984, 1987; Ndukwu, 2000; Ndukwu et al., 2005; Agbagwa, et al., 2007; Agbagwa and Ndukwu 2004) but reports on the anatomical features of *Zehneria* species is lacking.

This study was carried out with the aim of comparing the morphological and anatomical features of *Z. capillacea* and *Z. scabra* which are two wild cucurbits with little scientific report and to use these characters to improve the delimitation of the species.

MATERIALS AND METHODS

Experimental site

The experiment was carried out in the biosystematics and

taxonomy laboratory of the Department of Plant Science Biotechnology, University of Port-Harcourt, Choba, Rivers State, Nigeria (04° 54' 29.00"; 006° 55' 02.90").

Plant material

Fresh plant materials of *Z. capillacea* and *Z. scabra* were collected during field trips to various parts of Rivers State, Bayelsa State and the University of Port-Harcourt environment.

Morphological studies

Standard morphological descriptors for *Cucumis* germplasm prepared by The International Plant Genetic Resources Institute (IPGRI) Italy, was used to score vegetative and inflorescence characters (IPGRI, 2003). Overall morphology of the leaf, flowers and fruits were recorded by photography.

Epidermal studies

Foliar materials for epidermal studies were collected fresh from plants in the field. 5 mm – 1 cm square leaf cuttings were obtained from identical regions of each fresh leaf, generally from mid-way between the leaf base and apex of lamina including the mid-rib. The adaxial and abaxial epidermal peels were obtained using sharp pointed forceps. Peels were stained with 1% safranin or alcian blue rinsed with distilled water to remove excess stain and then mounted in a drop of pure glycerol on clean glass slides. A cover glass was placed over the drop and sealed with nail varnish to prevent dehydration (Okoli and Ndukwu, 1992). The epidermal features that were observed include: organization of the epidermis, arrangement of the epidermal cells, nature of trichomes, shape of epidermal cells and nature of the anticlinal cell wall of the leaf epidermis, stomatal types, density and index. The stomatal index (SI) was estimated based on Metcalfe and Chalk (1979) while the terminology for the stomatal type is taken after (Malvey, 2004). The mean and standard deviation were calculated using Microsoft excel 2010.

Stem and petiole anatomical studies

Specimens for anatomical analysis were obtained fresh from matured plants and fixed in FAA for 12 h. They were dehydrated, transferred to 70% ethanol and kept at room temperature until required. The stem and petiole were hand sectioned using sharp razor blades (Okoli and Ndukwu, 1992). The sections were stained in 1% safranin red for two minutes, viewed and micro-photographed using a photomicroscope.

RESULTS

The summary of the results of this study is presented in Tables 1 – 3 and Figures 1 – 4.

Habit, habitat and morphology of *Zehneria* species

The species grow in swampy, seasonal flood plain or moist soils. *Z. scabra* predominantly grows in sandy

Table 1. Morphological characteristics of *Z. capillacea* and *Z. scabra* evaluated in the wild.

Descriptors	Species	
	<i>Z. capillacea</i>	<i>Z. scabra</i>
Growth habit	Indeterminate	Indeterminate
Stem pubescence	Moderate	Dense
Leaf shape	Triangular with hastate base	Triangular with cordate base
Leaf color	Dark green	Green
Fruit shape	Elliptical	Oval (Globose)
Flower color	White	Yellow
Primary fruit color	Green	Light green
Secondary fruit color	Dark green	Dark green
Ripe fruit color	Green	Red
Seed coat	Light brown	Dark brown
Seed coat texture	Smooth	Rough
Inter-node length (cm)	4 - 6	6 - 7
Petiole length (cm)	2 - 4	3 – 5

Table 2. Epidermal characteristics of *Zehneria capillacea* and *Zehneria scabra*.

Epidermal character	<i>Z. capillacea</i>		<i>Z. scabra</i>	
	Adaxial	Abaxial	Adaxial	Abaxial
Shape of epidermal cell	Regular	Irregular	Regular	Irregular
Anticlinal cell wall pattern	Slightly straight or curved	Undulating but partly curved	Slightly straight or curved	Undulating
Stomata type	Anomocytic, tetracytic, isotricytic	Anomocytic, tetracytic	Anomocytic, tetracytic	Anomocytic, tetracytic
Stomata index (S.I)	2.47±0.058	13.68±0.022	3.24±0.125	19.72±0.199
Trichomes	+	+	+	+
Glandular	+	+	-	-
Eglandular	2 Type	2 Type	1 Types	1 Types

+ = Present; - = Absent.

Table 3. Anatomical characteristics of *Zehneria capillacea* and *Zehneria scabra*.

Anatomical character	<i>Z. capillacea</i>		<i>Z. scabra</i>	
	Stem	Petiole	Stem	Petiole
Shape	5-angled	U-shaped	5-angled	U-shaped
No. of vascular bundles	8	5	8	7
Nature of adaxial surface	NA	U-shaped or curved	NA	V-shaped

NA = not applicable.

beaches or soils while *Z. capillacea* grows on moist soil. The qualitative descriptors scored for morphological traits in the two species are shown in Table 1. The plant habit (creeping) was similar for both species (Figure 1). However, there was phenotypic variation in the vegetative and reproductive morphology of plants evaluated. The leaf size of *Z. scabra* was visually observed to be bigger in size and more hairy than *Z. capillacea* (Figure 1a-d). The flower color is yellow in *Z. scabra* and white in *Z. capillacea*. Other observed variation include stem and leaf pubescence, leaf shape,

fruit shape, fruit primary and secondary color and seed coat texture (Table 1 and Figure 1e-f).

Epidermal characters

The result of the leaf epidermal study for the two species revealed uniseriate epidermis. The two species are amphistomatic. Three stomata types were observed on the epidermal surfaces of these species. The stomata types include tetracytic (stoma completely surrounded by

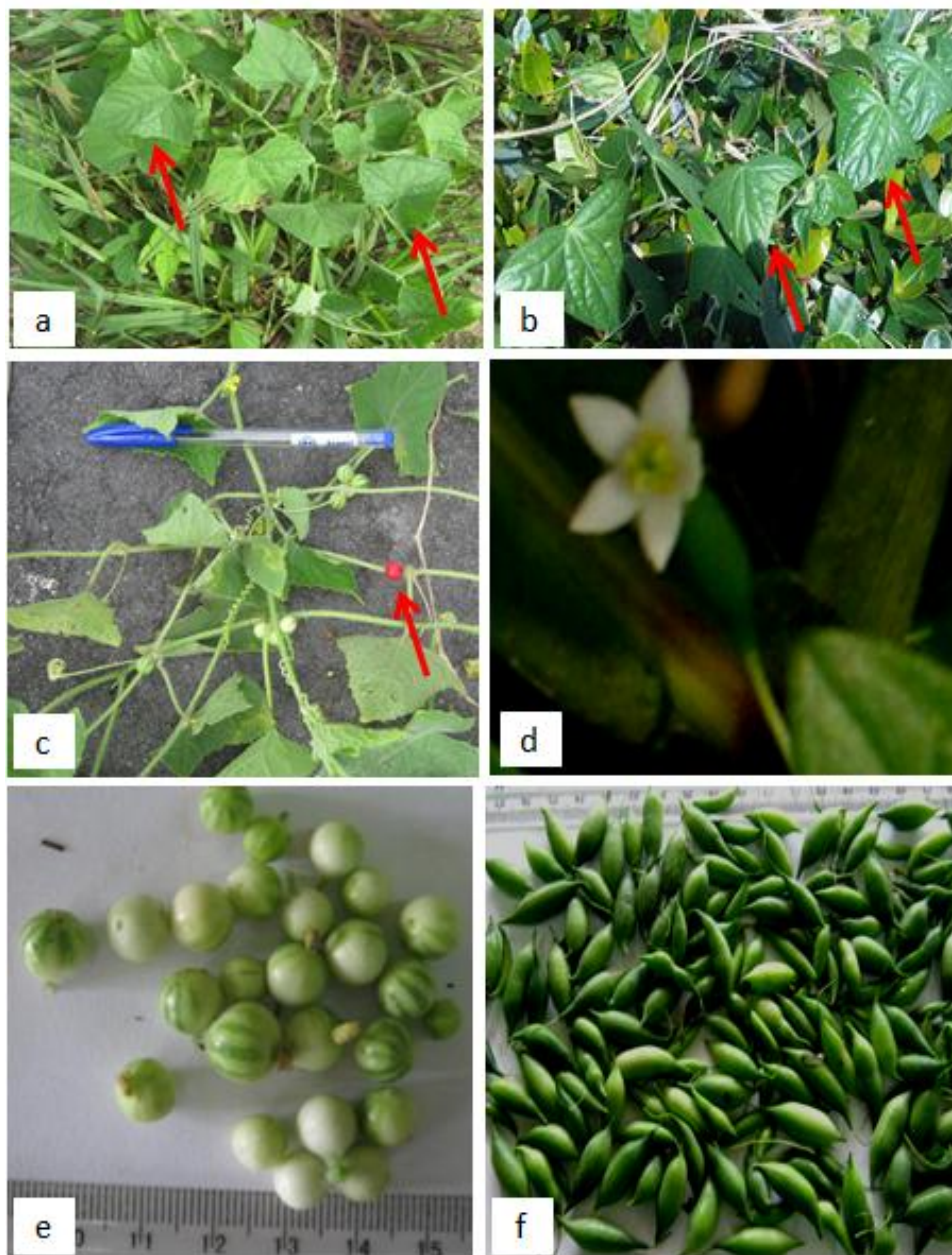


Figure 1. Habit and habitat of *Z. scabra* (a) and *Z. capillacea* (b). Arrows show the *Zehneria* species. Plate 2. Reproductive characters of *Zehneria* species: (c) *Z. scabra* showing yellow flowers, arrow shows ripe red fruit; (d) *Z. capillacea* showing white flowers; (e) Fruits of *Z. scabra* and (f) Fruits of *Z. capillacea*.

only 4 subsidiary cells, variable in size and shape, of which two are polar and two are lateral in position), anomocytic (stoma completely surrounded by only 4 or more subsidiary cells, variable in size and shape other than tetracytic and staurocytic types) and isotricytic (stoma completely surrounded by only 3 subsidiary cells, variable in position and shape but 3 of the subsidiary

cells are more or less of equal size) (Table 2). The data recorded revealed that epidermal characters such as stomatal density and stomatal index are more on the abaxial surface than the adaxial surface (Table 2). The stomatal index on the adaxial surface was 13.7 ± 0.021 and 19.7 ± 0.199 for *Z. capillacea* and *Z. scabra* respectively while on the abaxial surface, it was 2.47 ± 0.058

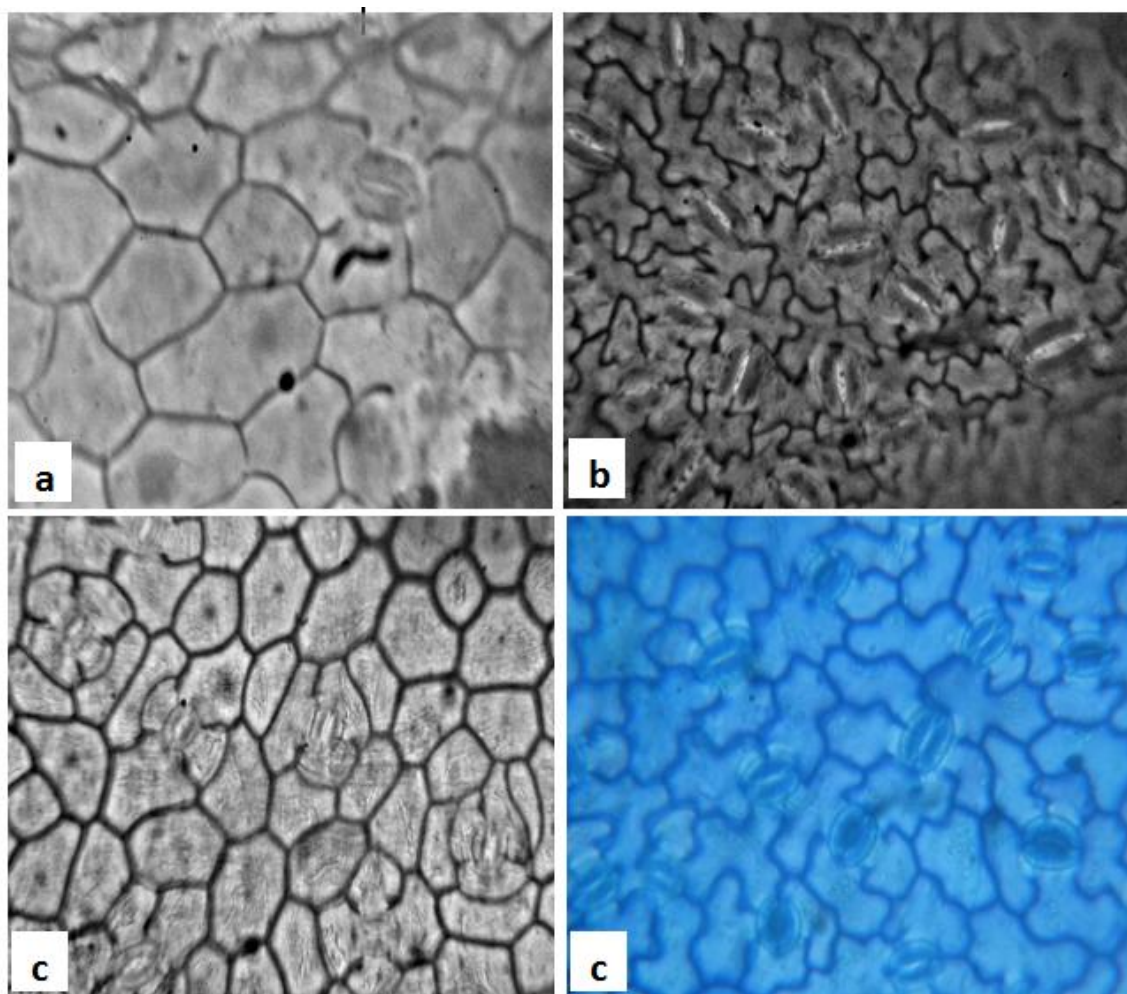


Figure 2. Epidermal features of *Zehneria* species: ((a). Adaxial surface of *Z. scabra* (x100); (b). Abaxial surface of *Z. scabra* (x100); (d). Adaxial surface of *Z. capillacea* (x40) and (e). Abaxial surface of *Z. capillacea* (x40). Note: polygonal shape of the upper epidermis and the irregular crented shape of the lower epidermis in both species.

and 3.24 ± 0.125 for *Z. capillacea* and *Z. scabra*, respectively. Variations were also observed in the shape of the epidermal cells. The abaxial cells are irregular, wavy or crenulated while the adaxial cells are more regular in shape (Figure 2).

Only uniseriate eglandular trichomes were observed in *Z. scabra* (Figure 3a) while uniseriate eglandular and multicellular glandular trichomes are present on leaf surfaces of *Z. capillacea* (Figure 3b-d). The two types of eglandular trichomes observed on the adaxial surface of *Z. capillacea* are a short thick walled eglandular trichome with an acute tip and a broad multicellular base (Figure 3c) and a short unbranched multicellular trichome (Figure 3d). The latter which is absent on the abaxial surface has 4-celled head, serrated short stalk and a spherical broad base.

Anatomical characters

The stem anatomy of the two species revealed a pentagonal-shaped or 5-angled stem with 8 bicollateral vascular bundles arranged in two major rings (Table 3 and Figure 4a-b). The five peripheral smaller bundles occur on each angle and alternate with the three bigger inner bundles which border on the pith cavity. The stem epidermal cells have uniseriate eglandular unbranched trichomes. Angular collenchyma cells are present below the epidermis and a broad band of perivascular fibres in the cortex.

Transverse section of the petiole showed single layered epidermis which consists of thin walled cells with conical uniseriate eglandular unbranched trichomes. It also revealed a free bundle vasculature pattern arranged in a

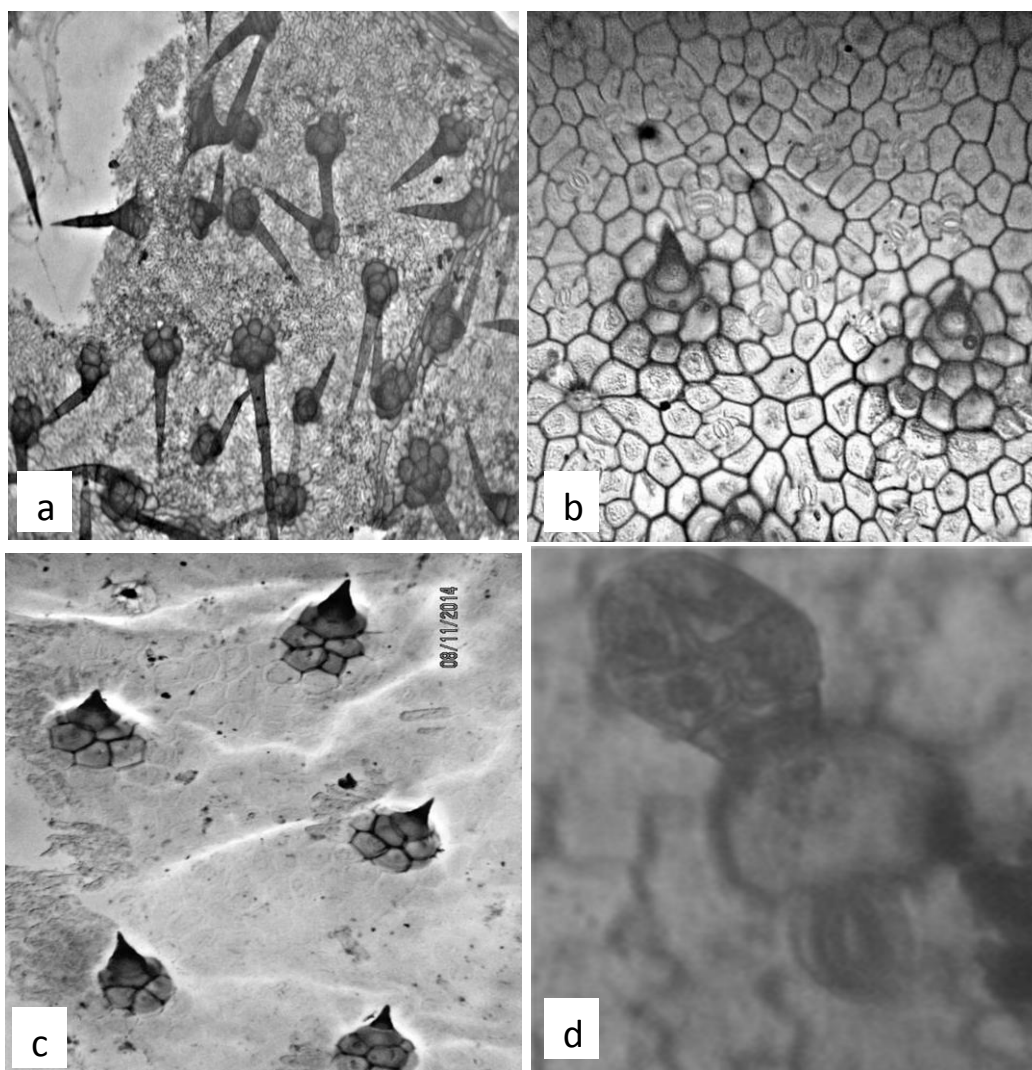


Figure 3. Trichomes in foliar epidermis of *Zehneria* species. (a) Uniserial eglanular trichome of *Z. scabra* with multicellular base; (b) Uniserial eglanular trichome of *Z. capillacea*; (c) Eglanular trichome of *Z. capillacea* with multicellular base only on adaxial surface and (d) Glandular trichome of *Z. capillacea* showing 4-celled head.

semi cylinder. There are 5 bicollateral vascular bundles present in *Z. capillacea* (Table 3 and Figure 4c) *Z. scabra* had 7 vascular bundles (Figure 4d). The abaxial surface of *Z. scabra* is V-shaped while that of *Z. capillacea* is curved or U-shaped (Figure 4c-d).

DISCUSSION

Most curcubits are dioecious (male and female flowers on different plants) and rarely monoecious (male and female flowers on the same plant). For example, *Citrullus lanatus* Thunb (watermelon) and *Cucurbita pepo* L. (pumpkin) are dioecious (Agbagwa and Ndukwu, 2004), *Cucumis*

sativus L. (cucumber) are monoecious (Ndukwu, 1988). Then, there are some special plants like limon-cetriolo, lemon-cucumber which has staminate (male) flowers but also hermaphroditic (Ndukwu and Okoli, 1992). Some cultivars of watermelon have male, female and hermaphrodite flowers on the same plant (Okoli, 1984). Flowers are small, white or yellow, monoecious or dioecious, rarely hermaphroditic. Male flowers are solitary or few to many in sessile or pedunculate racemiform or umbelliform clusters. The fruit is solitary or clustered, globose, ellipsoid or fusiform, red, whitish or green, smooth, sometimes finely pitted when dry (Hutchinson and Dalziel, 1954). The seeds are small, elliptic to broadly ovate in outline, compressed or flat (Hutchinson

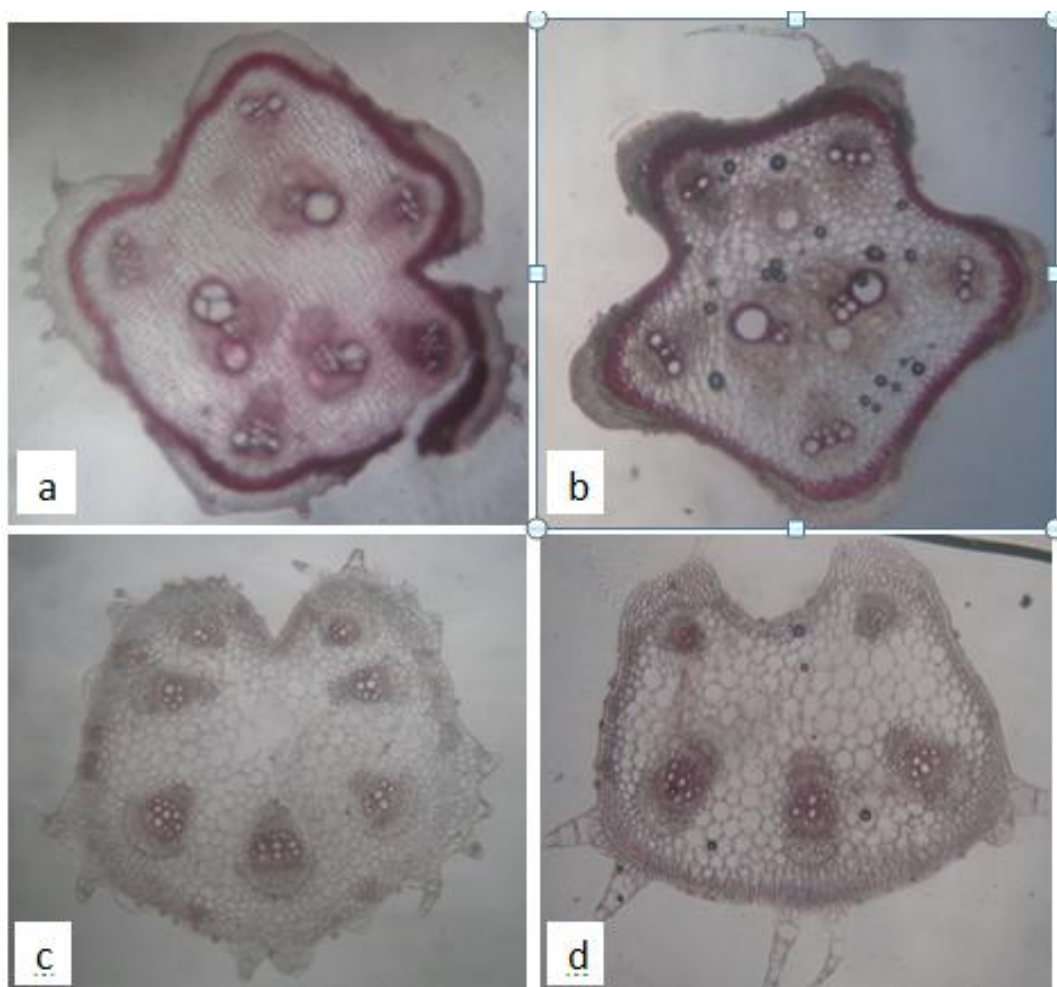


Figure 4. Anatomical features of transverse section (TS) of *Z. capillacea* and *Z. scabra*: (a) Stem of *Z. scabra*; (b) Stem of *Z. capillacea*; (c) Petiole of *Z. scabra* (V-shape of abaxial surface) and (d) Petiole of *Z. capillacea* (curved or U-shape of abaxial surface) Note: 5-angled (pentagonal) stem with similar vascular bundles (a and b) and the variation in the petiole anatomy and number of vascular bundles (c and d).

and Dalziel, 1954; Okoli 1984; Ndukwu, 1988; Ndukwu and Okoli, 1992; Jeffrey, 1990).

The variations that exist between the two species of *Zehneria* is of taxonomic value as observed in this study. The plant trailing growth habit, ecology, habitat and the vegetative morphological traits described are consistent with the description reported by Jeffrey (1990) but in contrast with the color of *Z. capillacea* fruits which was reported as red instead of green as observed in the current study. *Z. scabra* occurred predominantly in sandy soils while *Z. capillacea* can grow in any soil type.

The leaves of the species are amphistomatic with anomocytic, tetracytic and isotricytic types of stomata. Anomocytic type of stomata was the dominant stomata type observed in the species. It has been described in many plant species of the Cucurbitaceae (Okoli, 1989)

and it is the most common in angiosperms. However, isotricytic stoma was found only on the adaxial epidermal surface of *Z. capillacea*. This feature is distinctive and can be used to distinguish among the species studied. Furthermore, there are more stomata on the abaxial than the adaxial surface. This is expected since is in line with reports of Adebooye et al. (2012) on *Trichosanthes cucumerina*. A type of uniseriate eglandular trichome and one type of glandular trichome with 4-celled head were identified in *Z. capillacea* while only uniseriate eglandular trichome was observed in *Z. scabra*. The occurrence and types of these trichomes could be used to distinguish the species. For instance, the 4-celled head glandular trichome was only observed on the abaxial surface of *Z. capillacea*. Also, uniseriate eglandular trichome was found in both species but the ones in *Z. capillacea* are

short and thick, while the ones in *Z. scabra* are long and thin. This is consistent with the different types of glandular and eglandular trichomes that have been studied and described in cucurbits (Okoli, 1989; Kolb and Muller, 2004; Aguru and Okoli, 2012; Agbagwa and Ndukwu, 2001).

Morphology of plants is an important factor used in making useful taxonomic conclusion about plants but it cannot be solely used. Anatomical feature is also of great importance in taxonomy since they are less affected by environmental factors. In this study, the fruit shape and size, the vascular system of the petiole, the presence of isotricytic stoma and trichome types are all diagnostic. The diagnostic features of the two species of *Zehneria* therefore as belonging to the family Cucurbitaceae include the presence of bicollateral vascular bundles and arrangement of the vascular bundles in two rows. The observed anatomical similarities among the *Zehneria* species studied indicate phylogenetic relatedness of the taxa. The anatomical differences observed in each species must have been as a result of evolution, conferring heritable variation that could be exploited for taxonomic purposes.

Conclusion

Two species share the same ancestral gene pool as they have most characteristics in common. Based on anatomical features, the two species of *Zehneria* studied can be distinguished from one another based on morphological traits and variation in the number vascular bundles present in the petiole. This information is useful in identification and authentication of the species.

Conflict of Interests

The authors have not declared any conflict of interest.

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