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Anatomical Diversity among Certain Genera of Family Cucurbitaceae

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Abstract: *The anatomical characters of the Cucrbitaceae having long attracted the attention of botanists. In order to understand the evolution of anatomical diversity, anatomical features of five genera in the family Cucurbitaceae, (Colocynthis, Cucumis, Cucurbita, Citrullus, and Luffa) were investigated. Similarities in the distribution, tissue differentiation and number of layers of cells and tissues in the root, stem and leaf transverse sections were similar in all genera. However, there were variations in the vascular bundles in the roots where they were bicollateral in Cucumis sativus and Luffa aegyptiaca, while it consisted of four radial arms of primary xylem alternating with four arms of primary phloem in the other species.*

The Trichomes were multicellular, glandular and non-glandular types with a preponderance of non-glandular types with various shapes. The variation in number of tiers within these trichomes was taxonomically significance.

Keywords: *Diversity, Cucurbitaceae, Taxonomy, Anatomy.*

1. INTRODUCTION

Cucurbits belong to the family Cucurbitaceae and consist of about 130 genera and 800 species, according to the last taxonomic treatment of Jeffrey [1]. The family Cucurbitaceae belongs to the order Cucurbitales, class Magnoliopsida (subclass Rosidae). The family Cucurbitaceae is one of the most important in the Angiosperm taxa. This is especially so in Sudan where members of the family are used as fruits (water melon), food (pumpkin), soup condiment (egusi), vegetables (Fluted pumpkin), sponge (*Luffa spp*), water reservoirs (*Lageneria spp*) and as stew condiment (snake tomatoes) [2, 3]. Reference [4] attributed a lot of medicinal potency to the species of the family Cucurbitaceae. The most important cultivated genera are *Cucurbita* L., *Cucumis* L., *Citrullus* L., *Colocynthis* Mill, *Lagenaria* L., *Luffa* L., [5] Coccinia (Wight & Arn.), *Corallocarpus* (Welw), *Ctenolepis* (Hook), *Kedrostis* Medik. and *Momordica* L., [6, 7,8].

Literature searches revealed that the scientific importance and implications of anatomical features in different groups of plants have been indicated by different authors. These families include Dioscoreaceae, where certain anatomical features were used in the characterization of *D. alata* (L.) and *D. smilacifolia* L. [9]. In Costaceae, where differences in features of vegetative anatomy suggested a separate specific status for *C. afer* and *C. lucanusianus* as opposed to the conspecific treatment given to them by previous researchers [10]. In Leguminosae-Caesalpinoideae, where the nature of unicellular and multicellular trichomes are described in certain species of *Senna* (Tourn ex Mill) and *S. hirsuta* was reported to be diagonostic in acquisition of these two types of trichomes [11]. Other studies of interest relating to anatomy of different angiospermous groups could be found in Curcurbitaceae [12], Dicotyledon plants as a whole [13] Leguminosae-Papilionoideae [14].

The objective of this investigation is to describe the root, stem and leaf anatomical characters of the Cucurbit species and to assess the relevance of and the extent to which anatomical features could be

utilized in the biosystematic deliniation of the Cucurbit species in view of their perceived similarities in structural and reproductive biology.

2. MATERIALS AND METHODS

In order to study the variation of internal structural in the leaves, stems and roots of some cucurbits, microtome study was undertaken and microscopic anatomical observations were carried out following the method suggested by [15], [16], [17] and [18].

2.1. Plant Materials

Nine species of the family Cucurbitaceae were collected from different places in Khartoum State (Table 1). About 2.5 cm long parts were prepared using vegetative parts of roots, stems and leaves of the plant material**.**

2.2. Killing and Fixing

The specimens were killed and fixed in formalin acetic acid (FAA) for at least 24 hours (FAA: 10:50:5:35 proportion of formalin, alcohol, acetic acid and water) which is represented by the previous formula according to [16]. The plant specimens were washed in distilled water three times allowing a time of 20 minutes for each wash.

2.3. Dehydration

After killing and fixing the plant specimens in FAA solution, they were transferred to a series of different alcoholic concentrations (50 %, 70 %, 90 %, and 95%) and then were left overnight or more in each concentration.

2.4. Clearing

The plant specimens were cleared using two mixtures, with different ratios for different times: mixture I composed of absolute alcohol : cedar wood oil (100 : 0 for 24hr, 50 : 50 for 3hr, 25 : 75 for 3 hr and 0 : 100 for 24 hr) respectively, and mixture II composed of cedar wood oil : xylene (100 : 0 for 24hr, 50 : 50 for 3hr, 25 : 75 for 3 hr and 100 for 24 hr), respectively.

2.5. Embedding

Next to the clearing process, the plant specimens were embedded in wax with melting point of 60 º C. The plant specimens were placed in closed vials containing 1:1 xylene and melted wax and put in the oven for 45 minutes. The wax was later replaced by pure wax twice after 45 minutes. The vials were left open to get rid of the xylene vapour. The specimens were transferred from the vials to the mold containing pure melted wax. Each specimen was pressed gently against the peripheral part of the mold. The wax was left to consolidate.

2.6. Sectioning

The paraffin embedded specimens were sectioned with the help of a rotary microtome. The thickness of the stem sections was 14 micrometer (µm) while the leaves and the roots were 9 and 12 µm thick, respectively. The ribbons were mounted on slides flooded with distilled water and placed on a hot plate to flatten the sections.

2.7. Staining

The double staining process was employed for this purpose. The steps of this process included dewaxing, rehydration, staining, and dehydration of material. In the dewaxing process the slides were passed twice through xylene, each for 3-5 minutes. Rehydation was carried by passing the dewaxed slides through a series of different concentrations of ethanol in this order: absolute, absolute, 95%, 90%, 70% and 50% and were then immersed in safranine, each for 2-3 minutes. Dehydration was carried out by passing the slides through different concentrations of ethanol (50%, 70%, 90%, 95% and absolute) each for 2-3 minutes. The slides were then immersed in fast green stain for one and a half minutes. The slides were then cleaned by passing through absolute ethanol and xylene.

2.8. Mounting

The material was mounted in D.P.X and covered with cover slips. The slides were kept in an oven at 60 ºC and left for 3 days, before examining under a light microscope at x10.

Table1. *Plant accessions used in this study.*

No	Species	Distribution and description
1	*Colocynthis vulgaris*	Wild. Nile banks, wadis and valleys in Khartoum State. Fruit edible by camels; the pulp is extracted and used in medicine.
2	*Ctenolepis cerasiformis*	Wild. Nile banks, Khartoum State
3	*Cucumis melo* var *flexuous*	Widespread. Cultivated, Faculty of Agric, Dept of Horticulture. Fruits edible as salad or pickled
4	*Cucumis sativus*	Widespread. Cultivated, Faculty of Agric, Dept of Horticulture. Fruits edible as salad or pickled
5	*Cucumis melo* var *reticullatus*	Widespread. Cultivated, Faculty of Agric, Dept of Horticulture. The fresh flesh of the fruit is eaten as dessert.
6	*Luffa aegyptiaca*	Wild. Widespread in Khartoum State. Used as bath sponges and for cleaning purposes.
7	*Cucurbita pepo*	Widespread. Cultivated, Faculty of Agric, Dept of Horticulture. The immature fruits eaten as a fresh vegetable; mature fruits used for baking, making jam and for pies or forage for livestock.
8	*Citrullus lanatus*	Cultivated. Widespread in Khartoum State. Edible. Dried parched seeds are chewed.
9	*Cucurbita moschata*	Widespread. Cultivated, Faculty of Agric, Dept of Horticulture. The immature fruits eaten as a fresh vegetable; mature fruits used for baking, making jam and for pies or forage for livestock.

3. RESULTS AND DISCUSSION

The anatomical study features of the roots, stems and leaves of the some Cucurbit species have been presented in photomicrographs of the transverse sections of these parts. Trichome differences in the taxa were remarkable. Using the most comprehensive trichome terminology [19], [20] there were two basic hair types (glandular and non – glandular) and many variations among the species under study. All species showed multicellular and non-glandular trichomes with various shapes (Fig. 1). Reference [21] found glandular trichomes with various tiers in Momordica sp, *Lageneria* and *Telfairia* sp. The glandular trichomes have been implicated for storage [19]. Reference [22] pointed that the non-glandular trichomes were useful as defense organs for the plants that posses them.

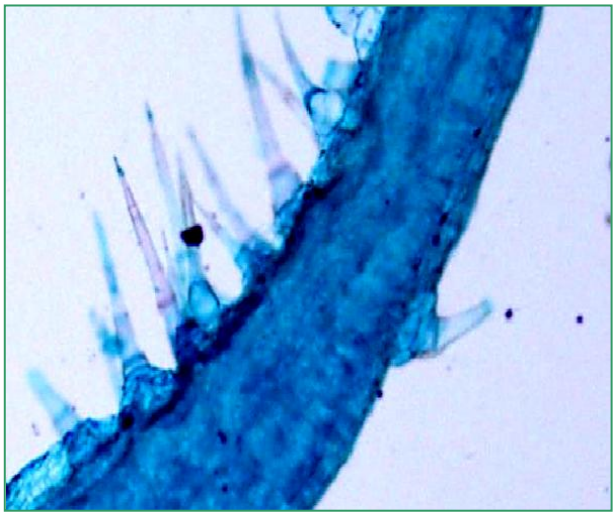

Fig1. *Leaf surface of Cucumis melo L. (x4). Acuminate trichome (the nonglandular multicellular trichomes).*

The roots usually consist of epidermis, cortex, endodermis and vascular bundles in most of the genera of the family Cucurbitaceae. Reference [23] reported that the structure and development of the *Cucurbita pepo* root is typical of dicotyledonous roots with limited secondary growth. Epidermis of the root of most genera is one cell thick. The root cortex collenchyma is 2 – 4 cells thick and cortex parenchyma is 5–7 cells thick in most of the genera. The endodermal and pericyclic layers are inconspicuous. The root vascular bundles consist of four radial arms of primary xylem alternating with four zones of primary phloem in most species except *Cucumis sativus* and *Luffa aegyptiaca* where the vascular bundles are bicollateral and arranged in a ring (Figs. 2, A and B).

Reference [24] found that the root epidermal layer of the *Hibiscus rosa sinensis* and *Abelmoschus esculenta* studied that the epidermal cells are in the form of short chains (kioned) small and numerous in *Hibiscus rosa sinensis* while they are of long chains, big and numerous in *Abelmoschus esculenta* . Similarly the cortex tissue shows the presence of small-sized parenchyma cells in *Hibiscus rosa sinensis* while in *Abelmoschus esculenta* the parenchyma cells are bigger in size. Both taxa show presence of angular collenchyma. The xylem vessels are numerous, circular in shape and are radially grouped in *Hibiscus rosa sinensis* while they are few and cuboidal in shape in *Abelmoschus esculenta*. Reference [25] reported the following information on the root anatomy of Cucurbits. The meristem consists of about seven layers of cambium-like cells, the number diminishing towards the periphery of the root apex. The tissue directly in the center forms the vascular cylinder, while adjacent cells form the cortical region. Approximate delimitation of the cortex and vascular cylinder is possible, because the cells of the innermost layer of the cortex continue to divide tangentially for a longer period of time than the adjacent cells of the vascular cylinder.

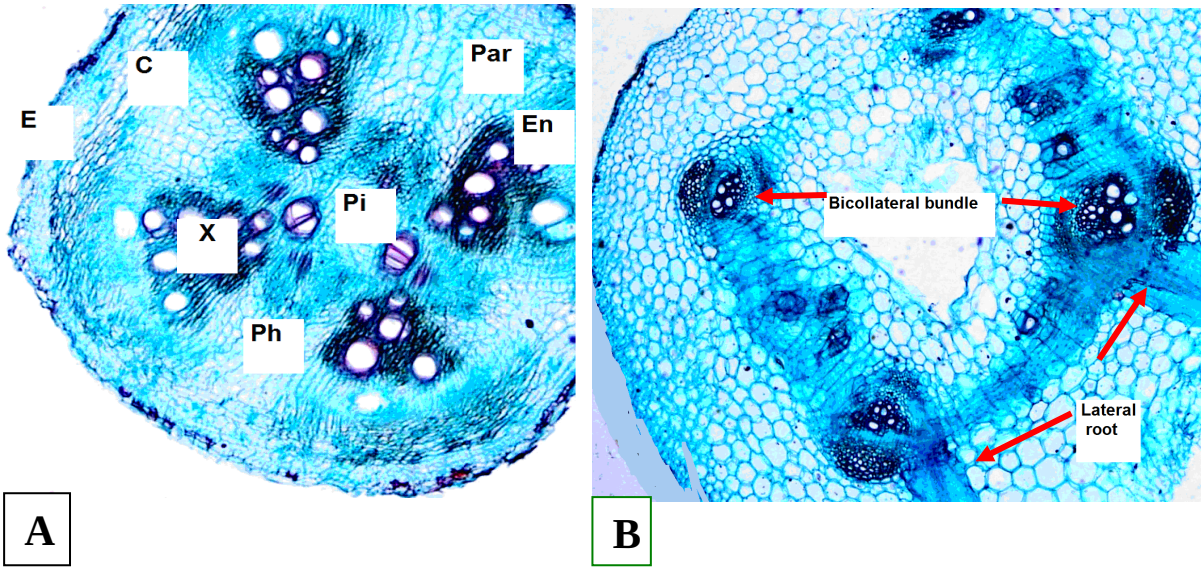

Fig2. ***(A)****: T. S of Root of Cucurbita moschata L. (X4).In general view with primary structure; Epidermis (E), Cortex (C), Parenchyma (Par), Endodermis (En), Xylem (X), Phloem (Ph) and Pith (Pi).* ***(B):*** *T. S of Root of Luffa aegyptiaca Mill. (X4). Vascular tissue (bicollateral bundle) and lateral root.*

The stem epidermis of Cucurbits is regular, with thin cells and scattered collenchyma cells in most of the species except *Cucurbita moschata* (Fig. 3A) where there are no scattered collenchyma. Stem cortex collenchyma is 4 - 6 cells thick in *Cucurbita moschata* while it consists of a narrow band of sclarenchyma cells in most of the other species eg. *Colocynthis vulgaris* (Fig.3D). Stem vascular bundles in most of species are arranged in two rings. The outer ring is composed of the often smaller bundles which are located at the angles of the stem. The inner ring contains the often larger bundles which alternate with those of the outer ring as in *Colocynthis vulgaris* (Fig.3C). However, in *Cucurbita moschata* the vascular bundles are arranged in one ring (Fig. 3B). The basic number of bundles is ten, each cycle consisting of five. Stem vascular bundles are bicollateral (Fig. 3A) have also been observed in Cucurbitaceae, Solanaceae and Asteraceae [26], [27], [28].

Leaves usually have a single layer of epidermal cells on both the upper and lower surfaces in all species with hairs present on both surfaces. Collenchyma tissues are seen in the median line of the upper surface of the leaf midrib. The thickness of the palisade parenchyma is not uniform in the different species. The spongy parenchyma consists of 2 to 6 layers of cells. This observation is in line with the work of reference [12], [10], [29] who used both the root and leaf anatomical features in the family Cucurbitaceae and Dioscoraceae in establishing relationship among taxa. The vascular bundles are bicollateral and arranged in different ways depending on the genera. The genera *Cucurbita* and *Citrullus* had seven bundles, the largest being the undermost and the six smaller ones lying above or on each side. The genus *Cucumis* has three bundles, which are arranged in a straight line, from above downwards; the uppermost bundle is the smallest, while the lowest is the largest. The genus *Luffa* has four bundles, being distinguished from the genus *Cucumis* by an additional small bundle near the upper part of the central bundle (Figs. 4 A and B).

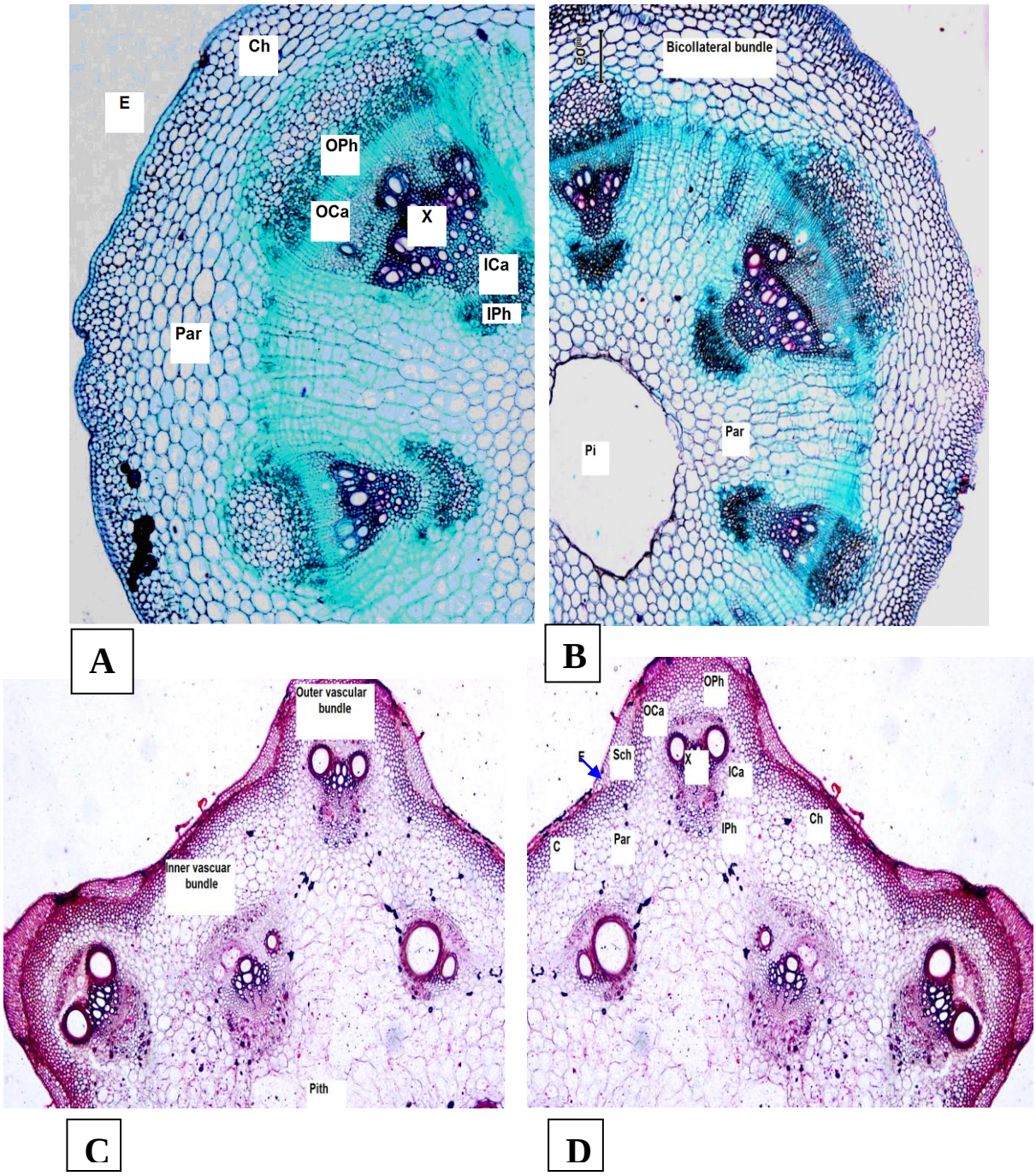

Fig2. ***(A)****. and* ***(D).*** *T. S of Stem of Cucurbita moschata L. and Colocynthis vulgaris Schrad. (X4).In general view with primary structure; Epidermis (E), Chollenchyma (Ch), Parenchyma (Par), Outer phloem (OPh), Outer cambium (OCa) Xylem (X),Inner cambium (ICa), Inner phloem (IPh), and Pith (Pi).* ***(B).*** *T. S of Stem of Cucurbita moschata L. (X4).Vascular tissue (bicollateral bundle) are arranged in one ring.* ***(C).*** *T. S of Stem of Colocynthis vulgaris Schrad. (X4). Vascular tissue (bicollateral bundle) are arranged in two rings.*

Various investigations have been made on the anatomy of Cucurbits, most of them prior to 1940 [30]. Although differences exist between Cucurbit species, Reference [31] had shown that, in general, there was considerable similarity among them.

There are some reports by [32], [33] on epidermal and vegetative characteristics of the three species *Cucurbita moschata*, *Cucurbita maxima* and *Cucurbita pepo* which agree with the results of this study.

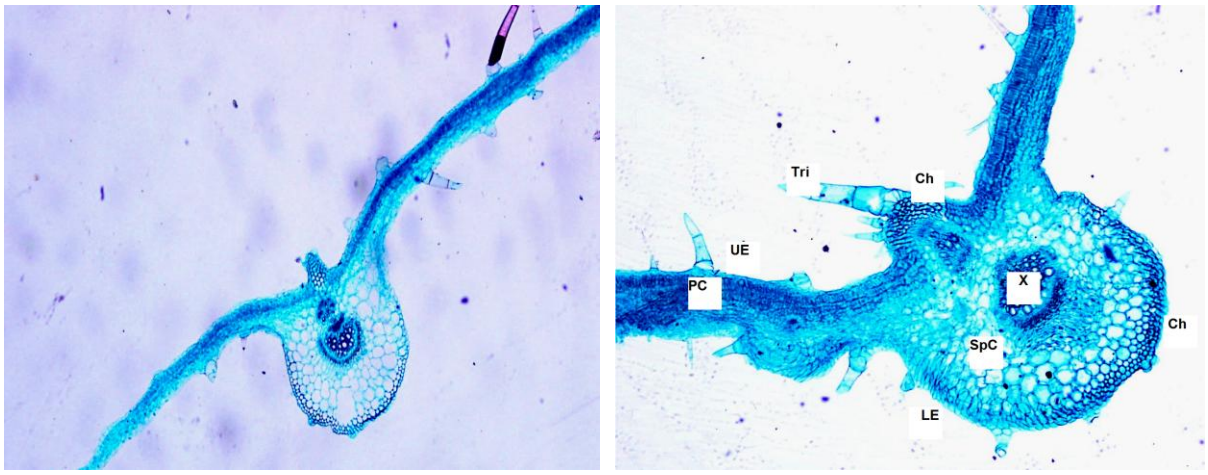

Fig4. ***(A).*** *T. S of Leaf of Cucumis sativus L. (X4). .In general view with primary structure; Upper epidermis (UE), Trichome (Tri), Palisade cells (PC), Spongy cells (SpC), Chollenchyma (Ch), Lower epidermis (LE) and Xylem (X).* ***(B).*** *T. S of Leaf of Luffa aegyptiaca Mill. (X4).Vascular tissue (bicollateral bundle).*

4. CONCLUSION

The data from these studies has provided further guidance for the taxonomic delimitation of species of Cucurbita, which are found in Sudan. It is hoped that field officers, botanists and farmers will find these information helpful in distinguishing the species. This study is therefore based on the principles that root, stem and leaf anatomy have played a major role in the identification, characterization and delimitation of plants. Hence the need to incorporate information from root, stem and leaf anatomy with data derived from other botanical disciplines remains vital when formulating conclusions on the systematics of the taxa investigated.

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