Comparative morphological and anatomical studies of *Boerhavia* (*Nyctaginaceae*) species in Nigeria

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Abstract. Comparative morphological and anatomical studies of stem, leaf, and petiole of the three common *Boerhavia* species found in Nigeria were carried out to determine the taxonomic significance of these characters. No clear intraspecific variation was observed among the studied species on the basis of their morphological attributes and trichome types. However, the epidermal and anatomical features were diagnostic. Differences in the stomata type made the species distinct. Also, the occurrence of raphide in their leaf lamina, the number of vascular bundles in midrib and stem, and the presence, shape, and clustering of macrosclereids in the leaf epidermis varied from one species to another.

Key words: Anatomy, *Boerhavia*, morphology, *Nyctaginaceae*, raphides, sclereids, trichomes

Introduction

Boerhavia L. belongs to *Nyctaginaceae* with other genera such as *Mirabilis* L., *Pisonia* L., and *Commicarpus* Standl. *Boerhavia* is found mainly in tropical America and in small numbers in the tropics and subtropics of the Old World (Hutchinson & Dalziel 1954). There are about 40 species worldwide but mostly occurring in the southwestern parts of North America (Douglas & Spellenberg 2010) and 16 species in Africa (Klopper & al. 2006; African Plant Database 2010). In West Africa, *Boerhavia* L. has 10 species as tropical or pantropical weeds (Hutchinson & Dalziel 1954). Four of these species commonly found in West Africa are *B. erecta* L., *B. coccinea* Mill., *B. diffusa* L., and *B. repens* L. (Edeoga & Ikem 2001). Taxonomy and identification of members of this genus are difficult and problematic due to their complex morphological attributes, which leads to their treament as complex groups, e.g. *B. diffusa-B. coccinea* complex (Thulin 1993; Spellenberg 2004; Chen & Wu 2007).

Among the Nigerian species of *Boerhavia,* extensive studies have been carried out into their morphology (Hutchinson & Dalziel 1954; Akobundu & al. 2017), photochemistry (Edoga & Ikem, 2001), pollens (Edoga & Ikem, 2002), ethnobotany (Gill 1992; Olukoya & al. 1993; Akaneme 2008), and anatomy of the photosynthetic pathway (Ajao & al. 2017). However, there have been no comprehensive studies into the comparative anatomy of the species. The aim of this work is to describe plant morphology, including anatomy of the stem, leaf, and petiole of these *Boerhavia* species commonly found in West Africa, and to report how significant taxonomically are these characters for the studied species.

Material and methods

The plant material used for this study was obtained from live specimens, namely *B. coccinea, B. diffusa* and *B. erecta*. They were collected from different parts in South Nigeria (Auchi in Edo State, Ado Ekiti in Ekiti State, Gboko in Benue State and Alakahia in Rivers State). The voucher specimens were deposited in the University of Port Harcourt Herbarium, Choba, Nigeria (Table 1).

Table 1: List of Voucher specimens studied.

Species name	Locality; Date of collection	Voucher number
B. coccinea Mill	Ado Ekiti, Ekiti State; 13/08/17	UPH/V/1164
	Wilberforce Island, Bayelsa State; 17/06/16	UPH/V/0874
	Alakahia in Rivers State; 28/09/17	UPH/V/1013
	Behind Faculty of Science building, University of Benin, Edo State; 25/09/17	UPH/V/1012
	Opposite DPR office, Imo State; 10/05/16	UPH/V/0912
B. diffusa L.	Auchi in Edo State; 25/04/15	UPH/V/0914
	Behind Civil Engineering Department, University of Port Harcourt;	UPH/V/1135
	Department of Plant Science and Biotechnology, Ekiti State University, Ekiti State; 15/08/17	UPH/V/1167
	Bonny, Rivers State; 09/11/17	UPH/V/1173
	Obiga-Asa; 20/10/15	UPH/V/1001
B. erecta L.	Gboko in Benue State; 13/03/17	UPH/V/1130
	Effuru, Delta State; 10/10/17	UPH/V/1174
	Izombe, Imo State; 13/08/18	UPH/V/1890
	Lekki, Lagos State; 19/12/13	UPH/V/0701
	Ida, Kogi State; 12/10/13	UPH/V/0673

Epidermal studies

Fresh leaf material was collected from plants growing in the wild. The adaxial and abaxial epidermal surfaces were peeled out, stained with Alcian blue and counterstained with 1 % Safranin O, rinsed in distilled water to remove the excess staining, mounted in a drop of pure glycerine on clean glass slides, and observed under the microscope (Okoli & Ndukwu 1992). The epidermal features and stomatal types were described according to Metcalfe & Chalk (1979) and Prabhakar (2004), respectively.

Anatomical studies

Sections of the petiole, midrib from mature leaves and young stems were cut and fixed in FAA (formaldehyde: glacial acetic acid: ethanol in the ratio 1:1:18 of 70 % ethanol v/v) for at least 48 hours. Then the samples were washed out in several changes of distilled water, dehydrated by alcohol series (30 %, 50 %, 70 %, and 100 %) solution for two hours each and embedded in wax. Sections between 15–20 μm were cut with a Leitz 1512 rotary microtome. The selected thin sections were de-waxed, stained with Alcian blue and counterstained with 1 % Safranin O, rinsed, mounted on slides, and photomicrographs of the anatomical sections were taken with Leitz Diaplan photomicroscope outfitted with a Leica WILD MPS 52 camera.

Results

The summary of the observed diagnostic features of the studied species is presented in Table 2.

Table 2. Summary of the diagnostic features observed in the studied *Boerhavia* species.

Character	Species name		
	B. ciccinea	***B. erecta***	***B. difussa***
Habit	Erect	Erect	Prostrate
Flower	Pinkish, small, 5–13 in a capitulum.	Pale pinkish-white, about 2–6 flowers in a capitulum	Deep purple or crimson, about 2–6 flowers in a capitulum
Stomata type	Stuarocytic	Isotricytic	Anisocytic
No. vascular bundles in stem	12	18	10
Layer of parenchymatous cell in stem	6	4–5	4–5
No. vascular bundle in the midrib	4 in open ring	2 forming parallel plate	3 in open arc
Palisade mesophyll	Loosely packed with intercellular spaces	Closely parked without intercellular space	Loosely packed with intercellular space
Raphides	Embedded in the spongy mesophyll or extending to the adaxial epidermis	Extends from palisade to spongy mesophyll, lying between the palisade and spongy mesophyll or vertically interspersed in the palisade mesophyll	Vertically interspersed in the palisade mesophyll, extending to the spongy mesophyll or lying between the palisade and spongy mesophyll
Papillae	Present	Present	Absent
Vascular bundle in the petiole	4 in open arc with two interspersed small ones and two accessory ones	3 in open arc with two accessory ones	6 in open arc with two accessory ones

Plant descriptions follow a certain format. Whenever the authors have consulted any taxonomy textbook, this format is explained and supported by examples (they can also consult any published plant description). However, since the manuscript deals with comparison, the descriptions of the three species have been compared and the differences between them are presented.

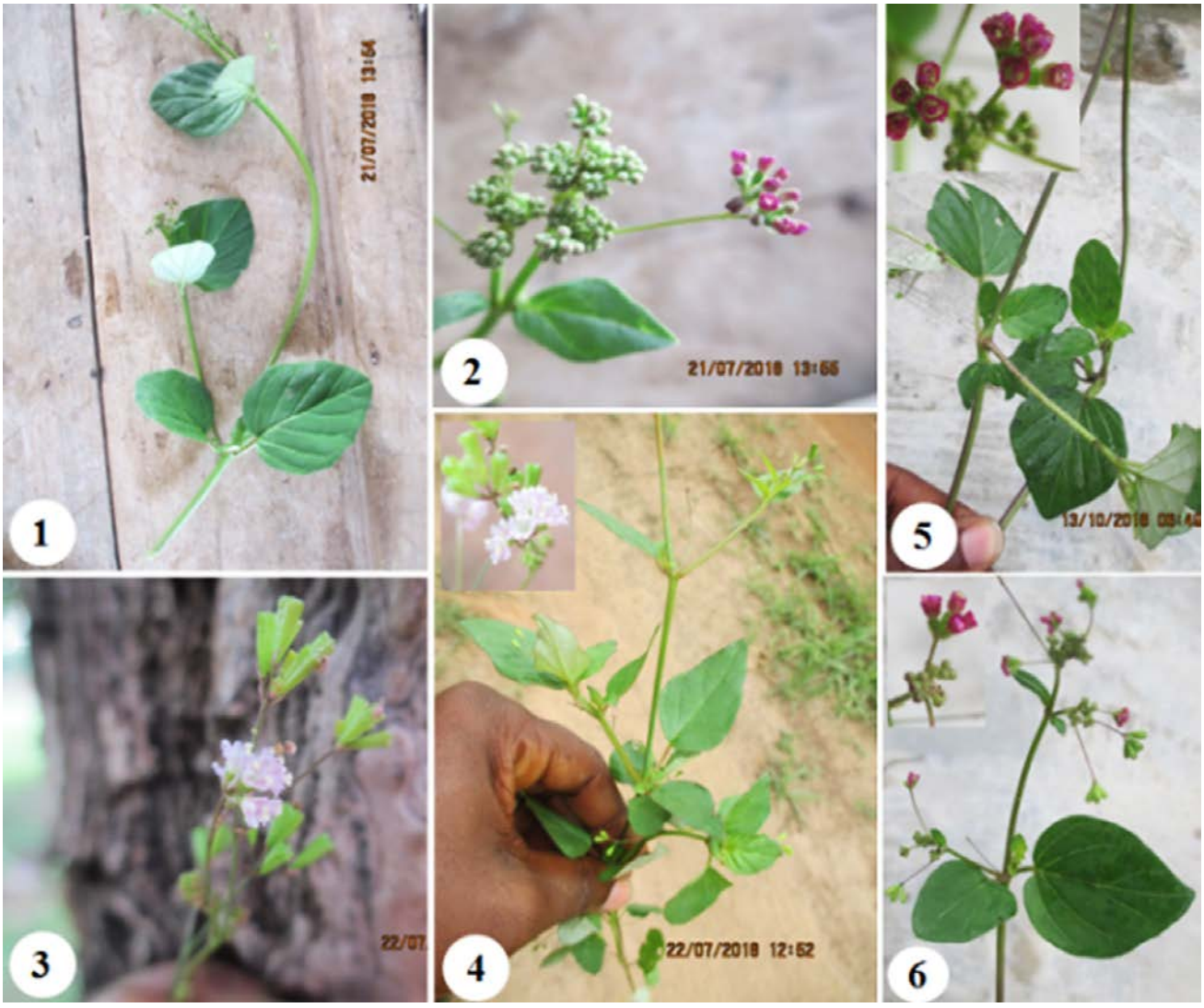
Plant morphology

B. coccinea

A partly erect perennial herb, about 45–60 cm high and reproducing by seeds. The stems are slender, jointed, woody towards the base, greenish or occasionally reddish, with branches at the base. The branches are hairy or glabrous, and sticky when with glandular hairs. The leaves are opposite, fleshy and sticky when squeezed. They are broadly ovate, about 2.5–4.5 cm long and 1.8–4.5 cm wide, margins entire, with petiole 1.2–3.1 cm long. The inflorescence is leafy, terminal/determinate, elongated with a slender-stalked cyme. The flowers are small, 5–13 in a capitulum, pinkish, and about 2 mm long. The fruits are achenes about 3.2 mm long, club-shaped, pentangular, and very sticky with glandular hairs (Figs 1 and 2).

B. erecta

A partly glabrous, erect perennial herb about 60–70 cm high, which reproduces by seeds. The stem is slender, jointed, and woody towards the base. It is greenish or occasionally purplish, branching, and sparingly or not sticky. The leaves are opposite, somewhat fleshy, broadly ovate, 2.6–4.3 cm long and 2.2–4.4 cm wide, with a



Figs 1–6. Vegetative and floral parts of the studied *Boerhavia* L. (1–2) *B. coccinea* (3–4) *B. erecta* and (5–6) *B. diffusa* L.

petiole 1.1–3 cm long, entire and smooth. The cyme is elongated, determinate, with numerous branches. The flowers are pale pinkish-white, with about 2–6 flowers in a capitulum. The fruit is a 1-seeded capsule, pentangular, about 3.2 mm long (Figs 3–4).

B. diffusa

A perennial herb, partly hairy, prostrate to partly prostrate, about 60–70 cm high, reproducing by seeds. The stem is slender, jointed, and woody at base. It is greenish or occasionally purplish, branching, and seldom sticky. The leaves are opposite, broadly ovate, 2.3–4.4 cm long and 1.8–4.2 cm wide, with a petiole 1.2–3 cm long, entire and smooth. The inflorescence is a long determinate cyme with many branches. The flowers are deep-purple or crimson, with about 2–6 flowers in a capitulum. The fruit is one-seeded sticky capsule, pentangular, about 3 mm long (Figs 5–6).

Epidermal description

Generally, all studied species have similar epidermal cells. They are amphistomatic, with polygonal adaxial epidermal cells and curved anticlinal walls, while the abaxial epidermal cells are irregular in shape, with undulating anticlinal walls (Table 1, Figs. 7–12). The stomata types identified among the studied species are: tetracytic (the stoma completely surrounded by only four subsidiary cells, variable in size and shape, of which two are polar and two are lateral in position), stuarocytic (the stoma completely surrounded by only four subsidiary cells, variable in size and shape, of which two are co-joined polar and two are lateral to the guard cells), anomocytic (the stoma completely surrounded by four or more subsidiary cells, variable in size and shape from the tetracytic and stuarocytic types), isotricytic (the stoma completely surrounded by only three subsidiary cells, variable in position and shape; however, three of the subsidiary cells are more or less equal in size), and anisocytic (the stoma completely surrounded by only three subsidiary cells, variable in position and size, but one of the subsidiary cells is distinctly small).

B. coccinea

This species is with stuarocytic, tetracytic and anomocytic stomata on the adaxial epidermis (Fig. 7), and anomocytic, tricytic and tetracytic stomata on the abaxial epidermis (Fig. 8).

B. erecta

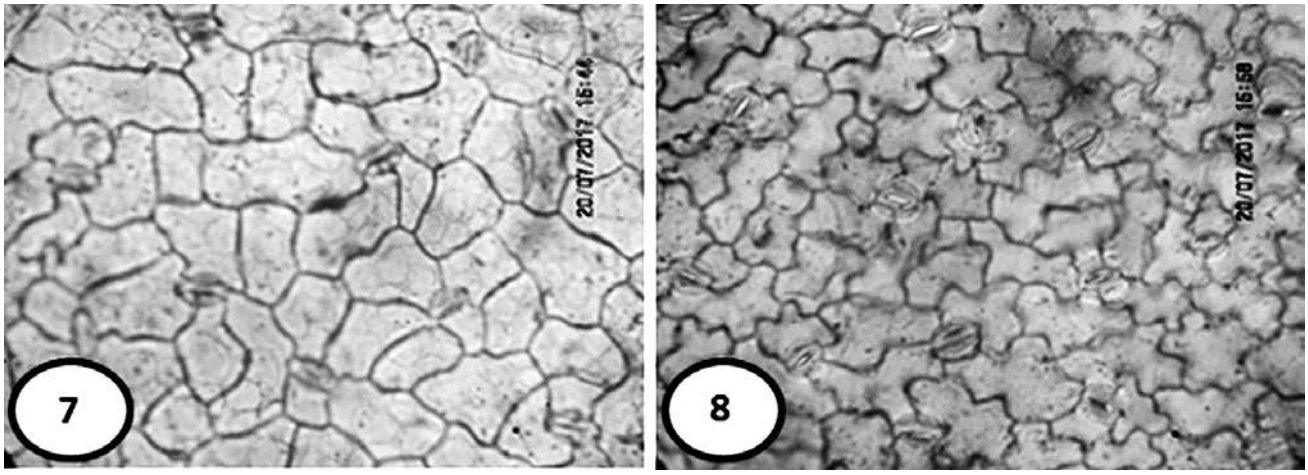
It has tetracytic, isotricytic, tricytic, and anomocytic stomata on the adaxial epidermis (Fig. 9), and anomocytic and tetracytic stomata on the abaxial epidermis (Fig. 10).

B. diffusa

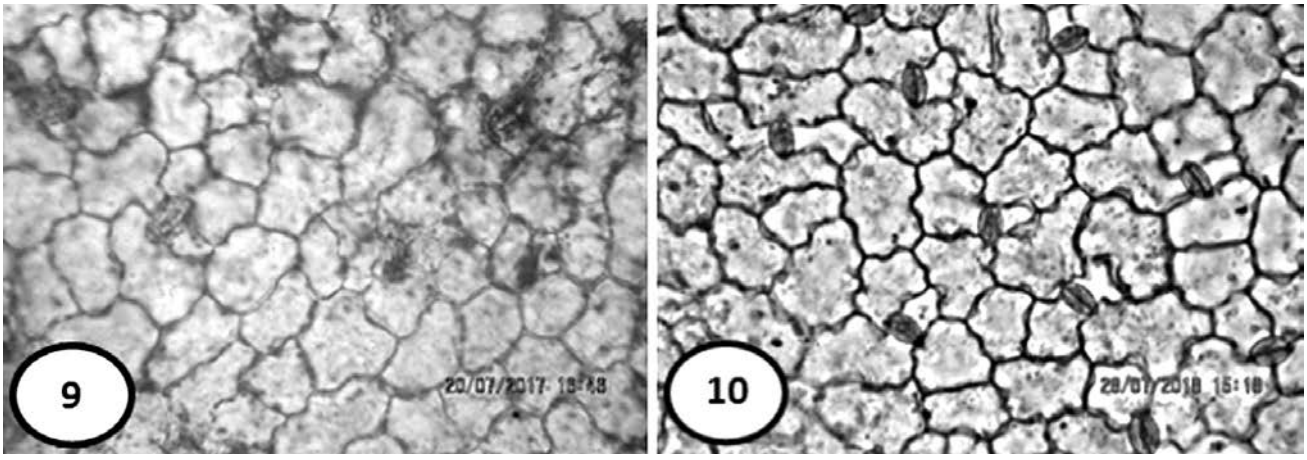
This species has tricytic and tetracytic stomata on the adaxial epidermis (Fig. 11), and anomocytic, tricytic, anisocytic, and tetracytic stomata on the abaxial epidermis (Fig. 12).

Trichome types

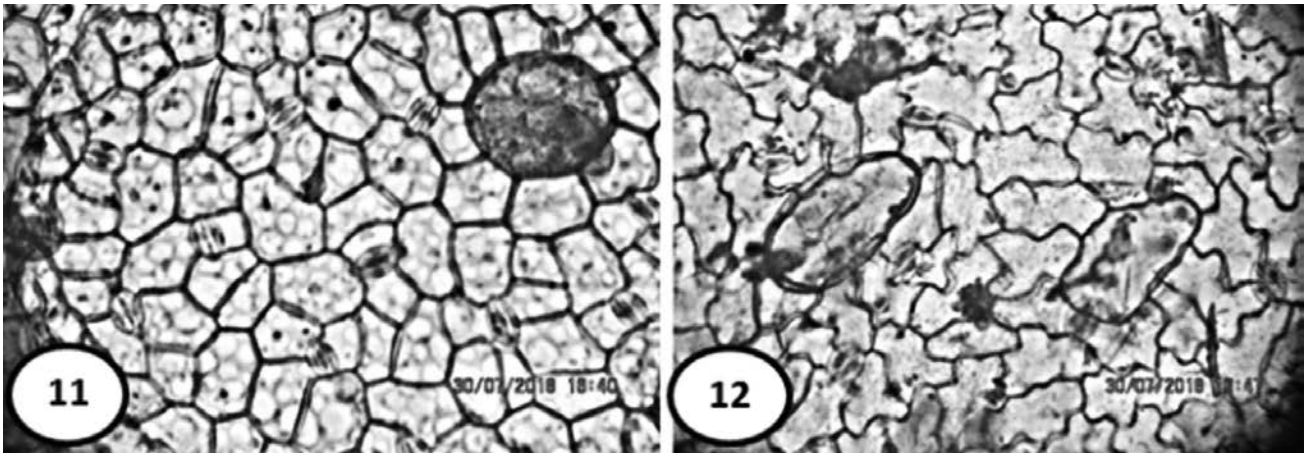
Trichomes in the *Boerhavia* species vary from glandular to non-glandular, unicellular to multicellular, and conical to elongated (Fig. 13). These trichomes are present on the leaves, petioles, midribs, and stems of all studied species. They vary in size,



Figs 7–8. Epidermal characteristics of *Boerhavia coccinea* (7 – adaxial epidermis and 8 – abaxial epidermis).



Figs 9–10. Epidermal characteristics of *Boerhavia erecta* (9 – adaxial epidermis and 10 – abaxial epidermis).



Figs 11–12. Epidermal characteristics of *Boerhavia diffusa* (11 – adaxial epidermis and 12 – abaxial epidermis).

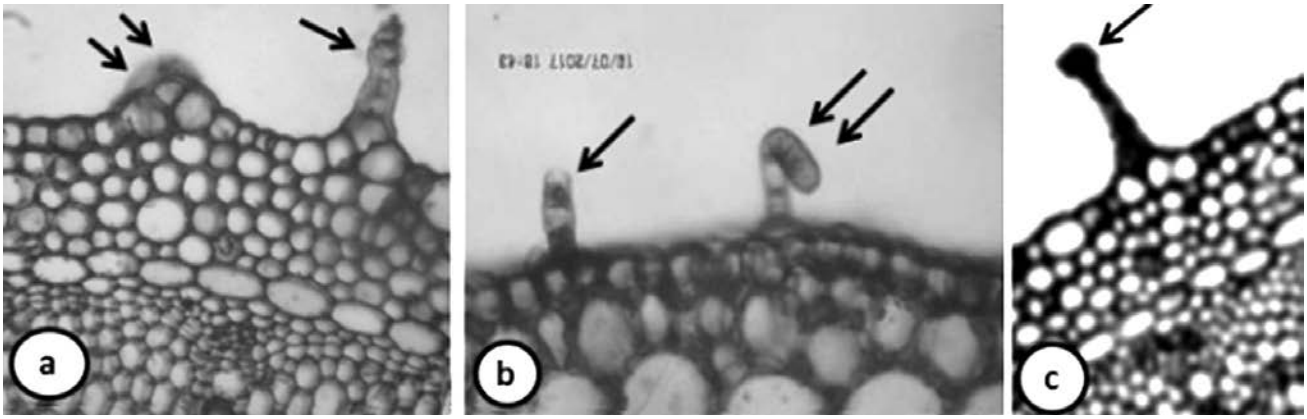


Fig. 13. Trichomes in the studied *Boerhavia* species: (**a**) double arrow – papilose, single arrow – non-glandular trichome, (**b**) glandular trichomes (single arrow – with clavate head; and double arrow – with club-head) and (**c**) glandular trichomes with globose head.

length, forms, abundance, and distribution. The different forms include non-glandular unicellular or multicellular trichomes (Fig. 13a), and glandular trichomes with clavate (Fig. 13b) or globose head (Fig. 13c).

Anatomical description

B. coccinea

The midrib has four vascular bundles in an open ring and a V-shaped adaxial cuticle outline (Fig. 14).

Kranz anatomy was observed in the palisade mesophyll around the minor veins (Figs. 15–18). The palisade mesophyll has one layer of cells and a spongy mesophyll of more than three layers of cells with intercellular spaces. Raphide bundles are embedded in the spongy mesophyll (Fig. 15); the palisade mesophyll extending to the adaxial epidermal cells (Fig. 18) and lamina have papillae on the adaxial epidermis (Fig. 17). There are single macrosclereides on the adaxial epidermis (Figs 42–48) and raphides on the abaxial epidermis (Fig. 49). The petiole has four vascular bundles in an open arc, with two interspersed ones and accessory vascular bundles (Fig. 29). The stem consists of 12 vascular bundles and a continuous ring of sclerenchyma (Fig. 31), with six layers of parenchymatous cells (Fig. 33).

B. erecta

Midrib has two parallel plates of the vascular bundle (Fig. 19); mesophyll is the same as in *B. coccinea* but the palisade mesophyll is closely parked, or without intercellular spaces. Raphide bundles extend from palisade to spongy mesophyll (Fig. 20) between the palisade and spongy mesophyll (Fig. 21), and are perpendicular in the palisade mesophyll (Fig. 22). The petiole is similar to *B. coccinea,* with three vascular bundles (Fig. 30). The stem shows similar anatomical features with that of *B. coccinea* but with 18 vascular bundles, including medullary ones (Fig. 35), and 4–5 layers of parenchymatous cortex (Fig. 36).

B. diffusa

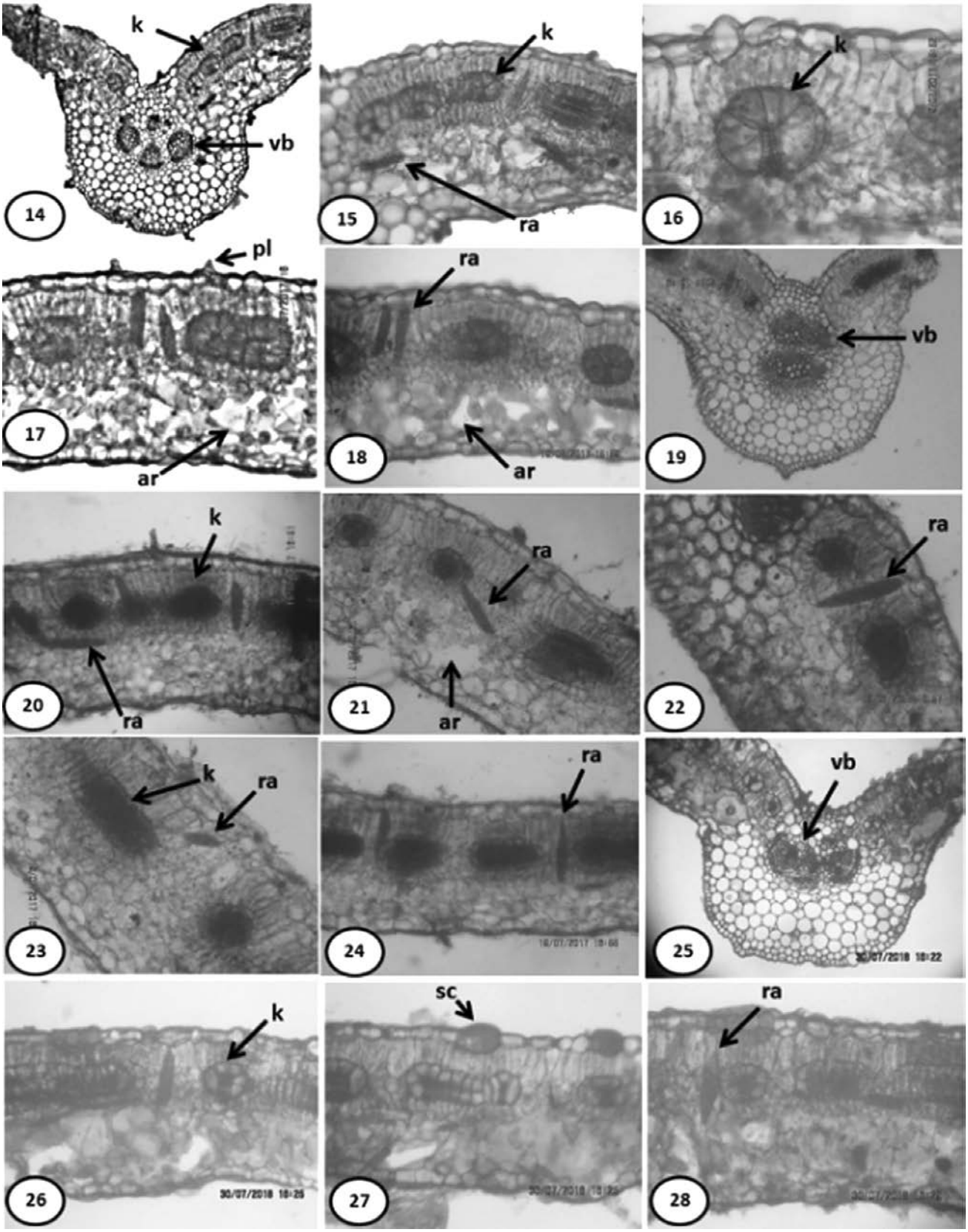
The structure of midrib and stem corresponds with that of *B. coccinea,* but with three vascular bundles in an arc (Fig. 25). Raphides are present vertically interspersed in the palisade mesophyll (Fig. 26) and extending to the spongy mesophyll, or between the palisade and spongy mesophyll (Fig. 28). Macrosclereids are enshrined between two adaxial epidermal cells (Fig. 26). The stem has 10 vascular bundles, including the medullary ones (Fig. 36), and 4–5 parenchymatous cell layers (Fig. 37).

Discussion

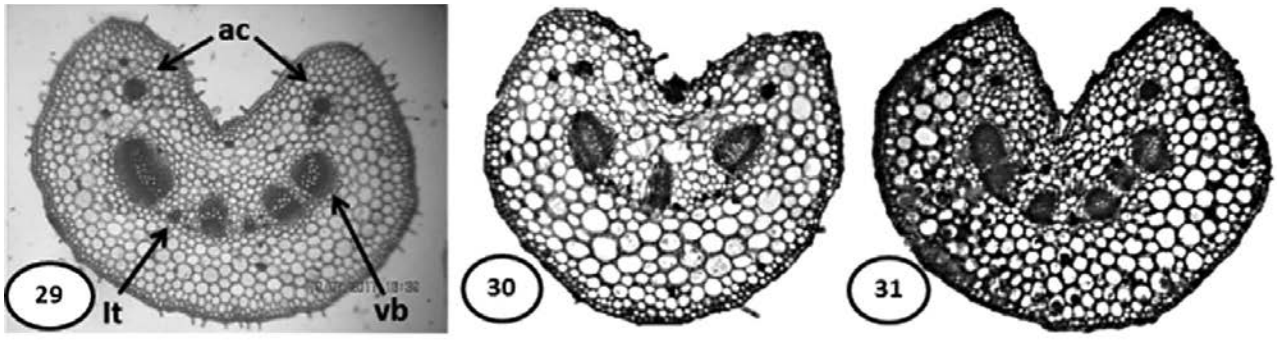
Morphology of *Nyctaginaceae* was described by Hutchinson & Dalziel (1954), anatomy – by Metcalfe & Chalk (1979; 1983) and Bittrich & Kühn (1993), and there are numerous works focused specifically on the anatomical features of *B. diffusa* (Edeoga & Ikem 2002; Carlquist 2004). The morphological characteristics of the species are similar with those in the works of Hutchinson & Dalziel (1954), Jordaan (2000) and Akobundu & al. (2017).

Among members of the *Boerhavia* species, stomata were present on both the adaxial and abaxial epidermises (amphistomatic), or only on the abaxial epidermis (hypostomatic) of the leaf (Metcalfe & Chalk 1950; Bittrich & Kühn 1993). Also, the presence of papillae has been reported as an important taxonomic feature and varies from one species to another (Metcalfe & Chalk 1979). All this is in concordance with the observations in our study. Furthermore, we have noted a variation in the stomata types on the leaf surfaces even in one and the same species and in the presence of papillae in *B. coccinea*. These characters were fairly diagnostic. The presence of staurocytic stomata in *B. coccinea,* isotricytic stomata in *B. erecta* and anisocytic stomata in *B. diffusa* distinguished them from each other. This finding supports the conclusions of Abdulrahaman & Oladele (2005), who reported that stomata types among members of the *Ocimum* L. could be used to differentiate them.

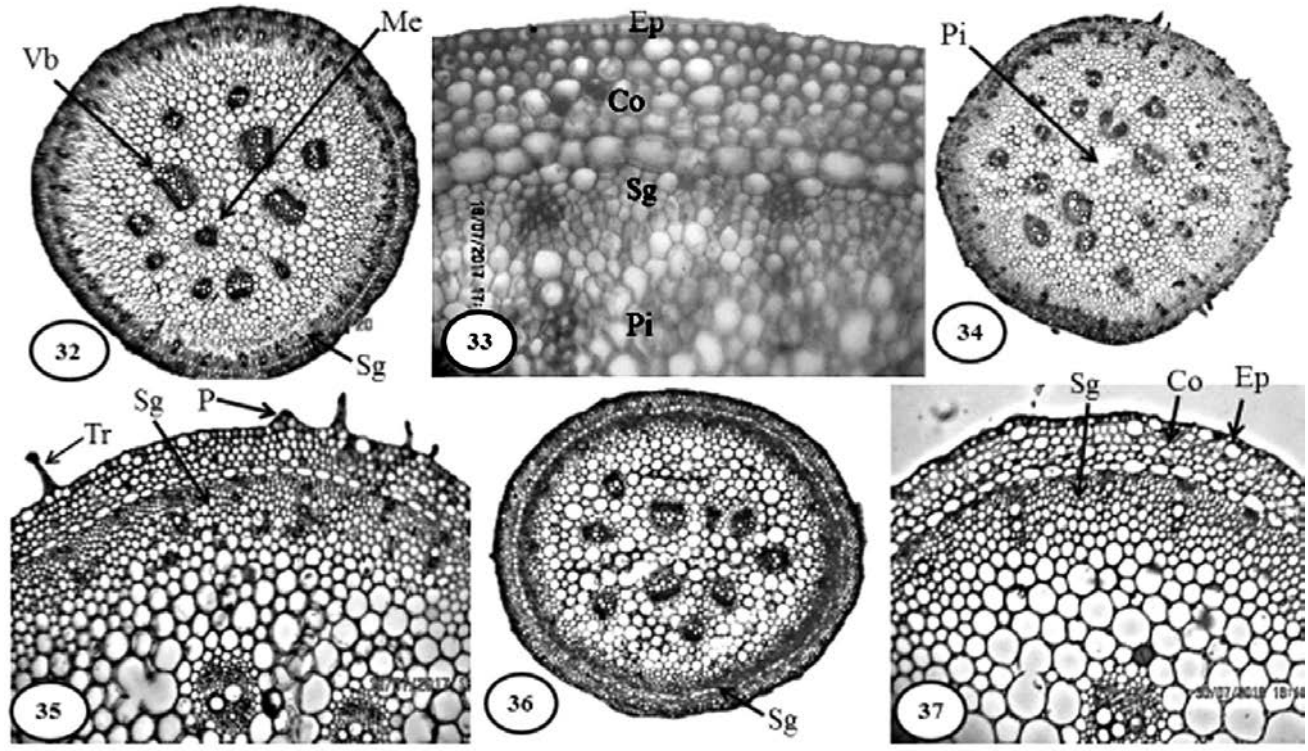
Several authors have reported that mesophyll in the leaves of *Boerhavia* species consists of palisade and spongy parenchyma and could be centric, dorsiventral or isobilateral with Kranz anatomy (Metcalfe & Chalk 1950; Carolin & al. 1978; Bittrich & Kühn 1993; Muhaidat & al., 2007). Furthermore, palisade cells increase the internal leaf surface and, therefore, the photosynthetic rate (Rhizopoulou & Psaras 2003), while the spongy mesophyll enhances the circulation of carbon dioxide across the leaf in order to maintain a high rate of photosynthesis (Mauseth 1988; Struwig & al. 2011; Ajao et al. 2017). It has been also suggested that large intercellular air spaces, such as found for *Boerhavia* L., may reduce leaf temperature by scattering the amount of infrared radiation that reaches the mesophyll (McClendon 1984). In our study, we have recorded similar observation in addition to the variation in the position of calcium oxalates (raphide bundles) in the leaf lamina. The latter feature is diagnostic among the studied species and is in line with the report of Ekeke & Agbagwa (2014) on the genus *Combretum* Loelf. stating that distribution of calcium oxalates in the leaf lamina of this genus varies from one species to another. Furthermore, the raphide bundles



Figs 14–28. Anatomy of leaf midrib and lamina (14–18) *B. coccinea*, (19–24) *B. erecta* and (25–28) *B. diffusa;* **vb** – vascular bundle, **sc** – macrosclerid, **ra** – raphide bundle, **ar** – intercellular air space, **k** – Kranz anatomy, **pl** – papillae.



Figs 29–31. Petiole anatomy: (29) *B. coccinea*, (30) *B. erecta*, (31) *B. diffusa*; it – interspersed vascular bundle, **vb** – vascular bundle, **ac** – accessary vascular bundle.

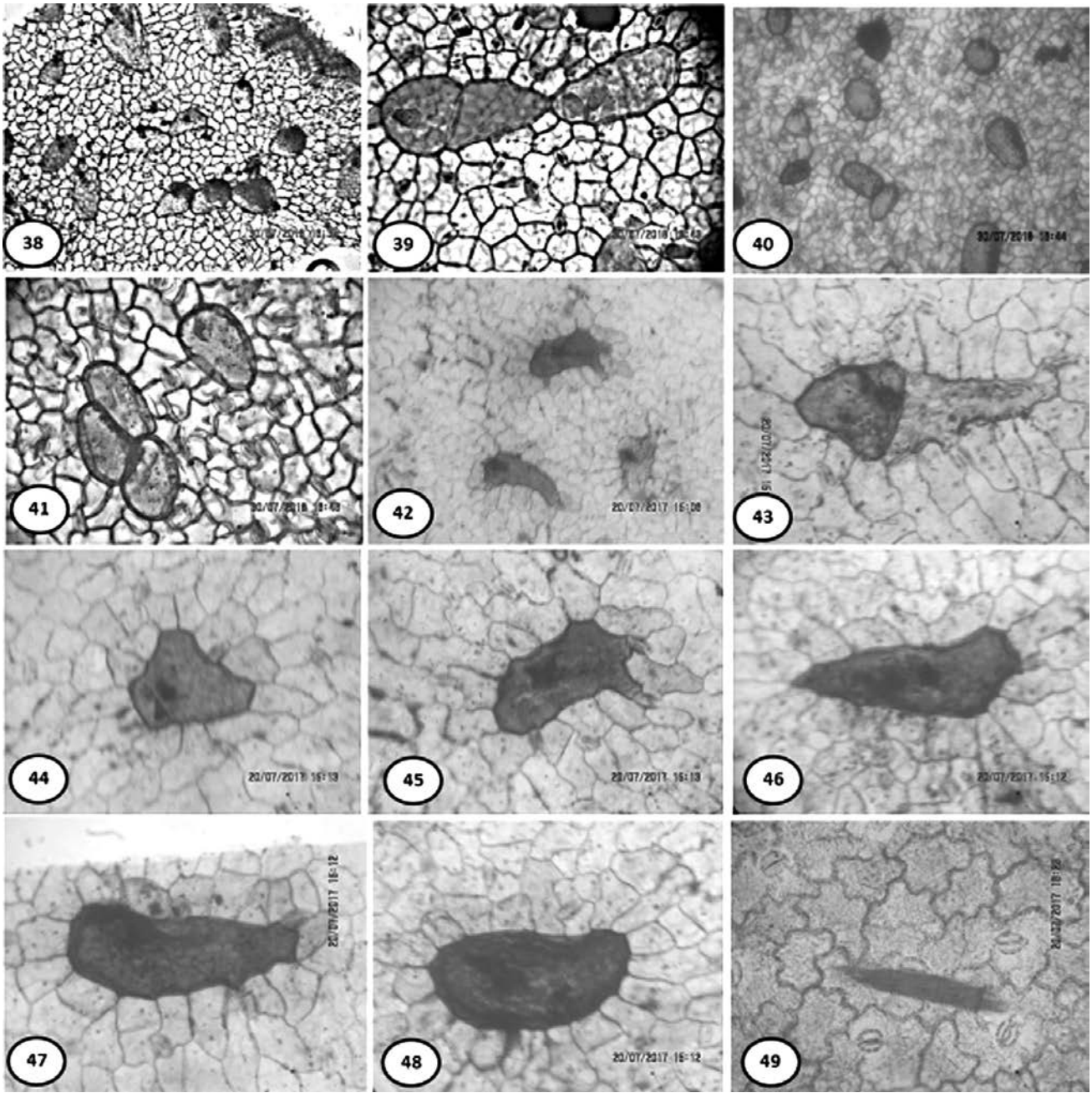


Figs 32–37. Stem anatomy of the studied *Boerhavia* species: (32–33) *B. coccinea*, (34–35) *B. erecta*, (36–37) *B. diffusa,* **vb** – vascular bundle, **me** – medullary bundle, **pi** – pith, **sg** – secondary growth, **tr** – trichome, **p** – papillae, **co** – cortex.

observed in the species are the same as those reported by Metcalfe & Chalk 1950; Bittrich & Kühn 1993; Edeoga & Ikem 2002, and are present in all studied species of *Boerhavia*.

The number and arrangement of vascular bundles in midrib have shown a remarkable difference and are diagnostic. This is in agreement with the reports of other authors, who have stated that the shape of midrib, the number and arrangement of vascular bundles in it vary among the species, genus or family and is a valuable character in plant identification in *Asteraceae* (Ekeke & Mensah 2015), *Parashorea* (Dipterocarpaceae) (Noraini & Cutler, 2009) and *Anthurium* section *Urospadix* subsection *Flavescentiviridia* (Araceae) (Mantovani & al. 2009).

We have noted an anomalous secondary growth in all studied *Boerhavia* species. This feature is not diagnostic among the species but the number of vascular bundles, layers of parenchymatous cortex and occurrence of papillae in the stem of the species have varied and could be used to identify the species. Members of *Nyctaginaceae* have a stem characterized by anomalous secondary growth. It entails formation of successive rings of cambia and the phenomenon has been studied by several authors (Metcalfe & Chalk 1950; Carlquist 2004).



Figs 38–49. Different forms of macrosclereides and raphide bundles in the leaf epidermis of studied *Boerhavia* species: (38–39) upper epidermis of *B. erecta*), (40–41) lower epidermis of *B. erecta*), (42–48 upper epidermis of *B. coccinea*), and (49 – lower epidermis of *B. coccinea;* arrow shows raphide bundle).

No clear intraspecific variation has been observed among the studied species on the basis of their morphological attributes and trichome types. However, the epidermal and anatomical features have shown some diagnostic characters. In our study, we have noted variation in the stomata types and presence of papillae in the leaf epidermis of *B. coccinea*. The presence of staurocytic stomata in *B. coccinea,* isotricytic stomata in *B. erecta* and anisocytic stomata in *B. diffusa* distingushed them from each other. Also, the occurrence of raphides in the leaf lamina, the number of vascular bundles in midrib and stem, and the presence, shape and clustering of macrosclereids in the leaf epidermis have varied from one species to another. These characters are diagnostic and could be used to distinguish the studied *Boerhavia* species. Most of these characters and their taxonomic importance have been

reported by different researchers (Solereder 1908; Hutchinson & Dalziel 1954; Metcalfe & Chalk, 1979; Fadeyi & al. 1989; Edeoga & Ikem, 2002; Muhaidat & al. 2007; Akobundu & al. 2017).

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