CHAPTER 5: ANATOMY

5.1 Introduction

5.1.1 Anatomy of the Nyctaginaceae

The anatomy of the Nyctaginaceae is summarized by Metcalfe & Chalk (1950; 1979; 1983) and Bittrich & Kühn (1993) and numerous studies have focused on specific anatomical features in a few species (Joshi & Rao, 1934; Kajale, 1938; Nair & Nair, 1961, 1963; Pant & Mehra, 1962; Sharma, 1963a,b; Wilson, 1974; Mikesell & Popham, 1976; Willson & Spellenberg, 1977; Stattler & Perlin, 1982; Louro *et al*., 1992; Vanvinckenroye *et al*., 1993; Rajput & Rao, 1998; Edeoga & Ikem, 2002; Carlquist, 2004).

The stem anatomy of the family is characterized by anomalous secondary growth, which is the result of the formation of successive rings of cambia, and numerous studies have focused on this phenomenon (Metcalfe & Chalk, 1950; Balfour, 1965; Rajput & Rao, 1998; Carlquist, 2004).

Stomata are present in both the adaxial and abaxial epidermis (amphistomatic) or only in the abaxial epidermis (hypostomatic) of the leaf (Metcalfe & Chalk, 1950; Bittrich & Kühn, 1993). The mesophyll is centric, dorsiventral or isobilateral and Kranz anatomy has been recorded for *Allionia* L., *Boerhavia* L., *Bougainvillea* Comm. ex Juss., *Phaeoptilum* Radlk. and *Okenia* Schltdl. & Cham. (Metcalfe & Chalk, 1950; Carolin *et al*., 1978; Bittrich & Kühn, 1993; Muhaidat *et al*., 2007).

The structure of the anthocarp differs amongst genera. The wall of the anthocarp can either have ribs (a protruding enlargement of the anthocarp wall) or wings (an enlarged proximal part of the rib), which are either smooth or bear warts (Willson & Spellenberg, 1977; Spellenberg, 2004; Douglas & Manos, 2007). The anthocarp wall is constructed of epidermis, sclerenchyma, parenchyma, vascular strands and columnar parenchyma cells (Willson & Spellenberg, 1977).

Calcium oxalate crystals are present throughout the plant body in the form of raphides or styloids, or a mixture of both, except for species from the tribe Leucasteraea, where crystals are absent (Metcalfe & Chalk, 1950; Bittrich & Kühn, 1993; Edeoga & Ikem, 2002). Tannin idioblasts occur in the epidermis of roots, stems and leaves of some genera, especially in *Boerhavia* and *Okenia* (Metcalfe & Chalk, 1950; Bittrich & Kühn, 1993; Edeoga & Ikem, 2002).

5.1.2 Anatomy of *Boerhavia* and *Commicarpus*

Boerhavia and *Commicarpus* can be distinguished from each other morphologically based on their growth habit, shape of the flower and anthocarp structure (Meikle, 1978). *Boerhavia* has a diffuse habit, the flowers have a bell-shaped perianth and the anthocarp has either 3–5 wings or five ribs, and the surface is either smooth or covered in multicellular trichomes (Stannard, 1988). *Commicarpus* has a scrambling or climbing habit, the flowers have a funnel-shaped perianth and the anthocarp has ten ribs covered in large, viscid and mucilaginous glands (Stannard, 1988).

The anatomy of the genus *Boerhavia* has been studied extensively, with *B. diffusa* L. var. *diffusa* mostly used for these studies (Joshi & Rao, 1934; Metcalfe & Chalk, 1950; Nair & Nair, 1961; Inandar, 1968; Carolin *et al.*, 1978; Sattler & Perlin, 1982; Rajput & Rao, 1998; Edeoga & Ikem, 2002). The anatomy of *Commicarpus*, however, was inadvertently studied during studies of *B. repanda* Willd. or *B. chinensis* (L.) Aschers. & Schweinf. Both names are currently regarded as synonyms for *C. chinensis* (L.) Heimerl. subsp. *chinensis* (Joshi & Rao, 1934; Kajale, 1938; Sharma, 1963a, b; Inandar, 1968; Das & Santakumari, 1978; Rajput & Rao, 1998).

Southern African *Boerhavia* and *Commicarpus* species have not been studied anatomically except for *B. diffusa* var. *diffusa,* which is a South American species introduced to southern Africa (Codd, 1966; Stannard, 1988; Bromilow, 2010). It is therefore not known whether there are any special anatomical characters found in southern African taxa that can be used to distinguish between these two genera and their species locally or internationally.

5.1.3 Anatomical adaptations

In southern Africa, 15 of the 16 *Boerhavia* and *Commicarpus* species occur in Namibia (Germishuizen & Meyer, 2003). Namibia is subject to a dry atmosphere and a mean annual rainfall of > 600 mm, intense solar radiation, high daytime temperatures (> 30 °C) and evapotranspiration (Mendelsohn *et al*., 2002). These arid conditions have been maintained for millions of years, resulting in shallow soils, low levels of soil nutrients and scarcity of water in most areas (Mendelsohn *et al*., 2002).

Plants that are adapted for survival in a dry habitat are known as xerophytes (Mauseth, 1988). Levitt (1956) classifies xerophytes as plants which are drought evaders, avoiders and tolerant of drought. Evaders are either ephemerals that survive the dry conditions as seeds, or perennials that survive the unfavourable conditions through dormancy as bulbs or tubers. Avoiders have morphological, anatomical or physiological adaptations which reduce water loss, increase water uptake, store excessive quantities of water or enhance water translocation. Tolerant plants can undergo dehydration without dying (Levitt, 1956). The southern African *Boerhavia* and *Commicarpus* species are postulated to be avoiders of desiccation.

5.2 Aim

The aim is to describe the stem, leaf and anthocarp anatomy of southern African *Boerhavia* and *Commicarpus* species for the first time; to report on the taxonomic significance of these characters; and to investigate the anatomical adaptations of the southern African *Boerhavia* and *Commicarpus* species to survive in arid environments.

5.3 Materials and Methods

5.3.1 Collecting of plant material

Stem, leaf and anthocarp material was collected *in situ* during 2009 and 2010 in Namibia and South Africa. Voucher specimens of the collected material were deposited in the A.P. Goossens Herbarium (PUC), Potchefstroom, South Africa, and duplicates of specimens collected in South Africa in the National Herbarium, Pretoria (PRE), South Africa and duplicates collected in Namibia in the National Herbarium (WIND), Windhoek, Namibia (Table 5.1).

5.3.2 Scanning electron microscopy

Freshly collected stem, leaf and anthocarp material was stored in 70% ethanol and dehydrated successively in 90% and twice in 100% ethanol for 10 min per treatment before critical point drying. Material was then mounted on specimen stubs and sputter-coated with gold/palladium. Materials collected from herbarium specimens were directly mounted on specimen stubs and sputter-coated with gold/palladium. In all cases specimens were examined and micrographs were taken with a FEI Quanta 200 environmental scanning electron microscope (ESEM).

5.3.3 X-ray micro-analysis

The composition of the crystals observed in the leaves and anthocarps was determined by element analysis on a FEI Quanta 200 ESEM with an integrated Oxford Inca X-sight EDS system operating at 15 kV. A representative specimen of a single *Boerhavia* and *Commicarpus* species was examined.

5.3.4 Light microscopy

Stem, leaf and anthocarp material was fixed in 4% aqueous paraformaldehyde (pH 7.2). Fixed material was then rinsed three times in 0.05 M cacodylate buffer for 15 min per treatement and followed by three rinses of 15 min each with distilled water. The material was dehydrated in an ethanol series of 50%, 70%, 90% and twice in 100% ethanol for 15 min each followed by 15 min in 100% resin (L.R. White™ Wirsam/London Resin Company). This was followed by two changes in resin for one hour each and then left overnight at 20 °C before being embedded and then polymerised overnight at 65 °C. Embedded material was cut with a Reichert-Jung Ultracut microtome and stained with aqueous 0.1% neufuchsin for 15 sec, aqueous 0.5% toluidine blue in 1% borax and aqueous 0.16% cresyl fast violet in water with 20% ethanol for 2 min each. Sections were also stained for pectinaceous substances in

0.02% ruthenium red in water for 10 min, and for lipids in Sudan black B in 70% ethanol for 30 min. Micrographs were taken at 40x, 60x and 100x magnification with a Nikon Digital Camera DXM 1200 F, fitted on a Nikon Eclipse E 800 and a Nikon Digital Sight camera fitted on a Nikon Eclipse 80i light microscope.

Sections of anthocarps, leaves and stems were made from mature plant material. Sections of stems were made from the centre of an internode towards the nodes. Anthocarps and leaves were sectioned from the centre towards the apexes.

5.4 Results

No interspecific variation in the arrangement of the organ tissues was evident within the genera. However, intergeneric variations were more pronounced and therefore only the anatomy of the two genera will be described and contrasted, and not the different species individually. Descriptions therefore apply to both genera except when stated otherwise.

5.4.1 Trichomes

Trichomes are present on the stems, leaves, flowers and anthocarps and vary in their size, length, distribution and abundance. The trichomes are uniseriate and multicellular. The trichomes terminate in a head which is either globose (Fig. 5.1A) or clavate (Fig. 5.1B). The walls of the trichomes are impregnated with numerous crystals (Fig. 5.1C). The trichome heads stain dark purple/blue with toluidine blue, which is an indication of dense cytoplasm and numerous small vacuoles (Fig. 5.1D). The trichomes secrete a substance which makes the organs extremely sticky.

5.4.2 Stem

5.4.2.1 *Boerhavia*

The cell walls of the epidermis are thickened, especially the outer periclinal wall, which is impregnated with crystals of varying thickness (Fig. 5.2A). The cuticle is thin. The epidermal cells are rounded and not of the same size, with some cells larger than others. The stomata are not sunken and the outer and inner periclinal walls of the guard cells are thickened but not cutinized. Small inner and large outer ledges are present. The collenchymatous hypodermis consists of one to three cell layers. The hypodermis does not form a continuous cylinder around the axis of the stem but is interrupted at the substomatal chambers. The chlorenchyma cells are spherical to brick-shaped and arranged in two to four rows with large and small intercellular spaces. The cells vary in size. The innermost cell layer of the cortex (starch sheath or endodermoid layer) consists of large cells with thickened walls (Fig. 5.2A). The primary xylem consists of vessel elements with a large diameter. All the stem sections show anomalous secondary growth. The secondary xylem and secondary phloem form clusters with thick walled fibres and conjunctive parenchyma in between (Fig. 5.2B). Consecutive cambial layers differentiate from phloem parenchyma cells which were formed by the preceding cambium. No rays are present. Medullary bundles form in the pith (Fig. 5.3). Bundles of raphide crystals are present throughout the stem in no specific pattern.

5.4.2.2 *Commicarpus*

The structure of the stem corresponds with that which was described for *Boerhavia*, except that the chlorenchyma cells are arranged in three to six rows (Fig. 5.4).

5.4.3 Leaves

5.4.3.1 *Boerhavia*

The epidermal cells are large, irregular in shape and the walls thickened, especially the dome-shaped outer periclinal wall, which is not cutinized (Fig. 5.5). The outer periclinal wall is impregnated with crystals which are more numerous on the abaxial surface than the adaxial surface. The cuticle is thin. Tannin idioblasts are sometimes present in the epidermis of either the adaxial or abaxial epidermal surfaces (Fig. 5.5C) or both. The tannins fill the entire cell and have a coarsely granular appearance (Fig. 5.5C). The stomata are present on both leaf surfaces (amphistomatic) and the structure is the same as described for the stem. The mesophyll consists of palisade and spongy mesophyll cells which are irregular in shape. The minor veins are surrounded by atriplicoid Kranz anatomy (that is, the veins are surrounded by a layer of Kranz cells which in turn are surrounded by palisade cells). The cells between the veins are sometimes also Kranz cells and not palisade cells (Fig. 5.5E, F). The main veins are not surrounded by Kranz anatomy but with palisade cells and the vessels have a large diameter. Throughout the mesophyll, bundles of raphide crystals are present in no specific pattern (Fig. 5.6A) and small druse crystals are present inside the palisade cells (Fig. 5.6B).

5.4.3.2 *Commicarpus*

The structure of the leaf is the same as described for *Boerhavia*, except that the minor veins are not surrounded by Kranz anatomy but with large parenchyma cells, and tannin idioblasts are absent from the epidermal cells (Fig. 5.7).

5.4.4 Anthocarp

5.4.4.1 *Boerhavia*

The anthocarps of the different species have five ribs (Fig. 5.8), except for *B. cordobensis,* which has three wings (Fig. 5.8B) and *B. erecta* which has five wings (Fig. 5.8E). The outer epidermal cells are irregularly brick-shaped to round. The outer periclinal wall of the epidermal cells is thickened and impregnated with a thick layer of crystals. The cuticle is thin. The epidermis overlays three to five rows of parenchyma cells which are followed by three to eight rows of sclerenchyma. Sclerenchyma occurs within the ribs and the area between the ribs. Below the epidermis of the ribs, columnar cells sometimes occur which becomes mucilaginous. Vascular bundles (5 or 6) occur near the sclerenchyma in the ribs (see Fig. 5.10C). The inner epidermal cells are brick shaped and the outer periclinal wall is thickened, although not as much as in the outer epidermis. Bundles of raphide crystals are present throughout the anthocarp in no specific pattern.

5.4.4.2 *Commicarpus*

The anthocarp has ten ribs (Fig. 5.9). The epidermis is followed by two to five rows of parenchyma cells. Columnar cells, which become mucilaginous, can sometimes occur. The sclerenchyma occurs in a bundle within the ribs and, unlike *Boerhavia*, is not present in the area between the ribs. The sclerenchyma bundle is either round or elongated to the sides in outline (Fig. 5.10A, B). Five vascular bundles occur in each of the ribs in contact with the sclerenchyma bundle, although it may appear as if bundles have fused (Fig. 5.10C). Towards the inside of the rib the sclerenchyma bundle is followed by three rows of parenchyma cells and the inner epidermis. The inner epidermal cells are brick-shaped and the outer periclinal walls are thickened, although not as much as those of the outer epidermis. The area between the ribs consists of three to six rows of parenchyma cells. Bundles of raphide crystals are present throughout the anthocarp in no specific pattern.

5.4.5 Crystals

The crystals occurring in the leaves and anthocarps of both the *Boerhavia* and *Commicarpus* species mainly consist of calcium, carbon and oxygen (Table 5.2). Small amounts of magnesium, aluminium, chlorine and potassium were present on the leaf surfaces of *Boerhavia* and *Commicarpus* species, with the latter having sulphur present rather than aluminium. Only one leaf and anthocarp of each genus was studied. This is important for the interpretation of the results.

5.5 Discussion

5.5.1 Taxonomic significance

Careful observations of the anatomy of the different species suggest that within a genus there is uniformity. Anantomical characters can therefore not be used to distinguish among the different species.

The trichomes of the Nyctaginaceae have been described as glandular and uniseriate with ellipsoidal, clavate or spherical terminal cells, or stellate, as in the tribe

Leucastereae, or branched, as in *Pisonia* (Metcalfe & Chalk, 1965; Solereder, 1908). Fadeyi *et al*. (1989) decribed the trichome morphology of four *Boerhavia* species which occur in Nigeria as variable in their morphology, distribution and abundance and they are generally uniseriate and multicellular with an acute apex or the trichomes terminate in a large apical cell. The trichomes of the southern African *Boerhavia* and *Commicarpus* are uniseriate and multicellular with globose or club-shaped heads and their size, length, distribution and abundance vary considerably within species. The trichomes could therefore not be used to distinguish between the two genera.

The structure of the stem and leaves of *Boerhavia* correspond to the descriptions given by Metcalfe & Chalk (1950; 1979; 1983). Current results show that *Commicarpus* has more rows of chlorenchyma than *Boerhavia*, but more individuals (more than two individuals per species) need to be examined to determine whether it is constant. The anatomy of the leaves of the genera differ as *Boerhavia* has Kranz anatomy around the minor veins, which is absent in *Commicarpus*. This confirms findings of Muhaidat *et al*. (2007) who investigated *C. plumbagineus*, *B. coccinea* and *B. dominii* Meikle & Hewson and only recorded this character for the two *Boerhavia* species. The presence or absence of Kranz anatomy is therefore a diagnostic character to distinguish between *Boerhavia* and *Commicarpus*.

Tannin idioblasts and raphides have been recorded for the family by various authors (Metcalfe & Chalk, 1950; Bittrich & Kühn, 1993; Edeoga & Ikem, 2002). The tannin idioblasts are absent from *Commicarpus* leaves, but due to them not always being present in the epidermal cells of *Boerhavia* leaves either, they cannot be used as a reliable character to distinguish between the two genera. The distribution of the raphides revealed no specific pattern and is considered of no taxonomic value.

Anthocarp morphology is the character by which most genera have hitherto been distinguished within the family (Willson & Spellenberg, 1977; Douglas & Manos, 2007) and, likewise, the anthocarp anatomy of the southern African *Boerhavia* and *Commicarpus* differs significantly. *Boerhavia* has five-ribbed or 3–5-winged anthocarps with sclerenchyma present within the ribs and the area between the ribs. *Commicarpus* has ten ribs with sclerenchyma only present within the ribs, and the sclerenchyma bundles can either be round (as in *C. decipiens, C. pentandrus, C.*

plumbagineus var. *plumbagineus* and *C. squarrosus*) or elongated (as in *C. chinensis* subsp. *natalensis, C. fallacissimus*, *C. fruticosus, C. pilosus* and *C. helenae* var. *helenae)* in outline. However, the reliability of the outline of the sclerenchyma bundles still needs to be investigated further at different developmental stages of the anthocarp before it can be considered a definite diagnostic character with which to divide the *Commicarpus* species into two groups.

The following key based on anatomical characters is therefore proposed to distinguish between the genera:

1a. Anthocarp 5-ribbed or 3-winged; sclerenchyma present within the ribs and the area between the ribs; minor veins of the leaves surrounded by Kranz anatomy ……………………………………………………………………………*Boerhavia*
1b. Anthocarp 10-ribbed; sclerenchyma only present in the rib area; minor veins of the leaves surrounded by parenchyma cells …..…………………………*Commicarpus*

5.5.2 Anatomical adaptations

Gross morphology of *Boerhavia* and *Commicarpus* species show few traditional xeromorphic characters. Relatively thin cuticles and uncutinized outer periclinal walls characterize the epidermal cells. Furthermore, the mesophyll of *Commicarpus* species has little mechanical support tissue and relatively large intercellular air spaces. Most of the features observed are typical of the family Nyctaginaceae. Nevertheless, the investigated species survive their extreme environment very well. The combination of typical family characteristics may confer some advantages to survive in a xeric environment. Two noteworthy features are Kranz-anatomy in the *Boerhavia* species and the presence of crystalline inclusions in the outer epidermal cell walls of *Boerhavia* and *Commicarpus* species. These crystals may play a significant role to reduce excessive heat loads as well as to reflect harmful ultraviolet light. Some workers are of the opinion that excess heat load may be more of a limiting factor in desert environments than lack of water. Similarly, C4 photosynthesis that occurs in *Boerhavia* species, as reflected by the presence of Kranz-anatomy, helps plants to prevent photorespiration during high temperatures (MacAdam, 2009). Therefore C4 photosynthesis is more an adaptation to high temperatures than to drought.

5.5.2.1 Trichomes

The presence of dense non-living hairs is often regarded as a xeromorphic feature. However, many xerophytes do not have a dense hair layer and seem to survive quite well without them (Cutler *et al*., 2008).

The *Boerhavia* and *Commicarpus* species do not have non-glandular trichomes but various densities of glandular trichomes. The trichomes of the species studied are present on both the stems and leaves, being most dense on the *Boerhavia* species. These trichomes produce a secretion which makes the stems and leaves sticky. Fernandes (1994) and Handley *et al*. (2005) reported that trichomes provide protection against herbivory and oviposition, as trichomes immobilize and trap insects and also lessen the ability of insect eggs to stick to the leaf surface. In an arid environment it is important to deter herbivores, as any injuries to plant parts may lead to water loss.

Non-glandular trichomes are traditionally considered to protect against water loss as they decrease air movement at the leaf surface and increase the thickness of the boundary layer (Fahn & Cutler, 1992; Hanley *et al*., 2007). The densely arranged glandular trichomes may have a similar effect and due to their significant length may increase the thickness of the boundary layer substantially.

Some authors attribute more value to the heat load reducing ability of non-glandular trichomes than to their ability to directly reduce transpiration by increasing the boundary layer (Metcalfe & Chalk, 1983; Fahn, 1986). Non-glandular trichomes rather reduce leaf temperature, and consequently indirectly the transpiration rate, by increasing the amount of sunlight reflected from the leaf surface (Fahn & Cutler, 1992; Gibson, 1998). Whether the glandular trichomes of *Boerhavia* and *Commicarpus* are capable of significantly reflecting sunlight is unknown. The secretory product itself may be of more value in this regard but needs further investigation. For instance, it is known that the resinous material secreted by glandular trichomes of some xerophytes forms a shiny layer that reflects sunlight (Dell & McComb, 1974; Werker & Fahn, 1981).

Trichomes in *Centaurea scabiosa* L. and *Leontodon hispidus* L. act as a calcium sink as a portion of the calcium that arrives at the epidermis is diverted into the trichomes (De Silva *et al*., 1996), a function that could also be attributed to the species studied as the walls of the trichomes are impregnated with crystals.

5.5.2.2 Crystals

Micro-analysis revealed that the crystals consist of up to 30% calcium. The calcium is probably derived from associated soils rich in $CaSO_4.H_2O$, a gypsum found mainly in arid to semi-arid climates with low precipitation (Grotzinger *et al*., 2007) The family is known to grow on soil rich in gypsum (Douglas & Manos, 2007) and Metcalfe & Chalk (1950) report that calcium oxalate crystals are found in the family. The crystals of the species studied are therefore also possibly of calcium oxalate composition.

Crystals have numerous functions and the functions that may be performed by those in the studied species are highlighted here. Crystals accumulate excess calcium (Franceschi, 2001), thereby maintaining ionic balance within the cells (Franceschi, 2001; Ruiz *et al*., 2003). The crystals also play a role in the defense mechanism against herbivores as they cause irritation, interfere with digestion and have an abrasive effect on insects' mandibles (Finely, 1999; Korth *et al*., 2006). The crystals in the epidermis may reflect sunlight, cooling the surface (Franceschi, 2001) and impede transmission of sunlight to the underlying tissue (Fahn & Cutler, 1992). Crystals in the epidermis could also give structural support (Franceschi & Horner, 1980) to the stems, leaves and anthocarps.

5.5.2.3 Stomata

The stomata of both the *Boerhavia* and *Commicarpus* species are similar and the outer and inner periclinal walls of the guard cells are thickened to reduce evaporation of water from the guard cells (Jordaan, 1989). The small inner and large outer ledges protrude towards the pore and therefore may lengthen the diffusion path through the guard cells to reduce water loss through transpiration (Mauseth, 1988). Stomata are present on both surfaces of the leaf, which shortens the distance of carbon dioxide diffusion to the mesophyll cells and therefore increases photosynthesis, especially under favourable environmental conditions (Mauseth, 1988; Gibson, 1998). The presence of stomata on both leaf surfaces may indicate a high rate of photosynthesis when sufficient moisture is available. Amphistomatous leaves are very common in plants that grow in arid environments (Parkhurst, 1978).

5.5.2.4 Collenchyma

Collenchyma gives mechanical support to growing organs as the walls are thickened and protect against sunlight and water loss (Cutter, 1978; Mauseth, 1988). A collenchymatous hypodermis is not regarded as a xeromorphic feature in this case as it is a basic support structure present in many young mesophytic stems as well. Older stems of *Boerhavia* and *Commicarpis* species that have undergone anomalous secondary growth have massive amounts of fibres and although a family feature, this may have ecological implications for the water economy of the plant. This is further discussed under section 5.5.2.7

5.5.2.5 Mesophyll

The mesophyll in the leaves of *Boerhavia* and *Commicarpus* species consists of palisade and spongy parenchyma. The 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 throughout the leaf to maintain a high rate of photosynthesis (Mauseth, 1988). It was also proposed that large intercellular air spaces, such as was found for *Boerhavia* and *Commicarpus*, may reduce leaf temperature as the amount of infrared radiation that reaches the mesophyll is scatttered (McClendon, 1984).

5.5.2.6 Tannins

Tannins occur only in the epidermal cells of the *Boerhavia* species and may protect the underlying mesophyll cells against excess sunlight and ultraviolet radiation (Salatino *et al*., 1988). Tannins can also deter herbivores as the protein-precipitating action of the tannins renders the plant indigestible, unpalatable and of poor nutritional value (Salatino *et al*., 1988).

5.5.2.7 Conducting tissue and secondary growth

Vessels in the vascular bundles of both *Boerhavia* and *Commicarpus* are wide, which increases conductivity (Zwieniecki *et al*., 2001). Such vessels are, however, vulnerable to cavitation, especially in an arid environment (Baas, 1986). The leaves of the *Boerhavia* species have Kranz anatomy around the minor veins, which coincides with C_4 photosynthesis (Fahn & Cutler, 1992). The C_4 photosynthesis enables species to increase their light, water, and nitrogen use and therefore productivity in arid conditions (Long, 1999). The stems of both *Boerhavia* and *Commicarpus* show anomalous secondary growth, which results in the production of massive numbers of fibres. The fibres give mechanical support to the stem. According to Gaff & Carr (1961), the apoplastic water stored in the thick cell walls and cell lumina may act as reservoirs and protect against dehydration by acting as a buffer to symplastic water loss. The phloem is also encapsulated by these fibres, which protect the phloem from drying. The phloem can therefore be active for many years (Mauseth, 1988).

5.5.2.8 Anthocarp – sclereids and mucoidal substance

The anthocarp of *Boerhavia* species has five ribs or 3–5 wings and *Commicarpus* species have ten ribs. The columnar cells of the ribs become mucilaginous and a mucoidal substance is released especially when the anthocarps are wet (Spellenberg, 2004). The mucoidal substance is sticky and may assist with seed dispersal by animals (Willson & Spellenberg, 1977; Spellenberg, 2004) or it might anchor the seed to the soil to allow the radicle to penetrate the soil (Van der Phijl, 1983). The ribs and the sclerenchyma present in the ribs give mechanical support to the anthocarp. The ribs and the sclerenchyma therefore prevent the structure of the anthocarp from collapsing when the mucoidal substance is released and protects the seed inside the fruit against damage and dehydration.

5.5.2.9 Phylogenetic implications

The Nyctaginaceae originated in the neotropics (Douglas & Manos, 2007), but genera occur in both tropical and desert habitats (Douglas & Manos, 2007; Douglas & Spellenberg, 2010). It is therefore uncertain as to whether the family originated in tropical or desert environments. It is therefore not possible to determine whether adaptations as discussed above are apomorphic or synapomorphic and further studies which investigate adaptations in the family is therefore necessary. *Phaeoptilum spinosum* Radlk., an endemic to southern Africa, is one of the basal lineages in the family (Douglas & Manos, 2007) and its anatomy should be compared with that of southern African *Boerhavia* and *Commicarpus* species to shed some light on possible synapomorphies. This will, however, require a detailed anatomical study of all three genera.

5.6 Summary

Leaf and anthocarp anatomy can be used to distinguish between *Boerhavia* and *Commicarpus*. The minor veins of the leaves of the *Boerhavia* species are surrounded by Kranz anatomy, while those of the *Commicarpus* species are surrounded by parenchyma cells. In the anthocarp of the *Boerhavia* species, sclerenchyma is present within the ribs and the area between the ribs, while in the *Commicarpus* species the sclerenchyma is only present in the rib area. The anatomy at infrageneric level is uniform and uninformative, and cannot be used to distinguish between the species. The number of chlorenchyma rows in the stems can also be a possible diagnostic character to distinguish between *Boerhavia* and *Commicarpus* and the shape of the sclerenchyma bundles in the anthocarp can possibly be used to divide *Commicarpus* into two groups (that is, a group with rounded sclerenchyma bundles and a group with elongated sclerenchyma bundles), which can aid with identification.

The stems, leaves and anthocarps of the *Boerhavia* and *Commicarpus* species have some anatomical adaptations which are beneficial to survive desert conditions by conserving water, reducing heat load, maintaining a high photosynthetic rate when water is available and aiding seed dispersal. It is still unknown how the cytoplasm of these species may withstand dehydration and extensive experimental work is needed to determine the efficiency in performing the functions ascribed to the different anatomical features. The southern African *Boerhavia* and *Commicarpus* species are, however, classified as drought avoiders.

Table 5.1. Specimens examined, as well as the material sectioned/examined for light microscopy (LM) and scanning electron microscopy (SM)

Taxon	Specimens examined	Material sectioned for LM	Material examined with SEM
Boerhavia coccinea var. *coccinea*	Struwig, M. 55	Anthocarp, leaf	Anthocarp, leaf, stem
	Struwig, M. 77	–	Anthocarp, leaf
	Struwig, M. 81	–	Anthocarp, leaf
	Struwig, M. 108	Anthocarp, leaf	–
	Struwig, M. 120	Anthocarp, leaf, stem	–
Boerhavia cordobensis	Gotze, A. 146	–	Anthocarp, leaf
	Straub, C.C. 808	–	Anthocarp, leaf
	Struwig, M. 82	–	Anthocarp, leaf
	Struwig, M. 112	Leaf, stem	–
	Struwig, M. 122	Anthocarp, leaf	–
	Struwig, M. 132	Anthocarp, leaf	–
Boerhavia deserticola	Struwig, M. 38	Anthocarp, stem	Anthocarp, leaf, stem
	Struwig, M. 42	Anthocarp, leaf	Anthocarp, leaf
	Struwig, M. 43	Anthocarp, leaf, stem	Anthocarp, leaf, stem
Boerhavia diffusa var. *diffusa*	Struwig, M. 70	–	Anthocarp, leaf
	Struwig, M. 88	Anthocarp, leaf, stem	Anthocarp, leaf, stem
	Struwig, M. 89	–	Anthocarp, leaf
	Struwig, M. 117	Anthocarp, stem	–
	Struwig, M. 125	Anthocarp, stem	–
Boerhavia erecta	Struwig, M. 23	Anthocarp, stem	–
	Struwig, M. 135	Anthocarp, leaf	–
	Struwig, M. 143	Anthocarp, leaf	–
	Siebert, S.J. 6964	–	Anthocarp, leaf
	De Lemos, F. & Macuacuo, L. 65	–	Anthocarp, leaf
	La Grange, M. & Lubbe, R. 11	–	Anthocarp, leaf
Boerhavia hereroensis	Struwig, M. 34	Anthocarp, leaf	Anthocarp, leaf, stem
	Struwig, M. 35	Anthocarp	Anthocarp, leaf, stem
	Struwig, M. 40	Anthocarp, stem	Anthocarp, leaf, stem
Boerhavia repens var. *repens*	Struwig, M. 168	Anthocarp, stem, leaf	–
	Struwig, M. 170	Anthocarp, leaf	–
	Acocks, J.P.H. 21788	–	Anthocarp, leaf
	Straub, C.C. 833	–	Anthocarp, leaf
Commicarpus chinensis subsp. *natalensis*	Struwig, M. 60	Stem	Stem, leaf
	Struwig, M. 61	Anthocarp, stem, leaf	Anthocarp, leaf, stem
	Struwig, M. 63	Anthocarp	Anthocarp, leaf, stem
Commicarpus decipiens e	Struwig, M. 47	Anthocarp, leaf, stem	Anthocarp,leaf, stem
	Struwig, M. 51	–	Anthocarp,leaf
	Struwig, M. 53	–	Leaf, stem
	Struwig, M. 176	Anthocarp,leaf	–
	Struwig, M. 181	Anthocarp,leaf	–
Commicarpus fallacissimus	Struwig, M.33	Anthocarp, leaf, stem	Anthocarp, leaf, stem
	Struwig, M. 46	Anthocarp, stem	Anthocarp, leaf, stem
	Germishuizen, G. 9553	–	Anthocarp, leaf
Commicarpus fruticosus	Struwig, M. 59	Anthocarp, leaf, stem	Anthocarp, leaf, stem
	Struwig, M.163	Anthocarp, leaf	–
	Struwig, M. 164	Anthocarp, leaf, stem	–
Commicarpus helenae var. *helenae*	Struwig, M. 44	Anthocarp, stem	Anthocarp, stem, leaf
	Struwig, M. 141	Leaf	Leaf, stem
	Struwig, M. 183	Anthocarp, leaf, stem	–
Commicarpus pentandrus	Kurzweg, S. 7141	Anthocarp, leaf	Anthocarp, leaf, stem
	Struwig, M. 48	–	Anthocarp, leaf, stem
	Struwig, M. 57	Anthocarp, stem, leaf	Anthocarp, leaf, stem
	Struwig, M. 131	Leaf	–
Commicarpus pilosus	Struwig, M. 109	Anthocarp, leaf	–
	Struwig, M. 111	Anthocarp, leaf, stem	–
	Struwig, M. 114	Anthocarp, leaf	–
	Leach, L.C. 10664	–	Anthocarp, leaf
	Seydel, R.H.W. 4488	–	Anthocarp, leaf
	Straub, C.C. 609	–	Anthocarp, stem, leaf
Commicarpus plumbagineus var. *plumbagineus*	Siebert, S.J. 3970	Anthocarp, leaf, stem	Anthocarp, leaf
	Struwig, M. 106	Anthocarp, leaf, stem	–
	Struwig, M. 126	Anthocarp, leaf	–
	Cole, D.T. 876	–	Anthocarp, leaf
Commicarpus squarrosus	Struwig, M. 36	Anthocarp	Anthocarp, leaf, stem
	Struwig, M. 39	Anthocarp, stem	Anthocarp, leaf, stem
	Struwig, M. 41	Anthocarp, leaf	Anthocarp, leaf, stem

Table 5.2. The composition of the crystals (% weight) occurring in the anthocarp and leaves of *Boerhavia* and *Commicarpus* species. (Al, aluminium; C, carbon; Ca, calcium; Cl, clorine; K, potassium; O, oxygen; S, sulphur; Si, silica).

Taxon and plant organ	**Element**	**% weight**
*Boerhavi*a sp. anthocarp	C	21
	Ca	13
	O	64
	K	1
Boerhavia sp. leaf	C	15
	Ca	30
	O	52
	Al	1
	Cl	1
	K	1
	Mg	1
Commicarpus sp. anthocarp	C	23
	Ca	19
	O	55
	K	1
	Si	1
Commicarpus sp. leaf	C	22
	Ca	12
	O	64
	K	1
	Cl	1
	Mg	1
	S	1

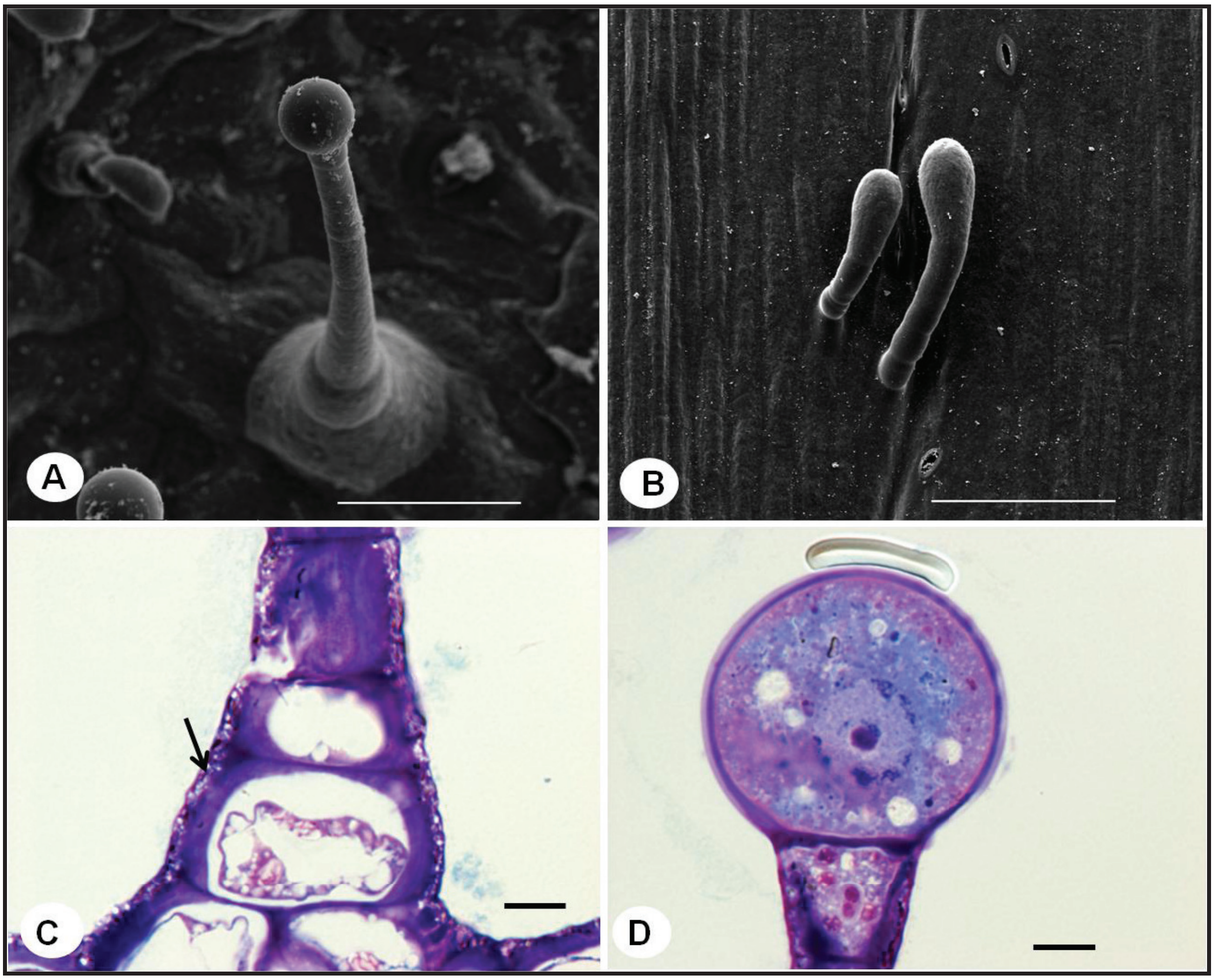


Figure 5.1: Scanning electron micrograph of: A: trichome with a globose head of *Boerhavia hereroensis* (*Struwig 34*). Scale bar 50 µm; B: trichomes with a clavate head of *Commicarpus fallacissimus* (*Struwig 33*). Scale bar 100 µm. Light micrograph of: C: crystals in the cell wall of a trichome of *B. hereroensis* (indicated by the arrow) (*Struwig 35*). Scale bar 10 µm; D: dense cytoplasm in the globose head of a trichome of *B. hereroensis* (*Struwig 35*). Scale bar 10 µm.

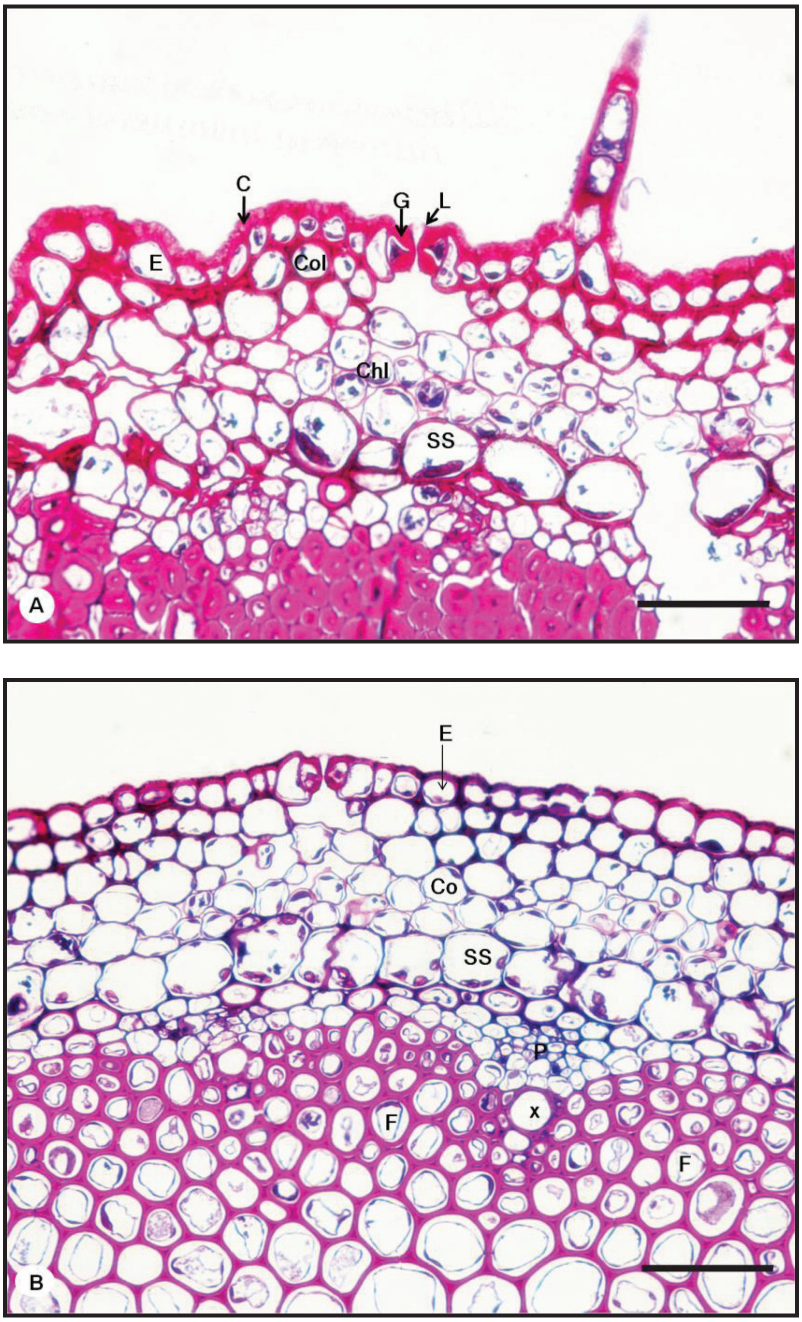


Figure 5.2: Light micrographs of: A: cross section through a portion of the stem of *Boerhavia deserticola* (*Struwig 43*) (C, crystals; Chl, chlorenchyma; Col, collenchyma; E, epidermis; G, guard cell of stoma; L, outer ledge of guard cell of stoma; SS, starch sheath); B: cross section through the stem of *B. diffusa* var. *diffusa* showing secondary growth (*Struwig 117*) (Co, cortex; E, epidermis; F, fibre; P, secondary phloem; SS, starch sheath; X, secondary xylem). Scale bars 50 µm.

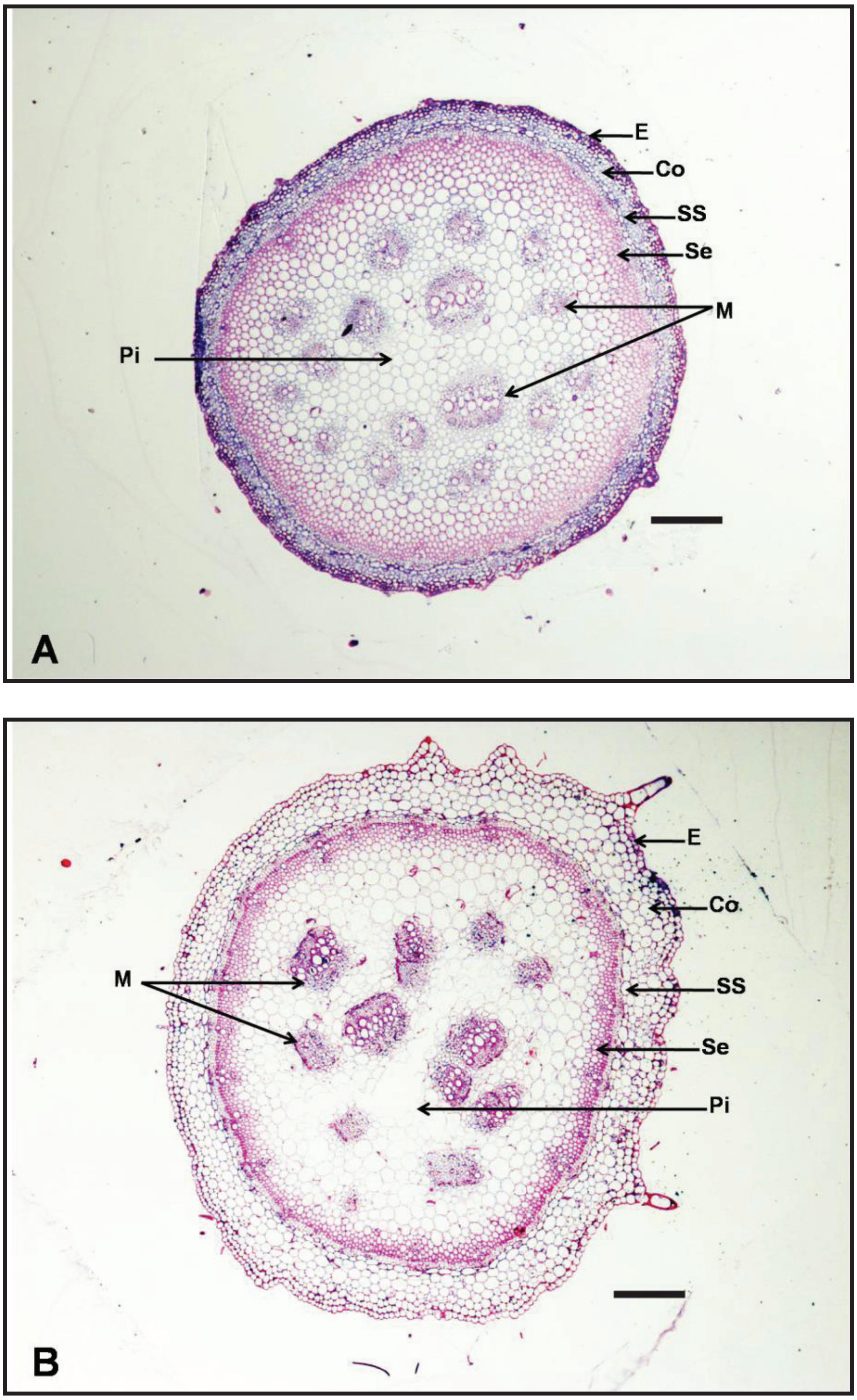


Figure 5.3: Light micrographs of cross sections through the stems of *Boerhavia* species. A: *B. coccinea* var. *coccinea* (*Struwig 120*); B: *B. cordobensis* (*Struwig 112*) (Co, cortex; E, epidermis; M, medullary bundles; Pi, pith; Se, secondary growth; SS, starch sheath). Scale bars 250 µm.

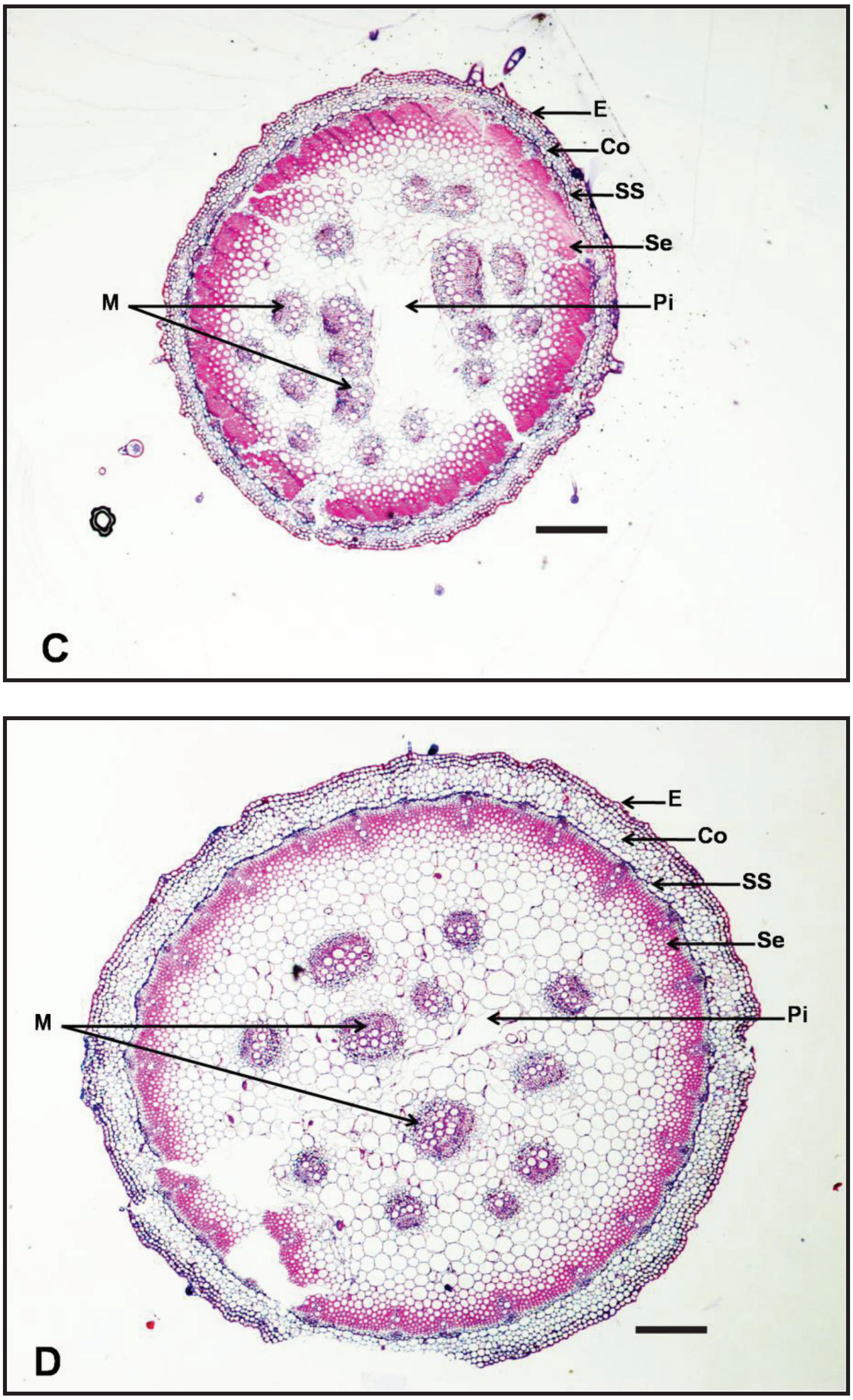


Figure 5.3: Light micrographs of cross sections through the stems of *Boerhavia* species. C: *B. deserticola* (*Struwig 43*); D: B. *diffusa* var. *diffusa* (*Struwig 117*) (Co, cortex; E, epidermis; M, medullary bundles; Pi, pith; Se, secondary growth; SS, starch sheath). Scale bars 250 µm.

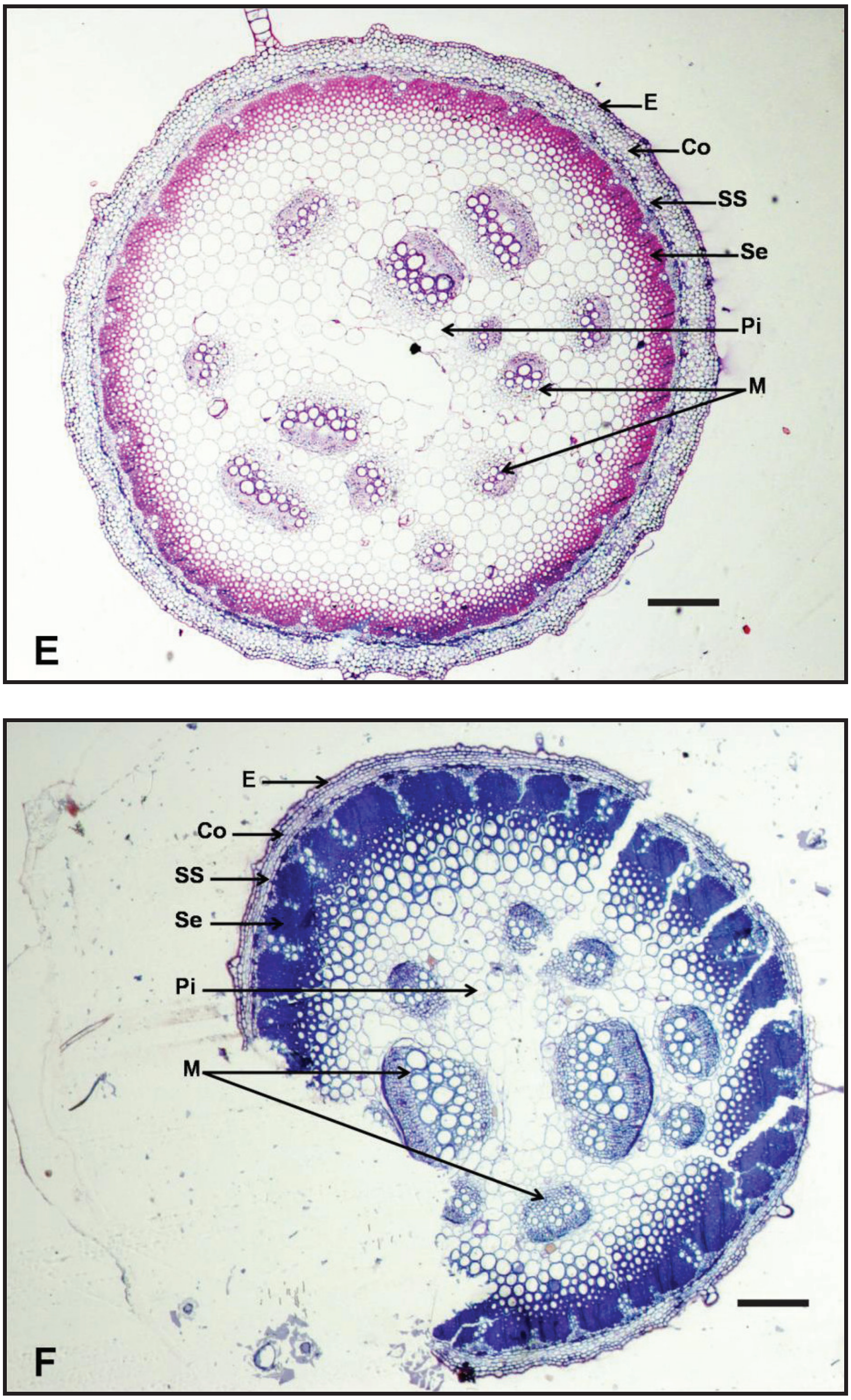


Figure 5.3: Light micrographs of cross sections through the stems of *Boerhavia* species. E: *B. erecta* (*Struwig 23*); F: *B. hereroensis* (*Struwig 40*) (Co, cortex; E, epidermis; M, medullary bundles; Pi, pith; Se, secondary growth; SS, starch sheath.) Scale bars 250 µm.

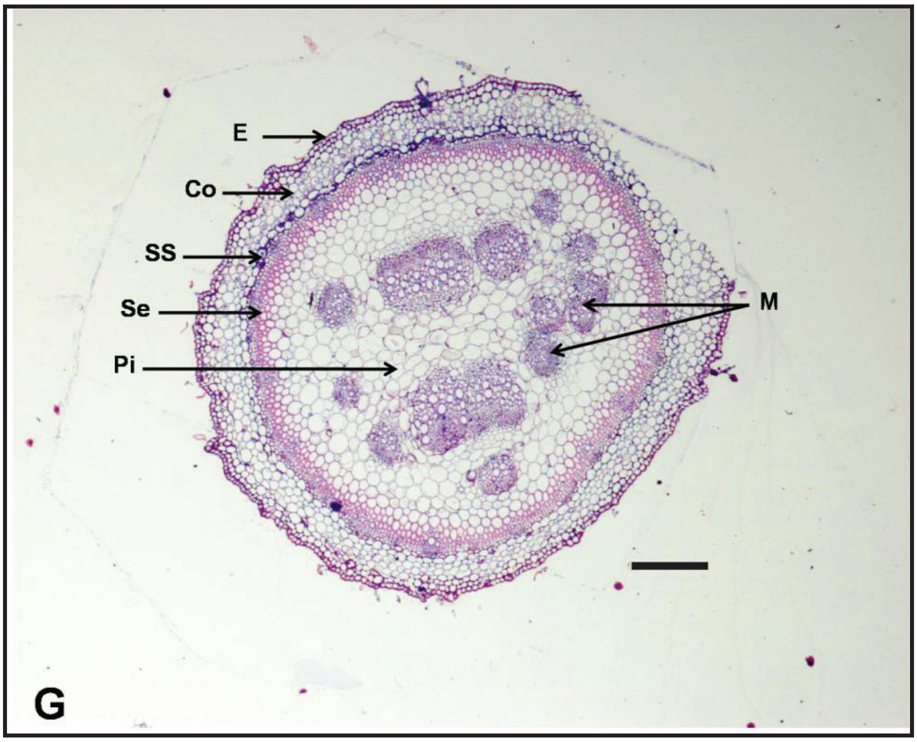


Figure 5.3: Light micrograph of a cross section through the stems of *Boerhavia* species. G: *B. repens* subsp. *repens* (*Struwig 168*) (Co, cortex; E, epidermis; M, medullary bundles; Pi, pith; Se, secondary growth; SS, starch sheath). Scale bars 250 µm.

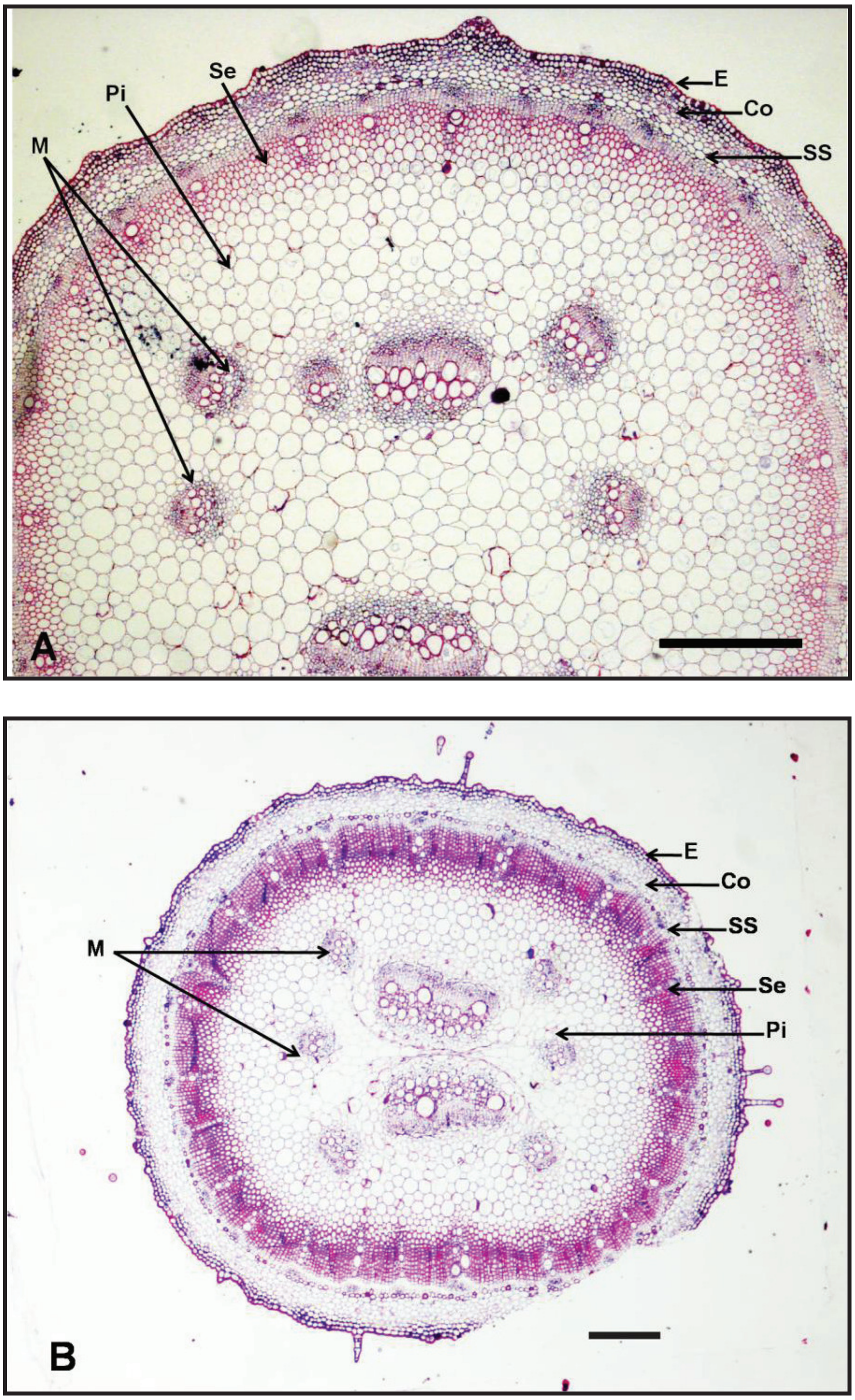


Figure 5.4: Light micrographs of cross sections through the stems of *Commicarpus* species. A: *C. chinensis* subsp. *natalensis* (*Struwig 60*). Scale bar 500 µm; B: *C. decipiens* (*Struwig 47*) Scale bar 250 µm. (Co, cortex; E, epidermis; M, medullary bundles; Pi, pith; Se, secondary growth; SS, starch sheath).

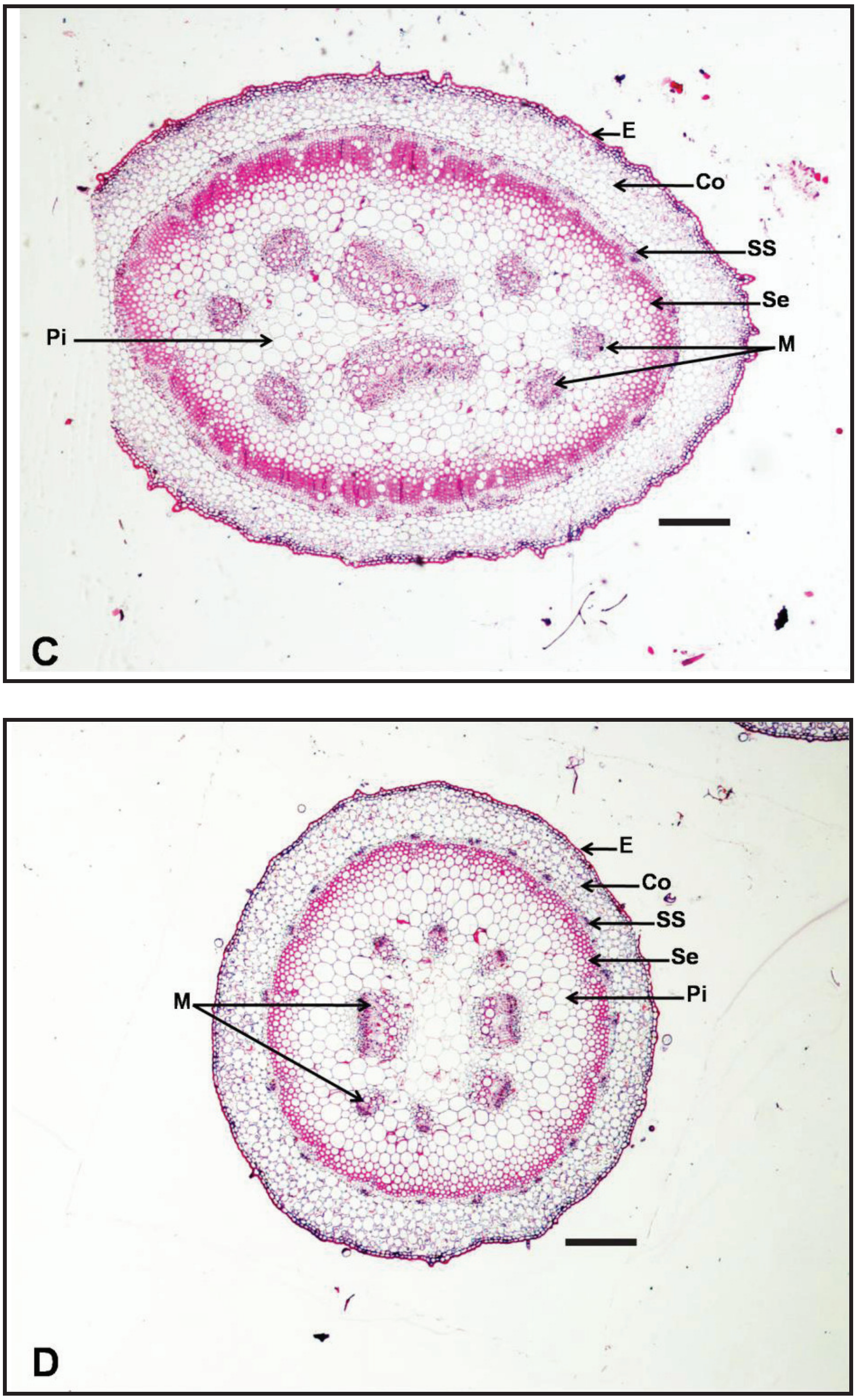


Figure 5.4: Light micrographs of cross sections through the stems of *Commicarpus* species. C: *C. fallacissimus* (*Struwig 46*); D: *C. fruticosus* (*Struwig 59*) (Co, cortex; E, epidermis; M, medullary bundles; Pi, pith; Se, secondary growth; SS, starch sheath). Scale bars 250 µm.

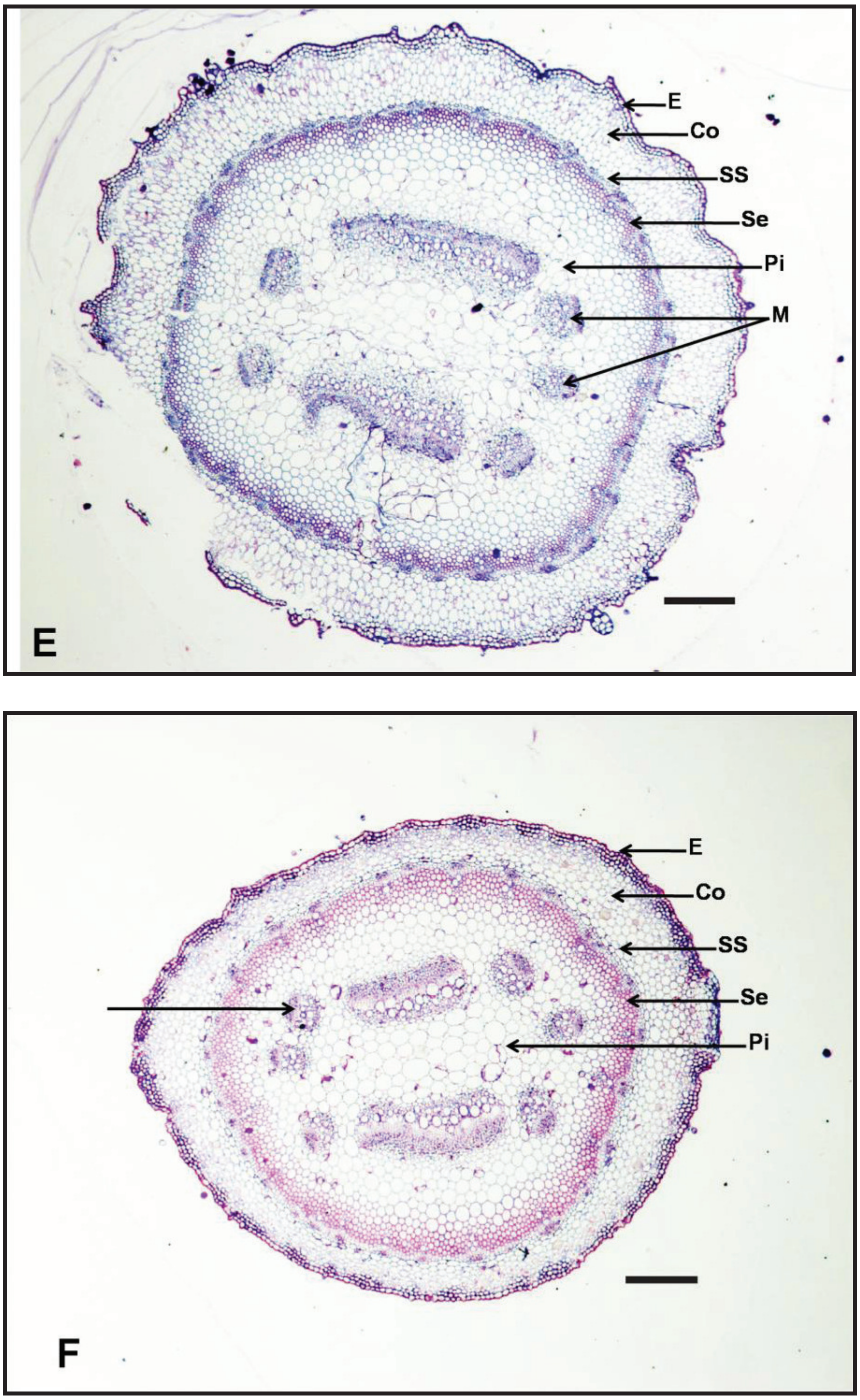
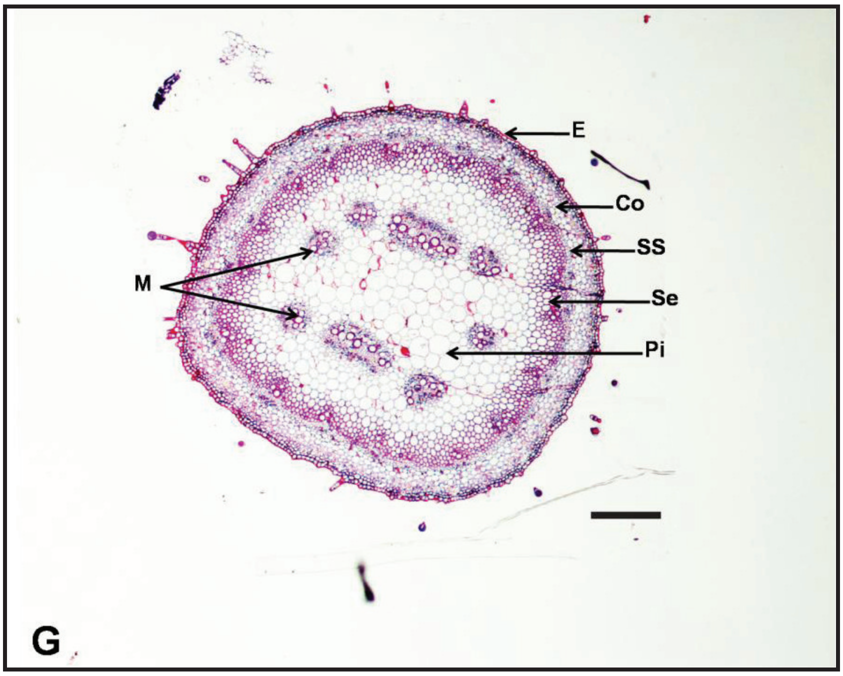


Figure 5.4: Light micrographs of cross sections through the stems of *Commicarpus* species. E: *C. helenae* var. *helenae* (*Struwig 183*); F: *C. pentandrus* (*Struwig 57*) (Co, cortex; E, epidermis; M, medullary bundles; Pi, pith; Se, secondary growth; SS, starch sheath). Scale bars 250 µm.



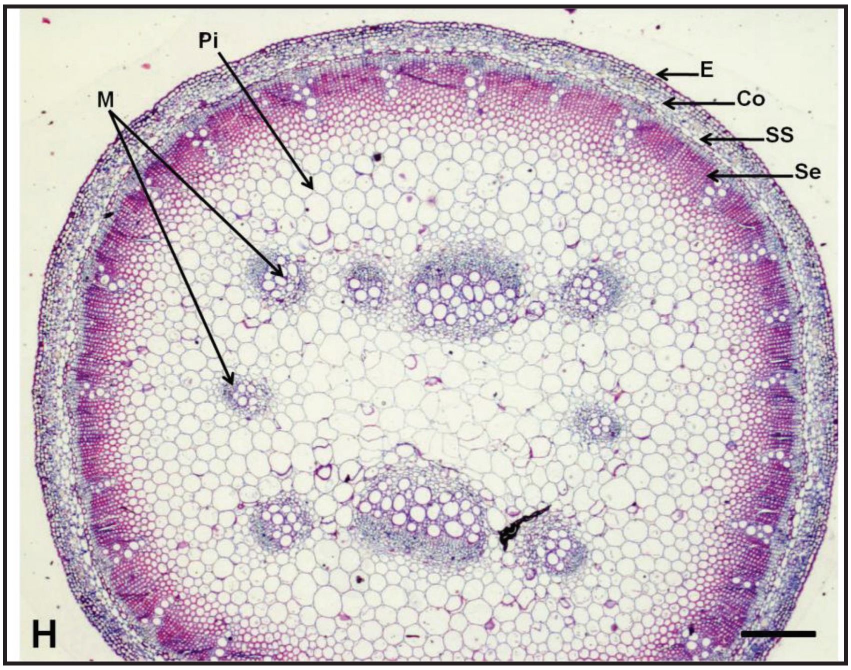


Figure 5.4: Light micrographs of cross sections through the stems of *Commicarpus* species. G: *C. pilosus* (*Struwig 111*); H: *C. plumbagineus* (*Siebert 3970*) (Co, cortex; E, epidermis; M, medullary bundles; Pi, pith; Se, secondary growth; SS, starch sheath). Scale bars 250 µm.

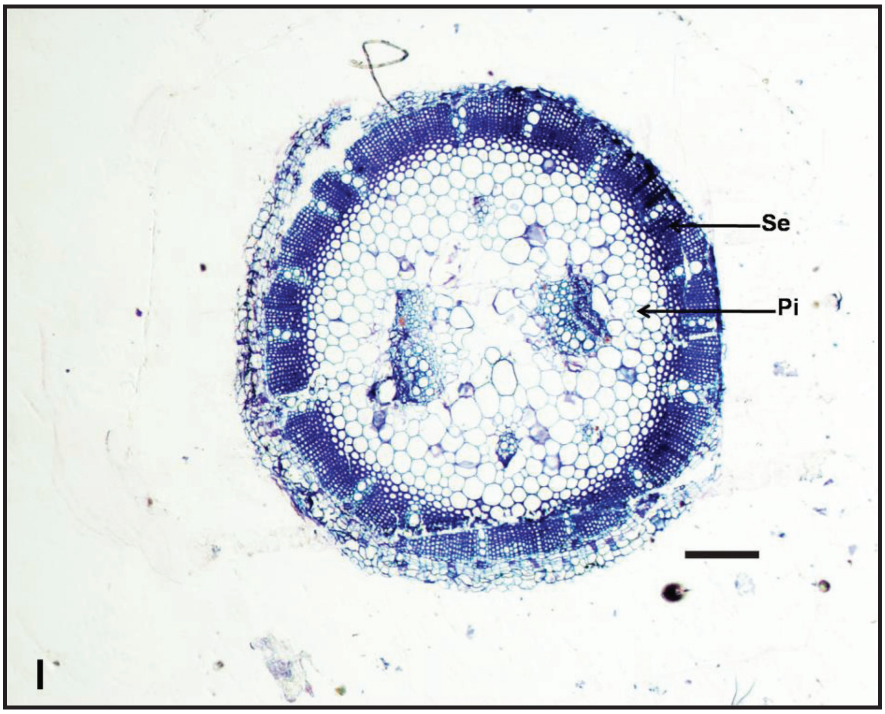


Figure 5.4: Light micrograph of cross sections through the stems of *Commicarpus* species. I: *C. squarrosus* (*Struwig 39*) (Pi, pith; Se, secondary growth). Scale bars 250 µm.

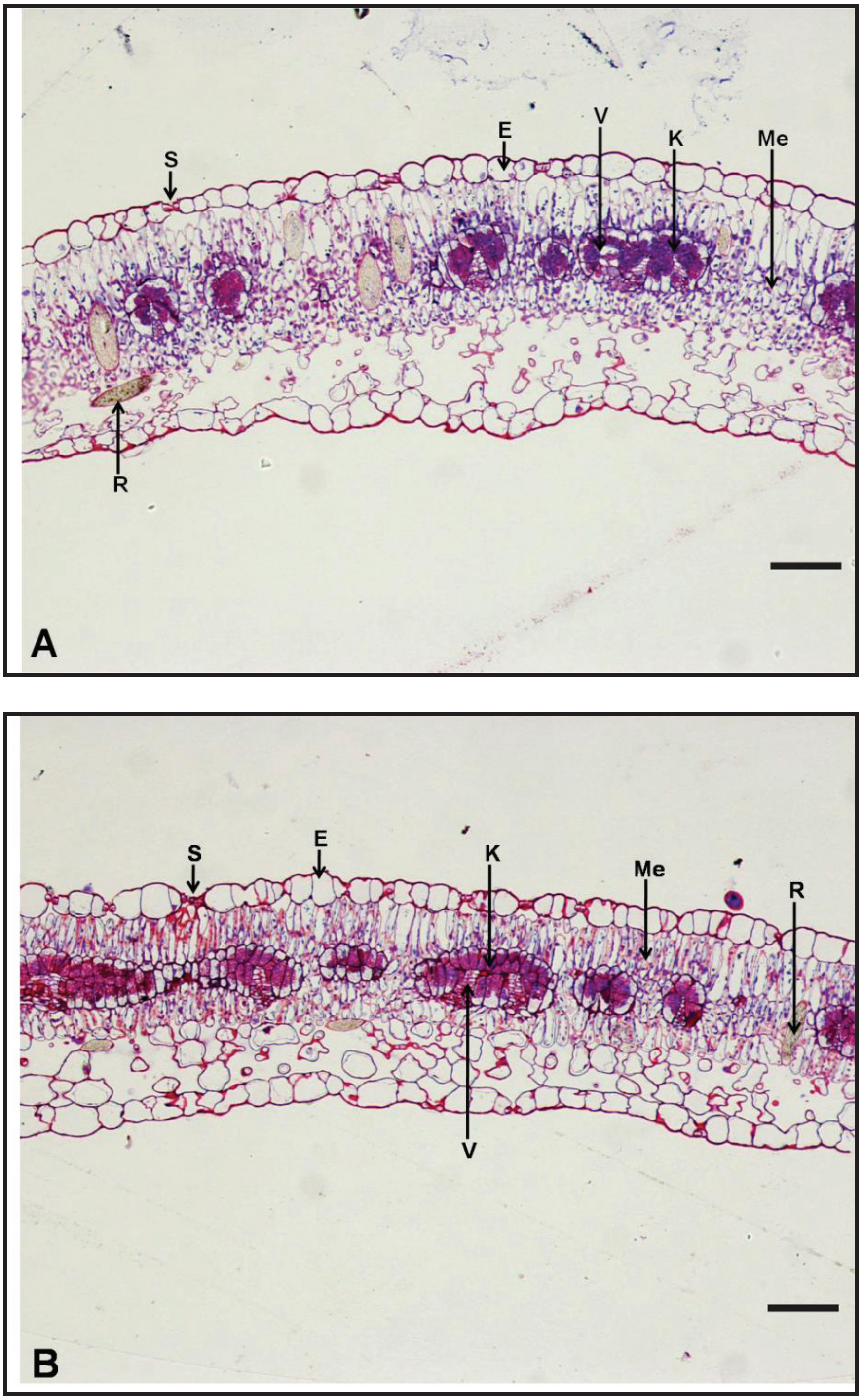


Figure 5.5: Light micrographs of cross sections through the leaves of the *Boerhavia* species. A: *B. coccinea* var. *coccinea* (*Struwig 55*); B: *B. cordobensis* (*Struwig 122*) (E, epidermis; K, Kranz anatomy; Me, mesophyll; R, raphide bundle; S, stoma; V, vascular bundle). Scale bars 100 µm.

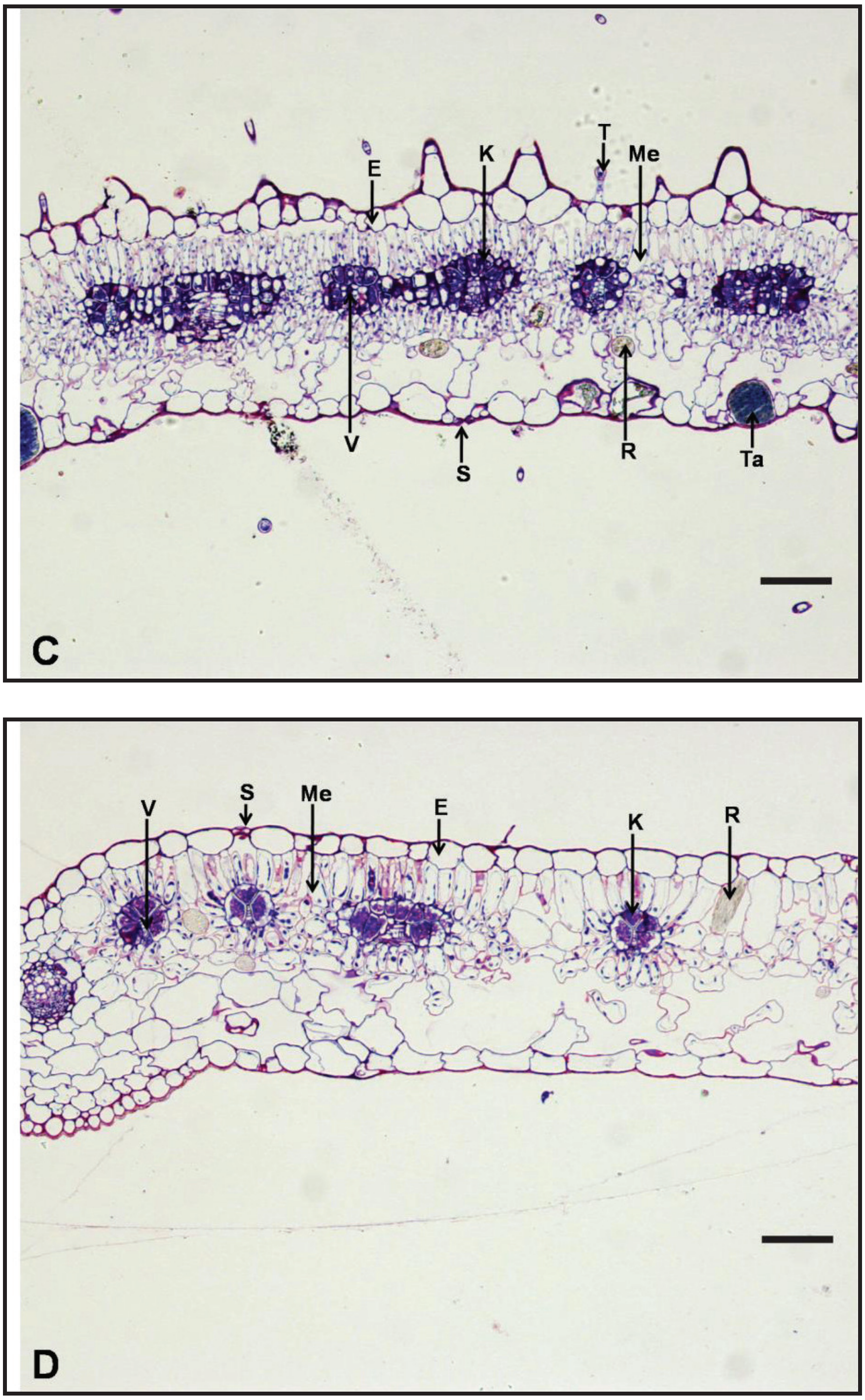


Figure 5.5: Light micrographs of cross sections through the leaves of the *Boerhavia* species. C: *B. deserticola* (*Struwig 42*); D: *B. diffusa* var. *diffusa* (*Struwig 88*) (E, epidermis; K, Kranz anatomy; Me, mesophyll; R, raphide bundle; S, stoma; V, vascular bundle). Scale bars 100 µm.

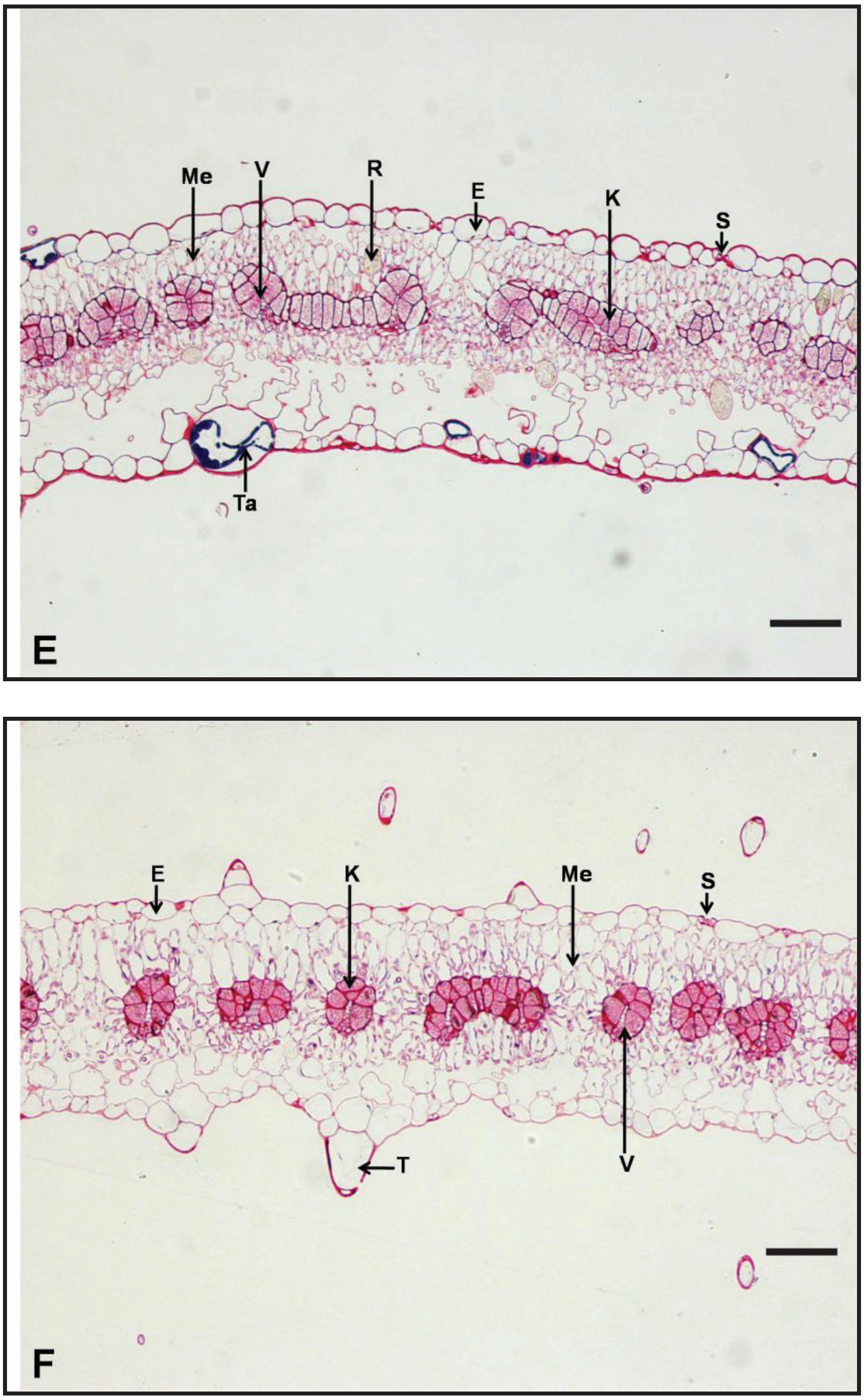


Figure 5.5: Light micrographs of cross sections through the leaves of the *Boerhavia* species. E: *B. erecta* (*Struwig 135*); F: *B. hereroensis* (*Struwig 34*) (E, epidermis; K, Kranz anatomy; Me, mesophyll; R, raphide bundle; S, stoma; T, trichome; V, vascular bundle). Scale bars 100 µm.

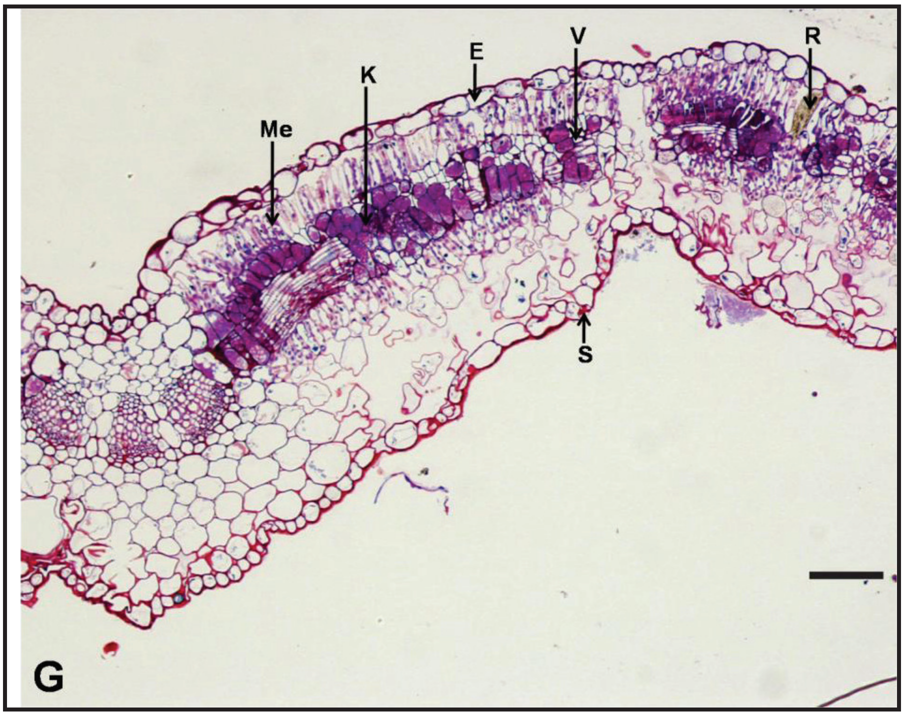


Figure 5.5: Light micrograph of cross sections through the leaves of the *Boerhavia* species. G: *B. repens* subsp. *repens* (*Struwig 168*) (E, epidermis; K, Kranz anatomy; Me, mesophyll; R, raphide bundle; S, stoma; V, vascular bundle). Scale bar 100 µm.

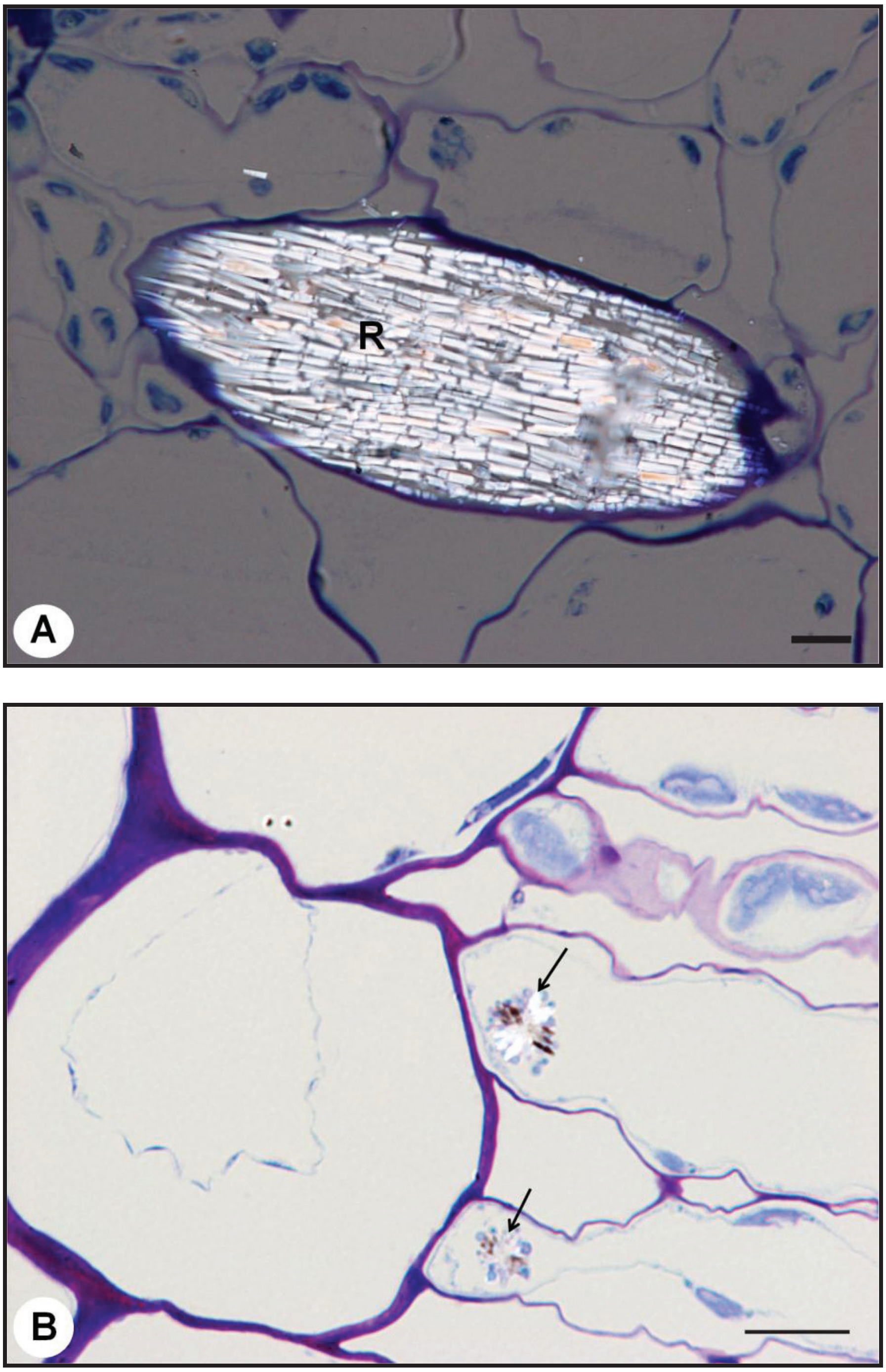


Figure 5.6: Light micrographs of: A: bundle of raphide crystals (R) and B: druse crystals (indicated by the arrows) in *Boerhavia hereroensis* (*Struwig 35*). Scale bars10 µm.

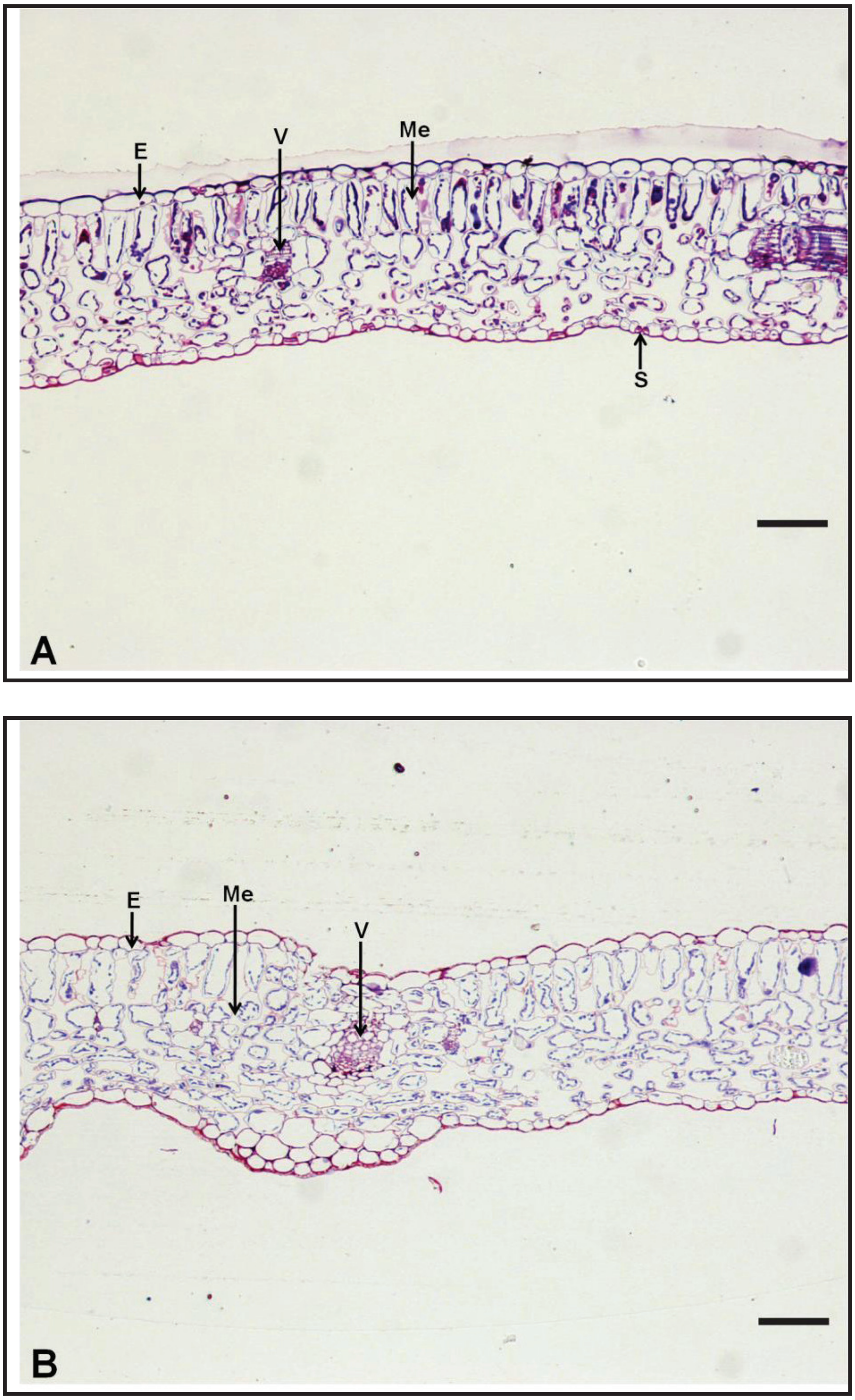


Figure 5.7: Light micrographs of cross sections through the leaves of *Commicarpus* species. A: *C. chinensis* subsp. *natalensis* (*Struwig 61*); B: *C. decipiens* (*Struwig 176*) (E, epidermis; Me, mesophyll; V, vascular bundle). Scale bars 100 µm.

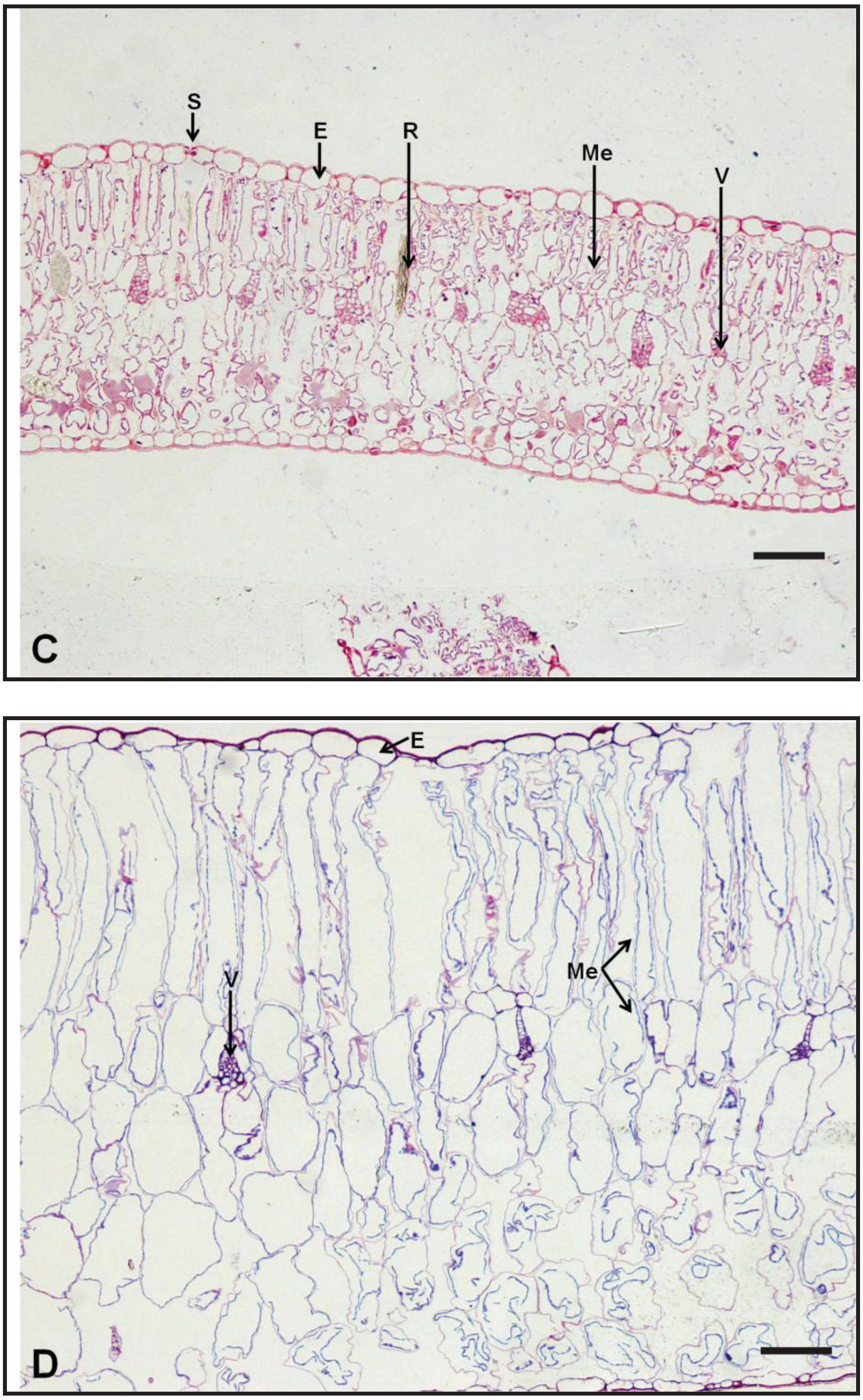


Figure 5.7: Light micrographs of cross sections through the leaves of *Commicarpus* species. C: *C. fallacissimus* (*Struwig 46*); D: *C. fruticosus* (*Struwig 163*) (E, epidermis; Me, mesophyll; V, vascular bundle). Scale bars 100 µm.

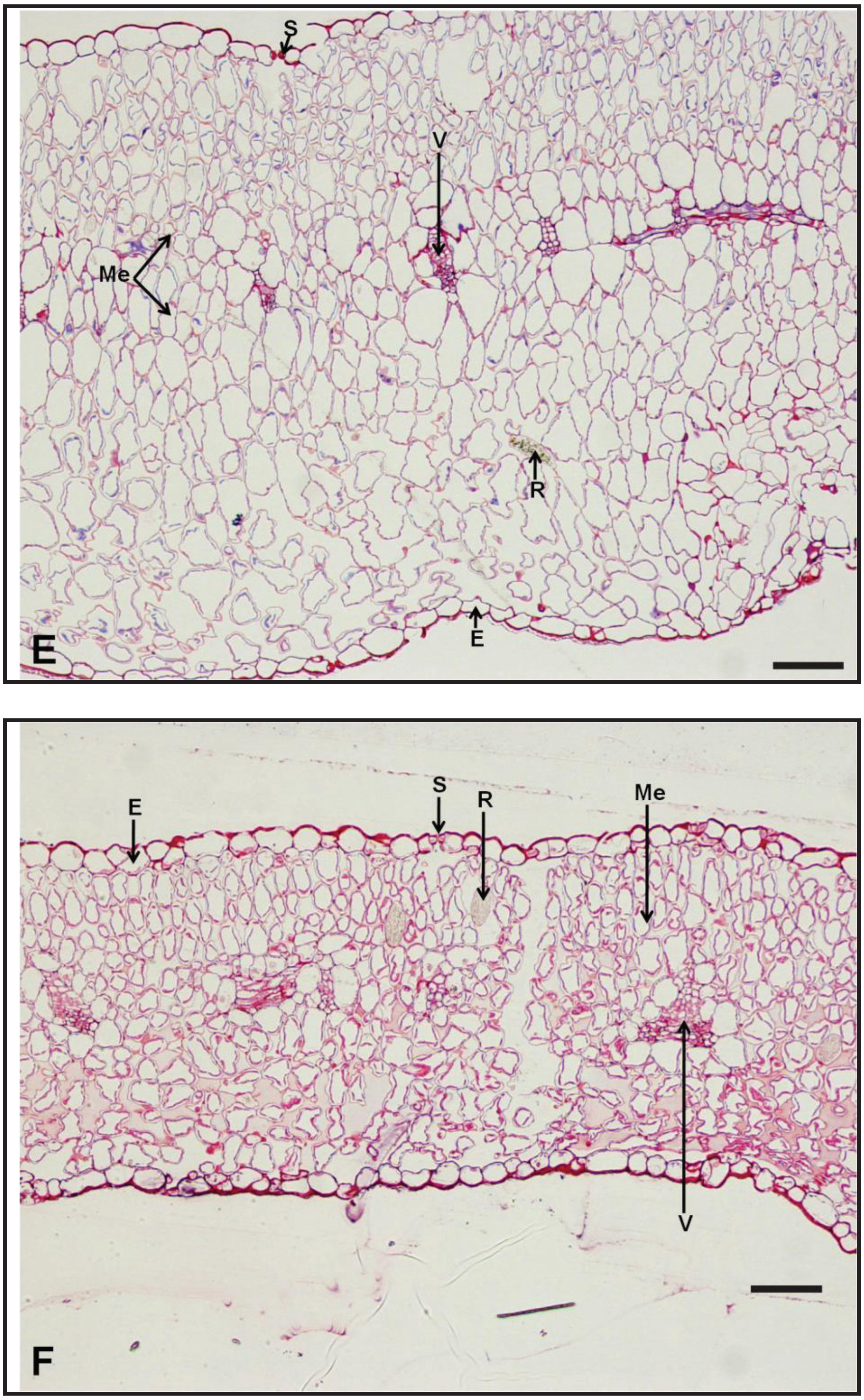
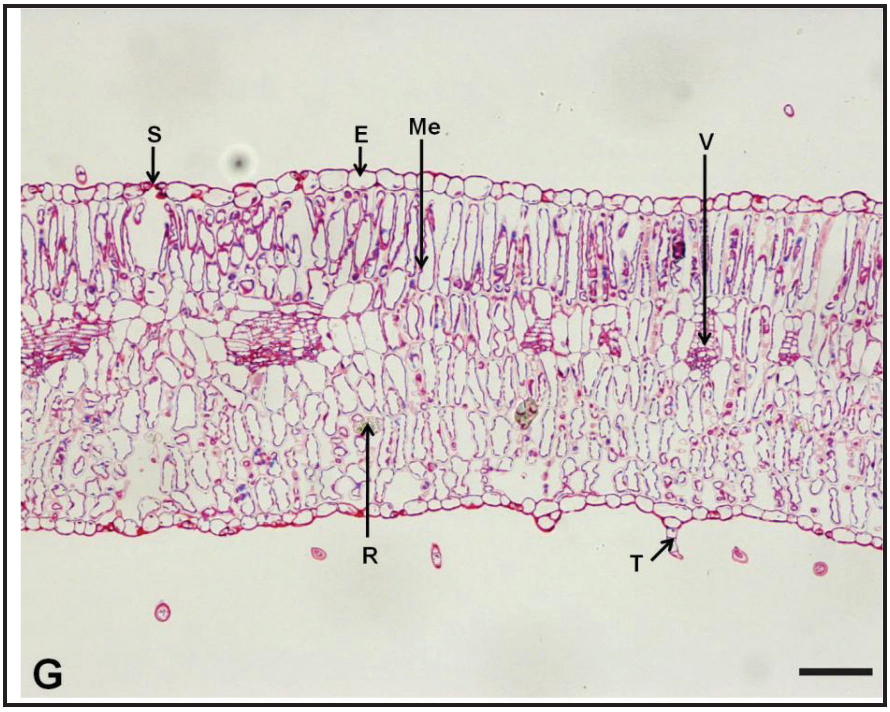


Figure 5.7: Light micrographs of cross sections through the leaves of *Commicarpus* species. E: *C. helenae* var. *helenae* (*Struwig 183*); F: *C. pentandrus* (*Struwig 57*) (E, epidermis; Me, mesophyll; R, raphide; S, stoma; V, vascular bundle). Scale bars 100 µm.



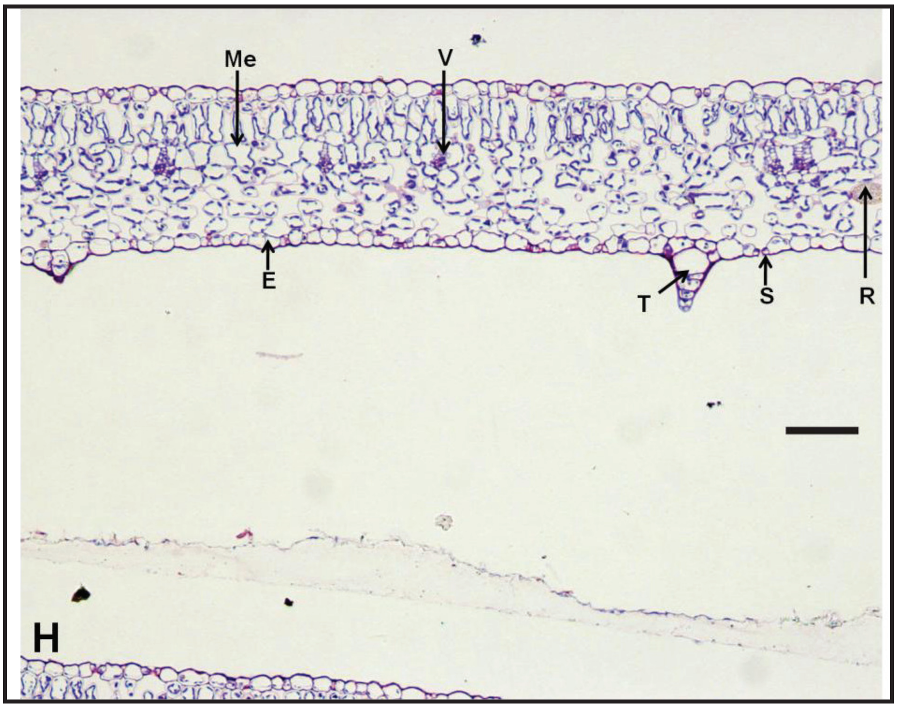


Figure 5.7: Light micrographs of cross sections through the leaves of *Commicarpus* species. G: *C. pilosus* (*Struwig 111*); H: *C. plumbagineus* (*Struwig 106*) (E, epidermis; Me, mesophyll; R, raphide; S, stoma; T, trichome; V, vascular bundle). Scale bars 100 µm.

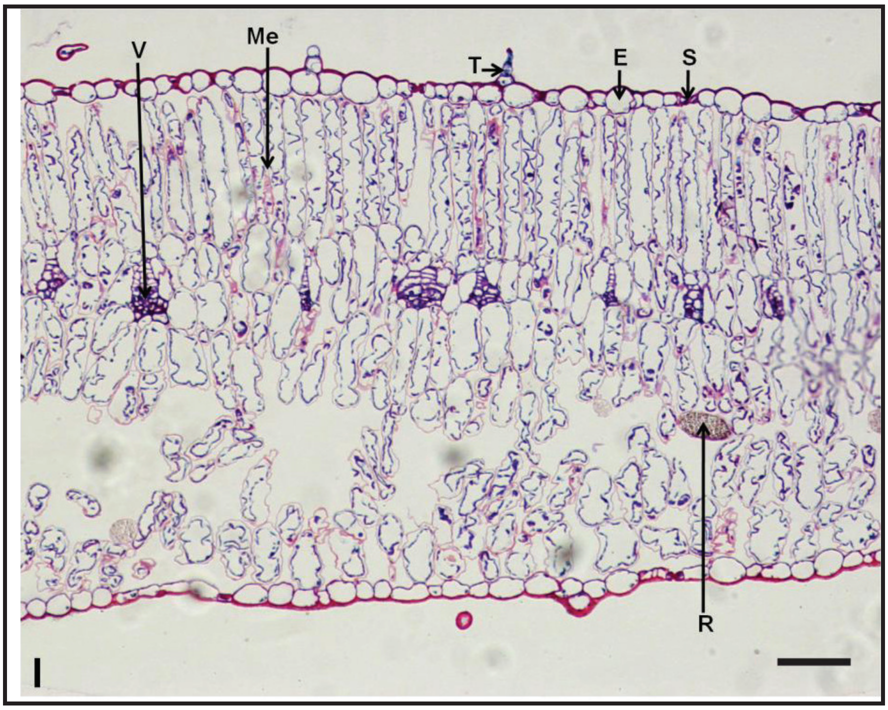
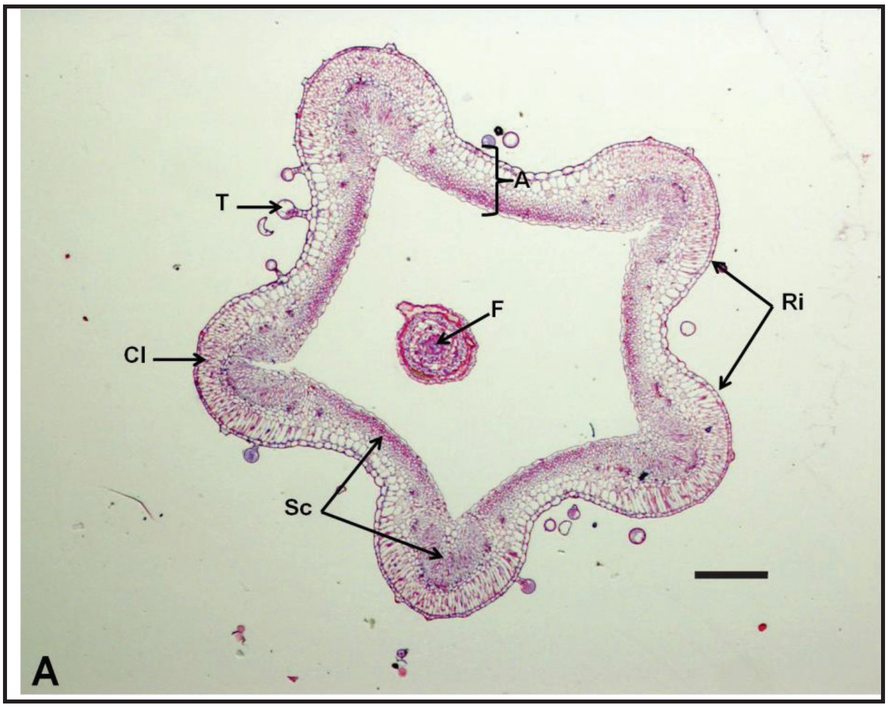


Figure 5.7: Light micrograph of cross sections through the leaves of *Commicarpus* species. I: *C. squarrosus* (*Struwig 41*) (E, epidermis; Me, mesophyll; R, raphide; S, stoma; T, trichome; V, vascular bundle). Scale bar 100 µm.



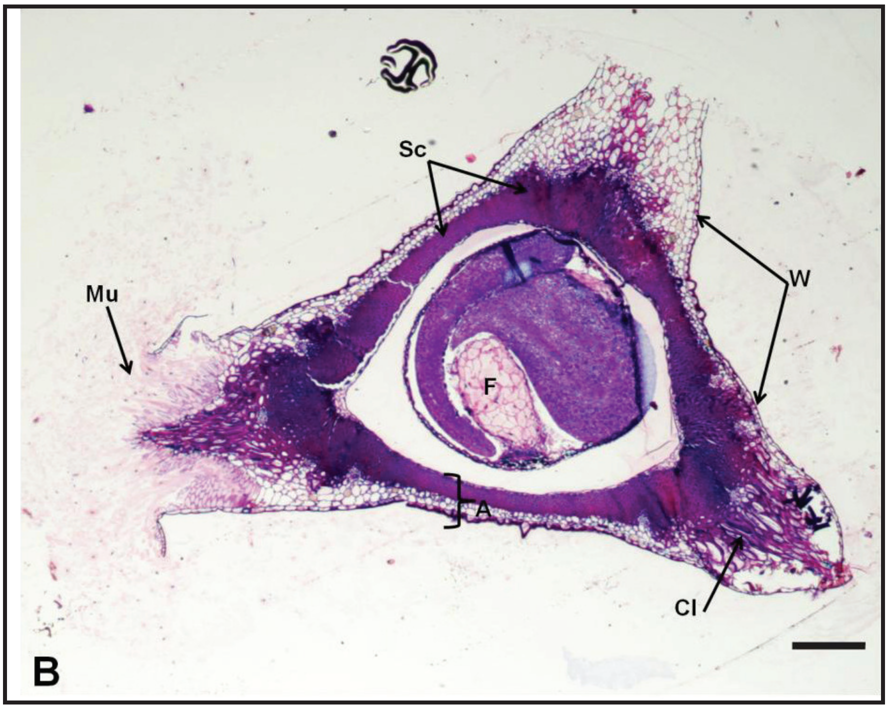


Figure 5.8: Light micrographs of cross sections through the anthocarps of *Boerhavia* species. A: *B. coccinea* var. *coccinea* (*Struwig 55*); B: *B. cordobensis* (*Struwig 132*) (A, anthocarp; Cl, columnar cells; F, fruit; Mu, mucoidal substance; Ri, rib; Sc, sclerenchyma; T, trichome; W, wings). Scale bars 250 µm.

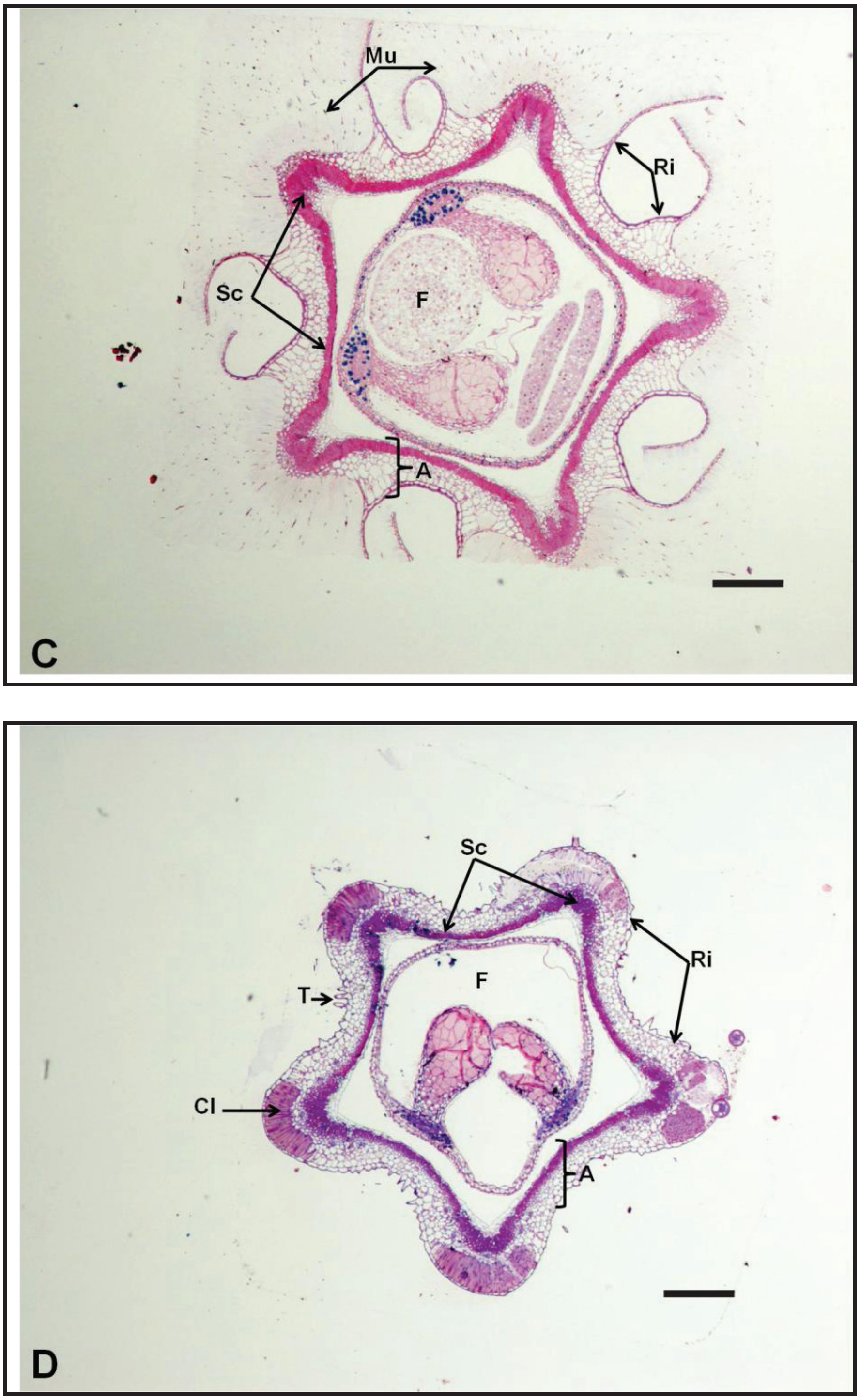
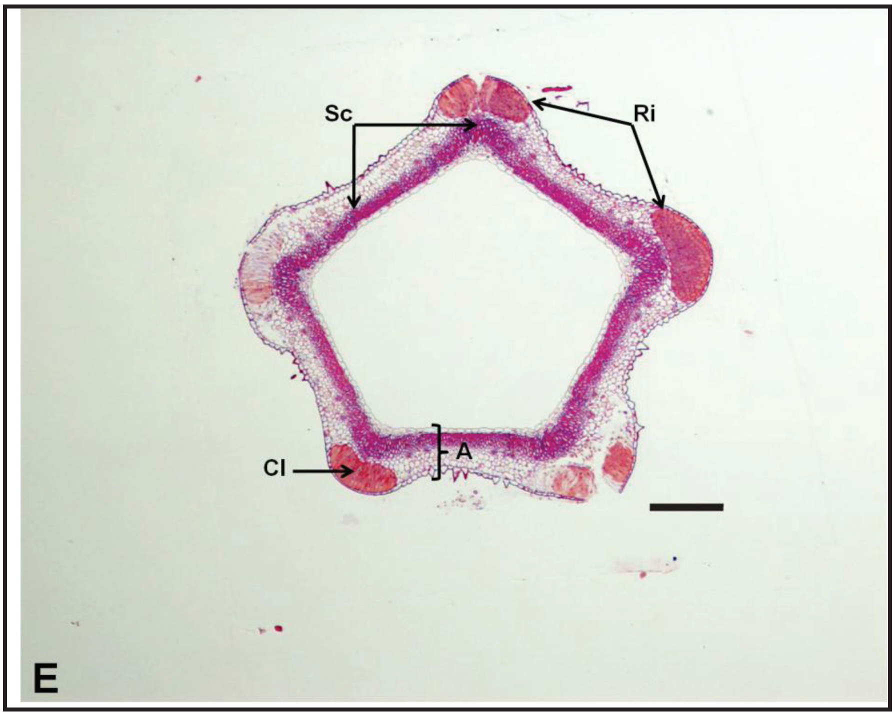


Figure 5.8: Light micrographs of cross sections through the anthocarps of *Boerhavia* species. C: *B. deserticola* (*Struwig 43*); D: B. *diffusa* var. *diffusa* (*Struwig 125*) (A, anthocarp; Cl, columnar cells; F, fruit; Mu, mucoidal substance; Ri, rib; Sc, sclerenchyma; T, trichome). Scale bars 250 µm.



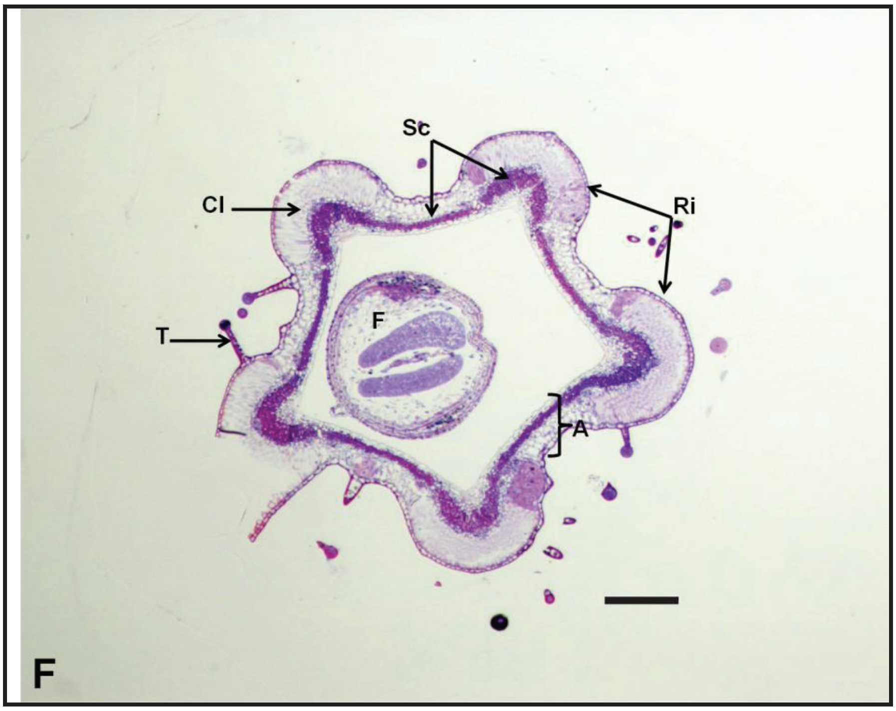


Figure 5.8: Light micrographs of cross sections through the anthocarps of *Boerhavia* species. E: *B. erecta* (*Struwig 143*); F: *B. hereroensis* (*Struwig 35*) (A, anthocarp; Cl, columnar cells; F, fruit; Ri, rib; Sc, sclerenchyma; T, trichome). Scale bars 250 µm.

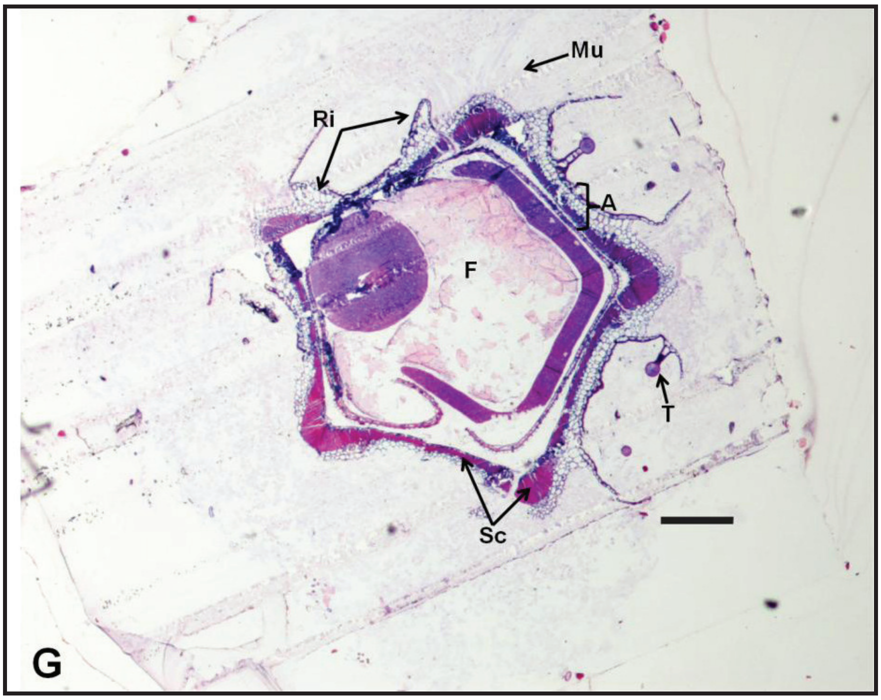


Figure 5.8: Light micrograph of cross sections through the anthocarps of *Boerhavia* species. G: *B. repens* subsp. *repens* (*Struwig 170*). (A, anthocarp; F, fruit; Mu, mucoidal substance; Ri, rib; Sc, sclerenchyma; T, trichome). Scale bar 250 µm.

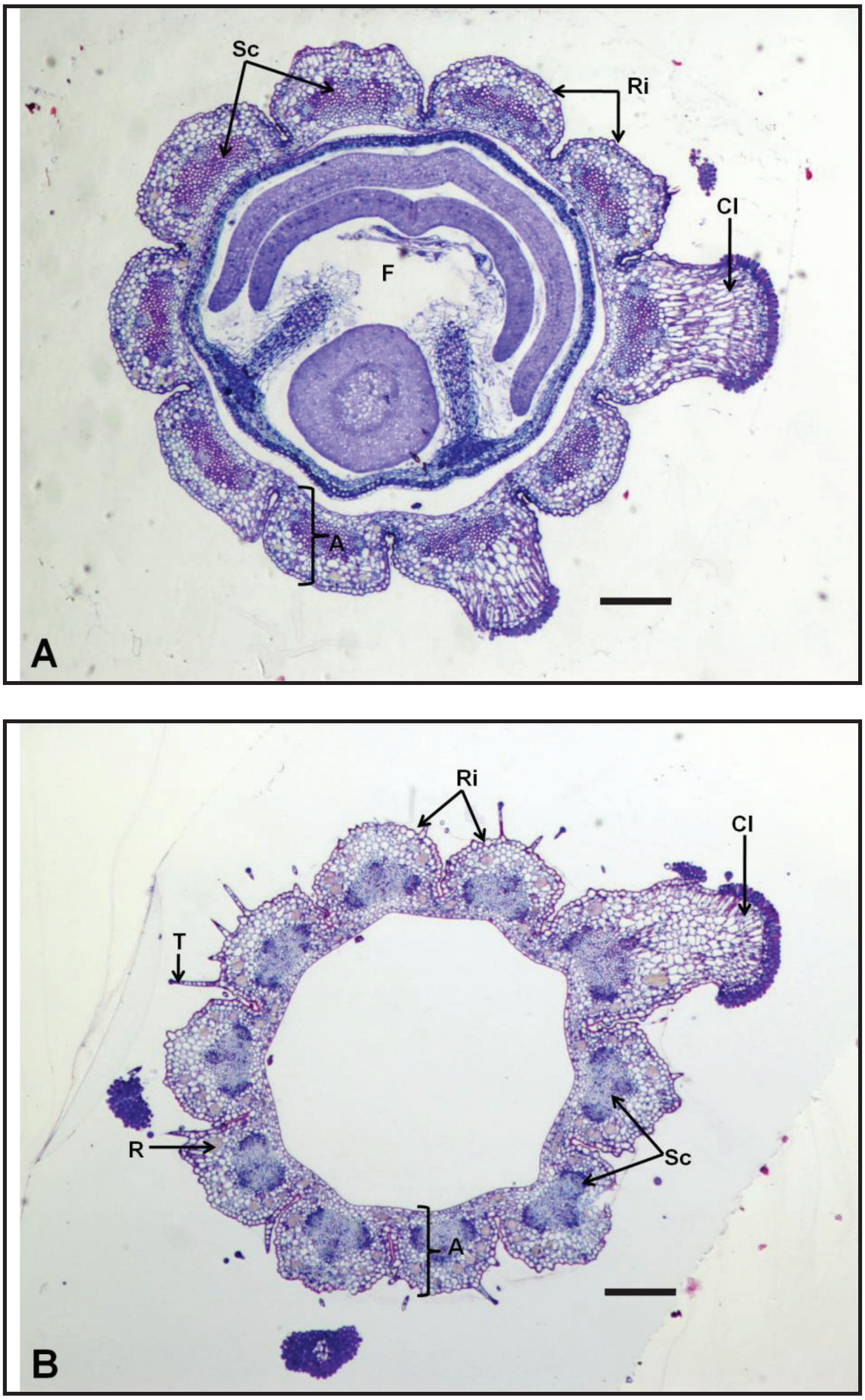


Figure 5.9: Light micrographs of cross sections through the anthocarps of *Commicarpus* species. A: *C. chinensis* subsp. *natalensis* (*Struwig 63*); B: *C. decipiens* (*Struwig 176*) (A, anthocarp; Cl, columnar cells; F, fruit; R, raphide bundle; Ri, rib; Sc, sclerenchyma; T, trichome). Scale bars 250 µm.

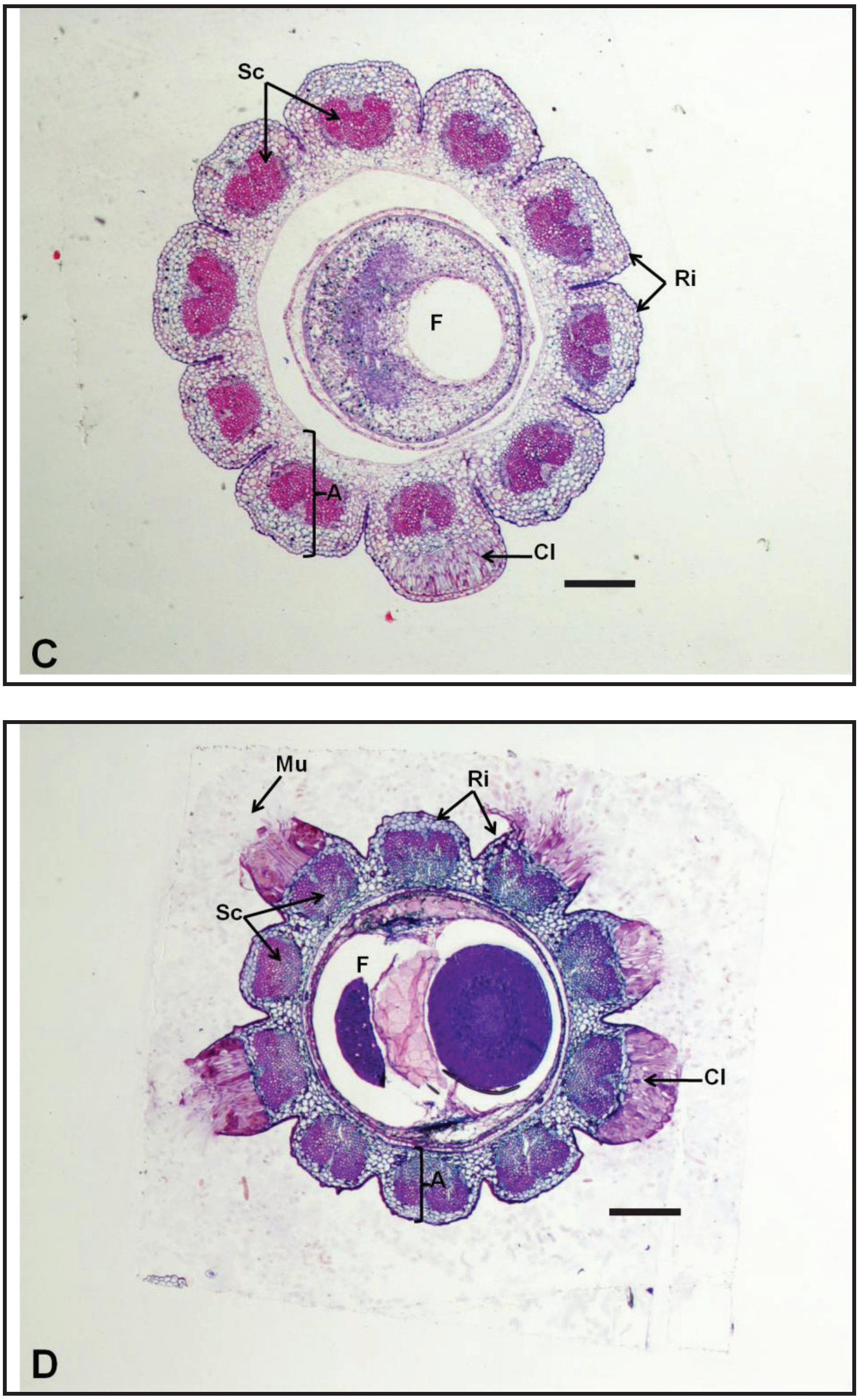


Figure 5.9: Light micrographs of cross sections through the anthocarps of *Commicarpus* species. C: *C. fallacissimus* (*Struwig 46*); D: *C. fruticosus* (*Struwig 164*) (A, anthocarp; Cl, columnar cells; F, fruit; Mu, mucoidal substance; Ri, rib; Sc, sclerenchyma). Scale bars 250 µm.

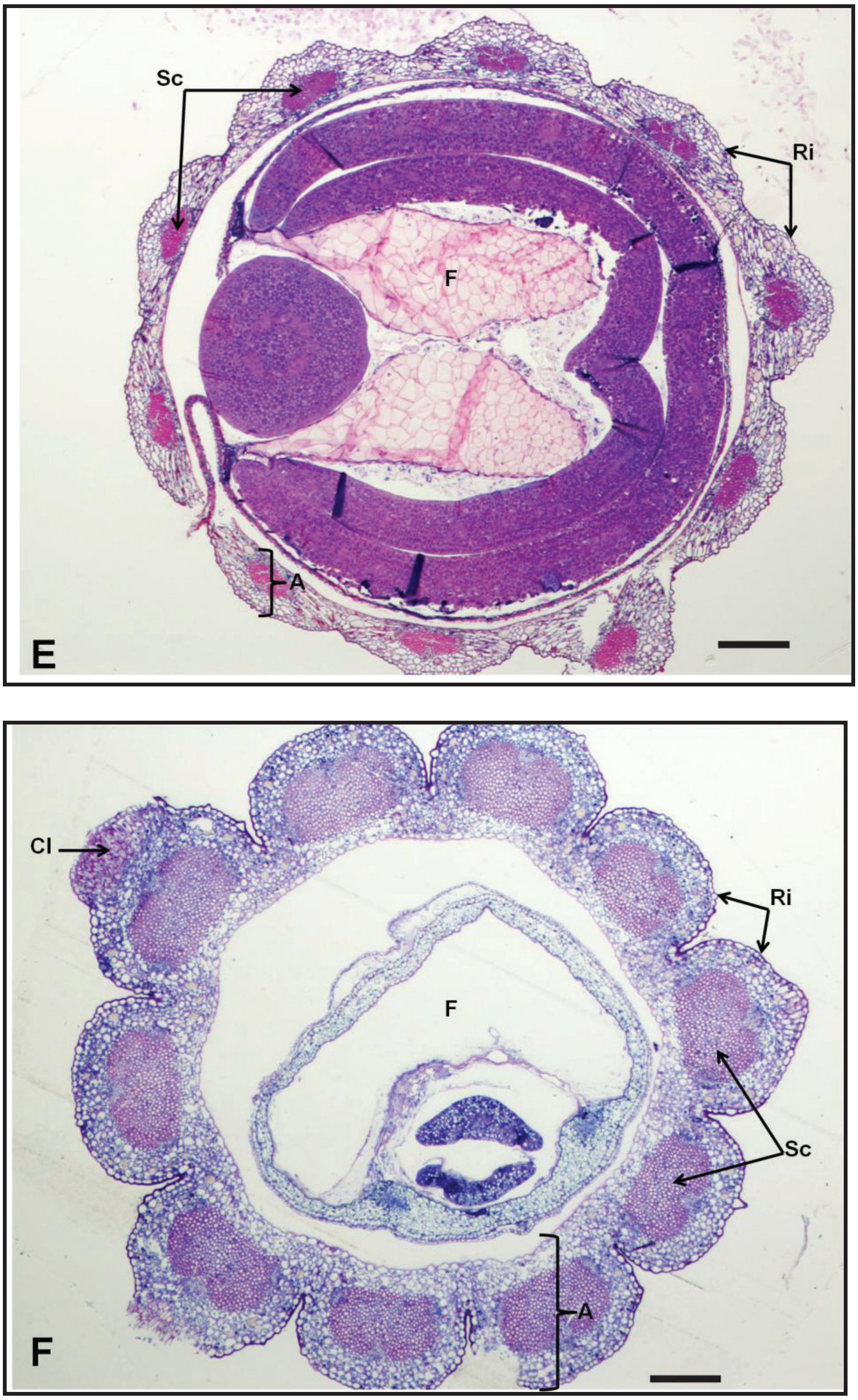


Figure 5.9: Light micrographs of cross sections through the anthocarps of *Commicarpus* species. E: *C. helenae* var. *helenae* (*Struwig 183*); F: *C. pentandrus* (*Kurzweg 7141*) (A, anthocarp; Cl, columnar cells; F, fruit; Ri, rib; Sc, sclerenchyma). Scale bars 250 µm.

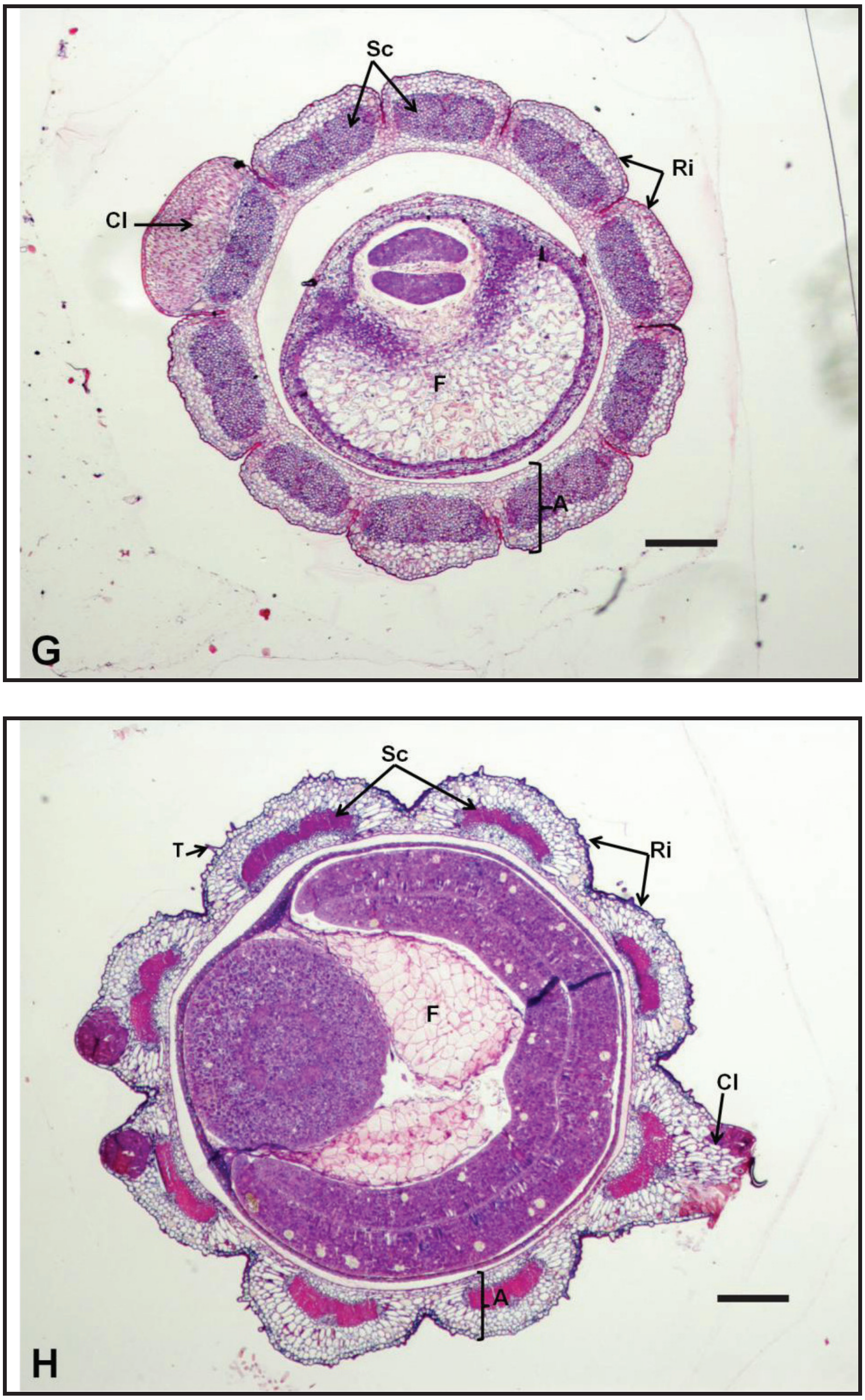


Figure 5.9: Light micrographs of cross sections through the anthocarps of *Commicarpus* species. G: *C. pilosus* (*Struwig 111*); H: *C. plumbagineus* (*Struwig 106*) (A, anthocarp; Cl, columnar cells; F, fruit; Ri, rib; Sc, sclerenchyma; T, trichome). Scale bars 250 µm.

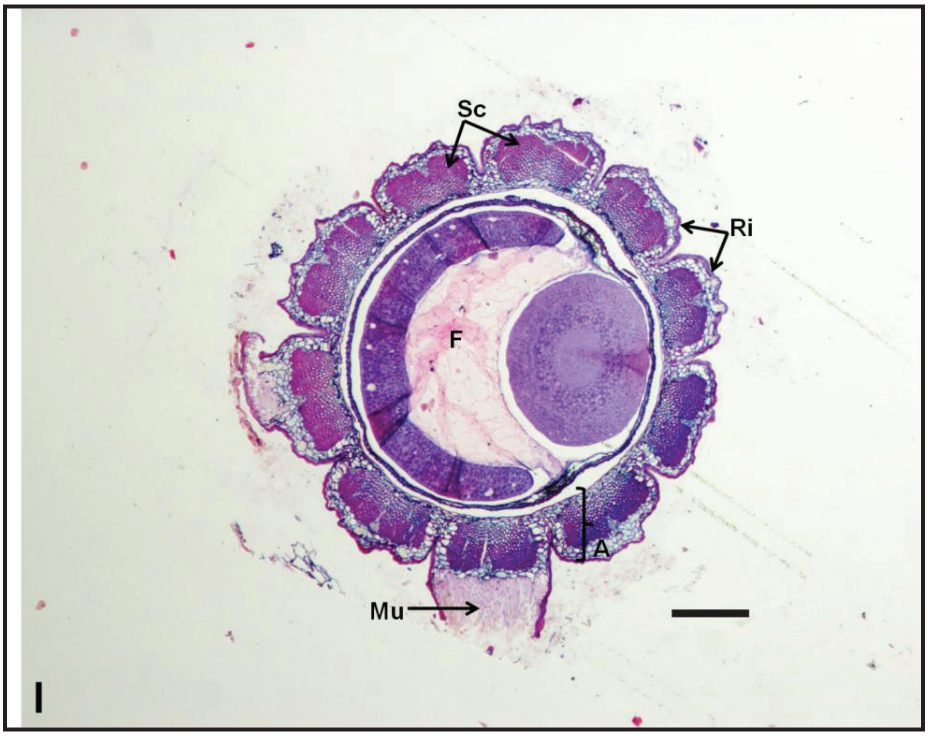


Figure 5.9: Light micrograph of cross sections through the anthocarps of *Commicarpus* species. I: *C. squarrosus* (*Struwig 39*) (A, anthocarp; F, fruit; Mu, mucoidal substance; Ri, rib; Sc, sclerenchyma). Scale bar 250 µm.

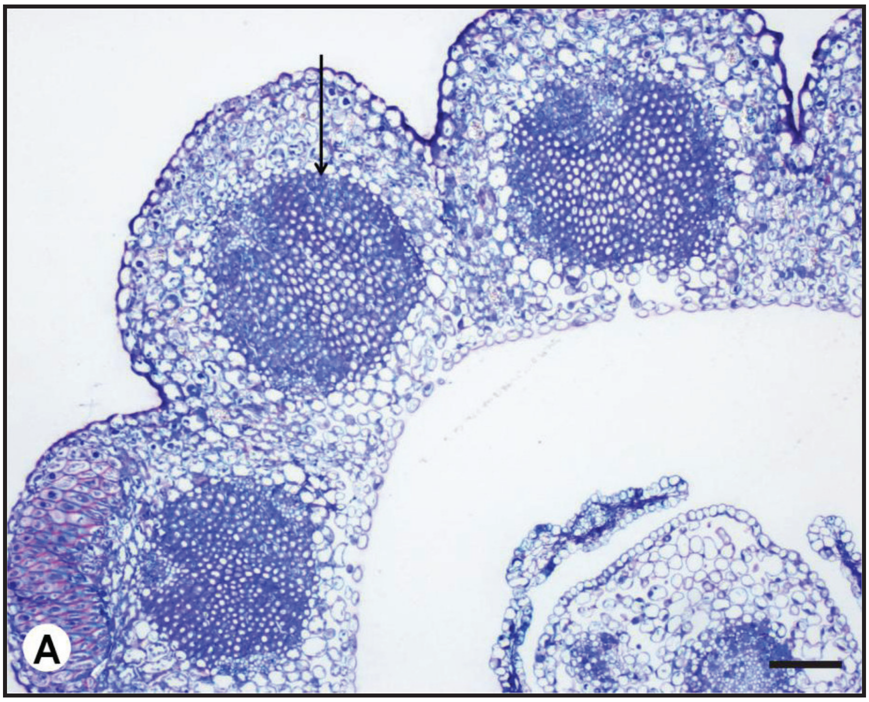


Figure 5.10: Light micrographs of a portion of cross sections through the anthocarp of *Commicarpus* species A: *C. pentandrus* showing the round outline of the sclerenchyma (indicated by the arrow) (*Kurzweg 7141*); B: *C. pilosus* showing the elongated outline of the sclerenchyma (indicated by the arrow) (*Struwig 111*). Scale bars 100 µm.

Figure 5.10: Light micrograph of: C: *C. chinensis* subsp. *natalensis* showing five vascular bundles which occur in each of the ribs near the sclerenchyma bundle, although it may appear as if bundles have fused (indicated by the arrows) (*Struwig 63*). Scale bar 100 µm.