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Botanical Study of *Orobanche minor* Smith (Orobanchaceae)

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ABSTRACT

Orobanche minor Smith is an obligate root parasite weed subsisting on *Trifollium* species. Many of *Orobanche* plants are well known to have different pharmacological actions such as anti-inflammatory, neuroprotective and hepatoprotective activities. Therefore, this work aimed to study the main morphological and anatomical characteristics of the leaf, stem, flower, fruit, bulb and haustorium of *Orobanche minor* contributing to provide more information about characterization and identification of this species growing in the Egyptian Clover fields, as this plant is considered one of the parasitic weeds used in folk medicine while it is still under biological and chemical studies. The main morphological features are the succulent hairy stem with sessile leaves and the fleshy underground bulb bearing haustoria. The plant is characterized microscopically by the epidermal cells with beaded anticlinal walls, anomocytic stomata and glandular hairs, starch granules with centric clefted hilum and ovoid dust-like microscopical seeds with lignified reticulated seed coat.

1. Introduction:

Orobanchaceae is considered as the largest family containing parasitic plants, including 90 genera and 2,060 species. Plants belonging to family Orobanchaceae are annual, biennial, or perennial parasitic herbs without chlorophyll where they derive the majority of their carbon and nitrogen requirements from the host plants' phloem and often classified as obligate holoparasites [1]. Stems of plants belonging to genus *Orobanche* are yellowish- brown fleshy, simple, non- branched or sometimes branched. The flowers are white or yellowish white, often with a purple tinge. In Orobanchaceae the flowering period is short, starting about 1 week following emergence, with seed release beginning about 1 month following emergence [2]. Plants of this family are characterized by the production of numerous dust-sized seeds (extremely small seeds measuring from 200 to 400 µm in size) which are easily dispersed [3]. Mature *O. minor* plant produce between 50,000 and 500,000 seed per generation [1]. *Orobanche* seeds are characterized by a long latency period, where it may remain dormant in the soil for many years until germination is stimulated by the release of chemical signals from a host plant and the germination takes place only in the presence of the

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host plant or of its root secretions. The radical that develops during germination can grow only to a limited distance of a few millimeters. If the germinated radical finds a host root nearby, the radical develops a "haustorium" (specific multicellular organ, from the Latin, haurire, which means "to drink") that penetrates the root of the host and establishes a vascular connection, but if it failed to meet a host root the seedling will die [4]. The plantlets stick in the hairy areas of the host plant rootsthrough the papillary cells of the haustorium apex that generate by repeated divisions, form the main haustorium. The haustoria tissues surround all the central cylinder of the host plant root. The haustoria presents in the sub terminal part a tuberized area (bulb- like thickening) from which lateral roots emerge exogenously. Subsequently, from these roots, secondary haustoria detach and these resemble metamorphosed roots [5].If the parasitic *Orobanche* affixes on an annual plant, it flowers at the end of the first year, but if it parasitizes on a perennial species, it can survive several years [6]. Both hemiparasites and holoparasites have to exhaust water and solutes from the host. Therefore, plant parasites seem to have a higher xylem conductance than their hosts, and the stomata of the parasites remain wider and, sometimes, continuously open (day and night) in comparison to their hosts [7,8]. *Orobanche minor* Smith (small broomrape) (Fig. 1A) is an annual predominant herbaceous parasitic herb belonging to family Orobanchaceae. It is classified as facultative or obligate root parasite devoid of chlorophyll and usually parasitic on the roots of *Trifolium* species. It belongs to phloem-feedingparasites that abstract their nutrition predominantly from the phloem of their host plant [1]. Although phloem-feeding parasites typically retain a xylem connection (Fig.1B), they derive the majority of their carbon and nitrogen requirements from phloem of the host plants phloem [3]. The habitat of *O. minor* is the winterrainfall area of the Mediterranean region. It is associated with the cropping of *Trifolium alexandrinum* (Egyptian Clover) Family Fabaceae (Fig. 1C) and is not generally found in wild vegetation [1]. Parasitic Orobanchaeae plantshave been used in folk medicines in various regions around the world and the experimentally studied species reported the presence of phytochemical constituents such as phenolic acids, phenylethanoid glycosides and flavonoids exhibiting a wide range of medicinal activities including anti-inflammatory, antioxidant, hepatoprotective and neuroprotective properties [9,10,11]. The present study aims to provide the detailed macroscopical and microscopical features of *Orobanche minor* to enable further identification of this plant in either single or herbal mixture.

2. Materials and Methods

2.1. Plant material

The plant material used in this work consists of the whole plant of *Orobanche minor* Smith obtained from Ezbet Almokabla, Benha, Qualyobia, Egypt. The plant material was collected in February 2021 during the flowering stage. The plant was authenticated by Prof. Dr. Ahmed Abd Alrazik Mubarak (Professor of Taxonomy, Botany Department, Faculty of Science, Benha University), and a voucher specimen was kept in the Pharmacognosy Department Herbarium, Faculty of Pharmacy, Zagazig University, under the number of (OR-13). The plant material used for the botanical study was taken from the fresh samples and preserved in alcohol (70%)-glycerin-water (1:1:1, *v*/*v*/*v*).

2.2. Reagents:

Reagents used in botanical study were potassium hydroxide solution (5%), phloroglucinol (3% alcoholic solution)/ Conc. HCl, saffranin and 60% sulphuric acid.

3. RESULTS AND DISCUSSION

3.1. Macro-morphology

The leaves of the plant are odourless, simple, scaly and sessile. They are small triangular flaps, alternate to the stem, measuring from 0.5 to 1.5 cm in length and 2-5 mm in width, with a yellowish-brown color (Fig. 2A). The leaves have acuminate apices, wide bases and parallel venation. The upper surface of the leaf is smooth while the lower is hairy.

The stem of the plant is simple, erect, succulent and cylindrical in shape with spike inflorescence. It is

characterized by hairy pale yellow with brownish to straw-colored surface. The stem measures 6-20 cm in length and 0.3-1.5 cm in diameter with alternate scaly sessile leaves (Fig.1A). Stem has a faint odour and a mucilaginous taste. Inflorescence of plant is a spike consisting of many (15-30) florets (Fig. 2B).

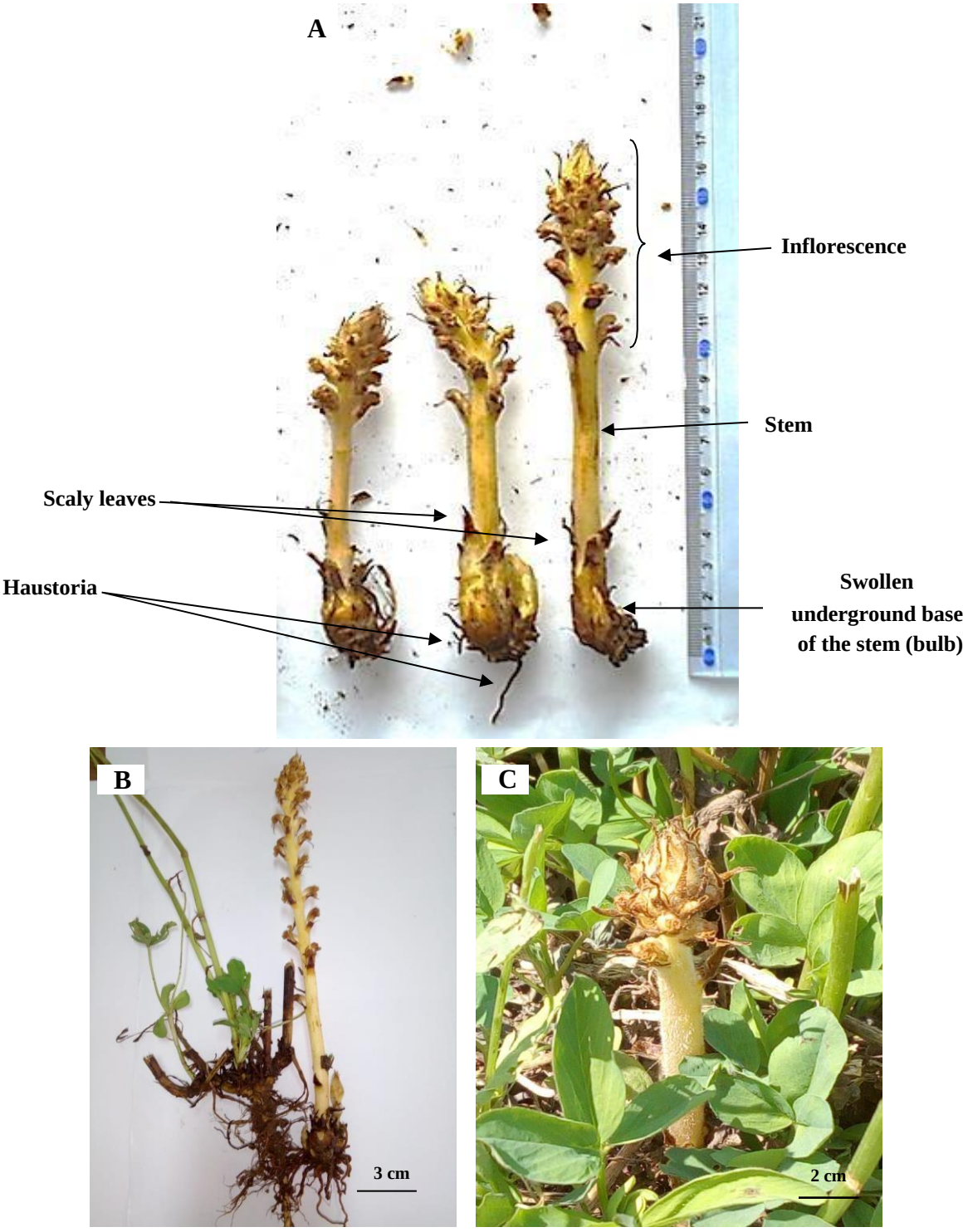

Figure 1: Photographs of *Orobanche minor* Smith. A: Whole plants, B: Host–parasite relationship between *Trifolium alexandrinum* (the host plant) and *Orobanche minor* Smith (the parasitic weed), C: Areal parts of Orobanche minor Smith in fields of the Egyptian Clover.

Florets are yellowish brown in color measuring 1-1.2 cm in length and 3-4 mm in width. Florets are sessile, hypogenous, actinomorphic, hermaphrodite. They are solitary in the axile in a terminal spike, crowded with a yellowish-brown bract.

The bract of *O. minor* is triangular in shape similar to the leaf and appearing simple lanceaolate with a wide base and acuminate apex, parallel venation, smooth upper surface and hairy lower surface. It measures 0.7-1 cm in length and 2-3 mm in width (Fig. 2C).

O. minor calyx is divided into two lateral halves. Calyx is composed of 4 sepals brownish yellow in color with outer hairy surface. Each 2 sepals are fused from the base and measuring together 5-7 mm in length and 1 mm in width. Corolla is very delicate and yellowish brown in color. It is composed of 4 fused petals forming a tube that measures 0.8-1 cm in length and 6-8 mm in diameter (Fig. 2C). The androecium is composed of 4 epipetalous stamens in one whorl attached to the corolla with off-white cylindrical filaments and very small bilobular didynamous yellow anthers.

The gynoecium is bicarpellary, unilocular ovary, with white to faint yellow color bearing a single style and bi-fid brown stigma. The style is measuring 0.5 cm in length while the stigma length is 1-2 mm. The ovary is measuring 6-7 mm in length and 2-3 mm in diameter containing numerous minute yellowish white ovules which changes into dark brown color seeds in the ripe fruit (Fig. 2D and E).

The whole life cycle of *O. minor* weed is about one month. At the end of the month, the ovary slightly shrinks and develops into a loculicidal smooth ovoid fruit capsule that measures 5-6 mm in length and 2-3 mm in diameter with persistent style which opens by 2 longitudinal valves releasing numerous dark brown dust like seeds which are profusely produced and easily dispersed (Fig. 2D and E). The floral diagram is shown in figure 2F, and the floral formula is represented by:

$$\oplus, \varphi^{\sigma}, K_4, \overset{\frown}{C_{(4)}, A_4}, \underline{G}_{(2)}$$

The basal underground part of *O. minor* (Fig.2G) is composed of a fleshy swollen, ball or egg like swelling of the stem "the bulb" covered with firm non smooth dark brown scales. The bulb is measuring from 1-3 cm in length and 1-2 cm in diameter. The haustoria are short, tapering, fleshy tubes developed from the bulb. From each bulb, 10-25 brown haustoria are emerged. Their rule is to drain water and nutrients from the host plant, where the length of each haustorium measures 1-2 cm and its diameter measures from 0.5-1 mm.

3.2. Micro-morphology

3.2.1. The leaf

A transverse section of the leaf appears linear in outline showing upper and lower epidermises covered with cuticle and enclosing between them parenchymatous ground tissue. The parenchyma of the ground tissue consists of 12-15 rows (Fig. 3A). Epidermis consists of polygonal elongated cells measuring 70-100 µm in length and 30-40 µm in width with straight beaded anticlinal walls covered with smooth cuticle (Fig. 3B). Lower epidermis is covered with glandular trichomes measuring 100-300 µm in length with multicellular uniseriate stalk and short terminal cell and multicellular globular head (Fig. 3C). The lower part of the ground tissue is interrupted by small vascular bundles showing spiral lignified xylem vessels measuring 15-17 µm in diameter.

3.2.2. The stem

A transverse section of the stem appears circular in outline (Fig. 4A). It shows an epidermis inclosing a wide cortex which consists of about 12-15 rows of parenchymatous cells. The endodermis is indistinct. The pericycle consists of parenchyma cells. The vascular tissue consists of a ring of 30-35 vascular bundles with outer phloem and an inner radiating xylem; both are traversed by medullary rays. The pith is formed of parenchyma cells containing starch granules and showing large air cavity (Fig.4B, C & D).

3.2.2.1. The epidermis

The epidermis of the stem is formed of one row of polygonal axially elongated cells of 25-35 µm in length and 10-15 µm in width with straight beaded anticlinal walls covered with a smooth cuticle. Epidermal cells show anomocytic stomata (Fig.5A) and numerous glandular trichomes with multicellular

stalks from 3 to 5 cells of unequal length and the head is globular multicellular (3-8 cells). Hairs exhibit different lengths ranging from 200 to 600 µm (Fig. 4B and Fig. 5B).

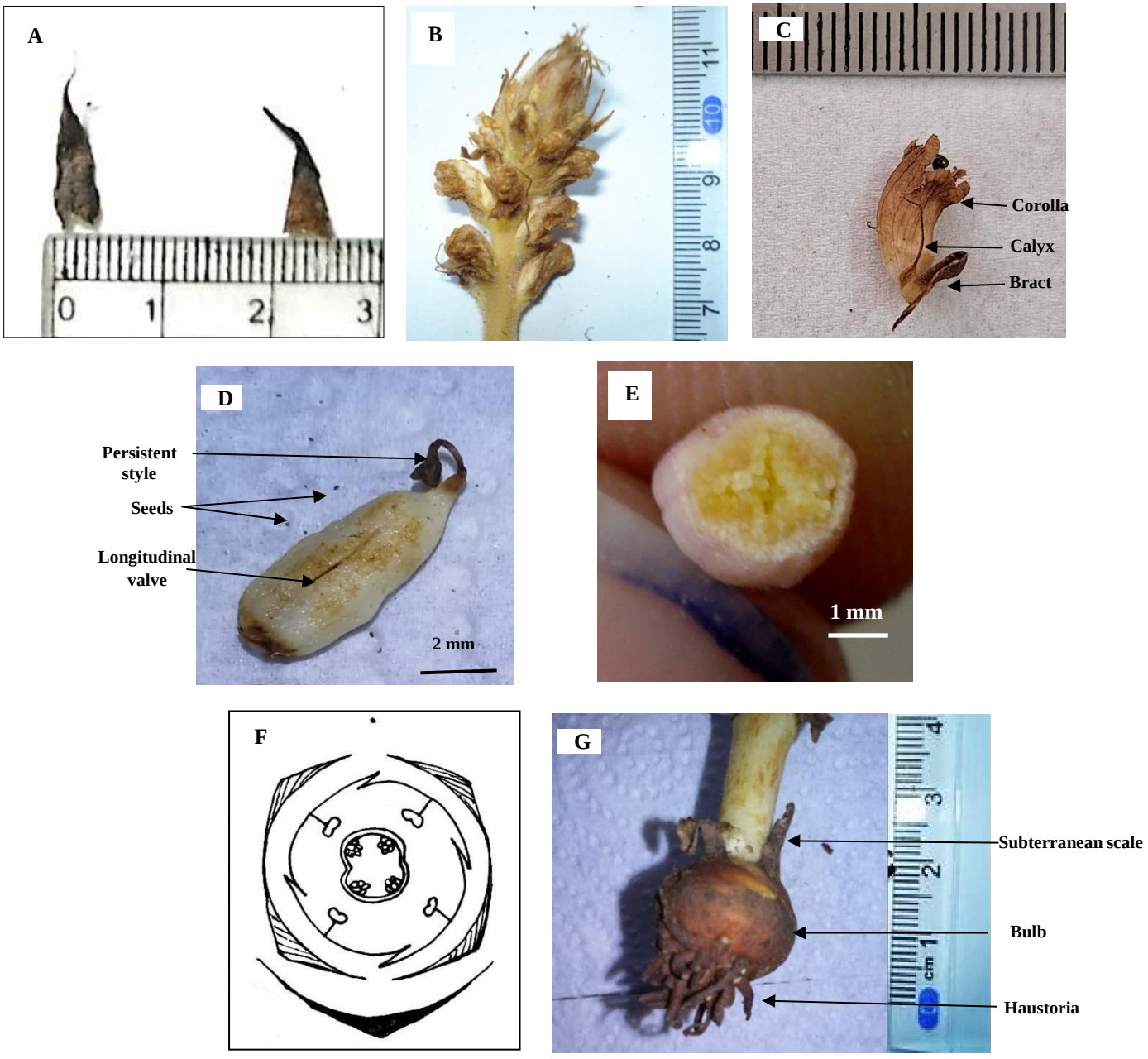

Figure 2: Macro-morphology of *Orobanche minor* Smith. A: Photograph of leaves, B: The whole inflorescence, C: The floret, D: fruit, E: Transverse cut in unripe capsule, F: The floral diagram, G: The underground parts**.**

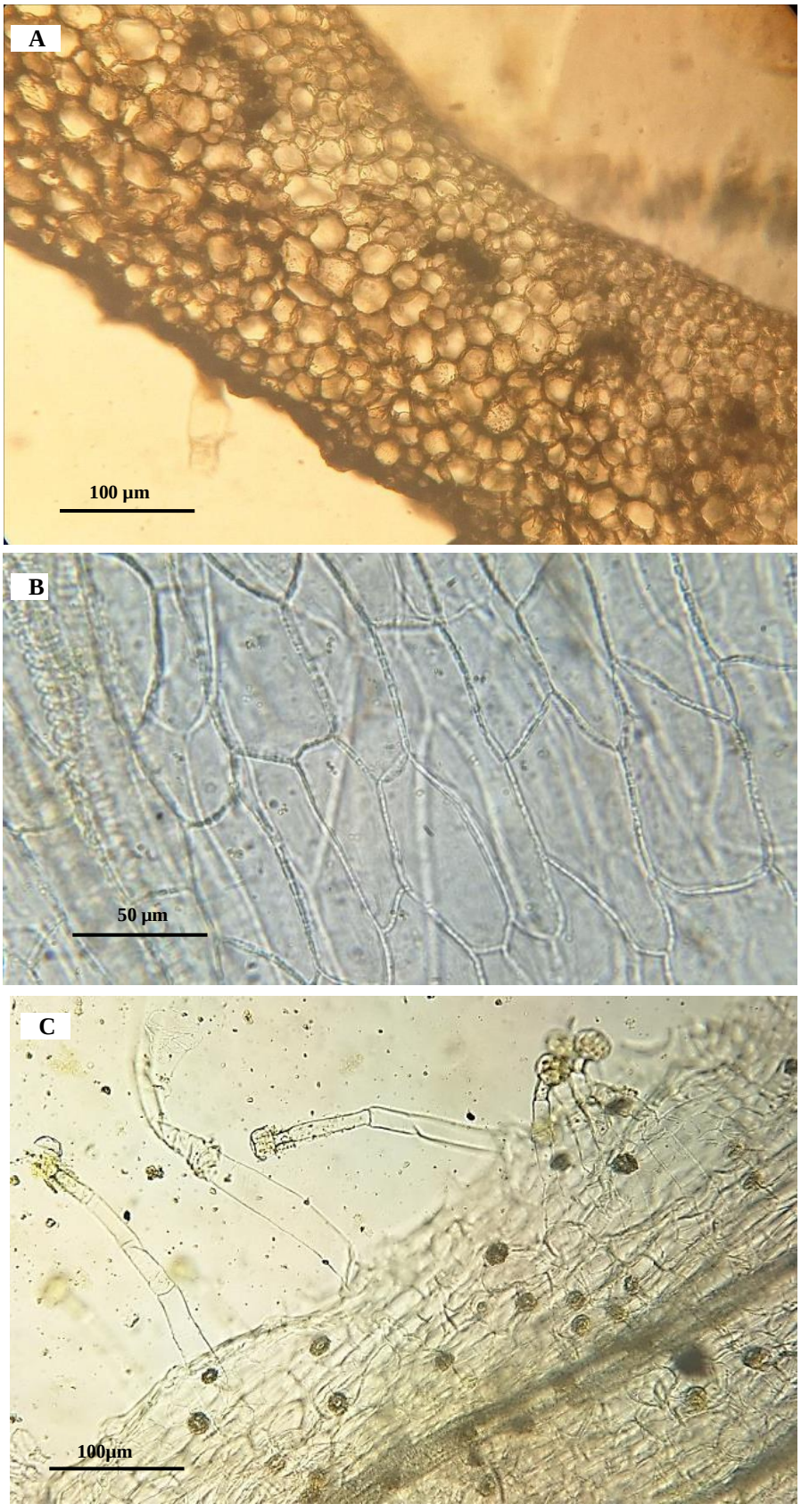

Figure 3: A: Transverse section in the leaf, B: Upper epidermis of leaf, C: Glandular hairs in lower epidermis.

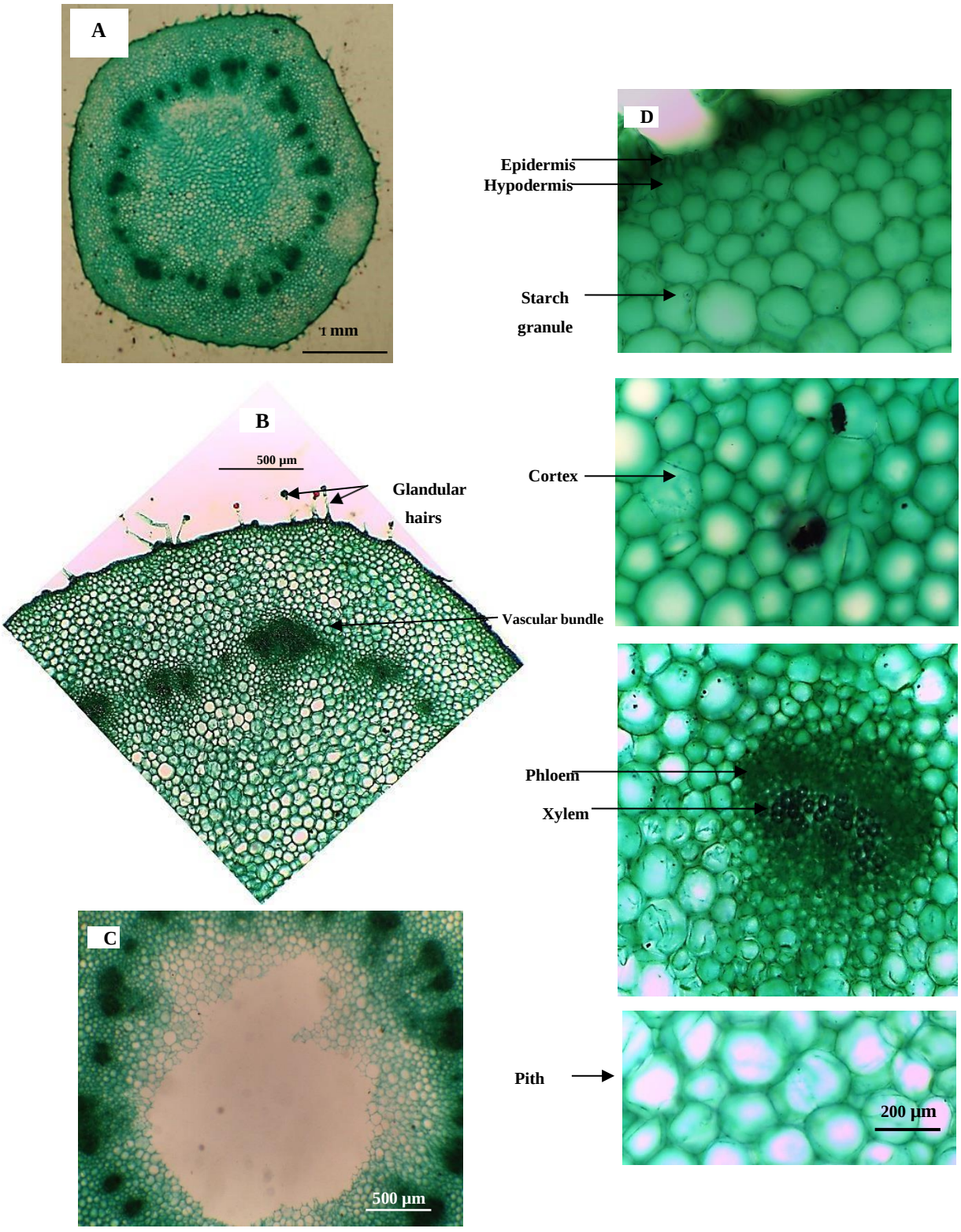

Figure 4: A: The whole sector of the stem, B: Transverse section, C: The old stem pith showing large air cavity, D: Detailed T.S.

3.2.2.2. The cortex

The cortex consists of about 12-15 rows of rounded to oval parenchymatous cells measuring 70-130 µm in diameter (Fig. 4B and D), having thin cellulosic walls with narrow intercellular spaces, containing starch granules at the lower zone of cortex. Endodermis is indistinguishable.

3.2.2.3. The pericycle

The pericycle is narrow indistinguishable. It is composed of few rows of parenchyma cells containing starch granules.

3.2.2.4. The vascular System

Vascular system consists of a ring of (30-35) vascular bundles of different sizes which are composed of outer phloem, cambium and inner radiating lignified xylem; both are traversed by medullary rays (Fig. 4B and D).

a. The phloem: The phloem is narrow and composed of thin-walled soft cellulosic elements which are sieve tubes, companion cells and phloem parenchyma (Fig. 4D)

b. The xylem: The xylem consists of a narrow zone of lignified thick-walled radially arranged elements traversed by rounded slightly elongated parenchymatous medullary rays with thin cellulosic walls (Fig. 4D). Elements of xylem include xylem vessels, tracheids and wood fibers. The xylem vessels measuring 20-30 µm in diameter are arranged in radial rows and show mainly spiral lignifications (Fig. 5C). The tracheids are slightly elongated with lignified pitted walls and rounded to blunt ends measuring 200-300 µm in length and 20-30 µm in width (Fig. 5D). The wood fibers measure 400-600 µm in length and 10-15 µm in width with slightly lignified walls. They are characterized by narrow lumens and acuminate ends (Fig.5E).

3.2.2.5. The medullary rays

The medullary rays are tetra- to hexa-seriate consisting of rounded parenchyma cells measures 30-60 µm in diameter with cellulosic walls in xylary and phloem regions (Fig. 4B).

3.2.2.6. The pith:

The pith is formed of a comparatively wide central zone of rounded to oval thin-walled parenchymatous cells with diameter from 70-130 µm which constitutes about 3/4 of the whole section (Fig. 4A). The cells neighboring to the primary xylem are much smaller and closely packed than that adjacent to the pre-medullary zone (Fig. 4B). In older stems the central part became partially hollow, resulting wide intercellular spaces with irregular shape that came together forming a central air cavity (Fig. 4C). The inner part of the pith is formed of thin-walled parenchyma cells with intercellular spaces (Fig. 4D and 5F).

3.2.3. The flower and the fruit

Isolated elements of the flower contain fragment of the epidermis of bract showing elongated polygonal cells with straight anticlinal walls and smooth cuticle measuring 20-30 µm in length and 7-10 µm in width bearing glandular trichomes (Fig. 6A). Epidermal cells of sepals show wavy beaded anticlinal walls measuring 30-40 µm in length and 10-15 µm in width bearing glandular trichomes (Fig 6 B and C). Epidermis of petals with cells appearing polygonal, axially elongated cells with wavy anticlinal walls covered with a smooth cuticle with glandular trichomes (Fig. 6D and E). Xylem vessels showing spiral lignifications (Fig. 6F).

Moreover, isolated elements of andorecium show epidermal cells of filament (Fig. 7A) and smooth spherical pollen grains with a diameter measuring 10-13 µm (Fig. 7B). The isolated elements of the gynoceium show fragments of the epidermis of ovary and style (Fig. 7C). In surface view, the epidermal cells of the stigma are polygonal, papillosed and covered with smooth cuticle (Fig. 7D). Stomata and trichomes are absent.

A transverse section in the fruit capsule is almost circular in outline (Fig. 8A). Pericarp is formed of two united carpels with one locule and parietal placentation. Trichomes are not observed in the surface of the fruit (Fig. 8A). The surface of fruit is smooth showing outer epicarp and inner endocarp, both consist of single layer of rectangular shaped cells measuring 60-70 µm in length and 30-40 µm in width enclosing from 4-6 layers of parenchymatous mesocarp which consists of parenchyma cells measuring 70-90 µm in diameter and transversed by 25-30 vascular strands (Fig. 8B and C).

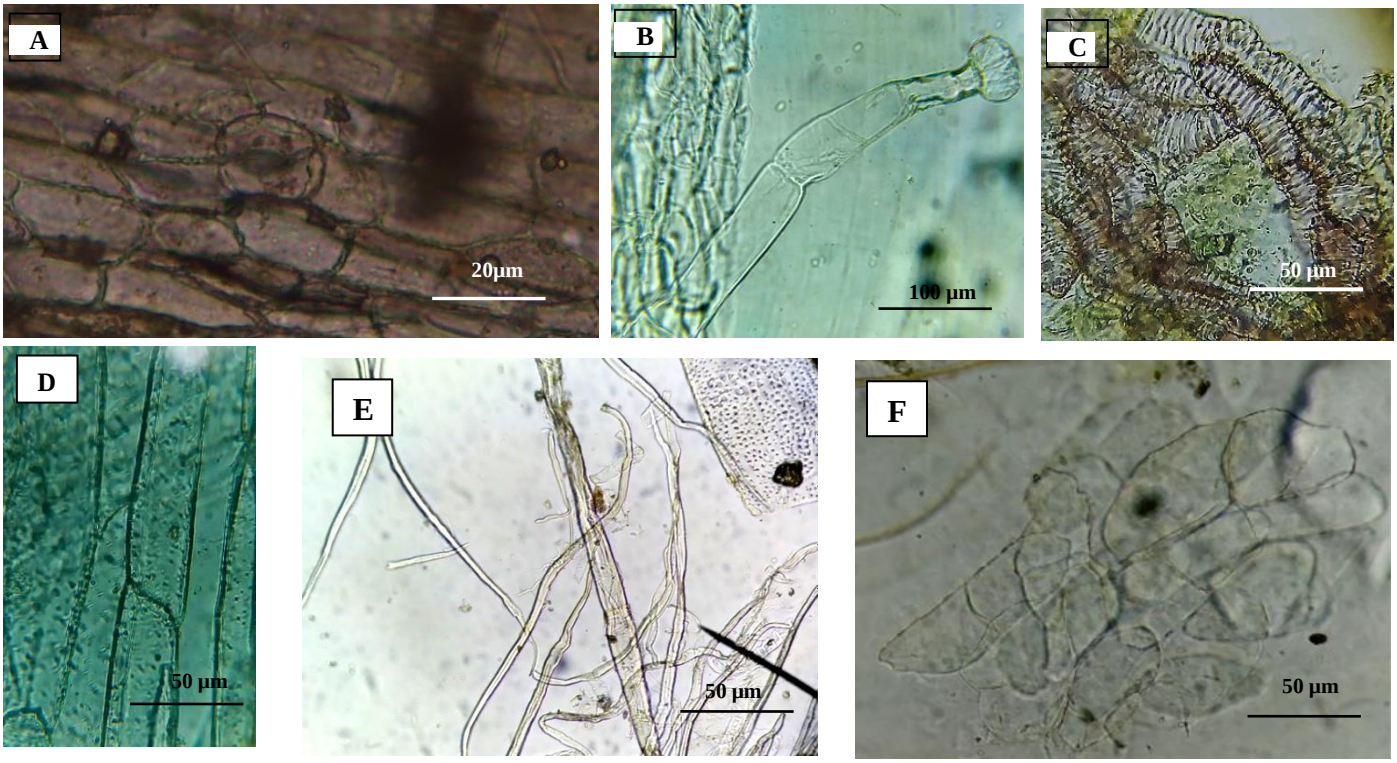

Figure 5: Isolated elements of the stem. A: Fragment of epidermis containing anomocytic stomata, B: Glandular hair, C: Xylem vessels, D: Tracheids, E: Wood fibers, F: Fragments of parenchyma cells of pith.

The seeds are numerous oblongoid tangentially elongated oval shaped of very small size 300-400 µm in length and 200-300 µm in width (Fig. 8D), the seeds have terminal funicular attachment. The seed coat is dark brown in color and composed of reticulated lignified polygonal cells (Fig.8E).

Epidermal seed coat cells are more or less isodiametric to tangentially elongated, anticlinal walls of medium depth and evenly thickened, with a narrow trough that is noticeable all around the edge of the cells or just at the vertices. Inner periclinal walls of epidermal seed coat cells have perforations of smaller diameter than the thickness of the walls that separate them, sometimes with a small central mammilla [12].

3.2.4. The bulb

The underground base of the stem is bulb-like structure. Although *O. minor* is belonging to dicotyleadons plants, but the structure of its bulb is extensively altered, resembling the one of the bulbs of several monocotyledonous [6]. A transverse section of the stem base (bulb) is more or less circular in outline (Fig. 9A) and show an outer metaderm followed by a parenchymatous tissue with scattered vascular bundles. Each protostele vascular bundle consists of central xylem surrounded by cellulosic phloem and a band of lignified parenchyma outside (Fig. 9B).

Metaderm: Bulb is surrounded from outside by irregularly arranged supersized cells derived from the outer raw of the cortex called metaderm. The metaderm (Fig. 9C) consists of few rows of radially arranged polygonal to rectangular cells with supersized walls and brown contents. Their diameter ranges from 50-100 µm.

Ground tissue: The T.S. is not differentiated into cortex and pith. Ground tissue is consisting of cellulosic parenchyma tangentially elongated cells with slightly thickened cellulosic walls. Cortical parenchyma is rich in starch grains. The cortical cells are larger than those of the aerial part of the stem. They measure 80 to 150 µm in diameter (Fig. 9C). The central part is composed of large rounded cells with thin cellulosic walls measures 80-100 µm in diameter filled with rounded starch granules measuring 8-15 µm in diameter with centric clefted hilum of size 3-6 µm.

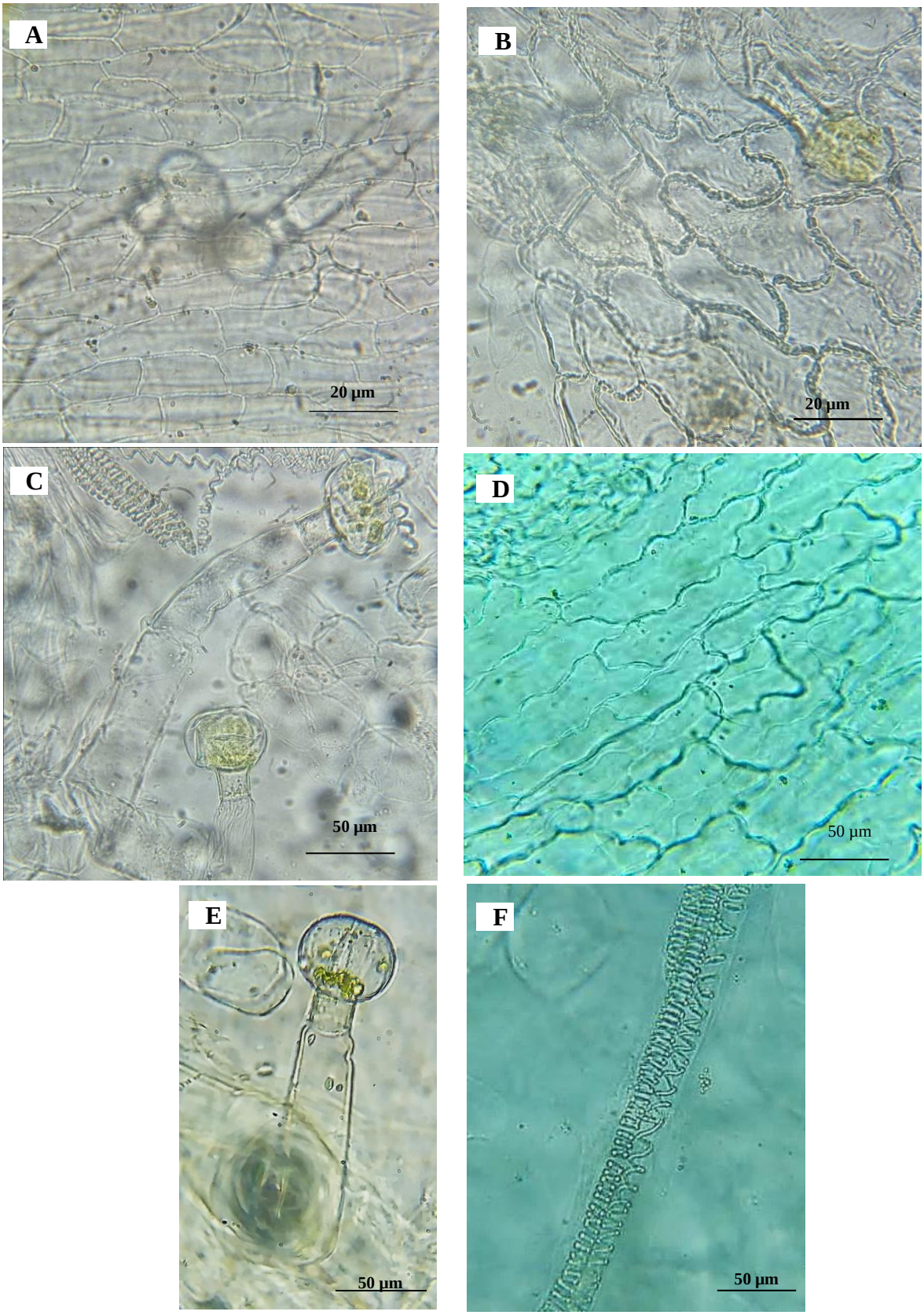

Figure 6: Isolated elements of the flower (bract, calyx and corolla). A: Epidermis of bract, B: Epidermis of sepal, C: Glandular hair of sepal, D: Epidermis of petal, E: Glandular hair of petal, F: Xylem vessels in corolla

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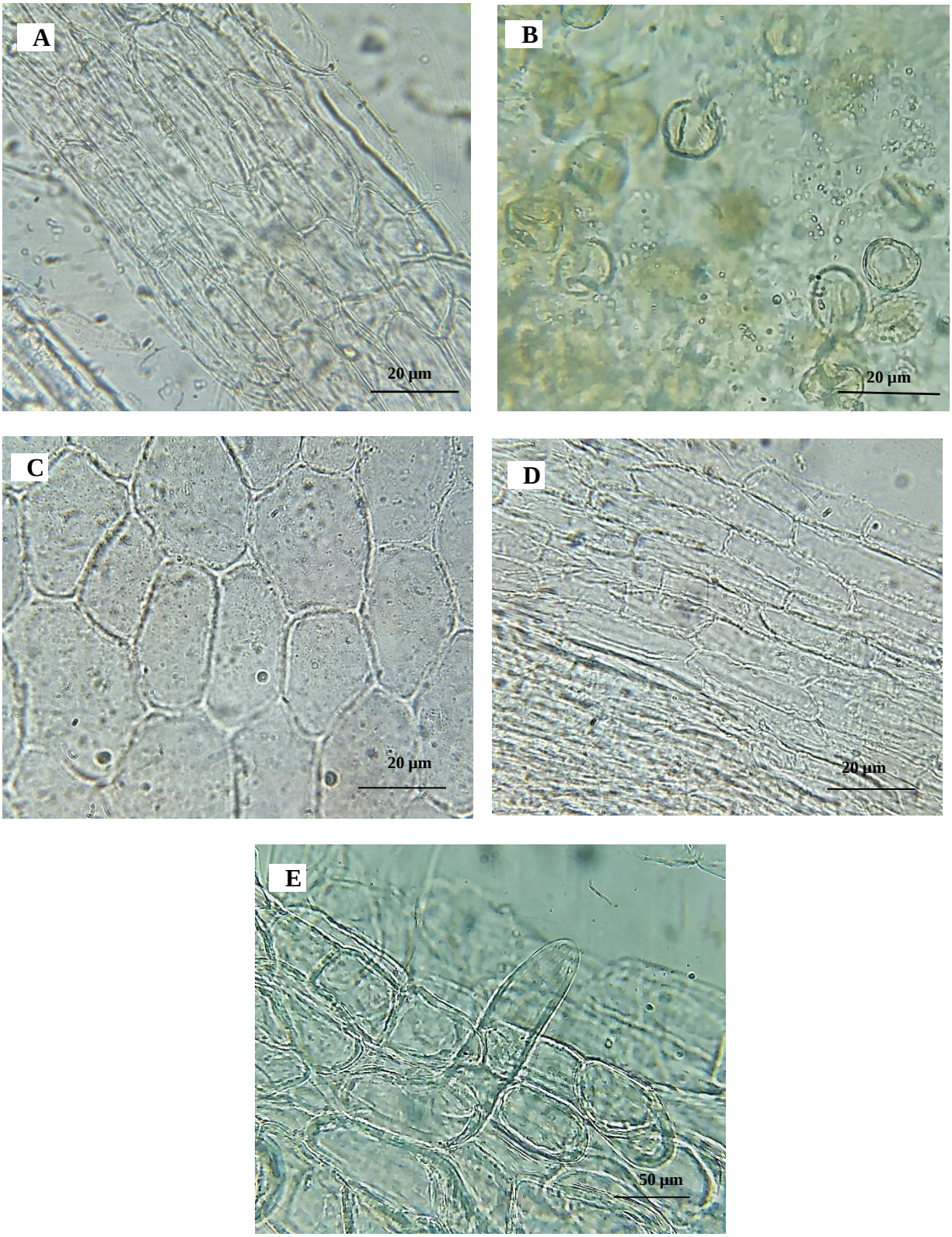

Figure 7: Isolated elements of flower (androceium and gynoceium). A: epidermis of anther, B: Pollen grains, C: Epidermis of ovary, D: Epidermis if style, E: Papillosed stigma.

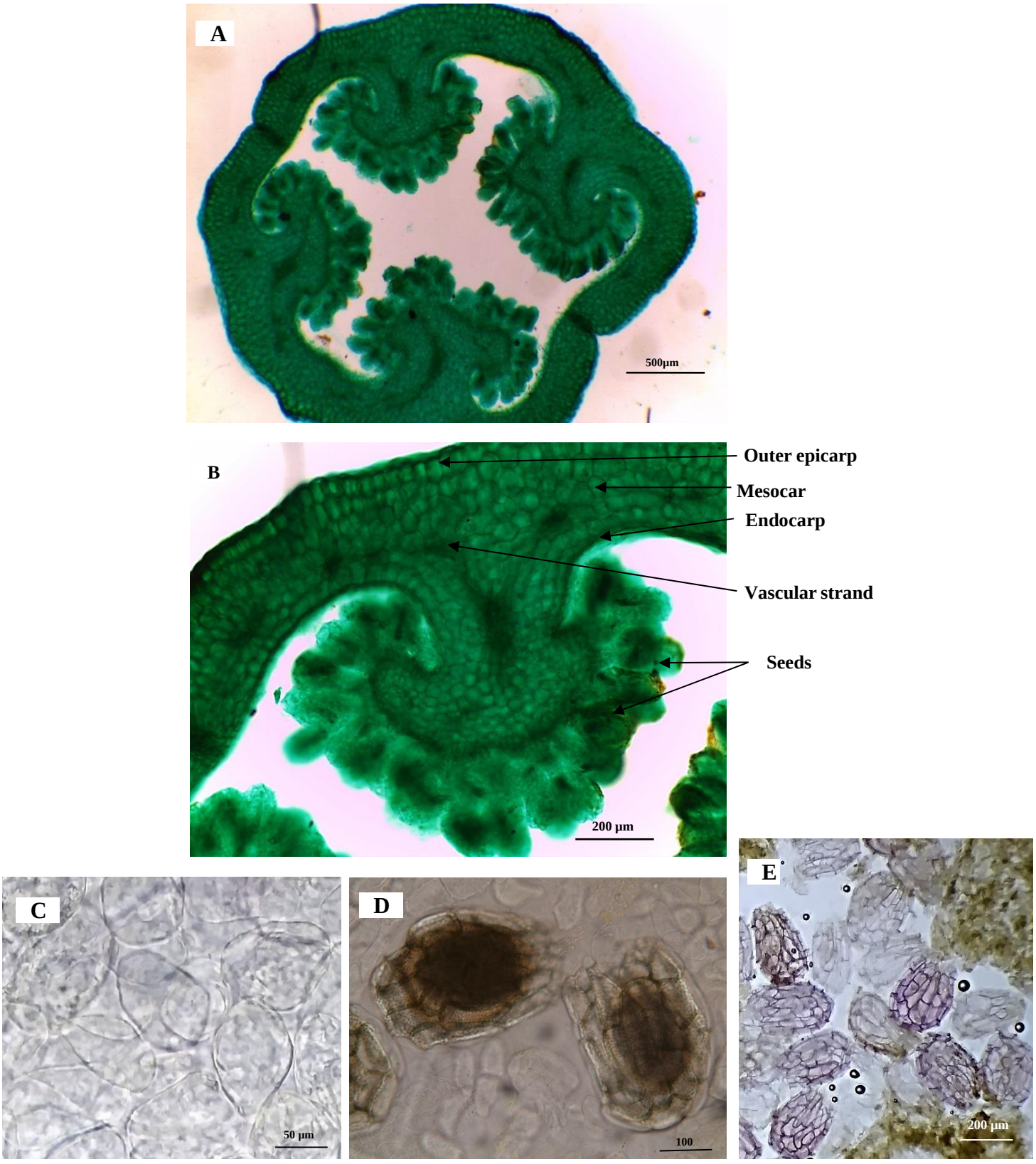

Figure 8: T.S. and Isolated elements of fruit. A: Whole sector, B: Detailed T.S., C: parenchyma of mesocarp, D: Seeds, E: Seeds stained with phloroglucinol and HCl.

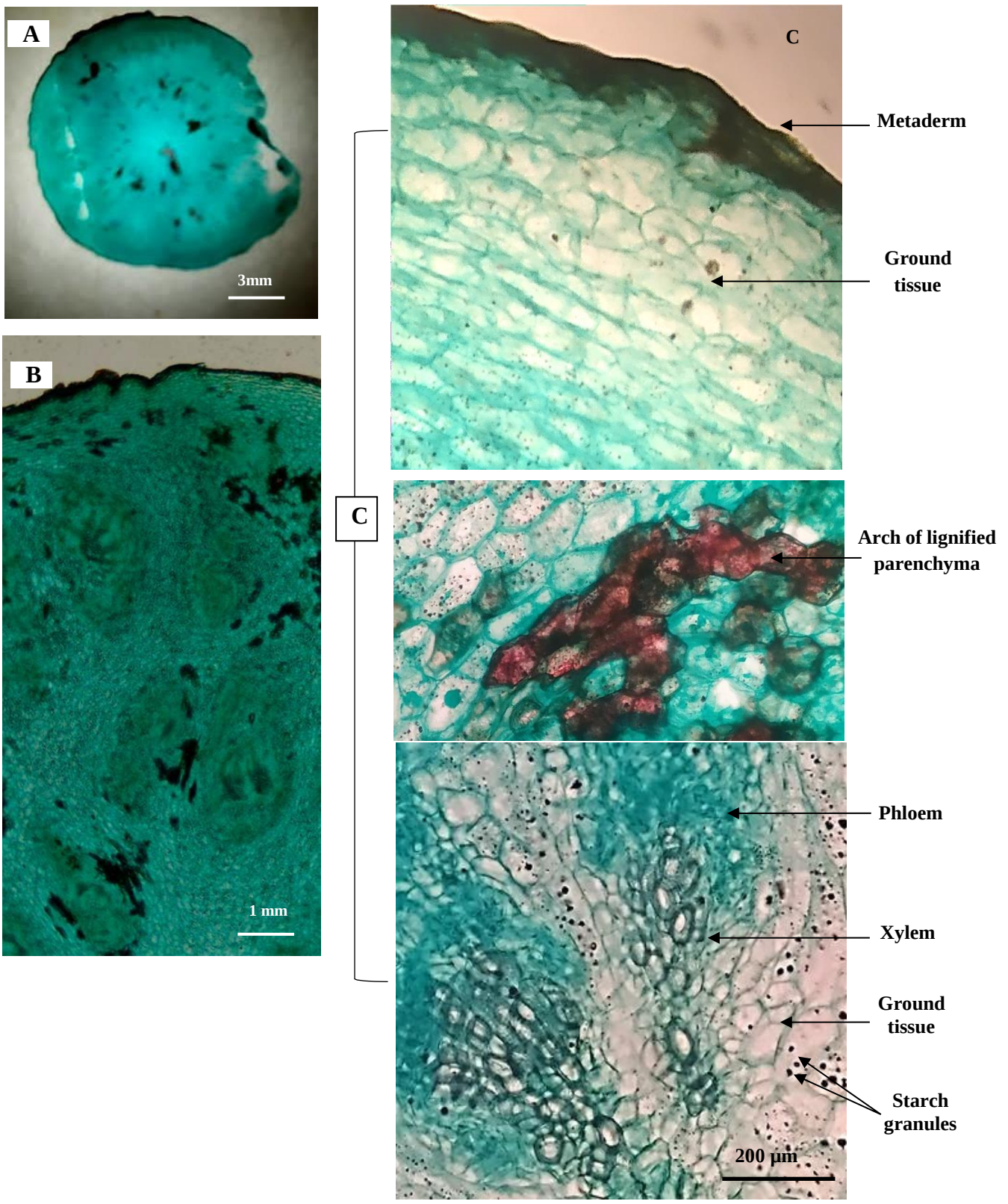

Figure 9: A: Diagrammatic whole sector of *O. minor* bulb, B: Part of the T.S. of bulb, C: Detailed T.S.

Vascular system: Parenchyma of the central cylinder shows numerous vascular bundles irregularly spread in the sector and have different positions, so that on cross section some of them appear longitudinally or obliquely sectioned and that is why the wood seems surrounded by phloem tissue. The phloem disorganizes, resulting that the xylem with vessels and cellulose parenchyma cells persist in the center, the phloem is rich in starch grains (Fig. 9C). In every woody bundle, among vessels, there can also be observed few elements of cellulosic woody parenchyma. Beside the typical xylem vessels, there are big parenchymatous cells with lignified walls forming arches at the internal sides of the few vessels' groups. All the bundles are disposed in a circle elongated radially within them being few xylem vessels which continue with a radial band of lignified parenchyma cells. Vascular bundles show the xylem almost completely lignified; only from place to place there are remain cells of cellulosic woody parenchyma. Unlike the aerial stem at this level all the parenchyma between the bundles remains of cellulosic type [6].

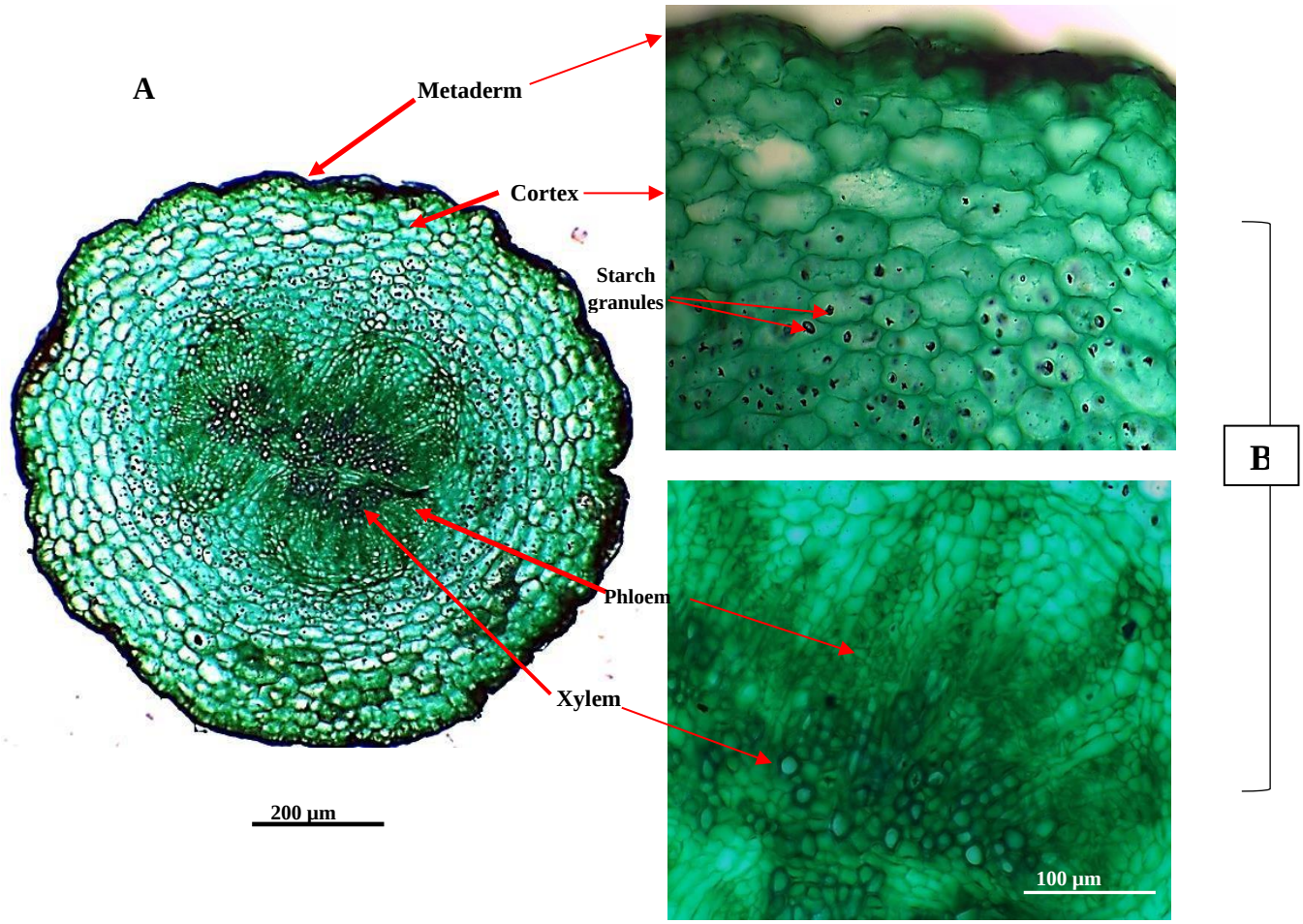

Figure 10: T.S. in haustorium A: Whole sector, B: Detailed T.S.

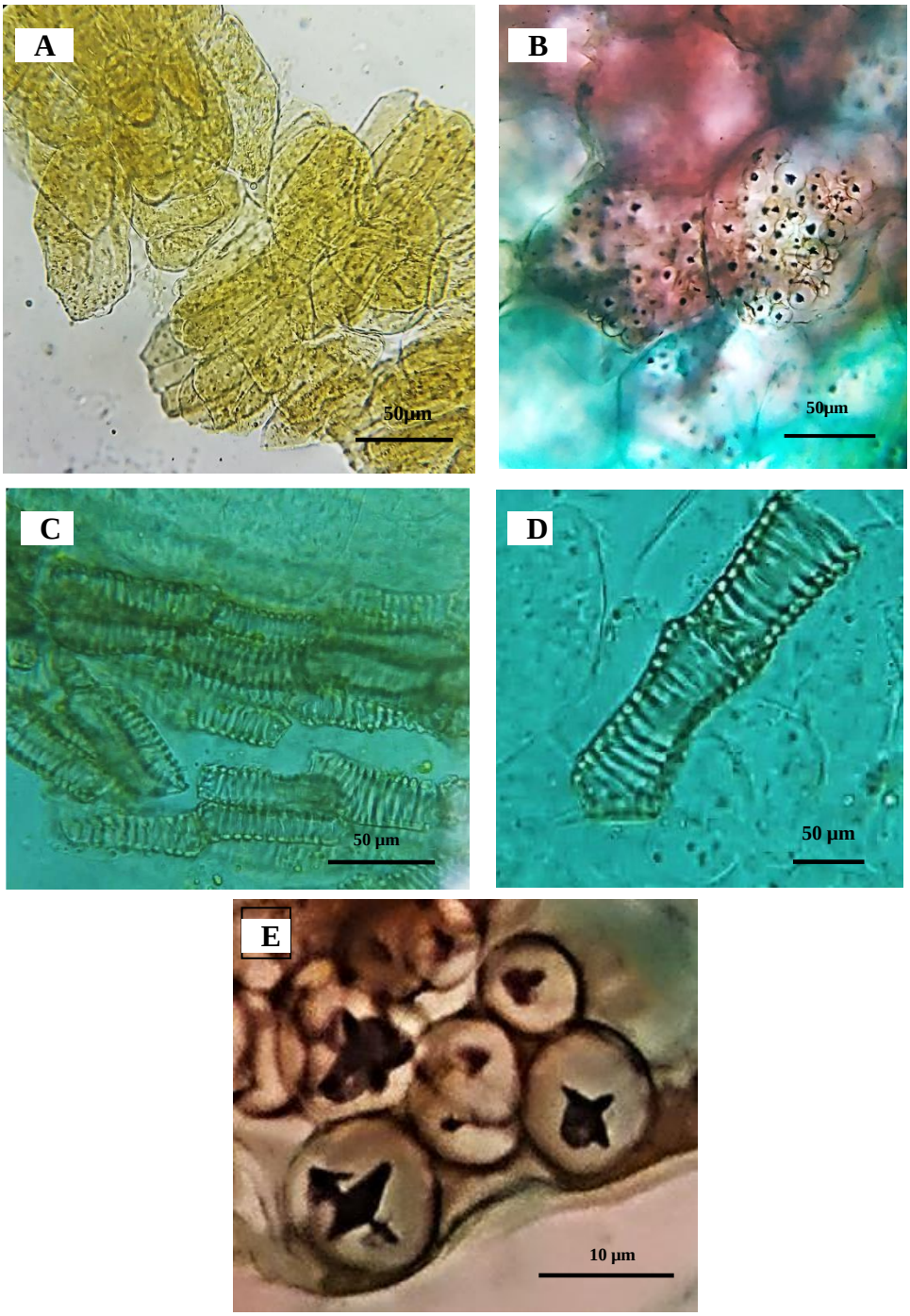

Figure 11: Isolated elements of the stem base and the haustoria. A: Metaderm, B: Parenchyma cells containing starch granules, C and D: Xylem vessels, E: Starch granules with centric clefted hilum.

3.2.5. The haustorium

A transverse section of the haustorium has a circular slightly zigzag outline (Fig 10A). It shows a central vascular region surrounded by a large cortex which is bounded by a few layers of metaderm. Root hairs are not developed and the roots are apparently non-functional. The sector is showing the following layers.

Metaderm: The outer external layer is composed of rounded or slightly elongated cells covered with external thick supersized wall adhered giving the section irregular outline. They measure 40-50 µm in diameter (Fig. 10B).

Cortex: Relatively thick area composed of 10- 12 layers of irregular rounded mostly large in size parenchyma cells measuring 50-60 µm in diameter

containing starch granules increasing in the central region with cells tangentially elongated (Fig. 10B).

Vascular system: Xylem is central and projects in a number of rays, appearing irregularly star-shaped in transverse section. A number of phloem strands lie outside the xylem, either alternating with the xylem rays or opposite to them. Radial rows of thin-walled cells separate phloem and xylem and extend in-between the xylem rays. The medullary rays are composed of few large cellulosic parenchymatous cells (Fig. 10B). The Xylem vessels have spiral lignifications measuring 30-50 µm in diameter (Fig. 10B).

Bulb and the haustorium isolated elements:

Cells of metaderm with supersized wall (Fig. 11A). Parenchyma cells of the cortex with starch granules (Fig. 11B). Xylem vessels with spiral lignifications (Fig. 11C and D). Starch granules with clefted hilum (Fig. 11E).

4. Conclusions

Morphology of Orobanche minor Smith is characterized by sessile triangular scaly leaves, succulent cylindrical hairy stem with spike inflorescence. The fruit is loculicidal ovoid capsule with persistent style containing numerous dust-like microscopic seeds. The plant exhibits a fleshy swollen underground base from which haustoria are emerged. Anatomical investigation showed some special characters of this parasitic weed. The plant is characterized by the presence of glandular trichomes with multicellular uniseriate stalk and short terminal cell and multicellular globular head detected in leaves, stem and flowers. The pith of the stem is formed of parenchyma cells containing starch granules with centric clefted hilum and enclosing large air cavity. The fruit is characterized by the presence of numerous seeds with reticulated lignified polygonal seed coat. The vascular tissue of the bulb contains a number of scattered vascular bundles; each one consists of central xylem surrounded by cellulosic phloem and a band of lignified parenchyma outside, while the haustorium shows a central vascular region surrounded by a large-celled cortex which is bounded by a few layers of metaderm. This botanical study aimed to be of helpful for identification of O. minor for medicinal use or phytochemical investigation.

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