Anthocerotae/Anthocerotopsida

Anthocerotae or hornwort is a distinct evolutionary group of thalloid liverworts, without an internal differentiation, and each cell having a single large chloroplast and an associated pyrenoid. The "horn" describes the sporophyte, a tapered structure of indeterminate growth.

Due to the presence of single large chloroplast with an associated pyrenoid per cell and indeterminate growth of sporophyte some authors consider hornworts to be entirely different from Bryophytes and elevate these to a new division Anthocerophyta of plant kingdom.

In this account hornworts are, however, treated at par with other classes; Hepaticeae and Musci.

In class Anthocerotae, there is an order Anthocerotales with one or two families. Commonly recognised genera are *Anthoceros*, *Megaceros*, *Dendroceros*, *Phaeoceros* and *Folioceros* (*Aspiromitus*) belonging to family Anthocerotaceae. Another genus *Notothylas* is included in a separate family Notothylaceae. A general opinion is to include all members in one family Anthocerotaceae.

All members of Anthocerotae are widely distributed in temperate as well as tropical regions - on moist soil of water banks and cliffs. *Megaceros* is quite common on splashed rocks of water streams and *Dendroceros* is common on tree trunks of humid forests. In temperate areas the plants are seasonal, but in milder climate of tropics the gametophytes are perennial.

In this account are included two representative genera, *Anthoceros* and *Notothylas*.

ANTHOCEROS

This hornwort with about 250 species is widely distributed in temperate as well as tropical regions of world. Over 25 species of this genus have been recorded from India. *A. erectus* is quite common in western Himalayas and this is so for *A. gemmulosus* in eastern Himalayas. *A. mangaloreus* is extensively distributed in hills of south India and extends up to western ghats. *A. khandalensis* abounds Khandala region of Deccan plateau. *A. erectus* is also found growing in plains of Bihar, U.P. and M.P. The genus is adapted to grow in mild climatic conditions of hilly areas and is not suited to dry conditions.

The plant body is a small dorsiventral dark green prostrate thallus; which is variously lobed (Fig. 3.1A, E), with a tendency towards dichotomous branching

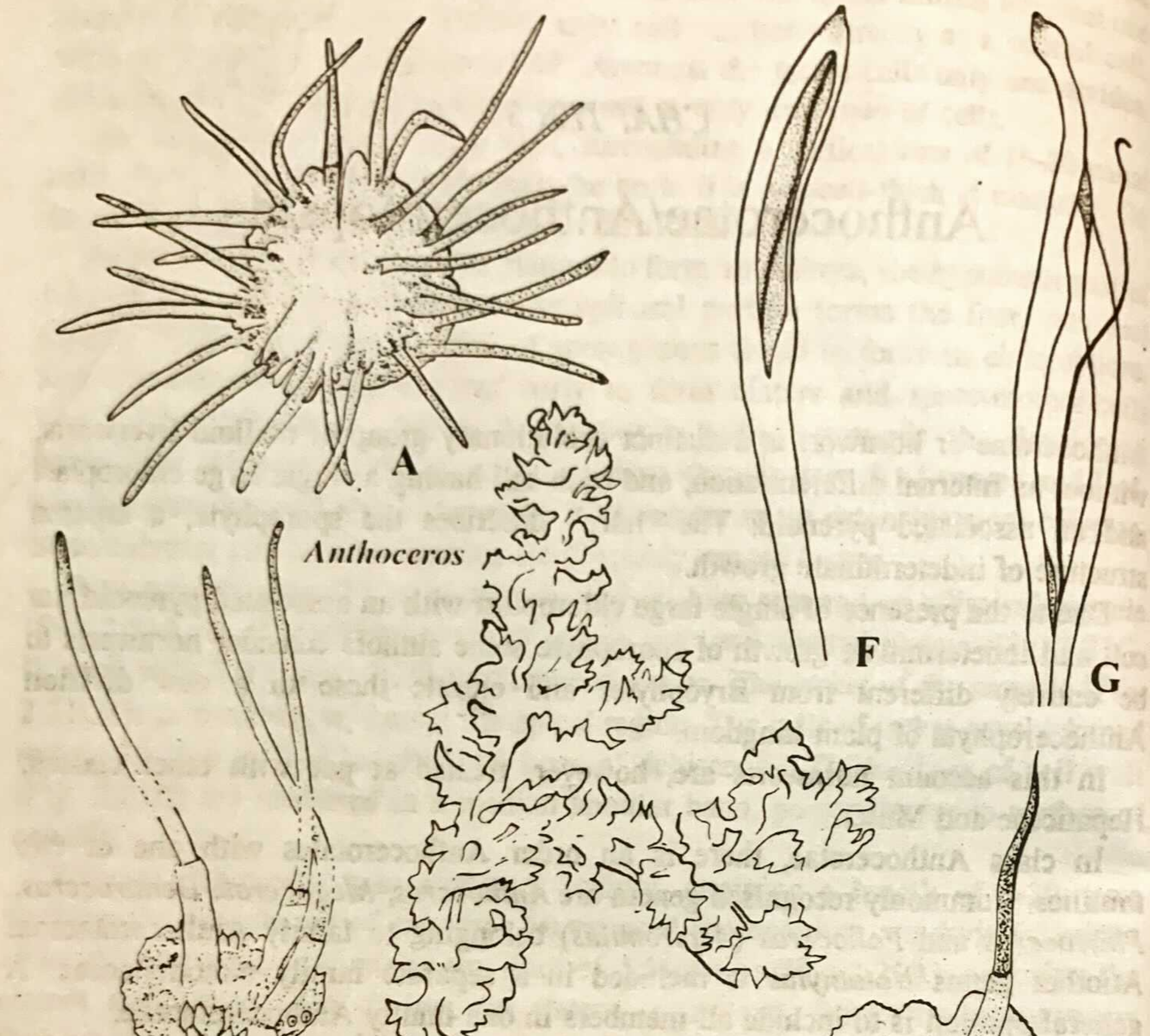

Fig. 3.1. *Anthoceros,* structure and reproduction.
A, fertile rosette of *A. crispulus.* B, a portion of *A. erectus,* enlarged. C, *A. gemmulosus* showing androecia and place of an archegonium showing mucilage mound. D, *Dendroceros,* a thallus with midrib and uniseriate lobes. F, G, stages in dehiscence of capsule.
(A, after Udar; B, C, F, G, after Bharadwaj; C, after Proskauer)

and forming orbicular or semiorbicular rosette. Sometimes many gametophytes are close together and due to irregular growth semierect lobes are formed. The thallus is raised in *A. erectus* (Fig. 3.1B). It is like a thick vertical or ascending stalk. The dorsal surface of thallus is smooth but on *A. crispulus* there are many flat-lobed lamellae which impart the plant a velvetty appearance. The thallus of *A. gemmulosus* (Fig. 3.1C) is spongy and bears a large number of marginal sub-spherical spongy bodies like a gemma.

The thallus of *Anthoceros* is several layers thick but it lacks a definite midrib. Only in *Dendroceros* there is midrib (Fig. 3.1D) from which uniseriate lobes or ruffles extend. In a thallus all cells are thin-walled (Fig. 3.2A). However in *Dendroceros* and *Megaceros* there are some elongated cells with slit-like pits, similar to pitted cells in Marchantiales, which are considered to assist in conduction. On the ventral surface of thallus are smooth colourless rhizoids. Stomata-like pores occur on the undersurface. Also on the undersurface of thallus are seen small dark bluish-green spots; which are colonies of cyanobacterium *Nostoc*.

On the undersurface of thallus, very early in development, are formed cavities (Fig. 3.2A). It is due to breakdown of cells and their replacement by mucilage. With the maturity of thallus, the mucilage in these cavities dries out resulting in air-filled chamber. The cyanobacterium *Nostoc* entering through mucilage pore forms a colony in these cavities (Fig. 3.2A). These cavities occur in *Anthoceros, Dendroceros* and *Notothylas* but are absent in *Megaceros* and *Phaeoceros*. The cyanobacterium-hornwort association is symbiotic; the thallus supplies carbohydrate, to the cyanobacterium and the latter in turn adds to nitrate nutrition by fixing atmospheric nitrogen.

Each cell of a thallus contains a single large chloroplast (Fig. 3.2B). The chloroplast of a superficial cell is larger than others and is lens-shaped. Each chloroplast encloses a body of 25-50 discrete units, the pyrenoid. The nucleus lies opposed to chloroplast, usually near the pyrenoid body. At times the chloroplast, gets folded to enclose the nucleus as well as pyrenoid. A single chloroplast per cell with its associated pyrenoid is unique to *Anthoceros* and unknown in any other bryophyte. Some species of *Anthoceros* have variable number of chloroplasts per cell. In *A. pearsoni* there are two chloroplasts in the inner cells and in *A. halli* the number may be even four. In *Dendroceros* there are up to 12 chloroplasts per cell.

The growth of thallus is due to single wedge-shaped apical cell of four cutting faces (Campbell, 1918) or a marginal meristem (Leitgeb 1879; Mehra & Handoo, 1953) where each cell has four cutting faces - dorsal, ventral and two lateral. Each of the segments produced by dorsal and ventral faces, divides into an outer and inner cell. The inner cell contributes to central tissue of thallus whereas the outer cell on dorsal surface contributes to upper epidermis and sex organs and the one on ventral surface contributes to lower epidermis and rhizoids. The lateral segments contribute to the expanse of thallus.

Asexual reproduction by progressive death and decay of older parts of thallus is not so common in *Anthoceros* as in other liverworts. Instead the thallus is capable of producing many tubers, which can withstand unfavourable conditions especially the drought to which most of the thallus is intolerant. These tubers readily

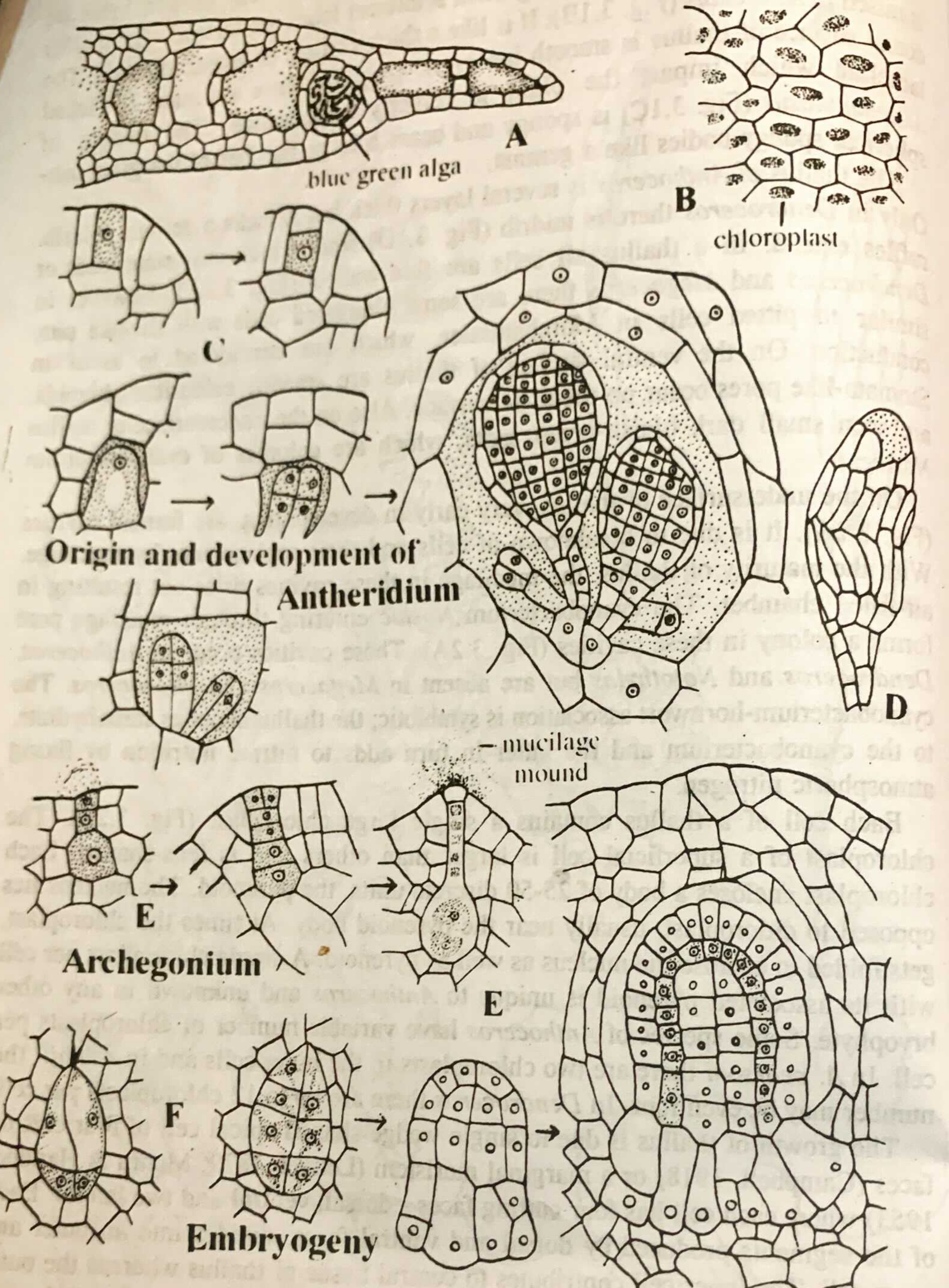

Fig. 3.2. *Anthoceros*, development of sex organs and embryogeny. A, section through thallus showing mucilage canals and a cyanobacterial colony. B, a part magnified to show single chloroplast per cell. C, stages in development of an antheridium and formation of secondary antheridia in an antheridial chamber. D, wholemount of an antheridium showing body cell, four tiered wall and stalk. E, stages in development of an archegonium. F, stages of embryogeny and development of sporophyte. (A, after Udar; B, D, after Bharadwaj)

regenerate on return of favourable conditions and thus make the thallus perennial. On the periphery of a tuber are 2-3 layers of corky hyaline cells. This layer surrounds the inner cells which contain starch, oil globules and aleurone grains.

Reproduction

Most of the hornworts are monoecious such as *A. fusiformis, A. punctatus* and *A. himalayensis.* The hornwort thalli are protandrous; and archegonia formation begins when antherozoids are mature. This is to prevent self-fertilization.

Some species of *Anthoceros* are also dioecious and heterothallic (Proskauer, 1948); the female plants have broader fertile lobes than male plants. The sex determination is genotypic, two spores of a tetrad develop into male plants and other two into female plants. These plants grow in close proximity. Dioecious species are *A. erectus, A. laevis, A. khandalensis* and *A. gemmulosus.* Both types of sex organs are initiated just behind the growing point, and are embedded in dorsal region of thallus. At a place there is usually a single archegonium embedded in the thallus. Contrary to it the antheridia are formed in groups, enclosed in chambers, visible as discrete areas known as androecia (Fig. 3.1C). The place of an archegonium can be identified by the presence of a mucilage mound (Fig. 3.1C, 3.2E) on the thallus.

The androecia occur in acropetal succession close behind the growing point, embedded in the thallus, externally appearing as scooped areas; as seen in *A. erectus.* However, in *A. gemmulosus* the androecia are hemispherical elevations on the thallus (Fig. 3.1C). Inside a chamber many antheridia (Fig. 3.2C) appear in acropetal succession. Normally there are four or less antheridia in a chamber but their number may go up to as many as 30 in *A. gemmulosus* and 22 in *A. erectus.*

A fundamental difference between Anthocerotae and Hepaticeae is that the antheridial initial is the inner daughter cell (Fig. 3.2C), arising out of a transverse division of a superficial cell near the growing point of thallus. This cell never becomes papillate. Following it there is separation of these two cells and the space so created gets filled with mucilage (Fig. 3.2C). The outer daughter cell - the wall initial - by periclinal divisions produces cells that result in a bistratose roof.

The antheridial initial divides to form four cells (Fig. 3.2C). This is followed by formation of an upper and lower tier (Proskauer, 1948; Mehra & Handoo, 1953) each having four cells. The lower four cells contribute to four-rowed stalk of an antheridium, which is several cells in length. The four upper cells divide to form an octant. Each cell of this octant divides periclinally to form 8 primary jacket cells towards outside and 8 primary androgonial cells towards inside. Further divisions in jacket cells and androgonial cells may be irregular as seen in *A. himalayensis* and *A. laevis* or in a regular manner as seen in *A. erectus* so that a single-layered-wall of an antheridium has 4 tiers of elongated rectangular cells. The last cell generation of androgonial cells are androcyte-mother-cells. These cells divide by oblique or longitudinal division (Bagchee, 1924) to form spermatocytes which metamorphose into biciliate antherozoids.

Presence of many antheridia in an antheridial chamber is possible due to (a) budding at the base of an old antheridium (Rink, 1935), these are secondary antheridia and (b) antheridial initial by its repeated vertical divisions form many

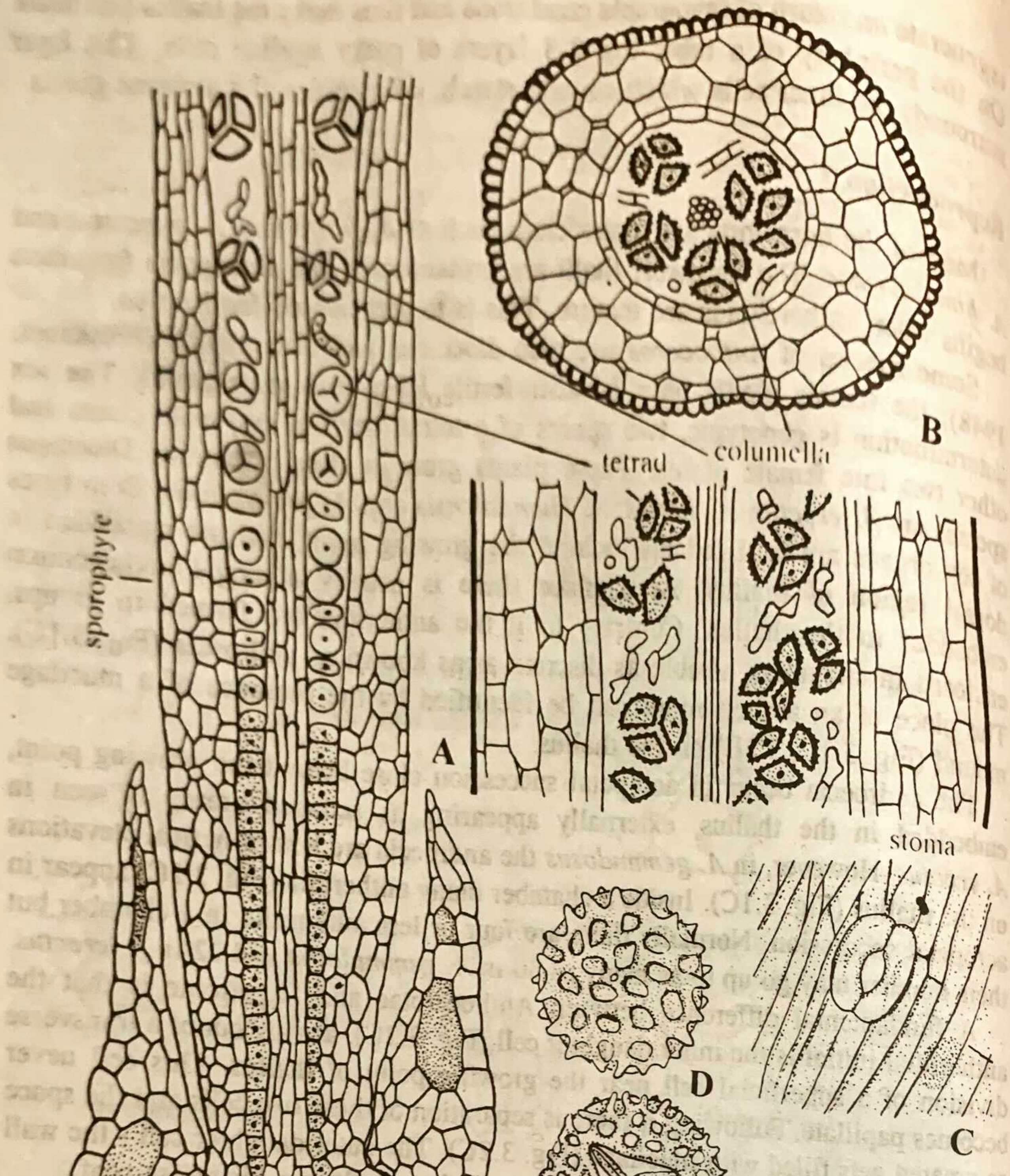

Fig. 3.3. *Anthoceros,* sporophyte structure and germination of spore.
A, longitudinal section of a sporophyte and an involucre, showing an haustorial foot, meristematic region and mature region. B, transverse and longisections of sporophyte from mature region, in these are seen central columella, spores and elaters. C, peel mount of sporophyte jacket to show a stoma. D, *A. erectus,* distal and proximal views of spores, in the former is seen reticulate exine and in the latter is seen a triradiate mark. E, stages in germination of a spore.
(A, after Udar; D, after Bharadwaj; E, after Mehra & Kachroo)

initials all of which form antheridia, these are all primary antheridia. Both these modes (a and b) are seen in *A. erectus* (Mehra & Handoo, 1953).

A mature antheridium is an orange-coloured club-shaped structure (Fig. 3.2D) with a multicellular stalk and a cylindrical body. The cells in unistratose wall are arranged in four tiers. The cells of lower three tiers are rectangular and elongated. The cells of uppermost tier are triangular with a narrow end, towards the apex. At the time of dehiscence these cells get deflexed and fall apart.

The dehiscence of a mature antheridium is due to its pressing against narrow opening of the roof (Pande, 1960), as a consequence the uppermost tier of triangular cells of antheridial jacket gaps apart releasing a cloud of antherozoids. The dispersal of sperms is also facilitated by falling rain drops. After dehiscence an antheridium loses its turgor and collapses. This is followed by another group of antheridia converging towards the opening and in this way a continuous stream of sperms is possible and it explains the large number of sporophytes in hornworts. The entire process of dehiscence can be observed microscopically on transfer of antheridia to a drop of water on a slide.

An archegonium arises from a dorsal segment of an apical cell, in its close proximity, and remains embedded in the thallus. New archegonia arise in acropetal succession. The archegonial initial can be identified by its dense contents. This initial cell undergoes transverse division to form an upper primary archegonial cell and lower primary stalk cell as described for *A. crispulus* and *A. gemmulosus.* However, in *A. erectus* the archegonial initial directly functions as primary archegonial cell. In the primary archegonial cell appear three vertical walls forming three peripheral cells - jacket initials - enclosing the primary axial cell.

The axial cell divides transversely to form two equal cells, the lower cell, forms primary venter cell. The upper cell undergoes transverse division forming upper primary cover cell and lower primary neck canal cell. The primary cover cell divides to form 2–4 cover cells. The primary neck canal cell divides to form 4-6 neck canal cells. The primary venter cell divides to form a venter canal cell and an egg.

The three jacket initials divide by vertical walls to form six cells. These cells divide transversely to form a jacket of six rows of cells.

A mature archegonium has 2-4 cover cells, a jacket enclosing 4-6 neck canal cells, a venter canal cell and an egg. At the region of venter the jacket cells divide peripherally to form bistratose jacket. The place of an archegonium on a thallus can be identified by the presence of a mucilage-mound (Fig. 3.2E). At the time of fertilization the cover cells are thrown off, the venter canal cells and cells of neck disintegrate releasing a mucilaginous mass which becomes continuous with the mucilage-mound on the thallus surface. The sperms caught in mucilage reach the egg. Prior to fusion, the egg enlarges and fills the venter. After entry within the egg the male nucleus, prior to fusion, can be seen along with female nucleus. Their fusion results into a zygote.

The first division of zygote is longitudinal (Fig. 3.2F), in this way hornworts differ from rest of bryophytes. Subsequent divisions result in a quadrant and then into an octant embryo. The lower cells of this are destined to form the foot (Fig.

3.2F) while the upper ones form the rest of sporophyte, which is a capsule without a seta. Early in the development of an embryo there is differentiation of amphithecium and endothecium, the endothecium contributes to the formation of central sterile region - columella - whereas amphithecium produces sporogenous tissue and jacket. At the base of capsule, early in development, differentiates an intercalary meristem, which contributes towards the continuous growth of capsule. In these features the sporophyte of *Anthoceros* differs from rest of liverworts.

A protective sheath or involucre encloses the developing sporophyte. The upper part of involucre is derived from the venter of archegonium and the lower part from cells of thallus. With the elongation of sporophyte, the involucre is pierced through its apex. A part of involucre remains at the apex of sporophyte - it is reminiscent of calyptra of mosses - and the lower part forms a collar around the base of sporophyte (Fig. 3.1B). This collar helps to retain moisture, which is needed at the base of capsule around the intercalary meristem. In this manner continued growth and differentiation of cells is possible in the sporophyte as long as conditions permit. These conditions are favourable weather as well as vigour of thallus. With age, the thallus becomes old, it tends to decompose and sporophytes are isolated.

The sporophyte is an elongate structure - giving the name 'hornwort' to plants with a bulging foot, that penetrates the thallus. The outer layer of foot is a superficial lining of elongated cells (Fig. 3.3A). These cells often produce short haustorial projections that push amongst the cells of thallus. The inner part of foot is made up of irregularly arranged vacuolated cells. The jacket of sporophyte is multistratose (Fig. 3.3B) and has stomata. The stomata are absent in *Dendroceros*, these are rudimentary or wanting in *Megaceros* and some species of *Anthoceros*. A stoma is typical, with a linear aperture and two elongated guard cells (Fig. 3.3C). The jacket is normally 4 to 5-cell-thick but exceptionally in *Megaceros* it is up to 16-cell-thick. The epidermal cells of jacket are thick-walled and in *Megaceros* inner linings of jacket cells have weakly defined transverse thickened bands. The epidermal cells have 2-4 shallow grooves, which mark the lines of dehiscence.

In the central region of sporophyte is columella of elongate cells, which assist in internal conduction. The columella cells may have spiral and annular thickenings in *Dendroceros*.

The young sporophyte has an archesporium (Fig. 3.3A) of single layer of cells which remain single-layered up to maturity in *A. erectus* and *A. crispulus*. However, in *A. gemmulosus* the archesporium becomes multilayered as the capsule advances to maturity. The archesporial cells can be identified by their small size and dense contents. Higher up in the capsule these cells differentiate (Fig. 3.3A) in an equal ratio into spherical to oval spore-mother-cells and elliptical to rectangular elater-mother-cells. Each spore-mother-cell on undergoing meiosis forms a spore tetrad. An elater-mother-cell forms a four-celled elater. Each elater is a compound structure homologous to a spore tetrad. The mature elaters are vermiform four-celled structures with tapering ends. Walls of elaters have irregularly arranged thick and thin areas. In *Dendroceros* and *Megaceros* the elater walls have a single spiral thickening.

The dehiscence of a capsule (Fig. 3.1F, G) is usually by two longitudinal lines, occasionally it is by a single line and rarely by four lines. In *A. crispulus* the blackened mature sporophyte splits near the tip, first along one line of dehiscence and then extends backwards. It is followed by splitting along other line of dehiscence forming two valves; still attached at the tip and exposing the columella, mass of spores and elaters. The elaters, as they dry out tend to twist. In this way elaters help to loosen the spores and allow them to disperse. Air currents also play a role in dispersal of spores.

The spores of hornworts are semi-circular with a conspicuous triradiate mark (Fig. 3.3D). They tend to remain as tetrads. Spores are always multicellular in *Dendroceros* and in species of *Anthoceros* the spores are unicellular as well as multicellular. In *Dendroceros* and *Megaceros* the spores are green. In the remaining genera the spores are golden yellow, dark brown to black. The spores are variously ornamented - vermicular ridged to spiny. These are viable for a long period and germinate readily to form thalli. The sporelings are of two types. In *Anthoceros,* on a short uniseriate germ-tube differentiates an apical cell that initiates the growth of thallus (Fig. 3.3E). In *Dendroceros,* the spores are chlorophyllous and multicellular; at the time of dispersal, and initiate the formation of thallus on release from exine.

NOTOTHYLAS

Another member of Anthocerotales is *Notothylas*, with some unique features. *Notothylas* (Fig. 3.4A) with about a dozen species is fairly widespread in temperate as well as tropical regions. It usually grows in a damp, shady place, either on moist soil or a rock or even on walls of old buildings.

Of the five species (*N. indica, N. levieri, N. chaudhurii, N. javanicus and N. pandei*) reported from India, the first two are quite common. Both the species are found, often, growing together in Himalayas (east as well as west). However, *N. indica* is also quite common on the plains, it can be seen growing on walls of old abandoned buildings along with other liverworts (*Cyathodium* and *Riccia*).

The two common species represent a variation of habit, possible in the genus. Thalli of *N. indica* form bluish-green rosettes (Fig. 3.4A) 2-5 mm in diameter, whereas the thalli of *N. levieri* are pale green elongated and irregularly branched. On the dorsal surface are cyanobacterial auricles and horizontally borne sporophytes (Fig. 3.4B). On the ventral surface are smooth rhizoids which fix the plant to soil, tuberculate rhizoids and scales are missing.

The gametophyte in its growth, anatomy, structure and development of sex organs (Fig. 3.4C) is similar to *Anthoceros*. The sporophyte, however, is quite variable and deserves a detailed account.

First division of zygote may be transverse as in *N. indica* and *N. orbicularis* or it may be longitudinal as in *N. javanicus* and *N. levieri.* Irrespective of the mode of first division, further divisions result in the formation of a three tiered embryo (Fig. 3.4E), having four cells in each tier. Of these three tiers the lower two produce the foot while the capsule is derived from the upper tier. In the upper tier, following periclinal division is the demarcation of zones of endothecium and amphithecium.

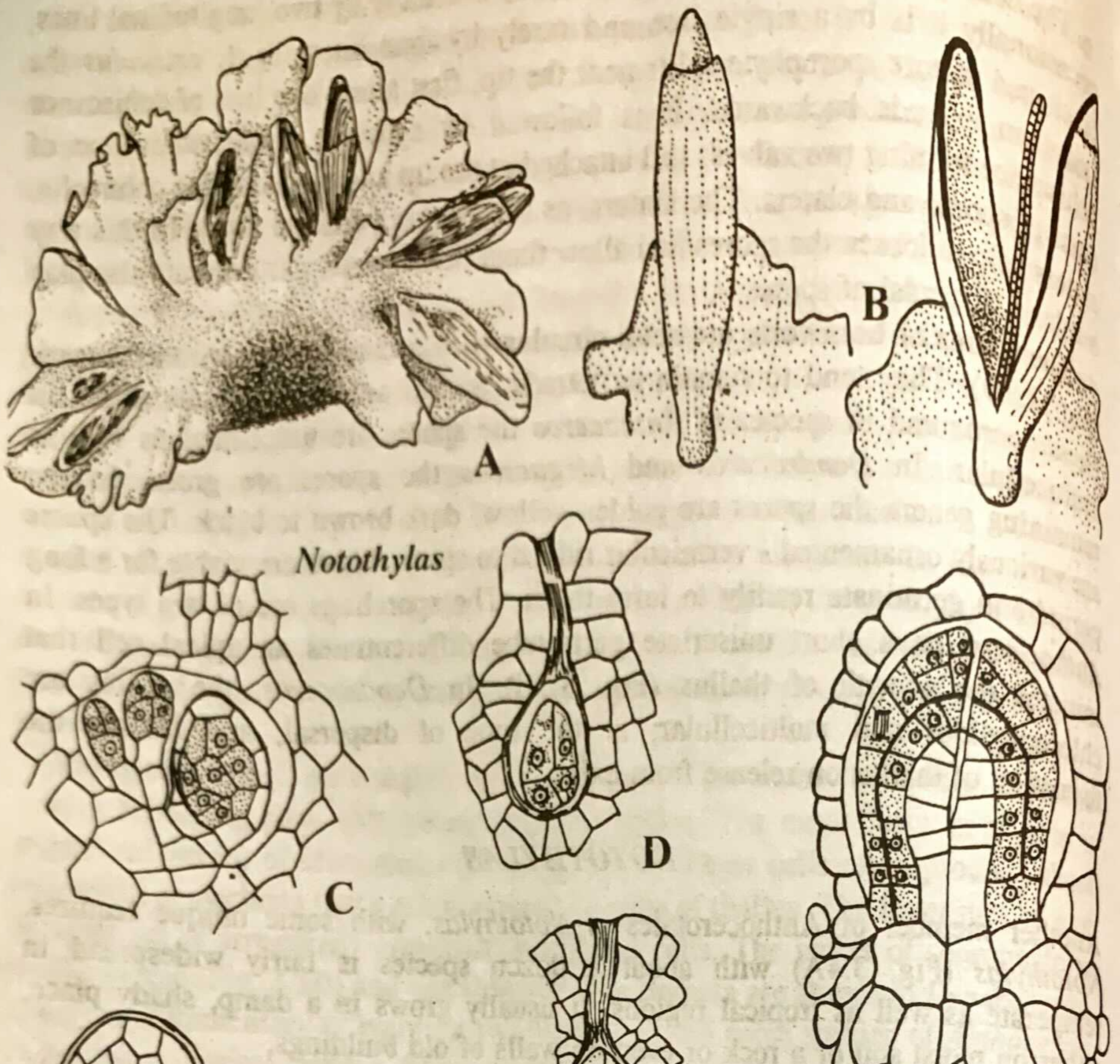

Fig. 3.4. *Notothylas*, structure and reproduction.
A, rosette of *N. indica* with horizontally borne sporophytes (B). C, antheridia in different stages of development. D, an archegonium bearing young embryo. E, three-tiered embryo. F-I, differentiation of amphithecium, endothecium and columella.
(A-H, after Udar)

Up to this stage the embryos are alike and following it the development becomes different in different species. In *N. indica* the cells of amphithecium further differentiate into outer amphithecium which forms the wall of capsule and inner amphithecium contributes to sporogenous tissue whereas endothecium forms the columella (Fig. 3.4F, G, H). On the other hand in *N. levieri* the entire amphithecium forms the wall of capsule and from endothecium is derived the sporogenous tissue. In brief, this sequence as seen in *N. indica* is similar to *Anthoceros* and rest of hornworts, whereas in *N. levieri* it is similar to other liverworts. Hence, *N. levieri* is a connecting link between Anthocerotae and Hepaticeae. Rather it strengthens the argument of positioning of Anthocerotae as an order (Anthocerotales) of Hepaticeae, instead of being a class or equivalent to it.

Presence or absence of columella results in different position of archesporium which affects the capsule structure in two groups. In *N. indica* archesporium is a layer of cells in between columella and capsule wall, it becomes several layered towards the apex. In *N. levieri* the archesporium is central in position due to absence of columella.

In archesporial cells are found two bands of tissues, sterile and fertile, forming elaters and spore-mother-cells, respectively. The elaters are unicellular short structures often curved with thickening bands on their walls. At the base of capsule can be seen a meristematic zone; the intercalary meristem that contributes to various types of tissues in the capsule. However, it is short-lived in its activity and hence, the sporophyte fails to achieve the growth as seen in *Anthoceros*.

A mature sporophyte is pointed cylindrical structure (Fig. 3.4I) with a massive foot, the basal cells of which elongate to form rhizoidal outgrowths. The wall of sporophyte is 4-cell-thick. Cells in the outermost layer are thickened without any stomata. With the maturation of sporophyte this layer becomes brown. One to four rows of thick-walled cells can be seen running along the entire length of capsule, meeting at its apex. These are lines of dehiscence.

The capsule opens like a follicle, dehisces along one, two or four lines, dispersing the spores. The spores are black and finely granulose. A spore germinates to form a mass of cells - a germ disc of either a quadrant or octant stage. One or two basal cells of this disc extend to form rhizoids, which are not separated by a wall. The growth of thallus is initiated by differentiation of a marginal meristem.