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Developmental Studies of Indian Laboulbeniales - *Dimeromyces anisolabis* (*Ascomycota, Laboulbeniomycetes*)

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ABSTRACT

The morphological development of the thallus of *Dimeromyces anisolabis* Thaxter is described and illustrated. It is a dioecious genus, collected from the common earwigs. The developmental stages of both the male and female thalli have been described. Supporting figures are camera lucida drawings and photographic illustrations.

Key words: *Ascomycota, Laboulbeniomycetes, Perithecium, Antheridi*

INTRODUCTION

Despite the laboulbeniomycetes having been known since 1850, there are very few publications dealing with the ontogeny of these unique and very interesting fungi. We have been carrying out extensive research on these fungi since last many years and during this study we were able to trace out different stages in the development of *Dimeromyces anisolabis*.

Dimeromyces is one of the largest genera among the Laboulbeniomycetes. About 108 species have been reported so far from various parts of the world, which is the highest number of species among the dioecious genera of these fungi. Although, most of these parasites are found on beetles (Coleoptera), few species are found to be associated with earwigs (Dermaptera), flies (Diptera), mites (Acarina), and thrips (Thysanoptera) (Rossi *et al.*, 2018). Few species of Dimeromyces reported are: *D. nanomasculus* (Proaño Castro and Rossi, 2008) on *Ardistomis venustulus* (Coleoptera, Carabidae), *D. osellae* (Rossi, 2010) on *Plesiomalota cotopaxiensis* (Coleoptera, Staphylinidae), *D. unguipes* (Bernardi *et al.*, 2014) from *Stilbus* sp. (Coleoptera, Phalacridae), *D. bubalinus* on *Coroicona* spp. (Coleoptera, Chrysomelidae), *D. perettiorum* on *Carcinophora americana* (Dermaptera, Carcinophoridae), *D. trilobatus* on *Apalotrius* sp. and *Physimerus* sp. (Coleoptera, Chrysomelidae), and *D. yasuniensis* on *Lenkous rossii* Ferrer (Coleoptera, Tenebrionidae). We have reported one species, *D. anisolabis* on *Labiduara riparia* from India (Kaur and Mukerji, 1996), the life cycle of which is described in this paper.

MATERIALS AND METHODS

Dimeromyces anisolabis is an exo-parasite on the dermapteran beetles *Euborellia annulipes*, *Nala lividpes*, and *Labiduara riparia*. The insects collected were fixed in an acidic alcohol fixative of glacial acetic acid and ethyl alcohol in 1:3 ratio for 24 hrs. After this, they were hydrolysed in 6N HC1 for five minutes only, and at room temperature, since the fungus is very small and fragile. Then the insect with the fungus still attached to it (otherwise it can be lost) was put in the acetocarmine stain for some time so that their ontogeny could be traced exactly. The fungus was then isolated by sticking the insect on the slide first, and then detaching the fungus from it with fine, sharp, needles.

OBSERVATIONS

Different stages in the life cycle of *Dimeromyces anisolabis* were observed. For describing the life cycle of the fungi, the

terminology used by Thaxter (1896) has been followed. Small case letters are used to designate the immature cells of the receptacle that have yet to undergo divisions. When the cells of the receptacle have reached the condition found in the mature fungus, Roman numerals are used to designate the cells. When the layer of the receptacle is single celled, the cell of that layer is referred to with the number of the layer in the text e.g. the cell of the single celled first layer is I.

RESULT

General description of the mature thallus: The fungus was found all over the body of Dermapteran earwigs of the species *Euborellia annulipes, Nala lividpes,* and *Labidura riparia.* The insects were found in trash dumps, damp soils and under leaf litter. The fungus was continuously collected over a long period of time. It was isolated from the tail pincers, the abdomen, the legs, and the thorax.

The fungus is dioecious having separate male and female thalli. The male thallus is small and hyaline to pale brown in colour. The thallus consists of receptacle and two compound antheridia. The receptacle has six to seven single celled layers, with the basal layers placed laterally with respect to each other due to the oblique septa between them. The compound antheridia are stout, flask-shaped, and the discharge tube has a pointed tip that is bent anteriorly.

The female thallus is bigger. The receptacle is eight to nine layered and each layer is single celled. The receptacle has a basal and a distal part. The basal part has oblique septa between the cells, as in male thalli, the distal part is cylindrical with straight, horizontal septa between cells. Perithecium is generally single sometimes there may also be two, formed laterally on the third layer of the receptacle. The male and the female thalli are found developing intermingled with each other on the insect part, where they are attached.

Development of the male thallus

The development begins with the ascospores. Eight ascospores are produced within each ascus. Each ascospore is spindle shaped, consisting of two unequal cells, and surrounded by a gelatinous sheath.

As per the orientation, the smaller cell is the apical cell and the bigger cell is the basal cell. The first division in the ascospore takes place in the basal cell which cuts off a black, conical foot at its basal end (**Figure1A, 2A**). The basal cell - cell 'b' then divides at its distal end and forms a small square shaped cell - cell 'iv' separated from it by a horizontal septum. The apical cell also divides by a horizontal septum and forms two cells. Later, the septum between the basal and apical cell, becomes thick and black (**Figure1A, 2B**). This septum thus separates what develops into the basal and distal parts of the receptacle. The basal cell of the receptacle, divides obliquely to form two small, flat cells. The basal cell is the single cell of the first layer. It is a large cell, rectangular in shape, getting broad distally. The flat cells it produces share an oblique septum and form the 2^{nd} and 3^{rd} single celled layers. The cell III of the third layer has a horizontal septum between itself and the cell of the fourth layer (i.e. cell 'iv'). The cell 'iv' thus, develops into cell IV of the fourth layer. The basal part of the receptacle thus has four cells, the three basal cells of which have oblique septa, and the fourth cell has horizontal septum (**Figure 1C**).

The distal end of the basal part of the receptacle has the dark septum beyond which is the distal part of the receptacle. The distal part consists of two cells formed from the apical cell of the spore. Another horizontal division results in three rectangular cells arranged in a series to form the cylindrical shaped distal part of the receptacle (**Figure1F, 2C**).

The cell III of the third layer divides obliquely at its lateral end and produces a small cell 'bc'. This cell is an initial and elongates and then divides at its distal end a horizontal division (**Figure 1D**). This apical cell also elongates and is slightly curved anteriorly. The basal cell then undergoes an oblique division to form a cell and then divides again to form another cell (**Figure 1E, 2C**). These two cells divide further longitudinally once each to form four cells. These four cells are small, and they

divide horizontally once to form four more cells which get arranged in a tier above the initial four cells. The bulk of these eight cells, are arranged in a circle and form the swollen base of the compound antheridium. The cells function as spermatia mother cells and form the spermatia which are released in the cavity of the antheridium and later released through the apical cell which forms the discharge tube. The discharge tube is slender and is bent anteriorly (Figure1F, 2D).

While the initial antheridiiferous cells are being formed, the cell of the second layer of the receptacle also cuts off a small cell which progresses in the same way to form another compound antheridium.

The male thallus thus consists of a small receptacle and two compound antheridia. The receptacle consists of a basal part consisting of four cells and a distal part consisting of three cells arranged in a cylindrical series. The basal part has cells arranged obliquely except the cell IV. The two parts are separated by a thick dark septum. The two compound antheridia are formed laterally on the 2nd and 3rd layer of the receptacle. The antheridia have a swollen base tapering to the slender discharge tube which is bent anteriorly.

Development of female thalli

The development of the female thallus also begins from the two celled ascospore. The smaller cell is the apical cell and the larger cell is the basal cell.

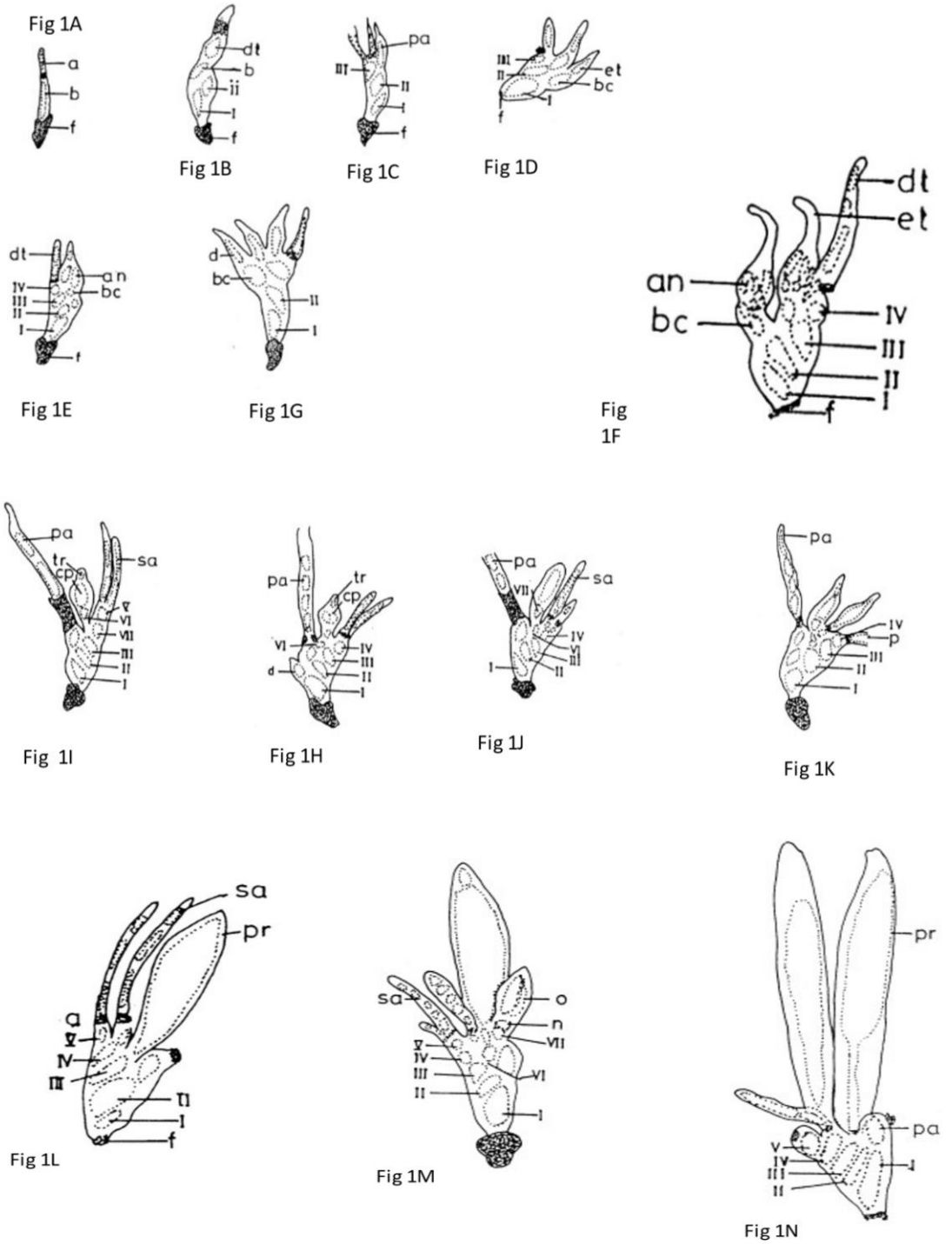
The basal cell cuts off a small foot at its basal end. The foot is conical in shape and darkens to become black. The septum between the basal and the apical cell becomes thick and black, and gets slightly constricted at the base. The septum thus separates the basal and the distal parts of the receptacle (**Figure 1A, 2A**). At the distal end of the basal cell 'b', a horizontal division takes place and a small, rectangular cell called cell 'iv' is cut off by a straight, horizontal septum (**Figure 1A**). Similarly in the apical cell also, a horizontal division occurs forming two cells in the distal part of the thallus.

The basal cell of the receptacle, divides by oblique divisions thrice to form four cells in a row separated by oblique septa. The cells thus become oriented obliquely. The fifth cell in the basal part of the receptacle is the cell 'iv' formed by the first division the basal cell (**Figure 1G**).

At the same time more horizontal divisions occur in the distal part of the receptacle forming, a cylindrical row of three rectangular cells with straight septa. The cells are all small, broader than long. In the basal part of the receptacle, the cell I is big, trapezoid, and gets broad distally when it ends in an oblique septum to give rise to the cell II. Cell II and Cell III form the single celled layers II and III of the receptacle. Both the cells are narrow, and have both their septa obliquely oriented. The cell IV which forms the fourth layer of the receptacle is a bigger cell but less longer than cell I. At its distal end the septum is horizontal instead of an oblique septum. It leads to the cell V of the fifth layer of the receptacle which is isodiametric in shape (**Figure 1H, 2E**).

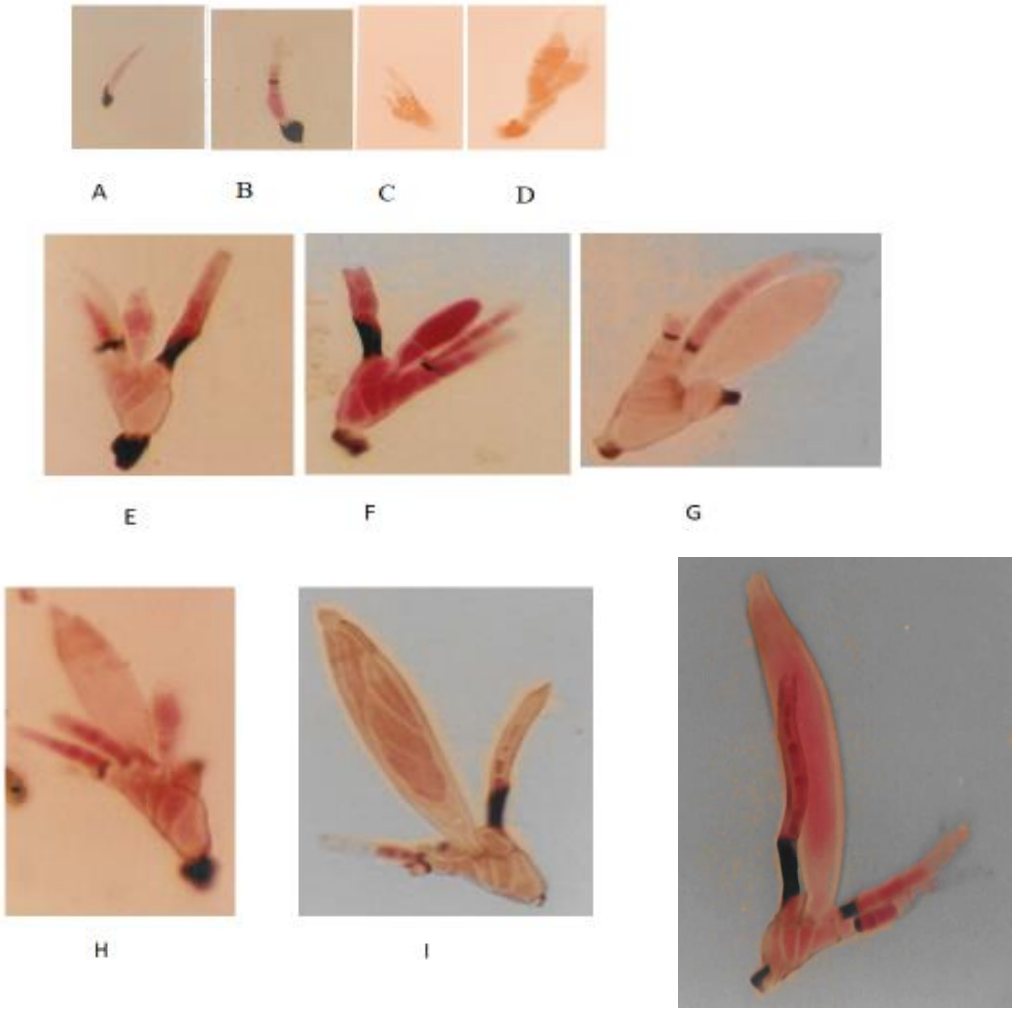
The cell III divides laterally to cut off a small square cell. This cell functions as the initial cell called cell 'd' for the perithecium (**Figure 1G**). The cell later elongates upwards and curves away from the receptacle. It gets swollen near the middle part. The cell II of the second layer of the receptacle also divides laterally and forms a small cell which elongates and forms a cylindrical cell. This cell then divides by a horizontal septum to form two cylindrical shaped cells. The septum is narrow and blackens early. This forms the primary appendage of the thallus (**Figure 1G**).

Meanwhile all the cells of the receptacle enlarge. In the basal part also, the cells enlarge including the cells II and III. Cell I remains the biggest cell, the receptacle is laterally oriented due to the oblique divisions. The fifth cell increases in size and becomes somewhat bell shaped with a broad base and tapering distally.



Figures 1 A-N: Developmental stages of *Dimeromyces anisolabis*. Camera lucida drawings. All figures X 400. Explanation and terminology are given in the text.

F, foot; I, first layer; II, second layer; III, third layer; IV, fourth layer; Ic, insertion cell; pa, primary appendages; sa, secondary appendage; d, perithecial primordium; h, basal cell; i, carpogonial initial; k, stalk cell primordium; j, secondary stalk cell primordium; m, n, and 'n", basal cells; VI, stalk cell; VII, secondary stalk cell; 'o', basal ties of outer wall cells; 'w' and 'w", upper tiers of outer wall cells; p, basal tier of inner wall cell; 'x' and 'x", upper tiers of inner wall cells; pr, perithecium; as, ascospore; ac, ascus; an, antheridium; bc, basal cell; d , distal part of receptacle



Figures 2 A-J: Developmental stages of female thalli of *Dimeromyces anisolabis*. Photographic illustrations. The first three stages are common with those of male thalli. All figures X 400. Explanation and terminology are given in the text.

The cells II, III, and IV also enlarge and maintain their oblique shapes (**Figure 1N**). The basal cell of the distal part of the receptacle enlarges and becomes swollen to assume an isodiametric shape. The rest of the cells remain cylindrical in shape.

The cell 'd' elongates and enlarges such that it has a small foot like part near the distal end of the cell III, this part then thins down to a slender strand as it elongates and then broadens at its distal end. The cell then divides by a horizontal septum at the distal end to form a triangular cell. The basal cell then functions as the stalk cell 'h' and the apical cell as the initial cell 'i' of the perithecium. The whole structure is oblong in shape with a greatly tapering base reaching down to the basal end of the cell near the layer III.

In the primary appendage, the cell divides to form another cell, which is also cylindrical in shape. The black septum widens and the basal cell of the appendage swells up. The cell IV of the fourth layer of the receptacle, also cuts off a cell, which again cuts off more cells to form

a second appendage (**Figure 1G**). The septum between the basal and the subbasal cell of the appendage blackens (**Figure 1H, 2E**). In the distal part of the receptacle, the cells divide further to form a row of three to four cells. All the cells are arranged in a cylindrical row.

The initial cell of the perithecium cell 'i' divides further to form the perithecium, while the stalk cell 'h' divides to form the basal cells. The cell 'h' divides horizontally to form a cell 'j' at its distal end, and cell 'k' at the base. The cell k divides and forms the stalk cell VI and a basal cell 'm'. The cell 'j' divides and forms the secondary stalk cell VII and two cells 'n' and 'n". Only one 'n' cell is visible in one plane.

Each of these three basal cells divide horizontally to form four cells in total. These four cells termed as the 'o' cells form the first tier in the outer wall cells. These curve around the carpogonial apparatus (**Figure 1I, 2E**).

The cell 'i' divides horizontally and forms the carpogenic cell 'cp' at the base and a common initial cell for the trichophore and the trichogyne apically (**Figure 1H**). The common cell later divides horizontally and thus three cells are formed in a row the carpogonium 'cp' at the base, the trichophoric cell 'tc' in the middle, and the cell of the trichogyne 'tr' apically. The trichogyne juts upwards and thus forms a stout**,** blunt, beak at the distal end of the obtriangular shaped structure (**Figure 1I, 2E**).

As the thallus, enlarges, the carpogonial apparatus also enlarges and elongates. The basal cells divide again to form four 'p' cells, which are placed, internally to the outer wall cells. These form the inner wall cells. All the wall cells divide horizontally form more tiers of the wall cells which enclose the carpogonial apparatus (**Figure 1K, 2F**). After fertilization, the trichogyne regresses, and, the wall cells close up around it. The outline of the perithelium shows very slight constriction where the stalk cell ends and the basal cells begin. The perithecium tapers towards the apex which is narrow but blunt, the perithecium is oblong in shape, with a narrow base, and a tapered apex (**Figure 1J, 2G**).

Both the appendages have four cells arranged in a cylindrical row. The septa between the basal and the sub-basal cells in both are darkened to black. The septum is much wider in the primary appendage and the basal cell is rectangular and swollen. The septum moves a bit to the side and gives an asymmetrical bulge to the cell. The cells of this appendage are comparatively broader than those in the other appendage (**Figure 1I**).

The perithecium enlarges further and is oblong with a swollen middle tapering both distally and basally. The apex is blunt. As the perithecium matures it elongates further and thins a bit to become almost cylindrical. Sometimes, another perithecium starts to develop on the fifth or the fourth cell of the receptacle. The pattern of development is the same (**Figure 1M, 2H**). At maturity, the four asci have formed eight ascospores each. When the ascospores are mature, the asci release them in the perithecial cavity, from where they are released out through the ostiole.

The mature thallus (**Figure 1N, 2I**) thus, consists of a basal conical foot, this gives rise to the basal part of the receptacle. The basal part looks oblique and consists of live layers of cell, each layer is unicellular. The first cell is the biggest, it is narrow at the base and becomes broad at its distal end. The first cell gives rise to the cell of the second layer through an oblique septum. The cells of the second, third, and, fourth layer, have oblique septa. The layers II and III have equal sized cells. They are both arranged obliquely and are about twice more broad than long.

The cell of the IV^{th} layer is much smaller in size, it is trapezoid in shape and leads to the cell of the V^{th} layer by a straight horizontal septum. The cell V of the V^{th} layer is a small, but swollen, isodiametric shaped cell. The fifth layer leads to the distal part of the receptacle. The septum between the two is black and constricted slightly. The distal part consists of five to seven superposed cylindrical cells.

There is a single anterior appendage, which arises from the second layer of the basal part of the receptacle (Cell II). It has a prominent basal cell which is swollen and looks asymmetrical due to its distal septum moving to the side. The septum is wide, blackened. The rest of the appendage has eight to nine superposed cells, rectangular in shape. One or two more appendages can arise on the other side of the perithecium from the cell IV. The septum between the basal and the sub-basal cell is blackened. The cells are rectangular, six to seven in number and narrower than the anterior appendage.

From the third layer of the basal part of the receptacle, arises the lateral perithecium. It is elongated, cylindrical with a blunt apex. It has a stalk cell which tapers to join the receptacle. Rest of the perithecium is free. The basal cells of the perithecium are indistinct. Very rarely, a second perithecium develops on the fourth layer of the basal part of receptacle.

DISCUSSION

The Laboulbeniomycetes are an interesting and unique group of fungi associated obligately with Arthropods. India with its range of climate and insects has a lot of potential for these fungi. We have started extensive research on taxonomic and ontogeny of these fungi. The developmental stages in the life cycle of a dioecious genus *Dimeromyces* have been studied. It was found that the

The male is not different from the female thallus. The male receptacle has many cells as does the female. The difference is only that instead of the perithecial initial, initials for the compound antheridia are formed. Two compound antheridia are found per thallus. They are made up of two tiers of four to five small cells arranged in a small barrel shaped structure tapering to a thin tube which is bent distally. The female thallus produces one rarely two perithecia.

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