

A MORPHOLOGICAL AND CYTOLOGICAL STUDY OF THE
GAMETOGENESIS AND OOSPORE FORMATION IN *ALBUGO*
SPECIES ON *IPOMOEA HEDERACEA*

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INTRODUCTION

Investigations on the gametogenesis and oospore formation in species of *Albugo* by different investigators for the last fifty years have revealed a remarkable and interesting diversity of nuclear cycle occurring during the sexual process. No other member of Peronosporales has afforded such an interesting cytological picture. Even as far back as 1896 and later 1899, Wager and Stevens had shown the occurrence of simple and compound oospheres in species of *Albugo* including the critical details. In recent studies cytological details like stage of zonation, coenocentrum, receptive papilla, etc., have been given taxonomic importance and are used in the differentiation of species.

Cytological investigations on species of *Albugo* occurring on Convolvulaceae in India have been carried out by Damle (1943), Thirumalachar *et al.* (1949), Safeeulla (1952) and Safeeulla and Thirumalachar (1951). Studies on *Albugo evolvoli* var. *merremiae* Safee. and Thirumal. have shown characteristic cytological differences in the gametogenesis and oospore formation in addition to variations in spore measurements. Another species parasitizing the shoots of *Ipomoea hederacea* Jacq., inciting large gall formations, was previously referred to *Albugo evolvoli* var. *mysorensis* Safee. by Safeeulla (1952). A reinvestigation of the morphology and cytology of the spore forms revealed that the fungus should be given a separate specific rank. The name *Albugo mysorensis* Safee. and Thirumal. nom. nov. is proposed for its accommodation and the description of the fungus will be given later in this paper.

SYMPTOMS OF THE DISEASE

The fungus is widely distributed in North and South India. On the leaves, pedicels and calyx lobes of the flowers, the fungus produces numerous white sori as in other species of *Albugo*, which on rupture erupt large masses of spores. The infected leaves and flowers show hypertrophy, and the diseased portions may turn

yellowish in colour. Detailed examination has revealed that no sex organs are developed in the infected leaves and flowers.

The oogonia, antheridia and oospores are produced on the large spherical to cerebriform galls (Fig. 1) in the leaf axils, stems and petioles up to 2 cm. in diameter. Because they are not accompanied by sporangia, they have been overlooked by earlier workers or taken for structures unconnected with the *Albugo* species. The galls appear as small protuberances at first and gradually enlarge developing nodular surface. Mature galls are yellowish, measuring up to 3 cm. in diameter.

MATERIAL AND METHODS

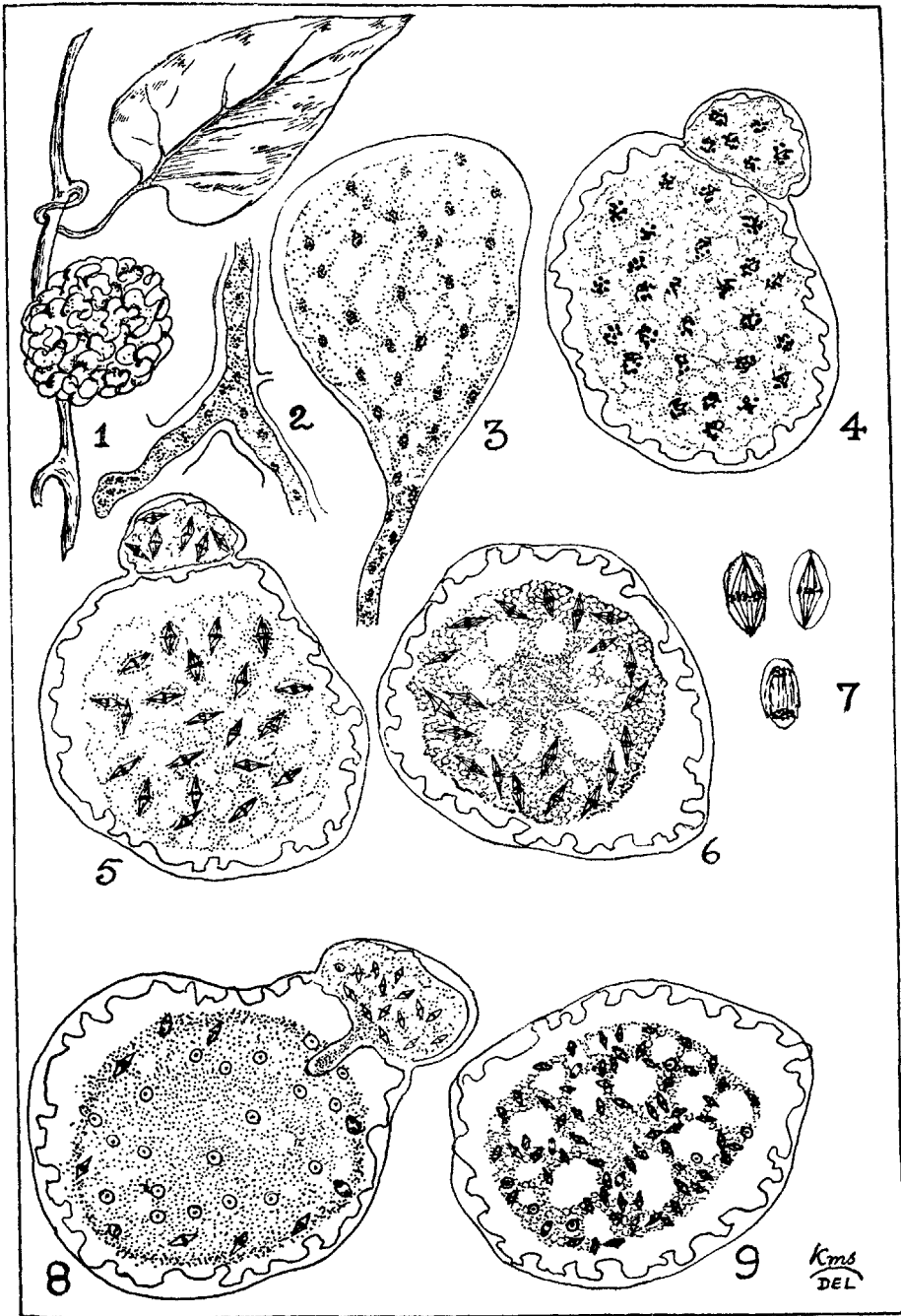
Material for cytological studies was fixed in formalin acetic alcohol or Allen's modification of Bouin's fluid. Sections of 6 to 10 μ were cut and stained with iodine gentian violet or by Heidenhain's iron-alum haematoxylin, with orange G as counter stain.

DEVELOPMENT OF ANTHERIDIUM AND OOGONIUM

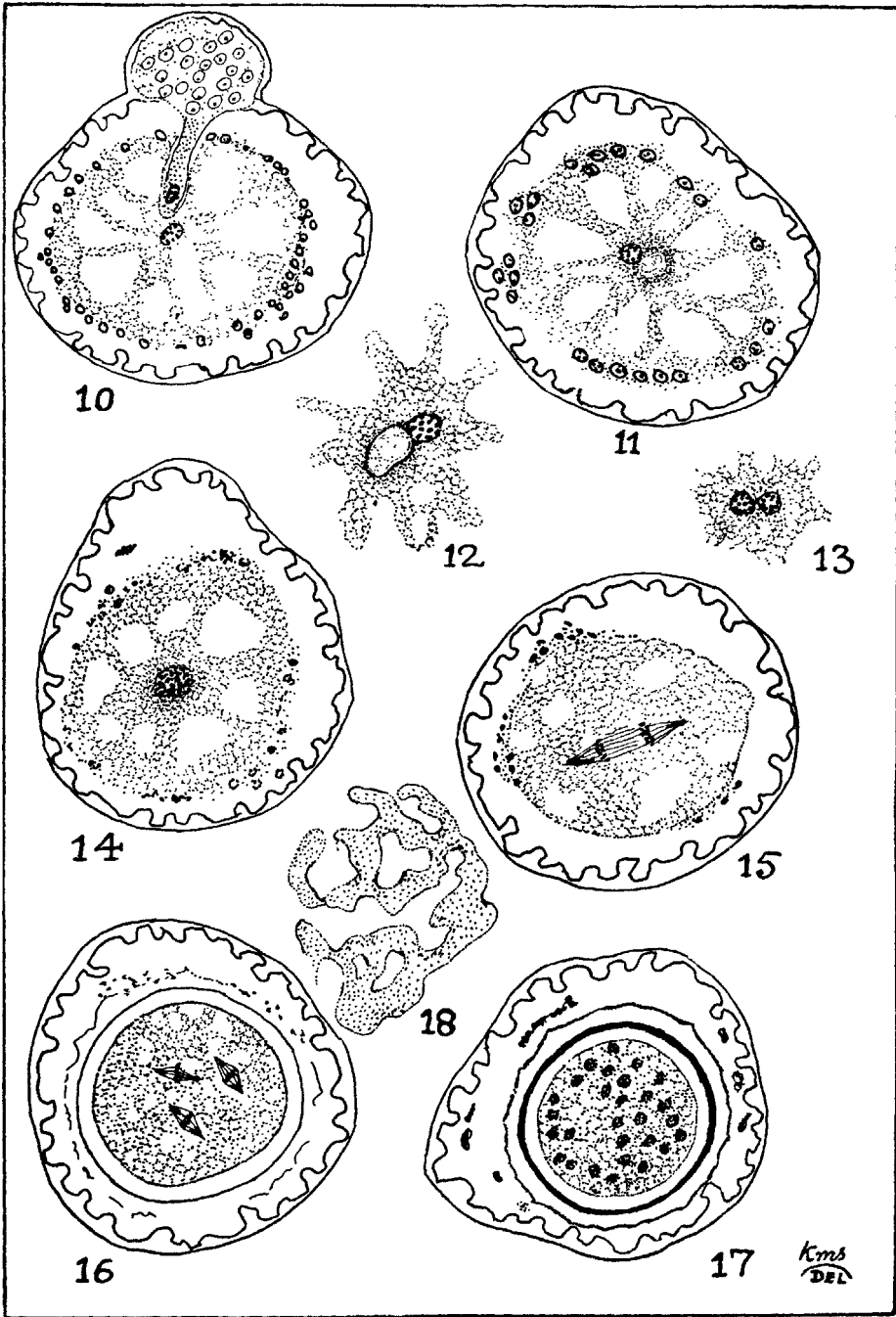
The mycelium is intercellular and coenocytic containing numerous nuclei (Fig. 2), developing small haustorial processes into the host cells. The differentiation of the oogonium at the tip of the swollen hyphae (Fig. 3) is similar to that described for other *Albugo* species previously. The antheridia are paragynous and remain closely adpressed to the oogonia. Young oogonia show 30 to 40 nuclei soon after differentiation, the nuclei being distributed uniformly within cytoplasm (Fig. 4). The first division of all the nuclei within the oogonium is simultaneous with that of the antheridium (Fig. 5). The spindles of the dividing nuclei are quite prominent, intranuclear (Fig. 7), and are not arranged in a definite manner to resemble the stage of zonation reported in species like *A. bliti*, *A. portulacae* and others. After this first nuclear division, all the nuclei migrate towards the periphery and the central region is devoid of all nuclei. At this stage, there is a differentiation of well developed (Fig. 6) coenocentrum (Figs. 11 and 12) by the accumulation of the cytoplasmic material and which stains very deeply with haematoxylin. There is a second simultaneous nuclear division in the oogonium of all the nuclei most of which are redistributed again towards the central region also (Fig. 9). The second nuclear division in the oogonium may or may not be accompanied by similar nuclear divisions within the antheridium. In many cases, the second nuclear division within the antheridium precedes that in the oogonium. During the second nuclear division in the oogonium as many as 80 to 90 dividing nuclei in their metaphase stage have been counted. The differentiation of the ooplasm and periplasm takes place by the migration of a single nucleus towards the centre. The rest of the nuclei move towards the periphery and degenerate. In the degeneration process, the chromatin network in the nucleus first disappears and finally the nuclear membrane and nucleoli disintegrate. No receptive papilla has been observed.

The antheridia show at first 6 to 8 nuclei, and after the two mitotic divisions 24 to 34 male nuclei are observed within the mature antheridium. As already stated, the first mitotic division of the nuclei within the antheridium is simultaneous with that of the oogonium while the second division sometimes takes place in advance of that in the oogonium (Fig. 8).

Soon after the differentiation of the oosphere, the antheridial tube enters penetrating the periplasm (Fig. 10). A single male nucleus is discharged into the oosphere, which during the process of migration appears slightly ovate or elongated, and becomes spherical again after reaching the egg nucleus (Fig. 13). The male and female nuclei fuse immediately and the fusion nucleus is surrounded by densely staining cytoplasm (Fig. 14). A wall is laid at the region of the periplasm and the fusion nucleus undergoes free nuclear divisions. The nuclear membrane disappears prior to the differentiation of the spindles and the divisions are extra



TEXT-FIG. 1



TEXT-FIG. 2

nuclear. During the metaphase of the nuclear division of the zygote nucleus, 24 chromosomes have been counted (Figs. 15 and 16). There are 24 to 32 nuclei within the mature oospore.

The wall of the mature oospore shows two layers (Fig. 17). The endospore is smooth and coloured and the exospore is thick and slightly irregular in outline. The wall of the oogonium is persistent and is closely adpressed with the oospore wall at certain places and appearing in surface view as being covered with tuberculate projections.

GENERAL DISCUSSION

Species of *Albugo* parasitizing members of the Convolvulaceae in India include *A. evolvuli* (Damle) Safee. and Thirumal. on *Evolvulus alsinoides* L., *A. ipomoeae-aquaticae* Sawada on *Ipomoea aquatica* Forsk. (*I. reptans* Poir), *A. evolvuli* var. *merremiae* Safee. and Thirumal. on *Merremia emarginata* Hall. and present species under study referred to *A. mysorensis* on *Ipomoea hederacea*. The collection on *Ipomoea eriocarpa* from Kashmir reported by Butler and Bisby (1931) has not been available to us for examination. All the above-mentioned species are easily differentiated from *Albugo ipomoeae-panduranae* (Schw.) Swingle in the sporangial stage by the lack of the characteristic equatorial thickenings present in the wall of the sporangium. The oospores are characterized by the persistent oogonial wall which forms the outermost envelope and resembles the condition present in the genus *Sclerospora*. In contrast, the oospore in *A. ipomoeae-panduranae* resembles those of other *Albugo* species in having a large space separating the oogonial wall and the mature oospore. From these considerations, it is apparent that there is as yet no authentic record of *A. ipomoeae-panduranae* in India. The other species reported on the members of the Convolvulaceae are *A. minor* Ciferri, *A. ipomoeae-pescarpe* Ciferri, and *A. ipomoeae-hardwickii* Sawada which are characterized by the presence of equatorial thickenings of the sporangia. The types of symptoms produced on the host are characteristic of the species concerned. In *A. evolvuli* and *A. evolvuli* var. *merremiae* there is no hypertrophy of the infected portion of the host. The infection is systemic in the shoots which strangely change from the prostrate to erect habit. In *A. ipomoeae-aquaticae* the fungus is confined to portions of plant which become hypertrophied. In all the three species mentioned above, the sporangia are formed first on the plant, and from the same hyphae in later stages the oospores are formed within the host tissues. In the species on *Ipomoea hederacea* on the other hand, infection is localized and the sporangia are produced on the leaves, shoots and flowers appearing as white erumpent pustules which incite slight hypertrophy of the host. The oosporic stage produced as a result of infection from the sporangia is formed on young axillary shoots and stems. It develops at first as tiny protuberances and gradually enlarge into large cerebriform galls of considerable size. There is no covering of sporangial pustules on these galls, and these would be overlooked if one has not followed the developmental cycle of the fungus. Even very mature stages of leaf and flower-bearing sporangial infection fail to develop the oospores. The seat of sporangial and oospore development are therefore separate from each other though both belong to the same cycle. A comparative account of measurements of sporangiophores, sporangia and oospores in the four species *A. evolvuli*, *A. evolvuli* var. *merremiae*, *A. ipomoeae-aquaticae* and *A. mysorensis* is presented in Table 1. An account of the differences in cytological details are also given for the four species.

The table indicates that, apart from the characteristic differences in the type of symptoms produced on the host, and size of the sporangia and oospores, the nuclear details in the gametogenesis and oospore formation vary with respect to the number of nuclei in the oogonium and antheridium and the presence of well developed coenocentrum, etc. To indicate these differences, the allocation of a separate species for the *Albugo* species on *Ipomoea hederacea* is justified.

TABLE I

<i>Albugo euvolvuli</i>	<i>Albugo euvolvuli</i> var. <i>merremiae</i>	<i>Albugo ipomoeae-aquaticae</i>	<i>Albugo mysorensis</i>
Infection systemic, non-hypertrophy of parts. Infection causing change from prostrate to erect habit.	Same as in <i>A. euvolvuli</i> .	Infection confined to portion of the shoots which are swollen.	Slight hypertrophy of sporangial infections and large oosporic galls in stems and axillary shoots.
Sex organs produced only in infected flowers and none in leaves and shoots.	Sporangia and oospores in both shoots and flowers.	Sporangia and oospores in the infected shoots.	Sporangia and oospores borne in separate infected loci.
Sporangiophores measure 37-44 × 13-16 μ and sporangia 13-15 × 11-13 μ without any equatorial thickening.	Sporangiophores 21-40 × 13-17 μ , sporangia 14-20 × 13-18 μ , without equatorial thickening.	Sporangiophores 27-45 × 15-21 μ , sporangia 18-23 × 16-20 μ without equatorial thickening.	Sporangiophores 33-41 × 13-18 μ , sporangia 15-19 × 13-16 μ , without equatorial thickening.
Oogonium before organization has 30-40 nuclei.	Oogonium before organization has 30-50 nuclei.	Oogonium before organization has 30-50 nuclei.	Oogonium before organization has 80-90 nuclei.
Mature antheridium has 6-8 nuclei.	Mature antheridium has 8-16 nuclei.	Mature antheridium has 8-16 nuclei.	Mature antheridium has 24-32 nuclei.
Coenocentrum well developed and prominent.	Coenocentrum not well developed.	Coenocentrum only faintly present.	Coenocentrum very conspicuous and well developed.
Mature oospore has 8-16 nuclei.	Mature oospore has 8-16 nuclei.	Mature oospore has 8-16 nuclei.	Mature oospore has 24-32 nuclei.
Receptive papilla present.	No receptive papilla.	Receptive papilla absent.	Receptive papilla not observed.
$2n$ number of chromosomes during meiotic division of zygote nucleus is 14-16.	$2n = 16$.	Number of chromosomes not known.	$2n = 24$.
Wall of oospore two layered, and the oogonial wall is firmly united with the outer wall of the oospore.	Oospore wall has three layers, the exospore, mesospore and endospore. The oogonial wall does not form such a firm envelope as in <i>Albugo euvolvuli</i> .	Wall of oospore is three layered, and the oogonial wall does form a firm envelope.	Oospore wall is two layered. The oogonial wall is confluent with the exospore only at certain points and not entirely as in <i>Albugo euvolvuli</i> .

Albugo mysorensis Safee. and Thirumal.

Sporangia produced on leaves, flowers and rarely on stems, inciting slight hypertrophy and paling of the leaves, white, erumpent and pulverulent. Sporangio-phores subepidermal, clavate-cylindric, $33-41 \times 13-18\mu$, producing in succession chains of sporangia. Mature sporangia cuboid to spherical, without equatorial thickenings, hyaline, smooth, measuring $15-19 \times 13-16\mu$. Sex organs produced in large cerebriform galls produced by transformation of axillary buds or tender stems. Oogonia spherical, $46-58\mu$ in diameter; antheridia paragynous, measuring $16-20 \times 10-13\mu$, cinnamon-yellow to pale brown, thick-walled, with an outer exospore and inner endospore. Wall of the oogonium persistent, with the tuberculate thickenings, confluent with oospore wall at certain places, measuring $44 \times 54\mu$.

Hab. on leaves, flower and shoots of *Ipomoea hederacea*, Hebbal, Bangalore, leg. M. J. Thirumalachar (Type).

Albugo mysorensis Safee. and Thirumal. nom. nov.

Sporangia producta in foliis, floribus atque raro in culmis, inducentia, erumpentia, pulverulenta. Sporangio-phori subepidermales, clavato-cylindrici, $33-41 \times 13-18\mu$, successive producentes sporangiorum catenules. Matura sporangia cuboidea vel sphaerica, absque spissatione equatoriali, hyalina, levia, magnit. $15-19 \times 13-16\mu$. Organa sexualia amplis cerebriformibus, gallae vero efformatur ex mutationem alabastrorum axillarum vel culmorum tenerum. Oogonia sphaerica, $46 \times 58\mu$ diam.; antheridia paragyna, magnit. $16-20 \times 10-13\mu$, cinnamomo-lutea vel pallide brunnea, crassis parietibus praedita; parietes vero constant exosporio externo et endosporio. Oogonii parietes persistentes vero constant exosporio externo et interno. Oogonii parietes persistentes, tuberculate spissati, confluentes cum oosporum parietibus locis denitis, magnitud. $44 \times 54\mu$.

Habitat in foliis, floribus et culmis *Ipomoeae hederaceae*, Typus lectus in loco Hebbal, Bangalore, a M. J. Thirumalachar.

ABSTRACT

A detailed morphological and cytological study of the gametogenesis and oospore formation in *Albugo* species on *Ipomoea hederacea* is made.

A comparative account of species of *Albugo* parasitizing the members of Convolvulaceae in India is presented.

Apart from the characteristic differences in the type of symptoms produced on the hosts, and size of sporangia and oospores, the nuclear details in the gametogenesis and oospore formation, an account of cytological details is also given to justify the allocation of a separate specific rank for *Albugo* species on *Ipomoea hederacea*.

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REFERENCES

- Butler, E. J., and Bisby, G. R. (1931). *The Fungi of India*.
 Damle, V. P. (1943). A new species of *Cystopus* on *Evolvulus alsenoides* Linn. *Jour. Ind. Bot. Soc.*, **22**, 133-136.
 Safeeulla, K. M. (1952). Morphological and cytological studies of *Albugo* species on *Ipomoea hederacea*. *Curr. Sci.*, **21**, 287-288.

- Safeella, K. M., and Thirumalachar, M. J. (1951). Morphological and cytological studies in *Albugo* species on *Ipomoea aquatica* and *Merremia emarginata*. *La Cellule*, tome LV, fascicule 2, 225-232.
- Stevens, F. L. (1899). The compound oosphere of *Albugo bliti*. *Bot. Gaz.*, 28, 149-176 and 225-245.
- (1904). Oogenesis and fertilization in *Albugo ipomoeae-panduranae*. *Bot. Gaz.*, 110, 487-491.
- Thirumalachar, M. J., Whitehead, M. D., and Boyle, J. S. (1949). Gametogenesis and oospore formation in *Cystopus (Albugo) evolvuli*. *Bot. Gaz.*, 110, 487-491.
- Wager, H. (1896). On the structure and reproduction of *Cystopus candidus* Lev. *Ann. Bot.*, 10, 295-342.

EXPLANATION OF FIGURES

Text-fig. 1. Figs. 1-9.

- FIG. 1. Showing the habit of diseased shoot, nat. size.
- „ 2. Intercellular hypha. $\times 1,350$.
- „ 3. Oogonial initial. $\times 1,350$.
- „ 4. Oogonium and antheridium. $\times 1,350$.
- „ 5. First simultaneous nuclear division in the antheridium and oogonium. $\times 1,350$.
- „ 6. First nuclear division in the oogonium with the dividing nuclei arranged near the periphery. $\times 1,350$.
- „ 7. Intranuclear spindles $\times 2,025$.
- „ 8. Second nuclear division in the antheridium alone, prior to the division of nuclei in the oogonium. $\times 1,350$.
- „ 9. Second nuclear division in the oogonium. $\times 1,350$.

Text-fig. 2. Figs. 10-18.

- FIG. 10. Uninucleate oosphere and antheridial tube before the male nucleus is discharged. $\times 1,350$.
- „ 11. Oosphere with coenocentrum. $\times 1,350$.
- „ 12. Same as in 11, enlarged. $\times 2,025$.
- „ 13. A portion of oosphere with male and female nuclei. $\times 1,350$.
- „ 14. Oosphere with fusion nucleus. $\times 1,350$.
- „ 15. First division of the fusion nucleus. $\times 1,350$.
- „ 16. Subsequent divisions in the oospore. $\times 1,350$.
- „ 17. Mature oospore. $\times 1,350$.
- „ 18. Areole. $\times 1,350$.

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