A REVISION OF THE GENUS SAPROLEGNIA

by

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TABLE OF CONTENTS

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INTRODUCTION

Presently defined species in the aquatic Phycomycetes are, in numerous instances, quite restricted and allow for little morphological variation. Identification of variants, therefore, may be extremely difficult, especially in the Saprolegniaceae. The number of taxa included in this family, however, indicates that the clarification of species concepts can be accompolished only by a systematic study of the individual genera. The beginning of such an arduous study was made by Johnson (1956), who presented a new account of the genus Achlya. Recently, Scott (1961) published the results of studies on the morphology and systematics of the species belonging to Aphanomyces. Thus, the present study grew out of a recognized need for a critical review of the species of Saprolegnia, the largest and most commonly known water mold genus in the family, on a world-wide basis.

Unlike the two previous studies mentioned above, this contribution is intended to present a new approach to the study of these fungi. The use of a chemically defined medium has played an important role in the preparation of species descriptions, supplemented with data obtained with the more generally employed substrate, hemp seed (Cannabis sativa L.). It is the author's hope that the use of a chemically defined medium on a universal scale may aid in clarifying species concepts within Saprolegnia, as well as in other genera of the Saprolegniaceae.

Morphological criteria are the principal basis for delimiting species in this monograph; these were selected from among those criteria that can be interpreted with comparative ease and reliability. Most of the taxonomic

conclusions as well as the descriptions herein are based on the study of over eight-hundred living isolates.

History of The Genus

The first indisputable reference to the genus dates back to 1821, when Gruithusen described a fungus, which he called Conferva ferax, on the remains of a dead snail. The characteristic saprolegnoid type of zoospore discharge and internal proliferation was illustrated for the first time.

In 1823 Nees von Esenbeck first separated the then known forms of water molds into two genera, Saprolegnia and Achlya. The genus Saprolegnia was thus established and based upon the following brief description: "Fila simplicia, articulata, sporas, per articulos sibi succedentes simplices motu praeditas, apargentes Saprolegnia". Nees called Gruithusen's fungus Saprolegnia molluscorum, but apparently Nees did not know or observe the sexual organs making it impossible to accertain the real identity of his species.

After brief and unimportant mention of these fungi in earlier papers, Meyen (1839) described the formation, release, and germination of Saprolegnia zoospores, which confirmed the opinion of many investigators, that these organisms were algae.

Kutzing (1843) included in his newly established family, Saprolegniaceae, three species of the type genus and described six additional forms in his "Species Algarum" (1849).

The following year Thuret (1850) described the biflagellate zoospores of a form he called Saprolegnia ferax and illustrated the oogonia for the

first time. No species had been previously described or illustrated as to be now recognizable. S. ferax has since been accepted as the type species of the genus and is designated as the nomenclatoral type.

Although published accounts of the genus Saprolegnia existed prior to 1851, it remained for deBary (1852) to make significant contributions to our knowledge and understanding of these fungi. The first investigator to use pure culture methods in this group, deBary substantiated the generic distinctions of von Esenbeck and demonstrated the necessity for utilizing sexual structures for delimiting species.

In 1858, Pringsheim contributed to the literature a concise description of the family, which he regarded as an algal taxon. This belief was shared by many of the early investigators until 1866 when deBary removed the family Saprolegniaceae from the algae and included the family in his classification of fungi.

Leitgeb (1868, 1869) observed diplanetic zoospores in an isolate of Saprolegnia and proposed the generic name Diplanes for fungi of this type. The latter genus was soon abandoned when the phenomenon of diplanetism was found to occur in other members of the family.

The posthumously published paper of deBary (1888) represented the single most comprehensive treatment of the genus to that date, in that descriptions of all known species of Saprolegnia were compiled in a single paper.

Mention should also be made of such indispensible treatises as those of Cornu (1872) and Fischer (1892) particularly with respect to older taxa of the genus. Appearing almost simultaneous with the latter publication was Humphrey's (1893) paper on the Saprolegniaceae of the United

States in which he included seven species of Saprolegnia.

Tiesenhausen (1912) extensively collected and studied the saprolegnias of Switzerland; Minden (1912, 1915) reviewed and redescribed many of the American and European species.

Kauffman (1921) established the genus Isoachlya to segregate three species of water molds from members of the genus Saprolegnia based upon cymose renewal of secondary sporangia. Johannes (1955) reduced Isoachlya and erected a new genus, Cladolegnia, to include those species of Saprolegnia and Isoachlya possessing 1-3 oospores and polyplanetic zoospores. Subsequent workers have not, however, recognized Johannes' Cladolegnia (Cejp, 1959; Dick, 1960; Sparrow, 1960).

Coker (1923) published a critical and well-illustrated analysis of the American species of Saprolegnia supplemented by descriptions of European forms known at the time, and a discussion of doubtful and invalid species. Our present day knowledge of many previously described species is based upon his descriptions and these have come to be the accepted interpretations.

Since 1923 several accounts, mostly in the form of regional monographs, have been published in various parts of the world. Ivimey-Cook and Forbes (1933), Ivimey-Cook and Morgan (1934) and Forbes (1935a, 1935b) in England, Apinis (1930) in Latvia, Cejp (1959) in Czechoslovakia, Lund (1934) in Denmark, Van Beverweijk (1948) in the Netherlands, Beneke and Rogers (1962) in Brazil, Crooks (1937) in Australia, Nagai (1931, 1933) and Ito (1936) in Japan, Ou (1940) in China, Chaudhuri, et.al. (1947) in India, Hohnk (1935), Stoll (1936) and Richter (1937) in

in Germany, and the Moreaus (1935) in France have contributed greatly to our knowledge of the genus.

The works of Coker and Matthews (1937), Gilman (1945) and Beneke (1948) represent the most recent compilations of American species belonging to the genus.

MATERIALS AND METHODS

Collection

The collection of species of Saprolegnia from water and soil is a relatively simple procedure and one which has been used, with slight modification by previous investigators. Samples of soil were collected individually in 35 ml, sterile, polypropylene, screw-cap bottles. For convenience in handling and culturing approximately a tablespoon of soil was found to be sufficient. Soils were secured from the profundal regions of lakes, rivers, swamps or other sites where the soil was consistantly wet or waterlogged.

The samples were returned to the laboratory within a few hours after collecting and plated out as follows: if the sample lacked sufficient water, sterile distilled water was added to the collecting bottle, then vigrously shaken until the soil was more or less in suspension, and dispensed into a sterile Petri dish. After the soil and detritus had settled, three or four split, autoclaved seeds of hemp (Cannabis sativa L.) were added as "bait" to each gross culture. Following "baiting", all cultures were maintained at 18°C. The "ephermeral" nature of zoosporangia of many of these fungi necessitated that the gross culture be examined regularly if the characteristic zoospore discharge was to be

observed. Starting with the second day of incubation, all cultures were examined with a stereoscopic dissecting microscope (15X oculars and a 6X objective). Within two to four days most of the baits were surrounded by a thick mat of white hyphae. With the formation and subsequent maturation of zoosporangia, the genera were determined by microscopic examination of the colony and species of Saprolegnia were selected for isolation. Probable and positive isolates were transferred to fresh Petri dishes containing sterile distilled water and additional hemp seed bait. From this beginning pure cultures were obtained in the manner to be discussed later. Dry or moderately moist soils yielded no isolates.

Collection data indicated that members of the genus were more abundantly represented in fresh waters. Isolates appeared more frequently, however, from those taken at the margin of the water, at a depth of 4-5 inches. Such collections were made from both lentic and lotic environments in 11 South-eastern and Midwestern states. Water samples were collected in 35 ml, sterile, polypropylene bottles.

The samples were returned to the laboratory, dispensed into sterile Petri dishes and baited in the manner previously described. Any debris in a sample was examined microscopically for the presence of zoosporangia; if such were found the material was transferred to separate Petri dishes and treated in the same manner as the original sample.

Isolation and Culture

Pure cultures upon known substrata are almost essential to the identification of species of Saprolegnia. Since the morphological responses to diverse nutrients and especially to the stimulation of

mixtures of other fungi and bacteria growing with any particular species is great, the study of each isolate in pure culture in media of known composition is of particular interest. Usually, only one or two species were recorded from any one soil or water sample, but up to six species have been obtained on a single piece of "bait". It was, therefore, necessary that each individual isolate be obtained in pure culture before any attempt was made to identify or describe them. Bacteria-free unifungal cultures were easily obtained using SPS agar and Rapers' ring technique (Raper, 1937), modified by Scott, et al. (1963). A sterile micro-pipette was placed opposite the discharge pore of a zoosporangium, and in a few seconds several hundred zoospores could be collected. These spores were deposited within a glass ring (18 X 20 mm.), secured by SPS agar in the center of a Petri dish. After a period of incubation, usually 12-24 hours, a dense mycelial growth was observed in the agar outside the ring. Two agar blocks, 0.5 cm^2, containing mycelium were cut at the periphery of the colony; one block was placed aseptically in a Petri dish containing 25 ml of sterile distilled water. This served to verify the correct identification of the isolate. The other block was transferred to an agar slant and subsequently submerged under oil and stored at 5°C until needed. One such culture was prepared for each fungus isolated. Occasionally when contaminants were particulary persistent, it was necessary to repeat the procedure. Sub-cultures were tested for purity by inoculating a liquid maltose-peptone medium, which rapidly became turbid in the event of any persisting bacterial contamiration.

There is a nearly limitless array of natural substrata for growing these fungi in water culture. Hemp seeds constitute one of the best and most widely used natural substratum and these were used to cultivate and study the fungi in what the writer somewhat arbitarily considers a "natural environment". That this environment was "normal" or at least apparently highly favorable was indicated by the fact that under such conditions species were generally constant in their morphology when compared to the same species grown on a chemically defined medium. After obtaining a suspension, in the manner previously described, the zoospores were inoculated into a Petri dish containing 25 ml of sterile distilled water. The autoclaved hemp seed halves were added aseptically to each dish and the culture incubated at 18°C.

When the study of a large number of fungi is undertaken, comparison of these fungi under controlled and reproducible conditions of growth is essential. Foremost among the conditions which must be standardized is the culture medium and/or substratum. All but two species examined in the present study were described in part from cultures grown on a chemically defined medium. The medium used was a modification of the one recommended by Scott, et al. (1963), which will henceforth be referred to as MSPS. The composition of MSPS is given in table 1. The requirements of different species and isolates, varied and frequently dilutions of the medium were made to meet the needs of the particular isolate under study. The object of this procedure was the same: to secure the maximum production of sexual structures. All dilutions reported were made prior to the addition of agar. Twenty-five-ml aliquots of medium were pipetted into

screw-capped "Kimble" culture tubes, autoclaved at 15 pounds pressure for 15 minutes and stored at 5°C until needed. All culture vessels used were 20 X 100 mm sterile, polystyrene Petri dishes containing 25 ml of medium.

Single zoospore cultures were prepared using inoculum taken from a stock culture and grown on MSPS with 2 per cent agar in a Petri dish. When the colony was approximately 3-5 cm in diameter, sporulation was initiated as previously described. Subsequently, the spore suspension was dropped onto a fresh agar plate and incubated at 18°C from 6 to 24 hours, or until the germ tubes were approximately 10-20 μ long, which insured the isolation of viable spores. Well-separated sporelings were located with the aid of a dissecting microscope and by using a micro-scapel fashioned out of a platnium wire, a small bit of agar, with a sporeling was removed and inoculated into a Petri dish containing the culture medium. All cultures were maintained at 18°C.

Preservation

Any fungus that is of value interest because it has been used in fundamental research should be preserved to insure its identity for subsequent use or reference. In the present study a serious attempt was made to maintain in a viable state all isolates studied.

The mineral oil method, as outlined by Buell and Weston (1947) and shown by Reicher (1949) and Goldie-Smith (1956) to be applicable for the preservation of saprolegniaceous fungi, was adopted as the standard method of preserving cultures of Saprolegnia.

Unifungal, bacteria-free isolates to be preserved were grown on

slants of MSPS agar in screw-capped "Kimble" culture tubes containing 8 to 10-ml. of medium. Normally, five days of growth was sufficient to insure a vigorously growing colony.

Fisher, U.S.P. paraffin oil (viscosity 335/350) was autoclaved in half-filled, 250-ml., cotton-stoppered, Erhlenmeyer flasks at 15 pounds pressure for 45 minutes. After "clearing" for one to two days, the oil was dispensed aseptically into a 1000-ml. sterile, separatory funnel as described by Buell and Weston (1947). The oil was then poured over the colony to a depth of about 1 cm. above the uppermost extent of the agar. All preserved cultures were stored upright and maintained at 5°C. Transfer from "oiled" cultures was easy and direct. A bit of inoculum on a piece of agar substrate was removed by means of a sharpened "spoonula" and planted onto a fresh agar slant, agar plate, or into distilled water. Although all isolates have remained viable for 26 months, transfers are made every 12 months. By this method a total of 864 isolates were obtained and are in the possession of the writer. Duplicate cultures have been deposited in the mycological culture collection at Virginia Polytechnic Institute.

Permanently preserved specimens are generally considered to be undesirable since the various mounting media may oftentimes distort valuable taxonomic characters. Ammans' and lacto-phenol have been used previously for preparing permanent mounts of saprolegniaceous fungi (Scott, 1961; Johnson, 1956). Permanent mounts, however well prepared, cannot under any condition replace carefully handled living cultures. Preliminary experiments on preserved material versus living cultures indicated that preserved specimens could be the source of

erroneous impressions concerning the diminsions, shape, and appearance of structures, as well as, the loss of the oil reserve in the mature oospore. The results confirmed the writer's opinion that permanent mounts are not reliable for descriptive data, regardless of how valuable they may be for indicating the distribution of species.

Johnson (1956) has maintained viable cultures of Achlya for periods of from 14 to 28 months on hemp seeds in quart jars two-thirds full of sterile distilled water. Although the method was tried and found worthy, the size of the resultant collection was prohibitive to its continuation in this investigation.

Material Examined

The systematic treatment presented here was based upon the comparative study of 864 isolates of Saprolegnia in culture. Of the 15 species now recognized within the limits of the genus, the writer has been able to examine living isolates of all but two species. Where it was necessary to utilize previously published descriptions in lieu of living specimens, the descriptions were made in the interest of conciseness and in keeping with the format of the descriptions compiled from living material.

The preparation of material for study was as follows: approximately 1 cm^2 block was cut from the culture surface and placed on a clean glass slide. Using a fine stream of water from a wash bottle the hyphae were spread out, a cover glass added, and the culture examined. Hemp seed cultures were treated in a similar manner, except the hyphae were cut from the seed before mounting. In all cases measurements were made with a Leitz "Ortholux" microscope using the Pl 40/0.65 objective and

10X oculars. On the basis of preliminary studies the measurement of 100 sexual structures was found to be enough to give a representative sample of a given isolate. Drawings were made with a camera lucida attachment and measurements, with a calibrated ocular micrometer.

MORPHOLOGY AND TAXONOMIC CRITERIA

The taxonomy of saprolegniaceous fungi is based entirely on the morphology of the sexual reproduction organs and, particularly, on the structure and shape of the oogonial wall, the origin of the antheridial branches, and the nature of the mature oospore. An understanding of the terminology involved is necessary before an attempt is made to delve into the descriptive portion of this paper. It is desirable, to elucidate the terminology and to discuss the morphology of each of the pertinent structures prior to the subsequent descriptive considerations.

Mycelium

The mycelium of Saprolegnia is easily visible in culture, where it forms a profuse mass of irregularly branched, cylindrical, multinucleate, coenocytic hyphae which develop radially from the submerged substrate and extend outward into the surrounding medium. Although capable of unlimited growth, the length of the extramatrical filaments is determined to a large extent by the environment. Different species and even different isolates of the same species vary greatly in the diameter of their hyphae; the range extends approximately from 10-250 microns or more in diameter. The hyaline hyphal walls give a cellulose

reaction upon treatment with chloroiodide of zinc. Young actively growing hyphae are usually densely packed with coarse, granular cytoplasm, the apices sometimes appearing light brown in mass. Scattered throughout the cytoplasm are small elongate vacuoles, numerous nuclei, and highly refractive oil droplets. In older filaments the cytoplasm is limited to a thin peripheral layer surrounding a large vacuole, and with age the hyphae appear empty. Although the hyphae are predominately coenocytic, septa are normally formed to delimit oogonial and antheridial cells, zoosporangia, gemmae, injuries, and infectious parasites such as Olpidiopsis. The gemmae are simply distended parts of the hyphae, of variable size and shape, within which occur an accumulation of protoplasm. Gemmae may eventually disarticulate but usually remain attached. Upon the advent of favorable conditions they germinate by the direct conversion into zoosporangia or by the formation of hyphae.

The vegetative mycelium of Saprolegnia has little to distinguish it from that of other water mold genera and, since there are innumerable variations in hyphal characteristics even within a single isolate, such features are of no taxonomic value.

Zoosporangium

In most species, asexual reproduction is by the development of zoospores produced within zoosporangia. The filaments destined to become zoosporangia are very similar in appearance to young, vigrously growing hyphae. There is an accumulation of protoplasm, apparently from the older portions of the hyphae, until the zoosporangial initial is filled with dense, granular protoplasm surrounding a reduced central

vacoule. At the base of the initial a septum is formed delimiting it from the supporting hypha.

The first indication that an initial is to produce zoospores is the appearance of irregular lines of cleavage extending outward from the central vacoule. These increase in number, connect with one another, and delimit the spore initials into irregular polygonal uninucleate masses. The number of polygonal masses cleaved out depends upon the number of nuclei in the sporangial initial at the time of delimitation. Appearing simultaneously with cleavage is the formation of an apical outgrowth, or papilla, on the sporangium. Cleavage is followed by a homogeneous stage in which the plasma membrane bursts, and part of the cell sap is expelled, accompanied by a loss of turgidity of the sporangium. At this point the spore initials absorb the expelled cell sap or water from the surrounding medium, and cleavage lines or extensions of the plasma membrane suddenly become visible due to the increase in size of the initials. Small vacoules appear and disappear in these initials, the contents of which become less granular. This entire process may proceed as a wave down the length of the sporangium and may be completed in two to three seconds. The small vacoules vanish, the cytoplasm again becomes granular, and the zoospore initials contract and separate along the old cleavage lines into rounded bodies.

As the spores acquire their ultimate shape, those near the apex of the zoosporangium exhibit a slow trembling motion. Upon discharge the primary zoospores emerge one by one in rapid succession, through an opening developed at the tip of the papilla, and swim away. This manner

of zoospore discharge (saprolegnoid) is typical for all species under normal environmental conditions and is the fundamental characteristic which serves to separate Saprolegnia from closely related genera.

Less frequently, under contaminated cultural conditions or when cultured upon semi-solid media, primary zoospores may fail to emerge and may encyst within the zoosporangium. Such spores may subsequently germinate by the formation of germ tubes, which protrude through the wall (aplanoid), or secondary zoospores may emerge through the sporangial wall, wherein a ladder-like network of empty cysts remain within the zoosporangium (dictyoid). Only in rare instances is the achlyoid type of discharge, characteristic of Achlya and Aphanomyces, observed. In the latter the primary spores emerge and encyst at the orifice in the form of a loose, spherical cluster.

Frequently after a zoosporangium has emptied, the hypha may grow up into the empty space, filling it, and becoming a secondary sporangium. The formation of sporangia by internal proliferation in such a manner may occur repeatedly and is a common method of zoosporangial renewal for most taxa. In some taxa, however, instead of proliferating, the hypha immediately below the emptied sporangium may branch laterally. The filament may grow upward to form a new sporangium parallel to the empty one. The sympodial formation of sporangia was previously used to distinguish Isolachlya from Saprolegnia. The separation of genera on such a variable and inconsistant characteristic is inexplicable. Coker (1923), who recognized Isolachlya, frequently illustrated species of Saprolegnia with sympodial sporangia, but retained them in the genus

Saprolegnia.

Primary Zoospore

The anteriorly biflagellate primary zoospores have a somewhat pyriform or "pip-shaped" body with two subapically attached flagella. The flagella are almost equal in length and are attached near the anterior end. One flagellum is directed backward and is of the whip-lash type, whereas the other is directed forward and is of the tinsel type (Couch, 1941). After swimming for some time, the spore begins to swim quickly in ever decreasing circles, suddenly stops, resorbs the flagella, and encysts (McKeen, 1963). After a period of encyst-ment lasting a few minutes to several hours, the primary cyst may germinate either by germ tube or the contents of the cyst may emerge as laterally biflagellated secondary spores. Meier and Webster (1954) have found that empty cyst membranes are essentially smooth-walled, although some bear tufts of radiate hairs that are believed to be the remains of flagella. The primary zoospores are very similar in size within any particular isolate, except those of Saprolegnia anisospora. In this species three size-groups of spores are to be found ranging from small (9 μ) to intermediate (14 μ), and 32 μ in "giant" spores. This characteristic appears to be constant in the isolates studied, but is of minor significance when compared with the combination of sexual characters. So far as it is known this is the only species in the family with variable-sized spores.

Secondary Zoospore

The secondary spore emerges from the cyst within two to three seconds and remains stationary just outside the empty membrane. Twenty to thirty minutes later the secondary spores begin to move convulsively, become more reniform and suddenly swim away. Both flagella arise from a shallow groove on the body; the anterior flagellum being distinctly shorter than it was on the primary spore and its tinsel hairs more conspicuous (Couch, 1941). The longer posterior flagellum is of the usual whiplash type. In contrast to the primary spore, the secondary spore is an extremely vigorous and durable swimmer. The reason for this may be the fin-like construction of the posterior flagellum (Manton, et al., 1951). The spore swims in a spiral path, rotating on its own axis. Couch (1941) points out that both flagella are active in contrast with the generally accepted idea that the posterior flagellum is dragged passively along behind and functions mainly as a rudder. However, McKeen (1962) is of the opinion that the posterior flagellum propels the spore and the anterior flagellum serves as a rudder. After swimming for a period of time, the secondary spore sheds the flagella (McKeen, 1962), may continue to jerk for a few seconds, encysts and may germinate by a germ tube or by forming another secondary spore. Since many of the so-called diplanetic forms are in reality "polyplanetic", due to repeated emergences (Weston, 1919; Salvin, 1940, 1941), Hohnk (1933) proposed the term "dimorphic" for these forms, placing emphasis on spore morphology. Johannes (1955) used polyplanetism as one characteristic to separate his genus, Cladolegnia, from "diplanetic" genera.

Since repeated emergences are controlled to a large extent by the environment, this character is of little diagnostic value. The secondary cyst membranes possess double-headed hooks scattered over the surface (Manton, et al., 1951; Meier and Webster, 1954). The latter investigators believe the hooks may assist in attaching the secondary cysts to the substrate. They also believe that more extensive study of cysts may show them to be of value in determining relationships within the family. The structure of the cyst of Saprolegnia ferax and S. diclina are very similar to cysts of Isolachlya eccentrica and I. unispora, whereas the cysts of closely related genera are distinctly different. The germination of the secondary cyst by a single germ tube to form a branched mycelium, completes the cycle of asexual reproduction.

Oogonium

Within a short time following the cessation of asexual reproduction or less frequently, simultaneously with zoospore formation, sexual reproductive structures appear on the somatic hyphae. This is the usual sequence of events in most species cultivated on hemp seed. However, under carefully controlled conditions it is possible to manipulate a culture so that the formation of sexual structures may occur with complete suppression of asexual reproduction. This is of particular interest when only a single spore isolate is being studied.

The "female" gametangia or oogonia are borne laterally on branches of varying length, intercalary or terminally on the somatic hyphae and are quite variable in size, shape, and wall morphology. Only in extremely

clear-cut cases have the first two features been relied upon for species determinations. The apex of the hypha, destined to become an oogonium, begins to enlarge until the tip is several times the diameter of the sub-attendant hypha. The enlarging oogonium is filled with densely packed alveolar cytoplasm and numerous nuclei which lie equidistant from one another. At this point the oogonial initial is cut off from its concomitant hypha by a transverse septum, the subtending portion then being termed the "oogonial stalk". In some species the septum may protrude upwards into the oogonium as a columella-like structure (pl. II, figs. 1-2), while in others it is formed at the base of the initial, or sometimes lower within the oogonial stalk. The shape of the oogonium often depends upon the position of the septum which is usually quite variable. As enlargement continues the central vacuole forces the multinucleate cytoplasm toward the periphery of the oogonial wall. Blunt furrows extending centrifugally from the vacuolar membrane eventually cleave the protoplast into a number of conical masses. These units of protoplasm begin to swell and become rounded to form oospheres which, when fully mature, usually appear spherical or less frequently ellipsoidal. The number of oospheres range from one to three in some species, to over forty in others. Utilizing the egg number as a fundamental taxonomic feature, Coker (1923) separated Saprolegnia into two natural subgenera: Eusaprolegnia, with more than two eggs per oogonium, and Pseudosaprolegnia, with only one or two eggs per oogonium. Although this character may well be used to separate the genus into two sub-taxa, it is much too variable in the writer's opinion

for this purpose. In species with single oospores, the protoplast, instead of developing centrifugally, proceeds centripetally toward the center of the initial leaving a clear area around the periphery of the wall. The coarse, granular appearance of the oosphere contents change, oil droplets begin to appear and the entire structure is surrounded by a thin membrane.

Frequently, during the thickening of the oogonial wall, circular unthickened areas or "pits" may remain scattered over the surface (pl. II, figs. 1-4). Such pits are usually conspicuous, but are often very difficult to discern. Moreover, in few species, isolates may show a gradation from forms with no pits to forms that are conspicuously pitted. Although pitting is not an absolute character, it is still of taxonomic value in separating closely related species.

Some species form oogonia whose outer walls are ornamented with blunt or rounded papillae (pl. I, figs. 1-9). These papillae vary considerably in size, shape, and length, but are quite consistant in their appearance in any one species. One species, S. terrestris exhibits an intermediate type of ornamentation, in which the oogonial wall is sometimes wavy or irregulary roughened or may be extended into short papillae (pl. III, fig. 2).

Antheridium

At approximately the same time, or soon after the formation of the oogonial initials, antheridial branches arise, rapidly elongate, and ultimately make contact with the oogonial wall in a manner charac-

teristic for the species concerned. The terminal portion of these antheridia enlarge slightly and become filled with a non-vacuolate mass of cytoplasm in which there are several nuclei. Shortly afterward a septum is formed delimiting the antheridial cell from its subtending branch. Both the origin of the antheridial branch and the method of antheridial cell attachment to the oogonium are important species characteristics. The antheridial cell may be attached along its entire length (laterally appressed, pl. II, fig. 3), or only by its apical end (apically appressed, pl. 1, fig. 6). In some species antheridia may be attached in both ways or even by foot-like projections (pl. III, fig. 4). Although variations occur among the different species and sometimes among isolates of the same species, the origin and morphology of the antheridial branch is, nevertheless, a diagnostic character. Based on their point of origin, four types may be distinguished as follows:

Androgynous: the antheridial branch arising from the oogonial stalk to which it is attached, but with the antheridial cell not a part of the oogonial stalk (pl. I, fig. 1).

Diclinous: with the oogonium and its attendant antheridial branch originating on different hyphae (pl. II, figs. 5-8).

Hypogynous: the antheridial branch lacking, but with the antheridial cell abstricted as a part of the oogonial stalk immediately below the oogonium and frequently extending up into the oogonial cavity (pl. II, figs. 1-2).

Monoclinous: the oogonium and its attending antheridial branch

originating on the same hyphae (pl. II, fig. 3).

The number of antheridia per oogonum has led to considerable confusion in delimiting species. In some species, such as S. ferax, antheridia may attend all oogonia, a percentage of the oogonia, or may be completely lacking, depending upon the cultural conditions. When antheridia are completely lacking the eggs apparently mature parthenogenetically (apogamously).

Following attachment the antheridial cell may develop one or more delicate fertilization tubes (pl. II, figs. 1-3) that pentrate the oogonial wall and make contact with one or more eggs. Whether or not fertilization always takes place if antheridia are present has been a matter of considerable discussion in the past (Coker, 1923). Apparently fertilization is effected by a migration of an antheridial nucleus, and possibly some of the cytoplasm, into the oosphere where the two gamete nuclei unite with each other.

Oospore

After fertilization each oospore develops a thick wall and one or more oil droplets appear in the finely granular cytoplasm. A. deBary (1881) was the first to realize the significance of the mature oospore and described two types, centric and eccentric. Coker (1923) observed intergradations between these two types and introduced the term subcentric. Dick (1960) proposed still another type which he called subeccentric. The arrangement of the oil droplets within the ooplasm of the mature oospore is of considerable taxonomic signifigance and was employed by Johnson (1959) to separate species of Achlya into sub-genera.

Briefly, the four types of oospores may be defined as follows:

Centric: with one or two peripheral layers of small oil droplets completely surrounding the central ooplasm (pl. II, fig. 8-9).

Eccentric: with one large oil droplet located on one side of the oospore and not entirely encircled by the ooplasm (pl. I, fig. 9, 11).

Subcentric: with one layer of small oil droplets on one side of the oospore and two or more on the opposite side (pl. I, fig. 7).

Subeccentric: with one layer of oil droplets on one side of the oospore forming a "cap" over the ooplasm (pl. I, fig. 9, 11).

Although it is generally believed that meiosis occurs at the time of oospore germination, Sansome (1962) recently reported meiosis occurring in the antheridia and oogonia of Pythium. Further investigation of this phenomenon in Saprolegnia may reveal some very interesting results.

The first visible indication of oospore germination is the breaking up of the oil droplets and a gradually darking of the ooplasm followed by a definite increase in the size of the egg. Several hours later the wall becomes quite thin and a large central vacuole develops. A small protrusion appears on the surface of the oospore, rapidly elongates and penetrates the oogonial wall either through a pit or by the breakdown of the wall itself. After passing to the outside, the tube either elongates and reestablishes the mycelium, or a cross-wall is formed delimiting a zoosporangium at the apex. Zoospore discharge is typically "saprolegnoid".

Before preceeding on to the systematic section of this paper, a note of explanation is required here concerning the treatment of the various taxa.

It is readily apparent to the student of the water molds that the concept of a population of Saprolegnia is extremely difficult, unless one wishes to consider all the individuals of a cosmopolitan species as constituting a population. Earlier students of these fungi frequently used such infraspecific entities as "variety" and "forms" in a quite indescriminate manner. Mostly such "forms" and "varieties" have been based on one or a few specimens, while others have been established solely on the basis of such variable characters as zoosporangia.

To use such taxa as "forms" or "varieties" within the context of modern population taxonomy is erroneous. These entities were estimated on the basis of a "type species" which cannot be considered as analogous to ecological or "genomic variants" of some well-studied seed plants. Such catagories are not acceptable for a taxonomic treatment of the lower phycomycetous fungi.

SYSTEMATIC TREATMENT

Saprolegnia C. G. Nees

Nova Acta Acad. Leop. -Carol., 11: 514. 1823

Diplanes Leitgeb, Jahrb. wiss. Bot. 7: 374. 1869.

Isoachlya Kauffman, Amer. J. Bot. 8: 231. 1921.

Cladolegnia Johannes, Repert. spec. nov. reg. veg. 58: 211. 1955.

Thalli monoecious. Hyphae stout or delicate, branched or unbranched,

straight or flexuous, gradually tapering from base to apex; variable in length and diameter. Gemmae, when present, formed by segmentation of the hyphae; variable in size and shape; functioning as zoosporangia or oogonia or germinating by one or more slender hyphae. Zoosporangia filiform, cylindrical, clavate, or irregular; terminal at first; renewed by internal proliferation, sympodially or by basipetalous development and cymose branching. Zoospores dimorphic; primary zoospores usually pyriform with two apical flagella; swimming away from the zoosporangial orifice upon discharge, soon afterward encysting; primary cyst germinating by secondary reniform, laterally biflagellate zoospores; encysted secondary cysts germinating by a slender hypha or by secondary zoospore; polyplanetic; in a few species, aplanoid or dictyoid discharge also present. Oogonia born variously, being lateral on stalks of variable length, terminal, intercalary, or sessile; variously shaped, predominantly spherical or pyriform. Oogonial walls with or without ornamentations; pitted or unpitted. Oospheres generally maturing. Oospores one to many; centric, subcentric, eccentric or subeccentric; variable in size; spherical or ellipsoidal. Antheridial branches, when present, diclinous, monoclinous, androgynous, or in one species hypogynous. Antheridial cells predominantly tubular and clavate; laterally or apically appressed to the oogonial wall or attached by finger-like projections; fertilization tubes usually present. Oospore germination, when present, either by hyphae or by a slender germ tube terminating in a zoosporangium.

Type species: Saprolegnia ferax (Gruith.) Thuret, Ann. Sci. Nat.

III, 14: 214, pl. 22. 1850.

Key to the Species of Saprolegnia

1. Oogonial wall densely ornamented on outer surface ..... S. asterophora, p. 33

1. Oogonial wall smooth or papillate 2

2. Oospores generally one or two 13

2. Oospores generally more than two 3

3. Oogonial wall prominently papillate; antheridial branches lacking ..... S. latvica, p. 36

3. Oogonial wall smooth; antheridial branches present or lacking 4

4. Mature oospores eccentric or subeccentric; zoospores of two sizes ..... S. anisospora, p. 37

4. Mature oospores centric or subcentric; zoospores nearly uniform in size 5

5. Antheridial branches lacking; antheridial cells hypogynous ..... S. hypogyna, p. 40

5. Antheridial branches present or lacking; antheridial cells never hypogynous 6

6. Antheridial branches completly lacking or present in variable numbers ..... S. ferax, p. 43

6. Antheridial branches present, never lacking 7

7. Antheridial branches predominantly diclinous, rarely androgynous or monoclinous 8

7. Antheridial branches predominantly androgynous, monoclinous, rarely diclinous 9

8. Mature oospores subcentric; oogonial wall unpitted ..... S. parasitica, p. 51

8. Mature oospores centric; oogonial wall pitted or unpitted ..... S. diclina, p. 56

9. Antheridial branches predominantly androgynous 10

9. Antheridial branches predominantly monoclinous, or diclinous, rarely androgynous ..... S. ferax, p. 43

10. Oogonial stalks 1-6 times coiled, frequently pendant ..... S. furcata, p. 61

10. Oogonial stalks, when present, straight or bent; never coiled 11

11. Oogonial wall thick, with numerous conspicuous pits; oogonia mostly lateral on short stalks..... S. turfosa, p. 64

11. Oogonial wall thin, with few or no pits; oogonia mostly terminal 12

12. Mature oospores subcentric; antheridial branches usually less than three ..... S. terrestris, p. 67

12. Mature oospores centric; antheridial branches usually numerous ..... S. litoralis, p. 69

13. Antheridial branches lacking 14

13. Antheridial branches present, never lacking 15

14. Mature oospores eccentric ..... S. eccentrica, p. 70

14. Mature oospores not eccentric ..... S. unispora, p. 72

15. Antheridial branches predominantly monoclinous; oogonial wall smooth ..... S. megasperma, p. 73

15. Antheridial branches predominantly androgynous; oogonial wall

often extended into one or more short papillae. S. intermedia, p. 74

Saprolegnia asterophora deBary

Jahrb. wiss. Bot. 2: 189, pl. 20, figs. 25-27. 1860

(Pl. I, Figs. 1-9)

Cladolegnia asterophora (deBary) Johannes, Repert. spec. nov. reg. veg. 58: 215. 1955.

Hyphae slender flaccid, often undulate, 10-40 μ in diameter, predominantly 12-14 μ, sparingly branched: Gemmae sparse or absent; infrequently clavate or irregular: Zoosporangia rare or abundant (see discussion); clavate or filiform, occasionally irregular; 120-821 μ long by 12-43 μ in diameter, predominantly 340-485 X 26-34 μ; renewed by internal proliferation or in basipetalous succession, occasionally lateral. Zoospore discharge saprolegnoid; encysted primary spores 12-14 μ in diameter. Oogonia moderately abundant; lateral, occasionally terminal, rarely intercalary; spherical or oval; 36-78 μ in diameter, predominantly 44-52 μ, inclusive of wall ornamentations. Oogonial wall unpitted; densely ornamentated, ornamentations short, (2-10 μ lmg) blunt or rounded papillae, occasionally apiculate (up to 20 μ). Oogonial stalks usually long; slender; straight, often branched, very rarely beset with few papillae. Oospheres maturing. Oospores subcentric or subeccentric; spherical or ovoid, filling the oogonium; 1-6 in number, usually 1-2; 20-36 μ in diameter, predominantly 29-32 μ; Antheridial stalks variable in frequency, usually numerous; androgynous, rarely monoclinous or very rarely diclinous; short; usually same diameter as supporting oogonial stalk; often branched and appearing contorted;

occasionally irregular; 1-2 on an oogonium. Antheridial cells simple, short-clavate, often tuberous, apically or laterally appressed; fertilization tubes not observed. Oospore germination not observed.

Material examined: Netherlands, A. L. Van Beverwijk (CBS); England, L. G. Willoughby; Virginia, R. Seymour.

Distribution: Coker (1923: pl. 19), North Carolina; Apinis (1930), Latvia; Van Beverwijk (1948), Netherlands; Beneke (1948), Illinois; Miller (1965) Virginia; Petersen (1909, 1910), Lund (1934), Denmark; Ivimey-Cook and Morgan (1934), M. W. Dick (1960: figs. 1,2,3M), Dick and Newby (1961), Dick (1962; 1963), Perrott (1960), Forbes (1935), Roberts (1963), Sparrow (1936), England; Hohnk (1935), Haryen (1928), Minden (1912: fig. 1f), deBary (loc. cit., 1881, 1888), Fischer, (1892), Germany; Humphrey (1893, legit Trelease), Massachusetts; Istvanffi (1895), USSR; Ookuba (1954), Japan, Cejp (1959: 253, fig. 96) Czechoslovakia.

Discussion: Saprolegnia asterophora deBary is easily recognized by its densely papillate oogonia, prevalence of one or two oospores in an oogonium, scarcity of zoosporangia, and by its thin, flaccid hyphae. In some respects, S. asterophora bears resemblence to S. latvica, which is also papillate and has thin, flacid hyphae. The former species, however, is readily separated on the basis of more numerous papillae and unpitted oogonial walls. Furthermore, these taxa may be distinguished by the antheridia which are lacking in S. latvica; and by the structure of the oospores, which are centric in the latter species. DeBary (loc. cit.) in his description reported centric oospores for

the "type" material. Fischer (1892) also reported such an oospore structure, as did Humphrey (1893). The latter investigator admittedly referred to deBary's description for some details, since he was observing preserved material obtained from Trelease. The illustrations of these authors give no indication as to the structure of the oospores. Since the term subcentric was not used until 1923 by Coker, and has been used by subsequent workers in describing the structure of the oospore, it is quite possible the earlier workers were also observing subcentric eggs.

Recently, Dick (1960) in his description of S. asterophora described his isolate as having subeccentric oospores (see page 28). His isolate is of particular interest in that it differs from the "typical" forms in abundance of zoosporangia, scarcity of antheridia, and in the above mentioned egg type. Several other different forms have been recently isolated by Dick and may represent specific rand (personal communication).

Based on S. asterophora, Coker (1923) erected the subgenus Pseudosaprolegnia. However, it is the opinion of the writer that the subgeneric concept is now little more than a convenience and since it shows no natural relationship it is excluded from consideration here. There is little uniformity of subgeneric concepts in related genera. Johnson (1959), for example, established subgenera based on oospore structure in Achlya and Scott (1961) used oogonial wall ornamentation in establishing subgenera of Aphanomyces.

It should be noted here for sake of clarity that Coker and Matthews

(1937) and Beneke (1948) incorrectly cited deBary's 1860 publication as 1859.

Judging from the majority of existing reports in the literature, most investigators regard S. asterophora as rare, although it is widespread in distribution. This writer has isolated the fungus only once from bog soil, whereas Dick (1960) found it not uncommonly in damp, strongly acid soils of England.

Saprolegnia latvica Apinis

Acta Horti Bot. Univ. Latv., 4: 211, pl. 1, figs. 1-12. 1930 Archilegnia latvica Apinis, Acta Horti Bot. Univ. Latv., 8: 103, pl. 1, figs. 1-15. 1935.

Hyphae slender and flaccid. Gemmae absent or sparse; globose or oval. Zoosporangia cylindrical; 65-210 μ long X 32-48 μ in diameter; renewed by internal proliferation. Zoospore discharge saprolegnoid; zoospores 12-20 μ in diameter. Oogonia terminal, lateral, occasionally intercalary; spherical or pyriform, cylindrical when intercalary; 30-110 μ in diameter. Oogonial stalks short; straight. Oogonial wall unpitted; moderately ornamented with papillate projections, 7-10 μ long, apex of ornamentations thin-walled. Oospores centric; spherical; 1-30 in number, generally 3-10; 20-28 μ in diameter, predominantly 24-26 μ. Antheridial branches lacking. Antheridial cells lacking. (Modified from Apinis, Acta Horti Bot. Univ. Latv., 4: 211. 1930 and Stpiczynska. Mono. Bot., 15: 423-424. 1963.)

Distribution: Apinis (loc. cit.), Latvia; Lund (1934: fig. 5), Denmark; Roberts (1963), Roberts (1963; legit Howarth), Apinis (personal

communication), England; Stpiczynska (1963: fig. 1), Poland.

Discussion: This very distinct species is presently known to only a few European workers and is apparently one of the rarest members of the genus. According to their reports, S. latvica has been collected exclusively from Sphagnum bogs or from highly acid waters. Extensive sampling of three Sphagnum bogs, in an effort to isolate this fungus, was unsuccessful. Unfortunately, Apinis, Roberts, and Stpiczynska (personal communication) have not maintained this species in culture. Consequently, the writer has not observed this species. The above description of Saprolegnia latvica has been compiled from the reports of Apinis (1929) and Stpiczynska (1963).

Apinis (1935) described as Archilegnia latvica, a saprolegniaceous fungus in which the eggs are said to be fertilized by minute uniflagellate free-swimming male gametes. As has been pointed out by Coker and Matthews (1937), recognition of such an aberrant organism must be withheld until a further study is made. From the figures given, it is entirely possible that the material carried a persistent monad infection (Sparrow, 1960). Apinis has recently (personal communication) concurred with this disposition of his fungus, Archilegnia latvica.

Saprolegnia anisospora deBary

Bot. Zeit. 46: 619, pl. 9, fig. 4. 1888

(Pl. I, Figs. 11-12)

Isoachlya anisospora (deBary) Coker, North Amer. Flora, 2(1): 26. 1937.

Isoachlya anisospora var. indica Saksena and Bhargava, Curr. Sci., 13: 79. 1944.

Hyphae slender, usually delicate, sparingly to moderately branched, 10-40 μ in diameter, predominantly 18-28 μ. Gemmae usually abundant; pyriform; occasionally irregular; terminal and often catenulate, rarely intercalary and single; functioning as zoosporangia. Zoosporangia abundant; cylindrical or clavate; generally curved or irregular; 60-195 μ long by 16-32 μ in diameter; renewed by internal proliferation, frequently cymosely or in basipetalous succession. Zoospore discharge saprolegnoid, rarely dictyoid; spores commonly in three size groups; large (25-33 μ X 10-12 μ), intermediate (20-24 μ X 10-13 μ), small (11-14 μ X 8-11 μ); usually in separate sporangia, rarely mixed; enapted primary spores 10-18 μ in diameter. Oogonia abundant; terminal, often intercalary, occasionally lateral or cymose; spherical or pyriform, doliform or lageniform when internal; 38-87 μ in diameter, predominantly 46-60 μ. Oogonial wall smooth; pitted only under point of attachment of antheridial cells, frequently obscure. Oogonial stalks usually less than the diameter of the oogonia length; stout; straight or sometimes slightly bent. Oospheres maturing. Oospores thick or thin-walled; eccentric or subeccentric; spherical, generally filling the oogonium; 1-18 in number, generally 4-6; 16-34 μ in diameter, predominantly ramerous; 18-24 μ. Antheridial branches diclinous, occasionally to extremely rarely monoclinous or androgynous; slender, often becoming inconspicuous; simple. Antheridial cells tubular or clavate; simple or branched; laterally appressed; fertilization tubes present, not persistent. Oospore germination not observed.

Material examined: Netherlands, A. L. Van Beverwijk (from the

Centraalbureau voor Schimmelcultures, Baarn); India, K. S. Bhargava; (sub-culture from the type material of S. anisospora var. indica).

Distribution: Coker (1923: pl. 7-10), North Carolina, Harvey (in Coker and Matthews, 1931: 27), Montana; Obel (1910a; 1910b, Denmark; Van Beverwijk (1948), Netherlands; Nagai (1931: 5, pl. 1, figs. 22-30), Japan; Fischer (1892), Minden (1915: 619, pl. 9, fig. 4), deBary (loc. cit.), Germany; Chaudhuri, et.al. (1947), Saksena and Bhargave (loc. cit.), India; Apinis (1930: 221, pl. 3, figs. 1-3), Latvia; Roberts (1963), Newby (1948), Dick and Newby (1961), Dick (1963), England; Cejp (1959: 193, figs. 67-68), Czechoslovakia.

Discussion: As the specific epithet implies, the zoospores of Saprolegnia anisospora deBary are of unequal size. The characteristic size differences among zoospores, together with diclinous antheridial branches and eccentric oospores, readily distinguishes Saprolegnia anisospora from S. diclina.

There has been some controversy as to what constitutes the structure of mature oospores in this species. Although deBary (loc. cit.) described and clearly illustrated eccentric oospores in the original diagnosis, Coker (1923) thought that deBary s observations were erroneous and that his figures showed oospores which were breaking down. This writer in agreement with Newby (1948) that Coker (1923) was probably observing immature oospores, which often appear to be centric. In 1937 Coker corrected his former statement and agreed with deBary that the oospores have an eccentric arrangement.

In many instances other conditions of the oospores have been

observed. Often two or more smaller oil droplets were present instead of a single large droplet, but were arranged upon an eccentric plan (see pl. 1, figs. 11, 13). Newby (1948) observed a similar condition, but suggested that since the walls of "multi-droplet" oospores are relatively thin they may represent immature stages in which several oil droplets have not yet coalesced. In an effort to avoid further confusion, this writer proposes the term subeccentric (see page 28) be used in connection with the "multi-droplet" oospores.

The frequent cymose renewal of secondary zoosporangia prompted Coker (in Coker, 1937) to transfer this species to the genus Isoachlya, a change already suggested by Apinis (1929). Since the genus Isoachlya is considered a synonymous entity, Isoachlya anisospora is hereby reduced to synonomy.

Saksena and Bhargava (1944) described, Isolachlya anisospora var. indica, which differs in the structure of the oospore by its centric or subcentric, but never eccentric form. I have examined an isolate of this variety, kindly furnished by Dr. K. S. Bhargava, which is no different from typical S. anisospora. Apparently these investigators committed the same error as Coker (1923) in describing immature oospores.

Saprolegnia hypogyna (Pringsheim) deBary

Bot. Zeit., 41: 56. 1883

(Pl. II, Figs. 1-2)

Saprolegnia ferax var. hypogyna Pringsheim, Jahrb. wiss. Bot., 9: 191, pl. 18, figs. 5, 9, 10. 1873.

Saprolegnia hypogyna var. II Maurizio, Flora, 79: 109, pl. 4-5, figs. 13-16. 1894.

Saprolegnia hypogyna var. III Maurizio, Ibid., figs. 17-20a.

Saprolegnia hypogyna var. IV Maurizio, Ibid., figs. 21-23.

Saprolegnia hypogyna var. V Maurizio, Ibid., figs. 24-27.

Saprolegnia intermedia Maurizio, Jahrb. wiss. Bot., 29: 97. 1896.

Saprolegnia hypogyna var. Coregoni Maurizio, Mittheil. Deutsch. Fischerei-Vereins, 7: 55. 1899.

Hyphae slender, sparingly branched, 18-55 μ in diameter. Gemmae sparse or abundant; pyriform or clavate, infrequently spherical; terminal, single or catenulate; functioning as zoosporangia. Zoosporangia abundant; filiform or clavate, straight or curved, occasionally irregular; 90-480 μ long by 13-38 μ in diameter, predominantly 100-265 X 18-27 μ; renewed by internal proliferation, rarely sympodially. Zoospore discharge saprolegnoid; encysted zoospores 10-13 μ in diameter. Oogonia abundant; terminal; infrequently intercalary, rarely lateral; spherical, infrequently pyriform, rarely doliform; 29-101 μ in diameter, predominantly 68-75 μ. Oogonial wall smooth; pitted. Oogonial stalks short or rather long when present; straight; slender. Oospheres maturing. Oospores centric; spherical; frequently filling only upper portion of oogonium; 1-20 in number, usually 7-10; 18-44 μ in diameter, predominantly 21 μ. Antheridial branches lacking. Hypogynous antheridial cells present as distal portion of oogonial stalk. Fertilization tubes arising apically from hypohynal cells; simple or branched, persistent. Oospore germination by a slender germ tube.

Material examined: England, L. G. Willoughby.

Distribution: Pringsheim (loc. cit.), deBary (loc. cit.; 1888:

615), Minden (1912: 520, fig.1f), Maurizio (loc. cit.), Germany; Tiesenhausen (1912: figs. 2-3), Switzerland; Gaumann (1918), Lapland; Lund (1934), Obel (1910a; 1910b), Petersen (1909; 1910), Denmark; Apinis (1930), Latvia; Cejp (1959: 215, fig. 77), Czechoslovakia; Kauffman (1908: 361, pl. 23), Perrott (1960), Michigan.

Discussion: Saprolegnia hypogyna (Pringsh.) deBary is the only species of Saprolegnia which has hypogynous (sub-Oogonial) antheridial cells (pl. 2, figs. 1-2).

This species was first described briefly by Pringsheim (loc. cit.) as Saprolegnia ferax, var. hypogyna, but was referred to as S. hypogyna in connection with his figures. Fifteen years later deBary (loc. cit.) isolated Pringsheim's var. hypogyna, which he considered worthy of specific rank and hence made the combination Saprolegnia hypogyna.

Maurizio (1894) described five varieties (I-V) as belonging to S. hypogyna. Although his five varieties have been reduced to synonomy, from the illustrations and measurements given, there seems to be no reason to believe that his variety I is at all distinct from Saprolegnia ferax. In the event the hypogynous antheridial cell is absent, the character which Maurizio based this variety upon, S. hypogyna var. I is indistinguishable from S. ferax. Furthermore, Lund (1934) reported S. hypogyna var. I from Denmark, apparently basing his identification on the absence of the hypogynous antheridial cell. There is little doubt that Maurizio s var. I and the var. I reported by Lund should not be regarded as S. ferax.

Saprolegnia intermedia, as described by Maurizio (1896), merely

represented a variant of S. hypogyna and was reduced to synonomy by Minden (1912). The latter author, however, overlooked Maurizio's (1899) later variety S. hypogyna var. Coregoni, which was subsequently considered synonomy by Cejp (1959).

The general morphology of the oogonia, i.e., size, shape and position, the large conspicuous pits, and the frequent basal ingrowth of the oogonial wall (pl. II, figs. 1-2) of S. hypogyna denote its close relationship to S. ferax.

Presently, S. hypogyna is widespread in Europe. The only account of this species outside Europe is that of Kauffman (1908).

Saprolegnia ferax (Gruith.) Thuret

Ann. Sci. Nat. 3, 14: 214, pl. 22. 1850

(Pl. II, Figs. 3-4)

Conferva ferax Gruithusen, Nova Acta Acad. Leop.-Carol., 10: 445. 1821.

Achlya prolifera Pringsh., Nova Acta Acad. Leop.-Carol., 23: 395, pl. 46, figs. 10, 15, pl. 50, figs. 1-5. 1851.

Saprolegnia monoica Pringsheim, Jahrb. wiss. Bot., 1: 292, pls. 19-20, 1858.

Saprolegnia dioica Pringsh., Jahrb. wiss. Bot., 2: 206, pl. 22, figs. 1-6, 1860.

Achlya intermedia Bail, Amtl. Ber. 35te Versamml. deutsch. naturf. Aerzte in Konigsberg (1860), 35: 257. 1861.

Diplanes saprolegnioides Leitgeb, Jahrb. wiss. Bot., 7: 385, pl. 24, 1869.

Saprolegnia dioica var. racemose de la Rue, Bull. Soc. Imp. Nat.

Moscou, 42 (1): 469. 1869.

Saprolegnia dioica Schroeter, in Schroeter and Schneider, Jahresb. Schles. Gesell. vaterl. Cultur, 1869, 47: 143. 1870.

Achlya ferax (Gruith.) Duncan, Proc. Roy. Soc. London, 25: 253. 1876.

Saprolegnia Thureti deBary, Abhandl. Senck. Naturforsch. Gesell., 12: 326, pl. 5, figs. 1-10. 1881. (Also in Morph. u. Phys. der Pilze, IV Riehe: 102. 1881)

Saprolegnia mixta deBary, Bot. Zeit., 41: 38, 54. 1883.

Saprolegnia monoica var. montana deBary, Bot. Zeit., 46: 617. 1888.

Saprolegnia hypogyna var. I Maurizio, Flora, 79: 109, pl. 4-5, figs. 5-12. 1894.

Saprolegnia esocina Maurizio, Jahrb. wiss. Bot., 29: 82, pl. 1, figs. 4-17. 1896.

Saprolegnia heterandro Maurizio, Jahrb. wiss. Bot., 29: 87, pl. 1, figs. 18-27. 1896.

Saprolegnia bodanica Maurizio, Jahrb. wiss. Bot., 29: 107, pl. 2, figs. 52-59a. 1896.

Saprolegnia paradoxa Maurizio, Mittheil. Deutsch. Fischerei-Vereins, 7, heft 1: 46, figs. 10-12. 1899.

Saprolegnia floccosa Maurizio, Mittheil. Deutsch. Fischerei-Vereins, 7: 50, figs. 16-17. 1899.

Saprolegnia semidioica Petersen, Bot. Tidsskrift, 29: 378. 1909. (Also in Ann. Mycol., 8: 519, fig. 1F. 1910.)

Saprolegnia monoica var. vexans Pieters, Bot. Gaz., 60: 489. 1915.

Saprolegnia var. Asplundii Gaumann, Botaniska Notiser, 1918-19: 155. 1918.

Saprolegnia lapponica Gaumann, ibid., p. 156. 1918.

Saprolegnia invaderis Davis and Lazar, Trans. Amer. Fish. Soc., 70: 267, figs. 1-6. 1940.

Saprolegnia monoica var. floccosa (Maur.) Cejp, Flora CSR. Oomycetes I, p. 234, fig. 86. 1959.

Saprolegnia ferax var. lapponica (Gaumann) Cejp, ibid., 245. 1959.

Saprolegnia ferax var. esocina (Maur.) Cejp, ibid., 246. 1959.

Material examined: W. R. Ivimey-Cook (from the Centraalbureau voor Schimmelcultures, Baarn; location unknown), Commonwealth Mycological Institute (location unknown); England, A. E. Apinis; Iowa, R. Crang; France, F. E. Moreau (from the C.B.S. as S. mixta); Utah, Virginia, West Virginia, A. H. O'Bier; Virginia, K. Nicely; Georgia, W. W. Scott; North Carolina, West Virginia, Ohio, Illinois, Virginia, R. Seymour.

Distribution: Thuret (loc. cit.), Schnetzler (1887), Blanc (1887) (?), Moreau and Moreau (1939: 227, 228, pl. 1, fig. 1-28), France; Pringsheim (1851; 1858; 1860), Bail (loc. cit.), Leitgeb (loc. cit.), Schroeter (loc. cit.), deBary (1881; 1883; 1888), Maurizio (1894; 1896; 1899), Pieters (1915b: 310), Hohnk (1958: 229), Germany; de la Rue (loc. cit.), Russia; Petersen (loc. cit.), Obel (1910a; 1910b), Lund (1934), Denmark; Gaumann (loc. cit.), Lapland; Cejp (loc. cit.), Czechoslovakia; Walentowicz (1885), Poland; Hohnk (1960), Strømø Island; Chaudhuri, et. al. (1947), India; Apinis (1930: 213; 214), Latvia; Van Beverwijk (1948: fig. 3), Netherlands; Rodway (1897), Hardy (1910), Johnson (1917; 1921), Crooks (1937: 211, fig. 1), Australia; Tiesenhausen (1912: 282, fig. 9), Switzerland; Boedijn (1921; 1923), Holland; Ou (1940), China; Ivimey-Cook and Morgan (1934: 347), Wales; Sparrow (1953: 15), Cuba; Nagai (1931:

6, pl. 2, figs. 2-15), Ookuba and Kobayasi (1955), Japan; Beneke and Rogers (1962), Brazil; Cejp (1932: 4, 5; 1934: 4, pl. 1, figs. 5-6; 1959b), Bohemia; Honk (1956), Greenland; Agersborg (1933), Apinis (1960; 1964), Berkeley (1864), Brown (1938: 163; 166, Text-fig. 1, Text-fig. 2, 4-6), Clinton (1894), Dick and Newby (1961), Dick (1962; 1963), Forbes (1935a: 224-225; 1935b: 8, fig. 1), Howarth (in Roberts, 1963), Ivimey-Cook and Forbes (1933), Morgan (1939), Murray (1885), Newby (1948: 266, figs. 1-6), Perrott (1960), Roberts (1963), Smith (1878), England; Humphrey (1893: 104-106, pl. 16, figs. 37-45), Alabama (legit Atkinson), Kentucky (legit Keller), Louisiana (legit Langlois), Massachusetts, Mississippi (legit White), Missouri (legit Trelease), Pennsylvania (legit Keller), Wisconsin (legit Trelease); Cooke and Bartsch (1959; 1960), Ohio, Maryland; Beneke (1948: 32, pl. 2), Bretsnyder (1943), Illinois; Beneke and Schmitt (1961), Schmitt and Beneke (1962), Harvey (1952), Ohio; Harvey (1942), California; Graff (1928), Montana; Harvey (1927; 1928a; 1928b; 1930), Wolf (1937; 1944: 53, pl. 1, figs. 3-4), Wisconsin; Poitras (1955), Pennsylvania; Wolf and Wolf (1941), Ziegler (1952: 7, pl. 2, fig. 4; 1958), Florida; Johnson (1956), Mississippi; Johnson (1950), Kauffman (1905; 1908), Pieters (1915a, 1915b), Michigan; Holland (1959), Miller (1965), Scott (1960), Scott and O'Bier (1962), Scott, et.al. (1963), Virginia; Coker (1923: 40, 46, pls. 11-12), Harvey (1925), North Carolina; Davis and Lazar (loc. cit.), Scott and O'Bier (1962), West Virginia.

Discussion: Saprolegnia ferax (Gruith.) Thruet, the most abundant and widespread species, encompasses a complex of morphological varients, many of which have been given taxonomic recognition. Within this complex

are several ill-defined phases, each of which is recognized by a series of morphological tendencies. However, none is sufficiently distinct for taxonomic recognition since the variation is neither great enough nor consistant enough to recognize more than one entity. It is, therefore, felt best to regard them as morphological extremes of a highly variable species.

There has been considerable discussion as to whether Saprolegnia ferax, S. mixta and S. monoica should be regarded as three distinct taxa, since S. ferax and S. monoica represent two morphological extremes. When considered by themselves, these two phases could be taken as two taxa, most probably at the varietal level. However, when they are considered in context with S. mixta or with the complete "population" of S. ferax, taxonomic recognition becomes impractical.

S. ferax has usually been described with antheridia on less than 10 per cent of the oogonia, S. mixta has antheridia on about 50 per cent of the oogonia, whereas in S. monoica all the oogonia are provided with antheridia. Klebs (1899) found that when he cultured S. mixta sensu deBary (1888) in a solution of hemoglobin, no antheridia were produced. On the other hand, Coker (1923) cultured a strain of S. ferax in which he was able to obtain 1 to 90 per cent of the oogonia bearing antheridia depending on the medium used. Pieters (1915) cultured S. monoica in 0.05 per cent hemoglobin solution and observed antheridia on 0 to 17 per cent of the oogonia. Kauffman (1908) described a form of S. mixta though 75-90 per cent of the oogonia were accompanied by antheridia, while later in the same paper (p. 369) he

described another form in which the antheridia were present only on 1 or 2 per cent of the oogonia. In experiments with the latter form, Kauffman (1908) was able to increase the number of antheridia to 25 per cent, but no more. A strict interpretation of the original description of S. mixta would exclude both forms with which Kauffman worked. O'Bier (1961) found that certain isolates when cultured on hempseeds showed characteristics of S. monoica in that nearly all the oogonia were accompanied by antheridia. The same isolates when cultured on small pieces of fish egg were almost entirely lacking antheridia.

Forbes (1935) suggested that the three species in question be regarded as different growth forms and referred to her isolate as Saprolegnia ferax f. mixta. Petersen (1909), Lund (1934), Van Beverwijk (1948), Johnson (1959) and O Bier (1960) have suggested similar disposition of these taxa. M.et Mme. Fernand Moreau (1939, p. 242) made the comment that these forms are often only separable by, "des caracteres de plus ou de moins".

If the original descriptions and illustrations of these taxa are compared, other features of similarity are apparent: oospore type, oogonial wall pitting, shape and position of oogonia, and number of oospores. Furthermore, there is a rather close intergradation of oogonia and oospore sizes of these taxa.

The results of a comparative study of 38 living isolates under controlled conditions, have shown that the taxonomy of this group is rather complex. While these taxa do exhibit (sensu strictu) minor differences there is a remarkable series of intermediates connecting

these three taxa. There are forms which have, for example, smaller or larger oospores and oogonia than are described from the types, but when a number of variant forms are examined, these size differences are insignificant.

Each of the three taxa previously discussed are represented by a small series of rather highly distinctive specimens, which are connected with typical S. ferax by a large series of intermediates. Of the specimens at hand, only a small proportion can definitely be regarded as characteristic of each phase; the bulk is composed of intermediates. Consequently, these forms are not recognized in this revision.

There is nothing in the measurements or illustrations of Saprolegnia heterandra Maurizio (1896) to lead one to believe this species is anything but another isolate of S. ferax. Coker (1923) asserted that this species may be best treated as a form of S. mixta, since Maurizio reported antheridia associated with about half the oogonia.

Saprolegnia mixta var. Asplundii proposed by Gaumann (loc. cit.) was reported to differ from the typical form of S. mixta sensu Maurizio (1895) only in the smaller oospores, which vary from 15-21 μ. Such a distinction is not a sufficient basis for discriminating species, let alone varieties.

Saprolegnia lapponica Gaumann (loc. cit.) was described, but not illustrated as a questionably new species, inasmuch as Gaumann could not be certain that his isolate was actually different from S. Thurteii (S. ferax). Gaumann's species was subsequently reduced to a variety of S. ferax by Cejp (1959). Such a reduction is inexplicable.

Pieters' variety vexans of Saprolegnia monoica (loc. cit.) differs

from S. monoica sensu Pringsheim in one respect only, i.e., the apparent loss of sexual structures only under the "stimulus of this special combination, leucin and levulose in concentration $\frac{M}{200}$ each". Saprolegnia monoica var. montana deBary (loc. cit.) does not have a single characteristic which is not common to many strains of this complex. The variety of S. monoica described by Schkorbatov (1923) as ocellata is nearly identical with several o' the Virginia isolates of S. ferax. Oogonial and oospore sizes are identical. Consequently, var. ocellata is reduced to synonomy.

There is nothing stated in the description of Saprolegnia esocina Maurizio (1896) to indicate that it is different from S. ferax, except in the size of the oospores, which are 21.5-25 μ in diameter. This condition has been observed frequently in certain isolates and as previously mentioned cannot be used as a specific or infraspecific characteristic. Saprolegnia bodanica also described by Maurizio (loc. cit.) was considered a synonym of Saprolegnia ferax by Coker (1923).

Maurizio (1899) based his description of Saprolegnia paradoxa upon a pecularility of the antheridial branches, which when arising from the base of an oogonium, may contain oospores. Brown (1938) reported S. paradoxa Maurizio from England, but did not observe any antheridia. Why she referred to S. paradoxa Maurizio is not clear.

Miss Forbes (1935) suggested that Saprolegnia paradoxa Maurizio was no more than a growth form of S. monoica and proposed the name S. monoica f. paradoxa for her isolate. However, she illustrated (fig. 1a) an antheridial branch bearing an oogonium, which is more

characteristic of Saprolegnia furcata sensu Maurizio (1899) than S. paradoxa sensu Maurizio (loc. cit.). Newby (1948) also isolated this fungus in England and made several attempts to stimulate the production of the egg-containing structures, but obtained no results. Van-Beverwijk (1948) observed several forms which possessed these curious structures, but found they became less in number after successive subculturing. She maintained that S. paradoxa was merely a variant within this complex.

Saprolegnia ferax has been misidentified or imperfectly described a number of times by some of the earlier investigators of the water molds. Pringsheim (1851) ascribed an isolate to Achlya prolifera, which, from his illustrations (loc. cit.) of the zoosporangia, is clearly a species of Saprolegnia. Coker (1923) referred Pringsheim's A. prolifera to Saprolegnia ferax. Achlya intermedia Bail, according to Lindsteldt (1879) a synonym of Diplanes saprolegnioides. D. Saprolegnioides merely represented a renaming of S. ferax. Duncan's Achlya ferax was also a renaming of S. ferax, since Duncan made no distinction between the two genera.

It is readily apparent that Saprolegnia ferax, as delimited here, encompasses several more or less divergent phases, each of which is characterized by its own aspect, which relates to a series of morphological tendencies. With the present information none of these phases can be sufficiently defined for taxonomic recognition.

Saprolegnia parasitica Coker

Saprolegniaceae, p. 57, pl. 18. 1923

Isoachlya parasitica (Coker) Nagai, Journ. Fac. Agr. Hokkaido Imp. Univ., 32: 12, pl. 2, figs. 27-34. 1931.

Saprolegnia parasitica var. Kochhari Chaudhuri, in Chaudhuri and Kochhar, Proc. Indian Acad. Sci., Sect. B, 2: 139. 1935.

Hyphae stout, moderately branched, 30-118 u in diameter. Gemmae abundant; clavate, pyriform, or irregular; terminal, single or frequently catenulate, occasionally intercalary and single; functioning as oogonia or zoosporangia. Zoosporangia abundant; cylindrical, clavate or irregular; straight, infrequently curved or irregular; 75-850 μ long by 20-80 μ in diameter, predominantly 150-200 μ X 30-45 μ in diameter; renewed by internal proliferation or laterally in basipetalous succession. Zoospore discharge saprolegnoid, rarely aplanoid; encysted primary spores 9-11 μ in diameter. Oogonia usually sparse, often formed only after prolonged period of time; terminal or infrequently intercalary; clavate, pyriform or irregular; 54-146 μ long X 18-72 μ in diameter, pyriform ones 62-75 μ in diameter. Oogonial wall unpitted, thin, smooth. Oospheres maturing. Oospores subcentric; spherical; filling the oogonium; 2-40 in number, usually 14-23; 16-28 μ in diameter, predominantly 18-24 μ; Antheridial branches diclinous, very rarely androgynous; simple, delicate; often wrapping about the oogonium and its hypha; persistent. Antheridial cells tubular or clavate; simple, occasionally branched; laterally appressed; fertilization tubes not observed. Oospore germination not observed.

Material examined: Nebraska, Utah, Maryland, West Virginia, Virginia, A. H. O'Bier; Netherlands (through Centraalbureau voor

Schimmelcultures), A. L. Van Beverwijk; Virginia, J. S. Wells, R. Seymour.

Distribution: Coker (1923: 58, pl. 18) (?), Scott and O'Bier (1962), Virginia; Beneke (1948) (?), Illinois; Beneke and Schmitt (1961) (?), Schmitt and Beneke (1962) (?), Bangham (1933) (?), Ohio; Graff (1928) (?), Montana; Johnson (1950: 397), Kanouse (1932: 433, pls. 12-13, figs. 1-32), Sparrow (1952) (?), Michigan; Johnson (1956: 186) (?), Mississippi; Tiffney and Wolf (1937: 298) (?), Massachusetts; Hoffman (1949) (?), Iowa; Estes (1957) (?), Alabama; Poitras (1955), Pennsylvania; Wolf (1944: 53, pl. 1, figs. 5-6) (?); Coker (1923: 58, pl. 18) (?), Washington, D. C.; Apinis (1930) (?), Latvia; Beneke and Rogers (1962) (?), Brazil; Nagai (loc. cit.) (?), Japan; Chaudhuri and Kochhar (loc. cit.) (?), Chaudhuri, et.al. (1947: 25; 27, figs. 6-7) (?), India; Cejp (1934: 3, pl. 1, figs. 1-4) (?), Bohemia.

Discussion: This species was not described in the modern sense (i.e., including sexual reproductive structures) until 1963, by Kanouse. We have no substantiating evidence that Kanouse's Saprolegnia parasitica was the same as Coker's, but since Kanouse chose to use the name originally applied by Coker, it must be retained.

Coker (loc. cit.) originally proposed the name Saprolegnia parasitica for non-fruiting strains associated with fish and fish eggs. The use of asexual characters and the parasitic habit as diagnostic features have made this taxon a depository for all non-fruiting forms of Saprolegnia. This practice is untenable since it has been shown by O'Bier (1960) that many non-fruiting strains may represent not one species, but a complex of two or more species. There is, however,

inconclusive evidence that heterothallism may be in part, an explanation for the absence of sexual structures in some strains of Saprolegnia (O'Bier, 1960). As yet, this phenomenon has not been demonstrated in Saprolegnia.

In view of this, many of the recorded collections are only tentatively included here inasmuch as no reference was made to sexual reproductive organs.

Saprolegnia parasitica sensu Kanouse (1932) is remarkably similar to Pieters (1915) S. Kauffmania. O'Bier (1960) was the first to suggest this relationship and maintained the separation of these taxa on oospore size alone. Although O'Bier (1960) observed sexual structures in his isolates, only the Utah and Virginia (J. S. Wells) isolate produced such structures. The two isolates examined do have characteristics almost identical with those of Saprolegnia Kauffmania Pieters (1915). Like features are: (1) sparse and infrequent production of sexual structures, (2) size and shape of oogonia, (3) unpitted oogonial walls, (4) number and origin of antheridial branches, and (5) oospore number. Although, Pieters (1915) did not describe the structure of the oospore, the only contrasting character appears to be the larger oospores reported for S. Kauffmania. In his description, Pieters (1915) reported "oospores average about 30 μ in diameter", as compared to Kanouse's 18-22 μ and Coker's average 23 μ for S. parasitica. The oospores of the Virginia isolate ranged in size from 22 to 33 μ, with an average diameter of 28 μ. The Utah isolate exhibited a range of 18-27.5 μ, with an average diameter of 24 μ.

Lacking conclusive evidence to the contrary, Saprolegnia parasitica sensu Kanouse is presently considered a valid species. I feel justified in restricting the name to only those isolates which consistently manifest these two features: Small (ave. 23 μ), subcentric oospores. When more isolates representing the Saprolegnia parasitic-S. Kauffmania complex are examined, they may from all indications, be considered synonymous, in which case, Saprolegnia Kauffmania would be the valid binomial.

Nagai (loc. cit.) transferred Saprolegnia parasitica to the genus Isolachlya making the combination Isoachlya parasitica (Coker) Nagai. Coker and Matthews (1937) subsequently reduced Nagai s combination, thus retaining Saprolegnia parasitica. This type of practice serves to reiterate the unstable characteristic upon which the genus Isolachlya was based.

Saprolegnia parasitica var. Kochhari presented as a new variety by Chaudhuri (loc. cit.), is ill-defined. According to his description, the zoosporangia often reached a length of 600 μ, which Chaudhuri considered characteristic of a "bigger class" and worthy of varietal distinction. According to Coker's original description (loc. cit.) of Saprolegnia parasitica the zoosporangia were at times up to 0.7 mm long. Chaudhuri (loc. cit.) did not observe sexual structures, previously described by Kanouse (1932), Tiffney (1939) reduced Saprolegnia parasitica var. Kochhari to synonomy.

Saprolegnia parasitica is easily distinguished from S. diclina by the often irregularly shaped oogonia, no pits, and subcentric oospores.

The reluctance to produce sexual reproductive structures, suggests a close connection with the latter species as do the diclinous antheridial branches. Saprolegnia anisospore clearly differs from S. parasitica by the eccentric structure of the mature oospore.

Saprolegnia diclina Humphrey

Trans. Amer. Phil. Soc. (N.S.), 17: 109, pl. 17, figs. 50-53. 1893

(Pl. II, Figs. 5-8)

Saprolegnia dioica deBary, Bot. Zeit., 46: 619, pl. 10, figs. 12-13. 1888.

Saprolegnia crustosa var. I Maurizio, Mittheil. Deutsch. Fischerei-Vereins, 7, heft 1: 52. 1899 (nomen ambiguum).

Saprolegnia crustosa var. II Maurizio, ibid., 53. 1899 (nomen ambiguum).

Saprolegnia crustosa var. III Maurizio, ibid., 54. 1899 (nomen ambiguum).

Saprolegnia delica Coker, Saprolegniaceae, p. 30, pls. 5, 6. 1923.

Saprolegnia pseudocrustosa Lund, Kgl. Danske Vidensk. Selsk. Skrift., Naturv. Math., Afd. IX, 6(1): 9, fig. 2. 1934.

Saprolegnia diclina var. numerosa Cejp, Flora CSR. Oomycetes I, p. 219. 1959.

Saprolegnia diclina var. minima Cejp, ibid., 219, fig. 78h. 1959.

Saprolegnia crustosa var. similis Cejp, ibid., 224. 1959.

Saprolegnia crustosa var. punctulata Cejp, ibid., 225. 1959.

Hyphae slender, sparingly to abundantly branched, 15-68 μ in diameter. Gemmae abundant or sparse; variable in shape, usually pyriform; terminal or intercalary, single or catenulate; occasionally disarticulating; function as zoosporangia. Zoosporangia abundant;

clavate or filiform; straight; 110-400 μ long by 25-45 μ in diameter; renewed by internal proliferation, rarely in basipetalous sucession. Zoospore discharge saprolegnoid; primary zoospores 10-12 μ in diameter. Oogonia usually abundant, often forming only after prolonged period of time or frequently lateral, not rarely intercalary or catenulate; spherical or pyriform, usually doloform when intercalary; 32-110 μ in diameter, predominantly 52-65 μ in diameter. Oogonial wall variable in pitting, usually pitted under point of attachment of antheridial, occasionally elsewhere or rarely unpitted; smooth. Oogonial stalks short or rather long; straight or slightly bent; unbranched. Oospheres maturing. Oospores centric or rarely subcentric; spherical, frequently filling the oogonium; 1-28 in number, generally 8-12; 12-36 μ in diameter, predominantly 23-26 μ. Antheridial branches almost always present; diclinous, rarely monoclinous, extremely rarely anderogynous; slender, irregular, infrequently branched; not persistent. Antheridial cells tubular or clavate, infrequently irregular; often branched; laterally, very rarely apically appressed; persistent; fertilization tubes present, not persistent. Mature oospore at germination forming slender, slightly irregular germ tube bearing a small, apically, clavate zoosporangia.

Material examined: England, L. G. Willoughby; Wales, W. R. Ivimey-Cook, Netherlands, A. Meurs (through Centraalbureau voor Schimmelcultures); Iowa, R. Crang; Virginia, Pennsylvania, Utah, Montana, A. H. O'Bier; Virginia, R. Seymour; North Carolina, S. E. Neff.

Distribution: Petersen (1909: 377, fig. 1c; 1910: 519, fig. 1c), Obel (1910a; 1910b), Lund (loc. cit.), Denmark; Apinis (1930: 221),

Latvia; Beneke and Rogers (1962), Brazil; Nagai (1931: 5, pl. 1, figs. 12-21), Japan; Tiesenhausen (1912: 273, figs. 4-5), Switzerland; Moreau and Moreau (1939: pl. 2), France; Cejp (1932), Bohemia; Cejp (loc. cit.), Czechoslovakia, Boedijn (1923), Holland; Ou (1940), China; Ivimey-Cook and Morgan (1934), Wales; Brown (1938: 164, 166, text-fig. 2, 1-3); Dick and Newby (1961), Dick (1963), Forbes (1935a: 226), Roberts (1963, Perrott (1960), Ivimey-Cook and Forbes (1933), England; Rossy-Valderama (1956), Puerto Rico; Humphrey (1893: 109, 110, pl. 17, figs. 50-53), Massachusetts, Alabama (legit Atkinson), Louisiana (legit Langlois), Pennsylvania (legit Keller); Hughes (1959; 1962), Georgia; Hoffman (1949) Iowa; Harvey (1952), Schmitt and Beneke (1962), Cooke and Bartsch (1959; 1960), Ohio; Graff (1928), Montana; Coker (1923: 26, 30, pls. 3-6), Ward (1939), Ziegler (1948: 18, pl. 2, figs. 5-6), North Carolina; Coker and Braxton (1927), South Carolina; Beneke (1948), Bretsnyder (1943), Illinois; Poitras (1955), Pennsylvania; Kauffman (1915: 1958; legit Pieters), Pieters (1915a), Johnson (1950), Sparrow (1952: 769), Michigan; Ziegler (1952: 14, pl. 2, figs. 1-2; 1958), Wolf and Wolf (1941), Florida; Johnson (1956: 185), Mississippi; Cooke and Bartsch (1959; 1960); Maryland, Matthews (1935), Scott (1960), Scott and O'Bier (1962), Scott, et.al. (1963), Virginia.

Discussion: Saprolegnia diclina Humphrey was first described by deBary (loc. cit.) under the binominal Saprolegnia dioica. In the original description of this species, deBary confused the nomenclature by using the specific epithet dioica; a name which Pringsheim (1860) had previously applied to another species of Saprolegnia ferax (see

page 43), Humphrey (loc. cit.) clearly pointed out that deBary's S. dioica should be rejected "in the interest of clearness and accuracy". According to the International Code of Botanical Nomenclature, Saprolegnia dioica deBary would now be considered illegimate under Article 61 and rejected as a later homonym. With the abolishment of S. dioica deBary, Humphrey (loc. cit.) applied the epithet diclina in keeping with the diclinous origin of the antheridial branches.

Some authors writing subsequent to the Humphrey paper (loc. cit.) that described his Saprolegnia diclina, have maintained deBary's name for isolates of their collections. The fungi described by Petersen (1909, 1910), Tiesenhausen (1912), Minden (1915), and Hohnk (1935, 1953) as Saprolegnia dioica deBary are regarded here as representatives of S. diclina Humphrey.

The Saprolegnia dioica described by Schroeter (1869) and S. dioica var. racemosa purposed by de-la-Rue (1874) were considered synonymous with S. ferax by Fischer (1892) and should not be confused with S. dioica deBary (loc. cit.).

Saprolegnia crustosa was described, but not illustrated, by Maurizio (loc. cit.) as a "Sammelspecies" consisting of three numerical varieties, none of which were designated as a type. Since Maurizio failed to give a species description for S. crustosa, it may prove best to designate it a nomen ambiguum in compliance with Article 62 of the International Code of Botanical Nomenclature. After a careful comparason of Maurizio's descriptions with data obtained from my observations on 31 isolates of Saprolegnia diclina, there seems to be no reason to

believe that Maurizio's varieties are at all distinct from S. diclina Humphrey. In any event there is no reason to warrent continued recognition of Saprolegnia crustosa and the species is accordingly rejected as a nomen ambiguum.

The fungi described by Lund (1934), Ivimey-Cook and Morgan (1934), Richter (1937), and Cejp (1959) as S. crustosa are here referred to S. diclina Humphrey.

Lund (1934) described S. pseudocrustosa, characterized by terminal, as well as lateral oogonia, wall furnished with few or numerous pits, antheridia diclinous and present on all oogonia, which is identical to Humphreys original description of S. diclina. Lund asserts, however, his species differs from S. diclina in that oogonia are never in chains and is more nearly allied to S. crustosa, but does not suggest a variety. Such a difference is untenable to retain S. pseudocrustosa as distinct from S. diclina.

Coker (loc. cit.) recognized the similarities of Saprolegnia delica and S. diclina, but held that they could be satisfactorily separated by the following differences: (1) abundant oogonia, averaging fewer eggs, (2) pitted oogonial walls, (3) presence of a few androgynous antheridia, (4) larger and more distinct antheridial branches which last longer, (5) fewer gemmae, and (6) a portion of oogonia on lateral branches. Recently, Johnson (1959) reported finding seven isolates which showed intermediate characteristics between the two fungi in question and suggested that they are probably varients of the same taxon. He further reported that one isolate was intermediate between S. diclina and S. crustosa.

An examination of 6 strains identified as S. delica by O'Bier (1961) and 25 isolates identified as S. diclina indicates that Johnson's observations are well founded and the structural features used in the separation of S. diclina and S. delica are not applicable when isolates are examined on a comparative basis.

Saprolegnia diclina seems to show affinities with S. anisospora on the basis of wall pitting and origin of the antheridial branches, but is readily separable on the structure of the mature oospore, which is eccentric in the latter species.

Saprolegnia furcata Maurizio

Mittheil. Deutsch. Fischerei-Vereins, 7, heft 1: 48, figs. 13-15. 1899

(Pl. II, Figs. 9-11)

Saprolegnia monoica var. glomerata Tiesenhausen, Arch. f. Hydrobiologie Und Planktonkunde, 7: 277, figs. 6-8. 1912.

Saprolegnia glomerata Lund, Kgl. Danske Vidensk. Selsk. Skrift., Naturv. Math., Afd. IV, 6(1): 14, fig. 4. 1934.

Hyphae slender, rather delicate; 5-46.8 μ in diameter; moderately branched; branches usually short, contorted, or knob-like, very rarely straight, often coiled. Gemmae sparse; filiform, globose or pyriform, clavate, occasionally irregular; functioning as a zoosporangium. Zoosporangia not abundant; cylindrical, clavate, occasionally fusiform; 45-560 μ long by 35-85 μ in diameter; renewed by internal proliferation. Zoospore discharge saprolegnoid, very rarely aplanoid; encysted primary spores 10-12 μ in diameter. Oogonia very abundant; lateral, occasionally terminal, very rarely intercalary; spherical or pyriform, infrequently

oval, doliform when intercalary; 24-98 μ in diameter; usually 42-50 μ. Oogonial wall smooth, 1.3-2 μ thick; pitting variable in frequency and size; when pitted, pits usually 7-10.4 μ in diameter; Oogonial stalks short or rather long; 1-6 times coiled, pendant, occasionally bent or crooked, very rarely straight, often unbranched with short, contorted or coiled branches; oospheres maturing. Oospores centric or rarely subcentric; spherical or rarely ovoid, usually filling the oogonium; 1-25 in number, generally 6-9; 13-32.5 μ in diameter, predominantly 19.5-22 μ; Antheridial stalks abundant; androgynous, occasionally monoclinous, rarely diclinous, often branching bearing a terminal oogonium; Antheridial cells simple; apically or laterally appressed; fertilization tubes sometimes formed, not persistent. Mature oospore at germination forming a short, simple germ tube bearing a small, terminal, cylindrical zoosporangium or germination into a new mycelium.

Material examined: England, A. E. Apinis, M. W. Dick; South Carolina, W. W. Scott.

Distribution: Dick and Newby (1961), England; Apinis (1929: 215, figs, 1-4), Latvia; Lund (loc. cit.), Denmark; Tiesenhausen (loc. cit.), Switzerland; Maurizio (loc. cit.), Germany.

Discussion: Saprolegnia furcata Maurizio is easily recognized by its coiled or often pendant oogonial stalks, contorted lateral hyphal branches, and predominantly androgynous antheridial branches.

In the original description and discussion, Maurizio (loc. cit.) made explicit note to a peculiar distinctive feature of S. furcata: the antheridial stalks frequently and constantly branch giving rise to

an oogonium. The three isolated of this species which were examined showed considerable variability in this respect and is in itself of limited taxonomic value.

Although Maurizio did not emphasize the appearance of the oogonial stalks, other than they were "much bent or wound", the stalks have been found to be quite characteristic in my isolates and afford a striking taxonomic feature.

Tiesenhausen (loc. cit.) apparently over-looked Maurizio's S. furcata when he described S. monoica var. glomerata, since he considered his variety to be closely allied to S. monoica (S. ferax) and to no other species, other than, perhaps, Cornu's (1872) ill-defined S. spiralis. The status of the latter species is doubtful (see page 83).

Apinis (1929) asserted that Tiesenhausen's variety was the same as Maurizio's S. furcata, and reduced S. monoica var. glomerata to synonomy. Lund (loc. cit.) recognized the similarity of these two taxa, but questioned Apinis' disposal of the variety glomerata solely on the basis that "according to Maurizio, the antheridia of S. furcata are always androgynous." Consequently, Lund regarded the variety as a distinct and separate species, S. glomerata, on the basis of some diclinous antheridial branches in his isolate and the lack of these in S. furcata.

The strains of the latter species which were examined by the writer, showed considerable variation in the percentages of androgynous, monoclinous, and diclinous antheridial branches present. In all isolates, those of androgynous origin predominated. Moreover, S. monoica var. glomerata sensu Tiesenhausen and Lund's S. glomerata are the same as

S. furcata in other major features; coiled or pendant oogonial stalks, size and number of oospores, variability of wall pitting, and the presence of contorted lateral hyphal branches. These taxa are synonomous.

The fungus described by Coker (1923) is not S. monoica var. glomerata sensu Tiesenhausen. (loc. cit.). Even a cursory examination of Coker"s description and illustrations (pl. 13) of the oogonia shows he was not dealing with Tiesenhausen s variety, but are more suggestive of those of S. monoica (S. ferax). Coker (1923) also suggested "there seems no doubt that our Chapel Hill plant and Humphrey's plant are the same as Tiesenhausen's variety glomerata". It is apparent that Humphrey (1892) was correct in his identification of S. monoica (S. ferax) and that Coker was also describing that species.

Scott's South Carolina collection establishes its occurrence in the United States.

Saprolegnia turfosa (Minden) Gaumann

Bot. Notiser, 1918: 154. 1918

(Pl. III, Figs. 3-4)

Saprolegnia sp. 2 Reinsch, Jahrb. wiss. Bot., II: 295, pl. 14, figs. 7-8, 11-13. 1877.

Saprolegnia paradoxa Petersen, Bot. Tidsskriff, 29: 379. 1909. (Also in Ann. Myc., 8: 520, fig. 1d, e. 1910).

Saprolegnia monoica var. turfosa Minden, Krypt.-fl. Mark Brandenburg, 5: 516. 1912.

Aplanes turfosus (Minden) Coker, Journ. Elisha Mitchell Sci. Soc., 42: 216. 1927.

Hyphae stout, sparingly branched, 14-43 μ in diameter, usually 19-25 μ. Gemmae lacking or sparse; cylindrical or pyriform, infrequently irregular; single and terminal, rarely intercalary; germination not observed. Zoosporangia sparse or abundant; filiform or clavate; 185-594 μ long X 20-35 μ in diameter. Zoospore discharge saprolegnoid; encysted spores 9-11 μ in diameter. Oogonia abundant; lateral, rarely terminal or intercalary; spherical, rarely oval; 18-92 μ. Oogonial wall up to 5 μ thick; smooth or rarely 1-3 short blunt, rounded extended into papillae; pits numerous and conspicuous. Oogonial stalks short, very rarely longer than the diameter of the oogonium, rarely lacking; stout; straight. Oospheres maturing. Oospores centric; spherical, occasionally ellipsoidal; filling the oogonium; 1-38 in number, generally 6-15; 20-31 μ in diameter, predominantly 26-28 μ. Antheridial branches androgynous, rarely monoclinous and arising near stalk; short; simple or branched. Antheridial cells long, tubular or cylindrical; simple or branched; laterally appressed, rarely attached by projections; fertilization tubes not observed. Oospore germination not observed.

Material examined: England, M. W. Dick; Virginia, R. Seymour; West Virginia, F. Marland, R. Seymour; Wales, W. R. Ivimey-Cook (from the Centraalbureau voor Schimmelcultures as Saprolegnia monoica).

Distribution: Dick and Newby (1961), Dick (1962; 1963), England; Gaumann (loc. cit.), Germany; Cejp (1959: 271, figs. 104a, b, 105, as Aplanes turfosus), Czechoslovakia; Petersen (loc. cit.), Denmark; Beneke (1948: 39, pl. 3), Illinois; Coker (1923: 79, pl. 20, as Aplanes treleaseanus; 1927), North Carolina.

Discussion: Saprolegnia turfosa has had a curious and confusing taxonomic history. It is unquestionably the same as Saprolegnia paradoxa, which Petersen (loc. cit.) described as a questionably new species, inasmuch as he did not observe zoospore discharge. His statement "At any rate as this species must be either an Saprolegnia or an Achlya, it deserves no doubt the name of paradoxa", is inexplicable. Although Petersen's name, S. paradoxa, has priority it must be rejected under Article 62 of the Botanical Code of Nomenclature as a later homonym of Maurizio's (1899) Saprolegnia paradoxa.

Minden (loc. cit.) apparently unaware of Petersen's species described the same form as Saprolegnia monoica var. turfosa. Gaumann (loc. cit.) later isolated a fungus, which he considered to be Minden's S. monoica var. turfosa, and raised it to specific rank.

Coker (1923) described a fungus which he considered to be Humphrey s Saprolegnia treleaseana. Coker's plant rarely formed zoosporangia and since this is the characteristic of deBary's genus Aplanes, he assigned Humphrey s species to it. The Aplanes treleaseanus (Humphrey) Coker was a misidentification, later corrected by him in his description of Aplanes turfosus (Coker, 1927).

In his 1927 paper, Coker reported the collection of an isolate similar to Saprolegnia turfosa (Minden) Gaumann and transferred Gaumann s species to the genus Aplanes, at the same time reducing S. turfosa (Minden) Gaumann to synonomy. In his description of this species Coker (1927) described internal proliferation of the zoosporangia, but did not observe zoospore discharge.

Recently, Beneke (1948) obtained several isolates from a Sphagnum

bog, which possessed all the characteristics of S. turfosa (Minden) Gaumann. Since his isolates repeatedly produced zoosporangia with saprolegnoid discharge, Beneke (loc. cit.) revived Saprolegnia turfosa as a valid entity.

The Aplanes treleaseana of Lund (1934) and Richter (1937) is based on Coker's description of Aplanes treleaseana in 1923 and clearly represents S. turfosa. Cejp's (1959) record of Aplanes turfosus is also included here, as it is the same species being described.

It is of interest to note that only Coker (1927) has described and illustrated papillate-warts on some oogonia. Of the 14 isolates, which were examined by the writer, one formed these structures consistantly, but never on more than 10 per cent of the oogonia. These papillae, however, were never as pronounced as those illustrated by Coker (1923: pl. 20, fig. 9).

It is of further interest to note that all previous investigators, except Beneke (1948), emphasized the paucity of zoosporangia. I have found these structures to be quite abundantly formed by all isolates examined, but only when an agar block containing mycelium was placed into distilled water. However, the same isolates, when cultured on hemp seed, rarely produced zoosporangia.

Saprolegnia turfosa is readily identified by the large, spherical, thick-walled oogonia, short oogonial stalks, and numerous conspicuous pits. The extremely short androgynous antheridial branches with long antheridial cells are further useful points of distinction.

Saprolegnia terrestris Cookson

Proc. Royal Soc. Victoria (N.S.), 49(2): 235, pl. 11, figs. 1-13. 1937

(Pl. III, Figs. 5-7)

Hyphae slender, 10-45 μ in diameter, sparingly branched. Gemmae, when present, filiform or pyriform, infrequently irregular. Zoosporangia abundant; filiform or clavate, frequently spherical, rarely irregular and contorted; 60-400 μ long by 16-48 μ in diameter; renewed by internal proliferation or cymosely. Zoospore discharge saprolegnoid; encysted spores 6-9 μ in diameter. Oogonia abundant; terminal or lateral, infrequently intercalary; spherical or pyriform; 35-91 μ in diameter, generally 60-65 μ. Oogonial wall pitted or unpitted; smooth or occasionally with very sparse papillate ornamentations. Oogonial stalks short or rather long; stout; straight. Oospheres maturing. Oospores subcentric or rarely centric; spherical or ellipsoidal; 1-12 in number, usually 3-6; 20-41 μ in diameter, generally 29-32 μ. Antheridial branches androgynous, arising from immediately below the oogonium; rarely branched; generally one to three in number. Antheridial cells simple; clavate, apically or laterally appressed, becoming inconspicuous; fertilization tubes present. Oospore germination not observed.

Material examined: Australia, I. Cookson (isolate from type culture deposited at the Centraalbureau voor Schimmelcultures).

Distribution: Cookson (loc. cit.), Australia.

Discussion: Saprolegnia terrestris is, at present, endemic to Australia. The prevalence of three or four oospores in an oogonium and the extremely short androgynous antheridial branches are the major distinctive features of Saprolegnia terrestris.

Saprolegnia litoralis Coker

Saprolegniaceae, p. 54, pls. 15, 16. 1923

(Pl. III, Figs. 1-2)

Hyphae stout, sparingly branched, reaching 55 μ in diameter. Gemmae abundant; spherical or pyriform; terminal or rarely intercalary, single or catenulate; germination not observed. Zoosporangia sparse; cylindrical, filiform or irregular; straight or infrequently bent; renewed by internal proliferation. Zoospore discharge saprolegnoid; primary zoospores 10-12 μ in diameter. Oogonia abundant; terminal, occasionally lateral on short branches, rarely intercalary; spherical or pyriform, doliform when intercalary; 42-80 μ in diameter, predominantly 53 μ in diameter. Oogonial wall sparsely, but conspicuously pitted, up to 10 μ in diameter, smooth, rarely with a single, terminal apiculus. Oogonial stalks short; stout; straight; frequently branched, often with a terminal swelling. Oospheres maturing. Oospores centric; spherical or ellipsoidal; filling the oogonium; 1-15, usually 2-6; 22-49 μ in diameter, predominantly 32 μ. Antheridial branches androgynous or frequently monoclinous when the oogonium is lateral; very rarely diclinous; slightly irregular; stout; rarely branched. Antheridial cells tubular; simple; laterally appressed; fertilization tubes rarely observed. Oospore germination not observed.

Material examined: Wales, W. R. Ivimey-Cook (through the Centraalbureau voor Schimmelcultures, Baarn); England (from the Commonwealth Mycological Institute); West Virginia, W. B. Cooke; Virginia, R. Seymour.

Distribution: Lund (1934: 13, 14, fig. 3c, e), Denmark; Apinis (1930: 216, fig. 2), Latvia; Moreau and Moreau (1939: 11, figs. 29-34); Perrott (1960), Dick and Newby (1961), Dick (1962; 1963), Roberts (1963), England; Cejp (1959: 238, fig. 87), Czechoslovakia; Coker (loc. cit.), Ward (1939), North Carolina; Coker and Braxton (1926), South Carolina; Matthews (1935), Miller (1965); Virginia; Hughes (1959; 1962), Georgia; Poitras (1955), Pennsylvania; Johnson (1956), Mississippi; Sparrow (1952), Michigan; Ziegler (1952: 14, pl. 2, figs. 5-6; 1958), Florida; Beneke (1948), Illinois.

Discussion: Although this species is closely related to Saprolegnia terrestris, the numerous androgynous antheridial branches, the large oospores, and the large conspicuous pits readily distinguish S. litoralis.

Saprolegnia eccentrica Coker, comb. nov.

(Pl. III, Figs. 10-11)

Isoachlya eccentrica Coker, Saprolegniaceae, p. 87, pl. 24. 1923.

Hyphae slender, delicate, sparingly branched, 6-21 μ in diameter, generally 12-15 μ. Gemmae abundant; clavate or pyriform, frequently irregular; functioning as zoosporangia. Zoosporangia abundant; filiform or clavate, infrequently pyriform; 130-380 μ long X 25-36 μ in diameter; renewed sympodially, occasionally in basipetalous succession, rarely by internal proliferation. Zoospore discharge saprolegnoid, rarely dictyoid; encysted spores 10-12 μ in diameter. Oogonia abundant; lateral, infrequently terminal; spherical, occasionally pyriform; single, rarely catenulate; 15-42 μ in diameter, predominantly 30-35 μ.

Oogonial wall smooth; pitted or unpitted. Oogonial stalks 1-2 times the diameter of the oogonium in length; stout; straight or curved, rarely branched or irregular. Oospheres maturing. Oospores eccentric; spherical or ellipsoidal, usually filling the oogonium; 1-4 in number, predomonantly 1; 12-32 μ in diameter, predominantly 24-28 μ. Antheridial branches and cells absent. Oospore germination not observed.

Material examined: Wales, W. R. Ivimey-Cook (through the Centraalbureau voor Schimmelcultures); Virginia, R. Seymour.

Distribution: Ivimey-Cook and Morgan (1934), Wales; Dick and Newby (1961), Dick (1962; 1963), England; Cejp (1959: 198, fig. 70), Czechoslovkia; Coker (loc. cit.), Harvey (1925; 1930), North Carolina; Matthews (1935), Virginia; Hughes (1959: 1962), Georgia; Ziegler (1958), Florida.

Discussion: In his original description and discussion, Coker (loc. cit.) proposed that this species be included as a new species of Isoachlya, I. eccentrica. It was primarily because of the method by which the secondary zoosporangia are renewed that the author referred this species to the genus Isoachlya. Since this writer feels justified in reducing the latter genus to synonomy, it becomes necessary to erect a new combination for this fungus. The binominal Saprolegnia eccentrica is proposed.

Saprolegnia eccentrica is easily separated from other species of Saprolegnia by the eccentric oospore. With the exception of Saprolegnia anisospora, no other presently known species has such an oospore structure. Saprolegnia eccentric is readily distinguished from S. anisospora by the prevalence of a single oospore in an oogonium.

This prevalence of a single oospore denotes a similarity to Saprolegnia megasperma, S. intermedia, S. asterophora, and S. unispora. It is perhaps more closely related to the latter species since both S. eccentrica and S. unispora lack antheridia.

Saprolegnia unispora Coker and Couch, comb. nov.

Isoachlya unispora Coker and Couch, In Coker, Saprolegniaceae, p. 85, pls. 22, 23. 1923.

Hyphae stout, irregular, sparingly branched, 10-36 μ in diameter. Gemmae variable in abundance; filiform, pyriform a spherical; terminal or intercalary, single or catenulate; functioning as zoosporangia. Zoosporangia abundant; fusiform, filiform or pyriform; variable in length and diameter; renewed internally a sympodially. Zoospore discharge saprolegnoid, rarely dictyoid; encysted spores 10-12 μ in diameter. Oogonia abundant; lateral, occasionally terminal, rarely intercalary; frequently clustered on a hypha; spherical or pyriform; 18-68 μ in diameter, predominantly 45-52 μ. Oogonial wall pitted; smooth; extremely rarely with one or more short papillae. Oogonial stalks variable in length; straight, curved or irregular; occasionally branched. Oospheres maturing. Oospores subcentric or centric; spherical; 1-4 in number, generally 1-2; 18-43 μ in diameter, predominantly 32-38 μ. Antheridial branches and cells absent. Mature oospore germination not observed.

Material examined: Wales, W. R. Ivimey-Cook (through the Centraalbureau voor Schimmelcultures); Virginia, North Carolina, R. Seymour.

Distribution: Ivimey-Cook and Morgan (1934), Wales; Apinis (1964),

England; Cejp (1959: 199), Czechoslovakia; Johnson and Surratt (1955), Johnson (1956), Mississippi; Hughes (1959; 1962), Georgia; Coker (loc. cit., legit Couch), North Carolina; Scott (1960), Matthews (1935), Virginia; Ziegler (1952: 8, pl. 2, fig. 10; 1958), Florida.

Discussion: This species differs from S. intermedia only in the absence of antheridial branches. Further discussion on their relationships is presented on page 77. After careful consideration, it would appear that no satisfactory disposition of this species is possible at this time. Saprolegnia unispora is hereby retained with reservation as a separate taxa distinguished by the key characters.

Saprolegnia megasperma Coker

Saprolegniaceae, p. 56, pl. 17. 1923

Hyphae stout; straight or irregular, 9-35 μ in diameter, predominantly 15-20 μ. Gemmae abundant; spherical, oval or irregular; functioning as zoosporangia. Zoosporangia abundant, variable in shape; 100-400 μ long X 15-45 μ in diameter; renewed internally or rarely cymosely. Oogonia moderately abundant; lateral, frequently clustered; 40-100 u in diameter. Oogonial wall pitted or unpitted; smooth. Oogonial stalks, short, less than the oogonium diameter in length. Oospores subcentric; spherical, not filling the oogonium; 1-10 in number, single in over 50 per cent of the oogonia; 30-52 μ in diameter. Antheridial branches monoclinous, frequently diclinous. Antheridial cells simple; apically appressed, rarely laterally appressed, rarely laterally appressed; fertilization tubes present. Mature oospore germination not observed. (Modified from Coker, loc. cit.)

Distribution: Apinis (1930: 218), Latvia; Dick and Newby (1961), Dick (1962: 1963), England; Poitras (1955), Pennsylvania; Beneke (1948), Illinois; Ziegler (1952: 14, pl. 2, fig. 3; 1958), Florida; Hughes (1959; 1962), Georgia; Coker (loc. cit.), North Carolina.

Discussion: Although this writer has not seen Saprolegnia megasperma, the simple, unbranched, monoclinous antheridial branches and the apically appressed antheridial cells appears to make it rather easily identifiable. Saprolegnia megasperma shows no close connection with other species in the genus, but its proximity to S. unispora and S. intermedia is indicated by the size, number and structure of the oospores.

Saprolegnia intermedia Coker and Harvey, comb. nov.

(Pl. III, Figs. 8-9)

Pythiopsis intermedia Coker and Harvey, in Harvey, Jour. Elisha Mitchell Sci. Soc. 41: 157. 1925.

Isoachlya subterranea Dissmann, Beih. Bot. Cent. 58: 110. 1931.

Isoachlya itoana Nagai, Jour. Fac. Agr. Hokkaido Imp. Univ. 32: 11, pl. 2, figs. 35-37; pl. 3, figs. 1-8. 1931.

Isoachlya glomerata Richter, Flora 131: 241. 1937.

Isoachlya intermedia (Coker and Harvey) Coker, in Coker and Matthews, North Amer. Flora 2 (1): 27. 1937.

Hyphae stout, moderately branched, 11-14 μ in diameter. Gemmae variable in abundance; filiform, spherical, pyriform, or irregular; terminal or intercalary, single or catenulate; functioning as zoosporangia. Zoosporangia abundant; fusiform, filiform, frequently bent,

curved or irregular, infrequently branched; variable in length and diameter; renewed internally or sympodially; internally proliferated ones always formed outside the discharged zoosporangium. Zoospore discharge saprolegnoid, rarely dictyoid; oogonia abundant; lateral, occasionally terminal, rarely intercalary; frequently clustered on a hypha; spherical or pyriform, rarely filiform; 19-60 μ in diameter, predominantly 40-50 μ. Oogonial wall pitted, unpitted, or pitted only under point of attachment of antheridial cells; smooth; rarely irregular on inner surface, occasionally with one or a few papillate projections. Oogonial stalks variable in length; straight, curved, bent, or irregular; occasionally branched, then bearing oogonia in glomeruli. Oospheres maturing. Oospores centric or subcentric; spherical; one, rarely two, extremely rarely three or four in number; 18-46 μ in diameter, predominantly 30-40 μ. Antheridial branches usually androgynous, occasionally monoclinous, infrequently or rarely diclinous; when androgynous, arising from any point along the length of the oogonial stalk, but occasionally very short; sparingly branched; lacking on some oogonia. Antheridial cells simple; laterally appressed, extremely rarely attached by projections or apically appressed; fertilization tubes present, persistent. Mature oospore germinating by a long, simple germ tube bearing a small, apical zoosporangium.

Material examined: New York, H. S. Vishniac (from the Centraalbureau voor Schimmelcultures); Georgia, W. W. Scott; North Carolina, Maryland, Virginia, R. Seymour.

Distribution: Ivimey-Cook and Morgan (1934), Wales; Dissmann

(loc. cit.), Richter (loc. cit.), Germany; Nagai (loc. cit.), Japan; Johnson and Surratt (1955: 124, figs. 1-34), Johnson (1956), Mississippi; Coker and Harvey (loc. cit.), Kelman (loc. cit.), North Carolina; Hughes (1959; 1962), Georgia; Beneke (1948), Illinois; Ziegler (1952: 8, pl. 2, fig. 9; 1958), Florida; Scott (1960), Virginia.

Discussion: Saprolegnia intermedia was first described by Coker and Harvey (1925) as Pythiopsis intermedia. Twelve years later, Coker (1937) transferred P. intermedia to the genus Isolachlya and made the combination I. intermedia (Coker and Harvey) Coker.

Dissmann, in 1931, described I. subterranea, which was followed one month later by Nagai's (1931) description of I. itoana. Richter (1937) described I. glomerata as a new species six years later. A critical review of the papers describing these fungi leads one to the conclusion that none of the three authors knew of or considered the previously described species. These taxa were later regarded by Johnson and Surratt (1955) to be synonymous, for which Isoachlya subterranea was the valid binonimal.

Johnson and Surratt (1955) regarded I. subterranea distinct from S. intermedia on the basis of a single characteristic "....the presence of some irregularly shaped oogonia having wavey or irregular walls which may, in extreme cases, be extended into short papillae". Although these investigators were quite explicit with regard to the presence of papillae in S. intermedia, it is clearly stated in their diagnosis of I. subterranea that the oogonial wall may "extremely rarely be with one or a few short papillate projections". There is now other mention of papillae in their discussion of S. subterranea, which gives one the

impression that S. subterranea is always smooth-walled.

By a comparative examination of several isolates of the I. subterranea-S. intermedia complex, it was found that features formerly considered to be exclusive to one or the other of these taxa are actually common to both. Isolates identified as S. intermedia (sensu strictu) were indistinguishable from I. subterranea sensu Johnson and Surratt, when grown on MSPS. The same isolates, however, when grown on MSPS 1:5 or on hempseed in distilled water were frequently papillate and easily recognized as S. intermedia in the strictest interpretation. Furthermore, other isolates, formed papillae on all substrata with varing degrees of frequency. Unfortunately, Johnson and Surratt (1955) based their findings upon cultures grown only on hempseeds in distilled water. Because a given isolate can vary greatly in its morphology, and since the character in this case is the only one used to delimit two taxa, it is indicative that the two taxa are too similar to justify their continued recognition as distinct entities. Saprolegnia intermedia, therefore, becomes the valid combination, inasmuch as it has priority. The description of S. intermedia is emended here to include S. subterrana and the forms of these which have been isolated.

The relationship between S. intermedia and S. unispora presents another complex taxonomic problem. These two taxa have formerly been separated soley on the basis of the presence or absence of antheridial branches. Johnson and Surratt (1955) reported that in certain isolates possessing antheridia the antheridial branches were found on 34 to 98 per cent of the oogonia is indicative, but not conclusive evidence that

the two taxa may be a single entity, since they agree in all other pertinent characters.

Although variation has been frequently recorded, this investigator has yet to observe any antheridial branches in isolates of S. unispora nor has he observed under the same conditions less than 45 per cent of the oogonia of S. intermedia lacking antheridial branches.

It seems temporarily desirable to retain the antheridial branched species separate from Saprolegnia unispora, although they are remarkably similar in other morphological features. For the present, opinion is reserved as to the true relationship of these taxa until such time as more isolates can be critically compared.

TAXA OF DOUBTFUL AFFINITIES

Saprolegnia torulosa deBary

Abh. Senck. Nat. Ges., 12: 255. 1881

Zoosporangia slender, cylindric, clavate. Oogonia irregularly spherical, elongate, pyriform or cylindric, rarely oval; catenulate, rarely single and terminal. Oogonial wall pitted or unpitted. Oospores centric. Antheridial branches and antheridial cells usually lacking; when present either androgynous or diclinous. Fertilization tubes present. (Modified from deBary, Bot. Zeit. 46: 618. 1888)

For a discussion of this species, see Saprolegnia monilifera (p. 79).

Isoachlya toruloides Kauffman and Coker

In Kauffman, Amer. J. Bot., 8: 231. 1921

Hyphae delicate moderately branched. Gemmae not abundant; pyriform or irregular; single or catenulate. Zoosporangia clavate, cylindrical irregular; renewed internally, less often laterally. Zoospore discharge saprolegnoid, rarely aplanoid. Oogonia abundant; spherical, oval or pyriform; terminal or lateral, often intercalary or catenulate. Oogonial wall smooth with 1 or 2 inconspicuous pits, often with no pits visible. Oospores centric; 1-6 in number, rarely 8-12; 11-33 μ in diameter, generally 22-30 μ. Antheridial branches absent or present on 1-45 per cent of the oogonia; diclinous; delicate, quickly disappearing after the formation of antheridial cells. Antheridial cells clavate or tuberous; laterally appressed; fertilization tubes present. (Modified from Kauffman and Coker, loc. cit.) For a discussion of this species, see Saprolegnia monilifera (p. 79).

Saprolegnia monilifera deBary

Bot. Zeit., 46: 629. 1888

Isoachlya monilifera (deBary) Kauffman, Amer. J. Bot., 8: 231. 1921.

Zoosporangia clavate; renewed cymosely, rarely internally. Oogonia spherical or clavate; catenulate, rarely single. Oogonial wall smooth; pitted or unpitted. Antheridia absent. Oospores 1-16 in number, usually 6-12; centric. (Emend. Fischer, Kryptogamen-Fl., 1(4): 342. 1892.)

Saprolegnia torulosa deBary, S. monilifera and Isoachlya toruloides Kauffman and Coker form an intricate complex of species, the relationships of which can be discussed only as a unit. Correct disposal of these taxa at present seems impossible, for although each exhibits features suggestive of its individuality, the three show even more remarkable

similarities. Too little is known about them, and the information that has been published is, in many instances, contradictory.

The following summary of the pertinent characteristics of Saprolegnia torulosa, I. toruloides, and S. monilifera shows their close interrelationship: (1) If the presence or absence of antheridial branches is considered indicative of the relationships of Saprolegnia torulosa, I. toruloides, and S. monilifera, the first two are similar since both exhibit these structures to varing degrees, while S. monilifera does not. Nagai (1931) and Lund (1934) however, reported antheridia to be present, but rare in S. monilifera. (2) Oospore numbers unites all three taxa. (3) I. toruloides and S. monilifera are identified with respect to the presence of cymose secondary zoosporangia to the exclusion of S. torulosa. (4) All three taxa are identical with respect to, oospore type, oogonial wall pitting and arrangement of oogonia in torulose chains. Furthermore, there is a close intergradation of oogonia and oospore sizes of these taxa.

After careful consideration, it would appear that no satisfactory disposition of these taxa is possible at this time. Since they are ill-defined -some descriptions being quite ambiguous and contradictory, - treating them as separate taxa and relegating them to the doubtful catagory seems justified. To avoid further confusion, I am retaining the name Isoachlya toruloides in lieu of making a doubtful combination. If, after further study, I. toruloides is considered a valid entity a new combination will be made.

Saprolegnia Kauffmania Pieters

Bot. Gaz. 60: 488, pl. 21. 1915

Saprolegnia Kauffmania has been relagated to this catagory pending further examination. The similarities of this taxon have been discussed elsewhere (see page 54) and need not be repeated here.

EXCLUDED TAXA

Saprolegnia androgyna Archer, Quart.

Jour. Mic. Sci. 7(N.S.): 123, pl. 6, fig. 1. 1867

This species is now assigned as a synonym of Aplanes androgynus (Archer) Humphrey.

Saprolegnia bernardensis Harvey

Journ. Elisha Mitchell Sci. Soc., 58: 22, pl. 3, figs. 1-7. 1942

This taxon is excluded as an illegitimate name, since it was not described with a Latin diagnosis.

Saprolegnia candida Kutzing

Species Algarum, Lipsiae. 1849

S. candida and three other names published by Kutzing at the same time, viz., S. tenius, S. saccata and S.Libertiae (Bory) Kutz., are too ill-defined to distinguish. Fischer (1892) believed the latter species Apodya lactea Cornu (Leptomitus lacteus).

Saprolegnia corcagiensis Hartog

Quart. J. Micro. Sci. 27: 429. 1887

From all indications Hartog (loc. cit.) was describing the zoosporangia of Leptomitus lacteus and pitted oogonia of an unknown

saprolegniaceous fungus. It has been recognized only by Hartog.

Saprolegnia deBaryi Walz

Bot. Zeit. 28: 537. 1870

Both Humphrey (1893) and Coker (1923) consider this to be a Pythium; not a Saprolegnia.

Saprolegnia elongata Massee

British Fungi, p. 217. 1891

This was described from a mixed culture of Pythium and Saprolegnia. Goldie-Smith (1952) has suggested the Pythium may be P. undulatum. The Saprolegnia is indistinguishable.

Saprolegnia exigena Murgoci-Antoniu

Acad. roumana, Bucharest secturniea

Stuntfica Bull., 30(3): 154. 1947

This species is excluded as an illegitimate name since the required Latin diagnosis was not included with the original description.

Saprolegnia minor Kutzing

Phycologia Generalis. Lipsiae. 1843

This species is probably an Entomophora. It is not saprolegnoid.

Saprolegnia mucopliage Smith

Gardners Chron., 22: 245, fig. 50. 1884

Humphrey (1893) and Coker (1923) considered Smith's fungus may be a Pythium, but not Saprolegnia.

Saprolegnia philomuckes Smith

Gardeners Chron., 22: 245. 1884

Like the preceeding species this is possible a Pythium.

Saprolegnia quisquiliarum Roumeg

Humphrey (1893) stated that this name was based on a specimen issued as No. 5932 of the "Fungi Gallici", but neither he nor A. B. Seymour could detect anything saprolegniaceous.

Saprolegnia schachtii Frank

Krankheiten der Pilzen, p. 384. Berlin. 1881

This is probably Pythium deBaryanum (Coker, 1923).

Saprolegnia siliquoeformis Reinsch

Jahrb. wiss. Bot., 11: 293. 1878

According to Fischer (1892) this is Monoblepharis prolifera.

Saprolegnia spiralis Cornu

Ann. Sci. Nat., Series 5, 15: 10, pl. 6, figs. 10-12; 15-17; pl. 7, figs. 1-4; 10. 1872

This species must be excluded on the grounds that the description was based on a mixed culture of infected material. Simultaneously with his description of S. spiralis, Cornu (loc. cit.) described the accompaning parasite as Rozella septigena (see Sparrow, 1963).

Saprolegnia xylophila Kutzing

Phycologia Generalis. Lipsiae. 1843

Like the previous species described by Kutzing (loc. cit.) S. xylophila is unidentifiable.

SUMMARY

The present investigation deals with a revisional study of the genus Saprolegnia, a group of water molds belonging to the family Saprolegniaceae. The data on which this revision is based, have been obtained from an examination of living material. The majority of this material was obtained from 864 collections of soil and water. Where information from living material was not available, the original and subsequent descriptions of the species were utilized.

All isolates studied were propagated on a chemically defined medium and on halves of sterilized hemp seed (Cannabis sativa). Observations of morphological characteristics were made as soon as mature reproductive structures were formed, ordinarily, within a period of one week.

Insofar as possible, the relationships of species have been indicated. This has necessitated finding specific, significant morphological features on which to base the separation of species.

As a result of this investigation, fifteen species are recognized as valid members of the genus Saprolegnia. Four species have been considered doubtful and eighteen taxa excluded from the genus.

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APPENDIX

Table 1. The Ingredients of MSPS.

1.	Chelation agent:		
	Ethylenediaminetetraacetic acid	0.2	grams/liter
2.	Buffer for pH 6.5		
	K_2HPO_4	0.087	"
	KH_2PO_4	0.068	"
3.	Inorganic nutrients:		
	$MgCl_2 \cdot 6H_2O$	0.16	"
	$CaCl_2 \cdot 6H_2O$	0.066	"
	$MnCl_2 \cdot 4H_2O$	0.075	"
	$ZnCl_2$	0.04	"
	$FeCl_2 \cdot 6H_2O$	0.0013	"
4.	Organic Nutrients:		
	D L Methionine	0.05	"
	Glucose	3.0	"
	L-Sodium glutamate (mono)	0.5	"

5. Dissolve the ingredients of steps 1, 2, 3 and 4 within one liter of predistilled ion-exchanged water and adjust the pH of the solution to 6.5-6.8 with KOH pellets.

6.	Agar (Difco Purified).	1.5	grams/liter

Add to the above solution.

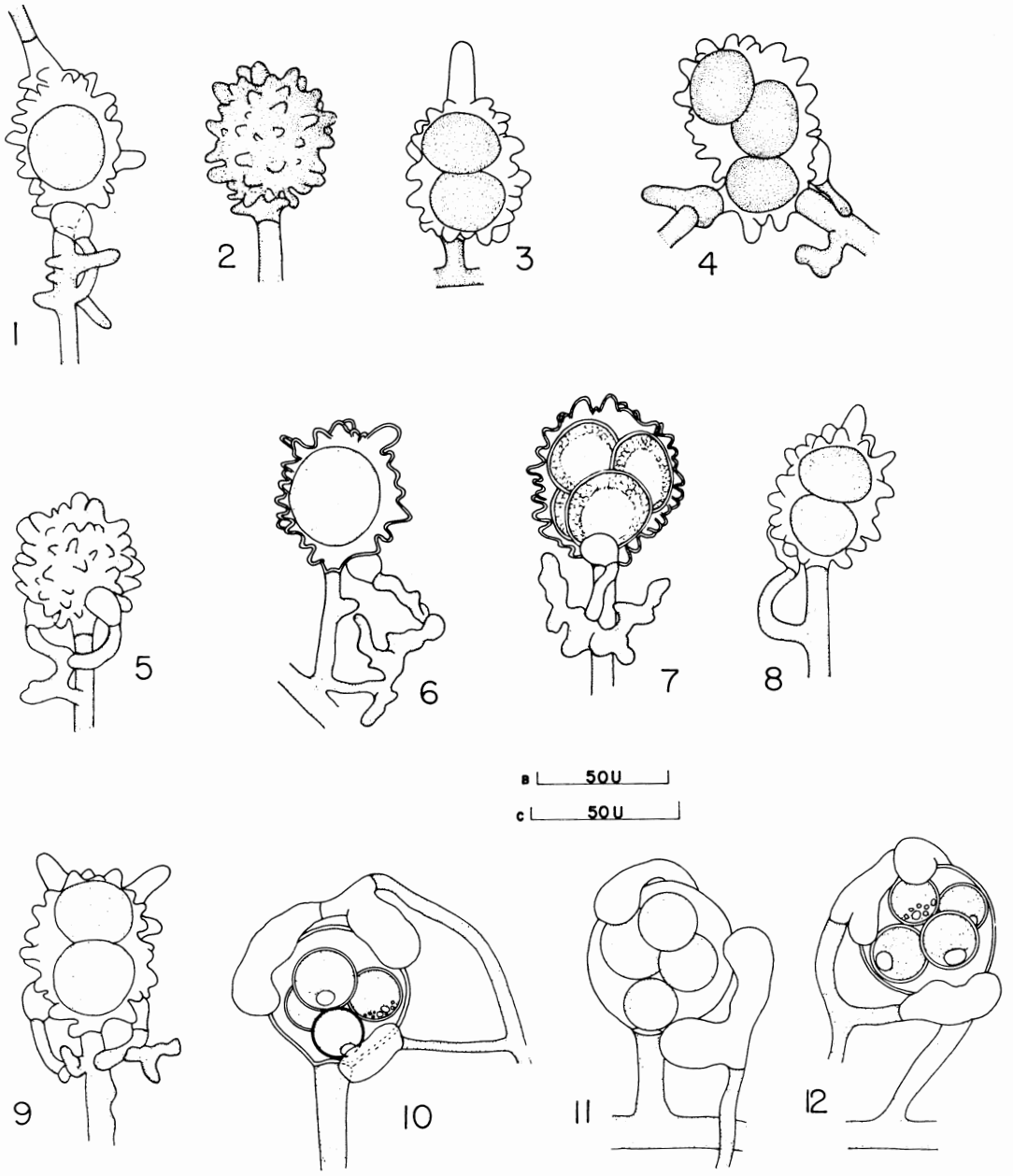
7. Autoclave the medium at 15 lbs. for 15 minutes.

Explanation of Plate I

Figs. 1-9. *Saprolegnia asterophora*: 1, intercalary oogonium; 2, immature papillate oogonium; 3, mature papillate oogonium; 4, oogonium containing three immature oospores; 5, immature oogonium with attendant androgynous antheridial branches; 6-7, oogonia with contorted androgynous antheridial branches; note the subcentric oospores in fig. 7; 8-10, papillate oogonia. All figures, scale c.

Figs. 11-13. *Saprolegnia anisospora*: 11, oogonium containing both eccentric and subeccentric oospores; 12, immature lateral oogonium; 13, mature lateral oogonium; note eccentric and subeccentric oospores. All figures, scale C.

Plate I



Explanation of Plate II

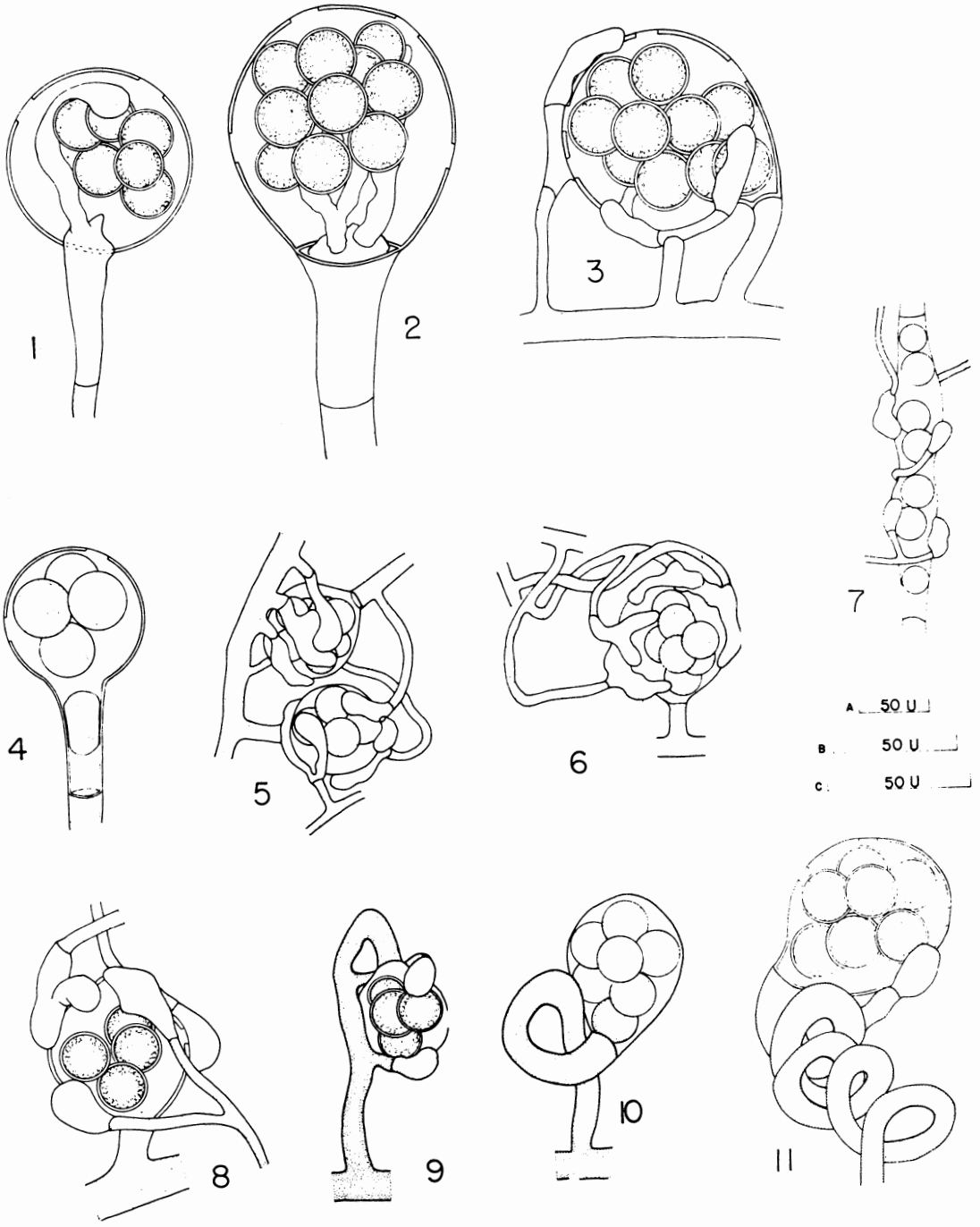
Figs. 1,2. Saprolegnia hypogyna: 1-2, oogonia with hypogynous antheridial cells and fertilization tubes; note columella. Both figures, scale C.

Figs. 3,4. Saprolegnia ferax: 1, lateral oogonium with mature centric oospores and monoclinous antheridial branch; 2, terminal pyriform oogonium lacking antheridia; note large pits in oogonial wall of both figures. Both figures scale B.

Figs. 5-8. Saprolegnia diclina: 5-8, oogonia with attendant diclinous antheridial branches. Figures 5-7, scale A; Figure 8, scale B.

Figs. 9-11. Saprolegnia furcata Maurizio: 9, lateral, pendant oogonium; 10, lateral oogonium with curled oogonial stalk; 11, oogonium on a coiled stalk, with an androgynous antheridial branch. All figures scale, B.

Plate II



Explanation of Plate III

Figs. 1,2. Saprolegnia litoralis: 1, terminal oogonium with several androgynous antheridial branches; 2, intercalary oogonium with centric oospores. Both figures, Scale B.

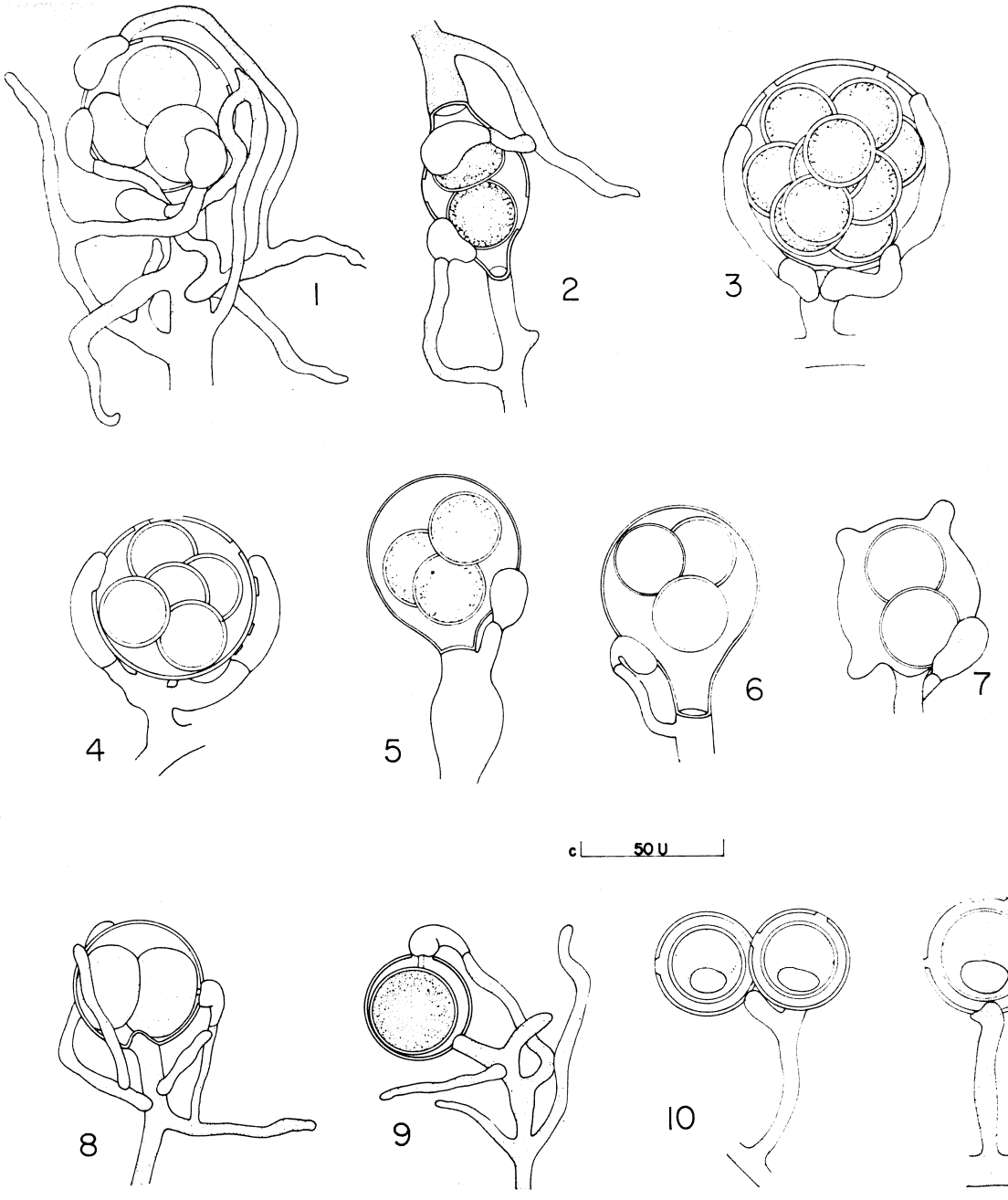
Figs. 3,4. Saprolegnia turfosa: 3, mature lateral oogonium containing mature oospores; note the thick oogonial wall with numerous pits; 4, lateral oogonium with androgynous antheridial branches showing one antheridial all attached with projections. Both figures, scale B.

Figs. 5-7. Saprolegnia terrestris; 5, 6, terminal oogonia, showing few antheridial branches; 7, papillate oogonium. All figures, scale B.

Figs, 8,9. Saprolegnia intermedia: 8, immature oogonium showing androgynous antheridial branches and small fertilization tube; 9, mature oogonium showing subcentric oospores. Both figures, scale C.

Figs. 10,11. Saprolegnia eccentrica: 10, 11, oogonia showing eccentric oospores. Both figures, scale C.

Plate III



ABSTRACT

The purpose of this investigation is to present a new account of the genus Saprolegnia, to bring together in one paper the published studies dealing with the morphology and taxonomy of the various species, and to add to this information conclusions based upon observations of living material. The majority of living specimens was obtained from 864 collections of water and soil made by the writer and others in eleven states and in Australia, England and India.

The procedures used in the isolation and propagation of these fungi is presented. All isolates studied were propagated on a chemically defined medium, as well as, on halves of sterilized hemp seed (Cannabis sativa).

Reliable taxonomic criteria are used in the keys provided for the identification of species.

As a result of this investigation, fifteen species are recognized as valid members. Three new combinations are proposed. Four species are doubtful and eighteen taxa are excluded from the genus.