ON A NEW SACCHAROMYCETE *MONOSPORELLA UNICUSPIDATA* GEN. N. NOM., N. SP., PARASITIC IN THE BODY CAVITY OF A DIPTEROUS LARVA (*DASYHELEA OBSCURA* WINNERTZ).

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(With Three Text-figures.)

The genus *Monospora* (= *Monosporella* renamed[1]) was founded by Metschnikoff in 1884 to designate a parasitic fungus which he discovered in *Daphnia magna*. This monocellular fungus, of which he described only one species, *Monospora bicuspidata*[2], lives free in the body cavity of its host, where it multiplies actively by budding in a yeast-like manner (Fig. I, 1, 2, 3).

When the body cavity of the host is entirely invaded by the parasites, these grow in size, become elongated, and form club- or sausage-shaped asci in each of which is developed a single needle-like spore having both ends pointed (Fig. I, 4, 5 and 6). When the parasitized host dies, it is filled with ripe spores, and healthy Daphnias, which feed on the detritus of their dead and diseased fellows, become infected by ingesting the asci. The latter, when they enter the host's alimentary canal, set free the needle-shaped spores which perforate the gut wall and penetrate in the body cavity (Fig. I, 8) where they germinate laterally, thus starting the new infection (Fig. I, 7).

Metschnikoff's studies on this parasite afford a striking instance of the phenomenon of phagocytosis. In his lectures on inflammation (1893, pp. 83–84), it is stated that directly the spore "appears outside the intestinal wall, it is attacked by leucocytes, which are carried to the spot by the blood-stream. The cells fix themselves on the spore, forming around it a collection of cells, which often fuse together into a plasmodium, which causes the spore to undergo a series of remarkable changes. On being enclosed in the leucocytes the spore

[1] The generic name *Monospora*, given by Metschnikoff to the parasite, is invalidated for the reason stated in the Appendix to this paper, p. 90.

[2] The species of *Monospora*, discovered by Metschnikoff, was described by him under the name *M. bicuspidata*, and under this name it is referred to in his various publications, nevertheless all the authors I have consulted (Zopf, Hansen, Dangeard, Guillermond, Lafar, Saccardo) wrongly name the species *M. cuspidata* Metschnikoff. I do not know who changed the specific name, but incline to the view that the error may have arisen through a misprint or misquotation, none of the authors mentioned having apparently referred to Metschnikoff's original papers.

first loses its regular contour, becomes sinuous, and finally breaks up into a mass of brownish granules...." By isolating infected *Daphnia*, Metschnikoff succeeded in restoring them to health "thanks to the destruction of the spores by their phagocytes. If on the other hand the phagocytic action is inadequate, owing to the continued increase in the number of spores swallowed or for any other reason, the latter begin to germinate and give rise to budding conidia."

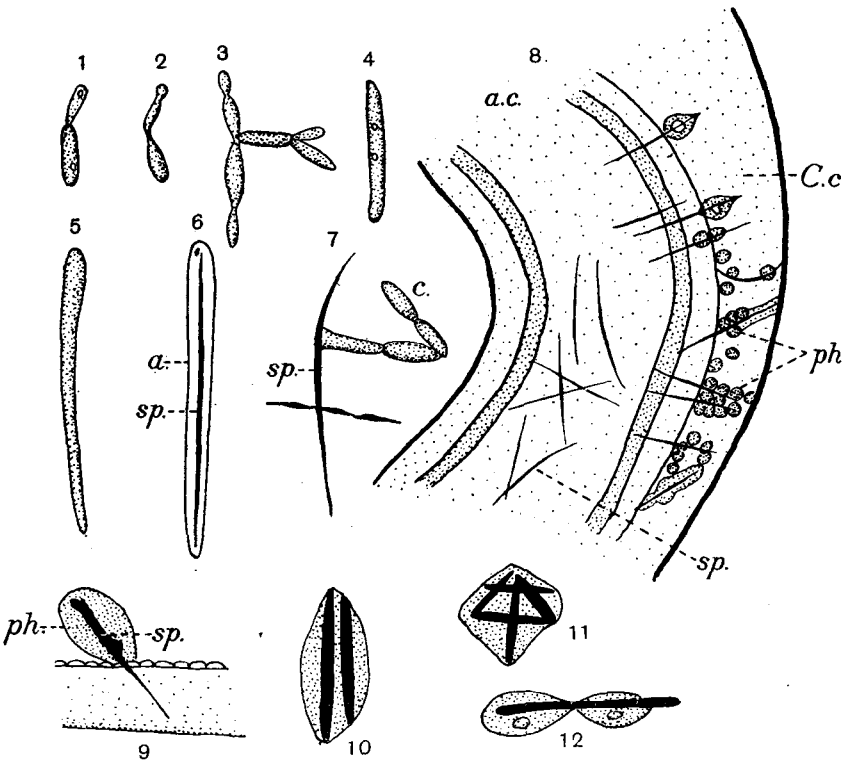

Fig. I. *Monosporella bicuspidata* (Metsch.), after Metschnikoff, slightly schematized. 1, 2 and 3, budding cells; 4 and 5, elongated cells developing into asci; 6, ascus (*a.*) with spore (*sp.*); 7, germination (*c*) of a spore (*sp.*) that has penetrated into the body cavity of the host; 8, anterior portion of *Daphnia* showing the spores (*sp.*) of *M. bicuspidata*, free in the alimentary canal (*a.c.*) or perforating its wall and penetrating into the body cavity (*C.c.*) where they are surrounded by phagocytes (*ph.*); 9, spore (*sp.*) penetrating into the body cavity, partly surrounded and digested by a phagocyte (*ph.*); 10, phagocyte containing two cells of parasite; 11, plasmodium of phagocytes containing several cells of *Monosporella*; 12, fungus cell surrounded by two phagocytes.

Following upon Metschnikoff, almost all authors (Zopf 1890, Hansen 1904, Dangeard 1907, Guillermond 1907, Lafar 1910 and Saccardo 1911) dealing with *Monospora* refer to Metschnikoff's observation. Chatton (1907), however, seems to be the only author who has actually seen the parasite; thus, in his general account of parasites and commensals living upon Cladocera he mentions (p. 807) that every year during the spring he used to find *Daphnia*

in abundance parasitized by *M. bicuspidata*. His material was derived from a tank at the Jardin des Plantes, Paris.

The genus *Monospora*, or, as we shall call it now, *Monosporella*, has hitherto comprised: (*a*) the species *bicuspidata* Metschnikoff and (*b*) a yeast-like fungus found by Bütchli (1876, p. 148, Pl. XIV, fig. 8) in the coelom of a free-living nematode, *Tylenchus pellicidus* Bast. Unfortunately this author's description and figures are insufficient for determining more than the genus to which the parasite belongs.

This summer (1919) I found a new species of *Monosporella*, which I propose to name *Monosporella unicuspidata*, living in a Dipterous larva: *Dasyhelea obscura* Winnertz[1]. The larvae of this Ceratopogonid live usually in the thick brown sap which fills the infected wounds of elm or horse-chestnut trees. Whilst larvae collected from the wounds of a horse-chestnut, standing on the grounds behind the School of Agriculture, Cambridge, harboured *Monosporella*, those taken from elms (at Newnham and along the Backs, Cambridge) were not infected by this fungus, they contained however other parasites which will be dealt with separately.

The proportion of larvae infected with *M. unicuspidata* appeared to be low, for but twenty out of several hundred forming the material examined by me were found to be infected. The actual proportion of infected individuals doubtless varies in nature and it must have been greater in this instance. Owing to the larvae being insufficiently transparent to permit the detection of the few parasites that may occur in mild forms of infection, some of these doubtless escaped notice. It appears probable, moreover, that a number of larvae may rid themselves of parasites by phagocytosis as some examples of *Daphnia* do when attacked by few *M. bicuspidata*. The parasitized larvae observed by me belonged to three successive generations of *Dasyhelea* and they were all heavily infected. A parasitized *Dasyhelea* larva is easily recognized by the milky appearance of the body and especially of its posterior segments. Examined microscopically, the larva shows an enormous number of elongated refractive cells, completely filling the body cavity, and in some cases so crowded together that they all take a direction parallel to the long axis of the body of the larva. In spite of the great number of parasites that are present, the larvae are able to move, the fat body seems to be the only organ which is completely destroyed, whereby the larva becomes more transparent and the parasites are better observed. Finally the larva dies and decomposes rapidly, thus setting free the resistant forms of the parasite. In the living larva, even when heavily infected, almost all developmental stages of the parasite are easily seen by cutting open the larva in a drop of normal salt solution or Amman's lactophenol[2].

[1] The identification of this Ceratopogonid I owe to the kindness of Mr F. W. Edwards of the British Museum.

[2] Amman's lactophenol, pure or mixed with 0·5 % of cotton-blue, which was used for the examination of the parasite, is highly recommended for similar purposes.

Description of *Monosporella unicuspidata* n. sp. (Fig. II, 1 to 17).

In the young stages, the parasite occurs in the form of small oval cells from 4μ to 10μ long, budding at one end. The buds are usually single (Fig. II, 1 to 6) but occasionally two or three buds are formed simultaneously (Fig. II,

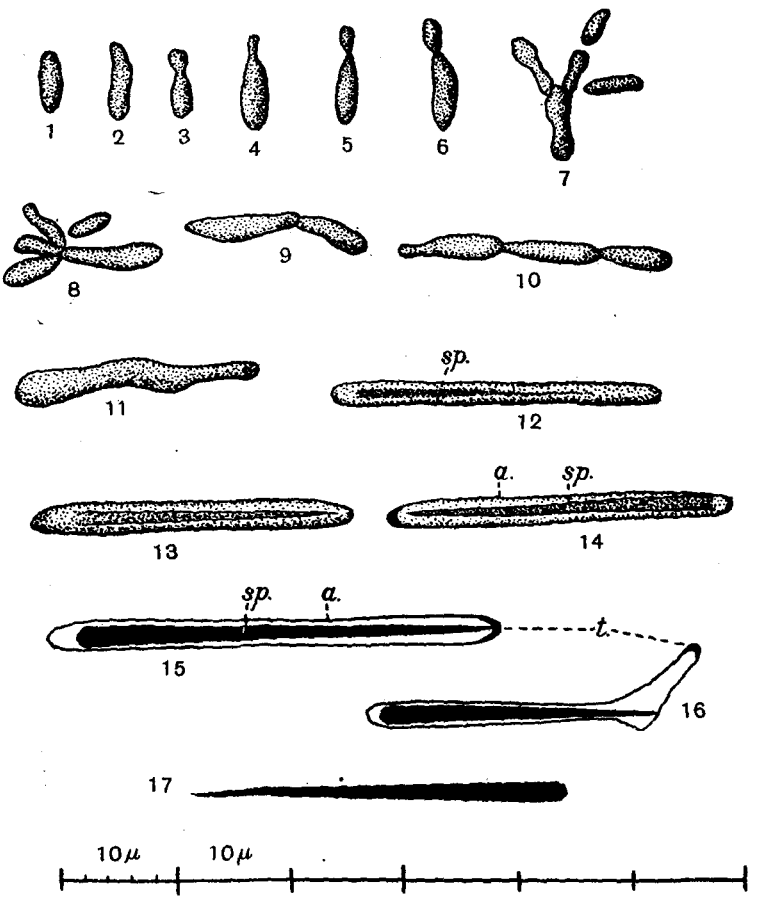

Fig. II. *Monosporella unicuspidata* n. sp. 1 to 6, different stages of budding cells; 7 and 8, rare cases of multiple budding; 9, ordinary budding; 10, chain with three cells; 11, elongated cell developing into ascus; 12, ascus with the beginning of the spore formation (*sp.*); 13 and 14, more advanced stages of spore formation; 15, ascus (*a.*) with well formed spore (*sp.*), *t*, thickened wall of the ascus; 16, deformed ascus; 17, spore. Figures drawn with camera lucida, slightly schematized, the refractory spores being represented in black. The scale of magnification is given beneath the figures.

7, 8). The new buds generally detach themselves very soon and begin to bud; in only a few cases did I find three cells joined to form a chain 24μ in length (Fig. II, 10). When the body of the larva is completely invaded, the parasites become elongated and acquire a uniform shape reaching 30μ in length and

2·5μ in width (Fig. II, 13, 14). These elongated forms correspond to asci and are at first transparent having finely granulated protoplasm. As development proceeds, the protoplasm near one end of the ascus begins to show a small triangular refractive body which gradually elongates until it occupies almost the whole length of ascus. The refractive body (Fig. II, *sp.* 14, 15) gradually becomes clearly defined and finally develops into a long needle-shaped unicellular spore with one end sharply pointed, the other end truncated. The space between the spore and walls of the ascus is filled with transparent fluid, and the end of the ascus facing the pointed portion of the spore is thickened. In some cases, after the death of the larva, when the asci escape into the fluid surrounding the insect, the ascus walls become deformed so that the thickened end is bent to one side of the spore (Fig. II, 16). The asci and spores vary in size, the asci measure 30μ to 40μ and the spores 24μ to 35μ in length; the truncated end of the spore usually measures 1·8μ across.

I have failed to observe the liberation of the spores from the asci and the germination of spores. In only one larva was the alimentary canal found to contain several free spores (Fig. II, 17), but unfortunately the larva was damaged during examination whereby further observation was precluded.

As the spores of *M. unicuspidata* have only one end pointed, whilst *M. bicuspidata* (Metsch.) has spores with both ends pointed, it is probable that the first named species has a poorer chance of perforating the alimentary canal of its host and this may account for the smaller proportion of infected hosts as compared to what has been observed with *M. bicuspidata* and *Daphnia*.

It is worthy of note that other Dipterous larvae (those of *Rhyphus fenestralis* Scop., *Mycetobia pallipes* Meig., *Aulacogaster rufitarsis* Mcq., *Phaonia cincta* Zett. and a few Eristalines, Drosophilids and Dolichopodids) living under the same conditions as *Dasyhelea obscura*, were not found to be infected with *Monosporella unicuspidata*.

The genus *Monosporella*, hitherto known as *Monospora* Metschnikoff, is often placed by systematists near to the genus *Nematospora* Peglion (Fig. III). The latter, which contains but one species *N. coryli*, discovered and named by Peglion (1901), is a parasite of the hazel-nut in Italy. It is a budding, yeast-like fungus with elongated cells; the ascus is sausage-shaped 65μ–70μ long by 6μ–8μ broad, and contains 8 spores in two longitudinally disposed bundles of 4, separated by an interval midway along the length of the ascus. These spores are elongate spindle-shaped, with a long flagellum at one end and measure 38μ–40μ without the flagellum which is about 35μ to 40μ long. Before germination, the spore loses its flagellum and broadens. Peglion succeeded in cultivating *N. coryli*, finding that it grew well on sterilized sugar-beet or meat-broth gelatin and badly in fluid media, where it formed only a mycelium.

The systematic position of the genera *Monosporella* and *Nematospora* is not yet clearly defined. Metschnikoff and Zopf placed *Monosporella* among

the true yeasts, and Peglion divided the Saccharomycetes into four genera, *i.e. Saccharomyces, Schizosaccharomyces, Monospora* (= *Monosporella*) and *Nematospora.*

On the other hand Hansen (1904), to whom we owe the recent classification of Saccharomycetes which is accepted by almost all mycologists, considers the last two genera as representing "doubtful Saccharomycetes," and he remarks that they are rather rare fungi which were observed only by the authors who discovered them[1], moreover, that he and his collaborators had searched for them in vain in the hope of obtaining material for purposes of study. Notwithstanding the great authority of Hansen, I cannot but

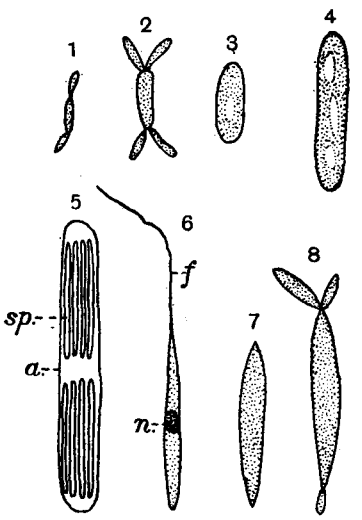

Fig. III. *Nematospora coryli* Pegl. after Peglion. 1 and 2, budding cells; 3–4, stages of growing cell; 5, ascus (*a.*) with eight spores (*sp.*); 6, spore stained with gentian violet, showing the nucleus (*n.*) and flagellum (*f.*); 7, spore after losing its flagellum; 8, budding spore.

believe that *Monosporella* and *Nematospora* have been sufficiently known for purposes of classification, and that they should without hesitation be placed in the family of Saccharomycetaceae. In fact all the characters of this family, as defined by Hansen himself, cover perfectly well the representatives of these two genera. Thus Hansen's classification, with the few modifications and details added by Lafar (1910), is as follows:

I shall omit the expressions (for the group A) "true Saccharomycetes" and (B) "doubtful Saccharomycetes" and shall add more details bearing on the genus *Monosporella.* In Hansen's classification the Saccharomycetaceae are widely separated from the genus *Schizosaccharomyces* for which he creates the family Schizosaccharomycetaceae.

[1] I would note that *M. bicuspidata* Metsch. was often observed by Chatton (1907) and cultures of *N. coryli* were sent by Peglion to the late Prof. A. Giard of Paris who tried to inoculate hazelnuts with the fungus.

Family **Saccharomycetaceae.** Monocellular, sporogenic budding fungi. Each cell is a potential sporogenic cell (or ascus). The number of spores in the ascus is usually 1 to 4, seldom 12. The spores are monocellular. Typical mycelium is formed by a few species.

A. Spore rounded, oval, pileate or lemon-shaped with or without projecting rim.

I. GROUP. In Saccharine nutrient liquids they furnish only sedimental yeast at the outset, surface films occur only at a later period, if at all. Films more or less mucilaginous. Spore smooth, globular or oval, with 1 or 2 membranes. Spore germinates either by gemmation or by producing a promycelium. Great majority produce alcoholic fermentation.

Genus 1. *Saccharomyces* Meyen. Spore with single membrane; germinates by ordinary gemmation. In addition to yeast cells a few of them produce mycelium with well defined septa.

Genus 2. *Zygosaccharomyces* Barker. Ascus is formed after cell fusion.

Genus 3. *Saccharomycodes* Hansen. Spore with a single membrane, germinates into a promycelium, the new cells, being incompletely separated, form a mycelium with well defined septa.

Genus 4. *Saccharomycopsis* Schiönning. Spores with two membranes.

II. GROUP. Film produced on the surface of nutrient solution immediately after the same has been inoculated. The film has a dry dull appearance due to the inclusion of air bubbles. Several species produce esters, and a few of them do not cause fermentation. Spore with a single membrane, of different shapes with or without projecting edge.

Genus 5. *Pichia* Hansen. Spore rounded, hemispherical or irregular and angular. A strong mycelium is formed. No fermentation.

Genus 6. *Willia* Hansen. Spore pileate, or lemon-shaped, with a projecting rim. Most of the species possess considerable ester-forming powers, but a few do not produce fermentation.

B. Spores acicular or spindle-shaped, parasitic fungi.

Genus 7. *Nematospora* Peglion. Elongated budding cells; sausage-like ascus containing eight unicellular spores in two bundles of four. Spore elongated, spindle-shaped with a long flagellum, which is lost before germination. Parasitic on hazel-nuts in Italy. Grows well on sugar-beet or meat-broth gelatin, badly in liquids where it forms mycelium only. One species: *N. coryli* Peglion (Fig. III).

Genus 8. *Monosporella* (= *Monospora* Metschnikoff 1884). Budding, yeast-like fungi, each cell a potential ascus. The latter is elongated and

produces one unicellular acicular spore. Parasite in the blood of invertebrates. Not yet cultivated. The genus comprises the following species:

(1) *M. bicuspidata* (Metschnikoff 1884). Asci slightly narrowing at one end; spores pointed at both ends. Parasite in the body cavity of *Daphnia magna* (Crustacea) (Fig. I).

(2) *M. unicuspidata* n. sp. Asci of elongated but regular form with the wall thickened at one pole. Spore pointed at one end, truncated at the other. Parasite in the body cavity of Ceratopogonid larvae: *Dasyhelea obscura* Winnertz. (Insecta: Diptera.) (Fig. II.)

(3) *M.* sp. Yeast-like fungi found by Bütschli (1876) in the coelom of a free-living nematode: *Tylenchus pellicidus* Bast. (Vermes.)

(4) *M.* (?) sp. Yeast-like fungi of elongated shape found by Caullery and Mesnil (1899 and 1911) in a Polychaete worm *Potamilla torelli*, where they seem to produce a special kind of tumour ("néoformation papillomateuse"). They consider this yeast to be related to *Monospora* although they did not succeed in finding the spores. They also mention a similar yeast occurring in a pelagic Copepod *Acartia*.

Family **Schizosaccharomycetaceae.** Endosporogenic, monocellular fungi, reproduce by fission which is preceded by the formation of a septum that at once commences to divide into two lamellae from outside. No budding occurs. Spores unicellular of which 1–8 occur in each ascus. In some cases formation of asci is preceded by fusion. Spores stained blue by a solution of iodine in potassium iodide. The cells never contain glycogen (a contrast to Saccharomycetaceae). Produce alcoholic fermentation; one genus *Schizosaccharomyces* Lindner.

Acknowledgment.

I am much indebted to Professor G. H. F. Nuttall for valuable suggestions in connection with this study.

APPENDIX.

Concerning the re-naming of Metschnikoff's genus Monospora.

According to Lafar (1910, p. 292), "the genus *Monospora* Metschnikoff ought really to be re-named, since this title has already been applied by Hochstetter, to one of the Flacourtiaceae."

This statement is correct. I would add that Hochstetter's name dates from 1841. However, in Warburg's monograph of the Flacourtiaceae (1894, in Engler and Prantl's *Die natürlichen Pflanzenfamilien*, Part III, p. 37) I find that the name *Monospora* Hochstetter is condemned as a synonym of *Trimeria* Harvey (1838).

On the other hand I find that the name *Monospora* was also given by Solier in 1845 to an Alga of the family Rhodomelaceae; the name was accepted by all specialists of the group and is actually quoted in botanical text-books.

The foregoing evidence serves unfortunately to condemn Metschnikoff's name *Monospora* as being preoccupied. This is regrettable because the name has long been associated with that author's early observations on the important phenomenon to which he gave the name of phagocytosis. I have endeavoured, however, to replace the original name by another which expresses the same meaning, and *Monosporella*, which I propose, seems most fitting, other suitable names being preoccupied.

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