Osborn Zoölogical Laboratory, Yale University,
and University College, New York University.

On certain parasites of Phacus and Euglena; *Sphaerita phaci*, sp. nov.

By

Theo. L. Jahn [1]).

(With plates 16 and 17.)

Within the last decade considerable attention has been drawn to the parasites of protozoa that are grouped under the genus *Sphaerita*. These parasites were first described by DANGEARD (1886, 1886 a) in Nuclearia, Heterophrys, and Euglena, and the descriptions were extended in later studies (DANGEARD, 1889 a, 1895). Since that time members of this genus have been described in other free-living rhizopods (CHATTON and BRODSKY, 1909; PENARD, 1912; MATTES, 1924; and perhaps IVANIĆ, 1925), in free-living flagellates (DANGEARD, 1889; de PUYMALY, 1927; SKVORTZOW, 1927; MITCHELL, 1928; MAINX, 1928; and perhaps NÄGLER, 1911), in intestinal amoebae (WENYON, 1907, 1926; CRAGG, 1919; DOBELL, 1919; NÖLLER, 1921; EPSTEIN, 1922; LWOFF, 1925; BECKER, 1926; BACIGALUPO, 1927; SASSUCHIN et al, 1930), in intestinal flagellates (WENYON, 1907, 1926; DA CUNHA and MUNIZ, 1923; SASSUCHIN et al, 1930), and in *Nyctotherus ovalis* (SASSUCHIN, 1928).

During the course of cytological studies on various euglenoid flagellates, two parasites of this genus have been observed by the writer. The present report is a description of an apparently new species of Sphaerita in *Phacus pleuronectes* and *P. longicauda*, and a confirmation of the report of *Sphaerita dangeardi* in American euglenae (MITCHELL, 1928).

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[1]) National Research Fellow in Zoology at Yale University.

The parasitized Phacus used in the present study were obtained from a pond in van Courtlandt Park, New York City, and the parasitized Euglena from a pond in the vicinity of Houston, Texas. Material was fixed in ZENKER'S or SCHAUDINN'S fluids and stained with haematoxylin by HEIDENHAIN'S method.

Sphaerita phaci, sp. nov.

The uninucleate stage as observed in *Phacus pleuronectes* is elongated (8—9 μ by 2 μ) and usually slightly curved, and contains a central nucleus and two vacuoles, one at each end of the cell (fig. 1). The organism is surrounded by a distinctly chromophobic area, apparently a vacuole in the cytoplasm of the host. The uninucleate stage is usually located close to the gullet, and this proximity is taken to indicate that infection occurs by way of the gullet, since it seems probable that entrance through the gullet would be much easier than through the rigid pellicle of the cell. However, neither mode of infection has been demonstrated. In one case (fig. 2) a mitotic figure was observed. This mitotic figure resembles rather closely those observed in other members of the order Chytridiales (e. g., WAGER, 1913, in *Polyphagus euglenae*) in that there is a clear area surrounding the amphiaster. This specimen was somewhat larger (11 μ in length) than other uninucleate specimens, and the vacuoles were apparently undergoing degeneration.

The young plasmodium (figs. 4, 6 and 8) is elongated, and as it increases in size it may become curved in various forms as shown in figs. 5, 7 and 9. The vacuoles disappear in the early growth stages, and the most mature plasmodia seen contained about thirty-five nuclei but no other visible structures.

Double and triple infections are common, and the damage to the host in these cases is manifest in a decreased stainability of the chromatophores and an apparent vacuolization of the cytoplasm. Loss of the original culture did not permit observations on the living organisms, but the present findings seem to be in agreement with the fading and disorganization of the chromatophores described by DANGEARD (1889) for *Phacus* and by de PUYMALY (1927) for *Euglena.*

This parasite differs from the other species of Sphaerita in the possession of a slightly elongated uninucleate stage, containing a vacuole at each end. The older uninucleate stages of *S. dangeardi* (figs. 11, 12 and 13) are elongated, but they lack vacuoles and are considerably larger (17—20 μ by 8—9 μ as compared to 8—9 μ by 2 μ). Uninucleate stages of other species are not elongated and do

not contain vacuoles. Plasmodial stages of other species are not nearly so elongated nor so curved as those of this species. The plasmodia of *Pseudosphaerita euglenae*, however, are curved but are larger in size and undergo precocious splitting.

In view of the differences between this and other species of Sphaerita, it is proposed to create a new species, *Sphaerita phaci*, to include this organism. The possibility arises that when the complete life history of *Sphaerita phaci* is known it may be necessary to transfer it to another genus, but at present it seems to resemble *S. dangeardi* more closely than other known species, and for that reason it is placed in the genus Sphaerita. There is also a possibility that this is the same organism as that seen by DANGEARD (1889, 1889 a) in *Phacus alata* and *P. pyrum*, but lack of adequate descriptions preclude a positive identification.

Sphaerita dangeardi CHATTON and BRODSKY, 1909.

The parasite observed in *Euglena gracilis* (?) seems to be the same as that described by DANGEARD (1895) and MITCHELL (1928) in various species of Euglena. The youngest stage found (fig. 10) is almost spherical and is 4—5 μ in diameter. The largest stage observed was 25 μ long and 16 μ wide. Intermediate stages are shown in figs. 11—13. The structure of the nucleus of these intermediate stages is seen in figs. 12 and 13. The endosome is slightly eccentric, and the nucleus possesses a peripheral ring of chromatin granules. These were not observed in earlier stages. In well destained specimens the endosome seems to be vacuolated. In the largest stage observed (fig. 14) the chromatin granules were scattered throughout the nucleus. It seems as if this might be an early stage of mitosis, but this could not be determined with certainty. These might be early stages of either of the two forms described by MITCHELL (1928) who stated that the early stages of his parasites were indistinguishable.

Discussion.

In his first description of *Sphaerita*, DANGEARD (1886) used the specific name *S. endogena* for the parasites of both rhizopods and flagellates which he considered to be of the same species. Later (1895) he described in more detail the *Sphaerita* of *Euglena* and still referred to it as *S. endogena*. He also found a second form in *Euglena* which differed from the first in that the young plasmodium underwent a precocious splitting before multiple fission. He sug-

gested that this might be a different parasite and also suggested that if such should be the case, it might be called *Pseudosphaerita euglenae.*

CHATTON and BRODSKY (1909) in their description of a species of Sphaerita in *Amoeba limax* point out that their parasite resembles very closely the parasite DANGEARD described in rhizopods and also that it differs from the *Sphaerita* of the euglenoids. They follow the suggestion of DANGEARD (1897) that the parasites of rhizopods and flagellates may be different, and on the basis of a comparison of their cytological observations on the parasite of *Amoeba* with the later studies by DANGEARD (1895) on those in *Euglena*, they separated the parasites into two species. Since the specific name *endogena* was first applied to the parasite of rhizopods, they proposed the name *S. dangeardi* for the parasite described by DANGEARD as *S. endogena* in *Euglena.* The writer has not been able to confirm the statement of MITCHELL (1928) that CHATTON and BRODSKY propose the name *Pseudosphaerita euglena* for this parasite; they propose *Sphaerita dangeardi* as stated above. The name *Pseudosphaerita euglenae* had previously been suggested by DANGEARD (1895) for a different organism.

MITCHELL (1928) discovered in various members of the genus *Euglena* two types of parasites, one of which had previously been described by DANGEARD (1895). MITCHELL does not cite the paper of DANGEARD (1895) in which the cytology of this form was described, but states that the parasites found by himself are not the same as those described by DANGEARD in earlier papers (DANGEARD, 1886, 1889 a). From a comparison of the figures of DANGEARD (1895) and MITCHELL (1928) it appears to the writer that the parasites found by the two workers are identical. The later paper of DANGEARD (1895) was a cytological study of the parasites which he had observed previously (1886, 1889 a), and it seems probable that the differences in the figures of the papers are due primarily to differences in magnification and to the improved staining methods used in the later studies. The form described by MITCHELL (1928) which has a multinucleate plasmodium (in *E. viridis*) is *Sphaerita dangeardi* CHATTON and BRODSKY, 1909, and is identical with one of the parasites described by DANGEARD (1895) as *S. endogena* in *Euglena sp.* The form described by MITCHELL (1928) which does not have a multinucleate plasmodium (in *E. caudata*), however, is not the same as any of the parasites described by DANGEARD and is apparently a new parasite or a new stage in the life history of *S. dangeardi.* DANGEARD described encysted stages of *S. dangeardi,*

and the possibility arises that the second form of MITCHELL might be precystic stages of this species. However, due to lack of evidence, this point cannot be determined.

The question of the motility of the zoöspores of *Sphaerita* is one of considerable importance in the taxonomy of the genus and one which has, in some cases, been overlooked. To the knowledge of the writer, the only descriptions of flagellated zoöspores are those of DANGEARD (1886, 1886 a, 1889 a, 1895) and of de PUYMALY (1927). In all of the other above mentioned papers on *Sphaerita* the authors state that a flagellated stage was not observed, or that it does not occur, or that the zoöspores are definitely non-motile. This has led to general statements in the literature that the spores of *Sphaerita* are non-motile (e. g., WENYON, 1926). DANGEARD (1886, 1886 a), however, observed the release of both flagellated and non-flagellated zoöspores and noted that the non-motile spores were apparently released prematurely, that is, before their development was complete. He observed the ingestion of non-motile spores by rhizopods but could not determine whether or not they underwent development. The flagellated spores, however, were seen to attach themselves to the pellicle of *Euglena*. These observations were partly repeated and extended by de PUYMALY (1927). Therefore, it seems certain that at least some species possess motile spores, but the possibility still remains that certain species may produce non-motile spores only.

Up to the present time nine species of *Sphaerita* have been described. These species, together with the hosts from which they were originally described, are as follows:

1. *S. endogena* DANGEARD, 1886, partim, CHATTONand BRODSKY, 1909, in *Nuclearia* and *Heterophrys*.
2. *S. dangeardi* CHATTON and BRODSKY, 1909. Found by DANGEARD (1886, 1895) and by MITCHELL (1928) in *Euglena*.
3. *S. minor* DA CUNHA and MUNIZ, 1923, in *Trichomonas*.
4. *S. amoebae* MATTES, 1924, in *Amoeba sphaeronucleolus*.
5. *S. plasmophaga* MATTES, 1924, in *A. sphaeronucleolus*.
6. *S. nucleophaga* MATTES, 1924, in *A. sphaeronucleolus* and *A. terricola*.
7. *S. normeti* LWOFF, 1925, in *Entamoeba coli* and *E. histolytica*.
8. *S. endamoebae* BECKER, 1926, in *Entamoeba citelli*.
9. *S. trachelomonadis* SKVORTZOW, 1927, in *Trachelomonas teres* and *T. swirenkoi*.

To this list of species it is proposed to add:

10. *S. phaci*, sp. nov., in *Phacus pleuronectes* and *P. longicauda*. Uninucleate stage elongated with a maximum size of about $10 \times 2\,\mu$, containing a vacuole at each end. Plasmodium elongated and curved. not round or oval as in other species. Flagellated stages not observed.

Summary.

1. *Sphaerita phaci*, sp. nov., from *Phacus pleuronectes* and *P. longicauda* is described.

2. The existence of *S. dangeardi* in American euglenae is confirmed, and the cytology of the uninucleate stages is described.

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Description of plates.

Plates 16 and 17.

Plate 16.

Sphaerita phaci, sp. nov.

Fig. 1. Uninucleate stage of *Sphaerita phaci* in *Phacus pleuronectes*. Parasite is 9 μ in length.

Fig. 2. Mitotic division of uninucleate stage.

Fig. 3. Uninucleate stage in *Phacus longicauda.*

Figs. 4 and 6. Young plasmodia.

Figs. 5, 7, 8 and 9. Older plasmodial stages. Double infection in fig. 7 and triple infections in the other figures.

Plate 17.

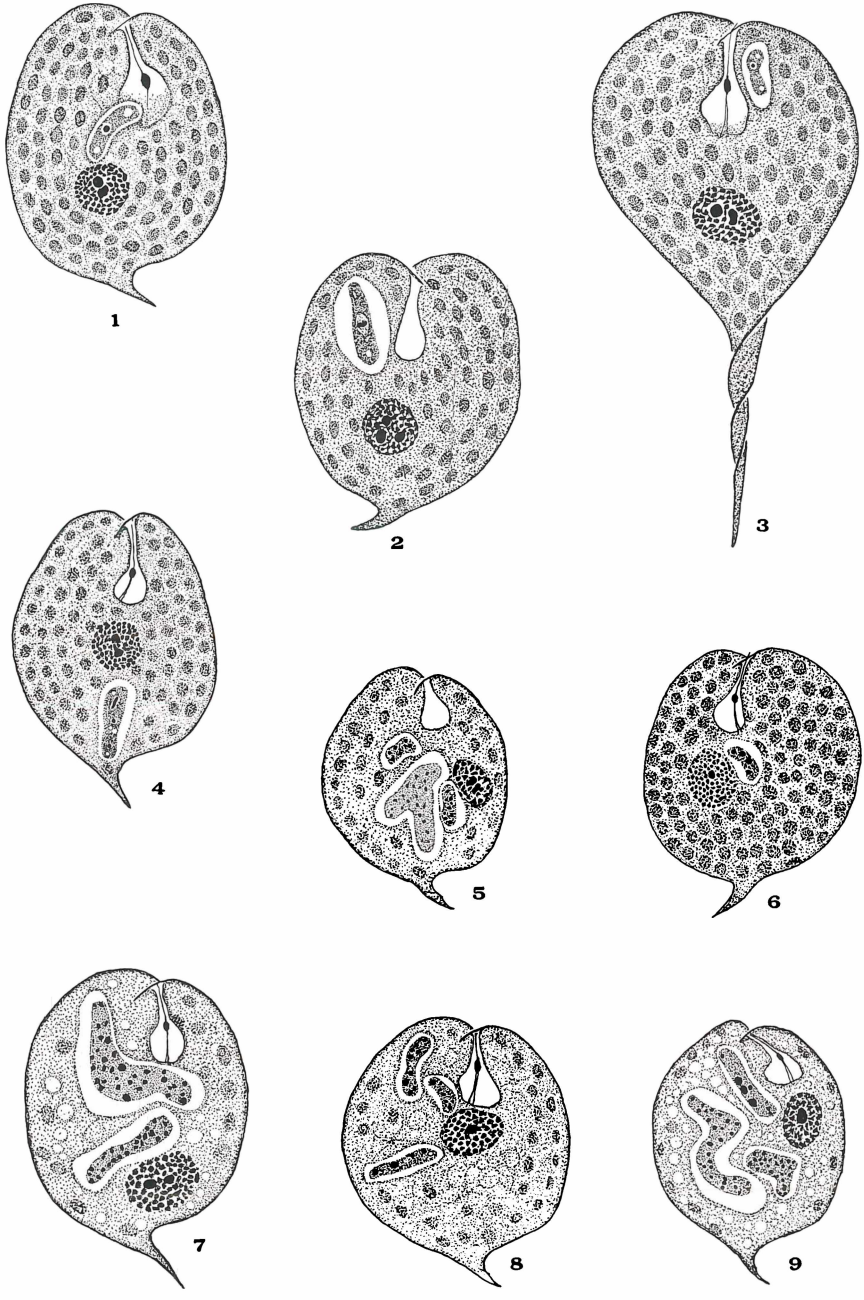
Sphaerita dangeardi Chatton and Brodsky in *Euglena gracilis* (?).

Fig. 10. Double infection showing very young stages.

Figs. 11—13. Older uninucleate stages, showing nuclear structure.

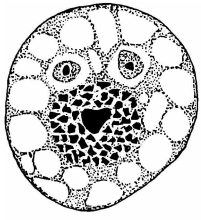
Fig. 14. Largest stage found, showing nuclear structure which might be indicative of a prophase stage of mitosis.

In figs. 11 and 13 the euglenoid nucleus is in a lower focal plane, and in figs. 12 and 14 it is not shown.



Jahn, Sphaerita phaci.

VERLAG VON GUSTAV FISCHER IN JENA



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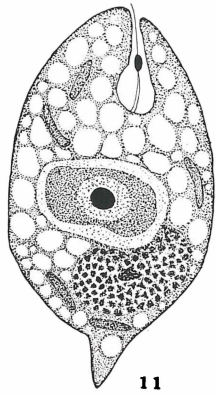

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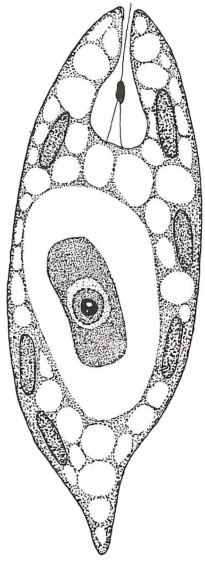

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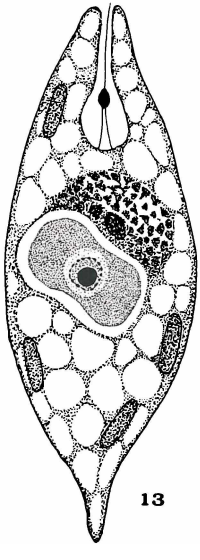

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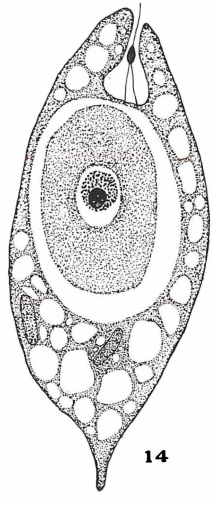

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Jahn, Sphaerita phaci.

VERLAG VON GUSTAV FISCHER IN JENA.