FIRST RECORD OF AN ECTOPARASITIC FLAGELLATE ON CILIATES : AN ULTRASTRUCTURAL INVESTIGATION OF THE MORPHOLOGY AND THE MODE OF ATTACHMENT OF SPIROMONAS GONDERI NOV. SPEC. (ZOOMASTIGOPHORA, SPIROMONADIDAE) INVADING THE PELLICLE OF CILIATES OF THE GENUS COLPODA (CILIOPHORA, COLPODIDAE)

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SUMMARY

A flagellate ectoparasitic on ciliates occurred spontaneously in a soil sample. It was first described by GONDER (1910) as "Colpoda-Parasit", but its flagellate nature was not recognized. Thus - as far as we know - this is the first reliable record of an ectoparasitic flagellate on ciliates. Its fine structure and mode of life show that it is related to the genus Spiromonas. We think it is a new species, Spiromonas gonderi nov. spec., because it is host-specific for ciliates of the genus Colpoda and does not have a rostrum. Heavily infested hosts move more slowly than normal; their cytoplasm becomes strongly vacuolated and then they die. Light - and electron microscopic examination reveals the following peculiarities, some of which are different from those described by BRUGEROLLE and MIGNOT (1979) for S. perforans: 1. The left flagellum arises from a periflagellar canal, the right one directly from the cell surface. By contrast, both flagella of S. perforans emerge from shallow depressions. 2. The pellicle consists of 3 unit membranes and is perforated by many micropores. There are subpellicular microtubules and a netlike fibro-granular layer on the surface of the pellicle. This surface coat is not demonstrable in S. perforans. 3. The anterior region of the parasite contains many micronemes. 4. The host-parasite connection is mediated by a complex "apical apparatus" that consists of a ring of 4-5 microtubules, a polar ring-like structure, and a unit membrane that separates the host from the parasite. The separating membrane is made of the inner electron-dense layers of the cell membrane of the ciliate and parasite respectively. We suppose that nutritive substances must pass through this membrane, because S. gonderi lacks a true cytostome and feeding is a long-lasting process in this species. Lytic enzymes released from the micronemes could be of importance during this procedure. No comparable "apical apparatus" has been described for S. perforans. 5. The cristae mitochondriales are tubular. 6. Unlike S. perforans, which has a contractile vacuole and trichocysts of the dinoflagellate type, S. gonderi did not exhibit such organelles. These fine structural peculiarities, especially the "apical apparatus", suggest a close affinity of Spiromonas to the Sporozoa. Thus one may speculate that this enigmatic creature might be either a developmental stage of a coccidian or a coccidian that has turned secondarily to an ectoparasitic mode of life. But if we consider the flagellated trophic stage of Spiromonas, it is more likely that the Spiromonadidae are nearer to the Zoomastigophora than to the Sporozoa.

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RÉSUMÉ

Spiromonas gonderi n. sp. qui fait l'objet de ce travail est le premier flagellé décrit qui soit ectoparasite de ciliés. Inféodé aux Colpoda, il a été antérieurement observé par GONDER (1910) mais cet auteur n'en a pas reconnu la nature. Fortement apparenté à Spiromonas perforans, ectoparasite de Chilomonas paramaecium étudié par BRUGEROLLE et MIGNOT (1979), il se distingue néanmoins de ce dernier par de nombreux caractères : 1. Le flagelle gauche émerge d'un canal périflagellaire, le droit de la surface cellulaire alors que les deux flagelles de S. perforans émergent de dépressions peu profondes. 2. Pellicule formée de 3 membranes unitaires et percée de nombreux micropores. Présence de microtubules sous-pelliculaires et d'un revêtement réticulé de texture granulo-fibrillaire à la surface de la pellicule. Ce revêtement superficiel est absent chez S. perforans. 3. Nombreux micronèmes dans la région antérieure du parasite. 4. Présence d'un « appareil apical » complexe formé d'un anneau de 4-5 microtubules, d'un anneau polaire et d'une membrane unitaires séparant l'hôte du parasite au niveau de la jonction de ces derniers. La membrane de séparation est faite des couches internes, denses aux électrons, des membranes cellulaires du cilié et du parasite. Le passage de substances nutritives à travers cette membrane est envisagé en raison de l'absence d'un véritable cytostome chez S. gonderi et de la lenteur de la nutrition chez cette espèce. Des enzymes lytiques, produits par les micronèmes pourraient intervenir dans ce dernier processus. Un tel « appareil apical » n'a pas été décrit chez S. perforans. 5. Crêtes mitochondriales tubulaires. 6. Absence de la vacuole contractile et des trichocystes de type dinoflagellé décrit chez S. perforans.

Ces particularités ultrastructurales et plus particulièrement l'« appareil apical » suggèrent une parenté étroite entre Spiromonas et les Sporozoa. On peut ainsi supposer que ce Protiste énigmatique serait soit le stade de développement d'un Coccidie, soit une Coccidie devenue secondairement un ectoparasite. Cependant, compte-tenu du stade trophique flagellé de Spiromonas, les Spiromonadidae sont probablement plus proches des Zoomastigophora que des Sporozoa.

I. - INTRODUCTION

In 1910 GONDER described an ectoparasite of *Colpoda cucullus*, which appeared spontaneously in a hay infusion and destroyed the *Colpoda* within one day. GONDER (1910) did not assign a name to the parasite because he could not determine its systematic position.

We have been fortunate enough to rediscover this evidently rare organism, and have examined it in the electron microscope. It proved to have many features in common with the flagellates *Spiromonas angusta* and *Bodo perforans*, described by ALEXEIEFF (1929) and HOLLANDE (1938). The latter species parasitizes flagellates of the genus *Chilomonas* and has been studied electron-microscopically by BRUGEROLLE and MIGNOT (1979). Because of its very unusual ultrastructural peculiarities they considered the possibility that it might be a link between the dinoflagellates and the sporozoans. Our investigation of the *Colpoda* parasite, which is evidently related to *Spiromonas (Bodo) perforans*, reveals new aspects that bear on this interesting question.

II. — MATERIAL AND METHODS

Spiromonas gonderi occurred spontaneously in a soil sample gathered on October 29, 1982 in the Seewinkel of the Burgenland, in the so-called "hell". The soils of this region are saline and are frequently inundated by ground water. The sample was air-dried for 2 months, moistened with distilled water, and investigated after 6 days.

FIGS. 1, 2. — Spiromonas gonderi : free, non-parasitizing individual and an old one that has been parasitizing for a long time. From observations of living cells.

FIG. 10. - Three-dimensional reconstruction of the organization of S. gonderi; ventral view. From light and electron micrographs.

FIGS. 11, 12. – Original illustrations of the "Colpoda-Parasit", from GONDER (1910).

FIGS. 3-5. — Changes in shape of S. gonderi after forcible separation from the host. From living cells.

FIG. 6. — Schematic illustration of the formation of the separating membrane between *Spiromonas* and *Colpoda*. From electron micrographs.

FIG. 7. – A Colpoda fastigata parasitized by many S. gonderi. From living cells.

FIG. 8. – A S. gonderi parasitizing on Paracolpoda steinii. Protargol impregnation.

 $F_{IG.}$ 9. — Schematic drawing of the organization of a parasitizing S. gonderi. Longitudinal section at the level of the anterior flagellum. From electron micrographs.

Abbreviations : AF = anterior flagellum, AI = alveolus of the pellicle of the ciliate, DB = dense body, DV = dense vesicle, E = epiplasm of the ciliate, ER = cisternae of the endoplasmic reticulum, F_2 = fibril system 2, G = Golgi body, IA = inner alveolar membrane of the ciliate, ILC = inner layer of the outer cell membrane of the ciliate, ILF = inner layer of the outer cell membrane of the flagellate, IM = inner cell membranes of the flagellate, L = netlike layer covering the outer cell membrane of the flagellate, LB = lamellar body, LV = empty vesicle, M = mitochondria of the flagellate, MC = cell membrane of the ciliate, Mi = mitochondria of the ciliate, MN = micronemes, MP = micropores, MR = microtubules of the attachment ring, Mt = subpellicular microtubules of the flagellate, N = nucleous, Nu = nucleolus, OA = outer alveolar membrane of the ciliate, OM = outer cell membrane of the flagellate, PC = periflagellar canal, R = ribosomes, SM = separating membrane.



The light-microscopic observations were performed with a microscope equipped with conventional and differential-interference-contrast optics. Protargol slides were prepared according to the method of FOISSNER (1982).

The electron-microscopy procedure was carried out according to the method of PECK *et al.* (1975). Ciliates parasitized by flagellates were fixed in a glutaraldehydeosmium mixture made by adding 3 parts of 2 % (v/v) glutaraldehyde, buffered with 0.05 M Na-cacodylate at pH 7.0, to 1 part of 4 % (w/v) OsO₄. After a 20-min fixation, a solution of 0.75 M Na-cacodylate at pH 7.0 was slowly added to the cells, which were subsequently rinsed in 4 changes of this solution. Total rinsing time was 3 hours. Cells were dehydrated in a graded series of unbuffered ethanol, transferred to propylene oxide, and embedded in Epon. Sections were cut with a Reichert Ultracut, stained with uranyl acetate and lead citrate, and examined either with a Philips EM 400 or with an AEI Corinth 500 electron microscope.

III. – RESULTS

1. Description of Spiromonas gonderi nov. spec.

Diagnosis : In vivo $5-12 \times 4-10 \,\mu\text{m}$ in size; egg-shaped to spherical *Spiromonas* with rounded anterior end. Flagella ca. 1.5-2 times the body length; the left (anterior) flagellum arises from a periflagellar canal, the right (posterior) directly from the cell surface.

Hosts : Colpoda spp.

Type location : In the soil of the "hell" between two lakes, the Neusiedlersee and the Oberer Stinkersee.

Type specimens : One slide of holotype specimens and one slide of paratype specimens have been deposited in the collection of microscopic slides of the Upper Austrian Museum in Linz.

Morphology according to light-microscopic examination (Figs. 1 -5, 7, 8, 13-18): Freely moving, not parasitizing individuals are egg-shaped, with the more pointed end anterior and the more rounded end posterior; size ca. $5-7 \times 4-6 \,\mu\text{m}$. Well-nourished parasitizing individuals are nearly spherical, measuring up to $10-12 \times 8-10 \,\mu\text{m}$, with circular cross section. Flagella about equal in lenght with insertion clearly subpolar; they beat vigorously but cause only a trembling, nearly undirected movement of the animal. Those of parasitizing individuals are extended backward, usually so as to form a figure-eight. Nucleus approximately in the middle of the body, with a large central nucleolus. The cytoplasm of free individuals and young parasites contains only a few minute granules, whereas older parasitizing individuals always have one large spherical to kidney-shaped inclusion at the posterior end. The latter is impregnated like the nucleus with protargol silver. No contractile vacuoles were seen, either in vivo or in the electron microscope.

Species comparison : We believe that our species is identical with the "parasite of Colpoda cucullus" described by GONDER (1910). This inference is based on the correspondence in mode of life, size and cytoplasmic organization, in particular the strongly refractive inclusion behind the nucleus (cf. Figs. 2 and 8 with the original illustrations of GONDER, Figs. 11 and 12). GONDER (1910), who presumably examined only animals prepared for histology, could find no flagella but suggested that 1 or 2 granules stained deep black by hematoxylin that were located at the anterior end could be the sites of origin of free flagella. The "fine structures of the pellicle" could be either artefacts or the subpellicular microtubules.

Spiromonas gonderi differs in body form and in host from S. angusta (ALEXEIEFF) and Bodo perforans HOL-LANDE; the latter was assigned by BRUGEROLLE and MIGNOT (1979) to the Spiromonadidae HOL-LANDE, 1952*, on the basis of its life cycle, manner of

^{*} BRUGEROLLE and MIGNOT (1979) proposed the same family, evidently overlooking the fact that it had already been created by HOLLANDE (1952) for S. angusta.

FIG. 13. — Colpoda fastigata parasitized by many Spiromonas gonderi. Living specimen as seen in differential interference contrast. Bar : 10 um.

FIG. 14. — Paracolpoda steinii parasitized by 3 S. gonderi. Living specimen as seen in differential interference contrast. Bar :10 μ m. FIGS. 15, 16. — Dying Paracolpoda steinii. The parasites are spherical and have a large posterior inclusion. Living specimens as seen in differential interference contrast. Bar : 10 μ m.

FIG. 17. - Colpoda fastigata parasitized by 2 S. gonderi. Protargol impregnation. Bar : 7 µm.

FIG. 18. – A S. gonderi parasitizing Paracolpoda steinii. Protargol impregnation. Bar : 7 μm.

FIG. 19. – Electron micrograph of S. gonderi. The arrow points to fibril system 2. Longitudinal section at the level of the anterior flagellum. Bar : 1 µm.

FIG. 20. – A Colpoda fastigata parasitized by 2 S. gonderi. The arrows point to highly vacualated parts of the cytoplasm of the host. Median section. Bar : 1 μ m.

Abbreviations : AF = anterior flagellum, DB = dense body, DV = dense vesicle, Fl = flagella, LB = lamellar body, LV = empty vesicle, Mi = mitochondria of the ciliate, N = nucleus, Nu = nucleolus, V = empty vacuoles in the ciliate.



feeding and ultrastructure. In both S. angusta and S. perforans the anterior end is elongated to form a rostrum. Spiromonas perforans is thought to parasitize only Chilomonas species; the host of S. angusta, unfortunately, is unknown.

2. Host-Parasite Relations, Life Cycle

Of the 25 ciliate species that developed in the soil sample, only Colpoda fastigata, C. inflata, C. cucullus, C. aspera and Paracolpoda (Colpoda) steinii were parasitized. Spiromonas gonderi is thus specialized for ciliates of the genus Colpoda, as the findings of GONDER (1910) had indicated.

About 70% of the *Colpoda* were parasitized, both trophonts and theronts. The number of parasites varied widely, some individuals having only one and others with as many as ca. 20. They attach most commonly to the posterior end of the host (Figs. 7, 13, 14, 17). Individuals only slightly parasitized display no conspicuous behavioral impairment, but those severely infested slow their locomotion and cease feeding. The cytoplasm then becomes highly vacuolated, and eventually the animals become rounded and die (Figs. 13, 15, 16).

Because attempts to culture the animals failed, few data on their life cycle are available. The free parasites presumably contact the host by chance. At first they are not tightly attached, for many of the small parasites fall off when the cover glass is put in place. By the time that the posterior inclusion can be discerned the attachment has become so firm that considerable force is required to separate parasite from host; when they are pulled away their anterior ends usually become frayed and sometimes a long thread of protoplasm forms, which finally breaks off and fuses with the body (Figs. 3-5). Food intake is evidently a slow, continuous process, for in 30 minutes of observation only a slight increase in size of the parasite is seen. Satiated parasites are spherical in shape and fall away from the host. On several occasions we had the impression that these were surrounded by a thicker membrane than are the young parasites, and that their flagella were shorter or absent (Fig. 2).

3. Fine Structure

All the electron-microscopic data presented here are based on attached individuals parasitizing *Colpoda fasti*gata or *Paracolpoda steinii*. Unfortunately, we found no free parasites.

Flagella and the associated fibrils : The flagella and their basal bodies have few unusual features. At the level of the rather large axosome the flagella are distinctly constricted. The proximal part of the left flagellum is seated in a periflagellar canal, closed at the proximal part and at the distal one tapering out as a shallow groove open ventrally. Its basal body is located ca. 0.5 µm further anterior than that of the right flagellum, which arises directly from the cell surface. The basal bodies are arranged approximately in parallel with the ventral surface, form roughly a right angle with one another and are joined proximally by a microfibrillar, cross-striated desmose measuring ca. 400 \times 80 nm. From the left basal body 2 fibril systems, presumably consisting of microtubules, extend to the level of the nucleus. System 1 arises near the interkinetosomal desmose and runs along tightly underneath the pellicle. System 2 arises at the left edge of the basal body and runs along the dorsal wall of the periflagellar canal (Figs. 9, 10, 19, 28, 31, 33).

Pellicle : The pellicle is ca. 25-30 nm thick and consists of 3 unit membranes. The outer membrane covers the whole animal, including the flagella, as a continuous sheet. The two inner membranes, which are very closely apposed and difficult to resolve, are absent in the tubular part of the periflagellar canal, at the micropores, and perhaps also in other small regions of the cell. Often one has the impression that they form flat alveoli, or that the innermost membrane is lacking in certain areas (Figs. 23, 24, 28). At many places in the pellicle there are tubular or saclike invaginations of the outer cell membrane; we follow BRUGEROLLE and MIGNOT (1979) in calling these "micropores". In cross section they have a bright lumen ca. 30-40 nm in diameter, surrounded by a ring of very electron-dense material about equal in width. The diameter of the entire organelle is thus ca. 100 nm (Figs. 9, 10, 24, 25, 28, 33). Just below the pellicle lie microtubules in a fairly regular arrangement; they begin at the level of the

FIGS. 21-25. - Spiromonas gonderi. Electron micrographs. Bars : 400 nm; Fig. 24 inset, 93 nm.

Figs. 21, 22. Sections through the surface at the level of the ring of microtubules. The arrows in Fig. 21 point to the separating membrane, and those in Fig. 22 show the boundaries of the microtubule ring.

Figs. 23, 24. Pellicle and cortical region. Inset : cross section through a micropore at the level of the inner membranes of the pellicle. Fig. 25. Midregion of the body with two dictyosomes.

Abbreviations : AF = anterior flagellum, DV = dense vesicle, ER = endoplasmic reticulum, G = dictyosomes of the Golgi body, IM = inner cell membranes of the flagellate, L = netlike layer covering the outer cell membrane of the flagellate, M = mitochondria of the flagellate, MI = mitochondria of the ciliate, MN = micronemes, MP = micropore, MR = microtubules of the attachment ring, Mt = subpellicular microtubules of the flagellate, MtC = subpellicular microtubules of the ciliate, OM = outer cell membrane of the flagellate, R = ribosomes.



fixation apparatus and extend about to the middle of the body—some, perhaps, as far as the posterior end. They appear to have a slightly spiral arrangement (Figs. 9, 10, 21, 22). In almost all parasites the pellicular surface is covered by a netlike fibro-granular structure 50-100 nm thick. This surface coat is absent only in the region of the periflagellar canal and the fixation apparatus (Figs. 21, 23, 24, 33).

Fixation apparatus : At the edge of the conically expanded zone where host and parasite are joined there are 4-5 $(\bar{x} = 4.6, n = 8)$ microtubules in an annular (perhaps spiral) arrangement. We could not determine whether this ring is formed of several specialized subpellicular microtubules curved at their anterior ends or whether it is an independent structure. The first interpretation is supported by sections in which both the microtubules of the ring and those of the body are cut in the same direction, longitudinally or across (Figs. 21, 22). The second possibility is supported by pictures in which the microtubules of the ring are cut across, and those of the body longitudinally or oblique (Figs. 9, 26). Above the ring there is a small, empty zone bounded by a unit membrane ca. 10 nm thick, which separates host and parasite. This separating membrane is formed in a remarkable manner (Figs. 6, 29, 30) : near the outermost microtubule of the ring the inner electron-dense layer of the outer cell membrane of the ciliate fuses with that of the flagellate. We do not know whether the membrane seal is a new formation or whether the original membranes of host and parasite contribute to some extent. The two inner membranes of the pellicle of the flagellate are usually joined to the outermost microtubule of the ring, appearing slightly more dense in this region (Figs. 29, 30). The resulting picture resembles the polar ring of the Coccidia (cf. SCHOLTYSECK, 1979; RUSSELL and BURNS, 1984). The membrane-bounded alveoli of the ciliate end in the vicinity of the separating membrane (Figs. 9, 26, 29).

Cytoplasmic organelles : In the anterior region of the parasite, chiefly in the vicinity of the periflagellar canal, there are many membrane-bounded, electron-dense, ampulla-shaped organelles; we adopt the term "microneme" for these, as proposed by BRUGEROLLE and MIGNOT (1979). Their thin, tubular endings usually point in the direction of the host. The micronemes are up to 1000 nm long, and their thickness is ca. 24 nm anteriorly and up to 120 nm posteriorly. In cross section they are circular, with a more electron-dense area in the center (Figs. 9, 10, 22, 26, 33, 35).

The cytoplasm of the parasite is frequently less electron-dense than that of the host (Fig. 20). It contains many ribosomes, which often form dense aggregations in the form of an irregular sphere up to 200 nm in diameter (Figs. 25, 26). The cisternae of the endoplasmic reticulum are usually rather indistinct; they tend to be just below the pellicle and are most abundant in the anterior region (Fig. 24). The Golgi apparatus is always very difficult to discern. There are probably 2 dictyosomes approximately at right angles to one another near the nucleus, each composed of a few thin cisternae (Figs. 9, 10, 25, 26). The nucleus has no special features (Figs. 10, 16, 18, 19, 20, 31).

In most of the preparations the section passes through many mitochondria. Some series, though incomplete, indicate that these are part of a single, highly branched, netlike giant mitochondrion. The cristae are tubular to vesicular, very irregularly distributed, and sometimes constricted at the base. The matrix is brighter than that of the host's mitochondria (Figs. 21, 22, 33).

The cytoplasm also contains the following inclusions :

(i) Membrane-bounded vesicles up to ca. 800 nm in diameter, which in general are clear though sometimes they contain several small, very electron-dense spheres (Figs. 9, 19, 34).

(ii) Membrane-bounded vesicles up to 1 000 nm in diameter, moderately electron-dense, which presumably contain polysaccharides inasmuch as they closely resemble those of *S. perforans* (BRUGEROLLE and MIGNOT, 1979) (Figs. 9, 10, 19, 21, 25, 32).

(iii) Irregularly spherical lamellar bodies up to 1 200 nm in diameter, which appear to be constructed of concentric membranes. These are found only in individuals that have been parasitizing for some time (Figs. 9, 10, 27, 31, 32). They could be lipid bodies in the process of formation (WANNER and KÖST, 1984).

(iv) A spherical to kidney-shaped inclusion in the posterior part of the body, up to 8 000 nm in size, which could also be identified in the light microscope (Figs. 2, 7,

FIGS. 26-30. - Spiromonas gonderi. Electron micrographs. Bars in Figs. 26-29 : 400 nm, in Fig. 30 : 91 nm.

Fig. 26. – Median section through the anterior part of the body. The small arrows point to the separating membrane between host and parasite, and the two large arrows mark the microtubule ring.

Fig. 27. - Detail from the midregion of the body of a satiated parasite. Three lamellar bodies are discernible.

Fig. 28. — Section through the ventral surface at the level of the flagella.

Figs. 29, 30. — Host-parasite transition region, highly magnified. The arrows point to the separating membrane between host and parasite. The triangles mark the membrane seal.

Abbreviations : AF = anterior flagellum, C = crystall in the cytoplasm of the ciliate, E = epiplasm of the ciliate, $F_{1,2}$ = fibril system 1 and 2, G = Golgi body, IK = interkinetosomal desmose, IM = inner cell membranes of the flagellates, LB = lamellar body, LV = empty vesicle, MP = micropore, MR = microtubules of the attachment ring, Mt = subpellicular microtubules of the flagellate, MiC = subpellicular microtubules of the ciliate, PF = posterior flagellum, R = ribosomes, V = empty vacuoles in the cytoplasm of the ciliate.



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8, 15, 18, 19, 20, 32, 34). It is especially well developed in older individuals that have been parasitizing for a long time and may well contain reserve substances or excreta, as its finely granular structure indicates. It is enclosed in two unit membranes and has a central more electron-dense region in the form of an irregular sphere. Here and in the brighter periphery are scattered membrane-bounded vesicles with undefinable content (Figs. 32, 34).

Reactions of the host: The parasite destroys the host's pellicle at the site of attachment. In some sections the cytoplasm of the ciliate near the separating membrane contains large numbers of small, apparently empty vacuoles (Figs. 20, 29). Probably these eventually fuse to produce the striking vacuolation, visible even in the light microscope, of the cytoplasm of a heavily infested host (Figs. 13, 16). In cases of severe infestation the cytoplasm is stained remarkably deep black after protargol impregnation, and few or no extrusomes (mucocysts) remain. Apart from these, the host exhibits no conspicuous changes. Its mitochondria and subpellicular microtubules, often closely apposed to the separating membrane, are normal in structure and unaltered in their staining behavior (Figs. 22, 26).

IV. - DISCUSSION

Spiromonas gonderi belongs unequivocally in the category of ectoparasites, according to the definitions of REIMER (1970) and PIEKARSKI (1975). Neither in the reviews of KIRBY (1941) and BALL (1969) nor in many smaller relevant papers have we found any report of flagellates being ectoparasitic upon ciliates. CHATTON and VILLENEUVE (1936) and PUYTORAC (1952, 1953) suggest that the unusual ectoparasites of the heterotrichous ciliate Fabrea salina and the astomate Anoplophrya cf. lumbrici could be flagellates, but as they observed no flagellated stage the systematic position of these animals remains open (GRASSÉ, 1952). Therefore we believe that our study provides the first indisputable evidence of this phenomenon. On the other hand, there is ample evidence of intracellular trypanosomatid flagellates of the genus Leptomonas in the macronucleus of Paramecium and Euplotes (GILLIES and HANSON, 1963; WILLE et al., 1981). Because these parasites have a kinetoplast and only a single flagellum, it is certain that they are not closely related to Spiromonas.

1. Comparison of Spiromonas gonderi and S. perforans

As regards their fine structure, these species resemble one another in the following respects (cf. BRUGEROLLE and MIGNOT 1979) : arrangement (orthogonal) and connection (microfibrillar desmose) of the basal bodies of the flagella, structure of the pellicle (3 unit membranes, micropores, subpellicular microtubules), micronemes at the level of the flagella, 2 dictyosomes (less conspicuous in S. gonderi), mitochondria (presumably only 1 highly branched giant mitochondrion with ampulla-shaped cristae), cytoplasmic inclusions (empty vacuoles, polysaccharide granules, large posterior vacuole with diverse inclusions). However, there are also many differences, which indicate that these species may not be congeneric : S. gonderi has no contractile vacuole, no microfibrils in the rostrum, no extrusomes, no chromation strands between nuclear membrane and nucleolus, presumably no lipid granules, and only one periflagellar canal. Features lacking in S. perforans include the fibro-granular netlike surface coat on the pellicle, the lamellar bodies and a fixation apparatus comparable to that of S. gonderi. The basal bodies of the flagella are further apart from one another and more antero-posteriorly shifted than in S. perforans. BRUGE-ROLLE and MIGNOT (1979) infer that in S. perforans both basal bodies are associated with microtubules, whereas in S. gonderi fibrils arise from only the left basal body.

2. Food Intake and Separating Membrane

Neither BRUGEROLLE and MIGNOT (1979) nor we have made complete observations of the feeding process. Spiromonas perforans sinks its rostrum into the body of the host, taking in the cytoplasm of the latter within 5 minutes. Like S. gonderi, it becomes spherical as it feeds, and the food accumulates behind the nucleus without being collected in a typical food vacuole (BRUGEROLLE and MIGNOT, 1979). As described by ALEXEIEFF (1929), the process is similar in S. angusta. Spiromonas gonderi, however, first brings itself into intimate contact with the host by way of a complicated fixation apparatus, develops a separating membrane, and takes in food very slowly. Two mechanisms merit consideration, as follows.

1. The separating membrane might open briefly now and then, allowing cellular components to be transferred

FIGS. 31-35. - Spiromonas gonderi. Electron micrographs. Bars in Figs. 31-34 : 1000 nm, in Fig. 35 : 111 nm.

- Fig. 31. Oblique section at the level of the nucleus (arrows).
- Fig. 32. Slightly oblique section in the region of the posterior end of the body.
- Fig. 33. Slightly oblique section in the region of the anterior end of the body.

Fig. 34. Part of a dense body (boundaries marked by black lines), highly magnified. The arrow points to a vacuole within the dense body.

Fig. 35. Region of the separating membrane (large arrows) with many micronemes (small arrows) in cross section.

Abbreviations : AF = anterior flagellum, DB = dense body, DV = dense vesicle, LB = lamellar body, LV = empty vesicle, M = mitochondria of the flagellate, MN = micronemes, MP = micropore, PF = posterior flagellum, R = ribosomes.

from the host into the parasite. Because none of our sections revealed intact or only slightly digested host organelles within the parasite, this hypothesis is not very plausible.

2. The parasite might take in only dissolved substances from the host, because it has very likely no true or permanent cytostome. In such a process substances (enzymes ?) released by the micronemes could play a role, by predigesting the host's cytoplasm. Because we observed neither gaps nor pinocytosis vesicles in the separating membrane, molecules that cross it must be either permeative or transportable. This hypothesis demands that the parasite be almost completely integrated with the host a degree of integration, for example, like that between mitochondrion and cytoplasm; it is supported by the observation that parasitization leads to marked vacuolation of the cytoplasm (Figs. 13, 20). But then the substances must be at least partially converted to others by the parasite, for the lamellar bodies have a very distinct structure (Figs. 27, 32).

No separating membrane develops between *S. perforans* and *Chilomonas paramaecium* BRUGEROLLE and MIGNOT, 1979). The reason may be that the partners in this case are so similar in size that the parasite is in no danger of being "assimilated" by its host. In the case of *S. gonderi* one might almost expect that the strong cyclosis in the host cytoplasm could drag the content of the parasite cell along with it.

It is highly unusual to find a separating membrane formed of the inner layers of the cell membranes of host and parasite (Fig. 6). We have not been able to find any report of a similar structure in the literature. Even among the gregarines and in Pyrsonympha no comparable junction between host and parasite has been found (ORMIÈRES and MAROUÈS, 1976; SCHOLTYSECK, 1979; COCHRANE et al., 1979). However, there is a similar relationship between the tentacles of the Suctoria and the pellicle of their prey; here the cell membranes of predator and prey fuse with one another and the membrane of the tentacle knob is continuous with the peritrophic membrane (BARDELE and GRELL, 1967; TUCKER, 1974; GRELL and MEISTER, 1982), though the details of this membrane seal have yet to be elucidated. It would be particularly interesting in this regard to study in the electron microscope certain ectoparasitic suctorians that cause their hosts to invaginate part of the body surface, forming a pocket within which the parasite can proceed with its destructive activity (DIECK-MANN, 1984).

3. Spiromonas : A Parasitic Flagellate or a Free-Living Coccidian ?

BRUGEROLLE and MIGNOT (1979) place *S. perforans* near the parasitic dinoflagellates, because it has similar trichocysts and a comparable flagellar insertion but lacks a conoid like that of the Sporozoa. They draw a comparison between the micropores and micronemes of this species and the thecapores and "dense bodies" described in *Oodinium cyprinodontum* and *Amyloodinium* sp. by LOM and LAWLER (1973). Because of these organelles, however, together with the typical structure of the pellicle and the animals' mode of life, they regard S. *perforans* as a possible link between the dinoflagellates and the coccidians.

We put less weight on the similarities with the dinoflagellates than do BRUGEROLLE and MIGNOT (1979), particularly in the case of S. gonderi, for here the complex of characteristics "apical apparatus-microneme-micropores" indicates so strongly a relationship to the Coccidia that one would assign them to that group without reservation if they lived intracellularly and had no flagella (Fig. 10). Moreover, S. gonderi has no dinoflagellate extrusomes, whereas it does - like many Sporozoa have a fibro-granular coat on the surface of the pellicle (VIVIER, 1979). The micronemes and micropores also closely resemble those of the Coccidia in both structure and size (SCHOLTYSECK, 1979; SPEER and DUBEY, 1981). If one imagines the anterior end of Sarcocystis fusiformis as being conically expanded, its apical complex, clearly described by HEYDORN et al. (1975), would be difficult to distinguish from the apical apparatus of S. gonderi (Fig. 10). The separating membrane and the inferred mechanism of food intake are reminiscent of the parasitophorous vacuole of the coccids (MEHLHORN and PIEKARSKI, 1981). Spiromonas could therefore be a developmental stage of a coccidian, or one that has secondarily converted to an ectoparasitic mode of life (FOISSNER and FOISSNER, 1984). The "neoteny" hypothesis is supported by the prevalence of a flagellated microgamete stage among the Coccidia. For example, the gametes of Eucoccidium dinophili, 5 µm long and bearing two flagella, are very like Spiromonas in appearance (BARDELE, 1966; GRELL, 1968).

One of the difficulties with these interpretations is the relative rarity of sporozoan infestation of ciliates (KIRBY, 1941; HOVASSE, 1950; BALL, 1969). However, KIRBY (1941) suspects that it may be much more common than the few reports suggest. A more severe problem lies in the fact that true tissue or body-cavity parasites - as all the Coccidia are - would probably find it extremely difficult to revert to a partially free-living, ectoparasitic mode of life. On the other hand, one also encounters severe problems in placing Spiromonas near the dinoflagellates. For one thing, it is unlikely that such a complicated constellation of characteristics as the apical apparatus, the microneme and the micropores could have evolved independently several times, by convergence - although in the case of single characteristics such as the micropores, which are also found in free-living dinoflagellates (SCHNEPF and DEICH-GRÄBER, 1972) and in the ciliates in the form of parasomal sacs (Corliss, 1979), convergent evolution almost certainly did occur. Furthermore, one must ask why Spiromonas does not have the characteristic nucleus of the dinoflagellates, a very conservative characteristic within this taxon; however, some exceptions exist (see BRUGEROLLE and MIGNOT, 1979). Finally, it remains unclear why S. gonderi possesses the fibro-granular pellicular coat typical of internal parasites.

A satisfactory explanation of this peculiar constellation of features would be that the Spiromonadidae occupy a position between the flagellates and the sporozoans. The successive acquisition of new structures (S. perforans: microneme; S. gonderi: apical apparatus) and progressive integration into the host (Spiromonas: ectoparasite; Eucoccidia: body-cavity and tissue parasites, see GRELL, 1962) could ultimately produce an obligate intracellular parasite (e.g., Sarcocystis). Because the Spiromonadidae — unlike the Sporozoa, in which only the gamete generation is flagellated — are equipped with flagella in the trophic phase, it seems reasonable to classify them as zooflagellates.

In a recently published paper LOM and SCHUBERT (1983) describe the stalk of the parasitic dinoflagellate *Piscinoo-dinium pillulare* as having a ring of microtubules similar to that of *S. gonderi*. In the former, however, it evidently does not serve for attachment to the host, because special rhizoids are available for that purpose. In other respects as well, the organization of this flagellate differs greatly from that of *S. gonderi* : it has chloroplasts as well as rhizocysts, $3-5 \mu m$ long and of complex structure, that penetrate the host and hence can hardly be considered as homologous with the small (1 μm at most) micronemes of *S. gonderi*.

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