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ORIGINAL PAPER

Fine structure of the bird parasites *Trichomonas gallinae* and *Tetratrichomonas gallinarum* from cultures

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Abstract The trophozoites of *Trichomonas gallinae* and *Tetratrichomonas gallinarum* were studied by means of light and electron microscopy after cloning and cultivating them axenically. *T. gallinae* trophozoites varied in shape reaching from ovoidal to pyriform and had a size of about 7–11 μm. They were provided with four free flagella and a fifth recurrent one, which did not become free at the posterior pole. The nucleus was ovoid, had a size of about 2.5–3 μm, and was situated closely below the basal bodies of the flagella. The axostyle consisted of a row of microtubules running from the region of the apical basal bodies to the posterior end of the cell. In addition to flagellated stages, which contained food vacuoles, hydrogenosomes, a costa-like structure, and glycogen granules besides lacunes of endoplasmic reticulum, spherical, non-flagellated, and cyst-like stages occurred. The trophozoites of *T. gallinarum* appeared mostly pear-shaped and ranged in size from 6 to 15 μm. They had also four free anterior flagella and a fifth recurrent one, which became free at the posterior pole in contrast to that of *T. gallinae*. Another clearly visible difference to *T. gallinae* was the occurrence of a sphere of lacunes of the endoplasmic reticulum surrounding in a regular distance the nucleus with its typical perinuclear membranes. Furthermore, the food vacuoles appeared very large. However, both species clearly differed from the trophozoites of *Histomonas meleagridis*.

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Introduction

Two flagellated protozoans may occur inside the digestive tract of poultry: *Trichomonas gallinae* (syn. *Trichomonas hepatica*, *Trichomonas columbae*), which is found worldwide in the mouth, pharynx, esophagus, and crop of a large variety of birds (especially in Columbiformes such as doves and pigeons) with pathogenic and nonpathogenic strains, was first described by Rivolta (1878). The specimens of pathogenic strains may become disseminated all over the body and thus gain also access to the liver, where they form caseous lesions similar to those of *H. meleagridis*.

The second species *Tetratrichomonas gallinarum* parasitizes mainly inside the ceca of chickens but also in other birds. This species was also first described by Rivolta (1878) and later better defined by Martin and Robertson (1911). Both species had been (under different names) the object of many studies since their small size, their broad spectrum of host birds, the occurrence of mixed infections, and their varying pathogenicity led to many discussions and misunderstandings. However, although both species may occur as additional pathogens in turkeys and chicken suffering from histomonosis due to infections with *H. meleagridis* (Knispel 2005; Grabensteiner et al. 2008), the clinical relevance of such double infections is discussed controversially (Lif. c. f. Norton 1997; Kemp and Reid 1964; Mehlhorn 2008).

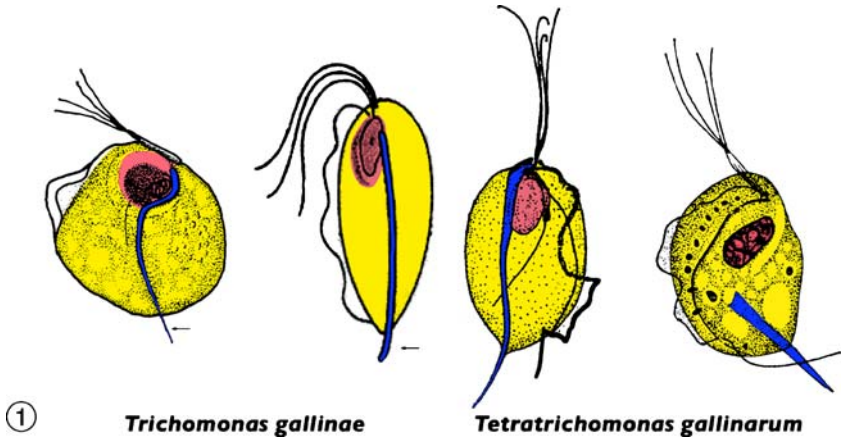


Fig. 1 Diagrammatic representations of trophozoites of *T. gallinae* and *T. gallinarum* (combined after descriptions and results of Cheng (1986), Soulsby (1986), and the present study)

Isolated flagellated stages of both species from different birds showed often true or dubious slight variations, so that different species had been described for those isolates. Levine (1973) reviewed *Tritrichomonas eberthi* in chicken and turkeys and *T. gallinae* for Columbiformes or *T. gallinarum* in chicken, while he claimed *Tritrichomonas anatis* to occur in ducks or *Tritrichomonas anseris* in goose, whereas other authors like Friedhoff (1982) believed that the host spectrum of *T. gallinae* comprises Columbiformes, chicken, predator birds, Passeriformes, sea gulls, and even parrots. Therefore, isolation and clonation followed by morphological and transmission studies of such stages are needed to clarify the situation and to find out their participation in diseases due to mixed infections in economically important host species such as turkey, chicken, goose, etc. Such approaches were successfully done by Hess et al. (2006), by Grabensteiner et al. (2007), and by Aziza et al. (2008), thus providing material for the present transmission electron microscopic (TEM) study.

Material and methods

Parasites

The *T. gallinarum* clones were isolated from turkeys, while clone *T. gallinae*/budgerigar/Austria/5895-C1 was isolated from a diseased budgerigar and the other clone of *T. gallinae* was isolated from a pigeon. Mono-eukaryotic cultures were established by micromanipulation following a recently described protocol. Axenization was obtained by adding certain antimicrobials. The clones of *T. gallinarum* were grown axenically in *Trichomonas vaginalis* medium (Glenn et al. 1985). The *T. gallinae* clones were grown in a modification of Hollander agar medium (Hollander 1976). The initial inoculum was 10^5 trophozoites per milliliter of medium. The samples for electron microscopic investigations were collected at 48 h postincubation. After this, the number of motile trophozoites in this culture ranged between 6 and 8×10^6 per milliliter of medium.

Fixation and preparation for electron microscopy

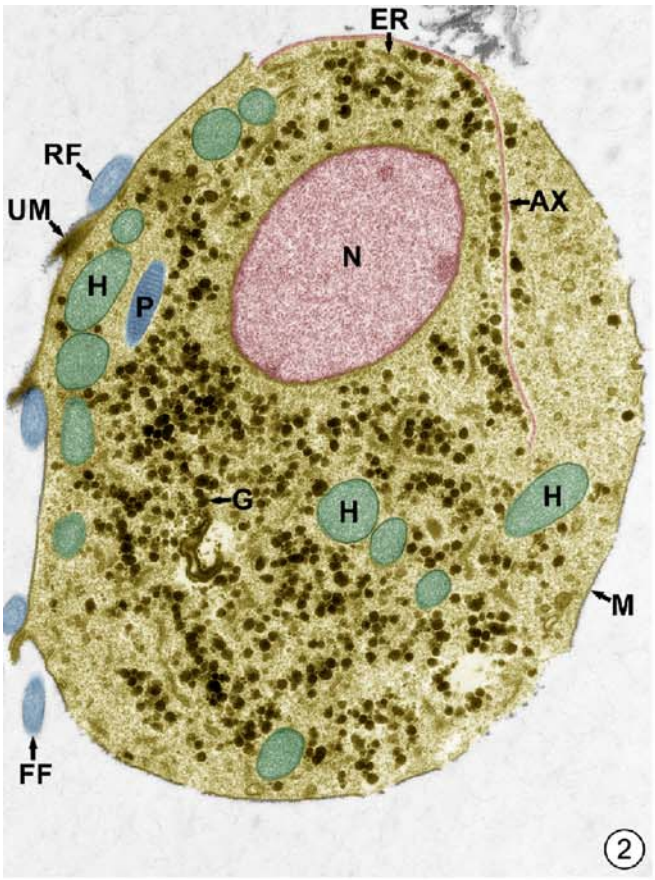
The fixation of pellets with trophozoites was done with 5% glutaraldehyde in 4°C Karnovsky buffer for at least 24 h prior to sending the probes with regular mail to the Düsseldorf Institute. The following processes of staining embedding sectioning and investigation were the same as described for stages from clones of *H. meleagridis* (Mielewczik et al. 2008).

Results

Trichomonas gallinae

The flagellated trophozoites of this species appear varying in shape (reaching from ovoidal to pyriform) with a mean size of about 7–11 μm. Their ovoid nucleus is situated closely to the basis of the apically situated basal apparatuses of the flagella, while at the opposite pole the axostyle proceeds into a short peak (Fig. 1). At the apical pole, four free flagella arise each from a typical basal body. A fifth flagellum originates as well from the apical pole but is closely attached to a slight off-folding of the surface thus giving rise to aspects of a "undulating membrane" (Figs. 2 and 3). However, this recurrent flagellum does not become free at the posterior end of the trophozoite but extends only to two thirds until three fourths of the cell surface,

Figs. 2–5 Transmission electron micrographs of sections through ▶ trophozoites of *T. gallinarum*. *AX*=axostyle, *C*=costa, *ER*=endoplasmic reticulum, *FF*=free flagellum, *G*=glycogen granule, *H*=hydrogenosome, *M*=cell membrane, *N*=nucleus, *NM*=nuclear membrane, *NU*=nucleolus, *P*=pelta-like, *RF*=recurrent flagellum, *RI*=ribosomes, *UM*=undulating membrane, *V*=food vacuole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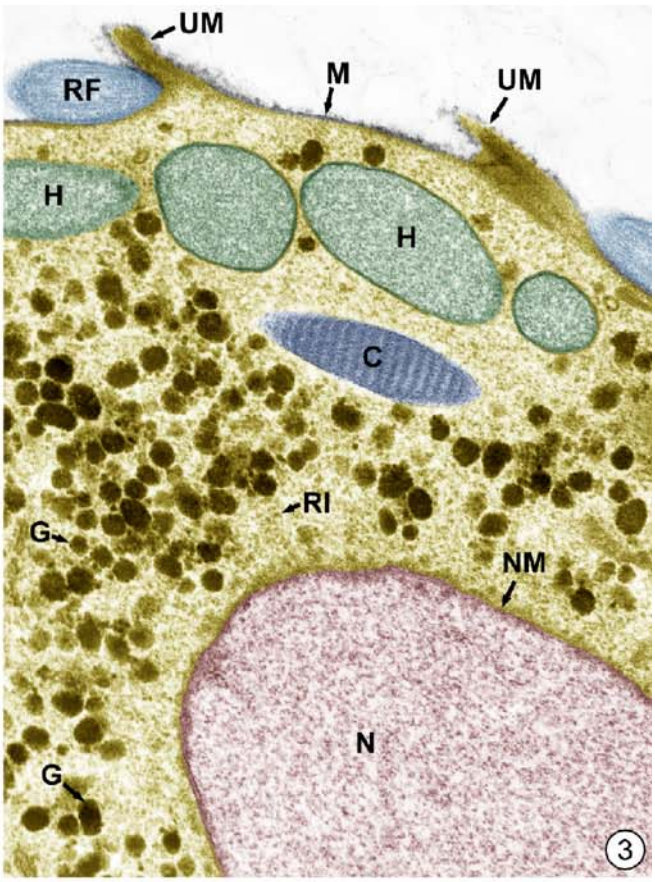
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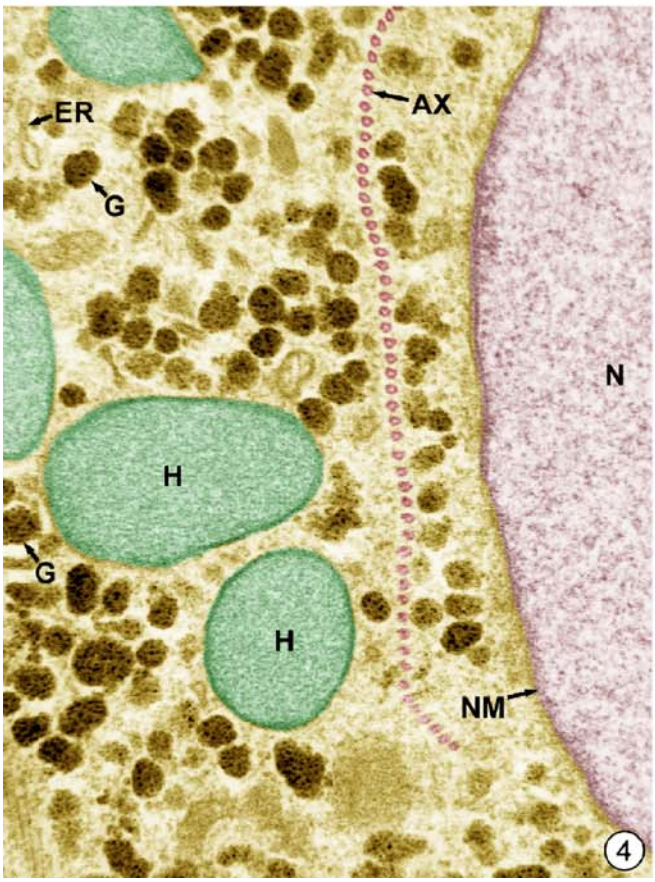
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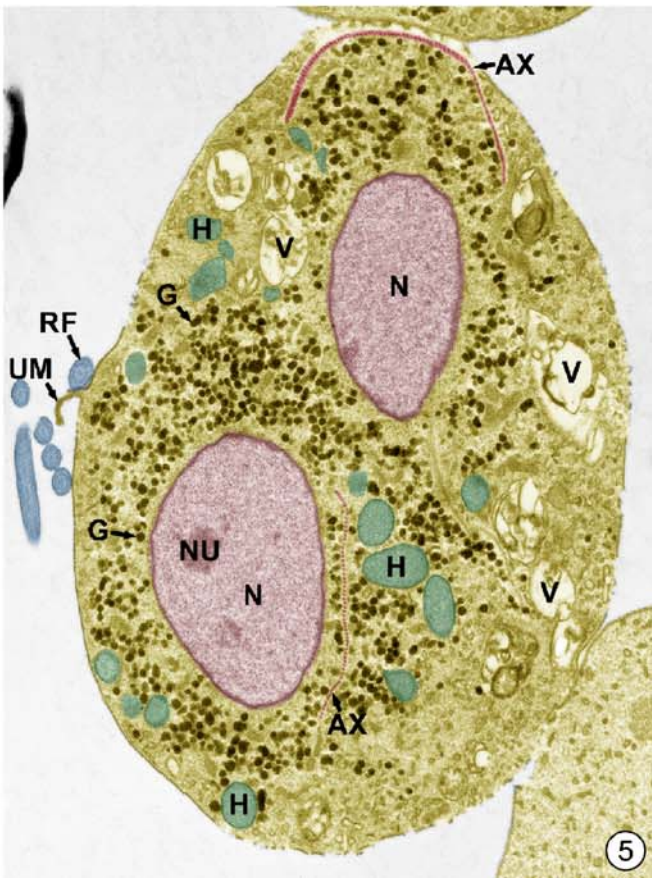
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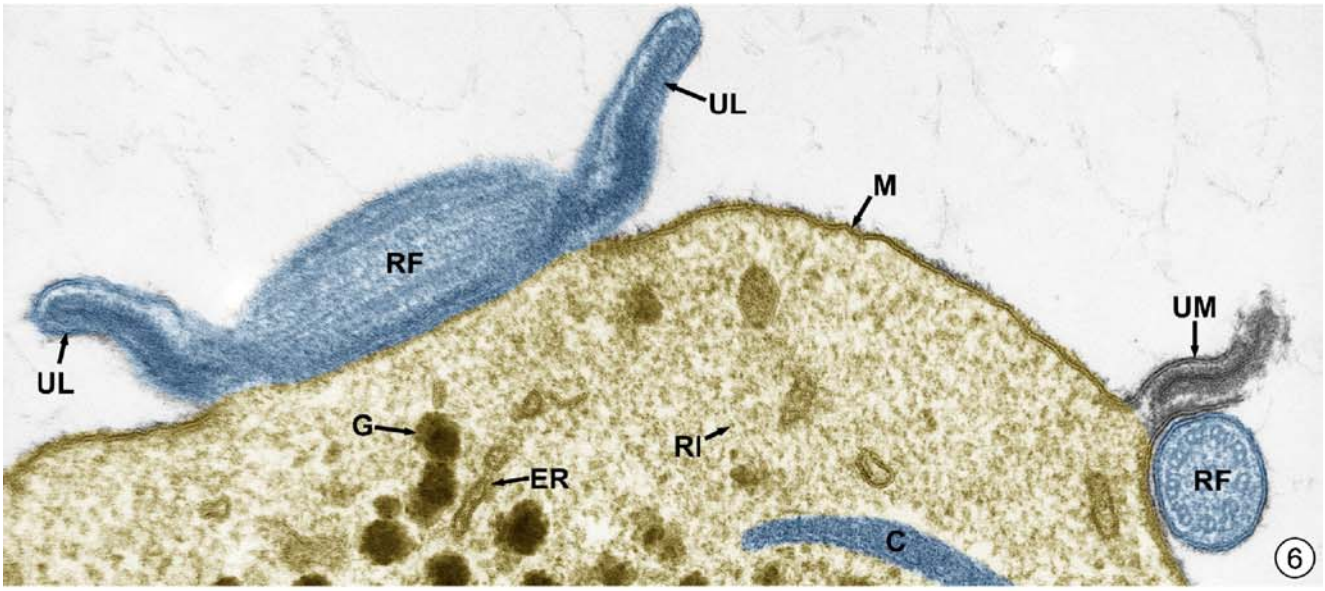
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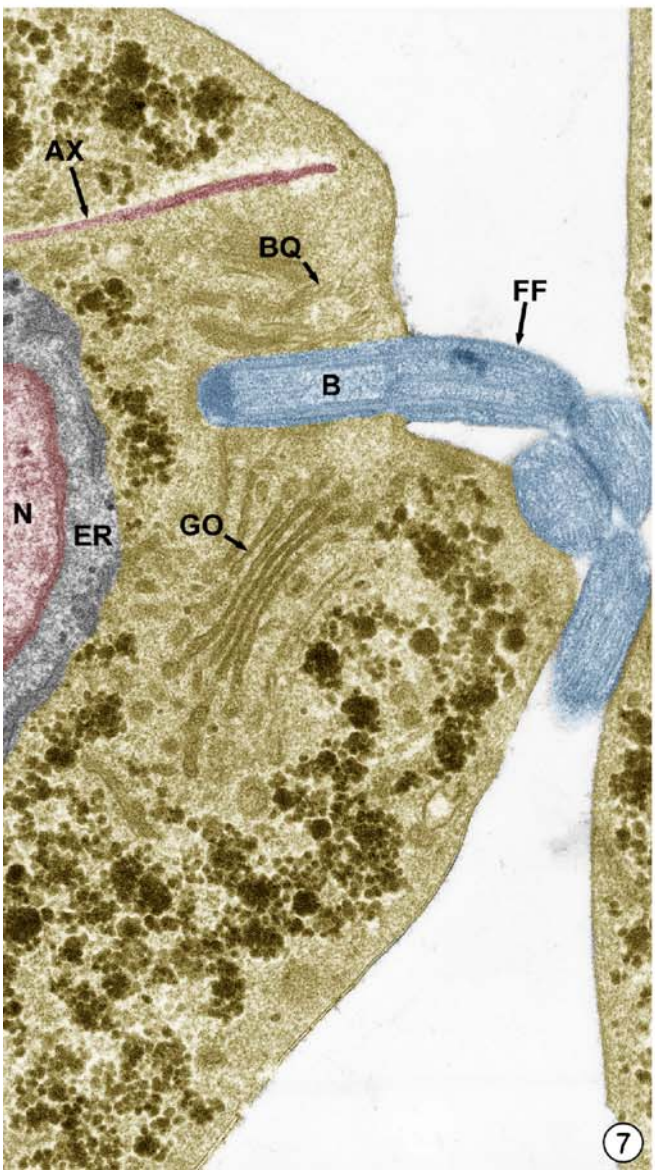
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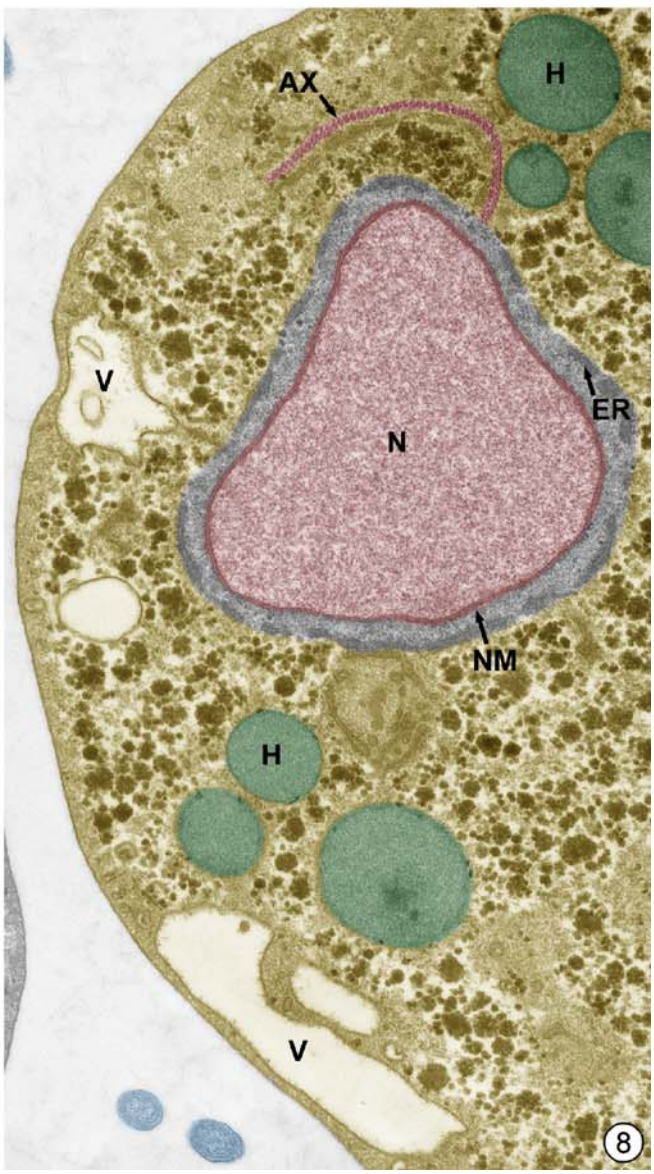
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◀ **Figs. 6–8** TEM of sections through trophozoites of *T. gallinarum*. Note that the nuclei in Figs. 7 and 8 are closely surrounded by a layer of lacunes of the endoplasmic reticulum. This zone is even visible in light microscopy (Fig. 1). *AX*=axostyle, *B*=basal body, *C*=costa, *BA*=basal body (cross-sectioned), *GO*=Golgi apparatus, *ER*=endoplasmic reticulum, *FF*=free flagellum, *M*=cell membrane, *N*=nucleus, *RF*=recurrent flagellum, *RI*=ribosomes, *UL*=underlying material, *UM*=undulating membrane, *V*=food vacuole

while the anterior four free flagella have a length of about 11–13 μm.

The trophozoites are bordered by a single cell membrane (Fig. 3), which does not give rise to a cell mouth (micropore, cytostome, etc.) but apparently forms exclusively pinocytotic vesicles or small food vacuoles, which engorge material from the culture fluid. The ovoid nucleus of the trophozoite has a longitudinal diameter of about 2.5–3 μm and is limited by a typical nuclear border consisting of two parallel membranes leaving gaps as pores (Figs. 3 and 4). The axostyle consists of a row of microtubules running from the region of the apical basal bodies to the posterior end of the cell (Figs. 2 and 4).

In the apical region, these microtubules cover the nucleus in a basket-like manner (Fig. 4).

Inside the cytoplasm, large amounts of electron-dense-appearing glycogen granules occur besides numerous typical hydrogenosomes and a dense cross-striated long structure (=costa), which apparently offers another fortification to the interior of the trophozoite (Figs. 2 and 3). The cytoplasm around the nucleus appears rather clear and no densification is seen (Figs. 3 and 4). The reproduction occurs as binary fission (Fig. 5), during which the spindle is situated within an extranuclear groove (as in other trichomonads). However, besides all these flagellated stages, a few spherical trophozoites occurred which did not show flagella in semithin and ultrathin sections.

Tetratrichomonas gallinarum

The trophozoites of this species have a size range between 6 and 15 μm and appear mostly pear-shaped (Fig. 1). They possess four free flagella at the apical pole and a fifth recurrent one, which, in contrast to *T. gallinae*, however, becomes free at the terminal pole, where the cell is pointed by the protruding axostyle (Fig. 1). The flagella possess typical basal bodies, which are lined by one or more typical Golgi apparatuses (Fig. 6). The protrusions of the surface membrane at the side of the recurrent flagellum are underlaid by dense material, thus offering a peculiar fortification of this "undulating membrane" (Fig. 7). The axostyle consists again (as in *T. gallinae*) of a single row of microtubules (Fig. 6). A pelta-like structure—also consisting of a single but curved row of microtubules—occurs close to the anterior pole of the nucleus (Fig. 7). The interior of the trophozoites contains large amounts of electron-dense granules of glycogen, large-sized hydrogenosomes, and the ovoid nucleus (Fig. 8). The appearance of the nucleus is characterized by a sphere of lacunes of the endoplasmic reticulum surrounding closely the whole nucleus in addition to the nuclear membranes. This peculiarity introduces an aspect similar to a second nuclear envelope (Figs. 6 and 8), which can be seen even in light microscopy. Furthermore, there occur rather large food vacuoles, which are seen often close to the surface of the trophozoite (Fig. 8).

Discussion

T. gallinae and *T. gallinarum* look very similar when isolated from their different infection sites in birds since they range in the same size and possess four free anterior flagella and a recurrent one, which runs along an upfolding of the cell membrane (McDowell 1953; Friedhoff et al. 1991). Although *T. gallinae* is mostly found in the upper intestinal tractus and *T. gallinarum* in lower intestinal region, both species can hardly be differentiated from each other in generalized infections with occurrence of parasites in many organs. The present TEM study being based on clonal cultures of both species, however, showed two clear characteristics which can be used for differential diagnosis. In *T. gallinae*, the recurrent flagellum never becomes free at the terminal pole of the cell, while the recurrent flagellum of *T. gallinarum* always surmounted the terminal pole. The latter species is furthermore characterized by the phenomenon in which lacunes of the endoplasmic reticulum encircle the nucleus at some distance of the nuclear membranes, thus creating a layer which can be seen even in light microscopy. Our transmission electron micrographs confirm with respect to the undulating membranes of both species the findings of Tasca and DeCarli (2003), when they used the scanning electron microscopy in studies on *T. gallinae*.

Both studies as well as that of Knispel (2005) brought clear evidence that the fifth flagellum, i.e., the recurrent one, does not become free at the terminal pole of the trophozoite of *T. gallinae*. This was also suggested by several previous authors, who stimulated Soulsby (1986) and Cheng (1986) to publish their drawings which were depicted and extended in the present study as Fig. 1. Also, the bright space around the nucleus seen in the present study in *T. gallinarum* is already drawn in their diagrammatic representations.

The method of the production of clonal cultures by micromanipulation used by Hess et al. (2006) has successfully proven that *T. gallinae* and *T. gallinarum* are clearly distinct species based on morphological criteria. Corresponding results were obtained by Knispel (2005)

with in vivo isolates when studying them with molecular biological methods using the restriction enzyme patterns. However, Knispel's results indicated that in different hosts considerable variations may exist, which might be due to further differentiation into races. Clonal reproduction of such isolations has to be studied in the future.

Some of the stages of *T. gallinae* showed apparently no flagella at all. This might be in correlation with the findings of Granger et al. (2000) or Tasca and DeCarli (2003), who described in their SEM studies that some spherical stages "engorged" (=redraw) their flagella and interpreted this phenomenon as formation of "pseudocyst" (young cysts), which might give shelter during the oral transmission of this parasite from host feces to a new host. Similar processes of a formation of cyst-like stages had been described by Munsch et al. (2009a, b) and Mielewczik et al. (2008) in clonal stages of *H. meleagridis*. Thus, such a cyst formation in all these intestinal parasites ameliorates their chance of transmission from one host to another and would enlarge the survival time outside of a host.

The general morphology of *T. gallinae* and *T. gallinarum* corresponds closely to that of other trichomonads, i.e., the axostyle, the costa, the pelta, flagella, and especially the hydrogenosomes look very similar. The latter apparently may divide and may appear with four membranes at places, where infoldings had occurred. This finding corresponds to the observations of Benchimol et al. (1996) in a broad spectrum of trichomonads.

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