FLAGELLATES, BACTERIA, AND Fungi ASSOCIATED WITH TERMITES: DIVERSITY AND FUNCTION IN NUTRITION – A REVIEW

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Abstract. Termites nutrition depends on a spectrum of symbiotic organisms. The members of the fungus-growing subfamily Macrotermitinae of the higher termites, i.e., the Termitidae, cultivate fungi of the genus Termitomyces, in special galleries. These fungi are of a high nutritional value and their erectores probably contribute to cellulose digestion, i.e., to varying degrees among different termite species. Lower termites dig cellulose with the help of symbiotic gut flagellates. After a partial breakdown by the termites own enzymes, the fungal cellulose is engulfed by the flagellates and processed further. The flagellate Thecatothricus acreatus is the major metabolic product for absorption by the termite hosts. Various bacteria also supply nutrients and warrant the maintenance of a specialist gut motility. There are phylogenetically ancient gut flagellates, i.e., dichotomons, hymenostomes, and xanthomonads, that are characterized by typical cell-segments. They all lack mitochondria, instead, dichotomons and xanthomonads possess hydrogenosomes that deliver ATP and reduce acceptors. Accepted 20 October 1999

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TERMITES

Termites are classified in the order Isoptera of the phylum of 'gnats'. Most of the approximately 2000 species are found in tropical and subtropical regions. They are abundant, with local densities exceeding 6000 specimens per m² and hemes (50-250 km²) but not infrequently surpassing those of grazing mammalian herbivores (Lee & Wood 1971). Collins & Wood (1984). Termites are regarded as harmful insects because of their ability to destroy all materials containing cellulose. They have a great economic impact on wood used in and around buildings. Living plants, including agricultural products, are also damaged by many species. In the United States, the annual cost for damage repair and control efforts probably exceed 300 million (Redek 1989). In their natural habitats, however, termites are impor- tant decomposers. They reduce organic material to small pieces, thereby improving the nutrient contents of topsoil soils, which are generally infertile due to nutrient poverty (Caveny et al. 1988, Park et al. 1994). They also improve soil aeration and drainage.

Termites are highly social insects. Hundreds to millions of individuals may coexist in one nest (Krishna & Weern De 1987, Geisel 1984). One colony consists of one or several pairs of reproductive termites and a large number of workers, soldiers, and larvae. The kings and queens of a termite colony reproduce, the workers forage and feed their nestmates, and the soldiers defend their home colony. Unlike the social Hymenoptera, termite societies contain individuals of both sexes, and they have a larva instead of an imaginal polymorph (Nielsen 1991). Moreover, lower termites can change their physical case during development, i.e., a case of temporal polymorphism (Nielsen 1991). Usually, termite castes contain countess subcastes of workers and drummers, and the spectacular hill-shaped colonies may rise to several meters. Wood-firing termites inhabit fire wood and rotten log or termite nests (Tennant, cockroaches, and praying mantises represent a monophyletic group. Molecular phylo- genetic data suggest a sister group relationship of the termites to a cockroach-manid complex (order Dec- tyoptera), and the Blattaria is a sister group of the Man- toidea (Thorne & Carpenter 1992, Rambaut 1995). Termites can be separated into seven families: the Macrotermitidae, Kalotermitidae, Hodotermiti- dae, Termitidae, Rhinotermitidae, Sertotermitidae, and Termitidae. The first six families are collective- ly referred to as the "lower termites." Lower termites, however, do not form a monophyletic taxon but...
an ancestral grade composed of a series of families sharing several plesiomorphic characters (Nisbet 1995). For cellulose digestion they all depend on intestinal flagellates. The Termitea, referred to as the "higher termites," are the largest family comprising about three-quarters of termite species. Higher termites do not possess symbiotic protozoa in their gut and display a more complex eutrichous and inter- nal anatomy and social organization. Phylogenetic trees based on family-level relationships inferred from molecular data and from morphological characters differ in some aspects. Mastotermesidae seem to be the basal lineage. According to molecular data, a clade comprised of Termitidae (higher termites) and Rhinotermitidae is the sister group of the Kaloter- minidae, and the Tentospondyls are arranged at a more basal position than the Kalotermitidae (Kamb- hampati et al. 1996). These results were partly sup- ported by Nisbet's (1995) proposal, based on a com- parative study of gut morphology, that the Termitea, Rhinotermitidae, and Seriotermitidae might be the sister group of Kalotermitidae. Morphological analyses by Krishna (1970), however, grouped the Kalotermitidae as a relatively basal group, implying a sister group relationship with the Mastotermesidae.

NUTRITION AND DIGESTION IN HIGHER TERMITES

Data, and their symbiotes, differ among lower and higher termites. The Termitidae ingest a wide range of materials, for example leaves, stems, grass, dung, and soil humus (Wood & Johnson 1992). There are two groups within the Termitea, fungo-calci- vating and non-fungo-calci-vating species. Non-fungo- calci-vating higher termites probably digest their food, including cellulose, by enzymes that are pro- duced by their own midgut and salivary glands (Staley 1992, Brozek & Bruce 1994). Thus, inocu- lated bacteria do not seem to play a major role in their cellulose digestion (Brozek & Bruce 1994). Cellu- losytic bacteria could indeed be isolated from gut con- tents, but their cellulolytic activity and growth rate on cellulose was very low and insignificant (Brozek & Bruce 1994). Cellulosytic genera are, e.g., Cellu- lomonas and Bacillus (Söding & Breuing 1997).

Termites of the fungus-calci-vating subfamily Mastotermesinae create large fungal gardens in their nests. These gardens are constructed by assembling partially digested plant material that is permeated and further digested by the fungal mycelium (Wood & Thomas 1989). For example, plant polysaccharides and lignin are partially digested within the comb (Bohrman & Rossmann 1986, Veizer et al. 1991). The garden fungi mostly belong to the genus Syn- nemospora and are exclusively found in termite nests. They are maintained and distributed by the ter- mites, who for their part depend on the high nutritio- nal value of the fungus. Fertile workers eat the fung- us combs, which includes Synnemospora mycelium and rendshah aerial spores (mycostem) (Crookall et al. 1996). In addition to the direct nutritional value, the ingested fungi may deliver maintaining enzymes essential for the completion of cellulose diges- tion (Marlin & Marlin 1978; Marlin 1987, 1991, 1992). This opinion, however, has become com- mercial (Staley 1992, Beggs et al. 1994, Crookall et al. 1996). The amount of enzymes delivered by fungi seems to depend on the termite species (Rou- land et al. 1991). In some groups of species the fungus supplies a relatively high enzymatic activity, while in others its contribution is very low when compared with the enzyme secretion of the termite midgut epithelium. In Mastotermes herveyi, for example, most cellulolytic activity endoglucanase, endo- β-glucanase, and β-glucosidase is located in the midgut and not in the mycostem of the fungus (Crookall et al. 1996). In another species of the same termite genus, M. natal- ensis, fungal enzymes seem to be necessary at least for the digestion of crystalline cellulose (Marlin & Marlin 1978; Marlin 1987, 1992). According to Staley (1992), however, the experimental results are not entirely convincing. Enzymes for hemiecellulose digestion, i.e., xyloses, may also be supplied by the fungus (Rouland et al. 1990). In any case, the na- ture and relative importance of ingested fungal en- zymes vary from species to species.

NUTRITION AND DIGESTION IN LOWER TERMITES

Many species of lower termites fail to digest woody material completely. Although this food is hard to digest and poor in nutrients, particularly nitrogen, the Pseudotermitinae (termites prefer wood that has been attacked by fungi), which is easier to utilize and richer in protein due to the presence of fungal mycelia. The following paragraphs describe in some detail the mechanisms by which lower termites manage to digest lignocellu- loses and extract their dietary requirements from such food. The following presentation focuses on the symbiotic relationships of termites with the intesti-
nal flagellates and bacteria contained in a large dilatation of their hindgut, the antrum (Fig. 1).

CONDITIONS OF LIFE IN THE TERMITE GUT ENVIRONMENT

The physicochemical conditions in the termite hindgut are influenced by the host as well as by the intestinal inhabitants. The pH values in the mid- and hindgut of lower and higher termites range typically around neutral, i.e., at pH = 6 to 7.5 (O’Brian & Slaytor 1982). Soil-feeding termites (Termiteidae) may have an extremely alkaline hindgut milieu (Bignell & Anderson 1980, Brune & Kühl 1990). While conditions in the midgut are anaerobic, oxygen concentrations are low in the hindgut. Oxygen conditions were examined by fixing the termites red-eyes and observing their color transformation in the gas (Nécœs et al. 1980), or by physiological measurements on intestinal homogenates (Bignell & Anderson 1980). Redox potentials ranging from ~230 to ~270 mV were registered, i.e., practically anaerobic conditions were revealed by this method (Bignell 1983). Recently, more precise measurements were obtained by employing microelectrodes (Bignell 1998). The rather simplistic concept of the termite hindgut as a purely anaerobic fermentor therefore has to be revisited. While the central portion was proven to be strictly anaerobic, the outer zone of the hindgut was shown to contain oxygen due to diffusion from outside. A steep oxygen gradient is maintained by the respiratory activity of facultatively and obligately aerobic members of the gut microorganisms (Brune 1998). An anaerobic milieu in the center of the antrum is essential for the obligately anaerobic symbiotic protozoa of the lower termites (Cleveland 1929a, 1929b). Furthermore, there is a radial hydrogen gradient in the hindgut. The highest concentration is found in the center, and hydrogen acts as a major hydrogen sink in the gut periphery (Brune 1998). The spatial arrangement of metabolic groups of microorganisms and protozoa in the termite gut is thus governed by different gradients of substrates which thereby depend on the activity of the symbionts.

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FIG. 1. Schematic view of the masticatory interactions in lower termites. Besides the digestion of cellulose by the termites’ own enzymes and phagoctytosing symbiotic flagellates, the metabolic roles of some groups of gut bacteria are sketched. The proportions of the termite and the sizes of its organs are altered in favor of an enlarged presentation of the hindgut processes.
THE ROLE OF BACTERIA

Although most gut bacteria are not directly involved in cellulose degradation in termites, they are essential for the survival of their hosts (Slátyr 1992). This is demonstrated, for example, by antibiotic deformation which reduces the termites' life expectancy to a couple of days only, and has a similar devastating effect on nutrition (Eisik et al. 1978). Some of the different roles of the gut bacteria have been identified (Fig. 1). Bacteria may, for example, protect the gut from invasion by foreign bacteria (Tashimaura 1995). Anaerobic and facultatively anaerobic bactes consume a portion of the acetate, and of the other organic acids, that have been released by the gut flagellates. Thus they compete with the termite tissues that also use these substances. However, the anaerobic bacteria use the oxygen diffusing into the gut for oxidation processes maintaining the anoxic status of the internal gut regions (Nötsch et al. 1982). A negative redox potential resulting from this activity is a prerequisite for the survival of the obligatly anaerobic symbiotic protozoa. Fermenting bacteria, e.g., of the genera Spiroplasma, Bacteroides, Faecalibacterium, and Lactobacillus, profit by the low amounts of soluble mono-, di-, and oligonucleotides liberated by the flagellates (Breznak 1983). Their metabolic and products, acetate and other organic acids, can be translocated across the gut wall. Other microbials release the H₂ and CO₂ that is released from the hydrogen donors of the protozoa. So, aceticogenic, CO₂-reducing bacteria produce acetate for use by the termites (Breznak et al. 1988). Species so far identified include Acetocella kowae (Kane & Breznak 1991) and Parvimonas sp. (Toden et al. 1997). Methanogenic bacteria (e.g., Methanobrevibacter-like species) primarily use CO₂ and the methyl group of acetate as electron acceptors (Odelson and Breznak 1985a; Breznak & Boone 1994). Acetococcus succinicus produces methanogenes as the major electron (H₂) sink in the hindgut fermentation of almost all lower termites, and in wood-feeding higher termites (Breznak & Switzen 1986). Since nitrogen compounds are scarce in wood, the ability of nitrogen-fixing bacteria (e.g., Enterobacter, Rhizobium, and Desulfovibrio) is also important for the symbiotic community (Lowéick et al. 1985, König & Becq 1997). These bacteria produce amino acids that are partly liberated and thus may be used by termites and flagellates. Other bacteria recycle nitrogen from urea acid. The metabolic role of the large sphaeroids (Figs. 2, 3) is complete-ly unknown. They belong to the family Pilleraceae (Balows et al. 1991) that is only found in termites and the cockroach Cryptocercus, and have a relatively complex ultrastructure (To et al. 1978) (Fig. 3). Some functions as motility symbions since they propel the flagellae cells to which they are attached, e.g., Mucorchea penadous (Clevered & Girman 1986). The gut bacteria are phylogenetically diverse (Odauma & Kudo 1996). The taxonomic affiliation of many of them has yet to be deter-mined neither are their functions for the gut ecosysstem completely understood. This is also true for the bacteria which are epi- or endobiotically asso-ciated with numerous species of gut flagellates (Ball 1969, Read et al. 1992).

SYMBIOTIC RELATIONSHIPS OF TERMITES AND FLAGELLATES

The pseudergates, i.e., older larvae that are workers in the lower termites, feed on wood with their man-dibles. The lignicolous particles are then ground by the curicular spines of the prevertermusc. Some hydrolyzing enzymes, including cellulolytic ones (endoglucanases and cellobiases), from the salivary glands and the midgut must digest the food in front of and midgut. The chief site of cellulase digest-ion, however, is the large dilatation of the hindgut, the pouch. Here, the extremely numerous sym-biotic flagellates ingest and digest small wood particles, and excrete metabolites for use by the termites. Pio-narium symbionts constitute one-third to one-seventh of the total body weight of, e.g., the nymphs of the termite Zootermopsis (Huang 1934).

The association of termites and flagellates is not only advantageous for the termites, but also for the unveretermusc (Radek & Hausmann 1991). The flagellates are supplied with food by their hosts, pre-tested from excrescences, and receive shelter in a constant environment. They are protected from desiccation, and changes in temperature are avoided by their hosts moving activity to suitable sites. However, life in an intestine also holds dangers, for example being ex- pelled from the host through the anus. When the pouch is filled to its capacity in contents are pushed into adjacent gut regions. An ectrotive valve prevents an overflow into the midgut. The termites may also actively empty part of their pouch content in order to supply, via prococol feeding, young larvae, sol-diers, reproducers, or nec-taries that have mated (Honigberg 1970). Since the hindgut has an eco-
dermal origin, the paunch is equipped with an intima. During molts the contents of the paunch are more or less wrapped by the old intima and get lost. The molted termites must be refurnished by procured feeding, or possibly by ingestion of the shed cuticle. Even though many of the ingested flagellates are damaged or digested during passage through the gut, sufficient numbers can reach the paunch to multiply again.

There is no convincing evidence for the formation of true cysts in termite flagellates (Himes 1919, Grose & Neison 1945, Forshager 1953a). This does not seem to be necessary since they are transmitted to their hosts by procured feeding. Morphological changes, however, occur during the molts of their hosts. The molting hormone, ecdysone, seems to be responsible for inducing the sexual cycles in some flagellates, e.g., A Mestinea paracanaliculata (Cleveland 1966a), Delabicinclus liaudiana (Cleveland 1966b), and Konga (Cleveland 1966c), which are symbionts of the primitive termite Mestinea davainiae. Hormone-induced sexuality was also reported from several other genera of ponerine living in termites (Cleveland 1965a, b), and in flagellates from the cockroach Cryptocercus (Cleveland 1953). However, parts of the life cycles described are uncertain, since individuals of different flagellate species seem to be put together in a common cycle. Stangberg (1970) suggested that the capacity for sexual reproduction was present in ancestral flagellates and may have been lost by many of the proterans during the process of evolution. The method of transmission of the flagellates by termites would render sexuality rather superfluous.

THE ROLE OF FLAGELLATES IN THE DIGESTION OF WOOD

Lower termites are incapable of digesting plant tissues consisting lignocellulose without the help of symbiotic flagellates. They die within two weeks despite continued feeding if the processa are killed (Stangberg 1970). Detoxification can be achieved, for example, by increasing temperature (Cleveland 1926) or by reducing pressure (Cleveland 1925a, b, 1928), by starvation of the termites (Cleveland 1925a, Yoshimura 1995), or by ultraviolet irradiation (House et al. 1977). It has been suggested that the associated symbiotic bacteria of the flagellates might be involved in enzyme production. However, experiments with bacteria-free, axenically cultured flagellates, e.g., Trichomonas sphaerica (Yamagami 1961) and Trichomonas termopsodis (Yamagiwa 1978, Yamagiwa & Tager 1979, Olden 1983), proved the capability of these species at least to decompose cellulose without bacterial aid. The hindgut flagellates ingests fresh food particles by phagocytosis (Figs. 4, 10). There is no special feeding apparatus or cytosomes. Ingestion can take place throughout the plasma membrane except the flagellated body surfaces. When the bolus is coated with symbiotic bacteria these are engulfed and remain attached to the membrane of the euglossium vacuole for some time (Bedek et al. 1992). Bacteria living in the gut fluid and occasionally small flagellates are also phagocytosed. Dissolved substances not be incorporated by phagocytosis (Hollands & Valer- tis 1969). Each flagellate species of a certain termite host has its specified role in digestion (Yoshimura et al. 1993, 1994; Yoshimura 1995). For example, the largest protistan in the termite Cryptocercus formosus, Pseudomonas formosus, is involved in the decomposition of highly polysaccharoidal cellulose, while the other two species, Holusamites harmoniaeni and Sphaeromonas limicola, can only use low molecular weight cellulose (Yoshimura 1995). Many small flagellate species do not ingest particles and may play a role in later stages of cellulose metabolism such as FIG. 3. Large sinuses of the family Pilla- cinea in the gut of the lower termite Kalotermes fasci- collis. FIG. 2. Several flagellates. Differential inter- ference contrast. Bar = 10 mm. FIG. 2. Ultra- rather cross-section of Pillaena sp. depicting the pro- plasma cylinder (pc), axial fibrils corresponding to flagellar (arrow), and a wavy outer membrane (om). Bar = 6.5 μm.
mechanogensesis (Yoshimura et al. 1980). The spe- cific function of each protenous species seems to be correlated with distribution in the gut, showing spe- cific niches for the respective flagellates (Yoshimura 1995).

The lignocellulosic food of the termites consists of the major fractions cellulose (28–50%), hemi- celluloses (20–30%), and lignin (18–30%) (Brennac & Brun 1994). Since covalent lignin-carbohydrate linkages impede the enzymatic degradation of ligno- cellulose, a high lignin content renders material hard to digest (Jeffries 1990). The published results on lignin degradation are somewhat ambiguous (see Brennac & Brun 1994; Vanma et al. 1994). The limited information obtained so far indicates that lignin is not totally excised during its passage through the termite gut. The breakdown of the aromatic ring system requires oxygen. Microorganisms near the anterior pouch epithelium may start the breakdown of lignin and continue digestion outside the gut (Vanma et al. 1994). Therefore repeated recycling of faeces may increase the efficiency of lignin digestion.

Hemicellulose is digested to a considerable degree (Mishra 1979), but our understanding of hemicell- ulose digestion in termites is still meager (Brennac & Brun 1994). Hemicellulose is a heterogeneous group of polysaccharides composed chiefly of D-xylose, D-mannose, L-arabinose, D-galactose, or of combinations of these. Xylanase activity by pro- tozoa has been demonstrated in the hindgut of lower termites (Oldenbo & Brennac 1985b; Inoue et al. 1997).

Considerable research attention has been paid to the digestion of cellulose, the most important com- ponent for termite nutrition. In general, enzymatic hydrolysis of cellulose to glucose occurs through the action of cellulases, a general term referring to a mix- ture of enzymes consisting of three major classes: en- doglucanases, cellobioglucanases, and cellubiose (Brennac & Brun 1994). Endoglucanases (endo-1,4-β-glucanases) cleave internal glycosidic bonds along the polygalactan chain. They are active against amorphous cellulose and water-soluble derivatives (e.g., carbo- synmethyliodextrin), and release glucose, cellulobiose, cellobiose, and other higher oligomers. However, they are less active against crystalline cellulose. En- doglucanases (cellodextrinhydrolyses and cellobiohydrolases) attack the termites of a polygalactan chain including highly crystalline cellulose, liberating cellulose or glucose from the non-reducing end. Cellulases (β- 1,4-glucanases) hydrolyse cellulose and water- soluble cellodextrins to glucose.

Lower termites are able to synthesize several cellulase components. Thus the cellulose and hemicell- ulose components of the wood particles ingested by the flagellates are already partially attacked (re- viewed by Brennac & Brun 1994). The enzymes, for example endoglucanases, cellobioglucanases and other carbo- hydrate-hydrolyzing enzymes, are produced in the urinary glands and the midgut. However, the flagel- lans are the major agents of wood cellulose hydro- lysis in lower termites (Högenberg 1978; Brennac 1982, 1983; O’Brien & Shlyar 1982; Oldenbo & Brennac 1985a). They play an important role in de- livering further enzymes, including those which cymi- vers microcrystalline cellulose to glucose (Yamitz & Trager 1977, Oldenbo & Brennac 1986). With this supply, most of the cellulose can be hydrolyzed. Recently, Itakura et al. (1997) found all three types of cellulases that are essential for the hydrolysis of natural cellulose in the urinary glands, formic, and midgut of the lower termite Coptotermes formosanus. Thus part of the cellulose degradation into glucose can clearly be performed by the termites’ own enzy-
mes. However, the highest level of activity, especially that of non-cellulololytic bacteria (87%), is found in the hindgut due to the pronomal fauna (Yoshimura 1995).

Microscopic investigations of symbiotic flagellates show a gradual decomposition of ingested wood cellulose within the food vacuoles, independent of bioclastic activity (Yoshimura et al. 1994, 1996). Indigestible residues, probably lignin, are released from the cells as a loose fibrous mat. The water-soluble nutrients, such as glucose and small sugar oligomers, are liberated into the cecropath. Glucose is stored in the protosac as glycogen. Only a low percentage of the glucose reaches the fluid of the hindgut. Most of it is physiologically transformed to pyruvate, and utilized for energy production by special organelles, the hydrogenosomes (Lindskog et al. 1989, Mäkile 1993). Hydrogenosomes are often classified as mitochondria or perichromatin organs that live in anoxic environments, such as aerobic citruses, corydalis/morey fungi, and termite flagellates (Muller 1993). In chitinomaiid and microtermitidae termite flagellates such organelles are spherical bodies measuring 0.5-3.0 mm in diameter (Kida et al. 1986). They are formed by two closely adjacent membranes and are filled with a granular matrix and dense amorphous or paracrystalline inclusions (Fig. 5). The pyruvateform fermentation and hydrogenosomes are enzyme systems in which hydrogenosomes (Muller 1993). Hydrogenosomes are often composed of ATP by an anaerobic substrate-level phosphorylation reaction, re-feeding O2, H2, and CH3H2 (Red & Stearn 1994). Aciotat is strictly oxygenated by the wall of the pouch and used in the major oxidizable energy source of the termites. As well as constituting an important biosynthetic precursor (Odeon & Breznak 1988). Anaiota is indeed the main source of energy in the hindgut fluid of lower termites. 15 ppm for 94-95 mole% (Odeon & Breznak 1983). Small amounts of other volatile fatty acids, such as propionate and butyrate, are also present.

SYMBIOTIC FLAGELLATES: TAXONOMICAL AND MORPHOLOGICAL CHARACTERISTICS

The symbiotic flagellates of lower termites belong to several phylogenetically ancient protozoa taxa. Their classification, however, differs from author to author. According to Lox (1985), termite flagellates are found in three orders: Chilomonadales, Hypermami- stigmata and Oxytricha, within the class Zoa- maminophorista. In more recent publications they are considered any termite species taxonomic rank. Bilimilium & Hau- man (1994) combined them with the phyla Pico- baalia and Trochida by Corriveau (1994) and Cavaleri- Smith (1967) respectively. The oomycetes are classified in the phylum Phoromorpha of the kingdom Archaea and receive the rank of a class, Oxy- tricha, by Corriveau (1994) and the rank of a sub- phylum, Parostralia, with the single class Oomyc- ophora by Cavaleri-Smith (1995).

The flagellate species are typical of the respective termite host species (Yamam 1979). Soluble populations of host termites may, however, contain slightly different flagellate communities, for example if the pair of reproductives that had bred the nest con- tained a different spectrum of species. Occasionally, the same flagellate species are found in differ- ent hosts. A termite species may contain only a few species or more. Several species can be found in the same host species, since only about 200 of the 4000 known species of lower termites have been examined so far.

A specific organellar of termite flagellates is a red compound of microbalsac, the astaxanthin. It extends from the anterior cell pole adjacent to the basal bodies of the flagella back to the posterior cell pole. In length varies according to the species. The microbalsac are arranged in a line on the spiral wound plate which forms a red flagellum (Fig. 6). The bridges that connect the microbalsac within and between adjacent plates have motor functions in some oomycetes, e.g., Xostix, Oomycetes, and Saccharomycetes, thus con- torting motility to the respective astaxanthins (Greenaw & Cleveland 1965, Michorob 1973, Mooreker & Tilney 1973). In the anterior body portion the astaxans- styles often occur to the nucleus and form extensions (called pellicles) that support anterior plasma membrane regions (Fig. 4). The Golgi apparatus (+ parahistidine apparatus) of chitinomaiid and hypermamaians is a special, very complex organelle. The membranous stacks of the depositions are composed of numerous

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FIG. 9. Silver staining with protargol of the trichomonad. *Trichomonas vaginalis* from *Nasrini, nasrini* (female). The recurrent flagellum forms an undulating membrane (arrow) with the body surface. A thal-like costa (co) subdivides the undulating membrane. The nucleus (n) with one nucleolus and the sole "kinetosome" (k) are also stained. Bar = 50 μm. FIG. 10. The surface of the hypermastigote *Kerarga bonita* from *Macrocrinum dimorphism* is densely covered with rows of flagella. Scanning electron micrograph. Bar = 20 μm. FIG. 11. The electron micrograph of the oxydornad *Macrocrinum dimorphism* from *Kerarga bonita* shows one pair of basal bodies connected to the paracystellar protoplasmic (arrow). g = glycogen granules, n = nucleus. Bar = 1 μm. FIG. 12. Several individuals of the oxydornad *Metahymenolobus reclinatus* are attached to the gut wall of *Cephalaspis dulcis* with a slender, anterior extension of the cell body, the rostellum (arrowheads). Bar = 20 μm.
Oxyrhynchs have one or more tritocerebral systems and nuclei. Each metadiencephalic system contains two flagella that are typically arranged in two pairs of two (Holland & Carpenter-Wilson 1970, Buegeroff 1981, Radik 1994). One of the four flagella is current. The basal bodies of the two pairs are connected by a paracrystalline structure, the paracrystal (Fig. 11). Many oxyrhynchs may attach to the wall of the pouch via an apical cell protrusion, a retractum, which is reinforced by microtubules of the associated flagella (Figs. 12) (Rathke et al. 1999). Oxyrhynchs differ from the lineage of the pseudorhynchs (trichomonads and hypernemids) in the flagellar apparatus morphology and organization, mitotic apparatus, and attachment to the host. Furthermore, they may have mitochondria or hydroxygenosomes, and a Golgi apparatus is missing.

**FUTURE PERSPECTIVES**

Many researchers have investigated the relationships of trematodes with the diverse pro- and eukaryotic intestinal and external symbiotic co-inhabitants. Nevertheless, various questions have been answered only partially, and many controversial results await clarification. This situation is double-ended due to the multitude of trematode species and the even higher species richness of the associated organisms. Hence, not only a large number of organisms remains to be investigated, but diverse evolutionary pathways leading to different symbiotic systems and strategies can be expected. Most of the flagellates and many of the bacteria have never been grown in culture; these severely restricting experimental investigation. Progress in molecular biology, notably the introduction of the polymerase chain reaction (PCR), now offers opportunities to characterize non-cultivable species at the genomic level. The complex, still puzzling interactions of trematodes, flagellates, bacteria, and fungi will remain a fascinating field of research.

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