

***Staurojoenina* and Other Symbionts in *Neotermes* from San Salvador Island, Bahamas**

MICHAEL DOLAN and LYNN MARGULIS*

Department of Biology, University of Massachusetts, Amherst, MA 01003-5810, USA. Tel. +413-545-3244, Fax. +413-545-3243

Received July 21, 1996; Accepted November 8, 1996

LIBRARY

JAN 11 2010

SAINT MARY'S UNIVERSITY
HALIFAX, CANADA

B3H 3C3

Abstract

Staurojoenina, a conspicuous hypermastigote protist (undocumented in any *Neotermes*) and other hindgut symbionts are reported for the first time in *Neotermes* nr. *jouteli*, a dry-wood-eating termite (Kalotermitidae), from the red mangroves at the northeast corner of San Salvador Island. Other distinctive protists (*Macrotrichomonas*, *Metadevescovina*, two morphotypes of small trichomonads) and bacteria (*Arthromitus*-type filamentous spore-formers) symbionts were also found in this termite. This *Staurojoenina* sp. replete with epibiotic bacterial symbionts is not distinguished from previously described species of *Staurojoenina*.

Keywords: Hypermastigote, termite, hydrogenosomes, devescovinid, trichomonad

1. Introduction

The distribution and evolutionary origin of the protist and bacterial symbionts of wood-eating termites remains poorly understood despite lifetimes of work by scientists such as L.R. Cleveland et al. (1934), Harold Kirby (1994), P.-P. Grassé, André Hollande (Hollande and Carruette-Valentin, 1971) and others. The most recent compilation of termites and their protist symbionts indicates that over 170 species of Kalotermitidae alone have never been

*The author to whom correspondence should be sent.

examined for their protist symbionts (Yamin, 1979). No coordinated effort to record variation in the hindgut community between geographically isolated populations of the same termite species has been made. Seasonal, caste or other long-term changes in population structure, if any, remain undocumented since these complex symbioses were first reported some 150 years ago.

Although a new species of *Neotermes* from Florida has been described (Nickle and Collins, 1989), its symbiotic microorganisms have not been reported. In this first study of *Neotermes* from this eastern-most Bahamian island north of the Tropic of Cancer, we identify the insect and its most conspicuous protist symbionts. The opportunity to collect termites was afforded by the celebration at the Bahamian Field Station of the tenth anniversary of the San Salvador Island's natural history society (Elliot et al., 1996).

2. Materials and Methods

Termites were collected by one of us (L. M.) from a decaying red mangrove stump during the expedition to San Salvador Island in June 1995. They were reared in the laboratory in their native wood and soil. Specimens were sacrificed and their hindguts broken open in a salt solution (Trager, 1934). Microbial symbionts were examined in live wet mounts, sealed with vaseline, or smeared on poly-L-lysine-treated coverslips and fixed in 1% glutaraldehyde or STF, Streck Tissue Fixative (Streck Laboratories, Inc., Omaha, NE). These fixed specimens were stained with Heidenhain's hematoxylin or with protargol (Duval and Margulis, 1995).

Live specimens, both the termites and the hindgut symbionts, were recorded on videotape using a Sony CCD camera mounted on a Nikon Fluorophot microscope. Still photographs were taken with TMAX 400 or 160 tungsten color slide film on the same microscope. Images were also captured using a SONY videographic printer.

3. Results

Samples of soldier and worker dry-wood-eating kalotermitid termites placed in 70% alcohol were sent to Dr. Rudy Scheffrahn of the Fort Lauderdale Research and Education Center. There they were kindly identified by his colleague Dr. Jan Křeček as *Neotermes* nr. *jouteli*, a close relative of *Neotermes luykxii* and of *Neotermes jouteli*.

All of the 20–25 termite specimens examined contained at least five species of protists: the large complex hypermastigote *Staurojoenina* sp. (Fig. 1b), two

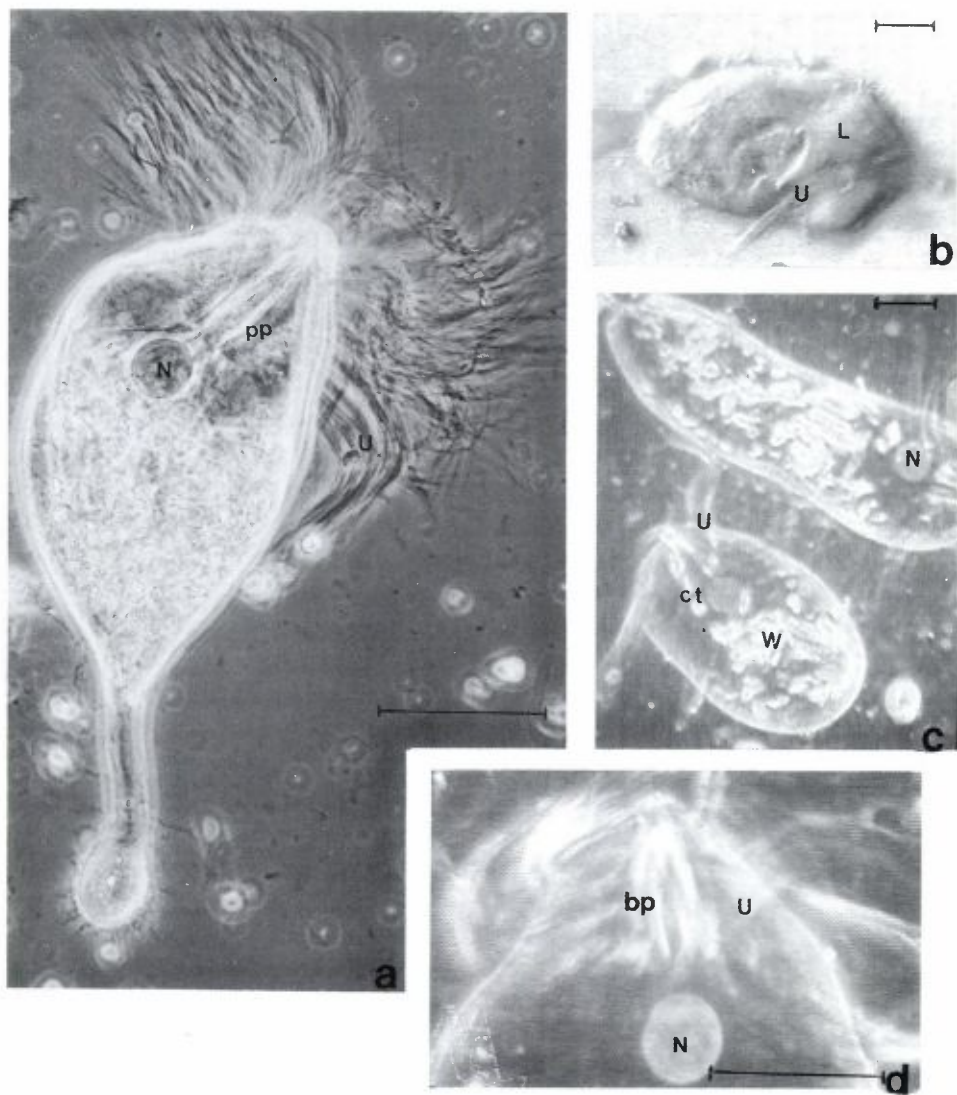


Figure 1. *Staurojoenina* a. *S. assimilis* from *Incisitermes minor*, Newbury Park CA, phase contrast microscopy by David Chase; b. *Staurojoenina* sp. from *Neotermes* nr. *jouteli* San Salvador Is. Bahamas, differential interference contrast microscopy; c. and d. *Staurojoenina* from *Bifiditermes condonensis*, light micrograph by L.R. Cleveland. pb = peripheral bands, L = lobes of cytoplasm covered by the epibiotic bacteria of Fig. 3, N = nucleus, U = bundled undulipodia and W = wood particles. Bars = 60 μ m.

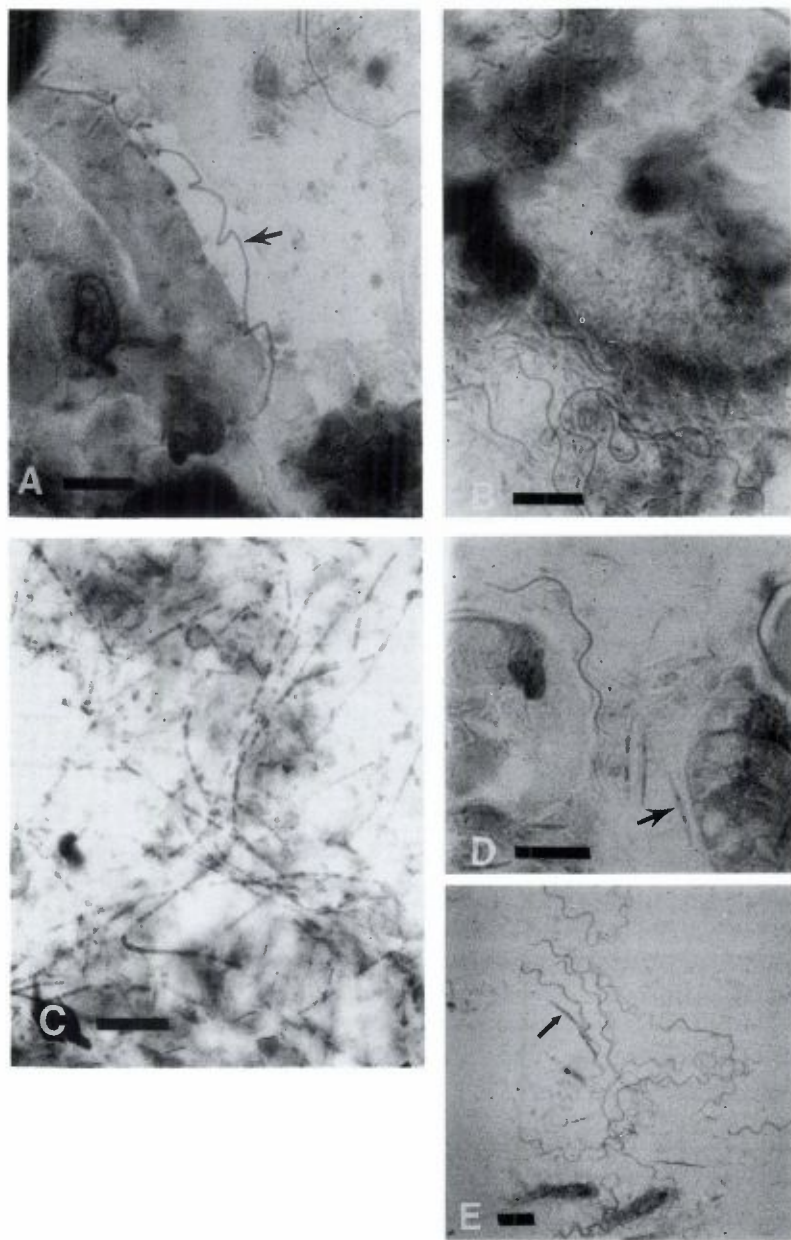


Figure 2. *Neotermes* nr. *jouteli* hindgut symbionts a. *Macrotrichomonas restis* with recurrent undulipodium (arrow), bar = 10 μ m; b. *Metadevescovina* sp. with long cresta (arrow), bar = 10 μ m; c. *Arihromitus* sp., bar = 10 μ m; d. unidentified "diplo" spore-forming bacterium (arrow), bar = 10 μ m; e. *Arthromitus* sp. (arrow) and spirochetes of the family Pillotinae. a,b,d,e stained with hematoxylin, c with protargol.

devescovinids: *Macrotrichomonas restis* (Fig. 2a), *Metadevescovina* sp. (Fig. 2b) and two small trichomonads. The four bands of undulipodia and the complex cytoskeleton characteristic for *Staurojoenina* are shown in Fig. 1. The distance from the posterior end of the nucleus to the anterior end of the atractophores was nearly a constant 60 μm , with a range of 59 to 62 μm ($n=20$). *Macrotrichomas restis*, with its distinctive recurrent undulipodium attached lengthwise to the cell, was generally free of spirochete epibionts while the *Metadevescovina* sp., with its long, curved cresta, had a dense covering of these bacteria at its posterior end. Many larger free-swimming spirochetes (Pillotinaceae, Bermudes et al., 1988) were seen (Fig. 2d), some over 50 μm long, in addition to the smaller epibiotic spirochetes.

Two groups of endospore-forming rod-like bacteria were seen. In one termite, stained with protargol, the hindgut was replete with long filaments (30–40 cells/filament) of spore-forming bacteria (Fig. 2c). Identified as *Arthromitus* sp. very similar to *A. chaseii* (Margulis et al., 1990), this bacterium was also seen in live preparations. A second "diplo" (2-celled) endospore-forming bacterium, commonly reported on and around termite hindgut protists (Ball, 1969), was also observed (Fig. 2d). The microbes identified from this termite are listed in the taxonomic summary of Table 1.

Table 1. Microbes identified in San Salvador *Neotermes* nr. *jouteli*

K.	Protoctista	
	Phylum Archaeoprotista	
	Class Parabasalia	
	Order Trichomonadida	
	Fam. Devescovinidae	
	<i>Macrotrichomonas restis</i>	
	<i>Metadevescovina</i> sp.	
	Order Hypermastigida	
	Fam. Staurojoenidae	
	<i>Staurojoenina</i> sp.	
K.	Bacteria	
	Phylum Spirochaeta	
	Fam. Pillotinaceae	
	<i>Pillotina-Hollandina</i> -like spirochetes that require ultrastructure for identification	
	Phylum Endospora	
	Fam. Arthromitaceae	
	<i>Arthromitus</i> sp. two-celled ("diplo") endospore former (formally undescribed)	

4. Discussion

Staurojoenina was conspicuously present in all the healthy termites examined (n=25). As is commonly the case only one "large" protist was present; *Trichonympha*, *Calonympha* and all other large parabasalids were conspicuously absent. At the level of light micrographic resolution available to us no differences were noted between this San Salvador *Staurojoenina* and that from *Incisitermes minor*, Newbury Park California (Fig. 1). Our unpublished collaboration with the late David G. Chase displays the remarkable complexity of the composite cell: the four bands of undulipodia alternate with four lobes of cytoplasm decorated with regularly-associated unidentified gram-negative bacteria (Fig. 3a). A microtubule subtends each bacterial attachment site (Fig. 3b). The defining features of the genus, the parabasal plates and peripheral bands, are composed of proteinaceous sheets of unknown composition. Both *Staurojoenina caulleryi* from *Postelectrotermes praecox* and *S. assimilis* from *Incisitermes minor* contain parabasal plates which define a space filled with membrane-bounded but cell-wall lacking organelles (Hollande and Valentin, 1968). These were identified as mitochondria by Hollande and Carruette-Valentin (1971). Given that mitochondria are absent in all parabasalids, now classified as Archaeprotista (Margulis 1996), these structures could be hydrogenosomes, bacteria-like organelles or a second bacterial symbiont comparable to that reported in termite protists (*Caduceia* sp.) by Tamm (1982) and the endobionts surrounded by endoplasmic reticulum in *Mixotricha paradoxa* from *Mastotermes darwiniensis* by Cleveland and Grimstone (1964). For discussion of hydrogenosomes and related organelles see Fenchel and Finlay (1995). These membrane-bounded structures in *Staurojoenina* were probably misidentified as mitochondria because of their comparable size, staining pattern, distribution in the protist and absence of cell walls.

Symbiotic bacteria in vacuoles or aligned densely and characteristically on the surface of certain protists are clearly visible in Tamm's (1982) micrographs. Our *Staurojoenina* symbionts differ from both the bacterial epibionts (the fusiform and the peritrichly flagellated rod) reported by Tamm (1982). The remarkable pattern of the fusiform alternating with the protist-moving peritrichly flagellated bacterium will be part of the taxonomic description of this devescovininid known colloquially as "Rubberneckia". As seen most clearly in Tamm's work the epibiotic bacteria are such integral components of these hindgut symbiotic protists that they need to be part of their proper description. In some cases this has occurred inadvertently as with *Devescovina striata*, whose "striations" are epibionts. *Staurojoenina* (itself an obligate symbiont of

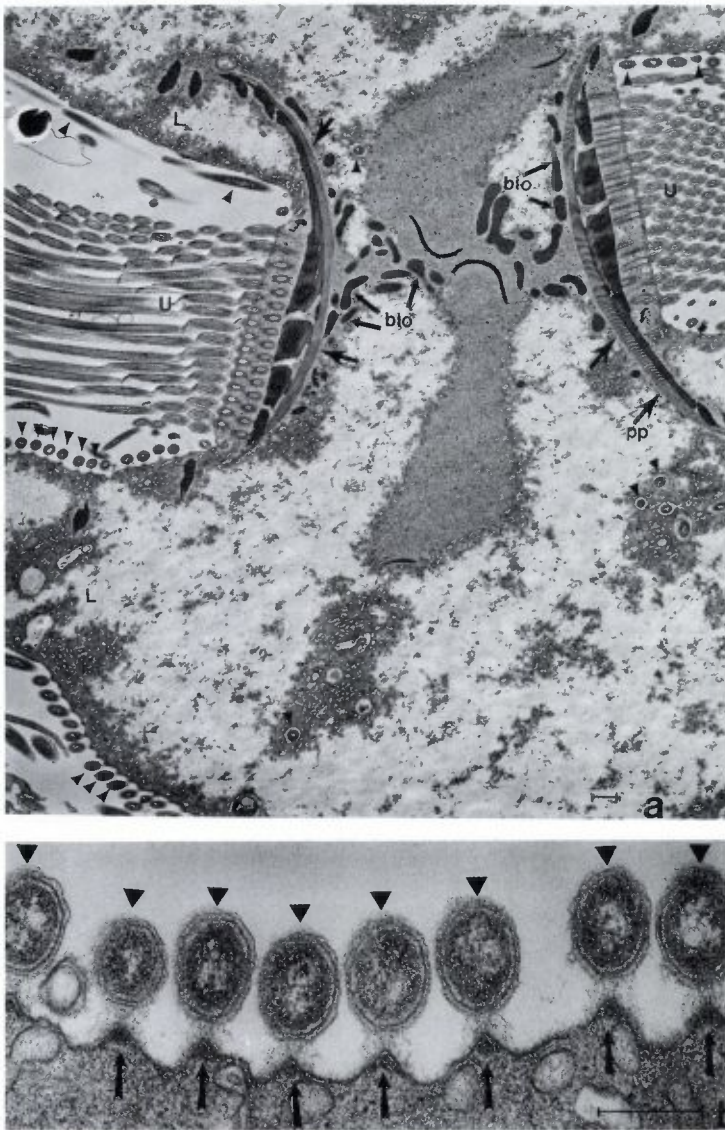


Figure 3. *Staurojoenina* a. *S. assimilis* from *Incisitermes minor*, Newbury Park CA showing two parabasal plates (arrows), epibiotic bacteria decorating the four lobes (L) of cytoplasm (arrowheads), bacteria-like organelles (blo) between the proteinaceous parabasal plates mistaken for mitochondria by Hollande and Carruette-Valentin (1971) and bundles of undulipodia (U) between the lobes of cytoplasm, bar = 1.0 μ m. b. The epibiotic bacteria (arrowheads) that decorate the four cytoplasmic lobes (L) of Fig. 1 above. Each epibiont (arrowhead) is associated with a cytoplasmic protrusion underlain by a single submembranous microtubule (arrow), bar = 0.5 μ m. Electron micrographs by David Chase.

dry-wood eating kalotermitid termites), is composed of at least three different symbiotic partners i.e., nucleocytoplasm, rod-shaped walled epibionts and other rod-shaped, wall-less endobiotic bacteria. The walled epibiotic bacterium is similar to rod-shaped epibionts of *Urinympa* from *Cryptocercus* (Bloodgood and Fitzharris, 1976).

This is the first report of *Staurojoenina* from *Neotermes*. The common dry wood *Neotermes* usually harbor devescovinids and calonymphids as their large hindgut protist symbionts. Of the twenty-five *Neotermes* species examined only five contain hypermatigotes. Fifty-seven species of this genus have not had their protists reported. Both devescovinids, *Macrotrichomonas restis* and *Metadevescovina* sp., are reported from Florida *Neotermes jouteli*, so their presence in this close Caribbean relative is not surprising.

Four different species of *Staurojoenina* have previously been described in five geographically separated termite genera (references from Yamin, 1979): *S. assimilis* Kirby in *Bifiditermes condonensis* (Sutherland, 1933) and in *Incisitermes minor*; *Staurojoenina* sp. in *Marginitermes hubbardi* (Kirby, 1926), *S. caulleryi* Grassé and Hollande in *Postelectrotermes* (formerly *Neotermes*) *praecox* (Grassé and Hollande, 1945), and *S. mirabilis* Grassi in *Epicaloertermes aethiopicus* (Grassi, 1917). The late David G. Chase analysed *S. assimilis* from *Incisitermes minor* in Newbury Park, California by electron microscopy. Apart from his work (Fig. 3) only one other species of *Staurojoenina* has been described at the ultrastructural level (Hollande and Valentin, 1968; Hollande and Carruette-Valentin, 1971). The distance between the posterior end of the nucleus and the anterior end of the atractophores (called centrolepharoplasts by Kirby) was nearly a constant 60 μm , the same measurement Kirby found for *S. assimilis*. He considered this a more consistent character than body length or shape as these can vary. Our light micrographs provide no reason to name a new species. A comparative ultrastructural study of all four species of this genus is needed.

Staurojoenina is limited only to the "lower" dry wood-eating termites of the family Kalotermitidae. *Idionympha*, with its four bands of undulipodia and presence in the wood-eating cockroach *Cryptocercus*, is the only other genus assigned (Cleveland et al. 1934) to the Staurojoenidae. The presence of *Idionympha* in the Appalachian *Cryptocercus* implies the existence of an ancestor common to *Idionympha* and *Staurojoenina*. The Staurojoenidae presumably evolved early during the diversification of termites from *Cryptocercus*-like wood-eating cockroaches. Its members may have been lost from all other subsequent termite lineages (Honigberg, 1970).

To describe the symbiotic community of the hindgut we must understand both the protists and the bacteria living on and in them. The physiological relations of termite symbionts (e.g., cellulolytic protists, acetogenic,

methanogenic and heterotrophic bacteria) with the animals were reviewed in Breznak and Brune (1994) and Nalepa (1994). Bacterial morphology (spirochete and bacillus) was reviewed by Bermudes et al. (1988), Margulis and Hinkle (1992) and Margulis et al. (1990). New molecular biological techniques may bridge the gap between physiological and morphological data by divulging the diversity of bacteria within a single termite (Ohkuma and Kudo, 1996) and by identification of the bacteria by *in situ* hybridization techniques (Paster et al., 1996). Any more complete reconstruction of the evolutionary history of wood-feeding insects and their symbionts requires the integration of natural history with cytological, physiological and molecular biological data.

Acknowledgements

We are grateful for aid from Dr. R. Scheffrahn, Dr. Jan Krecek, Dr. Mark Deyrup and his son Stephen, Jennifer Margulis. We thank Thomas H. Teal for lab and field work and Donna Reppard for help with manuscript preparation. For financial support we thank the Richard Lounsbery Foundation, NASA Space Sciences and the University of Massachusetts Graduate School. This research was in part supported by a grant from the Margaret E. and Howard E. Bigelow Gift to the University of Massachusetts Amherst.

REFERENCES

- Ball, G.H. 1969. Organisms living on and in protozoa. In: *Research in Protozoology*, vol. 3., T.-T. Chen, ed. Pergamon, Oxford, pp. 565-718.
- Bermudes, D., Chase, D., and Margulis, L. 1988. Morphology as a basis for taxonomy of large spirochetes symbiotic in wood-eating cockroaches and termites: *Pillotina* gen. nov., nom. rev.; *Pillotina calotermitidis* sp. nov., nom. rev.; *Diplocalyx* gen. nov., nom. rev.; *Diplocalyx calotermitidis* sp. nov., nom. rev.; *Hollandina* gen. nov., nom. rev.; *Hollandina pterotermitidis* sp. nov., nom. rev.; and *Clevelandina reticulitermitidis* gen. nov., sp. nov. *International Journal of Systematic Bacteriology* 38: 291-302.
- Bloodgood, R.A. and Fitzharris, T.P. 1976. Specific associations or prokaryotes with symbiotic flagellate Protozoa from the hindgut of the termite *Reticulitermes* and the wood-eating roach *Cryptocercus*. *Cytobios* 17: 103-122.
- Breznak, J.A. and Brune, A. 1994. Role of microorganisms in the digestion of lignocellulose by termites. *Annual Review of Entomology* 39: 453-487.
- Cleveland, L.R., Hall, S.R., Sanders, E.P., and Collier, J. 1934. The wood-feeding roach *Cryptocercus*, its protozoa, and the symbiosis between protozoa and roach. *Memoirs of the American Academy of Arts and Sciences* 17: 185-342.

- Cleveland, L.R. and Grimstone, A.V. 1964. The fine structure of the flagellate *Mixotricha paradoxa* and its associated micro-organisms. *Proceedings of the Royal Society Series B* **159**: 668–686.
- Duval, B. and Margulis, L. 1995. The microbial community of *Ophrydium versatile* colonies: endosymbionts, residents and tenants. *Symbiosis* **18**: 181–200.
- Elliot, N.B., Edwards, D.C., and Godfrey, P.J., eds. 1996. Proceedings of the 6th Symposium of the Natural History of the Bahamas, Bahamian Field Station, San Salvador, Bahamas.
- Fenchel, T. and Finlay, B.J. 1995. Ecology and Evolution in Anoxic Worlds. Oxford University Press, Oxford and New York.
- Grassé, P.-P. and Hollande, A. 1945. La structure d'une hypermastigine complexe *Staurojoenina caulleryi*. *Annales des Sciences Naturelles, Zoologie et Biologie Animale*, ser. 11., **7**: 147–158.
- Grassi, B. 1917. Flagellati viventi nei termiti. *Memorie della Reale Accademia dei Lincei*, ser. 5. **12**: 331–394.
- Hollande, A. and Valentin, J. 1968. Infrastructure du complexe rostral et origine du fuseau chez *Staurojoenina caulleryi*. *C. R. Acad. Sc. Paris* **266**: 1283–1286.
- Hollande, A. and Carruette-Valentin, J. 1971. Les atractophores, l'induction du fuseau et la division cellulaire chez les hypermastigines. Étude infrastructurale et révision systématique des trichonymphines et des spirotrichonymphines. *Protistologica* **7**: 5–100.
- Honigberg, B.M. 1970. Protozoa associated with termites and their role in digestion. In: *Biology of Termites*, vol. 2, K. Krishna and F.M. Weesner, eds. Academic Press, New York, pp. 1–35.
- Kirby, H. 1926. On *Staurojoenina assimilis* sp. nov., an intestinal flagellate from the termite, *Kaloterme minor* Hagen. *University of California Publications in Zoology* **29**: 25–102.
- Kirby, H., annotated by Margulis, L. 1994. Harold Kirby's symbionts of termites: Karyomastigont reproduction and calonymphid taxonomy. *Symbiosis* **16**: 7–63.
- Margulis, L., Olendzenski, L., and Afzelius, B.A. 1990. Endospore-forming filamentous bacteria symbiotic in termites: ultrastructure and growth in culture of *Arthromitus*. *Symbiosis* **8**: 95–116.
- Margulis, L. and Hinkle, G. 1992. Large symbiotic spirochetes: *Clevelandina*, *Cristispira*, *Diplocalyx*, *Hollandina*, and *Pillotina*. In: *The Prokaryotes*, Vol. 4. A. Balows, H.G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer, eds., Springer Verlag, New York, pp. 3965–3978.
- Margulis, L. 1996. Archaeal-eubacterial mergers in the origin of Eukarya: Phylogenetic classification of life. *Proceedings of the National Academy of Sciences, USA* **93**: 1071–1076.
- Nalepa, C.A. 1994. Nourishment and the origin of termite eusociality. In: *Nourishment and Evolution in Insect Societies*. J.H. Hunt, and C.A. Nalepa, eds., Westview Press, Boulder, pp. 57–104.
- Nickle, D.A. and Collins, M.S. 1989. Key to the Kalotermitidae of Eastern United States with a new *Neotermes* from Florida (Isoptera). *Proceedings of the Entomological Society of Washington* **91**: 269–285.

- Ohkuma, M. and Kudo, T. 1996. Phylogenetic diversity of the intestinal bacterial community in the termite *Reticulitermes speratus*. *Applied and Environmental Microbiology* **62**: 461-468.
- Paster, B.J., Dewhirst, F.E., Cooke, S.M., Fusing, V., Poulsen, L.K., and Breznak, J.A. 1996. Phylogeny of not-yet-cultured spirochetes from termite guts. *Applied and Environmental Microbiology* **62**: 347-352.
- Sutherland, J.L. 1933. Protozoa from Australian termites. *Quarterly Journal of Microscopical Science* **76**: 145-173.
- Tamm, S.L. 1982. Flagellated ectosymbiotic bacteria propel a eucaryotic cell. *Journal of Cell Biology* **94**: 697-709.
- Trager, W. 1934. The cultivation of a cellulose-digesting flagellate, *Trichomonas termopsidis*, and of certain other termite protozoa. *Biological Bulletin* **66**: 182-190.
- Yamin, M.A. 1979. Flagellates of the orders Trichomonadida Kirby, Oxymonadida Grassé, and Hypermastigida Grassi & Foà reported from lower termites (Isoptera families Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae and Serritermitidae) and from the wood-feeding roach *Cryptocercus* (Dictyoptera: Cryptocercidae). *Sociobiology* **4**: 1-120.