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# Antibiotics Remove *Caduceia versatilis* from the *Cryptotermes cavifrons* (Kalotermitidae: Isoptera) Hindgut and Increase Production of Calcium-Rich Crystals

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**Abstract**

The microbial community of the hindgut of the wood-eating termite *Cryptotermes cavifrons* contains many different bacterial types associated with the wood-digesting protists (*Caduceia versatilis*, and the multinucleate *Snyderella tabogae* and *Stephanonympha* sp.). Bacterial symbionts are common on the surface membranes, in the cytoplasm and in the nuclei of the protists. The bacterial distribution pattern is species-specific in these protists. *Cryptotermes cavifrons* fed a mixture of penicillin and streptomycin over one month were compared to untreated termites. After four weeks of treatment, no *C. versatilis* or *Stephanonympha* remained; *S. tabogae* persisted as the only large protist present. Calcium-rich organic crystals of unknown composition accumulated in far greater quantities in the guts of the antibiotic-treated termites relative to controls. *Snyderella tabogae* cells from termites living in the original wood sample were 10 percent shorter and had 30 percent fewer nuclei than those fed paper with or without antibiotics or NaCl.

Keywords: Trichomonads, termites, endosymbiotic bacteria, gut ecology

## 1. Introduction

As documented by Harold Kirby (1941), many of the protist symbionts of wood-eating termites are closely associated with bacteria, on their cell surface,

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in their cytoplasm and in their nuclei. Apart from methanogens, which are easily detected by their coenzyme M-based blue autofluorescence, the epi- or endosymbiotic bacteria remain mostly uncharacterized. Certain bacteria are vital to the microbial ecology of the termite's gut: they remove hydrogen gas in the formation of methane or acetate, they fix nitrogen and ferment organic matter (Breznak and Brune, 1994). Termites can be fed antibiotics to remove bacteria, some of which blanket the protists (Radek et al., 1996; Grosovsky and Margulis, 1982; Dyer and Khalsa, 1993).

The kalotermitid *Cryptotermes cavifrons*, readily available in the southeastern coast of the United States, can be kept in its native wood for several years and for months on filter paper in a Petri dish in the laboratory. *C. cavifrons* will persist in small groups of 10–15. Fewer individuals molt into alates, the winged reproductive form, which harbor fewer symbionts, each year in the laboratory than do individuals of *Cryptotermes brevis*. Individuals of this latter species mature into alates at the rate of 90 percent or more each year under the same laboratory conditions, making them more difficult to work with for a longer period of time (personal observation).

Seven protist symbionts of the phylum Archaeprotista (Margulis and Schwartz, 1998) live in the hindgut of *C. cavifrons*: six trichomonads, *Snyderella tabogae* Kirby 1929, *Stephanonympha* sp., *Caduceia versatilis* d'Ambrosio 1999; *Foaina reflexa* Kirby 1942, a second *Foaina*, *Tricercomitus divergens* Kirby 1930, and a species of *Oxymonas* [Class Metamonada]. Personal observation confirmed the published record (Yamin, 1979) and records of the Kirby Collection, American Museum of Natural History. Because the smaller trichomonads, the two species of *Foaina* and *T. divergens*, and *Oxymonas*, are less amenable to quantification, this study was limited to the three large trichomonads: *C. versatilis*, *Stephanonympha* sp., and *S. tabogae*. *Oxymonas* attaches to the hindgut wall and the smaller trichomonads clump together rendering these four protist populations inaccessible to the sampling method employed here.

*C. versatilis*, family Devescovinidae, is a monomastigont trichomonad with a mean length of 108 μm and a mean width of 59 μm. It displays a noticeable rotation of its anterior region (Tamm and Tamm, 1976). It harbors two types of epibiotic bacteria, endonuclear bacteria, and at least one type of endocytoplasmic bacteria, located in a distinct region called the bacterial cup, as revealed by DNA-binding stains and electron microscopy (d'Ambrosio et al., 1999; Tamm, 1980).

*Stephanonympha* sp. is a multinucleate trichomonad, as are all genera of the Calonymphidae. Its nuclei are arranged in karyomastigonts, organelle systems in which each nucleus is connected to four undulipodia (flagella), a Golgi complex, and a microtubular shaft, called an axostyle, which extends to the posterior of the cell. The species in *C. cavifrons* has a mean length of 54 μm and

a mean width of 39 μm. It has a rounded anterior and contains a row of three to five bacteria inside or associated with each axostyle (Kirby and Margulis, 1994).

*Snyderella tabogae*, is also a calonymphid, however its 50 or more nuclei are dispersed, unattached in the cytoplasm. Its undulipodia are arranged in typical trichomonad fashion (in groups of four with a Golgi complex and axostyle) over the cortex of the cell, but lack a nuclear connection and are called akaryomastigonts (Kirby, 1929). The cell is pyriform and on average 109 μm long and 73 μm wide. While its surface is usually covered with an undescribed rod bacterium and it has spirochetes attached to its posterior, *Snyderella* does not have a standard complement of endosymbiotic bacteria.

*C. cavifrons* hindguts have been observed to contain 20–50 μm long, translucent, rhomboid-shaped crystals, dispersed in the lumen with the symbionts and wood particles (personal observation). These crystals have been seen on occasion inside cells of *Snyderella*, presumably phagocytized as would be a piece of wood. This study aimed to determine which bacteria and protist species were susceptible to penicillin and streptomycin, to record changes in populations of hindgut protists with a series of feeding regimens and antibiotics treatments, and to quantify the presence of and characterize the previously unreported crystals that accumulate in the hindgut of *C. cavifrons*.

## 2. Materials and Methods

*C. cavifrons* was collected in southern Florida and kept in its native wood in the laboratory for two and a half years. Termites were extracted from a single colony in the wood and divided into four groups of ten. One group was immediately sacrificed. The other groups were placed in small plastic Petri dishes (Corning 33mm) and kept at room temperature, approximately 25°C, for four weeks. The second group was fed several, small, untreated pieces of Schleicher and Schuall No. 595 filter paper. The third group was fed the same filter paper saturated with a solution of 10,000 units penicillin and 10 mg streptomycin per ml of 0.9 percent NaCl. The fourth group was fed the same paper saturated with 0.9 percent NaCl (to control for NaCl in the antibiotics solution). At the end of four weeks all termites in the dishes were sacrificed and their hindgut contents examined. Over 100 termites, not included in the controlled experiment, were fed the same antibiotic-treated paper.

Hindguts of four termites from each treatment were individually extracted and broken open in 0.6 percent NaCl. The number of translucent, 20–50 μm long, rhomboidal crystals, in the gut were counted in this wet sample at 40× under the stereo-dissecting microscope. The contents of the gut were then pipetted into 1 ml of 1.0 percent glutaraldehyde in phosphate buffered saline (PSA) and

fixed for 15 minutes. Cells were centrifuged at 9 × g for two minutes, washed once in distilled water, resuspended in water and stained with 2 μM 4,6-diamidino-2-phenylindole (DAPI) for 30 minutes. After washing with water and centrifuging again, the cells were allowed to settle to the bottom of the tube and were pipetted to a slide to make a wet mount. Coverslips (16 mm × 16 mm) were sealed with fingernail polish. Slides were examined using a Nikon Fluorophot microscope with the UV filter cube (365 nm). One slide was made for each termite. By counting the number of protist cells in a whole gut preparation and the number of cells present using this method it had been determined that this method results in a wet mount that contains approximately half the total protist population in a hindgut.

A slide, as prepared and examined above, under 200×, can be said to consist of approximately 20 nonoverlapping lateral rows, each with a field diameter of 700 μm. To avoid observer bias in sampling the distribution of protists in the gut, every other row of the slide was scanned and the number of cells of each species counted. Since this preparation method provided samples equal to half the number of cells in the gut, and since half the cells in the sample were counted, this number was multiplied by four to estimate the total number of cells of each species in the hindgut. To quantify the length of *Snyderella tabogae* the entire slide was scanned and every fifth cell showing the typical pyriform morphology was measured using an eyepiece micrometer and examined for bacterial symbionts and nuclei based on DAPI fluorescence.

Additional plates of ten termites each given the same feeding regimens as above (plain paper, paper/ NaCl, paper/NaCl/antibiotics) were set up for four weeks. The termites were then sacrificed, their hindguts were broken open and the pH measured with Alkacid Test Paper (Fischer Scientific Catalog No. A-980).

Gut crystals were obtained from a different set of termites fed antibiotics. Hindguts were broken open in 0.6 percent NaCl on a glass slide, gut tissue and most of the gut contents, including the protists, were removed by pipette and the crystals were allowed to dry on the slide. The slides were washed once with distilled water and allowed to dry. They were then coated with carbon and examined with the Cameca SX50 scanning electron microprobe.

## 3. Results

The three trichomonads are numerous in the wood-fed termites (Table 1). *Stephanonympha* from wood-fed termites were always packed with wood particles throughout their cytoplasm, right up to the anterior karyomastigonts (Fig. 1) and were tan-colored in contrast to *S. tabogae* and *C. versatilis* cells, which were more loosely packed and translucent as seen with the dissecting

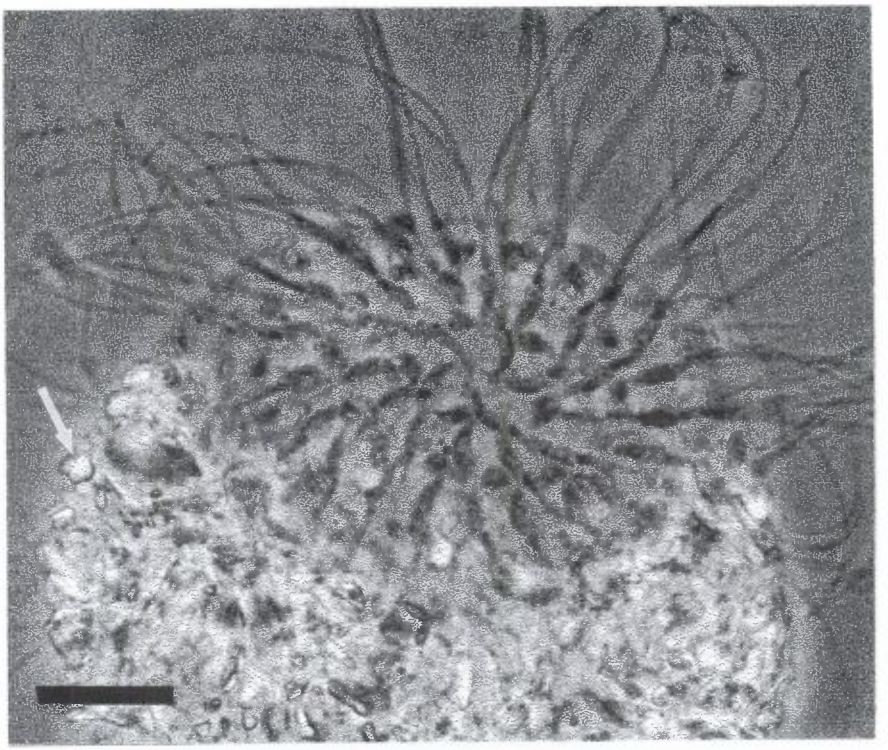

Figure 1. *Stephanonympha* sp. from *Cryptotermes cavifrons*. Note the cell is filled with wood particles (arrow). Phase contrast micrograph. Bar = 5 μm.

microscope. *C. versatilis* persisted in large numbers in the paper-fed termites. Two of the three trichomonads, *C. versatilis* and *Stephanonympha* sp., disappeared from the hindgut of the antibiotics-fed termites. The third trichomonad, *S. tabogae*, survived (Table 1). In over 100 antibiotics-fed termites, not included in the controlled experiment, *C. versatilis* was always absent. *Stephanonympha* was present at a rate of fewer than 10 cells per termite or none compared to the usual number of over 1000 per termite.

Also removed by the antibiotics were spirochetes and the long rod bacterium, which was usually attached by its tip to the posterior of *S. tabogae*. Most, if not all, of the small rods and coccoid bacteria from the surface of *S. tabogae* were also lost. Because *C. versatilis* and *Stephanonympha* sp. were removed by the antibiotics, I could not detect if their bacterial symbionts were differentially susceptible to the treatment. Free-swimming spirochetes were absent in the guts of antibiotic-fed termites.

Table 1. Number of large trichomonads and crystals in individual *Cryptotermes cavifrons* under four feeding regimens. Termites No. 9–12 were fed paper saturated with 0.9% NaCl. Termites 13–16 were fed paper saturated with antibiotics in 0.9% NaCl.

| | Treatment | *Snyderella* | *Stephanonympha* | *Caduceia* | Crystals |
|---|---|---|---|---|---|
| 1. | Wood only | 818 | 1046 | 300 | 0 |
| 2. | Wood only | 1014 | 1818 | 4726 | 0 |
| 3. | Wood only | 2468 | 1568 | 116 | 0 |
| 4. | Wood only | 436 | 2628 | 3500 | 1 |
| 5. | Paper only | 216 | 28 | 2060 | 0 |
| 6. | Paper only | 52 | 0 | 1264 | 120 |
| 7. | Paper only | 8 | 16 | 1976 | 0 |
| 8. | Paper only | 28 | 14 | 1944 | 73 |
| 9. | Paper/NaCl | 900 | 30 | 0 | 0 |
| 10. | Paper/NaCl | 464 | 8 | 0 | 104 |
| 11. | Paper/NaCl | 116 | 16 | 34 | 0 |
| 12. | Paper/NaCl | 792 | 0 | 208 | 10 |
| 13. | Paper/NaCl/ab | 16 | 0 | 0 | >200 |
| 14. | Paper/NaCl/ab | 138 | 0 | 0 | >300 |
| 15. | Paper/NaCl/ab | 650 | 0 | 0 | >200 |
| 16. | Paper/NaCl/ab | 266 | 0 | 0 | 100 |

Table 2. Length of *Snyderella tabogae* cells from *Cryptotermes cavifrons* under four feeding regimens. P values are from two-sample t tests, n=80 except for the antibiotics treatment where n=70.

| Treatment | Range (μm) | Mean | SD | SE | P 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|
| 1. Wood | 56–148 | 92 | 16.8 | 1.89 | 0.0001 | 0.012 | 0.001 |
| 2. Paper | 72–176 | 105 | 22.8 | 2.66 | | 0.075 | 0.288 |
| 3. 0.9% NaCl | 48–148 | 100 | 19.8 | 2.22 | | | 0.461 |
| 4. Antibiotics | 68–128 | 102 | 19.3 | 2.33 | | | |

Table 3. Nuclei per cell of *Snyderella tabogae* under four feeding regimens. P values are from two-sample t tests, n=80 except for the antibiotics treatment where n=70.

| Treatment | Range | Mean | SD | SE | P 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|
| 1. Wood | 12–55 | 35 | 9.5 | 1.06 | 0.0000 | 0.000 | 0.000 |
| 2. Paper | 21–120 | 58 | 20.2 | 2.26 | | 0.491 | 0.137 |
| 3. 0.9% NaCl | 17–125 | 60 | 22.3 | 2.49 | | | 0.041 |
| 4. Antibiotics | 17–105 | 53 | 22.3 | 2.66 | | | |

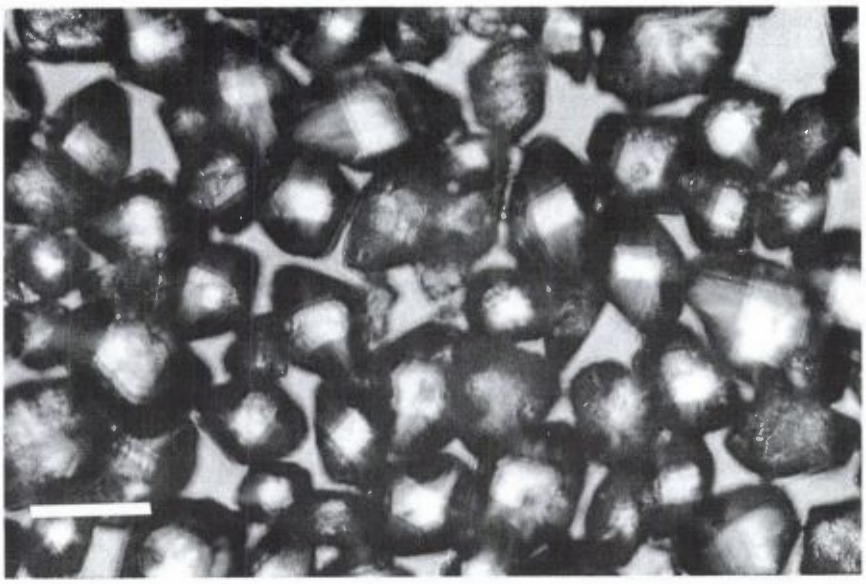

Figure 2. Rhomboidal crystals from *Cryptotermes cavifrons*. Brightfield micrograph. Bar = 40 μm.

The cell size of the wood-fed *Snyderella* was 10 percent shorter than that of cells fed under the other three, paper-based, regimens (two sample t-test, $p<0.05$) (Table 2). There was no difference in cell length between the three paper-fed groups. The nuclei per cell were 30 percent fewer (two sample t test, $p<0.01$) in the wood-fed termites than in those from the three paper-based treatments (Table 3). The number of nuclei per cell in *S. tabogae* between the three treatment groups that had been fed paper did not vary significantly.

Rhomboidal, translucent crystals, 20–50 μm long (Figs. 2 and 3), were found in all four treatment groups. They were most abundant in the antibiotics-fed termites (Table 1). Scanning electron microprobe analysis of these crystals (Fig. 4) indicated that they are rich in calcium. Because they did not dissolve in HCl, even after dipping in acetone, they are unlikely to be composed solely of standard $CaCO_3$. The hindgut pH of antibiotics-fed termites (n = 10) was slightly higher (7.5) compared to those not fed antibiotics (6.5).

## 4. Discussion

The devescovinid *C. versatilis* is the most sensitive of all the trichomonads in *C. cavifrons* to a mixture of penicillin and streptomycin in 0.9 percent NaCl.

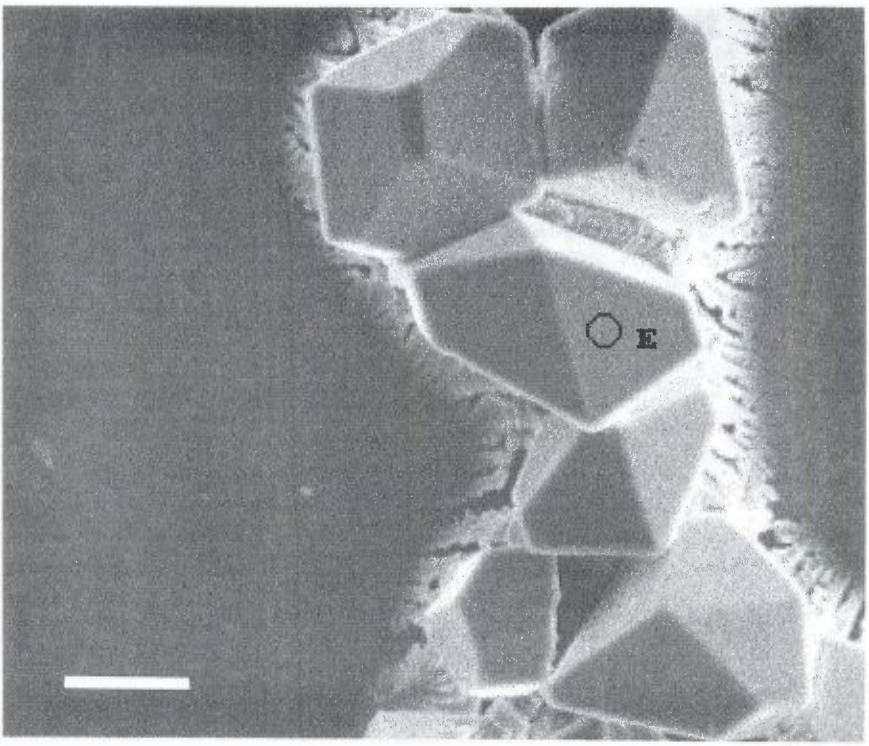


Figure 3. Rhomboidal crystals from *Cryptotermes cavifrons*. Scanning electron micrograph. Bar = 20 μm.

No cells of this trichomonad were present after four weeks of treatment. While *C. versatilis* was also affected by salt-treated paper, the complete absence of this species from over 100 antibiotic-fed termites suggests it is very sensitive to the antibiotics. These trichomonads harbor several morphotypes of bacteria (d'Ambrosio et al, 1999), including an epibiotic rod (a motility symbiont – its bacterial flagellar-based motility propels the trichomonad) (Tamm, 1982), an epibiotic fusiform bacterium, an undescribed type that forms the "bacterial cup" around *Caduceia*'s axostyle and intranuclear symbionts. One or more of these, at least four distinct symbiotic bacterial morphotypes, are apparently vital to *Caduceia*'s survival.

The *Stephanonympha* sp. found in *Cryptotermes cavifrons*, with a population of over 1000 cells per hindgut in wood-fed termites, is greatly reduced in all three groups of paper-fed termites. When freshly sacrificed termites from wood are examined, it is apparent that *Stephanonympha* cells are tan colored and are packed with wood particles in contrast to cells of

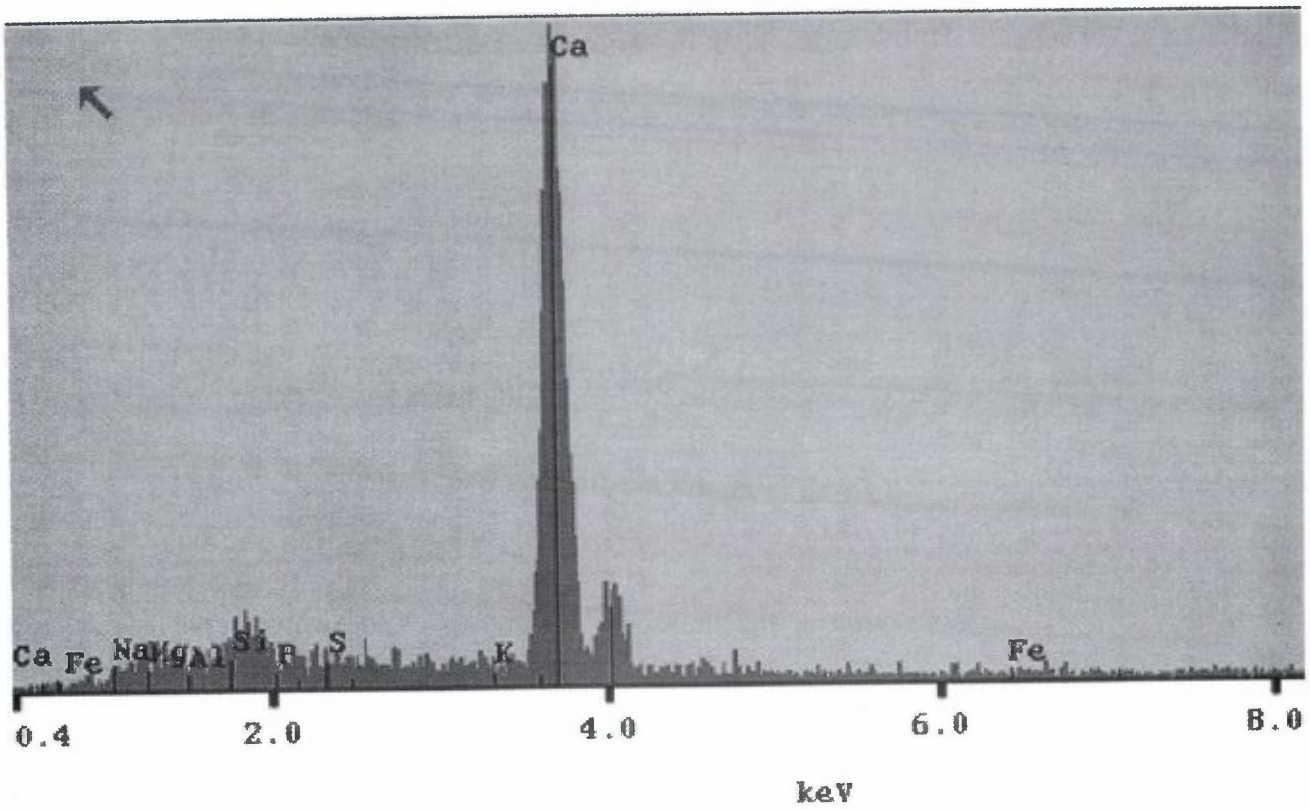


Figure 4. Elemental composition of *Cryptotermes cavifrons*' hindgut crystals. Scanning electron microprobe analysis.

*S. tabogae* and *C. versatilis*, which are more translucent and less densely packed. Since its population size declined so dramatically on paper in the absence of antibiotics, and on salt-treated paper, it cannot be concluded that the antibiotics affected *Stephanonympha*. These results are consistent with a requirement by *Stephanonympha* for other compounds in the wood beside cellulose.

*S. tabogae* was most resilient in the antibiotics-treated hindguts, surviving for at least four weeks while the other large trichomonads had been killed. Their length and number of nuclei per cell from the antibiotics-fed termites were no different from those of the other two paper-based termite regimens; however, the cells from all three groups fed paper were longer and had more nuclei per cell than did those fed wood. Since mitosing cells of *Snyderella* show synchronized division of all nuclei (Dolan et al., 2000), as in other calonymphids (Kirby, 1939; Kirby and Margulis, 1994), and since cytokinesis occurs infrequently in the termite gut protists, apparently associated with the termite's molting cycle (Andrew and Light, 1929) or with donation of proctodeal food to recently molted termites (Tamm and Tamm, 1980), there is no biological basis for increased frequency of karyokinesis over cytokinesis over this short period of time. However, budding, an unequal cytokinesis, which has been documented in another calonymphid, *Metacoronympha* (Dolan, 1999), would result in two different-sized offspring cells with different numbers of nuclei. The morphological differences between the wood- and paper-fed groups may then be due to the selection, for unknown reasons, of larger, more nucleated cells, and the death of smaller, less nucleated cells of *Snyderella*, under the paper-based regimens.

Many variables complicate an analysis of the symbioses of these cryptic social insects. All variables, which include caste within the colony, molting state of the individual, unknown nature of food consumed by protist species, unknown metabolism of protists, and unknown symbiotic bacteria and metabolism in and on the protists, cannot be controlled. While some of these questions will be answered, such as identification of bacterial symbionts by *in situ* DNA hybridization, a coherent account of the hindgut microbial community requires broader investigation of physiology, morphology and community ecology. Nevertheless, these hindgut microbes can be used to examine important questions of microbial evolution including the nature of cellular symbioses, and the origin of asexual species.

There have been no previous reports of the formation of these calcium-rich crystals in termites. Mound-building termites (Termitidae) have been shown to accumulate calcium carbonate at greater concentrations in their mounds than in surrounding soils (Pendleton, 1942). These termites do not contain the cellulose-digesting protists. They eat soil, so it is likely that the excess calcium in their mounds comes from the soil. Calcium-rich mineral precipitation in arthropod

digestive systems has been reported in an homopteran (Gouranton, 1968), a collembolan (Humbert, 1978), and crustaceans (Hopkin and Nott, 1979; Meyran et al., 1986), but in all cases these were submicron-sized, intracellular spherical concretions. These minerals were thought to function in excretion (Gouranton, 1968), detoxification (Humbert, 1978; Hopkin and Nott, 1979), and storage (Meyran et al., 1986).

Since the crystals were most numerous in the guts of termites that had lost one or more genera of protist symbionts, it is likely that the calcium, and perhaps other material in the crystals, came from the decomposed protist cells. The protists' death may have also caused the pH of the hindgut to rise slightly, since it is thought that the termites live off the volatile fatty acid waste-products of the cellulose fermentation (Odelson and Breznak, 1983). This could have led to the greater precipitation of the calcium-containing mineral, which is most likely calcium carbonate. Although the crystals did not dissolve in HCl, they may still contain calcium carbonate bound up in organic matter, which would make them difficult to dissolve. Since the crystals are found in untreated, wood-eating termites, they may be a normal physiological product involved in calcium excretion, detoxification or storage.

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## REFERENCES

Andrew, B.J. and Light, S.F. 1929. Natural and artificial production of so-called 'mitotic flares' in the intestinal flagellates of *Termopsis angusticollis*. *University of California Publications in Zoology* **31**: 433–440.

Breznak, J.A. and Brune, A. 1994. Role of microorganisms in the digestion of lignocellulose by termites. *Annual Review of Entomology* **39**: 453–487.

d'Ambrosio, U., Dolan, M., Wier, A.M., and Margulis, L. 1999. Devescovinid trichomonad with axostyle-based rotary motor ("Rubberneckia"): Taxonomic assignment as *Caduceia versatilis* sp. nov. *European Journal of Protistology* **35**: 327–337.

Dolan, M.F. 1999. Amitochondriate protists: Symbiotic trichomonads of dry-wood-eating-termites. Dissertation. University of Massachusetts, Amherst, Amherst, MA. 88p. Available on microfilm from Bell and Howell Information and Learning, Accession Number 9932306.

Dolan, M.F., d'Ambrosio, U., Wier, A.M., and Margulis, L. 2000. Surface kinetosomes and disconnected nuclei of a calonymphid: Ultrastructure and evolutionary significance of *Snyderella tabogae*. *Acta Protozoologica* (in press).

Dyer, B. and Khalsa, O. 1993. Surface bacteria of *Streblomastix strix* are sensory symbionts. *BioSystems* **31**: 169–180.

Gouranton, J. 1968. Composition, structure, et mode de formation des concrétions minérales dans l'intestin moyen des homoptéres cercopides. *Journal of Cell Biology* **37**: 316–328.

Grosovsky, B. and Margulis, L. 1982. Termite microbial communities. In: *Experimental Microbial Ecology*. R.G. Burns and J. H. Slater, eds. Blackwell, Oxford, pp. 519–532.

Hopkin, S.P. and Nott, J.A. 1979. Some observations on concentrically structured, intracellular granules in the hepatopancreas of the shore crab *Carcinus maenas* (L.). *Journal of the Marine Biological Association, UK* **59**: 867–877.

Humbert, W. 1978. Cytochemistry and x-ray microprobe analysis of the midgut of *Tomocerus minor* Lubbock (Insecta, Collembola) with special reference to the physiological significance of the mineral concretions. *Cell and Tissue Research* **187**: 397–416.

Kirby, H. 1929. *Snyderella* and *Coronympha*, two new genera of multinucleate flagellates from termites. *University of California Publications in Zoology* **31**: 417–432.

Kirby, H. 1939. Two new flagellates from termites in the Genera *Coronympha* Kirby, and *Metacoronympha* Kirby, new genus. *Proceedings of the California Academy of Sciences* **22**: 207–220.

Kirby, H. 1941. Organisms living on and in protozoa. In: *Protozoa in Biological Research*. G.N. Calkins and F.M. Summers, eds. Hafner, New York, pp. 1009–1113.

Kirby, H. and Margulis, L. 1994. Harold Kirby's symbionts of termites: Karyomastigont reproduction and calonymphid taxonomy. *Symbiosis* **16**: 7–63.

Margulis, L. and Schwartz, K.V. 1998. *Five Kingdoms*. 3rd ed. W.H. Freeman, New York.

Meyran, J.-C., Graf, F., and Nicaise, G. 1986. Pulse discharge of calcium through a demineralizing epithelium in the crustacean *Orchestia*: Ultrastructural cytochemistry and x-ray microanalysis. *Tissue & Cell* **18**: 267–283.

Odelson, D.A. and Breznak, J.A. 1983. Volatile fatty acid production by the hindgut microbiota of xylophagous termites. *Applied and Environmental Microbiology* **45**: 1602–1613.

Pendleton, R.L. 1942. Importance of termites in modifying certain Thailand soils. *Journal of the American Society of Agronomy* **34**: 340–344. Cited in Snyder, T.E. 1956. Annotated, subject heading bibliography of termites, 1350 B.C. to A.D. 1954. Smithsonian Miscellaneous Collections 130. Smithsonian Institution, Washington, DC.

Radek, R., Rösel, J., and Hausmann, K. 1996. Light and electron microscopic study of the bacterial adhesion to termite flagellates applying lectin cytochemistry. *Protoplasma* **193**: 105–122.

Tamm, S.L. 1980. The ultrastructure of prokaryotic-eukaryotic cell junctions. *Journal of Cell Science* **44**: 335–352.

Tamm, S.L. 1982. Flagellated ectosymbiotic bacteria propel a eucaryotic cell. *Journal of Cell Biology* **94**: 697–709.

Tamm, S.L. and Tamm, S. 1976. Rotary movements and fluid membranes in termite flagellates. *Journal of Cell Science* **20**: 619–639.

Tamm, S. and Tamm, S.L. 1980. Origin and development of free kinetosomes in the flagellates *Deltotrichonympha* and *Koruga*. *Journal of Cell Science* **42**: 189–205.

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.