

## Endospore-Forming Filamentous Bacteria Symbiotic in Termites: Ultrastructure and Growth in Culture of *Arthromitus*

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### Abstract

Many morphologically distinguishable filamentous spore-forming bacteria symbiotic in the paunch (hypertrophied hindguts) of wood-eating insects have been seen since *Arthromitus* was first described and named as a plant by Leidy in 1850. Previous descriptions were inadequate for acceptance of the group in modern bacteriological literature.

Twenty-two distinguishable arthromitids in nine different arthropod hosts are recorded on the basis of microscopic studies. Five are named, including two whose ultrastructure are detailed: *Arthromitus chasei* sp. nov. that lives in the damp wood-eating termite *Zootermopsis angusticollis* (from the west coast of North America) and *Arthromitus reticulitermitidis* sp. nov. from the subterranean west coast termite *Reticulitermes tibialis*. *A. pterotermitidis* from the desert termite *Pterotermitidis occidentis*; *A. zootermopsidis*, also from *Z. angusticollis*; and *A. cristatus* (Leidy, 1881) from *Reticulitermes flavipes* of eastern North America are also named here. Characterized by trichomes that show a morphogenetic sequence from no spores through immature spores to mature spores with spore filaments, *Arthromitus* symbionts can be identified as members of the genus by light microscopy and habitat. Electron microscopy reveals their remarkable complexity. They attach by spore filaments to various objects including the host gut wall; their maturation extends distally toward the termite lumen. By surface sterilization of the termite, maceration of the paunch, exposure to boiling temperatures and plating on soft acetate agar, the heat resistant

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nature of the spores and facultatively aerobic nature of *Arthromitus* sp. (from *Zootermopsis*) was demonstrated.

Keywords: anthrax, *Arthromitus*, *Arthromitus chasei*, *Arthromitus cristatus*, *Bacillus anthracis*, arthromitids, bacterial gut symbionts, branched filamentous bacteria, *Coleomitus*, endospores, facultative anaerobic symbionts, multicellular bacteria, spore attachment filaments, spore filaments, termites

## 1. Introduction

Writing about "the extraordinary parasitic community of the Termites thus accidentally discovered," Joseph Leidy (1881, p. 425) claims "I was led to the literature concerning these insects; but I found no description of the parasites." Leidy describes the genus he named as "delicate filamentous plants, found growing within the intestine of certain myriapods" (*Spirobolus marginatus* and *Polydesmus virginienensis*) and beetles (*Passalus cornutus*). The genus name *Arthromitus* derives from the Green *arthron*, a joint, and *mitos*, a thread. Leidy's description of *Arthromitus cristatus* from the subterranean termite from the east coast of North America, *Termes flavipes* (now renamed *Reticulitermes flavipes*), is fully consistent with our own observations (Fig. 1) except, of course, for the fact that he is referring to plants and not bacteria. His description of the genus is as current today as it was then:

"The characters of the genus *Arthromitus* are as follows: Plant [sic] in the form of exceedingly fine delicate filaments, usually attached by an attenuated extremity and growing isolated or in small divergent groups. Filaments always simple, cylindrical, of uniform diameter, homogeneous, and inarticulate, or more or less distinctly articulate, with the free end slightly expanded or narrowed, and rounded or truncated. Articuli mostly cylindrical, with little difference of length and breadth, sometimes feebly keg-shaped, homogeneous. Spores mostly in a series occupying the distal articuli, always single, oval or oblong, darkly outlined, translucent and homogeneous." (Leidy, 1881, p. 442)

In describing the particular example from his New Jersey termites, he explained that the trichomes

"are usually observed loose, and have been probably detached from their points of growth. When attached, they appear to spring singly or in groups of several together from a granule or minute round disk adherent to the epithelium of the intestine." (Leidy, 1881, p. 442)

The "minute round disk" can be easily inferred in Fig. 2: it is actually a space caused by convergence (c) of the basal ends of the *Arthromitus* trichomes when they attach to depressions in the chitinous wall of the termite intestine. Indeed,

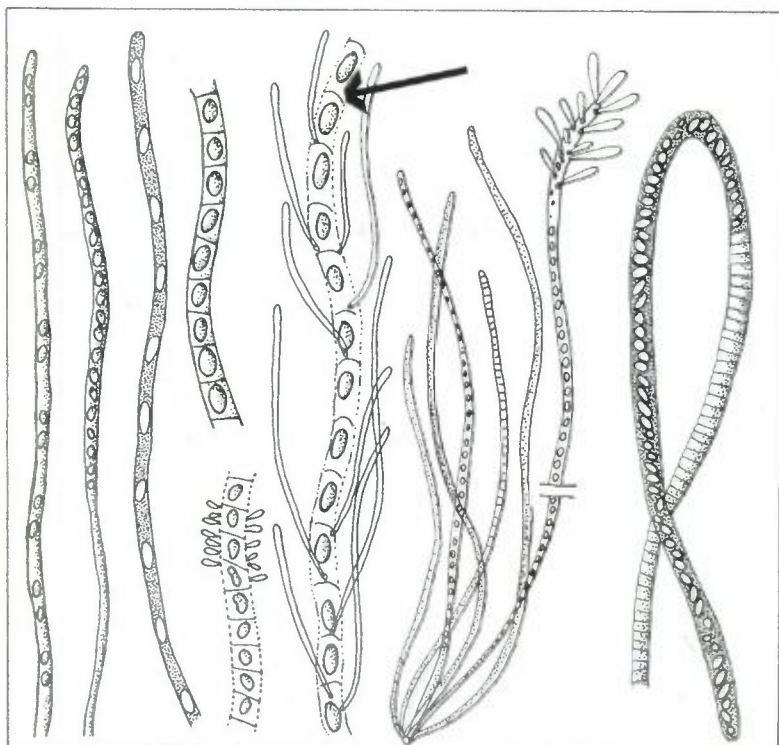


Figure 1. *Arthromitus* sp. redrawn from Leidy (1881; Figs. 32-38 on plate 52). *A. cristatus* (arrow)

we have observed Leidy's "*Arthromitus cristatus*" on numerous occasions in worker and soldier termites of *Reticulitermes flavipes* (both from Woods Hole, Massachusetts and Gulfport, Mississippi) just as depicted in his figures 28-32 reproduced here (Fig. 1). Furthermore, we have seen the same type of organism in many other termites and wood-eating insects. About these symbionts Starr and Skerman wrote,

"Nothing has appeared in recent literature on these genera [*Arthromitus*, *Coleomitus*] of intestinal parasites. As Pringsheim (1959) has remarked, 'it is difficult to assess the taxonomic relationships between the forms observed by the various authors.' This is likely to remain the case until further studies are initiated on these endosporegenous organisms."

(Starr and Skerman, 1965, p. 429)

Reference to the early literature was carried forward, e.g., *Arthromitus* appears in *Bergey's Manual*, eighth ed., 1974, Buchanan and Gibbons, eds., p. 531. In



Figure 2. Point of attachment (c) of clump of *Arthromitus* trichomes (t) in an intestinal pit (ip) illustrating Leidy's statement. Chitinous wall (ch) of termite, spirochetes (s) and a portion of an unidentified protist (p) can be seen in this electron micrograph of the hindgut of *Reticulitermes* from California. Bar = 5  $\mu$ m.



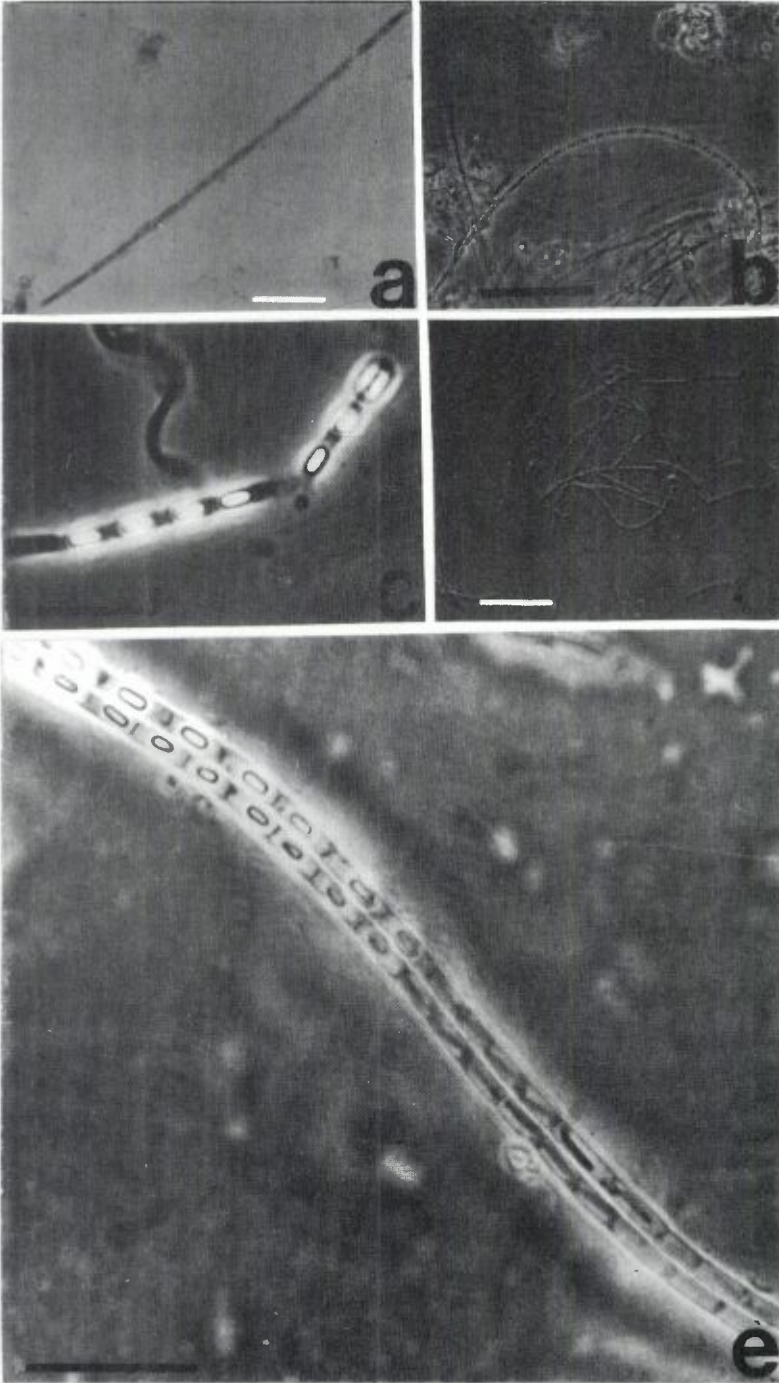
the most recent edition of *Bergey's*, they were placed under "Genera Incertae Sedis":

"The response of all these organisms to oxygen, their physiology and fine structure of their endospores is still unknown...their resistance to environmental stresses has not, however, been investigated." (Sneath, 1986, p. 1138)

No further study has been made of *Arthromitus* since Leidy's original description. The lack of recent work is our explanation for the absence of any reference to *Arthromitus* as a symbiotic filamentous spore former in the comprehensive compendium of bacteria, *The Prokaryotes* (Starr, et al., eds., 1981). Here, we resuscitate interest in these "arthromitids" by describing the symbiotic status and structure of two newly described *Arthromitus* species from microbial hindgut communities of subterranean and dampwood termites from the western United States. This description provides necessary background for the formal introduction into the modern bacteriological literature of the "Arthromitaceae."

The life history and ultrastructure of *Arthromitus*-like organisms symbiotic in the guts of mice and rats were beautifully described by Chase and Erlandsen (1976). Arthromitids from domestic ducks were also described (as *Anisomitus denisi* by Grassé, 1925). Those associated with the intestinal wall of fowl (unnamed by Fuller and Turvey, 1971) as well as the variants described in the drywood termite *Calotermes* sp. from Loyalty Island termites (named *Coleonema pruvoti* by Duboscq and Grassé, 1929) all seem to be related bacteria. Microbes variously labeled *Coleomitus*, *Arthromitus* or *Anisomitus* in insect hindguts are arthromitids deserving modern study. Certain intestinal symbionts of guinea pigs (Fig. 3e) and even many bacilli, we will show, are probably arthromitids. Arthromitids, by definition filamentous endospore forming bacteria symbiotic in the intestines of animals, have far more complexity than members of the genus *Bacillus*.

The profound changes wrought by bacteriologists both in concepts of nomenclature and in construction of higher taxa since Leidy described these "plants" render imperative a comprehensive taxonomic treatment of this huge group of symbiotic bacteria. For publication of new genera and species names in the international *Journal of Systematic Bacteriology* (manuscript in preparation), characterization of several species is required. Although standard microbial identifications are underway, we believe that this large group of filamentous spore-forming *Arthromitus*-like bacteria, with their complex developmental cycles, deserves to be better known to biologists, especially those interested in symbiotic associations. Arthromitids comprise a group of normal bacterial



← Figure 3. *Arthromitus* light micrographs

a. *Arthromitus* sp. from *Kalotermes schwartzi*, Southern Florida. Stained with Leifson's spore stain showing distribution of spores. Brightfield microscopy. In a, b, d, bar = 10  $\mu$ m.

b. The smaller *Arthromitus* sp. from *Pterotermes occidentis*, drywood Sonoran desert termite from Arizona in which the spores are symmetrically distributed apparently in pairs (see Fig. 8c). Phase contrast microscopy.

c. The larger *Arthromitus pterotermitidis* from *Pterotermes occidentis* in which the centrally located oval spores are evenly distributed. Phase contrast microscopy. Bar = 5  $\mu$ m.

d. *Arthromitus* sp. from *Zootermopsis angusticollis* cultivated in "sloppy agar." Differential interference contrast microscopy.

e. *Arthromitus*-like filamentous spore-forming bacterium from guinea pig caecum. Note the sequence of spore maturation: from generally-attached proximal portion lacking spores (lower right) to the intestinal lumen (generally distal, upper left) containing mature spores. Bar = 5  $\mu$ m.

symbionts. All members of the genus are morphologically modified from standard bacilli by their intimate association with the intestinal tissues of their host animals.

In their pioneer work on mammalian arthromitids, Chase and Erlandsen (1976) showed that these intestinal bacteria (very similar to our termite symbionts) are not *Streptobacillus moniliformis* (agents of rat bite fever), nor as Reimann (1965) had claimed, are they fungi. Rather, Chase and Erlandsen concluded that the spore and holdfast-forming filamentous bacteria in the ileum of mice and rats are "members of the Arthromitaceae, segmented spore-formers described many years ago from the intestines of certain arthropods and amphibians." These intestinal bacteria are absent from fetuses but appear in young rats and mice only 2-3 weeks after birth which suggests "the existence of a microbial stage capable of surviving outside the host" (Chase and Erlandsen, 1976, p. 581). Their data imply the existence of functionally resistant spores. Our goal is to document their conclusions by showing that the structures appearing to be spores inside the trichomes of the symbionts are indeed heat-resistant bacterial endospores.

## 2. Materials and Methods

### Collection

Termites and other wood-eating animals were either collected from fallen logs or they were obtained from colleagues. The sources and identifications of the animals are listed in Table 1.

Table 1. Occurrence of *Arthromitus*-like bacteria in arthropods.

Host animal	Location Animal identified by	Source Comments
<b>ARTHROPODA</b>		
<b>Blattaria (wood-eating cockroach)</b>		
<i>Cryptocercus punctatus</i>	Virginia, USA/ L.R. Cleveland	in 16 mm films (unpublished)*
<b>Isoptera (termites)</b>		
<b>Drywood</b>		
<i>Mastotermes darwiniensis</i>	Darwin, Australia/ L.R. Cleveland	in 16 mm films (unpublished)
<i>Pterotermes occidentis</i>	Arizona, USA W. Nutting	To et al., 1980 and this paper
<i>Calotermes**</i> sp.	Loyalty Islands, Pacific/O. Duboscq and P.-P. Grassé	called <i>Coleomitus</i> Duboscq and Grassé, 1929
<i>Kalotermes approximatus</i>	Florida, USA/ P. Luykx	L. Margulis (unpublished)
<i>Kalotermes schwarzi</i>	Florida, USA/ P. Luykx	L. Margulis (unpublished)
<i>Kalotermes minor</i>	California, USA/ D. Chase	L. Margulis (unpublished)
<i>Kalotermes flavicollis</i>	N.E. Spain, S. France/ A. Escarré	L. Margulis (unpublished)
<i>Kalotermes praecox</i>	Island of Madeira/ A. Hollande	A. Hollande, I. Gharozoglou (unpublished)
<b>Dampwood</b>		
<i>Zootermopsis angusticollis</i>	Friday Harbor, Washington, USA/ A. Stuart	B.A. Afzelius (this paper)
<i>Zootermopsis nevadensis</i>	California, USA/ D. Chase	D. Chase*
<b>Subterranean</b>		
<i>Reticulitermes flavipes</i>	Philadelphia, New Jersey, USA/J. Leidy	Leidy, 1850, 1861
<i>Reticulitermes hesperus</i>	California, Nevada/ D. Chase	Chase (this paper)
<i>Reticulitermes tibialis</i>	California/D. Chase	Chase (this paper)
<i>Coptotermes formosanus</i>	Honolulu, Hawaii/ J. Atema	To et al., 1980
<b>Coleoptera (beetles)</b>		
<i>Polydesmus</i> (unicorn wood-beetle)	Philadelphia, New Jersey, USA/J. Leidy	Leidy, 1881



Table 1. (Cont.)

Host animal	Location Animal identified by	Source Comments
<b>MYRIAPODA</b>		
<i>Spirobolus</i> centipede (unidentified)	Philadelphia, USA Woods Hole, MA, USA	Leidy, 1881 J. Buckley*
<b>CRUSTACEANS</b>		
<b>Isopoda</b>		
sow-bug (unidentified)	Woods Hole, MA, USA	D. Forest*

\* 16 mm film, video or micrographs on file, with L. Margulis.

\*\* *Calotermes* ≡ *Incistermes*; (Krishna, 1961) in the French literature is spelled *Kalotermes* in English or German

### *Light microscopy*

The insects were surface sterilized by dipping in absolute ethanol for a minute or two. Their intestines were then removed using two sets of forceps under a Nikon dissecting microscope equipped with laser illumination. The insects were held behind the head with coarse forceps while gentle pressure was applied with fine forceps to the protruding hindgut. Whole intestines were then placed in several drops of salt solution, either insect Ringer's or Trager's solution (To et al., 1978) and punctured to allow the escape of the symbionts. The filamentous spore-formers tend to comprise a conspicuous portion of the hindgut community and are visible upon inspection of the community at high power with phase or differential interference contrast optics. Light-micrographic images of the organisms were photographed either live or after staining with Leifson's spore stain as described in To et al. (1978).

### *Cultivation*

Spore formers were isolated onto soft agar plates using the following procedure. Whole hindguts from five or six termites were collected into a testtube of insect Ringer's solution and placed into a small mortar and pestle for grinding. After thorough grinding, gut material was suspended in fresh salt solution and the tubes were placed for 8–10 min in a water bath. Upon cooling to about 60°C, samples were inoculated onto the center of plates containing the medium indicated (Table 2) and incubated at either 31 or 38°C. Some plates were incubated anaerobically either in Brewer jars with nitrogen in the gas phase or in a Coy glove box with 7% H<sub>2</sub>, 13% CO<sub>2</sub> and 80% N<sub>2</sub>. Since vigorous overnight

Table 2. "Sloppy agar" growth medium

Component	% Quantity (wgt)
yeast extract	0.5
Bactopeptone (not Difco or proteose)	0.5
sodium acetate	0.01
agar	0.2

Procedure: The first three ingredients are dissolved in water and the pH adjusted to 7.4-7.6 with NaOH. Agar is then added and the medium is autoclaved for at least 20 min. Inoculate with *Arthromitus* spore suspension after boiling.

Anaerobic growth: Medium on plates or tubes incubated in Coy glove box with H<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub> in gas phase.

Transfer: May be transferred as spores or growing cultures, biweekly or weekly as preferred.

growth occurred on both anaerobically incubated plates and those exposed to air, further attempts to ensure strict anaerobiosis were limited to experimental protocol.

#### *Electron microscopy*

Entire hindguts were fixed in glutaraldehyde, post-fixed in osmium, sectioned and examined as described in To et al. (1978) and Chase and Erlandsen (1976).

### 3. Results and Discussion

#### *Species of Arthromitus*

Various *Arthromitus* sp. from south Florida and Sonoran desert termites are depicted in the light micrographs of figures 3a-c. Live *Arthromitus* was photographed from the sources described (Figs. 3b-e). Several distinguishable types of filamentous spore formers are seen in each termite, including two described earlier in our study on the hindgut community of the Sonoran desert termite (To et al., 1978). Measurements of the spore size and width of various *Arthromitus* specimens confirm our visual assessment of the presence of at least two distinct species in *Zootermopsis angusticollis*, one of which is *A. chasei* (Table 3). At least two different types are present in each *Reticulitermes* (data not shown; but see Figs. 7,8). *Arthromitus*, as long trichomes, tend to extend distally from the chitinous wall toward the lumen of the intestine (Fig. 3e).

Table 3. Measurements of *Arthromitus* from video light microscopy of *Zootermopsis angusticollis*

	Measurements ( $\mu\text{m}$ )	Organism
trichome width	1.1 + 0.08 (n=7)	<i>Arthromitus chasei</i>
spore length	2.9 + 0.41 (n=7)	
trichome width	2.6 + 0.17 (n=6)	<i>Arthromitus zootermopsidis</i>
spore length	3.7 + 0.29 (n=6)	
	6.9 + 0.60 (n=3)*	

\* Trichomes of *A. zootermopsidis* contain spores in two size classes. The larger spores are slender and oval in shape and may represent early stages of spore development. Measurements were taken from a video display of differential interference contrast light microscopic images taken with 40, 60 and 100 $\times$  objectives on a Nikon Microphot microscope.

Another species of *Arthromitus* may be present based on size and morphology but the above mentioned species comprise the most conspicuous and abundant size of classes of arthromitids in *Zootermopsis angusticollis*.

Here we describe two morphotypes, from different genera of termites. Two of the types of *Arthromitus* from *Zootermopsis* are in culture. Because the morphology is so distinctive and the presence of the bacterium in the animal intestine of an identifiable host so predictable, physiological characterization (which will form the basis of a future publication), is not required for identification of the following five species.

#### *Arthromitus cristatus* (Leidy, 1881)

Symbiotic spore-forming bacteria with characteristics of the genus from *Reticulitermes flavipes* from the east coast of North America, Fig. 1, arrow. Central spores; trichomes tend to have this filamentous symbiotic bacteria attached at joints. The other arthromitids depicted (e.g., Leidy's "*Arthromitus intestinalis*" and others in Fig. 1) require modern study prior to naming.

#### *Arthromitus chasei* sp. nov. (Figs. 4a-d, 5a-c, 6)

Named in memory of David G. Chase, American morphologist who performed ultrastructural studies of these insect symbionts and recognized their relation to mammalian gut inhabitants. Symbiotic filamentous spore-forming bacterium with characteristics of the genus (Leidy, 1881). From paunch (hyper-trophied hindgut) of the dampwood termite, *Zootermopsis angusticollis*, from Friday Harbor, Washington, USA. The individual cells of the millimeter-long



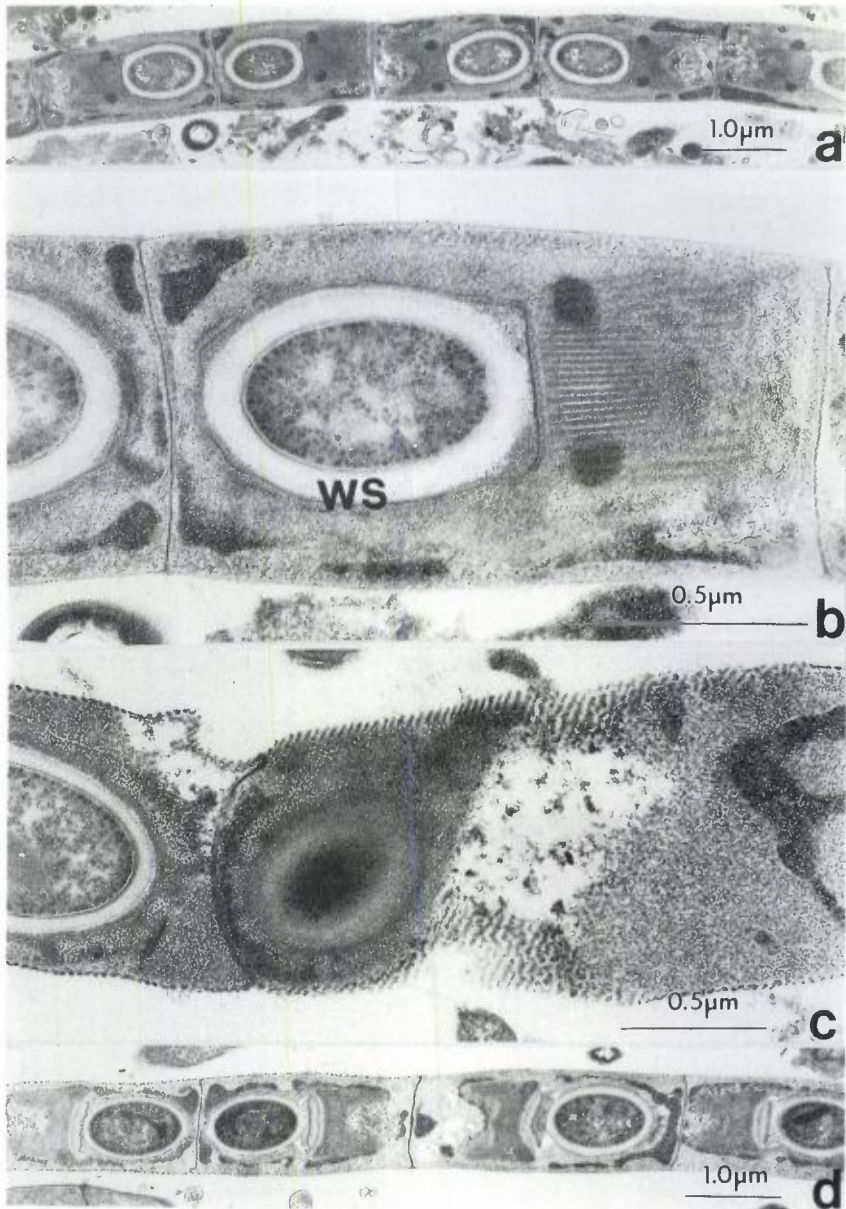


Figure 4. *Arthromitus chasci* from *Zootermopsis angusticollis* (type species)  
 a. Characteristic appearance of a trichome.  
 b. Parental cell with its walled spore (ws) with a smooth cell wall to which are attached spore filaments.  
 c. The parental wall, tangentially cut, is ornamented by ridges that extend helically around the cell.  
 d. An exceptional trichome in which two adjacent cells contain mature spores which have tandem polarity rather than the usual antiparallel (mirror plane symmetry) orientation. (e.g., Figs. 4a,b and left portion of 4d).



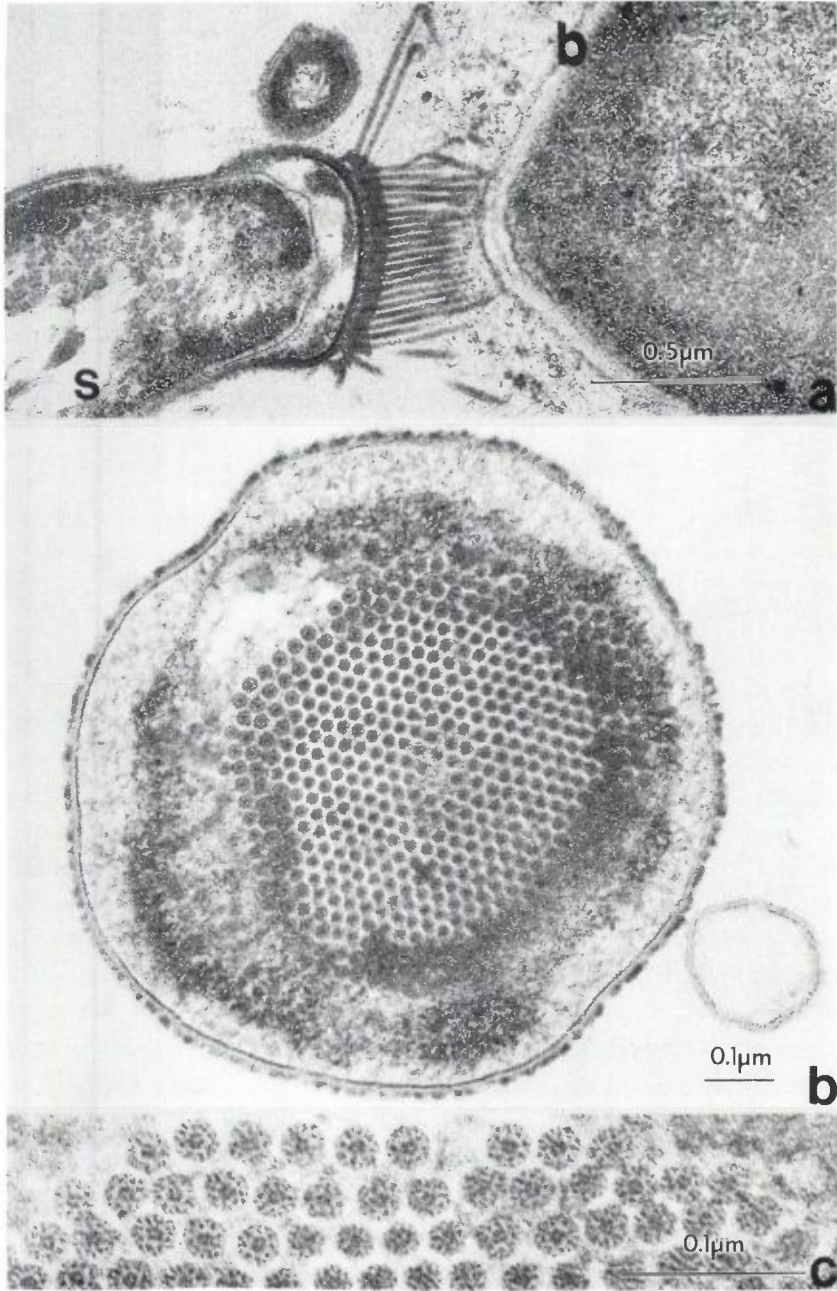


Figure 5. Spore filaments of *Arthromitus chasei*.

- a. A germinated spore (s) which was released from its parent cell and attached by filaments to another bacterium (b).
- b. *Arthromitus chasei* section that reveals over 400 spore filaments in transverse section.
- c. Around a dense center each filament has electron-dense and electron-translucent spots arranged in an 8-fold symmetrical array; higher magnification of a portion of the cell shown in 5b.

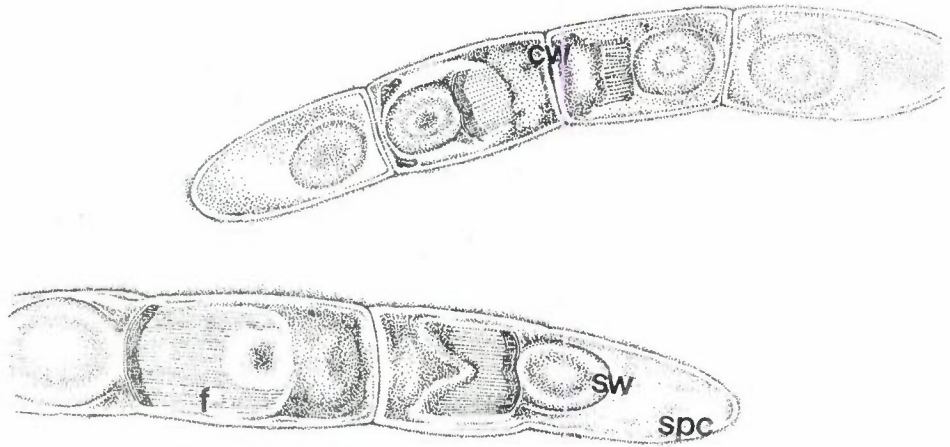


Figure 6. *Arthromitus chasei* from *Zootermopsis angusticollis*, showing salient features of the genus: trichomes with cross walls (cw), spore wall (sw), spore attachment filaments (f), nucleoid (n), spore parent cell (spc). Drawing by J. Steven Alexander.

trichomes are  $1\ \mu\text{m}$  wide and approaching  $3\ \mu\text{m}$  in length. In electron micrographs, the spore when contained in a cell, is close to  $1\ \mu\text{m}$  long (the  $0.1\ \mu\text{m}$  thick spore wall included) and  $0.5\ \mu\text{m}$  wide. The spore wall is rather electron-transparent although its outer  $10\ \text{nm}$  appears electron dense. Filaments which extend from the base of the thick wall in the spore parent cell were identified by David Chase (1934–1987) as spore filaments. Over 400 spore filaments, each  $0.6\ \mu\text{m}$  long and  $20\ \text{nm}$  thick, are attached to each spore (Fig. 5b). An 8-fold symmetry can be seen in cross-sections of the spore filament (Fig. 5c), in contrast, for example, to the 13-fold symmetry of eukaryotic microtubules. The spore filaments retain their attachment to the spore after the parent cell disintegrates. The wall, especially the electron-translucent portion, is largely dissolved in the released spores, although the spore filaments retain a base plate that is somewhat separate from the plasma membrane of the spore. Spore filaments probably have adhesive properties. Filaments on released spores often attach at their distal ends to bacteria or to other items (Fig. 5a).

The individual cells of some trichomes have smooth cell walls (Figs. 4a,b). The cell wall of others has been cut such that striations of some material which appears to be helically arranged around the cell are revealed (Fig. 4c). Only on a few occasions do adjoining cells seem to be oriented in tandem rather than with opposite polarity as determined by the position of spores and spore filaments (Fig. 4d). At least two *Arthromitus* species were distinguished in electron micrographs and videotapes of live material on the basis of size classes

(Table 3). *A. chasei* is the thinnest trichome with asymmetrically distributed spores. Probably three morphotypes co-exist in these termites.

*Arthromitus pterotermitidis* (To)

This organism, the larger of the *Arthromitus* types from the Sonoran desert termite, was described in To et al. (1978, her Figs. 9–10). The large centrally located spore nearly fills the parent cell (Fig. 3c), making the organism easily distinguishable from the second smaller *Arthromitus* with asymmetrically distributed spores (Fig. 3b). Sonoran arthromitids are often associated with a surface investment of rod bacteria originally depicted by Leidy (Figs. 1, 7a,b).

*Arthromitus reticulitermitidis* sp. nov. (Figs. 7c,8b–d)

Symbiotic filamentous spore-forming bacteria with characteristics of the genus (Leidy, 1850, 1881). From the hypertrophied gut of the southern California termite *Reticulitermes tibialis*. Spores, spherical to ovoid, measure approximately 3  $\mu\text{m}$  in length by 0.5  $\mu\text{m}$  in diameter. In longitudinal section, a mature spore is seen to have two prominent posteriorly oriented protrusions which may be a collar in three dimensions. More than 300 hollow filamentous attachment fibers are found per mature spore.

*Arthromitus zootermopsis* sp. nov.

Symbiotic filamentous spore-formed bacteria with characteristics of the genus from *Zootermopsis angusticollis*. Trichomes, approximately 2.6  $\mu\text{m}$  in diameter, bear evenly distributed spores. Spores are 3.5  $\mu\text{m}$  long and 1.7  $\mu\text{m}$  wide, measurements derived from video light microscopy of live material. Surface-associated rod shaped bacteria commonly seen especially at the distal ends of the trichomes. A very similar arthromitid has been observed in *Reticulitermes hesperus* (Fig. 7a,b).

*Cultivation of Arthromitus from Zootermopsis*

Inoculation of the heat resistant spores in suspension led to the appearance, within 24–28 hr after incubation, of four or five morphotypes of spore-forming bacteria on sloppy agar plates.

The *Arthromitus* sp. colonies in culture tend to be gelatinous, orangish and diffuse. Many termite bacilli and perhaps other spore formers may be isolated from hindgut by these methods. *Arthromitus* may be identified by their distinctive morphology. The length of the trichomes tend to be shorter in aerobically cultured bacteria than microbes observed either in anaerobic culture or in the



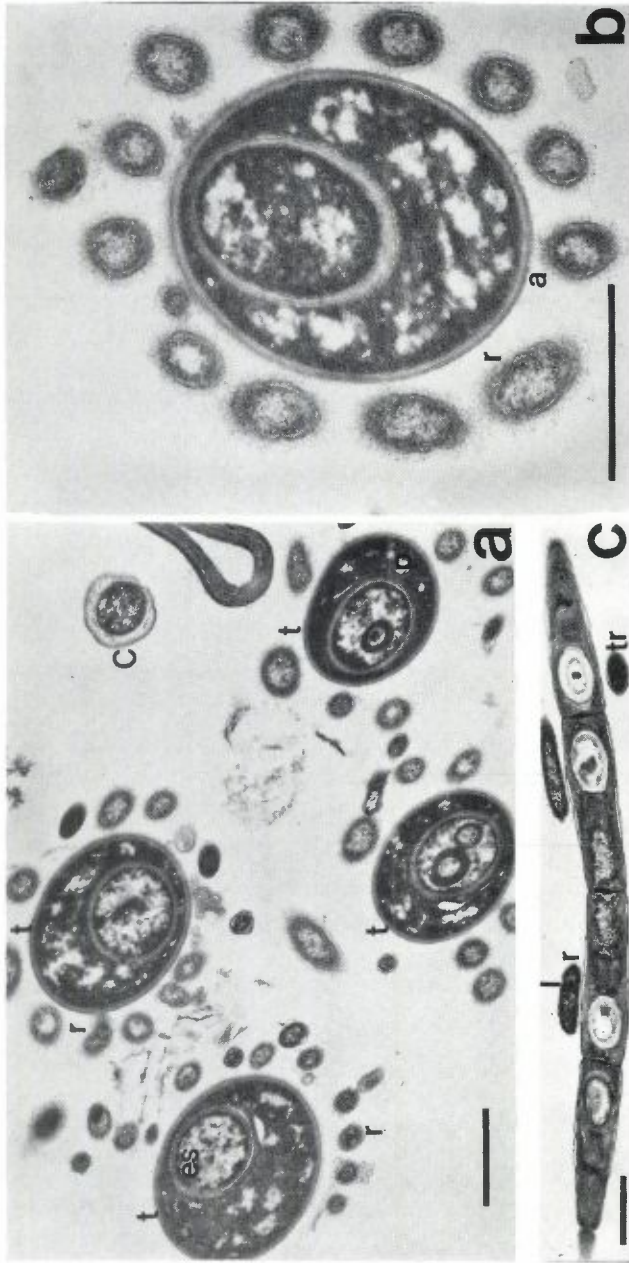


Figure 7. Epibionts of *Arthromitus* sp. from *R. hesperus* (a) Four trichomes (t) with presumptive endospores (es) in transverse section each surrounded by associated rod-shaped epibionts (r) attached. *Cleavelandina* sp., a morphologically complex large spirochete (c) is seen at the upper right. Early sporogenesis in walled material inside parental (p) cell. Bar = 1.0 μm.  
 b. The fuzzy, loose nature of the attachments (a) of rod-shaped *Arthromitus* (r) epibiotic symbionts (r) is seen at higher magnification. Bar = 1.0 μm.  
 c. Epibionts, unidentified rod-shaped bacteria (r) on *Arthromitus reticulitermitidis* from *Reticulitermes tibialis* intestine in transverse (tr) and longitudinal (l) section. Bar = 1.0 μm.



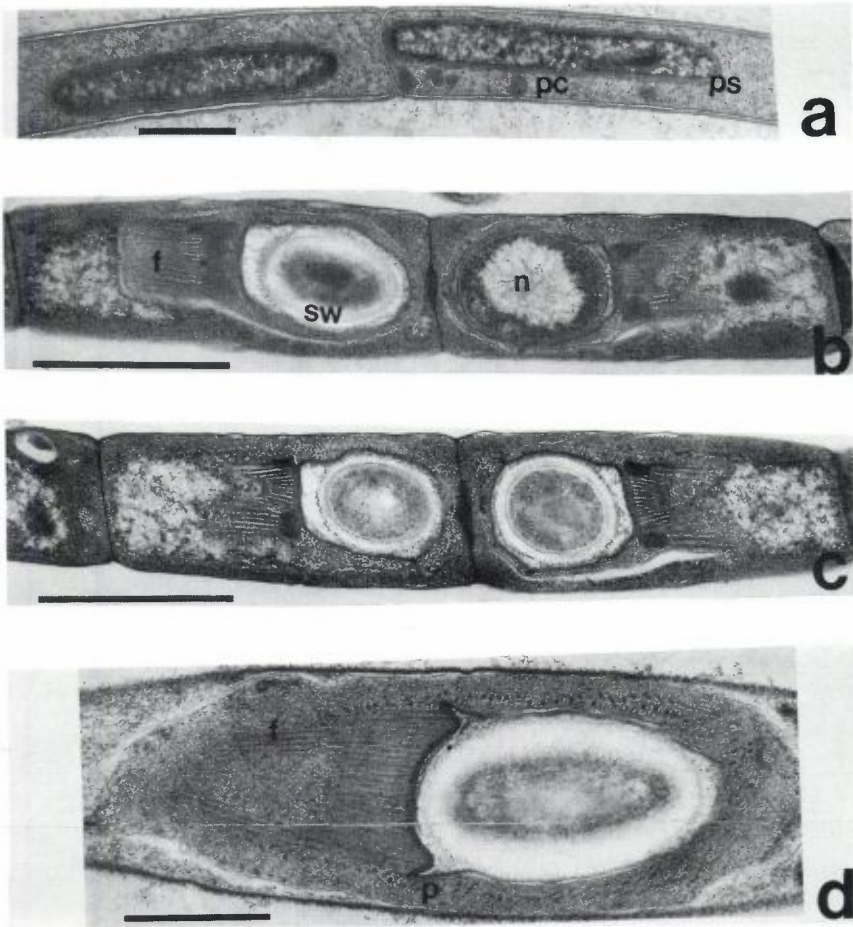


Figure 8. Spores of *Arthromitus* sp. from *Reticulitermes tibialis*.

a. Presumptive early oval spore (ps) is segregated by membrane from the parental cytoplasm (pc). Bar = 1.0  $\mu\text{m}$ .

b. *Arthromitus reticulitermitidis* maturing spores (younger on right), nucleoid (n), and spore attachment filaments (f). The more mature spore on the left has developed a spore wall (sw). Bar = 1.0  $\mu\text{m}$ .

c. Two mature spores (sp), one per cell, asymmetrically distributed showing why, on the light microscope level, trichomes seem to contain paired spores, two per cell (compare with Fig. 3b). Bar = 1.0  $\mu\text{m}$ .

d. Mature spores showing spore attachment filaments (f) and posterior spore protrusions (pr). Bar = 0.5  $\mu\text{m}$ .

termite hindgut (Fig. 3d). Since many bacilli but no *Arthromitus*-like bacteria ever appeared on hard agar, we assume that the arthromitids adjust to their oxygen or other gas concentration optima by growth to the appropriate level in less dense sloppy agar media. The optimal growth temperature, yet to be determined precisely, is approximately 31°C.

Overnight growth is apparent on plates inoculated with spores; either anaerobically or aerobically-grown cultures thrive. After desiccation of the agar plates, rewetting leads to resumption of growth. The *Arthromitus*-sp. from *Zootermopsis* has been transferred at least 3 times on 2 occasions demonstrating the ability of these termite symbionts to be grown in culture. That the cultured organisms were filamentous was determined by electron microscopy. The quality of the micrographs did not suffice, however, to assign the cultured *Arthromitus* to any particular species.

#### *Other arthromitids*

Arthromitids have been observed in many other insects (Table 1), including dampwood, drywood and subterranean termites, and unidentified beetles, sowbugs and millipedes. A larger *Arthromitus* sp. from *Reticulitermes hesperus* is conspicuously covered with rod-shaped epibionts attached to the trichome as seen in Leidy (1881) and To et al. (1980). Two isolates, one from a sowbug and another from *Reticulitermes flavipes*, of *Arthromitus*-like bacilli were grown in culture through three transfers for about two weeks each. Unlike termite spirochetes which have never been in cultivation (Bermudes et al., 1988), arthromitids grow easily *in vitro*. Because of their vigorous growth on sloppy agar medium detailed bacteriological characterization of these spore-formers should be obtainable relatively soon. The observation of the ease and vigor with which arthromitids grow on plates suggests these symbiotic organisms survive and have a rapidly growing phase in the soil. Indeed, many single-celled soil bacteria, e.g., *Bacillus anthracis*, may have an unstudied filamentous life-history stage in animal hosts.

*Bacillus anthracis* Weysbridge UM44-1 may either be classifiable as *Arthromitus* or belong to a closely related genus (Fig. 9). When carrying appropriate plasmids (i.e., pX01 encodes *B. anthracis* toxin synthesis and pX012 encodes fertility and parasporal crystal (c) formation, the cells take on an appearance comparable to *Arthromitus* sp. Possibly, like other arthromitids, *B. anthracis* lacking toxin is a benign symbiont which alternates a resistant spore-soil stage with a growing stage in the intestines of animals, including *Homo sapiens*.

Probably a vast number of different symbiotic arthromitids exist in the digestive tracts of animals. Because at least some have holdfasts and resistant spores, it is likely that many endure stages in the soil from which they are reingested as spores. That soil bacilli cyclically form associations with animal hosts and develop *Arthromitus* stages should not be overlooked by overzealous bacteriologists who tend to overemphasize growth in culture and undervalue cell morphology as a tool in bacterial identification. Filamentous bacilli especially deserve scrutiny in this regard (e.g., Pichinoty and Asselineau, 1984). Much detailed morphology is lost with the rapid growth typical of culture



Figure 9. *Bacillus anthracis* trichomes (t) from cultured material showing its tendency to form *Arthromitus*-like filaments. Parasporal crystals (c), a plasmid-borne inclusion, formed between spores (s). Courtesy of Prof. Curtis Thorne, Dept. of Microbiology, University of Massachusetts, Amherst. Bar = 5  $\mu\text{m}$ .

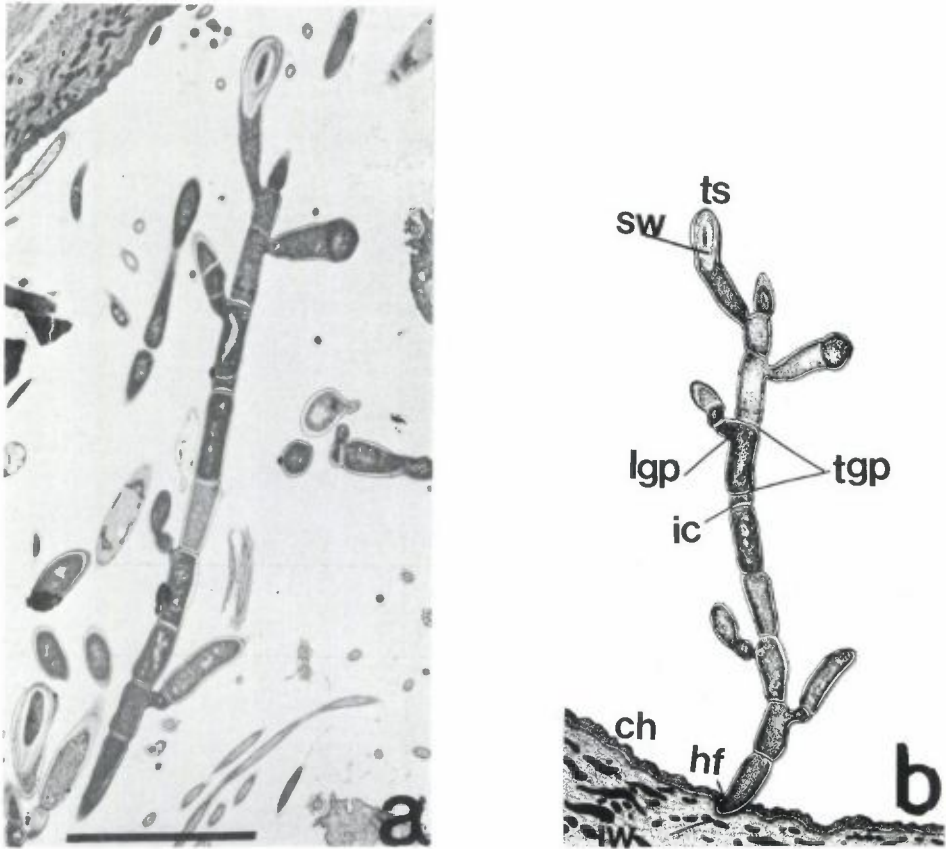


Figure 10. a. Branching *Arthromitus*-like filamentous spore-forming bacterium from *Reticulitermes hesperus*. Bar = 5  $\mu$ m.

b. Branched *Arthromitus*-like bacterium, two terminal (tgp) and one lateral (lgp) growth points per cells. Terminal spore (ts), spore wall (sw). Sporogenic parental cells may be adjacent to each other (as in the anterior cells) or separated by intercalary cells (ic). Holdfasts (hf) extends into the chitinous (ch) layer of the termite intestinal wall (iw). Drawing by Christie Lyons.



conditions. The possibility certainly exists that *Arthromitus*-like bacteria will be described that are even more complex than those shown in electron micrographs (Fig. 10a) and accompanying drawings (Fig. 10b). Outside the range of Leidy's original description, even branched filamentous forms of these bacteria exist. The branching pattern of the filamentous spore former that has two growth points per cell (Fig. 10) is ascribable, on the basis of morphology alone, to a second genus. These branching, filamentous bacteria which undergo cyclic differentiation of spores are among the most morphologically complex prokaryotes ever reported.

Like the complex symbiotic spirochete of wood-eating cockroaches and termites, large bacteria can be identified and classified on the basis of host range and microscopic morphology (e.g., termite spirochetes, Bermudes et al., 1988; and these arthromitids). As in the case of most cyanobacteria, no absolute requirement for *in vitro* cultivation exists because morphology and ecology are distinctive and sufficient for identification. The relative ease with which the arthromitid facultative aerobes can be cultured, their wide distribution in only distantly related animals and their large size makes them ideal microorganisms for study by those seeking rewarding microbiological research related to the analysis of associations between symbionts.

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