**Pasteuria penetrans** (ex Thorne, 1940) nom. rev., comb. n., sp. n., a Mycelial and Endospore-Forming Bacterium Parasitic in Plant-Parasitic Nematodes

**RICHARD M. SAYRE** and **MORTIMER P. STARR**

1 Nematology Laboratory, Plant Protection Institute, Beltsville Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705, and
2 Department of Botany and Microbiology, Arizona State University, Tempe, Arizona 85287

**ABSTRACT**: A spore-forming parasite of plant-parasitic nematodes, at first believed to be a sporozoan ("Dubosquio D. penetrans" Thorne, 1940), was later recognized to be a bacterium and was renamed "Bacillus penetrans" (Thorne, 1940) Mankau, 1975. Because "Bacillus penetrans" was not included in the 1980 "Approved Lists of Bacterial Names," it has no taxonomic standing. In effecting the formalities incident to reviving lapsed bacterial names, it became clear that "Bacillus penetrans" was misassigned to the bacterial genus *Bacillus* Cohn, 1872. Although the mode of formation and the structure of the endospore of the nematode parasite are similar to that described for members of the genus *Bacillus*, the organism differs from the description of that genus in cellular shape and size, motility, flagellation, sporangial shape and size, habitat, and nutritional requirements. The following traits of the nematode parasite suggest that it more properly belongs in the genus *Pasteuria* Metchnikoff, 1888: Primary vegetative colonies consist of a dichotomously branched, septate mycelium; daughter colonies, formed by fragmentation, gradually contain fewer but larger cells arranged predominantly in quartets; these larger vegetative cells differentiate into sporangia, arranged in quartets and doublets; eventually, single sporangia predominate in the nematode’s pseudocoelom; the sporangium consists of a conical stem, a swollen middle cell, and an endogenous spore; the mature endospores, released from the remnants of the nematode, attach to the cuticles of other host nematodes and germinate; then, the parasitic cycle is repeated. A description of *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n., and an emended description of the genus *Pasteuria* are presented.

**Archival Background**

The first report of an organism resembling "Bacillus penetrans" (Thorne, 1940) Mankau, 1975 was by Cobb (1906), who found numerous highly refractile spores infecting specimens of the nematode *Dorylaimus bulbiferous*. He erroneously viewed these spores as "perhaps monads" of a parasitic sporozoan. This error of placing in the protozoan an organism now known to be a bacterial parasite of nematodes was to persist for nearly 70 years. A more precise but still incorrect placement was suggested by Micoletzky (1925), who found a nematode parasite having spores similar in size and shape to those reported by Cobb; Micoletzky suggested that these spores belong to the genus *Dubosquio* Perez, 1908, again a sporozoan group (Perez, 1908). Later, Thorne (1940) described in detail a new parasite from *Pratylenchus pratensis* (de Man) Filipjev; on the assumption that this organism was similar to the nematode parasite described by Micoletzky, it also was assigned by Thorne (1940) to the protozoan genus *Dubosquio*, as *D. penetrans*.

Thorne’s description and nomenclature were to persist until 1975, even though other investigators (Williams, 1960; Canning, 1973), who examined this nematode parasite in some detail, questioned this placement. It was not until the nematode parasite was reexamined using electron microscopy that its true affinities to the bacteria rather than to the protozoan were recognized and the name "Bacillus penetrans" (Thorne, 1940) Mankau, 1975 was applied to it (Mankau, 1975a, b; Mankau and Imbriani, 1975; Imbriani and Mankau, 1977).

A different set of incorrect conclusions have until now inhibited reassignment of "Bacillus penetrans" to the genus *Pasteuria* Metchnikoff, 1888, where we believe it properly belongs. The situation, stated briefly, is that Metchnikoff (1888) described an endospore-forming bacterial parasite of cladocerans; he named this bacterium *Pasteuria ramosa* Metchnikoff, 1888. Metchnikoff presented drawings and photomicrographs of the life stages of this parasite as they occurred in the...
hemolymph of the water fleas, *Daphnia pulex* Leydig and *D. magna* Strauss; he was, however, unable to culture the organism in vitro.

Subsequent workers (Henrici and Johnson, 1935; Hirsch, 1972; Staley, 1973), who were looking in cladocerans for Metchnikoff’s unique bacterium, reported on a different bacterium with only superficial resemblance to certain life-stages of *P. ramosa*. Their investigations led to the axenic cultivation of a budding bacterium, which is occasionally found on the exterior surfaces of *Daphnia* sp. Unlike Metchnikoff’s organism, this budding bacterium does not form endospores, it is not mycelial, its staining reaction is Gram-negative, and it is not an endoparasite of cladocerans. After searching for, but not finding in water fleas, the bacterial endoparasite as described by Metchnikoff, the erroneous conclusion was reached that this budding bacterium, which occurs on the surfaces of *Daphnia* sp., was the organism Metchnikoff had described. Culminating this chain of errors, a budding bacterium (strain ATCC 27377) was mistakenly designated (Staley, 1973) as the type culture for *Pasteuria ramosa* Metchnikoff, 1888, the type (and, then, sole) species of the genus *Pasteuria*.

This confusion between Metchnikoff’s cladoceran parasite and the quite different budding bacterium was only recently resolved (Starr et al., 1983): The budding bacterium (with strain ATCC 27377 as its type culture) was named *Planctomyces staleyi* Starr, Sayre, and Schmidt, 1983 and conservation of the original description of *Pasteuria ramosa*, as updated, was recommended.

Using as a basis the conserved description of *Pasteuria ramosa* (i.e., Metchnikoff’s, as updated), we present here data supporting our view that this bacterial parasite of nematodes, “*Bacillus penetrans*” (Thorne, 1940) Mankau, 1975, properly belongs in the genus *Pasteuria* rather than in the genus *Bacillus*. Its traits suggest that “*B. penetrans*” should be referred to a new species of the genus *Pasteuria*. “*Bacillus penetrans*” was not included in the “Approved Lists of Bacterial Names” (Skerman et al., 1980), nor subsequently legitimized; hence, it now has no standing under the current rules as given in the “International Code of Nomenclature of Bacteria” (Lapage et al., 1975). A formal description of this organism, a legitimate name, *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n., and an emended description of the genus *Pasteuria* Metchnikoff, 1888 are presented.

### Materials and Methods

#### Bacterial specimens

The infectious spores of “*Bacillus penetrans*” (Fig. 1) were found adhering to the cuticles of larvae of *Meloidogyne incognita*, a sedentary nematode that parasitized roots of the ornamental pepper, *Capsicum annum* Linn., grown in a greenhouse soil bench. All attempts at axenic cultivation of the vegetative stages or spores of “*B. penetrans*” have been unsuccessful to date. Hence, its life cycle was followed in nematode hosts reared on the roots of tomato seedlings (Sayre and Wergin, 1977). The specimens of *Pasteuria ramosa* were found in the hemolymph of cladocerans (*Moina rectirostris*) collected from a pond near Beltsville, Maryland. Axenic cultivation of *P. ramosa* has not yet been achieved; consequently, material derived from two-membered laboratory systems of bacteria plus cladocerans was used. Laboratory-reared cladocerans served as hosts for *P. ramosa*; procedures for cultivating cladocerans and inoculating them with *P. ramosa* were as previously described (Sayre et al., 1979).

#### Light microscopy

The developmental stages of “*Bacillus penetrans*” were obtained by macerating infected tomato roots in a 50% (v/v) solution of Pectinol (Rohm and Haas, Philadelphia). The macerated roots were shaken in water to dislodge nematode larvae and adults. The freed nematodes were transferred to glass slides and examined. Nematodes parasitized by “*B. penetrans*” were crushed on slides, air-dried, and stained by Gram’s method. Cladocerans were filtered from an aquarium onto a 200-mesh screen, and then backwashed into a 150-mm plastic Petri dish provided with a counting grid. Excess water was removed with a pipette, leaving the cladocerans immobilized in a thin water film on the bottom of the dish. The dish, covered to prevent further evaporation of the water, was placed on the stage of a Leitz monot model S2 inverted microscope, and the contents of the cladocerans’ coeloms were examined at 250× for the presence of *Pasteuria ramosa*.

#### Transmission electron microscopy

Root galls containing nematodes infected with “*Bacillus penetrans*” were placed in a solution of 3.0% (w/v) glutaraldehyde in 0.05 M phosphate buffer (pH 6.8). Galls were cut into 2–3-mm segments and transferred to glass vials containing the buffered fixative. In addition, mature, parasitized female nematodes, about 30 days old, were dislodged from roots with a scalpel, handpicked with a Pasteur pipette, and crushed in molten 30.0% (w/v) agar solution at 50°C; this procedure caused the sporangia to disperse in agar, making for easier observation. The agar, containing gall and nematode material, was allowed to solidify; it was then placed in 3.0% (w/v) glutaraldehyde for 1.5 hr, washed in six changes of buffer over a period of 1 hr, postfixed in 2.0% (w/v) osmium tetroxide for 2 hr, dehydrated in an acetone series that began with 10% acetone and

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increased by 10% every 20 min until 100% acetone, and infiltrated with Spurr’s low-viscosity resin mixture. Cladocerans parasitized by Pasteuria ramosa were fixed in 3.0% (w/v) glutaraldehyde for 24 hr, washed in six changes of 0.05 M potassium phosphate buffer (pH 6.8) over a period of 1 hr, postfixed in 2.0% (w/v) osmium tetroxide for 2 hr, and dehydrated in the acetone series. Following dehydration, the specimens were infiltrated with Spurr’s low-viscosity resin mixture. The embedded material (root galls containing nematodes parasitized by “Bacillus penetrans”; cladocerans containing Pasteuria ramosa) were sectioned on a Sorvall MT-2 ultramicrotome with a diamond knife. Thin sections were mounted on uncoated copper grids (75 × 100-mesh), stained for 10 min with 2.0% (w/v) aqueous uranyl acetate, and then stained for 5 min with 2.66% (w/v) lead citrate. The sections were viewed with a Phillips model 200 transmission electron microscope operating at 60 kV with 20-μm apertures.

### Scanning electron microscopy

Second-stage larvae of the root-knot nematode encumbered with “Bacillus penetrans” spores were prepared for scanning electron microscopy by fixation in 3.0% (w/v) glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) for 1.5 hr and dehydration in an ethanol series (20, 40, 60, 80, and 100% anhydrous ethanol), followed by critical-point drying from liquid CO₂. Such preparations, containing stages of the two parasites, were examined with a Hitachi model HHS-2R scanning electron microscope operated at 15 or 20 kV. Preparations of Pasteuria ramosa were made for scanning electron microscopy by crushing parasitized female cladocerans on aluminum stubs. The liberated sporangia were air-dried before coating and examination.

### Results

When observed adhering to the cuticles of root-knot nematode larvae (Fig. 1) and parasitizing adult female nematodes, “Bacillus penetrans” is so distinctive in its morphology, ultrastructure, and life stages that these features alone served as reliable diagnostic traits.

### Morphological characteristics of vegetative cells

Mycelial colonies of “Bacillus penetrans” up to approximately 8 μm in diameter are formed in the pseudocoelom, where they are observed after the parasitized larvae penetrate the plant roots (Fig. 2). The dichotomously branched mycelium comprising the colony is septate (Figs. 2, 3). Measurements of the diameters of hyphal cells vary from approximately 0.2–0.5 μm; because of the sinuous pattern of mycelial growth, estimates of cell length would not be meaningful. Cells are bounded by a compound wall, about 0.12 μm thick, composed of an outer and an inner membrane. The inner membrane of the wall forms the septation and delineates individual cells. In addition, this membrane is continuous with a membrane complex or mesosome frequently associated with the septum (Fig. 3).

Sporulation of “B. penetrans” is a synchronously initiated process in the nematode host; it involves the terminal hyphal cells of the mycelium. As the process begins, the terminal cells bifurcate (Fig. 2) and enlarge from typical hyphal cells to ovate cells measuring about 2.0 by 4.0 μm. Structure and content of the cytoplasm change from a granular matrix, which contains numerous ribosomes as found in the hyphal cells, to one that lacks particulate organelles. During these changes, the developing sporangia separate from their parental hyphae, which stop growing and eventually degenerate.

After these early structural alterations, a membrane forms within the sporangium and separates the upper third of the cell or forespore from its lower or parasporal portion (Fig. 4). The granular matrix confined within the membrane then condenses into an electron-opaque body, 0.6 μm in diameter, which eventually becomes encircled by a multilayered wall. The discrete structure that ensues is an endospore (Fig. 5).

### Morphological characteristics of sporangia and endospores

Spores of “Bacillus penetrans” that measure about 3.8 μm and adhere to the surface of root-knot nematode larvae are considered mature (Figs. 1, 6; Imbiani and Mankau, 1977; Sayre and Wergin, 1977). Two distinct forms of these spores can be observed with the scanning electron microscope (Fig. 6). The surface of one form appears as a wrinkled membrane that encompasses the entire spore. This “membrane” is actually the exosporium, which is generally sloughed prior to germination. In the absence of the exosporium, the second form of the spore can be resolved into two distinct components: a spherical central endospore, 2.3 μm in diameter, and a peripheral matrix, 1.85 μm wide, which surrounds the endospore (Fig. 6).

Cross sections viewed by transmission electron microscopy (Imbiani and Mankau, 1977; Sayre and Wergin, 1977) that the “B. penetrans” endospore consists of a central, highly electron-opaque core surrounded by an inner and an outer wall composed of several distinct layers.
(Fig. 7). When observed with the transmission electron microscope, the peripheral matrix of the spore is fibrillar. Fine microfibrillar strands, about 1.5 nm thick, extend outward and downward from the sides of the endospore to the cuticle of the nematode, where they become more electron-dense.

**Morphological characteristics of parasporal structures**

Coincident with the formation of an endospore in "Bacillus penetrans" is the emergence of the parasporal fibers. These fine fibers, which form around the base of the spore, differentiate from an electron-translucent, granular substance. They appear to connect with and radiate from the external layer of the wall of the endospore (Fig. 8). During development of the parasporal fibers, the formation of another membrane, the exosporium, isolates the newly formed endospore within the sporangium. At this later stage of spore development, the granular content of the parasporal structure becomes less dense, degenerates, and disappears. As a result, the mature sporangium contains a fully developed endospore enclosed within the exosporium (Fig. 8). The cell wall of the "B. penetrans" sporangium remains intact until the nematode is disrupted, after which event the endospores are released. The exosporium apparently remains associated with the endospore until contact is made with a new nematode and the infection cycle restarts.

**Penetration of host**

Larvae of root-knot nematodes belonging to the genus *Meloidogyne*, as well as other nematode species from plants, are susceptible to attack by this parasite (Table 3). Only vermiform stages of nematodes are encumbered by the parasite as they migrate through spore-infected soils. A mature spore of "Bacillus penetrans" attaches to the surface of a nematode so that a basal ring of wall material lies flatly against the cuticle. The orientation is such that a median section through the endospore and perpendicular to the surface of the nematode would bisect this basal ring. As a result, the ring appears as two protruding pegs that are continuous with the outer layer of the spore wall and rest on the cuticular surface of the nematode (Fig. 9).

The peripheral fibers of the spore also are closely associated with the cuticle of the nematode (Fig. 10). The fibers, which encircle the endospore, lie along the surface of the nematode and follow the irregularities of the cuticular annuli. They apparently secure the spore to the nematode, but do not appear to penetrate the cuticle.

The germ-tube of the "B. penetrans" endospore emerges through the central opening of the basal ring, penetrates the cuticle of the nematode, and enters the hypodermal tissue (Fig. 10). Hyphae were initially encountered beneath the cuticle of the nematode near the site of germ-tube penetration. From this site, they apparently penetrate the hypodermal and muscle tissues and enter the pseudocoelom.

**Discussion**

Now that the aforementioned problem involving the type strain of *Pasteuria ramosa* has been resolved (Starr et al., 1983), and the status of the genus *Pasteuria* has thereby been returned to something akin to the original concept of Metchnikoff (1888), the way is now clear to discuss assigning the nematode parasite to the genus *Pasteuria*—in our view, a much more fitting generic repository than *Bacillus*, as is demonstrated in what follows.

**Morphological characteristics common to "Bacillus penetrans" and *Pasteuria ramosa***

A close relationship exists between "Bacillus penetrans" (Thorne, 1940) Mankau, 1975 and...
Figure 4. Section through a sporangium that has formed a membrane separating the anterior third of the spore or forespore (FS) from the parasporal segment (PS). Bar = 0.25 μm.

Figure 5. This median section through a sporangium illustrates an early stage of endospore development in Pasteuria penetrans. The electron-opaque body, which has formed within the forespore, is surrounded by membranes that will contribute to the multilayered wall of the mature endospore. Bar = 0.25 μm.

Figure 6. Scanning electron micrograph of endospores of Pasteuria penetrans attached to the cuticle along the lateral field of the larva of a root-knot nematode, Meloidogyne incognita. (A) Spore has retained its exosporium, resulting in the appearance of a crinkled or reticulated surface. (B) The exosporium has been sloughed, exposing the central dome of the endospore; the peripheral fibers can be distinguished. Bar = 0.5 μm.

Figure 7. Section through a sporangium of Pasteuria penetrans containing an almost developed endospore. The lateral regions (light areas marked by arrows) differentiate into parasporal fibers. Bar = 0.5 μm.
Figure 8. Median section through a sporangium of *Pasteuria penetrans* containing a fully developed endospore. The last stages of endospore differentiation include formation of an encircling membrane or exosporium (E) and emergence of parasporal fibers (F) within the granular material that lies laterally around the spore. Bar = 0.25 μm.

*Pasteuria ramosa* Metchnikoff, 1888, the type species of the genus *Pasteuria*. They share several distinctive morphological characteristics (Table 1), among which are the dichotomously branched mycelial microcolonies that give rise by fragmentation to sporangia arranged in quartets, in doublets, and eventually singly, and finally to endogenous spores formed within the old mother cell walls (Fig. 11B, C). Scanning and transmission electron microscopy reveal remarkable similarities at the ultrastructural level in the unique forms and sequences of life stages of the two organisms (Fig. 13).

Morphological characteristics of the genus *Bacillus* compared to “*Bacillus penetrans*”

The nematode parasite differs from the current concept of the genus *Bacillus* in many respects, particularly in cellular shape and size, motility, flagellation, sporangial shape and size, habitat, and nutritional requirements. The formation of a dichotomously branched mycelium by “*B. penetrans*” definitely excludes the nematode parasite from the family Bacillaceae Fischer, 1895 (we consider separately, below, its relationship to the mycelial actinomycetes). Most members of the genus *Bacillus* have flagella and are motile; by contrast, flagella and active motility have never been observed in “*B. penetrans*”; its microcolonies are carried passively by the currents of the hemolymph in the pseudocoelom of the nematode. *Bacillus* species are generally chemoheterotrophic and readily cultivated on laboratory media, whereas “*B. penetrans*” is a parasite that has resisted all efforts to culture it apart from the nematode host.

On the other hand, there is no doubt about the formation by the nematode parasite of endospores similar in origin, structure, and environmental resistance to the spores typical of mem-
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Vegetative growth

Vegetative growth

Fig. 11. Generalized bacterial endospore formation (upper row; A) is compared with sporogenous stages of Pasteuria ramosa (middle row; B) and Pasteuria penetrans (lower row; C). Aside from the differences in parasporal structures, the stages of the two Pasteuria species result in spores having characteristics very similar to those of other endospore-forming bacteria. The asterisk (*) indicates that the free spore of that species has neither been observed, nor has its mode of penetrating and initiating infections in cladocerans been determined.

Fig. 9. Cross section through an endospore of Pasteuria penetrans on the surface of a nematode. Parasporal fibers (F) appear to radiate outward from the lower half of the spore to the cuticle of the nematode. Short “hairs” (arrows) project outward from the surface of the fibers to give the crinkled or reticulated surface appearance shown in Figure 6. Bar = 0.25 μm.

Fig. 10. Cross section through a germinated spore of Pasteuria penetrans; the penetrating germ-tube follows a sinuous path as it traverses the cuticle (C) and hypodermis (H) of the nematode. Bar = 0.5 μm.

Affinities of the nematode parasite to the actinomycetes

Evidence has been presented (Cross, 1970; Cross and Unsworth, 1981) for true endospore formation in several genera of the Actinomycetales. “Bacillus penetrans” also has some characteristics consistent with placement in the Actinomycetales: (i) the Gram-positive staining characteristic; (ii) the slender vegetative cells, usually from 0.2 to 0.5 μm in diameter; (iii) the mycelial format, with terminal hyphae enlarging and then segmenting to yield club-shaped spo-
Table 1. Characteristics held in common by *Pasteuria ramosa* Metchnikoff, 1888 and *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n.

**MORPHOLOGICAL SIMILARITIES AS OBSERVED BY LIGHT MICROSCOPY**

**Vegetative Cells**
- Dichotomously branched mycelium gives rise to microcolonies
- Diameter of mycelial filaments similar (about 0.2–0.5 μm)
- Mycelial filaments seen only in early stages in host tissues
- Daughter microcolonies may be formed by lysis of intercalary "suicidal" cells, allowing mother colony to break internally
- Nearly all vegetative cells lyse, leaving only sporangia

**Stages of Sporogenesis**
- Only external or peripheral cells of the colony elongate and give rise to sporangia
- A single spore produced in each sporangium
- Spores are similar in size (about 4.0 μm)
- Refractility of spores increases as spores mature

**Staining Reaction**
- Gram-positive

**ULTRASTRUCTURAL SIMILARITIES**

**Vegetative Cells**
- Mycelial cell walls are typical of Gram-positive bacteria
- Mycelial filaments divided by septa
- Double-layered cell walls
- Mesosomes in both species are similar in appearance and seem to be associated with division and septum formation

**Stages of Sporogenesis**
- Typical endogenous spore formation (Fig. 11)
- Identical sequences of life stages in both organisms: (i) septa form within sporangia; (ii) sporangium cytoplasm condenses to form forespore; (iii) spore walls form; (iv) final spore matures; and (v) light areas adjacent to spore give rise to extrasporal fibers

**SIMILAR SEQUENCES OF LIFE STAGES** (Fig. 13)

**Microcolonies**
- Fragmentation of microcolonies
- Quartets of sporangia
- Doublets of sporangia
- Single sporangia
- Free endospores

**HOST-BACTERIUM RELATIONSHIPS**

- Both parasitize invertebrates
- Colonies first observed in the host are sedentary and located in the host's musculature
- Growth in muscle tissue eventually leads to fragmentation and entry of microcolonies into the coelom or pseudocoelom of the respective host
- Microcolonies carried passively by body fluids
- Colonization of hemolymph or pseudocoelomic fluid by the parasite is extensive
- Host ranges of the two bacteria are very narrow; moreover, *P. ramosa* occurs only in cladocerans and *P. penetrans* only in nematodes
- Bacteria appear to develop in synchrony with their host which, although parasitized, continues its developmental cycle for some time
- Host is completely utilized by the bacteria; in the end, the host becomes little more than a bag of bacterial endospores

**SURVIVAL MECHANISMS**

- Survives in field soils and in muck soils at bottom of ponds
- Resists desiccation
- Moderately resistant to heating

Although the aforementioned traits suggest that the genus *Pasteuria* (including the nematode parasite under consideration here) might possibly be placed in the Actinomycetales, further consideration of this point requires axenic cultures of these organisms and the usual phenotypic and genetic comparisons, one of our future research goals.
Table 2. Differences between Pasteuria penetrans (ex Thorne, 1940) nom. rev., comb. n., sp. n., and Pasteuria ramosa Metchnikoff, 1888.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Pasteuria ramosa</th>
<th>Pasteuria penetrans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony shape</td>
<td>Like cauliflower floret</td>
<td>Spherical, to cluster of elongated grapes</td>
</tr>
<tr>
<td>Sporangia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape</td>
<td>Teardrop-shaped</td>
<td>Cup-shaped</td>
</tr>
<tr>
<td>Diameter (μm)</td>
<td>3.3–4.1</td>
<td>3.5–4.0</td>
</tr>
<tr>
<td>Height (μm)</td>
<td>4.8–5.7</td>
<td>2.2–2.9</td>
</tr>
<tr>
<td>Central spore, diameter (μm)</td>
<td>2.1–2.4</td>
<td>1.6–1.7</td>
</tr>
<tr>
<td>Host</td>
<td>Cladocerans</td>
<td>Nematodes</td>
</tr>
<tr>
<td>Location in host</td>
<td>Hemocoele and musculature; sometimes found attached to coelom walls</td>
<td>Pseudocoelom and musculature; no attachment observed</td>
</tr>
<tr>
<td>Attachment of spores on host</td>
<td>Spores not observed to attach or accumulate on surface of cladoceran</td>
<td>Spores accumulate in large numbers on cuticular surface</td>
</tr>
<tr>
<td>Mode of penetration of host</td>
<td>Not known; suspected to occur through gut wall</td>
<td>Direct penetration of nematode cuticle by hyphal strand</td>
</tr>
<tr>
<td>Source of host</td>
<td>Pond mud, freshwater</td>
<td>Soil, plants</td>
</tr>
</tbody>
</table>

Relationships of “Bacillus penetrans” with nematodes

Over the years, numerous reports (e.g., Kuiper, 1958; Esser and Sobers, 1964; Boosalis and Man- kau, 1965; Williams, 1967; Prasad, 1971; Man- kau and Prasad, 1977) have appeared listing—from various geographical localities in a score of countries—the nematodes parasitized by the organism under consideration here, usually under one of its earlier synonyms such as Duboscquia penetrans (Table 3). According to these reports, “Bacillus penetrans” parasitizes some 50 nematode species belonging to some 30 genera from widely separated families of the phylum Nema- toda. These reports on the geographical occurrence of this bacterial parasite of nematodes suggest that any given isolate of “B. penetrans” might possibly be a nonspecific pathogen capable of parasitizing several nematode species at any one collection site. However, this is decidedly not the situation! For example, Dutky and Sayre (1978) have reported that the bacterial spores collected from Pratylenchus brachyurus attached to and parasitized only that nematode species and none of the other ten nematode species tested including Pratylenchus penetrans. Similarly, they found that spores of the bacterium from Meloidogyne incognita attached only to larvae of two additional species of root-knot nematodes but not to eight other nematode species, some of which had been reported as hosts of “B. penetrans”. Man- kau and Prasad (1977), working with “B. pene-
doparasitic nematodes. The original description by Thorne (1940) dealt with *Duboscquia penetrans* on a *Pratylenchus* species and, because we intend the present taxonomic action to result in revival of Thorne's name, our description similarly deals with those bacteria having spores of the smaller diameter when attached to *Pratylenchus* or *Meloidogyne* species.

"Bacillus penetrans" has been reported to parasitize not only plant-parasitic nematodes but also free-living and/or predaceous nematodes belonging to several genera (Table 4). A report by Thorne (1927), read in the context of the belief held for some 70 years that this spore-forming bacterium was a sporozoan, deals with a "sporozoan" parasite of *Mononchus parabrackyurus* causing a decline in the field population of that predaceous nematode. Thorne theorized that the mononch had acquired the "sporozoan" by ingesting prey nematodes encumbered with spores or actively infected by the microorganism. In his drawing of an infected nematode, the parasite can be seen to differ from "*B. penetrans*" in that it lacks both the central refractile area and the cup-shaped profile. Thorne's critical attitude might profitably be extended to papers (e.g., Steiner, 1938) reporting the occurrence of "*B. penetrans*" in free-living nematodes. The short life cycle of only a few days and the four rapidly successive molts characteristic of most free-living nematodes would provide little opportunity for the bacterium to penetrate the nematodes through their cuticles, if penetration occurred in the same way as it does in the case of root-knot nematode infections. If something like "*Bacillus penetrans*" does parasitize predaceous or free-living nematodes, there is good reason to suspect these bacteria will be new species or pathotypes, because their modes of penetration and infection would necessarily differ from the typical model of "*B. penetrans*" parasitization.

**Nomenclatural Formalities**

As has been detailed in the foregoing, "*Bacillus penetrans*" differs in important respects from members of the genus *Bacillus*. It does share many
Table 3. Geographical and host ranges of *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n.*

<table>
<thead>
<tr>
<th>Location</th>
<th>Nematode</th>
<th>Reference</th>
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<tr>
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<tr>
<td>California</td>
<td><em>Dolichodorus obtusus, Meloidogyne hapla, Meloidogyne javanica, Meloidogyne incognita, Pratylenchus scribneri,</em> and <em>Hoplolaimus sp.</em></td>
<td>Allen (1957), Boosalis and Mankau (1965), and Prasad and Mankau (1969)</td>
</tr>
<tr>
<td>Colorado</td>
<td><em>Tylenchorkynchus sp.</em></td>
<td>Prasad and Mankau (1969)</td>
</tr>
<tr>
<td>Florida</td>
<td><em>Belonolaimus gracilis, Helicotylenchus microlobus, Hoplolaimus sp.,</em> and <em>Pratylenchus sp.</em></td>
<td>Esser and Sobers (1964)</td>
</tr>
<tr>
<td>Georgia</td>
<td><em>Pratylenchus brachyurus</em></td>
<td>Thorne (1940)</td>
</tr>
<tr>
<td>Hawaii</td>
<td><em>Discolaimus bulbiferus</em></td>
<td>Cobb (1906)</td>
</tr>
<tr>
<td>Illinois</td>
<td><em>Pratylenchus sp.</em></td>
<td>Boosalis and Mankau (1965)</td>
</tr>
<tr>
<td>Maryland</td>
<td><em>Meloidogyne hapla, Meloidogyne incognita,</em> and <em>Pratylenchus brachyurus</em></td>
<td>Dutky and Sayre (1978), and Sayre and Wergin (1977)</td>
</tr>
<tr>
<td>Oregon</td>
<td><em>Pratylenchus sp.</em></td>
<td>Prasad (1971)</td>
</tr>
<tr>
<td>South Carolina</td>
<td><em>Pratylenchus brachyurus</em></td>
<td>Thorne (1940)</td>
</tr>
<tr>
<td>South Dakota</td>
<td><em>Aporcelaimus eurydorus, Laimydorus reversus, Nygolaimus parabrackyurus, Tylenchorkynchus nudus,</em> and <em>Xiphinema chambers</em></td>
<td>Thorne and Malek (1968), and Thorne (1974)</td>
</tr>
<tr>
<td>Utah</td>
<td><em>Megadorus megadorus</em></td>
<td>Allen (1941)</td>
</tr>
<tr>
<td>Australia</td>
<td><em>Meloidogyne javanica</em></td>
<td>Stirling and White (1982)</td>
</tr>
<tr>
<td>Belgium</td>
<td><em>Tylenchorkynchus dubius</em> and <em>Tylenchorkynchus nanus</em></td>
<td>Coomans (1962)</td>
</tr>
<tr>
<td>Ceylon</td>
<td><em>Xiphinema americana</em></td>
<td>Prasad (1971)</td>
</tr>
<tr>
<td>Congo</td>
<td><em>Discolaimus sp. and Xiphinema sp.</em></td>
<td>DeConinck (1962)</td>
</tr>
<tr>
<td>Germany</td>
<td><em>Rhopalolaimus similis</em></td>
<td>Thorne (1961)</td>
</tr>
<tr>
<td>India</td>
<td><em>Hoplolaimus indicus, Meloidogyne javanica,</em> and <em>Paralongidorus sali</em></td>
<td>Boosalis and Mankau (1965), and Siddiqi et al. (1963)</td>
</tr>
<tr>
<td>Italy</td>
<td><em>Dorylaimellus virginianus, Dorylaimus sp.,</em> and <em>Rotylenchus robustus</em></td>
<td>Allherr (1954)</td>
</tr>
<tr>
<td>Japan</td>
<td><em>Meloidogyne javanica</em></td>
<td>Allen (1941)</td>
</tr>
<tr>
<td>Mauritius</td>
<td><em>Meloidogyne incognita,</em> <em>Meloidogyne javanica,</em> and <em>Xiphinema elongatum</em></td>
<td>Williams (1960, 1967)</td>
</tr>
<tr>
<td>Netherlands</td>
<td><em>Hoplolaimus uniformis,</em> <em>Meloidogyne arenaria,</em> <em>Pratylenchus penetrans,</em> <em>Pratylenchus pratense,</em> <em>Rotylenchus robustus,</em> and <em>Tylenchorkynchus dubius</em></td>
<td>Kuiper (1958)</td>
</tr>
<tr>
<td>Nigeria</td>
<td><em>Heliocotylenchus sp., Isolaimus nigeriensis,</em> and <em>Scutellonema sp.</em></td>
<td>Prasad (1971), and Timm (1969)</td>
</tr>
<tr>
<td>Scotland</td>
<td><em>Eudorylaimus sp., Mononchus papillatus,</em> and <em>Tylenchorkynchus dubius</em></td>
<td>Prasad (1971)</td>
</tr>
<tr>
<td>South Africa</td>
<td><em>Discocircinematella mauritensis,</em> <em>Heliocotylenchus dihydraster,</em> <em>Heliocotylenchus krugeri,</em> <em>Histotylenchus histoides,</em> <em>Meloidogyne incognita,</em> <em>Pratylenchus zeae,</em> <em>Rotylenchus incultus,</em> <em>Rotylenchus unisexual,</em> <em>Scutellonema brachyurus,</em> <em>Scutellonema truncatum,</em> <em>Tylenchulus sp.,</em> <em>Xiphinema elongatum,</em> and <em>Xiphinema cf. imitator</em></td>
<td>Spaull (1981)</td>
</tr>
<tr>
<td>Sweden</td>
<td><em>Ironus ignavus</em></td>
<td>Allgen (1925)</td>
</tr>
<tr>
<td>Uganda</td>
<td><em>Mumtazium mumtazae</em></td>
<td>Siddiqi (1969)</td>
</tr>
<tr>
<td>Venezuela</td>
<td><em>Eudorylaimus morbida</em></td>
<td>Loof (1964)</td>
</tr>
</tbody>
</table>

* As related in the text, *Pasteuria penetrans* was referred to in the earliest literature as a “sporozoan” belonging to the genus *Duboscqia*; in later literature, the name “*Bacillus penetrans*” was sometimes used.
Table 4. Variation in size of *Pasteuria penetrans* spores occurring within nematodes or adhering to their cuticles.*

<table>
<thead>
<tr>
<th>Nematode host</th>
<th>Nematode size (μm)</th>
<th>Method of feeding†</th>
<th>Spore diam. (μm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length</td>
<td>Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rotylenchus incognita</em></td>
<td>360-580</td>
<td>14</td>
<td>ME</td>
<td>3.6</td>
</tr>
<tr>
<td><em>Meloidogyne incognita</em></td>
<td>395-466</td>
<td>16</td>
<td>ME</td>
<td>3.7</td>
</tr>
<tr>
<td><em>Pratylenchus sp.</em></td>
<td>—</td>
<td>—</td>
<td>ME</td>
<td>3.75</td>
</tr>
<tr>
<td><em>Meloidogyne hapla</em></td>
<td>360-393</td>
<td>12</td>
<td>ME</td>
<td>3.8</td>
</tr>
<tr>
<td><em>Tylenchus sp.</em></td>
<td>—</td>
<td>—</td>
<td>ME</td>
<td>3.9</td>
</tr>
<tr>
<td><em>Discocroconemella mauritiensis</em></td>
<td>—</td>
<td>—</td>
<td>EC</td>
<td>3.9</td>
</tr>
<tr>
<td><em>Helicotylenchus dihystera</em></td>
<td>550-640</td>
<td>26</td>
<td>EC</td>
<td>3.9</td>
</tr>
<tr>
<td><em>Meloidogyne incognita</em></td>
<td>360-393</td>
<td>12</td>
<td>SE</td>
<td>4.1</td>
</tr>
<tr>
<td><em>Meloidogyne incognita</em></td>
<td>360-393</td>
<td>12</td>
<td>SE</td>
<td>4.27</td>
</tr>
<tr>
<td><em>Meloidogyne javanica</em></td>
<td>340-400</td>
<td>14</td>
<td>SE</td>
<td>4.30</td>
</tr>
<tr>
<td><em>Xiphinema elongata</em></td>
<td>2,090</td>
<td>43</td>
<td>EC</td>
<td>4.5</td>
</tr>
<tr>
<td><em>Mumtazium mumtazae</em></td>
<td>670-880</td>
<td>24</td>
<td>PRED</td>
<td>4.55</td>
</tr>
<tr>
<td><em>Helicotylenchus krugeri</em></td>
<td>500-700</td>
<td>25</td>
<td>EC</td>
<td>4.7</td>
</tr>
<tr>
<td><em>Rotylenchus unisexus</em></td>
<td>700-1,040</td>
<td>32</td>
<td>EC</td>
<td>4.8</td>
</tr>
<tr>
<td><em>Scutellonema truncatum</em></td>
<td>610-750</td>
<td>27</td>
<td>EC</td>
<td>4.9</td>
</tr>
<tr>
<td><em>Dolichodorus obtusus</em></td>
<td>1,900-2,700</td>
<td>57</td>
<td>EC</td>
<td>4.9</td>
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<tr>
<td><em>Meloidogyne javanica</em></td>
<td>340-400</td>
<td>14</td>
<td>ES</td>
<td>5.0</td>
</tr>
<tr>
<td><em>Meloidogyne incognita</em></td>
<td>360-393</td>
<td>12</td>
<td>ES</td>
<td>5.0</td>
</tr>
<tr>
<td><em>Histotylenchus histoides</em></td>
<td>1,080-1,180</td>
<td>28</td>
<td>EC</td>
<td>5.1</td>
</tr>
<tr>
<td><em>Scutellonema brachyurum</em></td>
<td>720-890</td>
<td>36</td>
<td>EC</td>
<td>5.2</td>
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<tr>
<td><em>Isolaimus nigeriensis</em></td>
<td>2,730-3,530</td>
<td>62</td>
<td>FL</td>
<td>5.3</td>
</tr>
<tr>
<td><em>Xiphinema americanum</em></td>
<td>1,500-2,000</td>
<td>38</td>
<td>EC</td>
<td>5.3</td>
</tr>
<tr>
<td><em>Scutellonema bradyi</em></td>
<td>950-1,190</td>
<td>43</td>
<td>ME</td>
<td>5.5</td>
</tr>
<tr>
<td><em>Hoplolaimus indicus</em></td>
<td>950-1,400</td>
<td>44</td>
<td>EC</td>
<td>5.6</td>
</tr>
<tr>
<td><em>Xiphinema elongata</em></td>
<td>2,090</td>
<td>43</td>
<td>EC</td>
<td>5.7</td>
</tr>
<tr>
<td><em>Xiphinema cl. imitator</em></td>
<td>2,220</td>
<td>39</td>
<td>EC</td>
<td>6.0</td>
</tr>
<tr>
<td><em>Belonolaimus gracilis</em></td>
<td>2,150</td>
<td>43</td>
<td>EC</td>
<td>6.2</td>
</tr>
<tr>
<td><em>Rotylenchus incultus</em></td>
<td>710-840</td>
<td>26</td>
<td>EC</td>
<td>6.2</td>
</tr>
<tr>
<td><em>Xiphinema bakeri</em></td>
<td>4,050</td>
<td>63</td>
<td>EC</td>
<td>6.5</td>
</tr>
<tr>
<td><em>Longidorus sp.</em></td>
<td>—</td>
<td>—</td>
<td>EC</td>
<td>6.5</td>
</tr>
<tr>
<td><em>Paralongidorus salii</em></td>
<td>2,250-2,850</td>
<td>41</td>
<td>EC</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Xiphinema sp.</em></td>
<td>—</td>
<td>—</td>
<td>EC</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Discolaimus sp.</em></td>
<td>—</td>
<td>—</td>
<td>PRED</td>
<td>7.0</td>
</tr>
</tbody>
</table>

* Assembled from lists of Kuiper (1958), Williams (1967), Prasad (1971), Dutky and Sayre (1978), and Spaul (1981), with additions and corrections.
† ME = migratory endoparasite; SE = sedentary endoparasite; EC = ectoparasite; PRED = predator; and FL = free-living.
‡ Approximate mean of several determinations; variance usually was not over 0.5 μm.

* Pasteuria penetrans* was not included in the 1980 "Approved Lists of Bacterial Names" (Skerman et al., 1980), it presently has no taxonomic standing (Lapage et al., 1975). We now assign this organism to *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n. Under the "Code" (Lapage et al., 1975), "ex" indicates our belief that we are dealing here with Thorne’s organism, "nom. rev." shows that we are reviv-
Figure 13. Drawings of the life stages of *Pasteuria penetrans* (bottom row) based on electron micrographs are compared with those of *Pasteuria ramosa* (top row) as drawn by Metchnikoff (1888). Starting at the far left of the bottom row, a vegetative colony of *P. penetrans* is followed by daughter colonies, quartets of sporangia, doublets of sporangia, single sporangia, and finally (at the far right) the mature endospore within the old sporangial wall. The corresponding drawings of *P. ramosa* in the top row are placed in order of their occurrence in the life cycle of the parasite as reported by Metchnikoff (1888).

ing a lapsed bacterial name, "comb. n." refers to placement of Thorne's species in a new genus, and "sp. n." indicates that it is a new species as mandated by the revised bacteriological nomenclatural system in effect since January 1, 1980. The formal description of *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n., and an emended description of the genus *Pasteuria* Metchnikoff, 1888 follow.

**Genus Pasteuria** (Metchnikoff, 1888) emend. mut. char.


Gram-positive, dichotomously branching, septate mycelium, the terminal hyphae of which enlarge to form sporangia and eventually endospores. Maturing colonies are shaped like cauliflower florets or elongated grapes in clusters; daughter colonies are formed by fragmentation. The sporogenous cells at the periphery of the colonies are usually attached by narrow hyphae that lyse, causing arrangement of the developing sporangia in quartets, then in doublets, and finally as single, mature, teardrop- to cup-shaped sporangia. The rounded end of the sporangium encloses a single refractile endospore, 1.5–2.5 μm in diameter, slightly oval to spherical in shape, resistant to desiccation and elevated temperatures (one species has only limited heat tolerance). Nonmotile. Sporangia and microcolonies are parasitic in the bodies of freshwater, plant, and soil invertebrates. Has not been cultivated axenically, but can be grown in the laboratory with the invertebrate host.

**Type species:** *Pasteuria ramosa* Metchnikoff, 1888. Not *Pasteuria ramosa* Staley, 1973, a quite different bacterium (Gram-negative, nonmycelial, not endospore-forming, budding, nonprothecately appendaged, not endoparasitic in cladocerans) belonging to the Blastocaulis–Planctomyces group (Starr et al., 1983). Modern descriptions of *P. ramosa* Metchnikoff, 1888 can be found in papers by Sayre et al. (1979, 1983) and Starr et al. (1983).

*Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n.


Gram-positive vegetative cells. Mycelium is septate; hyphal strands, 0.2–0.5 μm in diameter,
branch dichotomously. Sporangia, formed by expansion of the hyphal tips, are pustule-like, about 1.6–2.5 μm by 3.7–4.3 μm, each divided into two unequal sections. The smaller proximal body is not as refractile as the larger, rounded, cup-shaped portion that encloses an endospore, 1.6–2.5 μm in diameter. Endospores seem to be of the kind typical of the genus Bacillus; they are resistant to both heat and desiccation. Sporangia and vegetative cells are found as parasites in pseudocoeloms of several species of plant-parasitic nematodes. Has not been cultivated axenically. Type descriptive material consists of the descriptions and illustrations in this paper and elsewhere (Sayre et al., 1979, 1983).

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Literature Cited


