Ann. Inst. Pasteur/Microbiol.
1988, **139**, 11-31

PASTEURIA THORNEI SP. NOV. AND *PASTEURIA PENETRANS SENSU STRICTO* EMEND., MYCELIAL AND ENDOSPORE-FORMING BACTERIA PARASITIC, RESPECTIVELY, ON PLANT-PARASITIC NEMATODES OF THE GENERA *PRATYLENCHUS* AND *MELOIDOGYNE*

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SUMMARY

Descriptions are presented of two members of the *Pasteuria penetrans* group of mycelial and endospore-forming bacteria, parasitic on plant-parasitic nematodes. In one case, the epithet *P. penetrans sensu stricto* emend. has now been limited to members of this group with cup-shaped sporangia and ellipsoidal endospores, parasitic primarily on the root-knot nematode *Meloidogyne incognita*. The second organism, with rhomboidal sporangia and nearly spherical endospores, which is parasitic primarily on the root-lesion nematode *Pratylenchus brachyurus*, is assigned to *P. thornei* sp. nov. An updated and emended description is offered of the genus *Pasteuria* Metchnikoff 1888 emend. The relationships are analysed among these two nematode parasites and the type species of this genus, *P. ramosa* Metchnikoff 1888, a parasite of cladoceran invertebrates. Because none of these microbes has been publicly reported to have been cultivated axenically, these relationships are based mainly on morphological, developmental, and pathological criteria.

KEY-WORDS: *Pasteuria penetrans sensu stricto, Pasteuria ramosa, Pasteuria thornei* sp. nov.; Nematodes, Descriptions.

Received, July 22, 1987. Dedicated to the memory of Elie Metchnikoff (1845-1916), whose discovery of *Pasteuria* was reported in these *Annales* a century ago.

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INTRODUCTION

The genus *Pasteuria* Metchnikoff 1888 was established [**13**] for a bacterium, *P. ramosa,* that parasitizes cladoceran invertebrates, water fleas of the genus *Daphnia.* We have related elsewhere [**20, 26**] the complex of errors [**3, 4, 25**] that had confused understanding of this genus for the half-century before it became the subject of various modern investigations [**18, 23**] that elucidated its correct nature as an unusual mycelial and endospore-forming bacterium. To make a long story short, for our present purposes, conservation of the original descriptions of the genus *Pasteuria* and of *P. ramosa,* as updated, was recommended [**26**] and approved in Opinion 61 of the Judicial Commission [**7**]. Neither *P. ramosa* nor any of its relatives has ever been publicly reported to be cultivated axenically; they are known only from associations with their invertebrate hosts.

Confusion has also reigned in the understanding of the nature of *P. penetrans* (ex Thorne 1940) Sayre and Starr 1985, a parasite of plant-parasitic nematodes and currently the only other validly named species of the genus *Pasteuria.* For some seventy years after the first report of this microorganism by Cobb in 1906 [**2**], it was believed to be a protozoan that was eventually named «*Duboscqia penetrans*» by Thorne [**30**]. It was not until this nematode parasite was re-examined using electron microscopy that its true affinities to the bacteria rather than to the protozoa were recognized and the name «*Bacillus penetrans*» (Thorne 1940) Mankau 1975 was applied to it [**5, 10, 11**]. «*B. penetrans*» was not included in the 1980 Approved Lists of Bacterial Names [**24**]; hence, it had no taxonomic standing. In any case, although «*B. penetrans*» forms endospores reminiscent of those found in members of the genus *Bacillus* Cohn 1872, its other traits (*e.g.* mycelial habit, developmental cycle, and endoparasitic associations with plant-parasitic nematodes) suggested it did not belong in the genus, *Bacillus.* Rather, because it is related to *P. ramosa,* it has more properly been assigned [**19**] to *Pasteuria* Metchnikoff 1888, and named *P. penetrans* (ex Thorne 1940) Sayre and Starr 1985.

Recent tabulations [**19, 20, 29**] show that members of the *P. penetrans* group (mainly under its earlier names, «*D. penetrans*» and «*B. penetrans*»), have been reported from about 175 different nematode species belonging to some 70 nematode genera, from at least a dozen states of the United States, and from some 40 other countries or other political units on five continents (as well as islands in the Atlantic, Pacific, and Indian Oceans). Up to now, most studies on these bacteria have involved members of the *P. penetrans* group capable of parasitizing root-knot nematodes of the genus *Meloidogyne;*

RKP = root-knot *Pasteuria, i.e. P. penetrans sensu stricto.*
RLP = root-lesion *Pasteuria, i.e. P. thornei* sp. nov.
WFP = water flea *Pasteuria, i.e. P. ramosa* Metchnikoff 1888.

works by Nishizawa [**14, 15**] with such bacteria from cyst nematodes of the genera *Heterodera* and *Globodera* and by Sayre *et al.* [**21**] with related bacteria from the root-lesion nematode *Pratylenchus brachyurus* being notable exceptions. The significance of this confined attention becomes clear from the fact that, as presently conceived, *P. penetrans* is by no means a uniform entity. Rather, it constitutes an assemblage of numerous pathotypes and morphotypes; it probably also comprises a multiplicity of taxa, though their boundaries and categorial levels are by no means clear [**19**]. To alleviate some of the confusion in referring to these organisms, we repeat here our prior recommendation [**19, 20**] to the effect that the name *P. penetrans* (or its earlier synonyms «*D. penetrans*» and «*B. penetrans*») might quite justifiably be considered — at least during the current transitional period — as meaning «member(s) of the *P. penetrans* group» or a similar locution.

The preserved material on which Thorne [**30**] had based his description of «*D. penetrans*» has been re-examined [**21**]. As related elsewhere [**21**], this material involves a member of the *P. penetrans* group parasitizing the root-lesion nematode *P. pratensis* (now more correctly labelled *P. brachyurus*). This bacterium from the archival material and also from current infections of *P. brachyurus* was recently demonstrated [**21**] to be substantially different in endospore size and shape, developmental cycles, and host specificity from the one parasitizing the root-knot nematode *M. incognita,* on which material Sayre and Starr [**19**] had based the description of *P. penetrans*.

Clearly, the time has come to clarify the nomenclature of these bacteria! We present here a recapitulation of the observed differences between the members of the *P. penetrans* group occurring on *P. brachyurus* and on *M. incognita,* and name them respectively *P. thornei* sp. nov. and *P. penetrans sensu stricto* emend. Also included is an updated description of the genus *Pasteuria* Metchnikoff 1888 emend.

MATERIALS AND METHODS

Material examined.

The host for one member of the bacterial group under consideration here was the plant-parasitic nematode *P. brachyurus.* This host nematode was collected from roots of peanut plants using the method suggested by Chapman [**1**]. The collected specimens were examined microscopically for the external and internal presence of members of the *P. penetrans* group. Those nematodes found colonized by bacteria of this sort were prepared for light and electron microscopy using methods previously detailed by us [**17, 19, 21, 22**]. For the bacteria from *M. incognita,* we used the extensive archival material already available on photographic film (USDA Electron Microscope Laboratory) or as preserved specimens (USDA Nematode Collection), some of which have figured in our other publications (*e.g.* [**19, 20, 21, 22**]. Similarly, no new material was prepared for the type species of the genus *Pasteuria, P. ramosa;* instead, we have relied on available archival material consisting of photographic films (USDA Insect Pathology Laboratory), some of which have also been included in our previous reports [**17, 18, 23, 26**].

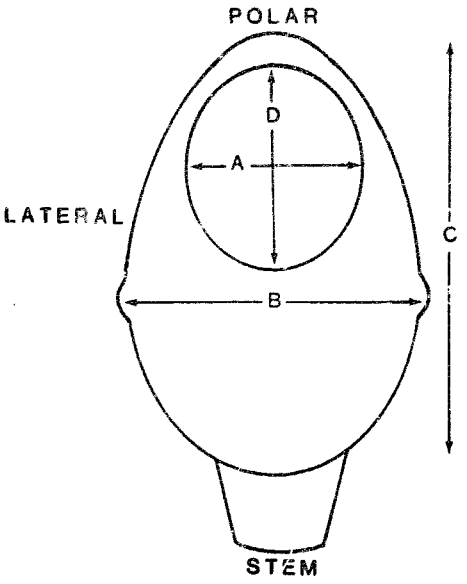


FIG. 1. — *Sketch of a sporangium of* P. ramosa, *type species of the genus* Pasteuria, *showing conventions used for obtaining standardized measurements and indicating orientation.*

A = width of endospore; B = diameter of sporangium; C = height of sporangium; D = height of endospore; « polar » refers to the apical end distal to the stem end; « stem » is the residual hyphal cell that remains attached to and carried away by the mature sporangium; « lateral » means situated at the side of the sporangium, 90° from the median line and perpendicular to the polar-stem axis.

Microscopy and measurements.

In collecting quantitative data on the microcolonies, we used as one indicator of a median section through a colony, the presence of one or more « sacrificial » intercalary cells showing lysis or separation. These cells are thought to be more or less central, in that they are responsible for the separation of a colony into two equal daughter colonies. Figure 1 indicates the conventions used for measuring and presenting the orientation of the mature *Pasteuria* sporangium and endospore.

The morphological features used to determine a median or true longitudinal section for measurements of *P. ramosa* were: (1) the presence of a complete stem cell (*i.e.* the residual hyphal cell that remained attached to and then was carried away by the mature sporangium), coupled with (2) central elliptical endospores, (3) positioned under high arching sporangial domes (fig. 11). Because stem cells were not usually attached to the mature sporangia of the members of the *P. penetrans* group, the central pore of the endospore was used as a partial indicator of the median plane in these nematode parasites. The criteria for a truly longitudinal section which could confidently be measured (fig. 19, 26) included: (1) visibility of the plane of the pore, (2) symmetry of endospores and parasporal fibres about the central axis, and (3) occurrence of the endospore under a polar arch where the multiple laminar spore coats were visible and well defined.

RESULTS AND DISCUSSION

The clear distinctions between the members of the *P. penetrans* group occuring on the root-lesion nematode *P. brachyurus* and the root-knot nematode *M. incognita* are summarized in table I. These bacteria differ significantly in sporangial shape and size, endospore shape and size, developmental cycles, and host specificity. To avoid cumbersome locutions, we will refer to the bacterium from the nematode *P. brachyurus* as RLP (root-lesion *Pasteuria*), to the bacterium from the nematode *M. incognita* as RKP (root-knot *Pasteuria*), and to the bacterium from the cladoceran *Moina rectirostris* as WFP (water flea *Pasteuria*).

Problems in quantitation of morphological features.

The quantitating morphological features of members of the *P. penetrans* group were not different from those belabouring most microscopists. These problems lay in the domains of specimen fixation, staining, and orientation. Early vegetative stages of the bacterial life cycle were often fleeting and simply not encountered in any of the sections examined. This difficulty was particularly apparent in the rapidly growing microcolonies of RLP; the formation of septa was rarely observed (fig. 22) and the usually accompanying mesosomes were not seen at all in RLP, albeit they have been reported in RKP [**19, 22**], and are illustrated here in WFP (fig. 8). Similarly, the «sacrificial» intercalary cells that lyse to yield daughter colonies were not seen in RLP, although they are shown here in RKP (fig. 15) and have been previously reported in WFP [**17**]. Because lysed «sacrificial» intercalary cells, the criterion for a median section of a microcolony, were not observed in any of our RLP preparations, accurate measurements of the central diameter of RLP microcolonies were not possible. With the onset of endosporogenesis, accurate measurements were more readily realized in all three organisms because of the distinctive and fixed morphology of these final developmental stages.

The methods used for light microscopy and scanning and transmission electron microscopy have involved different dehydration, fixation, and staining reagents. These different methodologies have considerable influence on the final sporangial dimensions and morphologies. The least sporangial shrinkage occurred during the very slow glycerine infiltration method used in light microscopy [**31**], the greatest shrinkage occurred during the ethanol dehydration used in scanning electron microscopy [**22**], while the treatments with glutaraldehyde and acetone used for transmission electron microscopy had an intermediate effect on sporangial dimensions. Essentially all the dimensions reported herein stem from data secured by transmission electron microscopy. Although systematic comparisons have not yet been made, dimen-

sions of some of the same material based on light microscopy and scanning electron microscopy can be found elsewhere [**19, 21**].

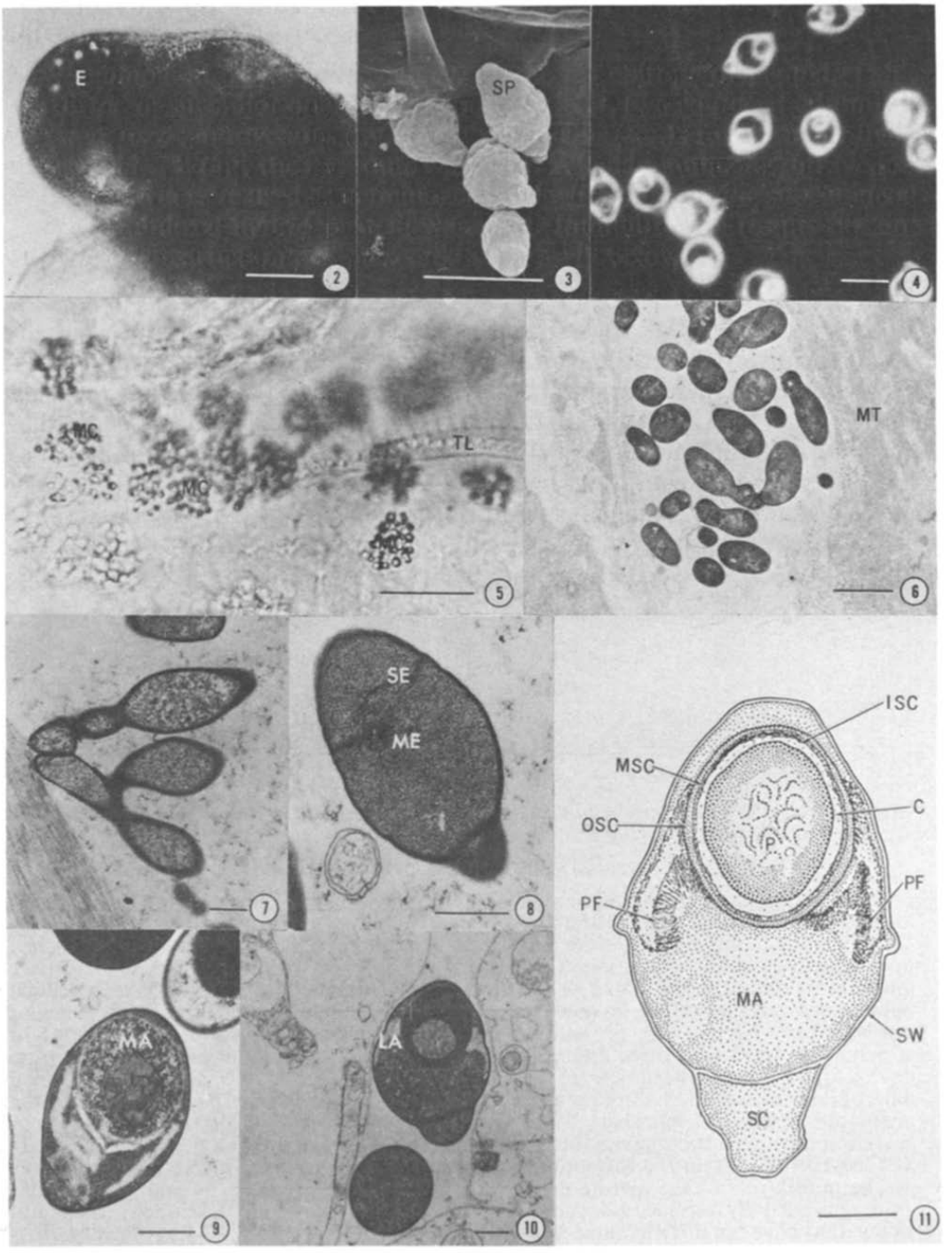
Sporangial shape and size.

In their initial developmental stages, the immature sporangia of WFP (fig. 6 and 7), RKP (fig. 16) and RLP (fig. 22) have much the same morphology *i.e.* the swollen and elongated distal cells of the microcolonies of all three bacteria will, under most circumstances, resemble one another. In fact, even the intermediate stages of the developing sporangia are similar in shape (fig. 8, 9, 17 and 23). It is only in the later stages of endosporogenesis that clear morphological differences become apparent. WFP has a distinctive three-part external marking corresponding to a conical stem cell, a swollen middle cell, and an apical endogenous spore; this tripartite marking was obvious in light micrographs (fig. 4) and in both scanning (fig. 3) and transmission (fig. 8, 9, 10 and 11) electron micrographs. The external features of the other two bacteria were much less obvious. In the scanning electron micrographs of RKP (fig. 12) and RLP (fig. 20), a two-part organization composed of a central dome or endospore surrounded by a circular ring of parasporal fibres was visible.

FIG. 2-11. — *Light micrographs, scanning and transmission electron micrographs, and a drawing showing life stages of* P. ramosa *parasitizing the water flea,* M. rectirostris.

Fig. 2. — Anterior of water flea parasitized by the bacterium showing its eye (E) virtually obscured by bacterial sporangia; bar = 100.0 μm. *Fig. 3* — Scanning electron micrograph of sporangia (SP) ruptured cuticle of a water flea, showing external endospore markings; bar = 5.0 μm. *Fig. 4.* — Dark-field micrograph of several sporangia showing the highly refractile endospores; bar = 5.0 μm. *Fig. 5.* — Micrograph of several vegetative microcolonies (MC) of *P. ramosa* in and adjacent to a thoracic leg (TL) of the water flea; bar = 10.0 μm. *Fig. 6.* — Section through muscle tissue (MT) delineating a channel in the haemocoel of the water flea and showing numerous individual and doublet sporangia; bar = 10.0 μm. *Fig. 7.* — Section through a mycelial colony attached to a muscular fibre bridging a sinus within the water flea; bar = 1.0 μm. *Fig. 8.* — Section through an expanding sporangium prior to endospore formation; a septu (SE) and mesosomes (ME) delineate the forespore area from the basal portion of the sporangium; bar = 1.0 μm. *Fig. 9.* — Section showing early stage of forespore formation; the granular matrix (MA) surrounding the central polar area has not formed a cortex or multilayered wall; bar = 1.0 μm.

Fig. 10. — Section of nearly mature sporangium showing initiation of cortex and multilayered wall; light areas (LA) adjacent to the endospore are possible precursors of parasporal appendages; bar = 1.0 μm. *Fig. 11.* — Drawing of a cross section through a typical sporangium of *P. ramosa,* detailing its morphological features. The central endospore contains a protoplast (P) showing stranded inclusions; the cortex (C), which surrounds the protoplast, is itself enveloped by inner (ISC), middle (MSC), and outer (OSC) spore coats; the parasporal fibres (PF) are attached to the outer spore coat and extend into the basal portion of the sporangium; a granular matrix (MA) is the predominant feature of the stem portion of the sporangium, which is entirely surrounded by the double-layered sporangial wall (SW); a stem cell (SC) remains attached to the sporangium. Bar = 1.0 μm.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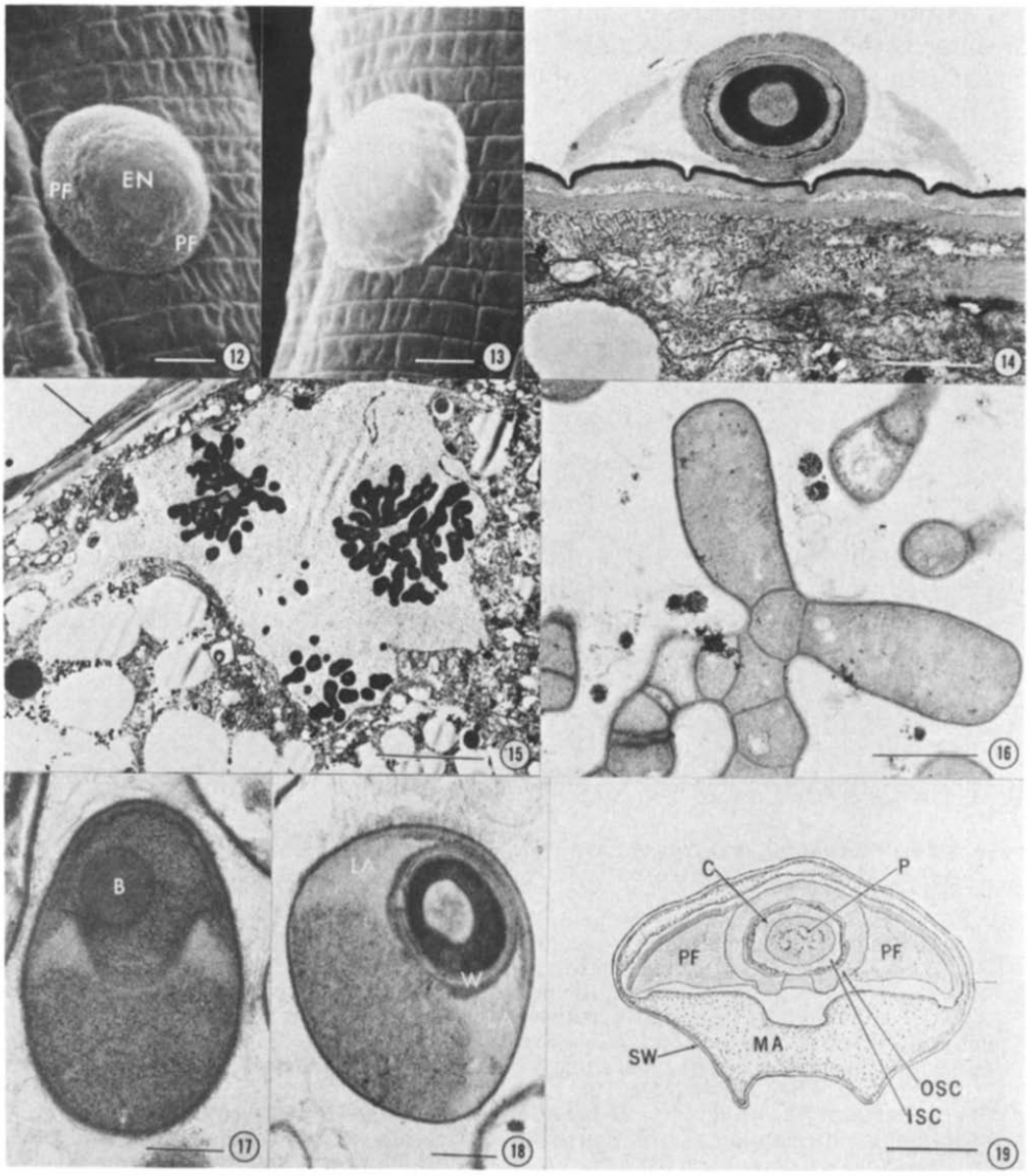
Careful examination of the transmission electron micrographs showed that the differences in sporangial morphologies among the three bacteria were largely the result of the arrangement of the parasporal structures, not the endospore itself. The manner in which the parasporal fibres were arranged about the endospore were particularly significant in determining sporangial shapes. The parasporal fibres of WFP were swept sharply downward from the endospore wall, and bent slightly inward on the matrix of the middle cell (fig. 10 and 11); this arrangement, and the attached basal stem cell, contribute to the teardrop shape of WFP sporangia, whose dimensions are 3.3 to 4.1 μm in diameter and 4.8 to 5.7 μm in height.

The role of the parasporal fibres in determining the final configurations of the sporangia of RKP (fig. 18 and 19) and RLP (fig. 24, 25 and 26) is similarly crucial. In RKP (fig. 18 and 19), the parasporal fibres only gradually arched downward from their points of attachment on the endospore wall, thus giving the RKP sporangia their typical cup-shaped appearance and their dimensions of 3.0 to 4.0 μm in diameter and 2.26 to 2.60 μm in height. The somewhat different parasporal fibres of RLP first bend sharply downward from the endospore wall, then remained nearly straight during their descent into the matrix. This arrangement contributed heavily to the rhomboidal shape of the RLP sporangia (fig. 25 and 26), which measure 2.22 to 2.70 μm in diameter and 1.96 to 2.34 μm in height.

FIG. 12-19. — *Light micrographs, scanning and transmission electron micrographs, and a drawing showing life stages of* P. penetrans sensu stricto *parasitizing the root-knot nematode,* M. incognita.

Fig. 12. — Scanning electron micrograph of a sporangium with central endospore (EN) attached to the cuticular surface of a nematode larva; note the surrounding parasporal fibres (PF); bar = 1.0 μm. *Fig. 13.* — Scanning electron micrograph of a sporangium attached to a nematode larva; a thin wrinkled wall, the exosporium, hides the central endospore and fibres; bar = 1.0 μm. *Fig. 14.* — Transmission electron micrograph of a longitudinal section through a nematode larva with an attached sporangium on the nematode's cuticular surface; bar = 1.0 μm. *Fig. 15.* — Transmission electron micrograph of a cross section of a nematode larva with three discrete microcolonies of the bacterium in the hypodermal layers directly under the cuticle (arrow); bar = 5.0 μm. *Fig. 16.* — Transmission electron micrograph of a section through a developing microcolony of the bacterium within the nematode's pseudocoelom; bar = 1.0 μm. *Fig. 17.* — Transmission electron micrograph of a section through a sporangium illustrating endospore development; a dark spherical body (B) has formed within the forespore; surrounding it is a granular matrix that condenses to the multilayers of the mature endospore; bar = 1.0 μm. *Fig. 18.* — Sporangium containing a partially formed endospore; the walls (W) are not fully developed; the lateral areas (LA) have not differentiated into fibres; bar = 1.0 μm. *Fig. 19.* — Drawing of a cross section through a mature sporangium of *P. penetrans sensu stricto* emend., the bacterial parasite of *M. incognita,* illustrating the ultrastructural features used in its identification: protoplast (P) containing stranded inclusions; multilayered structures including a cortex (C) and inner (ISC) and outer (OSC) spore coats; parasporal fibres (PF); matrix (MA); sporangial wall (SW). Bar = 1.0 μm.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Endospore shape and size.

The sizes and shapes of the endospores of the three bacteria are more nearly alike than those of their sporangia. All of the several stages of endosporogenesis have been observed in all three bacteria and some stages are illustrated here for WFP (fig. 8, 9, 10 and 11), RKP (fig. 17, 18 and 19), and RLP (fig. 23, 24, 25 and 26). Endospores are all oblate spheroids, *i.e.* ellipsoids which vary in shape in the following ways: RLP endospores are narrowly elliptic or almost circular in section with the major axis horizontal, *i.e.* parallel to the base of the sporangium (fig. 26); WFP endospores are also narrowly elliptic in section but the major axis is vertical, *i.e.* perpendicular to the base of the sporangium (fig. 11); RKP endospores are broadly elliptic in section with the major axis horizontal (fig. 19). The greater thickness of the endospore wall (table I) of RKP compared to WFP suggests one possible reason for the increased resistance of RKP endospores over WFP endospores. Characterization of the resistance of RLP endospores to heat and other environmental factors has not yet been determined. WFP has the largest endospores (2.1 to 2.4 μm in diameter); the smaller endospores of RKP and RLP measure 0.99 to 1.21 by 1.30 to 1.54 μm and 0.96 to 1.20 by 1.15 to 1.43 μm, respectively.

Developmental cycles.

The developmental cycles of the three bacteria are distinctively different with respect to duration. While much remains to be learned about this sub-

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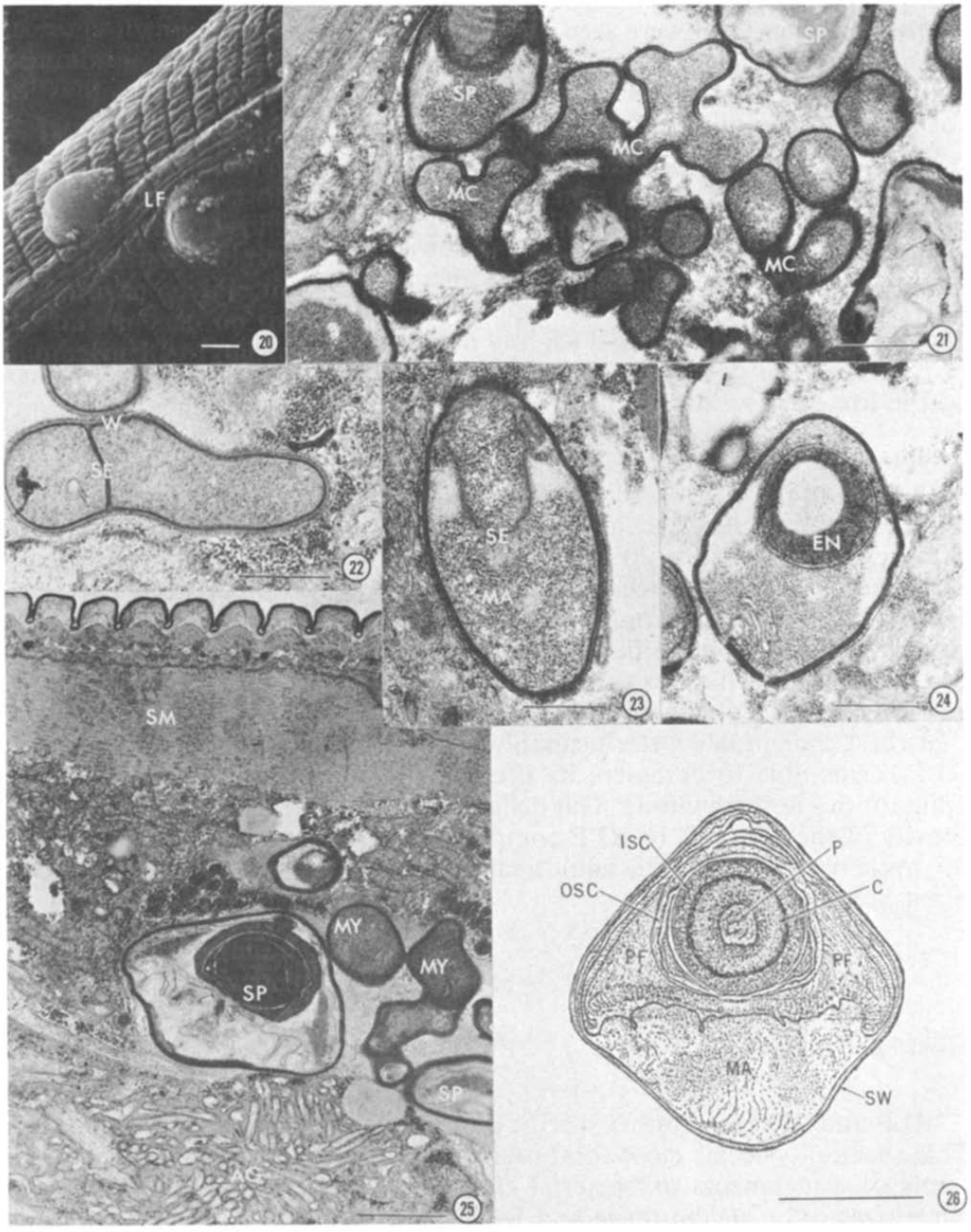
FIG. 20-26. — *Scanning and transmission electron micrographs and a drawing showing life stages of* P. thornei *sp. nov., parasitizing the root-lesion nematode* P. brachyurus.

Fig. 20. — Scanning electron micrograph of a nematode larva showing the nematode's lateral field (LF) slightly deformed by the presence of the attached bacterial sporangia; bar = 1 μm. *Fig. 21.* — Section of a nematode larva, showing vegetative microcolonies (MC) and sporangia (SP) of the bacterium in the nematode's pseudocoelomic cavity; bar = 1.0 μm. *Fig. 22.* — Section of bacterial vegetative mycelium, showing a septum (SE) and double cell wall (W); bar = 1.0 μm. *Fig. 23.* — Section of immature bacterial sporangium showing the developing septum (SE) separating the polar area that condenses into an endospore from the parasporal matrix (MA); the light areas sublateral to the polar area will develop into parasporal fibres; bar = 0.5 μm. *Fig. 24.* — Cross section of nearly mature bacterial sporangium; the light areas adjacent to the central thin-walled endospore (EN) will form the attachment fibres; bar = 0.5 μm. *Fig. 25.* — Longitudinal section of a nematode larva below the several cuticular annuluses and the [illegible] muscles (SM), bacterial vegetative mycelium (MY) and a mature sporangium (SP) formed in the pseudocoelom; the nematode's ampidial gland (AG) is located near the anterior end of the nematode; bar = 1.0 μm. *Fig. 26.* — Drawing of a cross section through a mature sporangium of *P. thornei* sp. nov., illustrating the ultrastructural features used in its identification: protoplast (P) containing stranded inclusions; multilayered structures including a cortex (C) and inner (ISC) and outer (OSC) spore coats; parasporal fibres (PF); matrix (MA); sporangial wall (SW). Bar = 1.0 μm.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ject, these temporal differences run along the following lines. WFP has the shortest life cycle. At 31°C, three days after adding dried preparations containing endospores into cultures of healthy water fleas *(M. rectirostris),* life stages of the bacteria were seen coursing through the haemocoeloms of the cladocerans (fig. 2 and 6). It was not possible to determine precisely whether fully mature sporangia were present, but the life cycle of WFP was only a few days in duration.

The relatively short life cycle of WFP contrasts sharply with the much longer life cycle of RKP. Sayre and Wergin [22] reported that the penetration of RKP into the larval host *(M. incognita)* was not observed until the eighth day after inoculation. The vegetative microcolonies were not formed until the fourteenth day, and mature sporangia were present only after about 30 days. Stirling [28], using a slightly higher temperature (30°C) and a different species of root-knot nematode *(M. javanica),* reported the duration of the life cycle as 20 days.

Although detailed studies have not yet been completed, our impression is that the life cycle of RLP is intermediate in duration between RKP and WFP. *Pratylenchus* species are known to moult four times and complete their life cycles in 30 days at 30°C [9]. All forms in the life cycle of RLP were observed in one or other of the migratory stages of *P. brachyurus* (fig. 21). During our light microscopic examinations of *P. brachyurus,* a few fourth-stage larvae were found filled with mature sporangia of RLP from the bases of their stylets to the tips of their tails. Because no normal tissues or food reserves (*e.g.* fat globules) were seen in these sporangia-laden larvae, we suspect that these individuals were incapable of again moulting to become adults. RLP seems able to complete its life cycle in any stage, larval as well as adult, of this host nematode. This behavior must have a bearing on the relative brevity of the life cycle of RLP compared to RKP; completion of the RKP life cycle occurs only in the adult nematode after it has begun feeding on its plant host.

Host specificity.

RLP and RKP have rather specific relationships with particular nematodes. This specificity seems close to absolute! When host specificity is scored in terms of attachments of bacterial endospores to nematode cuticles, RKP parasitizes only *Meloidogyne* and RLP parasitizes only *Pratylenchus,* and usually only one species of the indicated nematode genus (for details, see table 3 in [21], and table 3 in [20]). The only exception to this dogma in the literature is the case discussed elsewhere [21] of a RKP preparation from *M. javanica,* the endospores of which were reported [12] on one occasion to attach to *P. scribneri.*

Taxonomic considerations.

In establishing the species *P. penetrans,* Sayre and Starr [**19**] intended that this taxon be limited to bacteria of this sort with relatively small endospores, such as those found parasitizing nematodes of the genera *Pratylenchus* and *Meloidogyne.* Albeit the first clear description of a member of this group [**30**] dealt with a parasite of the root-lesion nematode *P. brachyurus,* most subsequent research and practically all of our current knowledge about these bacteria deal with them as parasites of various economically important root-knot nematodes of the genus *Meloidogyne.* Moreover, the descriptive type material [**19**] of *P. penetrans* refers exclusively to the bacteria as they occur in *M. incognita.* Since the nomenclatural code [**8**] requires that the name of a bacterial taxon be tied to the type material, the name *P. penetrans* in the strict sense must refer only to RKP, the parasite of *M. incognita.*

TABLE I. — **Comparison of *P. ramosa, P. penetrans sensu stricto* emend. and *P. thornei* sp. nov.**

Trait	*P. ramosa*	*P. penetrans*	*P. thornei*
Colony shape	Like cauliflower floret (fig. 5)	Spherical, to cluster of elongated grapes (fig. 15)	Small, elongate clusters (fig. 21)
Sporangia:			
— shape	Teardrop-shaped (fig. 11)	Cup-shaped (fig. 19)	Rhomboidal (fig. 26)
— diameter (µm)	3.3-4.1	3.0-4.0	2.22-2.70
— height (µm)	4.8-5.7	2.26-2.60	1.96-2.34
Sporangial wall's fate at maturity of endospore	Remains rigidly in place; external markings divide sporangium in three parts (fig. 3 and 4)	Basal portion collapses inward on the developed endospore; no clear external markings (fig. 19)	Remains essentially rigid, sometimes collapsing at bases; no clear external markings (fig. 26)
Exosporium	Not observed	Present (fig. 13)	Present (fig. 20)
Stem cell	Remains attached to most sporangia (fig. 2, 4, 8, 10 and 11)	Rarely seen; attachment of a second sporangium sometimes observed	Neither stem cell nor second sporangium seen
Endospore:			
— shape	Oblate spheroid, an ellipsoid, narrowly elliptic in section (fig. 11)	Oblate spheroid, an ellipsoid broadly elliptic in section (fig. 19)	Oblate spheroid, an ellipsoid sometimes almost spherical, narrowly elliptic in section (fig. 26)
Orientation of major axis to sporangium base	Vertical (fig. 11)	Horizontal (fig. 19)	Horizontal (fig. 26)
Dimensions (µm)	2.1-2.4	0.99-1.21 by 1.30-1.54	0.96-1.20 by 1.15-1.43
Wall thickness (µm)	0.17-0.23	0.28-0.34	0.22-0.26

Trait	*P. ramosa*	*P. penetrans*	*P. thornei*
Protoplast	Contains pronounced stranded inclusions (fig. 9 and 11)	Stranded inclusions sometimes seen (fig. 18 and 19)	Stranded inclusions observed (fig. 26)
Partial middle spore wall	Not observed	Surrounds endospore laterally, not in basal or polar areas (fig. 14 and 19)	Surrounds endospore somewhat sublaterally (fig. 25 and 26)
Pore:			
— presence	Absent	Present	Present
— characteristics	—	Basal annular opening formed from thickened outer wall (fig. 19)	Basal cortical wall thins to expose inner endospore (fig. 25 and 26)
— diameter (μm)	—	0.28 ± 0.11	0.13 ± 0.01
Parasporal structures:			
— fibres, origin and orientation	Long primary fibres arise laterally from cortical wall, bending sharply downward to yield numerous secondary fibres arrayed internally toward the granular matrix (fig. 11)	Fibres arise directly from cortical wall, gradually arching downward to form an attachment layer of numerous shorter fibres (fig. 19)	Long fibres arise directly from cortical wall, bending sharply downward to form an attachment layer of numerous shorter fibres (fig. 26)
— matrix, at maturity	Persists as fine granular material (fig. 11)	Becomes coarsely granular; lysis occurs; sporangial wall collapses; base is vacuolate (fig. 19)	Persists, but more granular; some strands are formed and partial collapse may occur (fig. 26)
Host	Cladocerans: *Daphnia; Moina* (fig. 2)	Nematodes: *Meloidogyne incognita* (fig. 12, 13 and 14)	Nematodes: *Pratylenchus brachyurus* (fig. 20 and 25)
Completes life cycle in nematode larvae	—	No, only in adult	Yes, in all larval stages and adult
Location in host	Haemocoel and musculature; sometimes found attached to coelom walls (fig. 7)	Pseudocoelom and musculature; no attachment to coelom walls seen (fig. 15)	Pseudocoelom and musculature; no attachment to coelom walls seen (fig. 21)
Attachment of spores on host	Spores not observed to attach or accumulate on surface of cladoceran	Spores accumulate in large numbers on cuticular surface (fig. 12, 13 and 14)	Spores accumulate in large numbers on cuticular surface (fig. 20)
Mode of penetration of host	Not known; suspected to occur through gut wall	Direct penetration of nematode cuticle by hyphal strand	Direct penetration suspected but not seen
Source of host	Pond mud, freshwater	Soil, plants	Soil, plants

TABLE II. — **Characteristics held in common by *P. ramosa* Metchnikoff 1888, *P. penetrans sensu stricto* emend. and *P. thornei* sp. nov.**

MORPHOLOGICAL SIMILARITIES AS OBSERVED BY LIGHT MICROSCOPY

Vegetative cells.
Microcolonies consist of dichotomously branched mycelium.
Diameter of mycelial filaments similar.
Mycelial filaments are seen in host tissues only during early stages of infection.
Daughter microcolonies seem to be formed by lysis of «sacrificial» intercalary cells.
Nearly all vegetative mycelium eventually lyses, leaving only sporangia and endospores.

Endospores.
Terminal hyphae or peripheral cells of the colony elongate and swell, giving rise to sporangia.
A single endospore is produced within each sporangium.
Endospores are in the same general size range.
Refractility of endospores, as observed in the light microscope, increases with maturity.

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.
Where they occur, mesosomes are similar in appearance and seem to be associated with division and septum formation.

Endospores.
Typical endogenous spore formation.
Identical sequences in endospore formation: (1) septa form within sporangia; (2) sporangium cytoplast condenses to form forespore; (3) endospore walls form; (4) final endospore matures; and (5) «light» areas adjacent to endospore give rise to extrasporal fibres.

SIMILAR SEQUENCES OF LIFE STAGES

Microcolonies.
Fragmentation of microcolonies.
Quartets of sporangia.
Doublets of sporangia.
Single sporangia.
Free endospores.

HOST-BACTERIUM RELATIONSHIPS

All 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 haemolymph or pseudocoelomic fluid by the parasite is extensive.
Host ranges are very narrow; for example, *P. ramosa* occurs only in cladoceran water fleas, *P. penetrans sensu stricto* only in the root-knot nematode *M. incognita,* and *P. thornei* sp. nov. only in the root-lesion nematode *P. brachyurus.*
Host is completely utilized by the bacteria; in the end, the host becomes little more than a bag of bacterial endospores.

SURVIVAL MECHANISMS

Survive in field soils and at bottoms of ponds.
Resist desiccation.
Moderately to strongly resistant to heat.

The members of the *P. penetrans* group from *P. brachyurus* and *M. incognita* under consideration here clearly belong in the genus *Pasteuria* Metchnikoff 1888 [**13**]. An updated and emended definition of this genus is given below. Table II summarizes the features of this genus, emphasizing the traits held in common among the type species of this genus, *P. ramosa* Metchnikoff 1888 [**13**], and the two related bacteria from nematodes (RLP and RKP) under consideration here.

RLP and RKP differ from each other and from *P. ramosa* Metchnikoff 1888 (table I). The several morphological and developmental differences between RLP and RLK summarized in the text and table 1, coupled with the clear host specificity, strongly suggest that these two members of the *P. penetrans* group should be placed in separate species. Because procedures for the axenic cultivation of members of the *P. penetrans* group have not yet been made public, many traits customarily employed in bacteriological classification are not now ascertainable. Hence, greater than normal dependence must be placed on the available morphological, developmental, and pathological features. However, we feel that carefully circumscribed taxa based on such features may help bridge the gap between our immediate need to delineate obviously different taxa in the *P. penetrans* group and our long-term expectation of reconciling such taxa into the mainstream of bacteriological taxonomy once procedures for the axenic cultivation of these bacteria become generally available.

Considerable reliance is placed on spore and sporangial morphology in the classification of other spore-forming bacteria. Comparable morphological

differences exist in the members of the *P. penetrans* group under consideration here. When these morphological dissimilarities are coupled with the demonstrated distinctions in host specificity and developmental cycles, delineation of these two distinct kinds of mycelial- and endospore-forming bacteria from *P. brachyurus* and *M. incognita* as separate species of the genus *Pasteuria* Metchnikoff 1888 seems to us to be fully warranted.

NOMENCLATURAL FORMALITIES

Genus *Pasteuria* Metchnikoff 1888, 166[AL] emend.

Genus *Pasteuria* Metchnikoff 1888, 166[AL] emend. Pas. teu' ri . a. M. L. gen. n. *Pasteuria* of Pasteur; named for Louis Pasteur, French savant and scientist. Archival synonyms include *Torula* or other yeast genera [**16**], *Duboscqia* [**30**] and other genera of microsporidian protozoa [**6, 32**], *Dermocystidium* [**27**], and *Bacillus* [**10**]. See also *Pasteuria* Metchnikoff 1888, 166[AL] emend. Sayre and Starr, 1985, 149[VP] [**19**]. Not *Pasteuria* in the sense of Henrici and Johnson [**3**], Hirsch [**4**], or Staley [**25**]; see [**26**].

Gram-positive, dichotomously branching, septate mycelium, the terminal hyphae of which enlarge to form sporangia and eventually endospores. Vegetative colonies are shaped like cauliflower florets or elongated grapes in clusters or small elongate clusters; daughter colonies are formed by fragmentation. The sporogenous cells at the periphery of the colonies are usually attached by «sacrificial» intercalary hyphae that lyse, causing arrangement of the developing sporangia in quartets, then in doublets, and finally as single, mature, teardrop-shaped or cup-shaped or rhomboidal sporangia. The rounded end of the sporangium encloses a single refractile endospore, 1.0 to 3.0 μm in major dimension, an oblate spheroid, ellipsoidal or almost spherical in shape, resistant to desiccation and elevated temperatures (one species has somewhat limited heat tolerance). Non-motile. Sporangia and microcolonies are endoparasitic 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, 166[AL]. Not *P. ramosa* Staley 1973, a quite different bacterium (not endospore-forming, nonmycelial, Gram-negative, not endoparasitic in cladocerans, budding, with non-prosthecate major appendages) belonging to the *Blastocaulis-Planctomyces* group [**26**]. Modern descriptions and illustrations of *P. ramosa* Metchnikoff 1888 can be found in publications by Sayre *et al.* [**17, 18, 21, 23**] and Starr *et al.* [**26**], and herein.

Pasteuria thornei **sp. nov.**

Pasteuria thornei sp. nov. thor' . ne . i. M. L. gen. n. named for Gerald Thorne, American nematologist, who described and named this *Pratylenchus* parasite as a protozoan, «*Duboscqia penetrans*».

Gram-positive vegetative cells. Mycelium is septate; hyphal strands, 0.2-0.5 μm in diameter, branch dichotomously. Sporangia, formed by expansion of hyphal tips, are rhomboidal in shape, about 2.22 to 2.70 μm in diameter and 1.96 to 2.34 μm in height. Each sporangium is divided into two almost equal units. The smaller unit, proximal to the mycelium, is not refractile and contains a granular matrix interspersed with many fibrillar strands. The refractile apical unit is cone-shaped; it encloses an ellipsoidal endospore, sometimes almost spherical, having axes of 0.96 to 1.20 by 1.15 to 1.43 μm, with cortical walls about 0.13 μm in thickness except for an additional inner sublateral wall that gives the endospore a somewhat triangular appearance in cross-section. The tapering outer cortical wall at the base of the endospore forms an opening approximately 0.13 μm in diameter. Sporangia and endospores are found as parasites of several root-lesion nematodes belonging to the genus *Pratylenchus;* they may also parasitize other kinds of plant-parasitic nematodes. Has not been cultivated axenically; the type descriptive material consists of the text and photographs in Sayre *et al.* [**21**] and herein.

Pasteuria penetrans **(ex Thorne 1940)** *sensu stricto* **emend.**

Pasteuria penetrans (ex Thorne 1940) *sensu stricto* emend. pen' . e . trans. L. v. *penetro,* present participle *penetrans* to enter. Synonyms: «*Duboscqia penetrans*» Thorne 1940 [**30**]; «*Bacillus penetrans*» (Thorne 1940) Mankau 1975 [**10**]; *Pasteuria penetrans* (ex Thorne 1940) Sayre and Starr 1985, 149^{VP} [**19**].

Gram-positive vegetative cells. Mycelium is septate; hyphal strands, 0.2 to 0.5 μm in diameter, branch dichotomously. The sporangia, formed by expansion of hyphal tips, are cup-shaped, about 2.26 to 2.60 μm in height with a diameter of 3.0 to 4.0 μm. Each sporangium is divided into two unequal sections. The smaller proximal body is not as refractile as the larger, rounded, cup-shaped portion which encloses an ellipsoidal endospore broadly elliptic in section having axes of 0.99 to 1.21 by 1.30 to 1.54 μm. 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 the pseudocoeloms of plant-parasitic nematodes. The epithet is now restricted to members of the *P. penetrans* group with cup-shaped sporangia and ellipsoidal endospores broadly elliptic in section occurring primarily as parasites of root-knot nematodes belonging to the genus *Meloidogyne,* particularly *M. incognita;* they also may parasitize other kinds of plant-parasitic nematodes. Has not been cultivated axenically; the type descriptive material consists of

the text and photographs in Sayre and Starr [19], Sayre *et al.* [21], and herein. *P. penetrans sensu stricto* differs from *P. thornei* sp. nov. and other members of the *P. penetrans* group in host specificity, in size and shape of sporangia and endospores, and in other morphological and developmental characteristics.

RÉSUMÉ

PASTEURIA THORNEI sp. nov. ET *PASTEURIA PENETRANS sensu stricto* emend., BACTÉRIES FORMANT DES ENDOSPORES ET DU MYCÉLIUM ET PARASITANT RESPECTIVEMENT LES NÉMATODES DU GENRE *PRATYLENCHUS* ET *MELOIDOGYNE*, PARASITES DES PLANTES

Dans ce travail sont présentés deux membres du groupe *Pasteuria penetrans*, bactéries formant des spores et du mycélium, et parasitant des nématodes parasites des plantes. Dans un cas, l'appellation *P. penetrans sensu stricto* emend. est maintenant limitée aux membres du groupe ayant un sporange en forme de coupe et des endospores ellipsoïdales, et qui sont les parasites principaux du nématode *Meloidogyne incognita* responsable d'une maladie des racines des plantes. Le 2e microorganisme, avec un sporange rhomboïdal et des spores quasiment sphériques et qui parasite surtout le nématode *Pratylenchus brachyurus* responsable de lésions des racines, est désigné comme *P. thorneii* sp. nov.

Une description revue et corrigée du genre *Pasteuria* Metchnikoff 1888 emend. est présentée. Les relations entre ces 2 parasites de nématodes et l'espèce-type du genre, *P. ramosa* Metchnikoff 1888, sont analysées. Etant donné qu'aucun de ces microorganismes n'est connu pour avoir été cultivé en milieu axénique, ces relations sont basées surtout sur des critères de morphologie, de croissance et de pathologie.

MOTS-CLÉS: *Pasteuria thornei* sp. nov., *Pasteuria penetrans sensu stricto, Pasteuria ramosa;* Taxonomie, Nematodes, Descriptions.

ACKNOWLEDGEMENTS

We are grateful to Dieter Sturhan for critical reading of the manuscript; to Phoebe Betty Starr for skilled bibliographic and redactional assistance; to William P. Wergin for advice and assistance in preparing the micrographs in figures 3, 12-18 and 20; to Jean Adams who helped prepare figures 6-10; to Burton Y. Endo for his contribution of figures 21-25; to Robert Ewing who drafted figures 1, 11 and 19; and to Chris Poole, Robert Reise and Sharon Ochs for proficient technical assistance.

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