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Observations on a *Pasteuria* Isolate Parasitic on *Hoplolaimus galeatus* in Peru[1]

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Abstract: A *Pasteuria* isolate associated with a population of the lance nematode *Hoplolaimus galeatus* was discovered in Peru. The infective propagules adhered to adult stages and juveniles and were found filling the bodies of males and females. The endospore and central core diameters measured 4.5 ± 0.4 µm and 1.9 ± 0.2 µm, respectively, which differed from those reported for other *Pasteuria* isolates found in North America on the same host. Examinations of endospore ultrastructure with scanning electron microscopy showed the presence of a thin layer of parasporal fibers surrounding the central core, a thin reduced layer of parasporal fibers in contact with the host's cuticle, and a putative basal core ring.

Key words: biodiversity, biological control, endospore, *Hoplolaimus galeatus*, lance nematode, nematode, parasitism, *Pasteuria penetrans*, Peru.

Pasteuria spp. (Actinomycetes) include specialized parasites that complete their life cycle inside nematode hosts (Ciancio, 1995; Sayre et al., 1988, 1991; Starr and Sayre, 1988; Stirling, 1991; Sturhan et al., 1994). In the soil environment and outside the nematode body, *Pasteuria* isolates occur as durable and resistant propagules perpetuating infection through passive host interception. *Pasteuria* isolates have shown promise as nematode biological control agents when introduced into soil (Stirling, 1991) and have given natural biological control or nematode suppression in the field (Weibelzahl-Fulton et al., 1996).

In recent years, several *Pasteuria* variants have been discovered in widely separated nematode taxa (Ciancio, 1995; Giblin-Davis et al., 1990, 1995; Jaffee et al., 1985: Sayre et al., 1988; Sturhan et al., 1994). However, the taxonomic criteria utilized for the identification and description of new species, e.g. endospore morphometrics and host genera, fit neither the isolates having different morphometrics and parasitizing nematodes within the same genera or species, nor the isolates having similar morphometrics but remarkably distant hosts (Ciancio, 1995; Giblin-Davis et al., 1990). The biodiversity observed at the phenotypic level suggests a complex process of host and parasite coevolution and speciation (Ciancio, 1995).

During a survey of plant-parasitic nematodes and microbial antagonists in Peru, a population of the lance nematode, *Hoplolaimus galeatus* (Cobb) Thorne, was observed to be infected with a new *Pasteuria* isolate. We illustrate the ultrastructure and morphology of this isolate and provide data on parasitism ecology and morphometrics.

Materials and Methods

Soil samples were collected near Juliaca, Puno, Peru, in August 1995. Nematodes were extracted from soil with a sieving and decanting technique, with 2-mm- and 45-µm-pore sieves, and stored in 4% formalin until examined. For identification and measurement with light microscopy at ×500–1,250, specimens of *H. galeatus* were handpicked from the suspension, dehydrated to glycerol by a slow method (Southey, 1970), and mounted in glycerol. For scanning electron microscopy (SEM), dehydrated specimens of *H. galeatus* with adhering endospores were cleared of excess glycerol, mounted on a metal stub, and sputter-coated with gold in vacuum (Sher and Bell, 1975). The nematodes were examined with a Stereoscan 360 SEM at 3 kV.

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Results and Discussion

A new geographic record is herein established for *H. galeatus* and an associated *Pasteuria* isolate in Peru. The nematode population was discovered in a small fallow field, located 2 km north of Juliaca, Puno, on the road to Lampa, at more than 3,800-m altitude. This field was cultivated the previous cropping season for production of oat and potato.

Specimens of *H. galeatus* were found in 72% of the Juliaca samples examined, with only one sample positive for *Pasteuria*. The nematode density in this sample was 82/liter soil. Endospores (Fig. 1C–E) were found adhering to males, females, and juveniles. The percentage of nematodes with adhering propagules was 30.2%, but only 1.2% of the males and females examined were filled with propagules (Fig. 1A,B). Thalli were observed as thin diffused mycelia, often visible in the cephalic or caudal regions (Fig. 1F). The parasite prevalence was 14.7%.

The endospores and central core diameters of propagules adhering to *H. galeatus*

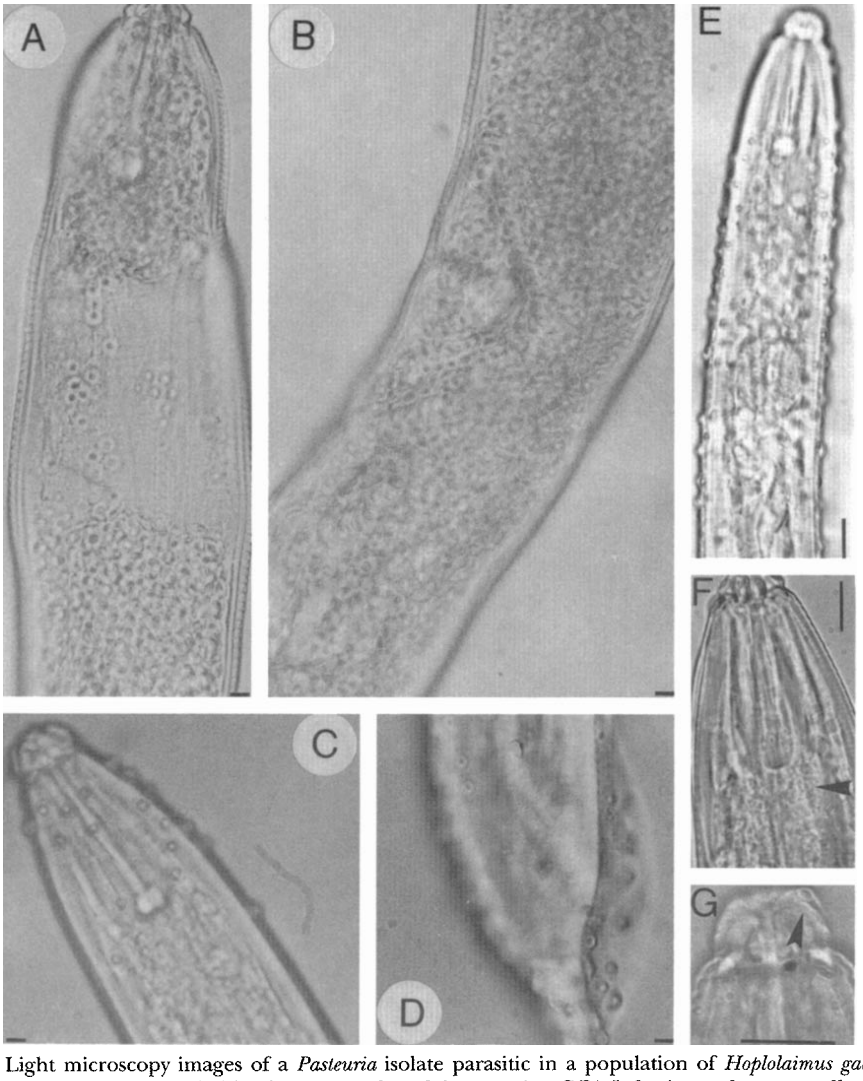

Fig. 1. Light microscopy images of a *Pasteuria* isolate parasitic in a population of *Hoplolaimus galeatus* from Peru. A,B) Female specimen filled by the propagules of the parasite. C,D) Infective endospores adhering to the cephalic region and bursa. E) Male encumbered with several infective propagules. F) *Pasteuria* vegetative thallus in the esophageal region of the host (arrowhead). G) Endospore adhering to the first labial annule (arrowhead). Scale bars: A, B = 6 μm; C, D = 3 μm; E–G = 10 μm.

were 4.5 ± 0.4 µm (mean ± SD), and 1.9 ± 0.2 µm, respectively, as measured with light microscopy. The spore height was 1.6 ± 0.3 µm. The thickness of the host cuticle and hypodermis, measured at midbody on nematodes with adhering endospores, was 5.8 ± 0.3 µm. The number of endospores per infested nematode was 1 to 25. Specimens with high numbers of endospores (>250) also were observed (Fig. 1E). The endospores adhered to all body regions, including the first labial annule and bursa (Figs. 1C–E,G; 2A,B). When examined by light microscopy, some adhering endospores appeared refractile, and a ring marking the central core boundary was visible (Fig. 1C,D). At the completion of the *Pasteuria* life cycle, adult nematodes appeared completely filled with mature endospores and the only body parts apparently not decom-

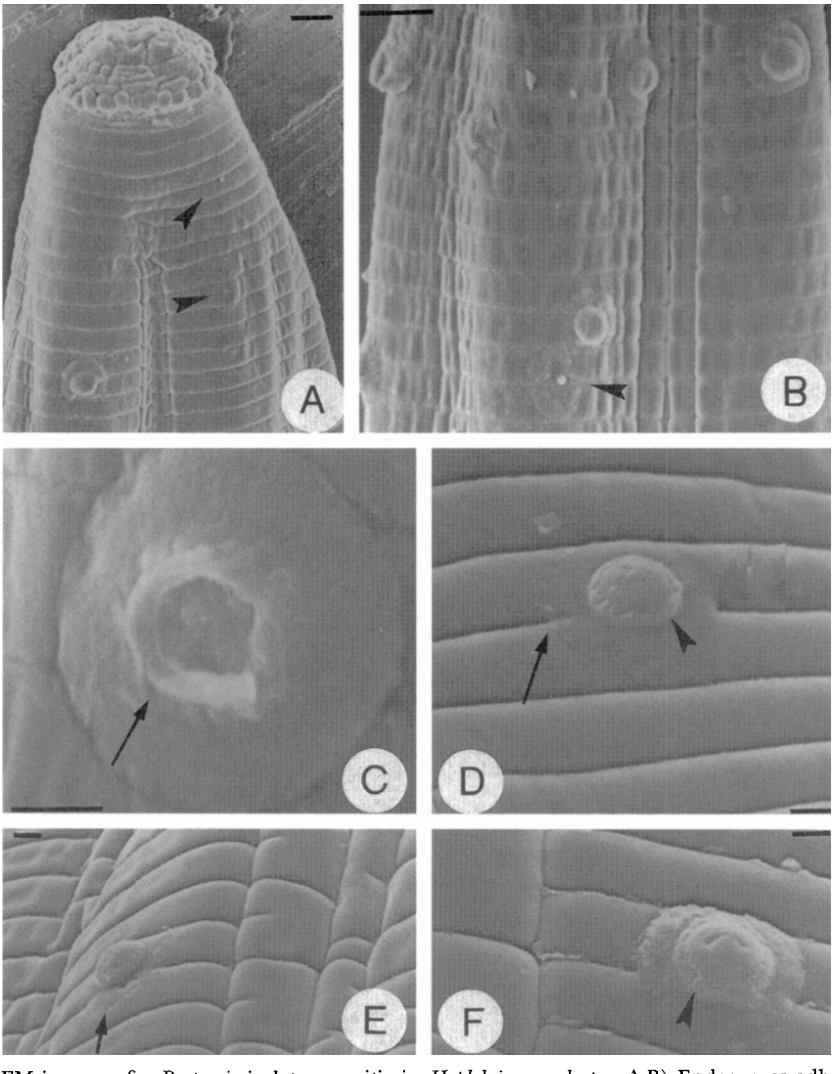

FIG. 2. SEM images of a *Pasteuria* isolate parasitic in *Hoplolaimus galeatus*. A,B) Endospores adhering to the nematode anterior region and cuticular marks attributable to previously adhering endospores (arrowheads). C) Collapsed endospore still covered by remnants of the exosporium, showing the occurrence of an inner ring surrounding the central core (arrow). D) Endospore showing a thin layer of adhering parasporal fibers following the profile contour of the host cuticle (arrow) and a further reduced layer of fibers surrounding the central core (arrowhead). E) Central endospore core sticking to the nematode cuticle with surrounding cuticular incisions (arrow) attributable to detached parasporal fibers. F) Partially collapsed endospore showing the occurrence of a higher external layer of parasporal fibers (arrowhead). Scale bars: A, B = 4 µm; C–F = 1 µm.

posed by the parasite were sclerotized organs, including the cuticle, stylet, and vulval lips (Fig. 1A,B).

The general structural organization of the infective propagules consisted of a central core and external surrounding fiber layers. Infective endospores appeared round and symmetric, with a central, globose, or hemispherical-shaped core, clearly distinct and emerging from a basal layer of parasporal fibers (Fig. 2A,B,D). In some cases, the core appeared empty or partially collapsed (Fig. 2C,F), showing a putative internal basal core ring (Fig. 2C). A low, external ring of parasporal fibers 0.3 μm high surrounding the central core also was observed (Fig. 2D,F). The layer of parasporal fibers in contact with the host cuticle appeared thin and delicate, in some cases following the profile contour of the cuticle annulations (Fig. 2D,F). In some SEM images the parasporal fibers were not present, whereas the central core remained attached, showing a circular mark or incisions on the cuticle area where fibers might have been attached previously (Fig. 2E). In other cases, a circular mark reminiscent of the shape and dimensions of detached endospores was clearly visible on the cuticle, with a central, 0.3-μm-wide spot, possibly the result of germ tube penetration or lytic activity related to germination (Fig. 2A,B).

These data suggest that, in the genus *Hoplolaimus,* a significant degree of *Pasteuria* morphometric diversity can be expected even among isolates parasitizing populations of the same host species. Two distinct *Pasteuria* isolates previously reported from *H. galeatus* in Florida (Giblin-Davis et al., 1990) showed endospore diameters respectively smaller and larger than those of the Peruvian isolate. The Peruvian isolate also had endospore diameters smaller than those reported for other *Pasteuria* isolates associated with two additional *H. galeatus* populations found in Florida and Pennsylvania (Jaffee et al., 1985). They were also smaller than the diameters observed for an isolate parasitizing *H. columbus* in South Carolina, whose endospores measured 5.7 ± 0.2 μm (D. Harshman, pers. comm.). Considering adhesion to the host cuticle as a selective barrier, these observations suggest that the endospore biodiversity observed might be related to differences in cuticle structure among these populations. *Hoplolaimus* spp. have a robust, thick cuticle associated with a high degree of resistance to temperature, chemical, and osmotic stresses. Morphological variations also occur in *H. galeatus,* with a high frequency of juvenile and female heteromorphism (Lewis and Fassuliotis, 1982).

Morphological differences among endospores appeared also significant. The infective propagule of the Peruvian *Pasteuria* isolate, although similar to other isolates in their general structure, showed two thin layers of adhesive fibers. A layer of fibers running externally around the base of the central core (Fig. 2D,F) has not been previously reported. A "double ring" endospore was observed with light microscopy in two distinct *Pasteuria* isolates parasitic in *H. galeatus* (Sayre et al., 1988). In *P. penetrans* and other *Pasteuria* isolates from lesion, spiral, or cyst nematodes, the parasporal fibers show two layers, visible with transmission electron microscopy (TEM) as a top matrix of elongated fibers connected to the cortical wall, or as a basal layer with shorter fibers oriented toward the host's cuticle (Ciancio, 1995; Sayre et al., 1991; Starr and Sayre, 1988; Stirling, 1991; Sturhan et al., 1994). In *P. nishizawae,* an additional basal layer was found (Sayre et al., 1991). It is possible that the small core collar of parasporal fibers observed results from the regression of the top fiber matrix, or that it develops to facilitate passive host interception. The presence of a regressed superior layer could account for the low height and thickness of the parasporal fibers in contact with the host cuticle surface (Fig. 2D,F).

The external ring of fibers matches an inner core ring observed in relief in collapsed endospores (Fig. 2A). In *P. penetrans,* an electron-dense basal core ring is visible in TEM sections. Rings present within or surrounding the core wall in basal-median locations were found in two *Pasteuria* isolates parasitizing *Belonolaimus longicaudatus* (Giblin-Davis et al., 1995) and *Xiphinema diversi-*

caudatum (A. Ciancio and R. Favre, unpubl.), respectively. However, in TEM sections of a large *Pasteuria* isolate parasitizing *H. galeatus* in Florida, no core rings were visible in or surrounding the core walls (Giblin-Davis et al., 1990). The function and occurrence of rings or thickenings surrounding the central core are not yet known. They could be related to the insertion of adhesive fibers onto the core wall, thus representing a structural and morphological component of taxonomic significance.

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