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Occurrence, hosts, morphology, and molecular characterisation of *Pasteuria* bacteria parasitic in nematodes of the family Plectidae

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Abstract

Parasitic bacteria of the genus *Pasteuria* are reported for three *Anaplectus* and four identified and several unidentified *Plectus* species found in eight countries in various habitats. The pasteurias from plectids agree in essential morphological characters of sporangia and endospores as well as in developmental cycle with those of the *Pasteuria* species and strains described from tylenchid nematodes, but appear to be mainly distinguished from these by absence of a distinct perisporium in the spores and the endospores obviously not being cup- or saucer-shaped. The wide range of measurements and morphological peculiarities of sporangia and endospores suggest that probably several *Pasteuria* species have to be distinguished as parasites in Plectidae. From an infected juvenile of an unidentified plectid species the 16S rRNA gene sequence of *Pasteuria* sp. was obtained. Substantial sequence divergence from described *Pasteuria* species and its phylogenetic position on molecular trees indicate that this *Pasteuria* sp. could be considered as a new species. Preliminary results of the analysis of DNA phylogeny of *Pasteuria* spp. and their nematode hosts provide evidence for incongruence of their phylogenetic history and of host switching events during evolution of the bacterial parasites.

Keywords: *Anaplectus*; Bacterial parasite; Coevolution; Development; Distribution; Hosts; Morphology; Nematodes; *Pasteuria*; Phylogeny; *Plectus*; 16S rRNA sequence

1. Introduction

Bacteria of the genus *Pasteuria* are common parasites of nematodes, not only of plant-parasitic nematodes, but of a wide variety of soil-inhabiting nematodes. The most recent list of hosts records more than 300 nematode taxa, the majority being members of the orders Tylenchida and Dorylaimida (Chen and Dickson, 1998). The four *Pasteuria* species described from nematodes are parasites of plant-parasitic Tylenchida: *Pasteuria penetrans* (*ex*Thorne 1940) Sayre and Starr 1986 from *Meloidogyne*, *Pasteuria thornei* (Sayre and Starr, 1988) from *Pratylenchus*, *Pasteuria nishizawae* Sayre et al., 1991 from *Heterodera* and *Pasteuria usgae* Giblin-Davis et al., 2003 from *Belonolaimus longicaudatus*. Additional *Pasteuria* forms, which have been studied more in detail, are also from Tylenchida, e.g., from *Hoplolaimus galeatus* (Giblin-Davis et al., 1990); *Heterodera goettingiana* (Sturhan et al., 1994; Winkelheide and Sturhan, 1993); *Heterodera avenae* (Davies et al., 1990); *Heterodera cajani* (Sharma and Davies, 1996); *Tylenchulus semipenetrans* (Kaplan, 1994); *Trophonema okamotoi* (Inserra et al., 1992); *Tylenchorhynchus cylindricus* (Galeano et al., 2003). Also, the

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recent molecular studies on *Pasteuria* are confined so far to *P. ramosa* Metchnikoff, 1888 from Cladocera and to described species and further isolates from Tylenchida (Anderson et al., 1999; Atibalentja et al., 2000; Bekal et al., 2001; Ebert et al., 1996; Leonetti et al., 2001; Preston et al., 2003; Wang et al., 2003).

Parasitism of Plectidae by *Pasteuria* has been reported a few times: for *Anaplectus granulosus* from Germany and Iceland and for *Plectus* sp. from Germany (Sturhan, 1985), for *Anaplectus grandepapillatus*, *Plectus acuminatus*, *Plectus cirratus* and *Plectus rhizophilus* from Germany (Sturhan in Sayre and Starr, 1988), for *P. acuminatus* and *Plectus parvus* from Russia (Subbotin et al., 1994). With the exception of a single photomicrograph showing sporangia in the body of *P. acuminatus* (Subbotin et al., 1994) no data on morphology, ultrastructure or dimensions of *Pasteuria* sp. in plectids have been published.

Some more specimens of various Plectidae species parasitised by *Pasteuria* were subsequently collected by the senior author in several countries. Despite this material is still scarce and no attempt could be made to do scanning or transmission electronic microscopic studies, light microscopic observations on the morphology of these bacteria, on the developmental cycle, on hosts and distribution are compiled in the present paper. The main reason for presenting these preliminary data, however, is the fact that DNA sequencing of the 16S rRNA gene of *Pasteuria* from an unidentified plectid nematode has been successful. The results of these first molecular studies on a *Pasteuria* form from a nematode taxon outside the Tylenchida are detailed in this paper.

2. Materials and methods

2.1. Nematode and Pasteuria material

Specimens of *Plectus* and *Anaplectus* species parasitised by *Pasteuria* were mostly found when nematode suspensions fixed with hot TAF (triethanolamine–formalin–distilled water) were analysed at higher microscopic magnifications. The nematodes had in general been isolated from soil or moss samples by the sieving-decanting method with final extraction through a Baermann funnel or by the centrifugation–flotation method with $MgSO_4$. The infected plectid specimens were handpicked from the suspensions and subsequently transferred to glycerin by a slow evaporation method and mounted on permanent slides for morphological studies. The nematode-*Pasteuria* samples and their origin are listed in Table 1. The numbers subsequently used in the text and in the legends of the figures refer to the numbers of nematodes/*Pasteuria* sources given in Table 1. The permanent microscopical slides of plectids with *Pasteuria* are deposited in the German Nematode Collection (DNST) at Biologische Bundesanstalt, Münster. One slide with infected *Plectus turricaudatus* specimens had been supplied by H.H. Zell (No. 17 in Table 1).

Table 1
Host, source, nematode stage, and infection site of *Pasteuria* used in the present study

No.	Nematode species	Source of nematodes	Nematode stage (number of specimens)	Infection site
1	*A. grandepapillatus*	Germany, coastal dunes	Female (1), juvenile (1)	Pseudocoelom
2	*A. granulosus*	Germany, grassland	Female (1)	Pseudocoelom
3	*A. granulosus*	Germany, grassland	Juvenile (2)	Cuticle, pseudocoelom
4	*A. granulosus*	Iceland, grassland	Juvenile (1)	Pseudocoelom
5	*A. granulosus*	USA, grassland	Female (1)	Pseudocoelom
6	*A. granulosus*	Dominica, grassland	Male (1)	Cuticle, pseudocoelom
			Female (1)	Cuticle
7	*A. granulosus*	New Zealand, bowling green	Male (2)	Cuticle
			Female (1)	Pseudocoelom
			Juvenile (1)	Cuticle, pseudocoelom
8	*A. granulosus*	New Zealand, bowling green	Female (2)	Pseudocoelom
			Juvenile (1)	Cuticle
9	*A. granulosus*	New Zealand, grassland	Juvenile (1)	Cuticle
10	*A. porosus*	Germany, grassland	Juvenile (1)	Pseudocoelom
11	*Anaplectus*/*Plectus* sp.	Germany, woodland	Juvenile (1)	Pseudocoelom
12	*P. acuminatus*	Germany, grassland	Juvenile (1)	Pseudocoelom
13	*P. cirratus*	Germany, grassland	Juvenile (2)	Pseudocoelom
14	*P. longicaudatus*	Germany, woodland	Female (1)	Pseudocoelom
15	*P. rhizophilus*	Germany, woodland	Female (1)	Pseudocoelom
16	*P. rhizophilus*	Germany, grassland	Female (1)	Pseudocoelom
17	*P. turricaudatus*	Finland, Sphagnum	Female (4)	Cuticle, pseudocoelom
18	*Plectus* sp.	Germany, woodland	Juvenile (2)	Cuticle, pseudocoelom
19	*Plectus* sp.	Germany, grassland	Female (1)	Pseudocoelom
20	*Plectus* sp.	Germany, river sediment	Female (1)	Pseudocoelom
21	*Plectus* sp.	Germany, moss	Female (1)	Cuticle, pseudocoelom
22	*Plectus* sp.	Madeira, vineyard	Juvenile (1)	Pseudocoelom

For the molecular studies a single "large" mobile plectid juvenile with its body filled with *Pasteuria* sporangia and several morphological similar uninfected juveniles were picked out from an unfixed nematode suspension isolated from woodland soil (No. 11 in Table 1) and air-dried in a small drop of water in an Eppendorf tube. The specimen was probably a member of the genus *Plectus*. Plectids identified in the same sample were *P. thornei*, *Plectus* sp., *Anaplectus granulosus* and *A. porosus*. In no additional plectid specimen *Pasteuria* attack was observed. Also in numerous soil samples subsequently taken at the same sampling site, each containing plectids, no further specimens with *Pasteuria* were found, but one specimen each of *Cylindrolaimus communis* and *Aporcelaimellus obtusicaudatus* with *Pasteuria* sporangia in the pseudocoelom.

2.2. Light microscopy

A total of only about 30 plectid specimens with *Pasteuria*, ranging from specimens with a single spore attached to the cuticle to specimens filled with mature sporangia were available. Because of this little material and the fact that all nematodes were fixed, no specimens could be squashed to release different *Pasteuria* developmental stages from the nematodes pseudocoelom. For the morphological observations, microphotography and measurements made under light microscopes with DIC optics were used.

Measurements were taken of sporangia which were considered as mature, when coats of the central body of the endospores were visible or the central bodies in sporangia within an infected nematode were of uniform diameter. Height and width of sporangia were measured when sporangia were distinctly in lateral position. The size of endospores within the sporangia could not be identified.

The terminology used in this paper is in agreement with that used or proposed by Sturhan et al. (1994). The translucent structures encircling the central highly refractile "central body" of the endospores are referred to as "perisporium", not as "parasporal" fibers (as used in most *Pasteuria* publications), because they are part of the spores. In bacteriology structures developing in a sporangium *outside* the endospore are generally designated as "parasporal" (Greek peri = around, para = besides). When coats can be distinguished, the inner part of the central body is called "core". Walls of the central body (= coats) and core are mostly difficult to observe in *Pasteuria* endospores from plectids but they are very distinct in germinated "empty" spores.

2.3. DNA extraction from Pasteuria sp., amplification and cloning of 16S rRNA gene

Total bacterial DNA was obtained from an unidentified plectid juvenile carrying *Pasteuria* endospores in pseudocoelom. Three hundred microlitres of decoating buffer (50 mM Tris, pH 9.5; 1% sodium dodecyl sulphate; 8 M urea; 50 mM dithiothreitol; and 10 mM EDTA) was added to the Eppendorf tube with the nematode sample, which was then incubated with shaking at 60 °C for 90 min. The suspension was centrifuged and washed three times in buffer (10 mM Tris–HCl, pH 8.0; 10 mM EDTA, and 150 mM NaCl). The pellet was lysed and the total DNA was purified by the method used for DNA isolation from *Bacillus* organisms (Cutting and Vander Horn, 1990). The 16S rRNA gene was amplified with forward 27F (5′-AGAGTTTGATCCTGGCT CAG-3′) and reverse 1522R (5′-AAGGAGGTGATC CARCCGCA-3′) primers using *Taq* polymerase and amplification buffer (Fermentas, Lithuania) in a GeneAmp PCR system 2400 (Perkin–Elmer Applied Biosystems) under the following conditions: DNA denaturation of 5 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 57 °C and 1 min 20 s extension at 72 °C, and a final extension at 72 °C for 7 min. Amplification products were purified with the PCR Purification Kit (QIAquick, Qiagen) and were cloned with a pGEM-T cloning vector (Promega, Madison, WI). Streamlined method to analyse 16S rRNA gene clone libraries was used (Vergin et al., 2001). The inserted DNA was re-amplified using 27F and P1R primers to search for *Pasteuria*-like 16S rRNA gene sequences. Primer P1R (5′-GATTCC TACTTCAYGCAGGC-3′) was designed for specific amplification of *Pasteuria*-like 16S rRNA gene sequences on the basis of unique and consensus sequences among *Pasteuria* and related sequences within the family Alicyclobacillaceae. Three clones carrying *Pasteuria*-like 16S rRNA gene were subjected for sequencing. Internal 357F (5′-CTCCTACGGGAGG CAGCAG-3′) and 1100R (5′-GGGTTGCGCTCG TTG-3′) primers as well as 27F and 1522R primers were used for sequence reactions.

2.4. DNA extraction from nematodes and amplification of D2–D3 expansion region of 28S gene

Detailed protocols for DNA extraction from nematodes and PCR are described by Tanha Maafi et al. (2003). The forward D2A (3′-ACAAGTACCGTGA GGGAAAGTTG-5′) and reverse D3B (3′-TCGGAA GGAACCAGCTACTA-5′) primers were used in the present study to amplify the fragment of 28S rDNA gene and for the subsequent sequence reactions.

2.5. DNA sequencing

DNA fragments were sequenced with a terminator cycle sequencing reaction kit (BigDye Perkin–Elmer Applied Biosystems, UK) according to the manufacturer's instructions. The resulting products were purified and run on a DNA sequencer (Model 377, PE Applied

Biosystems). Sequences of three clones of the 16S rRNA gene from *Pasteuria* sp. and the D2-D3 expansion fragment of 28S gene of unidentified plectid nematode were deposited in the GenBank under Accession Nos. AY652776–AY652778 and AY652779, respectively.

2.6. Alignment and phylogenetic analysis

The newly obtained sequences of the 16S rRNA gene of *Pasteuria* sp. were aligned with nearly full length sequenced 16S rRNA gene of *Pasteuria* spp. (Anderson et al., 1999; Atibalentja et al., 2000, unpublished; Ebert et al., 1996; Giblin-Davis et al., 2003) and partly sequenced clones of the 16S gene (ca. 600 bp) of *Pasteuria* spp. extracted from soil samples (Duan et al., 2003). The 16S rDNA sequences of *Thermoactinomyces dichotomicus* and *Alicyclobacillus hesperidum* were used as outgroups. The D2–D3 sequence of the unidentified plectid nematode was aligned with those for *Meloidogyne arenaria* (De Ley et al., unpublished), *Belonolaimus longicaudatus*, *Heterodera glycines*, *H. goettingiana* (Subbotin et al., unpublished) and *Daphnia magna* (Swain and Taylor, 2003). Sequence alignments were made by the computer program Clustal X1.64 with default options.

Sequence alignment was analysed with maximum likelihood (ML) and maximum parsimony (MP) methods using PAUP* 4.0b10 (Swofford, 2002). For MP heuristic search setting was used with ten replicates of random addition. Gaps were treated as missing data. For ML the appropriate substitution model of DNA evolution that best fitted the data set was determined by the Akaike Information Criterion with ModelTest 3.04 (Posada and Crandall, 1998). Estimates of support for clades were obtained by non-parametric bootstrap analysis with 100 replicates for ML and 1000 replicates for MP.

3. Results

3.1. Distribution and hosts

Parasitism of nematodes of the family Plectidae by *Pasteuria* is obviously not common. In hundreds of nematode suspensions, most of them with plectids, checked for the presence of *Pasteuria* attack, only rarely an infection of members of Plectidae was observed. Early developmental stages of the parasites may, however, be easily overlooked as well as an internal infection when no spores are attached to the nematodes cuticle. Mostly only a single or very few specimens were found infected among many plectids in a sample, e.g., in sample No. 3 of Table 1 two of 34 plectid specimens, in sample No. 11 only one specimen among more than 100.

Pasteuria attack of plectids was observed in seven countries (Table 1) and had been reported also for Russia (Subbotin et al., 1994). Most samples were from grassland and forests, with soil ranging from almost pure sand to very heavy clay; one sample each was from wet *Sphagnum* moss and from a moss sample from a tree trunk (Table 1). *Pasteuria* infection was observed in three species of *Anaplectus* and four identified species of *Plectus* (Table 1). The species identity of several *Plectus* samples could not be determined, because only juveniles were available or too few or only poorly preserved specimens to make precise identification. Both adults and juveniles served as *Pasteuria* hosts.

At 15 of the 22 sampling sites listed in Table 1 also other nematodes attacked by *Pasteuria* were found, among these seven times one additional taxon (besides the plectids), four times two more taxa, twice three more, and once each six and nine additional nematode taxa. In most of these taxa *Pasteuria* sporangia and endospores differed distinctly in morphology from those in members of the Plectidae (see also below).

3.2. Morphology and developmental cycle

In 14 of the total of 35 nematode specimens with *Pasteuria*, endospores were attached to the cuticle showing no preferred site of attachment. The maximum number of spores per nematode was 28 (No. 17 in Table 1). In five of the specimens with spores on the cuticle also an internal infection was observed. The remaining 21 specimens, most of them with their pseudocoelom filled with various *Pasteuria* developmental stages, had no endospores (left) on the body surface (cp. also Table 1).

Endospores attached to the nematodes cuticle are circular in apical view, with an evenly rounded central body. In lateral view the perisporium appears rather flat and the central body protruding (Figs. 1A and B). The perisporium is generally only weakly developed, often invisible in apical view and obviously dissolved soon after attachment (Fig. 1D). The central body shows mostly an indistinctly offset wall (coat), which varies from thin to rather thick (cp. measurements for *P. rhizophilus* and *A. porosus* in Table 2), but could not at all be discerned with the light microscope in the smallest spores observed (*Plectus* sp. at the bottom of Table 2). Walls of the central body and the diameter of the core of the central body are more easily discerned and measured in "empty" endospores attached to the nematodes cuticle, which were found to be mostly devoid of a perisporium. Occasionally the walls of such "empty" spores were collapsed (Fig. 1C). A germ tube penetrates from the basal side of the endospore through the nematodes cuticle (Figs. 1B and C). The germinal pore is generally visible in germinated spores (Figs. 1D and E), and penetration pore and penetration tube in the host cuticle was occasionally seen even when the spores had detached from the nematode cuticle (Fig. 1E, in centre between the three spores). Commonly "empty" spores (without

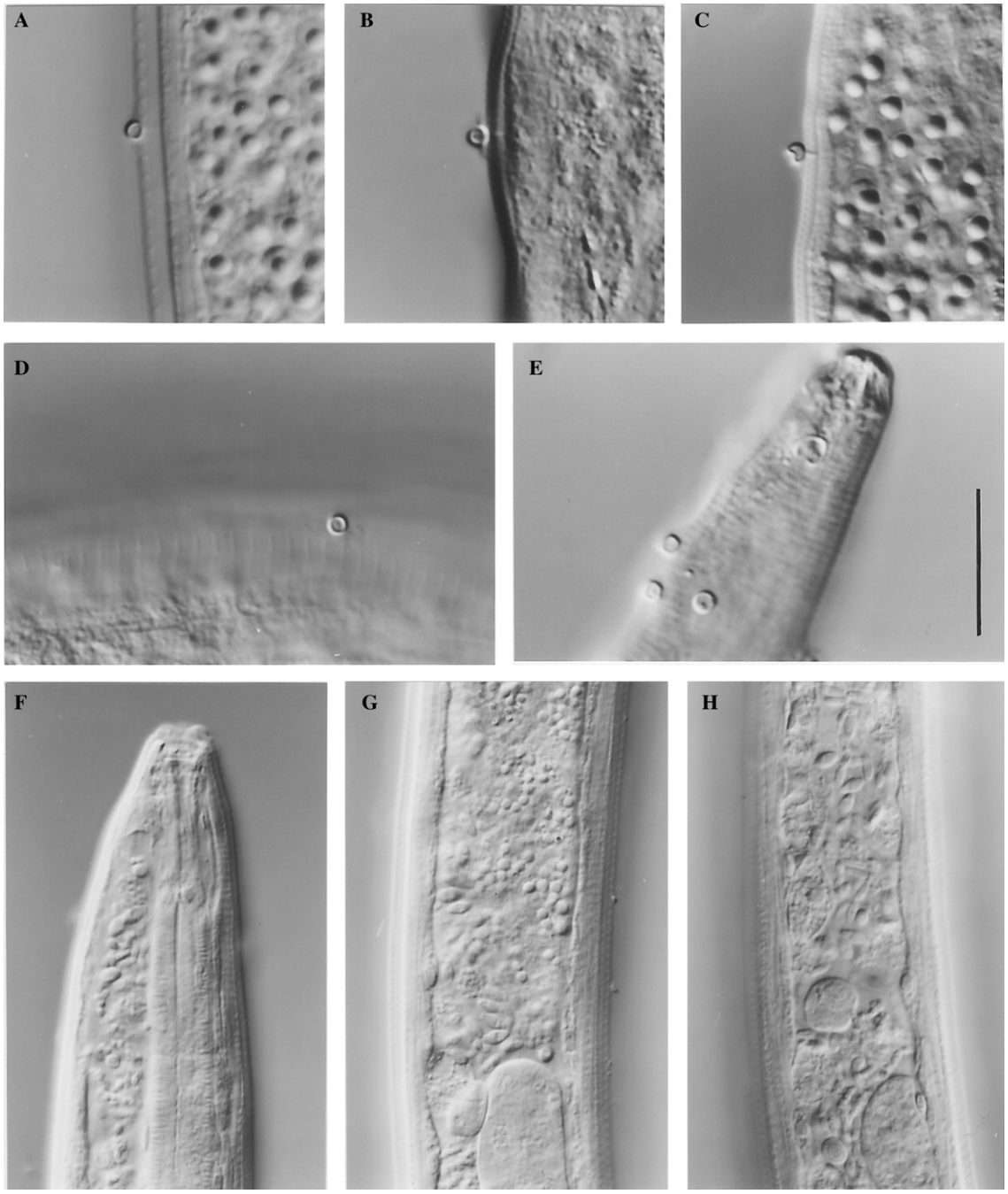

Fig. 1. *Pasteuria* in Plectidae. (A) Lateral view of central body of endospore on cuticle of *Anaplectus granulosus* (6); (B, C) endospores with penetration tube in lateral position in *Plectus turricaudatus* (17); (D) central body of endospore on *A. granulosus* cuticle, apical view, germinal pore visible (6); (E) central bodies of endospores on cuticle of *P. turricaudatus*, with germinal tube in between (17); (F) mycelial, other vegetative, and early sporogenesis stages in anterior end of *A. granulosus* (7); (G, H) mycelial, other vegetative, and sporogenesis stages in *A. grandepapillatus* (1) (scale bar: A–E = 40 μm, F–H = 20 μm).

protoplast) were observed on the surface of plectid specimens, where obviously no penetration of the cuticle and an internal infection took place.

In nematodes with internal infection *Pasteuria* developmental stages ranging from mycelial microcolonies and various vegetative stages to fully developed sporangia were found in the pseudocoelom throughout the host nematode, but often the pharyngeal bulb or the anterior extended part of the intestine served as a barrier preventing spread to the anterior or posterior regions of the body (Fig. 2A). Various developmental stages of the parasite commonly occurred simultaneously within the same host specimen. The microcolonies mostly appeared as elongate clusters, which attained a length up to 11 μm, but more often only chains or accumulations of separate very small globular vegetative particles were observed.

Table 2
Dimensions of *Pasteuria* sporangia from different host nematodes (for numbers cp. Table 1); grouped according to decreasing sporangium size (measurements given in μm)

Host nematode	*n*	Sporangium diameter, height × width	Central body (with coat)	Core of central body
P. rhizophilus (15)	10	5.2 (5.0–5.4) × 4.6 (4.4–5.0)	2.4 (2.2–2.5)	1.2 (1.2–1.3)
P. longicaudatus (14)	10	5.1 (4.7–5.4) × 4.8 (4.5–5.0)	1.9 (1.8–2.2)	1.1 (1.0–1.1)
P. turricaudatus (17)	5	4.7 (4.5–4.9) × 3.8 (3.6–3.9)	2.2 (2.1–2.3)	1.1 (1.1–1.2)
Plectus sp. (19)	10	4.4 (4.1–4.6) × 4.4 (4.2–4.5)	2.5 (2.4–2.5)	1.6 (1.4–1.9)
A. granulosus (8)	8	4.1 (4.0–4.5)	2.1 (1.9–2.3)	1.1 (1.0–1.3)
A. porosus (10)	5	4.0 (3.9–4.2)	2.5 (2.2–2.7)	2.1 (1.9–2.2)
P. cirratus (13)	10	3.9 (3.7–4.2)	2.2 (2.0–2.3)	1.5 (1.4–1.7)
A. granulosus (6)	10	3.9 (3.8–4.0)	2.2 (2.0–2.3)	1.2 (1.2–1.3)
A. grandepapillatus (1)	7	3.7 (3.4–3.9)	2.1 (2.0–2.2)	1.2 (1.1–1.3)
Plectus sp. (20)	10	3.5 (3.3–3.7) × 2.5 (2.4–2.8)	1.5 (1.4–1.5)	

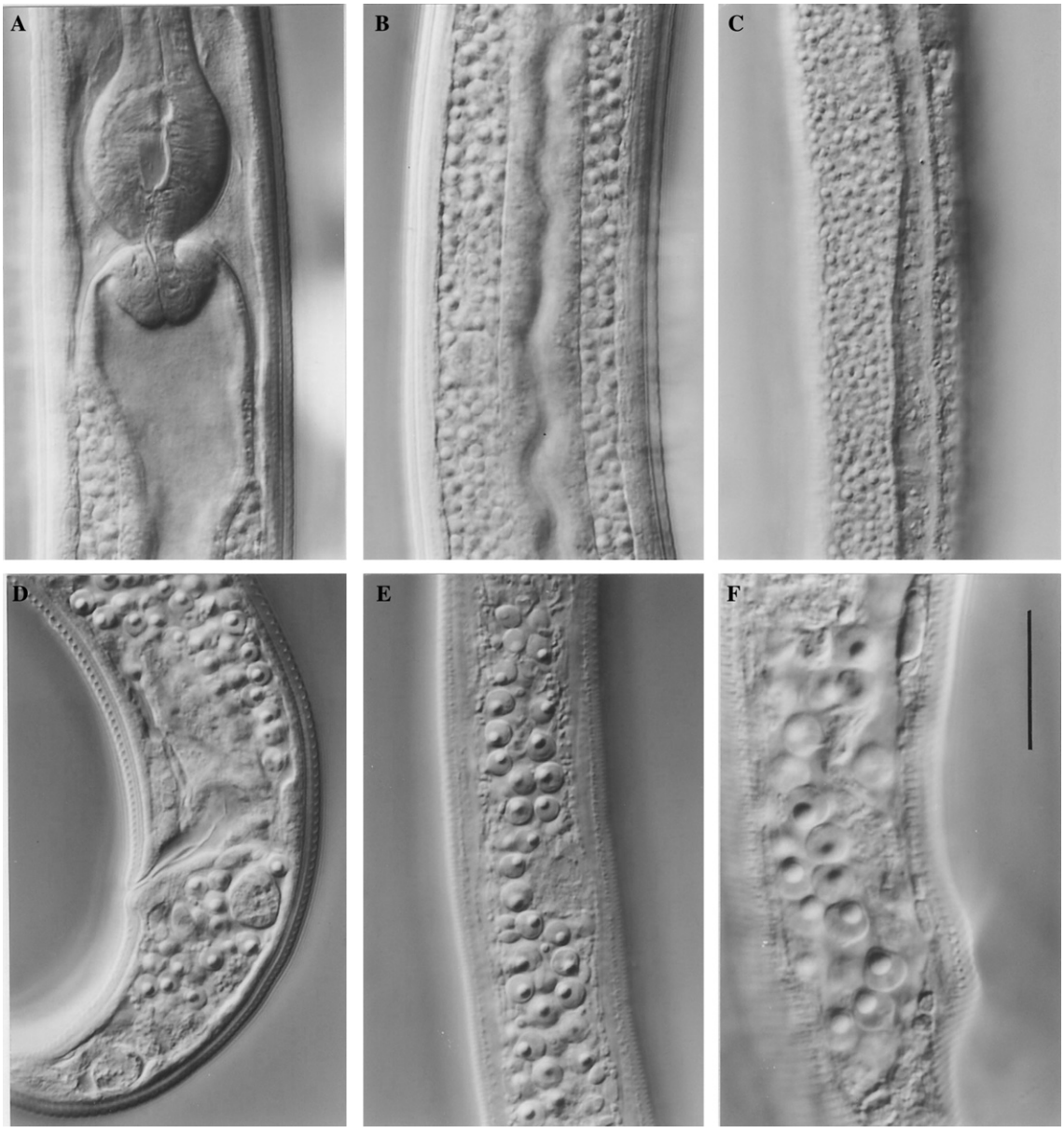

Fig. 2. *Pasteuria* in Plectidae. (A, B) Sporangia around intestine behind cardia and in midbody region of *A. grandepapillatus* (1); (C) sporangia in midbody region of *Plectus* sp. (20); (D) immature and mature sporangia in posterior end of *A. granulosus* (2); (E) mycelial and other vegetative stages, sporogenesis stages and mature sporangia in *P. longicaudatus* (14); (F) mature sporangia in *P. rhizophilus* (15) (scale bar: A–E = 20 μm, F = 40 μm).

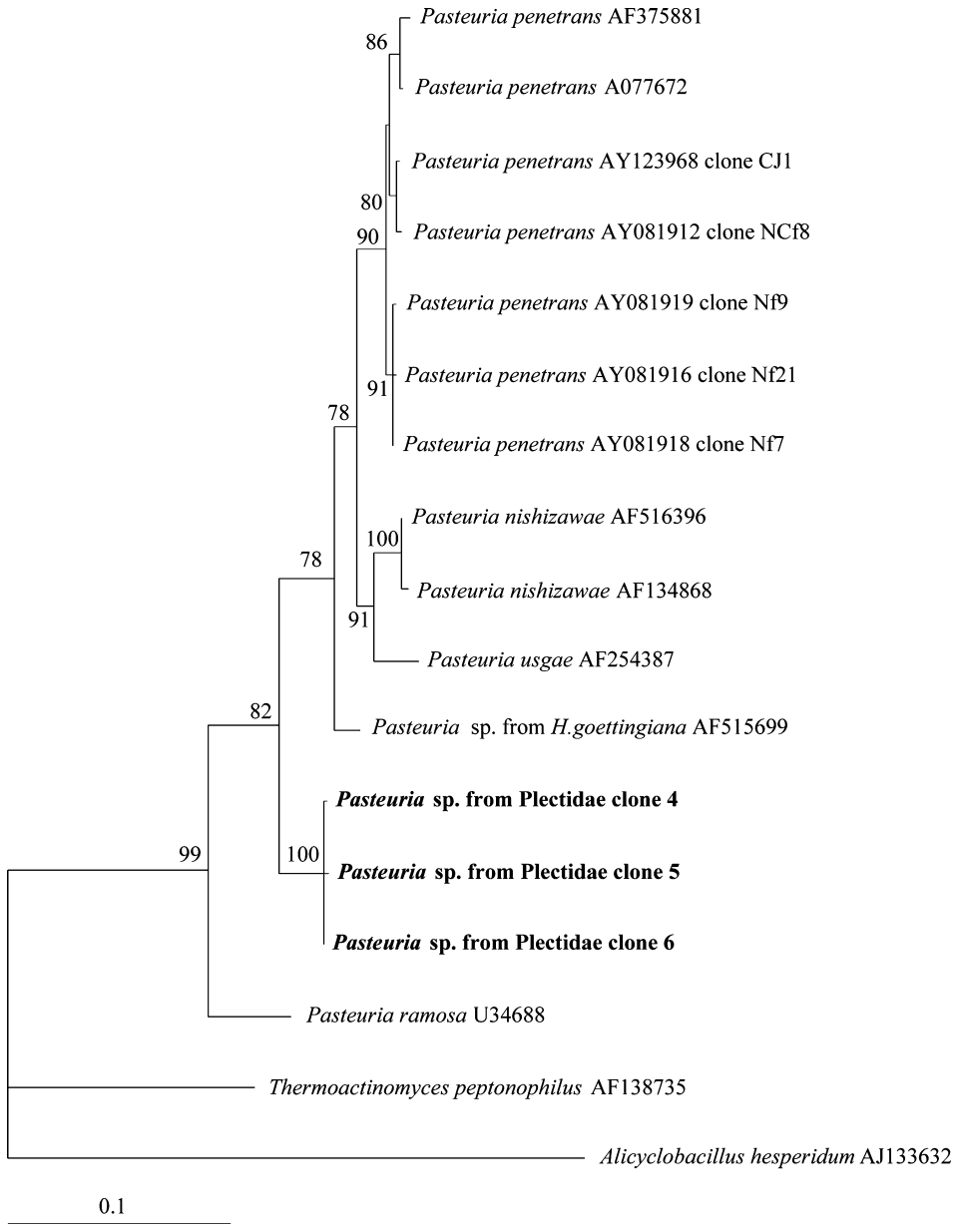

Fig. 3. Phylogenetic relationships among *Pasteuria* species as inferred from maximum likelihood analysis of the 16S rRNA gene sequences (alignment length = 1376 bp, ln $L = -4477.12$, model DNA evolution = GTR + I + G). Sequences of three clones of *Pasteuria* sp. from an unidentified plectid nematode are marked by bold. Bootstrap value more than 70% is given on appropriate clade.

Dichotomously branched and obviously flat "reticulate" mycelia (comparable to those shown by Sturhan et al., 1994; Fig. 6A) of up to 6 μm diameter were often seen (Figs. 1F and G), but no quartets or doublets were observed.

Early stages of sporogenesis were rod-like and up to 3.7 μm long. They grew in length up to 4.5 μm with initiation of septum and forespore formation (Figs. 1G and H). The sporangia are subsequently increasing in size and attaining an ovate to almost globular shape. The protoplast of the forespore is becoming more refractile, and finally more or less indistinct coats are mostly seen around the central body (of the endospore) within the sporangium. Sporangia were generally considered as "mature," when such walls had developed.

In lateral view mature sporangia mostly attained a rhomboidal shape, with the apical part generally slightly to distinctly conoid and the shape of the basal part ranging from hemispherically rounded to conoid (Figs. 2D, E, and F). A collapse of this basal part was never observed. The shape of the endospore within the sporangium could hardly be determined. The perisporium is mostly indistinct and fibers were only rarely visible with the light microscope. The central body is generally situated in the apical part of the sporangium (Figs. 2E and F); its shape varies from uniformly globular to slightly

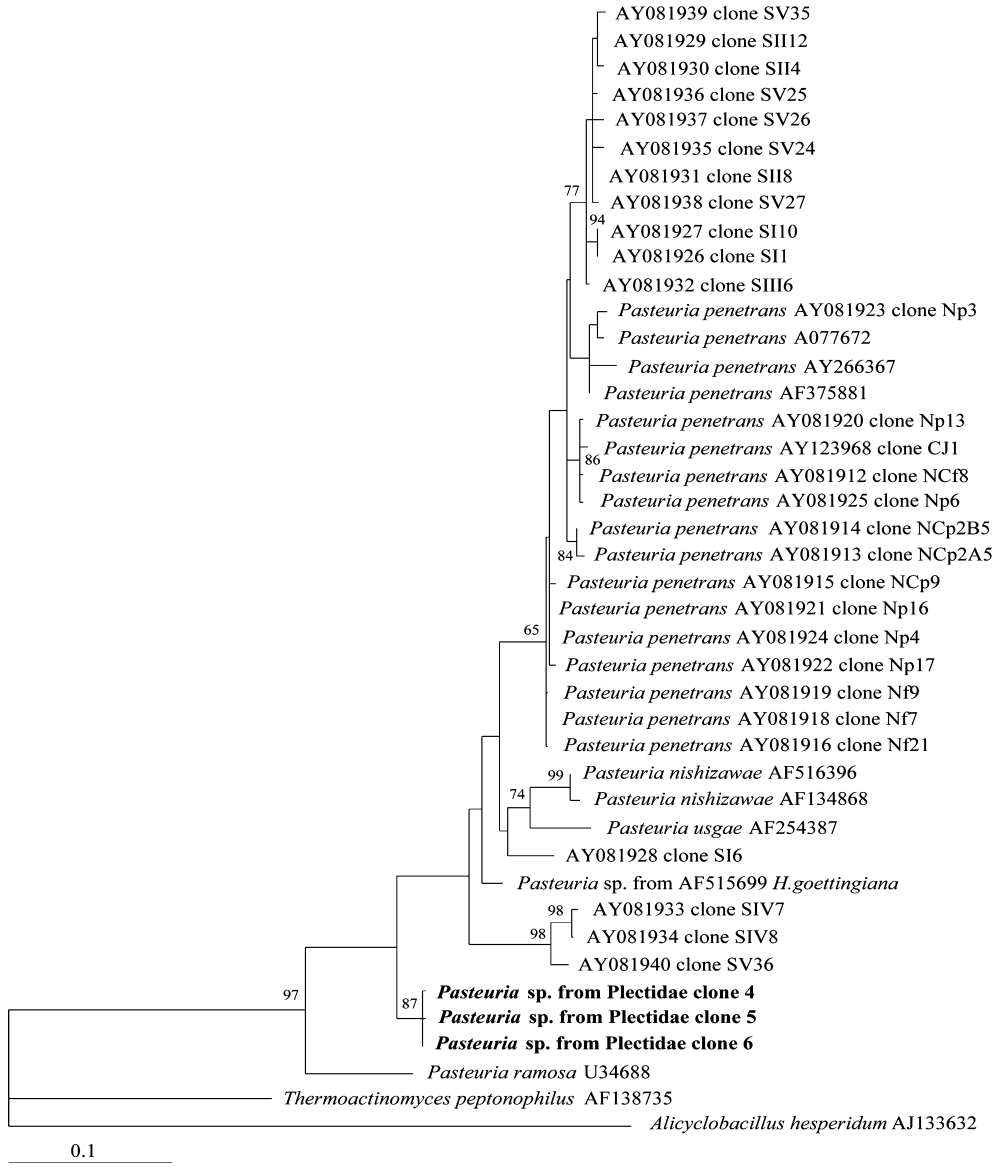

Fig. 4. Phylogenetic relationships within *Pasteuria* spp. as inferred from maximum likelihood analysis of the partly 16S rRNA gene sequences (alignment length = 638 bp, ln $L = -2976.38$, model DNA evolution = GTR + I + G). Sequences of three clones of *Pasteuria* sp. from an unidentified plectid nematode are marked by bold. Bootstrap value more than 60% is given on appropriate clade.

broader and flattened at its base. The diameter of endospores attached to the host cuticle was generally distinctly higher than the diameter of mature sporangia within the same host specimen, which indicates that the perisporium obviously expands in endospores released from the sporangia, while it is pressed together in spores still within the sporangia. There is no evidence that mature endospores attain a cup- or saucer-shape.

Measurements of sporangia, central body and core of central body (= central body without coats) of selected *Pasteuria* sources are given in Table 2. In cases where sporangia were found in exactly lateral position, both their height and width was measured. Figs. 2B–E show differences in sporangium size at the same magnification.

3.3. Molecular characterisation and relationships among Pasteuria species

Length of alignment of the 16S rRNA gene sequences for *Pasteuria* species and outgroup taxa was 1376 bp. Three clones of the 16S gene sequences from the *Pasteuria* infected plectid nematode differ in 2–5 nucleotides (0.15–0.37%) from each other and in 61–86 nucleotides (4.6–6.5%) from other *Pasteuria* sequences. In the ML tree obtained using full alignment, the *Pasteuria* from the plectid nematode occupies a basal position to all other *Pasteuria* spp. known from nematodes, with moderate bootstrap support (Fig. 3). ML analysis of the alignment of partial sequences of 16S rRNA gene

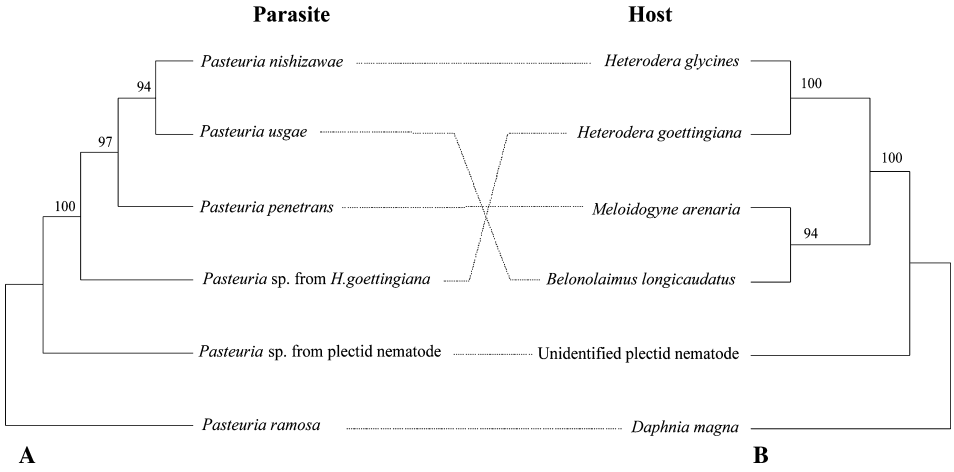

Fig. 5. Phylogenies for *Pasteuria* spp. and nematodes as inferred from maximum parsimony analysis of the 16S rRNA gene and D2–D3 expansion fragment of 28S rRNA gene sequences, respectively. (A) MP tree for *Pasteuria* spp. (number of informative characters = 51, tree length = 198); (B) MP tree for nematodes and *D. magna* (number of informative characters = 195, tree length = 722). Bootstrap value is given on appropriate clade.

resulted a not well resolved tree (Fig. 4). MP trees of *Pasteuria* spp. and the host nematodes were incongruent (Fig. 5), and this comparison appears to indicate differences in phylogenetic history of bacteria and their hosts.

4. Discussion

The data available so far on *Pasteuria* in Plectidae are indicating that the parasites occur worldwide in members of this family and that many species can serve as hosts. Pasteurias from plectids agree in essential morphological characters and in developmental cycle with those of the *Pasteuria* species described from tylenchid nematodes: endospore with highly refractile spheroid central body encircled by a transparent perisporium, attachment of the spores to the host cuticle, invasion of the bacterial protoplast into the host through a germinal pore at the basal side of the endospore and by forming a penetration tube through the nematodes cuticle, development of fragmenting mycelial stages, and other vegetative stages in the hosts pseudocoelom, sporogenesis starting from elongate cells to more or less globular sporangia, in which a single endospore is formed.

The little material available did not allow us to do detailed studies on morphology and to extend the studies to TEM observations. The remarkable wide range of measurements presented in Table 2, differences in shape of the sporangia and endospores, in development and thickness of coats around the central body are indicating that probably several *Pasteuria* species have to be distinguished as parasites in Plectidae. From the *Pasteuria* species described from Tylenchida and also, e.g., from such bacterial parasites found in Dorylaimida, pasteurias from Plectidae appear to be mainly distinguished by the indistinct perisporium and the endospores obviously not being cup- or saucer-shaped and that they appear to detach soon from the host cuticle; moreover, walls around the central body of the endospores are only poorly or not at all developed.

Nothing is known about host specificity of *Pasteuria* "forms" parasitising Plectidae species. At none of the sampling sites, where these bacterial parasites occurred on/in plectids (Table 1), more than one Plectidae species was found with spores on the cuticle or with an internal infection. *Pasteuria* endospores and sporangia, which were observed in other nematode taxa at the majority of the sampling sites with *Pasteuria* found on Plectidae (see above), differed in shape and other morphological characters of sporangia and endospores, in particular, from those described from Tylenchida and those observed in Dorylaimida. Thus, *Pasteuria* sporangia in the dorylaim *Aporcelaimellus obtusicaudatus* specimen from the sampling site, where the *Anaplectus*/*Plectus* specimen used for the molecular studies had been collected (11 in Table 1), are distinctly different from sporangia found in the plectids. However, *Pasteuria* sporangia in a female of the closer related *Cylindrolaimus communis* (Diplopeltoididae, Plectida) from the same site resembled those found in Plectidae species (diameter of sporangia 3.5 μm and of central body 2.0 μm; perisporium of endospore and coats of central body indistinct; endospores probably not cup-shaped). *Pasteuria* sporangia and endospores found in members of other taxa presently placed in the order Plectida (in the sense of De Ley and Blaxter, 2002), which had been collected in various countries worldwide, were also similar in their main morphological characteristics, as seen under the light microscope, with those observed in *Plectus* and *Anaplectus* species (Sturhan, unpublished).

The molecular analysis supports the evidence based on morphological characteristics that the *Pasteuria* from the single juvenile specimen of the unidentified plectid is

different from *Pasteuria* spp. from other nematodes and also from *P. ramosa* from Cladocera. Substantial sequence divergence and its phylogenetic position on the molecular trees obtained in the result of analyses of the full sequenced and partly sequenced 16S rRNA genes of the bacteria, including sequences of the environmental samples, strongly suggest that *Pasteuria* sp. from this plectid nematode could be considered as new species. Lack of more material, however, did not allow detailed morphological and other studies and to present a precise description of this species.

Determining the phylogenetic history of *Pasteuria* species can provide a first view toward understanding of speciation and host specialisation in these bacteria. Our primary results of the analysis of DNA phylogeny of *Pasteuria* species and isolates and of their hosts, based on limited data sets, provide evidence for incongruence of their phylogenetic history and indicate host switching events during bacterial evolution.

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