

# '*Candidatus Rhabdochlamydia porcellionis*', an intracellular bacterium from the hepatopancreas of the terrestrial isopod *Porcellio scaber* (Crustacea: Isopoda)

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Intracellular bacteria were observed in the hepatopancreas of the terrestrial isopod *Porcellio scaber*. Comparative 16S rRNA gene sequence analysis and electron microscopic observations were used to determine the taxonomic position of these intracellular bacteria. Phylogenetic analysis and a complex developmental cycle affiliate these bacteria to the order *Chlamydiales*, within which they form a distinctive lineage, close to the family *Simkaniaceae*. They share <92% 16S rRNA gene sequence similarity with their closest relative and <88% similarity with other members of the order *Chlamydiales*. A specific signature oligonucleotide sequence was identified and used as a probe, enabling the identification of intracellular bacteria in infected hepatopancreatic tissue. According to the distinctive morphology of their elementary bodies, which are rod-shaped rather than spherical and contain translucent oblong structures, their genomic properties and their crustacean host, the name '*Candidatus Rhabdochlamydia porcellionis*' is proposed for intracellular bacteria in the hepatopancreas of *P. scaber*.

## INTRODUCTION

As in many crustaceans (Fryer & Lannan, 1994), tissues of terrestrial isopods (Isopoda, Oniscidea) are often invaded by intracellular bacteria with a complex developmental cycle that is characteristic of chlamydiae and *Rickettsiella* spp. (Weiss *et al.*, 1984). As well as documented infections of terrestrial isopod tissue with *Rickettsiella grylli* (Vago *et al.*, 1970; Federici, 1984; Weiss *et al.*, 1984; Abd El-Aal & Holdich, 1987; Frutos *et al.*, 1994), another intracellular bacterium with a complex developmental cycle was observed in hepatopancreatic cells of the terrestrial isopod *Porcellio scaber* Latreille, 1804 (Shay *et al.*, 1985; Drobne *et al.*, 1999). Despite obvious similarities in the morphology of the described intracellular bacteria and the pathological changes in infected hepatopancreatic tissue, various authors affiliated these bacteria to different bacterial groups. Thus,

Drobne *et al.* (1999) related the intracellular bacteria to the genus *Rickettsiella*, due to the rod-like shape of their elementary bodies (Weiss *et al.*, 1984). This was in concert with prevailing reports on the occurrence of this bacterial group in terrestrial isopods (Federici, 1984; Weiss *et al.*, 1984; Abd El-Aal & Holdich, 1987). On the other hand, the condensation of nucleotide in the centre of the intermediate body, the complex developmental cycle and the positive Giménez staining of the elementary bodies led Shay *et al.* (1985) to propose that these bacteria were chlamydiae. As no cross-reactivity was observed between the intracellular hepatopancreatic bacteria from *P. scaber* with *Chlamydia trachomatis*- and *Chlamydia psittaci*-specific antisera, the name '*Chlamydia isopodii*' was proposed (Shay *et al.*, 1985). However, the description of the species was not validly published in any of the journals recommended by the International Committee for Systematic Bacteriology and the name has not been included in either the Approved Lists of Bacterial Names or the List of Bacterial Names with Standing in Nomenclature (Euzéby, 1997).

In order to clarify the phylogenetic position of the hepatopancreatic intracellular bacteria from *P. scaber*, electron microscopic observations and a molecular approach that included comparative 16S rRNA gene sequence analysis and fluorescence *in situ* hybridization with 16S rRNA-targeted

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Abbreviation: ECL, environmental chlamydiae lineage.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence determined in this study is AY223862.

Micrographs showing intracellular bacteria in infected hepatopancreatic cells of *P. scaber* are available as supplementary material in IJSEM Online.

oligonucleotide probes were performed. The results, which confirm the chlamydial origin of the intracellular hepatopancreatic bacteria from *P. scaber* and reveal that they represent a novel phylogenetic lineage among the order *Chlamydiales*, are described in this paper.

## METHODS

**Animals.** Terrestrial isopods (*P. scaber*) were identified according to the woodlouse determination key (Hopkin, 1991). Animals of both sexes were kept at 20 °C in glass tanks filled with soil, under conditions of high humidity and a 16/8 h day/night cycle. Animals were fed on leaf litter from an unpolluted site for at least 1 month prior to the experiment. Infected animals with macroscopically distinctive white spots in the hepatopancreas were used in the experiment.

**DNA extraction, PCR amplification and cloning.** Infected digestive glands were removed from the animals with fine-tipped sterile forceps. The glands were transferred to 0.5 ml sterile PBS (pH 7.4) and crushed by using a teflon homogenizer. Genomic DNA was extracted from the isolated digestive glands by a method described previously (Kostanjšek *et al.*, 2002). The universal forward primer 522-f (5'-CAGCAGCCGCGGTAATAC-3', *Escherichia coli* positions 522–536) (Giovannoni *et al.*, 1988) or the chlamydiae-specific forward primer 16S1 (5'-CGGATCCTGAGAATTTGATC-3', *E. coli* positions –2–18) (Pudjiatmoko *et al.*, 1997) were used, in combination with the universal reverse primer 1392-r (5'-GYACACACCG-CCCGT-3', *E. coli* positions 1392–1406) (Lane, 1991), for PCR amplification of the 16S rRNA gene (denaturation at 94 °C for 5 min, 30 cycles of 40 s at 95 °C, 30 s at 65 °C and 80 s at 72 °C, followed by a terminal extension of 10 min at 72 °C), which resulted in amplification products of approximately 870 or 1365 bp, respectively. Amplified DNA was checked by agarose electrophoresis and subsequently purified with a Qiaquick Gel Extraction kit (Qiagen). Purified PCR products were ligated into a p-BAD TOPO vector and cloned into *E. coli* TOP10 recipient cells by using the p-BAD TOPO TA Cloning kit (Invitrogen) according to the manufacturer's recommendations.

**Amplified rDNA restriction analysis and sequencing.** Plasmid DNA was isolated from *E. coli* recombinant cells by the 'mini-prep' method, using 5% (w/v) hexadecyltrimethylammonium bromide (CTAB) and ethanol precipitation (Sambrook *et al.*, 1989). Cloned amplicons were reamplified with primers 522-f and 1392-r as described above. These amplicons were then digested with restriction endonucleases *Hae*II and *Hha*I for 2 h at 37 °C and analysed by electrophoresis in 2% (w/v) agarose gel. Sequences of selected amplicons were retrieved by Mycosynth GmbH (Baglach, Switzerland) at our request, using 522-f and 1392-r as sequencing primers. To determine the sequence of the 5' region of the 16S rRNA gene, the chlamydiae-specific primer 16S1 was used, firstly to amplify the almost-complete gene sequence directly from genomic DNA from digestive glands of infected animals and, subsequently, as the primer for sequencing the obtained PCR product. Nucleotide sequence data have been deposited in GenBank under the accession no. AY223862.

**Phylogenetic analysis.** Retrieved 16S rRNA gene sequences were compared with ribosomal sequences from related bacteria that were deposited in the Ribosomal Database Project (Cole *et al.*, 2003), GenBank (NCBI) (Benson *et al.*, 2002) and EMBL (EBI) (Stoesser *et al.*, 2002) nucleotide sequence databases, in order to find the most closely related sequences. Sequences were aligned by using CLUSTAL X (Thompson *et al.*, 1997). The final alignment was inspected and corrected manually. A dataset of 45 unambiguously aligned 16S rRNA gene sequences with approximately 1370 nucleotide positions

was used for the construction of phylogenetic trees. Neighbour-joining (Saitou & Nei, 1987), parsimony (Fitch, 1977) and maximum-likelihood (Felsenstein, 1981) trees were constructed by using the DNADIST, NEIGHBOR, DNAPARS and DNAML programs from the PHYLIP package (version 3.6) (Felsenstein, 2002). Tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) by using SEQBOOT from the PHYLIP package on a set of 1000 resamplings.

**In situ hybridization.** Infected hepatopancreatic tissue was fixed in Carnoy-B fixative, dehydrated in a graded alcohol series and embedded in Paraplast, as described previously (Drobne *et al.*, 1999). Serial sections (5 µm) were mounted onto adhesive slides and rehydrated by immersing the slides in xylene, and then subsequently into 96, 80 and 50% ethanol, for 3 min each. Sections were hybridized in 18 µl hybridization buffer (Hahn *et al.*, 1992) and 2 µl fluorescent probe (50 pmol) at 46 °C for 4 h in sealed hybridization frames (MJ Research). The oligonucleotide probe was synthesized and labelled with the cyanine dye Cy3 by MWG-Biotech AG (Ebersberg, Germany). After hybridization, slides were washed in hybridization buffer for 20 min at 48 °C, rinsed with distilled water and air-dried. Sections were examined by using an Olympus BX50 fluorescent microscope.

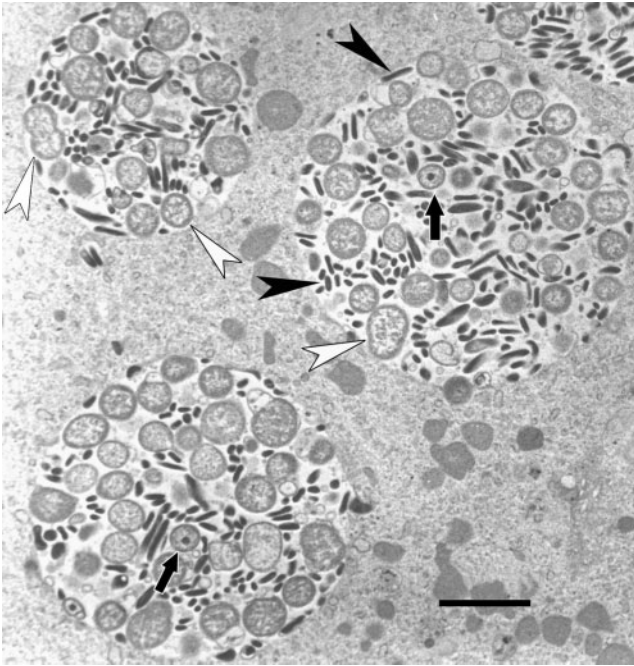
**Microscopy.** Light microscopy of Carnoy-B-fixed and haematoxylin/eosin-stained serial sections of infected cells was performed as described previously (Drobne *et al.*, 1999). Ultrastructural observations of the intracellular bacteria were performed by using transmission electron microscopy as described previously (Drobne *et al.*, 1999).

## RESULTS AND DISCUSSION

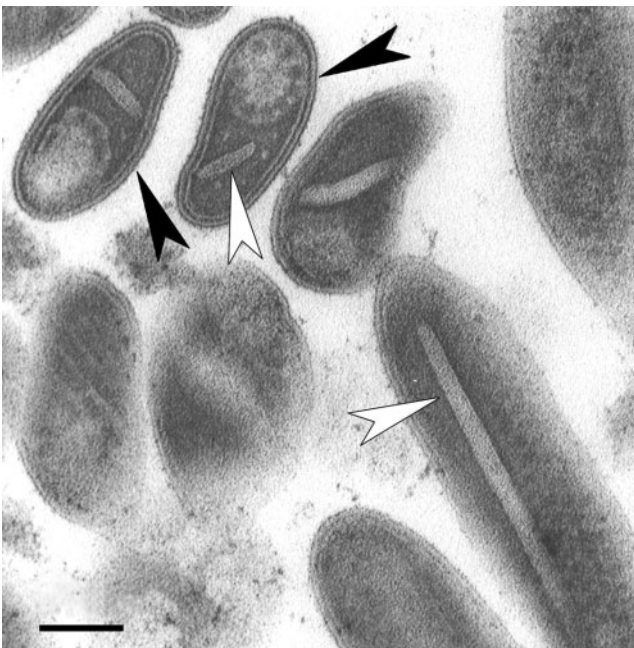
### Morphology and life cycle of the intracellular bacteria in hepatopancreatic tissue

Examination of electron micrographs revealed a chlamydia-like morphology and developmental cycle for the intracellular bacteria in the hepatopancreas of *P. scaber* (Fig. 1), as defined by Everett *et al.* (1999). Our observations were congruent with previous descriptions of intracellular bacteria that were given by Shay *et al.* (1985) and Drobne *et al.* (1999).

Three distinct forms of intracellular bacteria were observed in infected cells: spherical reticulate bodies, spherical early stages of elementary bodies (known as intermediate bodies) and rod-shaped mature elementary bodies (Fig. 1). Spherical reticulate bodies exhibited a network of fine filaments in the cytoplasm and a cell wall with three layers. They appeared as small reticulate bodies (up to 1 µm in diameter), in which binary fission was observed, and as larger reticulate bodies (1–4 µm in diameter) with electron-translucent granular material in the cytoplasm (Drobne *et al.*, 1999). Rod-shaped mature elementary bodies were approximately 250–700 nm long, with a diameter of 100–150 nm. Detailed observations of mature elementary bodies revealed the presence of a five-layered cell-wall structure, with approximately 16 nm thick, translucent, oblong structures in the electron-dense cytoplasm (Fig. 2). Transitional forms between reticulate and elementary bodies were spherical intermediate bodies (approx. 350–650 nm in diameter), with a characteristic electron-dense



**Fig. 1.** Transmission electron micrograph of intracellular bacteria in membranous vacuoles of an infected hepatopancreatic cell. Black arrowheads indicate rod-shaped mature elementary bodies; white arrowheads indicate spherical reticulate bodies; arrows indicate intermediate bodies. Bar, 2  $\mu$ m.



**Fig. 2.** Transmission electron micrograph of mature elementary bodies. Black arrowheads indicate a five-layered cell wall; white arrowheads indicate translucent oblong structures in electron-dense cytoplasm. Bar, 100 nm.

central area (Fig. 1). All forms of the hepatopancreatic intracellular bacteria showed the Gram-negative microscopic appearance of a cell wall, without a discernible peptidoglycan layer.

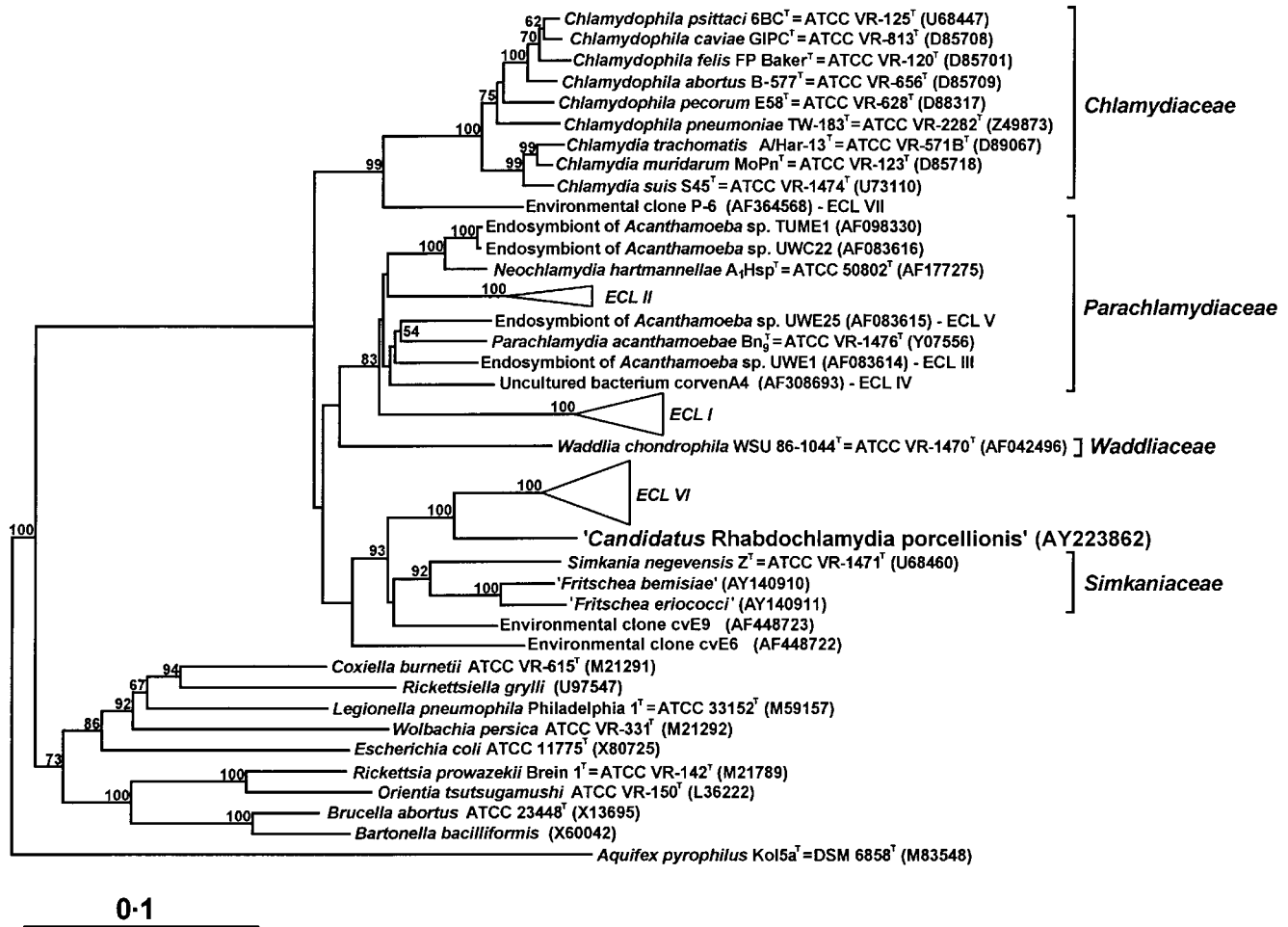
Bacteria in the cytoplasm of infected cells were packed in membrane-bound vacuoles (Fig. 1), which is characteristic of most members of the order *Chlamydiales* (Everett *et al.*, 1999). In the later stages of infection, vacuole size increased, due to multiplication of the intracellular bacteria (see Supplementary Figure in IJSEM Online). At this stage, vacuoles (up to 30  $\mu$ m in diameter) or aggregations of vacuoles (50–150  $\mu$ m in diameter) were seen as white spots in the infected hepatopancreas (Drobne *et al.*, 1999). Vacuoles, filled mainly with elementary bodies, were released into the lumen of the hepatopancreas, as described by Drobne *et al.* (1999). Although the feeding rate of infected animals was not affected, infected cells were damaged during the vacuole extrusion (Drobne *et al.*, 1999). It therefore appears to be appropriate that these bacteria should be described as intracellular parasites.

### Phylogenetic analysis

Intracellular bacteria with complex developmental cycles are found in phylogenetically distant bacterial groups. At first, attempts were made to amplify the ribosomal genes directly from DNA isolated from infected hepatopancreas tissue of *P. scaber* by using a universal primer that anneals at the start [positions 8–26 (Weisburg *et al.*, 1991)] of the 16S rRNA gene, in combination with the universal reverse primer 1392-r. As these attempts were unsuccessful, a universal primer that anneals further downstream (522-f) was used, enabling the amplification of a product of approximately 870 bp.

Restriction profiles, observed after digestion of ten randomly chosen cloned 16S rRNA amplicons with *Hae*III and *Hha*I, were identical, indicating the predominance of a single bacterial species in infected tissue (data not shown). The latter assumption was further supported by the sequencing of two randomly selected cloned amplicons, which provided an identical sequence. As preliminary phylogenetic analysis related the 870 bp sequences obtained to 16S rRNA gene sequences of bacteria in the order *Chlamydiales*, a chlamydiae-specific forward primer, 16S1, was used in combination with the universal primer 1392-r for amplification of a 1366 bp product.

Currently available 16S rRNA gene sequences with highest similarity to the sequence of the intracellular hepatopancreatic bacteria were retrieved from public databases. Pairwise alignments revealed that the sequence obtained is related most closely, with 90.5–91.7% similarity, to 16S rRNA gene sequences from the 'environmental chlamydiae lineage' (ECL) VI group, members of which were retrieved directly from activated sludge (Horn & Wagner, 2001). Similarity to ribosomal sequences from other members of the order *Chlamydiales* ranged from 83.1 to 87.2%.



**Fig. 3.** Phylogenetic tree showing relationships between the 16S rDNA sequences of 'Candidatus Rhabdochlamydia porcellionis' and other members of the order Chlamydiales (Everett *et al.*, 1999). The tree is based on neighbour-joining analysis of a 1366 nt region of 16S rDNA. Bootstrap values >50% are indicated at branching points. For tree construction, 16S rRNA gene sequences of clones that form the following ECLs, isolated from activated sludge (Horn & Wagner, 2001), were included: ECL I [clones P-4 (GenBank accession no. AF364575), P-7 (AF364564) and P-9 (AF364569)], ECL II [clones P-3 (AF364563) and P-5 (AF364565)] and ECL VI [clones P-2 (AF364561), P-8 (AF364574), P-11 (AF364577), P-13 (AF364562), P-16 (AF364570) and P-18 (AF364572)]. Bar, 10% estimated sequence divergence.

Phylogenetic trees, constructed with the parsimony and maximum-likelihood treeing methods, revealed in general the same topology as the neighbour-joining tree (presented in Fig. 3). The tree (Fig. 3) shows that the sequence of the intracellular hepatopancreatic bacteria forms an independent lineage within the order Chlamydiales, which is related most closely to cluster ECL VI and, somewhat more distantly, to *Simkania negevensis* Z<sup>T</sup> (=ATCC VR-1471<sup>T</sup>) from the family Simkaniaceae (Everett *et al.*, 1999), which shared 86.7% sequence similarity with the sequence of the intracellular hepatopancreatic bacteria.

### In situ hybridization

A specific oligonucleotide probe, named S-S-RhaC-0992-a-A-20 [according to the proposed standardization of

oligonucleotide probe nomenclature that was proposed by Alm *et al.* (1996)], was designed to be complementary to the characteristic 16S rRNA gene sequence region of the hepatopancreatic intracellular bacteria, positioned at nt 992–1011 (*E. coli* numbering). A probe-check program (Cole *et al.*, 2003) confirmed that the specific oligonucleotide differs in at least 4 nt from any of the 16S rRNA gene sequences of other chlamydia (Table 1). Fluorescence *in situ* hybridization was performed on serial sections of the infected hepatopancreas tissue by using this probe.

Fluorescent microscopy revealed a strong signal in the membrane-bound vacuoles inside infected cells [see Supplementary Figure (b) in IJSEM Online]. Hybridization with the universal probe 522-f (Giovannoni *et al.*, 1988), which was used as a positive control, was also positive

**Table 1.** Multiple sequence alignment of the S-S-RhaC-0992-a-A oligonucleotide probe sequence with target sequences from 16S rRNA genes of related bacteria

S-S-RhaC-0992-a-A-20 ( <i>E. coli</i> positions 992–1012)	GenBank accession no.	Sequence 3'–CTTTACGTTTCCTGTCGTAG–5'
' <i>Candidatus</i> Rhabdochlamydia porcellionis' ECL VI	AY223862 AF364561, AF364562, AF364570, AF364574, AF364577	5'–GAAATGCAAAGGACAGCATC–3' 5'–.....T..T.....T.T.–3'
<i>Simkania negevensis</i> Z <sup>T</sup> =ATCC VR-1471 <sup>T</sup> ECL III	U68460 AF083614	5'–...C...TT.G.....C.....–3' 5'–...C.....G.....T.CT–3'
<i>Parachlamydia acanthamoebae</i> Bn <sub>9</sub> <sup>T</sup> =ATCC VR-1476 <sup>T</sup>	Y07556	5'–...C.....G.A.....T.T–3'
<i>Chlamydia suis</i> S45 <sup>T</sup> =ATCC VR-1474 <sup>T</sup>	U73110	5'–...C.....T.T...C..GG.–3'
<i>Chlamydia muridarum</i> MoPn <sup>T</sup> =ATCC VR-123 <sup>T</sup>	D85718	5'–...C.....T.T...C..GG.–3'
<i>Chlamydia trachomatis</i> A/Har-13 <sup>T</sup> =ATCC VR-571B <sup>T</sup> ECL II	D89067 AF364563, AF364565	5'–...C...T.T.T...C..GG.–3' 5'–...C.....C...ATAT.A.–3'
ECL I	AF364564, AF364569, AF364575	5'–...C.....CTT...C.GC.–3'
<i>Chlamydomphila psittaci</i> 6BC <sup>T</sup> =ATCC VR-125 <sup>T</sup>	U68447	5'–...C...T.TTT...C..GG.–3'
<i>Chlamydomphila pecorum</i> E58 <sup>T</sup> =ATCC VR-628 <sup>T</sup>	D88317	5'–...C...T.TTT...C..GG.–3'
<i>Chlamydomphila caviae</i> GPIC <sup>T</sup> =ATCC VR-813 <sup>T</sup>	D85708	5'–...C...T.TTT...C..GG.–3'
<i>Chlamydomphila felis</i> FP Baker <sup>T</sup> =ATCC VR-120 <sup>T</sup>	D85701	5'–...C...T.TTT...C..GG.–3'
<i>Chlamydomphila abortus</i> B577 <sup>T</sup> =ATCC VR-656 <sup>T</sup>	AB001783	5'–...C...T.TTT...C..GG.–3'
<i>Neochlamydia hartmannellae</i> A <sub>1</sub> Hsp <sup>T</sup> =ATCC 50802 <sup>T</sup>	AF177275	5'–...C.....G.T..TC.TTA.–3'
<i>Waddlia chondrophila</i> WSU 86-1044 <sup>T</sup> =ATCC VR-1470 <sup>T</sup>	AF042496	5'–...C..AT.C.T...G..TCT–3'
<i>Chlamydomphila pneumoniae</i> TW-183 <sup>T</sup> =ATCC VR-2282 <sup>T</sup> ECL VII	Z49873 AF364568	5'–...C...T.TTT...A.T–GT–3' 5'–...C..ATCTTT...TGG.–3'
ECL V	U68599	5'–A.C.....CTT.GA..AT.A–3'
ECL IV	AF308693	5'–...C..A..GT...A.TT.–..–3'

(data not shown). After hybridization of infected hepatopancreas cells with a non-specific probe [Supplementary Figure (c)] and hybridization of uninfected hepatopancreatic cells with the specific probe S-S-RhaC-0992-a-A-20 [Supplementary Figure (d)], a fluorescent signal was not detected. The results thus clearly demonstrate the presence of the target sequences in the intracellular bacteria inside the membranous vacuoles of infected cells.

### Assignment of the intracellular hepatopancreatic bacteria to the order *Chlamydiales*

Although phylogenetic analysis of the intracellular bacteria did not rely on a complete 16S rRNA gene sequence, high similarity (>80%) with ribosomal sequences from members of the order *Chlamydiales*, as well as the chlamydia-like developmental cycle, clearly indicate that the hepatopancreatic bacteria of *P. scaber* belong to this order (Everett *et al.*, 1999). However, they cannot be assigned to any known genus or family within this order. Similarity between the sequence of the intracellular hepatopancreatic bacteria and ribosomal sequences of type strains in the order *Chlamydiales* was always <90% and, therefore, does not exceed the proposed 95% threshold value for distinction of genera in the order *Chlamydiales* (Everett *et al.*, 1999). Phylogenetic analyses of the retrieved sequences also clarified a reason for the unsuccessful

attempts to amplify the 16S rRNA gene of the intracellular bacteria with a standard universal primer that anneals at the 5' end of the gene. At least three mismatches can be found in most chlamydial 16S rRNA gene sequences within the otherwise conserved region at positions 8–26 (*E. coli* numbering), which is commonly used as target for PCR primer fd1 (Weisburg *et al.*, 1991). Affiliation of the intracellular hepatopancreatic bacteria to a novel chlamydial genus is further supported by the unique morphological characteristics of their elementary bodies, i.e. rod-like shape, five-layered cell wall and oblique cytoplasmic structures, which differ clearly from the spherical elementary bodies observed in other members of the order *Chlamydiales* (Everett *et al.*, 1999).

According to Everett *et al.* (1999), host range is also an important feature in chlamydial grouping. As all known members of the order *Chlamydiales* are recognized pathogens of vertebrates, protozoans (Moulder, 1984; Amann *et al.*, 1997; Fritsche *et al.*, 2000; Horn *et al.*, 2000; Horn & Wagner, 2001) and also, as recently described, insects (Zchori-Fein & Brown, 2002; Thao *et al.*, 2003), the fact that the isopod crustacean hosts intracellular hepatopancreatic bacteria supports the affiliation of these bacteria to a novel chlamydial genus. The crustacean host may also explain the relatively large sequence dissimilarity between the 16S rRNA gene of the intracellular hepatopancreatic bacteria and those of other chlamydiae.

*P. scaber* is probably the natural reservoir for these intracellular hepatopancreatic bacteria, as chlamydial infections are generally transferred without an alternative host (Everett *et al.*, 1999). This is supported by the lack of influence on the host feeding rate (Drobne *et al.*, 1999) and by the constant presence of infected animals in natural populations, indicating an asymptomatic state of coexistence of the intracellular bacteria within the host.

### Description of 'Candidatus Rhabdochlamydia porcellionis'

According to Murray & Stackebrandt (1995), properties of obligate symbionts of eukaryotic cells should be recorded by a 'Candidatus' designation. For this reason, it is proposed that the intracellular hepatopancreatic bacteria from *P. scaber* should be designated 'Candidatus Rhabdochlamydia porcellionis' [Rhab.do.chla.my'di.a. Gr. fem. n. *rhabdos* stick, rod; N.L. fem. n. *Chlamydia* taxonomic name of a bacterial genus; N.L. fem. n. *Rhabdochlamydia* rod-shaped chlamydiae, referring to the rod-like shape of elementary bodies; por.cel.li.o'nis. N.L. gen. n. *porcellionis* of *Porcellio*, pertaining to the taxonomic genus name of the host organism (a terrestrial isopod)].

'Candidatus Rhabdochlamydia porcellionis' comprises intracellular bacteria within membrane-bound vacuoles in the cytoplasm of hepatopancreatic cells of the terrestrial isopod *Porcellio scaber*. Cells are non-motile, non-cultivable on cell-free media and have the Gram-negative microscopic appearance of a cell wall without a discernible peptidoglycan layer. Morphology of 'Candidatus Rhabdochlamydia porcellionis' corresponds to the description of '*Chlamydia isopodii*' (Shay *et al.*, 1985) and a 'Rickettsiella-like organism' (Drobne *et al.*, 1999). Bacteria exhibit a chlamydia-like developmental cycle, in which the cells appear in three morphological forms (reticulate, intermediate and mature elementary bodies) that are packed in membrane-bound vacuoles in the cytoplasm of the host cell. Reticulate bodies with coccoid morphology can be small (up to 1 µm in diameter), which multiply by binary fission, or larger (1–4 µm in diameter), with granular inclusions in the cytoplasm. Morphology of intermediate bodies is coccoid (350–650 nm in diameter), with an electron-dense area in the centre of the cell. Mature elementary bodies have a five-layered cell wall, variable rod-shaped morphology (250–700 nm in length and 100–150 nm in diameter) and oblong structures in the cytoplasm. On the basis of its 16S rRNA gene sequence, 'Candidatus Rhabdochlamydia porcellionis' forms a distinctive lineage within the order *Chlamydiales*, close to the family *Simkaniaceae*, with an oligonucleotide that is complementary to a unique region of the 16S rRNA gene (5'-GAAATGCAAAGGACAGCATC-3'). Assignment to 'Candidatus Rhabdochlamydia porcellionis' is based on the sequence of the 16S rRNA gene (GenBank accession no. AY223862) and the distinctive morphology of the elementary bodies.

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