Characterization of a '*Bacteroidetes*' symbiont in *Encarsia* wasps (Hymenoptera: Aphelinidae): proposal of '*Candidatus* Cardinium hertigii'

Einat Zchori-Fein,[1] Steve J. Perlman,[2] Suzanne E. Kelly,[2] Nurit Katzir[1] and Martha S. Hunter[2]

[1]Department of Vegetable Crops, Agricultural Research Organization, Newe Ya'ar Research Center, Ramat Yishay, 30095, Israel

[2]Department of Entomology, 410 Forbes Building, University of Arizona, Tucson, AZ 85721, USA

Correspondence
Einat Zchori-Fein
einat@volcani.agri.gov.il

Previously, analysis of 16S rDNA sequences placed a newly discovered lineage of bacterial symbionts of arthropods in the '*Bacteroidetes*'. This symbiont lineage is associated with a number of diverse host reproductive manipulations, including induction of parthenogenesis in several *Encarsia* parasitoid wasps (Hymenoptera: Aphelinidae). In this study, electron microscopy and phylogenetic analysis of the 16S rRNA and *gyrB* genes of symbionts from *Encarsia hispida* and *Encarsia pergandiella* are used to describe and further characterize these bacteria. Phylogenetic analyses based on these two genes showed that the *Encarsia* symbionts are allied with the *Cytophaga aurantiaca* lineage within the '*Bacteroidetes*', with their closest described relative being the acanthamoeba symbiont '*Candidatus* Amoebophilus asiaticus'. The *Encarsia* symbionts share 97 % 16S rDNA sequence similarity with *Brevipalpus* mite and *Ixodes* tick symbionts and 88 % sequence similarity with '*Candidatus* A. asiaticus'. Electron microscopy revealed that many of the bacteria found in the ovaries of the two *Encarsia* species contained a regular, brush-like array of microfilament-like structures that appear to be characteristic of the symbiont. Finally, the role of this bacterium in parthenogenesis induction in *E. hispida* was confirmed. Based on phylogenetic analyses and electron microscopy, classification of the symbionts from *Encarsia* as '*Candidatus* Cardinium hertigii' is proposed.

INTRODUCTION

Recent molecular advances have revolutionized the description of unculturable micro-organisms in various environments, including those that live in close association with invertebrates. In recent years, molecular-based studies have confirmed long-standing observations that communities of symbiotic prokaryotes within arthropods are extremely diverse (e.g. Buchner, 1965; Aksoy, 2000; Dolan, 2001; Moran *et al.*, 2003). Whilst a few of these symbionts are reasonably well-characterized (Moran & Telang, 1998; Stouthamer *et al.*, 1999), many others are undescribed and little is known about their influence on the phenotype of their hosts.

Published online ahead of print on 9 January 2004 as DOI 10.1099/ijs.0.02957-0.

Abbreviations: CFB, *Cytophaga–Flexibacter–Bacteroides*; ML, maximum-likelihood; MLS, microfilament-like structure; MP, maximum-parsimony; NJ, neighbour-joining; TBR, tree bisection–reconnection.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *gyrB* gene sequences of '*Candidatus* Cardinium hertigii' are AY331187 and AY332003, respectively.

Bacterial symbionts that are transmitted strictly vertically from mother to offspring will increase in frequency if they directly enhance the fitness of their hosts or if they manipulate the reproduction of their hosts in ways that enhance their own transmission (Bull, 1983). The former include symbionts that supply nutrients that are missing from the host diet (Grenier *et al.*, 1994; Douglas, 1998), increase heat tolerance (Montllor *et al.*, 2002) or provide resistance to parasitoid wasps (Oliver *et al.*, 2003). The latter type, of which the α-group proteobacterium *Wolbachia* is the best-known example, has been shown to induce diverse reproductive alterations, such as: (i) cytoplasmic incompatibility, in which uninfected female hosts produce few or no offspring when mated with infected males; (ii) parthenogenesis induction, in which haploid host eggs are converted into viable diploid female offspring; (iii) feminization, in which genetic male hosts are converted into females; and (iv) male-killing, in which male hosts are killed during development (Stouthamer *et al.*, 1999). Recently, a novel lineage of bacteria has been shown to be associated with several reproductive disorders, including parthenogenesis in a number of parasitoid wasps in the genus

Encarsia (Zchori-Fein *et al.*, 2001), feminization in the mite *Brevipalpus phoenicis* (Weeks *et al.*, 2001) and cytoplasmic incompatibility in the wasp *Encarsia pergandiella* (Hunter *et al.*, 2003). Phylogenetic analysis of the 16S rRNA gene placed these bacteria in the '*Bacteroidetes*' group (= *Cytophaga–Flexibacter–Bacteroides* or CFB group) (Zchori-Fein *et al.*, 2001), with '*Candidatus* Amoebophilus asiaticus', an intracellular bacterium of acanthamoebae (Horn *et al.*, 2001), as their closest described relative (88 % similarity). This symbiont has provisionally been called the *Encarsia* bacterium (Zchori-Fein *et al.*, 2001), the CFB-BP (BP for *B. phoenicis*) (Weeks & Breeuwer, 2003) and the *Cytophaga*-like organism (Hunter *et al.*, 2003; Weeks *et al.*, 2003).

In this study, we characterize and describe this symbiont lineage by phylogenetic analyses of full-length 16S rDNA and *gyrB* sequences and electron microscopic observations of bacterial structure. Furthermore, we present evidence that the symbiont is the causal agent of parthenogenesis in *Encarsia hispida*.

METHODS

Parasitoid wasp-rearing. An asexual, symbiont-infected line of *E. pergandiella* was originally collected in Brazil from a *Bemisia* sp. whitefly in 1993. A sexual, symbiont-infected line of *E. pergandiella* was collected in the Rio Grande Valley, Texas, from *Bemisia tabaci* and was established in our laboratory in 2000. An asexual, symbiont-infected population of *E. hispida* was collected in 2002 in San Diego, California, from *Aleurodicus dugesii* on hibiscus in Balboa Park. The *E. hispida* population from California is probably conspecific with a European population that has also been called *Encarsia meritoria* (Polaszek *et al.*, 1992). European *E. hispida* is also parthenogenetic and infected with the symbiont and has been shown to produce males after antibiotic treatment (Hunter, 1999; Giorgini, 2001). All *Encarsia* species were reared in the laboratory at the University of Arizona on the sweet potato whitefly, *B. tabaci*, on cowpea (*Vigna unguiculata*) under fluorescent and natural light at approximately 25 °C with a photoperiod of 14 h light : 10 h dark.

Electron microscopy. Ovaries from adult, sexual *E. pergandiella* and *E. hispida* were fixed in 4 % glutaraldehyde in 0·1 M cacodylate buffer, pH 7·3, for 1 h. They were then embedded in 2 % agarose in 0·1 M cacodylate buffer, returned to the 4 % glutaraldehyde fixative and held overnight. Following post-fixation for 2 h in 2 % osmium tetroxide, specimens were washed and stained en bloc in 2 % uranyl acetate for 90 min. Subsequently, specimens were dehydrated serially in ethanol (50, 70, 95 and 100 %), transferred to propylene oxide and embedded in Epon. Thin sections were stained with saturated uranyl acetate, followed by Reynolds lead citrate, and examined with a JEOL 100CX II transmission electron microscope. Length and width of 139 bacteria from both host species were measured and recorded.

DNA amplification and sequencing. In order to obtain protein-encoding DNA sequences for further characterization and evolutionary studies of the *Encarsia* symbionts, partial sequences of the gene that encodes gyrase B (*gyrB*) were determined. A series of 'universal' primers for this highly conserved, informational gene has been developed (Yamamoto & Harayama, 1995) and, in conjunction with 16S rDNA, this gene has been found to be a useful marker of bacterial diversity (Huang, 1996).

Wasp host and bacterial symbiont DNA was extracted by using a Qiagen DNeasy tissue extraction kit (protocol for animal tissues). A fragment of the *gyrB* gene from the asexual population of *E. pergandiella* was PCR-amplified by using the conserved universal *gyrB* primers UP-1 (5′-GAAGTCATCATGACCGTTCTGC-AYGCNGGNGGNAARTTYGA-3′) and UP-2r (5′-AGCAGGGTA-CGGATGTGCGAGCCRTCNACRTCNGCRTCNGTCTA-3′), designed by Yamamoto & Harayama (1995). PCR conditions were as recommended in the Identification and Classification of Bacteria (ICB) database (http://seasquirt.mbio.co.jp/icb); a MasterTaq kit (Eppendorf) was used. The PCR product was cloned into *Escherichia coli* by using a pGEM-T Easy plasmid (Promega) according to the manufacturer's protocol; three different clones were sequenced both ways by an automatic sequencer (ABI 3700 DNA analyser; Macrogen). In order to obtain *gyrB* sequences for the symbionts from *E. hispida* and from the sexual population of *E. pergandiella*, the universal *gyrB* primers UP-1 and 2Tr-SR1 (5′-CCATAGCTGCGTAGCATTCA-TYTCNCCNARNCCYTT-3′) were used (http://seasquirt.mbio.co.jp/icb/). PCR conditions were as described above, except that the annealing temperature was 52 °C. This PCR product was then used as a template in a second PCR, using the internal sequencing primers gyr125F (5′-CCGTATACCGAAACGGAA-3′) and gyr1023R (5′-TTAGCAGTACCACCAGCA-3′), which were designed to amplify a 900 bp fragment of the *gyrB* gene.

In order to determine whether there were any other symbionts in *E. hispida*, the 16S rRNA gene was PCR-amplified by using the universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′ and 1513R (5′-ACGGYTACCTTGTTACGACTT-3′ (Weisburg *et al.*, 1991) from an extract of multiple *E. hispida* wasps. This single PCR product was cloned and 33 clones were sequenced, using methods described previously (Hunter *et al.*, 2003).

Phylogenetic analysis of the *Encarsia* symbionts using 16S rDNA and *gyrB*. In order to understand the relationships of the bacterial symbionts of *Encarsia* within the '*Bacteroidetes*' group, we constructed a phylogenetic tree by using full-length 16S rDNA sequences. We used full-length sequences from the bacterial symbionts of both the asexual and sexual populations of *E. pergandiella*, from *E. hispida* (obtained in this study) and from the arthropods *B. phoenicis* and *Ixodes scapularis*. In addition, we used sequences from a diverse assemblage of '*Bacteroidetes*' bacteria, representing all major divisions in this group. Finally, *Chlorobium limicola* was used as an outgroup.

Sequences were first aligned in CLUSTAL W (Thompson *et al.*, 1994) by using default settings and then aligned manually in MacClade 4.0.1 (Maddison & Maddison, 2001). A small number of internal sequences (three regions that were <20 bp and two that were <40 bp) that were difficult to align were removed. PAUP* 4.0b6 (Swofford, 2001) was used for all phylogenetic analyses. Under maximum-parsimony (MP), heuristic searches with TBR (tree bisection–reconnection) branch-swapping and 100 random-addition replicates were performed, with Maxtrees set to increase without limit. All characters were weighted equally. Clade robustness was assessed by bootstrap analysis (Felsenstein, 1985), using heuristic searches with 1000 replicates and a random-addition sequence with $n=1$. Distance methods to reconstruct the 16S rDNA phylogeny were also applied, using both the HKY85 model of DNA evolution and a general time-reversible model of nucleotide substitution, with rate heterogeneity between sites (GTR + Γ + I). We used neighbour-joining (NJ) trees to estimate the six nucleotide transition parameters, the gamma shape parameter for rate heterogeneity (Γ) and the proportion of invariable sites (I). We performed heuristic distance searches with TBR branch-swapping. We also performed a bootstrap analysis, using heuristic searches with 1000 replicates under the HKY85 model.

In addition, we constructed a phylogenetic tree of *gyrB* sequences that represented diverse '*Bacteroidetes*' taxa, with *Flavobacterium ferrugineum*

and *Chitinophaga pinensis* as outgroups. Phylogenetic analysis was done as described above for 16S rDNA, except that no internal sequences were removed. In addition to MP and distance analyses, we also performed a maximum-likelihood (ML) analysis, using the GTR+Γ+I model of nucleotide substitution with parameters estimated from NJ and MP trees. Under ML, we performed a heuristic search, using TBR branch-swapping, a starting tree obtained by neighbour-joining and the 'asis' stepwise-addition option.

Finally, we identified signature 16S rDNA sequences for the symbiont lineage. Nucleotide alignments were first scanned in MacClade, after which candidate sequences were checked in the Ribosomal Database Project (RDP II) (http://rdp.cme.msu.edu/html/) (Cole *et al.*, 2003) by using probe match and were also BLAST-searched for short, nearly exact matches in GenBank.

Parthenogenesis induction in *E. hispida* by the '*Bacteroidetes*' symbiont. *E. hispida* females, less than 48 h old, were held in vials for 48 h with honey only, or 50 mg rifampicin ml^{-1} in honey. Females were then placed individually into 35 mm Petri dish arenas, where a leaf disc bearing 20 third- to early fourth-instar *B. tabaci* whitefly nymphs was embedded in agar [for details, see Hunter *et al.* (2003)]. After 4 h, females were removed and the dishes were incubated at 27 °C, 65 % relative humidity, until pupation of the wasp progeny. Pupae were then removed and isolated in 1·2 ml glass vials until adult emergence. Adults were sexed, then killed in alcohol and stored until a PCR assay of infection status was conducted. Extraction and PCR methods were as described previously (Hunter *et al.*, 2003).

RESULTS AND DISCUSSION

Morphology

Electron microscopy revealed highly pleomorphic bacteria with parallel arrays of microfilament-like structures (MLSs) attached to their inner membrane (Fig. 1). Bacteria were located in the cytoplasm of all host ovarian cells examined, including nurse cells, follicle cells and oocytes of *E. hispida* and sexual and asexual *E. pergandiella* ovaries. These bacteria were morphologically indistinguishable from bacteria observed in the ovaries of the asexual *E. pergandiella*, as described previously (Zchori-Fein *et al.*, 2001). The bacteria appeared to vary greatly in size (range of lengths, 0·42–2·35 μm, $n=139$; range of widths, 0·31–0·66 μm,

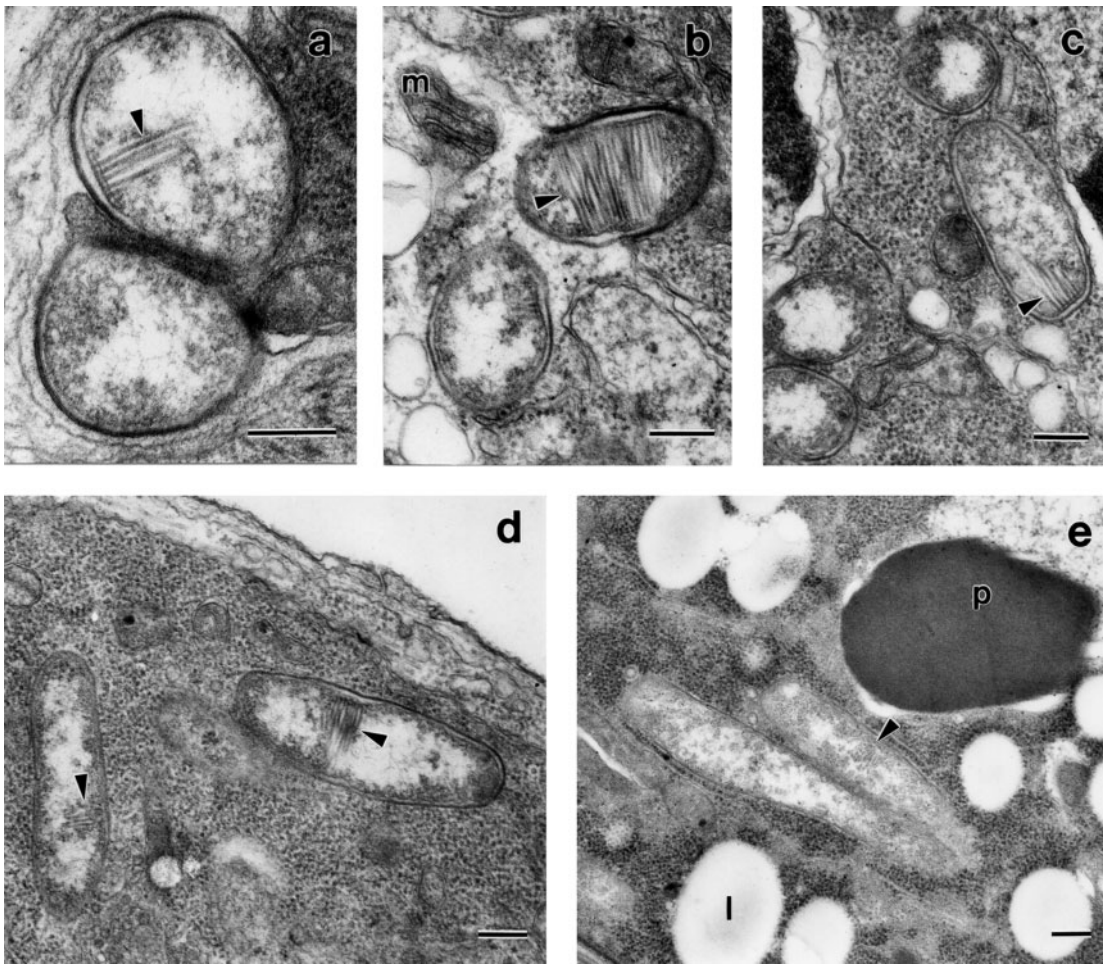

Fig. 1. Electron micrographs of '*Candidatus* Cardinium' in ovaries of *Encarsia* parasitoid wasps. (a) Bacteria in a follicle cell of *E. hispida*. (b–d) Bacteria in sexual *E. pergandiella* nurse cells. (e) Bacteria in an *E. hispida* oocyte. Arrowheads point to MLSs; l, lipid-filled vacuoles in the oocyte; m, mitochondrion; p, protein. Bars, 0·25 μm.

$n=139$). As serial sections were not examined, it was not possible to distinguish cross-sections of elongated individuals from spherical forms. The lack of serial sections also prevented us from establishing the mean dimensions of the bacterium.

Within bacteria found in the ovaries of a single female, MLSs are variable with respect to length, straightness and position within the cell. Whilst most MLSs observed are straight, parallel, perpendicular to the long dimension of the cell and reach only approximately halfway across the cell (Fig. 1a), a few issue from one end of the cell (Fig. 1c) and, in one bacterium, the MLSs are attached to two opposing sides, merging in the centre (Fig. 1b). MLSs could not be observed in all bacterial cells, although it is not clear whether cells are polymorphic or some MLSs were not detected because they were in a different plane.

MLSs have previously been observed only in several symbiotic bacteria of invertebrates, such as symbionts of the sweet potato whitefly, *B. tabaci* (Costa *et al.*, 1995), the predatory mite *Metaseiulus occidentalis* (Hess & Hoy, 1982), the soybean cyst nematode, *Heterodera glycines* (Endo, 1979), the leafhopper *Helochara communis* (Chang & Musgrave, 1972) and a symbiont of the tick *I. scapularis* (Kurtti *et al.*, 1996). In two of these host taxa, *B. tabaci* and *M. occidentalis*, a PCR product was amplified by using primers that were specific to the 16S rDNA of the novel symbiont lineage (Weeks & Breeuwer, 2003), and in a third, *I. scapularis*, the 16S rDNA GenBank sequence places it within the group. As yet, investigations of all symbionts with MLSs have yielded evidence that they are members of the novel symbiont lineage. It is at least possible, therefore, that the MLSs are not only characteristic, but distinguish this bacterium. Interestingly, the description of the sister lineage, '*Candidatus* A. asiaticus', does not mention or show the presence of similar structures. The function of the MLSs remains unknown.

Sequence and phylogenetic analysis

16S rDNA. We generated 1487 bp of the *E. hispida* symbiont 16S rRNA gene sequence (G+C content, 48 mol%). This sequence is 99 % similar to the sequence determined for symbionts in asexual and sexual *E. pergandiella*, 97 % similar to those of the *Brevipalpus* and *Ixodes* symbionts and 88 % similar to the sequence of the Acanthamoeba symbiont '*Candidatus* A. asiaticus'. Our aligned dataset was 1215–1221 bp long, except for the GenBank sequences of *Blattabacterium* sp. (1121 bp), *Cytophaga aurantiaca* (1163 bp) and *Flammeovirga aprica* (1170 bp). MP and distance analyses produced similar tree topologies (Fig. 2) and none of the nodes that differed in trees obtained by the different methods showed >50 % bootstrap support. MP analysis produced two most parsimonious trees (tree length, 3472; confidence interval, 0·30; 478 parsimony-informative characters out of a total of 1221 characters). Under ML and by using an NJ tree, the following nucleotide substitution parameters were estimated (nucleotide transition parameters): A–C, 0·81; A–G, 2·57; A–T, 1·65; C–G, 0·67; C–T, 3·69; and G–T, 1·00. The proportion of invariant sites was estimated to be 0·38 and the shape parameter of the gamma-distribution was estimated to be 0·63. The *Encarsia* symbionts form a monophyletic group with '*Candidatus* A. asiaticus' and the symbionts from *Brevipalpus* and *Ixodes*. This symbiont lineage is aligned with bacteria from the *C. aurantiaca* group, such as '*Microscilla sericea*' and '*Microscilla furvescens*' (Fig. 2).

We designate the following signature sequence for 16S rDNA for the *Encarsia* symbiont: 5′-GTATTTTGCTAC-TTTG-3′. The following 16S rDNA sequences are unique to the *Encarsia*+*Brevipalpus*+*Ixodes* symbiont clade (i.e. '*Candidatus* Cardinium'): 5′-GCGGTGTAAAATGAGC-GTG-3′; and at position 729: 5′-GGTCTTTAACTGAC-GCT-3′.

***gyrB*.** We generated a 1283 bp *gyrB* gene sequence from the asexual *E. pergandiella* symbiont (G+C content, 38 mol%), 924 bp from the sexual *E. pergandiella* symbiont (40 mol%) and 858 bp from the *E. hispida* symbiont (40 mol%). The symbionts from the two populations of *E. pergandiella* are 98 % similar and both are 95 % similar to the *E. hispida* symbiont. Our aligned dataset was 924–936 bp long, except for the *E. hispida* symbiont sequence. As in the 16S rDNA analysis, MP, ML and distance analyses produced similar tree topologies (Fig. 3) and none of the nodes that differed in trees obtained by the three methods showed >50 % bootstrap support. MP analysis produced three most parsimonious trees (tree length, 4854; confidence interval, 0·31; 618 parsimony-informative characters out of a total of 942 characters). Under ML and by using an NJ tree, the following parameters of nucleotide substitution were estimated (nucleotide transition parameters): A–C, 2·71; A–G, 3·84; A–T, 2·13; C–G, 2·23; C–T, 8·01; and G–T, 1·00. The proportion of invariant sites was estimated to be 0·25 and the shape parameter of the gamma-distribution was estimated to be 1·08.

Phylogenetic analysis of *gyrB* sequences confirms the affinity of the *Encarsia* symbionts with the *C. aurantiaca* group, aligned most closely with '*M. sericea*' and '*M. furvescens*' (Fig. 3). There are currently no available *gyrB* sequences for the '*Bacteroidetes*' symbionts of *Acanthamoeba*, *Brevipalpus* or *Ixodes*.

Parthenogenesis induction in *E. hispida* by the '*Bacteroidetes*' symbiont

Here, we provide additional evidence that parthenogenesis in *E. hispida* is caused by the '*Bacteroidetes*' symbiont. The attribution of effects on hosts to particular unculturable symbionts has necessarily been indirect. Stouthamer *et al.* (1999) suggested three types of evidence, ranked by increasing levels of stringency, that have been used to associate a phenotype with a particular symbiont. They

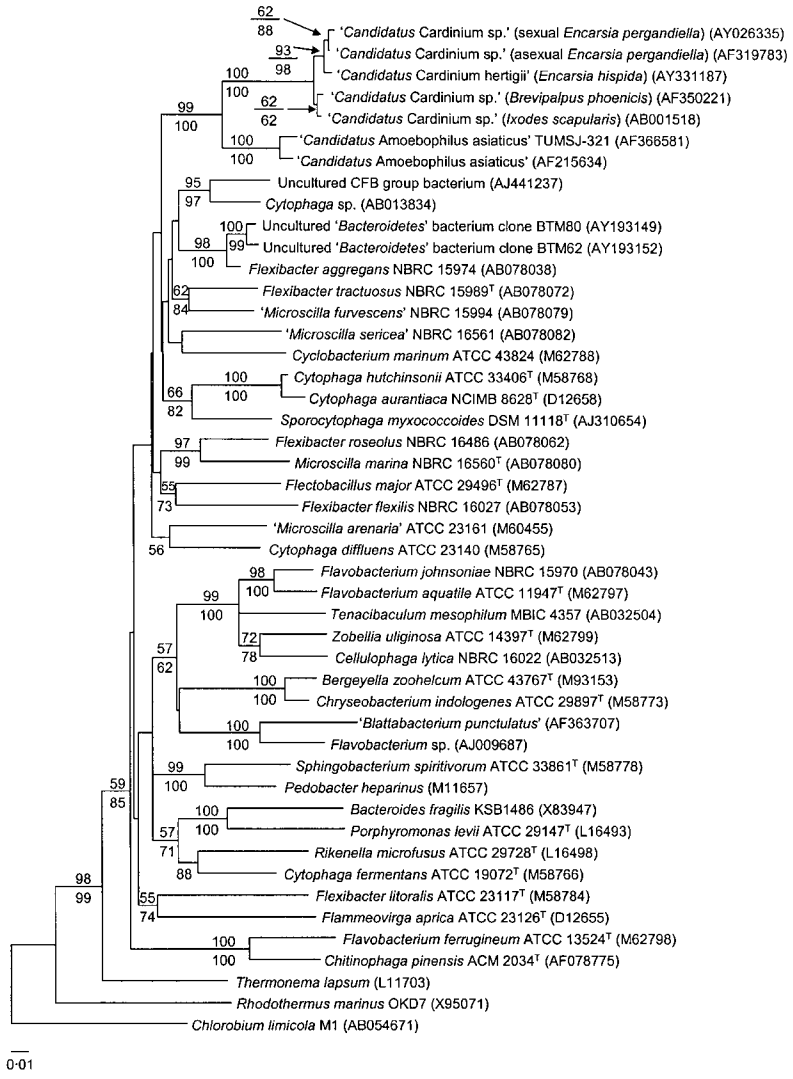

Fig. 2. Distance tree of the bacterium '*Candidatus* Cardinium hertigii', based on an HKY85 model of DNA evolution, using '*Bacteroidetes*' 16S rDNA sequences and rooted with the bacterium *Chlorobium limicola*. The topology is similar to those of MP trees. Numbers above branches indicate parsimony bootstrap values (%); numbers below indicate distance bootstrap values (%). Bar, 0·01 substitutions per site.

are: (i) an association between phenotype and infection; (ii) antibiotic treatment stops the expression of the phenotype and cures the infection; (iii) artificial inoculation from an infected to an uninfected host causes an infection and the phenotype in the new host. Zchori-Fein *et al.* (2001) established an association between the *Encarsia* symbiont and parthenogenesis induction in *Encarsia* and, in *E. hispida*, antibiotic treatment has been shown to cause male production in a European population (Hunter, 1999; Giorgini, 2001). Here, we examined the population of *E. hispida* from California in more detail. We found that whilst untreated females produced only daughters, antibiotic treatment of *E. hispida* females caused them to produce only sons. Antibiotic-treated females produced $0{\cdot}9 \pm 0{\cdot}31$ males (mean $\pm$ SE, $n=10$), whereas untreated females produced $2{\cdot}9 \pm 0{\cdot}31$ females (mean $\pm$ SE, $n=10$). PCR assays of these offspring showed that daughters of infected females were infected, but the sons of treated females were uninfected. We also extended the criteria of Stouthamer *et al.* (1999) to better exclude the possibility that other symbionts within a host could be responsible for the phenotype observed. Sequencing of 33 16S rDNA bacterial clones from *E. hispida* yielded 33 identical products of 1487 bp, with 99 % sequence identity to the symbiont of parthenogenetic *E. pergandiella* (GenBank accession no. AF319783). These lines of evidence suggest strongly that the symbiont described here is the causal agent of parthenogenesis in *E. hispida*. Interestingly, the picture is somewhat less clear in other infected parthenogenetic *Encarsia* spp. For example, *E. pergandiella* females treated with antibiotics produced almost no offspring and no males, possibly as a result of accumulation of deleterious mutations in male function (Zchori-Fein *et al.*, 2001).

Proposal of '*Candidatus* Cardinium hertigii'

In this paper, we describe a novel bacterial genus based on sequences of the *gyrB* and 16S rRNA genes, as well as electron microscopy observations. Results of both the *gyrB*- and the 16S rDNA-based phylogenies are broadly similar and suggest that the newly discovered symbiont lineage is allied with the *C. aurantiaca* group. 16S rDNA analysis shows the recently described 'Candidatus *A. asiaticus*', a

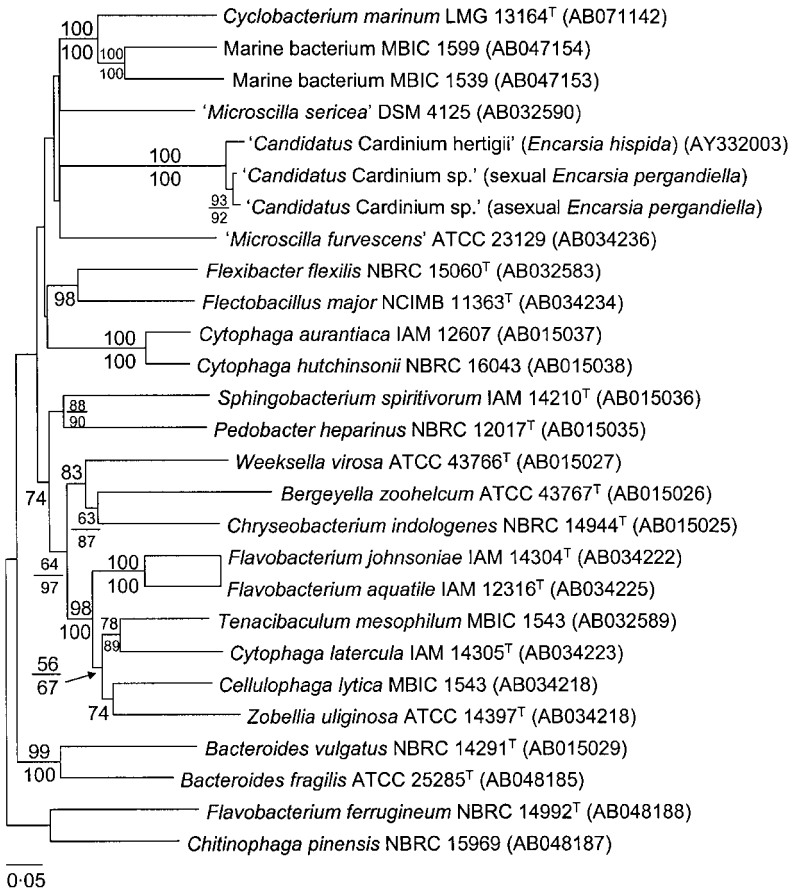

Fig. 3. Distance tree of the bacterium '*Candidatus* Cardinium hertigii,' based on an HKY85 model of DNA evolution, using '*Bacteroidetes*' *gyrB* sequences and rooted with the bacteria *Chitinophaga pinensis* and *Flavobacterium ferrugineum*. The topology is similar to those of MP and ML trees. Numbers above branches indicate parsimony bootstrap values (%); numbers below indicate distance bootstrap values (%). Bar, 0·05 substitutions per site.

symbiont of *Acanthamoeba* sp., to be the closest relative of the novel symbiont lineage, with 88 % sequence homology to the symbiont of *E. pergandiella*. As 95 % similarity of the 16S rRNA gene has been suggested as the limit under which bacteria genera are distinguished (Ludwig *et al.*, 1998), the symbionts of *Encarsia*, as well as the symbionts found in *I. scapularis* and *B. phoenicis*, both with 97 % sequence similarity to the *Encarsia* symbiont, should all be included in a novel bacterial genus.

In accordance with the recommendations of the International Committee on Systematic Bacteriology, unculturable prokaryotic symbionts that cannot be characterized by using the International Code of Nomenclature of Bacteria should be regarded as *Candidatus* (Murray & Stackebrandt, 1995). We therefore propose to name the bacterium associated with species of the parasitoid wasp *Encarsia* as '*Candidatus* Cardinium'. The genus name (Car.di′ni.um. N.L. n. *Cardinium*) is derived from *cardo* (gen. *cardinis*), the main axis of a Roman town, typically flanked by columns. The name pertains to the brush-like structure of MLSs observed within the bacteria. '*Candidatus* Cardinium' belongs to the phylum '*Bacteroidetes*' of bacteria, to the class '*Sphingobacteria*', to the order '*Sphingobacteriales*' and to the family '*Flexibacteraceae*' (Garrity & Holt, 2001) and is a Gram-negative, rod-shaped bacterium. '*Candidatus* Cardinium' is a transovarially transmitted, intracellular symbiont of eukaryotes. The genus is assigned based on the 16S rRNA gene sequence (GenBank accession no. AY331187), the *gyrB* gene sequence (accession no. AY332003) and the array of MLSs that are observed with an electron microscope.

At the time of writing, '*Candidatus* Cardinium' contains a single species, '*Candidatus* Cardinium hertigii'. The specific epithet (her.ti′gi.i. N.L. gen. masc. n. *hertigii*) refers to Marshall Hertig, the microbiologist who described *Wolbachia* (Hertig, 1936). The bacterium found in the parthenogenetic population of the parasitic wasp *E. hispida* from San Diego, California, is proposed as the type strain. The G+C content of 1487 bp of '*Candidatus* C. hertigii' 16S rDNA is 48 mol%, with the signature sequence 5′-GTATTTTGCTACTTTG-3′. The level of 16S rDNA sequence similarity between the *I. scapularis* and *B. phoenicis* symbionts and the *Encarsia* symbionts (97 %) places them all within the genus '*Candidatus* Cardinium', but this amount of sequence divergence between the taxa suggests that the tick and mite symbionts belong to a different, as yet undescribed species (Stackebrandt & Goebel, 1994).

In the brief period since its discovery, '*Candidatus* Cardinium' has been associated with diverse effects on its arthropod hosts. Surveys of its distribution suggest that it may infect 6–7 % of arthropods and be found most commonly in the Hymenoptera, Hemiptera and Acari (Weeks *et al.*, 2003). Whilst not as common as the same

authors' estimates of *Wolbachia* frequency (22–24 %), these estimates nonetheless suggest that this symbiont may be found in thousands of arthropod species and has only begun to be explored.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Drs V. Portnoy, J. Wilson and D. Bentley for invaluable help, Y. Gottlieb for the suggestion of the name and H. Trüper for advice on naming issues. This research was supported by grant no. 200276 from the United States–Israel Binational Science Foundation (BSF), Jerusalem, Israel, to E. Z.-F., N. K. and M. S. H. and a USDA NRI grant (2001-35302-10986) to M. S. H. Contribution no. 115/2003 from the ARO.

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