Taxonomic Note

Correspondence
Nathan Lo
nathan@usyd.edu.au

Taxonomic status of the intracellular bacterium *Wolbachia pipientis*

N. Lo,¹ C. Paraskevopoulos,² K. Bourtzis,² S. L. O'Neill,³ J. H. Werren,⁴ S. R. Bordenstein⁵ and C. Bandi⁶

¹School of Biological Sciences and Sydney University Biological Informatics and Technology Centre, The University of Sydney, NSW 2006, Australia

²Department of Environmental and Natural Resources Management, University of Ioannina, 2 Seferi St, 30100 Agrinio, Greece

³School of Integrative Biology, The University of Queensland, Brisbane, QLD 4072, Australia

⁴Department of Biology, University of Rochester, Rochester, NY, USA

⁵Josephine Bay Paul Center for Comparative Molecular Biology and Evolution - The Marine Biological Laboratory, Woods Hole, MA, USA

⁶Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria – Sezione di Patologia Generale e Parassitologia, Università degli Studi di Milano, Milano, Italy

Wolbachia pipientis is a maternally inherited, intracellular bacterium found in more than 20 % of all insects, as well as numerous other arthropods and filarial nematodes. It has been the subject of a growing number of studies in recent decades, because of the remarkable effects it has on its arthropod hosts, its potential as a tool for biological control of arthropods of agricultural and medical importance and its use as a target for treatment of filariasis. *W. pipientis* was originally discovered in cells of the mosquito *Culex pipiens* and is the only formally described member of the genus. Molecular sequence-based studies have revealed a number of phylogenetically diverse strains of *W. pipientis*. Owing to uncertainty about whether *W. pipientis* comprises more than one species, researchers in the field now commonly refer to *W. pipientis* simply as *Wolbachia*. In this note, we briefly review higher-level phylogenetic and recombination studies of *W. pipientis* and propose that all the intracellular symbionts known to cluster closely with the type strain of *W. pipientis*, including those in the currently recognized supergroups (A–H), are officially given this name.

Introduction

Wolbachia pipientis (class *Alphaproteobacteria*, order *Rickettsiales*) and its taxonomically undescribed close relatives are intracellular, maternally transmitted symbiotic bacteria found in more than 20 % of all insect species, numerous other arthropods and filarial nematodes (Bandi *et al.*, 1998; Jeyaprakash & Hoy, 2000; Plantard *et al.*, 1999; Taylor & Hoerauf, 1999; Wenseleers *et al.*, 1998; Werren *et al.*, 1995; Werren & Windsor, 2000). They are well known as manipulators of arthropod host reproduction, causing cytoplasmic incompatibility, feminization, parthenogenesis and male-killing (Stouthamer *et al.*, 1999; Werren, 1997), and have, more recently, been shown to act as obligate mutualists in filarial nematodes (Bandi *et al.*, 2001; Taylor & Hoerauf, 1999).

W. pipientis was discovered in 1924 in the cells of the mosquito *Culex pipiens* (Hertig & Wolbach, 1924) and was formally described in 1936 (Hertig, 1936). In recent decades,

the use of PCR and sequencing techniques has revealed a large number of bacteria with close phylogenetic affinity to *W. pipientis*. Although they form a clear monophyletic cluster with *W. pipientis*, many of these undescribed strains show significant differences in the 16S rRNA gene when compared to the type strain (i.e. more than 3 %). Due to some uncertainty about whether such bacteria might represent different species, most researchers in the field now refer to these strains simply as *Wolbachia*. This term, although convenient and well-accepted, is not taxonomically legitimate. Owing to the growing interest in the biology and applications of *W. pipientis* and its relatives, a uniform nomenclatural system for these bacteria is desirable. One way to resolve this issue is to recognize all closely related strains as *W. pipientis*; an alternative would be to split the group into two or more species. In this note, we argue for the former option, based on results from phylogenetic and recombination studies, as well as a taxonomic precedent in other symbiotic bacteria.

Molecular phylogenetic and recombination analyses of *W. pipientis*

No *W. pipientis* strains have yet been isolated in pure culture, largely because of their fastidious requirements. Traditional methods for bacterial species and strain determination, such as those involving DNA–DNA hybridization and biochemical parameters, have therefore not been feasible. Early phylogenetic analysis of 16S rRNA gene sequences assigned *W. pipientis* clearly to the *Alphaproteobacteria*, revealing a sister-group relationship with the genera *Ehrlichia*, *Anaplasma* and *Neorickettsia* (O'Neill *et al.*, 1992). This study showed that *W. pipientis sensu stricto* formed a monophyletic clade with other insect-associated microorganisms and suggested 'the classification of these bacteria as members of the same species' (O'Neill *et al.*, 1992).

Further 16S rRNA-based studies of intracellular bacteria from various arthropods and filarial nematodes showed that they formed a monophyletic group with *W. pipientis* (Rousset *et al.*, 1992; Sironi *et al.*, 1995; Stouthamer *et al.*, 1993) (see also Dumler *et al.*, 2001). A study of several different *W. pipientis* arthropod strains based on the more variable gene *ftsZ* showed that two divergent clades exist (A, B) (Werren *et al.*, 1995). The type strain (from *C. pipiens*) was found within clade B. A subsequent analysis of intracellular bacteria from filarial nematodes found that they formed two additional clades (C, D) (Bandi *et al.*, 1998). These clades have since been termed 'supergroups'; a schematic summary of currently recognized supergroups is shown in Fig. 1. A number of other supergroups have been proposed more recently, including supergroups E

(Collembola) (Czarnetzki & Tebbe, 2004; Vandekerckhove *et al.*, 1999), F (arthropods and filarial nematodes) (Campbell *et al.*, 1992; Casiraghi *et al.*, 2005; Lo *et al.*, 2002; Rasgon & Scott, 2004), G (spiders) (Rowley *et al.*, 2004) and H (termites) (Bordenstein & Rosengaus, 2005). A number of divergent lineages, including those from various flea species and the filarial nematode *Dirofilaria repens*, have yet to be designated supergroups (Casiraghi *et al.*, 2005; Gorham *et al.*, 2003).

Relationships among the various supergroups are not well understood, primarily because of the absence of a suitable outgroup for rooting the inferred trees (Lo *et al.*, 2002). The closest known relatives of *W. pipientis* are members of a clade including the genera *Anaplasma* and *Ehrlichia*, which have corrected 16S rRNA gene sequence identities of ~90 % with *W. pipientis*. The last common ancestor of *W. pipientis* and these outgroups is likely to have existed several hundred million years ago, which has led to the erosion of phylogenetic signal in many sequence characters. In a recent study (Casiraghi *et al.*, 2005), three protein-coding genes were combined and analysed, including their homologues in *Anaplasma* and *Ehrlichia* as outgroups. Using a statistical test that distinguishes between competing hypotheses (Shimodaira & Hasegawa, 1999), the scores of a number of different trees with differing outgroup positions were compared. It was found that no outgroup placement was significantly more likely than another. Without a reliable root for the tree, it is not possible to assign supergroups confidently to different, monophyletic species. A more prudent approach is to consider all strains as members of *W. pipientis*.

Fig. 1. Schematic diagram of *W. pipientis* phylogeny based on various phylogenetic studies of the genes *ftsZ*, *groEL*, *gltA* and *dnaA* (see text). Letters represent supergroups that have been confirmed on the basis of these four genes. The position of supergroup G is tentative since it was estimated using the *wsp* and 16S rRNA genes. Host species are indicated next to each clade or lineage. Two lineages, from *Dipetalonema gracile* and *Ctenocephalides felis*, have not yet been classified into supergroups. Bar, 0.1 substitutions per site (an approximation based on a concatenated gene analysis of these four genes).

A number of recent studies have shown that exchange of genetic information has occurred among supergroups. Evidence has been found for recombination of genes within and between supergroups A and B (Baldo *et al.*, 2005, 2006a; J. C. Dunning Hotopp and J. H. Werren, unpublished data). Exchange of genes/gene fragments within bacteriophages that infect different *W. pipientis* supergroups has also been demonstrated (Bordenstein & Wernegreen, 2004; Masui *et al.*, 2000). Genome sequencing studies show that *W. pipientis* has the genetic machinery for gene transfer (Wu *et al.*, 2004) and a number of arthropods have been shown to be infected by both A and B representatives (Werren *et al.*, 1995; Werren & Windsor, 2000), which would provide a means of gene flow. It has been clearly demonstrated that recombination in the *wsp* gene encoding an outer-surface protein is widespread among supergroup A and B bacteria (Baldo *et al.*, 2005; Jiggins *et al.*, 2001; Werren & Bartos, 2001). This gene should therefore be avoided when trying to understand relationships among *W. pipientis* strains. Due to the level of recombination in *W. pipientis* and close relatives, reliable strain identification requires a multilocus strain typing (MLST) approach (Maiden *et al.*, 1998). A comprehensive MLST system has been developed for *W. pipientis* using five standard house-keeping genes (Baldo *et al.*, 2006b; Paraskevopoulos *et al.*,

2006). Results reveal frequent recombination and a remarkable diversity among *W. pipientis*: 35 of 37 haplotypes were unique (Baldo *et al.*, 2006b). Such frequent recombination is consistent with *W. pipientis* and its closely related strains being members of one species.

Proposal to recognize all *Wolbachia* formally as members of the species *W. pipientis*

At present, the majority of strains that cluster with *W. pipientis* do not have a proper scientific name. We and other *W. pipientis* researchers frequently encounter dissatisfaction from the microbial community over this issue, particularly editors and reviewers who request the addition of '*pipientis*' to the title of manuscripts. As interest in these bacteria continues to increase, it is important to minimize taxonomic confusion within the broader community.

In the case of the aphid obligate primary symbionts, only one species name, *Buchnera aphidicola* (Munson *et al.*, 1991), is recognized and commonly used by researchers in the field. This is despite the fact that separation of the main lineages of these bacteria occurred a few hundred million years ago, and corrected genetic divergences in 16S rRNA typically exceed 8% (Moran *et al.*, 1993). Strains of *B. aphidicola* are simply known by the host aphid they infect.

On the basis of results from phylogenetic and recombination analyses, as well as the taxonomic precedent set by aphid-symbiotic bacteria, we propose that bacteria with a close phylogenetic affinity to *W. pipientis* be formally included in this species. This includes those bacteria currently within the recognized supergroups (A–H) and those that display a similar genetic distance from the type strain. This was the consensus reached at a recent international conference on *W. pipientis* (Heron Island, Australia, August 2004). We note that this formal recognition will not prevent researchers from continuing to use the common name '*Wolbachia*' in their papers. Without formal recognition of all strains as *W. pipientis*, the research community runs the risk of prematurely naming novel species in the genus. Because *W. pipientis* is so abundant in insects, with at least 2–6 million infected species (Werren *et al.*, 1995), naming species based on host associations would lead to an unmanageable proliferation of species names.

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