Wigglesworthia gen. nov. and *Wigglesworthia glossinidia* sp. nov., Taxa Consisting of the Mycetocyte-Associated, Primary Endosymbionts of Tsetse Flies

SERAP AKSOY

Department of Internal Medicine, Yale University School of Medicine,
New Haven, Connecticut 06510

The primary endosymbionts (P-endosymbionts) of tsetse flies (Diptera: Glossinidae) are harbored inside specialized cells (mycetocytes) in the anterior region of the gut, and these specialized cells form a white, U-shaped organelle called mycetome. The P-endosymbionts of five tsetse fly species belonging to the Glossinidae have been characterized morphologically, and their 16S ribosomal DNA sequences have been determined for phylogenetic analysis. These organisms were found to belong to a distinct lineage related to the family Enterobacteriaceae in the γ subdivision of Proteobacteria, which includes the secondary endosymbionts of various insects and *Escherichia coli*. These bacteria are also related to the P-endosymbionts of aphids, *Buchnera aphidicola*. Signature sequences in the 16S ribosomal DNA and genomic organizational differences which distinguish the tsetse fly P-endosymbionts from members of the Enterobacteriaceae and from the genus *Buchnera* are described in this paper. I propose that the P-endosymbionts of tsetse flies should be classified in a new genus, the genus *Wigglesworthia*, and a new species, *Wigglesworthia glossinidia*. The P-endosymbiont found in the mycetocytes of *Glossina morsitans morsitans* is designated the type strain of this species.

Many insects that have single food sources have developed obligatory associations with prokaryotic microorganisms (7, 9). All members of the family Glossinidae harbor primary endosymbionts (P-endosymbionts) in specialized organelles called mycetomes, which consist of cells (mycetocytes) that contain these microorganisms (7). In members of the genus *Glossina*, the mycetomes are white, U-shaped organelles that are localized in the anterior region of the gut. In addition to their mycetome-associated P-endosymbionts, many *Glossina* species carry secondary endosymbionts (S-endosymbionts) that are associated with their midgut cells (28). In 1907 Stuhlman found bacteria in the intestines of tsetse flies (31), and in 1919 Roubood described a mycetome-like organelle that he observed in the tsetse fly midgut (26), an observation which was confirmed by Wigglesworth in 1929 (35) and by Buxton in 1955 (8). In 1972, Reinhard et al. described the results of ultrastructural studies of large bacteroids (length, up to 8 μ m) which they found in tsetse fly mycetomes (25). In 1974 Huebner and Davey reported that bacteroids are present in tsetse fly ovary tissue (11), and Pinnock and Hess distinguished the mycetome organisms from the smaller endosymbionts found in midgut cells (24).

The endosymbionts are essential for the survival and well-being of their hosts in many cases (7, 9). In tsetse flies, experimental elimination of these organisms by prokaryote-specific antibiotic treatment (10, 24, 30) and lysozyme treatment (19) or by treatment with antibodies specifically raised against the endosymbionts (20, 21) results in retarded growth of the insect and a decrease in egg production, which in turn results in a loss of the ability of the aposymbiotic hosts to reproduce. The ability to reproduce can be partially restored if the aposymbiotic tsetse flies receive a bloodmeal that is supplemented with B-complex vitamins (thiamine, pantothenic acid, pyridoxine, folic acid, and biotin), suggesting that the endosymbionts probably play a role in metabolism that involves these compounds (22). The individual functions of the P- and S-endosymbionts are not known. It is thought that the tsetse fly gut organisms

are maternally transmitted to intrauterine larvae via milk secretions of the mother (13).

Recent advances in molecular techniques, as well as the use of nucleic acid sequences in phylogenetic reconstructions, have provided additional insight into the relationships among bacteria (37). The phylogenetic positions of the three distinct endosymbionts found in tsetse flies have been determined by a 16S ribosomal DNA (rDNA) sequence analysis of the P- and S-endosymbionts associated with gut tissues and the microorganisms found in the ovaries (1, 3, 23). A phylogenetic analysis of the tsetse fly S-endosymbiont 16S rDNA sequence has shown that this organism is a member of the family Enterobacteriaceae in the γ -3 subdivision of the Proteobacteria (3). In vitro cell-free cultures of these S-endosymbionts have also been established (3, 34). The microorganisms harbored in tsetse fly ovaries have also been characterized phylogenetically and have been found to belong to the genus *Wolbachia* in the α subdivision of the Proteobacteria (23). The P-endosymbionts of tsetse flies were isolated from mycetomes of five species of flies belonging to the three subgenera of the genus *Glossina* (1). A preliminary analysis of the 16S rDNA-encoding sequences of these organisms revealed that they are different from the S-endosymbionts and the bacteria that reside in ovaries and instead represent a distinct lineage in the γ subdivision of the Proteobacteria (1). Similarly, a 16S rDNA sequence comparison revealed that the P-endosymbionts of 12 different species of aphids belong to a distinct lineage in the γ subdivision of the Proteobacteria (15, 16, 32). These endosymbionts have been placed in the genus *Buchnera*, and *Buchnera aphidicola*, the endosymbiont of *Schizaphis graminum*, has been designated the type species of this genus (16).

The intracellular P-endosymbionts of tsetse flies have not been successfully cultivated in vitro or in tissue culture yet, but according to Rule 16a of the *International Code of Nomenclature of Bacteria*, in cases where a species has not been maintained in laboratory culture, a description or preserved specimen may serve as the type strain (12, 36). Thus, the obligate symbionts of cockroaches, the spirochetes found in

FIG. 1. Electron micrograph of *W. glossinidia* in a mycetome of *G. morsitans*. The endosymbionts are surrounded by a two-layer membrane. Bar = 2 μ m.

wood-eating cockroaches and termites, have been assigned to new genera and species (7). Similarly, it has not been possible to cultivate members of the genus *Wolbachia* which cause reproductive incompatibility in culture (33). Recently, the International Committee on Systematic Bacteriology has proposed a new prokaryotic category, the category *Candidatus*, for characterization of organisms that are difficult to cultivate (17). Clearly, *Candidatus* status instead of formal recognition of a taxon could lead to contentious discussions. In this paper, I describe a new genus, the genus *Wigglesworthia* (Wigglesworth i.a. M.L. fem. n. *Wigglesworthia*, named after the parasitologist V. B. Wigglesworth), and a new species, *Wigglesworthia glossinidia* (glossinidia i.a. M.L. fem. adj. *glossinidia*, referring to *Glossina*, the genus of tsetse flies) for the P-endosymbionts of *Glossina* species. The P-endosymbiont of *Glossina morsitans morsitans* is designated the type strain of this species. In addition to morphological characteristics, the 1,100-bp 16S rDNA sequences of the P-endosymbionts of five distantly related *Glossina* species were determined and phylogenetically analyzed (1). Signature sequences in the 16S rDNAs that distinguish these organisms from members of the family *Enterobacteriaceae* and the genus *Buchnera* and the genomic organization of the rDNA locus are discussed below.

Description of *Wigglesworthia* gen. nov. Mycetomes are packed with rod-shaped microorganisms that are up to 4 to 5 μ m long (Fig. 1). The microorganisms are in the cytoplasm of the specialized epithelial cells called mycetocytes. They have a typical gram-negative cell wall and lack flagella. The cells appear to divide by binary fission. These organisms have not been

cultivated outside their hosts, although it is possible to keep them metabolically active for at least 72 h under transient tissue culture conditions (2). It is possible to study the gene expression and protein transport in P-endosymbionts of tsetse flies by using transient tissue culture conditions, and in this way the major protein product has been identified as a groEL homolog by immunological cross-reactivity (2).

On the basis of their 16S rDNA sequences, members of this genus form a distinct lineage in the γ -3 subdivision of the *Proteobacteria*. As shown in the phylogenetic tree in Fig. 2, they are closely related to members of the genus *Buchnera*, to S-endosymbionts of other insects, and to *Escherichia coli*. Table 1 shows the sequence signatures in the 16S rDNA that distinguish the genus *Wigglesworthia* from members of the genus *Buchnera* and from other members of the γ -3 subgroup.

Description of *Wigglesworthia glossinidia* sp. nov. The morphological description is the same as that given above for the genus. The type strain is the primary endosymbiont of *G. morsitans morsitans*.

W. glossinidia 16S rDNA sequences have also been obtained from the following species and subspecies of tsetse flies: *Glossina tachinoides*, *Glossina palpalis palpalis*, *Glossina austeni*, and *Glossina brevipalpis*. These sequences were analyzed phylogenetically by performing a parsimony analysis (Fig. 2).

Additional information for differentiating *W. glossinidia* from other symbionts. The S-endosymbionts of tsetse flies harbored in midgut cells and the *Wolbachia*-like bacteria of tsetse flies harbored in ovary tissues are morphologically different from members of the genus *Wigglesworthia*. They are short rods (length, 1 to 2 μ m) and are inside cells surrounded by host membranes (25). There are clear lytic zones surrounding the cells that are reminiscent of the zones found with members of the family *Rickettsiaceae*, to which the genus *Wolbachia* is related (24, 29). Since a phylogenetic analysis of the S-endosymbionts from tsetse flies showed that these organisms are not related to rickettsiae, it is not clear what the clear lytic zones mean.

FIG. 2. Phylogenetic tree based on the 16S rDNA sequences of the endosymbionts of aphids, tsetse flies, and whiteflies and *E. coli*. -P, primary mycetocyte endosymbiont; -S, secondary endosymbiont. All of the names except *E. coli* are the names of the insect species that contain the endosymbionts. Abbreviations: *E. coli*, *Escherichia coli*; *A. pisum*, *Acyrtosiphon pisum*; *G. m. morsitans*, *Glossina morsitans morsitans*; *S. zeamais*, *Sitophilus zeamais*; *G. brevipalpis*, *Glossina brevipalpis*; *G. tachinoides*, *Glossina tachinoides*; *G. palpalis*, *Glossina palpalis*; *G. austeni*, *Glossina austeni*; *M. persica*, *Myzus persica*; *M. rhois*, *Melaphis rhois*; *T. vaporariorum*, *Trialeurodes vaporariorum*; *S. phyllireae*, *Siphoninus phyllireae*. This tree is the majority-rule consensus tree obtained after 100 bootstrap runs; the numbers are bootstrap indices for the levels of support of the nodes. There were 220 informative characters. Reprinted from *Insect Molecular Biology* (2) with permission of the publisher.

TABLE 1. Sequence signatures that distinguish *W. glossinidia* from *B. aphidicola* and other members of the γ -3 subgroup

Position ^a	Nucleoside in:		
	<i>Wigglesworthia</i> ^b	<i>Buchnera</i> ^c	γ -3 subgroup ^d
75	T	A	G
79	T	A	G
188	T	A	C
210	A	T	C
225	G	T	C
592	A	T	G
621	A	C	G
911	G	T	C

^a The numbers refer to positions in the *E. coli* sequence (37).^b Sequences of the P-endosymbionts obtained from *G. morsitans morsitans*, *G. tachinoides*, and *G. brevipalpis*.^c Sequences of the P-endosymbionts (*B. aphidicola*) obtained from the aphids *Myzus persicae*, *Acyrtosiphon pisum*, and *Melaphis rhois*.^d Sequences of *E. coli*, *Proteus vulgaris*, and the S-endosymbionts of *Acyrtosiphon pisum*, *Sitophilus zeamais*, and *G. morsitans morsitans* (18).

The organization and copy number of the 16S rDNA of the genus *Wigglesworthia* are also different from the organization and copy number of the 16S rDNA operons of *Buchnera* species and *E. coli*. The free-living bacterium *E. coli* (4, 5) and S-endosymbionts of tsetse flies (2) have multiple copies of rDNA operons encoding their 16S and 23S rDNA gene products. Members of the genus *Wigglesworthia* have single operons encoding their 16S rDNA sequences (2), like members of the genus *Buchnera* (32). While the copy number of the *Wigglesworthia* rDNA operon is similar to the copy number of the *Buchnera* rDNA operon, the genomic organization of the 16S and 23S rDNA genes in the genus *Wigglesworthia* is different. In the genus *Buchnera*, these genes are not genetically linked (14, 27), while in the genus *Wigglesworthia* they are encoded by the same operon (i.e., they are transcriptionally linked) (2). In terms of genetic organization, this is similar to the arrangement found in *E. coli* (6) and in the tsetse fly S-endosymbiont genome (unpublished data) (1).

Nucleotide sequence accession numbers. The GenBank accession numbers for the 16S rDNA sequences used to construct the phylogenetic tree are as follows: *E. coli*, V00348; S-endosymbiont of *Acyrtosiphon pisum*, M27040; S-endosymbiont of *G. morsitans morsitans*, M99060; S-endosymbiont of *Sitophilus zeamais*, M85269; P-endosymbiont of *G. brevipalpis*, L37341; P-endosymbiont of *G. tachinoides*, L37342; P-endosymbiont of *G. palpilis*, L37343; P-endosymbiont of *G. austeni*, L37340; P-endosymbiont of *G. morsitans morsitans*, L37339; P-endosymbiont of *Myzus persicae*, M63249; P-endosymbiont of *A. pisum*, M27049; P-endosymbiont of *Melaphis rhois*, M63255; P-endosymbiont of *Trialeurodes vaporariorum*, Z11928; and P-endosymbiont of *Siphoninus phillyreae*, Z11927.

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