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The Motility Symbiont of the Termite Gut Flagellate *Caduceia versatilis* Is a Member of the "*Synergistes*" Group▽†

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The flagellate *Caduceia versatilis* in the gut of the termite *Cryptotermes cavifrons* reportedly propels itself not by its own flagella but solely by the flagella of ectosymbiotic bacteria. Previous microscopic observations have revealed that the motility symbionts are flagellated rods partially embedded in the host cell surface and that, together with a fusiform type of ectosymbiotic bacteria without flagella, they cover almost the entire surface. To identify these ectosymbionts, we conducted 16S rRNA clone analyses of bacteria physically associated with the *Caduceia* cells. Two phylotypes were found to predominate in the clone library and were phylogenetically affiliated with the "*Synergistes*" phylum and the order *Bacteroidales* in the *Bacteroidetes* phylum. Probes specifically targeting 16S rRNAs of the respective phylotypes were designed, and fluorescence in situ hybridization (FISH) was performed. As a result, the "*Synergistes*" phylotype was identified as the motility symbiont; the *Bacteroidales* phylotype was the fusiform ectobiont. The "*Synergistes*" phylotype was a member of a cluster comprising exclusively uncultured clones from the guts of various termite species. Interestingly, four other phylotypes in this cluster, including the one sharing 95% sequence identity with the motility symbiont, were identified as nonectosymbiotic, or free-living, gut bacteria by FISH. We thus suggest that the motility ectosymbiont has evolved from a free-living gut bacterium within this termite-specific cluster. Based on these molecular and previous morphological data, we here propose a novel genus and species, "*Candidatus* Tammella caduceiae," for this unique motility ectosymbiont of *Caducaia versatilis*.

Termites harbor complex gut microbiota, which comprises unicellular eukaryotes, bacteria, and archaea. The majority of the symbiotic flagellates in termite guts are characterized by both their cellulolytic activity and an abundance of prokaryotic symbionts within and/or attached to the outsides of their cell bodies. Whereas the ecological roles that these physically associated symbionts fulfill for their host protists are mostly unknown, that of ectosymbionts of the flagellate *Caduceia versatilis* (family Devescovinidae, order Cristamonadida, phylum Parabasalia) in the gut of the termite *Cryptotermes cavifrons* was clearly demonstrated by Tamm in 1982; the ectosymbiotic bacteria confer motility on the host *Caduceia* protists with their flagella (36). Although the host protists possess their own flagella, their rapid gliding movement is provided solely by the flagellated ectosymbiotic bacteria and never by their own flagella. This arrangement is comparable to the only other known example of motility symbiosis between prokaryotes and eukaryotic hosts, that is, between the devescovinid flagellate *Mixotricha paradoxa* in the gut of the termite *Mastotermes darwiniensis* and its ectosymbiotic spirochetes (6, 19, 41).

Detailed microscopic observations have revealed that the motility ectosymbionts of *C. versatilis* are uniform rod-shaped bacteria partially embedded in the surfaces of the host protist cells and that they possess flagella only on the side exposed to the gut fluid (8, 36, 37) (Fig. 1). From 2,000 to 3,000 of the rod bacteria are aligned end to end in parallel rows that follow a helical path over the host cell surface. About 12 flagella from each rod bacterium form a bundle together with those from the adjacent rods, creating helical waves in synchrony. The spaces between the rod bacteria are covered by another type of ectosymbiont, fusiform bacteria without flagella (8, 36, 37) (Fig. 1).

In the present study, we aimed to identify this uncultured, unique motility symbiont by a combination of clone analyses and fluorescence in situ hybridization (FISH) analyses of 16S rRNA. Further, the symbiotic origin of this organism is discussed in the context of results from the molecular phylogeny analysis and FISH analyses of closely related phylotypes.

MATERIALS AND METHODS

Termites. The wood-feeding termites *C. cavifrons*, *Neotermes koshunensis* (family Kalotermitidae, order Isoptera), and *Reticulitermes speratus* (family Rhinotermitidae) were collected from Florida and from Okinawa and Saitama, Japan, respectively. They were kept in laboratories with the nest wood blocks before being used. Their entire guts were removed by sterile forceps and suspended in solution U (40). Then the gut suspensions were separated into three fractions, the gut wall, protists, and the remaining luminal content, by filtration through a nylon mesh (300-μm pore size) and low-speed centrifugations. These fractionated samples were used for FISH analyses.

WGA. The flagellated *C. versatilis* protists were collected from the gut of a *C. cavifrons* worker by using a micromanipulator, TransferMan NK2 (Eppendorf), and an inverted microscope, Leica DRM IRB. Whole *Caduceia* cells were used directly as templates for isothermal whole-genome amplification (WGA) with

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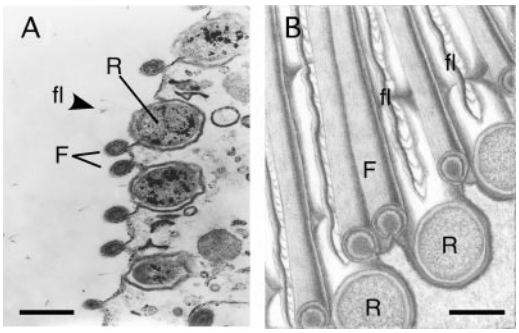

FIG. 1. Transmission electron micrograph of the cell surface area of *C. versatilis* (A) and view reconstructed from various microscopic observations (B). Reproduced with slight modifications from the *European Journal of Protistology* (8) with the permission of the publisher. The original scheme was published by Tamm (36). F, fusiform bacterium; R, rod bacterium; fl, flagellum or bundle of flagella. Bars, 0.5 μm.

the GenomiPhi HY amplification kit (GE Healthcare). The cells were suspended in a lysis buffer containing 0.4 N KOH, 10 mM EDTA, and 100 mM dithiothreitol. The amplification was performed for 2.5 h according to the manufacturer's instructions. The obtained products were purified by ethanol precipitation. By using WGA, we could repetitively analyze a small quantity of DNA samples, if necessary.

PCR amplification and cloning. The purified WGA products were diluted 1,000 times and used as templates for PCR amplifications with a proofreading DNA polymerase, Phusion (Finnzymes), and the *Bacteria*-specific primer pair 27F (5′-AGRGTTTGATYMTGGCTCAG) and 1492R (5′-GGHTACCTTGTTACGACTT) targeting 16S rRNA genes in the following program: an initial 45-s denaturation at 98°C; 15 cycles of denaturation (10 s at 98°C), annealing (1 min at 50°C), and extension (3 min at 72°C); and a final 10-min extension at 72°C. PTC-200 thermal cyclers (Bio-Rad) were used for PCRs. The products were purified using a MonoFas DNA purification kit (GL Sciences), and TA cloning was performed using a Zero Blunt Topo PCR cloning kit (Invitrogen).

For clone analysis of whole-gut microbiota, DNA was isolated using an Isoplant II kit (Nippon Gene Co.) and a DNeasy tissue kit (QIAGEN) as described previously (39). PCR with primers 27F and 1390R (38) for amplifications of 16S rRNA genes was performed using Ex-*Taq* polymerase with 12 cycles as described previously (14). The products were purified as described above, and TA cloning was performed using a TOPO TA cloning kit (Invitrogen).

Sequencing and phylogenetic analysis. Sequencing was performed using a BigDye Terminator cycle sequencing kit (Applied Biosystems) and an ABI 3700 genetic analyzer as described previously (16). All sequenced clones were evaluated for the identification of chimeric sequences by using the Bellerophon server (18) and the program Mallard (4) and by visually inspecting the aligned sequences. The detected chimeras were eliminated from the subsequent analyses. The remaining clones were sorted into phylotypes, defined by the criterion of 99.0% sequence identity, by using the program DOTUR v1.5 (31).

The sequences of clones were incorporated into the ARB database ssujun02 (21), modified in our previous studies (13–15), and the alignment was corrected manually. Closely related sequences, found by a BLAST search (2), and all termite gut clone sequences available in the public databases DDBJ, GenBank, and EMBL as of March 2007 were also added to the ARB database. An approximate taxonomic assignment was conducted using ARB (21) with its maximum-parsimony criterion. For detailed phylogenetic analysis, maximum-likelihood trees were constructed using the PHYML v2.4.4 program (12) with a general time-reversible nucleotide substitution model. The heterogeneity of nucleotide substitution rates among sites was approximated by a gamma distribution and an assumption of invariable sites.

FISH and enumeration. We designed oligonucleotide probes targeting 16S rRNA, specific to a phylotype or phylotypes (Table 1; also see Fig. S1 in the supplemental material), by using the probe-designing function in ARB (21). Each probe had two or more mismatches relative to any other sequences found in public databases, as confirmed with the Probe Match program in the Ribosomal Database Project II (23). The probes were labeled at the 5′ end with either Texas Red or 6-carboxyfluorescein (FAM). FISH analyses were performed at the hybridization temperature of 60°C for all probe sets as described previously (13, 28). It was demonstrated previously by FISH of 16S rRNA gene clones (32) that probes with expected melting temperatures similar to or higher than those of the probes used in this study never hybridized with sequences possessing two mismatches under identical conditions (13). The total number of prokaryotic cells in a whole termite gut was estimated, after the disruption of protist cells in sterile water, by counting immobilized cells on a black filter membrane (Millipore; pore size, 0.22 μm) stained with 4,6-diamidino-2-phenylindole (DAPI) as described previously (14). Enumerations of cells of a phylotype were performed, also after the disruption of protist cells in sterile water, basically as described previously (13). Briefly, cells immobilized on a silane-coated slide glass were hybridized with a *Bacteria*-specific probe mixture comprising EUB338 (3) and EUB338-II and EUB338-III (7) and simultaneously with a phylotype-specific probe and stained with DAPI. Then the number of specifically detected cells per approximately 400 to 1,400 DAPI-stained prokaryotic cells was calculated.

Nucleotide sequence accession numbers. The 16S rRNA sequences generated in this study have been deposited with DDBJ under accession numbers AB299516 to AB299568.

RESULTS

Clone analysis of bacteria associated with *Caduceia* protist cells. Two phylotypes predominated in the 16S rRNA clone library that was prepared from a mixture of 10 cells of *C. versatilis*. The phylotypes, CcCv-03 and CcCv-02, accounted for 57 and 28% of 94 analyzed clones and were phylogenetically affiliated with the order *Bacteroidales* in the phylum *Bacteroidetes* and the undescribed phylum "*Synergistes*," respectively. The *Bacteroidales* phylotype, CcCv-03, formed a monophyletic cluster with phylotypes NkD2-1 and CdD3-1 (Fig. 2), which were identified previously as ectosymbionts of the devescovinid protists *Devescovina* sp. from *N. koshunensis* guts and *Devescovina lemniscata* from *Cryptotermes domesticus* guts, respectively (26). Phylotype CcCv-03 showed 93 and 92% sequence identity to phylotypes NkD2-1 and CdD3-1, respectively. The other predominant phylotype, CcCv-02, in the "*Synergistes*" phylum, was affiliated with a cluster comprising exclusively uncultured clones from the guts of various termite species (Fig. 3). We designated this cluster termite cluster 2. The clones sorted into either the CcCv-03 or CcCv-02 phylotype showed almost no sequence variation within the respective phylotype. The remaining clones were dominated by a phylotype of the candidate phylum "Termite Group 1" (accounting for 9% of the clones), members of which are known as intracellular symbionts of various termite gut protists (30, 35).

Identification of motility symbionts by FISH. To identify the corresponding cells of phylotypes CcCv-03 and CcCv-02 in situ, FISH using specific probes (Table 1) was performed. The detection of the two phylotypes simultaneously by FISH clearly showed that the rod-shaped motility symbionts were the "*Synergistes*" phylotype CcCv-02 and that the fusiform ectobionts were the *Bacteroidales* phylotype CcCv-03 (Fig. 4A to D). The topologies of the bacteria were well exhibited

TABLE 1. FISH probes designed in this study

Probe	Sequence (5′→3′)	Targeted phylotype(s)[a]
CcCv-02-189	CTCCATCCTCTCACGCTC	CcCv-02, NkW01-046
Cc3-025-190	ACGGCACCCTCTCACGCT	Cc3-025
Cc3-074-191	CTATCCCTTTCACGCGCAA	Cc3-074
Rs-N28-190	ACATCACTGCCTTTCACGC	Rs-N28
CcCv-03-441	CACTCTTTACTCCCCTTC	CcCv-03

[a] Similarity to other sequences is shown in Fig. S1 in the supplemental material.

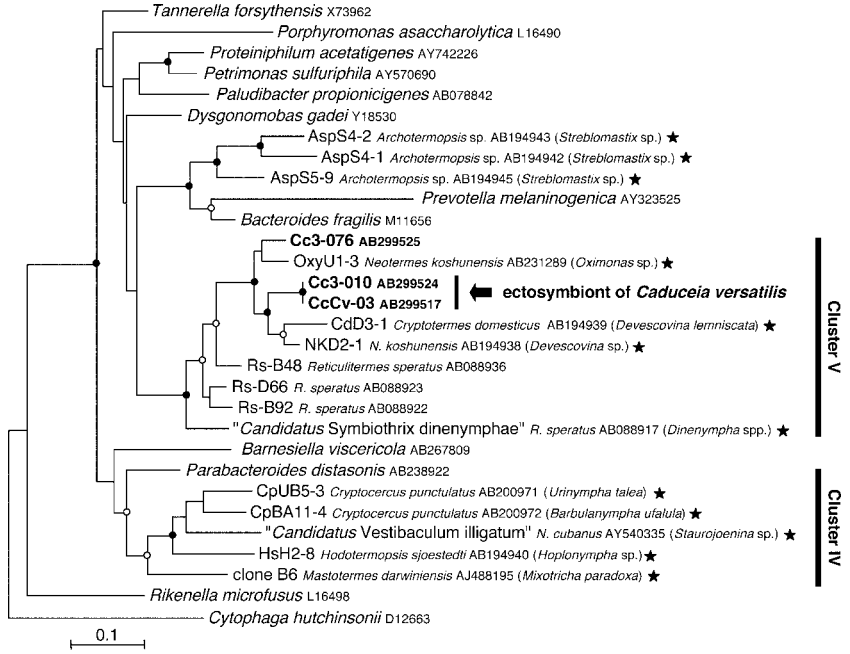

FIG. 2. Maximum-likelihood tree showing the phylogenetic position of the "*Bacteroidales*" ectosymbiont of *C. versatilis*, based on 16S rRNA sequences. Unambiguously aligned nucleotide sites (1,311) corresponding to positions 28 to 1389 in *Escherichia coli* (J01695) were used. Uncultured clones obtained from termite or cockroach guts are listed, along with their host species. Ectosymbionts of gut protists are indicated by stars, and their host protist species are given in parentheses. Cc3-010 is a clone belonging to phylotype CcCv-03 obtained from the clone analyses of the whole-gut microbiota. Clusters IV and V were designated in a previous study (29). A bootstrap test with 100 resamplings was performed. Open and closed circles at the nodes indicate bootstrap confidence values of 70 to 94 and 95 to 100, respectively.

in magnified micrographs (Fig. 4E to G), and their cell morphologies were more obvious in a DAPI-stained fragment of the protist cell surface area (Fig. 4H). Both FISH signals were detected almost exclusively from *Caduceia* cells; signals from clusters of bacteria, as shown in Fig. 4G, in luminal and gut wall fractions were observed rarely and most likely reflected the presence of surface fragments of crushed *Caduceia* cells. Although probe CcCv-02-189 can also potentially detect phylotype NkW01-046, this phylotype has been found exclusively in samples from *N. koshunensis* guts (25) and never in samples of cells of *C. versatilis* or the whole guts of *C. cavifrons* by clone analyses. Therefore, bacteria detected with this probe in *C. cavifrons* guts were considered to be exclusively phylotype CcCv-02.

Enumeration and size of symbiont cells. The frequency of cells of the "*Synergistes*" phylotype CcCv-02 was 3.2% ± 1.6% and 2.8% ± 1.4% of the prokaryotic cells detected in the whole-gut microbiota ($n = 3$) with a *Bacteria*-specific probe mixture and DAPI, respectively. The total number of DAPI-stained prokaryotic cells was estimated to be $6.3 \times 10^7 \pm 0.2 \times 10^7$ per gut ($n = 6$); the number of CcCv-02 cells was 1.8×10^6 per gut. This value is within the range from 2.8×10^5 to 1.2×10^7 that is expected based on the number of rod bacteria per host cell (approximately 2,500) (36) and the number of host cells per gut (110 to 4,700) (10). The *Bacteroidales* phylotype CcCv-03 accounted for 8.5% ± 0.5% and 7.6% ± 0.5% ($n = 3$) of cells in the whole-gut microbiota detected with the *Bacteria*-specific probe mixture and DAPI, respectively. The cell size of each phylotype is shown in Table 2. The lengths and widths of the cells were similar to those given in previous reports (8, 36, 37).

Finding of related "*Synergistes*" members. To test whether there are other phylotypes related to the motility symbiont CcCv-02 in *C. cavifrons* guts, we conducted a clone analysis of 16S rRNA from the whole-gut microbiota of *C. cavifrons* by using a *Bacteria*-specific primer pair. Among 112 analyzed clones, 51 phylotypes were recognized; the *Bacteroidales* phylotype CcCv-03 was the most dominant (17%), and the motility symbiont phylotype CcCv-02 was the second most dominant (14%). Besides phylotype CcCv-02, four phylotypes, each representing only one or two clones, were classified into the "*Synergistes*" phylum, and two of them, Cc3-025 and Cc3-074, were affiliated with termite cluster 2 (Fig. 3). Phylotype Cc3-025 was phylogenetically most closely related to phylotype CcCv-02, showing 94.9% sequence identity.

Localization of related "*Synergistes*" members. In order to locate the additionally found "*Synergistes*" phylotypes, Cc3-025 and Cc3-074, in *C. cavifrons* guts, FISH was performed using probes designed to be specific to each phylotype (Table 1). As a result, both phylotypes were specifically detected as nonectosymbiotic (hereinafter called free-living) gut bacteria (Fig. 5A to D). Further, phylotype NkW01-046 from *N. koshunensis* (25) and phylotype Rs-N28 from *R. speratus* (16) in termite cluster 2 (Fig. 3) were also specifically detected by FISH as free-living gut bacteria in the respective host termite species (Fig. 5E to H). All of these four free-living phylotypes in termite cluster 2 were found as rare components (frequency,

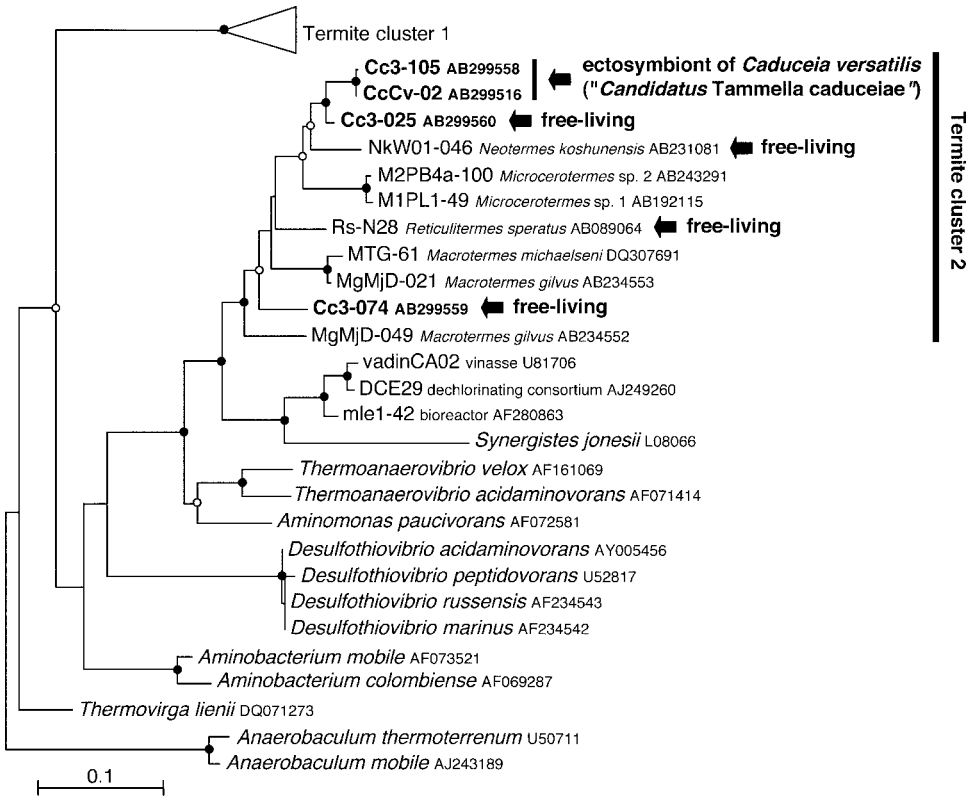

FIG. 3. Maximum-likelihood tree showing the relationship of members of the "*Synergistes*" phylum, based on 16S rRNA sequences. Unambiguously aligned nucleotide sites (1,257) corresponding to positions 28 to 1389 in *E. coli* (J01695) were used. Cc3-105 is a clone belonging to phylotype CcCv-02 obtained from the clone analyses of the whole-gut microbiota. Termite cluster 1 comprises 11 previously described phylotypes from termite guts (14–16) and two novel phylotypes, Cc3-068 and Cc3-109, obtained in the present study. Short sequences obtained from termite guts in a previous study (11), all of which belonged to termite cluster 1, were not included in this analysis. See the legend to Fig. 2 for further explanation.

<1%) of the respective gut microbiotas. Their morphological characteristics are summarized in Table 2.

DISCUSSION

Motility symbiosis between eukaryotes and prokaryotes in which the latter confer motility on the former is an extremely rare phenomenon; only two examples are known thus far. The first one was discovered by Cleveland and Grimstone in 1964; the termite gut flagellate *M. paradoxa* is propelled not by its own flagella but by its ectosymbiotic spirochetes (6). The second one, i.e., the present case, was first described by Tamm in 1982 in ecological and morphological detail (36) but without taxonomic assignment, which has been accomplished in the present study; the motility symbiont belongs to the phylum "*Synergistes*." Since the symbiont is considered to be confined to *C. versatilis* cells and a *C. versatilis* cell without this symbiont has never been observed, this symbiosis appears to be obligatory. The specialized attachment structures for the symbiont, as well as the specialized morphology of the symbiont, which has flagella only on the exposed side (8, 36, 37), also indicate the robust symbiotic relationship as a consequence of coevolution. This is the first report of an obligate symbiont from the "*Synergistes*" phylum that physically associates with a eukaryotic cell.

It was demonstrated in the present study that the motility symbiont is a member of a termite-specific cluster. This cluster, designated termite cluster 2, contains, in addition, four phylotypes from lower termites that harbor symbiotic gut flagellates and five clones or phylotypes from higher termites that generally lack gut flagellates. Since the former four were identified in this study as free-living gut bacteria by FISH and the latter have no candidate host protists, it is likely that this monophyletic cluster comprises exclusively free-living gut bacteria except for the motility symbiont. This idea implies that the motility symbiont has originated from an ancestral free-living gut bacterium within termite cluster 2. However, it is mysterious why this unique, unusual symbiont has evolved from this lineage. In the case of spirochetal motility symbionts of *M. paradoxa*, the evolutionary process is conceivable because there are plenty of examples of nonlocomotory ectosymbiotic spirochetes on diverse protists in termite guts, implying metabolic symbiosis with the host protists via, e.g., hydrogen molecules, which can be postulated from the physiological properties of isolated *Treponema* strains (20). Unfortunately, the physiological characteristics of termite cluster 2 bacteria of the "*Synergistes*" are unknown because no isolate has been obtained.

Clues to the physiology of termite cluster 2 bacteria may be found in features shared by isolates in the other clusters within the "*Synergistes*" phylum. This phylum contains 13 described

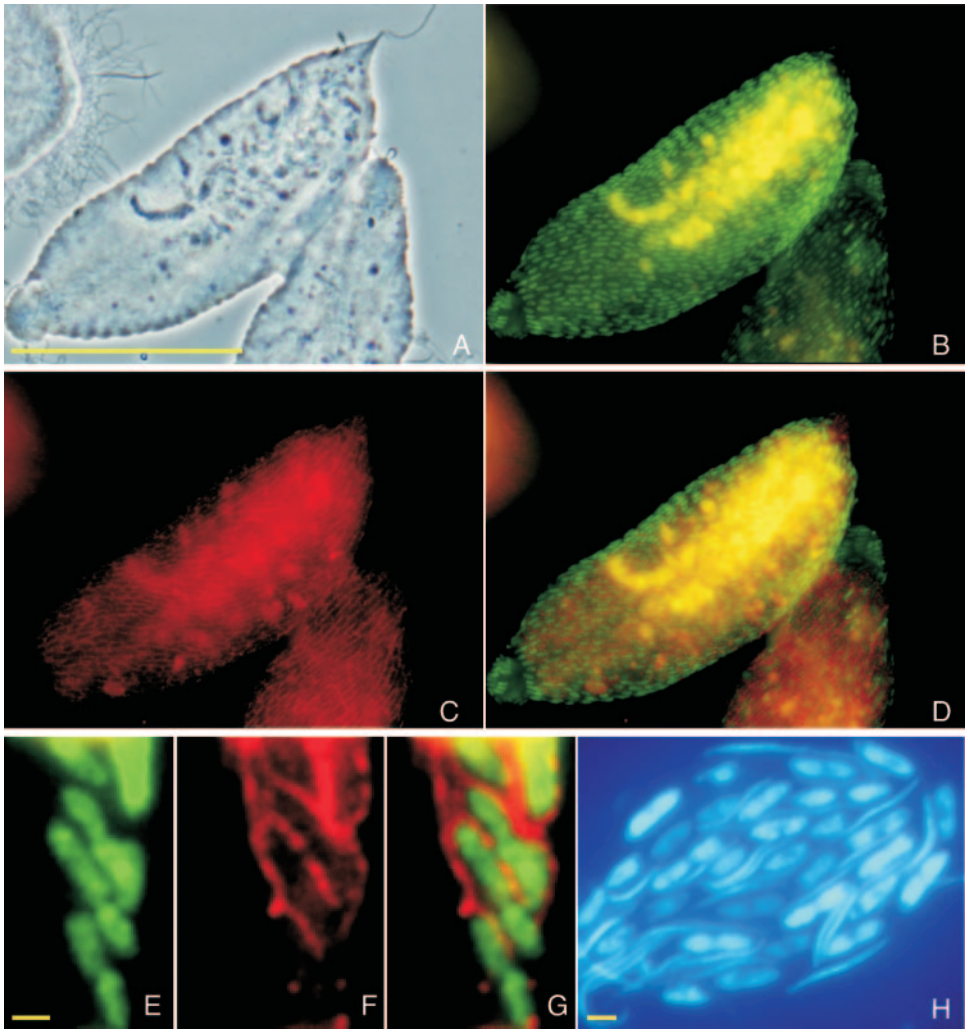

FIG. 4. In situ identification of ectosymbiotic bacteria of the flagellate *C. versatilis*. (A) Phase-contrast image of *C. versatilis*. (B) Specific detection of the "*Synergistes*" phylotype CcCv-02 with a FAM-labeled probe (green). The yellowish color was caused by the autofluorescence of ingested wood particles. (C) Specific detection of the *Bacteroidales* phylotype CcCv-03 with a Texas Red-labeled probe. (D) Overlaid FISH image of panels B and C. (E to G) Magnified images of the ectosymbionts on a fragment of the host cell surface as detected by FISH with the same combination of probes used for panels B to D. (H) DAPI-stained fragment of the host cell surface immobilized on a black filter membrane. The rods are the "*Synergistes*" motility symbionts; the fusiform cells are the *Bacteroidales* symbionts. Bars, 100 μm in panel A and 1 μm in panels E and H.

species of seven genera, as shown in Fig. 3. All of them are gram-negative rods or curved rods, are strictly anaerobic, and characteristically ferment amino acids as carbon, energy, and nitrogen sources into acetate, hydrogen, carbon dioxide, and other products (e.g., see references 1 and 22). Symbioses with methanogens via hydrogen molecules have been observed occasionally (e.g., see references 5 and 24). Nine species possess flagella, which is indicative of the presence of flagella in an

TABLE 2. Characteristics of phylotypes detected by FISH in this study

Phylotype	Phylum	Localization[a]	Dominant morphotype	Length (μm)	Width (μm)	Amplitude[b] (μm)
CcCv-02	"*Synergistes*"	Ectosymbiont	Straight rod	1.3–2.9	0.5–1.0	
Cc3-025	"*Synergistes*"	Free living	Curved rod	1.6–5.5	0.3–0.5	0.5–1.6
Cc3-074	"*Synergistes*"	Free living	Long or curved rod	1.9–9.5	0.3–0.5	0.5–0.8
NkW01-046	"*Synergistes*"	Free living	Slightly curved rod	1.5–16.7	0.2–0.4	0.4–0.7
Rs-N28	"*Synergistes*"	Free living	Straight rod	1.8–4.0	0.3–0.4	
CcCv-03	*Bacteroidetes*	Ectosymbiont	Fusiform	1.9–6.1	0.2–0.4	

[a] Free living means not ectosymbiotic with gut protists and found exclusively in the luminal and gut wall fractions.
[b] The amplitude is herein defined as the minimum distance from the peak of the rod curve to the straight line between the cell ends.

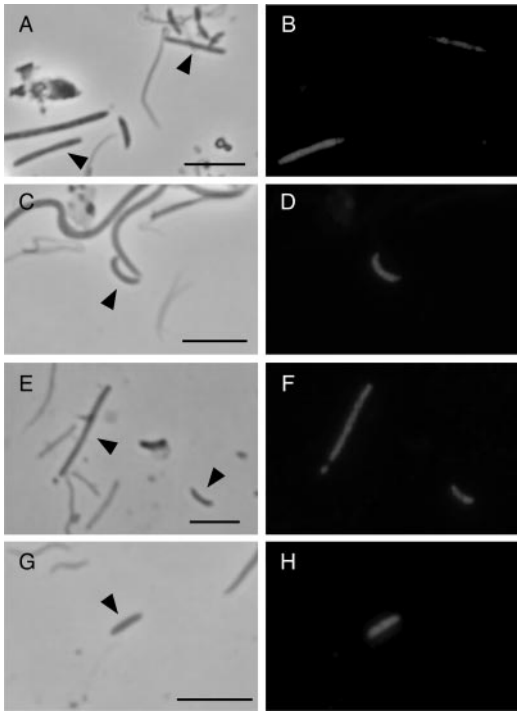

FIG. 5. In situ detection of phylotypes in termite cluster 2 of the "*Synergistes*" phylum. Panels A, C, E, and G are phase-contrast images; panels B, D, F and H are FISH images obtained by using phylotype-specific probes. (A and B) Phylotype Cc3-025 detected in a sample from a *C. cavifrons* gut. (C and D) Phylotype Cc3-074 from a *C. cavifrons* gut. In order to ensure the specific detection of these respective phylotypes, the specific probes (Texas Red-labeled Cc3-025-190 and FAM-labeled Cc3-074-191) were used simultaneously. (E and F) Phylotype NkW01-046 from an *N. koshunensis* gut. (G and H) Phylotype Rs-N28 from an *R. speratus* gut. Each of the Texas Red-labeled probes CcCv-02-189 and Rs-N28-190 was used with FAM-labeled probe TG3S1-168 (13) to distinguish nonspecific signals. Arrowheads indicate the detected cells. Bars, 5 μm.

ancestor of the motility symbiont. It is unclear if the ability to ferment amino acids would enhance the growth of the protists or termites. Given that 0.5 to 2.5 mM concentrations of several specific amino acids, such as asparagines and glutamate, exist in a termite hindgut (33), "*Synergistes*" bacteria are likely to survive on these amino acids with or without benefits to their hosts and other gut microbes. Nevertheless, "*Synergistes*" bacteria other than this motility symbiont have never been found as dominant members of termite gut microbiotas (e.g., see references 9, 11, 13, 14, 15 and 16).

As discussed previously (6), movement without a specific direction may not be problematic for the host protists inhabiting termite guts, where they rarely need to search for food or escape from unfavorable conditions and predators. Interactions via the exchange of metabolites between the "*Synergistes*" ectosymbionts and the host *Caduceia* protist, as well as between the "*Synergistes*" and *Bacteroidales* ectosymbionts, remain to be clarified. *Bacteroidales* ectosymbionts have been commonly and abundantly found in association with diverse protist species from the guts of various termite species and a cockroach (17, 26, 27, 34). Since *Bacteroidales* ectosymbionts, though members of a lineage distinct from that identified in the present study, have also been found on *M. paradoxa* cells that harbor spirochetal motility symbionts (Fig. 2) (6, 19, 41), the elucidation of the function of these *Bacteroidales* ectosymbionts may also be important for understanding the evolution and mechanism of the motility symbioses.

Based on the present molecular and previous morphological and ecological data (8, 36, 37), we propose a novel genus and species, "*Candidatus* Tammella caduceiae," for this motility symbiont of the flagellate *C. versatilis*.

Description of "*Candidatus* Tammella caduceiae." *Tammella caduceiae* (Tamme′lla. N.L. fem. dim. n. *Tammella*, name honoring Sidney L. Tamm, a contemporary American cytologist, for his discovery of the symbiosis in which this bacterium confers motility. Ca.du.ce.i′ae. N.L. gen. n. *caduceiae*, of *Caduceia*, referring to the genus name of the host protist). The bacteria are gram-negative, straight rods of 1.3 to 2.9 μm by 0.5 to 1.0 μm. They are specifically attached to the cell surface of the flagellate *Caduceia versatilis* in the gut of the termite *Cryptotermes cavifrons*. About two-thirds of the cell is embedded in the host cell surface and held by a specialized structure, with approximately 12 lateral flagella protruding from the exposed side. About 2,000 to 3,000 cells per host cell are aligned end to end in parallel rows over the host cell surface. Bundles formed by the flagella confer motility on the host protist. The classification was verified based on the 16S rRNA gene sequence and hybridization with a 16S rRNA-targeted oligonucleotide probe (5′-TTCACTCTCAAGTCGCCC). The organism has not been cultured thus far.

ACKNOWLEDGMENTS

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