The phylogenetic relationships of *Caulobacter*, *Asticcacaulis* and *Brevundimonas* species and their taxonomic implications

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The phylogenetic relationships among the species of *Caulobacter*, *Asticcacaulis* and *Brevundimonas* were studied by comparison of their 16S rDNA sequences. The analysis of almost complete sequences confirmed the early evolutionary divergence of the freshwater and marine species of *Caulobacter* reported previously [Stahl, D. A., Key, R., Flesher, B. & Smit, J. (1992). *J Bacteriol* 174, 2193–2198]. The freshwater species formed two distinct clusters. One cluster contained the species *Caulobacter bacteroides*, *Caulobacter crescentus*, *Caulobacter fusiformis* and *Caulobacter henricii*. *C. bacteroides* and *C. fusiformis* are very closely related (sequence identity 99.8%). The second cluster was not exclusive and contained the species *Caulobacter intermedius*, *Caulobacter subvibrioides* and *Caulobacter variabilis*, as well as *Brevundimonas diminuta* and *Brevundimonas vesicularis*. The marine species *Caulobacter halobacteroides* and *Caulobacter maris* were very closely related, with a sequence identity of 99.7%. These two species were most closely but distantly related to the marine hyphal/budding bacteria *Hyphomonas jannaschiana* and *Hirschia baltica*, which formed a deep phylogenetic line with *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*. *Caulobacter leidyia* is unrelated to the other species of *Caulobacter* and belongs to the alpha-4 subclass of the *Proteobacteria*, forming a distinct cluster with *Asticcacaulis excentricus* and *Asticcacaulis biprosthecium*. The taxonomic implications of the polyphyletic nature of the genus *Caulobacter* and the absence of a type culture for the type species of the genus, *Caulobacter vibrioides*, are discussed.

Keywords: 16S rRNA, phylogeny, *Caulobacter*, *Asticcacaulis*, *Brevundimonas*

INTRODUCTION

Current knowledge of the phylogenetic and evolutionary relationships of *Caulobacter* species is based on data from chemotaxonomy (Nikitin *et al.*, 1990), DNA–DNA hybridization (Moore *et al.*, 1978) and sequence identities of their 5S rRNA (Nikitin *et al.*, 1990) and 16S rRNA genes (Sly *et al.*, 1997; Stackebrandt *et al.*, 1988; Stahl *et al.*, 1992).

The EMBL accession numbers for the rDNA sequences from *Asticcacaulis biprosthecium* ACM 2498^T (= ATCC 27554^T), *Asticcacaulis excentricus* ACM 1263^T (= ATCC 15261^T), *Brevundimonas vesicularis* ACM 2862^T (= ATCC 11426^T), *Caulobacter intermedius* ACM 2608^T (= ATCC 15262^T), *Caulobacter fusiformis* ATCC 15257^T, *Caulobacter halobacteroides* ATCC 15269^T, *Caulobacter henricii* ATCC 15253^T, *Caulobacter leidyia* ATCC 15260^T, *Caulobacter maris* ATCC 15268^T and *Caulobacter variabilis* ATCC 15255^T are AJ007799–AJ007808, respectively.

The species of *Caulobacter* so far studied belong to the alpha-2 subclass of the *Proteobacteria* (Woese *et al.*, 1984). Stahl *et al.* (1992) undertook a phylogenetic study of several species of *Caulobacter* and a number of freshwater and marine isolates. These authors concluded that, on the basis of 16S rRNA sequence identities, the species of *Caulobacter* studied formed a diverse but coherent phylogenetic assemblage but that there had been an early evolutionary divergence of the freshwater and marine species. The one exception was *Caulobacter subvibrioides*, which was only peripherally related to the major *Caulobacter* group. It has since been shown, by resequencing of the 16S rDNA of the type strain of *C. subvibrioides*, ATCC 15264^T, that this species does in fact belong to the *Caulobacter* cluster comprising *Caulobacter bacteroides* and *Caulobacter crescentus*, the only species for which sequences of the type strains were available at the time (Sly *et al.*, 1997).

Stahl *et al.* (1992) also showed that the closest phylogenetic relative of the caulobacters is *Brevundimonas* (*Pseudomonas*) *diminuta*. These authors speculated that *B. diminuta*, a non-prosthecae, Gram-negative, motile bacterium with a polar flagellum (Segers *et al.*, 1994), may have lost the ability to form prosthecae during its evolution or may be permanently locked in the motile stage of its development cycle.

A full understanding of the relationships of the species of the genus *Caulobacter* has been limited by the lack of 16S rRNA sequences for all eleven species (Moore & Moore, 1992; Skerman *et al.*, 1980) and for related bacteria, including the second species of the genus *Brevundimonas*, *Brevundimonas vesicularis*. In this paper this situation has been rectified by the determination of 16S rDNA sequences for the available type strains of *Caulobacter* species and for *B. vesicularis*. Sequences have also been obtained for the two species of *Asticcacaulis* (*Asticcacaulis biprosthecium* and *Asticcacaulis excentricus*). The genus *Asticcacaulis* is treated taxonomically as a close relative of the genus *Caulobacter* (Poindexter, 1989, 1992; Schmidt, 1981), primarily because of its prosthecae morphology and rosette formation, but evidence to support a close phylogenetic relationship has been lacking.

METHODS

Bacterial strains. The type cultures of *A. biprosthecium* ACM 2498^T (= ATCC 27554^T), *A. excentricus* ACM 1263^T (= ATCC 15261^T), *B. vesicularis* ACM 2862^T (= ATCC 11426^T) and *Caulobacter intermedius* ACM 2608^T (= ATCC 15262^T) were obtained from the Australian Collection of Micro-organisms, Department of Microbiology, University of Queensland, Brisbane, Australia. The type cultures of the species *Caulobacter fusiformis* ATCC 15257^T, *Caulobacter halobacteroides* ATCC 15269^T, *Caulobacter henricii* ATCC 15253^T, *Caulobacter leidyia* ATCC 15260^T, *Caulobacter maris* ATCC 15268^T and *Caulobacter variabilis* ATCC 15255^T were obtained from the American Type Culture Collection, Manassas, VA, USA.

Extraction and purification of genomic DNA. Genomic DNA was purified as described by Rainey *et al.* (1992). The concentration and purity of DNA extracts was examined by electrophoresis of 5 µl purified DNA with 3 µl loading buffer (0.25%, w/v, bromophenol blue; 30%, v/v, glycerol in water) and a 1 kbp DNA ladder (Gibco-BRL) on a 1% agarose gel (1 × TAE buffer containing 5 µl of a 10 mg ethidium bromide ml⁻¹ solution per 100 ml).

PCR amplification of 16S rDNA. Each 100 µl reaction mixture contained 10 × PCR buffer (Biotech) (10 µl), 0.1 mM dNTPs, 1.5 mM Mg²⁺ (in the form of MgCl₂), universal primers 27f and 1492r (Lane, 1991), each at 2 µg l⁻¹, 1–2 µl genomic DNA template and sterile, deionized water (Milli-Q). The initial denaturation step was performed at 95 °C for 5 min in the PCR apparatus (PTC-100 Thermal Cycler; MJ Research). The denatured reaction mixture was held at 4 °C until 2.2 U *Tth* DNA polymerase (Biotech) was added to each tube to start the reaction. The thermal cycling program included 30 cycles of denaturation (94 °C, 1 min), annealing (48 °C, 1 min) and extension (72 °C, 2 min). Final annealing (48 °C, 1 min) and extension (72 °C, 5 min) steps were performed at the end of the 30 cycles. The PCR products

were purified by using Wizard PCR-Preps DNA purification system (Promega) as described by the manufacturer. The purity and fragment size of the purified preparations were estimated by electrophoresis of 2 µl of each PCR product on a 1% agarose gel with a low-molecular-mass ladder (Gibco-BRL) as described above for genomic DNA.

Sequencing of 16S rDNA. The PRISM Ready Reaction DyeDeoxy Terminator cycle-sequencing kit (Applied Biosystems) was used to sequence the purified 16S rDNA directly, as described by the manufacturer. The final concentrations of reagents in the 20 µl sequencing reactions were: 8 µl terminator ready reaction mix, 1–2 µl PCR product, 25 ng sequencing primer and sterile, deionized water (Milli-Q). Sequencing primers 27f, 357f, 530f, 803f, 1114f, 342r, 519r, 787r, 1100r and 1492r (Lane, 1991) were used to sequence the forward and reverse strands. The reaction mixtures were purified and automatically sequenced with an Applied Biosystems model 373A DNA sequencer as recommended by the manufacturer.

Phylogenetic analysis. The 16S rDNA sequences determined for *A. biprosthecium* ACM 2498^T (= ATCC 27554^T), *A. excentricus* ACM 1263^T (= ATCC 15261^T), *B. vesicularis* ACM 2862^T (= ATCC 11426^T), *C. fusiformis* ATCC 15257^T, *C. halobacteroides* ATCC 15269^T, *C. henricii* ATCC 15253^T, *C. intermedius* ACM 2608^T (= ATCC 15262^T), *C. leidyia* ATCC 15260^T, *C. maris* ATCC 15268^T and *C. variabilis* ATCC 15255^T were aligned manually with the sequence of *Escherichia coli* and other representative sequences of the *Proteobacteria* by using the ae2 sequence editor (Maidak *et al.*, 1997).

The sequences of the following strains were obtained from GenBank and the Ribosomal Database Project (RDP) (Maidak *et al.*, 1997): *Agrobacterium tumefaciens* IAM 13129^T (= ATCC 23308^T), accession no. D12784; *Blastomonas natatoria* ATCC 35951^T, Y13774; *Brevundimonas* (*Pseudomonas*) *diminuta* ATCC 11568^T, M59064; *C. bacteroides* ATCC 15254^T, M83796; *C. crescentus* CB2A^T (= ATCC 15252^T), X52281; *C. subvibrioides* ATCC 15264^T, X94470; *C. subvibrioides* CB81^T, M83797; *Caulobacter* sp. strain FWC2, M83803; *Caulobacter* sp. strain FWC14, M83800; *Caulobacter* sp. strain FWC17, M83801; *Caulobacter* sp. strain FWC18, M83802; *Caulobacter* sp. strain FWC26, M83804; *Caulobacter* sp. strain FWC38, M83805; *Caulobacter* sp. strain MCS6, M83811; *Caulobacter* sp. strain MCS10, M83807; *Caulobacter* sp. strain MCS17, M83808; *Caulobacter* sp. strain MCS18, M83809; *Caulobacter* sp. strain MCS24, M83810; *Erythrobacter longus* OCH101^T (= ATCC 33941^T), L01786; *Hirschia baltica* ATCC 49814^T, X52909; *Hyphomicrobium vulgare* ATCC 27500^T, X53182; *Hyphomonas jannaschiana* ATCC 33882^T, M83806; *Pedomicrobium ferrugineum* ACM 3037^T (= ATCC 33119^T), X97690; *Porphyrobacter neustonensis* ACM 2844^T, L01785; *Rhizomonas suberifaciens* IFO 15211^T (= ATCC 49355^T), D13737; *Rhodobacter capsulatus* ATCC 11166^T, D16428; *Rhodobacter sphaeroides* ATCC 17023^T, X53855; *Rhodocista centenaria* ATCC 43720^T, D12701; *Rhodomicrobium vannielii* ATCC 51194^T, M34127; *Rhodospirillum rubrum* ATCC 11170^T, M32020; *Sphingomonas adhaesiva* IFO 15099^T (= ATCC 51229^T), D16146; *Sphingomonas capsulata* GIFU 11526^T (= ATCC 14666^T), D16147; *Sphingomonas macrogoltabidus* IFO 15033^T (= ATCC 51380^T), D13723; *Sphingomonas parapaucimobilis* IFO 15100^T (= ATCC 51231^T), D13727; *Sphingomonas paucimobilis* GIFU 2395^T (ATCC 29837^T), D16144; *Sphingomonas sanguinis* IFO 13937^T (= ATCC 51382^T), D13726;

Sphingomonas terrae IFO 15098^T (= ATCC 51381^T), D13727; *Sphingomonas yanoikuyae* IFO 15102^T (= ATCC 51230^T), D13728; and *Zymomonas mobilis* ATCC 10988^T, accession *Zym.mobili* (RDP).

Pairwise evolutionary sequence similarities and distances (Jukes & Cantor, 1969) of the rDNA sequences were computed by using the DNADIST program in the PHYLIP 3.5 software package (Felsenstein, 1993). Phylogenetic trees were constructed by using the neighbour-joining method of Saitou & Nei (1987) in the program NEIGHBOR (Felsenstein, 1993). Bootstrap analysis of 100 data resamplings was performed with SEQBOOT and CONSENSE (Felsenstein, 1993) to determine the statistical confidence of branch points in the trees.

RESULTS AND DISCUSSION

Phylogenetic relationships

The phylogenetic analysis of almost complete 16S rDNA sequences (> 1380 nucleotides) for all available type strains confirmed the early evolutionary diver-

Fig. 1. Neighbour-joining tree showing the phylogenetic relationships among the species of *Caulobacter*, *Asticcacaulis*, *Brevundimonas* and other members of the α -Proteobacteria. The sequence of *Rhodospirillum rubrum* was used as the outgroup. The analysis included 1090 unambiguous nucleotide positions. Bootstrap values from 100 analyses are shown at the branch-points of the tree. The scale bar represents 10 nucleotide substitutions per 100 nucleotides of 16S rDNA sequence. Sequences of species shown in bold were determined in this study.

gence of the freshwater and marine species of *Caulobacter* reported by Stahl *et al.* (1992). The type strain of *Caulobacter vibrioides* is not extant (Skerman *et al.*, 1980) and therefore could not be included in the study.

In an analysis of 1090 nucleotides (Fig. 1), the freshwater species were all shown to belong to a single phylogenetic lineage strongly supported by a bootstrap value of 100%. Two clusters were evident in the lineage. The first cluster contained the species *C. bacteroides*, *C. crescentus*, *C. fusiformis* and *C. henricii*. Except for the species *C. bacteroides* and *C. fusiformis*, which were very closely related (99.8% sequence identity), members of this cluster had sequence identities in the range 97.2–97.8%. The second cluster was not exclusive and contained the species *C. intermedius*, *C. subvibrioides* and *C. variabilis*, as well as the two species of *Brevundimonas*. In this analysis, *B. diminuta* and *B. vesicularis* had a sequence identity of 97.5% but did not cluster together. Both species are most closely related to *C. intermedius*, which has 99% sequence identity to *B. vesicularis* and 96.9% to *B. diminuta*. The sequence identities of members of this cluster were in the range 95.7–99.0% and exhibited 94.2–96.1% sequence identity to the species in the first cluster.

The phylogenetic analysis showed that the marine species *C. halobacteroides* and *C. maris* were very closely related, with a sequence identity of 99.7%. These two species were most closely but distantly related to the marine hyphal/budding bacteria *Hyphomonas jannaschiana* and *Hirschia baltica*, which formed a deep phylogenetic line with *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*. Stahl *et al.* (1992) first showed the close relationship with *Rhodobacter* and this relationship has endured with the addition of sequences for all species of *Caulobacter*. The other freshwater members of the budding/hyphal bacteria, *Pedomicrobium*, *Rhodocyclum* and *Hyphomicrobium*, belong to a separate, deep branch.

C. leidya, isolated from the hind gut of a millipede, is unrelated to the other species of *Caulobacter*. *C. leidya* belongs to the alpha-4 subclass of the Proteobacteria, forming a distinct cluster with *A. excentricus* and *A. biprosthecium*. All three species are very closely related, having sequence identities in the range 99.7–99.9%. Interestingly, the closest relative of these species is the original sequence (M83797) of *C. subvibrioides*, C81, which should no longer be associated with this species (Sly *et al.*, 1997). Previously, this sequence was shown to be from a bacterium most closely related to *S. adhaesiva* (Sly *et al.*, 1997), but the inclusion of the sequences determined in the current study shows sequence M83797 to be most closely related to *C. leidya* (99.8% sequence identity).

A second phylogenetic analysis was undertaken to include the sequences obtained by Stahl *et al.* (1992) for a number of marine and freshwater isolates. This analysis included only 968 nucleotides, owing to shorter sequence lengths for some strains and an unsequenced gap of 99 nucleotides in the 509–610

Fig. 2. Neighbour-joining tree showing the phylogenetic relationships among the species of *Caulobacter*, *Brevundimonas* and several marine and freshwater isolates (Stahl *et al.*, 1992) of *Caulobacter*. The sequence of *Agrobacterium tumefaciens* was used as the outgroup. The analysis included 968 unambiguous nucleotide positions. Bootstrap values from 100 analyses are shown at the branch-points of the tree. The scale bar represents 10 nucleotide substitutions per 100 nucleotides of 16S rDNA sequence. Sequences of species shown in bold were determined in this study.

region of the sequences. The phylogenetic tree (Fig. 2) supports the general conclusions of Stahl *et al.* (1992) but the inclusion of sequences for all *Caulobacter* species allows some more definite affiliations to be inferred. The marine strain MCS10 is most closely related (sequence identity 99.3–99.5%) to *C. halobacteroides* and *C. maris*. However, because of the high sequence identity between these two species, DNA–DNA hybridization will be required to resolve these relationships (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987). The marine strains MCS6 and MCS18 form a strongly supported phylogenetic line with 96.4–96.7% identity to *C. halobacteroides* and *C. maris* and probably represent a new species.

Stahl *et al.* (1992) concluded that the freshwater isolates FWC2, FWC17, FWC18 and FWC26 were most closely related to *C. crescentus*. The inclusion of sequences for all available type strains of *Caulobacter* species has confirmed the close relationship of strain FWC26 with *C. crescentus* (99.8% sequence identity). Strains FWC17 and FWC18 are also most closely related to *C. crescentus* (99.1–99.3% identity), even though they cluster with low bootstrap support with *C. henricii*. Strain FWC2 has 98.5% sequence identity to both *C. crescentus* and *C. henricii*. The inclusion of the additional sequences in the analysis has also allowed more definite conclusions to be drawn about the identity of strains FWC14 and FWC38, isolated from water-treatment systems (MacRae & Smit, 1991). The sequence of strain FWC14 is most closely related to the sequences of *B. vesicularis* and *C. intermedius* de-

termined in this study (sequence identity 98.6%), while strain FWC38 remains unaffiliated and may represent a new species. Stahl *et al.* (1992) showed that two marine strains, MCS17 and MCS24, are affiliated to the freshwater line of descent. The analysis with additional reference strains confirms this and shows that these isolates are most closely related to *C. variabilis*. Interestingly, strain MCS24 is reported to be freshwater tolerant (Stahl *et al.*, 1992).

Taxonomic implications

Several of the phylogenetic relationships revealed in this study have taxonomic implications. The morphology of the budding and prosthecae bacteria has played an important part in their taxonomy (Henrici & Johnson, 1935; Poindexter, 1964, 1989, 1992; Schmidt, 1981; Staley & Fuerst, 1989) and the distinct nature of the prosthecae stalk of *Caulobacter* has been used as a primary characteristic for the assignment of species to the genus. However, the phylogenetic study of Stahl *et al.* (1992) provided the first evidence that the species assigned to the genus may be less related than expected by their distinctive morphology. These workers provided evidence of an early divergence of the marine and freshwater species, and the evolutionary significance of the divergence of the marine and freshwater species has been discussed at length by Stahl *et al.* (1992). The taxonomic implications were also noted by Stahl *et al.* (1992), who cautioned that taxonomic revision should await the availability of full sequences for all species. These are now available for all species except *C. vibrioides*, for which the type culture of the species is not extant (Skerman *et al.*, 1980).

Only one of the lineages of *Caulobacter* species can retain the generic name. Under Rule 39a,b of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992), the generic name must be retained for the genus that includes the type species. The lack of a type culture for the type species complicates this decision. Several avenues are available to resolve this issue. It would be reasonable to assume that the type species *C. vibrioides* would belong to the lineage of freshwater species, but it is possible that the two clusters of freshwater species may ultimately be assigned to separate genera. It is therefore more desirable to resolve the issue experimentally rather than theoretically. The possible strategies are to locate the type strain, to designate a neotype strain (Rule 18c) or alternatively to designate a new type species (Rule 20d). The original description of *C. vibrioides* by Henrici & Johnson (1935) was based solely on morphological characters determined in slide culture of natural communities. The description of *C. vibrioides* was emended by Bowers *et al.* (1954) after the study of an isolate from well water. Poindexter (1964) designated strain CB51^T as the neotype strain over strain CB-G from Bowers *et al.* (1954), on the grounds that the latter strain was limonoid in shape rather than vibrioid. Poindexter (1964) also found a significant number of phenotypic differences between strain CB-

G and strain CB51^T, and there is doubt as to whether Bowers *et al.* (1954) studied the species originally observed by Henrici & Johnson (1935). The neotype strain CB51^T was reconfirmed by Poindexter & Lewis (1966) and became the type strain upon publication of the Approved Lists of Bacterial Names (Skerman *et al.*, 1980), even though at that time it was recorded as not extant. Strain CB51^T is not available in any of the major service culture collections, although strain CB-G is available (NCIB 9082, ATCC 11764). It may not be necessary to have a viable culture of strain CB51^T in order to determine which species should bear the genus name. It might be possible to obtain an rDNA sequence for the phylogenetic placement of *C. vibrioides* from non-viable lyophilized material, as has been done for *Pedomicrobium australicum* (Cox & Sly, 1997). Should any researchers have suitable material, they are encouraged to pursue this course of action or to send the material to this laboratory for study.

There is one further piece of information which may influence the designation of the type culture of *C. vibrioides*. In 1978, Moore and co-workers reported a study of the DNA similarity among the freshwater species of the genus *Caulobacter* (Moore *et al.*, 1978). This study revealed that there was a high degree of similarity among the strains of *C. vibrioides* and *C. crescentus*. The respective type strains, CB51^T and CB2^T, were more closely related to each other than to any other strains used in the study, sharing 97% DNA–DNA similarity. At this level of relatedness, it could be considered that the two species are subjective synonyms. In the case that the two species were united, *C. vibrioides* would have priority (Rule 24b), but it could be argued that the type strain CB2^T of *C. crescentus*, having 97% DNA–DNA similarity with CB51^T, could be designated as the neotype culture of *C. vibrioides*. Such a course of action needs to be considered thoroughly in light of the phenotypic characteristics that have been used to differentiate the two species in the past. *C. crescentus* has long been considered a well-defined, separate species and is differentiated from *C. vibrioides* on the bases of the absence of growth-factor requirements in *C. crescentus*, pigmentation in *C. vibrioides* and differences in carbon source utilization. Unifying the two species would therefore change the description of *C. vibrioides*. Strain CB-G on the other hand exhibited only 60% similarity with strain CB51^T and 58% with CB2^T, confirming that it is not a typical member of either species and should not be considered as a potential neotype culture.

The discovery that *C. leidyia* belongs to the alpha-4 subclass of the *Proteobacteria* was unexpected from previous publications, which indicated that the species of *Caulobacter* were phylogenetically and taxonomically coherent. The further discovery that *A. excentricus* and *A. biprosthecium* are the nearest phylogenetic relatives of *C. leidyia* added weight to the finding of a new grouping. The presence of a polar stalk in *C. leidyia* and a single prosthecium in *A.*

excentricus and *A. biprosthecium* were confirmed. The occurrence of two prosthecae in *A. biprosthecium* is apparently an inconsistent character (Poindexter, 1989). Although these three species have very high 16S rDNA sequence identities, in the range 99.7–99.8%, DNA–DNA-hybridization studies indicate that they are distinct species that exhibit no homology with each other or with any other species of *Caulobacter* (Moore *et al.*, 1978).

Given the likelihood that the name *Caulobacter* will be assigned to one of the major clusters of freshwater species, it will be necessary to transfer *C. leidyia* to the genus *Asticcacaulis*. Currently, *Caulobacter* and *Asticcacaulis* are distinguished on the basis of constrictive cell division in *Caulobacter* and septation in *Asticcacaulis* and the absence of a holdfast function for the prosthecae of *Asticcacaulis*. A close examination of these features in the three species is warranted in light of the phylogenetic relationship revealed. Known members of the alpha-4 subclass of the *Proteobacteria* are characterized by the presence of α -hydroxymyristic acid in their cell membranes, and this feature should also be examined before emendation of the description of the genus *Asticcacaulis*. Andreev *et al.* (1986) predicted that the unusual fatty acid 11-methyl-*cis*-octadec-11-enoic acid may be a chemotaxonomic marker for the genus *Caulobacter*. This compound makes up 10.7% of the cellular fatty acid in *C. leidyia*, so it is possible that its occurrence is not restricted to the true caulobacters.

It will be necessary to circumscribe a new genus to include the marine species *C. halobacteroides* and *C. maris* and a new species to include the strains MCS6 and MCS18. However, as Stahl *et al.* (1992) have already observed, it would be premature to do this before 16S rRNA sequences for all species of *Hyphomonas* are available for phylogenetic analysis. Given the evolutionary divergences revealed by the inclusion of additional species in the phylogenetic analyses, we concur with this view.

Finally, it is necessary to consider the taxonomic future of the genus *Brevundimonas*. The two species, *B. diminuta* and *B. vesicularis*, fall within a cluster of *Caulobacter* species. There is no evidence of prosthecate formation in either species of *Brevundimonas* (Segers *et al.*, 1994). However, given the range of cellular morphologies and the influence of nutritional conditions such as phosphate limitation on prosthecum formation in *Caulobacter* (Poindexter, 1989), it would be advisable to examine *Brevundimonas* under similar growth conditions to verify the absence of this important morphological feature. Any decision on the taxonomic future of *Brevundimonas* should await these further studies.

ACKNOWLEDGEMENTS

We thank Helen Hosmer and Rick Webb from the Department of Microbiology and the Centre for Microscopy and Microanalysis for assistance with electron microscopy.

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