

# Phylogenetic relationships of the genera *Stella*, *Labrys* and *Angulomicrobium* within the 'Alphaproteobacteria' and description of *Angulomicrobium amanitifforme* sp. nov.

Ingo Fritz,† Carsten Strömpl and Wolf-Rainer Abraham

Correspondence  
Wolf-Rainer Abraham  
wab@gbf.de

GBF – National Research Center for Biotechnology, Department of Environmental Microbiology, Mascheroder Weg 1, 38124 Braunschweig, Germany

The unusually shaped bacteria of the genera *Stella*, *Labrys* and *Angulomicrobium* have been described based on their cell morphology and biochemistry. However, their phylogenetic relationships remain unresolved. An earlier study that was based on 5S rRNA gene sequences placed the genus *Stella* within the 'Alphaproteobacteria'. In the present report, polar lipids and 16S rRNA genes of the type strains of the two species in the genus *Stella*, *Stella humosa* DSM 5900<sup>T</sup> and *Stella vacuolata* DSM 5901<sup>T</sup>, are studied, as well as the type strains of the monospecific genera *Labrys* (*Labrys monachus* VKM B-1479<sup>T</sup>) and *Angulomicrobium* (*Angulomicrobium tetraedrale* DSM 5895<sup>T</sup>). It was found that the genus *Stella* belongs to the order *Rhodospirillales* in the family *Rhodospirillaceae*, and not to the *Acetobacteraceae*. Whilst the position of the genus *Angulomicrobium* in the family *Hyphomicrobiaceae* was confirmed, the genus *Labrys* could not be placed into any known family, but was adjacent to the family 'Beijerinckiaceae'. In addition, data were obtained for strain VKM B-1336, which was shown not to belong to the genus *Angulomicrobium*, and strain NCIMB 1785<sup>T</sup> (= DSM 15561<sup>T</sup>), for which the name *Angulomicrobium amanitifforme* sp. nov. is proposed.

## INTRODUCTION

Star-shaped cells have been observed in various aquatic environments and soil samples since 1966 (Hirsch & Schlesner, 1981). The first pure culture of star-shaped bacteria was obtained by Vasilyeva (1970) and was designated strain AUCM B-1137<sup>T</sup>. Vasilyeva (1985) reported ten additional strains of star-shaped bacteria that had been isolated from soil, compost, horse manure, sewage sludge and marine sediment and described two of these strains as monotypic species, *Stella humosa* AUCM B-1137<sup>T</sup> (= DSM 5900<sup>T</sup>) and *Stella vacuolata* INMI 229<sup>T</sup> (= DSM 5901<sup>T</sup>). DNA–DNA reassociation experiments showed low

DNA–DNA similarity between *S. humosa* DSM 5900<sup>T</sup> and *S. vacuolata* DSM 5901<sup>T</sup> (Reimer & Schlesner, 1989). These authors described the isolation of 11 additional strains of star-shaped bacteria from a variety of aquatic habitats and showed, by DNA–DNA hybridization experiments, that the two *Stella* species and the 11 isolates could be divided into at least five distinct groups. Their data suggested that, among the strains investigated, *S. humosa* DSM 5900<sup>T</sup> and *S. vacuolata* DSM 5901<sup>T</sup> were genetically the most remotely related, with the 11 additional strains being more or less closely related to one of the two type strains. In spite of *S. humosa* DSM 5900<sup>T</sup> being one of the first strains for which the 16S rRNA gene was sequenced (Schlesner *et al.*, 1990), to our knowledge, no 16S rRNA gene sequence data have been published so far and the affiliation of the genus *Stella* within the 'Alphaproteobacteria' has only been deduced from 5S rRNA gene sequence comparisons (Bomar & Stackebrandt, 1987) and 16S rRNA cataloguing (Fischer *et al.*, 1985). The pioneering 5S rRNA study of Bomar & Stackebrandt (1985) placed *S. humosa* within the 'Alphaproteobacteria', but the limited dataset available at that time only allowed comparison with very few other genera.

In 1984, Vasil'eva and Semenov described a strain of budding prosthecate bacteria that was isolated from silt of Lake Mustijärv in the former Estonian SSR (Vasil'eva & Semenov,

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†Present address: MPI, Max Planck Institute for Molecular Genetics, Department of Vertebrate Genomics, Ihnestr. 73, 14195 Berlin, Germany.

Abbreviations: CID, collision-induced dissociation; GPA, glycerophosphoric acid; MS, mass spectrometry.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of *Labrys monachus* VKM B-1479<sup>T</sup>, *Angulomicrobium tetraedrale* VKM B-1335<sup>T</sup>, *Angulomicrobium amanitifforme* NCIMB 1785<sup>T</sup>, *Stella humosa* DSM 5900<sup>T</sup> and *Stella vacuolata* DSM 5901<sup>T</sup> are AJ535707–AJ535711, respectively, and that for [*Angulomicrobium*] sp. VKM B-1336 is AJ542534.

1984). Based on radial cell symmetry, multiplication by budding and the presence of prosthecae, the authors placed this strain, VKM B-1479<sup>T</sup>, in a novel genus with the orthographically incorrect name '*Labrys monahos*' (Vasil'eva & Semenov, 1984). The name of this strain has been validly published as *Labrys monachus* (Vasil'eva & Semenov, 1985). Cells of this strain consisted of flat, triangular cells with prosthecae in two of the three corners, which allows clear morphological distinction from other genera of budding bacteria. So far, the phylogenetic position of *L. monachus* VKM B-1479<sup>T</sup> has not been determined.

The first 'mushroom-shaped', budding bacterium was described by Whittenbury & Nicoll (1971). The authors isolated strain NCIMB 1785<sup>T</sup> from fresh pond water; this strain differed from previously described budding bacteria in a number of properties, including dividing mode, cell morphology and fine structure (Whittenbury & Nicoll, 1971). This strain has not yet been assigned to a genus or species. A morphologically similar, non-motile, Gram-negative strain (Z-2821<sup>T</sup> = VKM B-1335<sup>T</sup> = DSM 5895<sup>T</sup>) was isolated in 1972 from a cumulative culture of methane-oxidizing bacteria that were sampled from a lowland marsh in Abramtsevo, near Moscow (Namsaraev & Zavarzin, 1973, 1974). Vasil'eva and co-workers (Lafitskaya & Vasil'eva, 1976; Vasil'eva *et al.*, 1980) reported an additional mushroom-shaped bacterial strain, Z-1109 (=VKM B-1336), and proposed a novel genus, *Angulomicrobium*, with the type species *Angulomicrobium tetraedrale* VKM B-1335<sup>T</sup> (Vasil'eva *et al.*, 1986). Despite certain morphological and physiological dissimilarities to strain VKM B-1335<sup>T</sup>, these authors included strain VKM B-1336 in the genus *Angulomicrobium* without attributing it to a particular species (Vasil'eva *et al.*, 1980). Two additional strains that resembled 'mushroom-shaped bacteria' were isolated by Stanley *et al.* (1976). So far, no data on the taxonomic affiliation of 'mushroom-shaped bacteria' have been published.

The aim of the present work was to determine the taxonomy of the type strains of the genera *Labrys*, *Stella* and *Angulomicrobium* by lipid analysis and comparison of 16S rRNA

gene sequences. Furthermore, the phylogenetic positions of strains NCIMB 1785<sup>T</sup> and VKM B-1336 have been determined, resulting in the proposal of *Angulomicrobium amantiforme* sp. nov., with the type strain NCIMB 1785<sup>T</sup>.

## METHODS

**Strains.** The strains used in this study and their origins are listed in Table 1. All strains were grown in freshwater *Caulobacter* medium PYEM [2 g peptone, 2 g yeast extract, 0.5 g NH<sub>4</sub>Cl, 1 l MilliQ water (Millipore)]. After autoclaving and cooling, 5 ml sterile-filtered riboflavin (0.2 mg ml<sup>-1</sup>), 2 ml 50% sterile glucose, 1 ml 20% sterile MgSO<sub>4</sub> and 1 ml 10% sterile CaCl<sub>2</sub> were added. For lipid analysis, strains were grown in 2 l flasks at 30 °C and 100 r.p.m. and biomass was harvested in the late-exponential phase after 72 h. Due to their very slow growth, *Stella* strains were harvested after 2 weeks.

**Spectroscopic DNA-DNA hybridization.** DNA was isolated by chromatography on hydroxyapatite, according to the procedure of Cashion *et al.* (1977). DNA-DNA hybridization in 2 × SSC + 10% (v/v) DMSO at 69 °C was carried out as described by De Ley (1967) with the modification described by Huss *et al.* (1983) and Escara & Hutton (1980), using a model 2600 spectrophotometer equipped with a model 2527-R thermoprogrammer and plotter (Gilford Instrument Laboratories). Renaturation rates were computed with the program TRANSFER.BAS (Jahnke, 1992).

**16S rDNA sequencing.** Almost-complete 16S rRNA genes were amplified by PCR from the strains listed in Table 1 and were sequenced as described previously (Abraham *et al.*, 1999). Resulting sequences were aligned with reference 16S rRNA gene sequences (Stoesser *et al.*, 2002; Cole *et al.*, 2003) by the ARB program package (Ludwig *et al.*, 2003), using the evolutionarily conserved primary sequence and secondary structure as references (Gutell *et al.*, 1985). Evolutionary distances (Jukes & Cantor, 1969) were calculated from pairwise similarities of complete sequences by using only homologous, unambiguously determined nucleotide positions. A phylogenetic tree was constructed by using the DNADIST and FITCH programs of the PHYLIP package (Felsenstein, 1989).

### Lipid analysis

**Polar lipid fatty acid analysis.** Lipids were extracted by using a modified Bligh-Dyer procedure (Bligh & Dyer, 1959) and fatty acid methyl esters were generated and analysed by GC, as described previously (Vancanneyt *et al.*, 1996).

**Table 1.** Strains used in this study and their origin

Strain	Origin
<i>Angulomicrobium</i> :	
<i>Angulomicrobium tetraedrale</i> DSM 5895 <sup>T</sup>	Lowland bog near Moscow, Russia
<i>Angulomicrobium tetraedrale</i> VKM B-1335 <sup>T</sup>	Lowland bog near Moscow, Russia
[ <i>Angulomicrobium</i> ] VKM B-1336	Peat bog soil near Moscow, Russia
Mushroom-like bacteria NCIMB 1785 <sup>T</sup>	Fresh pond water
<i>Labrys monachus</i> VKM B-1479 <sup>T</sup>	Lake Mustijärv, Estonia
<i>Stella</i> :	
<i>Stella humosa</i> DSM 5900 <sup>T</sup>	Virgin chernozem, A <sub>1</sub> layer, Krasnodar region, Russia
<i>Stella vacuolata</i> DSM 5901 <sup>T</sup>	Sewage sludge from pig farm, St Petersburg region, Russia

**Tandem mass spectrometry (MS).** Fast atom bombardment-MS in the negative mode was performed on the first of two mass spectrometers of a tandem, high-resolution instrument (JMS-HX/HX110A; JEOL) as described previously (Abraham *et al.*, 1997). A mixture of triethanolamine and tetramethylurea was used as the matrix. Negative daughter ion spectra were recorded by using all four sectors of the tandem mass spectrometer. Helium served as the collision gas for generating collision-induced dissociation (CID) for identification of prominent molecular ions.

## RESULTS AND DISCUSSION

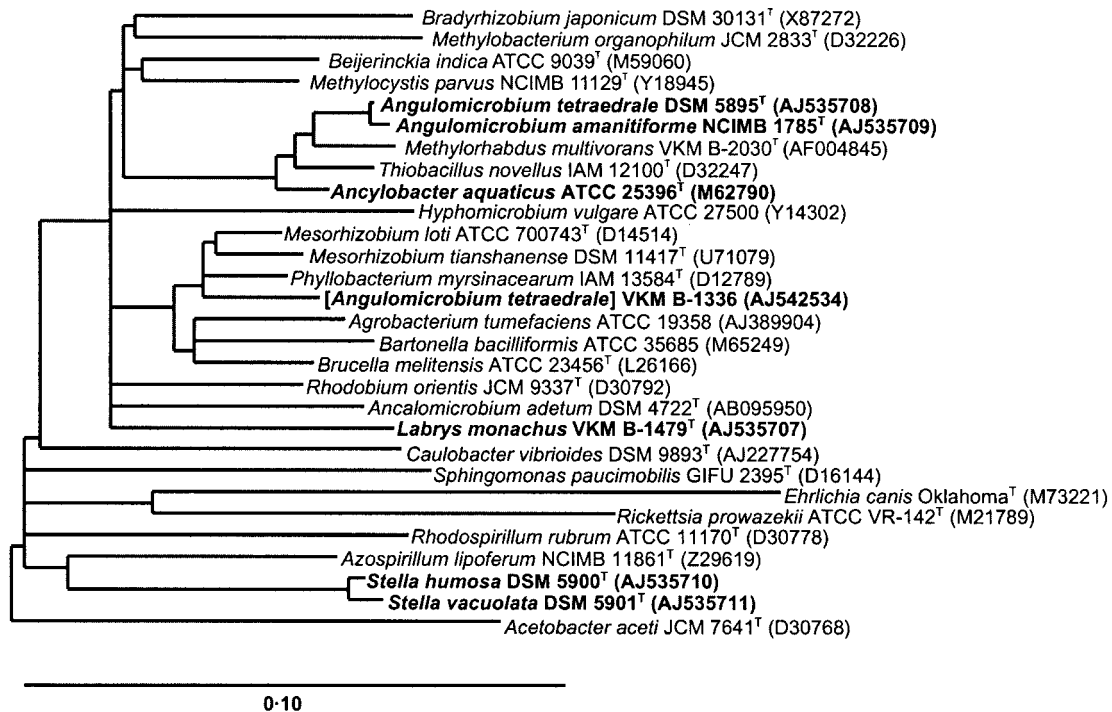
### 16S rRNA gene sequence analysis

As revealed by 16S rRNA gene sequence comparison, the strains of *Angulomicrobium*, *Labrys* and *Stella* that were studied were found to cluster with the 'Alphaproteobacteria' (Fig. 1). Within this subclass, the strains formed distinct branches that appeared to be well-separated from other bacterial genera. Despite their common radial symmetry and, with the exception of *Angulomicrobium* strains, the presence of prosthecae, these genera displayed no obvious closer relationships. This corroborates with the finding of

the polyphyletic origin of stalks in caulobacteria (Stahl *et al.*, 1992; Abraham *et al.*, 1999).

### Genus *Stella*

Both type strains of *Stella* species, DSM 5900<sup>T</sup> and DSM 5901<sup>T</sup>, were found to form a well-separated monophyletic group (Fig. 1) that showed only distant relationships to published 16S rDNA sequences of type strains of other bacterial genera. The 16S rRNA gene sequences from genera with validly published names that were most similar to those of the *Stella* cluster were from strains that belonged to the genera *Azospirillum*, *Magnetospirillum* and *Aquaspirillum*, with sequence similarities that ranged between 87 and 90%. This placed the genus *Stella* in the order *Rhodospirillales* in the family *Rhodospirillaceae* and not in the *Acetobacteraceae*, as has been assumed previously (Garrity *et al.*, 2001). Comparison of the 16S rRNA gene sequences revealed 99.0% similarity between *S. humosa* DSM 5900<sup>T</sup> and *S. vacuolata* DSM 5901<sup>T</sup>. This close 16S rRNA gene sequence similarity is not reflected by data from DNA–DNA hybridization experiments; Reimer & Schlesner (1989) determined a DNA–DNA similarity as low as 15% between the type



**Fig. 1.** Consensus dendrogram based on comparison of 16S rRNA gene sequences. The calculation was based on an initial dataset that consisted of representatives of all orders within the class 'Alphaproteobacteria', all families within the 'Rhizobiales' and *Rhodospirillales*, as listed by Garrity *et al.* (2001), and the closest relatives in the genera *Angulomicrobium*, *Labrys* and *Stella*. *Escherichia coli* was used as an outgroup. Trees were calculated with distance matrix (neighbour-joining and Fitch), maximum-parsimony and maximum-likelihood methods, as implemented in the ARB program package (Ludwig *et al.*, 2003). After a comparison of tree topologies, a consensus tree was constructed. Where it was not possible to resolve the branching order unambiguously [i.e. low bootstrap values (<50%) and/or inconsistent branching orders in dendrograms calculated with different treeing methods], ambiguous branch-points were displayed as multifurcations in the dendrogram.

strains of *S. humosa* and *S. vacuolata*, thus pointing to substantial differences in the genetic structure of these strains.

Contrary to DNA–DNA reassociation data, the genus *Stella* appears to be quite consistent in terms of morphology, physiology, nutrient requirements and DNA G + C content (approx. 67–73 mol%) (Hirsch & Schlesner, 1981; Vasilyeva, 1985). Major distinguishing traits are the occurrence of gas vacuoles in some strains and low DNA–DNA hybridization values that are reported between a number of strains.

Mass spectra of the phospholipid fractions from *Stella* spp. showed mainly molecular ions with even mass numbers, pointing to the presence of mainly nitrogen-bearing phospholipids. Although CID-MS for identification of compounds could not be run, due to scarcity of material, the recorded masses, together with the fatty acids found in the polar lipid fraction, strongly favour the presence of phosphatidylethylamine and phosphatidylcholine. Additionally, two unidentified lipids with molecular ions at  $m/z$  893 and 1042 were observed. Sittig & Schlesner (1993) reported the presence of phosphatidyl *N*-methylethylamine, phosphatidylcholine and cardiolipin for *Stella* strains; however, the latter could not be detected in the mass spectra. Furthermore, they found relatively high amounts of long-chain hydroxy fatty acids.

### Genus *Labrys*

*L. monachus* VKM V-1479<sup>T</sup> exhibited 16S rRNA gene sequence similarities of 91–92 % to the most closely related genera, namely species of *Mesorhizobium*, *Rhodopseudomonas* and *Azorhizobium* (Fig. 1). This confirmed the separation of *L. monachus* VKM B-1479<sup>T</sup> at the genus level, as has been suggested previously on the basis of morphological and physiological data (Vasil'eva & Semenov, 1984). 16S rRNA gene sequence data did not allow us to affiliate VKM B-1479<sup>T</sup> with any of the most closely related families that were suggested by Garrity *et al.* (2001), namely *Rhizobiaceae*, *Brucellaceae*, 'Phyllobacteriaceae', 'Methylocystaceae', 'Beijerinckiacaceae' and 'Bradyrhizobiaceae'. Additionally, *L. monachus* VKM B-1479<sup>T</sup> is not affiliated to the family *Hyphomicrobiaceae*, as has been suggested by Garrity *et al.* (2001). VKM B-1479<sup>T</sup> may be assigned to a separate family, 'Labryaceae', in the future. However, due to the availability of only one strain for characterization, it is too early to resolve the affiliation of *L. monachus* VKM B-1479<sup>T</sup> at family level.

Four different types of polar lipid could be detected in this strain: phosphatidylglycerol, phosphatidyl *N,N*-dimethylethylamine, phosphatidylcholine and a lipid with a mass of 827 Da that belonged to an unknown type of phospholipid. With the aid of CID-MS, most compounds were elucidated. CID of the (M-H)<sup>-</sup> ion yielded abundant carboxylate anions from both the *sn*-1 and the *sn*-2 position, thus allowing identification of the fatty acids attached to the different lipids. In addition, there were neutral losses of the *sn*-2 and *sn*-1 substituent as free carboxylic acid, as well as loss of each fatty acyl group as a substituted ketene. Furthermore, the

positions of the fatty acids at the glycerol backbone could be determined. For the fatty acid positioned at *sn*-2, neutral loss as free fatty acid, as well as substituted ketene, is more frequent than for that at *sn*-1 (Murphy & Harrison, 1994). By this method, the structure of the phospholipids was identified (Abraham *et al.*, 1997); Table 2 summarizes the results. Several differences in the structure of the phospholipids can be found between *L. monachus* VKM B-1479<sup>T</sup> and *A. tetradrale* DSM 5895<sup>T</sup> (Table 2), which can be used to differentiate between these two genera. Such differentiation was not possible on the basis of phospholipid types alone (Sittig & Schlesner, 1993).

Fatty acids found in the polar lipid fractions of *L. monachus* VKM B-1479<sup>T</sup>, *A. tetradrale* DSM 5895<sup>T</sup> and strain NCIMB 1785<sup>T</sup> are listed in Table 3, together with the fatty acids of the whole-cell hydrolysate of strain NCIMB 1785<sup>T</sup>. The fatty acids of *L. monachus* VKM B-1479<sup>T</sup> were similar to those of *Angulomicrobium* strains, but the amount of C<sub>18:1</sub>ω7 was rather low, whilst that of C<sub>19:0</sub>Δ8,9 was the highest of all strains in this study, confirming an earlier report by Sittig & Schlesner (1993).

### Genus *Angulomicrobium*

The 16S rRNA gene sequences of *A. tetradrale* VKM B-1335<sup>T</sup> and DSM 5895<sup>T</sup> were identical. 16S rRNA gene analysis of *A. tetradrale* VKM B-1335<sup>T</sup> (= DSM 5895<sup>T</sup>) confirmed the status of this taxon as a distinct genus and species and the placement of this genus in the family *Hyphomicrobiaceae*. The closest cultivated relatives of *A. tetradrale* as deduced by 16S rRNA gene sequencing were *Methylorhabdus multivorans*, *Starkeya novella* and *Ancylobacter aquaticus*, with sequence similarities that ranged between 96 and 97 %. Additionally, high sequence similarities (>96 %) to molecular clones of thiosulfate-oxidizers from rice fields (Stubner *et al.*, 1998) and to *Xanthobacter* spp. were detected. However, several traits permit distinction between members of the genus *Angulomicrobium* and their close relatives. *Angulomicrobium* differs from *Starkeya* in terms of cell morphology and replication, lack of a chemolithotrophic nutrition mode and utilization of the Entner–Doudoroff pathway for breakdown of sugars by the former (Vasil'eva *et al.*, 1980; Kelly *et al.*, 2000). Furthermore, *Angulomicrobium* can be distinguished from *Methylorhabdus* by its budding mode of replication, presence of oxidase activity, inability to reduce nitrate and by lower amounts of C<sub>16:0</sub>, but higher amounts of C<sub>14:0</sub> and C<sub>18:1</sub> in its cellular fatty acids (Vasil'eva *et al.*, 1980; Doronina *et al.*, 1995).

Strain VKM B-1336, which was originally proposed to be a member of the genus *Angulomicrobium*, was included in the genus description (Vasil'eva *et al.*, 1980) despite observed differences in morphology, substrate utilization, ultrastructure and mode of division. We determined overall 16S rRNA gene sequence similarity between strains VKM B-1336 and VKM B-1335<sup>T</sup> (= DSM 5895<sup>T</sup>) to be as low as 91 %. This points to a remote relationship above genus level between

**Table 2.** Polar lipids identified in cell extracts of *Angulomicrobium tetraedrale* DSM 5895<sup>T</sup>, strain NCIMB 1785<sup>T</sup> and *Labrys monachus* VKM B-1479<sup>T</sup>

Abbreviations: DME, phosphatidyl *N,N*-dimethylethylamine; PA, phosphatidyl acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; unknown, unknown phosphatidyl lipid.

Mass (Da)	Type	R <sub>1</sub> -COOH ( <i>sn</i> -1)	R <sub>2</sub> -COOH ( <i>sn</i> -2)	Strain
688	PA	C <sub>19:0</sub> Δ8,9	C <sub>16:0</sub>	VKM B-1479 <sup>T</sup>
714	PA	C <sub>19:0</sub> Δ8,9	C <sub>18:1</sub>	DSM 5895 <sup>T</sup> = NCIMB 1785 <sup>T</sup>
728	PA	C <sub>19:0</sub> Δ8,9	C <sub>19:0</sub> Δ8,9	VKM B-1479 <sup>T</sup>
740	Unknown	C <sub>19:0</sub> Δ8,9	C <sub>18:1</sub>	DSM 5895 <sup>T</sup>
785	DME	C <sub>18:1</sub>	C <sub>19:0</sub> Δ8,9	DSM 5895 <sup>T</sup> = NCIMB 1785 <sup>T</sup> = VKM B-1479 <sup>T</sup>
788	PG	C <sub>19:0</sub> Δ8,9	C <sub>18:1</sub>	DSM 5895 <sup>T</sup>
799	DME	C <sub>19:0</sub> Δ8,9	C <sub>19:0</sub> Δ8,9	DSM 5895 <sup>T</sup> = NCIMB 1785 <sup>T</sup> = VKM B-1479 <sup>T</sup>
799	DME	C <sub>18:1</sub>	C <sub>20:1</sub>	DSM 5895 <sup>T</sup>
802	PG	C <sub>19:0</sub> Δ8,9	C <sub>19:0</sub> Δ8,9	VKM B-1479 <sup>T</sup>
813	PC	C <sub>19:0</sub> Δ8,9	C <sub>19:0</sub> Δ8,9	DSM 5895 <sup>T</sup> = NCIMB 1785 <sup>T</sup>
815	DME	C <sub>19:0</sub> Δ8,9	C <sub>20:0</sub>	VKM B-1479 <sup>T</sup>
827	Unknown	C <sub>19:0</sub> Δ8,9	C <sub>19:0</sub> Δ8,9	VKM B-1479 <sup>T</sup>
932	Unknown	C <sub>18:1</sub>	C <sub>19:0</sub> Δ8,9	DSM 5895 <sup>T</sup> = NCIMB 1785 <sup>T</sup>
932	Unknown	C <sub>20:1</sub>	C <sub>17:1</sub>	DSM 5895 <sup>T</sup>
946	Unknown	C <sub>19:0</sub> Δ8,9	C <sub>19:0</sub>	NCIMB 1785 <sup>T</sup>

these strains. 16S rDNA sequence data suggest that strain VKM B-1336 is affiliated to the genus *Mesohizobium* (Fig. 1).

Strain NCIMB 1785<sup>T</sup> shared 99.4% 16S rDNA sequence similarity with strain VKM B-1335<sup>T</sup> (= DSM 5895<sup>T</sup>). This confirmed the affiliation of strain NCIMB 1785<sup>T</sup> with the genus *Angulomicrobium*, which was suggested by earlier morphological and physiological data (Whittenbury & Nicoll, 1971; Stanley *et al.*, 1976; Vasil'eva *et al.*, 1980). DNA–DNA hybridization between *A. tetraedrale* DSM 5895<sup>T</sup> and strain NCIMB 1785<sup>T</sup> gave 60.6% DNA–DNA

similarity. An accepted recommendation by Wayne *et al.* (1987) suggested that strains that share > 70% DNA–DNA similarity should be included in the same species. Therefore, we propose that strain NCIMB 1785<sup>T</sup> should be placed in a novel species, *Angulomicrobium amanitifforme* sp. nov.

Four different types of polar lipids were detected in strains DSM 5895<sup>T</sup> and NCIMB 1785<sup>T</sup>: phosphatidylglycerol, phosphatidyl *N,N*-dimethylethylamine, phosphatidylcholine and lipids that belonged to an unknown type of phospholipid (Table 2). Phosphatidylglycerol was only found in *A. tetraedrale* DSM 5895<sup>T</sup>; strain NCIMB 1785<sup>T</sup> lacked this class of phospholipids. From the structure of the different phospholipids, it is apparent that strain NCIMB 1785<sup>T</sup> prefers C<sub>19:0</sub>Δ8,9 cyclopropyl fatty acids more than strain DSM 5895<sup>T</sup>, and that all main lipids of strain NCIMB 1785<sup>T</sup> had this fatty acid. *Angulomicrobium* strains were the only strains in this study to possess unidentified lipids with masses of 932 and 946 Da. Their CID spectra all showed the formation of glycerophosphatic acid (GPA) ions, identifying this group of polar lipids as phospholipids. Analysis of GPAs revealed the fatty acids and their relative position at the glycerol backbone of these unknown phospholipids. Further studies are needed to identify these lipids, which may be glycerophospholipids.

In this study, it was demonstrated for the two type strains of *Stella* species that high 16S rDNA sequence similarity (≥ 99%) does not necessarily correspond to high DNA similarity [DNA–DNA hybridization value < 15%; data from Reimer & Schlesner (1989)]. This well-known phenomenon (Regenhardt *et al.*, 2002; Stackebrandt *et al.*, 2002 and references therein) also applies to the genus *Angulomicrobium*. Although strains NCIMB 1785<sup>T</sup> and VKM B-1335<sup>T</sup> share 99.4% 16S rRNA gene sequence similarity, they

**Table 3.** Fatty acid content (mean percentage of total) of whole-cell hydrolysate of strain NCIMB 1785<sup>T</sup> and fatty acids of phospholipids of *Angulomicrobium tetraedrale* DSM 5895<sup>T</sup>, strain NCIMB 1785<sup>T</sup> and *Labrys monachus* VKM B-1479<sup>T</sup>

Strains: 1, DSM 5895<sup>T</sup> (phospholipids); 2, VKM B-1479<sup>T</sup> (phospholipids); 3, NCIMB 1785<sup>T</sup> (phospholipids); 4, NCIMB 1785<sup>T</sup> (whole-cell hydrolysate). Fatty acids for which the amount for all taxa was < 1% are not given. TR, Trace amount (< 1%); –, not detected.

Fatty acid	1	2	3	4
C <sub>14:0</sub>	–	TR	–	11.2
C <sub>16:0</sub>	5.0	19.7	5.0	4.5
C <sub>18:1</sub> ω7	35.8	2.9	32.9	62.0
C <sub>18:0</sub>	10.5	13.0	8.9	2.0
ECL 18:063	4.1	4.2	4.0	–
ECL 18:586	2.8	2.9	2.7	–
ECL 18:793	1.5	1.4	1.4	–
C <sub>19:0</sub> Δ8,9	24.8	40.4	28.9	20.2
C <sub>20:1</sub> ω9	11.7	11.9	11.4	–

are not related at species level, as shown by DNA–DNA hybridization. Aside from this, strain NCIMB 1785<sup>T</sup> differs from *A. tetradrale* VKM B-1335<sup>T</sup> by the use of citrate, D-(–)-ribose and L-serine as substrates and the inability to utilize D-(+)-malate, D-(+)-mannose, D-(+)-melibiose, methylamine hydrochloride and L-(+) and D-(–)-tartrate as substrates.

### Description of *Angulomicrobium amanitifforme* sp. nov.

*Angulomicrobium amanitifforme* (a.ma.ni'ti.for.me. N.L. n. *Amanita* name of fungal genus; L. adj. suffix *-formis -is -e* -like, of the shape of; N.L. neut. adj. *amanitifforme* formed like a toadstool).

The description of the species is as that for the genus, with the following additions. Cells are non-motile, non-spore-forming, capsulated and irregularly shaped. They are 1.0 × 1.5 µm in size with radial symmetry and show tetrahedral cell morphology during the early stages of cell replication. A complex membranous system is not developed, but in addition to the cytoplasmic membrane, some peripherally aligned membranes and a membranous cell body at the site of cell division are present. The budding process is initiated by doubling the tube part of the cell, followed by enlargement of the tube part end-section. Finally, symmetric fission of the tube forms two mushroom-shaped cells. Colonies are white, round and mucous with a pearly shine. However, there is a second colony morphology with smaller, non-slimy colonies. Nutritional type is chemoorganotrophic. CO<sub>2</sub> in the presence of H<sub>2</sub> is not utilized as a sole carbon and energy source. Organic growth factors are not required, but do stimulate growth. Glucose, arabinose, galactose, fructose, mannitol, glycerol, formate, acetate, propionate, lactate, fumarate, succinate, citrate and glutarate are used as sole carbon and energy sources, whereas mannose, lactose, maltose, sucrose, dulcitol, butyrate, tartrate, urea, glycine and methanol are not. Contrary to VKM B-1335<sup>T</sup>, strain NCIMB 1785<sup>T</sup> utilizes citrate, D-(–)-ribose and L-serine and does not utilize D-(+)-malate, D-(+)-mannose, D-(+)-melibiose, methylamine hydrochloride or (L+)- or (D–)-tartrate. Metabolism is strictly aerobic. Catalase and oxidase activities are present. Nitrate, carbonate and sulfate are not utilized as electron acceptors. Cytochromes *a*, *b* and *c* are present. Acids are produced from sugar and sugar alcohols. Growth temperature ranges from 15 to 40 °C, with an optimum growth temperature between 28 and 30 °C. At 30 °C, growth occurs at pH values between 5.2 and 8.0, with an optimum at pH 6.8–7.0. Main phospholipids are phosphatidyl *N,N*-dimethylethylamine and phosphatidylcholine, containing at least one C<sub>19:0</sub>Δ<sup>8,9</sup> cyclopropyl fatty acid. DNA G+C content is 67.7 mol% for the type strain. 16S rRNA gene sequencing places this species in the '*Alpha-proteobacteria*'. DNA–DNA hybridization between the type strain and *A. tetradrale* DSM 5895<sup>T</sup> was 60.6 %.

The type strain of the species is NCIMB 1785<sup>T</sup> (=DSM 15561<sup>T</sup>).

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