Reclassification of *Sphingobacterium antarcticum* Shivaji et al. 1992 as *Pedobacter antarcticum* comb. nov. and *Pedobacter piscium* (Takeuchi and Yokota 1993) Steyn et al. 1998 as a later heterotypic synonym of *Pedobacter antarcticus*

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The taxonomic position of *Sphingobacterium antarcticum* has been revised by means of 16S rRNA gene sequences, DNA–DNA hybridization, and phenotypic and chemotaxonomic characteristics. All data previously reported, as well as the results of the present phylogenetic analysis, support that *Sphingobacterium antarcticum* is clearly a member of the genus *Pedobacter*, also affiliated with the family *Sphingobacteriaceae*. We propose that *Sphingobacterium antarcticum* (corrigh. Shivaji et al. 1992) should be reclassified as *Pedobacter antarcticus* comb. nov.

The family *Sphingobacteriaceae* (phylum *Bacteroidetes*) was initially described based on two genera, *Sphingobacterium* and *Pedobacter* (Steyn et al., 1998), and to date eight different genera (*Mucilaginibacter*, *Nubsella*, *Olivibacter*, *Parapedobacter*, *Pedobacter*, *Pseudosphingobacterium*, *Solitalea* and *Sphingobacterium*) have been described (Euzéby, 2013). The genus *Sphingobacterium* was established by Yabuuchi et al. (1983) to accommodate three species (*Sphingobacterium spiritivorum*, *Sphingobacterium multivorum* and *Sphingobacterium mizutaii*) and was distinguished from the genus *Flavobacterium* by the presence of high concentrations of sphingolipids (Yabuuchi et al., 1983). On the basis of this criterion, two species of *Flavobacterium* were reclassified as members of this new genus, *Sphingobacterium spiritivorum* (type species) and *Sphingobacterium multivorum*, and a novel species, *Sphingobacterium mizutaii*, was described (Yabuuchi et al., 1983), whose name was later corrected to *Sphingobacterium mizutaii* (Holmes et al., 1988; Choi & Lee, 2012). At the time of writing, the genus *Sphingobacterium* comprises 24 recognized species isolated from different origins, principally soil and compost (Euzéby, 2013). Phylogenetic knowledge of the genus *Sphingobacterium* has improved over the last two decades, with the application of molecular techniques that have facilitated the identification of novel species (18 in the last 7 years, 75%) and reclassification of others (*Pedobacter hepaurinus* and *Pedobacter piscium*) (Steyn et al., 1998; Euzéby, 2013). In a recent study describing a novel *Sphingobacterium* species, we obtained evidence for the misclassification of another species of this genus, *Sphingobacterium antarcticum* (Marqués et al., 2012). In the present report, the reclassification of *Sphingobacterium antarcticum* in its taxonomic position within the genus *Pedobacter*, also affiliated with the family *Sphingobacteriaceae*, is proposed.

*Sphingobacterium antarcticum* was first described from two pure cultures of bacteria (4BY and 6BY) isolated from soil samples collected in the Schirmacher Oasis of Antarctica (Shivaji et al., 1992). Both isolates were identified as belonging to the genus *Sphingobacterium* based on phenotypic and physiological tests, fatty acid profiles, the presence of sphingolipids and a low DNA G+C content (39.3 and 40.3 mol%). The two isolates differed in colony size, colour and antibiotic sensitivity pattern, but DNA–DNA hybridization indicated they represented the same species, with 100% relatedness (Shivaji et al., 1992). Additionally, these two Antarctic strains differed from all species of this genus known at the time (*Sphingobacterium spiritivorum*, *Sphingobacterium multivorum* and *Sphingobacterium mizutaii*) in being psychrotrophic (the others were mesophilic) and in their DNA–DNA relatedness results. DNA–DNA hybridizations showed only 10% hybridization with *Sphingobacterium multivorum* and about 5% with *Sphingobacterium spiritivorum*. Therefore, the two strains were recognized as representing a novel species, *Sphingobacterium antarcticus*, and the type strain, 4BY¹ (=MTCC 675¹) (Shivaji et al., 1992) was deposited in the Microbial Type Culture Collection (MTCC, India) and GenBank. Steyn et al. (1998) later proposed that the
two *Sphingobacterium* species be reclassified within a new genus, *Pedobacter*, but did not include *Sphingobacterium antarcticus* in their study. These authors described that *Sphingobacterium antarcticus* was not included in this study because no subspecies were available from any culture collection. Since the species is psychrotrophic and differs in many physiological characteristics, it can be considered as not belonging to the newly proposed *Pedobacter* taxa and may be distantly related to other *Sphingobacterium* species’ (Steyn et al., 1998). Euzéby (1998) proposed changing the specific epithet to *Sphingobacterium antarcticum* to agree in gender with the generic name. At present there are several culture collections containing strains of *Sphingobacterium antarcticum*: 4BY ( = ATCC 51969 = MTCC 675), 6BY ( = ATCC 51970) and 6B1Y ( = DSM 15311 = CECT 8499).

In the last decade, 16S rRNA gene sequences of *Sphingobacterium* have been used in studies of bacterial diversity or descriptions of novel *Sphingobacterium* species (Shivaji et al., 2004; Xiang et al., 2005; Kim et al., 2006; Marque’s et al., 2012), but not to clarify its taxonomic position within the genus *Sphingobacterium*. Five 16S rRNA gene sequences of this taxon are currently available in public sequence databases. The oldest sequence (GenBank accession number AJ576248, strain 6B1Y) comes from a study of Shivaji et al. (2004) on bacterial diversity, and was obtained by cloning the total 16S rRNA gene from a soil sample of the same geographical origin where this taxon was originally isolated. It is not clear whether the isolate named as 6B1Y in this study is the same as the one reported in the original species description as 6BY (Shivaji et al., 1992). Strain 6B1Y shared a high level of 16S rRNA gene sequence similarity (100 %) with the type strain of *Sphingobacterium antarcticum* (Shivaji et al., 2004). Later, two short sequences (GenBank accession number AY526660, strain Muzt-E11, 641 bp; and GenBank accession number AY526676, strain Muzt-F93, 661 bp), obtained by Xiang et al. (2005) from isolates recovered from different depths of the Muztag Ata Mountain glacier on the Pamirs Plateau (China), were assigned to *Sphingobacterium antarcticum*. Both sequences were compared with those available in the GenBank database using a BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and found to share 99 % 16S rRNA gene sequence similarity with the type strain of *Acinetobacter lwoffii* (family *Monaxellaceae*, class *Gammnaprotobacteria*, phylum *Proteobacteria*), so we excluded them from the present phylogenetic analysis. More recently, in 2010, two nearly complete 16S rRNA gene sequences of *Sphingobacterium antarcticum* were determined by reference laboratories and deposited in the GenBank database (GenBank accession number HM448033, strain ATCC 51969, 1468 bp; and GenBank accession number FR733711, strain DSM 15311, 1509 bp). These sequences were included in the ‘All-Species Living Tree Project’, a 16S rRNA-based phylogenetic tree of all sequenced type strains (Yarza et al., 2008, 2013).

We compared the three representative 16S rRNA gene sequences of *Sphingobacterium antarcticum* (GenBank accession numbers AJ576248, HM448033 and FR733711) with other 16S rRNA gene sequences available from the GenBank database of all currently described species of the family *Sphingobacteriaceae*. The phylogenetic analysis confirmed that *Sphingobacterium antarcticum* is not affiliated with the genus *Sphingobacterium* and the closest related genus was found to be *Pedobacter*, with a strong bootstrap support (100 %) (Fig. 1). When analysed with respect to all recognized *Pedobacter* species, *Sphingobacterium antarcticum* was grouped in a well-supported clade (100 % bootstrap value) with *Pedobacter piscium* (Fig. 2), and showing high levels of 16S rRNA gene sequence similarity (98–99 %). The phylogenetic analysis clearly demonstrated that *Sphingobacterium antarcticum* is very closely related to *Pedobacter piscium*, but that 16S rRNA gene sequence analysis was not sufficiently discriminatory at the species level. Other *Pedobacter* species showed a high level of 16S rRNA gene sequence similarity above threshold values for species delineation (97 % similarity or below). For example, the type strains of *Pedobacter caeni* and *Pedobacter steynii*, despite being phenotypically distinct, share 99 % 16S rRNA gene sequence similarity (Fig. 2). Thus, the 16S rRNA gene has limited use as a phylogenetic marker for the delineation of *Pedobacter* species.

The genus *Pedobacter* described by Steyn et al. (1998) contained two former species of the genus *Sphingobacterium*, *Pedobacter heparinus* comb. nov. and *Pedobacter piscium* comb. nov., and two novel species, *Pedobacter africanus* and *Pedobacter saltans* (Steyn et al., 1998; Takeuchi & Yokota, 1992). At the time of writing, the genus *Pedobacter* consists of 40 recognized species, most of which have been described in the last 10 years (Euzéby, 2013). The genera *Sphingobacterium* and *Pedobacter* share many common phenotypic and chemotaxonomic features, but *Pedobacter* can be distinguished by an ability to produce heparinase (except *Pedobacter piscium*) and acetoin, absence of urease activity, and the inability of most strains to produce acid from melibiose and to assimilate melezitose.

The genus *Sphingobacterium* is characterized by the presence of ω-fucosidase activity (except *Sphingobacterium mizutaii*) and greater amounts of iso-C_{15:0} (2-OH) (Steyn et al., 1998). Only a few phenotypic and genotypic characteristics can be used to differentiate between *Sphingobacterium antarcticum* and *Pedobacter piscium*, including urease and gelatinase activities, acid production from several carbon sources and DNA G+C content (Shivaji et al., 1992; Steyn et al., 1998). Distinctive characteristics of the two species are shown in Table 1. The variable results obtained by these studies could be explained by the use of different analytical methods for characterization (i.e. traditional methods or the API system) or because the two taxa are different species. The phenotypic characterization of *Sphingobacterium antarcticum* 6BY (= DSM 15311) and *Pedobacter piscium* DSM 11725 was confirmed in our laboratory, simultaneously and applying the same methodology. The following analytical procedures were performed. Growth at different temperatures (4–37 °C) was determined using trypticase soy
agar (TSA; Pronadisa) as the basal medium. Heparinase activity was studied according to the method of Zimmermann et al. (1990). Gelatinase activity was studied according to the methods of Pochon & Tardieux (1962) and Barrow & Feltham (1993). Urease activity was determined on urea broth (Difco). Acid production was studied with O/F basal medium (Difco). Single carbon-source utilization was determined as described by Bowman et al. (1996). All experiments were conducted in triplicate with incubation at 15 °C for 14 days. In contrast to previously published data, the results obtained showed very few differences between the two taxa (Table 1). Only heparinase activity and succinate assimilation gave divergent results, both being negative for Sphingobacterium antarcticum. Based on these data, it was unclear if both strains belonged to the same species or to two closely related species, so for further clarification DNA–DNA hybridization and determination of the DNA G + C content were performed by the BCCM/LMG Identification Service (Ghent, Belgium). Genomic DNA was isolated following the procedure of Wilson (1987) with some modifications (Cleenwerck et al., 2002). DNA–DNA hybridizations were performed in the presence of 50 % formamide at 38 °C following the method described by Ezaki et al. (1989) with some modifications (Goris et al., 1998; Cleenwerck et al., 2002). The DNA base composition was determined by HPLC (Mesbah et al., 1989). The G + C content of the chromosomal DNA was determined as the mean of three independent analyses. DNA–DNA relatedness between Sphingobacterium antarcticum DSM 15311 and

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (Kimura's two-parameter model, MEGA5 software), showing the position of strains of Sphingobacterium antarcticum (including the type strain 4BYT) with the type strains of other Sphingobacterium and related genera. Flavobacterium aquatile DSM 1132T was used as the outgroup. GenBank sequence accession numbers are given in parentheses. Bootstrap values (≥ 50 %) based on 1000 resamplings are shown at the nodes. Bar, 0.02 substitutions per nucleotide position.
**Fig. 2.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (Kimura’s two-parameter model, MEGA5 software), showing the position of strains of *Sphingobacterium antarcticum* with the type strains of other *Pedobacter* species. *Flavobacterium aquatile* DSM 1132T was used as the outgroup. GenBank sequence accession numbers are given in parentheses. Bootstrap values (≥50 %) based on 1000 resamplings are shown at the nodes. Bar, 0.02 substitutions per nucleotide position.

*Pedobacter piscium* DSM 11725T was 88 %, clearly higher than the 70 % DNA–DNA relatedness generally accepted as the limit for species delineation, and the DNA G + C content was identical in both taxa (40.1 mol%). These results therefore confirmed that both strains belong to the same species.

More recently, Yarza et al. (2013) described an initiative coordinated by the Living Tree Project (LTP) to obtain high-quality 16S rRNA gene sequences of the type strains of all species with validly published names. This study showed that in some cases the taxonomic placement based


Reclassification of <i>Sphingobacterium antarcticum</i>

Table 1. Differential characteristics between <i>Sphingobacterium antarcticum</i> and <i>Pedobacter piscium</i>

Data in the left-hand column are from the present study. +, positive; −, negative; +b, weakly positive; v, variable.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>&lt;i&gt;Sphingobacterium antarcticum&lt;/i&gt; DSM 15311</th>
<th>&lt;i&gt;Pedobacter piscium&lt;/i&gt; DSM 11725T</th>
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<tbody>
<tr>
<td>Urease</td>
<td>−</td>
<td>(+)b</td>
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<tr>
<td>Gelatinase</td>
<td>−</td>
<td>(+)b</td>
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<tr>
<td>Acid production from:</td>
<td>+</td>
<td>(−)b</td>
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<tr>
<td>Sucrose</td>
<td>−</td>
<td>(−)b</td>
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<td>Assimilation of:</td>
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<tr>
<td>Glycerol</td>
<td>−</td>
<td>(+)a</td>
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<tr>
<td>Malate</td>
<td>+w</td>
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<td>Melibiose</td>
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<tr>
<td>Pyruvate</td>
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<tr>
<td>Sorbitol</td>
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<td>(+)a</td>
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<tr>
<td>Succinate</td>
<td>−</td>
<td>(+)a</td>
</tr>
<tr>
<td>Starch</td>
<td>+w</td>
<td>(−)b</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>40.1</td>
<td>(39.3–40.3)a</td>
</tr>
</tbody>
</table>

Data in parentheses were obtained from: a, Shivaji et al. (1992); b, Takeuchi and Yokota (1992); c, Gallego et al. (2006).

on current names was not consistent with placement based on the 16S rRNA gene sequence comparisons. One of the examples was <i>Sphingobacterium antarcticum</i> ATCC 51969T, which showed an unexpected affiliation with <i>Pedobacter</i> (99.9% similarity with the type strain of <i>Pedobacter piscium</i>), thus supporting the need for a revision of its current taxonomic status (Yarza et al., 2013). On the basis of the phylogenetic analysis and molecular and phenotypic data, we propose that <i>Sphingobacterium antarcticum</i> be transferred to the genus <i>Pedobacter</i> as <i>Pedobacter antarcticus</i> comb. nov.

Description of <i>Pedobacter antarcticus</i> comb. nov.

<i>Pedobacter antarcticus</i> comb. nov. (ant.arc’ti.cus. L. masc. adj. antarcticus pertaining to Antarctica).


The description is identical to that given for <i>Sphingobacterium antarcticum</i> (corrig. Shivaji et al. 1992). Strains may be positive or negative for urease and gelatinase activities, starch hydrolysis, acid production from sucrose, and assimilation of glycerol, melibiose, pyruvate and succinate. The type strain is 4BYT (=ATCC 51969T = MTCC 675T).

Emended description of the genus <i>Pedobacter</i> Steyn et al. 1998

The description is as given by Steyn et al. (1998), Vanparys et al. (2005), Gallego et al. (2006), Hwang et al. (2006) and Zhou et al. (2012) with the following additions. <i>Pedobacter antarcticus</i> strains may be positive or negative for heparinase activity. The type species is <i>Pedobacter heparinus</i>.

Emended description of <i>Pedobacter piscium</i> (Takeuchi and Yokota 1993) Steyn et al. 1998

The description is as given by Steyn et al. (1998), with the following additions. The type strain (DSM 11725T) is positive for heparinase activity, acid production from melibiose and assimilation of melezitose, malate, mannotol, sorbitol and succinate. <i>Pedobacter piscium</i> is a later heterotypic synonym of <i>Pedobacter antarcticus</i>.

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References


