Reclassification of *Ruminococcus obeum* as *Blautia obeum* comb. nov.

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During our previous studies we reclassified *Clostridium coccoides* and a number of misclassified ruminococci into a novel genus *Blautia* within the family *Lachnospiraceae*. However, the Rules of the Bacteriological Code currently require that the types of all species and subspecies with new names (including new combinations) be deposited in two different collections in two different countries. The type strain of *Ruminococcus obeum* was, at that period in time, only deposited in the American Type Culture Collection (ATCC) and a second independent deposit, as required by the Code, was not available. Consequently, the transfer of this species to the genus *Blautia* could not be made, because the resulting species name would not conform to the Rules governing the valid publication of species names and deposit of type material (Rules 27 and 30) and consequently would not be considered to be validly published. This resulted in a nomenclatural and taxonomic anomaly with *R. obeum* being phylogenetically placed among members of the genus *Blautia* with 16S rRNA gene sequence similarities of between 91.8 and 96.6 %. In order to rectify this unsatisfactory situation, through our discussions with the ATCC, the deposit of strain *R. obeum* ATCC 29174T to the DSMZ as strain number DSM 25238T was completed. Hence, the transfer of *R. obeum* to the genus *Blautia* as *Blautia obeum* comb. nov. is now proposed. The type strain is ATCC 29174T (=DSM 25238T=KCTC 15206T).

A number of studies have shown that the genus *Ruminococcus* is not monophyletic and is phylogenetically heterogeneous (Rainey & Janssen, 1995; Willems & Collins, 1995). The type species, *Ruminococcus flavefaciens* along with *Ruminococcus albus*, *Ruminococcus callidus* and *Ruminococcus bromii* belong to the family *Ruminococcaceae* (formally designated clostridial rRNA cluster IV; Collins et al., 1994). The remainder of the species are phylogenetically removed and belong to the family *Lachnospiraceae* (formally known as rRNA cluster XIVa; Collins et al., 1994).

In our previous studies, on the basis of a polyphasic taxonomic investigation, we proposed that *Clostridium coccoides*, *Ruminococcus hansenii*, *Ruminococcus hydrogenotrophicus*, *Ruminococcus luti*, *Ruminococcus productus* and *Ruminococcus shinkii* be transferred to a novel genus as *Blautia coccoides* comb. nov., *Blautia hansenii* comb. nov., *Blautia hydrogenotrophica* comb. nov., *Blautia luti* comb. nov., *Blautia producta* comb. nov. and *Blautia shinkii* comb. nov. (Liu et al., 2008). The Rules of the Bacteriological Code require that the types of all species and subspecies with new names (including new combinations) be deposited in two different collections in two different countries. However, the type strain of *Ruminococcus obeum* was, at that period in time, only deposited in the American Type Culture Collection as ATCC 29174T and a second independent deposit, as required by the Code, was unable to be arranged. Consequently, the transfer of this species to the genus *Blautia* could not be made, because the resulting species name would not conform to the Rules governing the valid publication of species names and deposit of type material (Rules 27 and 30) and consequently would not be considered to be validly published. This situation resulted in a nomenclatural anomaly with *R. obeum* placed among members of the genus *Blautia*. Recently, Park et al. (2012, 2013) described two additional species, *Blautia stercoris* and *Blautia faecis*, with the authors highlighting the aforementioned nomenclatural and taxonomic inconsistency. Through continued discussions with the American Type Culture Collection, the deposit of strain *R. obeum* ATCC 29174T to DSMZ as strain number DSM 25238T was finally completed. Therefore, the transfer of *R. obeum* to the genus *Blautia* as *Blautia obeum* comb. nov. is now proposed. The type strain is ATCC 29174T (=DSM 25238T).

The 16S rRNA gene sequence of *R. obeum* ATCC 29174T was used to search for its nearest neighbours using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim
et al., 2012). These sequences and those of other related strains were then aligned using the program CLUSTAL W (Thompson et al., 1994) via the MEGA 6 program (Tamura et al., 2013). Phylogenetic reconstructions were performed in MEGA 6 (Tamura et al., 2013) using the neighbour-joining method using evolutionary genetic distances that had been calculated by the Kimura two-parameter model (Kimura, 1980). The phylogenetic analysis confirmed the position of R. obeum within the genus Blautia (16S rRNA gene sequence similarity values of 91.8–96.6%). The closest relatives were B. faecis (96.5%), Blautia glucerasea (95.9%) and B. luti (96.6%) (Fig. 1). It is pertinent to note that the branching node for members of the genus as a whole was not supported by a significant bootstrap value; this may suggest that a restructuring of the genus may be required at some future date.

**Fig. 1.** Phylogenetic tree showing the phylogenetic inter-relationships of Blautia obeum comb. nov. with members of the genus Blautia and some close relatives within the family Lachnospiraceae. The tree was reconstructed using the neighbour-joining method based on the pairwise comparison of approximately 1340 nt. Clostridium butyricum was used as the outgroup. Bar, 1% sequence divergence. Major branching orders and bootstrap values (>85%) expressed as a percentage of 1000 replications, are given at branching points.
### Table 1. Biochemical characteristics of members of the genus *Blautia* and *R. obeum*


<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
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</thead>
<tbody>
<tr>
<td><strong>End products of metabolism</strong>&lt;sup&gt;†&lt;/sup&gt;</td>
<td>A</td>
<td>A</td>
<td>S</td>
<td>L</td>
<td>A</td>
<td>A</td>
<td>F</td>
<td>L</td>
<td>A</td>
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<td>Arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>d</td>
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<td>Lactose</td>
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<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>d</td>
</tr>
<tr>
<td>Mannose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>d</td>
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<td>Sucrose</td>
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<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>−</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>d</td>
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<tr>
<td>α-Arabinosidase</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
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<tr>
<td>α-Fucosidase</td>
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<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>α-Mannosidase</td>
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<td>−</td>
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<td>−</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Alkaline phosphatase</td>
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<td>−</td>
<td>W</td>
<td>−</td>
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<td>−</td>
<td>−</td>
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<tr>
<td>N-Acetyl-β-glucosaminidase</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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</tr>
<tr>
<td>Major fatty acids (&gt;10%)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>C&lt;sub&gt;16:0&lt;/sub&gt; DMA; C&lt;sub&gt;16:1 cis11&lt;/sub&gt; DMA</td>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;12:0&lt;/sub&gt; DMA; C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>ND</td>
<td>ND</td>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>40–41</td>
<td>44.3</td>
<td>41.6</td>
<td>40.7</td>
<td>44–45</td>
<td>45</td>
<td>43.3</td>
<td>44–45</td>
<td>46–47</td>
<td>35.6</td>
<td>ND</td>
</tr>
<tr>
<td>Source</td>
<td>Human faeces</td>
<td>Mouse faeces</td>
<td>Human faeces</td>
<td>Dog faeces</td>
<td>Human faeces</td>
<td>Human faeces</td>
<td>Human faeces</td>
<td>Human faeces</td>
<td>Human faeces</td>
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</tr>
</tbody>
</table>

<sup>†</sup>A, acetic acid; L, lactic acid; S, succinic acid; E, ethanol.
In addition to our own studies, Bae and colleagues have described two additional species of the genus *Blautia* (*B. faecis* and *B. stercoris*) and during the course of these investigations have performed additional biochemical and chemotaxonomic analyses on *R. obeum* (Park et al., 2012, 2013). For cellular fatty acid analysis, biomass was collected from cells grown for 3 days on peptone-yeast-extract glucose (PYG) (Anaerobe Systems, CA) and extracted and analysed using the Sherlock Microbial Identification System (MIDI). The fatty acid profile included C12:0 (0.6 %), C14:0 (8.3 %), C14:1 DMA (2.6 %), C14:0 DMA (8.3 %), C16:0 ALDE (3.8 %), C15:0 (0.3 %), C16:1 cis7 (0.1 %), C16:1 cis9 (1.9 %), C16:0 (9.2 %), C16:1 cis7 DMA (7.8 %), C16:0 DMA (10.7 %), C18:0 ALDE (1.4 %), C18:1 cis9 (6.5 %), C18:0 (1.5 %), C18:1 cis9 DMA (9.2 %), C18:1 cis11 DMA (13.2 %) and C18:0 DMA (2.5 %). In addition small amounts of fatty acids were found in summed feature 1 (C13:0 cis12 and/or C14:0 ALDE; 1.4 %), summed feature 4 (unknown 14.762 and/or C15:2 2.2 %), summed feature 6 (C15:0 anteiso 3-OH and/or C16:1 cis7 DMA; 0.6 %), summed feature 7 (C17:2 and/or C17:1 cis8; 2.4 %), summed feature 8 (C17:2 cis9 and/or C17:2; 1.2 %) and summed feature 10 (C18:1 cis11 trans9 and/or or unknown 17.834; 2.9 %). The results of the API (bioMérieux) Rapid ID 32A, ZYM and 50CH test systems are given in the species description (Park et al., 2013).

In an earlier study, uncultured bacteria represented by sequences derived from faecal 16S rRNA gene clone libraries were found to be closely related to *R. obeum* (Suau et al., 1999). Zoetendal and colleagues (2002) followed up on these studies using a combination of fluorescent *in situ* hybridization and flow cytometry; several probes, one of which was designated Urobe63, were specific for these *R. obeum*-like sequences. Their data demonstrated that approximately 16 % of the total community belonged to the cluster XIVa (*C. coccoides* group) and of this 2.5 % belonged specifically to the *R. obeum*-like organisms; although this value varied between 1 and 6 % between individuals it demonstrated that *R. obeum* comprises a significant fraction of the faecal community. In addition, a recent publication by the Gordon Laboratory has shown that in *Vibrio cholerae*-associated acute diarrhoea (cholera) *R. obeum* is amongst a number of taxa that are significantly increased during the recovery phase in Bangladeshi children (Hsiao et al., 2014). The investigation demonstrated that *R. obeum* restricts *V. cholerae* colonization and future studies using gnotobiotic mice and newly isolated *R. obeum* strains will be conducted to elucidate the precise mechanisms involved. Therefore, with the increasing number of reports pertaining to the gastrointestinal microbiome demonstrating the presence of *R. obeum*, it is important that the transfer of *R. obeum* to the genus *Blautia* is completed, so enabling the correct nomenclature to be used avoiding future confusion.

Now that the Rules of the Bacteriological Code that currently require that the types of all species and subspecies with new names (including new combinations) be deposited in two different collections in two different countries is fulfilled, we propose the transfer of *R. obeum* to the genus *Blautia as Blautia obeum* comb. nov. The type strain is ATCC 29174T (=DSM 25238T). The characteristics that distinguish *R. obeum* from other members of the genus *Blautia* are summarized in Table 1.

### Description of *Blautia obeum* comb. nov.

*Blautia obeum* (o’be.um. Gr. n. obeum egg, referring to the ovoid shape of the cells).


In addition to the description given by Moore *et al.* (1976), using the API Rapid ID 32A kit positive reactions are observed for α-galactosidase and β-galactosidase. A weakly positive reaction is given with α-arabinosidase. No activity is detected for N-acetyl-β-glucosaminidase, alkaline phosphatase, arginine dihydrolase, α-fucosidase, β-galactosidase 6-phosphate, glutamic acid decarboxylase, glycine arylamidase, leucine arylamidase, mannose arylamidase, raffinose arylamidase or serine arylamidase. Using the API ZYM kit, positive reactions are obtained with acid phosphatase, alkaline phosphatase, α-galactosidase, β-galactosidase, α-glucosidase and naphthol-AS-BI-phosphohydrolase. Negative reactions are observed for N-acetyl-β-glucosaminidase, β-glucosidase and α-fucosidase. With the CH50 fermentation kit, positive reactions are observed for D-arabinose, methyl β-D-xylolide, methyl β-D-glucoside, N-acetyl-β-glucosamine, arbutin, salicin, L-fucose and turanoside. Negative reactions are given with L-arabinose, glycerol, glycogen and erythritol. The predominant fatty acids are C16:0 DMA and C18:1 cis11 DMA but C14:0 C16:0 C16:1 cis9 DMA and C18:1 cis9 DMA are also found in significant amounts.

Isolated from human faeces. The type strain is ATCC 29174T (=DSM 25238T =KCTC 15206T).

### Acknowledgements

We wish to thank Dr Tim B. Lilburn of the American Type Culture Collection for the deposit of *R. obeum* ATCC 29174T to the DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany.

### References


