Revision of the genus *Massilia* La Scola et al. 2000, with an emended description of the genus *Naxibacter* as new combinations, and proposal of *Massilia consociata* sp. nov.

Peter Kämpfer,1 Nicole Lodders,1 Karin Martin2 and Enevold Falsen3

1Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany
2Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie e. V., Hans-Knöll-Institut, D-07745 Jena, Germany
3Culture Collection University Göteborg, Dept of Clinical Bacteriology, S-41346 Göteborg, Sweden

A Gram-stain-negative, rod-shaped, non-spore-forming bacterium originating from a human clinical specimen was studied for its taxonomic position. 16S rRNA gene sequence similarity studies clearly allocated this strain (CCUG 58010T) to the class *Betaproteobacteria*, closely related to members of the genera *Massilia* and *Naxibacter*. *Naxibacter varians* was shown to be the most closely related species on the basis of 16S rRNA gene sequence similarity (97.5 %), followed by *Massilia niastensis* (96.8 %) and *Massilia aerilata* (96.4 %). Similarities to all other species of the genera *Naxibacter* and *Massilia* were in the range 93.9–96.2 %. Chemotaxonomic data (major ubiquinone: Q-8; major polar lipids: phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol; and major fatty acids: summed feature 3 (C₁₆:₁ω₇c and/or iso-C₁₅:₀ 2-OH), C₁₆:₀, C₁₈:₁ω₇c and C₁₂:₀, with C₁₀:₀ 3-OH as hydroxylated fatty acid) supported the affiliation of the isolate to these genera, which share these chemotaxonomic traits. DNA–DNA hybridization of strain CCUG 58010T with the type strain of *N. varians* CCUG 35299T resulted in a relatedness value of 39.2 % (reciprocal, 50 %) and physiological and biochemical tests also allowed phenotypic differentiation of the isolate from the most closely related species. There is currently no justification for a division of the genera *Massilia* and *Naxibacter* and for this reason a proposal is made to transfer all species of the genus *Naxibacter* to the genus *Massilia*, as *Massilia alkalitolerans* comb. nov., *Massilia varians* comb. nov., *Massilia haematophila* comb. nov. and *Massilia suwonensis* comb. nov. Strain CCUG 58010T represents a novel species, for which the name *Massilia consociata* sp. nov. is proposed, with the type strain CCUG 58010T (≡CCM 7792T).

The genus *Massilia* was proposed by La Scola et al. (1998), who described this new genus on the basis of a fastidious, slowly growing bacterium isolated from a culture of blood from a 25-year-old man with common variable immuno-deficiency. The name was validly published in 2000 (La Scola et al., 2000). At the time of writing, a total of 11 species with validly published names have been described (La Scola et al., 1998; Gallego et al., 2006; Zhang et al., 2006; Zul et al., 2008; Weon et al., 2008, 2009, 2010), isolated from various sources. Members of the genus are characterized as aerobic, Gram-negative, motile, non-spore-forming rods, all containing the fatty acids iso-C₁₅:₀ 2-OH and/or C₁₆:₁ω₇c, C₁₈:₁ω₇c, C₁₆:₀ and C₁₀:₀ 3-OH as characteristic fatty acids. In 2005, the genus *Naxibacter* was described by Xu et al. (2005) with the type species *Naxibacter alkalitolerans*. Phylogenetically it was placed in the vicinity of the genera *Massilia*, *Telluria*, *Duganella* and *Janthinobacterium*. One of the most striking characteristics of *N. alkalitolerans* was the report by Xu et al. (2005) of phosphatidylinositol mannosides in its polar lipid profile, which is usually a characteristic of Gram-positive bacteria. However, this result could not be confirmed in the subsequent study by Kämpfer et al. (2008), who found very similar polar lipid profiles for *Naxibacter varians*, *Naxibacter*...
haematophilus and N. alkalitolerans consisting of the major compounds phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol and moderate amounts of unknown lipids and aminolipids, but no glycolipids like phosphatidylinositol mannosides. Consequently the description of the genus Naxibacter was emended (Kämpfer et al., 2008). Also, Weon et al. (2010) could not detect phosphatidylinositol mannosides in the polar lipid profile of Naxibacter suwonensis.

In addition, species of the genera Massilia and Naxibacter are often grouped together on the basis of 16S rRNA gene sequence comparisons and are found intermixed depending on the mode of sequence comparison (Kämpfer et al., 2008; Weon et al., 2009, 2010). At the time of writing, the genus Naxibacter contains four species with validly published names: N. alkalitolerans (Xu et al., 2005), N. varians and N. haematophilus (Kämpfer et al., 2008) and N. suwonensis (Weon et al., 2010).

In 1996, strain CCUG 58010T was isolated in Göteborg, Sweden, from blood of a 48-year-old man. This strain was presumptively identified as Massilia–Naxibacter-like and showed a beige-coloured colony morphology on blood agar (Oxoid) and nutrient agar (Oxoid) at 37 °C. Subcultivation for further analyses was done on tryptone soy agar (TSA, Oxoid) at 28 °C for 48 h.

Gram-staining was performed as described by Gerhardt et al. (1994). Cell morphology was observed under a Zeiss light microscope at ×1000, with cells grown for 3 days at 28 °C on TSA. The 16S rRNA gene was analysed as described by Kämpfer et al. (2003). Phylogenetic analysis was performed using the ARB software package (version December 2007; Ludwig et al., 2004) after multiple alignment of data with the ARB alignment tool and with the SILVA SSURef 100 database (release August 2009; Prusse et al., 2007). Distances (pairwise distances) and tree calculation with the neighbour-joining method and the maximum-likelihood method with fast DNAML (Olsen et al., 1994) were performed with the ARB software package. Clustering with the neighbour-joining method was performed by using bootstrap values based on 1000 replications. The 16S rRNA gene sequence of the isolate (1438 bp) was compared to those of related species by using sequence similarity calculations. The results of these calculations indicated that the closest relatives of strain CCUG 58010T were the type strains of N. varians (97.5% similarity), Massilia niastensis (96.8%) and Massilia aerilata (96.4%). Similarities to all other species of the genera Naxibacter and Massilia were in the range 93.9–96.2%. Phylogenetic trees are shown in Fig. 1 and Fig. S1 (available in IJSEM Online). It is obvious that all species of the genus Naxibacter were grouped together with the majority of species of the genus Massilia, including the type species Massilia timonae; however, in both trees it can be seen that the two species of the genus Telluria also fell together with the four species of the genus Massilia, Massilia albidiflava, Massilia dura, Massilia lutea and Massilia plicata. Hence, it seems to be necessary to investigate the species of the genus Telluria again very carefully and, on the basis of the results of genotypic and chemotaxonomic investigations, a reclassification of this group may also be appropriate.

For quinone and polar lipid analyses, strain CCUG 58010T was grown for 48 h in nutrient broth in shake flasks at 180 r.p.m. at 28 °C. Respiratory quinones were extracted as described by Collins et al. (1977) and analysed by high-performance liquid chromatography (Groth et al., 1996). Polar lipids extracted by the method of Minnikin et al. (1979) were identified by two-dimensional thin-layer chromatography as described by Collins & Jones (1980). The polar lipid profile contained the major compounds phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol (Fig. 2). No significant differences could be detected when compared with the lipid profiles published for species of the genera Naxibacter and Massilia (Kämpfer et al., 2008; Weon et al., 2010). Again, phosphatidylinositol mannosides could not be detected.

The fatty acid profiles of all strains are shown in Table 1. No pronounced differences in fatty acid profiles were found; however, slight differences could be observed. All type strains of the genera Naxibacter and Massilia as well as strain CCUG 58010T had fatty acid profiles composed of summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH) and C16:0 as the major components, as well as C18:1ω7c, C10:0 3-OH and C12:0 in moderate amounts. Additionally, C12:0 2-OH, C14:0, C13:0 3-OH, anteiso-C15:0, iso-C16:0, C17:0 cyclo, iso-C17:0 3-OH, C17:0 9c and C20:0 could be found in some of the strains. However, the amount of these fatty acids was mostly <3%, except for C17:0 cyclo (present as 1.0–11.4% in seven Naxibacter and Massilia type strains), C14:0 2-OH (6.1% in M. plicata, <3% in five other Massilia type strains) and C12:0 2-OH (present as 1.5–3.1% in nine Naxibacter and Massilia type strains and in strain CCUG 58010T). It is interesting to note that the fatty acid C14:0 2-OH was found in the species M. albidiflava, M. dura, M. lutea and M. plicata, which grouped together with the species of the genus Telluria (Fig. 1 and S1) and may represent a separate line of variation.

Examinations based on almost entire 16S rRNA gene sequences showed affiliation of strain CCUG 58010T to the genera Naxibacter and Massilia. The strain also shared the characteristics of both genera, which are a fatty acid profile consisting of the major compounds summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH) and C16:0 as well as moderate amounts of C12:0, C18:1ω7c and the hydroxylated fatty acid C10:0 3-OH, a main quinone system with ubiquinone Q-8 and a polar lipid profile containing the major compounds phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol.

Results of the physiological characterization are given in the species description and in Table 2 (differential characters) with methods as described previously (Kämpfer et al., 1991). At the species level, strain CCUG 58010T showed, with 97.5% similarity, the highest 16S rRNA gene
sequence similarity to the type strain of *N. varians* and 97% similarity to all other type strains of the genera *Naxibacter* and *Massilia*. DNA–DNA hybridization of strain CCUG 58010^T^ with *N. varians* CCUG 35299^T^ (method according to Ziemke et al., 1998) resulted in a relatedness value of 39.2% (reciprocal, 50%). On the basis of these results as well as the phenotypic traits, we describe a novel species, for which the name *Massilia consociata* sp. nov. is proposed. Furthermore, we propose the reclassification of *Naxibacter alkalitolerans*, *N. varians*, *N. haematophilus* and *N. suwonensis* in the genus *Massilia* as *Massilia alkalitolerans* comb. nov., *M. varians* comb. nov., *M. haematophila* comb. nov. and *M. suwonensis* comb. nov.

**Emended description of the genus *Massilia* La Scola et al. 2000**

The description is that of La Scola et al. (1998), with the following additional features. Quinone system consists of ubiquinone Q-8. The major compounds in the polar lipid profile are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Oxidase reaction may be variable. May produce variable results for acid production from carbohydrates, arginine dihydrolase, urease and aesculin reactions as well as for gelatin hydrolysis. The G+C content of the DNA ranges from 62.4 to 68.9 mol%.

**Description of *Massilia consociata* sp. nov.**

*Massilia consociata* (con.so.ci.9.ta. L. part. fem. adj. consociata associated; intended to mean that the organism was associated with a human clinical case). Cells are weakly motile, non-spore-forming rods (approx. 2 μm in length and 1 μm in width). Gram-stain-negative, oxidase-positive, showing an oxidative metabolism. Good growth occurs on R2A agar, TSA, PYE agar, nutrient agar and MacConkey agar at 25–30 °C. Grows on TSA at temperatures ranging from 15 to 37 °C. No growth at 10 or 45 °C. Grows in TS broth at pH 5.5–10.5. Beige, translucent and shiny colonies with entire edges are formed within 24 h, with a diameter of approximately 2 mm. Quinone system consists of ubiquinone Q-8. The major compounds in the polar lipid profile are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol.
Fig. 2. Two-dimensional TLC of polar lipid extracts from strain CCUG 58010T, stained with molybdatophosphoric acid. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; AL, unknown aminolipid; PL1 to PL3, unknown phospholipids.

Moderate amounts of three unknown phospholipids and of one unknown aminolipid are present. The fatty acid profile is largely composed of summed feature 3 (C_{16:1\alpha7c} and/or iso-C_{15:0} 2-OH) and C_{16:0} as well as moderate amounts of C_{18:1\alpha9c}, C_{10:0} 3-OH and C_{12:0}. Carbon source utilization and hydrolysis of chromogenic substrates (including differentiating characters for all species of the genus *Naxibacter*) are indicated in Table 2. Assimilates D-glucose, L-arabinose, trisodium citrate, glycogen, propionic acid, 3-hydroxybutyric acid and L-proline, but not potassium gluconate, L-rhamnose, suberic acid, lactic acid, L-alanine, 3-hydroxybenzoic acid, L-serine or L-histidine. Positive for aesculin hydrolysis, but negative for nitrate reduction, urease and oxidase.

The type strain, CCUG 58010T (=CCM 7792T), was isolated in Göteborg, Sweden, from blood of a 48-year-old man.

**Description of Massilia alkalitolerans comb. nov.**


The description is identical to that given by Xu et al. (2005) with the emendment of Kämper et al. (2008).

The type strain is YIM 31775T (=CCTCC AA 204003T =KCTC 12194T).

**Table 1.** Cellular fatty acid compositions of species of the genera *Massilia* and *Naxibacter*

<table>
<thead>
<tr>
<th>Fatty acid</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>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{10:0} 3-OH</td>
<td>4.3</td>
<td>3.3 (6.2)</td>
<td>3.0 (4.1)</td>
<td>5.5</td>
<td>4.7</td>
<td>6.1</td>
<td>5.4</td>
<td>7.0</td>
<td>4.8</td>
<td>4.9</td>
<td>5.5</td>
<td>5.7</td>
<td>6.6</td>
<td>10.1</td>
<td>4.6</td>
<td>3.2</td>
</tr>
<tr>
<td>C_{12:0}</td>
<td>3.4</td>
<td>3.7 (4.5)</td>
<td>3.3 (3.8)</td>
<td>4.7</td>
<td>3.4</td>
<td>5.9</td>
<td>4.4</td>
<td>5.3</td>
<td>4.6</td>
<td>5.0</td>
<td>3.9</td>
<td>4.0</td>
<td>8.9</td>
<td>7.1</td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>C_{12:0} 2-OH</td>
<td>1.8</td>
<td>2.0 (3.1)</td>
<td>1.5 (2.1)</td>
<td>2.4</td>
<td>2.5</td>
<td>1.7</td>
<td>2.4</td>
<td>3.9</td>
<td>1.9</td>
<td>2.0</td>
<td>2.5</td>
<td>2.5</td>
<td>3.9</td>
<td>2.9</td>
<td>6.1</td>
<td>2.2</td>
</tr>
<tr>
<td>C_{14:0}</td>
<td>-</td>
<td>0.7</td>
<td>-</td>
<td>1.9</td>
<td>1.2</td>
<td>2.6</td>
<td>0.6</td>
<td>-</td>
<td>1.1</td>
<td>1.2</td>
<td>-</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C_{14:0} 2-OH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
<td>2.4</td>
<td>-</td>
<td>2.6</td>
<td>2.9</td>
<td>-</td>
<td>6.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anteiso-C_{15:0}</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C_{16:0}</td>
<td>21.5</td>
<td>28.5 (23.6)</td>
<td>26.2 (25.2)</td>
<td>26.9</td>
<td>30.6</td>
<td>22.5</td>
<td>28.8</td>
<td>23.4</td>
<td>36.8</td>
<td>23.0</td>
<td>27.5</td>
<td>26.6</td>
<td>23.6</td>
<td>25.1</td>
<td>30.5</td>
<td>26.6</td>
</tr>
<tr>
<td>iso-C_{16:0}</td>
<td>2.2</td>
<td>-</td>
<td>2.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C_{17:0} cyclo</td>
<td>-</td>
<td>-</td>
<td>2.7 (9.6)</td>
<td>3.4</td>
<td>6.1</td>
<td>1.0</td>
<td>2.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>iso-C_{17:0}</td>
<td>-</td>
<td>-</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>iso-C_{17:1\alpha9c}</td>
<td>-</td>
<td>-</td>
<td>1.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C_{18:1\alpha7c}</td>
<td>12.8</td>
<td>8.1 (8.0)</td>
<td>8.7 (10.4)</td>
<td>12.3</td>
<td>11.7</td>
<td>12.1</td>
<td>7.8</td>
<td>7.4</td>
<td>2.5</td>
<td>9.0</td>
<td>7.03</td>
<td>7.8</td>
<td>7.8</td>
<td>11.7</td>
<td>7.9</td>
<td>6.6</td>
</tr>
<tr>
<td>C_{20:0}</td>
<td>-</td>
<td>-</td>
<td>2.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Summed feature 3†</td>
<td>54.0</td>
<td>51.7 (41.0)</td>
<td>44.7 (43.1)</td>
<td>38.1</td>
<td>35.2</td>
<td>49.0</td>
<td>45.8</td>
<td>46.0</td>
<td>48.3</td>
<td>54.2</td>
<td>52.0</td>
<td>51.1</td>
<td>46.0</td>
<td>36.9</td>
<td>47.0</td>
<td>55.2</td>
</tr>
</tbody>
</table>

*Data in parentheses from Weon et al. (2010).
†Summed feature 3 included C_{16:1\alpha7c} and/or iso-C_{15:0} 2-OH.
Table 2. Differential properties of species of the genera Massilia and Naxibacter

<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>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidase</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydrolysis of aesculin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Assimilation of:

- D-Glucose: +
- 1-Arabinose: +
- Potassium gluconate: +
- Adipic acid: ND
- Trisodium citrate: +
- 1-Rhamnose: +
- Suberic acid: +
- Lactic acid: +
- 1-Alanine: +
- Glycogen: +
- 3-Hydroxybenzoic acid: +
- 1-Serine: +
- Propionic acid: +
- Valeric acid: ND
- 1-Histidine: -
- 3-Hydroxybutyric acid: +
- 4-Hydroxybenzoic acid: ND
- 1-Proline: +
- DNA G + C content (mol%): ND

*Data from: a, Zhang et al. (2006); b, Gallego et al. (2006); c, Zul et al. (2008); d, Weon et al. (2009); e, Lindquist et al. (2003); f, Xu et al. (2005); g, Kämpfer et al. (2008).

Description of *Massilia haematopila* comb nov.

*Massilia haematopila* (ha.e.ma.to.p’i.la’. Gr. n. haima-atos (Latin transliteration haema-atos), blood; N.L. adj. philus-atos (from Gr. adj. philos-ê-atos), friend, loving; N.L. fem. adj. haematopila loving blood, referring to the isolation of the type strain from blood).

Basonym: *Naxibacter haematophilus* Kämpfer et al. 2008.

The description is identical to that given by Kämpfer et al. (2008).

The type strain is CCUG 38318T (=CCM 4780T).

Description of *Massilia suwonensis* comb nov.

*Massilia suwonensis* (su.wo.nen’sis. N.L. fem. adj. suwonensis of or belonging to Suwon region in the Republic of Korea, where the type strain was found).

Basonym: *Naxibacter suwonensis* Weon et al., 2010.

The description is identical to that given by Weon et al. (2010).

The type strain is 5414S-25T (=KACC 12635T =DSM 21311T).

Description of *Massilia varians* comb. nov.

*Massilia varians* (va’ri.an.s. L. part. adj. varians varying, pertaining to the variable results in biochemical tests).

Basonym: *Naxibacter varians* Kämpfer et al., 2008.

The description is identical to that given by Kämpfer et al. (2008).

The type strain is CCUG 35299T (=CCM 7478T).
Acknowledgements

We are grateful to the Public Health Laboratory Blood Department for submitting the isolate and to the entire CCUG staff for their devoted work. We thank Dr Jean Euzeby for his advice with the nomenclature.

References


