Proposal of Histophilus somni gen. nov., sp. nov. for the three species incertae sedis ‘Haemophilus somnus’, ‘Haemophilus agni’ and ‘Histophilus ovis’

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Earlier investigations have shown that ‘Haemophilus somnus’, ‘Haemophilus agni’ and ‘Histophilus ovis’ represent the same species. In the present investigation, the taxonomic position of this species is explored further by sequencing the 16S rRNA and rpoB genes of strains that were investigated previously by DNA–DNA hybridization. These results clearly support the allocation of this species to a novel genus within the family Pasteurellaceae. The phenotypic separation of Histophilus somni gen. nov., sp. nov. from other members of the family can, for most strains, be based on capnophila, yellowish pigmentation and indole production. However, due to phenotypic variation, the use of a species-specific PCR test based on the 16S rRNA gene is included in the species description. This is justified by the high sequence similarity of the 16S rRNA gene within the species and the fact that the highest sequence similarity to any other taxon within the family is 93.4%. The type strain, 8025T = ATCC 43625T = CCUG 36157T, was isolated in the USA from a bovine brain with lesions of thromboembolic meningoencephalitis.

INTRODUCTION

Infectious thromboembolic meningoencephalitis (TME), a disease condition in feedlot cattle, was described by Griner et al. (1956). A disease with similar manifestations was observed a few years later; a Gram-negative rod was isolated from the cerebral lesions and the disease could be reproduced by intravenous injection (Kennedy et al., 1960). The bacterium was labelled ‘Haemophilus-like’, although it did not require X- or V-factor for growth. In 1969, the name ‘Haemophilus somnus’ was proposed for this bacterium (Bailie, 1969). Phenotypically similar bacteria had previously been isolated from sheep; for example, an organism named ‘Histophilus ovis’ had already been isolated (Mitchell, 1925), but this isolate is no longer available. In 1956, ‘Histophilus ovis’ was isolated from mastitis in sheep (Roberts, 1956) and has since been isolated from ovine septicaemia, synovitis and epididymitis and from ovine vaginas (Rahaley & White, 1977; Rahaley, 1978). In 1958, a bacterium was isolated from lambs with septicaemia in Australia and designated ‘Haemophilus agni’ (Kennedy et al., 1958).

DNA–DNA hybridization has shown that ‘Histophilus ovis’, ‘Haemophilus somnus’ and ‘Haemophilus agni’ should be regarded as the same species (Walker et al., 1985; Piechulla et al., 1986), supporting earlier investigations of the antigenic and cytochemical relationships among these taxa (Stephens et al., 1983). However, the taxonomic position of this potential species is uncertain, as it was not affiliated to the genus Haemophilus by DNA–DNA hybridization (Walker et al., 1985; Piechulla et al., 1986), DNA–rRNA hybridization (de Ley et al., 1990) or 16S rRNA gene sequencing (Dewhirst et al., 1993). It apparently represents a novel genus within the family Pasteurellaceae (Piechulla et al., 1986; Bisgaard, 1995). In the second edition of Bergey’s Manual of Systematic Bacteriology, ‘Haemophilus somnus’, ‘Haemophilus agni’ and ‘Histophilus ovis’ are listed under the genus Haemophilus as ‘other organisms’ (Kilian, 2003). ‘Haemophilus somnus’ is currently used as a convenient, though incorrect, name for these taxa.
Nevertheless, ‘Histophilus ovis’ is often preferred, e.g. by Australian authors. Furthermore, as the name ‘Haemophilus somni’ is grammatically incorrect, the name ‘Haemophilus somnis’ has also been suggested (Miles et al., 1972). Clarification of the taxonomic position and nomenclature related to this species is thus sorely needed. A formal description of the species has not been published and no type strain has yet been designated.

In the present investigation, phylogenetic analysis of 16S rDNA and rpoB sequences from a number of strains examined previously by DNA–DNA hybridization is presented and used as a basis for changing the taxonomic status of this organism. As Histophilus somni gen. nov., sp. nov. will be proposed as a new name for this species, this name will be used in the rest of the presentation when a common designation for ‘Haemophilus somni’, ‘Haemophilus agni’ and ‘Histophilus ovis’ is needed.

METHODS

Bacterial strains. Strains investigated are listed in Table 1. All strains have been lyophilized or stored at –80°C. Histophilus somni strains were grown on BA [Colombia blood agar base (Oxoid), supplemented with 5% bovine blood] at 37°C in atmospheric air that contained 10% CO₂ for 24 h. Twelve of the strains have previously been studied by DNA–DNA hybridization (Table 1). Nine strains that represent different rRNA types (A, D, F, G, H, I, K, N and P) according to Fussing & Wegener (1993) have previously been investigated with 16S rDNA sequencing (Table 1) and used as the basis for developing a species-specific PCR test (Angen et al., 1998).

Phenotypic characterization. Biochemical reactions and fermentative activities of the strains were examined according to Mannheim et al. (1980). Evaluation of nitrate reduction, urease activity and catalase formation took place within 24 h. Indole formation was observed in a quadruple procedure for 4 days and the final reading of the formation of acidic substance (indicating the presence of polyvalent alcohols [with bromothymol blue as indicator]) took place 8 days after inoculation. All tests were performed in duplicate.

16S rDNA sequencing. Strains were grown overnight at 37°C on BA in atmospheric air that contained 10% CO₂; one bacterial colony was suspended in water and lysed by boiling. Sequencing was performed on either an ABI 373A or an ABI 377 automatic sequencer (Applied Biosystems) by using the ABI Prism BigDye Terminator Cycle Sequencing kit (Applied Biosystems). After purification of sequencing products by ethanol precipitation, they were run on an ABI 3100 Genetic Analyser (Applied Biosystems). Sequences were edited and read in both directions by using Sequencher (Gene Codes). Phylogenetic relationships and trees were established by using Bionumerics version 2.50 (Applied Maths). Corrected distances were calculated by using the Jukes–Cantor algorithm and a tree was built by the neighbour-joining method. Bootstrap values were computed by using 500 repetitions. GenBank accession numbers for the rpoB sequences are listed in Table 1.

Phylogeny of Histophilus somni in relation to the Pasteurellaceae. Seventy-three sequences from GenBank were selected to represent 61 named and 12 unnamed taxa within the Pasteurellaceae (see supplementary phylogenetic tree in IJSEM Online). Only 16S rRNA gene sequences that contained fewer than ten ambiguous bases and where information was available on animal source and geographical origin were included in the analysis. Haemophilus paragallinarum, Haemophilus paraphrophthaemolyticus and Haemophilus parahaemolyticus were not included because of inadequate data quality. The ‘multiple-outgroup’ approach was used to determine the phylogeny of the family by inclusion of different taxa of the family as outgroups, with respect to suspect monophyletic groups (Wiley, 1981; Dalevi et al., 2001). The alignment included the region that corresponded to positions 27–1360 of the E. coli rpoB gene; 1061 positions remained after removal of ambiguous positions, with 211 distinct data patterns analysed. Maximum-likelihood analysis was performed by fastDNAm (Felsenstein, 1981; Olsen et al., 1994) on a Linux 7.2-compatible server with a transition/transversion ratio (T) of 1.5. The position of Histophilus somni in the phylogenetic tree was tested by including Histophilus somni as a sister group to major monophyletic groups until the highest log-likelihood value was reached. Bootstrap analysis was performed by a script supplied with the fastDNAm program on a SunOS 5.8 with high-performance computing and run 100 times. For each repetition, input sequences were randomized up to 100 times and the tree with the highest log-likelihood value was kept.

rpoB sequencing. For sequencing of the rpoB gene, primers adapted from Mollet et al. (1997) were used to PCR-amplify and sequence a 520 bp fragment of the gene. PCR products were purified with a High Pure PCR Product Purification kit (Roche Molecular Diagnostics) and sequenced directly with a dRhodamine Dye Terminator Cycle Sequencing kit (Applied Biosystems). After purification of sequencing products by ethanol precipitation, they were run on an ABI 3100 Genetic Analyser (Applied Biosystems). Sequences were edited and read in both directions by using Sequencher (Gene Codes). Phylogenetic relationships and trees were established by using Bionumerics version 2.50 (Applied Maths). Corrected distances were calculated by using the Jukes–Cantor algorithm and a tree was built by the neighbour-joining method. Bootstrap values were computed by using 500 repetitions. GenBank accession numbers for the rpoB sequences are listed in Table 1.

RESULTS

The phylogenetic position of the proposed type strain of Histophilus somni (8025T) in the family Pasteurellaceae, based on 16S rDNA sequencing, can be seen in the full phylogenetic tree available as supplementary material in IJSEM Online. By phylogenetic analysis of 16S rRNA gene sequences, 13 monophyletic groups and ten single taxa that were not affiliated with any of these groups were identified. The results mainly confirmed the monophyletic groups of Olsen et al. (2003), verifying the position of Histophilus somni within cluster 20 (Olsen et al., 2003). Histophilus somni does not show a close affiliation to any existing genera within the family. The highest 16S rDNA sequence similarity value observed was 93-4%, to Actinobacillus succinogenes. Deeper phylogenetic relationships were difficult to resolve. To simplify the dendrogram and indicate the lack of precision at deeper levels of the tree, branch-lengths that include zero within the 95% confidence interval (Felsenstein, 1981) have been set to zero in the tree. Constantly high maximum-likelihood values were never obtained when input sequences were randomized, because of the complexity of the tree; bootstrap values are probably underestimates.

Phylogenetic analysis of the 16S rDNA gene sequences of the strains listed in Table 1 is shown in Fig. 1. All strains that
### Table 1. Strains of *Histophilus somni* and reference strains of other species used in the present investigation

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Origin (reference)</th>
<th>DNA–DNA*</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>16S rDNA sequence</td>
<td><em>rpoB</em> sequence</td>
</tr>
<tr>
<td><em>Histophilus somni</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8025^T = HIM 734-8^T = CCUG 36157^T = ATCC 43625^T</td>
<td>USA, bovine, brain (Brown et al., 1972)</td>
<td></td>
<td>AF549387</td>
</tr>
<tr>
<td>2336 = CCUG 46769</td>
<td>USA, bovine, pneumonia (Corbeil et al., 1985)</td>
<td>1</td>
<td>AF549389†</td>
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<tr>
<td>CCUG 18779 = HIM 734-4 = Corboz 606</td>
<td>Switzerland, calf, lung (Corboz &amp; Wild, 1981)</td>
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<td>AF549389†</td>
</tr>
<tr>
<td>CCUG 12839 = HIM 734-5 = Corboz 791</td>
<td>Switzerland, bull, semen (Corboz &amp; Wild, 1981)</td>
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<td>AF549390†</td>
</tr>
<tr>
<td>CCUG 46775 = HIM 734-6 = Corboz 43</td>
<td>Switzerland, bovine, uterus (Corboz &amp; Wild, 1981)</td>
<td>1</td>
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</tr>
<tr>
<td>CCUG 18780 = HIM 734-7 = Corboz 679</td>
<td>Switzerland, calf, joint (Corboz &amp; Wild, 1981)</td>
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<td>AF549392</td>
</tr>
<tr>
<td>CCUC 46774 = HIM 849-1 = L. Stephens 43826</td>
<td>Canada, bovine, brain (Stephens et al., 1983)</td>
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<tr>
<td>CCUG 46770 = HIM 849-3 = APCC UQV 179</td>
<td>Australia, bovine, lung (Stephens et al., 1983)</td>
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<tr>
<td>CCUG 18777 = HIM 874-5 = L. Stephens 43803‡</td>
<td>Canada, ovine, brain (Stephens et al., 1983)</td>
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<td>Canada, ovine, vagina (Stephens et al., 1983)</td>
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<tr>
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<td>1, 2</td>
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<td>1, 2</td>
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<tr>
<td>CCUG 36158 = Sp 30/12</td>
<td>Germany, bovine, preputium</td>
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<td>CCUG 47046 = 3-85</td>
<td>Denmark, bovine, preputium (Fussing &amp; Wegener, 1993)</td>
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<td>CCUG 47048 = 189-83</td>
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<tr>
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<td>Denmark, bovine, pneumonia (Fussing &amp; Wegener, 1993)</td>
<td></td>
<td>AF549404†, AY170214</td>
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<td>CCUG 47050 = 1062-89</td>
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<td><em>Actinobacillus lignieresii</em> NCTC 4189^T = ATCC 49236^T</td>
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<td>M75068</td>
<td>AY170215</td>
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<tr>
<td><em>Haemophilus influenzae NCTC</em> 8143^T = ATCC 33391^T</td>
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<td>UK, human isolate</td>
<td>M35019</td>
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<td><em>Pasteurella multocida NCTC</em> 10322^T = ATCC 43137^T</td>
<td>Canada, porcine isolate</td>
<td>M35018</td>
<td>AY170216</td>
</tr>
<tr>
<td><em>Mannheimia haemolytica NCTC</em> 9580^T = ATCC 33396^T</td>
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<td>AF606699</td>
<td>AY170217</td>
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<tr>
<td><em>Lonepinella koalorum ATCC</em> 700131^T = ACM 3666^T</td>
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<td>Y17189</td>
<td>AY170218</td>
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<tr>
<td><em>Phocoenobacter uteri NCTC</em> 12872^T</td>
<td>UK, porpoise, uterus</td>
<td>X89379</td>
<td>AY170219</td>
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<td><em>Escherichia coli</em> K12 = MG 1655</td>
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<td>J01695</td>
<td>NC_000913</td>
</tr>
</tbody>
</table>

*Investigated by DNA–DNA hybridization: 1, Piechulla et al. (1986); 2, Walker et al. (1985).
†Strains that represent different ribotypes according to Fussing & Wegener (1993), used as the basis for developing the species-specific PCR test (Angen et al., 1998).
‡Previously designated *Histophilus ovis*.
§Previously designated *Haemophilus agni*.
||Strain Rd used for *rpoB* sequencing.
have been investigated previously by DNA–DNA hybridization are indicated in bold type. Sequence similarities between all investigated strains of *Histophilus somni* were >99.5%.

The relationship between the *rpoB* sequences of eight selected strains of *Histophilus somni* and the type species of existing genera within the family *Pasteurellaceae* is shown in Fig. 2. Strains of *Histophilus somni* showed a clear separation from other reference strains within the family. There was 15–20% sequence difference between the *Histophilus somni* *rpoB* sequence and those of other type species of the family *Pasteurellaceae*. Among the *Histophilus somni* strains, two clusters (separated by a sequence difference of approximately 6%) were observed (Fig. 2).

The investigated strains produced a yellowish pigment and showed increased growth in 10% CO₂ (capnophilia), but no dependence on X- or V-factor. All strains were positive in alkaline phosphatase, nitrate reduction, oxidase and ONPG tests and were negative for catalase production, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and gelatinase activities. Indole was produced by all strains except for CCUG 46772 (originally designated *Haemophilus agni*). All strains fermented D-glucose without gas production, whereas variable fermentation was observed for D-mannose. Acid was not produced from L-sorbose, adonitol, D-galactose, D-fructose, L-rhamnose, D-xylene, L-arabinose, D-sucrose, trehalose, maltose, D-lactose, raffinose, D-mannitol, D-sorbitol, dulcitol, *meso*-inositol, salicin or aesculin.

Table 2 shows phenotypic characters that separate *Histophilus somni* from the other genera of the family.

**DISCUSSION**

Earlier suggestions that *Histophilus somni* should represent a novel genus within the family *Pasteurellaceae* (de Ley et al., 1990; Bisgaard, 1995) are supported by the low affiliation found to the other taxa of the family in phylogenetic analysis based on 16S rDNA gene sequences. The full phylogenetic tree is available as supplementary material in IJSEM Online. Analysis also shows that this novel genus is monophyletic and, at present, contains only one species.

DNA–DNA hybridization has shown that *Histophilus ovis*, *Haemophilus somnis* and *Haemophilus agni* should be regarded as the same species (Walker et al., 1985; Piechulla et al., 1986). The strains marked in bold type in Fig. 1 were found to have DNA–DNA binding values >91% with each other when investigated by the spectrophotometric method (Piechulla et al., 1986). Consequently, these strains should

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**Fig. 1.** Phylogenetic analysis, based on 16S rDNA sequences, of *Histophilus somni* isolates and type species of genera in the family *Pasteurellaceae*. The tree was built with the neighbour-joining method, based on Jukes–Cantor-corrected distances. Bootstrap values (500 repetitions) are indicated as percentages at major branching-points. Strains in bold type have been investigated by DNA–DNA hybridization. Bar, 0.02 substitutions per nucleotide position.

**Fig. 2.** Phylogenetic analysis, based on partial *rpoB* sequences, of *Histophilus somni* isolates and type species of genera in the family *Pasteurellaceae*. The tree was built with the neighbour-joining method, based on Jukes–Cantor-corrected distances. Bootstrap values (500 repetitions) are indicated as percentages at major branching-points. Bar, 0.02 substitutions per nucleotide position.
be regarded as members of the same species. This conclusion is supported by the results of 16S rDNA sequence analysis in the present investigation (Fig. 1), as all investigated strains of Histophilus somni show 16S rDNA similarity values of >99.5% to each other. The 16S rDNA sequences of Histophilus somni are very homogeneous and show a very clear separation from all other described taxa. The 16S rDNA sequences of a collection of reference strains have, for this reason, previously been used as the basis for developing a species-specific PCR test (Ange et al., 1998).

Separation of Histophilus somni from other genera of the family was also found by analysis of rpoB gene sequences (Fig. 2). As expected, higher heterogeneity was found among strains of Histophilus somni in rpoB gene sequence than in 16S rDNA sequence, as the resolving power of the rpoB gene sequence is generally higher. This corresponds to earlier reports that state that the interspecies variability of the rpoB gene for Enterobacteriaceae is about three times higher than that of 16S rDNA (Mollet et al., 1997).

Busse et al. (1997) investigated the polyamine patterns of two strains of Histophilus somni, which also were included in the present investigation (CCUG 46772 and CCUG 46773). Similar polyamine patterns were observed for these two strains and for representatives of subclusters 3A and 3C according to Dewhirst et al. (1993). However, a close relationship between Histophilus somni and these groups has not been found by 16S rDNA sequencing, DNA–rRNA hybridization or DNA–DNA hybridization studies (Piechulla et al., 1986; de Ley et al., 1990; Dewhirst et al., 1993). On the other hand, the polyamine pattern of Histophilus somni was different from those of clusters 1C, 4A and 3B (Dewhirst et al., 1993), which contain the type species of the genera Haemophilus, Actinobacillus and Pasteurella, respectively. This supports the proposal that Histophilus somni should be allocated to a novel genus within the family.

We feel that it is important to resolve the present confusion in taxonomy and nomenclature for this species. Histophilus somni is of great clinical importance for cattle and sheep worldwide (Humphrey & Stephens, 1983; Harris & Janzen, 1989; Kwiecien & Little, 1992). Furthermore, it is an organism that receives a lot of attention in basic molecular studies (Inzana et al., 1992; Wu et al., 2000) and the total genome of strain 2336 is currently being sequenced (http://microgen.ouhs.edu/h_somnus/h_somnus_home.htm). As it is obvious that a new genus name must be proposed for ‘Histophilus ovis’, ‘Haemophilus somnius’ and ‘Haemophilus agni’, a natural choice is to use the previously proposed name Histophilus. This name has no current standing in bacterial nomenclature, but was proposed for similar bacteria (that no longer exist) by Mitchell (1925). For the species epithet, several possibilities are available. The name ‘Histophilus somnius’ cannot be used, as the word somnius cannot stand in apposition (Trüper & De’Clari, 1997) and should consequently be in the genitive case: hence Histophilus somni. ‘Histophilus ovis’ would be grammatically correct and is the oldest available name. However, most reports about this organism come from cattle, therefore the name would be slightly misleading and less likely to be readily accepted. ‘Histophilus somnifer’ is grammatically correct but is unnecessarily complicated in comparison to Histophilus somni. Consequently, Histophilus somni is proposed as the new name for this organism.
Phenotypic separation from existing genera is possible by using the criteria listed in Table 2. In this table, phenotypic data are restricted to taxa located in the 16S rDNA clusters that contain the type strains of the genera (Olsen et al., 2003). *Histophilus somni* can thus normally be differentiated from other genera by capnophilia, yellowish pigmentation and indole production. However, capnophilia has also been reported for other taxa, e.g. within the genus *Haemophilus* (Kilian, 2003), and, although newly isolated strains of *Histophilus somni* show CO₂-dependent growth, they can adapt gradually to fully aerobic growth (Biberstein, 1981). Variation in pigmentation has also been reported (Corboz, 1981). Indole production is observed for most strains; however, strains previously designated ‘*Haemophilus agni*’ do not produce indole. Furthermore, considerable phenotypic variability between strains and laboratories has been reported (Garcia-Delgado et al., 1977; Biberstein, 1981; Fussing & Wegener, 1993; Kilian, 2003), especially in carbohydrate fermentation. In contrast to the present investigation, production of acid from D-fructose, D-xylose, D-mannose, D-mannitol, D-sorbitol, L-arabinose, maltose and trehalose has been reported. *Histophilus somni* is a feeble-growing and has a variable phenotypic appearance, which complicates bacterial isolation and identification. Thiabdin monophosphate is required for growth (Asmussen & Baugh, 1981). The phenotypic variability observed, e.g. in carbohydrate fermentation, may relate to differences in composition of fermentation media.

To overcome the difficulties associated with phenotypic characterization, we suggest that a PCR test should be included in the criteria used for describing this species. This is in accordance with the report of the ad hoc committee for the re-evaluation of the species definition in bacteriology (Stackebrandt et al., 2002), where it is recommended that ‘species should be identifiable by readily available methods (phenotypic, genomic)’. PCR has become a readily available method for most diagnostic laboratories and consequently fulfils this criteria. A collection of 105 *Histophilus somni* strains from Denmark, Switzerland and the USA was rhibotyped by Fussing & Wegener (1993), demonstrating 16 different ribotypes. The 16S rDNA of strains that represent nine of these ribotypes was sequenced (Table 1) and the sequences were used as the basis for developing a species-specific PCR test (Angen et al., 1998). Species-specificity of the PCR test was evaluated by using a representative collection of strains within the family *Pasteurellaceae*. The appropriateness of using a PCR test based on the 16S rRNA gene is further supported by the clear separation from all other taxa observed in the phylogenetic analysis (see supplementary material in IJSEM Online) and high 16S rDNA sequence similarity within the species (Fig. 1). By including a PCR test among the criteria used in the species description, a quick and reliable identification method becomes available for diagnostic laboratories. The PCR test has been applied to clinical samples, whereby the detection rate of *Histophilus somni* in connection with calf pneumonia has been increased (Tegtmeier et al., 2000).

**Description of *Histophilus gen. nov.*

*Histophilus* (Hist.o'phi.lus. Gr. n. histos tissue; N.L. adj. philus from Gr. adj. philos friendly; N.L. masc. n. Histophilus the tissue friend).

*Histophilus* is a novel genus within the family *Pasteurellaceae* Pohl (1979) 1981. The genus consists of Gram-negative, non-motile rods or cocobacilli. Endospores are not formed. Growth is mesophilic and capnophilic and is not dependent on V-factor or X-factor. Glucose is fermented without gas production. Oxidase reaction is normally positive. Alkaline phosphatase test is positive and nitrate is reduced. Simmons’ citrate, Voges–Proskauer, arginine dihydrolase and ornithine decarboxylase tests are negative and there is no fermentation of adonitol or L-sorbose. Urease reaction is negative. Phenotypic characters that separate the genus *Histophilus* from other genera of the family *Pasteurellaceae* are given in Table 2. The genus is not closely phylogenetically related to any other taxon within the *Pasteurellaceae*. The type species of the genus is *Histophilus somni*.

**Description of *Histophilus somni* sp. nov.

*Histophilus somni* (som’ni. L. gen. n. somni of sleep, referring to one of the disease conditions associated with the bacterium).

Characteristics are as described for the genus. Most isolates produce indole and yellow pigment (*‘H. agni*’ has been described as indole-negative). Considerable phenotypic variability has been reported between strains and laboratories (Garcia-Delgado et al., 1977; Biberstein, 1981; Fussing & Wegener, 1993; Kilian, 2003). In the present investigation, strains of *Histophilus somni* did not ferment sucrose, D-galactose, D-fructose, maltose or trehalose (Table 2). Growth of most strains requires addition of 5–10% CO₂. Haemolysis may vary; can be non-haemolytic, α-haemolytic or β-haemolytic on calf blood agar. Colonies of most strains are pinpoint after 24 h and up to 1–1.5 mm in diameter after 48 h. Thiamin monophosphate is required for growth (Asmussen & Baugh, 1981). DNA G+C content of the type strain is 37.5 mol% (Piechulla et al., 1986). *Histophilus somni* can be identified by using a species-specific PCR test, as described by Angen et al. (1998): one bacterial colony is resuspended in 100 μl distilled water. Bacteria are lysed by boiling and the sample is diluted 1:100 before PCR amplification. A sample (2 μl) is pipetted into 48 μl prepared reaction mixture that contains 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 100 μM each dNTP, 65 ng each oligonucleotide primer (HS-453F: 5′-GAAGGCGATTAGTTTAAGAG-3′ and HS-860R: 5′-TTCGCGCAGCAAGTRTTCA-3′), 0.5 U Taq polymerase (PerkinElmer) and covered with 50 μl paraffin oil. Samples are subjected to an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, in a thermal cycler. Expected size of the amplicon is 400 bp. *Histophilus somni* has been described as an...
aetiological agent for a variety of diseases in cattle and sheep, including TME, pneumonia, septicaemia, mastitis, arthritis, myocarditis and reproductive disease (Humphrey & Stephens, 1983). It is also found as a commensal in the prepuces and semen (Humphrey et al., 1982; Krogh et al., 1983) and in the female genital tract (Kwicien & Little, 1992) and may also be part of the resident microbial flora of the upper respiratory tract (Humphrey & Stephens, 1983; Corbeil et al., 1986).

The type strain is 8025T (= ATCC 43625T = CCUG 36157T), which was isolated in the USA from a bovine brain with TME lesions (Brown et al., 1972).

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