Proposal of *Bisgaardia hudsonensis* gen. nov., sp. nov. and an additional genomospecies, isolated from seals, as new members of the family *Pasteurellaceae*

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Phenotypic and phylogenetic studies were performed on eight Gram-negative-staining, rod-shaped bacteria isolated from seals. Biochemical and physiological studies showed identical profiles for all of the isolates and indicated that they were related to the family *Pasteurellaceae*. 16S rRNA gene sequencing demonstrated that the organism represented a distinct cluster with two sublines within the family *Pasteurellaceae* with <96% sequence similarity to any recognized species. Multilocus sequence analysis (MLSA) including *rpoB*, *infB* and *recN* genes further confirmed these findings with the eight isolates forming a genus-like cluster with two branches. Genome relatedness as deduced from *recN* gene sequences suggested that the isolates represented a new genus with two species. On the basis of the results of the phylogenetic analysis and phenotypic criteria, it is proposed that these bacteria from seals are classified as *Bisgaardia hudsonensis* gen. nov., sp. nov. (the type species) and *Bisgaardia* genomospecies 1. The G+C content of the DNA was 39.5 mol%. The type strain of *Bisgaardia hudsonensis* gen. nov., sp. nov. is M327/99/2^T (=CCUG 43067^T=NCTC 13475^T=98-D-690B^T) and the reference strain of *Bisgaardia* genomospecies 1 is M1765/96/5 (=CCUG 59551=NCTC 13474).

The family *Pasteurellaceae* accommodates a broad group of Gram-negative, chemo-organotrophic, facultatively anaerobic and fermentative bacteria and currently contains 15 recognized genera and more than 60 species with validly published names (Christensen & Bisgaard, 2008; Kuhnert *et al.*, 2010). Members of the family *Pasteurellaceae* are generally inhabitants of the mucous membranes of man and animals. Many species of the family are known to be associated with and/or cause disease in many mammalian

species as well as in birds and tortoises (Bisgaard, 1993; Frederiksen, 1993). The range of hosts for these organisms has been extended with the publication of three novel species from marine mammals, namely *Actinobacillus delphinicola* (Foster *et al.*, 1996) from several species of cetacean, *Actinobacillus scotiae* (Foster *et al.*, 1998) from porpoises and *Phocoenobacter uteri* (Foster *et al.*, 2000) also from porpoises. Reports of the isolation of members of the family *Pasteurellaceae* from seals however are limited and in the examples that do exist, there is little information given regarding the methods used for their identification (Baker & Ross, 1992). In this paper, we report the results of an investigation using a polyphasic taxonomic approach of novel members of the family *Pasteurellaceae* recovered from seals in Canada and Scotland. A new genus, novel species and new genomospecies are described.

Investigations into the deaths of 21 ringed seals (*Phoca hispida*) near the communities of Umiujaq (56° 33' N 76° 33' W) and Sanikiluaq (56° 36' N 79° 12' W), Eastern Hudson Bay, Northern Quebec, Canada, in the autumn of 1998, lead to the recovery of three strains (M327/99/1,

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences determined for nine strains in this study are GU295083–GU295091. The GenBank accession number for the 16S rRNA gene sequence of strain M327/99/2^T is GU295084. Partial *rpoB* and *infB* gene sequences for nine strains have been deposited under the numbers GU295092–GU295100 and GU295101–GU295109, respectively. The partial *recN* gene sequences of ten strains have been deposited with accession numbers GU295074–GU295082 and GU321105.

Supplementary figures are available with the online version of this paper.

M327/99/2^T and M327/99/3) of an unknown Gram-negative, rod-shaped bacterium. The strains were isolated from the lungs of three out of four of the animals necropsied and were found to be phenotypically similar to members of the family *Pasteurellaceae*. One of these strains, M327/99/2^T (=98-D-690B^T), was sequenced and deposited in GenBank (GenBank accession no. AJ251339). Following the seal mortalities, two disease surveys took place in Hudson Bay and Ungava Bay in winter 1999 to determine the health status of ringed seals. Additional material was collected for bacteriological analysis as part of these studies. A further two similar isolates (M327/99/4 and M327/99/5) were cultured from the retro-mandibular lymph nodes of two adult seals harvested in Aupaluk (59° 45' N 69° 41' W) and from a lymph node and tonsil of a young adult seal killed near the community of Kuujuaq (58° 06' N 68° 24' W). Lungs and lymph nodes of the seals did not show any macroscopic or microscopic lesions that could have been associated with the bacterium.

In Scotland, seals reported as part of the Marine Mammal Strandings Scheme were necropsied as part of an investigation into the causes of death in marine mammals. Examinations were carried out in accordance with a nationally agreed protocol (Kuiken & Hartmann, 1991) and selected tissues were collected for bacteriological culture. Three strains that were similar in appearance to the isolates from ringed seals were recovered from two grey seals (*Halichoerus grypus*) in 1996 and 1997 and a harbour seal (*Phoca vitulina*) in 1998. Strain M1765/96/5 was recovered from the liver, lung and pre-scapular lymph node of a grey seal which was suspected to have died from septicaemia and from which *Arcanobacterium phocae* was also cultured. Strain M1718/97/2 was recovered from the lung and kidney of a further grey seal which appeared to be in good condition but had been shot and M2461/98/1 was recorded from only the lung of a harbour seal pup alongside *Arcanobacterium phocae*. The latter animal, weighing 14.5 kg had been losing body condition and had a midsternal blubber thickness of 9 mm. It had not been feeding and was ill with a significant parasitic pneumonia. Associated with this was haemorrhage from the lungs into the airways.

Primary isolations were made on Columbia agar (Oxoid) supplemented with 5% defibrinated sheep blood (CSBA) that was incubated at 37 °C in an aerobic atmosphere containing 10% CO₂. Isolates were subcultured to CSBA to provide inocula for biochemical tests. Catalase, oxidase, X and V factor requirement, indole, nitrate reduction, Voges–Proskauer, arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase, urease, acid production from carbohydrates and ability to grow in different atmospheres, at selected temperatures, without blood or serum and on MacConkey agar were determined as described previously (Foster *et al.*, 1996, 1998). The biochemical characteristics of the strains were also examined with the API ZYM system (bioMérieux) according to the manufacturer's instructions. Reactions were scored as 0, 1, 2, 3, 4 or 5 on the basis of the results of a comparison with the colour chart of the

manufacturer; scores of 3, 4 or 5 were considered strong positive reactions and scores of 1 or 2 were considered weak positive reactions. The G+C content of DNA was determined as described by Garvie (1978).

Colonies of the isolates grown on CSBA were circular, entire, low convex, grey, translucent or opaque, non-haemolytic and 0.5 mm in diameter after 24 h of aerobic incubation at 37 °C. Elevated levels of CO₂ were not required for subculture. The cells were Gram-negative-staining and rod-shaped. The organisms were catalase-positive, oxidase-positive and non-motile. They grew under anaerobic conditions on pre-reduced CSBA plates using the Oxoid Anaerogen system and on Columbia agar without blood or serum added. Growth did not occur on MacConkey agar with or without NaCl. They grew at 22 °C, but not at 42 °C. Nitrate was reduced to nitrite. The organisms were negative for the Voges–Proskauer reaction and in tests for urease, indole, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase. Acid was produced from D-glucose and other carbohydrates listed with the description of the species. Using the API ZYM system, strong positive reactions were observed for alkaline phosphatase, leucine arylamidase, acid phosphatase and α -fucosidase and a weak positive reaction was obtained for naphthol-AS-BI-phosphohydrolase.

The eight isolates could not be separated phenotypically and were suspected of belonging to the same species within the family *Pasteurellaceae*. This was also the case when testing the eight isolates on the Vitek 2 system (bioMérieux) using GN and NH cards and resulting in identical profiles with each panel. Positive reactions were obtained for arginine arylamidase, leucine arylamidase, phenylalanine arylamidase, phosphorylcholine, D-galactose, D-mannose, phosphatase and phenylphosphonate. Negative reactions were obtained for acid production from D-mannitol, sorbitol, sucrose and trehalose, while variable reactions were observed with D-glucose and maltose. Differences in acid production from some sugars observed between the commercial Vitek 2 system and the conventional tube assay have previously been seen and may be due to the fact that the Vitek 2 tests use large inocula and short incubation times, thereby not necessarily measuring fermentation but rather acidification of sugars (Kuhnert *et al.*, 2010). All other results obtained with the Vitek 2 system are provided in the species description. All of the strains were subjected to molecular characterization to determine their taxonomic status.

Phylogenetic analysis was performed for four different genes with all eight isolates. Sequences for partial 16S rRNA, *rpoB*, *infB* and *recN* genes were determined as described previously (Kuhnert *et al.*, 2002; Korczak *et al.*, 2004; Kuhnert & Korczak, 2006; Mayor *et al.*, 2006). The *infB* and *recN* gene sequences for the recently described genus *Chelonobacter* as well as the partial *recN* gene sequence of the related species [*Pasteurella*] *testudinis* were also determined in the framework of this study. For the partial *recN* gene sequence of *Chelonobacter oris* 1662^T,

primers *recN*_Ptes-2 (5'-CGGTCAATATCGAAGCGAT-GC-3') and *recN*_Chel-3 (5'-GTTGCTTCCTGTACTT-GAATC-3') were used in combination with *recN*-L and *recN*-R, respectively (Kuhnert & Korczak, 2006). The GenBank accession numbers for the gene sequences are given in the individual phylogenetic trees for each gene as presented in Supplementary Figs S1–S4 (available in IJSEM Online).

A combined tree of all four genes is given in Fig. 1. The eight strains formed a monophyletic, genus-like cluster within the family *Pasteurellaceae*, for which the new genus name *Bisgaardia* gen. nov. is proposed. The *Bisgaardia* gen. nov. cluster formed two distinct but homogeneous species-like branches. One branch comprised the five isolates from ringed seals (M327/99/1, M327/99/2^T, M327/99/3, M327/99/4, M327/99/5) and the other included the three isolates from the grey and harbour seal (M2461/98/1, M1765/96/5, M1718/97/2). The names *Bisgaardia hudsonensis* gen. nov., sp. nov. and *Bisgaardia* genomospecies 1 are proposed for these two lineages. The new genus clustered in the combined tree closest to *Histophilus somni* 8025^T. This clearly separated cluster with two branches was reflected in each individual tree (see Supplementary Figs S1–S4). In the 16S rRNA gene tree, the genus *Bisgaardia* gen. nov. formed a monophyletic branch unrelated to other groups (Supplementary Fig. S1). The 16S rRNA gene divergence of the genus *Bisgaardia* gen. nov. with other members of the family was at least 4% with '*Actinobacillus porcitosillarum*' showing highest absolute sequence similarity (95.5%). The two species of the genus *Bisgaardia* gen. nov. shared 16S rRNA gene sequence similarity of 98.5%. In the *rpoB* gene-based phylogenetic tree, the genus *Bisgaardia* gen. nov. clustered in the proximity of the genus *Pasteurella* (Supplementary Fig. S2). Highest sequence similarity was observed with *Pasteurella canis* CCUG 12400^T (86.5%) whereas the two species of the genus *Bisgaardia* gen. nov. shared 88.5% similarity within the partial *rpoB* gene. A similar observation was made in the *infB* gene-based analysis (Supplementary Fig. S3) where members of *Bisgaardia* gen. nov. also clustered closest to the genus *Pasteurella*, whereby *Bisgaardia* shared on average 81.5% sequence similarity with *Pasteurella canis* CCUG 12400^T. The *infB* gene sequence similarity between the two novel species of the genus *Bisgaardia* was 86.5%. Finally, in the *recN* gene-based phylogenetic tree (Supplementary Fig. S4), the closest genus was *Histophilus* sharing about 72% sequence similarity. The absolute sequence similarity of this partial gene between the species of the genus *Bisgaardia* gen. nov. was 88.5%.

Recently it was shown that *recN* gene sequence similarity is suitable for the deduction of genome similarities within the family *Pasteurellaceae* and can be used as an alternative to DNA–DNA hybridization (Kuhnert & Korczak, 2006). This approach has since contributed successfully to resolving the taxonomy of the family *Pasteurellaceae* (Kuhnert *et al.*, 2007; Bisgaard *et al.*, 2007, 2009; Kuhnert *et al.*, 2010). The genetic similarity of *Bisgaardia hudsonensis* sp. nov. was

calculated with all the type species of the 15 genera of the family *Pasteurellaceae* as well as with *Bisgaardia* genomospecies 1 (Table 1). The highest genetic relatedness was seen with *Histophilus somni* 8025^T, whereas the lowest genetic relatedness was observed with *Chelonobacter oris* 1662^T. This was also reflected in the combined tree (Fig. 1) as well as the *recN* gene-based phylogenetic tree (Supplementary Fig. S4). The 16S rRNA gene sequence similarity of >98% between the two novel *Bisgaardia* species did not allow clear species separation (Stackebrandt & Ebers, 2006). However, the *recN* gene-based similarity of 0.69 was clearly below the threshold of 0.85 normally observed for species of the family *Pasteurellaceae* and the MLSA phylogeny, as well as the tree topology of the individual trees (Supplementary Figs S1–S4), allowed the distinction of the two homogeneous species within the genus *Bisgaardia* gen. nov. On the other hand, the genetic similarity value was clearly above the threshold of 0.4 that allows species to be grouped within the same genus.

Therefore, based on phenotypic, phylogenetic and genetic analyses, we propose the new genus *Bisgaardia* gen. nov. with two species. Given the lack of phenotypic characteristics to separate the two species, we propose *Bisgaardia hudsonensis* gen. nov., sp. nov. and *Bisgaardia* genomospecies 1. The inability to distinguish between the two species using phenotypic tests presents difficulties for diagnostic laboratories encountering isolates of the genus *Bisgaardia* that are unable to perform sequencing. Differences with respect to host species was the only criterion observed between the strains of the two species examined in this study and additional investigations are necessary to examine further isolates from ringed and common seals as well as isolates from different geographical populations to determine whether host association is a useful indicator for the species of *Bisgaardia* gen. nov.

The newly characterized genus can be readily distinguished from currently described members of the genera *Pasteurella*, *Haemophilus*, *Actinobacillus*, *Mannheimia*, *Phocoenobacter*, *Bibersteinia*, *Lonepinella*, *Histophilus*, *Nicoletella*, *Gallibacterium*, *Avibacterium*, *Volucribacter*, *Aggregatibacter*, *Chelonobacter* and *Basfia* by the characteristics shown in Table 2.

Description of *Bisgaardia* gen. nov.

Bisgaardia (Bis.ga.ar'di.a. N.L. fem. n. *Bisgaardia* to honour Magne Bisgaard, a contemporary Danish microbiologist, for his contributions to the taxonomy of the family *Pasteurellaceae*).

Cells consist of pleomorphic, Gram-negative-staining rods. Non-motile and facultatively anaerobic. Neither X nor V factors are required for growth. Does not grow on MacConkey agar. Catalase-positive and oxidase-positive. Voges–Proskauer reaction and tests for arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase, urease and indole are negative. Nitrate is reduced to nitrite. Acid is produced from D-glucose, D-galactose, D-mannitol, D-mannose, D-sorbitol, sucrose and trehalose, but not from

Fig. 1. Combined tree based on 16S rRNA, *rpoB*, *infB* and *recN* gene sequences. *Escherichia coli* was included as an outgroup to root the tree. The distance matrix was calculated using Jukes–Cantor correction and the tree was built using neighbour-joining in Bionumerics v.5.1. Cophenic correlations are given, indicating the reliability of the branching compared with the actual genetic relatedness of the taxa. The accession numbers of the gene sequences used for tree construction are listed in the individual trees (see Supplementary Figs S1–S4 in IJSEM Online). Bar, 4 % sequence divergence.

Table 1. Genetic similarity of *Bisgaardia hudsonensis* gen. nov., sp. nov. to the type species of genera of the family *Pasteurellaceae* calculated based on *recN* gene sequences

Strain	<i>Bisgaardia hudsonensis</i> M327/99/2 ^T
<i>Bisgaardia</i> genomospecies 1 M1765/96/5	0.69
<i>Actinobacillus ligneresii</i> NCTC 4189 ^T	0.04
<i>Pasteurella multocida</i> subsp. <i>multocida</i> CCUG 17976 ^T	0.22
<i>Haemophilus influenzae</i> Rd KW20	0.26
<i>Mannheimia haemolytica</i> NCTC 9380 ^T	0.10
<i>Lonepinella koalarum</i> ATCC 700131 ^T	0.24
<i>Phocoenobacter uteri</i> NCTC 12872 ^T	0.11
<i>Gallibacterium anatis</i> CCUG 15563 ^T	0.01
<i>Volucrobacter psittacidia</i> JEO101	0.20
<i>Histophilus somni</i> 8025 ^T	0.32
<i>Avibacterium gallinarum</i> NCTC 11188 ^T	0.22
<i>Nicoletella semolina</i> CCUG 43639 ^T	0.05
<i>Bibersteinia trehalosi</i> NCTC 10370 ^T	0.08
<i>Aggregatibacter actinomycetemcomitans</i> HK1651	0.19
<i>Chelonobacter oris</i> 1662 ^T	0.00
<i>Basfia succiniciproducens</i> DSM 22022 ^T	0.21

D-xylose. Alkaline phosphatase and α -fucosidase are produced in large amounts, but α -glucosidase is not produced. The G + C content of the DNA is 39.5 mol%. Isolated from lungs and other tissues of seals. Phenotypic tests that separate the genus *Bisgaardia* from the other members of the family *Pasteurellaceae* are given in Table 2. The type species is *Bisgaardia hudsonensis*.

Description of *Bisgaardia hudsonensis* sp. nov.

Bisgaardia hudsonensis (hud.so.nen'sis. N.L. fem. adj. *hudsonensis* from Hudson Bay, the area of Canada where the strains originated).

Colonies on CSBA are circular, entire, low convex, smooth, grey and attain a diameter of 0.5 mm after 48 h of aerobic incubation at 37 °C. Non-haemolytic. Growth occurs on media without blood or serum. Growth occurs at 22 °C, but not at 42 °C. In addition to characteristics for the genus, acid is produced from *myo*-inositol and maltose, but not from adonitol, dulcitol, lactose, melibiose, raffinose or L-rhamnose. Leucine arylamidase and acid phosphatase are produced in large amounts and naphthol-AS-BI-phosphohydrolase is produced in lesser amounts. Valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, phosphoamidase, α -galactosidase, β -galactosidase, β -glucosidase,

Table 2. Key characteristics for differentiation of *Bisgaardia* gen. nov. from other genera of the family *Pasteurellaceae*

Genera: 1, *Actinobacillus sensu stricto* (data from Christensen & Bisgaard, 2004); 2, *Pasteurella sensu stricto* (Mutters *et al.*, 1985); 3, *Haemophilus sensu stricto* (includes *H. influenzae*, *H. haemolyticus* and *H. aegypticus* – results for *H. parainfluenzae* and *H. pittmaniae* are given in square brackets) (Nørskov-Lauritsen *et al.*, 2005); 4, *Mannheimia* (Angen *et al.*, 1999); 5, *Lonepinella* (Osawa *et al.*, 1995); 6, *Phocoenobacter* (Foster *et al.*, 2000); 7, *Gallibacterium* (Christensen *et al.*, 2003; Bisgaard *et al.*, 2009); 8, *Volucrobacter* (Christensen *et al.*, 2004); 9, *Histophilus* (Angen *et al.*, 2003); 10, *Avibacterium* (Blackall *et al.*, 2005); 11, *Nicoletella* (Kuhnert *et al.*, 2004); 12, *Bibersteinia* (Blackall *et al.*, 2007); 13, *Aggregatibacter* (Patel *et al.*, 2004; Nørskov-Lauritsen & Kilian, 2006); 14, *Chelonobacter* (Gregersen *et al.*, 2009); 15, *Basfia* (Kuhnert *et al.*, 2010); 16, *Bisgaardia* gen. nov. (this study). +, Positive; –, negative; v, variable; w, weak positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Haemolysis	v	–	v	v	–	–	+	–	v	–	–	+	v	+	–	–
Catalase	v	+	v	v	–	–	+	v	–	v	+	v	v	+	–	+
Urease	+	–*	+	[v]	–	–	–	–	–	–	+	–	–	–	–	–
Oxidase	+	+	+	+	–	+	+	v	+	+	+	v	–	+	+	+
Indole	–	+	v	–	–	–	–	–	+	–	–	–	–	–	–	–
D-Galactose	v	+	+	+	ND	ND	+	+	ND	v	–	–	v	ND	+	+
D-Mannitol	+†	v	–	+	–	–	+	–	ND	v	–	+	v	+	+	+
D-Mannose	v	+	–	[+]	–	+	+	+	ND	+	–	+	v	+	+	+
D-Sorbitol	v	v	–	v	–	–	v	–	v	v	–	+	–	–	+	+
Sucrose	+	+	–	[v]	+	v	–	+	–	+	–	+	v	+	+	+
Trehalose	v	v	–	–	–	–	v	–	–	v	–	+	v	+	+	+
D-Xylose	+‡	–§	v	+	ND	–	+	–	–	–	–	–	v	+	+	–
α -Fucosidase	–	–	–	v	–	ND	–	–	ND	–	ND	–	ND	–	ND	+
α -Glucosidase	v	+	–	–	–	ND	+	–	ND	+	ND	v	v	–	–	–

**Pasteurella dagmatis* positive.

†*Actinobacillus suis* negative.

‡*Actinobacillus ureae* negative.

§*Pasteurella multocida* variable.

β -glucuronidase, β -glucosaminidase, α -mannosidase, esterase (C4), esterase lipase (C8) and lipase (C14) are not produced. Using the Vitek 2 system, positive reactions are obtained for arginine arylamidase, leucine arylamidase, phenylalanine arylamidase, phosphorylcholine, D-galactose, D-mannose, phosphatase and phenylphosphonate. Negative reactions using Vitek 2 are obtained for adonitol, L-arabitol, Ala-Phe-Pro-arylamidase, maltotriose, β -galactopyranosidase, β -galactosidase, D-malate, L-pyrrolydonyl arylamidase, β -N-acetylglucosaminidase, γ -glutamyltransferase, glutamyl-arylamidase, β -glucosidase, β -xylosidase, β -alanine arylamidase, L-proline arylamidase, lipase, palatinose, citrate, L-malate, urease, H₂S, D-tagatose, malonate, L-lactate alkalization, succinate alkalization, β -N-acetylgalactosaminidase, glycine arylamidase, lysine decarboxylase, ornithine decarboxylase, L-histidine, β -glucuronidase, Glu-Gly-Arg-arylamidase, pyruvate, L-glutamine, cellobiose, 5-keto-D-gluconate, α -galactosidase and coumarate. Variable reactions were observed with α -arabinosidase, N-acetyl-D-glucosamine, D-ribose, tyrosine arylamidase, starch (glycogen), L-lysine arylamidase and Ellman's reagent.

The type strain is M327/99/2^T (=CCUG 43067^T=NCTC 13475^T=98-D-690B^T). Isolated from ringed seals. Pathological significance is unknown.

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References

- Angen, Ø., Muttters, R., Caugant, D. A., Olsen, J. E. & Bisgaard, M. (1999). Taxonomic relationships of the [*Pasteurella*] *haemolytica* complex as evaluated by DNA–DNA hybridizations and 16S rRNA sequencing with proposal of *Mannheimia haemolytica* gen. nov., comb. nov., *Mannheimia granulomatis* comb. nov., *Mannheimia glucosida* sp. nov., *Mannheimia ruminantis* sp. nov. and *Mannheimia varigena* sp. nov. *Int J Syst Bacteriol* **49**, 67–86.
- Angen, Ø., Ahrens, P., Kuhnert, P., Christensen, H. & Muttters, R. (2003). Proposal of *Histophilus somni* gen. nov., sp. nov. for the three species *incertae sedis* 'Haemophilus somnus', 'Haemophilus agni' and 'Histophilus ovis'. *Int J Syst Evol Microbiol* **53**, 1449–1456.
- Baker, J. R. & Ross, H. M. (1992). The role of bacteria in phocine distemper. *Sci Total Environ* **115**, 9–14.
- Bisgaard, M. (1993). Ecology and significance of *Pasteurellaceae* in animals. *Zentralbl Bacteriol* **279**, 7–26.
- Bisgaard, M., Christensen, J. P., Bojesen, A. M. & Christensen, H. (2007). *Avibacterium endocarditidis* sp. nov., isolated from valvular endocarditis in chickens. *Int J Syst Evol Microbiol* **57**, 1729–1734.
- Bisgaard, M., Korczak, B. M., Busse, H. J., Kuhnert, P., Bojesen, A. M. & Christensen, H. (2009). Classification of the taxon 2 and taxon 3 complex of Bisgaard within *Gallibacterium* and description of *Gallibacterium melopsittaci* sp. nov., *Gallibacterium trehalosifermentans* sp. nov. and *Gallibacterium salpingitidis* sp. nov. *Int J Syst Evol Microbiol* **59**, 735–744.
- Blackall, P. J., Christensen, H., Beckenham, T., Blackall, L. L. & Bisgaard, M. (2005). Reclassification of *Pasteurella gallinarum*, [*Haemophilus*] *paragallinarum*, *Pasteurella avium* and *Pasteurella volantium* as *Avibacterium gallinarum* gen. nov., comb. nov., *Avibacterium paragallinarum* comb. nov., *Avibacterium avium* comb. nov. and *Avibacterium volantium* comb. nov. *Int J Syst Evol Microbiol* **55**, 353–362.
- Blackall, P. J., Bojesen, A. M., Christensen, H. & Bisgaard, M. (2007). Reclassification of [*Pasteurella*] *trehalosi* as *Bibersteinia trehalosi* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **57**, 666–674.
- Christensen, H. & Bisgaard, M. (2004). Revised definition of *Actinobacillus sensu stricto* isolated from animals. A review with special emphasis on diagnosis. *Vet Microbiol* **99**, 13–30.
- Christensen, H. & Bisgaard, M. (2008). Taxonomy and biodiversity of members of *Pasteurellaceae*. In *Pasteurellaceae: Biology, Genomics and Molecular Aspects*, pp. 1–26. Edited by P. Kuhnert & H. Christensen. Norwich, UK: Caister Academic Press.
- Christensen, H., Bisgaard, M., Bojesen, A. M., Muttters, R. & Olsen, J. E. (2003). Genetic relationships among avian isolates classified as *Pasteurella haemolytica*, '*Actinobacillus salpingitidis*' or *Pasteurella anatis* with proposal of *Gallibacterium anatis* gen. nov., comb. nov. and description of additional genomospecies within *Gallibacterium* gen. nov. *Int J Syst Evol Microbiol* **53**, 275–287.
- Christensen, H., Bisgaard, M., Aalbak, B. & Olsen, J. E. (2004). Reclassification of Bisgaard taxon 33, with proposal of *Volucrobacter psittacidia* gen. nov., sp. nov. and *Volucrobacter amazonae* sp. nov. as new members of the *Pasteurellaceae*. *Int J Syst Evol Microbiol* **54**, 813–818.
- Foster, G., Ross, H. M., Malnick, H., Willems, A., Garcia, P., Reid, R. J. & Collins, M. D. (1996). *Actinobacillus delphinicola* sp. nov., a new member of the family *Pasteurellaceae* Pohl (1979) 1981 isolated from sea mammals. *Int J Syst Bacteriol* **46**, 648–652.
- Foster, G., Ross, H. M., Patterson, I. A. P., Hutson, R. A. & Collins, M. D. (1998). *Actinobacillus scotiae* sp. nov., a new member of the family *Pasteurellaceae* Pohl (1979) 1981 isolated from porpoises (*Phocoena phocoena*). *Int J Syst Bacteriol* **48**, 929–933.
- Foster, G., Ross, H. M., Malnick, H., Willems, A., Hutson, R. A., Reid, R. J. & Collins, M. D. (2000). *Phocobacter uteri* gen. nov., sp. nov., a new member of the family *Pasteurellaceae* Pohl (1979) 1981 isolated from a harbour porpoise (*Phocoena phocoena*). *Int J Syst Evol Microbiol* **50**, 135–139.
- Frederiksen, W. (1993). Ecology and significance of *Pasteurellaceae* in man – an update. *Zentralbl Bacteriol* **279**, 27–34.
- Garvie, E. I. (1978). *Streptococcus raffinolactis* Orla-Jensen and Hansen, a group N streptococcus found in raw milk. *Int J Syst Bacteriol* **28**, 190–193.
- Gregersen, R. H., Neubauer, C., Christensen, H., Bojesen, A. M., Hess, M. & Bisgaard, M. (2009). Comparative studies on [*Pasteurella*] *testudinis* and [*P.*] *testudinis*-like bacteria and proposal of *Chelonobacter oris* gen. nov., sp. nov. as a new member of the family *Pasteurellaceae*. *Int J Syst Evol Microbiol* **59**, 1583–1588.
- Korczak, B., Christensen, H., Emler, S., Frey, J. & Kuhnert, P. (2004). Phylogeny of the family *Pasteurellaceae* based on *rpoB* sequences. *Int J Syst Evol Microbiol* **54**, 1393–1399.
- Kuhnert, P. & Korczak, B. M. (2006). Prediction of whole-genome DNA–DNA similarity, determination of G+C content and phylogenetic analysis within the family *Pasteurellaceae* by multilocus sequence analysis (MLSA). *Microbiology* **152**, 2537–2548.
- Kuhnert, P., Frey, J., Lang, N. P. & Mayfield, L. (2002). Phylogenetic analysis of *Prevotella nigrescens*, *Prevotella intermedia* and *Porphyromonas gingivalis* clinical strains reveals a clear species clustering. *Int J Syst Evol Microbiol* **52**, 1391–1395.
- Kuhnert, P., Korczak, B., Falsen, E., Straub, R., Hoops, A., Boerlin, P., Frey, J. & Muttters, R. (2004). *Nicoletella semolina* gen. nov., sp. nov., a

new member of *Pasteurellaceae* isolated from horses with airway disease. *J Clin Microbiol* **42**, 5542–5548.

Kuhnert, P., Korczak, B. M., Christensen, H. & Bisgaard, M. (2007). Emended description of *Actinobacillus capsulatus* Arseculeratne 1962, 38^{AL}. *Int J Syst Evol Microbiol* **57**, 625–632.

Kuhnert, P., Scholten, E., Haefner, S., Mayor, D. & Frey, J. (2010). *Basfia succiniciproducens* gen. nov., sp. nov., a new member of the family *Pasteurellaceae* isolated from bovine rumen. *Int J Syst Evol Microbiol* **60**, 44–50.

Kuiken, T. & Hartmann, M. G. (1991). Cetacean pathology: dissection techniques and tissue sampling. *ECS Newsletter* No. 17 (special issue).

Mayor, D., Korczak, B. M., Christensen, H., Bisgaard, M., Frey, J. & Kuhnert, P. (2006). Distribution of RTX toxin genes in strains of [*Actinobacillus*] *rossii* and [*Pasteurella*] *mairii*. *Vet Microbiol* **116**, 194–201.

Mutters, R., Ihm, P., Pohl, S., Frederiksen, W. & Mannheim, W. (1985). Reclassification of the genus *Pasteurella* Trevisan 1887 on the basis of deoxyribonucleic acid homology, with proposals for the new species *Pasteurella dagmatis*, *Pasteurella canis*, *Pasteurella stomatis*, *Pasteurella anatis* and *Pasteurella langaa*. *Int J Syst Bacteriol* **35**, 309–322.

Nørskov-Lauritsen, N. & Kilian, M. (2006). Reclassification of *Actinobacillus actinomycetemcomitans*, *Haemophilus aphrophilus*, *Haemophilus paraphrophilus* and *Haemophilus segnis* as *Aggregatibacter actinomycetemcomitans* gen. nov., comb. nov., *Aggregatibacter aphrophilus* comb. nov. and *Aggregatibacter segnis* comb. nov., and emended description of *Aggregatibacter aphrophilus* to include V factor-dependent and V factor-independent isolates. *Int J Syst Evol Microbiol* **56**, 2135–2146.

Nørskov-Lauritsen, N., Bruun, B. & Kilian, M. (2005). Multilocus sequence phylogenetic study of the genus *Haemophilus* with description of *Haemophilus pittmaniae* sp. nov. *Int J Syst Evol Microbiol* **55**, 449–456.

Osawa, R., Rainey, F., Fujisawa, T., Lang, E., Busse, H. J., Walsh, T. P. & Stackebrandt, E. (1995). *Lonepinella koalarum* gen. nov., sp. nov., a new tannin-protein complex degrading bacterium. *Syst Appl Microbiol* **18**, 368–373.

Patel, S. M., Mo, J. H., Walker, M. T., Adley, B. & Noskin, G. A. (2004). Epidural abscess and osteomyelitis due to *Actinobacillus actinomycetemcomitans*. *Diagn Microbiol Infect Dis* **50**, 283–285.

Stackebrandt, E. & Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* **33**, 152–155.