Antonie van Leeuwenhoek (2014) 105:731–741
DOI 10.1007/s10482-014-0129-0

ORIGINAL PAPER

Frederiksenia canicola gen. nov., sp. nov. isolated from dogs and human dog-bite wounds

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Received: 19 November 2013 / Accepted: 28 January 2014 / Published online: 8 February 2014

Abstract Polyphasic analysis was done on 24 strains of Bisgaard taxon 16 from five European countries and mainly isolated from dogs and human dog-bite wounds. The isolates represented a phenotypically and genetically homogenous group within the family *Pasteurellaceae*. Their phenotypic profile was similar to members of the genus *Pasteurella*. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry clearly identified taxon 16 and separated it from all other genera of *Pasteurellaceae* showing a characteristic peak combination. Taxon 16 can be further separated and identified by a RecN protein signature sequence detectable by a specific PCR. In all phylogenetic analyses based on 16S rRNA, *rpoB*, *infB* and *recN* genes, taxon 16 formed a monophyletic branch with intraspecies sequence similarity of at least 99.1, 90.8, 96.8 and 97.2 %, respectively. Taxon 16 showed closest genetic relationship with *Bibersteinia trehalosi* as to the 16S rRNA gene (95.9 %), the *rpoB* (89.8 %) and the *recN* (74.4 %), and with *Actinobacillus lignieresii* for *infB* (84.9 %). Predicted genome similarity values based on the *recN* gene sequences between taxon 16 isolates and the type strains of known genera of *Pasteurellaceae* were below the genus level. Major whole cell fatty acids for the strain HPA 21^T are $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ and $C_{16:1}$ ω7c/$C_{15:0}$ iso 2OH. Major respiratory quinones are menaquinone-8, ubiquinone-8 and demethylmenaquinone-8. We propose to classify these organisms as a novel genus and species within the family of *Pasteurellaceae* named *Frederiksenia canicola* gen. nov., sp. nov. The type strain is HPA 21^T (= CCUG 62410^T = DSM 25797^T).

Keywords Bisgaard taxon 16 · Pasteurellaceae · Taxonomy · Phylogeny · Zoonosis

Electronic supplementary material The online version of this article (doi:10.1007/s10482-014-0129-0) contains supplementary material, which is available to authorized users.

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Introduction

Presently, the family *Pasteurellaceae* is one of the largest bacterial families, with many taxa still awaiting proper classification (Christensen and Bisgaard 2008; Gregersen et al. 2009; Kuhnert et al. 2010; Foster et al. 2011; Christensen et al. 2011; Hansen et al. 2012). Some members of the *Pasteurellaceae* are frequently

found in the oral cavity and upper respiratory tract of companion animals such as dogs and cats (Christensen and Bisgaard 2008). They are mainly considered commensals, however, under certain circumstances they may also act as opportunistic pathogens. Species obtained from dogs and cats include *Pasteurella multocida*, *Pasteurella dagmatis*, *Pasteurella stomatis* and *Pasteurella oralis*. Two additional species are more restricted in their host-specificity, *Pasteurella canis* with dogs and [*Haemophilus*] *felis* with cats (Mutters et al. 1985; Inzana et al. 1992; Christensen et al. 2012). In humans the aforementioned species may cause wound infections inflicted by dog- or cat bites/scratches (Abrahamian and Goldstein 2011).

Correct classification of these taxa has major impact on an unambiguous identification and is essential for proper medical treatment of patients, the development of preventive measures and the performance of epidemiological studies. Identification of these taxa, however, can be problematic as additional *Pasteurella*-like organisms have been reported from the same niche (Saphir and Carter 1976; Bisgaard and Mutters 1986; Ganiere et al. 1993; Muhairwa et al. 2001; Forsblom et al. 2002). A group of bacteria, tentatively named Bisgaard taxon 16, shows a phenotype, mol% G+C in DNA, genome size and cellular fatty acid composition similar to the genus *Pasteurella* and might be misidentified as *P. canis*, *P. stomatis* or *P. dagmatis* (Bisgaard and Mutters 1986; Forsblom et al. 2002). On the other hand, analysis of data provided by the DNA–DNA and DNA-rRNA hybridization (De Ley et al. 1990; Bisgaard and Mutters 1986), 16S rRNA sequencing (Olsen et al. 2005) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology (Kuhnert et al. 2012) showed that this taxon differed considerably from the other known genera within the family and should be classified as a new genus.

Using a polyphasic approach we investigated 24 isolates of taxon 16 that were diverse in geographical location and host/tissue source. Based on phenotypic, genetic, as well as phylogenetic characteristics, including biochemistry, chemotaxonomy, MALDI-TOF MS, amino-acid signature specific PCR, multilocus sequence analysis (MLSA), and *recN*-derived genome similarity data, we propose classification of Bisgaard taxon 16 as *Frederiksenia canicola* gen. nov., sp. nov., a new genus within the family of *Pasteurellaceae*. The type strain is HPA 21^T (= CCUG 62410^T = DSM 25797^T also deposited under the number CCUG 36444^T = Him932-7^T).

Materials and methods

Bacterial strains and phenotypic characterization

Most of the 24 strains investigated were obtained from dog, mainly from the upper respiratory tract or vagina (Table 1). Clinical cases included rhinitis, tracheitis, chronic tracheobronchitis, facial swelling and genital tract infection. Four strains were isolated from humans, three of them from dog-bite wounds while single isolates were obtained from cat, lion, hedgehog and banded mongoose (domesticated or zoo animals). Primary phenotypic characterization identified them as taxon 16 or atypical *P. canis*, *P. stomatis*, *P. dagmatis* or *Pasteurella* sp. All strains had been kept frozen at −80 °C for further investigation. The bacteria were subcultivated from frozen stocks on tryptone soya agar plates with sheep blood (TSA; Oxoid, Pratteln, Switzerland) for 24 h at 37 °C in an aerobic atmosphere. A number of different biochemical tests recommended for characterization of the phenotype of members of the *Pasteurellaceae* family were applied to all isolates as proposed in the "minimal standards" for the family (Christensen et al. 2007).

Chemotaxonomy, MALDI-TOF MS and amino acid signature specific PCR

Analysis of fatty acids and respiratory quinones of the strain HPA 21^T were carried out by the Identification Service of the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen), Braunschweig, Germany. The analysis of whole cell fatty acid content was done using the Sherlock Microbial Identification System (MIS) (MIDI, Microbial ID, Newark, DE 19711, USA) according to the previously published protocols (Miller 1982; Kuykendall et al. 1988; Kampfer and Kroppenstedt 1996). Analysis of respiratory quinones for the strain HPA 21^T were performed according to published protocols (Tindall 1990a, b).

MALDI-TOF MS was performed according to Kuhnert et al. (2012). *Frederiksenia canicola* (taxon 16) specific peaks were determined in Biotyper 3.0 software (Bruker Daltonik GmbH, Bremen, Germany). For diagnostic identification the direct plating

Table 1 *Frederiksenia canicola* (Bisgaard taxon 16) strains investigated

	Strain No.	Country	Initial phenotypic identification	Isolated from	Clinical signs	GenBank accession no.			
						16S rRNA	*infB*	*recN*	*rpoB*
1	HPA 21^T = CCUG 62410^T = DSM 25797^T also known as CCUG 36444^T and Him932-7^T	Denmark	*Pasteurella* sp.	Dog, pharynx	Healthy dog	JQ356598	JQ356622	JQ356650	JQ356678
2	Smith392 = P919 = CCUG 17204	UK	*Pasteurella* sp.	Dog	Facial swelling	JQ356599	JQ356623	JQ356651	JQ356679
3	M2500/96/3	UK	N.a.	Dog, eye	N.a.	JQ356600	JQ356624	JQ356652	JQ356680
4	D1949_98 = JF2157	Switzerland	*P. canis*	Dog, trachea	Trachitis	JQ356601	JQ356625	JQ356653	JQ356681
5	D2018_98	Switzerland	*P. canis*	Dog, nose	Rhinitis	JQ356602	JQ356626	JQ356654	JQ356682
6	D2941_98_JF2198	Switzerland	*Pasteurella* sp.	Dog, nose	Rhinitis	JQ356603	JQ356627	JQ356655	JQ356683
7	D227_99 = JF2221	France	*P. canis*	Dog, tracheo bronchial ichor	Chronical tracheobronchitis	JQ356604	JQ356628	JQ356656	JQ356684
8	D262_99 = JF2223	Switzerland	*P. canis*	Dog, nose	Rhinitis	JQ356605	JQ356629	JQ356657	JQ356685
9	D452-3_99 = JF2240	Switzerland	*P. canis*	Dog, nose	N.a.	JQ356606	JQ356630	JQ356658	JQ356686
10	D536-2_99 = JF2247	Switzerland	*P. canis*	Dog, tonsil	Chronical cough and tonsillitis	JQ356607	JQ356631	JQ356659	JQ356687
11	D597-2_99 = JF2247	Switzerland	*P. canis*	Dog, vagina	Brown discharge	JQ356608	JQ356632	JQ356660	JQ356688
12	D1071_99	Switzerland	*P. canis*	Dog, vagina	N.a.	JQ356609	JQ356633	JQ356661	JQ356689
13	KM1266_04 = JF4823	Switzerland	*Pasteurella* sp.	Dog, vagina	N.a.	JQ356610	JQ356634	JQ356662	JQ356690
14	KM1549_04	Switzerland	*P. stomatis*	Dog	N.a.	JQ356611	JQ356635	JQ356663	JQ356691
15	KM1721_06	Switzerland	*Pasteurella* sp.	Dog, nose	Rhinitis	JQ356612	JQ356636	JQ356664	JQ356692
16	KM555_08 = JF4826	Switzerland	*Pasteurella* sp.	Dog, nose	Rhinitis	JQ356613	JQ356637	JQ356665	JQ356693
17	CCUG 22043	Sweden		Human, nasopharynx	N.a.	JQ356614	JQ356638	JQ356666	JQ356694
18	P987	Denmark	*P. dagmatis*	Human	Dog-bite wound	JQ356615	JQ356639	JQ356667	JQ356695
19	P988	Denmark	*P. dagmatis*	Human	Dog-bite wound	JQ356616	JQ356640	JQ356668	JQ356696
20	P989	Denmark	*P. dagmatis*	Human	Dog-bite wound	JQ356617	JQ356641	JQ356669	JQ356697
21	HPA 172 = CCUG 17205	Denmark	*Pasteurella* sp.	Cat, pharynx	No lesions	JQ356618	JQ356642	JQ356670	JQ356698
22	T1A Past	Denmark	N.a.	Lion	No lesions	JQ356619	JQ356643	JQ356671	JQ356699
23	F21_hedgehog9	Denmark	N.a.	Hedgehog	No lesions	JQ356620	JQ356644	JQ356672	JQ356700
24	F12_denscaninum	Denmark	N.a.	Banded mongoose	No lesions	JQ356621	JQ356645	JQ356673	JQ356701

N.a. data not available

method was applied on the Bruker Microflex LT (Bruker Daltonik GmbH) using an emended Biotyper 3.0 database (Kuhnert et al. 2012).

For further identification of *F. canicola* and especially discrimination from other *Pasteurellaceae* isolated from dogs and cats a PCR based on a specific signature sequence in the *recN* gene was developed. Primers recN_Fred-1 CCACGCTCTATCAAACTATTCG and recN_first-R CCRCTAATYCCMACATCNACYTCATC amplifying a 749 bp fragment were used for the *F. canicola* specific PCR. A control PCR amplifying a 1394 base-pair fragment from other members of *Pasteurellaceae* can be included by addition of the primer recN_first-L ATGCTTANYCAWCTYACKATYAATMATTTTGC. The PCR can be used as single or as a multiplex PCR with the following conditions. The 30 µl PCR contained 0.4 µM of each primer, 1x FIREPol® Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia) and 1 µl of bacterial lysate (approximately 30 ng DNA). The lysate was prepared by dissolving a few bacterial colonies in 450 µl of lysis buffer (0.1 M Tris–HCl, pH 8.5, 0.05 % Tween-20, 240 µg/ml proteinase K) incubated at 60 °C for 1 h and heat inactivated at 94 °C for 15 min. The PCR was run in a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: 3 min of initial denaturation at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 50 °C, 1 min at 72 °C, and a final extension step of 7 min at 72 °C. The specific size of the PCR the amplicons were checked on a 1.5 % agarose gel.

Phylogenetic analyses

The partial sequences of the 16S rRNA, *rpoB* (encoding beta subunit of the DNA-dependent polymerase), *infB* (encoding translation initiation factor 2), and *recN* (encoding DNA repair protein) genes were generated for all 24 isolates of *F. canicola* according to previously described protocols (Kuhnert et al. 2002; Korczak et al. 2004; Kuhnert et al. 2004; Mayor et al. 2006). In contrast to the published protocols the PCRs were simplified by using FIREPol® Master Mix Ready to Load (Solis BioDyne). The 16S rRNA and *rpoB* gene fragments were amplified in a multiplex PCR using 16SUNI-L, 16SUNI-R, PasrpoB-L and RPOB-R primers (Online Resource Table S1). The *infB* fragments were obtained using infB-L and infB-R or infB-R1 primers. The *recN* was amplified using primers recN_first-L and recN_first-R. The annealing temperature for *recN* was 50 °C and for the other targets 54 °C. To check their quality the PCRs products were run on a 1.5 % agarose gel. Ten microliters of each amplicon were purified from residual deoxynucleotides and primers by adding 2.0 µl of rAPid Alkaline Phosphatase (1 U/µl; Roche Diagnostics, Rotkreuz, Switzerland), 0.4 µl of the corresponding buffer and 0.1 µl of exonuclease I (*Exo*I; New England Biolabs, Ipswich, MA, USA) and incubation at 37 °C for 20 min and subsequently at 80 °C for 20 min to inactivate the enzymes. In addition to *F. canicola* strains *infB*, *recN* and *rpoB* sequences from type strains of *Otariodibacter oris* Baika1^T (= CCUG 59994^T), *infB* and *recN* from *P. dagmatis* CCUG 12397^T, *P. stomatis* CCUG 17979^T and [*H.*] *felis* ATCC 49733^T, as well as 16S rRNA, *infB* and *recN* from *P. oralis* P683^T (= CCUG 19794^T) and finally *infB* from *Necropsobacter rosorum* CCUG 28028^T and *recN* from *Volucribacter psittacicida* ATCC 47536^T were also determined in this study.

Primers used for PCRs were also applied for sequencing (Online Resource Table S1). Internal primers for 16S rRNA and *infB* were universal for all strains while species specific primers were designed for *recN*. Five pmol of the appropriate primer was added to about 20 ng (1.0 µl) of purified PCR product and sequenced with the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) in a thermocycler with 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 1 min. After ethanol precipitation of the sequencing products, the samples were run on an ABI Prism 3130*xl* genetic analyser (Applied Biosystems). The sequences were edited using the Sequencher software version 5.0 (Gene Code Corporation, Ann Arbor, MI, USA).

BioNumerics version 6.6 (Applied Maths NV, Sint-Martens-Latem, Belgium) was used for phylogenetic analysis and calculating sequence similarity between the strains. Sequences obtained were submitted to GenBank (www.ncbi.nlm.nih.gov). The accession numbers of the sequences of investigated *F. canicola* isolates are given in Table 1 and for the other strains they are indicated in the Fig. 2 for 16S rRNA and in the Online Resource Figs. S2 to S4 for other target genes.

The *recN* sequences were further used for prediction of genome similarity values between the investigated strains and the type strains of type species of all

Table 2 Key characters for differentiation of genera within the family *Pasteurellaceae*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
β-haemolysis	−	d	−	−	−	d	−	+	d	−	−	−	−	d	+	−	−	−	n.a.
V-factor, β-NAD requirement	−	+ [d]	−[a]	−[b]	−	−	−	−	−	−	−	+[c]	d	−	−	−	−	−	−
X-factor, porphyrin requirement	−	+ [−]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Methyl red	−	n.a.	−	−	−	−	n.a.	w	n.a.	+	n.a.	−	n.a.	−	n.a.	+	+	n.a.	n.a.
Voges Proskauer	−	n.a.	−	−	+	−	+	−	−	−	n.a.	−	n.a.	−	n.a.	−	−	−	+
Urease	−	+ [d]	+	−[d]	−	−	−	d	−	−	+	−	−	−	−	−	−	−	d
Indol	+	d	−	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Phosphatase	+	+	+	+	−	+	+	+	+	+/w	d	+[e]	+	+	w	+	n.a.	+	+
Oxidase	+	+	d	d	−	+	+	d	+	d	+	+	−/w	d	+	+	+	+	+
Catalase	+	d	d	+	−	d	−	d	−	d	+	d	d	d	+	−	+	+	+
Acid from																			
Dulcitol	−	−	−	d	+	−	−	d	n.a.	−	−	−	n.a.	−	+	−	+	−	−
(−)-D-fructose	+	− [d]	+	+	+	+	n.a.	+	−	+	−	+	+	+	+	+	+	n.a.	n.a.
Glucose	+	n.a.	n.a.	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
Glucose gas	−	n.a.	n.a.	−	n.a.	−	n.a.	−	−	−	−	−	n.a.	−	−	−	+	n.a.	−
Maltose	+	+	+	−[f]	+	d	−	d	−	d	−	d	+	+	+	+	+	+	+
(−)-D-mannitol	−	−	+[g]	−[h]	−	+	−	+	n.a.	−	−	d	d	+	+	+	−	+	−
(+)-D-mannose	+	− [+]	d	+	+	−	−	+	n.a.	+	−	+	d	+	+	+	+	+	−
Sucrose	+	− [d]	+	+	d	+	−	+	−	+	−	+	d	+	+	+	+	+	−
D-Sorbitol	−	n.a.	d	−[d]	n.a.	n.a.	n.a.	n.a.	n.a.	−	n.a.	d	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	−
(+)-D-xylose	−	d	+[i]	d	n.a.	+	−	+	−	d	−	−	d	−	+	+	+	−	−
Dextrin	+	n.a.	+	−[f]	(+)	d	n.a.	n.a.	n.a.	d	n.a.	d	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
ODC	−	d	−	+[j]	n.a.	d	−	−	−	−	−	−	−	−	−	−	−	−	−

Genera: 1, *Frederiksenia* (this study); 2, *Haemophilus* (includes *H. influenzae, H. haemolyticus* and *H. aegypticus* – results for *H. parainfluenzae* and *H. pittmaniae* are given in [] (Kroppenstedt and Mannheim 1989; Norskov-Lauritsen et al. 2005); 3, *Actinobacillus* (Kroppenstedt and Mannheim 1989; Christensen and Bisgaard 2004); 4, *Pasteurella* (Mutters et al. 1985; Kainz et al. 2000); 5, *Lonepinella* (Osawa et al. 1995); 6, *Mannheimia* (Kroppenstedt and Mannheim 1989; Angen et al. 1999); 7, *Phocoenobacter* (Foster et al. 2000); 8, *Gallibacterium* (Engelhard et al. 1991; Christensen et al. 2003); 9, *Histophilus* (Mutters et al. 1993; Angen et al. 2003); 10, *Volucribacter* (Christensen et al. 2004); 11, *Nicoletella* (Kuhnert et al. 2004); 12, *Avibacterium* (Kroppenstedt and Mannheim 1989; Blackall et al. 2005); 13, *Aggregatibacter* (Kroppenstedt and Mannheim 1989; Mutters et al. 1993; Norskov-Lauritsen and Kilian 2006); 14, *Bibersteinia* (Mutters et al. 1993; Blackall et al. 2007); 15, *Chelonobacter* (Gregersen et al. 2009); 16, *Basfia* (Kuhnert et al. 2010); 17, *Necropsobacter* (Christensen et al. 2011); 18, *Bisgaardia* (Foster et al. 2011; Hansen et al. 2012); 19, *Otariodibacter* (Hansen et al. 2012)

Discrepancies are indicated by: [a]*Actinobacillus pleuropneumoniae* biovar 1 positive; [b]*P. multocida* might be positive; [c]*Avibacterium gallinarum* negative; some isolates of *Avibacterium paragallinarum* also negative (biovar 2); [d]*P. dagmatis* positive; [e]*A. paragallinarum* biovar 1 might be negative; [f]*P. dagmatis* and *P. oralis* positive; [g]*Actinobacillus suis* negative; [h]*P. multocida* positive; [i]*Actinobacillus urea* negative; [j]*P. dagmatis* and *P. stomatis* negative + 90 % or more of the strains positive within 1–2 days; − less than 10 % of the strains positive within 14 days; *d* positive or negative; *w* weak positive; n.a. data not available

currently known genera within the family *Pasteurellaceae* as previously described (Kuhnert and Korczak 2006).

Results and discussion

All *F. canicola* isolates were non-haemolytic, but most of them showed co-haemolysis with the beta-sphingomyelinase of *Staphylococcus aureus* CCUG 4151 known as the CAMP effect (Christie et al. 1944). After 24 h of aerobic incubation on TSA plates the colonies were 1.5–2 mm in diameter with circular, slightly raised and regular shape and they did not adhere to the agar. Their surface was smooth, shiny and opaque with grayish or rarely yellowish tinge. The cells were Gram-negative, non-motile, pleomorphic straight or curved rods. The isolates showed a uniform biochemical profile that was in a good concordance with the results previously published for taxon 16 (Bisgaard and Mutters 1986).

One or more physiological and biochemical characters separate *F. canicola* from the other genera of the *Pasteurellaceae* (Table 2). In this respect the genus *Pasteurella* is most closely related to *F. canicola*. Production of acid from maltose and dextrin distinguish *F. canicola* from *P. multocida, P. canis* and *P. stomatis,* and ornithine decarboxylase, production of acid from (+)-D-xylose and dulcitol separate *P. oralis* from *F. canicola*, while urease separates *P. dagmatis* from *F. canicola. Frederiksenia canicola* can be phenotypically identified through the simple scheme developed by Dousse et al. (2008) including maltose as an additional test. To support diagnostic identification a specific amino-acid signature sequence in the DNA repair protein (RecN) discriminating *F. canicola* from from other members of *Pasteurellaceae* in particular those found in dogs and cats can be detected by PCR (Fig. 1). The phenotypic signature covers three conserved amino-acids that are, based on the *P. multocida* PM70 RecN sequence, L247T, A251T and Q253R (Fig. 1a). The PCR can be used as a single or a multiplex PCR that contains an amplification control in the absence of the *F. canicola* specific product. This consists of an additional primer that results in amplification of a larger *recN* PCR product in other *Pasteurellaceae* species. Besides its use as a control this PCR product has an additional diagnostic value when being sequenced thereby allowing also identification of species other that *F. canicola* (Kuhnert and Korczak 2006). To validate the PCR, all 24 *F. canicola* isolates investigated in the study (Table 1) as well as eight *P. multocida* strains (CCUG 17976^T, CCUG 17977^T, CCUG 17978^T, M139-04, 40KM283, 10KM754, 10OD1096, 10M2394), six *P. canis* strains (CCUG 12400^T, 10KM116-1, D1779-99, D1281-99, D2980-98, D2899-98), four *P. stomatis* (CCUG 17979^T, CCUG 36589, D488-99, D753-99), and four *P. dagmatis* (CCUG 12397^T, CCUG 33474, KM540-07, KM1126-01), in addition to five *P. oralis* strains (KM1603-05, KM770-05, OD1456-07, D1927-98, D842-99) were used. With all these strains the PCR resulted in fragments of expected sizes and allowed clear separation between *F. canicola* and species of the genus *Pasteurella* (Fig. 1b).

Based on reference spectra analysis of MALDI-TOF MS data described by Kuhnert et al. (2012) combination of peaks at 5,244, 6,304, 6,377, 8,305 and 9,436 m/z was found to be characteristic for *F.*

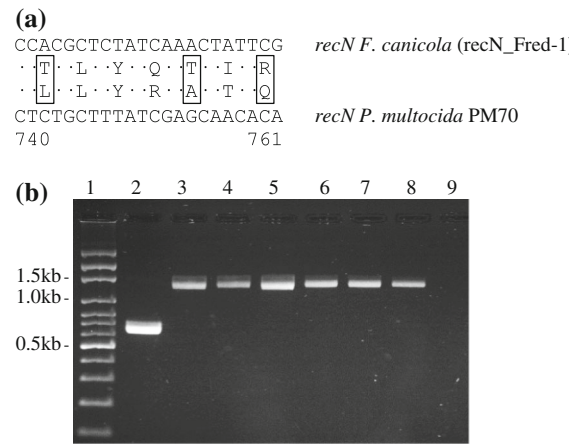

Fig. 1 *recN*-based identification of *Frederiksenia canicola* and differentiation from other members of *Pasteurellaceae* isolated from dogs and cats. **a** Signature sequence specific for identification of *F. canicola* in comparison to *Pasteurella sensu stricto*. The amino acids in brackets define conserved consensus amino acids in *F. canicola* and *Pasteurella sensu stricto*, respectively. **b** *F. canicola* specific PCR. The signature sequence can be detected by the primers recN_Fred-1/recN_first-R byPCR resulting in the 749 bp fragment. As an amplification control the additional primer recN-first-L was included in a multiplex PCR resulting in the 1,394 bp fragment for other species isolated from dogs and cats. *Lane 1* molecular weight standard; *lane 2 F. canicola* HPA 21^T; *lane 3 Pasteurella multocida* CCUG 17976^T; *lane 4 Pasteurella canis* CCUG 12400^T; *lane 5 Pasteurella stomatis* CCUG 17979^T; *lane 6 Pasteurella dagmatis* CCUG 12397^T; *lane 7 Pasteurella oralis* CCUG 19794^T; *lane 8* [*Haemophilus*] *felis* ATCC 49733^T; *lane 9* negative control

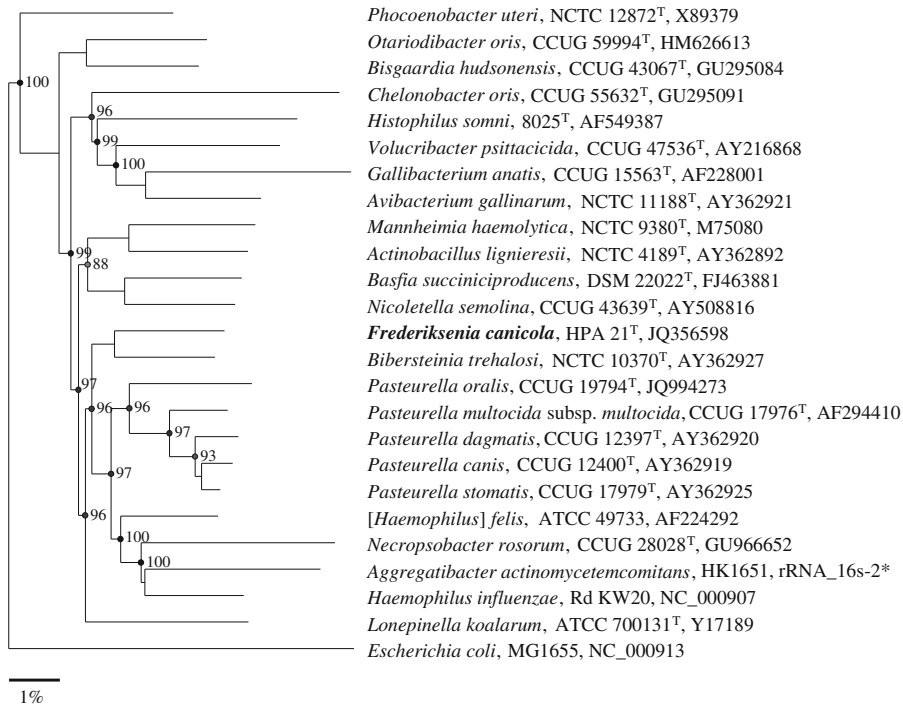

Fig. 2 Phylogenetic relationship of *Frederiksenia canicola* and other members of the family *Pasteurellaceae* based on Neighbor Joining tree of partial 16S rRNA sequences. *Escherichia coli* was included as an outgroup to root the dendrogram. The clustering was supported by cophenetic correlation. The scale bar indicates sequence differences between the taxa. *Gene sequence ID, Oralgen www.oralgen.lanl.gov

canicola (Fig. S1, available online). Fast and clear-cut identification of *F. canicola* and its separation from the other genera was possible with all 24 strains (Table 1) resulting in score values above 2.3 when using the emended Biotyper database for identification and they were in all cases below 2.0 with any other members of *Pasteurellaceae* (Kuhnert et al. 2012).

Major fatty acids for the strain HPA 21^T are $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and summed features $C_{16:1}$ ω7c/$C_{15:0}$ iso 2OH; minor are $C_{12:0}$ ALDE, $C_{12:0}$, $C_{12:0}$ 3OH, $C_{15:0}$, $C_{16:1}$ ω5c, $C_{17:0}$, $C_{18:2}$ ω6,9c/$C_{18:0}$ ANTE, $C_{18:1}$ ω7c, $C_{18:1}$ ω9c, $C_{20:0}$, $C_{20:1}$ ω7c, $C_{20:4}$ ω6,9,12,15c, and one unidentified fatty acid (Online Resource Table S2). These results were in accordance with observations previously made on other taxon 16 strains (Forsblom et al. 2002). Analysis of respiratory quinones for the strain HPA 21^T resulted in menaquinone-8 (MK-8), demethylmenaquinone 8 (DMK-8), ubiquinone-8 (Q-8) and minor amounts of ubiquinone-7 (Q-7). A comparison with other members is given in Online Resource Table S3 and *F. canicola* falls within the range of *Pasteurella*, *Phocoenobacter*, *Bisgaardia* and *Otariodibacter* all having chain length 8.

Phylogenetic analyses were performed using sequences of 16S rRNA gene, *rpoB*, *infB*, and *recN* all of which have been shown to be useful for establishing phylogenetic relationships within the *Pasteurellaceae* family (Korczak and Kuhnert 2008). Trees based on the alignment of sequences of individual target genes (Fig. 2; Online Resource Figs. S2–S4) as well as multilocus sequence analysis (MLSA) of the four concatenated genes (Fig. 3) placed *F. canicola* on a monophyletic, genus-like branch within the *Pasteurellaceae* confirming its relatedness to this family as a separate taxon in concordance with Olsen et al. (2005). 16S rRNA gene sequence comparison between the 24 strains of *F. canicola* showed a similarity of at least 99.1 %. The highest sequence similarity to other genera was to the type strain of the genus *Bibersteinia* with 95.9 %. Most of the 24 strains of *F. canicola* demonstrated *rpoB* similarities above 95.4 % within the group, confirming their close relatedness at species level. Two strains, HPA 172 isolated from a cat and D536-99 isolated from a dog, shared *rpoB* sequence and formed a separate branch within the *F. canicola* cluster in the phylogenetic tree (data not shown) but still closely

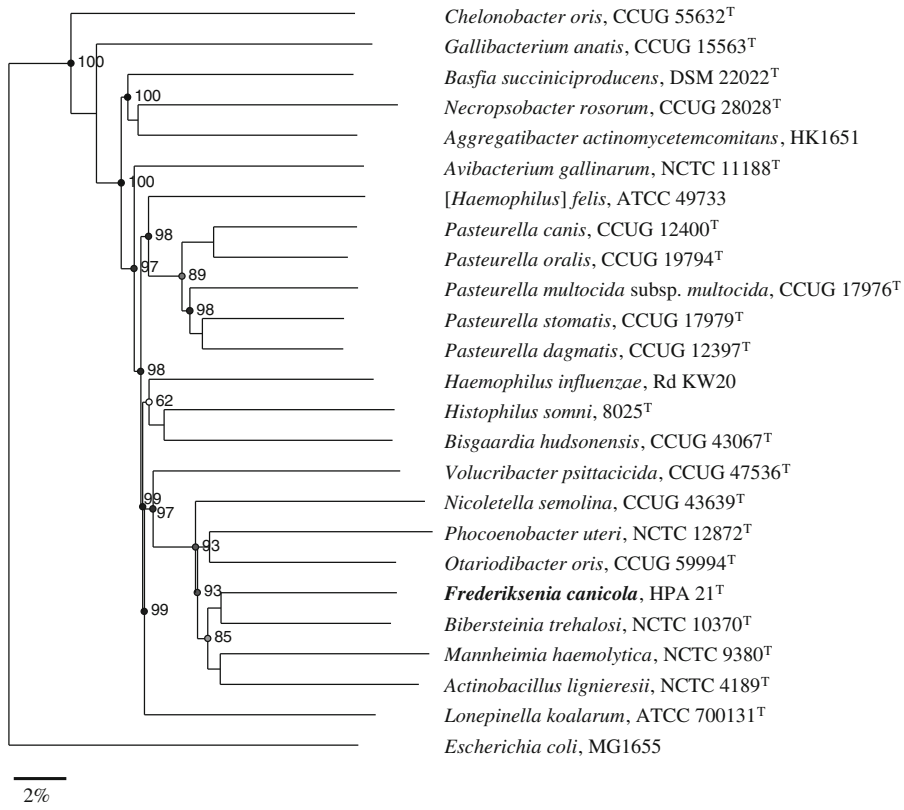

Fig. 3 Phylogenetic relationship of *Frederiksenia canicola* and other members of the family *Pasteurellaceae* based on Neighbor Joining tree of concatenated 16S rRNA, *infB*, *recN* and *rpoB* partial gene sequences. *Escherichia coli* was included as an outgroup to root the dendrogram. The reliability of the branching was supported by cophenetic correlation. The scale bar indicates the differences in concatenated sequences between the taxa

related to other *F. canicola* strains (90.8–92.5 % *rpoB* sequence similarity). Based on *rpoB* sequence similarity *F. canicola* showed the closest relatedness to genus *Bibersteinia* (89.8 %). Similarities of the *infB* sequences within *F. canicola* were higher than 96.8 % and did not exceed 84.9 % seen with *Actinobacillus lignieresii*. Finally, *recN*-based phylogeny also showed a clear separation of *F. canicola* from other genera with sequence similarities within the group of >97.2 %. The nearest sequence match of 74.4 % was again observed to genus *Bibersteinia*. Low similarities of *F. canicola* were observed when comparing the 16S rRNA gene, *rpoB*, *infB* and *recN* sequences derived from type strains of species representing the genus *Pasteurella* as well as [*H.*] *felis* (93.5–95.4, 83.5–87.9, 78.9–86.6 and 61.0–63.4 %, respectively). This makes DNA sequence based identification of *F. canicola*, either alone or as confirmation of phenotypic results, the method of choice, especially for differential diagnosis.

Calculation of genome similarity values based on *recN* sequences which represents an alternative to whole DNA–DNA hybridization within the *Pasteurellaceae* (Kuhnert and Korczak 2006; Bisgaard et al. 2007; Kuhnert et al. 2010; Foster et al. 2011) provided evidence that the 24 investigated strains belong to the same species representing a new genus. Values calculated for all *F. canicola* strains were ≥0.90 confirming their close relatedness as representatives of the same species (Table 3). Similar to whole DNA–DNA hybridizations (Bisgaard and Mutters 1986) comparison of predicted genome similarities between *F. canicola* and the genus *Pasteurella* as well as other genera of *Pasteurellaceae* did not show values allowing classification of taxon 16 within any currently known genus (Table 3). The highest genome similarity value of 0.37 was obtained with *B. trehalosi*. The G+C content for the suggested type strain of *F. canicola*

(HPA 21^T) is 43.5 mol% as determined by Bisgaard and Mutters (1986).

Description of *Frederiksenia* gen. nov.

Frederiksenia (Fre.de.rik.sen'ia N.L. fem. N. to honour Wilhelm C. Frederiksen, a Danish microbiologist, for his involvement in and contribution to research on the *Pasteurellaceae*).

Frederiksenia is a new genus within the *Pasteurellaceae* family. The cells are Gram-negative, non-motile, non-haemolytic, pleomorphic straight or curved rods. No exogenous V-factor (beta-NAD) or X-factor (porphyrin) is required for growth. Positive reactions are observed for oxidase, catalase and indol; negative tests for methyl red, Voges Proskauer, urease and ornithine decarboxylase. Acid is formed from sucrose, (−)-D-fructose, maltose, (+)-D-mannose and dextrin, but not from dulcitol, D-sorbitol and (−)-D-mannitol. Glucose is fermented without gas production. The phosphatase test is positive. Comparison of phenotypic characters separating the genus *Frederiksenia* from the other *Pasteurellaceae* are given in Table 2. The DNA G+C for the type strain of the type species is 43.5 mol%. The major fatty acids found in the type strain of the type species are $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and summed features $C_{16:1}$ ω7c/$C_{15:0}$ iso 2OH; minor fatty acids are shown in the Online Resource Table S2. The main respiratory quinones detected in the type strain of the type species are Q-8, DMK-8 and MK-8. *Frederiksenia canicola* is the type species of the genus.

Description of *Frederiksenia canicola* sp. nov.

Frederiksenia canicola (ca.ni.'co.la, L. n. *canis*, dog; L. n. *icola*, a dweller, inhabitant; N. L. fem. *canicola*, the inhabitant of dog).

After 24 h of aerobic incubation on blood agar colonies are 1.5–2 mm in diameter with a circular, slightly raised and regular shape and they do not adhere to the agar. Their surface is smooth, shiny and opaque with grayish or rarely yellowish tinge. Most strains, including the type strain, are CAMP positive. In addition to characteristics included in the genus description, acid is formed from (−)-D-ribose and (after more than 24 h) from (+)-D-galactose. Most of the strains, including the type strain, are able to produce acid from (+)-D-trehalose. No acid is produced from (+)-D-xylose. Weak positive reactions are observed for hydrolysis of (−)-D-arabinose. MALDI-TOF MS analysis of investigated strains shows combination of peaks characteristic for this taxon at 5,244, 6,304, 6,377, 8,305 and 9,436 m/z. Isolates

Table 3 Calculated genome similarity values of *Frederiksenia canicola* to type species of genera of *Pasteurellaceae* based on *recN* sequences

	Frederiksenia canicola HPA 21^T = CCUG 62410^T = DSM 25797^T
Frederiksenia canicola (24 investigated strains)	0.90–0.95
Bibersteinia trehalosi NCTC 10370^T	0.37
Otariodibacter oris CCUG 59994^T	0.34
Actinobacillus ligneresii NCTC 4189^T	0.30
Nicoletella semolina CCUG 43639^T	0.30
Mannheimia haemolytica NCTC 9380^T	0.28
Phocoenobacter uteri NCTC 12872^T	0.25
Haemophilus influenzae Rd KW20	0.12
Volucribacter psittacicida CCUG 47536^T	0.12
Basfia succiniciproducens DSM 22022^T	0.07
Bisgaardia hudsonensis CCUG 43067^T	0.07
Lonepinella koalarum ATCC 700131^T	0.07
Pasteurella multocida CCUG 17976^T	0.07
Aggregatibacter actinomycetemcomitans HK1651	0.05
Avibacterium gallinarum NCTC 11188^T	0.05
Histophilus somni HS8025^T	0.03
Chelonobacter oris CCUG 55632^T	0.00
Gallibacterium anatis CCUG 15563^T	0.00
Necropsobacter rosorum CCUG 28028^T	0.00

have mainly been obtained from the oral cavity of dogs and dog-bite wounds in humans, but also from cat, lion, hedgehog, banded mongoose.

The type species is *Frederiksenia canicola* HPA 21^T (= CCUG 62410^T = DSM 25797^T) isolated in 1983 from pharynx of a healthy dog in Denmark.

Acknowledgments We thank Geoffrey Foster for sending strain M2500/96/3 and Andreas Thomann for technical assistance.

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