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*

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A collection of 12 strains, isolated from diseased tortoises and tentatively identified as [*Pasteurella*] *testudinis*-like based on phenotypic characters, was compared with three reference strains of [*P.*] *testudinis*. All strains could be separated from the reference strains with respect to 16S rRNA gene sequences, partial sequences of the *rpoB* housekeeping gene and by phenotypic characters. Based upon differences in 16S rRNA and *rpoB* gene sequences, the new isolates are suggested to represent a novel species in a new genus of the family *Pasteurellaceae* Pohl 1981, for which the name *Chelonobacter oris* gen. nov., sp. nov. is proposed. The type strain is 1662^T (=CCUG 55632^T=DSM 21392^T). β -Haemolysis and acid production from (+)-L-arabinose, dulcitol, (–)-D-mannitol, (+)-D-mannose, trehalose and salicin separated the new strains from members of existing genera of the family *Pasteurellaceae*, in addition to the β -galactosidase, urease and α -glucosidase reactions. Differences in indole production, phosphatase, β -glucosidase and production of acid from dulcitol and trehalose separated *C. oris* from [*P.*] *testudinis*. Several phenotypic characters separated *C. oris* from Bisgaard's taxa 14 and 32.

A *Pasteurella* species subsequently classified as [*Pasteurella*] *testudinis* was consistently found to be associated with respiratory lesions in captive tortoises with signs of respiratory disease (Snipes & Biberstein, 1982). Previously, phenotypically related isolates have been found to be part of the gastrointestinal and nasal flora of healthy tortoises (Snipes *et al.*, 1980), indicating a commensal inhabitation of the mucosal membrane of the respiratory and alimentary tract with possible opportunistic pathogenic capabilities (Jang & Biberstein, 1991). The collection

of strains investigated by Snipes & Biberstein (1982) showed a significant degree of phenotypic diversity. Based on DNA–DNA hybridization data, Mutters *et al.* (1985) excluded [*P.*] *testudinis* from the genus *Pasteurella sensu stricto*. Subsequent studies of the 16S rRNA gene sequence-based phylogeny of the family *Pasteurellaceae* showed that [*P.*] *testudinis* formed a separate cluster, which branched deeper than all previously described taxa within the family (Dewhirst *et al.*, 1993). Finally, Christensen *et al.* (2003) reported that the avian taxa 14, 32 and 40 of Bisgaard were phylogenetically related to [*P.*] *testudinis*.

The aims of the present study were to characterize a collection of strains from tortoises that had been tentatively identified as [*P.*] *testudinis* and to evaluate the taxonomy of these organisms within the family *Pasteurellaceae*. Classification was based mainly on sequencing of 16S rRNA and *rpoB* genes. *rpoB* gene sequence-based classification has been used previously with the family *Pasteurellaceae* (Angen *et al.*, 2003; Korczak *et al.*, 2004). In addition, this gene has been recommended as one of the genes suitable for DNA sequence-based identification in cases where phenotypic identification is difficult (Christensen *et al.*, 2007).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 1662^T, 8484, 17123 and 18043 are EU331064, EU331065, EU331067 and EU331066, respectively; those for the *rpoB* gene sequences of strains 11321, 18043, 8484, 17123, 4n and 770 are EU331069, EU331070, EU331068, EU331073, EU331072 and EU331071, respectively.

Phylogenetic relationships between *Chelonobacter oris* gen. nov., sp. nov. and strains related to [*Pasteurella*] *testudinis* based on maximum-likelihood analysis of *rpoB* sequences (Fig. S1), a photomicrograph of *Chelonobacter oris* strain 1662^T after cultivation in brain-heart infusion broth (Fig. S2) and characteristics for differentiation of genera within the family *Pasteurellaceae* (Table S1) are available with the online version of this paper.

A total of 15 strains, 12 unclassified isolates from clinically affected tortoises and turtles and three reference strains of [*P.*] *testudinis*, including the type strain, was investigated (Table 1). The three reference strains were included in the original study on the classification of [*P.*] *testudinis* (Snipes & Biberstein, 1982). Unclassified isolates were originally classified as haemolytic members of the family *Pasteurellaceae* based upon colony morphology, Gram staining, motility tests, catalase and oxidase activities, and fermentative catabolism of glucose in Hugh and Leifson's medium (Bisgaard *et al.*, 1991).

Sequencing of the 16S rRNA genes of strains 1662^T, 8484, 17123 and 18043 was performed according to previous reports (Angen *et al.*, 2003; Christensen *et al.*, 2002). Accession numbers for the 16S rRNA gene sequences of the above-mentioned strains are given in Table 1 and Fig. 1.

A BLAST search (Altschul *et al.*, 1997) of GenBank (Benson *et al.*, 2006) was performed. Pairwise comparisons for similarity were performed by the program WATER included in EMBOSS (Rice *et al.*, 2000). Multiple alignment was performed by CLUSTAL_X (Thompson *et al.*, 1997). Maximum-likelihood analysis including bootstrap analysis was performed by FASTDNAML (Felsenstein, 1995; Olsen *et al.*, 1994) on a Linux 7.2-compatible server. The analysis was run with a transition/transversion ratio of 1.5.

The three strains 1662^T, 8484 and 18043 formed a monophyletic group in the 16S rRNA gene sequence analysis with 100% bootstrap support; 99.9% similarity was observed between strains 1662^T and 8484 and 98.6% between strains 1662^T and 18043. The group was only distantly related to other members of the family *Pasteurellaceae* (Fig. 1). Taxa 14 and 32, as well as [*P.*] *testudinis* CCUG 19802^T and strain 17123, were the most closely related, although the relationship was poorly defined and without bootstrap support. The highest sequence similarity (93.3%) to the group of strains 1662^T, 8484 and 18043 was observed with strain 17123, whereas 92.9% similarity was observed with the type strain of [*P.*] *testudinis* and with taxa 14 and 32. Since the 16S rRNA gene sequence similarities are predominantly below approximately 95% between genera of the family *Pasteurellaceae* (Christensen *et al.*, 2007), strains 1662^T, 8484 and 18043 clearly represent a distinct genus from the 16S rRNA gene sequence perspective, for which the name *Chelonobacter* gen. nov. is proposed. 16S rRNA gene sequence similarity between strain 17123 and the type strain of [*P.*] *testudinis* was 95.6%, indicating a genus-like relationship between these two strains.

The partial *rpoB* gene sequence was determined according to Mollet *et al.* (1997) and covered the region 509–680 (*Escherichia coli* positions) of the deduced protein sequence as reported previously (Angen *et al.*, 2003; Korczak *et al.*, 2004). In addition to published primers, the forward PCR primer *rpobfAp* (GCAGTGAAAGAGTTCTTYGGTTC) was used for PCR amplification and DNA sequencing since the published primers did not result in amplification.

Sequencing was performed by Macrogen (Seoul, Korea). The GenBank accession numbers of the *rpoB* gene sequences of strains 11321, 18043, 8484, 17123, 4n and 770 are listed in Table 1 and Supplementary Fig. S1 (available in IJSEM Online). The *rpoB* gene sequences of strains 266/6375, 1662^T, 10259, 10977, 11321, 14061, 14314, 15322 and 15875 were identical and only that of strain 11321 was deposited in GenBank. Strain 8484 was closely related to these sequences with 99.8% similarity. Strain HIM 768-4^T of [*P.*] *testudinis* shared the published sequence for this strain (ATCC 33688^T; GenBank accession no. AY362972) and was closely related to strains 4n and 770 with similarities of 99.3 and 99.7%, respectively. Strain 18043 was related to the *Chelonobacter* group with 95% similarity and to the other strains with 83–84% similarity. Strain 17123 was most closely related to the type strain of [*P.*] *testudinis* with 88% similarity. The lowest *rpoB* gene sequence similarity within the *Chelonobacter* group was 94.7% between strains 18043 and 8484 (Supplementary Fig. S1, available in IJSEM Online). At the protein level, all 12 strains within the *Chelonobacter* group shared identical sequences. Also, the two strains 4n and 770 shared identical protein sequences with the type strain of [*P.*] *testudinis*. In the phylogenetic analysis of *rpoB* gene sequences, the published sequence of the type strain of [*P.*] *testudinis* was included as well as that of *Pasteurella multocida*, representing an outgroup (Supplementary Fig. S1). Strains 18043 and 1662^T, representing the novel species *Chelonobacter oris* gen. nov., sp. nov., as well as strain 17123, had the closest relationship to [*P.*] *testudinis* according to a BLASTN search of GenBank, indicating no other published sequences for these bacteria. The relatedness of the strains within the *Chelonobacter* group was confirmed by the *rpoB* gene sequence analysis with a bootstrap support of 100% (Supplementary Fig. S1, available in IJSEM Online).

Previous studies with comparisons of the *rpoB* gene sequence concluded that genera of the family *Pasteurellaceae* showed similarities above 85–88% at the nucleotide sequence level (Korczak *et al.*, 2004; Nørskov-Lauritsen *et al.*, 2004). Observations based on *rpoB* gene sequence comparisons confirmed that 11 of the 12 unclassified isolates belong to a new genus, *Chelonobacter*, whereas the remaining isolate, strain 17123, was related to a genus-like group represented by [*P.*] *testudinis*.

The DNA G + C content of strain 1662^T was 47.2 mol%, as determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany) according to the procedure of Mesbah *et al.* (1989).

Classical phenotypic tests were performed as previously reported (Bisgaard *et al.*, 1991). In addition, Diatabs (Rosco diagnostica) were used according to the manufacturer's protocol. Diatabs have been evaluated previously for phenotypic characterization within the family *Pasteurellaceae* (Angen *et al.*, 2002).

Table 1. Strains investigated in this study

Taxon	GenBank accession no.		Source of isolation	Year of isolation	Disease associated with isolate
	16S rRNA gene sequence	<i>rpoB</i> gene sequence†			
[<i>Pasteurella</i>] <i>testudinis</i>					
ATCC 33688 ^T (=HIM 768-4 ^T =UCD 90-23-79n ^{T*})		AY362972	Nares, <i>Gopherus agassizi</i> , USA		
770*		EU331071	Mouth, <i>Gopherus agassizi</i> , USA		
4n*		EU331072	Nares, <i>Gopherus agassizi</i> , USA		
<i>Chelonobacter oris</i>					
1662 ^T	EU331064	id. EU331069	Mouth, choanae, <i>Testudo hermanni</i> , Austria	2007	Cachexy, soft carapace, redness of carapace (suspicious for bacterial infection), apathy
266/6375		id. EU331069	Abscess, tortoise, Belgium		
8484	EU331065	EU331068	Mouth, choanae, <i>Testudo hermanni</i> , Austria	2005	Vomitus, redness of carapace (suspicious for bacterial infection), vesicles on nasal mucosa
0259		id. EU331069	Mouth, <i>Testudo hermanni</i> , Austria	2006	‘Blocked’ nose, respiratory sounds, cachexy
10977		id. EU331069	Mouth, choanae, <i>Testudo hermanni</i> , Austria	2006	Apathy, respiratory sounds, hyperkeratosis
11321		EU331069	Mouth, <i>Testudo hermanni</i> , Austria	2006	Lameness
14061		id. EU331069	Conjunctiva, <i>Testudo hermanni</i> , Austria	2006	Infection of both eyes
14314		id. EU331069	Mouth, choanae, <i>Testudo hermanni</i> , Austria	2005	Apathy, tail injury, redness of carapace (suspicious for bacterial infection)
15322		id. EU331069	Mouth, <i>Testudo hermanni</i> , Austria	2006	Diarrhoea, cachexy
15875		id. EU331069	Mouth, choanae, <i>Testudo hermanni</i> , Austria	2005	Redness of carapace (suspicious for bacterial infection)
18043	EU331066	EU331070	Reptile, no further information, Austria		No information
Unclassified					
17123	EU331067	EU331073	Skin, <i>Trachemys scripta scripta</i> , Austria	2005	Inflammation of skin

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†id., DNA sequence identical to that of the accession number indicated.

Fig. 1. Phylogenetic relationships between *Chelonobacter oris* gen. nov., sp. nov. and representative members of the family *Pasteurellaceae* based on maximum-likelihood analysis of 16S rRNA gene sequences. Support for monophyletic groups by bootstrap analysis is indicated as numbers out of 100. The strains in bold have been sequenced in the present investigation. Bar, 0.01 evolutionary distance.

All isolates classified as *Chelonobacter oris* gen. nov., sp. nov. and unclassified strain 17123 stained Gram-negatively, were non-motile, pleomorphic rods (see Supplementary Fig. S2, available in IJSEM Online), were catalase- and oxidase-positive, and produced acid fermentatively from glucose without gas in Hugh and Leifson's medium. β -Haemolysis was observed with all strains when cultured on 5% bovine blood agar.

Phenotypic characteristics separating the strains investigated are given in Table 2. The three reference strains of [*P.*] *testudinis* showed identical reaction patterns. The 12 unclassified strains could be distinguished from the reference strains on the basis of indole production, phosphatase and formation of acid from dulcitol and trehalose. The single [*P.*] *testudinis*-like strain, 17123, differed only from the type strain in trehalose and β -glucosidase (NPG) activities and all strains except 17123 were positive for aesculin hydrolysis. Strain 18043 could be distinguished from strains of *Chelonobacter oris* gen. nov., sp. nov. based on indole production. The 12 unclassified strains could be separated from Bisgaard's taxa 14 and 32 (Christensen *et al.*, 2003) based on several characteristics, including β -haemolysis. At least two phenotypic characteristics separated the new genus from existing genera within the family *Pasteurellaceae* (Supplementary Table S1, available in IJSEM Online).

The results obtained from genotypic and phenotypic tests showed good correlation. The monophyly of the new genus

proposed as *Chelonobacter* gen. nov. was clearly indicated by 16S rRNA gene sequence analysis. Comparison of *rpoB* gene sequences supported this, since members of other genera within the family *Pasteurellaceae* have been found to show relatedness values above 85–88% at the DNA level (Angen *et al.*, 2003; Korczak *et al.* 2004; Nørskov-Lauritsen *et al.*, 2004; Kuhnert *et al.*, 2004). Furthermore, the proposed genus, *Chelonobacter* gen. nov., fulfils the

Table 2. Differential phenotypic characteristics of the strains investigated in this study

Taxa: 1, *Chelonobacter oris* gen. nov., sp. nov.; 2, [*P.*] *testudinis* UCD 90-23-79n^T; 3, strain 17123. +, Positive reaction within 1–2 days; (+), positive reaction within 3–14 days; w, weakly positive reaction; –, negative reaction after final reading.

Characteristic	1	2	3
Indole production	–*	+	+
Phosphatase	w†	–	–
Acid production from:			
Dulcitol	+‡	–	–
Trehalose	+	–	+
β -Glucosidase (NPG)	+	+	–

*Reaction for strain 18043 was +.

†Reactions observed for strains 8484 and 10259 were +.

‡Reaction for strain 18043 was (+).

suggested minimal standards for descriptions of members of the family *Pasteurellaceae*, including the number of strains investigated (Christensen *et al.*, 2007). The authors desist from the reclassification of [*P.*] *testudinis* and the single isolate 17123 since more strains are needed to demonstrate possible divergence within this group.

The low diversity within the genus *Chelonobacter* gen. nov. indicates the presence of only one species, for which the name *Chelonobacter oris* gen. nov., sp. nov. is proposed. The highest genotypic and phenotypic diversity was observed between a group of ten strains, including the type strain, and strain 18043. Divergence was observed in only one phenotypic characteristic (indole production) and low genotypic divergence between strain 18043 and the other ten strains was found with respect to both 16S rRNA and *rpoB* gene sequences.

Description of *Chelonobacter* gen. nov.

Chelonobacter (Che.lo.no.bac'ter. Gr. n. *chelone* -es a tortoise; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Chelonobacter* rod isolated from tortoise).

Members of the genus stain Gram-negatively and are non-motile, pleomorphic rods that are catalase- and oxidase-positive and produce acid fermentatively from glucose without gas in Hugh and Leifson's medium. After 24 h aerobic incubation at 37 °C, regular, slightly raised, circular colonies are formed with an entire margin surrounded by a zone of β -haemolysis. Positive for nitrate reduction and acid production from (+)-L-arabinose, dulcitol, (-)-D-mannitol, (-)-D-fructose, (+)-D-glucose, (+)-D-mannose, cellobiose, maltose, sucrose, trehalose and salicin. Positive for β -galactosidase (ONPG). Negative for X- and V-factor requirement, urease, lysine decarboxylase, ornithine decarboxylase, pigment formation, α -fucosidase, α -galactosidase, α -glucosidase (PNPG), β -glucuronidase (PGUA), α -mannosidase and β -xylosidase (ONPX). Strains have been isolated from diseased tortoises and turtles. The type species is *Chelonobacter oris* sp. nov.

Description of *Chelonobacter oris* sp. nov.

Chelonobacter oris (o'ris. L. n. *os oris* mouth; L. gen. n. *oris* off/from the mouth).

Colony and cellular morphologies are as given in the genus description. The surface of the colonies is smooth, shiny and opaque. Colonies are between 1.0 and 1.5 mm in diameter. The consistency of the colonies is unguent-like and adherence to the agar is not observed. In addition to the phenotypic characteristics given in the genus description, acid is produced from (+)-D-arabitol, (-)-D-ribose, (+)-D-xylose, (-)-D-fucose, raffinose and dextrin. Positive for aesculin hydrolysis and β -glucosidase (NPG). Negative for citrate utilization and production of acid from adonitol, (-)-D-sorbitol, lactose, melibiose and inulin. The type strain is indole-negative; a single isolate (18043) is indole-positive.

The type strain is 1662^T (=CCUG 55632^T=DSM 21392^T), isolated from a diseased tortoise. The DNA G + C content of the type strain is 47.2 mol%.

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References

- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Angen, Ø., Ahrens, P. & Bisgaard, M. (2002). Phenotypic and genotypic characterization of *Mannheimia* (*Pasteurella*) *haemolytica*-like strains isolated from diseased animals in Denmark. *Vet Microbiol* **84**, 103–114.
- Angen, Ø., Ahrens, P., Kuhnert, P., Christensen, H. & Muters, R. (2003). Proposal of *Histophilus somni* gen. nov., sp. nov. for the three species *incertae sedis* '*Haemophilus sommus*', '*Haemophilus agni*' and '*Histophilus ovis*'. *Int J Syst Evol Microbiol* **53**, 1449–1456.
- Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J. & Wheeler, D. L. (2006). GenBank. *Nucleic Acids Res* **34**, D16–D20.
- Bisgaard, M., Houghton, S. B., Muters, R. & Stenzel, A. (1991). Reclassification of German, British and Dutch isolates of so-called *Pasteurella multocida* obtained from pneumonic calf lungs. *Vet Microbiol* **26**, 115–124.
- Christensen, H., Bisgaard, M., Angen, Ø. & Olsen, J. E. (2002). Final classification of Bisgaard taxon 9 as *Actinobacillus arthritidis* sp. nov. and recognition of a novel genomospecies for equine strains of *Actinobacillus lignieresii*. *Int J Syst Evol Microbiol* **52**, 1239–1246.
- Christensen, H., Foster, G., Christensen, J. P., Pennycott, T., Olsen, J. E. & Bisgaard, M. (2003). Phylogenetic analysis by 16S rDNA gene sequence comparison of avian taxa of Bisgaard and characterization and description of two new taxa of *Pasteurellaceae*. *J Appl Microbiol* **95**, 354–363.
- Christensen, H., Kuhnert, P., Busse, H. J., Frederiksen, W. C. & Bisgaard, M. (2007). Proposed minimal standards for the description of genera, species and subspecies of the *Pasteurellaceae*. *Int J Syst Evol Microbiol* **57**, 166–178.
- Dewhirst, F. E., Paster, B. J., Olsen, I. & Fraser, G. J. (1993). Phylogeny of the *Pasteurellaceae* as determined by comparison of 16S ribosomal ribonucleic acid sequences. *Zentralbl Bakteriol* **279**, 35–44.
- Felsenstein, J. (1995). PHYLIP (phylogeny inference package) version 3.57c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Jang, S. S. & Biberstein, E. L. (1991). Observations on the occurrence of *Pasteurella testudinis* in clinical specimens from animals. *J Vet Diagn Invest* **3**, 174–176.
- 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., Falsen, E., Straub, R., Hoops, A., Boerlin, P., Frey, J. & Muters, 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.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Mollet, C., Drancourt, M. & Raoult, D. (1997). *rpoB* sequence analysis as a novel basis for bacterial identification. *Mol Microbiol* **26**, 1005–1011.

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., Christensen, H., Okkels, H., Kilian, M. & Bruun, B. (2004). Delineation of the genus *Actinobacillus* by comparison of partial *infB* sequences. *Int J Syst Evol Microbiol* **54**, 635–644.

Olsen, G. J., Matsuda, H., Hagstrom, R. & Overbeek, R. (1994). fastDNAm1: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Comput Appl Biosci* **10**, 41–48.

Rice, P., Longden, I. & Bleasby, A. (2000). EMBOSS: the European molecular biology open software suite. *Trends Genet* **16**, 276–277.

Snipes, K. P. & Biberstein, E. L. (1982). *Pasteurella testudinis* sp. nov.: a parasite of desert tortoises (*Gopherus agassizi*). *Int J Syst Bacteriol* **32**, 201–210.

Snipes, K. P., Biberstein, E. L. & Fowler, M. E. (1980). A *Pasteurella* sp. associated with respiratory disease in captive desert tortoises. *J Am Vet Med Assoc* **177**, 804–807.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.