**Pelistega suis** sp. nov., isolated from domestic and wild animals

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Biochemical and molecular genetic studies were performed on three novel Gram-stain-negative, catalase- and oxidase-positive, bacilli-shaped organisms isolated from the tonsils of two pigs and one wild boar. The micro-organism was identified as a species of the genus *Pelistega* based on its cellular morphological and biochemical tests. The closest phylogenetic relative of the novel bacilli was *Pelistega indica* HM-7T (98.2 % 16S rRNA gene sequence similarity to the type strain). *groEL* and *gyrB* sequence analysis showed interspecies divergence from the closest 16S rRNA gene phylogenetic relative, *P. indica* of 87.0.% and 69 %, respectively. The polyamine pattern contains predominantly putrescine and 2-hydroxyputrescine. The major quinone is ubiquinone Q-8 and in the polar lipid profile, phosphatidylethanolamine, phosphatidyglycerol, an unidentified aminolipid and an unidentified lipid are predominant. The novel bacterial isolate can be distinguished from *P. indica* by several biochemical characteristics, such as the production of L-pyrrolydonil arylamidase but not gamma-glutamyltransferase, and the utilization of different carbon sources. Based on both phenotypic and phylogenetic findings, the novel bacterium is classified as representing a novel species of the genus *Pelistega*, for which the name *Pelistega suis* sp. nov. is proposed. The type strain is 3340-03T (=CECT 8400T =CCUG 64465T).

The genus *Pelistega*, originally described by Vandamme *et al.* (1998), contains rod-shaped bacteria with variable morphological forms. At the time of writing, this genus embraces two species with validly published names, *Pelistega europaea* and *Pelistega indica* (Vandamme *et al.*, 1998; Prakash *et al.*, 2014). Isolates of *P. europaea* were obtained from diseased pigeons, while *P. indica* was isolated from human gut (Vandamme *et al.*, 1998; Prakash *et al.*, 2014). In this study, we report the phenotypic and phylogenetic features of three *Pelistega*-like organisms isolated from tonsil swab samples from two pigs (strains 3340-03T and DICM11-00060-1A) and one wild boar (strain ZTA10-00042-1C). Samples were collected, transported under refrigeration to the laboratory and processed for bacteriological analysis within 48 h. Samples were cultured on blood agar plates that were incubated at 37 °C for 24 h under aerobic and anaerobic conditions. On the basis of the phenotypic and phylogenetic results, a novel species of the genus *Pelistega* is proposed.

Abbreviations: MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; PFGE, pulsed-field gel electrophoresis.

The GenBank/EMBL/DDJB accession numbers for the 16S rRNA gene sequence of strains 3340-03T, DICM11-00060-1A and ZTA10-00042-1C are LN830267, LN830268 and LN830269, respectively.

Two supplementary figures are available with the online Supplementary Material.

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last cycle was followed by 10 min elongation at 72 °C. The amplified product was sequenced bidirectionally using universal primers pA (5’-AGAGTTTGATCTCCTAGTCAG-3’; positions 8–27, *E. coli* numbering), pB (5’-AAGGAGGTGATCCAGGGCA-3’; positions 1541–1522), antiIKK (5’-CGTCCAGCAGCGGTTGTAAT-3’; positions 517–537) and 3 (5’-GGTGCGCTGTTGCGGGACT-3’). Comparative analysis of the 16S rRNA gene sequences revealed 99.7 to 99.9 % sequence similarity between the isolates, thereby demonstrating their high genealogical relatedness. The identifications of phylogenetic neighbours and calculations of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (Kim et al., 2012; http://eztaxon-e.ezbiocloud.net/). Sequence searches revealed that the unknown bacilli were members of the genus *Pelistega*, being most closely related to *Pelistega indica* HM-7T (98.2 % 16S rRNA gene sequence similarity). The 16S rRNA gene sequence similarity of isolate 3340-03 T with the type strain of the only other species of the genus *Pelistega* (*Pelistega europaea* LMG 10982T) was 96.5 %. These sequences and those of other closely related strains were retrieved from the GenBank database and aligned with the newly determined sequences using the program SeqTools (Rasmussen, 2002). Phylogenetic trees were reconstructed according to three different algorithms: neighbour-joining (Saitou & Nei, 1987) using the programs SeqTools and TreeView (Page, 1996; Rasmussen, 2002), maximum-likelihood using the software package MEGA version 4 (Tamura et al., 2007), and maximum-likelihood using the PHYLML software (Guindon & Gascuel, 2003). Genetic distances for the neighbour-joining and maximum-likelihood algorithms were calculated by Kimura’s two-parameter method (Kimura, 1980), and close-neighbor-interchange (search level=2, random additions=100) was applied in the maximum-parsimony analysis. The stability of the groupings was estimated by bootstrap analysis (1000 replications). According to the analysis of the 16S rRNA gene sequences based on the neighbour-joining algorithm, the two currently recognized species of the genus *Pelistega* and isolate 3340-03 T were not grouped in a single clade (Fig. 1). isolate 3340-03 T and *P. indica* HM-7T formed a separate clade to that represented by *P. europaea* LMG 10982T which clustered with the type strains of *Taylorella asinigenitalis* and *Taylorella equigenitalis*. This clustering was supported by significant bootstrap resampling values and it was also obtained with the other two tree-making algorithms (Fig. 1). Therefore, based on 16S rRNA gene sequence data, the genus *Pelistega* seems to be paraphyletic.

Sequencing of multiple protein-encoding genes with higher resolution than 16S rRNA gene sequences has been proposed for delineation of prokaryotic species (Tindall et al., 2010). Sequence analysis of the genes *gyrB* (encoding the B-subunit of DNA gyrase) and *groEL* (encoding the 60 kDa heat-shock protein GroEL) has been demonstrated to be a useful tool for bacterial systematics (Yamamoto et al., 2000; Watanabe et al., 2001; Rasis et al., 2014; Li et al., 2015). In the present study, nucleotide sequences of *gyrB* and *groEL* genes were determined from isolate 3340-03 T, *P. indica* HM-7T, *P. europaea* LMG 10982T, *Advenerella incenata* CCUG 45225T, *Brackiella oedipodis* CCUG 47103T, *Oligella urethralis* CCUG 13463T and *Taylorella equigenitalis* CCUG 10786T. Partial sequences of *gyrB* (690 bp) and *groEL* (575 bp), chosen in conserved regions, were amplified using primer pairs *gyrBF* (5’-ATGACCGATGCGNGANTNGA-3’) and *gyrBR* (5’-ARYTGYCKGTGTTGATCTCCTA-3’), and *groELF* (5’-ATGCAATTGTGCGNGNTA-3’) and *groELR* (5’-ACATRCCTTCTCNGA-3’). The *gyrB* and *groEL* sequence similarities of strain 3340-03 T and its closest 16S rRNA gene phylogenetic relative *P. indica* DSM 27484T were 69 % and 87.0 %,
respectively. Strain 3340-03<sup>T</sup> exhibited a mean gyrB sequence similarity of 61.5 % with other 16S rRNA gene phylogenetic relatives, ranging from 57.8 % (A. incenata CCUG 45225<sup>T</sup>) to 73.0 % (P. europaea CCUG 39967<sup>T</sup>). The mean groEL sequence similarity was 80.1 %, ranging from 77.1 % (A. incenata CCUG 45225<sup>T</sup>) to 87.1 % (P. europaea CCUG 39967<sup>T</sup>). Evolutionary distances were calculated using Kimura’s two-parameter method (Kimura, 1980) and the resulting tree was reconstructed using the neighbour-joining algorithm using the software package MEGA version 4 (Tamura et al., 2007). The phylogenetic trees inferred from gyrB and groEL gene sequence comparisons revealed that P. indica (DSM 27484<sup>T</sup>) and P. europaea LMG 10982<sup>T</sup> grouped together with strain 3340-03<sup>T</sup> in a single clade, with the later strain forming a separate branch from the two species of the genus Pelistega (Fig. S1, available in the online Supplementary Material). Phylogenetic analysis based on concatenated gyrB, groEL and 16S rRNA gene sequences was also performed as described for individual genes. The resulting phylogenetic tree based on the concatenated sequences also places P. europaea CCUG 39967<sup>T</sup>, P. indica DSM 27484<sup>T</sup> and strain 3340-03<sup>T</sup> in a single and consistent clade with 100 % bootstrap, distinct from that formed by species of the genus Taylorella (Fig. 2). These results support the monophyletic grouping of the species of the genus Pelistega and position of strain 3340-03<sup>T</sup> as representative of a novel species of this genus.

The three novel isolates were Gram-stained and assessed for the presence of catalase. The haemolytic reaction was determined on Columbia agar containing 5 % desfibrinated sheep blood (bioMérieux) incubated aerobically at 37 °C for 24 and 48 h (Facklam & Elliott, 1995). Determination of the growth of at 4, 15, 22, 30, 37 and 42 °C was performed in brain heart infusion broth (Difco) with the pH adjusted to 7.5 (Facklam & Elliott, 1995). The ability of the isolates to tolerate the presence of 3.5, 4.5 and 6.5 % NaCl in brain heart infusion broth (Difco) was assessed as recommended by Facklam & Elliott (1995). The isolates were biochemically characterized using the API 20E, API 20NE and API ZYM systems (bioMérieux) according to the manufacturer’s instructions. Bacterial suspensions were also analysed with GN cards using the VITEK system (bioMérieux) following the manufacturer’s instructions. The three isolates exhibited identical biochemical characteristics. The phenotypic characteristics that differentiate the proposed species from other species of the genus Pelistega are shown in Table 1. Detailed descriptions of morphological, physiological and biochemical data for the novel species are given in the species description.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been considered a reliable identification method for species of the genus Pelistega (Prakash et al., 2014) and it was therefore used in the present study to characterize the potential novel species. Colonies from strain 3340-03<sup>T</sup> and from P. indica DSM 27484<sup>T</sup> were subjected to a protein extraction protocol according to the manufacturer’s instructions (SOP, Ethanol/Formic Acid Extraction Method; Bruker Daltonics) and were spotted in eight different spots [to test reproducibility and for the creation of a mass spectral profile (MSP)] on the MALDI-TOF MS sample plate and air-dried at room temperature. A 1 μl aliquot of matrix

![Table 1. Characteristics useful in differentiating Pelistega suis sp. nov. from the other two species of the genus Pelistega](http://ijs.microbiologyresearch.org)

<table>
<thead>
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<th>Characteristic</th>
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<tr>
<td>Growth on MacConkey agar</td>
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<td>Catalase</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Nitrate reductase</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>L-Pyrrolydonil arylamidase</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Gamma-glutamyl-transferase</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Citrate (sodium)</td>
<td>–</td>
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<td>Alkalization of:</td>
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<tr>
<td>L-Lactate</td>
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<td>Succinate</td>
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<td>Assimilation of:</td>
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<td>L-Malate</td>
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<td>L-Lactate</td>
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solution [saturated solution of α-cyanohydroxycinnamic acid (α-HCCA; Sigma-Aldrich)] dissolved in 50% acetonitrile, 47.5% water and 2.5% trifluoroacetic acid (Fluka; Sigma-Aldrich) was added onto each sample spot and again allowed to dry (Liu et al., 2007; Böhme et al., 2010). Mass spectra acquisition was performed on a Bruker UltraFleXtrem platform (Bruker Daltonics) using a mass range of 2–20 kDa. The Bruker Bacterial Test Standard (Escherichia coli DH5) was used as an external protein calibration mixture (Alispach et al., 2010). Mass spectra were smoothed, baseline corrected and peak detected using the FlexAnalysis software (version 3.4, Bruker Daltonics). Three biological replicates from each sample were created and analysed to test the reproducibility of the procedure. Generated profiles were compared with the bacterial database of Bruker Daltonics generated using type cultures of previously characterized bacteria. The results of MALDI-TOF for strain 3340-03T did not match with any bacterial species in the Bruker Daltonics database. MALDI-TOF data showed that the profile of both strain 3340-03T and P. indica DSM 27484T contained 70 peaks in the range of 3000 to 12 000 Da. However, comparison of the MALDI-TOF mass spectra of both strains showed differences (Fig. S2) in terms of quantity of proteins (number of spikes) and amount of expressed protein (intensity of peaks). MALDI-TOF mass spectra of strain 3340-03T was also different to that of P. europaea CCUG 39967T (data not shown). These MALDI-TOF data corroborate the phylogenetic results based on the 16S rRNA gene sequencing and indicate that strain 3340-03T is different from both currently recognized species of the genus Pelistega.

Preparation of high-molecular-mass DNA and DNA–DNA hybridization experiments were performed by the Identifi-
cation Service of the Leibniz Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). Genomic DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion et al. (1977). DNA–DNA hybridization was carried out in 2 x SSC at 68 °C by the Identification Service of the DSMZ using the method described by De Ley et al. (1970) under consideration of the modifications described by Huss et al. (1983) with a model Cary 100 Bio UV/VIS spec-
trophotometer equipped with a Peltier thermostated 6 x 6 multiecell changer and a temperature controller with in situ temperature probe (Varian). The DNA–DNA reassociation value between isolate 3340-03T and P. indica DSM 27484T was 10.4%, below the recommended threshold value of 70%, which confirms that this taxon merits its separate species status (Wayne et al., 1987).

Biomass subjected to chemotaxonomic analyses was grown in 3.3 x PYE broth (1% peptone from casein, 1% yeast extract, pH 7.2). Quinones and polar lipids were extracted from biomass harvested at the stationary growth phase and analysed applying the integrated procedure reported by Tindall (1990a, b) and Altenburger et al. (1996). Polyamines were extracted and analysed from cells harvested at the late exponential growth phase as recommended by Busse & Auling (1988). HPLC analysis was carried out using the equipment described by Stolz et al. (2007) slightly modified in the gradient as reported by Busse et al. (1997). The quione system of strain 3340-03T consisted predominantly of ubiquinone Q-8 (97%) and minor amounts of Q-9 (2%) and Q-7 (1%). The polar lipid profile contained the major lipids phosphatidylethanolamine, phosphatidylglycerol, an unidentified aminolipid (AL1) and an unidentified lipid (L1) only visible after staining for total lipids. Furthermore, moderate to minor amounts of another aminolipid (AL2) and four polar lipids (L2–L5) were detected (Fig. 3). The polyamine pattern of strain 3340-03T was composed of putrescine [31.6 μmol (g dry weight)]1], 2-hydroxyputrescine [31.3 μmol (g dry weight)]1] and minor amounts of 1,3-diaminopropane [0.2 μmol (g dry weight)]1], cadaverine [0.1 μmol (g dry weight)]1], spermine [3.1 μmol (g dry weight)]2] and spermidine [1.7 μmol (g dry weight)]2]. These chemotaxonomic traits are in line with those of other members of the family Alcaligenaceae (Kämpfer et al., 2006, 2010; Blümel et al., 2001; Busse et al., 1992; Stolz et al., 2005; Zhang et al., 2012).

The three strains from domestic and wild pigs were characterized by pulsed-field gel electrophoresis (PFGE) profiling of their genomic DNAs, after digestion with the restriction enzyme Bsp1201, according to previous specifications (García et al., 2011). Similarities between restriction endonuclease digestion profiles were based on visual comparisons of the band patterns of strains run in the same gel. Strains differing in at least one band were considered
different. The strains displayed three different PFGE restriction profiles (data not shown).

Overall, the results of the present polyphasic study demonstrate that the characteristics of the novel strains from pigs and wild boar match those described for the genus Pelistega, and the strains should be assigned to this genus. The phylogenetic, genotypic and phenotypic differences clearly demonstrate that the strains merit classification as a novel species of the genus, for which the name Pelistega suis sp. nov. is proposed.

Description of Pelistega suis sp. nov.

_Pelistega suis_ (su’is. L. gen. n. suis of the hog).

Cells are Gram-stain-negative, 0.75 μm wide by 1 μm long, non-spore-forming, non-motive and bacilli-shaped. Facultatively anaerobic and catalase- and oxidase-positive. Colonies are circular, smooth, entire and approximately 1 mm diameter on Columbia blood agar after incubation for 24 h at 37 °C. Growth does not occur on MacConkey agar. Cells are not able to grow in the presence of 3% NaCl. Produces acetoin at 37 °C. Does not produce acid from glucose and some other sugars. Nitrates are not reduced to nitrites. Gelatin and urea are not hydrolysed. Indole is produced but not H₂S. Positive (on Vitek 2 GN cards) for L-malate and L-lactate utilization and L-tryptophan arylamidase activity, but not for adonitol, sucrose, L-arabitol, D-glucose, maltose, D-mannitol, D-mannose, D-sorbitol, D-tagatose, trehalose, D-malate and citrate utilization, and Ala-Phe-Pro arylamidase, β-galactosidase _N_-acetyl-β-glucosaminidase, glutamyl arylamidase-p-nitroanilide γ-glutamyltransferase, β-glucosidase, β-xylanidase, γ- and alanie arylamidase-p-nitroanilide, L-proline arylamidase, lipase, palatinose, tyrosine arylamidase, _N_-acetyl-β-galactosaminidase, α-glucosidase, glycine arylamidase, ornithine decarboxylase, lysine decarboxylase and Glu-Gly-Arg arylamidase activities. Positive results with API ZYM kit for esterase (C4), leucine arylamidase and naphthol-AS-Bl-phosphohydrolase activities, but negative result for esterase lipase (C8), valine arylamidase, cysteine arylamidase, trypsin, _x_-chymotrypsin, _x_-glycosidase, β-glucosidase, _x_-mannosidase and _x_-fucosidase activities; the type strain does not produce alkaline phosphatase. The quinone system is composed predominantly of ubiquinone Q-8 and minor amounts of Q-7 and Q-9. The polar lipid profile contains predominantly phosphatidyethanolamine, phosphatidylglycerol, an unidentified aminolipid and four polar lipids are present. The polyamine pattern is characterized by the presence of the major components putrescine and 2-hydroxyputrescine, moderate amounts of spermidine and spermine and traces of 1,3-diaminopropane and cadaverine.

The type strain, 3340-03T (=CECT 8400T = CCUG 64465T) was isolated from the tonsil of a pig. Two additional strains of the species are DICM11-00060-1A and ZTA10-00042-1C, isolated from a pig and a wild boar, respectively.

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