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Proposal of *Lysobacter pythonis* sp. nov. isolated from royal pythons (*Python regius*)☆

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ABSTRACT

The bacterial strains 4284/11T and 812/17 isolated from the respiratory tract of two royal pythons in 2011 and 2017, respectively were subjected to taxonomic characterization. The 16S rRNA gene sequences of the two strains were identical and showed highest sequence similarities to *Lysobacter tolerans* UM1^T (97.2%) and *Luteimonas aestuarii* DSM 19680^T (96.7 %). The two strains were identical in the sequences of the 16S-23S rRNA internal transcribed spacer (ITS) and partial *groEL* gene sequences and almost identical in genomic fingerprints. In the ITS sequence *Ly. tolerans* DSM 28473^T and in the *groEL* nucleotide sequence *Luteimonas mephitis* DSM 12574^T showed the highest similarity. *In silico* DDH analyses using genome sequence based ANIb and gANI similarity coefficients demonstrated that strain 4284/11^T represents a novel species and revealed *Ly. tolerans* UM1^T as the next relative (ANIb = 76.2 %, gANI = 78.0 %). Based on the topology of a core gene phylogeny strain 4284/11^T could be assigned to the genus *Lysobacter*. Chemotaxonomic characteristics including polyamine pattern, quinone system, polar lipid profile and fatty acid profile were in accordance with the characteristics of the genera *Lysobacter* and *Luteimonas*. Strains 4284/11^T and 812/17 could be differentiated from the type strains of the most closely related species by several physiological tests. In conclusion we are here proposing the novel species *Lysobacter pythonis* sp. nov. The type strain is 4284/11^T (= CCM 8829^T = CCUG 72164^T = LMG 30630^T) and strain 812/17 (CCM 8830) is a second strain of this species.

The genera *Lysobacter* and *Luteimonas* were placed in the family "*Xanthomonadaceae*" by Saddler and Bradbury [39]. The name of the family *Xanthomonadaceae* is illegitimate because it contains the genus *Lysobacter* which is the type genus of the family *Lysobacteraceae* [47]. Phylogenetic studies indicate that both genera *Lysobacter* and *Luteimonas* are not monophyletic. For instance, *Lysobacter panaciterrae* is found next to several *Luteimonas* species [27,49,52] and might be reclassified in the genus *Luteimonas*. Unfortunately, for establishment of a stable taxonomy within this group chemotaxonomic characteristics such as fatty acids, polar lipids, quinones and polyamines are not useful because so far no characteristics were found distinguishing these genera.

During routine microbiological diagnosis of human and veterinary specimens often only the supposed causative agent of the diagnosed disease is identified whereas commensals detected after primary cultivation are usually ignored. However, for treatment of the disease identification of the commensals is of no relevance if the causative agent can be detected in the specimen. On the other hand, commensal isolates appear to be an interesting source of unrecognized bacterial species and hence they are most interesting for bacterial taxonomists.

In this study we are reporting on the taxonomic characterization of two bacterial strains isolated from royal python (*Python regius*). Strain 4284/11^T was isolated in 2011 from the trachea of a royal python suffering from respiratory tract infection after antibiotic treatment and strain 812/17 was isolated in 2017 from the tra-

☆ The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene, the groEL, the 16S-23S internal transcribed spacer sequences (ITS) and the genome assembly are: 16S rRNA gene LM994044 and MG760578; groEL LM994049, LM994050, LM994051, LM994052, MH105811 and MH105812; ITS LM994045, LM994046, LM994047, LM993048, MG760579 and MG760580; the Genbank accession of the genome of strain 4284/11T is GCA_003697345.1.

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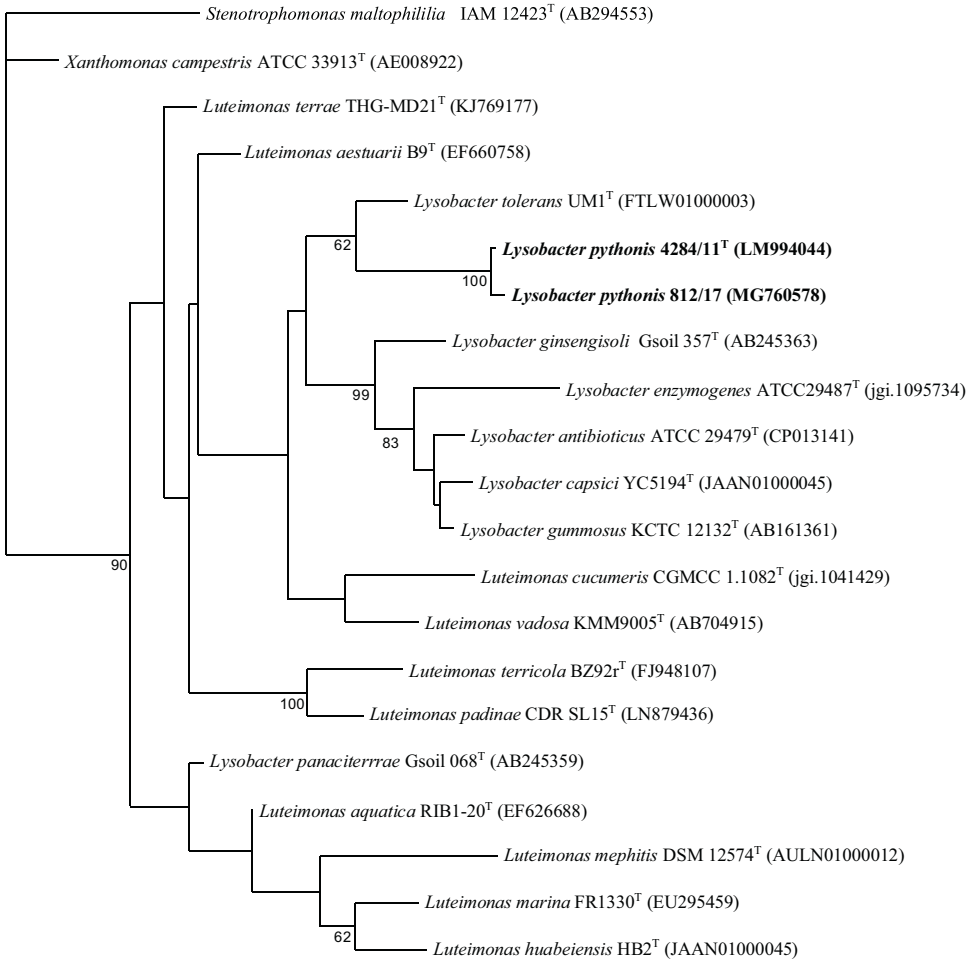

Fig. 1. Maximum-likelihood tree based on 16S rRNA gene-sequences showing the relatedness of the strains 8284/11 and 812/17 with reference species of the genera *Lysobacter* and *Luteimonas* sharing at least 95.0% 16S rRNA gene sequence similarity. *S. maltophilia* IAM 12423^T was used as an outgroup. Bootstrap values (%) >60 based on 100 replicates are given at nodes. Bar indicates 0.1 substitutions per nucleotide position.

chea of another royal python displaying symptoms of respiratory distress.

For preliminary classification, the 16S rRNA genes were amplified using universal primers 27f and 1494r [23]. The PCR reaction mix consisted of 30 μl REDTaq ReadyMix PCR Reaction Mix with $MgCl_2$ (Sigma–Aldrich), 37.5 pmol of each primer (Invitrogen), 2.50 μl DNA and 26.20 μl sterile water. Amplification started with an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1.5 min, annealing at 53 °C for 1.5 min and elongation at 72 °C for 5 min, and a final elongation at 72 °C for 10 min.

The PCR products were purified using Wizard® SV Gel und PCR Clean-Up System (Promega) according to the manufacturer's instructions and sequenced by LGC Genomics (Berlin) resulting in 16S rRNA gene sequences of strains 4284/11^T and 812/17 with length of 1447 and 1442 nucleotides. The two 16S rRNA gene sequences were 100% identical. Sequence comparisons were carried out using the EzTaxon-e server [50] and identified *Lysobacter tolerans* UM1^T [27,34] and *Luteimonas aestuarii* B9^T [37] as the next related species sharing 97.2 and 96.7% similarity, respectively. Sequence similarities with the type species of the genera *Lysobacter* and *Luteimonas*, *Lysobacter enzymogenes* DSM 2043^T and *Luteimonas mephitis* DSM 12574^T, were 95.7% and 93.3%, respectively. Surprisingly, among the species sharing in the 16S rRNA gene sequence more than 95.0% similarity nine *Luteimonas* species and six *Lysobacter* species were found. However, the higher sequence similarities of 4284/11^T and 812/17 to the type species of *Lysobacter* suggest that they could be assigned to this genus. For phylogenetic analyses the 16S rRNA gene sequences of strains 4284/11^T and 812/17 were aligned with sequences of type strains of all *Lysobacter* and *Luteimonas* species, which shared more than 95.0% similarity, and *Lu. mephitis* DSM 12574^T, *Stenotrophomonas maltophilia* IAM 12423^T and *Xanthomonas campestris* ATCC 33913^T using clustal_x [44]. The sequences were manually edited for ambiguous bases and gaps using Bioedit [14]. Phylogenetic trees were calculated applying the algorithms maximum likelihood, maximum parsimony and neighbor joining and standard adjustments implemented in the PHYLIP package [10] were applied. In the maximum likelihood tree (Fig. 1) the two strains formed, together with *Ly. tolerans* UM1^T, a lineage deeply branching from the neighboring lineage with *Ly. enzymogenes* ATCC 29487^T, and clearly separate from *Lu. mephitis* DSM 12574^T. Also, the phylogenetic trees calculated applying the maximum parsimony and neighbor joining algorithms showed these branching patterns (results not shown). On the other hand, phylogenetic analyses did not suggest a very close relatedness to the type species of the genera *Luteimonas* or *Lysobacter*. It is worth to mention that only a minority of branchings were supported from bootstrap values (>70%) in all trees calculated.

In order to gain more genetic information concerning genus affiliation of strain 4284/11^T the partial *groEL* sequence of the isolate and the reference species *Ly. tolerans* DSM 28473^T, *Lu. aestuarii* DSM 19680^T, *Lu. mephitis* CIP 107229^T and *Ly. enzymogenes*

LMG 8762^T were analyzed. Forward primer Lut-groELf (5′ GAA CCC GAT GGA YCT SAA RCG 3′) and reverse primer Lut-groELr (5′ CCA TGY CRC CCA TRC CRC C 3′) applied for amplification of partial *groEL* genes were constructed on the basis of corresponding gene sequences of members of the family "*Xanthomonadaceae*", including five *Xanthomonas*, one *Stenotrophomonas* and two *Pseudoxanthomonas* species, accessible from gene banks. The PCR reaction mix consisted of 12.5 μl REDTaq ReadyMix PCR Reaction Mix with $MgCl_2$ (Sigma–Aldrich), 0.5 μl of each primer (10 μM), 1.0 μl DNA and 10.5 μl sterile water. Amplification started with an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 65.6–70.0 °C for 0.5 min and elongation at 72 °C for 1.5 min, and a final elongation at 72 °C for 5 min. The resulting PCR product was purified and sequenced as described above. After alignment in clustal_x [44] and manual editing in Bioedit [14] each of the sequences of the four strains consisted of a continuous stretch of 813 nucleotides. Comparison of the partial *groEL* sequences resulted in 100% sequence similarity between strains 4284/11^T and 812/17 suggesting that both strains are members of a single species. They showed highest similarity to *Lu. mephitis* CIP 107229^T (90.5%) and 89.2%, 89.1%, and 87.6% with *Ly. tolerans* DSM 28473^T, *Lu. aestuarii* DSM 19680^T, and *Ly. enzymogenes* LMG 8762^T, respectively (Supplementary Table S1), not indicating a close relationship to any of the reference species. In the corresponding amino acid sequence *Ly. tolerans* DSM 28473^T showed the highest similarity (96.6%) with the two python isolates, followed by *Lu. mephitis* (95.9%), *Lu. aestuarii* DSM 19680^T (94.8%), and *Ly. enzymogenes* LMG 8762^T (90.7%). The analysis of the *groEL* sequences suggest that similarities in the gene sequence less than 94.0% and in the GroEL amino acid sequence less than 97.0% are indicative for different species.

Gonçalves and Rosato [12] analyzed the intergenic transcribed spacer (ITS) between the genes encoding for 16S and 23S rRNA of 17 *Xanthomonas* species. They found that the total length varies between species from 492 to 578 nucleotides consisting of three non-coding sequences (ITS1, ITS2, ITS3) and two genes encoding for tRNA^{Ala} and tRNA^{Ile} separated by ITS2. Hence, we decided to analyze the complete16S-23S rRNA ITS of strain 4284/11^T, strain 812/17 and the four reference species. For amplification of the internal 16S/23S spacer (ITS) region the forward primer Lut-ITSf 5′ GTT CCC GGG CCT TGT ACA 3′ [15] and the reverse primer Lut-ITSr 5′ GGG TTY CCC CAT TCR GA 3′ [33] were applied binding to flanking regions in the 16S rRNA (1347–1364 *Escherichia coli* numbering; [6]) and 23S rRNA (114–130 *E. coli* numbering; [5]) encoding genes.

The PCR reaction mix consisted of 12.5 μl REDTaq ReadyMix PCR Reaction Mix with $MgCl_2$ (Sigma–Aldrich), 0.5 μl of each primer (10 μM), 1.0 μl DNA and 10.5 μl sterile water. Amplification started with an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 0.5 min and elongation at 72 °C for 1 min, and a final elongation at 72 °C for 5 min. The resulting PCR product was purified and sequenced as described above. The resulting PCR products were approximately 800–840 bp in length (results not shown) which is in the expected size range. No additional amplicons were visible indicating that either only one rRNA operon is present in the genomes or the different operons do not differ in length of the 16S–23S rRNA sequence. Obviously, possible different operons also do not differ in the sequence composition of the ITS because no ambiguous nucleotides were obtained during sequencing. Like in xanthomonads [12] the 16S–23S rRNA ITS sequences each contained three non-coding sequences (ITS1, ITS2, ITS3) and two genes encoding for tRNA^{Ala} and tRNA^{Ile}, respectively and in strains 4284/11^T and 812/17, *Ly. tolerans* DSM 28473^T *Lu. mephitis* CIP 107229^T and *Ly. enzymogenes* LMG 8762^T the orientation in the 16S-23S rRNA ITS was 5′-ITS1- tRNA^{Ala}-ITS2- tRNA^{Ile}-ITS3 whereas in *Lu. aestuarii* DSM 19680^T the two tRNA encoding genes occurred in reverse order. For comparison the sequences were edited for gaps and ambiguous nucleotides in Bioedit [14] and the order of the two tRNA encoding genes in the sequence of *Lu. aestuarii* DSM 19680^T were substituted with each other to correspond to the order in the other strains. The ITS sequences of strains 4284/11^T and 812/17 were identical and shared with *Ly. tolerans* DSM 28473^T, *Ly. enzymogenes* LMG 8762^T, *Lu. mephitis* CIP 107229^T, and *Lu. aestuarii* DSM 19680^T 89.8, 79.9, 80.8 and 79.7% (68.3% native order) similarity, respectively (Supplementary Table S2). These data support the close relatedness between the two python isolates and *Ly. tolerans* and the more distant relatedness to the other reference strains.

Besides *groEL* and ITS sequence analyses, the genome of strain 4284/11^T was sequenced *de novo*. Extraction of genomic DNA was carried out using the Qiagen QIAamp DNA Mini kit (protocol for bacteria from plate cultures). Prior to whole genome

Table 1
Genome sequencing, assembly and annotation information of strain 4284/11^T.

Genome sequencing	
Library construction (MIGS-28)	Illumina paired-end library of category IS1 [16]
Library construction kit^M	Illumina TruSeq DNA PCR-free low throughput Library Prep Kit
Sequencing kit	Illumina MiSeq Reagent Kit v3 600-cycle
Sequencing platform^M (MIGS-29)	Illumina MiSeq
Read quality control and filtering	
Quality control tools	FastQC v0.11.4 and NGS QC Toolkit v2.2.3 [32]
Number of high-quality read pairs	780,188
Length of high-quality read pairs	2 × 225 nucleotides
Genome assembly	
Assembler^M (MIGS-30)	SPAdes v2.5.1 [4]
Sequencing depth^M (MIGS-31.2)	~120-fold
Number of contigs^M (MIGS-31.3)	74
Minimum contig size	533 bp
N50 value^M	158,432 bp
Maximum contig size	285,773 bp
Assembly size^M	2,929,486 bp
Number of ambiguous bases	0 bp
DNA G + C^M	65.65 mol%
Genome assembly completeness and contamination	
Validation tool	CheckM v1.0.13 [31]
Validation strategy	Lineage-specific marker sets
Estimated completeness	99.24%
Estimated contamination^M	0.80%
Finishing quality (MIGS-31)	High-quality draft
Genome annotation	
Gene prediction tool (MIGS-32)	Prodigal v2.6.2 [17] as part of Prokka v1.13.3 [40]
RNA prediction tools	ARAGORN v1.2 [24], Barrnap v0.7 and Infernal v1.1.1 [28] as part of Prokka
Protein-coding genes	2595
RNA genes	79
tRNAs	53
tmRNAs	1
rRNAs	2 (5S and 16S)
ncRNAs	23
CRISPR prediction tool	MinCED v2.0 as part of Prokka
CRISPR repeats	2

MIGS specifications [11] are in parentheses. The web references of the tools Barrnap, FastQC, and MinCED are https://github.com/tseemann/barrnap, https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ and https://github.com/ctSkennerton/minced, respectively.
^{M}—Mandatory information according to the minimal standards proposed by Chun et al. [9].

Table 2
All-versus-all matrix of ANIb (upper triangular) and gANI (lower triangular) genomic similarities of strain 4284/11^T and all available *Luteimonas*/*Lysobacter* type strains.

Strain		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
4284/11^T	(1)		**76.2**	75.0	74.6	73.7	74.9	73.9	74.1	74.9	74.1	74.2	73.7	73.4	74.2	74.0	73.2
Ly. tolerans UM1^T	(2)	**78.0**		75.1	74.8	74.2	74.6	74.6	74.6	75.0	74.5	74.8	74.2	74.5	75.4	74.8	74.0
Lu. mephitis DSM 12574^T	(3)	76.9	73.1		77.5	78.3	79.9	77.1	76.6	77.9	76.7	76.3	75.9	76.0	74.4	76.5	75.4
Ly. enzymogenes ATCC 29487^T	(4)	76.5	73.1	79.3		77.3	78.8	77.9	84.1	81.0	79.6	79.6	78.7	77.9	74.0	78.4	76.5
Lu. abyssi XH031^T	(5)	75.7	71.9	76.2	75.0		81.0	76.7	74.4	75.5	74.4	74.3	74.4	74.2	72.8	74.2	73.8
Lu. huabeiensis HB2^T	(6)	76.9	72.8	78.0	76.5	82.7		78.0	75.8	77.6	76.6	76.0	76.3	75.3	73.8	75.7	75.5
Lu. rhizosphaerae 4-12^T	(7)	76.0	72.1	79.3	75.7	78.7	79.8		75.1	76.0	75.0	74.6	74.3	74.8	73.2	75.0	73.9
Ly. antibioticus ATCC 29479^T	(8)	76.3	72.7	78.7	82.0	76.8	78.0	77.5		78.2	77.2	77.1	75.7	75.7	73.5	77.9	76.0
Ly. arseniciresistens ZS79^T	(9)	76.9	72.5	80.1	78.9	77.9	79.9	78.6	80.3		80.0	77.9	78.9	75.5	73.6	77.8	79.4
Ly. concretionis Ko07^T	(10)	75.9	72.1	78.7	77.5	76.8	78.6	77.4	79.5	82.2		76.7	77.5	74.7	73.1	76.6	78.1
Ly. daejeonensis GH1-9^T	(11)	76.1	72.5	78.3	77.7	76.7	77.9	77.1	79.3	80.1	78.8		75.8	75.1	73.2	77.2	75.8
Ly. defluvii DSM 18482^T	(12)	76.0	71.5	78.2	76.5	77.1	78.8	77.2	78.0	81.3	79.5	78.2		73.8	72.2	75.3	76.7
Ly. dokdonensis DS-58^T	(13)	75.6	71.9	77.9	76.3	76.3	77.4	77.2	77.6	77.9	76.8	77.3	76.4		73.2	75.9	73.7
Ly. silvestris AM20-91^T	(14)	76.3	72.9	76.4	75.8	75.1	75.6	75.5	75.7	76.1	75.2	75.5	74.9	75.5		73.5	72.3
Ly. soli KCTC 22011^T	(15)	76.0	72.4	78.6	80.4	76.6	77.8	77.4	80.0	80.1	78.8	79.4	77.8	78.0	75.8		75.4
Ly. spongiicola DSM 21749^T	(16)	75.3	71.5	77.7	78.6	76.3	77.8	76.6	78.3	81.6	80.1	78.2	79.0	76.2	74.9	78.1	

ANIb values were estimated using JSpeciesWS [36], whereas gANI values were calculated with the Microbial Species Identifier Software [48]. Values represent the average of bidirectional comparison. Comparison values of strain 4284/11^T to its most closely related species *Ly. tolerans* UM1^T are highlighted in bold.

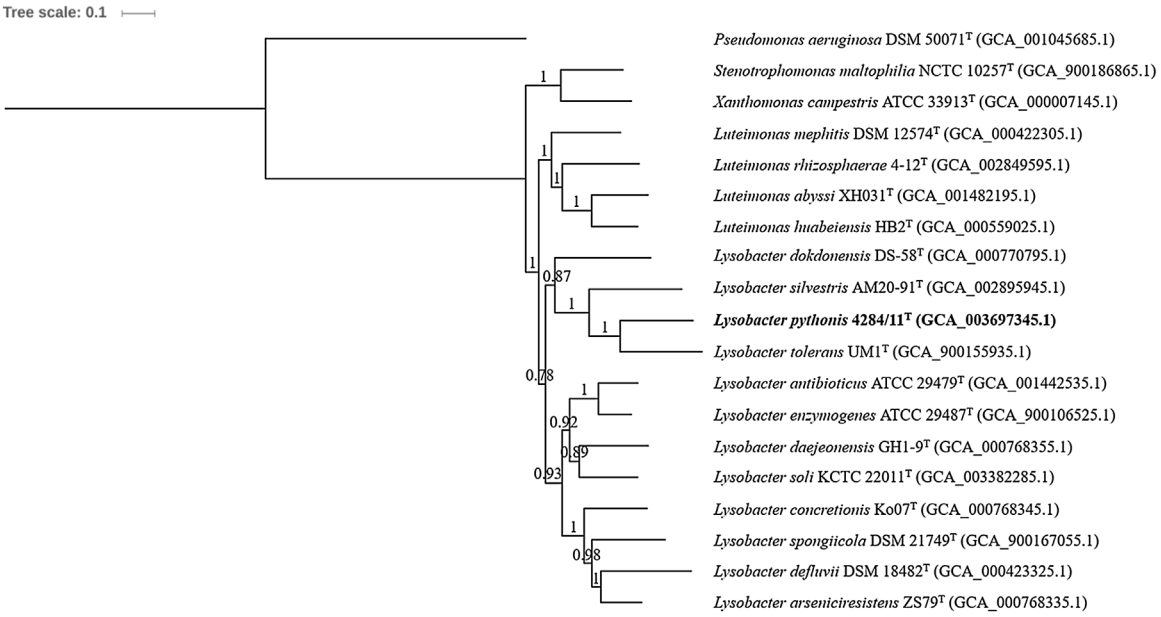

Fig. 2. Maximum-likelihood tree based on 133,256 polymorphic positions (not considering gaps and missing data) in the multiple sequence alignment of 267 predicted protein-coding core genes. The phylogeny shows the relatedness of strain 4284/11^T (bold) to all available *Luteimonas*/*Lysobacter* type strains as well as *S. maltophilia* NCTC 10257^T and *X. campestris* ATCC 33913^T, which are the type strains of the type species of genera *Stenotrophomonas* and *Xanthomonas*, respectively. GenBank assembly accessions are given in brackets. *P. aeruginosa* DSM 50071^T was used as outgroup. Phylogenetic reconstruction was performed with 200 bootstrap replicates. Bootstrap fractions are given at nodes. Bar indicates 0.1 substitutions per nucleotide position.

sequencing on the Illumina MiSeq system using a v3 600-cycle kit, IS1-library preparation was performed as described elsewhere [16]. Quality control, trimming and filtering of raw read data was done with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and the NGS QC Toolkit [32]. High quality read pairs of length 2 × 225 nts were assembled using SPAdes [4], reaching a sequencing depth of ~120-fold. The authenticity of the resulting draft genome assembly was confirmed by comparison of the 16S sequence obtained via Sanger sequencing (LM994044) to the 16S sequence extracted from the assembly by the use of RNAmmer [22]. With the help of the CheckM software [31] the completeness of the assembly and its degree of contamination was estimated at 99.2% and 0.8%, respectively. Consequently, the assembled genome is nearly complete exhibiting a very low amount of contamination. Furthermore, it is 2,929,486 bp in size, comprises 74 contigs with a N50 value of 158,432 bp and has a G + C-content of 65.65 mol%. Hence, the size of the genome of *Ly. tolerans* UM1^T is almost 0.4 Mbp smaller and its G + C-content is approximately 4 mol% smaller. Genome annotation with Prokka [40] predicted 2674 genes, of which 2595 are protein-coding and 79 correspond to RNA genes. A detailed summary on the sequencing, assembly and annotation of strain 4284/11^T is given in Table 1.

During the last decade experimental DNA-DNA hybridization (DDH) was increasingly replaced by *in silico* indices estimating genomic relatedness. Two such indices are the widely used Average Nucleotide Identity calculated by BLAST (ANIb) [2,13] and the genome-wide Average Nucleotide Identity (gANI), which is estimated based on all orthologous protein coding genes that are shared by any two prokaryotic isolates [48]. Comparisons of all available *Luteimonas*/*Lysobacter* type strain genomes in the NCBI Assembly database (as of September 06, 2018) to strain 4284/11^T clearly show no relatedness at species level, since resulting ANIb and gANI values (Table 2) are far below the proposed species delineation thresholds of 95–96% [35] and 96.5% [48], respectively. With

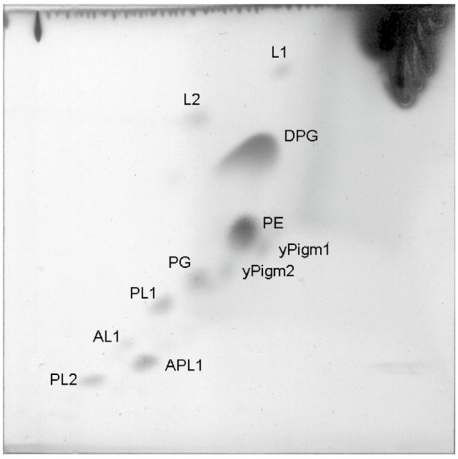

Fig. 3. Polar lipid profile of strain 4284/11^T after 2-dimensional thin layer chromatography (1st dimension: chloroform:methanol:water 65:25:4; chloroform:methanol:acetic acid:water 80:12:15:4) and staining with 5% ethanolic molybdatophosphoric acid.
Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL1, PL2, unidentified phospholipids; APL1, unidentified aminophospholipid; AL1, unidentified aminolipid; L1, L2, unidentified lacking a functional group; yPigm1, yPigm2, yellow pigments. yPigm2 contains an amino group.

similarities of 76.2% (ANIb) and 78% (gANI) strain 4284/11^T is most closely related to *Ly. tolerans* UM1^T. Therefore, based on ANIb and gANI analyses strain 4284/11^T represents a new genomospecies.

To infer the genus affiliation of strain 4284/11^T a core gene multiple sequence alignment based on Prokka [40] genome annotations of all available *Luteimonas/Lysobacter* type strain genomes including genome annotations of *S. maltophilia* NCTC 10257^T, *X. campestris* ATCC 33913^T and *Pseudomonas aeruginosa* DSM 50071^T was calculated with Roary [29]. The resulting alignment comprised 267 predicted protein-coding core genes. Sequence similarities among all investigated strains are depicted in Supplementary Table S3. At both nucleotide and protein sequence level strain 4284/11^T is almost equally similar to the type strains of the genera *Luteimonas* (80.9% and 81.8%, respectively) and *Lysobacter* (80.9% and 81.3%, respectively). After using SNP-Sites [30] to reduce the core gene alignment to contain polymorphic positions only, a maximum-likelihood phylogeny based on the general time-reversible model [43] with gamma distributed rate heterogeneity (5 categories) and a significant proportion of invariable sites (GTR + G + I) was reconstructed via the MEGA X software [21]. Tree visualization was done with the interactive Tree Of Life (iTOL) online tool [25] using *P. aeruginosa* DSM 50071^T to root the phylogeny (Fig. 2). In the maximum-likelihood phylogeny strain 4284/11^T is most closely related to *Ly. tolerans* UM1^T and located within the monophyletic group containing *Ly. enzymogenes* ATCC 29487^T. Both observations together with the fact that *Ly. tolerans* UM1^T was only recently reclassified to the genus *Lysobacter* based on phylogenetic analysis of 400 universally conserved proteins [27] clearly demonstrate the membership of strain 4284/11^T to the genus *Lysobacter*.

The data from *groEL* and ITS sequence analyses strongly suggest, that the isolates 4284/11^T and 812/17 are strains of the same species. In order to support the suggested relationship at species level, the two python isolates and *Ly. tolerans* DSM 28473^T were subjected to genomic fingerprinting after ERIC-, REP and RAPD-PCR. PCR conditions were described by Loncaric et al. [26]. The sequences of the RAPD primers were RAPD1: GTGGATGCGA and RAPD2: AGCGGGCCAA. ERIC-, REP- and RAPD1-PCR profiles of

Table 3
Polyamine patterns of strain 4284/11^T and the reference strains, *Lu. mephitis* CIP 107229^T, *Ly. enzymogenes* LMG 8762^T and *Lu. aestuarii* DSM 19680^T.

Strain	DAP	PUT	CAD	SPD	SPM
	μmol g (dry weight)$^{-1}$				
4284/11^T	0.1	0.1	0.1	49.2	2.4
812/17	–	0.1	–	64.5	1.3
Ly. tolerans DSM 28473^T	–	0.1	–	32.9	1.8
Lu. aestuarii DSM 19680^T	t	0.1	–	32.1	4.1
Lu. mephitis CIP 107229^T	2.7	0.1	0.1	36.6	1.7
Ly. enzymogenes LMG 8762^T	–	0.1	t	63.0	2.8

Abbreviations: DAP, 1,3-diaminopropane; PUT, putrescine; CAD, cadaverine; SPD, spermidine; SPM, spermine; t = traces [<0.05 μmol g (dry weight)$^{-1}$].

Table 4
Fatty acid patterns (percentages) of strain (1) 4284/11^T, (2) 812/17 and the reference strains, (3) *Lu. mephitis* CIP 107229^T, (4) *Ly. enzymogenes* LMG 8762^T, (5) *Lu. aestuarii* DSM 19680^T and (6) *Ly. tolerans* DSM 28473^T. All data from this study.

Fatty acids	1	2	3	4	5	6
iso-$C_{11:0}$	4.7	5.1	4.8		6.2	
iso-$C_{12:0}$	0.6					
iso-$C_{11:0}$ 3OH	6.4	6.2	5.7	5.7	5.5	6.2
$C_{11:0}$ 2OH				0.5		
iso-$C_{14:1}$ E				0.9		
iso-$C_{14:0}$	2.7	1.2	1.0			
$C_{14:0}$			0.7	0.7		
$C_{13:0}$ 2OH				7.7		
iso-$C_{13:0}$			0.5			
iso-$C_{15:1}$ F	2.3	2.2	3.2			1.2
iso-$C_{15:0}$	32.2	32.2	48.9	31.3	26.6	25.1
anteiso-$C_{15:0}$	0.9	0.9	3.4		5.1	
$C_{16:1}\omega7c$ alcohol				5.8		
$C_{16:0}$ N alcohol				1.6		
iso-$C_{16:0}$	15.9	10.9	2.8	0.5	7.7	10.8
$C_{16:0}$ 3OH			0.4			
Sum in feature 3[a]	0.8	0.8	1.2	17.0	1.0	
$C_{16:0}$	1.4	1.9	1.6	7.9	2.4	1.5
iso-$C_{17:1}\omega9c$	24.3	27.9	19.4	10.3	25.7	31.6
iso-$C_{17:0}$	7.8	11.4	6.3	7.1	18.7	23.5
anteiso-$C_{17:0}$					0.9	
$C_{18:1}\omega7c$				2.1		
$C_{18:0}$				0.8		

[a] Summed feature 3 comprises $C_{18:1}\omega7c$ and/or *iso*-$C_{15:0}$ 2OH.

strains 4284/11^T and 812/17 were undistinguishable but clearly different from the profile of *Ly. tolerans* DSM 28473^T (Supplementary Fig. S1). These data confirm the other results that strains 4284/11^T and 812/17 are members of the same species. However, in a RAPD 2-PCR the two python isolates could be distinguished based on the additional presence of faint bands at approximately 3 kb and 1.8 kb in the genomic fingerprint of strain 812/17 demonstrating that the two strains are not clonally related.

For analyses of quinones, polar lipids and polyamines cells were grown in PYE broth (0.3% peptone from casein, 0.3% yeast extract, pH 7.2) or 3.3xPYE broth (1% peptone from casein, 1% yeast extract, pH 7.2). Quinones and polar lipids were extracted from approximately 100 mg of lyophilized biomass from cells harvested at the stationary growth phase and analyzed as described by Tindall [45,46] and Altenburger et al. [1]. Lyophilized biomass subjected to polyamine extraction and analysis was harvested at the late exponential growth phase as recommended by Busse and Auling [7]. HPLC conditions applied for polyamine analyses were reported by Busse et al. [8]. HPLC equipment used for quinone and polyamine analyses was described by Stolz et al. [41]. Fatty acids were analyzed according to the standardized procedures described previously [19].

The quinone systems of strains 4284/11^T and 812/17 contained the major ubiquinone Q-8 and 1% Q-9 or 2% Q-7 which is most similar to that of *Ly. tolerans* [34], and other representatives of

Table 5
Physiological test results of strain (1) 4284/11^T and (2) 812/17 (this study) and the reference strains, (3) *Lu. mephitis* CIP 107229^T (this study), (4) *Ly. enzymogenes* LMG 8762^T (this study), (5) *Lu. aestuarii* DSM 19680^T (this study), (6) *Ly. tolerans* DSM 28473^T (this study), (7) *Lu. vadosa* KMM 9005^T [38], (8) *Lu. cucumeris* [38,42], (9) *Lu. terricola* BZ92r^T [51]. +, positive; –, negative; Nd, not determined.

	1	2	3	4	5	6	7	8	9
Hydrolysis of:									
pNP-α-D-Glucopyranoside (α-glucosidase)	–	–	–	–	+	–	–[a]	–[a]	–[a]
pNP-ß-D-Glucopyranoside (ß-glucosidase)	–	–	–	–	+	+	–[a]	+[a]	–[a]
pNP-ß-D-Xylopyranoside	–	–	–	–	+	–	Nd	Nd	Nd
pNP-Phenyl-phosphonate	+	+	+	–	+	+	Nd	Nd	Nd
pNP-Phosphoryl-choline	+	+	+	–	–	–	Nd	Nd	Nd
2-Deoxythymidine-5′-thymidine-pNP-phosphate	(+)	(+)	+	–	+	+	Nd	Nd	Nd
Esculin	–	–	–	–	–	–	+	+	–
Assimilation of:									
N-Acetyl-D-galactosamine	–	–	–	+	–	–	Nd	Nd	Nd
N-Acetyl-D-glucosamine	–	–	–	+	+	–	–	+	–
p-Arbutin	–	–	–	+	–	–	Nd	Nd	Nd
D-Cellobiose	–	–	–	+	+	–	Nd	Nd	Nd
D-Fructose	–	–	–	+	–	–	Nd	Nd	Nd
D-Glucose	–	–	–	+	+	+	–	±[b]	–
D-Mannose	–	–	–	+	+	–	–	–	–
D-Maltose	–	–	–	+	–	+	–	–	–
α-D-Melibiose	–	–	–	+	–	–	–	+	Nd
D-Ribose	–	–	–	–	+	–	–	Nd	Nd
Sucrose	–	–	–	+	–	–	–	Nd	Nd
Salicin	–	–	–	+	–	–	-	Nd	Nd
D-Trehalose	–	–	–	+	–	–	Nd	Nd	Nd
D-Xylose	–	–	–	–	+	–	Nd	Nd	Nd
Maltitol	–	–	–	+	–	+	Nd	Nd	Nd
Acetate	+	+	+	+	+	–	–	Nd	Nd
Propionate	–	–	–	–	+	–	Nd	Nd	Nd
trans-Aconitate	+	+	–	–	–	–	Nd	Nd	Nd
Citrate	(+)	(+)	–	+	–	–	–	±[b]	–
Fumarate	–	–	–	+	+	+	Nd	Nd	Nd
DL-Lactate	+	+	(+)	–	–	–	–	Nd	Nd
L-Malate	–	–	–	+	+	–	–	–	–
Mesaconate	–	–	–	–	–	–	Nd	Nd	Nd
Oxoglutarate	+	+	–	+	–	–	Nd	Nd	Nd
Pyruvate	+	+	(+)	+	–	–	Nd	Nd	Nd
L-Aspartate	–	–	–	+	–	–	Nd	Nd	Nd
L-Proline	–	–	–	+	+	+	–	+	Nd
L-Serine	–	–	–	+	+	+	–	Nd	Nd

The six strains analysed in this study were negative for: acid production from: glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, l-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl-D-glucoside, erythritol, melibiose, D-arabitol, and D-mannose; they were negative for the hydrolysis of: esculin, oNP-ß-D-galactopyranoside, pNP-ß-D-glucuronide, L-glutamate-γ-3-carboxy-pNA and L-proline-pNA and for the assimilation of: L-arabinose, D-galactose, gluconate, and L-rhamnose, D-mannitol, D-sorbitol, putrescine, D-adipate, 4-aminobutyrate, azelate, suberate, L-alanine, ß-alanine, Glutarate, DL-3-hydroxybutyrate, histidine, L-leucine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate, and L-phenylacetate. The four strains were positive for the hydrolysis of: bis-pNP-phosphate and L-alanine-pNA.

[a] Tested applying API-ZYM.
[b] Differing results reported by Romanenko et al. [38] and Sun et al. [42].

the genera *Lysobacter* and *Luteimononas*. The polar lipid profile of 4284/11^T consisted of the major compounds diphosphatidylglycerol and phosphatidylethanolamine, moderate amounts of phosphatidylglycerol and minor to trace amounts of two unidentified phospholipids (PL1, PL2), an unidentified aminophospholipid (APL1), an unidentified aminolipid (AL1), and two unidentified lipids (L1, L2) only detectable after total lipid staining indicating that a functional group is absent (Fig. 3). Furthermore, two spots with yellow pigmentation were detected (yPigm1, yPigm2). Since yPigm2 was also positive after staining with ninhydrin the presence of an aminogroup is indicated. The lipid profile of strain 812/17 was almost undistinguishable from that of 4284/11^T differing only in negligible relative amounts of some minor lipids (results not shown). Concerning lipids present in major or moderate amounts strains 4284/11^T and 812/17 were similar to *Ly. tolerans*, *Lu. mephitis* CIP 107229^T, *Ly. enzymogenes* LMG 8762^T and *Lu. aestuarii* DSM 19680^T, whereas several differences were detected in presence/absence of some minor lipids. Most striking differences were the presence of a glycolipid in *Lu. mephitis* CIP 107229^T and phosphatidylmonomethylethanolamine in *Ly. enzymogenes* LMG 8762^T (results not shown).

The polyamine patterns of strain 4284/11^T, 812/17 and the reference strains *Ly. tolerans* DSM 28473^T, *Lu. mephitis* CIP 107229^T, *Lu. aestuarii* DSM 19680^T and *Ly. enzymogenes* LMG 8762^T all exhibited a profile containing predominantly spermidine and minor to trace amounts of 1,3-diaminopropane, putrescine, cadaverine and/or spermine (Table 3). This polyamine pattern is rather similar to those of other members of the *Xanthomonadaceae* including species of the genera *Xanthomonas, Fulvimonas* and *Pseudofulvimonas* [3,20].

The fatty acid profile of strain 4284/11^T and 812/17 consisted mainly of iso-branched fatty acids, including *iso*-$C_{11:0}$ 3OH, *iso*-$C_{15:0}$, and *iso*-$C_{17:0}$ and the unsaturated fatty acid *iso*-$C_{17:1}\omega9c$ (Table 4). The fatty acid profiles of *Lu. mephitis*, *Lu. aestuarii*, *Ly. enzymogenes* and *Ly. tolerans* comprised also the major acids *iso*-$C_{11:0}$ 3OH, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*-$C_{17:0}$ and *iso*-$C_{17:1}$ ω9c. The relatively high content of *iso*-$C_{16:0}$ distinguished strains 4284/11^T and 812/17 from the reference species (Table 3) except *Ly. tolerans*. Absence of $C_{13:0}$ 2OH, $C_{16:1}$ ω7c alcohol, and low amounts of Sum in feature 3 and $C_{16:0}$ distinguished strains 4284/11^T and 812/17 from *Ly. enzymogenes* LMG 8762^T the type species of the genus to which they are next related. Differences in relative amounts of certain fatty acid

clearly distinguished strains 4284/11^T and 812/17 from *Ly. tolerans* including iso-$C_{17:0}$, *iso*-$C_{17:1}\omega 9c$ and *iso*-$C_{15:0}$.

Physiological phenotyping was carried out according to Kämpfer et al. [18]. The detailed results are given in Table 5 in comparison to the results of the type strains of the most closely related species. Several tests allowed a clear differentiation of strains 4284/11^T and 812/17 from the reference species. Growth on Sabouraud agar, MacConkey agar and Columbia agar with 5% sheep blood was studied using ready-to-use plates (Becton Dickinson). The SIM test was prepared and carried out according to the instructions of the manufacturer (Merck). For Gram-staining the Gram-color Stain set from Merck was used.

Phylogenetic analyses suggested a closer association of strains 4284/11^T and 812/17 with the type species of *Lysobacter*, *Ly. enzymogenes* LMG 8762^T, than with *Lu. mephitis* CIP 107229^T. *Ly. tolerans* DSM 28473^T appeared to be the next related species and the branching node was supported by a moderate bootstrap value (62%). This close relationship was supported by highest 16S rRNA gene sequence, 16S/23S ITS sequence similarity, the partial GroEL amino acid sequence and genomic similarity, as well. Only in the partial *groEL* nucleotide sequence *Lu. mephitis* CIP 107229^T showed a slightly higher similarity (90.5%) than *Ly. tolerans* DSM 28473^T (89.2%).

Quinone system, polar lipid profile and polyamine pattern are well in agreement with the characteristics of the reference species. However, in the polar lipid profiles strains 4284/11^T and 812/17 were most similar to *Ly. tolerans* DSM 28473^T and *Lu. aestuarii* DSM 19680^T whereas the presence of a glycolipid and phosphatidylethanolamine distinguished from *Lu. mephitis* CIP 107229^T and *Ly. enzymogenes* LMG 8762^T, respectively. The sum of data here presented indicate that strains 4284/11^T and 812/17 are strains of a novel species of the genus *Lysobacter* for which we propose the name *Lysobacter pythoni* sp. nov. with the type strain 4284/11^T (= CCM 8829^T = CCUG 72164^T = LMG 30630^T). A second strain of the species is 812/17 (= CCM 8830).

Description of *Lysobacter pythonis*

pythonis (py.tho'nis. N.L. gen. n. *pythonis* of the python, a constrictor snake genus).

Cells are Gram-stain negative rod. Colonies are yellow pigmented. Cells grow well on PYE agar and broth, PYE agar supplemented with 3% PYE agar supplemented with 3% sea salts (w/v) and 5% sheep blood agar, weakly on Sabouraud agar but not on MacConkey agar. Hemolysis is not observed. In the SIM test it is positive for motility and weakly for sulfide production but negative for indol production. Hydrolyses pNP-phenyl-phosphonate, pNP-phosphoryl-choline and 2-deoxythymidine-5′-thymidine-pNP-phosphate (weakly) but not pNP-α-D-glucopyranoside (α-glucosidase), pNP-ß-D-glucopyranoside (ß-glucosidase), pNP-ß-D-xylopyranoside and esculin. Acetate, *trans*-aconitate, citrate (weakly), dl-lactate, oxoglutarate and pyruvate are assimilated but not *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, p-arbutin, D-cellobiose, D-fructose, D-glucose, D-mannose, D-maltose, α-D-melibiose, D-ribose, sucrose, salicin, D-trehalose, D-xylose, maltitol, propionate, fumarate, L-malate, mesaconate, L-aspartate, L-proline or L-serine. Major fatty acids are *iso*-$C_{11:0}$ 3OH, iso-$C_{15:0}$, *iso*-$C_{17:0}$ and *iso*-$C_{17:1}\omega 9c$. The quinone system is ubiquinone Q-8. In the polyamine pattern spermidine is predominant. The polar lipid profile comprises the major compounds diphosphatidylglycerol and phosphatidylethanolamine, moderate amounts of phosphatidylglycerol and minor to trace amounts of two unidentified phospholipids (PL1, PL2), an unidentified aminophospholipid (APL1), an unidentified aminolipid and two unidentified lipids (L1, L2) lacking a functional group. Two yellow pigment spots are visible in the polar lipid profile as well.

The G + C-content is 65.65 mol% (genome)

Isolated from the respiratory tract of royal python.

The type strain is 4284/11^T (= CCM 8829^T = CCUG 72164^T = LMG 30630^T). A second strain of the species is 812/17 (= CCM 8830).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.syapm.2019.02.002.

References

[1] Altenburger, P., Kämpfer, P., Makristathis, A., Lubitz, W., Busse, H.-J. (1996) Classification of bacteria isolated from a medieval wall painting. J. Biotechnol. 47, 39–52.
[2] Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J.H., 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.
[3] Auling, G., Busse, H.-J., Pilz, F., Webb, L., Kneifel, H., Claus, D. (1991) Rapid differentiation, by polyamine analysis, of *Xanthomonas* strains from phytopathogenic pseudomonads and other members of the class *Proteobacteria* interacting with plants. Int. J. Syst. Bacteriol. 41, 223–228.
[4] Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477.
[5] Brosius, J., Dull, T.J., Noller, H.F. (1980) Complete nucleotide sequence of a 23S ribosomal RNA gene from *Escherichia coli*. Proc. Natl. Acad. Sci U. S. A. 77, 201–204.
[6] Brosius, J., Palmer, M.L., Kennedy, P.J., Noller, H.F. (1978) Complete nucleotide-sequence of a 16S ribosomal-RNA gene from *Escherichia coli*. Proc. Natl. Acad. Sci U. S. A. 75, 4801–4805.
[7] Busse, J., Auling, G. (1988) Polyamine pattern as a chemotaxonomic marker within the Proteobacteria. Syst. Appl. Microbiol. 11, 1–8.
[8] Busse, H.-J., Bunka, S., Hensel, A., Lubitz, W. (1997) Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. Int. J. Syst. Evol. Microbiol. 47, 698–708.
[9] Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D.R., da Costa, M.S., Rooney, A.P., Yi, H., Xu, X.W., De Meyer, S., Trujillo, M.E. (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int. J. Syst. Evol. Microbiol. 68, 461–466.
[10] Felsenstein, J. 2013 PHYLIP (Phylogeny Inference Package) Version 3.695, Department of Genome Sciences, University of Washington., Seattle, WA.
[11] Field, D., Garrity, G., Gray, T., Morrison, N., Selengut, J., Sterk, P., Tatusova, T., Thomson, N., Allen, M.J., Angiuoli, S.V., Ashburner, M., Axelrod, N., Baldauf, S., Ballard, S., Boore, J., Cochrane, G., Cole, J., Dawyndt, P., De Vos, P., DePamphilis, C., Edwards, R., Faruque, N., Feldman, R., Gilbert, J., Gilna, P., Glöckner, F.O., Goldstein, P., Guralnick, R., Haft, D., Hancock, D., Hermjakob, H., Hertz-Fowler, C., Hugenholtz, P., Joint, I., Kagan, L., Kane, M., Kennedy, J., Kowalchuk, G., Kottmann, R., Kolker, E., Kravitz, S., Kyrpides, N., Leebens-Mack, J., Lewis, S.E., Li, K., Lister, A.L., Lord, P., Maltsev, N., Markowitz, V., Martiny, J., Methe, B., Mizrachi, I., Moxon, R., Nelson, K., Parkhill, J., Proctor, L., White, O., Sansone, S.A., Spiers, A., Stevens, R., Swift, P., Taylor, C., Tateno, Y., Tett, A., Turner, S., Ussery, D., Vaughan, B., Ward, N., Whetzel, T., San Gil, I., Wilson, G., Wipat, A. (2008) The minimum information about a genome sequence (MIGS) specification. Nat. Biotechnol. 26, 541–547.
[12] Gonçalves, E.R., Rosato, Y.B. (2002) Phylogenetic analysis of *Xanthomonas* species based upon 16S–23S rDNA intergenic spacer sequences. Int. J. Syst. Evol. Microbiol. 52, 355–361.
[13] Goris, J., Konstantinidis, K.T., Klappenbach, J.A., Coenye, T., Vandamme, P., Tiedje, J.M. (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int. J. Syst. Evol. Microbiol. 57, 81–91.
[14] Hall, T.A. 1999 BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/ NT, 41, pp. , 95–98.
[15] Honeycutt, R.J., Sobral, B.W., McClelland, M. (1995) tRNA intergenic spacers reveal polymorphisms diagnostic for *Xanthomona albilineans*. Microbiology 141, 3229–3239.

[16] Huptas, C., Scherer, S., Wenning, M. (2016) Optimized illumina PCR-free library preparation for bacterial whole genome sequencing and analysis of factors influencing de novo assembly. BMC Res. Notes 9, 269.
[17] Hyatt, D., Chen, G.L., Locascio, P.F., Land, M.L., Larimer, F.W., Hauser, L.J. (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatic 11, 119.
[18] Kämpfer, P., Steiof, M., Dott, W. (1991) Microbiological characterisation of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. Microb. Ecol. 21, 227–251.
[19] Kämpfer, P., Kroppenstedt, R.M. (1996) Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. Can. J. Microbiol. 42, 989–1005.
[20] Kämpfer, P., Martin, E., Loddeers, N., Langer, S., Schumann, P., Jäckel, U., Busse, H.-J. (2009) *Pseudofulvimonas gallinarii* gen. nov., sp. nov., a new member of the family *Xanthomonadaceae*. Int. J. Syst. Evol. Microbiol. 60, 1427–1431.
[21] Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.
[22] Lagesen, K., Hallin, P., Rødland, E.A., Stærfeldt, H.-H., Rognes, T., Ussery, D.W. (2007) RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35, 3100–3108.
[23] Lane, D.J. (1991) 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), Nucleic Acid Techniques in Bacterial Systematics, John Wiley & Sons New York, pp. 115–175.
[24] Laslett, D., Canback, B. (2004) ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res. 32, 11–16.
[25] Letunic, I., Bork, P. (2016) Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res. 44, W242–W245.
[26] Loncaric, I., Heigl, H., Licek, E., Moosbeckhofer, R., Busse, H.-J., Rosengarten, R. (2009) Typing of *Pantoea agglomerans* isolated from colonies of honey bees (*Apis mellifera*) and culturability of selected strains from honey. Apidologie 40, 40–54.
[27] Margesin, R., Zhang, D.-C., Albuquerque, L., Froufe, H.J.C., Egas, C., da Costa, M.S. (2018) *Lysobacter silvestris* sp. nov., isolated from alpine forest soil, and reclassification of *Luteimonas tolerans* as *Lysobacter tolerans* comb. nov. Int. J. Syst. Evol. Microbiol. 68, 1571–1577.
[28] Nawrocki, E.P., Eddy, S.R. (2013) Infernal 1.1: 100-fold faster RNA homology searches. Bioinformatics 29, 2933–2935.
[29] Page, A.J., Cummins, C.A., Hunt, M., Wong, V.K., Reuter, S., Holden, M.T.G., Fookes, M., Falush, D., Keane, J.A., Parkhill, J. (2015) Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 31, 3691–3693.
[30] Page, A.J., Taylor, B., Delaney, A.J., Soares, J., Seemann, T., Keane, J.A., Harris, S.R. (2016) *SNP-sites*: rapid efficient extraction of SNPs from multi-FASTA alignments. Microb. Genom. 2, e000056.
[31] Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., Tyson, G.W. (2015) CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 25, 1043–1055.
[32] Patel, R.K., Jain, M. (2012) NGS QC Toolkit: a toolkit for quality control of next generation sequencing data. PLoS One 7, e30619.
[33] Quatrini, P., Scaglione, G., Cardinale, M., Caradonna, F., Puglia, A.M. (2002) *Bradyrhizobium* sp: nodulating the Mediterranean shrub Spanish broom (*Spartium junceum* L.). J. Appl. Microbiol. 92, 13–21.
[34] Rani, P., Mukherjee, U., Verma, H., Kamra, K., Lal, R. (2016) *Luteimonas tolerans* sp nov., isolated from hexachlorocyclohexane-contaminated soil. Int. J. Syst. Evol. Microbiol. 66, 1851–1856.
[35] Richter, M., Rosselló-Móra, R. (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc. Natl. Acad. Sci. U. S. A. 106, 19126–19131.
[36] Richter, M., Rosselló-Móra, R., Glöckner, O.F., Peplies, J. (2016) JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32, 929–931.
[37] Roh, S.W., Kim, K.-H., Nam, Y.-D., Chang, H.-W., Kim, M.-S., Yoon, J.-H., Oh, H.-M., Bae, J.-W. (2008) *Luteimonas aestuarii* sp nov., isolated from tidal flat sediment. J. Microbiol. 46, 525–529.
[38] Romanenko, L.A., Tanaka, N., Svetashev, V.I., Kurilenko, V.V., Mikhailov, V.V. (2013) *Luteimonas vadosa* sp nov., isolated from seashore sediment. Int. J. Syst. Evol. Microbiol. 63, 1261–1266.
[39] Saddler, G.S., Bradbury, J.F. (2014) Family I. Xanthomonadaceae fam. nov, in: Brenner, D.J., Krieg, N.R., Staley, J.T., Garrity, G.M. (Eds.), Bergey's Manual of Systematic Bacteriology, vol. 2, 2nd edn., p. 65.
[40] Seemann, T. (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069.
[41] Stolz, A., Busse, H.-J., Kämpfer, P. (2007) *Pseudomonas knackmussii* sp. nov. Int. J. Syst. Evol. Microbiol. 57, 572–576.
[42] Sun, Z.-B., Zhang, H., Yuan, X.-F., Wang, Y.-X., Feng, D.-M., Wang, Y.-H., Feng, Y.J. (2012) *Luteimonas cucumeris* sp. nov.: isolated a from cucumber leaf. Int. J. Syst. Evol. Microbiol. 62, 2916–2920.
[43] Tavaré, S. 1986 Some probabilistic and statistical problems in the analysis of DNA sequences Lectures on Mathematics in the Life Sciences, vol. 17, American Mathematical Society Providence, Rhode Island, pp. , 57–86.
[44] Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.
[45] Tindall, B.J. (1990) A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. Syst. Appl. Microbiol. 13, 128–130.
[46] Tindall, B.J. (1990) Lipid composition of *Halobacterium lacusprofundi*. FEMS Microbiol. Lett. 66, 199–202.
[47] Tindall, B.J. (2014) The family name *Solimonadaceae* Losey et al. 2013 is illegitimate, proposals to create the names '*Sinobacter soli*' comb. nov. and '*Sinobacter variicoloris*' contravene the Code, the family name *Xanthomonadaceae* Saddler and Bradbury 2005 and the order name *Xanthomonadales* Saddler and Bradbury 2005 are illegitimate and notes on the application of the family names *Solibacteraceae* Zhou et al. 2008, *Nevskiaceae* Henrici and Johnson 1935 (Approved Lists 1980) and *Lysobacteraceae* Christensen and Cook 1978 (Approved Lists 1980) and order name *Lysobacteriales* Christensen and Cook 1978 (Approved Lists 1980) with respect to the classification of the corresponding type genera *Solibacter* Zhou et al. 2008, *Nevskia* Famintzin 1892 (Approved Lists 1980) and *Lysobacter* Christensen and Cook 1978 (Approved Lists 1980) and importance of accurately expressing the link between a taxonomic name, its authors and the corresponding description/circumscription/emendation. Int. J. Syst. Evol. Microbiol. 64, 293–297.
[48] Varghese, N.J., Mukherjee, S., Ivanova, N., Konstantinidis, K.T., Mavrommatis, K., Kyrpides, N.C., Pati, A. (2015) Microbial species delineation using whole genome sequences. Nucleic Acids Res. 43, 6761–6771.
[49] Verma, A., Ojha, A.K., Kumari, P., Sundharam, S.S., Mayilraj, S., Krishnamurthi, S. (2016) Luteimonas padinae sp. nov., an epiphytic bacterium isolated from an intertidal macroalga. Int. J. Syst. Evol. Microbiol. 66, 5444–5451.
[50] Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J. (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA and whole genome assemblies. Int. J. Syst. Evol. Microbiol. 67, 1613–1617.
[51] Zhang, D.-C., Liu, H.-C., Xin, Y.-H., Zhou, Y.-G., Schinner, F., Margesin, R. (2010) *Luteimonas terricola* sp. nov., a psychrophilic bacterium isolated from soil. Int. J. Syst. Evol. Microbiol. 60, 1581–1584.
[52] Zhao, G.-Y., Shao, F., Zhang, M., Zhang, X.-J., Wang, J.-Y., Fan, S.-J., Dai, M.-X. (2018) *Luteimonas rhizosphaerae* sp. nov., isolated from the rhizosphere of *Triticum aestivum* L. Int. J. Syst. Evol. Microbiol. 68, 1198–1203.