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Title	Revising the taxonomy of the Acinetobacter lwoffii group: the description of Acinetobacter pseudolwoffii sp. nov. and emended description of Acinetobacter lwoffii
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Abstract

In 1986, Bouvet and Grimont delineated two related taxa of the genus Acinetobacter termed genospecies (GS) 8 and 9. They proposed the name Acinetobacter lwoffii for GS8, which included the supposed type strain (CIP 64.10). As the authenticity of CIP 64.10 was later questioned, this study aimed at reassessing the taxonomy of these genospecies. We investigated 52 strains of GS8 or GS9, including CIP 64.10 and the genuine type strain of A. lwoffii (NCTC 5866T). All strains were subjected to the genus-wide comparative analyses of MALDI-TOF whole-cell mass spectra, rpoB gene sequences and metabolic traits while whole-genome sequences were analysed for 16 strains. The strains were classified into two distinct groups corresponding to GS8 (n=15) and GS9 (n=37). CIP 64.10 fell within GS8 whereas NCTC 5866T belonged to GS9. Intraspecies ANIb values for the genomes of GS8 (n=6) and GS9 (n=10) were ≥96.1% and ≥95.4%, respectively, whereas the ANIb values between them were 86.8–88.6%. Based on core genome phylogeny, GS8 and GS9 formed a distinct clade within the genus, with two respective, strongly supported subclades. GS8 and GS9 were similar in physiological and catabolic properties but were clearly separable by MALDI-TOF MS. We conclude that the name A. lwoffii pertains to GS9 and not to GS8 as originally proposed and that these groups represent two distinct species. We propose the name Acinetobacter pseudolwoffii sp. nov. for GS8, with ANC 5044T (= CCM 8638T = CCUG 67963T) as the type strain, and provide the emended description of A. lwoffii.

Keywords	carbon source assimilation; core genome; MALDI-TOF MS; rpoB; whole genome sequence
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Corresponding Author	Alexandr Nemec
Corresponding Author's Institution	National Institute of Public Health
Order of Authors	Alexandr Nemec, Lenka Radolfova-Krizova, Martina Maixnerova, Matej Nemec, Dominique Clermont, Jaroslav Bzdil, Petr Ježek, Petra Spanelova
Suggested reviewers	Zhiyong Zong, Lenie Dijkshoorn, paul higgins, Ignasi Roca

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Dear editors of Systematic and Applied Microbiology,

We are submitting a manuscript entitled “Revising the taxonomy of the *Acinetobacter lwoffii* group: the description of *Acinetobacter pseudolwoffii* sp. nov. and emended description of *Acinetobacter lwoffii*” for your kind consideration for publication in the SAM.

In this taxonomic study, we aim at the clarification of the taxonomic confusion associated with the name *Acinetobacter lwoffii*, one of two oldest validly published names of the genus *Acinetobacter*. Our results show that the emended description of *A. lwoffii* by Bouvet and Grimont (1986) was based on an incorrect (type) strain and was associated with a taxon different from the species which includes the genuine type strain of *A. lwoffii*. We believe that this subject has a publication value given the medical and ecological importance of strains so far classified as *A. lwoffii*.

Yours faithfully,

Alexandr Nemec

Alexandr Nemec, PhD
Professor in medical microbiology
Laboratory of Bacterial Genetics
National Institute of Public Health
Srobarova 48, 10042 Prague
Czech Republic
www.szu.cz/anemec/anemec.htm

Revising the taxonomy of the *Acinetobacter lwoffii* group: the description of *Acinetobacter pseudolwoffii* sp. nov. and emended description of *Acinetobacter lwoffii* †

Alexandr Nemec[a,b,*], Lenka Radolfova-Krizova[a], Martina Maixnerova[a], Matej Nemec[a], Dominique Clermont[c], Jaroslav Bzdil[d], Petr Jezek[e], Petra Spanelova[f]

[a] *Laboratory of Bacterial Genetics, Centre for Epidemiology and Microbiology, National Institute of Public Health, Šrobárova 48, 100 42 Prague 10, Czech Republic*

[b] *Department of Laboratory Medicine, Third Faculty of Medicine, Charles University, Šrobárova 50, 100 34 Prague 10, Czech Republic*

[c] *Collection de l'Institut Pasteur, Institut Pasteur, Paris, France*

[d] *Department of Special Microbiology, State Veterinary Institute Olomouc, Jakoubka ze Stříbra 1, 779 00 Olomouc, Czech Republic*

[e] *Department of Clinical Microbiology and Parasitology, Příbram Regional Hospital, 261 01 Příbram I, Czech Republic*

[f] *Czech National Collection of Type Cultures, Centre for Epidemiology and Microbiology, National Institute of Public Health, Šrobárova 48, 100 42 Prague 10, Czech Republic*

Abbreviations: MALDI-TOF MS, Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight Mass Spectrometry; ANIb, average nucleotide identity based on BLAST; dDDH, digital DNA–DNA hybridization

† The GenBank/ENA /DDBJ accession numbers for the partial nucleotide sequences of the *rpoB* gene reported in this study are MG564139–MG564159. The whole genome shotgun projects for *Acinetobacter pseudolwoffii* ANC 5044^T, ANC 5347, ANC 5324 and ANC 5318 have been deposited at DDBJ/ENA/GenBank under the accession numbers PHRG00000000, PGOZ00000000, PGPA00000000 and PGPB00000000, respectively. The versions described in this paper are PHRG01000000, PGOZ01000000, PGPA01000000, and PGPB01000000.

* Corresponding author.

E-mail address: alexandr.nemec@szu.cz (A. Nemec)

Abstract

In 1986, Bouvet and Grimont delineated two related taxa of the genus *Acinetobacter* termed genospecies (GS) 8 and 9. They proposed the name *Acinetobacter lwoffii* for GS8, which included the supposed type strain (CIP 64.10). As the authenticity of CIP 64.10 was later questioned, this study aimed at reassessing the taxonomy of these genospecies. We investigated 52 strains of GS8 or GS9, including CIP 64.10 and the genuine type strain of *A. lwoffii* (NCTC 5866^T). All strains were subjected to the genus-wide comparative analyses of MALDI-TOF whole-cell mass spectra, *rpoB* gene sequences and metabolic traits while whole-genome sequences were analysed for 16 strains. The strains were classified into two distinct groups corresponding to GS8 (n=15) and GS9 (n=37). CIP 64.10 fell within GS8 whereas NCTC 5866^T belonged to GS9. Intraspecies ANIb values for the genomes of GS8 (n=6) and GS9 (n=10) were ≥96.1% and ≥95.4%, respectively, whereas the ANIb values between them were 86.8–88.6%. Based on core genome phylogeny, GS8 and GS9 formed a distinct clade within the genus, with two respective, strongly supported subclades. GS8 and GS9 were similar in physiological and catabolic properties but were clearly separable by MALDI-TOF MS. We conclude that the name *A. lwoffii* pertains to GS9 and not to GS8 as originally proposed and that these groups represent two distinct species. We propose the name *Acinetobacter pseudolwoffii* sp. nov. for GS8, with ANC 5044^T (= CCM 8638^T = CCUG 67963^T) as the type strain, and provide the emended description of *A. lwoffii*.

Keywords: carbon source assimilation; core genome; MALDI-TOF MS; *rpoB*; whole genome sequence

Introduction

In their landmark study of 1986 [2], Bouvet and Grimont laid down a basis for the classification of the genus *Acinetobacter* at the species level. Based on DNA-DNA hybridization (DDH) and comprehensive phenotypic testing, they delineated 12 taxa termed genospecies among 85 archive *Acinetobacter* strains. These genospecies were numbered from 1 to 12, with formal species names being proposed for six of them. Two of these genospecies, 8 and 9, were found to be more related to each other than to the other genospecies in terms of both DNA-DNA relatedness and biochemical characteristics. As genospecies 8 included strain CIP 64.10 assumed to be derived from NCTC 5866^T, the type strain of *Acinetobacter lwoffii* [(Audureau 1940 [1]) Brisou and Prévot 1954 [3]], this genospecies was assigned the name *A. lwoffii* in line with Rule 40b of the Bacteriological Code [11].

The authenticity of CIP 64.10 as a derivative of *A. lwoffii* NCTC 5866^T was, however, later questioned by Tjernberg and Ursing in their taxonomic study on clinical *Acinetobacter* isolates based on DDH [31]. They reported that their results for *A. lwoffii* NCTC 5866^T and four strains classified by Bouvet and Grimont [2] as genospecies 8 (ATCC 17925) or 9 (ATCC 17968, ATCC 9957, and ATCC 17910) were discordant with the data of Bouvet & Grimont [2] for CIP 64.10 and the same four ATCC strains. Nevertheless, as the DDH relatedness and ΔT_m values found for all these strains were close to the thresholds recommended for species delineation, they decided to lump them in a single taxonomic group [31]. The fusion of genospecies 8 and 9 into one taxon was followed in many taxonomic studies on *Acinetobacter* [4,6,7,33]. The problem of the identity of CIP 64.10 and *A. lwoffii* NCTC 5866^T was recently reopened by Touchon et al. [32] in their comparative analysis of the whole genome sequences of *Acinetobacter* species. They found that the average nucleotide identity value between the genome sequences of NCTC 5866^T and CIP 64.10 was as low as 88.3%, which indicates that these organisms differed at both the strain and species level of resolution.

Organisms identified as *A. lwoffii* have been commonly reported from human, animal, or environmental specimens [14,15,16,27] and were associated with opportunistic infections in humans [33]. Given this significant medical and ecological importance, we conducted the present study in order to resolve the taxonomic discrepancies associated with the name *A. lwoffii*.

Material and methods

Bacteria

Fifty-two strains of the *A. lwoffii* group investigated in the present study are listed in Table 1. They included CIP 64.10 and other seven strains studied by Bouvet and Grimont [2], the genuine type strain of *A. lwoffii* (CCM 8638^T derived from NCTC 5866^T) and 31 additional strains which were selected from the collection of the Laboratory of Bacterial Genetics to be as diverse in their origin and phenotypic and genotypic characteristics as possible. Representatives of all *Acinetobacter* distinct species with validly published names (except for *Acinetobacter piscicola*, which was unavailable at the time of our analyses) and several provisional taxa of the genus were included in the analyses. Three junior synonyms and *Acinetobacter lactucae* which is synonymous with *Acinetobacter dijkshoorniae*, [19,20,34] were not considered.

MALDI-TOF MS

Whole cell profiling by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS was performed as described previously [24], using a standard matrix based on alpha-cyano-4-hydroxycinnamic acid solution. All measurements and data processing were carried out using the Microflex LT instrument (Bruker Daltonics) and BioTyper software version 3.1 (Bruker Daltonics).

The rpoB *gene analysis*

Comparative nucleotide sequence analysis of the *rpoB* (RNA polymerase β-subunit) gene was carried out for an 861 bp region which corresponds to nucleotide positions 2915–3775 of the *rpoB* coding region of *Acinetobacter baumannii* CIP 70.34^T (GenBank/ENA /DDBJ accession no. DQ207471.1) as described previously [17,18]. Cluster analysis was performed using the neighbour-joining algorithm with Kimura's two-parameter substitution model. All analyses were carried out using the BioNumerics 7.6 software (Applied-Maths).

Whole-genome sequence analysis

Generation of DNA sequencing libraries and paired-end sequencing (250 bp × 2) on the Illumina MiSeq platform were carried out in the Genomics Core Facility (EMBL). Reads were assembled *de novo* using the Geneious 9.0.5 software (Biomatters), with only contigs >1000 bp included in the final genome sequences. Genome sequences were compared using the average nucleotide identity based on BLAST (ANIb) and digital DNA–DNA hybridization (dDDH) parameters. ANIb and dDDH values were calculated, respectively, using the JSpecies (http://www.imedea.uib.es/jspecies) [25] and GGDC 2.1 (http://ggdc.dsmz.de) [13] programs with the recommended parameters and/or default settings.

Core genome-based phylogenetic analysis

For phylogenetic analysis based on the *Acinetobacter* core genome, the procedure used by Popel et al. [23] was adopted. All genome sequences were annotated with Prokka [26], and predicted protein coding sequences were used further. To obtain core genes, OrthoMCL [12] was first run to identify orthologs from the genome sequences of five strains, i.e. *A. radioresistens* CIP 103788^{T}, *A.baylyi* CIP 107474^{T}, *A. baumannii* CIP 70.34^{T}, *A. calcoaceticus* CIP 81.8^{T}, and *A. nosocomialis* NIPH 2119^{T}. Potentially orthologous protein sequences were clustered into homologous groups and redundant sequences were removed to retain only one homologous sequence per genome in each group. Only groups including sequences from all the five strains were analysed further. Sequences within each group were aligned with MAFFT-LINSI [9], and a profile hidden Markov model (pHMM) was created from each alignment using the hmmbuild command of the HMMER3 software package (http://hmmer.janelia.org). HaMStR ortholog search [5] was then applied for the remaining genomes using pHMMs for all homologous groups. Protein sequences found in all genomes were used for phylogeny reconstruction. They were aligned for each group of orthologs with MAFFT-LINSI. Alignments were concatenated, and sites with gaps were trimmed using Phyutility [29]. Maximum Likelihood (ML) tree reconstruction on the resulting supermatrix was then conducted with RAxML 8.1.9 [30] using the PROTGAMMAILGF model for amino acid sequence evolution.

Phenotypic analysis

Metabolic and physiological features were assessed using a genus-targeted set of in-house, strictly standardized tests (Table 2) as described previously [10,18]. Assimilation tests were performed in fluid mineral medium supplemented with 0.1% (w/v) carbon source. In the case of auxotrophic strains, the medium was also supplemented with 15% (v/v) of the AUX medium used in the API 20NE system (bioMérieux). Temperature growth tests were carried out in brain-heart infusion broth (Oxoid) using a thermostatically controlled water bath. Except for the temperature growth tests, the culture temperature was 30 °C. The assimilation tests were interpreted after six days of culture and the other tests after three (haemolytic and gelatinase activities) or two (D-glucose acidification, temperature growth tests) days. Gram-staining and tests for oxidase, catalase, nitrate reduction, motility, and anaerobic growth were performed as described by Radolfova-Krizova et al. [24].

Results and Discussion

MALDI-TOF MS- and rpoB*-based classification*

The results of the genus-wide cluster analyses of MALDI-TOF whole-cell mass spectra and partial *rpoB* gene sequences are depicted in Fig. 1. Based on MALDI-TOF MS, the 52 strains of the *A. lwoffii* group formed a distinct cluster, with two well separated, internally cohesive subclusters. The larger subcluster (n=37) included *A. lwoffii* CCM 8638^T and all six strains classified by Bouvet and Grimont as GS9, whereas the smaller one (n=15) comprised CIP 64.10 and the other strain (CIP 70.17) classified by Bouvet and Grimont as GS8. This picture was mirrored, with the exception of two strains, in the *rpoB*-based phylogram. The partial *rpoB* sequences formed a well separated cluster within the genus, with a large and a small subcluster (respective intracluster sequence identities of ≥96.9% and ≥97.3%), which comprised, respectively, the strains classified by Bouvet and Grimont as GS9 and GS8. The two strains with inconsistent positions in the *rpoB* and MALDI-TOF MS dendrograms were NIPH 713, placed in between two main clusters in the *rpoB* tree, and ANC 5324, which grouped with the GS8 and GS9 strains in the MALDI-TOF MS and *rpoB* dendrograms, respectively. However, based on the analysis of whole genome sequences (Table 2, Fig. 2), these two strains could be allocated unequivocally to the GS8 group, and the same picture was obtained if the complete *rpoB* sequences derived from the whole genomes were compared. The inspection of the completete *rpoB* sequences of NIPH 713 and ANC 5324 revealed that while the major parts of these sequences were congruent with those of the GS8 strains, the regions used for the partial *rpoB* gene analysis (positions 2915–3775 of the *rpoB* coding region) were more similar, either completely (ANC 5324) or partly (NIPH 713), to those of the GS9 strains. This suggests that the *rpoB* sequences of these two strains underwent homologous recombination following the acquisition of the *rpoB* sequences from strains of the GS9 group.

Comparison of whole genome sequences

Whole genome sequences were analysed in six and 10 strains classified, respectively, as GS8 and GS9 by Bouvet and Grimont and/or by MALDI-TOF MS and *rpoB* sequencing (Table 2). Four of them (GenBank/ENA /DDBJ accession nos PHRG00000000.1, PGPB00000000.1, PGPA00000000.1, and PGOZ00000000.1) were determined in the present study, whereas the remaining 12 were published previously [22,32]. The basic features of these genome sequences are summarized in Table S1. The pairwise ANIb and dDDH values for the 16 genome sequences are shown in Table 2 while those between these strains and all hitherto described *Acinetobacter* species are summarized in Tables S2 and S3. The intraspecies ANIb/dDDH values for GS8 and GS9 were 96.14–97.44%/68.8–79.3% and 95.36–99.98%/64.7–99.3%, respectively, whereas the ANIb/dDDH values between GS8 and GS9 were

86.72–88.58%/32.3–36.8%. The ANIb and dDDH values between the genomes of GS8 or GS9 and those of the other species of the genus were ≤82.84% and ≤26.9%, respectively. In light of the recommended threshold values of ANIb (95–96%, [25]) and dDDH (70% [13]) for species circumscription, these values indicate that GS8 and GS9 are two distinct species. Although a high proportion of the pairwise dDDH values for GS9 were slightly below 70% (Table 2), it is of note that such dDDH values can also be found for the members of other ecologically ubiquitous species such as *Acinetobacter johnsonii* (unpublished results).

Core genome-based phylogeny

The results of the phylogenetic analysis of the 16 genome sequences of the *A. lwoffii* group within the whole genus are shown in Fig. 2. The resulting phylogram was reconstructed from the concatenated alignment of the of 54,871 amino acid residues based on 1,276 orthologous protein groups. The 16 genomes formed a distinct clade within the genus, with two monophyletic subbranches which, respectively, correspond to GS8 and GS9. This picture is consistent with the previously published phylogram [32] based on 1,590 protein families of the *Acinetobacter* core genome, which included 11 of the 16 genome sequences of the *A. lwoffii* group used in the present study.

Physiological and metabolic features

Phenotypic features assessed using the genus-targeted set of physiological and metabolic tests (Table 3) are presented in the standardized way as described in our previous nomenclatural proposals [10,21]. Table S4 shows the summarized data for GS8 and GS9, along with those for all known *Acinetobacter* species with validly published names, whereas Table 3 is a subset of these data, which compares the phenotypes of GS8 and GS9 with those of the species that are phylogenetically closest to them (*A. gandensis*, *A. indicus*, *A. schindleri*, *A. towneri*, and *A. variabilis*) according to the phylogram of Fig. 2. Overall, GS8 and GS9 belong, together with those phylogenetically close species, to catabolically less active members of the genus, with a limited number of characteristics that can differentiate between them. Even though no single diagnostic feature was identified which could discriminate unequivocally between the strains of GS8 and GS9, GS9 appeared to be more active than GS8 as exemplified by a higher proportion of GS9 strains to grow on adipate, 4-aminobutyrate, DL-lactate, and ethanol or by the ability of some GS9 strains to oxidize D-glucose (Table 3).

The type strain of A. lwoffii

To our best knowledge, the National Collection of Type Cultures preserves the oldest known lineage of the type strain of *A. lwoffii* (listed under no. NCTC 5866^{T}), deposited there by André Lwoff before

1939. NCTC 5866^T was later deposited in a number of culture collections including the Collection de l'Institut Pasteur (CIP 64.10) and the Czech Collection of Microorganisms (CCM 8638^T). The results of the present study showed that CIP 64.10 and CCM 8638^T differed at both the strain and species level. To shed more light on, we obtained another culture of NCTC 5866^T from the National Collection of Type Cultures, which has been deposited in the Collection de l'Institut Pasteur under no. CIP 110687^T. This new culture was studied along with CIP 64.10 and CCM 8638^T using macrorestriction analysis with ApaI, which corroborated the identity of CIP 110687^T and CCM 8638^T and distinctness of CIP 64.10 (data not shown). These observations are in line with those of Tjernberg and Ursing [31] and indicate that CIP 64.10 is a strain different from NCTC 5866^T. Although a mislabelling of strains is the most likely reason behind this, it must be emphasized that such a mislabelling was difficult to recognize at the time of the study of Bouvet and Grimont [2], given the high phenotypic similarity of the two strains (Table 3). To avoid further confusion, CIP 64.10 is not available from the Collection de l'Institut Pasteur any longer but has been deposited in the Czech National Collection of Type Cultures under no. CNCTC 7645.

Conclusions

The present study provides strong evidence that taxa GS8 and GS9 described by Bouvet and Grimont [2] are two phylogenetically related but taxonomically clearly distinct species of the genus *Acinetobacter*. This evidence is based on the congruence of genotypic and phenotypic results, with those based on the analyses of whole-genome sequences and whole-cell protein spectra being most conclusive. The two species are ecologically ubiquitous, as indicated by their occurrence in various human, animal, and environmental specimens. Despite their mutual resemblance in catabolic properties and possible problems with their identification based on single gene markers, the reliable differentiation between these species for routine diagnostics is achievable via MALDI-TOF MS. Our results further demonstrate that the genuine type strain of *A. lwoffii* [1,3] is a member of GS9 and not GS8, as assumed by Bouvet and Grimont [2] and that, therefore, the name *A. lwoffii* pertains to the former species. In light of these findings, we provide the emended description of *A. lwoffii* and propose the name *Acinetobacter pseudolwoffii* sp. nov. for GS8.

Emended description of *Acinetobacter lwoffii* (Audureau 1940) Brisou and Prévot 1954.

Gram-stain-negative, strictly aerobic, oxidase-negative, and catalase-positive coccobacilli typically occurring in pairs, incapable of dissimilative denitrification and swimming motility, and capable of growing in defined mineral media containing a single carbon and energy source and ammonia as the sole source of nitrogen. Rare strains are auxotrophic. Positive in the transformation assay of Juni [8].

Colonies on tryptic soy agar (Oxoid) after incubation at 30 °C for 24 h are 1.0–2.0 mm in diameter, grey–white, slightly opaque, circular, convex and smooth, with entire margins. Growth occurs in brain-heart infusion broth (Oxoid) at temperatures ranging from 15 to 37 °C, but not at 44 °C; most strains do not grow at 41 °C. Most strains do not produce acid from D-glucose. Gelatin is not hydrolysed. Neither haemolysis nor greenish discoloration is observed on agar media supplemented with sheep erythrocytes. Acetate, ethanol, and azelate are utilized as sole sources of carbon, with growth visible in 6 (mostly 2) days of culture at 30° C. Most strains grow on adipate, 4-aminobutyrate, benzoate, DL-lactate, and phenylacetate, whereas only rare strains grow on *trans*-aconitate, L-arginine, 2,3-butanediol, citrate (Simmons),L-glutamate, levulinate, D-malate, malonate, L-ornithine, and tricarballylate. No growth occurs on β-alanine, L-arabinose, L-aspartate, citraconate, gentisate, D-gluconate, D-glucose, glutarate, histamine, L-histidine, 4-hydroxybenzoate, L-leucine, L-phenylalanine, putrescine, D-ribose, L-tartrate, trigonelline, or tryptamine. DNA G+C content is 42.5–43.2 mol% (based on 10 genome sequences).

The type strain, CCM 5581^{T} (= NCTC 5866^{T} = CIP 110687^{T} = CNCTC 6167^{T} = NIPH 512^{T}), was deposited in the NCTC collection by André Lwoff before 1939. This strain grows on adipate, 4-aminobutyrate, benzoate, DL-lactate, and phenylacetate but not on *trans*-aconitate, L-arginine, 2,3-butanediol, citrate (Simmons),L-glutamate, levulinate, D-malate, malonate, L-ornithine, or tricarballylate. The whole genome sequence of the type strain is available under GenBank/EMBL/DDBJ accession no. AYHO00000000.1 (size: 3 382 003 bp, number of contigs: 16, number of proteins: 3 237, G+C content: 43.1%). This genome sequence contains five copies of the 16S rRNA gene, with three sequence variants, i.e. AYHO01000004 (locus_tag: P800_01948), AYHO01000005 (locus_tag: P800_02544 and P800_02592). The taxonumber of the digital protologue is TA00593.

Description of *Acinetobacter pseudolwoffii* sp. nov.

Acinetobacter pseudolwoffii (pseu.do.lwo.ff'i.i. Gr. adj. pseudes false; N.L. gen. masc. n. *lwoffii*, of Lwoff, named in honour of André Lwoff and specific epithet; N.L. masc. adj. *pseudolwoffii*, a false (*Acinetobacter*) *lwoffii*, referring to the high phenotypic similarity to and historical confusion with *A. lwoffii*).

Gram-stain-negative, strictly aerobic, oxidase-negative, and catalase-positive coccobacilli typically occurring in pairs, incapable of dissimilative denitrification and swimming motility, and capable of growing in defined mineral media containing a single carbon and energy source, and ammonia as the sole source of nitrogen. Positive in the transformation assay of Juni [8]. Colonies on tryptic soy agar (Oxoid) after incubation at 30 °C for 24 h are 1.0–2.0 mm in diameter, grey–white, slightly opaque, circular, convex and smooth, with entire margins. Growth occurs in brain-heart infusion broth (Oxoid) at temperatures ranging from 15 to 37 °C, but not at 41 °C. Acid is not produced from D-glucose. Gelatin is not hydrolysed. Neither haemolysis nor greenish discoloration is observed on agar media supplemented with sheep erythrocytes. Acetate is utilized as a sole source of carbon, with growth visible in 6 (mostly 2) days of culture at 30° C. Growth on adipate, azelate, benzoate, ethanol, DL-lactate, or phenylacetate is common while only rare strains grow on glutarate, 4-hydroxybenzoate, D-malate, malonate, or L-tartrate. No growth occurs on 4-aminobutyrate, *trans*-aconitate, β-alanine, L-arabinose, L-arginine, L-aspartate, 2,3-butanediol, citraconate, gentisate, citrate (Simmons), D-gluconate, D-glucose, L-glutamate, histamine, L-histidine, L-leucine, levulinate, L-ornithine, L-phenylalanine, putrescine, D-ribose, tricarballylate, trigonelline or tryptamine. DNA G+C content is 42.9–43.4 mol% (based on six genome sequences).

The type strain, ANC 5044^{T} (= CCM 8638^{T} = CCUG 67963^{T} = CNCTC 7472^{T}), was isolated from creek sediment in a protected deciduous forest (Natural reserve Mohelnička, the Czech Republic, GPS coordinates: 49.1043269°N 16.2148017°E) in September 2014. This strain grows on adipate, azelate, benzoate, ethanol, DL-lactate, phenylacetate, and L-tartrate but not on glutarate, D-malate, malonate, or 4-hydroxybenzoate. The whole genome sequence of the type strain is available under GenBank/EMBL/DDBJ accession no. PHRG00000000.1 (size: 3 105 311 bp, number of contigs: 30, number of proteins: 2 791, G+C content: 43.3%). The complete 16S rRNA gene sequence is available from the whole genome sequence (PHRG01000001.1, locus_tag: CWI32_02650). The taxonumber of the digital protologue is TA00592.

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Appendix A. Supplementary tables

Supplementary tables S1–S4 associated with this article, can be found, in the online version, at http://dx.doi.org/?.

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Figure legends

Fig. 1. Results of the clustering of the (A) MALDI-TOF mass spectra and (B) partial sequences of the *rpoB* gene of the of 37 strains of *Acinetobacter lwoffii* (genospecies 9), 15 strains of *Acinetobacter pseudolwoffii* sp. nov. (genospecies 8) and 56 type or reference strains representing the known species of the genus *Acinetobacter*. The MALDI-TOF MS analysis was carried out by using the Biotyper MSP Dendrogram Creation Standard Method (Bruker Daltonics) with the correlation distance measure and average linkage algorithm (UPGMA). Analysis of the *rpoB* sequences was carried out for nucleotide positions 2915–3775 (861 bp) of the coding region of the gene using the BioNumerics 7.6 software (Applied-Maths). Evolutionary distances were computed using Kimura's two-parameter model while the tree was reconstructed using the neighbour-joining algorithm with the sequence of *Pseudomonas aeruginosa* PAO1 (DDBJ/ENA/GenBank accession no. NC002516) as the outgroup. GenBank accession numbers for the *rpoB* sequences or whole-genome sequences from which the *rpoB* sequences were extracted are shown in parentheses. In the case of identical *rpoB* sequences, accession numbers are shown only for one representative. Bootstrap values (>80%) after 1000 resamplings are indicated at branch nodes; bar, 5% of change per nucleotide site. Squares and circles denote the strains studied using DNA–DNA hybridization by Bouvet and Grimont [2] and those analysed by whole genome sequencing in the present study, respectively; filled and empty figures indicate the strains allocated to *A. lwoffii* and *A. pseudolwoffii*, respectively.

Fig. 2. Core genome-based tree for the genus *Acinetobacter* showing the phylogenetic position of *A. lwoffii* (genospecies 9) and *A. pseudolwoffii* sp. nov. (genospecies 8). Included are the genomes of 10 and 6 strains of *A. lwoffii* and *A. pseudolwoffii*, respectively, and those of 54 type or reference strains representing the known species of the genus *Acinetobacter* (missing are only the genomes of *Acinetobacter halotolerans* and *Acinetobacter piscicola*, not available at the time of analysis). The tree was reconstructed using maximum likelihood with the PROTGAMMAILGF model for amino acid sequence evolution. Bootstrap values based on 100 replications are shown at the nodes of the tree. Bar, 0.05 amino acid substitutions per site.

Table 1

Strains of *A. lwoffii* (genospecies 9) and *A. pseudolwoffii* sp. nov. (genospecies 8).

Strain classification and designation	Specimen	Location and/or year of isolation	Donor and/or reference
Acinetobacter lwoffii (n = 37)			
CCM 5581^{T} = NIPH 512^{T} = CIP 110687^{T} = CNCTC 6167^{T} = NCTC 5866^{T} = Lwoff(1)^{T}	Unknown	Unknown	
NIPH 237	Blood (inpatient)	Příbram, CZ, 1994	[16]
NIPH 238	Vagina (outpatient)	Dobříš, CZ, 1993	[16]
NIPH 393	Throat (outpatient)	Příbram, CZ, 1996	[16]
NIPH 403	Throat (outpatient)	Příbram, CZ, 1996	[16]
NIPH 461	Gastric juices (inpatient)	Praha, CZ, 1996	A. Steinerová [16]
NIPH 473	Nose (outpatient)	Český Brod, CZ, 1996	E. Aldová [16]
NIPH 474	Urine (human)	České Budějovice, CZ, 1996	O. Hausner [16]
NIPH 478 = CIP 110447	Ear (outpatient)	Horní Planá, CZ, 1997	M. Horníková [16]
NIPH 486	Nose (outpatient)	Příbram, CZ, 1997	[16]
NIPH 616	Burn (human)	Praha, CZ, 1994	J. Vránková [16]
NIPH 666	Ear (outpatient)	Praha, CZ, 1997	J. Sobotková [16]
NIPH 671	Cannula (inpatient)	České Budějovice, CZ, 1997	O. Hausner [16]
NIPH 715 = CIP 110448	Pus (inpatient)	Příbram, CZ, 1997	[16]
NIPH 912	Ear (outpatient)	Příbram, CZ, 1998	[16]
NIPH 913	Nose (outpatient)	Sedlčany, CZ, 1998	[16]
NIPH 1094	Nose (outpatient)	Sedlčany, CZ, 1999	[16]
NIPH 2172 = CIP 70.31 = 62[b]	Gangrenous lesion (human)	IT, Before 1946	P. J. M. Bouvet [2]
NIPH 2175 = CIP A162 = 65[b]	Conjunctivitis	Before 1941	P. J. M. Bouvet [2]
NIPH 2176 = CIP 70.19 = 66[b]	Unknown	Unknown	P. J. M. Bouvet [2]
NIPH 2257 = LMG 10590 = 44[b]	Prostate secretion (human)	Malmö, SE, 1980-1981	I. Tjernberg [31]
NIPH 2266 = LMG 10599 = 202[b]	Urine (human)	Malmö, SE, 1980-1981	I. Tjernberg [31]
ANC 3906[c]	Mud (forest)	Lány forestland, CZ, 2010	
ANC 4203[c]	Mud (wetland)	Křečkov, CZ, 2011	
ANC 4217[c]	Clayey mud (drained pond)	Hostivice, CZ, 2012	
ANC 4305 = 67[b]	Pus	Unknown	P. J. M. Bouvet [2]
ANC 4309 = 64[b]	Sperm culture	Unknown	P. J. M. Bouvet [2]
ANC 4400 = SH145 = CCUG 57819	Hand (human)	Cologne, DE, 1994	[28]
ANC 4568 = CIP 51.11	Pleural pus (human)	FR, 1951	
ANC 4569 = CIP 102136	Sternum (human)	Paris, FR, 1986	
ANC 4570 = CIP 101966	Sputum (human)	Nevers, FR, 1985	
ANC 4571 = CIP 64.7 = 68[b]	Urine	Before 1964	P. J. M. Bouvet [2]
ANC 4897[c]	Water (forest well)	Bílichov, CZ, 2014	
ANC 5032[c]	Water (river)	Birecik, TR, 2014	
ANC 5055[c]	Water with organic debris (forest)	Mohelno, CZ, 2014	
ANC 5085[c]	Soil (dry creekbed)	Peçenek, TR, 2014	
ANC 5086[c]	Soil (dry creekbed)	Peçenek, TR, 2014	
Acinetobacter pseudolwoffii (n = 15)			
ANC 5044$^{T, c}$ = CCUG 67963^{T} = CCM 8638^{T}	Water with organic debris (forest)	Mohelno, CZ, 2014	
CIP 64.10 = ANC 4579 = CNCTC 7645	Unknown	Unknown	P. J. M. Bouvet [2]
NIPH 713 = CIP 110446	Vagina (inpatient)	Příbram, CZ, 1997	[16]
NIPH 746 = A46-1[b]	Soil (lakeshore)	Aquilasee, Westfalia, DE, 1990s	H. Seifert
NIPH 748 = A80-2[b]	Water (river)	Centa Albenga, IT, 1990s	H. Seifert
NIPH 831 = RUH 581[b]	Soil	Rotterdam, NL	L. Dijkshoorn [Q2]
NIPH 1041	Conjunctiva (outpatient)	Příbram, CZ, 1998	[16]
ANC 4683 = CIP 70.17 = 61[b]	Unknown	Before 1958	P. J. M. Bouvet [2]
ANC 5303	Nose (calf)	Rýmařov, CZ, 2015	
ANC 5307	Nose (cow)	Chválkovice, CZ, 2015	
ANC 5318	Nose (horse)	Valašské Meziříčí, CZ, 2015	
ANC 5320	Faeces (sheep)	Bělkovice, CZ, 2015	
ANC 5324	Nose (goat)	Kopřivnice, CZ, 2015	
ANC 5347	Rectum (guinea pig)	Ivanovice, CZ, 2015	
ANC 5504	Mud (wetland)	Olší, CZ, 2016	

Abbreviations: CCM, Czech Collection of Microorganisms, Brno, Czech Republic; CCUG, Culture Collection, University of Göteborg, Sweden; CIP, Collection de l'Institut Pasteur, Institut Pasteur, Paris, France; CNCTC, Czech National Collection of Type Cultures, Prague, Czech Republic; LMG, Bacteria Collection, Laboratorium voor Microbiologie Gent, Gent, Belgium; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, UK. ANC and NIPH, strain designation used by the Laboratory of Bacterial Genetics. Country abbreviations: CZ, Czech Republic; DE, Germany; FR, France; IT, Italy; NL, the Netherlands; SE, Sweden; TR, Turkey.

[a] If known hospitalized (inpatient) or ambulatory (outpatient) human patients are indicated.

[b] Strain designation used by the donor.

[c] GPS coordinates of sampling sites: ANC 3906 (50°6'56.602"N, 13°55'11.481"E), ANC 4203 (50°11.11837'N 15°6.88278'E), ANC 4217 (50°4'16.872"N, 14°15'15.190"E), ANC 4897 (50.2506431°N 13.9026414°E), ANC 5032 (37°02'42.0"N 37°58'58.2"E), ANC 5055 (49.1043269°N 16.2148017°E), ANC 5085 and ANC 5086 (37°21'50.5"N 41°47'09.5"E), and ANC 5044 (49.1043269°N 16.2148017°E).

Table 2. Average nucleotide identity based on BLAST (ANIb) and digital DNA-DNA hybridization (dDDH) values for the genome sequences of the *A. lwoffii* group

Genome	Strain	ANIb (%)/ dDDH (%)														
		AYHO01	APRU01	APRY01	APQT01	APRX01	APRV01	APOG01	APQU01	APOT01	ACPN01	PHRG01	PGPB01	PGPA01	PGOZ01	APQS01
***A. lwoffii* (genospecies 9)**																
AYHO00000000.1	NIPH 512^{T} (= CCM 5581^{T})															
APRU00000000.1	CIP 51.11 (= ANC 4568)	95.70/ 65.5														
APRY00000000.1	CIP 64.7 (= ANC 4571)	95.61/ 66.5	95.57/ 64.9													
APQT00000000.1	CIP 70.31 (= NIPH 2172)	95.65/ 66.9	95.79/ 66.3	96.75/ 74.2												
APRX00000000.1	CIP 101966 (= ANC 4570)	95.75/ 67.1	95.71/ 65.8	96.25/ 69.9	96.76/ 72.9											
APRV00000000.1	CIP 102136 (= ANC 4569)	95.69/ 66.7	95.62/ 65.4	96.23/ 70.2	96.71/ 72.6	96.55/ 71.6										
APOG00000000.1	CIP A162 (= NIPH 2175)	99.98/ 99.3	95.64/ 65.8	95.54/ 66.9	95.59/ 67.3	95.72/ 67.4	95.61/ 66.8									
APQU00000000.1	NIPH 478	95.64/ 66.0	95.95/ 67.2	95.36/ 64.7	95.75/ 66.1	95.60/ 65.3	95.59/ 66.0	95.62/ 66.2								
APOT00000000.1	NIPH 715	96.05/ 68.0	95.74/ 66.1	96.42/ 71.9	96.48/ 70.7	96.53/ 71.5	96.47/ 71.0	95.97/ 67.9	95.78/ 66.1							
ACPN00000000.1	SH145 (= ANC 4400)	95.94/ 67.4	95.57/ 65.1	96.45/ 71.6	96.70/ 72.1	96.38/ 70.8	96.47/ 71.5	95.97/ 67.5	95.69/ 66.1	96.33/ 69.9						
***A. pseudolwoffii* (genospecies 8)**																
PHRG00000000.1	ANC 5044^{T}	87.32/ 33.7	87.21/ 33.1	87.33/ 34.0	87.44/ 34.0	87.43/ 34.1	87.29/ 33.8	87.29/ 33.7	87.04/ 33.0	87.39/ 34.0	87.27/ 33.7					
PGPB00000000.1	ANC 5318	87.12/ 33.3	87.24/ 33.2	87.32/ 33.7	87.45/ 33.7	87.23/ 33.7	87.15/ 33.5	87.07/ 33.3	87.01/ 32.9	87.27/ 33.6	87.06/ 33.5	96.76/ 73.8				
PGPA00000000.1	ANC 5324	87.33/ 33.8	87.22/ 33.2	87.49/ 34.1	87.45/ 33.8	87.41/ 34.0	87.31/ 33.7	87.26/ 33.8	86.99/ 33.1	87.48/ 33.9	87.24/ 33.8	96.97/ 74.8	97.19/ 77.2			
PGOZ00000000.1	ANC 5347	87.59/ 34.2	87.17/ 33.2	87.62/ 34.6	87.77/ 34.6	87.64/ 34.6	87.48/ 34.2	87.56/ 34.2	87.33/ 33.7	87.67/ 34.6	87.58/ 34.6	97.10/ 76.4	97.04/ 75.9	97.04/ 75.3		
APQS00000000.1	CIP 64.10 (= ANC 4579)	88.33/ 36.0	88.06/ 35.1	88.54/ 36.8	88.57/ 36.7	88.37/ 36.6	88.22/ 36.4	88.24/ 36.0	87.97/ 35.3	88.58/ 36.7	88.41/ 36.6	96.55/ 72.0	96.14/ 68.8	96.51/ 70.6	96.83/ 73.5	
APRJ00000000.1	NIPH 713	86.90/ 33.0	86.96/ 32.7	87.08/ 33.3	87.13/ 33.2	87.03/ 33.2	86.99/ 33.0	86.96/ 33.0	86.72/ 32.3	87.08/ 33.2	86.93/ 33.0	97.39/ 78.6	97.32/ 77.0	97.23/ 76.4	97.44/ 79.3	96.53/ 72.6

Table 3. Metabolic and physiological properties of *Acinetobacter lwoffii* (genospecies 9), *Acinetobacter pseudolwoffii* sp. nov. (genospecies 8), and phylogenetically related species.

Characteristic	*A. lwoffii* (37)	*A. pseudolwoffii* (15)	*A. gandensis* (6)	*A. indicus* (2)	*A. schindleri* (22)	*A. towneri* (2)	*A. variabilis* (16)
Growth at 44 °C	-	-	-	D	-	-	31W (W)
Growth at 41 °C	11W (-)	-	-	+	+	+	+
Acidification of D-glucose	19 (-)	-	-	-	-	-	13 (-)
Utilization of							
trans-Aconitate	5 (-)	-	-	-	-	-	6 (-)
Adipate	76 (+)	40 (+)	-	-	41 (-)	-	69 (-)
4-Aminobutyrate	84 (+)	-	-	-	-	-	19 (-)
L-Arabinose	-	-	-	-	-	-	19 (-)
L-Arginine	5 (-)	-	-	-	-	-	19 (-)
Azelate	+	80 (+)	-	50 (-)	64 (-)	-	81 (+)
Benzoate	84 (+)	80 (+)	+	50 (+)	91 (+)	+	88 (+)
2,3-Butanediol	8 (-)	-	33 (-)	-	32 (-)	50 (-)	81 (+)
Citrate (Simmons)	8 (-)	-	50 (+)	-	59W (+)	-	25 (-)
Ethanol	+	73 (+)	+	+	95 (+)	+	+
Gentisate	-	-	-	-	41 (+)	-	-
L-Glutamate	8 (-)	-	33 (+)	50 (+)	- (D)	50 (+)	25 (-)
Glutarate	-	13 (-)	83 (+)	-	95 (+)	-	19 (-)
4-Hydroxybenzoate	-	7 (-)	-	-	64 (+)	-	-
DL-Lactate	84 (+)	40 (+)	+	+	+	+	6 (-)
Levulinate	3 (-)	-	-	-	-	-	-
D-Malate	11 (-)	7 (-)	-	-	95W (+)	50 (-)	13 (+)
Malonate	8 (-)	7 (-)	17 (-)	-	-	-	-
L-Ornithine	8 (-)	-	-	-	-	-	-
Phenylacetate	81 (+)	80 (+)	-	+	-	-	75 (+)
L-Phenylalanine	-	-	-	-	-	-	38 (-)
D-Ribose	-	-	-	-	-	-	13 (-)
L-Tartrate	-	13 (+)	-	-	18 (-)	-	-
Tricarballylate	8 (-)	-	-	-	45 (+)	-	-

The results were obtained either in this study or have been published previously [10,21]. All strains grew at 20-37 °C and on acetate. None of the strains liquefied gelatin, produced hemolysis on sheep blood agar, or grew on β-alanine, L-aspartate, citraconate, D-gluconate, D-glucose, histamine, L-histidine, L-leucine, putrescine, trigonelline, or tryptamine. *A. lwoffii* strains NIPH 2176, ANC 4305, and ANC 4309 were auxotrophic. +, All strains positive; -, all strains negative; D, (mostly) doubtful or irreproducible reactions; W, (mostly) weakly positive reactions. Numbers are percentages of strains with clearly positive reactions. For strain-dependent reactions, results for type strains are given in parentheses.

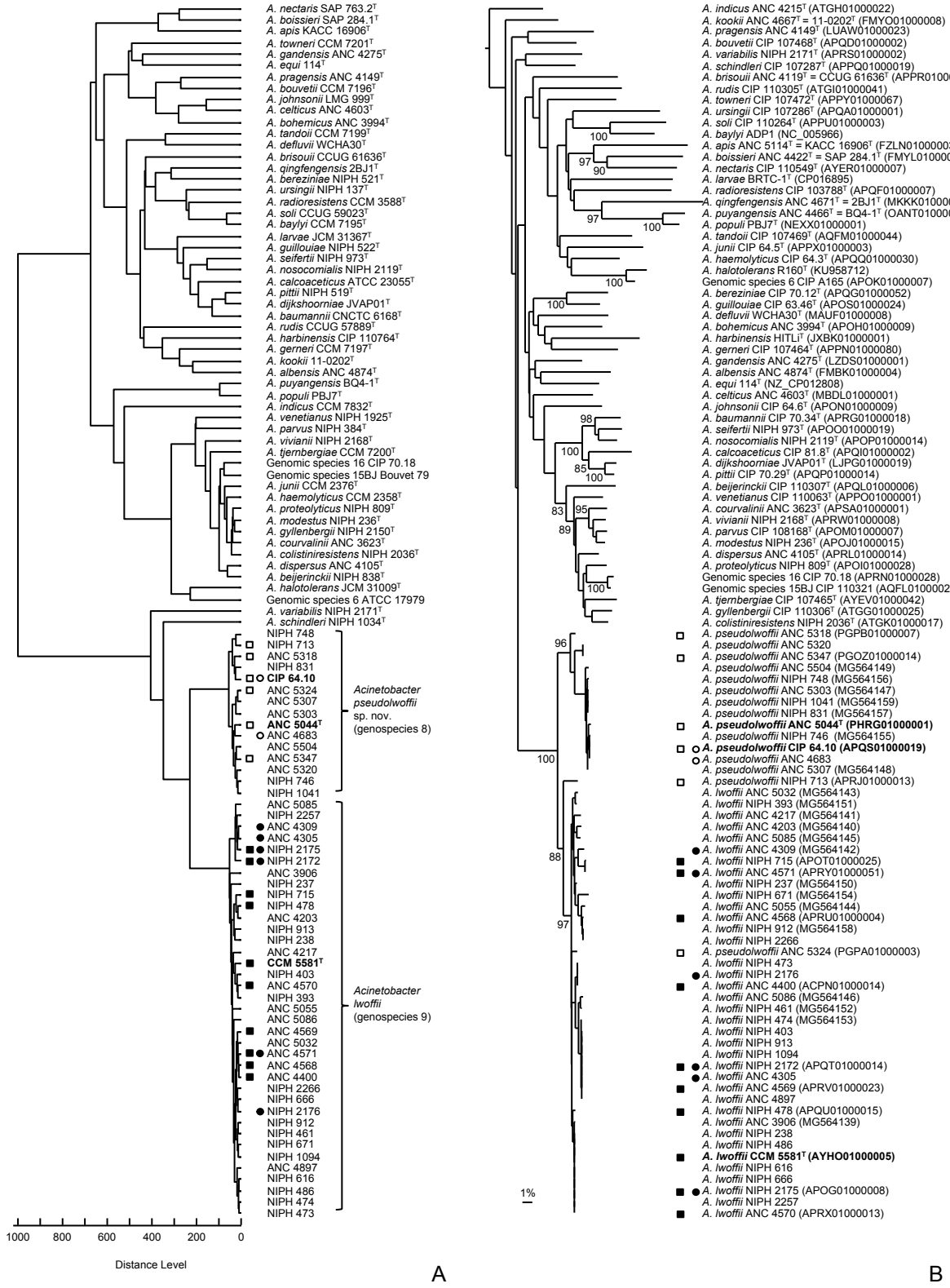
A. nectaris SAP 763.2T
A. boissieri SAP 284.1T
A. apis KACC 16906T
A. towneri CCM 7201T
A. gandensis ANC 4275T
A. equi 114T
A. pragensis ANC 4149T
A. bouvetii CCM 7196T
A. johnsonii LMG 999T
A. celticus ANC 4603T
A. bohemicus ANC 3994T
A. tandoii CCM 7199T
A. defluvii WCHA30T
A. brisouii CCUG 61636T
A. qingfengensis 2BJ1T
A. bereziniae NIPH 521T
A. ursingii NIPH 137T
A. radioresistens CCM 3588T
A. soli CCUG 59023T
A. baylyi CCM 7195T
A. larvae JCM 31367T
A. guillouiae NIPH 522T
A. seifertii NIPH 973T
A. nosocomialis NIPH 2119T
A. calcoaceticus ATCC 23055T
A. pittii NIPH 519T
A. dijkshoorniae JVAP01T
A. baumannii CNCTC 6168T
A. rudis CCUG 57889T
A. harbinensis CIP 110764T
A. gerneri CCM 7197T
A. kookii 11-0202T
A. albensis ANC 4874T
A. puyangensis BQ4-1T
A. populi PBJ7T
A. indicus CCM 7832T
A. venetianus NIPH 1925T
A. parvus NIPH 384T
A. vivianii NIPH 2168T
A. tjernbergiae CCM 7200T
Genomic species 16 CIP 70.18
Genomic species 15BJ Bouvet 79
A. junii CCM 2376T
A. haemolyticus CCM 2358T
A. proteolyticus NIPH 809T
A. modestus NIPH 236T
A. gyllenbergii NIPH 2150T
A. courvalinii ANC 3623T
A. colistiniresistens NIPH 2036T
A. dispersus ANC 4105T
A. beijerinckii NIPH 838T
A. halotolerans JCM 31009T
Genomic species 6 ATCC 17979
A. variabilis NIPH 2171T
A. schindleri NIPH 1034T
NIPH 748
NIPH 713
ANC 5318
NIPH 831
CIP 64.10
ANC 5324
ANC 5307
ANC 5303
ANC 5044T
ANC 4683
ANC 5504
ANC 5347
ANC 5320
NIPH 746
NIPH 1041
Acinetobacter pseudolwoffii sp. nov. (genospecies 8)
ANC 5085
NIPH 2257
ANC 4309
ANC 4305
NIPH 2175
NIPH 2172
ANC 3906
NIPH 237
NIPH 715
NIPH 478
ANC 4203
NIPH 913
NIPH 238
ANC 4217
CCM 5581T
NIPH 403
ANC 4570
NIPH 393
ANC 5055
ANC 5086
ANC 4569
ANC 5032
ANC 4571
ANC 4568
ANC 4400
NIPH 2266
NIPH 666
NIPH 2176
NIPH 912
NIPH 461
NIPH 671
NIPH 1094
ANC 4897
NIPH 616
NIPH 486
NIPH 474
NIPH 473
Acinetobacter lwoffii (genospecies 9)
1000
800
600
400
200
0
Distance Level
A
A. indicus ANC 4215T (ATGH01000022)
A. kookii ANC 4667T = 11-0202T (FMYO01000008)
A. pragensis ANC 4149T (LUAW01000023)
A. bouvetii CIP 107468T (APQD01000002)
A. variabilis NIPH 2171T (APRS01000002)
A. schindleri CIP 107287T (APPQ01000019)
A. brisouii ANC 4119T = CCUG 61636T (APPR01000012)
A. rudis CIP 110305T (ATGI01000041)
A. towneri CIP 107472T (APPY01000067)
A. ursingii CIP 107286T (APQA01000001)
A. soli CIP 110264T (APPU01000003)
A. baylyi ADP1 (NC_005966)
A. apis ANC 5114T = KACC 16906T (FZLN01000003)
A. boissieri ANC 4422T = SAP 284.1T (FMYL01000007)
A. nectaris CIP 110549T (AYER01000007)
A. larvae BRTC-1T (CP016895)
A. radioresistens CIP 103788T (APQF01000007)
A. qingfengensis ANC 4671T = 2BJ1T (MKKK01000012)
A. puyangensis ANC 4466T = BQ4-1T (OANT01000001)
A. populi PBJ7T (NEXX01000001)
A. tandoii CIP 107469T (AQFM01000044)
A. junii CIP 64.5T (APPX01000003)
A. haemolyticus CIP 64.3T (APQQ01000030)
A. halotolerans R16T (KU958712)
Genomic species 6 CIP A165 (APOK01000007)
A. bereziniae CIP 70.12T (APQG01000052)
A. guillouiae CIP 63.46T (APOS01000024)
A. defluvii WCHA30T (MAUF01000008)
A. bohemicus ANC 3994T (APOH01000009)
A. harbinensis HITLiT (JXBK01000001)
A. gerneri CIP 107464T (APPN01000080)
A. gandensis ANC 4275T (LZDS01000001)
A. albensis ANC 4874T (FMBK01000004)
A. equi 114T (NZ_CP012808)
A. celticus ANC 4603T (MBDL01000001)
A. johnsonii CIP 64.6T (APON01000009)
A. baumannii CIP 70.34T (APRG01000018)
A. seifertii NIPH 973T (APOO01000019)
A. nosocomialis NIPH 2119T (APOP01000014)
A. calcoaceticus CIP 81.8T (APQI01000002)
A. dijkshoorniae JVAP01T (LJPG01000019)
A. pittii CIP 70.29T (APQP01000014)
A. beijerinckii CIP 110307T (APQL01000006)
A. venetianus CIP 110063T (APPO01000001)
A. courvalinii ANC 3623T (APSA01000001)
A. vivianii NIPH 2168T (APRW01000008)
A. parvus CIP 108168T (APOM01000007)
A. modestus NIPH 236T (APOJ01000015)
A. dispersus ANC 4105T (APRL01000014)
A. proteolyticus NIPH 809T (APOI01000028)
Genomic species 16 CIP 70.18 (APRN01000028)
Genomic species 15BJ CIP 110321 (AQFL01000028)
A. tjernbergiae CIP 107465T (AYEV01000042)
A. gyllenbergii CIP 110306T (ATGG01000025)
A. colistiniresistens NIPH 2036T (ATGK01000017)
A. pseudolwoffii ANC 5318 (PGPB01000007)
A. pseudolwoffii ANC 5320
A. pseudolwoffii ANC 5347 (PGOZ01000014)
A. pseudolwoffii ANC 5504 (MG564149)
A. pseudolwoffii NIPH 748 (MG564156)
A. pseudolwoffii ANC 5303 (MG564147)
A. pseudolwoffii NIPH 1041 (MG564159)
A. pseudolwoffii NIPH 831 (MG564157)
A. pseudolwoffii ANC 5044T (PHRG01000001)
A. pseudolwoffii NIPH 746 (MG564155)
A. pseudolwoffii CIP 64.10 (APQS01000019)
A. pseudolwoffii ANC 4683
A. pseudolwoffii ANC 5307 (MG564148)
A. pseudolwoffii NIPH 713 (APRJ01000013)
A. lwoffii ANC 5032 (MG564143)
A. lwoffii NIPH 393 (MG564151)
A. lwoffii ANC 4217 (MG564141)
A. lwoffii ANC 4203 (MG564140)
A. lwoffii ANC 5085 (MG564145)
A. lwoffii ANC 4309 (MG564142)
A. lwoffii NIPH 715 (APOT01000025)
A. lwoffii ANC 4571 (APRY01000051)
A. lwoffii NIPH 237 (MG564150)
A. lwoffii NIPH 671 (MG564154)
A. lwoffii ANC 5055 (MG564144)
A. lwoffii ANC 4568 (APRU01000004)
A. lwoffii NIPH 912 (MG564158)
A. lwoffii NIPH 2266
A. pseudolwoffii ANC 5324 (PGPA01000003)
A. lwoffii NIPH 473
A. lwoffii NIPH 2176
A. lwoffii ANC 4400 (ACPN01000014)
A. lwoffii ANC 5086 (MG564146)
A. lwoffii NIPH 461 (MG564152)
A. lwoffii NIPH 474 (MG564153)
A. lwoffii NIPH 403
A. lwoffii NIPH 913
A. lwoffii NIPH 1094
A. lwoffii NIPH 2172 (APQT01000014)
A. lwoffii ANC 4305
A. lwoffii ANC 4569 (APRV01000023)
A. lwoffii ANC 4897
A. lwoffii NIPH 478 (APQU01000015)
A. lwoffii ANC 3906 (MG564139)
A. lwoffii NIPH 238
A. lwoffii NIPH 486
A. lwoffii CCM 5581T (AYHO01000005)
A. lwoffii NIPH 616
A. lwoffii NIPH 666
A. lwoffii NIPH 2175 (APOG01000008)
A. lwoffii NIPH 2257
A. lwoffii ANC 4570 (APRX01000013)
1%
B

Fig. 1

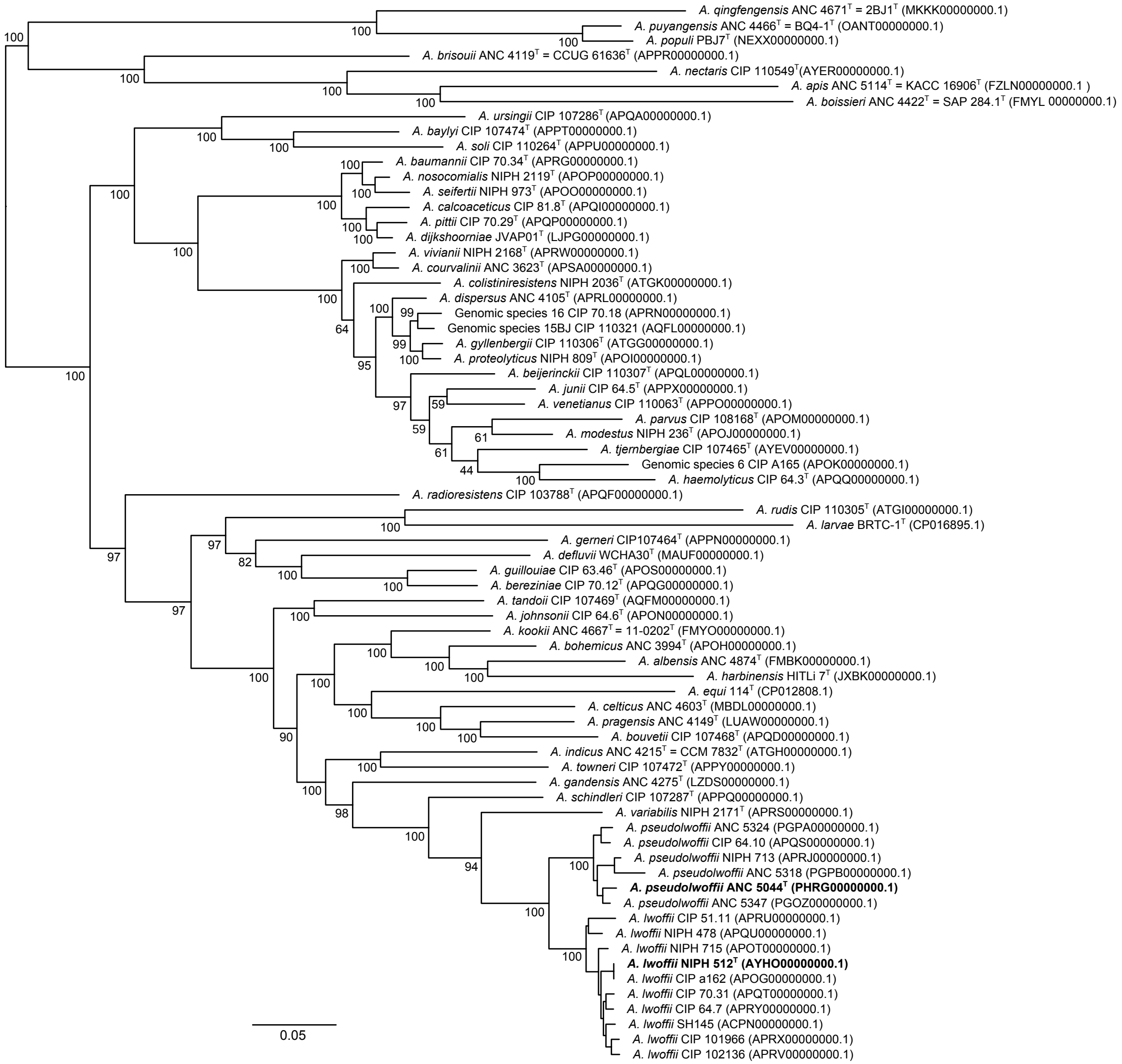
A. qingfengensis ANC 4671T = 2BJ1T (MKKK00000000.1)
A. puyangensis ANC 4466T = BQ4-1T (OANT00000000.1)
A. populi PBJ7T (NEXX00000000.1)
A. brisouii ANC 4119T = CCUG 61636T (APPR00000000.1)
A. nectaris CIP 110549T(AYER00000000.1)
A. apis ANC 5114T = KACC 16906T (FZLN00000000.1)
A. boissieri ANC 4422T = SAP 284.1T (FMYL 00000000.1)
A. ursingii CIP 107286T (APQA00000000.1)
A. baylyi CIP 107474T (APPT00000000.1)
A. soli CIP 110264T (APPU00000000.1)
A. baumannii CIP 70.34T (APRG00000000.1)
A. nosocomialis NIPH 2119T (APOP00000000.1)
A. seifertii NIPH 973T (APOO00000000.1)
A. calcoaceticus CIP 81.8T (APQI00000000.1)
A. pittii CIP 70.29T (APQP00000000.1)
A. dijkshoorniae JVAP01T (LJPG00000000.1)
A. vivianii NIPH 2168T (APRW00000000.1)
A. courvalinii ANC 3623T (APSA00000000.1)
A. colistiniresistens NIPH 2036T (ATGK00000000.1)
A. dispersus ANC 4105T (APRL00000000.1)
Genomic species 16 CIP 70.18 (APRN00000000.1)
Genomic species 15BJ CIP 110321 (AQFL00000000.1)
A. gyllenbergii CIP 110306T (ATGG00000000.1)
A. proteolyticus NIPH 809T (APOI00000000.1)
A. beijerinckii CIP 110307T (APQL00000000.1)
A. junii CIP 64.5T (APPX00000000.1)
A. venetianus CIP 110063T (APPO00000000.1)
A. parvus CIP 108168T (APOM00000000.1)
A. modestus NIPH 236T (APOJ00000000.1)
A. tjernbergiae CIP 107465T (AYEV00000000.1)
Genomic species 6 CIP A165 (APOK00000000.1)
A. haemolyticus CIP 64.3T (APQQ00000000.1)
A. radioresistens CIP 103788T (APQF00000000.1)
A. rudis CIP 110305T (ATGI00000000.1)
A. larvae BRTC-1T (CP016895.1)
A. gerneri CIP107464T (APPN00000000.1)
A. defluvii WCHA30T (MAUF00000000.1)
A. guillouiae CIP 63.46T (APOS00000000.1)
A. bereziniae CIP 70.12T (APQG00000000.1)
A. tandoii CIP 107469T (AQFM00000000.1)
A. johnsonii CIP 64.6T (APON00000000.1)
A. kookii ANC 4667T = 11-0202T (FMYO00000000.1)
A. bohemicus ANC 3994T (APOH00000000.1)
A. albensis ANC 4874T (FMBK00000000.1)
A. harbinensis HITLi 7T (JXBK00000000.1)
A. equi 114T (CP012808.1)
A. celticus ANC 4603T (MBDL00000000.1)
A. pragensis ANC 4149T (LUAW00000000.1)
A. bouvetii CIP 107468T (APQD00000000.1)
A. indicus ANC 4215T = CCM 7832T (ATGH00000000.1)
A. towneri CIP 107472T (APPY00000000.1)
A. gandensis ANC 4275T (LZDS00000000.1)
A. schindleri CIP 107287T (APPQ00000000.1)
A. variabilis NIPH 2171T (APRS00000000.1)
A. pseudolwoffii ANC 5324 (PGPA00000000.1)
A. pseudolwoffii CIP 64.10 (APQS00000000.1)
A. pseudolwoffii NIPH 713 (APRJ00000000.1)
A. pseudolwoffii ANC 5318 (PGPB00000000.1)
A. pseudolwoffii ANC 5044T (PHRG00000000.1)
A. pseudolwoffii ANC 5347 (PGOZ00000000.1)
A. lwoffii CIP 51.11 (APRU00000000.1)
A. lwoffii NIPH 478 (APQU00000000.1)
A. lwoffii NIPH 715 (APOT00000000.1)
A. lwoffii NIPH 512T (AYHO00000000.1)
A. lwoffii CIP a162 (APOG00000000.1)
A. lwoffii CIP 70.31 (APQT00000000.1)
A. lwoffii CIP 64.7 (APRY00000000.1)
A. lwoffii SH145 (ACPN00000000.1)
A. lwoffii CIP 101966 (APRX00000000.1)
A. lwoffii CIP 102136 (APRV00000000.1)
0.05

Fig. 2

Revising the taxonomy of the *Acinetobacter lwoffii* group: the description of *Acinetobacter pseudolwoffii* sp. nov. and emended description of *Acinetobacter lwoffii*

Alexandr Nemec, Lenka Radolfova-Krizova, Martina Maixnerova, Matej Nemec, Dominique Clermont, Jaroslav Bzdil, Petr Jezek, Petra Spanelova

Supplementary tables

Systematic and Applied Microbiology

Content

Table S1. Basic properties of the genome sequences of *Acinetobacter lwoffii* (genospecies 9) and *Acinetobacter pseudolwoffii* sp. nov. (genospecies 8)

Genome accession no.	Strain	Species	GC%	No. of contigs	Total length of contigs (bp)	No. of scaffolds	Total length of scaffolds (bp)	No. of proteins	Sequencing technology	Coverage	Reference
AYHO00000000.1	NIPH 512^T = CCM 5581^T = NCTC 5866^T	*A. lwoffii*	43.1	16	3382003	12	3384618	3237	Illumina	147.0x	Touchon *et al*. (2014)
ACPN00000000.1	SH145 = ANC 4400	*A. lwoffii*	42.8	250	3347001	76	3481004	3134	454	22.06x	Peleg *et al*. (2012)
APOG00000000.1	CIP A162 = NIPH 2175	*A. lwoffii*	43.0	12	3356935	7	3364056	3204	Illumina	92.0x	Touchon *et al*. (2014)
APOT00000000.1	NIPH 715 = CIP 110448	*A. lwoffii*	42.9	76	3400693	26	3454560	3314	Illumina	118.0x	Touchon *et al*. (2014)
APQT00000000.1	CIP 70.31 = NIPH 2172	*A. lwoffii*	43.0	79	3546109	12	3677680	3503	Illumina	74.0x	Touchon *et al*. (2014)
APQU00000000.1	NIPH 478 = CIP 110447	*A. lwoffii*	42.9	27	3276129	9	3301256	3088	Illumina	232.0x	Touchon *et al*. (2014)
APRU00000000.1	CIP 51.11 = ANC 4568	*A. lwoffii*	43.2	11	3133520	5	3143762	2899	Illumina	85.0x	Touchon *et al*. (2014)
APRV00000000.1	CIP 102136 = ANC 4569	*A. lwoffii*	42.5	51	3559292	20	3606614	3395	Illumina	82.0x	Touchon *et al*. (2014)
APRX00000000.1	CIP 101966 = ANC 4570	*A. lwoffii*	42.9	50	3485835	18	3528462	3424	Illumina	84.0x	Touchon *et al*. (2014)
APRY00000000.1	CIP 64.7 = ANC 4571	*A. lwoffii*	42.9	59	3608282	19	3657789	3550	Illumina	82.0x	Touchon *et al.* (2014)
PHRG00000000.1	ANC 5044^T = CCUG 67963^T = CCM 8638^T	*A. pseudolwoffii*	43.3	30	3105311	-	-	2791	Illumina Miseq	55x	This study
APQS00000000.1	CIP 64.10 = ANC 4579	*A. pseudolwoffii*	43.1	29	3206079	16	3218415	3019	Illumina	144.0x	Touchon *et al.* (2014)
APRJ00000000.1	NIPH 713 = CIP 110446	*A. pseudolwoffii*	43.3	19	3006240	12	3042530	2816	Illumina	88.0x	Touchon *et al.* (2014)
PGOZ00000000.1	ANC 5347	*A. pseudolwoffii*	42.9	55	3179951	-	-	2908	Illumina Miseq	51x	This study
PGPA00000000.1	ANC 5324	*A. pseudolwoffii*	43.4	19	2978620	-	-	2709	Illumina Miseq	50x	This study
PGPB00000000.1	ANC 5318	*A. pseudolwoffii*	43.4	40	2972456	-	-	2681	Illumina Miseq	45x	This study

Table S2. Average nucleotide identity based on BLAST values (%) between the whole genome sequences of the *A. lwoffii* group and those of the known *Acinetobacter* species.

The values were calculated by the JSpecies web program with default settings (www.imedea.uib.es/jspecies; Richter & Rosselló-Móra, 2009). Genomes are indicated by their DDBJ/ENA/GenBank accession numbers.

Species	Strain	Genome	AYHO	APRU	APRY	APQT	APRX	APRV	APOG	APQU	APOT	ACPN	PHRG	PGPB	PGPA	PGOZ	APQS	APRJ
A. lwoffii	**NIPH 512^T (= CCM 5581^T)**	**AYHO00000000**																
A. lwoffii	**CIP 51.11**	**APRU00000000**	95.7															
A. lwoffii	**CIP 64.7**	**APRY00000000**	95.61	95.57														
A. lwoffii	**CIP 70.31**	**APQT00000000**	95.65	95.79	96.75													
A. lwoffii	**CIP 101966**	**APRX00000000**	95.75	95.71	96.25	96.76												
A. lwoffii	**CIP 102136**	**APRV00000000**	95.69	95.62	96.23	96.71	96.55											
A. lwoffii	**CIP A162**	**APOG00000000**	99.98	95.64	95.54	95.59	95.72	95.61										
A. lwoffii	**NIPH 478**	**APQU00000000**	95.64	95.95	95.36	95.75	95.6	95.59	95.62									
A. lwoffii	**NIPH 715**	**APOT00000000**	96.05	95.74	96.42	96.48	96.53	96.47	95.97	95.78								
A. lwoffii	**SH145**	**ACPN00000000**	95.94	95.57	96.45	96.7	96.38	96.47	95.97	95.69	96.33							
A. pseudolwoffii	**ANC 5044^T**	**PHRG00000000**	87.32	87.21	87.33	87.44	87.43	87.29	87.29	87.04	87.39	87.27						
A. pseudolwoffii	**ANC 5318**	**PGPB00000000**	87.12	87.24	87.32	87.45	87.23	87.15	87.07	87.	87.27	87.06	96.76					
A. pseudolwoffii	**ANC 5324**	**PGPA00000000**	87.33	87.22	87.49	87.45	87.41	87.31	87.26	86.99	87.48	87.24	96.97	97.19				
A. pseudolwoffii	**ANC 5347**	**PGOZ00000000**	87.59	87.17	87.62	87.77	87.64	87.48	87.56	87.33	87.67	87.58	97.1	97.04	97.04			
A. pseudolwoffii	**CIP 64.10**	**APQS00000000**	88.33	88.06	88.54	88.57	88.37	88.22	88.24	87.97	88.58	88.41	96.55	96.14	96.51	96.83		
A. pseudolwoffii	**NIPH 713**	**APRJ00000000**	86.9	86.96	87.08	87.13	87.03	86.99	86.96	86.72	87.08	86.93	97.39	97.32	97.23	97.44	96.53	
A. albensis	ANC 4874^T	FMBK00000000	75.13	75.42	75.29	75.33	75.07	75.23	75.36	75.14	75.31	75.04	75.15	75.2	75.22	75.22	75.19	75.18
A. apis	ANC 5114^T (= KACC 16906^T)	FZLN00000000	70.93	71.14	70.91	70.89	70.78	70.89	70.92	70.74	71.04	70.62	70.57	70.66	70.59	70.59	71.06	70.88
A. baumannii	CIP 70.34^T	APRG00000000	73.37	73.45	73.45	73.34	73.19	73.16	73.44	73.17	73.43	72.91	72.99	73.13	72.97	72.97	73.26	73.09
A. baylyi	CIP 107474^T	APPT00000000	73.15	73.34	73.16	73.22	73.2	73.15	73.35	73.0	73.16	73.06	73.0	73.12	73.13	73.13	73.25	73.2
A. beijerinckii	CIP 110307^T	APQL00000000	73.27	73.43	73.32	73.29	73.1	73.32	73.34	73.08	73.35	73.11	72.84	72.93	72.95	72.95	73.12	73.0
A. bereziniae	CIP 70.12^T	APQG00000000	74.54	74.44	74.93	74.6	74.61	74.76	74.75	74.55	75.	74.29	74.11	73.97	73.87	73.87	74.36	73.9
A. bohemicus	ANC 3994^T	APOH00000000	76.02	76.17	76.11	76.19	76.08	76.06	76.24	76.03	76.26	75.87	76.02	76.1	75.94	75.94	76.1	76.02
A. boissieri	ANC 4422^T (= SAP 284.1^T)	FMYL00000000	70.83	71.02	70.85	70.76	70.51	70.77	70.87	70.48	71.07	70.53	70.39	70.55	70.32	70.32	70.86	70.74
A. bouvetii	CIP 107468^T	APQD00000000	75.72	76.0	75.77	75.9	75.71	75.77	75.99	75.72	75.98	75.63	75.76	75.68	75.6	75.6	75.78	75.79
A. brisouii	ANC 4119^T (= CCUG 61636^T)	APPR00000000	73.38	73.7	73.56	73.54	73.44	73.5	73.51	73.52	73.7	73.23	73.44	73.42	73.32	73.32	73.67	73.46
A. calcoaceticus	CIP 81.8^T	APQI00000000	73.19	73.43	73.24	73.26	73.13	73.12	73.36	73.02	73.36	72.94	72.99	72.99	72.91	72.91	73.13	73.07
A. celticus	ANC 4603^T	MBDL00000000	75.73	75.9	75.87	75.92	75.68	75.74	76.02	75.64	75.76	75.57	75.67	75.62	75.62	75.62	75.78	75.75
A. colistiniresistens	NIPH 2036^T (= 1191^T)	ATGK00000000	74.16	73.85	74.57	74.65	74.26	74.82	74.37	74.	74.46	74.21	73.74	73.67	73.68	73.68	74.22	73.67
A. courvalinii	NIPH 1847^T (= 382^T)	APRR00000000	74.19	74.34	74.	73.99	73.93	73.91	74.23	73.89	74.05	73.95	73.94	73.81	73.93	73.93	73.96	73.92
A. defluvii	WCHA30^T	MAUF00000000	74.76	74.78	75.17	75.57	75.07	75.16	74.92	74.68	75.37	74.67	74.46	74.45	74.5	74.5	74.92	74.6
A. dijkshoorniae	JVAP01^T	LJPG00000000	73.25	73.46	73.19	73.21	73.08	73.12	73.35	73.	73.25	72.94	73.08	73.03	72.9	72.9	73.12	73.1
A. dispersus	ANC 4105^T	APRL00000000	73.64	73.75	73.61	73.56	73.48	73.52	73.79	73.52	73.67	73.46	73.41	73.48	73.37	73.37	73.6	73.52
A. equi	114^T	CP012808	75.04	75.08	75.12	75.05	74.98	74.96	75.17	74.75	75.2	74.86	74.76	74.72	74.74	74.74	74.93	74.86
A. gandensis	ANC 4275^T (= UG 60467^T)	LZDS00000000	76.44	76.34	76.38	76.38	76.19	76.47	76.58	75.94	76.37	75.82	75.94	75.94	75.87	75.87	76.1	76.06
A. gerneri	CIP 107464^T	APPN00000000	74.45	74.26	74.75	74.71	74.61	74.66	74.57	74.16	74.63	74.1	73.95	73.85	73.95	73.95	74.5	73.96
A. guillouiae	CIP 63.46^T	APOS00000000	74.54	75.07	74.7	74.65	74.69	74.77	74.69	74.5	74.75	74.5	74.32	74.56	74.21	74.21	74.53	74.35
A. gyllenbergii	CIP 110306^T	ATGG00000000	73.63	73.82	73.73	73.65	73.62	73.53	73.84	73.44	73.74	73.51	73.5	73.51	73.43	73.43	73.63	73.57
A. haemolyticus	CIP 64.3^T	APQQ00000000	73.36	73.3	73.72	74.25	73.52	73.61	73.58	73.24	73.89	73.29	73.02	73.13	73.02	73.02	73.32	73.18
A. harbinensis	HITLi7^T	JXBK00000000	75.38	75.22	75.8	75.66	75.48	75.67	75.27	75.51	75.68	75.25	75.03	74.96	75.08	75.08	75.28	75.16
A. indicus	ANC 4215^T (= CCM 7832^T)	ATGH00000000	77.76	77.97	78.33	78.69	78.58	78.62	77.69	77.67	78.28	78.08	77.55	77.34	77.32	77.32	77.95	77.34
A. johnsonii	CIP 64.6^T	APON00000000	76.31	76.03	76.92	77.22	76.4	76.83	76.42	76.04	77.12	76.43	76.03	75.92	75.73	75.73	76.22	75.76
A. junii	CIP 64.5^T	APPX00000000	73.82	73.6	73.83	74.19	73.68	73.71	73.93	73.42	73.85	73.57	72.91	72.89	72.82	72.82	73.45	72.98
A. kookii	ANC 4667^T (= KCTC 32033^T)	FMYO00000000	77.23	77.24	77.38	77.39	77.45	77.27	77.44	77.24	77.42	77.05	77.24	76.9	77.03	77.03	77.05	77.09
A. larvae	BRTC-1^T	CP016895	72.57	72.76	72.66	72.7	72.42	72.47	72.77	72.42	72.8	72.39	72.47	72.48	72.33	72.33	72.8	72.54
A. modestus	NIPH 236^T	APOJ00000000	73.76	74.17	73.92	73.96	73.63	73.88	73.78	73.77	74.0	73.72	73.09	73.53	73.23	73.23	73.49	73.2
A. nectaris	CIP 110549^T	AYER00000000	71.13	71.4	71.24	71.06	71.05	71.16	71.15	70.99	71.44	70.92	71.0	71.15	70.79	70.79	71.38	71.21
A. nosocomialis	NIPH 2119^T	APOP00000000	73.32	73.46	73.3	73.2	73.18	73.18	73.41	73.11	73.25	73.0	73.06	73.08	72.98	72.98	73.18	73.1
A. parvus	CIP 108168^T	APOM00000000	74.79	74.57	75.41	75.63	75.03	75.43	74.75	74.49	75.46	75.	74.04	73.97	73.94	73.94	74.81	74.03
A. pittii	CIP 70.29^T	APQP00000000	73.23	73.49	73.28	73.28	73.23	73.23	73.37	73.11	73.3	73.07	73.05	73.05	72.95	72.95	73.19	73.12
A. populi	ANC 5415^T (= CFCC 11170^T)	NEXX00000000	72.04	72.1	71.91	71.93	71.83	72.08	72.12	71.68	72.11	71.73	71.76	71.69	71.63	71.63	72.16	71.64
A. pragensis	ANC 4149^T	LUAW00000000	75.64	75.9	75.76	75.93	75.74	75.76	75.95	75.59	75.89	75.6	75.63	75.48	75.62	75.62	75.78	75.7
A. proteolyticus	NIPH 809^T	APOI00000000	73.82	73.94	73.79	73.75	73.68	73.64	73.97	73.6	73.89	73.58	73.59	73.6	73.49	73.49	73.76	73.69
A. puyangensis	ANC 4466^T (= BQ4-1^T)	OANT00000000	71.62	71.93	71.84	71.7	71.51	71.73	71.85	71.61	71.85	71.5	71.58	71.53	71.48	71.48	71.81	71.48
A. qingfengensis	ANC 4671^T (= 2BJ1^T)	MKKK00000000	71.12	71.44	71.36	71.32	70.87	71.24	71.38	71.21	71.35	71.06	70.85	70.88	70.95	70.95	71.21	71.06
A. radioresistens	CIP 103788^T	APQF00000000	74.5	74.82	74.99	75.17	75.33	75.35	74.47	74.61	75.07	75.14	74.	73.91	74.02	74.02	74.35	73.97
A. rudis	CIP 110305^T	ATGI00000000	72.84	72.89	72.61	72.86	72.72	72.66	72.87	72.69	73.04	72.6	72.62	72.52	72.41	72.41	72.89	72.66
A. seifertii	NIPH 973^T	APOO00000000	73.89	74.05	74.04	74.32	74.07	74.15	74.03	73.63	74.13	73.59	73.24	73.53	73.13	73.13	73.75	73.3
A. schindleri	CIP 107287^T	APPQ00000000	79.75	79.72	80.12	80.3	80.32	79.94	79.75	79.8	80.05	79.68	79.02	78.99	79.07	79.07	79.31	79.06
A. soli	CIP 110264^T	APPU00000000	73.13	73.29	73.06	73.07	73.05	73.09	73.24	73.05	73.04	72.92	72.91	72.95	72.99	72.99	73.11	73.03
A. tandoii	CIP 107469^T	AQFM00000000	75.32	75.67	75.79	75.83	75.47	75.74	75.54	75.42	75.9	75.34	75.28	75.33	75.08	75.08	75.14	75.09
A. tjernbergiae	CIP 107465^T	AYEV00000000	73.45	73.6	73.65	73.72	73.47	73.51	73.69	73.26	73.73	73.37	73.23	73.3	73.28	73.28	73.32	73.24
A. towneri	CIP 107472^T	APPY00000000	76.69	76.84	77.28	77.58	77.26	77.28	76.88	76.54	77.28	76.68	76.87	76.55	76.81	76.81	76.79	76.55
A. ursingii	CIP 107286^T	APQA00000000	74.63	74.45	74.76	74.93	74.61	74.65	74.52	74.28	75.04	74.3	73.9	74.02	73.86	73.86	74.51	73.86
A. variabilis	NIPH 2171^T	APRS00000000	82.02	82.03	82.67	82.84	82.64	82.54	81.93	82.35	82.58	82.39	81.16	81.11	81.1	81.1	81.69	80.96
A. venetianus	CIP 110063^T	APPO00000000	73.37	73.48	73.51	73.6	73.45	73.31	73.53	73.19	73.48	73.24	73.06	73.09	73.17	73.17	73.33	73.2
A. vivianii	NIPH 2168^T	APRW00000000	73.98	74.1	73.84	73.8	73.87	73.72	74.05	73.67	73.92	73.69	73.72	73.68	73.69	73.69	73.77	73.7
Genomic sp. 6	CIP a165	APOK00000000	74.	74.08	74.13	73.91	73.86	73.9	74.25	73.84	74.23	73.69	73.66	73.55	73.53	73.53	73.83	73.61
Genomic sp. 15BJ	CIP 110321	AQFL00000000	74.08	74.2	74.61	74.12	74.16	73.9	74.19	73.9	74.34	74.02	73.64	73.8	73.51	73.51	74.1	73.6
Genomic sp. 16	CIP 70.18	APRN00000000	73.89	73.87	74.68	74.58	74.12	74.33	73.95	73.8	74.19	73.98	73.39	73.46	73.47	73.47	73.9	73.47

Table S3. Digital DNA-DNA hybridization values (%) between the whole genome sequences of the *A. lwoffii* group and those of the known *Acinetobacter* species.

The values were calculated by the GGDC 2.1 web program (http://ggdc.dsmz.de; Meier-Kolthoff *et al*., 2013). Genomes are indicated by their DDBJ/ENA/GenBank accession numbers.

Species	Strain	Genome	AYHO	APRU	APRY	APQT	APRX	APRV	APOG	APQU	APOT	ACPN	PHRG	PGPB	PGPA	PGOZ	APQS	APRJ
A. lwoffii	**NIPH 512^T (= CCM 5581^T)**	**AYHO000000000**																
A. lwoffii	**CIP 51.11**	**APRU00000000**	65.50															
A. lwoffii	**CIP 64.7**	**APRY00000000**	66.50	64.90														
A. lwoffii	**CIP 70.31**	**APQT00000000**	66.90	66.30	74.20													
A. lwoffii	**CIP 101966**	**APRX00000000**	67.10	65.80	69.90	72.90												
A. lwoffii	**CIP 102136**	**APRV00000000**	66.70	65.40	70.20	72.60	71.60											
A. lwoffii	**CIP A162**	**APOG00000000**	99.30	65.80	66.90	67.30	67.40	66.80										
A. lwoffii	**NIPH 478**	**APQU00000000**	66.00	67.20	64.70	66.10	65.30	66.00	66.20									
A. lwoffii	**NIPH 715**	**APOT00000000**	68.00	66.10	71.90	70.70	71.50	71.00	67.90	66.10								
A. lwoffii	**SH145**	**ACPN00000000**	67.40	65.10	71.60	72.10	70.80	71.50	67.50	66.10	69.90							
A. pseudolwoffii	ANC 5044^T	PHRG00000000	33.70	33.10	34.00	34.00	34.10	33.80	33.70	33.00	34.00	33.70						
A. pseudolwoffii	ANC 5318	PGPB00000000	33.30	33.20	33.70	33.70	33.70	33.50	33.30	32.90	33.60	33.50	73.80					
A. pseudolwoffii	ANC 5324	PGPA00000000	33.80	33.20	34.10	33.80	34.00	33.70	33.80	33.10	33.90	33.80	74.80	77.20				
A. pseudolwoffii	ANC 5347	PGOZ00000000	34.20	33.20	34.60	34.60	34.60	34.20	34.20	33.70	34.60	34.60	76.40	75.90	75.30			
A. pseudolwoffii	CIP 64.10	APQS00000000	36.00	35.10	36.80	36.70	36.60	36.40	36.00	35.30	36.70	36.60	72.00	68.80	70.60	73.50		
A. pseudolwoffii	NIPH 713	APRJ00000000	33.00	32.70	33.30	33.20	33.20	33.00	33.00	32.30	33.20	33.00	78.60	77.00	76.40	79.30	72.60	
A. albensis	ANC 4874^T	FMBK00000000	20.50	20.50	20.60	20.60	20.50	20.50	20.60	20.50	20.30	20.40	20.40	20.50	20.30	20.30	20.50	20.50
A. apis	ANC 5114^T (= KACC 16906^T)	FZLN00000000	21.10	21.40	21.40	21.40	20.70	20.90	21.50	21.10	21.00	19.80	20.30	20.70	20.40	20.30	20.80	21.00
A. baumannii	CIP 70.34^T	APRG00000000	21.50	21.10	21.70	21.60	21.40	21.50	21.50	21.50	21.00	20.80	20.70	20.80	20.60	20.80	21.10	20.90
A. baylyi	CIP 107474^T	APPT00000000	20.70	20.50	20.80	20.60	20.70	20.40	20.70	20.70	20.20	20.10	20.50	20.30	20.30	20.30	20.60	20.60
A. beijerinckii	CIP 110307^T	APQL00000000	21.00	21.60	21.30	21.40	21.10	21.50	21.10	21.00	20.90	21.00	20.20	20.20	20.00	20.20	20.60	20.30
A. bereziniae	CIP 70.12^T	APQG00000000	22.80	22.00	23.80	23.00	23.00	23.10	22.70	22.80	22.10	22.40	21.50	21.40	21.30	22.00	22.30	21.30
A. bohemicus	ANC 3994^T	APOH00000000	21.50	21.50	21.60	21.60	21.60	21.70	21.60	21.50	21.30	21.60	21.40	21.10	21.10	21.40	21.60	21.30
A. boissieri	ANC 4422^T (= SAP 284.1^T)	FMYL00000000	21.40	21.40	22.30	21.90	21.10	21.50	21.80	21.40	21.00	20.60	21.00	20.80	20.70	20.90	22.20	21.50
A. bouvetii	CIP 107468^T	APQD00000000	20.80	20.90	21.10	21.00	21.00	21.00	20.80	20.80	20.80	21.00	21.00	21.10	20.90	20.90	21.00	20.90
A. brisouii	ANC 4119^T (= CCUG 61636^T)	APPR00000000	21.30	21.50	21.40	21.10	21.90	21.50	21.40	21.30	21.20	21.30	20.90	20.90	21.10	20.90	21.30	21.10
A. calcoaceticus	CIP 81.8^T	APQI00000000	21.30	21.30	21.20	21.30	21.00	21.10	21.40	21.30	21.10	21.10	20.40	20.50	20.30	20.40	20.90	20.60
A. celticus	ANC 4603^T	MBDL00000000	21.30	20.90	20.70	20.70	20.70	20.80	20.70	20.60	20.60	20.70	20.80	20.80	20.70	20.70	20.90	20.90
A. colistiniresistens	NIPH 2036^T (= 1191^T)	ATGK00000000	21.30	21.50	23.20	23.50	23.50	24.10	23.00	22.80	22.00	22.40	21.40	20.90	21.30	22.40	22.60	21.20
A. courvalinii	NIPH 1847^T (= 382^T)	APRR00000000	21.30	21.40	21.30	21.10	21.00	21.20	21.20	21.10	21.10	20.80	20.90	20.80	20.70	20.80	21.10	21.10
A. defluvii	WCHA30^T	MAUF00000000	21.80	21.60	22.90	23.00	22.70	22.50	21.70	21.80	21.50	21.90	21.00	21.30	21.10	21.50	22.20	21.00
A. dijkshoorniae	JVAP01^T	LJPG00000000	20.40	20.70	20.40	20.30	20.40	20.40	20.50	20.40	20.00	20.20	20.10	20.00	20.00	20.00	20.50	20.30
A. dispersus	ANC 4105^T	APRL00000000	21.40	21.40	21.30	21.20	21.30	21.30	21.40	21.40	21.00	21.00	20.50	20.40	20.40	20.50	20.60	20.60
A. equi	114^T	CP012808	21.50	21.50	21.80	21.70	21.70	21.60	21.60	21.50	21.40	21.40	21.50	21.40	21.30	21.50	21.80	21.40
A. gandensis	ANC 4275^T (= UG 60467^T)	LZDS00000000	21.80	21.60	21.90	21.90	21.80	22.00	21.90	21.80	21.20	21.30	21.10	21.50	21.00	21.30	21.40	21.20
A. gerneri	CIP 107464^T	APPN00000000	22.40	21.80	22.90	22.80	22.70	23.40	22.40	22.40	21.70	22.20	22.20	22.00	22.30	23.20	22.90	21.60
A. guillouiae	CIP 63.46^T	APOS00000000	22.20	22.90	22.70	22.70	23.00	22.60	22.00	22.20	22.00	21.80	21.80	22.40	21.60	21.90	22.20	21.60
A. gyllenbergii	CIP 110306^T	ATGG00000000	20.70	20.90	21.00	21.00	20.80	20.90	20.70	20.70	20.70	20.50	20.40	20.30	20.20	20.50	20.80	20.40
A. haemolyticus	CIP 64.3^T	APQQ00000000	22.60	21.90	24.40	25.10	23.40	23.90	22.80	22.60	21.70	22.70	21.20	21.40	21.40	22.00	22.40	21.60
A. harbinensis	HITLi7^T	JXBK00000000	21.10	20.80	21.90	21.50	21.60	21.60	20.80	21.10	21.20	21.30	21.20	20.30	20.30	20.80	21.10	20.40
A. indicus	ANC 4215^T (= CCM 7832^T)	ATGH00000000	23.00	22.70	23.40	23.90	23.50	23.80	22.80	23.00	22.70	23.20	22.30	22.10	22.20	22.20	22.90	22.00
A. johnsonii	CIP 64.6^T	APON00000000	22.90	22.10	23.80	24.20	23.60	23.70	22.80	22.90	22.30	23.10	22.00	21.90	21.80	22.20	22.50	21.50
A. junii	CIP 64.5^T	APPX00000000	23.30	22.00	23.40	23.70	23.40	23.40	23.10	23.30	22.30	22.90	21.00	20.80	20.80	21.80	22.50	21.10
A. kookii	ANC 4667^T (= KCTC 32033^T)	FMYO00000000	21.80	21.70	22.00	21.90	21.90	22.10	21.80	21.50	21.80	21.60	21.40	21.40	21.30	21.30	21.50	21.30
A. larvae	BRTC-1^T	CP016895	21.50	21.80	21.70	21.50	21.60	21.70	21.70	21.90	21.30	21.20	21.30	20.90	21.00	21.10	21.60	21.50
A. modestus	NIPH 236^T	APOJ00000000	21.90	23.20	22.00	22.10	22.00	21.70	21.20	21.10	22.20	21.40	20.70	21.10	20.30	20.30	21.50	20.60
A. nectaris	CIP 110549^T	AYER00000000	21.10	21.30	21.00	21.00	20.90	21.40	21.40	21.20	20.60	20.40	20.70	20.70	20.50	20.90	20.90	20.70
A. nosocomialis	NIPH 2119^T	APOP00000000	21.20	21.20	21.50	21.40	21.30	21.30	21.40	23.70	20.80	21.00	20.60	20.40	20.60	20.50	20.90	20.70
A. parvus	CIP 108168^T	APOM00000000	23.70	22.20	25.20	17.00	24.80	24.70	23.40	21.00	22.60	24.30	21.10	21.70	21.20	22.10	23.10	21.30
A. pittii	CIP 70.29^T	APQP00000000	21.00	21.20	21.10	21.40	20.80	21.00	21.10	21.60	20.80	20.90	20.90	20.60	20.70	20.70	21.10	21.20
A. populi	ANC 5415^T (= CFCC 11170^T)	NEXX00000000	21.60	22.00	22.30	21.70	21.70	21.90	21.80	20.70	20.80	21.30	20.90	21.00	21.00	21.30	22.20	21.40
A. pragensis	ANC 4149^T	LUAW00000000	20.70	20.90	21.00	21.00	21.00	20.90	20.80	21.20	20.80	20.90	20.80	20.90	20.80	20.80	21.00	20.90
A. proteolyticus	NIPH 809^T	APOI00000000	21.20	21.20	21.30	21.40	21.40	20.90	21.30	21.80	21.00	20.80	20.90	20.50	20.80	20.80	21.10	20.90
A. puyangensis	ANC 4466^T (= BQ4-1^T)	OANT00000000	21.80	22.20	21.40	21.10	21.20	21.50	22.00	21.60	21.10	20.80	20.80	20.40	20.70	20.90	21.60	21.20
A. qingfengensis	ANC 4671^T (= 2BJ1^T)	MKKK00000000	21.60	22.40	22.30	21.70	21.40	22.10	22.00	22.40	22.20	20.90	20.80	21.20	20.90	20.90	21.80	21.40
A. radioresistens	CIP 103788^T	APQF00000000	22.40	22.90	23.40	23.40	24.40	24.30	22.00	21.80	22.40	23.90	21.40	21.10	21.20	21.70	22.10	21.00
A. rudis	CIP 110305^T	ATGI00000000	21.80	22.20	21.90	21.80	21.50	21.70	22.00	23.20	21.50	21.60	21.20	20.80	20.80	20.80	21.50	21.40
A. seifertii	NIPH 973^T	APOO00000000	23.20	22.50	24.20	24.20	24.00	24.40	23.20	23.70	22.40	23.30	21.40	21.90	21.40	22.00	22.60	21.50
A. schindleri	CIP 107287^T	APPQ00000000	23.70	23.40	24.20	24.30	24.50	24.00	23.70	20.90	23.60	23.60	21.40	22.60	22.60	22.80	23.20	22.70
A. soli	CIP 110264^T	APPU00000000	20.90	21.00	20.90	20.90	20.80	20.90	21.00	21.50	20.70	20.80	20.40	20.50	20.70	20.50	20.90	20.70
A. tandoii	CIP 107469^T	AQFM00000000	21.50	21.80	22.00	22.00	21.80	21.90	21.40	20.80	21.40	21.40	21.20	21.20	20.90	20.90	21.30	20.70
A. tjernbergiae	CIP 107465^T	AYEV00000000	20.80	21.50	21.10	21.20	21.30	21.00	20.80	22.10	20.60	20.80	20.10	20.00	19.90	20.30	20.50	20.20
A. towneri	CIP 107472^T	APPY00000000	22.10	22.10	22.90	23.10	22.80	23.00	22.20	22.70	21.70	22.00	22.00	21.90	21.80	22.00	22.40	21.70
A. ursingii	CIP 107286^T	APQA00000000	22.70	22.10	23.20	23.10	23.30	22.80	22.00	25.90	22.30	22.10	21.00	21.20	20.80	21.70	22.00	20.60
A. variabilis	NIPH 2171^T	APRS00000000	25.90	25.40	26.90	26.90	26.70	26.70	25.80	21.20	26.00	26.50	24.30	24.50	24.40	24.90	25.30	24.20
A. venetianus	CIP 110063^T	APPO00000000	21.20	21.10	21.30	21.30	21.00	21.20	21.30	21.20	21.10	21.10	20.50	20.30	20.40	20.50	20.80	20.70
A. vivianii	NIPH 2168^T	APRW00000000	21.00	21.40	21.10	21.40	21.10	20.90	21.10	15.90	20.90	20.60	20.80	20.70	20.70	20.60	21.20	20.80
Genomic sp. 6	CIP a165	APOK00000000	21.00	22.80	25.40	25.30	24.70	24.70	23.20	23.70	22.60	23.20	22.00	21.50	21.70	23.60	23.20	21.70
Genomic sp. 15BJ	CIP 110321	AQFL00000000	22.60	22.00	22.90	22.50	22.40	22.70	22.70	22.60	21.80	21.70	20.90	20.80	20.80	21.30	21.50	21.00
Genomic sp. 16	CIP 70.18	APRN00000000	21.80	22.00	23.10	21.90	22.70	22.10	21.90	21.80	21.10	22.10	20.90	20.90	20.50	21.60	21.70	20.60

Table S4. Phenotypic properties of *Acinetobacter lwoffii* (genospecies 9), *Acinetobacter pseudolwoffii* sp. nov. (genospecies 8), and the *Acinetobacter* species with validly published names.

Listed are all species with validly published names except for *A. boissieri* and *A. nectaris*, which are negative in the assimilation tests shown (they utilize other substrates such as sucrose or D-fructose), *A. grimontii*, *A. guangdongensis* and *A. pakistanensis*, which are the later synonyms of *A. junii* (Vaneechoutte *et al*., 2008), *A. indicus* (Nemec & Radolfova-Krizova, 2017) and *A. bohemicus* (Nemec & Radolfova-Krizova, 2016), respectively, and *A. lactucae*, which is synonymous with *A. dijkshoorniae* (Dunlap & Rooney, 2018). Each species includes a type strain. All the results were obtained in the Laboratory of Bacterial Genetics in Prague using an array of strictly standardized, in-house tests as described by Nemec *et al*. (2009) and Krizova *et al*. (2015). All the data were published previously (Krizova *et al*., 2015; Nemec *et al*., 2016, 2017; Radolfova-Krizova *et al*., 2016a,b) except for those for the type strains of *A. halotolerans*, *A. larvae*, *A. piscicola* and an additional strain of *A. kookii*. Except for temperature-dependent tests, the culture temperature was 25 °C for A. celticus and 30 °C for the other species. Assimilation/growth tests were interpreted after 6 days of culture, other tests after 3 (haemolytic and gelatinase activities) or 2 (D-glucose acidification, temperature growth tests) days. +, All strains positive; -, all strains negative; !, results which are opposite to those published in original nomenclatural proposals; D, (mostly) doubtful or irreproducible reactions; W, (mostly) weak positive reactions; ND, not determined. Numbers are percentages of strains with clearly positive reactions; for strain-dependent reactions, results for type strains are given in parentheses. Numbers of strains of individual species are indicated in parentheses after the species names.

Characteristic	*A. lwoffii* (37)	*A. pseudolwoffii* (15)	*A. albensis* (8)	*A. apis* (1)	*A. baumannii* (25)	*A. baylyi* (5)	*A. beijerinckii* (15)	*A. berezinae* (16)	*A. bohemicus* (25)	*A. bouvetii* (1)	*A. brisouii* (1)	*A. calcoaceticus* (11)	*A. celticus* (6)	*A. colistiniresistens* (24)	*A. courvalinii* (9)	*A. defluvii* (1)	*A. dispersus* (9)	*A. dijkshoorniae* (5)	*A. equi* (5)	*A. gandensis* (6)	*A. gerneri* (1)	*A. guillouiae* (17)	*A. gyllenbergii* (9)	*A. haemolyticus* (16)	*A. halotolerans* (1)	*A. harbinensis* (1)	*A. indicus* (2)
Growth at 44 °C	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	60 (+)	-	-	-	-	-	-	-	-	D
Growth at 41 °C	11W (-)	-	-	-	+	60 (-)	-	-	-	-	-	9 (-)	-	-	50W (D)	-	-	+	D	-	-	-	-	94 (+)	- !	-	+
Growth at 37 °C	+	+	-	-	+	+	+	+	-	D	+	91 (-)	-	82 (+)	+	+	+	+	+	+	+	D	+	+	+	-	+
Growth at 35 °C	+	+	-	-	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	- !	+
Growth at 32 °C	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acidification of D-glucos	19 (-)	-	-	+	+	+	-	88 (+)	-	-	-	91 (-)	-	+	+	-	-	+	-	-	+	-	-	75 (+)	+	-	-
Hemolysis of sheep blo	-	-	-	-	-	-	+	-	80W (W)	-	-	-	-	+	89 (+)	- !	+	-	-	-	-	-	+	+	+	-	-
Liquefaction of gelatin	-	-	-	-	-	-	13 (-)	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	+	94 (+)	+	-	-
Assimilation of																											
Acetate	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
trans-Aconitate	5 (-)	-	-	-	92 (+)	+	-	38 (-)	-	-	+	+	-	-	44 (-)	-	11 (-)	+	-	-	-	12 (-)	-	63 (+)	+	-	On
Adipate	76 (+)	40 (+)	88 (+)	-	88 (+)	+	-	63 (+)	-	-	-	+	-	-	+	-	22 (-)	+	+	-	+	+	+	-	-	-	-
β-Alanine	-	-	-	-	+	-	-	+	-	-	+	91 (-)	-	17 (-)	89 (+)	- !	+	+	-	-	+	94 (+)	+	-	-	- !	-
4-Aminobutyrate	84 (+)	-	-	-	+	+	+	+	+	-	+	+	-	-	+	- !	+	+	-	-	+	88 (D)	D	+	+	-	-
L-Arabinose	-	-	-	-	84 (+)	-	-	-	-	-	-	27 (-)	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
L-Arginine	5 (-)	-	-	-	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	-	-	-	+	94 (+)	+	-	-
L-Aspartate	-	-	+	-	+	+	+	+	+	-	+	+	+	-	11 (-)	-	-	+	+	-	D	+	-	31 (-)	+	-	-
Azelate	+	80 (+)	88 (+)	-	88 (+)	+	-	63 (+)	-	-	-	+	-	-	+	-	22 (-)	+	-	-	+	+	+	-	-	- !	50 (-)
Benzoate	84 (+)	80 (+)	D (+)	-	84 (+)	+	-	+	92 (+)	+	+	+	+	17 (-)	+	+	+	+	+	+	+	88 (+)	+	-	-	+ !	50 (+)
2,3-Butanediol	8 (-)	-	-	-	+	+	-	+	+	-	+	+	-	-	-	-	-	+	-	33 (-)	+	+	-	-	-	-	-
Citraconate	-	-	-	-	40 (+)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate (Simmons)	8 (-)	-	+	+	+	+	+	+	-	+	+	91 (D)	-	+	+	-	+	+	+	50 (+)	+	+	+	75 (+)	+	-	-
Ethanol	+	73 (+)	-	-	96 (+)	+	+	+	+	+	+	91 (+)	+	17 (-)	-	+	11 (-)	+	80	+	+	+	22 (-)	94 (+)	+	+	+
Gentisate	-	-	50 (+)	-	4 (-)	-	-	-	4 (-)	-	-	-	-	88 (+)	-	-	33 (-)	+	+	-	-	18 (-)	11 (+)	81 (+)	-	-	-
D-Gluconate	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-Glucose	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-Glutamate	8 (-)	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+ !	+	+	+	33 (+)	+	+	+	+	+	- !	50 (+)
Glutarate	-	13 (-)	-	-	96 (+)	D	-	+	-	+	+	91 (D)	-	-	+	+ !	+	+	-	83 (+)	+	+	D (-)	-	-	-	-
Histamine	-	-	-	-	-	-	-	63 (+)	-	-	-	-	-	-	11 (-)	-	-	-	-	-	-	65 (+)	-	-	-	-	-
L-Histidine	-	-	-	-	96 (+)	-	+	94 (+)	+	+	-	+	-	+	+	-	+	+	+	-	-	94 (+)	+	+	+	-	-
4-Hydroxybenzoate	-	7 (-)	88 (+)	-	92 (+)	+	-	88 (+)	92 (+)	-	+	91 (+)	-	83 (+)	+	-	+	+	+	-	+	88 (+)	89 (+)	81 (+)	+	-	-
DL-Lactate	84 (+)	40 (+)	+	-	+	+	-	+	+	+	+	+	+	96 (+)	+	+	+	+	+	+	+	+	+	-	-	+	+
L-Leucine	-	-	-	-	88 (+)	-	93 (+)	-	-	-	-	91 (-)	-	13 (-)	+	-	+	+	-	-	-	-	+	88 (+)	+	-	-
Levulinate	3 (-)	-	-	-	24 (-)	-	-	-	-	-	-	91 (-)	50 (-)	-	11 (-)	-	-	60 (-)	-	-	-	-	-	-	-	-	-
D-Malate	11 (-)	7 (-)	38W (W)	-	92 (+)	D (+)	+	88 (+)	D	-	+	D (-)	-	92 (+)	89 (+)	-	+	+	-	-	-	94 (+)	+	88 (+)	+	-	-
Malonate	8 (-)	7 (-)	+	-	88 (+)	+	+	-	+	-	+	+	+	8 (-)	+	-	22 (-)	+	-	17 (-)	-	18 (-)	78 (+)	-	-	+	-
L-Ornithine	8 (-)	-	-	-	76 (-)	-	-	-	-	-	+	+	-	-	89 (+)	-	89 (D)	+	-	-	-	-	56 (+)	-	-	-	-
Phenylacetate	81 (+)	80 (+)	-	-	84 (+)	-	-	25 (-)	-	-	+	+	67 (+)	96 (+)	+	- !	89 (+)	+	-	-	+	65 (+)	+	-	-	-	+
L-Phenylalanine	-	-	-	-	84 (+)	-	-	-	-	-	-	+	-	96 (+)	+	-	89 (+)	+	-	-	+	-	89 (+)	-	-	-	-
Putrescine	-	-	-	-	96 (+)	-	-	-	-	-	D	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-
D-Ribose	-	-	-	-	76 (+)	-	-	-	-	-	-	45 (-)	-	-	-	-	-	80 (+)	-	-	-	-	-	-	-	-	-
L-Tartrate	-	13 (+)	-	-	32 (-)	-	-	-	16 (-)	-	+	9 (-)	-	-	22 (-)	-	-	-	-	-	-	-	-	-	-	-	-
Tricarballylate	8 (-)	-	+	-	92 (+)	80 (+)	-	38 (-)	4 (-)	-	+	+	-	-	44 (-)	-	11 (-)	+	80	-	-	12 (-)	-	-	-	-	-
Trigonelline	-	-	-	-	60 (+)	+	-	+	-	-	-	9 (-)	-	33 (-)	67 (+)	- !	-	60 (+)	-	-	-	59 (-)	-	-	-	-	-
Tryptamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22 (D)	-	11 (+)	D	-	-	+	82 (+)	-	-	-	-	-

Table S4. Phenotypic properties of *Acinetobacter lwoffii*, *Acinetobacter pseudolwoffii* sp. nov. and the *Acinetobacter* species with validly published names. – *continued*

Characteristic	*A. johnsonii* (30)	*A. junii* (14)	*A. kookii* (2)	*A. larvae* (1)	*A. modestus* (7)	*A. nosocomialis* (20)	*A. parvus* (10)	*A. pisciola* (1)	*A. pittii* (20)	*A. populi* (3)	*A. pragensis* (7)	*A. proteolyticus* (6)	*A. puyangensis* (2)	*A. qingfengensis* (2)	*A. radioresistens* (12)	*A. rudis* (3)	*A. schindleri* (22)	*A. seifertii* (16)	*A. soli* (5)	*A. tandoii* (1)	*A. tjernbergiae* (2)	*A. towneri* (2)	*A. ursingii* (29)	*A. variabilis* (16)	*A. venetianus* (5)	*A. vivianii* (9)
Growth at 44 °C	-	-	-	- !	-	95 (+)	-	-	10 (-)	-	-	-	-	-	-	-	-	13 (-)	-	-	-	-	-	31W (W)	-	-
Growth at 41 °C	-	93 (+)	+	- !	-	+	-	-	+	+	-	-	-	- !	+	-	+	94 (+)	80 (+)	-	-	+	-	+	-	-
Growth at 37 °C	10W (D)	+	+	+	+ (W)	+	90 (+)	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Growth at 35 °C	+	+	+	+	+	+	+	-	+	+	D	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Growth at 32 °C	+	+	+	+	+	+	+	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acidification of D-glucos	-	-	-	-	-	+	-	-	95 (+)	-	-	-	-	- !	-	-	-	+	+	-	-	-	-	13 (-)	-	89 (+)
Hemolysis of sheep blo	60W (-)	50 (-)	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	60 (+)	-	+	-	-	-	+	+
Liquefaction of gelatin	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	80 (+)	-
Assimilation of																										
Acetate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
trans -Aconitate	-	-	-	- !	-	60 (+)	-	-	+	-	-	+	-	- !	-	-	-	+	+	+	-	-	-	6 (-)	-	56 (-)
Adipate	-	-	-	- !	14 (-)	95 (+)	-	-	+	33 (+)	-	67 (-)	-	-	+	-	41 (-)	63 (-)	+	-	-	-	+	69 (-)	20 (-)	89 (+)
β-Alanine	-	-	-	+	-	85 (+)	-	- !	90 (+)	-	-	+	-	-	-	+	-	88 (-)	-	-	-	-	-	-	-	+
4-Aminobutyrate	83 (+)	86 (+)	+	+	-	+	-	-	+	+	-	D (-)	50 (-) !	+	+	+	-	+	+	+	-	-	-	19 (-)	+	+
L-Arabinose	-	-	-	-	-	+	-	-	85 (+)	-	-	-	-	- !	-	-	-	-	-	-	-	-	-	19 (-)	-	-
L-Arginine	83 (+)	93 (+)	-	- !	+	+	-	+	+	-	-	+	-	- !	83 (+)	-	-	+	+	+	50 (+)	-	-	19 (-)	+	+
L-Aspartate	83 (+)	21 (+)	-	+	-	+	-	-	+	+	-	-	50 (-) !	50 (-) !	-	-	-	+	+	+	-	-	97W (+)	-	-	11 (D)
Azelate	-	-	-	-	-	95 (+)	-	- !	+	-	-	67 (-)	-	-	+	-	64 (-)	63 (-)	+	-	-	-	+	81 (+)	20 (-)	89 (+)
Benzoate	97 (+)	79 (+)	+	- !	+	90 (+)	-	+ !	90 (+)	-	+	+	D	-	+	+	91 (+)	94 (+)	+	+	-	+	52 (-)	88 (+)	+	+
2,3-Butanediol	67 (-)	-	+	+	14 (-)	90 (+)	-	-	85 (+)	+	29 (+)	-	+ !	+ !	+	+	32 (-)	+	+	+	-	50 (-)	-	81 (+)	-	-
Citraconate	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate (Simmons)	90 (-)	79 (+)	-	+	-	+	-	+	+	-	+	+	- !	50 (-) !	-	+	59W (+)	+	+	+	-	-	+	25 (-)	+	+
Ethanol	+	93 (+)	+	+	+	+	+	+ !	+	+	+	-	+	+	+	+	95 (+)	+	+	+	+	+	+	+	+	-
Gentisate	-	-	-	-	-	10 (-)	-	-	25 (-)	-	43 (+)	+	-	-	-	-	41 (+)	75 (+)	-	-	-	-	-	-	-	89 (+)
D-Gluconate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
D-Glucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
L-Glutamate	+	+	-	+	+	+	-	+	+	+	86 (D)	+	+	+	+	+	- (D)	+	+	+	+	50 (+)	+	25 (-)	+	+
Glutarate	-	-	-	+	-	95 (+)	-	- !	90 (+)	+	+	33 (-)	50 (+) !	-	+	+	95 (+)	+	+	-	-	-	97 (+)	19 (-)	-	89 (+)
Histamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-Histidine	-	93 (+)	-	+	+	+	-	-	+	-	-	+	-	-	-	+	-	94 (+)	+	+	+	-	-	-	+	+
4-Hydroxybenzoate	20 (-)	-	-	- !	-	80 (+)	-	-	+	-	+	+	+	+	-	+	64 (+)	94 (+)	+	+	-	-	97 (+)	-	-	+
DL-Lactate	+	93 (+)	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	6 (-)	-	+
L-Leucine	-	14 (-)	-	-	-	95 (+)	-	-	95 (+)	-	-	83 (-)	-	-	92 (+)	+	-	94 (-)	-	+	-	-	-	-	+	+
Levulinate	-	-	-	-	-	5 (-)	-	-	5 (-)	-	-	-	-	-	-	-	-	6 (-)	-	-	-	-	-	-	-	-
D-Malate	10W (-)	79 (D)	-	- !	D	+	-	- !	95 (+)	67 (+)	-	+	-	-	-	-	95W (+)	88 (+)	80 (D)	+	-	50 (-)	+W	13 (+)	+	+
Malonate	73 (-)	-	-	+	-	20 (+)	-	-	95 (+)	-	+	67 (+)	-	+W	+	+	-	75 (+)	+	+	-	-	-	-	+	+
L-Ornithine	-	-	-	- !	-	95 (+)	20 (-)	-	95 (+)	-	-	+	-	-	-	-	-	81 (-)	-	+	-	-	-	-	-	+
Phenylacetate	-	-	50 (+)	-	-	85 (+)	-	-	75 (+)	33 (-)	71 (+)	83 (-)	- !	- !	+	+	-	88 (+)	+	+	-	-	-	75 (+)	-	+
L-Phenylalanine	-	-	-	-	-	85 (+)	-	-	75 (+)	-	-	+	- !	-	92 (+)	+	-	88 (+)	+	+	-	-	-	38 (-)	-	+
Putrescine	-	-	-	+	-	95 (+)	-	-	+	-	-	-	-	- !	92 (+)	-	-	81 (-)	-	+	-	-	-	-	-	78 (+)
D-Ribose	-	-	-	-	-	80 (+)	-	-	35 (-)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13 (-)	-	22 (+)
L-Tartrate	37 (-)	-	-	-	-	-	-	-	85 (+)	-	+	-	-	-	-	-	18 (-)	31 (+)	20 (-)	+	-	-	-	-	-	-
Tricarballylate	-	-	-	-	-	95 (+)	-	-	+	-	71 (-)	+	-	-	-	-	45 (+)	+	+	+	-	-	-	-	-	56 (-)
Trigonelline	-	-	-	-	+	20 (-)	-	-	20 (+)	-	-	-	50 (-) !	+	-	-	-	-	+	-	50 (+)	-	-	-	-	+
Tryptamine	-	-	-	-	-	-	-	-	-	-	57 (-)	50 (+)	-	-	-	+	-	6 (-)	-	-	-	-	-	-	-	D

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