Acinetobacter portensis sp. nov. and *Acinetobacter guerrae* sp. nov., isolated from raw meat

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Abstract

The taxonomic status of six strains of *Acinetobacter* obtained from meat samples, collected from supermarkets in Porto, Portugal, was investigated using polyphasic analysis. Partial *rpoB* sequence similarities lower than 95% to other *Acinetobacter* species with validly published names led to the hypothesis that these strains represented novel species. This was confirmed based on comparative multilocus sequence analysis, which included the *gyrB*, *recA* and 16S rRNA genes, revealing that these strains represented two coherent lineages that were distinct from each other and from all known species. The names *Acinetobacter portensis* sp. nov. (comprising four strains) and *Acinetobacter guerrae* sp. nov. (comprising two strains) are proposed for these novel species. The species status of these two groups was confirmed by low (below 95%) whole-genome sequence average nucleotide identity values and low (below 70%) digital DNA–DNA hybridization similarities between the whole-genome sequences of the proposed type strains of each novel species and the representatives of the known *Acinetobacter* species. Phylogenomic treeing from core genome analysis supported these results. The coherence of each new species lineage was supported by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry differentiation of the species at the protein level, by cellular fatty acid profiles, and by unique and differential combinations of metabolic and physiological properties shared by each novel species. The type strain of *A. portensis* sp. nov. is AC 877^T (=CCUG 68672^T=CCM 8789^T) and the type strain of *A. guerrae* sp. nov. is AC 1271^T (=CCUG 68674^T=CCM 8791^T).

The genus *Acinetobacter* was described initially by Brisou and Prévot [1] and currently comprises 63 species with validly published names (www.szu.cz/anemec/Classification.pdf), including four pairs of heterotypic synonyms. There are also four genomospecies delineated by DNA–DNA similarity [2–4]. Most of these species were proposed based on the descriptions of clinical and environmental isolates while descriptions of strains derived from food are less common (www.szu.cz/anemec/Classification.pdf). However, during a study focused on the diversity of *Acinetobacter* species in samples of meat (beef, pork, chicken and turkey), we identified novel genomospecies clearly distinct from all known species and other genomospecies of the genus [5].

This study carried out comprehensive characterizations and assessed the properties and taxonomic and phylogenetic positions of these strains, using polyphasic taxonomic methods relevant for the genus *Acinetobacter* [6, 7]. Our results indicate that these bacteria represent two novel

Table 1. Strains of *Acinetobacter portensis* sp. nov. and *Acinetobacter guerrae* sp. nov.

Strain designation	Specimen	Locality and date of isolation	16S rRNA gene	*rpoB* gene	*gyrB* gene	*recA* gene	Genome sequence
			Accession no.	Accession no.	Accession no.	Accession no.	Accession no.
Acinetobacter portensis (*n*=4)							
AC 877[T] (=CCUG 68672[T]=CCM 8789[T])	Raw pork meat	Porto, Portugal. September 2014	KX870877	KX885208	KX885192	KX885200	LWRV00000000.1
AC 1335 (=CCUG 68677)	Raw turkey meat	Porto, Portugal. September 2014	ND	KX885213	KX885197	KX885205	ND
AC 1301 (=CCUG 68676)	Raw chicken meat	Porto, Portugal. September 2014	ND	KX885212	KX885196	KX885204	ND
AC 1123 (=CCUG 68673=CCM 8790)	Raw beef meat	Porto, Portugal. September 2014	ND	KX885209	KX885193	KX885201	ND
Acinetobacter guerrae (*n*=2)							
AC 1271[T] (=CCUG 68674[T] = CCM 8791[T])	Raw chicken meat	Porto, Portugal. September 2014	KX870878	KX885210	KX885194	KX885202	LXGN00000000.1
AC 1272 (=CCUG 68675=CCM 8792)	Raw chicken meat	Porto, Portugal. September 2014	ND	KX885211	KX885195	KX885203	ND

CCM, Czech Collection of Microorganisms, Brno, Czech Republic; CCUG, Culture Collection University of Gothenburg, Gothenburg, Sweden; ND, not determined.

species genotypically and phylogenetically coherent and distinct from each other and from all known species. The names *Acinetobacter portensis* sp. nov. and *Acinetobacter guerrae* sp. nov. are proposed for these two taxonomic lineages.

ISOLATION AND ECOLOGY

A total of six strains of *A. portensis* sp. nov. (*n*=4) and *A. guerrae* sp. nov. (*n*=2) (Table 1) were isolated from raw meat samples (purchased from supermarkets in Porto, Portugal), using enrichment cultivation in Dijkshoorn's medium, at 30 °C, for 24 h, followed by plating on CHRO-Magar *Acinetobacter* at 30 °C, for 24–48 h, according to Carvalheira *et al.* [5]. The typical red colonies of *Acinetobacter* species were identified to the genus level on the basis of phenotypic (Gram-negative, coccobacilli, oxidase-negative and catalase-positive) and genotypic (16S rRNA gene sequence signatures and similarities) characteristics according to Vanbroekhoven *et al.* [8].

Macrorestriction profile analysis of genomic DNA was performed by pulsed-field gel electrophoresis according to Carvalheira *et al.* [5] to verify the diversity of the isolates at the strain level and the results showed that these strains yielded unique macrorestriction patterns (Fig. S1). Additionally, the genotypic heterogeneity of the strains studied was supported by differences in DNA-directed RNA polymerase β-subunit (*rpoB*) gene sequences [5], which is the best studied single gene taxonomic and phylogenetic marker for *Acinetobacter* species [9–14].

16S rRNA GENE PHYLOGENY

Strains AC 877[T] (=CCUG 68672[T]=CCM 8789[T]) and AC 1271[T] (=CCUG 68674[T]=CCM 8791[T]) were selected, respectively, as the designated type strains of the species *A. portensis* sp. nov. and *A. guerrae* sp. nov. To determine the 16S rRNA gene sequences, the primers 16F28 (5′-AGAGTTTGATCKTG-GCTCAG-3′) and 16R1494 (5′-NTACGGYTACCTTGTT ACGAC-3′) were used for PCR amplification, and primers 16F63 (5′-AGGCCTAACACATGCAAGTC-3′), 16SF1103 (5′-TGTTGGGTTAAGTCCCGCAAC-3′), 16SR806 (5′-GGACTACCAGGGTATCTAAT-3′) and 16R1494 were used for Sanger sequencing. PCR amplification of biomarker genes was performed in volumes of 25 µl, with 1.25 U Taq PCR Master Mix Kit 250 Units (Qiagen), 1 µM of each amplification primer and 5 µl bacterial genomic DNA. After initial denaturation, at 95 °C, for 2 min, 35 amplification cycles were performed according to the format: 30 s at 95 °C; 1 min at 55 °C; 2 min at 72 °C; and a final extension of 10 min at 72 °C. PCR products were purified and sequenced (GATC Biotech AG). The PCR-amplification primers target the region corresponding to positions 28–1494 in the *Escherichia coli* 16S rRNA gene sequence numbering (NCBI accession no. J01859).

The full-length 16S rRNA gene sequences were extracted from the available whole-genome sequences of the type strains of *Acinetobacter* species and reference strains of genomospecies (Table S1, available in the online version of this article) to compare the sequences of the putative new species within the context of all known species of the genus. For *Acinetobacter*

modestus and *Acinetobacter piscicola*, only incomplete 16S rRNA gene sequences were available in the genome sequence assemblies. Thus, alternative publicly available, nearly complete sequences were used (Table S1).

The analysis was carried out using a region of 1353 nucleotide positions corresponding to positions 105 through 1457 of the 16S rRNA gene sequence of *E. coli*. Evolutionary distances were calculated using the maximum composite likelihood method [15] with MEGA 6 software [16] and a phylogenetic tree was reconstructed using the neighbour-joining method [17] (Fig. S2). The most closely related species to the type strain of *A. portensis* sp. nov. was observed to be *Acinetobacter celticus* ANC 4603[T] (97.7%) and *Acinetobacter cumulans* WCHAc060092[T] (97.6%). The most closely related species to the type strain of *A. guerrae* sp. nov. was *Acinetobacter ursingii* CIP 107286[T] (99.1%) and *Acinetobacter beijerinckii* CIP 110307[T] (97.3%). Notably, in the case of the comparative analyses of *A. portensis* sp. nov., the similarity values are below 98.5%, which was proposed by Kim *et al.* [18] as the threshold for delineating prokaryotic species, confirming that this proposed novel species is distinct from all known species. Concerning *A. guerrae* sp. nov., the 16S rRNA gene sequence similarity value is above the threshold of 98.5%; therefore, the species status was confirmed based on housekeeping gene sequence analyses and whole-genome sequences analysis. The lowest 16S rRNA gene sequence similarities to other *Acinetobacter* species between the type strains of *A. portensis* sp. nov. and *A. guerrae* sp. nov. were with the 16S rRNA gene sequence of *Acinetobacter apis* ANC 5114[T] (94.2 and 94.7%, respectively; Fig. S2).

GENOME SEQUENCE DETERMINATION

Representatives of all *Acinetobacter* species, including known genomospecies, except *Acinetobacter grimontii*, a later synonym of *Acinetobacter junii* [19], *Acinetobacter pakistanensis*, a later synonym of *Acinetobacter bohemicus* [20], *Acinetobacter guangdongensis*, a later synonym of *Acinetobacter indicus* [21] and *Acinetobacter dijkshoorniae*, a later synonym of *Acinetobacter lactucae* [22], were included in the genomic analyses (Figs. 1 and 2, S3). Accession numbers are presented in Table S1.

The type strains of *A. portensis* sp. nov. AC 877[T] and *A. guerrae* sp. nov. AC 1271[T] were cultivated overnight, on Columbia agar base with 5% of defibrinated horse blood, at 30 °C. Cells were lysed in CLB buffer (1 mM Tris, 0.1 mM EDTA, pH 8.0) supplemented with proteinase K (1 mg ml⁻¹), incubating at 56 °C for 1 h. Afterwards, genomic DNA was isolated using a MagNA Pure Compact Nucleic Acid Isolation Kit version I (Roche Diagnostics). Libraries for the whole-genome sequencing were prepared using the TruSeq DNA Nano kit (Illumina) with a mean fragment length of 900 bp. Libraries were sequenced on the Illumina MiSeq platform version 3 chemistry, 2×300 bp (SciLifeLab). Subsequently, sequence reads were trimmed and assembled *de novo* using CLC Genomics Workbench version 8.5.1 (CLC bio). The genome assembly of *A. portensis* sp. nov. AC 877[T] yielded 123 contigs

of a total size of 2.89 Mb and a G+C content of 36.6 mol%. The genome assembly of *A. guerrae* sp. nov. AC 1271[T] yielded 34 contigs of a total size of 3.41 Mb and a G+C content of 39.2 mol%. The genome sequences of *A. portensis* sp. nov. AC 877[T] and *A. guerrae* sp. nov. AC 1271[T] were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [23], revealing 2595 and 3104 coding sequences, respectively.

OVERALL GENOME RELATEDNESS INDICES

The average nucleotide identities based on BLAST (ANIb) [24] were determined between the genome sequences of *A. portensis* sp. nov. AC 877[T], *A. guerrae* sp. nov. AC 1271[T] and those of the type strains of nearly all *Acinetobacter* species and representatives of genomospecies (Table S2). The ANIb values were determined using the JSpeciesWS server (http://jspecies.ribohost.com/jspeciesws) [25]. The ANIb values (means of reciprocal values; Tables 2 and S2) of the genome sequences of *A. portensis* sp. nov. AC 877[T] and *A. guerrae* sp. nov. AC 1271[T] against those of all other species of *Acinetobacter* ranged from 71.26% (*Acinetobacter qingfengensis* ANC 4671[T]) to 78.23% (*Acinetobacter equi* 114[T]) and from 71.52% (*Acinetobacter boissieri* ANC 4422[T]) to 86.70% (*A. ursingii* CIP 107286[T]), respectively. These values are much lower than the suggested threshold of 94–96% sequence similarity proposed to distinguish bacterial species [26, 27] and further supports the genomic distinction of *A. portensis* sp. nov. and *A. guerrae* sp. nov. at the species level. These findings were supported by the similarity values from digital DNA–DNA hybridization (dDDH) analyses (Tables 2 and S2), which were determined using the Genome-to-Genome Distance Calculator (GGDC) web server [28]. The dDDH values ranged between 19.2–23% for *A. portensis* sp. nov. AC 877[T] and other species of *Acinetobacter* and between 19.1–32.6% for *A. guerrae* sp. nov. AC 1271[T] and other species of *Acinetobacter*, all well below the threshold of 70% for belonging to the same species.

RELATEDNESS WITH PUBLICLY AVAILABLE GENOME SEQUENCES

All genome sequences labelled as *Acinetobacter* species that were available in NCBI RefSeq [29] on 12 January 2020, were downloaded. Subsequently, ANIb values between *A. portensis* AC 877[T], *A. guerrae* AC 1271[T] and the downloaded genome sequences were calculated using JSpeciesWS [25]. The analyses revealed two genome sequences of strains matching *A. guerrae*: TUM15565 (GenBank accession number BKYM00000000; ANIb value 97.90%) and WCHAc060096 (GenBank accession number RAXU00000000; ANIb value 98.26%).

Strain TUM15565 was isolated from human sputum in Kanagawa, Japan, in 2013; the genome sequence has a total length of 3.86 Mb, G+C content of 39.5 mol% and 3465 coding sequences. Strain WCHAc060096 was isolated from hospital sewage in Sichuan, PR China, in 2018; the genome sequence has a total length of 3.54 Mb, G+C content of 39.3 mol% and 3252 coding sequences. Strains of *A. guerrae* described in
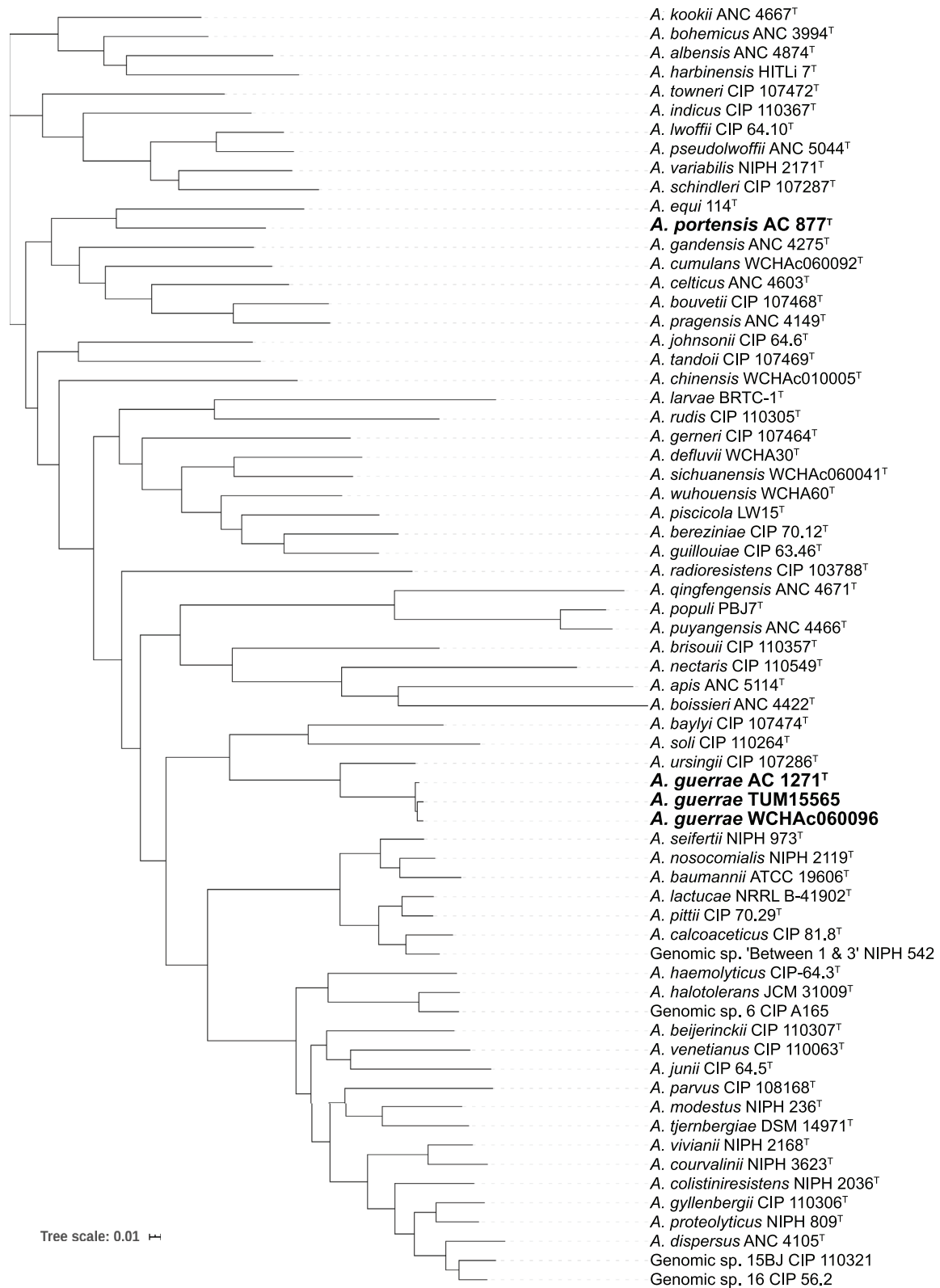
Fig. 1. Core genome-based phylogenomic tree based on the alignment of 1007 core genes. Taxa in bold highlight the two proposed novel species.
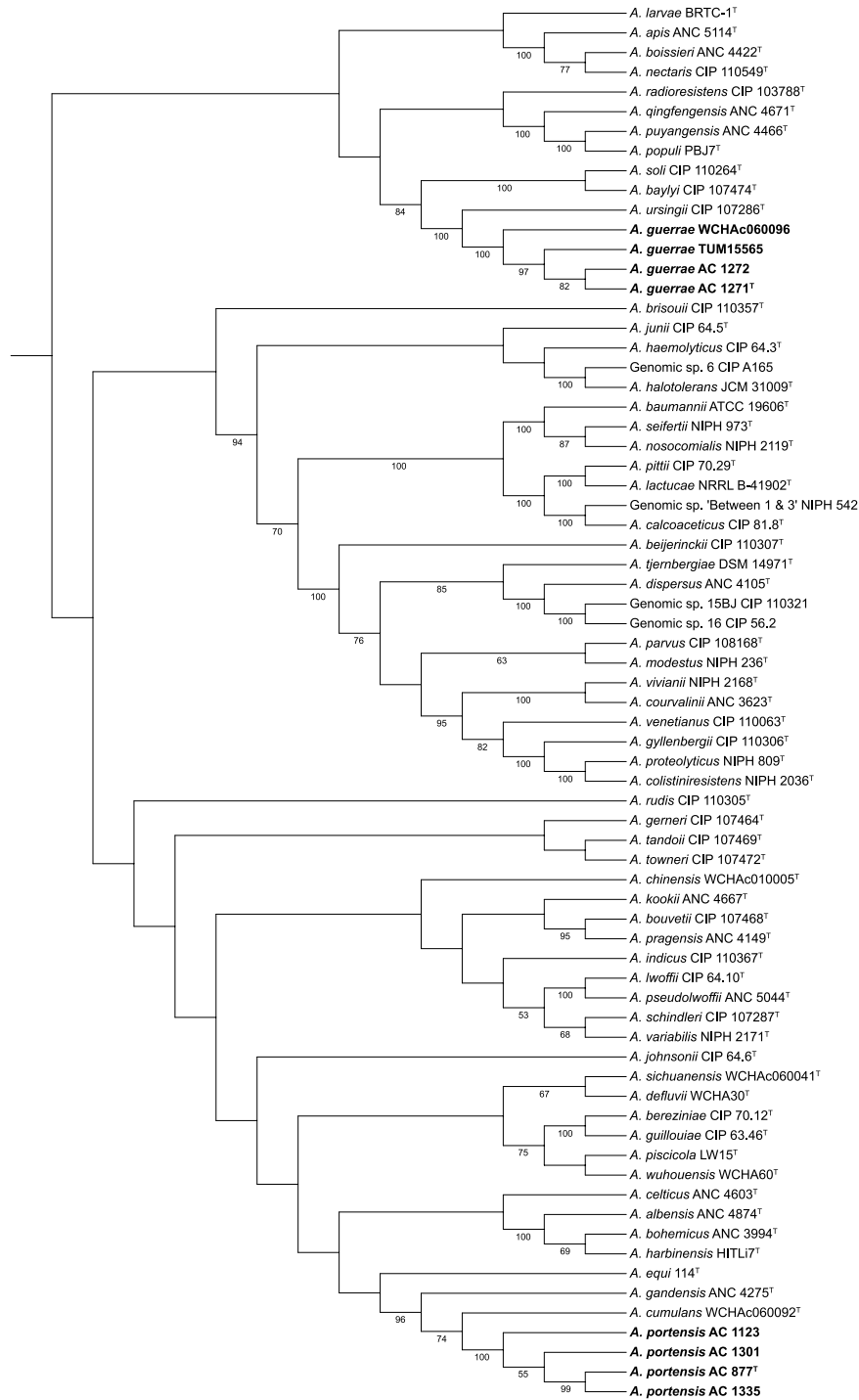
Fig. 2. Neighbour-joining phylogram based on the concatenated partial *rpoB*, *recA* and *gyrB* gene sequences of four strains of *Acinetobacter portensis* sp. nov., four strains of *Acinetobacter guerrae* sp. nov., the type strains of the 63 species with validly published names of the genus *Acinetobacter*, and four genomospecies of the genus. The evolutionary distances were computed using the maximum composite likelihood method [15]. Bootstrap values (>50%) after 1000 simulations are shown at branch nodes. All calculations were conducted in MEGA 6 [16].

Table 2. Highest similarity values between 16S rRNA gene, concatenated *rpoB*, *recA*, *gyrB* gene sequences and whole genome sequences of the strains of *Acinetobacter portensis* sp. nov., *Acinetobacter guerrae* sp. nov. and the type or reference strains of known species of the genus *Acinetobacter*.

	Highest similarity values			
Strain designation	**16S rRNA gene**	**Concatenated *rpoB*, *recA*, *gyrB* gene**	**ANIb**	**dDDH**
Acinetobacter portensis (n=4)				
AC 877^T (=CCUG 68672^T=CCM 8789^T)	97.7% *A. celticus*	86.6% *A. cumulans*	78.2% *A. equi*	23% *A. cumulans*
AC 1335 (=CCUG 68677)		86.6% *A. cumulans*		
AC 1301 (=CCUG 68676)		86.7% *A. cumulans*		
AC 1123 (=CCUG 68673=CCM 8790)		86.9% *A. cumulans*		
Acinetobacter guerrae (n-2)				
AC 1271^T (=CCUG 68674^T=CCM 8791^T)	99.1% *A. ursingii*	87% *A. ursingii*	86.7% *A. ursingii*	32.6% *A. ursingii*
AC 1272 (=CCUG 68675=CCM 8792)		86.7% *A. ursingii*		

the present study were isolated from raw meat in Portugal. However, the finding of these two additional strains isolated from human sputum and hospital sewage in Asia suggests that the species has a broader geographical and ecological distribution and that the public health risk of *A. guerrae* strains should not be neglected. Therefore, these two additional *A. guerrae* strains (TUM15565 and WCHAc060096) were also included in the genotypic analyses (Figs. 1 and 2, S3).

CORE GENOME-BASED PHYLOGENOMIC ANALYSIS

The core genome for the dataset of the novel genome sequences and the reference genomes (listed in Table S1) was determined using the Roary pan-genome analysis pipeline [30] with an amino acid identity threshold of 50%.

The resulting core genome alignment, based on 1007 core genes, was used for reconstruction of a dendrogram of phylogenetic relationships using FastTree [31]. The core genome tree supported other phylogenetic analyses in that *A. portensis* was observed to be most closely related to *A. equi* while *A. guerrae* is most closely related to *A. ursingii*, sharing an evolutionary clade with *Acinetobacter baylyi* and *Acinetobacter soli* (Fig. 1).

MULTILOCUS SEQUENCE ANALYSIS (MLSA)

The intraspecies genotypic coherence of the strains of *A. portensis* sp. nov. and *A. guerrae* sp. nov. was assessed, using MLSA based on the concatenated sequences of genes encoding *rpoB*, recombinase subunit-A (*recA*) and DNA gyrase subunit-B (*gyrB*).

The partial sequences of *rpoB* of *A. portensis* sp. nov. and *A. guerrae* sp. nov. strains were obtained as previously described [5, 10]. Likewise, the partial sequences of *recA* were amplified with the primers, RA1 and RA2, as described previously [32], and the partial sequences of *gyrB* were obtained using newly developed primers, Aci_gyrB-F4:5′-AHHGAAAARGCDTATGATTCYTC-3′

and Aci_gyrB-R2:5′-SWHCCRCCHGCNGARTCRC-3′) under the same PCR-amplification and Sanger-sequencing conditions described above for 16S rRNA gene analyses. Sequence accession numbers are listed in Table S1. We obtained complete or nearly complete *rpoB*, *recA* and *gyrB* gene sequences from GenBank or by extracting them from the available whole-genome sequences of the type strains of *Acinetobacter* species and reference strains of genomospecies to compare these sequence data within the context of the genus (Table S1). The three gene sequences were also extracted from the two additional database whole-genome sequences of strains of *A. guerrae* and included in the analysis.

The nucleotide sequences of *rpoB*, *recA* and *gyrB* were individually aligned by MEGA 6 software [16] and further concatenated, using the Join Alignments tool of the CLC Genomics Workbench version 12 (Qiagen). The phylogenetic tree and evolutionary distances were inferred by MEGA 6 software [16] as described above for 16S rRNA gene sequences. Gene sequence similarity calculations and cluster analyses were carried out for 717 bp, 388 bp and 773/776 bp segments, corresponding to nucleotide positions 3041–3757, 210–597 and 425–1200 of the *rpoB*, *recA* and *gyrB* coding regions of *Acinetobacter baumannii* ATCC 19606^T (NCBI accession no. ACQB00000000.1), respectively. Fig. 2 shows the results of cluster analysis for the concatenated *rpoB*, *recA* and *gyrB* sequences of the strains of *A. portensis* sp. nov. and *A. guerrae* sp. nov., and type strains of all species and genomospecies of *Acinetobacter*.

As shown in Fig. 2, the strains of *A. portensis* sp. nov. and *A. guerrae* sp. nov. grouped into two respective clusters, distinct from all known *Acinetobacter* species, which was supported by high bootstrap values. The intraspecies similarity values (expressed as the percentages of identical nucleotides in corresponding positions in two aligned sequences) of the concatenated sequences of the strains of *A. portensis* sp. nov. ranged from 99.8 to 100 % while the similarity values for the strains of *A. guerrae* sp. nov. ranged from 98.4 to 99 %. The similarity values between the strains of *A. portensis* sp.

nov. and *A. guerrae* sp. nov. with the other species of the genus ranged from 72.4% (*A. radioresistens* CIP 103788[T]) to 86.9% (*A. cumulans* WCHAc060092[T]), and from 72.8% (*A. apis* ANC 5114[T]) to 87% (*A. ursingii* CIP 107286[T]), respectively (Table 2). High intraspecies similarities were also reflected in *rpoB* individual sequences: 99.4–100% and 99.4–99.6% for the *A. portensis* sp. nov. and *A. guerrae* sp. nov. strains, respectively. The similarity values between *rpoB* individual sequences of *A. portensis* sp. nov. and *A. guerrae* sp. nov. strains with the other species of the genus ranged from 70.7% (*Acinetobacter puyangensis* ANC 4466[T]) to 92.0% (*Acinetobacter wuhouensis* WCHA60[T]), and from 74% (*Acinetobacter kookii* ANC 4667[T]) to 92.2% (*A. ursingii* NIPH 107286[T]), respectively (Fig. S3). These results are in agreement with the results of other studies of *rpoB* sequence analyses of *Acinetobacter* species, wherein *rpoB* interspecies similarity values are observed to be below 95% [9, 10, 20, 21, 32]. Therefore, these data further support the distinctness of these two novel group at the species level.
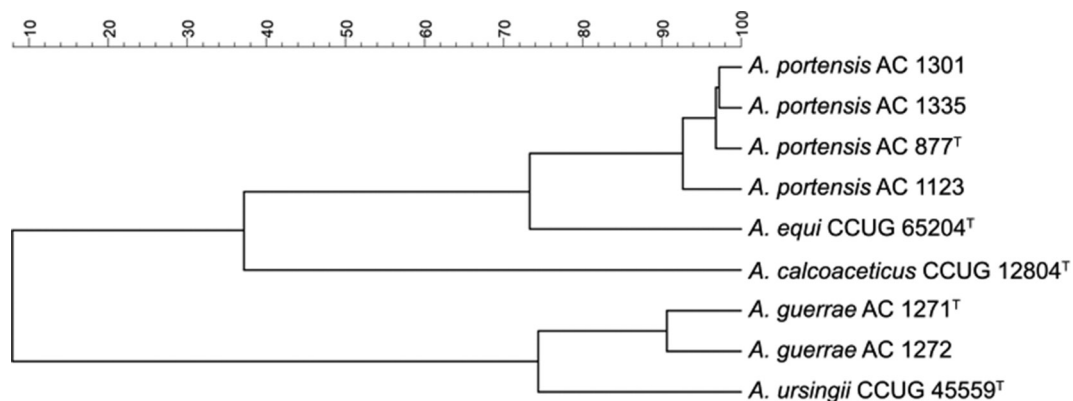
MALDI-TOF MS

Colonies from fresh cultures (overnight growth on Columbia II agar base, BD BBL, with 5% defibrinated horse blood, at 30 °C) of each strain, as well as the type strains of the most closely related species (i.e., based on the core genome-based phylogenomic tree analyses) and the type strain of the type species of the genus (i.e. *Acinetobacter calcoaceticus*) were smeared in duplicate, onto disposable target plates and covered with 1 µl α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution for MALDI-TOF MS analysis. The MALDI-TOF MS analysis were carried out on a VITEK MS RUO system ('Research Use Only' configuration; bioMérieux), using standard settings for routine identifications, within a mass range of 2–20 kDa. Spectra were acquired in automatic mode by accumulating 100 profiles of five laser shot cycles each, using the auto quality control of Launchpad 2.9. External calibration of the mass spectra was performed, using *E. coli* (CCUG 10979). The MALDI-TOF MS-based dendrogram was generated using the

correlation distance measure with the mean linkage algorithm of the BioNumerics software platform, version 7.5 (Applied Maths). Cluster analysis demonstrated clear separation of the respective mass spectra of the strains of *A. portensis* sp. nov. and *A. guerrae* sp. nov. from those of the representatives of the closest-related members of the genus based on the core genome phylogenetic tree analyses (Fig. 3).

CHEMOTAXONOMY AND PHYSIOLOGY

Cellular fatty acid-fatty acid methyl ester (CFA-FAME) analyses of two strains of each novel species (*A. portensis* sp. nov., AC 877[T] and AC 1301, *A. guerrae* sp. nov., AC 1271[T] and AC 1272) were determined from strain cultures grown for 24 h, at 30 °C under aerobic conditions on Columbia II agar base (BD BBL) with 5% defibrinated horse blood. The bacterial biomass was harvested in stationary phase and treated chemically by alkaline methanolysis afterwards, following a modified version [33] of the method proposed by MIDI in order to extract the fatty acid methyl esters (FAMEs) present in the samples [34]. CFA-FAMEs were identified by gas chromatography by means of P 5890A Series II apparatus (Hewlett-Packard). The predominant CFA-FAMEs of *A. portensis* strains were observed to be $C_{16:1}$ ω7*c* and $C_{16:0}$, while the fatty acids, $C_{18:1}$ ω9*c* and $C_{16:1}$ ω7*c* predominated in the CFA composition of *A. guerrae* strains, as well as those of the type strains of related species (Table S3). Moreover, the novel species could be distinguished from the type strains of the most related species (based on the core genome-based phylogenomic tree analyses) by their CFA profiles. Indeed, *A. portensis* strains could be distinguished from *A. equi* CCUG 65204[T] by the higher proportions of the fatty acids $C_{16:1}$ ω7*c* and $C_{16:0}$ and lower proportions of the fatty acids $C_{12:0}$ and $C_{12:0}$ 3-OH and the presence of the fatty acid $C_{17:1}$ ω8*c*. *A. guerrae* strains could be distinguished from *A. ursingii* CCUG 45559[T] by higher proportions of the fatty acid $C_{16:1}$ ω7*c*, lower proportions of the fatty acid $C_{18:1}$ ω9*c* and the absence of $C_{14:0}$ and $C_{16:1}$ ω9*c* (Table S3).

Fig. 3. Dendrogram based on the MALDI-TOF MS mass spectra of four strains of *Acinetobacter portensis* sp. nov., two strains of *Acinetobacter guerrae* sp. nov. and the type strains of the most closely related species of the genus *Acinetobacter* (based on the core genome phylogenomic tree). The dendrogram was constructed using the correlation distance measure with the average linkage algorithm (UPGMA).

Metabolic and physiological features of the strains were assessed using a set of tests described by Nemec *et al.* [14], with some modifications and additions. Unless stated otherwise, the cultivation temperature was 30 °C and results were evaluated after 2 days incubation. Liquefaction of gelatin was performed using the Kohn gelatin test. Haemolytic activity was tested on Columbia II agar base (BD BBL) supplemented with 5% defibrinated horse blood. Growth tests at 20, 30, 37 and 42 °C were performed in tubes containing 3 ml brain heart infusion broth (Acumedia) and each tube was inoculated with a drop (30 µl) of cell suspension of standardised turbidity (~10^8 c.f.u. ml^{-1}) prepared in saline from an overnight blood agar culture at 30 °C. NaCl tolerance was assessed in culture medium supplemented with 0.5–6.0 % NaCl (w/v), as well as growth on Reasoner's 2A (R2A) agar (Oxoid) and trypticsoy agar (TSA) (Acumedia) was also tested at 30 °C. Utilization of citrate was tested on Simmons' citrate agar (Scharlab) and was recorded after 2, 4 and 6 days incubation. Tests for the assimilation of the other 27 carbon sources were performed using the basal mineral medium of Cruze *et al.* [35] supplemented with 0.1% (w/v) carbon source. Tubes containing the supplemented medium (3 ml) were inoculated as in the growth-temperature tests and growth on carbon sources was evaluated after 2, 4, 6 and 10 days by visual comparisons between inoculated tubes containing carbon sources and control tubes containing only inoculated basal medium.

The phenotypic characteristics of *A. portensis* sp. nov., *A. guerrae* sp. nov. and the reference strains of closely related members of the genus according to the core genome-based tree analyses (Table 3). The four strains of *A. portensis* were seen to have nearly identical metabolic features, as were the two strains *A. guerrae* seen to exhibit nearly identical metabolic profiles. *A. portensis* sp. nov. differs from *A. equi* 114^T in four characteristics (growth at 37 °C, assimilation of adipate, benzoate and 4-hydroxybenzoate) and from *A. calcoaceticus* CIP 81.8^T in 11 characteristics (assimilation of *trans*-aconitate, adipate, 4-aminobutyrate, L-arginine,

Table 3. Phenotypic characteristics of the strains of *Acinetobacter portensis* sp. nov. (AC 877^T, AC 1335, AC 1301, AC 1123), *Acinetobacter guerrae* sp. nov. (AC 1271^T, AC 1272), type strains of the most closely related species (based on the core genome phylogenomic tree) and *Acinetobacter calcoaceticus*, the type species of the genus *Acinetobacter*.

The results of the type strains of *A. equi*, *A. ursingii* and *A. calcoaceticus* were obtained from the study of Hu *et al.* [38]. All strains grow on acetate, L-aspartate, L-glutamate and DL-lactate, whereas no strain liquefies gelatin, acid is not produced from D-glucose or grows on *β*-alanine, L-arabinose, D-gluconate, D-glucose, histamine, L-leucine, D-ribose, L-tartrate or tryptamine. +, Positive; −, negative; numbers, percentages of strains giving a positive reaction; D, mostly doubtful or irreproducible reactions; ND, not determined.

Characteristic	A. portensis	A. guerrae	A. equi	A. ursingii	A. calcoaceticus
Growth at 37 °C	−	+	+	+	−
Growth at 42 °C	−	−	ND	−	−
Haemolysis of horse blood	−	−	ND	ND	ND
Haemolysis of sheep blood	ND	ND	−	−	−
Assimilation of:					
trans-Aconitate	−	−	−	−	+
Adipate	−	+	+	+	+
4-Aminobutyrate	−	−	−	−	+
L-Arginine	−	−	−	−	+
Benzoate	−	+	+	−	+
2,3-Butanediol	−	50 (+)	−	−	+
Citrate (Simmons)	+	+	+	+	D
Ethanol	75 (+)	+	−	+	+
Gentisate	25 (−)	+	+	−	−
L-Histidine	+	−	+	−	+
4-Hydroxybenzoate	−	50 (−)	+	+	+
Malonate	−	−	−	−	+
L-Ornithine	−	−	−	−	+
L-Phenylalanine	−	−	−	−	+
Putrescine	−	−	−	−	+

benzoate, 2,3-butanediol, 4-hydroxybenzoate, malonate, L-ornithine, L-phenylalanine and putrescine). While, *A. guerrae* sp. nov. differed from *A. ursingii* CIP 107286[T] in two characteristics (assimilation of benzoate and gentisate) and from *A. calcoaceticus* CIP 81.8[T] in 10 characteristics (growth at 37 °C, assimilation of *trans*-aconitate, 4-aminobutyrate, L-arginine, gentisate, L-histidine, malonate, L-ornithine, L-phenylalanine and putrescine; Tables 3 and S4).

The metabolic capabilities of *A. portensis* sp. nov. and *A. guerrae* sp. nov. were compared with those from all *Acinetobacter* species (http://apps.szu.cz/anemec/Phenotype.pdf) and the most useful combinations of characteristics for differentiating *A. portensis* from other members of the genus includes growth on L-aspartate and L-histidine and absence of growth on L-arginine and 2,3-butanediol and at 37 °C (Table S4). *A. guerrae* strains could be differentiated from other members of the genus by the combination of growth at 37 °C, assimilation of L-aspartate and gentisate and absence of assimilation of β-alanine, L-arginine and L-histidine. Although differentiation of *A. guerrae* strains from *A. guillouiae* is not so evident, the type strain of *A. guillouiae* can be differentiated by growth on β-alanine and L-histidine and no growth on gentisate (Table S4).

Altogether, these data are congruent with the accepted characterization of bacterial species as a group of strains that shows a high degree of overall similarity and differs considerably from related strain groups with respect to many independent characteristics [36].

DESCRIPTION OF *ACINETOBACTER PORTENSIS* SP. NOV.

Acinetobacter portensis (por.ten'sis. N.L. masc. n *portensis* pertaining to Porto, a city of Portugal where the first four strains of this species were isolated).

The description is based on the characterization of four strains (Table 1). Phenotypic characteristics correspond to those of the genus [37], i.e. cells are Gram-negative, strictly aerobic, oxidase-negative, catalase-positive, non-motile coccobacilli, capable of growth in mineral media with acetate as the sole carbon source and ammonia as the sole source of nitrogen but incapable of denitrification. Colonies on Columbia II agar base with 5% defibrinated horse blood after 24 h incubation at 30 °C are approximately 0.5–1.0 mm in diameter, circular, convex, smooth and bright with entire margins. Growth occurs at temperatures ranging from 20 to 30 °C, but not at 37 °C. Growth occurs also on R2A and TSA media, as well as in the presence of NaCl concentrations as high 4.0%. Haemolysis of horse blood is not observed after 24 h at 30 °C. Acid is not produced from D-glucose and gelatin is not hydrolysed. Acetate, L-aspartate, citrate (Simmons), ethanol (three strains), gentisate (one strain), L-glutamate, L-histidine and DL-lactate are utilized as sole sources of carbon, with growth becoming visible after 2 days of incubation. No growth occurs on *trans*-aconitate, adipate, β-alanine, 4-aminobutyrate, L-arabinose,

L-arginine, benzoate, 2,3-butanediol, D-gluconate, histamine, 4-hydroxybenzoate, L-leucine, malonate, L-ornithine, L-phenylalanine, putrescine, D-ribose, L-tartrate or tryptamine within 10 days (Tables 3 and S4). The predominant cellular fatty acids are $C_{16:1}\,\omega 7c$, $C_{16:0}$ and $C_{12:0}$ 3-OH.

The strains studied were isolated from raw beef, pork, chicken and turkey meat (Table 1). The type strain is AC 877[T] (=CCUG 68672[T]=CCM 8789[T]), isolated in September 2014 from raw pork meat. The type strain does not grow on gentisate. The GenBank/EMBL/DDBJ accession numbers for the genome, partial 16S rRNA, *rpoB*, *gyrB* and *recA* gene sequences of AC 877[T] are LWRV00000000, KX870877, KX885208, KX885192 and KX885200, respectively.

DESCRIPTION OF *ACINETOBACTER GUERRAE* SP. NOV.

Acinetobacter guerrae (*guer'rae* N.L. gen. n. *guerrae* of Guerra, named after Francisco Carvalho Guerra, a Portuguese biochemist, for his contributions to the foundation of Escola Superior Biotecnologia of Universidade Católica Portuguesa).

The description is based on the characterization of two strains (Table 1). Phenotypic characteristics correspond to those of the genus [37], i.e. cells are Gram-negative, strictly aerobic, oxidase-negative, catalase-positive, non-motile coccobacilli, capable of growth in mineral media with acetate as the sole carbon source and ammonia as the sole source of nitrogen but incapable of denitrification. Colonies on Columbia II agar base with 5% defibrinated horse blood after 24 h incubation at 30 °C are approximately 0.5–1.0 mm in diameter, circular, convex, smooth and bright with entire margins. Growth occurs at temperatures ranging from 20 to 37 °C, but not at 42 °C. Growth occurs also on R2A and TSA media, as well as in the presence of up to 4.5% of NaCl. Haemolysis of horse blood is not observed after 24 h at 30 °C. Acid is not produced from D-glucose and gelatin is not hydrolysed by strains. Acetate, adipate, L-aspartate, benzoate, 2,3-butanediol (one strain), citrate (Simmons), ethanol, gentisate, L-glutamate, 4-hydroxybenzoate (one strain) and DL-lactate are utilized as sole sources of carbon, with growth becoming visible after 2 days incubation. No growth occurs on *trans*-aconitate, β-alanine, 4-aminobutyrate, L-arabinose, L-arginine, D-gluconate, histamine, L-histidine, L-leucine, malonate, L-ornithine, L-phenylalanine, putrescine, D-ribose, L-tartrate or tryptamine within 10 days (Tables 3 and S4). The predominant cellular fatty acids are $C_{18:1}\,\omega 9c$, $C_{16:1}\,\omega 7c$ and $C_{16:0}$.

The strains studied were isolated from raw chicken meat and the type strain is AC 1271[T] (=CCUG 68674[T]=CCM 8791[T]), isolated in September 2014 (Table 1). The type strain grows on 2,3-butanediol but does not grow on 4-hydroxybenzoate. The GenBank/EMBL/DDBJ accession numbers for the genome, partial 16S rRNA, *rpoB*, *gyrB* and *recA* gene sequences of AC 1271[T] are LXGN00000000, KX870878, KX885210, KX885194 and KX885202, respectively.

Conflicts of interest
R.K. is affiliated with a company, Nanoxis Consulting AB. The company did not have any influence on the conception, elaboration or decision to submit the present article.

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