Acinetobacter bohemicus sp. nov.,

widespread in natural soil and water ecosystems in the Czech Republic

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AIM

To define the taxonomic status of a phenetically distinct group of 25 environmental *Acinetobacter* strains which did not belong to any of the known species.

STRAINS

The 25 strains were isolated from soil and water samples collected in natural ecosystems of the Czech Republic (Fig. 1) via selective enrichment in a vigorously aerated mineral medium with acetate. All strains were genotypically unique at the strain level, as revealed by ApaI macrorestriction analysis.

An additional large set of reference strains belonging to all known validly named species and genomic species was used in comparative analyses.

RESULTS and CONCLUSION

The **25 strains formed a genotypically and phenotypically coherent group** clearly distinct from the other *Acinetobacter* species based on the results of the following analyses:

- comparative sequence analyses of the ***rpoB*** and ***gyrB*** genes (Fig. 2 and Fig. 3)
- whole-cell **MALDI-TOF MS** profiling (Fig. 4 and Fig. 5)
- **nutritional** and **physiological** characterization (Table 1).

Three selected strains formed a distinct branch within the genus based on **16S rRNA cluster analysis** (Fig. 6).

BLAST-based **average nucleotide identity** (ANIb) values (calculated using JSpecies V1.2.1; www.imedea.uib.es/jspecies) between the whole genome sequence of ANC 3994^T (NCBI accession no. APOH00000000) and those derived from other 32 *Acinetobacter* (genomic) spp. were ≤77.3%.

All strains could be identified as the same new species using both phenotypic testing and MALDI-TOF MS profiling (Table 1, Fig. 4, and Fig. 5).

We conclude that the **25 strains represent a novel environmental species**, for which the name ***Acinetobacter bohemicus* sp. nov.** is proposed.

Fig. 2. Rooted neighbour-joining tree based on the partial nucleotide sequence of the *rpoB* gene (positions 2915-3775) of 25 *A. bohemicus* sp. nov. strains and the representatives of all known *Acinetobacter* spp. Bootstrap percentages (>70%) after 1000 simulations are shown. Bar, 5% sequence divergence.

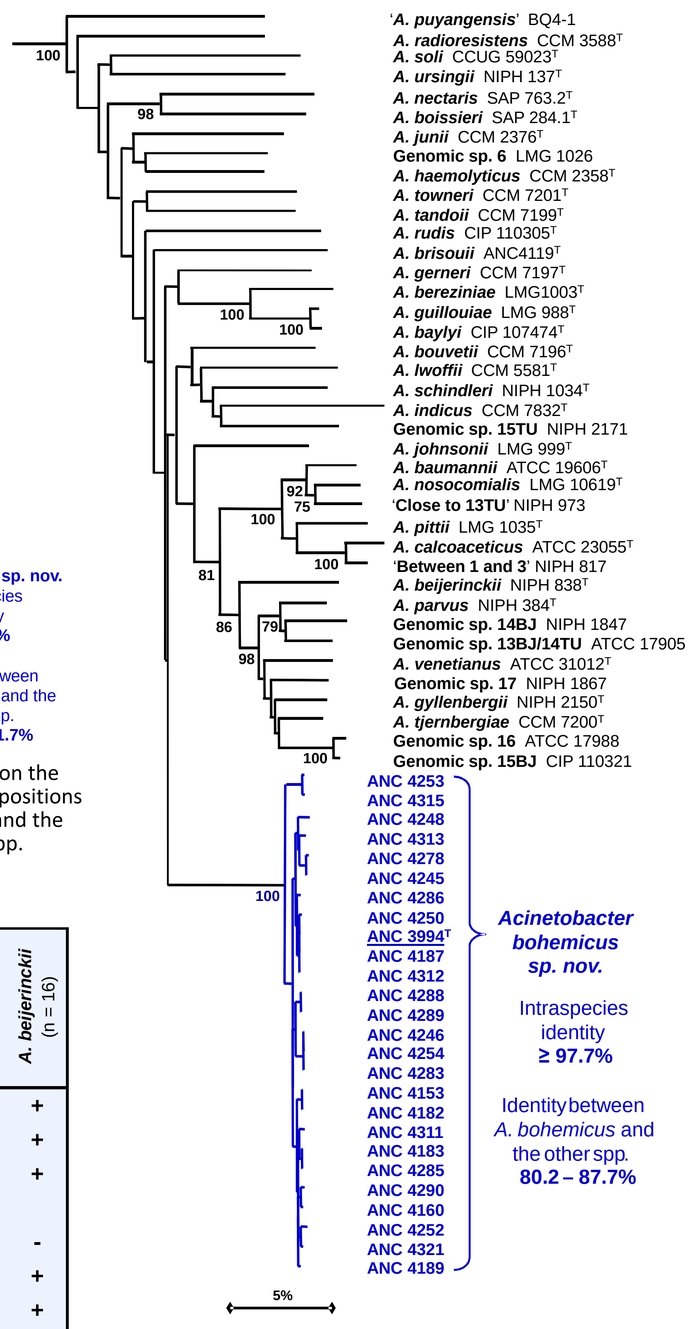

Fig. 3. Unrooted neighbour-joining tree based on the partial nucleotide sequence of the *gyrB* gene (positions 435-1190) of 25 *A. bohemicus* sp. nov. strains and the representatives of most other *Acinetobacter* spp.

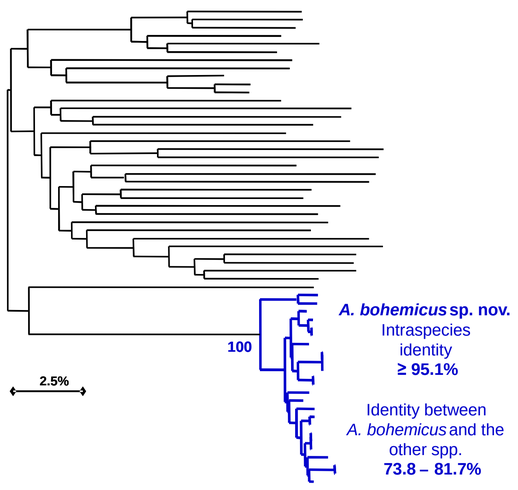

Fig. 4. Dendogram derived from the MALDI-TOF mass spectra of 25 *A. bohemicus* sp. nov. strains and the type/ reference strains of other *Acinetobacter* spp. Six additional *A. johnsonii* strains recovered from *A. bohemicus*-positive environmental samples are included.

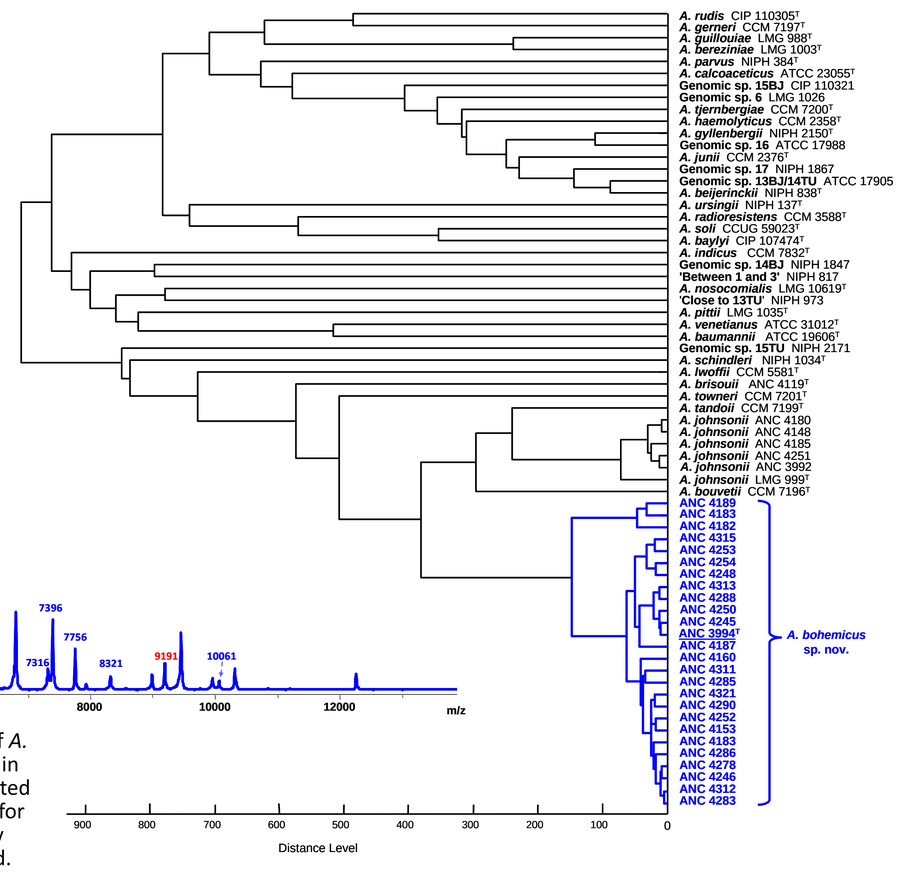

Fig. 5. MALDI-TOF mass spectra of *A. bohemicus* sp. nov. Peaks present in all *A. bohemicus* strains are indicated by molecular mass, those unique for *A. bohemicus* (not detected in any other *Acinetobacter* sp.) are in red.

Fig. 6. Rooted neighbor-joining tree based on 16S rRNA gene sequences, showing the relationships between three strains of *A. bohemicus* sp. nov. and other *Acinetobacter* spp.

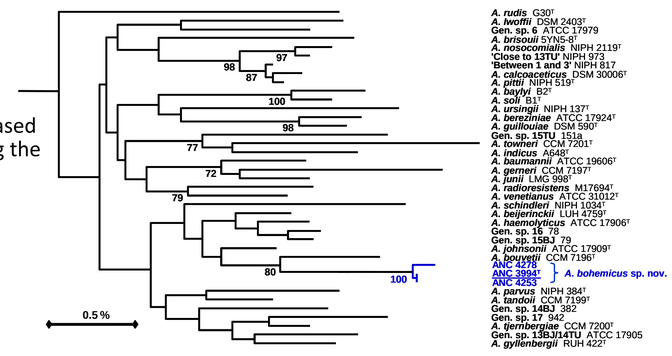

Table 1. Phenotypic properties useful for the differentiation of *A. bohemicus* sp. nov. from phenotypically most similar non-glucose-oxidizing *Acinetobacter* spp.

Tests were performed according to Nemec et al. (IJSEM 59:118–24). Utilization tests were evaluated after six days of incubation at 30°C, the other tests after two (temperature tests) or three days (hemolysis of sheep blood). +, All strains positive; -, all strains negative; numbers, percentages of strains giving a positive reaction.

Characteristic	*A. bohemicus* (n = 25)	*A. johnsonii* (n = 20)	*A. lwoffii* (n = 14)	*A. junii* (n = 15)	*A. beijerinckii* (n = 16)
Growth at 37 °C	-	25	+	+	+
Growth at 35 °C	-	+	+	+	+
Hemolysis	70	25	-	47	+
Utilization of					
DL-Lactate	+	+	93	93	-
Citrate (Simmons)	-	85	14	80	+
L-Aspartate	+	75	-	27	+
Azelate	-	-	+	-	-
L-Histidine	+	-	-	93	+
Malonate	+	50	7	-	+
Phenylacetate	-	-	71	-	-
4-Hydroxybenzoate	88	15	-	-	-
L-Arginine	+	70	-	93	-
L-Leucine	-	-	-	20	88
2,3-Butanediol	+	60	7	-	-
Benzoate	92	95	86	87	-
Adipate	-	-	79	-	-

Acinetobacter bohemicus sp. nov.

Acinetobacter bohemicus (bo.he'mi.cus. N.L. masc. adj. bohemicus, **pertaining to Bohemia** (a major historical region of the Czech Republic), where multiple strains of this organism were isolated.

The species description is based on the characterization of 25 strains isolated from soil or water in natural ecosystems. Colonies on Tryptic Soy Agar (Oxoid) after 24 h incubation at 30°C are 1.0–2.0 mm in diameter, circular, convex, smooth and slightly opaque with entire margins. Growth occurs in Brain-Heart Infusion (Oxoid) at temperatures ranging from 25°C to 30°C but not at 35°C. Acid is not produced from D-glucose and gelatin is not hydrolysed. Weak haemolysis on agar media supplemented with sheep erythrocytes is observed in most strains. Acetate, 4-aminobutyrate, L-arginine, L-aspartate, 2,3-butanediol, ethanol, L-glutamate, L-histidine, DL-lactate, and malonate are utilized as sole sources of carbon with growth visible in 4 days of incubation. No growth on *trans*-aconitate, adipate, β-alanine, L-arabinose, azelate, citraconate, D-gluconate, D-glucose, glutarate, histamine, phenylacetate, L-phenylalanine, L-leucine, levulinate, L-ornithine, putrescine, D-ribose, tricarballylate, or trigonelline occurs in 10 days. Various numbers of strains utilize benzoate (93% of the strains), gentisate (4%), 4-hydroxybenzoate (89%), L-tartrate (11%), or tricarballylate (4%). Tests of citrate (Simmons) or D-malate utilization are either negative, weakly positive or difficult to interpret.

The type strain is ANC 3994^T (= CIP 110496^T = CCM 8462^T = CCUG 63842^T), isolated from the wetland mud of a deciduous forest in Křivoklátsko (a national protected landscape area and UNESCO biosphere reserve, located in the central Bohemia; GPS coordinates: 50°6'23.215"N, 13°56'39.027"E) in May 2011. The strain is weakly hemolytic and grows on benzoate and 4-hydroxybenzoate but does not assimilate gentisate, L-tartrate or tricarballylate.

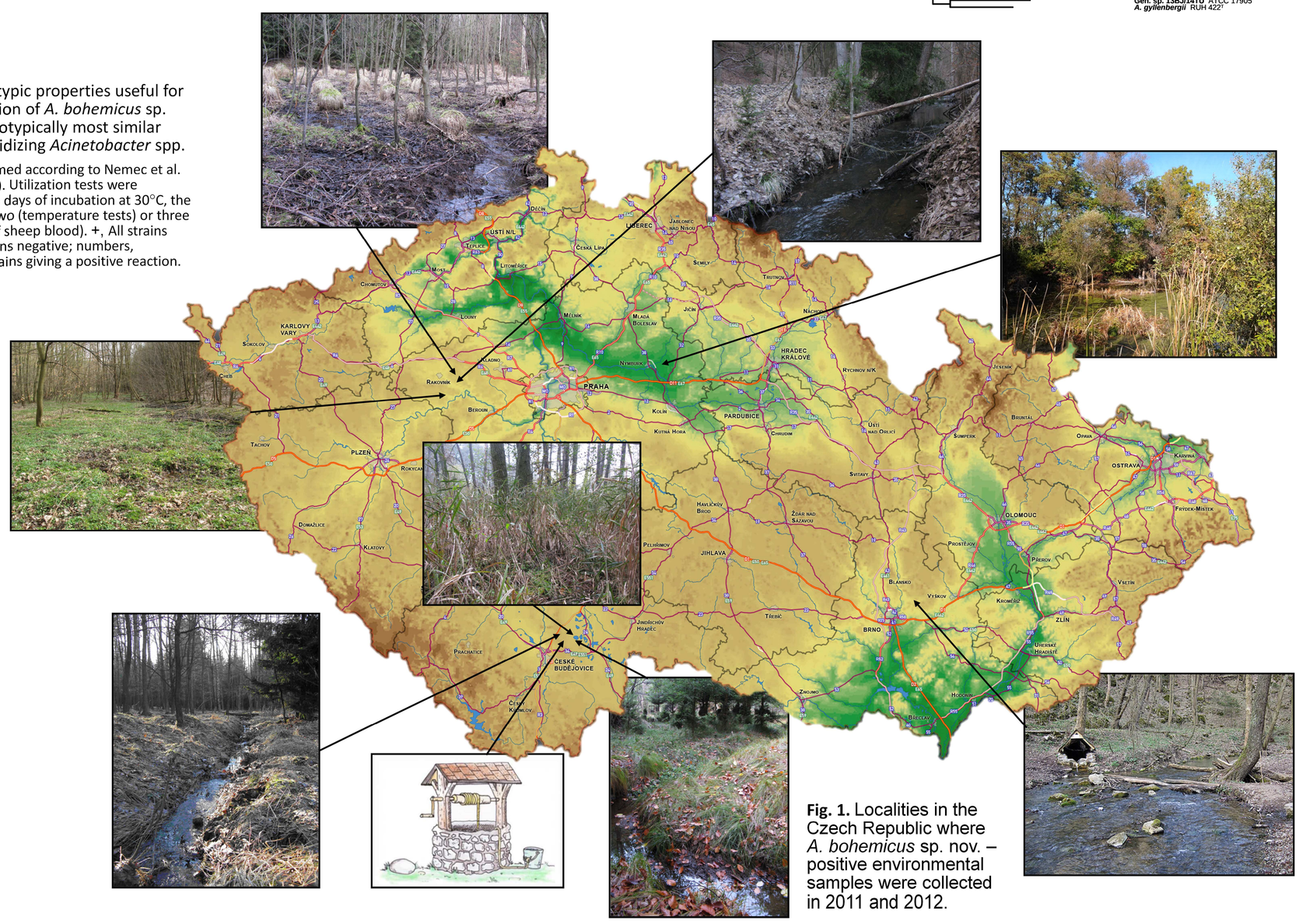

Fig. 1. Localities in the Czech Republic where *A. bohemicus* sp. nov. – positive environmental samples were collected in 2011 and 2012.

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