Natural soil and water ecosystems as a source of a high taxonomic diversity of *Acinetobacter*

Lenka Křížová, Martina Maixnerová, Alexandr Nemec

Laboratory of Bacterial Genetics, National Institute of Public Health, Prague, Czech Republic

AIM

To gain insight into the diversity of culturable *Acinetobacter* strains from the natural ecosystems.

SAMPLES

A total of 114 soil and water samples were gathered from well-protected landscape areas in the Czech Republic (**Fig. 1**) encompassing deciduous or mixed temperate forests. The choice of localities was done with due caution to minimize the possibility of secondary contamination by human activities. The altitude of the sampling sites ranged from 188 to 1180 m above MSL.

The samples were cultured at 25°C in a mineral medium supplemented with sodium acetate[1]. The grown-up cultures were streaked onto both non-selective and acetate agar plates (**Fig. 2**).

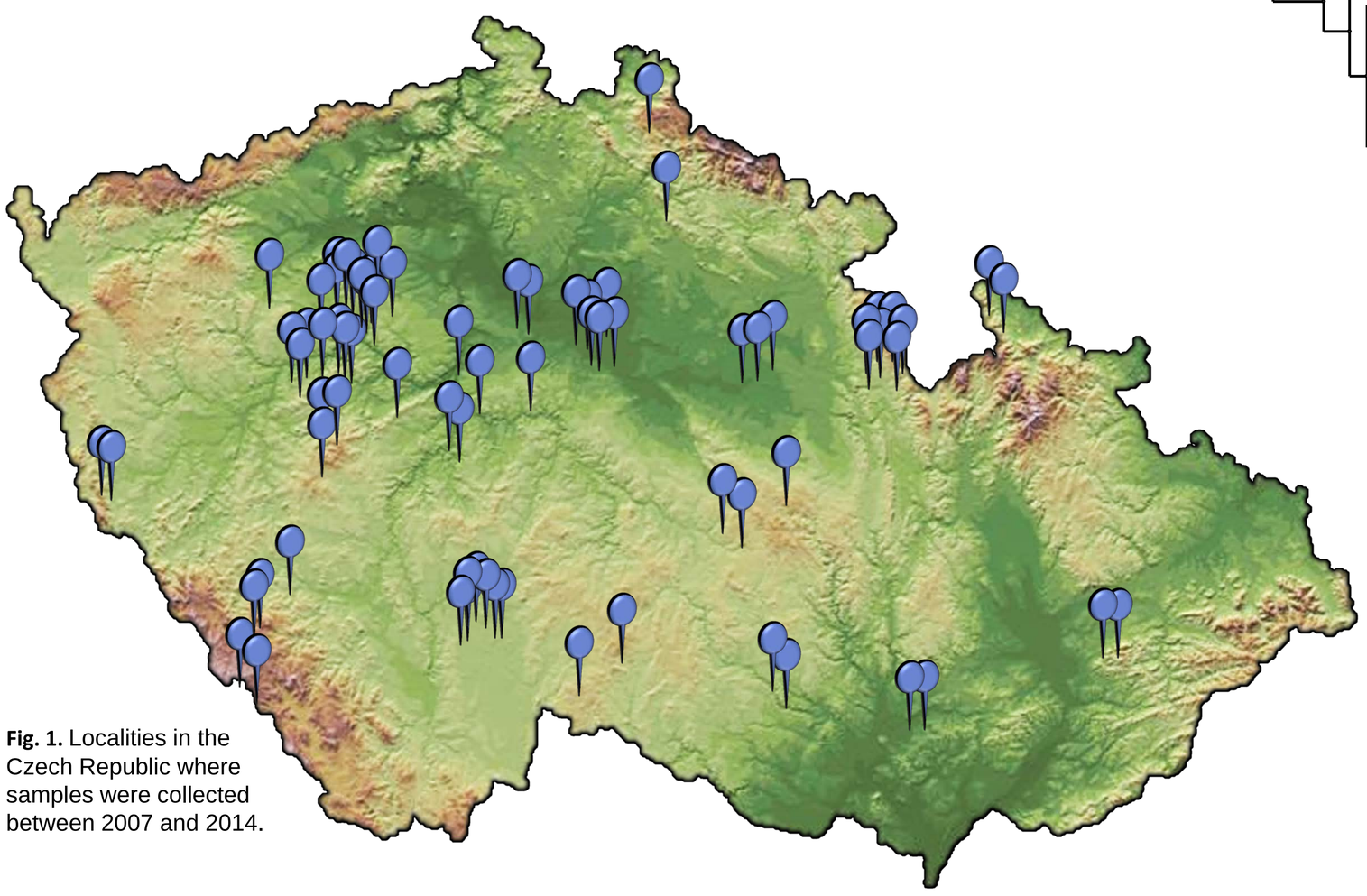

Fig. 1. Localities in the Czech Republic where samples were collected between 2007 and 2014.

STRAINS

Acinetobacter colonies were identified based on the genus-specific phenotypic properties (A) and/or whole-cell profiling by MALDI-TOF MS (B) (**Fig. 2**) with the current Bruker Daltonics database supplemented with homemade entries representing all known and provisional *Acinetobacter* spp.[2]

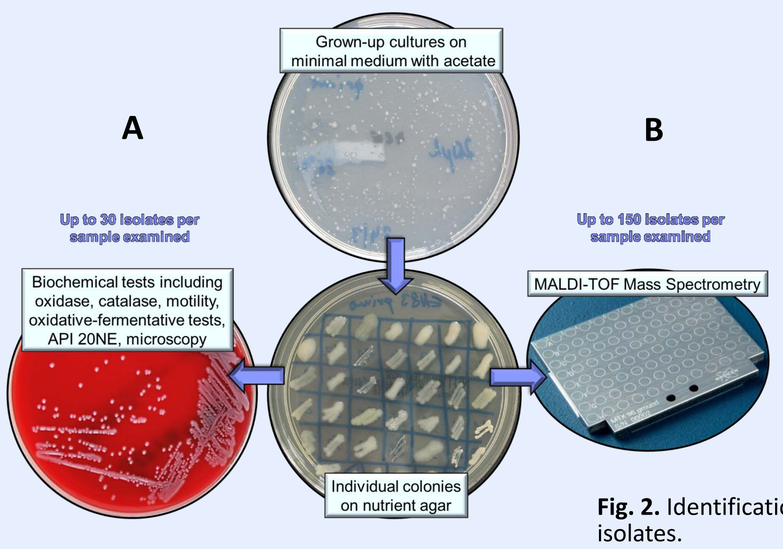

Fig. 2. Identification of *Acinetobacter* isolates.

The uniqueness of isolates at the strain level was verified by RAPD or macrorestriction analysis. The final classification of strains at the species level was based on comparative analysis of the *rpoB* gene, MALDI-TOF MS and comprehensive physiological and metabolic testing (**Fig. 3**).

CONCLUSION

The diversity of environmental *Acinetobacter* isolates at the species level appears to be unpredictably high and warrants further systematic investigation to provide a taxonomically precise basis for ecological studies.

RESULTS

Of the 114 samples, 80 (**70%**) **were positive for *Acinetobacter***, with **254 distinct strains** recovered.

Among these strains, 179 (70%) were allocated to 16 known (genomic) species with the predomination of ***A. bohemicus*** (n=46), ***A. guillouiae*** (n=42), ***A. johnsonii*** (n=39), ***A. calcoaceticus*** (n=27) and ***A. lwoffii*** (n=6).

Less than four strains were found for each of the following species: *A. baumannii*, *A. beijerinckii, A. bouvetii, A. gandensis*, *A.* genomic species 17, *A. junii*, *A. kookii, A. pittii, A. schindleri, A. tandoii* and *A. towneri*.

The remaining 75 (**30%**) **strains** were classified into 19 **novel taxonomic groups** or as 17 **taxonomically unique strains**, which are likely to represent as yet unknown species (**Fig. 3**).

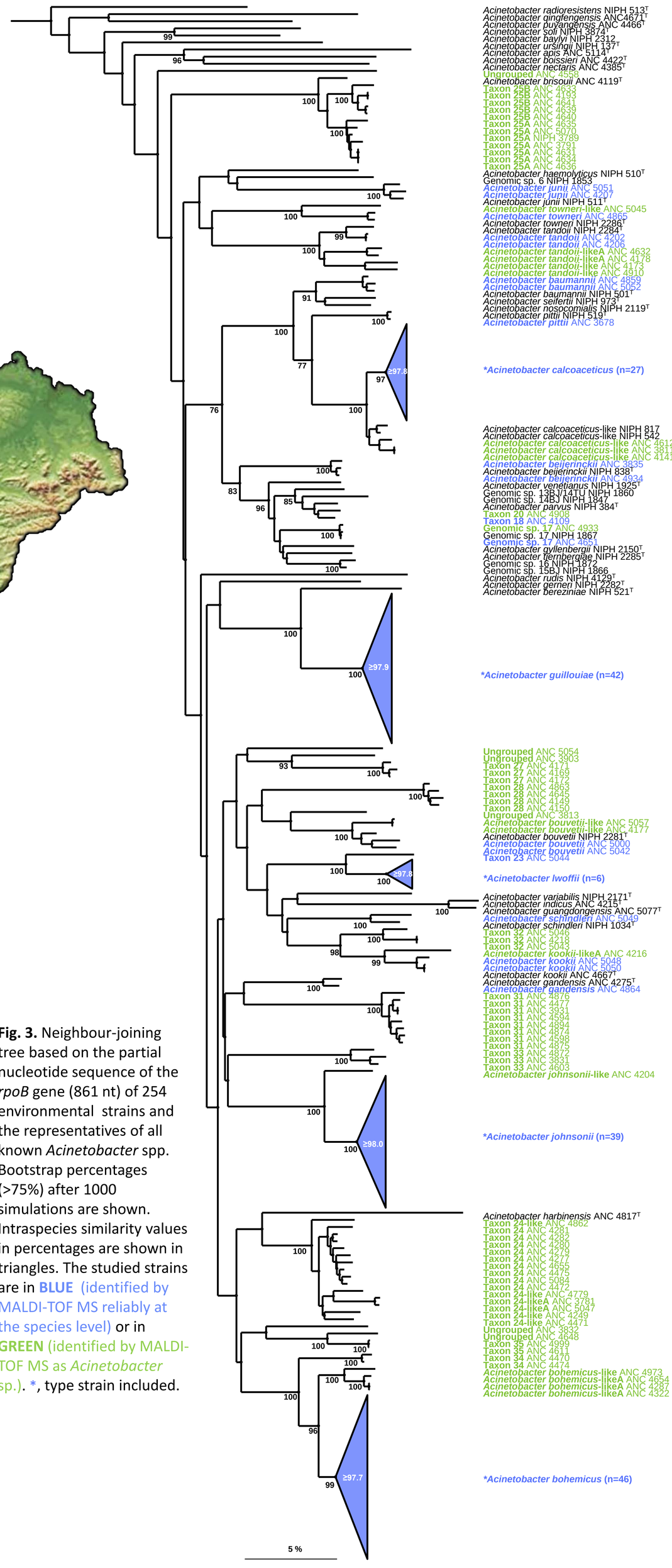

Fig. 3. Neighbour-joining tree based on the partial nucleotide sequence of the *rpoB* gene (861 nt) of 254 environmental strains and the representatives of all known *Acinetobacter* spp. Bootstrap percentages (>75%) after 1000 simulations are shown. Intraspecies similarity values in percentages are shown in triangles. The studied strains are in **BLUE** (identified by MALDI-TOF MS reliably at the species level) or in **GREEN** (identified by MALDI-TOF MS as *Acinetobacter* sp.). *, type strain included.

References: [1]Krizova *et al*. 2014; *Syst Appl Microbiol* 37: 467–473. [2]Touchon et al. 2014; *Genome Biol Evol* 6: 2866-2882. **Correspondence:** krizova.work@gmail.com. Presented as **Poster P 6B at the 10th International Symposium on the Biology of *Acinetobacter***, June 3–5, 2015, Athens, Greece. **Funding:** Project no. 13-26693S of the Czech Science Foundation.