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## **P1-4: Delineation of a novel environmental phylogroup of the genus *Acinetobacter* encompassing *Acinetobacter terrae* sp. nov., *Acinetobacter terrestris* sp. nov. and three tentative species**

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In May 2019, the genus *Acinetobacter* comprised 60 validly published species names (including four pairs of heterotypic synonyms) and two effectively published species names awaiting validation (<https://apps.szu.cz/anemec/Classification.pdf>). Even though this classification sufficiently covers *Acinetobacter* spp. isolated from humans, the knowledge of the taxonomic diversity of acinetobacters confined to non-human environments is limited.

This study aimed to define the taxonomic position and structure of a novel, phenetically unique group of 26 *Acinetobacter* strains, provisionally designated Taxon 24 (T24). The strains were recovered from soil and freshwater ecosystems (n=21) or animals (n=5) in Czechia, England, Germany, the Netherlands and Turkey between 1993 and 2015. The strains were non-glucose-acidifying, nonhemolytic, nonproteolytic, growing at 32°C and on acetate and ethanol as single carbon sources, but not on 4-hydroxybenzoate and mostly not at 37°C. Their whole-genome sequences were 3.0–3.7 Mb in size, with GC contents of 39.8–41.3%. Based on core genome phylogenetic analysis, the 26 strains formed a distinct clade within the genus *Acinetobacter*, with strongly supported subclades termed T24A (n=11), T24B (n=8), T24C (n=2), T24D (n=3) and T24E (n=2). Internal genomic ANIb values for these subclades were >94.7%, while the ANIb values between them were <92.3%. The results of MALDI-TOF MS-based analyses were in good but not complete agreement with this classification. The five subclades differed from each other in the results of 1–6 carbon source assimilation tests.

Given the genomic and phenotypic distinctness and internal coherence, quantity and geographically diverse origin of T24A and T24B, we propose the respective names *Acinetobacter terrae* sp. nov. and *Acinetobacter terrestris* sp. nov. for these taxa. We conclude that these two species together with the other T24 strains represent a novel, widely dispersed *Acinetobacter* clade primarily associated with terrestrial ecosystems.

# Delineation of a novel environmental phylogroup of the genus *Acinetobacter* encompassing *Acinetobacter terrae* sp. nov., *Acinetobacter terrestris* sp. nov. and three tentative species

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## AIM

As of September 2019, the genus *Acinetobacter* comprises 62 validly published species names (<https://apps.szu.cz/anemec/Classification.pdf>), including four pairs of heterotypic synonyms. Even though this classification sufficiently covers *Acinetobacter* spp. isolated from humans, the taxonomic diversity of acinetobacters confined to non-human environments is little understood.

The aim was to define the taxonomic position and structure of a phenetically novel group of 26 *Acinetobacter* strains, provisionally designated Taxon 24 (T24).

## CONCLUSION

Given the genomic and phenotypic distinctness and internal coherence, numerousness and geographically diverse origin of T24A and T24B, we propose the respective names *Acinetobacter terrae* sp. nov. (type strain: ANC 4282<sup>T</sup> = CCM 8986<sup>T</sup>) and *Acinetobacter terrestris* sp. nov. (type strain: ANC 4471<sup>T</sup> = CCM 8985<sup>T</sup>) for these taxa. These two species together with the other T24 strains form a novel *Acinetobacter* clade, dispersed in terrestrial ecosystems.

## RESULTS

- Twenty-six T24 strains were recovered from soil and freshwater ecosystems (n=21) or animals (n=5) in Czechia, England, Germany, the Netherlands or Turkey between 1993 and 2015 [Table 1].
- Their whole-genome sequences are 3.0–3.7 Mb in size, with GC contents of 39.8–41.3% [Table 1].
- Based on core genome phylogenetic analysis, the 26 strains formed a distinct clade within the genus *Acinetobacter*, with strongly supported subclades termed T24A (n=11), T24B (n=8), T24C (n=2), T24D (n=3) and T24E (n=2) [Fig. 1].
- Internal genomic ANIb values for these subclades were ≥94.8%, while the ANIb values between them were ≤92.3% [Table 2].
- The results of whole-cell profiling based on MALDI-TOF MS agreed with this classification [Fig. 2].
- The strains were non-glucose-acidifying, nonhemolytic, nonproteolytic, growing at 32°C and on acetate and ethanol, but not on 4-hydroxybenzoate and mostly not at 37°C. T24A–T24E differed from each other in the ability to grow on 1–6 C-sources [Table 3].

Table 1. Origin and genome features of the strains of Taxon 24

Strain classification and designation	Specimen	Location and/or year of isolation	Genome	Total length of contigs (nt)	GC mol%
<b>T24A (<i>Acinetobacter terrae</i> sp. nov.)</b>					
ANC 4282 <sup>T</sup> = CCM 8986 <sup>T</sup> <sup>c</sup>	Mud (forest creek bank)	Křivoklátsko forestland, Czechia, 2012	This study	3,275,105	40.2
ANC 4281 <sup>c</sup>	Mud (forest creek bank)	Křivoklátsko forestland, Czechia, 2012	SJOA01000000	3,454,887	40.1
ANC 4655	Mud (seasonal forest creek)	Křivoklátsko forestland, Czechia, 2013	NEG01000000	3,372,974	40.1
SFC (= ANC 6055)	Sheep	United Kingdom, 2014	LSZG01000000	3,346,544	39.9
NIPH 763 (= A117c) <sup>a</sup>	Soil (walking path)	Bensberg, Germany, 1993–1994	This study	3,202,930	40.3
NIPH 799 (= A107c) <sup>a</sup>	Soil (flower field)	Cologne, Germany, 1993–1994	This study	3,008,766	40.4
ANC 4277 <sup>c</sup>	Mud (forest creek bank)	Křivoklátsko forestland, Czechia, 2012	This study	3,305,281	40.2
ANC 4279 <sup>c</sup>	Mud (forest creek bank)	Křivoklátsko forestland, Czechia, 2012	This study	3,404,939	40.3
ANC 4280 <sup>c</sup>	Mud (forest creek bank)	Křivoklátsko forestland, Czechia, 2012	This study	3,374,638	40.2
ANC 4475 <sup>d</sup>	Limestone surface (cave)	Moravský Kras, Czechia, 2013	This study	3,213,522	40.4
ANC 5380	Mud (seasonal forest creek)	Dobříš, Czechia, 2015	This study	3,697,753	39.8
<b>T24B (<i>Acinetobacter terrestris</i> sp. nov.)</b>					
ANC 4471 <sup>T</sup> = CCM 8985 <sup>T</sup> <sup>d</sup>	Limestone surface (cave)	Moravský Kras, Czechia, 2013	SJNZ01000000	3,287,055	41.0
ANC 4472 <sup>d</sup>	Limestone surface (cave)	Moravský Kras, Czechia, 2013	SJNY01000000	3,393,586	41.0
ANC 4249	Mud (forest wetland)	Křivoklátsko forestland, Czechia, 2012	SJOB01000000	3,312,852	41.0
NIPH 2377 (= LUH 8567) <sup>b</sup>	Faeces (cow)	Wageningen, the Netherlands, 2003	This study	3,371,116	41.0
NIPH 2378 (= LUH 8642) <sup>b</sup>	Faeces (horse)	Leidschenveen, the Netherlands, 2004	This study	3,241,384	41.3
NIPH 2379 (= LUH 8611) <sup>b</sup>	Rectum (chicken)	Voorburg, the Netherlands, 2003	This study	3,156,332	41.3
ANC 5062	Soil (rocky pastureland)	Yağmur, Turkey, 2014	This study	3,384,805	41.1
ANC 5084	Soil (dry creekbed)	Peçenek, Turkey, 2014	This study	3,343,945	41.0
<b>T24C</b>					
ANC 4779	Mud (forest creek bank)	Křivoklátsko forestland, Czechia, 2014	SJNU01000000	3,385,630	40.3
SFB (= ANC 6054)	Sheep	United Kingdom, 2014	LSZH01000000	3,307,724	40.3
<b>T24D</b>					
ANC 4862	Water with detritus (forest pool)	Šnepov, Czechia, 2014	SJXH01000000	3,321,581	40.3
ANC 5378	Mud (seasonal forest creek)	Dobříš, Czechia, 2015	This study	3,215,218	40.3
ANC 5414	Soil (forest pool bank)	Dobříš, Czechia, 2015	This study	3,302,340	40.4
<b>T24E</b>					
ANC 3781	Soil (beech forest)	Bělýšov Reserve, Czechia, 2009	SJOH01000000	3,387,669	40.3
ANC 5047	Water with detritus (forest creek)	Mohelnická reserve, Czechia, 2014	This study	3,227,501	40.4

<sup>a,b</sup> Strains kindly provided by H. Seifert (A117c and A107c) and L. Dijkshoorn (LUH 8567, LUH 8642, and LUH 8611), respectively.

<sup>c,d</sup> Strains recovered from single samples but yielding different genomic macrorestriction patterns.

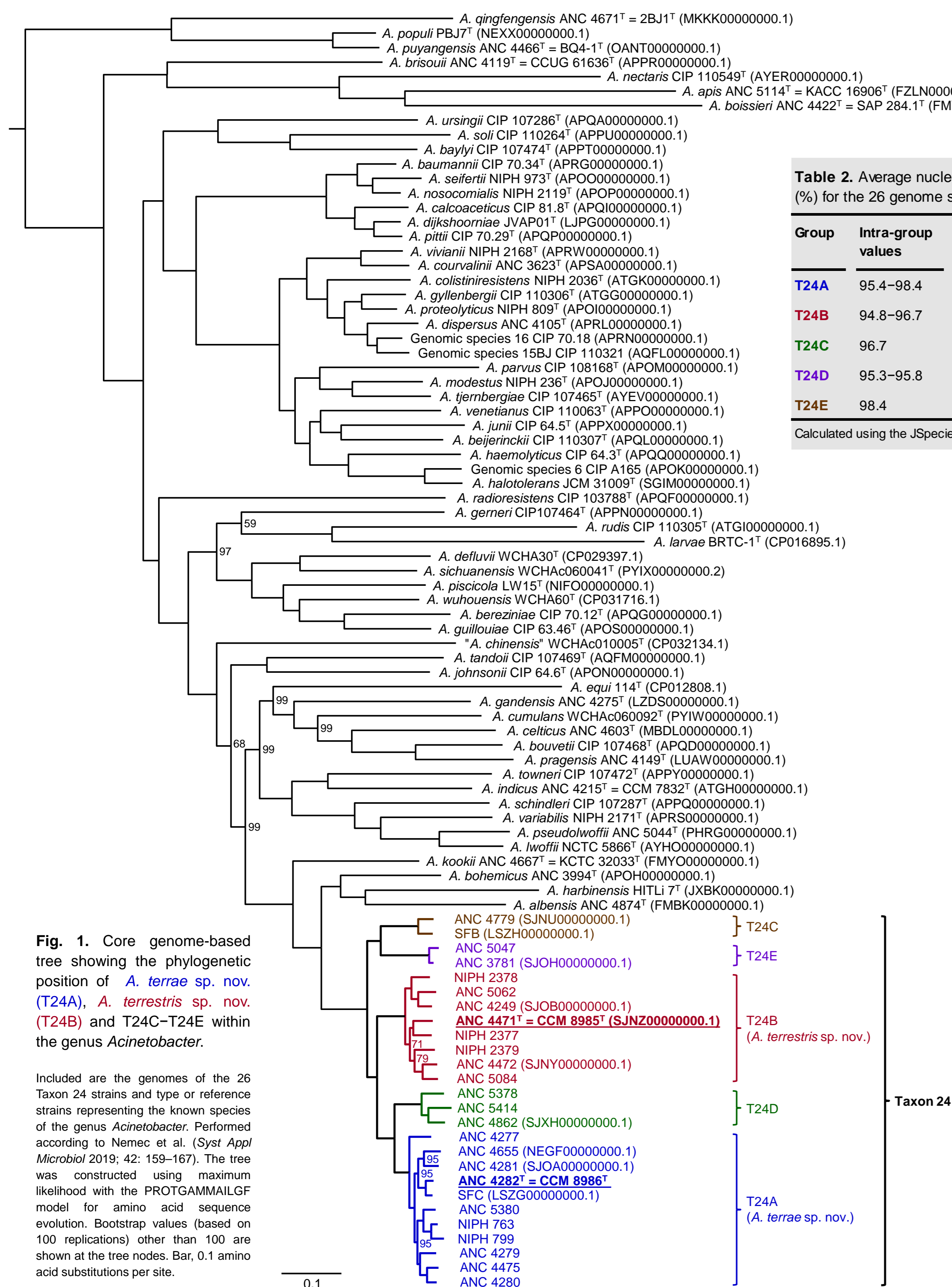


Fig. 1. Core genome-based phylogenetic tree showing the phylogenetic position of *A. terrae* sp. nov. (T24A), *A. terrestris* sp. nov. (T24B) and T24C–T24E within the genus *Acinetobacter*.

Included are the genomes of the 26 Taxon 24 strains and type or reference strains representing the known species of the genus *Acinetobacter*. Performed according to Nemeč et al. (*Syst Appl Microbiol* 2019; 42: 159–167). The tree was constructed using maximum likelihood with the PROTGAMMAILGF model for amino acid sequence evolution. Bootstrap values (based on 100 replications) other than 100 are shown at the tree nodes. Bar, 0.1 amino acid substitutions per site.

Table 2. Average nucleotide identity based on BLAST (ANIb) values (%) for the 26 genome sequences of Taxon 24

Group	Intra-group values	Inter-group values			
		T24A	T24B	T24C	T24D
T24A	95.4–98.4				
T24B	94.8–96.7	90.0–90.4			
T24C	96.7	88.4–89	88.2–88.7		
T24D	95.3–95.8	91.9–92.3	88.8–89.3	87.9–88.2	
T24E	98.4	88–88.4	87.5–87.8	89.2–89.5	87.8–88.2

Calculated using the JSpecies (www.imedea.uib.es/jspecies).

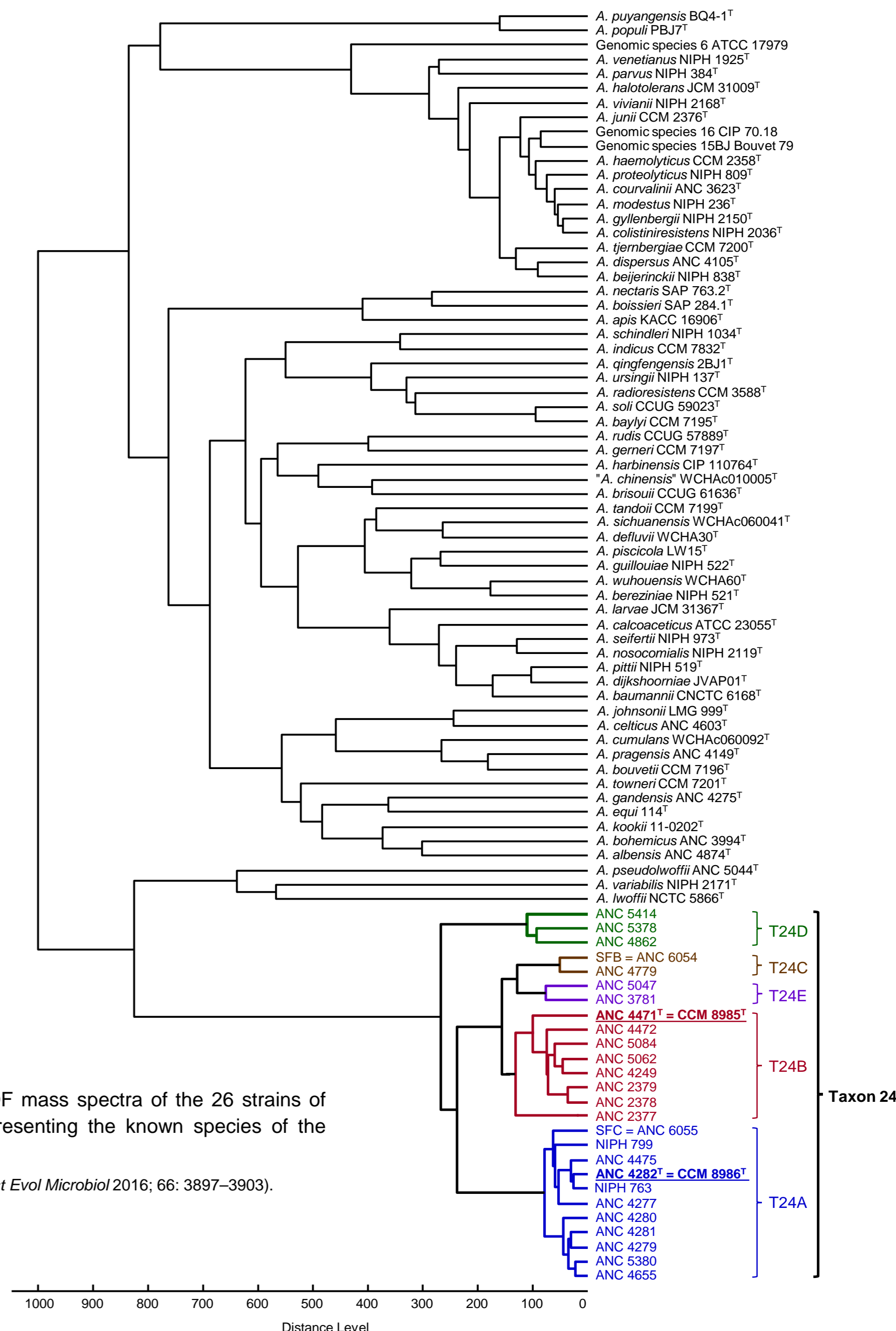


Fig. 2. Dendrogram based on the MALDI-TOF mass spectra of the 26 strains of Taxon 24 and type or reference strains representing the known species of the genus *Acinetobacter*.

Performed according to Radolfová-Křížová et al. (*Int J Syst Evol Microbiol* 2016; 66: 3897–3903).

Table 3. Metabolic and physiological properties of Taxon 24

Characteristic	T24A (n=11)	T24B (n=8)	T24C (n=2)	T24D (n=3)	T24E (n=2)
Growth at 37 °C	-	13	-	-	50
Growth at 35 °C	91	87	50	+	+
Utilization of					
Adipate	+	+	+	-	+
4-Aminobutyrate	-	-	-	-	+
L-Aspartate	-	+	+	-	+
Azolate	-	+	50	-	+
Benzoate	91	88	+	+	+
2,3-Butanediol	-	63	-	-	-
Citrate (Simmons)	-	+	-	-	-
Gentisate	-	13	-	-	-
L-Glutamate	-	-	+	-	+
Glutarate	55	88	50	-	+
DL-Lactate	45	+	50	+	+
D-Malate	9	-	-	-	-
Malonate	-	-	50	-	-
Phenylacetate	91	63	+	+	+
Tricarballic acid	9	-	-	-	-

All strains grew on acetate, ethanol and at 15–32°C. None of the strains acidified D-glucose, liquefied gelatin, hemolysed sheep blood agar, grew at 41°C or utilized trans-aconitate, β-alanine, L-arabiose, L-arginine, citrate, D-gluconate, D-glucose, histamine, L-histidine, 4-hydroxybenzoate, L-leucine, levulinic, L-ornithine, L-phenylalanine, putrescine, D-ribose, L-tartrate, trigonelline or tryptamine. +, all strains positive; -, all strains negative. Numbers are percentages of strains with positive reactions. Performed according to Nemeč et al. (*Int J Syst Evol Microbiol* 2009; 59: 118–24).