Abstract book
Acinetobacter 2017

11th International Symposium on the Biology of Acinetobacter

September 20-22, 2017
Seville, Spain

www.acinetobacter2017.com

Cleaning up the nomenclatural chaos in the genus *Acinetobacter*: the effectively but not validly published names *'Acinetobacter oryzae'* Chaudhary et al. 2012, *'Acinetobacter plantarum'* De et al. 2016, *'Acinetobacter refrigeratoris'* Feng et al. 2014 and *'Acinetobacter seohaensis'* Yoon et al. 2007 are synonymous with the validly published names of well-established species.

Lenka Radolfova-Krizova, **Paul G. Higgins (Germany),** Alexandr Nemec.

Proposals of novel species names based on the description of only a single strain predominate in the current bacterial taxonomy despite the theoretical and practical limitations of this approach. Such proposals are regularly associated with taxonomically unconvincing or erroneous data. In the present study, we illustrate this problem on examples of four proposals of *Acinetobacter* species names which were effectively published outside the International Journal of Systematic and Evolutionary Microbiology. Genus-wide, whole-genome comparative analysis using the average nucleotide identity based on BLAST (ANIb) parameter revealed that the strains '*Acinetobacter* oryzae' B23, '*Acinetobacter plantarum*' KCTC 42611, '*Acinetobacter refrigeratoris*' KCTC 42011 and '*Acinetobacter seohaensis*' CCUG 56483 could be assigned to *Acinetobacter* johnsonii (ANIb of 95.4-96.0%), *Acinetobacter* junii (ANIb of 97.4-98.2%), Acinetobacter variabilis (ANIb of 95.3-96.0%) and *Acinetobacter* towneri (ANIb of 97.1%), respectively. Congruent with these assignments were the results of the genus-wide analyses of whole-cell mass fingerprints generated by matrix-assisted laser desorption/ionization-time-of-flight MS, and of metabolic and physiological features. Inspection of the original nomenclatural proposals revealed a number of inconsistencies in both genotypic and phenotypic data as compared with those obtained by us. To improve the quality of the circumscription of novel species, we emphasize the importance of using the genus-wide comparative analysis of results based on genus-targeted taxonomic methods applied on multiple strains of a novel taxon.

Cleaning up the nomenclatural chaos in the genus *Acinetobacter*:

the effectively but not validly published names
‘*Acinetobacter oryzae*’ Chaudhary et al. 2012,
‘*Acinetobacter plantarum*’ Du et al. 2016,
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are synonymous with the validly published names of well-established species

L. Radolfová-Křížová, P. G. Higgins & A. Nemec

What‘s the point?

- To build phylogenetic trees to investigate evolution
- Aids in communication between scientists
- Vital for
    - Clinicians
    - Food processing industry
    - Agriculture
    - Bioremediation
    - Public health
    - Environmental science
    - Biosafety

Species *versus* species names

- **Lack of generally accepted, biologically sound species concept (ontological status).**
- **Species definitions are principally based on discontinuities in the distribution of bacterial characteristics in a taxonomic space.**
- ***A monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity with respect to many independent characteristics ...* (Rossello-Mora & Amann 2001).**
- ***The adequacy of characterization of a bacterium is a reflection of time; it should be as full as modern techniques make possible. Unfortunately, one now regarded as adequate is likely, in 10 years time, to be hopelessly inadequate!* (Cowan 1965).**
- **Unlike classification, nomenclature is strictly regulated by the International Code of Nomenclature of Bacteria.**

Classification of *Acinetobacter** (September 2017)

57 validly published species names including these pairs of synonyms:

A. bohemicus = *A. pakistanensis* (later syn.) Nemec & Radolfova-Krizova 2016
A. indicus = *A. guangdongensis* (later syn.) Nemec & Radolfova-Krizova 2017
A. junii = *A. grimontii* (later syn.) Vanechoutte et al. 2008
A. dijkshoorniae = *A. lactucae* Unpublished

>> 53 different species with validly published names.

9 effectively but not validly published species names

Tentative species with provisional designations (N = >30)

Genomic species 6, 15BJ and 17 or Taxon 21, 22 and 23 etc, *“A. x-like”*.

A high number of taxonomically unique single strains (N = >20)

*** http://apps.szu.cz/anemec/Classification.pdf**

Effectively but not validly published names

Name*	Source	Reference
'A. antiviralis'	**Tobacco**	Lee et al. *J Microbiol Biotechnol* 2009
'A. kyonggiensis'	**Sewage**	Lee & Lee *J Microbiol* 2010
'A. marinus'	**Sea water**	Yoon et al. *J Microbiol Biotechnol* 200
'A. oleivorans'	**Soil**	Kang et al. *J Microbiol* 2011
'A. oryzae'	**Rice**	Chaudhary et al. *Microb Ecol* 2011
'A. plantarum'	**Wheat**	Du et al. *Arch Microbiol* 2016
'A. refrigeratoris'	**Refrigerator**	Feng et al. *Curr Microbiol* 2014
'A. seohaensis'	**Sea water**	Yoon et al. *J Microbiol Biotechnol* 2007
'A. septicus'	**Human blood**	Kilic et al. *J Clin Microbiol* 2007

All names proposed based on* ***a single isolate*

Taxonomic positions based on whole genome comparison (ANIb):

'A. kyonggiensis'	**Closest to *A. bohemicus* (92.7%)**
'A. marinus'	**Unique**
'A. oleivorans'	**Closest to *A. calcoaceticus* (91.4-91.8%)**
'A. septicus'	**Synonym of *A. ursingii* (96.5-97.5%)** Nemec *et al*. 2007

Aim

To define the taxonomic position of four strains for which binomial names were effectively but not validly published

Strains

‘*A. oryzae*’ B23

‘*A. plantarum*’ KCTC 42611

‘*A. refrigeratoris*’ KCTC 42011

‘*A. seohaensis*’ CCUG 56483

Methods*

Genus-wide comparative analyses of

- **Whole-cell patterns obtained by MALDI-TOF MS**
- **Partial *rpoB* gene sequences**
- **Whole-genome sequences**
- **Genus-targeted metabolic and physiological features**

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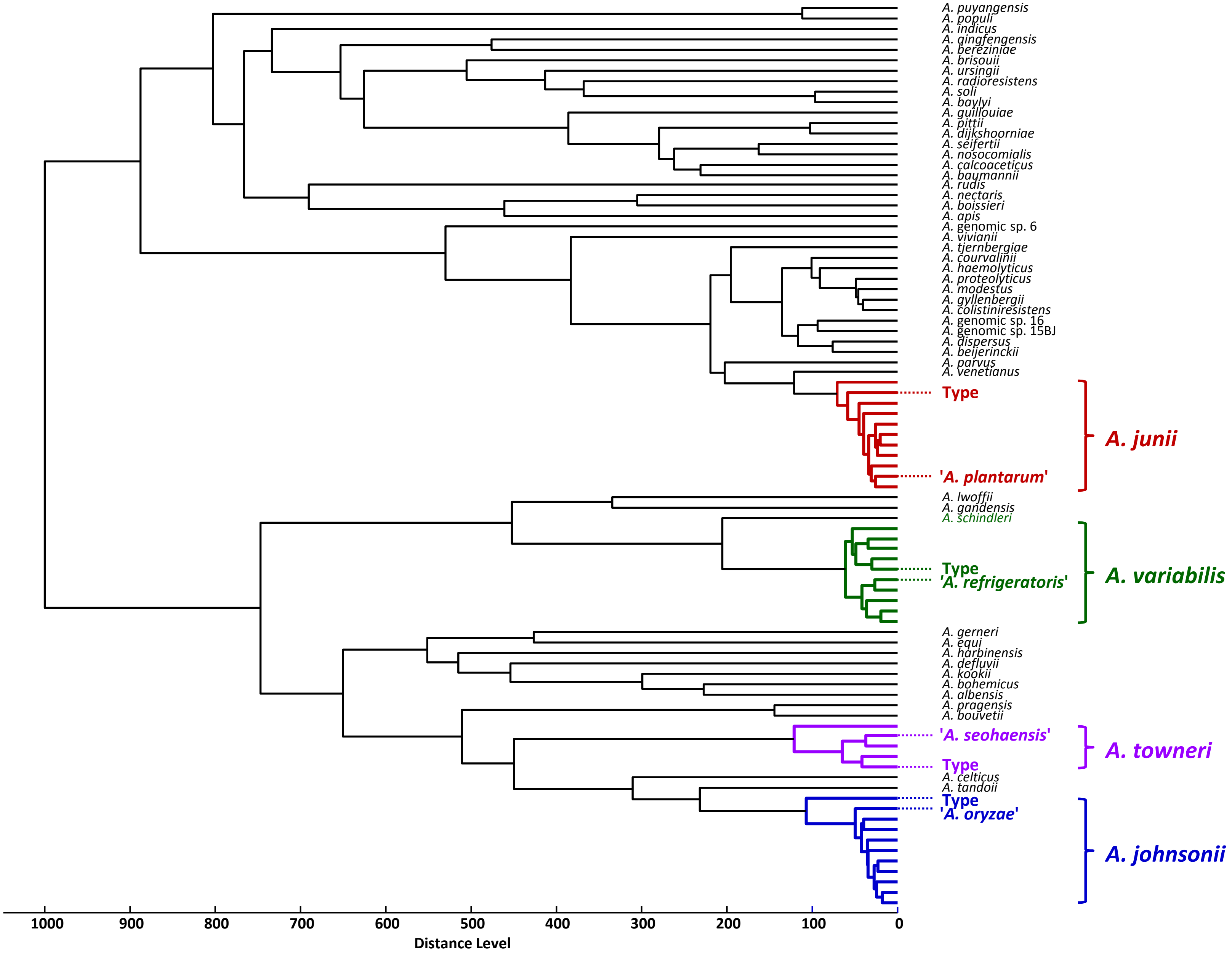
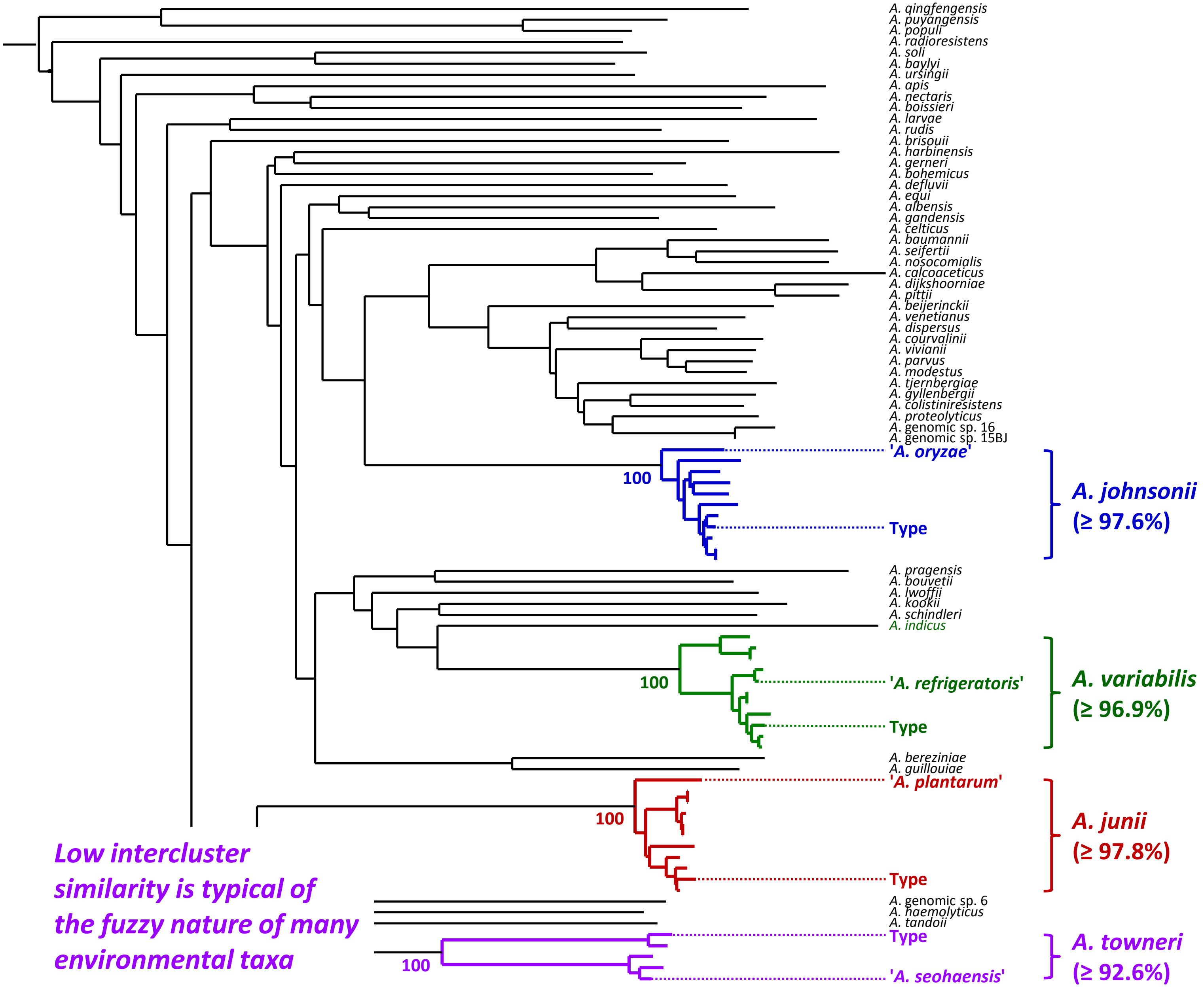
*Krizova 2014 , SAM Vol 37, 467-473

MALDI-TOF MS


A. puyangensis
A. populi
A. indicus
A. qingfengensis
A. bereziniae
A. brisouii
A. ursingii
A. radioresistens
A. soli
A. baylyi
A. guillouiae
A. pittii
A. dijkshoorniae
A. seifertii
A. nosocomialis
A. calcoaceticus
A. baumannii
A. rudis
A. nectaris
A. boissieri
A. apis
A. genomic sp. 6
A. vivianii
A. tjernbergiae
A. courvalinii
A. haemolyticus
A. proteolyticus
A. modestus
A. gyllenbergii
A. colistiniresistens
A. genomic sp. 16
A. genomic sp. 15BJ
A. dispersus
A. beijerinckii
A. parvus
A. venetianus
Type
'A. plantarum'
A. junii
A. lwoffii
A. gandensis
A. schindleri
Type
'A. refrigeratoris'
A. variabilis
A. gerneri
A. equi
A. harbinensis
A. defluvii
A. kookii
A. bohemicus
A. albensis
A. pragensis
A. bouvetii
'A. seohaensis'
Type
A. towneri
A. celticus
A. tandoii
Type
'A. oryzae'
A. johnsonii
1000
900
800
700
600
500
400
300
200
100
0
Distance Level

Partial *rpoB* gene sequencing



Low intercluster similarity is typical of the fuzzy nature of many environmental taxa

Whole genome comparison

Strain (genome)	**Compared with**	**ANIb* (%)**	**dDDH** (%)**
‘A. oryzae’ (NEWE00000000.1)	***A. johnsonii*** (Type + 6 strains)	**95.4–96.0**	**64.4–67.5**
‘A. plantarum’ (NGKN00000000.1)	***A. junii*** (Type + 4 strains)	**97.5–98.2**	**79.2-84.7**
‘A. refrigeratoris’ (JGI Id: 1102394)	***A. variabilis*** (Type + 2 strains)	**95.3-96.0**	**65.2–69.4**
‘A. seohaensis’ (JGI Id: 1102376)	***A. towneri*** (Type + 1 strain)	**97.1**	**77.3**

	ANIb (%)	**dDDH (%)**
Recommended approximate intraspecies values	**≥ 95**	**≥ 70**
Intraspecies values found for *Acinetobacter* spp.	**≥ 94.4**	**≥ 58.3**

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* **http://imedea.uib-csic.es/jspecies**

** **http://ggdc.dsmz.de/distcalc2.php**

Phenotyping

In-house metabolic and physiological tests*

- **Gelatinase production**
- **D-glucose acidification**
- **Hemolysis**
- **Growth at different temperatures**
- **Growth on 36 different substrates as single carbon and energy sources**

Results for all four strains consistent with the characteristics of the respective species

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* http://apps.szu.cz/anemec/Phenotype.pdf

Conclusion 1

- **The four strains with effectively published novel species names belong to known species:**

  ‘A. oryzae’ **>** ***A. johnsonii*** Bouvet & Grimont 1986
  ‘A. plantarum’ **>** ***A. junii*** Bouvet & Grimont 1986
  ‘A. refrigeratoris’ **>** ***A. variabilis*** Krizova et al. 2015
  ‘A. seohaensis’ **>** ***A. towneri*** Carr et al. 2003

- **5/9 effectively but not validly published names are synonymous to already known species with validly published names**

  (‘A. septicus’ = ***A. ursingii)***

Result comparison

16S rRNA	**Sequence difference: real v published**
‘A. oryzae’	**0.6%** (identity: 1429/1438 nt) v **1.6%**
‘A. plantarum’	**1.5%** (identity: 1424/1450 nt) v **2.1%**
‘A. refrigeratoris’	**0.5%** (identity: 1489/1497 nt)
‘A. seohaensis’	**0.1%** (identity: 971/972 nt) v **2%**

Errors in 16s sequences are common

rpoB	**Sequence difference**
‘A. plantarum’	**0%** (identity: 861/861 nt)
‘A. refrigeratoris’	**7.1%** (identity: 800/861 nt)

‘A. plantarum’ **sequence was correct but the 16s and DDH value against *A. junii* were erroneous**

DNA-DNA hybridization	**Experimental**	**Digital**
‘A. oryzae’ X ***A. johnsonii*** Type	**32–41%**	**64.4%**
‘A. plantarum’ X ***A. junii*** Type	**41.3%**	**79.2%**
‘A. seohaensis’ X ***A. towneri*** Type	**8.5–16.7%**	**77.9%**

Phenotype	**Numerous discrepancies**

Sources of problems

Species description/circumscription based on a single isolate

- **Contradictory to the current, population-based definition of bacterial species**
- **Impossible to assess phenotypic intraspecies variability and species-specific diagnostic traits**

16S rRNA

- **Unreliable as a primary criterion of relationship between species within a genus**
- **Unreliable data in GenBank (up to an error of 3.5% per sequence)**

Phenotype

- **Using tests with low or no discriminatory value for the genus**
- **Comparison only with a few (type) strains selected based on 16S rRNA sequences**

DNA-DNA hybridization

- **Poor reproducibility** (impossibility to check during peer-review)
- **Practical impossibility to perform genus-wide comparisons**
- **Technocratic, non-contextual application of taxonomic cut-offs**

Erroneous data

Scientific ethics

Conclusion 2

- **Current bacterial taxonomy suffers from undue emphasis on creating novel species names without sound taxonomic evidence that they mark biologically meaningful entities.**
- **A formal species name should be proposed mainly for a phylogenetically distinctive group which encompasses multiple strains of diverse origin and which is both genotypically and phenotypically internally coherent and, at the same time, different from known taxa at the species level of resolution.**
- **Any such proposal should be the very last step in comprehensive, context-wide taxonomic analysis in which as much publicly available data and biological material as possible are taken into account.**
- **Genus-wide comparative analysis based on WGS should be mandatory**

This is a prerequisite for reaching a meaningful and understandable formal classification of bacteria