

Rahnella perminowiae sp. nov., Rahnella bonaserana sp. nov., Rahnella rivi sp. nov. and Rahnella ecdela sp. nov., isolated from diverse environmental sources, and emended description of the genus Rahnella

by Brady, C., Asselin, J.A., Beer, S., Brurberg, M.B.,
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Brady, C., Asselin, J.A., Beer, S., Brurberg, M.B., Crampton, B., Venter, S., Arnold, D. and Denman, S. (2022) 'Rahnella perminowiae sp. nov., Rahnella bonaserana sp. nov., Rahnella rivi sp. nov. and Rahnella ecdela sp. nov., isolated from diverse environmental sources, and emended description of the genus Rahnella', *International Journal of Systematic and Evolutionary Microbiology*, 72(1).

1 **Description of *Rahnella perminowiae* sp. nov., *Rahnella bonaserana***
2 **sp. nov., *Rahnella rivi* sp. nov. and *Rahnella ecdela* sp. nov. from**
3 **diverse environmental sources and the emended description of the**
4 **genus *Rahnella*.**

5
6 **1.1 Author names**

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29
30 **1.4 Keyword**

31 *Rahnella*, *Yersiniaceae*, bacterial decay, onion, Acute Oak Decline

32 1.5 Repositories:

33 The GenBank/EMBL/DDBJ accession numbers are as follows: MW715676 - MW715683 (16S rRNA);
34 MW699050 - MW699063 (*atpD*); MW699064 - MW699077 (*gyrB*); MW699078 - MW699091 (*infB*);
35 MW699092 - MW699105 (*rpoB*); JAFMOS0000000000 - JAFMPD0000000000 (whole genome)

37 ABSTRACT

38 Bacteria isolated from onion bulbs suffering from bacterial decay in the United States and Norway
39 were previously shown to belong to the genus *Rahnella* based on partial housekeeping gene
40 sequences and/or fatty acid analysis. However, many strains could not be assigned to any existing
41 *Rahnella* species. Additionally, strains isolated from creek water and oak as well as a strain with
42 bioremediation properties were assigned to *Rahnella* based on partial housekeeping gene sequences.
43 The taxonomic status of these 21 strains was investigated using multilocus sequence analysis, whole
44 genome analyses, phenotypic assays and fatty acid analysis. Phylogenetic and phylogenomic analyses
45 separated the strains into five clusters, one of which corresponded to *Rahnella aceris*. The remaining
46 four clusters could be differentiated both genotypically and phenotypically from each other and
47 existing *Rahnella* species. Based on these results, we propose the description of four novel species:
48 *Rahnella perminowiae* sp. nov. (type strain SL6^T = LMG 32257^T = DSM 112609^T), *Rahnella bonaserana*
49 sp. nov. (type strain H11b^T = LMG 32256^T = DSM 112610^T), *Rahnella rivi* sp. nov. (type strain FC061912-
50 K^T = LMG 32259^T = DSM 112611^T) and *Rahnella ecdela* sp. nov. (type strain FRB 231^T = LMG 32255^T =
51 DSM 112612^T).

54 *Rahnella* is a genus of environmentally-linked species in the family *Yersiniaceae* [1]. For many years
55 *Rahnella aquatilis* was the only validly described species in the genus *Rahnella* [2], although two
56 genomospecies were proposed containing strains that could not be phenotypically differentiated from
57 *R. aquatilis* [3]. *R. aquatilis* has long been acknowledged as a truly ubiquitous bacterium and has been
58 isolated from a diverse range of sources, both environmental and clinical [4]. The genus *Rahnella* has
59 expanded exponentially in recent years with the description of six novel species from a range of
60 ecological niches and the elevation of the two genomospecies to validly described species [5–7]. These
61 eight species contributed to the existing diversity of *Rahnella* with isolations of *Rahnella victoriana*,
62 *Rahnella variigena* and *Rahnella inusitata* from bleeding cankers of oak; *R. victoriana*, *R. variigena*
63 and *R. woolbedingensis* from asymptomatic alder and walnut; *Rahnella bruchi* from the gut of the
64 *Agilus biguttatus* beetle; *Rahnella aceris* and *Rahnella laticis* from sap of *Acer pictum* and *Rahnella*
65 *contaminans* as a contaminant from MRSA agar plates [5–7]. In addition to their isolation from the

66 natural environment, *Rahnella* species have been linked to nitrogen-fixation [8], metal and
67 radionuclide sequestration [9] and biological control [10]; and more recently as possible pathogens of
68 oak [11], poplar [12] and onion [13].

69

70 A study by Asselin *et al.* [13] indicated the existence of several potential novel *Rahnella* species,
71 isolated over a number of years from onion bulbs with signs of bacterial decay in the United States
72 and Norway, and from creek water in the United States. Multilocus sequence analysis of a selection
73 of onion isolates placed them in four separate clusters without reference strains in the *Rahnella* genus,
74 suggesting they belong to four novel taxa [13]. A further potential novel *Rahnella* taxon was identified
75 in this study following *gyrB* gene sequencing of a strain previously isolated from a *Quercus* species
76 displaying symptoms of Acute Oak Decline (AOD) in the Netherlands. The above mentioned strains
77 were examined using a polyphasic approach based on genotypic, phenotypic, genomic and fatty acid
78 assays to clarify their taxonomic position. Based on the results, we propose four novel *Rahnella*
79 species: *Rahnella perminowiae* sp. nov., *Rahnella bonaserana* sp. nov., *Rahnella rivi* sp. nov. and
80 *Rahnella ecdela* sp. nov.

81

82

83 **Isolation and Ecology**

84 Bacterial strains were previously isolated from onion bulbs in the United States (New York State and
85 Oregon) and Norway (Vestfold, Østfold, Oppland and Hedmark) as described in Asselin *et al.* 2019 [13],
86 either directly from onion tissue or following soaking and crushing in buffer or sterile water. Strain
87 FC061912-K^T was isolated from creek water following high-speed centrifugation and culturing. A
88 *Rahnella* strain Y9602 able to sequester metals was isolated from a mixed-waste-contaminated
89 subsurface in Tennessee, United States [14]. FRB 231^T was isolated from the bleeding lesion on a
90 symptomatic oak in the Netherlands displaying symptoms of Acute Oak Decline (AOD). A swab was
91 taken from the lesion, suspended in sterile Ringers solution and the resulting suspension plated onto
92 Luria-Bertani (LB) agar. All strains can be routinely cultured on LB agar or in LB broth incubated at 28
93 °C, and stored in 40 % glycerol at -80 °C. See Suppl. Table S1 for a list of strains investigated in this
94 study.

95

96

97 **Genotypic characterisation**

98 DNA for all PCR reactions was extracted using alkaline lysis [15] and stored at -20 °C. Multilocus
99 sequence analysis (MLSA) was performed on strains which weren't included in the study by Asselin *et*

100 *al.* [13], by amplification and sequencing of the *gyrB*, *rpoB*, *infB* and *atpD* housekeeping genes as
101 previously described [16]. The following modifications were used: annealing temperature of 46 °C for
102 the *gyrB* PCR, alternative *rpoB* amplification and sequencing primers designed for *Rahnella* species
103 [13] and an alternative *atpD* reverse sequencing primer atpD-08R 5' CCCAGAAGTGCGGACACTTC 3'.
104 Almost complete 16S rRNA gene sequencing was performed on a selection of strains (AR20, L31-1-12,
105 C60, SL6^T, H11b^T, FC061912-K^T and FRB 231^T) using the primers from Coenye *et al.* [17] and standard
106 amplification cycles with an annealing temperature of 55 °C. Additional sequences for the closest
107 phylogenetic relatives were downloaded from GenBank and added to the datasets which were aligned
108 and trimmed in BioEdit v7.2.5 [18] to the following lengths: *gyrB* – 741 bp, *rpoB* – 636 bp, *infB* – 615
109 bp, *atpD* – 642 bp and 16S rRNA – 1346 bp. Following concatenation of the four housekeeping genes,
110 Smart Model Selection (SMS) [19] was performed on both the MLSA and 16S rRNA gene datasets
111 before maximum likelihood phylogenetic analysis using PhyML 3.0 [20]. Reliability of the generated
112 clusters was assessed with 1000 bootstrap replicates.

113

114 In the maximum likelihood phylogenetic tree based on concatenated multilocus gene sequences (Fig.
115 1), the strains isolated from onion bulbs in the United States and Norway were separated into three
116 clusters. The first cluster (*Rahnella* clade 1) comprised eight strains isolated from onion in the United
117 States and Norway, strain Y9602 which can sequester heavy metals and the type strain of a recently
118 described *Rahnella* species, *R. aceris* [6]. As the cluster was strongly supported with a bootstrap value
119 of 100 % and there was little sequence variation amongst the strains, it was concluded that strains in
120 this cluster belonged to *R. aceris*. The remaining isolates from onion bulbs did not cluster with any
121 reference or type strains. *Rahnella* clade 2, another large, well-supported group of nine strains from
122 onion in the United States and Norway, clustered on the border of the type species, *R. aquatilis*, but
123 was clearly separated and constituted a potential novel species. The remaining strain isolated from
124 onion, H11b^T (*Rahnella* clade 3) was situated on a separate branch between *R. victoriana* and *R.*
125 *variigena*. Strains FRB 231^T, isolated from *Quercus* sp. (*Rahnella* clade 4), and FC061912-K^T (*Rahnella*
126 clade 5), isolated from creek water adjacent to an onion field, also had separate positions within the
127 phylogenetic tree with no close association to a type strain indicating these three strains belonged to
128 further novel *Rahnella* species.

129

130 The 16S rRNA gene sequence pairwise similarity for the selected strains was calculated using
131 EZBioCloud [21], and was 99.3 % to *R. aceris* and *R. aquatilis* for strains from *Rahnella* clade 2; 99.2 –
132 99.5 % similar to *R. variigena*, *R. bruchi*, *R. wooldbedingensis* and *R. victoriana* for H11b^T, FRB 231^T and
133 FC061912-K^T (*Rahnella* clades 3, 4 and 5). As expected and as previously observed [5–7], due to the

134 recognised high degree of homogeneity in the 16S rRNA gene of genera in the *Enterobacterales*, the
135 taxonomic position of the potential novel species was not clearly or reliably represented by the 16S
136 rRNA gene phylogenetic tree (Suppl. Fig. S1). The existing *Rahnella* species did not form a
137 monophyletic clade and are interspersed by *Rouxiiella* species and *Ewingella americana*.

138

139 BOX and ERIC PCR (repetitive element-based PCR) were performed on all isolates to examine their
140 genetic diversity using the primers BOX-A1R and ERIC-2 and -1R, respectively [22]. Included in the
141 analyses were two representative strains from each existing *Rahnella* species. Amplicons were
142 separated in 1.5 % agarose at 50 V for ~3 h. BOX PCR provided the best resolution for all strains tested
143 and allowed the differentiation of the four potential novel species, not only from each other but also
144 from existing *Rahnella* species (Suppl. Fig. S2). Although the fingerprint patterns for strains from
145 *Rahnella* clade 2 had similar patterns, they were isolated from onion bulbs in different areas and
146 countries and therefore cannot be clones.

147

148

149 **Genome features**

150 The whole genome sequences of nine strains isolated from symptomatic onion bulbs (*Rahnella* clade
151 1: AR20, F57b, L31-1-12, R92a; *Rahnella* clade 2: C60, L72c, L151-1A, SL6^T and *Rahnella* clade 3: H11b^T)
152 and two single strains (*Rahnella* clades 4 and 5) from *Quercus* sp. (FRB 231^T) and creek water (FC06191-
153 K^T) were sequenced by Microbes NG (Birmingham, UK) on the Illumina HiSeq platform, following DNA
154 extraction by cell lysis and DNA purification with SPRI (Solid Phase Reversible Immobilization) beads.
155 Reads were adapter trimmed using Trimmomatic 0.30 with a sliding window quality cutoff of Q15 [23].
156 *De novo* assembly was performed using SPAdes version 3.11.1 [24] and the resulting contigs were
157 annotated in Prokka 1.11 [25]. Genome sizes from 5.40 to 5.75 Mbp and DNA G + C contents ranging
158 from 51.4 to 53.2 mol % were observed for the sequenced strains. The genome sequences were
159 submitted to Genbank under the BioProject number PRJNA706176); genome features and assembly
160 accessions are listed in Suppl. Table S2. The 16S rRNA sequences derived by genome sequencing of
161 the above strains were compared to those obtained with Sanger sequencing to ensure there was no
162 contamination of the whole genome sequences.

163

164 To infer the phylogenomic position of the strains, pairwise comparisons between the genomes were
165 conducted using Genome Blast Distance Phylogeny (GBDP) and accurate intergenomic distances
166 inferred under the algorithm 'trimming' and distance formula d_5 [26] with 100 distance replicates
167 each. The resulting intergenomic distances were used to construct a balanced minimum evolution tree

168 including Subtree Pruning and Regrafting (SPR) post-processing using FASTME 2.1.6.1 [27]. Branch
169 support was inferred from 100 pseudo-bootstrap replicates and the tree was rooted at the midpoint
170 [28]. In the resulting phylogenomic tree (Fig. 2), all representative *Rahnella* strains from the present
171 study formed a robust clade with existing *Rahnella* species with 100 % bootstrap support. The
172 clustering of the strains agreed with that observed in the MLSA phylogenetic tree (Fig. 1), with five
173 strains assigned to the recently validated species *R. aceris* and four strains isolated from onion forming
174 a separate cluster representing a novel taxon. The remaining representative strains from various
175 sources had unique positions within the *Rahnella* clade confirming their taxonomic status as three
176 novel species.

177

178 Whole genome comparisons were performed between representative *Rahnella* strains from the
179 present study and existing *Rahnella* species using average nucleotide identity with FastANI [29].
180 Strains from *Rahnella* clade 1 shared ANI values of 98.2 to 99.7 % with each other and 98.4 to 99.4 %
181 with the type strain of *R. aceris* SAP-19^T (Suppl. Table S3). These values are above the suggested
182 species limit of 95 % [30] confirming that the strains from onion and strain Y9602 belong to the same
183 taxon, *R. aceris*. ANI values ranging from 99.1 – 99.3 % were observed amongst strains from *Rahnella*
184 clade 2 confirming they belong to a single taxon. The strains from the three single-strain species
185 (*Rahnella* clades 3 – 5) exhibited ANI values of less than 91.2 % to each other, and to strains from
186 *Rahnella* clade 2. Furthermore, representative strains from *Rahnella* clades 2 – 5 were less than 94 %
187 related in terms of ANI to all existing *Rahnella* species (Table 1). The conclusions drawn from ANI
188 analysis were confirmed by *in silico* DNA-DNA hybridisation (*is*DDH) using the genome-to-genome
189 distance calculator (GGDC) [26] and are also presented in Table 1.

190

191 **Physiology and Chemotaxonomy**

192 Cell size, morphology and motility were determined using light microscopy and the microscopy
193 imaging software CellSens version 1.11 (Olympus Life Science, Tokyo, Japan). Flagella arrangement for
194 all proposed species, and existing *Rahnella* species (except *R. contaminans* and *R. laticis*) was observed
195 by transmission electron microscopy (FEI Tecnai 12 120kV BioTwin Spirit TEM) following negative
196 staining. Briefly, grids were floated on mid-log phase bacterial suspensions for 2 mins, washed 3 times
197 in distilled water, stained with 3 % uranyl acetate for 30 sec and washed again 3 times before wicking
198 away excess liquid and air drying. Colony morphology was examined following growth on tryptone
199 soya agar (TSA, Sigma) incubated at 28 °C for 48 h, while the growth temperature range was
200 determined on TSA incubated at 4, 10, 25, 28, 30, 37 and 41 °C in triplicate. Ranges for pH were tested
201 in triplicate in tryptone soya broth (TSB, Oxoid) with the pH adjusted to 4 – 10 (in increments of 1)

202 with sodium acetate/acetic acid and carbonate/bicarbonate buffers. Salt tolerance was tested in
203 saline-free nutrient broth (3 g l⁻¹ beef extract, 5 g l⁻¹ peptone) with the salt concentration adjusted to
204 1 – 7 % (in increments of 1 % w/v) by supplemented NaCl. These were incubated overnight at 28 °C
205 with shaking. Included in the temperature, pH and salt tolerance tests were the type strains for
206 existing *Rahnella* species (except *R. aceris*, *R. contaminans* and *R. laticis*). Catalase and oxidase activity
207 were determined by bubble production in 3 % v/v H₂O₂ and staining with Kovács reagent (1 % tetra-
208 methyl-*p*-phenylenediamine dihydrochloride), respectively.

209

210 Cells from all strains are straight rods with an average size of 0.6 x 1.6 µm. They occur singly, or in
211 pairs and are motile by peritrichous flagella (Fig. 3). Members of the order *Enterobacterales* are known
212 for their motility by several peritrichous flagella, and a recent study confirmed that all examined
213 *Rahnella* species possess the primary peritrichous flagella locus (*flag-1*), with most strains of *R.*
214 *variigena* encoding an additional secondary predicted peritrichous locus (*flag-3b*) [31]. However, the
215 recently described *R. aceris*, *R. contaminans* and *R. laticis* are indicated to be motile by a single, polar
216 flagellum [6, 7] and it was suggested that as all *Rahnella* species have similar flagella gene profiles,
217 they could all be motile by a polar flagellum [7]. The original description of the genus *Rahnella*
218 describes *R. aquatilis* as motile by peritrichous flagella, although electron microscopy images were not
219 published [2, 4]. To clarify the flagella arrangement of the existing *Rahnella* species, strains of these
220 were also imaged by TEM as described above (with the exception of *R. contaminans* and *R. laticis*). All
221 species examined clearly displayed multiple flagella on the surface of the cells, not at the poles,
222 providing evidence that the majority of *Rahnella* species are motile by peritrichous flagella (Suppl. Fig.
223 S3). Additionally, the genomes of all existing *Rahnella* species and the proposed four novel species
224 were screened for the presence of *flag* loci. All species were found to possess the primary peritrichous
225 flagella locus (*flag-1*), while the additional secondary *flag-3b* locus was encoded in the genome of
226 strain H11b^T (*Rahnella* clade 3) along with the *flag-1* locus (data not shown).

227

228 Following growth on TSA for 48 h, the resulting colonies are cream-coloured, round, slightly convex,
229 smooth with entire margins and 2 – 3 mm in diameter. All strains tested grew at 4, 10, 25, 28, 30 and
230 37 °C but not at 41 °C. Growth for all *Rahnella* strains included in this study was observed in the pH
231 range of 5 to 8, with weak growth at pH 9. Strains from the four proposed novel species and existing
232 *Rahnella* species grew well in nutrient broth supplemented with up to 6 % NaCl, while weak growth
233 was observed at 7 % NaCl. All strains from the proposed novel species are oxidase negative and
234 catalase positive.

235

236 Phenotypic testing was performed on a selection of strains from *Rahnella aceris* (AR20, C1b, F57b,
237 L31-1-12, L172-1A, R92a and Y9602), *Rahnella* clade 2 (A66, C60, L72c, L51-1-12, L151-1A, L173-1B
238 and SL6^T) and the three strains from clades 3 – 5 (H11b^T, FRB 231^T and FC061912-K^T) using the
239 commercial assays API 20E, API 50 CH/B (bioMérieux) and GEN III GN/GP microplates (Biolog). These
240 were performed according to the manufacturer's instructions. GEN III plates were scored after 6 h and
241 again after 24 h, while API 20 E and 50CH/B galleries were read after 24 h and 24 - 48 h, respectively.
242 The type strain of *R. aquatilis*, LMG 2794^T, was included as a positive control in the API 20E and 50
243 CH/B tests. Due to a lack of phenotypic data for existing *Rahnella* species based on the GEN III
244 microplate system, the type strains and reference strains for these were included in the GEN III assays
245 (with the exception of the type strains of *R. aceris*, *R. contaminans* and *R. laticis*).

246

247 Strains from the four proposed novel species were clearly differentiated from each other, and from
248 the existing species in the genus *Rahnella* based on phenotypes. Even the three proposed single-strain
249 species have clearly distinguishable phenotypic profiles. The most useful phenotypic characteristics
250 for species differentiation are listed in Table 2. The full phenotypic profiles for each proposed species
251 are described in the below protologues. It is acknowledged that the phenotypic profiles for the single-
252 strain species may change as further strains belonging to these taxa are isolated. Differing results for
253 several phenotypic characteristics for existing *Rahnella* species were observed by Jeon *et al.* [7], such
254 as citrate utilization, arginine dihydrolase, gelatinase and acetoin production. The phenotypic data
255 generated in the present study and previous studies [5, 6] were obtained following the manufacturer's
256 instructions for incubation temperature and time, whereas the data presented by Jeon *et al.* was
257 generated under different incubation conditions. This could account for the differences observed
258 between the studies.

259

260 Fatty acid methyl ester (FAME) analysis was performed on selected strains from *Rahnella* clade 1, now
261 confirmed as belonging to *R. aceris* (AR20, L31-1-12, R92a and Y9602), clade 2 (C60, L72c, L151-1A and
262 SL6^T) and the three strains from clades 3 - 5 (H11b^T, FRB 231^T and FC061912-K^T) by Fera Science Ltd.
263 (York, UK). Strains were cultivated on TSA at 28 °C for 24 h and the protocol followed was based on
264 the Sherlock Microbial Identification System Version 6.4 (MIDI Inc.). The results obtained were
265 compared against the library RTSBA6 6.21. The fatty acid profiles obtained for all strains were similar
266 in composition to those of existing *Rahnella* species [5, 6]. Complete fatty acid profiles for all *Rahnella*
267 species are presented in Table 3.

268

269

270 In the past eight years, the genus *Rahnella* has evolved from a monotypic genus to a genus comprising
271 species from a diverse range of hosts, sources and locations. The description of four novel *Rahnella*
272 species contributes to an already extensive list of environmental niches and highlights a possible role
273 for several species in bacterial decay of onion and AOD. Additionally, a large number of strains from
274 onion bulb decay in the USA and Norway have been assigned to *R. aceris*, along with strain Y9602 that
275 has the ability to sequester heavy metals, enhancing the description of this former single-strain
276 species.

277

278 Based on the genotypic, genomic, phenotypic and chemotaxonomic data generated in this study, we
279 conclude that the strains represent four novel species and propose the description of: *Rahnella*
280 *perminowiae* sp. nov. (type strain SL6^T = LMG 32257^T = DSM 112609^T), *Rahnella bonaserana* sp. nov.
281 (type strain = H11b^T = LMG 32256^T = DSM 112610^T), *Rahnella rivi* sp. nov. (type strain = FC061912-K^T
282 = LMG 32259^T = DSM 112611^T) and *Rahnella ecdela* sp. nov. (type strain = FRB 231^T = LMG 32255^T =
283 DSM 112612^T).

284

285 **Description of *Rahnella perminowiae* sp. nov.**

286 *Rahnella perminowiae* (per.mi.no'wi.ae. N.L. gen. fem. n. *perminowiae*, pertaining to Perminow,
287 named after Juliana I.S. Perminow for her work on bacterial plant diseases, including diseases of onion,
288 at the Norwegian Institute of Bioeconomy Research since 1993).

289

290 Gram-negative rods (0.6 – 0.8 x 1.5 – 1.8 µm) which occur singly or in pairs and are motile. Colonies
291 are cream on tryptone soya agar, round, convex and smooth with entire margins, facultatively
292 anaerobic, oxidase negative and catalase positive. Optimum growth is at 28 °C, although strains can
293 grow within the range 4 – 37 °C. Strains grow well at pH 5 - 8 and in broth supplemented with NaCl up
294 to 6 %. Positive for β-galactosidase, arginine dihydrolase and citrate utilization. Negative for lysine
295 decarboxylase, ornithine decarboxylase, H₂S, urease, tryptophan deaminase, indole production,
296 acetoin production and gelatinase. Nitrate is reduced to nitrite. Acid is produced from: glycerol, L-
297 arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, dulcitol,
298 D-mannitol, D-sorbitol, *N*-acetylglucosamine, methyl-αD-glucopyranoside, arbutin, esculin ferric
299 citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-
300 raffinose, gentiobiose, D-fucose and L-fucose (API 50CHB/E). Dextrin, D-maltose, D-trehalose, D-
301 cellobiose, gentiobiose, sucrose, D-raffinose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, D-
302 salicin, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, α-D-glucose, D-mannose, D-fructose, D-

303 galactose, D-fucose, L-fucose, L-rhamnose, D-sorbitol, D-mannitol, glycerol, α -D-glucose-6-phosphate,
304 β -D-fructose-6-phosphate, glycyl-L-proline, L-alanine, L-aspartic acid, L-glutamic acid, L-histidine, L-
305 serine, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid,
306 glucuronamide, mucic acid, quinic acid, D-saccharic acid, methyl pyruvate, citric acid, L-malic acid,
307 bromosuccinic acid, acetic acid and formic acid are utilised (Biolog GEN III). L-lactic acid and α -
308 ketoglutaric acid are variable (type strain is weakly positive). Major fatty acids include C_{16:0}, C_{18:1} ω 7c,
309 C_{17:0} cyclo, summed feature 2 (iso-C_{16:1} and/or C_{14:0} 3-OH) and summed feature 3 (C_{16:1} ω 7c and /or iso-
310 C_{15:0} 2-OH).

311 The DNA G + C content of the type strain is 51.8 mol %.

312 The type strain SL6^T (= LMG 32257^T = DSM 112609^T) was isolated from onion in Hedmark, Norway.

313

314 **Description of *Rahnella bonaserana* sp. nov.**

315 *Rahnella bonaserana* (bo.na.se.ra'na. N.L. fem. adj. *bonaserana*, pertaining to Bonasera,
316 named after Jean M. Bonasera for her work on bacterial plant diseases at Cornell University over 22
317 years developing culturing and identification techniques)

318

319 Gram-negative rods (0.5 – 0.6 x 1.3 – 1.6 μ m) which occur singly or in pairs and are motile. Colonies
320 are cream on tryptone soya agar, round, convex and smooth with entire margins, facultatively
321 anaerobic, oxidase negative and catalase positive. Optimum growth is at 28 °C, although strains can
322 grow within the range 4 – 37 °C. Strains grow well at pH 5 - 8 and in broth supplemented with NaCl up
323 to 6 %. Positive for β -galactosidase, arginine dihydrolase and citrate utilization. Negative for lysine
324 decarboxylase, ornithine decarboxylase, H₂S, urease, tryptophan deaminase, indole production,
325 acetoin production and gelatinase. Nitrate is reduced to nitrite. Acid is produced from: glycerol, L-
326 arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, dulcitol,
327 D-mannitol, D-sorbitol, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-
328 melibiose, D-saccharose, D-trehalose, D-raffinose, D-fucose and L-fucose (API 50CHB/E). Dextrin, D-
329 maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-raffinose, α -D-lactose, D-melibiose, β -
330 methyl-D-glucoside, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl- β -D-mannosamine, *N*-acetyl
331 neuraminic acid, α -D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, D-fucose, L-
332 fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, glycerol, α -D-glucose-6-phosphate, β -D-fructose-
333 6-phosphate, D-aspartic acid, D-serine, glycyl-L-proline, L-alanine, L-aspartic acid, L-glutamic acid, L-
334 histidine, L-serine, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic
335 acid, glucuronamide, mucic acid, quinic acid, D-saccharic acid, methyl pyruvate, L-lactic acid, citric

336 acid, L-malic acid, acetic acid and formic acid are utilised (Biolog GEN III). Major fatty acids include C_{16:0}
337 and C_{17:0} cyclo.

338 The DNA G + C content of the type strain is 51.9 mol %.

339 The type strain H11b^T (= LMG 32256^T = DSM 112610^T) was isolated from onion in New York State, USA.

340

341 **Description of *Rahnella rivi* sp. nov.**

342 *Rahnella rivi* (ri'vi. L. gen. n. *rivi*, of a river or creek, referring to the
343 isolation source of the type strain)

344

345 Gram-negative rods (0.6 – 0.7 x 1.5 – 1.8 µm) which occur singly or in pairs and are motile. Colonies
346 are cream on tryptone soya agar, round, convex and smooth with entire margins, facultatively
347 anaerobic, oxidase negative and catalase positive. Optimum growth is at 28 °C, although strains can
348 grow within the range 4 – 37 °C. Strains grow well at pH 5 - 8 and in broth supplemented with NaCl up
349 to 6 %. Positive for β-galactosidase, arginine dihydrolase and acetoin production. Negative for lysine
350 decarboxylase, ornithine decarboxylase, citrate utilization, H₂S, urease, tryptophan deaminase, indole
351 production and gelatinase. Nitrate is reduced to nitrite. Acid is produced from: glycerol, L-arabinose,
352 D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, dulcitol, D-mannitol,
353 methyl-α-D-glucopyranoside, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose,
354 D-melibiose, D-saccharose, D-trehalose, D-raffinose, gentiobiose and D-turanose (API 50CHB/E).
355 Dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, D-
356 raffinose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, D-salicin, N-acetyl-D-glucosamine, N-
357 acetyl-β-D-mannosamine, N-acetyl neuraminic acid, α-D-glucose, D-mannose, D-fructose, D-
358 galactose, D-fucose, L-fucose, L-rhamnose, inosine, D-mannitol, glycerol, α-D-glucose-6-phosphate, β-
359 D-fructose-6-phosphate, D-aspartic acid, D-serine, glycyl-L-proline, L-alanine, L-aspartic acid, L-
360 glutamic acid, L-histidine, L-serine, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic
361 acid, D-glucuronic acid, glucuronamide, mucic acid, D-saccharic acid, L-lactic acid, citric acid, L-malic
362 acid, tween 40, acetoacetic acid, acetic acid and formic acid are utilised (Biolog GEN III). Major fatty
363 acids include C_{16:0}, C_{18:1} ω7c, C_{17:0} cyclo, summed feature 2 (iso-C_{16:1} and/or C_{14:0} 3-OH) and summed
364 feature 3 (C_{16:1} ω7c and /or iso-C_{15:0} 2-OH).

365 The DNA G + C content of the type strain is 53.2 mol %.

366 The type strain FC061912-K^T (= LMG 32259^T = DSM 112611^T) was isolated from river water in New
367 York State, USA.

368

369

370 **Description of *Rahnella ecdela* sp. nov.**

371 *Rahnella ecdela* (ec.de'la. N.L. fem. adj. *ecdela* from Gr. adj. *ekdélōs* meaning clear or manifest,
372 referring to the clear separation from other species in this genus)

373

374 Gram-negative rods (0.5 – 0.6 x 1.3 – 1.5 μm) which occur singly or in pairs and are motile. Colonies
375 are cream on tryptone soya agar, round, convex and smooth with entire margins, facultatively
376 anaerobic, oxidase negative and catalase positive. Optimum growth is at 28 °C, although strains can
377 grow within the range 4 – 37 °C. Strains grow well at pH 5 - 8 and in broth supplemented with NaCl up
378 to 6 %. Positive for β -galactosidase. Negative for arginine dihydrolase, lysine decarboxylase, ornithine
379 decarboxylase, citrate utilization, H₂S, urease, tryptophan deaminase, indole production, acetoin
380 production and gelatinase. Nitrate is reduced to nitrite. Acid is produced from: glycerol, L-arabinose,
381 D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, dulcitol, D-mannitol,
382 *N*-acetylglucosamine, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-
383 melibiose, D-saccharose, D-trehalose, D-raffinose, gentiobiose, D-fucose, L-fucose and D-arabitol (API
384 50CHB/E). Dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-raffinose, α -D-
385 lactose, D-melibiose, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl- β -D-mannosamine, *N*-acetyl-D-
386 galactosamine, α -D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, D-fucose, L-
387 fucose, L-rhamnose, inosine, D-mannitol, D-arabitol, glycerol, α -D-glucose-6-phosphate, β -D-fructose-
388 6-phosphate, D-aspartic acid, D-serine, glycyl-L-proline, L-alanine, L-aspartic acid, L-glutamic acid, L-
389 histidine, L-serine, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic
390 acid, glucuronamide, mucic acid, quinic acid, D-saccharic acid, L-lactic acid, citric acid, L-malic acid,
391 tween 40, acetoacetic acid, acetic acid and formic acid are utilised (Biolog GEN III). Major fatty acids
392 include C_{16:0}, C_{17:0} cyclo and summed feature 2.

393 The DNA G + C content of the type strain is 51.9 mol %.

394 The type strain FRB 231^T (= LMG 32255^T = DSM 112612^T) was isolated from *Quercus* spp. exhibiting
395 AOD symptoms in the Netherlands.

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403 **Emended description of the genus *Rahnella* (Izard et al. 1981 emend. Brady et al. 2014, Jeon et al.**
404 **2021)**

405

406 *Rahnella* (Rah.nel'la. N.L. dim. ending *-ella*; N.L. fem n. *Rahnella* named after Otto Rahn, the German-
407 American microbiologist who proposed the name *Enterobacteriaceae* in 1937)

408

409 The description is based on the data from Brenner *et al.* 1998 [3], Kämpfer, 2005 [4], Brady *et al.* 2014
410 [5], Lee *et al.* 2020 [6], Jeon *et al.* 2021 [7] and this study.

411 Gram-negative straight rods (0.5 – 1.0 x 1.0 – 3.0 μm), facultatively anaerobic, oxidase negative and
412 catalase positive. Cells occur singly or in pairs and are motile by peritrichous flagella when grown at
413 25 °C, although some species possess a single flagellum. Colonies are white to cream on nutrient or
414 tryptone soya agar, round, slightly convex and smooth with entire margins. Strains can grow at
415 temperatures between 4 and 30 °C with optimum growth at 28 – 30 °C, growth at 37 °C varies
416 depending on the species. Strains can grow within the pH range 5 – 8 and in the presence of 0 – 6 %
417 (w/v) NaCl. Positive for β -galactosidase activity but negative for H₂S, urease and indole production.
418 Lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activity are all negative.
419 Arginine dihydrolase, citrate utilization, acetoin and gelatinase production are variable. Nitrate is
420 reduced to nitrite. Acid is produced from: L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-
421 fructose, D-mannose, L-rhamnose, D-mannitol, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-
422 maltose, D-lactose, D-melibiose and D-trehalose. The following carbon sources are utilized at 28 °C:
423 D-maltose, *N*-acetyl-D-glucosamine, α -D-glucose, D-mannose.

424 Frequently isolated from fresh water and various environmental habitats including soils, the
425 rhizosphere, woody tissues of oak, alder and walnut, tree sap and onion bulbs. Also found in the
426 intestines of snails and insects such as beetles and moths. Can be isolated from foods or human clinical
427 specimens, especially from immunocompromised patients. Major fatty acids include C_{16:0} and C_{17:0}
428 cyclo. The presence of C_{18:1} ω 7c, summed feature 2 (iso-C_{16:1} and/or C_{14:0} 3-OH) and summed feature 3
429 (C_{16:1} ω 7c and /or iso-C_{15:0} 2-OH) as major fatty acids is variable.

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431 The G + C content ranges from 51.3 to 53.7 mol %.

432 The type species is *Rahnella aquatilis*.

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AUTHOR STATEMENTS

1.6 Authors and contributors

CB was involved in the conceptualisation, data curation, formal analysis, investigation, methodology, validation, visualisation, writing, reviewing and editing of the work. JA, SB, MB, BC and SV were involved in the provision of resources and the conceptualisation, writing, reviewing and editing of the manuscript. DA and SD were responsible for funding acquisition and the conceptualisation, writing, reviewing and editing of the manuscript.

1.7 Conflicts of interest and disclaimers

The authors declare that there are no conflicts of interest.
Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

1.8 Funding information

This research was supported by the UK Research and Innovation's (UKRI) Strategic Priorities Fund (SPF) programme on Bacterial Plant Diseases (grant BB/T010886/1) funded by the Biotechnology and Biological Sciences Research Council (BBSRC), the Department for Environment, Food and Rural Affairs (Defra), the Natural Environment Research Council (NERC) and the Scottish Government. CB received additional funding from Woodland Heritage, BC and SD received funding from the Forestry Commission.

1.9 Acknowledgements

The authors would like to thank Dr Maria Chuvochina for assisting with the etymology of the novel species, Angeliki Savvantoglou for discussions on Greek translations and Dr Dann Turner for discussions on genome analyses and electron microscopy. We gratefully acknowledge the Wolfson Bioimaging Facility, especially Dr Lorna Hodgson, for assistance with the electron microscopy as well as the National Reference Centre (NRC) of the Netherlands Food and Consumer Product Safety Authority (NVWA) for the provision of the samples from symptomatic *Quercus*. Genome sequencing was provided by MicrobesNG (<http://www.microbesng.uk>) which is supported by the BBSRC (grant number BB/L024209/1).

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558 **FIGURES AND TABLES**

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560 **Table 1:** Percentages of average nucleotide identity (fastANI – lower left, orange) and *in silico* DNA-DNA hybridization (*is*DDH – upper right, blue) between
 561 *Rahnella perminowiae* sp. nov., *Rahnella bonaserana* sp. nov., *Rahnella rivi* sp. nov., *Rahnella ecdela* sp. nov. and existing species of the genus *Rahnella* (type
 562 strain columns are shaded in grey). Percentages above cut-off value for species delimitation (>95 % for ANI and >70 % for *is*DDH) are shaded.

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<i>is</i> DDH fastANI	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	100	96.2	93.4	93.9	32.2	28.2	31.6	49.2	42.3	31.4	28.6	25.3	28.2	32.8	33.9	31.6
2	99.3	100	93.1	93.6	31.7	27.8	31.2	45.0	41.8	31.1	28.3	26.5	27.9	32.4	33.8	31.2
3	99.1	99.1	100	94.4	31.8	27.7	31.2	44.8	41.8	31.0	28.3	26.5	27.8	32.4	33.7	31.2
4	99.2	99.1	99.3	100	31.9	27.9	31.3	45.1	42.0	31.3	28.4	26.6	28.1	32.6	33.8	31.3
5	87.4	87.8	87.8	87.8	100	28.8	42.8	34.1	34.1	42.1	29.9	26.0	28.9	54.8	33.2	42.2
6	85.5	86.4	86.3	86.4	86.3	100	28.6	29.0	28.7	28.5	27.8	31.7	27.8	29.2	28.8	28.7
7	87.1	87.5	87.5	87.6	91.2	86.2	100	33.3	33.2	53.5	29.6	25.4	28.7	44.3	33.2	47.6
8	91.7	91.6	91.6	91.6	88.4	86.3	88.0	100	49.2	32.9	29.8	25.5	29.5	34.4	37.5	33.1
9	90.6	90.6	90.7	90.7	88.6	86.1	88.1	92.9	100	33.0	29.4	27.4	28.9	35.3	36.5	33.2
10	86.9	86.9	86.6	86.9	90.7	86.1	93.8	87.9	87.9	100	29.6	25.9	28.7	42.8	33.0	53.0
11	85.7	85.6	85.7	85.6	87.0	85.8	86.9	86.5	86.4	86.8	100	27.5	60.8	30.4	30.0	29.8
12	84.6	84.5	84.6	84.6	85.2	88.0	85.4	85.4	85.3	85.2	85.5	100	27.9	25.8	26.6	25.7

13	85.5	85.4	85.4	85.4	86.2	85.8	86.3	86.5	86.2	86.2	95.3	85.6	100	29.4	29.5	28.8
14	87.6	87.7	87.6	87.7	94.0	86.6	91.6	88.5	89.2	91.2	87.3	85.7	86.8	100	34.3	43.6
15	88.1	88.0	88.1	88.0	88.7	86.4	88.2	90.0	89.2	88.1	86.8	85.8	86.5	89.3	100	33.4
16	87.1	87.0	86.9	87.0	91.0	86.2	92.5	88.1	88.0	93.6	86.8	85.5	86.3	91.5	88.2	100

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565 (1) *Rahnella perminowiae* SL6^T (GCA_019049755.1), (2) *Rahnella perminowiae* C60 (GCA_019049695.1), (3) *Rahnella perminowiae* L72c (GCA_019049715.1),
566 (4) *Rahnella perminowiae* L151-1A (GCA_019049735.1), (5) *Rahnella bonaserana* H11b^T (GCA_019049675.1), (6) *Rahnella rivi* FC061912-K^T
567 (GCA_019049655.1), (7) *Rahnella ecdela* FRB 231^T (GCA_019049625.1), (8) *Rahnella aquatilis* CIP 78.65^T (GCA_000241955), (9) *Rahnella aceris* SAP-19^T
568 (GCA_011684115), (10) *Rahnella bruchi* DSM 27398^T (GCA_003614975), (11) *Rahnella contaminans* Lac-M11^T (GCA_011065485), (12) *Rahnella inusitata* DSM
569 30078^T (GCA_003602055), (13) *Rahnella laticis* SAP-17^T (GCF_015644585), (14) *Rahnella variigena* CIP 105588^T (GCA_003602185), (15) *Rahnella*
570 *victoriana* DSM 27397^T (GCA_004330295), (16) *Rahnella woolbedingensis* DSM 27399^T (GCA_003602095).

571 ^T = type strain.

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582 **Table 2:** Phenotypic characteristics allowing differentiation of *Rahnella perminowiae* sp. nov., *Rahnella bonaserana* sp. nov., *Rahnella rivi* sp. nov. and
 583 *Rahnella ecdela* sp. nov. from each other and existing *Rahnella* species

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585 1 = *Rahnella perminowiae* sp. nov. (*n* = 7), 2 = *Rahnella bonaserana* sp. nov. (*n* = 1), 3 = *Rahnella rivi* sp. nov. (*n* = 1), 4 = *Rahnella ecdela* sp. nov. (*n* = 1), 5 =
 586 *Rahnella aquatilis* (*n* = 1), 6 = *Rahnella aceris* (*n* = 7, type strain not included), 7 = *Rahnella bruchi* (*n* = 3), 8 = *Rahnella contaminans* (*n* = 1), 9 = *Rahnella*
 587 *inuitata* (*n* = 3), 10 = *Rahnella laticis* (*n* = 3), 11 = *Rahnella variigena* (*n* = 5), 12 = *Rahnella victoriana* (*n* = 7), 13 = *Rahnella woolbedingensis* (*n* = 3). Data for
 588 type strain of 6 taken from [6] and data for 5, 7, 9, 11 – 13 taken from [5] except for carbohydrate utilisation data which was obtained from the present
 589 study. *n* = number of strains.

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	1	2	3	4	5	6	7	8	9	10	11	12	13
Arginine dihydrolase	+	+	+	-	-	+ ^a	-	-	-	-	-	-	-
Acetoin production	-	-	+	-	+ ^a	-	-	-	+ ^a	-	+	+ ^a	-
Gelatinase production	-	-	-	-	-	-	+	-	+ ^b	-	+ ^b	w+	w+
Acid from:													
D-sorbitol	+	+	-	-	+	+	v ^c	+	v ^c	-	+	+	-
methyl- α -D-glucopyranoside	+	-	+	-	-	v	v ^c	-	v ^c	-	-	-	-
N-acetylglucosamine	(+)	-	-	+	+	+	+	+	+	v ^c	+	+	+
D-melezitose	-	-	-	-	-	+	-	-	-	-	-	-	-
gentiobiose	(+)	-	+	+	+	(+)	+	+	+	+	+	+	+

D-turanose	-	-	+	-	-	+	-	-	v ^c	-	-	-	-
D-fucose	+	+	-	+	+ ^a	(+) ^d	-	-	-	-	(+) ^d	+	-
D-arabitol	-	-	-	+	-	-	+	+	+	-	-	-	-
Utilisation of:													
D-turanose	-	-	+	-	-	+	v	ND	+	ND	-	-	-
stachyose	-	-	+	-	-	v	+	ND	+	ND	v	-	-
<i>N</i> -acetyl-D-galactosamine	-	-	w+	+	w+	-	+	ND	-	ND	-	-	+
<i>N</i> -acetyl neuraminic acid	-	+	+	-	w+	-	+	ND	+	ND	-	+	+
3-methyl glucose	-	+	-	+	+	-	+	ND	-	ND	-	-	+
D-serine	-	+	+	+	+	-	+	ND	+	ND	+	+	+
D-sorbitol	+	+	-	-	+	+	-	ND	v	ND	+	+	-
D-arabitol	-	-	-	+	-	-	+	ND	+	ND	-	-	-
D-aspartic acid	-	+	+	+	+	+	+	ND	-	ND	+	v	+
D-serine	-	+	+	+	+	-	+	ND	-	ND	+	+	+
minocycline	-	+	-	-	-	-	-	ND	-	ND	+	-	-
quinic acid	+	+	-	+	+	+	+	ND	-	ND	+	+	+
tween 40	-	-	+	+	+	v	+	ND	+	ND	-	-	+
acetoacetic acid	-	-	+	+	+	-	+	ND	-	ND	-	+	+
sodium butyrate	-	+	-	+	-	-	-	ND	-	ND	+	-	-

591 +, 90 – 100 % strains +; (+), 70 – 89 % strains +; -, 91 – 100 % strains -; w+, weakly positive; v, variable; ND, not determined

592 ^a Differs from Jeon *et al.*, 2021

593 ^b Late reaction for type strain

594 ^c Positive for type strain

595 ^d Negative for type strain

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613 **Table 3:** Fatty acid composition (percentage of peak areas) of *Rahnella* species.

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615 1 = *Rahnella perminowiae* sp. nov. (n = 4), 2 = *Rahnella bonaserana* sp. nov. (n = 1), 3 = *Rahnella rivi* sp. nov. (n = 1), 4 = *Rahnella ecdela* sp. nov. (n = 1), 5 =
 616 *Rahnella aquatilis* (n = 1), 6 = *Rahnella aceris* (n = 4, type strain not included), 7 = *Rahnella bruchi* (n=3), 8 = *Rahnella contaminans* (n = 1), 9 = *Rahnella inusitata*
 617 (n=3), 10 = *Rahnella laticis* (n = 2), 11 = *Rahnella variigena* (n=4), 12 = *Rahnella victoriana* (n=4), 13 = *Rahnella woolbedingensis* (n=3). Values are expressed
 618 as the average if more than one strain per species were investigated, with the standard deviation shown in parentheses. Data for 5, 7, 9, 11 – 13 taken from
 619 [5], data for 8, 10 taken from [7] . n = number of strains.

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Fatty acid	1	2	3	4	5	6	7	8*	9	10*	11	12	13
Saturated fatty acids													
C _{12:0}	4.1 (± 0.1)	3.9	3.9	4.5	4.2	4.1 (± 0.0)	3.5 (± 0.0)	3.2	3.6 (± 0.2)	3.4 (± 0.07)	3.6 (± 0.1)	3.6 (± 0.3)	3.2 (± 0.0)
C _{14:0}	5.5 (± 0.1)	5.7	5.8	6.1	6.4	5.5 (± 0.1)	6.0 (± 0.3)	7.0	6.2 (± 0.2)	6.6 (± 0.8)	6.6 (± 0.3)	6.6 (± 0.3)	6.4 (± 0.1)
C _{16:0}	33.2 (± 0.4)	34.6	33.0	38.5	33.1	33.6 (± 0.3)	34.1 (± 0.5)	31.1	34.4 (± 1.4)	30.9 (± 5.9)	34.4 (± 2.1)	34.8 (± 0.3)	34.2 (± 1.1)
Unsaturated fatty acids													
C _{18:1 ω7c}	9.3 (± 0.9)	4.6	12.1	7.3	8.4	9.0 (± 0.2)	2.6 (± 0.9)	8.1	9.6 (± 0.8)	10.1 (± 3.4)	5.4 (± 1.1)	6.9 (± 0.5)	8.2 (± 1.3)
Cyclopropane fatty acids													
C _{17:0}	27.7 (± 1.3)	29.7	19.8	28.7	28.7	23.7 (± 0.5)	30.7 (± 0.7)	28.0	24.2 (± 2.4)	24.2 (± 3.1)	30.3 (± 0.5)	28.1 (± 0.6)	28.4 (± 1.2)
C _{19:0 ω8c}	2.6 (± 0.2)	4.7	0.3	5.7	2.3	1.6 (± 0.1)	7.9 (± 0.7)	2.4	1.0 (± 0.4)	1.6 (± 0.1)	5.0 (± 0.5)	3.6 (± 0.6)	3.9 (± 0.5)
Summed features													

2: C _{14:0} 3-OH and/or iso-C _{16:1}	9.5 (± 0.2)	8.9	9.5	9.5	6.9	9.3 (± 0.1)	9.0 (± 0.1)	9.0	9.1 (± 0.4)	8.5 (± 1.9)	8.9 (± 0.2)	8.8 (± 0.3)	8.7 (± 0.1)
3: C _{16:1} ω7c and/or C _{16:1} ω6c	8.4 (± 1.3)	4.2	12.6	4.4	8.1	9.9 (± 0.8)	1.8 (± 0.2)	7.2	10.1 (± 1.8)	10.2 (± 5.2)	2.9 (± 0.7)	5.0 (± 0.6)	4.2 (± 1.4)

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622 * Fatty acid analysis for these two species was performed by Jeon *et al.* [7] following growth on nutrient agar, whereas the remaining *Rahnella* species were
623 cultured on TSA prior to analysis by Fera Science Ltd. (York, UK).

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640 **Figure 1:** Maximum likelihood tree based on concatenated partial *gyrB*, *rpoB*, *atpD* and *infB* gene
641 sequences of proposed novel *Rahnella* species, existing *Rahnella* species and the closest phylogenetic
642 neighbours. Bootstrap values after 1000 replicates are expressed as percentages (values > 50 %
643 shown). *Hafni aalvei* (ATCC 13337^T) is included as an outgroup. The scale bar indicates the fraction of
644 substitutions per site. ^T = type strain

645

646 **Figure 2:** Phylogenomic tree of proposed novel *Rahnella* species, existing *Rahnella* species and the
647 closest phylogenetic neighbours. GBDP pseudo-bootstrap support values > 60 % shown at the nodes
648 (from 100 replicates), with an average branch support of 85.4 %. The branch lengths are scaled in
649 terms of GBDP distance formula d_5 . The tree is rooted at the midpoint. GenBank assembly and
650 accession numbers are given in parentheses. ^T = type strain

651

652 **Figure 3:** Transmission electron microscopy of proposed novel *Rahnella* species displaying their
653 flagella arrangement: (a) *Rahnella perminowiae* SL6^T, (b) *Rahnella bonaserana* H11b^T, (c) *Rahnella rivi*
654 FC061912-K^T, (d) *Rahnella ecdela* FRB 231^T. Scale bar, 1 μm .

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