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**Peptoniphilus nemausensis sp. nov. a new  
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clinical samples, an emendated description of the genus  
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names**

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1 ***Peptoniphilus nemausensis* sp. nov. a new Gram-positive anaerobic coccus isolated from**  
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4 **names.**

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26

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29

30 The GenBank/ENA/DDBJ accession numbers for the 16S rRNA, gene sequences of  
31 *Peptoniphilus nemausensis* sp. nov. type strain 1804121828<sup>T</sup> (= LMG 31466<sup>T</sup> = CECT 9935<sup>T</sup>)  
32 is MK945758. Whole genome sequences of *Peptoniphilus nemausensis* 1804121828<sup>T</sup> and  
33 *Peptoniphilus coxii* CCUG 59622<sup>T</sup> (BioProject number PRJEB38837 ; BioSample numbers  
34 SAMEA6943338 and SAMEA6943337, respectively), and *Peptoniphilus tyrrelliae* CCUG  
35 59621<sup>T</sup> (BioProject number PRJEB38837 ; BioSample number SAMEA8918417) were  
36 deposited in the National Center for Biotechnology Information (NCBI)'s reference sequence  
37 database under respective accession numbers CAIJCS000000000, CAIJCT000000000 and  
38 CAJUZH000000000. The respective Sequence Read Archive accession numbers are  
39 ERS4653225, ERS4653224 and ERS6601290.

40

41 Declarations of interest: none.

42

43

44

45

46 **Abstract**

47 A Gram-positive, anaerobic coccus isolated from a human surgical site infection was  
48 previously shown to belong to an unknown species of the genus *Peptoniphilus* initially  
49 proposed as '*Peptoniphilus nemausus*' sp. nov., based on both 16S rRNA gene sequence  
50 identity of 97.9% with the most closely related species *Peptoniphilus coxii* and an  
51 individualized phylogenetic branching within the genus *Peptoniphilus*. A polyphasic  
52 characterization of the novel species is proposed herein. Whole genome sequence analysis  
53 showed an average nucleotide identity value of 84.75% and digital DNA-DNA hybridization  
54 value of 28.9% against *P. coxii* type strain. The strain displayed unique features among  
55 members of the genus *Peptoniphilus*, as it was able to hydrolyze aesculin, and produced  
56 acetate as the major metabolic end-product without associated production of butyrate. Growth  
57 was observed under microaerophilic conditions. From all these data, the isolate is confirmed  
58 as belonging to a new *Peptoniphilus* species, for which the name *Peptoniphilus nemausensis*  
59 sp. nov. is proposed. The type strain is 1804121828<sup>T</sup> (= LMG 31466<sup>T</sup> = CECT 9935<sup>T</sup>). A  
60 database survey using a highly polymorphic partial sequence of the 16S rRNA gene of *P.*  
61 *nemausensis* revealed *P. nemausensis* to be a particularly rare skin-associated species in  
62 humans. An emended description of the *Peptoniphilus* genus is proposed based on a review  
63 of the characteristics of the 12 new species with validly published names since the genus  
64 description in 2001 and of *P. nemausensis*. Finally, the relationships between members of the  
65 genus *Peptoniphilus* were explored based on whole genome sequence analysis in order to  
66 clarify the taxonomic status of not yet validly published species showing that three pairs of  
67 species should be considered as synonyms: *Peptoniphilus timonensis* and '*Peptoniphilus*  
68 *phoceensis*', *Peptoniphilus lacydonensis* and '*Peptoniphilus rhinitidis*', *Peptoniphilus*  
69 *tyrrelliae* and *Peptoniphilus senegalensis*.

70 **Keywords:** *Peptoniphilus*, polyphasic taxonomy, diversity, clinical isolate, human skin.

## 71 **Introduction**

72           In 2001, the genus *Peptoniphilus* was proposed to accommodate strictly anaerobic  
73 Gram-positive cocci previously classified in the genus *Peptostreptococcus*. They were non-  
74 saccharolytic, butyrate-producers and used peptone and amino acids as major energy sources  
75 [20] unlike the saccharolytic, butyrate-producers which are members of the genus  
76 *Anaerococcus*. The genus *Peptoniphilus* belongs to the Firmicutes phylum and is classified in  
77 the *Tissierellia* class, the *Tissierellales* order and the *Peptoniphilaceae* family. Since the  
78 initial reclassification of five species from the genus *Peptostreptococcus* to the genus  
79 *Peptoniphilus*, i.e., *P. asaccharolyticus*, *P. lacrimalis*, *P. harei*, *P. indolicus* and *P. ivorii* [20];  
80 12 new species have been successively validated [6, 10, 23, 36, 38, 47, 51, 52, 55]. Currently,  
81 17 species names have been validly published (<https://lpsn.dsmz.de/genus/peptoniphilus>) [48]  
82 while 10 others species have not validly published names (February, 6<sup>th</sup>, 2021): ‘*P.*  
83 *grossensis*’ in 2012 [35], ‘*P. rhinitidis*’ in 2012 [25, 26], ‘*P. obesi*’ in 2013 [37], ‘*P.*  
84 *mikwangii*’ in 2015 [12], ‘*P. phoceensis*’ in 2016 [39], ‘*P. urinimassiliensis*’ in 2017 [7], ‘*P.*  
85 *pacaensis*’, ‘*P. raoultii*’ and ‘*P. vaginalis*’ in 2019 [17] and ‘*P. nemausus*’ in 2020 [18].  
86 Among the genus, species with validly published names can be distinguished by biochemical  
87 traits, metabolic profile, cellular fatty acid content and 16S rRNA gene and/or whole genome  
88 sequence analyses. The members of the genus *Peptoniphilus* are important representatives of  
89 human microbiota, which can also act as opportunistic pathogens in humans and may display  
90 notable resistance towards antimicrobial agents [40, 42].

91 The aim of this study was to perform a polyphasic characterization of strain 1804121828<sup>T</sup>,  
92 which we previously proposed as the type strain of a new species within the genus  
93 *Peptoniphilus* [18]. Initially proposed as ‘*P. nemausus*’, the name of this novel species has  
94 been changed to *Peptoniphilus nemausensis* for correct nomenclature. An emended  
95 description of the genus *Peptoniphilus* is proposed based on both the unique characteristics

96 displayed by the *P. nemausensis* strain and a review of the literature analyzing features  
97 reported for species with validly published names since the genus was first described. Lastly,  
98 the relationships among members of the genus were also explored in order to clarify the  
99 taxonomic status of not validly published species with available genomic data.

100

## 101 **Materials and methods**

### 102 *Strains*

103 Strain 1804121828<sup>T</sup> is representative of isolates of an, anaerobic, Gram-positive coccus that  
104 was recovered in pure culture from five human clinical samples, a periprosthetic fluid and  
105 four tissue specimens, sampled during the investigation of a surgical site infection in a patient  
106 who had undergone revision of a total hip prosthesis. Detailed clinical and microbiological  
107 information (initial culture, identification including 16S rRNA gene sequencing and  
108 phylogenetic analysis, and antimicrobial susceptibility pattern) is available in the publication  
109 by Enault *et al.* [18]. Briefly, the highest 16S rRNA gene sequence identity (97.9%) was  
110 observed with the type strain of *P. coxii* and neighbour-joining (NJ)-based phylogenetic  
111 analysis showed a clearly individualized branching within the genus *Peptoniphilus*; both  
112 observations supported the inclusion of the isolate in a new species [18].

113 Strain 1804121828<sup>T</sup> from a tissue sample is here submitted to polyphasic investigation  
114 comparative to the type strain of *Peptoniphilus coxii*, *P. coxii* CCUG 59622<sup>T</sup>.

115

### 116 *16S rRNA gene-based genetic and phylogenetic analyses*

117 The 16S rRNA gene sequence and NJ phylogenetic analyses have been described in Enault *et*  
118 *al.* [18]. A database survey was made using the nearly complete (1390 base pairs (bp)) 16S  
119 rRNA gene of *P. nemausensis* type strain. The GenBank database was searched using the  
120 Megablast program and the RDP database was investigated using the Seqmatch tool

121 (<http://rdp.cme.msu.edu/>). Sequences with a level of similarity to *P. nemausensis* of at least  
122 98.7 % and a minimum length of 1300 bp were selected. To consider shorter sequences,  
123 analysis was also conducted with a 145-bp fragment (position 74 to 202, *E. coli* numbering),  
124 found as highly specific for *P. nemausensis*. To evaluate the ecological relevance of *P.*  
125 *nemausensis*, both the 1390-bp and the 145-bp sequences were used for searching against the  
126 Microbiome Database (MDB) (<https://db.cngb.org/microbiome/>). Finally, metagenomic  
127 databases were searched using the Integrated Microbial Next Generation Sequencing  
128 (IGMNGS) platform, a comprehensive open source tool for processed 16S rRNA microbial  
129 profiles for ecology and diversity studies (<https://www.imngs.org/>) on April 23, 2021 [30]. A  
130 partial 16S rRNA gene sequence of 338 bp (including the 145 bp-sequence specific for *P.*  
131 *nemausensis* type strain and flanking regions - position 59 to 362, *E. coli* numbering - and  
132 displaying less than 99% of identity (92.5%) with that of *P. coxii* type strain) was used at the  
133 99% similarity threshold level proposed to represent the species for the search according to a  
134 strategy previously published [1]. Briefly, to determine the host or type of metagenome range  
135 of colonization without consideration of relative sequence abundances, the “report.0.tab” file  
136 was used (report of positive samples per sample category - e.g., human gut metagenome,  
137 human lung metagenome, pig gut metagenome, etc. – for which a single hit is sufficient for a  
138 sample to be considered as positive). To determine the levels of relative abundance, the  
139 reports “report.0.1.tab” excluding rare (> 0.1% threshold) and “report.0.01.tab” including  
140 only abundant (> 1%) sequences were respectively used. These three exported spreadsheet  
141 files with the number of samples that were positive for the presence of the queried taxonomy  
142 for each sample category were manually curated by removal of all samples that did not  
143 include “metagenome” in their designation and of those with no or ambiguous host  
144 information.

145 A maximum-likelihood (ML) (algorithm general time-reversible (GTR) as the substitution  
146 model with the addition of invariant sites and a gamma distribution of rates across sites)  
147 evolutionary tree was reconstructed using phylogenetic analyses available on  
148 <http://www.phylogeny.fr> [16]. Branch supports were assessed by bootstrap analysis (100 re-  
149 samplings).

150

#### 151 *Whole genome sequencing and analysis*

152 DNA of *P. nemausensis* 1804121828<sup>T</sup> and *P. coxii* CCUG59622<sup>T</sup> were extracted with the  
153 MasterPure DNA purification kit (Epicentre Biotechnologies, Madison WI, USA). Libraries  
154 were constructed with the Nextera XT DNA library preparation kit (Illumina, San Diego, CA,  
155 USA) and sequenced on a NextSeq 500 instrument with a 2 × 150-bp paired-end (PE)  
156 protocol. All sequenced PE reads were clipped and trimmed with AlienTrimmer v. 0.4.0 [14],  
157 corrected with Musket v. 1.1 [31], and subjected to a digital normalization procedure with  
158 Khmer v. 1.3 software [15]. For each sample, the remaining processed reads were assembled  
159 and scaffolded with SPAdes v. 3.11.0 [5]. All programs were used with their default settings.

160 Average nucleotide identity (ANI) based on BLAST (ANIb) and digital DNA–DNA  
161 hybridization (dDDH) were calculated between pairs of type strain genomes using  
162 JSpeciesWS (<http://jspecies.ribohost.com/jspeciesws/>) [49] and GGDC (<http://ggdc.dsmz.de>)  
163 [34] online services, respectively.

164 The Bacterial Pan Genome Analysis tool (BPGA) V1.3 [11] was used with default settings to  
165 identify orthologous groups and determine the set of genes shared by *P. nemausensis* and *P.*  
166 *coxii* type strains. BPGA was also used for functional annotation of Cluster of Orthologous  
167 group (COG) categories and the Kyoto Encyclopedia of Genes and Genomes (KEGG)  
168 pathways. The circular map of the genomes was generated using the BLAST Ring Image  
169 Generator (BRIG) [2]. Antibiotic resistance genes were identified using ResFinder version 3.2



170 [56]. The VFAnalyzer tool of the virulence factor database (VFDB,  
171 <http://www.mgc.ac.cn/VFs/>) was used to identify virulence genes using available *Firmicutes*  
172 genera as a reference [32]. Presence of Integrative Conjugative Elements (ICEs) was  
173 investigated by homology searches using BLAST against the ICEberg database (version 2.0)  
174 [33]. The PHASTER (PHAge Search Tool Enhanced Release) program was used to identify  
175 prophage sequences [4].

176

### 177 *Phenotypic characterization*

178 Both strains, 1804121828<sup>T</sup> and *P. coxii* CCUG 59622<sup>T</sup>, were studied according to the  
179 procedures described in the VPI Anaerobe Laboratory Manual [22]. Susceptibility to special-  
180 potency discs was determined as previously described [24]. The ability to grow in  
181 microaerophilic conditions was tested using CampyGen<sup>TM</sup> Compact (Oxoid). Enzymatic  
182 activity profiles were studied with rapid ID 32 A (bioMérieux). Metabolic end-products were  
183 assayed by quantitative Gas Chromatography (GC) as previously described [8]. For further  
184 biochemical characterization, the strain was grown anaerobically in trypticase-glucose-yeast  
185 extract-hemin (tryptone peptone, 30 g/liter; glucose, 5 g/liter; yeast extract, 20 g/liter; hemin,  
186 5 mg/liter; and cysteine HCl, 0.5 g/liter; pH: 7.6) broth and incubated at 37 °C for 72 h in an  
187 Anoxomat<sup>TM</sup> (MART Microbiology) anaerobic jar containing 5% H<sub>2</sub>, 5% CO<sub>2</sub> and 90% N<sub>2</sub>  
188 (by vol.). Antimicrobial susceptibility testing was performed and interpreted according to the  
189 2020 recommendations of the Antibiogram Committee of the French Society for  
190 Microbiology (CA-SFM) for Gram-positive anaerobes ([https://www.sfm-](https://www.sfm-microbiologie.org/wp-content/uploads/2020/04/CASFM2020_Avril2020_V1.1.pdf)  
191 [microbiologie.org/wp-content/uploads/2020/04/CASFM2020\\_Avril2020\\_V1.1.pdf](https://www.sfm-microbiologie.org/wp-content/uploads/2020/04/CASFM2020_Avril2020_V1.1.pdf)).

192

## 193 **Results and Discussion**

### 194 ***Peptoniphilus nemausensis* sp. nov., isolated from human clinical samples**

195 *16S rRNA gene-based assessment of the taxonomic position of P. nemausensis*

196 Previously published results [18] showed a highest 16S rRNA gene sequence identity of  
197 97.9% over 1390 bp with the most closely related species, *P. coxii* CCUG 59622<sup>T</sup>, a value  
198 below the threshold of 98.65-98.7% of 16S rRNA gene identity delineating species [27,53]. In  
199 addition, it should be noted that nearly all the divergent nucleotides were located in the 5'-part  
200 of the 16S rRNA gene sequence encompassing the variable region V1 and part of the V2  
201 (positions 74 to 202 according to *E. coli* numbering, 82% sequence identity with *P. coxii* for  
202 this 145-bp sequence). The level of 16S rRNA gene sequence identity with other members of  
203 the genus *Peptoniphilus* ranged from 85.7% with *P. olsenii* to 94.6% with *P. ivorii* (up to  
204 97.3% with yet non validated '*P. pacaensis*' strain KHD5<sup>T</sup>). Percentages were calculated  
205 based on an alignment of 1377 bp except for the pairwise comparisons with *P. senegalensis*  
206 (1330 nt), *P. catoniae* (1281 nt) and *P. timonensis* (1229 nt) type strains based on the length  
207 of sequences deposited.

208 The ML 16S rRNA gene-based tree (Supplementary Figure 1) was congruent with the NJ tree  
209 previously published [18], revealing that strain 1804121828<sup>T</sup> represents an independent robust  
210 branch within a clade grouping *P. coxii* and *P. ivorii* type strains, and supporting its  
211 assignation to a new species.

212

213 *Whole genome sequence-based taxonomy and distinctive characteristics from P. coxii*

214 The draft assembly of the genome sequence of strain 1804121828<sup>T</sup> was composed of 83  
215 contigs giving a total genome size of 1,805,580 bp (N50 = 185,257, L50 = 3) with a digital  
216 DNA G+C content of 45.70 mol%. The total number of PE reads was 1,188,781 for a genome  
217 sequencing coverage depth of 130×. The whole genome was annotated to include 1,708  
218 protein-coding sequences. Comparatively, the genome sequence of strain *P. coxii* CCUG

219 59622<sup>T</sup> (35 contigs, 1,722,467 PE reads) had a total genome size of 1,849,763 bp with a  
220 digital DNA G+C content of 45.70 mol% and included 1,689 proteins.

221 ANIb of strain 1804121828<sup>T</sup> genome against other *Peptoniphilus* type strain genomes ranged  
222 from 64.18% (*P. indolicus*) to 84.75% (*P. coxii*) (Supplementary Table 1). dDDH ranged  
223 from 28.9% (*P. coxii*) to 63.4% (*P. senegalensis*). All these values are below the respective  
224 thresholds of 95-96% for ANIb and 70% for dDDH used to delineate distinct species,  
225 therefore supporting the *P. nemausensis* taxonomic status as a new species [13, 29].

226 Overall genome sequence identity between strain 1804121828<sup>T</sup> and *P. coxii* CCUG59622<sup>T</sup> is  
227 shown in Figure 1. *P. nemausensis* and *P. coxii* type strains shared 1,395 genes while 266 and  
228 265 genes were respectively unique to each strain. The KEGG pathway analysis and COG  
229 distribution were similar for both species (Supplementary Figure 2). Differences were  
230 especially noted for virulence factor- and antimicrobial resistance-encoding genes, and  
231 prophage content. Both species harboured a panel of virulence factor-encoding genes  
232 putatively involved in important steps of pathogenesis with five genes (VFDB classes  
233 enzyme, protease, adherence, toxin, antiphagocytosis and secretion system) differentially  
234 identified in *P. nemausensis* and *P. coxii* as summarized in Supplementary Table 2. Regarding  
235 antimicrobial resistance-encoding genes, *ermA* (99.73% similarity with *Streptococcus*  
236 *pyogenes* erythromycin resistance methylase gene with accession number AF002716) was  
237 present in the *P. nemausensis* genome, and *tetM* was detected in both *P. nemausensis* and *P.*  
238 *coxii* type strains' genomes (99.9% similarity with *Neisseria meningitidis tetM* gene with  
239 accession number X75073 and 99.84% similarity with *Enterococcus faecalis* strain F01 *tetM*  
240 gene with accession number X92947). *P. nemausensis tetM* gene was carried by a Tn916-like  
241 transposon (17.8 kbp) for which the highest similarity (99.6%) in the ICEberg database was  
242 obtained with *Streptococcus pneumoniae* Tn5152 (FJ711160). *tetM* gene was also carried by  
243 a Tn916-like transposon in the *P. coxii* genome (93.3% similarity with that of *P.*

244 *nemausensis*). The Tn916-like transposon appeared widely distributed among members of the  
245 *Peptoniphilus* genus as we also detected similar sequences in *P. lacrimalis*, *P. senegalensis*,  
246 *P. hareii*, and *P. duerdenii* type strains' genomes (98.7, 98.6, 94.6, 93.3 and 92% similarity  
247 with that of *P. nemausensis*, respectively). Finally, the *P. nemausensis* genome included five  
248 prophage regions of which two regions were intact and three regions were incomplete (for a  
249 total of about 200 kbp) while a unique incomplete prophage region of 23 kbp was found in the  
250 *P. coxii* genome ([Supplementary Table 3](#)).

251

### 252 *Phenotypic characterization*

253 The 1804121828<sup>T</sup> strain is an anaerobic Gram-positive coccus, which is non-motile and non-  
254 spore-forming. Cells are 0.5-1  $\mu\text{m}$  in diameter with no particular arrangement. Colonies on  
255 *Brucella* blood agar after 5 days of incubation are 1 mm in diameter, circular, entire, greyish,  
256 opaque, shiny and non-haemolytic. After prolonged incubation, colonies, which were initially  
257 flat or raised, became umbonate. Growth in microaerophilic conditions was observed after 5  
258 days of incubation. No gaz was produced. Nitrate and nitrite were not reduced. Catalase,  
259 indole, gelatinase and urease were not produced. Aesculin was hydrolyzed. Milk was not  
260 fermented. The strain was mostly asaccharolytic except for acid production noted from  
261 aesculin whereas acid was not produced from fructose, glucose, lactose, maltose, mannose or  
262 sucrose. Metabolic end-products from TGY were acetic acid (7.22  $\text{mmol l}^{-1}$ ), 3-  
263 phenylpropionic acid (4.64  $\text{mmol l}^{-1}$ ), lactic acid (2.8  $\text{mmol l}^{-1}$ ), succinic acid (2.03  $\text{mmol l}^{-1}$ )  
264 and isovaleric acid (0.71  $\text{mmol l}^{-1}$ ) with traces of 2-hydroxybutyric acid (0.14  $\text{mmol l}^{-1}$ ) and  
265 2-hydroxyvaleric acid (0.006  $\text{mmol l}^{-1}$ ). Enzymic profiles obtained with rapid ID 32 A  
266 (bioMérieux) showed proline arylamidase activity and a weak alanine arylamidase activity.  
267 A susceptibility pattern to special-potency disks was resistance to colistin (10  $\mu\text{g}$ ), and  
268 susceptibility to vancomycin (5  $\mu\text{g}$ ), kanamycin (1 mg), and metronidazole (5  $\mu\text{g}$ ). Results of

269 antimicrobial susceptibility testing are shown in [Supplementary Table 4](#) showing overall  
270 susceptibility to antimicrobial agents tested except for fluoroquinolones. Distinctive  
271 characteristics between type strains of *P. nemausensis* and *P. coxii* were noted for the colistin  
272 special-potency disk and activity of fluoroquinolones. Despite no breakpoints being available  
273 for cyclines, we noted distinct antimicrobial susceptibility results for both species harboring  
274 *tetM* determinants.

275

276 The characteristics that differentiate the strain 1804121828<sup>T</sup> from other species related to the  
277 genus *Peptoniphilus* are summarized in [Table 1](#).

278

279 Genetic, phylogenetic and unique metabolic characteristics supported the proposition of a new  
280 species of the genus *Peptoniphilus* for which the name *Peptoniphilus nemausensis* sp. nov.  
281 was proposed in reference to the city of Nîmes, in the south of France, where the type strain  
282 was isolated.

283

#### 284 *Habitat and lifestyle of P. nemausensis from the database survey*

285 The database survey was performed to learn more about the habitat and lifestyle of *P.*  
286 *nemausensis*. Only 76 sequences with at least 98.7 % similarity to the nearly complete 16S  
287 rRNA gene of *P. nemausensis* type strain (MK945758) and a minimum length of 1300 bp  
288 matched with the reference sequence of *P. nemausensis* and all corresponded to uncultured  
289 clones ([Table 2](#)). All except one sequence came from different skin samples [[21](#), [28](#), [43](#), [50](#)]  
290 and the majority of sequences (n=65) came from a single study [[28](#)], in which *P. nemausensis*  
291 was detected in 11 of the 23 patients included (48%) and represented 65 of the 151,924 near  
292 full-length 16S ribosomal RNA sequences (0.55%) only. Search with the 145-bp fragment  
293 (position 74 to 202, *E. coli* numbering), highly specific for *P. nemausensis*, revealed three

294 additional clones from dust, coral and anemones [41, 46] (Table 2). No additional sequences  
295 were obtained in the RDP and Microbiome databases. Metagenomic database screening using  
296 the IMNGS platform found the presence of 16S rRNA gene sequences displaying close  
297 identity to that of the *P. nemausensis* type strain in a narrow panel of metagenomes congruent  
298 with the results of BLAST search (human skin metagenome; indoor and dust metagenomes).  
299 The metagenomes for which associated information was available, albeit imprecise for some  
300 of them, are presented in Table 3. This approach showed the species to be also detected in  
301 mouse skin metagenome and, to a lesser extent, in unspecified bovine metagenome.  
302 Regarding the relative abundance of *P. nemausensis* sequences in these metagenomes, *P.*  
303 *nemausensis* hits mostly represented rare sequences below 0.1% of the total sequences (Table  
304 3). Overall, the database survey suggested that *P. nemausensis* was skin-associated in  
305 humans, congruent with the isolation source of the type strain, but was a particularly rare  
306 species.

307

### 308 **Data supporting the need for an emended description of the genus *Peptoniphilus* Ezaki** 309 **2001**

310 Since the initial description of the genus by Ezaki *et al.* in 2001 based on the characteristics of  
311 the five reclassified species, 12 new species names have been successively validated. Some of  
312 these species showed features distinct from those included in the genus description. They are  
313 hereby reviewed to propose an emended description of the genus based on these  
314 characteristics and those of *P. nemausensis*. Despite some data were not gathered under  
315 strictly the same nutrient availability although continuity of the proteinacious substrates  
316 provided, notable differences from the initial genus description (“*obligately anaerobic cocci;*  
317 *the major cellular fatty acid is C<sub>18:1</sub> [19]; carbohydrates are not fermented; the major*  
318 *metabolic end-product from peptone/yeast extract/glucose [PYG] medium is butyric acid.*”)

319 were noted for major cellular fatty acid and metabolic-end products while some exceptions  
320 were noted regarding the use of carbohydrates and the ability to grow in microaerophilic  
321 conditions compared with the initial description. Divergences between studies regarding  
322 cellular fatty acid composition were also noted, particularly regarding the type species *P.*  
323 *asaccharolyticus* [23, 47, 51]. However, C<sub>16:00</sub>, C<sub>18:1</sub>ω<sub>9c</sub> and/or C<sub>18:2</sub>ω<sub>6,9c</sub>/anteiso-C<sub>18:0</sub> were  
324 the major metabolic end-products noted for several species [23, 47, 51], and C<sub>14:00</sub> was found  
325 to be the major metabolic end-product for *P. stercorisuis* [23]. The major metabolic end-  
326 product was acetate for *P. methionivorax* [51], *P. gorbachii* [52] and *P. nemausensis* [this  
327 study]. Mostly described as unable to ferment carbohydrates, we showed herein that a  
328 member of the genus *Peptoniphilus* is able to ferment aesculin. Finally, the ability to grow in  
329 microaerophilic conditions was noted for *P. lacydonensis* and *P. nemausensis* [6, this study].  
330 The description of the genus *Peptoniphilus* as published by Ezaki in 2001 [20] therefore needs  
331 to be emendated to include the following information: The major cellular fatty acids are  
332 C<sub>16:00</sub>, C<sub>18:1</sub>ω<sub>9c</sub>, C<sub>18:2</sub>ω<sub>6,9c</sub>/anteiso-C<sub>18:0</sub> and/or C<sub>14:00</sub>. The major metabolic end-products are  
333 butyric acid and/or acetic acid. Carbohydrates are generally not utilized. Growth is mostly  
334 obligately anaerobic but growth in microaerophilic conditions may be observed.

335

336 **Taxonomic status of published species with available genomic data but not validly**  
337 **published names**

338 16S rRNA gene sequence analysis and phylogeny in the genus *Peptoniphilus* revealed three  
339 pairs of closely related species (>98.7% of sequence identity), with names either validly  
340 published or not, *i.e.*, *P. tyrrelliae* and *P. senegalensis*; ‘*P. rhinitidis*’ and *P. lacydonensis*;  
341 and ‘*P. phoceensis*’ and ‘*P. timonensis*’ (Figure 2). All other pairs of species did not share  
342 more than 98.1% of 16S rRNA gene sequence identity supporting the discriminative power of  
343 16S rRNA gene in the genus *Peptoniphilus*. Both taxonomic issues of later synonym species

344 and of the accumulation of effectively published names of prokaryotic taxa never validated, as  
345 recently discussed by Oren et al. [45] prompt us to check the taxonomic status of the three  
346 above pairs of closely related species. At the time of writing, 18 Whole Genome Sequences  
347 (WGS) were available for type strains of *Peptoniphilus* species (nine for species with validly  
348 published names, nine for species with not validly published names including '*P. nemausus*')  
349 (Supplementary Table 1). As the WGS was not available for the type strain of *P. tyrrelliae*,  
350 we sequenced *P. tyrrelliae* CCUG 59621<sup>T</sup> genome to allow comparison for the couple of  
351 species *P. tyrrelliae* and *P. senegalensis* whose type strains share 99.5% of 16S rRNA gene  
352 sequence identity and were closely related according to 16S rRNA gene-based phylogeny  
353 (Figure 2). *P. senegalensis* was proposed in 2013 without any reference to *P. tyrrelliae*  
354 published in 2012 and Mishra et al. stated that *P. gorbachii* was the most closely related  
355 species to *P. senegalensis* with 97.4% of 16S rRNA gene sequence pairwise identity [38].  
356 This questioned the real existence of two different species that should have been considered  
357 before validation of *P. senegalensis* name in 2016 [44]. Here, we confirmed that *P.*  
358 *senegalensis* is a later synonym for *P. tyrrelliae*, which should not have any place in the  
359 nomenclature. Indeed, we found ANIb values of 96.88% when comparing the genomes of the  
360 strains CCUG 59621<sup>T</sup> and JC140<sup>T</sup>, that are above the recommended species delineation  
361 threshold value (95-96%), thereby supporting the affiliation of both strains to a unique species  
362 [13, 29], confirmed by a dDDH value of 75.70%.

363 The proposed '*P. rhinitidis*' species name had never been validated after effective publication  
364 in 2014 and availability of the genome sequence of strain 1-13<sup>T</sup> in 2012 [25, 26]. Four years  
365 later, in 2018, *P. lacydoensis*, whose type strain EL1<sup>T</sup> had 99.6% of 16S rRNA gene sequence  
366 pairwise identity with '*P. rhinitidis*' strain 1-13<sup>T</sup>, has been effectively published and name  
367 validated without any reference to '*P. rhinitidis*' [6]. We found ANIb values of 98.6 and  
368 98.79% and a dDDH value of 91.9% when comparing the genomes of the strains 1-13<sup>T</sup> and



369 EL1<sup>T</sup>, supporting the affiliation of both strains to a unique species [13, 29]. Although *P.*  
370 *lacydoensis* should have been a later synonym for '*P. rhinitidis*', the fact that authors  
371 proposing '*P. rhinitidis*' had never taken the necessary steps to validate the proposed species  
372 name has given '*P. rhinitidis*' no standing in the nomenclature. This illustrates the need for  
373 authors of taxon descriptions to validate the name of proposed new species - unless a distinct  
374 nomenclature can be validated by other authors for the same species - to avoid adding  
375 confusion to the taxonomy.

376 *P. timonensis* and '*P. phoceensis*' were respectively proposed in 2012 by Mishra *et al.* and in  
377 2016 by Mourembou *et al.* [36, 39]. Proposed type strains shared 99.6% of their 16S rRNA  
378 gene sequence and genome sequence analysis revealed ANI<sub>b</sub> of 96.31% and dDDH of 71.1%,  
379 incompatible with the existence of two different species. Interestingly, the same team that  
380 published *P. timonensis* species four years before proposed '*P. phoceensis*'. The authors  
381 stated that the closest species to '*P. phoceensis*' was '*P. grossensis*' (96.95% of 16S rRNA  
382 gene identity), a fact that was clearly not congruent with their phylogenetic analysis showing  
383 that '*P. phoceensis*' formed a tight group with *P. timonensis* [39].

384 In summary, three pairs of species should be considered as synonyms: *Peptoniphilus*  
385 *timonensis* and '*Peptoniphilus phoceensis*', *Peptoniphilus lacydonensis* and '*Peptoniphilus*  
386 *rhinitidis*', *Peptoniphilus tyrrelliae* and *Peptoniphilus senegalensis*. Our observations on a  
387 limited number of proposed new species in a definitive genus have revealed several  
388 inconsistencies that promote confusing taxonomy in the genus. They also highlight the fact  
389 that new species proposals should be more carefully monitored, not only by the authors  
390 proposing these new species but also by the reviewers and editors of the corresponding  
391 publications, and *in fine* by the International Committee on Systematics of Prokaryotes  
392 validating species names published in journals other than the International Journal of  
393 Systematic and Evolutionary Microbiology [54].

394 Considering the above remarks, we reviewed data for the two species with non-validated  
395 names in the clade formed by the proposed *P. nemausensis*, the species *P. coxii* and *P. ivorii*,  
396 and '*P. paccaensis*' and '*P. urinimassiliens*'. The latter shared 98% of their 16S rRNA gene  
397 sequence and genome sequence analysis supported that both species names could be validly  
398 published (ANIb 91.01% and 91.29% and dDDH 45.0%) [17]. The validity of other species  
399 names with available WGS was all supported by ANIb and dDDH values observed in our  
400 study.

401

#### 402 **Description of *Peptoniphilus nemausensis* sp. nov.**

403 The description is reported in [Table 4](#).

404

#### 405 **Emendated description of the genus *Peptoniphilus* Ezaki, 2001**

406 The description of the genus *Peptoniphilus* is as described by Ezaki (2001) with the following  
407 modifications: the major cellular fatty acids are C<sub>16:00</sub>, C<sub>18:1ω9c</sub>, C<sub>18:2ω6,9c</sub>/anteiso-C<sub>18:0</sub>  
408 and/or C<sub>14:00</sub>. The major metabolic end-products from PYG medium are butyric acid and/or  
409 acetic acid. Carbohydrates are generally not utilized. Cocci are mostly obligately anaerobic  
410 but growth in microaerophilic conditions may also be observed.

411

#### 412 **Sample CRediT author statement**

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414 Chistelle Mazuet: Methodology - phenotypic data acquisition, writing - revision & editing.

415 Alexis Criscuolo: Methodology - genomic data acquisition, data curation, writing - revision &  
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423 original draft.

424

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432 **References**

- 433 [1] Alauzet, C., Aujoulat, F., Lozniewski, A., Ben Brahim, S., Domenjod, C., Enault, C.,  
434 Lavigne, J.-P., Marchandin, H. (2021) A new look at the genus *Solobacterium*: a retrospective  
435 analysis of twenty-seven cases of infection involving *S. moorei* and a review of sequence  
436 databases and literature. *Microorganisms*. 9, 1229.  
437 <https://doi.org/10.3390/microorganisms9061229>.
- 438 [2] Alikhan, N.F., Petty, N.K., Ben Zakour, N.L., Beatson, S.A. (2011) BLAST Ring Image  
439 Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics*. 12, 402. DOI:  
440 10.1186/1471-2164-12-402.
- 441 [3] Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman,  
442 D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search  
443 programs. *Nucleic Acids Res.* 25, 3389–3402. DOI: 10.1093/nar/25.17.3389.
- 444 [4] Arndt, D., Grant, J., Marcu, A., Sajed, T., Pon, A., Liang, Y., Wishart, D.S. (2016)  
445 PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res.* 44,  
446 W16–21. DOI: 10.1093/nar/gkw387.
- 447 [5] Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin,  
448 V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N.,  
449 Tesler, G., Alekseyev, M.A., Pevzner, P.A. (2012) SPAdes: a new genome assembly  
450 algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19, 455–477.  
451 DOI:10.1089/cmb.2012.0021.
- 452 [6] Beye, M., Bakour, S., Le Dault, E., Rathored, J., Michelle, C., Cadoret, F., Raoult, D.,  
453 Fournier, P.E. (2018) *Peptoniphilus lacydonensis* sp. nov., a new human-associated species  
454 isolated from a patient with chronic refractory sinusitis. *New Microbes New Infect.* 23, 61–69.  
455 DOI: 10.1016/j.nmni.2018.02.007.

- 456 [7] Brahimi, S., Cadoret, F., Fournier, P.E., Moal, V., Raoult, D. (2017) '*Peptoniphilus*  
457 *urinimassiliensis*' sp. nov., a new bacterial species isolated from a human urine sample after  
458 de novo kidney transplantation. *New Microbes New Infect.* 16, 49–50. DOI:  
459 10.1016/j.nmni.2017.01.001.
- 460 [8] Carlier, J.P. (1985) Gas chromatography of fermentation products: its application in  
461 diagnosis of anaerobic bacteria. *Bull. Inst. Pasteur.* 83, 57–69.
- 462 [9] Carlier, J.P., Marchandin, H., Jumas-Bilak, E., Lorin, V., Henry, C., Carrière, C., Jean-  
463 Pierre, H. (2002) *Anaeroglobus geminatus* gen. nov., sp. nov., a novel member of the family  
464 *Veillonellaceae*. *Int. J. Syst. Evol. Microbiol.* 52, 983–986. DOI: 10.1099/00207713-52-3-  
465 983.
- 466 [10] Citron, D.M., Tyrrell, K.L., Goldstein, E.J. (2012) *Peptoniphilus coxii* sp. nov. and  
467 *Peptoniphilus tyrrelliae* sp. nov. isolated from human clinical infections. *Anaerobe.* 18, 244–  
468 248. DOI: 10.1016/j.anaerobe.2011.11.008.
- 469 [11] Chaudhari, N.M., Gupta, V.K., Dutta, C. (2016) BPGA- an ultra-fast pan-genome  
470 analysis pipeline. *Sci. Rep.* 6, 24373. DOI: 10.1038/srep24373.
- 471 [12] Cho, E., Park, S.N., Shin, Y., Lim, Y.K., Paek, J., Kim, H.K., Hwang, C.H., Jo, E., Jin,  
472 D., Chang, Y.H., Kook, J.K. (2015) *Peptoniphilus mikwangii* sp. nov., isolated from a clinical  
473 specimen of human origin. *Curr. Microbiol.* 70, 260–266. DOI: 10.1007/s00284-014-0712-7.
- 474 [13] Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D.R., da Costa, M.S., Rooney,  
475 A.P., Yi, H., Xu, X.W., De Meyer, S., Trujillo, M.E. (2018) Proposed minimal standards for  
476 the use of genome data for the taxonomy of prokaryotes. *Int. J. Syst. Evol. Microbiol.* 68,  
477 461–466. DOI: 10.1099/ijsem.0.002516.

478 [14] Criscuolo, A., Brisse, S. (2013) AlienTrimmer: a tool to quickly and accurately trim off  
479 multiple short contaminant sequences from high-throughput sequencing reads. *Genomics*.  
480 102, 500–506. DOI:10.1016/j.ygeno.2013.07.011.

481 [15] Crusoe, M.R., Alameldin, H.F., Awad, S., Boucher, E., Caldwell, A., Cartwright, R.,  
482 Charbonneau, A., Constantinides, B., Edvenson, G., Fay, S., Fenton, J., Fenzl, T., Fish, J.,  
483 Garcia-Gutierrez, L., Garland, P., Gluck, J., González, I., Guermond, S., Guo, J., Gupta, A.,  
484 Herr, J.R., Howe, A., Hyer, A., Härpfer, A., Irber, L., Kidd, R., Lin, D., Lippi, J., Mansour,  
485 T., McA’Nulty, P., McDonald, E., Mizzi, J., Murray, K.D., Nahum, J.R., Nanlohy, K.,  
486 Nederbragt, A.J., Ortiz-Zuazaga, H., Ory, J., Pell, J., Pepe-Ranney, C., Russ, Z.N., Schwarz,  
487 E., Scott, C., Seaman, J., Sievert, S., Simpson, J., Skennerton, C.T., Spencer, J., Srinivasan,  
488 R., Standage, D., Stapleton, J.A., Steinman, S.R., Stein, J., Taylor, B., Trimble, W., Wiencko,  
489 H.L., Wright, M., Wyss, B., Zhang, Q., Zyme, E., Brown, C.T. (2015) The khmer software  
490 package: enabling efficient nucleotide sequence analysis. *F1000Res*. 4, 900. DOI:  
491 10.12688/f1000research.6924.1.

492 [16] Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.F.,  
493 Guindon, S., Lefort, V., Lescot, M., Claverie, J.M., Gascuel, O. (2008) Phylogeny.fr: robust  
494 phylogenetic analysis for the non-specialist. *Nucleic Acids Res*. 36, W465–469. DOI:  
495 10.1093/nar/gkn180.

496 [17] Diop, K., Diop, A., Michelle, C., Richez, M., Rathored, J., Bretelle, F., Fournier, P.E.,  
497 Fenollar, F. (2019) Description of three new *Peptoniphilus* species cultured in the vaginal  
498 fluid of a woman diagnosed with bacterial vaginosis: *Peptoniphilus pacaensis* sp. nov.,  
499 *Peptoniphilus raoultii* sp. nov., and *Peptoniphilus vaginalis* sp. nov. *Microbiologyopen*. 8,  
500 e00661. DOI: 10.1002/mbo3.661.

501 [18] Enault, C., Aujoulat, F., Pantel, A., Cellier, N., Lechiche, C., Mégy, B., Lavigne, J.P.,  
502 Marchandin, H. (2020) Surgical site infection after hip replacement due to a novel  
503 *Peptoniphilus* species, provisionally named '*Peptoniphilus nemausus*' sp. nov. *Anaerobe*. 61,  
504 102071. doi: 10.1016/j.anaerobe.2019.102071.

505 [19] Ezaki, T., Yamamoto, H., Ninomiya, K., Suzuki, S. & Yabuuchi, E. (1983). Transfer of  
506 *Peptococcus indolicus*, *Peptococcus asaccharolyticus*, *Peptococcus prevotii*, and *Peptococcus*  
507 *magnus* to the genus *Peptostreptococcus* and proposal of *Peptostreptococcus tetradius* sp.  
508 nov. *Int. J. Syst. Evol. Microbiol.* 33, 683-698. DOI: 10.1099/00207713-33-4-683.

509 [20] Ezaki, T., Kawamura, Y., Li, N., Li, Z.Y., Zhao, L., Shu, S. (2001) Proposal of the  
510 genera *Anaerococcus* gen. nov., *Peptoniphilus* gen. nov. and *Gallicola* gen. nov. for members  
511 of the genus *Peptostreptococcus*. *Int. J. Syst. Evol. Microbiol.* 51, 1521–1528. DOI:  
512 10.1099/00207713-51-4-1521.

513 [21] Grice, E.A., Kong, H.H., Conlan, S., Deming, C.B., Davis, J., Young, A.C., NISC  
514 Comparative Sequencing Program, Bouffard, G.G., Blakesley, R.W., Murray, P.R., Green,  
515 E.D., Turner, M.L., Segre, J.A. (2009) Topographical and temporal diversity of the human  
516 skin microbiome. *Science*. 324, 1190–1192. DOI: 10.1126/science.1171700.

517 [22] Holdeman, L.V., Cato, E.P., Moore, W.E.C. (1977) *Anaerobe Laboratory Manual*, 4th  
518 edn. Blacksburg, VA: Virginia Polytechnic Institute and State University.

519 [23] Johnson, C.N., Whitehead, T.R., Cotta, M.A., Rhoades, R.E., Lawson, P.A. (2014)  
520 *Peptoniphilus stercorisuis* sp. nov., isolated from a swine manure storage tank and description  
521 of *Peptoniphilaceae* fam. nov. *Int. J. Syst. Evol. Microbiol.* 64, 3538–3545. DOI:  
522 10.1099/ijs.0.058941-0.

523 [24] Jousimies-Somer, H.R., Summanen, P., Citron, D.M., Baron, E.J., Wexler, H.M.,  
524 Finegold, S.M. (2002) Wadworth-KTL Anaerobic Bacteriology Manual, 6th edn. Belmont,  
525 CA, USA: Star Publishing Company.

526 [25] Jung, M.Y., Cho, J.H., Shin, Y., Paek, J., Park, I.S., Kim, J.S., Kim, W., Ma, J.Y., Park,  
527 S.J., Chang, Y.H. (2014) *Peptoniphilus rhinitidis* sp. nov., isolated from specimens of chronic  
528 rhinosinusitis. *Anaerobe*. 30, 30–34. DOI: 10.1016/j.anaerobe.2014.07.005.

529 [26] Kim, D.S., Jung, M.Y., Kang, A., Cho, J., Sin, Y., Paek, J., Kim, D.W., Kim, R.N., Nam,  
530 S.H., Kim, A., Park, H.S., Choi, S.H., Chang, Y.H. (2012) Genome sequence of *Peptoniphilus*  
531 *rhinitidis* 1-13T, an anaerobic coccus strain isolated from clinical specimens. *J. Bacteriol.*  
532 194, 2405–2406. DOI: 10.1128/JB.00192-12.

533 [27] Kim, M., Oh, H.S., Park, S.C., Chun, J. (2014) Towards a taxonomic coherence between  
534 average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation  
535 of prokaryotes. *Int. J. Syst. Evol. Microbiol.* 64, 346–351. DOI: 10.1099/ijs.0.059774-0.

536 [28] Kong, H.H., Oh, J., Deming, C., Conlan, S., Grice, E.A., Beatson, M.A., Nomicos, E.,  
537 Polley, E.C., Komarow, H.D., NISC Comparative Sequence Program, Murray, P.R., Turner,  
538 M.L., Segre, J.A. (2012) Temporal shifts in the skin microbiome associated with disease flares  
539 and treatment in children with atopic dermatitis. *Genome Res.* 22, 850–859. DOI:  
540 10.1101/gr.131029.111.

541 [29] Konstantinidis, K.T., Tiedje, J.M. (2005) Genomic insights that advance the species  
542 definition for prokaryotes. *Proc. Natl. Acad. Sci. USA.* 102, 2567–2572. DOI:  
543 10.1073/pnas.0409727102.

544 [30] Lagkouvardos, I., Joseph, D., Kapfhammer, M., Giritli, S., Horn, M., Haller, D., Clavel;  
545 T. (2016) IMNGS: A comprehensive open resource of processed 16S rRNA microbial  
546 profiles for ecology and diversity studies. *Sci. Rep.* 6, 33721. doi: 10.1038/srep33721.



547 [31] Liu, Y., Schröder, J., Schmidt, B. (2013) Musket: a multistage k-mer spectrum-based  
548 error corrector for Illumina sequence data. *Bioinformatics*. 29, 308–315.  
549 DOI:10.1093/bioinformatics/bts690.

550 [32] Liu, B., Zheng, D.D., Jin, Q., Chen, L.H., Yang, J. (2019) VFDB 2019: a comparative  
551 pathogenomic platform with an interactive web interface. *Nucleic Acids Res.* 47, D687-D692.  
552 DOI: 10.1093/nar/gky1080.

553 [33] Liu, M., Li, X., Xie, Y., Bi, D., Sun, J., Li, J., Tai, C., Deng, Z., Ou, H.Y. (2019)  
554 ICEberg 2.0: an updated database of bacterial integrative and conjugative elements. *Nucleic*  
555 *Acids Res.* 47, D660-D665. DOI: 10.1093/nar/gky1123.

556 [34] Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.P., Göker, M. (2013) Genome sequence-  
557 based species delimitation with confidence intervals and improved distance functions. *BMC*  
558 *Bioinformatics*. 14, 60. DOI: 10.1186/1471-2105-14-60.

559 [35] Mishra, A.K., Hugon, P., Robert, C., Raoult, D., Fournier, P.E. (2012) Non contiguous-  
560 finished genome sequence and description of *Peptoniphilus grossensis* sp. nov. *Stand.*  
561 *Genomic Sci.* 7, 320–330. DOI: 10.4056/sigs.3076460.

562 [36] Mishra, A.K., Lagier, J.C., Robert, C., Raoult, D., Fournier, P.E. (2012) Non contiguous-  
563 finished genome sequence and description of *Peptoniphilus timonensis* sp. nov. *Stand.*  
564 *Genomic Sci.* 7, 1–11. DOI: 10.4056/sigs.2956294.

565 [37] Mishra, A.K., Hugon, P., Lagier, J.C., Nguyen, T.T., Robert, C., Couderc, C., Raoult, D.,  
566 Fournier, P.E. (2013) Non contiguous-finished genome sequence and description of  
567 *Peptoniphilus obesi* sp. nov. *Stand. Genomic Sci.* 7, 357–369. DOI: 10.4056/sigs.3276687.

568 [38] Mishra, A.K., Lagier, J.C., Nguyen, T.T., Raoult, D., Fournier, P.E. (2013) Non  
569 contiguous-finished genome sequence and description of *Peptoniphilus senegalensis* sp. nov.  
570 *Stand. Genomic Sci.* 7, 370–381. DOI: 10.4056/sigs.3366764.

571 [39] Mourembou, G., Traore, S.I., Rathored, J., Khelaifia, S., Lagier, J.C., Michelle, C.,  
572 Raoult, D., Million, M. (2016) Isolation of new species "*Peptoniphilus phoceensis*" from  
573 human gut. *New Microbes New Infect.* 12, 52–53. DOI: 10.1016/j.nmni.2016.04.002.

574 [40] Murdoch, D.A. (1998) Gram-positive anaerobic cocci. *Clin. Microbiol. Rev.* 11, 81–120.  
575 DOI: 10.1128/CMR.11.1.81.

576 [41] Muller, E.M., Fine, M., Ritchie, K.B. (2016) The stable microbiome of inter and sub-  
577 tidal anemone species under increasing pCO<sub>2</sub>. *Sci. Rep.* 6, 37387. DOI: 10.1038/srep37387.

578 [42] Murphy, E.C., Frick, I.M. (2013) Gram-positive anaerobic cocci--commensals and  
579 opportunistic pathogens. *FEMS Microbiol. Rev.* 37, 520–553. DOI: 10.1111/1574-  
580 6976.12005.

581 [43] Oh, J., Freeman, A.F., NISC Comparative Sequencing Program, Park, M., Sokolic, R.,  
582 Candotti, F., Holland, S.M., Segre, J.A., Kong, H.H. (2013) The altered landscape of the  
583 human skin microbiome in patients with primary immunodeficiencies. *Genome Res.* 23,  
584 2103–2114. DOI: 10.1101/gr.159467.113.

585 [44] Oren, A., Garrity, G.M. (2016) Validation list no. 170. List of new names and new  
586 combinations previously effectively, but not validly, published. *Int. J. Syst. Evol. Microbiol.*  
587 66, 2463–2466. DOI: 10.1099/ijsem.0.001149.

588 [45] Oren, A., Garrity, G.M., Parte, A.C. (2018) Why are so many effectively published  
589 names of prokaryotic taxa never validated? *Int. J. Syst. Evol. Microbiol.* 68, 2125–2129. DOI:  
590 10.1099/ijsem.0.002851.

591 [46] Pakarinen, J., Hyvärinen, A., Salkinoja-Salonen, M., Laitinen, S., Nevalainen, A.,  
592 Mäkelä, M.J., Haahtela, T., von Hertzen, L. (2008) Predominance of Gram-positive bacteria  
593 in house dust in the low-allergy risk Russian Karelia. *Environ. Microbiol.* 10, 3317–3325.  
594 DOI: 10.1111/j.1462-2920.2008.01723.x.

595 [47] Patel, N.B., Tito, R.Y., Obregón-Tito, A.J., O'Neal, L., Trujillo-Villaroel, O., Marin-  
596 Reyes, L., Troncoso-Corzo, L., Guija-Poma, E., Lewis, C.M., Lawson, P.A. (2016)  
597 *Peptoniphilus cationiae* sp. nov., isolated from a human faecal sample from a traditional  
598 Peruvian coastal community. Int. J. Syst. Evol. Microbiol. 66, 2019–2024. DOI:  
599 10.1099/ijsem.0.000985.

600 [48] Parte, A.C., Sardà Carbasse, J., Meier-Kolthoff, J.P., Reimer, L.C., Göker; M. (2020)  
601 List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. Int. J.  
602 Syst. Evol. Microbiol. 70, 5607–5612. DOI: 10.1099/ijsem.0.004332.

603 [49] Richter, M., Rosselló-Móra, R., Glöckner, F.O., Peplies, J. (2016) JSpeciesWS: a web  
604 server for prokaryotic species circumscription based on pairwise genome comparison.  
605 Bioinformatics. 32(6):929–931. DOI: 10.1093/bioinformatics/btv681.

606 [50] Rintala, H., Pitkäranta, M., Toivola, M., Paulin, L., Nevalainen, A. (2008) Diversity and  
607 seasonal dynamics of bacterial community in indoor environment. BMC Microbiol. 8, 56.  
608 DOI: 10.1186/1471-2180-8-56.

609 [51] Rooney, A.P., Swezey, J.L., Pukall, R., Schumann, P., Spring, S. (2011) *Peptoniphilus*  
610 *methioninivorax* sp. nov., a Gram-positive anaerobic coccus isolated from retail ground beef.  
611 Int. J. Syst. Evol. Microbiol. 61, 1962–1967. DOI: 10.1099/ijs.0.024232-0.

612 [52] Song, Y., Liu, C., Finegold, S.M. (2007) *Peptoniphilus gorbachii* sp. nov., *Peptoniphilus*  
613 *olsenii* sp. nov., and *Anaerococcus murdochii* sp. nov. isolated from clinical specimens of  
614 human origin. J. Clin. Microbiol. 45, 1746–1752. DOI: 10.1128/JCM.00213-07.

615 [53] Stackebrandt, E., Ebers, J. (2016) Taxonomic parameters revisited: tarnished gold  
616 standards. Microbiology Today. 33, 152–155.

617 [54] Trujillo, M.E., Oren, A., Garrity, G.M. (2019) Preparation of the Validation Lists and the  
618 role of the List Editors. Int. J. Syst. Evol. Microbiol. 69, 3–4. DOI: 10.1099/ijsem.0.003106.

619 [55] Ulger-Toprak, N., Lawson, P.A., Summanen, P., O'Neal, L., Finegold, S.M. (2012)  
620 *Peptoniphilus duerdenii* sp. nov. and *Peptoniphilus koenoeniae* sp. nov., isolated from  
621 human clinical specimens. *Int. J. Syst. Evol. Microbiol.* 62, 2336–2341. DOI:  
622 10.1099/ijjs.0.031997-0.

623 [56] Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O.,  
624 Aarestrup, F.M., Larsen, M.V. (2012) Identification of acquired antimicrobial resistance  
625 genes. *J. Antimicrob. Chemother.* 67, 2640–2644. DOI: 10.1093/jac/dks261.

626

627 **Figure legends**

628

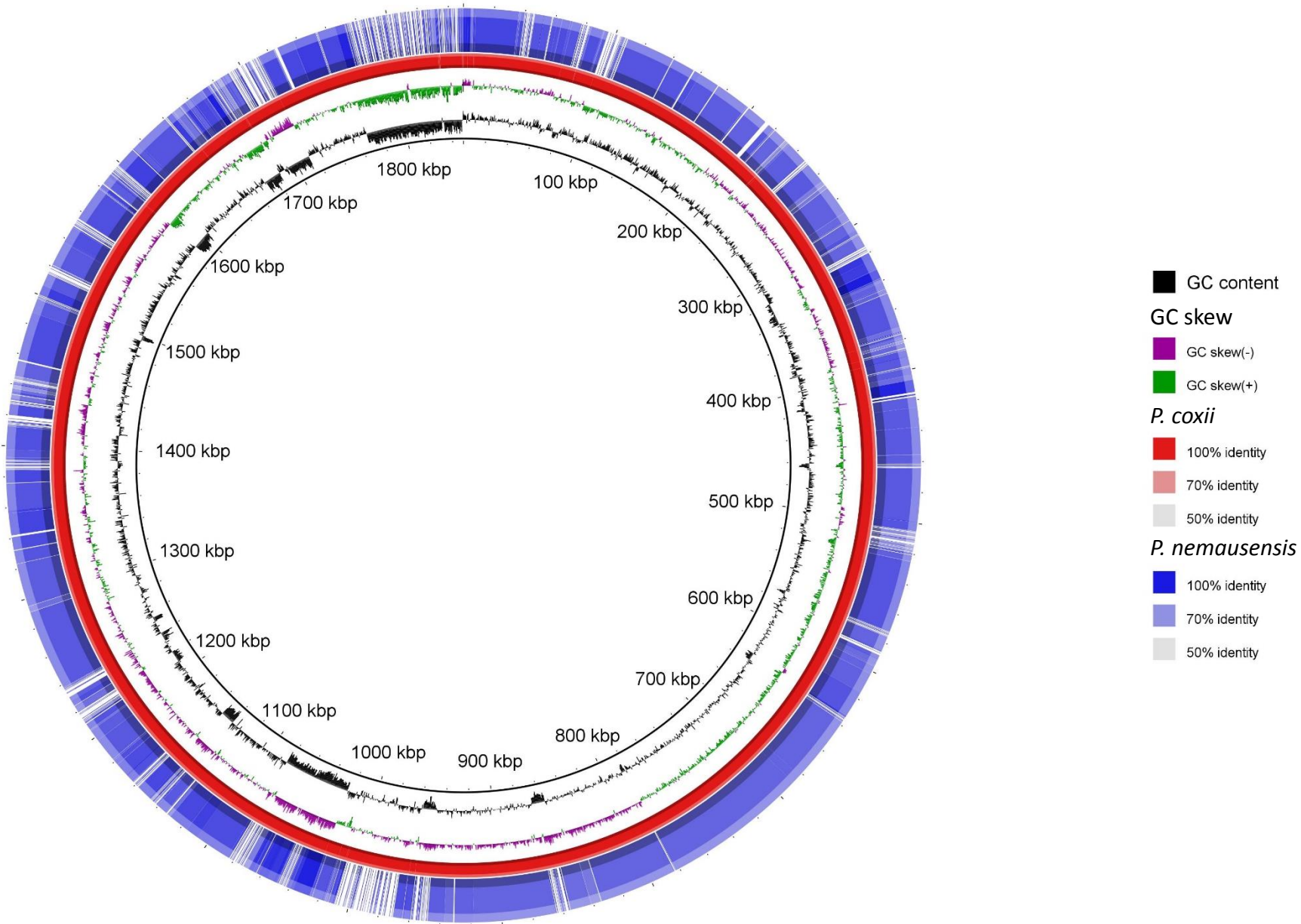
629 **Fig. 1.** Overall genome sequence identity distribution between *Peptoniphilus nemausensis*  
630 strain 1804121828<sup>T</sup> and *Peptoniphilus coxii* CCUG59622<sup>T</sup>. Location in the reference genome  
631 is indicated by numeration on the inside of the ring. GC content in the reference genome is  
632 indicated by the black bar graphs between the genomic coordinates and the colored ring. The  
633 graphical view of the alignments was rendered using the BLAST Ring Image Generator  
634 (BRIG) [2].

635

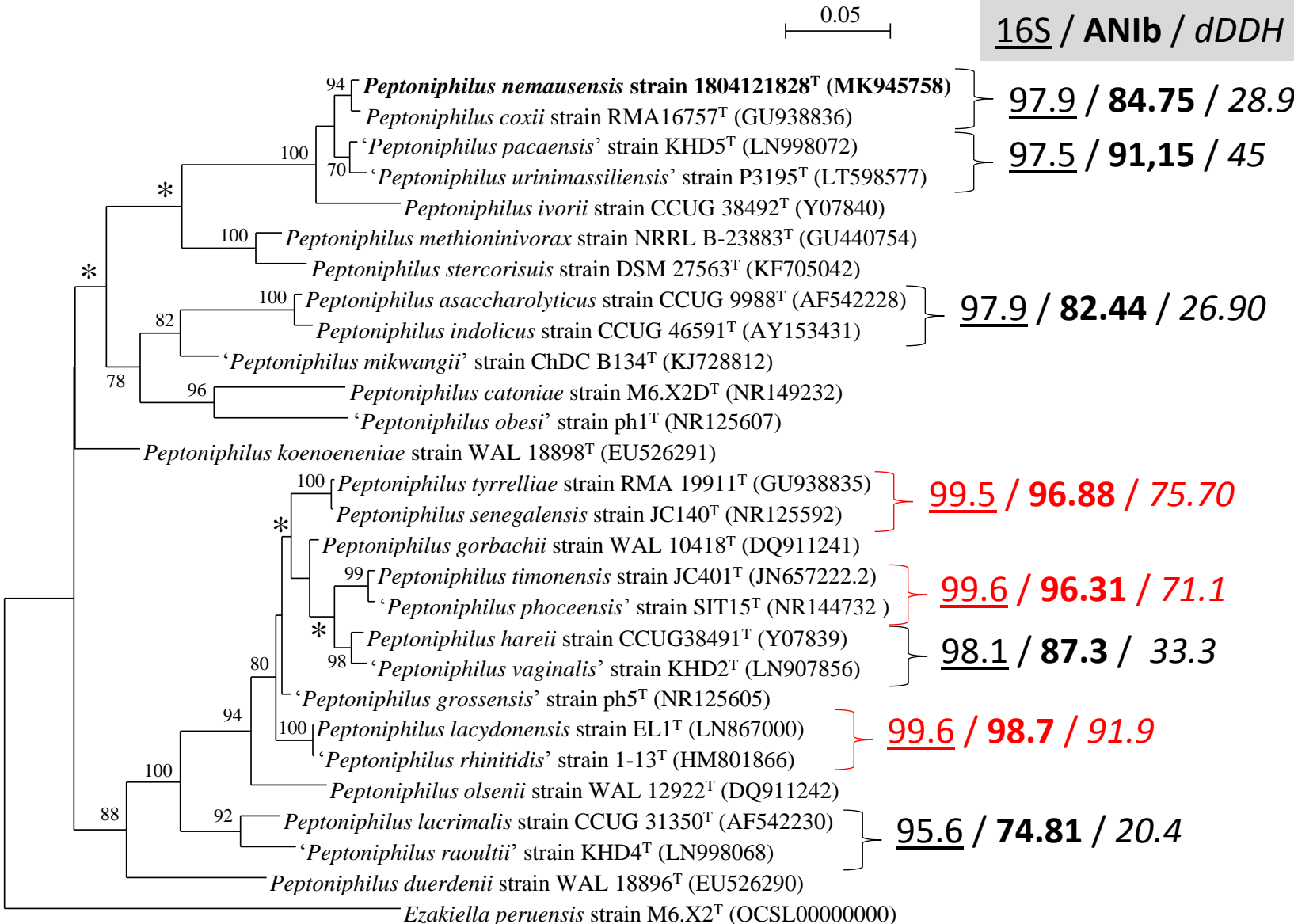
636 **Fig. 2.** Maximum likelihood phylogenetic tree showing the relationships between the 16S  
637 rRNA gene sequences of *Peptoniphilus nemausensis* sp. nov. type strain 1804121828<sup>T</sup> and  
638 type strains of other species, either validly published or not, in the genus *Peptoniphilus*.  
639 Names for effectively published but not validated species are indicated between quotes.  
640 GenBank accession numbers are indicated in parentheses. Alignment length was 1229 nt.  
641 Bootstrap supports were computed based on 100 replicates. Bootstrap values are indicated at  
642 the corresponding nodes for values of at least 70%. Star indicates node not observed in the  
643 Maximum Likelihood analysis. *Ezakiella peruensis* strain M6.X2<sup>T</sup> was used as an outgroup.  
644 Bar, 0.02 substitutions per site. Right part of the figure: for pairs of related strains (including  
645 species with uncertain taxonomic status *P. senegalensis* and *P. tyrrelliae*, *P. lacydoensis* and  
646 '*P. rhinitis*', and *P. timonensis* and '*P. phoceensis*' further discussed in the text) are  
647 successively indicated from left to right: 16S rRNA gene pairwise sequence identity (16S, %,   
648 underlined), overall genome relatedness index: Average nucleotide identity based on BLAST  
649 algorithm (ANIb, %, bold type), and digital DNA-DNA Hybridization (dDDH, %, italics);  
650 values indicated in red are above the respective thresholds of 98.7% for 16S rRNA gene

651 sequence identity, 95-96% for ANIb and 70% for dDDH used to delineate distinct species  
652 [\[13, 27, 29, 53\]](#). NA, not available.

Figure 1



**Figure 2**





**Table 1. Characteristics useful in the differentiation of *P. nemausensis* strain 1804121828<sup>T</sup> from close relatives *Peptoniphilus* species\***

	<i>P. nemausensis</i> 1804121828 <sup>T**</sup>	<i>P. coxii</i> RMA 16757 <sup>T**</sup>	<i>P. ivorii</i> DSM 10022 <sup>T</sup>	<i>P. methionivorax</i> NRRL B-23883 <sup>T</sup>	<i>P. stercorisuis</i> SF-S1 <sup>T</sup>
Production of:					
Alkaline phosphatase	-	-	-	+	-
Arginine dihydrolase	-	-	-	+	+
Major terminal volatile fatty acids A		NR	B	A (B, p)	B, A, L
Activity of:					
Tyrosine arylamidase	-	-	-	+	+
Alanine arylamidase	w	-	-	w	+
Arginine arylamidase	-	-	-	NR	+
Proline arylamidase	+	+	+	+	-
Phenylalanine arylamidase	-	-	-	w	+
Leucine arylamidase	-	V	-	+	+
Glycine arylamidase	-	-	-	w	+
Histidine arylamidase	-	-	-	NR	+
Serine arylamidase	-	-	-	w	+

\* Information is not all gathered under the same nutrient availability as PYG or TGYH media were used according to the study.

\*\* Additional characteristics differentiating *P. nemausensis* and *P. coxii* type strains (not available for the 3 other species included in the table) reported in the text are:

- susceptibility (*P. coxii*) / resistance (*P. nemausensis*) to the colistin special-potency disk (10 µg),
- aesculin hydrolysis by *P. nemausus* only (while aesculin fermentation was noted for both species).

w, weak; +, positive; -, negative.

A, acetic acid; B, butyric acid; L, lactic acid; p, propionic acid.

NR: not reported.

Data were from species description publications as follows: *P. coxii* [10]; *P. ivorii* [20]; *P. methionivorax* [51]; *P. stercorisuis* [23]; or were taken from www.CCUG.se.

V, negative reaction from the original publication and positive reaction from www.CCUG.se.

**Table 2. Database survey performed using the nearly complete 16S rRNA gene or a 145-bp region of *Peptoniphilus nemausensis* type strain sequence**

Sequence use for BLAST (accession number, length)	Number of sequence(s) with identity > 98.7%	Representative accession number	Sequence identity (%) with <i>P.</i> <i>nemausensis</i>	Source	Reference
MK945758 (1390 bp)	65	HM248706	99-99.9	Human skin (popliteal fossa, volar forearm, antecubital fossa...)	[28]
MK945758 (1390 bp)	6	GQ001519	99.3-99.6	Human skin (manubrium, gluteal crease, buttock, antecubital fossa, popliteal fossa)	[21]
MK945758 (1390 bp)	4	KF082872	99.5-99.6	Human skin (volar forearm, retroauricular crease)	[43]
MK945758 (1390 bp)	1	AM696714	99.1	Indoor dust	[51]
partial MK945758 (145 bp)	1	MG488499	99.3	<i>Acropora cervicornis</i> (coral)	-
partial MK945758 (145 bp)	1	KX150339	99.3	<i>Actinia</i> (anemone)	[41]
partial MK945758 (145 bp)	1	AM942981	99.3	House dust	[46]

**Table 3. Metagenomes containing *P. nemausensis* sequences\***

Type of metagenome	Samples (n)	Positive samples (n)		
		All abundances (%)	Excluding rare abundance <0.1%	Abundance >1% only
<b><i>Human-associated metagenomes</i></b>				
<i>Homo sapiens</i>	16511	67 (0.41%)	7 (0.04%)	-
human metagenome	19843	50 (0.25%)	35 (0.18%)	-
human skin metagenome	3235	<b>9 (0.28%)</b>	7 (0.22%)	-
human lung metagenome	4410	2 (0.05%)	1 (0.02%)	-
human nasopharyngeal metagenome	2037	1 (0.05%)	-	-
<b><i>Non human host-associated metagenomes</i></b>				
bovine gut metagenome	9908	5 (0.05%)	-	-
bovine metagenome	1101	5 (0.45%)	-	-
mouse gut metagenome	19703	1 (<0.01%)	-	-
mouse skin metagenome	685	<b>24 (3.5%)</b>	7 (1.02%)	-
<b><i>Unspecified host-associated metagenomes</i></b>				
gut metagenome	42163	19 (0.05%)	1 (<0.01%)	-
feces metagenome	7724	17 (0.22%)	2 (0.03%)	-
skin metagenome	1977	1 (0.05%)	-	-
<b><i>Environmental source &amp; other metagenomes</i></b>				
soil metagenome	67790	7 (0.01%)	-	-
marine metagenome	37438	1 (<0.01%)	-	-
freshwater metagenome	14593	2 (0.01%)	-	-
food metagenome	2119	1 (0.05%)	-	-
dust metagenome	1018	5 (0.5%)	-	-
indoor metagenome	719	<b>12 (1.67%)</b>	12 (1.67%)	-
hot springs metagenome	419	1 (0.24%)	-	-
food production metagenome	376	1 (0.27%)	-	-
hospital metagenome	63	1 (1.59%)	-	-

\* according to the search through the Integrated Microbial Next Generation Sequencing (IGMNGS) platform [30].

A sample corresponds to an SRA (sequence read archive)-derived sample; a positive sample corresponds to a sample in which at least one sequence corresponding to *P. nemausensis* (threshold of sequence identity with the 145-bp sequence specific for *P. nemausensis* : 99%) was detected (all abundances). The numbers of samples with relative abundance of *P. nemausensis*-related sequence >0.1% and >1% are given according to the type of metagenome.

-: no sample in this category.

Bold type indicates the type of metagenome with precise origin available in each category that includes the highest percent of hits corresponding to *P. nemausensis*.

**Table 4.** Description of *Peptoniphilus nemausensis* sp. nov.

<b>Genus name</b>	<i>Peptoniphilus</i>
<b>Species name</b>	<i>Peptoniphilus nemausensis</i>
<b>Specific epithet</b>	<i>nemausensis</i>
<b>Species status</b>	sp. nov.
<b>Species etymology</b>	(ne.mau.sen'sis. L. masc. adj. <i>nemausensis</i> pertaining to Nemausis, the Latin name of Nîmes, an ancient Roman city in the south of France where the type strain was isolated)
<b>Description of the new taxon and diagnostic traits</b>	<p>Cells are Gram-stain positive cocci of 0.5-1 µm in diameter. Non-spore-forming. Anaerobic and microaerophilic. Grown on blood agar, colonies are 1 mm in diameter after 5 days of incubation, circular, entire, greyish, opaque, shiny and non-haemolytic. After prolonged incubation, colonies initially flat or raised become umbonate. Gaz is not produced. Nitrate and nitrite are not reduced. Catalase-, urease-, and gelatinase-negative. Nitrate is not reduced and indole is not produced. Aesculin is hydrolyzed. Milk is not fermented. Carbohydrates are generally not fermented with the exception of aesculin. Major metabolic end product from TGY was acetic acid with production of lower amounts of 3-phenylpropionic acid, lactic acid, and succinic acid. Enzymic profiles obtained by using the API Rapid ID 32A show proline arylamidase activity and a weak alanine arylamidase activity. Resistance to colistin (10 µg), and susceptibility to vancomycin (5 µg), kanamycin (1 mg), and metronidazole (5 µg) is noted using special-potency disks. Susceptible to penicillin G, amoxicillin, coamoxiclav., imipenem, rifampin, metronidazole, linezolid, clindamycin, minocycline, tetracycline, and moxifloxacin, and resistant to ofloxacin, levofloxacin and ciprofloxacin</p> <p>The type strain 1804121828<sup>T</sup> (= LMG 31466<sup>T</sup> = CECT 9935<sup>T</sup>) was isolated from a human tissue sample of a patient with surgical site infection.</p>
<b>Country of origin</b>	France
<b>Region of origin</b>	Nîmes - Occitanie
<b>Date of isolation (dd/mm/yyyy)</b>	19/04/2018
<b>Source of isolation</b>	Human tissue sample of a patient with surgical site infection (Enault et al., 2018)
<b>Sampling date (dd/mm/yyyy)</b>	12/04/2018
<b>Latitude (xx°xx'xx"N/S)</b>	43°50'12.116"N
<b>Longitude (xx°xx'xx"E/W)</b>	4°21'36.194"E
<b>Altitude (meters above sea level)</b>	39 m
<b>16S rRNA gene accession nr.</b>	MK945758
<b>Genome accession number [RefSeq; EMBL; ...]</b>	RefSeq: NZ_CAIJCS000000000
<b>Genome status</b>	Draft
<b>Genome size</b>	1,805,580
<b>GC mol%</b>	45.7
<b>Number of strains in study</b>	1
<b>Designation of the Type Strain</b>	<i>Peptoniphilus nemausensis</i> type strain 1804121828 <sup>T</sup>
<b>Strain Collection Numbers</b>	= LMG 31466 <sup>T</sup> = CECT 9935 <sup>T</sup>