



**Peptoniphilus nemausensis sp. nov. a new  
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names**

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***Peptoniphilus nemausensis* sp. nov. a new Gram-positive anaerobic coccus isolated from human clinical samples, an emendated description of the genus *Peptoniphilus* and an evaluation of the taxonomic status of *Peptoniphilus* species with not validly published names.**

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The GenBank/ENA/DDBJ accession numbers for the 16S rRNA, gene sequences of *Peptoniphilus nemausensis* sp. nov. type strain 1804121828<sup>T</sup> (= LMG 31466<sup>T</sup> = CECT 9935<sup>T</sup>) is MK945758. Whole genome sequences of *Peptoniphilus nemausensis* 1804121828<sup>T</sup> and *Peptoniphilus coxii* CCUG 59622<sup>T</sup> (BioProject number PRJEB38837 ; BioSample numbers SAMEA6943338 and SAMEA6943337, respectively), and *Peptoniphilus tyrrelliae* CCUG 59621<sup>T</sup> (BioProject number PRJEB38837 ; BioSample number SAMEA8918417) were deposited in the National Center for Biotechnology Information (NCBI)'s reference sequence database under respective accession numbers CAIJCS0000000000, CAIJCT0000000000 and CAJUZH0000000000. The respective Sequence Read Archive accession numbers are ERS4653225, ERS4653224 and ERS6601290.

Declarations of interest: none.

## Abstract

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

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

## Introduction

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

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

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

## Materials and methods

### *Strains*

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

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

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

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

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

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

#### *Whole genome sequencing and analysis*

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

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

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



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

### *Phenotypic characterization*

Both strains, 1804121828<sup>T</sup> and *P. coxii* CCUG 59622<sup>T</sup>, were studied according to the procedures described in the VPI Anaerobe Laboratory Manual [22]. Susceptibility to special-potency discs was determined as previously described [24]. The ability to grow in microaerophilic conditions was tested using CampyGen<sup>TM</sup> Compact (Oxoid). Enzymatic activity profiles were studied with rapid ID 32 A (bioMérieux). Metabolic end-products were assayed by quantitative Gas Chromatography (GC) as previously described [8]. For further biochemical characterization, the strain was grown anaerobically in trypticase-glucose-yeast extract-hemin (tryptone peptone, 30 g/liter; glucose, 5 g/liter; yeast extract, 20 g/liter; hemin, 5 mg/liter; and cysteine HCl, 0.5 g/liter; pH: 7.6) broth and incubated at 37 °C for 72 h in an 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> (by vol.). Antimicrobial susceptibility testing was performed and interpreted according to the 2020 recommendations of the AntibioGram Committee of the French Society for Microbiology (CA-SFM) for Gram-positive anaerobes ([https://www.sfm-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)).

## **Results and Discussion**

### ***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

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

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

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

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

### *Phenotypic characterization*

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

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

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

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

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

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

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

#### **Data supporting the need for an emendated description of the genus *Peptoniphilus* Ezaki 2001**

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

were noted for major cellular fatty acid and metabolic-end products while some exceptions were noted regarding the use of carbohydrates and the ability to grow in microaerophilic conditions compared with the initial description. Divergences between studies regarding cellular fatty acid composition were also noted, particularly regarding the type species *P. 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 the major metabolic end-products noted for several species [23, 47, 51], and C<sub>14:00</sub> was found to be the major metabolic end-product for *P. stercorisuis* [23]. The major metabolic end-product was acetate for *P. methioninivorax* [51], *P. gorbachii* [52] and *P. nemausensis* [this study]. Mostly described as unable to ferment carbohydrates, we showed herein that a member of the genus *Peptoniphilus* is able to ferment aesculin. Finally, the ability to grow in microaerophilic conditions was noted for *P. lacydonensis* and *P. nemausensis* [6, this study]. The description of the genus *Peptoniphilus* as published by Ezaki in 2001 [20] therefore needs to be emendated to include the following information: The major cellular fatty acids are 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 butyric acid and/or acetic acid. Carbohydrates are generally not utilized. Growth is mostly obligately anaerobic but growth in microaerophilic conditions may be observed.

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

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

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

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



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

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

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

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

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

The description is reported in Table 4.

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

The description of the genus *Peptoniphilus* is as described by Ezaki (2001) with the following 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> and/or C<sub>14:00</sub>. The major metabolic end-products from PYG medium are butyric acid and/or acetic acid. Carbohydrates are generally not utilized. Cocci are mostly obligately anaerobic but growth in microaerophilic conditions may also be observed.

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

Fabien Aujoulat: Investigation - genomic data analysis, data curation, writing - original draft.

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

Alexis Criscuolo: Methodology - genomic data acquisition, data curation, writing - revision & editing.

Michel R. Popoff: Methodology - phenotypic data acquisition, writing - revision & editing.

Laure Diancourt: Methodology - phenotypic and genomic data acquisition.

419 Cécilia Enault: Investigation - strain isolation and genetic data analysis.  
420 Estelle Jumas-Bilak: Writing - revision & editing.  
421 Jean-Philippe Lavigne: Writing - revision & editing.  
422 Hélène Marchandin: Conceptualization, project administration and supervision, writing -  
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## Figure legends

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

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

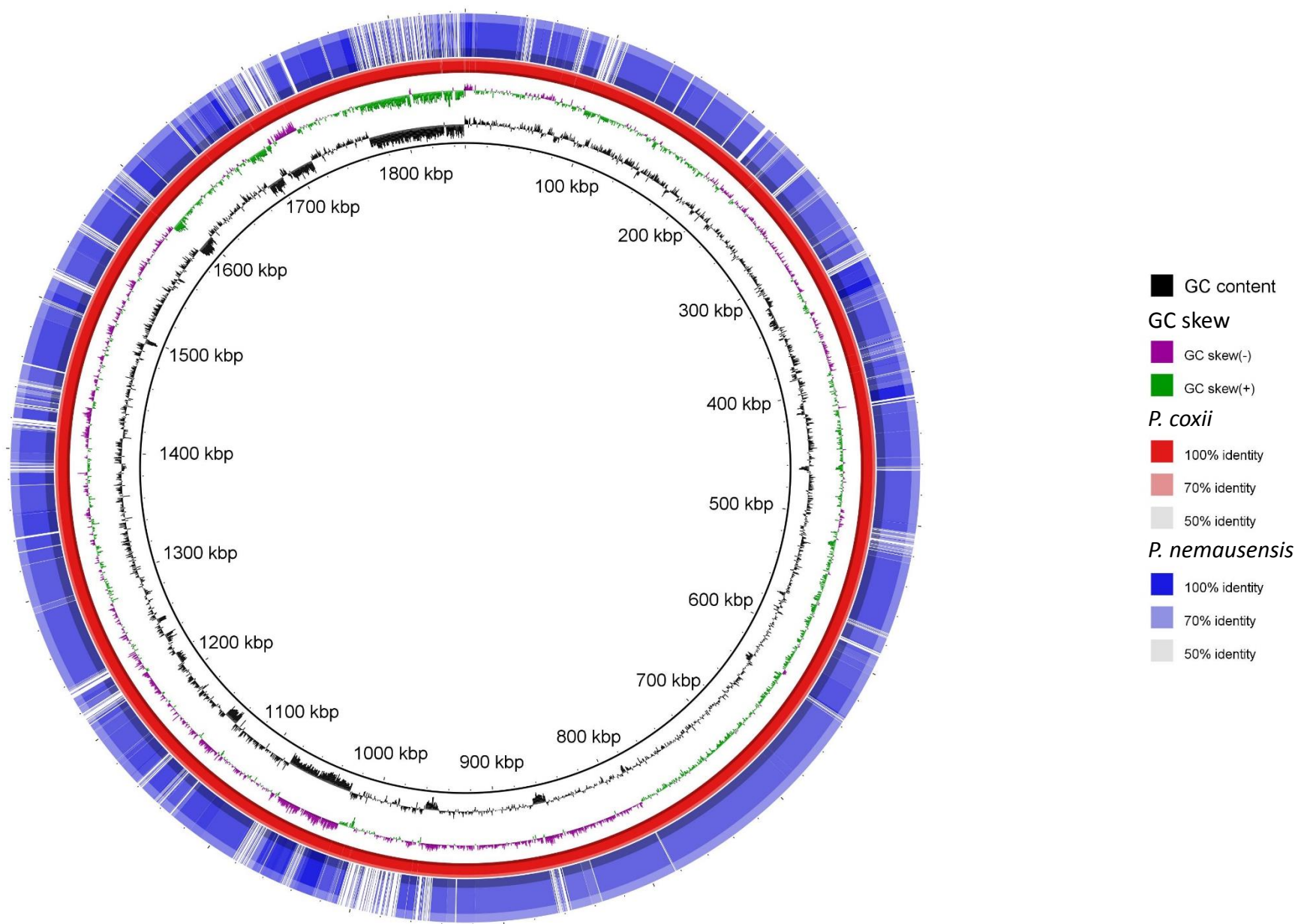
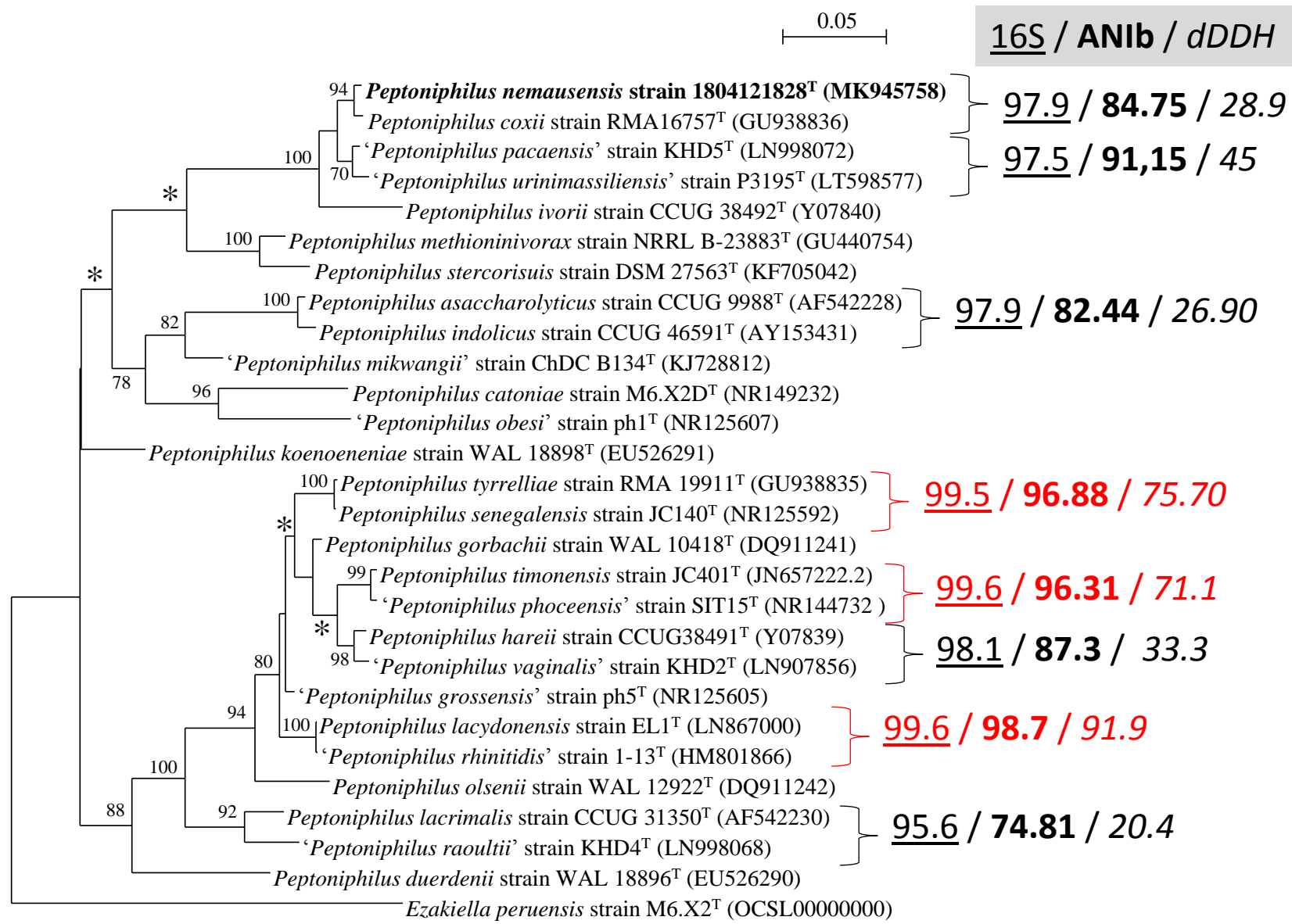


Figure 2





**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. methioninivorax</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.

\*\* Additionnal characteristics differenciating *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. methioninivorax* [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...)	<a href="#">[28]</a>
MK945758 (1390 bp)	6	GQ001519	99.3-99.6	Human skin (manubrium, gluteal crease, buttock, antecubital fossa, popliteal fossa)	<a href="#">[21]</a>
MK945758 (1390 bp)	4	KF082872	99.5-99.6	Human skin (volar forearm, retroauricular crease)	<a href="#">[43]</a>
MK945758 (1390 bp)	1	AM696714	99.1	Indoor dust	<a href="#">[51]</a>
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)	<a href="#">[41]</a>
partial MK945758 (145 bp)	1	AM942981	99.3	House dust	<a href="#">[46]</a>

**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>Human-associated metagenomes</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>Non human host-associated metagenomes</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>Unspecified host-associated metagenomes</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>Environmental source &amp; other metagenomes</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>