

# *Yersinia artesianana* sp. nov., *Yersinia proxima* sp. nov., *Yersinia alsatica* sp. nov., *Yersinia vastinensis* sp. nov., *Yersinia thracica* sp. nov. and *Yersinia occitanica* sp. nov., isolated from humans and animals

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## Abstract

Thirty-three *Yersinia* strains previously characterized by the French *Yersinia* National Reference Laboratory (YNRL) and isolated from humans and animals were suspected to belong to six novel species by a recently described core genome multilocus sequence typing scheme. These strains and five additional strains from the YNRL were characterized using a polyphasic taxonomic approach including a phylogenetic analysis based on 500 core genes, determination of average nucleotide identity (ANI), determination of DNA G+C content and identification of phenotypic features. Phylogenetic analysis confirmed that the 38 studied strains formed six well-demarcated clades. ANI values between these clades and their closest relatives were <94.7% and ANI values within each putative novel species were >97.5%. Distinctive biochemical characteristics were identified in five out of the six novel species. All of these data demonstrated that the 38 strains belong to six novel species of the genus *Yersinia*: *Yersinia artesianana* sp. nov., type strain IP42281<sup>T</sup> (=CIP 111845<sup>T</sup>=DSM 110725<sup>T</sup>); *Yersinia proxima* sp. nov., type strain IP37424<sup>T</sup> (=CIP 111847<sup>T</sup>=DSM 110727<sup>T</sup>); *Yersinia alsatica* sp. nov., type strain IP38850<sup>T</sup> (=CIP 111848<sup>T</sup>=DSM 110726<sup>T</sup>); *Yersinia vastinensis* sp. nov., type strain IP38594<sup>T</sup> (=CIP 111844<sup>T</sup>=DSM 110738<sup>T</sup>); *Yersinia thracica* sp. nov., type strain IP34646<sup>T</sup> (=CIP 111842<sup>T</sup>=DSM 110736<sup>T</sup>); and *Yersinia occitanica* sp. nov., type strain IP35638<sup>T</sup> (=CIP 111843<sup>T</sup>=DSM 110739<sup>T</sup>).

The genus *Yersinia* is a member of the order *Enterobacteriales* and belongs to the family *Yersiniaceae* [1]. The genus *Yersinia* includes three prominent human and animal pathogens: *Yersinia pestis*, the causative agent of plague [2], *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* (biotypes 1B, 2, 3, 4 and 5), responsible for enteric yersiniosis [3], and the putatively pathogenic species *Yersinia wautersii* [4]. Two species are pathogenic only for animals: *Yersinia ruckeri* is the causative agent of enteric redmouth disease in salmonid fish [5] and *Yersinia entomophaga* is known to cause disease

in larvae of the New Zealand grass grub [6]. In addition, 14 non-pathogenic species have been described since the 1980s: *Yersinia aldovae* [7], *Yersinia aleksiciae* [8], *Yersinia bercovieri* [9], *Yersinia frederiksenii* [10], *Yersinia intermedia* [11], *Yersinia kristensenii* [12], *Yersinia massiliensis* [13], *Yersinia mollaretii* [9], *Yersinia nurmii* [14], *Yersinia pekkanenii* [15], *Yersinia rohdei* [16], *Yersinia similis* [17] and the most recently described *Yersinia hibernica* [18] and *Yersinia canariae* [19].

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**Abbreviations:** ANI, average nucleotide identity; cgMLST, core genome multilocus sequence typing; CIN, cefsulodin-irgasan-novobiocine; MMN, mannitol motility nitrate; TSA, trypticase soy agar; YNRL, *Yersinia* National Reference Laboratory.

The GenBank/ENA accession numbers for 16S rRNA gene sequences of strains IP42281<sup>T</sup>, IP34646<sup>T</sup>, IP35638<sup>T</sup>, IP37424<sup>T</sup>, IP38594<sup>T</sup> and IP38850<sup>T</sup> are LR745664, LR745665, LR745666, LR745667, LR745669 and LR745670, respectively. The GenBank/ENA accession numbers for whole genome sequences of the type strains IP42281<sup>T</sup>, IP34646<sup>T</sup>, IP35638<sup>T</sup>, IP37424<sup>T</sup>, IP38594<sup>T</sup> and IP38850<sup>T</sup> are GCA\_902726545, GCA\_902170565.1, GCA\_902170605.1, GCA\_902170785.1, GCA\_902726565 and GCA\_902170305.1, respectively. The Genbank/ENA accession numbers for whole genome sequences of strains IP41384, IP42750 and IP42199, are GCA\_902726525, GCA\_902726535 and GCA\_902726555, respectively.

Two supplementary tables are available with the online version of this article.

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Seven putative novel species were identified from 1348 *Yersinia* genomes during the recent investigation of the genetic diversity and population structure of the genus *Yersinia* [20]. The seven clades were named *Yersinia frederiksenii* 2, *Yersinia frederiksenii* 3, NEW 2, *Yersinia kristensenii* 2, *Yersinia kristensenii* 3, NEW 3 and NEW 4 [20]. NEW 2 corresponds to *Yersinia canariae*, the species recently described by Nguyen *et al.* [19]. The present study aimed to determine the taxonomic status of the six remaining putative novel species, using a polyphasic approach.

In order to increase the number of strains included in each clade, we added three, one and one strains to NEW 3, *Y. kristensenii* 2 and *Y. frederiksenii* 3 clades, respectively. A total of 38 strains belonging to these six putative novel species were studied (Table 1). All strains belong to the collection of the French *Yersinia* National Reference Laboratory (YNRL) for plague and other yersiniosis. They were isolated in France from clinical samples and in Germany, Bulgaria and Italy from animals. They were initially identified as *Y. enterocolitica* biotype 1A (strains of NEW 3 and NEW 4), *Y. frederiksenii* (strains of *Y. frederiksenii* 2 and 3) and *Y. kristensenii* (strains of *Y. kristensenii* 2 and 3) by phenotypic characterization and considered as new taxa after applying the recently described *Yersinia*-core genome multilocus sequence typing (cgMLST) [20] (Table 1).

## GENOMIC FEATURES AND 16S rRNA GENE

Genome sequences of strains were determined as previously described by Savin *et al.* [20]. All genomes were sequenced using a NextSeq 500 instrument (Illumina) and sequencing libraries were prepared using a Nextera XT DNA library preparation kit (Illumina). Paired-end reads of 150 nucleotides were obtained using the Mid Output or High Output kits (Illumina). A *de novo* assembly was performed using SPAdes version 3.12.0 [21]. A minimum sequencing depth of 50× was obtained for each genome. On average, genomes of the 38 studied strains were assembled into 116 contigs (min, 20; max, 419) with a total size of 4.57 Mb (min, 4.12; max, 5.25) and with an N50 value of 149 309 (min, 29 468; max, 490 559; Table S1, available in the online version of this article). The ENA accession numbers of the nucleotide sequences are listed in Table 1. DNA G+C content was determined from the whole genome sequence (Table S1). The presence of plasmid in the genomes was investigated *in silico* using PlasmidSeeker [22].

The phylogenetic analysis included the concatenated amino acid sequences of the 500 core genes selected for the *Yersinia* cgMLST scheme described by Savin *et al.* in 2019 [20]. The concatenated sequences of the five strains sequenced for this study together with the *Y. hibernica* type strain (CFS1934<sup>T</sup>) were compared to those of the 236 strains from the Savin *et al.* study leading to a total of 242 strains belonging to the 20 described species and the six undescribed taxa. A maximum-likelihood phylogenetic reconstruction was performed using RAxML 8.2.8 [23] (Fig. 1). The two strains IP37834 and IP38017 falling into to the NEW 2 clade were named *Y. canariae* as they clustered with the type strain in

the phylogenetic tree generated by Nguyen *et al.* [19]. The phylogenetic analysis confirmed that the six undescribed clades are strongly demarcated from the already known species. The 38 strains studied here fell into those six clades: NEW 3 (IP39904, IP41384, IP42281 and IP42750); NEW 4 (IP37424, IP37838, IP38046, IP38191, IP38663, IP38819, IP38868, IP38950, IP39432 and IP39924); *Y. frederiksenii* 2 (IP35553, IP37124, IP37802, IP38166, IP38403, IP38767, IP38850, IP39458 and IP39797); *Y. frederiksenii* 3 (IP37831, IP38006, IP38178, IP38594 and IP38831); *Y. kristensenii* 2 (IP6945, IP34646, IP35448 and IP42199); and *Y. kristensenii* 3 (IP28581, IP35638, IP37484, IP38487, IP38810 and IP38921).

The average nucleotide identity (ANI) values were calculated using fastANI version 1.1 [24]. ANI values between the six groups and their closest relatives were determined by Savin *et al.* in 2019 and were <94.7% [20]. ANI values obtained within each clade in our study by adding the genome of the five sequenced strains were all >97.5% (Table 2). These observations are concordant with the species status of these six groups according to the proposed delineation cut-off of 95–96% [25].

The 16S rRNA gene sequences of six strains (IP42281, IP37424, IP38850, IP38594, IP34646 and IP35638) were amplified using primers designed by Janvier and Grimont [26]. Amplicons were purified by QIAquick PCR purification kit (Qiagen) and sequenced by Eurofins, resulting in 1477–1491 bp products. Nucleotide sequences of 16S rRNA gene for the seven strains were deposited in GenBank. Accession numbers for IP42281<sup>T</sup>, IP34646<sup>T</sup>, IP35638<sup>T</sup>, IP37424<sup>T</sup>, IP38594<sup>T</sup> and IP38850<sup>T</sup> are LR745664, LR745665, LR745666, LR745667, LR745669 and LR745670, respectively. Sequences were compared to the sequences extracted from the whole genome assembly. Similarity between the product sequences and the extracted sequences was 100% for the six type strains, ensuring the authenticity of the genome data.

## PHENOTYPIC CHARACTERISTICS

Cells were cultured aerobically for 48 h at 28 °C on TSA (trypticase soy agar) and CIN (cefsulodin–irgasan–novobiocin) agar plates. Gram staining of cells was carried out from colonies on TSA and cell morphology was observed using an optical microscope. Motility and nitrate reductase were tested on mannitol motility nitrate (MMN) semisolid medium (Biorad). Catalase activity was determined by observing bubble production in a 3% (v/v) hydrogen peroxide solution. Oxidase activity was evaluated using discs impregnated with tetramethyl-*p*-phenyldiamine (Biorad). Tween esterase activity was detected using agar medium containing Tween 80, a positive reaction indicated by the production of aggregates in the media [27]. Pyrazinamidase activity was evaluated using agar medium with pyrazinamide, a positive reaction indicated by a brownish colour in the presence of ferrous salts [28]. Other biochemical characteristics were obtained using API 20E kits (bioMérieux): β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H<sub>2</sub>S production, urease, tryptophan deaminase,

**Table 1.** Strains belonging to the six novel species

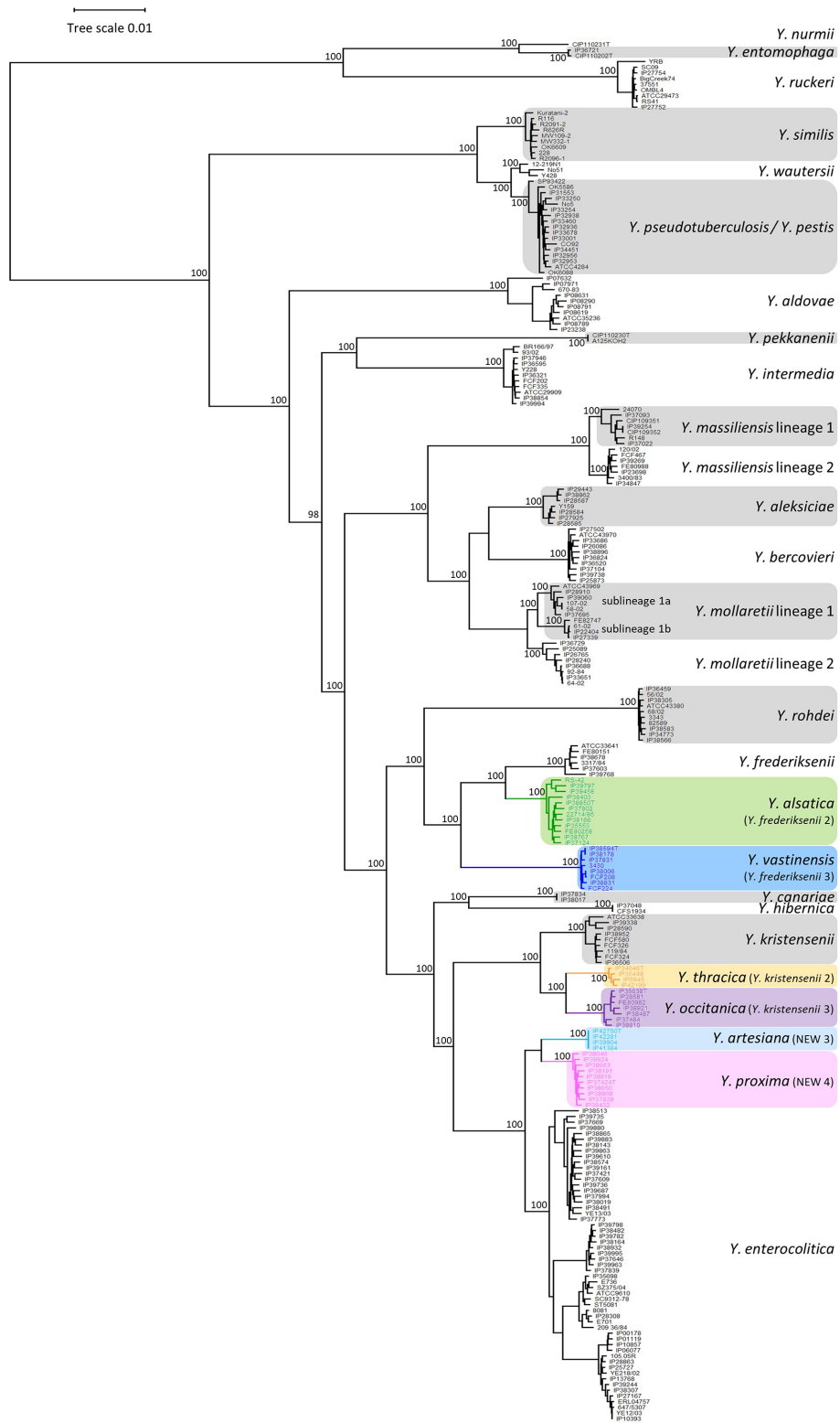
cgMLST clade <sup>a</sup>	Proposed novel species	Strain	Phenotypic characterization		Assembly accession number	Isolation			Year
			Species	Serotype		Source	Material	Country	
NEW 3	<i>Yersinia artesiiana</i>	IP39904	<i>Yersinia enterocolitica</i> biotype 1A	Not typeable	Human	Stool	France	Halennes les Hautbourdin	2018
		IP41384		Not typeable	Human	Stool	France	Le Haillan	2019
		IP42281 <sup>T</sup>		Not typeable	Human	Stool	France	Barlin	2019
		IP42750		Not typeable	Human	Stool	France	Volckerinckhove	2019
		IP37424 <sup>T</sup>		O:10-34	Human	Stool	France	Saint Laurent de la Salanque	2016
		IP37838		O:10-34	Human	Stool	France	Chalon sur Saône	2016
		IP38046		O:10-34	Human	Stool	France	Limoges	2016
		IP38191		O:10	Human	Stool	France	Besançon	2016
		IP38663		O:10-34	Human	Stool	France	Toulouse	2017
		IP38819		O:10	Human	Stool	France	Cholet	2017
<i>Yersinia frederiksenii</i> 2	<i>Yersinia alsatica</i>	IP38868	O:34	Human	Stool	France	Levallois Perret	2017	
		IP38950	O:10-34	Human	Stool	France	Levallois Perret	2017	
		IP39432	O:6,31	Human	Stool	France	Angoulême	2017	
		IP39924	O:10-34	Human	Stool	France	Metz	2016	
		IP35553	O:52-52,53	Human	Stool	France	Le Puy en Velay	2013	
		IP37124	O:16-16,29	Human	Stool	France	Levallois Perret	2015	
		IP37802	O:16-16,29	Human	Stool	France	Strasbourg	2016	
		IP38166	Not typeable	Human	Stool	France	Levallois Perret	2016	
		IP38403	Not typeable	Human	Stool	France	Strasbourg	2016	
		IP38767	Not typeable	Human	Stool	France	Nancy	2017	
NEW 4	<i>Yersinia proxima</i>	IP38850 <sup>T</sup>	O:40	Human	Stool	France	Strasbourg	2017	
		IP39458	Not typeable	Human	Stool	France	Brumath	2017	
		IP39797	Not typeable	Human	Stool	France	Strasbourg	2017	

Continued

Table 1. Continued

cgMLST clade <sup>a</sup>	Proposed novel species	Strain	Phenotypic characterization		Assembly accession number		Isolation			
			Species	Serotype	Species	Serotype	Source	Material	Country	Area
<i>Yersinia frederiksenii</i> 3	<i>Yersinia vastinensis</i>	IP37831	<i>Yersinia frederiksenii</i>	O:16-16,29	GCA_902170405.1	Human	Stool	France	Romilly sur Seine	2016
		IP38006		O:16-16,29	GCA_902170245.1	Human	Stool	France	Rodez	2016
		IP38178		O:16-16,29	GCA_902170255.1	Human	Stool	France	Brumath	2016
		IP38594 <sup>T</sup>		O:16-16,29	GCA_902726565 <sup>b</sup>	Human	Stool	France	Nemours	2017
		IP38831		O:16-16,29	GCA_902170295.1	Human	Stool	France	Rethel	2017
<i>Yersinia kristensenii</i> 2	<i>Yersinia thracica</i>	IP6945	<i>Yersinia kristensenii</i>	O:16	GCA_001123825.1	Pig	Stool	Germany	Unknown	1977
		IP34646 <sup>T</sup>		O:16-16,29	GCA_902170565.1	Fish	Unknown	Bulgaria	Sofia	2012
		IP35448		O:16-16,29	GCA_902170455.1	Bird	Unknown	Bulgaria	Sofia	2013
		IP42199		Not typeable	GCA_902726555 <sup>b</sup>	Wild boar	Stool	Italy	Parma	2019
<i>Yersinia kristensenii</i> 3	<i>Yersinia occitamica</i>	IP28581	<i>Yersinia kristensenii</i>	O:12,25-12,26	GCA_902170535.1	Unknown	Unknown	Unknown	Unknown	2005
		IP35638 <sup>T</sup>		O:12,25-12,26	GCA_902170605.1	Human	Stool	France	Rodez	2014
		IP37484		O:12,25-12,26	GCA_902170505.1	Human	Stool	France	Draguignan	2014
		IP38487		O:12,25-12,26	GCA_902170595.1	Human	Stool	France	Saint Mandé	2016
		IP38810		O:12,25-12,26	GCA_902170465.1	Human	Stool	France	Romilly sur Seine	2017
	IP38921		O:12,25-12,26	GCA_902170475.1	Human	Stool	France	Saumur	2017	

a, According to Savin et al. [20]; b, this study.



**Fig. 1.** Maximum-likelihood phylogenetic tree of the genus *Yersinia* (242 strains) based on 500 concatenated multiple sequence alignments. Bootstrap support values are shown close to the branches. Bar, 0.01 amino acid substitutions per character.

Table 2. Average nucleotide identity (ANI) matrix for the 38 Yersinia genomes: nine Y. frederiksenii 2 strains; five Y. frederiksenii 3 strains; four Y. kristensenii 2 strains; six Y. kristensenii 3 strains; four NEW 3 strains, 10 NEW 4 strains

Table with columns for Species, Strain, and ANI percentages for various Yersinia species and clades (NEW 3, NEW 4). Rows include Y. vastnisensis, Y. alaskica, Y. thracica, Y. occidentalis, Y. artesiana, and Y. pseudotuberculosis.

\*Clade name according to Savin et al. [20].

indole production, acetoin production (Voges-Proskauer) and gelatinase, and using API 50CH strips (bioMérieux) for testing sugar fermentation. Tests were performed at 28°C. Strains were serotyped with a set of 47 O:antigen-specific rabbit antisera. The characteristics of the six novel species are listed below in the species description.

Distinctive phenotypic features between the six putative novel species and the other described Yersinia species and subspecies, except Y. pestis, were obtained by comparison to type strains and other strains characterized at the YNRL by the phenotypic method. Strains are listed in Table S2. The results are shown in Table 3. Strains from clades NEW 3,

Table 3. Distinctive biochemical characteristics between the six novel species and the other Yersinia species and subspecies (except Y. pestis)

BT, biotype; +, 90% or more strains positive; -, 90% or more strains negative; d, 11–89% of strains positive.

Table showing biochemical characteristics for various Yersinia species and clades (NEW 3, NEW 4). Columns include species names and specific tests like API 20E results, API 50CH results, Lipase activity, and Motility at 28°C.



NEW 4, *Y. kristensenii* 2 and 3 can be distinguished from their closest relatives by the following biochemical tests: indole and acetoin production, L-fucose, D-arabitol and potassium 2- and 5-ketogluconate fermentation and lipase activity. In contrast, strains belonging to the clades *Y. frederiksenii* 2 and 3 cannot be distinguished from their closest relative *Y. frederiksenii* by the biochemical tests.

Three strains from each clade were also characterized using MALDI-ToF MS. The protein patterns of all the strains tested matched with patterns of already known *Yersinia* species with high score values above 2.3 (database version 8.0.0.0-7311-7854; RUO), threshold of reliable species identification. Therefore, these strains cannot be distinguished from their genetically closest relatives using this technique.

On the basis of the genetic and phenotypic results presented here, we concluded that the 38 isolates should be assigned to the following six novel species: NEW 3 clade as *Yersinia artesiiana* sp. nov. (type strain IP42281<sup>T</sup>), NEW 4 clade as *Yersinia proxima* sp. nov. (type strain IP37424<sup>T</sup>), *Y. frederiksenii* 2 clade as *Yersinia alsatica* sp. nov. (type strain IP38850<sup>T</sup>), *Y. frederiksenii* 3 clade as *Yersinia vastinensis* sp. nov. (type strain IP38594<sup>T</sup>), *Y. kristensenii* 2 as *Yersinia thracica* sp. nov. (type strain IP34646<sup>T</sup>) and *Y. kristensenii* 3 as *Yersinia occitanica* sp. nov. (type strain IP35638<sup>T</sup>).

#### DESCRIPTION OF *YERSINIA ARTESIANA* SP. NOV.

*Yersinia artesiiana* (ar.te.si.a'na. M.L. fem. adj. *artesiiana*, pertaining to Artois county in France where the type strain IP42281<sup>T</sup> was isolated).

Cells are short Gram-stain-negative rods. Colonies on CIN agar are small, circular, 3.5 mm diameter and have a deep-red centre surrounded by a transparent pale border. Colonies on TSA are beige, smooth and convex. The four strains are not motile on MMN semisolid medium and reduce nitrate. Oxidase is negative. Catalase is positive. Tween esterase is negative and pyrazinamidase is positive. In API20E tests, all strains are positive for β-galactosidase, urease, indole and acetoin production (Voges–Proskauer); and negative for arginine dihydrolase, lysine decarboxylase, citrate utilization, H<sub>2</sub>S production, tryptophan deaminase and gelatinase for all strains. Three out of the four strains, including the type strain, are negative for ornithine decarboxylase. In API 50CH tests, acid production from glycerol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, L-sorbose, inositol, mannitol, sorbitol, N-acetylglucosamine, arbutin, aesculin, salicin, cellobiose, maltose, lactose, sucrose, trehalose, gentiobiose, D-arabitol, gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate is positive; no acid production from erythritol, D-arabinose, L-xylose, adonitol, methyl β-D-xylopyranoside, rhamnose, dulcitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, melibiose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose or L-arabitol. All strains are not typeable for the O:antigen.

The type strain, IP42281<sup>T</sup> (=CIP 111845<sup>T</sup>=DSM 110725<sup>T</sup>), as well as strains IP39904, IP41384 and IP42750, were isolated from human stool. The complete genome of IP42281<sup>T</sup> has been deposited into ENA (accession number GCA\_902726545). The DNA G+C content of the type strain is 47.8mol%.

#### DESCRIPTION OF *YERSINIA PROXIMA* SP. NOV.

*Yersinia proxima* (pro'xi.ma. L. fem. adj. *proxima* closest, referring to its genetic closeness to *Y. enterocolitica*).

Cells are short Gram-stain-negative rods. Colonies on CIN agar are small, circular, 3.5 mm diameter and have a deep-red centre surrounded by a transparent pale border. Colonies on TSA are beige, smooth and convex. The ten strains are motile on MMN semisolid medium and reduce nitrate. Oxidase is negative. Catalase is positive. Tween esterase and pyrazinamidase are positive. In API20E tests, all strains are positive for β-galactosidase, urease, indole and acetoin production (Voges–Proskauer); negative for arginine dihydrolase, lysine decarboxylase, H<sub>2</sub>S production, tryptophan deaminase and gelatinase. Seven out of the ten strains, including the type strain, are negative for ornithine decarboxylase and five strains, including the type strain, are positive for citrate. In API 50CH tests, acid production from glycerol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, L-sorbose, inositol, mannitol, sorbitol, N-acetylglucosamine, arbutin, aesculin, salicin, cellobiose, maltose, lactose, sucrose, trehalose, gentiobiose, D-arabitol, gluconate and potassium 5-ketogluconate is positive; no acid production from erythritol, D-arabinose, L-xylose, adonitol, methyl β-D-xylopyranoside, rhamnose, dulcitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, melibiose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol and potassium 2-ketogluconate. Nine out of the ten strains, including the type strain, belong to the O:10–34 serotype.

The type strain, IP37424<sup>T</sup> (=CIP 111847<sup>T</sup>=DSM 110727<sup>T</sup>), and strains IP37838, IP38046, IP38191, IP38663, IP38819, IP38868, IP38950, IP39432 and IP39924 were isolated from human stool. The complete genome of IP37424<sup>T</sup> has been deposited into ENA (accession number GCA\_902170785). The DNA G+C content of the type strain is 47mol%.

#### DESCRIPTION OF *YERSINIA ALSATICA* SP. NOV.

*Yersinia alsatica* (al.sa'ti.ca. M.L. fem. adj. *alsatica* from Alsace, the region in the eastern part of France where the type strain IP38850<sup>T</sup> was isolated).

Cells are short Gram-stain-negative rods. Colonies on CIN agar are small, circular, 5 mm diameter and have a deep-red centre surrounded by a transparent pale border. Colonies on TSA are beige, smooth and convex. The nine strains are motile on MMN semisolid medium. Only eight strains, including the type strain, reduce nitrate. Oxidase is negative. Catalase

is positive. Tween esterase and pyrazinamidase are positive. In API20E tests, all strains are positive for  $\beta$ -galactosidase, urease, indole and acetoin production (Voges–Proskauer); negative for arginine dihydrolase, lysine decarboxylase,  $H_2S$  production, tryptophan deaminase and gelatinase. Five out of the nine strains, including the type strain, are positive for ornithine decarboxylase; and four strains, including the type strain, are positive for citrate. In API 50CH tests, all strains are positive for acid production from glycerol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, L-sorbose, rhamnose, inositol, mannitol, sorbitol, N-acetylglucosamine, arbutin, aesculin, salicin, cellobiose, maltose, sucrose, trehalose, gentiobiose, L-fucose, D-arabitol, gluconate and potassium 5-ketogluconate; and negative for acid production from erythritol, D-arabinose, L-xylose, adonitol, methyl  $\beta$ -D-xylopyranoside, dulcitol, methyl  $\alpha$ -D-mannoside, methyl  $\alpha$ -D-glucoside, amygdalin, lactose, melibiose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-arabitol and potassium 2-ketogluconate. The type strain belongs to the O:40 serotype.

The type strain, IP38850<sup>T</sup> (=CIP 111848<sup>T</sup>=DSM 110726<sup>T</sup>), as well as strains IP35553, IP37124, IP37802, IP38166, IP38403, IP38767, IP39458, and IP39797, were isolated from human stool. The complete genome of IP38850<sup>T</sup> has been deposited into ENA (accession number GCA\_902170305). The DNA G+C content of the type strain is 47.6mol%.

## DESCRIPTION OF *YERSINIA VASTINENSIS* SP. NOV.

*Yersinia vastinensis* (vas.ti.nen'sis. M.L. fem. adj. *vastinensis* pertaining to Gatinais county in France where the type strain IP38594<sup>T</sup> was isolated).

Cells are short Gram-stain-negative rods. Colonies on CIN agar are small, circular, 2.5 mm diameter and have a deep-red centre surrounded by a transparent pale border. Colonies on TSA are beige, smooth and convex. Two out of the five strains, including the type strain, are not motile on MMN semisolid medium and all strains reduce nitrate. Oxidase is negative. Catalase is positive. Tween esterase is negative in four strains, including the type strain and pyrazinamidase is positive in all strains. In API20E tests, all strains are positive for  $\beta$ -galactosidase, urease, indole and acetoin production (Voges–Proskauer) and negative for arginine dihydrolase, lysine decarboxylase, citrate,  $H_2S$  production, tryptophan deaminase and gelatinase. Four out of the five strains, including the type strain, are positive for ornithine decarboxylase. In API 50CH tests, acid production from glycerol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, L-sorbose, rhamnose, inositol, mannitol, sorbitol, N-acetylglucosamine, arbutin, aesculin, salicin, cellobiose, maltose, lactose, sucrose, trehalose, gentiobiose, L-fucose, D-arabitol, gluconate and potassium 5-ketogluconate is positive; no acid production from erythritol, D-arabinose, L-xylose, adonitol, methyl  $\beta$ -D-xylopyranoside, dulcitol,

methyl  $\alpha$ -D-mannoside, methyl  $\alpha$ -D-glucoside, amygdalin, melibiose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-arabitol and potassium 2-ketogluconate. All strains belong to the O:16–16,29 serotype.

The type strain, IP38594<sup>T</sup> (=CIP 111844<sup>T</sup>=DSM 110738<sup>T</sup>), as well as strains IP37831, IP38006, IP38178 and IP38831, were isolated from human stool. The complete genome of IP38594<sup>T</sup> has been deposited into ENA (accession number GCA\_902726565). The DNA G+C content of the type strain is 46.9mol%.

## DESCRIPTION OF *YERSINIA THRACICA* SP. NOV.

*Yersinia thracica* (thra.ci.ca. L. fem. adj. *thracica* referring to Thrace, the ancient province including Bulgaria where the type strain IP34646<sup>T</sup> was isolated).

Cells are short Gram-stain-negative rods. Colonies on CIN agar are small, circular, 2.5 mm diameter and have a deep-red centre surrounded by a transparent pale border. Colonies on TSA are beige, smooth and convex. Two out of the four strains, including the type strain, are not motile on MMN semisolid medium. All strains reduce nitrate. Oxidase is negative. Catalase and pyrazinamidase are positive. Tween esterase is negative in three strains including the type strain. In API20E tests, all strains are positive for urease; and negative for arginine dihydrolase, lysine decarboxylase, citrate,  $H_2S$  production, tryptophan deaminase, indole and acetoin production (Voges–Proskauer), and gelatinase. Three out of the four strains, including the type strain, are positive for  $\beta$ -galactosidase and ornithine decarboxylase. In API 50CH tests, acid production from glycerol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, L-sorbose, mannitol, sorbitol, N-acetylglucosamine, cellobiose, maltose, trehalose and gentiobiose is positive; no acid production from erythritol, D-arabinose, L-xylose, adonitol, methyl  $\beta$ -D-xylopyranoside, rhamnose, dulcitol, methyl  $\alpha$ -D-mannoside, methyl  $\alpha$ -D-glucoside, amygdalin, aesculin, salicin, melibiose, sucrose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium 2-ketogluconate and potassium 5-ketogluconate. Only the type strain is negative for inositol. Three out of the four strains, including the type strain, are positive for arbutin and negative for lactose and gluconate. Three out of the four strains, including the type strain, belong to the O:16 serotype and one strain is not typeable.

Strains of this novel species were isolated only in animals. The type strain, IP34646<sup>T</sup> (=CIP 111842<sup>T</sup>=DSM 110736<sup>T</sup>) was isolated from diseased rainbow trouts (*Onchorhynchus mykiss*) in Bulgaria. Strains IP6945, IP35448 and IP42199 were isolated from pig stool, bird and wild boar, respectively. The complete genome of IP34646<sup>T</sup> has been deposited into ENA (accession number GCA\_902170565). The DNA G+C content of the type strain is 47.4mol%.



## DESCRIPTION OF *YERSINIA OCCITANICA* SP. NOV.

*Yersinia occitanica* (oc.ci.ta'ni.ca. M.L. fem. adj. *occitanica*, referring to Occitanie province in France where the type strain IP35638<sup>T</sup> was isolated).

Cells are short Gram-stain-negative rods. Colonies on CIN agar are small, circular, 5 mm diameter and have a deep-red centre surrounded by a transparent pale border. Colonies on TSA are beige, smooth and convex. Four out of the six strains, including the type strain, are motile on MMN semisolid medium and all strains reduce nitrate. Oxidase is negative. Catalase is positive. Tween esterase is negative and pyrazinamidase is positive. In API20E tests, all strains are positive for  $\beta$ -galactosidase, ornithine decarboxylase, urease; negative for arginine dihydrolase, lysine decarboxylase, H<sub>2</sub>S production, tryptophan deaminase, acetoin production (Voges–Proskauer) and gelatinase. Four out of the six strains, including the type strain, are negative for citrate and five strains, including the type strain, produce indole. In API 50CH tests, acid production from glycerol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, mannitol, sorbitol, N-acetylglucosamine, cellobiose, maltose, trehalose, gentiobiose, gluconate and potassium 5-ketogluconate is positive; no acid production from erythritol, D-arabinose, L-xylose, adonitol, methyl  $\beta$ -D-xylopyranoside, rhamnose, dulcitol, methyl  $\alpha$ -D-mannoside, methyl  $\alpha$ -D-glucoside, amygdalin, aesculin, salicin, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol and L-arabitol. Five out of the six strains, including the type strain, are L-sorbose-positive; 50% of the strains, including the type strain are negative for inositol, arbutin and lactose fermentation and four strains, including the type strain are negative for potassium 2-ketogluconate. All strains belong to the O:12,25–12,26 serotype.

The type strain, IP35638<sup>T</sup> (=CIP 111843<sup>T</sup>=DSM 110739<sup>T</sup>), as well as strains IP37484, IP38487, IP38810 and IP38921, were isolated from human stool. The complete genome of IP35638<sup>T</sup> has been deposited into ENA (accession number GCA\_902170605). The DNA G+C content of the type strain is 47.8mol%.

IP38810 hosts a plasmid similar to the pKpN01-COL plasmid, with 99.97% identity and 100% coverage.

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### Conflicts of interest

The authors declare that there are no conflicts of interest.

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