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Clarifying the taxonomy of the causal agent of bacterial leaf spot of lettuce through a polyphasic approach reveals that *Xanthomonas cynarae* Trébaol et al. 2000 emend. Timilsina et al. 2019 is a later heterotypic synonym of *Xanthomonas hortorum* Vauterin et al. 1995

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1 **Clarifying the taxonomy of the causal agent of bacterial leaf spot**
2 **of lettuce through a polyphasic approach leads to combine**
3 ***Xanthomonas hortorum* Vauterin *et al.* 1995 and *Xanthomonas***
4 ***cynarae* Trébaol 2000 emend. Timilsina *et al.* 2019**

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15 **Keywords:** *Xanthomonas hortorum*; *Xanthomonas cynarae*; pathovar *vitians*; bacterial leaf
16 spot of lettuce; overall genome relatedness indices; phylogenomic

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30 **ABSTRACT**

31 Assessment of the taxonomy and diversity of *Xanthomonas* strains causing bacterial
32 leaf spot of lettuce (BLSL), commonly referred to as *Xanthomonas campestris* pv. *vitians*, has
33 been a long-lasting issue which held back the global efforts made to understand this pathogen.
34 In order to provide a sound basis essential to its study, we conducted a polyphasic approach
35 on strains obtained through sampling campaigns or acquired from collections. Results of a
36 multilocus sequence analysis crossed with phenotypic assays revealed that the pathotype
37 strain does not match the description of the nomenclature provided by Brown in 1918.
38 However, strain LMG 938 = CFBP 8686 does fit this description. Therefore, we propose that
39 it replaces LMG 937 = CFBP 2538 as pathotype strain of *X. campestris* pv. *vitians*.

40 Then, whole-genome based phylogenies and overall genome relatedness indices
41 calculated on taxonomically relevant strains exhibited the intermediate position of *X.*
42 *campestris* pv. *vitians* between closely related species *Xanthomonas hortorum* and
43 *Xanthomonas cynarae*. Phenotypic profiles characterized using Biolog microplates did not
44 reveal stable diagnostic traits legitimizing their distinction. Therefore, we propose that *X.*
45 *cynarae* Trébaol *et al.* 2000 emend. Timilsina *et al.* 2019 is a later heterotypic synonym of *X.*
46 *hortorum*, to reclassify *X. campestris* pv. *vitians* as *X. hortorum* pv. *vitians* comb. nov. and to
47 transfer *X. cynarae* pathovars in *X. hortorum* as *X. hortorum* pv. *cynarae* comb. nov. and *X.*
48 *hortorum* pv. *gardneri* comb. nov. An emended description of *X. hortorum* is provided,
49 making this extended species a promising model for the study of *Xanthomonas* quick
50 adaptation to different hosts.

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57 INTRODUCTION

58 Bacterial leaf spot of lettuce (BLSL) is a foliar disease occurring on all types of the
59 cultivated lettuce *Lactuca sativa* L. Its presence has been reported around the world where
60 lettuce is grown [1,39,65,74,81] and has become a preoccupying threat for lettuce producers
61 over the last decades in the absence of efficient means to control the disease [10,20,25].
62 Typical symptoms of BLSL starts with small water-soaked lesions on the outer leaves which
63 later become necrotic and surrounded by a chlorotic halo, before coalescing into large
64 necrotic patches under humid and warm conditions [11]. Although not directly lethal for the
65 plant, this damage will reduce the quality and yield, sometimes dramatically, and have been
66 suggested to increase the susceptibility to more severe fungal pathogens [65]. All major
67 commercial lettuce types can be infected, even though relative resistances in some types or
68 cultivars have been observed [9,25]. It has also been reported that on lettuce seed crops,
69 symptoms can develop on stems and flower bracts as brown to black longitudinal lesions
70 [32,53]. The pathogen is thought to be seedborne [32,69,81] and able to move systematically
71 within the stems of lettuce plants [4]. It can also remain viable for months in buried plant
72 debris or surface irrigation water, and was shown to maintain high epiphytic populations on a
73 wide variety on weeds [5,18,66].

74 The bacterium responsible was first isolated in 1916 in South Carolina and named
75 *Bacterium vitians* Brown 1918 [7] before being reclassified as *Xanthomonas vitians* Dowson
76 1943 at the creation of the genus [14]. Following the revision of the *International Code of*
77 *Nomenclature of Bacteria* (1980), which created stricter rules for naming bacterial species, it
78 was integrated into the polytypic species *X. campestris* [77]. A new infraspecies designation,
79 the “pathovar”, used to describe a group of organisms within a species with a particular host
80 range or causing distinct disease symptoms, was introduced upon this occasion. The species
81 epithet *vitians* was used as the new pathovar epithet to maintain continuity between literature

82 published before and after this change. Finally, DNA-DNA hybridization experiments showed
83 that strains of *X. campestris* pv. *vitians* (Brown 1918) Dye 1978 clustered into two different
84 species [72]. The pathotype strain CFBP 2538^{PT} (= NCPPB 976^{PT} = LMG 937^{PT}) was
85 grouped with *X. axonopodis* Vauterin *et al.* 1995 as *X. axonopodis* pv. *vitians*. It is important
86 to note that CFBP 2538^{PT} is not pathogenic on lettuce but weakly pathogenic on tomato and
87 pepper [26,53,54]. Another commonly used representative strain of BLSL, LMG 938
88 (= CFBP 8686 = NCPPB 2248), was included into the newly formed species *X. hortorum*
89 Vauterin *et al.* 1995 with four other pathovars (*hederae*, *pelargonii*, *taraxaci*, *carotae*).
90 Additional studies on the genetic diversity of strains associated with BLSL demonstrated that
91 strains that cause BLSL were all similar to LMG 938 and genetically distant from
92 CFBP 2538^{PT} [3,53]. However, the name “*X. hortorum* pv. *vitians* Vauterin *et al.* 1995” was
93 not valid according to the International Society of Plant Pathology Committee on the
94 Taxonomy of Plant Pathogenic Bacteria (ISPP-CTPPB, the governing body of pathovar
95 nomenclature) because there was no description given for the new taxon based on a proposed
96 pathotype strain [79]. As a result, the pathogen is still referred to as *X. campestris* pv. *vitians*
97 type A referring to the pathotype strain CFBP 2538^{PT} only, or *X. campestris* pv. *vitians* type B
98 for all the BLSL-causing strains including LMG 938 [72]. The lack of a valid pathovar name
99 for this taxon within *X. hortorum* has generated confusion and is a source of mistakes and
100 misunderstandings [41,43,57,76]. This is compounded by the fact that
101 *X. campestris* pv. *vitians* CFBP 2538^{PT} does not fit the description of the pathovar provided
102 by Brown 1918 and therefore is unsuitable to serve as the pathotype. According to standard
103 11 of the *International Standards for Naming Pathovars of Phytopathogenic Bacteria* [15], ‘If
104 a pathotype or neopathotype strain has become unsuitable due to changes in its characters or
105 for other reasons, then the matter should be referred to the Taxonomy Committee, which may
106 decide to take action leading to replacement of the strain.’ Thus, we sent a letter to the ISPP-

107 CTPPB on October the 29th 2019 to request replacement of this pathotype strain and to
108 propose strain LMG 938^{neoPT} = CFBP 8686^{neoPT} = NCPPB 2248^{neoPT} as a neopathotype strain
109 of *X. campestris* pv. *vitians*. In this manuscript we make two proposals that will resolve the
110 nomenclatural issues regarding the BLSL pathogens in *X. hortorum* and that will maintain the
111 priority (Brown 1918) for the pathogen causing BLSL. First, we propose the replacement of
112 the pathotype strain of *X. campestris* pv. *vitians*, then the transfer of this pathovar in *X.*
113 *hortorum* as *X. hortorum* pv. *vitians* comb. nov.

114 Another layer of complexity to the taxonomy of *X. hortorum* pathovars is that on one
115 hand they are genetically heterogeneous and that on the other hand some of them are highly
116 genetically related to *X. cynarae* pv. *cynarae* (pathogenic on artichoke) and *X. cynarae*
117 pv. *gardneri* (pathogenic on tomato and pepper) [29,68,78]. These pathovar names result from
118 the recent proposal of synonymy between *X. cynarae* and *X. gardneri* [64]. Thus, *X. hortorum*
119 is a paraphyletic species and a comprehensive taxonomic study of *X. hortorum* pathovars
120 including their nearest phylogenetic neighbors is needed to resolve their classification.

121 The present study aims at resolving formally the taxonomy of the causal agent of
122 BLSL and its close relatives within the comprehensive framework of the global structure of
123 the genus *Xanthomonas*. In order to investigate the genetic diversity of strains associated with
124 BLSL, we conducted sampling campaigns in the Rhône-Alpes region, France, and completed
125 our set with historical collection strains from various origins. Then, an extensive polyphasic
126 approach was conducted based on pathogenicity assays, multilocus sequence analysis (MLSA)
127 on three housekeeping genes, *de novo* whole-genome sequencing, phylogenomic tree
128 reconstruction, overall genome relatedness indices (OGRIs) calculations and standardized
129 biochemical phenotypic profiling. The results obtained support our proposals to replace the
130 pathotype strain of *X. campestris* pv. *vitians*, to transfer this pathovar in *X. hortorum* as *X.*
131 *hortorum* pv. *vitians* comb. nov. and to propose the synonymy between *X. hortorum* and

132 *X. cynarae*, reclassifying former pathovars of *X. cynarae* as *X. hortorum* pv. *cynarae* comb.
133 nov. and *X. hortorum* pv. *gardneri* comb. nov.

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135 **EXPERIMENTAL PROCEDURES**

136 **Bacterial strains, isolation procedure and growth conditions**

137 All the strains used in this study and their related information are listed in Table 1. The
138 collection consists of 55 strains of *X. campestris* pv. *vitians* including the pathotype strain, the
139 type, pathotype or representative strains of the four pathovars of *X. hortorum* (pvs. *pelargonii*,
140 *hederae*, *carotae* and *taraxaci*), four strains of *X. cynarae* including the type strain and the
141 pathotype strain of *X. cynarae* pv. *gardneri*. Strains were either obtained from the
142 CIRM-CFBP (International Center for Microbial Resources-French Collection of Plant
143 Associated Bacteria, Angers, France), the BCCM/LMG (Belgian Co-ordinated Collections of
144 Micro-organisms / Laboratory of Microbiology, Ghent, Belgium) or collected during two
145 sampling campaigns in summers 2016 and 2017 in the Rhône-Alpes region, France. Diseased
146 lettuce and weed samples were harvested and grinded in sterile deionized water, then plated
147 on semi-selective MMG medium [67] supplemented with cycloheximide at 50 µg/mL. After 4
148 days of incubation at 28°C, typical colonies of *Xanthomonas* were isolated and identified by
149 MLSA as described below. For long-term storage, strains were mixed in 1/10th tryptic soy
150 broth (TSB) containing 30 % glycerol and stored at -80°C. Bacterial cultures were made in
151 1/10th tryptic soy broth or on 1/10th tryptic soy agar (TSA) plates and cultivated 24 to 48 h at
152 28°C.

153 **Pathogenicity tests**

154 Pathogenicity of all bacterial strains was tested on leaf lettuce cv. Météore and oakleaf
155 lettuce cv. Kirinia. Plants were grown in a greenhouse in 8-cm pots containing TS3 mold
156 (Klasmann-Deilmann, Geeste, Germany) during 3 to 4 weeks. Overnight bacterial cultures

157 (0.8 to 1.0 OD_{600nm}) were spectrophotometrically adjusted to 0.2 OD_{600nm} in sterile deionized
158 water, corresponding to approximately 10⁸ CFU/mL with Tween 80 added at 0.08 %. Fifty
159 mL of the resulting suspensions were inoculated with a hand sprayer until run-off on 8 plants
160 per strain per cultivar. Eight plants sprayed with sterile deionized water supplemented with
161 Tween 80 at 0.08 % served as a negative control. Plants were incubated in a Fitoclima 10.000
162 EH environmental chamber (Aralab, Rio de Mouro, Portugal) at 25°C with at least 90%
163 relative humidity and an 18 h photoperiod. After 48 h, relative humidity was adjusted to 70 %
164 until the end of the experiment. Disease severity was measured every 2 – 3 days on each plant
165 for three weeks using the 5-point scale disease index described by Bull and Koike [11].
166 Following the same procedure, a subset of strains was assayed for pathogenicity on tomato cv.
167 Marmande, including the three strains of *X. cynarae* pv. *gardneri*, reference strain of
168 *X. hortorum* pv. *carotae*, pathotype strain of *X. hortorum* pv. *taraxaci*, and seven strains of
169 *X. campestris* pv. *vitians*.

170 **Multilocus sequence analysis**

171 Multilocus sequence analysis using three housekeeping genes (*gyrB*, *rpoD* and *gapA*) was
172 performed on all the strains studied. Loci *gyrB* (DNA gyrase β subunit) and *rpoD* (RNA
173 polymerase σ70 factor) were chosen among the 7 genes used in a sequence-based study of
174 *X. arboricola* [56], and *gapA* (glyceraldehyde-3-phosphate dehydrogenase A) was added
175 because of its efficiency to discriminate *X. campestris* pv. *vitians* strains as demonstrated in a
176 previous study [19]. Primer sequences, annealing temperatures and resulting fragment lengths
177 after trimming are displayed in Table S1. Colony-PCRs were performed in a total volume of
178 50 μL adjusted with ultra-pure water and consisting of 1X reaction buffer, 1.5 mM of MgCl₂,
179 0.2 mM of each dNTP, 5 % of DMSO, 0.2 mM of each primer and 5 U/mL of Taq'Ozyme
180 (Ozyme, Montigny-Le-Bretonneux, France). PCR amplifications were performed in a
181 Biometra Tone thermocycler (Analytik Jena, Jena, Germany) using the following program: 5

182 min of initial denaturation at 94°C, 30 cycles of 30 s at 94°C, 30 s at appropriate annealing
183 temperature, 1 min at 72°C, and a final extension for 5 min at 72°C. PCR products were then
184 purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany).
185 DNA quality and concentrations were assessed with a Nanophotometer NP80 (Implen,
186 Munich, Germany) before Sanger sequencing of one strand at Genoscreen (Lille, France) or
187 GATC Biotech (Konstanz, Germany) with primers XgyrB1F (*gyrB*), rpoDXSoR6 (*rpoD*) or
188 gap1F (*gapA*). Sequences of *gyrB* and *rpoD* loci of the CFBP strains were provided by the
189 CIRM-CFBP. Sequences were aligned, trimmed and concatenated following the alphabetical
190 order using CLUSTALΩ [60], resulting in a 2,331 bp sequence. The best-fitted nucleotide
191 substitution model was evaluated in MEGA 7.0.26 based both on the corrected Akaike
192 information criterion (AICc) and the maximized log likelihood (lnL). Maximum likelihood
193 phylogeny was constructed in MEGA 7.0.26 [33] using the Generalized Time Reversible
194 substitution model (GTR) with a gamma distribution of rate variation among sites (shape
195 parameter = 4) and acceptance of invariant sites (I). Branch support was assessed with 500
196 bootstrap replicates. *X. populi* CFBP 1817^T was chosen as an outgroup. All the sequences
197 generated were deposited in the National Center for Biotechnology Information GenBank
198 under accession numbers MK610462 to MK610526 for *gapA*, MK610527 to MK610591 for
199 *gyrB* and MK610592 to MK610656 for *rpoD*. The full concatenated alignment is provided
200 along with the supplementary material.

201 **Genome sequencing, assembly, annotation and acquisition**

202 Genomic DNAs were extracted and purified from 25 ml 1/10th TSB overnight cultures using
203 the standard phenol-chloroform method [55]. The genomes of five strains of *X. campestris*
204 pv. *vitians* type B (LM 16388, LM 16735, CFBP 498, CFBP 499 and CFBP 3978), pathotype
205 strain *X. campestris* pv. *vitians* type A CFBP 2538^{PT}, pathotype strains of *X. hortorum*
206 pv. *taraxaci* CFBP 410^{PT}, *X. hortorum* pv. *pelargonii* CFBP 2533^{PT} and *X. cynarae*

207 *pv. gardneri* CFBP 8163^{PT} were sequenced with Illumina technology in HiSeq paired-end
208 2*150 bp at GATC Biotech (Konstanz, Germany). Additionally, historical representative
209 strain of *X. campestris pv. vitians* type B LMG 938 was sequenced in Illumina MiSeq 2*250
210 bp at Penn State University. Paired-end reads were assembled in contigs using UNICYCLER
211 v0.4.2 [73] with a minimum contig size of 200 bp, then annotated with PROKKA v1.14 [58].
212 Additionally, 46 genome assemblies representing the taxonomic diversity of *Xanthomonas*
213 clade II as reported by Jacques *et al.* [28] with the emended propositions of Constantin *et al.*
214 [13] were acquired from the NCBI Genbank database. Genome characteristics, GenBank
215 accession numbers and quality statistics are listed in Table S2.

216 **Phylogenomic analysis**

217 Phylogenomic tree reconstruction was performed on all the genomes of type, pathotype and
218 representative strains of *Xanthomonas* clade II using PHYLOPHLAN v1.10 [59]. Type strain of
219 *Stenotrophomonas maltophilia* CFBP 3035^T was selected as an outgroup. A core of ~350
220 single-copy full-length predicted proteins from the PhyloPhlan database were retrieved in the
221 genomes with USEARCH v8.0.1623 [16], then aligned and concatenated with MUSCLE v3.8.31
222 [17]. Finally, a phylogenomic tree was built with FASTTREE 2.1.10 SSE3 [44] and robustness
223 was locally assessed by the Shimodaira-Hasegawa test (SH) using 1,000 resamples.
224 Additionally, a phylogeny was also constructed on all the available genomes of *X. campestris*
225 *pv. vitians* type B, *X. hortorum* and *X. cynarae* with *X. populi* as an outgroup following the
226 same procedure.

227 **Overall Genome Relatedness Indices (OGRI) calculation**

228 All OGRI values were calculated on a subset containing the genomes of all the type,
229 pathotype or representative strains of *X. campestris pv. vitians*, *X. hortorum*, *X. cynarae*, and
230 type strains of *X. populi* and *X. citri*. Pairwise MUMMER-driven average nucleotide identities
231 (ANIm) and tetranucleotide frequencies (Tetra) were calculated using the web server

232 JSPECIESWS v3.0.20 (<http://jspecies.ribohost.com/jspeciesws>) [47] whereas average
233 nucleotide identities based on BLAST+ alignments (ANIb) were obtained with ORTHOANI
234 v0.93.1 [34]. *In silico* DNA-DNA hybridization values (*is*DDH) [36] were determined at the
235 web server of GGDC v2.1 (Genome-to-Genome Distance Calculator) available at the DSMZ
236 website (<http://ggdc.dsmz.de/ggdc.php>).

237 **Phenotypic profiling**

238 Phenotypes of *Xanthomonas* strains were characterized using Biolog GEN III (carbon
239 sources and chemical resistances), PM02A (additional carbon sources) and PM03B (nitrogen
240 sources) microplates (Biolog, Hayward, United States). Experiments were repeated in
241 triplicates for GEN III microplates and duplicates for PM02A and PM03B microplates. All
242 strains were subcultured from frozen stock cultures on TSA 1/10th at 28°C for 72 h, then
243 spread on TSA plates for 20 - 24 h at 28°C. For GEN III microplates, tubes of fluid IF-A were
244 inoculated with sterile swabs to obtain transmittance values ranging from 92 to 96 %. For
245 PM02A and PM03B microplates, inoculation fluid IF-0 1x was prepared by addition of
246 manufacturer's Dye Mix A at 1.2 %. Finally, a 50x stock solution composed of sodium
247 succinate 1M and ferric citrate 100 µM was prepared and sterilized by filtration on 0.2 µm
248 filters, then added in the inoculation fluid only for PM03B microplates at a 1x final
249 concentration. Fluids were then inoculated following the same procedure as for the GEN III
250 microplates to obtain to transmittance values of 85 %. Plates were subsequently filled with
251 100 µL per well and incubated 5 days at 25°C in an OmniLog system. Raw data were
252 imported in R and analyzed using the opm package [70]. The maximum heights of the curves
253 (parameter A) were discretized using empirical cutoffs of 75 and 125 omnilog arbitrary units
254 for weak and positive reaction respectively.

255 Some of the phenotypic features reported in Brown's first description of the pathogen
256 responsible for bacterial leaf spot of lettuce [7] were also tested to check the authenticity of

257 the pathotype strain. Gram staining were performed by staining with crystal violet for 1 min
258 followed by Lugol for 1 min, then decolorized with 70 % ethanol for 20 s and finally stained
259 with safranin for 1 min. Starch hydrolysis was recorded after 5 days of growth on starch agar
260 plates containing 3 g/L of beef extract, 10 g/L of soluble starch and 12 g/L of bacteriological
261 agar by flooding the plates with Lugol's iodine solution. Gelatin hydrolysis was assayed every
262 day for 7 days in stab-inoculated 15-mL Falcon tubes filled with 5 mL of 5 g/L of
263 bacteriological peptone, 3 g/L of beef extract and 120 g/L of gelatin. Motility, hydrogen
264 sulfide and indole production were tested in stab-inoculated 15-mL Falcon tubes filled with 5
265 mL of Sulfate Indole Motility (SIM) medium made of 20 g/L of tryptone, 6.1 g/L of
266 bacteriological peptone, 0.13 g/L of anhydrous sodium thiosulfate, 0.2 g/L of hexahydrate
267 ferrous ammonium sulfate and 3.5 g/L of bacteriological agar. Indole production was assayed
268 5 days after inoculation using James reagent (Biomérieux, Marcy-l'Etoile, France). Litmus
269 milk was prepared with 100 g/L of skimmed milk powder, 0.5 g/L of sodium sulfite and 0.2 %
270 (w/v) of litmus and 10 mL were dispensed in 15-mL Falcon tubes. Reactions in the medium
271 were recorded after 7 days.

272 **RESULTS**

273 **Pathogenicity assays**

274 All strains were tested for their pathogenicity on two lettuce cultivars (oakleaf cv. Kirinia and
275 leaf lettuce cv. Météore), and 11 strains on tomato cv. Marmande. Strains able to induce a
276 mean disease severity ≥ 2 on the 8 plants at the end of the three-week monitoring period were
277 considered pathogenic, whereas strains were determined non-pathogenic when mean disease
278 severity was < 2 . When mean disease severity was ≥ 2 , few variations of disease severity were
279 observed between strains or cultivars using our five-point scale disease index (*data not*
280 *shown*). All strains labelled as *X. hortorum* pv. *vitians* were able to induce typical BLSL
281 symptoms on the two lettuce cultivars (Table 1), and therefore considered pathogenic on

282 lettuce. Consistent with previous reports [3,53], pathotype strain of *X. campestris* pv. *vitians*
283 CFBP 2538^{PT} was non-pathogenic on lettuce. (cvs. Kirinia and Météore). All other pathovars
284 tested were neither pathogenic, except strain *X. cynarae* pv. *gardneri* CFBP 7999 which was
285 surprisingly highly virulent on the two lettuce cultivars and produced unmistakable BLSL
286 symptoms. As expected, the three strains of *X. cynarae* pv. *gardneri* were pathogenic on
287 tomato cv. Marmande, and apart from *X. campestris* pv. *vitians* LM 16735 which was weakly
288 pathogenic, none of the other strains tested were able to induce symptoms on tomato (Table 1).

289 **Multilocus sequence analysis**

290 Assessment of the genetic diversity of a large panel of 57 BLSL-inducing strains, mostly
291 isolated in France and some in USA and Zimbabwe, was performed using multilocus
292 sequence analysis (MLSA) on housekeeping genes *gapA*, *gyrB* and *rpoD*. Three major
293 phylogenetic groups and six sequence types (STs) were identified based on the concatenated
294 sequences (Figure 1). Each MLSA group (referred to as A, B and C in this study) were
295 composed of two different STs, respectively A1, A2, B1, B2, C1 and C2. Six different alleles
296 were observed for *gapA* gene, 4 for *gyrB* and only one for *rpoD*. The three MLSA groups
297 were also obtained with *gapA* alone, whereas *gyrB* alone can only discriminate group B
298 (Figure S1). *rpoD* being identical for all the strains, it was not useful for assessing the intra-
299 pathovar diversity, although it allowed to discriminate most of *X. hortorum* and *X. cynarae*
300 pathovars (Figure S2). The two isolates included into the minor ST B2 were isolated in the
301 same field at the same date, making them potential clones rather than real populations. It was
302 the same situation for the 3 isolates of ST C2. For all the other STs described, no pattern of
303 repartition of the strains depending on their geographical origin or year of isolation could be
304 identified. The BLSL strains from USA grouped in STs A1 and B1 and it should be noted that
305 strain LMG 938 from Zimbabwe belonged to major ST C1.

306 **Whole-genome based phylogenies**

307 Two phylogenomic trees were built with PHYLOPHLAN: the first one on a set of genomes
308 representing the known diversity of *Xanthomonas* clade II (Figure 2a) and the second one on a
309 subset containing all available or newly-sequenced genomes of *X. hortorum*, *X. cynarae* and
310 *X. campestris* pv. *vitians* type B (Figure 2b). Analysis of the clade-scaled phylogeny revealed
311 that the pathotype strain of *X. campestris* pv. *vitians*, currently labeled as *X. axonopodis*
312 pv. *vitians* CFBP 2538^{PT} was highly related to the type strain of *X. citri* CFBP 3369^T (Figure
313 2a). This phylogeny also highlighted the genetic relatedness of strains labeled as *X. hortorum*,
314 *X. cynarae* and BLSL-causing *X. campestris* pv. *vitians*. Moreover, a clear cut-off between
315 this group and the closest species *X. populi* was obvious (Figure 2a). Within this group, *X.*
316 *hortorum* pvs. *carotae* and *hederae* were more genetically similar to each other than to the
317 other pathovars (Figure 2b). *X. hortorum* pv. *pelargonii* was the most divergent. The large
318 number of genomes available for *X. cynarae* pv. *gardneri* allowed us to evidence the narrow
319 diversity between the strains in this pathovar. Likewise, all sequenced strains of *X. hortorum*
320 pv. *vitians* had low genetic diversity except for strains LM 16388 and CFBP 499 which
321 clustered together and seemed to have diverged earlier than the others. Surprisingly, the strain
322 CFBP 7999 of *X. cynarae* pv. *gardneri* was more related to BLSL-causing strains of
323 *X. campestris* pv. *vitians* than to strains of *X. cynarae* pv. *gardneri*.

324 **Pairwise overall genome relatedness indices comparisons**

325 Four OGRI values (ANIm, ANIb, *is*DDH and Tetra) were calculated on a subset composed of
326 the genomes of all type, pathotype or representative strains of *X. campestris* pv. *vitians* type A
327 and B, *X. hortorum*, *X. cynarae*, and type strains of *X. populi*, *X. axonopodis* and *X. citri*.
328 ANIm and *is*DDH similarity matrices are displayed in Table 2, while ANIb and Tetra results
329 are presented in Table S3. As ANIb and Tetra values gave similar results to ANIm and *is*DDH,
330 they will not be discussed here. Unsurprisingly, *X. campestris* pv. *vitians* type A
331 CFBP 2538^{PT} presented only 88.2 % ANIm and 34.4 % *is*DDH values compared pairwise

332 with *X. campestris* pv. *vitians* type B LMG 938 but was highly similar to type strain of *X. citri*
333 CFBP 3369^T (ANIm = 98.7 % and *isDDH* = 89.5 %), confirming the observations made on
334 the genus-scaled phylogeny (Figure 2a). The comparison with the type strain of *X. axonopodis*
335 CFBP 4924^T (83.4 % ANIm and 52.6 % *isDDH*) showed that CFBP 2538 was actually
336 genetically closer to the type strain of *X. citri* than to the one of *X. axonopodis*.
337 *Xanthomonas cynarae* pvs. *cynarae* CFBP 4188^T and *gardneri* CFBP 8163^{PT} differed little on
338 their genomic content, with ANIm and *isDDH* reaching 99.3 % and 94.9 % respectively. By
339 contrast, between the several pathovars of *X. hortorum*, values were less substantial but still
340 higher or in the range of ANI > 95~96 % and *isDDH* > 60~70 %. Indeed, ANIm varied from
341 95.4 to 96.5 % and *isDDH* from 64.3 to 71.1 %. The lowest values were always obtained
342 when comparing *X. hortorum* pv. *pelargonii* CFBP 2533^{PT} and pv. *taraxaci* CFBP 410^{PT} and
343 the highest between pv. *hederae* CFBP 5858^T and pv. *carotae* CFBP 7900. Exploring the
344 relationships among these two species and *X. campestris* pv. *vitians* led to interesting
345 observations. The two *X. cynarae* strains, *X. campestris* pv. *vitians* LMG 938 and
346 *X. hortorum* pv. *taraxaci* CFBP 410^{PT} were robustly grouped together by all parameters, with
347 ANIm > 97 % and *isDDH* > 78 %, indicating unequivocally that they should belong the same
348 species. However, if *X. campestris* pv. *vitians* LMG 938 shared 98.4 % ANIm and 87.3 %
349 *isDDH* with type strain of *X. cynarae* CFBP 4188^T, it also shared 96.6 % ANIm and 68.5 %
350 *isDDH* with *X. hortorum* CFBP 5858^T. As a matter of fact, comparing type strains of
351 *X. cynarae* and *X. hortorum* revealed ANIm and *isDDH* values of 96.1 % and 68.5 %,
352 implying that genomic data could support their combination into one single species. Overall,
353 OGRI calculations revealed that all the strains of *X. hortorum*, *X. cynarae* and BLSL-causing
354 *X. campestris* pv. *vitians* type B formed a coherent genomic group as no clear cut-off in the
355 distribution of values could be observed. However, such a gap was distinctly observed
356 between the previously described group and the nearest species *X. populi* CFBP 1817^T, as

357 ANIm and *is*DDH values drastically fell from above 95 % ANIm and 60 % *is*DDH to
358 ~91 % and ~45 % respectively.

359 **Phenotype**

360 Resistance to chemical compounds and carbon and nitrogen sources utilization were
361 tested using Biolog GEN III, PM02A and PM03B microplates. The global phenotypic profiles
362 obtained with standardized tests and GEN III microplates are displayed in Table 3, while
363 PM02A and PM03B results are provided in Tables S4 and S5 respectively. Exhaustive
364 phenotypic profiles are depicted in the protologues.

365 The original description of *X. campestris* pv. *vitians* provided by Brown in 1918 [7]
366 and reworked by Burkholder in Bergey's *Manual of Determinative Bacteriology* of 1957 [6]
367 depicts the pathogen as a Gram-negative motile rod which is feebly amyolytic, liquefies
368 gelatin slowly, produces hydrogen sulfide, feebly produces indole and produces an alkaline
369 reaction in litmus milk with litmus reduction, casein hydrolysis and precipitation.
370 Reproducing these tests (Table 3), we observed that pathotype strain CFBP 2538 differs from
371 the previous descriptions as it was strongly amyolytic, did not liquefy gelatin after 7 days,
372 had a strong production of indole 5 days after inoculation and did not reduce litmus in litmus
373 milk, neither did it hydrolyze casein after 7 days. On the other hand, strain LMG 938 fits
374 completely the previously described characteristics, except for starch hydrolysis as no
375 reaction was observed 4 days after inoculation on starch agar plates. Indeed, none of the
376 strains of *X. hortorum* or *X. cynarae* hydrolyzed starch, all presented a slight production of
377 indole and gave the same reduction reaction in litmus milk.

378 Moreover, Biolog GEN III microplate assays showed that CFBP 2538 used Dextrin
379 and D-Maltose as carbon sources when none of other strains tested did. Overall, strains of
380 *X. hortorum*, *X. cynarae* and *X. campestris* pv. *vitians* type B exhibited a strong stable core
381 phenotype of 21 highly used carbon sources and 4 chemical resistances, all the other reactions

382 tested being either negative for all strains or highly variable among biological replicates for at
383 least one strain. No stable discriminative trait between *X. cynarae* and *X. hortorum* could be
384 observed in our analyses.

385 **DISCUSSION**

386 In this research we investigated all of the taxonomic relationships between all the
387 taxonomically relevant members of *X. hortorum*, *X. cynarae* and *X. campestris* pv. *vitians*
388 type A and B, which until this work, have never been explored in a single study. Though,
389 previous studies revealed the high genetic proximity between *X. hortorum* and *X. cynarae*
390 [64,78].

391 The *X. hortorum* Vauterin *et al.* 1995 species was proposed mainly on the basis of
392 DNA-DNA reassociation experiments and grouped together *X. campestris* pvs. *hederae*,
393 *taraxaci*, *carotae*, *pelargonii* and *vitians* type B strains [72]. Later, *X. cynarae* Trébaol *et al.*
394 2000 was described on artichoke and considered to be a new species because none of the
395 DNA-DNA hybridizations conducted against several other *Xanthomonas* allowed to bind it to
396 a previously described species at a level higher than the species threshold [68]. Unfortunately,
397 the type strain of *X. hortorum* pv. *hederae* CFBP 5858^T (= CFBP 4925^T = LMG 733^T) was
398 not tested as it should have been and only *X. hortorum* pv. *pelargonii* CFBP 2533^{PT}
399 (= LMG 7314^{PT}) was included and presented a 49 % reassociation value against *X. cynarae*
400 CFBP 4188^T (= ICMP 16775^T). *Xanthomonas gardneri* Jones *et al.* 2006 was created based
401 on DNA-DNA hybridization experiments conducted on strains of *Xanthomonas* pathogenic
402 towards tomato and pepper and on representative strains of the *Xanthomonas* diversity [29].
403 Again, the type strain of *X. hortorum* was not included in that study and the other *X. hortorum*
404 pathovars yielded reassociation values between 53 to 65 % towards *X. gardneri* CFBP 8163^T
405 (= LMG 962^T = ATCC 19865^T) except for *X. hortorum* pv. *taraxaci* CFBP 410^{PT}
406 (= LMG 870^{PT}) for which reassociation values reached 71-75 %. However, no reclassification

407 of this latter pathovar was proposed. It is noticeable that in 1990 and 1993, work by
408 Hildebrand *et al.* [27] and Palleroni *et al.* [42] classified '*X. gardneri*', whose name was not
409 valid at that time, in the same homology group as *X. hortorum* pathovars *pelargonii*, *carotae*
410 and *taraxaci* due to reassociation values ranging from 64 to 100% (highest values retrieved
411 for pv. *taraxaci*). Finally, using whole genome sequence comparisons, *X. gardneri* Jones *et al.*
412 2006 has been shown to be a later heterotypic synonym of *X. cynarae* Trébaol *et al.* 2000 and
413 was transferred in this species as *X. cynarae* pv. *gardneri* Timilsina *et al.* 2019 [64]. Despite
414 their genetic relatedness evidenced by wet-lab and *in silico* DNA-DNA hybridizations, no
415 comprehensive investigation of the taxonomic relationships between *X. hortorum*, *X. cynarae*
416 and their pathovars, using the relevant type and pathotype strains has been conducted to date.

417 Our comprehensive study yielded many OGRI values around the threshold
418 defined for bacterial species delineation [46], including the comparison between *X. hortorum*
419 and *X. cynarae* type strains. The *in silico* experiments revealed that *X. cynarae* pvs. *cynarae*
420 and *gardneri* and *X. hortorum* pvs. *taraxaci* and *vitians* belong undoubtedly to the same
421 species as ANI and *isDDH* values are well above 97 % and 70 % respectively. The
422 relationships between the previous cluster and *X. hortorum* pvs. *hederae*, *carotae* and
423 *pelargonii* may be more ambiguous as ANI and *isDDH* scores falls in a 'transition zone' [46]
424 with ANI ranging from ~95 to 96.5 % and *isDDH* from ~64 to 68 %. The lowest OGRI was
425 *isDDH* scores of 64~65 % obtained between pv. *taraxaci* and the three pathovars *hederae*,
426 *carotae* and *pelargonii*, yet the other OGRIs calculated and its central phylogenetic position
427 demonstrate clearly it belongs to the same species. Tetranucleotide signature frequencies
428 endorsed the hypothesis of one species only as all the pathovars mentioned compared pairwise
429 presented very high values of this correlation coefficient, above 0.999 for most and 0.998 at
430 least, while the determined threshold for species delineation is considered to be 0.990 [46,47].
431 These results are in accordance with previous wet-lab DDH values ; even though some wet-

432 lab DDH values might seem lower than the threshold, they should be considered with caution.
433 Indeed, although DNA-DNA hybridizations had been considered for years as the taxonomic
434 “gold standard” in the scientific community, it has been repeatedly criticized as a
435 cumbersome method, subjected to high standard deviations, sensitive to DNA quality and to
436 the methodology used to measure DNA relatedness, as different laboratories using different
437 methodologies could produce different results for the same comparisons [21,23,46].
438 Measurement of thermal stability of reassociated DNA (ΔT_m) was recommended to
439 complement DDH and to overcome its drawbacks [22] and it was not unusual to find low
440 ΔT_m associated to DDH around 50%. Moreover, the usual threshold of 70 % DDH was
441 recommended by the *ad hoc* committee as an approximate cut-off for species delineation and
442 not meant to be a strict boundary [38]. Indeed, its strict application might lead to the division
443 of taxa into different species without real biological significance. In fact, there is a “transition
444 zone” within 60 to 70 % DDH and 93 to 96 % ANI where the choice to merge or separate
445 species must be led by other criteria such as stable phenotypic diagnostic features and
446 phylogenetic relationships [46,52].

447 The phenotypic profiles we described endorsed the proposition of synonymy as no
448 stable phenotypes allowed to differentiate strains of *X. cynarae* from strains of *X. hortorum*. It
449 was rather observed that they all shared an invariable core phenotype of 21 highly used
450 carbon sources and 4 chemical resistances. The variable phenotypic traits were strain-specific,
451 unstable between replicates for at least one strain and consisted mostly of weakly used carbon
452 sources and resistances to chemical compounds, which can be considered as accessory
453 phenotypic features irrelevant for taxonomical purposes. Nitrogen source utilization profiles
454 were way more diverse and highly variable, as there was not a single common nitrogen source
455 for all the strains tested. It appeared surprisingly that *X. hortorum* pv. *pelargonii*
456 CFBP 2533^{PT} seems to use a large number of different nitrogen sources, maybe related to

457 some particular ecological lifestyle features. On the other hand, the proposed *X. hortorum*
458 emend. can easily be distinguished from its closest species *X. populi* as the latter is able to use
459 dextrin, cannot metabolize D-cellobiose, D-melibiose, L-fucose, gelatin, L-alanine, L-
460 glutamic acid, L-serine and propionic acid, is not able to grow in presence of 1 % NaCl, does
461 not liquefies gelatin in tube tests neither does it produces indole, and has an unusual optimum
462 growth temperature between 20 to 23°C, as systematically and consistently reported in
463 previous descriptions of this species [48,71,72].

464 To explore the phylogenetic relatedness between *X. hortorum* and *X. cynarae*, we
465 both built a genus-scaled phylogeny using available and reliable type or pathotype strain
466 genomes and another phylogeny focused on all available genomes of *X. hortorum* and
467 *X. cynarae*. Genome-based phylogenetic reconstructions have proven to be robust useful
468 methods to investigate the evolutionary relationships and infer taxonomic assignments for
469 *Xanthomonas* species [37,51]. In this study, we used a recently developed automated pipeline
470 which has been proven to be robust towards horizontal gene transfer (HGT) events [59]. The
471 resulting phylogenetic trees have strong statistical supports (Figure 2) and, as previously seen
472 by MLSA [78], revealed that *X. hortorum* represent a paraphyletic species that needs
473 taxonomic reclassification. Grouping pathovars of *X. hortorum* and *X. cynarae* in a single
474 species fulfills the monophyly criteria of the species concept and produces a species cluster
475 within which the evolutionary distances are in the range of those observed in other
476 polymorphic *Xanthomonas* species (Figure 2a).

477 Nevertheless, in order to achieve these taxonomic proposals formally, we need to
478 address the status of the pathotype strain *X. campestris* pv. *vitians* type A CFBP 2538^{PT}
479 (= NCPPB 976^{PT} = LMG 937^{PT} = ATCC 19320^{PT} = ICMP 336^{PT}). This particular strain was
480 reported many times in the past to exhibit unusual features compared to other BLSL-causing
481 strains [3,53,62,72]: different colony morphology, protein pattern, rep-PCR or RFLP

482 (restriction fragment length polymorphism) profiles, DNA homology group. However, the
483 most striking aberrant characteristic of this pathotype strain is its absence of pathogenicity
484 towards lettuce [26,53], confirmed by our study on two different lettuce cultivars. In the
485 original description of the South Carolina lettuce disease made by Nellie Brown in 1918, the
486 strain fulfilled the Koch's postulates and was clearly virulent on lettuce though, even a year
487 after its isolation in the field [7]. According to standard 11 of the *International Standards for*
488 *Naming Pathovars of Phytopathogenic Bacteria* [15], this strain has therefore become
489 unsuitable as the pathotype strain of *X. campestris* pv. *vitians*, as its primary character, *i.e.*
490 pathogenicity towards lettuce, has drastically changed. In addition to confirming that
491 *X. campestris* pv. *vitians* CFBP 2538^{PT} is not pathogenic on lettuce we demonstrate that it
492 differs from the original description provided by Brown 1918 [7] and complemented by
493 Burkholder in the *Bergey's Manual of Determinative Bacteriology* of 1957 [6] in the
494 following: starch hydrolysis, gelatin liquefaction, indole production and litmus milk reaction.
495 Unfortunately, only one strain was supposedly conserved from this original description and
496 none of the duplicates preserved in culture collections fits the original description. On the
497 other hand, reference strain LMG 938 is undoubtedly pathogenic towards lettuce, and the
498 symptoms it causes are identical to those described by Brown in 1918. Moreover, we
499 demonstrate that this strain matches the description of Brown 1918 and Burkholder 1957 for
500 the following: is a Gram-negative motile rod, liquefies gelatin, produces hydrogen sulfide,
501 slightly produces indole, provokes an alkaline reaction in litmus milk with litmus reduction,
502 casein hydrolysis and precipitation. The only feature differing from Brown's description is
503 that it does not hydrolyzes starch, which was reported to be feebly positive. However, potato
504 starch hydrolysis tests performed in 1918 differ significantly from the starch hydrolysis tests
505 available today and this likely accounts for this difference. In conclusion, to resolve this long-
506 lasting issue, we propose in accordance with standard 11 and 9-4 to officially replace the

507 pathotype strain CFBP 2538^{PT} (= NCPPB 976^{PT} = LMG 937^{PT} = ATCC 19320^{PT} = ICMP
508 336^{PT}) by strain LMG 938 as a neopathotype. In our opinion, the most probable hypothesis of
509 what rendered the pathotype of *X. campestris* pv. *vitians* unsuitable is that the original strain
510 deposited was mixed and/or exchanged with a *Xanthomonas* sp. strain at some point during its
511 long history of transfer and before distribution to culture collections.

512 The data presented here support the transfer of *X. campestris* pv. *vitians* into *X.*
513 *hortorum* as previously proposed by Vauterin *et al.* based on DNA-DNA hybridizations
514 experiments [72]. The comparison of strain LMG 938 with type strain of *X. hortorum*
515 confirms they belong to the same species, as they shared 96.1 % ANIm, 68.2 % *isDDH* and
516 resulted in a Tetra score of 0.99872. Moreover, the pathogenicity tests we performed
517 demonstrated that none of the existing pathovars of *X. hortorum* nor *X. cynarae* were
518 pathogenic on lettuce, eliminating the possibility of a pathovar synonymy. We therefore
519 propose its transfer in *X. hortorum* as *X. hortorum* pv. *vitians* comb. nov. with strain LMG
520 938 acting as the neopathotype.

521 The species-scaled phylogeny revealed that *X. hortorum* pv. *vitians* is most
522 probably divided in two genomic groups which might be further subdivided into close sub-
523 populations. *X. hortorum* pv. *vitians* strains representing MLSA groups A and C formed a
524 tight cluster whereas strains LM 16388 and CFBP 499 (MLSA group B) were slightly
525 divergent (Figure 2b). The three MLSA clusters identified in this study are identical to those
526 described recently by Fayette *et al.* [19] in the United States, as representative strains L43,
527 JF196 and L7 of Fayette's study appeared to belong to our major sequence types A1, B1 and
528 C1 based on the comparison of partial *gapA* sequences only (*data not shown*). In both MLSA
529 studies, the clustering is dependent of *gapA* and *gyrB* as other loci show no or little
530 polymorphism. The *gyrB*-based tree resulted in two groups seemingly similar to those

531 obtained using whole-genome alignments, whereas *gapA* is the only locus that discriminates
532 group C strains.

533 Overall, the evolutionary picture depicted by the species-level phylogeny (Figure
534 2b) clearly distinguished the different pathovars, highlighting recent specializations of closely
535 related organisms towards different hosts. *X. hortorum* pv. *pelargonii* branched at the root of
536 the species and pathovars *gardneri* and *cynarae* were the most highly related depicting a
537 recent divergence as demonstrated recently [64]. Apart from the exception discussed below,
538 none of the pathovars have been, to our knowledge, reported to be isolated on another plant
539 than their described hosts in natural conditions [35]. In addition, recent work made on the
540 comparison of host ranges of *X. cynarae* and *X. hortorum* pathovars by cross-inoculations in
541 artificial conditions revealed that they all differ by at least one host and cannot therefore be
542 considered as synonymous pathovars [26]. It would be of great interest to add to this
543 phylogeny and evaluate the experimental host ranges of some newly identified pathogenic
544 isolates which reportedly belong to *X. hortorum* based on partial *gyrB* sequencing or MLSA.
545 These new variants were isolated on radicchio (*Cichorium intybus*) [80], annual wormwood
546 (*Artemisia annua*) [61], English lavender (*Lavandula angustifolia*) [49], peony (*Paeonia* spp.)
547 [31], poinsettia (*Euphorbia pulcherrima*) [50], pot marigold (*Calendula officinalis*) and
548 avocado (*Persea americana*) [43]. It has also been reported that *X. campestris*
549 pv. *nigromaculans*, pathogenic on greater burdock (*Arctium lappa*) should belong to
550 *X. hortorum* emend. [29,43].

551 The last interesting point raised in this article is the status of strain CFBP 7999
552 (= ICMP 7383) from pv. *gardneri* for which the taxonomic singularity has been already
553 depicted [24,63]. According to our phylogenetic analyses and pathogenicity tests, it appears
554 that this strain belongs unequivocally to *X. hortorum* pv. *vitians* rather than pathovar *gardneri*.
555 Indeed, it was demonstrated by its high virulence on the two lettuce cultivars tested resulting

556 in typical BLSL symptoms. Its high virulence on tomato was an unusual feature for a pv.
557 *vitians* strain though. If several strains of *X. hortorum* pv. *vitians* have been already reported
558 to be weakly pathogenic on tomato and pepper [2,53,54], like strain LM16735 from this study,
559 none was shown to possess such level of aggressiveness on both hosts. While most of the
560 strains we investigated held only one plasmid, preliminary investigations demonstrated that
561 this peculiar strain possess three (*data not shown*). *In silico* comparisons revealed that plasmid
562 pICMP7383.2 resembles highly to the typical plasmid of *X. hortorum* pv. *vitians*, yet
563 plasmids pICMP7383.1 and pICMP7383.3 were found to be strikingly akin to pJS749-3.1 and
564 pJS749-3.2 of *X. hortorum* pv. *gardneri* CFBP 8588 (= JS749-3) [45]. Plasmid pICMP7383.1
565 was found also to be similar to pLMG911.1 of type strain of *X. vesicatoria* LMG 911^T
566 (= CFBP 2537^T), another *Xanthomonas* pathogenic towards tomato and pepper. This
567 particular plasmid feature raises questions about the role of plasmids in the adaptation to
568 different hosts in *Xanthomonas* and should therefore be investigated further. Regardless, it
569 may be involved in the singular phenotype which resulted in the erroneous taxonomic
570 affiliation of this particular strain.

571 As a conclusion, this polyphasic study has led us to propose to replace the
572 inappropriate pathotype strain CFBP 2538^{PT} (= NCPPB 976^{PT} = LMG 937^{PT} = ATCC
573 19320^{PT} = ICMP 336^{PT}) of *X. campestris* pv. *vitians* by the neopathotype strain LMG 938^{neoPT}
574 and to transfer this pathovar in *X. hortorum* emend. as *X. hortorum* pv. *vitians* comb. nov.
575 (proposed neopathotype LMG 938^{neoPT} = CFBP 8686^{neoPT} = NCPPB 2248^{neoPT}). This
576 proposition allows to maintain Brown's pathovar epithet priority. If these changes should be
577 rejected by the ISPP-Committee on the Taxonomy of Plant Pathogenic Bacteria, we still
578 propose the creation of a new pathovar named *X. hortorum* pv. *vitians* pv. nov. having the
579 same description and with LMG 938^{PT} being the pathotype strain. In addition, the
580 phylogenetic, genomic and phenotypic data presented in this work all support that *X. cynarae*

581 is a later heterotypic synonym of *X. hortorum* and reclassification of *X. cynarae* pv. *cynarae*
582 and *X. cynarae* pv. *gardneri* into *X. hortorum* as *X. hortorum* pv. *cynarae* comb. nov.
583 (pathotype strain CFBP 4188^{PT}) and *X. hortorum* pv. *gardneri* comb. nov. (pathotype strain
584 CFBP 8163^{PT}). An emended description of *X. hortorum* (type strain CFBP 5858^T) is provided.
585 Resolving these taxonomic issues will aid in further investigations into the biology and
586 epidemiology of these pathogens. We are currently investigating the race structure of
587 *X. hortorum* pv. *vitians* [8,25]. In future work, we will investigate the molecular determinants
588 underlying the intriguing host-specificity pattern of these pathovars on phylogenetically-
589 distant plants.

590

591 **TAXONOMY**

592 **Emended description of *Xanthomonas hortorum* Vauterin et al. 1995**

593 *Xanthomonas hortorum* (hor.to'rum. L. masc. gen. n. *hortorum*, from gardens)

594 The general characteristics are as depicted in the first description of the species [72],
595 emended with data from the present study. Based on Biolog GEN III MicroPlates assays,
596 strain CFBP 5858^T is undoubtedly able to utilize D-trehalose, D-cellobiose, sucrose,
597 D-melibiose, N-acetyl-D-glucosamine, D-glucose, D-mannose, D-fructose, D-galactose,
598 L-fucose, gelatin, L-alanine, L-glutamic acid, L-serine, methyl pyruvate, citric acid,
599 α -keto-glutaric acid, L-malic acid, bromo-succinic acid, propionic acid and acetic acid. Strain
600 CFBP 5858^T also undoubtedly grow at pH 6, in presence of 1 % NaCl, 1% sodium lactate and
601 lincomycin. Additionally, the Biolog PM03B MicroPlates revealed that pathovars of
602 *X. hortorum* presents highly variable profiles of nitrogen sources utilization.

603 The type strain is CFBP 5858^T = CFBP 4925^T = LMG 733^T = NCPPB 939^T =
604 ICMP 453^T. The GenBank accession number for its genome assembly is GCA_002940005.1
605 and its 16S rRNA gene accession number is NR_026386. The species has a G+C mole %
606 value between 63.3 to 63.9 %. The species includes, so far, the following pathovars based on
607 their phytopathogenic specialization: *X. hortorum* pv. *hederae*, *X. hortorum* pv. *pelargonii*,
608 *X. hortorum* pv. *carotae*, *X. hortorum* pv. *taraxaci*, *X. hortorum* pv. *cynarae*, *X. hortorum* pv.
609 *gardneri* and *X. hortorum* pv. *vitians*.

610 The closest species described is *X. populi* (ex-Ridé 1958) van den Mooter and Swings
611 1990 [71]. According to the characteristics of *X. populi* described in the valid description, in
612 Ridé and Ridé 1992 [48] and Vauterin *et al.* 1995 [72], *X. hortorum* strains can be
613 distinguished from *X. populi* by their incapacity to use dextrin, ability to use D-cellobiose, D-
614 melibiose, L-fucose, gelatin, L-alanine, L-glutamic acid, L-serine and propionic acid, to grow
615 in presence of 1 % NaCl, to liquefy gelatin and produce indole. Moreover, *X. populi* is a
616 fastidiously cultivable bacteria which has an optimal growth temperature of 20 to 23°C and
617 cannot grow at 28°C, which is the optimal growth temperature of *X. hortorum*. Finally,
618 *X. hortorum* can be phylogenetically discriminated from other *Xanthomonas* species using the
619 4-genes MLSA scheme proposed by Young *et al.* [78].

620 Strains reportedly identified as *X. hortorum* using partial *gyrB* sequencing were
621 isolated from symptomatic radicchio (*Cichorium intybus*) [80], annual wormwood
622 (*Artemisia annua*) [61], English lavender (*Lavandula angustifolia*) [49], peony (*Paeonia* spp.)
623 [31], poinsettia (*Euphorbia pulcherrima*) [50], pot marigold (*Calendula officinalis*) and
624 avocado (*Persea americana*) [43]. It has been also suggested that
625 *X. campestris* pv. *nigromaculans*, pathogenic on greater burdock (*Arctium lappa*), might
626 belong to *X. hortorum* [43].

627 ***X. hortorum* pv. *vitians* (Brown 1918) comb. nov.**

628 = *X. campestris* pv. *vitians* (Brown 1918) Dye 1978

629 The description is the same as the species. Additionally, the Biolog GEN III and PM02A
630 MicroPlates revealed the ability of strain LMG 938^{neoPT} to utilize β -gentiobiose, glycerol,
631 pectin, L-lactic acid, tween 40, α -keto-butyric acid, acetoacetic acid, sodium formate, gelatin,
632 laminarin, D-raffinose, N-acetyl-L-glutamic acid, weakly utilize L-tartaric acid, and grow in
633 presence of 4 % NaCl, tetrazolium violet, tetrazolium blue, potassium tellurite and sodium
634 bromate. The Biolog PM03B MicroPlates indicated that L-alanine, L-arginine, L-glutamic
635 acid, L-glutamine, L-ornithine, glucuronamide, D-glucosamine, N-acetyl-D-glucosamine,
636 guanine, xanthosine, uric acid, alanyl-glutamine, alanyl-glutamic acid and glycyl-glutamic
637 acid can be used as nitrogen sources.

638 The primary host is the lettuce (*Lactuca sativa* L.). The pathotype strain of *X. campestris* pv.
639 *vitians* (Brown 1918) Vauterin *et al.* 1995 CFBP 2538^{PT} = LMG 937^{PT} = NCPPB 976^{PT} =
640 ICMP 336^{PT} = ATCC 19320^{PT} has been proven to be non-pathogenic on lettuce,
641 phenotypically and genotypically different from all other bacterial leaf spot of lettuce-related
642 strains, as discussed in the present study and in previous ones [3,53,62]. Therefore, in
643 accordance with Standards 9-4 and 11 of the *International Standards for Naming Pathovars*

644 of *Phytopathogenic Bacteria* [15,75], we propose the replacement of this pathotype by
645 neopathotype strain LMG 938^{neoPT} = CFBP 8686^{neoPT} = NCPPB 2248^{neoPT}. We propose that its
646 subsequent transfer to *X. hortorum* be as *X. hortorum* pv. *vitians* (Brown 1918) Vauterin *et al.*
647 1995 comb. nov. in part to ensure the conservation of the priority established by Nellie Brown.
648 However, if the neopathotype is rejected, we propose that this same name be established as *X.*
649 *hortorum* pv. *vitians* pv. nov. The GenBank accession number for its genome assembly is
650 SMED000000000.

651 ***X. hortorum* pv. *cynarae* (Trébaol *et al.* 2000) comb. nov.**

652 = *X. cynarae* Trébaol *et al.* 2000

653 = *X. cynarae* pv. *cynarae* (Trébaol *et al.* 2000) Timilsina *et al.* 2019

654 The description is the same as the species. Additionally, the Biolog GEN III and PM02A
655 MicroPlates revealed the ability of strain CFBP 4188^T to utilize glycerol and L-histidine,
656 weakly utilize L-lactic acid and acetoacetic acid, and grow in presence of guanidine
657 hydrochloride and tetrazolium blue. The Biolog PM03B MicroPlates indicated that
658 N-phthaloyl-L-glutamic acid, guanine and alloxan can be used as nitrogen sources.

659 The primary host is the common artichoke (*Cynara scolymus* L.). The pathotype strain is
660 CFBP 4188^{PT} = ICMP 16775^{PT}, the former type strain of *X. cynarae* Trébaol 2000 emend.
661 Timilsina *et al.* 2019. The GenBank accession number for its genome assembly is
662 GCA_002939985.1.

663 ***X. hortorum* pv. *gardneri* (Jones *et al.* 2006) comb. nov.**

664 = *X. gardneri* (ex-Sutić 1957) Jones *et al.* 2006

665 = *X. cynarae* pv. *gardneri* (Jones *et al.* 2006) Timilsina *et al.* 2019

666 The description is the same as the species. Additionally, the Biolog GEN III and PM02A
667 MicroPlates revealed the ability of strain CFBP 8163^{PT} to utilize α -D-lactose, L-aspartic acid,
668 L-histidine, pectin, L-lactic acid, tween 40, α -keto-butyric acid, acetoacetic acid, sodium
669 formate, arbutin, D-raffinose, succinamic acid, D-tartaric acid, N-acetyl-L-glutamic acid and
670 L-homoserine, and grow in presence of 4 % NaCl, tetrazolium violet and tetrazolium blue.
671 The Biolog PM03B MicroPlates indicated that L-arginine, L-aspartic acid, L-glutamic acid,
672 L-leucine, N-acetyl-L-glutamic acid, D-glucosamine, N-acetyl-D-glucosamine, guanine,
673 xanthosine, alloxan, parabanic acid, alanyl-glycine, alanyl-threonine, glycyl-glutamine and
674 glycyl-glutamic acid can be used as nitrogen sources.

675 The primary hosts are tomato (*Solanum lycopersicon* L.) and pepper (*Capsicum annuum* L.).

676 The pathotype strain is CFBP 8163^{PT} = NCPPB 881^{PT} = ATCC 19865^{PT}. The GenBank

677 accession numbers for the two versions its genome assembly are SMDW00000000 and
678 GCA_000192065.2.

679

680 *In order to provide taxonomic data ready to use and easily comparable, and because the*
681 *original protologues may be difficult to find, we recall hereby the characteristics of the other*
682 *pathovars of X. hortorum complemented with data from our study:*

683

684 ***X. hortorum* pv. *hederae* (Arnaud 1920) Vauterin et al. 1995**

685 = *X. campestris* pv. *hederae* (Arnaud 1920) Dye 1978

686 The description is the same as the species. Additionally, the Biolog GEN III and PM02A
687 MicroPlates revealed the ability of strain CFBP 5858^T to utilize pectin, glycerol, L-lactic acid,
688 α -keto-butyric acid, acetoacetic acid, weakly utilize tween 40, and grow in presence of
689 guanidine hydrochloride and tetrazolium blue. The Biolog PM03B MicroPlates indicated that
690 N-phthaloyl-L-glutamic acid can be used as a nitrogen source. It should be noted that previous
691 studies on multiple strains of *X. hortorum* pv. *hederae* showed their ability hydrolyze starch
692 and gelatin [40], making these features strain-dependent for CFBP 5858^T.

693 The primary host is the common ivy (*Hedera helix* L.), yet strains of *X. hortorum* pv. *hederae*
694 have been reported to be pathogenic on other Araliaceae plants such as umbrella tree
695 (*Schefflera actinophylla*), dwarf umbrella tree (*Schefflera arboricola*), Japanese aralia (*Fatsia*
696 *japonica*), false aralia (*Pterandra elegantissima*) and ming aralia (*Polyscias fruticosa*) [12,40].
697 The pathotype strain is also the type strain of *X. hortorum* CFBP 5858^T (= CFBP 4925^T =
698 LMG 733^T = NCPPB 939^T = ICMP 453^T).

699 ***X. hortorum* pv. *pelargonii* (Brown 1923) Vauterin et al. 1995**

700 = *X. campestris* pv. *pelargonii* (Brown 1923) Dye 1978

701 The description is the same as the species. Additionally, the Biolog GEN III and PM02A
702 MicroPlates revealed the ability of strain CFBP 2533^{PT} to utilize β -gentiobiose, glycerol,
703 L-histidine, pectin, L-lactic acid, tween 40, acetoacetic acid, sodium formate, gelatin,
704 laminarin, amygdalin, arbutin, L-alaninamide, N-acetyl-L-glutamic acid, L-homoserine,
705 weakly utilize malonic acid and 3-O- β -D-galactopyranosyl-D-arabinose, and grow in
706 presence of 4 % NaCl, tetrazolium blue, tetrazolium violet, potassium tellurite and sodium
707 bromate. The Biolog PM03B MicroPlates indicated that ammonia, sodium nitrite, sodium
708 nitrate, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-glutamic acid, L-glutamine,
709 glycine, L-histidine, L-isoleucine, L-lysine, L-methionine, L-phenylalanine, L-proline,
710 L-threonine, L-tryptophan, L-valine, D-alanine, D-asparagine, L-citrulline, L-ornithine,

711 N-acetyl-L-glutamic acid, N-phthaloyl-L-glutamic acid, glucuronamide, D-glucosamine,
712 D-mannosamine, N-acetyl-D-glucosamine, adenosine, cytosine, guanine, xanthosine, uric acid,
713 alloxan, allantoin, parabanic acid, γ -amino-n-butyric acid, alanyl-aspartic acid,
714 alanyl-glutamine, alanyl-glutamic acid, alanyl-glycine, alanyl-histidine, alanyl-leucine,
715 alanyl-threonine, glycyl-asparagine, glycyl-glutamine, glycyl-glutamic acid,
716 glycyl-methionine and methionyl-alanine can be used as nitrogen sources.

717 The primary host is the ivy-leaved geranium (*Pelargonium peltatum* L'Hér.). The pathotype
718 strain is CFBP 2533^{PT} = LMG 7314^{PT} = NCPPB 2985^{PT} = ICMP 4321^{PT}. The GenBank
719 accession number for its genome assembly is SMDX000000000.

720 ***X. hortorum* pv. *carotae* (Kendrick 1934) Vauterin et al. 1995**

721 = *X. campestris* pv. *carotae* (Kendrick 1934) Dye 1978

722 The description is the same as the species. According to Kendrick (1934), acid is produced
723 from dextrose and glycerin, litmus milk is cleared in 7 days, and strains were pathogenic on
724 leaves, stems and floral parts of *Daucus carota* L. var. *sativa* DC. The pathotype strain CFBP
725 4997^{PT} = LMG 8646^{PT} = NCPPB 1422^{PT} = ICMP 5723^{PT} has been reported many times to be
726 unsuitable as it is not a *Xanthomonas* [75]. However, it was revealed that pathogenic strain
727 CFBP 7900 was able to utilize β -gentiobiose, glycerol, L-aspartic acid, pectin, tween 40,
728 gelatin, D-raffinose, D-tartaric acid, N-acetyl-L-glutamic acid, weakly utilize
729 L-hydroxyproline, and to grow in presence of 4 % NaCl, rifamycin SV, guanidine
730 hydrochloride, niaproof, tetrazolium violet, tetrazolium blue, lithium chloride and potassium
731 tellurite according to Biolog GEN III and PM02A MicroPlates. The Biolog PM03B
732 MicroPlates indicated that L-aspartic acid, L-glutamic acid, L-glutamine, N-acetyl-L-glutamic
733 acid, glucuronamide, D-glucosamine, N-acetyl-D-glucosamine, alloxan, alanyl-glutamine,
734 alanyl-glutamic acid and glycyl-glutamic acid can be used as nitrogen sources.

735 The primary host is the wild carrot (*Daucus carota* L.). Another pathotype strain must be
736 formally described. We used CFBP 7900 = M081 [30] as a pathogenic representative strain
737 because it will be formally proposed as a neopathotype in the near future (MA Jacques
738 personal communication). The GenBank accession number for its genome assembly is
739 GCA_000505565.1.

740 ***X. hortorum* pv. *taraxaci* (Niederhauser 1943) Vauterin et al. 1995**

741 The description is the same as the species. Additionally, the Biolog GEN III and PM02A
742 MicroPlates revealed the ability of strain CFBP 410^{PT} to utilize β -gentiobiose, glycerol,
743 L-histidine, pectin, acetoacetic acid, gelatin, arbutin, D-tartaric acid and L-homoserine. The
744 Biolog PM03B MicroPlates indicated that ammonia, sodium nitrate, L-alanine, L-arginine,

745 L-asparagine, L-aspartic acid, L-glutamic acid, L-glutamine, glycine, L-leucine, L-lysine,
746 L-proline, D-alanine, L-ornithine, N-acetyl-L-glutamic acid, glucuronamide,
747 N-acetyl-D-glucosamine, adenosine, cytosine, guanine, xanthosine, uric acid, parabanic acid,
748 alanyl-asparagine, alanyl-glutamine, alanyl-glutamic acid, alanyl-glycine, alanyl-leucine,
749 glycyL-asparagine, glycyL-glutamine and glycyL-glutamic acid can be used as nitrogen sources.
750 The primary host is the Kazakh dandelion (*Taraxacum kok-saghyz* Rodin). The pathotype
751 strain is CFBP 410^{PT} = LMG 870^{PT} = NCPPB 940^{PT} = ICMP 579^{PT} = ATCC 19318^{PT}. The
752 GenBank accession number for its genome assembly is SMDY000000000.

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TABLE 1. Bacterial strains used in this study

Proposed nomenclature ^a	Strain no.	Other collection no.	Former nomenclature ^b	Host of isolation	Geographic origin	Year of isolation	Pathogenicity ^y			Reference
							Lettuce cv. Kirinia	Lettuce cv. Météore	Tomato cv. Marmande	
<i>X. citri</i>	CFBP 2538 [*]	ATCC 19320 ICMP 336 LMG 937 NCPBP 976	<i>X. campestris</i> pv. <i>vitiens</i> (Brown 1918) Dye 1978 <i>X. axonopodis</i> pv. <i>vitiens</i> (Brown 1918) Vauterin <i>et al.</i> 1995	<i>Lactuca sp.</i>	United States	1917	-	-	NA	[5]
<i>X. hortorum</i> pv. <i>hederae</i>	CFBP 5858^T	CFBP 4925 LMG 733 NCPBP 939 ICMP 453		<i>Hedera helix</i>	United States	1944	-	-	NA	[63]
<i>X. hortorum</i> pv. <i>carotae</i>	CFBP 7900 ⁺	M081		<i>Daucus carota</i>	United States	2011	-	-	-	[24]
<i>X. hortorum</i> pv. <i>cynarae</i>	CFBP 4188^{PT}	ICMP 16775	<i>X. cynarae</i> Trébaol <i>et al.</i> 2000 <i>X. cynarae</i> pv. <i>cynarae</i> (Trébaol <i>et al.</i> 2000) Timilsina <i>et al.</i> 2019	<i>Cynara scolymus</i>	Bretagne, France	1996	-	-	NA	[60]
<i>X. hortorum</i> pv. <i>gardneri</i>	CFBP 8163^{PT}	ATCC 19865 NCPBP 881	<i>X. gardneri</i> (ex Šutič 1957) Jones <i>et al.</i> 2006 <i>X. cynarae</i> pv. <i>gardneri</i> (Jones <i>et al.</i> 2006) Timilsina <i>et al.</i> 2019	<i>Solanum lycopersicum</i>	Yugoslavia	1953	-	-	+	[23]
<i>X. hortorum</i> pv. <i>pelargonii</i>	CFBP 8588	JS749-3		<i>Solanum lycopersicum</i>	La Réunion, France	1997	-	-	+	[56]
	CFBP 2533^{PT}	LMG 7314 NCPBP 2985 ICMP 4321		<i>Pelargonium peltatum</i>	New Zealand	1974	-	-	NA	[63]
<i>X. hortorum</i> pv. <i>taraxaci</i>	CFBP 410^{PT}	ATCC 19318 LMG 870 NCPBP 940		<i>Taraxacum kok-sahgyz</i>	United States	1942	-	-	-	[63]
<i>X. hortorum</i> pv. <i>vitiens</i>	LM 16389	CFBP 8644		<i>Taraxacum sp.</i>	Isère, France	2016	-	-	NA	This study
	LMG 938^{neoPT}	CFBP 8686 NCPBP 2248	<i>X. campestris</i> pv. <i>vitiens</i> (Brown 1918) Dye 1978 “ <i>X. hortorum</i> pv. <i>vitiens</i> (Brown 1918) Vauterin <i>et al.</i> 1995”	<i>Lactuca sativa</i>	Zimbabwe	1966	+	+	NA	[63]
	CFBP 498	NCPBP 232		<i>Lactuca sp.</i>	United States	1949	+	+	NA	This study
	CFBP 499	NCPBP 969		<i>Lactuca scariola</i>	United States	1961	+	+	NA	This study
	CFBP 500	NCPBP 992		<i>Lactuca sp.</i>	United States	1949	+	+	NA	This study
	CFBP 3971			<i>Lactuca sativa</i>	France	1994	+	+	NA	This study
	CFBP 3973			<i>Lactuca sativa</i>	France	1994	+	+	NA	This study
	CFBP 3975			<i>Lactuca sativa</i>	France	1994	+	+	NA	This study
	CFBP 3976			<i>Lactuca sativa</i>	France	1994	+	+	NA	This study
	CFBP 3978			<i>Lactuca sativa</i>	France	1994	+	+	NA	This study
	CFBP 3980			<i>Lactuca sativa</i>	Isère, France	1995	+	+	NA	This study
	CFBP 3983			<i>Lactuca sativa</i>	Jura, France	1995	+	+	NA	This study
	CFBP 3984			<i>Lactuca sativa</i>	Vaucluse, France	1994	+	+	NA	This study
	CFBP 3985			<i>Lactuca sativa</i>	Rhône, France	1995	+	+	NA	This study
	CFBP 3986			<i>Lactuca sativa</i>	France	1994	+	+	NA	This study
	CFBP 3987			<i>Lactuca sativa</i>	France	1994	+	+	NA	This study
	CFBP 3990			<i>Lactuca sativa</i>	France	1995	+	+	NA	This study
CFBP 3993			<i>Lactuca sativa</i>	Loiret, France	1995	+	+	NA	This study	
CFBP 3995			<i>Lactuca sativa</i>	Isère, France	1996	+	+	NA	This study	

CFBP 3996			<i>Lactuca sativa</i>	Isère, France	1996	+	+	NA	This study
CFBP 7999	ICMP 7383	<i>X. gardneri</i> (ex Šutič 1957) Jones et al. 2006	<i>Solanum lycopersicum</i>	New Zealand	1980	+	+	+	[56]
LM 16382			<i>Lactuca sativa</i> cv. Escale	Isère, France	2016	+	+	NA	This study
LM 16383			<i>Lactuca sativa</i> cv. Escale	Isère, France	2016	+	+	NA	This study
LM 16384			<i>Lactuca sativa</i> cv. Escale	Isère, France	2016	+	+	NA	This study
LM 16386			<i>Lactuca sativa</i> cv. Escale	Isère, France	2016	+	+	NA	This study
LM 16387			<i>Lactuca sativa</i> cv. Minestrone	Isère, France	2016	+	+	NA	This study
LM 16388	CFBP 8640		<i>Lactuca sativa</i> cv. Minestrone	Isère, France	2016	+	+	-	This study
LM 16734	CFBP 8638		<i>Lactuca sativa</i> cv. Pattrinice	Savoie, France	2016	+	+	-	This study
LM 16735	CFBP 8639		<i>Lactuca sativa</i> cv. Almagro	Savoie, France	2016	+	+	w	This study
LM 16736			<i>Lactuca sativa</i> cv. Almagro	Savoie, France	2016	+	+	NA	This study
LM 16011A	CFBP 8641		<i>Lactuca sativa</i>	Ain, France	2016	+	+	-	This study
LM 16012			<i>Lactuca sativa</i>	Ain, France	2016	+	+	NA	This study
LM 16013			<i>Lactuca sativa</i>	Ain, France	2016	+	+	NA	This study
LM 16014			<i>Lactuca sativa</i>	Ain, France	2016	+	+	NA	This study
LM 16691	CFBP 8642		<i>Lactuca sativa</i> cv. Funride	Rhône, France	2016	+	+	NA	This study
LM 17421			<i>Lactuca sativa</i> cv. Almagro	Loire, France	2017	+	+	NA	This study
LM 17422			<i>Lactuca sativa</i> cv. Celesti	Loire, France	2017	+	+	NA	This study
LM 17423			<i>Lactuca sativa</i> cv. Almagro	Loire, France	2017	+	+	NA	This study
LM 17691			<i>Lactuca sativa</i> cv. Olana	Rhône, France	2017	+	+	NA	This study
LM 17692			<i>Lactuca sativa</i> cv. Olana	Rhône, France	2017	+	+	NA	This study
LM 17694			<i>Lactuca sativa</i>	Rhône, France	2017	+	+	NA	This study
LM 17695			<i>Lactuca sativa</i> cv. Kisheri	Rhône, France	2017	+	+	-	This study
LM 17696			<i>Lactuca sativa</i> cv. Oseka	Rhône, France	2017	+	+	NA	This study
LM 17697			<i>Lactuca sativa</i> cv. Analota	Rhône, France	2017	+	+	NA	This study
LM 17381			<i>Taraxacum</i> sp.	Isère, France	2017	+	+	-	This study
LM 17382			<i>Lactuca sativa</i> cv. Impression	Isère, France	2017	+	+	NA	This study
LM 17384			<i>Lactuca sativa</i> cv. Lilybel	Isère, France	2017	+	+	NA	This study
LM 17385			<i>Lactuca sativa</i> cv. Tourbillon	Isère, France	2017	+	+	NA	This study
LM 17388			<i>Lactuca sativa</i> cv. Tourbillon	Isère, France	2017	+	+	NA	This study
LM 173810			<i>Lactuca sativa</i> cv. Julena	Isère, France	2017	+	+	NA	This study
LM 173811			<i>Lactuca sativa</i> cv. Kisheri	Isère, France	2017	+	+	NA	This study
LM 173812			<i>Lactuca sativa</i> cv. Kisheri	Isère, France	2017	+	+	-	This study
LM 17011			<i>Lactuca sativa</i>	Ain, France	2017	+	+	NA	This study
LM 17012			<i>Lactuca sativa</i>	Ain, France	2017	+	+	NA	This study
LM 17013			<i>Lactuca sativa</i>	Ain, France	2017	+	+	NA	This study
LM 17014			<i>Lactuca sativa</i>	Ain, France	2017	+	+	NA	This study
LM 18071			<i>Lactuca sativa</i>	Ardèche, France	2018	+	+	NA	This study

LM 18072

Lactuca sativa

Ardèche, France

2018

+

+

NA

This study

^a Proposed nomenclature in accordance with the *International Code of Nomenclature of Prokaryotes* and the *International Standards for Naming Pathovars of Phytopathogenic Bacteria*

^b Two different nomenclatures may be displayed in order to respect validly published names and assure continuity through literature by adding the ones sometimes still used by researchers

^c Cancellation of CFBP 2538 as the pathotype strain of the *vitiensis* pathovar and proposal of LMG 938 as the neopathotype were submitted by letter to the Committee on the Taxonomy of Plant Pathogenic Bacteria

^d Representative strain CFBP 7900 of *X. hortorum* pv. *carotae* was chosen as the actual pathotype CFBP 4997 is known to be inconsistent

^e Pathogenicity assays conducted in this study: + = pathogenic, - = non-pathogenic, w = weakly pathogenic, NA = non-tested

TABLE 2. Pairwise ANIm and *isDDH* values among draft whole genome sequences of type, pathotype and representative strains of *X. hortorum* and *X. cynarae*. *X. populi* was chosen as an outgroup and type strain of *X. citri* and *X. axonopodis* were added for means of comparison to pathotype strain of *X. campestris* pv. *vitians*. ANIm values (%) are displayed in the lower triangle and *isDDH* values (%) in the upper triangle. Number in brackets indicates the percentage of aligned sequences used for calculation of ANIm between two genomes, and differences between ANIm reciprocal values were < 0.1 % in all comparisons. *isDDH* values are the point estimate plus the 95% model-based confidence intervals obtained with formula 2 as recommended at the GGDC web-server.

Current nomenclature	Proposed nomenclature	1	2	3	4	5	6	7	8	9	10	11
<i>X. cynarae</i> pv. <i>cynarae</i> CFBP 4188 ^T	1 ^a <i>X. hortorum</i> pv. <i>cynarae</i> CFBP 4188 ^T	-	94.9	87.3	79.5	68.5	68.6	67.3	44.7	34.4	34.4	34.9
<i>X. cynarae</i> pv. <i>gardneri</i> CFBP 8163 ^{PT}	2 ^a <i>X. hortorum</i> pv. <i>gardneri</i> CFBP 8163 ^{PT}	99.3 [93.3]	-	85.9	78.6	68	68.4	67.2	44.7	34.3	34.4	34.9
<i>X. campestris</i> pv. <i>vitians</i> LMG 938	3 <i>X. hortorum</i> pv. <i>vitians</i> LMG 938 ^{neopt}	98.4 [93.2]	98.3 [93.4]	-	80.8	68.2	68	67.1	44.5	34.4	34.3	34.9
<i>X. hortorum</i> pv. <i>taraxaci</i> CFBP 410 ^{PT}	4 <i>idem</i>	97.5 [92.9]	97.4 [93.2]	97.6 [94.0]	-	64.8	65	64.3	44.4	34.5	34.4	35.0
<i>X. hortorum</i> pv. <i>hederae</i> CFBP 5858 ^T	5 <i>idem</i>	96.1 [80.6]	96.0 [81.3]	96.1 [81.1]	95.6 [80.9]	-	71.1	68.8	44.4	34.8	34.5	35.0
<i>X. hortorum</i> pv. <i>carotae</i> CFBP 7900	6 <i>idem</i>	96.2 [86.8]	96.2 [87.8]	96.2 [87.0]	95.7 [86.3]	96.5 [85.1]	-	68.5	44.7	34.4	34.5	34.9
<i>X. hortorum</i> pv. <i>pelargonii</i> CFBP 2533 ^{PT}	7 <i>idem</i>	95.8 [86.0]	95.8 [85.8]	95.9 [85.8]	95.4 [89.7]	96.1 [83.4]	96.2 [86.5]	-	44.9	34.3	34.5	35.0
<i>X. populi</i> CFBP 1817 ^T	8 <i>idem</i>	91.2 [80.4]	91.4 [80.4]	91.3 [79.4]	91.3 [79.5]	91.3 [79.4]	91.4 [78.2]	91.4 [81.2]	-	33.5	33.6	33.9
<i>X. axonopodis</i> pv. <i>vitians</i> CFBP 2538 ^{PT}	9 <i>X. citri</i> CFBP 2538	88.2 [64.3]	88.2 [66.5]	88.2 [66.1]	88.2 [65.5]	88.3 [66.8]	88.2 [66.0]	88.1 [66.7]	87.7 [55.0]	-	52.6	89.5
<i>X. axonopodis</i> pv. <i>axonopodis</i> CFBP 4924 ^T	10 <i>idem</i>	88.1 [67.4]	88.1 [67.5]	88.1 [68.0]	88.1 [67.9]	88.1 [67.9]	88.0 [68.1]	88.1 [68.2]	87.63 [57.9]	93.4 [86.1]	-	53.5
<i>X. citri</i> CFBP 3369 ^T	11 <i>idem</i>	88.3 [64.3]	88.3 [64.6]	88.3 [64.8]	88.4 [64.4]	88.3 [63.4]	88.3 [64.6]	88.3 [64.5]	87.8 [52.6]	98.7 [89.8]	93.5 [85.2]	-

^T = type strain, ^{PT} = pathotype strain, ^{neopt} = neopathotype
^a = nomenclature *sensu* Timilsina *et al.* 2019

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TABLE 3. Phenotypic profiles of the different studied *Xanthomonas* using standard phenotypic tests and Biolog GEN III microplates with biological triplicates. Discriminative traits of *X. populi* reported in literature allowing to differentiate from *X. hortorum* emend. are highlighted in red. For Biolog GEN III results, characters negative for all strains tested are not displayed, resistance phenotypes are displayed in italic and shared stable traits for all *X. hortorum* emend. strains are highlighted in green.

Species ^a	1	2	3	4	5	6	7	8	9 [#]
Phenotypic tests									
Gram staining	rod negative	rod negative	rod negative	rod negative	rod negative	rod negative	rod negative	rod negative	rod negative
Optimum growth temperature	na	25 - 28°C	25 - 28°C	25 - 28°C	25 - 28°C	25 - 28°C	25 - 28°C	25 - 28°C	20 - 23°C
Starch hydrolysis	+	-	-	-	-	-	-	-	v
Gelatin hydrolysis	-	+	+	-	+	+	+	+	-
Motility	+	+	+	+	+	+	+	+	+
Hydrogen sulfide production	+	+	+	+	w	+	+	w	+
Indole production	+	w	w	w	w	w	w	w	-
Litmus milk									
Litmus reduction	-	+	+	+	+	+	+	+	v
Casein hydrolysatation	-	+	+	+	+	+	+	+	na
Casein precipitation	+	+	+	+	+	+	+	+	na
Biolog GEN III microplates^b									
Dextrin	+	-	-	w(-)	-	-	-	-	+
D-Maltose	+	-	-	-	-	-	-	-	na
D-Trehalose	+	+	+	+	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	+	+	+	-
β-Gentiobiose	+	+	+	-(+)	+	+	-	-	v
Sucrose	+	+	+	+	+	+	+	+	+
pH 6	+	+	+	+	+	+	+	+	+
<i>pH 5</i>	-	-	-(+)	-	-	-	-(+)	-	na
D-Raffinose	-	-	-	+(+)	-	-	-	-	-
α-D-Lactose	+	-	-	+(+)	-	+(+)	-	+	-
D-Melibiose	+	+	+	+	+	+	+	+	-
N-Acetyl-D-Glucosamine	+	+	+	+	+	+	+	+	v
1% NaCl	+	+	+	+	+	+	+	+	-
<i>4% NaCl</i>	w(+)	+	+	w(-)	-	+	v	+	-
<i>8% NaCl</i>	-	-	-	-	-	-(+)	-	-	-
D-Glucose	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+
D-Fucose	-	-	-	w(-)	-	-	-	-	na
L-Fucose	+	+	+	+	+	+	+	+	-
1% Sodium Lactate	+	+	+	+	+	+	+	+	na
D-Serine #2	-	-	-	-	-	+(+)	-	-	-
Glycerol	+	+	+	+	+	+	+	-	v
D-Fructose-6-Phosphate	w(-)	-	-	-	-	-	-	-	-
<i>Troleandomycin</i>	-	-(+)	v	-	-	-	-	-	na
<i>Rifamycin SV</i>	+(+)	-	+	-(+)	-(+)	-(+)	+(+)	-	na
Gelatin	+	+	+	+	+	+	+	+	-
Gly-Pro	+	+	+	+	+	+	+	v	na
L-Alanine	+	+	+	+	+	+	w	+	-
L-Aspartic Acid	-	-	+	w(-)	w(-)	v	w(-)	+	-
L-Glutamic Acid	+	+	+	+	+	+	+	+	-
L-Histidine	+	+	-	+	+	-(+)	w(+)	+	-
L-Serine	+	+	+	+	+	+	+	+	-
Lincomycin	+	+	+	+	+	+	+	+	na
<i>Guanidine Hydrochloride</i>	w(-)	+(+)	+	+	+(+)	+(+)	+	+(+)	na
<i>Niaproof</i>	-	+(+)	+	w(-)	v	w(-)	v	-	na
Pectin	+	+	+	+	+	+	-	+	na
D-Glucuronic Acid	-	-	-	w(-)	-	-	-	-	v
<i>Vancomycin</i>	-	v	w(-)	-	-	-	-	-	na
<i>Tetrazolium Violet</i>	+	+	+	-(+)	+(+)	+	+(+)	+	na
<i>Tetrazolium Blue</i>	+	+	+	+	+(+)	+	+	+	na
Methyl Pyruvate	+	+	+	+	+	+	+	+	v
L-Lactic Acid	+	+	w(-)	+	+(+)	+	w	+	na
Citric Acid	+	+	+	+	+	+	+	+	v
α-Keto-Glutaric Acid	+	+	+	+	+	+	+	+	v
L-Malic Acid	+	+	+	+	+	+	+	+	na
Bromo-Succinic Acid	+	w(+)	+	+	+	+	+	+	v
<i>Lithium Chloride</i>	+	+(+)	+	+(+)	-(+)	+(+)	+(+)	w(-)	na
<i>Potassium Tellurite</i>	-	+	+	w(+)	-	+	-	-	na
Tween 40	-	w(+)	+	w	w(+)	+	w(-)	+	-

α -Hydroxy-Butyric Acid	w(-)	-	w(-)	w(-)	v	-	-	w(-)	-
α -Keto-Butyric Acid	+	-	+(-)	+	-(+)	+	v	+	-
Acetoacetic Acid	+	w(+)	-	+	+	w(+)	w	+	na
Propionic Acid	+	+	+	+	+	+	+	+	-
Acetic Acid	+	+	+	+	+	+	+	+	v
Sodium Formate	+	+	+(-)	-(+)	+(-)	w(+)	+(-)	+	na
Aztreonam	+	+	+	+	+	+	+	+(-)	na
Sodium Bromate	-	+	v	-	-	w(+)	v	w(-)	na

^a Species (names as proposed in this study) :

1 = *X. citri* CFBP 2538, **2** = *X. hortorum* pv. *pelargonii* CFBP 2533^{PT}, **3** = *X. hortorum* pv. *carotae* CFBP 7900, **4** = *X. hortorum* pv. *hederae* CFBP 5858^T,
5 = *X. hortorum* pv. *taraxaci* CFBP 410^{PT}, **6** = *X. hortorum* pv. *vitians* LMG 938^{neOPT}, **7** = *X. hortorum* pv. *cynarae* CFBP 4188^{PT},
8 = *X. hortorum* pv. *gardneri* CFBP 8163^{PT}, **9** = *X. populi* as reported in Van den Mooter and Swings 1990 [63], Ridé and Ridé 1992 [42] and Vauterin *et al.* 1995 [62]

^b Reactions were classified as positive (+), weak (w) or negative (-). Management of triplicates was achieved using the following equivalences :

+ = (+/+)+ or (+/+w), w = (w/w/w), - = (-/-/-) or (-/-/w), +(-) = (+/+/-), -(+) = (-/-/+), w(+) = (w/w/+), w(-) = (w/w/-), v = (-/w/+)

^c For *X. populi*, (-) and (+) mean that characteristics were either negative or positive in the three studies where data were extracted, (na) that these tests were not conducted and (v) that variable results were described in the different studies.





