Studholme et al. Open Letter to the editor of Phytopathology

1 Transfer of Xanthomonas campestris pv. arecae, and Xanthomonas campestris pv. musacearum to

2 *Xanthomonas vasicola* (Vauterin) as *Xanthomonas vasicola* pv. *arecae* comb. nov., and *Xanthomonas*

3 *vasicola* pv. *musacearum* comb. nov. and description of *Xanthomonas vasicola* pv. *vasculorum* pv. nov.

4 AUTHORS

- David J. Studholme, Emmanuel Wicker, Sadik Muzemil Abrare, Andrew Aspin, Adam Bogdanove, Kirk
 Broders, Zoe Dubrow, Murray Grant, Jeffrey B. Jones, Georgina Karamura, Jillian Lang, Jan Leach,
 George Mahuku, Gloria Valentine Nakato, Teresa Coutinho, Julian Smith, Carolee T. Bull
- 8
- 9 Corresponding author: David J. Studholme (<u>d.j.studholme@exeter.ac.uk</u>)
- 10

11 **ABSTRACT**

12 Recent DNA sequence and other data indicated that several important bacterial pathogens should be 13 transferred into the species Xanthomonas vasicola Vauterin 1995. The first objective of this letter is 14 to propose the transfer of X. campestris pv. musacearum (Yirgou and Bradbury 1968) Dye 1978 to X. vasicola Vauterin 1995. The second objective is to give a clear overview of the different evolutionary 15 16 lineages that constitute the species X. vasicola, in the light of recent genomics analyses. These analyses 17 also indicate that strains described as [X. campestris pv. zeae] (Qhobela, Claflin, and Nowell 1990; 18 Coutinho and Wallis 1991) fall within the species X. vasicola Vauterin 1995. Furthermore, the sequence of its gyrB gene suggested that X. campestris pv. arecae (Rao and Mohan 1970) Dye 1978 is 19 20 closely related to the type strain of X. vasicola. Note that in this manuscript pathovar names that have 21 no valid standing in nomenclature are presented with square brackets as is standard.

Studholme et al. Open Letter to the editor of Phytopathology

22

23 AUTHOR ADDRESSES

24

24		
25	•	David J. Studholme: Biosciences, University of Exeter, Exeter, United Kingdom
26	٠	Emmanuel Wicker: UMR IPME, Univ Montpellier, CIRAD, IRD, Montpellier, France
27	•	Sadik Muzemiln Abrar: Southern Agricultural Research Institute (SARI), Areka Agricultural
28		Research Center, Areka, Ethiopia
29	•	Andrew Aspin: Fera Science Ltd. York, UK
30	٠	Adam Bogdanove: Plant Pathology and Plant-Microbe Biology Section, School of Integrative
31		Plant Science, Cornell University, 334 Plant Science Building, Ithaca, NY 14853, USA
32	•	Kirk Broders: Department of Bioagricultural Sciences and Pest Management, Colorado State
33		University
34	٠	Zoe Dubrow: Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant
35		Science, Cornell University, 334 Plant Science Building, Ithaca, NY 14853, USA
36	٠	Murray Grant: School of Life Sciences, Gibbet Hill, University of Warwick, Coventry, CV4 7AL,
37		UK
38	٠	Jeffrey B. Jones: University of Florida, Plant Patholgoy Dept., 1453 Fifield Hall, Gainesville,
39		Florida, United States , 32611
40	٠	Georgina Karamura: Bioversity International, Uganda
41	٠	Jillian Lang: Department of Bioagricultural Sciences and Pest Management, Colorado State
42		University, Fort Collins, CO 80523
43	٠	Jan Leach: Bioagricultural Sciences and Pest Management, Colorado State University, Ft
44		Collins, CO 80523
45	٠	George Mahuku: International Institute of Tropical Agiculture (IITA), East Africa Hub, IITA-
46		Tanzania P.O. Box 34441, Dar es Salaam, Tanzania
47	٠	Gloria Valentine Nakato: International Institute of Tropical Agiculture (IITA), Plot 15B, Naguru
48		East Road, Upper Naguru, P.O. Box 7878, Kampala, Uganda
49	٠	Teresa Coutinho: Department of Microbiology and Plant Pathology, Centre for Microbial
50		Ecology and Genomics (CMEG), Forestry and Agricultural Biotechnology Institute (FABI),
51		University of Pretoria, Private Bag X28, Pretoria 0028, South Africa
52	٠	Julian Smith: Fera Science Ltd. York, UK
53	٠	Carolee T. Bull: Department of Plant Pathology and Environmental Microbiology, Penn State

54 University, University Park, Pennsylvania, USA

Studholme et al. Open Letter to the editor of Phytopathology

56 **LETTER TO THE EDITOR**

Members of the genus Xanthomonas, within the gamma-Proteobacteria, collectively cause disease on 57 more than 400 plant species (Hayward 1993), though some members are apparently non-pathogenic 58 59 (Vauterin et al. 1996) and some have been isolated from clinical samples such as skin microbiota (Seité, 60 Zelenkova, and Martin 2017). Historically, taxonomy of *Xanthomonas* was tied to the plant host of isolation (Starr 1981; Wernham 1948), with the genus being split into large numbers of species, each 61 defined by this single phenotypic feature (Dye 1962). Subsequently, most of the species were 62 63 transferred (lumped) into a single species, X. campestris, and designated as nomenspecies because 64 the organisms could not be distinguished from one another by phenotypic and physiological tests 65 (Lapage et al. 1992; Dye and Lelliott 1974). As a temporary solution, and to help to maintain a connection with the historical and plant pathological literature, these nomenspecies were designated 66 67 as pathovars within X. campestris, each defined by host range or disease syndrome (Dye et al. 1980). 68 More recently, DNA sequence comparisons and biochemical approaches revealed that some of the 69 host ranges of pathovars of X. campestris were not correlated with inferred phylogenies (Parkinson et 70 al. 2007, 2009; Rodriguez-R et al. 2012). There have been heroic advances to improve the taxonomy 71 of the genus as a whole (Vauterin et al. 1990; Vauterin, Rademaker, and Swings 2000; Rademaker et 72 al. 2005; Vauterin et al. 1995) and individual taxa (da Gama et al. 2018; Constantin et al. 2016; Trébaol 73 et al. 2000; Timilsina et al. 2019; Jones et al. 2004), based on phenotypic, chemotaxonomic and 74 genotypic analyses, but in a number of taxa there remain issues not fully resolved.

The bacterial pathogen *X. campestris* pv. *musacearum* (Yirgou and Bradbury 1968) Dye 1978 presents a major threat to cultivation of banana and enset crops in central and eastern Africa, where it causes banana Xanthomonas wilt (BXW) and enset Xanthomonas wilt (EXW). Originally described as *X. musacearum* (Yirgou and Bradbury 1968), this pathogen was first isolated from enset and banana in Ethiopia in the 1960s and early 1970s, respectively (Yirgou and Bradbury 1968, 1974), although

Studholme et al. Open Letter to the editor of *Phytopathology*

symptoms consistent with EXW were reported for Ethiopia as early as the 1930s (Castellani 1939).
However, only in the 21st century did the disease establish in the banana-growing areas of Burundi,
Democratic Republic of Congo, Kenya, Rwanda, Tanzania and Uganda, (Biruma et al. 2007;
Tushemereirwe et al. 2004; Ndungo et al. 2006; Reeder et al. 2007; Carter et al. 2010). In this region
around the Great Lakes of eastern and central Africa, BXW disease severely challenges livelihoods and
food security (Blomme et al. 2017; Shimwela et al. 2016; Tinzaara et al. 2016; Blomme et al. 2013;
Biruma et al. 2007; Nakato, Mahuku, and Coutinho 2018).

87 There is confusion in the literature about the taxonomy of this bacterium. Since its assignment to X. 88 campestris (Young et al. 1978), molecular sequence and biochemical data indicate that this pathogen 89 is more closely related to X. vasicola (Parkinson et al. 2007; Aritua et al. 2007) as detailed below. Thus, 90 the first objective of this letter is to propose the transfer of X. campestris pv. musacearum (Yirgou and 91 Bradbury 1968) Dye 1978 to X. vasicola Vauterin 1995. The second objective is to give a clear overview 92 of the different evolutionary lineages that constitute the species X. vasicola, in the light of recent 93 genomics analyses. These analyses also indicate that strains described as [X. campestris pv. zeae] 94 (Qhobela, Claflin, and Nowell 1990; Coutinho and Wallis 1991) fall within a clade of X. campestris pv. 95 vasculorum (Cobb 1894) Dye 1978 that belongs within the species X. vasicola Vauterin 1995. 96 Furthermore, the sequence of its gyrB gene suggested that X. campestris pv. arecae (Rao and Mohan 97 1970) Dye 1978 is closely related to the type strain of X. vasicola (Parkinson et al. 2009). Note that in 98 this manuscript pathovar names that have no valid standing in nomenclature are presented with 99 square brackets as is standard (Bull et al. 2012).

The species *X. vasicola* (Vauterin et al. 1995) was created to encompass *X. campestris* pv. *holcicola* (Elliott 1930) Dye 1978 and a subset of strains (not including the pathotype) of *X. campestris* pv.
 vasculorum (Cobb 1894) Dye 1978 (Young et al. 1978; Vauterin et al. 1995). Taxonomic studies
 revealed that *X. campestris* pv. *vasculorum* contained groups of strains that are clearly distinguishable

Studholme et al. Open Letter to the editor of *Phytopathology*

104 from the pathotype strain (of X. campestris pv. vasculorum) by phenotypic and molecular traits despite 105 their shared host ranges (Vauterin et al. 1992; Péros et al. 1994; Dookun, Stead, and Autrey 2000; 106 Stead 1989; Vauterin et al. 1995; Destéfano et al. 2003). Vauterin's type-B strains are distinguished 107 from type-A strains (to which the pathotype strain of X. campestris pv. vasculorum belongs) by SDS-108 PAGE of proteins, gas chromatography of fatty acid methyl esters and DNA-DNA hybridization (Yang 109 et al. 1993). Type-A and type-B strains can also be distinguished by PCR-RFLP analysis (Destéfano et 110 al. 2003). Table 1 lists examples of X. campestris pv. vasculorum (Cobb 1894) Dye 1978 strains that 111 were classified in one or more of those studies. Vauterin and colleagues assigned type-A strains, along 112 with the pathotype, to X. axonopodis pv. vasculorum (Cobb) Vauterin, Hoste, Kersters & Swings and 113 type-B to [X. vasicola pv. vasculorum] (Vauterin et al. 1995). However, we note that this pathovar is 114 invalid because of the lack of a formal proposal differentiating it from other pathovars (Young et al. 115 2004) and no designation of a pathotype strain. For a given organism, competing classifications and 116 invalid names result in three different valid species names, X. campestris, X. axonopodis and X. 117 vasicola, having been used in the literature. For example, various authors have referred to strain 118 NCPPB 1326 as X. campestris pv. vasculorum, X. axonopodis pv. vasculorum (to which the strain clearly 119 does not belong) or [X. vasicola pv. vasculorum] (Wasukira et al. 2014; Lewis Ivey, Tusiime, and Miller 120 2010; Qhobela, Claflin, and Nowell 1990; Qhobela and Claflin 1992). Type-B strains NCPPB 702, NCPPB 121 1326 and NCPPB 206 were erroneously described as X. axonopodis pv. vasculorum (Lewis Ivey, 122 Tusiime, and Miller 2010) though they are clearly members of X. vasicola. However, we acknowledge 123 that examples of mistakes such as these will not likely be resolved by transfer of the pathovars from 124 X. campestris into X. vasicola.

A further source of confusion is the status of strains isolated from maize for which some authors use the invalid name [*X. campestris* pv. *zeae*] (Qhobela, Claflin, and Nowell 1990; Coutinho and Wallis 127 1991). Adding to the confusion, at least one strain of *X. campestris* pv. *vasculorum* (NCPPB 206) isolated from maize has the fatty-acid type characteristic of *X. vasicola* (Dookun, Stead, and Autrey

Studholme et al. Open Letter to the editor of *Phytopathology*

129 2000); consistent with this, on the basis of phylogenetic analysis of DNA sequence, NCPPB 206 falls 130 among strains assigned to Vauterin's invalid [X. vasicola pv. vasculorum] (Wasukira et al. 2014). A 131 useful nomenclature for this group has become more pressing since the recent outbreak of leaf streak 132 on corn in the USA, caused by bacteria very closely related to strains previously described as [X. 133 campestris pv. zeae]. One of these strains, NCPPB 4614 (=SAM119), has been suggested to be the 134 eventual pathotype strain of X. vasicola pv. vasculorum though no valid proposal has been made (Lang 135 et al. 2017; Korus et al. 2017). Although [X. vasicola pv. vasculorum] (Vauterin et al. 1995) is invalid, 136 this name has come to be understood by the community to represent a meaningful biological reality; 137 that is a set of X. campestris pv. vasculorum strains that are biochemically and phylogenetically similar 138 to X. vasicola. Therefore, below we propose a formal description of X. vasicola pv. vasculorum pv. 139 nov. that should be considered valid to harmonize the formal nomenclature with that which is in use. 140 Further, consistent with the previous suggestion (Lang et al. 2017) that strains classified to [X. vasicola 141 pv. vasculorum] and [X. campestris pv. zeae] (Vauterin et al. 1995) are insufficiently distinct to warrant separate pathovars, we therefore propose that [X. vasicola pv. vasculorum] group B, [X. campestris 142 143 pv. zeae] and phylogenetically closely related strains isolated from sugarcane and maize be assigned 144 into the newly described X. vasicola pv. vasculorum pv. nov.

145 Vauterin et al. (1995) designated the pathotype strain of X. vasicola pv. holcicola (LMG 736, NCPPB 146 2417, ICMP 3103 and CFBP 2543) as the type strain of X. vasicola, although they did not use the pathovar epithet for the specific epithet of the species as is most appropriate to indicate this 147 148 relationship. The natural host range of X. vasicola pv. holcicola includes the cereal crops millet and 149 sorghum on which it causes bacterial leaf streak (Table 2). The host range of the strains that Vauterin 150 et al. (1995) called [X. vasicola pv. vasculorum] is less well defined because in most of the relevant 151 pre-1995 literature it is impossible to distinguish between type-A and type-B of X. campestris pv. 152 vasculorum and therefore between X. axonopodis pv. vasculorum and strains belonging to X. vasicola. 153 However, X. campestris pv. vasculorum type-B strains (that is, members of X. vasicola) have been

Studholme et al. Open Letter to the editor of *Phytopathology*

isolated from sugarcane and maize and shown to infect these hosts on artificial inoculation (Vauterinet al. 1995; Karamura et al. 2015).

156 Previous studies suggested a close relationship between X. campestris pv. musacearum (Yirgou and 157 Bradbury 1968) Dye 1978b and X. vasicola py. holcicola (Elliott 1930) Vauterin et al. 1995 based on fatty acid methyl ester analysis, genomic fingerprinting using rep-PCR and partial nucleotide 158 159 sequencing of the gyrB gene (Aritua et al. 2007; Parkinson et al. 2009). Draft or complete sequence 160 assemblies are now available for more than a thousand Xanthomonas genomes, including those of 161 type strains for most species and pathotypes for most pathovars. Genome-wide sequence data can 162 offer some advantages, such as generally applicable threshold values for species delineation (Glaeser 163 and Kämpfer 2015; Meier-Kolthoff et al. 2013; Meier-Kolthoff, Klenk, and Göker 2014; Richter and Rosselló-Móra 2009). Therefore, we further explored relationships among these organisms using 164 165 whole genome sequences. We calculated pairwise average nucleotide identity (ANI) between X. 166 campestris pv. musacearum and representative Xanthomonas strains, including all available species 167 type strains and relevant pathotype strains. A representative subset of these pairwise ANI percentages 168 is tabulated in Figure 1. This revealed that the pathotype strain of X. campestris pv. musacearum 169 (Yirgou and Bradbury 1968) Dye 1978b shares 98.43 % ANI with the type strain of X. vasicola but only 170 87.27 % with the type strain of X. campestris. As expected, strains of X. vasicola pv. holcicola share 171 high ANI (> 99.6 %) with the X. vasicola type strain NCPPB 2417, which is also the pathotype strain of X. vasicola pv. holcicola (Elliott 1930) Vauterin et al. 1995. Also as expected, strains of X. campestris 172 173 pv. vasculorum previously called [X. vasicola pv. vasculorum] or [X. campestris pv. zeae], including 174 sequenced strain SAM119 (=NCPPB 4614) from corn isolated by T. Coutinho (Qhobela, Claflin, and 175 Nowell 1990), share > 98.5 % ANI with the type strain of X. vasicola, supporting the need to transfer 176 these strains to this species. Furthermore, unclassified strains NCPPB 902, NCPPB 1394, NCPPB 1395 177 and NCPPB 1396, from *Tripsacum laxum* (Mulder 1961) and the pathotype strain of X. *campestris* pv. 178 arecae (Rao and Mohan 1970) Dye 1978 (NCPPB 2649) all share more than 98 % ANI with the type

Studholme et al. Open Letter to the editor of *Phytopathology*

strain of *X. vasicola*, which places them unambiguously within *X. vasicola*. The next-nearest species to *X. vasicola* is *X. oryzae*; ANI between the respective type strains of these two species is 91.7%. It has been proposed that the boundary of a prokaryotic species can be delimited by 95 to 96% (Richter and Rosselló-Móra 2009). By this criterion, *X. campestris* pv. *arecae*, *X. campestris* pv. *musacearum* and strains from corn that are referred to by the invalid name [*Xanthomonas vasicola* pv. *zeae*] clearly fall within *X. vasicola* and outside *X. campestris*.

The high ANI levels clearly delineate a genomo-species that includes the type strain *X. vasicola* NCPPB 2417. Nevertheless, despite the usefulness of ANI for delimiting species boundaries, it does not include any model of molecular evolution and thus is unsuited for phylogenetic reconstruction. Therefore, we used RaxML via the RealPhy pipeline (Bertels et al. 2014; Stamatakis, Ludwig, and Meier 2005) to elucidate phylogenetic relationships, based on genome-wide sequencing data. This approach has the additional advantage of being based on sequence reads rather than on genome assemblies, where the latter may be of variable quality and completeness (Bertels et al. 2014).

192 Figure 2 depicts the phylogeny of X. vasicola based on RealPhy analysis of genome-wide sequence 193 data. Pathovars X. vasicola pv. holcicola and X. campestris pv. musacearum are monophyletic, 194 comprising well supported clades within the X. vasicola genomospecies. A third well supported clade includes the four "Xanthomonas sp." strains originating from Tripsacum laxum. A fourth clade consists 195 196 of mostly X. campestris pv. vasculorum strains isolated from sugarcane but also includes X. campestris 197 pv. vasculorum strain NCPPB 206 isolated from maize and several strains from maize attributed to the 198 invalid name [X. campestris pv. zeae]. This indicates that sequenced strains of [X. campestris pv. zeae] 199 from corn (Sanko et al. 2018; Lang et al. 2017; Qhobela, Claflin, and Nowell 1990; Coutinho and Wallis 200 1991) are monophyletic and fall within the clade containing type-B strains of X. campestris pv. 201 vasculorum (Figure 2). The single sequenced pathotype strain of X. campestris pv. arecae falls

Studholme et al. Open Letter to the editor of *Phytopathology*

immediately adjacent to the *X. vasicola* clade containing strains from corn and *X. campestris* pv. *vasculorum* type B strains (Figure 2).

204 Overall, our molecular sequence analyses strongly point to the existence of a phylogenetically 205 coherent species X. vasicola that includes strains previously assigned to X. campestris pathovars 206 musacearum, arecae, and some strains of X. campestris pv. vasculorum and strains collected from corn 207 and T. laxum grass that have not been previously assigned to species nor pathovar. Here we propose 208 that the pathovar Xanthomonas vasicola pv. vasculorum pv. nov. includes strains formerly classified 209 as X. campestris pv. vasculorum but distinguishable from X. axonopodis pv. vasculorum (Cobb) 210 Vauterin, Hoste, Kersters & Swings by protein SDS-PAGE, fatty acid methyl esterase (FAME) analysis 211 and DNA hybridisation (Vauterin et al. 1992; Yang et al. 1993; Vauterin et al. 1995). Our analyses also 212 support the transfer of X. campestris pv. arecae (Rao and Mohan 1970) Dye 1978 to X. vasicola. 213 Although only a single genome of this pathovar has been sequenced, that genome belongs to the 214 pathotype strain for this pathovar (Rao and Mohan 1970; C.T. Bull et al. 2010).

215 Our results are consistent with previous evidence for similarity between X. campestris pv. 216 musacearum (Yirgou and Bradbury 1968) Dye 1978 and strains of X. vasicola, based on FAME, genomic fingerprinting with rep-PCR and gyrB sequencing (Aritua et al. 2007; Parkinson et al. 2007). The formal 217 species description for X. vasicola states that this species can be clearly distinguished by its FAME 218 219 profiles (Vauterin et al. 1995). Pathogenicity studies demonstrated phenotypic distinctiveness of X. 220 campestris pv. musacearum (Yirgou and Bradbury 1968) Dye 1978 on banana; X. campestris pv. 221 musacearum produces severe disease whereas X. vasicola pv. holcicola NCPPB 2417 and X. campestris pv. vasculorum NCPPB 702 (which belongs to X. vasicola) showed no symptoms (Aritua et al. 2007). 222 223 The species description (Vauterin et al. 1995) also states that X. vasicola is characterised by metabolic 224 activity on the carbon substrates D-psicose and L-glutamic acid, and by a lack of metabolic activity on 225 the carbon substrates N-acetyl-D-galactosamine, L-arabinose, a-D-lactose, D-melibiose, P-methyl-D-

Studholme et al. Open Letter to the editor of Phytopathology

glucoside, L-rhamnose, D-sorbitol, formic add, D-galactonic acid lactone, D-galacturonic acid, Dgluconic acid, D-glucuronic acid, p-hydroxyphenylacetic acid, a-ketovaleric acid, quinic acid,
glucuronamide, L-asparagine, L-histidine, L-phenylalanine, urocanic acid, inosine, uridine, thymidine,
DL-a-glycerol phosphate, glucose 1-phosphate, and glucose 6-phosphate. We are not aware that these
metabolic activities have been tested for *X. campestris* pv. *arecae*, *X. campestris* pv. *musacearum* and
strains referred to as [*X. campestris* pv. *zeae*]; it is possible that the species description may need to
be amended to accommodate any deviation from this definition among the repositioned pathovars.

233 Overall, it seems that the species X. vasicola (including X. vasicola pv. holcicola, X. campestris pv. 234 vasculorum type-B strains, [X. campestris pv. zeae] strains, X. campestris pv. arecae and some strains 235 isolated from T. laxum) is almost exclusively associated with monocot plants of the families Palmae 236 and Gramineae. In this respect, it is similar to its closest sibling species X. oryzae, whose host range is 237 limited to Gramineae (Bradbury 1986). The exception is a report of leaf blight and dieback in 238 Eucalyptus caused by X. vasicola (Coutinho et al. 2015), remarkable given the phylogenetic distance 239 between this dicot plant and the usual monocot hosts of X. vasicola; the infected South African 240 plantation was in an area where sugarcane is grown.

241 In conclusion, analysis of available genome sequence data, combined with published pathogenicity 242 and biochemical data, strongly support the transfer of X. campestris pathovars arecae and 243 musacearum to the species X. vasicola as X. vasicola pv. musacearum comb. nov. with NCPPB 2005 as 244 the pathotype strain (being the type strain of X. musacearum and pathotype strain of X. campestris 245 pv. musacearum) and X. vasicola pv. arecae comb. nov with NCPPB 2649 as the pathotype strain (being 246 the type strain of X. arecae and pathotype strain of X. campestris pv. arecae). Strains NCPPB 206, 247 NCPPB 702, NCPPB 795, NCPPB 890, NCPPB 895, NCPPB 1326, NCPPB 1381, and NCPPB 4614 form a 248 phylogenetically and phenotypically coherent group with a distinctive host range causing symptoms 249 on maize and sugarcane but not on banana (Aritua et al. 2007; Karamura et al. 2015). We designate

Studholme et al. Open Letter to the editor of Phytopathology

250 the pathotype strain for X. vasicola pv. vasculorum pv. nov. as NCPPB 4614. This strain was previously proposed as the pathotype of X. vasicola pv. vasculorum (Lang et al. 2017) and causes disease 251 252 symptoms on maize and sugarcane (Lang et al. 2017) but not on banana (unpublished observation, Z. Dubrow and A. Bogdanove). Furthermore, given that strains from corn formerly described by the 253 invalid name [X. campestris pv. zeae] are members of X. vasicola and have host ranges that can not be 254 255 distinguished from the pathotype strain of X. vasicola pv. vasculorum, we propose that these strains 256 are members of this pathovar. Phylogenetic data support this as the corn strains represent a sub-clade 257 within strains of *X. campestris* pv. *vasculorum* that fall within the emended *X. vasicola*.

258 EMENDED DESCRIPTION OF XANTHOMONAS VASICOLA VAUTERIN ET.

259 AL., 1995.

The characteristics are as described for the genus and the species (Vauterin et al., 1995) extended 260 261 with phylogenetic data from this study. The species can be clearly distinguished from other 262 xanthomonads by MLSA and whole genome sequence analysis with members having more than 98 % 263 ANI with the type strain. SDS-PAGE protein and FAME profiles have been shown to be distinguishing 264 for some pathovars (Yang et al. 1993; Vauterin et al. 1992; Aritua et al. 2007), by the presence of 265 metabolic activity on the carbon substrates D-psicose and L-glutamic acid, and by a lack of metabolic 266 activity on the carbon substrates N-acetyl-D-galactosamine, L-arabinose, a-D-lactose, D-melibiose, P-267 methyl-D- glucoside, L-rhamnose, D-sorbitol, formic add, D-galactonic acid lactone, D-galacturonic 268 acid, D-gluconic acid, D-glucuronic acid, p-hydroxyphenylacetic acid, a-ketovaleric acid, quinic acid, 269 glucuronamide, L-asparagine, L-histidine, L-phenylalanine, urocanic acid, inosine, uridine, thymidine, 270 DL-a-glycerol phosphate, glucose 1-phosphate, and glucose 6-phosphate. The G+C content is between 271 63.1 and 63.6 mol % as calculated from whole-genome sequence data. The type strain is X. vasicola 272 pv. holcicola LMG 736 (= NCPPB 2417 = ICMP 3103 = CFBP 2543).

273

X. vasicola pv. holcicola Vauterin et al., 1995.

274 = *X. campestris* pv. *holcicola* (Elliott) Dye 1978.

275 Description is as presented by Vauterin et al., (1995). The pathovar is distinguished on the 276 basis of phytopathogenic specialization. As shown here and elsewhere (Lang et al. 2017), the 277 pathovar is distinct from other pathovars by MLSA and genome-wide sequence analysis. 278 According to Bradbury (1986), gelatin and starch are hydrolysed by most isolates examined. 279 The natural host range includes: *Panicum miliaceum, Sorghum* spp., *S. almum, S. bicolor (S.*

Studholme et al. Open Letter to the editor of Phytopathology

vulgare), S. caffrorum, S. durra, S. halepense, S. sudanense, S. technicum (S. bicolor var.
technicus), Zea mays. The artificial host range (by inoculation) includes Echinochloa

282 frumentacea, Pennisetum typhoides, Setaria italica.

283 Pathotype strain: PDDCC 3103; NCPPB 2417.

284 X. vasicola pv. vasculorum pv. nov.

Description as for the species and this pathovar is distinguished on the basis of 285 286 phytopathogenic specialization and includes the strains of the former taxon X. campestris pv. 287 vasculorum type B and pathogens from corn. The pathovar is identified to species and 288 distinguished from other pathovars by its gyrB gene sequence (Parkinson et al. 2009) and 289 genome-wide sequence analysis. It is not known whether the strains being transferred to this 290 taxon conform to the species description for metabolic activity. According to previously 291 published work (Coutinho et al. 2015; Aritua et al. 2007; Karamura et al. 2015; Hayward 292 1962)(unpublished observation, S. Dubrow and A. Bogdanove) the natural host range 293 includes: Saccharum spp., Zea mays, Eucalyptus grandis.

294 Pathotype strain: NCPPB 4614; SAM119.

295 X. vasicola pv. arecae (Rao & Mohan) Dye 1978 comb. nov.

296 = *X. campestris* pv. *arecae* (Rao & Mohan) Dye 1978.

297 Description as for the species and this pathovar is distinguished on the basis of phytopathogenic specialization. The pathovar is identified to species and distinguished from 298 299 other pathovars by its gyrB gene sequence (Parkinson et al. 2009) and by genome-wide 300 sequence analysis. According to Bradbury (1980) the natural host range includes: Areca 301 catechu (areca nut). Bradbury (1986) reports the artificial host range to include: Cocos nucifera 302 (coconut). Needle prick into sugar cane produced limited streaks, but the bacteria did multiply 303 to some extent and could be re-isolated. Disease: leaf stripe. Long, narrow water-soaked 304 lesions, becoming dark brown or black with age. It is not known if the strains being transferred 305 to this taxon conform to the species description for metabolic activity.

306 Pathotype strain: NCPPB 2649; PDDCC 5791.

307 *X. vasicola* pv. *musacearum* (Yirgou & Bradbury) Dye 1978 comb. nov.

308 = *X. campestris* pv. *musacearum* (Yirgou & Bradbury) Dye 1978.

309 Description as for the species and this pathovar is identified to species and distinguished on 310 the basis of phytopathogenic specialization and is distinct from other pathovars by its *gyrB* 311 gene sequence (Parkinson et al. 2009) and genome-wide sequence analysis. Gelatin slowly 312 liquefied, starch not hydrolysed. Growth quite rapid and very mucoid. According to Bradbury

- 313 (1986), the natural hosts include: *Ensete ventricosum* (enset), *Musa* spp. (banana). Additional
- hosts by inoculation: *Saccharum* sp. (sugarcane), *Zea mays* (maize) and disease is exhibited as
- 315 a bacterial wilt where leaves wilt and wither; yellowish bacterial masses are found in vascular
- 316 tissue and parenchyma. It is not known if the strains being transferred to this taxon conform
- 317 to the species description for metabolic activity.
- 318 Pathotype strain: NCPPB 2005; PDDCC 2870.
- 319
- 320

Studholme et al. Open Letter to the editor of Phytopathology

321

322 **REFERENCES**

- Aritua, V., Parkinson, N., Thwaites, R., Heeney, J. V, Jones, D. R., Tushemereirwe, W., et al. 2007.
- 325 Characterization of the *Xanthomonas* sp. causing wilt of enset and banana and its proposed
- 326 reclassification as a strain of *X. vasicola*. Plant Pathol. 57:170–177.
- 327 Bertels, F., Silander, O. K., Pachkov, M., Rainey, P. B., and van Nimwegen, E. 2014. Automated 328 reconstruction of whole-genome phylogenies from short-sequence reads. Mol. Biol. Evol. 31:1077– 329 88.
- Biruma, M., Pillay, M., Tripathi, L., Blomme, G., Abele, S., Mwangi, M., et al. 2007. Banana *Xanthomonas* wilt: A review of the disease, management strategies and future research directions.
 African J. Biotechnol. 6:953–962.
- Blomme, G., Dita, M., Jacobsen, K., Pérez-Vicente, L., Molina, A., Ocimati, W., et al. 2017. Bacterial
 diseases of bananas and enset: current state of knowledge and integrated approaches towards
 sustainable management. Front. Plant Sci. 8:1290.
- Blomme, G., Ploetz, R., Jones, D., De Langhe, E., Price, N., Gold, C., et al. 2013. A historical overview of
- the appearance and spread of *Musa* pests and pathogens on the African continent: highlighting the
- importance of clean *Musa* planting materials and quarantine measures. Ann. Appl. Biol. 162:4–26.
- 339 Bradbury, J. F. 1986. *Guide to plant pathogenic bacteria*. CAB International.
- Bull, C. T., De Boer, S. H., Denny, T. P., Firrao, G., Fischer-Le Saux, M., Saddler, G. S., et al. 2012. List of
 new names of plant pathogenic bacteria (2008-2010). J. Plant Pathol. 94:21–27.
- 342 C.T. Bull, 1, S.H. De Boer, 2, T.P. Denny, 3, et al. 2010. Comprehensive list of names of plant pathogenic
- 343 bacteria, 1980-2007. J. Plant Pathol. 92:551–592.

Studholme et al. Open Letter to the editor of Phytopathology

- 344 Carter, B. A., Reeder, R., Mgenzi, S. R., Kinyua, Z. M., Mbaka, J. N., Doyle, K., et al. 2010. Identification
- 345 of Xanthomonas vasicola (formerly X. campestris pv. musacearum), causative organism of banana
- 346 xanthomonas wilt, in Tanzania, Kenya and Burundi. Plant Pathol. 59:403.
- 347 Castellani, E. 1939. Su un marciume dell'*Ensete*. L'Agricoltura Colon. 33:297–300.
- 348 Cobb, N. A. 1894. Plant diseases and their remedies. Diseases of the sugarcane. Agric. Gaz. N. S. W.
- 349 4:808-833.
- 350 Constantin, E. C., Cleenwerck, I., Maes, M., Baeyen, S., Van Malderghem, C., De Vos, P., et al. 2016.
- 351 Genetic characterization of strains named as Xanthomonas axonopodis pv. dieffenbachiae leads to a
- 352 taxonomic revision of the *X. axonopodis* species complex. Plant Pathol. 65:792–806.
- Coutinho, T. A., and Wallis, F. M. 1991. Bacterial Streak Disease of Maize (*Zea mays* L.) in South Africa.
 J. Phytopathol. 133:112–112.
- 355 Coutinho, T. A., van der Westhuizen, L., Roux, J., Mcfarlane, S. A., and Venter, S. N. 2015. Significant
- 356 host jump of *Xanthomonas vasicola* from sugarcane to a *Eucalyptus grandis* clone in South Africa.
- 357 Plant Pathol. 64:576–581.
- Destéfano, S. A. L., Almeida, I. M. G., Rodrigues Neto, J., Ferreira, M., and Balani, D. M. 2003.
 Differentiation of *Xanthomonas* species pathogenic to sugarcane by PCR-RFLP analysis. Eur. J. Plant
 Pathol. 109:283–288.
- Dookun, A., Stead, D. E., and Autrey, L. J. 2000. Variation among strains of Xanthomonas campestris
 pv. vasculorum from Mauritius and other countries based on fatty acid analysis. Syst. Appl. Microbiol.
 23:148–55.
- Dye, D., and Lelliott, R. 1974. Genus II. *Xanthomonas* Dowson 1939. In *Bergey's Manual of Determinative Bacteriology*, eds. R. E. Buchanan and N. E. Gibbons. Williams and Wilkins Co.,
 Baltimore, U.S.A., p. 243–249.

- 367 Dye, D. W. 1962. The inadequacy of the usual determinative tests for the identification of 368 *Xanthomonas* spp. New Zeal. J. Sci. 5:393–416.
- 369 Dye, D. W., Bradbury, J. F., Goto, M., Hayward, A. C., Lelliott, R. A., and Schroth, M. N. 1980.
- 370 International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar
- and pathotype strains. Rev. Plant Pathol. 59:153–168.
- 372 Elliott, C. 1930. Bacterial streak disease of Sorghums. J. Agric. Res. 40:963–976 pp.
- da Gama, M. A. S., Mariano, R. de L. R., da Silva Júnior, W. J., de Farias, A. R. G., Barbosa, M. A. G.,
- 374 Ferreira, M. Á. da S. V., et al. 2018. Taxonomic Repositioning of *Xanthomonas campestris* pv. viticola
- 375 (Nayudu 1972) Dye 1978 as Xanthomonas citri pv. viticola (Nayudu 1972) Dye 1978 comb. nov. and
- 376 Emendation of the Description of *Xanthomonas citri* pv. <i>anac. Phytopathology. 108:1143–1153.
- 377 Glaeser, S. P., and Kämpfer, P. 2015. Multilocus sequence analysis (MLSA) in prokaryotic taxonomy.
- 378 Syst. Appl. Microbiol. 38:237–45.
- Harrison, J., and Studholme, D. J. 2014. Draft genome sequence of *Xanthomonas axonopodis* pathovar
 vasculorum NCPPB 900. FEMS Microbiol. Lett. 360:113–6.
- Hayward, A. C. 1962. *Studies on Bacterial Pathogens of Sugar Cane*. Mauritius Sugar Industry Research
 Institute.
- Hayward, A. C. 1993. The hosts of *Xanthomonas*. In *Xanthomonas*, eds. J G Swings and E L Civerolo.
 Dordrecht: Springer Netherlands, p. 1–119.
- Jacques, M. A., Bolot, S., Charbit, E., Darrasse, A., Briand, M., Arlat, M., et al. 2013. High-quality draft
 genome sequence of *Xanthomonas alfalfae* subsp. *alfalfae* strain CFBP 3836. Genome Announc.
 1:e01035-13-e01035-13.
- 388 Jones, J. B., Lacy, G. H., Bouzar, H., Stall, R. E., and Schaad, N. W. 2004. Reclassification of the

- Xanthomonads Associated with Bacterial Spot Disease of Tomato and Pepper. Syst. Appl. Microbiol.
 27:755–762.
- 391 Karamura, G., Smith, J., Studholme, D., Kubiriba, J., and Karamura, E. 2015. Comparative pathogenicity
- 392 studies of the Xanthomonas vasicola species on maize, sugarcane and banana. African J. Plant Sci.
- Korus, K., Lang, J. M., Adesemoye, A. O., Block, C. C., Pal, N., Leach, J. E., et al. 2017. First Report of
 Xanthomonas vasicola causing bacterial leaf streak on corn in the United States. Plant Dis. 101:1030.
- 396 Kumar, S. N. S. 1983. Epidemiology of bacterial leaf stripe disease of arecanut palm. Trop. Pest Manag.
- 397 29:249-252.
- Kumar, S. N. S. 1993. Perpetuation and host range of *Xanthomonas campestris* pv. *arecae* incitant of
 bacterial leaf stripe disease of arecanut palm. Adv. Hortic. For. 3:99–103.
- 400 Lang, J. M., DuCharme, E., Ibarra Caballero, J., Luna, E., Hartman, T., Ortiz-Castro, M., et al. 2017.
- 401 Detection and characterization of *Xanthomonas vasicola* pv. *vasculorum* (Cobb 1894) comb. nov.
- 402 causing bacterial leaf streak of corn in the United States. Phytopathology. 107:1312–1321.
- 403 Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R., and Clark, W. A., eds.
- 404 1992. International Code of Nomenclature of Bacteria: Bacteriological Code, 1990 Revision.
 405 Washington (DC).
- 406 Leinonen, R., Sugawara, H., and Shumway, M. 2011. The Sequence Read Archive. Nucleic Acids Res.
 407 39:D19–D21.
- 408 Lewis Ivey, M. L., Tusiime, G., and Miller, S. A. 2010. A polymerase chain reaction assay for the 409 detection of *Xanthomonas campestris* pv. *musacearum* in banana. Plant Dis. 94:109–114.
- 410 Marçais, G., Delcher, A. L., Phillippy, A. M., Coston, R., Salzberg, S. L., and Zimin, A. 2018. MUMmer4:

- 411 A fast and versatile genome alignment system ed. Aaron E. Darling. PLOS Comput. Biol. 14:e1005944.
- 412 Meier-Kolthoff, J. P., Auch, A. F., Klenk, H.-P., and Göker, M. 2013. Genome sequence-based species
- 413 delimitation with confidence intervals and improved distance functions. BMC Bioinformatics. 14:60.
- 414 Meier-Kolthoff, J. P., Klenk, H.-P., and Göker, M. 2014. Taxonomic use of DNA G+C content and DNA-
- 415 DNA hybridization in the genomic age. Int. J. Syst. Evol. Microbiol. 64:352–6.
- 416 Mulder, D. 1961. A Bacterial Disease of Guatemala Grass. Tea Q. 32:143–144.
- 417 Nakato, V., Mahuku, G., and Coutinho, T. 2018. *Xanthomonas campestris* pv. *musacearum*: a major
- 418 constraint to banana, plantain and enset production in central and east Africa over the past decade.
- 419 Mol. Plant Pathol. 19:525–536.
- Ndungo, V., Eden-Green, S., Blomme, G., Crozier, J., and Smith, J. J. 2006. Presence of banana
 xanthomonas wilt (*Xanthomonas campestris* pv. *musacearum*) in the Democratic Republic of Congo
 (DRC). Plant Pathol. 55:294.
- Parkinson, N., Aritua, V., Heeney, J., Cowie, C., Bew, J., and Stead, D. 2007. Phylogenetic analysis of *Xanthomonas* species by comparison of partial gyrase B gene sequences. Int. J. Syst. Evol. Microbiol.
 57:2881–7.
- 426 Parkinson, N., Cowie, C., Heeney, J., and Stead, D. 2009. Phylogenetic structure of *Xanthomonas*427 determined by comparison of *qyrB* sequences. Int. J. Syst. Evol. Microbiol. 59:264–74.
- 428 Péros, J. P., Girard, J. C., Lombard, H., Janse, J. D., and Berthier, Y. 1994. Variability of *Xanthomonas*
- 429 *campestris* pv. *vasculorum* from sugarcane and other Gramineae in Reunion Island. Characterization
- 430 of a different xanthomonad. J. Phytopathol. 142:177–188.
- 431 Potnis, N., Krasileva, K., Chow, V., Almeida, N. F., Patil, P. B., Ryan, R. P., et al. 2011. Comparative
- 432 genomics reveals diversity among xanthomonads infecting tomato and pepper. BMC Genomics.

Studholme et al. Open Letter to the editor of Phytopathology

433 12:146.

- 434 Qhobela, M., and Claflin, L. E. 1992. Eastern and southern African strains of *Xanthomonas campestris*
- 435 pv. vasculorum are distinguishable by restriction fragment length polymorphism of DNA and
- 436 polyacrylamide gel electrophoresis of membrane proteins. Plant Pathol. 41:113–121.
- 437 Qhobela, M., Claflin, L. E., and Nowell, D. C. 1990. Evidence that *Xanthomonas campestris* pv. zeae can
- 438 be distinguished from other pathovars capable of infecting maize by restriction fragment length
- 439 polymorphism of genomic DNA. Can. J. Plant Pathol. 12:183–186.
- 440 Rademaker, J. L. W., Louws, F. J., Schultz, M. H., Rossbach, U., Vauterin, L., Swings, J., et al. 2005. A
- 441 comprehensive species to strain taxonomic framework for *Xanthomonas*. Phytopathology. 95:1098–

442 111.

- Rao, Y. P., and Mohan, S. K. 1970. A new bacterial leaf stripe disease of arecanut (*Areca catechu*) in
 Mysore State. Indian Phytopathol. 23:702–704.
- 445 Reeder, R. H., Muhinyuza, J. B., Opolot, O., Aritua, V., Crozier, J., and Smith, J. 2007. Presence of banana
- 446 bacterial wilt (*Xanthomonas campestris* pv. *musacearum*) in Rwanda. Plant Pathol. 56:1038.
- 447 Richter, M., and Rosselló-Móra, R. 2009. Shifting the genomic gold standard for the prokaryotic species
- 448 definition. Proc. Natl. Acad. Sci. U. S. A. 106:19126–31.
- 449 Rodriguez-R, L. M., Grajales, A., Arrieta-Ortiz, M., Salazar, C., Restrepo, S., and Bernal, A. 2012.
- 450 Genomes-based phylogeny of the genus *Xanthomonas*. BMC Microbiol. 12:43.
- 451 Sanko, T. J., Kraemer, A. S., Niemann, N., Gupta, A. K., Flett, B. C., Mienie, C., et al. 2018. Draft genome
- 452 assemblages of 10 Xanthomonas vasicola pv. zeae strains, pathogens causing leaf streak disease of
- 453 maize in South Africa. Genome Announc. 6:1–2.
- 454 Seité, S., Zelenkova, H., and Martin, R. 2017. Clinical efficacy of emollients in atopic dermatitis patients

- 455 relationship with the skin microbiota modification. Clin. Cosmet. Investig. Dermatol. 10:25–33.
- 456 Shimwela, M. M., Ploetz, R. C., Beed, F. D., Jones, J. B., Blackburn, J. K., Mkulila, S. I., et al. 2016. Banana
- 457 xanthomonas wilt continues to spread in Tanzania despite an intensive symptomatic plant removal
- 458 campaign: an impending socio-economic and ecological disaster. Food Secur. 8:939–951.
- da Silva, A. C. R., Ferro, J. A., Reinach, F. C., Farah, C. S., Furlan, L. R., Quaggio, R. B., et al. 2002.
- 460 Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. Nature.
- 461 417:459–63.
- 462 Stamatakis, A., Ludwig, T., and Meier, H. 2005. RAxML-III: a fast program for maximum likelihood-
- 463 based inference of large phylogenetic trees. Bioinformatics. 21:456–63.
- Starr, M. P. 1981. The genus *Xanthomonas*. In *The Prokaryotes*, eds. Mortimer P. Starr, Heinz Stolp,
 Hans G. Trüper, Albert Balows, and Hans G. Schlegel. Berlin, Heidelberg: Springer Berlin Heidelberg, p.
 742–763.
- Stead, D. E. 1989. Grouping of *Xanthomonas campestris* pathovars of cereals and grasses by fatty acid
 profiling. EPPO Bull. 19:57–68.
- Studholme, D. J., Kemen, E., MacLean, D., Schornack, S., Aritua, V., Thwaites, R., et al. 2010. Genomewide sequencing data reveals virulence factors implicated in banana *Xanthomonas* wilt. FEMS
 Microbiol. Lett. 310:182–192.
- Timilsina, S., Kara, S., Jacques, M. A., Potnis, N., Minsavage, G. V., Vallad, G. E., et al. 2019.
 Reclassification of *Xanthomonas gardneri* (ex Šutič 1957) Jones et al. 2006 as a later heterotypic
 synonym of *Xanthomonas cynarae* Trébaol et al. 2000 and description of *X. cynarae* pv. *cynarae* and *X. cynarae* pv.<i>gardner. Int. J. Syst. Evol. Microbiol. 69:343–349.
- 476 Tinzaara, W., Karamura, E. B., Kubiriba, J., Ochola, D., Ocimati, W., Blomme, G., et al. 2016. The banana
- 477 Xanthomonas wilt epidemic in east and central Africa: current research and development efforts. Acta

Studholme et al. Open Letter to the editor of Phytopathology

478 Hortic. :267–274.

- 479 Trébaol, G., Gardan, L., Manceau, C., Tanguy, J. L., Tirilly, Y., and Boury, S. 2000. Genomic and
- 480 phenotypic characterization of *Xanthomonas cynarae* sp. nov., a new species that causes bacterial
- 481 bract spot of artichoke (*Cynara scolymus* L.). Int. J. Syst. Evol. Microbiol. 50 Pt 4:1471–8.
- 482 Tushemereirwe, W., Kangire, A., Ssekiwoko, F., Offord, L. C., Crozier, J., Boa, E., et al. 2004. First report
- 483 of *Xanthomonas campestris* pv. *musacearum* on banana in Uganda. Plant Pathol. 53:802–802.
- 484 Vauterin, L., Hoste, B., Kersters, K., and Swings, J. 1995. Reclassification of *Xanthomonas*. Int. J. Syst.
- 485 Bacteriol. 45:472–489.
- 486 Vauterin, L., Rademaker, J., and Swings, J. 2000. Synopsis on the taxonomy of the genus *Xanthomonas*.
- 487 Phytopathology. 90:677–82.
- Vauterin, L., Swings, J., Kersters, K., Gillis, M., Mew, T. W., Schroth, M. N., et al. 1990. Towards an
 Improved Taxonomy of *Xanthomonas*. Int. J. Syst. Bacteriol. 40:312–316.
- 490 Vauterin, L., Yang, P., Alvarez, A., Takikawa, Y., Roth, D. A., Vidaver, A. K., et al. 1996. Identification of
- 491 non-pathogenic *Xanthomonas* strains associated with plants. Syst. Appl. Microbiol. 19:96–105.
- 492 Vauterin, L., Yang, P., Hoste, B., Pot, B., Swings, J., and Kersters, K. 1992. Taxonomy of xanthomonads
- 493 from cereals and grasses based on SDS-PAGE of proteins, fatty acid analysis and DNA hybridization. J.

494 Gen. Microbiol. 138:1467–1477.

- 495 Vicente, J. G., Rothwell, S., Holub, E. B., and Studholme, D. J. 2017. Pathogenic, phenotypic and
- 496 molecular characterisation of *Xanthomonas nasturtii* sp. nov. and *Xanthomonas floridensis* sp. nov.,
- 497 new species of *Xanthomonas* associated with watercress production in Florida. Int. J. Syst. Evol.
- 498 Microbiol. 67:3645–3654.
- 499 Wasukira, A., Coulter, M., Al-Sowayeh, N., Thwaites, R., Paszkiewicz, K., Kubiriba, J., et al. 2014.

Studholme et al. Open Letter to the editor of Phytopathology

- 500 Genome sequencing of *Xanthomonas vasicola* pathovar *vasculorum* reveals variation in plasmids and
- 501 genes encoding lipopolysaccharide synthesis, type-IV pilus and type-III secretion effectors. Pathog.
- 502 (Basel, Switzerland). 3:211–37.
- 503 Wasukira, A., Tayebwa, J., Thwaites, R., Paszkiewicz, K., Aritua, V., Kubiriba, J., et al. 2012. Genome-
- 504 wide sequencing reveals two major sub-lineages in the genetically monomorphic pathogen
- 505 *Xanthomonas campestris* pathovar *musacearum*. Genes (Basel). 3:361–377.
- 506 Wernham, C. C. 1948. The species value of pathogenicity in the genus *Xanthomonas*. Am. Phytopathol.
- 507 Soc. 38:283–291.
- 508 Yang, P., Vauterin, L., Vancanneyt, M., Swings, J., and Kersters, K. 1993. Application of Fatty Acid
- 509 Methyl Esters for the Taxonomic Analysis of the Genus *Xanthomonas*. Syst. Appl. Microbiol. 16:47–71.
- 510 Yirgou, D., and Bradbury, J. F. 1974. A note on wilt of banana caused by the enset wilt organism
- 511 *Xanthomonas musacearum*. East African Agric. For. J. 40:111–114.
- 512 Yirgou, D., and Bradbury, J. F. 1968. Bacterial wilt of enset (*Ensete ventricosum*) incited by 513 *Xanthomonas musacearum* sp. Phytopathology. 58:111–112.
- Young, J. M., Bull, C. T., De Boer, S. H., Firrao, G., Saddler, G. E., Stead, D. E., et al. 2004. Names of Plant
 Pathogenic Bacteria , 1864 2004. Rev. Plant Pathol. 75:721–763.
- 516 Young, J. M., Dye, D. W., Bradbury, J. F., Panagopoulos, C. G., and Robbs, C. F. 1978. A proposed
- 517 nomenclature and classification for plant pathogenic bacteria. New Zeal. J. Agric. Res. 21:153–177.

518

Studholme et al. Open Letter to the editor of Phytopathology

520

521 Table 1. Classification of strains previously assigned to *X. campestris* pv. *vasculorum*.

Strain	Vauterin (Vauterin et al. 1992, 1995)	Dookun (Dookun, Stead, and Autrey 2000)	Péros (Péros et al. 1994)	Current species assignation			
NCPPB 186	Туре А	Group A	n/a	X. axonopodis			
NCPPB 891	Туре А	Group A	G1	X. axonopodis			
NCPPB 892	n/a	Group A	n/a	X. axonopodis			
NCPPB 893	n/a	Group A	n/a	X. axonopodis			
NCPPB 181	Туре А	Group B	n/a	X. axonopodis			
NCPPB 796 PT	Туре А	Group B	n/a	X. axonopodis			
NCPPB 899	n/a	Group D	n/a	X. axonopodis			
NCPPB 900	n/a	Group D	n/a	X. axonopodis			
NCPPB 795	Туре В	Group C	n/a	X. vasicola			
NCPPB 889	Туре В	Group C	n/a	X. vasicola			
NCPPB 206	n/a	Group C	n/a	X. vasicola			
NCPPB 702	n/a	Group C	n/a	X. vasicola			
NCPPB 795	n/a	Group C	n/a	X. vasicola			
NCPPB 889	n/a	Group C	n/a	X. vasicola			
NCPPB 890	n/a	Group C	n/a	X. vasicola			
NCPPB 895	n/a	Group C	n/a	X. vasicola			
NCPPB 1326	n/a	Group C	n/a	X. vasicola			
NCPPB 1381	n/a	Group C	n/a	X. vasicola			

522

^zIn this table, the superscript PT indicates the pathotype strain of *X. campestris* pv. *vasculorum*

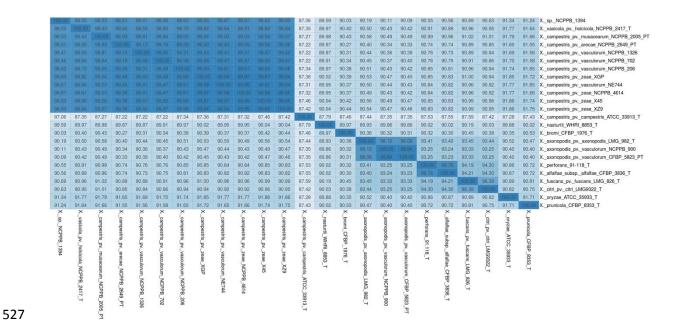
Studholme et al. Open Letter to the editor of Phytopathology

524 **Table 2. Host ranges of the taxa discussed in this letter.**

Current taxon	Proposed taxon	Pathotype or Type strains	Additional strains in NCPPB known to be part of the newly proposed taxon	Natural hosts	Hosts by inoculation
X. campestris pv. arecae (Rao and Mohan 1970) Dye 1978	X. vasicola pv. arecae pv. nov.	NCPPB 2649 = ICMP 5719 = LMG 533	None	Areca catechu (Bradbury 1986; Kumar 1993, 1983)	Cocos nucifera, Saccharum sp. (Bradbury 1986)
X. campestris pv. musacearum (Yirgou and Bradbury 1968) Dye 1978	X. vasicola pv. musacearum pv. nov	NCPPB 2005 = ATCC 49084 = CFBP 7123 = ICMP 2870 = LMG 785	NCPPB 2251; NCPPB 4378; NCPPB 4379; NCPPB 4380; NCPPB 4381; NCPPB 4383; NCPPB 4384; NCPPB 4386; NCPPB 4387; NCPPB 4388; NCPPB 4389; NCPPB 4390; NCPPB 4391; NCPPB 4392; NCPPB 4393; NCPPB 4394; NCPPB 4395; NCPPB 4433; NCPPB 4434	Ensete ventricosum, Musa sp. (Bradbury 1986), Tripsacum sp. (Unpublished observation, E. Wicker),	Saccharum sp., (Karamura et al. 2015), Zea mays (Karamura et al. 2015; Aritua et al. 2007)
[Xanthomonas vasicola pv. zeae Coutinho and Wallis 1990]	X. vasicola pv. vasculorum pv. nov.	NCPPB 4614 = SAM119	None	Zea mays (Coutinho and Wallis 1991)	Sorghum sp. (Lang et al. 2017)
[<i>Xanthomonas</i> <i>vasicola</i> pv. <i>zeae</i> Qhobela et al 1990]					
X. vasicola pv. holcicola (Elliott 1930) (Elliott 1930) Vauterin <i>et al.</i> 1995 (synonym of X. campestris pv. holcicola)	X. vasicola pv. holcicola (Elliott 1930) Vauterin et al. 1995	NCPPB 2417 = CFBP 2543 = ICMP 3103 = LMG 736	NCPPB 989; NCPPB 1060; NCPPB 1241; NCPPB 2417; NCPPB 2930; NCPPB 3162	Panicum miliaceum, Sorghum spp., Zea mays (Bradbury 1986)	Echinochloa frumentacea, Pennisetum typhoides, Setaria italica (Bradbury 1986)
X. campestris pv. vasculorum type B = [X. vasicola pv. vasculorum (Vauterin et al., 1995)]	X. vasicola pv. vasculorum pv. nov.	NCPPB 4614 = SAM119	NCPPB 206; NCPPB 702; NCPPB 795; NCPPB 889; NCPPB 890; NCPPB 895; NCPPB 1326; NCPPB 1381; NCPPB 4614	Saccharum spp., Zea mays, Eucalyptus grandis (Coutinho et al. 2015; Bradbury 1986; Vauterin et al. 1995)	Saccharum spp., Zea mays (Karamura et al. 2015)
Xanthomonas sp.	The taxonomic placement of these strains requires further study.	Not applicable	NCPPB 1394; NCPPB 1395; NCPPB 1396; NCPPB 902	Tripsacum laxum (Mulder 1961), Vetiveria zizanoides (Kumar 1993, 1983)	Not known

525

Studholme et al. Open Letter to the editor of Phytopathology

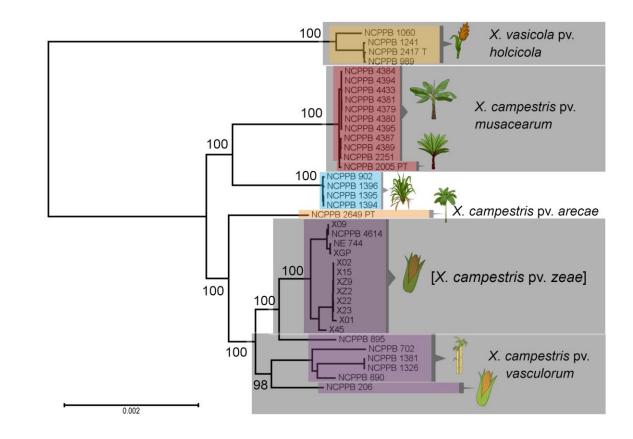


528 Figure 1. Average nucleotide identity (ANI) with type strains of Xanthomonas species. Genome 529 sequence assemblies were obtained from GenBank and aligned against each other and ANI was 530 calculated using the *dnadiff* function in MUMmer version 4 (Marcais et al. 2018). Accession numbers of assemblies: GCA_000774005.1, GCA_000772705.1, GCA 000277875.1, 531 the genome GCA 000770355.1, GCA 000277995.1, GCA 000159795.2, GCA 000278035.1, GCA 003111865.1, 532 GCA 002191965.1, GCA 002191955.1, GCA 003111905.1, GCA 003111825.1, GCA 000007145.1, 533 GCA 001660815.1, GCA 002939755.1, GCA 001401595.1, GCA 002939725.1, GCA 000724905.2, 534 535 GCA 000192045.3, GCA 000488955.1, GCA 001401605.1, GCA 002018575.1, GCA 000482445.1 536 and GCA_002846205.1 (Studholme et al. 2010; Wasukira et al. 2014, 2012; Lang et al. 2017; Sanko et al. 2018; 537 al. 2018; da Silva et al. 2002; Vicente et al. 2017; Harrison and Studholme 2014; Potnis et al. 2011; 538 Jacques et al. 2013).

539

Studholme et al. Open Letter to the editor of Phytopathology

541



543 Figure 2. Maximum-likelihood phylogenetic tree based on genomic sequencing reads. The maximum likelihood tree was generated using RealPhy (Bertels et al. 2014) and RaxML (Stamatakis, Ludwig, and 544 545 Meier 2005). Bootstrap values are expressed as percentages of 500 trials. Type and pathotype strains are indicated by 'P' and 'PT' respectively. Whole-genome shotgun sequence reads were obtained from 546 the Sequence Read Archive (Leinonen, Sugawara, and Shumway 2011) via BioProjects PRJNA73853, 547 548 PRJNA163305, PRJNA163307, PRJNA31213, PRJNA374510, PRJNA374557, PRJNA439013, 549 PRJNA439327, PRJNA439328, PRJNA439329 and PRJNA449864 (Lang et al. 2017; Wasukira et al. 2014, 550 2012; Sanko et al. 2018).

551

100.00					98.46	98.62	98.65	98.67	96.67	98.63	98.60	87.06	89.59	90.03	90.19	90.11	90.09	90.55	90.56	90.69	90.63	91.34	91.24	X. sp. NCPPB 1394
98.03		98.43	98.60	98.55	96.60	98.73		98.84	98.81	98.80	98.84	87.35	89.97	90.40	90.50	90.43	90.42	90.91	90.89	90.96	90.95	91.77	91.64	X. vasicola pv. holcicola NCPPB 2417 T
98.33	98.43		98.93	98,81	98.84			99.03		99.06		87.27	89.98	90.43	90.58	90.49	90.49	90.99	90.96	91.02	91.01	91.79	91.66	X. campestris pv. musacearum NCPPB 2005 PT
98.61	98.60											87.22	89.87	90.27	90.40	90.34	90.33	90.74	90.74	90.89	90.85	91.65	91.55	X. campestris pv. arecae NCPPB 2649 PT
98.41	98.55	98.81										87.22	89.87	90.31	90.44	90.36	90.39	90.76	90.73	90.89	90.84	91.69	91.56	X. campestris pv. vasculorum NCPPB 1326
98.46	98.60											87.22	89.91	90.34	90.45	90.37	90.40	90.76	90.75	90.91	90.86	91.70	91.58	X. campestris pv. vasculorum NCPPB 702
98.62	98.73											87.34	89.97	90.38	90.51	90.43	90.42	90.85	90.81	90.96	90.94	91.74	91.65	X. campestris pv. vasculorum NCPPB 206
98.65	98.82	99:04										87.36	90.02	90.39	90.53	90.47	90.45	90.85	90.83	91.00	90.94	91.85	91.72	Xcampestris_pvzeae_XGP
98.67	98.84	99.03										87.31	89.95	90.37	90.50	90.44	90.43	90.84	90.82	90.96	90.92	91.77	91.65	X. campestris_pv. vasculorum_NE744
98.67	98.81											87.32	89.95	90.37	90.49	90.43	90.42	90.84	90.82	90.96	90.92	91.77	91.66	X. campestris pv. zeae NCPPB 4614
98.63	98.80											87.46	90.04	90.42	90.56	90.49	90.47	90.85	90.83	90.99	90.96	91.86	91.74	X. campestris pv. zeae X45
98.60	98.84	99.07	99.36	99.42	99.47	99.58	99.94	99.84	99.85	99.89	100.00	87.42	90.04	90.44	90.54	90.47	90.46	90.83	90.82	90.99	90.95	91.86	91.75	X. campestris pv. zeae XZ9
87.06	87.35	87.27	87.22	87.22	87.22	87.34	87.36	87.31	87.32	87.46	87.42	100.00	87.79	87.46	87.44	87.35	87.35	87.53	87.55	87.59	87.42	87.28	87.43	X. campestris pv. campestris ATCC 33913 T
89.59	89.97	89.98	89.87	89.87	89.91	89.97	90.02	89.95	89.95	90.04	90.04	87.79		89.97	89.93	89.86	89.86	90.02	90.02	90.19	90.03	89.88	90.02	Xnasturti_WHRI_8853_T
90.03	90.40	90.43	90.27	90.31	90.34	90.38	90.39	90.37	90.37	90.42	90.44	87.46	89.97	100.00	90.36	90.32	90.31	90.32	90.30	90.45	90.38	90.35	90.53	X. bromi_CFBP_1976_T
90.19	90.50	90.58	90.40	90.44	90.45	90.51	90.53	90.50	90.49	90.56	90.54	87.44	89.93	90.36		98.12	98.08	93.41	93.40	93.45	93.44	90.52	90.47	X. axonopodis pv. axonopodis LMG 982 T
90.11	90.43	90.49	90.34	90.36	90.37	90.43	90.47	90.44	90.43	90.49	90.47	87.35	89.86	90.32	98.12			93.25	93.24	93.33	93.25	90,40	90.40	X. axonopodis pv. vasculorum NCPPB 900
90.09	90.42	90.49	90.33	90.39	90.40	90.42	90.45	90.43	90.42	90.47	90.46	87.35	89.86	90.31	98.08	99.84	100.00	93.25	93.23	93.33	93.25	90.40	90.40	X. axonopodis pv. vasculorum CFBP 5823 PT
90.55	90.91	90.99	90.74	90.76	90.76	90.85	90.85	90.84	90.84	90.85	90.83	87.53	90.02	90.32	93.41	93.25	93.25		98.76	94.19	94.30	90.86	90.72	X. perforans_91-118_T
90.56	90.89	90.96	90.74	90.73	90.75	90.81	90.83	90.82	90.82	90.83	90.82	87.55	90.02	90.30	93.40	93.24	93.23	98.76		94.21	94.30	90.87		X. alfalfae_subsp. alfalfae_CFBP_3836_T
90.69	90.96	91.02	90.89	90.89	90.91	90.96	91.00	90.96	90.96	90.99	90.99	87.59	90.19	90.45	93.45	93.33	93.33	94.19	94.21		96.39	90.89		X. fuscans pv. fuscans LMG 826 T
90.63	90.95	91.01	90.85	90.84	90.86	90.94	90.94	90.92	90.92	90.96	90.95	87.42	90.03	90.38	93.44	93.25	93.25	94.30	94.30	96.39	100.08	90.82		X. citri pv. citri LMG9322 T
91.34	91.77	91.79	91.65	91.69	91.70	91.74	91.85	91.77	91.77	91.86	91.86	87.28	89.88	90.35	90.52	90.40	90.40	90,86	90.87	90.89	90.82	100.00		X. oryzae ATCC 35933 T
91.24	91.64	91.66	91.55	91.56	91.58	91.65	91.72	91.65	91.66	91.74	91.75	87.43	90.02	90.53	90.47	90.40	90.40	90.72	90.72	90.81	90.75	91.71	100.00	X. prunicola CFBP 8353 T
×	×	×	č	č	×	×	×	č	č	×	×	č	č	×	×	č	č	č	×	×	č	×	×	
spNCPP8_1394	vasicola pv. holcicola NCPPB 2417 T	campestris pv. musacearum NCPPB 2005 P	campestris_pv_arecae_NCPPB_2649_PT	campestrls_pv_vasculorum_NCPPB_1326	campestris pv. vasculorum NCPPB 702	campestris pv. vasculorum NCPPB 206	campestris pv. zeae XGP	campestrls_pv_vasculorum_NE744	campestris_pv_zeae_NCPPB_4614	campestris pv. zeae X45	campestris pv. zece XZ9	campestris_pv_campestris_ATCC_33913_T	nasturtiLWHRI_8853_T	bromi CEBP 1976 T	axonopodis pv. avanopodis LMG 982 T	accropodis_pv_vasculorum_NCPPB_900	accropodis_pv_vasculorum_CFBP_5823_PT	perforans_91.118_T	alfalfae subsp. alfalfae CFBP 3836 T	tuscans pv. tuscans LMG 826 T	_dtri_pw_cttrl_LMG9022_T	oryzae_ATCC_35933_T	prunicola CFBP 8353 T	

