The phytopathogenic nature of *Dickeya aquatica* 174/2 and the dynamic early evolution of *Dickeya* pathogenicity

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25 Originality-Significance statement

26 Although the reach of large-scale comparative studies has spread exponentially over the years, the
 27 phytopathogenic *Dickeya* group remains overlooked. In this work, we sequence the complete
 28 genome of *Dickeya aquatica* type strain, a species isolated from water that was first assumed to be
 29 non-phytopathogenic. We show that the proteome of *D. aquatica* contains a wide number of
 30 proteins involved in *Dickeya* virulence, including plant cell wall degrading enzymes, suggesting that
 31 this species could be in fact pathogenic. Using experimental approaches, we confirm this
 32 prediction and uncover the particular affinity of *D. aquatica* for acidic fruits. In-depth phylogenomic
 33 analyses reveal that *Dickeya* species display a great degree of genetic plasticity in the
 34 pathogenicity determinants, explaining how this bacterial group was able to colonize a wide variety
 35 of plants growing in different climates. These observations greatly advance our understanding of
 36 how bacteria adapt to new ecological niches.

37

38 Summary

39 *Dickeya* is a genus of phytopathogenic enterobacterales causing soft rot in a variety of plants (e.g.
 40 potato, chicory, maize). Among the species affiliated to this genus, *Dickeya aquatica*, described in
 41 2014, remained particularly mysterious because it had no known host. Furthermore, while *D.*
 42 *aquatica* was proposed to represent a deep-branching species among *Dickeya* genus, its precise
 43 phylogenetic position remained elusive.
 44 Here, we report the complete genome sequence of the *D. aquatica* type strain 174/2. We
 45 demonstrate the affinity of *D. aquatica*^T for acidic fruits such as tomato and cucumber, and show
 46 that exposure of this bacterium to acidic pH induces twitching motility. An in-depth phylogenomic
 47 analysis of all available *Dickeya* proteomes pinpoints *D. aquatica* as the second deepest branching
 48 lineage within this genus and reclassifies two lineages that likely correspond to new
 49 genomospecies (gs.): *Dickeya* gs. poaceaephila (*Dickeya* sp NCPPB 569) and *Dickeya* gs.
 50 undicola (*Dickeya* sp 2B12), together with a new putative genus, tentatively named *Prodigiosinella*.
 51 Finally, from comparative analyses of *Dickeya* proteomes we infer the complex evolutionary history
 52 of this genus, paving the way to study the adaptive patterns and processes of *Dickeya* to different

environmental niches and hosts. In particular, we hypothesize that the lack of xylanases and xylose degradation pathways in *D. aquatica* could reflect adaptation to aquatic charophyte hosts which, in contrast to land plants, do not contain xyloglucans.

Introduction

Enterobacterales represent one of the most studied orders of *Gammaproteobacteria*. According to current systematics, *Enterobacterales* are divided into eight families: *Enterobacteriaceae*, *Yersiniaceae*, *Thorselliaceae*, *Hafniaceae*, *Morganellaceae*, *Budviciaceae*, *Erwiniaceae*, and *Pectobacteriaceae*. *Enterobacterales* are widespread, being found in very different environments such as soils, fresh water, ocean, sediments, and many of them are associated with plants and animals, including insects and humans (Brenner and Farmer III, 2005). *Enterobacterales* include also important model organisms such as *Escherichia coli*, human pathogens such as *Salmonella*, *Shigella*, and *Yersinia* (Dekker and Frank, 2015), and plant pathogens such as *Erwiniaceae* (e.g. *Erwinia*, *Pantoea*, *Phaseolibacter*) and *Pectobacteriaceae* (e.g. *Pectobacterium*, *Dickeya*, *Brenneria*, *Lonsdalea*) (Hauben et al., 1998; Samson et al., 2005). These phytopathogens share virulence genes with zoopathogens such as type III secretion systems (T3SS) that inject effector proteins in eukaryotic cells to suppress the host innate immune defence (Buttner, 2016). They also produce specialized plant virulence factors such as pectinases found in pectinolytic bacteria (Hugouvieux-Cotte-Pattat et al., 2014).

Among pectinolytic enterobacterales, *Dickeya* is the causative agent of soft rot in a wide variety of plants including economically important crops (e.g. potato, chicory, maize, rice, tomato, sugar beet, pineapple, banana) and many ornamental plants (Ma et al., 2007). This causes substantial production losses amounting, for instance for potato, to tens of millions of Euros/year in Europe (Toth et al., 2011). The *Dickeya* genus was first described by Samson et al. (2005), who initially distinguished six species: *Dickeya dadantii*, *Dickeya dieffenbachiae*, *Dickeya chrysanthemi*, *Dickeya paradisiaca*, *Dickeya zeae*, and *Dickeya dianthicola*. Subsequently, *Dickeya dieffenbachiae* has been reclassified as *D. dadantii* subsp. *dieffenbachiae* (Brady et al., 2012). More recently, three additional *Dickeya* species have been described: *D. solani*, a highly virulent

species isolated from potatoes (Van der Wolf et al., 2014), *Dickeya aquatica* from freshwater rivers (Parkinson et al., 2014), and *Dickeya fangzhongdai* from pear trees displaying symptoms of bleeding canker in China (Tian et al., 2016). Thus, the *Dickeya* genus now comprises eight species with distinctive phenotypic features (Supplementary Table S1). Virulence mechanisms have been extensively studied in *Dickeya dadantii*. During infection, the bacterium enters the host using natural openings and multiplies in intercellular spaces without causing major damage. Then, it suddenly induces the production of aggression factors, such as pectinases, that break down the plant cell wall pectin, causing the macroscopic symptom called soft rot (Reverchon and Nasser, 2013). *Dickeya* populations were initially considered to be restricted to tropical and subtropical plant hosts and areas (Perombelon, 1990). This assumption was called into question following the identification of *D. dianthicola* strains from potato plants in Western Europe (Janse and Ruissen, 1988) and the first isolation of *D. solani* strains in France, Finland, Poland, the Netherlands, and Israel (Czajkowski et al., 2011; van der Wolf et al., 2014). It was postulated that the pathogen was introduced in Europe via the international trade of potato seeds (van der Wolf et al., 2014). Strains of *D. solani* have also been found in hyacinth, which was interpreted as a possible recent transfer from hyacinth to potato, possibly via contaminated irrigation waters (Sławiak et al., 2009). This highlights the high capacity of adaptation and dissemination of *Dickeya* to new geographic areas and to new hosts.

According to a large phylogenomic analysis of 895 single copy gene families, *D. paradisiaca* was pinpointed as the first diverging species within *Dickeya*, while other species formed two groups, referred hereafter as to clusters I and II (Zhang et al., 2016). Cluster I encompassed *D. zeae* and *D. chrysanthemi*, while in cluster II, *D. solani* and *D. dadantii* formed the sister-lineage of *D. dianthicola* (Zhang et al., 2016). Species corresponding to these two clusters display different behaviours. For example, in temperate climate, *D. chrysanthemi* and *D. zeae* were frequently isolated from water and were rarely associated to potato infections, contrarily to *D. dianthicola* and *D. solani* (Potrykus et al. 2016). It is noteworthy, that in this study, strains M074 and M005, annotated as *D. chrysanthemi* and *D. solani*, respectively, branch in-between *D. dianthicola* and the *D. solani* / *D. dadantii* group within Cluster II, thus far away from the type strains of their

species (Zhang et al., 2016). *D. fangzhongdai* branched at the base of the Cluster II in a tree based on seven housekeeping genes (Tian et al., 2016). Finally, according to a tree based on six housekeeping genes, *D. aquatica* emerge just after the divergence of *D. paradisiaca* but before the divergence of Cluster I and Cluster II (Parkinson et al., 2014). Yet, the associated bootstrap values were weak (Parkinson et al., 2014), meaning that the relative order of emergence of *D. paradisiaca* and *D. aquatica* remained to determine. Among *Dickeya*, *D. aquatica* is remarkable because all the three known strains 174/2^T, 181/2, and Dw0440 have been isolated from waterways and, in contrast to other species, have no known vegetal host (Supplementary Table S1) (Parkinson et al., 2014; Ma et al., 2007). These features and its early-branching position within *Dickeya* make *D. aquatica* very interesting to study the origin and the early steps of the diversification of *Dickeya*, and in particular, the emergence of their virulence factors and capacity to infect plants. These important questions were the focus of this study. For this purpose, we sequence the complete genome of the *D. aquatica* 174/2 type strain. Based on the phylogenetic analysis of 1,341 single copy core protein families, we show that *D. aquatica* represents the second deepest branching lineage within *Dickeya*, reclassify two lineages that likely correspond to new genomospecies (gs.): *Dickeya* gs. poaceaeaphila and *Dickeya* gs. undicola, and identify a new lineage, tentatively called *Prodigiosinella*, that likely represents the closest relative of *Dickeya*. We also highlight the presence of different virulence factors, suggesting that *D. aquatica* 174/2 could be pathogenic. Stimulated by these findings, we explore the pathogenic potential of *D. aquatica* 174/2. Surprisingly, we identify the acidic fruits tomato (pH 4.8) and cucumber (pH 5.1) as potential hosts for *D. aquatica*, reflecting the potential of this aquatic species to flexibly adapt to a new ecological niche provided by acidic fruits with a high water content. Accordingly, we show that this strain displays a specific induction of twitching motility under acidic conditions. Finally, using phylogenomic approaches, we trace back the origin and evolution of key factors associated with virulence and host specificity in *Dickeya*, including *D. aquatica*. From this study we infer the evolution of gene repertoires along the diversification of *Dickeya* and highlight a remarkable general tendency toward proteome reduction in all *Dickeya* species.

136

137 **Results and discussion**

138 *General genomic features of D. aquatica 174/2 type strain*

139 The *D. aquatica* 174/2^T genome consists in a single circular chromosome of 4,501,560 base pairs
 140 in size, with a GC content of 54.6%. The sequence was submitted to European Nucleotide Archive
 141 (accession GCA_900095885). This genome contains a total of 4,202 genes including 4,080
 142 protein-coding DNA sequences (CDS), 22 ribosomal RNA-coding genes organized into seven
 143 operons, 76 tRNA-coding genes, and 23 non-coding RNA genes identified by sequence similarity
 144 with known functional RNAs entries in the RFAM database (Daub et al., 2015) (Figure 1). These
 145 features are typical of *Dickeya* species (Supplementary Table S2). The origin of chromosome
 146 replication (*oriC* position 1-8 bp) was predicted between *mnmG/gidA* and *mioC* as observed in
 147 *Escherichia coli* (Wolanski et al., 2014) and other enterobacterales, including *Dickeya* (Glasner et
 148 al., 2011; Zhou et al., 2015; Khayi et al., 2016). The terminus of replication is predicted between
 149 positions 2,220,802 and 2,220,829 bp with the *D. aquatica* 174/2^T Dif site
 150 (GGTTCGCATAATGTATATTATGTTAAAT) differing from the *E. coli* K-12 Dif site by only one
 151 nucleotide substitution (GGTGCGCATAATGTATATTATGTTAAAT). The distance from *oriC* to the
 152 predicted terminus was nearly equal for the two halves of the chromosome. It corresponds to a
 153 region near an inflection point in a slight strand-specific nucleotide compositional bias (Figure 1).
 154 The protein coding density is 85% of the genome with a slight preference for the leading strand
 155 utilization (57%). This density of predicted ORFs (slightly less than one per kilobase) is typical for
 156 *Enterobacterales*, including *Dickeya* species (Supplementary Table S2) (Glasner et al., 2011; Zhou
 157 et al., 2015; Khayi et al., 2016). Thus, despite its different ecological niche, *D. aquatica* shows
 158 genomic organisation very similar to other species of the *Dickeya* genus.

159

160 ***Deciphering the early evolution of Dickeya***

161 The massive release of *Dickeya* genomic sequences (7 complete genomes and 41 draft genomes)
 162 in public databases (Supplementary Table S3), in addition to the complete genome of *D. aquatica*
 163 174/2^T reported in this study, provided an interesting resource to explore the evolutionary history of
 164 this genus. We investigated the phylogeny of *Dickeya* using 51 ribosomal proteins (rprots), as

these were shown to be well suited to study the systematics of prokaryotes, especially *Proteobacteria* (Yutin et al., 2012; Ramulu et al., 2014), on the one hand, and 1,341 core protein families (core-pf), on the other hand. The maximum likelihood (ML) rprots tree, rooted with *Pectobacterium* (*Pectobacteriaceae*) and *Serratia* (*Yersiniaceae*), recovered the monophyly of *Dickeya* (Bootstrap Value (BV) = 100%, Supplementary Figure S1). Surprisingly, *Serratia* strains, used as outgroup to root the phylogeny of *Dickeya* together with *Pectobacterium*, did not form a monophyletic group, due to the robust clustering of *Serratia* sp. ATCC 39006 with *Dickeya* (BV = 99%). A similar grouping was also observed in trees based on the RNA components of the small and large subunits of the ribosome (SSU and LSU rRNA, respectively) (Supplementary Figure S2), suggesting that strain ATCC 39006 represents the sister-lineage of *Dickeya*, and was wrongly affiliated to the *Serratia*, based on its capacity to synthesize prodigiosin, a red pigment secondary metabolite with antimicrobial, anticancer, and immunosuppressant properties, characteristic of *Serratia* species (Thomson et al., 2000). The sister-relationship of ATCC 39006 and *Dickeya* is consistent with the close evolutionary link between the pectinolytic gene clusters (*pel*, *paeY*, *pemA*) shared by these taxa (Duprey et al., 2016b), and with the presence, in *Serratia* sp. ATCC 39006, of an homologue of the Vfm quorum sensing system previously reported as specific to *Dickeya* species (Supplementary Table S4 and Figure S3) (Nasser et al., 2013). The phylogenetic analysis of the genes involved in the biosynthesis of the prodigiosin showed that strain ATCC 39006 genes emerge in a cluster gathering sequences from unrelated taxa: *Serratia* (*Yersiniaceae*), *Hahella* (*Hahellaceae*), and *Streptomyces* (*Actinobacteria*), suggesting that the prodigiosin gene cluster spread among these lineages (including ATCC 39006 strain) through horizontal gene transfer (HGT) (Supplementary Figure S3). Interestingly, strain ATCC 39006, like the early-diverging *D. paradisiaca* species, was deprived of the *indABC* gene cluster responsible for the characteristic production of blue-pigmented indigoidine in *Dickeya* spp. (Supplementary Table S4) (Reverchon et al., 2002; Lee and Yu, 2006). Phylogenetic analyses showed that *Dickeya* IndA, IndB, and IndC proteins are closed to sequences from unrelated bacteria (Supplementary Figure S3), suggesting that indigoidine genes underwent HGT and have been secondarily acquired in *Dickeya* after the divergence with *D. paradisiaca*. The evolutionary link between strain ATCC 39006 and *Dickeya* is

also consistent with comparison of their proteomes. In fact, strain ATCC 39006 share in average more proteins families with *Dickeya* (2,554/4,221 – 60.5%), compared to *Pectobacterium* (2,387/4,221 - 56.5%) or other *Serratia* species (2,162/4,221 - 51.2%) (Supplementary Table S5). Yet, the large evolutionary distance between ATCC 39006 strain and *Dickeya* (Supplementary Figure S1), its lower GC content (49.2% vs 52.6 - 56.9% in *Dickeya*) (Supplementary Table S3), and the lower number of protein families shared between ATCC 39006 and *Dickeya* (60.5%) compared to between *Dickeya* species (64.7% - 89.6%, Supplementary Table S5), suggested that strain ATCC 39006 does not belong to *Dickeya* and represents rather a close but distinct lineage, possibly a new genus, that we propose to call *Prodigiosinella*, with strain ATCC 39006 being reclassified as *Prodigiosinella confusarubida*. By using leBIBI^{QBPP} phylogenetic SSU rRNA-based positioning tool (Flandrois et al., 2015), we detected two *Erwinia* sp. Strains: MK01 (SSU accession number at the NCBI: AY690711.1) and MK09 (SSU accession number at the NCBI: AY690717.1), isolated from the rhizosphere of *Phragmites communis*, that were very closely related to ATCC 39006 and could belong to *P. confusarubida*.

According to these results and to decrease computation time, the core-pf phylogeny was inferred using *P. confusarubida* ATCC 39006 as outgroup. The ML core-pf and rprots trees displayed similar topologies (Figure 2 and Supplementary Figure S1), excepted regarding *D. chrysanthemi*, *D. sp.* NCPPB 569, and *D. solani* (see below). Worth to note, the rprots tree was overall less resolved than the core-pf tree (i.e. the former displayed lower BV than the latter). Both trees supported the monophyly of *D. paradisiaca*, *D. aquatica*, *D. zeae*, *D. dianthicola*, *D. dadantii*, and *D. solani* (BV ≥ 84%). The monophyly of *D. chrysanthemi* was recovered with core-pf (BV = 100%) but not with rprots. In fact, while the relationships among *D. chrysanthemi* strains were strongly supported in the core-pf tree, they were mostly unresolved (i.e. associated to weak BV) with rprots, meaning that these markers did not contain enough phylogenetic signal to resolved the *D. chrysanthemi* phylogeny. A composite group composed of strains annotated as *D. sp.*, *D. solani*, and *D. chrysanthemi*: M074, M005, B16, MK7, S1, NCPPB 3274, and ND14b (formerly misidentified as *Cedecea neteri* and transferred recently to *D. solani*) was present in both trees (BV ≥ 95%). By using leBIBI^{QBPP} tool (Flandrois et al., 2015), we identified strains B16, MK7, and S1 as

221 *D. fangzhongdai*, suggesting that the whole clade could correspond to *D. fangzhongdai* (Alič et al.,
222 2018).

223 Cluster I (*D. zeae* and *D. chrysanthemi*) and cluster II (*D. dianthicola*, *D. solani*, *D. dadantii*, *D.*
224 *fangzhongdai* and *D. undicola*) were monophyletic (core-pf: both BV $\geq$ 100% and rprots: both BV $\geq$
225 80%, respectively). Within cluster II, *D. fangzhongdai* diverged first (BV = 100%), while *D. solani*
226 represented either the sister-group of *D. dadantii* (core-pf: BV = 100%) or *D. dianthicola* (rprots: BV
227 = 98%). The conflicting position of *D. solani* was supported by high BV in both trees, indicating a
228 real inconsistency between the two trees. To go further, AU tests performed on individual core-pf
229 and rprots by using the core-pf and the rprots ML topologies (Supplementary Table S6). These
230 tests showed that 1,063 out of 1,341 (79.3%) core-pf protein families reject the rprots topology,
231 while 853 out of 1,341 (63.6%) core-pf protein families do not reject the core-pf topology. In
232 contrast, 31.4% of the rprots do not reject the core-pf topology and 29.4% do not reject the rprots
233 topology. Thus, a large majority of core-pf protein families favour the core-pf topology, while an
234 equal number of rprots supports either one or the other topology. Accordingly, the rprots topology
235 and in particular the grouping of *D. solani* with *D. dianthicola* could be questioned.

236 Regarding the first divergences within *Dickeya*, both trees pinpointed *D. paradisiaca* and *D.*
237 *aquatica*, as early diverging lineages (core-pf: BV = 100%, rprots: BV = 86%), with *D. paradisiaca*
238 emerging first (core-pf: BV = 100%, rprots: BV = 65%). Finally, the core-pf placed robustly *D. sp.*
239 NCPPB 569, a strain isolated from sugarcane in Australia (Supplementary Table S3), at the base
240 of Cluster I (BV = 100%), while its position was unresolved in the rprots tree (i.e. unsupported
241 grouping with *D. aquatica*, BV = 46%). Irrespective of its position, the evolutionary distances
242 between strain NCPPB 569 and the eight *Dickeya* species were roughly similar, suggesting
243 NCPPB 569 could correspond to an independent lineage within *Dickeya*. This observation was in
244 agreement with a previous work based on the phylogenetic analysis of *recA* (Parkinson et al.
245 2009), and led us to propose that strain NCPPB 569 could represent a distinct genomospecies, we
246 proposed to name *Dickeya* gs. poaceaephila. This hypothesis was strengthened by the fact that
247 strain NCPPB 569 could be distinguished from other species according to shared protein families
248 (Figure 2 and Supplementary Figure S4). Among cluster II, *Dickeya* sp. 2B12 deserved attention.

249 In fact, this strain branched as a distant sister-lineage of *D. fangzhongdai* (core-pf: BV = 100% and
250 rprots: BV = 95%). This suggested that this strain could also represent a new genomospecies that
251 we have tentatively called *Dickeya* gs. undicola.

252 Altogether, the analysis of more than one thousand core protein families and ribosomal proteins
253 allows to resolve most of the speciation events within *Dickeya* and provides a solid framework to
254 investigate the origin and the evolution of important *Dickeya* biological features. As expected, both
255 trees were largely consistent, even if the rprots tree was globally less resolved, in particular
256 regarding *D. chrysanthemi* and *Dickeya* gs. poaceaephila. The main inconsistency concerned the
257 relative order of divergence of *D. dadantii*, *D. dianthicola*, and *D. solani* within cluster II. AU tests
258 suggested that rprots could contain a mix phylogenetic signal, and thus that the rprots tree could
259 be less reliable than the core-pf tree. Determining the origin of the conflicting signal in rprots would
260 require more investigations that are beyond the scope of the study. Because *D. solani* is a
261 relatively late diverging lineage within *Dickeya*, we anticipated that this will not impact significantly
262 inferences on the ancient *Dickeya* evolution. Nevertheless, in all analyses (e.g. evolution of
263 virulence related systems, inference of ancestral gene repertoires, see below), we used the
264 species tree based on core-pf, but by considering both possible placements for *D. solani*: either as
265 the sister-group of *D. dadantii* or *D. dianthicola*. Regarding *D. aquatica*, the core-pf tree suggested
266 that this species represent the second diverging lineage with *Dickeya*, while its position was
267 unresolved according to rprots. Our analyses also reclassified strain ATCC 39006 as
268 *Prodigiosinella confusarubida*, which could represent the closest relative of *Dickeya* genus. Better
269 characterization of the biology and diversity of *Prodigiosinella* will provide important clues about
270 their evolutionary links with *Dickeya* and the emergence of *Dickeya* as a genus.

271

272 **Virulence-associated phenotypes of *D. aquatica***

273 While most *Dickeya* species have known vegetal hosts, all the identified strains of *D. aquatica*
274 (174/2^T, 181/2 and Dw0440) have been isolated from waterways (Supplementary Table S1)
275 (Parkinson et al., 2014). To our knowledge, their pathogenicity has never been demonstrated. The
276 *in silico* survey of the *D. aquatica* 174/2^T genome for transcriptional regulator binding sites revealed

the presence of conserved key regulators of virulence (Supplementary Table S7). More precisely, all the regulators (KdgR, PecS, PecT, CRP, Fis, H-NS, GacA-GacS, RsmA-RsmB, MfbR) controlling virulence gene expression in *D. dadantii* (Reverchon et al., 2016) are present in *D. aquatica* 174/2^T. The global regulators FNR and CRP displayed the highest number (approximately 80) of targets, followed by Fur, CpxR, ArcA, Lrp and KdgR each with more than 30 targets. The activator CRP and the repressor KdgR have a key role in *Dickeya* virulence (Duprey et al., 2016a), as they tightly control the pectin degradation pathway and are involved in coupling central metabolism to pectinase gene expression (Nasser et al., 1997), while Fur acts as a repressor of pectinase genes (Franza et al., 2002). In addition, Fur represses genes involved in the metabolism of iron, a metal that plays an important role as a virulence regulatory signal in *Dickeya* (Franza et al., 2002).

The presence of regulators controlling the virulence in *D. aquatica* 174/2^T was puzzling and prompted us to investigate its pathogenicity. For this, we compared the pathogenic potential of *D. aquatica* 174/2^T and *D. dadantii* 3937 on various infection models such as chicory leaves, potato tubers, cucumbers and tomato fruits (Figure 3). *D. aquatica* showed little efficiency in rotting potato and chicory. Indeed, only a small rotten area was observed near the entry point, opposed to *D. dadantii*, which spread inside the tuber or the leaf (Figure 3). Surprisingly, *D. aquatica* appeared particularly efficient on tomatoes and cucumbers, showing a generalised infection and fruit opening after 42 hours. By contrast, *D. dadantii* was much less efficient in infecting tomatoes and cucumbers. Importantly, tomatoes and cucumbers are acidic fruits (pH 4.8 and pH 5.1, respectively), whereas potato tubers and chicory leaves display higher pH (around 6.0 and 6.5, respectively), suggesting that *D. aquatica* could be more efficient on acidic fruits. Yet, neither *D. aquatica* nor *D. dadantii* are able to induce pathogenic symptoms on very acidic fruits such as pineapple (pH 4) or kiwi (pH 3) (Figure 3B). Previous studies have shown that some unrelated *Dickeya* species (i.e. *D. chrysanthemi*, *D. dianthicola*) are also capable of infecting tomatoes (Supplementary Table S1), supporting the hypothesis that recurrent adaptations to similar hosts occurred independently during the evolution of *Dickeya* genus.

305 ***Stress resistance phenotypes of D. aquatica***

306 **Osmotic stress**

307 Depending on the plant host, *Dickeya* species encounter various stresses during the infectious
 308 process (Reverchon et al., 2016). We therefore assessed stress resistance of *D. aquatica* 174/2^T
 309 (Figure 4). This strain proved to be very sensitive to osmotic stress, as it displayed a 50% growth
 310 rate reduction on 0.3 M NaCl while *D. dadantii* was only slightly affected (20% growth rate
 311 reduction). This effect was even more pronounced on 0.5 M NaCl with a growth rate reduction of
 312 90 % for *D. aquatica* and 43% for *D. dadantii* (Figure 4A). This sensitivity to osmotic stress could
 313 be linked to the lack of two osmoprotectant biosynthetic pathways in *D. aquatica*: glycine betaine
 314 biosynthesis (*betA-betB* gene cluster) and phosphoglycosyl glycerate biosynthesis (*pggS-pggP*
 315 gene cluster) (Jiang et al., 2016) (Supplementary Table S4). The *pggSP* gene cluster was present
 316 in *P. confusarubida* and all *Dickeya* species except *D. aquatica*. The phylogeny of the two genes
 317 was consistent with the phylogeny of species (Supplementary Figure S3), indicating that their
 318 absence in *D. aquatica* results from a specific and secondary loss in this taxon. In contrast, the
 319 *betABI* gene cluster was absent in *D. paradisiaca*, *D. aquatica*, *D. dianthicola* and Cluster I species
 320 (Supplementary Table S4). Their phylogenies suggested that the *betABI* gene cluster could have
 321 been present in the ancestor of *P. confusarubida* and *Dickeya*, and secondarily and independently
 322 lost in the *Dickeya* lineages mentioned above (Supplementary Figure S3). Finally, the *ousA* gene
 323 encoding the major osmoprotectant uptake system in *D. dadantii* is missing in *D. aquatica*, as well
 324 as in most other *Dickeya* species, suggesting either a recent acquisition by a few species or
 325 multiple losses of an ancestral system (Supplementary Table S4). Interestingly, *D. solani* and *D.*
 326 *dianthicola*, both lacking the *ousA* gene, are also more sensitive to osmotic stress than *D. dadantii*
 327 (Figure 4B).

328 **Oxidative stress**

329 *D. aquatica* 174/2^T was also very sensitive to oxidative stress, displaying longer lag time than *D.*
 330 *dadantii* in presence of 75 μ M H₂O₂ (Figure 4C). This is consistent with the lack of the periplasmic
 331 superoxide dismutase SodC, whose gene was likely acquired in *Dickeya* after the divergence of *D.*
 332 *paradisiaca* and *D. aquatica* (Supplementary Figure S3), and the lack of the Fe-S cluster assembly

SUF system (Supplementary Table S4). Iron-sulfur (Fe-S) clusters are fundamental to numerous biological processes in most organisms, but these protein cofactors can be prone to damage by various oxidants (e.g., O₂, reactive oxygen species, and reactive nitrogen species) (Roche et al., 2013). In addition, the release of free iron in the bacterial cytoplasm amplifies the oxidative stress through the Fenton reaction, producing the highly toxic and reactive hydroxyl radical OH[•]. Most gammaproteobacteria have two Fe-S cluster biogenesis systems SUF and ISC (Roche et al., 2013). For example, *Escherichia coli* cells switch from the ISC to the SUF system under oxidative stress as OxyR, a sensor of oxidative stress, acts as an activator of *suf* operon expression (Lee et al., 2004). Both the SUF and ISC systems were shown to be required for *D. dadantii* growth in the plant host environment that is continually changing in terms of iron availability and redox conditions (Nachin et al., 2001; Expert et al., 2008; Rincon-Enriquez et al., 2008). While both the ISC and SUF systems were likely present in the ancestor of *Dickeya* and *P. confusarubida*, phylogeny and taxonomic distribution suggested that SUF genes were secondarily lost in *D. paradisiaca*, *D. aquatica*, *D. gs. poaceaephila*, and *D. zeae* (Supplementary Figure S3). The absence of both the SUF system and SodC in *D. aquatica* could be an important factor limiting its host range (Supplementary Figure S3). Finally, the presence of the DNA-binding protein Dps, which has a protective function against a wide range of stresses, in *P. confusarubida* and all *Dickeya* excepted *D. paradisiaca* suggested a specific loss in this latter lineage (Supplementary Figure S3).

Regarding other oxidative stress sources, the NorWVR system responsible for nitrite oxide detoxification was absent in the first diverging *Dickeya* species (Supplementary Table S4). The phylogenies of the corresponding proteins suggested secondary losses in *P. confusarubida*, *D. paradisiaca*, and *D. aquatica*, as these proteins were present in *Pectobacterium*, a closely related genus of *Dickeya* (Supplementary Figure S3). Finally, the ascorbate degradation pathway encoded by the *ula* genes was present in the *D. paradisiaca*, *D. aquatica*, *D. zeae*, and *D. chrysanthemi* species, suggesting that the pathway was also present in the ancestor of *Dickeya* and secondarily lost in *D. gs. poaceaephila* and in the ancestor of Cluster II (Supplementary Figure S3). Ascorbic acid, the major and probably the only antioxidant buffer in the plant apoplast, becomes oxidised during pathogen attack (Pignocchi and Foyer, 2003). Modification of the apoplastic redox state

modulates receptor activity and signal transduction to regulate plant defence and growth (Pignocchi and Foyer, 2003). The capacity of some *Dickeya* species to catabolize ascorbate could be a strategy to weaken the plant defence.

Acidic stress

Regarding pH sensitivity, both *D. aquatica* 174/2^T and *D. dadantii* showed optimum growth at pH 7.0. *D. aquatica* 174/2^T displayed no significant growth rate reduction down to pH 5.3 while *D. dadantii* was slightly affected (20% growth rate reduction at pH 5.3 compared to optimum pH 7.0) (Figure 4D). Below pH 4.9, the growth of *D. aquatica* abruptly diminished compared to that of *D. dadantii* indicating that *D. aquatica* growth was much more impacted by low pH (Figure 4D). Bacterial response to acid stress involves evasion of cell damage and adaptation of the enzymatic profile by reducing reactions producing protons and promoting those consuming protons (Bearson et al., 1997). Concerning the protection of proteins against pH damage, *Dickeya* strains, including *D. aquatica* strain 174/2^T, encode the lysine-rich protein Asr acid-inducible periplasmic chaperone, known to protect the proteins by sequestering protons due to its high basic amino acids composition (Seputiene et al., 2003). In *Dickeya*, the corresponding gene is located downstream the *rstAB* gene cluster, known to control the *asr* gene expression (Ogasawara et al., 2007). Yet, due to their atypical amino acid composition these proteins were often wrongly annotated as histone proteins (e.g. in *D. dadantii* NCPPB 898, *D. dianthicola* NCPPB 3534, *D. dianthicola* NCPPB 453, *D. dianthicola* GBBC 2039, and *D. chrysanthemi* ATCC 11663). In terms of metabolic adaptation, a major strategy for bacterial pH homeostasis consists in using decarboxylases to remove cytoplasmic protons (Krulwich et al., 2011). The glutamate-, arginine-, and lysine-inducible decarboxylases classically found in enteric bacteria (Foster, 2004) are absent in *Dickeya* species. However they contain some organic acid decarboxylases such as oxalate and malonate decarboxylases (Supplementary Table S4). Some *Dickeya* species, including *D. dadantii*, have two oxalate decarboxylation pathways: the *frc-oxc* pathway, being involved in the acid tolerance response in *E. coli* (Fontenot et al., 2013) and the OxdD decarboxylase pathway. Most *Dickeya* species harbour at least one of these oxalate decarboxylation pathways, except *D. aquatica*, *D. gs. poaceaeaphila*, *D. chrysanthemi*, and a few *D. zeae* strains, which were deprived of both pathways

(Supplementary Table S4 and Supplementary Figure S3). The lack of the two oxalate-decarboxylation pathways in *D. aquatica* could contribute to its sensitivity to acidic pH. Finally, in contrast with *P. confusarubida* and most *Dickeya* species, *D. aquatica* and a few *D. zeae* strains were also devoid of malonate decarboxylation pathway (mdcABCDEFGHR) (Maderbocus *et al.*, 2017), which consumes protons (Supplementary Table S4), suggesting secondary gene losses in these strains, a hypothesis that was confirmed by phylogenetic analyses (Supplementary Figure S3).

396 **Twitching motility**

397 Interestingly, colony shape changes were observed when *D. aquatica* was grown at low pH in
398 presence of malic acid (Figure 5). More precisely, colonies became wider (2-3 folds increase in
399 diameter compared to unstressed *D. aquatica*) with a morphology characteristic of twitching motility
400 (Henrichsen, 1972). Correspondingly, we detected genes encoding a complete type IV pilus
401 assembly responsible for twitching motility in *D. aquatica* genome and named them *pil* genes
402 according the *Pseudomonas aeruginosa* nomenclature (Maier and Wong, 2015). Among the
403 collection of twenty-six *Dickeya* strains representing different species, only eight showed twitching
404 at acidic pH (Figure 5). Interestingly, this phenotype appeared strain-dependent rather than
405 species-dependent, as *D. solani*, strain RNS07.7.3B showed twitching motility at acidic pH, while
406 *D. solani* strain PP09019 did not (Figure 5). As the *pil* genes, inherited by *Dickeya* from the
407 common ancestor shared with *P. confusarubida*, were conserved in all species (Supplementary
408 Table S4 and Figure S3), twitching was probably linked to strain-specific induction of these genes
409 under acidic pH. It is noteworthy that twitching motility is strongly influenced by changes in the
410 environment (Henrichsen, 1975). In plant pathogens, the contributions of type IV pilus to virulence
411 have been investigated mainly in vascular pathogens, such as *Ralstonia* and *Xylella*, where they
412 were proposed to contribute to bacterial colonization and spread in the xylem through cell
413 attachment, biofilm formation, and twitching motility (Burdman *et al.*, 2011). However, type IV
414 piliation was also shown to be important for initial adhesion and colonization of leaves in a few non-
415 vascular bacteria such as *Xanthomonas oryzae* *pv.* *oryzicola*, *Pseudomonas syringae* *pv.* *tabaci*
416 and *Pseudomonas syringae* *pv.* *syringae* (Burdman *et al.*, 2011). The importance of type IV pilus in

417 *Dickeya* pathogenicity is therefore an interesting question for the future studies.

418 **Antimicrobial peptides**

419 In response to infection, plants produce antimicrobial peptides (AMPs) to limit pathogen
420 propagation. To overcome AMPs, bacteria remodel their envelope, and more precisely, they
421 modify their LPS to decrease interaction with positively charged AMPs. Among the genes involved
422 in LPS modification, the operons *arnABCDEFT* and *dltXABCD* turned out to be ancestral and
423 conserved in all *Dickeya* species (Supplementary Figure S3), while other genes (i.e. *eptAB*, *pagP*,
424 *lpxO* and *lpxT*) displayed different taxonomic distributions and evolutionary histories
425 (Supplementary Table S4 and Figure S3). For example, *D. aquatica* 174/2^T is lacking *pagP*, *lpxO*
426 and *lpxT* genes possibly contributing to a greater sensitivity to AMPs.

427 To conclude, our data indicate that, *D. aquatica* 174/2^T has an unanticipated phytopathogenic
428 capacity similar to that of other *Dickeya* species. Particular stress resistance profiles and induction
429 of twitching at acidic pH may contribute to its restricted host range. Based on this observation, we
430 decided to compare the *D. aquatica* 174/2^T and other *Dickeya* species proteomes with a special
431 focus on the virulence determinants including plant cell wall degrading enzymes, secretion
432 systems, iron metabolism, plant adhesion elements and secondary metabolism.

433

434 **Distribution of plant cell wall degrading enzymes in *Dickeya***

435 The plant cell wall is a complex and dynamic meshwork of polymers (cellulose, hemicellulose,
436 pectin, structural glycoproteins) (Pauly and Keegstra, 2016). Among these polymers, pectin is the
437 most complex and includes both linear regions composed of polygalacturonan and ramified regions
438 (RGI and RGII, respectively). RGI contains a rhamnogalacturonan backbone and various lateral
439 chains such as galactan, arabinan and galacturonan (Caffall and Mohnen, 2009). RGII contains a
440 short galacturonan backbone, carrying four side chains, with a diversity of rare monosaccharides
441 (O'Neill et al., 2004). The carboxylic groups of D-galacturonate residues are methyl-esterified to
442 various degrees (up to 80%) and these residues are, to a lesser extent, acetylated at the C2 and/or
443 C3 positions. Feruloyl esters are a type of modification commonly found in arabinan and galactan
444 chains of ramified regions (Ishii, 1997). The virulence of *Dickeya* is correlated with their ability to

445 synthesize and secrete plant cell wall degrading enzymes, including a full set of pectinases
 446 (Hugouvieux-Cotte-Pattat et al., 2014), xylanases and xylosidases (Keen et al., 1996) proteases
 447 PrtA, PrtB, PrtC, PrtG (Wandersman et al., 1987), and the cellulase Cel5Z (Py et al., 1991). The
 448 presence of these virulence factors varies depending on the species (Matsumoto et al., 2003;
 449 Duprey et al., 2016b). To obtain a comprehensive view of this phenomenon, we explored the “Plant
 450 cell wall degradosome” of *Dickeya* species including *D. aquatica* (**Figure 6**).

451

452 The pectinasome

453 The *Dickeya* pectinasome includes multiple pectate lyases (PelA, PelB, PelC, PelD, PelE, PelL,
 454 PelI, PelN, PelW, PelX, PelZ, Pel10), pectin lyases (PnlG, PnlH), polygalacturonases (PehK,
 455 PehN, PehV, PehW, PehX), pectin methyl esterases (PemA, PemB), pectin acetyl esterases
 456 (PaeX, PaeY), feruloyl esterases (FaeD, FaeT), rhamnogalacturonate lyases (RhiE, RhiF), and
 457 one periplasmic endogalactanase (GanA) (Figure 6) (Hugouvieux-Cotte-Pattat et al., 2014). *D. gs.*
 458 *poaceaephila* NCPPB 569 was the genospecies with the poorest pectinase content. According to
 459 their taxonomic distribution and phylogeny, most of these proteins could be inferred in the ancestor
 460 of *Dickeya* and in its sister lineage *P. confusarubida*. Regarding the *peIAED* cluster, while *P.*
 461 *confusarubida* has a single gene, the ancestor of *Dickeya* had two copies, *peIA* and an
 462 undifferentiated *peIDE* pectate lyase-coding gene (Duprey et al., 2016b), suggesting that a
 463 duplication event occurred in the stem of *Dickeya*. This undifferentiated *peIDE* has undergone a
 464 duplication event after the emergence of *D. paradisiaca*, giving rise to *peID* and *peIE*
 465 (Supplementary Figure S5) (Duprey et al., 2016b). *D. aquatica* has then lost *peIE*, while *D.*
 466 *poaceaephila* has lost both *peIA* and *peIE*. The *peIA* gene was also lost in *D. dianthicola*, while
 467 *peIE* was lost in some *D. chrysanthemi* strains (Figure 6, Supplementary Figure S5). The *peIBC*
 468 cluster was also likely present in the *Dickeya* ancestor, and conserved in most *Dickeya* species.
 469 However, *peIB* was lost in *D. gs. poaceaephila*, and *D. dadantii subsp. dieffenbachiae* NCPPB
 470 2976 (Supplementary Figure S3). The pectate lyase PelI was present in *Pectobacterium* and in all
 471 *Dickeya*, except *D. paradisiaca*, and in *P. confusarubida*, suggesting that it could be ancestral in
 472 *Dickeya*. Similarly, the phylogeny of pectin methyl esterase PemB indicated that the protein was

473 present in *P. confusarubida* and *Pectobacterium*, suggesting an ancestral presence in *Dickeya*.
 474 Accordingly, its absence in *D. paradisiaca*, *D. aquatica*, *D. gs. poaceaephila*, and *D. zea* strains
 475 likely reflected secondary losses. The phylogeny of this protein also suggested that the *pemB* gene
 476 found in *D. chrysanthemi* was acquired by HGT from Cluster II species (Supplementary Figure S3).
 477 Regarding the polygalacturonase PehN, a similar scenario could be inferred except that the
 478 corresponding gene was also lost in *P. confusarubida*, and that a few *D. zea* strains seemed to
 479 have reacquired a PehN from Cluster II species by HGT (Supplementary Figure S3). Regarding
 480 the *pehVWX* cluster, the three genes were derived from a single *pehX* gene that was present in the
 481 ancestor of *P. confusarubida* and *Dickeya*. A gene duplication event occurred in the ancestor of *D.*
 482 *dianthicola*, *D. solani* and *D. dadantii* leading to PehV. A second event led to the divergence of
 483 PehW in the ancestor of *D. solani* and *D. dadantii* (Supplementary Figure S5). The
 484 polygalacturonase PehK was likely present in the ancestor of all *Dickeya* and secondarily lost in *D.*
 485 *gs. poaceaephila*, *D. solani*, and *D. dianthicola* (Supplementary Figure S3). The pectin lyase PnlG
 486 was likely present in the ancestor shared by *Dickeya* and *P. confusarubida*, and secondarily lost in
 487 *D. aquatica*, *D. gs. poaceaephila*, *D. chrysanthemi*, *D. dianthicola* and in some *D. zea* strains
 488 (Supplementary Figure S3). Finally, the rare pectate lyase Pel10 and pectin lyase PnlH displayed
 489 patchy taxonomic distributions (Figure 6). Their phylogenies suggested that they spread through
 490 HGT in *Dickeya* (Supplementary Figure S3).
 491 The saturated and unsaturated digalacturonates resulting from pectin degradation by pectinases
 492 are converted into monogalacturonate and 5-keto-4-deoxyuronate by the oligogalacturonate lyase
 493 Ogl, which is present in all *Dickeya* species (Figure 6). The phylogenies of the *gan* gene cluster
 494 responsible for degradation of galactan chains in pectin-ramified regions and the
 495 rhamnogalacturonate lyase RhiE involved in degradation of RGI pectin-ramified regions indicated
 496 they were likely present in the ancestor of *P. confusarubida* and *Dickeya*, and secondarily lost in
 497 the basal *D. paradisiaca* species, in *D. gs. poaceaephila*, and in a few other strains (Figure 6). The
 498 ferulate esterases FaeT and FaeD were absent in *P. confusarubida* and were acquired in the basal
 499 *D. paradisiaca* and *D. aquatica* species. The FaeT enzyme was then secondarily lost in *D. gs.*
 500 *poaceaephila*, and *D. dadantii subsp dieffenbachiae* (Supplementary Figure S3). Altogether, the

501 variability observed in the pectinasome among *Dickeya* species and even among different strains
502 likely reflects the dynamic evolution of the corresponding gene families involving gene acquisitions
503 and losses. More compellingly, this variability indicates that the pectinasome cannot be used to
504 distinguish among various species.

505

506 **Cellulases and Xylanases**

507 Two cellulases Cel5Z and CelY were likely ancestral in *Dickeya* species except in *D. gs.*
508 poaceaephila, which has lost Cel5Z. While Cel5Z was involved in cellulose degradation, CelY
509 belonged to the *bcs* gene cluster responsible for cellulose fiber formation (Prigent-Combaret et al.,
510 2012). Xylanases (XynA, XynB) and xylosidases (XynC, XynD) cleave xylan and xyloglucan, which
511 belong to the hemicelluloses. The β 1,4 xylan is mainly present in plant cell wall of monocots (Pena
512 et al., 2016) and is further decorated, often by acetyl, arabinosyl, and glucuronosyl side-chain
513 substitutions. Notably, the xylan substitution patterns depend on the plant species and are distinct
514 in gymnosperms and angiosperms (Busse-Wicher et al., 2016). Xyloglucan is a β -1,4 glucan that
515 can be substituted with a diverse array of glycosyl and nonglycosyl residues. The type and order of
516 xyloglycan substituents depend on the plant species (Pauly and Keegstra, 2016). Xyloglucan
517 polymers fall into one of two general types. In one type, three out of four backbone glucosyl
518 residues are xylosylated, leading to an XXXG-type xyloglycan, which is predominant in most
519 dicots. Another type of xyloglycan exhibits reduced xylosylation in that only two out of the four or
520 more backbone glucosyl residues are xylosylated, resulting in the XXGGn-type xyloglucan, which
521 is present in early land plants such as liverworts, mosses, lycophytes, and ferns of the order
522 Polypodiales. This type of xyloglucan seems to be absent from the gymnosperms and
523 angiosperms, with the exception of the grasses (Poales) and plants from the order Solanales, such
524 as potato, tobacco and tomato (Pauly and Keegstra, 2016). Thus, it is tempting to hypothesize that
525 the xylanase and xylosidase content of *Dickeya* species could be correlated with their plant host.
526 Interestingly, all *Dickeya* species as well as *P. confusarubida* contained at least one XynA, XynB,
527 XynC, or XynD coding gene (Figure 6). The phylogeny of XynA indicated that the corresponding
528 gene was likely present in the ancestor of *P. confusarubida* and *Dickeya*, and secondarily lost in *D.*

529 *dianthicola*, *D. gs. undicola*, *D. chrysanthemi*, *D. gs. poaceaephila*, and *D. aquatica*
530 (Supplementary Figure S3). The XynB phylogeny suggested a secondary acquisition in *Dickeya*
531 after the divergence of *P. confusarubida*, *D. paradisiaca*, and *D. aquatica*, followed by losses in *D.*
532 *dianthicola*, *D. chrysanthemi*, and *D. gs. undicola* (Supplementary Figure S3). While the mode of
533 action of the xylanase XynB was not studied, XynA is a glucuronoxylanase (CAZy family GH30)
534 hydrolyzing the xylan backbone adjacent to each glucuronosyl side-chain (Urbanikova et al., 2011).
535 *D. aquatica*, *D. chrysanthemi*, *D. gs. undicola*, and *D. dianthicola* were deprived of both XynA and
536 XynB (Figure 6) suggesting that these species would preferentially infect dicots as xylan is mainly
537 present in plant cell wall of monocots. However, this is probably a tendency since *D. chrysanthemi*
538 strain Ech1591 was isolated from maize, which is a monocot. The phylogeny of Xylosidase XynD
539 suggested that the corresponding gene has been acquired by HGT in *D. aquatica* and *D.*
540 *chrysanthemi*, (Supplementary Figure S3). A secondary acquisition via HGT could also be
541 hypothesized for XynC (Supplementary Figure S3), as it is absent in *P. confusarubida* and the
542 basal *Dickeya* species (i.e. *D. paradisiaca*, and *D. aquatica*), as well as in *D. gs. poaceaephila*, *D.*
543 *zeae* and some *D. chrysanthemi* strains. Strikingly, the xylose degradation pathway (XylABFGHR),
544 present in *P. confusarubida* and in all *Dickeya*, was absent in *D. aquatica*, indicating clearly a
545 specific loss in this species (Supplementary Figure S3). The absence of the xylanases XynA and
546 XynB, and xylosidase XynC as well as the xylose degradation pathway in *D. aquatica* could
547 contribute to its restricted host range.

548

549 **Proteases**

550 Finally, the proteases PrtA, PrtB, PrtC, and PrtG, resulting from specific duplications that occurred
551 during the diversification of *Dickeya*, and the associated type I protease secretion system PrtDEF
552 were absent in *P. confusarubida* and the basal branching *D. paradisiaca*. Yet, *Pectobacterium*
553 harbour closely related homologues of PrtDEF and a single protease-coding gene closely related
554 to *Dickeya* PrtA, PrtB, PrtC and PrtG. This suggested that the whole system could have been
555 present in the common ancestor they shared with *Dickeya*, and then secondarily lost in *P.*
556 *confusarubida* and *D. paradisiaca* (Supplementary Figure S3). PrtG was specifically lost in *D.*

557 *chrysanthemi*, *D. dianthicola* *D. gs. undicola* and *D. gs. poaceaephila*. This later genomospecies
558 conserved only one protease PrtC whereas *D. dianthicola* strains RNS04-9 and NCPB453
559 contained a *prtA* pseudogene and retained two proteases PrtC, PrtB (Figure 6).

560

561 **Other factors**

562 In addition to plant cell wall degrading enzymes, *Dickeya* use several other factors to colonize plant
563 tissue and enhance the progression of disease. Such factors include the extracellular necrosis
564 inducing protein NipE and the two paralogous proteins AvrL and AvrM. NipE and AvrL are
565 conserved in *Pectobacterium* and in most *Dickeya* species, except in *D. paradisiaca*, *D. gs.*
566 *poaceaephila*, and *P. confusarubida*, suggesting secondary losses in these lineages
567 (Supplementary Figure S3). AvrM was also absent in *D. zea*, some *D. chrysanthemi*, *D. gs.*
568 *undicola*, and *D. dianthicola*. Interestingly *D. zea* strains isolated from rice were the only *Dickeya*
569 to be devoid of Avr proteins (Supplementary Table S4). Altogether, our data indicate that the host
570 range specificity of the various *Dickeya* species is probably linked to the particular combination of
571 plant cell wall degrading enzymes and accessory toxins they produce.

572

573 **Distribution of secretion systems in *Dickeya* species**

574 For all *Dickeya* species, possession of secretion systems allowing them to actively secrete
575 virulence factors is of crucial importance. Unsurprisingly, different protein secretion systems (T1SS
576 to T6SS) are present in *Dickeya* species (Supplementary Table S4). As previously mentioned, the
577 type I protease secretion system PrtDEF was present in the ancestor of all *Dickeya* species and
578 lost in the basal *D. paradisiaca* species and in *P. confusarubida*. All *Dickeya* species are equipped
579 with the Out specific T2SS responsible for the secretion of most pectinases and the cellulase
580 Cel5Z. The phylogeny of the components of the Out system indicated it was likely present in the
581 ancestor shared by *Pectobacterium*, *P. confusarubida*, and *Dickeya*, and was conserved during the
582 diversification of *Dickeya* (Supplementary Figure S3). A second Stt specific T2SS, allows secretion
583 of the pectin lyase PnlH (Ferrandez and Condemine, 2008). Accordingly, the taxonomic
584 distributions of the pectin lyase PnlH and the Stt T2SS components were similar, being present in

585 *D. dianthicola*, *D. chrysanthemi*, and some *D. dadantii* strains, (Supplementary Table S4 and
 586 Figure 6). In *D. gs. poaceaephila*, the Stt T2SS was also present but not associated with PnlH,
 587 thus its function in this strain remains to be determined (Supplementary Table S4 and Figure 6).
 588 Interestingly, a T3SS is present in *Pectobacterium*, *P. confusarubida* and in all *Dickeya* species,
 589 except *D. paradisiaca* and *D. gs. poaceaephila* deprived of the T3SS and the associated DspE
 590 effector, and thus suggesting secondary losses (Supplementary Table S4). Therefore, these latter
 591 species are probably unable to suppress the plant immune response (see below). Interestingly,
 592 phylogenies of these proteins disclosed a close relationship with two bacterial phytopathogens,
 593 *Erwinia* and *Pseudomonas syringae*, suggesting that HGT occurred among these lineages
 594 (Supplementary Figure S3). In fact, the effector DspE belongs to the AvrE superfamily of Type III
 595 effectors (T3Es) (Degraeve et al., 2015). The AvrE family is the only family of T3Es present in all
 596 type III-dependent, agriculturally important phyto bacterial lineages that belong to the unrelated
 597 *Enterobacteriales*, *Xanthomonadales*, *Pseudomonadales* and *Ralstonia* taxa. This indicates that
 598 HGT of these effectors occurred in the ancestors of these important plant pathogen lineages
 599 (Jacobs et al., 2013). Recent studies indicated that AvrE-type effectors alter the sphingolipid
 600 pathway *in planta* by inhibiting the serine palmitoyl transferase (Siamer et al., 2014). The
 601 sphingolipid biosynthetic pathway is induced during the plant hypersensitive response that blocks
 602 pathogen attack at the site of infection (Berkey et al., 2012). Therefore, inhibition of this pathway
 603 delays hypersensitive response-dependent cell death and allows bacterial development *in planta*
 604 (Degraeve et al., 2015). In *Dickeya* species, the T3SS genes are in synteny with the *plcA* gene
 605 encoding a phospholipase. These genes were probably acquired during the same event since *D.*
 606 *paradisiaca* and *D. gs. poaceaephila* that were deprived of the T3SS are also deprived of PlcA
 607 (Figure 6).
 608 All *Dickeya* species and *P. confusarubida* were found to possess a two-partner secretion system
 609 (T5SS) CdiB-CdiA mediating bacterial intercellular competition. Their phylogenies clearly indicate
 610 an ancestral presence in both lineages (Supplementary Figure S3). CdiB is a transport protein that
 611 exports and presents CdiA proteins on the cell surface (Willett et al., 2015). The Cdi system is
 612 involved in contact-dependent growth inhibition (CDI) by delivering the C-terminal toxin domain of

613 CdiA (CdiA-CT) to target bacteria (Aoki et al., 2010). Some *Dickeya* strains are equipped with two
 614 CdiA proteins, for example, *D. dadantii* 3937, which produces two different CdiA-CT toxins: the first
 615 one being a tRNase and the second one harbouring DNase activity (Aoki et al., 2010; Ruhe et al.,
 616 2013). Each Cdi system also encodes a specific CdiI antitoxin that interacts with the cognate CdiA-
 617 CT toxin and prevents auto-inhibition (Willett et al., 2015).

618 The most striking feature in *D. aquatica* was the absence of both type IV (T4SS) and type VI
 619 (T6SS) secretion systems, a trait that was shared with *D. paradisiaca*, *D. gs. poaceaeaphila* and *P.*
 620 *confusarubida*. Yet, the presence of closely related T4SS and T6SS in *Pectobacterium* and other
 621 *Dickeya* species suggests that both systems were present in the ancestor of *Dickeya* and
 622 *Prodigiosinella*, and secondarily lost in the three mentioned species (Supplementary Figure S3).
 623 T4SS systems were used to transport a variety of biomolecules (DNA or proteins) across the
 624 bacterial envelope (Chandran Darbari and Waksman, 2015). Most T4SS detected in *Dickeya*
 625 species are associated with conjugal transfer proteins and thus, correspond likely to conjugative
 626 T4SS that transferred DNA (de la Cruz et al., 2010; Ilangovan et al., 2015). This process is
 627 instrumental in bacterial adaptation to environmental changes (Thomas and Nielsen, 2005). T6SS
 628 is used for interaction with the host and for inter-bacterial competition (Poole et al., 2011).
 629 Unfortunately, while effectors associated to the *Dickeya* T6SS carry C-terminal nuclease domains
 630 that degrade target cell DNA, little is known concerning their function in virulence (Ryu, 2015),
 631 limiting the interpretation of its absence in *D. aquatica*.

632

633 ***Distribution of plant adherence elements in Dickeya species***

634 A chaperone–usher pilus assembly pathway, associated with type I fimbriae (*fimEAICDFGHB*),
 635 was present in *D. aquatica* but not in any other *Dickeya* species (Supplementary Table S4).
 636 Phylogenetic analyses suggested an acquisition from *Morganellaceae* or *Enterobacteriaceae*,
 637 through HGT (Supplementary Figure S3). The adhesin FimH, a two-domain protein at the tip of
 638 type I fimbriae is known to recognize mannoside structures and to be responsible for adhesion to
 639 both animal epithelial cells and plant surface (Haahtela et al., 1985; Sauer et al., 2000). Therefore,
 640 we can hypothesize that type I fimbriae could be involved in adherence of *D. aquatica* to plant

641 surface. Interestingly, in *Xylella fastidiosa*, the Fim system is known to be an antagonist of
 642 twitching motility caused by type IV pili (De La Fuente et al., 2007). In *D. aquatica*, the mutually
 643 exclusive production of type I fimbriae and type IV pilus could be linked to the specific pH
 644 regulation of type IV pilus. When *D. aquatica* penetrates into the intercellular apoplast, which is an
 645 acidic compartment, twitching motility would be induced to favour bacteria dissemination in plant
 646 tissues, while the type I fimbriae would be no longer required during the colonization. This
 647 regulation of twitching would thus contribute to the efficiency of *D. aquatica* for infecting tomatoes,
 648 cucumbers, and probably other acidic fruits.

649 While the presence of type I fimbriae is a specific feature of *D. aquatica* among *Dickeya*, the
 650 Flp/Tad pilus, involved in plant surface adherence (Nykryri et al., 2013), is restricted to *D.*
 651 *chrysanthemi* likely as the consequence of a HGT from another proteobacterium (Supplementary
 652 Table S4 and Supplementary Figure S3). An operon encoding a multi-repeat adhesin
 653 (Dda3937_01477) associated to a T1SS secretion pathway was found in the genome of *D. dadantii*
 654 (Supplementary Table S4). This protein contains multiple cadherin-homologous domains and is
 655 likely involved in plant adhesion. Indeed, in *Pectobacterium atrosepticum*, such a multi-repeat
 656 adhesin secreted by a type I pathway was shown to be required for binding to the host plant
 657 (Perez-Mendoza et al., 2011). This adhesion and its secretion system were absent in the basal
 658 *Dickeya* species as well as in *D. zeae* and *D. chrysanthemi*. Phylogenetic analyses indicated an
 659 acquisition in the ancestor of Cluster II, followed by a secondary loss in *D. dianthicola*
 660 (Supplementary Figure S3).

661 From this analysis, it appears that the different *Dickeya* species retained distinct strategies to
 662 adhere to plant surfaces and that HGT played an essential role in the acquisition of the involved
 663 genes.

664

665 ***Distribution of iron assimilation systems in Dickeya species***

666 Iron acquisition by *Dickeya* is required for the systemic progression of maceration symptoms in the
 667 plant hosts (Enard et al., 1988, Dellagi et al., 2005, Franza et al., 2005). To chelate iron from the
 668 surroundings, most *Dickeya* species synthesize and excrete two siderophores: the

hydroxycarboxylate achromobactin encoded by the *acsABCDEF* cluster (Munzinger et al., 2000), and the catecholate chrysobactin encoded by the *cbsABCEFP* genes (Persmark et al., 1989). The *Dickeya* strain EC16 produces dichrysobactin and linear/cyclic trichrysobactin in addition to the monomeric siderophore chrysobactin (Sandy and Butler, 2011). These siderophores form a complex with Fe(III) designated as ferric-siderophore (Franza and Expert, 2013). The ferric-siderophores are specifically recognized by outer membrane transporters (Acr for ferric-achromobactin; Fct for ferric-chrysobactin). These transporters are gated-channels energized by the cytoplasmic membrane-generated proton motive force transduced by the TonB protein and its auxiliary proteins ExbB and ExbD (Franza and Expert, 2013). Two pairs of ExbB and ExbD proteins are present in most *Dickeya* species. Transport of a ferric-siderophore across the inner membrane involves a specific ABC permease (CbrABCD for ferric achromobactin; CbuBCDG for ferric chrysobactin). Interestingly, the achromobactin genes (*acs* gene cluster) and related transport system (*cbr* gene cluster) were absent in *P. confusarubida* and *D. paradisiaca*, suggesting they were acquired by HGT in *Dickeya* after the divergence of these two lineages. *Dickeya* genes are closely related to *Pseudomonas fulva* and *P. syringae* sequences, suggesting an HGT between these plant-associated bacteria (Supplementary Figure S3). By contrast the chrysobactin genes (*cbs* gene cluster) and related transport system (*cbu* gene cluster) were likely present in the ancestor of *Dickeya* and *P. confusarubida* (Supplementary Figure S3), and then specifically lost in *D. dadantii* subspecies *dieffenbachiae*. In addition to ferric-siderophores, various other iron uptake systems are present in *Dickeya*. The ferrous iron transport systems FeoAB and EfeUOB can be inferred as ancestral in all *Dickeya* species and *P. confusarubida* (Supplementary Figure S3). In contrast, the taxonomic distribution and the phylogeny of the YfeABCD permease that can import both iron and manganese, suggested an acquisition through HGT by *D. chrysanthemi* and Cluster II species, except *D. gs. undicola* (Supplementary Table S4 and Supplementary Figure S3). The haem transport Hmu system was present in most *Dickeya*. Its phylogeny suggested an ancestral presence in *Dickeya*, followed by secondary losses in *D. dianthicola*, *D. gs. undicola*, *D. zaeae*, and *D. aquatica* (Supplementary Figure S3, Supplementary Table S4). Although variable combinations of iron assimilation systems exist in *Dickeya* species, at

697 least four systems were present in each species. This multiplicity underscores the fact that
698 competition for this essential metal is critical for the outcome of the plant-*Dickeya* interaction.

699

700 ***Biosynthesis of secondary metabolites in Dickeya species***

701 In addition to siderophores, some *Dickeya* species produce secondary metabolites such as the
702 phytotoxin zeamine and the antifungal compound oocydin via non-ribosomal peptide synthases
703 (NRPS) and polyketide synthases (PKS) (Zhou et al., 2011a; Matilla et al., 2012). To evaluate the
704 diversity of secondary metabolites produced by the *Dickeya* genus, we screened the eight
705 complete genomes (*D. paradisiaca* Ech703, *D. aquatica* 174/2, *D. zeae* EC1, *D. zeae* Ech586, *D.*
706 *chrysanthemi* Ech1591, *D. solani* IPO2222, *D. fangzhongdai* N14b, *D. dadantii* 3937) and the three
707 partial genomes (*D. dianthicola* RNS04.9, *D. gs. poaceaephila* Ech569, *D. gs. undicola* 2B12) for
708 gene clusters encoding NRPS or/and PKS. Then, we analysed the evolutionary history of these
709 genes clusters among the 49 *Dickeya* genomes. The *oocBCDEFGJKLMNOPQRSTUVWXYZ* gene
710 cluster coding for oocydin biosynthesis proteins was present in *D. paradisiaca*, *D. zeae* strains
711 isolated from rice, *D. chrysanthemi* subspecies *chrysanthemi*, *D. solani*, *D. dianthicola*, and *D.*
712 *fangzhongdai* strain NCPPB 3274 (Supplementary Table S4). This could suggest an ancestral
713 presence in *Dickeya* accompanied by losses in *D. aquatica*, *D. gs. poaceaephila*, *D. dadantii*, *D.*
714 *gs. undicola*, most *D. fangzhongdai* strains, and some Cluster I strains (Supplementary Figure S3).
715 However, the hypothesis of acquisition and spreading through HGT within *Dickeya* could not be
716 excluded. The *zmsABCDEFGHIJKLMNPQRS* gene cluster directing zeamine biosynthesis was
717 restricted to *D. zeae* strains isolated from rice, *D. fangzhongdai* and *D. solani* (Supplementary
718 Table S4), suggesting secondary acquisition by HGT (Supplementary Figure S3). In addition,
719 genes involved in coronafic acid biosynthesis, a phytotoxin classically produced by *Pseudomonas*
720 *syringae* (Bender et al., 1999), were present only in *D. gs. poaceaephila* and *D. dadantii*
721 subspecies *dieffenbachiae* (Supplementary Table S4), suggesting specific acquisition via HGT. We
722 detected four additional gene clusters encoding NRPS and PKS, (i) cluster 1 was specific to *D.*
723 *paradisiaca* and *P. confusarubida*, (ii) cluster 2 was specific to *D. paradisiaca*, (iii) cluster 3 was
724 specific to *D. aquatica*, suggesting recent acquisitions by these species, while (iv) cluster 4 was

more widely distributed, being detected in *D. aquatica*, *D. gs. poaceaephila*, *D. fangzhongdai*, *D. solani*, some *D. dadantii* strains, and *D. zea* except the strains isolated from rice (Supplementary Table S4). To conclude, each *Dickeya* species was characterized by a specific combination of large gene clusters possibly involved in the production and secretion of toxic secondary metabolites. These clusters were likely acquired from unrelated bacteria through HGT and could have been selected based on the constraints imposed by host or environmental factors. For example, the *D. zea* strains can be subdivided in two groups, the strains isolated from rice, which produce both zeamine and oocydin, while the other strains infecting other crops produce the fourth-type metabolite encoded by cluster 4. This difference between *D. zea* strains was used by Zhou et al. (2015) to define the distinct pathovar linked to rice as *D. zea* subsp. *oryzae*.

Evolution of *Dickeya* gene repertoires

The 49 *Dickeya* proteomes used in this study contained in average 4,022 proteins, ranging in size from 3,533 (*D. gs. poaceaephila*) up-to 4,352 (*D. fangzhongdai* NCPPB 3274) proteins, representing a difference of 819 proteins (Supplementary Table S3). Comparison of the 197,073 proteins contained in the 49 *Dickeya* proteomes led to the delineation of 11,566 protein families (Figure 7). These protein families correspond to the pan-proteome of *Dickeya*. Among these protein families, 13.9% (1,604) were present at least in one copy in all *Dickeya* proteomes (Figure 7) and defined the core-proteome of this genus. Yet, the size of both pan- and core-proteomes could be slightly underestimated because the proteomes of some strains were deduced from draft genomes. Nevertheless, this meant that in average, ~39.9% of the proteins of any *Dickeya* proteome belonged to the core proteome, while a given *Dickeya* proteome encompassed only ~34.8% of the pan-proteome of this genus. Random taxonomic sampling-based rarefaction curves indicated that sequencing more *Dickeya* genomes will probably not change significantly the estimated size of the core-proteome, while it appears that the pan-proteome is far from being fully disclosed (Figure 7B). This highlights the high diversity and plasticity of the gene repertoires in *Dickeya*. This observation coupled to the great diversity of the virulence, stress resistance, metabolism, and secretion systems imply that none of the *Dickeya* strains could be regarded as a

753 representative model for this genus. Core protein families could be punctually lost in a given strain,
 754 while some protein families can be present transiently in a few strains. Thus, it is also relevant to
 755 consider the persistent (i.e. protein families present in more than 90% of the strains) and volatile
 756 proteomes (i.e. protein families present in less than 10% of the strains) (Touchon et al., 2009). In
 757 *Dickeya*, most protein families could be classified either as persistent (2,714 protein families,
 758 23.5%) or volatile (6,426 protein families, 55.6%) (Figure 7A). Unsurprisingly, the persistent
 759 proteome was enriched in proteins with known functions, while volatile proteome encompassed
 760 mostly hypothetical proteins, prophage elements, and transposases. It is tempting to consider the
 761 genes encoding for the volatile proteome as a reservoir of functional innovations, yet the adaptive
 762 potential of these genes remains a matter of debate (Touchon et al., 2009).

763 Among the 11,566 protein families inferred in *Dickeya*, 3,452 corresponded to strain specific
 764 families (i.e. being present in a single proteome) (Figure 7A and Supplementary Table S8). The
 765 number of strain specific protein families ranged from 336 in *D. aquatica* 174/2^T and 304 in *D. gs.*
 766 *Poaceaephila*, down to zero in *Dickeya solani* strains MK16, PPO9134 and RNS0773B
 767 (Supplementary Table S8). To determine the origin of these strain specific protein families, we
 768 used them as seeds to query with BLASTP (e-value cut-off 10⁻⁴) a local database gathering 3,104
 769 complete prokaryotic proteomes, including the 49 *Dickeya* and *P. confusarubida* ATCC 39006
 770 proteomes. Results indicated that 1,051 (30.4%) *Dickeya* strain specific protein families displayed
 771 best hit in one of the other 48 *Dickeya* strains, meaning that those sequences were wrongly
 772 considered as strain specific because they did not satisfy the coverage and identity parameters
 773 used to delineate the protein families. This was not surprising because some protein families could
 774 be fast-evolving, meaning that applying uniform parameters can fail to delineate correctly these
 775 protein families and lead to an overestimation of the strain specific protein families. In contrast,
 776 1,625 (47.1%) *Dickeya* strain specific protein families displayed best hits in non-*Dickeya*
 777 proteomes, meaning that the corresponding genes were likely acquired by HGT from non-*Dickeya*
 778 donors. The taxonomic distribution of the corresponding sequences pinpointed *Proteobacteria*
 779 (especially *Enterobacteriales*), and to a less extent *Firmicutes* as major donors (Supplementary
 780 Table S8 B-C). Yet, the contribution of these two phyla is likely overestimated due to their

overrepresentation in sequence databases compared to other lineages. Accordingly, these results should be interpreted as general trends but additional data would be required to precisely estimate the real contribution of *Firmicutes* and *Proteobacteria*. Finally, 776 (22.5%) *Dickeya* strain specific protein families displayed no significant hits or no hits at all, indicating that the corresponding genes were truly strain specific or corresponded to annotation errors (false positives).

At the species level proteome size variation ranged from 435 (*D. aquatica*) to 120 (*D. paradisiaca*) proteins (Supplementary Table S8). The taxonomic distribution of the species-specific protein families displayed overall similar pictures, with most of them being present in all strains of the species (Supplementary Figure S6). This revealed a relative homogeneity of proteomes within species. Interestingly, a few strains diverged from this general trend, such as strain NCPBB 3274 within *D. fangzhongdai*, *D. aquatica* 174/2^T, *D. chrysanthemi* NCPPB 402, *D. dadantii* NCPPB 2976, and *D. solani* RNS 0512A. This is consistent with some previous studies. For instance, *D. dadantii* NCPPB 2976 is part of the *dieffenbachiae* subspecies and has been shown to be clearly different from the other *D. dadantii* subsp *dadantii* strains based on ANI values (Zhang et al., 2016). Among, *D. solani*, strain RNS 0512A was proposed to define a novel *D. solani* sub-group based on the high number and wide distribution of nucleotide variations compared to other *D. solani* strains (Khayati et al., 2015).

Using COUNT (Csuros, 2010) on the 12,660 protein families built with SILIX and the topology of the core-pf tree, we inferred the ancestral protein repertoires at each node of the *Dickeya* core-pf phylogeny (Figure 8). COUNT provided similar results for different *Dickeya* species, irrespectively of the position of *D. solani*, as either the sister-group of *D. dadantii* or *D. dianthicola* (Supplementary Table S9), as suggested by core-pf and rprots phylogenetic analyses (see above).

The main difference lies in the numbers of gains and losses at the base of *Dickeya*.

We inferred 4,627 protein families in the ancestor of *Dickeya*, while in average 3,921 protein families are contained in present-day *Dickeya* proteomes. This corresponds to a global loss of 18% of the protein families. Interestingly, loss of protein families dominated over gains and affected all *Dickeya* species (Figure 8). Highest protein losses were observed on the stems leading to *D. gs. poaceaeaphila* and *D. aquatica* and to a lesser extent in *D. paradisiaca*, *D. gs. undicola*, and *D.*

809 *dianthicola*. These losses were only partially compensated by protein family gains. Surprisingly,
810 more gains were observed in *D. aquatica* 174/2^T, compared to the two other *D. aquatica* strains
811 (Figure 8). We assume that this was not due to biases in the annotation process by RAST,
812 because very similar results were obtained when using PROKKA (Seeman, 2014). In fact, this may
813 reflect the fact that the genomes of DW 0440 and CSL RW240 strains were not completed, being
814 reported as draft genomes. The general trends observed were robust irrespectly of the postion of
815 *D. solani* relatively to *D. dadantii* and *D. dianthicola* (Supplementary Table S9).

816

817 *Characterization of the D. aquatica mobilome*

818 Mobile Genetic Elements (MGEs) are the main actors of the HGT and include plasmids, viruses
819 (phages and prophages) and transposons (Jackson et al., 2011). They are often localised within
820 genomic islands on chromosomes. The mobilome of a strain is the repertoire of all the genes
821 associated with MGEs. Using both PHAST and IslandViewer, we detected seven phage elements
822 and ten genomic islands in *D. aquatica* 174/2^T (Figure 1, Supplementary Table S10). Most of the
823 genomic islands contained genes of transposases, integrases or mobile elements that were likely
824 remnants of HGT. They also contain 105 of the 336 ORFAN genes detected in *D. aquatica* 174/2^T
825 strain. Among the seven detected prophages, P2, P6 and P7 were related to transposable Mu-
826 phage. These were also present in some *D. zeae* strains isolated from river as well as *D.*
827 *dianthicola* strains (Supplementary Table S10). P3 and P5 were specific to some *D. aquatica*
828 strains, while P1, a defective prophage with only few conserved genes, and P4 were widespread in
829 all *Dickeya* species (Supplementary Table S10). Among the ten genomic islands detected in *D.*
830 *aquatica* 174/2^T, seven (GI1, GI2, GI4, GI5, GI6, GI7, GI9) were mainly composed of mobile
831 elements and small hypothetical proteins, GI2 also contained a type III restriction-modification
832 system, whereas GI4 included a type I restriction-modification system and a toxin-antitoxin system
833 (Supplementary Table S10). Similarly, GI6 contained a toxin-antitoxin system as well as an
834 isolated non-ribosomal peptide synthase, which was also found in *D. gs. poaceaephila* and *D. zeae*
835 (Supplementary Table S10). GI7 contained some metabolic proteins, including the previously
836 mentioned cluster 3 encoding NRPS and PKS, as well as transporters, notably a cobalt/nickel ABC

837 transporter (Supplementary Table S10). Excluding the mobile elements, GI1, GI2, GI4, GI5, GI6
838 GI7, and GI9 were specific to *D. aquatica* strains, even if a few genes composing these GIs can be
839 punctually detected in some others strains (Supplementary Table S10). The three other genomic
840 islands (GI3, GI8, GI10) were metabolic islands (Supplementary Table S10). GI3 has been laterally
841 transferred between *Erwinia pyrofolia* and *D. aquatica* (Supplementary Figure S3). GI8 that
842 included proteins related to fatty acid metabolism, was conserved in *D. zeae* strains isolated from
843 rice or originated from China (Supplementary Table S10). GI10 contained proteins related to the
844 complete carbapenem biosynthetic pathway CarABCDE and the associated resistance proteins
845 CarFG (Supplementary Table S10). *P. confusarubida* contained genes coding for CarABCDE as
846 well as CarFG, in agreement with its capacity to synthesize the carbapenem antibiotic (carbapen-
847 2-em-3-carboxylic acid) (Thomson et al., 2000; Coulthurst et al., 2005). This cluster was conserved
848 in *D. undicola*, *D. dadantii* subspecies *dieffenbachiae*, some *D. chrysanthemi* strains and in the *D.*
849 *zeae* CSL-RW192 strain isolated from water (Supplementary Table S10). *D. paradisiaca* only
850 contained the *carFG* resistance genes but was deprived of the biosynthetic genes (Supplementary
851 Table S9). The phylogeny of CarABCDE and CarFG shows that relationships among the *Dickeya*
852 sequences are inconsistent with the species phylogeny, suggesting a secondary acquisition
853 through HGT in this genus (Supplementary Figure S3).

854 Overall, phages and genomic islands from *D. aquatica* are mostly species specific suggesting a
855 wide genomic plasticity in the *Dickeya* genus.

856

857 *Concluding remarks*

858 In this work, we sequenced the complete genome of *D. aquatica* 174/2 and showed that unlike
859 initially supposed, this bacterium, similarly to other *Dickeya* species, is a phytopathogen. Who is
860 the natural host of *D. aquatica* and why is this bacterium found in aquatic environments rather than
861 associated with plants as are its close relatives? Although the data at hand are not sufficient to
862 answer these questions, we speculate that *D. aquatica* can be pathogenic for aquatic plants such
863 as charophytes, which are a family of complex-structured algae living in a variety of wetland and
864 freshwater habitats including those, from which the *D. aquatica* strains have been isolated.

Charophytes are thought to be the closest ancestor of land plants. The cells of these algae are surrounded by polysaccharide-based cell walls. However, their cell walls are thin and cannot be distinguished as primary or secondary cell walls. Furthermore, they lack xyloglucans, which are common in most land plants (Sarkar et al., 2009). Intriguingly, *D. aquatica* lacks the xylanases and xylose degradation pathways and we hypothesize that this could reflect an evolutionary adaptation to charophyte hosts.

The comparison of *D. aquatica* 174/2 and *Dickeya* strain proteomes available in public databases showed that the *Dickeya* genus displays a remarkable diversity featuring many unique protein families and emphasizing that our knowledge of this genus is still limited. The real size of the pan-proteome is an open question encouraging further exploratory studies on these bacteria, the success of which will depend on the collection of new strains isolated from various environments and the sequencing of the genomes of newly identified representatives of the genus. Remarkably, we observed an enormous degree of genetic plasticity in the pathogenicity determinants that enable various *Dickeya* species to colonize a wide range of plant hosts. Furthermore, in this work, we have postulated the existence of a sister genus of *Dickeya*, *Prodigiosinella*, and characterized two new *Dickeya* genomospecies. The reconstruction of ancestral genomes allowed us to gain new insights into the evolutionary history of this genus and highlighted an evolutionary trajectory dominated by the loss of protein families.

Experimental procedures

Genome sequencing, assembly and annotation

DNA for sequencing of the *D. aquatica* type strain (174/2) genome was extracted from overnight broth culture using Promega bacterial genomic DNA kit. PacBio sequencing to > 350X coverage was performed by Eurofins Genomics (<https://www.eurofinsgenomics.eu/>). Reads were assembled using CANU (Koren et al., 2017). The annotation was performed automatically with RAST (Aziz et al., 2008), then expertly reviewed using MAGE (Vallenet et al., 2013) and literature data. The expert review allowed to assign 2,457 gene names, correct 547 annotations and add 5 CDS missed by RAST. The replication origin (oriC) was predicted by OriFinder

893 (<http://tubic.tju.edu.cn/Ori-Finder>) (Gao and Zhang, 2008). The presence of mobile genetic
894 elements in the *D. aquatica* 174/2 genome was investigated by the following online tools:
895 IslandViewer (<http://pathogenomics.sfu.ca/islandviewer>) (Dhillon et al., 2015) for the GI regions,
896 CRISPRDetect (http://brownlabtools.otago.ac.nz/CRISPRDetect/predict_crispr_array.html) (Biswas
897 et al., 2016) for CRISPR arrays, whereas putative prophage sequences were identified by PHAST
898 and PHASTER analysis (<http://phast.wishartlab.com/>) (Zhou et al., 2011b; Arndt et al., 2016). The
899 genome sequence has been submitted to EMBL database under accession number
900 GCA_900095885 (<http://www.ebi.ac.uk/>).

901

902 *Virulence assays on various hosts*

903 Bacterial cultures were grown in M63G minimal medium (M63 + 0.2% w/v glucose) (Miller 1972)
904 and diluted to a given OD₆₀₀ depending on the host: 0.2 (chicory) or 1 (potato, cucumber, tomato,
905 pineapple and kiwi). For chicory, 5 µL of bacterial suspension were injected into a 2 cm incision at
906 the center of the leaf. For potato, cucumber and tomato, 5, 100 and 200 µL of bacterial suspension
907 were injected into the vegetable, respectively. 200 µL of bacterial suspension were also injected
908 into pineapple and kiwi fruits. Plants were incubated at 30°C with 100% humidity for 18 h (chicory)
909 or 42 h (potato, cucumber and tomato) or 78 h (pineapple and kiwi). The soft rot mass is used to
910 quantify virulence.

911

912 *Stress resistance assays*

913 Bacteria were cultured at 30°C in 96 well plates using M63G (M63 + 0.2% w/v glucose) pH 7.0 as
914 minimal medium. Bacterial growth (OD_{600nm}) was monitored for 48 h using an Infinite® 200 PRO -
915 Tecan instrument. Resistance to osmotic stress was analysed using M63G enriched in 0.05 to 0.5
916 M NaCl. Resistance to oxidative stress was analysed in the same medium by adding H₂O₂
917 concentrations ranging from 25 to 200 µM. The pH effect was analyzed using the same M63G
918 medium buffered with malic acid at different pH ranging from 3.7 to 7.0.

919

920 *Proteome database construction*

921 We built a local database (DickeyaDB) gathering the proteomes of *Serratia* sp. ATCC 39006, 48
922 *Dickeya* available at the NCBI (by January 2017), and *D. aquatica* type strain 174/2
923 (Supplementary Table S3). A second database (prokaDB) containing 3,104 proteomes of
924 prokaryotes, including the 50 proteomes of DickeyaDB, was also built.

925

926 *Assembly of Dickeya and Serratia ATCC 39006 protein families*

927 *Dickeya* and *Serratia* ATCC 39006 protein families were assembled with SILIX version 1.2.9 (Miele
928 et al., 2011). More precisely, pairwise comparisons of protein sequences contained in DickeyaDB
929 were performed using the BLASTP program version 2.2.26 with default parameters (Altschul et al.,
930 1997). Proteins in a pair providing HSP (High-scoring Segment Pairs) with identity over 60% and
931 covering at least 80% of the protein lengths were gathered in the same family. This led to the
932 assembly of 12,660 protein families, among which 1,493 were present in at least one copy in all
933 DickeyaDB proteomes. In contrast, considering the 49 *Dickeya* proteomes without taking into
934 account *Serratia* ATCC 39006 led to the assembly of 11,566 protein families, among which 1,604
935 were present at least in one copy in all *Dickeya* and *Serratia* ATCC 39006 proteomes, and 1,420 in
936 exactly one copy.

937

938 *Inference of reference phylogenies of Dickeya*

939 Reference phylogenies of *Dickeya* were inferred using ribosomal proteins (rprots) on the one hand
940 and core protein families (core-pf) on the other hand. The rprots phylogenetic tree was rooted with
941 sequences from *Serratia* ATCC 39006, together with three *Pectobacterium* species
942 (*Pectobacterium carotovorum* PC1, *Pectobacterium atrosepticum* SCRI1043, and *Pectobacterium*
943 *wasabia* WPP163) and five additional *Serratia* species (*Serratia marcescens* FGI94, *Serratia*
944 *fonticola* DSMZ4576, *Serratia liquefaciens* ATCC27592, *Serratia proteamaculans* 568, and
945 *Serratia plymuthica* AS9), while the core-pf phylogenetic tree was rooted with *Serratia* ATCC
946 39006 (see results).

947 Rprots sequences were extracted from the DickeyaDB using the engine of the riboDB database
948 (Jauffrit et al., 2016). Briefly, the riboDB engine allows retrieving rprots sequences through a

double approach combining reciprocal best-blast-hits and hidden Markov model (HMM) profiles searches.

Starting from the 1,420 core-pf protein families containing exactly one copy in each proteome of the DickeyaDB and 51 rprots, we applied several quality controls. First, within protein families extremely short sequences (<30% of the median length of the family) were discarded. Then, we used FastTreeMP (Price et al., 2010), and PhyloMCOA (de Vienne et al., 2012) to detect and discard outlier sequences, on the basis of nodal and patristic distances. At the end of the quality control process, 1,341 protein families present in more than 35 (70%) out of the 50 considered proteomes and 51 rprots were kept. For each of these protein families, multiple alignments were built using the CLUSTAL-Omega-1.1.0 program (Sievers et al., 2011) and trimmed using GBLOCKS (Castresana, 2000) with parameters set to a minimal trimming. The trimmed multiple alignments corresponding to 1,341 core-pf on the one hand and the 51 rprots on the other hand have been combined using Seaview-4.5.4 (Gouy et al., 2010) to build the core-pf and the rprots supermatrices containing 414,696 and 6,295 amino acid positions, respectively.

Maximum likelihood (ML) phylogenies of these supermatrices have been inferred with IQ-TREE-1.5.3 (Nguyen et al., 2015) with a C60 profile mixture of the Le and Gascuel evolutionary model (Le and Gascuel 2008) and a gamma distribution with four site categories (Γ_4) to model the heterogeneity of evolutionary rates across sites, as proposed by the model testing tool (Kalyaanamoorthy et al., 2017) available in IQ-TREE. The robustness of the inferred ML trees was estimated with the non-parametric bootstrap procedure implemented in IQ-TREE-1.5.3 for the rprots supermatrix (100 replicates of the original alignments) and the ultrafast bootstrap approach for the core-pf supermatrix (1,000 replicates).

971

Phylogenetic analysis of single markers

947 SSU rRNA and 125 LSU rRNA complete sequences from *Dickeya*, *Serratia* (including strain ATCC 39006), and *Pectobacterium* available in public databases were retrieved and aligned with MAFFT v7.222. The resulting multiple alignments were trimmed using BMGE-1.1 (default parameters). The phylogeny of the 947 SSU rRNA sequences was inferred using FastTree-2.1.9

977 (Price et al., 2010), with the GTR + gamma + cat 4 model, while the LSU rRNA tree was inferred
978 with IQ-TREE with the TIM3+F+I+Γ4 model according to the BIC criterion, as suggested by the
979 propose model tool available in IQ-TREE.

980 Homologues of proteins of interest were identified in the prokaDB with BLASTP. The first 75 HSP
981 hits with evalule smaller than 10^{-4} were kept. The retrieved sequences, together with the seed were
982 aligned using MAFFT v7.222, alignments were trimmed using BMGE-1.1 (default parameters). ML
983 phylogenies were built using IQTREE-1.5.3 (Nguyen *et al.*, 2015) with the LG+Γ4+I+F model. In
984 order to identify the origin and evolution of these proteins in *Dickeya*, their phylogenies were
985 compared and reconciliated with the *Dickeya* species phylogenies, by considering the two
986 alternative positions of *D. solani*: as either sister-group of *D. dadantii* or *D. dianthicola*.

987

988 *Identification of the D. fangzhongdai phylogenetic cluster*

989 To determine whether some sequenced strains are related to *D. fangzhongdai*, we used all the *D.*
990 *fangzhongdai* genes available in public databases SSU-rDNA (KT992690.1), *dnaX* (KT992713.1),
991 *fusA* (KT992697.1), *purA* (KT992705.1), *recA* (KT992693.1), *gapA* (KT992701.1) and *rplB*
992 (KT992709.1) genes, we extracted the corresponding genes from the DickeyaDB database and
993 then used these sequences as input for phylogenetic analyses using leBIBI^{QBPP} phylogenetic
994 positioning tool (Flandrois et al., 2015). We found that the strains B16, MK7 and S1 were
995 systematically affiliated with *D. fangzhongdai*.

996

997 *Ancestral gene content*

998 The program COUNT (Csuros, 2010) was used for gene families evolutionary reconstruction in
999 *Dickeya* species using the topology of the core-pf tree as reference. All the generated 12,660
1000 families were submitted to COUNT, which can perform ancestral genome reconstruction by
1001 posterior probabilities in a phylogenetic birth-and-death model. Rates were optimized using a gain–
1002 loss–duplication model and three discrete gamma categories capturing rate variation across
1003 families, with other parameters set at default and allowing different gain–loss and duplication–loss
1004 rates for different branches. One hundred rounds of optimization were computed. COUNT was run

1005 twice: first with *D. solani* as sister-group of *D. dadantii* and then as sister-group of *D. dianthicola*.

1006

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1013

1014 **Conflict of interest statement**

1015 The authors declare that no conflicting interests exist.

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1017 **References**

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1375 **Figure legends**

1376

1377 **Figure 1. Genomic organisation of *Dickeya aquatica* 174/2^T chromosome**

1378 The chromosome is represented as a wheel and the origin of replication (OriC) and terminus (Ter)
1379 are indicated. The circles from outside to inside represent protein-coding sequences (CDS) on the
1380 forward strand, CDS on the reverse strand, distribution of ribosomal RNA operons (four on the right
1381 replichore and three on the left replichore), distribution of tRNA genes, and then distribution of
1382 ncRNA. The blue areas correspond to phage elements detected using PHAST. The thin black lines
1383 correspond to four CRISPR arrays and the red areas represent the ten genomic islands predicted
1384 using IslandViewer. The next circle (black) indicates the GC content and the central circle
1385 (green/purple) shows the GC-skew. The window size of the GC content and GC-skew is 100
1386 nucleotides. Figure 1 was drawn using Gview <https://server.gview.ca/>.

1387

1388 **Figure 2. Phylogeny and proteome comparison of *Dickeya***

1389 Maximal likelihood phylogeny (left) of 1,341 *Dickeya* single copy protein families (50 sequences,
1390 414,696 amino acid positions). The tree was computed with IQ-TREE with the LG+C60 model and
1391 rooted using *Serratia* ATCC 39006. Numbers associated to branches correspond to ultrafast
1392 bootstrap values. The scale bar corresponds to evolutionary distance (i.e. the average number of
1393 the substitutions inferred per site). The table (right) corresponds to the S_{AB} association coefficient
1394 computed for each pair of strains as $S_{AB} = (100 \times 2N_{AB}) / (N_A + N_B)$, in which N_A is the number of
1395 protein families present in strain A, N_B is the number of protein families present in strain B and N_{AB}
1396 is the number of protein families shared by strain A and strain B. This coefficient ranged from 0
1397 when both strains do not share any gene family to 100 when all the families present in strain A
1398 were also present in strain B. The figure was generated using Evolview (He et al., 2016).

1399

1400 **Figure 3. Virulence of *D. aquatica* on potato, chicory, cucumber and tomato.** Bacterial
1401 cultures were grown in M63G minimal medium (M63 + 0.2% w/v glucose) and diluted to a given
1402 OD₆₀₀ depending on the host: 0.2 (chicory) or 1 (potato, cucumber and tomato). For chicory, 5 µL

of bacterial suspension were injected into a 2 cm incision at the center of the leaf. For potato, cucumber and tomato, 5, 100 or 200 μ L of bacterial suspension were injected into the vegetable, respectively. Plants were incubated at 30°C with 100% humidity for 18 h (chicory) or 42 h (potato, cucumber and tomato). **A)** Picture of representative specimens of infected plants after incubation. Note that the rotten area was removed for potato. **B)** Quantification of the soft rot mass. Data is represented as mean $\pm$ SD of 6 replicates. No soft rot symptoms were detected after 78 h for pineapple and kiwi fruits infected with 200 μ L of bacterial suspension at OD₆₀₀ 1.

1410

Figure 4. *D. aquatica* stress resistance. Bacteria were cultured at 30°C in 96 well plates using M63G (M63 + 0.2% w/v glucose) pH 7.0 as minimal medium. Bacterial growth (OD_{600nm}) was monitored for 48 h using an Infinite® 200 PRO - Tecan instrument. A and B) Resistance to osmotic stress was analysed using M63G enriched in 0.05 to 0.5 M NaCl (abscissa) and growth rates (ordinate) were determined. C) Resistance to oxidative stress was analysed in the same medium by adding H₂O₂ concentrations ranging from 25 to 200 μ M (abscissa). The lag time (ordinate) is represented instead of the growth rate because after the degradation of H₂O₂ by bacterial catalases, the growth rates are similar. D) The pH effect on growth rate (ordinate) was analyzed using the same M63G medium buffered with malic acid at different pH ranging from 3.7 to 7.0 (abscissa).

1421

Figure 5. Twitching motility induced under acidic condition in *Dickeya* species. Colony morphologies of various *Dickeya* strains grown on M63G pH 7.0 and M63G buffered with malic acid at pH 5 in agar plates. The strains corresponding to colony numbers and their twitching phenotypes are indicated on the right.

1426

Figure 6. Distribution of plant cell wall degrading enzymes in *Dickeya*. The type of cell wall degrading enzyme and the number of homologues of each enzyme are indicated for each of the strains. The phylogeny on the left corresponds the core-pf ML tree. The figure was generated using

1430 Evolveview (He et al., 2016).

1431

1432 **Figure 7: The core-, pan-, versatile-, and persistent-proteomes of *Dickeya* genus**

1433 The delineation of core-, pan-, versatile-, and persistent-proteomes was based on the taxonomic
 1434 distribution of the 11,566 proteins families identified by analyzing the 49 proteomes of *Dickeya*.
 1435 The core-proteome is defined by the protein families present at least in one copy in all 49 *Dickeya*
 1436 proteomes, the pan-proteome is defined by the 11,566 protein families, while the versatile- and the
 1437 persistent-proteomes are defined as the protein families present in less than 10% and in more than
 1438 90% of the 49 *Dickeya* proteomes, respectively.

1439 **(A) Distribution 11,566 protein families across the 49 *Dickeya* proteomes.**

1440 The 3,452 protein families present in a single *Dickeya* proteome (i.e. strain specific families) are on
 1441 the left of the x-axis, while the 1,604 protein families defining the core-proteome are on the right of
 1442 the x-axis.

1443 **(B) Estimation of the *Dickeya* core and pan-genomes**

1444 The graph shows the estimated sizes of the core- and pan-proteomes of *Dickeya* according to the
 1445 number of considered strains. The curves were computed by calculating the core- and the pan-
 1446 proteomes for an increasing number of strains randomly selected among the 49 *Dickeya* strains
 1447 (100 replicates at each point). When all the 49 *Dickeya* strains were considered, the core- and the
 1448 pan-proteomes encompassed 1,604 and 11,566 protein families.

1449

1450 **Figure 8. Evolution of protein family repertoires along the *Dickeya* phylogeny**

1451 The number of protein family repertoires, gains, and losses inferred by COUNT are mapped on the
 1452 topology of the core-pf reference phylogeny of *Dickeya*. Values mapped above the branches
 1453 correspond to gains / losses, while values below branches correspond to the number of protein
 1454 families inferred. Considering the alternative placement of *D. solani* as sister-group of *D.*
 1455 *dianthicola* provided very similar results (see Supplementary Table S9).

1456

1457 **Supplementary materials**

1458

1459 **Supplementary Figure S1: Maximum Likelihood phylogenetic tree of *Dickeya* strains based**
 1460 **on the Fr-protein supermatrix gathering 51 ribosomal proteins** (LG+C60, 58 sequences, 6,295
 1461 amino acid positions).

1462 Numbers at branch correspond to bootstrap values (100 replicates of the original data set). The
 1463 scale bar indicates the average number of substitutions per site. The figure was generated using
 1464 Evolvview (He et al., 2016).

1465

1466 **Supplementary Figure S2: Phylogenetic position of “*Serratia* sp. ATCC 39006” based on**
 1467 **LSU and SSU rDNA trees**

1468 ML trees of the *Dickeya* genus inferred with a collection of (A) 947 SSU rDNA and (B) 125 LSU
 1469 rDNA sequences retrieved from public databases. The trees were rooted using *Pectobacterium*
 1470 and *Serratia* sequences. The scale bars represent the estimated average number of substitution
 1471 per site. Numbers at nodes represent ultrafast bootstrap values (A) and bootstrap values (B).
 1472 *Pectobacterium* sequences are in pink, *Serratia* sequences in purple, and *Dickeya* sequences in
 1473 black. Worth to note, LSU and SSU rDNA sequences from *Serratia* sp. 39006 robustly branch with
 1474 *Dickeya* sequences and not within the *Serratia* genus. The trees were drawn using the iTOL
 1475 webserver (Letunic and Bork, 2016).

1476

1477 **Supplementary Figure S3 - Single gene phylogenies**

1478 Maximum Likelihood phylogenetic trees of 912 proteins of interest. Numbers associated with each
 1479 branch correspond to ultrafast bootstrap values (1000 replicates of the original data set). The scale
 1480 bars indicate the average number of substitutions per site. For each tree, the name and the
 1481 annotation of the seed is provided in red.

1482

1483 **Supplementary Figure S4 – Non-metric multi-dimensional scaling (NMDS) plot of *Dickeya***
 1484 **genomes according to their gene content.** A Bray distance similarity matrix was calculated
 1485 based on the presence/absence profiles of 8,115 gene families (present in at least 2 genomes) for

the 49 analyzed genomes and used to generate NMDS coordinates for each strain. The shorter distance linking two genomes indicates higher similarities between these genomes. Genomes from different species are indicated in different colors.

Supplementary Figure S5: Phylogenies and genetic organisation of *peIAED* and *pehVWX* clusters.

Maximum likelihood trees of *peIAED* and *pehVWX* clusters together with genetic organisation of these clusters are shown. Numbers associated with each branch correspond to ultrafast bootstrap values (1000 replicates of the original data set). The scale bar indicates the average number of substitutions per site. For each tree, the name and the annotation of the seed is provided in red.

Supplementary Figure S6: The number of shared protein families between and within *Dickeya* species. Figures were generated using the package UpSetR (Conway et al. 2017).

Supplementary Table S1: Phenotypic differentiation of species within the genus *Dickeya*.

Table showing the phenotypic features of *Dickeya* species based on Samson et al. (2005), Parkinson et al. (2014), van der Wolf et al. (2014) and Tian et al. (2016).

Supplementary Table S2: Genomic features of the *Dickeya* strains with completely sequenced genomes

Supplementary Table S3: List of the 49 *Dickeya* genomes used in this study indicating the plant host or habitat and the geographical origin of each strain

Supplementary Table S4: Distribution of genes with a role in pathogenicity or in adaptation to plant niche in *Dickeya* species

Genes of *Dickeya* species were considered as present if identity of the encoded protein was higher than 60% of full-length amino acids sequence. If local alignments were too short with regard to the

length of similar sequences, we performed a nucleotide BLAST on full-length DNA sequences with similar thresholds (10^{-5} e-value, 80% identity of full length sequence). This allowed us to eliminate false genes. Note that because some genomes represent only drafts, false negatives may occur.

1517

Supplementary Table S5: Protein family distribution within proteomes from *Dickeya*, *Pectobacterium* and *Serratia*.

Considered strains were *Dickeya aquatica* 174/2, *Dickeya dadantii* 3937, *Dickeya paradisiaca* NCPPB 2511, *Dickeya solani* IPO 2222, *Dickeya zeae* EC1, *Pectobacterium atrosepticum* SCRI1043, *Pectobacterium carotovorum* subsp *carotovorum* PC1, *Pectobacterium wasabiae* WPP163, *Serratia fonticola*, *Serratia liquefaciens* ATCC27592, *Serratia marcescens* FGI94, *Serratia plymuthica* AS9, *Serratia proteamaculans* 568 and *Serratia* sp ATCC39006. Families were built using SILIX version 1.2.9 with 60% of sequence identity and 80% of sequence coverage.

1526

Supplementary Table S6: Results of the AU tests performed on core-pf and rprots topologies using the 1,341 core-pf and the 51 rprots.

1529

Supplementary Table S7: Distribution of Transcription factor-binding sites in *D. aquatica* 174/2 genome. Binding sites for 56 transcriptional regulators were predicted in *D. aquatica* using nhmmer (Wheeler and Eddy, 2013).

1533

Supplementary Table S8: Protein family distribution within the proteomes of the 49 *Dickeya* strains.

Families were computed with SILIX version 1.2.9 using 60% of sequence identity and 80% of sequence coverage.

1538

Supplementary Table S9: Comparison of inferences of ancestral gene repertoires in *Dickeya* considering the two possible alternative positions for *D. solani*

1541

Supplementary Table S10: Characterization of the mobilome of *D. aquatica* 174/2 and its conservation in other *Dickeya* species

Genes of *Dickeya* species were considered as present if identity of the encoded protein was higher than 60% of full-length amino acids sequence. If local alignments were too short with regard to the length of similar sequences, we performed a nucleotide BLAST on full-length DNA sequences with similar thresholds (10^{-5} e-value, 80% identity of full length sequence). This allowed us to eliminate false genes. Note that because some genomes represent drafts, false negatives may occur.

Dickeya aquatica 174/2 genome

CLUSTER II

CLUSTER I

100	91.45	90.78	88.68	88.94	88.45	89.17	88.09	88.22	88.08	88.93	88.14	88.65	88.61	87.03	82.89	82.03	82.67	83.23	83.01	87.44	86.77	86.43	85.3	86.78	86.12	85.95	84.28	81.81	80.96	79.83	79.85	80.08	83.73	81.92	82.09	83.27	82.92	81.84	82.55	83.85	84	83.45	73.2	73.66	73.62	68.58	72.6	73.71	63.78	
91.45	100	89.5	88.06	89.74	89.19	89.92	88.7	88.86	88.06	89.7	89	89.52	89.45	87.85	81.87	80.8	80.75	82.45	82.32	87.32	86.64	86.56	86.1	86.8	86.4	86.71	83.98	82.33	80.65	79.12	79.34	79.55	83.58	81.93	82.84	83.5	83.18	80.25	81.25	82.02	82.15	82	72.55	73.43	73.36	68.31	72.53	73.62	64.12	
90.78	89.5	100	86.57	87.15	86.57	87.36	86.3	86.53	86.6	87.14	86.52	86.97	86.96	85.37	80.15	80.37	79.96	81.58	81.36	86.65	85.8	84.92	83.65	85.64	85.02	83.95	83.01	80.99	79.95	77.54	78.28	78.54	80.68	80.41	81.42	81.81	81.36	79.9	80.91	81.59	81.75	81.52	72.41	72.49	72.33	67.47	71.49	72.46	62.46	
88.68	88.06	86.57	100	87.14	86.69	87.36	86.32	86.52	86.5	87.21	86.36	86.81	86.72	85.17	81.56	80.61	81.03	82.35	82.15	85.84	85.35	85.18	84.32	84.72	84.09	85.83	82.7	81.48	80.96	79.51	80.68	80.9	82.39	81.1	81.99	81.55	81.27	80.87	80.64	81.73	82.03	81.89	74.63	74.18	74.29	69.22	72.6	73.68	63.9	
88.94	89.74	87.15	87.14	100	96.98	96.98	96.54	97.95	95.36	99.37	88.04	96.58	96.62	93.48	82.8	82.52	81.87	84.18	83.96	86.29	87.63	87.47	87.16	88.19	87.59	87.47	85.03	83.33	81.28	80.62	80.78	81.03	83.59	82.24	83.12	84.38	83.61	81.88	82.22	82.48	82.49	81.79	73.44	73.76	73.75	68.81	72.97	74.07	64.76	
88.45	89.19	86.57	86.69	96.98	100	96.03	97.97	97.46	95.78	98.79	87.42	97.94	97.98	92.86	82.27	82	81.4	83.73	83.48	87.69	87.09	86.88	86.66	87.57	87.04	86.79	84.6	82.82	80.72	80.17	80.33	80.58	83.02	81.79	82.64	83.9	83.08	80.94	81.79	81.85	82.08	81.42	73.06	73.42	68.31	72.08	73.68	64.76		
89.17	89.92	87.36	87.38	99.07	99.03	100	98.43	97.95	96.51	99.59	98.22	98.8	98.81	93.69	83.09	82.79	82.09	84.47	84.25	88.54	87.89	87.75	87.35	88.36	87.83	87.69	85.23	83.54	81.47	80.84	81.01	81.25	83.83	82.51	83.36	84.65	83.85	81.52	82.44	82.52	82.7	81.98	73.66	73.98	73.96	69.01	73.14	74.25	64.95	
88.09	88.7	86.3	86.32	96.34	97.93	96.43	100	99.3	95.93	98.16	96.49	97.4	97.44	92.49	81.99	81.69	81.14	83.39	83.19	87.35	86.67	86.57	86.28	87.23	86.64	86.47	84.23	82.53	80.53	79.9	80.3	80.36	82.81	81.48	82.38	83.64	82.84	80.74	81.48	81.14	81.74	81.03	73.07	73.14	73.08	68.11	72.51	73.54	64.25	
88.22	88.86	86.53	86.52	97.86	97.46	97.95	99.3	100	95.92	97.88	96.5	97.1	97.08	92.52	82.15	81.82	81.34	83.53	83.33	87.58	86.93	86.79	86.49	87.46	86.89	86.52	84.43	82.85	80.83	80.1	80.31	80.54	83.05	81.73	82.64	83.91	83.08	80.85	81.03	81.72	81.95	81.31	73.41	73.36	73.3	68.32	72.7	73.74	64.47	
88.08	89.06	86.6	86.5	96.36	95.78	96.51	95.53	95.52	100	96.31	96.89	97.51	97.5	93.09	82.16	81.78	81.25	83.48	84.03	87.68	87.09	86.88	86.66	87.57	87.04	86.79	84.6	82.82	82.92	81.09	80.26	80.42	80.85	83.07	82.05	82.82	84	83.21	80.88	82.26	82.48	82.14	81.66	73.44	73.91	74.24	69.03	72.8	74.27	65.03
88.93	89.7	87.14	87.21	99.37	98.79	98.59	98.16	97.68	96.31	100	97.94	98.5	98.52	93.44	82.86	82.53	81.87	84.26	84.02	88.28	87.6	87.48	87.15	88.15	87.58	87.48	85.12	83.38	81.31	80.68	80.84	81.06	83.68	82.3	83.18	84.44	83.67	81.33	82.23	82.36	82.59	81.89	73.54	73.87	73.63	68.81	72.97	74.09	64.82	
88.14	89	86.52	86.36	98.04	97.45	98.23	98.26	98.5	96.89	97.94	100	99.27	99.21	93.95	82.44	82.14	81.43	83.77	84.28	87.71	87.85	87.73	87.36	87.61	87.84	86.8	85.21	82.89	80.8	80.18	80.34	80.56	83.16	81.76	82.66	83.94	83.09	80.91	82.42	82.3	82	81.42	73	73.55	73.9	68.8	72.76	74.22	66.67	
88.65	89.52	86.97	86.81	98.58	97.84	98.8	97.4	97.1	97.51	98.3	99.27	100	99.88	94.57	82.92	82.6	81.94	84.26	84.78	88.2	86.36	86.23	86.49	88.09	88.33	87.32	85.73	83.41	81.27	80.66	80.82	81.05	83.7	82.29	83.19	84.49	83.64	81.32	82.9	82.81	82.48	81.88	73.49	74.03	74.38	69.25	73.21	74.64	65.06	
88.61	89.45	86.96	86.72	98.62	97.86	98.81	97.44	97.08	97.5	98.52	99.31	99.88	100	94.53	82.86	82.56	81.87	84.19	84.74	88.13	86.37	86.24	87.8	88.05	88.33	87.32	85.73	83.41	81.27	80.66	80.82	81.05	83.7	82.29	83.19	84.49	83.64	81.32	82.9	82.81	82.48	81.88	73.49	74.03	74.38	69.25	73.21	74.64	65.06	
87.03	87.85	85.37	85.17	93.49	92.86	93.69	92.48	92.52	93.09	93.43	93.95	94.57	94.53	100	81.13	80.85	82.46	82.31	82.36	86.68	86.89	86.61	86.51	86.48	86.86	86.86	85.76	80.45	81.59	79.56	80.1	79.33	81.78	80.94	81.67	82.47	82.04	79.84	81.71	81.05	80.73	80.52	72.34	72.65	73.5	68.51	71.76	73.14	64.66	
82.89	81.87	80.15	81.56	82.48	82.27	83.06	81.96	82.15	82.16	82.86	82.44	82.92	82.86	81.13	100	92.71	91.82	93.37	92.35	90.36	80.48	79.73	79.83	79.13	79.28	78.2	80.74	78.01	80.58	79.34	78.99	81.45	80.54	81.58	81.73	80.74	73.74	73.14	73.91	69	70.89	72.06	64.63							
82.03	90.8	90.37	80.61	82.52	82	82.79	81.69	81.82	81.78	82.53	82.14	82.6	82.56	80.65	92.71	100	92.42	93.37	92.35	90.44	80.01	79.76	79.52	80.61	80.25	79.79	80.49	79.32	79.72	78.03	78.12	78.34	79.49	78.1	79.02	79.96	78.65	81.27	80.64	82	82.16	80.61	72.78	74.22	74.05	69.17	70.83	72.01	63.82	
82.67	80.45	79.96	81.03	81.87	81.4	82.08	81.14	81.34	81.25	81.87	81.43	81.94	81.87	80.38	91.82	92.42	100	91.68	90.8	90.16	79.79	79.4	78.86	79.95	79.55	79.67	80.46	78.73	79.04	77.62	77.45	77.68	79.59	77.04	78.63	78.7	78.02	81.05	80.15	80.85	81	80.89	72.47	73.73	73.66	68.75	70.66	71.83	63.61	
83.23	82.45	81.35	84.18	83.73	84.47	83.39	83.53	83.48	84.78	84.19	82.46	83.37	83.37	91.68	100	90.85	91.83	81.43	81.05	80.93	81.22	81.3	82.08	80.46	80.63	78.97	80.18	80.36	80.85	79.05	79.33	80.63	79.96	83.61	81.66	82.61	82.77	82.27	83.34	73.73	73.68	68.66	72.35	73.52	64.81					
83.01	82.32	81.36	82.15	83.96	83.46	84.25	83.18	83.33	84.03	84.02	84.26	84.78	84.74	82.91	92.36	92.33	90.8	90.05	100	91.63	81.89	81.52	81.47	81.47	81.71	81.06	82.64	80.36	80.47	78.73	79.89	80.12	80.72	78.89	79.12	80.47	78.86	82.25	82.04	82.82	82.54	82.11	73.12	73.67	73.93	68.77	72.32	73.76	64.84	
87.44	87.32	86.65	85.84	88.29	87.89	88.54	87.93	87.98	88.68	88.28	87.7	88.2	88.13	86.68	80.96	80.44	80.16	81.63	81.63	100	97.79	93.66	93.6	91.99	90.51	99.31	85.04	82.06	81.32	79.88	79.7	79.92	82.16	81.07	81.99	82.88	82.51	79.53	80.8	80.93	80.72	72.53	73.65	71.6	71.37	72.44	64.12			
86.7	86.64	85.8	85.35	87.63	87.06	87.89	86.67	86	86.69	86.81	87.4	87.08	86.58	86.37	86.49	80.79	81.43	81.69	87.79	100	94.36	90.66	90.67	88.37	85.17	81.44	80.77	79.36	79.22	79.45	81.58	80.49	81.4	81.67	81.89	79.93	81.06	80.4	83.33	80.21	72.21	73.28	73.61	68.55	71.14	72.59	63.99			
86.43	86.56	84.92	85.18	87.47	86.88	87.79	86.57	86.79	87.79	87.87	87.38	86.23	86.24	86.51	79.98	79.76	79.4	81.05	81.52	86.66	94.36	100	90.37	90.38	90.6	87.84	81.63	80.74	79.49	79.46	79.6	79.83	81.62	81.1	81.36	82.22	81.92	79.06	80.81	81.31	80.89	80.8	71.76	73.35	68.02	71.27	72.73	63.72		
85.3	86.1	83.65	84.34	87.16	86.68	87.35	86.28	86.49	87.46	87.15	87.38	87.87	87.8	86.51	79.85	79.52	78.86	80.93	81.47	90.6	90.38	100	92.96	93.38	93.73	84.16	81.27	80.85	79.26	79.16	79.38	81.41	81.14	80.38	81.67	81.47	79.41	80.35	80.39	80.02	79.59	72.65	73.34	73.7	68.48	70.96	72.43	63.12		
87.88	86.8	85.64	84.72	88.19	87.57	88.36	87.23	87.46	87.59	88.15	87.81	86.89	86.05	86.48	80.61	80.61	79.95	81.47	81.47	91.59	90.66	90.38	92.98	100	96.73	88.37	84.71	82.51	82.1	80.66	80.1	80.35	80.28	81.07	81.69	82.83	82.58	79.67	81.1	81.4	81.56	80.83	74	74.71	74.58	69.25	72.59	73.91	64	

A

Chicory (pH 6.5)

Potato (pH 6.0)

Cucumber (pH 5.1)

Tomato (pH 4.8)

*D. dadantii**D. aquatica**D. dadantii**D. aquatica**D. dadantii**D. aquatica**D. dadantii**D. aquatica***B**

Rotten mass (g)	<i>D. dadantii</i>	<i>D. aquatica</i>
Chicory (leaf)	0.9 ± 0.5	0.02 ± 0.04
Potato (tuber)	5.7 ± 2.4	1.7 ± 0.2
Cucumber (fruit)	0.38 ± 0.49	11.6 ± 10.6
Tomato (fruit)	8.0 ± 4.6	36 ± 16
Pineapple (fruit)	0.0 ± 0.0	0.0 ± 0.0
Kiwi (fruit)	0.0 ± 0.0	0.0 ± 0.0

Colony	Strain	Twitching
1	<i>D. dadantii</i> 3937	-
2	<i>D. dadantii</i> 3937	-
3	<i>D. dadantii</i> NCPPB 3537	-
4	<i>D. dadantii</i> NCPPB 898	-
5	<i>D. aquatica</i> 174/2	+
6	<i>D. fangzhongdai</i> NCPPB 3274	+
7	<i>D. fangzhongdai</i> B16	+
8	<i>D. fangzhongdai</i> NCPPB 3274	+
9	<i>D. undicola</i> 2B12	+
10	<i>D. poaceaphila</i> NCPPB 569	-
11	<i>D. zeae</i> NCPPB 3532	+
12	<i>D. zeae</i> NCPPB 2547	-
13	<i>D. zeae</i> NCPPB 2538	-
14	<i>D. chrysanthemi</i> NCPPB 402	-
15	<i>D. chrysanthemi</i> NCPPB 3533	+
16	<i>D. dianthicola</i> RNS04.9	-
17	<i>D. dianthicola</i> CFBP 1888	-
18	<i>D. dianthicola</i> CFBP 2982	-
19	<i>D. dianthicola</i> NCPPB 453	-
20	<i>D. paradisiaca</i> NCPPB 2511	-
21	<i>D. solani</i> IPO2222	-
22	<i>D. solani</i> PP09019	-
23	<i>D. solani</i> RNS07.7.3B	+
24	<i>D. solani</i> GBBC2040	-
25	<i>D. solani</i> Ds0432-1	+
26	<i>D. solani</i> RNS05.1.2A	-
27	<i>D. dadantii</i> subsp. <i>dieffenbachiae</i> CFBP3694	-
28	<i>D. dadantii</i> subsp. <i>dieffenbachiae</i> NCPPB 2976	-