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Pectobacterium zantedeschiae sp. nov. a new species of a soft rot pathogen isolated from Calla lily (*Zantedeschia* spp.)[☆]

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ABSTRACT

Four Gram-negative, rod-shaped pectinolytic bacterial strains designated as 2M, 9M, DPMP599 and DPMP600 were subjected to polyphasic analyses that revealed their distinctiveness from the other *Pectobacterium* species. Strains 2M and 9M were isolated from Calla lily bulbs cultivated in Central Poland. DPMP599 and DPMP600 strains were isolated from Calla lily leaves from plants grown in Serbia. Phylogenetic analyses based on nine housekeeping genes (*gapA, gyrA, icdA, pgi, proA, recA, recN, rpoA*, and *rpoS*), as well as phylogeny based on the 381 most conserved universal proteins confirmed that *Pectobacterium zantedeschiae* strains were distantly related to the other *Pectobacterium*, and indicated *Pectobacterium atrosepticum*, *Pectobacterium betavasculorum, Pectobacterium parmentieri* and *Pectobacterium wasabiae* as the closest relatives. Moreover, the analysis revealed that *Pectobacterium zantedeschiae* strains are not akin to *Pectobacterium aroidearum* strains, which were likewise isolated from Calla lily.

The genome sequencing of the strains 2M, 9M and DPMP600 and their comparison with whole genome sequences of other *Pectobacterium* type strains confirmed their distinctiveness and separate species status within the genus based on parameters of *in silico* DNA–DNA hybridization and average nucleotide identity (ANI) values. The MALDI-TOF MS proteomic profile supported the proposition of delineation of the *P. zantedeschiae* and additionally confirmed the individuality of the studied strains. Based on of all of these data, it is proposed that the strains 2M, 9M, DPMP599, and DPMP600 isolated from Calla lily, previously assigned as *P. atrosepticum* should be reclassified as *Pectobacterium zantedeschiae* sp. nov. with the strain 9M^T (PCM2893 = DSM105717 = IFB9009) as the type strain.

[☆] The GenBank/EMBL/DDBJ accession numbers of genome sequences are PESL00000000 for 2M, NWTM00000000 for 9M^T and QETE00000000 for DPMP600 (=PC2). The accession numbers for 16S rDNA sequences of studied strains: 2M — MG761827, 9M T — MG761828, DPMP599 — MH165159, DPMP600 — MH165170; rpoA sequences: 2M MH367219, 9M — MH367220, DPMP599 — MH367222, DPMP600 — MH367221, gyrA sequences: 2M — MH367223, 9M MH367224, DPMP599 — MH367226, DPMP600 — MH367225, recN sequences: 2M — MH367227, 9M — MH367228, DPMP599- MH367229, DPMP600 — MH36730, rpoS sequences: 2M — MH367231, 9M — MH367232, DPMP599- MH367234, DPMP600 — MH36733, gapA sequences: 2M — MH367235, 9M — MH367236, DPMP599 — MH367237, DPMP600 — MH36738, recA sequences: 2M MH367239, 9M — MH367240, DPMP599- MH367242, DPMP600 — MH36741, icdA sequences: 2M - MH367243, 9M — MH367244, DPMP599- MH367245, DPMP600 — MH36746, pgi sequences: 2M MH367249, 9M — MH367248, DPMP599- MH367250, DPMP600 — MH36747, proA sequences: 2M — MH367251, 9M — MH367252, DPMP599 — MH367254, DPMP600 — MH367253.

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Bacteria belonging to the genus *Pectobacterium* are Gram-negative rod-shaped phytopathogens. Due to the presence of the considerable set of pectinolytic and cellulolytic enzymes they cause diseases like soft rot and blackleg with symptoms of severely damaged plant tissues [32]. *Pectobacterium* species infect both mono- and dicotyledonous plant hosts such as chicory, potatoes, carrots, squash, chrysanthemum, beets, sunflowers and many more [20]. However, bacteria representing this genus were also isolated from soil and water samples, as well as from invertebrates' gastrointestinal tracts [13].

Recently, the taxonomy of *Pectobacterium* underwent extensive remodelling. Firstly, the family *Pectobacteriaceae* was extracted from the *Enterobacteriaceae* [1]. Studies based on the genomic data have reported the need for reclassification of several strains [36,55]. Khayi et al. reported a transfer of *Pectobacterium wasabiae* strains isolated from potato to *Pectobacterium parmentieri* [18]. The delineation of a new species *Pectobacterium polaris* was proposed by Dees et al. [11]. We also proposed transfer of *Pectobacterium carotovorum* subsp. *carotovorum* strains from high altitudes to the new species *Pectobacterium peruviense* [50] but it has not been yet validly published under the rules of the International Code of Nomenclature of Bacteria (Bacteriological Code).

A new species *Pectobacterium aroidearum* that infects predominantly monocotyledonous hosts was proposed; and strains of this taxon were also occasionally isolated from dicotyledonous [28]. Strains isolated from monocotyledonous plants e.g. Calla lily are extremely pathogenic [43,54]. Soft rot of Calla lily tubers (*Zantedeschia aethiopica*) caused by *Pectobacterium* is the most important bacterial disease of these popular ornamental plants [53,45,46,9]. Until now, pectinolytic bacteria isolated from Calla lily have been identified not only as *P. aroidearum* [28] but also as *P. c.* subsp. *carotovorum* [43,53,46] and *Pectobacterium atrosepticum* [25,34]. In the present study we analyzed two *Pectobacterium* isolates 9M and 2M derived from Calla lily tubers obtained from commercial plantations in Skierniewice, the central Poland region (Table S1) and two strains DPMP599 and DPMP600 (originally named as PC1 and PC2, respectively) isolated from necrotic lesions on Calla lily leaves grown in greenhouse in Kraljevo area, Serbia, previously assigned to the species *P. atrosepticum* [25,34].

Here, based on biochemical tests, fatty acid profiling and molecular methods such as multi-locus sequence analysis (MLSA), *in silico* DNA–DNA hybridization (*is*DDH), the average nucleotide identity (ANI), and whole-cell MALDI-TOF mass spectrometry (MS) [21,24,38,39] we propose the delineation of Polish and Serbian isolates from Calla lily as a novel species *Pectobacterium zantedeschiae* sp. nov. with 9M^T (=PCM2893 = DSM105717 = IFB9009) as a type strain.

We performed 16S rDNA amplification and sequencing with primers 27F [37] and 1492R [51]. The alignment of the 16S rRNA gene of four *P. zantedeschiae* strains and type strains of *Pectobacterium* species and subspecies was performed using the MUSCLE algorithm with the default settings in Geneious Pro 9.1.6 (www.geneious.com) [17]. A phylogenetic tree was constructed using MEGA 7 software package (www.megasoftware.net) with the neighbour-joining method and the Jukes–Cantor as the best nucleotide substitution model [47]. A consensus tree was built on the basis of 1000 bootstraps. The obtained 16S rDNA sequences (1440 bp) of strains 2M, 9M, DPMP599 and DPMP600 are identical

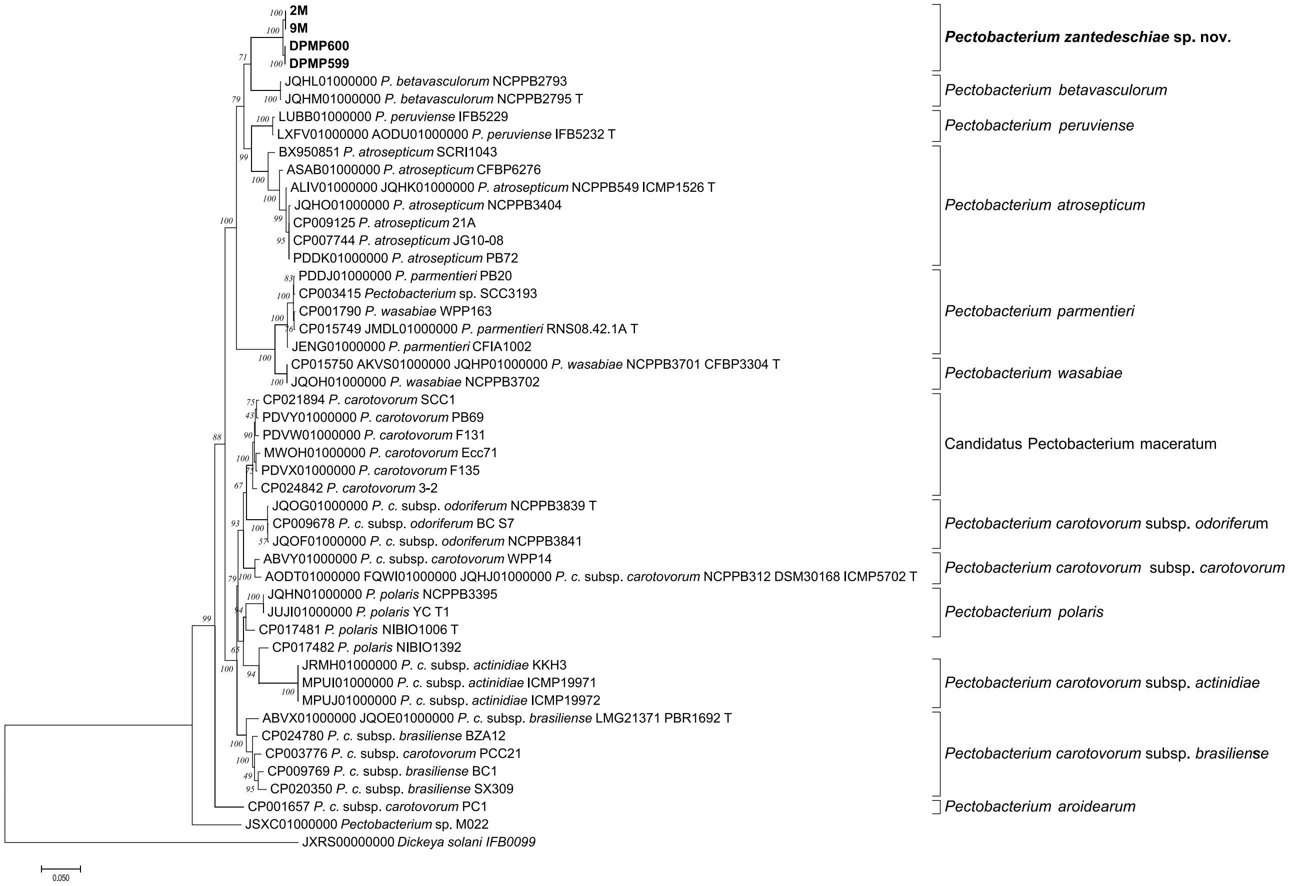

Fig. 1. Maximum Likelihood tree showing the phylogenetic relationship between *P. zantedeschiae* 2M, 9M^T, DPMP599, DPMP600 and other closely related members of the genus *Pectobacterium*, based on concatenated sequences (6539 bp) of amplified fragment of genes: *icdA* (1–553 nt), *gapA* (554–1033 nt), *gyrA* (1034–2055nt), *pgi* (2056–2596 nt), *proA* (2597–3277 nt), *recA* (3272–4008 nt), *recN* (4009–4881nt), *rpoA* (4882–5761 nt) and *rpoS* (5762–6539 bp). The gene sequences of *D. solani* IFB0099 (JXRS00000000) were used as an outgroup. The bootstrap consensus was inferred from 1000 replicates.

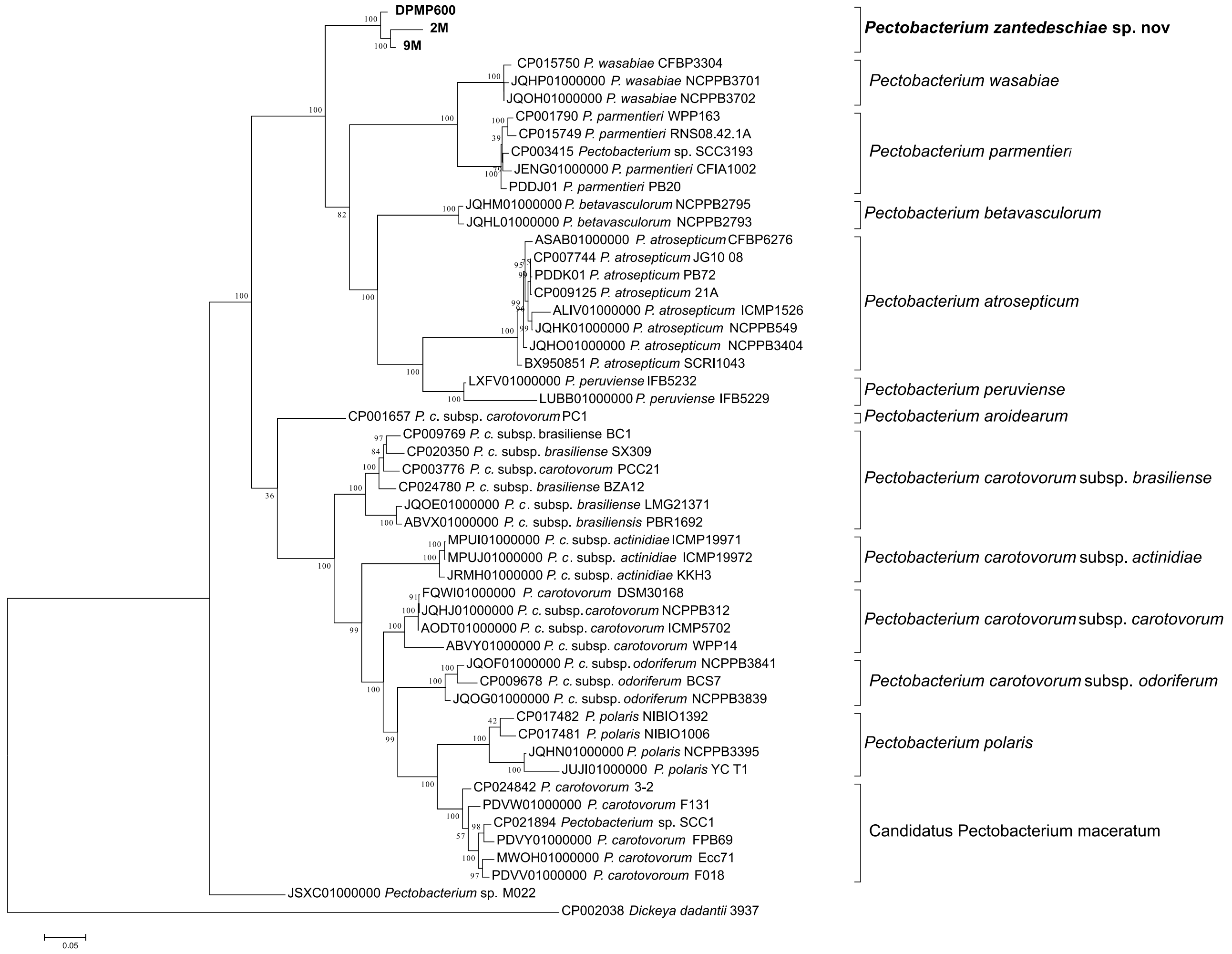

Fig. 2. The phylogenomic analysis of *P. zantedeschiae* strains, 2M, 9M^T, DPMP600 and other closely related members of the genus *Pectobacterium*, based on 381 most conserved universal proteins. The Maximum Likelihood tree was constructed using PhyloPhlAn computational pipeline (https://huttenhower.sph.harvard.edu/phylophlan) [40]. The gene sequences of *D. dadantii* 3937 (CP002038) were used as an outgroup. *P. c.* subsp. *brasiliense* neither is *P. c.* subsp. *actinidae* as well as *P. peruviense* and *P. polaris* are not yet a validly published taxon. While *P. maceratum* has been proposed as a new candidate genomospecies.

and cluster together as a separate group. Their closest relatives are type strains of *P. betavasculorum* (99.24% sequence similarity), *P. peruviense* (98.89–99.1%) and *P. atrosepticum* (98.89–98.96%) (Fig. S1).

In order to classify *P. zantedeschiae* strains, the MLSA analysis based on nine housekeeping genes (*icdA, gapA, gyrA, pgi, proA, recA, recN, rpoA*, and *rpoS*) was performed. All of these loci were previously used for MLSA of *Pectobacterium* species [20,26,27,49,50]. The sequences of these genes from strains 2M, 9M, DPMP599, and DPMP600 were compared with sequences retrieved from *Pectobacterium* genomes available at GenBank (20 April 2018) using the MUSCLE algorithm with the default settings in Geneious Pro 9.1.6 [17]. Phylogenetic analyses were performed on individual gene sequences: *gapA* — 480 bp, *gyrA* — 1022 bp, *icdA* — 553 bp, *pgi* — 541 bp, *proA* — 681 bp, *recA* — 731 bp, *recN* — 873 bp, *rpoA* — 880 bp and *rpoS* — 778 bp as well as on the concatenated data of 9 loci (6539 bp) with use of MEGA 7 with the Maximum Likelihood method and the General Time Reversible as the best nucleotide substitution model [47]. Bootstrapping was executed with 1000 replications. The gene sequences of *Dickeya solani* IFB0099 (JXRS00000000) were used as an outgroup. Phylogenetic analyzes were carried out on the basis of sequences retrieved from genomes of *Pectobacterium* species that are available at GenBank, whereas in the case of *P. carotovorum* subspecies, the sequences of type strains and those obtained from completely sequenced genomes were chosen so that the obtained trees were balanced. It is important to note that *P. c.* subsp. *brasiliense*, *P. c.* subsp. *actinidiae, P. peruviense* and *P. polaris* are not yet validly published taxa, while *P. maceratum* has been proposed recently as a new candidate genomospecies [42].

The *P. zantedeschiae* strains 2M, 9M^T, DPMP599 and DPMP600 possessed significantly similar sequences for each locus studied and formed a separate phylogenetic lineage in the case of every gene as well as after joining into concatenated data set. The clade formed by *P. zantedeschiae* strains is definitely distant from other *Pectobacterium* species, especially *P. carotovorum*. The most closely related species is *P. betavasculorum* and more distant are *P. atrosepticum, P. peruviense, P. wasabiae* and *P. parmentieri* (Fig. 1 and Supplementary Figs. S2–S10). Subsequently, to settle the taxonomic position of *P. zantedeschiae* strains we performed the phylogenomic analysis using PhyloPhlAn computational pipeline (https://huttenhower.sph.harvard.edu/phylophlan), which uses the 381 most conserved universal proteins from full proteomes to extract the phylogenetic signal [40]. In this case the phylogenomic tree was build based on 381 protein sequences.

The topology of ML tree based on core genome confirmed that *P. zantedeschiae* strains form a separate phylogenetic lineage with 100% bootstrap support and are distantly related to the other *Pectobacterium* species (Fig. 2).

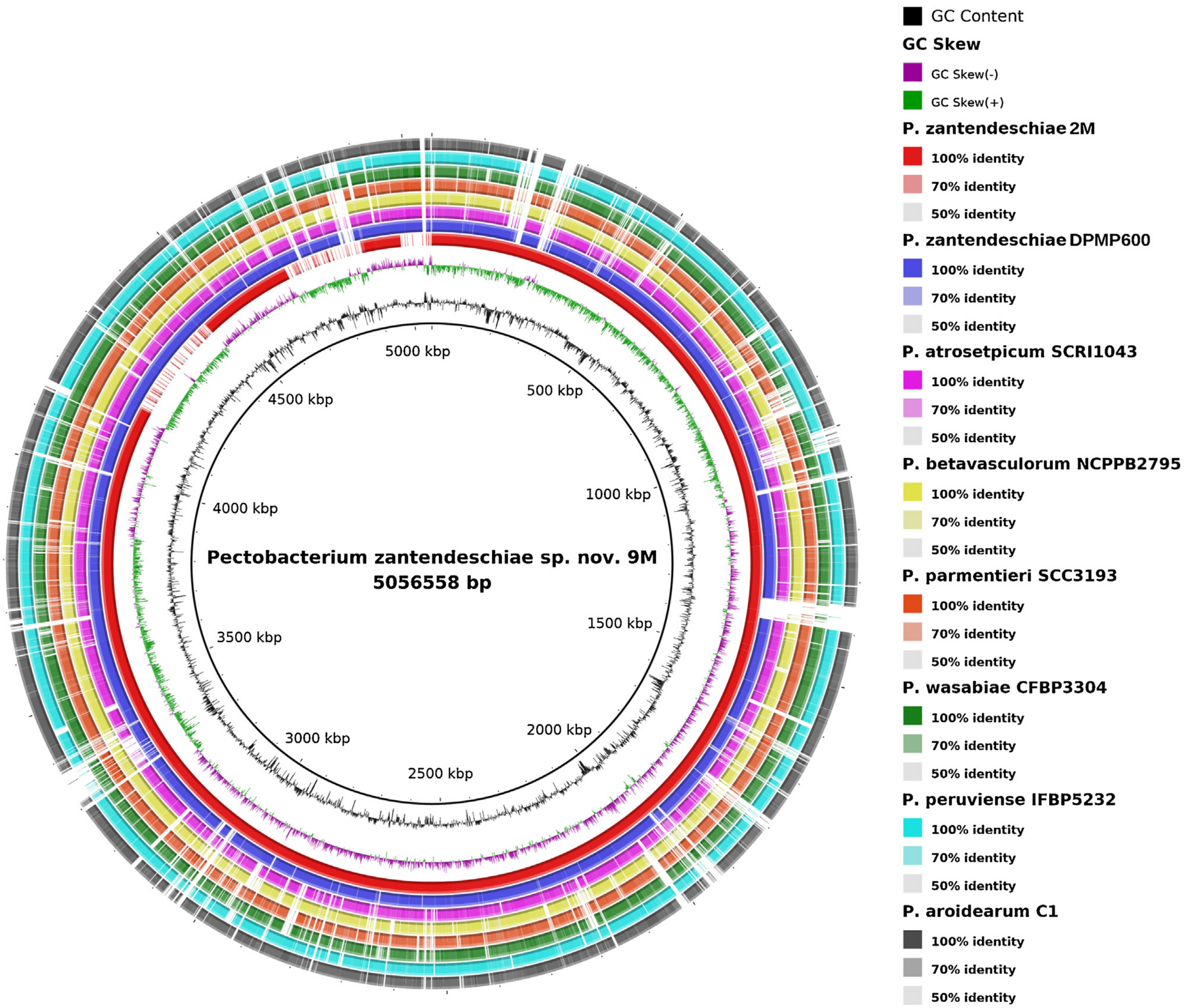

Fig. 3. Circular representation of whole-genome sequences of strains: *P. zantedeschiae* strains 2M $9M^T$, and DPMP600, *P. atrosepticum* SCRI1043, *P. parmentieri* SCC3193, *P. wasabiae* CFBP 3304^T, *P. betavasculorum* NCPPB 2795^T and *P. aroidearum* PC1. The inner ring portrays the reference *P zantedeschiae* $9M^T$ genome with corresponding genetic coordinates. The colored rings (from inner to outer ring) portray: GC% skew, GC content skew and whole-genome sequences of strains *P. zantedeschiae* 2M (PESL00000000) – red ring, *P. zantedeschiae* DPMP600 (QETE00000000) – dark blue ring, *P. atrosepticum* SCRI1043 (BX950851) – pink ring, *P. betavasculorum* NCPPB 2795^T (JQHM00000000) – yellow ring, *P. parmentieri* SCC3193 (CP003415) – orange ring and *P. wasabiae* CFBP 3304^T (CP015750) – green ring, *P. peruviense* $IFB5232^T$ (LXFV00000000) – turquise ring and *P. aroidearum* C1 (CP001657) – grey ring. Comparison created using BRIG platform application [3]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The genomes of three analysed strains $9M^T$, 2M and DPMP600 were sequenced on the Illumina HiSeq 2000 platform and assembled *de novo* by using Spades v3.10.1 [6] and Newbler v2.6 [22]. The assembly was evaluated using Quast v4.5 software [15].

The obtained sequences have been deposited at DDBJ/EMBL/GenBank under the accession numbers NWTM00000000 (*P. zantedeschiae* $9M^T$), PESL00000000 (*P. zantedeschiae* 2M) and QETE00000000 (*P. zantedeschiae* DPMP600). The genome of *P. zantedeschiae* $9M^T$ has a total of 5056558 bp and GC content of 50.74%, the genome of strain 2M has 4985156 bp and GC content 50.59%. While the genome of strain DPMP600 has a total of 5094003 bp and GC content is 50.37%.

Among the three *P. zantedeschiae* isolates only $9M^T$ harbours two small cryptic plasmids, p9Ma (9500 bp), p9Mb (3222 bp). Scaffolds corresponding to plasmids were successfully joined by PCR. The scaffold 12 corresponding to plasmid p9Ma contains sequences for integrase/recombinase, trimethoprim-resistant dihydrofolate reductase DfrB3, class A beta-lactamase GES-7, AacA4 family aminoglycoside N(6')-acetyltransferase, replication proteins A and C, relaxase, plasmid mobilization relaxosome protein MobC and a hypothetical protein. The plasmid is similar in 100% with plasmid IncQ2 type – pQ7 (FJ696404) harboring a class 3 integron-associated extended-spectrum beta-lactamase gene blaGES-1 from *Escherichia coli* [33] and plasmid pEMB1 (KJ631731) from natural environments carrying an ampicillin resistance-conferring beta-lactamase [19,35] or quinolone resistance gene *qnrS2* [52]. The scaffold 13 corresponding to plasmid p9Mb contains sequences for QacE family quaternary ammonium compound efflux SMR transporter, plasmid mobilization relaxosome protein MobC, nuclease, replication initiation protein and a hypothetical protein. The plasmid is similar in 100% with pIGMS32 (DQ298019), a ColE1-like plasmid from *Klebsiella pneumoniae* [44] and in 99% with numerous plasmids from cephalosporin-resistant or carbapenem-resistant members of the family *Enterobacteriaceae* isolates from rivers or other natural environments [2,41,23]. As well, plasmid p9Mb is in 90% similar with the non-self-transmissible plasmid pSCC1

(CP021895) from *P. carotovorum*, which was recently described by Ref. [30]. A similar small cryptic plasmid pEC3 (D45188) has been described earlier in the *P. c.* subsp. *carotovorum* strain IFO3380 [31].

The complete genome of investigated *P. zantedeschiae* strains was automatically annotated by the RAST server [5]. The strains 9M^T, 2M and DPMP600 showed 4597, 4583 and 4488 protein-coding genes respectively. Using KOALA (KEGG Orthology And Links Annotation) server [16], 2627, 2885 and 2895 genes have been annotated and functionally characterized for strains 9M^T, 2M, and DPMP600 respectively (Table S2).

Visual comparison of genome homology was done by using BRIG (BLAST Ring Image Generator) [3]. *P. zantedeschiae* 9M^T was used as the reference genome and was compared to the genomes of *P. zantedeschiae* 2M and DPMP600, *P. aroidearum* PC1 (CP001657), *P. atrosepticum* SCRI1043 (BX950851), *P. betavasculorum* NCPPB2793 (JQHL00000000), *P. parmentieri* SCC3193 (CP003415), *P. peruviense* IFB5232^T (LXFV00000000) and *P. wasabiae* CFBP3304^T (CP015750). The analysis was done with default settings. The BRIG analysis showed that most regions within the analyzed genomes were conserved between genomes of *P. zantedeschiae* strains 2M, 9M^T and DPMP600, however, there were clear differences between them and other analyzed *Pectobacterium* species (Fig. 3).

To evaluate assignment of the *P. zantedeschiae* sp. nov. isolates studied here to novel species the *in silico* DNA–DNA hybridization (*is*DDH) and the average nucleotide identity (ANI) were calculated between genomes of strains 2M, 9M^T and DPMP600, and genomes of *P. aroidearum* PC1 (CP001657), *P. atrosepticum* SCRI1043 (BX950851), CFBP6276 (ASAB00000000), NCPPB3404 (JQHO00000000), ICMP1526^T (ALIV00000000), JG10-08 (CP007744), 21A (CP009125), *P. betavasculorum* NCPPB2793 (JQHL00000000), NCPPB 2795^T (JQHM00000000), *P. peruviense* IFB5232^T (LXFV00000000), IFB5229 (LUBB0000000), *P. parmentieri* SCC3193 (CP003415), CFIA1002 (JENG00000000), RNS42.1A^T (CP015749), WPP163 (CP001790), and *P. wasabiae* CFBP3304^T (CP015750), NCPPB 3702 (JQOH00000000).

In silico DDH comparison was performed employing the Genome-to-Genome Distance Calculator (GGDC 2.0; http://ggdc.dsmz.de/distcalc2.php) using the recommended BLAST+ alignment and formula 2 (identities/HSP length) [4]. The estimated DDH was 99.9% between *P. zantedeschiae* 9M^T, 2M and DPMP600 strains and 87.4% between Polish and Serbian strains, but it dropped significantly (below 45%) when *P. aroidearum*, *P. atrosepticum*, *P. betavasculorum*, *P. peruviense*, *P. parmentieri* or *P. wasabiae* were used as queries (Table 1). Therefore, according to a threshold DDH value of 70%, *P. zantedeschiae* can be clearly distinguished from other *Pectobacterium* species considering DDH estimations.

The ANI values (99.4% between strains 9M^T, 2M and 98.6% between 9M, 2M and DPMP600) were calculated with the JSpecies (http://imedea.uib-csic.es/jspecies/about.html) [38] listed in Table 1 also supported the proposition of delineation of the *P. zantedeschiae* as a novel species according to a threshold of 95% proposed for the species definition [14,24]. The ANI value between *P. zantedeschiae* strains and the remaining species were in the range of 89 and 91%.

The internal strain variation of the novel species and distinctiveness from closely related species were clarified using whole-cell MALDI-TOF MS. This spectral analysis was done on strains grown on tryptic soy broth agar (TSA) plates at 28 °C for 48 h. Protein mass fingerprints were obtained using the Ultraflex III MALDI-TOF spectrometer (BrukerDaltonics, Bremen, Germany). The spectra were exported to the MALDI Biotyper Compass Explorer 4.1.60 software for data processing and evaluation by cluster analysis. The obtained protein profiles for *P. zantedeschiae* isolates 2M, 9M^T DPMP599 and DPMP600 were species-specific with several species-unique peaks (Figs. S11 and S12) that showed their distinctiveness from other species of the genus *Pectobacterium* as shown by hierarchical clus-

Table 1
ANI and isDDH values between *P. zantedeschiae* 9M, 2M and DPMP600 strains and related members of the genus *Pectobacterium*. The lower triangle shows the DDH values, and the upper triangle displays ANI values.

		P. zantedeschiae sp. nov.			*P. atrosepticum*						*P. peruviense*		*P. betavasculorum*		*P. wasabiae*		*P. parmentieri*				*P. aroidearum*
		1 2M	2 9M^T	3 DPMP600* (=PC2)	4 ASAB01.1	5 BX950851	6 JQHO01.1	7 ALIV01.1	8 CP007744	9 CP009125	10 IFB5232	11 IFB5229	12 JQHL01.1	13 JQHM01.1	14 CP015750	15 JQOH01.1	16 CP003415	17 CP015749	18 JENG01.1	19 CP001790	20 CP001657
1	2M	–	99.42	98.64	91.15	91.1	89.12	91.15	91.16	91.45	91.37	90.57	91.42	91.35	89.41	89.37	89.27	91.63	89.3	89.27	89.2
2	9M T	99.9	–	98.61	91.1	91	91.1	91.04	91.42	91.5	91.22	90.13	91.24	91.15	89.5	89.25	89.31	91.63	89.3	89.3	89.73
3	PC2	87.4	87.4	–	91.49	91.43	91.48	91.49	91.5	91.75	91.59	91.52	91.73	91.71	89.71	89.72	89.71	89.61	89.3	89.58	89.36
4	ASAB01.1	44.2	44	43.9	–	89.59	99.02	99.13	99.02	99.17	93.48	91.93	91.9	91.88	90.1	90.02	89.95	89.84	89.99	89.92	88.98
5	BX950851	43.6	43.8	43.5	90.6	–	98.39	98.43	98.42	98.55	93.45	91.86	91.86	91.86	90.01	89.96	89.82	89.78	89.85	89.83	88.92
6	JQHO01.1	43.7	43.8	43.7	95.6	89.9	–	98.87	99.01	98.86	93.39	91.9	91.82	91.78	90.11	89.96	89.88	92.78	89.89	89.82	89.78
7	ALIV01.1	43.7	43.8	43.7	96	90	94.8	–	99.11	99.32	93.43	91.96	91.84	91.8	90.05	89.97	89.82	89.76	89.85	89.79	88.92
8	CP007744	43.9	43.9	43.9	94.4	89.5	94.8	94.8	–	99.76	93.52	91.92	91.91	91.89	90.06	90.01	89.87	89.83	89.94	89.88	88.91
9	CP009125	43.8	44.9	44.9	94	89.3	96	96	97.2	–	92.13	91.91	92.37	92.32	90.22	90.2	90.02	89.98	89.98	89.99	89.13
10	IFB5232	44	44.2	44.2	54.2	54.1	54	54	54.3	55.5	–	95.37	91.91	91.83	90.02	89.98	89.72	94.23	89.9	89.7	88.78
11	IFB5229	43.8	43.9	43.9	55.3	55.2	55.2	55.2	55.5	56.6	96	–	91.92	91.86	90.06	89.98	89.73	89.72	89.82	89.72	88.86
12	JQHL01.1	44.4	44.6	44.5	46.9	46.8	46.9	46.9	46.9	48.4	47.4	47.5	–	98.48	89.53	89.45	89.35	90.91	89.36	89.34	88.92
13	JQHM01.1	44.3	44.4	44.2	47.1	46.9	46.9	47	47	48.7	47.3	47.5	95.1	–	89.55	89.46	89.36	90.76	89.31	89.39	88.88
14	CP015750	37.3	38	38	40.2	40	40	40.1	40.1	40.8	40	40.1	38.4	38.5	–	99.62	93.7	93.6	93.76	93.79	88.51
15	JQOH01.1	37.6	37.9	37.9	40.1	39.9	39.9	40	40	40.5	39.8	40	38.3	38.4	89.2	–	93.55	93.54	93.65	93.61	88.42
16	CP003415	37.7	37.9	37.9	39.4	39.4	39.3	39.5	39.5	39.9	39.3	39.4	37.7	37.9	54.6	54.5	–	99.86	98.64	98.45	88.48
17	CP015749	37.5	37.7	37.7	39.6	39.4	39.4	39.5	39.7	39.9	39.3	39.4	37.8	37.8	54.6	54.5	91.1	–	99.71	98.55	88.44
18	JENG01.1	37.3	37.6	37.7	39.6	39.4	39.4	39.5	39.7	39.9	39.6	39.6	37.9	37.8	54.8	54.7	90.5	90.9	–	98.73	88.63
19	CP001790	37.4	37.6	37.7	39.3	39.3	39.3	39.3	39.4	39.7	39.2	39.3	37.8	38	79.5	79.3	90.2	90.7	91.4	–	88.51
20	CP001657	36.6	37	36.8	35.5	35.5	36,6	36,6	36,7	37.4	36,6	36,7	36.7	36.8	35.5	35,6	35.5	35.5	35.7	35.6	–

* Strain DPMP600 was originally named as PC2 [34].

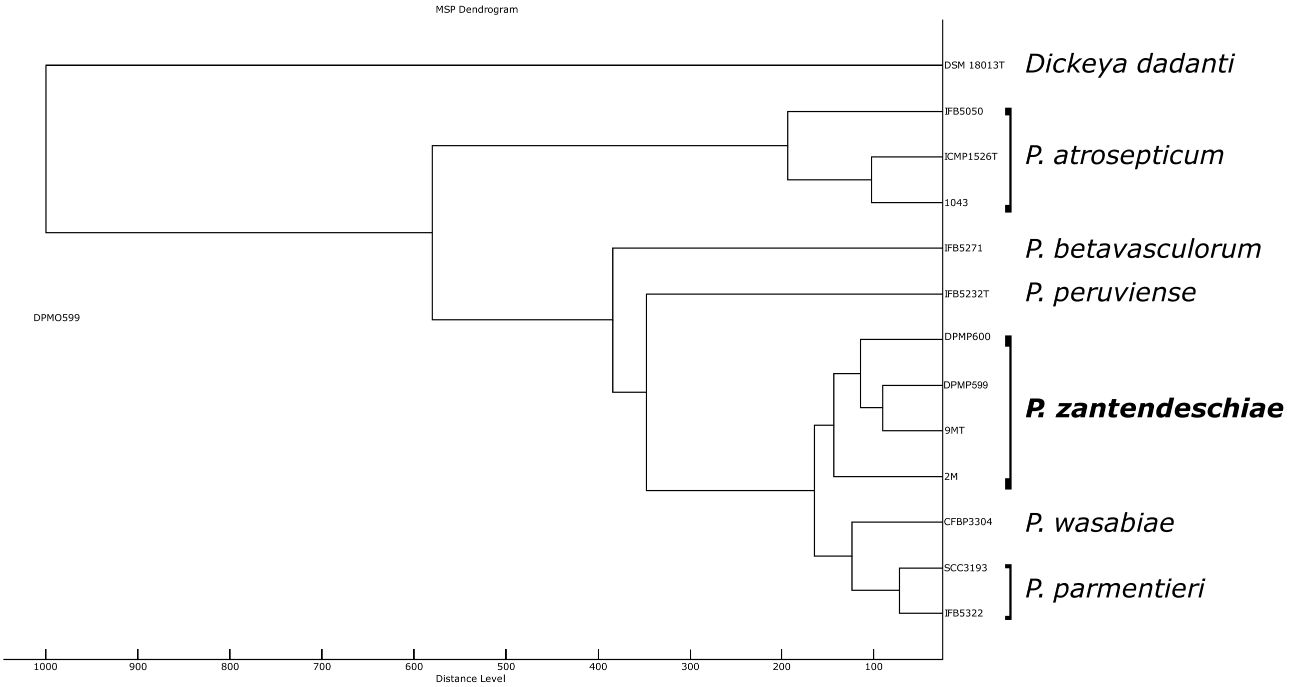

Fig. 4. The MPS dendrogram derived from whole cell MALDI-TOF MS protein mass fingerprints of *P. zantedeschiae* strains and other closely related members of the genus *Pectobacterium*. Protein mass fingerprint patterns obtained after running independently in two replicates per strain at two different evaluation times were reduced to a consensus spectrum in which peaks were reproducible in at least half of the measurements. Similarity distances are displayed in relative units.

ter analysis. A discrete variation between strains of *P. zantedeschiae* excluded the possibility of a clonal origin (Fig. 4).

To complete the proteomic profile of *P. zantedeschiae*, we investigated the genetic diversity with a repetitive element palindromic PCR (REP-PCR) fingerprinting using ERIC primers according to the procedure by Versalovic et al. [48]. Analysis with BioNumerics V6.6 (http://www.applied-maths.com) showed a discrete variation between Polish and Serbian *P. zantedeschiae* strains as well it confirmed their distinctiveness from other *Pectobacterium* species (Fig. 5). It should be noted that strains of 2M and 9M^T have the same fingerprint profile even though they are not clonal as the latter strain carries 2 plasmids.

Physiological and biochemical tests were performed for *P. zantedeschiae* sp. nov. strains 9M^T, 2M, DPMP599 and DPMP600 and six closely related species: *P. atrosepticum* (ICMP1526^T, SCRI1043, IFB5050), *P. aroidearum* (LMG2417^T), *P. betavasculorum* (IFB5271), *P. peruviense* (IFB5232^T and IFB9229), *P. parmentieri* (SCC3193, IFB5322), and *P. wasabiae* (CFBP3304^T) using BIOLOG GEN III plates (Table S3). Additionally, the production of acid from α-methyl glucoside or reducing substances from sucrose, utilization of citrate, dulcitol and dextrose as the only carbon source, fermentation of 10 sugars, inulin, mannitol and sorbitol and production of 17 enzymes using the DIATABS^{TM}, Diagnostic Tablets (RoscoDiagnostica A/S) were assessed (Table S4). The new species cannot be easily differentiated from other closely related species by phenotypic characteristics as summarized in Tables S3 and S4. However, the *P. zantedeschiae* strains can be discriminated from the most closely related species *P. atrosepticum* and *P. betavasculorum* by a few of phenotypic characteristics as summarized in Table 2.

Fatty acids (FA) of two *P. zantedeschiae* strains 2M and 9M^T were determined after growth on minimal medium M9 [12] for 48 h at 28 °C. Fatty acids were extracted according to previously described protocol [50]. Individual FAs were expressed as percentages of the total FAs present in the chromatogram. For comparison, the type strains of the closely related species, *P. atrosepticum* (SCRI1043, ICMP1526^T and IFB5050), *P. aroidearum* (LMG2417^T), *P. betavasculorum* (IFB5271), *P. peruviense* (IFB5232^T and IFB9229), *P. parmentieri* (SCC3193, IFB5322) and *P. wasabiae* (CFBP3304^T) were also included. The fatty acid profile of *P. zantedeschiae* strains 2M and 9M^T is very similar to that of related *Pectobacterium* species (Table 3) with the most important components 16:0, 16:1 and C18:1 isomers; minor components are C14:0, C15:0, C17:0 and C18:0.

Table 2
Phenotypic characters that differentiate *Pectobacterium zantedeschiae* sp. nov. from other closely related members of the genus. Species: 1 — *P. zantedeschiae* (9M T, 2M, DPMP599 and DPMP600), 2 — *P. atrosepticum* (SCRI1043, ICMP1526^T and IFB5050), 3 — *P. betavasculorum* (CFBP2122^T, IFB5271), 4 — *P. parmentieri* (SCC3193, IFB5322).

Characteristic	1	2	3	4
Utilization of D-serine[a]	+	–	–	–
Utilization of α-ketoglutaric acid[a]	+	–	–	–
Resistance to nalidicxic acid[a]	–	+	+	+
Acid production from α-methyl glucoside	+	+	+	–
Reducing sugars from sucrose	–	+	–	–
Fermentation of:				
Maltose	–	+	+	+
Trehalose	–	+	+	–
Sucrose	+	+	+	–
Raffinose	+	+	+	–
Rhamnose	+	+	–	–
Melibiose	+	+	–	–
Mannose	+	+	+	–
Mannitol	+	+	+	–
D-Xylose	+	–	–	–
Sorbitol	–	+	+	–

[a] Result from BIOLOG assay.

In conclusion, the four analysed *P. zantedeschiae* strains form a separate group in the genotypic, phenotypic and MALDI-TOF MS analyses. The strains 2M, 9M^T, DPMP599 and DPMP600 can be distinguished from related *Pectobacterium* species and therefore we propose classifying them as a new species, *Pectobacterium zantedeschiae* sp. nov. with the strain 9M^T (PCM2893 = DSM105717 = IFB9009) as the type strain. The full

Table 3
Fatty acid composition of 2 strains of *Pectobacterium zantedeschiae* sp. nov. and related species, *P. aroidearum, P. atrosepticum, P. betavasculorum, P. peruviense, P. parmentieri* and *P. wasabiae* analyzed in this study.

Fatty acid	*P. zantedeschiae* sp. nov.		*P. atrosepticum*			*P. aroidearum*	*P. betavasculorum*	*P. peruviense*		*P. parmentieri*		*P. wasabiae*
	2M	9M^T	ICMP1526^T	SCRI1043	IFB5050	LMG2417^T	IFB5271	IFB5232^T	IFB5229	IFB5322	SCC3193	CFBP3304^T
C14:0	1.09	1.04	2.61	1.20	1.31	0.75	1.17	1.81	1.49	1.05	0.82	0.46
C15:0	0.53	0.66	0.68	0.15	0.16	0.29	0.39	0.19	0.27	0.17	0.11	0.08
C16:0	36.88	36.43	41.99	37.07	42.15	39.62	36.39	37.17	35.32	35.94	33.48	33.77
C16:1	36.69	37.49	42.01	45.93	42.27	33.72	41.77	39.85	42.32	41.14	42.64	38.04
C17:0	0.45	0.53	0.19	0.11	0.13	0.27	0.15	0.23	0.20	0.15	0.12	0.13
C18:0	0.91	1.31	0.43	0.39	0.26	0.99	0.44	0.51	0.44	0.61	0.36	0.63
C18:1 isomers	23.45	22.54	12.09	15.15	13.73	23.75	19.69	19.96	19.26	20.94	22.48	26.89

n = 2. 14:0, lauric acid, 15:0, myrisitc acid; pentadecylic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 17:0, margaric acid.18:0, stearic acid; 18:1 isomers, cumulative value of peaks we hypothesized to be isomers of 18:1.

Table 4
Protologue for *Pectobacterium zantedeschiae* sp. nov.

Date Created	2018-01-08 21:19:57
Taxonumber	TA00379
Species name	*Pectobacterium zantedeschiae*
Genus name	*Pectobacterium*
Specific epithet	*zantedeschiae*
Species status	sp. nov.
Species etymology	*Pectobacterium zantedeschiae* sp. nov. (zan.te.des'chi.ae N.L. neut. adj. zantedeschiae, referring to the generic epithet of calla lily, *Zantedeschia aethiopica* (L.) Spreng (Alismatales: Araceae), the plant from which the strains were isolated)
Authors	Malgorzata Waleron, Agnieszka Misztak, Michal Waleron, Martyna Franczuk, Joanna Jonca, Bartosz Wielgomas, Artur Mikiciński, Tatjana Popovic and Krzysztof Waleron
Corresponding author	Malgorzata Waleron
E-mail of the corresponding author	malgorzata.waleron@biotech.ug.edu.pl
Designation of the type strain	9M
Strain collection numbers	PCM2893 = DSM105717 = IFB9009
16S rRNA gene accession number	MG761828
Alternative housekeeping genes	*rpoA* [MH367220], *gyrA* [MH367224], *rpoS* [MH367232], *gapA* [MH367236], *recA* [MH367240], *icdA* [MH367244], *pgi* [MH367248], *proA* [MH367252]
Genome accession number	NWTM00000000
Genome status	draft
Genome size	5,065,874 bp
GC mol %	50.76%
Country of origin	POL
Region of origin	Central Poland, Skierniewice
Date of isolation	30 Aug 2005
Source of isolation	Calla lily tubers, *Zantedeschia* spp.
Sampling date	01 Aug 2005
Source of isolation of non-type strains	Calla lily tubers, *Zantedeschia* spp.
Growth medium, incubation conditions used for standard cultivation	TSB/TSA, 28 °C, pH = 7
Conditions of preservation	−80 °C in 40% glycerol stock
Gram stain	NEGATIVE
Cell shape	Rod
Temperature optimum	20–35
Lowest pH for growth	4
Highest pH for growth	11
pH optimum	6–8
Highest NaCl concentration for growth	8%
Relationship to O_2	Facultative aerobe
Positive tests with BIOLOG	Dextrin, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-raffinose, stachyose, D-lactose, D-melibiose, β-methyl-D-glucoside, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, α-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, D-fucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, myo-inositol, glycerol, D-glucose-6-PO_4, D-fructose-6-PO_4, D-aspartic acid, L-aspartic acid, D-serine, glycyl-L-proline, L-alanine, D-galacturonic acid, D-gluconic acid, L-glutamic acid, L-galactonic acid lactone, L-lactic acid, α-ketoglutaric acid, L-malic acid, mucic acid, D-saccharic acid, acetic acid, citric acid, formic acid,
Negative tests with BIOLOG	*N*-acetyl-D-galactosamine, *N*-acetyl neuraminic acid, gelatin, L-arginine, L-histidine, L-serine, L-pyroglutamic acid, D-glucuronic acid, p-hydroxyphenylacetic acid, quinic acid, D-lactic acid methyl ester, methyl pyruvate, D-lactic acid, methyl ester, D-malic acid, Tween 40, γ-aminobutyric acid, α-hydroxybutyric acid, β-hydroxy-D,L-butyric acid, α-ketobutyric acid, acetoacetic acid, propionic acid
Acid formation from carbohydrates (all positive)	α-Methyl glucoside, arabinose, glucose, mannose, mannitol, sucrose, and raffinose
Acid formation for carbohydrates (all negative)	Dulcitol, maltose, melibiose, rhamnose, sorbitol, trehalose, and d-xylose
Oxidase	Negative
Catalase	Positive
Negative tests	Urease, indol, phosphatase, utilisation of citrate, reducing sugars from sucrose
Major fatty acids (FAME)	Palmitic acid (36.43–36.88%) Palmitoleic acid (36.69–37.49%), Isomers of octadecenoic acid (22.54–23.45%). Lauric acid (1.04–1.09%), Myristic acid (0.53–0.66%), Margaric acid (0.45–0.53%), Stearic acids (0.91–1.31%)

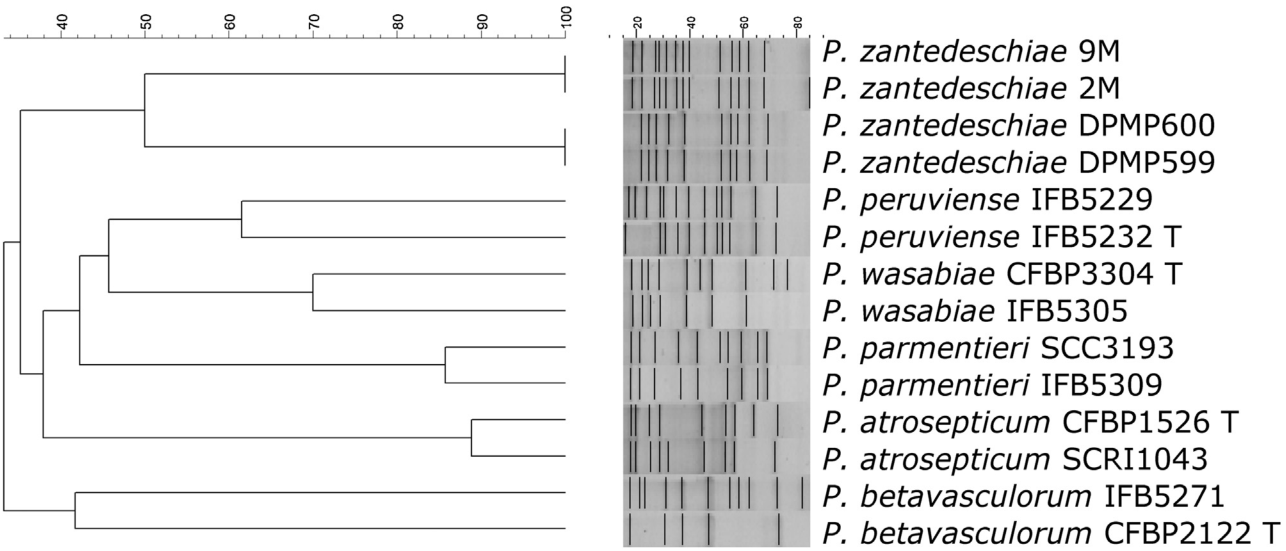

Fig. 5. Electrophoretic patterns obtained after REP-PCR with ERIC primer. 1 — *P. betavasculorum* IFB5271, 2 — *P. betavasculorum* CFBP2122^T, 3 — *P. zantedeschiae* 2M, 4 — *P. zantedeschiae* 9M, 5 — *P. zantedeschiae* DPMP599, 6 — *P. zantedeschiae* DPMP600, 6 — *P. atrosepticum* ICMP1525T, 7 — *P. atrosepticum* SCRI1043, 8 — *P. parmentieri* SCC3193, *P. parmentieri* IFB5322, *P. parmentieri* IFB5309, 9 — *P. wasabiae* CFBP 3304^T, 10 — *P. wasabiae* IFB5305 and M — marker GeneRuler *1 kb DNA Ladder* (Thermo Scientific, #SM0312). The UPGMA cladogram was created with band base Jaccard coefficient.

description of the new taxon is given in Table 4 with the unique Taxonumber TA00379.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.syapm.2018.08.004.

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Further reading

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