Reclassification of *Donghaeana dokdonensis* Yoon *et al.* 2006 as *Persicivirga dokdonensis* comb. nov. and emended descriptions of the genus *Persicivirga* and of *Persicivirga xylanidelens* O'Sullivan *et al.* 2006

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The accurate taxonomic position of *Donghaeana dokdonensis* DSW-6^T was investigated. Phylogenetic analysis revealed that the 16S rRNA gene sequences of the strain studied and of *Persicivirga xylanidelens* SW256^T shared 98.2 % similarity. On the basis of phylogenetic evidence, phenotypic data and DNA–DNA reassociation values, it is proposed that strain DSW-6^T be reclassified as *Persicivirga dokdonensis* comb. nov. Emended descriptions of the genus *Persicivirga* and of *P. xylanidelens* are also proposed.

The genus *Persicivirga* was proposed to accommodate Gram-negative, strictly aerobic, heterotrophic, peach-orange-pigmented, oxidase- and catalase-negative marine bacteria that belong to the family *Flavobacteriaceae*, phylum 'Bacteroidetes' (O'Sullivan *et al.*, 2006). The genus comprises a single species, *Persicivirga xylanidelens*; the type and only strain, designated SW256^T, was isolated from a coastal seawater sample in the North Sea (O'Sullivan *et al.*, 2006). At about the same time, another genus and species, *Donghaeana dokdonensis*, was proposed to accommodate strain DSW-6^T, isolated from surface water of the East Sea (Yoon *et al.*, 2006). Shortly after the genera *Persicivirga* and *Donghaeana* were described, their very close phylogenetic relationship became evident.

In the present work, the precise taxonomic positions of strains SW256^T and DSW-6^T were investigated by using a polyphasic approach. The results of phylogenetic, genomic and phenotypic analyses supported the affiliation of the two strains to the same genus. The name *Persicivirga* has priority over the name *Donghaeana* due to the earlier publication of its description (Rule 24b of Principle 6; Lapage *et al.*, 1992). Hence, it is proposed that strain DSW-6^T be transferred to the genus *Persicivirga* as a distinct species.

A phylogenetic analysis based on published 16S rRNA gene sequences was conducted according to the procedure described previously (Nedashkovskaya *et al.*, 2007). An unambiguously aligned 1211 bp sequence fragment was compared between members of closely related genera retrieved from GenBank. *P. xylanidelens* SW256^T was the

closest relative of *D. dokdonensis* DSW-6^T, with sequence similarity of 98.2 %. This value supports the allocation of strain DSW-6^T to the genus *Persicivirga* (O'Sullivan *et al.*, 2006). 16S rRNA gene sequence similarities between strain DSW-6^T and its other neighbours, members of the genera *Nonlabens*, *Sandarakinotalea* and *Stenothermobacter*, ranged from 93.1 to 94.3 %. The phylogenetic analysis revealed that strain DSW-6^T forms a cluster with the representatives of these genera, but forms a distinct phyletic line with strain SW256^T that is supported by a high bootstrap value (Fig. 1). In addition, Fig. 1 revealed that the genera *Nonlabens* and *Sandarakinotalea* also display high 16S rRNA gene sequence similarity (99.8 %) and should be reclassified (Fig. 1).

The DNA of the two strains was isolated following the method of Marmur (1961) and the DNA G+C content was determined by the thermal-denaturation method (Marmur & Doty, 1962). The DNA G+C contents of *D. dokdonensis* DSW-6^T and *P. xylanidelens* SW256^T were 35.9 and 35.5 mol%, respectively. These values are close to those obtained by using the HPLC technique (O'Sullivan *et al.*, 2006; Yoon *et al.*, 2006).

The DNA–DNA relatedness between the two strains was determined spectrophotometrically and initial renaturation rates were recorded as described by De Ley *et al.* (1970). The level of DNA–DNA relatedness between strains DSW-6^T and SW256^T was 33 %, a value that justifies the allocation of the two strains to two different species in the genus *Persicivirga* according to the proposal of Wayne *et al.* (1987).

Fig. 1. Rooted neighbour-joining tree based on 16S rRNA gene sequences showing the relationship between *Persicivirga dokdonensis* DSW-6^T and closely related genera of the family *Flavobacteriaceae*. Bootstrap values >50 % of 1000 replications are shown at nodes (upper value, Jukes–Cantor/Neighbour joining; lower value, maximum likelihood/parimony). *Maribacter ulvicola* KMM 3951^T (AY271626) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Physiological and biochemical features of strains DSW-6^T and SW256^T were tested as described previously (Nedashkovskaya *et al.*, 2004) and by using API 20E, API 20NE and API ZYM galleries (bioMérieux) according to the manufacturer's instructions, except that the incubation temperature was 28 °C.

Strains DSW-6^T and SW256^T share many phenotypic features. They are aerobic, non-gliding bacteria that possess alkaline phosphatase, caseinase and DNase activities. In contrast to previously reported data (O'Sullivan *et al.*, 2006), this study revealed that strain SW256^T is able to produce cytochrome oxidase and catalase and to hydrolyse aesculin, in line with strain DSW-6^T. However, the two strains differ from each other by several phenotypic characteristics such as hydrolysis of gelatin, starch and Tween 80, utilization of mannitol and production of several enzymes (Table 1).

Therefore, the phylogenetic evidence and similarity in fatty acid composition (Table 2) and phenotypic features (Table 1) provide a sound basis for reclassification of *D. dokdonensis* in the genus *Persicivirga*. Differences in several phenotypic characteristics and a low level of DNA–DNA relatedness between the two strains justify the classification of strain DSW-6^T as *Persicivirga dokdonensis* comb. nov. Emended descriptions of the genus *Persicivirga* and of *P. xylanidelens* are also proposed to include new data obtained in this study. Table 3 lists the phenotypic properties distinguishing members of the genus *Persicivirga* from their close relatives.

Emended description of the genus *Persicivirga* O'Sullivan *et al.* 2006

Persicivirga (Per.si.ci.vir'ga. L. neut. n. *persicum* peach; L. fem. n. *virga* rod; N.L. fem. n. *Persicivirga* a peach-coloured rod).

Cells are rod-shaped, non-motile, strictly aerobic and Gram-negative. Chemo-organotrophic. Endospores are not formed. Some strains can produce flexirubin-type pigments. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. Na⁺ ions are required for growth. The predominant cellular fatty acids are anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{15:0} 3-OH, C_{15:0} and summed

feature 3 (consisting of iso-C_{15:0} 2-OH and/or C_{16:1}ω7c). The major isoprenoid quinone is MK-6. As determined by 16S rRNA gene sequence analysis, the genus *Persicivirga* is a member of the family *Flavobacteriaceae*, phylum 'Bacteroidetes'. The type species is *Persicivirga xylanidelens*.

Table 1. Differential characteristics of *Persicivirga* species

Species: 1, *Persicivirga xylanidelens* SW256^T; 2, *Persicivirga dokdonensis* DSW-6^T. Both strains were positive for the following characteristics: respiratory metabolism, presence of esterase (C4), esterase lipase (C8), leucine and valine arylamidase, acid and alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, oxidase and catalase activities and hydrolysis of aesculin, casein, DNA and Tweens 20 and 40. All strains were negative for the following characteristics: gliding motility, presence of cystine arylamidase, α-chymotrypsin, α- and β-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities, hydrolysis of agar, cellulose (CM-cellulose and filter paper), urea and chitin, acid production from carbohydrates, utilization of arabinose, glucose, lactose, mannose, sucrose, inositol, sorbitol, adipate, citrate, caprate, gluconate, malate and phenylacetate, nitrate reduction and production of indole and acetoin (Voges–Proskauer reaction). Data are from O'Sullivan *et al.* (2006), Yoon *et al.* (2006) and this study.

Characteristic	1	2
Flexirubin-type pigments	+	–
H ₂ S production	+	–
Range of:		
Temperatures for growth (°C)	4–28	4–32
Salinities for growth (% NaCl)	2–6	1–8
Hydrolysis of:		
Gelatin	+	–
Starch	–	+
Tween 80	+	–
Utilization of mannitol	–	+
Production of lipase (C14), trypsin and β-glucosidase	+	–
DNA G + C content (mol%) as determined by:		
HPLC	34.7	36.9
Thermal denaturation	35.5	35.9

Table 2. Cellular fatty acid content (%) of *Persicivirga* species

Species: 1, *Persicivirga xylanidelens* SW256^T; 2, *Persicivirga dokdonensis* DSW-6^T. Fatty acids amounting to <1 % of the total fatty acids in the two strains are not listed. Both strains were grown for 3 days on marine agar 2216. Strain SW256^T was grown at 20 °C, whereas strain DSW-6^T was grown at 25 °C. Tr, trace (<1 %); –, not detected. Data are from O'Sullivan *et al.* (2006) and Yoon *et al.* (2006).

Fatty acid	1	2
iso-C _{13:1}	1.9	–
iso-C _{14:0}	3.2	–
anteiso-C _{14:0}	2.6	–
iso-C _{15:0}	18.1	19.2
anteiso-C _{15:0}	21.6	11.1
C _{15:0}	5.5	10.2
C _{15:1}	1.4	3.7
iso-C _{16:1}	1.4	1.3
iso-C _{16:0}	3.3	1.5
C _{16:0}	Tr	1.5
iso-C _{15:0} 3-OH	6.9	6.7
anteiso-C _{15:0} 3-OH	2.2	–
C _{15:0} 3-OH	–	3.3
C _{15:0} 2-OH	–	1.8
iso-C _{17:1} ω9c	8.5	3.5
C _{17:1} ω6c	–	2.9
iso-C _{16:0} 3-OH	4.7	3.5
C _{16:0} 2-OH	–	1.5
iso-C _{17:0} 3-OH	7.1	10.5
anteiso-C _{17:0} 3-OH	4.9	–
C _{17:0} 2-OH	–	3.0
C _{18:0} ω5c	–	1.7
C _{18:0}	–	2.0
Summed feature 3*	5.3	7.1

*Summed feature 3 consisted of iso-C_{15:0} 2-OH and/or C_{16:1}ω7c that could not be separated by GLC using the MIDI system.

Emended description of *Persicivirga xylanidelens* O'Sullivan *et al.* 2006

Persicivirga xylanidelens (xy.la.ni.del'ens. N.L. n. *xylanum* xylan; L. part. adj. *delens* destroying; N.L. part. adj. *xylanidelens* xylan-destroying).

Displays the following properties in addition to those listed in the emended description of the genus and those given by O'Sullivan *et al.* (2006). Growth occurs with 2–6 % NaCl. Decomposes aesculin, casein, DNA and Tweens 20 and 40. Does not hydrolyse agar or chitin. Acid is not produced from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannose, melibiose, raffinose, L-rhamnose, ribose, sucrose, trehalose, D-xylose, N-acetylglucosamine, citrate, glycerol or mannitol. L-Arabinose, D-glucose, D-lactose, D-mannose, sucrose, inositol, mannitol, sorbitol and citrate are not utilized. According to the API ZYM gallery, esterase (C4), esterase lipase (C8), lipase (C14), leucine and valine arylamidase, trypsin, acid phosphatase, β-glucosidase and naphthol-AS-

Table 3. Differential characteristics of members of the genus *Persicivirga* and related genera of the family *Flavobacteriaceae*

Genera: 1, *Persicivirga* (two species); 2, *Nonlabens* (one species); 3, *Sandarakinotalea* (one species); 4, *Stenothermobacter* (one species). All strains were positive for the following characteristics: respiratory metabolism and presence of catalase, oxidase and alkaline phosphatase activities. All strains were negative for the following characteristics: nitrate reduction, hydrolysis of agar, cellulose (CM-cellulose and filter paper), urea and chitin, acid production from carbohydrates, utilization of lactose and production of indole. +, Positive; –, negative; ND, no data available. Data are from Khan *et al.* (2006), Lau *et al.* (2005, 2006), O'Sullivan *et al.* (2006), Yoon *et al.* (2006) and this study.

Characteristic	1	2	3	4
Gliding motility	–	–	–	+
Acetoin production	–	+	ND	+
Range of:				
Temperatures for growth (°C)	4–32	12–44	10–40	20–36
Salinities for growth (% NaCl)	1–8	2–4	0.3–2.5	2–6
Hydrolysis of:				
Aesculin	+	–	–	–
Casein	+	–	ND	–
DNA	+	+	+	–
Tween 40	+	+	–	+
Acid production from carbohydrates	–	–	+	–
Utilization of:				
D-Arabinose	–	–	–	+
D-Glucose	–	+	–	+
D-Lactose	–	+	–	–
Sucrose	–	+	–	+
Sorbitol	–	+	–	+
Production of:				
α-Chymotrypsin, cystine arylamidase	–	+	ND	+
β-Galactosidase, α-glucosidase	–	–	ND	+
DNA G + C content (mol%)	34–37	33.6	35–37	41.0

BI-phosphohydrolase activities are present, but cystine arylamidase, α-chymotrypsin, α- and β-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. H₂S is produced. The predominant fatty acids are anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{17:1}ω9c, iso-C_{17:0} 3-OH, iso-C_{15:0} 3-OH, C_{15:0}, summed feature 3 (consisting of iso-C_{15:0} 2-OH and/or C_{16:1}ω7c), anteiso-C_{17:0} 3-OH and iso-C_{16:0} 3-OH.

The type strain, SW256^T (=NCIMB 14027^T=DSM 16809^T), was isolated from coastal North Sea water, Hope Cove, near Plymouth, UK.

Description of *Persicivirga dokdonensis* comb. nov.

Persicivirga dokdonensis (dok.do.nen'sis. N.L. fem. adj. *dokdonensis* of Dokdo, an island in the East Sea where the type strain was isolated).

Basonym: *Donghaeana dokdonensis* Yoon *et al.* 2006.

Displays the following properties in addition to those listed in the emended description of the genus and those given by Yoon *et al.* (2006). Mannitol is utilized, but L-arabinose, D-lactose, D-mannose, adipate, caprate, citrate, gluconate, malate, phenylacetate, inositol and sorbitol are not. DNA is hydrolysed.

The type strain, DSW-6^T (=DSM 17205^T=KCTC 12402^T), was isolated from sea water of the East Sea, also known as the Sea of Japan.

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