Neptunomonas concharum sp. nov., isolated from a dead ark clam, and emended description of the genus *Neptunomonas*

Hae-Won Lee, Na-Ri Shin, Jina Lee, Seong Woon Roh, Tae Woong Whon and Jin-Woo Bae

Correspondence
Jin-Woo Bae
baejw@khu.ac.kr

Department of Life and Nanopharmaceutical Sciences and Department of Biology, Kyung Hee University, Seoul 130-701, Republic of Korea

A novel Gram-staining-negative, facultatively anaerobic, motile and rod-shaped bacterium, designated strain LHW37^T, was isolated from a dead ark clam collected on the south coast of Korea. The novel strain grew optimally at 37 °C, at pH 7.0–8.0 and with 2 % (w/v) NaCl. The predominant cellular fatty acids were $C_{18:1}\omega7c$ and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH). The major isoprenoid quinone was ubiquinone-8 (Q-8) and the predominant polar lipids were phosphatidylethanolamine and phosphatidylglycerol. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the novel strain was most closely related to *Neptunomonas japonica* JAMM 0745^T (97.1 % sequence similarity). The genomic DNA G+C content of strain LHW37^T was 48.2 mol%. The DNA–DNA relatedness values recorded in hybridization experiments between the novel strain and its closest known relative were $\leqslant$18 %. Based on the phenotypic, genotypic and phylogenetic data, strain LHW37^T represents a novel species belonging to the genus *Neptunomonas* for which the name *Neptunomonas concharum* sp. nov. is proposed. The type strain is LHW37^T (=KACC 15543^T =JCM 17730^T). An emended description of the genus *Neptunomonas* is also provided.

The genus *Neptunomonas*, within the family *Oceanospirillaceae*, was first described by Hedlund *et al.* (1999) to accommodate a species of marine bacterium that degrades polycyclic aromatic hydrocarbons. At the time of writing, three species found in marine sediment or aquatic environments are included within the genus: *Neptunomonas naphthovorans* (Hedlund *et al.*, 1999), *Neptunomonas antarctica* (Zhang *et al.*, 2010) and *Neptunomonas japonica* (Miyazaki *et al.*, 2008). All are Gram-staining-negative, aerobic, motile microbes that contain Q-8 as the major ubiquinone. A novel *Neptunomonas*-like bacterium, designated strain LHW37^T, was isolated during a study of the bacterial community within an ark clam farm in South Korea. The taxonomic position of this novel strain has now been investigated by following a polyphasic approach.

Strain LHW37^T was isolated from a dead ark clam collected from a cage-cultured ark clam farm in Gang-jin bay, South Korea. The soft tissues of the dead clam were homogenized and diluted with sterile PBS. The diluted sample was then spread on plates of pleuropneumonia-like organisms agar (PPLOA; BBL) and incubated at 37 °C. Strain LHW37^T was isolated from one of these plates and then repeatedly subcultured under aerobic conditions, on marine agar (MA; BBL) at 37 °C, to obtain a pure culture. The isolate was then suspended in marine broth (MB; BBL) containing 40 % (v/v) glycerol and stored at −80 °C.

Growth at different temperatures (4, 10, 15, 20, 25, 30, 37, 45 and 65 °C) was examined in MB. Growth at pH 4.0−11.0 (at intervals of 1.0 pH unit) was investigated in the same medium, with the pH adjusted using 10 mM MES (pH 4–6), 10 mM TAPS (pH 7–9) or 10 mM Na_2HPO_4 (pH 10–11). Growth with 0, 1, 2, 3, 4, 5, 8, 10, 12, 15 and 20 % (w/v) NaCl was investigated in a medium made using the formula for NaCl-free MB. The optical density at 600 nm of each culture was measured, in a Synergy Mx spectrophotometer (BioTek) after incubation for 6, 12, 24 and 48 h and 7 days. Gram staining was performed using a Gram staining kit (bioMérieux) according to the manufacturer's instructions. The morphologies of the colonies and cells were determined after incubation for 2 days on MA at 37 °C. Cell morphology was investigated under a light microscope (Eclipse 50*i*; Nikon). Motility tests were conducted in semi-solid MA (Tittsler & Sandholzer, 1936). Anaerobic growth on MA was determined after incubation at 37 °C for 7 days in an anaerobic chamber filled with $N_2/CO_2/H_2$ (90 : 5 : 5, by vol.). Strain LHW37^T grew at pH 6.0–10.0 (optimum pH 7.0–8.0), at 10–45 °C (optimum 37 °C), and with 1–8 % (w/v) NaCl

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LHW37^T is JF748732.

A supplementary figure is available with the online version of this paper.

Table 1. Differential biochemical and physiological characteristics of strain LHW37^T and established species in the genus *Neptunomonas*

Strains: 1, LHW37^T; 2, *N. japonica* JAMM 0745^T; 3, *N. antarctica* S3-22^T; 4, *Neptunomonas naphthovorans* NAG-2N-126^T. All data are from this study unless stated otherwise. All four strains were positive for the assimilation of stachyose, acetic acid, α-ketovaleric acid and succinic acid monomethyl ester (on GN2 MicroPlates), for acid production from D-tagatose and 5-ketogluconate (on API 50CHB/E test strips) and for alkaline phosphatase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase activities (on API ZYM test strips). +, Positive; −, negative; ND, no data.

Characteristic	1	2	3	4
Growth temperature (°C)				
Range	10–45	5–25*	4–25†	4–24‡
Optimum	37	20*	15†	ND
NaCl for growth (%, w/v)				
Range	1.0–8.0	1.0–5.0*	0.5–5.0†	1.8–7.0‡
Optimum	2	2*	2–3†	ND
Growth pH				
Range	6.0–10.0	7.0–8.5*	6.0–8.0†	6.5–8.5‡
Optimum	7.0–8.0	7.5*	6.5–7.0†	7.5‡
Enzyme activity				
DNase	+	+	−	−
Esterase lipase (C8)	−	−	+	+
Acid phosphatase	+	+	− (+†)	+
Reduction of nitrates to nitrites	+	− (+*)	+	+ (−‡)
Indole production	−	−	−	+
Glucose fermentation	+	+	−	−
Gelatin hydrolysis	+	− (+*)	−	−
Acid production from:				
D-Arabinose	+	−	−	−
L-Arabinose	+	−	−	−
D-Ribose	+	+	−	+
D-Xylose	+	−	−	−
L-Xylose	+	−	−	−
D-Fructose	+	−	− (+†)	−
L-Sorbose	+	−	−	−
Aesculin	+	+	−	−
Salicin	+	−	−	−
Turanose	+	−	−	−
D-Lyxose	+	−	−	+
2-Ketogluconate	−	−	+	−
Assimilation of:				
Inulin	+	−	+	−
N-Acetyl-β-D-mannosamine	−	−	−	+
D-Arabitol	−	−	−	+
Cellobiose	−	−	−	+
L-Fucose	−	−	−	+
α-D-Glucose	−	−	−	+
Melibiose	−	−	−	+
3-Methyl D-glucose	+	−	+	+
Methyl α-D-glucoside	−	−	−	+
Methyl β-D-glucoside	+	+	−	+
Methyl α-D-mannoside	−	−	−	+
Palatinose	−	−	−	+
L-Rhamnose	−	−	−	+
D-Ribose	−	−	−	+
D-Sorbitol	−	−	−	+
Turanose	−	+	−	+
Xylitol	−	+	+	+
β-Hydroxybutyric acid	+	+	−	+
γ-Hydroxybutyric acid	−	−	−	+
p-Hydroxyphenylacetic acid	−	−	−	+
Lactamide	−	−	−	+
D-Lactic acid methyl ester	−	−	+	+
D-Malic acid	−	−	+	−
L-Malic acid	+	+	−	+
Pyruvic acid methyl ester	+	−	−	+
Propionic acid	−	−	−	+
Pyruvic acid	−	−	−	+
Succinamic acid	+	−	−	+
N-Acetyl-L-glutamic acid	+	−	−	+
L-Alanyl glycine	+	+	−	−
L-Asparagine	+	+	+	−
L-Glutamic acid	+	−	−	+
Glycyl L-glutamic acid	+	+	−	−
L-Serine	−	−	+	−
Putrescine	−	+	−	−
2,3-Butanediol	−	−	−	+
Glycerol	−	+	−	+
2′-Deoxyadenosine	−	−	−	+
Uridine	−	+	−	−
D-Fructose 6-phosphate	−	−	−	+

*Data from Miyazaki *et al.* (2008).
†Data from Zhang *et al.* (2010).
‡Data from Hedlund *et al.* (1999).

(optimum 2 %). The cells were motile, Gram-staining-negative rods (1.0–1.2 × 0.5–0.6 μm). After 2 days on MA at 37 °C, strain LHW37^T formed cream-coloured, circular, smooth and convex colonies. Growth occurred under anaerobic conditions.

Oxidase and catalase activity were evaluated using 1 % (v/v) *p*-tetramethyl phenylenediamine (bioMérieux) and 3 % (w/v) H_2O_2, respectively. DNase activity and H_2S production were tested on DNase test agar (BBL) and Kligler's iron agar (BBL), respectively. Acid production was tested using API 50CH strips (bioMérieux) with 50 CHB/E medium (bioMérieux), according to the manufacturer's instructions. Sole carbon source assimilation was determined using GN2 MicroPlates (Biolog) and inoculating fluid (Biolog) supplemented with 4 % (w/v) sea salts (Sigma-Aldrich), according to the manufacturer's instructions. API 20NE and API ZYM strips (bioMérieux) were used to characterize enzyme activity, again according to the manufacturer's instructions. Strain LHW37^T was found to

be catalase-, oxidase- and DNase-positive but negative for H_2S production. Acid was produced from D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-fructose, L-sorbose, aesculin, salicin, turanose, D-lyxose, D-tagatose and potassium 5-ketogluconate (API 50CHB). Inulin, 3-methyl glucose, methyl β-D-glucoside, stachyose, acetic acid, β-hydroxybutyric acid, α-ketovaleric acid, L-malic acid, pyruvic acid methyl ester, succinic acid monomethyl ester, succinamic acid, *N*-acetyl-L-glutamic acid, L-alanyl glycine, L-asparagine, L-glutamic acid and glycyl L-glutamic acid were all assimilated (GN2 MicroPlate). In API ZYM tests, the novel strain was positive for alkaline phosphatase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities but negative for esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. Strain LHW37^T was positive for the reduction of nitrate to nitrite, D-glucose fermentation and gelatin hydrolysis (API 20NE). Closely related species within the genus *Neptunomonas* gave markedly different results in the biochemical tests (Table 1).

For cellular fatty acid analysis, strain LHW37^T and three closely related reference strains (*N. japonica* JAMM 0745^T, *N. antarctica* S3-22^T and *N. naphthovorans* NAG-2N-126^T) were cultivated on MA supplemented with 2 % (w/v) NaCl at 20 °C for 2 days. Cellular fatty acids were extracted using the standard protocol of the Microbial Identification System (MIDI) and identified using the Microbial Identification software package (Sasser, 1990). Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v) according to the method of Collins & Jones (1981a), purified by one-dimensional TLC on a silica gel 60 F_{254} plate (Farcot *et al.*, 1988) and identified by HPLC (Collins & Jones, 1981b) using a 250 × 4.6 mm reverse-phase column (Hypersil ODS, Thermo Scientific). Polar lipids were extracted according to the method of Xin *et al.* (2000), separated by two-dimensional TLC (Farcot *et al.*, 1988) and detected using the appropriate spray reagents, as described by Tindall (1990). The predominant cellular fatty acids were identified as $C_{18:1}\omega7c$ (39.9 %) and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 38.0 %) (Table 2). The cellular fatty acid pattern of strain LHW37^T was similar to those of the established species in the genus *Neptunomonas*. The predominant ubiquinone of strain LHW37^T, Q-8, is also a major quinone of *N. japonica* JAMM 0745^T (Miyazaki *et al.*, 2008) and *N. antarctica* S3-22^T (Zhang *et al.*, 2010). The polar lipid profile of strain LHW37^T comprised phosphatidylethanolamine, phosphatidylglycerol and two unidentified lipids (Fig. S1, available in IJSEM Online).

The 16S rRNA gene of strain LHW37^T was amplified by colony PCR using a PCR pre-mix (WizBio) and the two universal primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1522R (5′-AAGGAGGTGATCCAGCCGC-3′). The PCR product was purified using a QIAquick PCR purification kit (Qiagen) and then sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) and an ABI PRISM 3730xl automated DNA analyser (Applied Biosystems). The sequence fragments were assembled, using the SeqMan software package (DNASTAR), to obtain a nearly full-length 16S rRNA gene sequence. This sequence was then compared with the 16S rRNA gene sequences of type strains of established species, using the EzTaxon-e server (Kim *et al.*, 2012). It was then aligned with the 16S rRNA gene sequences of the species that appeared to be most closely related to strain LHW37^T, using CLUSTAL W (Thompson *et al.*, 1994). The quality of the 16S rRNA gene sequences was confirmed using the BioEdit software package (Hall, 1999). Phylogenetic trees based on 16S rRNA gene sequences were generated using MEGA5 (Tamura *et al.*, 2011) and the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood methods (Felsenstein, 1981). The tree topologies were evaluated using bootstrap analyses, with 1000 replications. A distance matrix was determined using the two-parameter model of Kimura (1980). Strain LHW37^T was found to be most closely related to *N. japonica* JAMM 0745^T (97.1 % 16S rRNA gene sequence similarity), *N. antarctica* S3-22^T (96.3 %) and *N. naphthovorans* NAG-2N-126^T (95.3 %). In the phylogenetic trees, the novel strain clustered with these three established species belonging to the genus *Neptunomonas* (Fig. 1).

For the determination of G+C contents and/or for DNA–DNA hybridization experiments, genomic DNA was extracted from strain LHW37^T and its closest relative (*N. japonica* JAMM 0745^T) by using the G-spin genomic DNA extraction kit (iNtRON Biotechnology). DNA–DNA hybridization experiments were conducted using genome-probing microarrays (Bae *et al.*, 2005; Chang *et al.*, 2008), with DNA–DNA relatedness values calculated from the

Table 2. Cellular fatty acid contents (%) of strain LHW37^T and established species in the genus *Neptunomonas*

Strains: 1, LHW37^T; 2, *N. japonica* JAMM 0745^T; 3, *N. antarctica* S3-22^T; 4, *N. naphthovorans* NAG-2N-126^T. All data from this study. TR, trace (<1.0 %); ND, not detected; ECL, equivalent chain-length.

Fatty acid	1	2	3	4
$C_{10:0}$	2.9	2.9	TR	ND
$C_{10:0}$ 3-OH	5.1	4.8	4.5	6.1
$C_{12:0}$	1.3	1.7	TR	3.7
$C_{16:0}$	8.7	7.0	14.7	17.1
$C_{17:0}$ cyclo	ND	ND	ND	1.5
$C_{18:1}\omega7c$	39.9	32.5	9.9	34.3
10-Methyl $C_{19:0}$	1.4	2.4	ND	ND
Summed feature 3*	38.0	45.2	64.0	37.9
Unknown ECL 11.799	2.8	4.3	4.5	2.0

*Summed features represent groups of two or more fatty acids that could not be separated by GLC with the Microbial Identification System. Summed feature 3 contained $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

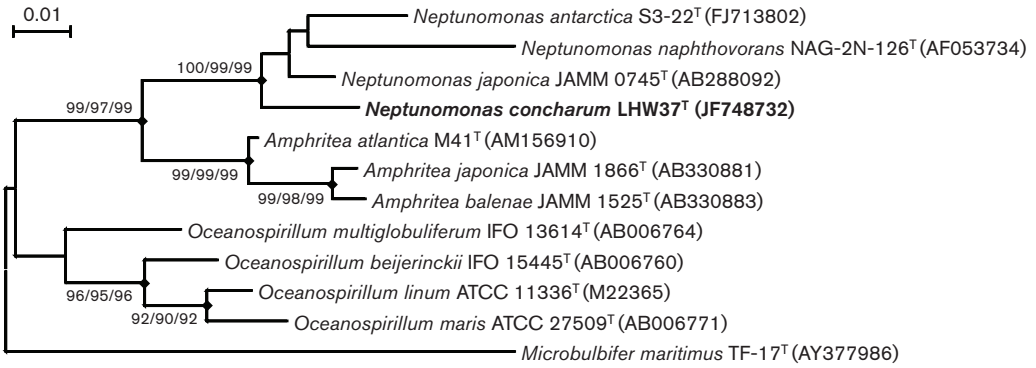

Fig. 1. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain LHW37^T and its phylogenetic neighbours, including the established species in the genus *Neptunomonas*. Bootstrap values (neighbour-joining/maximum-parsimony/maximum-likelihood) >70 % (each based on 1000 replications) are shown at branch points. Filled diamonds indicate that the corresponding nodes were obtained using all three tree-making methods. *Microbulbifer maritimus* TF-17^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

signal-to-noise ratios of the probes (Loy *et al.*, 2005). The mean DNA–DNA relatedness values recorded between strain LHW37^T and *N. japonica* JAMM 0745^T – 18 % and, in the reciprocal hybridization, 6 % – fell well below the threshold value of 70 % (Stackebrandt & Goebel, 1994), that might indicate that these two strains belonged to the same species. The genomic DNA G+C content of the novel strain was determined using SYBR Gold I (Life Technologies) and a real-time PCR thermocycler (Gonzalez & Saiz-Jimenez, 2002), with samples of genomic DNA from *Escherichia coli* K-12, *Ruegeria pomeroyi* DSS-3^T and *Ruminococcus obeum* ATCC 29174^T used for reference. The genomic DNA G+C content of strain LHW37^T, 48.2 mol%, fell within the range shown by established species belonging to the genus *Neptunomonas* (Hedlund *et al.*, 1999).

Based on the results of phenotypic, genotypic and phylogenetic analyses, strain LHW37^T represents a novel species within the genus *Neptunomonas*, for which the name *Neptunomonas concharum* sp. nov. is proposed.

Emended description of the genus *Neptunomonas*

The description of the genus *Neptunomonas* is based on that given previously by Hedlund *et al.* (1999) but with the following change. The major polar lipids include phosphatidylethanolamine and phosphatidylglycerol.

Description of *Neptunomonas concharum* sp. nov.

Neptunomonas concharum (con.cha′rum. L. gen. pl. n. *concharum* of bivalves, of shellfish, indicating the type of animal from which the species was isolated).

The cells are motile, Gram-staining-negative, facultatively anaerobic rods that measure 1.0–1.2 × 0.5–0.6 μm. Colonies formed after incubation for 2 days on MA at 37 °C are cream-coloured, circular, 0.5–2.0 mm in diameter, smooth and convex. Optimal growth occurs at pH 7.0–8.0, in the presence of 2 % (w/v) NaCl, and at 37 °C. Positive for catalase, oxidase and DNase activities and for the assimilation of inulin, 3-methyl glucose, methyl β-D-glucoside, stachyose, acetic acid, β-hydroxybutyric acid, α-ketovaleric acid, L-malic acid, pyruvic acid methyl ester, succinic acid monomethyl ester, succinamic acid, *N*-acetyl-L-glutamic acid, L-alanyl glycine, L-asparagine, L-glutamic acid and glycyl L-glutamic acid. Positive (API 20NE and API ZYM) for alkaline phosphatase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and acid phosphatase activities, reduction of nitrate to nitrite, D-glucose fermentation and gelatin hydrolysis. Acid is produced (in API 50CHB tests) from D-ribose, D-fructose, L-sorbose, D-tagatose, D-arabinose, L-arabinose, D-xylose, L-xylose, aesculin, salicin, turanose, D-lyxose and potassium 5-ketogluconate. The major cellular fatty acids are $C_{18:1}\omega7c$ and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH). The predominant quinone is Q-8. The polar lipid profile comprises phosphatidylethanolamine, phosphatidylglycerol and two unidentified lipids.

The type strain, LHW37^T (=KACC 15543^T =JCM 17730^T), was isolated from a dead ark clam collected on the southern coast of Korea. The genomic DNA G+C content of strain LHW37^T is 48.2 mol%.

Acknowledgements

We thank Dr J. P. Euzéby (École Nationale Vétérinaire, France) for his advice on nomenclature. This work was supported by the Republic of Korea's National Institute of Biological Resources and the National Research Foundation of Korea's Mid-career Researcher Program (2011-0028854). The National Research Foundation of Korea is itself funded by the Ministry of Education, Science and Technology.

References

Bae, J. W., Rhee, S. K., Park, J. R., Chung, W. H., Nam, Y. D., Lee, I., Kim, H. & Park, Y. H. (2005). Development and evaluation of genome-probing microarrays for monitoring lactic acid bacteria. *Appl Environ Microbiol* **71**, 8825–8835.

Chang, H. W., Nam, Y. D., Jung, M. Y., Kim, K. H., Roh, S. W., Kim, M. S., Jeon, C. O., Yoon, J. H. & Bae, J. W. (2008). Statistical superiority of genome-probing microarrays as genomic DNA-DNA hybridization in revealing the bacterial phylogenetic relationship compared to conventional methods. *J Microbiol Methods* **75**, 523–530.

Collins, M. D. & Jones, D. (1981a). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* **45**, 316–354.

Collins, M. D. & Jones, D. (1981b). A note on the separation of natural mixtures of bacterial ubiquinones using reverse-phase partition thin-layer chromatography and high performance liquid chromatography. *J Appl Bacteriol* **51**, 129–134.

Farcot, J. M., Mercky, F., Tritschler, J. L. & Schaeffer, F. (1988). Percutaneous cervical chordotomy in primary or secondary chest cancer pains (apropos of 19 cases). *Agressologie* **29**, 87–89 (in French).

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Gonzalez, J. M. & Saiz-Jimenez, C. (2002). A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* **4**, 770–773.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

Hedlund, B. P., Geiselbrecht, A. D., Bair, T. J. & Staley, J. T. (1999). Polycyclic aromatic hydrocarbon degradation by a new marine bacterium, *Neptunomonas naphthovorans* gen. nov., sp. nov. *Appl Environ Microbiol* **65**, 251–259.

Kim, O.-S., Cho, Y.-J., Lee, K., Yoon, S.-H., Kim, M., Na, H., Park, S.-C, Jeon, Y. S., Lee, J.-H., Yi, H., Won, S. & Chun, J. (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.

Loy, A., Schulz, C., Lücker, S., Schöpfer-Wendels, A., Stoecker, K., Baranyi, C., Lehner, A. & Wagner, M. (2005). 16S rRNA gene-based oligonucleotide microarray for environmental monitoring of the betaproteobacterial order "Rhodocyclales". *Appl Environ Microbiol* **71**, 1373–1386.

Miyazaki, M., Nogi, Y., Fujiwara, Y., Kawato, M., Kubokawa, K. & Horikoshi, K. (2008). *Neptunomonas japonica* sp. nov., an *Osedax japonicus* symbiont-like bacterium isolated from sediment adjacent to sperm whale carcasses off Kagoshima, Japan. *Int J Syst Evol Microbiol* **58**, 866–871.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Tindall, B. J. (1990). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Tittsler, R. P. & Sandholzer, L. A. (1936). The use of semi-solid agar for the detection of bacterial motility. *J Bacteriol* **31**, 575–580.

Xin, H., Itoh, T., Zhou, P., Suzuki, K., Kamekura, M. & Nakase, T. (2000). *Natrinema versiforme* sp. nov., an extremely halophilic archaeon from Aibi salt lake, Xinjiang, China. *Int J Syst Evol Microbiol* **50**, 1297–1303.

Zhang, X. Y., Zhang, Y. J., Yu, Y., Li, H. J., Gao, Z. M., Chen, X. L., Chen, B. & Zhang, Y. Z. (2010). *Neptunomonas antarctica* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* **60**, 1958–1961.