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TAXONOMIC DESCRIPTION

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Nonlabens ponticola sp. nov., isolated from seawater and reclassification of *Nonlabens sediminis* as a later heterotypic synonym of *Nonlabens tegetincola*

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Abstract

A Gram-stain-negative, orange-pigmented and strictly aerobic bacterium, designated strain MJ115^T, was isolated from seawater in Pohang, South Korea. Cells were non-motile rods and showed positive reactions for catalase and oxidase tests. Growth of strain MJ115^T was observed at 4–35 °C (optimum, 30 °C), pH 6.0–7.0 (optimum, pH 6.5) and in the presence of 0–8.0% (w/v) NaCl (optimum, 2.0%). Strain MJ115^T contained iso-$C_{15:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:1}$ ω9*c*, $C_{17:0}$ 2-OH, iso-$C_{16:0}$ 3-OH, iso-$C_{17:0}$ 3-OH and summed feature 3 ($C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*) as major cellular fatty acids and menaquinone-6 as the major respiratory quinone. Phosphatidylethanolamine, two unidentified aminolipids and four unidentified lipids were detected as major polar lipids. The G+C content of the genomic DNA was 40.7 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain MJ115^T formed a phyletic lineage with *Nonlabens marinus* S1-08^T, *Nonlabens agnitus* JC2678^T and *Nonlabens antarcticus* AKS 622^T within the genus *Nonlabens*. Strain MJ115^T was also most closely related to *N. marinus* S1-08^T, *N. agnitus* JC2678^T and *N. antarcticus* AKS 622^T with 96.5, 96.4 and 96.0% 16S rRNA sequence similarities, respectively. Here it is proposed that strain MJ115^T represents a new species of the genus *Nonlabens*, for which the name *Nonlabens ponticola* sp. nov. is proposed. The type strain is MJ115^T (=KCTC 72237^T=NBRC 113963^T). In addition, the comparison of the whole genome sequences and phenotypic features suggested that *Nonlabens tegetincola* and *Nonlabens sediminis* belong to the same species. Therefore, it is proposed that *N. sediminis* is reclassified as a later heterotypic synonym of *N. tegetincola*.

The genus *Nonlabens*, a member of the family *Flavobacteriaceae*, was first proposed in 2005 with *Nonlabens tegenticola* as type species isolated from estuarine microbial mat [1]. In 2012, the description of the genus *Nonlabens* has been emended to include members of three genera, *Persicivirga* [2], *Sandarakinotalea* [3] and *Stenothermobacter* [4] by Yi and Chun [5]. At the time of writing, the genus *Nonlabens* comprises 12 valid published species. All of them were isolated from various marine environments such as seawater [5, 6], glacier core [7], seashore sand [8], reclaimed land soil [9], tidal flat [10] and marine organisms [5, 11]. Members of the genus *Nonlabens* generally feature Gram-negative, orange-pigmented, catalase- and oxidase-positive, rod shaped and contain MK-6 as the major respiratory quinone. In this study, we isolated a putative new species of the genus *Nonlabens* from seawater and taxonomically characterized it using a polyphasic approach. The putative new species may be used as a model microorganism to study energy metabolisms of marine prokaryotes because the isolate harboured two rhodopsin genes for ATP synthesis like other *Nonlabens* species [12, 13]. In addition, it is proposed that *Nonlabens sediminis* should be reclassified as a later heterotypic synonym of *Nonlabens tegetincola* based on the genomic sequences and phenotypic features.

ISOLATION AND ECOLOGY

Strain MJ115^T was isolated from a seawater sample collected from Pohang in South Korea (35°52'40.3"N 129°31'18.1"E). The seawater sample was serially diluted in artificial seawater (ASW; 20 g NaCl, 2.9 g $MgSO_4$, 4.53 g $MgCl_2{\cdot}6H_2O$, 0.64 g KCl and 1.75 g $CaCl_2{\cdot}2H_2O$ per litre) and aliquots of each

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Abbreviations: MA, marine agar; MB, marine broth; MK-6, menaquinone-6; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbor-joining; PE, phosphatidylethanolamine; TLC, thin-layer chromatography.
The GenBank accession numbers for the 16S rRNA gene and genome sequences of strain MJ115^T are MH712297 and CP034549, respectively.
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Four supplementary figures and one supplementary table are available with the online version of this article.

serial dilution were spread on marine agar (MA; BD, USA) and incubated at 30 °C for 5 days. The 16S rRNA genes of colonies grown on MA were amplified and sequenced using universal PCR primers F1 (5′-AGA GTT TGA TCM TGG CTC AG-3′) and R13 (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) [14]. The resulting 16S rRNA gene sequences were compared with those of all type strains using the Nucleotide Similarity Search program in the EzBioCloud (http://www.ezbiocloud.net/identify) [15] and strain MJ115^T, a putative novel strain of the genus *Nonlabens*, was obtained from the analysis and routinely cultured on MA for 3 days at 30 °C and stored at −80 °C in marine broth (MB; BD, USA) containing 15% (v/v) glycerol for a long-term preservation. *Nonlabens marinus* KCTC 23432^T, *Nonlabens agnitus* KACC 14155^T and *Nonlabens antarcticus* JCM 14068^T were used as reference strains for the comparisons of phenotypic properties and fatty acid compositions.

PHYLOGENY OF 16S rRNA GENE SEQUENCES

The sequences obtained by the primers F1, 518R, 805F and R13 were assembled to get an almost complete 16S rRNA gene sequence (1460 nucleotides), as described previously [14]. Sequence similarities of 16S rRNA genes of strain MJ115^T and closely related type strains were calculated using the EzBioCloud server. The 16S rRNA gene sequences of strain MJ115^T and closely related type strains were aligned using the fast secondary-structure aware infernal aligner available in the Ribosomal Database Project and phylogenetic trees with bootstrap values (1000 replications) were constructed [16]. The maximum composite likelihood model, the nearest-neighbour-interchange heuristic search method and the complete deletion options were used for the tree constructions based on neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) algorithms in the MEGA7 software [17].

Phylogenetic analysis using the NJ, ML and MP algorithms revealed that strain MJ115^T formed a phylogenetic lineage with *Nonlabens marinus* S1-08^T, *Nonlabens agnitus* JC2678^T and *Nonlabens antarcticus* AKS 622^T within the genus *Nonlabens* (Figs 1 and S1 available in the online version of this article). Comparative analysis of 16S rRNA gene sequences also showed that strain MJ115^T was most closely related to *N. marinus* S1-08^T, *N. agnitus* JC2678^T and *N. antarcticus* AKS 622^T with 96.4, 96.4, 95.9% sequence similarities, respectively.

GENOME FEATURES

The genomic DNAs of strain MJ115^T and *N. antarcticus* JCM 14068^T were extracted using a Wizard Genomic DNA purification kit (Promega, USA), according to the manufacturer's instruction and sequenced using a combination of PacBio P6C4 chemistry in an eight-well SMART Cell v3 in PacBio RSII and Illumina Hiseq 2500 sequencing and by Illumina Hiseq X instrument, respectively at Macrogen (Republic of Korea), as describe previously [18]. The PacBio sequencing reads of strain MJ115^T were assembled with PacBio SMRT Analysis 2.3.0 using the HGAP2 protocol [19], which generated one circular chromosome with an average genome

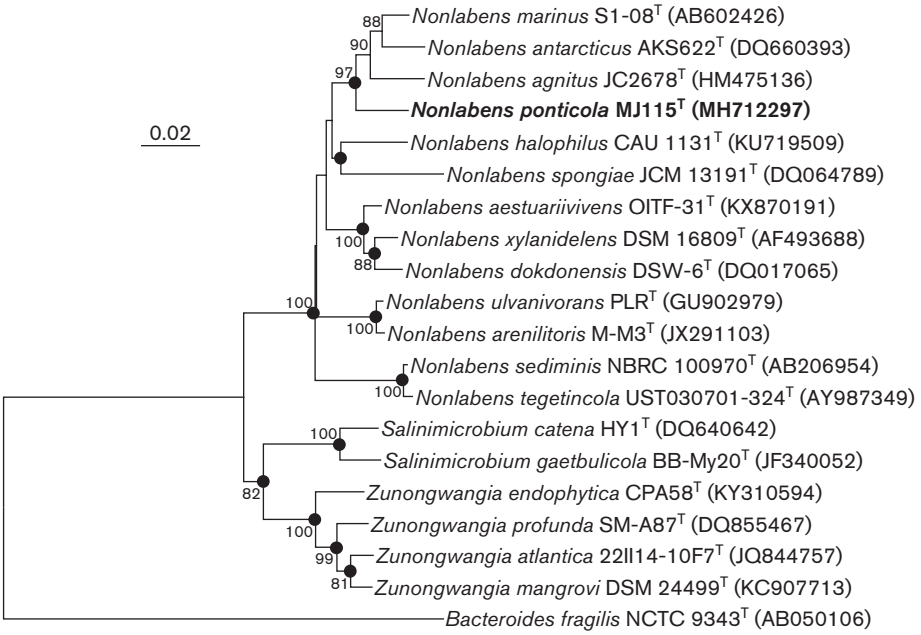

Fig. 1. A neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of strain MJ115^T and closely related taxa. Bootstrap values above 70% are shown on nodes in percentages of 1000 replicates. Filled circles indicate the corresponding nodes that were also recovered in the trees generated with maximum-likelihood and maximum-parsimony algorithms. *Bacteroides fragilis* NCTC 9343^T (AB050106) was used as an outgroup. Scale bar equals 0.02 changes per nucleotide position.

coverage of 398.6×. Paired-end sequences with 2046 Mb of 151 bp reads (712.4×average genome coverage) derived from the Illumina sequencing were mapped on the complete genome sequence derived from the PacBio sequencing reads for error corrections. The complete genome of strain MJ115^T was 2872100 bp in size and contained a total of 2633 predicted genes, 2572 protein coding sequences, 36 tRNA encoding 20 amino acids and two complete rRNA operons (16, 23 and 5S rRNA genes). Whereas the sequencing reads of *N. antarcticus* JCM 14068^T were *de novo*-assembled using SOAPdenovo2 program [20], which resulted in 66 contigs with an N50 of 415 Kb and sequencing coverage of 151.0×. The draft genome of *N. antarcticus* JCM 14068^T was 3270668 bp in size and contained a total of 2978 predicted genes, 2905 protein coding sequences, 34 tRNA encoding 20 amino acids and three rRNA genes.

The qualities of the resulting genomes of strain MJ115^T and *N. antarcticus* JCM 14068^T were assessed based on their completeness and contamination rates using CheckM software (version 1.0.4) [21]. The completeness and contamination rates of the genomes of strain MJ115^T and *N. antarcticus* JCM 14068^T were 99.7 and 0.7% and 99.9 and 0.0%, respectively, which clearly satisfied the criteria (≥90 and ≤10%, respectively) to be considered as a high quality genome [21]. In addition, it was confirmed that the 16S rRNA gene sequences of strain MJ115^T and *N. antarcticus* JCM 14068^T obtained by Sanger sequencing and from the genomes were identical. The genomic DNA G+C contents calculated from the whole genomes of strain MJ115^T and *N. antarcticus* JCM 14068^T were 40.7 and 38.5%, respectively, which are within the DNA G+C content range of *Nonlabens* species [1–10]. The general genomic features of strain MJ115^T and *N. antarcticus* JCM 14068^T were compared with those of closely related reference strains (Table 1). The genome sequences of strain MJ115^T and *N. antarcticus* JCM 14068^T were deposited in GenBank under the accession numbers CP034549 and JADFCO000000000, respectively.

For the genome-based phylogenomic analysis, 92 core genes of strain MJ115^T and closely related *Nonlabens* strains with available genomes were obtained and a phylogenomic tree with 1000 replicate bootstrap values was constructed based on the maximum-likelihood algorithm using the UBCG program [22] (Fig. S2). The phylogenomic tree clearly showed that strain MJ115^T formed a distinct phylogenetic lineage within the genus *Nonlabens*. Comparative genome analyses of strain MJ115^T and closely related *Nonlabens* strains showed that 483 proteins are specific to strain MJ115^T (50% amino acid identity cutoff) and strain MJ115^T harbours two rhodopsin genes (EJ995_00765 and EJ995_12215). Average nucleotide identity (ANI) and *in silico* DNA–DNA hybridization (DDH) values between strain MJ115^T and other related taxa were calculated using the Orthologous Average Nucleotide Identity Tool (OAT) software available in the EzBioCloud server (www.ezbiocloud.net/sw/oat) [23] and the Genome-to-Genome Distance Calculator ver. 2.1 (http://ggdc.dsmz.de/distcalc2.php) [24], respectively. ANI and *in silico* DDH values between strain MJ115^T and closely related *Nonlabens* strains were clearly lower than thresholds (<95% ANI and <70% DDH) for prokaryotic species delineation (Table 2) [25, 26], suggesting that strain MJ115^T represents a new species of the genus *Nonlabens*.

PHYSIOLOGY AND CHEMOTAXONOMY

Growth of strain MJ115^T was examined for 5 days at 30 °C on R2A agar, tryptic soy agar (BD, USA), nutrient agar (BD, USA), marine agar (BD, USA) and laboratory prepared LB agar. Growth of strain MJ115^T at different temperatures (4, 10, 15, 20, 25, 30, 37, 40 and 45 °C) and pH values (5.0–11.0

Table 1. General genomic features of strain MJ115^T and the type strains of closely related *Nonlabens* species

Taxa: 1, strain MJ115^T; 2, *N. marinus* S1-08^T; 3, *N. agnitus* JCM 17109^T; 4, *N. antarcticus* JCM 14068^T. The general features of the genomes were analysed using the NCBI prokaryotic genome annotation pipeline www.ncbi.nlm.nih.gov/genome/annotation_prok/.

Characteristic	1*	2	3	4*
Status status† (no of contig)	C (1)	C (2)	D (4)	D (66)
Genome size (Mb)	2.87	2.92	3.22	3.27
G+C contents (mol%)	40.7	39.7	40.9	38.5
No. of genes	2633	2693	2929	2978
No. of protein-coding genes	2572	2626	2878	2905
No. of tRNA	36	36	38	34
No. of pseudo genes	15	21	25	32
No. of rRNA operons (16S, 23S, 5S)	2	2	–	–
GenBank accession number	CP034549	AP014548	MQUC00000000	JADFCO000000000

*Genomes sequenced in this study.
†Genome status: C, complete genome sequence; D, draft genome sequence.

Table 2. Pair-wise average nucleotide identity (ANI) and *in silico* DNA–DNA hybridization (DDH) values among strain MJ115^T and the type strains of closely related *Nonlabens* species. The upper matrix represents ANI values, while the lower matrix in bold represents *in silico* DDH values

Taxa; 1, strain MJ115^T (CP034549); 2, *N. marinus* S1-08^T (AP014548); 3, *N. agnitus* JCM 17109^T (MQUC00000000); 4, *N. antarcticus* JCM 14068^T (JADFCO000000000); 5, *N. xylanidelens* DSM 16809^T (MQVW00000000); 6, *N. ulvanivorans* PLR^T(JPJI01000000); 7, *N. arenilitoris* KCTC 32109^T (MTPW01000000); 8, *N. dokdonensis* DSW-6^T (NC_020156); 9, *N. spongiae* JCM 13191^T (NZ_CP019344–5); 10, *N. tegetincola* JCM 12886^T (MQVV00000000); 11, *N. sediminis* NBRC 100970^T (CP019342).

Taxa	1	2	3	4	5	6	7	8	9	10	11
1	–	72.2	72.9	71.7	70.7	70.3	70.7	70.9	70.4	70.0	69.7
2	**18.1**	–	74.8	74.1	70.9	70.6	70.8	71.3	70.6	69.7	69.5
3	**18.1**	**18.4**	–	75.5	70.7	70.5	70.5	71.1	70.0	69.4	69.6
4	**18.0**	**19.1**	**18.6**	–	70.9	69.3	70.5	71.0	70.3	69.4	69.3
5	**18.6**	**18.8**	**17.7**	**18.8**	–	74.5	71.2	77.3	70.6	71.3	71.1
6	**18.1**	**18.1**	**17.9**	**17.8**	**21.2**	–	87.9	73.8	69.9	71.0	71.0
7	**19.2**	**18.9**	**18.4**	**18.9**	**20.2**	**34.0**	–	73.6	70.0	71.2	71.2
8	**18.1**	**19.3**	**18.4**	**17.6**	**20.4**	**20.5**	**20.1**	–	70.8	71.5	71.2
9	**19.3**	**21.6**	**18.8**	**21.8**	**18.6**	**18.5**	**19.7**	**18.9**	–	69.9	69.7
10	**20.1**	**20.1**	**19.5**	**18.8**	**18.2**	**17.9**	**19.1**	**19.7**	**19.1**	–	97.5
11	**18.1**	**18.5**	**18.6**	**17.8**	**18.2**	**17.7**	**18.0**	**17.9**	**19.4**	**79.0**	–

at 0.5 pH unit intervals) was evaluated on MA and in MB, respectively, for 5 days. MB with pH 5.0–5.5, pH 6.0–7.0 and pH 8.0–11.0 were prepared using sodium citrate buffer, phosphate buffer and Tris-HCl buffers, respectively [27]. The pH values of the MB were adjusted again after autoclaving. Growth of strain MJ115^T at different NaCl concentrations (0–10% at 1.0% intervals, w/v) was tested in MB prepared in the laboratory, according to the BD formula. The following biochemical tests and physiological analyses of strain MJ115^T were conducted using cells grown on MA for 5 days at 30 °C. Gram-staining was performed using the Gram-stain kit (bioMérieux, France), according to the manufacturer's instructions. Cell morphology of strain MJ115^T was investigated using phase-contrast microscopy (Carl Zeiss, Germany) and transmission electron microscopy (JEM–1010; JEOL, Japan). Oxidase activity was evaluated by oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine (Merck, USA) and catalase activity was tested by production of oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution [28]. Anaerobic growth was assessed on MA under anaerobic conditions using the GasPak Plus system (BBL, USA) at 30 °C for 21 days. The following properties of strain MJ115^T and three reference strains were investigated in parallel under the same conditions. The production of flexirubin-type pigments was evaluated using the KOH as described previously [29]. Antibiotic susceptibility was assayed using Neo-Sensitabs (Rosco) containing the following antibiotics: ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixan (30 µg), penicillin (10 U), rifampicin (5 µg), streptomycin (10 µg), tetracyclines (30 µg) and vancomycin (30 µg). Hydrolysis of tyrosine, casein, aesculin, gelatin, starch, Tween 20, Tween 80 and urea was tested on MA following the methods described by Smibert and Krieg [28] and Lányí [30]. Additional biochemical features and enzymatic activities were tested using the API 50CH, API 20NE and API ZYM systems (bioMérieux, France), respectively, according to the manufacturers' instructions.

Strain MJ115^T grew well on MA, but did not grow on tryptic soy agar, LB agar, R2A agar and nutrient agar. Cells of strain MJ115^T were Gram-stain-negative, non-motile rods with approximately 0.3–0.59 µm in width and 0.6–1.0 µm in length (Fig. S3). Anaerobic growth was not observed after 21 days of incubation on MA at 30 °C and flexirubin-type pigmets were absent (KOH-negative). Strain MJ115^T was sensitive to ampicillin, chloramphenicol, erythromycin, nalidixan, penicillin, rifampicin and vancomycin, but resistant to gentamicin, kanamycin, streptomycin and tetracycline. Common and different phenotypic characteristics between strain MJ115^T and closely related strains were summarized in Tables 3 and S1.

Polar lipids of strain MJ115^T were extracted from cells harvested during the exponential growth phase and analysed by two-dimensional thin-layer chromatography (TLC), according to the procedure described by Minnikin *et al.* [31]. The following reagents were used to identify different polar lipids: 10% ethanolic molybdophosphoric acid (for total polar lipids), ninhydrin (for aminolipids), Dittmer-Lester reagent (for phospholipids) and α-naphthol (for glycolipids). For cellular fatty acid analysis, strain MJ115^T and three reference strains were cultivated in MB at their optimal temperatures and their microbial cells were harvested at the same growth stage (exponential phase, optical density, OD_{600}=0.8). Cellular fatty acids of

Table 3. Comparison of phenotype characteristics of strain MJ115^T, most closely related *Nonlabens* species, and the type species of the genus *Nonlabens*. Strains: 1, strain MJ115^T (this study); 2, *N. marinus* KCTC 23432^T [6]; 3, *N. agnitus* KACC 14155^T [5]; 4, *N. antarcticus* JCM 14068^T [7]; 5, *N. tegetincola* UST030701-324^T [1, 5]; 6, *N. sediminis* CKA-5^T [3, 5]. All strains are positive for the following characteristics: activity* of catalase, oxidase, alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. All strains are negative for the following characteristics: flexirubin-type pigment production*, hydrolysis* of Tween 80, Tween 20 and tyrosine and activity* of α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Nitrate reduction* and indole production* are negative for strains MJ115^T, KCTC 23432^T, KACC 14155^T and JCM 14068^T, but the data are not available in *N. Tegetincola* and *N. Sediminis*. Symbols: +, positive; –, negative; ND, no data

Characteristic	1	2	3	4	5	6
Isolation source	Seawater	Pacific ocean	Seawater	Glacier core	Microbial mat	Sediment
Colony colour	Bright-orange	Orange	Orange	Orange	Orange	Orange
Gliding motility*	–	–	+	+	–	–
Growth at*:						
Temperature (°C) (optimum)	4–35 (30)	10–30 (25)	4–35 (30)	4–25	12–44	10–40
NaCl (%, w/v) (optimum)	0–8(2)	1–7 (2–3)	0–8(2.5)	0–4(2)	2–4	ND
pH (optimum)	6–7(6.5)	6–7(6.5)	6–9(7.5)	6–8(7)	5–10	ND
Hydrolysis* of:						
Casein	+	–	+	+	+	+
Aesculin	+	–	+	–	–	–
Starch	+	–	–	+	+	+
Urea	+	+	+	+	–	–
Gelatin	–	–	–	–	+	+
Enzyme activity (API ZYM)* of:						
Esterase (C4), crystine arylamidase	+	+	+	+	+	–
Esterase lipase (C8), lipase	+	+	+	–	+	–
Trypsin	–	+	+	–	+	+
α-Chymotrypsin	–	+	+	–	+	ND
α-Glucosidase, β-glucosidase	+	–	+	–	–	–
Polar lipids†	PE, 2AL	PE, 2AL, 2GL	PE, 4AL, GL	PE, 3AL, 4GL	GL, PE, 3AL	GL, PE, 4AL
DNA G+C content (mol%)‡	40.7	39.5	40.9	38.5	35.5	35.7

*These analyses were conducted in this study except for *N. tegetincola* and *N. sediminis*^{T}.
†AL, unidentified aminolipid; GL, unidentified glycolipid; PE, phosphatidylethanolamine.
‡The DNA G+C contents were calculated based on their genome sequences in this study.

microbial cells were saponified, methylated and extracted using the standard MIDI protocol. Fatty acid methyl esters were analysed by a gas chromatography (Hewlett Packard 6890) and identified by using the RTSBA6 database of the Microbial Identification System (Sherlock ver. 6.0B) [32]. Isoprenoid quinones were extracted, according to the method of Minnikin *et al.* [33] and analysed using a model LC-20A HPLC system (Shimadzu) equipped with a diode array detector (SPD-M20A; Shimadzu) and a reversed-phase column (250×4.6 mm, Kromasil; Akzo Nobel).

Phosphatidylethanolamine, two unidentified aminolipids and four unidentified lipids were detected as major polar lipids (Fig. S4), which had some differences with other closely related members of the genus *Nonlabens*. For instance, one of the reference strains, *N. antarcticus* JCM 14068^T, contained phosphatidylethanolamine, three unidentified aminolipids, four unidentified glycolipids and an unidentified lipid, but no glycolipid was identified from strain MJ115^T. Iso-$C_{15:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:1}$ *ω9c*, $C_{17:0}$ 2-OH, iso-$C_{16:0}$ 3-OH, iso-$C_{17:0}$ 3-OH and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) were identified from strain MJ115^T as major cellular fatty acids (>5% of the total fatty acids). The overall fatty acid profile of strain MJ115^T was similar to those of the closely related reference strains and type species of genus *Nonlabens*, but there were some differences in some components

Table 4. Comparison of cellular fatty acid compositions (%) of strain MJ115^T, and the most closely related *Nonlabens* type strains

Taxa: 1, strain MJ115^T; 2, *N. marinus* KCTC 23432^T; 3. *N. agnitus* KACC 14155^T; 4, *N. antarcticus* JCM 14068^T. All data were from this study. Data are expressed as percentages of the total fatty acids and fatty acids amounting to less than 0.5% in all strains are not shown. Major components (>5.0%) are highlighted in bold; TR, trace amount (<1.0 %); –, not detected.

Fatty acid	1	2	3	4
Saturated:				
$C_{15:0}$	1.1	2.2	3.9	2.7
$C_{16:0}$	–	–	1.5	–
Saturated branched-chain:				
iso-$C_{14:0}$	1.3	–	1.0	1.2
iso-$C_{15:0}$	**16.1**	**20.3**	**26.1**	**5.2**
anteiso-$C_{15:0}$	**19.2**	**14.4**	**6.4**	**28.7**
iso-$C_{16:0}$	3.8	3.9	**7.0**	**8.5**
iso-$C_{17:0}$	TR	TR	1.3	–
iso-$C_{16:1}$ h	TR	1.4	0.5	TR
Unsaturated branched-chain:				
anteiso-$C_{17:1}$ $\omega 9c$	**5.1**	**5.1**	–	**7.1**
$C_{17:1}$ $\omega 6c$	–	1.1	–	1.1
Hydroxy:				
$C_{15:0}$ 2-OH	2.9	2.0	1.5	3.5
$C_{16:0}$ 3-OH	TR	TR	TR	1.2
$C_{17:0}$ 2-OH	**14.7**	**8.5**	4.8	**9.7**
iso-$C_{15:0}$ 3-OH	3.1	3.4	4.3	TR
iso-$C_{16:0}$ 3-OH	**6.9**	4.6	4.9	**8.2**
iso-$C_{17:0}$ 3-OH	**11.2**	**13.9**	**20.0**	2.5
Summed feature*:				
3	**7.3**	**6.8**	4.5	**8.6**
9	3.0	**8.0**	**7.4**	2.0

*Summed features represent groups of two or more than two fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3, $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; summed feature 9, iso-$C_{17:1}$ $\omega 9$c and/or 10-methyl $C_{16:0}$.

(Table 4). For example, iso-$C_{14:0}$ was identified from strain MJ115^T, but it was not detected from *N. marinus* and anteiso-$C_{17:1}$ $\omega 9c$ was detected abundantly except for *N. agnitus*, but it was not detected from *N. agnitus*. The only respiratory quinone identified in strain MJ115^T was MK-6, which was in agreement with those of other species of the genus *Nonlabens*. In conclusion, phylogenetic, physiological and chemotaxonomic features support that strain MJ115^T represents a novel species of the genus *Nonlabens*, for which the name *Nonlabens ponticola* sp. nov. is proposed.

RECLASSIFICATION OF *NONLABENS SEDIMINIS* AS A LATER HETEROTYPIC SYNONYM OF *NONLABENS TEGETINCOLA*

The 16S rRNA gene sequence-based NJ, ML and MP trees and genome-based phylogenomic tree clearly showed that two type strains proposed differently as *N. tegetincola* and *N. sediminis* [1, 3] formed a tight phylogenetic lineage with 100% bootstrap value (Figs 1, S1 and S2). The 16S rRNA gene sequence similarity between the type strains was 99.6%. The ANI and *in silico* DDH values between the genomes of *N. tegetincola* JCM 12886^T (MQVV00000000) and *N. sediminis* NBRC 100970^T (CP019342) were 97.5 and 79.0%, respectively, which were clearly higher than the thresholds for prokaryotic species delineation (Table 2) [25, 26]. In addition, the comparison of phenotypyic properties showd the two species had almost identical phenotypic charateristics, including hydrolysis and enzyme activities, polar lipids, and G+C contents (Tables 3 and S1). The results suggested that *N. tegetincola* and *N. sediminis* belong to the same species and thus in this study it is proposed that *N. sediminis* is reclassified as a later heterotypic synonym of *N. tegetincola*.

DESCRIPTION OF *NONLABENS PONTICOLA* SP. NOV.

Nonlabens ponticola [pon.ti'co.la. L. masc. n. *pontus* the sea; L. masc. or fem. n. *incola* a dweller, inhabitant; N.L. masc. or fem. n. *ponticola* a dweller of sea].

Cells are Gram-stain-negative, orange-pigmented, strictly aerobic and non-motile rods. Gliding motility is negative. Catalase and oxidase reactions are positive. Growth occurs at 4–35 °C (optimum, 30 °C) and pH 6.0–7.0 (optimum, pH 6.5) and in the presence of 0–8.0% NaCl (optimum, 2.0 %). Does not produce indole and flexirubin-type pigments. Hydrolyses casein, aesculin, starch and urea, but not Tween 20, Tween 80, tyrosine and gelatin. Nitrate is not reduced to nitrite. In API ZYM, it is positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase and urease activities and negative for trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. In API 20NE, assimilation of D-glucose and potassium gluconate is positive, but assimilation of L-arabinose, D-mannose, D-mannitol, maltose, *N*-acetyl-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate and phenyl acetate is negative. In API 50CH, acid is produced from D-glucose, aesculin, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate, but acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-β-D-xylopyranoside,

D-galactose, D-fructose. D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentibiose, turanose, D-lyxose, D-tagatose, D-fuctose, D-arabitol and L-arabitol. Phosphatidylethanolamine, two unidentified aminolipids and four unidentified lipids are detected as major polar lipids. Major cellular fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:1}$ $\omega9c$, $C_{17:0}$ 2-OH, iso-$C_{16:0}$ 3-OH, iso-$C_{17:0}$ 3-OH and summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$). Only MK-6 is detected as the respiratory quinone. The type strain is MJ115^T (=KCTC 72237^T=NBRC 113963^T), isolated from seawater sample obtained from South Korea. The DNA G+C content of the type strain is 40.7 mol% (genome). The GenBank accession numbers of the 16S rRNA gene and genome sequences of strain MJ115^T are MH712297 and CP034549, respectively.

EMENDED DESCRIPTION OF *NONLABENS TEGETINCOLA* LAU *ET AL*. 2005

Basonym: *Sandarakinotalea sediminis* Khan *et al*. 2006.

Heterotypic synonym: *Nonlabens sedinimis* Yi and Chun 2012.

The species description is as given for *Sandarakinotalea sediminis* [3] and *Nonlabens sedinimis* [5] with the following modifications. The DNA G+C content of the type strain is 35.5 mol% (genome). The type strains is UST030701-324^T (=NRRL B-41136^T=JCM 12886^T). The GenBank accession numbers of the 16S rRNA gene and genome sequences are AY987349 and MQVV00000000, respectively.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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