INTERNATIONAL JOURNAL OF
SYSTEMATIC AND EVOLUTIONARY
MICROBIOLOGY

TAXONOMIC DESCRIPTION
He *et al.*, *Int. J. Syst. Evol. Microbiol.*
DOI 10.1099/ijsem.0.005998

MICROBIOLOGY SOCIETY

Paracoccus albicereus sp. nov., isolated from the shallow-sea hydrothermal system off Kueishantao Island

Yajie He, Dan Lin, Beihan Chen, Xinzhu Liu, Jianing Ye, Jia Sun and Kai Tang*

Abstract

Strain designated TK19116^T was isolated from the shallow-sea hydrothermal systems off Kueishantao Island in Taiwan, China. The bacterium was Gram-stain-negative, aerobic, oxidase-positive and catalase-positive. Cells of the strain TK19116^T were short-rod-shaped and non-motile. The results of phylogenetic analysis of 16S rRNA gene sequences indicated that strain TK19116^T belonged to the genus *Paracoccus*, with the highest sequence similarity to *Paracoccus alkanivorans* 4-2^T (97.1%). The average nucleotide identity values between the strain TK19116^T with *Paracoccus alkanivorans* 4-2^T, *Paracoccus zhejiangensis* J6^T, *Paracoccus siganidrum* M26^T and *Paracoccus tegillarcae* BM15^T were 75.3, 76.7, 76.7 and 75.8%, respectively. The digital DNA–DNA hybridization value between the strain TK19116^T with *Paracoccus alkanivorans* 4-2^T, *Paracoccus zhejiangensis* J6^T, *Paracoccus siganidrum* M26^T and *Paracoccus tegillarcae* BM15^T were 19.7, 20.3, 20.5 and 20.0%, respectively. The main respiratory quinone of strain TK19116^T was ubiquinone 10. The polar lipids include aminolipid, phosphatidylcholine, diphosphatidylglycerol, glycolipid, phosphatidylglycerol and phospholipid. The principal fatty acid of strain TK19116^T was summed feature 8 ($C_{18:1}\,\omega 6c$ and/or $C_{18:1}\,\omega 7c$). The G+C content of the chromosomal DNA was 64.2%. The combination of the results of the phylogenetic, phenotypic and chemotaxonomic analysis, strain TK19116^T represents a novel species of the genus *Paracoccus*, for which the name *Paracoccus albicereus* sp. nov. is proposed. The type strain is TK19116^T (= MCCC 1K08025^T=JCM 35527^T).

INTRODUCTION

The genus *Paracoccus* was first proposed by Davis *et al.* in 1969 [1], and is a clade of *Rhodobacteraceae* within the class *Alphaproteobacteria*. At the time of writing, the genus *Paracoccus* has contained 78 recognized species with validly published names (search time: 25 February 2023, http://www.bacterio.net/paracoccus.html) [2]. Species of genus *Paracoccus* were isolated from various environments, including freshwater [3], seawater [4], air [5], soil [6], tidal flat sediment [7], activated sludge [8], sediment [9], plant [10] and Tibetan antelope [11]. The members of *Paracoccus* share some common features, including having ubiquinone 10 as the dominant isoprenoid quinone, $C_{18:1}\,\omega 7c$ as the major fatty acid [12], and DNA G+C content between 64–70% [13]. It has been reported that some aerobic denitrifiers strains in the genus *Paracoccus* can be applicated in aquaculture, waste water processing and bio-degradation of organic pollutants [14, 15]. In this study we used a polyphasic taxonomic approach to clarify the taxonomic position of strain TK19116^T, and the results showed that it represents a novel species of the genus *Paracoccus*.

ISOLATION AND ECOLOGY

The seawater sample was collected from the intermediate seawater of yellow vent in the shallow-sea hydrothermal system located near Kueishantao Island (24.83560° N 121.96339° E), Taiwan, China in June 2019. The *in situ* temperature was 25.9 °C, the salinity was 34.4‰ and pH was 6.3. Seawater sample supplemented with 15% (v/v) glycerol was preserved in 4 °C before laboratory experiment. The seawater sample was diluted ten times and spread on marine agar 2216 (Haibo). After 6 days aerobic incubation at 28 °C, agar plate formed diverse colonies. A light yellow-pigmented colonies named TK19116^T showed relatively low 16S rRNA

Author affiliations: [1]State Key Laboratory of Marine Environmental Science, College of Ocean and Earth Science, Fujian Key Laboratory of Marine Carbon Sequestration, Xiamen University, Xiamen, PR China.
***Correspondence:** Kai Tang, tangkai@xmu.edu.cn
Keywords: *Paracoccus albicereus*; shallow-sea hydrothermal system; polyphasic taxonomy.
The GenBank accession number for the 16S rRNA gene sequence and the draft genome sequence of strain TK19116^T are OM838396 and JAKZEU000000000 respectively.
Three supplementary figures and two supplementary tables are available with the online version of this article.

005998 © 2023 The Authors

gene sequence similarity to other species. Subsequently strain TK19116^T was purified and cultivated in marine broth 2216 (Haibo) at 28 °C for 2 days. The culture was preserved at −80 °C with 15% (v/v) glycerol for long-term preservation.

16S rRNA PHYLOGENY

The 16S rRNA gene of strain TK19116^T was amplified by PCR using the universal primers 27F and 1492R [16]. PCR amplification programme was executed under the following conditions: 94 °C for 5 min; 30 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 90 s, followed by an elongation at 72 °C for 10 min. The PCR product was purified by PAGE and Sanger-sequenced. The 16S rRNA gene sequence of strain TK19116^T was compared with sequences available in the EzBioCloud database [17] and NCBI database to determine its closest matches. Phylogenetic trees were reconstructed by the neighbour-joining algorithms with the model Kimura two-parameter [18] and maximum-likelihood algorithms with the model K2 +G+I [19] in the MEGA version 7.0 with a bootstrap analysis of 1000 replications [20].

The PCR-amplified 16S rRNA gene sequence was 1458 bp and the extracted complete sequence was 1461 bp. Strain TK19116^T exhibited highest 16S rRNA gene sequence similarity of 97.1% with *Paracoccus alkanivorans* 4-2^T [21], 97.0% with *Paracoccus siganidrum* M26^T [22], 97.0% with *Paracoccus stylophorae* KTW-16^T [23] and less than 97% similarity to all other type strains of the genus *Paracoccus*. Phylogenetic analysis was based on neighbour-joining and maximum-likelihood algorithms. In the phylogenetic trees, strain TK19116^T formed a distinct branch within the genus *Paracoccus* (Figs 1 and S1, available in the online version of this article). *Paracoccus zhejiangensis* J6^T (=CCTCC AB2012031^T), *Paracoccus siganidrum* M26^T (=CCTCC AB2012865^T) and *Paracoccus tegillarcae* BM15^T (=KCTC 72032^T) obtained from China Centre for Type Culture Collection and Korean Collection for Type Cultures, are used as reference strains in the following taxonomic study of strain TK19116^T.

GENOME FEATURES

Genomic DNA of strain TK19116^T was extracted using a GENEray Bacteria DNA kit (Generay) according to the manufacturer's instructions. Genome sequencing of strain TK19116^T was carried out on an Illumina HiSeq Nova platform (Illumina, USA). The raw data was cleaned by using the quality statistics software cutadapt (v1.9.1). The clean reads were assembled using velvet, gapfilled with SSPACE and GapFiller [24–28]. The chromosomal DNA G+C content of strain TK19116^T was calculated according to the draft genome sequence. Under a Linux virtual machine, the up-to-date bacterial core gene (UBCG) phylogenetic analysis based on 92 core genes were run. The executable files and manuals can be found in https://www.ezbiocloud.net/tools/ubcg [29]. We extracted 92 UBCGs for multiple alignment and concatenated them. Then we used RAxML [30] and FastTree [31] for the phylogenetic tree analysis with GTR+CAT parameters and 1000 bootstrap replicates on the basis of 35 genomes. The average nucleotide identity (ANI) values were calculated by using the updated online ANI calculator in Ezbiocloud (https://www.ezbiocloud.net/tools/ani) [32]. The digital DNA–DNA hybridization (dDDH) estimates were calculated using the Genome-to-Genome Distance Calculator (GGDC 3.0) online service with the recommended formula two (https://ggdc.dsmz.de/distcalc2.php) [33]. Strains assembled genome sequences for UBCG phylogenic tree analysis, ANI analysis and dDDH estimate were available in NCBI (https://www.ncbi.nlm.nih.gov/home/download/).

The draft genome sequence of strain TK19116^T resulted in 20 scaffolds. Scaffolds varied in length from 1009 to 1098392 bp (N50=536683) and the sequencing depth of coverage was 1361×. The accession number for the whole genome sequence of strain TK19116^T is JAKZEU000000000. The DNA G+C content of strain TK19116^T calculated from the draft genome was 64.2%. The assembled genome size was 3660938 bp. The genome contains a total of 3525 protein coding genes, three rRNA genes, 42 tRNA genes and 21 other non-coding RNA genes. A total of 2859 genes (81.1%) were annotated into COG database. Approximately 21 COG functions were identified in strain TK19116^T. The top three abundant COG function genes were category R (general function prediction only), category E (amino acid transport and metabolism) and category S (function unknown), and these three categories percentages of the genome were 11.0, 9.8 and 9.7%, respectively. The draft genome of strain TK19116^T was used as a query to search against similar sequences in *Paracoccus denitrificans* and other strains' genomes found in the KEGG (Kyoto Encyclopaedia of Genes and Genomes) database with the BBH (bi-directional best hit) method. According to the results of the annotation in the KEGG, a series of genes for the nicotinate and nicotinamide metabolism was found and it illustrated that the strain TK19116^T may have the ability of nicotine degradation. UBCG analysis indicated that strain TK19116^T formed a novel linage compared to close relatives within the genus *Paracoccus* (Fig. 2). The details of the strains used for constructing the UBCG evolutionary tree was listed in the Table S2. The genomic relatedness between strain TK19116^T and four closely related strains was estimated by ANI and dDDH. ANI values of strain TK19116^T compared with *Paracoccus zhejiangensis* J6^T, *Paracoccus siganidrum* M26^T and *Paracoccus tegillarcae* BM15^T were 76.7, 76.7 and 75.8% respectively, clearly below the suggested ANI value (95–96%) for demarcating genomic species [34]. The estimated digital DDH values between strain TK19116^T and its closely related species (*Paracoccus zhejiangensis* J6^T, *Paracoccus siganidrum* M26^T and *Paracoccus tegillarcae* BM15^T) were 20.3, 20.5 and 20.0%, respectively. This was far below the standard cut-off value (70%) [35], which confirmed that strain TK19116^T represents a novel species of the genus *Paracoccus*. G+C content of strain TK19116^T were similar with the other strains in genus *Paracoccus*.

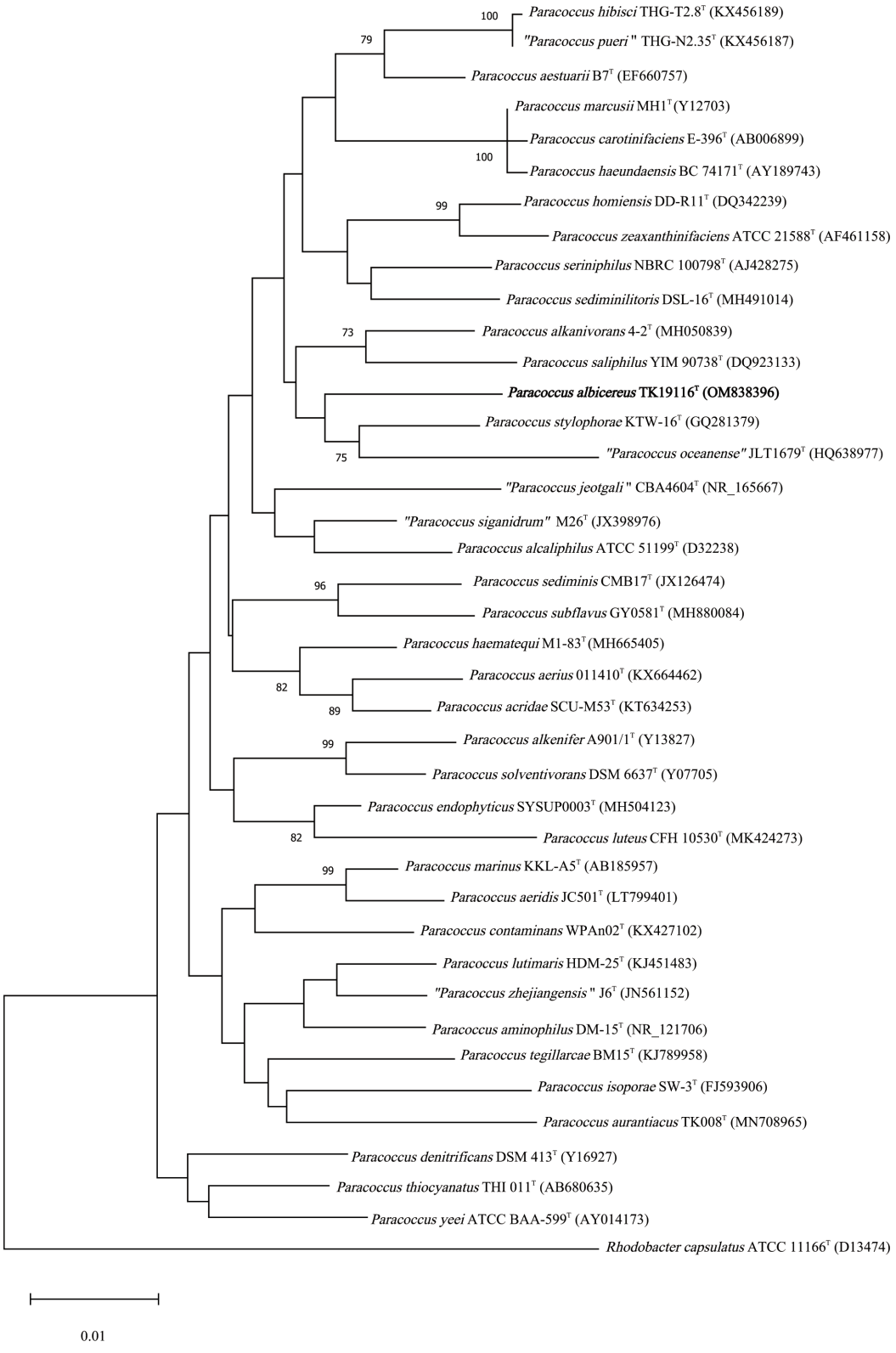


Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain TK19116^{T} among species of the Paracoccus. Rhodobacter capsulatus ATCC 11166^{T} was used as an outlier. Numbers at branch points indicate bootstrap percentages (based on 1000 replicates); only values >70% are indicated. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the neighbour-joining and maximum-likelihood algorithms. Bar, 0.01 substitutions per nucleotide position.

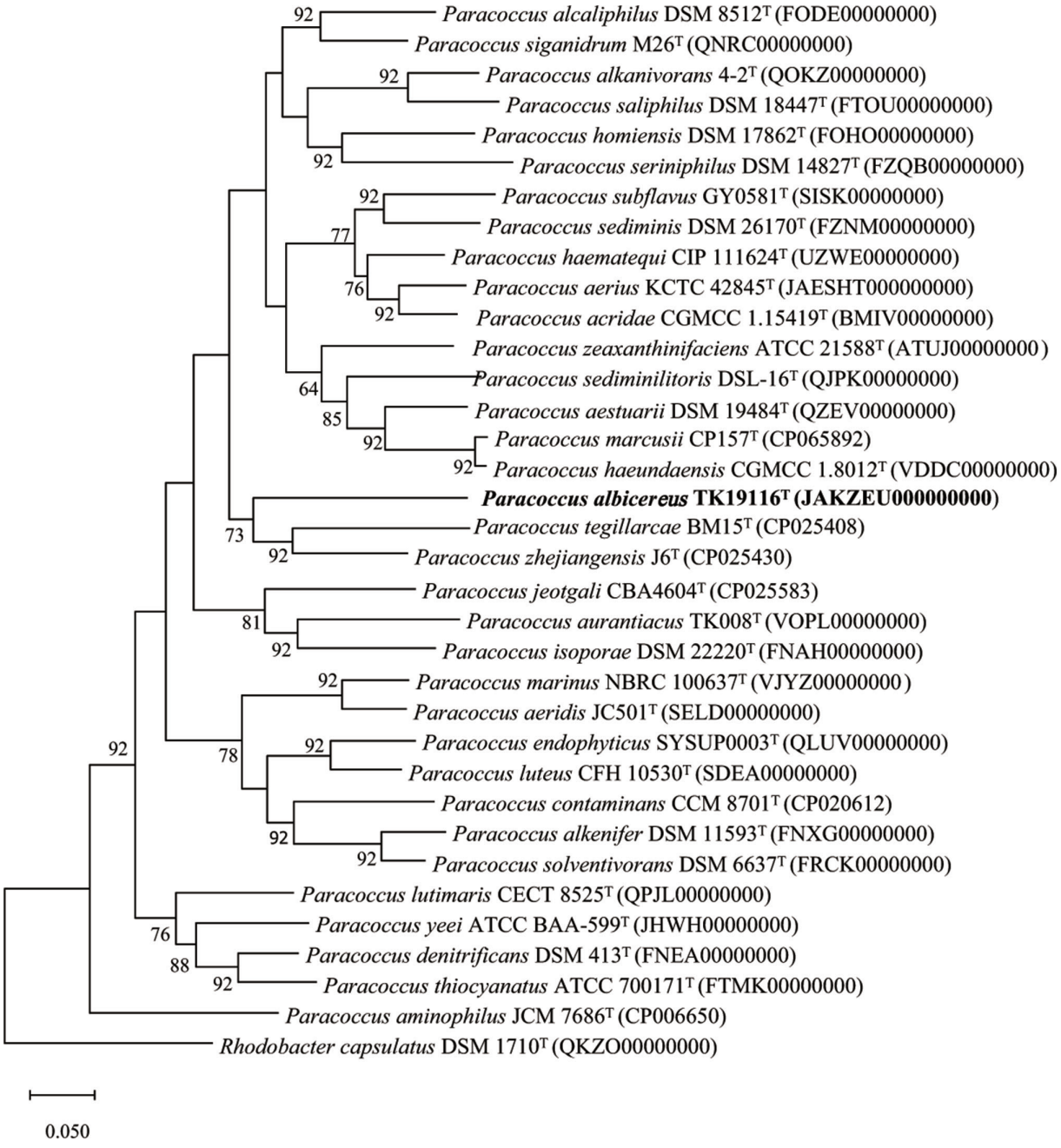


Fig. 2. RAxML-based UBCG phylogenomic tree of strain TK19116^T and related type strains. Genome of *Rhodobacter capsulatus* was used as an out group. Values lower than 70% are hidden. Shown in parentheses are GenBank accession numbers of whole genomes. Bar, 0.050 nucleotide substitution per position.

PHYSIOLOGY AND CHEMOTAXONOMY

Gram-reaction was performed using a Gram-stain kit (Haibo) according to the manufacturer's instructions. Cellular morphology and size were studied by transmission electron microscopy (Tecnai Spirit G2; FEI). Motility and growth under anaerobic conditions were determined by the semi-solid agar puncture method. Cells used for tests were all at exponential and stationary phases of growth. NaCl requirement and tolerance were tested in in rich organic (RO) medium at 28 °C for 2 days with NaCl concentrations ranging from 0–15% (intervals of 1%). The range of temperature (4, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C) for growth was tested in 2216E liquid medium (Hopebio, China). Growth at different pH was evaluated RO medium [36] (intervals of 1.0 pH unit, pH 3.0–12.0, Na_2HPO_4/citric acid for pH 3.0–8.0, glycine/NaOH for pH 9.0–10.0, Na_2HPO_4/NaOH for pH 11.0–12.0) at 28 °C for 2 days. To achieve sterility, the medium for pH range testing of strain TK19116^T was filtered through 0.22 µm pore-size

Table 1. Differential characteristics between strain TK19116^T and the type strains of its closest phylogenetic relatives. Strains: 1, Strain TK19116^T; 2, *Paracoccus siganidrum* M26^T; 3, *Paracoccus tegillarcae* BM15^T; 4, *Paracoccus zhejiangensis* J6^T

Characteristic	1	2	3	4
Colony colour	pale yellow	white or pale yellow[a]	pale yellow†	pale yellow‡
Source of isolation	seawater	fish gastrointestinal tract[a]	gastrointestinal tract of a blood cockle†	wastewater treatment system‡
Temperature range (°C)	4–40	20-40*	10-30†	10-37‡
NaCl range % (w/v)	0–11	0-10*	0-8†	0-5‡
pH range	5–8	5-10*	5-9†	6-8‡
API ZYM				
cystine arylamidase	+	−	+	−
α-galactosidase	−	−	−	+
N-acetyl-β-glucosaminidase	−	−	−	+
API 20NE				
D-glucose fermentation	−	+	+	+
urease	+	−	−	−
hydrolysis of gelatin	−	−	−	+
4-nitrophenyl-β-D-galactopyranoside	−	−	+	−
glucose	−	+	−	−
arabinose	−	+	−	−
mannose	−	+	−	−
mannitol	−	+	−	−
N-acetylglucosamine	−	+	−	−
maltose	−	+	−	−
gluconate	−	+	−	−
malte	−	+	−	−
phenylacetate	−	+	−	−
Major polar lipids	AL, PC, PG, DPG, GL1-2, PL1-3	PC, PG, AL, GL1-2, PL1-2*	PC, PE, PG, GL, L1-2†	PE, PG, DPG, PC, L1-3, PL1-2‡

*Data taken from Ying *et al.* [22]
†Data taken from Jung *et al.* [43]
‡Data taken from Zhi *et al.* [44]

polycarbonate filters (Millipore). The utilization of carbohydrates or amino acids as sole carbon and energy sources was assessed by using Biolog GEN III microplates with cells suspended in IF-A (Biolog). In the following experiments, *Paracoccus zhejiangensis* J6^T, *Paracoccus siganidrum* M26^T and *Paracoccus tegillarcae* BM15^T were tested under the same conditions in parallel as reference strains. Activities of catalase and oxidase were tested according to the methods described of Smibert and Krieg [37]. Other biochemical and enzymatic characteristics of strain TK19116^T were determined in API ZYM and API 20NE (bioMérieux) systems according to the manufacturer's instructions.

Detailed phenotypic properties of strain TK19116^T were shown in Table 1. Cells were about 0.5–0.8 µm wide and 1.1–2.0 µm long under transmission electron microscope observation (Fig. S2). After incubation for 2 days, cells formed light yellow and convex circular colonies. Cells of strain TK19116^T were Gram-stain-negative, strictly aerobic and non-motile. Strain TK19116^T grew well at 4–40 °C (optimum, 30 °C) and pH 5.0–8.0 (optimum, 6.0). The optimum growth occurred at 2% (w/v) NaCl with the tolerance of 0–11.0%. In Biolog GEN III Microplate system, the following substrates were utilized as carbon sources: gelatin, fructose, arabitol, L-alanine, L-galactonic acid lactone, L-arginine, L-lactic acid, β-hydroxy-D, L-butyric acid, L-aspartic acid, citric acid,

glucose-6-phosphate, L-glutamic acid, acetoacetic acid, L-histidine, malic acid, L-malic acid, serine, L-serine, bromo-succinic acid, formic acid; all other substrates included cannot be utilized. In assays with API ZYM strips, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *α*-glucosidase were present, but lipase, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *β*-glucosidase, *α*-mannosidase, *α*-fucosidase and N-acetyl-*β*-glucosaminidase were absent. Cells were positive for nitrate reduction, urease, hydrolysis of aesculin, but negative for indole production, D-glucose fermentation, arginine dihydrolase, hydrolysis of gelatin, and 4-nitrophenyl-*β*-D-galactopyranoside and assimilation of glucose, arabinose, mannose, mannitol, N-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate in API 20NE test strips (Table 1).

For fatty acid analysis, the biomass of strain TK19116^T and the three closely reference strains was harvested after culturing in marine agar for 48 h at 28 °C. Then they were saponified, extracted and methylated based on the standard midi protocol (Sherlock Microbial Identification System, version 6.0 and the library was TSBA6 6.00) [38]. Isoprenoid quinones were extracted from freeze-dried cells material, then separated into their different classes by thin-layer chromatography (TLC) on silica gel [39, 40], and analysed by the high performance liquid chromatography (HPLC). Subsequently, the molecular weight was measured by using mass spectrometry to identify the isoprenoid quinone. Polar lipids were extracted as described by Kates [41] using a $C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c* chloroform/methanol system and analysed using two-dimensional TLC. Polar lipids were detected by spraying with four reagents including molybdatophosphoric acid (for total lipids), phosphomolybdic acid (for phospholipids), ninhydrin (for aminolipids) and naphthol–sulfuric acid reagent (for glycolipids) [42].

The major fatty acid (>10%) of strain TK19116^T was summed feature 8 ($C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c* (Table S1), similar to the genus *Paracoccus*. In terms of the polar lipid composition, phosphatidylcholine and phosphatidylglycerol were detected in strain TK19116^T, *Paracoccus siganidrum* M26^T, *Paracoccus tegillarcae* BM15^T and *Paracoccus zhejiangensis* J6^T. Polar lipids of strain TK19116^T are composed of aminolipid (AL), phosphatidylcholine (PC), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), glycolipid (GL1-2) and phospholipid (PL1-3) (Fig. S3). The main respiratory quinone detected in strain TK19116^T was ubiquinone 10 (86.4%), with minor amounts of Q-9 (6.1%), Q8 (3.9%) and Q11 (3.4%), which is similar to other species of *Paracoccus*.

TAXONOMIC CONCLUSION

The morphological, physiological and biochemical characteristics of strain TK19116^T are given in the above analysis. The results of the phylogenetic analysis and chemotaxonomic studies indicate that strain TK19116^T has some common characteristics with species of the genus of *Paracoccus,* including DNA G+C content, the major fatty acid and the major respiratory quinone. These results support the view that strain TK19116^T should be assigned to the genus *Paracoccus*. However, the strain TK19116^T has the low 16S rRNA gene sequence similarity and differences to its reference strains. These analysis results collectively indicate that strain TK19116^T represents a novel species of the genus *Paracoccus*, for which the name *Paracoccus albicereus* sp. nov. is proposed.

DESCRIPTION OF *PARACOCCUS ALBICEREUS* SP. NOV.

Paracoccus albicereus (al.bi.ce're.us. L. masc. adj. *albus*, white; L masc. adj. *cereus*, wax-coloured; N.L. masc. adj. *albicereus* pale yellow coloured).

Cells are aerobic, non-motile, non-flagellated, short-rod-shaped, about 0.5–0.8 μm wide and 1.1–2.0 μm long. Cells are Gram-stain-negative, and catalase-positive and oxidase-positive. Colonies are yellow, convex circular and smooth on marine agar after incubation for 2 days on marine agar 2216 at 28 °C. Growth occurs at 4–40 °C (optimum, 30 °C), at pH 5–8 (optimum, 6) and in the presence of 0–11.0% (w/v) NaCl (optimum, 2%). In assays with API ZYM strips, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *α*-glucosidase were present, but lipase, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *β*-glucosidase, *α*-mannosidase, *α*-fucosidase and N-acetyl-*β*-glucosaminidase were absent. Cells were positive for nitrate reduction, urease, hydrolysis of aesculin, but negative for indole production, glucose fermentation, arginine dihydrolase, hydrolysis of gelatin, and 4-nitrophenyl-*β*-D-galactopyranoside and assimilation of glucose, arabinose, mannose, mannitol, N-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate in API 20NE test strips. In Biolog GEN III tests, the following substrates are utilized as carbon sources: gelatin, fructose, arabitol, L-alanine, L-galactonic acid lactone, L-arginine, L-lactic acid, *β*-hydroxy-D, L-butyric acid, L-aspartic acid, citric acid, glucose-6-phosphate, L-glutamic acid, acetoacetic acid, L-histidine, malic acid, L-malic acid, serine, L-serine, bromo-succinic acid, formic acid; all other substrates included cannot be utilized. The major isoprenoid quinone is ubiquinone 10. The major fatty acid is summed feature 8 ($C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*). The polar lipids are AL, PC, PG, DPG, GL1-2 and PL1-3.

The type strain, TK19116^T (= MCCC 1K08025^T=JCM 35527^T), was isolated from seawater in shallow-sea hydrothermal systems of Kueishantao Island, Taiwan, China. The DNA G+C content of the type strain is 64.2%. The GenBank accession numbers for the 16S rRNA gene sequence and the draft genome sequence of strain TK19116^T are OM838396 and JAKZEU000000000, respectively.

Funding information
This study was supported by the National Natural Science Foundation of China (42188102, 42276120), and International Science Partnership Programme of the Chinese Academy of Sciences (121311KYSB20190029).

Author contributions
Y.H., B.C., X.L., J.S., and J.Y. designed and performed the experiments. Y.H., K.T. analysed the data. Y.H., K.T. and D.L. wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of interest
Authors declare that there are no conflicts of interest.

References

1. Davis DH, Doudoroff M, Stanier RY, Mandel M. Proposal to reject the genus *Hydrogenomonas*: taxonomic implications. *Int J Syst Bacteriol* 1969;19:375–390.
2. Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M. List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *Int J Syst Evol Microbiol* 2020;70:5607–5612.
3. Sheu S-Y, Hsieh T-Y, Young C-C, Chen W-M. *Paracoccus fontiphilus* sp. nov., isolated from a freshwater spring. *Int J Syst Evol Microbiol* 2018;68:2054–2060.
4. Lyu L, Zhi B, Lai Q, Shao Z, Yu Z. *Paracoccus xiamenensis* sp. nov., isolated from seawater on the Xiamen. *Int J Syst Evol Microbiol* 2020;70:4285–4290.
5. Xue H, Piao C, Guo M, Wang L, Li Y. *Paracoccus aerius* sp. nov., isolated from air. *Int J Syst Evol Microbiol* 2017;67:2586–2591.
6. Dong X, Zhang G, Xiong Q, Liu D, Wang D, *et al*. *Paracoccus salipaludis* sp. nov., isolated from saline-alkaline soil. *Int J Syst Evol Microbiol* 2018;68:3812–3817.
7. Jung Y-T, Park S, Lee J-S, Yoon J-H. *Paracoccus lutimaris* sp. nov., isolated from a tidal flat sediment. *Int J Syst Evol Microbiol* 2014;64:2763–2769.
8. Liu X-Y, Wang B-J, Jiang C-Y, Liu S-J. *Paracoccus sulfuroxidans* sp. nov., a sulfur oxidizer from activated sludge. *Int J Syst Evol Microbiol* 2006;56:2693–2695.
9. Pan J, Sun C, Zhang X-Q, Huo Y-Y, Zhu X-F, *et al*. *Paracoccus sediminis* sp. nov., isolated from Pacific Ocean marine sediment. *Int J Syst Evol Microbiol* 2014;64:2512–2516.
10. Zhang H, Li Y-Q, Xiao M, Fang B-Z, Alkhalifah DHM, *et al*. Description of *Paracoccus endophyticus* sp. nov., isolated from Gastrodia elata Blume. *Int J Syst Evol Microbiol* 2019;69:261–265.
11. Li J, Lu S, Jin D, Yang J, Lai X-H, *et al*. *Paracoccus liaowanqingii* sp. nov., isolated from Tibetan antelope (*Pantholops hodgsonii*). *Int J Syst Evol Microbiol* 2020;70:744–750.
12. Sheu S-Y, Hsieh T-Y, Young C-C, Chen W-M. *Paracoccus fontiphilus* sp. nov., isolated from a freshwater spring. *Int J Syst Evol Microbiol* 2018;68:2054–2060.
13. Chen W-M, Li Y-S, Young C-C, Sheu S-Y. *Paracoccus mangrovi* sp. nov., isolated from a mangrove. *Int J Syst Evol Microbiol* 2017;67:2689–2695.
14. Pan D, Huang Q, Chen W. Screening and identification of two heterotrophic nitrifying bacteria and characterization of their capacity for nitrogen removal. *Wei Sheng Wu Xue Bao* 2011;51:1382–1389.
15. Wang W, Cai Z, Zhong W, Wang G. Research advances in aerobic denitrifiers. *Ying Yong Sheng Tai Xue Bao* 2007;18:2618–2625.
16. Lane. 16S/23S rRNA sequencing. 1991.
17. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, *et al*. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
18. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
19. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
20. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
21. Zhang Y-X, Li X, Li F-L, Ma S-C, Zheng G-D, *et al*. *Paracoccus alkanivorans* sp. nov., isolated from a deep well with oil reservoir water. *Int J Syst Evol Microbiol* 2020;70:2312–2317.
22. Liu Y, Xie Q, Hong K, Li L, Zhao Y, *et al*. *Paracoccus siganidrum* sp. nov., isolated from fish gastrointestinal tract. *Antonie van Leeuwenhoek* 2013;103:1133–1139.
23. Sheu S-Y, Jiang S-R, Chen CA, Wang J-T, Chen W-M. *Paracoccus stylophorae* sp. nov., isolated from the reef-building coral *Stylophora pistillata*. *Int J Syst Evol Microbiol* 2011;61:2221–2226.
24. Hunt M, Newbold C, Berriman M, Otto TD. A comprehensive evaluation of assembly scaffolding tools. *Genome Biol* 2014;15:R42.
25. Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. Scaffolding pre-assembled contigs using SSPACE. *Bioinformatics* 2011;27:578–579.
26. Boetzer M, Pirovano W. Toward almost closed genomes with GapFiller. *Genome Biol* 2012;13:R56.
27. Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 2008;18:821–829.
28. Zerbino DR, McEwen GK, Margulies EH, Birney E. Pebble and rock band: heuristic resolution of repeats and scaffolding in the velvet short-read de novo assembler. *PLoS One* 2009;4:e8407.
29. Na S-I, Kim YO, Yoon S-H, Ha S-M, Baek I, *et al*. UBCG: up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. *J Microbiol* 2018;56:280–285.
30. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
31. Price MN, Dehal PS, Arkin AP. FastTree 2--approximately maximum-likelihood trees for large alignments. *PLoS One* 2010;5:e9490.
32. Yoon S-H, Ha S-M, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
33. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Res* 2022;50:D801–D807.
34. Lee I, Ouk Kim Y, Park S-C, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 2016;66:1100–1103.
35. Moore WEC, Stackebrandt E, Kandler O, Colwell RR, Krichevsky MI, *et al*. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Evol Microbiol* 1987;37:463–464.
36. Yurkov VV, Krieger S, Stackebrandt E, Beatty JT. Citromicrobium bathyomarinum, a novel aerobic bacterium isolated from deep-sea hydrothermal vent plume waters that contains photosynthetic pigment-protein complexes. *J Bacteriol* 1999;181:4517–4525.
37. Gerhardt, P.*et al*., Methods for general and molecular bacteriology. 1994.
38. Komagata K. *[Methods in Microbiology] Current Methods for Classification and Identification of Microorganisms Volume 19 || 4 Lipid and Cell-Wall Analysis in Bacterial Systematics*. 1988, pp. 161–207.
39. Tindall BJ. A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* 1990;13:128–130.

40. Tindall B. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.
41. Henry H H, Jennifer L W. Lipid extraction procedures. *Methods Mol Biol* 1986;2468.
42. Costa MSD*et al*. *The Identification of Polar Lipids in Prokaryotes - ScienceDirect*. 2011, pp. 165–181.
43. Lee J-Y, Hyun D-W, Yun J-H, Jung M-J, Shin N-R, *et al*. *Paracoccus tegillarcae* sp. nov., isolated from the gastrointestinal tract of a blood cockle (*Tegillarca granosa*). *Int J Syst Evol Microbiol* 2019;69:2815–2822.
44. Wu Z-G, Zhang D-F, Liu Y-L, Wang F, Jiang X, *et al*. *Paracoccus zhejiangensis* sp. nov., isolated from activated sludge in wastewater-treatment system. *Antonie van Leeuwenhoek* 2013;104:123–128.