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TAXONOMIC DESCRIPTION

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Marinicaulis flavus gen. nov., sp. nov., a novel stalked bacterium of the family *Parvularculaceae*

Xiao-Yun Yu,[1] Xiao-Dong Yu,[1] Ge-Yi Fu,[2] Zhe Zhao,[1] Xia Shen,[1] Cong Sun[3,*] and Min Wu[1,*]

Abstract

A Gram-stain-negative, aerobic, ovoid or short rod-shaped bacterium with prosthecates and flagella, designated SY-3-19^T, was isolated from the surface seawater of the South China Sea, and subjected to a polyphasic taxonomic study. The isolate grew at 4–40 °C and pH 5.0–9.0 (optimum 28 °C and pH 6.5–7.5), and with 0.5–16.0 % (w/v) NaCl (optimum 4 %). It was positive for oxidase and catalase activity. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain SY-3-19^T constituted a separate branch in the family *Parvularculaceae*, sharing the highest sequence similarities to the genera *Aquisalinus* (91.9 %), *Amphiplicatus* (91.1 %) and *Parvularcula* (91.0–89.4 %). The sole respiratory quinone was ubiquinone-10 and the principal fatty acids (>10 %) were summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), 11 methyl $C_{18:1}\omega7c$ and $C_{16:0}$. The polar lipids of strain SY-3-19^T consisted of phosphatidylglycerol, nine unidentified glycolipids and four unidentified lipids. The DNA G+C content was 60.9 mol%. On the basis of morphological, physiological and chemotaxonomic characteristics, together with the results of phylogenetic analysis, strain SY-3-19^T is described as a novel species in a novel genus, for which the name *Marinicaulis flavus* gen. nov., sp. nov. (type strain SY-3-19^T=MCCC 1K03432^T=KCTC 62156^T) is proposed.

The family *Parvularculaceae*, the sole family of the order *Parvularculales* within the class *Alphaproteobacteria*, was established by Cho and Giovannoni [1]. At the time of writing, this family comprises three genera (*Amphiplicatus*, *Aquisalinus* and *Parvularcula*) and six species with validly published names: *Amphiplicatus metriothermophilus* GU51^T [2], *Aquisalinus flavus* KCTC 42673^T [3], *Parvularcula bermudensis* KCTC 12087^T [1], *Parvularcula lutaonensis* KCTC 22245^T [4], *Parvularcula dongshanensis* MCCC 1A06534^T [5] and *Parvularcula flava* NH6-79^T [6] (www.bacterio.net/). Until now, all members of this family were isolated from saline environments: seawater [1, 6], saline lake [3], salty hot spring [2, 4] and soft coral [5], respectively. Members of the family *Parvularculaceae* are characterized as Gram-stain-negative, aerobic, coccoid to short rod-shaped, chemoheterotrophic, oxidase-positive, non-spore-forming and motile by means of a single polar flagellum, and additionally, containing summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) as the major fatty acid and ubiquinone-10 (Q-10) as the major respiratory quinone [3, 6]. In this study, we present a polyphasic investigation describing a novel bacterium, strain SY-3-19^T, which belongs to a novel genus and species of the family *Parvularculaceae*.

A seawater sample was collected on 22 September 2012 from the South China Sea, and used as the source for the isolation of bacterial strains. Strain SY-3-19^T was isolated on 12 January 2016 by the standard dilution plating technique at 20 °C on modified marine agar 2216 (MA; BD Difco) [7], and cultivated routinely on MA or in marine broth 2216 (MB; Difco) at 28 °C. The yellow purified strain was maintained on MA at 4 °C for short-term storage and at −80 °C with 25 % (v/v) glycerol and by lyophilization with 20 % (w/v) skimmed milk for long-term preservation. Four type strains, *Aquisalinus flavus* KCTC 42673^T, *Amphiplicatus metriothermophilus* GU51^T, *P. flava* NH6-79^T and *P. bermudensis* KCTC 12087^T were selected as references for physiological and chemotaxonomic analysis. *Aquisalinus flavus* KCTC 42673^T and *P. bermudensis* KCTC 12087^T were purchased from the KCTC (Korean Collection for Type Cultures), and the other two strains were obtained from our laboratory.

Author affiliations: [1]College of Life Sciences, Zhejiang University, Hangzhou 310058, PR China; [2]Ocean College, Zhejiang University, Zhoushan 316000, PR China; [3]College of Life Sciences, Zhejiang Sci-Tech University, Hangzhou 310018, PR China.
***Correspondence:** Cong Sun, michael_sc@sina.com; Min Wu, wumin@zju.edu.cn
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Abbreviations: GL, unidentified glycolipid; PG, phosphatidylglycerol; Q-10, ubiquinone-10.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SY-3-19^T is KY861741. The Whole Genome Shotgun project of strain SY-3-19^T has been deposited at DDBJ/ENA/GenBank under the accession PJCH00000000. The version described in this paper is version PJCH01000000.
One supplementary table and two supplementary figures are available with the online version of this article.

Cell morphology, Gram reaction, motility, temperature and pH range for growth were performed as described by Yu *et al.* [8]. Cultures incubated for 3 days were used to determine the optimal growth while those incubated for 14 days were used to determine the growth limits. A UV/visible spectrophotometer (Ultrospec 6300 Pro, Amersham Biosciences) was used to measure the $OD_{600\ nm}$. Growth at various concentrations of NaCl (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 and 6.0–20.0 %, in increments of 2.0 %, w/v) was determined in the NaCl-free MB (prepared according to the MB formula except that NaCl was added at appropriate concentrations). Anaerobic growth was examined in an anaerobic jar (MGC) with an AnaeroPack (MGC) on modified MA supplemented with sodium thiosulfate (20 mM), sodium sulfite (5 mM), sodium sulfate (20 mM), sodium nitrite (5 mM), sodium nitrate (20 mM) and L-arginine (5.0 g l^{-1}) as potential electron acceptors for 30 days. Antibiotic susceptibility was tested on MA plates with discs containing the following antibiotics (μg per disc unless stated otherwise): chloramphenicol (30); rifampicin (5); kanamycin (30); vancomycin (30); streptomycin (10); gentamycin (10); and erythromycin (15). The inhibition zones were measured from the edges of susceptibility discs to the edges of the clear zones; <2 mm, 2–5 mm and >5 mm of the inhibition zones represented resistance, weak resistance and susceptibility, respectively [9].

Oxidase activity and catalase activity were examined as described previously [8]. Tests for nitrate and nitrite reduction, hydrolysis of starch (0.2 %, w/v), gelatin (1.0 %, w/v), skim milk (2.0 %, w/v), and Tweens 20, 40, 60 and 80 (1.0 %, v/v), were performed according to Dong and Cai [10]. H_2S production was measured as described previously [6]. Hydrolysis of CM-cellulose (0.2 %, w/v) was tested as described previously [2]. Indole production, methyl red and Voges–Proskauer tests were assayed according to Zhang *et al.* [11]. Single carbon source assimilation tests were performed using GN2 MicroPlates (Biolog) according to the manufacturer's instructions and as described by Park *et al.* [12], except that cells were suspended in GN2 medium supplemented with 2.0 % (w/v) sea salts. API ZYM and API 20NE tests (bioMérieux) were used to determine additional physiological and biochemical characteristics according to the manufacturer's instructions. As a modification, cells suspended in 3.0 % (w/v) NaCl solution were used for inoculation. The results of the API 20NE and API ZYM strips were observed after 48 h and 4 h, respectively. *Aquisalinus flavus* KCTC 42673^T was used as parallel comparison under identical conditions in the above tests. Unless otherwise stated, the data of other three reference strains were obtained from our previous work [2, 6].

Cells of strain SY-3-19^T and the four reference strains used for respiratory quinone, fatty acid and polar lipid analyses were obtained from cultures incubated in MB until exponential phase and then free-dried. All strains were cultured at their optimal temperatures. Strain SY-3-19^T, *Aquisalinus flavus* KCTC 42673^T, *Amphiplicatus metriothermophilus* GU51^T, *P. flava* NH6-79^T and *P. bermudensis* KCTC 12087^T were cultured at 28, 30, 50, 37 and 30 °C, respectively. Isoprenoid quinones were extracted as described by Komagata and Suzuki [13] and analysed by liquid chromatography–mass spectrometry (Agilent) [14]. Cellular fatty acids methyl esters were extracted as described by Kuykendall *et al.* [15] and analysed according to the instructions of the Microbial Identification System (MIDI; Microbial ID). The polar lipids were extracted and separated by two-dimensional thin-layer chromatography (silica-gel plates 60, Merck 5554), and identified using standard procedures [16, 17]. Four kinds of spray reagents were used to detect the corresponding lipids, comprising molybdophosphoric acid for total lipids, molybdenum blue for phospholipids, ninhydrin reagent for lipids containing free aminolipids and *p*-anisaldehyde reagent for glycolipids.

The 16S rRNA gene of SY-3-19^T was amplified using universal primers 27F/1492R [18]. The nearly complete 16S rRNA sequence was subjected to pairwise sequence alignment by the EzTaxon-e server [19]. Phylogenetic analysis

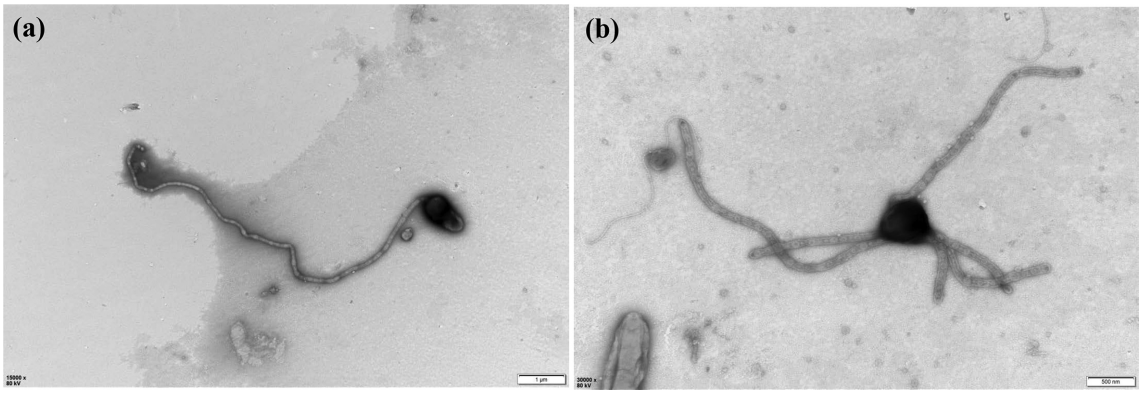

Fig. 1. Transmission electron micrographs of strain SY-3-19^T cultivated on MA for 5 days at 28 °C. (a) and (b) Cells with one and six prosthecates, respectively. Bars, 1 μm (a); and 0.5 μm (b).

was performed in ARB release 6.0.2 [20] in the All-Species Living Tree Project database (LTPs128) [21]. Based on the obtained phylogenetic resolution and the EzTaxon-e results, further trees were reconstructed by using the MEGA version 5.0 software package [22]. Sequence data were aligned with CLUSTAL_W [23]. Phylogenetic trees were reconstructed by the neighbour-joining [24], maximum-parsimony [25] and maximum-likelihood [26] methods. Evolutionary distances were calculated according to the algorithm of the Kimura two-parameter model [27] for the neighbour-joining method. All bootstrap analyses were based on 1000 replications. The genome of strain SY-3-19^T was sequenced by Solexa PE150 sequencing technology with the HiSeq platform (Novogene Technology). The sequencing generated approximate 1.43 Gb of clean data (approximately 250-fold genome coverage). The *de novo* assembly of the reads was performed using ABySS 2.0.2 [28]. The assembly *k*-value was tested from 32 to 64 to find the optimal *k*-value using abyss-pe script. The quality of microbial genomes was assessed using the bioinformatic tool CheckM [29].

Cells of strain SY-3-19^T were Gram-stain-negative, aerobic, non-spore-forming, slightly motile, and had three different

Table 1. Differential phenotypic characteristics of strain SY-3-19^T and the type strains of the reference strains

Strains: 1, SY-3-19^T; 2, *Aquisalinus flavus* KCTC 42673^T; 3, *Amphiplicatus metriothermophilus* GU51^T; 4, *Parvularcula flava* NH6-79^T; 5, *Parvularcula bermudensis* KCTC 12087^T; 6, *Hyphobacterium vulgare* WM6^T (Sun *et al.* [30]); 7, '*Aestuarium zhoushanense*' G7 (Yu *et al.* [31]). Data for taxa 1–2 were generated in this study; data for taxa 3–6, and 8 were taken from our previously work [2, 6, 30, 31]. +, Positive; –, negative; W, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Isolation source	Sea water	Saline lake water*	Hot spring water	Sea water	Sea water	Sea water	Tidal flat
Colony colour	Yellow	Yellow	Light yellow	Yellow	Yellowish-brown	Whitish yellow	Greyish yellow
Prosthecae	+	–*	+	–	–	+	–
Catalase activity	+	+	+	+	–	+	+
Temperature range (optimum) (°C)	4–40 (28)	10–35 (30)*	37–65 (48–50)	4–42 (37)	10–37 (30)	10–45 (30)	4–40 (28)
pH range (optimum)	5.0–9.0 (6.5–7.5)	6.0–10.5 (8.0)*	6.0–9.0 (7.5)	6.0–8.5 (7.0)	6.0–9.0 (8.0)	6.5–9.0 (7.5–8.5)	6.0–9.0 (7.5)
NaCl tolerance (optimum) (%, w/v)	0.5–16.0 (4.0)	0–14.0 (2.0)*	0.5–7.5 (2.0–3.0)	0.5-11(4.5)	0.75–25 (3.0)	1.0–6.0 (1.0–2.0)	0–7.0 (1.0)
H_2S production	+	–	+	+	–	–	ND
Nitrate reduction	+	–	+	–	+	–	–
Voges–Proskauer	–	+	ND	+	–	ND	ND
Hydrolysis of:							
Gelatin	–	+	+	+	+	+	+
Starch	–	W	–	–	–	–	–
Skim milk	–	–	–	–	+	–	–
Tween 20	+	–	+	+	–	+	–
Tween 40	+	–	–	+	–	–	+
Tween 60	+	+	–	+	–	–	+
Tween 80	+	+	–	+	–	–	–
Major fatty acid(s) (>10 %)	$C_{16:0}$, summed feature 8 ($C_{18:1}$ $\omega7c$/$C_{18:1}\omega6c$), summed feature 3 ($C_{16:1}\omega7c$/$C_{16:1}\omega6c$), 11 methyl $C_{18:1}\omega7c$	$C_{16:0}$, summed feature 8 ($C_{18:1}$ $\omega7c$/$C_{18:1}\omega6c$), $C_{19:0}$ Cyclo $\omega8c$	$C_{16:0}$, summed feature 8 ($C_{18:1}\omega7c$/$C_{18:1}\omega6c$)	$C_{16:0}$, summed feature 8 ($C_{18:1}\omega7c$/$C_{18:1}\omega6c$)	Summed feature 8 ($C_{18:1}\omega7c$/$C_{18:1}\omega6c$)	$C_{18:1}$ $\omega7c$	$C_{16:0}$, $C_{18:1}\omega7c$, summed feature 3 ($C_{16:1}\omega7c$/$C_{16:1}\omega6c$)
Major quinone(s)	Q-10	Q-10, Q-11*	Q-10	Q-10	Q-10	Q-10	Q-10
Major polar lipids	PG, 3 GLs	3GLs	DPG, PG, 2 GLs	DPG, 3 GLs, PGL	PG, 3 GLs, PGL	PG, GUDG, MGDG, SQDG.	PG, PE, PC
DNA G+C content (mol%)	60.9 (by genome)	59*	66.7	60.7	60.8	59.8	56.7

*Data from Zhong *et al.* [3].

†DPG, diphosphatidylglycerol; GL, unidentified glycolipid; GUDG, glucuronopyranosyldiglyceride; MGDG, monoglycosyldiglyceride; PC, phosphatidylcholine; PG, phosphatidylglycerol; PGL, unidentified phosphoglycolipid; PE, phosphatidylethanolamine; SQDG, sulfo-quinovosyl diacylglycerol.

morphology with one to three flagella including: (1) coccoid-shaped (0.5–1.0 µm in diameter) or ovoid-shaped with one to six prosthecates; (2) short rod- or rod-shaped (0.3–0.7 µm wide and 0.5–0.8 µm long) with no to one prosthecate; and (3) spiral-shaped or curved rod-shaped (2.0–5.0 µm long) with no prosthecate (Fig. 1). Colonies were 0.5–1.0 mm in diameter, yellow, slightly dry, convex and circular with entire margins after incubation on MA at 28 °C for 5 days. Cells were flocculated in liquid medium. Under the optimal growth conditions, cells reached the logarithmic phase in MB at the third day and on MA at the fifth day. The temperature range for growth was 4–40 °C (optimum, 28 °C). Growth occurred at pH 5.0–9.0 (optimum, pH 6.5–7.5) and with 0.5–16 % (w/v) NaCl (optimum, 4.0 %). No growth was observed without NaCl. Strain SY-3-19^T was positive for catalase, oxidase, nitrate reduction, H_2S production and hydrolysis of Tweens 20, 40, 60 and 80. The isolate was susceptible to chloramphenicol, rifampicin, kanamycin, vancomycin, streptomycin and gentamycin, but was resistant to erythromycin. Detailed physiological and biochemical characteristics are given in the species description, Tables 1 and S1 (available in the online version of this article).

The nearly complete 16S rRNA gene sequence (1424 nt) of strain SY-3-19^T was obtained. On the basis of 16S rRNA gene sequence alignment, strain SY-3-19^T exhibited the highest similarities to the genera *Aquisalinus* (91.9 %), *Amphiplicatus* (91.1 %) and *Parvularcula* (91.0–89.4 %). The All-Species Living Tree indicated that strain SY-3-19^T formed a clade in the family *Parvularculaceae* (Fig. S1). The phylogenetic trees reconstructed by using the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms showed that strain SY-3-19^T constituted an independent lineage within the family *Parvularculaceae*, and formed a robust cluster with *Amphiplicatus metriothermophilus* GU51^T (Fig. 2). The genome completeness of strain SY-3-19^T was 98.3 with 1.0 % contamination. Genome sequences estimated to be of ≥95 % completeness, and with ≤5 % contamination, were considered as excellent reference genomes for deeper analyses [29]. The genome sequences of strain SY-3-19^T was constituted of 17 contigs. The numbers of N50 and L50 values were 576 605 and 3, respectively. The DNA G+C nucleotide content of the bacterium calculated from the draft genome sequence was 60.9 %, a value similar to those of members of the genera *Aquisalinus* (59 %), *Amphiplicatus* (66.7 %) and *Parvularcula* (59.0–61.8 %) [1–6].

The chemotaxonomic data supported that strain SY-3-19^T was similar to the members within the family *Parvularculaceae*. Respiratory quinone composition analysis showed that Q-10 was the sole quinone in strain SY-3-19^T, which was in accordance with all members of the family *Parvularculaceae*

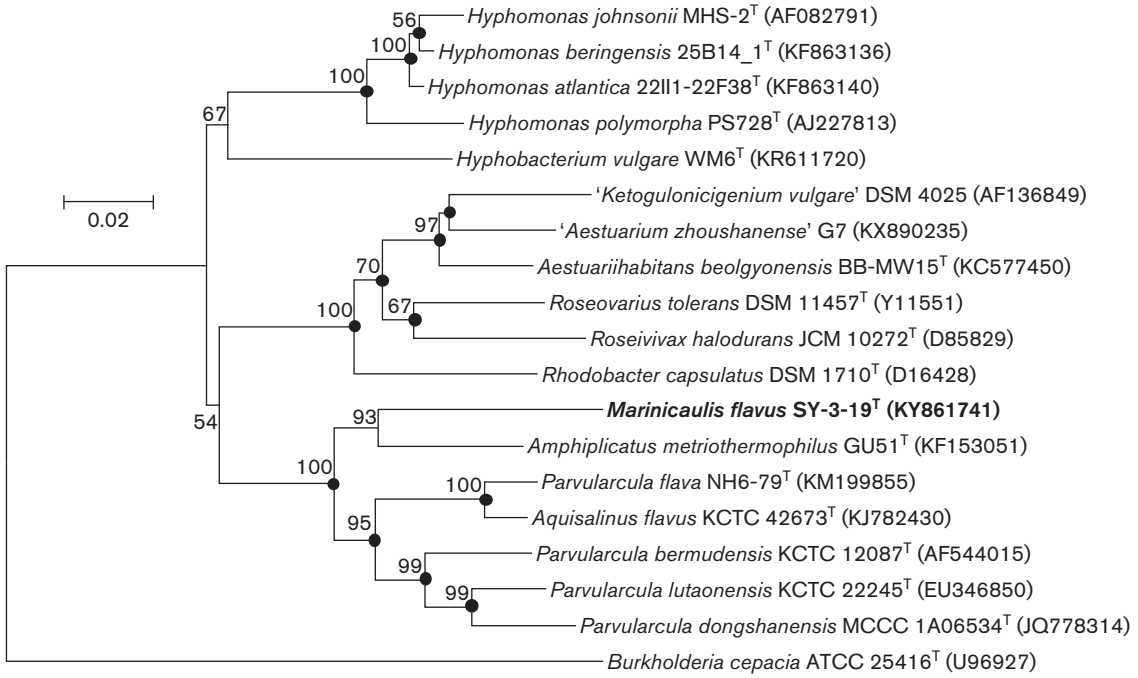

Fig. 2. Neighbour-joining tree using the Kimura two-parameter model based on the 16S rRNA gene sequences, showing the phylogenetic relationships of strain SY-3-19^T and related genera in family *Parvularculaceae*, *Hyphomonadaceae* and *Rhodobacteraceae*. Numbers at branching points represent bootstrap values (%) from 1000 replicates; only values ≥50 % are shown. Solid circles indicate that the corresponding nodes were also recovered in maximum-likelihood and maximum-parsimony trees. *Burkholderia cepacia* ATCC 25416^T (U96927) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

[3]. Fatty acid analysis of strain SY-3-19^T revealed that summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$, 41.7 %), summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, 13.1 %), 11 methyl $C_{18:1}\omega7c$ (15.6 %) and $C_{16:0}$ (14.3 %) were the major cellular fatty acids (>10.0 %) (Table 2). These data were similar to all the other members of the family *Parvularculaceae*, and possessed summed feature 8 and $C_{16:0}$ as the major fatty acids. The polar lipid profiles of strain SY-3-19^T contained phosphatidylglycerol (PG) and three unidentified glycolipids (GL1–GL3) as the major polar lipids, and six additional unidentified glycolipids and four uncharacterized lipids as the minor components (Fig. S2). GL1, GL2 and GL3 are the major polar lipids for all members of the family *Parvularculaceae* [2, 3, 6].

The chemotaxonomic results also showed clear differences in fatty acid compositions and polar lipid profiles between strain SY-3-19^T and its closely related genera. In detail, two fatty acids, 11 methyl $C_{18:1}\omega7c$ and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), were the major fatty acids of strains SY-3-19^T, but they were detected in strikingly lower amounts in the reference strains *Aquisalinus flavus* KCTC 42673^T (5.1 and 0.2 %, respectively), *Amphiplicatus metriothermophilus* GU51^T (not detected and 5.2 %, respectively), *P. flava* NH6-79^T (2.9 and 1.2 %, respectively) and *P. bermudensis* KCTC 12087^T (not detected and 0.6 %, respectively). In addition, the fatty acids iso-$C_{17:1}\omega5c$ and iso-$C_{18:0}$ were found in strain SY-3-19^T (1.7 and 3.1 %, respectively), but not detected in other reference strains. $C_{18:0}$ was present in the four reference strains, but absent in strain SY-3-19^T (Table 2). For the polar lipid profiles, the presence of PG in strain SY-3-19^T was a significant feature to distinguish the isolate from *Aquisalinus flavus* KCTC 42673^T (Fig. S2). Meanwhile, the existence of several unidentified glycolipids (GL4–GL9) and absence of diphosphatidylglycerol in strain SY-3-19^T clearly distinguished it from the genera *Amphiplicatus* and *Parvularcula* (Fig. S2, Zhang *et al.* [2], and Zhang *et al.* [6]). The fatty acid and polar lipid data suggested that strain SY-3-19^T may represent a novel species of a new genus in the family *Parvularculaceae*.

Besides the cellular fatty acid, polar lipid and phylogenetic analyses, strain SY-3-19^T could be definitively differentiated from the members of the genera *Aquisalinus*, *Amphiplicatus* and *Parvularcula* based on several physiological and phenotypic characteristics. For example, strain SY-3-19^T grown at 4–40 °C, could not grow without NaCl and had a wide tolerance range of 0.5–16.0 % (w/v) NaCl (optimum 4 %), which differed from all type species within the three genera in the family *Parvularculaceae*. In addition, hydrolysis of gelatin, assimilation of formic acid, γ-hydroxybutyric acid, itaconic acid, bromosuccinic acid and putrescine could also be used to distinguish it from the related reference strains (Tables 1 and S1). Especially, all members of family *Parvularculaceae* were motile with one flagellum, but more prosthecates and flagella were observed in the isolate. The low level of 16S rRNA gene sequence similarity between strain SY-3-19^T and all other members of the family *Parvularculaceae*, together with the differential properties described above, suggest that the isolate represents a novel species of a new genus within the family *Parvularculaceae*, for which the name *Marinicaulis flavus* gen. nov., sp. nov. is proposed.

DESCRIPTION OF *MARINICAULIS* GEN. NOV.

Marinicaulis (Ma.ri.ni. cau' lis. L. adj. *marinus* of the sea; L. masc. n. *caulis* stalk; N.L. masc. n. *Marinicaulis* a stalk from the sea).

Cells are Gram-stain-negative, aerobic, ovoid or short rod-shaped, non-spore-forming, slightly motile with

Table 2. Cellular fatty acid profile (%) of strain SY-3-19^T and the type strains of related species

Strains: 1, strain SY-3-19^T; 2, *Aquisalinus flavus* KCTC 42673^T; 3, *Amphiplicatus metriothermophilus* GU51^T; 4, *Parvularcula flava* NH6-79^T; 5, *Parvularcula bermudensis* KCTC 12087^T. Fatty acids that represented <1.0 % in all strains were omitted. Fatty acids that represented >10 % are in bold. –, Not detected. All data from this study.

Fatty acid	1	2	3	4	5
Straight chain:					
$C_{12:0}$	0.8	–	0.7	–	5.1
$C_{14:0}$	0.5	–	0.8	0.3	2.9
$C_{16:0}$	**14.3**	**12.7**	**21.3**	**36.2**	6.5
$C_{17:0}$	0.3	1.1	0.7	1.0	–
$C_{18:0}$	–	8.6	1.8	1.8	1.8
Branched:					
iso-$C_{15:0}$	2.0	–	1.0	–	1.4
iso-$C_{16:0}$	0.6	–	–	–	6.5
iso-$C_{17:0}$	0.7	0.1	1.3	0.4	–
iso-$C_{18:0}$	3.1	–	–	–	–
anteiso- $C_{15:0}$	–	–	–	–	3.5
Unsaturated:					
$C_{15:1}\omega5c$	1.6	–	0.7	–	–
11 methyl $C_{18:1}\omega7c$	**15.6**	5.1	–	2.9	–
iso-$C_{17:1}\omega5c$	1.7	–	–	–	–
$C_{19:0}$ Cyclo $\omega8c$	–	**12.7**	0.8	2.1	–
Hydroxy:					
$C_{10:0}$ 3-OH	–	0.5	–	1.3	–
$C_{14:0}$ 2-OH	1.2	–	2.1	–	–
Summed features:*					
3	**13.1**	0.2	5.2	1.2	0.6
6	–	–	–	7.5	–
7	–	2.7	1.7	3.5	–
8	**41.7**	**55.2**	**58.5**	**40.8**	**77.2**
9	–	–	2.4	–	–

*Summed features represent one or more fatty acids that cannot be separated by gas–liquid chromatography with the MIDI system. Summed feature 3 comprises $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$; summed feature 6 comprises $C_{19:1}\omega9c$ and/or $C_{19:1}\omega11c$; summed feature 7 comprises $C_{19:1}\omega6c$ and/or $\omega7c$ and/or $C_{19:0}$ cyclo $\omega10c$; summed feature 8 comprises $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$; summed feature 9 comprises $C_{16:0}$ 10-methyl and/or $C_{17:1}$ iso $\omega9c$.

prosthecates and flagella. Growth occurs in a wide range of NaCl concentrations and cells cannot grow without NaCl. Catalase- and oxidase-positive. The major isoprenoid quinone is Q-10. The principal cellular fatty acids (>10 %) are summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), $C_{16:0}$, 11 methyl $C_{18:1}\omega7c$ and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). The major polar lipids include PG and three GLs. The DNA G+C content of the type strain of the type species is 60.9 mol% (by genome). The genus is a member of the family *Parvularculaceae*. The type species is *Marinicaulis flavus*.

DESCRIPTION OF *MARINICAULIS FLAVUS* SP. NOV.

Marinicaulis flavus (fla′vus. L. masc. adj. *flavus* yellow, the colour of colonies or pigment that the bacterium produces).

Has the following characteristics in addition to those described for the genus. Cells have three different morphologies with one to three flagella including: (1) coccoid-shaped (0.5–1.0 µm in diameter) or ovoid-shaped with one to six prosthecates; (2) short rod- or rod-shaped (0.3–0.7 µm wide and 0.5–0.8 µm long) with no to one prosthecate; and (3) spiral-shaped or curved rod-shaped (2.0–5.0 µm long) with no prosthecate (Fig. 1). Colonies are 0.5–1.0 mm in diameter, yellow, slightly dry, convex and circular with entire margins after incubation on MA at 28 °C for 5 days. Cells are flocculated in MB. Growth occurs at 4–40 °C (optimum, 28 °C). pH 5.0–9.0 (optimum, pH 6.5–7.5) in MB and with 0.5–16 % (w/v) NaCl (optimum, 4.0 %) in modified MB. NaCl is necessary for growth. Nitrate can be reduced to nitrite, but not to nitrogen. Positive results in tests for H_2S production and hydrolysis of Tweens 20, 40, 60 and 80. Hydrolysis of starch, skim milk, gelatin and CM-cellulose are negative, as well as indole production, methyl red and Voges–Proskaur tests. The polar lipids are PG, nine GLs and four unidentified lipids.

The type strain is SY-3-19^T (=MCCC 1K03432^T=KCTC 62156^T), isolated from a surface seawater sample of South China Sea. The DNA G+C content was 60.9 mol% (by genome).

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

Ethical statement
This article does not contain any studies with human participants or animals performed by any of the authors.

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