A novel Gram-stain-negative, horseshoe-shaped, non-motile bacterium, designated strain KD51T, forming colonies coloured pink by carotenoid pigments, was isolated from aggregates of the alga *Chlorella autotrophica* collected from the coastal sea off the city of Xiamen, Fujian Province, China. 16S rRNA gene sequence comparison showed that strain KD51T was a member of the genus *Cyclobacterium*, forming a distinct lineage with *Cyclobacterium lianum* HY9T. The 16S rRNA gene sequence similarity between strain KD51T and the type strains of species of the genus *Cyclobacterium* ranged from 92.1% to 95.2%. Growth occurred at 4–40 °C (optimum, 28 °C), in the presence of 3–9% NaCl (optimum, 3–5%) and at pH 6–10 (optimum, pH 7.5). The dominant fatty acids (>20%) of strain KD51T were iso-C15:0 (32.2%) and summed feature 3 (comprising C16:1v7c and/or C16:1v9c; 22.2%). The DNA G+C content was 41.7 mol% and the only respiratory quinone was menaquinone-7. On the basis of phenotypic data and phylogenetic inference, strain KD51T represents a novel species of the genus *Cyclobacterium*, for which the name *Cyclobacterium xiamenense* sp. nov. is proposed. The type strain is KD51T (=CGMCC 1.12432T =KCTC 32253T). An emended description of the genus *Cyclobacterium* is also proposed.

The genus *Cyclobacterium*, type genus of the family *Cyclobacteriaceae* (phylum *Bacteroidetes*), was established by Raj & Maloy (1990) and its description has subsequently been emended by Ying et al., (2006). Members of the genus *Cyclobacterium*, displaying a unique ring-like or horseshoe-shaped cellular morphology, are common constituents of marine environments. At the time of writing, the genus *Cyclobacterium* contains five species with validly published names isolated from a variety of marine environments: *Cyclobacterium marinum* (Raj & Maloy, 1990), *C. amurskyense* (Nedashkovskaya et al., 2005), *C. lianum* (Ying et al., 2006), *C. qasimii* (Shivaji et al., 2012) and *C. caemipelagi* (Jung et al., 2013). In the present study, we characterized and determined the exact taxonomic position of a novel pink-pigmented bacterial strain with ring-like and horseshoe-shaped cells by using a polyphasic approach (Vandamme et al., 1996). Our data showed that the isolate represents a novel species of the genus *Cyclobacterium*.

In the course of a study on bacterial communities in marine algae off the coast of Xiamen city, Fujian Province, China (Zhou et al., 2013), we isolated strain KD51T in 2012 from aggregates of the alga *Chlorella autotrophica*. For isolation, 100 μl of a serially diluted sample was spread onto fresh marine agar 2216 (MA; Difco) and incubated at 28 °C for 7 days. A pink-pigmented colony, designated strain KD51T, was selected, subcultured on MA three times and stored at −80 °C in marine broth 2216 (MB; Difco) supplemented with 10% (v/v) glycerol.

The genomic DNA of strain KD51T was extracted according to the method of Ausubel et al. (1995) and the 16S rRNA gene was amplified by PCR using the primer pair P27F and P1492R (DeLong, 1992). Purification of the PCR product was carried out using a TIANquick Midi purification kit (TIANGEN) and the purified PCR product was cloned into vector pMD19-T and sequenced. Sequences of related taxa were downloaded from the GenBank database and the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/);
Kim et al., 2012). Phylogenetic analysis was performed using MEGA software version 4 (Tamura et al., 2007) after multiple alignment of data by DNAMAN (version 5.1). Evolutionary distances and clustering were performed by using the neighbour-joining method (Saitou & Nei, 1987). The resulting tree topology was evaluated by using bootstrap analysis, based on 1000 replicates.

Preliminary comparison of the 16S rRNA gene sequence of strain KD51T (1444 bp) with other sequences indicated that the novel isolate was closely related to the type strains of Cyclobacterium lianum, C. marinum, C. qasimii, C. amurskyense and C. caenipelagi, with pairwise sequence similarities of 95.2 %, 94.3 %, 94.0 %, 93.6 % and 92.1 %, respectively. Sequence similarities between strain KD51T and other members of the family Cyclobacteriaceae were significantly lower. As shown in Fig. 1, strain KD51T clustered together with C. lianum HY9T. Consequently, Cyclobacterium qasimii KCTC 23011T, C. amurskyense KCTC 12363T, C. lianum HY9T and C. marinum KCTC 2917T were obtained from culture collections, grown under the same conditions as strain KD51T and used as reference strains for a number of phenotypic tests.

Cell morphology and motility were observed by using transmission electron microscopy (JEM-2100HC; JEOL) and phase-contrast light microscopy (50i; Nikon), with cells from the early exponential phase grown on MA at 28 °C. Colony morphology was examined from cultures grown on MA for 7 days. Gliding motility was investigated as described by Bowman (2000). The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer’s instructions. Anaerobic growth was assessed on MA that was autoclaved and cooled to room temperature under a nitrogen atmosphere (99.999 % purity). Triplicate cultures were grown in 50 ml anaerobic serum bottles sealed with thick butyl rubber stoppers and aluminium caps, and incubated statically in the dark at 28 °C for 21 days. Growth in MB was tested at 4, 16, 20, 28, 30, 37 and 40 °C and at pH 3.0–10.0 (at 1 pH unit intervals). The pH of MB was adjusted prior to sterilization using the following buffers: citric acid/sodium citrate (for pH 3.0–6.0), Na2HPO4/citric acid (for pH 7.0–8.0) and lysine/NaOH (for pH 9.0–10.0). Verification of the pH values after autoclaving revealed only minor changes (Su et al., 2013). The NaCl concentration range and optimum for growth were determined in NaCl-free MB (containing 1 L distilled water: 5.0 g tryptone and 1.0 g yeast extract, pH 7.6–7.8), supplemented with 0–7 % (at 1 % intervals) and 9.0–13.0 % (at 2 % intervals) NaCl (w/v). Catalase and oxidase activities were assessed by the addition of 3 %

### Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of Cyclobacterium xiamenense sp.nov. KD51T with species of the genus Cyclobacterium and representative members of the family Cyclobacteriaceae. Bootstrap values >70 % (expressed as percentages of 1000 replications) are given at nodes. Wandonia haliotis Haldis-1T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.
hydrogen peroxide to exponential-phase colonies and by using oxidase reagent (bioMérieux), respectively. Hydrolysis of starch, Tweens 20, 40, 60 and 80, casein, aesculin, gelatin, tyrosine, urea, chitin, and agar was tested using MA supplemented with 0.5 % (w/v) starch and 1 % (w/v) other substrates. DNA hydrolysis was assessed on toluidine Blue DNase agar (Dong & Cai, 2001). Cellulose hydrolysis was tested both by using cellulose overlay plates (1 % CM-cellulose) and by examining strips of filter paper in liquid bacterial cultures for dissolution (Smibert & Krieg, 1994). The aforementioned tests were carried out on strain KD51T only, while the following tests were performed on strain KD51T and the four reference strains. Biochemical tests were carried out using API 20NE, API 20E and API ZYM strips (bioMérieux) according to the manufacturer’s instructions, except that the NaCl concentration in all tests was adjusted to 3.0 %. Susceptibility to antibiotics was tested both by using cellulose overlay plates (1 % CM-cellulose) and by examining strips of filter paper in liquid bacterial cultures for dissolution (Smibert & Krieg, 1994). The aforementioned tests were carried out on strain KD51T only, while the following tests were performed on strain KD51T and the four reference strains. Biochemical tests were carried out using API 20NE, API 20E and API ZYM strips (bioMérieux) according to the manufacturer’s instructions, except that the NaCl concentration in all tests was adjusted to 3.0 %. Susceptibility to antibiotics was tested both by using cellulose overlay plates (1 % CM-cellulose) and by examining strips of filter paper in liquid bacterial cultures for dissolution (Smibert & Krieg, 1994). All aforementioned tests were incubated at 28 °C for 5 days by using filter-paper discs (OXOID) containing various antibiotics (Lányi, 1987; Smibert & Krieg, 1994). All aforementioned tests were incubated at 28 °C. The novel isolate displayed the basic characteristics of members of the genus Cyclobacterium, e.g. pink-pigmented colonies and curved, ring-like or horseshoe-shaped cells (Fig. 2). Other phenotypic properties of strain KD51T are given in the species description and in Table 1.

Flexirubin-type pigments were absent from strain KD51T, as shown by the negative KOH test (Bernardet et al., 2002; Li et al., 2013). For pigment extraction, strain KD51T was inoculated into 1.5 ml MB, grown until exponential phase, and centrifuged at 6000 g at 4 °C for 10 min. The supernatant was discarded and the pellet was resuspended in 1.5 ml ethanol, mixed thoroughly on a vortex mixer and recentrifuged. The absorption spectrum of the supernatant was measured at 400–760 nm on a Lambda 35 spectrometer (Perkin Elmer) using ethanol as a blank (Shivaji et al., 2012). The broad peak with a maximum around 480 nm in the spectrum of the ethanol extract of strain KD51T was indicative of carotenoid pigments (Asker et al., 2007), as previously reported for C. qasimii M12-11B T (Shivaji et al., 2012).

For cellular fatty acid analysis, the fatty acids of strain KD51 T and the four reference strains grown on MA at 28 °C for 5 days were saponified, methylated and extracted using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0B). The fatty acids were analysed by GC (Agilent Technologies 6850) and identified by using the TSBA6 database of the Microbial Identification System (Sasser, 1990). The five strains had similar growth rates at 28 °C and the same physiological age at the time they were harvested. The dominant fatty acids (>20 %) of strain KD51 T were iso-C15 : 0 (32.2 %) and summed feature 3 (comprising C16 : 1ω7c and/or C16 : 1ω6c; 22.2 %). Significant amounts (>7 %) of anteiso-C15 : 0 and summed feature 9 (comprising iso-C17 : 1ω9c and/or C16 : 10-methyl) were also present. The fatty acid compositions of the four reference strains were similar, with minor differences in the respective proportions of some fatty acids. Strain KD51T mainly differed from C. qasimii KCTC 23011 T by the absence of iso-C11 : 0 and from C. amurskyense KCTC 12363 T and C. lianum HY9 T by the presence of C16 : 1ω5c and iso-C15 : 1 G, respectively (Table 2).

The G+C content of the DNA of strain KD51T was 41.7 mol%, as determined by thermal denaturation (Seidler & Mandel, 1971), a value within the range reported for members of the genus Cyclobacterium (Table 1). Respiratory quinones were also analysed at the CICC (China Center of Industry Culture Collection) on LDC Analytical HPLC (Thermo Separation Products) fitted with a reverse-phase column (Macherey-Nagel, 2 mm × 125 mm, 3 μm, RP18) using methanol/heptane (9 : 1, v/v) as the eluent. The only menaquinone present in strain KD51 T was menaquinone-7 (MK-7; 100 %), in line with other species of the genus Cyclobacterium (Jung et al., 2013; Shivaji et al., 2012).

On the basis of morphological, physiological and chemotaxonomic characteristics, as well as phylogenetic inference, strain KD51T represents a novel species of the genus Cyclobacterium, for which the name Cyclobacterium xiamenense sp. nov. is proposed. An emended description of the genus

**Fig. 2.** Transmission electron micrographs of cells of strain KD51 T grown on MA for 5 days at 28 °C. Bars, 1 μm.
Table 1. Differential characteristics between strain KD51^T and members of the genus *Cyclobacterium*

Strains: 1. KD51^T; 2. *C. qasimii* KCTC 23011^T; 3. *C. amurskyense* KCTC 12363^T; 4. *C. lianum* HY9^T; 5. *C. marinum* KCTC 2917^T. Data from this study except where otherwise indicated. All strains were ring-like and horseshoe-shaped, non-motile rods. All strains were positive for production of carotenoid pigments; catalase, alkaline and acid phosphatase, leucine and valine arylamidase and β-galactosidase activities; aesculin hydrolysis; utilization of citrate and D-maltose. All strains were susceptible to ampicillin, carbenicillin, cefazolin, cephradine, chloramphenicol, ciprofloxacin, erythromycin, minomycin, norfloxacin, novobiocin, ofloxacin, oxacillin, rifampicin, vancomycin, doxycycline and cephalaxin. All strains were weakly positive for lipase C14, cysteine arylamidase and trypsin activities. All strains were negative for production of flexirubin pigments; hydrolysis of gelatin, urea, chitin, starch, casein, filter paper and Tween 80; nitrate reduction; α-chymotrypsin and α-fucosidase and tryptophan deaminase activities; production of H2S and indole; utilization of citrate, potassium gluconate, capric acid and phenylacetic acid. All strains were resistant to clindamycin, metronidazole, neomycin, polymyxin B and streptomycin. +, Positive; –, negative; W, weakly positive; ND, no data available.

<table>
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*Data from Shivaji et al. (2012).
†Data from Nedashkovskaya et al. (2005a).
‡Data from Ying et al. (2006).
§Data from Raj & Maloy (1990).

Table 2. Cellular fatty acid content of strain KD51T and other members of the genus Cyclobacterium

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<td>–</td>
<td>–</td>
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<tr>
<td>C17 : 10:06c</td>
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<td>1.3</td>
<td>0.9</td>
<td>0.9</td>
<td>1.7</td>
</tr>
<tr>
<td>iso-C17 : 0 3-OH</td>
<td>4.7</td>
<td>5.9</td>
<td>6.1</td>
<td>5.6</td>
<td>4.8</td>
</tr>
<tr>
<td>C17 : 0 2-OH</td>
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<td>1.0</td>
<td>1.7</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>anteiso-C17 : 0 9c</td>
<td>0.9</td>
<td>0.6</td>
<td>1.0</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>C18 : 0</td>
<td>TR 0.6</td>
<td>1.3</td>
<td>TR –</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>C18 : 10:09c</td>
<td>TR 1.1</td>
<td>2.0</td>
<td>TR –</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Summed features*</td>
<td>3</td>
<td>22.2</td>
<td>19.6</td>
<td>12.1</td>
<td>23.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.1</td>
<td>2.7</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1.3</td>
<td>1.4</td>
<td>4.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>7.9</td>
<td>4.3</td>
<td>5.6</td>
<td>10.8</td>
</tr>
</tbody>
</table>

*As indicated by Montero-Calasanz et al. (2013), summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete equivalent chain-lengths (ECLs) as well as those where the ECLs are not reported separately. Summed feature 3 was listed as C16:0:7c and/or C16:0:6c; summed feature 4 was listed as iso-C17:1:1 and/or anteiso-C17:1:1 B; summed feature 8 was listed as C18:1:0:7c and/or C18:1:0:6c; summed feature 9 was listed as iso-C17:1:0:9c and/or C16:0:10-methyl.

Cyclobacterium xiamenense sp. nov.

Description of Cyclobacterium xiamenense sp. nov.

Cyclobacterium xiamenense (xia.men.e’n.se. N.L. neutr. adj. xiamenense of Xiamen, a city in Fujian Province, China, where the type strain was isolated).

Cells are Gram-stain-negative, non-motile, strictly aerobic and heterotrophic, curved, horseshoe-shaped or ring-like, 1.5–2.0 μm in length and 0.4–0.6 μm in diameter. Colonies grown for 5 days on MA are pink, circular with entire margins, 2–3 mm in diameter, smooth, translucent and raised. Carotenoid pigments are produced but flexirubin pigments are not. Growth occurs at 4–40 °C (optimum, 28 °C), with 3–9% (w/v) NaCl (optimum, 3–5%) and at pH 6–10 (optimum, pH 7.5). Tweens 20, 40 and 60 and aesculin are hydrolysed; starch and DNA are weakly hydrolysed; Tween 80, tyrosine, urea, chitin, gelatin, agar, filter paper and CM-cellulose are not hydrolysed. Catalase-positive and oxidase-negative. In the API ZYM strip, alkaline and acid phosphatases, esterase C8, leucine arylamidase, valine arylamidase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase activities are present; weak esterase C4, lipase C14, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase activities are present; trypsin, α-chymotrypsin, β-glucuronidase and α-fucosidase activities are absent. All tests in the API 20 NE strip are negative, except β-glucosidase activity (aesculin hydrolysis) and maltose assimilation. All tests in the API 20 E strip are negative except arginine dihydrolase and gelatinase activities and utilization of citrate. Susceptible to (μg per disc unless otherwise indicated): ampicillin (10), carbenicillin (100), cefazolin (30), cephradin (30), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), kanamycin (30), minomycin (30), norfloxacin (10), novobiocin (5), ofloxacin (5), oxacillin (1), penicillin G (10), piperacillin (100), polymyxin B (30 IU), rifampicin (5), vancomycin (30), ceftazidime (30), doxycycline (30), cefalexin (30) and sulphonmethoxazole (25)/trimethoprim (5). Resistant to clindamycin (2), gentamicin (10), metronidazole (5), neomycin (30), streptomycin (10), sulphafurazole (300) and tetracycline (30). The major fatty acids (>20%) are

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iso-C_{15:0} and summed feature 3 (comprising C_{16:1ω7c} and/or C_{16:1ω6c}). The complete fatty acid composition is given in Table 2. The only respiratory quinone is MK-7.

The type strain is KD51^T (=CGMCC 1.12432^T=KCTC 32253^T), isolated from aggregates of Chlorella autotrophica in Xiamen, China. The DNA G+C content of the type strain is 41.7 mol%.

**Emended description of the genus Cyclobacterium**

This emended description of the genus *Cyclobacterium* is based on the description of *Cyclobacterium caeniophilia* published by Jung et al. (2013) and on this study.

Cells are Gram-stain-negative, strictly aerobic, curved, ring-like or horseshoe-shaped, non-flagellated and non-motile. Colonies on MA are pink- or orangish-red-pigmented and shiny. Carotenoid pigments are produced but flexirubin pigments are not. Catalase-positive. The major or only respiratory quinone is MK-7. The major cellular fatty acids (>10 %) are iso-C_{15:0} and summed feature 3 (comprising C_{16:1ω7c} and/or C_{16:1ω6c}). The DNA G+C content is 40.5–45.2 mol%.

**Acknowledgements**

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**References**


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oilfield in the South China Sea, and emended description of the genus 

a member of the family *Cryomorphaceae* isolated from the 
marine alga *Phaeocystis globosa*, and emended description of 
1148.