**TAXONOMIC DESCRIPTION**

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**Nannocystis konarekensis** sp. nov., a novel myxobacterium from an Iranian desert

Kathrin I. Mohr, Azam Moradi, Stefanie P. Glaeser, Peter Kämpfer, Katja Gemperlein, Ulrich Nübel, Peter Schumann, Rolf Müller and Joachim Wink

Abstract

An orange-coloured myxobacterium, MNA11734\(^T\), was isolated from desert in Iran. MNA11734\(^T\) had rod-shaped vegetative cells, moved by gliding and was bacteriolytic. No real fruiting body formation could be observed, but sporangioles were produced on water agar. The strain was mesophilic, strictly aerobic and chemoheterotrophic. 16S rRNA gene analyses revealed that MNA11734\(^T\) belonged to the family Nannocystaceae, genus Nannocystis and was closely related to *Nannocystis pusilla* Na p29\(^T\) (DSM 14622\(^T\)) and *Nannocystis exedens* Na e1\(^T\) (DSM 71\(^T\)), with 97.8 and 97.6 % 16S rRNA gene sequence similarity, respectively. Laboratory-measured DNA–DNA hybridization showed only 9.5/15.7 % (reciprocal) similarity between the novel strain and *N. pusilla* Na p29\(^T\), and 14.1/20.4 % between the strain and *N. exedens* Na e1\(^T\), whereas DNA–DNA hybridization estimates derived from draft genome sequences were 21.8–23.0 % and 22.2–23.7 %, respectively, depending on the calculation method. The G+C content of DNA from *Nannocystis konarekensis* MNA11734\(^T\) was 73.3 mol%, for *N. pusilla* Nap29\(^T\) it was 71.8 mol% and for *N. exedens* Nae1\(^T\) it was 72.2 mol%. The major fatty acids of the new strain were C\(_{16:1}\) (56.2 %), iso-C\(_{17:0}\) (14.4 %), C\(_{14:0}\) (8.2 %), C\(_{16:0}\) (6.6 %) and iso-C\(_{15:0}\) (5.9 %). Strain MNA11734\(^T\) exhibited phylogenetic and physiological similarities to the two other species of *Nannocystis*, i.e. *N. pusilla* and *N. exedens*, but the differences were sufficient enough to represent a novel species, for which the name *Nannocystis konarekensis* sp. nov. is proposed. The type strain is MNA11734\(^T\) (=DSM 104509\(^T\)=NCCB 100618\(^T\)).

Myxobacteria are Gram-negative, unicellular bacteria with rod-shaped, vegetative cells. With the exception of *Anaeromyxobacter dehalogenans* [1] and some strains which may possibly represent the facultative anaerobes [2], myxobacteria are strictly aerobic organotrophs. They are common in top soil, on dung, on decaying plants and on the bark of living and dead trees [3]. Myxobacteria are able to glide over solid surfaces by secreting slime and show some interesting features such as an almost social behaviour. In times of nutrient deficiency, vegetative cells can aggregate and form often brightly coloured, species-specific fruiting bodies, which are reminiscent of eukaryotic myxomycetes or fungi rather than bacteria [4]. During fruiting body morphogenesis, vegetative cells are often clustered in sporangioles. Within these sporangioles some vegetative cells shorten and fatten and convert into nonmotile, desiccation-resistant myxospores. These structures can be disseminated by wind or animals and under more favourable environmental conditions spores can germinate. To date, myxobacteria are distinguished into two groups based on nutritional activity: micro-predators and cellulose-decomposers. Myxobacteria belong to the Deltaproteobacteria and within this group they form a relatively homogeneous cluster based on 16S rRNA gene analyses [5, 6]. Currently, they are divided into

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**Keywords:** Nannocystis; new species.

**Abbreviations:** BCFA, branched-chain fatty acids; FA(s), fatty acid(s); SCFA, short-chain fatty acids.

The GenBank/EMBL/DBJ accession numbers of the 16S rRNA gene sequence, the partial 23S rRNA gene, and partial *pyrG* and *rpoB* genes of strain MNA11734\(^T\) are KY381122, MF774778, MF77481, and MF77484, respectively. Partial 23S rRNA gene, partial *pyrG* and *rpoB* genes of strains *Nannocystis exedens* Na e1\(^T\) and *Nannocystis pusilla* Na p29\(^T\) are MF774779, MF774782, MF774785, and MF774780, and MF774783, and MF774786, respectively. Genome sequence data from MNA11734\(^T\), *Nannocystis exedens* Na e1\(^T\), and *Nannocystis pusilla* Na p29\(^T\) were deposited in the European Nucleotide Archive (https://www.ebi.ac.uk/ena) under study accession number PRJEB23840.

Three supplementary tables are available with the online version of this article.
three suborders, 10 families, 28 genera and 56 species. The family Nannocystaceae comprises four genera: *Nannocystis, Plesiocystis, Enhygromyxa* and *Pseudoenhygromyxa*, whereby the type strains of the latter three were isolated from marine environments and an estuarine marsh [7, 8, 9]. Two species belong to the genus *Nannocystis: Nannocystis exedens* [10] and *Nannocystis pusilla* [11, 12]. Strains which belong to *N. exedens* were isolated from a variety of soils collected from widely separated areas in different climate zones. This species was assigned to be a common member of the soil microflora [10]. The *N. pusilla* type strain was isolated from soil with decaying plant material collected in California, USA, and was described by Reichenbach in 1970 [11] and validated in 2007 [12]. Myxobacteria are not only fascinating because of their extraordinary lifestyle, they are also well known as producers of a variety of bioactive compounds such as polyketides, linear and cyclic peptides, and heterocyclic molecules [13, 14, 15]. Many of these compounds show interesting bioactivity. There have been only slight changes made to the established and successfully applied methods to isolate myxobacteria over the last 30 years. However, with these standard methods and a sample from a habitat common for myxobacteria, we successfully discovered a novel myxobacterium showing 97.8 and 97.6 % similarity on the basis of 16S rRNA gene sequencing to the closest related and only type species of the genus *Nannocystis, N. pusilla* Na p29T and *N. exedens* Na e1T, respectively. In this study, we investigated the taxonomic characteristics and on the basis of the phylogenetic and chemotaxonomic data we propose to classify strain MNa11734T (DSM 104509T=NCCB 100618T) as a representative of the novel species, *Nannocystis konarekensis* sp. nov.

Strain MNa11734T was isolated at the Helmholtz Centre for Infection Research (HZI) in autumn 2015 from a soil sample collected in Konarak County, Sistan and Baluchestan Province, Iran (coordinates: 25° 21’ 37” N 60° 23’ 58” E). Konarak is situated on the western coast of Chabahar Bay, on the Makran coast, Gulf of Oman. It has hot, humid weather in the summer and warm weather in the winter, giving it a hot desert climate. There is virtually no rainfall during the year in Konarak. The temperature averages 25.9 °C. It has an average maximum temperature of 34 °C and an average minimum temperature of 21.5 °C. The strain was isolated on water agar with *E. coli* bait [3], purified according to the methods of Reichenbach and Dworkin [16], and maintained in myxovirescin medium (1.0 % casein peptone, 0.005 % CaCl2, 0.025 % MgSO4, 1 mg l−1 CoCl2, 100 mM HEPES, pH 7.0) with 10 % A medium (0.8 % starch, 0.4 % soy flour, 0.2 % yeast, 0.1 % CaCl2, 0.1 % MgSO4, 100 mM Heps, 1 ml l−1 Fe-EDTA, 4 ml l−1 glycemin, pH 7.4) and vitamin B12 (final concentration, 500 µg l−1). Incubation temperature was 30 °C. Liquid cultures were incubated on a rotary shaker at 160 r.p.m. for 7 days. Morphology of culture, swarming behaviour and agar degradation were studied on CY [3], VY/2 [3], P [17] and water agar with *E. coli* bait [3] (all with 1.6 % agar) for a period of several weeks and photographed using a stereomicroscope (Olympus SZX12), while cells were observed by phase-contrast microscopy (Zeiss AX10), photographed using an Axiocam MRC (Zeiss) camera and further analysed by using AxioVision LE software.

Strain MNa11734T showed typical myxobacterial gliding and circular swarming activity on solid agar surfaces (Fig. 1a, b). On nutritious P agar, swarming was less distinctive in comparison to lean VY/2 and water agar, but cell mass production was lush. The colour of colonies was bright orange on P and CY agar (Fig. 1b). This is common for myxobacteria, because carotenoids are the main pigments in several myxobacteria. Therefore yellow/red pigmentation is widespread in this group, whereby *Nannocystis* contains mainly aromatic carotenoids and no glycosides [3]. Due to lower cell mass production, the swarm grew thinly and therefore appeared transparent on VY/2 and water agar. On CY agar, the strain grew in circular, agar-corroding veins (Fig. 1a, b). Agar corrosion was extensive, especially on CY and water agar, as already described for *Nannocystis* [3]. The outstanding trait of the myxobacteria is their ability to form multicellular fruiting bodies and sporangioles, which serve as housing for resistant resting cells, the myxospores [3]. Knobbled orange sporangioles could be observed on water agar with *E. coli* bait after 2 weeks of incubation (Fig. 1c). Most of the sporangioles were solitary and scattered on top of the agar, which has been also described for other members of the genus *Nannocystis* [3]. Sporangioles were up to 40 µm in diameter. A photograph of sporangioles, picked from VY/2 agar after about 100 days of incubation at 30 °C, is shown in Fig. 1d. However, no clear wall around the sporangioles, as described and shown for *N. exedens* by Reichenbach (1970) or for Na p29T, was visible. Myxospores were produced on water agar with *E. coli* and were 0.8–0.9 µm wide and 1.7–1.8 µm long (Fig. 1e). Vegetative cells were short and rod-shaped with blunt, rounded ends (Fig. 1f). They were 1.1–1.5 µm wide and 2.0–3.25 µm long. Cells of this morphology are typical for several members of the genera *Sorangium* and *Nannocystis*.

All differences between MNa11734T and the other two *Nannocystis* type strains, Na e1T (Fig. 1g) and Na p29T (Fig. 1h), are shown in Table 1.

Cellulose degradation was tested on Stan 21 agar with filter paper [3]. No cellulose degradation, only described for genera *Sorangium* and *Byssavorax*, could be observed. Starch degradation was tested on P agar inoculated with 100 µl liquid culture. After incubation, the 4-week-old culture was flooded with 2 ml 0.01 N iodine solution. Weak starch degradation was visible as small clear zones around the colonies. Microaerophilic growth was tested in 10 ml CY and P prick agar tubes. Growth was detected in the upper part of the puncture channel after 2 weeks of incubation. To date, all cultured myxobacteria belong to the aerobic group [3] except for the genus *Anaeromyxobacter* [1] and some novel isolates, probably representing the facultative anaerobes [2, 18]. Catalase was tested with 3 % H2O2 (w/v) [19]. Oxidase was tested with test strips (Bactident Oxidase, Merck) with...
100 µl liquid culture grown in CY-H medium (50 % CY [3] and 50 % H medium. H (per litre): 0.2 % soy flour, 0.2 % glucose, 0.8 % starch, 0.2 % yeast extract, 0.1 % CaCl₂, 0.1 % MgSO₄, 50 mM HEPES, 8 mg Fe EDTA, pH 7.4). Vegetative cells of strain MNa11734ᵀ were catalase- and oxidase-positive. Escherichia coli and baker’s yeast Saccharomyces cerevisiae (50 % autoclaved, yeast suspension) as food bait were tested on water agar supplemented with vitamins [3]. The bait was cross-streaked and an inverted overgrown piece of VY/2 agar (approx. 5 mm²) with the Nannocystis strains placed at the intersection and incubated at 30 °C for 1 week. The ability of MNa11734ᵀ to prey E. coli and S. cerevisiae as bait was distinctive. Transparent growth with swarming activity can be detected on water agar with living and dead E. coli cells. Lytic action of autoclaved yeast cells was also tested on VY/2 agar and could be observed as a weak clearing zone under and around the culture.
Strain MNa11734 grew at mesophilic temperatures and within a narrow pH range. Growth at different temperatures was tested with an inverted overgrown VY/2 agar block on VY/2 agar. Plates were incubated at 4, 18, 25, 30, 37, and 44 °C, respectively, and the swarm diameter was measured after 13 days. Growth of the three strains was observed between 25 and 37 °C, but strain Na e1 grew weakly at 44 °C. MNa11734 grew best at 37 °C, which distinguishes this strain from the other Nannocystis type strains which show optimum growth at 30 °C. No strain could grow on agar plates at 4 and 18 °C. In addition, MNa11734 and Na p29 were not able to grow at 44 °C. At 25 °C, all strains grew weakly.

Growth at different pH values (4.5–10.0) was tested in 0.5 pH units on VY/2 agar inoculated with an inverted overgrown VY/2 agar block. VY/2 was buffered with MES for pH below 6.5, HEPES for 6.5–8.0 and Tris for pH 8.0–10.0. The largest diameter of each strain was equated with value 100. If the diameter was larger than two thirds of the largest diameter, growth was evaluated with +++; 1/3–2/3 diameters were evaluated with ++ and growth up to one third of the largest diameter was evaluated with +. Very little growth, (+); no growth, –. Temperature, pH and antibiotic resistance was tested on VY/2 agar.

### Table 1. Differential phenotypical and physiological characteristics of strain MNa11734 and the two type strains of the genus Nannocystis, N. exedens (Na e1) and N. pusilla (Na p29)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MNa11734&lt;sup&gt;T&lt;/sup&gt;</th>
<th>Na e1&lt;sup&gt;T&lt;/sup&gt;</th>
<th>Na p29&lt;sup&gt;T&lt;/sup&gt;</th>
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<tr>
<td>Temperature-dependent growth at:</td>
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<tr>
<td>25 °C</td>
<td>(+)</td>
<td>+</td>
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<tr>
<td>30 °C</td>
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<td>+++</td>
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<tr>
<td>37 °C</td>
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<td>+</td>
</tr>
<tr>
<td>44 °C</td>
<td>–</td>
<td>(+)</td>
<td>–</td>
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<tr>
<td>Optimal temperature (°C)</td>
<td>37</td>
<td>30</td>
<td>30</td>
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<tr>
<td>Degradation of (after 9 days at 30 °C):</td>
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<tr>
<td>Starch (clearing zone on P agar)</td>
<td>–</td>
<td>(+)</td>
<td>–</td>
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<tr>
<td>Yeast (on water agar with S. cerevisiae bait)</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>pH-dependent growth at:</td>
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<tr>
<td>5.5</td>
<td>–</td>
<td>+</td>
<td>++</td>
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<tr>
<td>6.0</td>
<td>–</td>
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<td>++</td>
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<td>6.5</td>
<td>+</td>
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<tr>
<td>7.0</td>
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<td>10.0</td>
<td>+++</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Antibiotic resistance (50 µg ml&lt;sup&gt;–1&lt;/sup&gt;):</td>
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<tr>
<td>Chloramphenicol</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Gentamicin</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Hygromycin</td>
<td>e</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Polymyxin</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Spectinomycin</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Thiotrepton</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Enzyme activity (API ZYM):</td>
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<tr>
<td>Esterase (C4)</td>
<td>w</td>
<td>w</td>
<td>+</td>
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<tr>
<td>Esterase lipase (C8)</td>
<td>w</td>
<td>w</td>
<td>+</td>
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<tr>
<td>Leucine arylamidase</td>
<td>+</td>
<td>+</td>
<td>w</td>
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<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Cystine arylamidase</td>
<td>w</td>
<td>w</td>
<td>–</td>
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<tr>
<td>Trypsin</td>
<td>w</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>w</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Genome characteristics:</td>
<td></td>
<td></td>
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<tr>
<td>DNA G+C content (mol%) based on data of genome sequencing</td>
<td>73.3</td>
<td>72.2</td>
<td>71.8</td>
</tr>
<tr>
<td>Approximate length (total contig length, Mbp)</td>
<td>11.1</td>
<td>11.6</td>
<td>12.0</td>
</tr>
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</table>

8.5–10.0. PH of agar plates was checked using pH strips (pH-Fix, Roth, 4.5–10.0) before inoculation. The swarm diameter was measured after 19 days. MNa11734T was tolerant to pH 6.5–10.0 and grew optimally at pH 8.0–10.0. However, no growth was observed at values lower than pH 6.5. This also distinguishes MNa11734T from the other two Nannocystis type strains, which already grew at pH 5.5 and also up to pH 10.0. This is in accordance with Reichenbach, who mentioned growth tolerance between pH 5.5–10.0 for N. exedens Na e1T [10]. Antibiotic resistance against 13 antibiotics was tested on VY/2 agar as described at Mohr et al. [20]. The final concentration was set to 50 µg ml⁻¹. For reference, growth was also tested on VY/2 agar without antibiotics.

Plates were incubated for 1 week. Strain MNa11734T is sensitive to bacitracin (BC), oxytetracycline (OTC) and thioestreptone (THIOS). Growth, comparable to those on the plate without antibiotics (diameter of swarm), could be observed with ampicillin (ABPC), gentamicin (GM), polymyxin (PL), kanamycin (KM), spectinomycin (SPCM), cephalosporin (CEP), fusidic acid (FSA), trimethoprim (TMP), and chloramphenicol (CP). On plates with hygromycin (HYG), the strain hardly grew. Against six antibiotics (the six plus THIOS), Na p29T showed the same growth behaviour. However, Na e1T and Na p29T showed 10 matches (the six plus CP, HYG, PL and SPCM) with regard to antibiotic susceptibility.

In the API-ZYM test, alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, and naphtol-ASBL-phosphohydrolase activities were strong. Esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin and chymotrypsin activities were weak. No activity of α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosi-dase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities could be detected. The enzymatic activity pattern of MNa11724T was identical to that of Na e1T and both differed from Na p29T with regard to the activity of valine arylaminidase and weakly regarding the six enzymes.

For fatty acid (FA) analysis, strains MNa11734T, N. exedens Na e1T and N. pusilla Na p29T were cultivated in a 300 ml flask containing 50 ml M medium (per litre 1 % soy peptone, 1 % maltose, 0.1 % CaCl₂, 0.1 % MgSO₄, 50 mM HEPES, 8 mg Fe EDTA, pH 7.2), shaken at 180 r.p.m. for 5 days at 30 °C. The cultures were harvested by centrifugation. FA extraction was done by the fatty acid methyl ester method [21]. Gas chromatography–mass spectrometry analysis and identification of FAs was performed according to Gemperlein et al. [22]. FAs were expressed as percentages using the value obtained from the integrated signal. The major FAs of the novel myxobacterium MNa11734T with nearly 5 % or more of the total were straight-chained C₁₅:₀ (56.2 %), C₁₄:₀ (8.2 %) and C₁₆:₀ (6.6 %), and branched-chained iso-C₁₇:₀ (14.4 %), and iso-C₁₅:₀ (5.9 %). The main differences between N. exedens Na e1T and the novel strain were the absence of the fatty acid iso-C₁₇:₁ isomer 1 and the smaller amount of C₁₄:₀ dimethyl aldehyde (Table S1, available in the online version of this article). No hydroxy-type fatty acids were detected, which is one of the most remarkable characteristic of the family Nannocystaceae, shared with Haliangiaceae and Kofleriaceae, and also a distinguishing landmark of the suborder Nannocystineae [23].

![Score oriented dendrogram generated by the BioTyper software](image-url)
analyses revealed that strain MNa11734 was located within the clade comprising the two species of the genus Nannocystis, N. pusilla Na p29\(^\dagger\) (DSM 14622\(^\dagger\)) and N. exedens Na e1\(^\dagger\) (DSM 71\(^\dagger\)).

Both trees showed that strain MNa11734\(^\dagger\) was located within the clade comprising the two species of the genus Nannocystis, N. pusilla Na p29\(^\dagger\) (DSM 14622\(^\dagger\)) and N. exedens Na e1\(^\dagger\) (DSM 71\(^\dagger\)). With the new strain exhibited 97.8 and 97.6 % sequence similarity values, respectively.

For a higher phylogenetic resolution in addition to 16S rRNA gene analyses, further phylogenetic analyses were performed based on partial 23S rRNA gene and partial nucleotide and amino acid sequences of two protein coding genes, the orotidine-5′-phosphate decarboxylase gene pyrG and the rpoB gene, which encodes the β subunit of bacterial RNA polymerase. PCR amplification and Sanger sequencing was performed according to Chen et al. [36]. Reference sequences of type strains of the next related species were obtained from published genome sequences (accession numbers are given in Table S3). Analysis was performed in MEGA 7 [37]. Nucleotide sequences of protein-coding genes were aligned according to the deduced amino acid sequences with CLUSTAL W [38]. Phylogenetic trees were constructed using the maximum-likelihood method with using a discrete general time resolved (GTR) model by using RAxML version 7.04 [33] with GTR-GAMMA and rapid bootstrap analysis (100 replications) and the neighbour-joining method using the ARB neighbour-joining method and the Juke–Cantor correction [34] and bootstrap analysis [35] based on 1000 replications.

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gamma distribution (+G) with five rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+I) for nucleotide sequences and the Jones–Thornton–Taylor model+G+I for amino acid sequences [39]. Pairwise sequence similarities were calculated for the set of sequences applied for tree construction based on the calculation of the number of nucleotide or amino acid differences per sequence position (p-distance methods, MEGA7). All positions containing gaps and missing data were thereby eliminated. All phylogenetic trees showed the formation of a monophyletic cluster by strains MNa11734\(^T\), N. exedens Na e1\(^T\) and N. pusilla Na p29\(^T\) distinct to the next related genera Enhygromyxa and Plesiocystis within the Nannocystaceae (Fig. 4). Phylogenetic trees and pairwise sequence similarities (Table 2) furthermore indicated the distinction of strain MNa11734\(^T\) from N. exedens Na e1\(^T\) and N. pusilla Na p29\(^T\) and the close phylogenetic relationship of N. exedens Na e1\(^T\) and N. pusilla Na p29\(^T\).

However, the 16S rRNA gene sequence similarity between MNa11734\(^T\) and N. pusilla/N. exedens is 97.8 and 97.6 %, which alone is sufficient to suggest different species allocations, in consideration of the threshold of 98.7–99.0 % mentioned by Stackebrandt and Ebers [40] or the threshold of 98.65 %, mentioned by Kim et al. [41]. In addition, the branching out within the 23S, rpoB and pyrG phylogenetic trees from the known species and the clear distinction within the MALDI–TOF dendrogram indicate that strain MNa11734\(^T\) represents a novel species.

DNA–DNA hybridization (DDH) experiments were performed between strain MNa11734\(^T\) and N. exedens Na e1\(^T\), and N. pusilla Na p29\(^T\), respectively, as described at Moradi et al. [42]. Genomic DNA was sheared before DDH analysis using ultrasonication (Bandelin-Sonopuls GM70). The new strain MNa11734\(^T\) showed DNA homology of 9.5 % (reciprocal, 15.7 %) to N. pusilla Na p29\(^T\) and 14.1 % (reciprocal, 20.4 %) to N. exedens Na e1\(^T\). Illumina sequencing libraries

Fig. 4. Phylogenetic analysis based on partial 23S rRNA gene sequences and nucleotide and amino acid based pyrG and rpoB sequences. For the 23S rRNA, pyrG and rpoB genes, 838, 377 and 936 nucleotide positions were considered, for PyrG and RpoB 125 and 312 amino acids, respectively. Maximum-likelihood trees were calculated in MEGA7 based on 100 bootstraps. Numbers at nodes represent bootstrap values of 70 % and above. Bar, 0.05 exchanges per nucleotide or amino acid sequence position, respectively. The GenBank/EMBL/DDBJ accession numbers of the partial 23S rRNA gene, and partial pyrG and rpoB genes of strain MNa11734\(^T\) are MF774778, MF77481, and MF77486, respectively. Partial 23S rRNA gene, partial pyrG and rpoB genes of strains Nannocystis exedens Na e1\(^T\) and Nannocystis pusilla Na p29\(^T\) are MF774779, MF774782, MF774785, and MF774780, MF774783, and MF774786, respectively.
were prepared from genomic DNA from type strains of the three Nannocystis species according to a recently published protocol [43] and sequenced to at least 35-fold average coverage on an Illumina NextSeq machine applying a 2 × 150 bp Mid-Output kit (Illumina). Sequencing reads were assembled into sequence contigs by applying SPAdes version 3.11.0 [44]. The summed-up total contig lengths for each of the three type strains, G+C contents of the DNA sequences and digital estimates for DNA–DNA hybridization values were computed by using the online software tool GBPD2_BLASTPLUS available at http://ggdc.dsmz.de/ [45]. The results are summarized in Table 1. Estimated DNA–DNA hybridization values between 22 and 24%, depending on the calculation method applied, and differences in G+C contents of 1.1 and 1.5%, respectively, confirmed that strain MNA11734T represents a species that is clearly distinct from N. exedens and N. pusilla.

Thus, we propose the creation of a novel species of the genus Nannocystis, and suggest the name Nannocystis konarekensis sp. nov. for the species represented by strain MNA11734T described here.

**DESCRIPTION OF NANNOCYSTIS KONAREKENSIS SP. NOV.**

Nannocystis konarekensis (ko.na.rek.en’sis. N.L. fem. adj. konarekensis pertaining to Konarek, a county in Iran, where the soil sample was collected from which the strain has been isolated).

The vegetative cells are Gram-negative, rod-shaped, short with blunt, rounded ends (Nannocystis type) and non-motile, 1.1–1.5 µm in diameter and 2.0–3.25 µm long. Cells can move by gliding on solid surfaces. Myxospores are 0.8–0.9 µm wide and 1.7–1.8 µm long. The colour of colonies is bright orange on P and CY agar and in liquid medium. On VY/2 agar, transparent, distinctive swimming activity. On CY and water agar, agar corrosion is extensive but the strain cannot liquefy agar. Orange-coloured scattered sporangioles can be observed on water agar with E. coli bait. Growth temperature is between 25–37 °C (optimum 37 °C); no growth at 44 °C. Growth occurs at pH between 6.5–10.0 (optimum 8.0–10.0). Catalase- and oxidase-positive. Nannocystis konarekensis is susceptible to bacitracin, oxytetracycline and thiostrptom, but not to ampicillin, cephalosporin, chloramphenicol, fusidic acid, gentamicin, kanamycin, polymyxin, spectinomycin and trimethoprim.

In the API-ZYM test, alkaline phosphatase, leucin arylamidase, valine ary lamidase, acid phosphatase and naphtol-AS-BI-phosphohydrolase activities are strong. Esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, tryptase and chymotrypsin activities are weak. α-Galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities could not be detected. Major fatty acids (>5% of total fatty acids) are C15:0, iso-C17:0, cyclo-C14:n0, C16:0 and iso-C15:0. The mol DNA G+C of MNA11734T is 73.3 mol% based on data of genome sequencing. The type strain, MNA11734T (DSM 104509T=NCCB 100618T), was isolated from soil collected in Konarak County, Sistan and Baluchestan Province, Iran. The phylogenetic position is in the genus Nannocystis, family Nannocystaceae, suborder Nannocystineae, order Myxococcales.

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**References**


