

Article

Whole Genome Sequence-Based Classification of *Nonomuraea marmarensis* sp. nov., Isolated from Island Soil

Ahmet Ridvan Topkara ^{1,2} , Hayrettin Saygin ³ , Salih Saricaoglu ² , Aysel Veyisoglu ⁴ , Ali Tokatli ^{2,5},
Kiyimet Guven ⁶ , Demet Cetin ⁷  and Kamil Isik ^{2,*}

¹ Central Research Laboratory Application and Research Center, Çankırı Karatekin University, Çankırı 18100, Türkiye; ahmetridvantopkara@karatekin.edu.tr

² Department of Biology, Faculty of Science, Ondokuz Mayıs University, Samsun 55139, Türkiye; salihsaricaoglu@omu.edu.tr (S.S.); alitokatli@karatekin.edu.tr (A.T.)

³ Department of Molecular Biology and Genetics, Faculty of Science, Ondokuz Mayıs University, Samsun 55139, Türkiye; hayrettin.saygin@omu.edu.tr

⁴ Department of Medical Laboratory Techniques, Vocational School of Health Services, Sinop University, Sinop 57000, Türkiye; aveyisoglu@sinop.edu.tr

⁵ Technology Transfer Office, Çankırı Karatekin University, Çankırı 18100, Türkiye

⁶ Department of Biology, Faculty of Science, Eskisehir Technical University, Eskisehir 26555, Türkiye; kguven@eskisehir.edu.tr

⁷ Division of Science Education, Department of Mathematics and Science Education, Gazi University, Ankara 06500, Türkiye; demetcetin@gazi.edu.tr

* Correspondence: kamilis@omu.edu.tr; Tel.: +90-3623121919 (ext. 5438)

Abstract: Actinomycetes are known to produce a vast array of bioactive secondary metabolites with potential therapeutic applications, including antimicrobials, anticancer agents, and enzyme inhibitors. Among these, members of the genus *Nonomuraea* have received much attention due to their broad ecological importance in nutrient cycling in soil and their ability to produce new bioactive compounds. A novel actinomycetes, designated strain M3C6^T, was isolated from soil samples collected on Marmara Island, located in the Istanbul province, aiming to explore the microbial diversity of unexplored habitats, and characterized using a polyphasic approach. The isolate showed chemotaxonomic and morphological features consistent with members of the genus *Nonomuraea*. The 16S rRNA gene sequence analysis revealed that strain M3C6^T shared the highest similarity, at 98.7% sequence identity, to *Nonomuraea basaltis* 160415^T and *Nonomuraea turkmeniaca* DSM 43926^T. However, the ANI and dDDH values between strain M3C6^T and these reference strains were fairly low, ranging from 84.0 to 84.6% and 31.8 to 33.7%, respectively, below the generally accepted cutoffs for ANI and DDH that delineate different prokaryotic species. Genomic analysis of strain M3C6^T showed that it had a genome size of 10.38 Mbp and a DNA G+C content of 69.5 mol%. Based on these chemotaxonomic, phenotypic, and genomic data, strain M3C6^T is classified as a novel species within the genus *Nonomuraea*, for which the name *Nonomuraea marmarensis* sp. nov. is proposed. The type strain is M3C6^T (= KCTC 49983^T = CGMCC 4.8035^T). Genomic analyses confirmed the high potential of M3C6^T to produce specialized secondary metabolites.

Keywords: *Actinomycetes*; *Nonomuraea*; island soil; biosynthetic gene clusters; whole genome analysis; 16S rRNA gene



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1. Introduction

The genus *Nonomuria* [sic] was initially described by Zhang et al. [1], who classified it within the family *Streptosporangiaceae*, and then, due to a nomenclatural error,

Chiba et al. [2] subsequently corrected the genus name to *Nonomuraea*. At the time of writing, there are 68 species and 2 subspecies in the genus *Nonomuraea* that have validly published names (www.bacterio.net/nonomuraea.html; accessed on 11 December 2024). The type species is *Nonomuraea pusilla* [1,3]. Species of the genus *Nonomuraea* have been described as aerobic, Gram-positive bacteria that are non-acid-alcohol-fast and filamentous, with a well-developed, extensively branched substrate mycelium and aerial hyphae, giving them a highly organized structural appearance. The optimal temperature range for growth in members of *Nonomuraea* lies within a range of 20 °C to 45 °C, hence reflecting their mesophilic nature. Surprisingly, some strains show the capability to grow even at temperatures as high as 55 °C. The cell walls of *Nonomuraea* species generally contain the well-known *meso*-diaminopimelic acid (*meso*-A₂pm). Whole-organism hydrolysates contain madurose, which corresponds to a cell wall type III/B described by Lechevalier and Lechevalier [4]. The respiratory menaquinone in this genus is MK-9 [H₀, H₂, H₄]. Other properties characteristic of *Nonomuraea* are the presence of glucosamine-containing lipids, including phosphatidylethanolamine, phosphatidylmethylethanolamine, diphosphatidylglycerol, and phosphatidylinositol, which reflect the phospholipid type pattern IV [5]. According to Kroppenstedt [6], the major fatty acids in *Nonomuraea* species are C_{17:0} 10-methyl, and *iso*-16-branched components, which correspond to pattern 3c [7]. Branched-chain fatty acids of this type play an important role in membrane fluidity and resilience under fluctuating environmental stresses and thus provide enhanced adaptability to these bacteria [8].

Ecologically, most members of the genus *Nonomuraea* were isolated from soil environments [9,10], playing important roles in nutrient cycling due to their abilities to degrade a variety of complex organic substrates into easily available carbon and nitrogen forms through the secretion of various extracellular enzymes such as those degrading cellulose, lignin, and chitin [11–14]. This activity not only supports soil fertility and promotes healthy plant growth but also helps maintain soil structure and water-holding capacity. Their importance in soil health and ecosystem stability underlines their ecological significance [15]. In addition, members of *Nonomuraea* have been isolated from very different ecosystems, such as sediment [16], cave [17], stem [18], and root [19].

The metabolic diversity of *Nonomuraea* also represents an important resource for biotechnological research. For this reason, the discovery of novel species belonging to this genus opens new frontiers in basic and applied research. The most promising feature of the genus *Nonomuraea* is its production of bioactive compounds [20]. Included are antibiotics such as maduramycin and pradimicin U, active against resistant bacterial strains. Anticancer agents synthesized by *Nonomuraea* species include akazamicin and brartemicin, while antimalarial and anti-tuberculosis agents include pradimicin U, which is effective against infectious agents such as *Plasmodium falciparum* and *Mycobacterium tuberculosis* [21,22]. Moreover, genomic analyses have revealed that the members of the genus *Nonomuraea* possess a large number of biosynthetic gene clusters responsible for the biosynthesis of secondary metabolites [23,24]. Their high diversity presents many opportunities for discovering new and biologically active compounds, which signifies the potential for producing these structurally and functionally significant metabolites for pharmaceutical and industrial applications.

The aim of this study is to determine the taxonomic status of the strain M3C6^T by using a polyphasic approach and reveal the biotechnological potential based on the genome sequence of the novel strain. This study also highlights the potential for discovering novel actinobacterial species in unexplored environmental habitats such as island soil.

2. Materials and Methods

2.1. Bacterial Isolation and Cultivation

The M3C6^T strain was isolated from soil samples collected from Marmara Island in Istanbul province (GPS coordinates 40°34′43.0″ N and 27°34′18.3″ E) in November 2020. Geographically, Marmara Island is the largest island in the Sea of Marmara, and the second biggest island in Turkey [25]. The island measures an area of 117 km², with the highest elevation on the island at 700 m above sea level [26]. It obtains its name from the abundant white marble deposits found in it, known as marmor in Latin. The flora of Marmara Island is quite diverse, having at least 475 species of vascular plants [27]. The main vegetation on the island consists of Mediterranean forest trees, along with typical maquis and frigana plant communities, which altogether give it a rich ecological and botanical diversity [28].

The soil sample taken from the island was air-dried at room temperature for 14 days prior to analysis via a conventional dilution plate procedure. Dilutions of the soil suspension were inoculated onto Czapek's Dox medium according to Weyland [29], amended with cycloheximide, neomycin sulfate, nystatin, and rifampicin, all filter-sterilized, at 50 µg mL⁻¹, 4 µg mL⁻¹, 50 µg mL⁻¹, and 5 µg mL⁻¹, respectively. Incubation was performed at 28 °C for 4 weeks. The strain was maintained and purified on yeast extract–malt extract agar (ISP 2) [30] at room temperature, and long-term stored as mycelial fragments and spore suspensions in glycerol solution (20%, *v/v*) at −20 °C.

2.2. 16S rRNA Gene Phylogeny

For strain M3C6^T, genomic DNA was extracted and then subjected to PCR amplification and sequencing of the 16S rRNA gene according to Chun and Goodfellow [31]. Sequencing was performed using an ABI PRISM 3730 XL (Applied Biosystems, Waltham, MA, USA) automatic sequencer. The nearly complete 16S rRNA gene sequence of strain M3C6^T, containing 1485 nucleotides, was submitted to the EzBioCloud database [32]. Neighbor-joining [33], maximum-likelihood [34], and maximum-parsimony [35] approaches were performed using the software package MEGA version X [36]. Genetic distances between the sequences were estimated according to the Jukes and Cantor [37] model. Phylogenetic tree stability was estimated by bootstrap analysis with 1000 resamplings, as outlined by Felsenstein [38]. For rooting the phylogenetic trees, *Thermopolyspora flexuosa* DSM 43186^T (AY039253) was used as an outgroup.

2.3. Phylogeny and Genome Features Based on Whole-Genome Sequencing

The whole genome of M3C6^T was sequenced on an Illumina HiSeq 2500 (Illumina, San Diego, CA, USA) platform with 250 bp paired-end reads at Microbes NG using its standard protocols. The in silico genome assembly was performed using SPAdes version 3.13 on Bacterial and Viral Bioinformatics Resource Center (BV-BRC) [39]. The TYGS server (<http://tygs.dsmz.de>; accessed on 14 January 2025) was employed to construct a whole-genome phylogeny [40]. The genome sequences were annotated using the SEED viewer [41] after RAST annotation [42]. Digital DNA–DNA hybridization (dDDH) similarities between the genome of strain M3C6^T and the type strains of *Nonomuraea basaltis* and *Nonomuraea turkmeniaca* were computed using formula 2 from the Genome-to-Genome Distance Calculator (GGDC) server [43]. Average nucleotide identity (ANI) values were calculated among the genomes of strain M3C6^T and closely related type strains using the ANI-Blast (ANIB) algorithm implemented within the JSpeciesWS web service [44]. Later, the draft genome of the strain M3C6^T was then analyzed for the presence of secondary metabolite-biosynthetic gene clusters (BGCs) with strict detection using antiSMASH v.7.0 [45] and BAGEL4 [46].

2.4. Morphological, Cultural, Physiological and Biochemical Characteristics

The spore morphology of strain M3C6^T cultivated on ISP 3 medium [30] at 28 °C for 14 days was observed by scanning electron microscopy (JEOL JSM 6060). Strain M3C6^T and the type strains of *N. basaltis*, *N. turkmeniaca*, and *N. phyllanthi* were tested for cultural and physiological characteristics. The cultural characteristics, which included growth capacity, colony coloration, and formation of soluble pigments, were assessed on different ISP media (ISP 2-7) [30], modified Bennett's agar (MBA) [47], tryptic soy agar (TSA; Difco), Czapek's agar (CA) [48], and nutrient agar (NA) [49], using the NBS/IBCC color chart [50] for color description. Tolerances for temperature, pH, and NaCl were determined using ISP 2 (pH 7.2) as the basal medium. Growth at various temperatures (4, 10, 20, 28, 37, 40, 45, 50, and 55 °C) was observed following a 14-day incubation period at pH 7.2. NaCl concentrations ranging from 0 to 10% NaCl (*w/v*) in increments of 1% were maintained at 28 °C for 14 days, while pH tolerance was tested from 4.0 to 12.0 in increments of 1.0 pH unit under the same incubation temperature, with KH₂PO₄/HCl, KH₂PO₄/K₂HPO₄, and K₂HPO₄/NaOH buffer systems (50 mM) maintaining the pH of the medium. Hydrolysis studies for adenine, casein, guanine, hypoxanthine, Tweens (20, 40, and 80), and xanthine were conducted based on the procedures reported by Williams et al. [51]. Reduction (nitrate) and degradation (arbutin, allantoin, and urea) tests were determined following methods reported by Collins et al. [52]. The sole carbon source was tested on ISP 9 (pH 9) with 1% (*w/v*) carbohydrate [30], and a nitrogen source at 0.1% (*w/v*) was evaluated using the basal medium proposed by Williams et al. [51].

2.5. Chemotaxonomy

Biomass for chemotaxonomic analyses was generated by cultivating strain M3C6^T in N-Z-Amine broth (DSMZ Medium No: 554) in shake flasks at 200 rpm and 28 °C for a duration of 10 days. Cells from the cultured centrifuged mass were extracted and washed twice with sterile distilled water before freeze-drying. Fatty acids were extracted from the cells, methylated, and separated using gas chromatography on an Agilent Technologies 6890 N (Agilent Technologies, Santa Clara, CA, USA) instrument. This process followed the standard protocol of the Sherlock Microbial Identification-MIDI system [53,54]. The resulting peaks of fatty acid methyl esters were quantified using the TSBA 5.0 database. Isoprenoid quinones were extracted and purified based on the methodology of Collins et al. [55] and were analyzed using high-performance liquid chromatography (HPLC) as per Kroppenstedt [56]. The whole-cell hydrolysates were prepared for the isomer determination of diaminopimelic acid and sugar following the methods described by Lechevalier and Lechevalier [4]. These hydrolysates were subsequently analyzed using thin-layer chromatography in accordance with the methods described by Stanek and Roberts [57]. Polar lipids were extracted and examined using the procedure of Minnikin et al. [58], incorporating modifications introduced by Kroppenstedt and Goodfellow [59].

3. Results and Discussion

3.1. Genotypic and Phylogenetic Analysis

The nearly complete 16S rRNA gene sequence of strain M3C6^T (1485 bp) has been deposited in the GenBank/EMBL/DDBJ database with accession number OQ520334. Strain M3C6^T was closely related to the genus *Nonomuraea* based on the 16S rRNA gene sequence analysis carried out in EzBioCloud, with the highest 16S rRNA gene sequence similarity to *N. basaltis* 160415^T (98.68%) and *N. turkmeniaca* DSM 43926^T (98.66%). On the other hand, the phylogenetic tree based on 16S rRNA gene sequences showed that strain M3C6^T formed a monophyletic clade with *N. phyllanthi* PA1-10^T (98.06%) (Figure 1). This relationship was

supported by the corresponding maximum-likelihood and maximum-parsimony-based trees (Supplementary Figures S1 and S2).

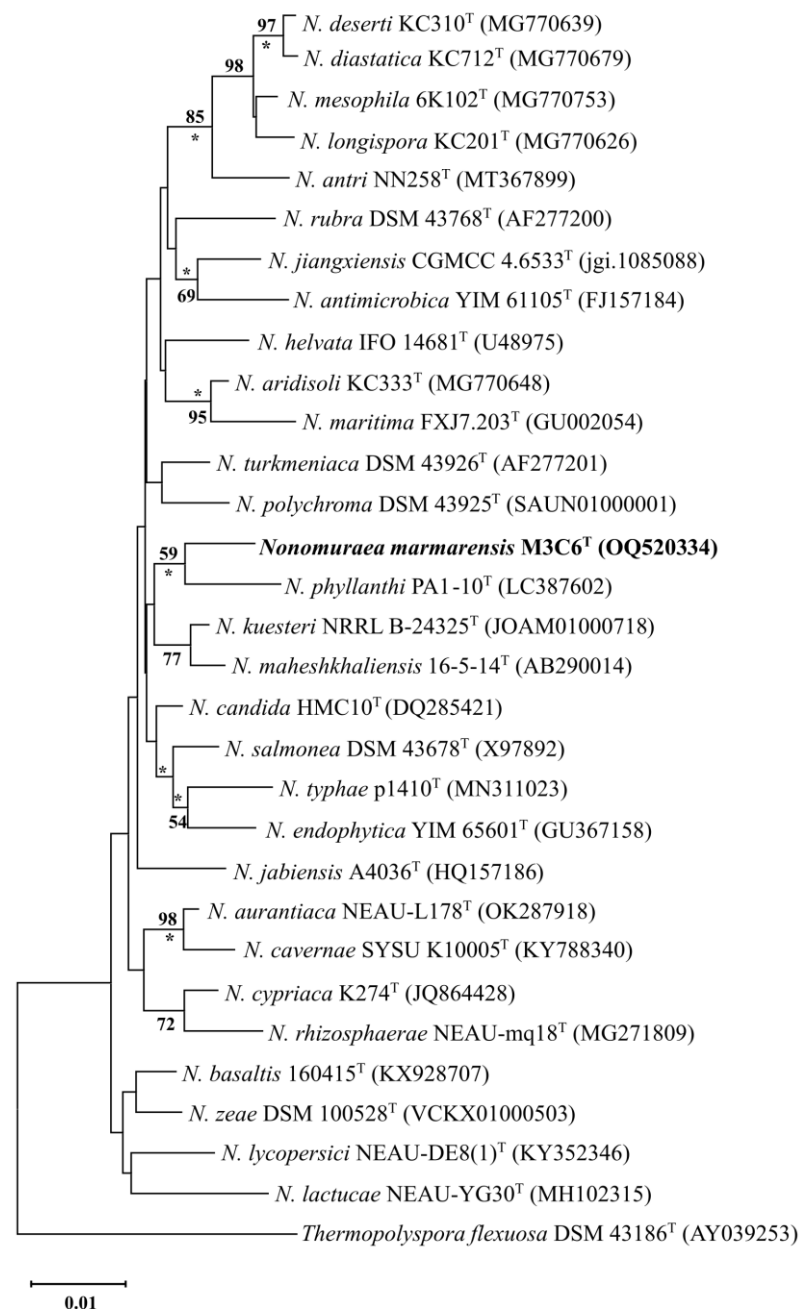


Figure 1. Neighbor-joining tree [33] based on almost complete 16S rRNA gene sequences showing the position of strain M3C6^T and amongst their phylogenetic neighbors. Asterisks (*) indicate branches of the tree that were also recovered using the maximum-likelihood [34] and maximum-parsimony [35] tree-making algorithms. Numbers at the nodes indicate the levels of bootstrap support (%); only values $\geq 50\%$ are shown. GenBank accession numbers are given in parentheses. Bar, 0.01 substitutions per site.

The draft genome sequencing of strain M3C6^T yielded a genome of 10,380,745 bp, assembled into 142 contigs with 10,377 coding sequences, an L50 of 15, and an N50 of 213,670 bp. The G + C content of strain M3C6^T was determined to be 69.5 mol%. Genomic features of strain M3C6^T and its closest type strains are summarized in Table 1. The dDDH value for comparing strain M3C6^T with *N. basaltis* 160415^T and *N. turkmeniaca* DSM 43926^T was found to be 33.7 and 31.8%, respectively. The ANIb values were 84.6% and

84.0% between strain M3C6^T with *N. basaltis* 160415^T and *N. turkmeniaca* DSM 43926^T, respectively. The phylogenomic tree revealed that the strain M3C6^T formed a cluster with *Nonomuraea jiangxiensis* CGMCC 4.6533^T with 100% bootstrap value (Figure 2). The highest dDDH and ANIb values of the strain M3C6^T were determined with *N. jiangxiensis* CGMCC 4.6533^T as 34.6% and 85.3%. All these values were below the threshold for bacterial species delineation [60], and these results confirm that strain M3C6^T represents a new taxon within the genus *Nonomuraea*. These results clearly show that the screening of unexplored habitats such as Marmara Island has a positive impact on revealing the biodiversity and distribution of this genus.

Table 1. General features of the genomes of strain M3C6^T and closely related type strains used in this study. Strains: **1**, M3C6^T; **2**, *N. basaltis* 160415^T; **3**, *N. turkmeniaca* DSM 43926^T; **4**, *N. phyllanthi* PA1-10^T; **5**, *N. jiangxiensis* CGMCC 4.6533^T.

	1	2	3	4	5
GenBank number	JBICRM000000000	VCJS000000000	VCKY000000000	VDLX000000000	FNDJ000000000
Genome size (bp)	10,380,745	14,334,648	11,350,886	11,027,144	11,808,550
Number of contigs	142	671	592	64	68
Genome coverage	90×	30×	30×	300×	67×
G+C content (%)	69.5	69.6	69.7	71.2	70.6
Genes (RNA)	87	81	75	78	87
Genes (total)	10,066	13,994	10,944	10,232	10,925
Genes (coding)	9827	13,067	10,275	9727	10,711
Longest contig size	618,571	176,753	143,656	1,156,183	759,783
N50 value	213,670	50,765	38,021	409,871	367,974
L50 value	15	86	96	9	12

Fourteen BGCs were detected in the genome of the strain M3C6^T. These biosynthetic gene clusters belonged to several cluster categories, such as lanthipeptide, NI-siderophore, NRPS, T1PKS, T3PKS, and terpene. Three out of the eighteen BGCs (Region 9.2, Region 24.1, and Region 63.1) detected in the genome of strain M3C6^T were putatively novel as these gene clusters do not show any similarity to any known compounds. The remaining ones were predicted to encode different compounds, including lysolipin I (43% gene identity), a lipophilic antibiotic for the treatment of Gram-positive and Gram-negative bacteria [61], 2-methylisoborneol (100% gene identity), a semi-volatile terpenoid compounds [62], and peucechelin (15% gene identity), a macrolide antibiotic exhibiting antibacterial activity [63]. The low similarities to known compounds for these BGCs highlight the potential of strain M3C6^T as a source of novel natural compounds. However, when interpreting the low similarity of BGCs located in the genome of strain M3C6^T to known compounds, the limitations of KnownClusterBlast and the insufficiencies of the current antiSMASH database should be taken into account. Further experimental validation is needed in order to confirm the biosynthetic potential of these gene clusters.

Genomic analysis of strain M3C6^T revealed 14 biosynthetic gene clusters responsible for the synthesis of bioactive compounds, indicating that the species could be a valuable resource for pharmaceutical and industrial applications. Screening of the type strain of this novel species, named *Nonomuraea marmarensis*, for new bioactive compounds could contribute to the solution of global problems such as antimicrobial resistance.

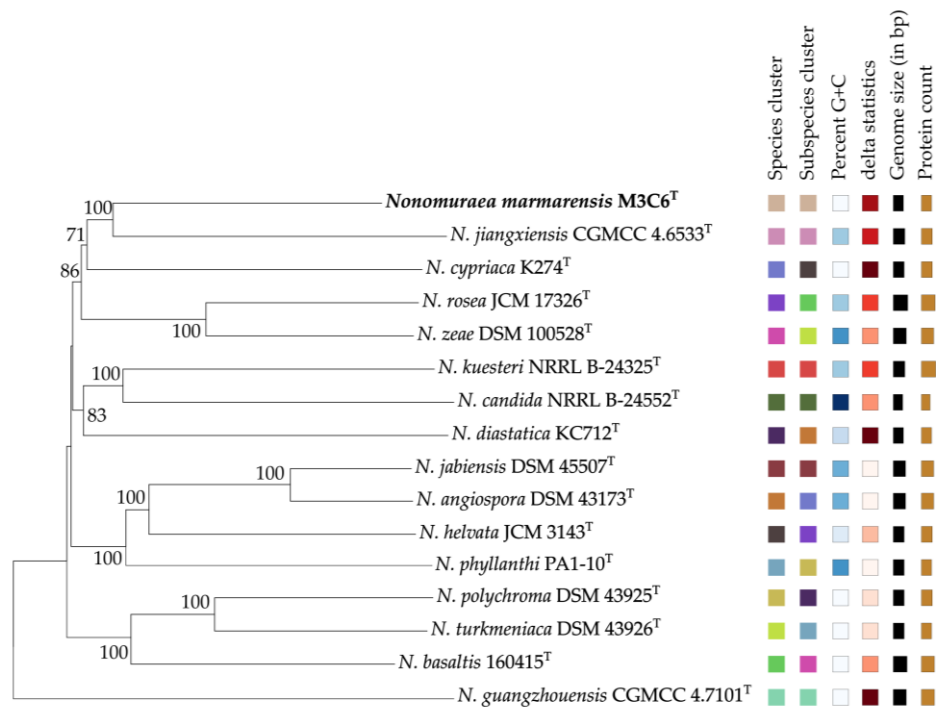


Figure 2. Phylogenetic tree based on whole-genome sequences of strain M3C6^T and related strains in the genus *Nonomuraea*. Tree inferred with FastME 2.1.6.1 [64] from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of the GBDP distance formula d5. The numbers above branches are GBDP pseudo-bootstrap support values > 60% from 100 replications, with an average branch support of 85.0%. The tree was rooted at the midpoint [65].

3.2. Morphology, Physiology and Biochemical Analysis

The electron micrograph demonstrates that isolate M3C6^T is capable of forming extensive substrate mycelium as well as aerial mycelium (Figure 3). Strain M3C6^T exhibited good growth on ISP 2, ISP 3, ISP 4, MBA, TSA, and NA, moderate growth on ISP 6 and CA, but poor growth on ISP 5 and ISP 7 media. The diffusible pigment was observed on ISP 2, ISP 5, and ISP 7 as claret red (Supplementary Table S1). Strain M3C6^T was found to grow at 28–37 °C (optimum, 28 °C), pH 5–11 (optimum, pH 7), and in the presence of 0–1% (*w/v*) NaCl. The isolate showed positive hydrolytic activity for arbutin, guanine, and urea. On the contrary, no hydrolysis was obtained for allantoin, adenine, casein, hypoxanthine, Tweens (20, 40, and 80), and xanthine. Detailed physiological and biochemical characteristics are given in the species description and in Table 2.

Table 2. Differential characteristics of strain M3C6^T and the type strain of closely related species of the genus *Nonomuraea*. Strains: **1**, M3C6^T; **2**, *N. basaltis* 160415^T; **3**, *N. turkmeniaca* DSM 43926^T; **4**, *N. phyllanthi* PA1-10^T; **5**, *N. jiangxiensis* CGMCC 4.6533^T.

	1	2	3	4	5
NaCl tolerance (% <i>, w/v</i>)	0–1	0–2	0–1	0–4	0–5
pH tolerance	5–11	6–9	7–9	5–9	6–10
Temperature range (°C)	28–37	10–40	20–40	20–40	28–37
Allantoin hydrolysis	–	+	–	–	–
Urea hydrolysis	+	–	+	+	–
Nitrate reduction	–	+	–	+	+
Nitrogen source utilization (0.1% <i>, w/v</i>)					
L-Arginine	+	–	+	+	+

Table 2. Cont.

	1	2	3	4	5
Glycine	+	—	+	+	+
L-Histidine	+	—	—	+	—
L-Proline	—	+	+	+	+
L-Threonine	—	+	+	—	+
Carbon source utilization (1.0%, w/v)					
D-Arabinose	+	+	—	—	—
D-Cellobiose	+	+	+	—	+
D-Fructose	+	—	+	—	+
D-Mannose	+	+	—	+	—
D-Melibiose	—	+	+	+	—
L-Sorbose	—	—	+	+	—
L-Rhamnose	+	+	—	—	—
Xylitol	—	—	+	—	+
Degradation of (% , w/v)					
Guanine (0.05%)	+	+	—	—	—
Hypoxanthine (0.4%)	—	+	—	+	+
Tween 20 (1.0%)	—	—	+	—	—
Tween 40 (1.0%)	—	—	+	+	—
Tween 80 (1.0%)	—	—	+	—	—
Xanthine (0.4%)	—	—	+	—	—
Major menaquinones (>10%)	MK-9(H ₄)	MK-9(H ₄), MK-9(H ₆) *	ND	MK-9(H ₄), MK-9(H ₂) #	MK-9(H ₄), MK-9(H ₆) ^γ
Major polar lipids	DPG, PE, OH-PE, 2OH-PE, PG, PI, PME	DPG, PG *	ND	DPG, PE, lyso-PE, PG, PIM, PME#	DPG, PE, PG, PI, PME, OH-PME ^γ
Major fatty acids (>10%)	<i>iso</i> C _{16:0} , <i>iso</i> C _{16:0} 2OH	<i>iso</i> C _{16:0} , C _{17:0} 10-methyl, <i>iso</i> C _{16:1} G	<i>iso</i> C _{16:0} , C _{17:0} 10-methyl, <i>iso</i> C _{16:0} 2OH	<i>iso</i> C _{15:0} , <i>iso</i> C _{16:0} , C _{17:0} 10-methyl	<i>iso</i> C _{16:0} , <i>iso</i> C _{16:1} G, C _{17:1} ω6c ^γ

Abbreviations: + positive, — negative; ND, not determined; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; OH-PE, hydroxy-phosphatidylethanolamine; 2OH-PE, dihydroxy-phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylmonomethylethanolamine, PIM, phosphatidyl inositol mannoside; lyso-PE, lyso-phosphatidylethanolamine. # Data taken from Klykleung et al. [66]. * Data taken from Saricaoglu et al. [67]. ^γ Data taken from Li et al. [68].

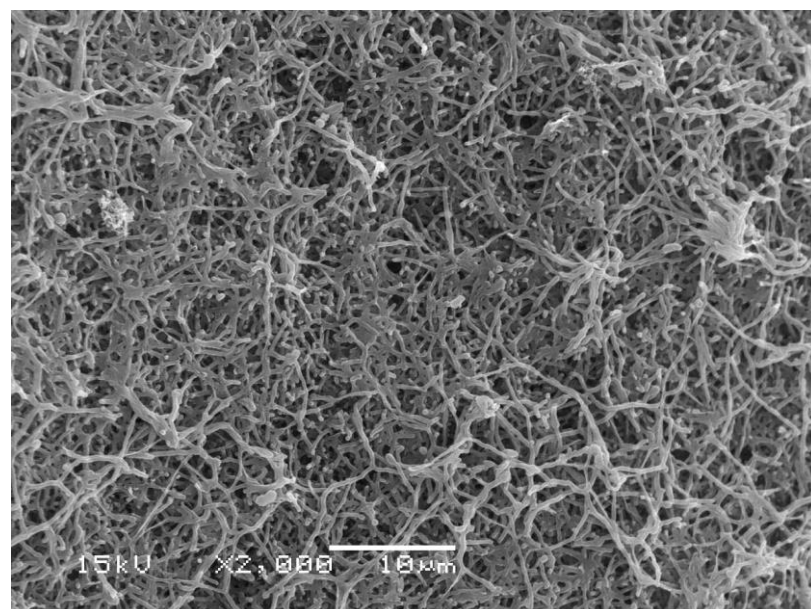


Figure 3. Scanning electronic micrograph of strain M3C6^T grown on ISP 3 at 28 °C for two weeks (magnification: 2000×).

3.3. Chemotaxonomic Characterization

Strain M3C6^T was observed to contain *meso*-diaminopimelic acid in the cell wall di-amino acid. Whole-cell hydrolysates contained glucose, madurose, and traces of arabinose and ribose. The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylinositol, hydroxy-phosphatidylethanolamine and dihydroxy-phosphatidylethanolamine, four unidentified glycerophospholipids, one uncharacterized glycolipid, one uncharacterized aminolipid, and three uncharacterized lipids (Supplementary Figure S3). The predominant menaquinone was MK-9(H₄) (71.3%) and minor menaquinones were MK-9(H₂) (9.6%), MK-9(H₈) (5.3%) and MK-9(H₆) (3.3%). Major cellular fatty acids were composed of *iso* C_{16:0} (35.9%) and *iso* C_{16:0} 2OH (11.9%) and minor fatty acids were C_{15:0} (9.5%), C_{17:0} 10-methyl (8.7%), C_{15:0} (5.4%), C_{16:0} 10-methyl (4.9%), C_{14:0} (4.4%), C_{16:0} (4.4%), *iso* C_{16:1} G (3.3%), *iso* C_{14:0} (1.7%), C_{17:0} (1.3%), and unknown 16.048 (1.8%) (Supplementary Table S2).

4. Conclusions

The comparison of cultural, chemotaxonomic, and phenotypic properties revealed distinct differences between strain M3C6^T and its closest phylogenetic neighbors, as summarized in Table 2. Based on the results of this polyphasic approach and genomic evidence, it is clear that strain M3C6^T is a novel species in the genus *Nonomuraea*, for which the name *Nonomuraea marmarensis* sp. nov. is herein proposed.

Further studies could be performed on the identification of bioactive compounds produced by this novel species and its ecological role. This will involve experimental validation of the BGCs identified in its genome. Studies on the contribution of the isolate to nutrient cycling and its interaction with other soil microbiota will be of interest. This study, also, underlines Marmara Island and other island ecosystems as promising sources for the discovery of novel bacterial species and stimulates further scientific exploration in such an environment.

Description of *Nonomuraea marmarensis* sp. nov.

Nonomuraea marmarensis (mar.ma.ren'sis. N.L. fem. adj. *marmarensis*, pertaining to Marmara Island in Istanbul province, from where the type strain was isolated).

Aerobic, Gram-positive, non-acid-fast, filamentous bacteria. Grows well on ISP 2, ISP 3, ISP 4, modified Bennett's, nutrient, and tryptic soy agars. The color of the colonies varies from cream to claret red. Growth occurs from 28 to 37 °C (optimum at 28 °C), pH 5 to 11 (optimum at pH 7), and in the presence of 0–1% (*w/v*) NaCl. Positive for hydrolysis of arbutin, guanine, and urea, but negative for allantoin, adenine, casein, hypoxanthine, Tweens (20, 40, 80), or xanthine. Nitrate is not reduced. It utilizes D-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, L-rhamnose, maltose, and xylose as sole carbon sources, but D-melibiose, L-sorbose, or xylitol are not utilized. L-Alanine, L-arginine, L-asparagine, glycine, L-histidine, α-isoleucine, L-methionine, and L-serine are used as sole nitrogen sources, but L-threonine and L-proline are not used. *Meso*-diaminopimelic acid is present in the cell wall and the whole-cell sugars are glucose, and madurose, with traces of arabinose and ribose. The major menaquinone is MK-9(H₄). The main polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, dihydroxy-phosphatidylethanolamine, phosphatidylinositol, and phosphatidylmethylethanolamine. The predominant components of the cellular fatty acids are *iso* C_{16:0} and *iso* C_{16:0} 2OH. The genomic DNA of the type strain contains 69.5 mol% G + C.

The type strain M3C6^T (= KCTC 49983^T = CGMCC 4.8035^T) was isolated from a soil sample collected from Marmara Island in Istanbul, Turkey. The GenBank/EMBL/DDBJ

accession number for the 16S rRNA gene and the genome sequence of the strain *Nonomuraea marmarensis* M3C6^T are OQ520334 and JBICRM000000000, respectively.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/taxonomy5010005/s1>, Figure S1: Maximum-likelihood tree based on almost complete 16S rRNA gene sequences showing the position of strain M3C6^T amongst its phylogenetic neighbors; Figure S2: Maximum-parsimony tree based on almost complete 16S rRNA gene sequences showing the position of strain M3C6^T amongst its phylogenetic neighbors; Figure S3: The phospholipids of strain M3C6^T. Diposphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), hydroxy-phosphatidylethanolamine (OH-PE), dihydroxy-phosphatidylethanolamine (2OH-PE), phosphatidylmethylethanolamine (PME), four unidentified glycerophospholipids (GPL₁₋₄), an unidentified glycolipid (GL), an unidentified aminolipid (AL), and three unidentified lipids (L₁₋₃); Table S1: Cultural characteristics of the M3C6^T and closely related type strains grown at 28 °C for 14 days; Table S2: Fatty acids profiles (%) of strain M3C6^T and type strains of closely related species. Fatty acids amounting to less than 1.0% in all strains were omitted.

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