**Lacinutrix gracilariae** sp. nov., isolated from the surface of a marine red alga *Gracilaria* sp.

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A Gram-stain-negative, aerobic, non-flagellated, rod-shaped bacterium, designated strain Lxc1T, was isolated from the surface of a marine red alga, *Gracilaria* sp., which was collected from the coastal regions in Jinjiang, Fujian Province, China. Colonies of the strain were orange–yellow, circular and smooth. The 16S rRNA gene of strain Lxc1T had maximum sequence similarity with *Lacinutrix himadriensis* E4-9aT (97.1 %), followed by *Lacinutrix jangbogonensis* PAMC 27137T, *Lacinutrix copepodicola* DJ3T, *Lacinutrix algicola* AKS293T and *Lacinutrix mariniflava* AKS 432T (similarities <96.4 %). Phylogenetic analysis showed strain Lxc1T formed a tight cluster with *L. himadriensis* E4-9aT and *L. copepodicola* DJ3T, but represented a novel lineage belonging to the genus *Lacinutrix*. The predominant fatty acids were iso-C15:0 3-OH (18.3 %), iso-C15:0 (16.7 %), iso-C17:0 3-0H (10.6 %) and iso-C15:0 3-OH (8.6 %). Menaquinone-6 (MK-6) was the only respiratory quinone present. The DNA G+C content of strain Lxc1T was 31.7 mol%. Combining the results above, it was ascertained that strain Lxc1T represented a novel species of the genus *Lacinutrix*, for which the name *Lacinutrix gracilariae* sp. nov. is proposed. The type strain is Lxc1T (=MCCC 1A01567T=KCTC 42808).  

The genus *Lacinutrix*, belonging to the family *Flavobacteriaceae*, order *Flavobacteriales* in the class *Flavobacteria* of the phylum *Bacteroidetes* was first coined and characterized by Bowman & Nichols (2005), with the description of the type species *Lacinutrix copepodicola* (type strain DJ3T), isolated from the lake-dwelling calanoid copepod, *Paralabidocera antarctica*. After that, six novel species were subsequently isolated and identified. These include *Lacinutrix algicola* and *Lacinutrix mariniflava*, which were isolated from a red alga belonging to the family *Gigartinaceae* growing in subtidal zone in Antarctica (Nedashkovskaya et al., 2008). *Lacinutrix himadriensis* was isolated from a marine sediment sample at a depth of 276 m in the Arctic Ocean (Srinivas et al., 2013), and *Lacinutrix jangbogonensis* was isolated from Antarctic marine sediment at a depth of 156 m (Lee et al., 2014). *Lacinutrix venerupis* was isolated from marine clams (*Venerupis decussate* and *Venerupis philippinarum*) on the Galician coast (Lasca et al., 2015), and

*Lacinutrix undariae* was isolated from a brown algae reservoir in the South Sea in South Korea (Park et al., 2015).

During our investigation of pigmented bacteria associated with the surface of marine alga, a bacterium forming orange–yellow colonies was isolated and designated strain Lxc1T. To determine the exact taxonomic status of strain Lxc1T, polyphasic characterization including phenotypic characteristics, biochemical and physiological properties, and phylogenetic analysis based on the 16S rRNA gene were carried out in this study.

The first author wishes to acknowledge Dr. Zongze Shao, who provided valuable assistance and guidance during the entire process of this study. The authors are also grateful to Dr. Li Gu and Dr. Guizhen Li for their valuable suggestions. This work was supported by grants from the National Natural Science Foundation of China (41606130), the Innovation Program of Fujian Academy of Sciences (2014J003), the Key Projects of Science and Technology of Fujian Provincial Department of Education (JAT16103), and the National Key R & D Program of China (2016YFA0502702).
Isoprenoid quinone-containing extracts of strain Lxc1T were analysed using the universal primers Eubac27F and 1492R (DeLong, 1992) with the Ex Taq kit (TaKaRa). The resulting PCR product was ligated into the PMD 18-T vector (TaKaRa) and transferred into chemically competent *Escherichia coli* DH5α. Positive clones were screened for the insert fragment using colony PCR with the vector primer RV-M and M13-47. Sequences of the two positive clones were determined from both ends using the vector primers RV-M and M13-47 with an ABI 3730 sequencer (Shanghai Majorbio Bio-Pharm Technology). The full-length 16S rRNA gene (1444 nt) was retrieved after removal of the vector sequence and the PCR primer.

The closest taxa based on high 16S rRNA gene sequence similarities were retrieved by searching recognised species in the EzTaxon database (Kim et al., 2012). The sequences were aligned using the **Clustal W** program implemented in the MEGA 6.0 software package (Tamura et al., 2013), and the phylogenetic tree was reconstructed using three different algorithms, neighbour-joining, maximum-likelihood and maximum-parsimony. The best nucleotide substitution model for the maximum-likelihood method was selected with the lowest BIC scores (Bayesian Information Criterion). The tree topology was evaluated by bootstrap analysis with 1000 replicates in each phylogenetic analysis.

The 16S rRNA gene sequence of strain Lxc1T had 97.1% similarity with *L. himadriensis* E4-9aT. The novel strain was more distant to other species of the genus *Lacinutrix* deposited in EzTaxon database, with 16S rRNA gene sequence similarities of 96.4% or lower. Phylogenetic analysis showed that strain Lxc1T formed a tight cluster with *L. himadriensis* E4-9aT and *L. copepodica* DJ3T (Fig. 1 and Figs S1 and S2, available in the online Supplementary Material) within the genus *Lacinutrix*, but in a separate lineage, indicating that strain Lxc1T may represent a novel bacterial species.

The draft genome of strain Lxc1T was determined using an Illumina Hiseq 2500 platform (Shanghai Majorbio Bio-Pharm Technology) following the manufacturer’s instructions. The raw reads were cleaned using FASTX _Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html) to remove bases below Q20 and shorter than 100 bp. The sequence was assembled using SPAdes version 3.5.0 with the default parameter for assembling pair end reads of 150 bp (Bankevich et al., 2012). The genomic DNA G+C content was determined to be 31.7 mol% after using **QUAST** (Gurevich et al., 2013), which is in accordance with the G+C content range of the genus *Lacinutrix* (29–37 mol%; Srinivas et al., 2013).

Isoprenoid quinone-containing extracts of strain Lxc1T were analysed using reversed-phase HPLC as previously described (Komagata & Suzuki, 1987), revealing that menaquinone-6 (MK-6) was the only respiratory quinone present. This corresponds with data obtained for the genus *Lacinutrix* (Srinivas et al., 2013) and is the quinone found for all members of the family *Flavobacteriaceae* (Bernardet et al., 2002).

Cell morphology was observed using light microscopy and by transmission electron microscopy (JEM-1230; JEOL).

The growth temperature of strain Lxc1T was determined in marine broth 2216 (MB; Difco, BD), testing at temperatures of 5, 10, 15, 25, 28, 35, 40 and 45 °C. The optimal salinity was determined using the basic medium Luria–Bertani broth supplemented with NaCl concentrations of 0, 0.5, 1, 2, and 3 % (w/v), and also by using MB supplemented with NaCl concentrations of 4, 5, 6, 7, 8, 9, 10, 12, 15, 18 and 20 % (w/v). The pH growth range was tested in MB ranging from pH 3 to pH 11 (at one unit intervals), adjusting the pH with citrate/phosphate buffer (pH 3–7), Tris/HCl buffer (pH 8–9) or sodium carbonate/sodium bicarbonate buffer (pH 10–11).

For comparison, the type strains, *L. himadriensis* E4-9aT, *L. algicola* AKS293T and *L. mariniflava* AKS432T were selected as reference cultures, and retrieved from the Korean Collection for Type Cultures (KCTC), South Korea. Unfortunately, the type strain, *L. copepodica* DJ3T was not available in KCTC and could not be accessed in the Institut Pasteur’s Biological Resources Center (CRBIP, France) either. Thus, this strain could not be used in this study. These type strains were cultured on MA plates, and suspensions prepared using sterile seawater to inoculate into API 20NE, API 20E and API ZYM (bioMérieux) test strips according to the manufacturer’s instructions. Test strips were cultured at 15 °C for comparing the physiological properties. For the substrate degradation tests, all strains were inoculated into the basal medium, MA 2216, containing soluble starch, xylan, skimmed milk, Tweens 20, 40, 60 and 80, casein or tributyrin (Table 1). To detect the agar and soluble starch hydrolysis, plates were flooded with Lugol’s iodine solution. Otherwise, hydrolysis was directly observed if there was a transparent zone around growth on the agar surface. Cellular fatty acids were extracted from cells grown on MA at 25 °C for 48 h and identified following the standard MIDI protocol (Sherlock Microbial Identification System, version 6B). The predominant fatty acids of strain Lxc1T were iso-C15:1 G (18.3 %), iso-C15:0 (16.7 %), iso-C17:0 3-OH (10.6 %), and iso-C15:0 3-OH (8.6 %). The detailed fatty acids composition is shown in Table 2.

The results of phylogenetic analysis of the 16S rRNA gene combined with the biochemical and physiological characterization showed that strain Lxc1T represents a novel species of the genus *Lacinutrix* (Figs 1, S1 and S2), for which the name *Lacinutrix gracilariae* sp. nov. is proposed. The closest taxa are *L. himadriensis* E4-9aT and *L. copepodica* DJ3T. It was observed that the topology of the phylogenetic tree places five species of the genus *Lacinutrix*, *L. algicola*, *L. mariniflava*, *L. jangbogonensis*, ‘*L. venerisp*’ and *L. undariae*, into a separate cluster.

**Description of Lacinutrix gracilariae** sp. nov.

*Lacinutrix gracilariae* (gra.ci.la’ri.ae. N.L. gen. n. gracilariae of the red alga *Gracilaria*).

Cells are Gram-stain-negative, non-flagellated and rod-shaped, 1.4–1.9 μm long and 0.5 μm wide (Fig. S3).
Winogradskyella undariae WS-MY5T (KC261665)
Winogradskyella pacifica KMM 6019T (GQ181061)
Winogradskyella psychrotolerans RS-3T (ATMR01000166)
Winogradskyella thalassocella KMM 3907T (AY521223)
Winogradskyella arenosi R807 (AB483962)
Winogradskyella rapida SCB36 (U64013)
Winogradskyella epiphytica KMM 3906T (AY521224)
Winogradskyella multivorans T-Y1T (JQ354979)
Winogradskyella lutea A73T (FJ919968)
Winogradskyella damuponensis F081-2T (HQ336488)
Winogradskyella eximia KMM 3944T (AY521225)
Winogradskyella echinorum KMM 6211T (EU727254)
Winogradskyella pulchriflava EM106T (JN865998)
Winogradskyella ukae KMM 6390T (HQ456127)
Winogradskyella exilis 022-2-26T (FJ595484)
Winogradskyella poriferorum UST030701-295T (AY848823)
Winogradskyella aquimaris DPG-24T (HM368527)
Winogradskyella litorisediminis DPS-3T (JQ432561)
Winogradskyella wandonensis WD-2-2T (KF768343)
Winogradskyella jejunensis CP27T (JF820844)

Lacinutrix gracilariae sp. nov.

Flavobacterium fluvii H7T (EU109724)
Colonies on MA are orange–yellow, circular (less than 1 mm in diameter), and glossy. Growth occurs in media containing 0.5–8 % (w/v) NaCl; the optimal salinity for growth is about 2 % (w/v) NaCl. The growth temperature ranges from 5 °C to 35 °C, with the optimal temperature at approximately 28 °C. Growth occurs at pH 6.0–9.0 with optimal growth at pH 7.0. The isoprenoid quinone is MK-6. The predominant fatty acids are iso-C_{15}:1 G, iso-C_{15:0}, iso-C_{17:0} 3-OH and iso-C_{15:0} 3-OH. Hydrolyses skimmed milk, agar and Tween 20, but not soluble starch, xylan, Tween40, Tween 60, Tween 80, casein or tributyrin. Positive result in tests for alkaline phosphatase, lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase and trypsin activities; weakly positive for esterase (C4), leucine aminopeptidase, valine arylamidase, cystine aminopeptidase and a-glucosidase activities; and negative for lipase (C14), a-chymotrypsin, a-galactosidase, b-galactosidase, b-glucuronidase, b-glucosidase, N-acetyl-b-glucosaminidase, a-mannosidase and a-fucosidase activities. Produces gelatinase, but cannot produce b-galactosidase, arginine dihydrolase, lysole decarboxylase, ornithine decarboxylase, urease or tryptophan deaminase. The Voges–Proskauer reaction is positive, but citrate utilization, oxidase activity, H_{2}S production and indole production tests are negative.

### Table 1. Phenotypic characteristics of strain Lxc1\textsuperscript{T} and type strains of the species of the genus Lacinutrix

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>4</th>
<th>5</th>
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<td>Hydrolysis of</td>
<td></td>
<td></td>
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<tr>
<td>Skimmed milk</td>
<td>+</td>
<td>++</td>
<td>++</td>
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<td>ND</td>
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<tr>
<td>Agar</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Tween 20</td>
<td>++</td>
<td>+</td>
<td>++</td>
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<td>ND</td>
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<tr>
<td>Enzyme activity</td>
<td></td>
<td></td>
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<tr>
<td>Esterase (C4)</td>
<td>w</td>
<td>-</td>
<td>w</td>
<td>w</td>
<td>ND</td>
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<tr>
<td>Lipase (C8)</td>
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<td>-</td>
<td>w</td>
<td>+</td>
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</tr>
<tr>
<td>Lipase (C14)</td>
<td>-</td>
<td>-</td>
<td>w</td>
<td>-</td>
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<td>w</td>
<td>+</td>
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<td>Valine arylamidase</td>
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<td>+</td>
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<td>Cystine aminopeptidase</td>
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<td>+</td>
<td>w</td>
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<td>-</td>
<td>ND</td>
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<tr>
<td>a-Glucosidase</td>
<td>w</td>
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<td>w</td>
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<td>Mannitol fermentation</td>
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<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Aesculin hydrolysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>4-Nitrophenyl-b-D-galactopyranoside</td>
<td>w</td>
<td>-</td>
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<td>Utilization of</td>
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<tr>
<td>D-Mannose</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Maltose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>31.7</td>
<td>29.8</td>
<td>37.0</td>
<td>34.7</td>
<td>37.0</td>
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Table 2. Fatty acid contents (%) of strain Lxc1T and type strains of species of the genus Lacinutrix

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<th>Fatty acid</th>
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<td>C13 : 1</td>
<td>ND</td>
<td>1.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>iso-C13 : 0</td>
<td>TR</td>
<td>2.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>anteiso-C13 : 0</td>
<td>TR</td>
<td>2.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>iso-C14 : 0</td>
<td>1.3</td>
<td>ND</td>
<td>4.1</td>
<td>TR</td>
<td>ND</td>
</tr>
<tr>
<td>C15 : 0</td>
<td>ND</td>
<td>ND</td>
<td>6.7</td>
<td>1.6</td>
<td>3.7</td>
</tr>
<tr>
<td>C15 : 0 2-OH</td>
<td>1.8</td>
<td>TR</td>
<td>1.9</td>
<td>2.5</td>
<td>ND</td>
</tr>
<tr>
<td>C15 : 0 3-OH</td>
<td>1.2</td>
<td>ND</td>
<td>1.1</td>
<td>ND</td>
<td>0.2</td>
</tr>
<tr>
<td>iso-C15 : 0</td>
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<td>13.8</td>
<td>12.5</td>
<td>10.6</td>
<td>19.9</td>
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<td>4.6</td>
<td>12.5</td>
<td>18.2</td>
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<td>iso-C15 : 0 3-OH</td>
<td>8.6</td>
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<td>10.0</td>
<td>12.2</td>
<td>3.1</td>
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<tr>
<td>C15 : 0:06c</td>
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<td>3.0</td>
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<td>1.4</td>
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<tr>
<td>iso-C15 : 1 G</td>
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<td>21.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>anteiso-C15 : 1 A</td>
<td>2.9</td>
<td>18.6</td>
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<tr>
<td>C16 : 0</td>
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<td>1.0</td>
<td>TR</td>
<td>2.9</td>
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<tr>
<td>iso-C16 : 1 G</td>
<td>1.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>iso-C16 : 0</td>
<td>1.7</td>
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<td>3.4</td>
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<td>iso-C16 : 0 3-OH</td>
<td>4.4</td>
<td>ND</td>
<td>14.4</td>
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<td>iso-C17 : 0 3-OH</td>
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<td>3.6</td>
<td>7.6</td>
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<td>C17 : 0 2-OH</td>
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<td>TR</td>
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<tr>
<td>anteiso-C17 : 0</td>
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<td>ND</td>
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<td>iso-C18 : 0:09c</td>
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<td>ND</td>
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<td>C18 : 0</td>
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<td>ND</td>
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<tr>
<td>Summed*</td>
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<td>3.6</td>
<td>2.0</td>
<td>9.2</td>
<td>5.8</td>
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<tr>
<td>8</td>
<td>4.8</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>1.9</td>
<td>1.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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</table>

*Summed features represent groups of fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 comprises C14 : 10:7c and/or C16 : 10:6c; summed feature 8 comprises C18 : 10:6c and/or C18 : 10:6c; summed feature 9 comprises iso-C17 : 10:9c and/or C16 : 0 10-methyl.

Acknowledgements

This work was financially supported by the project of Xiamen Southern Oceanographic Center (14CZP034HJ08), Public Welfare Project of SOA (201005032) and National Infrastructure of Microbial Resources of China (NIMR-2015-9).

References


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