The genus *Geobacillus* was proposed by Nazina et al. (2001), since when descriptions of several novel species have been published (Fortina et al., 2001; Sung et al., 2002; Banat et al., 2004; Nazina et al., 2004; Kuisiene et al., 2004; Schäffer et al., 2004; Miñana-Galbis et al., 2010; Dinsdale et al., 2011; Poli et al., 2011; Coorevits et al., 2012). At the time of writing, the genus *Geobacillus* comprises 15 species with validly published names.

During the course of research into unique microbial communities living in the Valley of Geysers, Kamchatka, Russia, we selected microbiological samples from bottom sediments of hydrothermal outlets. One novel thermophilic strain of spore-forming bacteria was isolated. The aim of this study was to provide a comprehensive characterization of the strain. A polyphasic taxonomic study, including phylogenetic analysis of 16S rRNA and *spo0A* gene sequences, the strain is considered to represent a novel species of the genus *Geobacillus*, for which the name *Geobacillus icigianus* sp. nov. is proposed. The type strain is G1w1^T (=VKM B-2853^T=DSM 28325^T).

A Gram-reaction-positive, motile, thermophilic spore-forming strain, G1w1^T, was isolated from a hot spring of the Valley of Geysers, Kamchatka (Russia). Based on data from the present polyphasic taxonomic study, including phylogenetic analysis of 16S rRNA and *spo0A* gene sequences, the strain is considered to represent a novel species of the genus *Geobacillus*, for which the name *Geobacillus icigianus* sp. nov. is proposed. The type strain is G1w1^T (=VKM B-2853^T=DSM 28325^T).

Strain G1w1^T was isolated from sludge samples of an unnamed explosive hydrothermal (97 °C) outlet situated near the Troinoy geyser (Valley of Geysers, Kronotsky Nature Reserve). A microbial community comprising a deep-green incrustation on pink sludge sediment was clearly visible to the naked eye. Water and sludge samples were collected using a sterile sampler into sterile containers and stored at 4 °C. Some samples were fixed using an equal volume of 96% ethanol. Fermentation of the microbial community was carried out on Luria–Bertani (LB) medium at 45–70 °C for 1–3 days (Gerhardt et al., 1994). Isolation and purification of the strain were conducted on LB agar medium at 55–65 °C.

The reference strains used in this study were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (*Geobacillus stearothermophilus* DSM 22^T, *Geobacillus thermoglucosidasius* DSM 2542^T) and from the All-Russian Collection of Microorganisms-VKM (*Geobacillus jurassicus* B-2301^T, *Geobacillus thermodenitrificans* B-1259^T). Strains were grown on LB agar medium at 55–65 °C. Biomass for chemical and molecular systematic studies was obtained by cultivating the organisms in shake flasks containing liquid LB medium at 55–60 °C for 12–24 h. Bacterial growth in the medium was monitored by measuring the optical density of the culture at 600 nm using an automatic multifunctional Epoch Microplate Spectrophotometer (BioTek). Medium was supplemented with 5 mg MnSO₄ 1⁻¹ to encourage sporulation.

The shape and size of cells were determined using light and electron microscopes (Axioskop 2 Plus, Axioskop A1; Zeiss), at the Interinstitutional Shared Center for Microscopic Analysis of Biological Objects SB RAS. Bacterial samples were prepared by conventional methods (Netrusov et al., 2005). The Gram reaction was determined using a Gram stain kit (Sintakon) according to the manufacturer’s recommended protocol. Characteristics of strain G1w1^T such as temperature and pH ranges for growth, NaCl tolerance, catalase, urease and oxidase activity, anaerobic growth, starch and casein degradation, gelatin liquefaction, citrate utilization, nitrate reduction, acid production and growth with various carbohydrates were tested according to Netrusov et al. (2005) and Logan & De Vos (2009). Most of the tests were carried out using reagents and kits produced by Lachema, DIA-M and Sigma. Tests were performed in triplicate.

**Abbreviations:** ANI, average nucleotide identity; ME, minimum-evolution.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *spo0A* gene sequences of strain G1w1^T are KF631430.1 and KF631431.1, respectively. The full genome sequence of strain G1w1^T has been deposited at DDBJ/EMBL/GenBank under accession number JPYA00000000.1.

One supplementary figure is available with the online Supplementary Material.
Microbial DNA was extracted using standard methods with phenol (Maniatis et al., 1982). Amplification of the 16S rRNA gene was conducted with universal bacterial primers 16S-8-f-B (5′-AGRGTGTATTGCTTGCTCA-3′) and 16S-1350-r-B (5′-GACGGCCGTTGTTACAG-3′). The fragment of the spo0A gene was amplified with primers GEOSPO-20F73 (5′-CAGCCGGAATGGAATGTGAT-3′) and GEOSPO-20R696 (5′-GACCGTATAGGCCAACAG-CG-3′) (Kuisiene et al., 2009). The repressor feed contained 1.5 mM MgCl$_2$, 65 mM Tris/HCl (pH 8.8), 16 mM  

To determine the taxonomic affinity of strain G1w1T we analyse full-length sequences of the aforementioned genes. The 16S rRNA gene sequence determined for strain G1w1T was 3692 nt long. Strain G1w1T are given in Table 1. Strain G1w1T was able to utilize a variety of sugars, carboxylic acids and hydrocarbons.

The main fatty acid of strain G1w1T was iso-C$_{15:0}$. The fatty acid profile of strain G1w1T included iso-C$_{15:0}$ (39.7 %), iso-C$_{17:0}$ (26.9 %), anteiso-C$_{17:0}$ (11.6 %), C$_{16:0}$ (7.7 %), anteiso-C$_{15:0}$ (6.8 %), anteiso-C$_{14:0}$ (2.4 %) and C$_{18:0}$ (1.1 %). No other fatty acids were present at more than 1 %.

A phylogenetic tree was reconstructed using 165 rRNA gene sequences of members of the genus Geobacillus from the GenBank database (Fig. 1). Based on 16s rRNA gene sequences, strain G1w1T is not a member of any recognized species of the genus Geobacillus.

The DNA G+C content of strain G1w1T was 52 mol%. This value is in accordance with values reported for the genus Geobacillus, which are in the range 49.0–58.0 mol% (Nazina et al., 2001).

For a number of strains belonging to the genus Geobacillus, full genomic sequences are known, although the molecular identification of this genus has been limited to use of 16s rRNA gene sequences, as data on other sequences are unavailable for most type strains. Kuisiene et al. (2009) studied the taxonomy of the genus Geobacillus based on spo0A gene sequences – this is the main regulator of the sporulation process, which controls more than 500 genes. The authors showed that by using this marker it was possible to identify reliably only G. thermodenitrificans, G. stearothermophilus and G. jurassicus. They therefore concluded that spo0A gene sequences cannot be used as a phylogenetic marker within this genus (Kuisiene et al., 2009).

Despite this, for a number species of the genus Geobacillus, the spo0A gene remains the only one to be sequenced. We have reconstructed a phylogenetic tree based on spo0A gene sequences (Fig. 2). This again shows that strain G1w1T is separate from all other representatives of the genus.

The following ANI scores were obtained when comparing strain G1w1T with the type strains of Geobacillus species: 86.41 % for G. stearothermophilus ATCC 7953$^T$, 87.75 % for G. kaustophilus NBRC 102445$^T$ and 85.47 % for G. thermodenitrificans DSM 465$^T$. These values are well below the threshold range of 95–96 %, indicating that strain G1w1T represents a distinct species (Kim et al., 2014).
Thus, we concluded strain G1w1T is a member of the genus *Geobacillus*, but differs markedly based on biochemical and molecular genetic characteristics. Therefore, we suggest that strain G1w1T represents a novel species of the genus *Geobacillus*, for which the name *Geobacillus icigianus* sp. nov. is proposed.
**Fig. 1.** ME phylogenetic tree reconstructed based on 16S rRNA gene sequences. Numbers near the branches are ME bootstrap support values. Bar, 0.005 changes per nucleotide position.

**Fig. 2.** ME phylogenetic tree reconstructed based on spo0A gene sequences. Numbers near the branches are ME bootstrap support values. Bar, 0.02 changes per nucleotide position.
Description of Geobacillus icigianus sp. nov.

Geobacillus icigianus [i.ci.gi.a’nus. N.L. masc. adj. icigianus referring to the Institute of Cytology and Genetics (IGG) at Novosibirsk where the type strain was isolated and described].

The description is based on a single strain. Cells are Gram-reaction-positive, motile rods, 0.5–1.0 × 3.5–6.0 μm, with terminal ellipsoidal or cylindrical endospores 1.5 × 3.0 μm in size. Sporulation is extremely rare. Swollen sporangia are not observed. Aerobic or facultatively anaerobic. After 24 h of incubation at 60 °C forms circular cream-coloured colonies with smooth or slightly curved edges, 3–5 mm in diameter. Grows at 50 and 75 °C, and optimally at 60–65 °C. Grows between pH 5.0 and 9.0; optimum growth at pH 6.5–7.0. Growth is rapid in medium with 1% (w/v) NaCl and is inhibited in the presence of 5% NaCl. Oxidase-negative, but catalase- and urease-positive. Aesculin and casein are hydrolysed, but starch and ONPG are not. Growth occurs on yeast extract; glucose and xylose can be utilized as sole carbon sources. Gelatin hydrolysis and nitrate reduction are positive and the Voges–Proskauer reaction is weak, but citrate utilization, and arginine, lysine, ornithine and malonate production are negative. Utilization of adonitol, sorbitol and dulcitol is weak. Acid is produced from D-xylose but not from lactose or inositol. Production of acids from cellobiose, glucose, mannitol, melibiose, raffinose, rhamnose, sucrose and trehalose is weak.

The type strain is G1w1T (=VKM B-2853T =DSM 28325T), which was originally isolated from a water–sludge sample of the hydrothermal (97 °C) outlets of the Troinoy geyser (Valley of Geysers, Kamchatka). The DNA G+C content of the type strain is 52 mol%.

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