Characterization of clinical and environmental isolates of *Vibrio cidicii* sp. nov., a close relative of *Vibrio navarrensis*

Fabini D. Orata,¹ Yue Xu,¹ Lori M. Gladney,²,³ Lavanya Rishishwar,⁴,⁵,⁶ Rebecca J. Case,¹ Yan Boucher,¹ I. King Jordan⁴,⁵,⁶† and Cheryl L. Tarr²†

¹Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada
²Enteric Diseases Laboratory Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA
³IHRC, Incorporated, Atlanta, GA, USA
⁴School of Biology, Georgia Institute of Technology, Atlanta, GA, USA
⁵Applied Bioinformatics Laboratory, Atlanta, GA, USA
⁶PanAmerican Bioinformatics Institute, Santiago de Cali, Valle del Cauca, Colombia

Four *Vibrio* spp. isolates from the historical culture collection at the Centers for Disease Control and Prevention, obtained from human blood specimens (*n*=3) and river water (*n*=1), show characteristics distinct from those of isolates of the most closely related species, *Vibrio navarrensis* and *Vibrio vulnificus*, based on phenotypic and genotypic tests. They are specifically adapted to survival in both freshwater and seawater, being able to grow in rich media without added salts as well as salinities above that of seawater. Phenotypically, these isolates resemble *V. navarrensis*, their closest known relative with a validly published name, but the group of isolates is distinguished from *V. navarrensis* by the ability to utilize L-rhamnose. Average nucleotide identity and percent DNA–DNA hybridization values obtained from the pairwise comparisons of whole-genome sequences of these isolates to *V. navarrensis* range from 95.4–95.8% and 61.9–64.3%, respectively, suggesting that the group represents a different species. Phylogenetic analysis of the core genome, including four protein-coding housekeeping genes (pyrH, recA, rpoA and rpoB), places these four isolates into their own monophyletic clade, distinct from *V. navarrensis* and *V. vulnificus*. Based on these differences, we propose these isolates represent a novel species of the genus *Vibrio*, for which the name *Vibrio cidicii* sp. nov. is proposed; strain LMG 29267T (=CIP 111013T=2756-81T), isolated from river water, is the type strain.

The genus *Vibrio* consists of over 100 species of bacteria autochthonous to the aquatic environment (Gomez-Gil et al., 2014). Species of clinical significance, such as *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*, have

---

**Correspondence**

Yan Boucher
yboucher@ualberta.ca

**Abbreviations:** ANI, average nucleotide identity; API, Analytical Profile Index; CDC, Centers for Disease Control and Prevention; COG, Clusters of Orthologous Groups of proteins; DDH, DNA-DNA hybridization; GGDC, Genome-to-Genome Distance Calculator; MLSA, multilocus sequence analysis; PM1, Phenotype MicroArray 1; TCBS, thiosulfate citrate bile salts sucrose; TSB, tryptic soy broth.

The GenBank/EMBL/DDJB accession numbers for the whole-genome sequences of *Vibrio cidicii* LMG 29267T, 1048-83, 2423-01 and 2538-88 are LOMK0000000, LOBP00000000, LOBO00000000 and LOR00000000, respectively, under BioProject accession number PRJNA304180. Accession numbers for the *rpoB* sequences of the same strains are KU593643, KU593646, KU593645 and KU593644, respectively, and for the *rpoB* sequences of *Vibrio navarrensis* LMG 15976T, 2232, 0053-83 and 08-2462 are KU593635, KU593636, KU593629 and KU593637, respectively. The accession number for the 16S rRNA gene sequence of *V. cidicii* LMG 29267T is KJ807108.

Seven supplementary tables and two supplementary figures are available with the online Supplementary Material.
been studied in depth, as clear identification of pathogens is recognized as essential for the treatment of the disease and epidemiologic surveillance. *Vibrio navarrensis*, a species not previously associated with human illness, has received little attention since the original description of isolates from sewage in 1991 (Urdaci et al., 1991). However, this species has recently been the focus of investigation after the identification of *V. navarrensis* by the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) among clinical isolates submitted for routine characterization (Gladney et al., 2014; Gladney & Tarr, 2014). Constructing an evolutionary framework that included contemporary *V. navarrensis* and phenotypically similar historical isolates led to the discovery of four isolates that could represent a novel species of *Vibrio* closely related to *V. navarrensis*. These isolates, three of which were recovered from human clinical specimens, are genetically distinct from *V. navarrensis* and *V. vulnificus* based on phylogenetic analysis of housekeeping gene sequences (Gladney & Tarr, 2014). The closest known relative of *V. navarrensis* with a validly published name is *V. vulnificus* (Gomez-Gil et al., 2014; Thompson et al., 2005). The latter is an opportunistic pathogen mainly associated with deaths related to seafood consumption, and it causes a fatality rate of over 50% in patients with septicemia (Jones & Oliver, 2009).

In this study, we employed a polyphasic approach to describe the novel species, such as extensive metabolic profiling of the four isolates, comparative genomic analysis to determine DNA–DNA relatedness, and multilocus sequence analysis (MLSA) of core genes. The name *Vibrio cidicii* sp. nov. is proposed for the new species.

Isolates of *V. cidicii* sp. nov. and *V. navarrensis* used in this study were obtained from the CDC (Table 1). Phenotypic characterization was performed on the four isolates of *V. cidicii* sp. nov. and three isolates of *V. navarrensis* (Tables 2 and S1, available in the online Supplementary Material). The isolates were streaked on tryptic soy broth (TSB; Becton Dickinson) with 1.0% NaCl (BDH), yielding a final concentration of 1.5% NaCl and 1.5% agar (Becton Dickinson). Alternatively, the isolates were streaked on tryosulfate citrate bile salts sucrose (TCBS) agar (Becton Dickinson). The cultures were then incubated overnight (TSB agar) or for two days (TCBS agar) at 30°C. Single colonies from the TSB agar cultures were tested using the Analytical Profile Index (API) 20 NE (bioMérieux) and the Phenotype MicroArray 1 (PM1) MicroPlate (Biolog) according to the instructions of the manufacturers. A minor modification of the PM1 test was the addition of 1.0% NaCl to the inoculating fluid (Biolog) to obtain a final concentration of 1.5% NaCl. The API 20 NE strips and PM1 plates were incubated for 42 or 18 h, respectively, at 30°C. Additional standard phenotypic tests for the routine identification of *Vibrio* not covered by API 20 NE and PM1 were also performed, including fermentation using various substrates, citrate (in Simons agar), DNase (at 25°C), H2S production (in peptone iron agar and triple sugar iron agar), malonate utilization, methyl red, lysine and ornithine decarboxylase (in Moeller medium), motility (swimming and swarming), ONPG (o-nitrophenyl β-D-galactopyranoside), oxidase, phenylalanine deaminase, tyrosine clearing, and Voges–Proskauer (Farmer et al., 2005; Tarr et al., 2015). Permissive growth temperatures were determined in TSB with a final concentration of 1.5% NaCl and incubation at a range of 4–45°C, whereas permissive salinity concentrations were determined in TSB at 30°C in a range of 0–10% NaCl. TSB without NaCl was prepared with 17.0 g l⁻¹ pancreatic digest of casein (Becton Dickinson), 3.0 g l⁻¹ papic digest of soybean (Becton Dickinson), 2.5 g l⁻¹ dextrose (Fisher Scientific) and 2.5 g l⁻¹ dipotassium phosphate (BDH). Gram staining was conducted on the isolates of *V. cidicii* sp. nov. following the protocol of Claus (1992) and viewing under a light microscope (Carl Zeiss) at ×1000 magnification.

For genotypic characterization, genomic DNA was extracted from overnight TSB cultures of the isolates of *V. cidicii* sp. nov. with the ArchivePure DNA Cell/Tissue Kit (5 PRIME). Whole-genome sequencing and assembly of the environmental isolate LMG 29267 (=CIP 111013T=2756-81T) were performed with the PacBio RS and the SMRT (Single-Molecule, Real-Time) Analysis software 2.0 (Pacific Biosciences), respectively, as previously described (Gladney et al., 2014). For the clinical isolates 1048-83 and 2538-88, 150-bp paired-end reads were generated on the MiSeq platform (Illumina), as previously described (Gladney et al., 2014). The genome for clinical isolate 2423-01 was sequenced using 454 sequencing on the Genome Sequencer FLX System (454 Life Sciences) and also on the Genome Analyzer Ix platform (Illumina), generating 70-bp single-end reads. De novo assemblies of the sequences from the clinical isolates were performed using the CG-Pipeline 0.4.1 (Kislyuk et al., 2010).

From the *V. cidicii* sp. nov. and *V. navarrensis* whole-genome sequences (Table 1), the G+C content was determined using Geneious 8.1.2 (Kearse et al., 2012). Pairwise average nucleotide identity (ANI) was calculated using the dndiff program in MUMmer 3.0 (ANIm; Kurtz et al., 2004). Pairwise percent DNA–DNA hybridization (DDH) was also calculated in *silico* using the Genome-to-Genome Distance Calculator 2.0 (GGDC; http://ggdc.dsmz.de/distcalc2.php; Meier-Kolthoff et al., 2013). The genome sequences were annotated with RAST 2.0 (Rapid Annotation Using Subsystem Technology; Aziz et al., 2008) and Prodigal 1.2 (Prokaryotic Dynamic Programming Genefinding Algorithm; Hyatt et al., 2010). Orthologous protein-coding gene families were determined from the annotated genomes by pairwise bidirectional BLASTp (Altschul et al., 1990) using the OrthoMCL pipeline 2.0 (Li et al., 2003) with 30% identity cut-off (Rost, 1999). The gene families unique to *V. cidicii* sp. nov. or *V. navarrensis* were subsequently determined using Intella 1.7.0 (https://www.vound-software.com). The predicted functions of these gene families were determined based on the Clusters of Orthologous Groups of proteins (COG) database (Tatusov et al., 2000) and by sequence similarity search in the GenBank database (National Center for Biotechnology Information) using BLASTp (Altschul et al., 1990).
With additional genome sequences from closely related species of the genus _Vibrio_ obtained from the GenBank database (Table S2), single-copy, protein-coding core gene families were determined using OrthoMCL 2.0 (Li et al., 2003). The sequences were aligned using ClustalW 2.1 (Larkin et al., 2007), and the alignments were concatenated, stripping columns with at least one gap, using Geneious 8.1.2 (Kearse et al., 2005). From the partial DNA sequences, a concatenated alignment of 2313 bp was obtained and used to reconstruct a maximum-likelihood tree with RAxML 8.2.8 (Stamatakis, 2014) using the GTR (general time reversible) nucleotide substitution model and gamma distribution pattern. Robustness of branching was estimated with 100 bootstrap replicates. Moreover, a subset of four housekeeping genes was selected for MLSA – _pyrH_, _recA_, _rpoA_ and _rpoB_ (Gladney & Tarr, 2014; Urdaci et al., 1991). From the partial DNA sequences, a concatenated alignment of 2313 bp was obtained and used to reconstruct a maximum-likelihood tree, as described above. Patristic distances between species, the sum of the lengths of the branches that link two terminal nodes in a tree, were calculated from the latter tree using Geneious 8.1.2 (Kearse et al., 2012). In addition, whole-genome phylogeny was also reconstructed based on genomic similarity (ANI) between each pair of genome sequences. First, pairwise ANI was computed using MUMmer 3.0 (Kurtz et al., 2004). The similarity obtained was then converted into average nucleotide distances (=100–ANI). The resulting distance matrix was utilized to quantify all against all pairwise species distances as well as in the reconstruction of a neighbour-joining tree (Saitou & Nei, 1987) using the software _MEGA_ 7.0 (Kumar et al., 2016).

All the isolates of _V. cidicii_ sp. nov. and _V. navarrensis_ studied exhibited growth in TSB without NaCl (Tables 2 and S1). This is contrary to a previous report of these isolates not exhibiting growth in nutrient broth without NaCl (Gladney & Tarr, 2014). This is possibly due to differences in the media used in both studies, as test conditions will dictate salt requirement (Farmer et al., 2005). Urdaci et al. (1991) reported seven out of ten isolates of _V. navarrensis_ grew weakly in peptone water without NaCl. Growth in medium without NaCl was previously reported for a few species of the genus _Vibrio_, including _V. cholerae_ and _Vibrio mimicus_ (Farmer et al., 2005; Gomez-Gil et al., 2014), two species of great clinical significance. The ability of bacteria to survive in freshwater makes it more likely to come in contact with humans through ingestion (Boucher et al., 2015). The isolation of strains of _V. cidicii_ sp. nov. and _V. navarrensis_ from river water and sewage, respectively, suggests these species are also able to survive in low salt environments. Furthermore, both species are also able to survive at 40 °C, a trait observed mostly in pathogenic vibrios that can survive inside the human body (Farmer et al., 2005; Gomez-Gil et al., 2014).

_Vibrio cidicii_ sp. nov. resembles _V. navarrensis_ in the majority of phenotypic characteristics tested (123 of 158 tests or 78 %; Table 2 and Table S1). However, a single phenotypic feature distinguished _V. cidicii_ sp. nov. from _V. navarrensis_:

*BioProject accession numbers: PRJNA304180 (_V. cidicii_ sp. nov.) and PRJNA242769 (_V. navarrensis_).
it tested positive for the utilization of L-rhamnose as the sole carbon and energy source in both fermentation and assimilation tests. Although previous reports support our result of an L-rhamnose-negative *V. navarrensis* (Farmer *et al.*, 2005; Gomez-Gil *et al.*, 2014), a recent study reported one isolate of *V. navarrensis* also capable of utilizing L-rhamnose (Gladden & Tarr, 2014). To our knowledge, this is the only reported isolate of *V. navarrensis* that is L-rhamnose-positive. *Vibrio vulnificus* is also not able to utilize this substrate (Farmer *et al.*, 2005; Gomez-Gil *et al.*, 2014). Other species of the genus *Vibrio* that are capable of utilizing this substrate are *Vibrio hispanicus*, *Vibrio natriegens* and *Vibrio pectenicida*, all of which are very distantly related to *V. cidicii* sp. nov. and *V. navarrensis* (Gomez-Gil *et al.*, 2014; Thompson *et al.*, 2005). Comparison of the annotated genomes of isolates of *V. cidicii* sp. nov. and *V. navarrensis* revealed four genes found only in the former, encoding proteins involved in L-rhamnose transport and metabolism: L-rhamnose isomerase, L-rhamnose mutarotase, L-rhamnose-proton symporter and rhamnulose-1-phosphate aldolase (Table S3; Ryu *et al.*, 2004; Sawada & Takagi, 1964; Wilson & Ajl, 1957). L-Rhamnose is produced in high levels by diatoms (Brown, 1991), a group of phytoplankton found in both marine and freshwater environments, where *V. cidicii* sp. nov. was also found. This suggests a physiological differentiation with *V. navarrensis*, in which *V. cidicii* sp. nov. is adapted to living on or near algae and exploiting their carbon exudates. An additional 24 genes with predicted functions were found in *V. cidicii* sp. nov. but were absent from *V. navarrensis* (Table S3). These could encode other distinguishing characteristics for the species. However, no physiological tests were available to test the phenotypes they are predicted to encode.

Three additional characteristics distinguish *V. cidicii* sp. nov. from its closest relatives, although not universal among isolates (Tables 2 and S1). First, two of the four isolates of *V. cidicii* sp. nov. exhibited swarming on marine agar. Many vibrios have been reported to exhibit swarming motility, including *Vibrio cincinnatiensis* and *Vibrio proteolyticus*. However, *V. navarrensis* and *V. vulnificus* are negative for the phenotype (Farmer *et al.*, 2005; Gomez-Gil *et al.*, 2014). Second, two of the four isolates of *V. cidicii* sp. nov. tested positive for salicin fermentation, while none of the *V. navarrensis* isolates did. This trait is also a key differentiating characteristic between *V. navarrensis* and *V. vulnificus*, with the latter also able to utilize the substrate (Farmer *et al.*, 2005). Lastly, three of the four isolates of *V. cidicii* sp. nov. are negative for the utilization of sodium citrate (citrate

**Table 2. Summary of phenotypic test results for *V. cidicii* sp. nov., *V. navarrensis* and *V. vulnificus***

<table>
<thead>
<tr>
<th>Phenotypic test/substrate tested</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate (Simmons agar)</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lysine decarboxylase (Moeller medium)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Ornithine decarboxylase (Moeller medium)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Swarming (marine agar, 25°C)</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Salicin</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinose*</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>–</td>
</tr>
<tr>
<td>Glycerol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth in TSB (at 30°C) with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 % NaCl</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>1.5 % NaCl</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth in TSB (with 1.5 % NaCl) at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

*Assimilation of L-arabinose (for *V. cidicii* sp. nov. and *V. navarrensis* isolates): positive with PM1, negative with API 20 NE.
†Results for *V. vulnificus* were obtained from Farmer *et al.* (2005) and Gomez-Gil *et al.* (2014).
test), whereas both *V. navarrensis* and *V. vulnificus* are able to utilize the substrate (Farmer *et al.*, 2005). Both *V. cidicii* sp. nov. and *V. navarrensis* can be differentiated from *V. vulnificus* by two characteristics; they test negative for lysine and ornithine decarboxylase (Farmer *et al.*, 2005; Gomez-Gil *et al.*, 2014).

We observed contradicting results between the PM1 and API 20 NE tests we conducted for the assimilation of l-arabinose, where all isolates of *V. cidicii* sp. nov. and *V. navarrensis* tested positive with the PM1 system and negative with the API 20 NE system. This difference can be attributed to the differences in methods between the tests (e.g. incubation period, NADH production and redox dye chemistry detection versus turbidity detection). We conclude that this test is not reliable for the identification of the species *V. cidicii* sp. nov. and *V. navarrensis*. Additionally, we observed a difference between our assimilation and fermentation tests (positive for all isolates with the former and negative with the latter) with two other substrates, glycercol and d-xylene. This difference is due to the different attributes being measured (i.e. NADH production versus acid production), suggesting that both species are capable of utilizing the substrates aerobically but not via fermentation.

Based on their whole-genome sequences, the G+C content of the four isolates of *V. cidicii* sp. nov. range from 47.9–48.2 mol%, which is within the known range for the genus *Vibrio* (38.0–51.0 mol%; Farmer *et al.*, 2005). This eliminates the assignment of the isolates to other genera in the family *Vibrionaceae* such as *Al livivibrio* (38.0–42.0 mol%; Urbanczyk *et al.*, 2007), *Photobacterium* (39.0–44.0 mol%; Thyssen & Oleveier, 2005), and *Salinivibrio* (49.4–50.5 mol%; Ventosa, 2005), and most genera in the family *Enterobactereaceae* (50.0–67.0 mol%; Brenner & Farmer, 2005).

Various tools to measure DNA–DNA relatedness in silico are available to replace the traditional method of DDH (Goris *et al.*, 2007; Konstantinidis & Tiedje, 2005; Meier-Kolthoff *et al.*, 2013; Richter & Rosselló-Móra, 2009). Here, we determined relatedness of organisms using ANI and DDH by pairwise comparisons of whole-genome sequences. The ANI between isolates within the species *V. cidicii* sp. nov. or *V. navarrensis* range from 97.4–100.0 % (Fig. 1 and Table S4). In contrast, the ANI between *V. cidicii* sp. nov. and *V. navarrensis* range from 95.4–95.8 %. Since the results are close to the cut-off of 96 % ANI for two genomes to belong to the same species (Richter & Rosselló-Móra, 2009), we complemented our ANI results and suggesting the two groups to be distinct from each other.

MLSA further supports our proposal of a novel species. Single-copy, protein-coding core genes are used as alternatives to 16S rRNA gene sequences for the identification and phylogenetic analysis of various species of the genus *Vibrio*, since there is a lack of species-level resolution using 16S rRNA gene sequences (Gladney & Tarr, 2014; Thompson *et al.*, 2005). A core genome tree was reconstructed from 586 single-copy core genes that are shared by all strains used in this study (Vernikos *et al.*, 2015). The four isolates of *V. cidicii* sp. nov. form a monophyletic clade that is distinct from the *V. navarrensis* and *V. vulnificus* clades, with 100 % bootstrap support (Fig. S1).

Since recombination is also apparent within the core genome and can occur at a high rate for very closely related species (Orata *et al.*, 2015), we examined a subset of four housekeeping genes (*pyrH, recA, rpoA* and *rpoB*) that do not exhibit recombination among the isolates of *V. cidicii* sp. nov., *V. navarrensis* and *V. vulnificus*. These genes have been shown to be reliable for the taxonomic characterization of vibrios (Gladney & Tarr, 2014; Tarr *et al.*, 2007; Thompson *et al.*, 2005). Phylogenetic analysis using the four housekeeping genes also distinguishes *V. cidicii* sp. nov. from *V. navarrensis* and *V. vulnificus* (Fig. 2). The average patristic distance calculated from this tree between the *V. cidicii* sp. nov. isolates and the *V. navarrensis* isolates is 0.066, while lower average distances of 0.005 and 0.007 are obtained when comparing isolates within the species *V. cidicii* sp. nov. or *V. navarrensis*, respectively (Table S6). To further demonstrate this distinction, a phylogeny was reconstructed based on whole-genome comparisons to account for whole-genome variation between isolates that would otherwise be excluded from the core genome (Fig. S2). This phylogeny also shows the distinct clustering of the isolates of *V. cidicii* sp. nov. from *V. navarrensis*. The average nucleotide distances calculated for this tree (Table S7) show that the diversity within *V. cidicii* sp. nov. (1.780) or *V. navarrensis* (2.160) is much lower than the diversity between the two species (4.414). Our phylogenetic analyses placed the *V. cidicii* sp. nov. lineage into the context of a larger *Vibrio* phylogeny, showing that the novel species is distinct from all *Vibrionaceae* that have been characterized to date (Figs 2, S1 and S2; Gladney & Tarr, 2014). On the other hand, the use of the 16S rRNA gene did not clearly distinguish *V. cidicii* sp. nov. and *V. navarrensis* (Gladney & Tarr, 2014).

Overall, phylogenetic analyses confirm the position of *V. cidicii* sp. nov. in the genus *Vibrio*, which forms a monophyletic clade distinct from *V. navarrensis* and *V. vulnificus*, supporting its identification as a novel species of the genus *Vibrio*. This distinction is further confirmed by ANI and percent DDH below 96 % and 70 %, respectively, between species. The ability of *V. cidicii* sp. nov. to utilize L-rhamnose could be a feature that drove its speciation from a common ancestor shared with *V. navarrensis*. Further studies are needed to determine the prevalence of *V.
cidicii sp. nov. in various environments. The isolation of strains from human blood suggests it is capable of infecting humans and can be pathogenic. Two additional isolates were recovered since 2014 after the commencement of this study, underscoring the need to identify and characterize isolates of this pathogen. It will be important to study the pathogenicity and epidemiology of this novel species for control, treatment and prevention of disease.

**Description of Vibrio cidicii sp. nov.**

Vibrio cidicii (ci.d’ci.i. N.L. gen. n. cidicii from the phonetics of the CDC, the acronym for the Centers for Disease Control and Prevention, where the species was initially identified).

Cells are Gram-negative, curved, motile rods, 0.64–0.78×1.48–1.68 µm in size, which produce convex, smooth, circular, entire, cream colonies on TSB agar and yellow colonies (sucrose-fermenting) on TCBS agar. Growth is observed in TSB at 30 °C with salt concentrations in the range of 0–6.5 % NaCl, and up to 8 % for some isolates (three out of four tested); no growth occurs in the presence of 10 % NaCl. Growth is also observed in TSB with 1.5 % total NaCl concentration at a temperature range of 30–40 °C, and no growth occurs at 4 °C and 45 °C. The ability to utilize L-rhamnose as the sole carbon and energy source distinguishes V. cidicii sp. nov. from V. navarrensis, its closest relative. In addition, the following characteristics are variable across isolates: swarming on marine agar (two positive out of four tested; negative for V. navarrensis), salicin fermentation (two positive out of four tested; negative for V. navarrensis), and sodium citrate utilization (three negative out of four tested; positive for V. navarrensis). Positive results in tests for: indole production; methyl red test; phenylalanine deaminase; gelatin and aesculin hydrolysis; reduction of nitrate to nitrite; oxidase and DNase; and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose. Negative results in tests for: Voges–Proskauer reaction; H₂S production; urea hydrolysis; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase; malonate utilization; gas production from D-glucose and acid production from D-glucose, cellobiose, maltose, mannitol, mannose, L-rhamnose, sucrose and trehalose.
fumaric acid, gelatin, D-gluconic acid, D-glucose, glucose 1-phosphate, glucose 6-phosphate, L-glutamic acid, L-glutamine, glycerol, DL-a-glycerol phosphate, glycyl L-aspartic acid, glycyl L-glutamic acid, glycyl L-proline, a-hydroxybutyric acid, a-hydroxyglutaric acid-g-lactone, inosine, a-ketobutyric acid, a-ketoglutaric acid, L-lactic acid, L-lyxose, D-malic acid, L-malic acid, DL-malic acid, D-maltose, maltotriose, D-mannitol, D-mannose, methyl pyruvate, methyl B-D-glucoside, monomethyl succinate, phenylalanine, potassium gluconate, L-proline, propionic acid, D-psicose, pyruvic acid, L-rhamnose, D-ribose, L-serine, succinic acid, sucrose, L-threonine, thymidine, trehalose, L-tryptophan, Tween 40, Tween 80, uridine and D-xylose. The following substrates are not utilized (in four tested isolates): adipic acid, 2-aminoethanol, D-arabitol, L-arginine, D-aspartic acid, capric acid, erythritol, L-fucose, L-galactonic acid-g-lactone, D-galacturonic acid, glucuronamide, D-glucosaminic acid, glyoxylic acid, m-hydroxyphenylacetic acid, p-hydroxyphenylacetic acid, myo-inositol, lysine, malonate, a-methyl-D-galactoside, ornithine, phenylacetic acid, phenylethylamine, 1,2-propanediol, raffinose, D-serine, m-tartaric acid, D-threonine, tricarballylic acid, trisodium citrate, tyramine, and urea.

The type strain is LMG 29267T (=CIP 111013T=2756-81T) isolated from river water in 1981 (country of origin unknown). The type strain displays all of the properties given above for the species. The G+C content of the type strain is 47.9 mol% based on whole-genome sequencing.

Acknowledgements

F. D. O. was supported by Alberta Innovates – Technology Futures. R. J. C. and Y. B. were supported by the Natural Sciences and Engineering Research Council of Canada. Y. B. was also supported by the Canadian Institute for Advanced Research. L. R. and I. K. J. were supported by the Georgia Institute of Technology (GIT) Bioinformatics Graduate Program and the IHRC-GIT Applied Bioinformatics Laboratory.

References

