Taxonomic reassessment of the genus *Elizabethkingia* using whole-genome sequencing: *Elizabethkingia endophytica* Kämpfer et al. 2015 is a later subjective synonym of *Elizabethkingia anophelis* Kämpfer et al. 2011

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The taxonomic status of all species of the genus *Elizabethkingia* was re-evaluated by comparative genomics based on whole-genome sequencing. From these results it is clear that *Elizabethkingia endophytica* is a later subjective synonym of *Elizabethkingia anophelis*. In addition, genome-based analysis revealed the misidentification of isolates previously identified by traditional approaches and indicates the presence of two more species. We propose a more rapid identification scheme on the basis of an *in silico* PCR assay derived from comparative genomics of whole-genome sequences (WGS) from 29 well-curated strains.

The genus *Elizabethkingia* was established in 2005 based on an analysis of 16S rRNA gene sequences from strains of the Chryseobacterium–Bergeyella–Riemerella group within the family Flavobacteriaceae (Kim et al., 2005). This led to Chryseobacterium meningosepticum and Chryseobacterium miricola being placed in a separate genus that was designated *Elizabethkingia*. Subsequently, two further species: *Elizabethkingia anophelis*, isolated from the midgut of the mosquito (Kämpfer et al., 2011), and *Elizabethkingia endophytica* (Kämpfer et al., 2015), isolated from the stem of Zea mays, were added to this genus. Thus, the genus *Elizabethkingia* presently comprises four species, three of which are known to be human pathogens, but it is not known yet if *E. endophytica* is a pathogen. Isolates of members of the genus *Elizabethkingia* are ubiquitously present in hospital environments and can cause nosocomial infections. Immunocompromised individuals are particularly at risk of developing severe infections, including bacteremia, and isolates are generally resistant to multiple antimicrobials (Lau et al., 2015, 2016). In an ongoing outbreak in the state of Wisconsin, in the USA, *E. anophelis* has been associated with more than 59 cases of infections and more than 18 deaths, suggesting epidemiological potential (WDHS, 2016). Members of the genus *Elizabethkingia* are non-fermentative Gram-negative bacteria and are differentiated based on morphological differences and biochemical characteristics. The four species show more than 97% 16S rRNA gene sequence similarity (Kämpfer et al., 2015).

Recently, whole-genome sequencing (WGS) has been employed as a taxonomic tool to determine relatedness based on *in silico* DNA–DNA hybridization (is DDH). Two methods viz. ‘Genome to Genome Distance Calculator’ (GGDC) and ‘Average Nucleotide Identity’ (ANI) are extensively used for precise bacterial taxonomic classification. These techniques have been used to resolve conflicts in complex taxonomic situations and recent studies have revealed misclassification among closely related bacteria, e.g. *Escherichia coli* (Meier-Kolthoff et al., 2013), *Bacillus* (Colston et al., 2014) and *Aeromonas* (Colston et al., 2014), which were previously classified using the polyphasic taxonomical approach. Accurate identification of pathogenic bacteria is of prime importance and determines the treatment strategy. In the case of the genus *Elizabethkingia*, the current identification is mainly based on morphological and biochemical assays as well as the 16S rRNA gene sequence analysis. This has led to misidentification and

Abbreviations: GGDC, Genome to Genome Distance Calculator; is DDH, *in silico* DNA–DNA hybridization; WGS, whole-genome sequencing.

The GenBank/EMBL/DDBJ accession number of the genome sequences of strains *E. anophelis*, *E. endophytica*, *E. miricola* and *E. meningoseptica* are FLST01000000, FLSU01000000, FLSS01000000 and FLSV01000000 respectively.
misinterpretation of the species involved in *Elizabethkingia*-related infections (Lau *et al.*, 2015; Matyi *et al.*, 2015). Phenotypic tests currently being used distinguishing the species are not reliable (Lau *et al.*, 2015). Therefore, there is an immediate need for accurate and validated identification of the species of the genus *Elizabethkingia*.

The genomes of the type strains of all four species, *Elizabethkingia meningoseptica* CCUG 214\textsuperscript{T}, *Elizabethkingia miricola* KCTC 12492\textsuperscript{T}, *Elizabethkingia anophelis* R26\textsuperscript{T} and *Elizabethkingia endophytica* JM-87\textsuperscript{T} were sequenced and the taxonomy of the members of the genus *Elizabethkingia* was re-examined. We used 29 (as of 1st April 2016) publicly available genomes of members of the genus *Elizabethkingia*, including a representative of the clonal strain involved in the Wisconsin outbreak for our analysis. Genomes were analyzed by GGDC formula two (<70 % cut-off value for novel species) (Auch *et al.*, 2010a, b) and JSpecies (<95 % cut-off value for novel species, which is equivalent to 70 % DNA–DNA hybridization) (Richter & Rosselló-Móra, 2009). Based on these cutoffs, species were defined (species–WGS). For better visualization, the DNA–DNA hybridization matrix was visualized by constructing a UPGMA-based dendrogram. Both the is DDH methods exhibited similar results and are represented in Fig. 1.

![Fig. 1. A UPGMA dendrogram based on the in silico DNA–DNA (is DDH) hybridization scores obtained by Genome to Genome Distance Calculator (GGDC). A 70 % is DDH value represents the cut-off value for novel species (equivalent to 95 % ANI value of JSpecies). Filled circles (●) represent misclassified species based on species–WGS. ‘★’ represents type strains. *Chryseobacterium indologenes* NBRC 14944\textsuperscript{T} was used for out-grouping purposes. (Note: the topology of the dendrogram derived from average nucleotide identity by JSpecies was exactly the same as that generated using GGDC).](image-url)
Fig. 2. 16S rRNA gene sequence similarity of all the 28/29 strains considered in this study (16S rRNA genes could not be extracted by ‘RNAmr 1.2’ from *E. anophelis* 12012–2 PRCM, Acc. No. LPXG000000000). Top, neighbor-joining tree based on 16S rRNA gene (1505 bp) sequences. Filled circles (●) represent misclassified species based on the species-WGS; ‘★’
Several interesting observations were made at the species level. Based on is DDH cut-off values, the genus Elizabethkingia is comprised of a total of five species (Fig. 1). Two strains, ATCC 33958 and EM_CHUV are misclassified as members of the species E. miricola, but actually seem to represent novel species. In addition, the GGDC and ANI values for E. endophytica type strain JM87T were 77 and 97 %, respectively with regard to the E. anophelis type strain R26T suggesting that E. endophytica JM87T represents a strain of E. anophelis. The main criteria for the proposal of the species E. endophytica (Kämpfer et al., 2015) was the moderate experimental DDH value (51/54 %) between E. endophytica and E. anophelis as well as some physiological differentiating parameters (Kämpfer et al., 2015). This indicates that DDH analysis results have to be evaluated critically if values are low but 16S rRNA gene sequence similarities are high.

The study indicates that several clinically important strains of the species Elizabethkingia are misclassified. More specifically, E. meningoheptica ‘FMS007’ which was isolated from T-cell non-Hodgkin’s lymphoma; ‘502’ obtained from the wound from an improvised explosive device (IED) and ‘endophthalmitis’ from a postoperative endophthalmitis in a patient who suffered the loss of vision in one eye, are in fact E. anophelis and have been misclassified as E. meningoseptica. Strains E. miricola ATCC 33958 and EM_CHUV isolated from contaminated industrial enzyme preparation and endotracheal secretions of a human respectively, seem to represent novel species within the genus Elizabethkingia. It should be noted that both ATCC 33958 and DSM 1471T are considered to represent isolates of E. miricola. However, they differ in a number of phenotypic tests (Kim et al., 2005). Based on its is DDH score (52 % by GGDC and 92.88 % ANI), ATCC 33958 seem to represent a novel species of the genus Elizabethkingia.

The 16S rRNA gene sequences of the 29 genomes considered in this study were compared. We observed that 16S rRNA gene sequence identity was higher than 98.65 (Fig. 2). This is in agreement with the results of Kim et al. (2014) who also suggested a species cut-off value of 98.65 %, based on comparative analysis of genome data and 16S rRNA gene sequence similarities. Hence, values below this cut-off value are a good indication of a presence of a novel species within the genus.

To enable rapid identification of individual species, an in silico PCR assay based on comparative genomics of the 29 WGS-taxonomically curated strains was developed. The specific primer sequences provided for general use are detailed in Table 1.

Our data clarifies the current taxonomic status of the species belonging to the genus Elizabethkingia. The genus comprises at present three species with validly published names: E. anophelis, E. meningoseptica and E. miricola. Two novel species indicated by the data reported here remain presently unnamed. A comprehensive polyphasic taxonomic study should be performed on these strains in order to confirm their species status. In addition, the species E. endophytica should not be considered as a separate species of the genus Elizabethkingia. Without clear assignments to species, it will be difficult to perform studies on the population structure of this genus and to carry out epidemiological studies as well as to identify those species associated with infections.

Emended description of Elizabethkingia anophelis (Kämpfer et al. 2011)

The description is that Kämpfer et al. (2011) with the following modifications: Growth on MacConkey agar, acid production form D-melibiose and D-cellobiose and urea.

Table 1. PCR primers to distinguish five species-WGS The 29 publicly available genomes (as of 30th March 2016) defining five species.WGS of the genus Elizabethkingia were analyzed by ‘Gegenees’ to obtain the unique genomic regions of each species (http://www.gegenees.org/). The sequences of the unique region, primers were designed by the ‘primer 3’ server (http://bioinfo.ut.ee/primer3-0.4.0/)

<table>
<thead>
<tr>
<th>Species</th>
<th>Primers for the regions uniquely present in the species</th>
<th>Amplicon size</th>
</tr>
</thead>
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<tr>
<td>Elizabethkingia meningoseptica</td>
<td>5'-GTCCTCCGATACCTGCCACAT-3'</td>
<td>352</td>
</tr>
<tr>
<td></td>
<td>5'-CTGTTGATATGGCCATGCTG-3'</td>
<td></td>
</tr>
<tr>
<td>Unidentified novel Elizabethkingia species B</td>
<td>5'-ACGGTTTAAATCTGCCATG-3'</td>
<td>487</td>
</tr>
<tr>
<td></td>
<td>5'-TGTGCTGGAATATGGCTTT-3'</td>
<td></td>
</tr>
<tr>
<td>Elizabethkingia miricola</td>
<td>5'-GCCACCAATGGAGATCAGTT-3'</td>
<td>562</td>
</tr>
<tr>
<td></td>
<td>5'-AATTGAAATCCGGTGCTGTCG-3'</td>
<td></td>
</tr>
<tr>
<td>Unidentified novel Elizabethkingia species A</td>
<td>5'-CAGGTAAGCCCGATTTGATG-3'</td>
<td>610</td>
</tr>
<tr>
<td></td>
<td>5'-AAAAAGCATACCGGCAACAC-3'</td>
<td></td>
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<tr>
<td>Elizabethkingia anophelis</td>
<td>5'-CAACCGGAACAGCTTACACA-3'</td>
<td>758</td>
</tr>
<tr>
<td></td>
<td>5'-GTCACTCCAGTTCCTCCACGT-3'</td>
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hydrolysis are variable. The strain *Elizabethkingia endophytica* JM-87 is an additional strain of *E. anophelis*.

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**References**


