Development of *Kaptive* databases for *Vibrio parahaemolyticus* O- and K-antigen serotyping

Authors

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Running title: Development of *V. parahaemolyticus* O- and K-antigen Kaptive databases

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Abstract

Vibrio parahaemolyticus is an important food-borne human pathogen and is divided in 16 O-serotypes and 71 K-serotypes. Agglutination tests are still the gold standard for serotyping, but many *V. parahaemolyticus* isolates are not typable by agglutination. An alternative for agglutination tests is serotyping using genome sequence data. In this study, we manually identified all known O- and K-loci from *V. parahaemolyticus* isolates which we serotyped and sequenced, and extracted additional O- and K-loci from publicly available genomes. We developed *Kaptive* databases for all O- and K-loci after manual curation of the loci. These *Kaptive* databases with the identified *V. parahaemolyticus* O- and K -loci can be used to identify the O- and K-serotypes of *V. parahaemolyticus* isolates from genome sequences.

Introduction

Vibrio parahaemolyticus (*V. parahaemolyticus*) is an important food-borne human pathogen that naturally inhabits marine environments worldwide, and can cause acute gastroenteritis and septicemia in human (M. Chen et al. 2012; Guo et al. 2017). *V. parahaemolyticus* is typically serotyped on the basis of its heat-stable somatic antigen (O) and its capsular antigen (K) and classified into 16 O-serotypes and 71 K-serotypes.

Serotyping of *V. parahaemolyticus* is important for pathogen detection and epidemiological surveillance. Many *V. parahaemolyticus* serotypes have been identified as pandemic clones, and certain serotypes, for example O3:K6, O1:KUT and O4:K68, are generally considered to be more virulent than others (Jones et al. 2012; Nair et al. 2007). Agglutination tests are the gold standard for *V. parahaemolyticus* serotyping but frequently, *V. parahaemolyticus* strains are non-typable with agglutination tests in routine screening (Gavilan, Zamudio, and Martinez-Urtaza 2013; Gil et al. 2007; Hashii et al. 2000; Jones et al. 2012). For several pathogenic bacterial species, e.g. several pathogenic *E. coli* serogroups (Wang et al. 2010) and *Vibrio cholerae* serogroup O1 and O139 (Albert et al. 1997; Hoshino et al. 1998), PCR methods are developed to detect the specific serogroups, but no PCR methods are available for *V. parahaemolyticus* serotyping.

Recently, the software tool *Kaptive* was developed for rapid O- and K-loci typing of *Klebsiella* strains from whole genome sequences (Wyres et al. 2016). This tool includes an option to use other, self-created databases. The genomic regions associated with the somatic synthesis locus (O-locus) and capsule synthesis locus (K-locus) for *V. parahaemolyticus* are generally found on the same genomic location (Okura et al. 2008; Y. Chen et al. 2010; Guo et al. 2017), which makes it possible to locate and extract the O- and K-loci nucleotide sequences from whole genome sequencing data of *V. parahaemolyticus* strains.

56 The aim of this study was to identify all O- and K-loci of *V. parahaemolyticus* by serotyping, sequencing
57 and analyzing in-house isolates and investigating public data of serotyped strains and manually curate
58 reference databases for O- and K-loci, which can be used to determine the O- and K-serotype of *V.*
59 *parahaemolyticus* strains from whole genome sequencing data.
60

61 **Materials and Method**

62

63 *Sequences of* V. parahaemolyticus

64 A total of 1602 *V. parahaemolyticus* genomes were used in this study, of which 775 genomes were
65 publicly available and downloaded from NCBI Genbank, 770 additional genomes were downloaded
66 from PATRIC (Wattam et al. 2014) and 57 isolates were in-house sequenced and serotyped. For strains
67 sequenced in this study, DNA was extracted with TIANamp Bacteria DNA Kit (Tiagen Biotech (Beijing)
68 CO., LTD, Beijing, China) according to the manufacturer's recommendation and DNA quality control
69 was performed using agarose gel electrophoresis and the Qubit dsDNA HS Assay Kit. DNA libraries and
70 DNA nanoballs (DNB) were constructed on BGISP-100 platform (WuHan MGI Tech Co.,Ltd, Wuhan,
71 China) with input of 150ng DNA. 50 bp single-end reads were generated with BGISEQ-50 sequencer
72 (WuHan MGI Tech Co.,Ltd, Wuhan, China) and assembled with SPAdes v3.12 (Bankevich et al. 2012).
73 All sequence reads are available under accession PRJEB39490 and PRJNA483379 of the ENA short read
74 archive.

75

76 *Serotyping of* V. parahaemolyticus *strains*

77 Serotyping of the 57 in-house sequenced strains was performed by slide agglutination according to
78 the manufacturer's instructions, using the 65 type-K serum set and 11 type-O serum set (Denka Seiken
79 CO., LTD, Tokyo, Japan).

80

81 *Development* Kaptive *database*

82 I. Identification of O-loci

83 *V. parahaemolyticus* is divided into 16 O-serotypes (M. Chen et al. 2012; Guo et al. 2017). The O-locus
84 has been defined between *dgkA* and *gmhD* genes, which encode a diacylglylcerol-kinase and an
85 epimerase respectively (Guo et al. 2017). With BlastN (minimum coverage of 80% and minimum

86 identity of 80%), the flanking genes of the O-region, *dgkA* and *gmhD*, were searched in all 1602
87 genome sequences and the O-loci sequences were extracted from the genomes manually.
88
89 II. Identification of K-loci

90 *V. parahaemolyticus* is divided in 71 K-serotypes (Guo et al. 2017). The K-locus has been defined
91 between *gmhD* and *rjg* or *ugd* or *gtaB* genes, which encode an epimerase and metallo-hydrolase,
92 respectively (Y. Chen et al. 2010). With BlastN (minimum coverage of 80% and minimum identity of
93 80%), the flanking genes of the K-region, *gmhD* and *rjg,* were searched in all 1602 genome sequences.
94 In some cases where the *rjg* gene was not found, gene *ugd* or gene *gtaB* was selected as flanking gene.
95 The K-loci sequences were extracted from the whole genome sequences manually.
96

97 III. Development of the *Kaptive* databases

98 For the development of the Kaptive databases, the set of 775 genomes from Genbank and 57 in-house
99 sequenced genomes were used, and PATRIC genomes were excluded, since the PATRIC database did
100 not contain metadata with the serotypes of the genomes. The extracted O- and K-loci sequences were
101 annotated using Prokka (Seemann 2014). For annotating of the O-loci, a custom database was used
102 which was built with the annotations of the previous described O1-O16 loci of *V. parahaemolyticus*
103 (M. Chen et al. 2012; Guo et al. 2017), and K-loci sequences were annotated with the default Prokka
104 database. Genes of the O- and K-loci respectively were clustered using Roary with an 80% cutoff on
105 amino acid identity (Page et al. 2015). The Roary gene presence absence table was used to create gene
106 presence-absence clusters of the O- and K-loci respectively. Of the total set of 832 genomes from
107 Genbank (n=775) and in-house sequenced genomes (n=57), 449 genomes had a known O-serotype
108 and 291 genomes had a known K-serotype. The known serotypes were linked to unique gene
109 presence-absence clusters, and the clusters that contained only genomes with the same specific
110 serotypes were selected. Of each unique cluster, one genome was selected as reference and the

111 nucleotide sequences of the O- or K-locus of the selected genomes were added to the *Kaptive*
112 database file and curated manually. The selected genomes that were used as reference for each O-
113 and K-serotype are shown in Supplemental Table 1.The *Kaptive* databases were tested with all 832 *V.*
114 *parahaemolyticus* genomes with default settings and with a minimum gene coverage of and a
115 minimum gene identity of 90%.
116 After the development of the *Kaptive* databases with the genomes with the known serotypes, the set
117 with 770 PATRIC genomes were analyzed with the databases, and genomes with a novel K-locus were
118 annotated and added to the *Kaptive* database as KUK (K-UnKnown).
119 The *V. parahaemolyticus* O-locus and K-locus *Kaptive* databases are available on the following Github
120 page: https://github.com/aldertzomer/vibrio_parahaemolyticus_genomoserotyping.
121
122 *Comparison of genes in O- and K-loci*
123 Genes of the selected O- and K-loci of the *Kaptive* databases were clustered using Roary with 80%
124 identity (Page et al. 2015). Phylogenetic trees were built from the Roary gene presence absence table
125 using RAxML with a BINCAT model, doing 100 bootstrap searches, 20 ML searches and returning the
126 best scoring ML tree. Schematic representations of the O- and K-loci were made with Geneious
127 (Biomatters, Auckland, New Zealand). Gene cluster comparisons are made with Clinker (Gilchrist and
128 Chooi 2020).
129

130 **Results and Discussion**

131

132 *Development of* Kaptive *databases for V. parahaemolyticus O- and K-loci*

133 Gene patterns of each O-locus could be linked to a specific O-serotype for O1 to O16, except O3 and
134 O13, since they share the same genes (M. Chen et al. 2012). Therefore O3 and O13 could not be
135 distinguished based on gene presence and are assigned in the *Kaptive* O-database as "O3 or O13". It
136 is possible that a second cluster modifies the O-antigen, similar to what has been described for *Shigella*
137 (Lehane, Korres, and Verma 2005). For O-serotype 4, two different gene patterns are found, and
138 therefore, serotype O4 has two gene patterns in the database, assigned as "O4" and "O4-a".

139

140 For the K-locus, several gene patterns could not be assigned to a specific K-serotype, because not all
141 K-serotypes of the genomes were known. We nearly doubled the number of observed unique K-
142 antigen encoding loci in the genomes. These unknown K-locus gene patterns (n=67) are assigned as
143 KUK (**K**-serotype **U**n**K**nown) in the *Kaptive* database. Eight gene patterns of K-Untypable (KUT) strains
144 previously described were found and these patterns are assigned in the *Kaptive* database as KUT
145 followed by subsequent numbers 1-8. No isolate or genome was available for K-serotype 71, and this
146 serotype is therefore not included in the database (or possibly assigned as KUK). For K-serotypes 20,
147 29, and 68, two different gene patterns were found, and therefore, these K-serotypes have two gene
148 patterns included in the database, distinguished with the addition of "-a" to the K-serotype. For K-
149 serotype 12, serotype K12-a is included in the database, which contain a similar gene pattern, but with
150 different sequence identity.

151

152 Examination of the phylogenetic tree of the isolates from which the loci were extracts shows that the
153 K-locus clearly clusters with bacterial clone and MLST type (Supplemental Figure 1), except for MLST
154 ST3 strains, as they appear to be quite comparable but have different K- and O-loci.

155

156

157 *Gene composition of O- and K-loci*

158 Phylogenetic trees of the O-loci and K-loci based on gene presence/absence are shown in Figure 1, the
159 representations of the gene content of each locus is shown in Supplemental Figure 2 and gene cluster
160 comparisons are shown in Supplemental Figure 3.

161 For the O-loci, two main clusters were found, with O14 having an unique organization of genes in the
162 O-locus compared to the other O-loci. The phylogenetic tree of the gene presence absences of the K-
163 loci showed moderate variability of the gene content of the identified K-loci, with many genes in
164 appearing in different organizations in the loci (Supplemental Figure 2), however, loci K3, K22 and K28
165 clustered separately from the other K-loci (Figure 1). In these three K-loci, a region with genes involved
166 in sulfate assimilation (genes *cysN, cysD* and *cysC*) as well as genes encoding a type II secretion system
167 (genes *xcpT, xpsE, epsF, gspK, xspD* and *bdbD*) are inserted between two *wecA* genes (Supplemental
168 Figure 2). Interestingly, the loci for K15 and K17 appear to be nearly identical (Figure 1, Supplemental
169 Figure 2).

170

171 *Testing* Kaptive *databases on NCBI and in house sequences genomes*

172 To develop the *Kaptive* databases, Roary gene clusters consisting of genomes with the same specific
173 serotypes were selected to be included in the databases, and genomes in clusters consisting of
174 different serotypes were excluded. To test the *Kaptive* databases, all 449 genomes with a known O-
175 serotype and 291 genomes with a known K-serotype were used. Of the 449 known O-serotypes, 403
176 O-serotypes were correctly identified (Figure 2A, Supplemental Table 1) and 257 of the 291 known K-
177 serotypes were correctly identified (Figure 2B, Supplemental Table 1). The mis-identified O- and K-
178 serotype genomes were all genomes downloaded from NCBI. Since we only have the genomes and
179 not the strains, we cannot check if these strains were serotyped correctly. The O-serotype O14 has
180 been recently described (Guo et al. 2017) and only one genome with this O-serotype is available. In
181 our dataset of 832 genomes, 14 NCBI genomes with serotype O5 are identified with the *Kaptive*
182 database as the new serotype O14. It is very likely that these genomes are mis-serotyped, because the

183 serum for serotype O14 was not available when these genomes were serotyped. Low performance is
184 also noted for K15 and K17, however it appears that these have identical gene content (Figure 2B,
185 Supplemental Figure 2B) and they may be misclassified.
186
187 For several K-serotypes, only one genome sequence was available and we could not determine if there
188 is variation in these K-loci gene content. Furthermore, several of the genomes sequenced in this study
189 were sequenced with a 50-bp single-end BGI sequencer, resulting in a higher number of short contigs,
190 therefore for several of these genomes, the K-locus was not assembled on one single contig. For these
191 genomes, the contigs with flanking genes were selected and concatenated manually. It is possible that
192 some genes are missing in these K-locus reference sequences, although these likely represent repeat
193 sequences. If more *V. parahaemolyticus* genome sequences with closed K-locus become available, the
194 *Kaptive* K-serotype database will be updated with the closed K-locus sequence of these K-serotypes.
195
196 **Conclusions**
197 The in this study developed *Kaptive* databases with the identified 16 O- and 70 K -loci can be used to
198 identify the O- and K-serotypes of *V. parahaemolyticus* isolates from whole genome sequencing data.
199 The variation of K-antigen loci is much higher than expected as we identified 67 new K-locus variants.
200
201 **Funding**
202 Sequencing of isolates was supported by the National Science and Technology Major Project of China
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281 **Figures and Tables**

282

283 **Figure 1**. Phylogenetic trees from the Roary gene presence absence table for (A) O-serotypes and (B)

284 K-serotypes

285

286 **Figure 2. Performance of the *Kaptive* database on genomes with known (A) O- and (B) K-serotypes.**

287 In blue the percentage of correctly typed genomes, in red the percentage incorrectly typed genomes.

288 The number in the bars represent the number of genomes. ***:** These are likely O14 serotype genomes

289 incorrectly agglutination serotyped because O14 has recently been described. #: The genetic

290 organization of K15 and K17 is identical. Likely these have been misclassified.

291

292 **Supplemental Figure 1.** Phylogenetic tree based on core genome analysis of O- and K-representative

293 isolates. The O and K loci and MLST STs are given.

294

295 **Supplemental Figure 2.** Schematic representations of gene organization of the (A) O-loci and (B) K-

296 loci.

297

298 **Supplemental Figure 3**. Gene cluster comparisons of the (A) O-loci and (B) K-loci.

299

300 **Supplemental Table 1.** Selected genomes used for the O- and K-serotype Kaptive databases.

301

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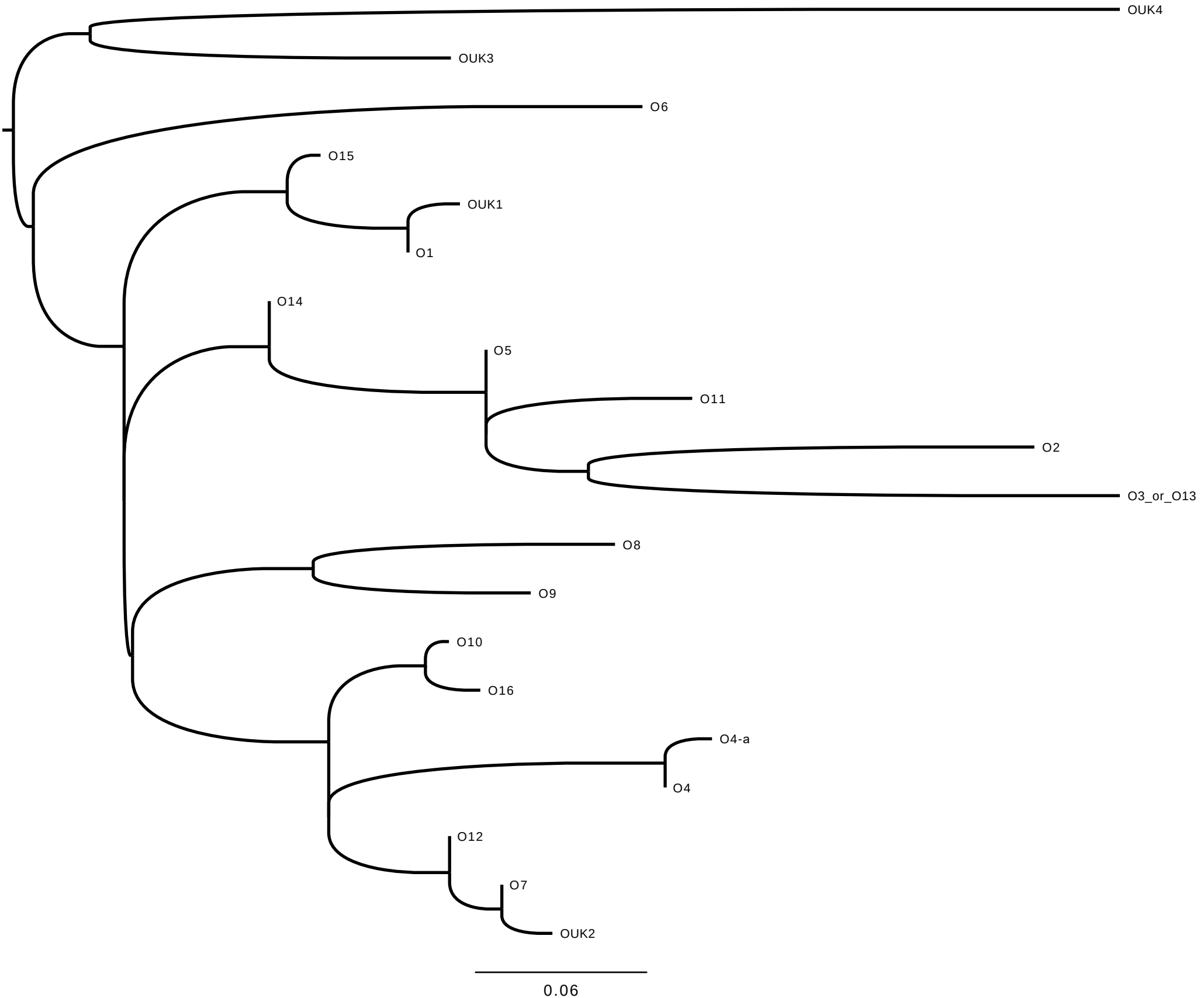
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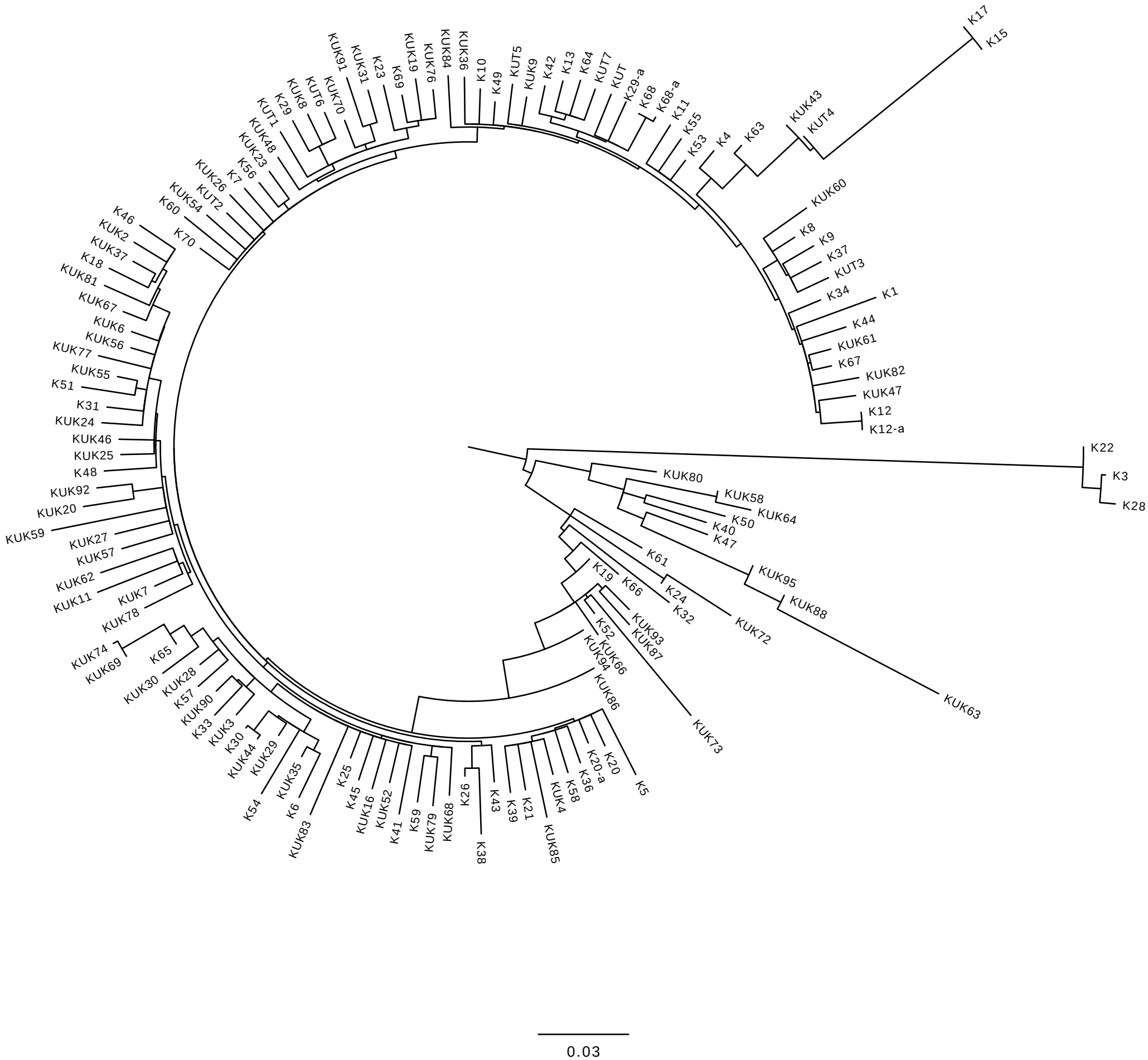
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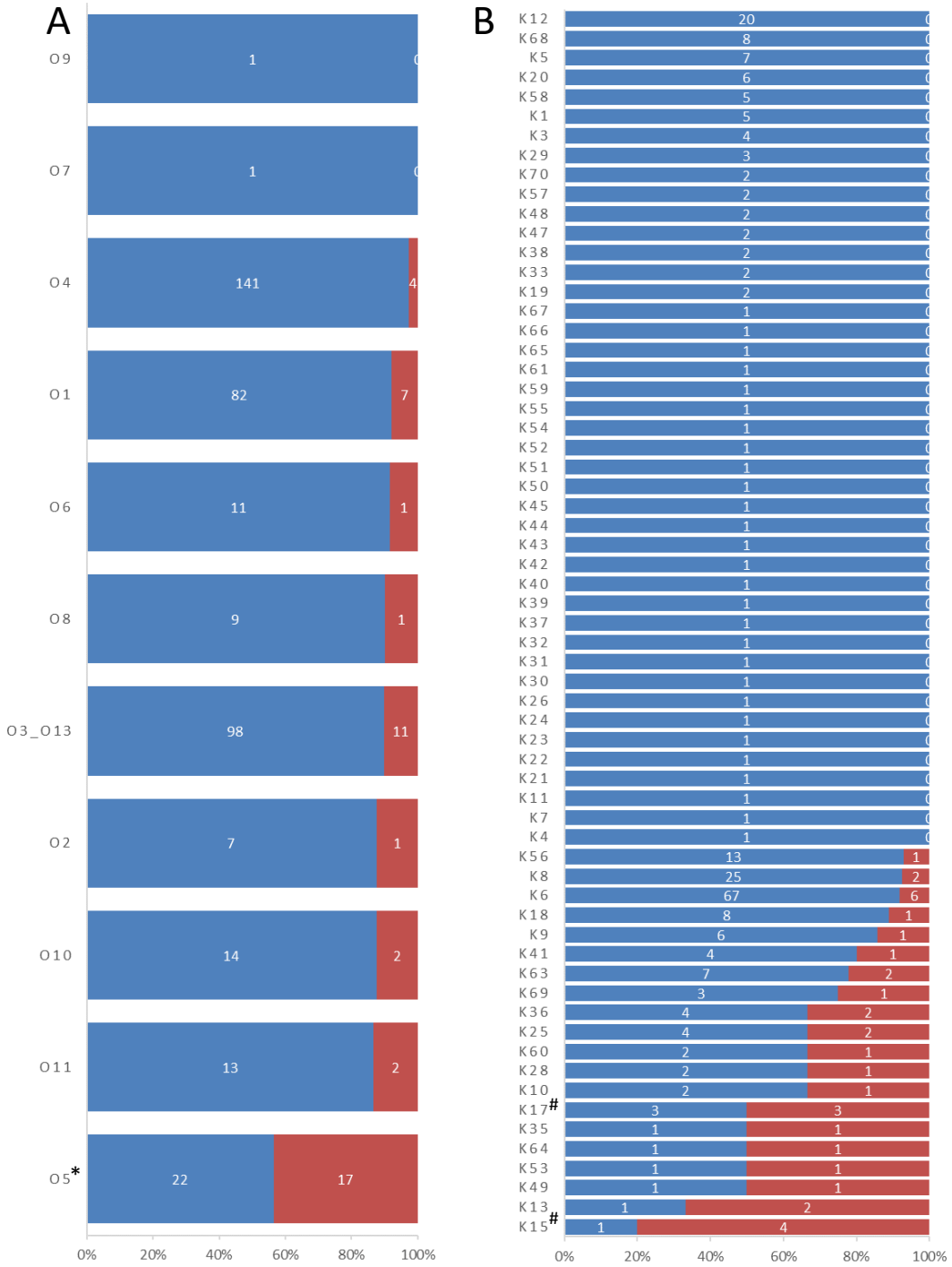
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