

Comparative genomic analyses provide clues to capsule switch in *Streptococcus suis*

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21 **Abstract**

22 *Streptococcus suis* (*S. suis*) is a major bacterial pathogen in swine industry and also an emerging
23 zoonotic agent. *S. suis* produces an important extracellular component, capsular polysaccharides
24 (CPS). Based on which, dozens of serotypes have been identified. Through virulence genotyping,
25 we uncovered the relatedness between proportions of SS2, SS3 and SS7 strains despite their
26 differences in serotypes. Multi-locus sequence typing (MLST) was used to characterize whole *S.*
27 *suis* population, revealing that there is capsule switch between *S. suis* strains. Importantly,
28 capsule switch occurred in SS2, 3 and 7 strains belonging to CC28 and CC29, which is
29 phylogenetically distinct from the main CC1 SS2 lineage. To further explore capsule switch in *S.*
30 *suis*, comparative genomic analyses were performed using available *S. suis* complete genomes.
31 Phylogenetic analyses suggested that SS2 strains can be divided into two clades (1 and 2), and
32 those classified into clade 2 are colocalized with SS3 and SS7 strains, which is in accordance
33 with above virulence genotyping and MLST analyses. Clade 2 SS2 strains presented high genetic
34 similarity with SS3 and SS7 and shared common competence and defensive elements, but are
35 significantly different from Clade 1 SS2 strains. Notably, although the *cps* locus shared by Clade
36 1 and 2 SS2 strains is almost the same, a specific region in *cps* locus of strain NSUI002 (Clade 2
37 SS2) can be found in SS3 *cps* locus, but not in Clade 1 SS2 strain. These data indicated that SS2
38 strains appeared in CC28 and CC29 might acquire *cps* locus through capsule switch, which could
39 well explain the distinction of genetic lineages within SS2 population.

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41 **Key words:** *Streptococcus suis*; Capsule switch; *S. suis* serotype 2; *S. suis* serotype 3; *S. suis*
42 serotype 7; MLST; Comparative genomic analyses.

43

44 **1.Introduction**

45 *Streptococcus suis* (*S. suis*) is a major bacterial pathogen causing global economic losses to
46 swine industry. It is also a serious zoonotic pathogen in countries with intensive swine
47 production. Capsule polysaccharide (CPS) is the key virulence determinant in *S. suis*, which
48 contributes to the bacterial resistance to host immunity (Fittipaldi et al., 2012). Sequence
49 analyses of *S. suis* genomes revealed a *cps* locus with variable lengths and a series of genes
50 specific to CPS production (Okura et al., 2013). It is thought that CPS of *S. suis* is synthesized
51 and exported through the Wzx/Wzy pathway, which is a mechanism commonly used in
52 *Streptococcus pneumoniae* (*S. pneumoniae*) and *Streptococcus agalactiae* (*S. agalactiae*)
53 capsular biosynthesis (Yother, 2011). The difference in *cps* locus would lead to the difference in
54 components and structures of CPS, and importantly, the serum antigenicity. In the 1980s and
55 1990s, 35 serotypes (types 1 to 34 and type 1/2) having been described based on CPS antisera
56 coagglutination test (Higgins et al., 1995). Later on, serotypes 20, 22, 26, 32, 33 and 34 are
57 suggested to be removed from *S. suis* species (Hill et al., 2005; Tien et al., 2013). More recently,
58 a novel variant serotype Chz and other 8 novel *cps* loci harboring specific *wzy* polymerase genes
59 and *wzx* flippase genes were identified (Pan et al., 2015; Qiu et al., 2016), revealing the high
60 diversity of *cps* locus in *S. suis* genomes.

61 Among the known serotypes worldwide, *S. suis* serotypes 2, 3, 9, 7, 8, 4 and 1 are the most
62 prevalent serotypes linked with infection of swine, especially serotype 2 (SS2) (Goyette-
63 Desjardins et al., 2014). In North America, serotype 2 and 3 are the two most prevalent serotypes
64 isolated from clinical pig cases (Goyette-Desjardins et al., 2014). In Asia, the most prevalent
65 serotypes in infected pigs are serotypes 2, 3, 4, 7 and 8 (Goyette-Desjardins et al., 2014; Wei et
66 al., 2009). In Europe, serotype 9 and 2 are more frequently found in clinical pig cases, followed

67 by serotypes 7, 8, 3 and 1 (Goyette-Desjardins et al., 2014). As for the infection of human,
68 serotypes 2, 4, 5, 9, 14, 16, 21 and 24 have already been reported, of which SS2 is the
69 predominate serotype (Goyette-Desjardins et al., 2014; Kerdsin et al., 2017). Therefore, most
70 studies in *S. suis* field are focusing on SS2 due to its close link with diseases. However,
71 heterogenicity in SS2 population has been observed, indicating serotyping alone is not sufficient
72 to characterize *S. suis* strains.

73 In addition to capsule serotyping, multilocus sequence typing (MLST) is most the widely used
74 typing method in epidemiological studies of *S. suis*. Analyses of MLST data from different
75 sources determined the sequence types (STs) of stains, which could be further clustered into
76 clonal complexes (CCs) (King et al., 2002). Interestingly, MLST analyses of *S. suis* isolates
77 suggested that SS2 population can be divided into two major lineages, not only in terms of
78 geographical and genetic background, but also virulence phenotype (Fittipaldi et al., 2011;
79 Goyette-Desjardins et al., 2014; Yao et al., 2015; Zhu et al., 2013). Among SS2 isolates,
80 although ST1/ST7 (CC1), ST25 (CC29) and ST28 (CC28) strains have been isolated in both
81 Asia and North American, CC1 strains are more prevalent in Asia, while CC28 and CC29 strains
82 are more commonly found in North America (Goyette-Desjardins et al., 2014). Importantly,
83 ST1/ST7 strains are significantly more virulent than ST25 and ST28 strains (Athey et al., 2015;
84 Fittipaldi et al., 2011; Guo et al., 2020). Although the distinctions of genetic and virulence
85 phenotypic lineages within SS2 population have been found, the way it was formed are not yet
86 fully understood.

87 Capsule switch, a change of serotype of a single clone by alteration or exchange of its *cps* locus,
88 has been identified in streptococcal species including *Streptococcus iniae* (*S. iniae*) (Heath et al.,
89 2016), *S. pneumoniae* (Wyres et al., 2013) and *S. agalactiae* (Martins et al., 2010). Development

90 of MLST greatly promote the studies on capsular switch, which can be more easily identified by
91 detecting strains of different serotypes sharing the same ST. Although capsular switch has been
92 found in *S. suis* isolates (King et al., 2002), the effects of this phenomena on *S. suis* population
93 structure has not been demonstrated. Previously, we showed that the virulence related genes are
94 differently distributed in strains of two SS2 clusters (Dong et al., 2015), which is in accordance
95 with the known SS2 sequence type classification (Fittipaldi et al., 2011; Zhu et al., 2013). In this
96 study, we combined MLST analysis, virulence genotyping and whole genome analysis, to
97 explore the capsular switch in *S. suis*, and highlight its potential role of in shaping SS2
98 population.

99 **2. Materials and methods**

100 **2.1 Bacteria strains and culture conditions**

101 All *S. suis* serotype 3 (SS3) and *S. suis* serotype 7 (SS7) strains are the field strains isolated from
102 China from 2004 to 2018 and were stored in our laboratory. The typical virulent SS2 strain
103 ZY0719 was isolated from a diseased pig during an outbreak in China. *S. suis* was grown in
104 Todd–Hewitt broth medium (THB; Becton Dickinson, Sparks, MD, USA) at 37°C overnight.
105 The antibiotics including spectinomycin (100 µg/ml) and chloramphenicol (5 µg/ml) were
106 added into the medium if it is needed. The plasmids for *Streptococcus* pSET-2::cat was used in
107 this experiment for DNA template. A detailed information for bacterial strains used in this study
108 listed in Table S1.

109 **2.2 PCR assays**

110 A previously established species-specific polymerase chain reaction (PCR) based on the *gdh* and
111 *recN* genes were performed to confirm the identification of *S. suis* (Okwumabua et al., 2003).
112 The serotype-specific PCR was used to identify SS3 and SS7 strains among the collected *S. suis*

113 isolates (Kerdsin et al., 2014). In the virulence genotyping assay, 19 *S. suis* virulence-associated
114 genes, including *mrp*, *epf*, *sly*, *rgg*, *ofs*, *srtA*, *pgdA*, *gapdh*, *iga*, *endoD*, *ciaRH*, *salKR*, *manN*,
115 *purD*, *dppIV*, *neuB*, *dltA*, *comR* and *scnF* were detected by individual PCR as previously
116 described (Dong et al., 2015; Dong et al., 2017). In MLST assays, seven housekeeping genes
117 *aroA*, *mutS*, *cpn60*, *dpr*, *recA*, *thrA* and *gki* were amplified by PCR as described previously
118 (King et al., 2002), and the amplification fragments were sequenced.

119 **2.3 Clustering analysis**

120 For MLST, allele numbers and sequence types (STs) were identified in MLST database
121 (<http://ssuis.mlst.net/>). The eBURST (<http://eburst.mlst.net>) program was used to determine
122 population structures through identifying potential clonal complexes (CCs) and founders. For
123 virulence genotyping, BioNumerics (version 6.6, Applied Maths, Kortrijk, Belgium) was used to
124 analyze the profiles of virulence related genes as previously described (Dong et al., 2015; Dong
125 et al., 2017; Mateus et al., 2013; Zhu et al., 2017): the resemblance was computed with simple
126 matching coefficients, and agglomerative clustering was performed using the unweighted
127 average linkage (UPGMA). The profiling of SS2 virulence related genes used in this clustering
128 analysis is acquired from a previous study (Dong et al., 2015).

129 **2.4 Phylogenetic analysis**

130 The complete genome sequences of 25 *S. suis* isolates of different serotypes were obtained from
131 NCBI GenBank and used for phylogenetic analyses, including the 3 *S. suis* genomes provided by
132 our research group (ZY05719, T15 and SC070731). A phylogenetic tree based on the 1373
133 single copy orthology clusters generated from clustering of the 25 strains was constructed using
134 neighbor-Joining (NJ) method. The information of strains were listed in table S2.

135 **2.5 Multi-genome alignment analysis**

136 Comparative analysis of alignments among *S. suis* genomes were conducted using the
137 progressive alignment option of the Mauve software (Darling et al., 2004). ZY05719 was
138 selected as virulent SS2 strains, NSUI002 and NSUI060 were the typical avirulent SS2 strains.
139 Genomes of SS3 strains YB51 and ST3, SS7 strain D9 were also used in genome alignments.

140 **2.6 Average nucleotide identity analysis**

141 The genomic similarity between SS2, SS3 and SS7 strains was evaluated by average nucleotide
142 identity (ANI) method. The algorithm implemented at the EzGenome server was used To
143 calculate the ANI value (www.ezbiocloud.net/tools/ani). The proposed and generally accepted
144 species boundary for ANI value are 95~96% (Richter and Rossello-Mora, 2009).

145 **2.7 Defense elements analysis**

146 Bacterial defense systems were ancient elements that confers resistance to foreign genetic
147 elements, including Restriction-Modification (RM) system and Clustered Regularly Interspaced
148 Short Palindromic Repeats (CRISPR) system. RM elements and CRISPR components in *S. suis*
149 were determined through CRISPRs finder (<http://crispr.u-psud.fr/>) and web-service REBASE
150 (<http://rebase.neb.com/rebase/rebase.html>), representatively. The structures of RM and CRISPR
151 systems are achieved through performing an all-to-all BLASTN search in the NCBI nucleotide
152 database. Consensus sequences of repeat sequences in defense systems were determined using
153 MegAlign.

154 **2.8 Competence system analysis**

155 *S. suis* harbors a natural competence system, which greatly facilitates the DNA exchange
156 through horizontal genetic transfer. In our previous study, we identified that ComRS-ComX
157 competence system varies in different genotype background *S. suis* strains (Zhu et al., 2019).

158 Through BLASTN search, competence systems were identified in representative SS2, SS3, SS7
159 genomes. The amino acid sequence of competence systems was visualized by DNAMAN
160 software. To experimentally validate the functional difference between *S. suis* strains, a
161 transformation efficiency test was performed between 8 selected SS2, SS3 and SS7 field strains
162 stored in our laboratory. Briefly, two different synthetic competence peptides XIP (type
163 A:GNWGTWVEE and type B: LGDENWWVK) were added to the bacterial culture with
164 template DNA (plasmid pSET-2::cat) to induce the natural transformation. Competency was
165 calculated based on the amount of transformants that grew in THB plates with spectinomycin
166 and chloramphenicol selection.

167 **2.9 Analysis of *cps* locus**

168 The diversity serotypes are based on the variation of genetic locus harboring capsular
169 polysaccharide related genes (*cps* locus). In general, strains with a same serotype would present
170 highly similar genomic sequence in *cps* locus. The sequence of *cps* locus in genomes of CC1
171 SS2 strain ZY05719, CC28 SS2 strain NSUI002, and CC28 SS3 strain ST3 were determined
172 using BLAST. Corresponding sequences and gene annotation information were obtained from
173 obtained from NCBI GenBank. The homologous analysis was performed using Easyfig software.

174 **3. Results**

175 **3.1 Virulence genotyping of field strains revealed relatedness of a SS2 subpopulation with** 176 **SS3 and SS7**

177 Virulence genotyping is a powerful tool to study pathogenic bacteria, which can contribute to
178 screen for specific disease-associated virulence genes (Gerjets et al., 2011; Rasmussen et al.,
179 2013) or uncover the relatedness between isolates (Dong et al., 2017; Mateus et al., 2013).
180 Previously, we applied a virulence genotyping strategy by detecting a set of virulence related

181 genes in SS2 isolates, and revealed that SS2 strains can be divided into two clusters due to the
182 different distribution of genes *epf*, *sly*, *endoD*, *rgg* and *scnF* (Dong et al., 2015). In this study, we
183 further detected the presence of virulence related genes in SS3 and SS7 isolates by PCR, and
184 performed clustering analysis based on the gene profiles of SS2 (n=62), SS3 (n=17) and SS7
185 (n=9) isolates (Figure 1). In accordance with what we have showed previously (Dong et al.,
186 2015), SS2 strains could be divided into two clusters ((I and II) with a different prevalence of
187 virulence related genes. Interestingly, SS3 and SS7 isolates were classified into cluster II
188 together with one SS2 subpopulation, suggesting a relatedness of SS3 and SS7 with that SS2
189 subpopulation (Figure 1). For the gene distribution, 8 genes were detected in all *S. suis* field
190 isolates, namely *srtA*, *pgdA*, *dltA*, *iga*, *sspA*, *manN*, *ciaHR* and *gapdh*; whereas gene *epf* and *rgg*
191 were only detected in cluster I but not in cluster II. According to virulence genotyping result in
192 this study and previous knowledge (Dong et al., 2015; Dong et al., 2017; Kobayashi et al., 2013;
193 Mateus et al., 2013), we hypothesized that the presence of similar virulence genotypes reflects
194 phylogenetic relatedness of SS2, SS3 and SS7. Therefore, we performed genetic analyses in *S.*
195 *suis* population to further test our hypothesis.

196 **3.2 MLST analysis demonstrated capsule switch in *S. suis* between SS2, SS3 and SS7**

197 MLST is an important and widely used molecular method in studying *S. suis* epidemiology, in
198 which seven housekeeping genes are sequenced to assess the genomic variation and define
199 Sequence Types (STs). The availability of updated information in *S. suis* MLST database
200 (<http://ssuis.mlst.net/>) makes it possible to apply a grouping approach for identification of Clonal
201 Complex (CC) and perform comparative analysis with strains of different serotypes. A total of
202 1528 *S. suis* strains (701 STs) from MLST database was diagramed by eBURST on the basis of
203 their allelic profiles. The eBURST analysis revealed 9 major clonal complexes (Figure 2A). CC1

204 (629/1528) is the predominant clonal complexes in *S. suis*, followed by CC16 (120/1528), CC28
205 (71/1528), CC29 (62/1528) and CC104 (30/1528), whereas CC94, CC528, CC423 and CC201
206 are much smaller schemes.

207 The MLST analysis revealed capsular switch in *S. suis*, that strains of different serotypes sharing
208 the same ST. In *S. suis*, capsular switch was firstly reported in 2002 (King et al., 2002), which
209 analyzed the capsular switch events from ST1 to ST92, and showed it occurred in ST1, 13, 16,
210 17, 27, 28, 29, 65 and 76. Our updated analysis identified novel STs with capsular switch,
211 including ST15, 89, 94, 105, 136, 156, 243 and 297 (Table 1). Importantly, there is capsular
212 switch within SS2, SS3 and SS7 strains. ST27 from CC28 has both SS2 and SS3 strains, and
213 ST29 from CC29 has SS2, SS3 and SS7 strains (Table 1 and Figure 2B). CC28 and CC29 have
214 already been demonstrated to be important SS2 lineages (Athey et al., 2015; Fittipaldi et al.,
215 2011). Interestingly, as shown in our eBURST analysis, CC28 harbors a SS3 major population,
216 and CC29 harbors a SS7 major population (Figure 2B). Those results suggested that a sub-
217 population of SS2 strains of CC28 and CC29 are more closely related to SS3 and SS7 strains,
218 and there is capsular switch in those two clonal complexes.

219 **3.3 Whole genome phylogenetic analysis of different serotype strains deciphered two** 220 **distinct clades in SS2**

221 To investigate the phylogenetic relationships among *S.suis* strains, we used 25 complete
222 genomes of different serotypes from GenBank dataset. The phylogenetic tree, constructed using
223 neighbor-joining (NJ) method (Figure 3), showed that *S.suis* strains of different serotypes can be
224 classified into three major clades (Clade 1, 2 and 3). SS1 strain, SS4 strain and SS2 avirulent
225 strain T15 appeared to be phylogenetically independent, whereas SS16 strain and SS9 strains are
226 located together in Clade 3.

227 SS14 strain JS14 and SS1/2 strain SS12 are grouped together with ten SS2 strains, and formed
228 the largest clade (Clade 1) on phylogenetic tree. SS2 strains in this clade presented a short
229 evolutionary distance from each other, suggesting that these strains were probably derived from a
230 recent common ancestor. This result is in accordance with the MLST analysis, that all SS2
231 strains in Clade 1 belong to CC1. SS14 strain JS14 and SS1/2 strain SS12 also presented CC1
232 related STs, indicating a close link with Clade 1 SS2 strains.

233 Clade 2 included SS7 strain D9, SS3 strains ST3 and YB51, and four SS2 strains. These SS2
234 strains therefore showed a large divergence from Clade1 SS2. SS2 strain NSUI060 is a strain of
235 ST25 (CC29), and is assigned in a same branch with ST29 (CC29) strain D9 on phylogenetic
236 tree. SS2 strains 90-1330, 05HAS68 and NSUI002 belongs to ST28 (CC28), and are grouped in
237 a same branch with two ST 35 (CC28) SS3 strains. Above result indicated that CC28/29 SS2
238 strains in this clade were more closely related with SS7 strain and SS3 strains rather than CC1
239 SS2 strains in Clade 1. This is consistent with the virulence genotyping result, and also reflecting
240 the capsule switch identified by MLST within SS2, SS3 and SS7strains. Therefore, we further
241 performed whole-genome comparative analysis to decipher capsule switch in *S. suis*.

242 **3.4 Clade 2 SS2 had a higher genomic similarity with SS3 and SS7 than Clade 1 SS2**

243 The arrangement and collinearity of Clade 1 and Clade 2 *S. suis* genomes were investigated
244 using Mauve program. We first used CC28 SS2 strain NSUI002 as reference genome, to
245 compare clade 1 CC1 SS2 strain ZY05719, as well as CC28 SS3 strains ST3 and YB51 that
246 colocalized with NSUI002 in Clade 2 sub-branch. Mauve analysis (Figure 4) of those strains
247 indicated that rearrangements occurred in genomes of those strains, but the overall genomic
248 organizations are relatively comparable. The NSUI002 and YB51 genomes showed more
249 collinear than ZY05719, suggesting that NSUI002 and SS3 strains have a higher genomic

250 similarity. We further compared the genomic organization of SS2 strain NSUI060 and SS7 strain
251 D9 (Figure S1), which are colocalized in a subgroup of Clade 2 as well. Alignment of the
252 genome sequences of NSUI060 and D9 revealed a high level of genomic rearrangement,
253 including large-scale deletion, insertion, translocation, and inversion. However, the genomes of
254 NSUI060 and D9 still shared a more similar sequence synteny with each other, but significantly
255 different from ZY05719 with respect to both collinearity and genome structure. Those results
256 show that Clade 2 SS2 strains and SS3/7 strains has the smaller scale of arrangements and higher
257 level of synteny compared with Clade 1 SS2.

258 The genomic relationship between Clade 1 isolates and Clade 2 isolates was further evaluated by
259 nucleotide sequence similarity. The average nucleotide identity (ANI) were calculated (Figure 5)
260 using the web-based EZ BioCloud platform (www.ezbiocloud.net/tools/ani). The ANI values
261 between the Clade 1 SS2 isolates are very high, ranged from 99.54 to 99.97%, whereas the
262 ANI values between Clade 1 SS2 isolates and Clade 2 SS2 is only from 96.28 to 96.94, which is
263 close to the cut-off values recommended for species delineation (95–96%) (Richter and
264 Rossello-Mora, 2009). The Clade 2 SS2, SS3 and SS7 strains have ANI values above 98, and can
265 be further divided into two sub-clusters, which is in accordance with the phylogenetic analysis
266 and Mauve result. Especially, the ANI values between three Clade 2 SS2 isolates, namely 90-
267 1330, 05HAS68 and NSUI002, and SS3 strains are from 99.57 to 99.70, while the ANI value
268 between SS2 strain NSUI060 and SS7 strain D9 is 99.50. Thus, these results revealed the
269 genomic dissimilarity of Clade 1 SS2 isolates and Clade 2 SS2 isolates, supporting the
270 conclusion that Clade 2 SS2 isolates have a high relatedness with SS3 and SS7 strains, as
271 implied by the above whole genome comparison results.

272 **3.5 SS3, SS7 and Clade 2 SS2 shared defense systems different from Clade 1 SS2**

273 The defense elements, including RM system and CRISPR/Cas system, were detected in *S. suis*
274 genomes. Both of the systems have a role in protecting bacteria against invading exogenous
275 DNA, but the mechanism is different (Dupuis et al., 2013). The type I RM system uses HsdS, a
276 single protein can respond to the methylation of target sequence, to determine the specificity of
277 both restriction and methylation by the action of endonuclease HsdR and methyltransferase
278 HsdM (Willemse and Schultsz, 2016). In *S. suis*, a total of three type I RM systems are detected
279 in *S. suis* (Figure 6A). The Type A and Type B RM systems specifically appear in the Clade 1
280 SS2 strain, but not in Clade 2 strains. Type C RM system is present and conserved in strains of
281 both clades. However, Clade 2 strains have an inserted *fic* gene between the *hsd* genes, which is
282 different from Clade 1 strains. Another defense element, CRISPR/cas system, provides acquired
283 immunity in prokaryotic organisms. It integrates short sequences of invading exogenous DNA
284 between CRISPR repeats, and cleaves reinvading foreign DNA when recognize same sequences
285 (Wiedenheft et al., 2012). CRISPR analysis result showed that the CRISPR components are
286 absent in Clade 1 strains but present in all Clade2 strains. The CRISPR repeat in Clade 2 strains
287 is 36 bp in length (GTTTTACTGTTACTTAAATCTTGAGAGTACAAA AAC), but SS7 strain
288 D9 has an additional variant form with an additional TTA at the end of the repeat (Figure 6B and
289 C). Above data is in accordance with a previous report (Okura et al., 2017), that SS3 and SS7
290 strains shared defense elements with Clade 2 SS2 strains, which is different from Clade 1 SS2
291 strains.

292 **3.6 SS3, SS7 and Clade 2 SS2 shared a competence system different from Clade 1 SS2**

293 *S. suis* is a bacterium with natural transformation ability, which depends on the ComRS
294 competence systems. Natural transformation contributes to the horizontal gene transfer in *S. suis*
295 and increase the diversity of *S. suis* genomes, which confers a unique advantage for capsule

296 switch. Previously, we have identified three types of ComRS systems with specific competence
297 pheromone in *S. suis* (Zhu et al., 2019). BLAST search results suggested that all Clade 1 isolates
298 harbors Type A ComRS system, while all Clade 2 strains harbors Type B ComRS system (Figure
299 6C). We further detected the distribution of ComRS systems in field strains used in virulence
300 genotyping (Figure 1). Results showed that all field strains that classified into cluster I in
301 virulence genotyping have Type A ComRS system, whereas all strains classified into cluster II,
302 including SS3 and SS7 isolates, harbor Type B ComRS system (data not shown). To
303 experimentally test the transformation efficiencies of strains stimulated with noncognate
304 synthetic competence pheromones, we randomly selected two cluster II SS2 strains
305 (ZJX0908005 and ZJ92091101), two cluster II SS3 strains (128-1-2 and 129-1-3), two cluster II
306 SS7 strains (SH59 and SH04815) and two representative cluster I SS2 strains (ZY05719 and
307 P1/7). Type A XIP exclusively induces competence in cluster I SS2 strains, and Type B XIP
308 induces competence in cluster II SS2 strains, SS3 strains and SS7 strains (Figure 7). Although
309 belonging to serotype 2, cluster II SS2 shared a same bacterial communication language with
310 SS3 and SS7, but could not respond to the pheromone from cluster I SS2 strains, which is
311 consistent with phylogenetic analyses results.

312 **3.7 Analysis of *cps* locus provided evidence for capsule switch between SS3 and Clade 2 SS2**

313 To precisely characterize the capsular switch between SS2, SS3 and SS7, we compared the *cps*
314 genes locus of the representative *S.suis* strains. Although SS7 strain D9 is closely related to ST25
315 SS2 strain NSUI060, BLAST search on the D9 genome showed that the *cps* locus is disrupted
316 due to genomic rearrangement, and the *cps* genes are separated and translocated to different
317 genomic segments (data not shown). Therefore, here we only compared representative SS2 and
318 SS3 strains, including SS3 strain ST3, Clade 1 SS2 strain ZY05719 and Clade 2 strain NSUI002

319 (Figure 8). Sequence alignment of *cps* locus and its flanking regions of these strains revealed that
320 the 7.2 kb-long upstream flanking sequence (*tetR* to *yaaA* and *cpsABCD*) and the 12.6 kb-long
321 downstream flanking sequence (*aroA* to *asnS*) are extremely conserved among *S. suis* isolates.
322 This suggested that the potential capsule switch event may occur through homologous
323 recombination in these regions probably independently of the overall genetic background. Except
324 for a translocated insertion of transposon element (red arrow), the SS2 *cps* locus of NSUI002 is
325 almost identical to that of ZY05719, and differs from SS3 *cps* locus. However, in the ending
326 region of SS2 *cps* locus, genes *cps2T*, *cps2U* and *cps2V* are absent from the *cps* locus of
327 NSUI002. Instead, NSUI002 has a fragment shared high identity with sequence from SS3 *cps*
328 locus containing *cps3O*, *cps3P* and *tnp3-4* (red line box). It is worth noting this unique region
329 has no homologous sequence in the genome of ZY05719. Thus, this trait in the *cps* locus of
330 NSUI002 strongly supports the hypothesis of capsular switch between SS2 and SS3, and favors a
331 possibility that the potential capsular switch between SS3 and SS2 might result from a
332 recombinational crossover point located ahead of *cps3O*. In summary, we find detailed evidence
333 for capsule switch within *S.suis*, at least and as expected, from the analysis of *cps* locus in CC28
334 SS2 and SS3 strains.

335 **4. Discussion**

336 Serotype 2 is the most prevalent serotype of *S. suis* worldwide. Among the major evolutionary
337 lineages revealed by genetics analyses, ST1 and 7 (CC1) SS2 are generally associated with
338 diseases (Goyette-Desjardins et al., 2014). However, ST25 (CC29) and ST28 (CC28), accounting
339 for larger proportions of SS2 strains in North American, present less virulence potentials in
340 animal model (Athey et al., 2015; Fittipaldi et al., 2011). In accordance, ST28 strains in China
341 are also regard as representative avirulent strains (Guo et al., 2020; Ma et al., 2020; Wang et al.,

342 2017). Those reports suggested that CC28 and CC29 SS2, which are phylogenetically distinct
343 from CC1 SS2, are the pool of strains with lower virulence levels. In this study, we report that
344 capsule switch exists in *S. suis* population, notably in CC28 and CC29 between SS2, SS3 and
345 SS7. This finding may explain the genetic and phenotypic differences between CC1 SS2 and
346 CC28/29 SS2, and indicate a possibility that CC28/29 SS2 was derived from an ancestor
347 unrelated with CC1 SS2 through capsule switch, which has often been overshadowed by simple
348 serotyping.

349 Capsule switch has been identified in other extracellular pathogens harboring polysaccharide
350 capsule and natural competence, such as *Neisseria meningitidis* (*N. meningitidis*), *S. agalactiae*
351 and *S. pneumoniae*. Among which, the capsule switch of *S. pneumoniae* is the most
352 representative and well-studied. Pneumococcal capsule switch can be achieved through gradual
353 evolution with a combination of minor mutation, deletion and recombination in *cps* locus. For
354 example, pneumococcal serotype 6A, 6B, 6C and 6D have near identity of *cps* locus, which only
355 differ in *wciP* gene (Song et al., 2011). A similar case in *S. suis* is the capsule switch between
356 SS1, SS2 and SS1/2 in CC1. Serotype 1/2 cannot be differentiated from serotype 1 and 2 by
357 serum antigenicity (Perch et al., 1983), and genetic analysis of the *cps* locus demonstrated
358 serotypes 1, 1/2 and 2 share the high genetic identities (Okura et al., 2013). On the other hand,
359 capsule switch can occur through the exchange of large genomic fragment containing full *cps*
360 locus (Bellais et al., 2012; Wyres et al., 2013), which is a suitable model to be applied in the
361 capsule switch between SS 2, 3, 7 strains in CC28 and 29. Given the fact that *cps* locus is high
362 diverse and variable between different *S. suis* serotypes (Okura et al., 2013), it is not logical to
363 keep *cps* locus conserved or even identical when genetic backbone is subject to high degree of
364 genetic rearrangement. Therefore, our data strongly supports a hypothesis that serotype 2, 3, 7

365 capsule switch results from the exchange of large *cps* locus, leads to similar genetic backbones
366 sharing common competence systems and defensive systems.

367 Importantly, the upstream and downstream flanking sequences of *cps* locus are almost identical
368 between different *S. suis* serotypes (Okura et al., 2013), providing potential recombination sites
369 for capsule switch. Furthermore, we studied the sequence of *cps* locus and flanking region
370 between a CC1 SS2 ZY05719, CC28 SS2 strain NSUI002 and SS3 strain ST3 in detail. A
371 genetic fragment was found to be conserved in the *cps* locus of CC28 SS3 and SS2, but absent at
372 that location in CC1 SS2, which suggests a potential recombination event occurred between
373 CC28 SS2 and SS3. However, based on current information, we cannot determine the temporal
374 relationship between strains, namely whether one is derived from another, or there is a common
375 ancestor. In addition, although ST27 (a ST harboring both SS2 and SS3), ST28 (including
376 NSUI002, SS2) and ST35 (including YB51 and ST3, SS3) are phylogenetically related and
377 clustered together in CC28, ST35 SS2 or ST28 SS3 have not yet been observed, indicating that
378 additional events occurred after capsule switch in this common evolutionary lineage. More
379 collected isolates and sequenced genomes in the future would be helpful to address those issues.

380 In fact, the core of capsule switch is to increase diversity in the population and enhance fitness in
381 certain environments, such as increasing antibiotic resistance or escaping herd immunity.
382 Accumulated studies have demonstrated that capsule switch in *S. pneumoniae* can be attributed to
383 the selection pressure from the use of vaccines targeting capsule (Martins et al., 2010). Similarly,
384 the change of CPS composition caused by *cpsG* mutation in *S. iniae* and capsule switch (from
385 type III to IV) in CC17 *S. agalactiae* contributes to vaccine escape (Bellais et al., 2012; Heath et
386 al., 2016). Besides that, capsule switch is also involved in bacterial pathogenicity. Pneumococcal
387 capsule switch from 6A to 6C promotes the resistance to complement system and presents

388 enhanced virulence for respiratory tract infection (Sabharwal et al., 2014). Artificial capsule
389 switch in *S. pneumonia* is able to alter virulence and infection outcomes in a mouse model (Kelly
390 et al., 1994; Trzcinski et al., 2003). Although the role of capsule switch in *S. suis* is not clear, it is
391 possible that the replacement of *cps* locus would cause phenotypic changes due to the alteration
392 of bacterial surface architecture. For instance, *NeuB*, an enzyme existing in serotype 1/2, 2 and
393 14, is essential for sialic acid biosynthesis (Feng et al., 2012). Therefore, acquiring NeuB may
394 increase bacterial resistance to complement system and phagocytosis (Feng et al., 2012;
395 Uchiyama et al., 2019). In fact, a recent study highlighted that experimentally switching capsule
396 type 2 to 3 in *S. suis* leads to defective whole blood survival and bacterial virulence (Okura et al.,
397 2020). More studies in the future are needed to clarify the phenotypic features of different *S. suis*
398 capsules, and what benefits bacteria may obtain from capsule switch.

399 **Acknowledgements**

400 This work was funded by the Natural Science Foundation of China (31972650), Shanghai
401 Agriculture Applied Technology Development Program (S0201700386), the Key Project of
402 Independent Innovation of the Fundamental Research Fund for the Central Universities of
403 Nanjing Agricultural University (KJQN201932) and the Priority Academic Program
404 Development of Jiangsu Higher Education Institutions (PAPD). We would like to thank Dr.
405 Qiang Li for his help in bioinformatic analyses.

406 **Conflict of interest**

407 The authors declare no conflicts of interest.

408 **Data availability statement**

409 The data that support the findings of this study are available from public database GENBANK,
410 and the accession number is listed in Table. S2

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413 **Ethical approval**

414 No animal experiments were performed thus ethical statement is not applicable in this study.

415 **References**

- 416 Athey, T.B., Auger, J.P., Teatero, S., Dumesnil, A., Takamatsu, D., Wasserscheid, J., Dewar, K.,
417 Gottschalk, M., Fittipaldi, N., 2015. Complex Population Structure and Virulence
418 Differences among Serotype 2 *Streptococcus suis* Strains Belonging to Sequence Type 28.
419 PLoS One 10, e0137760.
- 420 Bellais, S., Six, A., Fouet, A., Longo, M., Dmytruk, N., Glaser, P., Trieu-Cuot, P., Poyart, C.,
421 2012. Capsular switching in group B *Streptococcus* CC17 hypervirulent clone: a future
422 challenge for polysaccharide vaccine development. *J Infect Dis* 206, 1745-1752.
- 423 Darling, A.C., Mau, B., Blattner, F.R., Perna, N.T., 2004. Mauve: multiple alignment of
424 conserved genomic sequence with rearrangements. *Genome Res* 14, 1394-1403.
- 425 Dong, W., Ma, J., Zhu, Y., Zhu, J., Yuan, L., Wang, Y., Xu, J., Pan, Z., Wu, Z., Zhang, W., Lu,
426 C., Yao, H., 2015. Virulence genotyping and population analysis of *Streptococcus suis*
427 serotype 2 isolates from China. *Infect Genet Evol* 36, 483-489.
- 428 Dong, W., Zhu, Y., Ma, Y., Ma, J., Zhang, Y., Yuan, L., Pan, Z., Wu, Z., Yao, H., 2017.
429 Multilocus sequence typing and virulence genotyping of *Streptococcus suis* serotype 9
430 isolates revealed high genetic and virulence diversity. *FEMS Microbiol Lett* 364.
- 431 Dupuis, M.E., Villion, M., Magadan, A.H., Moineau, S., 2013. CRISPR-Cas and restriction-
432 modification systems are compatible and increase phage resistance. *Nat Commun* 4, 2087.
- 433 Feng, Y., Cao, M., Shi, J., Zhang, H., Hu, D., Zhu, J., Zhang, X., Geng, M., Zheng, F., Pan, X.,
434 Li, X., Hu, F., Tang, J., Wang, C., 2012. Attenuation of *Streptococcus suis* virulence by
435 the alteration of bacterial surface architecture. *Sci Rep* 2, 710.
- 436 Fittipaldi, N., Segura, M., Grenier, D., Gottschalk, M., 2012. Virulence factors involved in the
437 pathogenesis of the infection caused by the swine pathogen and zoonotic agent
438 *Streptococcus suis*. *Future Microbiol* 7, 259-279.
- 439 Fittipaldi, N., Xu, J., Lacouture, S., Tharavichitkul, P., Osaki, M., Sekizaki, T., Takamatsu, D.,
440 Gottschalk, M., 2011. Lineage and virulence of *Streptococcus suis* serotype 2 isolates
441 from North America. *Emerg Infect Dis* 17, 2239-2244.
- 442 Gerjets, I., Traulsen, I., Reiners, K., Kemper, N., 2011. Comparison of virulence gene profiles of
443 *Escherichia coli* isolates from sows with coliform mastitis and healthy sows. *Veterinary*
444 *microbiology* 152, 361-367.

- 445 Goyette-Desjardins, G., Auger, J.P., Xu, J., Segura, M., Gottschalk, M., 2014. *Streptococcus suis*,
446 an important pig pathogen and emerging zoonotic agent-an update on the worldwide
447 distribution based on serotyping and sequence typing. *Emerg Microbes Infect* 3, e45.
- 448 Guo, G., Du, D., Yu, Y., Zhang, Y., Qian, Y., Zhang, W., 2020. Pan-genome analysis of
449 *Streptococcus suis* serotype 2 revealed genomic diversity among strains of different
450 virulence. *Transbound Emerg Dis*.
- 451 Heath, C., Gillen, C.M., Chrysanthopoulos, P., Walker, M.J., Barnes, A.C., 2016. *Streptococcus*
452 *iniae* cpsG alters capsular carbohydrate composition and is a cause of serotype switching
453 in vaccinated fish. *Vet Microbiol* 193, 116-124.
- 454 Higgins, R., Gottschalk, M., Boudreau, M., Lebrun, A., Henrichsen, J., 1995. Description of six
455 new capsular types (29-34) of *Streptococcus suis*. *J Vet Diagn Invest* 7, 405-406.
- 456 Hill, J.E., Gottschalk, M., Brousseau, R., Harel, J., Hemmingsen, S.M., Goh, S.H., 2005.
457 Biochemical analysis, cpn60 and 16S rDNA sequence data indicate that *Streptococcus*
458 *suis* serotypes 32 and 34, isolated from pigs, are *Streptococcus orisratti*. *Vet Microbiol*
459 107, 63-69.
- 460 Kelly, T., Dillard, J.P., Yother, J., 1994. Effect of genetic switching of capsular type on virulence
461 of *Streptococcus pneumoniae*. *Infect Immun* 62, 1813-1819.
- 462 Kerdsin, A., Akeda, Y., Hatrongjit, R., Detchawna, U., Sekizaki, T., Hamada, S., Gottschalk, M.,
463 Oishi, K., 2014. *Streptococcus suis* serotyping by a new multiplex PCR. *J Med Microbiol*
464 63, 824-830.
- 465 Kerdsin, A., Hatrongjit, R., Gottschalk, M., Takeuchi, D., Hamada, S., Akeda, Y., Oishi, K.,
466 2017. Emergence of *Streptococcus suis* serotype 9 infection in humans. *J Microbiol*
467 *Immunol Infect* 50, 545-546.
- 468 King, S.J., Leigh, J.A., Heath, P.J., Luque, I., Tarradas, C., Dowson, C.G., Whatmore, A.M.,
469 2002. Development of a multilocus sequence typing scheme for the pig pathogen
470 *Streptococcus suis*: identification of virulent clones and potential capsular serotype
471 exchange. *J Clin Microbiol* 40, 3671-3680.
- 472 Kobayashi, N., Lee, K., Yamazaki, A., Saito, S., Furukawa, I., Kono, T., Maeda, E., Isobe, J.,
473 Sugita-Konishi, Y., Hara-Kudo, Y., 2013. Virulence gene profiles and population genetic
474 analysis for exploration of pathogenic serogroups of Shiga toxin-producing *Escherichia*
475 *coli*. *J Clin Microbiol* 51, 4022-4028.
- 476 Ma, J., Zhang, Z., Pan, Z., Bai, Q., Zhong, X., Zhu, Y., Zhang, Y., Wu, Z., Liu, G., Yao, H.,
477 2020. *Streptococcus suis* Uptakes Carbohydrate Source from Host Glycoproteins by N-
478 glycan Degradation System for Optimal Survival and Full Virulence during Infection.
479 *Pathogens* 9.
- 480 Martins, E.R., Melo-Cristino, J., Ramirez, M., 2010. Evidence for rare capsular switching in
481 *Streptococcus agalactiae*. *J Bacteriol* 192, 1361-1369.
- 482 Mateus, L., Henriques, S., Merino, C., Pomba, C., Lopes da Costa, L., Silva, E., 2013. Virulence
483 genotypes of *Escherichia coli* canine isolates from pyometra, cystitis and fecal origin. *Vet*
484 *Microbiol* 166, 590-594.
- 485 Okura, M., Auger, J.-P., Shibahara, T., Goyette-Desjardins, G., Calsteren, M.-R.V., Maruyama,
486 F., Kawai, M., Osaki, M., Segura, M., Gottschalk, M., Takamatsu, D., 2020. Capsular
487 polysaccharide switching in *Streptococcus suis* modulates host cell interactions and
488 virulence. *bioRxiv*, 2020.2010.2012.336958.
- 489 Okura, M., Nozawa, T., Watanabe, T., Murase, K., Nakagawa, I., Takamatsu, D., Osaki, M.,
490 Sekizaki, T., Gottschalk, M., Hamada, S., Maruyama, F., 2017. A Locus Encoding

- 491 Variable Defense Systems against Invading DNA Identified in *Streptococcus suis*.
492 *Genome Biol Evol* 9, 1000-1012.
- 493 Okura, M., Takamatsu, D., Maruyama, F., Nozawa, T., Nakagawa, I., Osaki, M., Sekizaki, T.,
494 Gottschalk, M., Kumagai, Y., Hamada, S., 2013. Genetic analysis of capsular
495 polysaccharide synthesis gene clusters from all serotypes of *Streptococcus suis*: potential
496 mechanisms for generation of capsular variation. *Appl Environ Microbiol* 79, 2796-2806.
- 497 Okwumabua, O., O'Connor, M., Shull, E., 2003. A polymerase chain reaction (PCR) assay
498 specific for *Streptococcus suis* based on the gene encoding the glutamate dehydrogenase.
499 *FEMS Microbiol Lett* 218, 79-84.
- 500 Pan, Z., Ma, J., Dong, W., Song, W., Wang, K., Lu, C., Yao, H., 2015. Novel variant serotype of
501 *streptococcus suis* isolated from piglets with meningitis. *Appl Environ Microbiol* 81,
502 976-985.
- 503 Perch, B., Pedersen, K.B., Henrichsen, J., 1983. Serology of capsulated streptococci pathogenic
504 for pigs: six new serotypes of *Streptococcus suis*. *J Clin Microbiol* 17, 993-996.
- 505 Qiu, X., Bai, X., Lan, R., Zheng, H., Xu, J., 2016. Novel Capsular Polysaccharide Loci and New
506 Diagnostic Tools for High-Throughput Capsular Gene Typing in *Streptococcus suis*.
507 *Appl Environ Microbiol* 82, 7102-7112.
- 508 Rasmussen, G., Monecke, S., Ehricht, R., Soderquist, B., 2013. Prevalence of clonal complexes
509 and virulence genes among commensal and invasive *Staphylococcus aureus* isolates in
510 Sweden. *PloS one* 8, e77477.
- 511 Richter, M., Rossello-Mora, R., 2009. Shifting the genomic gold standard for the prokaryotic
512 species definition. *Proc Natl Acad Sci U S A* 106, 19126-19131.
- 513 Sabharwal, V., Stevenson, A., Figueira, M., Orthopoulos, G., Trzcinski, K., Pelton, S.I., 2014.
514 Capsular switching as a strategy to increase pneumococcal virulence in experimental
515 otitis media model. *Microbes Infect* 16, 292-299.
- 516 Song, J.H., Baek, J.Y., Ko, K.S., 2011. Comparison of capsular genes of *Streptococcus*
517 *pneumoniae* serotype 6A, 6B, 6C, and 6D isolates. *J Clin Microbiol* 49, 1758-1764.
- 518 Tien, L.H.T., Nishibori, T., Nishitani, Y., Nomoto, R., Osawa, R., 2013. Reappraisal of the
519 taxonomy of *Streptococcus suis* serotypes 20, 22, 26, and 33 based on DNA-DNA
520 homology and *sodA* and *recN* phylogenies. *Vet Microbiol* 162, 842-849.
- 521 Trzcinski, K., Thompson, C.M., Lipsitch, M., 2003. Construction of otherwise isogenic serotype
522 6B, 7F, 14, and 19F capsular variants of *Streptococcus pneumoniae* strain TIGR4. *Appl*
523 *Environ Microbiol* 69, 7364-7370.
- 524 Uchiyama, S., Sun, J., Fukahori, K., Ando, N., Wu, M., Schwarz, F., Siddiqui, S.S., Varki, A.,
525 Marth, J.D., Nizet, V., 2019. Dual actions of group B *Streptococcus* capsular sialic acid
526 provide resistance to platelet-mediated antimicrobial killing. *Proc Natl Acad Sci U S A*
527 116, 7465-7470.
- 528 Wang, J., Feng, Y., Wang, C., Zheng, F., Hassan, B., Zhi, L., Li, W., Yao, Y., He, E., Jiang, S.,
529 2017. Genome-wide analysis of a avirulent and reveal the strain induces pro-protective
530 immunity against challenge with virulent *Streptococcus suis* Serotype 2. *BMC*
531 *microbiology* 17, 67.
- 532 Wei, Z., Li, R., Zhang, A., He, H., Hua, Y., Xia, J., Cai, X., Chen, H., Jin, M., 2009.
533 Characterization of *Streptococcus suis* isolates from the diseased pigs in China between
534 2003 and 2007. *Vet Microbiol* 137, 196-201.
- 535 Wiedenheft, B., Sternberg, S.H., Doudna, J.A., 2012. RNA-guided genetic silencing systems in
536 bacteria and archaea. *Nature* 482, 331-338.

- 537 Willemsse, N., Schultsz, C., 2016. Distribution of Type I Restriction-Modification Systems in
538 *Streptococcus suis*: An Outlook. *Pathogens* 5.
- 539 Wyres, K.L., Lambertsen, L.M., Croucher, N.J., McGee, L., von Gottberg, A., Linares, J., Jacobs,
540 M.R., Kristinsson, K.G., Beall, B.W., Klugman, K.P., Parkhill, J., Hakenbeck, R.,
541 Bentley, S.D., Brueggemann, A.B., 2013. Pneumococcal capsular switching: a historical
542 perspective. *J Infect Dis* 207, 439-449.
- 543 Yao, X., Li, M., Wang, J., Wang, C., Hu, D., Zheng, F., Pan, X., Tan, Y., Zhao, Y., Hu, L., Tang,
544 J., Hu, F., 2015. Isolation and characterization of a native avirulent strain of
545 *Streptococcus suis* serotype 2: a perspective for vaccine development. *Sci Rep* 5, 9835.
- 546 Yother, J., 2011. Capsules of *Streptococcus pneumoniae* and other bacteria: paradigms for
547 polysaccharide biosynthesis and regulation. *Annu Rev Microbiol* 65, 563-581.
- 548 Zhu, W., Wu, C., Sun, X., Zhang, A., Zhu, J., Hua, Y., Chen, H., Jin, M., 2013. Characterization
549 of *Streptococcus suis* serotype 2 isolates from China. *Vet Microbiol* 166, 527-534.
- 550 Zhu, Y., Dong, W., Ma, J., Yuan, L., Hejair, H.M., Pan, Z., Liu, G., Yao, H., 2017.
551 Characterization and virulence clustering analysis of extraintestinal pathogenic
552 *Escherichia coli* isolated from swine in China. *BMC Vet Res* 13, 94.
- 553 Zhu, Y., Dong, W., Ma, J., Zhang, Y., Pan, Z., Yao, H., 2019. Utilization of the ComRS system
554 for the rapid markerless deletion of chromosomal genes in *Streptococcus suis*. *Future*
555 *Microbiol* 14, 207-222.

556

557

558 **Figure legends**

559 **Figure 1. Clustering of SS2, SS3 and SS7 isolates based on the profiles of virulence related**

560 **genes.** The lateral axis of the matrix is the set of 19 genes and the vertical axis is the set of 88
561 strains. Green color covers SS2 strains, yellow covers SS3 strains and pink covers SS7 strains.
562 Each black square refers to a positive detection of a specific virulence related gene in a single *S.*
563 *suis* strain. Agglomerative clustering was performed using the unweighted average linkage
564 (UPGMA) with the BioNumerics software.

565 **Figure 2. eBURST analysis of *S. suis* MLST data. (A)** eBURST software was used to analyze

566 the MLST data of whole *S. suis* population. A total of 9 major CCs were identified, including
567 CC1, CC16, CC28, CC29, CC94, CC104, CC201, CC423 and CC528. **(B)** Detailed analysis all
568 SS2, SS3 and SS7 strains. SS2 strains are colored in back, SS3 in green and SS7 in purple. ST1

569 and ST7 SS2 are clustered into CC1. CC28 includes both SS2 and SS3 strains, whereas CC29
570 contains SS2 and SS7 strains.

571 **Figure 3. Phylogenetic tree of 25 *S. suis* strains based on orthologous gene clusters.** The
572 phylogenetic tree was constructed using neighbor-joining (NJ) method. *S. suis* strains of different
573 serotypes can be classified into 3 major clades. Clade 1 appeared to be phylogenetic distinct from
574 Clade 2. Clade 1 harbors virulent SS2 strains, and Clade 2 SS3 strains, SS7 strain and SS2 strains
575 with lower virulence level.

576 **Figure 4. Multigenome comparison between Clade 1 and Clade 2 *S. suis* strains obtained by**
577 **Mauve tool.** Each colored region refers to a locally collinear block (LCB). Colors are arbitrarily
578 assigned by software to each LCB. The vertical peaks in each LCB denotes the variance of
579 conservation. The LCBs below the center line of genomes are in reverse complement orientation.
580 As reference genome, Clade 2 SS2 strain NSUI002 are compared with SS3 strains (ST3 and
581 YB51) and Clade 1 SS2 strain ZY05719. The Multigenome comparison of SS2 strain ZY05719,
582 NSUI060 and SS7 strain D9 is shown in Figure S1.

583 **Figure 5. Heat map based on ANI values between every two genome sequences.** The average
584 nucleotide identity (ANI) were calculated using the web-based EZ BioCloud platform. Heatmaps
585 based on ANI values were generated with HemI 1.0 (Heatmap Illustrator software, version 1.0).

586 **Figure 6. The structures of defense systems in *S. suis* isolates. (A)** Three types of type I RM
587 systems are found in *S. suis*. Type A and B RM systems only present in Clade 1 *S. suis* strains,
588 and Type C was common RM system appearing in all of *S. suis* strains (Both Clade 1 and Clade
589 2). **(B)** Sequences of CRISPR repeats in representative *S. suis* strains. **(C)** The structures of
590 competence and CRISPR elements in *S. suis*. Only Clade 2 strains harbors CRISPRs. Clade 1

591 and Clade 2 strains have different sequences in ComRS competence systems. The ComX
592 regulator are conserved in *S. suis* strains, but Clade 2 strains have one more copy of ComX.

593 **Figure 7. *S. suis* transformation induced by two different XIPs.** Peptide XIP induces *S. suis*
594 competence to uptake exogenous DNA. The competence efficiency can be assessed by positive
595 transformants grow on THB (*spc:cm+*). Type A XIP is only able to induce transformation in
596 Clade 1 SS2 strains. Type B XIP induces transformation in SS2, SS3, SS7 strains belonging to
597 Clade 2.

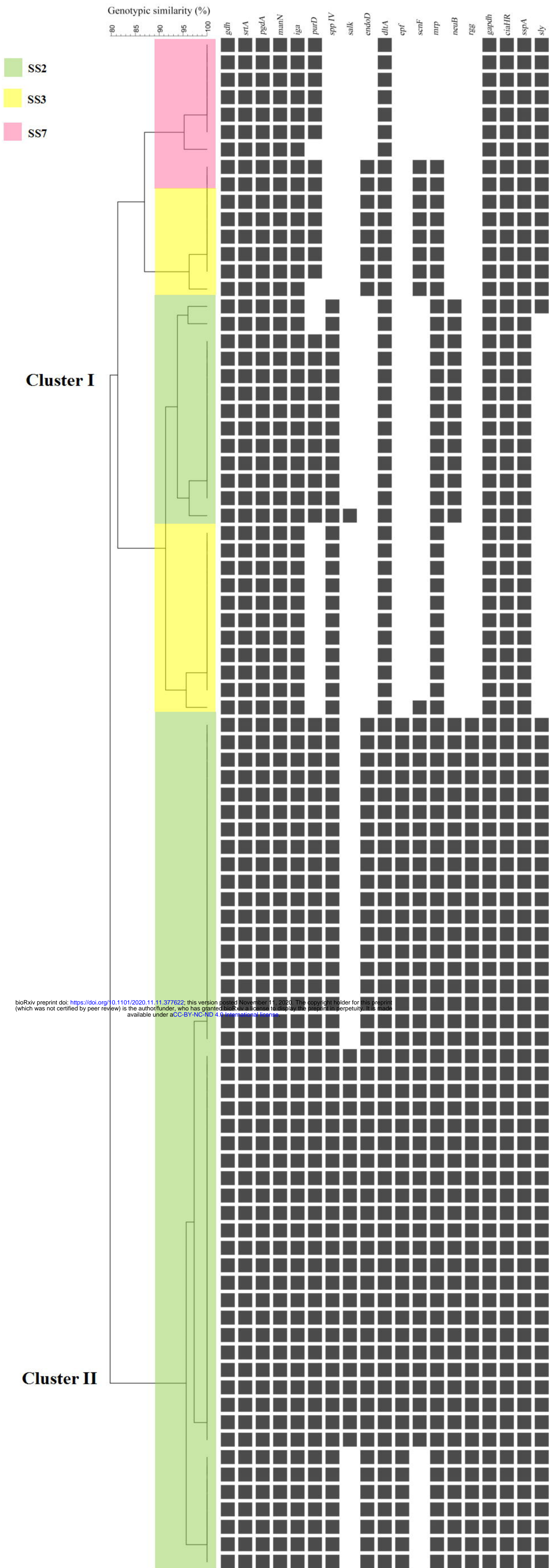
598 **Figure 8. Schematic representations of *cps* locus in SS2 and SS3 strains.** The *cps* locus of
599 Clade 2 SS2 strain NSUI002, Clade 2 SS3 strain ST3 and Clade 1 SS2 strain ZY05719 were
600 compared. The flanking regions of *cps* locus reveals high similarity. Red arrow indicates a
601 translocation event. Red line box shows a region uniquely present in the *cps* locus of both ST3
602 and NSUI002.

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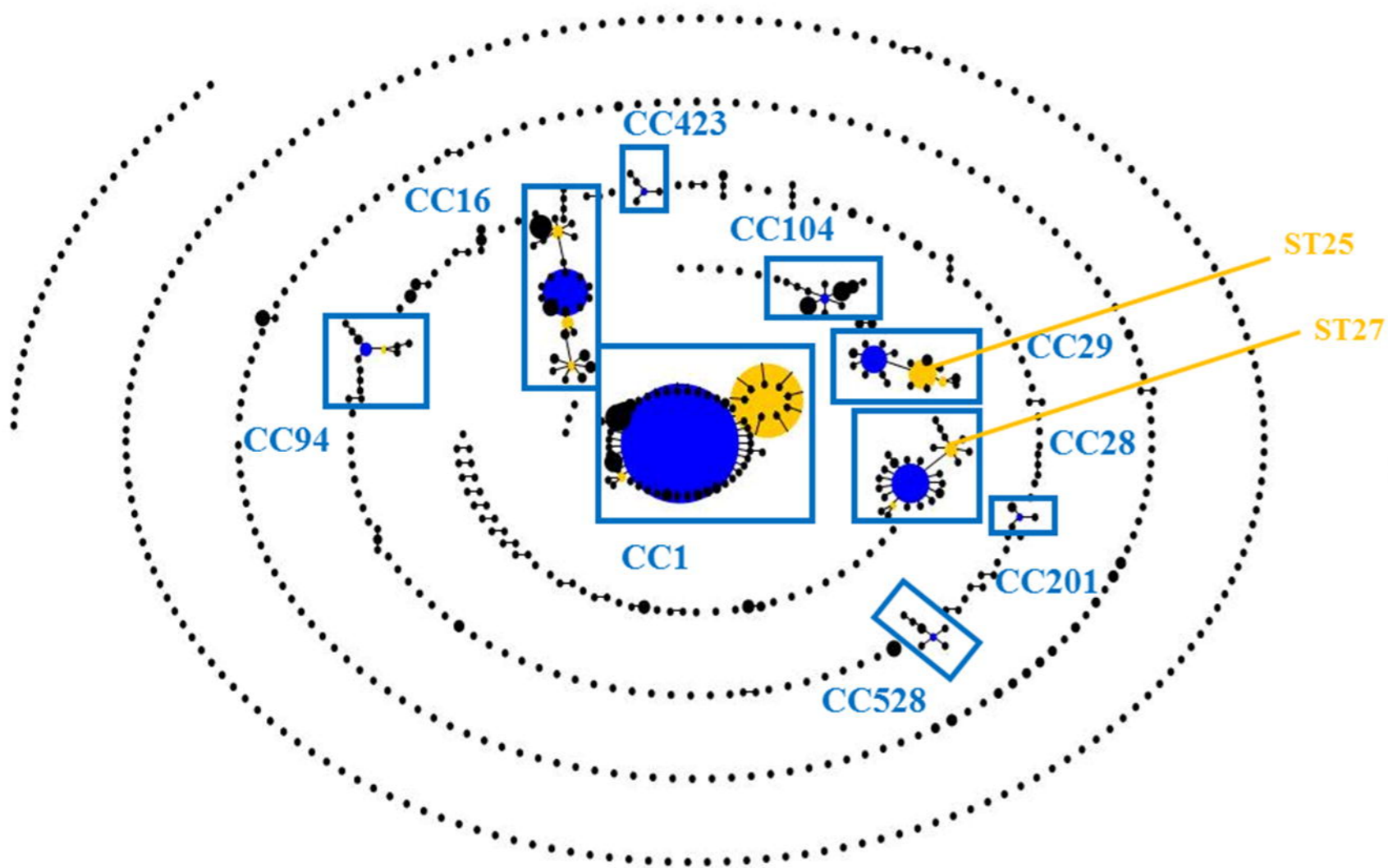
604 **Table 1** Existing capsule switch in *S. suis*

<i>S. suis</i> sequence type (ST)	<i>S. suis</i> serotypes
ST1	SS1/2, SS1, SS2, SS8, SS9, SS14
ST13	SS1, SS14
ST15	SS3, SS9
ST16	SS4, SS9
ST17	SS4, SS5
ST27	SS2, SS3
ST28	SS1/2, SS2
ST29	SS2, SS3, SS7
ST65	SS15, SS27

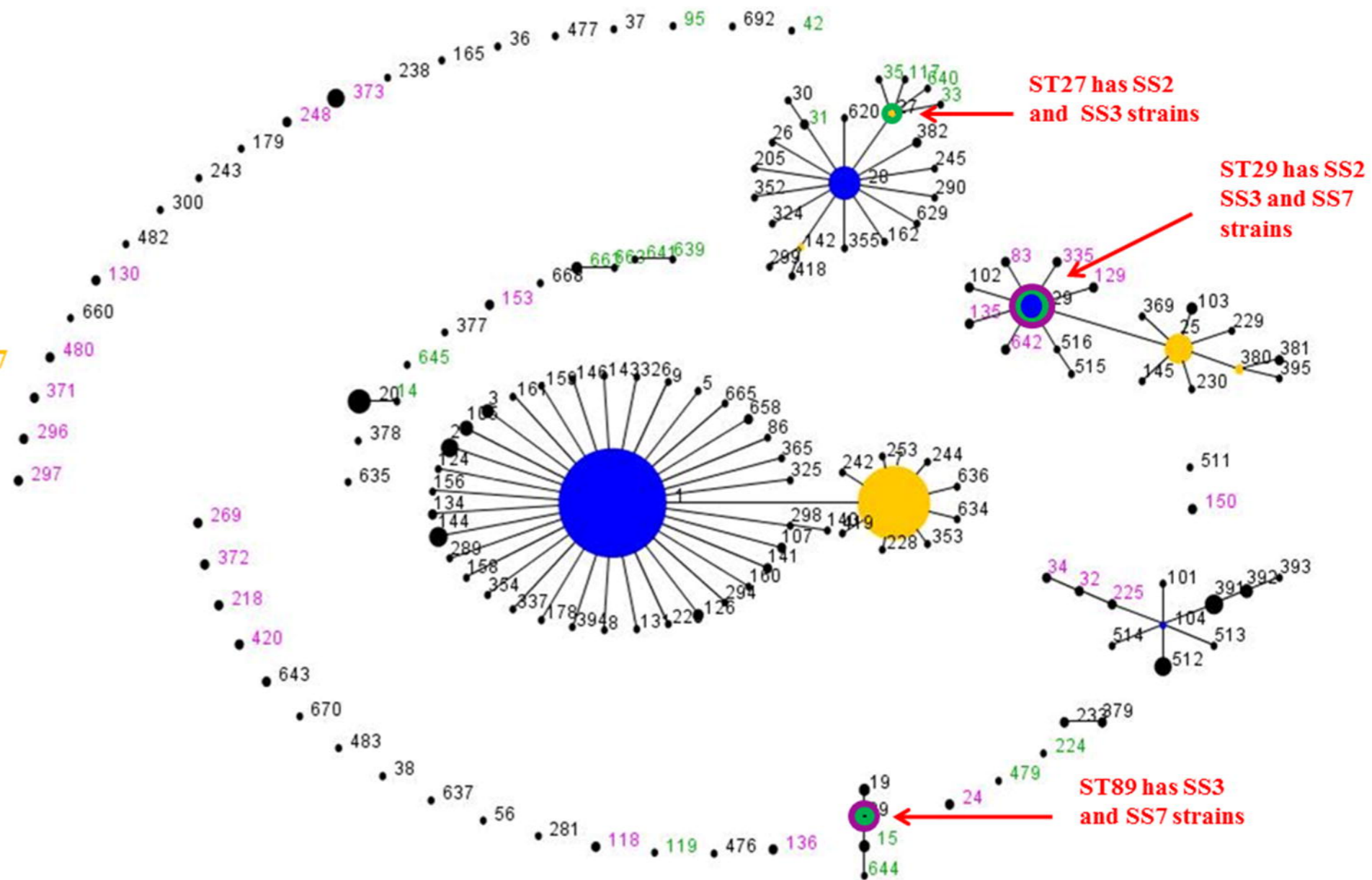
ST76	SS17, SS19
ST89	SS3, SS7
ST94	SS4, SS16
ST105	SS2, SS14
ST136	SS7, SS9
ST156	SS1, SS2
ST243	SS2, SS9
ST297	SS7, SS9

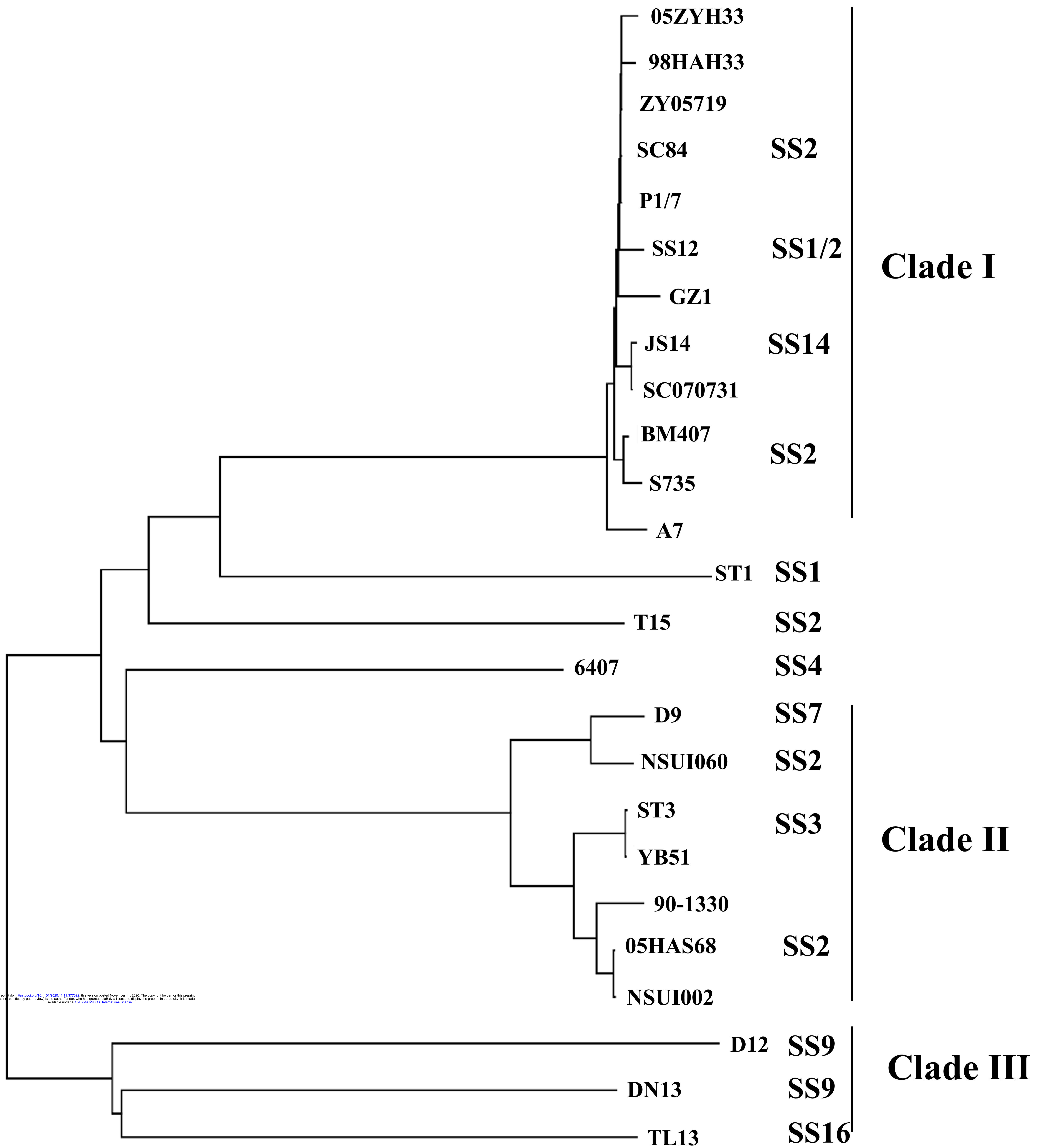


A



B



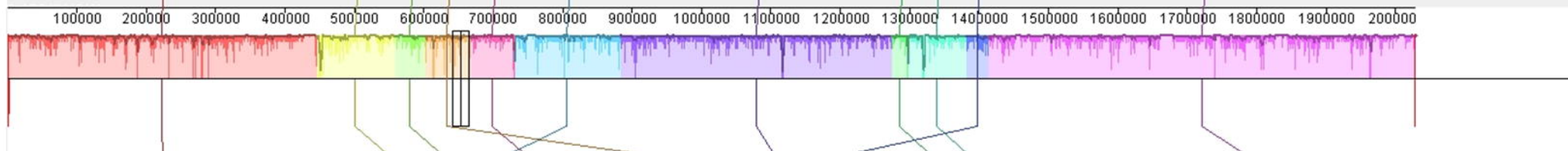


0.0050

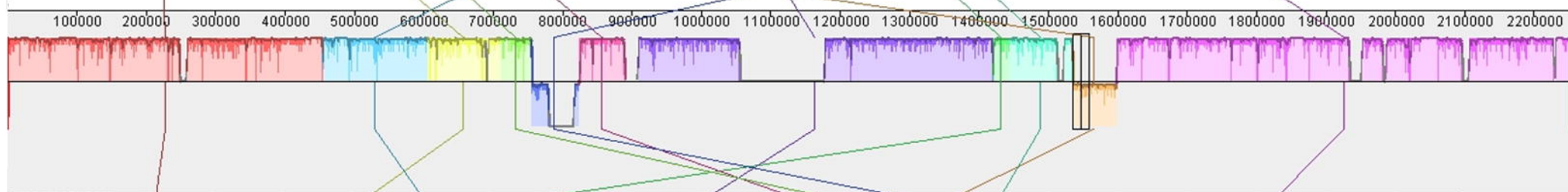
SS3 YB51



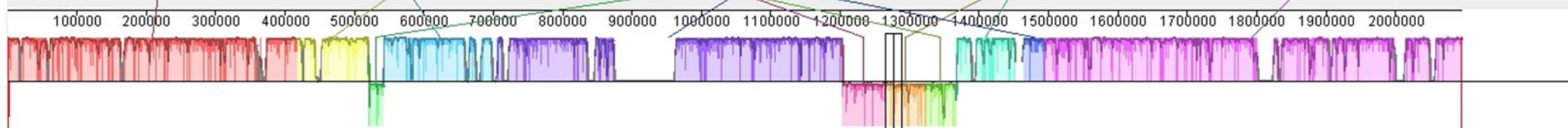
SS3 ST3



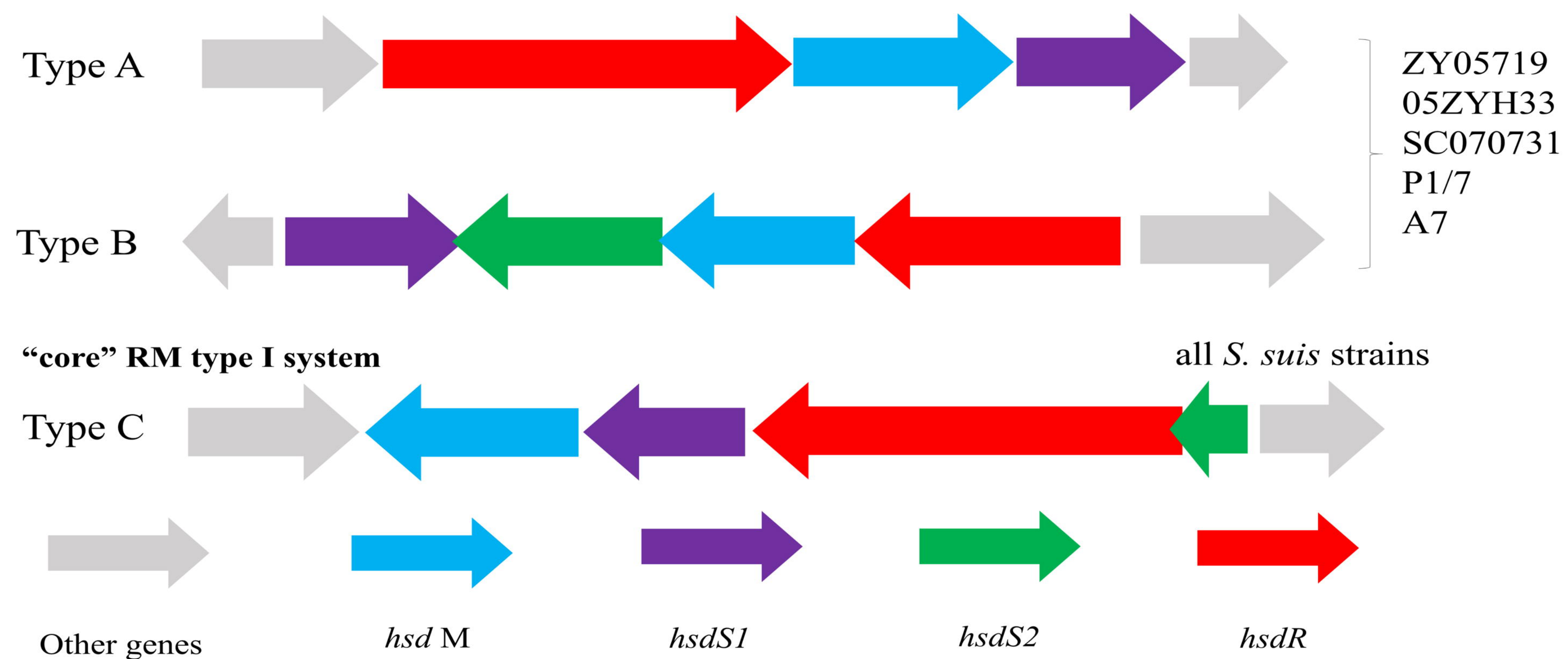
SS2 NSUI002



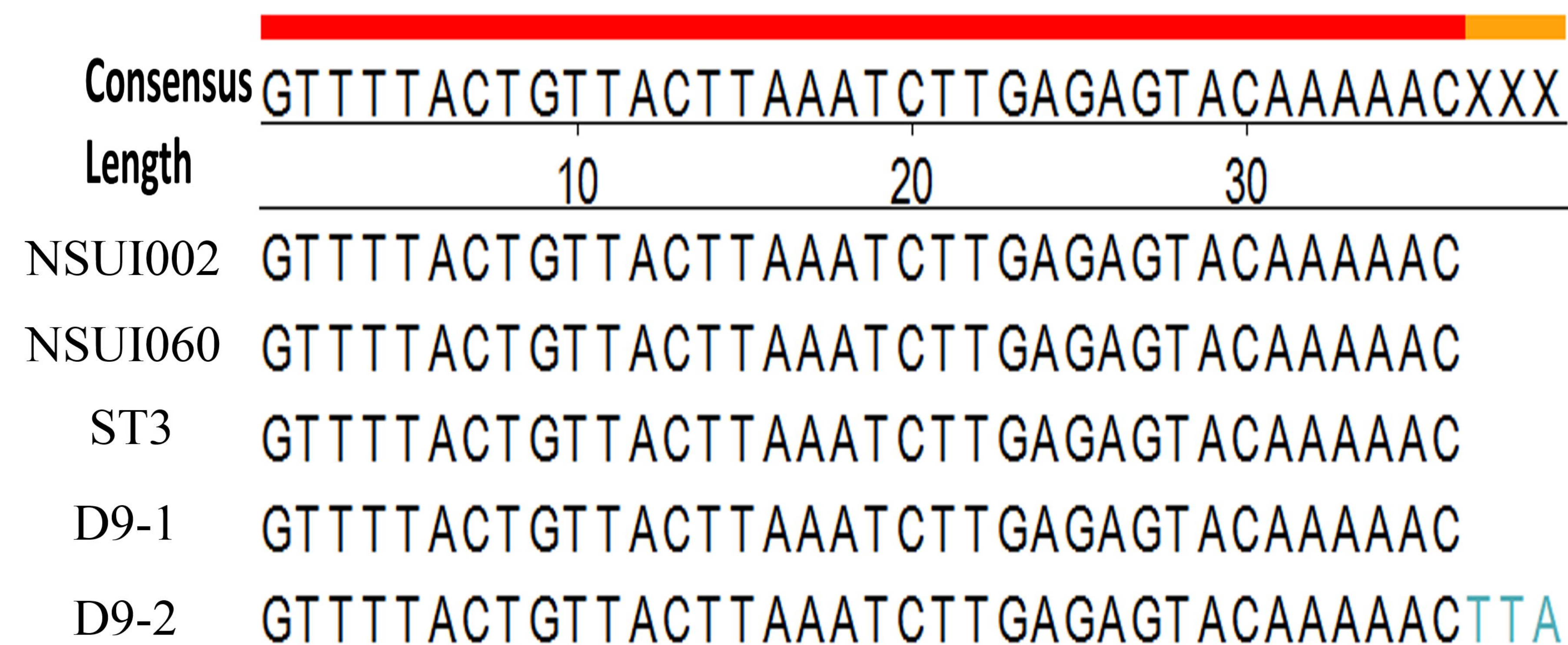
SS2 ZY05719



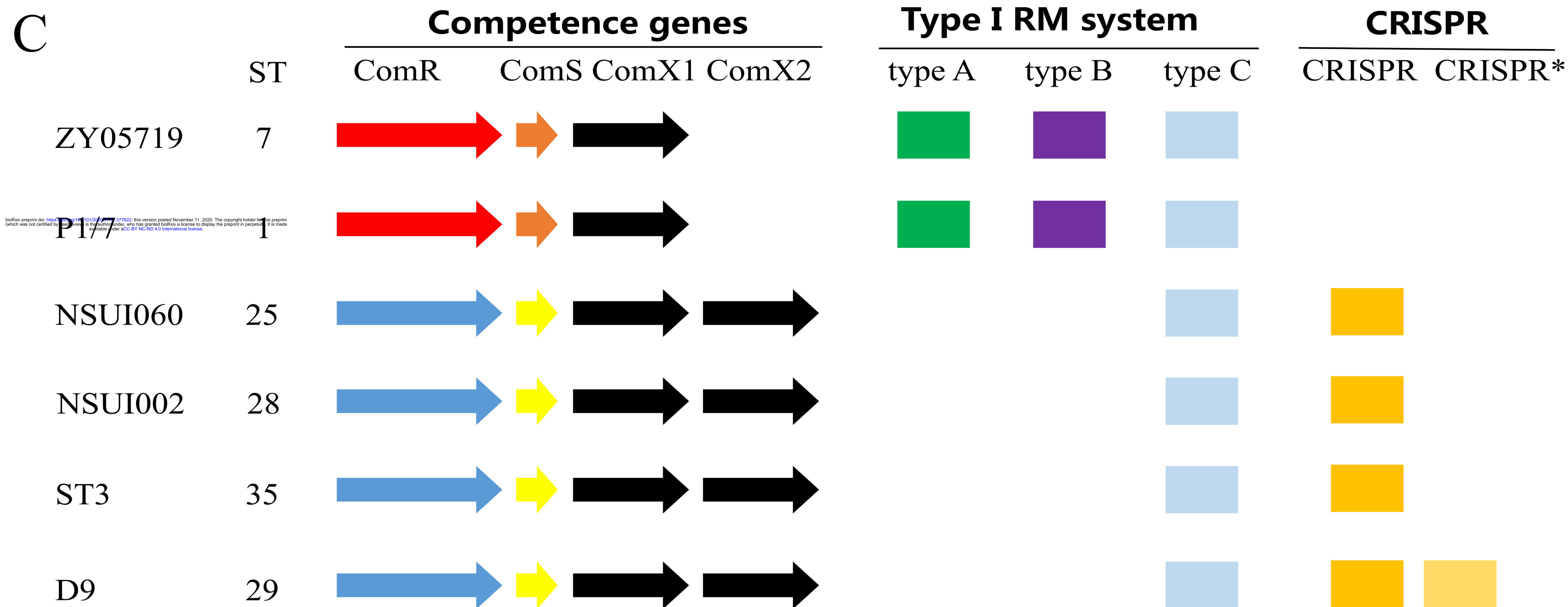
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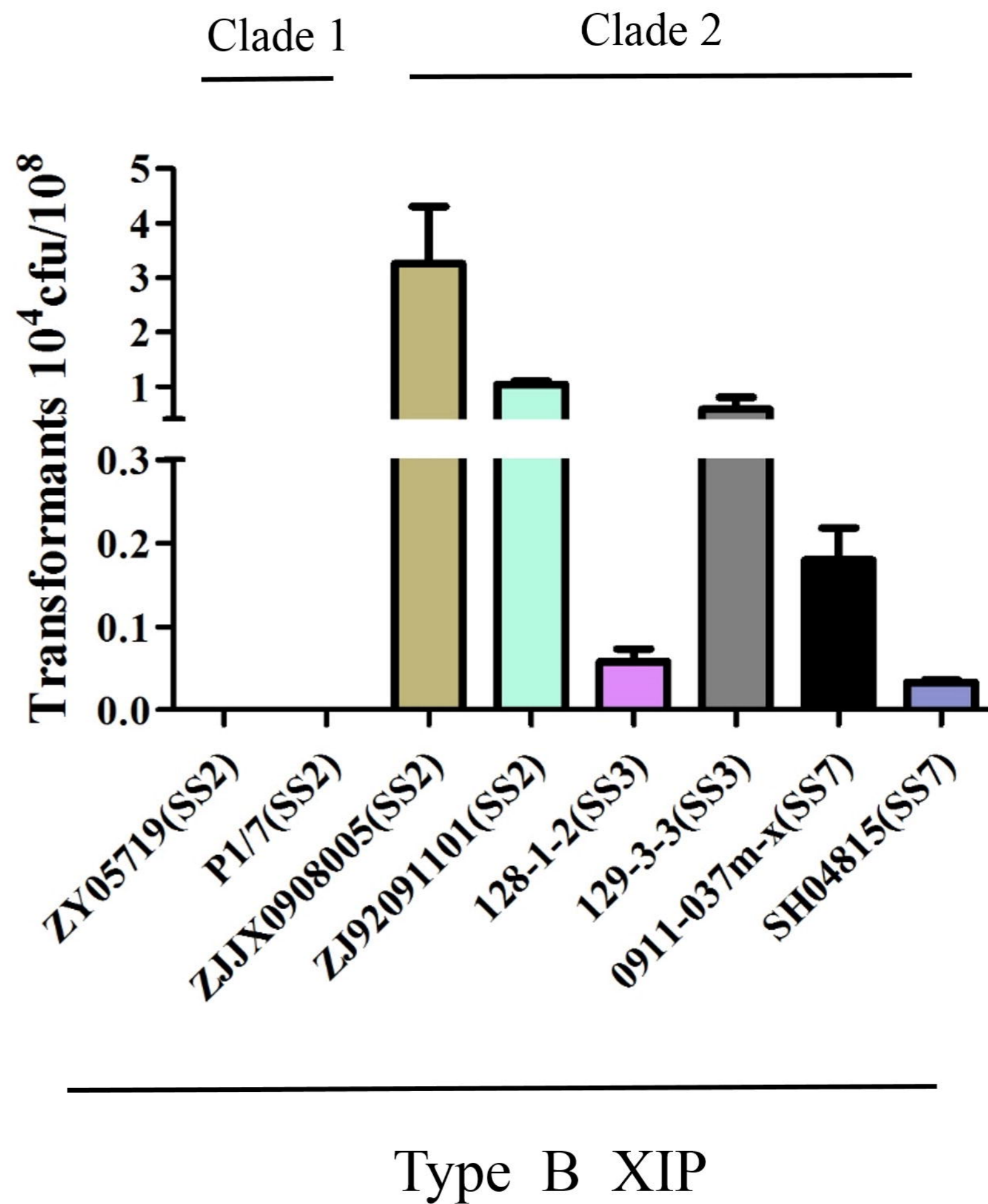
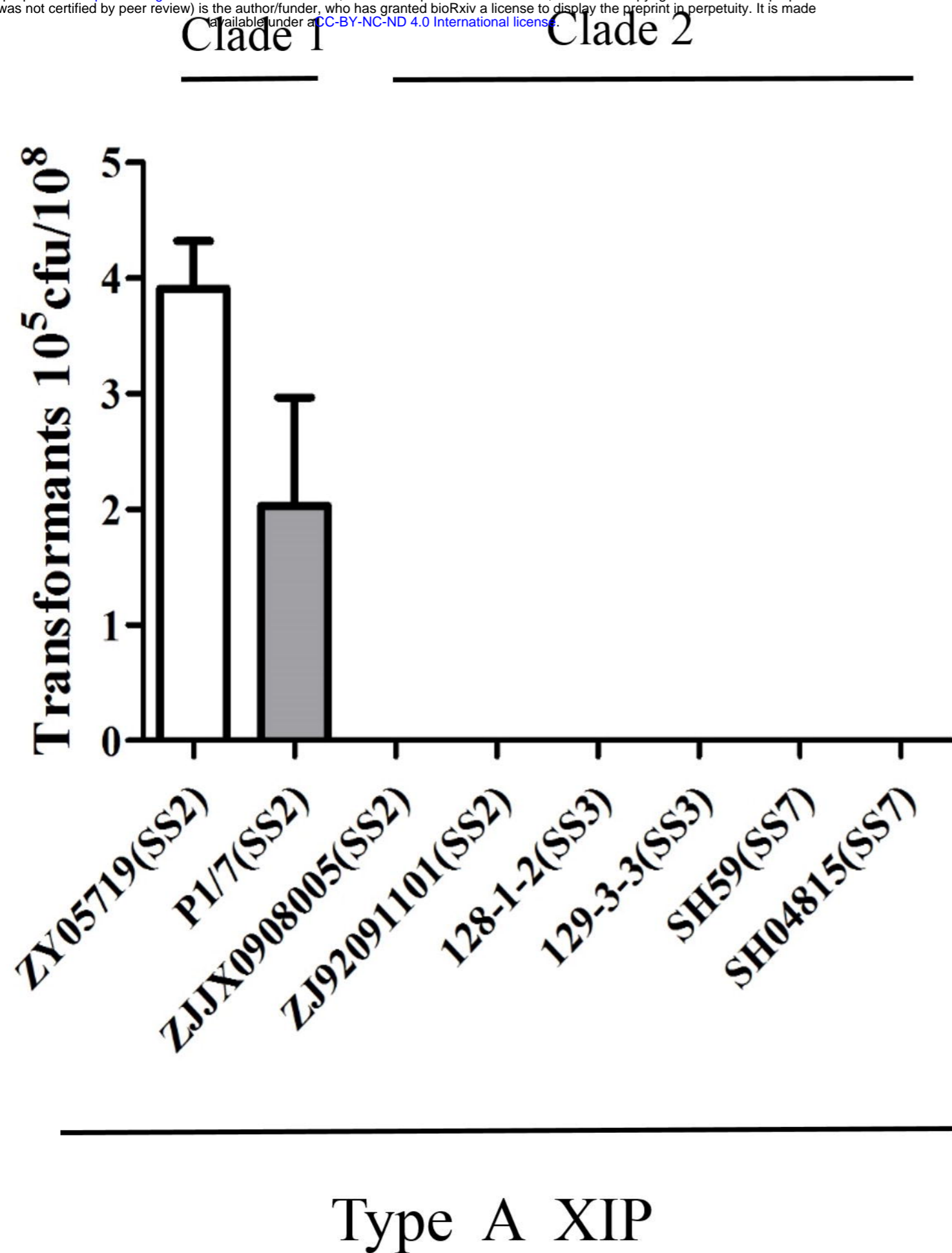
B

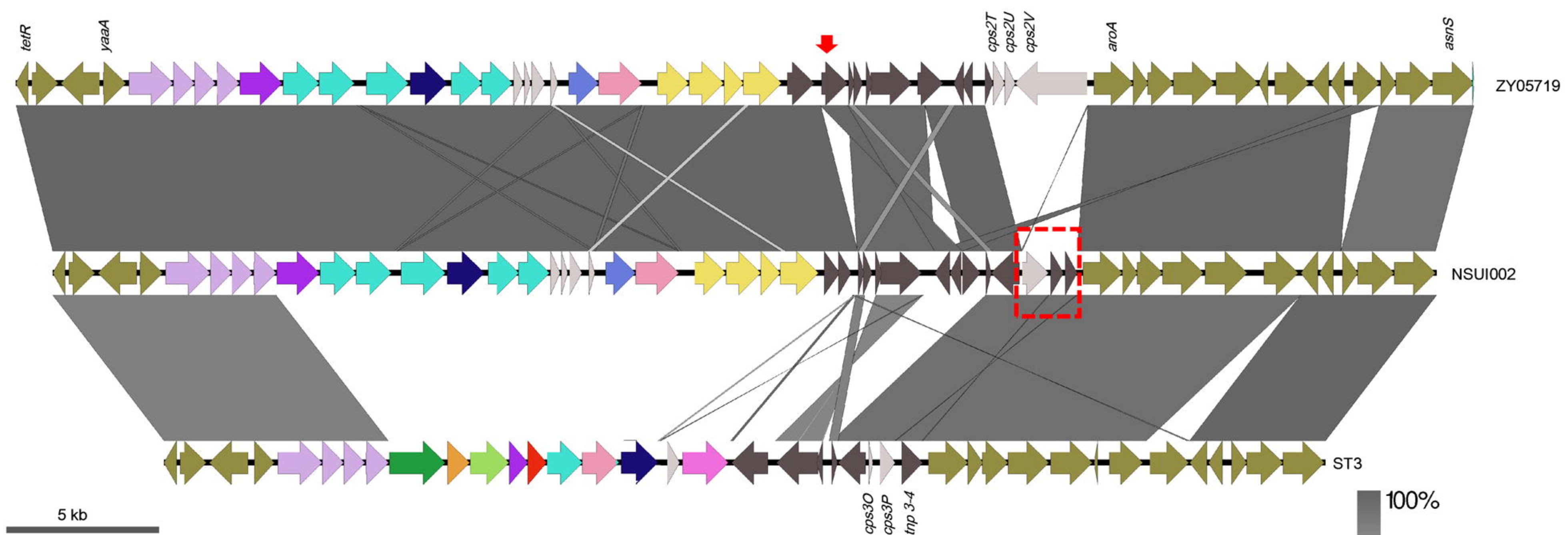


C



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- ➔ Regulation and processing cps ABCD
- ➔ Initial sugar transferase
- ➔ glycosyltransferase
- ➔ Wzy polymerase
- ➔ NeuAc transferase
- ➔ Wzy flippase
- ➔ Synthesis of NeuAc
- ➔ Transposase or integrase
- ➔ Hypothetical protein
- ➔ Sugar epimerase
- ➔ Fic family protein
- ➔ Aminotransferase
- ➔ Acetyltransferase/ acyltransferase
- ➔ Sugar dehydrogenase
- ➔ Cps flanking region

