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1
2 **SME-4 producing *Serratia marcescens* from Argentina belonging to the clade 2 of the *S.***
3 ***marcescens* phylogeny.**

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26

27 **Synopsis**

28 **Background:** SME serine carbapenemase are increasingly reported, especially from North
29 and South America. Here, we describe an SME-4-producing *Serratia marcescens* (SME-*Sm*)
30 clinical isolate from Argentina and compared its genome with other SME-*Sm* recovered from
31 different countries.

32 **Methods:** *S. marcescens* isolates were characterized by WGS using Illumina technology,
33 susceptibility testing and MIC determination. Carbapenemase activity was revealed by
34 biochemical tests based on imipenem hydrolysis. Additionally, WGS of *S. marcescens*
35 containing or not *bla*_{SME} genes were retrieved from Genbank database. A whole-genome
36 phylogenetic analysis based on non-recombinant core SNPs was inferred for *S. marcescens*
37 complete genomes and for those encoding any *bla*_{SME} variant.

38 **Results:** *S. marcescens* 163 was resistant to temocillin, aztreonam and carbapenems,
39 remaining susceptible to expanded-spectrum cephalosporins. Analysis of WGS data of *S.*
40 *marcescens* 163 revealed a genome of 5,139,329 bp, and a chromosomally-encoded class A
41 *bla*_{SME-4} carbapenemase gene, which was located on a genomic island closely related to
42 SmarG1-1 of *S. marcescens* N11-02820. The comparison of the *S. marcescens* genomes
43 revealed that all SME-*Sm* isolates possess this genomic island inserted at the same loci, form
44 a well-defined subcluster of cluster I of *S. marcescens* clade 1, while *S. marcescens* 163
45 belonged to clade 2, suggesting that the SME-encoding genomic island may have been
46 transferred between isolates from different clades.

47 **Conclusions:** To the best of our knowledge this is the first report of a SME-4-*Sm* from
48 Argentina. *Bla*_{SME-4} gene is located on a SmarG1-1-like genomic island. The genome of *S.*
49 *marcescens* 163 belongs to clade 2, unlike all the other SME-*Sm* isolates that belong to a
50 subcluster of clade I.

51

52 **Introduction**

53 Currently, β -lactamase-mediated resistance does not spare even the newest and most
54 powerful β -lactams (carbapenems), whose activity is challenged by carbapenemases.¹ Class A
55 carbapenemases may be chromosomally encoded (SME, NmcA, SFC-1, BIC-1, PenA, FPH-1,
56 SHV-38), plasmid-encoded (FRI-1) or both (KPC, GES, IMI).² Chromosomally-encoded
57 class A carbapenemases have been identified in rare Gram-negative species that appeared
58 sporadically in clinical or environmental samples since their first discovery, more than 20
59 years ago.² SME-1 (for “*Serratia marcescens* enzyme”) was first detected in England from
60 two *S. marcescens* isolates collected in 1982.^{3,4} SME-1 has a broad hydrolysis spectrum that
61 includes penicillins, cephalosporins, aztreonam, and carbapenems.^{1,4} The SME enzymes have
62 been found exclusively in *S. marcescens* and the five variants differing by one or two amino
63 acid substitutions have been found infrequently and sporadically in UK, across North and
64 south America, in Switzerland and in Mexico.^{2,3,5-9} Recently, analysis of the genetic
65 environment of *bla*_{SME} genes in three Canadian *S. marcescens* isolates revealed that it is
66 located on a novel cryptic prophage genomic island, SmarGI1-1. This genomic island can be
67 excised and circularized, which probably contributes to its dissemination amongst *S.*
68 *marcescens*.¹⁰

69 In this work, we have characterized the genome of an SME-4-producing *S. marcescens*
70 (SME-4-*Sm*) from a clinical isolate recovered from an University Hospital in Buenos Aires,
71 Argentina, and compared it to the genomes of SME-*Sm* isolates either sequenced in this study
72 or downloaded from the Genbank database.

73

74 **Materials and Methods**

75 **Bacterial strains.** *S. marcescens* 163 (Sm163) was identified with MALDI-TOF
76 (MALDI Biotyper CA system, Bruker Daltonics, Billerica, MA, USA). *Escherichia coli*

77 TOP10 (Invitrogen, Saint-Aubin, France) was used for cloning experiments. *Bla*_{SME} gene
78 harbouring *Serratia marcescens*, *S. marcescens* S6 (SmS6),^{3,4} *S. marcescens* S8 (SmS8),^{3,4} *S.*
79 *marcescens* CHE4 (SmCHE4),⁸ *S. marcescens* AW (SmAW),⁵ *S. marcescens* USA-1
80 (SmUSA-1) and *S. marcescens* Mex-1 (SmMEX-1) were also used in this study. The presence
81 of SME was systematically assessed on all the genome data of *S. marcescens* deposited on the
82 NCBI genome database (December 2017). Genomes of 323 *S. marcescens* isolates were
83 downloaded from Public databases including seven SME-*Sm* and 24 fully-sequenced
84 *S. marcescens* strains (Tables S1 and S2). The sequences of the SmarGI1-1 and flanking
85 regions from *S. marcescens* N10-0408, N11-2820 and N12-0620 were from accession
86 numbers KF445086, KF318367 and KF615855, respectively, in the GenBank database.¹⁰

87 **Antimicrobial agents, susceptibility testing.** Antimicrobial susceptibilities and MIC
88 values were determined as previously reported¹¹ and interpreted according to the EUCAST
89 breakpoints (16 May 2018; http://www.eucast.org/clinical_breakpoints/).

90 **Biochemical and molecular detection of carbapenemase-producing**
91 **Enterobacteria.** Carba NP test and β Carba Test were used for carbapenemase detection
92 (BioRad, Marnes-la-Coquette, France) as previously described.^{12,13} Genomic DNA of *S.*
93 *marcescens* 163 isolate extracted using the Qiagen DNAamp kit (Qiagen) was used as
94 template for amplification of the most prevalent carbapenemases (*bla*_{OXA-48}, *bla*_{KPC}, *bla*_{NDM},
95 *bla*_{VIM}, *bla*_{IMP}).¹⁴

96 **Whole genome sequencing and bioinformatic analysis.** The whole genome of
97 *Serratia marcescens* 163 was sequenced using Illumina technology, as previously reported.¹¹
98 The acquired antimicrobial resistance genes were identified by uploading assembled genomes
99 to the Resfinder server v2.1 (<http://cge.cbs.dtu.dk/services/ResFinder-2.1>).¹⁵

100 ProgressiveMauve and the Mauve distributed script stripSubsetLCBs¹⁶ were used to
101 extract all the regions with a minimum length of 500 bp shared by all the strains, generating a

102 2.877.168 bp core genome alignment. Recombinant variable positions were removed with
103 Gubbins (<https://github.com/sanger-pathogens/gubbins>), and a Maximum Likelihood
104 phylogeny was inferred for the alignment of the 631207 non-recombinant variable sites with
105 RAxML using a General Time Reversible (GTR) model incorporating a gamma distribution
106 rate among sites.¹⁷ .In parallel a whole genome phylogeny was estimated for all the *S.*
107 *marcescens* retrieved from public databases with kSNP3¹⁸ (Supplementary Material and
108 methods). All genomes were annotated with Prokka,¹⁹ and graphic representations were
109 generated with the genoplots²⁰ library in R.

110 **Nucleotide sequence accession number.** The whole genome sequences generated in
111 the study have been submitted to the Genbank nucleotide sequence database under the
112 accession number detailed in Table S1. The genomic islands constructed from whole genome
113 sequences available at Genbank nucleotide sequence database where submitted to the
114 Genbank Third Party Annotation database under accession numbers BK010577-BK010585
115 (Table S1). The nucleotide sequence of *bla*_{SRT-3} gene has been submitted to the
116 EMBL/Genbank nucleotide sequence database under the accession number MG234451.

117

118 **Results and discussion**

119 **Clinical case.** A 62-year-old man with diffuse large B cell lymphoma was admitted at
120 the University hospital in Buenos Aires in July 2016 for his third cycle of chemotherapy.
121 During previous hospital stays in the same year he had received antimicrobial therapy with
122 meropenem, colistin and vancomycin. During his 3rd stay the patient presented fevers and
123 developed pneumonia. He was treated with meropenem and oseltamivir, but cultures
124 remained negative. Twenty days after admission, a carbapenem-resistant *S. marcescens*
125 isolate was recovered from both a surveillance rectal swab and a catheter blood culture,
126 whereas the peripheral blood cultures remained negative. Further carbapenem-resistant *S.*

127 *marcescens* isolates were recovered, from the same patient, in subsequent surveillance
128 samples for 3 weeks. The patient was discharged after having completed the 4th cycle of
129 chemotherapy for his underlying disease and he did not suffer from subsequent infections.

130 **Characteristics of *S. marcescens* 163.** *S. marcescens* 163 was resistant to amoxicillin
131 (MIC >256 mg/ml), temocillin (MIC of 24 mg/L) aztreonam (MIC >256 mg/ml) and
132 carbapenems (MIC for imipenem, meropenem and ertapenem >32 mg/L), remaining
133 susceptible to expanded-spectrum cephalosporins (MIC for ceftazidime 1 mg/L). *S.*
134 *marcescens* 163 also showed an MIC of 64 mg/L for amoxicillin+clavulanic acid. (Carba NP
135 and β Carba tests gave positive results indicating the presence of a carbapenemase. PCRs
136 detecting the five most prevalent carbapenemases (*bla*_{OXA-48}, *bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP})
137 were negative. High level of resistance to amoxicillin and carbapenems, decrease MIC values
138 in presence of clavulanic acid and susceptibility to expanded-spectrum cephalosporins
139 suggested the presence of a *bla*_{SME} gene.

140 WGS of *S. marcescens* 163 revealed a genome of 5,139,329 bp, with a 68 X coverage.
141 Resistome was determined by searching acquired genes and point mutations likely involved in
142 resistance. According to <https://cge.cbs.dtu.dk/services/>, the isolate presented two β -lactamase
143 genes, the chromosomal and natural *bla*_{STR-3} gene coding for cephalosporinase and *bla*_{SME-4}
144 gene coding for a chromosomal class A carbapenemase (Table 1S). The isolate also harboured
145 the *S. marcescens* intrinsic *aac*(6')-Ic gene conferring resistance to amikacin and
146 gentamicin,²¹ and the tetracycline resistance gene *tet*(41).²² Isolates SmS6 and SmS8 also
147 harboured the streptomycin/spectinomycin resistance gene *aadA1* and the trimethoprim
148 resistant gene *dfrA1*.

149 WGS of *S. marcescens* 163, revealed that the *bla*_{SME-4} gene was inserted into a
150 SmarGI1-1 prophage-like genomic island initially described in three SME-1 producing *S.*
151 *marcescens* N11-02820, N10-00408 and N12-00620 isolates (accession numbers KF318367,

152 KF445086, and KF615855, respectively).¹⁰ This genomic island found in Sm163 is ~28.4 kb
153 in size and bracketed by 25 bp imperfect direct repeats (Figure 1A). While the upstream
154 sequences of the SmarGI1-1 were identical between Sm163 and N11-02820 isolates,
155 downstream of the *attR* site the sequences were similar except for an 8-kb deletion in *S.*
156 *marcescens* 163 that included genes coding for intCPΔ, GNAT family, RES domain,
157 Helicase, ATP-dependent OLD family protein (Figure 1A).

158 **Phylogeny of SME-producing *S. marcescens*.** Phylogeny of the *S. marcescens* from
159 this study along with the 323 *S. marcescens* assemblies retrieved from the NCBI genome
160 database using KSNP3, revealed the two clades, with clade 1 representing 80% of the isolates
161 (Figure S1). Sixteen phylogenetic clusters were defined using ClusterPicker. 6 clusters were
162 located in Clade 1, with cluster 1 being the main one. In Clade 2, 10 clusters were identified,
163 with Sm163 being not related to any cluster. The presence of *bla*_{SME} as well as other
164 carbapenemase genes was screened in the 323 *S. marcescens* assemblies retrieved from the
165 NCBI genome database (Figure 1S). In total seven *bla*_{SME} encoding isolates were found
166 (Table S1 and S2) that were added to the seven SME producers sequenced in the present
167 study. A core genome SNP-based Maximum Likelihood phylogenetic tree was obtained for
168 the fourteen SME-producing strains and 24 completed *S. marcescens* genomes retrieved from
169 the NCBI database (Table S1), using the *S. marcescens* fgi94 genome as outgroup. Two main
170 clades, with a mean genetic distance between them of 25.302 non-recombinant SNPs, were
171 revealed in the *S. marcescens* phylogeny (Figure 2, Table S1). The cluster numbers were
172 those from Figure S1 (Figure S1 and 2). Thirteen out of the fourteen SME-producers were
173 found on the clade 1, eleven of them formed a single sub-cluster of SME producers (hereafter
174 named SME sub-cluster within the cluster I), and the two remaining, MGH315 is located
175 outside the SME-sub-cluster but still in Cluster I, while BIDMC44 belongs to cluster III. On

176 the other hand, *S. marcescens* 163, remains as the single strain encoding SME in clade 2
177 (Figure 2).

178 Eleven isolates encoded other carbapenemases than *bla*_{SME} genes, 5 of them encoded
179 *bla*_{KPC} carbapenemase genes, 4 for *bla*_{NDM-1} genes, one encoded *bla*_{IMP-1} gene and one *bla*_{VIM-1}
180 gene (Figure S1, and Table S2). All of them were found in the clade 1, leaving *Sm*163 as the
181 only carbapenemase producing isolate from the clade 2.

182 **Genetic environment of the *bla*_{SME} genes.** In all cases the *bla*_{SME} gene was encoded
183 within a conserved SmarGI1-1-like genomic island that varies in size from c.a. 27.2 (in
184 *Sm*S6) to 28.5-Kb (in BIDMC44) and that is integrated after the *ssrA* gene (Figure 1A and
185 1B, and Figure S2). *ssrA* has been identified as a hotspot for integration of mobile genetic
186 elements in enterobacteria.¹⁰ Three different flanking regions have been identified
187 downstream of the *attR* site among the 14 SME producers (Figure 1B). The first one
188 possesses a 2.8-kb long inserted region observed in the *S. marcescens* strains N12-00620 (and
189 N10-00408, data not shown)¹⁰ that is shared by the strains that form the SME subcluster and
190 the strain MGH315 (Figure 1B and Figure S2). The second one possesses an 11.3-kb long
191 region as observed in the strain N11-02820. Finally, the third one has been described in the
192 *Sm*163 (Figure 1B), and in the strain BIDMC44, that is a member of clade 1 (Figure 2),
193 suggesting that the latter might be the donor of this structure.

194 The region downstream the *ssrA* gene was also analysed in the strains *umh3* and
195 *umh9*, both of them are basal to the SME subcluster (Figure 2). In the strain *umh3* an
196 incomplete SmarGI1-1 was identified (Figure 1C). It was structurally similar to the first 12.4
197 Kb of the SmarGI1-1, but the hypothetical protein downstream the *intA1* integrase gene was
198 substituted by three completely different genes coding for hypothetical proteins (Figure 1C).
199 No traces of the second half of the SmarGI1-1 could be identified in the *umh3* genome; however,
200 the *attL* and *attR* sites were present (Figure 1C). Not a single trace of SmarGI1-1 nor of the

201 truncated version of it was found in the strain umh9 basal to both, umh3 and SME subcluster.
202 No traces for SmarGI1-1 were detected in the closest strains to the other SME-producers
203 analysed in the present study. Despite that the incomplete SmarGI1-1-like in strain umh3
204 shares a structural similarity with the first 12.4 kb of the SmarGI1-1, the sequence identity is
205 low (about 92%), thus, ruling out the possibility that they share a recent ancestor. In fact,
206 there are a lot of recombination and exchanges between phages and other MGEs, which often
207 makes it impossible to build an evolutionary scenario.

208 Interestingly, in the phylogeny performed with the 323 sequenced *S. marcescens*, a
209 single isolate GCF_000739215 lacking the SmarGI1-1 genomic island was found within the
210 the SME-subcluster (Figure 1S). In fact, in the isolate GCF_000739215, the insertion site
211 AttB was empty as identified in isolate umh9.

212

213 **Conclusion**

214 This is the first report of SME-4-*Sm* in Argentina. Until now, SME-4 had only been
215 reported in Brazil,⁷ and in one single SME-4 producing *S. marcescens* from US, as reported in
216 GenBank under the accession number KF481967, but not published yet. The increase in the
217 use of carbapenems in Argentina,²³ together with the *S. marcescens* natural resistant to
218 polymyxin, could be factors that helped to select SME-producing *S. marcescens* isolates.
219 Finally, as increasing numbers of the SME carbapenemases are being reported from *S.*
220 *marcescens*, especially from North and South America, rapid detection systems based on
221 multiplex PCR analyses should also target *bla*_{SME} genes in addition to the big 5
222 carbapenemases: KPC and OXA serine carbapenemases, and the IMP, VIM and NDM
223 metallo- β -lactamases, as was previously suggested by Bush *et al.*²⁴ Thus, underdetection of
224 SME-producers may favor their silent spread.

225 Finally, the phylogeny of all the available *S. marcescens* genomes assemblies revealed
226 that all SME-*Sm* isolates possess a SmarGI1-1 inserted at the same loci, that they form a well-
227 defined subcluster of cluster I of *S. marcescens* clade 1, while *S. marcescens* 163 belonged to
228 clade 2, suggesting that the SmarGI1-1 may have been transferred between isolates from
229 different clades.

230

231

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235

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243

244 **Transparency declarations**

245 No competing interests to declare.

246

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Figure legends

Figure 1. (A). Schematic representation of the genetic structure of SmarGI1-1 of *S. marcescens* N11-02820 (KF318367) and *S. marcescens* 163 and flanking regions. Common structures are highlighted in gray. Genes and putative functions of genes are shown; H means hypothetical protein. **(B).** Comparison of the SmarGI1-1 and its genetic environments among the different *bla*_{SME}-encoding strains. **(C).** Comparison of the insertion site for SmarGI1-1 in the SME subcluster and its basal strains. Numbers below the representative SME cluster represent: 1. Phage regulatory protein, 2. Phage capsid protein, 3. AlpA phage transcriptional regulator, CI repressor-like protein, 5. primase. H above the CDS B) AttL and AttR sequences comparison between umh3 and SmarGI1-1 (UCI-88).

Arrows colored in blue, green, purple, red and yellow correspond to genomic CDS, variable genomic CDS, TmRNA²⁵, *Bla*_{SME} gene, and genomic island CDS, respectively. Horizontal bars (I) in black and in red represents, Att (L and R) sites and contig boundaries, respectively. Small light green boxes (□) represent RNA genes. Insertion sequences are indicated by an arrow in a rectangle light green for *ISEc31* and *ISSm3*, and grey for the interrupted *ISEc14*.

Figure 2. *S. marcescens* Maximum Likelihood tree obtained from core non-recombinant SNPs. All *bla*_{SME}-encoding strains were bolded and capital letters. Those sequences in this study are highlighted in grey, and *bla*_{SME-2}-expressing isolates are underlined. *S. marcescens* isolates not carrying *bla*_{SME} gene are represented in lower case letters.

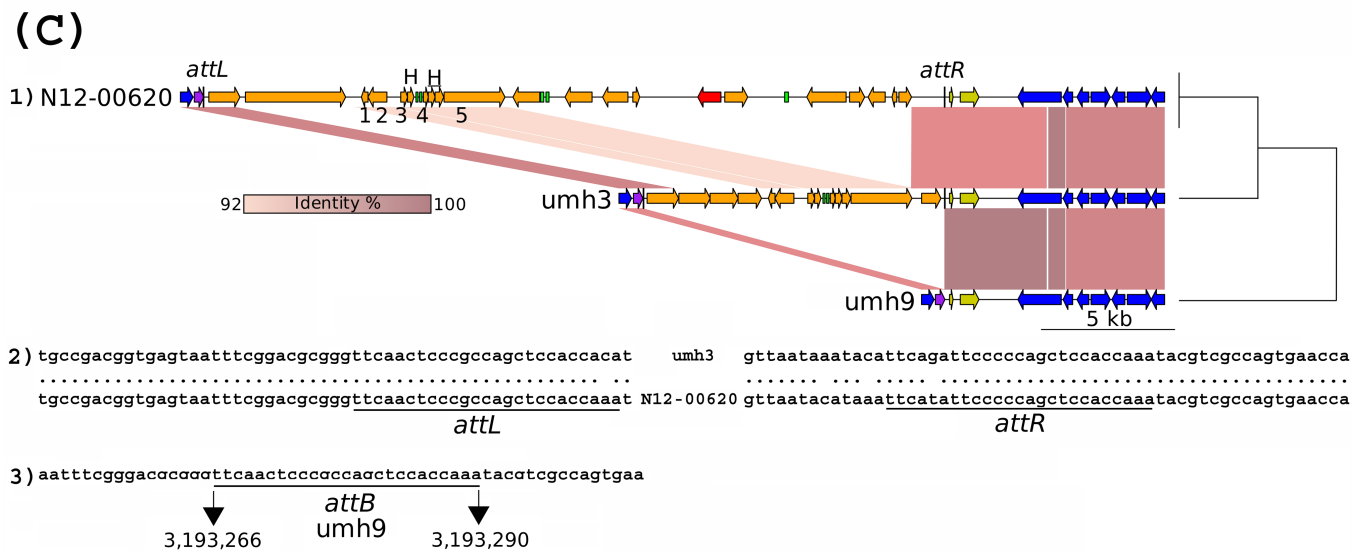
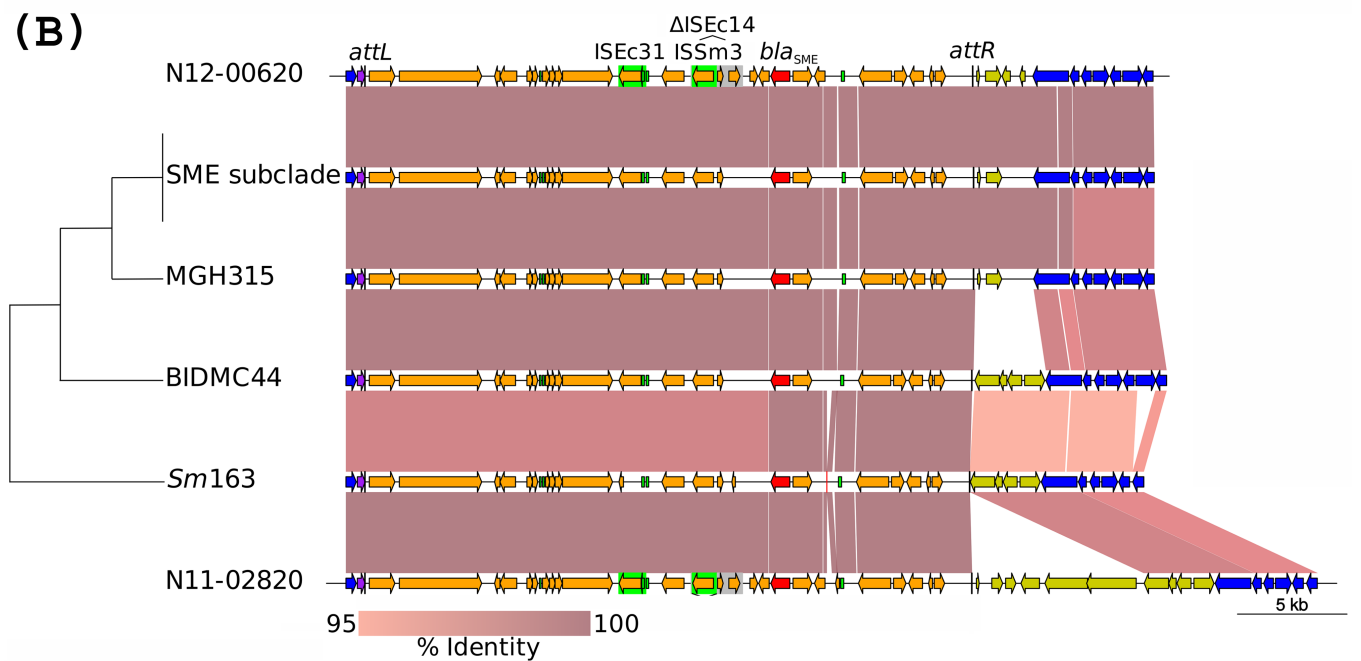
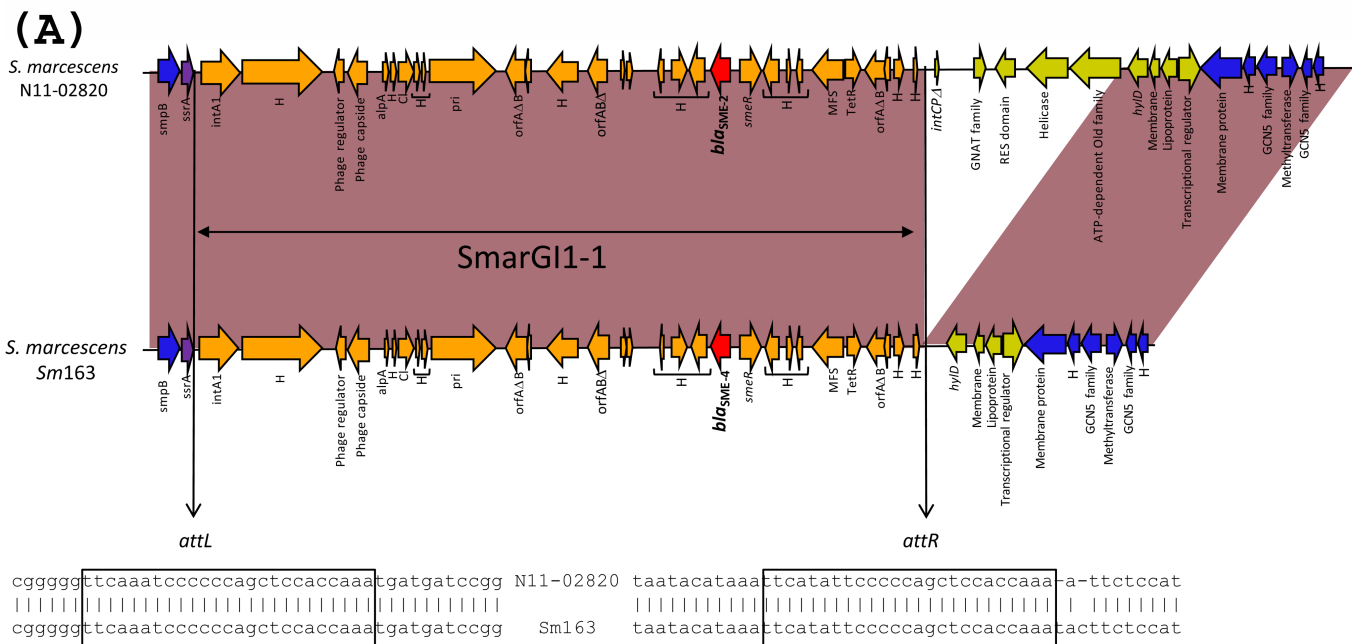


Fig. 1

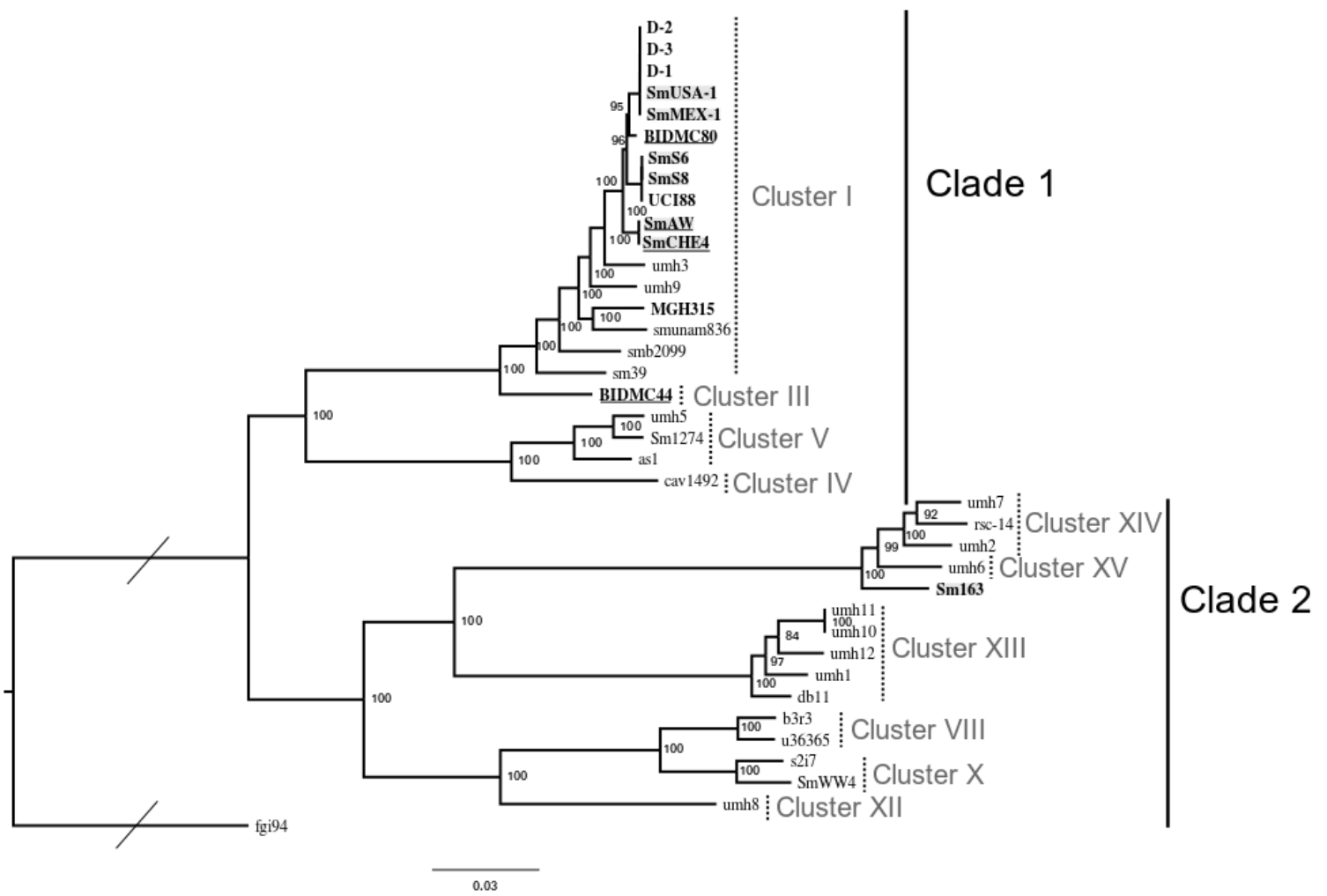


Fig. 2