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1 Mobile genetic elements provide evidence for a bovine origin of clonal complex 17 of
2 *Streptococcus agalactiae*

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20

21 This work was presented in part at the XVIth Lancefield International Symposium on
22 Streptococci and Streptococcal Diseases, Cairns, 25-29 September 2005.

23 We sought an explanation for epidemiological changes in *Streptococcus*
24 *agalactiae* infections, by investigating the link between ecological niches of the
25 bacterium, by determining the prevalence of 11 mobile genetic elements. The
26 prevalence of nine of these elements differed significantly according to the human or
27 bovine origin of the isolate. Correlating this distribution with the phylogeny obtained by
28 multilocus sequence analysis, we observed that human isolates harboring GBSi1, a clear
29 marker of the bovine niche, clustered in clonal complex 17. Our results are thus
30 consistent with the emergence of this virulent human clone from a bovine ancestor.

31 Epidemiological changes in the pattern of *Streptococcus agalactiae* infections
32 over the last six decades, from predominantly bovine infections (13), to a leading cause
33 of neonatal infections (25, 26) and, more recently, a cause of invasive disease in elderly
34 adults (6), requires explanation. Genomic lineages grouping the most invasive isolates
35 have been described by multilocus enzyme electrophoresis (17, 23), by pulsed-field gel
36 electrophoresis (1, 24) and more recently by the analysis of the polymorphism in the
37 allelic profiles of housekeeping genes (11), but are insufficient to account for the
38 emergence of *S. agalactiae* from such diverse ecosystems. A link between the bovine
39 and human niches has been proposed (8, 19), but remains a matter of debate. Indeed, it
40 was recently suggested that human and bovine *S. agalactiae* isolates are largely
41 unrelated (3) and represent distinct populations or subtypes (5, 27). Conversely, another
42 study provided evidence to suggest that the major hyperinvasive ST-17 clone implicated
43 in neonatal invasive diseases had arisen from bovine lineage ST-61 (2), whereas others
44 suggested that the genome of the common ancestor was closer to that of human ST-17
45 strains than to that of the ST-61 strains of bovine origin (4). We hypothesized that
46 mobile genetic elements (MGEs), such as insertion sequences (ISs) or group II introns,
47 which drive bacterial evolution (12, 30), might provide insight into changes in the
48 epidemiology of *S. agalactiae* infections.

49 We tested this hypothesis by analyzing a collection of 98 epidemiologically
50 unrelated isolates, mostly collected in France (3 isolates from Germany and 1 from
51 Malaysia) between 1966 and 2003: 63 human isolates from colonized individuals, adults
52 with invasive diseases and neonates with meningitis (52 of which were previously typed
53 by MLST and studied for the prevalence of IS1548, IS861, IS1381, ISSa4 and GBSi1)
54 (10), and 35 bovine isolates obtained from cows with clinical evidence of udder
55 infection.

56 The prevalence of 11 MGEs [one group II intron (GBSi1), and ten IS elements
57 corresponding to six insertion sequences (*IS1548*, *IS861*, *IS1381*, *ISSa4*, *ISSag1* and
58 *ISSag2*), and four transposase genes corresponding to remnants of IS elements (*ISSag9*,
59 *sag0448*, *sag1241*, and *sag0610*, relating to the identification of the ORFs in strains
60 A909 and 2605V/R) (28, 29)], was evaluated by PCR. *IS1548*, *IS861*, *IS1381*, *ISSa4*,
61 *ISSag1*, *ISSag2* and *GBSi1* were detected as previously described (10). Other
62 transposase genes were detected with the primers listed in Table 1. The prevalence of all
63 the MGEs except *IS861* and *ISSag2* differed significantly as a function of the original
64 reservoir (Fig. 1). This was particularly true for *IS1381*, *IS1548*, *ISSag1* and *ISSag9*,
65 which were mostly present in human isolates, and for *GBSi1* and *ISSa4*, which were
66 mostly present in bovine isolates (Fig. 1).

67 Multilocus sequence analysis, carried out with the standard multilocus sequence
68 typing (MLST) scheme described by Jones *et al.* (11), resolved the 98 isolates into 47
69 STs. A phylogenetic tree was generated, based on the nucleotide sequences of the
70 supergene obtained by concatenation of the seven loci for the 98 isolates, using MEGA
71 software version 3.1 (www.megasoftware.net/) with the neighbor joining (NJ) method
72 (14). Clonal complexes (CCs) (isolates sharing six or seven identical alleles) were
73 defined by eBURST analysis (<http://eburst.mlst.net/>) (7). Seven main NJ phylogenetic
74 divisions were identified, closely matching the seven main CCs identified by eBURST
75 analysis (Fig. 2): subCC19 (n=21), subCC2 (n=11), CC10 (n=12), CC7 (n=6), CC17
76 (n=13), CC67 (n=15) and CC23 (n=10). Three of these CCs were “host-specific”: CC17
77 and subCC19 consisted almost exclusively of human isolates (100% and 95% of the
78 isolates, respectively), whereas CC67 was almost exclusively composed of bovine
79 isolates (93.8% of the isolates). Moreover, the NJ dendrogram shows that human

80 lineage CC17 arose from bovine lineage CC67 (Fig. 2), consistent with the assertions of
81 Bisharat *et al.* (2).

82 A factorial analysis of correspondence (FAC) was performed on the whole data
83 set, with the 11 MGEs as active variables and the three main clonal complexes (CC19,
84 CC17 and CC67) and the bovine or human origins of the strains as illustrative variables.
85 Computations were performed with SPAD.N software (Centre International de
86 Statistiques et Informatique Appliquées, St Mandé, France), as previously described
87 (22). The projections of the active and illustrative variables and of the strains on the
88 F1/F2 plane accounting for 40.97 % of the total variance (Fig. 3), separated two groups
89 of variables and of strains. GBSi1 and ISSa4 (and to a lesser degree *sag0448* and
90 *sag1241*), bovine origin, CC17 and CC67 and 94.3% of bovine isolates were projected
91 onto the negative values of F1. IS1548, IS1381, ISSag9 and ISSag1 (and to a lesser
92 degree *sag0610*), human origin, CC19 and 79.4% of human isolates were projected onto
93 the positive values of F1. These findings confirm those of Luan *et al.* in that the two
94 major human *S. agalactiae* lineages are each marked by one MGE: subCC19 (defined as
95 CC19 by Luan *et al.*) (16), is characterized by IS1548, whereas CC17 is characterized
96 by GBSi1. All but one of the human isolates projected with the bovine isolates and
97 variable CC67 onto the negative values of F1 belonged to CC17, highlighting the
98 stronger linkage of CC17 with the bovine lineage CC67 than with any of the other
99 human lineages (Fig. 3). Moreover, our data also suggest that the presence of GBSi1 in
100 the bovine reservoir predates its presence in the human reservoir because (1) all our
101 bovine isolates harbored GBSi1 whereas this element was present in less than one third
102 of human isolates (almost all of which belonged to CC17) and (2) the bovine isolates
103 had higher copy numbers and more complex patterns for GBSi1 than the human isolates
104 (data not shown), consistent with an increase in IS pattern complexity over time (18).

105 MGEs therefore provide evidence for a bovine ancestor of the human clonal lineage
106 CC17, all the isolates of which harbor GBSi1. This statement is consistent with the
107 proposed evolutionary scheme of MGE acquisition, in which GBSi1 is thought to have
108 been acquired recently by human isolates (10).

109 The particular distribution of MGEs among bovine and human isolates (Fig. 3) is
110 also consistent with a physical barrier reducing the exchange of genetic material
111 between the bovine and human reservoirs. The human isolate H14, which occupies a
112 prominent position on the NJ dendrogram, between the bovine CC67 and the human
113 CC17 (Fig. 2), displayed two particular features. First, the copy number of GBSi1 in
114 this isolate was much higher than that in other isolates (human isolates had a median of
115 3 GBSi1, whereas H14 harbored 12 copies — data not shown). Parkhill *et al.* suggested
116 that IS expansion occurs during evolutionary bottlenecks (20, 21). They argued that in
117 *Yersinia*, IS expansion coincided with a change in the ecological niche of the species,
118 from commensal organism of the gut to systemic pathogen (21). Second, isolate H14
119 harbored both IS1548 and GBSi1. In our study, as in previous studies (9, 15), very few
120 isolates harbored both GBSi1 and IS1548 (see Fig. 2). Granlund *et al.* explained this
121 mutually exclusive distribution by the presence of a closed insertion site for the two
122 MGEs on the bacterial chromosome (9), but this distribution could also be explained by
123 IS1548 and GBSi1 originating from two different ecological niches. Thus, human clone
124 CC17 seems to have evolved from the bovine lineage containing GBSi1, and has
125 retained the GBSi1 genetic marker from its bovine donor.

126 In conclusion, our results provide at least a partial explanation of the emergence
127 of *S. agalactiae* as a major cause of neonatal meningitis, despite its original
128 identification as the causal agent of bovine mastitis. Assuming that CC17 evolved from
129 a bovine ancestor, as shown both by MLST analysis and by the presence of the bovine

130 marker GBSi1, the isolates of this clonal complex may have genetic features different
131 from those of other *S. agalactiae* isolates that have adapted to their human hosts over a
132 longer period of time. These features probably concern genes not present in the core
133 genome, as recently defined by Tettelin *et al.*, but that have been acquired by horizontal
134 exchange due to the environmental gene pool available in other ecosystems (28).
135 Indeed, we recently reported a link between prophage DNA fragments and *S. agalactiae*
136 isolates from neonatal cases of meningitis, suggesting the presence of additional genetic
137 material in these strains (10).

138

139

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140 We would like to thank Nicolas Jones, who manages the MLST website database,
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143

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270

271 TABLE 1. Primers used for the amplification of transposase genes from the
 272 *S. agalactiae* isolates.

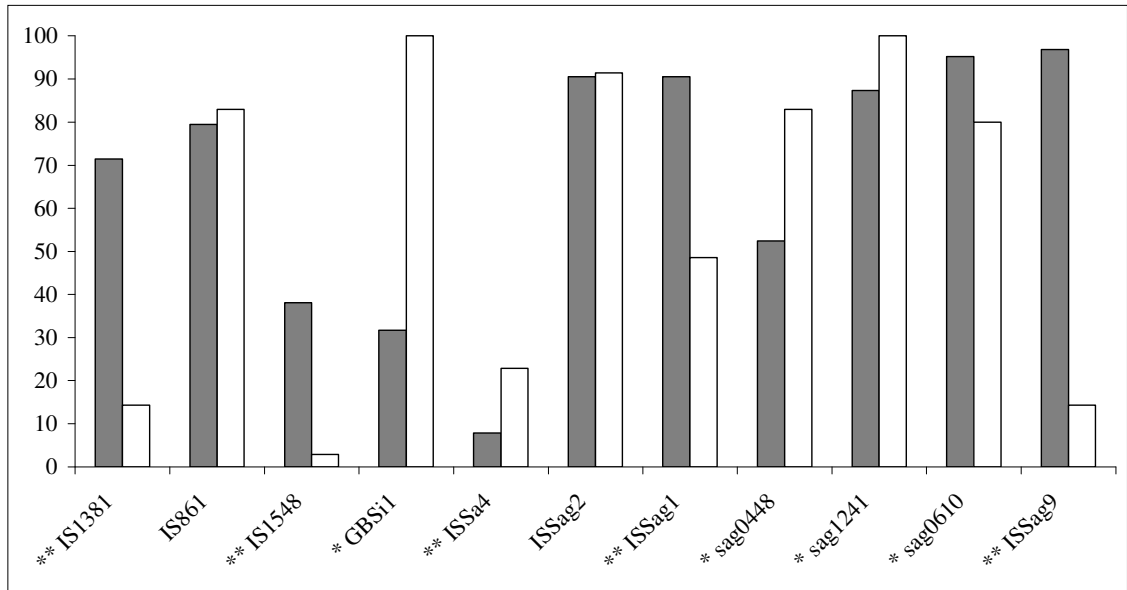
273

Target	Genbank accession no. ^a	Forward primer (5'-3')	Reverse primer (5'-3')	Tm (°C)	Amplicon (bp)
ISSag9	NC_004116	gaccgtaatgacatccaatctgg	ggcttgaaatggacacgggt	50	600
<i>sag0448</i>	NC_004116	ccacagaattacttaacttcttagc	cgaccaaccttattgaatcact	50	994
<i>sag1241</i> (<i>orfA</i>)	NC_004116	ctcttgtaacctttatcaaactgg	cctaaaaaagcaagtgccca	50	218
<i>sag0610</i>	NC_004116	tactccacattagctaaagaacagg	ggcctgatttaaccgagatt	50	394

274

275 ^a NC_004116 refers to strain 2603 V/R (29)

276 FIG. 1. Prevalence of the mobile genetic elements within the human (filled bars) and the
 277 bovine (open bars) *S. agalactiae* isolates. Significant differences (without correction for
 278 bias due to multiple comparisons) are indicated by one asterisk for p values below 0.05
 279 and two asterisks for p values below 0.001.
 280



281

282

283 FIG. 2. Relatedness of the 98 isolates of *S. agalactiae* analyzed by the NJ method, using
284 MEGA v3.1 software and the Kimura 2-parameter mutation model of genetic distance.
285 The nucleotide differences between the supergene sequences obtained in multilocus
286 sequence analysis, as described by Jones *et al.* (11), were used to infer phylogenetic
287 relationships between isolates. We used eBURST v3 software to group the 98 isolates
288 into clonal complexes (CC). For each isolate, sequence type (ST), β -hemolysis, serotype
289 and presence/absence of the 11 mobile genetic elements (MGEs) are indicated. Clonal
290 complexes are also indicated on the right of the figure (several isolates were not
291 attributed a CC number by the Start software and are considered “singletons”).

292

293

294

295

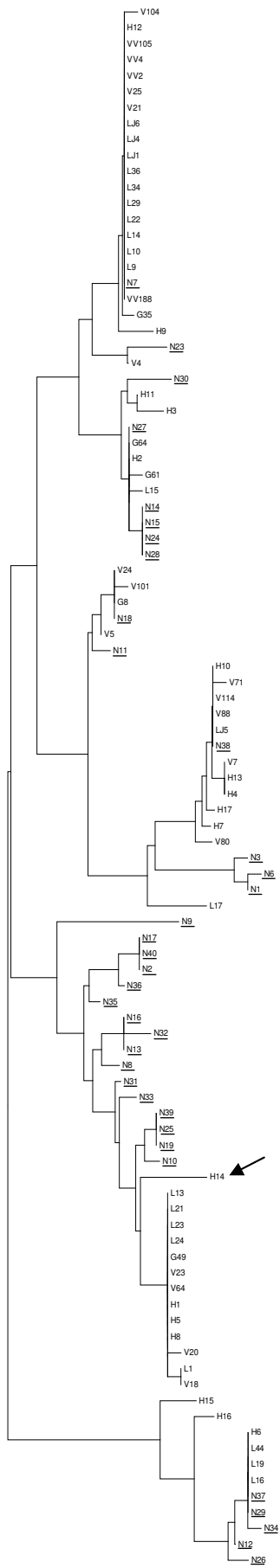
296

297 The particular position of isolate H14 is indicated by a black arrowhead.

298 The human isolates are named L or LJ (meningocerebral fluid), H (adult), V or VV
299 (vaginal carriage) or G (gastric fluid); the bovine isolates are named N (bovine isolates
300 are underlined).

301 NT: non typable

302 FIG. 3. Factorial analysis of correspondence of 98 *S. agalactiae* isolates based on the
303 mobile genetic element (MGE) data. Eleven active variables [the MGEs (▲) *IS1381*,
304 *IS861*, *IS1548*, *GBSi1*, *ISSa4*, *ISSag2*, *ISSag1*, *sag0448*, *sag1241*, *sag0610* and
305 *ISSag9*], and five illustrative variables [the main clonal complexes CC19 (◆), CC17 (◆)
306 and CC67 (◆), and the human (●) and bovine (■) origins of the isolates], and the 98
307 isolates are projected onto the F1/F2 plane. This plane, obtained by computation, is
308 defined by the two principal axes of the analysis; the first axis, F1, explains most of the
309 variance, and the second axis, F2 (orthogonal to F1), explains most of the variance not
310 explained by F1. Together, F1 and F2 accounted for 40.97% of the total variance.
311 Isolates of CC17 (n=13) are shown in red, isolates of CC67 (n=15) in green, and isolates
312 of subCC19 (n=21) in pink.
313



Isolate	Sequence Type	β-haemolysis	Serotype	MGEs										
				GBS1	IS1381	IS861	IS1548	ISSa4	ISSag2	ISSag1	sag0448	sag1241	sag0610	ISSag9
V104	ST 28	+	II	+	+	+	-	-	+	+	+	+	+	+
H12	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
VV105	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
VV4	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
VV2	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
V25	ST 19	+	NT	+	+	+	-	-	+	-	+	+	+	+
V21	ST 19	+	II	+	+	+	-	-	+	-	+	+	+	+
LJ6	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
LJ4	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
LJ1	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
L36	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
L34	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
L29	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
L22	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
L14	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
L10	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
L9	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
NZ	ST 19	+	III	+	+	+	+	-	-	+	+	+	+	-
VV188	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+
G35	ST 197	+	NT	+	+	+	+	-	-	+	+	-	+	+
H9	ST 131	+	V	+	+	+	+	-	-	+	+	+	+	+
N23	ST 226	+	II	+	-	-	-	-	-	+	+	+	+	+
V4	ST 366	+	Ia	-	-	-	+	-	-	+	+	+	+	-
N30	ST 261	+	IV	-	-	-	-	-	-	+	+	+	+	-
H11	ST 1	+	NT	-	-	-	-	-	-	+	+	+	+	+
H3	ST 186	+	NT	-	+	-	-	-	-	+	+	-	+	+
N27	ST 2	-	II	+	+	+	-	+	+	+	+	+	+	+
G64	ST 2	+	II	+	+	+	-	+	+	+	+	+	+	+
H2	ST 2	+	NT	+	+	-	-	-	-	+	+	-	+	+
G61	ST 202	+	V	-	-	-	-	-	-	+	+	+	+	+
L15	ST 196	+	IV	-	+	-	-	-	-	+	+	+	+	+
N15	ST 250	+	NT	+	+	-	-	-	-	+	+	+	+	-
N14	ST 250	+	NT	+	+	-	-	-	-	+	+	+	+	-
N24	ST 357	+	NT	+	+	+	+	+	+	+	+	+	+	-
N28	ST 250	+	NT	+	+	+	-	-	-	+	+	+	+	+
V24	ST 7	+	IV	-	+	+	-	-	-	+	+	+	+	+
V101	ST 41	+	V	-	+	+	-	-	-	+	+	+	+	+
G8	ST 7	+	Ia	+	+	+	-	-	-	+	+	+	+	+
N18	ST 7	+	Ia	+	+	+	-	-	-	+	+	+	+	+
V5	ST 6	+	Ib	+	+	-	-	-	-	+	+	+	+	+
N11	ST 354	+	Ib	+	+	+	-	-	-	+	+	+	+	+
H10	ST 10	+	Ib	+	+	+	-	-	-	+	+	+	+	+
V71	ST 8	+	Ib	-	+	+	-	-	-	+	+	+	+	+
V114	ST 10	+	Ib	-	+	+	+	-	-	+	+	+	+	+
V88	ST 10	+	II	-	+	+	-	-	-	+	+	+	+	-
LJ5	ST 10	+	Ib	-	+	+	-	-	-	+	+	+	+	+
N38	ST 10	+	Ib	+	-	+	-	-	-	+	+	+	+	-
V7	ST 12	+	Ib	-	+	+	-	-	-	+	+	+	+	+
H13	ST 12	+	Ib	-	+	+	-	-	-	+	+	+	+	+
H4	ST 12	+	Ib	-	+	+	-	-	-	+	+	+	+	+
H17	ST 200	+	Ib	-	+	+	-	-	-	+	+	+	+	+
H7	ST 10	+	II	-	+	+	+	-	-	+	+	+	+	+
V80	ST 12	+	II	-	+	+	+	+	+	+	+	+	+	+
N3	ST 952	-	NT	+	-	-	-	-	-	+	+	+	+	+
N6	ST 353	-	NT	+	-	+	-	-	-	+	+	+	+	-
N1	ST 350	+	NT	+	-	+	-	-	-	+	+	+	+	-
L17	ST 195	+	III	+	+	+	-	-	-	+	+	+	+	+
N9	ST 26	+	V	+	+	+	-	-	-	+	+	+	+	+
N17	ST 351	-	II	+	-	+	-	-	-	+	+	+	+	-
N40	ST 351	-	NT	+	-	+	-	-	-	+	+	+	+	-
N2	ST 351	-	II	+	-	+	-	-	-	+	+	+	+	-
N36	ST 365	+	II	+	-	+	-	-	-	+	+	+	+	-
N35	ST 364	-	NT	+	-	+	-	-	-	+	+	+	+	-
N16	ST 67	+	NT	+	+	-	-	-	-	+	+	+	+	-
N32	ST 85	+	NT	+	+	-	-	-	-	+	+	+	+	-
N13	ST 67	+	NT	+	+	-	-	-	-	+	+	+	+	-
N8	ST 64	-	II	+	-	+	-	-	-	+	+	+	+	-
N31	ST 361	-	III	+	-	+	-	-	-	+	+	+	+	-
N33	ST 362	-	III	+	-	+	-	-	-	+	+	+	+	-
N39	ST 61	+	NT	+	-	+	-	-	-	+	+	+	+	-
N25	ST 61	+	III	+	+	-	-	-	-	+	+	+	+	-
N19	ST 61	+	NT	+	+	-	-	-	-	+	+	+	+	-
N10	ST 63	+	II	+	+	+	-	-	-	+	+	+	+	-
H14	ST 22	+	II	+	+	+	-	-	-	+	+	+	+	-
L13	ST 17	+	III	+	+	+	-	-	-	+	+	+	+	+
L21	ST 17	+	III	+	+	+	-	-	-	+	+	+	+	+
L23	ST 17	+	III	+	+	+	-	-	-	+	+	+	+	+
L24	ST 17	+	III	+	+	+	-	-	-	+	+	+	+	+
G49	ST 17	+	III	+	+	+	-	-	-	+	+	+	+	+
V23	ST 17	+	NT	+	+	+	+	-	-	+	+	+	+	+
V64	ST 17	+	III	+	+	+	-	-	-	+	+	+	+	+
H1	ST 17	+	III	+	+	+	-	-	-	+	+	+	+	+
H5	ST 17	+	III	+	+	+	-	-	-	+	+	+	+	+
H8	ST 17	+	III	+	+	+	-	-	-	+	+	+	+	+
V20	ST 367	+	III	+	-	+	-	-	-	+	+	+	+	+
L1	ST 201	+	III	+	+	+	-	-	-	+	+	+	+	-
V18	ST 201	+	III	+	+	+	-	-	-	+	+	+	+	-
H15	ST 198	+	III	-	-	-	-	-	-	+	+	+	+	+
H16	ST 199	+	III	-	-	-	-	-	-	+	+	+	+	+
H6	ST 23	+	III	-	-	-	-	-	-	+	+	+	+	+
L44	ST 23	+	Ia	+	+	+	-	-	-	+	+	+	+	+
L19	ST 23	+	Ia	+	+	-	-	-	-	+	+	+	+	+
L16	ST 23	+	III	-	-	-	-	-	-	+	+	+	+	+
N37	ST 23	+	III	+	+	+	-	-	-	+	+	+	+	-
N29	ST 23	+	III	+	-	+	-	-	-	+	+	+	+	-
N34	ST 363	+	III	+	-	+	-	-	-	+	+	+	+	-
N12	ST 355	+	III	+	+	+	-	-	-	+	+	+	+	+
N26	ST 358	+	III	+	-	-	-	-	-	+	+	+	+	+

subCC19

subCC2

CC7

CC10

CC67

CC17

CC23

0.001

Axis F2

