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1 **Title**

2 *Echinococcus multilocularis* in Svalbard, Norway: microsatellite genotyping to investigate the
3 origin of a highly focal contamination.

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28 **ABSTRACT**

29 *Echinococcus multilocularis* is a threatening cestode involved in the human alveolar
30 echinococcosis. The parasite, mainly described in temperate regions of the northern
31 hemisphere was described for the first time in 1999 in the High Arctic Svalbard archipelago,
32 Norway. The origin of this contamination could be due to an anthropogenic introduction from
33 mainland Europe by domestic dogs or with the introduction of the sibling vole, perhaps from
34 mainland Russia (St Petersburg area), or with roaming Arctic foxes, known as the main
35 definitive host of the parasite in Arctic regions. The genetic diversity of *E. multilocularis* in
36 Svalbard was investigated here for the first time by genotyping using EmsB microsatellite and
37 compared to other genotyped populations in the main worldwide endemic areas. We found
38 low polymorphism amongst the 27 metacestode isolates from sibling voles trapped in the core
39 of the distribution area of the vole on Svalbard. *E. multilocularis* arctic populations, using the
40 Arctic fox as the definitive host, were genetically separated from European temperate
41 populations that use the red fox, but closely related to Saint Lawrence Island samples from
42 Alaska. The result is inconsistent with the hypothesis of an anthropogenic introduction from
43 mainland Europe, but can be seen as consistent with the hypothesis that Arctic foxes
44 introduced *E. multilocularis* to Svalbard.

45

46 **Keywords**

47 *Echinococcus multilocularis*

48 Microsatellite EmsB

49 Parasite dynamics

50 Arctic fox

51 Sibling vole

52

53 **1. Introduction**

54 The taeniid parasite *Echinococcus multilocularis* is the causative agent of Alveolar
55 Echinococcosis (AE), one of the most health-threatening helminthic zoonosis occurring on the

56 Northern hemisphere (Fig. 1). The parasite, mostly described in Europe, some parts of North
57 America, and Asia (Vuitton et al., 2003), requires two mammalian hosts to achieve its life
58 cycle: a carnivore definitive host such as the red fox (*Vulpes vulpes* Linnaeus 1758), or the
59 Arctic fox (*Vulpes lagopus* Linnaeus 1758) in Northern latitudes and a rodent intermediate
60 host, of which many species appear susceptible (Rausch, 1995; Vuitton et al., 2003). The
61 recent establishment of the parasite transmission on the Norwegian Arctic archipelago
62 Svalbard, (Henttonen et al., 2001) was made possible by the anthropogenic introduction of the
63 only available intermediate host species to the islands, the sibling vole (*Microtus levis* Miller
64 1908), certainly from mainland Russia. This introduction probably occurred in the first half of
65 the 20th century, with mining and maritime activities (Henttonen et al., 2001). The core
66 rodent habitat on Svalbard is along an 8 km stretch of bird cliffs along the coast in the
67 Grumant area, 10 km west of the main Norwegian settlement, Longyearbyen (78° 13' N, 15°
68 38' E) (Fuglei et al., 2008; Stien et al., 2010). In the Grumant area *E. multilocularis* has been
69 found with high prevalence in the rodents (26%; 59 infected of 224 trapped rodents
70 (Henttonen et al., 2001)), the Arctic foxes (35%; 13 infected of 37 trapped foxes (Stien et al.,
71 2010)), and in fox faeces (60%; 135 infected of 224 fox faeces sampled (Fuglei et al., 2008)).
72 While the introduction of the intermediate host was a prerequisite for the establishment of the
73 parasite life-cycle, the oncoming of the parasite has been suggested to be due to large scale
74 roaming on sea ice by Arctic foxes between Svalbard and *E. multilocularis*-endemic areas in
75 Siberia (Henttonen et al., 2001). An alternative hypothesis is the possible introduction of the
76 parasite with the sibling voles, which are likely to originate from the St Petersburg area in
77 Russia. Finally, a third alternative hypothesis is that the parasite has been anthropogenically
78 introduced from mainland Europe, in association with the substantial movement of humans
79 and possibly dogs between Europe and Svalbard due to tourism, mining and scientific
80 explorations in the 20th century.

81 *E. multilocularis* present a genetic homogeneity with nuclear and mitochondrial targets (Haag
82 et al., 1997), but fast evolution markers, such as the repeated microsatellite EmsB, are readily
83 available to detect variations at regional and local scales (Bart et al., 2006; Knapp et al., 2007;
84 Knapp et al., 2008). Recently, a European study has documented the presence of several
85 EmsB profiles distributed in the historical endemic area in the European Alps with surprising
86 patterns of genetic diversity and evidence for spread to peripheral regions as a mainland-
87 island system (Knapp et al., 2009). From this study, a catalogue of EmsB profiles, described
88 in Europe and in some of the main worldwide endemic areas including China (Tibetan

89 plateau), Alaska (Saint Lawrence Island) and Japan (Hokkaido), is now available and allows
90 us to characterize the parasite diversity. Here we have tested whether *E. multilocularis* on
91 Svalbard has been introduced from the European mainland by comparing the EmsB profiles
92 of samples from Svalbard with samples from the European mainland. In addition we evaluate
93 the genetic identity of the Svalbard samples in relation to the other endemic areas in the
94 northern hemisphere with genetic samples available to improve our description of patterns in
95 the genetic diversity of the parasite.

96

97

98 **2. Materials and methods**

99 *2.1. Samples collection and DNA extraction*

100 A sample collection of 27 metacestodes was constituted from sibling voles (*Microtus levis*)
101 trapped in August 2004 - 2006 in the Grumant area (Stien et al., 2010) (Fig. 2). Total DNA
102 was extracted from metacestode tissue or isolated protoscoleces (Stieger et al., 2002).

103 *2.2. Nuclear targets, PCR amplification and fragment size analyses*

104 PCR amplification was performed for the nuclear targets EmsB, as previously described (Bart
105 et al., 2006) and for the partial sequences of the protein coding genes of ezrin-radixin-moesin-
106 like protein (*Elp*), calreticulin (*Cal*) and thioredoxin peroxidase (*Th*), as recommended by
107 authors (Saarma et al., 2009). The EmsB target was studied for the polymorphism of the
108 tandemly repeated microsatellite by fragment size analysis, on 3730 ABI sequencer (Applied
109 Biosystem, Foster City, CA) and analysed on GeneMapper 3.0. The amplified samples were
110 compared with a worldwide *E. multilocularis* sample collection, containing aggregated
111 samples (from fields about 500m² in Switzerland and 100m² in Alaska) (Knapp et al., 2007))
112 and non correlated isolates from Europe (Switzerland, Czech Republic, Slovakia, Austria,
113 Germany, Netherlands, Poland and France; n=42), North America (St Lawrence Island,
114 Alaska and Canada, n=11), China (Tibetan plateau; n=5) and Japan (Hokkaido; n=6). The
115 available *E. multilocularis* samples closest to the putative starting point of the sibling voles
116 introduced to Svalbard were the North-East Polish specimens (900 km south of St
117 Petersburg). Two *E. granulosus* sensu lato was chosen as outgroups (Knapp et al., 2007) and
118 a hierarchical clustering analysis (HCA), using the Euclidean distance and the UPGMA

119 method in the R software was performed. A genetic threshold previously described on the
120 stability on time of EmsB profiles allowed us to discriminate similar and distinct isolates
121 (Knapp et al., 2007). The nuclear targets *elp* (1023 bp), *cal* (1368 bp) and *th* (583 bp),
122 previously sequenced for the currently described *Echinococcus* species (Saarma et al., 2009),
123 were chosen to investigate the worldwide genetic diversity of the parasite. The targets were
124 sequenced for *E. multilocularis* isolates from Central Europe (Switzerland), Canada (an
125 isolate from a rodent, exact geographical position unknown), Alaska (St Lawrence Island) and
126 Japan, and compared to one randomly chosen Svalbard sample using BioEdit (Hall, 1999).

127

128 **3. Results**

129 In comparison to referenced *E. multilocularis* sequences (GenBank Ref. FN582291;
130 FR820773 to FR820783), 4 mutations were described on *Elp* and *Th* for the Svalbard isolate
131 (none in *Cal*) (FR820594 to FR820596), two of these were common with the Canadian
132 specimen and one was common with the European specimen (Table 1). However, this low
133 genetic diversity did not allow us to conclude a divergence among the *E. multilocularis*
134 samples. The pattern of the EmsB electrophoregram profiles were first compared to the
135 others, and were found similar to Alaskan samples by the presence on the electrophoregram
136 of a 209 bp fragment (Fig. 3(a)) never found in the other samples so far. From the HCA (Fig.
137 3(b)) performed by EmsB data computing, the European samples were clustered in a group,
138 somewhat associated with the Chinese samples and distinct from the Japanese samples. The
139 Svalbard samples were all clustered together, in the same group; the genetic distance amongst
140 all the Svalbard samples was under the threshold of 0.08 (Fig 3(b)) and allowed us to
141 considered them as identical to each other. Moreover they were closely related to St Lawrence
142 Island samples from Alaska, and formed the most distant group from the other Eurasian
143 samples (Fig. 3 (b)). These Arctic samples clustered clearly in a separate group from the
144 samples from temperate Europe, China, Japan and Canada.

145 **4. Discussion**

146 In comparison with the high polymorphism of the EmsB target found in multiple European *E.*
147 *multilocularis* populations, an extremely low diversity seemed to occur in the isolated Arctic
148 islands of St Lawrence, Alaska (Knapp et al., 2007) and Svalbard. Such a pattern has
149 previously been observed in samples from small areas such as a field in Fribourg

150 (Switzerland) and by the Savoonga air-strip on St Lawrence (Alaska) (Knapp et al., 2007).
151 These findings emphasize a putative clonal distribution of the parasite on restricted areas, as
152 an expected result of an initial contamination by a small number of foxes (Knapp et al., 2007).
153 In addition, the low genetic polymorphism of the parasite in the investigated Arctic sites
154 could be linked to (i) the occurrence of one or few *E. multilocularis* populations in
155 circumpolar areas, after a founder event in a mainland-island context, (ii) these islands could
156 be considered as metapopulations connected by sea ice, explaining the genetic similarity
157 between insular samples or (iii) in this context a low diversity of intermediate and definitive
158 host species exists when compared to European sites. The Arctic fox appears as a genetically
159 homogenous species, except for a few isolated islands not connected by sea ice in winter
160 (Norén et al., 2011). From a co-evolution point of view, one could hypothesize that the
161 parasite may evolve more slowly in an insular environment than in the temperate mainland
162 situation where many species of potential intermediate host rodent species occur
163 simultaneously. As such, these findings indicate particularities in the circumpolar *E.*
164 *multilocularis* life cycle, which seems to have low or no connection with *E. multilocularis* life
165 cycles from temperate regions.

166 Our microsatellite EmsB analysis suggests that Arctic *E. multilocularis* populations have a
167 common history. Indeed, a high genetic similarity was observed between the geographically
168 distant samples from Svalbard and Alaska, and a wide dissimilarity with the clustered
169 European samples, even from North Europe was found (Fig. 3(b)). This may suggest an
170 epidemiological connection between polar populations. Most likely, this could be due to the
171 behavioral characteristics of the Arctic fox, a species with a circumpolar distribution and long
172 distance movements (Norén et al., 2011). From our samples, we can reject the hypothesis of
173 an anthropogenic introduction of *E. multilocularis* from mainland Europe, while the
174 hypothesis of an Arctic fox driven immigration from Siberia to Svalbard is likely. Concerning
175 the hypothesis of a strain coming from the Russian sibling vole we can speculate that the
176 Russian *E. multilocularis* could be similar to North-East Europe as the Polish parasites were
177 sampled 900 Km from St Petersburg. However, more samples of *E. multilocularis* from
178 Russia are needed to further evaluate this hypothesis.

179

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186

187 Dr. Knapp completed her PhD at the University of Franche-Comté and is a clinical research
188 engineer in the University hospital of Besançon, France. Her major research focus is the
189 molecular epidemiology of echinococcosis.

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191

192 Table and figure legends

193 Fig. 1. Worldwide dispersion of *E. multilocularis* (orange areas) and distribution of the world
194 collection samples, clustered by local position (black stars) studied for EmsB profiles. The
195 exact geographical position of the Canadian sample is unknown (From (Eckert et al., 2011;
196 Osterman Lind et al., 2011)).

197 Fig. 2. Map of Svalbard and the details of the Grumant area (dotted line) where the sibling
198 voles (*Microtus levis*) infected by *E. multilocularis* were sampled in August 2004 - 2006.

199 Fig. 3 (a) EmsB electrophoregrams of an Alaskan sample (3AL-r), a Svalbard sample
200 (Sval14-r), an Austrian sample (278A-F) and a German sample (101D-F), size in base pair.
201 (b) *E. multilocularis* hierarchical clustering analysis, based on EmsB microsatellite results.
202 The genetic threshold at 0.08 described similar and distinct EmsB profiles (for details on the
203 construction see Knapp et al., 2007). The sample codes used are: Sval: Svalbard; AL: Alaska;
204 CND: Canada; J: Japan; CH: Switzerland; CZ: Czech Rep., SK: Slovakia; A: Austria; D:
205 Germany; H: Netherlands; PL: Poland; F: France; PRC: Peoples' Rep. of China; hosts codes:
206 -r: rodent; -h: human; -m: monkey; -F: fox. Isolates 500AUB to 502AUB are one *E.*
207 *multilocularis* strain maintained in vivo by several passages in *Meriones unguiculatus*; the
208 grey scares represent the *E. multilocularis* regional groups; 116Eg (from Mauritanian camel)
209 and 539Eg (from Algerian sheep) are *E. granulosus* outgroup controls. The approximately
210 unbiased *P* values (numbers on nodes, in percent) were calculated with a multiscale bootstrap
211 resampling ($B = 1000$).

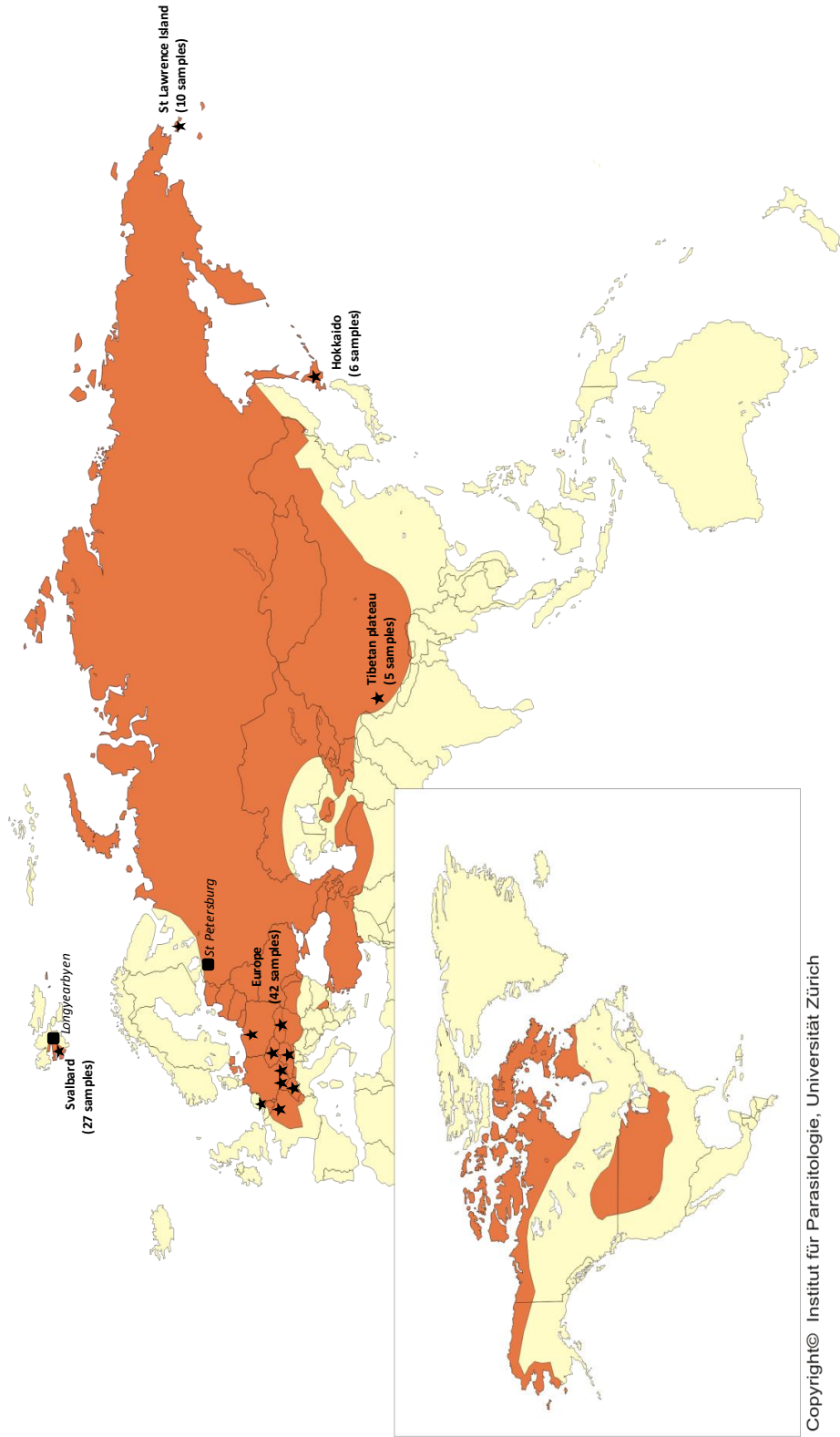
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214 Table 1: Comparison of the *E. multilocularis* *Elp* and *Th* gene fragments from different
215 origins.

<i>E. multilocularis</i> origin	<i>Elp</i> (bp)			<i>Th</i> (bp)
	323	459	631	328
Svalbard	C	A	T	C
St Lawrence Island	T	C	C	T
Canada	C	C	C	C
Switzerland	T	C	C	C
Hokkaido	T	C	C	T

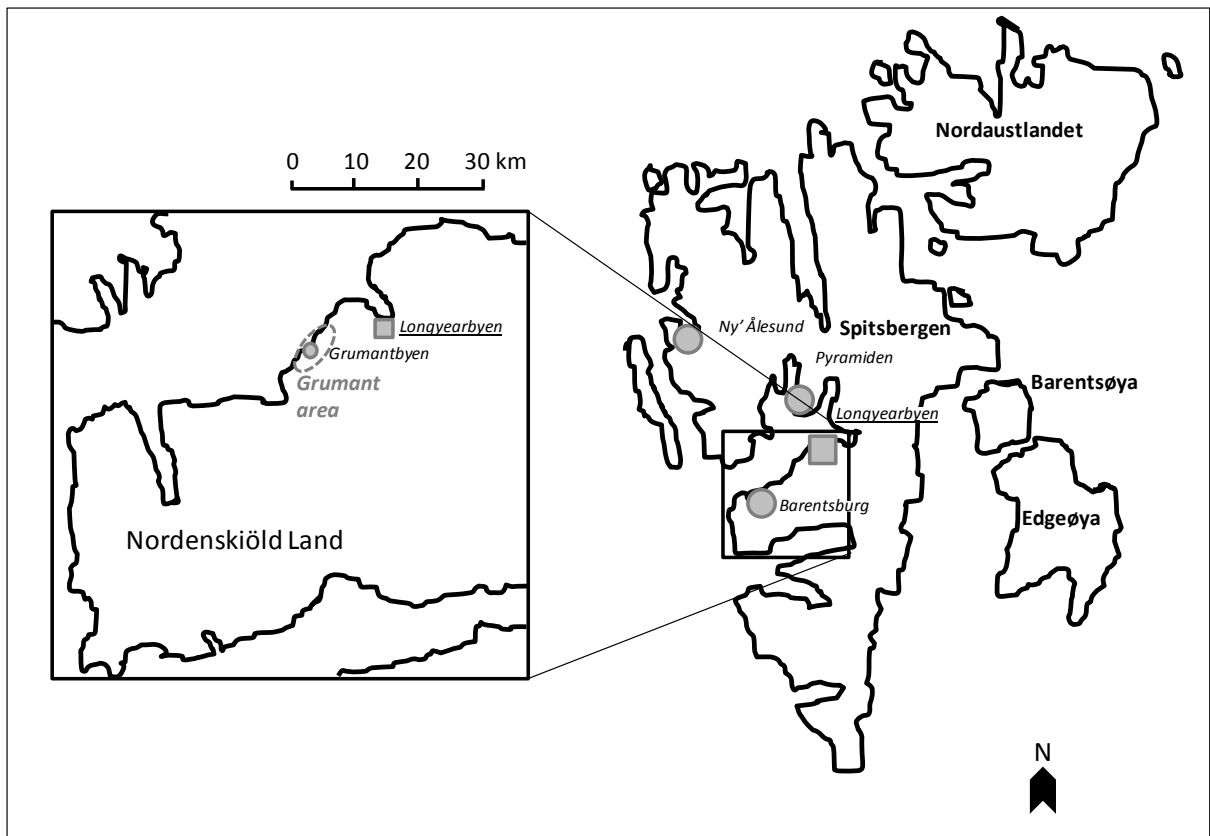
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218 Fig. 1

219



220

221 Fig. 2

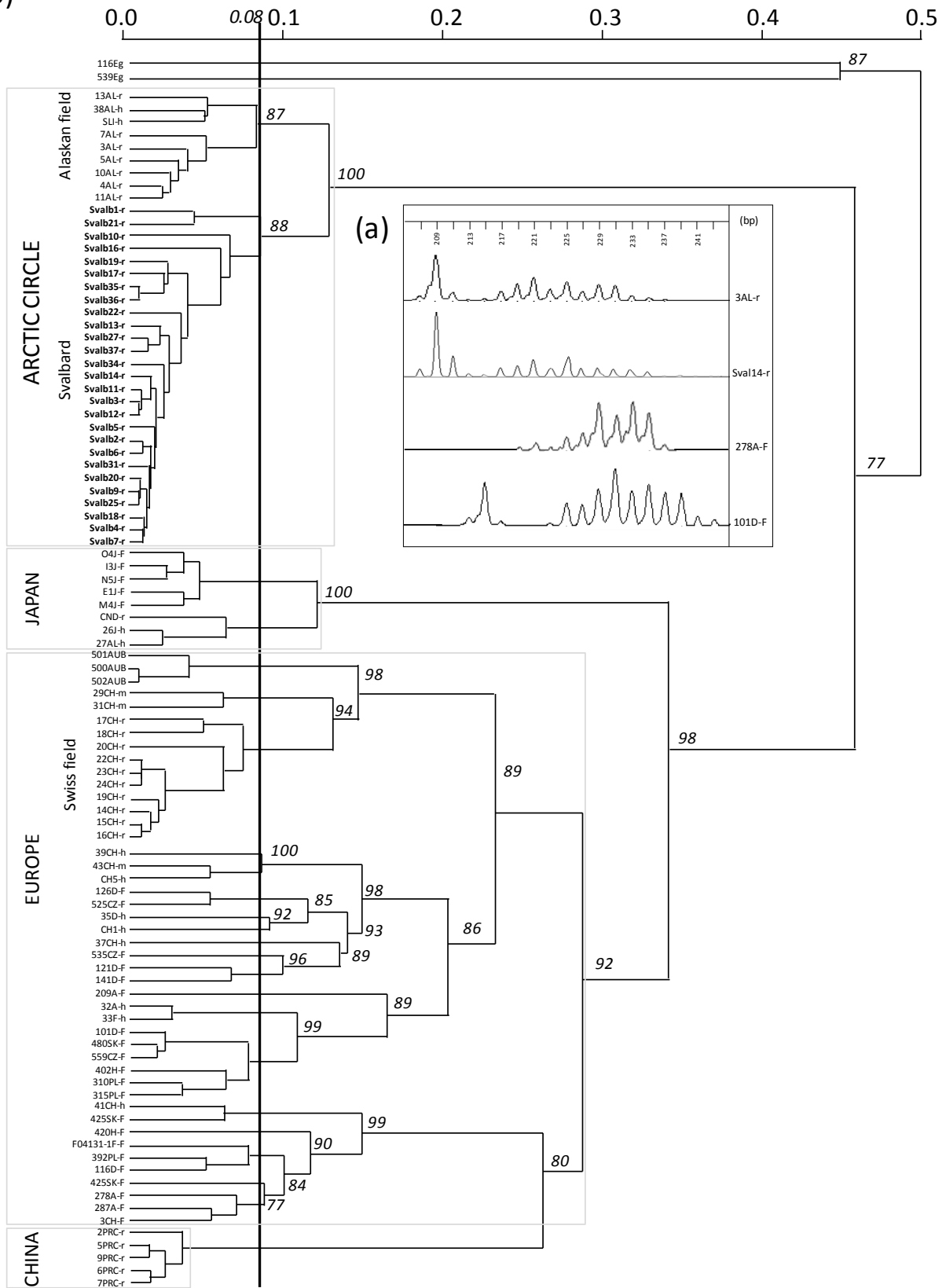
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Genetic distance

(b)



225

226 Fig. 3

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