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1 Original Research Paper

2 ***Echinococcus multilocularis* genetic diversity in Swiss domestic pigs assessed by EmsB**
3 **microsatellite analyzes**

4

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13

14 Abstract

15 Assessing the genetic diversity of the parasite *Echinococcus multilocularis* provides key information
16 about the temporal and spatial strain flow in a given area. Previous studies indicated that a historical
17 endemic area conventionally presents a relatively high genetic diversity, whereas peripheral or newly
18 endemic areas exhibit a more restricted variability of the parasite. The Swiss plateau region is part of
19 the European historically endemic area, and the genetic diversity has already been investigated by
20 assessing either human metacestode isolates or adult worms from foxes. To date, there have been
21 no studies covering the whole geographical area affected by the parasite. The aim of the present
22 study was to make use of the domestic pig to investigate the genetic diversity of *E. multilocularis* in
23 relation to spatial distribution. A total of 55 *E. multilocularis*-induced hepatic lesions from

24 slaughtered pigs from Switzerland were studied using EmsB microsatellite analyzes, and findings
25 were compared to already published data (originating from human, primate, foxes, and rodent
26 samples). A total of 12 EmsB profiles were described among the domestic pigs, some of them
27 presenting a clear spatial organization in the Swiss plateau, with three of the main profiles
28 geographically separated. One of the 12 EmsB profiles has been newly identified for Switzerland in
29 this study, while the other 11 profiles had been previously described in other Swiss *E. multilocularis*
30 isolates from other hosts. Overall, a total of 18 EmsB profiles have so far been described within the
31 Swiss endemic area. Six profiles appeared only among human, primate, rodent, and fox samples.
32 Based on a richness and diversity accumulation analysis, the sampling efficiency for the whole
33 studied area has now been improved considerably by compilation of 178 *E. multilocularis* specimens
34 obtained from four different intermediate and one definitive host species in Switzerland.

35

36 Keywords: *Echinococcus multilocularis*; domestic pigs; EmsB microsatellite; parasite dispersion

37 1. Introduction

38 The cestode *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis (AE), a
39 zoonosis largely distributed in the northern hemisphere (Baumann et al., 2019). The parasite
40 perpetuates itself by the development of the adult stage in the definitive host, mainly red foxes in
41 Europe, but also domestic dogs and some other carnivores, with compulsory cycling through the
42 metacestode stage in intermediate hosts, mainly small rodents. Some other mammalians can
43 become aberrant hosts for the larval stage, such as humans, primates, and even dogs and pigs,
44 among others (Vuitton et al., 2003; Conraths and Deplazes, 2015; Oksanen et al., 2016). In humans,
45 an estimation of 18,000 new AE cases occur worldwide per year (Torgerson et al., 2010), with 1,600
46 cases in Europe and 16,400 in China. AE still represents a neglected disease, and despite relevant
47 progress in control and prevention, the incidence of human AE in Europe has constantly increased
48 (Gottstein et al., 2015; Craig et al., 2017). Diagnoses made on aberrant hosts can provide interesting
49 information on the distribution of infection sources and modes of transmission of the parasite.
50 Firstly, with increasing prevalences of *E. multilocularis* in fox populations in many European
51 countries, the richness of intermediate and definitive hosts involved in its life cycle seems to increase
52 as well (Deplazes et al., 2017). Secondly, even if most of the aberrant hosts seem to play no or only a
53 minor role in *E. multilocularis* transmission, they can nevertheless represent a good proxy to study
54 the environmental infection pressure with parasite eggs (Oksanen et al., 2016). Pigs can develop
55 macroscopically visible liver lesions, are slaughtered and subject to routine meat and organ
56 inspection in large numbers, thus making it relatively easy to collect a large number of lesions in a
57 short time. Clinical descriptions of *E. multilocularis* infection in domestic pigs in various husbandry
58 systems were made in different countries in Europe, such as Germany (Böttcher et al., 2013),
59 Lithuania (Bruzinskaite et al., 2009), Poland (Karamon et al., 2012), and Switzerland (Sydler et al.,
60 1998). In Switzerland, a prevalence of 10% (9/90 animals) among animals from free-ranging pigs was
61 described. A large-scale surveillance study, combining cross-sectional and case-control studies, was
62 carried out in six slaughterhouses over 12 successive months. It encompassed 85% of all Swiss pigs

63 and based on the routine inspection of pig livers for *E. multilocularis* infection, a minimal prevalence
64 among Swiss pigs was estimated as 0.009% (200 of 2,143,996 animals), with 0.11% minimal
65 prevalence in breeding pigs (22 of 20,454 animals) (Meyer et al., 2020). The presence of foxes in pig
66 sheds and feeding of grass emerged as risk factors for *E. multilocularis* infection in pigs, among other
67 factors. Moreover, this study concluded that the presence of *E. multilocularis* eggs in the
68 environment seems to occur in the larger part of the Swiss territory north of the Alps, contradicting
69 the hypothesis of geographical clusters in this area. From all of the porcine cases reported so far, all
70 showed exclusively hepatic localization of the parasite, with no extra-hepatic lesions documented.
71 Experimental *E. multilocularis* infections in domestic pigs showed that macroscopic lesions occurred
72 between 3 to 6 months post-infection (Pfister and Franck, 1988; Deplazes et al., 2005). Such lesions
73 were generally small (<1 cm), with calcified necrotic centers (Deplazes et al., 2005). Lesions found in
74 natural cases, as diagnosed in wild boars or domestic pigs, presented fractured laminated layers and
75 occasionally isolated germinal cells, but usually the complete absence of a germinal layer, and no
76 protoscoleces development (Pfister et al., 1993; Deplazes et al., 2005; Böttcher et al., 2013; Meyer et
77 al., 2020). Consequently, pigs represent a dead-end host for the parasite. From a serological point of
78 view, pigs can either have a pronounced antibody reaction, usually associated with millimeter
79 lesions, or a reduced humoral reaction, associated with larger lesions (Deplazes et al., 2005).

80 Overall, the description of the parasite in such aberrant hosts may function as a good epidemiological
81 indicator to study the contamination pressure with infectious *E. multilocularis* eggs in the
82 environment. Thus, the diversity of the parasite through genetic patterns prevalent in such an
83 environment can be assessed upon investigating parasite hosts, accordingly sampled in a given area.
84 Due to the parasite exhibiting hermaphroditical mating, it presents a relatively low biological
85 polymorphism (Haag et al., 1997; Nakao et al., 2007; Spotin et al., 2018). Therefore, to efficiently
86 assess genetic diversity at a local geographical scale, the tandemly repeated microsatellite EmsB
87 appears appropriate. This marker has already proven its suitability at the local and regional scales in
88 intermediate and definitive hosts, (Knapp et al., 2007, 2008, 2009, 2012, 2017, 2020; Casulli et al.,

89 2009; Jenkins et al., 2012; Umhang et al., 2014, 2017; Afonso et al., 2015), with the highest
90 discriminatory power obtained from microsatellites ever tested for studies performed on *E.*
91 *multilocularis* diversity (Knapp et al., 2007). Briefly, the EmsB microsatellite was initially characterized
92 from *Echinococcus granulosus* (Bart et al., 2006), and subsequently described through in silico PCR in
93 several copies (about 40) also in the *E. multilocularis* genome (Valot et al., 2015). Methodically, the
94 amplification of respective parasite DNA sequences by PCR is followed by a PCR-product fragment
95 size analysis with a sequencer machine (Knapp et al., 2007). The analysis of the fragment size analysis
96 provides an electrophoretic diagram, corresponding to the genetic signature of a given sample
97 (Knapp et al., 2017). The electrophoregram is composed of a series of peaks, spaced from 2 bp linked
98 to the microsatellite pattern (CA)_n (GA)_n, depending on the number of repetitions and corresponding
99 to alleles. The position and the height of each peak are used to compare samples to each other, by
100 calculating Euclidean distances among tested samples.

101 The overall aim of the study was to conclusively characterize the genetic diversity of *E. multilocularis*
102 based on parasite hosts as domestic pigs. All samples were derived from a combined cross-sectional
103 and case-control study on *E. multilocularis* infection in pigs in Switzerland (Meyer et al., 2020), and
104 used to produce microsatellite EmsB profiles. These new results, related to a representative spatial
105 distribution of the parasite on the Swiss territory, were compared to EmsB data from already existing
106 Swiss specimens from other host species, available from the publicly accessible EmsB Website for
107 *Echinococcus* Typing (EWET) database (Knapp et al., 2017).

108

109 2. Materials and methods

110 2.1. *Parasite material*

111 All *E. multilocularis* specimens were isolated from domestic pigs slaughtered in six abattoirs in
112 Switzerland (detailed information available in Meyer et al., 2020). Briefly, of the 2.57 million pigs
113 slaughtered annually in Switzerland (Data from the Swiss Interprofessional meat sector Proviande,
114 2018), a total of 2,143,996 pigs (2,123,542 fattening pigs and 20,454 breeding pigs), i.e.
115 approximately 85% of the total annual production, were slaughtered at these six participating
116 facilities. The sampling period was from April 2017 to March 2018. For cost reasons, a random liver
117 sample was collected from each positive pig batch, exhibiting lesions suspicious for *E. multilocularis*.
118 All samples were stored at 4 °C and sent with overnight express to the Institute of Parasitology in
119 Bern, Switzerland within a week of slaughter. For each submitted liver, lesions were dissected from
120 liver tissue and then divided into sub-samples for processing for PCR, for storage at -20 °C as a
121 backup specimen, and for fixation in formalin for histopathological analyzes.

122 2.2. *DNA extraction and E. multilocularis diagnosis*

123 DNA from *E. multilocularis* specimens was isolated from approximately 20 mg of tissue, using Qiagen
124 DNeasy Blood & Tissue Extraction Kit (Qiagen, Hilden, Germany), following the manufacturer's
125 protocol. DNA was eluted in 200 µl elution buffer. DNA concentration was assessed by a NanoDrop
126 2000 spectrophotometer apparatus (Thermo Fisher Scientific, Waltham, MA) and the DNA
127 concentrations were standardized at 100 ng/µl. For the initial screening of samples performed by
128 Meyer et al. (2020), the *E. multilocularis* diagnosis was performed by applying the taeniid multiplex
129 PCR (Trachsel et al., 2007). The PCR products containing fragments of the genes *nad1* and *rrnS* were
130 subjected to electrophoresis on a 2% agarose gel to detect the presence of the expected product size
131 at 395 bp, specific to *E. multilocularis*, or for a differential diagnosis at 117 bp for *E. granulosus* and
132 267 bp for *Taenia* spp.

133 2.3. *Selection of isolates for EmsB analysis*

134 The samples positive for the taeniid multiplex PCR were subject to a second PCR. They were tested
135 by the EMH15/H17 *E. multilocularis*-specific PCR (Stieger et al., 2002; Georges et al., 2004). The
136 expected size of the PCR product, at 200 bp was checked by electrophoresis on a 1.5% agarose gel.
137 Only *E. multilocularis* EMH15/H17 PCR-positive isolates were selected for the subsequent EmsB-PCR,
138 and then fragment size analysis by polyacrylamide electrophoresis.

139 *2.4. PCR conditions*

140 The EmsB PCR reaction mixture was performed in a final volume of 18 µl, the Multiplex PCR master
141 mix containing 2.8 U of HotStarTaq DNA polymerase (Qiagen, Hilden, Germany), 0.1 µM of each
142 primer final concentration (EmsB A: 5'-FAM-GTGTGGATGAGTGTGCCATC-3' and EmsB C: 5'-
143 CCACCTTCCCTACTG-CAATC-3'), 3% DMSO, and up to 100 ng of purified DNA. PCRs were performed in
144 a Biometra T3 thermocycler (Whatman Biometra, Goettingen, Germany) as described by Knapp and
145 co-workers (Knapp et al., 2020). For each of these runs, the EmsB calibrator – a plasmid construction
146 made with four EmsB microsatellites – was used to check and compare the reliability of the fragment
147 size analysis (Knapp et al., 2017).

148 The fragment size analyzes were performed from the dyed PCR products by capillary electrophoresis
149 on an Applied Biosystems 3130 Genetics Analyzer (Applied Biosystems, Foster City, CA).
150 Electrophoregrams were analyzed on GeneMapper 4.1 (Life Technologies, Foster City, CA).

151 *2.5. Hierarchical clustering analysis*

152 The presence or absence of peaks and their associated fluorescence intensity heights were recorded
153 from the fragment size analysis data as previously described (Knapp et al., 2017). These were used to
154 assess the genetic diversity of the parasite from pig lesion isolates and to establish profiles by
155 hierarchical clustering analysis, using the Euclidean distance and the unweighted pair group method
156 with arithmetic mean (UPGMA) (Legendre and Legendre, 2012). To assess the stability of the clusters,
157 a multiscale bootstrap resampling (B = 1,000) was performed, providing the approximately unbiased

158 p-values (Shimodaira, 2004, 2002). Clustering analyzes were performed by using the RStudio
159 software (R version 3.5.1) (RStudio Team, 2016) and package pvclust (Suzuki and Shimodaira, 2006).
160 The genetic distance threshold was applied at 0.1 after modifications of the protocol as previously
161 described by Knapp and co-workers (Knapp et al., 2020). EmsB data publicly available on the EWET
162 project (<https://ewet-db.univ-fcomte.fr>) were added to the dendrogram construction (Knapp et al.,
163 2017), to assess the genetic diversity of the parasite within Switzerland. This collection was
164 composed of 123 specimens, 23 from human lesions (Knapp et al., 2020), 84 from fox worms (Knapp
165 et al., 2009), 11 from rodents, and five from captive monkeys (Knapp et al., 2007).

166 *2.6. Geographical distribution of samples and EmsB profiles*

167 The *E. multilocularis* lesions from pigs, humans, monkeys, foxes and rodents were mapped using the
168 Quantum GIS software version 3.6.0 (QGIS, Open Source Geospatial Foundation Project,
169 <http://qgis.osgeo.org>), and Eurostat map bases
170 ([https://ec.europa.eu/eurostat/fr/web/gisco/geodata/reference-data/administrative-units-
171 statistical-units/countries](https://ec.europa.eu/eurostat/fr/web/gisco/geodata/reference-data/administrative-units-statistical-units/countries)). Details of the geographical location of pigs were obtained from Meyer et
172 al. (2020), for humans at the Canton level (Knapp et al., 2020), foxes were all located in the Canton of
173 Zürich and the centroid of the Canton was attributed to them, for monkeys and rodents, the WGS84
174 position was available (Knapp et al., 2007). The distribution of the *E. multilocularis* EmsB profiles
175 were then graphically represented through the host geographical position (Supplementary data S1).

176 *2.7. Richness and diversity analyzes*

177 The richness and diversity of EmsB profiles were assessed first on the whole studied area, and
178 secondly by dividing the area into three parts as Jura-Western plateau (A), Central plateau (B), and
179 Eastern-plateau (C) in terms of the sampling effort made on pigs only or considering the whole Swiss
180 sampling. The sampling effort was then assessed through species accumulation curves, made by
181 plotting the cumulative number of EmsB profiles (genetic richness) from different areas (Magurran,
182 2004). The curve reaches an asymptote when the sampling is sufficient to catch the actual number of

183 genetic profiles in a given area. If an asymptote was not reached, the extrapolated richness was
184 assessed by the first order Jackknife estimator of species richness, taking into account the profiles
185 that occur in one pig only (Burnham and Overton, 1979) and the Bootstrap estimator, considering the
186 frequency of all profiles (Smith and van Belle, 1984), with the help of RStudio software and the vegan
187 and pgirmess packages (<http://cran.r-project.org/>).

188

189 3. Results

190 3.1. Selection of pig samples and their spatial distribution

191 From the overall collection of 186 *E. multilocularis* isolates from pigs, 55 samples appeared suitable
192 for subsequent analysis of the EmsB profiles (50 fattening pigs and 5 breeding pigs). Suitability
193 selection was based on findings obtained through EMH15/H17 PCR and electrophoresis. The intensity
194 of the expected PCR product band on the agarose gel allowed us to select samples to obtain EmsB
195 electrophoregrams allowing further processing. The samples with the weakest bands were excluded
196 from the analysis (Fig. 1). The final sample collection selection consisted of samples originating from
197 14 Swiss Cantons (Fig. 2A), with 1 to 13 samples per Canton. The collection therefore geographically
198 matches the previous large-scale surveillance study from which the isolates were derived (14 on the
199 18 Cantons) (Meyer et al., 2020).

200 3.2. Hierarchical clustering analysis and spatial distribution of the EmsB profiles

201 The 55 *E. multilocularis* pig isolates were classified into EmsB profiles by using a hierarchical
202 clustering analysis (Fig. 3). The isolates were clustered into a dendrogram together with profiles from
203 123 Swiss human patients, monkeys from Swiss zoos, rodents and foxes from the Canton of Zürich
204 (Fig. 2B). In total, 12 EmsB profiles were generated and clustered within 46 pig isolates. Nine other
205 isolates have been classified outside of any groups (Table 1). The most frequent profiles in pigs were
206 the EmsB profiles P2 (27.3%) present in four Cantons, P5 (12.7%) present in five Cantons, and P7
207 (16.4%) present in seven Cantons. In the whole collection, P2 (19.7%) and P7 (9.0%) were the most
208 frequent profiles among the different hosts. The geographical distribution of the profiles is shown in
209 Fig. 4A for pigs only and Fig. 4B for the whole Swiss collection. The pig samples, all located on the
210 Swiss plateau, were arbitrarily divided into three zones, each with about the same number of
211 genotype samples: the Jura-Western plateau (zone A, n=18), the Central plateau (zone B, n=21), and
212 the Eastern plateau (zone C, n=16). P2 was predominantly distributed among samples in the western
213 part of Switzerland (Cantons of Vaud, Genève, Fribourg, and West of Bern), corresponding to the

214 Jura-Western Swiss plateau (zone A), whereas P5 was mainly distributed on the northwestern part of
215 the country (Cantons of Jura, Aargau, Neuchâtel, Bern, and Solothurn), approximately corresponding
216 to the Central plateau (zone B). P7 was the most widespread profile, distributed within seven
217 Cantons (Aargau, Bern, Solothurn, Thurgau, Nidwalden, Luzern, and Saint Gallen), corresponding to
218 the Central (zone B) and Eastern plateau (zone C), but not in the Western plateau. This geographical
219 description of the three main profiles was also observed in the whole collection, except for P5 found
220 further east in foxes from Zürich.

221 The analysis was completed with the best possible selection of other Swiss samples available
222 (published in the literature) originating from humans, monkeys, rodents, and foxes. In total, 18
223 profiles were described among the 178 isolates, 13 isolates presenting six additional profiles different
224 from those described in pigs. Two human samples previously classified as “out” (Knapp et al., 2020),
225 were now clustered with monkey or fox samples (profiles P13 and P15). All but one (P8) of the
226 profiles described in pigs were shared among pig and human, monkey, rodent, or fox samples (Table
227 2). In the Canton of Fribourg, the profile P2 was found in pigs and the 11 rodent samples. Among
228 pigs, the profile P9 was exclusively described in a pig from the Canton of Ticino and shared with one
229 fox worm from the Canton of Zürich. Among the profiles described in the other hosts, the profile P13
230 was found exclusively in the Canton of Zürich among human, monkey, and fox isolates
231 (Supplementary data S1). The contribution of the host species in the occurrence and sharing of EmsB
232 profiles on the Swiss territory is shown in a Venn diagram (Fig. 5A). Pigs and foxes both presented 12
233 EmsB profiles, and humans, rodents, and monkeys together presented 9 profiles. Two profiles (P3
234 and P6) are shared by all the different hosts. Foxes and pigs shared the largest number of profiles (n
235 = 6). From a zone illustration, zone C represented the most important number of profiles (n=14), with
236 6 profiles described only in this zone, (Table 2 and Fig. 5B).

237 *3.3. Rarefaction curve, richness and diversity analyses of EmsB profiles*

238 For the pig collection, the rarefaction curve did not reach a plateau over all the studied areas and the
239 different subregions (Zones A, B, C) (Fig. 6A and B). The rarefaction curve analysis performed with all
240 the 164 samples with a profile attributed and information about geographical location reached a
241 plateau (Fig. 6C), but considering the 3 zones separately, no plateau was reached (Fig. 6D). Jackknife
242 and Bootstrap estimators are exposed in Table 3 and provide the extrapolated richness in each area.
243 They were all greater than the observed richness values, except for the analysis made on the whole
244 samples from the Swiss plateau. Considering the Swiss pig collection (46 isolates with attributed
245 EmsB profiles), the observed profile richness corresponded to 12 EmsB profiles, and diversity through
246 the inverse Simpson index (1-D) was 0.84 on the whole studied area (Table 3). The observed species
247 richness was 5, 8, and 6 and the diversity 0.51, 0.87, and 0.76 in the zones A, B, and C, respectively.
248 For the whole Swiss sample collection (164 isolates with attributed EmsB profiles), the richness
249 corresponded to 18 profiles, and the (1-D) index 0.90. The species richness was 6, 8, and 14 and the
250 diversity 0.43, 0.87, and 0.89 in the zones A, B, and C, respectively.

251 4. Discussion

252 Domestic pigs are hosts susceptible to infection with *E. multilocularis*. They can be classified, like
253 humans as “dead-end” hosts, because they generally exhibit abortive lesions, and no fertile
254 metacestode tissue has been described in this species so far. Moreover, slaughtered pigs in
255 Switzerland are not predated by dogs or foxes. Nonetheless, they appear as a very suitable species to
256 assess the environmental presence and distribution of parasite eggs. Most of the Swiss pig samples
257 were geographically distributed on the large Swiss plateau, which has also the highest human
258 population density. We propose that this study, encompassing multiple *E. multilocularis* isolates from
259 varying hosts, currently reflects the best way to address the local geographical diversity of the
260 parasite. Although a limited number of samples were studied, tendencies can be highlighted, as
261 demonstrated by the dominant profiles P2, located exclusively on the Jura-Western plateau, and the
262 P7 dispersed in seven Cantons on the Central and Western plateaus. Moreover, the three main

263 profiles did not overlap geographically, highlighting a circulation of the parasite in limited areas,
264 among circumscribed parasite life cycles. To complement the previous study performed by Meyer et
265 al. (2020), the present study goes further in the description of the *E. multilocularis* presence in
266 Switzerland. Indeed, no geographical clusters were previously described within the Swiss plateau,
267 emphasizing a likely homogeneous distribution of the parasite eggs through the Swiss environment.
268 But this genetic approach, studying the EmsB microsatellite, allowed us to investigate the circulation
269 pattern of the parasite and to highlight for the first time local distribution of a profile (profile P2)
270 involving different hosts, both free-roaming and captive. Nevertheless, the origin of some EmsB
271 profiles remains to be investigated, as the profile P8, only described in pigs from two distinct
272 Cantons, and the origin of the contamination occurring in a pig from the Canton of Ticino (profile P9),
273 clustered with a fox worm from the Canton of Zürich.

274 Different hosts have to be taken into consideration to characterize the genetic diversity of *E.*
275 *multilocularis* in a given study area. Pigs and foxes are largely involved in the EmsB profile diversity
276 illustration, as seen in the Venn diagram. Based on the research of profiles and the rarefaction curve
277 analysis findings, this present study contributes to expand knowledge of the complete EmsB genetic
278 diversity of a given area. Additional efforts must be undertaken, such as active genetic surveillance
279 that could markedly contribute to enriching existing databases. Moreover, from a technical point of
280 view, a better screening method has to be developed to select the best suitable samples for analysis,
281 as a dedicated RT-qPCR technique or a ddPCR techniques, in order to assess the parasite DNA
282 quantity. For a better follow-up of the parasite geographical distribution, precise coordinates are also
283 important to collect. A better recruitment would be particularly important for the Canton of Zürich,
284 where 14 profiles were described in this study, with both local profiles and over-dispersed profiles.
285 The analysis was conducted on identified EmsB profiles, a profile in our definition clustering at least
286 two isolates. Considering all the available Swiss samples, the maximum richness seems to be reached
287 within the Swiss territory. However, samples with no profile identified in the present study could
288 present rare profiles which remain to be characterized. Indeed, EmsB profiles previously described in

289 humans in single specimens were clustered here with lesions from other host species. Even if a
290 sample is not clustered with another, it remains important to keep the genotyping information in a
291 database for further investigations and strive to get closer to the completeness of *E. multilocularis*
292 genetic diversity. With additional efforts in sampling and genotyping, combined with more precise
293 geographical information, the population structure of the parasite in Switzerland and in Europe could
294 be further studied in order to trace back the evolution history of the parasite.

295

296 5. Conclusion

297 The investigation of a captive host to study the genetic diversity of the parasite *E. multilocularis*
298 allowed us to highlight local or micro-local circulation of the parasite, dispersed by foxes but also
299 potentially by feeding grass, harvested either from the farm or bought from agricultural suppliers.
300 This should be taken into consideration in terms of the dispersion of the parasite when drawing up
301 prevention strategies for professional and public health information.

302

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305 University Hospital of Besançon. Special thanks to participating Swiss farms, slaughterhouse staff,
306 veterinarians, and meat inspectors.

307

308 Figure legends.

309 Table 1. EmsB profiles described among the 55 *Echinococcus multilocularis* isolates from Swiss pigs.

310

311 Table 2. Number of samples with the EmsB profiles described among the 178 *Echinococcus*
312 *multilocularis* isolates from Swiss pig, human, monkey, rodent and fox specimens and number of
313 samples per zone, number of identified EmsB profiles per zone in brackets; n.d. for no data on the
314 geographical location.

315

316 Table 3. Sample size, number of observed and extrapolated richness in EmsB profile, and genetic
317 diversity in *Echinococcus multilocularis* isolates from Swiss pigs, humans, monkeys, rodents and
318 foxes.

319

320 Fig. 1. Analysis of *Echinococcus multilocularis* DNA amplification for the EMH15/H17 PCR products
321 (mtDNA 12S target) on agarose gel (1.5% agarose, tris-acetate-EDTA 1X) for the selection of EmsB
322 amplification, lanes 1 to 11 (1: 18D032, 2: 17D2659, 3; 17D4939, 4: 18D1021, 5: 18D0390*, 6:
323 18D0392*, 7: 18D0394*, 8: 18D0398, 9: 18D0466, 10: 17D4371, 11: 18D0470*; *samples not
324 retained for the analysis). M for size marker. Examples of EmsB fragment size analysis obtained for
325 three isolates amplified on the mtDNA 12S target in lane 1 (isolate 18D0302 retained for the
326 analysis), 5 (isolate 18D0390 with an unexpected profile, according to previous EmsB studies, was
327 excluded from the analysis) and 11 (isolate 18D0470 without EmsB amplification). On
328 electrophoregram, the x-axis for size of peaks in bp, the y-axis for fluorescence intensity.

329

330 Fig. 2. Geographical distribution of the 178 Swiss alveolar echinococcosis isolates, (A) the 55
331 domestic Swiss pigs by farm location, (B) the 178 Swiss isolates, including the 55 isolates from pigs
332 and 123 from humans, rodents, monkeys and foxes. Canton codes: AG: Aargau, BE: Bern, GE:
333 Genève, FR: Fribourg, JU: Jura, LU: Luzern, NE: Neuchâtel, NW: Nidwalden, SG: Saint-Gallen, SO:
334 Solothurn, TG: Thurgau, TI: Ticino, VD: Vaud, VS: Wallis, ZH: Zürich. Zones defined for the rarefaction
335 curve analysis, A: Jura-Western plateau, B: Central plateau, C: Eastern plateau.

336

337 Fig. 3. Dendrogram constructed from EmsB amplification data from the Swiss pig isolates, Swiss AE
338 patients, captive monkeys from Swiss zoos, rodents from the Canton of Fribourg and *Echinococcus*
339 *multilocularis* worms from Zürich foxes by hierarchical clustering analysis on an Euclidean distance
340 and unweighted pair group method. Approximately unbiased (au) p-values are indicated at tree
341 nodes as percentages calculated by multiscale bootstrapping (B = 1000). *Echinococcus granulosus*
342 sensu stricto (G1 strain, originating from an Algerian sheep) was used as a control outgroup. A
343 genetic distance threshold of 0.1 was used to distinguish EmsB profiles. A representative EmsB
344 electrophoregram from pig isolates is provided for each profile.

345

346 Fig. 4. Geographical distribution of the EmsB profiles of *Echinococcus multilocularis* lesions from the
347 55 EmsB genotyped Swiss pigs (A) and from the whole 178 EmsB genotyped Swiss specimen
348 collection (B). *no data available on the geographical position, the isolates were positioned with the
349 geographical coordinates of Switzerland (centroid of the country).

350

351 Fig. 5. Venn diagram of EmsB profiles from host (A): pigs, foxes, and the group humans, monkeys,
352 and rodent (sample size in brackets) and by geographical zone (B).

353

354 Fig. 6. Richness and diversity accumulation analysis. (A) analyzes performed on the Swiss pigs isolates
355 only (n=46 samples with a profile attributed) on the whole Swiss territory, and (B) on the three zones
356 in the Swiss plateau. (C) analyzes performed on the human isolates (n=21 samples with a profile
357 attributed), monkey isolates (n=2 samples with a profile attributed and information about
358 geographical location), rodent isolates (n=11 samples with a profile attributed), and fox isolates
359 (n=84) on the whole Swiss territory, and (D) on three zones in the Swiss plateau.

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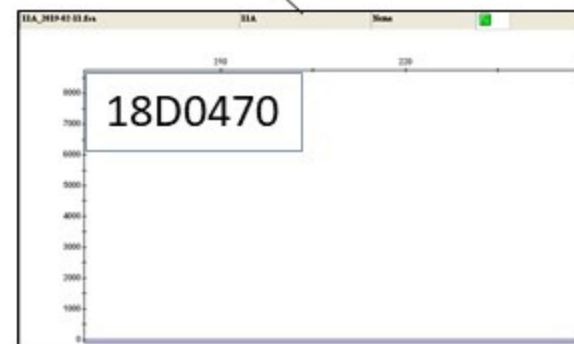
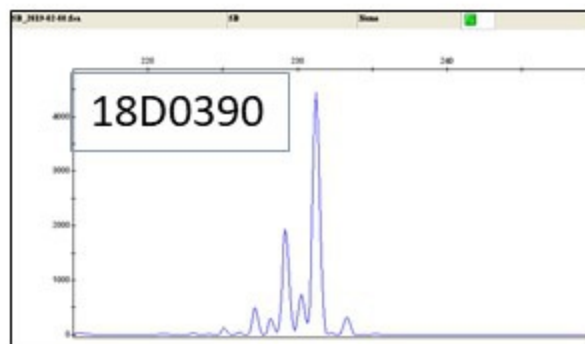
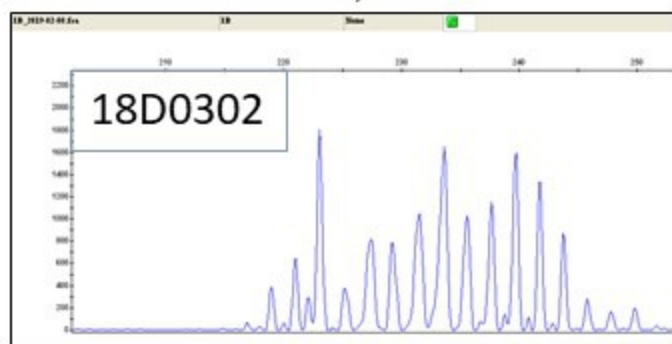
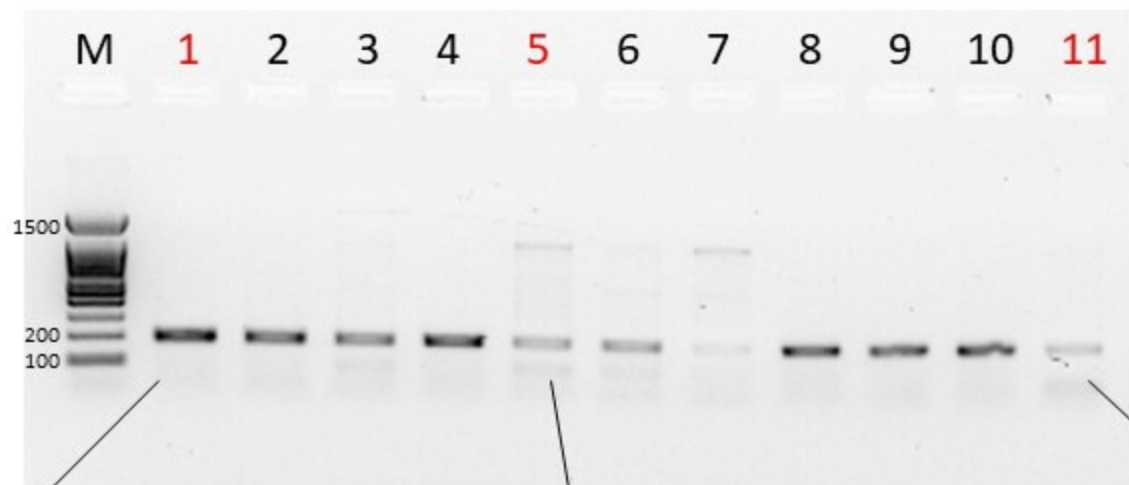
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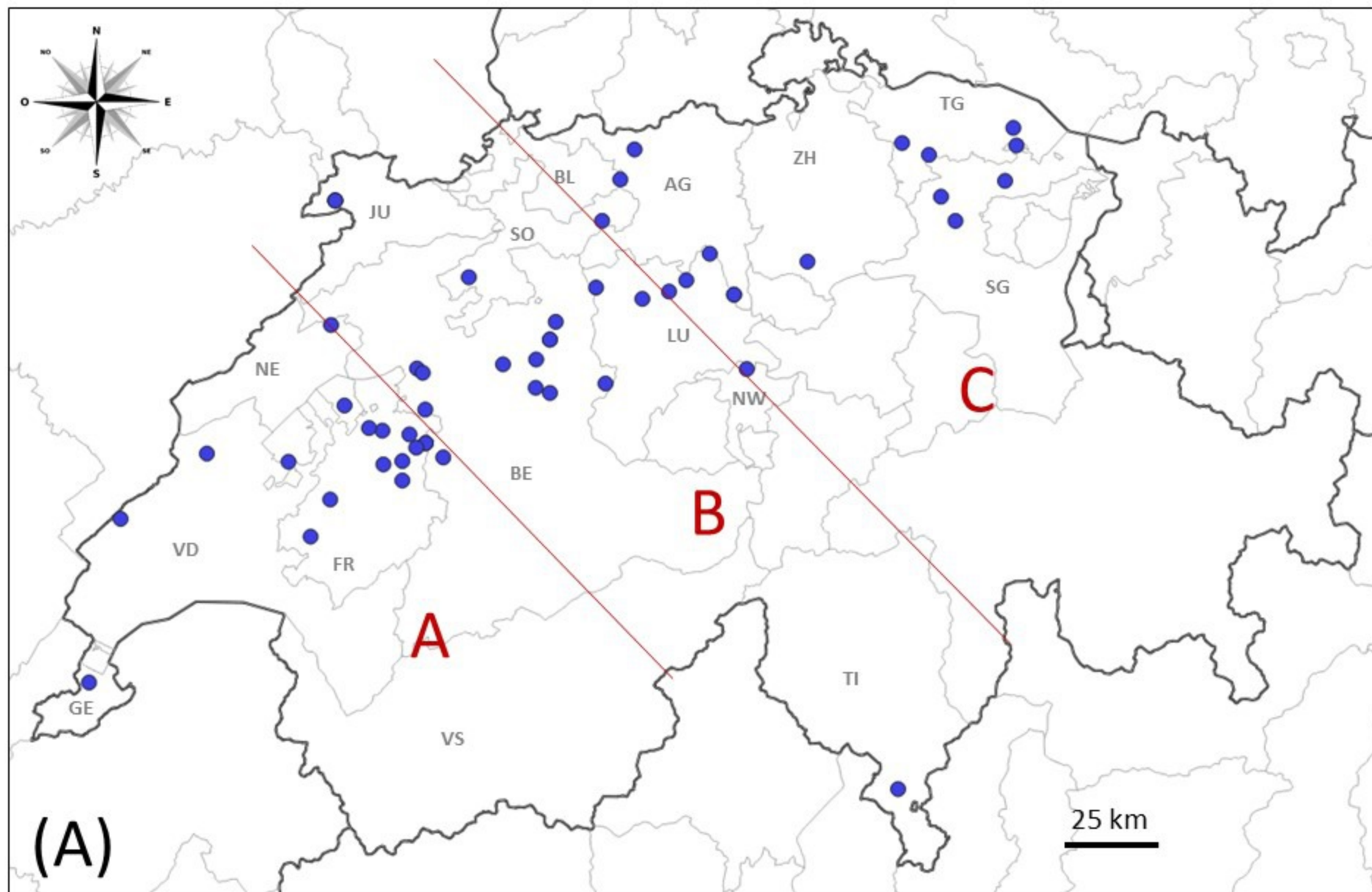
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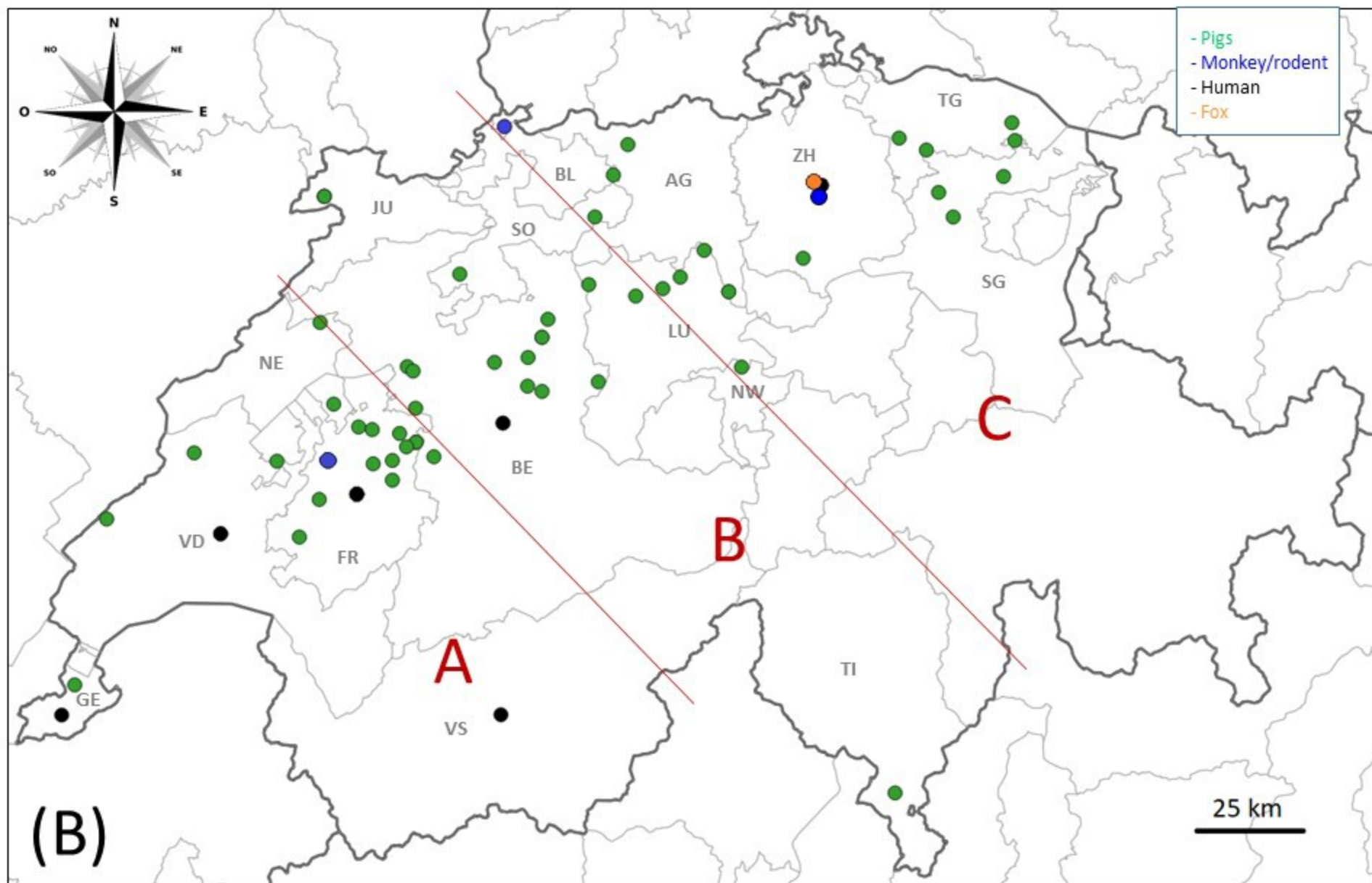
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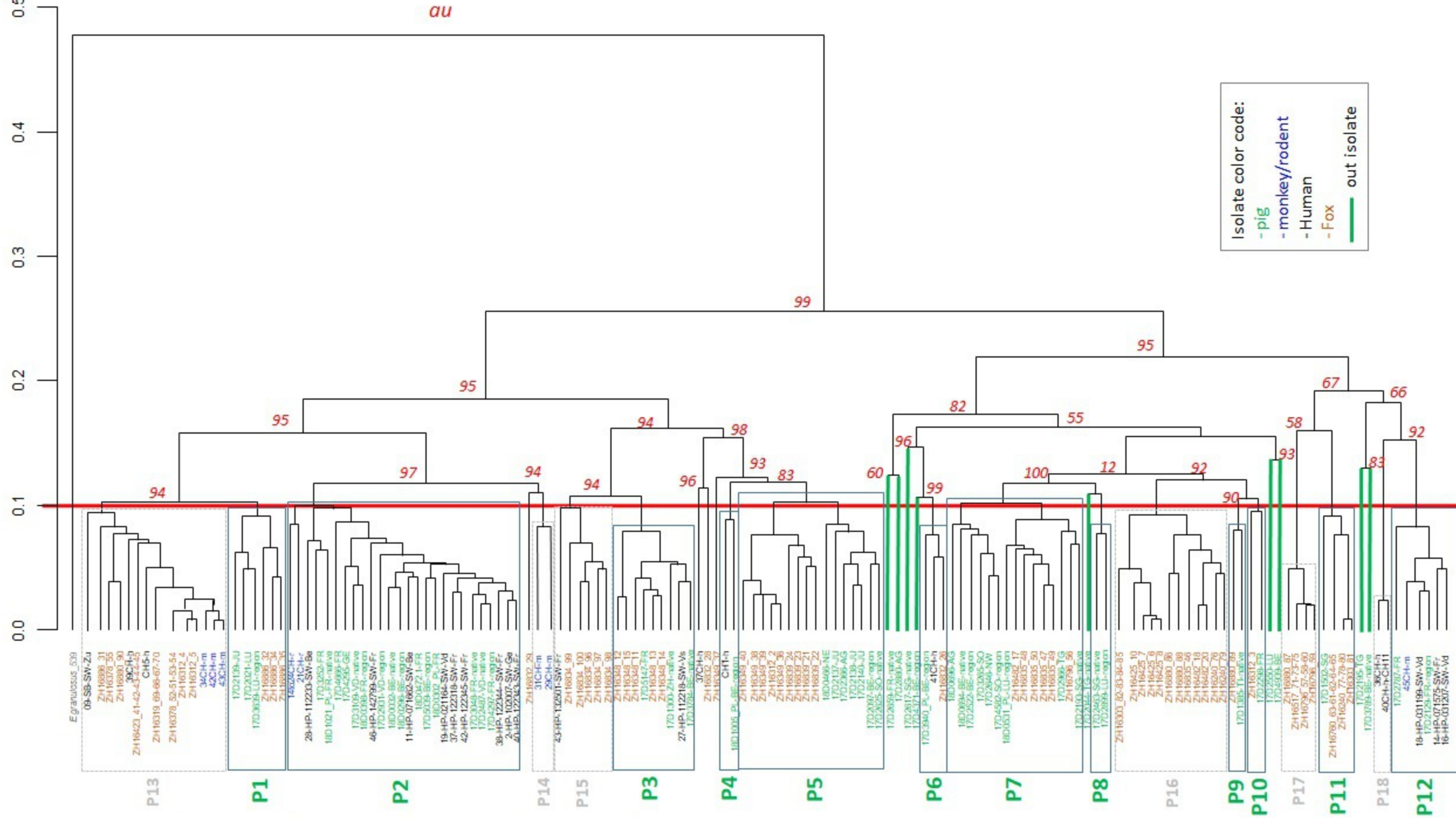
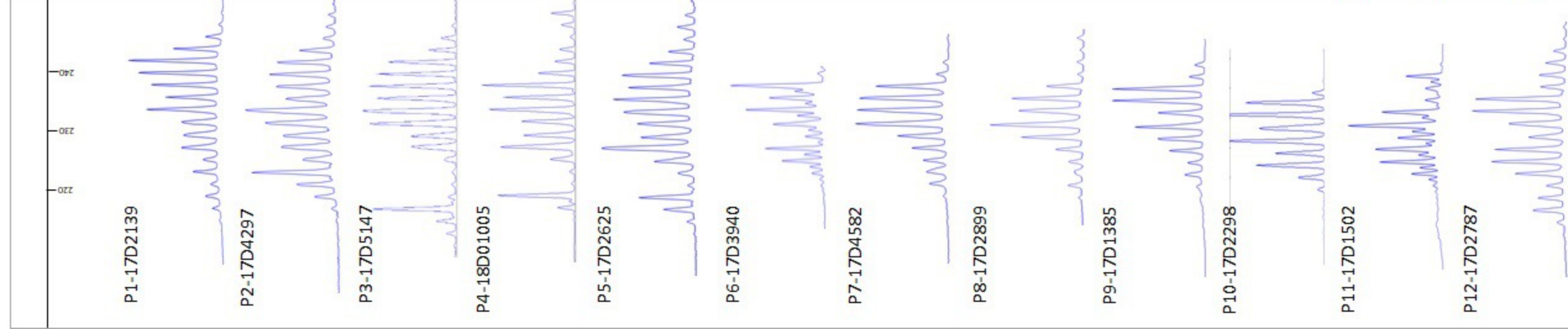
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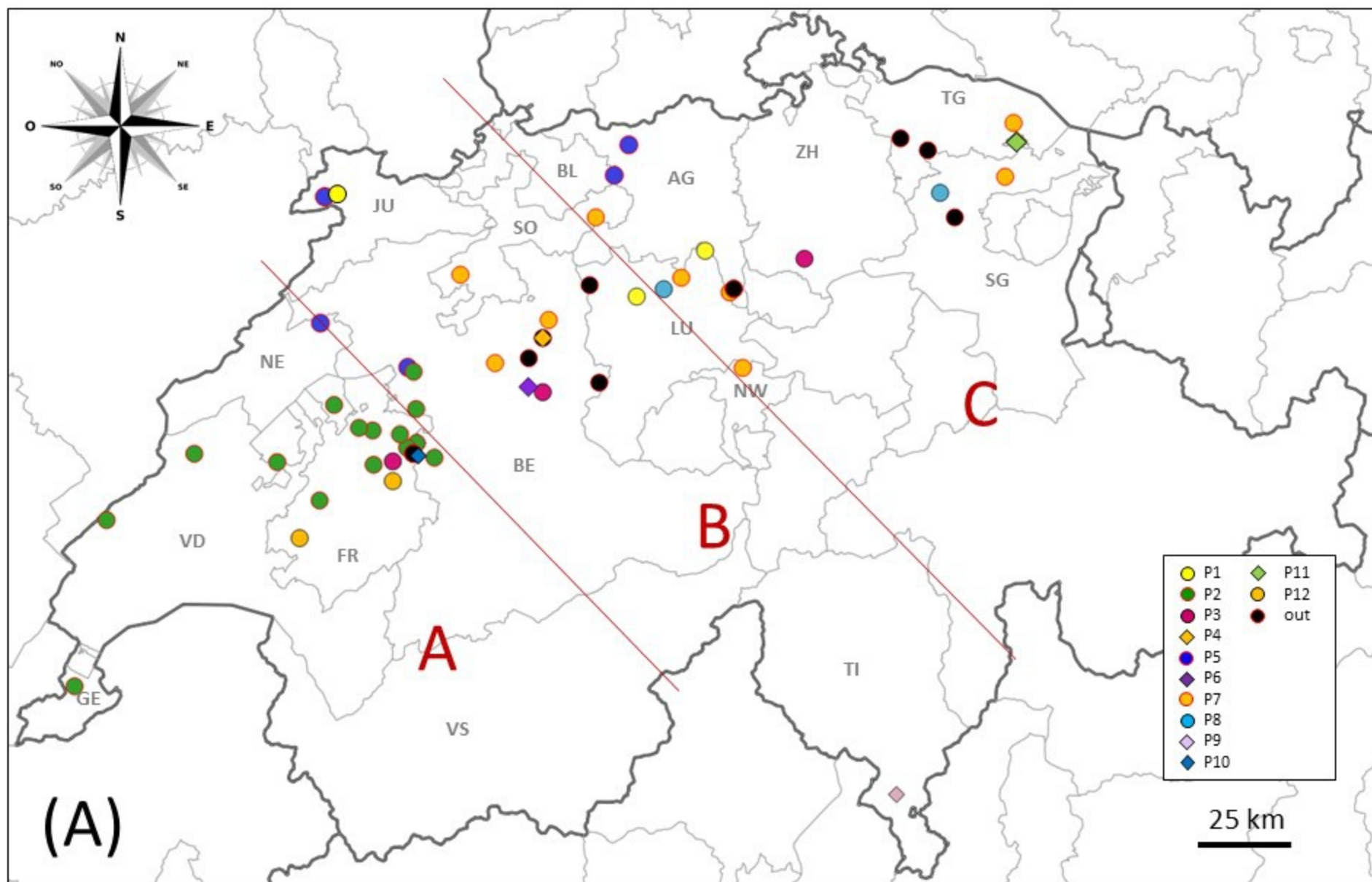


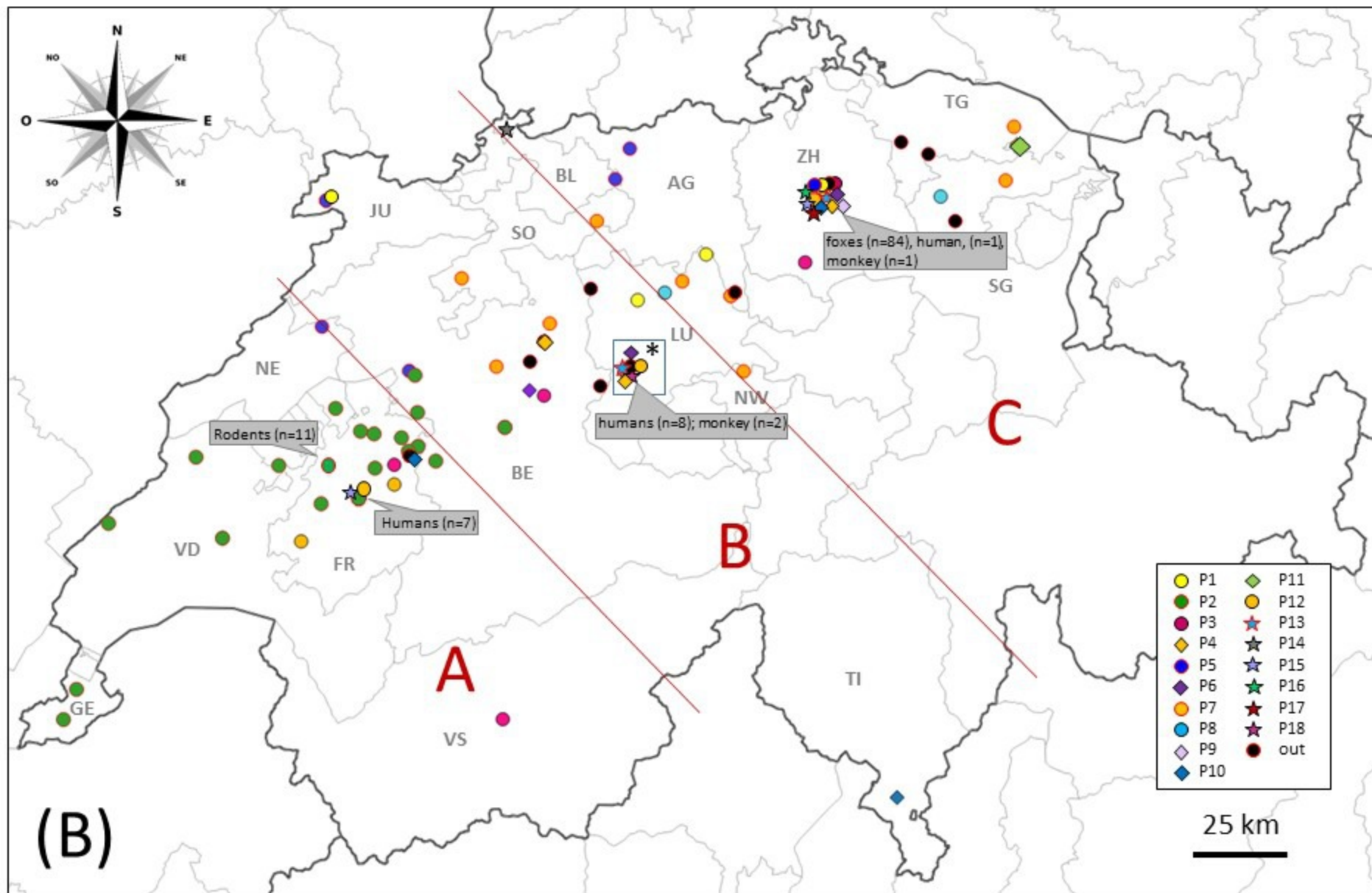




Fragment size (bp)

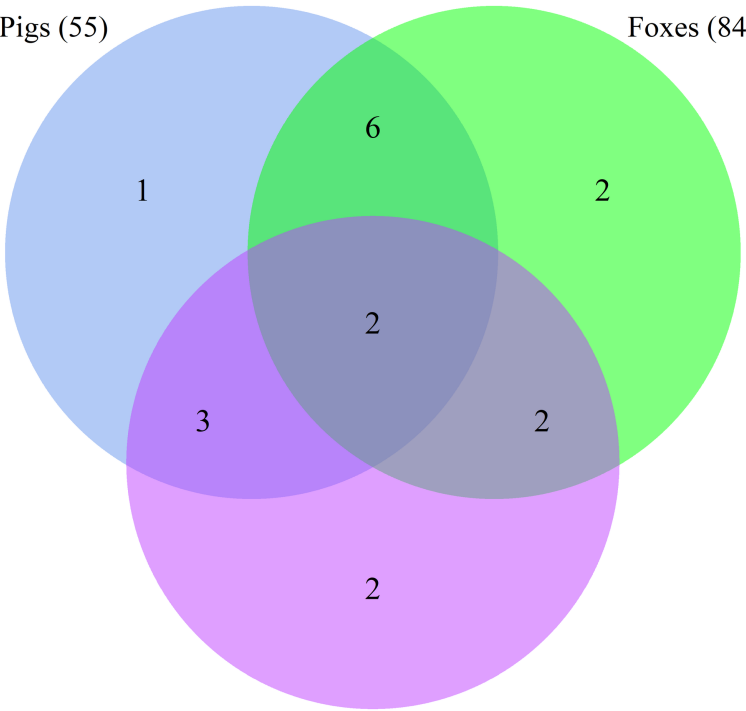






Pigs (55)

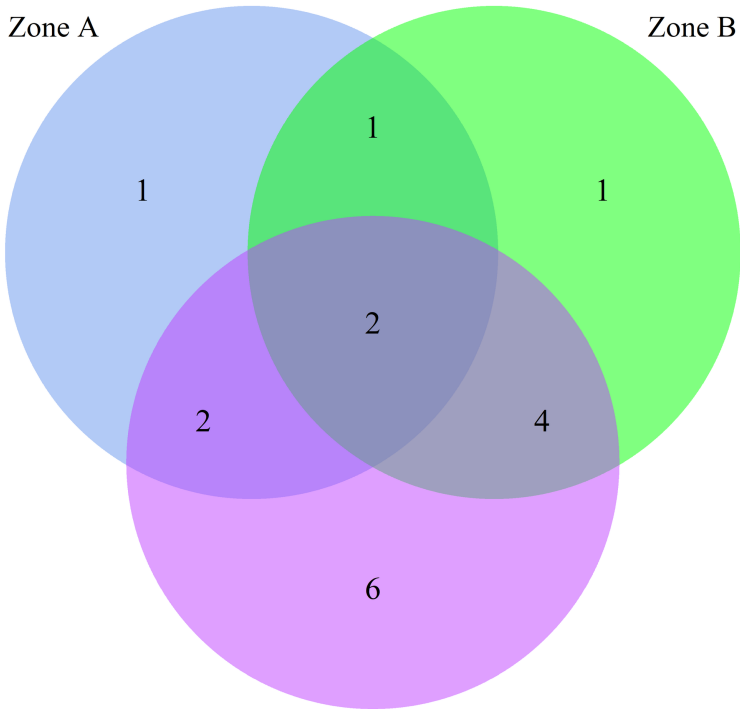
Foxes (84)



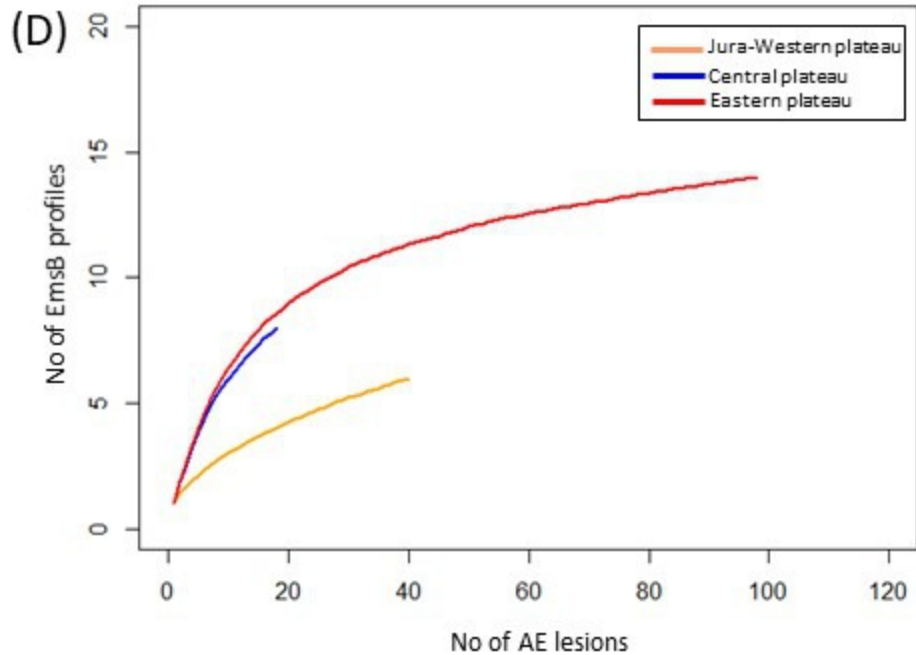
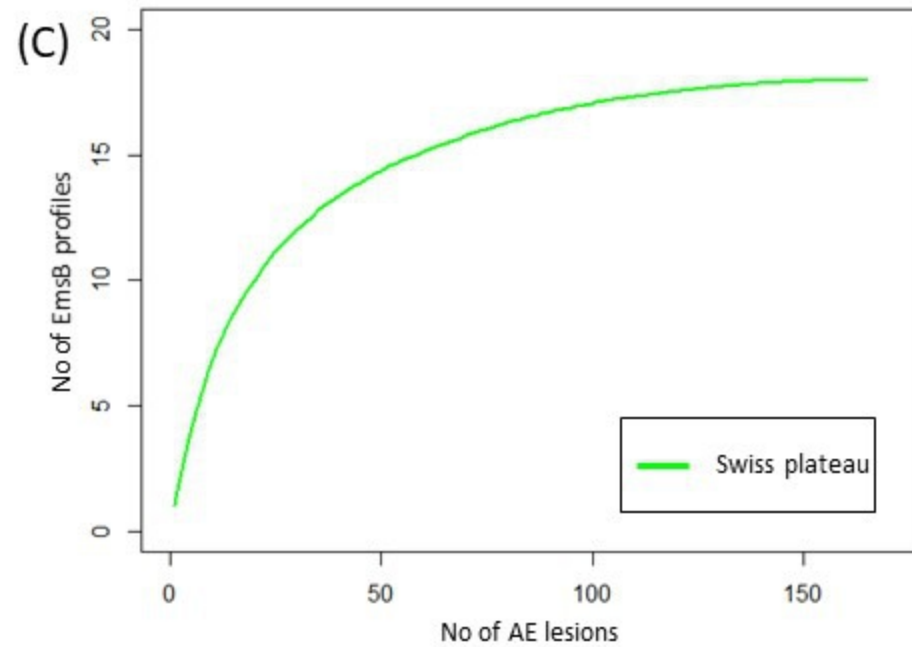
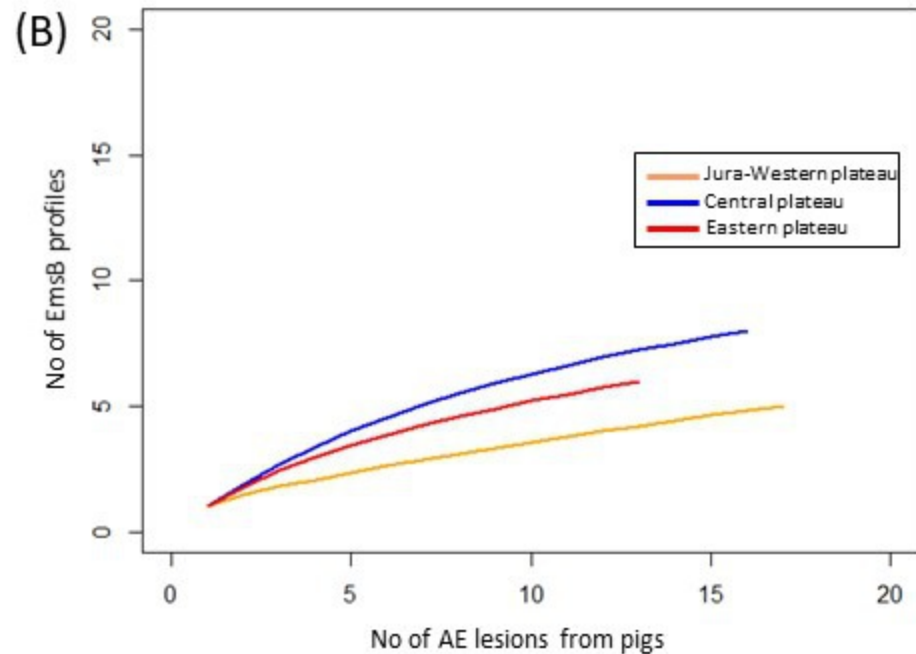
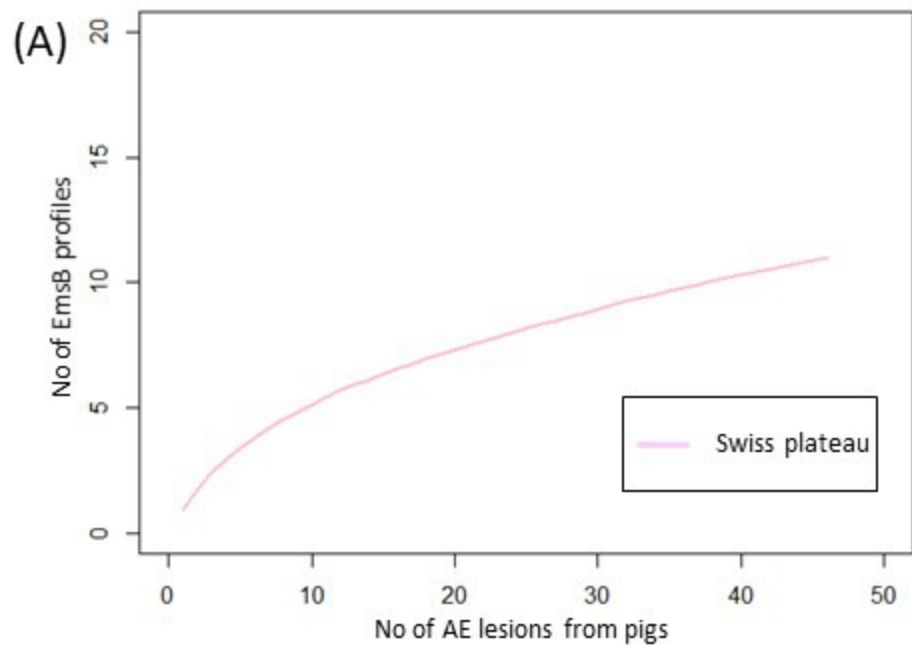
Monkeys/Humans/Rodents (39)

Zone A

Zone B



Zone C



1 Table 1. EmsB profiles described among the 55 *Echinococcus multilocularis* isolates from Swiss pigs.

2

Zone*	A	A	A	A	B	B	B	B	C	B	C	C	C	C	C	C	No. of isolate per Canton (%)
EmsB profile/Canton**	GE	VD	FR	NE	BE	JU	TI	SO- W	SO- E	LU- W	LU- E	NW	AG	ZH	SG	TG	
P1	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	3 (5.5%)
P2	1	3	8	0	3	0	0	0	0	0	0	0	0	0	0	0	15 (27.3%)
P3	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	3 (5.5%)
P4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1 (1.8%)
P5	0	0	0	1	1	3	0	1	0	0	0	0	1	0	0	0	7 (12.7%)
P6	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1 (1.8%)
P7	0	0	0	0	2	0	0	1	1	0	1	1	1	0	1	1	9 (16.4%)
P8	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2 (3.6%)
P9	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1 (1.8%)
P10	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.8%)
P11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1 (1.8%)
P12	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2 (3.6%)
out	0	0	1	0	3	0	0	0	0	1	0	0	1	0	1	2	9 (16.4%)
Total	1	3	13	1	12	4	1	2	1	2	3	1	3	1	4	3	55 (100%)

3 * The Swiss plateau was divided into 3 zones, A: Western plateau, B: Central plateau and C: Eastern
4 plateau.

5 ** Swiss Canton codes: AG: Aargau, BE: Bern, GE: Genève, FR: Fribourg, JU: Jura, LU: Luzern, NE:
6 Neuchâtel, NW: Nidwalden, SG: Saint-Gallen, SO: Solothurn, TG: Thurgau, TI: Ticino, VD: Vaud, ZH:
7 Zürich. Zones defined for the rarefaction curve analysis, A: Jura-Western plateau, B: Central plateau,
8 C: Eastern plateau.

9

Table 2. Number of samples with the EmsB profiles described among the 178 *Echinococcus multilocularis* isolates from Swiss pig, human, monkey, rodent and fox specimens and number of samples per zone, number of identified EmsB profiles per zone in brackets; n.d. for no data on the geographical location.

EmsB profile	Host					Total No. of isolates (%)	Zone* - No of samples (No of profiles)			
	pig	human	monkey	rodent	fox		A	B	C	n.d.
P1	3	0	0	0	3	6 (3.4%)	0	2	4	0
P2	15	9	0	11	0	35 (19.7%)	30	5	0	0
P3	3	1	0	0	5	9 (5.1%)	2	1	6	0
P4	1	1	0	0	0	2 (1.1%)	0	1	0	1
P5	7	0	0	0	9	16 (9.0%)	1	4	11	0
P6	1	1	0	0	1	3 (1.7%)	0	1	1	1
P7	9	0	0	0	6	15 (8.4%)	0	3	12	0
P8	2	0	0	0	0	2 (1.1%)	0	0	2	0
P9	1	0	0	0	1	2 (1.1%)	0	1	1	0
P10	1	0	0	0	1	2 (1.1%)	1	0	1	0
P11	1	0	0	0	9	10 (5.6%)	0	0	10	0
P12	2	3	1	0	0	6 (3.4%)	5	0	0	1
P13	0	4	2	0	18	24 (13.5%)	0	0	20	4
P14	0	0	2	0	0	2 (1.1%)	0	0	2	0
P15	0	1	0	0	5	6 (3.4%)	1	0	5	0
P16	0	0	0	0	15	15 (8.4%)	0	0	15	0
P17	0	0	0	0	8	8 (4.5%)	0	0	8	0
P18	0	2	0	0	0	2 (1.1%)	0	0	0	2
out	9	1	0	0	3	13 (7.3%)	1	4	7	1
Total	55	23	5	11	84	178 (100%)	41 (6)	22 (8)	105 (14)	10 (5)

* The Swiss plateau was divided into 3 zones, A: Western plateau, B: Central plateau and C: Eastern plateau.

Table 3. Sample size, number of observed and extrapolated richness in EmsB profile, and genetic diversity in *Echinococcus multilocularis* isolates from Swiss pigs, humans, monkeys, rodents and foxes.

Collection	Zone*	No. of samples	No. of profiles (observed richness)	Total No. of profiles estimated by Jackknife 1st order (std. error)	Total No. of profiles estimated by Bootstrap order (std. error)	Simpson diversity (1-D)
Swiss pigs	Swiss plateau	46	12	16.89 (2.19)	14.17 (1.15)	0.84
	A	17	5	7.82 (1.63)	6.19 (0.84)	0.51
	B	16	8	11.75 (1.88)	9.62 (0.94)	0.87
	C	13	6	8.77 (1.60)	7.29 (0.85)	0.76
Whole Swiss samples	Swiss plateau	165	18	18 (0)	18.86 (0.86)	0.90
	A	40	6	8.93 (1.69)	7.22 (0.88)	0.43
	B	18	8	11.78 (1.89)	9.60 (0.95)	0.87
	C	98	14	16.97 (1.71)	15.39 (0.96)	0.89

* The Swiss plateau was divided into 3 zones, A: Western plateau, B: Central plateau and C: Eastern plateau.

