1 **Species turnover between age groups of horses and positive network of co-**

2 **occurrences define the structure of horse strongylid communities: a meta-**

3 **analysis**

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19   **Abstract**

20   Grazing horses are infected by a wide range of strongylid species mostly located in the large intestine.

21   Despite their impact on equine health and the emergence of drug resistant isolates, the phenology of

22   these nematodes has been poorly characterized and the rules structuring their assembly as a

23   community are not understood. Here, we compiled data on 46 equine strongylid species collected

24   worldwide at the regional or horse scales (upon deworming or after necropsy) to analyse their richness,

25   diversity and associated factors of variation. Worldwide, twelve species from the *Cylicocyclus* (n = 4),

26   *Cylicostephanus* (n = 3), *Coronocyclus* (n = 2) and *Cyathostomum* (n = 2) genera were found in at least

27   75% of sites. Geoclimatic conditions had a limited effect on strongylid communities, but reduced

28   species richness was found under the temperate European area. The recovery method did not affect

29   species richness and differences on the temporal and sampling effort scales between studies applying

30   either methods underpinned heterogeneous variances in community diversity.

31   At the horse level, rarefaction curves correlated poorly to parasite egg excretion, suggesting little

32   contribution of community diversity to this trait. Using a diversity partitioning approach, we found that

33   within-host diversity represented half of overall diversity underscoring the importance of host density

34   and environmental contamination to the diversity of strongylid communities. While this is expected to

35   erase diversity across communities, species turnover between age classes was the second most

36   important contributor to overall diversity (23.9%). This was associated with a network of positive co-

37   occurrences between the four most prevalent genera that we resolved at the anatomical niche level.

38   Altogether this pattern of β-diversity maintenance across age classes combined with positive co-

39   occurrences may be grounded by priority effects between major species. Our findings set the first

40   assembly rules of equine strongylid communities.

41   **Keywords**: community, co-occurrence, diversity, horse, parasite, strongylid

42 1. **Introduction**

43 Grazing horses are naturally infected by a diverse parasitic fauna including Strongylidae (Taylor et al.,

44 2012). They encompass subfamilies Cyathostominae and Strongylinae (Lichtenfels et al., 2008). The

45 use of anthelmintics has resulted in prevalence levels for *Strongylus* sp. that has the highest fatality

46 rate among Strongylinae (Herd, 1990), but has led to the emergence of resistant cyathostomin isolates

47 worldwide (Matthews, 2014). affects horse growth and the massive emergence of encysted larvae can

48 lead to fatal cases of cyathostomosis, the main cause of parasite mortality in young horses in

49 Normandy, France (Sallé et al., 2020). Despite their importance in veterinary medicine, the phenology

50 and the rules structuring the assembly of remain largely undefined due in part to the complexity of

51 collecting data from large mammalian hosts under experimental conditions.

52 Inter-individual variation exists in host susceptibility to infection, with significant variation across age

53 groups (Debeffe et al., 2016; Kornaś et al., 2015; Relf et al., 2013; Wood et al., 2013). In addition,

54 climatic conditions affect the development of the free-living stages on pastures (Nielsen et al., 2007)

55 and species reappearance following anthelmintic drug treatment varies across species (Kooyman et

56 al., 2016) suggesting that combinations of their phenology or genetic diversity increase their fitness.

57 Despite knowledge, variations in community structure in anatomical niches or environmental conditions

58 remain largely uncharacterised.

59 Better defining the patterns of variation in helminth communities could however greatly improve their

60 management in the field, i.e. by preventing the emergence of subdominant species following

61 elimination of more dominant ones (Lello et al., 2004) or anticipating consequences of infections by

62 other pathogens (Brosschot et al., 2021; Sweeny et al., 2020; Telfer et al., 2010). Data on helminth

63 community assembly have been gathered across a wide range of host species including humans

64 (Tchuem Tchuenté et al., 2003), rabbit (Lello et al., 2004), rodent (Behnke, 2008; Dallas et al., 2019),

65 fishes (Poulin and Valtonen, 2002) with evidence of significant interactions between parasites. In

66 horses, community structure has been determined from necropsy reports or after deworming(Gawor,

67 1995; Kornas et al., 2011; Kuzmina et al., 2005; Kuzmina et al., 2011; Kuzmina et al., 2016; Lind et al.,

3

68 2003; Mfitilodze and Hutchinson, 1990; Ogbourne, 1976; Reinemeyer et al., 1984). However, these

69 reports remain often descriptive and limited exploration of the relationship between community

70 structure and host and environmental variables been conducted. For instance, variation in species

71 richness and diversity along the intestinal tract of horses has been reported (Collobert-Laugier et al.,

72 2002; Gawor, 1995; Ogbourne, 1976) and recent meta-analysis reported the impact of parasite

73 recovery method and geographical region on cyathostomin abundance or prevalence (Bellaw and

74 Nielsen, 2020). These studies however did not address how community structure, richness and

75 diversity varied with environmental factors of interest. In addition, co-occurrence patterns between

76 equine parasite species remain poorly characterized with contradictory findings reported (Sallé et al.,

77 2018; Stancampiano et al., 2010).

78 Here, we performed an exhaustive literature search to evaluate how equine strongylid communities

79 were shaped by environmental and host factors. In addition, we re-analyzed community data gathered

80 from individual horses to partition diversity across environmental factors and to build co-occurrence

81 networks at the anatomical niche level. Altogether, our results expand previous reports while opening

82 new perspectives on the parasite composition and structure.

83

84 2. **Materials and methods**

85 The python and R scripts used to collect and analyse the data and associated datasets will be

86 deposited at: https://github.com/MichelBoisseau37/script_meta_analyse   upon   manuscript

87 acceptance.

88

89 **2.1. Literature search and inclusion criteria**

90 Studies were gathered from the Medline database (https://pubmed.ncbi.nlm.nih.gov/) using "strongyl*

91 horse" as keywords, yielding a database of 1,032 articles. Article titles and abstracts were

92 subsequently screened for particular terms including all possible combinations of the following:

93 "intensity", "Intensity", "Abundance", "abundance", "Prevalence", "prevalence", "communit",

94   "Communit", "Helminth" or "helminth". To identify studies reporting strongylid burden, we also

95   included the main genera ("*Cylicocyclus*", "*Cyathostomum*", "*Cylicostephanus*") as keywords. This

96   filtering step retained 300 papers. For these, titles and abstracts were read to determine whether

97   findings of strongylid species prevalence and abundance would be available.

98   To broaden our database, we also run manual search on google (https://scholar.google.com/) using

99   the keywords "prevalence abundance Cyathostominae horses" and "prevalence abundance

100   Strongylidae horse". This query identified six additional studies. Additional studies were identified after

101   inspection of the references cited in three papers (Gawor, 1995; Ogbourne, 1976) that yielded

102   prevalence data from 13 additional studies.

103   To be included in the meta-analysis, reported articles had to meet several criteria. First, studies were

104   included in the dataset if they reported parasite prevalence (proportion of infected host), abundance

105   (the number of parasite individuals per host, including uninfected individuals) or relative abundance.

106   Second, published data were only included if the number of horses sampled was reported.

107   Finally, 39 papers met the aforementioned selection criteria (Supplementary Table 1). Among these, 16

108   papers presented both abundance and prevalence data, while others either only reported abundance

109   (n = 5) or prevalence (n = 18) data. Papers from Africa (n = 2) and Australia (n = 3) were included to

110   determine average relative abundance and prevalence across region-based studies but were not

111   considered in any further analyses as too few replicates were available to test for environmental effects.

112   For analytical purposes, papers were further divided into studies to account for the distinct

113   experimental groups considered. This process yielded 69 community sets for 46 species encountered

114   in at least one study.

115

116   **2.2. Parasite, environmental and host variables**

117   When available, metadata related to environmental factors were collected. Horse age was not

118   considered for the continent-wide study as only scarce and inaccurate data were available. The same

119   applied for horse breed or management type (wild or managed horses) that were reported in three

120 studies only. Following edition, information regarding continent, country, sampling effort (number of

121 horses sampled), the recovery method used (necropsy or deworming) were included.

122 Climate conditions were determined using the Köppen-Geiger climate classification

123 (http://hanschen.org/koppen/#data) matching the sampling area. The Köppen-Geiger climate

124 classification is encoded by a three- or two-letter system corresponding to the type of climate, rainfall

125 pattern and temperature range respectively. For studies with no precise location indicated, the closest

126 main city (state or country capital city) coordinates were used as a proxy (Supplementary Table 1). The

127 different climates were clustered into broad climatic categories, namely continental, tropical, temperate

128 and arid.

129

130 **2.3. Statistical analysis**

131 Data were analysed with R v. 3.6.3 and v. 4.0.2.

132 2.3.1. Analysis of worldwide strongylid community diversity

133 2.3.1.1. Considered regional community sets

134 Prevalence and relative abundance matrices were merged together into a single presence/absence

135 matrix (noted 0 and 1 for absence and presence respectively). Analyses were run on the species

136 presence/absence matrix for two reasons. First, this approach makes use of every available study (no

137 matter of the reported measure) thereby providing greater statistical power than an approach

138 considering abundance or prevalence data only. Second, most studies ($n = 29$ out of the 39 considered)

139 did not report any dispersion parameter of parasite abundance and prevalence preventing any

140 implementation of meta-analysis frameworks. In addition, because count data usually follow a negative

141 binomial distribution, reported arithmetic mean values can be poor descriptors of the parasite

142 population. This also speaks against sound estimation.

143 Because of the data structure, geoclimatic conditions (aggregated continent and climate zone) and the

144 recovery method (deworming or necropsy) were confounded. In addition, some conditions were under-

145 represented (less than five community sets) and not considered further. This included the community

6

146   sets from Asia (n = 1), Australia (n = 3), or sets from temperate Europe collected after deworming (n =

147   3).

148   As the deworming strategy was only applied in Europe, a continental European subset was considered

149   (*n* = 33 communities) to evaluate how the recovery method could affect the strongylid diversity.

150   Similarly, the geoclimatic zone effect was tested on communities recovered under the same necropsy

151   framework leaving 30 community sets that fell in temperate America (n = 8), tropical America (n = 7),

152   temperate Europe (n = 11) or continental Europe (n = 5).

153

154   2.3.1.2.   Diversity analyses

155   Species richness was taken as an α-diversity estimator, and the β-diversity index was estimated with

156   the Jaccard index using the vegan R package. (version2.5-7). Inter-sample distances were visualized

157   using the Non-Metric Scaling (NMDS) ordination method through the "metaMDS" function in the vegan

158   R package. This method generated fictitious axes maximizing the variance and allowing an optimal

159   visualization of distances. NMDS is the most appropriate representation because it minimizes

160   dissimilarities between similar communities and vice versa for more remote communities. NMDS was

161   run on the whole set of communities to establish how environmental factors (aggregated factor of the

162   geoclimatic zone and the recovery method), the sampling effort (number of horses) and the year of

163   publication were related to community structures.

164   A permutational multivariate analysis of variance (PERMANOVA) using distance matrix (adonis function

165   of the vegan R package) was then applied to identify the factors that would influence most the

166   community structure and to obtain the percentage of variance explained by the factors of interested,

167   i.e. sampling effort (number of horses), the geoclimatic zone and the recovery method using

168   appropriate subsets to control for environmental conditions.

169   The respective effects of the recovery method (tested on the European subset) or the geoclimatic area

170   (tested on the necropsy-based subset) on species richness were estimated using a linear model, fitting

171   sample size, year of publication and the factor of interest as fixed effects.

172

173   2.3.1.3. Effect of environmental factors on parasite prevalence across region-

174   based community sets

175 Last, we aimed to identify strongylid species whose presence or absence in a given set would be

176 associated with variation in the recovery method or the geoclimatic area. This was applied to the

177 aforementioned community subsets to account for the data structure. In each case, the presence or

178 absence of worm strongylid species was modelled using a logistic regression model, fitting an

179 interaction term between worm species and either the recovery method (European continental subset)

180 or the geoclimatic area (necropsy-based data). Because quasi-complete separation of species with

181 environmental factors of interest occurred (identified with the "detect_separation" function of the

182 *detectseparation* v0.1 package), we restricted this analysis to ten species. In addition, model

183 convergence could not be achieved in case the community set was fitted as a random effect. To deal

184 with this while accounting for inter-sets variation, we fitted the year of publication, sampling effort and

185 the species richness of the community as covariates.

186

187   2.3.2. Analysis of diversity at the horse level

188 These analyses were run on horse-based strongylid community data from three published studies,

189 either collected after the necropsy of 46 horses in France (Collobert-Laugier et al., 2002) or the

190 deworming of 48 horses in Poland (Sallé et al., 2018) and or 197 horses Ukraine (Kuzmina et al., 2016)

191 respectively.

192

193   2.3.2.1. Relationship between diversity and faecal egg count at the horse level

194 To evaluate how strongylid community diversity could relate to measured Faecal Egg Count (FEC) in

195 their host, we estimated Spearman's correlations between FEC and species richness, or FEC and the

196 Gini-Simpson index which corresponds to the slope of the species accumulation curve at its basis and

8

197 relates to species dominance (Chase and Knight, 2013). This was not done on the necropsy data as

198 FEC measures were not available.

199

200   2.3.2.2.   Impact of horse age on strongylid community diversity

201 To study the interactions between strongylid species within their hosts, we re-analyzed individual

202 necropsy report data collected in Normandy (Collobert-Laugier et al., 2002). Abundances of 20

203 strongylid species and other non- strongylid species (*Anoplocephala* sp*., Oxyuris sp*.) were quantified

204 within the caecum, ventral- and dorsal-colon of 46 horses. The data from 36 horses that had their age

205 registered and were infected by at least one parasite species were kept. To avoid spurious signals

206 associated with rare observations, we focused on the 15 species achieving at least 10% prevalence.

207 To capture the strongylid community assembly pattern across anatomical niches and age group, we

208 applied the hierarchical Bayesian joint species distribution model (Ovaskainen et al., 2017; Tikhonov et

209 al., 2017) implemented in the HMSC R package (v. 3.0-10) (Tikhonov et al., 2020). This model offers an

210 integrated framework to explicitly model species-species interactions while simultaneously accounting

211 for environmental covariates, species traits or their phylogenetic relationships (Ovaskainen et al., 2017;

212 Tikhonov et al., 2017). We used the same approach as described elsewhere (Abrego et al., 2020), fitting

213 two models that either accounted for the worm burden measured at the horse level or for additional

214 fixed effects including the anatomical niche and horse age. In both cases, horse and horse *x* anatomical

215 niche were fitted as random effects to account for inter-horse variation in species pairs co-occurrences

216 and for sample level variation not accounted for otherwise (Abrego et al., 2020). A hurdle-type model

217 was considered to account for the overdispersed nature of the count data, modeling log-transformed

218 positive species counts as normal and the binary presence or absence status using a probit-link

219 function (Abrego et al., 2020). Model predictive power was assessed by Area Under the Curve (AUC)

220 and R^2 parameters averaged across species for presence-absence and abundance data. Parameters

221 were estimated from 4,000 posterior samples collected every 100 iterations from four Markov Chain

222 Monte Carlo (MCMC) run for 150,000 iterations with a third discarded as burn-in.

223

224   2.3.3. Analysis at the anatomical niche level: species co-occurrences from necropsy

225   data

226 To study the interactions between strongylid species within their hosts, we re-analyzed individual

227 necropsy report data collected in Normandy (Collobert-Laugier et al., 2002). Abundances of 20

228 strongylid species and other non- strongylid species (*Anoplocephala* sp*.*, *Oxyuris sp*.) were quantified

229 within the caecum, ventral- and dorsal-colon of 46 horses. The data from 36 horses that had their age

230 registered and were infected by at least one parasite species were kept. To avoid spurious signals

231 associated with rare observations, we focused on the 15 species achieving at least 10% prevalence.

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239 fixed effects including the anatomical niche and horse age. In both cases, horse and horse *x* anatomical

240 niche were fitted as random effects to account for inter-horse variation in species pairs co-occurrences

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244 function (Abrego et al., 2020). Model predictive power was assessed by Area Under the Curve (AUC)

245 and R^2 parameters averaged across species for presence-absence and abundance data. Parameters

246 were estimated from 4,000 posterior samples collected every 100 iterations from four Markov Chain

247 Monte Carlo (MCMC) run for 150,000 iterations with a third discarded as burn-in.

248

249 3. **Results**

250 **3.1. Descriptive features of strongylid communities**

251 The breakdown of publication counts for factors of interest is provided in Supplementary Table 2. As

252 can be seen in Figure 1A, studies using deworming were mostly carried out in Eastern Europe. Our

253 data showed an average number of horses per study of 28.65, ranging between 2 and 150 individuals.

254 Strongylid communities were dominated by a small number of species, *i.e. Cylicocyclus nassatus,*

255 *Cylicostephanus longibursatus, Cyathostomum catinatum* (Figure 1B, supplementary table 3) with an

256 average prevalence of 83.97% ± 3.38. Along these three dominant species, a set of eight subdominant

257 species with an average prevalence of 54.96% ± 12.68. On the other hand, 31 rare species had an

258 average prevalence of 8.93% ± 6.70. These mean prevalence levels reported within horse populations

259 were slightly corroborated when considering the presence-absence pattern at the regional scale

260 (Figure 1C). Specifically, the dominant and subdominant species defined by an average prevalence of

261 25% or more at the horse population level were present in 75% of studies. Contrasting this broad

262 pattern, *Cylicostephanus goldi* was relatively frequent among horses but its presence varied widely

263 across studies (Figure 1C).

264 At the horse level, strongylid community diversity varied widely as illustrated by species accumulation

265 curves derived from the three available studies. The sampling of 95% of available diversity, required

266 22 horses (46% of total horse sample) for necropsy-based data from France (Collobert-Laugier et al.,

267 2002). On the contrary, strongylid communities gathered upon deworming reached the same threshold

268 for 25% of available horses (12 and 50 horses in the studies in Poland or Ukraine communities).

269 More diverse strongylid communities may reflect reduced drug usage and might be associated with

270 higher parasite egg excretion. However, limited agreement was found between Faecal Egg Count and

271 community diversity as measured by the rarefaction curves. Significant correlation was found between

272 FEC and species richness (Supplementary Figure 1) in strongylid communities gathered in Poland

273 (Spearman's ρ = 0.31, P = 9x10^{-3}) but not in Ukraine (Spearman's ρ = 0.03, P = 0.69). Correlation

274 between FEC and the rarefaction curve at its basis (Gini index) was not significant in any case

275   (Spearman's ρ = -0.14, P = 0.06 and ρ = 0.24, P = 0.1 in Polish and Ukrainian data respectively),

276   suggesting that species dominance does not affect FEC.

277

278   **3.2. Diversity analysis of worldwide strongylid communities**

279   As a first step, an exploratory multivariate analysis was applied to our meta-community matrix (Figure

280   2). This analysis highlighted the outstanding contribution of the strongylid recovery method on

281   community diversity (Figure 2). This clustering also closely matched a gradient defined by differences

282   in the temporal and the sampling effort scales. Publication times ranged between 2005 and 2018 or

283   between 1935 and 2016 for deworming- and necropsy-based sets respectively. In addition,

284   significantly more horses were sampled in necropsy-based studies (36.84 and 18.9 horses sampled

285   respectively, P = 2.2 x 10^{-16}).

286   The clear distinction between considered recovery methods was supported by the PERMANOVA (P =

287   3 x 10^{-4}) run on the strongylid communities gathered under the same geo-climatic conditions, i.e.

288   European continental climate. This was however associated with higher variance in deworming-based

289   methods ($F_{1,31}$ = 6.51, P = 0.02, supplementary Figure 2).

290   Logistic regression models found significant variation for *Triodontophorus serratus* and *Strongylus*

291   *vulgaris* that were less often recovered in the five considered necropsy-based sets relative to the 28

292   deworming studies performed under the same geoclimatic conditions (interaction terms equal to -4.1

293   ± 1.96, P=0.03 and -4.62 ± 2.00, P = 0.02). Of note, quasi-complete separation with the recovery

294   method was found for 13 species, either showing complete absence (*Cylicostephanus. bidentatus*) or

295   complete presence (*Cylicocyclus ashworthi*, *Gyalocephalus capitatus*, *Cylicodontophorus bicoronatus*,

296   *Coronocyclus coronatus*, *Cylicocyclus. elongatus*, *Coronocyclus labratus*, *Poteriostomum*

297   *imparidentatum*, *Petrovinema poculatum*, *Parapoteriostomum mettami*, *Poteriostomum ratzi*,

298   *Cylicocyclus Radiatus, Triodontophorus tenuicollis*) in studies practicing necropsy (Supplementary

299   Figure 3). The species richness was however not significantly different between the recovery methods

300   (P = 0.6).

301 On the contrary, the ordination analysis found limited clustering associated with geoclimatic factors

302 (Figure 2). This was corroborated by PERMANOVA on the set of communities collected after necropsy

303 (P = 1.9 x 10^{-3}). In that case, species richness was lower in the temperate European area (-6.57, P =

304 5×10^{-3}). Apart from the 15 species with quasi-complete separation in one of the considered geoclimatic

305 zones (Supplementary Figure 4), we did not find significant evidence of variation in their prevalence

306 pattern across geoclimatic conditions.

307 To sum up, the recovery method was not associated with variation in species richness but it was

308 associated with significant turnover across communities, likely mirroring scale differences between the

309 considered sets. On the contrary, species turnover across geoclimatic areas was limited and reduced

310 richness was found under temperate Europe.

311

312 ### 3.3. Strongylid community dynamic across age groups

313 Partitioning of overall γ-diversity across Ukraine operations revealed an outstanding contribution of

314 within-horse α-diversity (46.5% of overall diversity) relative to species turnover coefficients (Figure 3).

315 The observed value was however lower than under null model expectations (22.6% difference, P=10^{-}

316 ^{3}). Species turnover between horse age-group was the second most important contributor to γ-diversity

317 (23.9% of γ-diversity), significantly higher than that expected from a null model (P=10^{-3}, Figure 3) and

318 higher than that explained by between-farm variation (20% of γ-diversity, Figure 3). Of note, observed

319 between-horse turnover did not depart from the simulated null distribution (0.06% difference, P=0.4,

320 Figure 3).

321 Because of the importance of age group in driving strongylid community structure, we also searched

322 for indicator species that would preferentially be associated with a given age group. This analysis found

323 that *C. labiatus* and *C. ashworthi* were preferentially encountered in two-years old horses, irrespective

324 of the considered coefficient (indicator values of 0.659, P=7 x 10^{-3}, and 0.585, P = 0.04 or association

325 coefficient equal to 0.42, P = 0.01 and 0.41, P = 0.01 for *C. labiatus* and *C. ashworthi,* respectively).

13

326 Altogether these results suggest that horse-level community diversity and between age-class turnover

327 are the major contributors driving measured diversity under the European continental conditions

328 encountered across Ukraine operations.

329

330 3.4. Co-occurrence of species within different anatomical niches

331 Anatomical niche was the last scale examined to determine factors driving strongylid community

332 structure. The predictive power of the modeling approach was high with a mean AUC of 0.88 and R^2

333 of 0.79 for models based on presence-absence or positive abundance data.

334 Raw or residual co-occurrences only supported positive interactions between the considered

335 strongylid species (Figure 4). The correction for environmental covariates erased around half of

336 observed co-occurrences in both abundance (11 out of 26) and presence-absence (21 out of 42)

337 models. This was mostly due to correction for the anatomical niche that accounted for a third of total

338 variance while another third was equally shared across horse age and infection intensity (Table 1). Of

339 note, the presence of *C. goldi* was largely dependent on inter-horse variation but not its abundance

340 (supplementary Figures 5 and 6) suggesting limited expansion abilities once established in a host.

341 Residual models shared 12 positive co-occurrences involving *C. nassatus*, *C. labratus*, *C. coronatus*,

342 *Cylicostephanus minutus* and *C. catinatum* (Figure 4). However, more co-occurrences were found with

343 the presence-absence data. This suggests that strongylid species tend to have similar co-infection

344 patterns but their simultaneous presence does not necessarily affect their respective relative

345 abundances.

346

347 4. Discussion

348 In this meta-analysis, we have explored the principles of strongylid community structuring across

349 various scales, ranging from regional down to anatomical resolutions. Our findings define a community

350 with little rearrangements across contrasted geoclimatic conditions, whose diversity is mostly

351 grounded on within-host richness and turnover across-age groups. At the anatomical scale, strongylid

352   communities are structured around positive co-occurrences fluctuating across anatomical niches. As

353   such, our results complement and expand the past work by Bellaw and Nielsen (Bellaw and Nielsen,

354   2020) with additional horse-based data and different approaches and interpretations of the findings.

355   While we tried to account for common sources of variation at the regional scale, this was often limited

356   by the paucity of available metadata or by the data structure of past published work. For instance, age

357   group was poorly annotated across studies and horse sex was seldom reported despite significant

358   species turnover described between both sexes (Sallé et al., 2018). In addition, the lack of appropriate

359   summary statistics in past published work constrained our analysis to a presence-absence framework

360   that certainly overlooks finer grain patterns found with abundance data (Brian and Aldridge, 2020). This

361   is mostly the result of the existing asymmetry between presence of the parasite - a single infected

362   individual is enough - and absence - that requires the whole species to be absent (Chase et al., 2019).

363   Reliance on this binary matrix defined at the regional level also prevented the use of other statistics

364   like the effective number of species (Chase and Knight, 2013) or individual based rarefaction (Chase et

365   al., 2018) to better account for differences in scales (geographical and temporal) across the considered

366   species sets. As a result, the significant species turnover across the recovery methods that we

367   observed as other authors (Bellaw and Nielsen, 2020) may simply reflect scale differences between

368   studies. We took care to correct for differences in sampling effort and restricted the analysis to the

369   same geoclimatic conditions. But the wider diversity variance found for strongylid communities

370   examined after horse deworming is also likely to reflect the higher abundance of this type of data

371   relative to the necropsy-based communities as the more communities are sampled, the more likely

372   that species recovery will vary (Chase et al., 2019).

373   Overall, our results highlighted a small group of abundant and widespread species *C. nassatus*, *C.*

374   *longibursatus* and *C. catinatum* that clearly separated from other species. Of note, these species were

375   consistently associated with drug resistance reports suggesting their phenology may have favoured

376   higher fitness towards the use of modern anthelmintics (van Doorn et al., 2014). It is unclear however

377   what phenotypic trait underpins their higher abundance and prevalence in horse populations. Their

15

378   respective fecundities estimated from the number of eggs found in female worms *utero* was among

379   the lowest suggesting their dispersal is unlikely to define a better ability to colonize their hosts (Sallé et

380   al., 2018).

381   In addition, analysis of horse-based data expanded our understanding of strongylid communities. First,

382   we found limited correlation between parasite egg excretion, a trait used to monitor infection in horses,

383   and the strongylid community diversity. This was against our hypothesis that more permissive hosts

384   would tolerate higher parasite burden, thereby increasing the community species richness. This lack

385   of linear relationship may mirror variations in respective strongylid species fecundities (Kuzmina et al.,

386   2012) and reflect possible density dependence effects that are known to occur in trichostrongylid

387   species infecting ruminants (Bishop and Stear, 2001).

388   Second, we found that overall diversity was mostly partitioned across within-host richness and species

389   turnover across horse age groups, and to a lesser-extent between farm variation. Species richness is

390   conditional upon availability of host resources, leading to higher richness in larger animal species or

391   for higher host density (Kamiya et al., 2014). Richness also depends on environment saturation in

392   infective larvae (Shmida and Wilson, 1985). The latter two aspects underscore the importance of

393   pasture hygiene and limited stocking density for the management of strongylid infection in grazing

394   livestock. Of note, environmental saturation is usually expected to erase species turnover among hosts

395   (Vannette and Fukami, 2017) but we identified a significant turnover between age groups. This turnover

396   remains to be fully dissected to disentangle between the respective effects of selection applied through

397   the mounting of an effective immune response as horses age (Debeffe et al., 2016; Kornaś et al., 2015;

398   Relf et al., 2013; Wood et al., 2013) or the drift arising from putative competitive processes between

399   species. To this respect, the increased species turnover across age groups may arise from priority

400   effects between strongylid species that would counterbalance the effect of environmental saturation in

401   eggs and infective larvae (Vannette and Fukami, 2017). Such priority effect would in turn yield a network

402   of positive co-occurrence between species as found for mixed infection of rodents by malaria species

403   (Ramiro et al., 2016).

404 Using data with anatomical niche resolution, we performed a first investigation of co-occurrence

405 between strongylid species. This analysis did not reveal any negative effects thereby corroborating

406 past results obtained after horse deworming in Poland conditions (Sallé et al., 2018). Of note, a few co-

407 occurrences were dependent on associated covariates including the anatomical niche or horse age

408 that may define optimal environmental conditions shared across parasite species. However, a core

409 network of positive interactions remained after accounting for environmental variations between

410 species from four distinct genera, namely *Cyathostomum (C. catinatum)*, *Cylicocyclus* (*C. nassatus* and

411 *C. insigne*), *Cylicostephanus (C. minutus)* and *Coronocyclus* (*C. coronatus* and *C. labratus*). This would

412 be compatible with facilitative relationships between more divergent species that should be less prone

413 to resource competition (MacArthur and Pianka, 1966) although co-existence of the *Cylicocyclus* and

414 *Coronocyclus* members depart from this expectation. While the complexity of underpinning biological

415 processes may obscure the observed co-occurrences patterns (Blanchet et al., 2020), these

416 interactions were found across different parasite communities and with the lowest possible resolution

417 thereby supporting their biological meanings (Behnke, 2008). However, covariates like past infections

418 and treatment history were not accounted for and may contribute to this network. Besides, the

419 contribution of the resident gut microbiota may play a role in the definition of this particular structuring

420 as significant interactions between microbial and helminth communities are known to occur in horses

421 (Clark et al., 2018; Peachey et al., 2019; Walshe et al., 2020).

422 Altogether, priority effects between equine strongylid species in young horses may contribute to

423 enhance the species turnover found between age groups and to define the network of positive co-

424 occurrences between the most dominant genera. The simultaneous monitoring of both the microbial

425 and parasite communities using the latest barcoding approaches (Poissant et al., 2020) should provide

426 valuable contributions to challenge these hypotheses.

427

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431

432 **Figure and table legends**

433 **Figure 1. Descriptive features of worldwide equine strongylid communities**

434 A: World map of study distribution coloured by worm collection method, either following deworming

435 (green) or necropsy (orange).

436 B: Strongylid species (colored by their respective genera) relative abundances and prevalences

437 averaged across published mean estimates. Dotted lines distinguish between rare, subdominant and

438 dominant species in the left, middle and right quadrants respectively.

439 C: Comparison of observed species prevalence across studies (from the presence-absence matrix)

440 against the average published prevalence estimates. Colour and dotted lines match that used in B.

441

442 **Figure 2. Ordination applied to strongylid meta-community with fitted environmental variables**

443 Figure shows the first two axes from a NonMetric Dimensional Scaling (NMDS) ordination applied to

444 published metacommunity data. The colours correspond to the different types of environment

445 considered, coded as Continent – Climate – Recovery method. For visualization, species names were

446 abbreviated with correspondence given in Supplementary Table 3.

447

448 **Figure 3. Hierarchical diversity partitioning of overall diversity**

449 Within host alpha diversity and β-diversity (turnover) for the scales of interest (host, age class and farm),

450 observed (purple) and simulated (green) estimates are given in percentage of overall diversity

451 explained. Error bars materialize a 95% confidence interval of the simulated data.

452

453 **Figure 4. Raw and residual strongylid species co-occurrence matrices estimated from presence-**

454 **absence data or positive abundance data**

18

455 Co-occurrence matrices derived from presence-absence (left) or positive abundance (right) data are

456 represented for 15 strongylid species collected identified in 36 necropsied horses from Normandy,

457 France. In each case, off-diagonal elements describe the raw (above) and residual (below) co-

458 occurrences.

459

460 **Table 1. Proportion of variance explained by considered covariates in the residual co-occurrence**

461 **model**

462 For each covariate of interest (fixed or random effect), the proportion of variance explained is given for

463 the two model types considered using positive abundance data or presence-absence data.

464

Effect type	Covariate	Abundance data (%)	Occurrence data (%)
	Anatomical niche	31.2	32.7
Fixed effect	Horse age	14.6	12.9
	Worm burden	14.1	10.8
Random effect	Horse	12.8	33.4
	Horse x niche	27.3	19.1

465

466

467

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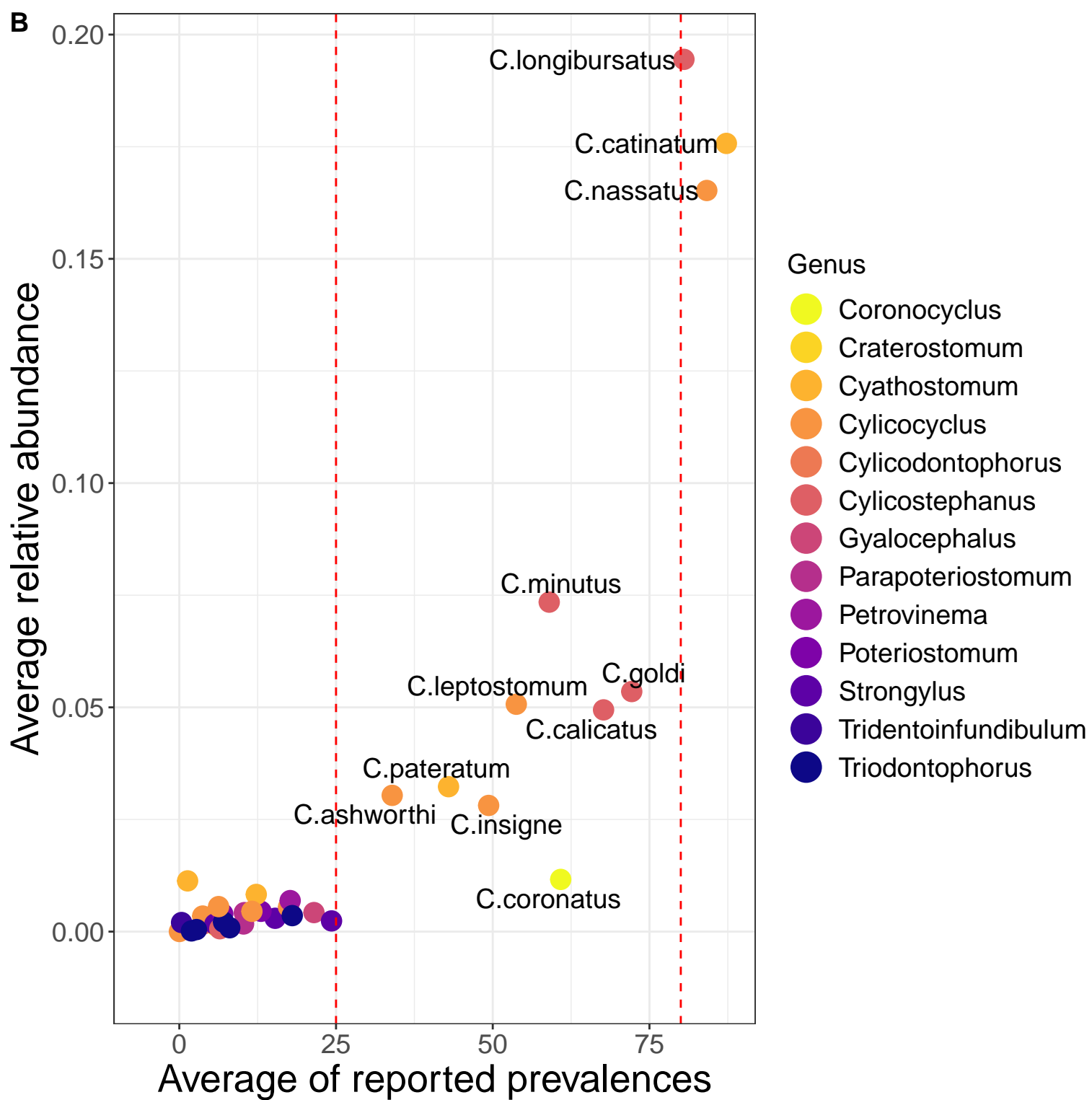
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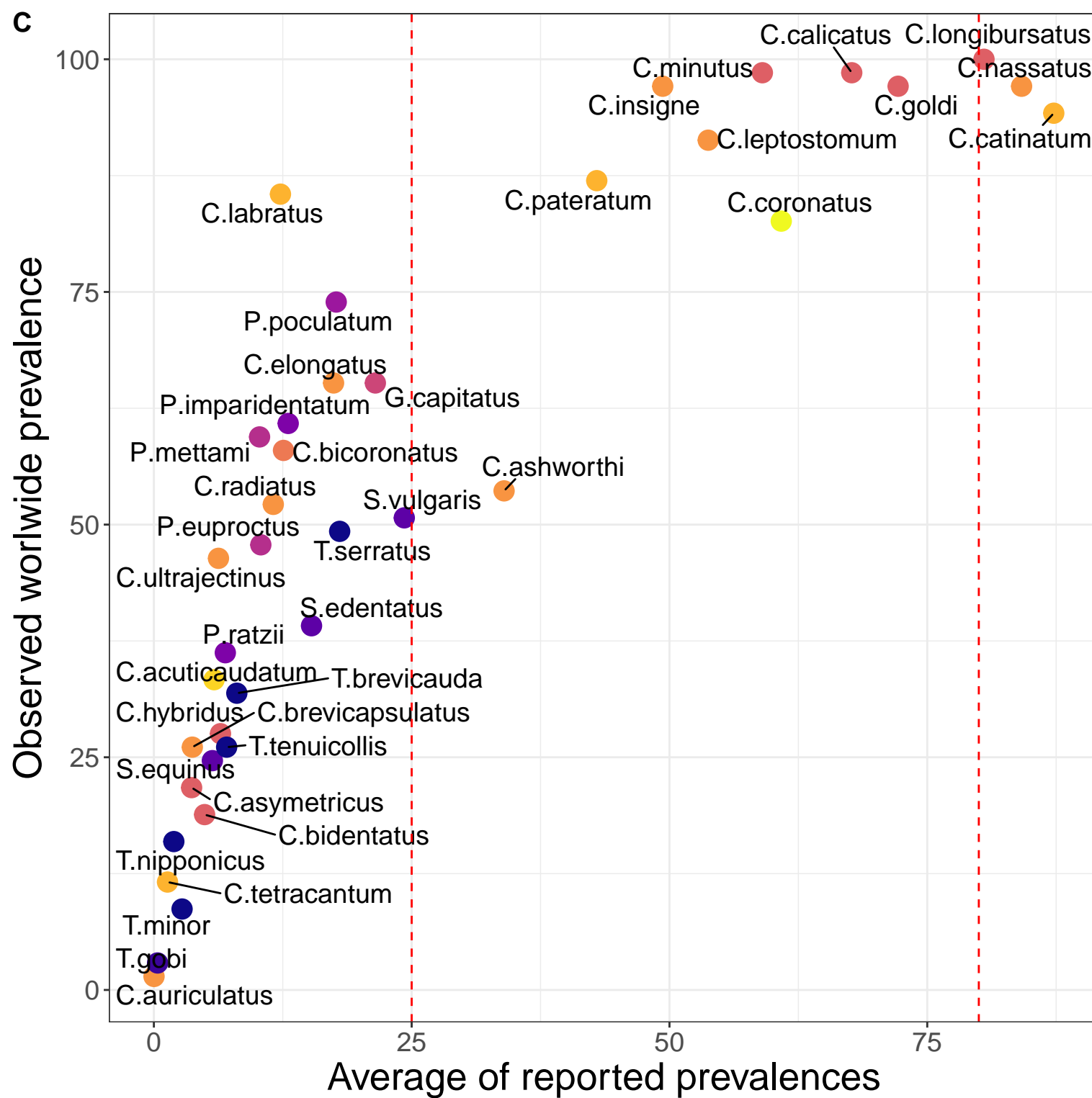
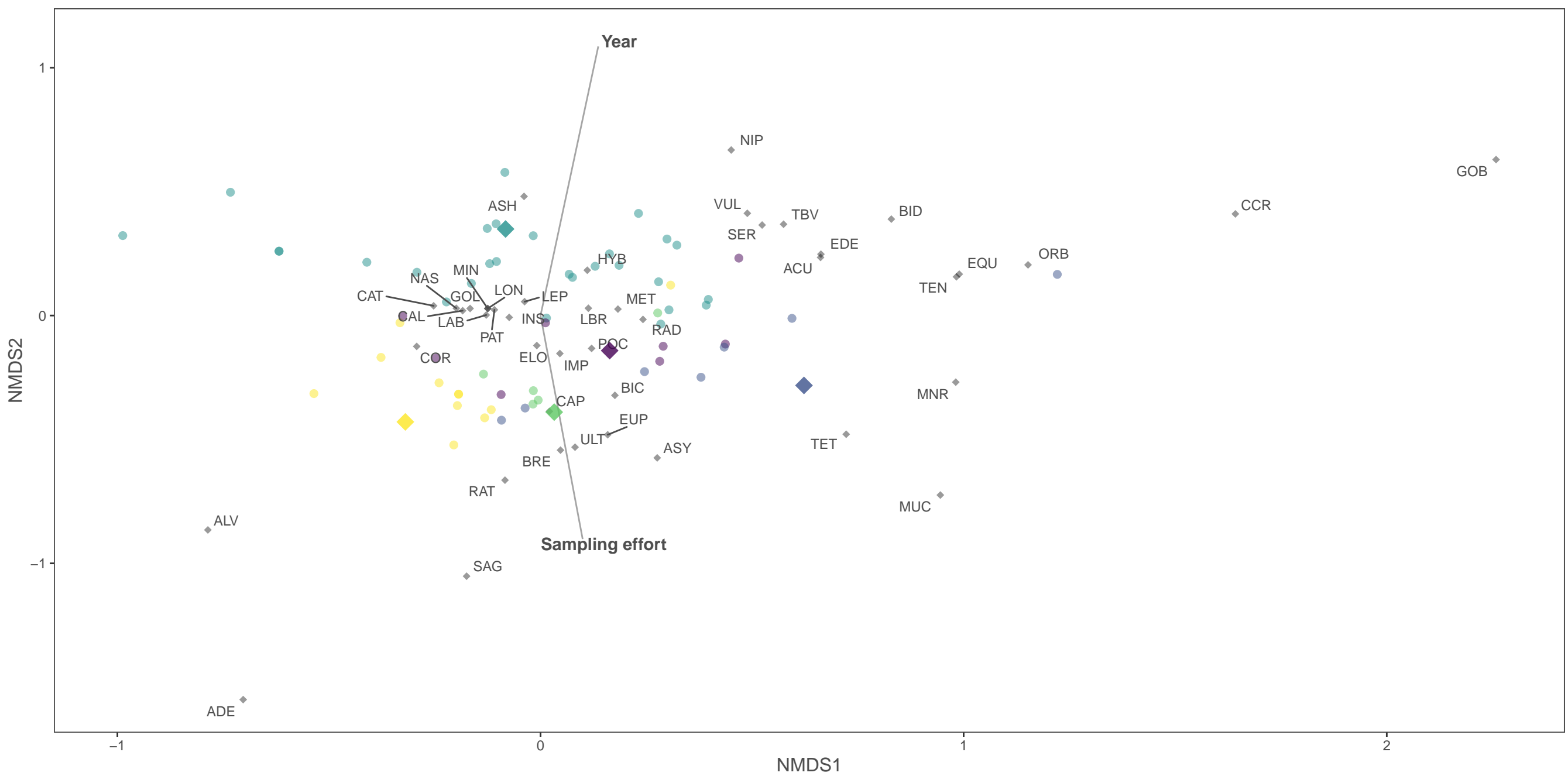603   seasonal variation. Parasitology 140, 115-128.

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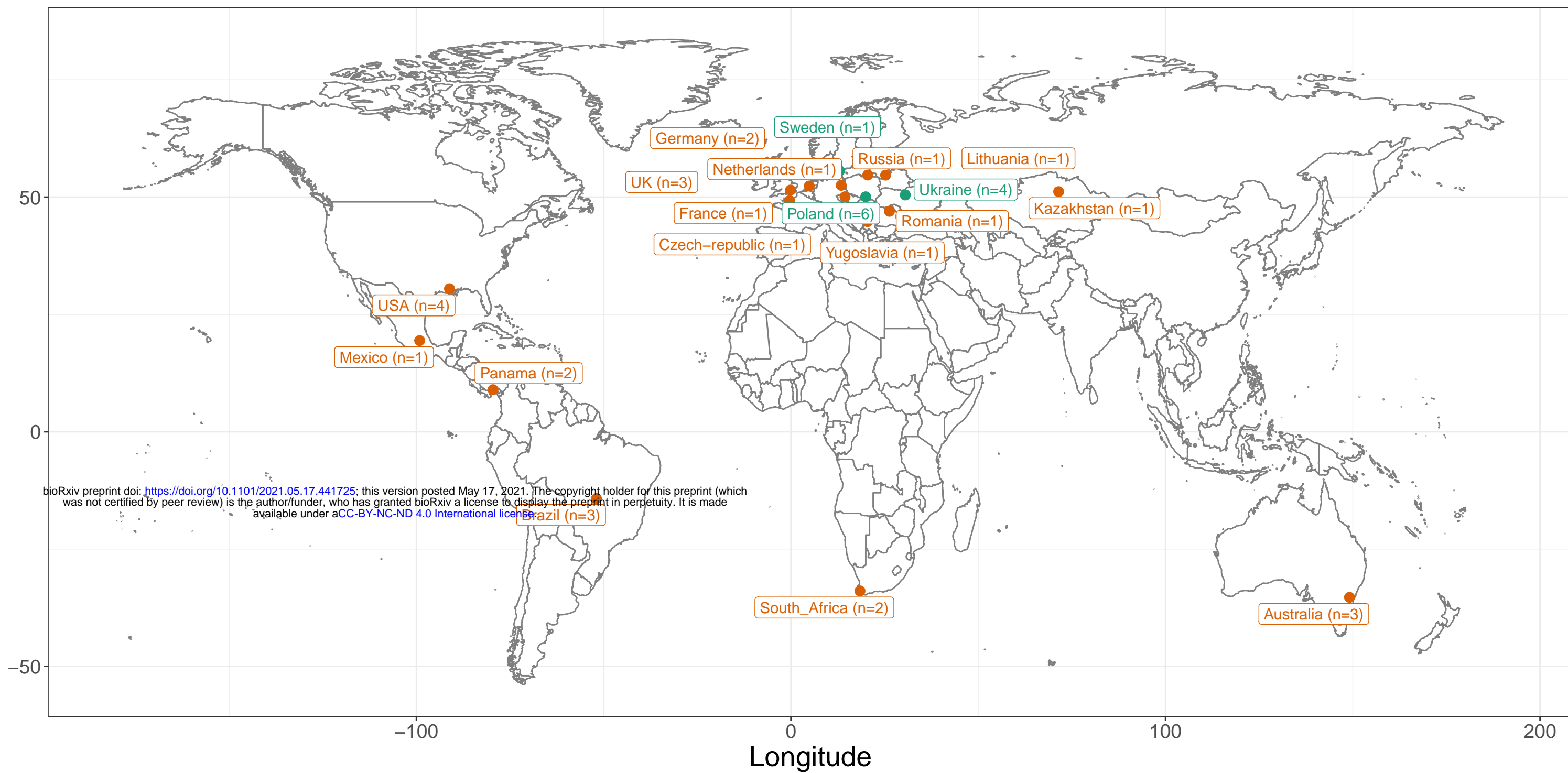
Legend:
- America – Temperate – Necropsy
- America – Tropical – Necropsy
- Europe – Temperate – Necropsy
- Europe – Continental – Necropsy
- Europe – Continental – Deworming