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## ► To cite this version:

Michel Boisseau, Núria Mach, Marta Basiaga, Slawomir Kornaś, Tetiana Kuzmina, et al.. Species turnover between age groups of horses and positive network of co-occurrences define the structure of horse strongylid communities: a meta-analysis. 2022. hal-03794670

**HAL Id: hal-03794670**

**<https://hal.inrae.fr/hal-03794670>**

Preprint submitted on 3 Oct 2022

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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  $\beta$ -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 Tchuente 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.,

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](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

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  $\alpha$ -diversity estimator, and the  $\beta$ -diversity index was estimated with  
156 the Jaccard index using the vegan R package. (version 2.5-7). 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 interest,  
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

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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235 integrated framework to explicitly model species-species interactions while simultaneously accounting  
236 for environmental covariates, species traits or their phylogenetic relationships (Ovaskainen et al., 2017;  
237 Tikhonov et al., 2017). We used the same approach as described elsewhere (Abrego et al., 2020), fitting  
238 two models that either accounted for the worm burden measured at the horse level or for additional  
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  
241 and for sample level variation not accounted for otherwise (Abrego et al., 2020). A hurdle-type model  
242 was considered to account for the overdispersed nature of the count data, modeling log-transformed  
243 positive species counts as normal and the binary presence or absence status using a probit-link  
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  $83.97\% \pm 3.38$ . Along these three dominant species, a set of eight subdominant  
257 species with an average prevalence of  $54.96\% \pm 12.68$ . On the other hand, 31 rare species had an  
258 average prevalence of  $8.93\% \pm 6.70$ . 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  $\rho = 0.31$ ,  $P = 9 \times 10^{-3}$ ) but not in Ukraine (Spearman's  $\rho = 0.03$ ,  $P = 0.69$ ). Correlation  
274 between FEC and the rarefaction curve at its basis (Gini index) was not significant in any case

275 (Spearman's  $\rho = -0.14$ ,  $P = 0.06$  and  $\rho = 0.24$ ,  $P = 0.1$  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,  $P = 2.2 \times 10^{-16}$ ).

286 The clear distinction between considered recovery methods was supported by the PERMANOVA ( $P =$   
287  $3 \times 10^{-4}$ ) 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  $\pm 1.96$ ,  $P=0.03$  and  $-4.62 \pm 2.00$ ,  $P = 0.02$ ). 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 ( $P = 1.9 \times 10^{-3}$ ). In that case, species richness was lower in the temperate European area ( $-6.57$ ,  $P =$   
304  $5 \times 10^{-3}$ ). 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  $\gamma$ -diversity across Ukraine operations revealed an outstanding contribution of  
314 within-horse  $\alpha$ -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,  $P=10^{-3}$ )  
316 <sup>3</sup>). Species turnover between horse age-group was the second most important contributor to  $\gamma$ -diversity  
317 (23.9% of  $\gamma$ -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  $\gamma$ -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,  $P=7 \times 10^{-3}$ , 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).

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 strongyloid 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 strongyloid 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 strongyloid 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 strongyloid 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, strongyloid

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



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

## 428 **Acknowledgements**

429 MBo and this work was supported by the Institut Français du Cheval et de l'Équitation (IFCE) and  
430 Fonds Éperon grant.

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  $\beta$ -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**

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

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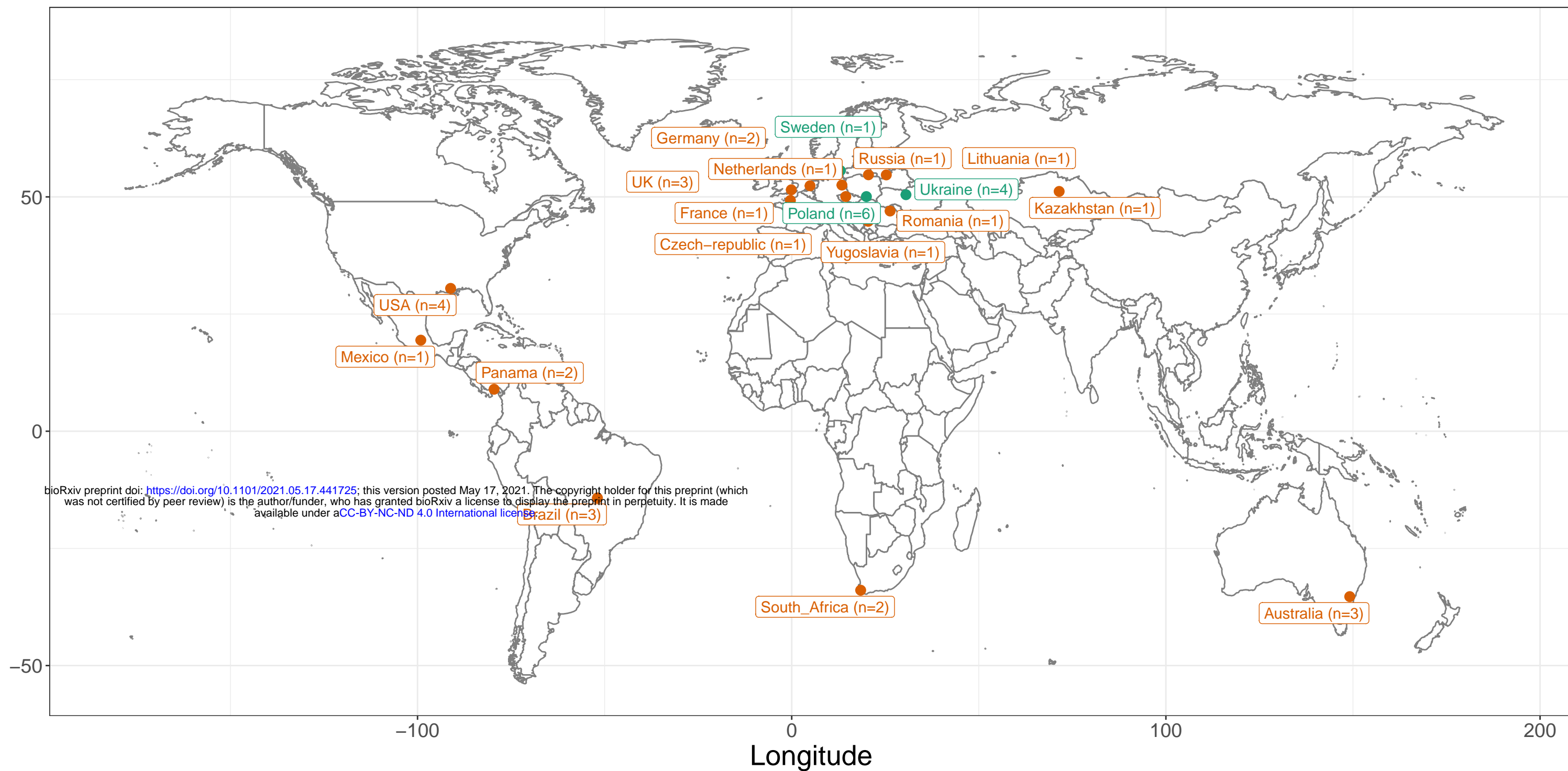
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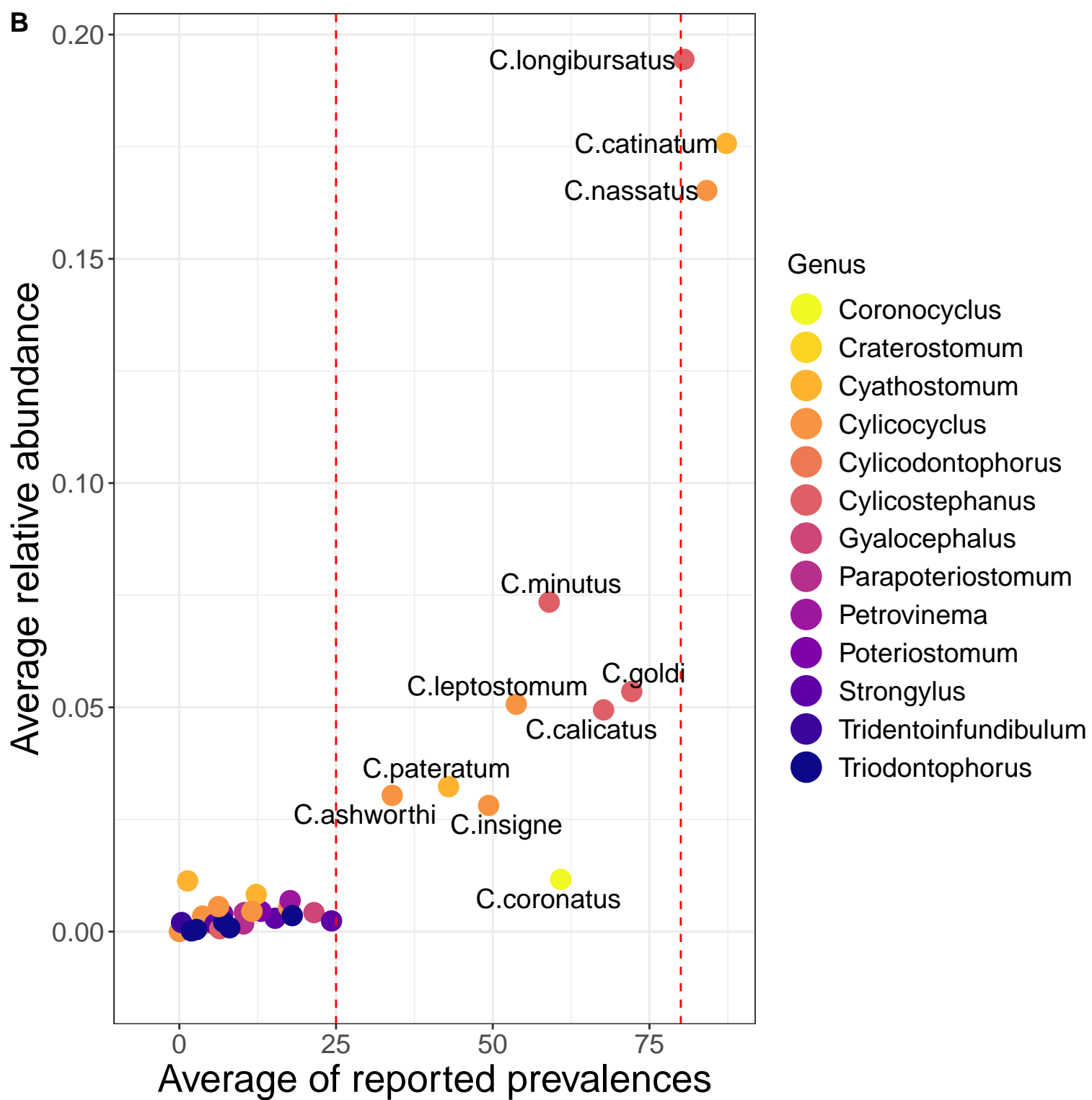
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