Spatial distribution and spread potential of sixteen *Leptospira* serovars in a subtropical region of Brazil

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Running Title:

Leptospira distribution in subtropical regions

Summary

Leptospirosis is a bacterial disease that represents a major problem in animal and public health due to its high prevalence and widespread distribution. This zoonotic disease is most prevalent in

tropical environments where conditions favor pathogen survival. The ecological preferences of *Leptospira* serovars are poorly understood, limiting our knowledge of where and when outbreaks can occur, which may result in misinformed prevention and control plans. While the disease can occur consistently in time and space in tropical regions, research on the ecology of Leptospirosis remains limited in subtropical regions. This research gap regarding *Leptospira* ecology brings public and veterinary health problems, impacting local economies. To fill this gap of knowledge, we propose to assess geographic and ecological features among *Leptospira* serovars in a subtropical area of Brazil where Leptospirosis is endemic to (i) highlight environmental conditions that facilitate or limit *Leptospira* spread and survival and (ii) reconstruct its geographical distribution. An ecological niche modeling framework was used to characterize and compare *Leptospira* serovars in both geographical and environmental space. Our results show that, despite the geographic overlap exhibited by the different serovars assessed, we found ecological divergence among their occupied ecological niches. Ecological divergences were expressed as ranges of potential distributions and environmental conditions found suitably by serovar, being Sejroe the most asymmetric. Most important predictors for the potential distribution of most serovars were soil pH (31.7%) and landscape temperature (24.2%). Identification of environmental preferences will allow epidemiologists to better infer the presence of a serovar based on the environmental characteristics of regions rather than inferences based solely on historical epidemiological records. Including geographic and ecological ranges of serovars also may help to forecast transmission potential of *Leptospira* in public health and the food animal practice.

45 **KEYWORDS:** Brazil, disease distribution, ecological similarities, environmental, *Leptospira*,

46 NicheA, spatial analysis, spread, zoonotic

INTRODUCTION

Leptospirosis is a major public health issue due to its high incidence and worldwide distribution (Bharti et al., 2003; Abela-Ridder et al., 2010; Adler and Moctezuma, 2010). Leptospirosis is a zoonotic disease endemic in tropical regions where environmental conditions favor the survival of the bacteria along the year and outside the host (Bharti et al., 2003). Tropical regions often concentrate the highest density of domestic, wild animals and humans (Stevens, 1989; Morand and Poulin, 1998; Gaston, 2000), facilitating interspecies transmission of *Leptospira*, the causative agent of leptospirosis (Adler and Moctezuma, 2010). *Leptospira* serovars have showed to be highly influenced by environmental conditions (Lau et al., 2010; Ivanova et al., 2012). For example, temperature and precipitation (Desvars et al., 2011; Chadsuthi et al., 2012), high humidity and heavy rainfall (Barcellos and Sabroza, 2001; Goarant et al., 2009), runoffs, soil pH, and primary productivity, all have been associated with *Leptospira* occurrence (Smith et al., 1961; Fajriyah et al., 2017; Rahayu et al., 2018).

Approximately 1.03 million cases of leptospirosis are reported globally each year, from which 58,900 are deaths (Costa et al., 2015). Likewise, the global burden estimated in Disability Adjusted Life Years (DALY) per annum for this disease was 2.9 million, showing the great economic impact of leptospirosis worldwide (Torgerson et al., 2015). Leptospirosis is no longer listed among the neglected tropical diseases prioritized by the world health organization (Molyneux et al., 2017). Instead, it is now considered a re-emerging infectious disease linked to a combination of factors, including intensification of livestock production, and limited access to health provision for animals and humans, and environmental change (Mwachui et al., 2015; Hotez, 2016; Goarant et al., 2019). For example, leptospirosis risk is amplified by the frequency

of extreme climatic events and major changes in land use (Pappas et al., 2008; Picardeau, 2015). Within these environmental parameters, Brazil is among the top 17 countries in the world with the highest incidence of human leptospirosis (Pappas et al., 2008).

In subtropical regions, the ecology of leptospirosis is generally assumed to be consistent with tropical regions. However, subtropical regions may have considerable environmental differences that may limit effectiveness of control strategies developed for tropical conditions. The Brazilian state of Rio Grande do Sul is located in a subtropical region of southern Brazil. This state has a dense livestock production and one of the highest horse populations in South America (SEAPI-RS., 2018). Additionally, Rio Grande do Sul has the 5/26 highest incidence of human leptospirosis in Brazil (4.7 cases per 10,000 habitants; Ministério da Saúde do Brasil, 2018), representing ~15% of the total cases (Pacheco and Caldas, 2012). Rio Grande do Sul was also identified among the top five states where improvement on leptospirosis surveillance, control, and elimination must be prioritized in Brazil (Baquero and Machado, 2018). Thus, there is a critical need to identify and anticipate areas and conditions more likely suitable for leptospirosis in this subtropical region.

A comprehensive understanding of the geographic distribution and environmental factors that facilitate *Leptospira* infections will help to inform intervention and prevention strategies for humans and animals (Grooms, 2006; Lilenbaum and Martins, 2014; Sánchez-Montes et al., 2015; Zhao et al., 2016). Such assessments have been widely applied in epidemiology through ecological niche modeling (ENM) in disease ecology. ENM explores geographic and ecological patterns of vectors, hosts or pathogens distribution, and transmission (Peterson, 2006). This approach has shown effectiveness under diverse applications to fundamental ecological questions

91 such as areas at risk of disease infection (Machado et al., 2018), likely pathogen spillover to
92 humans (Peterson, Martínez-Campos, Nakazawa, & Martínez-Meyer, 2005; Samy, Thomas,
93 Wahed, Cohoon, & Peterson, 2016) and environmental factors linked to infectious diseases (Jia
94 and Joyner, 2015; Sallam et al., 2017). Thus, ENM has proven to be a powerful approach to
95 reconstruct the likely factors shaping infectious diseases distributions.
96 This study aims to identify the geographic and ecological conditions where *Leptospira*
97 serovars occur under subtropical conditions. Using an ENM framework, we characterized and
98 compared the environmental features of *Leptospira* serovars to determine their potential
99 geographic distribution, environmental preferences, and likely hotspots of serovars diversity in
100 the study area. Our approach has the potential to facilitate the development of intelligence-based
101 based leptospirosis surveillance for public health and veterinary epidemiology in this and other
102 subtropical regions.
103
104 **MATERIAL AND METHODS**
105 The study design included a modeling framework based on the chosen study area for model
106 calibration, selection, and evaluation, followed by data collection, curation, and standardization
107 (i.e., field work to collect samples, laboratory work for serovar identification, and environmental
108 variables collection and management) (Fig. 1).
109
110 **Figure 1. Workflow of the modeling process. A**) Data collection in the modeling area defined
111 based on biogeographic barriers (**M** region *sensu* (Soberon and Peterson, 2005)), where black
112 dots represent the *Leptospira* occurrence, while the red color shows the study area ; **B**)

environmental variables relevant for *Leptospira* survival and transmission; **C**) Reduction of number and correlation among environmental variables, model selection, final model calibration; **D**) Assessments of ecological similarity among *Leptospira* serovars, where and the two maps exemplify the potential distribution of two serovars in the geographic space, while 3D plot in the environmental space. Histogram shows ENM comparison between the two serovars based on their environmental space; **E**) Identification of most important environmental for each serovar. NDVI= Normalized Difference Vegetation Index. Red lines represent the response curves that show how each environmental variable affects the ENM prediction for each *Leptospira* serovars, where environmental variables (x axis) and suitability values (y axis) are described for each serovar (left column).

Data collection

Rio Grande do Sul has ~103,000 registered horse farms, with a population of more than 550,000 horses (SEAPI-RS., 2018). Our primary dataset comes from a cross-sectional study where 1,010 animals were sampled from 341 farms randomly selected across the state. In each farm, horses were blood sampled to detect previous *Leptospira* exposure. Details of the sampling and laboratory analyses conducted are available elsewhere (Weiblen et al., 2016). Briefly, farms were randomly selected from the total number of farms that had at least one equid older than six months of age (n = 103,170), the number of farms to sample was stratified according to the horse population present in each of the administrative regions of the state (Weiblen et al., 2016). Samples were tested for *Leptospira* antibodies using the microscopic agglutination test (MAT) based on live antigens (Faine et al., 1999; Adler, 2014). Briefly, five 2-fold dilutions of serum

135 samples from 1:25 to 1:4000 were used. Samples were tested for 16 serovars: *Leptospira*
136 *interrogans* serovar Australis (Australis), *Leptospira interrogans* serovar Autumnalis
137 (Autumnalis), *Leptospira interrogans* serovar Sejroe (Sejroe), *Leptospira interrogans* serovar
138 Canicola (Canicola), *Leptospira interrogans* serovar Ballum (Ballum), *Leptospira interrogans*
139 serovar Celledoni (Celledoni), *Leptospira interrogans* serovar Copenhageni (Copenhageni),
140 *Leptospira borgpetersenii* Javanica (Javanica), *Leptospira interrogans* serovar Grippotyphosa
141 (Grippotyphosa), *Leptospira interrogans* serovar Hardjo (Hardjo), *Leptospira interrogans*
142 serovar Hebdomadis (Hebdomadis), *Leptospira interrogans* serovar Icterohaemorrhagiae
143 (Icterohaemorrhagiae), *Leptospira interrogans* serovar Pomona (Pomona), *Leptospira*
144 *interrogans* serovar Pyrogenes (Pyrogenes), *Leptospira interrogans* serovar Tarassovi
145 (Tarassovi) and *Leptospira interrogans* serovar Wolffi (Wolffi) (Adler, 2014; Filho et al., 2014;
146 Alves et al., 2016; Dreyfus et al., 2018). The antigens were stored at 28°C from 5 to 10 days in
147 EMJH (Ellinghausen and MCcullough, 1965) culture (Difco®-USA) that was enriched with
148 bovine albumin fraction V (Inlab®- Brasil) (Ellinghausen and McCullough, 1965). Serum
149 samples were considered positive when MAT titers were ≥ 100. The ultimate reactive serovar
150 was determined by the election of the highest titer that was presented. In the presence of
151 coagglutinations, all serovars that were involved were considered positive (see Fig. S1 to see the
152 spatial information related to positive farms per serovar).
153
154 ***Selection of the model calibration region***
155 To define the study area extent for model calibration for each *Leptospira* serovar, we followed
156 the framework proposed by (Soberon and Peterson, 2005), which restricts the ENM to ecological

157 features of plausible biological relevance for the organism in question, the resolution of the
158 environmental variables based on the geographic error, and the extent of the region where the
159 organisms are able to disperse based on biogeographic barriers (see **M** in the BAM framework in
160 (Soberon and Peterson, 2005). We assumed a geographic error < 30 m considering that we used
161 GPS devices to estimate the coordinates of each sample and biome regions as biogeographic
162 barriers since they represent homogeneous climatic and landscape composition (Lomolino et al.,
163 2010; Soberón, 2010). This resulted in an **M** in the ecoregion of the Uruguayan savanna (Olson
164 et al., 2001) (see Fig. 1A). This geographic delimitation, **M**, allows to determine the spread
165 potential of the *Leptospira* populations in the study area. Thus, our models are representative of
166 this study area extent.
167
168 ***Ecological Niche Models (ENMs)***
169 The environmental variables used to estimate the distribution of *Leptospira* were selected based
170 on the described requirements of the bacterium, including survival in specific landscapes with
171 suitable temperature and humidity and presence of livestock (Wint and Robinson, 2007). To
172 reconstruct the landscape structure we used Normalized Difference Vegetation Index (NDVI), a
173 satellite-derived variable resembling vegetation phenology and primary productivity commonly
174 used in ENM (Cook et al., 2008; Fajriyah et al., 2017). We also used annual mean temperature,
175 precipitation, runoff (index that quantity of water discharged in surface streams), and wetness
176 index (defined as a steady-state wetness index), since higher incidences of leptospirosis are
177 related to warmer temperatures (Lau et al., 2010; Desvars et al., 2011; Chadsuthi et al., 2012)
178 and humid environments (Barcellos and Sabroza, 2001; Pappachan et al., 2004; Desvars et al.,

179 2011; Ivanova et al., 2012). In addition, we also included soil pH, since previous studies have
180 explored the importance of this variable in the survival of the bacteria outside the host (Smith et
181 al., 1961; Saito et al., 2013; Schneider et al., 2018). Environmental variables were used at ~5 km
182 of spatial resolution at the equator (see Table S1 for details). Livestock presence was represented
183 by density of horses, cattle, and pigs (Gilbert et al., 2018), which are known reservoirs and
184 amplifiers of leptospirosis (Lo et al., 2006). To mitigate multicollinearity between environmental
185 variables, we used VIF (Variance Inflation Factors) implemented in the "usdm" R-package
186 (Naimi and Araújo, 2016); excluding highly correlated variables from the model (VIF > 7), since
187 this a signal of strong collinearity (Chatterjee and Hadi, 2015). A detailed description of each
188 environmental variables, such as, description, source, reference and VIF value are presented in
189 (Table S1).

190 ENMs were developed using a presence-background method that estimates
191 environmental suitability via an index of similarity that resembles a heterogeneous occurrence
192 process or logistic regression function (Phillips et al., 2006; Phillips and Dudík, 2008).
193 Specifically, we used Maxent algorithm with clamping and extrapolation turned off (i.e., no
194 prediction outside the range of environmental conditions used during calibration) (Elith et al.,
195 2010; Anderson, 2013; Owens et al., 2013). To determine the model parametrization with the
196 best fit to the data available, we assessed Maxent models for each serovar under different
197 regularization multiplier values (0.1, 0.3, 0.5, 0.7, 0.9, 1.3, 1.5, 1.7, 1.9 and 2) (Warren and
198 Seifert, 2011). At the same time, we explored all feature combinations ranging from a single
199 feature, linear (L), quadratic (Q), product (P), threshold (T) and hinge (H) (Muscarella et al.,

200 2014), to all feature combinations possible (i.e., 5!). Models were selected based on Akaike
201 Information Criterion (AIC) values, specifically ΔAICc=0.
202 To facilitate interpretations of final models, we used the logistic output as a proxy of
203 environmental suitability (Phillips and Dudík, 2008), which we normalized in the final models to
204 suitability ranging from 0 to 100 for easier visualization of values. Additionally, suitable areas
205 for each serovar were estimated as a Boolean (presence/absence) map that was thresholded based
206 on the minimum training presence method to generate binary maps without omission error
207 (Pearson et al., 2006). Serological results of serovars were pooled to general genus-level models
208 (see Table S2 for the best for each set of occurrence data). These models were generated
209 following the protocols described above but focused on the percentage of the variable
210 importance estimated by Maxent. To determine the hotspots of *Leptospira* serovars richness, we
211 ensembled all binary models by using Spatial Analysis in Macroecology (SAM) software
212 (Rangel et al., 2010), (available at https://ecoevol.ufg.br/sam).
213 In addition, we showed how each environmental variable affects the Maxent ENM
214 prediction for each serovar, representing how the predicted probability of presence changes as
215 each environmental variable is varied.
216
217 ***Ecological niche similarity among serovars***
218 We assessed ecological similarities among models of *Leptospira* serovars by using four methods
219 based on geographic and environmental dimensions. First, similarity was measured using the
220 Schoener's *D* index (Schoener, 1968) that measures similarity between two ENMs in geographic
221 space based on probabilities outputs being similar in terms of the environmental conditions

222 available to them (Rödder and Engler, 2011). Schoener's *D* was estimated by comparing
223 Maxent-generated ENMs against a null distribution of default Maxent models, resulting in
224 similarity values ranging from 0, non-similar, to 1, highly similar. We followed the protocols
225 described by (Warren et al., 2008) and (Warren et al., 2010). Second, we used the Jaccard
226 similarity index (Jaccard, 1912) that assesses similarity between two ENMs in environmental
227 space by measuring the volume and overlap of two ENMs (Escobar et al., 2015). Volume of
228 environmental space occupied by each serovar was estimated in three forms to capture variability
229 among estimates. First, volume of ENMs was estimated in NicheA software (Qiao et al., 2016),
230 available at http://nichea.sourceforge.net/function_niche_overlap.html. Briefly, original
231 environmental variables were collapsed into three environmental dimensions to reduce
232 redundancy and dimensionality. Then, volume was measured based on the environments
233 occupied by each *Leptospira* serovar in terms of a minimum-volume ellipsoid and a convex-
234 polyhedron. Finally, volume was estimated for all serovars combinations using "hypervolume"
235 package in the R software (Blonder et al., 2014). This method relies on a Gaussian kernel density
236 estimation procedure based on the Silverman method (Silverman, 1986), measuring the geometry
237 of the multidimensional hypervolume from the original variables standardized (Blonder et al.,
238 2014). The Jaccard similarity index based on NicheA and hypervolume values provides an
239 accurate measure of the geometrical relationships between serovars distribution in a
240 multidimensional space (Goodall, 1966; Real and Vargas, 1996). In summary, we generated one
241 similarity estimation in geographic space (Schoener's *D*) and three estimations in environmental
242 space (Jaccard indices from the minimum-volume ellipsoid, convex-polyhedron, and Gaussian
243 kernel density).

RESULTS

Spatial patterns of Leptospira serovars distribution

Approximately 45% of the total *Leptospira* serovars circulating in the study area were Hebdomadis, Tarassovi, Pyrogenes. (Fig. S1). Occurrence of the 16 *Leptospira* serovars showed considerable asymmetries among their geographic distribution (Fig. S1).

Ecological niche models (ENMs)

ENM results showed that central-northern areas in this study had suitable conditions for Autumnalis, Canicola, Copenhageni, Hardjo, Icterohaemorrhagiae, and Wolfii. Contrarily, central-western areas were suitable for Calledoni, Grippotyphosa, Javanica, Hebdomadis, and Pyrogenes were most common. Australis, Pomona and Tarassovi were found to prefer the northern area, while Serjroe, and Castellonis concentrated its potential distribution in the western and eastern regions, respectively (Fig 2).

Figure 2. Ecological niche model (ENM) predictions of *Leptospira* serovars in Southern Brazil. Warmer colors show areas with higher probability of presence. Background layer represents the earth in true color based on NASA's Terra satellite image for better visualization. Source https://neo.sci.gsfc.nasa.gov/.

These distributional differences among serovars were also reflected in the geographic patterns observed in the hotspot areas per serovar. Similarly, the model ensemble comprised specific

areas of agreement of *Leptospira* suitability. For example, the region bordering northern Argentina and areas between Caxias do Sul and Taquari River, were hotspot for *Leptospira* likely exposure. Additional areas of *Leptospira* exposure-risk were found in Taquarombo, Uruguay, and Lagoa Mirim, between Brazil and northeastern Uruguay (Fig. 3A). The visualization of the ensembled model also in a multidimensional environmental space, revealed that *Leptospira* occurred under consistent and trackable environmental conditions, however, available conditions were more diverse and broader than those occupied by the pathogen (Fig. 3B).

Figure 3. Model ensembled of *Leptospira* serovars in a subtropical region. A) Model ensemble in geographic space based on binary models summed to identify areas of highly (dark blue) and low (light blue) agreement among models. Dark areas denote areas found consistently suitable for *Leptospira* and therefore, for plausible exposure infection. **B**) Model ensemble in environmental space based on binary models summed to identify the environmental conditions occupied by the sero-positive cases (yellow convex polyhedron). Grey dots represent the environmental available conditions in **M**, axes are the first principal components from the original environmental variables (see Table S1).

Environmental drivers of serovars potential distribution

The final *Leptospira* ENM showed that soil pH (31.7%) and mean annual temperature (24.2%) were the most influential predictors associated with *Leptospira* sero-positivity (Table 1). Response curves also suggested that as pH and temperature increased linearly the suitability

288 index for *Leptospira* presence (Fig. 4). We identified the importance of variables related with
289 humidity wetness; index; runoff and precipitation in the distribution of Australis, Autumnalis,
290 Canicola, Celledoni, Pomona, Wolffi, Sejroe, and Tarassovi. Likewise, NDVI was the main
291 predictor for Grippotyphosa sero-positivity, while livestock production was observed to be the
292 most important predictor for serovar Sejroe sero-positivity (Table 1).
293
294 **Table 1. Variable importance for all *Leptospira* and for each serovar.** Warmer colors
295 represent higher levels of importance (%).

Serovar	Temperature	Wetness index	Soil pH	NDVI	Runoff	Precipitation	Livestock
Leptospira (all)	24.2	9.2	31.7	4.4	6.2	12.0	12.3
Australis	22.1	2.8	19.9	8.2	42.4	0.5	4.1
Autumnalis	3.6	20.8	35.9	7.4	10.7	11.5	10.1
Canicola	0.0	27.7	55.4	2.5	0.0	0.3	14.1
Castellonis	7.8	12.0	56.7	4.7	6.3	9.2	3.3
Celledoni	0.0	0.0	54.7	0.0	20.2	25.1	0.0
Copenhageni	5.7	0.0	66.5	0.0	0.0	6.7	21.1
Grippotyphosa	14.7	0.0	25.9	59.4	0.0	0.0	0.0
Hardjo	32.8	13.7	39.8	0.0	0.0	0.0	13.7
Hebdomadis	10.4	13.9	63.1	0.7	11.2	0.0	0.7
Icterohaemorrhagiae	0.0	2.6	72.2	4.4	0.0	0.9	19.9
Javanica	12.3	18.1	51.2	5.1	0.0	10.6	2.7
Pomona	0.0	24.3	44.1	0.7	0.0	30.9	0.0
Pyrogenes	34.7	14.5	42.6	0.0	1.6	3.1	3.5
Sejroe	0.1	0.1	0.2	0.1	8.9	44.8	45.9
Tarassovi	10.3	0.1	20.0	5.5	59.9	2.2	2.1
Wolffi	21.4	26.9	40.9	2.3	0.0	6.6	1.9

296
297 **Figure 4. Response curves of the different environmental variables by *Leptospira* serovars.**
298 Response curves (red line) estimated based on Maxent ENM predictions. Environmental
299 variables (x axis) and suitability values (y axis) are described for each serovar (left column).
300 Units, source, and details of each variable are found in Table 1.

301

302 ***Ecological similarities among Leptospira serovars***

303 The observed values of Schoener's (*D*) similarity tests showed niche similarity of all serovars,
304 which tend to overlap on average 0.68 ± 0.2, ranging from 0.09 (Sejroe and Castellonis) to 0.97
305 (Icterohaemorrhagiae and Javanica). In all cases, the serovar that showed the most asymmetric
306 results was Sejroe, where the highest observed similarity values were under 0.15 (Fig. 5A and
307 Table S3). These considerable variations in the ecological niches between serovars were also
308 observed in the NicheA results, showing that the ecological niche of *Leptospira* is characterized
309 by asymmetries in the distribution of the different serovars in the environmental space. The niche
310 overlap was on average 0.16 ± 0.08 "Convex polyhedron" (CP) (Fig. 5B, Table S4) and 0.16 ±
311 0.09 "Minimum Volume Ellipsoid" (MVE) (Fig. 5C, Table S5). These values ranged from 0.01
312 (CP= Sejroe and Pomona, MVE= Sejroe and Grippotyphosa) to 0.3 (CP= Copenhageni and
313 Tarassovi) and 0.38 (MVE= Tarassovi and Wolffi). Similarly to what was previously observed,
314 large asymmetries were observed by the "hypervolume" approach. Serovars tend to be similar on
315 average 0.11 ± 0.09, with values ranging from 0 (Grippotyphosa with Copenhageni, Hardjo,
316 Icterohaemorrhagiae, Javanica, Pomona and Sejroe) to 0.26 (Copenhageni and Pomona) (Fig. 5D
317 and Table S6).

318 Overall, higher asymmetries in the ecological niche were evidenced between the majority
319 of *Leptospira* serovars, these results were highly contrasted with the results based on the
320 geographical space (Schoener's (*D*) index) (Fig. 5A and Table S3), while, the lowest similarity
321 values were observed in the comparison based on convex polyhedron (Fig. 4B).

322

323 **Figure 5. Ecological niche similarity between *Leptospira* serovars based on four model**
324 **similarity metrics. A**) Schoener's *D* index, **B**) convex polyhedron, **C**) Minimum Volume
325 Ellipsoid and **D**) hypervolume.
326
327 **DISCUSSION**
328 This study combines geographic and ecological approaches to characterize eco-epidemiology
329 patterns of *Leptospira* sero-positivity in horses at serovar level. This cross-sectional study
330 allowed for the identification of geographic and ecological preferences of *Leptospira* serovars.
331 Despite the geographic similarities exhibited by each serovar, they showed different
332 environmental preferences, evidencing the diversity of environmental conditions where
333 *Leptospira* exposure can occur. Recent efforts have been made to understand the environmental
334 tolerances of *Leptospira* at serovar level (Fouts et al., 2016; Guernier et al., 2017; Jaeger et al.,
335 2018; Zarantonelli et al., 2018), as well as to identify potential risk areas for future leptospirosis
336 outbreaks (Sánchez-Montes et al., 2015; Zhao et al., 2016). However, there have been no studies
337 able to forecast regions where high-transmission risk exists and where disease surveillance and
338 control strategies (e.g., vaccination) would have better impact. Our multidimensional approach
339 (i.e., geographic and environmental dimensions) represents an important stepping-stone in the
340 study and understanding of *Leptospira* ecology not only for identifying risk areas for different
341 serovars but also for the development of new strategies to understand the ecological drivers of
342 *Leptospira* presence.
343 Our results showed considerable differences in the ecological landscape features of the
344 distribution of each *Leptospira* serovar. This could be explained by the fact that different

345 *Leptospira* lineages can survive and adapt to different environmental conditions. Historically,
346 leptospirosis has been widely associated to warm and humid conditions (Barcellos and Sabroza,
347 2001; Trueba et al., 2004; Ivanova et al., 2012; Saito et al., 2013; Schneider et al., 2018). Our
348 results support these previous findings since the variables related to temperature and
349 precipitation were considered highly important predictors for the potential distribution of most of
350 the serovars, especially for Australis, Hardjo, Pyrogenes and Tarassovi. Looking into detail, our
351 results showed a positive relationship between precipitation and a higher probability of presence
352 of *Leptospira*. However, we also found negative relationship of *Leptospira* presence with soil
353 humidity and runoff, which could be explained by the type of soil in the area: bentonite clay,
354 which is an amplifier of this pathogen since it absorbs half of *Leptospira* in suspension (Smith et
355 al., 1961). On the other hand, responses curves of each serovar associated to temperature did not
356 show a clear, consistent pattern.

357 Recently, Schneider et al., (2018), highlighted the importance of pH in the survival of this
358 bacteria in soil, which was strongly supported by our findings. We found that soil pH was the
359 main predictor for 12 of the 16 serovars examined. Response curves evidenced a positive
360 relationship between soil pH and the probability of presence of most of *Leptospira* serovars,
361 except for Autumnalis, Canicola, Hebdomadis and Javanica.

362 Niche similarity tests based on environmental space (convex polyhedron, MVE, and
363 hypervolume) revealed high asymmetries between the majorities of *Leptospira* serovars. These
364 results were highly contrasted with what we observed in the geographical space (Schoener's *D*
365 index), where most of the serovars tends to overlap their distributions. The niche similarity tests
366 offer biological realism to the different models by giving access to a broader perspective that

367 support the idea of phylogenetic niche conservatism among the *Leptospira* lineages studied
368 (Escobar, Qiao, Phelps, Wagner, & Larkin, 2016; Martinez-Meyer, Diaz-Porras, Peterson, &
369 Yanez-Arenas, 2012; Yañez-Arenas, Peterson, Mokondoko, Rojas-Soto, & Martínez-Meyer,
370 2014). The importance and significance of the use of these similarity tests at serovar level relies
371 in the fact that disease transmission is the product of complex interactions that involves
372 ecological, evolutionary, and epidemiological processes (Fountain-Jones et al., 2018; Galvani,
373 2003; Peterson, 2006).

374 Risk of horizontal gene transmission can occur between serovars (Ren et al., 2003; Haake
375 et al., 2004), which can facilitate shifts in virulence (Dzidic and Bedekovic', 2003; Khairani-
376 Bejo et al., 2004; Salyers et al., 2004; Adler, 2014). Thus, the possibility of multiple serovars
377 cohabiting in the same location increases the possibility of gene transfer making our serovar
378 richness maps informative to design *Leptospira* monitoring plans if areas of higher disease-
379 emergence risk (Fig. 3).

380 ENM is used to characterize environmental requirements of species and their potential
381 distribution s (Peterson, 2014; Peterson & Vieglais, 2001; Qiao et al., 2018). These analyses
382 have been applied for a wide variety of epidemiological purposes such as the prediction of
383 species invasions into novel areas (Benedict et al., 2007; Machado et al., 2018), anticipation of
384 disease emergence (Peterson, Bauer, & Mills, 2004; Williams & Peterson, 2009), and forecast of
385 the impact of climate change on future emerging disease distributions (González et al., 2010;
386 Gálvez et al., 2011; Daszak et al., 2013; De Oliveira et al., 2017; Baquero and Machado, 2018).
387 Our approach represents a novel application of ENM aimed to generate new knowledge about
388 the ecology of *Leptospira* at serovar level. However, more efforts are necessary to determine if

389 our findings are consistent in different biogeographic regions (e.g., tropical, temperate). Finally,
390 we faced limitations through the development of this study, mainly related to the species
391 sampled. More specifically, to obtain the most accurate representation of *Leptospira* circulation
392 in the landscape there would be necessary to assess the presence of *Leptospira* serovars in
393 wildlife and the environment to provide and integrative estimation of the geographic and
394 environmental risk (Albert et al., 2009).
395
396 **CONCLUSION**
397 In this study, we identified the geographic and environmental signatures of *Leptospira* serovars
398 in a subtropical region in southern Brazil. We determined the geographic and ecological
399 characteristics influencing the current and potential distributions of all *Leptospira* serovars tested
400 providing new ecological and epidemiological knowledge about *Leptospira* lineages circulation
401 in animal populations. We found specific environmental preferences of serovars, most serovars
402 were limited by soil pH and mean annual temperature. The maps generated in this study also
403 denote the local and regional hotspots of disease transmission risk, useful to design evidence-
404 based disease prevention strategies for effective surveillance and vaccination.
405
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Supporting information

Supplementary Tables

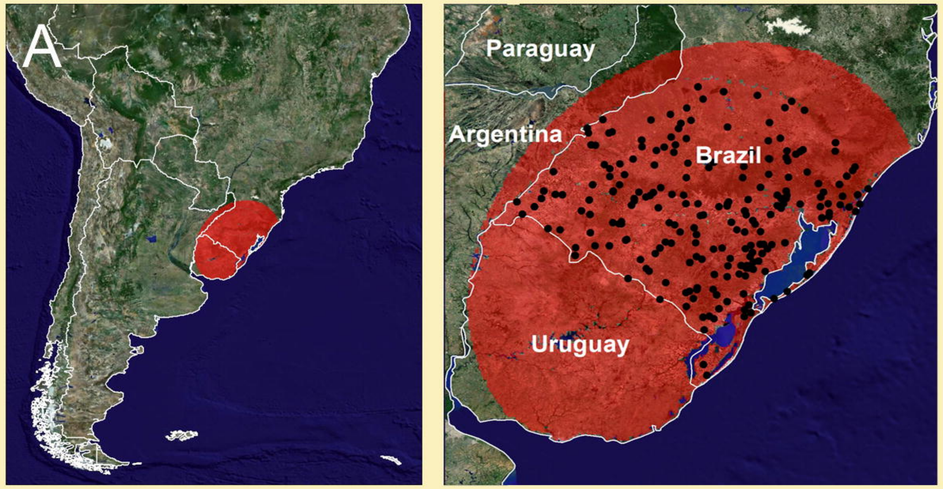
Table S1. Variable selection and Variance Inflation Factor analysis (VIF) to assess spatial multicollinearity.

Table S2. Model evaluation results based on Akaike's information criteria (ΔAICc = 0). Considering a variety of feature classes (linear= L, product= P, quadratic= Q, threshold= T and hinge= H) and regularization multiplier. AUC=Area Under Curve (Variance).

Table S3. Ecological niche similarity between *Leptospira* serovars (based on Schoener's D index) for *Leptospira* serovars, where Aus = australis, Aut = autumnalis, Can = canicola, Cas = castellonis, Cel = celledoni, Cop = copenhageni, Grip = grippotyphosa, Har = hardjo, Heb = hebdomadis, Ict = icterohaemorrhagiae, Jav = javanica, Pom = pomona, Pyr = pyrogenes, Ser = sejroe, Tar = tarassovi and Wol = wolffi. Warmer colors represent higher similarity values.

Table S4. Ecological niche similarity between *Leptospira* serovars (based on Hypervolume approach) for *Leptospira* serovars, where Aus = australis, Aut = autumnalis, Can = canicola, Cas

741 = castellonis, Cel = celledoni, Cop = copenhageni, Grip = grippotyphosa, Har = hardjo, Heb =
742 hebdomadis, Ict = icterohaemorrhagiae, Jav = javanica, Pom = pomona, Pyr = pyrogenes, Ser =
743 sejroe, Tar = tarassovi and Wol = wolffi. Warmer colors represent higher similarity values.
744
745 **Table S5.** Environmental niche comparison matrix (Jaccard index) based on minimum volume
746 ellipsoid for *Leptospira serovars*, where Aus = australis, Aut = autumnalis, Can = canicola, Cas
747 = castellonis, Cel = celledoni, Cop = copenhageni, Grip = grippotyphosa, Har = hardjo, Heb =
748 hebdomadis, Ict = icterohaemorrhagiae, Jav = javanica, Pom = pomona, Pyr = pyrogenes, Ser =
749 sejroe, Tar = tarassovi and Wol = wolffi. Warmer colors represent higher similarity values.
750
751 **Table S6**. Environmental niche comparison matrix (Jaccard index) based on convex polyhedron
752 for Leptospira serovars, where Aus = australis, Aut = autumnalis, Can = canicola, Cas =
753 castellonis, Cel = celledoni, Cop = copenhageni, Grip = grippotyphosa, Har = hardjo, Heb =
754 hebdomadis, Ict = icterohaemorrhagiae, Jav = javanica, Pom = pomona, Pyr = pyrogenes, Ser =
755 sejroe, Tar = tarassovi and Wol = wolffi. Warmer colors represent higher similarity values.
756
757 **Supplementary figures**
758 **Figure S1.** Geographic occurrences of *Leptospira* serovars in Southern Brazil.
759
760 **Figure S2.** Binary ENM predictions of *Leptospira* serovars in Southern Brazil.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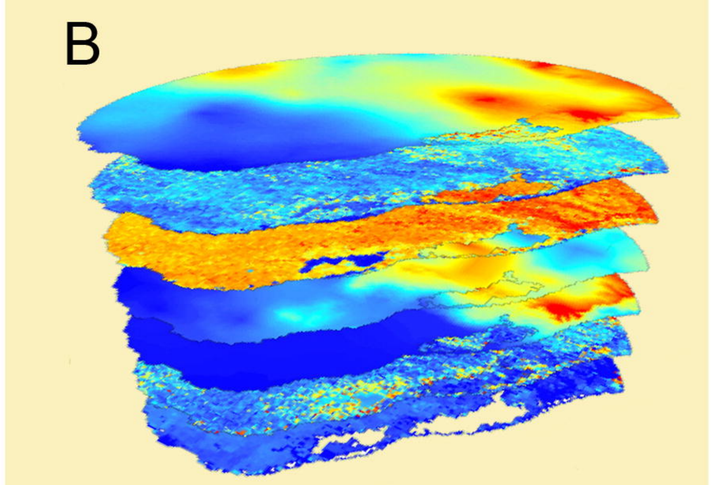
Paraguay
Argentina
Brazil
Uruguay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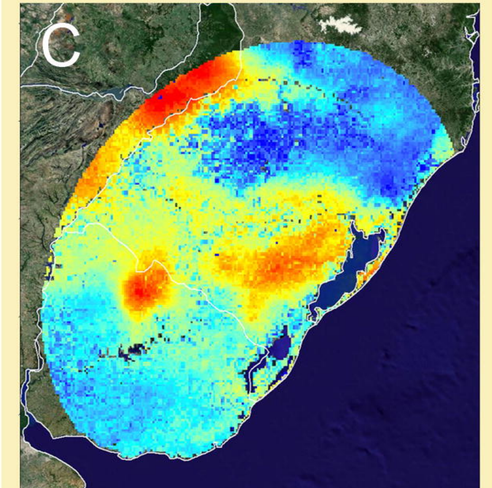
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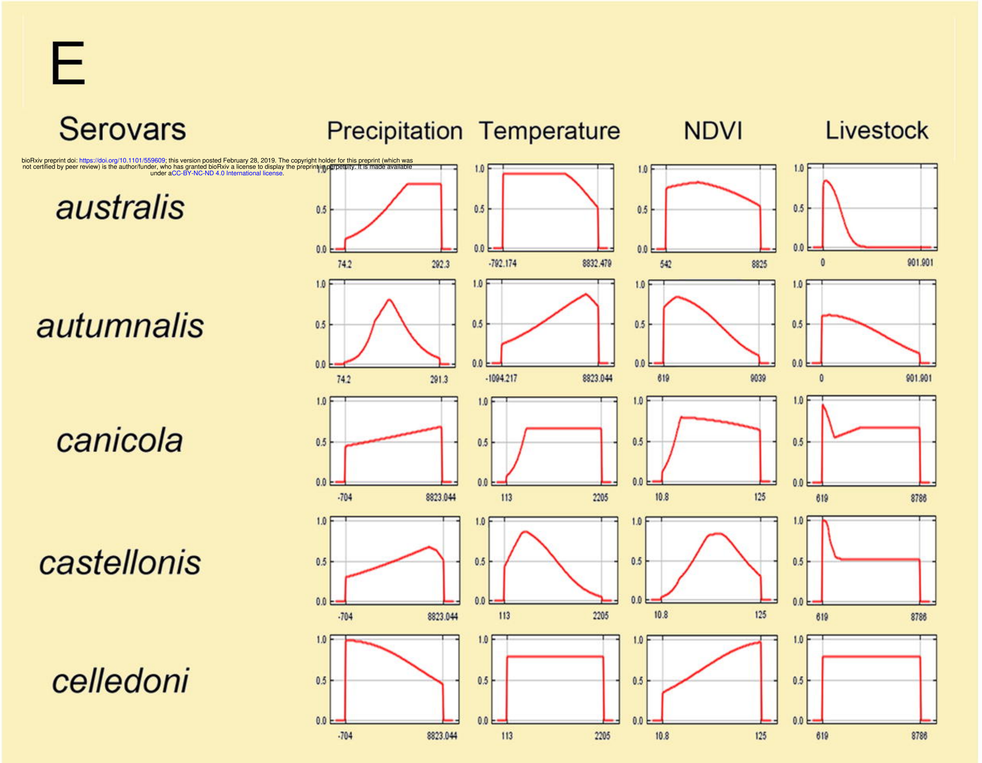
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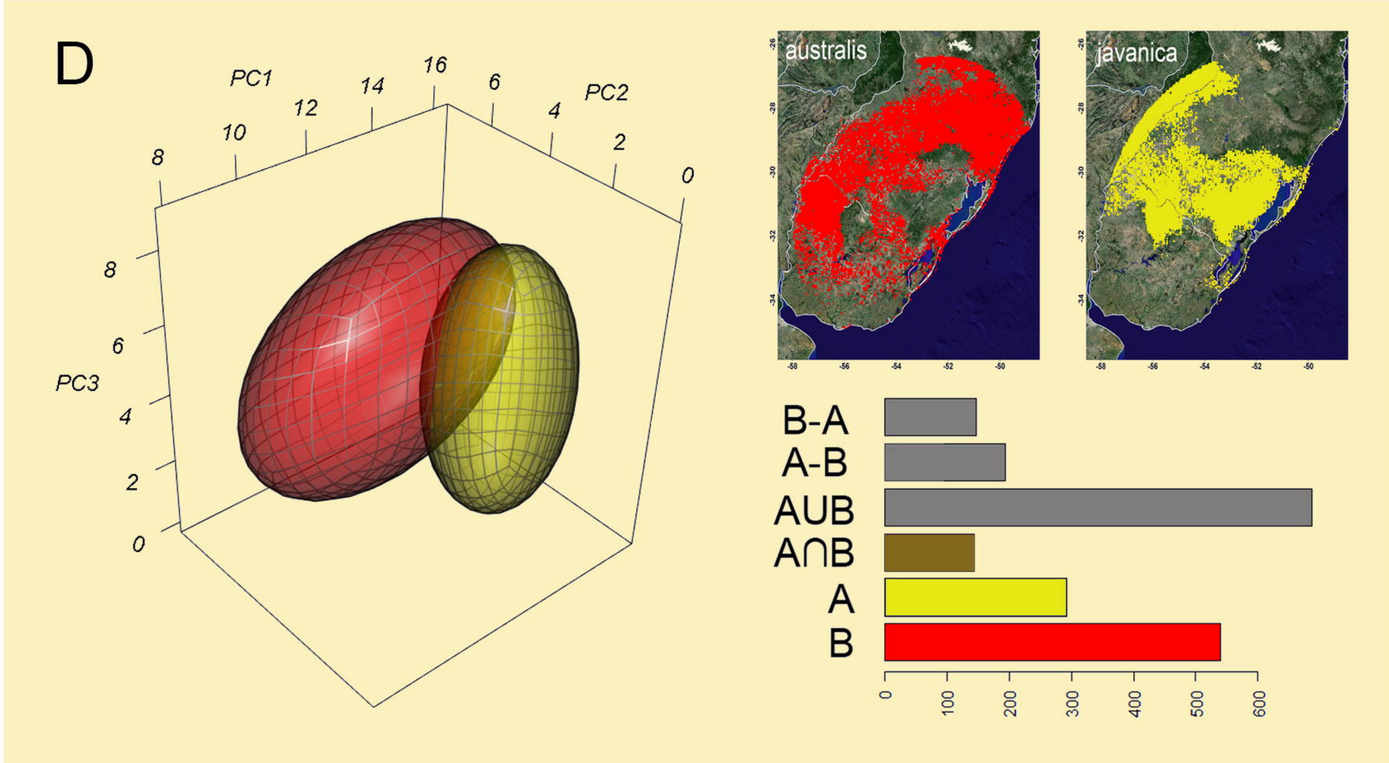
Serovars
Precipitation
Temperature
NDVI
Livestock
australis
autumnalis
canicola
castellonis
celledoni


D
PC1
PC2
PC3
australis
javanica
B-A
A-B
AUB
A∩B
A
B
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100
200
300
400
500
600

australis

autumnalis

canicola

castellonis

celledoni

copenhagen

grippotyphosa

hardjo

hebdomadis

icterohaemorrhagiae

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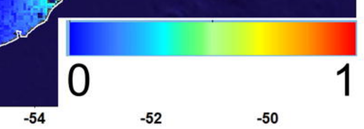
pomona

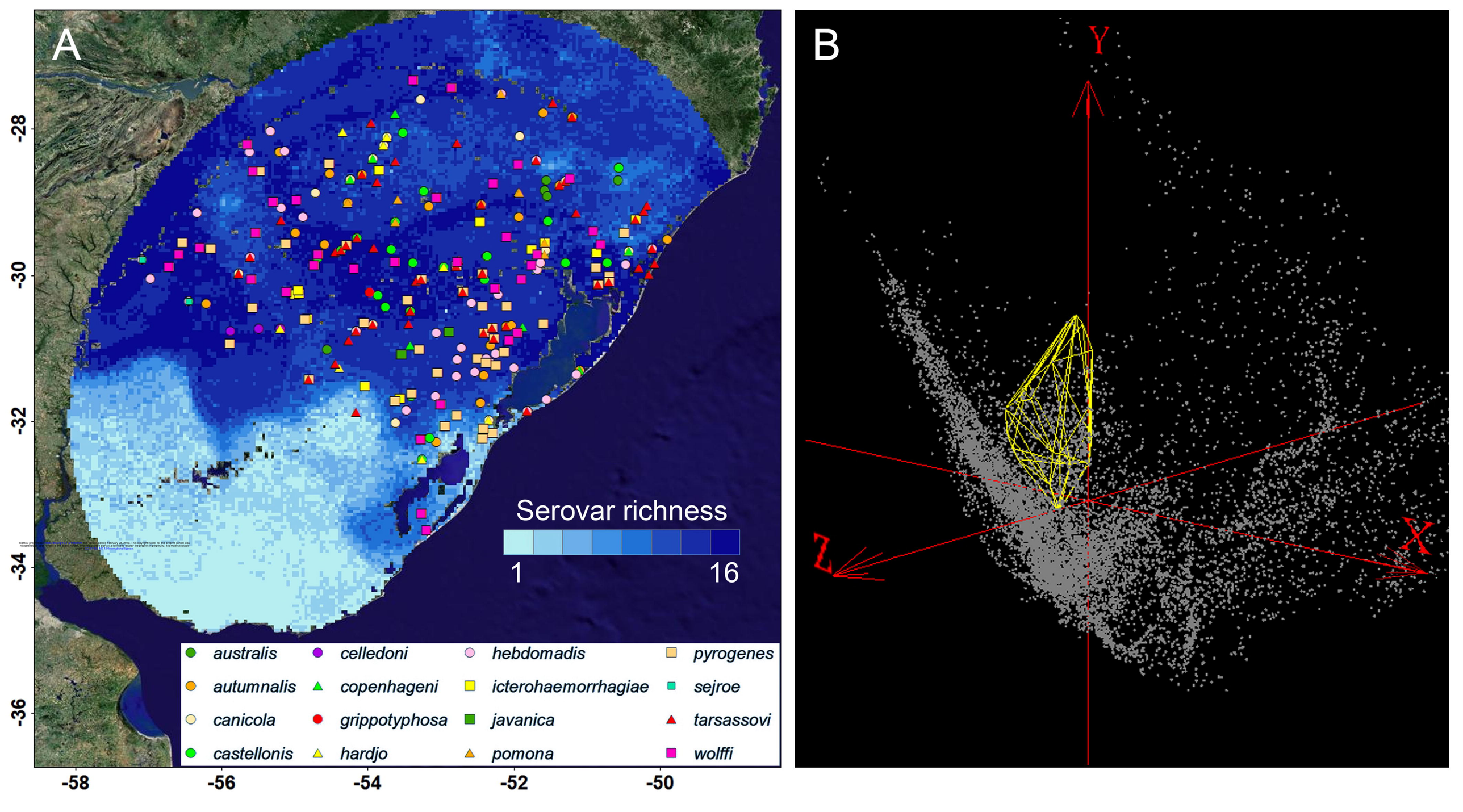
pyrogenes

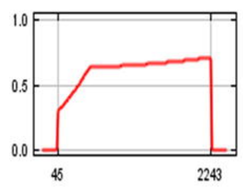
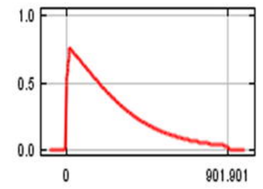
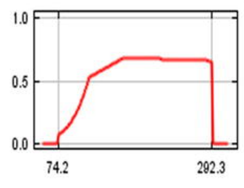
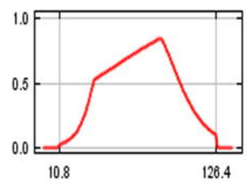
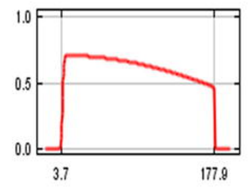
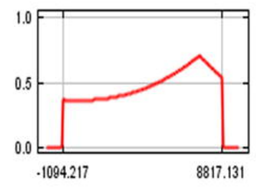
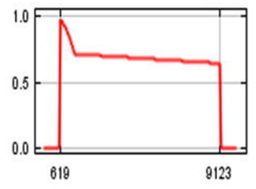
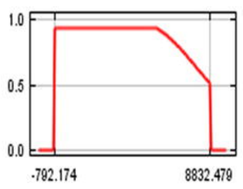
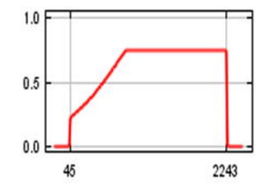
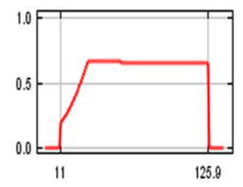
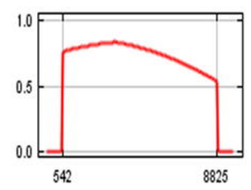
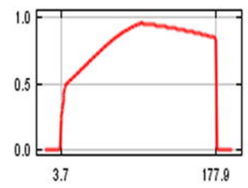
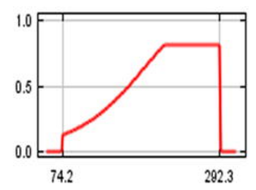
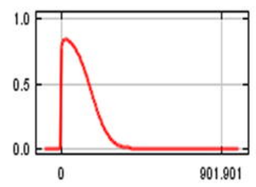
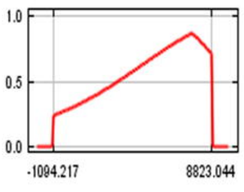
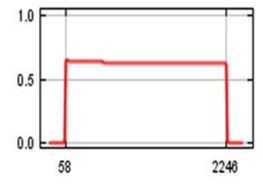
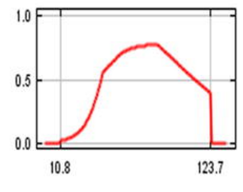
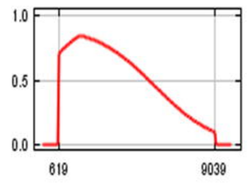
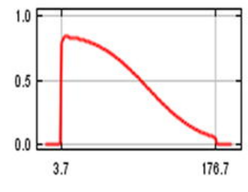
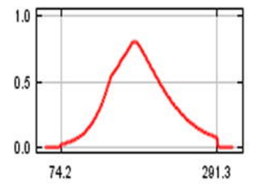
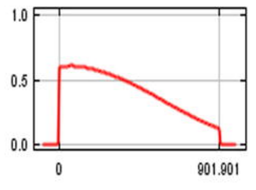
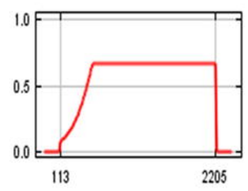
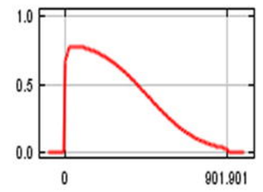
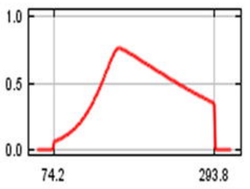
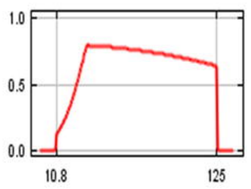
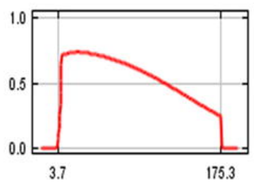
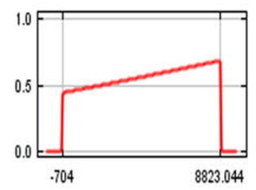
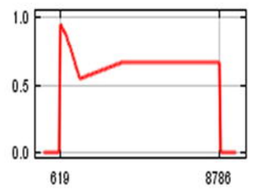
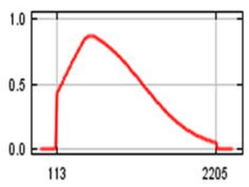
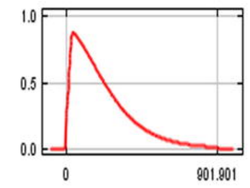
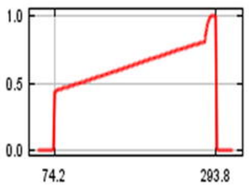
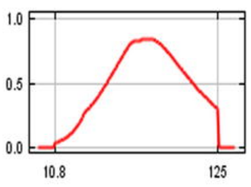
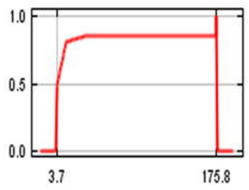
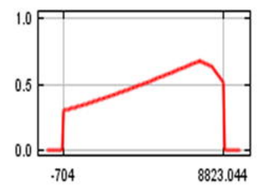
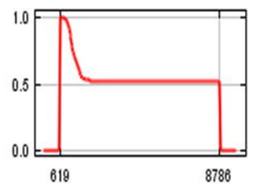
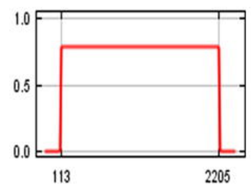
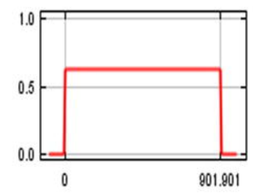
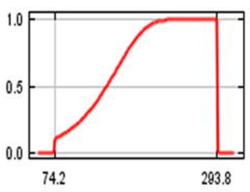
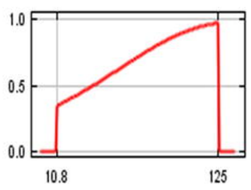
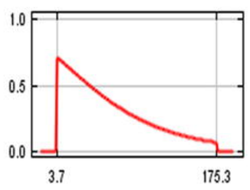
sejroe

tarassovi

wolffi




A
B
Y
X
Z
Serovar richness
1
16
-28
-30
-32
-34
-36
-58
-56
-54
-52
-50
australis
autumnalis
canicola
castellonis
celledoni
copenhageni
grippotyphosa
hardjo
hebdomadis
icterohaemorrhagiae
javanica
pomona
pyrogenes
sejroe
tarsassovi
wolffi

	Temperature	Wetness index	Soil pH	NDVI	Runoff	Precipitation	Livestock
Leptospira (all)							
Australis							
Autumnalis							
Canicola							
Castellonis							
Celledoni							
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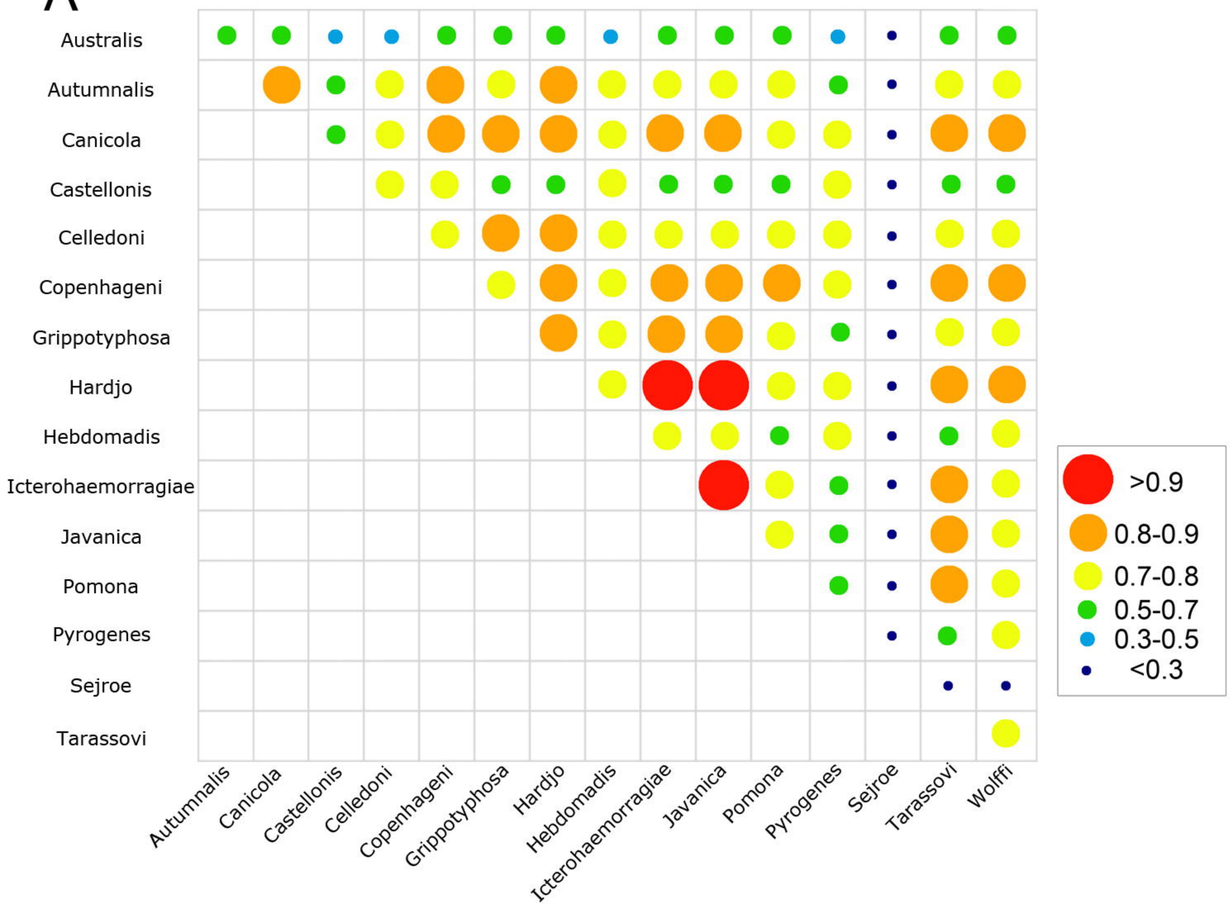

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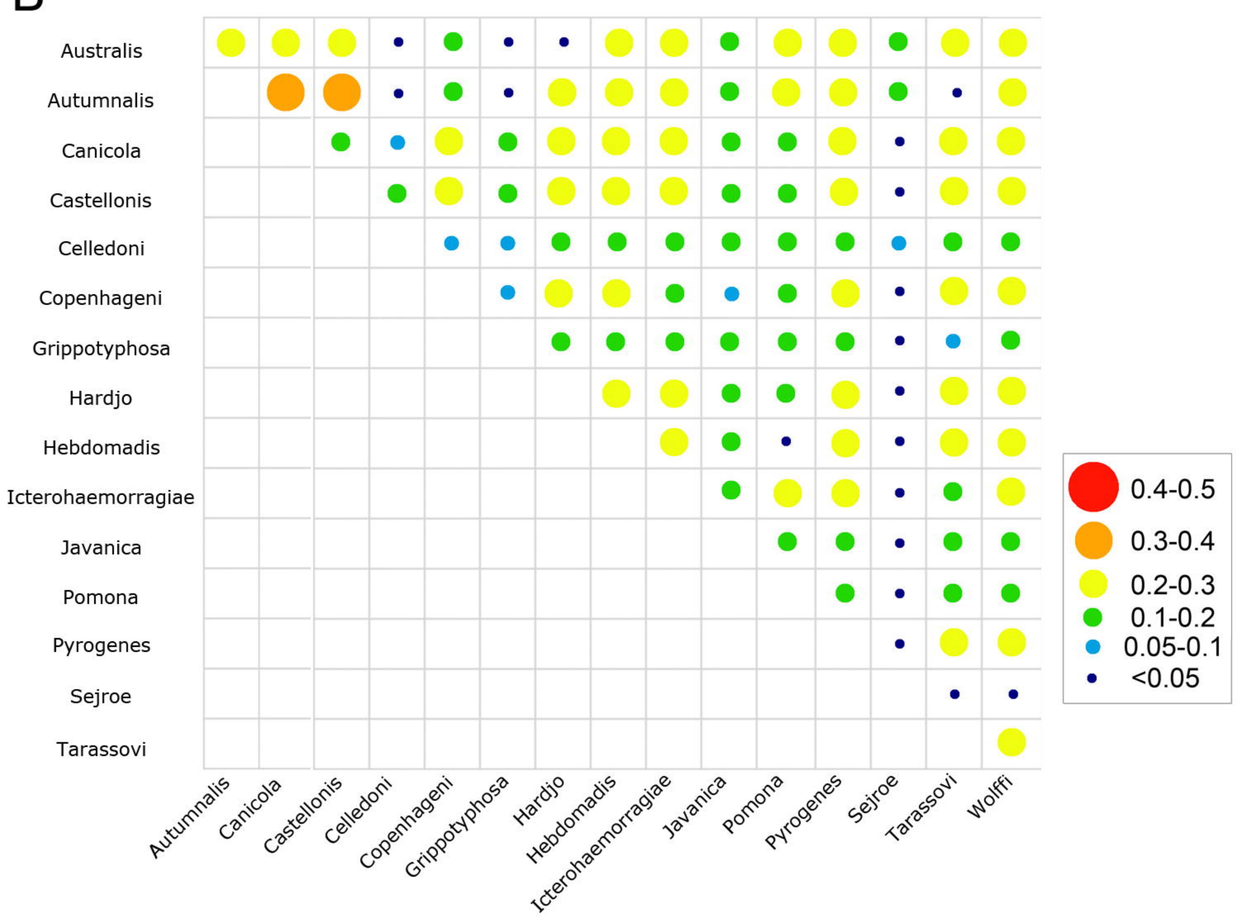

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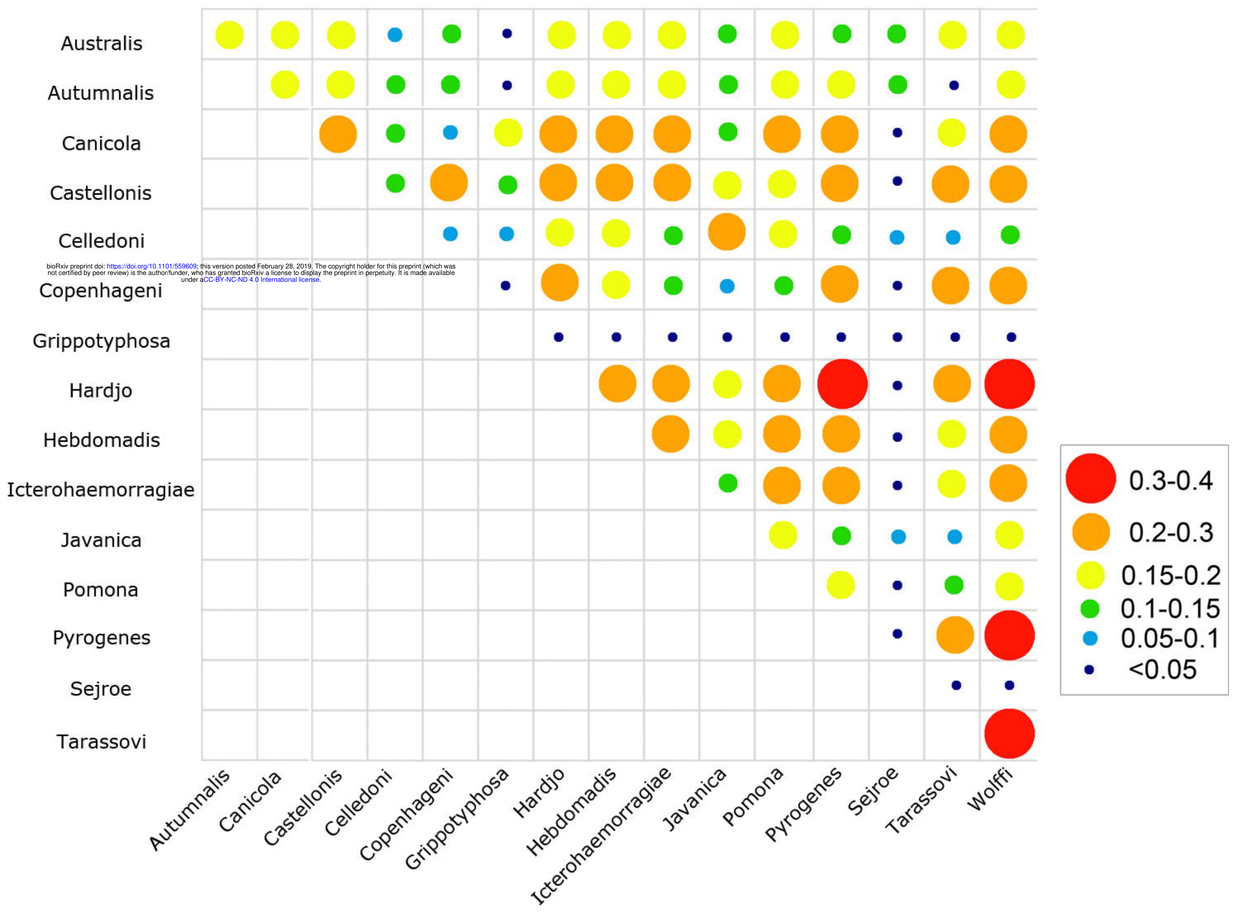

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