Porcine reproductive and respiratory syndrome virus dissemination across pig production systems in the United States

Running title: Evolutionary epidemiology of PRRSV

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Summary

Porcine reproductive and respiratory syndrome virus (PRRSV) remains widespread in the North American pig population. Despite improvements in virus characterization, it is unclear whether PRRSV infections are a product of viral circulation within a farm, within production systems (local) or across production systems (external). Here we examined the dissemination dynamics of PRRSV and the processes facilitating its spread within and among pig farms in three production systems. Overall, PRRSV genetic diversity declined since 2018, while phylodynamic results support frequent transmission across-production systems. We found that PRRSV dissemination occurred mostly through transmission between farms of different production companies, which were predominant for several months, especially from November until May when PRRSV tends to peak in the studied region. Within production systems, dissemination occurred mainly through regular pig flow (from sow to nursery and then to finisher farms); nevertheless, an important flux of PRRSV dissemination from finisher to sow and nursery farms highlighted the importance of downstream farms as sources of the virus. Farms at areas with pig density from 500 to 1000 pig/km^2 and farms located at a range within 0.5 km and 0.7 km from major roads were

more likely to infect by PRRSV, whereas farms at elevation between 41 and 61 meters and denser vegetation acted as dissemination barriers. Although remains a challenge, there is a need to disentangle the route of PRRSV transmission, results evidenced that dissemination among commercially unrelated pig production systems was intense, reinforcing the importance of farm proximity on PRRSV spread. Thus, consideration of farm location and their geographic characteristics may help to forecast dissemination. The understanding of PRRSV transmission routes has the potential to inform targeted strategies for its prevention and control. Further studies are needed to quantify the relative contribution of PRRSV transmission routes.

Keywords: molecular epidemiology, phylodynamics, virus dispersal, between-farm dissemination.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) remains among the most costly diseases in North America (Neumann et al., 2005; Holtkamp et al., 2013; Pileri and Mateu, 2016). Despite the great advances in reducing the incidence of PRRSv in some regions (Rathkjen and Dall, 2017) by enhanced biosecurity (Silva et al., 2019) and immunization strategies (Corzo et al., 2010), every year approximately 20-30% of US breeding herds still become infected with PRRSV (MSHMP, 2020).

PRRS is caused by an enveloped positive single-stranded RNA virus in the family Arteriviridae, order Nidovirales (Cavanagh et al., 1990). The genome of PRRSV is ~15 kb and consists of 9 open reading frames (ORFs) that encode seven structural proteins and 14 non-structural (Dokland, 2010). The mutation rate of PRRSV hinders the effectiveness of immune response against newly introduced variant strains, especially in farms with recurrent outbreaks (Linhares et al., 2014; Sanhueza et al., 2019). Likewise, recombination between North American wild-type virus strains and modified-live vaccines represents an important mechanism in the historical evolution and spread of PRRSV (Yuan et al., 1999).

The phylodynamic patterns of PRRSV spread and dissemination among pig systems and farm types have been previously described (Shi et al., 2010; Franzo et al., 2015; Alkhamis et al., 2016; Alkhamis et al., 2017; Sun et al., 2019). While there was support for between-production system

53 dissemination (Alkhamis et al., 2016), the relevance of transmission in the maintenance of local
54 incidence, in comparison with within-production system circulation, remains unknown. Thus, the
55 question of whether PRRSV circulation is the product of local dissemination between farms that are part
56 of a common commercial network (production system) versus the result of external viral introductions
57 from nearby infected farms of distinct production systems still remains (Arruda et al., 2018).
58 Bayesian phylodynamic approaches have provided a breakthrough in tracking pathogen
59 dissemination dynamics (Baele et al., 2018; Faria et al., 2019; Hicks et al., 2020). Structured coalescent
60 models and other phylodynamic approaches allow the estimation of relative contribution of local
61 dissemination versus external introductions to the overall incidence of disease (Rasmussen et al., 2018;
62 Yang et al., 2019; Hicks et al., 2020). Novel methods have been developed to integrate spatial and
63 environmental covariates in phylodynamic analysis, to determine their influence on the spread of
64 epidemics (Dudas et al., 2017; Fountain-Jones et al., 2018; Rasmussen et al., 2018; Dellicour et al., 2019;
65 Yang et al., 2019). Thus far, such approaches have not been widely implemented to study the
66 dissemination of diseases in domestic animals, except for Foot-and-Mouth virus (Duchatel et al., 2019).
67 In this study, we used phylodynamic methods to determine the viral dissemination rates among
68 pig production systems to assess whether different PRRSV variants are the product of local transmission
69 or external introductions and identified the factors that facilitate or impede PRRSV spread.

70 **Material and methods**

71 **Data collection**

72 In total, our dataset comprises 4,970 ORF-5 sequences collected from 2014 to 2019, obtained from three
73 commercially unrelated pig production systems (here coded as A, B, and C), in the US. Production
74 system A, B and C do not shared feed truck or traded pig among themselves. Sequences were generated
75 through outbreak investigation and from regular surveillance activities performed by each production
76 system and shared with the Morrison Swine Health Monitoring Program (MSHMP). Each sequence was
77 attributed to a farm type (e.g., sow, nursery, finisher, and boar stud), geographic location, pig production
78 system (A, B, and C), and the date when the sequence was reported.

Preliminary phylogenetic analysis

Sequences were aligned using Mega X, available at www.megasoftware.net (Kumar et al., 2018). The recombination detection program (RDP) v5.3 was used to search for recombination within our dataset (Martin et al., 2015). The alignment was screened using five methods (BootScan, Chimaera, MaxChi, RDP, and SiScan), evidencing 63 recombinant sequences, which were removed from the downstream analysis (Table S1). To determine whether there was a sufficient temporal molecular evolutionary signal, we used TempEst v1.5 (Rambaut et al., 2016). To calculate the *P*-values associated with the phylogenetic signal analysis, we followed Murray et al. (2016) using 1,000 random permutations of the sequence sampling dates (Navascués et al., 2010). The relationship found between phylogenetic tree root-to-tip divergence and sampling dates (years) supported the molecular clock analysis for this study ($P < 0.05$).

Discrete and continuous phylogeographical analysis

Phylogenies were estimated by Bayesian inference through Markov chain Monte Carlo (MCMC), implemented in BEAST v2.5.0 (Bouckaert et al., 2014). By using ModelFinder (Kalyaanamoorthy et al., 2017) built into IQ-Tree version 1.6.1 (Nguyen et al., 2015) Supplementary Table S2, we compared different substitution models to find the best fit for our genetic dataset. The marginal likelihood value supported the use of the general time-reversible model (GTR) with gamma-distributed rate heterogeneity plus a proportion of invariable sites (GTR+G+I) (Tavaré, 1986). To reconstruct PRRSV dissemination across systems and between farm types (i.e., sow, nursery, finisher, boar stud), we used the discrete-trait phylogeographic models implemented in BEASTv2.5.0 to reconstruct ancestral states (Bouckaert et al., 2014). To explore the most important historical dissemination routes for PRRSV spread across farms, as well as among pig systems, we used Bayesian stochastic search variable selection (BSSVS) (Lemey et al., 2009). Through BSSVS, we identified the non-zero rates of change between each pair of discrete traits (production system and farm types) based on its Bayes factor value (lower than 3). To perform this analysis, an asymmetric migration rate matrix was assumed. To infer the intensity of directional transitions (forward and backward) within the migration matrix, we used a Markov jump approach. To evaluate oversampling and possible selection bias towards location and/or farm types with

105 disproportionate amounts of samples (i.e., sow farms), we downsampled on each system to match the
106 number of samples between the three systems, maintaining the same proportion of farm type (system A -
107 89%, B =-39.5%, and C=31.4 %).
108 To analyze the spatiotemporal spread of PRRSV, we performed a Bayesian continuous
109 phylogeographic analysis, where the nucleotide substitution model was similar to the discrete model
110 mentioned above. In addition, we used an uncorrelated relaxed molecular clock model with lognormal
111 distribution (Drummond et al., 2006). The coalescent tree prior used was Bayesian SkyGrid with
112 covariates (Gill et al., 2016). Phylogeographic inference of ancestral locations was performed under a
113 relaxed random clock model, considering a lognormal distribution. All analyses were run for 200 million
114 generations, sampling every 10,000th generation and removing 10% of the chain as burn-in. To estimate
115 the relative genetic diversity, we used skygrid which is based on a nonparametric coalescent model, and is
116 utilized to calculate the effective population size over time (Hill and Baele, 2019). To visualize the
117 spatiotemporal spread of PRRSV, we used Spatial Phylogenetic Reconstruction of Evolutionary
118 Dynamics using Data-Driven Documents (D3) SPREAD3 software (Bielejec et al., 2016). To evaluate the
119 impact generated by the imbalance distribution of sequences among systems and production types, a
120 sensitivity analysis was performed. A subsample taken from the systems and farm types with the highest
121 number of sequences was used to match the sample size in the systems with the least number of
122 sequenced, thus this smaller sample was reanalyzed (Supplementary material S6).
123 **Spatiotemporal epidemiological statistics**
124 To summarize PRRSV diffusion over time and space we analyzed data from each production system
125 separately and all the sequences as a whole, using the R package "seraphim" version 1.0 (Dellicour et al.,
126 2016). Spatiotemporal information about lineage locations was extracted from 100 input trees sampled at
127 regular intervals from the post-burn-in posterior distribution to account for phylogenetic uncertainty
128 (Dellicour, Rose and Pybus, 2016). Each phylogeny branch was summarized and represented as a distinct
129 vector defined by its starting geographic location, ending geographic location and dates (Dellicour, Rose
130 and Pybus, 2016; Dellicour et al., 2016). Each branch vector represents an independent viral lineage

131 dissemination event (Pybus et al., 2012; Laenen et al., 2016). Each vector was assigned to a diffusion
132 coefficient value (*D*) for subsequent visualization: as described by (Pybus et al., 2012), which accounts
133 for the time (in years) during which each specific vector (lineage) moved from its initial location until the
134 end location of each lineage (measured from the location of the tree root). These vectors were also used to
135 assess the invasion velocity of the sampled lineages. To determine and compare the PRRSV diffusivity
136 associated with the overall data and for each production system, we estimated the spatial diffusion
137 coefficient using *D*-weighted statistics (Trovão et al., 2015) since this index is less sensitive to extreme
138 values of short branches. These results were displayed via: i) dissemination epidemiological statistics that
139 estimated the mean branch dissemination velocity, and ii) kernel plots showing the lineage diffusion
140 coefficients of the mean and variation on the highest posterior density (HPD).

141 **Between-production systems transmission: the contribution of within-system PRRSV circulation**
142 **(local) versus external introduction of virus from a commercially unrelated pig farm.**

143 We estimated the contribution of local transmission (i.e., between farms within a production system)
144 versus external introductions (i.e., between farms across production systems) using a structured
145 coalescent model. Due to computational demand, we used PRRSV alignment from MEGA software
146 (Kumar et al., 2008) to produce a phylogenetic tree using PhyML version 3.0 (Guindon et al., 2010),
147 using a substitution rate = 0.00672 (estimated), and considering an HKY Hasegawa-Kishino-Yano + Γ4
148 (Hasegawa et al., 1985; Yang, 1994), which was dated using Least Squares Dating (LSD) version 0.3 (To
149 et al., 2016). To infer the contribution of local versus external introductions the migration rates between
150 different pig production systems were estimated using the Marginal Approximation to the Structured
151 Coalescent (MASCOT) package (Müller et al., 2017, 2018) implemented in Beast2 (Bouckaert et al.,
152 2014). To infer the evolutionary rate from the time-calibrated tree, we used a strict clock model. Using
153 MASCOT, we estimated the effective population size in the local and external population and the
154 migration rates between them, which was allowed to be asymmetric. This analysis was replicated three
155 times, each time using one of the three pig systems as local and the other two as external. We also

156 calculated the posterior probability of each internal node in the tree being in each state (i.e., local or
157 external) using the forwards/backwards approach implemented in MASCOT (Müller et al., 2018).
158 All analyses were performed under 100 million MCMC runs, states were sampled every 10,000 and the
159 first 10% of samples were discarded as burn-in using TreeAnnotator v. 2.3.0 (Rambaut and Drummond,
160 2016). We reconstructed the number of migration events through time using the ancestral locations
161 reconstructed by MASCOT. A migration event was assumed to occur when the most probable location of
162 a lineage changed between its parent and child node, with the time of the migration event assumed to be
163 the date of the daughter node. Migration events (local and external) per month were plotted using "baltic"
164 (https://github.com/blab/baltic) in Python Software Foundation version 3.7, available at
165 http://www.python.org. Finally, we compared the differences in local vs external transmission between
166 PRRSV season and off-season through two-sample Wilcoxon test (Hogg et al., 2010), PRRSV season was
167 determined by the exponentially weighted moving average proposed by MSHMP (MSHMP, 2020). In the
168 same way as we described in the section above, a sensitivity analysis with a subsample was evaluated for
169 consistency of the results (Supplementary material S8).

170 **Factors promoting or restricting PRRSv spread**

171 To determine the effect of different predictors on the spread of PRRSV, we analyzed the results of the
172 continuous phylogeographic analysis using the R package "seraphim" version 1.0 (Dellicour et al., 2016),
173 considering a continuous location approach (based on the geographic coordinate location of each pig
174 farm) and a random walk diffusion model. Spatiotemporal information was extracted from 100 trees
175 sampled at regular intervals from the posterior distribution, after burn-in, to account for phylogenetic
176 uncertainty. Each phylogenetic branch was considered a vector defined by its start and end location (e.g.
177 latitude and longitude), and its start and end dates. Statistical significance for the correlation between
178 phylogenetic and predictive factors was tested using 100 trees generated and expressed in the form of
179 Bayes factors (BF) (Dellicour et al., 2016). Based on the extracted spatio-temporal information contained
180 in each phylogenetic tree described above, we calculated the distances based on predictor variables
181 associated with every branch of each tree, which were used to determine the correlation between the

duration of each branch in the phylogeny and its related predictor variable distance, including the annual temperature, annual precipitation, elevation, runoff (an index that quantity of water discharged in surface streams), soil humidity (commonly used to quantify topographic control on hydrological processes) (Sørensen et al., 2005), enhanced vegetation index (EVI), which can be used to quantify vegetation greenness, as well as the pig density, and distance to the main roads (Fig. 1 and Supplementary Table S3 for more details). Briefly, the distance between each farm (geographic coordinates) to the nearest main road was calculated based on the Euclidean distance. We calculated the statistic *Q*, which represents how much variation in lineage movement is explained with spatial heterogeneity when each predictor variable is considered (Dellicour, Rose and Pybus, 2016). Here, $Q = R^2_{env} - R^2_{null}$ where R^2_{env} is the coefficient of determination obtained from the regression between branch duration against predictor (factors) distances, while R^2_{null} represents the coefficient of determination for the regression between branch duration against predictor distances, which were replaced by a null raster. Thus, the factors that were considered for further Bayes factor (BF) analysis, were the ones that obtained positive *Q* values in at least 90% of the tested trees (Jacquot et al., 2017). We approximated a BF value for all predictors tested that passed the above-mentioned *Q* statistic. Predictive factors were treated as a conductance (variables that promote the spread of the disease) or a resistance factor (variables that impede its spread). For the interpretation of BF, values between 3-20 were considered "positively" supported, values between 20-150 were considered "strong", and values >150 were considered " overwhelming support" (Kass and Raftery, 1995). For all variables that showed a significant support either as conductance of resistance factors (considering *Q* statistic and Bayes factor) (Supplementary Table S10), each raster was subdivided into four alternative ranges based on Jenks natural breaks, for example, for elevation raster values below 20 meters constituted the low elevation areas, from 21-40 meters medium-low elevation, 41-60 medium-high elevation and >61 high elevation (Supplementary Table S11). The same continuous phylogeographic analysis using the R package "seraphim" was applied individually for each range raster as detailed above.

Results

Study population

208 A total of 63 recombinant PRRSV sequences were identified and removed from the database. Most
209 recombinants (52%) belonged to system B with the highest number of recombinants (82%) belonging to
210 sow farms (Supplementary Table S1). Thus, 4,753 sequences remained for further discrete and continuous
211 phylogeographic analyses. Most sequences originated from system A = 3,748 (79%), while B had 626
212 (13%), and C had 379 (8%). Overall, 2,958 sequences (62.2%) belonged to sow farms, 909 (19.1%) to
213 finishers, 873 (18.4%) to nurseries, and 13 (0.3%) to boar studs (Supplementary Table S1). The nearest-
214 neighbor distances among farms of distinct production systems had a median value of 7.6 km (1st
215 quartile= 3.6 km and 3rd quartile= 19.3 km), the closest related production systems were A and C with
216 median distance among all pairwise farms of 1.8 km (1st quartile= 1.2 km and 3rd quartile= 2.5 km), and
217 finisher farms of system A and C were the closest with median of 1.9 km (1st quartile= 1.3 km, 3rd
218 quartile= 3.2 km) (Supplementary Table S4 and S5).

219 **Discrete phylogeography analysis**

220 The results of PRRSV ancestral reconstruction showed that the most likely center of origin was
221 production system A with a root state posterior probability (RSPP) = 0.84, coming from a sow farm
222 (RSPP = 0.75). From there, it spread heterogeneously across the other systems, unrestricted to farm types
223 (Fig. 2A). The reconstructed phylogenetic relationship did not show evidence of any cluster related to
224 production systems. However, we observed a clear dominance of PRRSV originating from system A over
225 the other systems (Fig. 2A). Our sensitivity analysis on the downsample supported these results
226 (Supplementary Table S6). The relative genetic diversity expressed as the effective population size (e.g.
227 number of individuals contributing to a new PRRSV infection) over time showed a plateau until 2012.
228 After that time, we captured a steep increment that lasted until approximately 2016, when relative genetic
229 diversity decreased through June 2019 (Fig. 2B), the last month of data available for our study. The
230 temporal pattern shown by the sampled PRRSV sequences evidenced the typical annual trend of seasonal
231 outbreaks (Fig. 2B) (MSHMP, 2020). Phylogeographic results of system A showed that its most likely
232 center of origin was a sow farm (RSPP = 0.47), followed by an almost equal probability for origins in
233 finisher farm (RSPP = 0.27) and nursery farm (RSPP = 0.26), the boar stud farms were negligible (RSPP

234 < 0.01) (Fig. 3A). In recent years, there was an evident increase in the proportion of PRRSV positive
235 finisher farms, reaching a similar level of representation than sow farms for some clusters (Fig. 3A).
236 SkyGrid plot revealed a relatively constant trend of relative genetic diversity until ~2011, showing a
237 linear increase in population size, which reached its highest levels ~2016 (Fig. 3B). We observed a
238 similar trend when we analyzed all systems together (Fig. 3B), 2015 and 2018 were the years with more
239 samples sent to be sequenced (Fig. 3B).

240 For production system B, a sow farm (RSPP = 0.4) was also the most likely origin; however, a
241 high probability was also linked to a nursery farm (RSPP = 0.33) (Fig. 4A). The temporal pattern shown
242 by the effective population size was static until 2012, after which it increased rapidly until 2015-2016,
243 when it started a linear decrease until June 2019, similar to the seasonal pattern shown by the number of
244 sequences (Fig. 4B). As observed in systems A and B, the most likely center of origin for system C
245 occurred inside a sow farm (RSPP = 0.52), followed by a high probability for originating in a finisher
246 farm (RSPP = 0.4). In contrast to the other systems, we observed a low probability of origin related to a
247 nursery farm (RSPP > 0.1) (Fig. 5A). The temporal trend in the number of sampled sequences showed the
248 mentioned annual seasonal pattern, with peak occurrence ~2015. SkyGrid plot evidenced a more stable
249 pattern, with higher levels of variation in effective population size during the period 2013-2014, then
250 decreasing until 2018, when it showed a small peak and then decreased through June 2019 (Fig. 5B).

251 **Phylogeographic diffusion analysis and spread statistics**

252 The reconstructed spatiotemporal diffusion of PRRSV was characterized by continuous expansion in the
253 geographic space, accompanied by diversification events. The comparison between each system and all
254 samples together showed two noticeable patterns. The first one was characterized by a radial expansion
255 over time, found when all samples were analyzed together and within samples of system A. In contrast,
256 systems B and C evidenced a directional trend, since for both systems the spread started in the southwest
257 region. In the case of system B, PRRSV spread to the northeast, while for system C, the spread was
258 mainly to the north, though spread did occur in all directions (Supplementary Figs. S1-3).

The spatiotemporal analysis showed that when all sequences were analyzed together, the estimated median value for the mean branch velocity was 39 km/year (95% HPD = 31.2-61.1). This median value was lower than that shown by system A (49.2 km/year, 95% HPD= 42.9-60.4), and higher than the values observed in systems B and C (32.7 km/year, 95% HPD= 29.1-43.3), and (26.4 km/year, 95% HPD= 20.1-41.0), respectively. Estimated diffusion coefficients were asymmetric for each system and when all samples were analyzed together. System A showed the highest diffusivity (243.8 km^2/year); however, its diffusion variation among branches was the lowest registered. The overall results (all sequences) evidenced the highest among-branch variation, while its diffusion coefficient (150 km^2/year) was lower than system A and substantially higher than the other systems. Systems B and C showed a similar pattern in terms of diffusion coefficient variation among branches; however, B was higher than C (97 km^2/year) and (51 km^2/year), respectively (Supplementary Fig. S4).

PRRSv dissemination among pig systems and farm types

Bayesian stochastic search variable selection (BSSVS) identified that the most significant disseminations were highly related to system A, evidencing strong support by a Bayes Factor score > 20. The statistical support or evidence for system A sourcing most PRRSV strains to system B, BF = 102.1 (average relative migration rate "ARMR"= 1.79), followed by dissemination from A to C, BF = 47.8 (ARMR = 0.46); of less importance was from B to A, BF= 27.4 (ARMR = 0.35). All the other disseminations between production systems were also positive, but to a lesser degree than the ones mentioned above (Table 1). In addition, a sensitivity analysis aimed to evaluate the bias related to oversampling showed that while numerically it was a difference the interpretation remains the same, but we noticed that in the subsample there was even more support for the dissemination from company B to C and company C to A (Supplementary Table S7 for more details). Our analysis reveals that when comparing PRRSV dissemination between types of farms, the most frequent routes were linked with sow farms, especially when the direction of spread was from sow to nursery (BF > 42). The highest support for this route occurred when we analyzed all systems combined (BF = 109). The second most well-supported dissemination occurred from sow to finisher farms (BF > 17.9), and as in the previous case, was best

285 supported when we analyzed all systems together. (BF = 80.5). Likewise, the dissemination between
286 nursery and finisher farms (in both directions) also showed positive support (BF > 6). However, all routes
287 of dissemination that involved boar stud farms were rare events (BF < 3) (Table 2). Furthermore, the
288 sensitivity analysis showed similar trends, where the results of the equal sample sizes these were less
289 sharp than the one using the original dataset. However, the transmissions that involved BoarStud were all
290 negligible (Supplementary Table S8 for more details).

291 **Within-production systems circulation (local) versus introduction of PRRSV from commercially**
292 **unrelated farms (external)**

293 We tracked the origin of the migration events leading to the current PRRSV lineages circulating among
294 the three pig systems, to determine whether detected PRRSV strains originated from within the same
295 system (local) or from farms of non-commercially related pig systems (external introductions). For this
296 analysis, we considered each coalescent event in the tree as a proxy for the timing of a transmission event
297 and then classified each event as either a local transmission event if the parent and child lineages were
298 both reconstructed to be in the local population or an external introduction if the parent lineage resided
299 outside the local population. Overall, results showed a clear dominance of external transmission events
300 that were observed in all pig companies; this tendency was constant during the whole period of study
301 (Fig. 6). System C predominantly had external introductions which peaked in 2015 with 94.7% of the
302 transmission events, while systems A and B exhibited 82.0% in 2018 and 82.4% in 2017, respectively
303 (Table 3). In addition, our results show that overall, the external introduction events tend to increase over
304 time (Table 3). We also observed that coinciding with the month of higher incidence of PRRSV
305 (MSHMP, 2020) (Fig. 6 and Supplementary Table S9). Two-sample Wilcoxon test indicated that the
306 difference between the within farm transmission and external introductions were statistically significantly
307 higher during PRRSV season (mean= 69.3) than the rest of the year (mean= 60.1) (Z= -9.9, p< 0.001).

308 **Factors promoting or restricting PRRSV dissemination direction**

309 To quantify the impact of each factor on PRRSV distribution, we calculated the association between
310 lineage duration and each covariate scaled distance through the Q statistic method. We found strong

311 support (*Q* value was positive) for pig density and distance to main roads treated as conductance factors,
312 while elevation and vegetation presented strong support as resistance factors (Supplementary Table S10
313 and Supplementary Figs. S5 and S6 for more details). These results also received statistical support
314 provided by Bayes factors (BF) since we observed that farms located in areas with a high density of pig
315 farms were more exposed to the introduction of PRRSV (BF > 12). Likewise, the distance between the
316 farm and the nearest major road also showed a positive association with PRRSV diffusion (BF > 7). In
317 contrast, the factors identified as negative drivers, variables that act as barriers for the spread of PRRSV,
318 were higher elevation and denser vegetation (BF > 3) (Supplementary Table S10). With respect to the
319 analysis of the variables with significantly supported as conductance, analyzed as ranges (Supplementary
320 Table S11), the density of pigs ranging from 501-1.000 number of pigs/km^2 was sufficient to facilitate the
321 spread of PRRSV when all sequences were analyzed together and for system A, while farms at B and C
322 were more likely infected when pig density was above 1.000 pigs/km^2. Likewise, farms located at a
323 distance range of 1 km to 2 km of main roads showed to favor PRRSV introductions, while when systems
324 were analyzed in separately the dangerous distance was between 0.5 km and 1 km (Supplementary Table
325 S11). On the other hand, for variables acting as resistance factors, our results were consistent in which at
326 elevation above 61 meters was sufficient to slower PRRSV movement, but for system C range of 41-61
327 meter was sufficient. The results of vegetation index were in disagreement, while for all systems together
328 there was no analytical support, but farms at system A, B and C an EVI index between 41 and 45 acted as
329 a resistance range (Supplementary Table S11).

330 **Discussion**

331 This analysis detailed the evolutionary epidemiology of PRRSV among commercially related and
332 unrelated farms through continuous and discrete phylogeographic analyses. Overall, a decline in PRRSV
333 genetic diversity was observed over time. Such findings could have different explanations, one being a
334 rapid and efficient disseminating strain that was introduced into sow farms which consequently was
335 transferred to growing pig sites through regular pig movement. Another potential explanation could be
336 related to the efficacy of current interventions that local production systems have implemented, which

337 reduced the volume and frequency of newly emerging strains, suggesting a more optimistic scenario than
338 proposed in a similar study done in another US region (Alkhamis et al., 2017). Given the economic
339 impact and herd health relevance of new PRRSV strain introductions, it is imperative to evaluate how
340 often PRRSV spreads among pig production systems, determine the role of each farm type, and identify
341 factors that may facilitate or keep PRRSV from spreading. We demonstrated that PRRSV circulation has
342 been mostly originated by transmission from outside of each pig production system, and this tendency is
343 more predominant during the PRRSV season (from November until May) (MSHMP, 2020).

344 Our findings suggest that virus transmission between pig systems is common. It is not well
345 understood which transmission mechanism plays a role in the dissemination across systems. Airborne
346 spread has been suggested as a potential route (Dee et al., 2009; Otake et al., 2010). However, Arruda et
347 al., (2018, 2019) did not reach that same conclusion. Pig farm density as expected arises as a risk factor in
348 several studies and in our study, our phylodynamic findings agree with a regional spread as an important
349 factor for PRRSV dissemination among pig farms. PRRSV spread events appear to be relatively common
350 in farms located in highly dense pig areas and near main roads, while farms at higher elevations and
351 surrounded by denser vegetation were significantly less likely to report new cases of PRRSv (Arruda et
352 al., 2017). It is important to mention that the source of our sequence data represented a limitation for our
353 phylodynamic analysis since it was a convenience sample that can introduce bias into our analysis as the
354 number of sequences does not directly represent all PRRSV cases circulating in this study region. There
355 was important asymmetry in the number of sequences from boar studs, thus results of this farm type
356 should be interpreted with caution.

357 **PRRSV spatiotemporal dynamics**

358 The spatiotemporal dynamic pattern exhibited by PRRSV is the result of its transmission dynamics and
359 how circulating strains have been affected by viral selection (Pybus and Rambaut, 2009; Volz et al.,
360 2013). This can be observed in the shape of PRRSV phylodynamic patterns over the course of this study,
361 which shows a clear directional selection, which can result in the loss of genetic variation (Volz et al.,
362 2013). Based on MSHMP data (MSHMP, 2020), vaccine usage increased between 2012 and 2014, which

363 could have impacted PRRSV genetic diversity for system A after 2012. But the increment in the number
364 of farms using vaccines during 2014 did not show a noticeable effect on PRRSV genetic variation.
365 However, the increment in the application of MLV's in mid-2015 showed an undeniable positive effect
366 on decreasing PRRSV genetic diversity (as seen in Figs. 2-5), similarly to the observations made by other
367 authors (Sun et al., 2012; Jeong et al., 2018). However, to increase the robustness of future studies it is
368 necessary to increase the farm coverage as well as the timeframe, not only related to the PRRSV genetic
369 information but also to the vaccination data.

370 The estimated speed in which PRRSV was spreading was 39 km/year which was slightly higher
371 than previous observations in a study that used data from one production system in another U.S. region
372 (34 km/year) (Alkhamis et al., 2017). This difference may be related to pig farm densities, being our
373 study region more populated with an average of 3 farms per 5 km^2, and a more loyal representation of
374 PRRSV dynamics since we compiled samples of more than 92% of all farms in our study area..
375 Consequently, there are important aspects that have to be considered while analyzing the speed at which
376 PRRSV spreads, for example, those related with the overall pig health (i.e., absence of coinfections) and
377 factors related with on-farm biosecurity (i.e., presence of a line of separation, cleaning, and disinfection
378 station) (Rappole et al., 2006; Altizer et al., 2011; Lycett et al., 2019). Thus, further studies are needed to
379 evaluate how biosecurity practices and overall pig health may contribute in slowing or accelerating
380 PRRSV spread, results of these assessments can be used to predict the most effective biosecurity
381 measures and optimize control strategies that go beyond PRRSV (Silva et al., 2019), not to mention
382 current diseases that pose large-scale biosecurity threats to the U.S swine health such as African Swine
383 Fever.

384 **PRRSV spread among and within farms of commercially unrelated production systems**

385 Our analysis found relevant similarities in the spatiotemporal patterns of PRRSV spread among all farm
386 types of all production systems (Table 1, 2), which was similar to previously reported findings that used
387 data from one production system in the U.S. (Alkhamis et al., 2016). At farm type levels, sow farms were
388 by far the biggest spreader (Plain and Laurence, 2003) which was expected as they wean piglets on a

389 weekly basis and these can be transported several miles away to an off-site growing pig facility.
390 Phylogenetic results also showed that PRRSV introductions into sow and nursery farms likely originated
391 from finisher farms, which could be partially related to gilt development units sending replacement gilts
392 to sow farms (Dee, 1995; Perez et al., 2015). Despite the substantial efforts to improve farms' biosecurity
393 and immunization through the implementation of strategies such as, sow herd air filtration, gilt
394 acclimation, virus elimination strategies (i.e., load-close-expose and rollover) which successfully reduced
395 the number of PRRSV outbreaks (Corzo et al., 2010; Alonso et al., 2012; Velasova et al., 2012; Alonso et
396 al., 2013; Blanchette, 2015; Silva et al., 2019), we continue to observe significant PRRSv dissemination
397 by indirect routes (Corzo et al., 2010; Otake et al., 2010). Therefore, it is expected that changes in
398 immunization protocols, such as varying vaccine brands at farms of one specific system may have a
399 serious impact on PRRSV spread.

400 **Within-production systems circulation (local) versus introduction of PRRSV from commercially**
401 **unrelated farms (external)**

402 Between-farm animal movement has historically been associated with disease spread (Augusta et al.,
403 2019; Moon et al., 2019; Machado et al., 2020). Here, we used structured coalescent models to infer
404 migration rates among commercially unrelated pig farms, calculating PRRSV dissemination not related to
405 between-farm pig movement. Overall, PRRSV dissemination in this region has been mostly characterized
406 by transmission between non-commercially related farms (external introductions) rather than within a
407 production system (local). In addition, we observed that during PRRSV "season" (November-May), the
408 external transmissions tended to be greater than during the rest of the year. While our analyses was not
409 aimed to explore the route of transmission events or how viral lineages first entered a population
410 (Rasmussen et al., 2018), they reinforce the importance of PRRSV collateral spread and open the question
411 of how this dissemination is happening. Indeed, a recent work (Galvis et al., 2020) showed that for sow
412 farms more than 50% of the between-farm transmission occurred by proximity among infected and
413 susceptible farms, while 20% and 53% of the nurseries and finisher farms were infect by the same route,
414 respectively. While little is known about this collateral PRRSV propagations, at some level the external

415 introduction have been associated with contaminated vehicles that service multiple production systems
416 (i.e., general maintenance, vaccination, cleaning or loading) (Otake et al., 2003; Dee et al., 2004), shared
417 equipment (Pitkin et al., 2009), non-pig animal vectors (Otake et al., 2002; Schurrer et al., 2005),
418 transport of semen (Christopher-Hennings et al., 1995) and airborne transmission (Lager and Mengeling,
419 2000; Trincado et al., 2004; Cho et al., 2007; Dee et al., 2009). At some degree, a contracted farm will
420 start producing pigs under a new production system, thus it is possible that a resident virus would be
421 introduced and become widespread within the new production system network. Knowledge gaps
422 regarding local dissemination of PRRSV still exist, hence, studies that consider phylodynamics coupled
423 with epidemiological models such as susceptible–infected–recovered to assess the relative contribution of
424 between-farm PRRSV transmission routes are needed. In addition, the proposed modeling should allowed
425 not only to answer "how it can spread" questions but also "what for", i.e. determining what effort is
426 required for reducing transmission (Beaunée et al., 2017).

427 **Factors promoting or restricting PRRSV dissemination direction**

428 In our PRRSV dissemination models, directions of lineages revealed the potential importance of two
429 factors that limited the spread of PRRSV. Vegetation density and high elevation, which are similar to
430 previous observations made by Mahesh et al., (2015), Arruda et al., (2017), and (Balka et al., 2018).
431 These authors also highlighted the important role that topography may play in mitigating the airborne
432 spread of PRRSV. In the same way, a study on foot-and-mouth disease, which also used continuous
433 phylogeographic analysis, identified that the presence of pure cropland or mixed land use both had a
434 negative impact on virus diffusion (Duchatel et al., 2019). Numerous studies have shown multiple
435 positive impacts of having vegetation barriers surrounding farms (i.e., trees, shrubs) over the airborne
436 spread of different pathogens (Malone, 2004; Patterson et al., 2008; Van Ryswyk et al., 2019), thus it
437 should be recognized as a possible biosecurity strategy. This study provided the first look at which
438 amount of healthy vegetation needed to protect farms from new introductions, consistently EVI index
439 between 41 and 45 showed to be sufficient. These values correspond to a dense tree coverage that is
440 highly similar to the values exhibited by evergreen broadleaf forest, trees within this category are live oak

441 (*Quercus virginiana*), laurel oak (*Quercus laurifolia*) and loblolly pine (*Pinus taeda*) (Zhang et al., 2017).
442 Similarly, high elevated areas have proven to act as a natural topographic barrier for the local spread of
443 pathogens (Jacquot et al., 2017), where the slope of the terrain was found to have an association with a
444 lower PRRSV incidence (Arruda et al., 2017). We were able to identify a safe range of elevation that was
445 consistent above 61 meter from the sea level, while this can help the local production systems, other
446 regions of the US would need to be analyzed separately due to the differences possible elevation ranges.
447 The identification of the main drivers associated with PRRSV dissemination could help in deciding about
448 new farm locations, especially for systems located in highly dense pig areas (Yang et al., 2019). Farms
449 located in areas with higher farm density were more likely to sustain new PRRSV introductions, we
450 highlight areas in which the volume of pigs by km^2 above 500 animals, as has been previously described
451 by Firkins and Weigel, (2004), Arruda et al., (2017) and Alkhamis et al., (2017, 2018). In addition, our
452 results identified distance to the main road as associated with increased chances for PRRSV circulation.
453 This has led concern by the swine industry (Reicks, 2019), since vehicle movement has demonstrated to
454 have a significant impact on increasing the transmission of several other diseases, for example, African
455 Swine Fever (ASF) (Mur et al., 2012), Classical Swine Fever (CSF) (Bronsvoort et al., 2008), and for
456 Foot and Mouth Disease (Muroga et al., 2013). Our findings indicate that farms located at 0.5 km to 0.7
457 km from major roads were more easily exposed to PRRSV, therefore this distance could be used to make
458 decisions about routes used for pig movement, the enhancement of on-farm biosecurity of sites within this
459 range of distances to major roads, and in future expansions of operations new buildings could be built
460 beyond 0.7 km from the main road.

461 Here, we showed that continuous phylogeographic reconstructions represent a useful tool to
462 describe and analyze the dissemination of PRRSV spread. Although the outcomes of phylodynamic
463 analyses can be exploited to investigate the impact of relevant factors on lineage dissemination diffusion,
464 it is important to note that such analyses depend on inferred viral lineage movement and thus, on the
465 spatial distribution of the sampled sequences (Dellicour et al., 2019). Our samples represent the
466 circulating PRRS viral population, as data comes from ~ 92% of pig farms in one highly dense pig region,

467 therefore can be directly applied in the region. Given that most commercial pig operation in North
468 America are similar but not the same our finding could also help the swine industry decision making on
469 other parts of the county, conditional to data availability this analysis can be replicated at each relevant
470 region or production system.

471 Our study was subject to a variety of limitations. The results of distance to main roads as a proxy
472 for the exposure to PRRSV was inconsistent thus need to be interpreted with caution, when we analyzed
473 all production systems together the distance of 1 km to 2 km was the only dangerous range, even though
474 when analysis of individual systems showed that 0.7 km was enough this result need to the interpreted
475 with caution. In the same why swine densities above > 1000 pigs/km^2 were not supported as conductance
476 while range of 500- 1000 pigs/km^2, in both cases distance to main road and swine density, one reason for
477 both inconsistence results could be related to the number of farm in such range intervals. For that reason,
478 there is an urgent need to expand the current phylodynamic framework to allow for the calculation of
479 point estimates of the likelihood of PRRSV spread driven by one and more predictor variables more
480 directly, which would also considered categorical variables allowing us to determine relative safe or
481 dangerous distances to main roads, for example (Dellicour et al., 2020).

482 **Conclusion**

483 Our study revealed significant asymmetries in the phylodynamic patterns among farms of three pig
484 production systems. As a result of interventions that could be related to immunization and on-farm
485 biosecurity, PRRSV genetic diversity has consistently declined in this region, especially in the last two
486 years, which could explain why 2019 has had one of the lowest counts of PRRSV cases during its typical
487 high season in the US (Sanhueza et al., 2019). Our results evidenced that PRRSV transmission across
488 each system is most likely to originate from outside production systems. These results reiterate the
489 importance of PRRSV farm proximity as a relevant route for virus dissemination. We highlight
490 characteristics of locations to which PRRSV is more easily dispersed: highly dense pig population and
491 proximity to main roads. On the protective side, higher elevation and more vegetation would be preferred.
492 Thus, reinforcement of biosecurity may be the best tool. However, it raises the question of how much and

493 which biosecurity practices have relevant impact on PRRSV spread. Answering this question requires
494 further studies capable of quantifying how biosecurity practices impede or reduce PRRSV dissemination.
495

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503 **Authors' contributions**

504 MJ and GM conceived the paper ideas. MJ, DR, and GM participated in the design of the study. CC
505 coordinated the PRRSV data collection. MJ conducted data processing and cleaning. MJ performed the
506 phylodynamic analysis. MJ, DR, CAC, and GM wrote and edited the manuscript. All authors discussed
507 the results and critically reviewed the manuscript.

508 **Conflict of interest**

509 All authors confirm that there are no conflicts of interest to declare

510 **Ethical statement**

511 The authors confirm the ethical policies of the journal, as noted on the journal's author guidelines page.
512 Since this work did not involve animal sampling nor questionnaire data collection by the researchers there
513 was no need for ethics permits.

514 **Data Availability Statement**

515 The data that support the findings of this study are not publicly available and are protected by confidential
516 agreements, therefore, are not available.
517

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843

844 **Table list**

845 **Table 1**. PRRSV dissemination between systems. Bayes factor results represent the level of support in the
846 rates of the spread between systems, where BF > 20 is strong support, BF = 3-19 is positive support and
847 BF< 3 is negligible.

Origin	Destination	Average relative migration rate	Bayes factor	Interpretation
A	B	1.79 ± 0.92	102.1	Strong support
A	C	0.46 ± 0.39	47.8	Strong support
B	A	0.35 ± 0.37	27.4	Strong support
B	C	0.33 ± 0.13	7.9	Positive support
C	A	0.41 ± 0.22	14.7	Positive support
C	B	0.39 ± 0.16	10.3	Positive support

848

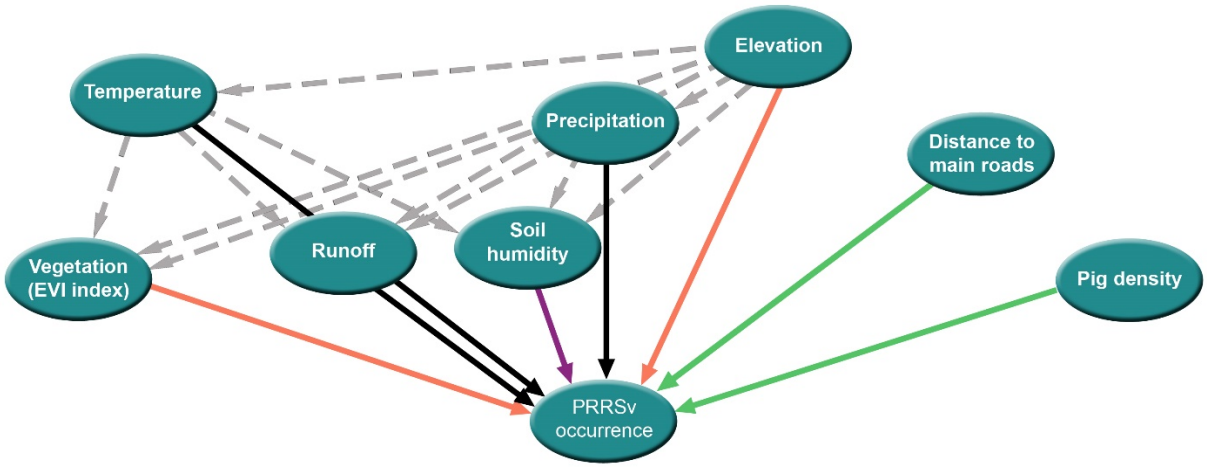
849 **Table 2.** PRRSV dissemination between types of farms. Bayes factor results represent the level of support
850 in the rates of the spread between types of farms, where BF > 20 is strong support, BF = 3-19 is positive
851 support and BF< 3 is negligible.

Farm type of origin	Farm type of destination	All systems (Bayes factor)	System A (Bayes factor)	System B (Bayes factor)	System C (Bayes factor)
Sow	Nursery	109	56.2	42.2	51.7
Sow	Finisher	80.5	36.6	28.1	17.9
Sow	Boar stud	0.1	2.2	0.6	1.1
Nursery	Sow	7.3	5.8	4.9	4.6
Nursery	Finisher	27.5	33.7	10.9	16.6
Nursery	Boar stud	1.9	0.1	0.7	1.3
Finisher	Sow	18.9	10.9	14.1	5.3
Finisher	Nursery	12	16.7	13.2	6.4
Finisher	Boar stud	0.2	1.4	0.1	0.6
Boar stud	Sow	0.5	1.1	0.3	0.1
Boar stud	Nursery	3.9	2.5	2.1	2.4
Boar stud	Finisher	0.4	2	0.8	1.5

Table 3. Number of transmission events that have been originated in farms that belong to the same pig system (local) compared to the events coming from farms integrated into another pig system (external).

System	Year	Local		External		Total
		Events	%	Events	%	
A	2014	349	25.9%	996	74.1%	1345
	2015	294	25.9%	840	74.1%	1134
	2016	292	25.8%	839	74.2%	1131
	2017	330	28.3%	836	71.7%	1166
	2018	109	18.0%	498	82.0%	607
B	2014	299	22.1%	1055	77.9%	1354
	2015	310	27.5%	819	72.5%	1129
	2016	262	23.1%	871	76.9%	1133
	2017	205	17.6%	963	82.4%	1168
	2018	117	18.5%	517	81.5%	634
C	2014	228	16.9%	1125	83.1%	1353
	2015	60	5.3%	1075	94.7%	1135
	2016	126	11.2%	1002	88.8%	1128
	2017	80	6.8%	1088	93.2%	1168
	2018	54	8.9%	551	91.1%	605

859 **Figure legend**



860

861 **Figure 1.** Diagram of factors promoting or restricting PRRSV spread. Arrows represent the relationship
862 between the predictor and PRRSV occurrence based on Bayes factor analysis, where green and orange
863 lines represent positive and negative drivers respectively. Black represent negligible support, and dashed
864 lines show relatedness between environmental predictors affecting PRRSV through other variables.

865

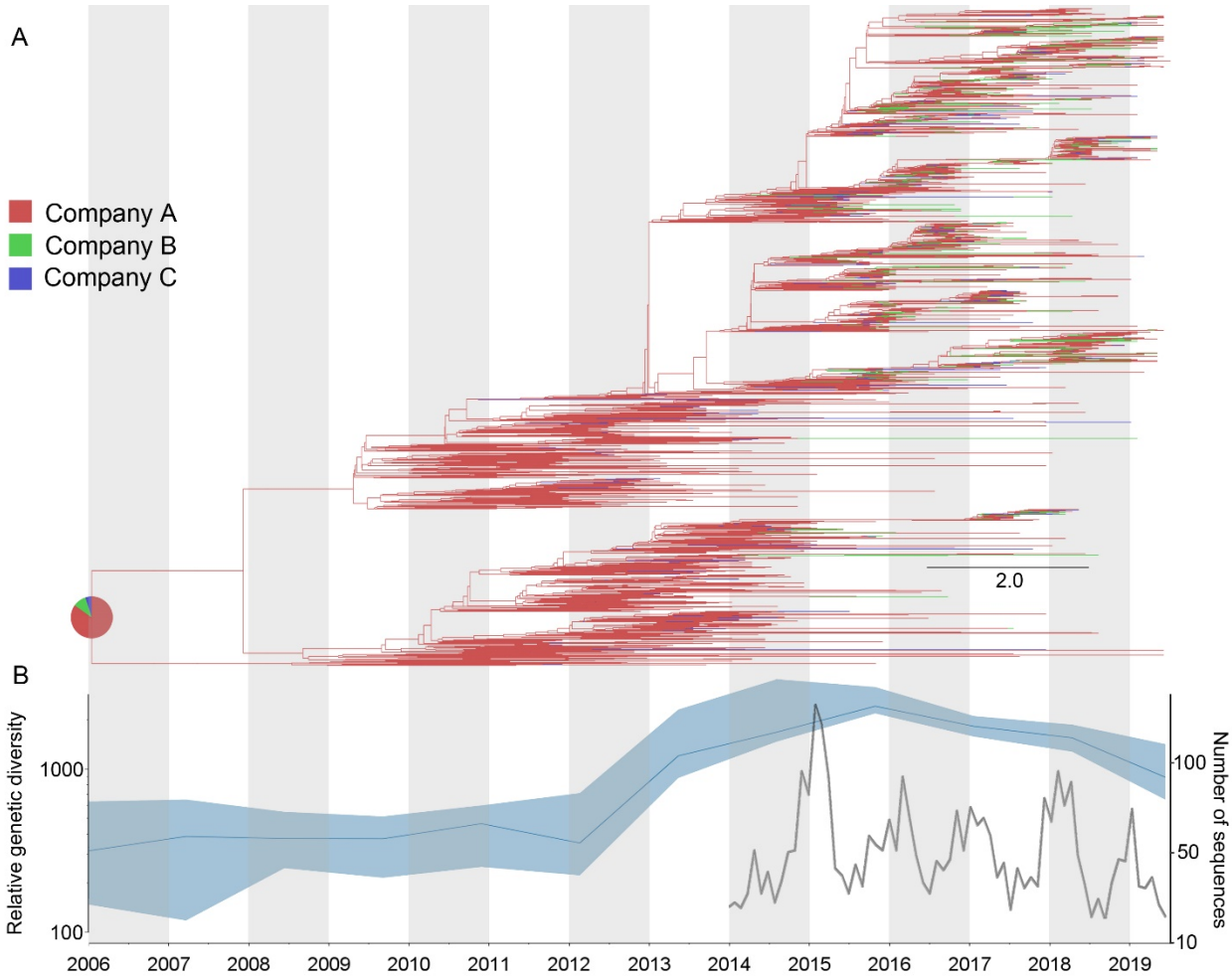


Figure 2. Dissemination history of PRRSV, inferred by discrete phylogeographic analysis. A) Maximum clade credibility phylogeny, colored according to pig production systems (A, B, C). The probabilities of ancestral states (inferred from the Bayesian discrete trait analysis) are shown in pie charts on each node, representing the most likely type of farms. B) Spatiotemporal patterns in the relative genetic diversity represented through the Bayesian SkyGrid plot and the number of sequences per month. The left y-axis summarizes the effective population size over time, the mean estimate is represented by the dark blue line, while the shaded light blue regions correspond to the 95% highest posterior density (HPD), and in the right y-axis shows the number of sampled sequences over time.

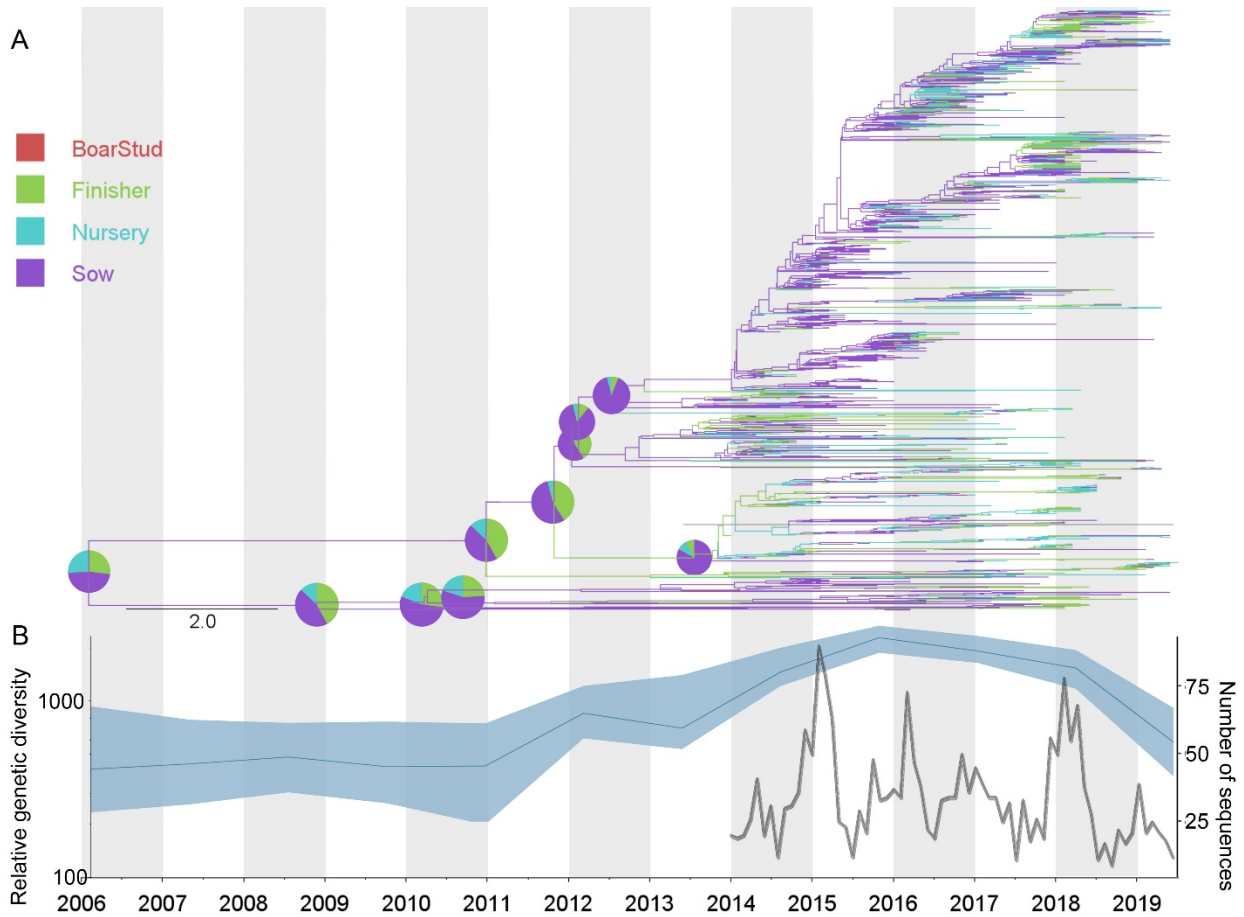


Figure 3. PRRSV dissemination within system A. Panel A) Maximum clade credibility phylogeny colored according to the different farm types (sow, nursery, finisher, and boar stud). Probabilities of ancestral states shown in pie charts in nodes, representing the most likely type of farm origin. Panel B) Spatiotemporal patterns in the relative genetic variation through Bayesian SkyGrid plot and number of sequences per month. The left y-axis summarizes the effective population size over time. Mean estimate is represented by the dark blue line. Shaded light blue regions correspond to the 95% highest posterior density (HPD), and in the right y-axis shows the number of sampled sequences over time.

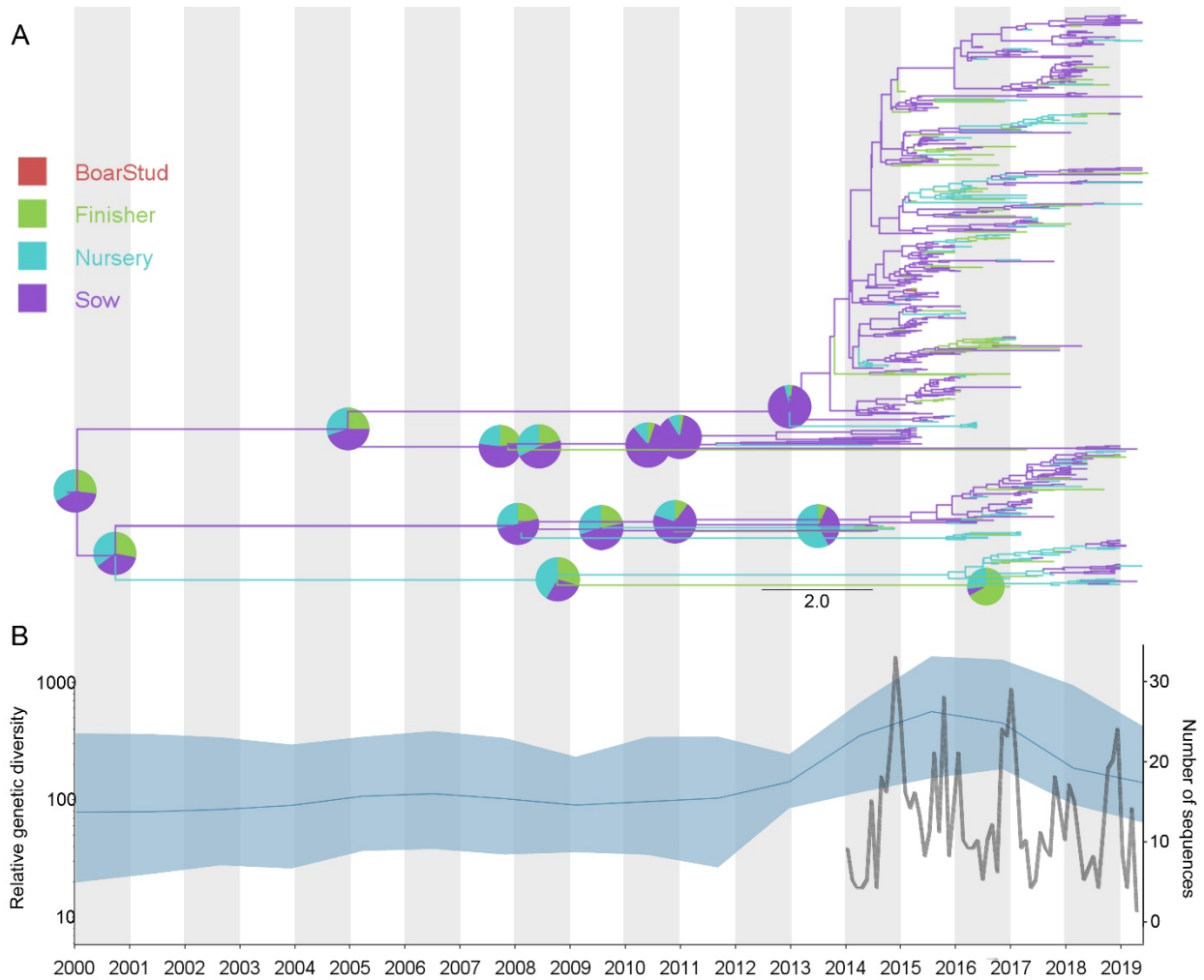


Figure 4. PRRSV dissemination within system B. Panel A) Maximum clade credibility phylogeny colored according to the different farm types (sow, nursery, finisher, and boar stud). Probabilities of ancestral states shown in pie charts in nodes, representing the most likely type of farm origin. Panel B) Spatiotemporal patterns in the relative genetic variation through Bayesian SkyGrid plot and number of sequences per month. The left y-axis summarizes the effective population size over time. Mean estimate is represented by the dark blue line. Shaded light blue regions correspond to the 95% highest posterior density (HPD), and in the right y-axis shows the number of sampled sequences over time.

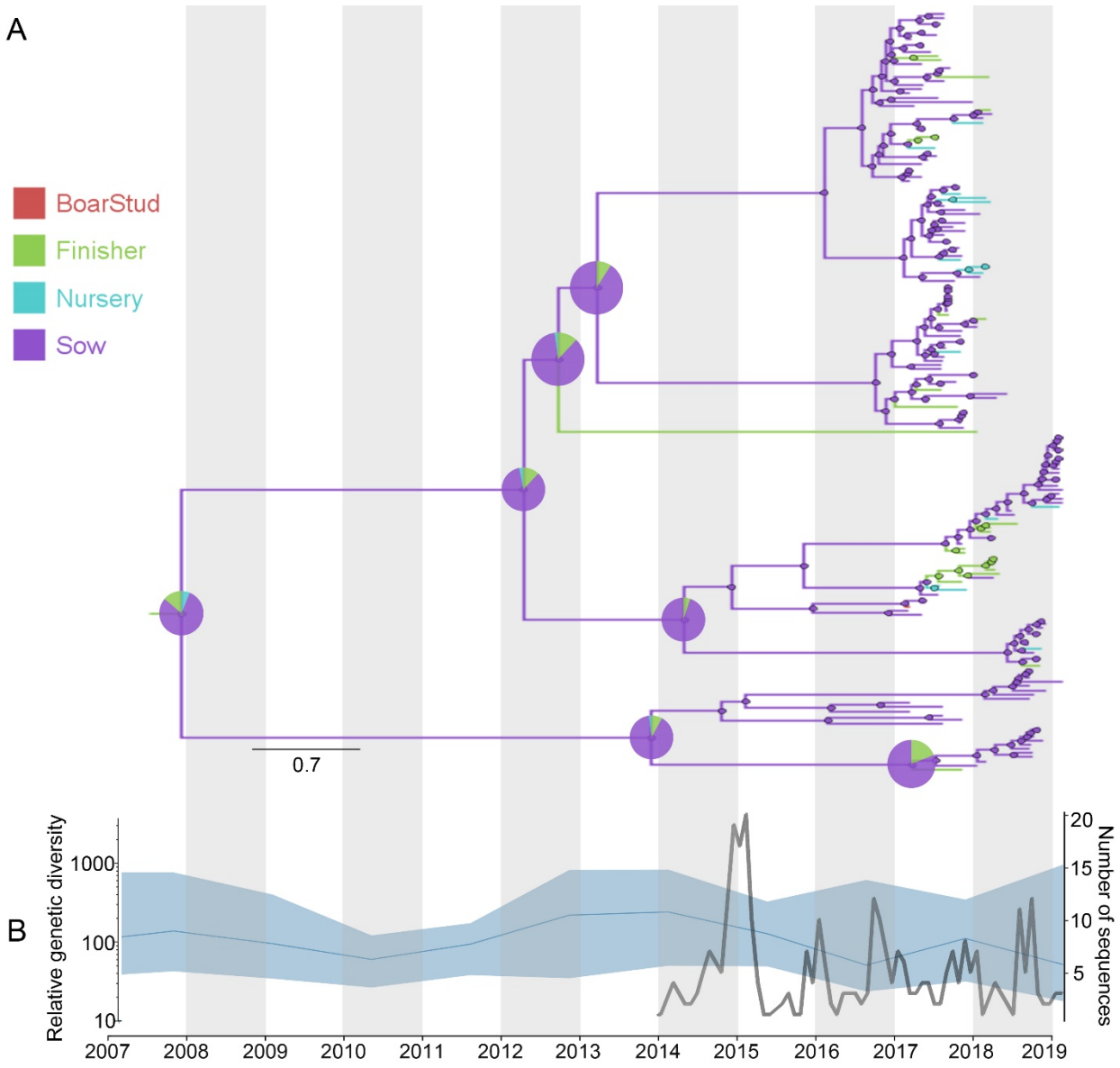


Figure 5. PRRSV dissemination within system C. Panel A) Maximum clade credibility phylogeny colored according to the different farm types (sow, nursery, finisher, and boar stud). Probabilities of ancestral states shown in pie charts in nodes, representing the most likely type of farm origin. Panel B) Spatiotemporal patterns in the relative genetic variation through Bayesian SkyGrid plot and number of sequences per month. The left y-axis summarizes the effective population size over time. Mean estimate is represented by the dark blue line. Shaded light blue regions correspond to the 95% highest posterior density (HPD), and in the right y-axis shows the number of sampled sequences over time.

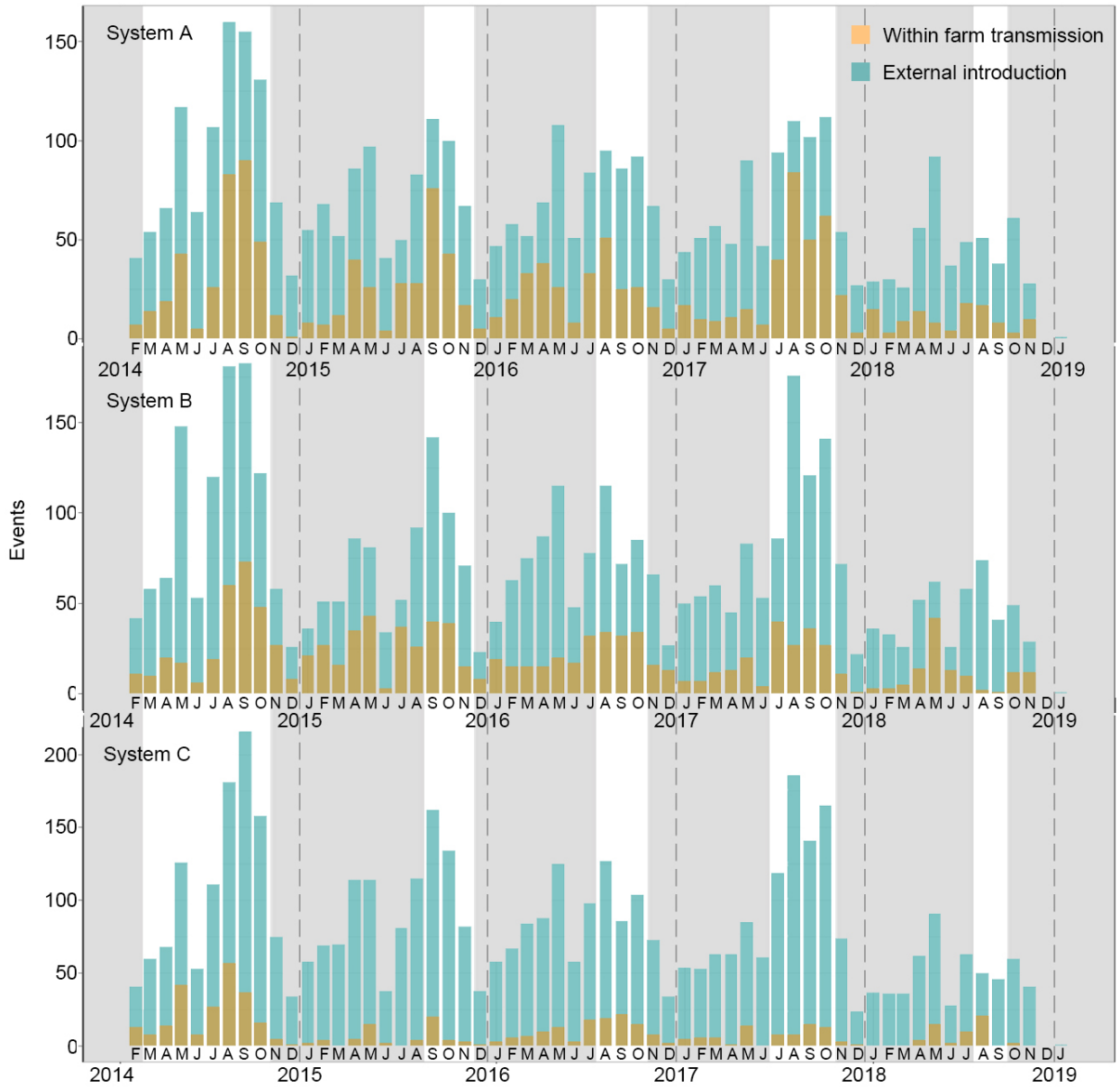


Figure 6. PRRSV migration events among pig systems are inferred through a structured coalescent model. Number of dissemination events originated in farms from the same pig system compared to events from farms integrated into another pig system. Grey background represents the months where the exponentially weighted moving average epidemic monitor surpassed the established PRRSv epidemic threshold based on the February 2020 MSHMP report (MSHMP, 2020), while dashed lines represent the beginning of each year.