A Microbial Tale of Farming, Invasion and Conservation: On the Gut Bacteria of European and American Mink in Western Europe

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Research Article

Keywords: mink, microbiota, invasion, phylosymbiosis, genetic diversity, mustelids

Posted Date: October 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-912230/v1

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Running title: Gut bacteria of European and American mink in Western Europe

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Abstract:

One of the threats that the critically endangered European mink (*Mustela lutreola*) faces throughout its relict range, including the occidental population, is the impact of the American mink (*Mustela vison*) invasion in its natural habitat. We aimed to explore the differences in microbiota and genetic diversity between European and American mink to test phylosymbiosis theory. We investigated the gut microbiota composition of European and American mink in a controlled environment (captive breeding compounds and fur farms respectively) to account for the impact of the environment on gut bacterial composition. We compared them to the gut microbiota of both mink species in the natural environment across multiple habitats. Our exploratory results showed differences between free-ranging and captive individuals, with more extreme changes in American mink

compared to European mink. However, feral American mink from a long-established population exhibited gut bacterial composition closer to the free-ranging native species compared to more recently established feral populations. This result could be explained by dietary shifts in the area sampled based on prey availability through different landscape, but also to a lesser extent due to greater genetic differentiation. This exploratory work contributes to the scarce literature currently available on the dynamics between gut microbiota and mammal invasion.

Keywords: mink, microbiota, invasion, phylosymbiosis, genetic diversity, mustelids

Declarations

Funding: Funding was supported by the NSERC CREATE grant, ReNewZoo and the Conversation Genetics Laboratory of the University of Liège

Conflict of interest: All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Data availability: Supporting information has been made available online. Final DNA sequences, ASV table, taxonomy table and mapping file have been uploaded: Dryad link TBD.

Authors' contributions: J.M., A.S.H. and P.V.L. planned and designed the study. C. A., C.F.C. F.U-M, and P.C. provided samples and contact for other sample access. P.V.L. performed the sample preparation for sequencing. J.M. provided sequencing services and

45 together with A.S.H. advised on laboratory and sampling procedures. P.V.L. performed
46 bioinformatics, statistical analyses and the interpretation of results with feedbacks provided
47 by A.S.H. and J.M.. P.V.L. wrote the manuscript with input from all authors.

48

49 **Introduction**

50 Invasive alien species have been widely recognized as one of the major threats to
51 biodiversity due to anthropogenic changes at both global and local scales (Lockwood et al.,
52 2007). Invasive species can directly impact the habitat and ecology of native species they
53 interact with as they affect native species' population sizes and habitat ranges (Genovesi
54 et al., 2012; Zalewski et al., 2010). An example of such a successful invader is the
55 American mink (*Mustela vison*) in Europe, which was introduced from North America for
56 fur farming in the early 20th century. Following accidental escapes, as well as intentional
57 releases, the mink became established in 28 European countries (Bonesi & Palazon, 2007;
58 Reid et al., 2016). This species is also present as an invasive species in parts of South
59 America and Asia (Mora et al., 2018; Shimatani et al., 2010).
60 The generalist and opportunistic aspects of this mustelid's diet strongly impacted
61 populations of 47 reported native species, reducing prey species of seabirds (Nordström et
62 al., 2002), voles (Banks et al., 2005), and crustaceans (Fischer et al., 2009), six of them
63 being included in the IUCN Red List categories near threatened, vulnerable, endangered
64 and critically endangered (Genovesi et al., 2012). One of them is the critically endangered
65 European mink (*Mustela lutreola*), with evidence of direct aggression from the invader
66 towards the native species involved in competition for resources (Melero et al., 2008;
67 Sidorovich et al., 2010; Podra et al., 2013). Both species have similar ecological niches,

being carnivorous mammals in riparian ecosystems and predating on both aquatic and terrestrial prey. The presence of the American mink in the native species habitat was shown to reduce the diet of European mink so that it becomes more specialized, while the American mink's diet became more generalist (Sidorovich et al., 2010).

The American mink can also play a role in disease transmission among native species, as they are capable of carrying the Aleutian Disease Virus (ADV), the Canine Distemper Virus (CDV) as well as many eukaryote parasites that can be transmitted to other mustelids, feral cats, and even humans (Leimann et al., 2015; Martínez-Rondán et al., 2017; Torres et al., 2007). Studying the invasive success of a carrier species like the American mink becomes even more critical, especially because mink, feral and/or in farms, interact with many other species and humans.

In France, the American mink was introduced in the 1920s in the Eastern side of the country; in the 1950s, many farms moved to the western side where access to fish by-products for mink feeding was readily available (Léger et al., 2005). A long-term monitoring study from 2000 to 2015 recorded evidence of the expansion of the American mink over the Atlantic coast in France with multiple established populations, including: (1) the historical region of Brittany, Normandy and Pays de la Loire, (2) the western region of the Pyrenees up to northern Aquitaine, and (3) the Eastern region of the Pyrenees (Léger et al, 2018). In contrast, the western distribution of the European mink is reduced to seven departments of southwestern France (Maizeret et al., 2002) and to northern Spain, mainly in Navarre, La Rioja and some neighbouring communities in the Upper Ebro Basin (Põdra & Gomez, 2018). Moreover, French populations are probably highly fragmented, especially in departments where the invasive species is abundant.

The low density of individuals in these regions and low genetic diversity of the Western population perhaps due to a bottleneck event (Cabria et al., 2015; Michaux et al., 2005) encouraged the creation of a captive breeding program in Spain at the Fundación para la Investigación en Etología y Biodiversidad (FIEB), with individuals originating from free-ranging populations in Spain.

Before a species establishes and expands to become a successful invader, the colonization of new habitats represents a challenge through a variety of new selective pressures encountered that can be highly costly from an adaptative lens. Therefore, host-associated microbes can play a critical role in the invasive success of an exotic species in a new habitat. These microorganisms (bacteria, archea, virus, fungi and protozoa) range from parasites to obligate mutualists (McKenney et al., 2018; West et al., 2019). This large range of interactions, often coupled with complex historical and introduction events, can result in a wide variety of ecological dynamics.

Within the last decade, we have begun to understand the underlying processes driving host-associated microbial community dynamics. The external environment of the host has been reported to be one of the main drivers of variation (Koskella et al., 2017; Spor et al., 2011). Housing facilities such as fur farms and captive breeding facilities in zoos provide intense veterinary care, sanitized enclosures, a standardized diet, and reduced social interactions. Hence, captivity has been shown to alter the microbiota of animals compared to their free-ranging counterparts (Clayton et al., 2016; Wasimuddin et al., 2017; van Leeuwen et al., 2020). The majority of these studies show similar trends: a decrease in bacterial phylotype richness (or α-diversity) among captive individuals compared to their free-ranging conspecifics, as well as differences in community composition (or β-diversity) between the

groups. However, some host species show the opposite pattern (McKenzie et al., 2017; Greene et al., 2019; Frankel et al., 2019), postulating that the gut microbiota respond differently to captivity according to host taxa, mainly through their feeding strategy and gut physiology. Differences observed in gut microbial communities have largely been attributed to altered diets in captivity that can also lead to the extinction of microbial niches and functions in the host's gut over multiple generations in captivity (Sonnenburg et al., 2016; van Leeuwen et al., 2020). Diet has therefore been reported to be the most important influence on the mammalian gut microbiota (Reese & Dunn, 2018; Martinez-Mota et al., 2019).

Despite the strong impact of the host environment on its gut microbial community, the genetics and biology of the host should also be taken into account to fully understand the complex dynamics that occur in these systems (Koskella et al., 2017; Spor et al., 2011). Phylosymbiosis is described as an increase in compositional similarity between bacterial communities colonizing closely related hosts compared with distantly related hosts (Groussin et al., 2017; Lim & Bordenstein, 2020). Many investigated mammals have supported this pattern, such as bats, apes and rodents (Brooks et al., 2016; Ochman et al., 2010; Kohl et al., 2018; Knowles et al., 2019), as well as other animal taxa (Pollock et al., 2018; Sevellec et al., 2019; van Opstal & Bordenstein, 2019); however, other studies have not detected signals of phylosymbiosis in some mammals (Baxter et al., 2015; Greene et al., 2019; Grond et al., 2020). Groussin et al. (2017) also suggested that the tight associations between some host taxa and some of their associated gut microbes might not generalize to the entire gut microbial community, hence the strong environmental effects on gut microbial composition. No study to date has examined phylosymbiosis in the

context of invasion ecology in carnivores. Carnivores have short transit time and digestive tracts, so the gut microbiota are potentially less impacted by diet (Reese & Dunn, 2018; Ley et al., 2008). From the current literature, mustelids are known to harbor relatively low diversity and abundance of gut microbes (Compo et al., 2018; Bahl et al., 2017). Moreover, large interindividual variation in gut bacterial communities' composition has been observed in farmed American mink, being generally dominated by the phylum *Firmicutes*, but in some cases also *Proteobacteria* and *Fusobacteria* (Compo et al., 2018; Bahl et al., 2017). At the class level, the average bacterial composition was dominated by *Clostridia*, *Gammaproteobacteria*, and *Fusobacteria*.

The purpose of this study was to understand the relationships between the gut microbiota of related invasive and native host species (specifically European and American mink) sharing similar ecological niches. We were interested in: (i) if the environment (free-ranging or captive) had a stronger influence than species or population identity for gut microbial diversity and composition, (ii) if there were fewer differences in abundance of microbial taxa within mink species than between them across multiple populations, and (iii) if genetic relationships between host populations were reflected in terms of gut microbial compositional similarity. To study these questions, we examined gut bacterial species (or phylotype) richness, gut microbiota structure, and composition differences between American and European mink in captive settings (fur farm and captive breeding program), and in the natural environment across three different habitats in western France and Spain (Brittany region, the Nive basin in the Pyrénées-Atlantiques departement, and Navarra). To test for a phylosymbiosis signal, we also investigated the genetic diversity

and structure of those mink populations using previously collected data through neutral microsatellite markers analysis.

Methods:

2.1 Sample collection and study sites

Fecal samples and rectal contents were collected from live or dead animals from five different populations. For free-ranging populations, six European mink were sampled in the Navarra region (Spain), twelve American mink were sampled in the Nive watershed (Southwest, Pyrénées-Atlantiques, France) and sixteen American mink from Brittany (Tomé island and close mainland; Figure 1). In order to investigate habitat variation from each free-ranging sampled populations, a map was created using QGIS 3.16.6-Hanover with GPS coordinates for each sample. Layers documenting landscape use, were simplified to agricultural, built, natural and water surfaces from datasets originating from IDENA (Spatial Data Infrastructure of Navarre) and data.gouv.fr from Open Street Map (Alexandre Lexman). For captive populations, ten European mink were sampled in captive settings at the Fundación para la Investigación en Etología y Biodiversidad breeding center (FIEB) and fourteen American mink from a fur farm in the Pyrénées-Atlantiques department (Southwest of France). All samples were collected using sterile tweezers and placed in sterilized microcentrifuge tubes filled with 96% ethanol. Those tubes were stored in a -20°C freezer until further processing (Asangba et al., 2019).

2.2 DNA extraction and sequencing

Gene amplicon sequencing was used to study the bacterial communities. DNA extractions from the fecal samples collected were conducted using the QiaAmp Mini Kit with Inhibitex

(Qiagen) following the manufacturer's instructions. Two blank extractions were made to control for contamination during the extraction process. A mock community sample (HM-783D, BEI resources) containing genomic DNA from 20 bacterial strains at concentrations ranging from 0.6 to 1400 pg/µl was also added in each library to confirm the reliability of our method. After DNA extraction, the targeted gene for taxonomic affiliation (16S rRNA gene – 515F & 806R) was amplified through polymerase chain reactions (PCRs). The library preparation and sequencing were performed by Novogene UK. Using their designated library protocol, 2 × 250 bp paired-end sequencing was completed using broad bacterial primers of the region V4 of the 16S rRNA gene using an Illumina NovaSeq platform in 100k reads/samples depth (Illumina Biotechnology Co.).

2.3 Bioinformatics

The quality controls of the paired-end sequence reads were performed through the software FastQC (Andrews, 2010). Sequence reads demultiplexing, denoising and amplicon sequence variants (ASVs) picking steps were done with the QIIME2 tool (Bolyen et al., 2019; v. 2020.8), using the DADA2 pipeline (Callahan, McMurdie, & Holmes, 2017; Callahan et al., 2016). ASVs –or also referred to as bacterial phylotypes– were then screened to the 97% 16S rRNA gene full-length reference sequences from the Silva RDP v.138.1 database (Pruesse et al., 2007) for taxonomical association using the VSEARCH classifier implemented in QIIME2 (Bokulich et al., 2018). Sequence alignment and phylogeny building were also conducted in QIIME2.

Analysis of a negative control showed the presence of bacterial sequences that probably derived from contamination during laboratory sample handling. However, the diversity of this control was dissimilar from those of all mink samples (Bray-Curtis dissimilarity >

70.8%). For subsequent analysis of sequences associated with mink samples, negative control sequences were trimmed from the whole dataset. The cumulative sum scaling (CSS) method was used to normalize the data using the *metagenomeSeq* package (Paulson et al., 2013) in R (R version 3.5.2, R Core Team, 2018). It can decrease the fold difference in sampling depth and avoid the rarefying of counts (Weiss et al., 2017).

2.4 Statistical analysis for comparison of α-diversity of gut bacteria between groups

After CSS normalization, mink groups were divided as followed: European mink in captivity (EM Breeding Center; n=7), American mink in captivity (AM Farm; n=14), free-ranging European mink (EM Spain; n=6); and free-ranging American mink distinct populations in Brittany and Nive (AM Brittany; n=15 and AM Nive; n=10; Figure 1). All statistical analyses were conducted in R (R version 3.5.2, R Core Team, 2018) using the *phyloseq* (McMurdie & Holmes, 2013) and *microbiome* packages (Lahti, 2017) for manipulation of data. Chao1, Shannon indexes and Faith's PD in each sample were used as metrics to measure and compare the α-diversity of gut bacteria between groups. Chao1 is an indicator for overall bacterial species richness, the Shannon index characterizes both the abundance and richness of bacterial phylotypes, and Faith's PD is a measure for phylogenetic diversity. Differences in the index values according to mink population, host species, host environment (wild or captive settings) and sex were investigated using a non-parametric Kruskall-Wallis rank sum test followed by Dunn test (1964) of Kruskal-Wallis multiple comparisons with Benjamini & Hochberg (1995) for p-value correction. The significance cutoff was set to p-value<0.05 for each test.

2.5 Statistical analysis for comparison of β-diversity of gut bacteria between groups and differential abundance

Unweighted and weighted UniFrac distance matrices between samples (Lozupone et al., 2010) were used to investigate differences in gut microbial communities between population, host sex, host environment, and host species with all bacterial taxa. A PERMANOVA model Adonis from the *vegan* package was constructed with 9,999 permutations with reported F, R2, and *p*-values to determine whether there were significant differences between the mink populations, host species, and sex as main effects (Oksanen et al., 2019) after testing the homogeneity of groups variances using the *betadisper* function from the same package. Pairwise PERMANOVAs were then conducted to investigate variations between groups with 9,999 permutations. A principal component analysis (PCoA) using Unifrac distance measures between samples was conducted to visualize the potential similarities between groups. Finally, a UPGMA dendrogram was constructed using the *qiime diversity beta-rarefaction* function in QIIME 2 by mink populations with weighted Unifrac distances with 20 iterations with mean ceiling at 10,000 sequences rarefaction.

The differential abundance analysis was conducted on the raw ASVs count, using the *DESeq2* package (Love et al., 2014), with a negative binomial Wald test to test significance between each group. Only phylotypes with a significance level (α) below 0.001 after false discovery rate (FDR) corrections were considered using the Benjamin–Hochberg method. All phylotypes were tested in contrast, meaning that differential abundance was done pairwise between each mink population. ASVs below the significance level and with a negative log2 fold change had thus their abundance significantly lower in the first group tested, and a positive log2 fold change indicated that the phylotype was significantly higher in the first group compared to the other group. We conducted this

analysis to test differential abundance first, between captive and free-ranging populations within mink species and second between free-ranging populations between and within mink species.

2.6 Microsatellite markers genotyping, and analysis

A total of 94 hair and tissue samples were extracted from a larger dataset of samples from European and American mink over a ten-year period between 2000 and 2019 (unpublished data). All samples derived from the same population that the fecal samples originated from, but many from different individuals. Eighteen free-ranging American mink were sampled in Brittany (Côtes d'Armor), thirty American mink were sampled in the Pyrenees Atlantiques (Southwest region of France), as well as thirty individuals from the same fur farm in the southwest of France. Finally, ten captive European mink were sampled in captive settings at the FIEB breeding center and six free-ranging European mink were sampled in Navarra (Spain).

Genomic DNA was isolated using the DNeasy Blood and Tissue Kit (QIAGEN) from tissue and hair samples. Negative controls were also used. Multilocus genotypes were obtained by PCR amplification of 10 autosomal microsatellites (Fleming et al., 1999; Cabria et al., 2007). The forward primer of each locus was 5'-end labeled with a fluorescent dye. The following multiplex sets were designed: mix 1 (MLUT 25, MLUT 27, Mvis 099) and mix 2 (MLUT 04, MER009, Mvis075, Mvis072, MER41, MER022). PCR and genotyping steps were carried out following Pigneur et al. (2019). Length variation determination (alleles and genotypes) was performed using Genemapper 4.0 (Applied Biosystems). To construct consensus multilocus genotypes, an allele was only accepted if

observed at least twice. We thus accepted heterozygous genotypes that were observed twice. A homozygote was accepted after three positive PCRs gave the same single allele. The genetic structure of the mink populations was inferred using Bayesian clustering analysis with Structure 2.3 software (Pritchard et al., 2000). We ran 10 iterations for each K value from 1 to 10 using the admixture model. A total of 10^6 MCMC repetitions were performed after a burn-in period of 20%. The results of the 10 iterations for each K value were summarized and averaged using the Clumpp method (Jakobsson & Rosenberg, 2007). The optimal number of clusters was investigated using the ΔK method (Evanno et al., 2005). For subsequent analyses, individuals were sorted according to their geographic origin (sorted into 5 main populations: Brittany, Nive basin, Navarra, Farm and Breeding Center, Figure 1). Mean allelic richness by locus (Ar), the expected (He) and observed (Ho) heterozygosity were calculated for each defined group using *diveRsity* (Keenan et al., 2013). An Euclidian distance matrix was constructed using GenAlEx 6.5 (Peakall and Smouse 2006), and a PERMANOVA model Adonis was conducted in a similar manner to β-diversity gut bacterial analysis with species and population. A UPGMA dendrogram was also constructed based with average linkage based on Fst values between mink populations.

Results

3.1 Microsatellite markers analysis

Overall, the three American mink populations had greater total allele count (A), percentage of heterozygous locus (%H), allelic richness (Ar), observed heterozygosity (Ho) and expected heterozygosity (He) than the European mink populations (Table 2). This suggests greater heterozygosity and genetic diversity in neutral markers for the American mink, and we observed even higher for American mink in the fur farm compared to feral conspecifics.

Bayesian clustering assignment recovered three distinct genetic clusters within our populations (Figure 2; Table S1); the European mink individuals form one cluster (K2), American mink from the farm and the Nive basin another (K1), and individuals from Brittany overlap on 2 clusters (K1 and K3; Figures 2& 3A). Only three American mink had admixture patterns between the two American mink clusters and belong to the fur farm population. Genetic distances between individuals' analysis through a PERMANOVA model indicated significantly greater distance between mink species than within, as well as according to mink population (Adonis: $F=7.6547$; $R^2=0.07206$; $p=0.0009$; $F=3.1927$; $R^2=0.09016$; $p=0.0089$, respectively). Finally, a dendrogram based on Fst distances between populations revealed that the mink population sampled had lower distance within species than between species (Figure 3A).

3.2. Comparison of α-diversity in gut bacterial

Samples of a mock community containing known concentrations of genomic DNA from 20 bacterial strains were sequenced. Nineteen of the twenty different strains originally included in the sample were detected. Therefore, our protocol allowed bacterial DNA detection and identification to the genus level as long as its concentration in the DNA extract was at least 2.8 pg/µl, and provided that the sequence was included in the reference database. Following the raw data processing, we obtained 1,947,964 sequences belonging to 3,036 distinct bacterial phylotypes (ASVs) after removal of negative control sequences, for 52 samples (other samples were removed during CSS normalization due to low sequencing depth).

Gut bacterial phylotypes richness did not significantly vary according to host species or host sex, when considering three richness measures (Table 2). However, both captive mink

species tend to have lower bacterial phylogenetic diversity and lower Shannon indexes compared to conspecific free-ranging mink (χ^2=10.59, p-value=0.001137; and χ^2=2.9118, p-value=0.08793, respectively; Figure 4B). The Shannon index also significantly varied according to mink populations (χ^2=11.681, p-value=0.01989). When conducting a Dunn test for multiple comparisons with Benjamin & Hochberg correction for p-values, captivity seemed to have a strong negative impact on gut bacterial richness for both host species, especially compared to the American mink population from Brittany (Figure 4A&B).

3.3. Comparison of β-diversity of gut bacteria between groups

As expected in mustelid gastrointestinal tracts, all samples were dominated by the *Firmicutes* and *Proteobacteria* phyla, mostly belonging to the *Clostridiaceae* and *Peptostreptococcaceae* families (Figure 5; Compo et al., 2018; Bahl et al., 2017). The gut bacterial community composition of male and female mink for both species considered in the study (Adonis: F=0.314; R^2=0.0058; p=0.725) were not significantly different and explained around 0.5% of the variation. Thus, males and females were not treated separately in subsequent statistical analyses. Host species did not have a significant effect on gut community composition, as it explained 1.75% of the community variation (Adonis: F=0.938; R^2=0.0175; p=0.2827). However, 20.9% of gut bacterial composition variation was explained by mink belonging to the different populations in both weighted and unweighted Unifrac distances (Adonis: F=3.3127; R^2=0.20949; p=0.003996; and F=1.859; R^2=0.07478; p=0.005994, respectively, Figure 6). The variation seemed to be mainly explained by free-ranging or captive conditions (Figure S1). We did observe significantly greater distances between feral American mink in Brittany and other American mink groups, but no differences were detected between both captive and free-ranging European

342 mink and American mink in Brittany (Figures 6&S1). A wide interindividual variation in
343 gut bacterial composition was also observed in free-ranging European mink (Figure S1).
344 Overall, feral American mink in Brittany and free-ranging European mink had lower β-
345 diversity between them than any other mink populations (Figure 3B).

346 *3.4. Differential bacterial abundance analysis*

347 The assessment of the differential abundance of bacterial phylotypes using a negative
348 binomial Wald test was conducted on the core microbiota of 391 phylotypes. From those,
349 141 phylotypes from nine phyla varied significantly among the mink populations with 82%
350 belonging to *Firmicutes* and *Proteobacteria*. When comparing captive and free-ranging
351 populations within mink species, feral American mink had phylotypes differentially
352 abundant to captive conspecifics, from 100 to 65 ASVs, most of them decreasing (Table
353 S2). Feral American mink had a ratio of 1.77 and 2.6, expressing more decreases than
354 increases in taxa abundance in the natural environment compared to captive conspecifics.
355 This decrease in taxon abundance between free-ranging and captive populations is higher
356 in American than European mink (0.7). A large portion of those phylotypes belonged to
357 the *Bacteroida* (families *Flavobacteriaceae, Muribacculaceae* and *Chitinobacteraceae*)
358 and *Clostridia* (genera *Rhomboustia* mostly) classes (Table S2). However, when
359 comparing free-ranging populations of both species, we observed more taxa abundance
360 variation between the two populations of free-ranging American mink (64 taxa
361 differentially aunbdant), than variation between American and European mink (53 taxa for
362 the Nive basin and 42 taxa for Brittany). Feral American mink in Brittany had more
363 phylotypes abundances in common with free-ranging native European mink than its
364 conspecifics from the Nive basin (Table S2). Most of the abundance variation was

attributed to reduction in ASVs belonging to the *Firmicutes* phylum (*Lactobacillus*, *Clostridium* genera and *Peptostreptococcaceae* family) and *Gammaproteobacteria* class between the two American mink populations.

Discussion

4.1. On the influence of human impacts on the mink gut microbiota

This study is the first to examine how the gut bacteria of riparian carnivores vary between related species with similar ecological niches in the context of farming, invasion, and conservation. Our results did not find any support for phylosymbiosis, as genetic relationships were not reflected in the composition of the gut microbiota (Figure 3). There was also a reduction in the richness of the bacterial community in captivity that surpassed any host species differences. A similar pattern was further observed in β-diversity measures. This trend has already been observed in other host taxa with a carnivorous diet (*Canidae*, McKenzie et al., 2017). It is currently well established that animals living in captivity experience a range of changes that can influence their gut bacteria, from diet change, veterinary care, specific and uniform environmental substrates, as well as reduced contact with conspecifics and other species. While most of the current literature compared free-ranging animals to individuals kept in zoos (Clayton et al., 2016; Borbón-García et al., 2017; Wasimuddin et al., 2017), the same trend is expected between feral and farmed mink.

We also observed differentially abundant taxa in free-ranging mink compared to captive conspecifics. In addition, bacterial loss was stronger in the invasive American mink than the native European species when comparing free-ranging populations to captive

388 conspecifics. In this regard, feral American mink would have experienced less
389 recolonization from gut bacteria in natural habitats than the European mink, when
390 compared to their captive conspecifics. By nestedness and turnover of bacterial
391 communities, feral American mink would have left a subset of captive gut microbes during
392 the invasion process, potentially leading to pathogen loss. However, many successful
393 invasions have occurred without any pathogen loss and further investigation on targeted
394 bacteria would be required (Ansellem et al., 2017).
395 There are three potential ways that can explain a stronger pattern of differentiation in gut
396 bacteria communities between feral to captive settings in the American mink compared to
397 the European mink. First, the two species have very different conditions in captivity.
398 Farmed American mink are held in individual and open-air elevated cages with minimal
399 substrate and enrichment, whereas European mink are held in an enclosure with access to
400 a pond, natural substrates and enrichment (branches, vegetation, mud). Moreover, the diet
401 of the American mink is composed of processed fish and chicken, whereas the diet of the
402 European mink consists of whole fish, chicken and mice. Those differences in captive
403 conditions could explain the significant difference in the bacterial communities between
404 wild and captive American mink, compared to the European mink.
405 Second, when considering free-ranging European mink in their natural habitat, they could
406 be more likely to select specific gut bacterial taxa because of their shared coevolutionary
407 history with the environmental microbes in western France (Bankers et al., 2021). On the
408 other hand, the invasive American mink may lack host-microbes coevolutionary history
409 with native bacteria and would thus be less likely to retain newly acquired microbes when
410 becoming feral. It is worth noting that the estimated divergence time between the two mink

411 species is 8.28 million years ago (Hedges et al., 2006), and further research with other
412 native mustelids such as the European polecat (*Mustela putorius*), that diverged more
413 recently from the European mink could give more insight into gut bacteria colonization
414 from wild to captive settings.
415 The third explanation relies on the evolutionary history of the American mink itself. The
416 domestication process of this species started in the 1860s in Canada (Morris et al., 2020),
417 as humans selected animals with dense, soft and shiny fur, as well as increased fertility to
418 maximize their revenue. Docility, also termed confidence towards humans, was another
419 behavioural trait that many European breeders favoured to facilitate daily handling and
420 improved welfare (Thirstrup et al., 2019). Thus, genetic and phenotypic differences have
421 already been observed between free-ranging and farmed American mink, including smaller
422 brain size, longer transit time and increased nitrogen metabolism in farmed animals (Morris
423 et al., 2020; Bowman et al., 2017; Gugolek et al., 2012; Kruska, 1996). This explanation
424 seems consistent with the high genetic diversity in mink from the fur farm observed in this
425 study compared to free-ranging American mink populations. There is increasing evidence
426 of the important interactions between the gut microbiota and the gut-brain axis in many
427 species, including farm animals (Collins et al., 2012; Kraimi et al., 2019). It would be likely
428 that artificial selection might have impacted the overall gut microbiota composition of the
429 American mink through morphological and physiological variation, and thus changes in
430 the gut-brain-axis, compared to the European mink that has not experienced domestication.
431 The adverse effects of domestication on gut bacteria have already been observed in other
432 mammals (Prabhu et al., 2020). However, to confirm either of both explanations on those
433 exploratory results, further investigation with larger sampling size should be conducted.

4.2. No phylosymbiosis signal observed in mink

In general, our results did not support the phylosymbiosis hypothesis, and it was observed that the host environment had a strong influence on the mink gut microbiota. First, neither gut bacterial α- or β-diversity varied according to host species. Second, the feral American mink groups were more distinct from one another than with the free-ranging European mink, despite belonging to the same species. Furthermore, feral American mink in the Nive basin had less similarly abundant bacterial taxa in common with free-ranging European mink than feral American mink in Brittany. The absence of a phylosymbiosis signal is consistent with the fact that despite not being the most diverse population genetically, the invasive American mink from Brittany are the most genetically differentiated from the other American mink populations, being composed of at least three different genetic clusters. Three genetic pools have already been documented in this long-established population due to accidental releases over multiple introduction events, fostering diversity but also genetic drift (Bifolchi et al., 2010).

Similar to formation of a distinct population through genetic drift within farms, an analogous concept termed ecological drift might have occurred in gut microbes between mink populations, in relation with the ecology of the host (Kohl, 2020). These shifts in bacterial composition between free-ranging mink species could be explained by variation in prey availability due to habitat differences between the areas sampled. Studies in other parts of Europe showed that the American mink has a plastic diet (Maran et al., 1998; Zalewski & Bartoszewicz, 2012; Chibowski et al., 2019). When found in agricultural landscapes, the mink tend to feed on ground-dwelling small mammals, such as *Microtus* sp that are highly abundant in rural habitats (Krawczyk et al., 2013). Considering the

variation in landscapes in our study (Figure 1), the Côtes d'Armor area in Brittany is more subject to anthropogenic activities compared to the Nive watershed in the Southwest. The latter is mainly composed of forests (48%) and meadows (30%; MNHN, 2015), while the Côtes d'Armor landscape was dominated in 2015 by agricultural areas (56%), then forests (21%) and very few meadows (9%; DRAAF Bretagne, 2021). A study conducted in northeastern Spain observed that the free-ranging American mink mostly predated on crayfish and this might be reflective of the mink diet in the Nive watershed (Melero et al., 2008). Alternatively, it is possible that feral American mink in Brittany have a similar diet to the mink from agricultural landscapes in Poland, preying on the available ground-dwelling rodents (Krawczyk et al., 2013). This difference in diet related to landscape variation between the two American mink populations could thus be reflected in the different composition of the gut microbial communities (Reese & Dunn, 2018; San Juan et al., 2020).

Regarding the free-ranging European mink habitat, the land uses of Navarra in 2015 was primarily agricultural areas (34.8%) and forests (28.2%), followed by meadows (15.7%; Vicente et al., 2005). The greatest proportion of agricultural lands in both Navarra and Brittany could thus indicate similar prey availability compared to the Nive watershed. Palazon et al. (2004) observed that the European mink diet in Navarra and La Rioja was predominantly composed of small mammals and fish, thus supporting the hypothesis that gut microbial composition of both mink species according to prey availability based on land occupation. To date, little is known about the diet of each mink species where our samples originated, but further work on their diet and gut bacteria, as well as prey surveys in mink territory could validate this hypothesis.

In conclusion, this study provides insight into the relationship between the gut bacteria of invasive and native carnivorous mammal hosts, with no observable signals of phylosymbiosis due to the strong influence of the environment and diet of the host on its associated microbes. Studying gut microbiota differences between mink farms in multiple countries, as well as individuals in their native habitat could also give more insight into the effects of domestication on microbe-host relationships.

Acknowledgments

FIEB (Ciprian Petrescu), INRAE (Olivier Lorvelec, Patricia le-Quilliec), Madis Podra, OFB (Christelle Bellanger, Maylis Fayet), Augustin Granel, Alice Mouton, Jasmine Veitch, Adrien André, EEP program for the European mink, Tiit Maran, the government of Navarra, and the Ministry for the Ecological Transition and the Demographic Challenge (Spain).

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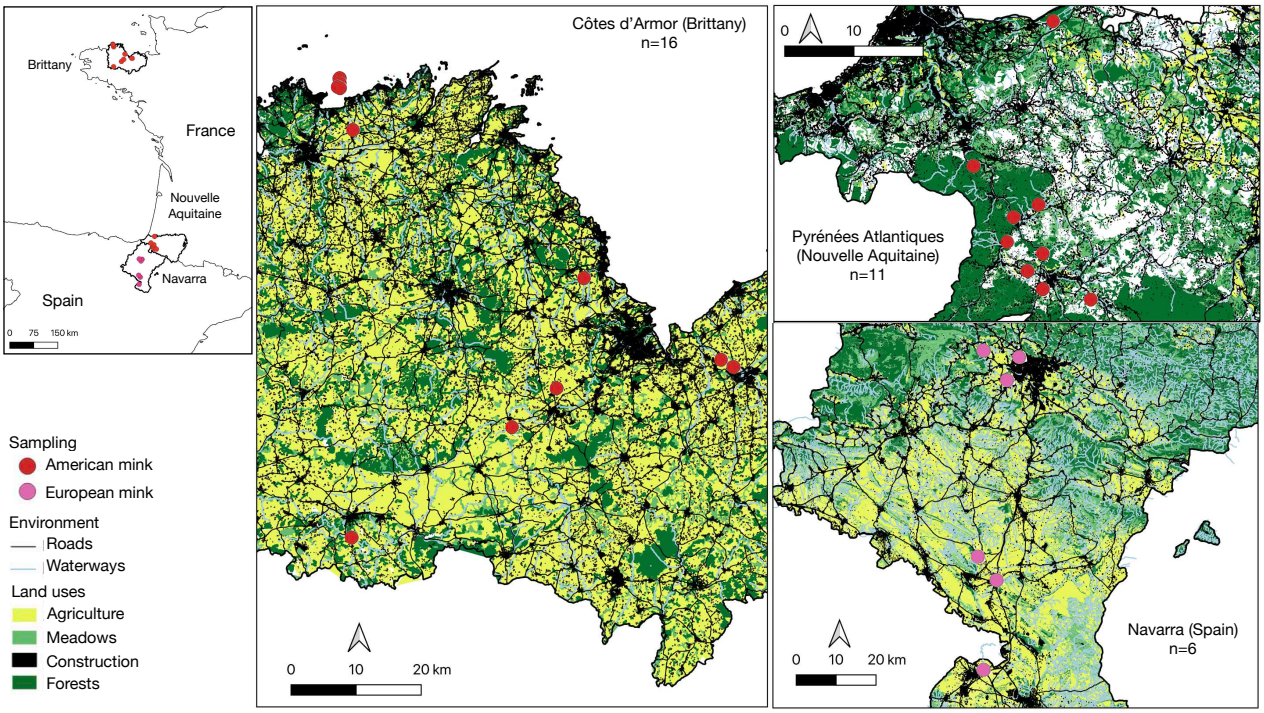

Figure 1. Map of free-ranging mink sampling sites with land uses.

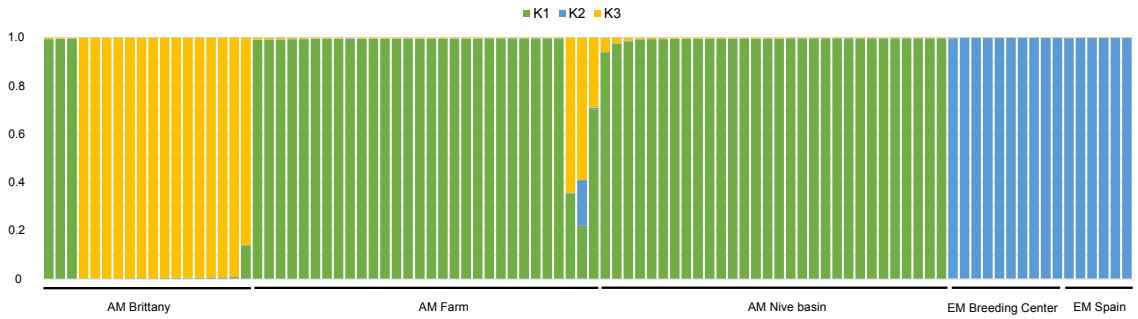

Figure 2. Individual assignment for each mink sampled according to Bayesian clustering following Evanno Best K method (K=3) based on microsatellite data.

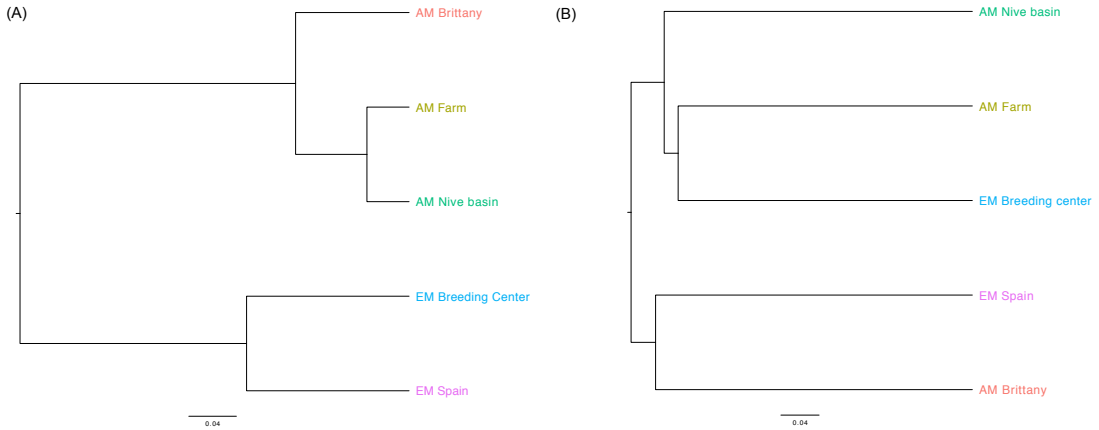

Figure 3. (A) UPGMA dendrogram constructed with Fst values from microsatellite data between mink population sampled, and (B) from weighted Unifrac distance matrix based on mean ceiling of each sample grouped by mink populations for gut microbial β-diversity.

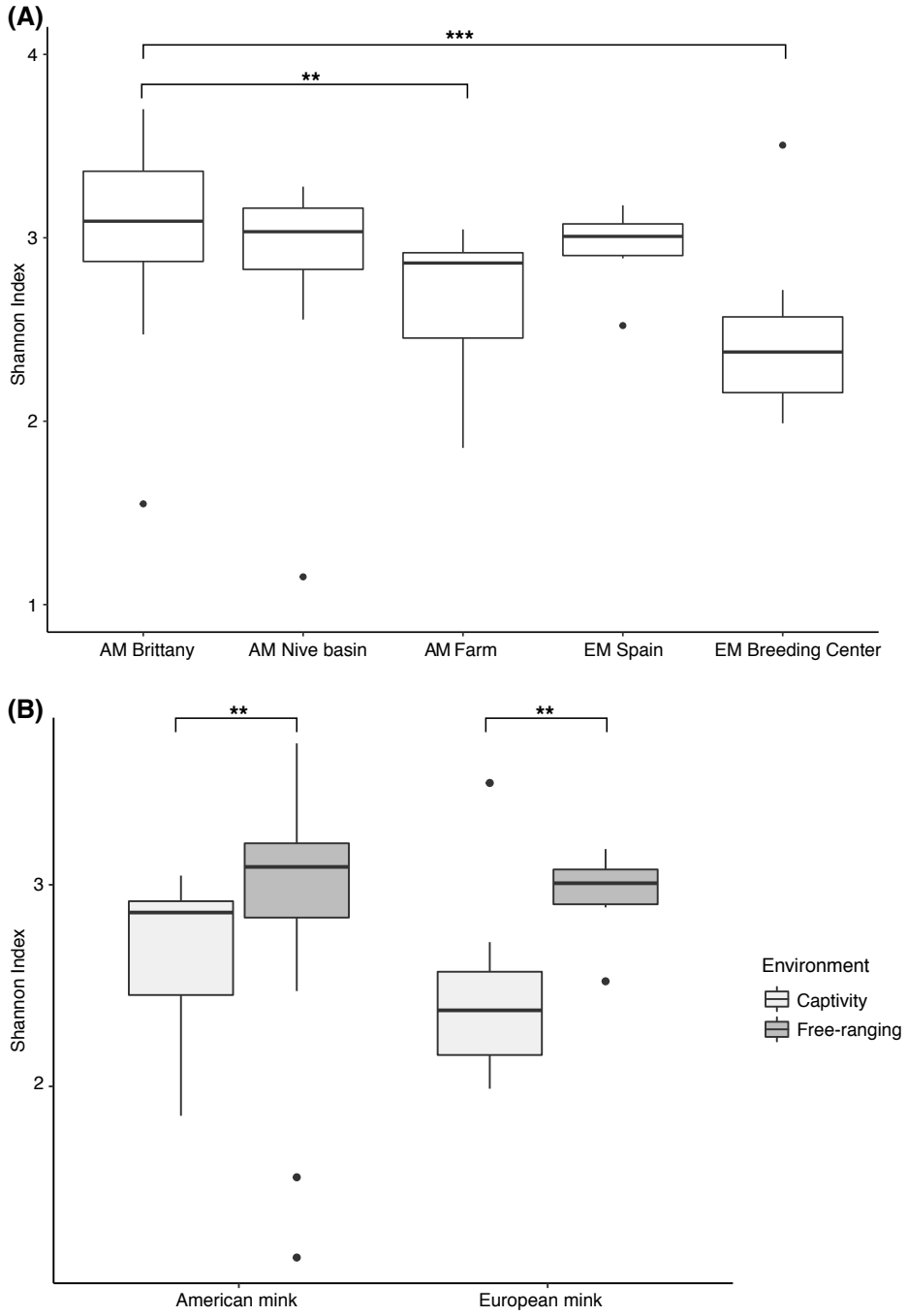

Figure 4. Boxplots representing Shannon Index variation of the gut microbiota depending on (A) host's environmental group, ** represents the p-value meeting the standard cutoff $p<0.01$ and *** $p<0.001$ from by Dunn test of Kruskal-Wallis multiple comparisons with Benjamini & Hochberg correction, and depending on (B) host's environment for both mink species.

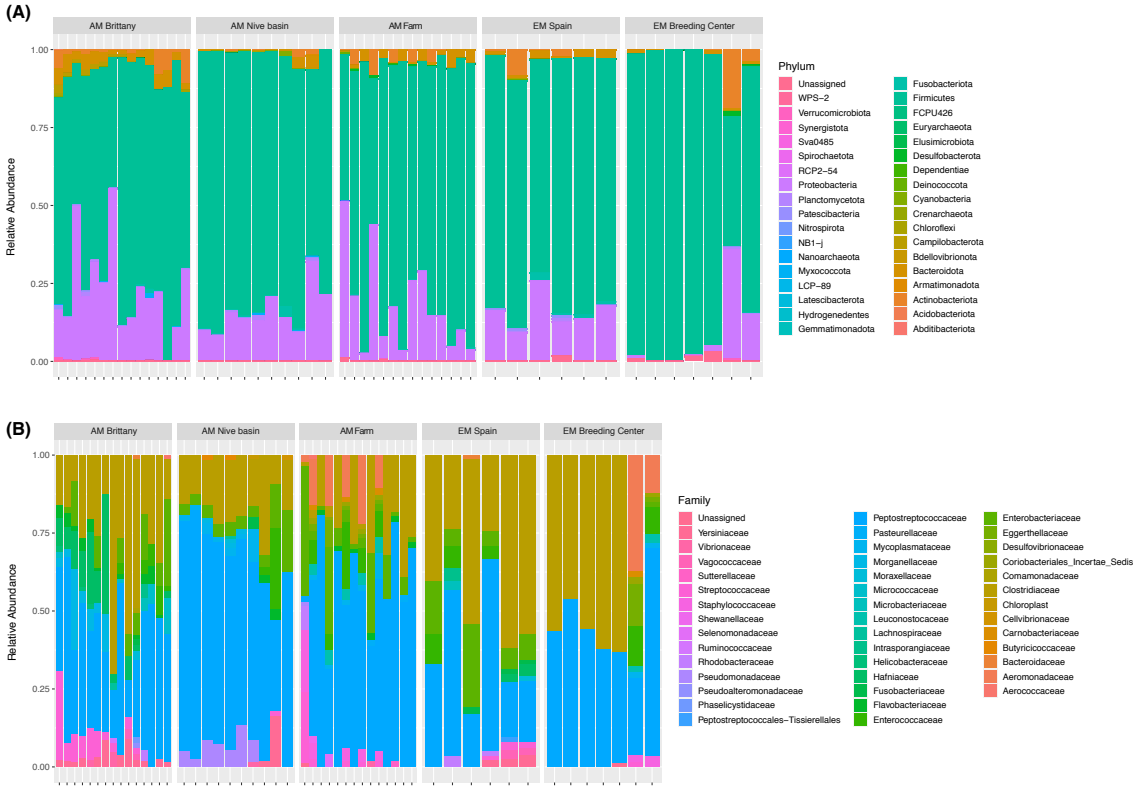

Figure 5. Compared relative abundance of bacterial taxa for each group of mink in the study (taxa showing less than 0.1% of relative abundance were not included). In each group, samples are sorted by individual. Stacked barplot showing the relative abundance at the (A) phylum and (B) family levels for gut bacteria.

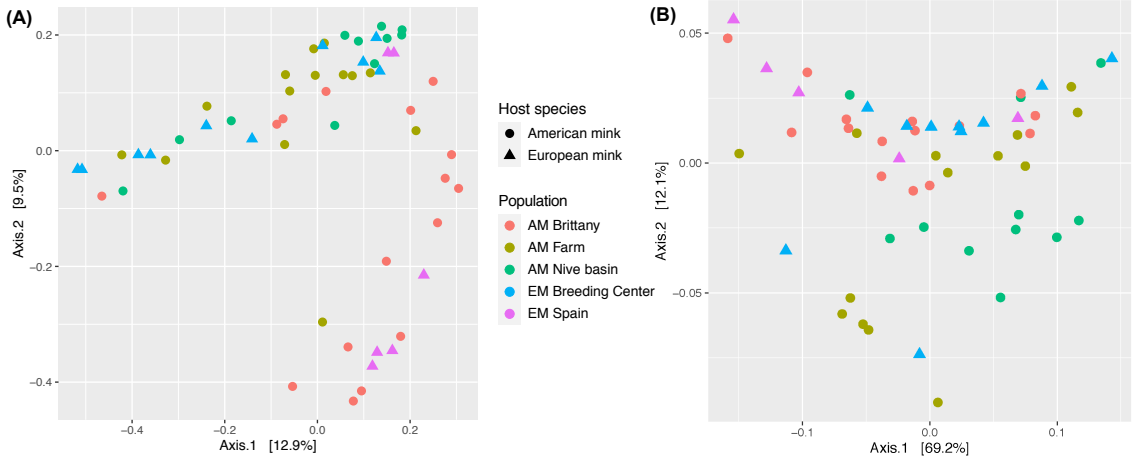

Figure 6. PCoA on (A) unweighted and (B) weighted Unifrac metric between samples. Unifrac metric calculated between samples for all gut bacterial taxa. Colors represent host population and shape the host species.

	American mink			European mink	
	Brittany	Nive basin	Farm	Spain	Breeding Center
N	17.6	27.9	25.4	5.8	9.7
A	66	80	97	18	22
%H	41.69	49.8	59.7	11.95	14.8
Ar	3.92	4.15	4.83	1.63	2.03
Ho	0.39	0.48	0.61	0.23	0.49
He	0.66	0.7	0.77	0.19	0.35
Fis	0.4043	0.3142	0.2144	-0.1869	-0.3894
Fis_Low	0.2886	0.2412	0.139	-0.468	-0.5148
Fis_High	0.5032	0.3935	0.2868	0.0324	-0.2669

Table 1. Sample size without missing data (N), total allele count (A), percentage of heterozygous locus (%H), allelic richness (Ar), observed heterozygosity (Ho), expected heterozygosity (He), and inbreeding coefficient (Fis) for each mink population.

	Chao1		Shannon index		Faith's PD	
	χ^2	p-value	χ^2	p-value	χ^2	p-value
Host species	0.472	0.492	1.634	0.201	0.169	0.680
Host sex	1.651	0.198	0.178	0.673	1.946	0.163
Mink population	3.527	0.474	*11.681*	*0.019*	5.829	0.212
Host environment	1.680	0.195	*10.59*	*0.001*	2.919	0.088

Table 2. Kruskal-Wallis chi-squared and p-values from tests for each alpha diversity metrics according to each variable investigated. Italicized values meet the standard cut-off for statistical significancy.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS2deseqresults.xlsx
- minkinvasionphylossuppaugust.docx