ARTICLE

Bacterial pathogens of non-native rodents and shrews of Busol Watershed and Forest Reservation, Baguio City

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ABSTRACT

In the Philippines, small mammal infections are poorly studied and are limited to zoonotic pathogens. To assess potential infectious threats to native non-volant mammal species within the Busol Watershed and Forest Reservation (BWFR), the pathogenic bacteria in synanthropic rodents and shrews were investigated using 16S rRNA gene sequencing. Potential transmission pathways of these pathogens were also studied through phylogenetic analyses and literature review. Twenty-seven non-native rodents and shrews were captured (12 *Suncus murinus*, 11 *Rattus exulans*, and four (4) *R. tanezumi*) and screened for putative bacterial pathogens. Eight (8) pathogenic bacterial phylotypes were identified: *Morganella morganii*, *Ehrlichia japonica*, *Exiguobacterium aurantiacum*, *Clostridium neonatale*, *Corynebacterium phoceense*, *Campylobacter curvus*, *Spodiobacter cordis*, and *Ralstonia* sp. The *S. cordis* and *Ralstonia* sp. were detected in the pooled blood and fecal samples, respectively. The presence of *Ralstonia* in feces suggests transmission through environmental exposure. Statistical tests also showed that the pathogen prevalence and dissimilarity had no association with host species, sex, and maturity, indicating that these are not linked to higher infection risks. This was also supported by the phylogenetic analyses wherein obtained sequences did not form distinct clades with genotypes associated with single host species. This study posits that synanthropic rodents and shrews in BWFR harbor pathogenic bacteria capable of cross-species transmission. Investigating these transmissions is important in predicting infection dynamics among small, non-volant mammal and human populations and the pathogen exposure risk of native mammal species within the BWFR.

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INTRODUCTION

The association of rodents and shrews with pathogens is an important area of study for both wildlife and human epidemiology since these share many characteristics with bats that are known to harbor high loads of microparasites, implying the potential of these species as hosts (Luis et al. 2013). Additionally, given that rodents comprise the most diverse taxon of mammals in terms of species and life-history strategies and the current extent of microbial analysis done in their populations in the wild, there is a significant probability of underrepresenting their occurring pathogens (Rabiee et al. 2018; Han et al. 2015, Luis et al. 2013). In support of this, one may look into the context of the Philippines. Despite the significant progress in small mammal studies, many areas characterized by a high degree of species endemism and richness remain to be surveyed extensively (Reginaldo and de Guia 2014). Thus, the greater extent of occurrence (species-specific association, distribution range/pattern) of pathogenic bacteria in wild populations of Philippine rodents and shrews remains to be explored. Furthermore, the relatively scarce number of studies on the pathogenic bacteria occurring in Philippine small, non-volant mammal species have primarily limited their scope to microbial

KEYWORDS

16S rRNA gene sequencing, bacterial pathogens, cross-species transmission, phylogenetic analysis, rodents, shrews, synanthropic

species significant to human epidemiology such as those belonging to the genera *Leptospira* and *Rickettsia*.

The synanthropic nature of non-native rodent and shrew species in the highly fragmented landscape of the Busol Watershed and Forest Reservation (BWFR) in Baguio City supports interactions with both human and wildlife populations. The resulting increase in contact rates also increases the opportunities for cross-species transmission of pathogens. Therefore, synanthropic, non-native rodent and shrew populations likely play a significant role in the transmission of bacterial pathogens from humans to native populations of small, non-volant mammals, and vice versa. As such, this study aimed to describe the occurrence of putative pathogens in non-native rodents and shrews present in synanthropic areas within the BWFR and their likely infection factors and transmission pathways.

MATERIALS AND METHODS

Rodent and shrew sampling

Sampling was done around the identified residential areas within the BWFR. Ground trapping using snap and cage traps was implemented using 45-m line transects. The trapping period spanned from October to November 2023, yielding 1,280 total trap nights.

As a prerequisite for trapping, a Memorandum of Agreement was signed with the Baguio Water District, the stakeholder primarily responsible for the BWFR's management. Additionally, an animal research permit (AR - 2023 - 0249) was obtained through the Cordillera Center for Animal Research and Development.

The sex, age class, body weight, morphometric measurements, and other diagnostic characteristics of the trapped individuals were recorded and consequently used for species identification.

Biological sampling

Oropharyngeal and rectal swabs, feces, blood, kidneys, lungs, and spleens were obtained from the rodents/shrews, and were preserved in 95% EtOH at -50°C until the DNA extraction. Mock sampling was performed by sweeping unused swabs over each of the traps that were able to capture rodents/shrews (Meldgaard, Bollen & Finsen 2004). Negative controls were also included, accounting for the false-positive results from the DNA extraction and PCR amplification. Phylotypes associated with the mock and negative samples were treated as potential contaminants.

Pathogenic bacteria identification

The DNA was extracted from the biological samples and the 95% EtOH eluents using the Hot Sodium Hydroxide and Tris (HotSHOT) technique by Alasaad et al. (2011), modified by extending the incubation time. The resulting DNA was processed for Polymerase Chain reaction (PCR) using the primer sets 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') to amplify the V4-V5 hypervariable regions of the bacterial 16S rRNA gene (Xiong et al., 2012). PCR products were processed for initial cleanup and then sent to Macrogen, Inc. for DNA sequencing.

Using RStudio (version 2023.06.1+524), the sequence outputs were subjected to quality filtering, de-noising, and chimera checking via a DADA2-based script adapted from Callahan et al. (2016). The DADA2 pipeline operationally defines units known as amplicon sequence variants (ASVs) through error corrections of input sequences. In addition to the quality filtering, ASVs with short sequences (<150 base pairs) were also removed in preparation for the taxonomic assignment (Chen et al. 2024).

Inference of the taxonomic identification of the ASVs was initiated by identifying the most similar 16S rRNA gene sequences across different databases (i.e., European Molecular Biology-European Bioinformatics Institute (EMBL-EBI), Genome Taxonomy Database (GTDB) R214, National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST), Ribosomal Database Project (RDP) version 16, SepsiTest BLAST, and SILVA SSU Ref NR 99). The percentage of identity match values generated was used for preliminary taxonomic identification of the ASVs as guided by the thresholds presented by Kim et al. (2014) and Yarza et al. (2014) in their studies.

The sequences of the obtained ASVs were further binned into phylotypes based on the biodiversity unit (i.e., candidate taxonomic unit, CTUs) proposed by Yarza et al. in 2014. Additionally, given that it assigned the most taxa down to the genus and species levels out of all the databases checked, the NCBI BLAST was used for the initial taxonomic definition of the ASVs.

Finally, a list of phylotypes with putative pathogenicity was curated based on the literature. The classification of a phylotype's pathogenic potential was based on (1) the resolution of their taxonomic assignment (i.e., at least down the genus level) and (2) the presence of published reports implicating their role in the development of pathological conditions in mammals.

Bacterial diversity and infection prevalence estimation and analyses

The α-diversity indices of the pathogenic bacterial phylotypes associated with identified host attributes (species, sex, and maturity) were calculated (Table 2). The Shannon diversity index (H) was compared across the host attributes using Hutcheson'sdiversity t-test (Hutcheson, 1970). The Simpson's (1-D) index was also obtained to evaluate species evenness and dominance. To test whether differences in 1-D are significant, a t-test following the method of Keefe and Bergerson (1977) was done.

The Bray-Curtis distance was used for dissimilarity assessment in the overall pathogen composition (β-diversity) between different host characteristics. The nonmetric multidimensional scaling (nMDS) was then used to visualize the community structure of detected pathogens according to host species, sex, and maturity. Afterward, similarity percentage (SIMPER) was used to determine the key contributing phylotypes responsible for the observed dissimilarities. The prevalence of the detected putative pathogens was also calculated for each subgroup based on the identified host attributes and biological sample used.

All diversity analyses were implemented in the Paleontological Statistics (PAST) software version 4.03, considering a p-value < 0.05 to be significant for the statistical tests.

Infection drivers and transmission characteristics inference

Multiple generalized linear models (GLMs) using RStudio's lme4 package were fitted to test whether host species, sex, maturity, or a combination thereof explained pathogen infection status among the rodents and shrews sampled. The best model was selected based on the least change in the corrected Akaike information criterion (ΔAICc).

Pathogen detection from the pooled biological samples was used to suggest potential transmission through exposure to these biological agents. Phylogenetic patterns of host specificity of the identified pathogens were also used to infer cross-species

transmissions. Clustering of the obtained sequences with other sequences, or the absence thereof, was used to infer potential transmission routes.

RESULTS AND DISCUSSION

Synanthropic rodent and shrew species within the BWFR

All recorded species in this study (Figure 1 A) were noted as Philippine non-natives, except for *R. everetti* which is endemic to the country. Given the scope of the study, the trapped *R. everetti* was excluded from the succeeding sampling and analyses.

The species accumulation curve (Figure 1A) indicates that no new species had been documented after the 11th day of trapping. However, the sample-based rarefaction curve (Figure 1B) suggests the possibility of recording a few more species with more extensive trapping efforts. Nevertheless, both the species accumulation and sample-based rarefaction curves suggest the representation of most, if not all, possible small, non-volant mammal species in the study area.

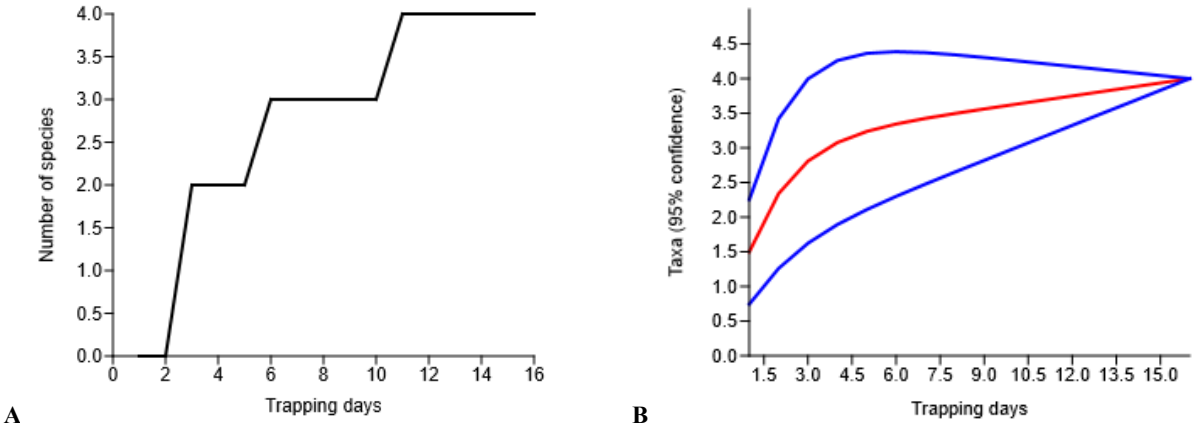

Figure 1: (A) Species accumulation and (B) sample-based rarefaction curves of small, non-volant mammal species based on daily captures.

Bacterial pathogen occurrence and prevalence

Analyses revealed eight (8) phylotypes of pathogenic bacteria, namely *Morganella morganii*, *Ehrlichia japonica*, *Exiguobacterium aurantiacum*, *Clostridium neonatale*, *Corynebacterium phoceense*, *Campylobacter curvus*, *Spodiobacter cordis*, and *Ralstonia* sp. However, the achieved sequencing depth underestimated the infecting pathogens as suggested by the species accumulation and sample-based rarefaction curves (Figure 2). In the case of sample pooling, prevalence could not be determined for some pathogens. This is exemplified with *S. cordis* and *Ralstonia* sp. which were identified from pooled blood and fecal samples, respectively. Nevertheless, the analyses showed that the synanthropic community of non-native rodents and shrews in the BWFR potentially harbors numerous pathogenic bacterial species.

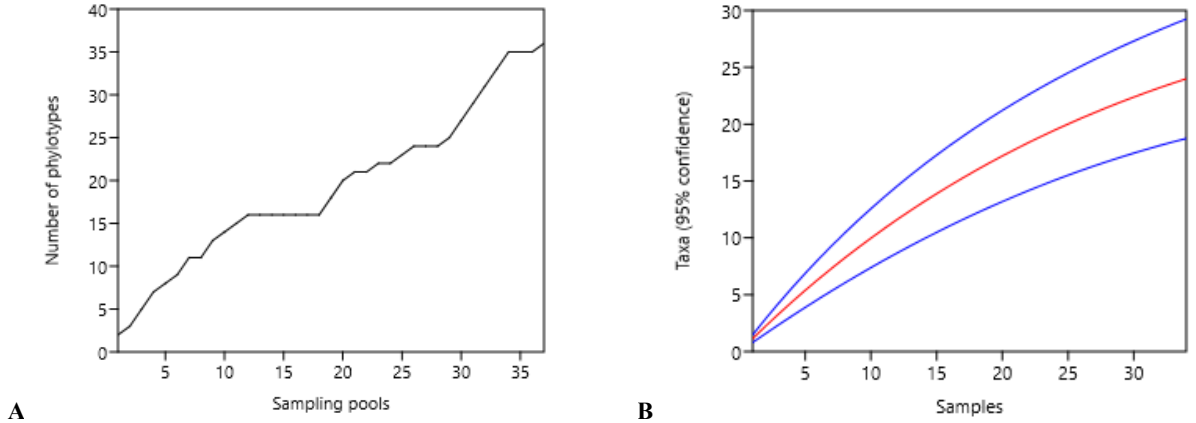

Figure 2: (A) Species accumulation and (B) sample-based rarefaction curves of bacterial phylotypes based on sequenced samples.

Several studies (e.g., Lee et al. 2008) identified *M. morganii* as an intestinal and respiratory commensal of rats. Despite being reported as part of the normal microbial flora of rodents, *M. morganii* has also been reported in a few disease cases (e.g., Vandenberge et al. 2013). Despite its low virulence, *M. morganii* is an emerging pathogen of epidemiological significance due to its antibiotic resistance (Ayyal et al. 2019). In this study, the prevalence of *M. morganii* was computed to be 3.70% . This prevalence was slightly higher than the reported rate (1.66%) in a study by Ayyal et al. (2019) on *R. rattus* captured from Baghdad City, Iraq. The prevalence rate of *M. morganii* indicated in this study was also comparable to prevalence reports of *M. morganii* in rats cohabitating with poultry in Nigeria (1.25-3.75%) as presented by Jemilehin et al. (2016).

E. japonica on the other hand is a highly virulent, obligate intracellular (macrophage) pathogen involved in diseases of major importance in humans and other mammals including rodents and dogs (e.g., Ismail et al. 2022; Lin et al. 2021; Zhang et al. 2024). This study recorded a low prevalence rate of 3.70%. Whether this value is high or low relative to other areas cannot be ascertained since no published reports on the prevalence rate specific to *E. japonica* have been found thus far.

Bacteria previously unreported in small, non-volant mammals

were also detected: *E. aurantiacum*, *C. neonatale*, *C. phoceense*, and *C. curvus*. The *E. aurantiacum* is part of the human body's commensal microflora (Inbakandan et al. 2010). It has also been found in environmental samples (e.g., soil, natural bodies of water) and marine species. While not widely documented in rodents and shrews, the emerging significance of *E. aurantiacum* in wildlife pathogenesis is proposed given its documented resistance to several antibiotics (Jain and Kamble 2018). On the other hand, *C. neonatale* is associated with necrosis-causing enterocolitis in human neonates. Specifically, the phylotype was detected from a juvenile female *R. tanezumi*. Attention to the occurrence of *C. neonatale* in small mammal species should also be sought due to its potential involvement in rodent intestinal pathogenesis and its resistance to clindamycin (Bouvet et al., 2014). *Corynebacterium* species are also considered as emerging pathogens (Barberis et al. 2021). *C. phoceense* in particular was reported to have several virulence factors, including the SpaD-type pili (Giannattasio-Ferraz et al., 2021). Additionally, *C. phoceense* has been reported in urinary tract and bloodstream infections of humans, cattle, and heifers (Barberis et al. 2021; Dubourg et al. 2018; Giannattasio-Ferraz et al. 2021). In this study, *C. phoceense* was detected from two (2) species, *R. exulans,* and *R. tanezumi*, with a prevalence of 7.41%. Aside from its infectious potential, its reported resistance to the antibiotics' nitrofurantoin and metronidazole (Cresci et al. 2016) is also an epidemiological concern. *C. curvus* is another pathogen associated with diseases and infections in humans and animals. It has been incriminated in periodontal diseases and extra-oral infections such as empyema, and liver and lung abscesses (Abbott et al. 2005; Horio et al. 2017; Iraola et al. 2014). Although other *Campylobacter* species such as *C. jejuni* have been identified in small, non-volant mammal species (e.g., Olkkola et al., 2021), records specific to *C. curvus* were not found. However, the potential spillover of *Campylobacter* species from humans to animals has been put forward by Zhang et al. (2023). In this study, its prevalence was calculated to be 3.70%.

No significant difference in infection prevalence of the pathogens described earlier was observed across different host species, sex, or age (Table 1). This suggests that the identified pathogens may be present in the environment regardless of the specific host characteristics examined.

Table 1: Pathogen infection prevalence across different host attributes

Putative pathogen	Host species			Host sex		Host maturity	
	R. exulans (N = 11)	*R. tanezumi* (N = 4)	*S. murinus* (N = 13)	Female (N = 13)	Male (N = 14)	Juvenile (N = 14)	Adult (N = 13)
M. morganii	9.09%	-	-	-	7.14%	-	7.69%
E. japonica	9.09%	-	-	-	7.14%	-	7.69%
E. aurantiacum	-	-	8.33%	7.69%	-	-	7.69%
C. neonatale	-	25.00%	-	7.69%	-	7.14%	-
C. phoceense	9.09%	25.00%	-	7.69%	7.14%	7.14%	7.69%
C. curvus	9.09%	-	-	-	7.14%	-	7.69%
Overall	27.27%	50.00%	8.33%	23.08%	21.43%	7.14%	30.77%

The sequence analyses in this study also revealed an adult male *R. exulans* co-infected with *M. morganii* and *E. japonica*, suggesting the potential for multiple pathogen exposure within the sampling population. More in-depth research is warranted to explore the frequency and implications of such co-infections. Results also show that *R. exulans* carry more than two (2) pathogenic species. Specifically, four (4) pathogens were identified from this host species: *M. morganii*, *E. japonica*, *C. phoceense*, and *C. curvus*. The potential status of *R. exulans* as a hyper-reservoir of pathogens infecting native, small, non-volant mammal populations in the BWFR is proposed. However, this will need to be further corroborated by evidence that (1) the said pathogens are circulating within the native mammal population, and that (2) these infections in the native population are not sustained with the elimination of all transmission routes with the non-native populations.

Pathogen diversity and community composition in non-native hosts

Although likely underestimated, the H values show a significantly higher species richness of bacterial pathogens in *R. exulans* than *S. murinus* (Figure 3; see also Chao1 estimate for *R. exulans* in Table 2). However, pathogen species richness did not differ between host sex or maturity. This reveals that host species is the most important determinant of pathogen α-diversity. Additionally, the results suggest that *R. exulans* may be favored for pathogen transmission, especially human-associated bacteria. Rodent species generally have been known to be one of the most commensal synanthropic species (Heaney et al. 2016; Jahan et al. 2021). *R. exulans* has been documented to prefer households over other disturbed habitats. This is in contrast with other rodent species such as *R. tanezumi* which have also been known to be abundant in disturbed habitats but have exhibited lower habitat specificity/preference (Heaney et al. 2016; Ivanova et al. 2012; Jahan et al. 2021; Neves et al. 2017). Additionally, *R. exulans* and *S. murinus* greatly differ in their diet with the former consuming a wider breadth of food items while the latter mainly feeding on invertebrates such as insects (Heaney et al. 2016; Jahan et al. 2021). These generalist characteristics in terms of habitat and diet preference might explain why in this study, more pathogen phylotypes have been detected in *R. exulans* than in *S. murinus*. Investigating these factors could provide insights into pathogen transmission dynamics. Such investigations could include determining the habitat use patterns/overlaps, and the gut pathogen load of *R. exulans*, *R. tanezumi*, and *S. murinus* within the BWFR.

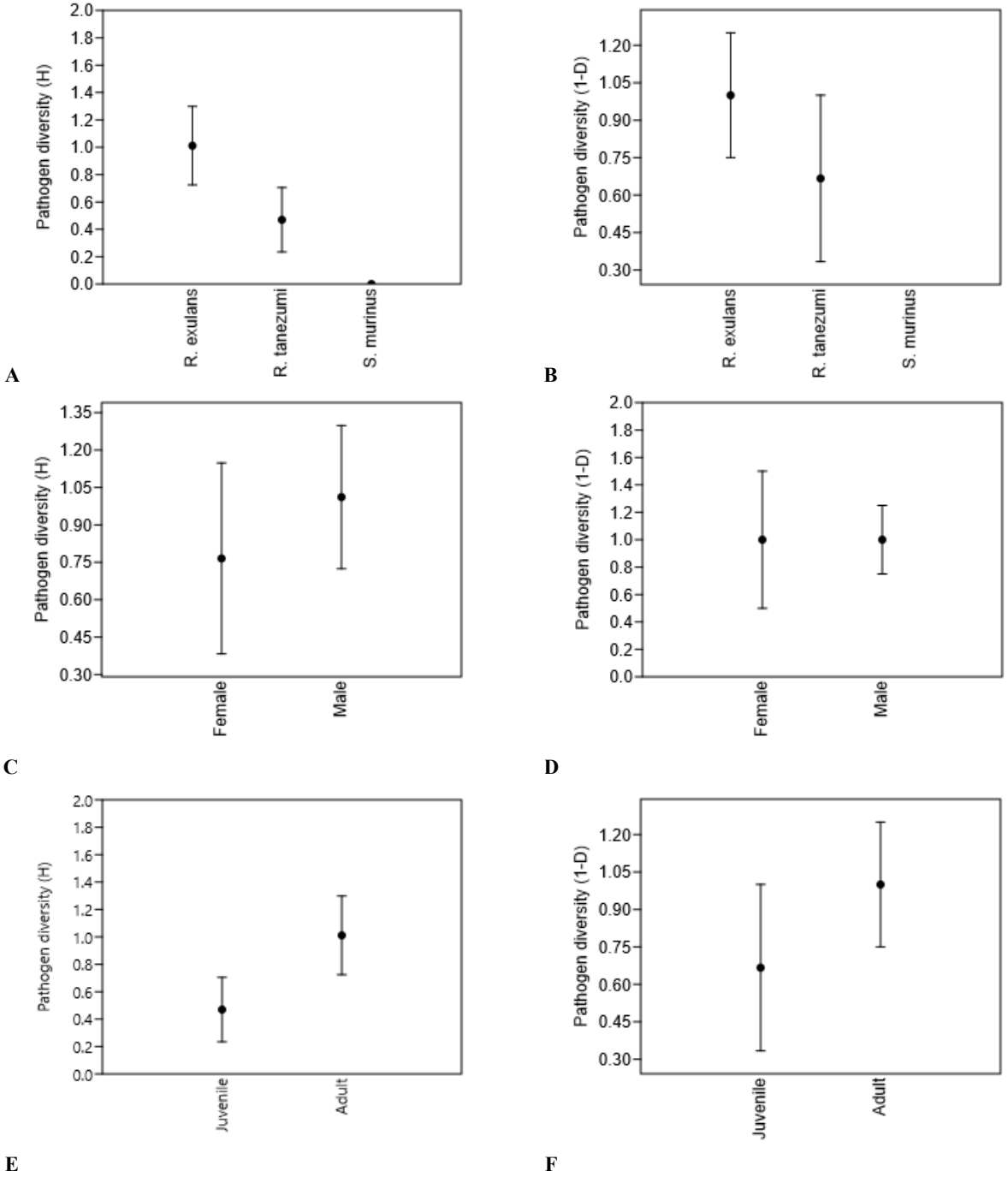

Figure 3: Diversity profiles of pathogenic bacteria between (A-B) host species, (C-D) host sex, and (E-F) host maturity.

Table 2: Diversity indices for pathogenic bacteria detected across different host attributes

Diversity measure	Host species			Host sex		Host maturity	
	R. exulans	*R. tanezumi*	*S. murinus*	Female	Male	Juvenile	Adult
Species richness							
Number of recorded phylotypes (S)	*4*	*2*	*1*	*3*	*4*	*2*	*5*
Chao1	*10* ± 4.00	2 ± 0.50	1 ± 0.00	6 ± 2.50	10 ± 4.00	2 ± 0.50	10 ± 4.00

Shannon index (H)	1.01 ± 0.29	0.47 ± 0.23	0 ± 0.00	0.78 ± 0.38	1.01 ± 0.29	0.47 ± 0.23	1.01 ± 0.29
Species evenness and dominance							
Simpson's index (1-D)	*1*	*0.67*	*0*	*1*	*1*	*0.67*	*1*

High dissimilarity in overall pathogen community structure was also found between *R. exulans* and *R. tanezumi*, having an average Bray-Curtis dissimilarity of 71.43%. Based on SIMPER analysis, *C. phoceense* and *C. neonatale* were predominantly associated with *R. tanezumi*, contributing to 62.50% of the dissimilarity. On the other hand, there was 100.00% dissimilarity within the rest of the host species (i.e., *R. exulans* vs. *S. murinus*, *R. tanezumi* vs. *S. murinus*). The *E. aurantiacum* phylotype was associated with *S. murinus*, but not with *R. exulans* or *R. tanezumi*. This translated to 44.44% and 41.67% contribution to dissimilarity with *R. exulans* and *R. tanezumi*, respectively. Pathogenic bacterial communities within different host sexes were also highly dissimilar (75.00%), predominantly driven by the presence of *C. phoceense*, *C. curvus* in the males, and *E. aurantiacum* in the females. Juvenile and adult hosts also exhibited 75.00% dissimilarity with *C. phoceense* and *C. neonatale* predominantly associated with the juvenile hosts.

As seen in the nMDS plot (Figure 4), no points were ordinated closely to each other, corroborating the high dissimilarity calculated. To have ordination with a better fit, the inclusion of other infection factors might be necessary.

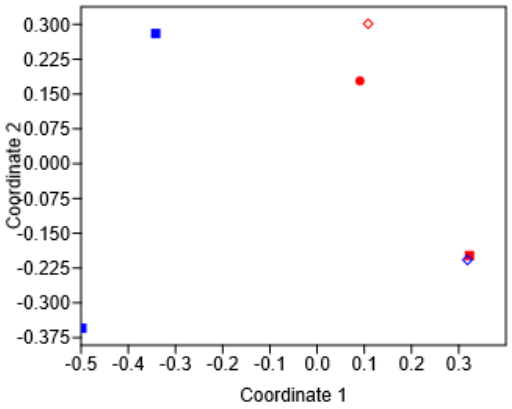

Figure 4: Non-metric multidimensional scaling (nMDS) of bacterial pathogenic communities associated with the non-native, synanthropic rodents and shrews within the Busol Watershed and Forest Reservation (BWFR (Rattus exulans - squares; Rattus tanezumi - diamonds; Suncus murinus - circles; male - blue; female - red; adult - filled; juvenile - hollow).

Potential infection drivers and transmission characteristics

The model, including the effect of host species and host maturity interaction, best fits the data (Table 3), indicating that pathogen infection status varies most dramatically when considering the interaction between both host attributes. However, these model predictions are not significant at a p-value of 0.05. This finding from the GLM indicates that although host characteristics play a role in pathogen infection, other confounding factors related to host species and maturity might have a more significant effect on the infection status of a synanthropic rodent/shrew in the BWFR.

Table 3: Model criteria scores of generalized linear models (GLM) of infection status

	Predictor variables	AICc	ΔAICc	AICc weight	Cumulative weight
Model 1	(Intercept only)	47.38	2.88	0.10	0.83
Model 2	Host species	46.24	1.74	0.17	0.57
Model 3	Host sex	49.11	4.61	0.04	0.98
Model 4	Host maturity	48.76	4.26	0.05	0.94
Model 5	Host species + host sex	48.38	3.88	0.06	0.89
Model 6	Host species + host maturity	44.50	0	0.40	0.40
Model 7	Host sex + host maturity	50.42	5.92	0.02	1.00
Model 8	Host species + host sex + host maturity	46.31	0.07	0.16	0.74

In the context of pathogen transmission, the wide distribution and lack of clear host and location clustering of the identified pathogens (Figures 5-10) suggest a potential for broad-host range or environmental transmission routes. This conclusion aligns with the hypothesis posited earlier that other factors, such as habitat and feeding preference of the host species, might significantly influence the transmission of the identified pathogens. Given that all the identified pathogenic phylotypes have been known to infect humans, it is proposed that these pathogens tend to be transmitted to host species that have closer association with humans (i.e., *R. exulans*). The synanthropic nature of these non-native mammal species allows them to have direct and indirect environmental exposures to pathogens carried by humans and their commensal species (e.g., canines, bovines,

and swine). The latter is also likely the case for *Ralstonia* sp. which was detected from the pooled fecal samples.

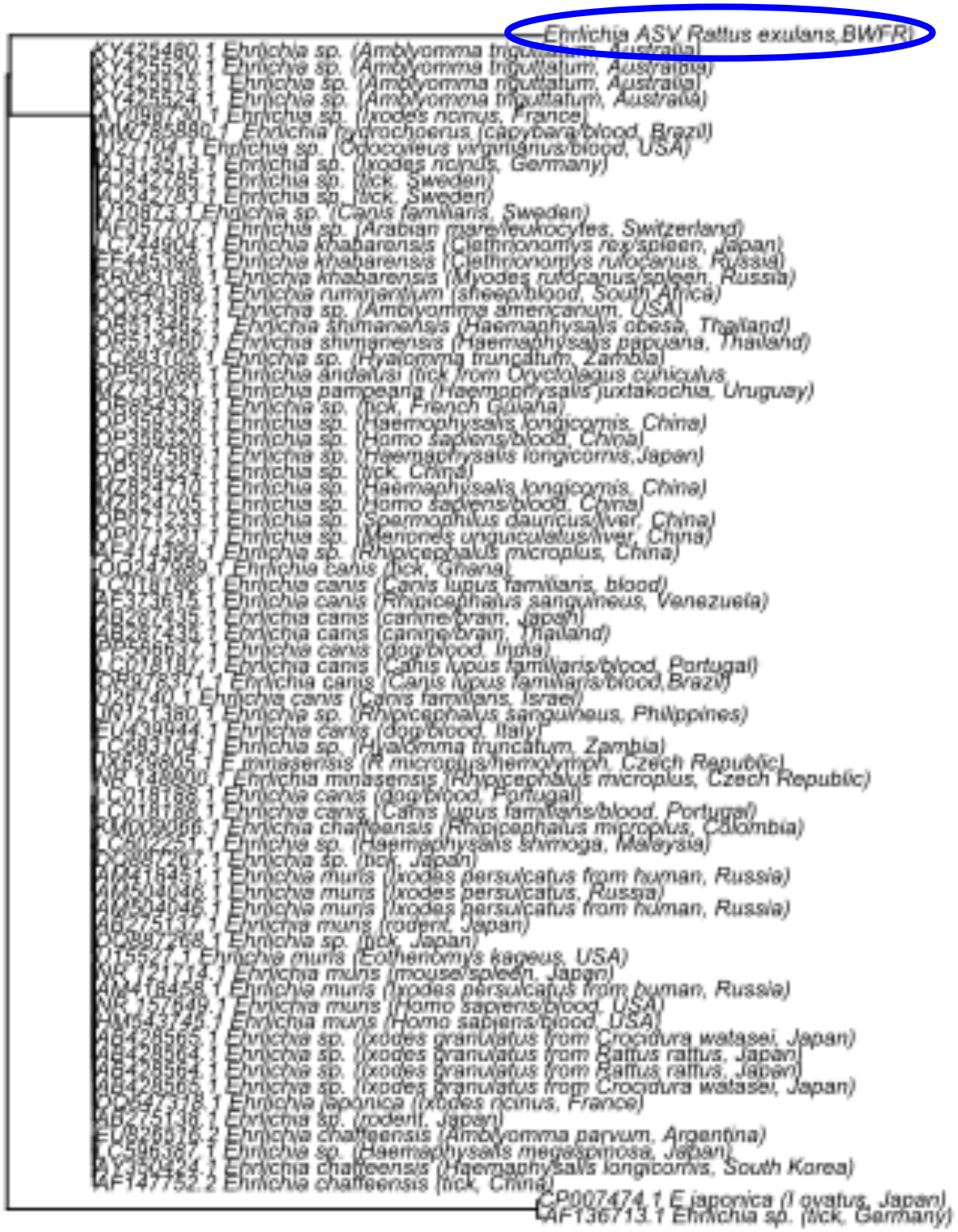

Figure 5: Optimized Phylogenetic Maximum Likelihood (PML) tree topologies of the 16S rRNA gene sequences of the detected *Morganella morganii* phylotype and of the related genotypes from the GenBank generated using the RStudio.

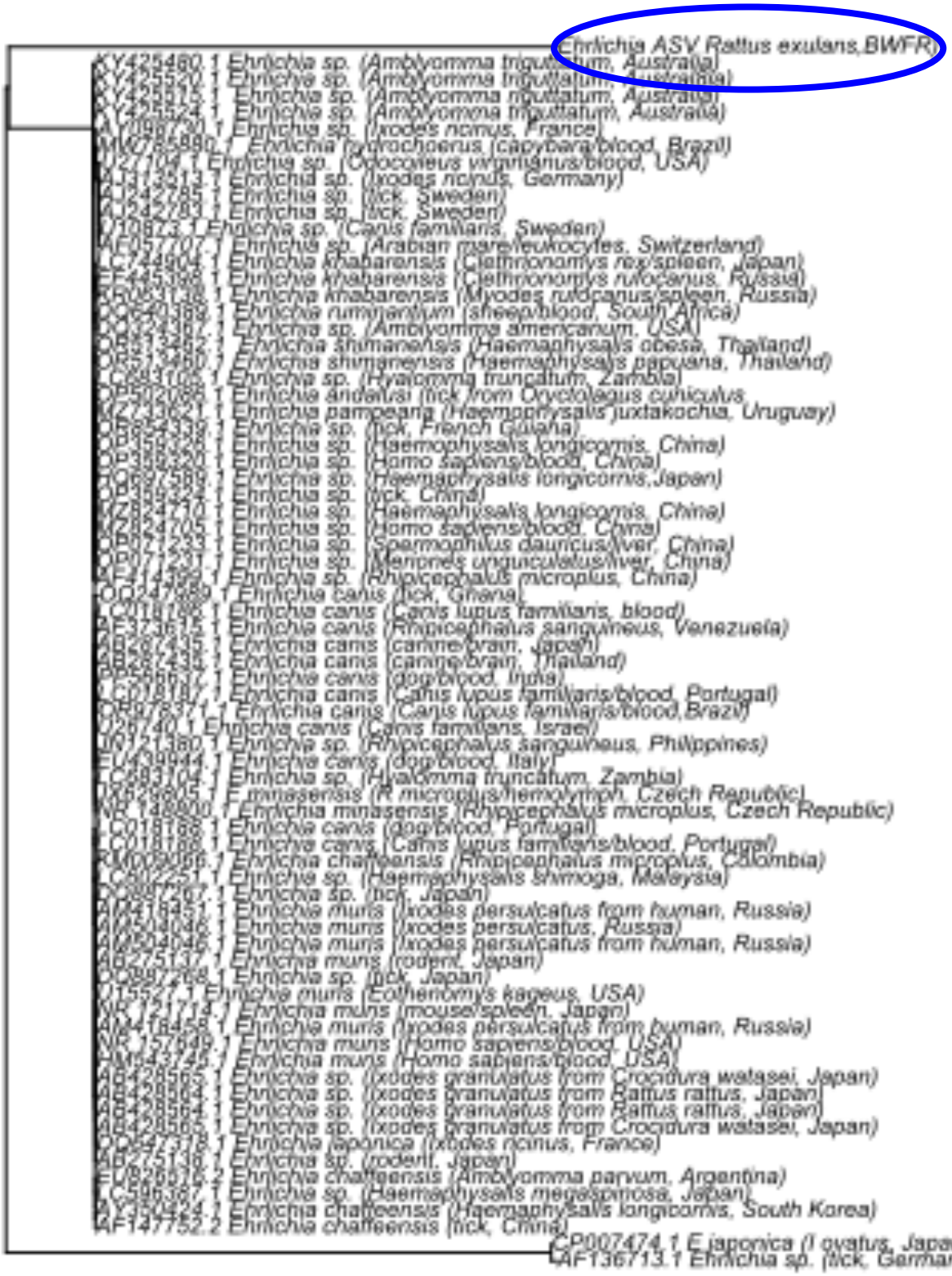

Figure 6: Optimized Phylogenetic Maximum Likelihood (PML) tree topologies of the 16S rRNA gene sequences of the detected *Ehrlichia japonica* phylotype and of the related genotypes from the GenBank generated using the RStudio.

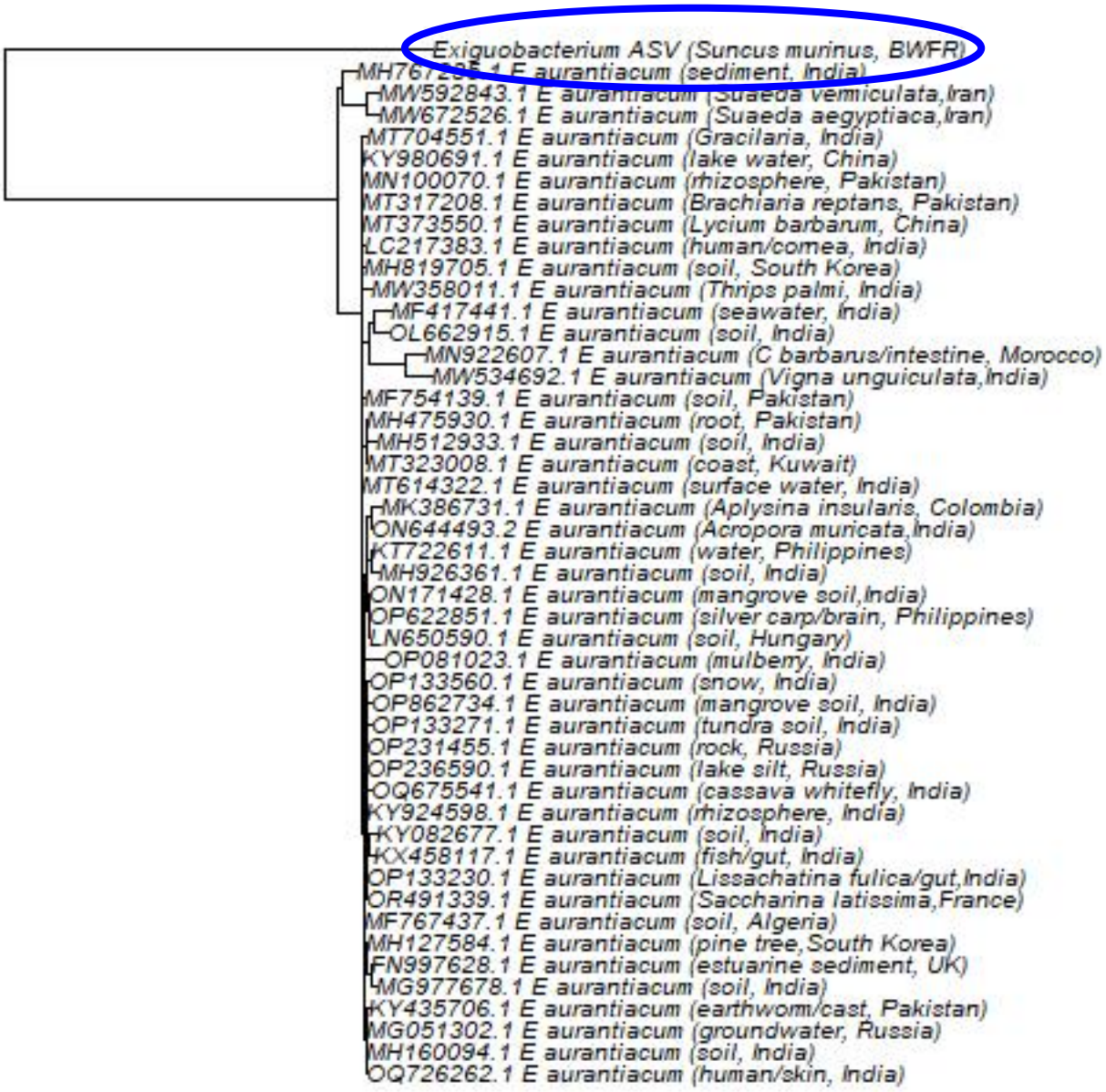

Figure 7: Optimized Phylogenetic Maximum Likelihood (PML) tree topologies of the 16S rRNA gene sequences of the detected *Exiguobacterium aurantiacum* phylotype and of the related genotypes from the GenBank generated using the RStudio.

Figure 8: Optimized Phylogenetic Maximum Likelihood (PML) tree topologies of the 16S rRNA gene sequences of the detected *Clostridium neonatale* phylotype and of the related genotypes from the GenBank generated using the RStudio.

Figure 9: Optimized Phylogenetic Maximum Likelihood (PML) tree topologies of the 16S rRNA gene sequences of the detected *Campylobacter curvus* phylotype and of the related genotypes from the GenBank generated using the RStudio.

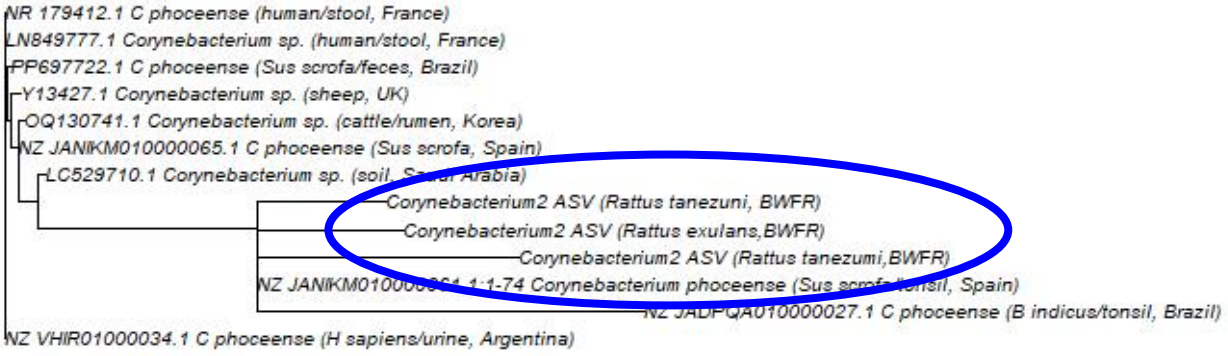

Figure 10: Optimized Phylogenetic Maximum Likelihood (PML) tree topologies of the 16S rRNA gene sequences of the detected *Corynebacterium phoceense* phylotype and of the related genotypes from the GenBank generated using the RStudio.

Implications of research findings on wildlife conservation efforts in the BWFR

Given the knowledge gaps on active infections of non-native rodent and shrew populations in the BWFR, the identification of bacterial pathogens these mammals harbor, along with the initial understanding of how these pathogens spread among their non-native hosts will contribute to the implementation of data-driven interventions even before a potential disease wildlife disease outbreak occurs. For instance, identifying *R. exulans* as a potential reservoir host necessitates targeted surveillance and prompt detection of infections that can jeopardize native rodents in the BWFR. Additionally, zoonotic pathogen identification presents collaborative opportunities with public health agencies for management strategies complementing the One Health framework, such as the delineation of buffer zones.

CONCLUSION

To the authors' knowledge, this study is the first investigation of pathogenic bacteria carried by non-native, synanthropic rodents/shrews in the BWFR. The following pathogenic bacteria were identified in the rodent (*R. exulans* and *R. tanezumi*) and shrew (*S. murinus*) populations in the area: *M. morganii*, *E. japonica*, *E. aurantiacum*, *C. neonatale*, *C. phoceense*, *C. curvus*, *S. cordis*, and *Ralstonia* sp. Results also reveal the need for a more robust detection of these bacteria in both native and non-native mammal populations in the BWFR. Given the data obtained in this study, no significant difference in infection prevalence across different host species, sex, or maturity was detected. However, *R. exulans* exhibited a higher pathogen richness than *S. murinus*. With this, the potential role of *R. exulans* as a multi-pathogen reservoir host species for the small mammal populations in the BWFR is suggested.

High dissimilarities in pathogen communities (>71.43%) were also observed across all host attributes Additionally, the nMDS plot does not show any tight clustering of data points, indicating that differences in host species, sex, and maturity do not sufficiently explain the measured dissimilarities. This finding highlights the importance of investigating other infection factors in the study population. This is corroborated by the GLM wherein the interaction between host species and maturity was shown to have the strongest influence on infection status in the study population, but showed no statistically significant effects.

The identified pathogens have also been shown to have a wide geographic distribution and several documented host species as shown by the absence of clustering of several genotypes according to isolation source (geographic area and host species). Apart from the broad host range, the results might also suggest the transmission of the identified pathogens through environmental routes (e.g., fecal exposure to *Ralstonia* sp.). Interactions with humans and other commensal, including domesticated mammals, are also likely responsible for the occurrence of pathogens in the study population.

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CONFLICT OF INTEREST

KP Paglingayen, RM Gutierrez, and RJ Calugay declare they have no conflicts of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

The conceptual framework and design were developed by KP Paglingayen and RM Gutierrez. Data acquisition, analysis, and interpretation were done by KP Paglingayen. The article was drafted by KP Paglingayen, with critical revisions from RM Gutierrez and RJ Calugay. The version to be published was approved by KP Paglingayen, RM Gutierrez, and RJ Calugay.

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