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

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ABSTRACT

n 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, Campylobacter Corynebacterium phoceense, 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

*Corresponding author Email Address: kppaglingayen@up.edu.ph Date received: 17 July 2024 Dates revised: 03 October 2024 Date accepted: 08 December 2025 DOI: https://doi.org/10.54645/202518SupLFF-84

.//doi.org/10.54645/202518SUPLFF-8

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.

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, crossspecies 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 a-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 (Δ AICc).

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.



Bacterial pathogen occurrence and prevalence

Analyses revealed eight (8) phylotypes of pathogenic bacteria, namely Morganella Ehrlichia morganii, japonica, Exiguobacterium Clostridium neonatale. aurantiacum, Corynebacterium Campylobacter phoceense, 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.

| | Host species | | | Host sex | | Host maturity | |
|-------------------|---------------------|----------------------------|----------------------------|--------------------|------------------|----------------------|-------------------|
| Putative pathogen | R. exulans (N = 11) | <i>R. tanezumi</i> (N = 4) | <i>S. murinus</i> (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% |

Table 1: Pathogen infection prevalence across different host attributes

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, nonvolant 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 nonnative 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 Chaol 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 adiversity. Additionally, the results suggest that R. exulans may be favored for pathogen transmission, especially humanassociated 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.



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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

| | Host species | | | Host sex | | Host maturity | |
|-----------------------------------|-------------------------|-----------------|-----------------|-----------------|------------------|-----------------|------------------|
| Diversity measure | 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 | <i>10</i> <u>+</u> 4.00 | 2 <u>+</u> 0.50 | 1 <u>+</u> 0.00 | 6 <u>+</u> 2.50 | 10 <u>+</u> 4.00 | 2 <u>+</u> 0.50 | 10 <u>+</u> 4.00 |

| Shannon index (H) | 1.01 <u>+</u> 0.29 | 0.47 <u>+</u> 0.23 | 0 <u>+</u> 0.00 | 0.78 <u>+</u> 0.38 | 1.01 <u>+</u> 0.29 | 0.47 <u>+</u> 0.23 | 1.01 <u>+</u> 0.29 |
|-------------------------------|--------------------|--------------------|-----------------|--------------------|-----------------------|--------------------|-----------------------|
| Species evenness and dominant | се | | | | | | |
| 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.

| | Predictor variables | AICc | ΔAICe | 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 |

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

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.

| Ehrlichia ASV Rattus exulans BWFRI |
|--|
| KY425480.1 Ehrlichia sp. (Amblyomma trigunation: Australia) Ky425520.1 Ehrlichia sp. (Amblyomma trigunation: Australia) |
| SY425515 1 Ehrlichia sp. (Amplyomma riguttatum, Australia) |
| A Y098730,1 Enrichia sp. (Ampiyomina Inputatum, Australia) |
| MMC785880.1 Ehrlichia hydrochoerus (capybara/blood, Brazil) |
| AJ3(3513.1 Ebrichia sp. (lyodas rojnus, Germany) |
| AJ242783.1 Enrichia sp. (lick, Sweden) AJ242783.1 Enrichia sp. liick, Sweden) |
| (/10873.1_Endichia sp. (Canis familians, Sweden) |
| (C744904.1 Ehrlichia khabarensis (Ciethrionomys reg/spieen, Japan) |
| EE4453388, T Ehmchia kriabarensis (Clefinrionoritys rutocanus, Russia) KR053138, T Ehrlichia kriabarensis (Myodes rutocanus/spleen, Russia) |
| 29640389.1 Ehrlichia ruminantium (sheep/blood, South Africa) |
| QB513462 1 Ehrlichia shimanahsis (Haamaphysalis obesa, Thailand) |
| C683105.1 Enrichia shimanensis (Haemaphysalis papuana, Thailand) C683105.1 Enrichia sp. (Hvalomma truncatum, Zambia) |
| OP502086 1 Ehrlichia Andalusi (tick from Oryctolagus cuhiculus, |
| QB854339.1 Ebrilchia sp. (lick, French Gulana). |
| OP359320.1 Enrichia sp. (Haemophysalis longicomis, China) OP359320.1 Enrichia sp. (Homo sapiens/blood, China) |
| 19697589.1 Ehrlichia sp. (Haemaphysalis longicomis, Japan) |
| 0Z824210 1 Ehrlichia sp. (Haemaphysalis longicomis, China) |
| OP071233.1 Enrichia sp. (Spermoghilus dauricus/liver, China) |
| OP071231.1 Enrichia sp. [Meriphe's unguiculatus/liver, China) |
| OQ247989 1 Ehrlichia canis (lick, Ghana) |
| AE323515 1 Ebritchia canis (Canis lubus ramitans, biobd) |
| 4B287435,1 Ehrlichia canis (carine/brain, Japah) 4B287435,1 Ehrlichia canis (carine/brain, Thailand) |
| PP566637.1 Ehrlichia canis (dog/blood, India) |
| QB978371_1 Enrichia canis (Carlis lupus familiaris/blood,Brazil) |
| U26740.1 Ehrlichia canis (Canis familians, Israel) UN121380.1 Ehrlichia sp. (Rhipicephalus sanouiheus, Philippines) |
| EU439944.1 Ehrlichia carits (dog/blood. Italy) CC593204.1 Ehrlichia sa, UHva/Smina Inunchtum, Zamhia) |
| 28629805.1 E minasensis (R micropus/hemolymph, Czech Republic) |
| C018188.1 Ehrlichia minasensis (Anipicephalus micropius, Czech Republic) |
| C018188,1 Ehrlichia canis, Canis Jupus familiaris/blood, Portugal) RM009066,1 Ehrlichia chatteensis (Ethinioenhalus microclus, Chlombia) |
| C602251,1 Ehrlichia sp. (Haemaphysalis shimoga, Malaysia) |
| AM418451, 1 Ehrlichia muris (bodes persulcatus from human, Russia) |
| AM504046 1 Ehrlichia muris (Ixodes persuicatus, Russia) AM504046 1 Ehrlichia muris (Ixodes persuicatus from human, Russia) |
| AB275137 1 Ehrlichia munis (rodent, Japan) |
| 015527.1 Elylichia multis (Eothenomy's kageus, USA) |
| AM418458.1 Ehrlichia muns (Ixodes persuicatus from buman, Russia) |
| NR 157649.1 Ehrlichia muris (Homo sabiens/blood, USA) HMR43746.1 Ehrlichia muris (Homo sabiens/blood, USA) |
| AB428565 1 Ehrlichia sp. (Drodes granulatus from Grocidura watasei, Japan) |
| A6428564.1 Etvicola sp. (ixodas granulatus from Rattus rattus, Japan) |
| 00547318.1 Ehrichia sp. (Ixodes granulatus from Crocidura watasai, Japan) |
| AB275138 1 Ehrlichia 80. (rodent, Japan) ET(82636 2 Ehrlichia chattaeosis (Alexandro pacyum, Argentina) |
| C595387, T Ehrlichia sp. (Haemaphysails megaspinosa, Japan) |
| AF147752.2 Enrichia chatteensis (Haemaphysalls longicornis, South Korea) |
| CP007474.1 E japonica (l ovatus, Japan) |
| The root root is introduced and contrology. |

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.

| Ehrlichia ASV Rattus exulans 8WER |
|---|
| (Y425480.1 Ehrlichle sp. (Amblyomma trigutatum, Australia) |
| KY425515.1 Ehrlichia šp. (Ambiyomma ingultatum, Australia) |
| Ky425524 1, Ehrlichia sp. [Amblyomma triguttatum, Australia) |
| MW785890,1 Ebdichia hydrochoerus (capybara/blood, Brazil) |
| U2/104.1 Endichia sp. (Odocovieus vi/ginianus/blood, USA) |
| 4J242785 [Ebrlichia sp. (lick. Sweden) |
| U10873.1 Ehrlichia sp. (ICK, Sweden) U10873.1 Ehrlichia sp. (Canis familiaris, Sweden) |
| AE057707.1 Ehrlichia sp. [Arabian mare/leukocytes, Switzerland] |
| EE445398.1 Ehmichia khabarensis (Clethrionontivs rulocanus, Russia) |
| (R063138.1 Ehrlichia khabarensis (Myodes rufdcanus/spleen, Russia) DOF40389.1 Ehrlichia niminantium (sheen/hknut, South Africa) |
| 20324367.1 Ebrichia sp. (Ambiyomma americanum, USA) |
| OR51,3452.7 Enrichia shimanensis (Haemannysais obesa, Thaland) OR51,3460.1 Enrichia shimanensis (Haemanhusalis naruana, Thaland) |
| C683105 1 Ehrlichia sp. (Hyalomma truncatum, Zambla) |
| MZ733021.1 Ehrlichia pampearia (Haemophysalis juxtakochia, Uruguay) |
| OB854339.1 Ehrlichia'sp. (fick, French Guiaha) OB159326.1 Ehrlichia sp. (Haerconhusais, Ionnicomis, China) |
| OP359320 1 Ehrlichia sp. (Homo sapiens/blood, China) |
| OP359324.1 Envictiva sp. (Haemaphysails longicomis,Japan) |
| VZ824710 1 Etinichia sp. (Haemaphi/salis longicomis, China) |
| OP021233 1 Ebritchia sp. (Spermophilus dauricus)(ver, China) |
| 0P071231.1 Enrichia sp. (Menones unguiculatus/liver, China) 4F414399.1 Enrichia sp. (Phiniceobalus micronius, China) |
| OQ247989 1 Ehrlichia cahis (lick, Ghana) |
| AE373615.1 Ebritchia canis (Caris lubus ramilians, biodd) AE373615.1 Ebritchia canis (Rhipicephalus sanguineus, Venezuela) |
| AB287435.1 Etrilicitia canis (canine/brain, Japah) AB287435.1 Ebdebia canis (canine/brain, Thailand) |
| PE566637, 1 Ehrlichia canis (dografood, India) |
| OR978371,1 Enrichia canis (Canis lupus familiaris/biood, Ponugal) |
| (26740 1 Ehrlichia canis (Canis familians, Israel) |
| EU439944.1 Ehrlicha caris (dog/blood, Italy) |
| LC583104.1 Enrichia sp. (Hyaiomma truncatum, Zampia) DX629805.1 E minasensis (R microplus/hemolymph, Czech Republic) |
| NR 148800,1 Ehrlichia minasensis (Rhipicephalus microplus, Czech Republic) |
| C018188, 1 Elvichia carva, (Carva Jupus familiaris/blood, Portugal) |
| C602251.1 Ehrlichia so, iHaemanhysais khimooa, Malaysia) |
| DOUBT267.1 Ehrlichia Sp. (Bok, Japan) |
| AM504046 1 Ehrlichia munis (Ixodas persulcatus, Russia) |
| AM504045.1 Ehrlichia muris (Ixodes persuicatus from human, Russia) AB275137.1 Ehrlichia muris (mdeol. Janan) |
| OC887268_[Ehrlichia sp. (jjck, Japan) |
| VR 121714.1 Ehrlichia muns (mouse/spleen, Japan) |
| AM418458 1 Ehrlichia munis (Ixodes persulcatus from buman, Russia) NR 157649 1 Ehrlichia munis (Homo sapieros/blood, USA) |
| HM643745.1 Ehrlichia muris (Homo sabiens/blood, USA) |
| AB428564.1 Ebrilcola sp. (ixodes granulatus from Battus rattus, Japan) |
| AB428564.1 Ebrüchia sp. (Ixodes branulatus from Rattus rattus, Japan) AB428565.1 Ebrüchia sp. (Ixodes branulatus from Crocidura wataseu Japan) |
| 20547318 1 Ehrlichia Japonica (Ixodes ricinus, France) |
| EU826516.2 Ehrlichia chatteensis (Amblyomma parvum, Argantina) |
| C596387.1 Ehrlichia sp. (Haemaphysalls megaspinosa, Japan) |
| AF147752.2 Ehrlichla chalfeensis (lick, Chine) |
| AF136713 1 Ebrichia sp. tilck. Germani |

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.

| | Exiquobacterium ASV (Suncus murinus BWER) |
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| 2 C C C C C C C C C C C C C C C C C C C | MH76 2015 1 E aurantiacum (sediment India) |
| | MM/5929424 (Lauranniacum (Securitori, Montioulata kan) |
| | MWV322043. (E aurantiacum (Suaeda Vennicurata, Iran) |
| 1.00 | HWW6/2526.1 E aurantracum [Suaeda aegyptiaca, ran) |
| 2 | IM1704551.1 E aurantiacum (Gracilaria, India) |
| | KY980691.1 E aurantiacum (lake water, China) |
| | MN100070.1 E aurantiacum (rhizosphere, Pakistan) |
| | MT317208.1 E aurantiacum (Brachiaria reptans, Pakistan) |
| | MT373550.1 E aurantiacum (Lycium barbarum, China) |
| | LC217383.1 E aurantiacum (human/comea, India) |
| | MH819705 1 E aurantiacum (soil South Korea) |
| | MW3580111 F aurantiacum (Thrips palmi, India) |
| 8.23 | -ME4174411 E aurantiacum (napawater India) |
| | CC2015 1 E aurantiacum (seal hadia) |
| | - CE062910. TE auranitacum (son, india) |
| | WN922607.1 E aurantiacum (C barbarus/intestine, Morocco) |
| | |
| | ME754139.1 E aurantiacum (soil, Pakistan) |
| | MH475930.1 E aurantiacum (root, Pakistan) |
| | HMH512933.1 E aurantiacum (soil, India) |
| | MT323008.1 E aurantiacum (coast, Kuwait) |
| | MT614322.1 E aurantiacum (surface water, India) |
| | MK386731.1 E aurantiacum (Aplysina insularis, Colombia) |
| | ON644493.2 E aurantiacum (Acropora muricata India) |
| | KT722611.1 E aurantiacum (water, Philippines) |
| | MH926361 1 E aurantiacum (soil India) |
| | ON171428 1 E aurantiacum (manorove soil India) |
| | OP622851 1 E aurantiacum (silver cam/brain, Philippines) |
| | N650590 1 E aurantiacum (soil Hungary) |
| | -OP081023 1 E aurantiacum (mulhemy India) |
| | OP133560 1 E aurantiacum (snow India) |
| | OP862734 1 E aurantiacum (mangrove soil India) |
| | OP1022754.1 E aurantiacum (mandro coil, mula) |
| | OP2214554 E supertissum (mak, Dussis) |
| | OP231455.1 E aurantiacum (lock, Russia) |
| | OP236390.1 E aurantiacum (rake sirt, Russia) |
| | OQ675541.1 E aurantiacum (cassava whiteriy, india) |
| | KY924598.1 E aurantiacum (mizosphere, India) |
| | KY082677.1 E aurantiacum (soil, India) |
| | HCX458117.1 E aurantiacum (fish/gut, India) |
| | OP133230.1 E aurantiacum (Lissachatina fulica/gut,India) |
| | OR491339.1 E aurantiacum (Saccharina latissima, France) |
| | MF767437.1 E aurantiacum (soil, Algeria) |
| | MH127584.1 E aurantiacum (pine tree, South Korea) |
| | FN997628.1 E aurantiacum (estuarine sediment, UK) |
| | MG977678.1 E aurantiacum (soil, India) |
| | KY435706.1 E aurantiacum (earthworm/cast. Pakistan) |
| | MG051302.1 E aurantiacum (groundwater, Russia) |
| | MH160094.1 E aurantiacum (soil India) |
| | OQ726262 1 E aurantiacum (human/skin India) |
| | server a bull hour promotion, and a |

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.

| LN624411.1 Clostridium sp. (sediment, Spain) LT801263.1 Clostridium sp. (soil, Japan) LT801253.1 Clostridium sp. (mouse/feces, China) PP512885.1 Clostridium sp. (mouse/feces, China) LC744592.1 Clostridium sp. (soil, Japan) LN998063.1 Clostridium sp. (soil, Japan) LN998063.1 Clostridium sp. (human/stool, France) OR555923.1 Clostridium sp. (human/feces, China) OP970227.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (human/feces, China) LK021118.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) ON103471.1 Clostridium sp. (soil, China) K2254751.1 Clostridium sp. (soil, China) K2254751.1 Clostridium sp. (soil, China) K25592.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) | LT801245.1 Clostridium sp. (human gut, Belgium) |
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| L T801263.1 Clostridium sp. (soil, Japan) L T801253.1 Clostridium sp. (human gut, Belgium) PP512885.1 Clostridium sp. (mouse/feces, China) LC744592.1 Clostridium sp. (mouse/feces, China) LC744592.1 Clostridium sp. (soil, Japan) LN998063.1 Clostridium sp. (human/stool, France) OR555923.1 Clostridium sp. (human/feces, China) OP970227.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (human/feces, China) LK021118.1 Clostridium sp. (human/feces, India) LK021118.1 Clostridium sp. (human/feces, India) DN556635.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) ON103477.1 Clostridium sp. (soil, China) MZ2547551.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) | LN624411.1 Clostridium sp. (sediment, Spain) |
| LT801253.1 Clostridium sp. (human gut, Belgium) PP512885.1 Clostridium sp. (mouse/feces, China) PP512905.1 Clostridium sp. (mouse/feces, China) LC744592.1 Clostridium sp. (soil, Japan) LN998063.1 Clostridium sp. (human/stool, France) OR555923.1 Clostridium sp. (human/feces, China) OP970227.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (human/feces, China) LK021118.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) ON103471.1 Clostridium sp. (human/feces, India) ON103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) | LT801263.1 Clostridium sp. (soil, Japan) |
| PP512885.1 Clostridium sp. (mouse/feces, China) PP512905.1 Clostridium sp. (mouse/feces, China) LC744592.1 Clostridium sp. (soil, Japan) LN998063.1 Clostridium sp. (human/feces, China) OP555923.1 Clostridium sp. (human/feces, China) OP970227.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (human/feces, China) LK021118.1 Clostridium sp. (human/feces, India) DN556635.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) LT161894.1 Clostridium sp. (human/feces, India) DN556635.1 Clostridium sp. (human/feces, India) DN556635.1 Clostridium sp. (human/feces, India) DN556635.1 Clostridium sp. (human/feces, India) EU161894.1 Clostridium sp. (human/feces, India) DN103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555522.1 Clostridium sp. (human/feces, China) | LT801253.1 Clostridium sp. (human gut, Belgium) |
| PP512905.1 Clostridium sp. (mouse/feces, China) LC744592.1 Clostridium sp. (soil, Japan) LN998063.1 Clostridium sp. (human/stool, France) OR555923.1 Clostridium sp. (human/feces, China) OP970227.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (human/feces, China) LK021118.1 Clostridium sp. (human/feces, China) LK021118.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) LT161894.1 Clostridium sp. (human/feces, India) ON103471.1 Clostridium sp. (human/fees, India) ON103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium sp. (soil, China) ON103471.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) | PP512885.1 Clostridium sp. (mouse/feces, China) |
| LC744592.1 Clostridium sp. (soil, Japan) LN998063.1 Clostridium sp. (human/stool, France) OR555923.1 Clostridium sp. (human/feces, China) OP970227.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (human/feces, China) LK021118.1 Clostridium sp. (human/stool, France) AM117583.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (human/feces, India) LT161894.1 Clostridium sp. (feces-contaminated soil, India) LT161894.1 Clostridium sp. (human/stool, France) AM117582.1 Clostridium sp. (human/stool, France) AM117582.1 Clostridium sp. (human/feces, India) DN103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) KZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) | PP512905.1 Clostridium sp. (mouse/feces, China) |
| LN998063.1 Clostridium sp. (human/stool, France) OR555923.1 Clostridium sp. (human/feces, China) OP970227.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (human/feces, China) LK021118.1 Clostridium sp. (human/stool, France) AM117583.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (feces-contaminated soil, India) LT161894.1 Clostridium sp. (feces-contaminated soil, India) LT161894.1 Clostridium sp. (human/stool, France) AM117582.1 Clostridium sp. (human/fees, India) ON103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) AF275949.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) | LC744592.1 Clostridium sp. (soil, Japan) |
| OR555923.1 Clostridium sp.(human/feces, China) OP970227.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (human/feces, China) LK021118.1 Clostridium sp. (human/feces, India) AM117583.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (feces-contaminated soil, India) LT161894.1 Clostridium sp. (feces-contaminated soil, India) LT161894.1 Clostridium sp. (human/stool, France) AM117582.1 Clostridium sp. (human/fees, India) ON103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) | LN998063.1 Clostridium sp. (human/stool, France) |
| OP970227.1 Clostridium sp. (soil, Turkey) OR544016.1 Clostridium sp. (human/feces, China) LK021118.1 Clostridium sp. (human/feces, India) AM117583.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (feces-contaminated soil, India) LT161894.1 Clostridium sp. (human/stool, France) AM117582.1 Clostridium sp. (human/fees, India) ON103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) | OR555923.1 Clostridium sp.(human/feces, China) |
| OR544016.1 Clostridium sp. (human/feces, China) LK021118.1 Clostridium sp. (human/feces, India) AM117583.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (feces-contaminated soil, India) LT161894.1 Clostridium sp. (human/stool, France) AM117582.1 Clostridium sp. (human/fees, India) ON103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) | OP970227.1 Clostridium sp. (soil, Turkey) |
| LK021118.1 Clostridium sp. (human/stool, France) AM117583.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (feces-contaminated soil, India) LT161894.1 Clostridium sp. (human/stool, France) AM117582.1 Clostridium sp. (human/fees, India) ON103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (cattle/manure, Sweden) MG030671.1 C neonatale (Human/blood, Canada) MG030671.1 C neonatale (Human/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) | OR544016.1 Clostridium sp. (human/feces, China) |
| AM117583.1 Clostridium sp. (human/feces, India) ON556635.1 Clostridium sp. (feces-contaminated soil, India) LT161894.1 Clostridium sp. (human/stool, France) AM117582.1 Clostridium sp. (human/fees, India) ON103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) | LK021118.1 Clostridium sp. (human/stool, France) |
| ON556635.1 Clostridium sp. (feces-contaminated soil, India) LT161894.1 Clostridium sp. (human/stool, France) AM117582.1 Clostridium sp. (human/fees, India) ON103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) | AM117583.1 Clostridium sp. (human/feces, India) |
| LT161894.1 Clostridium sp. (human/stool, France) AM117582.1 Clostridium sp. (human/fees, India) ON103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) LN846907.1 Clostridium sp. (human/stool, France) | ON556635.1 Clostridium sp. (feces-contaminated soil, India) |
| AM117582.1 Clostridium sp. (human/fees, India) ON103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) LN846907.1 Clostridium sp. (human/stool, France) | LT161894.1 Clostridium sp. (human/stool, France) |
| ON103471.1 Clostridium sp. (soil, China) MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/stool, France) LN846907.1 Clostridium sp. (human/stool, France) | AM117582.1 Clostridium sp. (human/fees, India) |
| MZ254751.1 Clostridium sp. (soil, China) EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/stool, France) LN846907.1 Clostridium sp. (human/stool, France) | ON103471.1 Clostridium sp. (soil, China) |
| EU869244.1 Clostridium neonatale (cattle/manure, Sweden) AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) LN846907.1 Stochidium sp. (human/stool, France) | MZ254751.1 Clostridium sp. (soil, China) |
| AF275949.1 Clostridium neonatale (human/blood, Canada) MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) LN846907.1 Clostridium sp. (human/stool, France) | EU869244.1 Clostridium neonatale (cattle/manure, Sweden) |
| MG030671.1 C neonatale (Homo sapiens/stool, Canada) OR555922.1 Clostridium sp. (human/feces, China) LN846907.1 Clostridium op. (human/stool, France) | AF275949.1 Clostridium neonatale (human/blood, Canada) |
| OR555922.1 Clostridium sp. (human/feces, China) LN846907.1 Slostridium sp. (human/stool, France) | MG030671.1 C neonatale (Homo sapiens/stool, Canada) |
| LN846907 1 Clostridium op. (human/stool, France) | OR555922.1 Clostridium sp. (human/feces, China) |
| | LN846907 1 Closhidium op. (human/stool, France) |
| | |

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.

| GU416533.1 C curvus (Homo saprens/oral, USA) |
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| LT801198.1 Campylobacter sp. (human/gut, Belgium) |
| GU416532.1 C curvus (Homo sapiens/oral, USA) |
| GU416531.1 C curvus (Homo sapiens/oral, USA) |
| KU550133.1 C curvus (Homo sapiens/oral, Iran) |
| GU416534.1 C curvus (Homo sapiens/oral,USA) |
| NZ LIWU01000016.1 C curvus (H sapiens/oral, USA) |
| MW433774.1 C curvus (H sapiens/oral, South Korea) |
| KY952171.1 C curvus (human/stool, Turkey) |
| PP266706.1 Campylobacter sp. (rat/intestine, India) |
| MW342695.1 Campylobacter sp. (S xanthoprymnus/feces, Turkey, |
| MW342694.1 Campylobacter sp. (S xanthoprymnus, Turkey) |
| MW342692.1 Campylobacter sp. (S xanthoprymnus, Turkey) |
| MW725221.1 Campylobacter sp. (H sapiens/oral, South Korea) |
| MW157375.1 Campylobacter sp. (cow, USA) |
| MW157377.1 Campylobacter sp. (cow, USA) |
| MW157376.1 Campylobacter sp. (cow, USA) |
| MZ542779.1 Campylobacter sp. (human/stool, iraq) |
| AM117593.1 Campylobacter sp. (human/feces, India) |
| PP266702.1 Campylobacter sp. (rat/intestine, India) |
| AM117594.1 Campylobacter sp. (human/feces, India) |
| DQ211919.1 C curvus (human/feces, California) |
| DQ211921.1 C curvus (human/feces, California) |
| DQ211920.1 C curvus (human/feces, California) |

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.

| R 179412.1 C phoceense (human/stool, France) |
|---|
| V849777.1 Corynebacterium sp. (human/stool, France) |
| P697722.1 C phoceense (Sus scrofa/feces, Brazil) |
| Y13427.1 Corynebacterium sp. (sheep, UK) |
| OQ130741.1 Corynebacterium sp. (cattle/rumen, Korea) |
| NZ JANIKM01000065.1 C phoceense (Sus scrofa, Spain) |
| rLC529710.1 Corynebacterium sp. (soil, Securi Arabia) |
| Corynebacterium2 ASV (Rattus tanezuni, BWFR) |
| Corynebacterium2 ASV (Rattus exulans,BWFR) |
| Corynebacterium2 ASV (Rattus tanezumi, BWFR) |
| NZ JANIKM010000001 1:1-74 Corynebacterium phoceense (Sus some Constit, Spain) |
| NZ JADPQA010000027.1 C phoceense (B indicus/tonsil, Brazil) |

NZ VHIR01000034.1 C phoceense (H sapiens/urine, Argentina)

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 nonnative 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.

ACKNOWLEDGMENT

The authors sincerely thank Dr. Roland M. Hipol for allowing the use of his laboratory facilities and resources. They also thank the Baguio Water District for all their fieldwork assistance and the Cordillera Center for Animal Research and Development for their guidance during the animal research clearance application.

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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