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Research Article

Genetic Diversity and Population Structure of *Rattus rattus* and *Rattus tanezumi* in Sporadic Leptospirosis Areas of Central Java, Indonesia

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ABSTRACT

Introduction: Rattus rattus and *Rattus tanezumi* are important vectors of leptospirosis, a zoonotic disease of public health concern in Indonesia. This study investigated the genetic diversity and population structure of these two rat species in Purworejo Regency, Central Java, an area that experienced a leptospirosis outbreak in 2023 with the highest case fatality rate in the province.

Method: Rats were trapped in two villages with contrasting ecological settings: Dadirejo (hilly terrain with plantations) and Kutoarjo (densely populated urban area). Mitochondrial cytochrome b gene sequences were analyzed to assess phylogenetic relationships, genetic diversity, and population structure.

Results: Phylogenetic analysis revealed distinct evolutionary lineages for *R. rattus* and *R. tanezumi*, with *R. tanezumi* exhibiting greater intraspecific genetic diversity. Haplotype and nucleotide diversity indices indicated higher genetic variation in *R. tanezumi* compared to *R. rattus*. Analysis of molecular variance (AMOVA) showed strong genetic differentiation among *R. rattus* populations (F_{ST} = 0.973), suggesting limited gene flow and potential multiple introduction events. In contrast, *R. tanezumi* displayed greater connectivity among central Java populations but substantial differentiation from Jakarta and Sulawesi populations. These distinct genetic profiles may influence the spatial heterogeneity of leptospirosis cases across Purworejo's sub-districts. The higher genetic diversity in *R. tanezumi* could enhance its adaptability and reservoir potential, while the genetic isolation of *R. rattus* populations may lead to location-specific pathogen strains.

Conclusion: These findings underscore the importance of considering rat population genetics in understanding leptospirosis transmission dynamics and designing effective control strategies.

Keywords: *R. rattus*, *R. tanezumi*, Population Genetics, Haplotype, Genetic Diversity

Introduction

Rodents, particularly rats, are highly adaptable animals widely distributed in environments associated with human activities.[1,2] In Indonesia, two of the most commonly found species are *Rattus rattus* and *Rattus tanezumi*.[3] Both are important vectors in the transmission of various zoonotic diseases, including leptospirosis, a disease caused by *Leptospira* spp. bacteria and transmitted through direct contact with infected animal urine or contaminated environments.[1,4]

Leptospirosis has long been a public health concern in Indonesia and is classified as a Neglected Tropical Disease (NTD).[5] In the past five years, leptospirosis cases in Indonesia have shown an increasing trend, with national case numbers reaching 1,419 cases in 2022 (Case Fatality Rate (CFR): 9.8%). Central Java Province is the main contributor to leptospirosis cases in Indonesia, with a significant surge from 265 cases in 2021 to 502 cases in 2022.[6]

Based on data from the Purworejo Regency Health Office (2023), Purworejo Regency in Central Java experienced a leptospirosis outbreak in 2023, with the highest CFR in the province at 15% (130 cases with 19 deaths). Previously, no leptospirosis cases were found in 2021, and only 18 cases were recorded in 2022. This sharp increase indicates potential changes in the vector population dynamics of the region. The distribution of cases in 2023 was widespread across all sub-districts, with Bagelen Sub-district recording the highest number of cases (23 cases), followed by Bruno, Bener, Purwodadi, and Kemiri. Meanwhile, Kutoarjo Sub-district ranked as having the highest CFR at 60%, despite having fewer cases.[7] Interestingly, these two sub-districts have vastly different geographical characteristics, Kutoarjo is a densely populated area, whereas Bagelen is dominated by hills and garden areas.

The gender-based distribution of cases shows that 78.46% of cases occurred in males, most of whom are field or rice paddy workers who have potential direct contact with environments contaminated with *Leptospira* spp., especially if open wounds are present on exposed body parts.[7]

Considering the potential role of rats as the main reservoir of *Leptospira* spp., it is crucial to understand the population structure and genetic dynamics of *Rattus* species in endemic areas.[1,8] Population genetics studies using haplotype analysis approaches, Analysis of Molecular Variance (AMOVA), and genetic differentiation coefficients between populations (fixation indices) can provide insights into genetic variation, gene flow, and potential distribution patterns of rat species in a region.[9,10] This research aims to explore the population structure and genetic variation of *R. rattus* and *R. tanezumi* in Purworejo Regency, as well as review their implications for the spread of zoonotic diseases, particularly leptospirosis.

Materials and Method

Ethics Statement

This study has been approved by the Health Research Ethics Commission under the reference number 368/EA/KEPK-FKM/2022. No additional animals were used in this study. All treatment of rats followed protocols designed to minimise suffering and distress, including anaesthesia, after which the rats were buried in an area far from settlements and water sources.

Sampling Site and Rat Trapping

This type of research is descriptive observational using a molecular approach conducted from April, 2024 to August, 2024. This study was conducted in Purworejo Regency, Central Java Province, Indonesia, a region that reported a leptospirosis outbreak in 2023 with the highest CFR in the province. Two villages, Dadirejo in Bagelen Sub-district and Kutoarjo in Kutoarjo Sub-district, were selected as sampling sites based on prior leptospirosis case reports and differences in ecological settings. Dadirejo Village in Bagelen Sub-district is dominated by hilly terrain with extensive plantation coverage, and Kutoarjo Village in Kutoarjo Sub-district is a densely populated urban settlement (Figure 1).

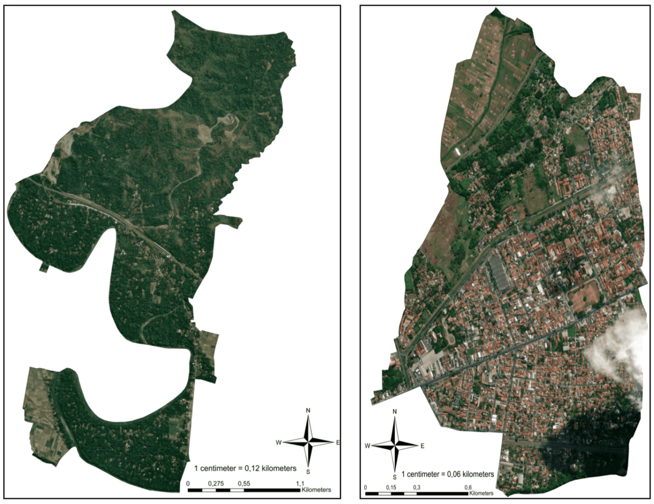

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Figure 1.Satellite Imagery of Rodent Sampling Sites in Purworejo Regency, Central Java Province, Indonesia. (a) Dadirejo Village (Bagelen Sub-district). (b) Kutoarjo Village (Kutoarjo Sub-district). Scale bars indicate spatial reference.

Rodent trapping was conducted over two consecutive nights in each site using a total of 75 live traps per location. Traps were strategically placed in areas with high rodent activity, such as inside and around human dwellings, including kitchens, storage areas, and peri-domestic environments. The traps were open for at least three days, checked daily and re-baited each morning. Captured rodents were transported to a field laboratory for species identification and tissue sampling. The trapping success rate was calculated by dividing the number of rodents captured by the total number of traps deployed. All the rats sampled were identified to the species level via external morphological criteria and molecular biology. Five linear measurements were considered: head and body length, tail length, hind-foot length, ear length, and head length. All measurements were performed with a ruler (precision 1 mm) except for head length, which was measured with a caliper (precision 0.1 mm). Kidney tissue was excised from each specimen and stored at -20 °C in absolute ethanol until DNA extraction.

DNA Extraction, Amplification, and Sequencing

Genomic DNA was extracted from kidney tissue using the Zymo Quick-DNA™ MiniPrep Kit (Zymo Research, Irvine, CA, USA) following the manufacturer's protocol. A 1,140 bp fragment of the mitochondrial cytochrome b (*cytb*) gene was amplified using primers L14727-SP (5'-GAC AGG AAA AAT CAT CGT TG-3') and H15915-SP (5'-TTC ATT ACT GGT TTA CAA GAC-3').[11] PCR reactions were performed in a 25 µL mixture containing 2 µL of DNA template, 12.5 µL of GoTaq® Green Master Mix (Promega, Madison, WI, USA), 1 µL of each primer (10 µM), and 8.5 µL of nuclease-free water.

Thermal cycling conditions included initial denaturation at 95 °C for 3 minutes; 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 50 °C for 30 seconds, and extension at 72 °C for 90 seconds; followed by a final extension at 72 °C for 10 minutes. PCR products were visualised using 1.5% agarose gel electrophoresis stained with SYBR™ Safe DNA Gel Stain (Invitrogen, Carlsbad, CA, USA). Amplified products with clear bands were purified and sequenced using the Sanger method.

Molecular Data Processing and Phylogenetic Analysis

The chromatograms of *cytb* sequences were manually edited using MEGA 12.[12] The obtained *cytb* sequences were compared to the reference sequences from Southeast Asia using the Basic Local Alignment Search Tool (BLAST) against the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) in order to verify species identity. Multiple sequence alignment was conducted using ClustalW.[13]

Phylogenetic analyses were conducted using the Maximum Likelihood (ML) method in the MEGA 12 software package, with evolutionary distances calculated according to the Tamura–Nei substitution model.[12] Statistical support for nodes was assessed through 1000 bootstrap iterations, and an appropriate outgroup taxon was incorporated to establish proper tree polarity. To investigate phylogeographic relationships, we analysed mitochondrial cytochrome b (*cytb*) sequence data obtained from Purworejo specimens in comparison with homologous sequences representing various Southeast Asian populations accessed through the GenBank database. Following sequence alignment, phylogenetic reconstruction was performed employing the parameters described above to elucidate evolutionary relationships and geographic structuring among the sampled populations.

Population Structure Visualisation and Statistical Analysis

Genetic diversity indices, including number of haplotypes (H), haplotype diversity (Hd), and nucleotide diversity (π), were calculated for each *Rattus* species using DnaSP v6.12.03.[14] We performed multiple statistical tests, including Tajima's D, Fu and Li's D* and F*, and Fu's Fs, to assess demographic history and selective neutrality.[15–17]

Haplotype relationships were visualised through median-joining networks constructed in PopART v1.7, with epsilon parameter set to 0.[18] We implemented AMOVA using Arlequin v3.5.2.2 in order to elucidate the hierarchical partitioning of genetic variation and assess population differentiation in *R. rattus* and *R. tanezumi*.[19] The analysis was conducted on mitochondrial cytochrome b (*cytb*) sequence data derived from six geographically distinct populations of *R. rattus* and five populations of *R. tanezumi*.

Genetic variance components were computed based on pairwise nucleotide differences between haplotypes, with statistical significance assessed through 1,023 permutation replicates. This approach enabled quantification of genetic variation distributed within and among populations, with fixation indices (F_{ST}) calculated to determine the degree of population subdivision. For each species, we constructed distance matrices comprising Euclidean square distances to facilitate comparative population genetic analyses.

We calculated pairwise F_{ST} values among all population pairs for each species to assess interpopulation differentiation patterns at a finer scale. Statistical significance of these values was determined through 1,023 permutation tests, with significance thresholds set at $p < 0.05$. We followed Wright's guidelines (1978) for interpretation of F_{ST} values, where F_{ST} values of 0–0.05 indicate negligible genetic differentiation, 0.05–0.15 indicate moderate differentiation, 0.15–0.25 show substantial differentiation, and values exceeding 0.25 indicate pronounced population structuring.[20] Notably, negative F_{ST} values, which can occur when within-population variation exceeds between-population variation, were interpreted as evidence of genetic homogeneity or extensive gene flow between populations.[19]

Results

Rat Trapping

A total of 32 rodents were captured from Dadirejo (Bagelen Sub-district) and Kutoarjo (Kutoarjo Sub-district). Molecular identification based on mitochondrial *cytb* gene sequences confirmed the presence of two *Rattus* species: *R. rattus* and *R. tanezumi*. The trapping success rate was 9% in Bagelen and 12% in Kutoarjo, with higher capture frequencies observed in Kutoarjo. The higher trap success on day two in both locations suggests a potential adaptation of the trapping methodology or increased rat activity on the second day (Table 1). Two types of baits were used on the first day: roasted coconut and cheese, while the baits used on the second day were roasted coconut, cheese, and a mixture of mackerel and tofu.

Table 1.Trapping Success and Capture Frequencies of *Rattus* Species across Two Study Sites in Purworejo

Location	Code	Capture Time	Live Trap	Rats Captured	Success Trap (%)
Dadirejo	DD	Day 1	75	4	5
		Day 2	75	10	13
		Total	150	14	9
Kutoarjo	KT	Day 1	75	6	8
		Day 2	75	12	16
		Total	150	18	12

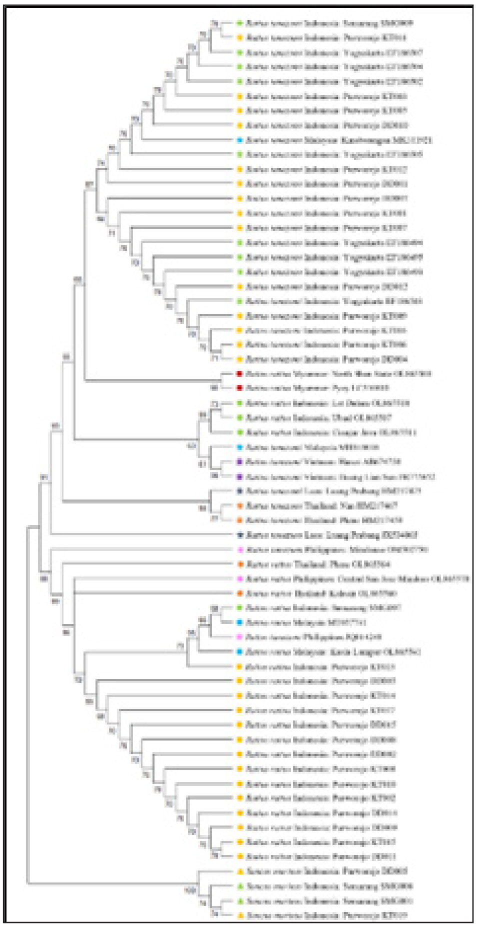

Figure 2.Phylogenetic Tree Based on Mitochondrial *cytb* Gene Sequences of *Rattus rattus* and *Rattus tanezumi*.

Phylogenetic Analysis

The maximum likelihood phylogenetic tree based on mitochondrial cytochrome b sequences revealed distinct evolutionary relationships among *R. rattus* and *R. tanezumi* populations. The analysis demonstrated a clear bifurcation between these two species, supported by high bootstrap values (> 80%) at the primary divergence points. *R. tanezumi* samples from Purworejo (indicated by yellow stars) formed multiple clusters within the broader *R. tanezumi* clade, exhibiting close genetic relatedness with other Indonesian populations, particularly those from Yogyakarta. These Purworejo *R. tanezumi* specimens were distributed across several subclades with generally robust bootstrap support (70–71%), indicating some level of genetic substructuring within this geographic region. Notably, *R. rattus* samples from Purworejo (marked by yellow dots) constituted a monophyletic group with strong bootstrap support (70–99%), positioned distinctly from *R. rattus* populations from other localities, including Malaysia, Thailand, and the Philippines (Figure 2).

The phylogenetic reconstruction demonstrated that both *Rattus* species maintain their genetic integrity despite sympatric distribution in Purworejo, with no evidence of intermixing between species-specific clades. The tree topology confirmed that Purworejo harbours genetically distinct populations of both rat species, with *R. tanezumi* displaying greater intraspecific genetic diversity as evidenced by its distribution across multiple subclades. The *R. rattus* samples from Purworejo exhibited a more cohesive genetic structure, forming a well-supported monophyletic assembly with other Indonesian *R. rattus* specimens. This phylogenetic pattern aligns with established taxonomic boundaries between these morphologically similar but genetically distinct *Rattus* species, while also revealing fine-scale population structure within the Purworejo sampling area. The outgroup positioning of *Suncus murinus* with 100% bootstrap support further validated the robustness of the phylogenetic reconstruction.

Sequences marked with yellow dots and yellow stars are sequences yielded from this study, whereas the rest are sequences from GenBank. The GenBank accession number is given after each isolate's name. The phylogenetic tree was constructed using the maximum likelihood (ML) method and the Tamura–Nei model in Mega 12. Bootstraps were tested at 1,000 replicates to assess the ML tree reliability. *Suncus murinus* was used as an outgroup in this analysis.

Genetic Diversity and Haplotype Relationship

The genetic analysis of *R. rattus* and *R. tanezumi* from Purworejo Regency revealed distinctive population structures that may influence their roles as disease vectors. As shown in Table 2, the examination of 20 *R. rattus* sequences identified 74 Single Nucleotide Polymorphisms (SNPs) forming 9 distinct haplotypes, while 34 *R. tanezumi* sequences yielded 75 SNPs constituting 13 haplotypes. Despite similar numbers of SNPs, *R. tanezumi* demonstrated significantly higher haplotype diversity (Hd = 0.856 ± 0.039) compared to *R. rattus* (Hd = 0.653 ± 0.122), suggesting greater genetic variation at the haplotype level. Interestingly, nucleotide diversity values remained comparable between species (*R. rattus*: π = 0.027 ± 0.006; *R. tanezumi*: π = 0.029 ± 0.004), indicating similar levels of genetic variation at the nucleotide level despite differences in haplotype distribution. Neutrality tests revealed contrasting evolutionary patterns, with *R. rattus* showing positive values across Tajima's D (0.806) and Fu and Li's D* (1.037) and F* (1.129), potentially indicating balancing selection or population subdivision. Conversely, *R. tanezumi* exhibited a more neutral Tajima's D (0.133) and negative values for Fu and Li's statistics (D* = -0.131, F* = -0.829), which could suggest purifying selection or slight population expansion, although values were not statistically significant. The positive Fu's Fs values for *R. rattus* and *R. tanezumi* (7.507 and 5.789, respectively) argue against recent population expansions. These distinct genetic profiles may explain the spatial heterogeneity of leptospirosis cases across Purworejo Regency's sub-districts, particularly between densely populated Kutoarjo and the hillier Bagelen region. The higher genetic diversity in *R. tanezumi* likely reflects a more complex population history, possibly involving multiple introduction events or longer establishment in the region, which could enhance its adaptability to diverse environments and potentially influence its capacity as a disease reservoir by promoting genetic diversity in hosted pathogen populations. These findings underscore the importance of species-specific approaches to rodent control in leptospirosis management strategies, as the two predominant *Rattus* species demonstrate different population genetic characteristics that may affect their ecology, distribution, and vector competence.

The haplotype network analysis of mitochondrial *cytb* gene sequences (Figure 3) revealed distinct genetic patterns among *R. rattus* and *R. tanezumi* populations across Indonesian localities. *R. rattus* exhibited a star-like network topology comprising 9 haplotypes from 20 sequences, with haplotype H1 (Purworejo) representing the dominant haplogroup (12/20 sequences, 60%). The remaining eight haplotypes (H2–H9) each consisted of a single sequence, distributed across various geographic locations (Purworejo, Semarang, Yogyakarta, Cianjur, Maluku, and Gianyar). The network structure displayed considerable mutational distances between certain peripheral haplotypes, particularly along the horizontal branches. In contrast, *R. tanezumi* showed higher haplotype diversity with 13 distinct haplotypes from 34 sequences. Two predominant haplotypes, H1 and H2, each containing 9 sequences (26.5% each), formed central nodes in the network, with H1 distributed across three locations (Purworejo, Semarang, and Yogyakarta) and H2 found in two locations (Purworejo and Yogyakarta). The *R. tanezumi* network exhibited a more complex reticulate structure with geographic clustering, particularly evident in Jakarta haplotypes (H8–H11, n = 9), forming a distinct cluster separated by multiple mutation steps from central haplotypes. Additionally, Sulawesi haplotypes (H12–H13) appeared as peripheral nodes connected to the Jakarta cluster. This haplotype showed differential geographic distribution patterns between these species, with *R. tanezumi* showing more extensive haplotype sharing across localities as compared to the more location-specific distribution observed in *R. rattus*.

Table 2.Genetic Diversity Indices and Neutrality Test Values of *Rattus rattus* and *Rattus tanezumi* Based on Mitochondrial *cytb* Gene Sequences

Species	No. of Sequences	SNPs	No. of Haplotypes	Diversity ± SD		Tajima's D	Fu and Li's		Fu's Fs
				Haplotype (Hd)	Nucleotide (π)		D*	F*	
Rattus rattus	20	74	9	0.653 ± 0.122	0.027 ± 0.006	0.806	1.037	1.129	7.507
Rattus tanezumi	34	75	13	0.856 ± 0.039	0.029 ± 0.004	0.133	-0.131	-0.829	5.789

SNPs: Single Nucleotide Polymorphisms, SD: Standard Deviation

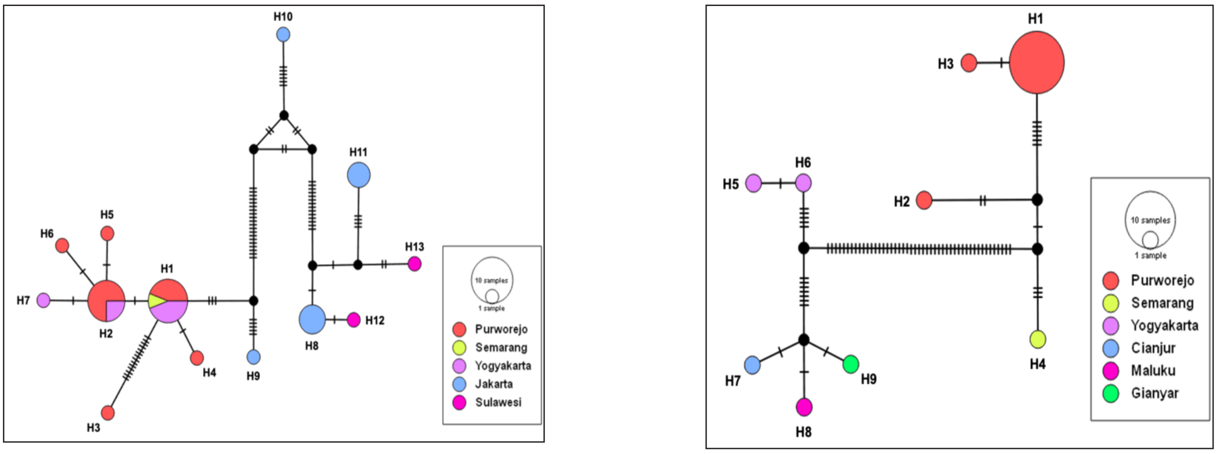

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Figure 3.Haplotype Networks Based on Mitochondrial cytb Gene Sequences of *Rattus rattus* and *Rattus tanezumi*. (a) *R. rattus*. (b) *R. tanezumi*

Table 3.Analysis of Molecular Variance (AMOVA) Results of *Rattus rattus* and *Rattus tanezumi* from Indonesian Populations Based on Mitochondrial *cytb* Gene Sequences

Species	Source of Variation	df	Sum of Squares	Variance Components	Percentage of Variation	Fixation Index (F_{ST})
Rattus rattus	Among populations	5	228.443	22.988 Va	97.32	0.973***
	Within populations	14	8.875	0.633 Vb	2.68	
	Total	19	237.300	23.620		

Rattus tanezumi	Among populations	4	226.273	8.993 Va	74.93	0.749***
	Within populations	29	87.256	3.009 Vb	25.07	
	Total	33	313.529	12.002		

df = degrees of freedom

Table 4.Pairwise Comparisons of Fixation Index (F_{ST}) among *Rattus rattus* Populations from Different Regions in Indonesia Based on Mitochondrial *cytb* Gene Sequences

Location	Purworejo	Semarang	Yogyakarta	Cianjur	Maluku	Gianyar
Purworejo	0.000	-	-	-	-	-
Semarang	0.869	0.000	-	-	-	-
Yogyakarta	0.978*	0.982	0.000	-	-	-
Cianjur	0.978	1.000	0.926	0.000	-	-
Maluku	0.978	1.000	0.926	1.000	0.000	-
Gianyar	0.978	1.000	0.926	1.000	1.000	0.000

*p < 0.05

Table 5.Pairwise Comparisons of Fixation Index (F_{ST}) among *Rattus tanezumi* Populations from Different Regions in Indonesia Based on Mitochondrial cytb Gene Sequences

Location	Purworejo	Semarang	Yogyakarta	Jakarta	Sulawesi
Purworejo	0.000	-	-	-	-
Semarang	-0.655	0.000	-	-	-
Yogyakarta	-0.314	-0.052	0.000	-	-
Jakarta	0.555	0.780**	0.759**	0.000	-
Sulawesi	0.872	0.923*	0.965*	-0.173	0.000

* p < 0.05; ** p < 0.01

Population Structure

F_{ST} denotes genetic differentiation between populations, quantifying the proportion of total genetic variance attributable to among-population differentiation. *R. rattus* populations (6) correspond to the following geographic localities: Purworejo, Semarang, Yogyakarta, Cianjur, Maluku, and Gianyar. *R. tanezumi* populations (5) correspond to the following geographic localities: Purworejo, Semarang, Yogyakarta, Jakarta, and Sulawesi. Va and Vb are the associated variance components; ***p < 0.001.

The AMOVA results shown in Table 3 revealed important patterns in the genetic makeup of the two rat species. For the black rat (*R. rattus*), 97.32% of genetic variation occurred among different populations, with only 2.68% observed within each population. This extremely high population differentiation (F_{ST} = 0.973) indicates that black rat populations are almost completely isolated from each other genetically.

For the Asian house rat (*R. tanezumi*), a similar but less extreme pattern was observed. About 75% of genetic variation occurred among populations, with 25% found within populations. This substantial but lower differentiation (F_{ST} = 0.749) suggests that while Asian house rat populations are distinct, they maintain somewhat more genetic connectivity than black rat populations.

These findings indicate that both species have strongly structured populations, meaning rats in different areas are genetically quite distinct from each other. This could result from multiple separate introductions from different source populations, limited movement between established populations, or differences in the species' invasion histories.

The pairwise F_{ST} table for *R. rattus* (Table 4) shows the genetic differentiation among rat populations from different Indonesian locations (Purworejo, Semarang, Yogyakarta, Cianjur, Maluku, and Gianyar). The F_{ST} values range from 0.869 to 1.000, indicating extremely high genetic differentiation among all population pairs. F_{ST}

values above 0.25 typically indicate very strong population differentiation, and values near 1.0 suggest almost complete genetic isolation. Here, most values exceed 0.90, with several reaching the maximum possible value of 1.000 (between Semarang-Cianjur, Semarang-Maluku, Semarang-Gianyar, Cianjur-Maluku, Cianjur-Gianyar, and Maluku-Gianyar). This means that the *R. rattus* populations across these Indonesian locations are genetically distinct and highly isolated from each other, with minimal gene flow occurring among them. The lowest differentiation (F_{ST} = 0.869) was observed between Purworejo and Semarang, suggesting that these populations may have slightly more genetic exchange than the others, but even this value indicates strong isolation. These findings support the earlier AMOVA results and provide additional evidence that *R. rattus* populations likely resulted from multiple separate introductions from different source populations, with very limited subsequent movement of rats between established populations. For practical pest management, these data suggest that control strategies may need to be tailored to the specific genetic characteristics of each local rat population.

Pairwise comparisons of the fixation index (F_{ST}) revealed low and negative values among populations of *R. tanezumi* from Purworejo (Central Java), Semarang (Central Java), and Yogyakarta (D. I. Yogyakarta), indicating weak genetic differentiation and substantial gene flow across these contiguous regions in Java. Conversely, significant and elevated F_{ST} values were observed between Jakarta and the Javanese populations (0.759–0.780, $p < 0.01$), as well as between Sulawesi and Semarang (F_{ST} = 0.923, $p < 0.05$) and Yogyakarta (F_{ST} = 0.965, $p < 0.05$), suggesting pronounced genetic differentiation (Table 5). Although the F_{ST} between Purworejo and Sulawesi was relatively high (0.872), it did not reach statistical significance, precluding confirmation of distinct population structuring. Notably, the comparison between Jakarta and Sulawesi yielded a negative F_{ST} (–0.173), indicating unexpected genetic similarity.

Discussion

The comprehensive genetic analysis of *R. rattus* and *R. tanezumi* populations in Purworejo Regency in Central Java revealed distinctive evolutionary histories and population structures that likely influence their roles as leptospirosis reservoirs in the region. These findings contribute to understanding how genetic diversity in rodent populations may shape the epidemiological landscape of this important zoonotic disease.

Phylogenetic Relationships and Evolutionary History

The phylogenetic reconstruction based on mitochondrial cytochrome b sequences demonstrated a clear evolutionary separation between *R. rattus* and *R. tanezumi* despite their sympatric distribution in Purworejo. The robust bifurcation in the phylogeny, supported by high bootstrap values (> 80%), not only confirmed the taxonomic validity of these morphologically similar species but also revealed complex population structures within each lineage. This genetic distinction between species in sympatry aligns with Aplin et al.'s comprehensive Indo-Pacific survey, which similarly documented clear genetic boundaries between these species even in zones of geographic overlap.[21]

Particularly notable is how *R. tanezumi* exhibits substantially greater genetic diversity across multiple subclades, suggesting a deeper evolutionary history in the Indonesian archipelago compared to the more genetically cohesive *R. rattus* populations. This pattern is consistent with Pagès et al.'s observation that *R. tanezumi* typically harbours more genetic diversity than *R. rattus* within shared territories, likely reflecting its longer establishment in Southeast Asia.[3] The strong monophyletic clustering of local *R. rattus* specimens (bootstrap support 70–99%) corresponds with Thomson et al.'s findings regarding population structure in island and isolated mainland populations, while the absence of genetic intermixing between species supports Pergams' observation that natural hybridisation remains exceptionally rare despite potential opportunities for interbreeding.[22,23]

Genetic Diversity and Population Structure

The detailed genetic analysis further illuminates the population dynamics of these two important rodent species. Despite similar numbers of SNPs (74 in *R. rattus* and 75 in *R. tanezumi*), *R. tanezumi* demonstrated significantly higher haplotype diversity (Hd = 0.856 ± 0.039) as compared to *R. rattus* (Hd = 0.653 ± 0.122). This suggests greater genetic variation at the haplotype level in *R. tanezumi*, despite comparable nucleotide diversity values (*R. rattus*: $\pi = 0.027 \pm 0.006$; *R. tanezumi*: $\pi = 0.029 \pm 0.004$). These findings align with those of Kosoy et al., who documented similar patterns of differential genetic diversity in *R. tanezumi* across Southeast Asian populations, attributing this to its longer evolutionary history in the region.[4]

The striking contrast in population structure between these species is further emphasised by the F_{ST} analysis. *R. rattus* showed remarkably strong genetic separation between locations, with F_{ST} values reaching as high as 0.973. The AMOVA results indicate that nearly all genetic variation in this species exists among populations rather than within them, suggesting that *R. rattus* populations likely originated from different source populations and have experienced limited gene flow since their introduction. This pattern of strong genetic isolation among *R. rattus* populations has been previously documented by Aplin et al. and Lack et al., who attributed it to multiple independent introduction events associated with human maritime activities throughout Indonesia's history.[9,21]

In contrast, *R. tanezumi* exhibits a more fluid population structure, particularly among populations in central Java (Purworejo, Semarang, and Yogyakarta), indicating greater connectivity and potential ongoing gene flow. This genetic cohesiveness suggests that these populations likely share a common ancestry and may still experience movement between regions. However, greater genetic differentiation is observed when comparing these central Javanese populations with those from Jakarta and Sulawesi. Interestingly, the genetic similarity between Jakarta and Sulawesi *R. tanezumi* populations potentially reflects human-mediated dispersal through established transportation networks, a phenomenon also noted by Lack et al. in their study of commensal rodent movements in Southeast Asia.[9]

Evolutionary Forces and Population History

The contrasting neutrality test results provide further insights into the evolutionary forces shaping these rodent populations. *R. rattus* exhibits positive values across Tajima's D (0.806), and Fu and Li's D* (1.037) and F* (1.129), potentially indicating balancing selection or population subdivision. Conversely, *R. tanezumi* shows a more neutral Tajima's D (0.133) and slightly negative values for Fu and Li's statistics (D* = -0.131, F* = -0.829), which could suggest purifying selection or slight population expansion, though these values are not statistically significant. These distinct patterns mirror observations made by Aplin et al., who found different demographic histories between these cryptic species throughout Indonesia.[21]

The positive Fu's Fs values observed for *R. rattus* and *R. tanezumi* (7.507 and 5.789, respectively) contradict expectations of recent population expansions, supporting Lack et al.'s conclusion that established commensal rodent populations in agricultural landscapes often exhibit genetic signatures of demographic stability rather than expansion.[9] This suggests that both species have maintained relatively stable populations in Purworejo Regency, despite the potential for rapid population growth typical of rodent species.

Implications for Leptospirosis Transmission

The distinct genetic profiles and population structures observed have important implications for understanding leptospirosis epidemiology in Central Java. The higher genetic diversity in *R. tanezumi* likely reflects a more complex population history, possibly involving multiple introduction events or longer establishment in the region. This enhanced genetic diversity may confer greater adaptability to diverse environments and potentially influence its capacity as a disease reservoir by promoting genetic diversity in hosted pathogen populations, as suggested by Pagès et al. in their study of pathogen prevalence in sympatric *Rattus* species.[3]

The strong genetic isolation observed among *R. rattus* populations suggests that these rats may carry genetically distinct strains of pathogens like *Leptospira* in different locations, potentially leading to spatial heterogeneity in disease risk across the landscape. These results complement the work of Costa et al., who demonstrated that rodent genetic diversity can significantly influence pathogen transmission dynamics and subsequently affect the spatial epidemiology of zoonotic diseases across varied landscapes.[24] This may explain the observed differences in leptospirosis case distribution between densely populated Kutoarjo and the hillier Bagelen region of Purworejo Regency.

Conclusion

This study revealed significant differences in the genetic diversity and population structure of *R. rattus* and *R. tanezumi* in Purworejo Regency, Central Java. These morphologically similar but genetically distinct species maintain their genetic integrity despite sympatric distribution, with markedly different population genetic characteristics that likely influence their roles in leptospirosis ecology. *R. rattus* populations exhibited strong genetic isolation between locations (F_{ST} values up to 0.973), suggesting multiple independent introductions with limited gene flow. In contrast, *R. tanezumi* displayed greater connectivity among Central Java populations, indicating ongoing movement between regions. These distinct genetic profiles may explain the spatial heterogeneity of leptospirosis cases across Purworejo's sub-districts, with isolated *R. rattus* populations potentially harbouring location-specific pathogen strains, while the higher genetic diversity in *R. tanezumi* may enhance its adaptability and disease reservoir capacity. Future research should expand geographic sampling and incorporate pathogen screening to further elucidate the relationship between rodent population genetics and leptospirosis transmission in Indonesia.

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