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Flea borne Rickettsioses Associated with Rats in Palabuhanratu Port, Sukabumi, West Java, Indonesia

(RICKETTSIOSIS YANG DITULARKAN OLEH PINJAL YANG MENGINFESTASI TIKUS-TIKUS DI PELABUHAN PALABUHANRATU, SUKABUMI, JAWA BARAT, INDONESIA)

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ABSTRACT

Flea borne rickettsiosis remains unrecognized and often overlooked in many tropical regions, particularly in high-risk areas such as ports with dense human activity that potentially support the circulation of zoonotic pathogens. This study aimed to detect the presence of *Rickettsia* spp. in fleas infesting rats at Palabuhanratu Port, Sukabumi, West Java, Indonesia. Flea samples were collected from rats captured using single live traps baited with roasted coconut, set purposively in fish markets, offices, warehouses, and food processing facilities from July to October 2025. Identification of rats and fleas was based on morphological characteristics, and then the fleas were pooled based on flea and host species before DNA extraction. Detection of *Rickettsia* spp. was performed using the Polymerase Chain Reaction (PCR) method targeting the 17 kDa and *gltA* genes. A total of 131 rats were captured, consisting of 100 *Rattus norvegicus* (76.3%) and 31 *Rattus tanezumi* (23.7%), with flea infestation found in 29 rats (22.1%). The 69 fleas collected consisted of *Xenopsylla cheopis* (97.1%) and *Ctenocephalides felis* (2.1%), and were grouped into 29 pools. PCR results showed that 89.7% of the pool samples were positive for *Rickettsia* based on the 17 kDa gene and 65.5% based on the *gltA* gene. Phylogenetic analysis of both genes confirmed that the detected *Rickettsia typhi* belongs to the Typhus Group. These findings provide molecular evidence for the circulation of murine typhus in rat fleas in the port of Palabuhanratu, West Java, Indonesia and emphasize the importance of ongoing vector and reservoir surveillance to support public health risk control.

Keywords: Rickettsia; fleas; rats; Palabuhanratu port

ABSTRAK

Rickettsiosis yang ditularkan oleh pinjal masih kurang dikenali dan sering terabaikan di banyak wilayah tropis, terutama pada kawasan berisiko tinggi seperti pelabuhan yang memiliki aktivitas manusia yang padat sehingga berpotensi mendukung sirkulasi patogen zoonotik. Penelitian ini bertujuan untuk mendeteksi keberadaan *Rickettsia* spp. pada pinjal yang menginfestasi tikus di pelabuhan Palabuhanratu, Sukabumi, Jawa Barat, Indonesia. Sampel pinjal dikoleksi dari hasil penangkapan tikus menggunakan perangkap hidup tunggal dengan umpan kelapa bakar yang dipasang secara purposive sampling di area pasar ikan, perkantoran, gudang, dan tempat pengelolaan pangan dari bulan Juli hingga Oktober 2025. Identifikasi tikus dan pinjal dilakukan berdasarkan karakteristik morfologi, kemudian pinjal dipool berdasarkan spesies pinjal dan spesies inang sebelum dilakukan ekstraksi DNA. Deteksi Rickettsia dilakukan menggunakan metode *Polymerase Chain Reaction* (PCR) yang menargetkan gen 17 kDa dan *gltA*. Sebanyak 131 ekor tikus berhasil ditangkap, terdiri atas 100 ekor *Rattus norvegicus* (76,3%) dan 31 ekor *Rattus tanezumi* (23,7%), dengan infestasi pinjal ditemukan pada 29 ekor tikus (22,1%). Sebanyak 69 pinjal yang dikoleksi terdiri dari *Xenopsylla cheopis* (97,1%) dan *Ctenocephalides felis* (2,1%), dan dikelompokan kedalam 29 pool. Hasil PCR menunjukkan bahwa 89,7% sampel pool positif *Rickettsia* berdasarkan gen 17 kDa dan 65,5% berdasarkan gen *gltA*. Analisis filogenetik dari kedua gen tersebut meneguhkan bahwa *Rickettsia typhi* yang terdeteksi, termasuk dalam kelompok *Typhus Group*. Temuan ini memberikan bukti molekuler adanya sirkulasi murine typhus pada pinjal tikus di pelabuhan Palabuhanratu, Jawa Barat, Indonesia dan menekankan pentingnya surveilans vektor dan binatang pembawa penyakit secara berkelanjutan untuk mendukung pengendalian risiko kesehatan masyarakat.

Kata-kata kunci: Rickettsia; pinjal; tikus; Pelabuhan Palabuhanratu

INTRODUCTION

Rickettsiae are obligate intracellular bacteria that cannot live independently and depend on vertebrate or arthropod hosts for their survival (Helminiak *et al*., 2022). Various hematophagous arthropods, such as mites, ticks, fleas and lice, act as vectors and reservoirs, while small mammals such as rodents contribute to the maintenance of these bacteria in nature (Liu, 2015). Rickettsial infections or rickettsioses occur in urban and rural areas worldwide (Jiang *et al*., 2021; Blanton, 2023), with transmission primarily through arthropod bites or skin/mucosal contamination by infected vector excreta. Humans are incidental hosts and play no role in the natural transmission cycle (Helminiak *et al*., 2022). Based on the disease presentation, the antigenicity and vectors involved, rickettsioses are categorized into three major groups, which are (1) The Typhus Group (TG), caused by *Rickettsia prowazekii* and *R. typhi*; (2) The Spotted Fever Group (SFG), caused by *R. ricktesii*, *R. conorii*, *R. africae*, *R. felis*, *R. australis*, *R. japonica*, etc; and (3) The Scrub Typhus Group (STG) caused by *Orientia tsutsugamushi* (Jiang *et al*., 2021). Murine typhus is one of the rickettsioses, caused by *R. typhi*, a member of the Typhus Group.

Oriental rat flea, *Xenopsylla cheopis*, is the primary vector for murine typhus transmission in humans in most regions of the world, while *Ctenocephalides felis* is the primary vector in suburban areas of the United States (Pramestuti *et al*., 2022). Several cases of murine typhus have been reported from Southeast Asia, North America, Latin America, the Mediterranean, Northern Africa and Europe (Bhengsri *et al*., 2016; Rauch *et al*., 2018; Doppler and Newton, 2020; Grouteau *et al*., 2020; Labropoulou *et al*., 2021; Robaina bordón *et al*., 2021; Faccini Martinez *et al*., 2022).

Indonesia is endemic for murine typhus. Serological evidence of murine typhus in humans has been reported from various regions in Indonesia, with prevalence rates of 6.5% in Northern Lampung (Hadi *et al*., 1986), 6.5% in Jakarta (Denis *et al*., 1981), 7% in Semarang (Gasem *et al*., 2009), and 2.1% in Papua (Richards *et al*., 2003). In addition, a study in the Greater Bandung area reported a seroprevalence of 40.3% among hospitalized patients with fever (Riswari *et al*., 2023). This disease is a significant but often neglected common cause of acute febrile illness, frequently misdiagnosed due to nonspecific symptoms that are similar to or even overlap with other febrile syndromes such as dengue fever and typhoid fever, as well as limited diagnostic capability (Herwanto *et al*., 2025).

The molecular detection of *R. typhi* in rat fleas from various Indonesian regions, including West Java and East Kalimantan (Barbara *et al*., 2010), Semarang, Kupang, and Maumere (Joharina *et al*., 2016) and Banjarnegara (Pramestuti *et al*., 2018), showed evidence of an active enzootic cycle involving commensal rodents and their ectoparasites. The potential for the transmission of the disease, murine typhus, is especially concerning in urban areas, especially at seaports, where there are large populations of urban rodents like *Rattus rattus* and *R. norvegicus* (Chiang *et al*., 2022; Blanton, 2023).

Palabuhanratu Port is a fishing port that features a fish market with a high rodent density (Santi *et al*., 2023), which is ideal for the maintenance of the enzootic cycles of murine typhus. However, Rickettsia infection in rat fleas from this area has not been reported. Therefore, this study was aimed to detect Rickettsia in fleas associated with rats captured at Palabuhanratu Port through the polymerase chain reaction (PCR) method. The findings will provide essential baseline data to support strengthened vector monitoring, enhance early warning systems, and improve public health preparedness against rodent borne zoonoses in Indonesian port environments.

RESEARCH METHODS

Collection Method

Flea samples were collected from rats captured in the Palabuhanratu Port from July to October 2025. Rat capture was performed using purposive sampling with a single live trap baited with grilled coconut. The collected rats were conducted according to ethical clearance number 358/KEH/SKE/ VIII/2025 issued by the Animal Ethics Committee, School of Veterinary Medicine and Biomedical Sciences, IPB University.

A total of 100 single live traps were set daily both inside and outside buildings including fish markets, offices, warehouses and food handling facilities, for four consecutive days each month. The traps were set in the afternoon between 4:00 and 6:00 PM and were checked the following morning between 6:00 and 7:00 AM. Captured rats were anesthetized and euthanized with a ketamine–xylazine combination; flea samples were collected by combing the fur. Rats were identified to species level based on external morphological measurements (body, tail, hind foot and ear length), body weight, nipple counts in females, and pelage characteristics (Balitbangkes, 2017). Fleas were morphologically identified using fleas identification keys (CDC, 1966) and preserved in DNA/RNA Shield for molecular analysis at the Laboratory of Veterinary and Medical Entomology, IPB University.

DNA Extraction and PCR Amplification

Fleas were pooled according to flea and host species. In total, 29 pools were obtained from 69 fleas for DNA extraction. The DNA was extracted using the Genomic DNA Mini Kit (Tissue) Geneaid® (Geneaid Biotech Ltd., New Taipei City, Taiwan), according to the manufacturer's instructions. Detection of *Rickettsia* spp., was performed by Polymerase chain reaction (PCR) targeting both the 17 kDa gene and the *gltA* gene (Supriyono *et al*., 2019) (Table 1). For the 17 kDa gene, PCR conditions were: predenaturation at 95°C for 5 min; 40 cycles of denaturation at 94°C for 30 s, annealing at 46.5°C for 30 s, and extension at 72°C for 30 s; followed by a final extension at 72°C for 7 min. For the *gltA* gene, PCR conditions were: predenaturation at 95°C for 5 min; 40 cycles of denaturation at 94°C for 45 s, annealing at 52°C for 30 s, and extension at 72°C for 45 s. The PCR products were resolved on 2% agarose gels with ethidium bromide and visualized under UV light using a Gel Documentation System, (Cleaver Scientific gelONE®, Cleaver Scientific Ltd., Rugby, Warwickshire, United Kingdom). A selected number of positive samples were reamplified (50 µL) and submitted for sequencing to confirm *Rickettsia* species identity.

Phylogenetic and BLAST Analysis

Sequencing results were analyzed using SnapGene Viewer (GSL Biotech LLC) and edited with MEGA version 12. The DNA sequences were compared with reference sequences available in GenBank using Basic Local Alignment Search Tool for nucleotides (BLASTn). Phylogenetic trees were constructed using the Neighbor Joining method with the Kimura 2 parameter model (Supriyono *et al*., 2019). All DNA sequences were deposited in GenBank with accession numbers PX963820 to PX963823.

RESULTS AND DISCUSSION

In this study, a total of 131 rats were collected from several areas, including fish markets, offices, warehouses, and food handling facilities. The trap placement areas are shown in Fig. 1. During the four-month observation period, the number of rats caught showed a decreasing trend from July to October, with the highest number in July (48 rats) and the lowest in October (22 rats). However, the number of rats infested with fleas did not follow the same pattern. Flea infestation showed a higher level in August and October than in other months (Fig. 2).

The trapped rats consisted of 100 *R. norvegicus* and 31 *R. tanezumi*. Of the total trapped rats, 29 (22.1%) were infested with fleas. The *R. tanezumi* has a higher flea prevalence than *R. norvegicus* (Table 2). A total of 69 fleas were collected from the trapped rats, belonging to two species (67 *X. cheopis* and 2 *C. felis*) (Fig. 3).

Detection of *Rickettsia* spp., in *X. cheopis* showed the highest prevalence in *R. norvegicus*, at 94.7% using the 17 kDa gene and 73.7% using the *gltA* gene. Overall, of the total pool tested, 89.7% were positive based on the 17 kDa gene, and 65.5% were positive based on the *gltA* gene. Meanwhile, one pool of *C. felis* examined only showed positive for the 17 kDa gene, with a prevalence of 100%, but was negative for *gltA* (Table 3).

From the samples that showed positive for *Rickettsia*, three samples were selected for sequencing due to showing better quality bands (pool numbers 13, 14 and 15). Sequencing of the 17 kDa gene revealed that pools 13 and 14 have a similarity of 100% with *R. typhi* Accession numbers PQ625781 and AE017197, respectively. For the *gltA* gene, pool number 13 showed 100% similarity with *R. typhi* (Accession number MW 631932), while pool 14 showed 94,85% similarity with *R. typhi* (Accession number JF448474). In contrast, pool 15 did not produce analyzable sequences for both the 17 kDa and *gltA* genes, due to the poor quality.

Phylogenetic analysis based on the 17 kDa and *gltA* genes consistently showed that both sequenced samples (13 and 14) clustered within the Typhus Group. In the 17 kDa gene, Pool 13 and 14 were in a cluster with *R. typhi* strains originating from Yucatán (Mexico), Texas (USA), and Thailand, supported by high bootstrap values indicating a strong evolutionary relationship (Fig. 4). Meanwhile, in the *gltA* gene, both samples were close to *R. typhi* strains from India, Tanzania (Africa), Thailand, South Korea, as well as a strain from Semarang, Indonesia, which was previously also reported to originate from *X. cheopis* (Fig.).

Table 1. Primer sequences and annealing temperatures used to detect *Rickettsia* spp.

Target gene	Primer name	Nucleotide sequence (5'-3')	Annealing T (°C)	Product length (bp)
17 kDa	R1_F	TCAATTCACAACTTGCCATT	46.5	488
	R2_R	TTTACAAAATTCTAAAAACC		
gltA	RpCs877p_F	GGGGGCCTGCTCACGGCGG	52	381
	RpCs1258n_R	ATTGCAAAAAGTACAGTGAAC		

Abbreviation: F, forward; R, reverse; T, temperature

Table 2. Rodent species trapped, and fleas collected from Palabuhanratu Port

Rodet species	No. Collec Ted rodent	No. flea infes Ted rodent	Prevalen ce (%)	Flea species (%)	
				X. cheopis	*C. felis*
R. norvegicus	100	20	20	57 (96.6)	2 (3.4)
R. tanezumi	31	9	29	10 (100)	0 (0)
Total	131	29	22,1	67 (97.1)	2 (2.9)

Table 3. Rickettsia prevalence detected on fleas infested rats in Palabuhanratu Port during July to October 2025

Flea species	Host	No of sample*	No. of positive samples* (Prevalence in %)	
			17 kDa	*gltA*
X. cheopis	*R. norvegicus*	19	18 (94.7)	14 (73.7)
	R. tanezumi	9	7 (77.8)	5 (55.6)
C. felis	*R. norvegicus*	1	1 (100)	-
Total		29	26 (89.7)	19 (65.5)

*) Pool

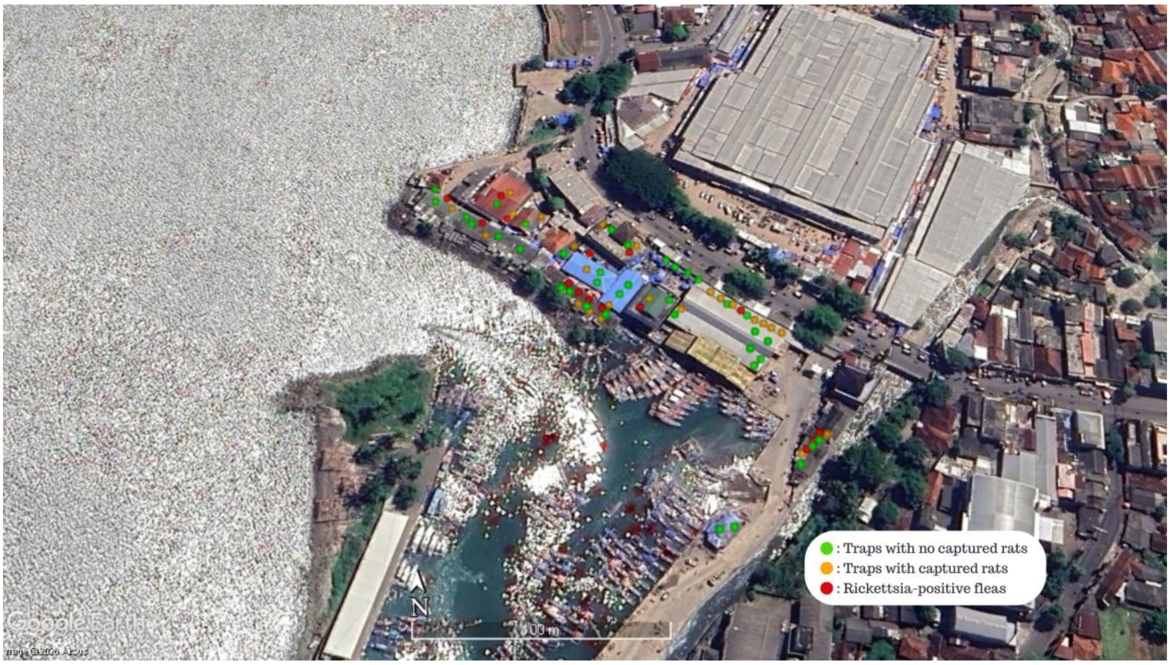

Figure 1. Sampling site Palabuhanratu Port, Sukabumi, West Java, Indonesia

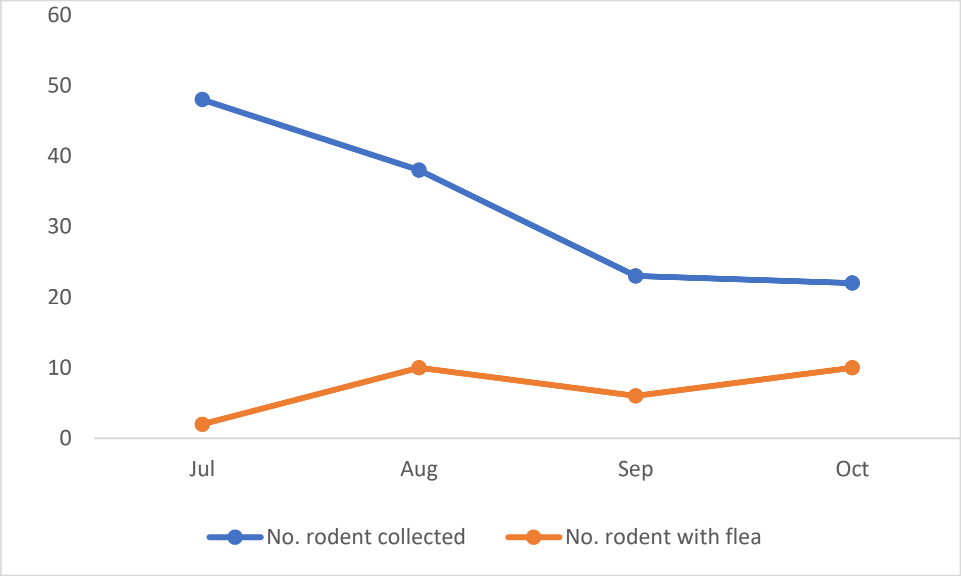

Figure 2. Monthly fluctuation of trapped rats and flea infested rats in Palabuhanratu Port from July to October 2025.

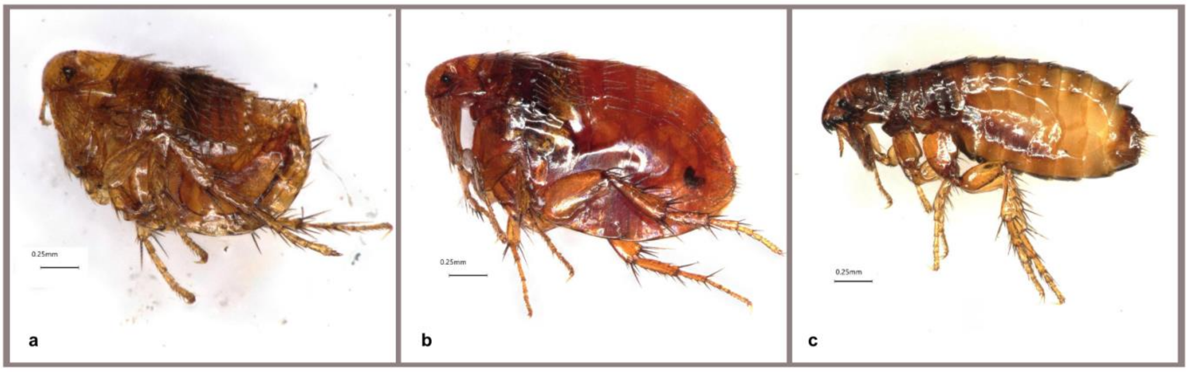

Figure 3. Fleas collected from Palabuhanratu Port (a) Male *X. cheopis*; (b) female *X. cheopis*; (c) female *C.felis.*

The consistency of clustering in these two gene targets confirms that the samples have a strong genetic kinship with *R. typhi* circulating globally.

This study trapped rats over four months, indicating that the environment of Palabuhanratu Port provides highly favorable conditions for rat presence. The port's function influences the high rat density in this area as a center of fishery activities, the presence of a fish market, high human activity and poor sanitation, all of which indirectly provide abundant food resources that support rat population growth. *Rattus tanezumi* and *R. norvegicus* are commensal rat species that are highly adaptive and able to thrive in environments rich in food sources and harborage sites (Byers *et al.*, 2019). Previous research conducted at ports in Indonesia, including Cilacap (Priyotomo *et al.*, 2015) and Manokwari (Manyullei *et al.*, 2020), as well as in other countries such as Taiwan (Chiang *et al.*, 2022), showed that *R. norvegicus* and *R. tanezumi* are species commonly found in port areas. Rats were known to be carriers and transmitters of diseases to humans, including leptospirosis and hantavirus infection (Griffiths *et al.*, 2022). In addition to harboring pathogens directly, rats also serve as primary hosts for ectoparasites such as fleas, which can act as vectors of diseases including plague, bartonellosis and rickettsiosis (Bai *et al.*, 2017).

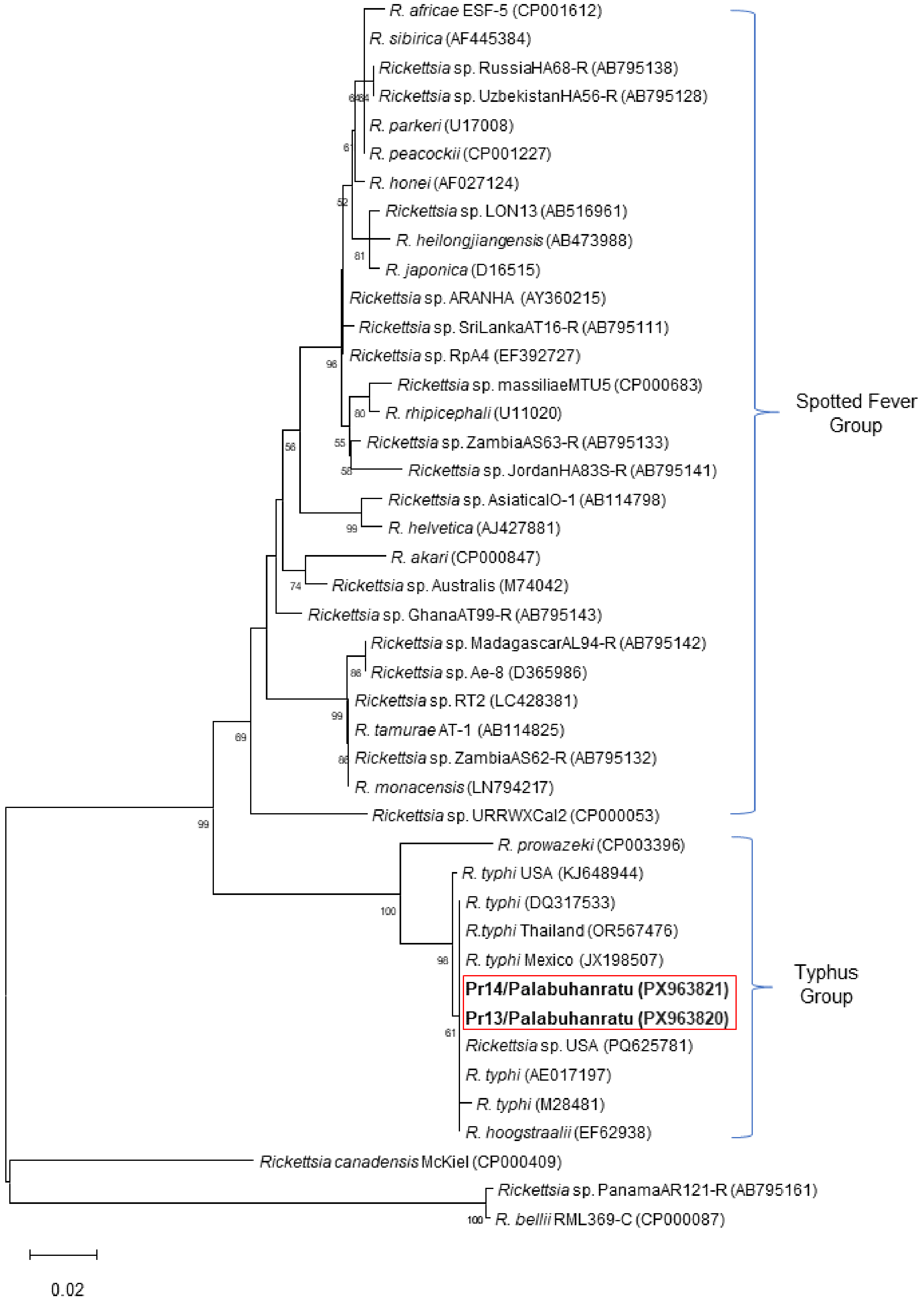

Figure 4. Phylogenetic tree of the 17 kDa gene using the neighbor joining tree with a bootstrap value of 1000.

Figure 5. Phylogenetic tree of the *gltA* gene using the neighbor joining tree with a bootstrap value of 1000.

Xenopsylla cheopis is the most common flea species found in rats captured in Palabuhanratu Port. This ectoparasitic organism is the most prevalent infesting rats within tropical regions and is considered cosmopolitan (Widiastuti *et al*., 2018). The wide distribution and adaptability of *X. cheopis* to various environments underscore its role as a major vector in the transmission of zoonotic diseases (Schaub, 2024).

In this study, *X. cheopis* was found infesting both *R. tanezumi* and *R. norvegicus*. Previous studies have reported *R. tanezumi* and *R. norvegicus* as hosts of *X. cheopis* (Zhao *et al.*, 2018; Susanna *et al.*, 2021; Cahyaning rum *et al*., 2024). The *X. cheopis* is highly mobile and can easily move from one host to another, either within the same or different species. The Norway rat, *R. norvegicus*, is a peridomestic species and is therefore likely to acquire *X. cheopis* from house rats (Zhao *et al*., 2018). Although *R. norvegicus* was the most frequently caught species, this study showed that the prevalence of flea infestation in *R. tanezumi* was higher. This high prevalence indicates that flea infestation is more influenced by host characteristics and dry nest microhabitats, rather than host density. The *R. tanezumi* typically nests in dry, warm indoor microhabitats that provide favorable conditions for the development of flea life stages. In contrast, *R. norvegicus* often inhabits damp areas such as drains, sewers and burrows near water, where moisture and fluctuating temperatures reduce flea survival (Panti May *et al*., 2016).

This study also found the presence of cat fleas (*C. felis*) on *R. norvegicus*, although only one individual rat was infested. The presence of cat fleas on rats, although rare, indicates interactions and flea transfer between different host species in the port environment. The *C. felis* has a broad host range that includes cats, dogs and other mammals, and can move to different hosts if necessary. The *C. felis* parasitizing rats has also been reported in Cyprus (Psaroulaki *et al*., 2006).

Detection of *Rickettsia* in fleas in Palabuhanratu Port showed a very high prevalence. The prevalence of fleas infected with Rickettsia in this study was highest in *R. norvegicus*, as this rodent species was the most commonly found at the study sites. The differences observed between PCR results using the 17 kDa and *gltA* gene targets may be attributed to primer characteristics, specifically specificity and sensitivity. The primer targeting the 17 kDa gene has higher specificity for detecting Rickettsia from the Spotted Fever Group (SFG), while the primer targeting the *gltA* gene has higher sensitivity for detecting Rickettsia from both the SFG and Typhus Group (Supriyono *et al*., 2019).

This study showed a very high prevalence of *Rickettsia* spp., in *X. cheopis*, with sequencing confirming *R. typhi* as the dominant species in the two sample pools analyzed. This finding indicates a potentially significant risk of murine typhus transmission in the port environment.

The identified Rickettsia, *R. typhi*, is the causative agent of murine typhus, an acute zoonotic disease transmitted primarily by the rat flea *X. cheopis*.

The *R. typhi* is a Gram negative, obligate intracellular bacterium that replicates in the flea's mesenteric epithelium. It is excreted in flea feces and can infect humans through inoculation into broken skin, scratching at the bite site (Blanton and Walker, 2017). The *R. typhi* can survive in dried flea feces for more than 100 days and does not decrease the fitness of its vector, allowing fleas to remain infective throughout their lives (Pramestuti *et al*., 2022). The ability to transmit between generations through transovarial transmission (Liu, 2015) also allows the bacterium to persist in flea populations despite less than ideal environmental conditions.

Although murine typhus is often considered a relatively mild disease, evidence suggests that it remains largely under recognized and often undiagnosed due to its nonspecific symptoms, which mimic other acute febrile illnesses. A cohort study of hospitalized patients from seven hospitals in Indonesia showed that *R. typhi* was one of the three most common microbiological etiologies in fatal acute febrile cases, with a mortality rate of 6.8% (Gasem *et al*., 2020). Global findings also confirm that delayed diagnosis results in prolonged fever duration (12–22 days), organ complications (lung, kidney, central nervous system and high hospitalization rates (50–84%) (Doppler and Newton, 2020). Even in untreated cases, murine typhus still causes significant morbidity. Furthermore, the clinical burden and cost of care increase substantially when the diagnosis is delayed or unrecognized.

Thus, the presence of *R. typhi* in *X. cheopis* in this study, combined with clinical evidence that murine typhus is often undiagnosed and can cause severe morbidity, reinforces the urgency of strengthening vector and reservoir surveillance and enhancing diagnostic capacity for rickettsiosis in ports. Early detection in both vectors and humans is crucial to prevent more severe cases, reduce the disease burden and support zoonotic preparedness in Indonesian port areas.

CONCLUSION

This study provides molecular evidence of *R. typhi* circulates in *X. cheopis* infested *R. norvegicus* and *R. tanezumi* at Palabuhanratu Port. This indicating a potential risk of murine typhus transmission to people working or living in the port area.

SUGGESTION

Strengthened vector and reservoir surveillance, improved environmental sanitation, vector control measures and community education are recommended to reduce the risk of murine typhus transmission in the port environment.

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