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Prevalence and diversity of mite ectoparasites of house mice (*Mus musculus* L.) in urban environments in New Jersey, USA

Running title: Ectoparasites of house mice

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Abstract

BACKGROUND

House mice, *Mus musculus* L., carry ectoparasites that cause dermatitis and can vector human diseases. Nevertheless, there is little information on the prevalence and diversity of ectoparasites from house mice living in urban environments. We collected and identified ectoparasites from house mice trapped in apartment buildings and a chicken breeding building in three cities in New Jersey, USA. Two blood-sucking mite species were genetically barcoded to facilitate future species determination.

RESULTS

A total of 189 house mice were trapped in apartment buildings while 113 mice were trapped in or around a chicken breeding building. Mites were the only ectoparasites found on house mice. The mite infestation rates ranged between 25–89%, and the mite species richness was between 1.0 ± 0.0–2.2 ± 0.1 per mouse among the study sites. The four mite species identified were *Myocoptes musculinus* (Koch), *Myobia murismusculi* (Schrank), *Echinonyssus butantanensis* (Fonseca), and the house mouse mite (*Liponyssoides sanguineus* [Hirst]). The house mouse mite was the only medically important mite found in this study. It was found only in apartment buildings with a prevalence between 30–79% and a mean density between 2.6 ± 0.6–8.5 ± 1.2 per host.

CONCLUSION

This study represents the first records of both *E. butantanensis* and the house mouse mite on house mice in New Jersey. This is significant as the house mouse mite has been documented to present a potential human health risk when there is a high prevalence in occupied buildings.

Keywords: ectoparasite, house mouse, urban pest, survey, mites, *Liponyssoides sanguineus*

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1 Introduction

The house mouse (*Mus musculus* L.) is a rodent species well adapted to a wide range of habitats globally. In urban environments, house mice primarily live indoors and are commonly found in residential areas.[1-3] House mice are major urban pests causing significant economic loss due to their potential for serious contamination of food supplies with urine and feces.[4] Mice produce allergens that can cause human allergic reactions.[5,6] House mice are also reservoir hosts for zoonotic pathogens such as *Salmonella* spp., *Yersinia* spp., lymphocytic choriomeningitis virus, and *Leptospira* spp.[7-12] and have the capacity to transmit diseases to humans.

House mice carry a range of ectoparasites such as mites, lice, fleas, and ticks. They may bite people, cause skin dermatitis, and vector disease pathogens.[13-15] In the United States, ectoparasites on house mice have been studied in Mississippi, Georgia, Indiana, South Carolina, and Iowa states.[16-21] Mites were found to be the major ectoparasite group on house mice. Some mite species localize to the base of the mouse's hair, consuming the skin debris or tissue fluid (e.g., *Myocoptes* spp. and *Myobia* spp.).[22] Some blood-sucking mite species may leave the host after feeding, resting in the host's nesting materials, and foraging around the nest for their next blood meal (e.g., mites in *Dermanyssidae*). Their foraging behavior creates opportunities for encountering humans and biting people. The house mouse mite, *Liponyssoides sanguineus* (Hirst), has been reported to transmit *Rickettsia akari* from mice to humans, leading to Rickettsialpox disease.[23] The house mouse mite was responsible for several Rickettsialpox outbreaks in the U.S.[24] Indeed, it is a species of mite that needs public attention for human health risks.

A recent survey found that house mice were the 2nd most common indoor pest in low-income apartment buildings in the northeastern U.S.[25] Yet, only a few studies have focused on ectoparasites on house mice within the last two decades.[18,26-32] Lacking updated knowledge on the prevalence and diversity of ectoparasites on house mice makes it challenging to predict their potential public health threats. This study aimed to investigate the prevalence of ectoparasites on house mice in apartment buildings and an urban farm building. We also developed genetic barcodes for two blood-sucking mite species to aid in future species identification.

2 Materials and Methods

2.1 Collection sites

Apartment buildings and a farm building located in three cities (Paterson, New Brunswick, and Trenton) in New Jersey, USA, were selected (Fig. 1). These sites have known high rates of house mouse infestations. The Paterson site included two high-rise apartment buildings 2 km apart, totaling 192 apartments. The New Brunswick site

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included six two-story garden-style apartment buildings in a community with 48 apartments, and each building had four crawl spaces. The Trenton site included four three-story garden-style apartment buildings in a community with 156 apartments and eight crawl spaces. These apartment buildings were managed by their respective housing authorities in each city.

The Farm site is an educational farm located on a university campus in New Brunswick city. A chicken breeding building (30 m × 10 m) which had chronic house mouse infestations is included in the study. This building had 10 cubicles separated by brick walls and contained chickens and their feeds during the study period. It was surrounded by other buildings, including classroom buildings, office buildings, and a farm store. Hence, it is considered an urban environment.

2.2 House mouse collection

All procedures for trapping and handling animals followed the guidelines of Rutgers University Institutional Animal Care and Use Committee (IACUC) Protocol No. PROTO202000189. House mice were collected during the following periods: Paterson (July to August 2022), New Brunswick (June to July 2021), Trenton (May to June 2022), and Farm (September to October 2021). Sherman live traps (7.6 cm × 8.9 cm × 22.9 cm; H.B. Sherman Inc., Tallahassee, FL, USA) and extended trigger mouse snap traps (Woodstream Co., Lititz, PA, USA) were used to capture mice. Sherman traps were baited with a slice of fresh potato, chocolate spread, and oatmeal flakes. Two snap traps were baited with chocolate spread and placed in a TrapRite box to prevent injury to pests and humans (Anstar Products, Inc., Niles, IL, USA). At Paterson, New Brunswick, and Trenton sites, two baited Sherman traps and two TrapRite boxes were placed in the kitchen, the living room, or the utility room per apartment with mouse activities. In addition, baited traps were placed in common areas including crawl spaces, boiler rooms, compactor rooms, and staff offices. At the Farm site, a total of 17 snap traps were placed outside the building and 10 Sherman traps plus 12 snap traps were placed inside the building. In contrast to apartments, two snap traps were placed in each PROTECTA EVO Express Bait Station (Bell Laboratories, Inc., Windsor, WI, USA) inside the building. All traps were inspected within 24 h after setup. Trapped live mice were transported to the laboratory and euthanized with carbon dioxide, followed by cervical dislocation. Dead mice were stored individually in plastic zip-lock bags. The trapped mice were identified to species and only house mice were used in this study. All mouse carcasses were stored at -15 °C for further ectoparasite inspection.

2.3 Ectoparasite inspection and identification

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Ectoparasites were collected using the Henry and McKeever[33] method with minor modifications. House mouse carcasses were placed in a mason jar (diameter: 9.5 cm, height: 17.5 cm) with 400 mL water and 3 mL detergent for the first washing. The mason jar was agitated for 5 minutes using a Blair Tornado II Electronic paint shaker (Blair Co., Swartz Creek, MI, USA). The mouse carcass was then transferred to a second mason jar with 400 mL water for a second washing. If there were bubbles during the washing period, a small amount of 95% ethanol was used to reduce them. After shaking, the water was filtered using Fisher brand P8 filter paper (9 cm diameter; Fisher Scientific, Waltham, MA, USA) and a Buchner funnel. After two washes, each mouse was systematically inspected for the presence of ectoparasites, especially on ears, eyes, and leg joints under a Leica stereo microscope S8AP0 (Leica Microsystems Inc., Deerfield, IL, USA).

The ectoparasites on the filter paper were examined under a Leica stereo microscope. For each species, a few specimens were prepared on slides using Hoyer's mounting medium (Hempstead Halide, Galveston, TX, USA) to facilitate identification. Ectoparasites were identified using taxonomic keys[22,34,35] and saved in 95% ethanol at -20 °C until DNA extraction. Voucher specimens are kept in the Department of Entomology at Rutgers University and in the University of Michigan Museum of Zoology. Images were taken using a Zeiss Axio Observer Z1 microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) equipped with Fluar 5x, EC Plan-Neofluar 10x, and LD Plan-Neofluar 20x objective lens and a Zen 3.5 software.

2.4 Mite DNA extraction, amplification, and phylogenetic analysis

Genetic fingerprinting of the two blood-sucking mite species found on house mice was analyzed to facilitate future species determination. DNA extraction was conducted using the HotSHOT method[36] with some modifications. An individual mite was placed in a 0.2 mL tube containing 20 µL of fresh Alkaline Lysis Buffer. The mite was crushed using an insect pin under a stereo microscope and incubated 30 min at 95 °C followed by 15 min at 4 °C. After incubation, 20 µL of neutralization buffer (40 mM Tri-HCl) was added immediately to the tube and mixed briefly. The DNA preparation was purified using Zymo Genomic DNA Clean and Concentration kit (Zymo Research Co., Freiburg, Germany) following the manufacturer's instructions.

Five microliters of template DNA were used to amplify the cytochrome c oxidase I (*COI*) using a Phusion High Fidelity PCR Kit (New England Biolabs, MA, USA) and primers of LCO1490 and HCO2198.[37] The cycle conditions were 98 °C for 30 s; 35 cycles of 98 °C for 5 s, 60 °C for 20 s, 72 °C for 15 s, and a final extension at 72 °C for 7 min. All PCR products were purified using QIAquick PCR Purification kit (Qiagen, Germantown, MD, USA). The purified PCR products (2 µL) were

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reamplified using a second primer (Forward: 5'-ATTGGAGGWTTTGGWAAYTGA-3'; Reverse: 5'-ATGTTGRTATAARATDGGATCYCC-3'). The cycle conditions for 2nd PCR were 98 °C for 30 s; 35 cycles of 98 °C for 5 s, 57 °C for 20 s, 72 °C for 15 s, and a final extension at 72 °C for 7 min. The final products were run and visualized on a 1% agarose gel. The DNA fragment was excised from the gel and purified using a QIAquick Gel Extraction kit (Qiagen, Germantown, MD, USA). Purified PCR amplicons were Sanger sequenced for the positive and negative strands at Genewiz (Azenta Life Sciences, South Plainfield, NJ, USA).

Sequences were submitted to GenBank, and accession numbers PQ299817 were assigned for *E. butantanensis* and PQ299818 for *L. sanguineus*. The evolutionary distances were calculated using the Maximum Composite Likelihood method[38] after all ambiguous positions were removed for each sequence pair. There were 472 positions in the final dataset. Trees were created using a Neighbor-Joining method[39] with 1,500 replicates using MEGA 11.[40] The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1500 replicates) are shown next to the branches.[41]

2.5 Data analysis

Mite infestation rate (percent of house mice with mites) among four sites was analyzed using chi-square tests. A one-way analysis of variance (ANOVA) was applied to compare the differences in species richness (mean number of mite species per mouse) among the four study sites, followed by a Tukey's test. Mean mite density (number of mites per mouse) was calculated for each mite species. Prevalence was also shown to demonstrate the percentage of each mite species present on house mice. Kruskal-Wallis analysis was conducted to determine the differences in mean mite density and prevalence among the three study sites (excluded Paterson site due to the small sample size), followed by Dunn's test. Welch's two-sample *t*-test was used to compare the mean density and prevalence of *M. musculinus* between New Brunswick and the Farm site. Relative abundance (number of mites of an individual species divided by the total number of collected mites) of each mite species was also reported. All statistical analyses were conducted using R version 4.3.1.[42]

3 Results

3.1 House mice collection

A total of 177 adults (108 females, 69 males) and 125 juveniles (40 females, 85 males) house mice were trapped and examined. Among them, 114 mice were trapped by Sherman traps, and 188 mice were trapped by snap traps (Table 1). All the mice from Sherman traps were alive when the researchers collected. At Paterson site, house

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mice were collected from 11 units (8 apartments, 1 compactor room, 1 recycling room, 1 staff office); at New Brunswick site, house mice were collected from 36 units (20 apartments, 3 boiler rooms, 13 crawl spaces); at Trenton site, house mice were collected from 36 units (34 apartments, 2 crawl spaces) (Table 1). At the Farm site, 56 house mice were caught by Sherman traps (inside the chicken breeding building), and 57 house mice were caught by snap traps (7 from outside the chicken breeding building, 50 from inside the chicken breeding building). One white-footed mouse (*Peromyscus leucopus* (Rafinesque)) was collected outside the chicken building, but the data was excluded from this study. Other commensal rodents, such as Norway rat (*Rattus norvegicus* (Berkenhout)) and the roof rat (*Rattus rattus* L.), were not found at any of the sampled sites during the study period.

3.2 Mite infestation rates, species identification, and their phylogenetics

Mites exclusively accounted for all the house mouse ectoparasites found in this study at all sites. The mite infestation rate of Sherman-trapped mice was 25–84%, and the infestation rate of snap-trapped mice was 47–92% from four sites (Table 1). Mite infestation rates of Sherman-trapped mice ($\chi^2 = 25.78$, df = 3, $p < 0.001$) and snap-trapped mice ($\chi^2 = 21.23$, df = 3, $p < 0.001$) varied significantly among sites. The Farm site had higher mite species richness than the other sites (Table 1).

Four species of mites were identified, including two fur mites: *Myocoptes musculinus* (Koch) (Sarcoptiformes: Myocopitidae) and *Myobia murismusculi* (Schrank) (Trombidiformes: Myobiidae) (previously known as *Myobia musculi*); one laelapid mite: *Echinonyssus butantanensis* (Fonseca) (Mesostigmata: Laelapidae [or Hirstionyssidae]); and the house moues mite *Liponyssoides sanguineus* (Hirst) (Mesostigmata: Dermanyssidae) (Fig. 2). In addition, the identification of five specimens could not be morphologically determined.

Myocoptes musculinus has modified, hook-like legs (third pair in males and larvae or both third and fourth pair of legs in females and nymphs) for grasping the host hair (Fig. 2A).[43] *Myobia murismusculi* has a modified first pair of legs adapted to grasp hairs (Fig. 2B)[44], and its body has characteristic bulges between the legs. *Echinonyssus butantanensis* has a jug-shaped epigynial plate and an elongated peritreme (Fig. 2C).[45] *Liponyssoides sanguineus* features a pair of very long chelicerae with serrated tips and a characteristic anal plate (Fig. 2D).[46]

Echinonyssus butantanensis and *L. sanguineus* mites were genetically barcoded in this study. We Sanger sequenced a 452 bp fragment of *COI.* The sequences were deposited in GenBank with accession numbers PQ299817 for *E. butantanensis* and PQ299818 for *L. sanguineus*. Their phylogenetic relations with other mite species were inferred in a phylogenetic tree (Fig. 3). *Echinonyssus butantanensis* clustered

with other species of the same genus, and *L. sanguineus* clustered with *Dermanyssus* species, which is expected as they all belong to the family Dermanyssidae.

3.3 Species distribution patterns

A total of 1,535 mites were collected when all three residential site buildings in this study were combined (Table 2). In contrast, 19,165 mites were collected from the Farm site. *Myobia murismusculi* and *L. sanguineus* were both common in apartment sites, whereas *M. musculinus* was the dominant species at the Farm site (Table 2).

In a preliminary analysis, the mean density of each mite species collected from Sherman-trapped mice was similar to that from snap-trapped mice. Hence, data from the two trap types were combined to calculate the mean density of each mite species per mouse (Fig. 4). *Myocoptes musculinus* was found only at the New Brunswick and the Farm sites (Fig. 4). It occurred at significantly higher density (t = -8.05, df = 96.20, $p < 0.001$) and prevalence (t = 24.01, df = 142.45, $p < 0.001$) at the Farm site than at New Brunswick. *Myobia murismusculi* was found in all study sites. Among New Brunswick, Trenton, and the Farm sites, *M. murismusculi* was found with similar density (χ^2 = 4.37; df =2; p = 0.113) (Fig. 4). Paterson was excluded from the Dunn's test of mite density because there were only three *M. murismusculi* on three mice. The Farm site had the highest prevalence of *M. murismusculi* among the four sites (F = 14.59, df = 3, 298, $p < 0.001$). Interestingly, *E. butantanensis* was found only at the Farm site while the house mouse mite was found only in apartment buildings. The mean density of the house mouse mite varied significantly among Paterson, New Brunswick, and Trenton (χ^2 = 13.19; df =2; $p < 0.001$). Mice from Trenton had a significantly higher mean density of the house mouse mite per mouse than those from New Brunswick (Dunn's test, $p < 0.01$) (Fig. 4).

Due to the medically important role of the house mouse mite, we documented its occurrence patterns within apartment buildings. A total of 34 and 40 mice were collected from apartments and crawl spaces at the New Brunswick site. Among them, 26% and 50% were infested with house mouse mites, respectively. The mean house mouse mite density was 3.2 ± 1.0 and 2.4 ± 0.3 per mouse, respectively. We also compared mite distribution patterns between kitchens and living rooms at the Trenton site, where had a large number of mice. A total of 54 and 20 mice were collected from kitchens and living rooms, respectively. Among them, 80% and 70% were infested with house mouse mites, respectively. The mean house mouse mite density was 9.9 ± 1.6 and 7.6 ± 2.1 per mouse, respectively. We did not conduct a statistical analysis comparing kitchens and living rooms because mice were not always from the same apartments. At the Paterson site, only 18 mice were caught. Among them, 28% had house mouse mites, and the mean density was 2.2 ± 0.7 per mouse.

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4 Discussion

In this study, mites were the only ectoparasite group found on house mice including *M. musculinus*, *M. murismusculi*, *E. butantanensis*, and the house mouse mite. Similarly, Clark[47] surveyed ectoparasites on 66 house mice in an inhabited building using snap traps in Indiana in the U.S. Only mites were recovered from the trapped house mice. Allymehr *et al.*[48] caught 77 house mice in poultry houses in northwest Iran and found three species of mites and one species of louse (*Polyplax serrata* (Burmeister)). Hornok *et al.*[30] trapped 37 house mice in farm buildings and houses in Hungary and found one flea species (*Leptopsylla segnis* (Schonherr)) and one mite species (*Laelaps algericus* Hirst). López-Pérez *et al.*[28] did a survey in a wetland in the Mojave Desert, California, and found mites, fleas, and a tick species from 18 trapped house mice. Compared to these surveys, our study's lower diversity of ectoparasites might be related to the location and the habitat (primarily apartment buildings in urban environments).

The house mouse mite is the only medically important mite found in this study. It is a blood-sucking mite that primarily parasitizes house mice[22] and Norway rats.[46,49] The house mouse mite tends to feed every three to four days, and each time requires one to two hours. However, it has been observed that it can survive for six weeks without blood access.[50] After feeding with blood meal, the females leave the hosts and can oviposit around rodent nests or pathways.[22] The house mouse mite can also be found in warmer areas of buildings such as walls and ceilings of incinerator rooms and radiators.[50] This mite had a developmental time of two to three weeks from egg to adult. The house mouse mite has been reported in several states in the U.S. including Connecticut, New York, Massachusetts, Pennsylvania, Indiana, Ohio, Arizona, South Carolina, Utah, Hawaii, and Illinois.[18,46,49,51] In this study, we documented the occurrence of the house mouse mite for the first time in New Jersey. In 1946, the house mouse mite was first recognized as a zoonotic disease vector after a Rickettsialpox outbreak in New York.[52] More than 80 cases were reported, triggering an epidemiological investigation. The Rickettsia bacteria, *Rickettsia akari* Huebner, was confirmed as the disease agent of Rickettsialpox, with the house mouse mite as the vector and the house mouse as the reservoir. Since the early 1950s, more cases of Rickettsialpox have been documented in major cities in the eastern U.S.[24] Notably, Rickettsialpox may be underreported in public health cases because it is a self-limiting infection causing only mild symptoms. Apart from *R. akari*, the house mouse mite exhibited the ability to transmit *Coxiella burnetii* (Derrick), the agent of Q fever, from birds to white mice under experimental conditions.[53] Although humans are not typical hosts of house mouse mites, building occupants with mouse infestations are

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potentially exposed to house mouse mites. The Trenton site had the highest abundance of house mouse mites, with 79% of the trapped mice infested and a mean density of 8.5 ± 1.2 mites per host. Further monitoring human pathogens from mites associated with house mice is essential for predicting potential human health risks due to mouse infestations.

Echinonyssus butantanensis is a blood-sucking mite of house mice and Norway rats (*R. norvegicus*)[45], but it has also been reported on Eastern chipmunks (*Tamias striatus* (L.)).[54] It was previously described under the genus *Hirstionyssus* Fonseca but was transferred to *Echinonyssus* Hirst in 1979.[55] In the continental U.S., *E. butantanensis* has been reported in Indiana[56], Massachusetts[54] and Iowa (University of Michigan Library Digital Collections. https://quod.lib.umich.edu/i/insect2ic/x-ummzi-232888/1) and as its junior synonym, *Hirstionyssus latiscutatus* (deMeillon & Lavoipierre) from Massachusetts, Mississippi, Missouri, Oregon, Texas, and Utah.[45] Out of the continental U.S., *E. butantanensis* was also documented in Hawaii in the 1980s.[57,58] It was found to have low density (1 mite per host) in previous studies. In the current study, *E. butantanensis* was found with a mean density of 4.5 ± 0.9 and a prevalence of 35% at the Farm site. To our knowledge, this study is the first record of *E. butantanensis* in New Jersey. *Echinonyssus butantanensis* is not currently considered a vector for any human pathogens.[22,59]

Myocoptes musculinus is a common ectoparasite on laboratory-maintained mice as well as free-ranging mice.[60] It stays at the base of the host's hair, feeding on skin debris and tissue fluid, and can be transmitted between hosts by direct contact. A survey in a pet store found *M. musculinus* present in 93.3% of the cage-reared mice.[18] In contrast, Smith[20] trapped house mice from different types of buildings, such as farm buildings and apartment buildings in Mississippi. He found that 37% of trapped mice were infested with *M. musculinus,* with a mean density of 5.9 mites per mouse. Whitaker[21] reported a 4–7% prevalence of *M. musculinus* in feral house mice, although there is no detailed description of the collection sites. In the current study, *M. musculinus* showed a 6% prevalence with a mean density of 2.4 ± 0.7 per mouse at the New Brunswick site. However, it was found on 86% of the mice with a mean density of 190.2 ± 23.3 per mouse at the Farm site. In this study, 113 mice were caught at the Farm site within 20 days, while 84 mice were caught at the New Brunswick site in three months. The large number of mice trapped in the farm site in a short period suggests that the Farm site has a higher density of mice than the New Brunswick site. The high density of mice would facilitate the direct transmission of *M. musculinus* and explain the differences in *M. musculinus* density between sites.

Myobia murismusculi is a host-specific mite of house mice. *Myobia murismusculi* is also known as myobiid fur mite, which grasps tightly on the hair

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shafts and skin surface of the hosts and is easily overlooked when examined.[61] It feeds on the host's tissue fluid but does not feed on their blood. Smith[20] documented an average number of 4.4 *M. murismusculi* and a 45.4% infestation rate on trapped house mice; Clark[47] reported that *M. murismusculi* had a 36% infestation rate among feral house mice. Compared to previous studies, *M. murismusculi* was found at a higher density on house mice in the current survey, except for the Paterson site. Although *M. murismusculi* has not been reported as a human disease vector, it is associated with atopic dermatitis, amyloidosis, inflammatory responses, immune system alterations in mice, and possibly facilitating other arthropod infestation.[62-65]

Live traps and snap traps are commonly used devices for ectoparasite surveys on small mammals. Live traps are more costly, bulky, and take more time to set up. In contrast, snap traps are cheaper and easier to carry and set up. The disadvantage is that ectoparasites could leave the hosts shortly after the host dies.[30] However, we did not find a lower percentage of mite infestation on snap-trapped mice than on live-trapped mice (Table 1). In addition, snap traps exhibited better effectiveness in capturing small mammals than live traps in previous surveys.[66,67] Therefore, snap traps can also be used to survey ectoparasites of small mammals in the future. However, it remains unclear whether snap-trapped mice are useful for surveying fleas and other ectoparasites.

The molecular identification of mites using tools such as *COI* barcoding can complement and provide an alternative to morphological identification. The morphological identification of mites is a laborious process that requires a tricky slide preparation step and an expert acarologist to determine the species. In this study, we reported the first partial *COI* sequence for the two blood-sucking mites identified in this study, *E. butantanensis* and the house mouse mite, that can facilitate mite species identification in subsequent studies. The phylogenetic tree is well supported by the values for each node. Both mite genera are classified in the same family, Dermanyssidae, and grouped together with other mesostigmata mites. Remarkably, our *COI* sequence for the house mouse mite is the only sequence available for the species and for the genus *Liponyssoides*.

Considering the prevalence (30–79%) and abundance (2.6–8.5 per mouse) of the house mouse mite in apartment buildings, as well as the high rate of mice infestations found in buildings in some communities, the incidence of close contact and accidental bites from mites will likely be high.[68] The tropical rat mite, *Ornithonyssus bacoti* (Hirst), is often found in structures and is an ectoparasite of house mice and rats.[22,68,69] However, it was not found in this study. This may be because tropical rat mite prefers feeding on rats, particularly roof rats.[22] These results reveal a strong need for adopting more effective house mouse monitoring and management programs to mitigate the

health risks from house mouse infestations.

5 Conclusion

Four mite species were identified from house mice trapped in apartment buildings and a chicken farm building over a one-year period. Among four species, *E. butantanensis* and the house mouse mite were the only blood-sucking mites and were the first records in New Jersey. Additionally, the relative abundance of the mite species from the three apartment sites was different from the Farm site. The abundance of house mouse mites in apartment buildings indicates potential human health risks from ectoparasites associated with house mouse infestations. We also developed genetic barcodes of *E. butantanensis* and the house mouse mite to facilitate future identification of blood-sucking mites on mice. This study highlights the need for periodic monitoring of house mice infestations and adopting more effective house mouse management programs. Further investigations of the associated zoonotic pathogens will be valuable to public health workers for making informed decisions to prevent disease epidemics resulting from rodent infestations in urban environments.

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Conflict of Interest Declaration

The authors declare no competing financial interest.

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Figure legends

Figure 1. New Jersey county boundary map showing the locations of the four sampling sites.

Figure 2. Pictures of the four species of mite collected from house mice. (A) female *Myocoptes musculinus* with hook-like third and fourth pair of legs, (B) male *Myocoptes musculinus* with hook-like third pair of legs, (C) *Myobia murismusculi* with modified first pair of legs and the body bulges between the legs, (D) *Echinonyssus butantanensis* with jug-shaped epigynial plate (dotted frame) and three setae on each side (see arrows), (E) *Liponyssoides sanguineus* with a pair of long chelicerae (see arrows).

Figure 3. Neighbor-Joining phylogenetic tree showing the evolutionary relations of *E. butantanensis* and *L. sanguineus*. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1500 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method.

Figure 4. Mean density and prevalence of each mite species carried by house mice from four study sites. Mean density is the number of mites carried by each mouse per species. Prevalence is described as the percentage of mice carrying that mite species. For *M murismusculi* and *L. sanguineus*, numbers followed by different letters indicated significant differences in mean density or prevalence within each species among sites (Kruskal-Wallis with the Dunn's test, $p < 0.05$). The Paterson site was excluded from Dunn's test due to small sample size. For *M. musculinus*, numbers followed by different letters were significantly different between the New Brunswick and Farm sites (Welch's two-sample *t*-test, $p < 0.05$).

Graphical Abstract Text:

Ectoparasites were examined from house mice collected from apartment buildings and a farm in urban cities in New Jersey. *Myocoptes musculinus*, *Myobia murismusculi*, *Echinonyssus butantanensis*, and *Liponyssoides sanguineus* were found with different density and prevalence among sites.

Table 1. Diversity and prevalence of mites found on house mice caught by two types of traps from four study sites in New Jersey, USA.

Site	Sherman traps					
	Units with mice*	Number of mice	Mite infestation (%)	Species richness (Mean ± SE)**	Units with mice*	Numb of mi
Paterson	4	6	33	1.0 ± 0.0 a	8	17
New Brunswick	14	20	25	1.0 ± 0.0 a	29	64
Trenton	21	32	66	1.1 ± 0.1 a	27	50
Farm	1	56	84	2.2 ± 0.1 b	1	57
Statistics			$\chi^2 = 25.78$, df = 3, $p < 0.001$	F (3, 71) = 15.72, $p < 0.001$		

* The units could be apartments or other common areas including crawl spaces, boiler rooms, compactor rooms, and staff offices.

** Numbers within the same column followed by different letters are significantly different (ANOVA with Tukey's test, $p < 0.05$).

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Table 2. Relative abundance of four mite species on house mice

Species	Apartment sites (total = 1,535 mites)*	the Farm site (total = 19,165 mites)*
Myocoptes musculinus	0.7%	96.2%
Myobia murismusculi	55.8%	2.8%
Echinonyssus butantanensis	0%	0.9%
Liponyssoides sanguineus	43.4%	0%

* Two mites from apartment sites and three mites from the Farm site were not identified and excluded from the total abundance.

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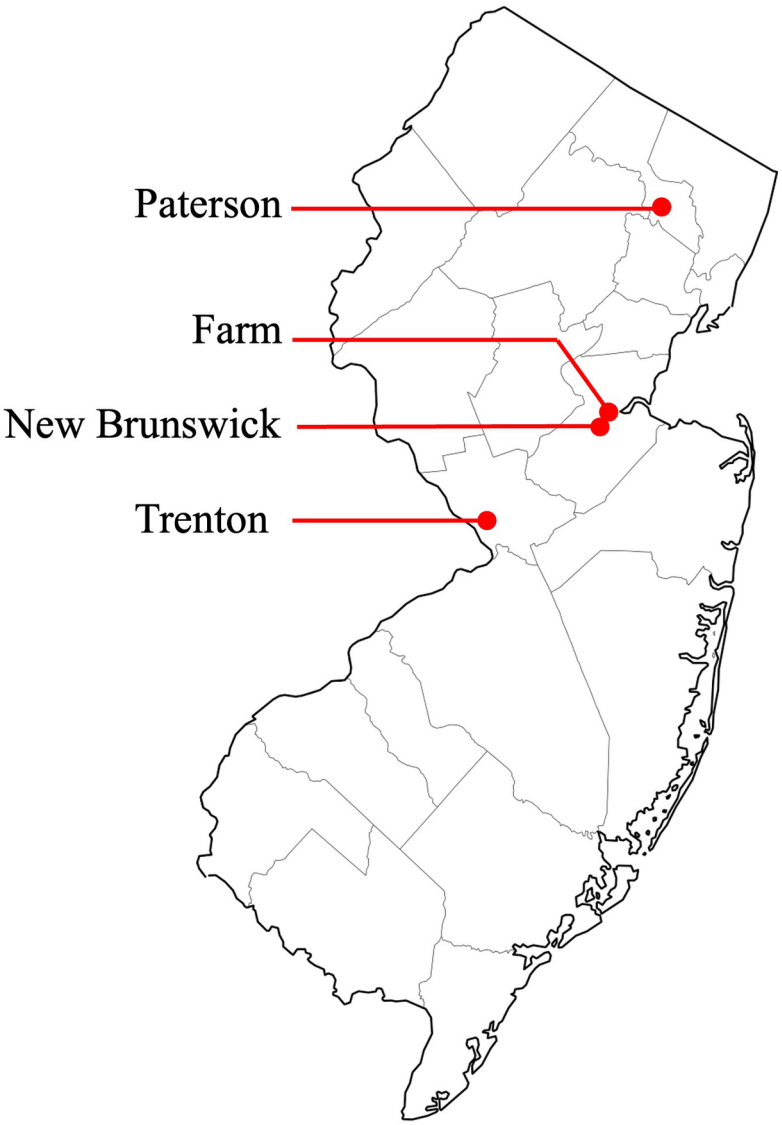
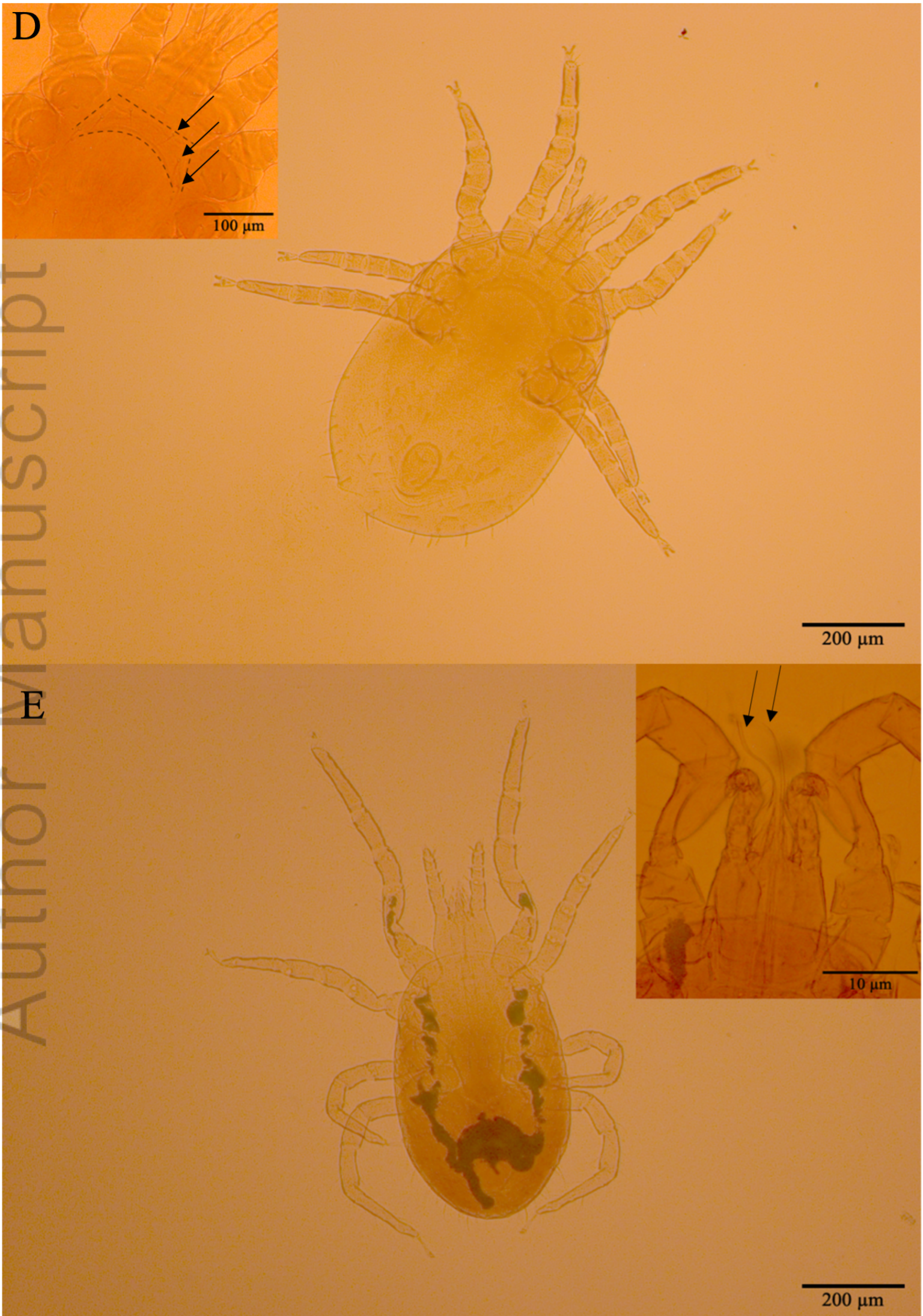


Figure 1.tif



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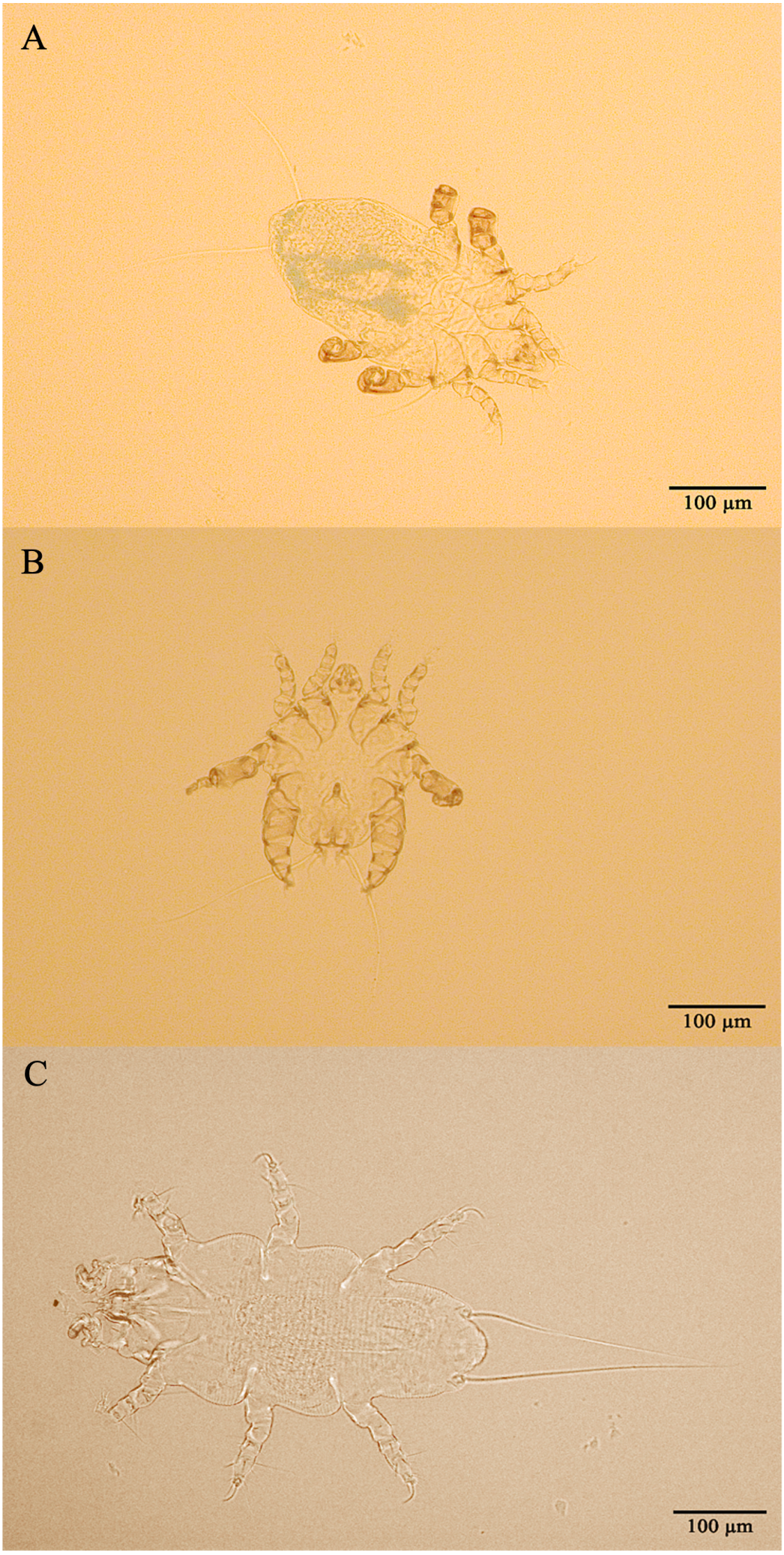


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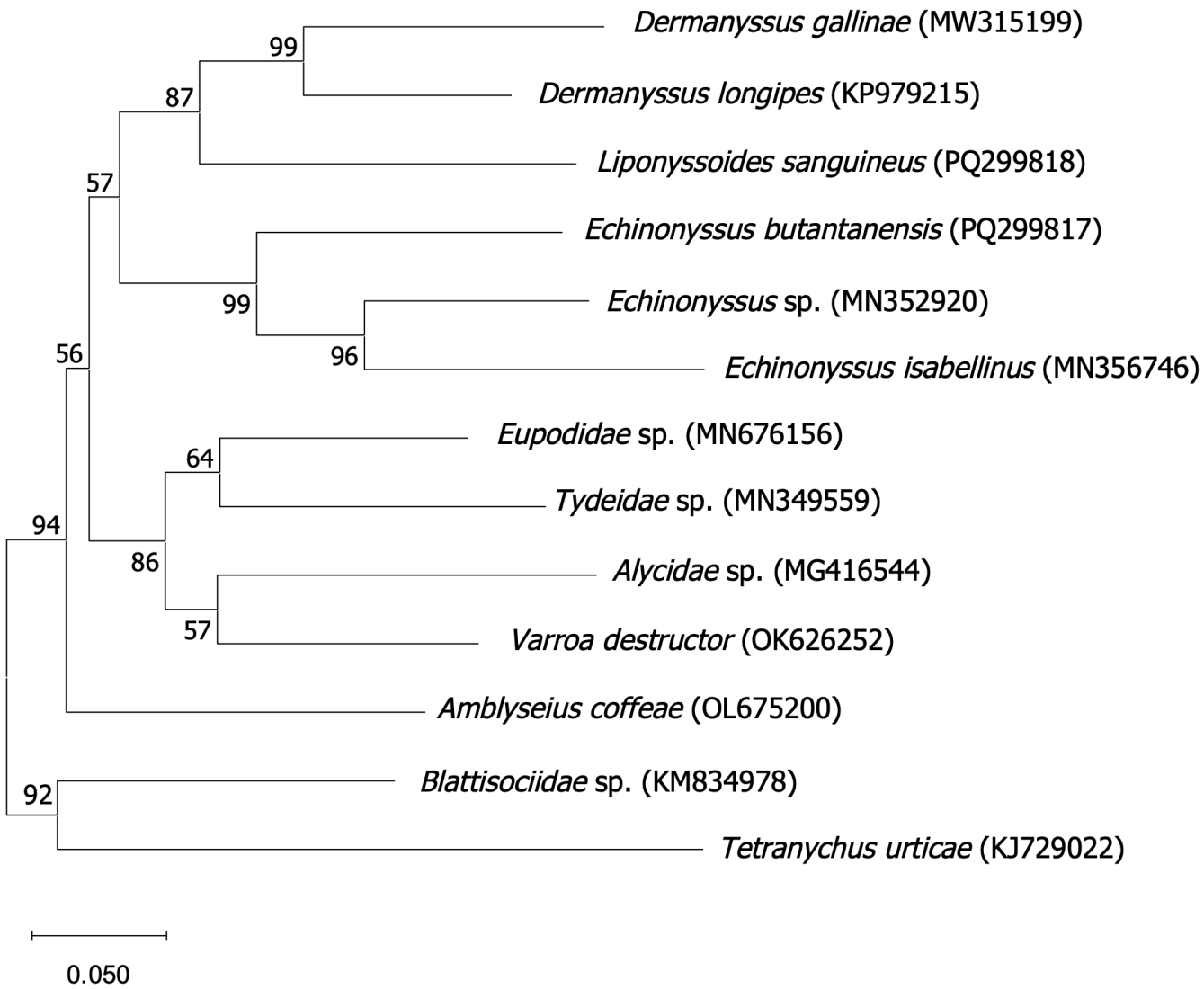


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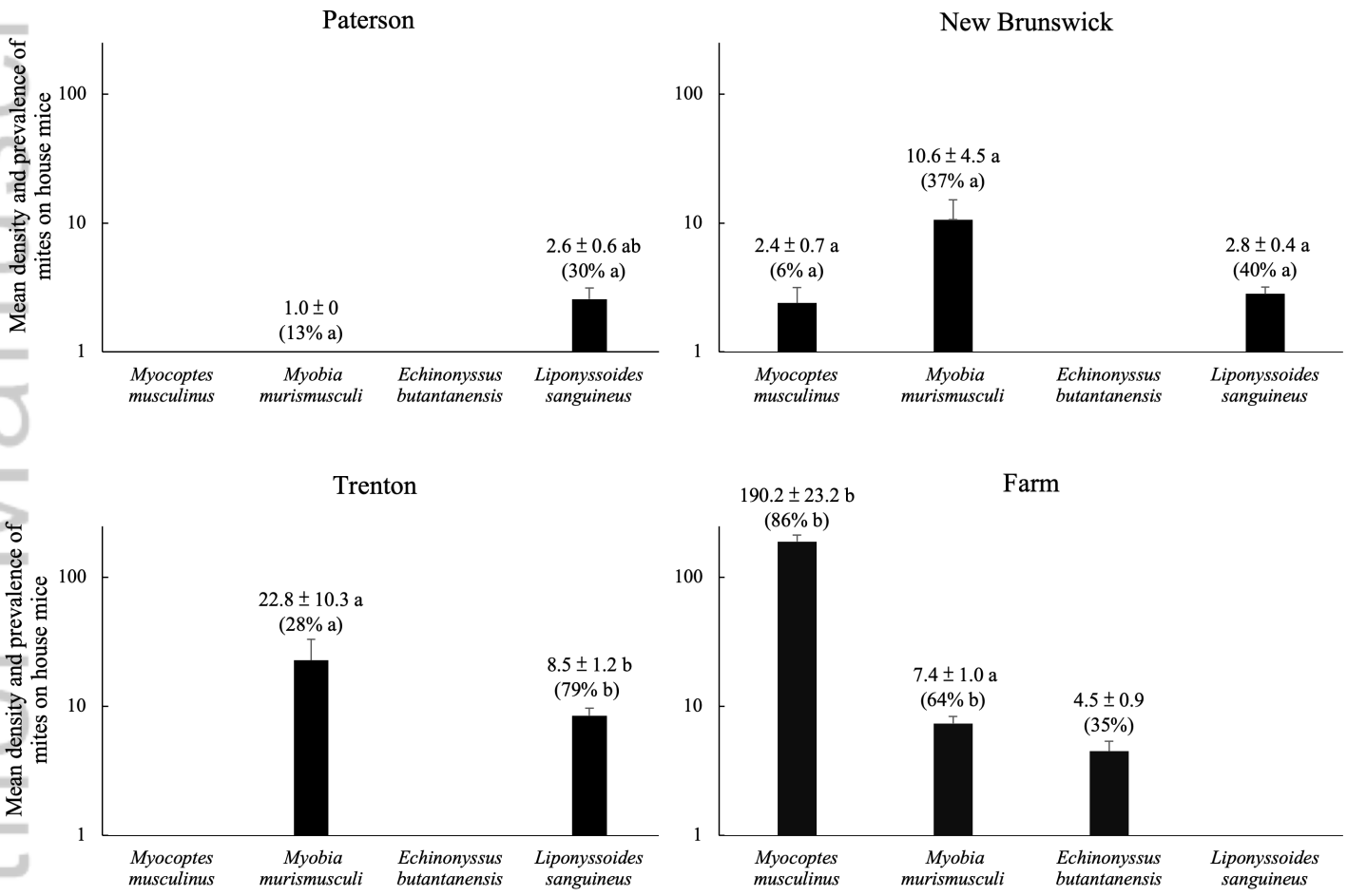
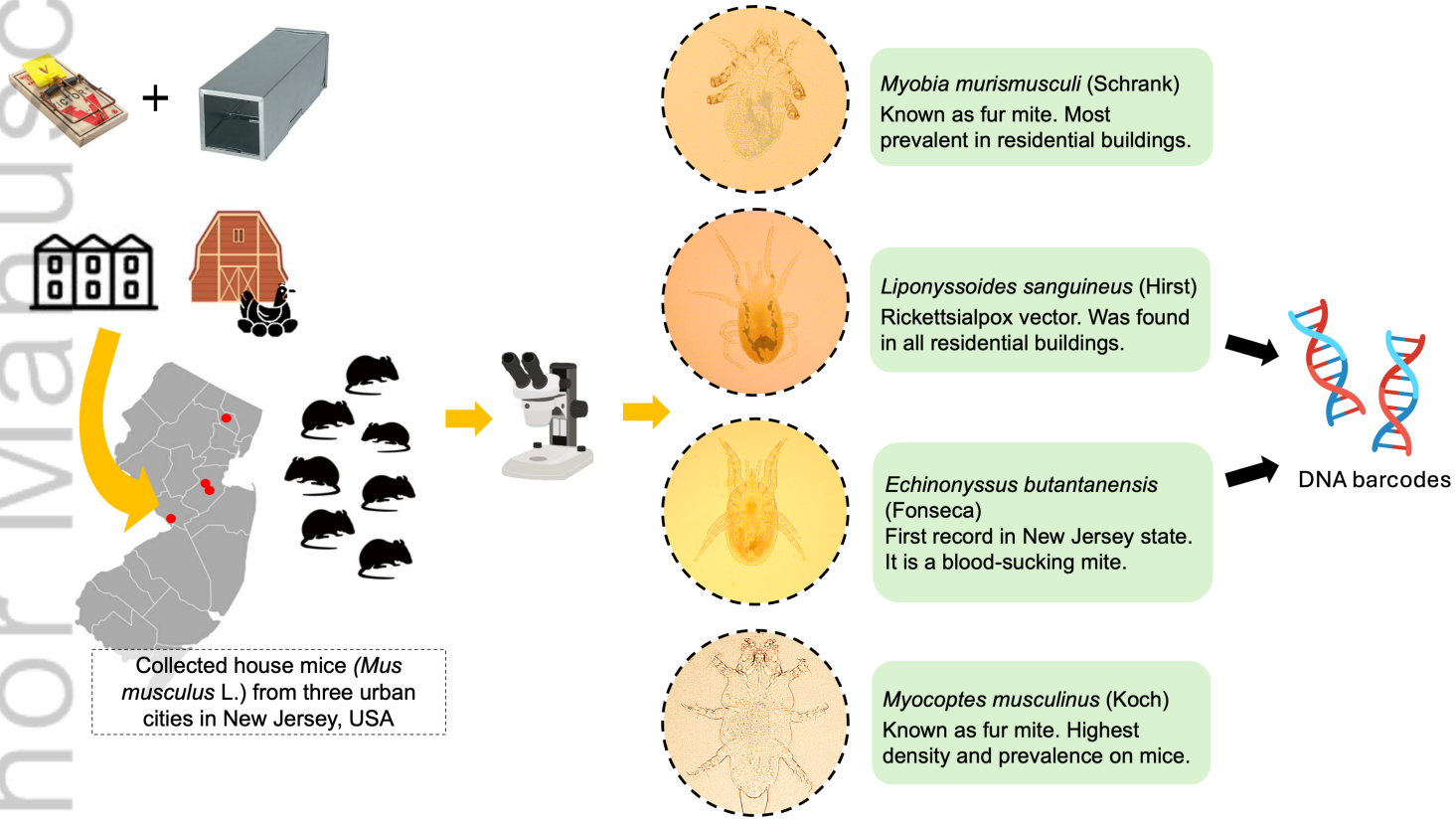


Figure 4-R1.tif

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