The diagnosis and vector potential of Ornithonyssus bacoti tropical rat mites in northern Europe.

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Abstract

The mesostigmatid tropical rat mite, *Ornithonyssus bacoti*, is an important cause of disease in small rodents, and of gamasoidosis in humans when they come into contact with infestations. Most reports of *O. bacoti* infestations are from warmer parts of the Americas, southern Europe and Asia; and infection has only rarely been recorded in northern Europe. In 2021 and 2024, two separate cases of gamasoidosis were identified in student flats in the city of Edinburgh, UK. Further investigation highlighted the value of combining conventional morphological and 16S ribosomal DNA sequencing methods in establishing the species identity of the mites; hence confirming the diagnosis of gamasoidosis. The bacterial microbiome associated with the mites was explored by conventional culture and metabarcoding microbiome sequencing of the ribosomal16S v3-v4 hypervariable region. The results highlight the utility of the mixed approach; and show the presence of potentially pathogenic bacteria and recognised causes of opportunistic nosocomial infections, along with known mite gut and intracellular symbionts. The results indicate the potential for *O. bacoti* mites as vectors of bacterial infections. The clinical presentation of gamasoidosis is indistinguishable from non-specific arthropod-bite reactions; and the cause is seldom confirmed because the temporarily parasitic mites spend most of their time in the environment. The two confirmed index cases may, therefore, represent a more widespread emerging problem; putatively associated with an increase in urban rodent populations.

Keywords: *Ornithonyssus bacoti*; tropical rat mite; gamasoidosis; hamster; morphological identification; next generation molecular speciation

1. Introduction

Gamasid mites (sub-order Mesostigmata) are morphologically delineated by the presence of stigmata above the coxae of the second, third, or fourth pairs of legs. The genera *Ornithonyssus* Sambon, 1928 (family Macronyssidae) and *Dermanyssus* Dugès, 1834 (family Dermanyssidae) are of veterinary and public health importance worldwide. *Dermanyssus gallinae* (poultry and pigeon red mites), *Ornithonyssus sylviarum* (northern fowl mites) and *Ornithonyssus bursa* (tropical fowl mites) are primarily parasites of birds; whereas *Ornithonyssus bacoti* (tropical rat mites) are primarily parasites of small rodents. Nymph and adult mite stages of both sexes are haematophagous, usually feeding at night and spending most of their life history in their hosts' nests, or in nearby dark crevices or detritus. Adult females normally take a single blood meal every two to three days, before egg laying in the environment. The life cycle can be completed in as little as one week, and mite populations can expand rapidly (Sparagano et al., 2014). *D. gallinae*, *O. sylviarum* and *O. bursa* infestations are a major cause of production loss in commercial poultry farming worldwide (Sparagano et al., 2009). *O. bacoti* infestations are a nuisance in laboratory colonies (French, 1987) and in pet rodents (Creel et al., 2003).

Adult gamasid mites can survive for several months without feeding, allowing populations to persist in the absence of preferred hosts. Populations of adult mites that have not fed for several weeks can seek and infest alternative hosts, including humans (Fuentes et al., 2009). Zoonotic transmission may also occur opportunistically following human skin contact with unfed mites. Gamasoidosis is an important occupational disease in poultry workers (Cafiero et al., 2011) and in laboratory rodent facilities (Fox, 1982; Tika-Ram et al., 1986; Cole et al., 2005). Urban gamasoidosis occurs where wild birds nest in close proximity to human homes and workplaces (Bassini-Silva et al., 2019; Mesquita-Sousa et al., 2020; Sargison et al., 2020); in association with rodent population expansion (Alsoubani et al., 2023); or through close contact with pet rodents (Beck, 2008).

Feeding on often different hosts every two to three days creates an opportunity for gamasid mites to be vectors of pathogens (Kowalski and Sokol, 2009; Sparagano et al., 2014). *D. gallinae* can acquire and transmit bacterial (Cocciolo et al., 2020) and viral (Sommer et al., 2016) pathogens within poultry flocks. Studies have shown that mites can acquire *Salmonella* spp. by feeding on contaminated blood, or by cuticular contact between the mite and the bacterium, and that birds can then acquire this bacterium from ingesting contaminated *D. gallinae* (Valiente Moro et al., 2007; 2009). The possibility that *O. bacoti* mites might act as vectors of zoonotic pathogens has been implied (Reeves et al., 2007; Bradley et al., 2014). The potential for zoonotic transmission of endemic typhus, *Bartonella* spp., Q fever, tularemia, epidemic hemorrhagic fever, coxsackievirus, *Yersinia pestis*, and rickettsial pox has been described (Alsoubani et al., 2023).

Providing empirical support for vector transmission of zoonotic pathogens is challenging, due to issues of mite containment and cross-contamination in traditional field-type or *in vivo* studies. Nevertheless, the demonstration of pathogens in association with mites can inform research questions concerning their role as vectors. The development of an Illumina MiSeq method to re-sequence bacterial 16S DNA derived from mites (Ponnusamy et al., 2018; Guo et al., 2020) provides an opportunity to identify pathogenic bacteria that might be transmitted by gamasid mites. This report describes the identification of potentially pathogenic bacteria in macerated *O. bacoti* extracts, in the context of the prolonged healing of gamasoidosis lesions.

This report describes the occurrence of *O. bacoti* mites in two separate Edinburgh flats, in association with gamasoidosis lesions in the occupants. Few reports implying links

between *O. bacoti* infestations and zoonotic disease transmission are supported by accurate speciation of the gamasid mites involved. Nevertheless, accurate identification of gamasid mites is necessary to identify the source of the infestation, and instigate targeted preventive management. The first objective of the work was, therefore, to provide robust morphological and molecular confirmation of the species identity of *O. bacoti* mites. Gamasid mites tend to feed at night when they are unnoticed; hence are seldom identified on the body in direct association with gamasoidosis lesions. The second objective of the work was, therefore, to describe the bacteria associated with the mites in order to explain why *O. bacoti* mites were the most probable aetiological agents of human arthropod bite-like pruritic excoriated papules and urticaria lesions.

2. Materials and Methods

2.1. Problem history

Case 1: During autumn 2021, three residents of an old student flat in Edinburgh developed multiple 1 to 2 mm diameter, erythematous papules and urticaria on their upper arms and legs (Figure 1A,B,C). The lesions took several weeks to heal. *Cimex hemipterus* bed bugs were at first suspected, but were not found. No other ectopartasites were found on the bodies of the affected residents. Small white arthropods, about 1mm long and barely visible to the naked eye, were identified by their movement on the kitchen floor, and were collected using clear adhesive tape. Microscopic examination confirmed their identity as mesostigmatid (gamasid) mites; supporting a provisional diagnosis of gamasoidosis as the cause of the human skin lesions. The block of flats had a history of rodent infestation, which had recently been controlled.

Case 2: In Spring 2024, the resident of a modern student flat in Edinburgh first developed similar pruritic papular lesions on her arms (Figure 1D). No ectoparasites were found on the body of the affected resident. The lesions developed shortly after changing the resident's pet hamster's bedding. About 3 weeks later, several similar small white arthropods were identified for the first time by their movement on the coat of her pet hamster (Figure 1E). These were collected using clear adhesive tape, and microscopic examination confirmed their identity as gamasid mites; supporting a provisional diagnosis of gamasoidosis as the cause of the human skin lesions. Closer inspection of the hamster's wood shavings bedding revealed numerous dark spots, presumed to be engorged mites. The resident and her hamster had lived in the same flat for the previous 8 months; and no other residents of the same building were affected. All wooden materials in the hamster's cage, along with its bedding were removed, and the hamster was treated with a spot on formulation containing 25 µg ivermectin (Anti-Parasite Spot On; Beaphar). The human lesions resolved quickly after treatment of the hamster, and re-infection did not occur.

2.2. Morphological mite identification

Adult mites were soaked in 10% sodium hydroxide for two days to allow unimpeded observation of cuticular structures at different planes of microscopic focus. They were then mounted directly and examined by stereomicroscopy. Species identification was based on published morphological identification keys and descriptors (Pratt, 1963; Krantz and Walter, 2009; Nieri-Bastos et al., 2011; Di Palma et al., 2012).

2.3. Molecular mite speciation

DNA was extracted from individual whole mites (separate from those used for morphological speciation) and a ~ 460 bp ribosomal16S fragment was amplified. Published primers designed for Metastriata tick species (Mangold et al., 1998) that had been tested on *Ornithonyssus* spp. (Nieri-Bastos, 2011) and previously described conditions (Sargison et al., 2020) were used. DNA templates for direct sequencing of the 16S rDNA fragment of five individual mites were cleaned using a QIAquick PCR Purification Kit (Qiagen) following the manufacturers' protocols. Amplicons were Sanger sequenced using the same primers used for PCR amplification (the forward primer was used for *Case 1* and the reverse primer for *Case 2*). The resulting rDNA 16s sequences were aligned to NCBI GenBank through the Basic Local Alignment Search Tool (BLAST) to confirm the species identities. All the available sequences of that species were downloaded from the GenBank and were used for comparison through alignment to the field sequences and phylogenetic analysis. The field sequences of 16S rDNA were edited to remove primers and poor-quality sequence on both ends using Geneious Prime (Kearse et al., 2012). Geneious Prime was also used for their alignment and the construction of phylogenetic tree, employing the Tamura-Nei genetic distance model (Tamura and Nei, 1993) and the neighbour-joining method (Saitou and Nei, 1987). To ensure the robustness of the tree nodes, bootstrapping was performed with 1,000 replicates and a 50% threshold was used for the branches.

2.4. Bacteriological investigation

The possibility that the mites might harbour bacterial infections, and that this might account for the inflammation and slow healing associated with the human lesions was investigated for *case 1*. Mites were macerated and disrupted in sterile water before extracts were used for bacterial culture and microbiome sequencing.

2.4.1. Bacterial culture

Four mite preparations were plated onto Columbia Blood Agar with 5% (v/v) sheep blood, and incubated at 37°C, statically, overnight. Samples from 12 colonies, representing four morphotypes (designated as *White*, *Black*, *Grey* and *Yellow*) were collected using a sterile loop and resuspended in 1x phosphate buffered saline (PBS). These were plated again onto Columbia Blood Agar and incubated at 37°C, statically, overnight to purify the individual colony growth. The purified bacterial colonies were resuspended in saline as templates for molecular speciation.

Classical microbiological assays were used on selected colonies, which were subjected to Gram staining, catalase and oxidase tests. Catalase tests were performed by suspending a single colony in 3% (v/v) hydrogen peroxide, and confirmed by the presence of effervescence. Oxidase tests were performed using Oxidase test disks (Sigma) following the manufacturer’s instructions.

2.4.2. Microbiome sequencing

DNA was extracted from 12 macerated individual mites and three each of the *Black*, *White*, *Yellow*, and *Grey* bacterial colony samples. Templates were incubated at 60°C for two hours in a lysis solution of 5% 1M dithiothreitol (DDT) and 5% proteinase K in DirectPCR Lysis Reagent (Viagen Biotech) followed by 15 minutes at 85°C to inactivate the proteinase K. The lysates were stored at -20°C until further use.

Previously published primers (Klindworth et al., 2013), that have been developed and used to describe mite microbiomes (Ponnusamy et al., 2018), were used to amplify a ~460 bp fragment of 16S v3-v4 hypervariable region of bacterial DNA. The PCR reaction conditions were as previously described (Sargison et al., 2022). The thermocycling parameters were: an initial denaturation step for 2 min at 95°C, followed by 35 cycles of denaturation for 20 sec at 98°C, primer annealing for 15 sec at 55°C and primer extension for 15 sec at 72°C; and a final extension for 2 min at 72°C.

PCR products were purified using AMPure XP magnetic beads (Beckman Coulter) according to the manufacturer's protocol. A second round PCR was performed to add unique barcode combinations to each sample using a previously described method (Rehman et al., 2020). Finally, the samples were pooled and purified using a Qiagen gel extraction and purification kit, followed by further purification through AMPure XP magnetic beads. 20 µl of the pooled sample was included in an Illumina MiSeq run.

The FASTQ files obtained from the post-run Illumina MiSeq processing, representing full-length sequences present in each index-recognised sample, were analysed following the adapted Illumina MiSeq SOPs in Mothur v1.39.5 (Schloss et al., 2009). The steps involved joining paired forward and reverse reads and screening sequences shorter than 200 bp (to allow for deletions in some of the sequences), longer than 465 bp, or with any ambiguous bases. An *Escherichia coli* 16S gene was downloaded and sequence reads on either side of the primer binding sites were trimmed. The filtered sequences were aligned to a bespoke v3-v4 hypervariable region reference sequence library containing 12,884 bacterial reference sequences. The sequences were then classified into different species/groups according to the taxonomy file of the reference library using the k-Nearest Neighbor algorithm (knn) method, as previously described (Sargison et al., 2022). Finally, a summary file was created, showing a total of ~900,000 aligned sequences (~40,000 reads per sample) belonging to different bacteria species, or group in each sample. Any species that had less than 1,000 reads (~2.5% of the total reads per sample) was removed to negate the effects of contamination or index hopping during the sequencing process. A total of 822,053 reads (average 35,153; range 13,248 – 53,460 reads per sample) were used to calculate the proportional abundances of the bacterial species that were identified.

3. Results

3.1. Morphological mite speciation

Stereomicroscopic examination of each of the six mites examined from both *Case 1* and *Case 2* confirmed that the mites were mesostigmatid, with respiratory openings (stigmata) above the coxae of the third pairs of legs (Figure 2A). They were identified as *Ornithonyssus* spp. on the basis of: elongated chelicerae (Figure 2B); a single large posteriorly tapering, dorsal sclerotised shield; a ventral shield narrowed posteriorly (Figure 2D); and a distinct anal shield with a cranially positioned anus (Figure 2E). The relatively narrow shape of the sternal shield, containing just two pairs of setae (Figure 2C) and the

position of the setae on the anal shield (Figure 2E) supported the species identity of *O. bacoti* (Nieri-Bastos et al., 2011).

3.2. Molecular mite speciation

The 16S rDNA fragment was successfully amplified from three mites derived from *Case 1* and one mite derived from *Case 2*. The sequenced DNA fragments were aligned with seven sequences of 16S rDNA of *Ornithonyssus* spp. deposited in the NCBI GenBank database (Figure 3). The *Ornithonyssus* spp. 16S rDNA sequences from GenBank comprised of seven unique haplotypes. The sequences derived from the Edinburgh flats were identical and comparison with the previously published *O. bacoti* revealed that they formed a distinct haplotype, along with a GenBank sequence derived from a French National Museum of Natural History laboratory strain (Roy et al., 2009).

3.3. Bacteriology

3.3.1. Bacterial cultures

Four distinct colony morphotypes were identified in the mite preparations that were cultured on Columbia agar plates containing 5% sheep blood: large, white, β-haemolytic opaque colonies (denoted as *White*); non-haemolytic black colonies (denoted as *Black*); α-haemolytic, punctiform, grey colonies (denoted as *Grey*); and very small, abundant, yellow colonies (denoted as *Yellow*) (Figure 4). As secondary validation, two colonies (*White* and *Black*) were selected for further examination. Gram staining and catalase testing indicated that the β-haemolytic *White* colonies were Gram positive, catalase positive bacteria, aligning with *Staphylococcus* spp.. The *Black* colonies showed Gram-negative rods that were oxidase positive, aligning with bacteria such as *Pseudomonas* spp. or *Pasteruella* spp..

3.3.2. Bacterial culture re-sequencing

A mean of 35,114 (range 13,284 – 53,460) sequence reads was generated from 11 individual colonies (Table 1). The sequences derived from one of the *Black* colonies aligned with *Delftia* spp; and those of the other aligned with *Pseudomonas* spp.. Most of the sequences derived from each of the *White* colonies aligned with *Staphylococcus* spp.. All of the sequences derived from each of the *Grey* colonies aligned with *Delftia* spp.. Most of the sequences derived from two of the *Yellow* colonies aligned with *Micrococcus* spp., along with *Brevundimonas* spp. and closely related *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* spp.. The sequences derived from the third *Yellow* colony aligned with *Micrococcus* spp., *Staphylococcus* spp. and *Delftia* spp.; implying that the *Yellow* colonies were mixed (Figure 5A).

3.3.3. Microbiome re-sequencing of DNA extracted from mites

A mean of 35,190 (range 15,905 – 43,374) filtered sequence reads was generated from each of 12 macerated whole mites (Table 1). These aligned with 8 bacterial genera (considering the closely related *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* spp. as a single genus complex). *Brevundimonas* was the predominant genus identified in 11 of

the 12 mites (Figure 5B). Less than 1,000 sequence reads aligning with *Corynebacterium* spp., *Streptococcus* spp., *Cutibacterium* spp., and unclassified Burkholderiaceae, Corynebacteriales, Moraxellacea and Alphaproteobacteria, were each present in multiple mite samples.

4. Discussion

The identify of *O. bacoti*, tropical rat mites as the most probable cause of gamasoidosis in residents of two student flats in the city of Edinburgh, Scotland was confirmed using morphological and molecular methods. The parasite has a worldwide distribution, but there are few reports from northern Europe. The earliest example of *O. bacoti* in the UK was found in 1933, believed to have originated from ship rats at a port (*cited by* Fox et al., 2004). *O. bacoti* was first reported in a domestic rodent (pet hamster) in the UK in 2002 following the investigation of arthropod bite-like lesions in family members (Fox et al., 2004). *O. bacoti* was reported in the following year, coincidentally by Edinburgh based vets, in a number of pet shop and pet rats; with the mites also infesting the household dogs and the owner (Pizzi et al., 2004). Subsequent reports from the UK are anecdotal. The scarcity of reports may simply reflect inherent difficulties in diagnosing the cause of arthropod bite-like lesions in people; whereby the parasites spend most of their time in the environment and are seldom, if ever, found feeding on the body. In both cases that are described in this report, gamasoidosis occurred in veterinary students who knew how to investigate such problems. Various reports of *O. bacoti* from around the world are supported by images of whole mites. However these are not readily distinguishable from other gamasid mites which can infest mammals, in particular *O. sylviarum* and *O. bursa*. This report, therefore, highlights the importance of detailed morphological speciation, and of the additional value of 16S rDNA sequencing where subtle or overlapping morphological differences exist between species.

Molecular phylogenetic analysis of the partial 16S rDNA gene showed that the mites recovered from Edinburgh, Scotland, in this report were indistinguishable from a French reference laboratory strain; but clearly differed from those originating from North America and Asia. Unfortunately, the small number of GenBank sequences, coupled to the unavoidable need to trim poorly Sanger sequenced regions distant from the primer binding sites limit the utility of the findings. Furthermore, doubt has been cast over the *O. bacoti* species identity of the phylogenetically distant Genbank sequences originating from Georgia, USA (Nieri-Bastos et al., 2011).

The slow lesion healing and appearance of secondary infection in *Case 1* raised suspicions that *O. bacoti* may have been a vector of bacterial pathogens. Vector transmission of *Salmonella* spp. by *D. gallinae* mites on poultry farms has been implied (Valiente Moro et al., 2009; Pugliese et al., 2019) and shown under experimental conditions (Valiente Moro et al., 2007; Cocciolo et al, 2020). The potential for *O. bacoti* mites to act as vectors of disease has been proposed (Hopla, 1951); but the definitive proof of vector transmission depends on animal challenge experiments, which were beyond the scope of this report. Isolation of bacteria from the gamasoidosis lesions was also beyond the scope of this report. Nevertheless, the demonstration of potential bacterial pathogens on the surface of, or within mites is an important first step in the investigation of vector transmission.

In this report, Illumina MiSeq metabarcoding re-sequencing of the bacterial 16S v3-v4 hypervariable region was used to show the bacterial biomes associated with *O. bacoti*

mites recovered from *Case 1*. Variation between sequences in the v3-v4 hypervariable region reference sequence library was insufficient to differentiate between bacterial species belonging to the same genus, or group. Nevertheless, the generation of between 1,009 and 49,635 taxon-specific sequence reads per sample confirmed the identity of nine genera, and one closely related group of bacteria. The presence of a further seven genera, or groups was shown, but could not be confirmed due to the generation of less than 1,000 sequence reads. With hindsight, deeper sequencing of the bacterial biome may have been achieved by first depleting the mite extracts of host nucleic acid (Price et al., 2019).

Classical microbiological culturing techniques are traditionally the most used practice for bacterial speciation. However, bacterial strains that are slower growing, less abundant, anaerobic, or metabolically restricted are typically underrepresented. A study of human patients with pulmonary infections, showed that more bacterial pathogens were positively identified using metagenomic next generation re-sequencing than using bacterial culture methods; highlighting the lack of representation using traditional methods (Huang *et al.,* 2020). In the present study, standard bacteriology mostly aligned with the traditional speciation methods; albeit there were some discrepancies. Individual colony sequencing of the *Yellow* colonies alluded to a mixed culture. Although care was taken in the extraction of colonies, this could be a result of a contaminant, but could also be due to the presence of multiple *Yellow* colony phenotypes on the blood agar plates. Indeed, retrospective analysis of the *Yellow* colonies identified two distinct, phenotypes, possibly explaining the presence of a mixed culture. Interestingly, *Micrococcus* spp. and *Staphylococcus* spp. were identified from single colonies, but not in the microbiome samples. All of the bacterial species, or groups that were identified in the microbiome samples were Gram-negative; whereas *Micrococcus* spp. and *Staphylococcus* spp. are both Gram-positive. Gram-positive pathogens typically require enzymatic lysis to enable sufficient yields of DNA to be detected (Ring et al, 2023), hence their underrepresentation in the microbiome samples could be due to poor yields from the lysis method. The same extraction method was used for sequencing of the colonies and microbiome samples. Nevertheless, the large numbers of bacteria in the colony samples would have allowed for small yields of DNA to be extracted and amplified; while lower representation of these species in microbiome samples would be expected due to their lower abundance. Together, these observations highlight the advantages of using traditional methods of bacterial speciation alongside next generation DNA sequencing, to provide a full picture of the bacterial species present in the study of vector borne diseases.

Most of the bacteria that were identified by metabarcoding Illumina MiSeq resequencing of the hypervariable 16S region of the ribosomal cistron are not considered to be pathogenic. Some of these, may be mite intracellular symbionts, for example, *Arsenophonus* spp. (Nováková et al., 2009), or part of the gut microbiota, for example *Sphingomonas* spp. (Lima-Barbero et al., 2019). Others are common environmental bacteria that might have been present on the surface of the mites, or acquired during feeding. Some of the environmental bacterial species that were identified, for example, *Micrococcus* spp. are found as commensals on the skin, and have been reported to cause skin reactions in immunocompromised people (Nuñez, 2014). Staphylococci cause skin and systemic disease in immune-compromised patients in hospital setting; and a growing number of strains are showing resistance to certain antibiotics. *Stenotrophomonas* spp., *Delftia* spp. and *Pseudomonas* spp. can also cause opportunistic nosocomial infections (Bilgin et al., 2015; Lara-Oya, 2022).

The likely source of the *O. bacoti* mites in *Case 1* arose from a rodent infestation of the older block of flats, which had recently been controlled. Typically, people are bitten when trapping, poisoning, or disease of the normal rodent host means that they are no longer available to the mites (Pratt, 1663; Beck and Fölster-Holst, 2009). There was no known rodent infestation in the modern block of flats in *Case 2*. The hamster had shown no signs of infestation for several months beforehand, and the possibility was considered that the acquisition of *O. bacoti* mites by visiting cats, that may have caught infested rodents, might have contributed to the spread of the parasites (Theis et al., 1980; Beck and Fölster-Holst, 2009). Outbreaks of gamasoidosis have been attributed to such indirect human contact with urban rodents (Baumstark et al., 2007; Alsoubani et al., 2023). It is predicted that urban rodent populations will continue to expand as a result of climate change and anthropomorphic activities (Dammhaln et al., 2020), increasing the spread of *O. bacoti* and risk of disease in humans and domestic pets.

Figures

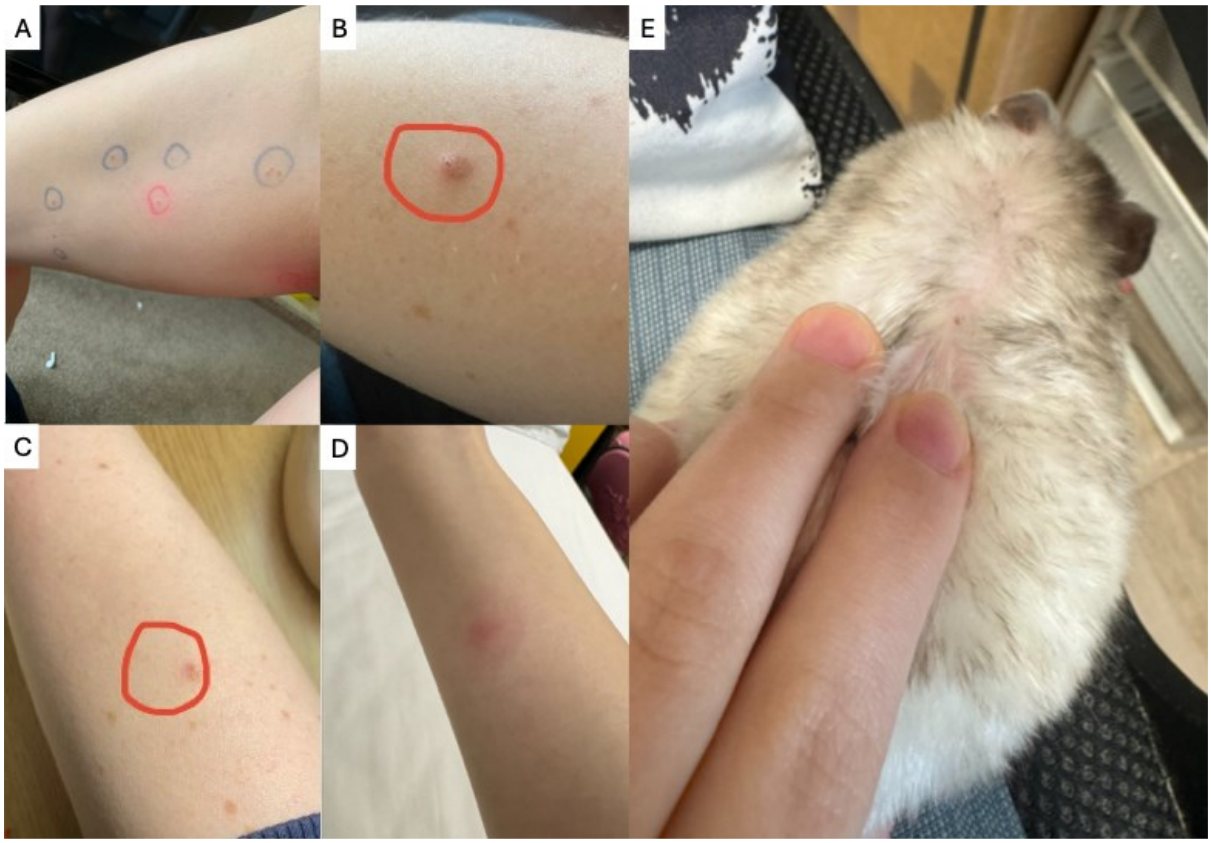

Figure 1. Gamasoidosis lesions: (A) Case 1: erythematous papular lesion on a resident of a student flat in Edinburgh, November 6th, 2021; (B) and (C) Case 1: progression of the same lesion on November 27th, 2021, and January 13th, 2022; (D) Case 2: pruritic papular lesion on a different resident of a different student flat, March 29th, 2024; (E) Case 2: gamasid mites on the hamster's fur, collected with adhesive tape.

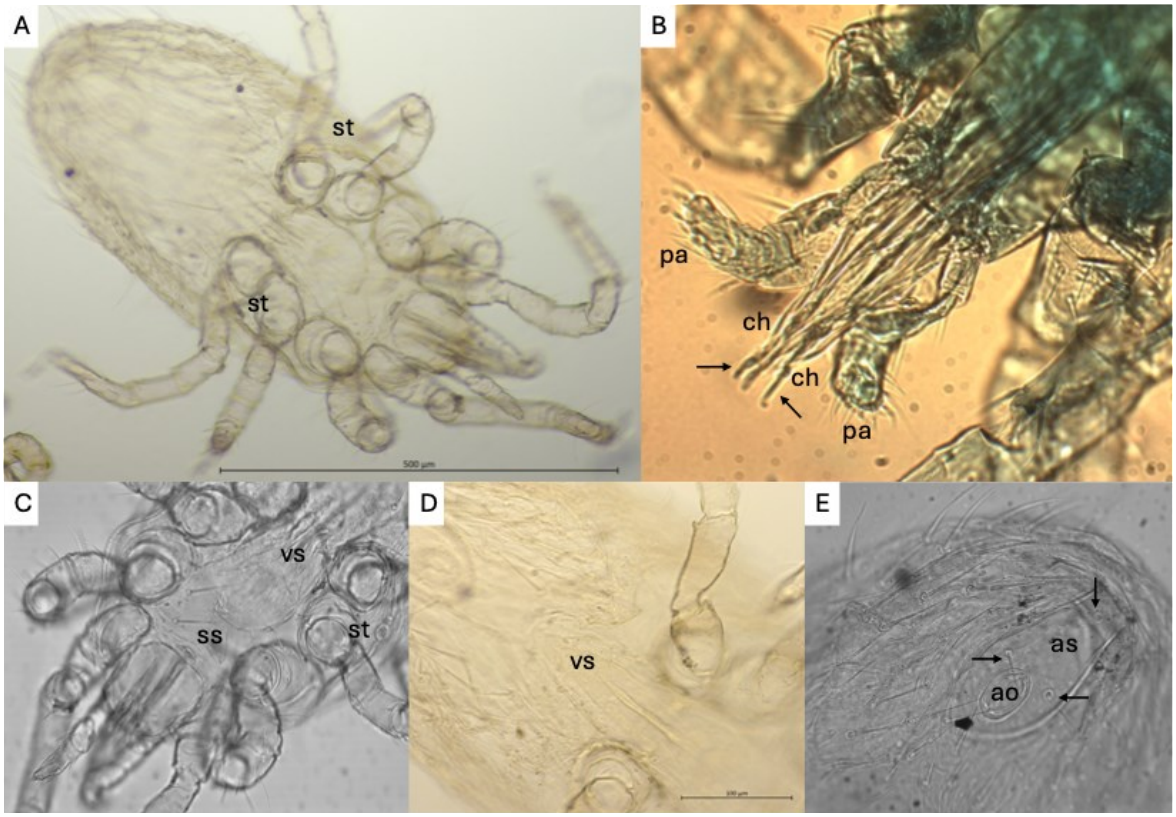

Figure 2. Microscopic images of *Ornithonyssus bacoti* mite collected from *Case 1* (A, C, D, E) and *Case 2* (B). (A) General view of a female mite. (B) Detail of mouthparts, with arrows pointing to movable digits at the tips of the chelicerae. (C) Detail of the sternum and stigmata. (D) Detail of the ventral (genital) shield, narrowing posteriorly. (E) Detail of the opisthosoma, with arrows denoting three anal setae posterior to the anal opening. Abbreviations: ao, anal opening; as, anal shield, pa, pedipalps; ss, sternal shield; st, stigmata; vs, genital shield.

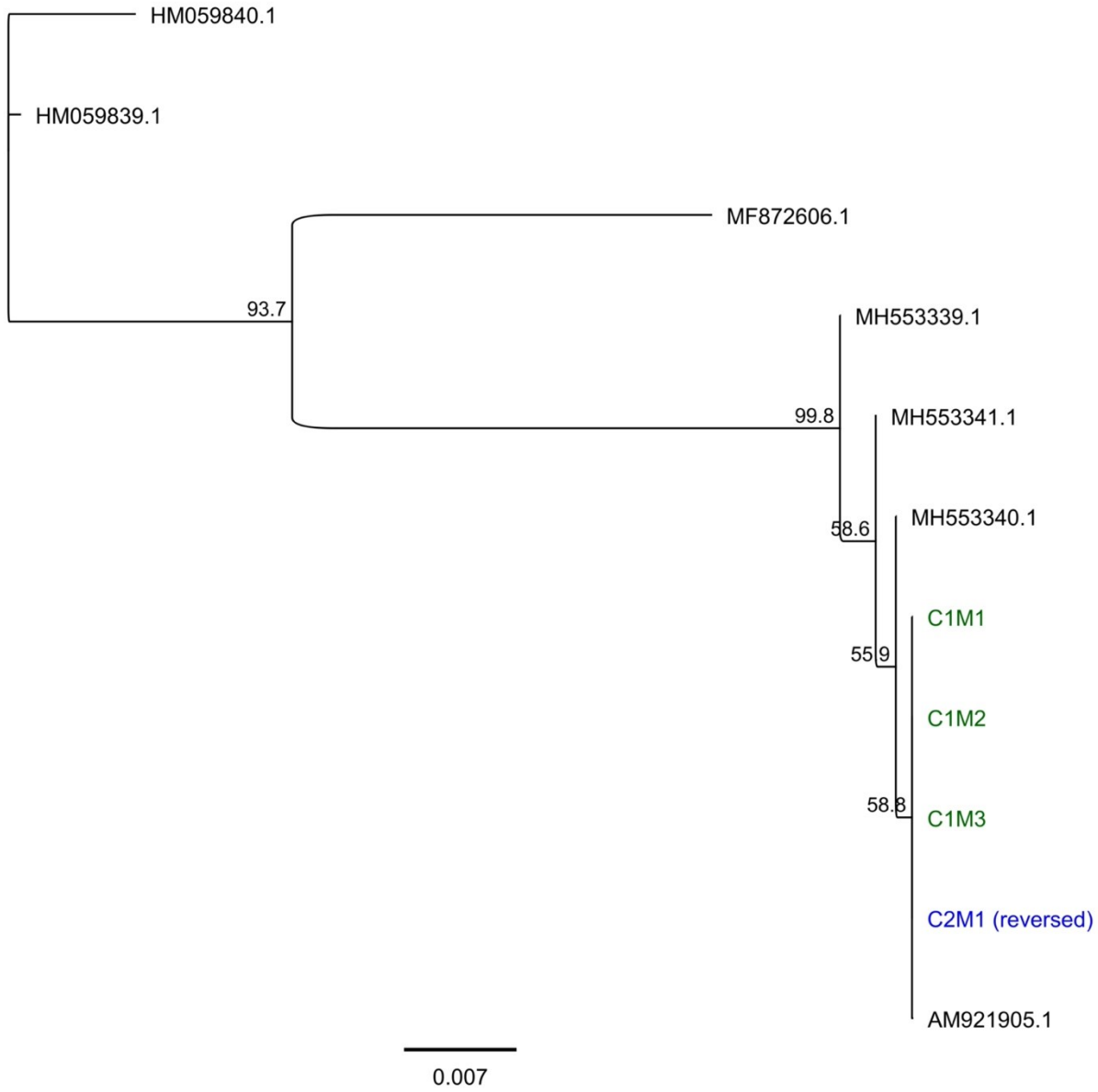

Figure 3. Molecular phylogenetic analysis using a genetic distance model and Neighbour-Joining method of the partial 16S rDNA gene from GenBank sequences of *Ornithonyssus bacoti* and mites recovered from *Case 1* (C1M1, C1M2, C1M3) and *Case 2* (C2M1). The GenBank sequences originated from: Georgia, USA (HM059839.1, HM059840.1); California, USA (MF872606.1); China (MF553339.1, MF553340.1, MF553341.1); Paris museum collection, France (AM921905.1). Poorly sequenced regions distant from the primer binding sites, which were opposite for *Case 1* and *Case 2*, were removed to allow complete sequence alignment.

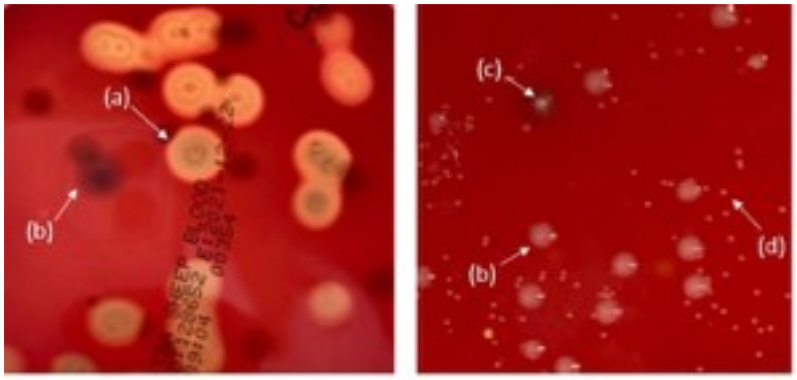

Figure 4. Bacterial colonies cultured on Columbia agar plates containing 5% sheep blood, viewed from below (left) and above (right). Colonies were selected for microbiome sequencing. (a) Beta-haemolytic *White* colonies. (b) Large *Black* opaque colonies. (c) Alpha-haemolytic *Grey* colonies. (d) Small abundant *Yellow* colonies.

Figure 5. Proportions of sequence reads aligned to different bacterial genera derived from Illumina MiSeq resequencing of DNA extracted from: (A) agar plate bacterial colonies; and (B) macerated whole mites.

436 **Table**
437
438 Table 1. Comparison of numbers of unfiltered sequence reads generated from 12 macerated
439 mites and 11 bacterial colony samples.
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Sequence alignment	**Mites**	**Bacterial colonies**
Brevundimonas	205,256	13,069
Delftia	34,481	141,128
Staphylococcus	1,132	154,197
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	80,456	6,895
Micrococcus		71,205
Pseudomonas	36,301	13,284
Sphingomonas	34,994	
Arsenophonus	24,506	
Variovorax	16,100	
Stenotrophomonas	15,734	
Unclassified Corynebacteriales	4,341	
Unclassified bacteria	3,178	
Unclassified unknown	2,430	
*Corynebacterium*_1	2,067	
Streptococcus	1,825	

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