*https://doi.org/10.17576/serangga-2025-3003-21*

**SHORT COMMUNICATION**

# CHALLENGES IN THE MOLECULAR IDENTIFICATION OF SOIL MITES ASSOCIATED WITH MASS MORTALITY EVENTS

**Abby K. Jones[1], Nurul Azmiera[1], Chong Chin Heo[1,3], Van Lun Low[2], Razuin Rahimi[3,4], Jamal Houssaini[1,3] & Farah Roslinda Mohd Rustam[1]***

[1]Department of Medical Microbiology and Parasitology,
Faculty of Medicine,
Universiti Teknologi MARA,
Sungai Buloh Campus, Jalan Hospital,
47000 Sungai Buloh, Selangor, Malaysia
[2]Tropical Infectious Diseases Research & Education Centre (TIDREC),
High Impact Research (HIR) Building,
Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[3]Cardiovascular Advancement and Research Excellence Institute,
Universiti Teknologi MARA,
Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia
[4]Department of Forensic Pathology,
Hospital Al-Sultan Abdullah,
Universiti Teknologi MARA,
42300 Bandar Puncak Alam, Selangor, Malaysia
*Corresponding authors: *farah7757@uitm.edu.my*

Received: 15 March 2025; Accepted: 16 November 2025; Published: 15 December 2025

## ABSTRACT

Forensic acarology is an emerging field of science which utilizes mites in death investigations, particularly in cases where entomological evidence is limited. Mites are found in various locations and can withstand many harsh conditions including mummification, burial, and low temperatures. However, acarological information is lacking in forensic cases, which is further exacerbated by the lack of acarology experts and difficult morphological keys. Our research initially aimed to evaluate the diversity and species composition of mites in soil at previous simulated mass mortality events. Unfortunately, we were never able to conclusively receive solid molecular results. Therefore, the objective of this paper is to discuss the technical difficulties and challenges associated with molecular mite analysis, specifically focusing on Oribatida. After testing numerous primers, only ITS1&4 resulted in oribatid identifications. However, query cover was low (<23%) and percent similarity was less than 90%. This outcome could be due to poor DNA yield, the lack of optimized or standardized molecular protocols, and the limited availability of reference sequences, as the specimens may represent undescribed or novel taxa. In this article, we discuss different protocols for Oribatida molecular analyses,

our challenges, and our future suggestions and recommendations for improvements to future forensic acarology research.

**Keywords:** Forensic science; mass mortality event; molecular identification; *Oppiella nova;* Oribatida

## ABSTRAK

Akarologi forensik merupakan bidang sains yang semakin berkembang yang menggunakan hama dalam penyiasatan kematian, terutamanya bagi kes di mana bukti berkaitan entomologi adalah terhad. Hama boleh ditemui di pelbagai lokasi dan dapat bertahan dalam keadaan persekitaran yang ekstrem seperti mumifikasi, pengebumian dalam tanah, dan suhu rendah. Walau bagaimanapun, terdapat kekurangan data akarologi dalam kes forensik, ditambah pula dengan ketidakcukupan pakar akarologi, serta proses identifikasi menggunakan kunci morfologi yang mencabar. Kajian kami pada asalnya bertujuan untuk menilai kepelbagaian dan komposisi spesies hama di dalam tanah sebagai simulasi bagi peristiwa kematian besar-besaran. Malangnya, kami tidak berjaya memperoleh keputusan molekular yang kukuh. Oleh itu, objektif penulisan ini adalah untuk membincangkan kesukaran teknikal dan cabaran yang berkaitan dengan analisis molekul hama, dengan memberi tumpuan khusus kepada Oribatida. Selepas menguji pelbagai primer, hanya ITS1 dan 4 dapat menghasilkan pengecaman oribatid. Walau bagaimanapun, liputan kueri adalah rendah (<23%) dan peratus persamaan adalah kurang daripada 90%. Hasil ini mungkin disebabkan oleh hasil dapatan DNA yang rendah, kekurangan protokol molekular yang optimum dan seragam, dan ketersediaan data jujukan yang terhad untuk dijadikan rujukan, dan berkemungkinan specimen kami mewakili taksa yang belum pernah dikenalpasti. Dalam artikel ini, kami membincangkan protokol yang berbeza untuk analisis molekular Oribatida, cabaran yang dihadapi dan cadangan untuk penambahbaikan kepada penyelidikan akarologi forensik pada masa hadapan.

**Kata kunci:** Sains forensic; peristiwa kematian beramai-ramai; pengenalpastian molekul; *Oppiella nova*; Oribatida

Forensic acarology is an emerging area of forensic science which focuses on the use of mites in death investigations. Generally, forensic entomology focuses on forensically important Diptera present in the first few weeks of decomposition (Mégnin 1894; Bornemissza 1957; Byrd & Castner 2000) and may focus on beetles for human remains with longer postmortem interval periods (Mégnin 1894; Smith 1986; Byrd & Castner 2000). However, with forensic acarology, mites can be utilized in the later stages of decomposition, particularly when other species are unavailable or not prevalent (Perotti et al. 2009). Mites have been utilized in forensic investigations since as early as 1894, when Mégnin applied *Tyrophagus longior* (Gervais 1844) (Astigmata: Acaridae) to estimate the postmortem interval of mummified human remains (Mégnin 1894; Bornemissza 1957; Perotti 2009; WoRMS Editorial Board 2025). Mites are also useful outside of typical postmortem interval calculations. For example, *Dermatophagoides* spp. feed on shed human skin flakes in which human DNA has been successfully extracted from, allowing forensic scientists to use household dust mites to obtain victim and suspect DNA profiles (Çakan et al. 2015).

Recently, they have been used in several death investigations, including identifying the scene of death, offering details about the circumstances of death, establishing the minimum post-mortem interval (mPMI), and determining the stages of the dead body's decomposition (Perotti 2009; Rai et al. 2020; Saloña et al. 2010; Szelecz et al. 2018). Mites are particularly

useful in forensic investigations when environmental factors such as low temperatures, concealment and burial, or body condition such as mummification hinder other species, as mites are capable of surviving in a wide range of conditions (Braig & Perotti 2009; Perotti & Braig 2009; Rai et al. 2022; Walter & Proctor 2013). Mites arrive at carrion in various ways from phoresy (Braig & Perotti 2009; Perotti & Braig 2009) to being already present in the soil (Braig & Perotti 2009; Goff 1991; Rai et al. 2020; Szelecz et al. 2018). In particular, it is possible that soil mites could be affected further by the presence of multiple carrion such as in the case of mass mortality events. Mass mortality events are defined as large die-offs that result in a mortality rate higher than the average background mortality of the population (Fey et al. 2015; y Juárez et al. 2012). Many factors can lead to these death events including disease, biotoxicity, climate change, and more (Fey et al. 2015). One such extreme event occurred in 2015 when over 200,000 saiga antelopes perished due to increased temperatures and humidity resulting in a deadly year for *Hemorrhagic septicemia* (Fey et al. 2015; Kock et al. 2018).

With many deceased animals present at mass mortality, as well as an increase in insect presence, many mites might arrive at the scene due to phoresy. Phoretic associations of mites with forensic entomological specimens such as flies and beetles can provide valuable information on mPMI and death location or relocation (Azmiera et al. 2019; González Medina et al. 2013; Kamaruzaman et al. 2018; Szelecz et al. 2018). It is well known that mesostigmatid mites perform phoresy on forensic fly species (Perotti & Braig 2009; Pereira Sato et al. 2018) as well as astigmatid mites (Krantz & Walter 2009; Perotti & Braig 2009; OConnor 2009). Recently in Malaysia, Histiostomatidae (Acari: Astigmata) mites were found phoretically on the blow fly species *Chrysomya villeneuvi* (Patton 1922) (Diptera: Calliphoridae) (Azmiera et al. 2019; Patton 1922). Astigmata and Mesostigmata mite specimens have also been located in old graves containing partly or fully skeletonized bodies, including in association with phoresy (Rai et al. 2020).

However, mites are not often used for a myriad of reasons. First, there are difficulties with morphological identification due to the small size, difficult morphological keys, and lack of acarology experts (Krantz & Walter 2009). Therefore, it is important to assess other ways of identifying associated mites. Molecular identification of mites has been proposed as an alternative method (Krantz & Walter 2009). Molecular work can be used to identify mites as forensic indicators more accurately, assuming there are proper techniques and previously identified DNA sequences to associate for future use (Perotti et al. 2009).

Oribatids are particularly important as they are decomposers associated with plant detritus and litter (Norton & Ermilov 2014; Rai et al. 2021). These mites are primarily found in the soil, with different species being found in soils without carrion and soils underneath carrion (Braig & Perotti 2009; Rai et al. 2021). There can often be a decrease in Oribatid in carrion associated soils compared to those without decomposition (Saloña et al. 2010). However, certain species such as *Platynothrus peltifer* (Koch 1839) have been found in carrion associated soils (GBIF 2023; Saloña et al. 2010). Oribatids are present in the soil at all stages of decomposition, with families differing during the course of succession (Rai et al. 2021). More research is needed on the relation of different Oribatida to carrion decomposition.

The initial aim of this study was to molecularly identify mites associated with mass mortality events. However, despite our efforts, we encountered significant challenges with testing and the accuracy of the results. Therefore, this paper aims to discuss the techniques employed, the issues faced, and potential solutions for future researchers.

Soil arthropod samples were collected from the United States of America in native grasslands surrounded by rangelands, with shallow soils in fields in Love County, southern Oklahoma (34°11'8.75"N, 97°5'12.42"W) at field sites provided by provided by the Noble Research Institute. Feral swine carrion (*Sus scrofa*) was donated by landowners who were conducting feral swine eradications to prevent property damage and were not culled for the purposes of any experiments. Both mass mortality (~360 kg) and non-carrion plots were replicated four times during the same two-year period and were sampled throughout, with samples selected from the following dates: 21 April 2019 (1 week postmortem), 20 July 2019 (~3 months postmortem), and 18 May 2020 (~1 year postmortem) (see Jones 2022). Soil arthropods were extracted using Berlese funnels (Barberena-Arias et al. 2012). Samples were then stored in 70% isopropyl alcohol until molecular work were conducted.

Mite samples were first identified by their dominant suborders as seen in the six different treatments (non-carrion plots vs MMEs across three dates postmortem [1 week, 3 months, 1 year]). Mites were sampled to achieve a subsampling between 7 to 15% of all mites per sample, 10 to 20% of the suborder used (Alkathiry et al. 2022; Chaisiri et al. 2019). Due to no significant differences *(P>0.05*; Figure 1) across community data (of all soil arthropods) across replicates based on non-metric multidimensional scaling (NMDS; Bray-Curtis) and PermANOVA, replicates were combined (6 total sampling pools; MMEs at three time points, non-carrion plots at three time points).

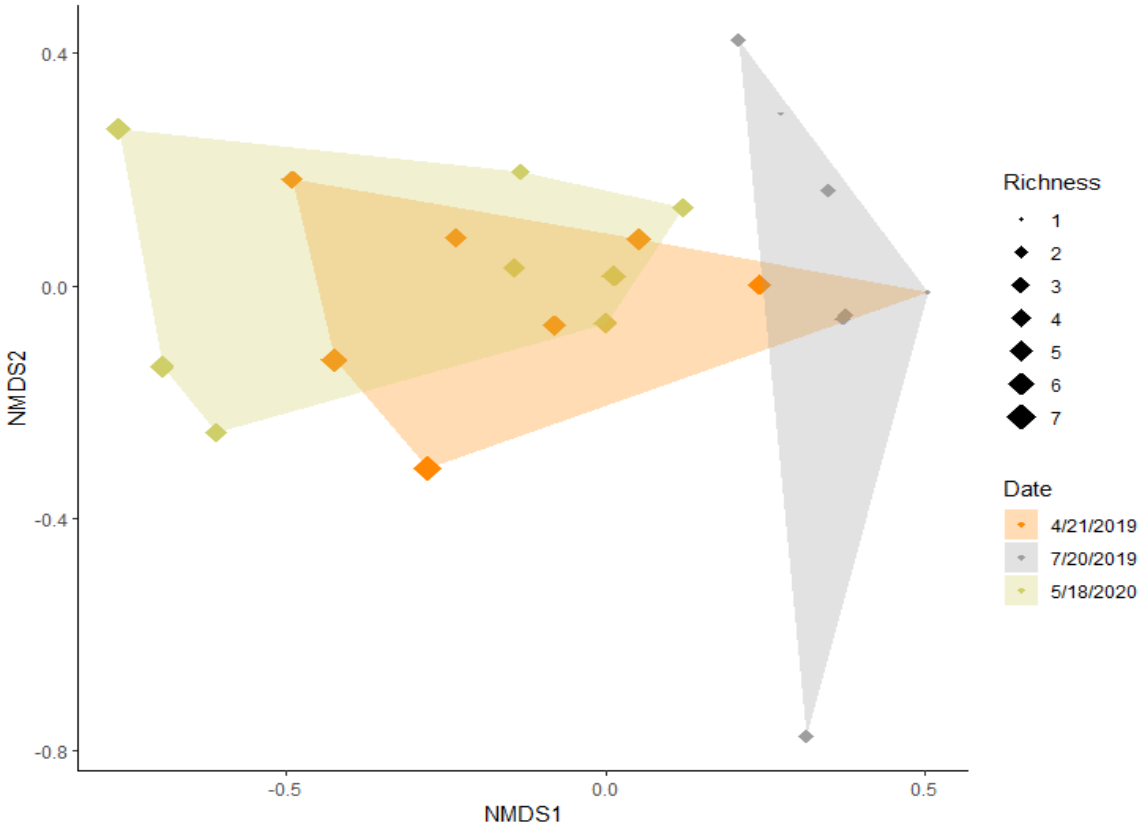


Figure 1. This non-metric dimensional scaling compares the replicate sites across different dates for all soil arthropods. PermANOVA shows that replicate samples are not significantly different (adonis2(formula = data.com ~ Site+Date +Treatment+Biomass, data = perm, permutations = 999, method = "bray")), (Df=3, F=1.3378, *P=0.238*).

Molecular analysis of the mites was conducted in Malaysia. DNA was extracted from individual mite specimens (without pooling) to identify the species. We used the QIAMP DNA Micro Kit (<10mg) for all extractions. The extraction was performed according to the manufacturer's specifications. Extracted DNA was first checked for purity and the concentration of DNA using a SpectraMax QuickDrop UV-Vis spectrophotometer (Molecular Devices, USA) at A260/A280. Afterwards, Polymerase Chain Reaction (PCR) was performed on the extracted DNA. LCOI primers were Universal LCO-1490 Forward primer (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO-2198 Reverse primer (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') with modifications from Tuccia et al. (2016) for the amplification of Oribatida and Mesostigmata mites. The initial heat activation step was 95°C for 10 minutes followed by 40 cycles of 95°C for 1 minute, 56°C for 1 minute, and 72°C for 1 minute, with a final extension of 72°C for 10 minutes. DNA was also amplified using ITS1_F (5'-TCCGTAGGTGAACCTGCGGA-3') and ITS4_R (5'-TCCTCCGCTTATTGATATGC-3') primers. Denaturation was performed at 94°C for 3 minutes followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, followed by a final extension of 72°C for 7 minutes.

Subsequent PCR products were tested for successful DNA amplification using 1% gel electrophoresis (1.5 uL Fluorosafe DNA stain) with the ladder at 377 bp and 421 bp (Gomez-Puerta et al. 2022). Once DNA is successfully visualized, the remaining PCR product (>20 uL) was sent for sequencing to Apical Sdn. Bhd. The resulting sequence data were examined via NCBI BLAST (nucleotide) to determine the family and/or species of the mites based on sequences amongst known and unknown mite taxa (Madden 2013). Due to low coverage, BLASTN was used to identified somewhat similar sequences as no highly similar sequences could be detected. Taxid ID 66551 (and taxid 386855) was used for the Oribatid mites. Due to technical difficulties, no further parameters were issued.

Astigmata, Mesostigmata, and Oribatida mites were all attempted with sequencing. However, only some Oribatida mites yielded sequences. Further optimization with pooling mites, different annealing temperatures, and QIAGEN Isopropanol precipitation of DNA did not improve results (Table 1). Oribatid mites were the only successfully tested suborder, with DNA bands present only when using ITS1 and ITS4 primers. Therefore, Oribatid mites were sampled as previously stated in the methods and sent for sequencing. However, despite sequences being returned, none of the results were conclusive with BLAST sequencing as query cover was very low in all samples (<23%) (Table 2).

Table 1. Several primer pairs were used to attempt the identification of mites molecularly, including Oribatida mites. This table includes the primers, the PCR processes used, and the citations information was based on. Some protocols were taken directly from papers while others were modified

| Target Region | Primer Name | Primer Sequence (5'–3') | PCR Conditions | Reference(s) |
|---|---|---|---|---|
| **COI** | LCO1490 (F)<br>HCO2198 (R) | LCO:<br>GGTCAACAAATCATAAAGATATTGG<br>HCO:<br>TAAACTTCAGGGTGACCAAAAAATCA | (1) Initial denaturation: 95 °C, 10 min; 40 cycles: 95 °C, 1 min / 56 °C, 1 min / 72 °C, 1 min; final extension: 72 °C, 10 min (2) Initial denaturation: 95 °C, 15 min; 34 cycles: 95 °C, 30 s / 40 °C, 45 s / 72 °C, 30 s; final extension: 72 °C, 10 min | Kreipe et al. (2015); Tuccia et al. (2016) |
| **COI** | COI (F, R) | F: GTTTTGGGATATCTCTCATAC R: GAGCAACAACATAATAAGTATC | (1) 95 °C, 3 min; 45 cycles: 95 °C, 30 s / 56 °C, 30 s / 72 °C, 1 min; final: 72 °C, 7 min (2) 95 °C, 5 min; 40 cycles: 95 °C, 40 s / 47 °C, 40 s / 72 °C, 30 s; final: 72 °C, 5 min | Prior optimization Gebrezgiher et al. (2023) |
| **ITS2** | ITS (F, R) | F: CGACTTTCGAACGCATATTGC R: GCTTAAATTCAGGGGGTAATCTCG | (1) 94 °C, 2 min; 35 cycles: 94 °C, 30 s / 56 °C, 30 s / 72 °C, 50 s; final: 72 °C, 7 min (2) same cycling, final: 72 °C, 3 min | Wong et al. (2011); Shafique et al. (2018) |
| **ITS1–ITS4** | ITS1 (F), ITS4 (R) | ITS1: TCCGTAGGTGAACCTGCGGA<br>ITS4: TCCTCCGCTTATTGATATGC | 94 °C, 3 min; 40 cycles: 94 °C, 30 s / 55 °C, 30 s / 72 °C, 1 min; final: 72 °C, 7 min | Cruickshank (2002) |
| **COI (Cox1)** | COIarch1 (F)<br>COIarch2 (R) | COIarch1:<br>GGTCAACAAATCATAAAGAYATYG<br>COIarch2:<br>TAAACTTCAGGGTGACCAAAAAATCA | 95 °C, 15 min; 34 cycles: 95 °C, 45 s / 51 °C, 1 min / 72 °C, 55 s; final: 72 °C, 10 min | Folmer et al. (1994); Domes et al. (2008) |

Table 2. Received sequences and results from BLASTN analysis (see methods for details). This does not include samples sent for analysis that did not result in sequences, hence the missing of some sample identifications under certain treatments. Samples were sent in batches

| Carrion Treatment | Date Collected | Sample ID | Supposed Species | Percent Similarity | Query Cover |
|---|---|---|---|---|---|
| Mass Mortality | 21 April 2019 (1 week postmortem) | Oribatid 2 | NA | No similarity found in suborder | NA |
| | | Oribatid 3 | Inconclusive | 75% Oppia nitens RNA | 6% |
| | | Oribatid 4 | *Oppiella nova* | 6.21% (3e-10) | 10% |
| | | Oribatid 5 | *Oppiella nova* | 85.42% (8e-06) | 7% |
| | | Oribatid 6 | *Oppiella nova* | 84.75% (6e-08) | 22% |
| | | Oribatid 7 | *Oppiella nova* | 86.0% (7e-07) | 7% |
| | | Oribatid 8 | *Oppiella nova* | 85.96% (2e-09) | 13% |
| | | Oribatid 9 | *Platynothrus peltifer* | 69.72% (4e-04) | 22% |
| | | Oribatid 10 | *Oppiella nova* | 69.72% (4e-04) | 4% |
| No Carrion | | Oribatid 1 | *Oppiella nova* | 84.48% (2e-07) | 22% |
| | | Oribatid 2 | *Oppiella nova* | 83.61% (6e-08) | 23% |
| | | Oribatid 3 | *Oppiella nova* | 85.96% (2e-09) | 7% |
| | | Oribatid 4 | *Oppiella nova* | 85.71% (3e-06) | 21% |
| | | Oribatid 5 | *Oppiella nova* | 5.96% (1e-09) | 9% |
| | | Oribatid 8 | *Oppiella nova* | 84.21% (6e-08) | 9% |
| Mass Mortality | 20 July 2019 (~3 months postmortem) | Oribatid 1 | *Oppiella nova* | 86.0% (7e-07) | 17% |
| | | Oribatid 2 | *Oppiella nova* | 75.86% (2e-07) | 20% |
| No Carrion | | Oribatid 1 | *Oppiella nova* | 6.0% (7e-07) | 7% |
| | | Oribatid 2 | *Oppiella nova* | 82.76% (4e-06) | 5% |
| Mass Mortality | 18 May 2020 (~1 year postmortem) | Oribatid 1 | *Oppiella nova* | 85.71% (2e-06) | 7% |
| | | Oribatid 2 | *Oppiella nova* | 82.76% (3e-06) | 8% |
| | | Oribatid 3 | *Oppiella nova* | 85.96% (1e-09) | 9% |
| | | Oribatid 5 | *Oppiella nova* | 83.05% (8e-07) | 16% |

| | | | | |
|---|---|---|---|---|
| | Oribatid 6 | NA | No similarity found in suborder; potential prostigmata unlikely (23% query) | NA |
| | Oribatid 7 | *Oppiella nova* | 84.75% (6e-08) | 8% |
| No Carrion | Oribatid 1 | Unknown - *Oppiella nova* possible | NA | NA |
| | Oribatid 2 | NA | No similarity found in suborder | NA |
| | Oribatid 3 | NA | No similarity found in suborder | NA |
| | Oribatid 4 | *Medioppia subpectinata* | 91.53% (0.005) | 4% |
| | Oribatid 5 | NA | No similarity found in suborder | NA |
| | Oribatid 6 | *Oppiella nova* | 85.71% (5e-09) | 8% |
| | Oribatid 7 | *Oppiella nova* | 82.76% (3e-06) | 9% |
| | Oribatid 8 | NA | No similarity found in suborder | NA |

While DNA was successfully sequenced, percent similarity for all specimens ranged from ~75 to 89% for those that potentially matched Oribatida species. The query cover was also only between 4 and 23%. Sequences were additionally assessed with Chromas Sequence Scanner Programming, showing that there was background noise present in these specimens. Overall, molecular analyses found nine potential *Oppiella nova* (Oudemans 1902) (60%) out of the fifteen successfully identified Oribatida, with one potential *Medioppia subpectinata* (Oudemans 1900) (6.7%), and five unknown specimens (33.3%) in the plots with no carrion (across all three time points) (GBIF 2023). At mass mortality plots (across all three dates), there were fifteen potential *Oppiella nova* (78.9%) out of nineteen total, with four unknown specimens (21.1%).

Our results suggest that many of the collected Oribatid mites were the same species, *Oppiella nova* in both MME and non-carrion plots. Unfortunately, these results cannot be confirmed due to low similarity (<90%) and limited query cover (<23%). Another possible explanation for these results is the limited availability of mite sequences in the database, likely due to low PCR amplification success. Consequently, accurate identification is challenging, and many of the sequences obtained could represent taxa that are new to science.

While molecular work can be beneficial, especially when lacking morphological expertise and access, there are also several difficulties such as potential cost, optimization of primers, finding the appropriate methodology, and poor DNA yields. Not including cost, molecular analyses can be challenging in terms of DNA amplification. Many of these samples had prominent background noise. This could be for numerous reasons. First, it is possible that the ITS1 and ITS4 primers are not optimal for oribatid identifications. Indeed, these primers have been more successful in other groups by comparison, such as fungi associated with

oribatids (Renker et al. 2005) and Phytoseiidae (Paavanen-Huhtala et al. 1999; Yli-Mattila et al. 2000), though oribatid DNA bands were present on all gel electrophoresis. Second, it is possible that the samples were degraded prior to DNA extraction.

Various primers and protocols have been used for different suborders of mites. Oribatida is one suborder that has little information on appropriate primers and protocols for DNA amplification, indicating further gaps in databases and research. Different genetic markers may be more useful in different scenarios. In Navajas et al. (1999), ITS primers were most useful in species level identifications compared to family level (Family: Phytoseiidae). Different primer pairs for one species may be different or less effective at amplifying the ITS region compared to other species (Yli-Mattila et al. 2000). Unfortunately, none of the tested primers worked in this study except for ITS1&4, which had low query cover and less ideal similarity percentages. It is also possible that all mite DNA may have suffered degradation.

Our results for molecular identifications were inconclusive and showed no difference in specimens between mass mortality and non-carrion soils. However, there were numerous difficulties discovered when attempting to molecularly identify mites, both with DNA preservation and primer amplification. However, when taking a more holistic approach (i.e., community analysis), there are issues with accurate molecular identifications. Therefore, studies optimizing different mite PCR amplification protocols and primers could provide a great benefit to the scientific community and the future of acarology. Despite complications, this is the first attempt at identifying mites associated with mass mortality events. Therefore, more studies are necessary to understand the role of mites associated with carrion, particularly in mass mortality events, to enhance our knowledge of mite ecology and forensic acarology, as well as to advance molecular studies of mites.

We have several recommendations for future studies. First, specimens should be stored in ethanol and subsequently placed in a -20°C freezer to best preserve mite DNA (Cruickshank 2002). While ethanol has been shown to yield DNA for several years after collection, freezing will aid in the preservation. Additionally, mites that are dried for shipment may also see the destruction of some specimens as these mites may have experienced (Cruickshank 2002). Therefore, mites collected for DNA analysis should be completed as soon as possible. Mite samples that are up to several years old may be difficult to molecularly identify.

## ACKNOWLEDGMENTS

The authors would like to acknowledge the Noble Research Institute and those who worked on the previous project involving the collection of soil arthropods from simulated mass mortality events (see Jones 2022). The authors would also like to thank the Faculty of Medicine, Universiti Teknologi MARA for funding this research as well as the Institute of Medical Molecular Biotechnology (IMMB) for providing laboratory space and support.

## AUTHORS DECLARATION

**Funding Statement**
This work was supported by the Faculty of Medicine Research Grant (600-TNCPI 5/3/DDF (MEDIC) (004/2022)).

**Conflict of Interest**
The authors have no relevant financial or non-financial interests to disclose.

**Ethics Approval**
This study requires no ethical approval.

**Data Availability Statement**
The datasets generated and/or analyzed during this study are available upon request from the corresponding author.

**Author Contributions**
**Abby K. Jones:** Methodology, formal analysis and investigation, writing (original draft preparation), writing (review and editing); **Nural Azmiera:** Methodology, formal analysis and investigation, writing (review and editing); **Chong Chin Heo:** Conceptualization, methodology, writing (review and editing), funding acquisition, supervision; **Raziun Rahimi:** Conceptualization, methodology, writing (review and editing), funding acquisition; **Jamal Hussaini:** Conceptualization, methodology, writing (review and editing), funding acquisition; **Van Lun Low:** Conceptualization, methodology, writing (review and editing), funding acquisition; **Farah Roslinda Mohd Rustam:** Conceptualization, methodology, writing (review and editing), funding acquisition. All authors approve this final published version and agree to be held accountable for all aspects of the work.

## REFERENCES

Alkathiry, H., Al-Rofaai, A., Ya'cob, Z., Cutmore, T.S., Mohd-Azami, S.N.I., Husin, N.A., Lim, F.S., Koosakulnirand, S., Mahfodz, N.H., Ishak, S.N. & Loong, S.K. 2022. Habitat and season drive chigger mite diversity and abundance on small mammals in Peninsular Malaysia. *Pathogens* 11(10): 1087.

Azmiera, N., Mariana, A. & Heo, C.C. 2019. First report of *Histiostoma* sp. (Astigmata: Histiostomatidae) phoretic on a forensically important blowfly, *Chrysomya villeneuvi* (Diptera: Calliphoridae). *Tropical biomedicine* 36(4): 1099-1104.

Barberena-Arias, M.F., González, G. & Cuevas, E. 2012. Quantifying variation of soil arthropods using different sampling protocols: Is diversity affected? In. Sudarshana, P., Nageswara-Rao, M. & Soneji, J.R. (eds.). *Tropical Forests,* pp. 51-70. Intech (online publisher).

Bornemissza, G.F. 1957. An analysis of arthropod succession in carrion and the effect of its decomposition on the soil fauna. *Australian Journal of Zoology* 5(1): 1-12.

Braig, H.R. & Perotti, M.A. 2009. Carcases and mites. *Experimental and Applied Acarology* 49(1): 45-84.

Byrd, J.H. & Tomberlin, J.K. 2000. *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. Boca Raton: CRC press.

Çakan, H., Güven, K., Çevik, F.E., Demirci, M. & Saribas, S. 2015. Investigation of human DNA profiles in house dust mites: Implications in forensic acarology. *Romanian Society of Legal Medicine* 23: 187-92.

Chaisiri, K., Gill, A.C., Stekolnikov, A.A., Hinjoy, S., McGarry, J.W., Darby, A.C., Morand, S. & Makepeace, B.L. 2019. Ecological and microbiological diversity of chigger mites, including vectors of scrub typhus, on small mammals across stratified habitats in Thailand. *Animal Microbiome* 1(1): 18.

Cruickshank, R.H. 2002. Molecular markers for the phylogenetics of mites and ticks. *Systematic and Applied Acarology* 7(1): 3-14.

Domes, K., Maraun, M., Scheu, S. & Cameron, S.L. 2008. The complete mitochondrial genome of the sexual oribatid mite *Steganacarus magnus:* Genome rearrangements and loss of tRNAs. *BMC genomics* 9(1): 532.

Fey, S.B., Siepielski, A.M., Nusslé, S., Cervantes-Yoshida, K., Hwan, J.L., Huber, E.R., Fey, M.J., Catenazzi, A. & Carlson, S.M. 2015. Recent shifts in the occurrence, cause, and magnitude of animal mass mortality events. *Proceedings of the National Academy of Sciences* 112(4): 1083-1088.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3(4): 294-299.

GBIF Secretariat. 2023. GBIF Backbone Taxonomy. Checklist Dataset. https://doi.org/10.15468/39omei [21 January 2025]

Gebrezgiher, G.B., Makundi, R.H., Katakweba, A.A., Belmain, S.R., Lyimo, C.M. & Meheretu, Y. 2023. Arthropod ectoparasites of two rodent species occurring in varied elevations on Tanzania's second highest mountain. *Biology* 12(3): 394.

Goff, M.L. 1991. Use of Acari in establishing a postmortem interval in a homicide case on the island of Oahu, Hawaii. *Modern Acarology. Volume I. Proceedings of the VIII International Congress of Acarology, held in České Budějovice, Czechoslovakia, 6-11 August 1990,* pp. 439-442.

Gomez-Puerta, L.A., Pacheco, J.I., Angulo-Tisoc, J.M., García, W., Castillo, H., Lopez-Urbina, M.T. & Gonzalez, A.E. 2022. Prevalence and molecular characterization of *Sarcoptes scabiei* from vicuñas (*Vicugna vicugna*) from Southern Peruvian Andes. *Parasitology* 149(5): 581-586.

González Medina, A., González Herrera, L., Perotti, M.A. & Jiménez Ríos, G. 2013. Occurrence of *Poecilochirus austroasiaticus* (Acari: Parasitidae) in forensic autopsies and its application on postmortem interval estimation. *Experimental and Applied Acarology* 59(3): 297-305.

Jones, A.K. 2022. Effects of mass death on community structure and ecosystem function. PhD Dissertation, Mississippi State University.

Kamaruzaman, N.A.C., Mašán, P., Velásquez, Y., González-Medina, A., Lindström, A., Braig, H.R. & Perotti, M.A. 2018. *Macrocheles* species (Acari: Macrochelidae) associated with human corpses in Europe. *Experimental and Applied Acarology* 76(4): 453-471.

Kock, R.A., Orynbayev, M., Robinson, S., Zuther, S., Singh, N.J., Beauvais, W., Morgan, E.R., Kerimbayev, A., Khomenko, S., Martineau, H.M. & Rystaeva, R. 2018. Saigas on the brink: Multidisciplinary analysis of the factors influencing mass mortality events. *Science advances* 4(1): 2314.

Krantz, G.W. & Walter, D.E. 2009. *A Manual of Acarology*. Third Edition. Lubbock: Texas Tech University Press.

Kreipe, V., Corral-Hernández, E., Scheu, S., Schaefer, I. & Maraun, M. 2015. Phylogeny and species delineation in European species of the genus *Steganacarus* (Acari, Oribatida) using mitochondrial and nuclear markers. *Experimental and Applied Acarology* 66(2): 173-186.

Madden, T. 2013. The BLAST sequence analysis tool, Chapter 16. In. McEntyre & Ostell (eds.). *The NCBI Handbook.* pp. 425-436. Bethesda (MD): National Library of Medicine (US).

Mégnin, P. 1894. *La Faune Des Cadavres: Application De L'entomologie À La Médecine Légale*.Volume 101. Paris: G. Masson: Gauthier-Villars et fils.

Navajas, M., Lagnel, J., Fauvel, G. & De Moraes, G. 1999. Sequence variation of ribosomal internal transcribed spacers (ITS) in commercially important Phytoseiidae mites. *Experimental & Applied Acarology* 23(11): 851-859.

Norton, R.A. & Ermilov, S.G. 2014. Catalogue and historical overview of juvenile instars of oribatid mites (Acari: Oribatida). *Zootaxa* 3833(1): 1-132.

OConnor, B.M. 2009. Astigmatid mites (Acari: Sarcoptiformes) of forensic interest. *Experimental and Applied Acarology* 49(1): 125-133.

Paavanen-Huhtala, S., Avikainen, H. & Yli-Mattila, T. 2000. Development of strain-specific primers for a strain of *Gliocladium catenulatum* used in biological control. *European Journal of Plant Pathology* 106(2): 187-198.

Patton, W.S. 1922. Some Notes on Indian Calliphorinae. Part IV. *Chrysomyia albiceps*,Wied. (rufifacies, Froggatt), one of the Australian Sheep Maggot Flies, and *Chrysomyia. villeneuvii*, sp. nov. *Indian Journal of Medical Research* 9(3): 561-569.

Pereira Sato, T., Leal Caetano, R., Carriço, C., Ferreira da Silva, A., Salles Gazeta, G. & Teixeira Pinto, Z. 2018. First record of phoretic and parasitc mites (Arachnida: Acari) associated with necrophagous flies in Brazil. *Revista Colombiana de Entomología* 44(1): 48-52.

Perotti, M.A. 2009. Mégnin re-analysed: The case of the newborn baby girl, Paris, 1878. *Experimental and Applied Acarology* 49(1): 37-44.

Perotti, M.A. & Braig, H.R. 2009. Phoretic mites associated with animal and human decomposition. *Experimental and Applied Acarology* 49(1): 85-124.

Perotti, M.A., Goff, L.M., Baker, A.S., Turner, B.D. & Braig, H.R. 2009. Forensic acarology: An introduction. *Experimental and Applied Acarology* 49(1): 3-13.

Rai, J.K., Amendt, J., Bernhardt, V., Pasquerault, T., Lindström, A. & Perotti, M.A. 2020.Mites (Acari) as a relevant tool in trace evidence and postmortem analyses of buried corpses. *Journal of Forensic Sciences* 65(6): 2174-2183.

Rai, J.K., Pickles, B.J. & Perotti, M.A. 2021. Assemblages of Acari in shallow burials: Mites as markers of the burial environment, of the stage of decay and of body-cadaver regions. *Experimental and Applied Acarology* 85(2): 247-276.

Rai, J.K., Pickles, B.J. & Perotti, M.A. 2022. The impact of the decomposition process of shallow graves on soil mite abundance. *Journal of forensic sciences* 67(2): 605-618.

Renker, C., Otto, P., Schneider, K., Zimdars, B., Maraun, M. & Buscot, F. 2005. Oribatid mites as potential vectors for soil microfungi: Study of mite-associated fungal species. *Microbial Ecology* 50(4): 518-528.

Saloña, M.I., Moraza, M.L., Carles-Tolrá, M., Iraola, V., Bahillo, P., Yélamos, T., Outerelo, R. & Alcaraz, R. 2010. Searching the soil: Forensic importance of edaphic fauna after the removal of a corpse. *Journal of Forensic Sciences* 55(6): 1652-1655.

Shafique, R.H., Akhter, S., Abbas, S. & Ismail, M. 2018. Sensitivity to house dust mite allergens and prevalence of allergy-causing house dust mite species in Pothwar, Pakistan. *Experimental and Applied Acarology* 74(4): 415-426.

Smith, K.G.V. 1986. *A Manual of Forensic Entomology*. London UK: British museum (Natural History).

Szelecz, I., Lösch, S., Seppey, C.V., Lara, E., Singer, D., Sorge, F., Tschui, J., Perotti, M.A. & Mitchell, E.A. 2018. Comparative analysis of bones, mites, soil chemistry, nematodes and soil micro-eukaryotes from a suspected homicide to estimate the post-mortem interval. *Scientific reports* 8(1): 25.

Tuccia, F., Giordani, G. & Vanin, S. 2016. A combined protocol for identification of maggots of forensic interest. *Science & Justice* 56(4): 264-268.

Walter, D.E. & Proctor, H.C. 2013. Mites in soil and litter systems. In. Walter & Proctor (eds.). *Mites: Ecology, Evolution & Behaviour: Life at a Microscale*, pp. 161-228. Dordrecht: Springer Netherlands.

Wong, S.F., Chong, A.L., Mak, J.W., Tan, J., Ling, S.J. & Ho, T.M. 2011. Molecular identification of house dust mites and storage mites. *Experimental and Applied Acarology* 55(2): 123-133.

WoRMS Editorial Board. 2025. World Register of Marine Species. Checklist Dataset. https://doi.org/10.14284/170 [21 January 2025].

y Juárez, E.I.A., Mace, G.M., Cowlishaw, G. & Pettorelli, N. 2012. Natural population die offs: Causes and consequences for terrestrial mammals. *Trends In Ecology & Evolution* 27(5): 272-277.

Yli-Mattila, T., Paavanen-Huhtala, S., Fenton, B. & Tuovinen, T. 2000. Species and strain identification of the predatory mite *Euseius finlandicus* by RAPD-PCR and ITS sequences. *Experimental & applied acarology* 24(10): 863-880.