Soil bulb mites as trace evidence for the location of buried money

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Highlights

- *Rhizoglyphus howensis* Manson mites recovered from buried money.
- *Rhizoglyphus* mites are soil dwellers, root/tubercle associated mites.
- *R. howensis* are unique to Australasia, directing the money search to this region.
- This is the first record of *R. howensis* Manson in Europe and from banknotes.
- Immediate collection and preservation of invertebrate traces is essential.

Abstract

This study reports for the first time the use of soil micro-invertebrates, mites, as trace evidence to localise buried objects such as money.
The case relates to a crime in Germany, where a large sum of banknotes had been hidden in an unknown location, likely abroad. In 2016, part of the money (approx. €500,000 in €500 value notes) was confiscated by the police and once analysed in the forensic lab, it was discovered that the notes were covered with small particles of a sort of debris, later identified as specimens of *Rhizoglyphus howensis* Manson, a non-European, nor Mediterranean species of root or bulb mites (Acaridae: Rhizoglyphinae).
The restricted biogeographic distribution of *R. howensis,* in unspoiled forest soil in the Australasian region limited the search for the money to the areas visited by the perpetrators during their trips into the region.
R. howensis biology can provide even with more clues on the whereabouts of the banknotes, as they are specialist plant feeders, exclusively feeding on seeds of palm trees and on roots of *Quercus patula* in the Australian region.
This report aims to highlight the importance of the correct identification of the microscopic organisms associated with a crime scene and the immediate retrieval of micro-invertebrate trace evidence.
This is the first record of *R. howensis* from Europe, and from banknotes.

Keywords: Forensic; illegal; money; bill; contraband; concealment; Acaridae; Astigmata; *Rhizoglyphus*.

Introduction

Criminal activities often involve the hiding and concealment of corpses as well as illegal merchandises, drugs, weapons, stolen and valuable objects and money. The act of hiding these items seems to respond to the culprits' desire of forgetting the crime, and avoiding visualization of evidence and in the case of valuables, allowing them to return at a later stage to retrieve them [1-4].

One of the most notorious, still unsolved cases of hidden money in FBI history is known as NORJAK (for Northwest Hijacking). According to the FBI official webpage, in November 1971 a man named Dan Cooper took a plane from Northwest to Seattle, Washington. During the flight, Cooper threatened the crew by pretending that he was carrying a bomb and in exchange for the life of the 36 passengers he demanded, in addition to a parachute, a $200,000 ransom, all in $20 unmarked bills. Cooper received the money upon landing and the flight was re-directed to Mexico City. Somewhere on the way, the hijacker jumped off the plane with the $200,000. Leaving no evidence behind, the case remains a mystery. In 1980, a young boy found a concealed rotten package covered with soil, containing $5,800, all in $20 notes, around a suspected area. The money bills matched the serial numbers of the money taken by Cooper. Until today, the culprit and the rest of the money remain at large [5, 6].

Soil is frequently used in forensic analysis [1-3], especially when important pieces of evidence from a crime scene, stolen or illegal goods and cadavers, are hidden within walls, under the ground, [7], or come in contact with soil [2, 3]. Another, more recent criminal case involved an attempt of robbery when the suspect tried to gain access to the property, by crawling through an opening in the ground covered by dirt and soil. The only trace evidence that linked the perpetrator to the crime scene was particles of soil found on the inside of his waistband. The suspect was not wearing a shirt, and this facilitated the accumulation of soil around his waist when he was crawling. The suspect claimed that at the time of the robbery he was playing baseball in a different area. However, the soil on him linked him directly to the source of origin, after which he pleaded guilty [2].

Every type of soil has distinctive compounds [1-4, 8-10], abiotic ones such as minerals, chemical substances [4, 11], accompanied by unique fauna and flora [10-19]. The forensic analysis of soil may also include living microorganisms, for example, testate amoebae [9, 20, 21]. Swindles and Ruffell [20] reanalyzed soil samples from a 10 years old cold case. They looked at the possibility of solving murder cases by using testate amoebae found in dry soil that was collected from the victim's clothing [20]. Slightly bigger in size, micro-arthropods represent the most diverse and dominant mesofauna of all soils [22-27]. Oribatida mites are one of the most prevalent components of soils, in both spoiled and unspoiled habitats, their numbers can reach hundreds in a single square meter of soil [25, 28]. They feed on organic matters such as litter, low growing grass and surface leaves, shoots, and twigs of trees; oribatids are habitat specific and could offer reliable evidence [25]. Mites from other taxa, Mesostigmata, Astigmata and Trombidiformes are also an important part of the soil mite community. They live either as parasites on vertebrates, on invertebrates, as living predators, as detritivorous, or consumers of decay dwelling in soil [29-32]. Species from the Acaridae family (within Astigmata) are specialized in

soil, feed on grains, mold, decaying tree litter, bulbs and roots [32, 33]. Thus, they are frequent and numerous soil dwellers, particularly those from the Rhizoglyphinae sub-family. The genus *Rhizoglyphus* comprise mites known as bulb-mites because they live and dwell in cultivated bulbs and corms of many crops and flowers, in farms, agricultural lands as well as in greenhouses and pristine habitats [33]. They feed on living tissue (e.g. roots) and some of the species from this genus, like the cosmopolitan *Rhizoglyphus robini* Claparède*,* are more generalistic pests in terms of host plant specificity [33, 34]. However, specificity is common too in this genus, and one good example is *Rhizoglyphus howensis* Manson, a highly specific species, uniquely associated with unspoiled forest soil, feeding on seeds of palm trees or on trees and roots of *Quercus patula* (an early synonym of *Quercus garryana* Dougl. ex Hook.) [33].

These 'biological' properties of soil due to its unique microfauna, make it one of the most important sources of evidential clues [3, 4]. Due to their high habitat specificity, some soil components such as mites can therefore become useful markers of location [35]. Soils and their biological components can act like markers of high resolution due to their distinctive characters and unique biological traits.

Until present and particularly for mites, researchers have explored the faunal diversity below the ground mainly in association with soil productivity [22, 36-39]. For instance, an assessment of micro-arthropod richness, Oribatida abundance, and other Acari communities' composition in undisturbed forest soils was conducted in 2006 by John and colleagues, showing that there are great differences between soil habitats [40]. According to Lindo and Winchester, the micro-arthropods and mites' abundances are considerably greater in forests soils when compared with the canopy layers of the same environments [41]. Despite these efforts, only a handful of studies have focused on soil mite fauna, especially pristine environments and its role as evidence in crime scenes [12, 42-53].

Knowledge of the local soil fauna diversity is crucial when soil becomes a piece of evidence in a crime investigation [3, 4, 11]. Mites used as trace evidence, when found on goods, weapons, human trafficking as well as suspects [54-56] are markers of location of origin. They can offer reliable information of the environment where a crime originated, on the new environment after relocation, and their biology can assist with details of the crime scene itself, e.g. explaining circumstances related to a killing, timing, etc [35, 48, 57-59]. Despite their importance as valuable trace evidence, mites have not yet been adopted in routine investigations of crime scenes involving soil. This might be due to the lack of case reports offering examples of interpretation of trace evidence based on acarological findings. By analyzing a crime involving the hiding of a large sum of money from Germany, this work aimed to provide, i) a basic protocol for the easy recovery and preparation of micro-arthropod evidence -that usually goes unspotted, and ii) to bring light to the potential evidential value of micro-arthropods frequently associated with buried objects.

Materials and methods

Case History

Police in Germany restrained a perpetrator who was carrying concealed packages, containing a large amount of money (up to €500,000) all in €500 banknotes. The confiscated money was wrapped in plastic bags; most of the banknotes were discolored and partially destroyed. According to the initial culprits' statement, the money was kept in the plastic bags under the ground somewhere in Spain.

The confiscated banknotes were initially taken to a bank for counting with the help of an automated counting-machine. After determining the exact amount of the money found, the packages with money inside were handed over to the forensic investigator (UT) from the Kriminaltechnisches Institut for further examination. Evidence was needed to confirm the location in which the money was buried and to reconstruct the case. The primary examination of the notes at the lab showed that the paper of the notes was partially degraded with a distinct stain of brownish-red coloration of minute particles, and parts of the notes were gluing together possibly due to moisture (Fig. 1). Other parts of the money notes were perforated showing signs of galleries and burrows, similar to those produced by insects. Debris were collected from the inside of the plastic bags and the primary examination indicated that they were mainly composed of small paper particles. The use of a stereomicroscope (LEICA MZ 16) allowed the close examination of the layers of notes and the debris. No outside material was found between the layers of the bank notes, this is unfortunately, due to the elimination of all potential trace evidence caused by the automated counter. Single mineral grains and other particles were found in the debris of paper along side 20 to 30 dead and/or partially decomposed mites. The mites were gently brushed off the banknotes into an Eppendorf tube containing 70% (v/v) solution of ethanol, using an extra fine artist paint brush (10/0) with only a few hairs remaining to avoid further damage of the mites. Around 15 mites were sent to the experts (acarologists, MH and MAP) for their identification and further analysis.

Procedures followed for the preparation of mites for identification

Preparation of samples

The mites were partially damaged and dehydrated; some of them were completely fragmented likely due to the counting-process, damaged by the machines. This has predominantly happened due to the lack of immediate preservation of valuable trace evidence. Only five out of the 15 fragmented mites recovered were prepared and mounted onto three different slides with the voucher numbers -M.C.G.1, -M.C.G.2 and -M.C.G.3. They are kept in the collection of the Forensic Acarology Lab, University of Reading. The rest of specimens (eight) are kept in 70% (v/v) solution of ethanol for further potential work. Two specimens were originally very fragmented and were lost after exposure to 50% (v/v) lactic acid solution, and two out of the five mounted specimens were sent back to the Kriminaltechnisches Institut.

At the Forensic Acarology Lab., using a stereo-microscope (LEICA M125) the mites samples were gently rehydrated by submerging them into a 2% (v/v) TWEEN 20 solution for 5min. Five mites were selected and cleared in 50% (v/v) lactic acid solution [60]. The acid macerates the inside of the mite by digesting the internal organs and particularly the muscles, without damaging the exoskeleton [61-63]; aiding their identification based on morphologies determined by the undissolved exoskeleton (cuticle). The mites in lactic acid were kept in a dry bath incubator overnight at 45 ^{0}C; incubation in lactic acid can vary from 1 hour to 1 day, depending

on the thickness of the mite cuticle, and has to be monitored during the process, otherwise the mites can disintegrate. The mites were removed from the lactic acid solution and thoroughly washed with distilled water, twice [62, 63].

Finally, the mites were kept partially dehydrated in 70% (v/v) ethanol solution for 20 minutes, then preserved in 96% Ethanol. The clear and dehydrated mites were carefully placed on clean mounting slides. 20µl of Hoyer's mounting medium was added before a cover slip was placed on the top to permanently mount and preserve the mites [60, 61, 64, 65]; the coverslips were later sealed with a sealing primer (insulating enamel 1201Q) produced by Glyptal®.

Identification of mites

The transparency of mites is essential for the microscopic examination when phase contrast microscopy and microphotography is applied [62, 63]. This method can be used (by mounting the mites) when evidence is not expected to leave the forensic lab. Consequently the untrained acarologists may take photographs of the diagnostic characters (guided by the acarologist in the distance) and send the images for an accurate identification of species.

For the identification of mites, a phase contrast (Nikon Optiphot) microscope was used with magnification lenses up to 1500X (15X ocular used with a 100X objective). All specimens mounted were adult female mites [33, 37]. The adult stage in mites facilitates the identification of specimens, as the majority of described species are in adult stage.

The primary identification to the genus level was done using keys by Hughes (1976). Yet, the identification to the species level was difficult due to the fragile conditions of the specimens. Despite that, the main diagnostic features were well preserved in 3 specimens. For species identification, descriptions and keys from different sources were used [33, 34, 37, 60, 66-72].

The main diagnostic characters for adult females of the genus (Fig. 2) were used and these include: color and length of idiosoma; setae on the dorsum of the idiosoma; Grandjean organ; legs pigmentation and setae [37, 66, 72].

Results and discussion

The mites collected from the illegal money, belong to the Acaridae family (Astigmata). A widely spread taxon, specially synanthropic in terms of habitat exploitation [33]. More in detail, the specimens belonged to subfamily Rhizoglyphinae [33, 37], genus *Rhizoglyphus* which are bulb or root plant mites, found in soil at shallow depths [33, 37, 68, 73]. Mites from the genus *Rhizoglyphus* are soil dwellers, they are considered as pests that attack the roots of most plants, but they are mostly linked to bulbs, among which are garlic, onions, and other bulbs of flowers such as lilies [66].

Following descriptions and keys for the identification of European and North African species, there was no match with any described species of *Rhizoglyphus*. Therefore, the book "Revision of *Rhizoglyphus* Claparède (Acari: Acaridae) of Australasia and Oceania" [33] was used. As many of the originally described species of *Rhizoglyphus* are now considered cosmopolitan, this book has become more or less a cosmopolitan

key for *Rhizoglyphus* species. The mites of this case were identified as *Rhizoglyphus howensis* Manson (Fig. 2 and 3) [33]. *R. howensis* adult female unique morphological features include pointy and simple cheliceral setae; the prodorsal shield is punctuated and the Grandjean's organ has a forked tip with two uneven branches [33, 72].

Although the females are strikingly similar to *R. robini*, in a general view, according to Fan and Zhang [33], there were clear and large differences in the following diagnostic morphologies:
In the Genu of leg I of the questioned specimens, setae *cG* has an average length of 20.94µm (N = 4, SD= 1.78µm) (Fig. 3), coinciding with the description of *R. howensis* Manson. While in *R. robini* Claparède, setae *cG* ranges between 12µm and 14µm in length. Setae *mG* has an average lengh of 16.68µm (N = 4, SD = 1.44 µm) and its shape is acuminate and simple (Fig. 3), consistent with the description of *R. howensis* Manson by Fan and Zhang [33]. Although in *R. robini* Claparède, setae *mG* ranges in a similar length as in *R. howensis* Manson (between 14µm and 17µm), its shape is distinctively different. It is spine-like in *R. robini* Claparède and acuminate/simple in *R. howensis* Manson. There is a variation in the shape of solenidion ω_1 in the questioned specimen. ω_1 is expanded at its tips to form a spatulate shape (Fig. 3); different from *R. robini*. The number of pairs of setae around the anal opening of the contended specimens is only one pair, ps_3, consistent with *R. howensis* Manson; while for *R. robini* Claparède it is six (6) pairs of setae. The distance between sclerites of oviducts is roughly 21.47µm (N = 4, SD = 1.01 µm) in the studied specimens, as it is in *R. howensis* Manson, and only ranges between 6µm and 8µm in *R. robini* Claparède. The copulatory opening and duct of spermatheca were not observed in these specimens (due to the state of the specimens); therefore, they could not be compared with *R. howensis* Manson in Fan and Zhang [33]. In *R. robini* Claparède the copulatory opening has an incomplete circular shield and the spermathical duct is slender and long.

Mite's distribution and Habitat
Acari from the genus *Rhizoglyphus* commonly thrive under the ground and burrow into corms and bulbs of many ornamental plants and crops [33]. They feed on living as well as damaged tissue of the host plants; some of them graze on fungal material and are capable of ingesting it [38, 67, 73]. So far, since its description, *R. howensis* Manson has only been associated with *Quercus garryana* Dougl. ex Hook. seeds in Australia and palm seeds on the Lord Howe Island, in Australia [33]. Yet, these mites have been lately validated as new pests found on *Persea americana* Mill in Northland, New Zealand, as per the Ministry for Primary Industries (MPI) of New Zealand in 2016. Despite routine checks on plants traded around the globe this species has been only reported in these three close areas in the Australasian region. Unfortunately, due to its rarity not much is known yet on the biology of this species.

Interpretation of R. howensis association with money notes
Plants infested by *Rhizoglyphus howensis* Manson are unique to Australia [33], and, according to the MPI of New Zealand, in 2016 it has been newly recorded in the Northland of New Zealand [74].
The bank notes might have come in direct or indirect contact with one or both types of seeds (Palm or *Quercus garryana* Dougl. ex Hook. seeds) or with *Persea americana* Mill, in the Australasian region. The restricted geographical distribution of the plants and mites, strongly suggests Australasia. The contact could have occured

directly with the seeds, with the plants of these seeds, with the soil or with any other object that was kept close to these plants and, therefore, triggered material transfer, following 'Locards' postulate, contaminating the banknotes by this mite species [75, 76].

Initially, the possibility that the suspect/s travelled to Australia or New Zealand or somewhere else in the Australasian region was considered, and it might have came into contact with these materials; therefore, the mites travelled on the bank notes to Germany. However, the bank notes themselves do not offer the optimal medium for the development of such big colony of mites, indicating that the only way that such a massive colony developed was with the money buried. Access to soil debris of the plants and moisture inside bags (or a similar container) of money would have enhanced the growth of fungal material, on which the mites primarily feed. These, coupled with absence of light seemed to have provided perfect conditions for *R. howensis* mites to thrive into a considerable big colony. According to the forensic scientist, hundred of particles were observed inside bags/containers and between the banknotes, before counting the notes. Despite the counting process of the banknotes by machines, which has caused a great loss of material of forensic importance (e.g. fibers, minerals and mites), a few mites were still present among the debris left in the bags.

Interestingly, in a later, final confession to the police, the offender declared that the money was buried in Australasia, somewhere in Thailand. By following the lead of mite trace evidence remaining on banknotes, it was possible to correctly unravel that the money was: i) buried under the ground, specifically in soil, and ii) buried in, or associated with soil in the Australasian region.

At present, in western Europe, expertise on invertebrate taxa of utility in forensic investigations is scarce, and gradually, and unfortunately disappearing. The considerable lack of forensic protocols and reports for the collection and preparation of these invertebrates for examination exacerbates the problem, leading to the potential disregard or loss of valuable trace evidence. This report aims to encourage new enquiries into trace analysis by forensic practitioners, highlighting the importance of the preservation of any minute traces, and, by providing a basic protocol to prepare mite specimens. The mites could come from any habitat, not necessarily soil, for their further analysis and consultation with experts. The hope is to facilitate and speed up the interpretation of micro-arthropod trace evidence found in a variety of crime scenes.

Declarations of interest: none

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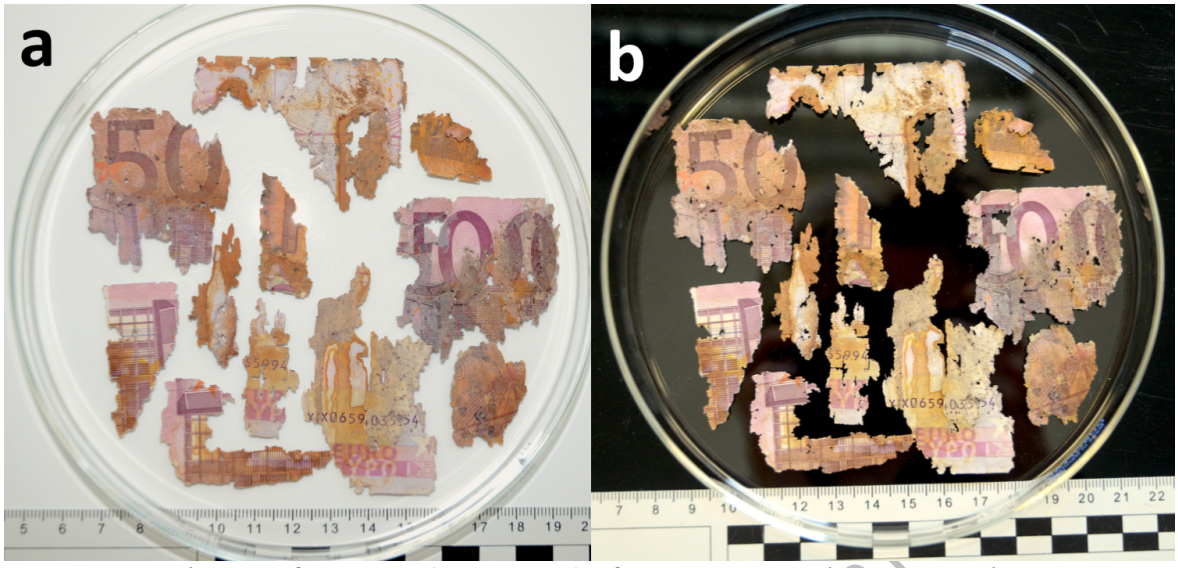

Figure 1. Banknotes' fragments (€500 notes) after exposure to the automated counting machine, when most mites were already lost. The banknotes were 'cleaned' of valuable trace evidence: a) white background; b) black background.

Figure 2. *Rhizoglyphus howensis* Manson (adult female), from this study. Ventral view of Gnathosoma (mouth parts showing the robust base and the translucent tips of the chelicera) and Propodosoma (1st two pair of walking legs).

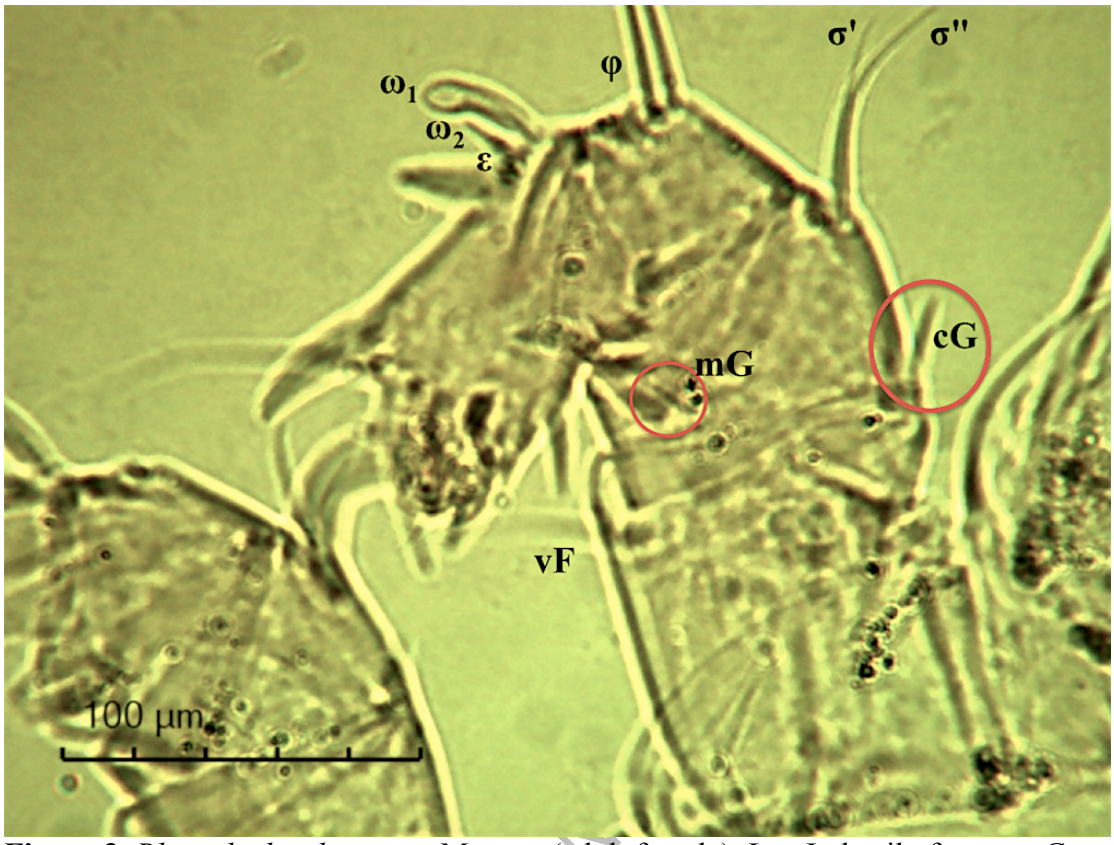

Figure 3. *Rhizoglyphus howensis* Manson (adult female). Leg I, detail of setae *mG, cG, vF,* ω_1, ω_2, ε, φ, σ'and σ".