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CASE REPORT

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PATHOLOGY/BIOLOGY

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Mites (Acari) as a Relevant Tool in Trace Evidence and Postmortem Analyses of Buried Corpses

ABSTRACT: This report interprets the presence of mite species in three clandestine graves in Europe, evaluating their potential use as trace evidence or markers. Grave 1 (Sweden): Two mite species *Rhizoglyphus robini* Claparède, 1869 and *Parasitus loricatus* (Wankel, 1861) were recovered from the surface of a body buried in a shallow grave in an area surrounded by trees, in close vicinity to house gardens. Grave 2 (Germany): Phoretic deutonymphs of *Gamasodes spiniger* (Trägårdh, 1910) were attached to an adult fly (Diptera: Sphaeroceridae) found within a shallow grave containing two human bodies covered in soil and dung. Grave 3 (France): *P. loricatus* were recovered from the soil around a body buried in a deep grave (80 cm under). In graves 1 and 3 both corpses were undergoing advanced decay and skeletization, the locations match with the subterranean habit of *P. loricatus*, highlighting the value of this species as a marker of graves or burials in soil and during late decomposition. *R. robini* is a soil mite that feeds on decayed roots and bulbs; this mite species confirms the location of the corpse within top soil, agreeing with a more specific type of superficial burial, a shallow grave. In case 2, the presence of both coprophiles, the mite *G. spiniger* and the carrier fly confirm association of remains with dung or animal feces. The three mite species are reported for the first time in human graves. There are no previous records of *R. robini* from Sweden.

KEYWORDS: trace evidence, burial, clandestine grave, soil mite, decomposition, marker of decomposition, corpse, Acari, Acaridae, Parasitidae

To conceal a murder (homicide), perpetrators often bury their victims. Such clandestine graves are typically shallow, use a mixture of plant materials and soil and are <50 cm in depth (1,2). VanLaerhoven and Anderson already stated 30 cm as the most common depth for clandestine burials (3). However, in rarer cases illegal graves may also be at much greater depths (4). As decomposition of the body progresses through the five most frequently recognized stages of cadaver decay: fresh, bloated, active, advanced, and dry/remains, it forms a rich source of organic material that is able to sustain a large community of arthropod scavengers (5,6). A number of early studies already showed that arthropods arrive at a carcass in a relatively predictive and successive pattern; different species are attracted to different stages of decay. Analysis of the composition of the arthropod community associated with each decomposition stage and the rate of decay can be used for estimation of the minimum

postmortem interval (PMI min) (5–7) or as trace evidence. Of the great variety of animals accessing corpses in soil, insects such as Diptera and Coleoptera and minute arachnids such as Acari (mites) are often the most abundant and diverse (3,8).

The majority of PMI estimations of exposed corpses utilize necrophagous dipterans, frequently blow flies, as they can colonize a corpse within minutes after death and are therefore important markers of time (6). Estimating the PMI is crucial in every murder investigation. However, it is a challenging task because a decomposing body represents such a rapidly changing and ephemeral habitat. A major factor that can influence the decomposition rate and the succession, diversity, and abundance of decomposer arthropod communities in and around a cadaver is burial (3). Concealment of a carcass results in reduced insect activity which significantly decreases the rate of decay (3,9). Accordingly, the diversity of species, the ecological succession, and the colonizing time periods of major forensic insects are significantly altered or even prevented in a grave environment (2,3,10,11). In such circumstances, the acarological fauna (mites) may become useful as forensic indicator. Mites are a major part of the carrion fauna in outdoor decomposition, particularly those species sheltering in soil (8,12) but are often unnoticed or ignored because of their small size and difficulties in identification. Nevertheless, they are present through all stages of vertebrate decomposition and therefore have huge potential in interpreting a crime scene (13–21).

The vertical distribution of mites in soils (22) means that they can rapidly colonize a buried carcass at varying depths to feed on

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cadaverous tissue as well as predate on micro-organisms, insect larvae, micro-arthropods, and nematodes already inhabiting the carcass or the neighboring soil (8). Mites will also arrive at a buried carcass phoretically, carried by specific dipteran and coleopteran species that can access the corpse through cavities in the soil (23). Phoresy is the dispersal of one organism (the phoront) through the attachment to a host organism (24,25). This relationship is often transient and is displayed by many species of mites during ontogenesis to rapidly exploit ephemeral habitats, such as dung heaps and carrion (23,26). The host-phoront relationship between mites and insects is sometimes highly specific; where the choice of host is restricted to a single or a handful of species.

Therefore, a forensic acarologist can reconstruct the presence of the carrier species even in its absence, from analyzing the species of mites found at the crime scene (18). Mites may also be introduced on a carcass through material transfer on the victim or the perpetrator from an entirely different location and the habitat specificity of mites can be valuable as trace evidence (20,21,27). Jean Pierre Mégnin, the founder of Forensic Acarology, was the first to place mites along with insects and other arthropods throughout the 8 waves of arthropod colonization of exposed cadavers, where the 6th wave was composed entirely of mites (28). Mégnin listed mites as part of the 4 waves of arthropods associated with buried cadavers along with Diptera, Coleoptera, and Lepidoptera (28). In 1898, Motter reviewed bodies buried in coffins up to 150 cm in depth, mites were the most abundant arthropods, and *Uropoda depressa* (described by Mégnin) was the most common species (8,29). Recent analyses of buried carcasses have demonstrated that mites are plentiful in human graves though mites are unidentified or their role mainly unknown (8,14,22,30).

The main aim of this work is to document the mite species occurring in graves in three different biogeographical locations in countries in Europe: Sweden, Germany, and France, as well as to interpret the occurrence of certain species as markers of specific "burial" environments.

Materials and Methods

Description of Studied Graves

Grave 1

During construction work in Central Sweden, the remains of a male were discovered in a shallow grave (<50 cm) on 17 March 2015. The body was found in a small grove near an old manor surrounded by several houses and gardens. Homicide was suspected, and on 24 March 2015, the remains were autopsied. The corpse was partly skeletonized and the abdomen had a layer of adipocere. The internal organs were partly decomposed but relatively intact and the head was almost completely skeletonized. Ten individual mites were collected directly from the clothing during the autopsy and preserved in 70% ethanol. The sample containing the mite specimens were later sent to the Acarology laboratory, University of Reading (U.K.), for identification and interpretation of the acarological evidence. Insect fauna was also collected from the grave. It consisted of Phoridae adults, Piophilidae larvae, and Muscidae pupae. Several individuals of *Rhizophagus parallelocollis* (graveyard beetle—several millimeters in length) indicated a PMI of 10–24 months.

Grave 2

The bodies of 2 individuals were discovered on a horse ranch in a rural area in Germany in June 2014 (Fig. 1). The bodies were positioned horizontally adjacent to each other in a shallow grave of approximately 30 cm depth and was covered with horse manure and soil. The 2 individuals displayed evidence of gunshot wounds. A small adult Diptera was recovered from the samples taken from the grave soil carrying two phoretic mites grasped dorsally to the fly. The Dipteran was identified to the family Sphaeroceridae, *Spelobia* sp., because of the minute size (approximately 1–2 mm) and a characteristically thickened tarsomere of the posterior leg. All specimens were preserved in 70% ethanol. No more insect evidence was present. Mites were sent to the Acarology laboratory, University of Reading (U.K.), for identification and interpretation of the acarological evidence. Based on the clarified identity of the dead and the case reconstruction, a six-week PMI could be assumed.

Grave 3

A skeletonized body was exhumed from a deep soil grave (approximately 80 cm) in the west of France in 2004 (Fig. 2) (4). The soil was mainly clay based, and the body was covered by 40cm of farm quicklime. Entomological evidence was collected: Larvae, pupae, and empty puparia of Heleomyzidae (Diptera) (at 40–60 cm); pupae and empty puparia of scuttle fly *Megaselia* sp. (Diptera: Phoridae)—in hair, reaching 90 cm in depth; adults of unidentified lesser dung flies (Diptera: Sphaeroceridae) at 40–60 cm and adults of rove beetles (Coleoptera: Staphylinidae)



FIG. 1—Grave site of remains of two individuals on discovery in June 2014 in Germany (grave depth approximately 30 cm).

Aleochara sp. The absence of Calliphoridae and Sarcophagidae confirmed burial shortly after death. It was suspected that the victim was killed 6 months before the discovery of the body, during early autumn when the temperatures were likely to be favorable. A few mite specimens were also recovered from the grave, preserved in 70% ethanol, and were sent to the Acarology laboratory, University of Reading (UK), for identification and interpretation of the acarological fauna.

Identification of Mites

The clearing and mounting of mites was based on previously described methods (31). A Nikon Optiphot phase contrast light microscope was used for identification (objectives used were 10 \times , 40 \times and 100 \times). Images were captured with Motic Image Plus 3.0. Several taxonomical keys were used for the identification of mites. For case study 1, key for Astigmata species by Hughes (1976) was mainly used for identification to the genus and species level (32). A number of other keys and descriptions of Astigmata, Acaridae were also used (33–36). For identification of the Mesostigmata, Parasitidae, for cases 1, 2, and 3, a key to Mesostigmata families was first used to identify the mite to the family level (Parasitidae) (37) followed by a key to Parasitidae species (38).

Results and Discussion

Grave 1

Of the sample received, five individuals were identified as the bulb mite species *Rhizoglyphus robini* Claparède, 1869 (Fig. 3). All individuals of *R. robini* were in the hypopial stage, a heteromorphic deutonymph adapted to phoresy (Fig. 4a,b). The rest of the five specimens were identified as adults of the Parasitidae species *Parasitus loricatus* (Wankel, 1861) (Figs 5 and 6).

The hypopi of the genus *Rhizoglyphus* are similar in morphology to those of *Caloglyphus* (*Sancassania*) (Astigmata: Acaridae). Differences can be found in some morphologies such as minute pits evident on the dorsal surface of *R. robini*, the presence of shorter legs, and a transverse line separating the sternal and ventral shield. They also show similarities to *Acarus farris* hypopi (Astigmata: Acaridae).



FIG. 2—Case study 3. Grave site of remains of an individual in France in 2008 (grave depth, approximately 80 cm).



FIG. 3—Hypopus of *Rhizoglyphus robini* (ventral).

However, apodemes IV do not curve or run parallel for a short while as in *A. farris*, but rather meet at a point. Some diagnostic characteristics of *R. robini* are (i) the protrusion of the rostrum covers the entire gnathosoma, (ii) the apodemes do not reach the posterior edge, and (iii) the sucker plate, almost identical to the diagrammatic description shown by Fan and Zhang in 2004 (36), with 2 large central suckers with 6 smaller bordering suckers that are equal in size. In the contested specimens, vertical dorsal setae were not as distinguishable as expected, however, are expected to be relatively short in *R. robini*. Legs IV were slightly longer than expected and visible when viewed dorsally. The morphology of *R. robini* is closely related to *R. echinopus* (Astigmata: Acaridae). However, a number of diagnostic characters unique to *R. robini* rather than *R. echinopus* were identified (33,39–41). For example, these specimens show a gnathosoma entirely covered by the rostrum and not visible dorsally, agreeing with Radwan and co-authors (42).

This is the first report of *R. robini* in Sweden, although the species has a cosmopolitan distribution worldwide and is frequently reported in synanthropic habitats such as greenhouses and gardens in Europe (Table 1). Species of the family Acaridae are important pests of agricultural plants. Within the Acaridae family, bulb mites from the genus *Rhizoglyphus* typically attack bulbs, tubers, or corms of potato, carrot, onion, and garlic plants among other vegetables, as well as flower bulbs in greenhouses and fields (34–36,43). Among the broad variety of plants that *Rhizoglyphus* mites damage, they are most commonly associated with members of the Liliaceae family, one of the largest families of (garden) plants (35). The bulb mite undergoes 6 stages during

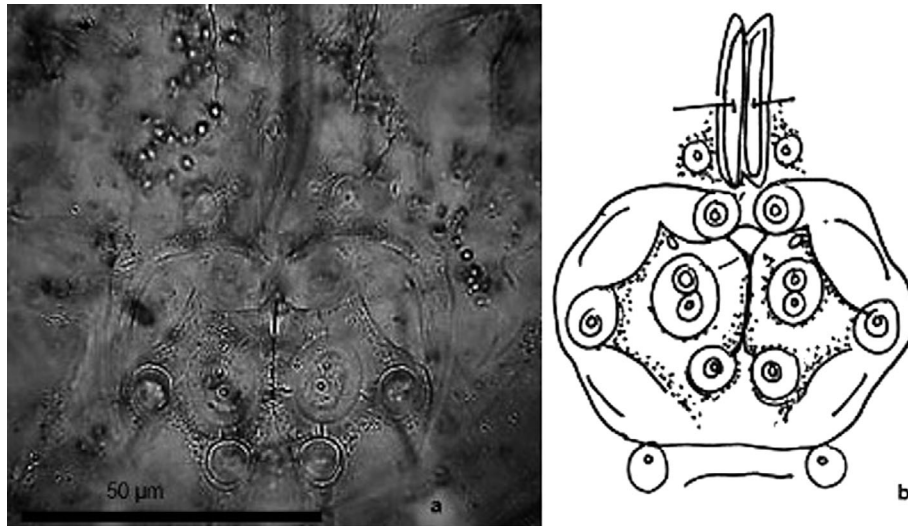


FIG. 4—Ventral sucker plate of *Rhizoglyphus robini* hypopus of case specimens. b. Schematic drawing of the sucker plate of *R. robini*, adapted from previous work (32,36), showing a pair of large central suckers and 3 pairs of bordering suckers that are roughly equal in size.



FIG. 5—*Parasitus loricatus* female (ventral).

its life cycle: egg, larva, protonymph, deutonymph, tritonymph, and adult (44).

Astigmata mites transform into the hypopi (nonfeeding, phoretic deutonymph) in response to deteriorating environmental conditions such as extremes of temperature and humidity and poor food quantity and quality (26). *Rhizoglyphus* hypopi are known

to attach to several species of Diptera and Coleoptera and have been found phoretically associated with Scarab beetles, including *Osmoderma eremicola*, *Bothynus gibbosus*, and *Phyllophaga* spp., which are opportunistic colonizers of animal and human remains (45,46). The abundance of *Rhizoglyphus* hypopi found within populations in the field is generally low since most individuals will molt directly from a protonymph to a tritonymph if there is food available (35).

Only one past study has recovered *R. robini* from soil associated with decomposing surface animal remains, and no previous study has documented its occurrence in graves. Anderson and VanLaerhoven found *R. robini* in the soil beneath surface pig carcasses, along with Dipterans and Coleopterans, in a rural farming area of British Columbia (47). The life stage was not noted. Between one to 10 individuals were found in the soil when the pigs were undergoing the dry remains stage. Considering the location of the case, a rural area surrounded by some houses and gardens, *R. robini* places the origin of the corpse in the environment where it was found. *Rhizoglyphus robini* are considered to favor living plant matter such as the bulbs of common garden plants and ornamentals, to decomposing matter (34). However, the soil surrounding the body was devoid of such vegetation, and this had triggered the production of hypopi. The occurrence of *R. robini* is supported by the presence of a population of *R. parallellocollis* in the grave, which is a small (approximately 4 mm) root eating beetle that feeds on buried organic matter, commonly found in gardens and compost heaps as well as buried corpses (48).

Other 5 mite specimens of *P. loricatus* were recovered from the corpse, 3 females and 2 males. The females (Fig. 5) show the typical roughly triangular opisthonotal shield with the genital shield sharply pointed anteriorly, and the presence of the metasternal shield (38). The lack of diffusion between the genital and opisthogastric plates helped distinguished them from a closer species *P. fimetorum*. The males bear the specific diagnostic characters of the species, such as the leg apophyses (protrusions) on legs II (Fig. 6), a deeply bifid and V-shaped spur of femur II (Fig. 6a), and a clefted corniculli (Fig 6b) (38).

Parasitus loricatus is not restricted to isolated or secluded habitats and has been reported from a wide variety of biotopes

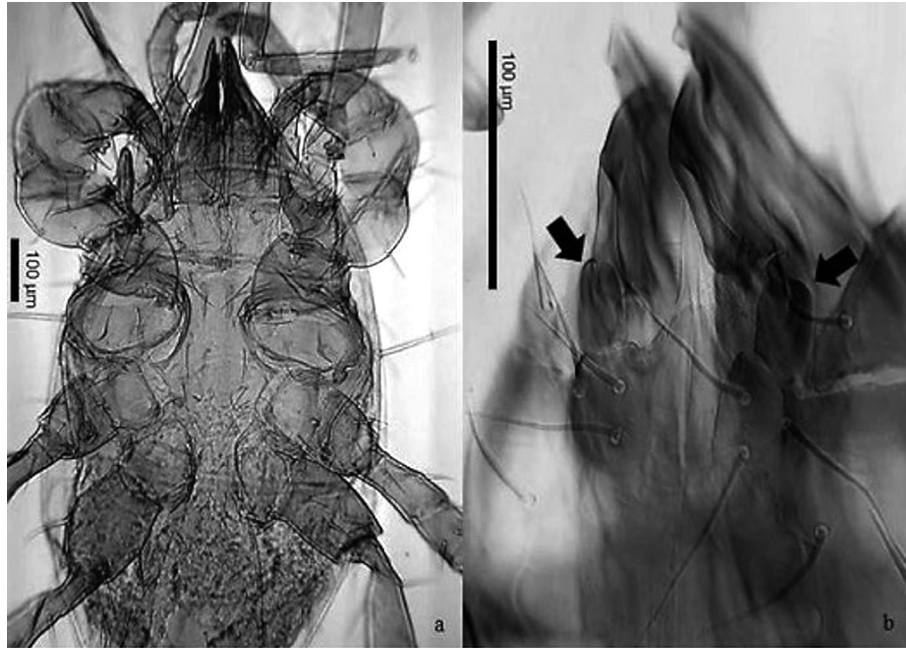


FIG. 6—(a) *Parasitus loricatus* male (ventral); (b) Clefted corniculi of male *P. loricatus* (arrows).

TABLE 1—Occurrence of *Rhizoglyphus robini* in European countries: habitat type, host, and life stage.

Location	Habitat	Host	Life Stage	Reference
Hungary	Unknown	Sour cherry tree	Unknown	(61)
Poland	Unknown	Bird Nests	Unknown	(62)
Poland	Garden	Onions	Ad	(42)
Canada	Rural Farm	Soil beneath pig carcass	Ad	(47)
Norway	Agriculture	Onion dust	Unknown	(63)
Holland	Lily fields	Lily plants	Ad	(64)
Poland	Dwelling	Dust samples	Unknown	(65)
Denmark	Beech woodland soil	Soil	Unknown	(66)
Denmark	Dwelling	Dust from a mattress (<i>Rhizoglyphus</i> sp.) (Unspecified)	Unknown	(67)
United Kingdom		Unknown	<i>Freesia</i> sp., (68)	
	<i>Narcissus</i> sp.	Unknown		
Poland	Rye field	Rye	Ad	(69)
Greece	Unknown	<i>Dahlia</i> sp.	Unknown	(33)*
Holland	Unknown	<i>Amaryllis</i> , <i>Gladiolus</i> sp., <i>Iris</i> sp., <i>Lilium</i> sp.	Unknown	(33)*
United Kingdom		House (70)	Stored products	
Italy	Unknown	Bulbs	Unknown	(71)*
Austria		Bulbs	Unknown	(71)*

Ad, Adult.

*Review paper.

such as forest soil, nests of birds and mammals and semi-aquatic habitats such fish pond litter (Table 2). During analysis of the existent literature on *P. loricatus*, a common and major problem in acarology became apparent. The majority of reports that cite this species describe it as a eu-troglophile species; assuming its origin is from caves. However, the original publication by

Wankel in 1861, written in Dutch, describes the species as a soil dwelling mite found in underground tunnels, often associated with micro nests of small mammals and arthropods (49). The species is found in subterranean habitats such as below-ground nests of rodents (50), justifying its occurrence in graves and on surface terrains such as compost, bird nests and in excavations like graves (this study). There is no past documentation of the association of *P. loricatus* with buried or surface cadavers and this is the first report of this species from a human grave. This species is frequently found in Europe, especially in Southern Sweden, Baltic Island of Gotland, and Norway and is often the most common species of caves (Table 2).

Grave 2

Two mites were found attached to the dorsal surface of a *Spelobia* fly (Sphaeroceridae) and were identified as *Gamasodes spiniger* (Trägårdh, 1910) deutonymphs (Figs 7 and 8) (38). *G. spiniger* deutonymphs are characterized by the presence of spurs on Leg II, on femur, genu, tibia, and tarsus, where femur and tibia bear one spur each (Fig. 7b). The femur spur is thumb shaped with a curved tip, the genu has a shorter more pointed spur, the tibia a rounded spur and the tarsus a short conical spur. Presternal shields are wide and elongated, and the sternal shield is characteristically outlined and partly punctate (i.e., bearing holes). The dorsal setae are mainly short where more than two pairs of dorsal setae are stouter and pilose; the opisthonotal shield (dorsal) bears 14 pairs of setae, where setae Z1, Z3, and J5 are stouter and pilose.

The sternal and opisthogastric setae are typically fine and slender. The specimens differ slightly from the description in Hyatt (1980) (38) in the shape of the sternal shield and lateral spines of the tectum, with dentate lateral margins. This species is a saprophile (associated with dead or decaying matter) and a coprophile (associated with dung); therefore, it is also frequently found in dung or manure (Table 3). The deutonymphs of *G. spiniger* are known to be phoretic with Coleoptera such

TABLE 2—Occurrence of *Parasitus loricatus* in European countries, habitat type, and life stage.

Location	Habitat	Life Stage	Reference
Poland	Caves	Unknown	(72)
Belgium	Subterranean cavities	Ad	(73)
Italy	Caves and subterranean cavities	Unknown	(74)
Slovakia	Fields, surrounded by farms.	Ad, Dt	(50)
	Subterranean nests of mound-building mouse		
Romania	Mountain soil	Unknown	(75,76)
Western Slovakia	Nests of Anseriformes and Passeriformes	Unknown	(77)
South West Slovakia	Forest soil	Unknown	(78)
Poland	Fur of Voles	Dt	(79)
Slovakia	Soil and litter of fishponds and Mallard nests	Unknown	(80)
Northern Slovakia	Caves	Unknown	(81)
Slovakia	Bat dung and soil/sediment of caves	Unknown	(82)
Hungary	Cave	Unknown	(82)
Slovakia	Caves	Unknown	(83)
Sweden	Caves	Ad, Dt	(84)
United Kingdom	Yew	Unknown	(85)
Poland	Grassland	Unknown	(86)

Ad; Adult, Dt; Deutonymph.

as *Copris hispanus* (Coleoptera, Scarabaeidae) as well as Diptera (51), especially small specimens, for example, sciariid flies, which are well known pests of greenhouses (52) (Table 4). Many *Gamasodes* species are predators, existing as parasitic and free-living mites and practice phoretic activity for dispersal into bird nests and the nests of small mammals (38). *Gamasodes* species have also been found phoretically associated with several species of dung beetles (53). *G. spiniger* is a common soil dwelling species in European countries and is also

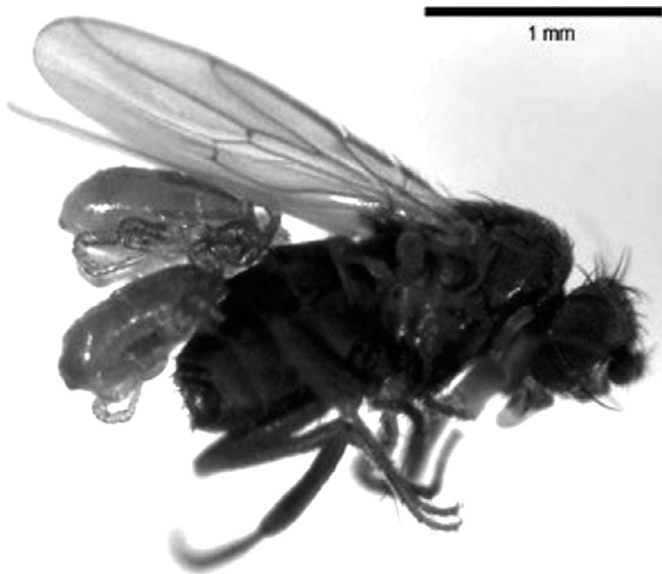


FIG. 8—Two deutonymphs of *Gamasodes spiniger* attached to dipteran (*Spelobia species*).

frequently found inhabiting nests of mammals and birds (Table 3).

There are only three documented cases of *Gamasodes* species associated with animal carcasses. *Gamasodes spiniger* was collected from beneath exposed pig carcasses in a rural farming area during the very early fresh stage of decomposition, with no further occurrence of this species throughout the rest of decomposition (47). Mesostigmatid mites were found in high abundance in the soil directly associated with decaying surface rabbit carcasses in Malaysia, with Macrochelidae species occurring throughout decomposition and Parasitidae mites such as *Gamasodes* sp. (unidentified species) dominating in the late stages of decomposition (46).

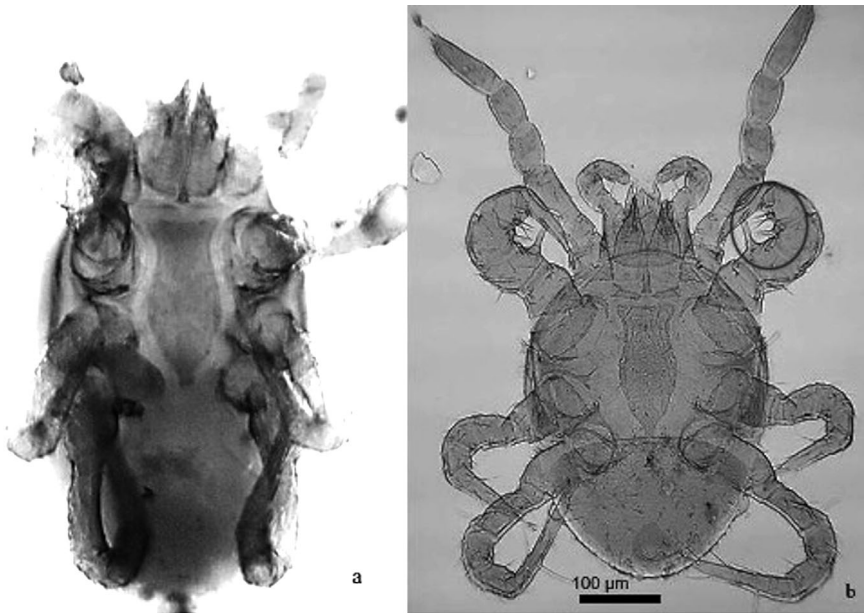


FIG. 7—*Gamasodes spiniger* deutonymph (ventral). (a) Image of specimen from case study. (b) Example of *G. spiniger* (deutonymph), using another specimen not-related to this case, to show the diagnostic features such as the sternal shield and presternal shields which have a characteristic shape, and spurs on femur and genu on Leg 2 (circled).

TABLE 3—Occurrence of *Gamasodes spiniger* in European countries, habitat type, and the life stage.

Location	Habitat	Host	Life Stage	Reference
Romania	Forest	Soil	Unknown	(75)
Belgium	Underground cavities		Dt	(73)
Slovakia	Fields, surrounded by farms	Subterranean nests of mound-building mouse	Ad, Dt	(50)
Poland	Forest	Nests of black stork	Ad, Dt	(87)
Slovakia		Bearded tit	Unknown	(88)
Italy		Bearded tit	Unknown	(88)
Austria		Bearded tit	Unknown	(88)
Southern Sweden	Garden Lawn	Fly (Sphaeroceridae)	Dt	(56)
Northern Ireland	Bramley apple orchard	Leaf and pitfall	Unknown	(89)
Eastern Germany	Sterile soil	Soil	Unknown	(90)
Latvia	Strawberry field	Leaf and pitfall	Unknown	(91)
Poland	Nests of white stork	Diptera	Dt	(56)
Poland and Czech Republic	Mountain range	Bank vole and common Vole	Unknown	(79)
Slovakia	Fishponds and Mallard nests	Soil and litter of fishponds and Mallard nests	Unknown	(80)
Slovakia	Farmland	Nests of Red-backed shrike	Ad, Dt	(92)
England and Wales	Grassland, garden	Yew	Unknown	(85)
United Kingdom	Grassland	Slurry	Unknown	(93)
United Kingdom	Farm	Poultry litter	Unknown	(94)

Ad; Adult, Dt; Deutonymph.

TABLE 4—Literature citing deutonymphs of *Gamasodes spiniger* with associated arthropod host.

Location	Habitat	Host	Reference
Southern Sweden	Garden Lawn	Sphaeroceridae (Diptera)	(56)
Poland	Nests of white stork	Diptera	(55)
Spain	Forest, on pig carrion bait	Flying insects	(54)
Czech republic	Unknown	Sphaeroceridae (in dung)	(52)
Israel	Forest	<i>Copris hispanus</i> (Coleoptera: Scarabaeidae)	(51)

Deutonymphs of *G. spiniger*, phoretic with flying insects, were recovered at irregular intervals colonizing unconcealed pig carrion baits placed on forest soil in North Spain (54). This however is the first report of *G. spiniger* from a human grave.

Phoresy of *G. spiniger* with dipterans has been previously documented but these handful studies have not always indicated the species of Diptera. For example, *G. spiniger* has been found in the nests of white storks in Poland; thought to have arrived through phoretic activity, attached to dipterans, however, the species were not identified (55). There are previous records of

deutonymphs of *G. spiniger* associated with flies of Sphaeroceridae; and of unattached *G. spiniger* deutonymphs found along with Sphaeroceridae flies in manure; but the flies were never identified to species (52). In another study on phoront-host associations between mites and insects in a garden lawn in Southern Sweden, in 1998, a single *G. spiniger* mite was found attached with its chelicera to the abdomen of a Sphaeroceridae fly, at day 100 of a study. More so, a further 17 deutonymphs of *G. spiniger* were collected during the same study (56). However, none of the records identified the species of flies. This is the first confirmation of *G. spiniger* traveling on *Spelobia* species (Sphaeroceridae).

The association of *G. spiniger* with Sphaeroceridae is interesting from the forensic point of view. This is a family of Diptera with a global distribution, occurring in most terrestrial habitats, commonly known as lesser dung flies, which thrive in dung, but also feed on dead animal matter (57). Sphaeroceridae are generally less abundant on vertebrate carrion than Calliphoridae (blow flies). However, in cases where blow flies cannot access corpses, as in the case of burials and particularly if the environment of the grave contains animal dung, the smaller Sphaeroceridae are more adapted to detect and colonize such remains than Calliphoridae. In a study of buried and surface pigs in Michigan, larvae of Sphaeroceridae were recovered from pigs buried at 30 cm but not from pigs buried at 60 cm, 60 days after burial. In the same study, no Sphaeroceridae were found colonizing the surface pigs (2). The puparia of Sphaeroceridae were found in the lead coffin graves of Archbishop Greenfield, buried in 1315 (48) and in 1968, Payne found Sphaeroceridae colonizing pigs buried 50–100 cm in soil during bloating and active decay (58). Interestingly, the species *Spelobia luteilabris* has been previously reported among the dominating dipteran species in both open habitats and forests in Southern Germany feeding on various forms of carrion baits, and in exposed and buried up to 5 cm (57). *Spelobia* species have been collected from exposed pigs in a meadow undergoing late fresh stage during winter in Germany (59). The studied grave in the present work is located in Germany.

In this case, both the fly, *Spelobia* sp. and *G. spiniger* mites were likely attracted to the horse manure that was used to conceal the grave. The occurrence of *G. spiniger* during mid to late stage of decay of the corpses in this case study is not surprising as the species predate on other soil-inhabiting micro-arthropod decomposers of cadavers such as bacteria, fungi, nematodes, and other micro-arthropods. *Spelobia* sp. along with *G. spiniger* seemed to have arrived shortly before the bodies were discovered due to the recovery of a low number of specimens of both species. The simultaneous occurrence of the two species is indicative of late decomposition in graves in livestock-related environments or habitats.

Grave 3

Of the five mites recovered from this corpse, three were identified as *P. loricatus* (male, female, and deutonymph). The other two specimens were relatively fragmented, which prevented their preparation for identification; however, they still showed general similarities with the species. The presence of both adults and deutonymphs suggests at least a single life cycle within the grave, indicating that the decomposition process of the body might have occurred within the isolated grave; information was also complemented by the absence of Calliphoridae and Sarcophagidae flies. This is the first report documenting the

occurrence of *P. loricatus* in a human grave of approximately 80 cm depth, which is considered a deep grave (4). Small size arthropods mainly occupy upper horizons of soils, due to the decreasing porosity of the soil from the surface to deep layers. The fauna of deeper layers of soil is typically scarce and opportunistic (60). This case highlights the value of *P. loricatus* as markers of deep burials. The corpse in this case was undergoing advanced decay with some skeletal remains, similar stage to case study, grave 2. With further studies on the species, it might be possible to define its role in advanced and/or late decomposition within the deep grave environment.

The three case studies confirm the association of mites with decomposing human remains in graves, shallow, and deep and at different stages of decomposition. Exposure to a variety of environments, such as garden soil or dung, allows more information on specificity to habitats, which helps identify specific markers of decomposition, locations, or a stage of decay. This is particularly important when investigating homicide cases and there is little or no insect activity.

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