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Research article

Association of *Myianoetus muscarum* (Acari: Histiostomatidae) With *Synthesiomyia nudiseta* (Wulp) (Diptera: Muscidae) on Human Remains

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Abstract

Synthesiomyia nudiseta (Wulp) (Diptera: Muscidae) was identified during the course of three indoor medicolegal forensic entomology investigations in the state of Texas, one in 2011 from Hayes County, TX, and two in 2015 from Harris County, TX. In all cases, mites were found in association with the sample and subsequently identified as *Myianoetus muscarum* (L., 1758) (Acariformes: Histiostomatidae). This report represents the first records of a mite associated with *S. nudiseta* in the continental United States. In particular, this association is believed to be of potential future value in forensic investigations, as it lends new insight into the community structure of colonizers on human remains in indoor environments.

Key words: forensic entomology, Acari, phoresy, *Myianoetus*

Numerous groups of mites (Acari) are intimately associated with patchy and ephemeral decomposition habitats such as fungi, dung, decaying vegetation, and carrion (Lebrun et al. 1991). In these patches, mites play essential roles in recycling associated nutrients from organic resource pulses back into the ecosystem (Heneghan et al. 1999, Pramanik et al. 2001). These roles include inoculating organic material with fungal spores, fungivory (consumption of fungi), consumption of nematodes, regulation of microbial interactions, and increasing the rate of soft tissue mass loss (Santos and Whitford 1981, Heneghan et al. 1999).

Mites are often present on and around decomposing vertebrate remains, including human, giving this group of arachnids potential use as evidence in death investigations (Goff 1989, 1991; Perotti et al. 2009). In particular, their biology and ecology may be useful in determining a time of colonization (TOC), which could translate to a minimum postmortem interval (m-PMI), assuming colonization occurred soon after death (Tomberlin et al. 2011). Development of arthropods, particularly blow flies (Diptera: Calliphoridae), associated with decomposing human remains currently represents one of the more reliable methods for estimating the m-PMI, which can give insight into the time of death of an individual. Researchers have also been developing tools to use successional patterns, or changes in carrion arthropod community over time, to estimate PMI (Early and Goff 1986, YuKun et al. 2000, Segura et al. 2009). Little attention has been paid to the utility of mites despite the fact they may represent the first and/or final ecological seres (waves) associated with carrion decomposition due to the success associated with blow fly biology in m-PMI estimation (Braig and Perotti 2009, Tomberlin et al. 2011). We report here a fly–mite relationship discovered in the course of routine forensic casework that may aid the integration of information about the Acari into modern casework.

Case Histories

Myianoetus muscarum (L.) (Acariformes: Histiostomatidae) was found in association with *Synthesiomyia nudiseta* Wulp (Diptera: Muscidae) in three medicolegal death investigations involving natural deaths indoors. The first case originated in Hayes County, TX, and was investigated by the Hayes County Sherriff's Office, Dripping Springs, TX, and the Forensic Laboratory for Investigative Entomological Sciences (FLIES) Facility at Texas A&M University, College Station, TX, in April 2011. The other two cases were investigated by the Harris County Institute of Forensic Sciences (HCIFS),

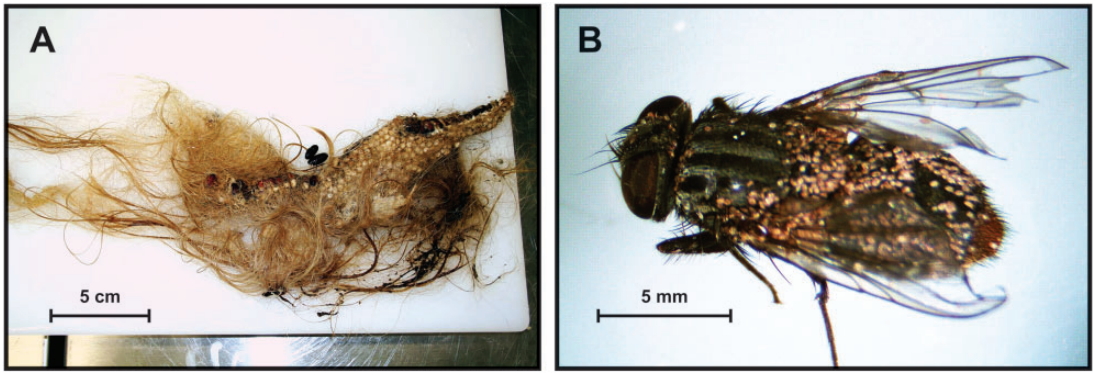

Fig. 1. Samples collected during the investigation of Case #1 from Hayes County. (**A**) Matted hair mass with large cluster of *S. nudiseta* pupae. (**B**) An exemplar of the *S. nudiseta* flies which emerged from the hair mass, immediately after being frozen, demonstrating the extent of phoresy.

Houston, TX, in February 2015 and were located ~40 km apart in Harris County, TX, and 307 km from the Hayes County Case.

Case #1

In April of 2011, during a residential wellness check, law enforcement personnel discovered the individual in question deceased in his bedroom, clothed, under the sheets and blankets of his bed in Hayes County, TX. The bedroom window was open at the time of the wellness check, though no damage to the screen covering the window was evidenced in either the crime scene photos or the police report. Other than clothing, the only organic materials in the room were two unfinished sleeves of crackers. The remains appeared to be in an advanced stage of decomposition, with most of the soft tissue dried and mummified. At autopsy, dipteran larvae and pupae were removed from the body, and pupae were recovered from the decedent's hair and scalp. Pupae were packed tightly in a honeycomb arrangement, with tangled hair between each pupa (Fig. 1A). Larvae and pupae removed from the body were divided into two samples: half were preserved in isopropanol and half were kept alive. The pupal mass from the hair was retained alive, so it could be reared to the adult stage for identification purposes. These entomological samples were sent to the FLIES Facility at Texas A&M University, College Station, TX, for further processing. Voucher specimens of muscid flies and some associated mites are deposited in the TAMU Insect Collection under voucher number 710. Voucher specimens of mites are deposited in the University of Michigan Museum of Zoology under Voucher number BMOC 11-1219-001. Sarcophagid fly specimens were destroyed by the law enforcement agency per their standard operating procedure before identification could be made (see section below).

Entomological Analysis

The preserved samples were identified to the family level while the live samples were placed in a mosquito-breeding container (Mosquito Breeder, BioQuip, Rancho Dominguez, CA) in an environmental growth chamber (136LLVL Percival, Percival Scientific Inc., Perry, IA) at 27°C, 70% RH, and a photoperiod of 12:12 (L:D) h to allow for adult emergence. The preserved larvae were slightly decomposed, making species level identification difficult. However, based on spiracular morphology as decribed by Stojanovich et al. (1962), the samples were tentatively identified as a mixed sample containing individuals belonging to three dipteran families: Calliphoridae, Sarcophagidae, and Muscidae. Further identification to a lower taxonomic unit was not possible given the degradation of the sample. Additionally, the Sarcophagidae larvae were not identifiable, as keys are only available for adult males (Pape and Dahlem 2010), and molecular based identification infeasible due to cost constraints and standard operating procedure. The pupal samples collected from the body of the decedent began yielding adult flies 16 d after discovery of the remains. Emergent adults were identified as *S. nudiseta* (Cole 1969) and Sarcophagidae (Dodge 1958). None of the emergent adults from the body of the decedent were associated with mites. Adults began emerging from hair sample pupae 15 d after discovery and all were identified as *S. nudiseta*. Emergent adults from the hair sample exhibited large (>100/ fly) populations of mites (Fig. 1B).

Meteorological Information and Time of Colonization

Hourly weather data were obtained from the National Oceanic and Atmospheric Administration servers for Austin-Bergstrom Airport (WBAN # 13904), ~36.8 km from the decedent's home. The nearest weather data, from the Dripping Springs weather station (COOP # 412585; ~8.7 km away), only reports manually recorded daily maxima and minima. When average temperatures were compared over the period April 1–April 17, Austin-Bergstrom averaged 0.48°C warmer, which was accounted for during Accumulated Degree Day/ Accumulated Degree Hour calculations. A morgue storage temperature of no more than 5°C was assumed for the period between pickup and autopsy.

The biology of *S. nudiseta* has been well studied and data sets are available (Siddons and Roy 1942, Rabinovich 1970, Baharudin et al. 1994, Devi et al. 2011). Therefore, TOC estimates were calculated using *S. nudiseta*. Due to the difficulties inherent in identification of flies in the family Sarcophagidae, as they require molecular identification or specialized techniques and keys, there are limited data sets available (Knipling 1936, Pape and Dahlem 2010). As a result, utilization of sarcophagid specimens for TOC estimates can be challenging. Based on development (Kumara et al. 2009, Devi et al. 2011) and weather data, *S. nudiseta* is estimated to have begun colonizing the remains no less than 7–8 d prior to discovery. The adult flies reared from the hair mass were also covered in mites in various stages of development, with many in the dispersing deutonymphal stage (Fig. 2). No mites were observed on the adult sarcophagid flies which eclosed from the pupae collected from the body of the decedent.

Case #2

This scene consisted of a one-bedroom apartment located on the ground floor of a multiunit apartment building in Harris County, TX. The apartment was locked and secured with no obvious points of entry for insects other than around closed doors and windows.

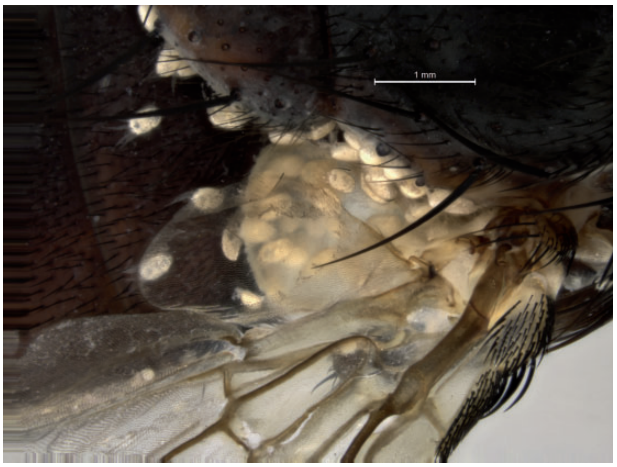

Fig. 2. Close up of deutonymphyal mites around the calypters of an *S. nudiseta* fly that emerged from the hair mass collected from Case #1.

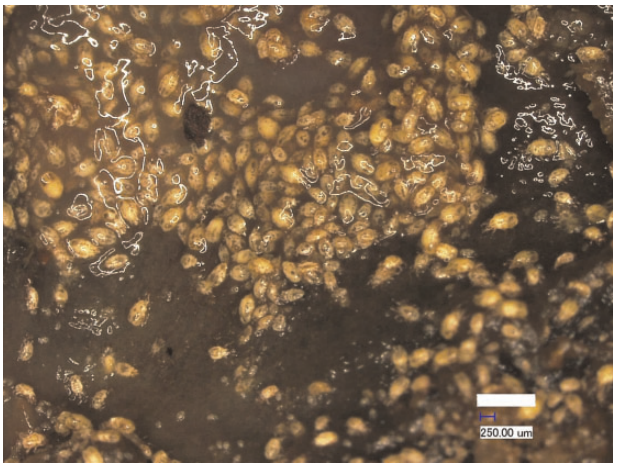

Fig. 3. Large number of mites found accumulated on defrosted beef liver in rearing container containing specimens of *S. nudiseta* larvae reared to the adult stage from Case #2.

The male decedent was located in the bedroom on the bed. He was found in a moderate state of decomposition (similar to the bloat stage as in Reed 1958, Rodriguez and Bass 1983) lying prone on the bed. He was clothed in a t-shirt and socks and the lower part of his body was partially covered by a bed sheet. An electric heating pad was observed under the decedent's left torso, but the electricity to the apartment had been shut off at the time of scene investigation. Small brown pupae consistent in appearance with scuttle flies, Phoridae, were observed on the decedent's buttocks and larval insect activity was abundant under the decedent. A small maggot mass was observed under the left shoulder. Additional scuttle fly pupae and partially completed silken cases, consistent in appearance with *S. nudiseta* (Ferrar 1980), were observed in the folds of the sheet next to the decedent's right upper body. Representative samples of the larval and pupal insect activity were collected at the scene following HCIFS standard operating procedures for forensic entomology, during the middle of the day on 5 February 2015.

The thermostat in the apartment was observed to be set to 76°F (24.5°C); however, the electricity to the apartment had been shut off for an unknown period of time prior to scene investigation and the ambient temperature recorded inside the apartment was recorded as 62.4°F (16.9°C). The temperature observed at the nearest weather station, located at William P. Hobby Airport (Station #12918), at this same time was 9.1°C cooler. The TOC estimate for this case was based on the formation of pupal *Megaselia scalaris* Loew (Diptera: Phoridae) (Greenberg 1991) and suggested a TOC of mid-January 2015. The TOC for the *S. nudiseta* was estimated to be ~10 d later based on development data through the completion of larval stages at 20.0°C and base 3.0°C (Velásquez et al. 2013).

HCIFS standard operating procedures for forensic entomology require that when sufficient samples are collected of representative specimens, a portion of those specimens are reared to the adult stage (or as far as is possible) to ensure accurate identification. Specimens were reared on defrosted beef liver on a sand substrate in a Bioquip mini-mosquito breeder in a Percival I-30VL incubator set to 27.0°C during day and 25.0°C during night, with a photoperiod of 12:12 (L:D) h. At the conclusion of rearing the *S. nudiseta* specimens, a very large number of mites were observed in the bottom of the rearing container on the remaining beef liver (Fig. 3). The container had been frozen to kill and preserve the adult flies that emerged from the rearing. Representative mite specimens were preserved and shipped to BMOC for confirmation of identification as *M. muscarum*. Mites were not observed on the fly specimens which emerged from this rearing.

Case #3

This scene consisted of a two-story multibedroom single-family residence where the decedent had resided alone also in Harris County, TX. The house was closed, with no signs of forced entry, open windows, or doors that would allow for obvious insect access. The male decedent was found in an advanced state of decomposition (similar to the decay stage as in Reed 1958, Rodriguez and Bass 1983) on the floor of his bathroom. He was lying prone and was clothed in a long-sleeved pajama shirt, t-shirt, and socks. Insect activity consisted mainly of pupae and pupal exuvia covering the body and surrounding the body on the floor. Silky material was observed covering many of the pupal cases, which was consistent in appearance with the fly, *S. nudiseta* (Ferrar 1980). These pupae were present under the body in a pattern consistent with the decedent's position on the floor. Small brown pupae, consistent in appearance with scuttle fly, Phoridae (Diptera), were observed on the decedent's shirt. Representative specimens were collected by the forensic investigator at the scene during the middle of the day on 17 February 2015. This sample included pupae, pupal exuvia and adult flies that were slowly moving on the decedent's upper left arm. Specimens were stored in the morgue cooler (at ~4.6°C) for several days before being transferred to the forensic entomologist; therefore, no rearing was performed.

The ambient temperature recorded at the scene was 68.2°F (20.1°C), which was ~9.0°C warmer than the temperature recorded outside at the nearest weather station, located at Hooks Memorial Airport (Station #53910). The thermostat settings were not documented in this case, but an assumption of warmer indoor temperatures was made to estimate the TOC based on the formation of pupal *S. nudiseta* as late January 2015 (based on rearing at 20.0°C and base 3.0°C; Velásquez et al. 2013). In addition to the *S. nudiseta* pupae, pupal *Chrysomya rufifacies* Macquart (Diptera: Calliphoridae) were collected that indicated a more recent colonization time. The adult flies that were so slow moving at the scene as to be collected by hand, were identified as *S. nudiseta* and were observed to be carrying a number of mites (Fig. 4). These mites were later identified as *M. muscarum* by BMO.

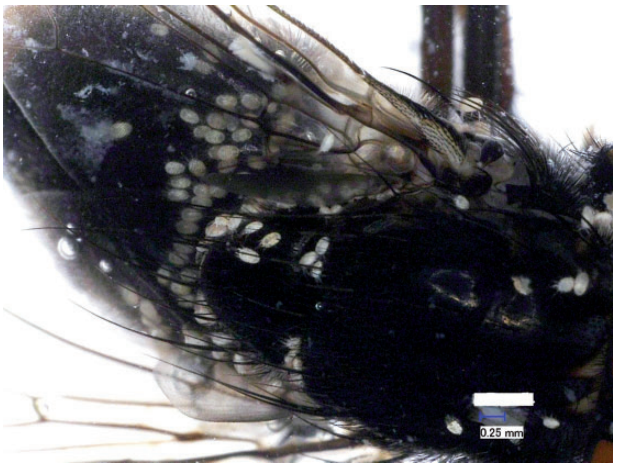

Fig. 4. Adult *Synthesiomyia nudiseta* with abundant *Myianoetus muscarum* collected from decedent in advanced state of decomposition, indoors in February 2015 from Harris County, TX. Details are described in Case #3.

Synthesiomyia nudiseta

Synthesiomyia nudiseta is a cosmopolitan species found throughout tropical and subtropical zones (Rabinovich 1970, Devi et al. 2011). In North America, this taxon can be found from California (Cole 1969) to Texas (Wells and Greenberg 1994), and from North Carolina to Florida (Byrd and Castner 2010). Aside from decomposing vertebrate remains, this species also breeds in decomposing vegetable matter, vertebrate waste, garbage, and refuse (James 1947, Cole 1969) and has been a documented myiasis-causing agent in humans (Siddons and Roy 1942, James 1947). *Synthesiomyia nudiseta* larvae also display predatory tendencies on carcasses, and have even been found to consume larvae of the hairy maggot blow fly, *C. rufifacies*, which is also a facultative predator (Kumara et al. 2009). Larval morphology of this species is similar to other muscids, with a creamy white appearance and lack of posterior tubercles (James 1947). However, the larvae of *S. nudiseta* produce a silk-like material to form a cocoon enveloping the puparium (James 1947). This material often adheres the pupa to a substrate after it hardens. *Synthesiomyia nudiseta* adults superficially resemble flesh flies (Diptera: Sarcophagidae), as they are large (7–10 mm) and possess a tessellated abdomen. However, flesh flies possess three longitudinal stripes on the thorax and generally a red apical abdominal segment, while *S. nudiseta* possesses four longitudinal stripes and a yellow or orange apical abdominal segment (James 1947). This species can also be differentiated from other muscids, as it lacks setation on the antennal arista (Cole 1969).

The variable habits of *S. nudiseta* make this species relevant to forensic investigations involving human remains and cases of human myiasis. This taxon arrives at a decomposing vertebrate resource concurrently with flesh flies but may exhibit variable developmental rates that delay pupation so that it will also occur with later seres of flies. This taxon has been documented to occur on a decomposing vertebrate resource from the fresh stage (Wells and Greenberg 1994) to skeletonized remains (Segura et al. 2009). Since this species can also be associated with garbage and refuse, remains exposed to an environment containing such materials (e.g., a human residence) may experience colonization by this fly or other garbage-breeding flies. This species of fly has been documented to colonize human remains in indoor environments, particularly in Malaysia (Baharudin et al. 1994, Kumara et al. 2009, Devi et al. 2011, Syamsa et al. 2012). Other than descriptive works, however, little is known about the ecology of *S. nudiseta* and the impact of interspecific interactions with arthropods other than flies on its development or behavior.

Myianoetus spp

The mites in this case were originally identified as an unknown species in the genus *Myianoetus* (Acariformes: Histiostomatidae). This genus includes species that specialize on fleeting resources, such as dung (Eraky 1993), and vertebrate carrion including human remains (Russell et al. 2004, OConnor 2009). Species in this genus are phoretic on flies belonging to several dipteran families, such as Sphaeroceridae (Fain et al. 1980, Samšiňák 1989), Ceratopogonidae (Fain and Domrow 1980), Muscidae (Greenberg and Carpenter 1960, Nuorteva 1971, Negm and Alatawi 2011), and Calliphoridae (Greenberg and Carpenter 1960, Miranda and Bermudez 2008). Like most other free-living astigmatid mites, these mites exhibit a morphologically and behaviorally specialized deutonymphal instar that facilitates dispersal by permitting persistent attachment to appropriate hosts (Krantz and Walter 2009). Under laboratory conditions, a species identified as *Myianoetus muscarum* (L.) exhibited attraction to *Muscina stabulans* Fallen (Diptera: Muscidae), *Stomoxys calcitrans* L. (Diptera: Muscidae), *Lucilia sericata* Meigen (Diptera: Calliphoridae), and *Musca domestica* Linnaeus (Diptera: Muscidae) only after a puparium had been formed and only if the mites were in their deutonymphal stage (Greenberg and Carpenter 1960). The taxonomy of *Myianoetus* suffers from inadequate descriptions of species and lost or nonexistent type and voucher specimens, making the identity of taxa mentioned in prior works questionable (e.g., Greenberg and Carpenter 1960, Nuorteva 1971) (OConnor et al. 2015).

Identification of Mites

Comparison of specimens collected in this case with other species described in this genus yielded some slight challenges: the adults appeared clearly different from any other described species in the genus. Characteristics of the collected adults that had not been previously noted within the genus *Myianoetus* include branched paraproctal setae in the female, a large dorsal hysteronotal sclerite in the male, and rows of sclerotized tubercles along the ventral midline of the body and first tarsus of the male.

While the adults appeared distinct from any described species, deutonymphs were identical to those described as *M. muscarum* by Hughes and Jackson in their revision of the Histiostomatidae (1958). The deutonymphs illustrated in this work were based on specimens collected from *M. stabulans* from London, Ontario, Canada. Other host and locality records for "*M. muscarum*" were also given. Some of these are from the older European literature when the number of species in *Myianoetus* was few and most were simply referred to as *M. muscarum*. Those authors also reported this species from North America collected from "houseflies" in Berkeley, California, *Calliphora terraenovae* Macquart from Pullman, Washington, USA, and *S. nudiseta* from San Juan, Puerto Rico. Illustrations of adult *M. muscarum* were reprinted from figures from an older paper that indicated adults were reared from deutonymphs (Cooreman 1947); however, deutonymphs were not illustrated in this paper. The adults from the Cooreman paper appeared clearly distinct from those collected as evidence in this case. The specimens from Canada and California housed in the US National Museum collection have been examined and found to be conspecific with the deutonymphs collected in this case. Surprisingly, when we examined the voucher specimens used by Cooreman (1947) for his redescription of *M. muscarum*, and the deutonymphs from which he reared

those adults, housed in l'Institut royal des Sciences Naturelles in Brussels, Belgium, both turned out to be identical to our material from Texas. Cooreman's published description and figures of the adults contain numerous errors and omissions, notably the female paraproctal setae are branched, not simple as illustrated, and the single, damaged male has tubercles on the anterior tarsi (dorsal sclerotization and ventral idiosomal tubercles could not be observed due to the damage to the specimen). To stabilize the identity of *M. muscarum*, the species has been redescribed and a neotype has been designated from among Cooreman's specimens (OConnor et al. 2015).

Discussion

Phoresy is common among mites and carrion insects. Because mites will attach to primary colonizers, such as blow flies and house flies, they have the potential to become some of the first arthropods on remains. The presence of mites on a resource may have important implications to the forensic entomologist, as the mites can arrive early in decomposition and potentially prey on eggs and larvae of primary colonizers (Blackman 1997). The coevolution of mites and their insect hosts has led to a dual succession: as insect species replace each other temporally throughout decomposition, mite species associated with those insects also change over time. Acarological succession involving both incidental soil mite communities and phoretic mites hold great potential with regard to estimating TOC in forensic investigations (Byrd and Tomberlin 2010). For example, some astigmatic mites, such as species of Lardoglyphidae, tend to arrive later in decomposition and consume dry tissue, fungi, and various microbes (Perotti and Braig 2009). Saprophagus mites display life history traits conducive to survival in environments that rapidly change, giving them the potential to generate large populations over short periods of time (OConnor 2009). The presence and abundance of specific mite species could help to establish more accurate TOC estimations in later stages of decay.

A small number of case studies involving late stage human decomposition utilize mite evidence in order to estimate an approximate TOC. Mite evidence collected from soil associated with the skeletal remains of a child on the island of Oahu, HI, was used to support PMI estimations based on insect evidence (Goff 1991). In this case, successional data of mites on carcasses from a previous study (Goff 1989) were utilized in order to narrow the estimation, which corresponded to the actual date of death of the child (confession of murderer). Mites in the genus *Myianoetus,* along with other genera, were also associated with a child's remains stored inside a plastic bag in the basement of a residence in Germany (Russell et al. 2004). These researchers utilized mite community structure and estimated number of generations on and around the remains to state that the remains had been decomposing between 1.0–1.5 yr. Perhaps one of the most famous examples of acarological evidence applied to a death investigation is that of the mummified newborn in Paris, France, by Mégnin (1894). Not only were mites used to estimate PMI in this case, but this case serves as one of the first recorded instances of applied forensic entomology. Mégnin originally gave an estimation of 5 mo; however, modern analysis of the evidence and drawings produced by Mégnin place the PMI around 8 mo (Perotti 2009).

In the present cases, large numbers (what appeared to be in the thousands) of *M. muscarum* were associated with the forensically important fly *S. nudiseta*, which had been sustained on human remains found indoors. No known association between the host and the phoront exists in continental North America. The previous record of "*Myianoetus muscarum*" from *S. nudiseta* in Puerto Rico cannot be verified in the absence of voucher specimens and the previous confusion over the use of this name. This finding represents both a new biological record and a hypothesized ecological relationship with forensic potential. Given the limited data on the associations and relationships of mites, flies, and carrion, caution should be exercised in using these relationships to estimate the TOC as related to an m-PMI in an applied forensic context. Further work is necessary to document the species, conditions, and host–phoresy associations found within the carrion ecosystem in both indoor and outdoor cases.

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