EXTRACTION EFFICIENCY AND IDENTIFICATION GUIDE TO COMMON HOUSE DUST AND STORAGE MITES

by

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ABSTRACT

House dust mites (HDMs) and storage mites are serious indoor pests because they produce allergens that cause issues with allergy and asthma in humans. In order to study these animals they must be extracted from the urban landscape. Two methods of extraction were tested using *D. pteronyssinus*. The heat escape and flotation techniques were examined using samples of known numbers of mites directly from cultures, in dust, and kapok. Extraction efficiency was overall low, but the flotation method provided better efficiency than heat escape. An introductory identification guide and key was created using images from light, confocal, and SEM microscopy to illustrate common species of HDMs and storage mites.

INDEX WORDS: House dust mites, *Dermatophagoides*, extraction efficiencies, kapok, identification guide, storage mites

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DEDICATION

For my parents

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CHAPTER 1

INTRODUCTION

House dust mites (HDMs) and storage mites are groups of synanthropic mites. These small mites are found worldwide and can sometimes build up to large numbers (Chew et al. 1999, Arlian and Morgan 2003, Colloff 2009). The major concerns with these mites are the allergens that they produce and release into their environment, often through their waste products (Colloff 2009). House dust mite allergens are a serious problem, with some studies reporting that 27.5% of people have a positive skin reaction to them (Arbes et al. 2005). Sensitization can lead to issues such as asthma, allergy, hay fevers, and atopic dermatitis (Nadchatram 2005). Asthma in particular is a major issue in industrialized countries. It is the number one cause of childhood visits to the hospital in the United States (Bonnefoy et al. 2008).

There are several species of HDMs and storage mites that may be found within the home. House dust mites are members of the family Pyroglyphidae, examples include *Dermatophagoides pteronyssinus* and *D. farinae*. Storage mites are non-pyroglyphid mites found within the home, such as *Blomia tropicalis*, *Suidasia* sp., and *Tyrophagus* sp.

Studies of HDMs that are done either in the field or laboratory need a reliable estimate of the quantity of mites that can be extracted from household fabrics (Mehl 1998). The goals for this project are:

1. Find the connection between the weight of pure samples of *D. pteronyssinus* and *D. farinae* mites and the actual number of mites in these samples.

2. Find the percentage of mites that can be extracted from clean samples, dust, and kapok fibers using two different extraction methods – heat escape and flotation.

This information will make it easier to find the number of mites in a sample using weight instead of individually counting these small mites. Finding the extraction efficiency for removing mites from dust, the substrate where these mites are usually found, and from kapok fibers, a fiber often used in mattresses, will aid in estimating the number of mites within a home.

Another issue with these mites is their identification. The typical house has multiple species present in it. Thus, the goal of the second project was to develop an identification guide that can be used by those with or without a science background for the most common species of mites found within the home.

References Cited

Arbes, S. J., P. J. Gergen, L. Elliott, and D. C. Zeldin. 2005. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. J Allergy Clin Immunol 116: 377-383.

Arlian, L. G., and M. S. Morgan. 2003. Biology, ecology, and prevalence of dust mites. Immunol Allergy Clin North Am 23: 443-468.

Bonnefoy, X., H. Kampen, and K. Sweeney. 2008. Public health significance of urban pests, vol. World Health Organization, Copenhagen, Denmark.

Chew, F. T., L. Zhang, T. M. Ho, and B. W. Lee. 1999. House dust mite fauna of tropical Singapore. Clin. Exp. Allergy 29: 201-206.

Colloff, M. J. 2009. Dust mites, 1 ed, vol. CSIRO Publishing and Springer, Collingwood, Australia.

Mehl, R. 1998. Occurrence of mites in Norway and the rest of Scandinavia. Allergy 53: 28-35.

Nadchatram, M. 2005. House dust mites, our intimate associates. Trop Biomed 22: 23-37.

CHAPTER 2

LITERATURE REVIEW

Introduction

House dust mites (HDMs) and storage mites can be found within the urban environment and are important sources of indoor allergens (Arlian and Morgan 2003, Colloff 2009). These mites are very small (240-435 µm in length and 3.5-13.0 µg in weight), and are able to survive in household dust and fabric material, like carpet, bedding, and furniture (Arlian and Morgan 2003, Bonnefoy et al. 2008). They live in their food source, which consists of shed human skin, pollen, spores of microorganisms, fungal mycelia, and bacteria (Hay et al. 1992, Bonnefoy et al. 2008). The allergens that these mites produce are able to accumulate in the home, which can negatively affect sensitized humans (Colloff 2009).

House Dust Mite Taxonomy

Mites are eight-legged arthropods found in the class Arachnida, which also includes spiders, scorpions, and harvestmen (Krantz et al. 2009). Furthermore, mites are found in the subclass Acari, which also contain ticks (Krantz et al. 2009). HDMs and storage mites are classified within the order Astigmata (Colloff 1991, Arlian and Morgan 2003). Astigmatid mites do not have a respiratory system and instead use their cuticle for gas exchange (Arlian 1989) this leaving them susceptible to changes in humidity (Warner et al. 1999).

HDMs are in the family Pyroglyphidae (van Bronswijk 1981). There are 46 species in this family, not all of which are found within the home (Fain et al. 1990). Many pyroglyphid species inhabit niches that put them in close proximity to birds (van Bronswijk 1981). The two

most widely distributed HDMs are *Dermatophagoides pteronyssinus* and *D. farinae*, commonly known as the European and American house dust mites, respectively (Arlian et al. 2002). Despite the region-specific common names, both of these mites occur worldwide (Colloff 2009).

The functional group known as storage mites is composed of species that may be predominant within homes (Arlian and Morgan 2003). These mites are known as storage mites because they are often found in facilities such as barns, silos, and other agricultural facilities (Warner et al. 1999). Among the species that are considered storage mites is *Blomia tropicalis* of the family Echimyopodidae (Bonnefoy et al. 2008, Krantz et al. 2009), which is known to inhabit homes in tropical regions (Arlian et al. 1993). In addition, mites in the genus *Suidasia* (family Suidasiidae) are also commonly found in house dust and stored food products in warmer climates (Krantz et al. 2009). The family Acaridae contains *Tyrophagus* sp., which can be found in stored products (Warner et al. 1999).

House Dust Mite Lifecycle

Mites pass through several developmental stages before they reach adulthood. A molt, as with all arthropods, is required in order for a mite to move from one stage to the next (Colloff 2009). The number of molts and stages that a mite passes through is dependent on the species, temperature, and humidity (Colloff 2009). The general life stages of a mite include the egg, larva, nymph, and adult stages (Bonnefoy et al. 2008). Larval mites are easily identified because they have 3 pairs of legs whereas nymphal and adult mites have 4 pairs (Colloff 2009). Species of HDMs can have a quiescent or inactive phase, similar to a hypopus stage in other mites, if they develop under optimal conditions (Arlian et al. 1990, Arlian and Dippold 1996, Colloff 2009). Mites undergo metamorphosis during the quiescent stage within the previous stage's exoskeleton (Arlian et al. 1990, Arlian and Dippold 1996). The hypopus stage may be present in

glycyphagoid and acaroids mites, and it allows for dispersal or survival in non-ideal conditions (Colloff 2009). The entire lifecycle, from egg to adult, for *D. pteronyssinus* lasts 122.8 ± 14.5 days (Arlian et al. 1990), while *D. farinae* has a mean lifespan of 140.1 ±14.7 days (Arlian and Dippold 1996). *Dermatophagoides pteronyssinus* and *D. farinae* females mate more than once during their lifetime (Alexander et al. 2002). *Dermatophagoides pteronyssinus* females lay a mean of 2.5 ± 0.7 eggs/day/female (Arlian et al. 1990), while *D. farinae* females lay a mean of 2.2 ± 0.1 eggs/day/female (Arlian and Dippold 1996, Alexander et al. 2002).

Medical Importance

HDMs and storage mites are microscopic, yet they produce allergens that may have major health effects on humans including respiratory conditions such as allergy, hay fever, rhinitis, asthma, and even atopic dermatitis (Nadchatram 2005). Respiratory problems appear in sensitized humans when mites – dead or alive -, their fecal pellets, or their byproducts are inhaled (Nadchatram 2005). The typical fecal pellet of a HDM is between 10-40 µm in diameter, and can easily be aerosolized and inhaled (Tovey et al. 1981).

Allergy is an exceptionally common ailment in most parts of the world (Platts-Mills and de Weck 1989). About 23.2 million homes in the United States contain the high allergen levels associated with the development of asthma (Zeldin et al. 2001). Worldwide, asthma leads to about 500,000 hospitalizations each year, of which 34.6% involve children (Sharma et al. 2011).

Mite allergens are found throughout the home. High allergen levels are most often found in areas inhabited by HDM's and include mattresses, carpets, corners of rooms, and under beds (Nadchatram 2005). The bed has the highest concentration of HDMs due to human skin dander accumulating in the bedding during sleep (Nadchatram 2005). Mite allergens are grouped according to biochemical composition, homology, and molecular weight (Diego et al. 2011).

Group 1 and Group 2 HDM allergens have been widely studied (Arlian 2002). Group 1 (Der f 1 and Der p 1) allergens are glycoproteins with cysteine proteases that are produced by cells in the intestinal track of mites (Strom et al. 1980, Tovey and Marks 1990, Thomas et al. 1991). Therefore, Der f 1 and Der p 1 are the allergens found in fecal material of the mites (Strom et al. 1980, Tovey and Marks 1990, Thomas et al. 1991). Group 2 allergens are nonglycosylated proteins which are either produced in the male reproductive tract or are associated with molting proteins and are found in the shed skin or dead mites (Lind 1985, Yasueda et al. 1986, Heymann et al. 1989, Thomas and Smith 1998).

It should be noted that HDMs are not the only agent that can leave allergens in the home. Others include cockroaches, cats, dogs, fungi, nitrogen dioxide, domestic birds, endotoxins, pollen, and environmental tobacco smoke (2000). One study found that 43% of people in the United States are allergic to at least one allergen found within homes (Arbes et al. 2005). Another showed that dust mites were the most common allergen that people had a positive reaction to, at 27.5% (Arbes et al. 2005). The German cockroach *Blatella germanica*, a common indoor arthopod, is also responsible for a large portion (18.1%) of allergic reactions (Arbes et al. 2005). The prevalence of cockroach allergies in children in inner cities of the United States was 36.8% as indicated by a positive skin reaction (the testing was done with a mixture of *Blattella germanica* and *Blattella americana*), while HDMs produced a reaction in 34.5% (Rosenstreich et al. 1997).

Discovery of HDMs as a source of allergens

A correlation between house dust and asthma has been known for a long time (Major 1953). A *Treatise on Asthma* published in 1698 by Floyer mentioned health issues that appeared in asthmatics when house dust was swept (Andrews 1976). It has been known since 1922 that

allergens can lead to asthma attacks (Coca and Cooke 1923), but the responsible components in house dust were not known (Kern 1921). As a result, there were attempts to decrease the levels of dust within homes (Van Leeuwen 1924). In 1923, it was noticed that a species of mite, *Pyemotes centricosus*, found in stored grain could produce an allergic reaction in people (Colloff 1991). Mites within the home, however, were first found in 1928 by brushing bedding and using a microscope to look at the dust (Dekker 1928). These mites were studied by Voorhorst and Spiekma-Boezeman who hypothesized that the mites may be the source of house dust allergy (1964). The mites they found were *D. pteronyssinus*, first described in 1864 by Bogdanov, which before this that was not considered a common species (Voorhorst et al. 1964). *Dermatophagoides pteronyssinus* was confirmed as a source of allergens in house dust in 1967 (Voorhorst et al.). The allergen produced by *D. farinae* was described in 1968 (Miyamoto et al.).

Control of HDMs

The most intuitive option for decreasing allergic symptoms is to reduce the amount of allergens, and therefore the amount of mites within a home (Nadchatram 2005). It is critical that live and dead mites be removed because both can cause allergenic reactions (Nadchatram 2005). There are several methods for reducing the level of mites and mite allergens within the home including: reducing relative humidity, using mattress covers, vacuuming, and acaricides (Korsgaard 1991, Potter 2000, Nadchatram 2005, Apperson and Waldvogel 2008).

HDMs need high relative humidity to survive due to their need to acquire water from the air (Wharton 1976). Mites consume dead skin, however this substrate is not suitable for them to eat until mold or fungi colonize and moisten it (Nadchatram 2005). Molds, including *Aspergillus*, thrive in warm and humid conditions (Nadchatram 2005). Thus, mites need a high relative humidity not only to be able to consume food but also to acquire water. Studies have shown that

increasing ventilation within the home decreases indoor relative humidity, with a corresponding decrease in mite numbers (Korsgaard 1991).

Vacuuming is used to remove both live and dead mites in addition to their feces (Nadchatram 2005). Vacuuming, to be effective, must be thorough by going over every fold and depression in a mattress (Nadchatram 2005). The main drawback of vacuuming is that it cannot penetrate a mattress deep enough to reach all mites (Nadchatram 2005). This is a reason why vacuuming is not effective for long-term control because mites inside the mattress will continue to reproduce (Wassenaar 1988, Hart and Whitehead 1990, Nadchatram 2005). Vacuum cleaners can actually increase the amount of allergens in the air (Tovey 1992). It is therefore recommended that a HEPA filter be used (Tovey 1992). Care also should be taken to wash clothes and bedding, in addition to vacuuming, to assist in killing and removing mites and their allergens (Nadchatram 2005).

Encasing mattresses and pillows also can reduce allergen buildup, with permeable plastic covers considered the most comfortable option (Nadchatram 2005). Plastic covers reduce humidity and also stop buildup of shed skin cells on a mattress (Nadchatram 2005). Replacing carpet with tile or hardwood flooring is recommended because of the large populations of mites found in carpet (Nadchatram 2005).

Acaricides are pesticides used to kill Acari (ticks and mites) (Mullen et al. 2009). They are able to kill HDMs, but they are not always able to penetrate deep into mattresses (Mitchell et al. 1985). Acaricides tested in laboratory bioassays can identify effective agents, but it is more difficult to kill sufficient numbers of mites within the home (Nadchatram 2005). Extension services have recommended acaricides that contain benzyl benzoate (Acarosan™) or tannic acid

(Allergy Control™) for severe infestations, but not as a first line of defense against HDMs (Potter 2000, Apperson and Waldvogel 2008).

HDM related health issues also can be treated with a more expensive option, allergy immunotherapy, where the patient is exposed to extracts of mite antigens or house dust that contain mites (Nadchatram 2005).

House Dust Mite Keys and Mite Micrography

There are several keys to HDMs that have been published in the last 20 years (van Bronswijk and Sinha 1971, Wharton 1976, Fain et al. 1988, 1990, Colloff and Spieksma 1992, Colloff 2009). The majority of these keys are picture-keys (van Bronswijk and Sinha 1971, Colloff and Spieksma 1992, Colloff 2009), along with several dichotomous keys (Wharton 1976). The most current key for identifying HDMs was published by Colloff in 2009, and it lists mites found in the home by life stage and sex. Two keys by Fain et al., from 1988 and 1990, concentrate on subfamilies of the pyroglyphid HDMs. The pictorial key by van Bronswijk and Sinha (1971) concentrates on HDMs, while the key by Colloff and Spieksma (1992) examines domestic mites in general.

Keys for storage mites are available and include a key to families of storage mites published by Hughes in 1976. An illustrative key by Gorham in 1991 entitled, "Insect and Mite Pests in Food" contains both storage and HDMs (1991).

One mite identification guide that uses SEM photography was published in 2003 by Lofarma (2003).

Light Microscopy

The typical way that mite identification is accomplished is by using a light microscope with specimens that are 'cleared' to facilitate examination of the internal genitalia (Krantz et al.

2009). Clearing involves removing fat body and pigments in order to make the mite more transparent (Moreira et al. 2012). Mites can be cleared with lactic acid (Colloff 1989) or semi-permanent mounting media such as Hoyer's or de Faure's (Krantz et al. 2009). However, because the latter are gum chloral-based, they tend to dry out if the cover slip is not sealed, and sometimes specimens are damaged when the medium crystallizes (Upton 1993). Alternatives include a modification of polyvinyl alcohol in lactophenol (Heinze 1952, Colloff 2009).

Confocal Microscopy and HDM

An alternative way to view HDMs is with confocal microscopy. Confocal microscopy is known to improve out-of-focus interference because the microscope focuses on a limited section of the specimen at one time and combines images as it moves over time through/over the subject (Amos et al. 1987). Confocal microscopy therefore allows stacking of multiple images from a specimen that can be assembled into a 3-dimensional clearly focused figure (Klaus and Schawaroch 2006). Confocal microscopy has been used to image arthropods utilizing autofluorescent tissues in the cuticle and has the advantage of not 'destroying' specimens by mounting in a permanent medium (Klaus and Schawaroch 2006).

Confocal microscopy has been used to look at the central nervous system in *Phytoseiulus persimilis* (van Wijk et al. 2006), and examine the taxonomy of a new genus of water mite, *Vagabundia* (Valdecasas 2008). One species of HDM that has been subject to confocal microscopy is *Lepidoglyphus destructor* (van Hage-Hamsten et al. 1995). In that study the confocal microscope was used to localize major allergens by immunohistochemical staining (van Hage-Hamsten et al. 1995).

SEM Microscopy and House Dust Mites

Scanning electron microscopy (SEM) has been used to look at mite ultrastructure (Alberti and Coons 1999, Kennaway et al. 2004), including the storage mite *Suidasia pontifica* (Ahamad et al. 2011), *Dermatophagoides farinae*, and *D. pteronyssinus* (Walzl 1992). SEM is time consuming and expensive due to having to fix and otherwise prepare mites prior to imaging (Glauert 1987, Glauert and Lewis 1998). Mites are killed in liquid nitrogen, 5% KOH, or boiling water (Evans 1972) then fixed in gluteraldehyde or osmium tetroxide (Klaus and Schawaroch 2006) and lastly coated with gold, gold-palladium, platinum, or carbon prior to examination with the microscope (Krantz et al. 2009).

Collecting and Extraction Methods

The foundation of our understanding of HDM biology is based on our ability to collect mites by extracting them from substrates such as fabrics and dust. A realistic assessment of the number of mites found within homes should be the basis for determining their impact on indoor air quality and therefore human health issues including asthma.

Heat Escape Extraction Method

Mites can be extracted using the heat escape method which uses the mites' avoidance of desiccation in order to collect them (Bischoff et al. 1992). One technique uses a heat mat placed under a carpeted area (Bischoff et al. 1992). The heat is slowly increased over a period of 30 minutes, which drives mites out and onto an adhesive (Bischoff et al. 1992). An alternative is to place a heated item on the surface of the textile (such as a warm water bottle), causing the mites to move toward the heat because the heat is not hot enough to decrease humidity, but warm enough that they will move to it (Colloff 1991). The mites are then trapped on a piece of adhesive tape (Colloff 1991).

Flotation Extraction Method

The flotation extraction method is commonly used in HDM surveys (Malainual et al. 1995, Boquete et al. 2006, Caplin et al. 2009). It has the advantage of removing both live and dead mites from the substrate (Hart and Fain 1987). The flotation method is accomplished by placing the sampled substrate in ethanol for several hours and then moving the substrate to a saturated NaCl solution for 10 minutes (Hart and Fain 1987). The assumption is that the mites will rise to the top of NaCl solution and can be collected (Hart and Fain 1987). Mites float to the top of the saturated NaCl solution because the water in their bodies is replaced by the ethanol which lowers their density to 0.86 g/ml, which is above the density of NaCl (1.2 g/ml) (Hart and Fain 1987). There are alternatives to using NaCl, such as using CCl_4 (Oshima 1970); however, CCl_4 is toxic (Hart and Fain 1987).

Conclusion

Pyroglyphid HDMs and non-pyroglyphid storage mites contribute allergens to the indoor environment. They are common within homes and can cause allergy and asthma issues in individuals. There is a need for a clear way to identify these mites. Each mite species produces a different allergen, thus knowing what species are present in the house is important. Past identification guides have concentrated on using drawings or dichotomous keys. An easy to use guide to the species of HDM with worldwide distributions that uses images from a variety of microscopes to identify key characteristics would help health officials, researchers and citizens recognize the mites found within homes. Studies have used both heat escape and flotation in order to extract mites from the indoor human habitat. Finding the efficiency of these methods will help in determining realistic numbers of mites in the home.

References Cited

2000. Clearing the Air: Asthma and Indoor Air Exposures, pp. 438. Institute of Medicine, Washington, D.C.

2003. Mites & Allergy: Notes on House Dust and Storage Mites, vol. Lofarmaa, Milano, Italy.

Ahamad, M., S. R. Louis, Z. Hamid, and T. M. Ho. 2011. Scanning electron micrographs of medically important dust mite, *Suidasia pontifica* (Acari: Astigmata: Saproglyphidae) in Malaysia. Trop Biomed 28: 275-282.

Alberti, G., and B. Coons. 1999. Mites, pp. 515-1265. *In* H. F.W. and F. R. (eds.), Microscopic anatomy of invertebrates: Chelicerate arthropoda, vol. 8C. Wiley-Liss, New York.

Alexander, A., N. Fall, and L. Arlian. 2002. Mating and fecundity of *Dermatophagoides farinae*. Exp Appl Acarol 26: 79-86.

Amos, W. B., J. G. White, and M. Fordham. 1987. Use of confocal imaging in the study of biological structures. Appl Opt 26: 3239-3243.

Andrews, M. 1976. The life that lives on man, vol. Faber and Faber, London.

Apperson, C. S., and M. Waldvogel. 2008. Mites that "Bug" People, Insect Note - ENT/rsc-2 Department of Entomology, North Carolina Cooperative Extension. http://www.ces.ncsu.edu/depts/ent/notes/Urban/mites.htm.

Arbes, S. J., P. J. Gergen, L. Elliott, and D. C. Zeldin. 2005. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. J Allergy Clin Immunol 116: 377-383.

Arlian, L. G. 1989. Biology and ecology of house dust mites, *Dermatophagoides* sp. and *Euroglyphus* sp. Immunol Allergy Clin North Am 9: 339-356.

Arlian, L. G. 2002. Arthropod allergens and human health. Annu Rev Entomol 47: 395-433.

Arlian, L. G., and J. S. Dippold. 1996. Development and fecundity of *Dermatophagoides farinae* (Acari: Pyroglyphidae). J Med Entomol 33: 257-260.

Arlian, L. G., and M. S. Morgan. 2003. Biology, ecology, and prevalence of dust mites. Immunol Allergy Clin North Am 23: 443-468.

Arlian, L. G., C. M. Rapp, and S. G. Ahmed. 1990. Development of *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). J Med Entomol 27: 1035-1040.

Arlian, L. G., D. A. L. Vyszenski-Moher, and E. Fernandez-Caldas. 1993. Allergenicity of the mite, *Blomia tropicalis*. J Allergy Clin Immunol 91: 1042-1050.

Arlian, L. G., M. S. Morgan, and J. S. Neal. 2002. Dust mite allergens: ecology and distribution. Curr Allergy Asthma Rep 2: 401-411.

Bischoff, E. R. C., A. Fischer, and B. Liebenberg. 1992. Assessment of mite numbers: new methods and results. Exp Appl Acarol 16: 1-14.

Bonnefoy, X., H. Kampen, and K. Sweeney. 2008. Public health significance of urban pests, vol. World Health Organization, Copenhagen, Denmark.

Boquete, M., V. Iraola, E. Fernández-Caldas, L. A. Villaroel, F. J. Carballada, C. G. de la Cuesta, M. L. Lopez-Rico, R. N. Orjales, A. Parra, and M. T. Soto-Mera. 2006. House dust mite species and allergen levels in Galicia, Spain: a cross-sectional, multicenter, comparative study. J Investig Allergol Clin Immunol 16: 169.

Caplin, J., A. Capriles-Hulett, V. Iraola, H. Pinto, M. Sanchez-Borges, G. de los Santos, and E. Fernandez-Caldas. 2009. Allergic sensitization to domestic mites in Corpus Christi, Texas. Allergy Asthma Proc 30: 166-170.

Coca, A. F., and R. A. Cooke. 1923. On the classification of the phenomena of hypersensitiveness. J Immunol 8: 163-182.

Colloff, M. J. 1989. A new and rapid method of making permanent preparations of large numbers of house-dust mites for light microscopy. Exp Appl Acarol 7: 323-326.

Colloff, M. J. 1991. Practical and theoretical aspects of the ecology of house dust mites (Acari: Pyroglyphidae) in relation to the study of mite-mediated allergy. Rev Med Vet Entomol 79: 611-630.

Colloff, M. J. 2009. Dust mites, 1 ed, vol. CSIRO Publishing and Springer, Collingwood, Australia.

Colloff, M. J., and F. T. M. Spieksma. 1992. Pictorial keys for the identification of domestic mites. Clin Exp Allergy 22: 823-830.

Dekker, H. 1928. Asthma and milben. Munchener Med Wochensch 12.

Diego, O., A. O. Deise, F. C. Jorge, G. J. Meimei, F. Hamilton, H. Leandro, O. Rafael, D. O. Janethe, J. Sun-Sang, and R. S. Gesmar. 2011. Serum and Salivary IgE, IgA, and IgG 4 Antibodies to *Dermatophagoides pteronyssinus* and Its Major Allergens, Der p1 and Der p2, in Allergic and Nonallergic Children. Clin Dev Immunol 2011: 1-11.

Evans, G. O. 1972. Scanning electron microscopy in the study of the gnathosoma of the Acari, vol. Potchefstroomse Universiteit vir C.H.O., Potchefstroom, South Africa.

Fain, A., B. Guérin, and B. J. Hart. 1988. Acariens et allergies, vol. Allerbio, Varennes en Argonne.

Fain, A., B. Guérin, and B. J. Hart. 1990. Mites and allergic disease, vol. Allerbio, Varennes en Argonne.

Glauert, A. M. 1987. Fixation, dehydration and embedding of biological specimens - Cytological staining, pp. 5-110, Practical methods in electron microscopy, vol. 3. Elsevier, Amsterdam.

Glauert, A. M., and P. R. Lewis. 1998. Biological specimen preparation for transmission electron microscopy - Embedding methods., pp. 147-69, Practical methods in electron microscopy, vol. 16. Portland Press, London.

Gorham, J. R. 1991. Insect and mite pests in food: An illustrated key, vol. 1. U.S. Dept. of Agriculture, Agricultural Research Service : U.S. Dept. of Health and Human Services, Public Health Service, Food and Drug Administration, Washington, D.C.

Hart, B. J., and A. Fain. 1987. A new technique for isolation of mites exploiting the difference in density between ethanol and saturated NaCl: qualitative and quantitative studies. Acarol. 28: 251-254.

Hart, B. J., and L. Whitehead. 1990. Ecology of house dust mites in Oxfordshire. Clin Exp Allergy 20: 203-209.

Hay, D. B., B. J. Hart, R. B. Pearce, Z. Kozakiewicz, and A. E. Douglas. 1992. How relevant are house dust mite-fungal interactions in laboratory culture to the natural dust system? Exp Appl Acarol 16: 37-47.

Heinze, K. 1952. Polyvinylalkohol-Lactophenol-Gemisch als Einbettungsmittel für Blattläuse. Naturwissenschaften 39: 285-286.

Heymann, P. W., M. D. Chapman, R. C. Aalberse, J. W. Fox, and T. A. E. Platts-Mills. 1989. Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (*Dermatophagoides* sp). J Allergy Clin Immunol 83: 1055-1067.

Kennaway, G. M., A. S. Baker, and A. D. Ball. 2004. A method for preparing lightly sclerotized mites for examination by transmission electron microscopy. Systematic and Applied Acarology 9: 3-10.

Kern, R. A. 1921. Dust sensitization in bronchial asthma. Med Clin North Am 5: 751-758.

Klaus, A. V., and V. Schawaroch. 2006. Novel methodology utilizing confocal laser scanning microscopy for systematic analysis in arthropods (Insecta). Integr Comp Biol 46: 207-214.

Korsgaard, J. 1991. Mechanical ventilation and house dust mites: a controlled investigation. Dust mite allergens and asthma. Brussels: UCB Institute of Allergy: 87-9.

Krantz, G. W., D. E. Walter, and V. Behan-Pelletier. 2009. A manual of acarology, 3 ed, vol. Texas Tech University Press.

Lind, P. 1985. Purification and partial characterization of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. J Allergy Clin Immunol 76: 753-761.

Major, R. H. 1953. A note on the history of asthma. *In* E. A. Underwood (ed.), Science, medicine, and history, vol. 2. Oxford University Press.

Malainual, N., P. Vichyanond, and P. Phan-Urai. 1995. House dust mite fauna in Thailand. Clin. Exp. Allergy 25: 554-560.

Mitchell, E., S. Wilkins, M. Deighton, and T. A. E. Platts-Mills. 1985. Reduction of house dust mite allergen levels in the home: use of the acaricide, pirimiphos methyl. Clin Exp Allergy 15: 235-240.

Miyamoto, T., S. Oshima, T. Ishizaki, and S. Sato. 1968. Allergenic identity between the common floor mite (*Dermatophagoides farinae* Hughes, 1961) and house dust as a causative antigen in bronchial asthma. J Allergy 42: 14-28.

Moreira, F. M. S. M. S., E. J. J. Huising, and D. E. E. Bignell. 2012. A Handbook of Tropical Soil Biology: Sampling and Characterization of Below-ground Biodiversity, vol. Taylor & Francis.

Mullen, G., G. R. Mullen, and L. Durden. 2009. Medical and veterinary entomology, 2 ed, vol. Academic Press.

Nadchatram, M. 2005. House dust mites, our intimate associates. Trop Biomed 22: 23-37.

Oshima, S. 1970. Studies on the mite fauna of the house-dust of Japan and Taiwan with special reference to house-dust allergy. Jap J Sanit Zool 21: 1-17.

Platts-Mills, T. A. E., and A. L. de Weck. 1989. Dust mite allergens and asthma—a worldwide problem. J Allergy Clin Immunol 83: 416-427.

Potter, M. F. 2000. House Dust Mites. University of Kentucky College of Agriculture.

Rosenstreich, D. L., P. Eggleston, M. Kattan, D. Baker, R. G. Slavin, P. Gergen, H. Mitchell, K. McNiff-Mortimer, H. Lynn, and D. Ownby. 1997. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med 336: 1356-1363.

Sharma, S., M. Sood, and A. Sood. 2011. Environmental risk factors in relation to childhood asthma in rural area. Pediatr Res 15: 29-32.

Strom, G. B., R. N. Boxwell, and R. L. Jacobs. 1980. Imported fire ant (IFA) sting sensitivity: correlation between venom and whole body extract (WBE) testing. J Allergy Clin Immunol 65.

Thomas, B., P. Heap, and F. Carswell. 1991. Ultrastructural localization of the allergen Der p1 in the guy of the house dust mite *Dermatophagoides pteronyssinus*. Int Arch Allergy Appl Immunol 94: 365-67.

Thomas, W. R., and W. Smith. 1998. House dust mite allergens. Allergy 53: 821-832.

Tovey, E., and G. Marks. 1990. Localization of anigens and allergens in thin sections of the house dust mite, *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). J Med Entomol 27: 368-76.

Tovey, E. R. 1992. Allergen exposure and control. Exp Appl Acarol 16: 181-202.

Tovey, E. R., M. D. Chapman, and T. A. E. Platts-Mills. 1981. Mite faeces are a major source of house dust allergens. Nature 289: 592-593.

Upton, M. S. 1993. Aqueous gum-chloral slide mounting media: an historical review. Bull Entomol Res 83: 267-267.

Valdecasas, A. G. 2008. Confocal microscopy applied to water mite taxonomy with the description of a new genus of Axonopsinae (Acari, Parasitengona, Hydrachnidia) from Central America. Zootaxa 1820: 41-48.

van Bronswijk, J. 1981. House dust biology for allergists, acarologists and mycologists, vol. van Bronswijk, J., University of Michigan.

van Bronswijk, J., and R. N. Sinha. 1971. Pyroglyphid mites (Acari) and house dust allergy. J Allergy 47: 31-52.

van Hage-Hamsten, M., S. Olsson, A. Emilson, B. Härfast, A. Svensson, and A. Scheynius. 1995. Localization of major allergens in the dust mite *Lepidoglyphus destructor* with confocal laser scanning microscopy. Clin Exp Allergy 25: 536-542.

Van Leeuwen, W. S. 1924. Bronchial asthma in relation to climate. Proc R Soc Med 17: 19-26.

van Wijk, M., W. J. Wadman, and M. W. Sabelis. 2006. Gross morphology of the central nervous system of a phytoseiid mite. Exp Appl Acarol 40: 205-216.

Voorhorst, R., M. I. Spieksma-Boezeman, and F. T. Spieksma. 1964. Is a mite (*Dermatophagoides* sp.) the producer of the house-dust allergen? Allerg Asthma 10: 329.

Voorhorst, R., F. T. M. Spieksma, H. Varekamp, M. J. Leupen, and A. W. Lyklema. 1967. The house-dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identify with the house-dust allergen. J Allergy 39: 325-339.

Walzl, M. G. 1992. Ultrastructure of the reproductive system of the house dust mites *Dermatophagoides farinae* and *D. pteronyssinus* (Acari, Pyroglyphidae) with remarks on spermatogenesis and oogenesis. Exp Appl Acarol 16: 85.

Warner, A., S. Boström, C. Möller, and N. I.-M. Kjellman. 1999. Mite fauna in the home and sensitivity to house-dust and storage mites. Allergy 54: 681-690.

Wassenaar, D. P. J. 1988. Effectiveness of vacuum cleaning and wet cleaning in reducing house-dust mites, fungi and mite allergen in a cotton carpet: a case study. Exp Appl Acarol 4: 53-62.

Wharton, G. W. 1976. House dust mites. J Med Entomol 12: 577-621.

Yasueda, H., H. Mita, Y. Yui, and T. Shida. 1986. Isolation and characterization of two allergens from *Dermatophagoides farinae* Int Arch Allergy Appl Immunol 81: 214-223.

Zeldin, P. J. Vojta, W. Friedman, J. Zhou, E. Schuett, R. Cohn, H. Burge, M. Muilenberg, and P. S. Thorne. 2001. National Survey Examines Factors Related to High Levels of Dust Mite and Cockroach Allergen in Beds. National Institute of Environmental Health Sciences - National Institute of Health. http://www.niehs.nih.gov/news/newsroom/releases/news-archive/2001/may22/index.cfm.

CHAPTER 3

HOUSE DUST MITE COUNT ESTIMATE AND EXTRACTION USING HEAT ESCAPE AND FLOTATION[1]

[1] Roden, A.E. and B.T. Forschler. To be submitted to Experimental & Applied Acarology

Abstract

House dust mites (HDM) are a functional group of mites that are found within homes. These mites produce allergens that can lead to allergies. Information from HDM surveys of human habitations are used to determine the species distribution and number of mites. In this study, the correlation between the weight of two common species of HDMs, *Dermatophagoides pteronyssinus* and *D. farinae*, and the actual number of mites in that sample were determined. In addition, two extraction methods, heat escape and flotation, were examined to determine efficiency. These methods were found to provide low extraction efficiencies, especially if mites were combined with a substrate such as dust or fiber (kapok).

Keywords: House dust mites, Dermatophagoides pteronyssinus, farinae, extraction efficiencies, kapok

Introduction

House dust mites (HDM) are small arthropods (100-500 μm in length) that are associated with human urban habitats (Arlian and Morgan 2003). These mites are commonly found in dust and fabrics of the home, such as carpets, pillowcases, and linens (Bonnefoy et al. 2008). Mites have been found in amounts up to 10,000 mites per gram of household dust (Chew et al. 1999).

The most common mite species found worldwide within homes include *Dermatophagoides farinae, D. pteronyssinus*, and *Euroglyphus maynei* (Arlian and Morgan 2003). These species are from the family Pyroglyphidae, which includes 18 described genera and 46 species (Wharton 1976, Fain et al. 1990, Arlian et al. 2002). Most of the pyroglyphid mites are not found within homes, but in other habitats such as around bird and mammal nests or in stored products (van Bronswijk 1981).

The major problems with HDMs are the allergens found within their bodies and feces that lead to allergies and asthma (Sporik et al. 1990). Asthma is evidenced by chronic inflammation of the airways, and is considered a serious worldwide problem that contributes to around 500,000 hospitalizations each year, 34.5% of which involve children (Sharma et al. 2011). The sensitization rate to HDM allergens may be 15-20% of people in industrialized countries (Zock et al. 2006).

The association between HDMs and allergic reactions and asthma demands that laboratory and field studies have a reliable estimate of the number of mites extracted from the landscape (Mehl 1998). Many survey studies look at the number and density of mites found within homes, and thus have to use an extraction method that may result in not all of the mites being recovered (Racewicz 2001, Irigoyen and del Hoy 2002, Boquete et al. 2006). Therefore, the correlation between the weight of *D. pteronyssinus* and *D. farinae* mites and the number of

mites in that sample was tested as the essential preliminary to examination of extraction methods used in HDM surveys. The percentage of *D. pteronyssinus* mites that can be extracted from clean samples, dust, and kapok was also determined using the heat escape method and flotation extraction.

Materials and Methods

Mites were obtained from cultures maintained at Mahidol University, Department of Parasitology at Siriraj Hospital in Bangkok, Thailand. Mites were extracted from their culture media by using a heat-escape method that causes the mites to leave their food source and travel through a sieve (Mahakittikun et al. 2006).

Estimate of counting accuracy for Dermatophagoides pteronyssinus and D. farinae

The correlation between live weights and number of mites was examined using *Dermatophagoides pteronyssinus* and *D. farinae.* Mites were removed from cultures and weighed at three different weight classes: 1.0 mg, 5.0 mg, and 10.0 mg on a digital scale. Each weight class was replicated 10 times for *D. pteronyssinus* and 5 times for *D. farinae*. All mites from each replicate for every weight class were placed on a slide, mounted with Hoyer's solution and covered with a slide cover that contained a grid. All mites on each slide were counted in each grid section to obtain a total number of mites for each replicate of every weight class. Counting was started in the upper right-hand corner of the grid and each grid section was counted separately. If a mite was in any way in a grid section, it was counted for that section. If the mite was seen again in another section, it would not be counted since part of it reached into a previous section and thus it could be assumed that it had already been counted. A regression line and R^2 were determined using all weight classes and mite counts with SAS 9.2 software (2010).

The mean number of mites per gram was found for each weight class by dividing the number of mites per replicate by the number of replicates for each weight class.

Heat escape extraction

The efficiency of the heat escape method using *D. pteronyssinus* was obtained by placing a known weight of mites in an extraction device. Five samples of mites that weighed 2.0 mg each were placed in an extraction device (Figure 3- 1). The device was constructed using 3 plexiglass rings (2.6-cm in diameter; 1.5-mm wide), with a 1.0-cm hole in the center. Two plexiglass rings were placed over a standard microscope slide and the topmost ring was covered with a piece of gauze that was folded over on itself. This arrangement was intended to hold the mites while allowing them to escape the heat by traveling downward to the glass slide. The gauze was then covered by another plexiglass ring to hold the gauze in place. Mites were placed on the gauze wrapped around the second (middle) plexiglass ring, and a 60-watt incandescent light bulb placed 9.6-10.0 cm from the top of the uppermost plexiglass ring. The temperature at the top plexiglass ring was 48.0 – 55.0° C. Mite samples were kept under the lamp for 15 minutes. The mites that escaped to the glass slide were mounted in Hoyer's solution and counted. The estimated number of mites in the original 2.0 mg was found using the formula from the linear regression line found with the weight-number experiments. The percent recovery was found by dividing the number of mites recovered by the number of mites that were estimated to be in the original sample.

The heat escape method was also conducted using a second device using only the 2.0 mg weight class replicated 5 times (Figure 3- 2). The second device had the previously described device placed on top of a tube (4.0-cm length) half filled with 70% ethanol. However, instead of gauze, the device contained a metal grid that functioned as a platform for the mites to be placed

on. A known weight of live mites, 2.0 mg, was placed under a 60- watt incandescent light bulb 5.0 cm from the top of the device and left for 15 minutes (Figure 3- 3). The mites were pipetted out of the second device from the tube and mounted in Hoyer's medium. The percent recovery was found by dividing the number of mites recovered by the number of mites that were estimated to be in the original sample.

Flotation extraction

The efficiency of extracting *D. pteronyssinus* using the flotation method was also determined. Mites taken directly from cultures were placed on an electric scale and weighed into 2 weight classes, 1.0 mg and 5.0 mg. Mites were then added to one of the following: 70% EtOH (controls), 100.0 mg of dust, or 100.0 mg kapok fiber. Kapok is a seed fiber found in a pod around the seeds of the kapok tree, *Ceiba pentandra* that is sometimes used to stuff mattresses (Trevillian et al. 2003, Nadchatram 2005). These fibers are 18 mm in length and 20-30 µm in diameter, on average (Robertson and Grieve 1999). Past studies have shown that HDMs are found in this material (Abbott et al. 1981).

Each weight and substrate combinations was replicated 5 times. The method used to extract mites from either substrate followed the technique outlined by Hart and Fain (1987). In our study, we placed mites from each replicate into a glass vial containing 70% ethanol for 24 hours, after which the EtOH supernatant was removed by pipette and a saturated (6 M) NaCl solution added for 10 minutes, as is typically done (Sun and Lue 2000, Bao et al. 2005) The NaCl solution was pippetted in and out along with the settled dust or kapok. The 10 minutes allowed for the dust or kapok to settle to the bottom of the test tube while the mites floated to the top. The NaCl solution that contained the floating mites was then filtered through a #1 Whatman filter paper using vacuum in a Büchner funnel. Vials were rinsed three times with the NaCl

solution. Filter papers were placed in a 100x 15-mm Petri dish with a lid that contained a grid. All mites on the filter paper were counted under a binocular dissecting microscope using the grid as a reference. The percent recovery was found by dividing the number of mites recovered by the number of mites estimated to be in the original sample. The mean and standard deviation for each material were found using SAS 9.2 software (2010).

Results

Estimate of mite counts and weights for Dermatophagoides pteronyssinus and D. farinae

The linear regression line using the weights and number of mites from *Dermatophagoides pteronyssinus* (Figure 3- 4) and *D. farinae* (Figure 3- 5) provided a strong correlation for the live weight mites. The average number of mites per gram can be found in Table 3- 1.

Heat escape extraction

The first heat escape method had an extraction efficiency rate between 0.16% and 16.00%, as seen in Figure 3- 6. The mean number of mites recovered by this method was 46.67 mites, while the average percent recovered was 6.97% (std dev =5.54%).

The second heat escape method had an extraction rate between 1.65 and 8.35%, which also can be seen in Figure 3- 6. The mean number of mites recovered using this method was 29.0 mites, while the average percent recovered was 4.34% (std dev =3.45%)

Analysis of variance between the two test types found no significant difference between the means of the two heat extract methods (F=2.58, df=5,4, P=0.3795).

Flotation extraction

Two weight classes were examined for mite extraction efficiency using the flotation extraction method. The percent extracted from the 1.0 mg weight class initially placed in 70%

ethanol ranged from 41.28% to 96.40% with a mean of 59.80% (std dev= 21.44%) (Figure 3- 7). Mites from the 5.0 mg weight class that were initially placed in ethanol provided a range of efficiency from 44.71% - 53.40% with a mean of 48.98% (std dev= 3.47%) (Figure 3- 7).

The 1.0 mg weight class placed in 100.0 mg of dust gave a range of extraction efficiencies between 2.34 to 15.55%, with a mean of 6.53% (std dev= 5.30%) (Figure 3- 7). The 5.0 mg weight class of mites placed in 100.0 mg of dust provided a range from 2.24% to 16.12%, and a mean percent mites extracted of 6.85% (std dev=6.91%) (Figure 3- 7).

The 1.0 mg of mites placed on the fabric kapok gave a range of extraction efficiencies between 0.0% to 1.17% and a mean of 0.52% (std dev=0.46%) (Figure 3- 7). The range of percentages of mites extracted from kapok in the 5.0 mg weight class was 0.06% to 0.45%, while the percentage of mites removed was 0.27% (std dev=0.24%) (Figure 3- 7).

Discussion

The strong correlation that was found for both *D. pteronyssinus* and *D. farinae* weight of mites from the Siriraj cultures and the number was the basis for the accurate estimate of extraction efficiencies using initial sample weight. The weights used were live weights, and could be changed if just dead mites were used or if there was a mixture of live and dead mites.

All extraction methods used in this study had low rates of HDM recovery. For the two heat escape methods used, the extraction efficiency from the first (mean 6.97%) was higher than the second method (mean 4.34%); however, these means were not statistically different. One of the main uses of the heat escape method is to remove live mites from cultures that contain a substrate. These low extraction rates indicate that only a small percentage of mites are being removed from the substrate, and thus the initial number of mites within the substrate is much

greater. Further testing could look at different levels of light and heat, along with bulb wattage, in order to optimize this method.

The flotation method had an extraction efficiency for the EtOH controls of about 50-60% (Figure 3- 7). When this method was used with substrates such as dust, which is the most common substrate from which HDMs are usually collected during survey studies, extraction efficiency was much lower than expected, at around 6.6% (Figure 3- 7). For example, one study found on average 100 mites per gram of dust in an Atlanta, GA home, with a range of between 9 to 650 mites per gram of dust (Smith et al. 1985). If the extreme situation of 650 mites/gram of dust is considered with the flotation extraction data from this study, only about 6.6% of the mites in this dust were able to be extracted. This means that there may have been up to 9848.5 mites per gram of dust.

When mites were added to kapok fibers, the extraction efficacy was even lower at less than 1.0% (Figure 3- 7). This experiment utilized up to 1800 HDMs per 0.1 g of kapok in order to examine extraction efficacy. If a mattress contains about 6000 grams of kapok fibers, then there is a potential that thousands of mites may be unable to be removed from this material.

Additional experiments could be done in order to optimize the flotation method. Increasing the diameter of the test tubes that contained the mites while they were soaking in saturated NaCl and ethanol, along with increasing or decreasing the amount of time of letting the mites soak in these solutions could increase or decrease extraction efficiency. Centrifuging the container with the mites and saturated NaCl solution could remove some of the mites that are trapped in the substrate and thus increasing extraction efficiency.

The two methods in this study, the flotation and heat escape, have been used to estimate numbers of HDM within urban environments. The extraction efficiency of these methods will

help researchers determine an accurate assessment of the number of mites within a home. Studies that look at the abundance of mites inside a home may be underestimating the actual number the mites in that habitat. Mites will get trapped in dust and in fibers when trying to remove them, thus removing all the mites from a sample may be impossible.

References Cited

2010. Base SAS® 9.3 Procedures Guide computer program, version SAS Institute Inc., Cary, NC.

Abbott, J., J. Cameron, and B. Taylor. 1981. House dust mite counts in different types of mattresses, sheepskins and carpets, and a comparison of brushing and vacuuming collection methods. Clin Exp Allergy 11: 589-595.

Arlian, L. G., and M. S. Morgan. 2003. Biology, ecology, and prevalence of dust mites. Immunol Allergy Clin North Am 23: 443-468.

Arlian, L. G., M. S. Morgan, and J. S. Neal. 2002. Dust mite allergens: ecology and distribution. Curr Allergy Asthma Rep 2: 401-411.

Bao, P., Y. Cui, Q. Jiang, and H. Zu. 2005. Domestic mites in dormitories and results of skin prick test on students with *Dermatophagoides farinae* in Huainan, China. Journal of US-China Medical Science 2: 11-15.

Bonnefoy, X., H. Kampen, and K. Sweeney. 2008. Public health significance of urban pests, vol. World Health Organization, Copenhagen, Denmark.

Boquete, M., V. Iraola, E. Fernández-Caldas, L. A. Villaroel, F. J. Carballada, C. G. de la Cuesta, M. L. Lopez-Rico, R. N. Orjales, A. Parra, and M. T. Soto-Mera. 2006. House dust mite species and allergen levels in Galicia, Spain: a cross-sectional, multicenter, comparative study. J Investig Allergol Clin Immunol 16: 169.

Chew, F. T., L. Zhang, T. M. Ho, and B. W. Lee. 1999. House dust mite fauna of tropical Singapore. Clin. Exp. Allergy 29: 201-206.

Fain, A., B. Guérin, and B. J. Hart. 1990. Mites and allergic disease, vol. Allerbio, Varennes en Argonne.

Hart, B. J., and A. Fain. 1987. A new technique for isolation of mites exploiting the difference in density between ethanol and saturated NaCl: qualitative and quantitative studies. Acarol. 28: 251-254.

Illi, S., E. von Mutius, S. Lau, B. Niggemann, C. Grüber, and U. Wahn. 2006. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. The Lancet 368: 763-770.

Irigoyen, J., and J. del Hoy. 2002. Skin sensitization to mites and mite identification in the homes of an allergic population in the Huelva province. Alergología e Inmunología Clínica 17: 61-71.

Mahakittikun, V., J. J. Boitano, E. Tovey, C. Bunnag, P. Ninsanit, T. Matsumoto, and C. Andre. 2006. Mite penetration of different types of material claimed as mite proof by the Siriraj chamber method. J Allergy Clin Immunol 118: 1164-1168.

Mehl, R. 1998. Occurrence of mites in Norway and the rest of Scandinavia. Allergy 53: 28-35.

Nadchatram, M. 2005. House dust mites, our intimate associates. Tropical Biomedicine 22: 23.

Racewicz, M. 2001. House dust mites (Acari: Pyroglyphidae) in the cities of Gdańsk and Gdynia (northern Poland). Ann Agric Environ Med 8: 33.

Robertson, J., and M. C. Grieve. 1999. Forensic Examination of Fibres, vol. CRC Press.

Sharma, S., M. Sood, and A. Sood. 2011. Environmental risk factors in relation to childhood asthma in rural area. Pediatr Res 15: 29-32.

Smith, T. F., L. B. Kelly, P. W. Heymann, S. R. Wilkins, and T. A. E. Platts-Mills. 1985. Natural exposure and serum antibodies to house dust mite of mite-allergic children with asthma in Atlanta. J Allergy Clin Immunol 76: 782-788.

Sun, H. L., and K. H. Lue. 2000. Household distribution of house dust mite in central Taiwan. J Microbiol Immunol Infect 33: 233-236.

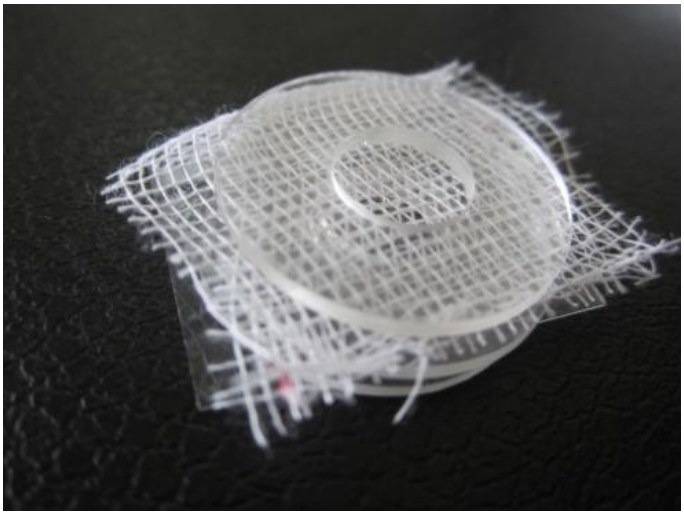
Sporik, R., S. T. Holgate, T. A. E. Platts-Mills, and J. J. Cogswell. 1990. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. N Engl J Med 323: 502-507.

Trevillian, L. F., A. L. Ponsonby, T. Dwyer, L. L. Y. Lim, A. Kemp, J. Cochrane, and A. Carmichael. 2003. An association between plastic mattress covers and sheepskin underbedding use in infancy and house dust mite sensitization in childhood: a prospective study. Clin Exp Allergy 33: 483-489.

van Bronswijk, J. 1981. House dust biology for allergists, acarologists and mycologists, vol. van Bronswijk, J., University of Michigan.

Wharton, G. W. 1976. House dust mites. J Med Entomol 12: 577-621.

Zock, J. P., J. Heinrich, D. Jarvis, G. Verlato, D. Norbäck, E. Plana, J. Sunyer, S. Chinn, M. Olivieri, and A. Soon. 2006. Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II. J Allergy Clin Immunol 118: 682-690.



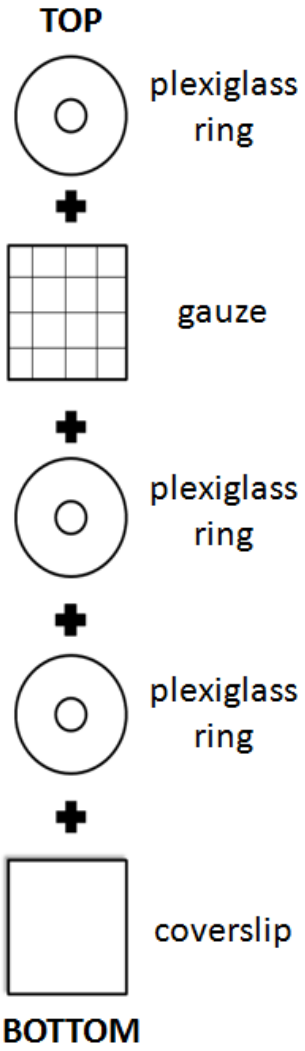


Figure 3- 1. Setup for First Heat Escape Extraction Method. On the left is an image of the actual device. The right contains a breakdown of the exact parts of the device starting with the top layer and moving downwards.

Figure 3- 2. Setup for Second Heat Escape Extraction Method. This heat escape extraction setup contained two plexiglass rings on top of wire mesh and an additional plexiglass ring on top of a tube.

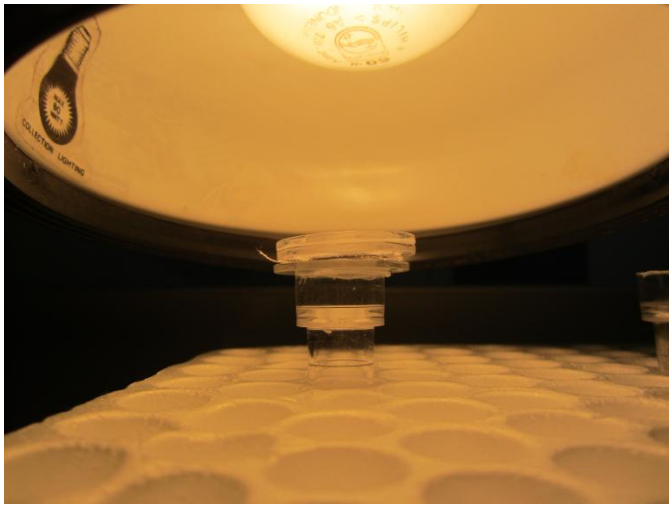


Figure 3- 3. Second mite extraction device under heat source.

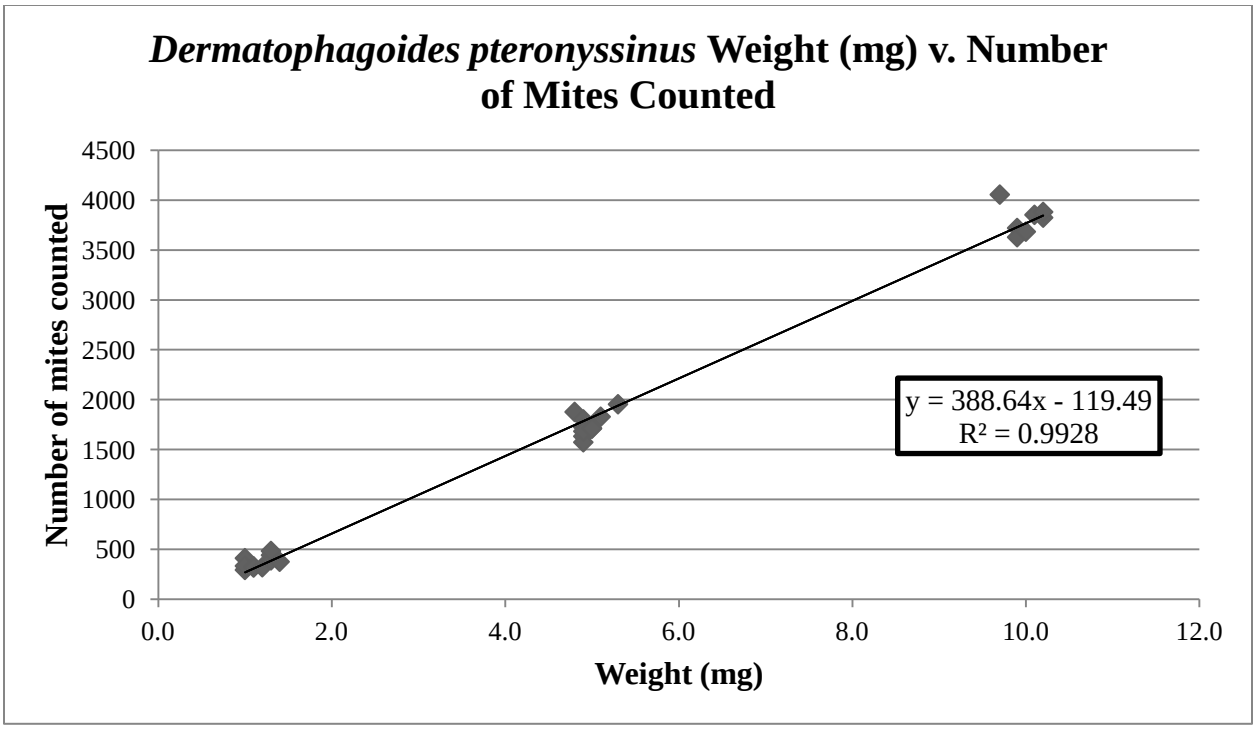


Figure 3- 4. Fresh weights (mg) of mites plotted over of number of *D. pteronyssinus* mites counted at 1.0 mg, 5.0 mg, and 10.0 mg, along with linear regression and R^2 value.

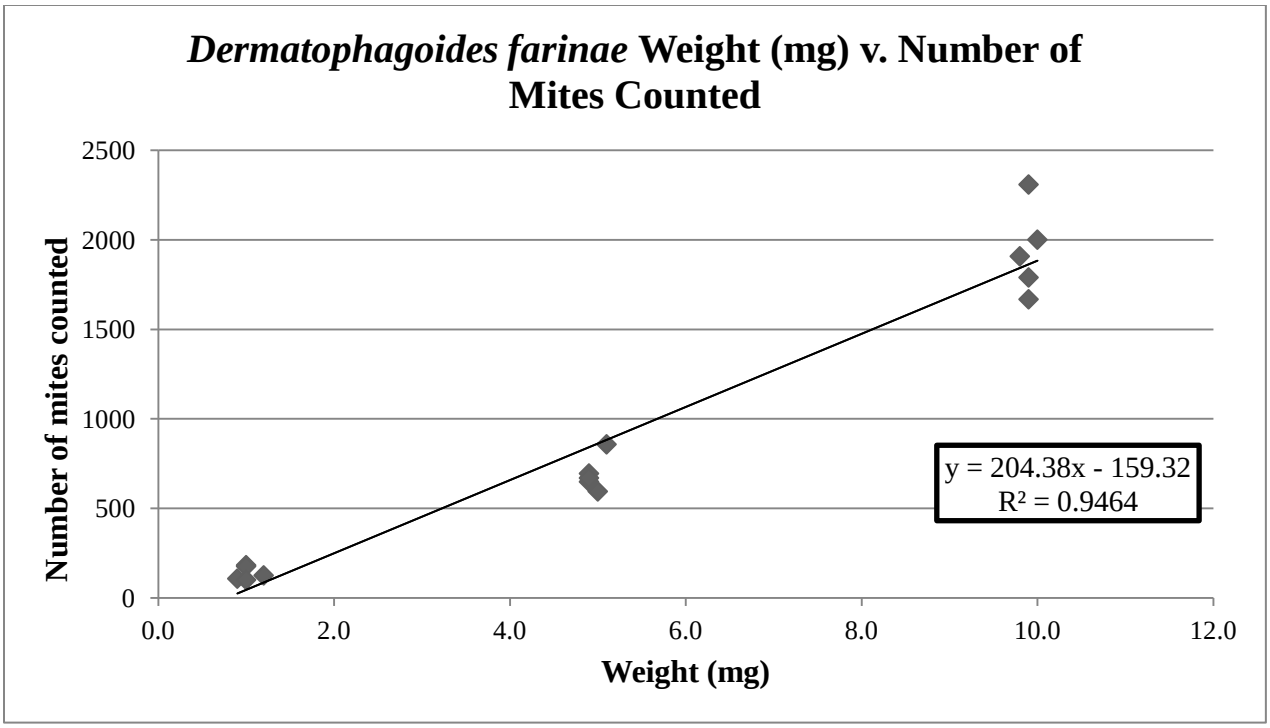


Figure 3- 5. Fresh weights (mg) of mites plotted over of number of *D. farinae* mites counted at 1.0 mg, 5.0 mg, and 10.0 mg, along with linear regression and R^2 value.

Table 3- 1. Number of *Dermatophagoides farinae* and *D. pteronyssinus* per gram

	Mean weight of mites used (g)	***Mean number of mites per mg***	***Standard Error***	***Mites/gram***
Dermatophagoides farinae	0.0053	922.53	189.85	174062.26
Dermatophagoides pteronyssinus	0.0049	1771.89	118.65	361610.20

The mean weight of all the replicates for each species along with the mean number of mites counted from all the replicates. This information was used to determine the number of mites from each species that would be found in each gram.

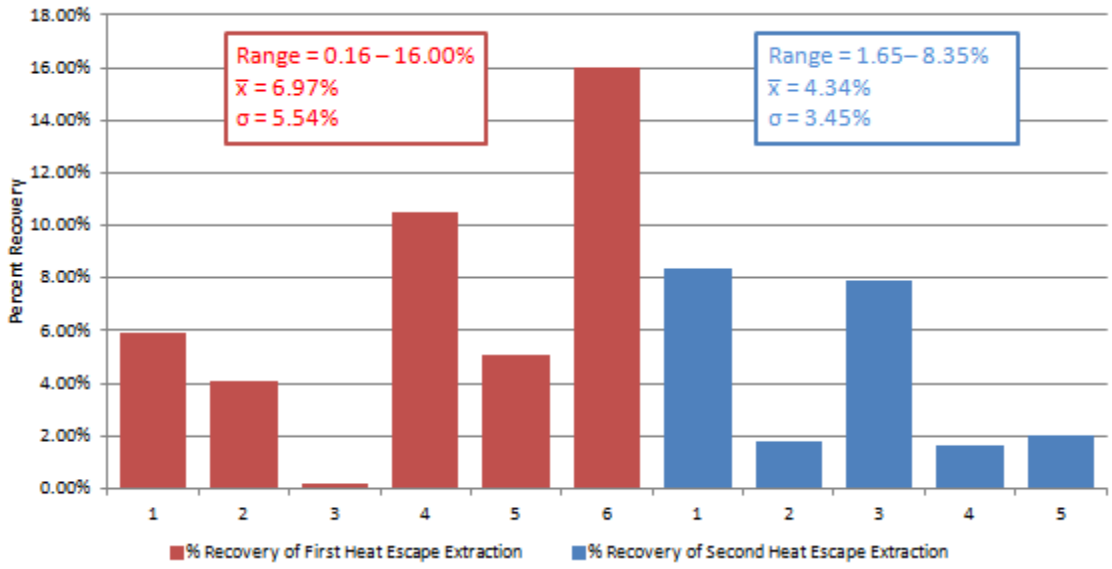


Figure 3- 6. Percentage of *D. pteronyssinus* recovered using heat escape extraction methods. Two different heat escape methods were used; these two methods are represented by two different colors in the chart. The red bars are each replicate of the first method, while the blue bars are each replicate of the second method. The numbers below each bar represent the replicate number. The first method, in the red, had 6 replicate, and the second method, in blue, had five replicates.

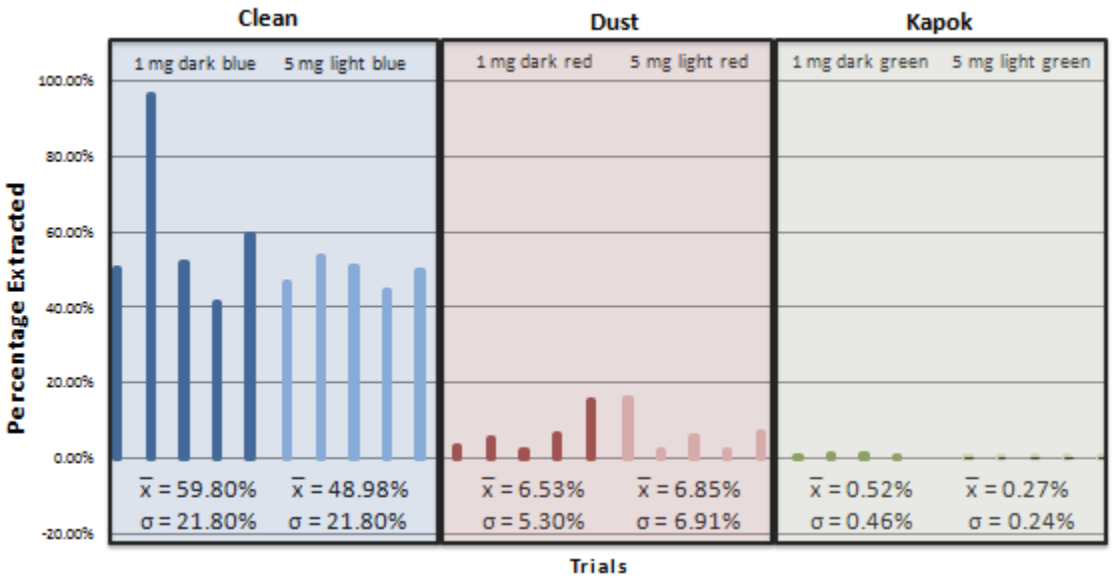


Figure 3- 7. Percentage of *D. pteronyssinus* recovered using the flotation extraction method. Mites were extracted from samples only containing EtOH (clean), dust, and the fiber kapok.

CHAPTER 4

A GUIDE TO COMMON HOUSE DUST MITES AND STORAGE MITES FOUND WITHIN THE HOME

Introduction

The terms house dust mites (HDMs) and storage mites refer to two groups of mite species that colonize the human living space. Together these mites are considered domestic mites. These mites are extremely small and typically found within household fabrics and stored products, respectively. This identification guide is intended for use by anyone interested in identifying mites found in close association with the human habitat and includes basic information on the taxonomy and biology of selected HDMs and storage mites. While there are many other mites that are found within the home, this guide is presented as a tool for identifying the adults of five of the most common mite types of mites found worldwide: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae, Blomia tropicalis, Suidasia* sp., and *Tyrophagus* sp.

House Dust Mites and Storage Mites

There are about 300 species of mites that can be found within household dust, furniture, carpets, fabrics, stored products, and other places in the home (Colloff 2009). This booklet focuses on two main categories – storage mites and house dust mites (HDMs). HDMs are classified in the family Pyroglyphidae and occur within household dust, carpets, bedding fabrics and furniture (Arlian and Morgan 2003). The most commonly encountered mites in this group include *Dermatophagoides farinae* and *D. pteronyssinus* (Arlian and Morgan 2003). Storage mites are classified in various families such as Echimyopodidae, Suidasiidae, and Acaridae, and

are found in products such as grain, hay, and straw in addition to household dust (Arlian and Morgan 2003). The most widely distributed and encountered storage mites include *Blomia tropicalis* (Caplin et al. 2009), *Tyrophagus* sp. (Arlian 1989, van Hage-Hamsten and Johansson 1992) , and *Suidasia* sp. (Hughes 1976, OConnor 1982).

The Problem of Mites in the Home

These animals are small, but they can cause health problems in humans. House dust mite feces and bodies contain proteins that are considered allergens because they can lead to allergies and asthma in humans. It is thought that allergy to HDMs occurs in 1-2% of the world's population (Colloff 2009). Asthma is the result of an allergic reaction that causes the airways within the body to become inflamed and is an extremely common aliment in children (Colloff 2009). Studies have shown a link between exposure to HDM allergens and the development of asthma (Sporik et al. 1990). One study found that a higher level of HDM allergens within a child's bed was linked to early expression of asthma symptoms (Sporik et al. 1990). However, other studies have not found a convincing link between severity of asthma and levels of HDM allergens found within the home. For example, one study found that rural families, involved in farming were less sensitized to HDMs (Schram-Bijkerk et al. 2006). This indicates that there are likely more factors responsible for expression of an allergic reaction than simple direct allergen exposure, but most clinicians consider HDMs a serious threat because of their known potential to trigger allergic reactions (Colloff 2009).

Taxonomy

Mites belong to the class Arachnida along with spiders, scorpions, and ticks (Walter and Proctor 1999). Mites and ticks are classified in the subclass Acari which contains approximately 45,000 discovered species (Walter and Proctor 1999). The oldest mite fossils date to 400 million

years ago (Bernini 1991). Estimates put the number of mites that remain to be discovered - and named - at over a million (Walter and Proctor 1999). Those estimates of mite diversity indicate that these small animals represent a vast number of lifestyles and ecological roles that may be important in understanding our planet's biodiversity and such topics as nutrient cycles and ecosystem stability. This guide illustrates the few species that are currently known from mites in the human habitat.

All the mites discussed in this identification guide are classified in the order Astigmata (Arlian and Morgan 2003). Astigmatid mites do not have a structured respiratory system and therefore do not have any external openings on their body that are used for breathing (Arlian and Morgan 2003). Instead, these mites exchange respiratory gas through their skin or exoskeleton (Arlian 1989). Most of the mites in this order are parasites of birds and mammals (OConnor 1994).

The family Pyroglyphidae contains 18 genera and 46 species. Mites in this family are found on and around birds, mammals, and in stored products, with the majority having an association with bird nests (Arlian and Morgan 2003). A large percentage of Pyroglyphid mites, about 73%, are found outside of homes (Fain et al. 1990). The remaining 29% includes three species commonly collected within the home: *Dermatophagoides pteronyssinus*, *D. farinae*, and *Euroglyphus maynei* (Wharton 1976, van Bronswijk 1981, Fain et al. 1990). It is thought that these mites made the shift from bird nests to homes only a few thousand years ago (Colloff 1991). House dust and bird nests do have some similarities, such as the availability of keratin found in bird feathers and human skin cells as well as high levels of heat and humidity produced by the host animal (Colloff 1991). HDMs are the only species that have a cosmopolitan distribution (Colloff 2009).

The storage mites featured in this booklet are found in a number of taxonomic families. *Blomia tropicalis* is classified in the family Echimyopodidae. This family contains 5 genera and 32 species (Krantz et al. 2009). *Tyrophagus* sp. is in the family Acaridae and this genus is found in many different habitats, including insect colonies and straw (Samšiňàk 1962, Hughes 1976). The genus *Suidasia* sp., in the family Suidasiidae, contains 18 species (Krantz et al. 2009) and can be found in storage products such as milk powder (Ho 1996), rice (Mariana et al. 2009), wheat bran, cowpeas, and peanuts (Gorham 1991). This genus is found worldwide, however they are more commonly found in countries with tropical climates such as India, Taiwan, and Thailand (Chmielewski 2009).

A summary of the taxonomic classification of the mites included in this identification guide is featured in Figure 4- 1.

Mite Collection and Preparation for Microscopic Examination

Mites are typically collected from houses using vacuum sampling (Colloff 2009). A dust collecting device attached to the vacuum hose is the most commonly used technique (Colloff 2009). This device is typically a sleeve that contains a wire mesh screen to collect the dust (Colloff 2009). This technique can be as simple as inserting nylon gauze into the vacuum hose (Colloff 2009).

In order to remove mites from the dust sample, they can be picked out with a fine needle or small hair (such as one from an eyebrow) attached to a handle. Mites will stick to the needle or hair and can be placed in ethanol for storage. Another option is to use the flotation method. In this method, the dust sample is soaked in 70% ethanol for 24 hours (Hart and Fain 1987). The sample is then moved to a saturated NaCl solution for 10 minutes (Hart and Fain 1987). During this time the mites will float to the top of NaCl solution, and can be picked off or filtered through

a vacuum filtration system (Hart and Fain 1987). The mites can then be placed in ethanol for storage.

In order to view mites under a light microscope, they are typically placed on a microscope slide with a mounting media such as Hoyer's (Krantz et al. 2009). This allows for the mite to 'clear', exposing the internal genitalia. Lactic acid or de Faure's mounting media are alternatives for clearing mites for light microscopy (Krantz et al. 2009). Once the mite it in the mounting media, a coverslip is applied and the mites can be viewed.

In order to view the mites under a confocal microscope, you prepare the mites the same way as for light microscope. As an alternative, if an inverted confocal microscope is used, the mites may be placed in a chamber that contains a coverslip bottom. This chamber can be filled with ethanol in order to keep the mites hydrated.

Mites have to be fixed and prepared in a specific manner before they can be used viewed using a SEM (Glauert 1987, Glauert and Lewis 1998).). Mites usually fixed in gluteraldehyde or osmium tetroxide (Klaus and Schawaroch 2006). After this process, they are coated with gold, gold-palladium, platinum, or carbon in order to allow examination with the microscope (Krantz et al. 2009).

The Mite Body and Its Parts

The body of a mite is divided into two main regions. The front, or head region, is known as the gnathosoma and the second, tail-end, region is called the idiosoma, (Krantz et al. 2009). The gnathosoma contains structures used in feeding called the chelicerae and palps (Krantz et al. 2009). These two structures allow the mite to acquire and process food prior to swallowing (Krantz et al. 2009). The idiosoma, found directly behind the gnathosoma, is similar to the thorax and abdomen in insects and is the body region that contains the legs, majority of the digestive

tract, and sex organs (Krantz et al. 2009). Figure 4- 3 provides illustrations of the mite body, regions and main body parts most often used in mite identification. There is also a glossary that defines common mite body parts at the end of this document.

Lifecycle of Mites

Mites pass through several life stages before they reach adulthood. Mites go from one stage to the next by shedding their skin, called the exoskeleton (Colloff 2009). The number of stages and the length of each stage depends on the species of mite and environmental conditions (mostly temperature and humidity) (Colloff 2009). Mites that have just hatched from an egg are considered to be in the larval stage (Colloff 2009). This stage can be readily identified because larval mites have a total of 6 legs (Colloff 2009). Following this first stage are two stages where the mites are called nymphs. Mites in this stage have eight legs, but they are still considered immature (Colloff 2009). The nymphal stages are followed by the adult stage signified when the mite has developed functional sex organs (genitalia) (Colloff 2009). Adults mites live for approximately 4-6 weeks (Colloff 2009). Figure 4- 2 shows a typical mite life cycle, including the egg, larval, two nymphal stages (protonymph and tritonymph), along with the adult stage.

Keys for Identifying House Dust and Storage Mites

Recently published keys include one by Colloff (2009) for common house dust mites and a key by the United States Department of Agriculture and Department of Health and Human Services for storage mites (Gorham 1991). These keys can help identify HDMs and storage mites beyond the common species included in this guide and are useful, in conjuncture with this booklet, for verifying identification of mite species. A key that includes just the mites found in this guide is located at the end of the document. This guide provides information for identifying HDMs and storage mites first to the taxonomic level of genus and then to differentiate a few of

the most common mites to species. It includes pictures taken from several different microscopes which provide contrasting views of certain characters that are found on these mites. It has been derived from the key created by Colloff (2009).

Distinguishing Sex and Developmental Stage

There are several characteristics used to distinguish between sex and developmental stages. This guide focuses on adult specimens; however it is useful to know the characteristics of other developmental stages. An outline of these stages is found in Figure 4- 2. The eggs are oblong egg-shaped structures found in samples of these mites. The next stage is the larval stage which has 3 pairs of legs instead of 4. There are two nymphal stages that may be present in samples – the tritonymph and the protonymph. These mites will have 4 pairs of legs like adult mites; however they will not have adult genitalia. Instead, they will have genital papillae is there a description or image for reference. Protonymphs have one pair of genital papillae, while tritonymphs have two pairs of genital papillae.

Adult mites have genitalia along with 4 pairs of legs. Females will have a v-shaped structure between the second and third legs, known as the vulva which is the site where eggs are laid. Above the vulva is the U-shaped epigynium. Male mites display an aedeagus. Some mites, such as *D. farinae*, *D. pteronyssinus*, *Suidasia* spp., and *Tyrophagus* spp., have anal suckers. A line drawing of these features is found in Figure 4- 3.

Species Included in this Guide

This guide includes the most common mites found within house dust. This includes *Dermatophagoides pteronyssinus*, *D. farinae*, *Blomia tropicalis*, *Suidasia* sp., and *Tyrophagus* sp. This section contains an overview of each of these mites.

Dermatophagoides pteronyssinus

Dermatophagoides pteronyssinus, called the European Dust Mite, is found in homes throughout the world (Arlian et al. 2002). It is the species of HDM that is most often identified in surveys (Colloff 2009).

D. pteronyssinus can complete its life cycle at a wide range of temperatures 16 - 35°C (Arlian et al. 1990). The males usually live about 77 days, while unmated females live 45 days but once mated live less – about 31 days (Colloff 1987). Females can lay between 40 to 80 eggs (about 2-3 a day) during their lifespan (Colloff 1987).

Dermatophagoides farinae

Dermatophagoides farinae is known by the common name American Dust Mite (Arlian et al. 2002). However, it has been discovered that this mite has a cosmopolitan distribution (Arlian et al. 1992, Arlian et al. 2002).

D. farinae has an optimum range of developmental temperatures between 23-30° C (Arlian and Dippold 1996). Outside of this temperature *D. farinae* eggs do not hatch (Arlian and Dippold 1996). This species prefers a relative humidity of 75% (Arlian and Dippold 1996). Adult females reach a size of 390-440 µm (Colloff 2009). Females live longer than the females of other HDM species – about 100 days –and they lay 40-80 egg during their lifespan (Bonnefoy et al. 2008).

Blomia tropicalis

Blomia tropicalis is considered a storage mite that is found in household dust, and produces an allergen that can affect sensitized individuals (Stanaland et al. 1994, Stanaland et al. 1996). These mites have been recovered within homes at levels as high as 8,934 mites per gram of dust (Mariana 2002).

It is common in tropical and subtropical areas (Caplin et al. 2009). In the United States, this includes humid areas such as Florida, Texas, and the Gulf Coast (Fernandez-Caldas et al. 1990, Arlian et al. 1992, Caplin et al. 2009), but in general this mite is found 30° to either side of the equator (Colloff 2009). This species ranges in size between 0.23-0.47 mm in length (Mariana et al. 2007). Mated females live, on average, 32.2 days, and male live on average 30.9 days (Mariana et al. 1996).

Suidasia spp.

Storage mite in the genus *Suidasia* produce allergens that can cause allergic reactions in humans (Chmielewski 2009). They are found less often in HDM surveys than other species (Vargas and Mairena 1991, Fernandez-Caldas et al. 1993, Mariana and Ho 1996, Chew et al. 1999). The genus has a cosmopolitan distribution, and although it is thought to be mainly tropical, it has been surveyed in more temperate areas such as Poland, Germany, and Great Britain (Chmielewski 2009). It can survive and thrive at a temperature of 20° C and 85% relative humidity (Chmielewski 2009).

The life cycle is short, taking about 12.6 days to progress from egg to adult (Mercado et al. 2001). Females can lay up to 111.6 eggs during a mean lifespan of 48.6 ±13 days (Mercado et al. 2001). Males have a mean lifespan of 49.1 ±20 days (Mercado et al. 2001).

Tyrophagus spp.

Tyrophagus sp. is one type of storage mite known for infesting stored grains and finished grain products (Arlian 1989). It also is a cause of allergies (Liao et al. 2010). This species is found throughout the world (Wharton 1976).

Tyrophagus sp. mites prefer a temperature between 25-30° C with a relative humidity of 80% (van Hage-Hamsten and Johansson 1992). Members of this genus can produce between 100 to 700 eggs during their lifetime (Fan and Zhang 2007).

Identifying the genus *Dermatophagoides*

A defining physical character for the genus *Dermatophagoides* involves setae on the dorsal side of the idiosoma. The dorsal seta *sce*, is 5x longer than seta *sci* (Figure 4- 5)(Arlian 1989). This genus also has cuticle that shows fine striations appearing as ridges and grooves (Figure 4- 4).

Identifying *Dermatophagoides pteronyssinus*

Figure 4- 9 shows an adult female *Dermatophagoides pteronyssinus*, and Figure 4- 11 shows an adult male. The receptaculum seminis of the adult female has a ‘sombrero’ or ‘hat’ shape (Figure 4- 6 and Figure 4- 7). The epigynum of this species has a greater arc when compared to *D. farinae* (Figure 4- 8, Figure 4- 9, and Figure 4- 10). Males can be separated from similar species of mites because the first pair of legs because are not enlarged, and the epimeres of coxae I do not form a Y or V shape (Figure 4- 11 and Figure 4- 12).

Identifying *Dermatophagoides farinae*

Figure 4- 9 shows an adult female *Dermatophagoides farinae*, and Figure 4- 11shows an adult male. The shape of the receptaculum seminis is cup-shaped in females of this species (Figure 4- 6 and Figure 4- 7). They also have an epigynum that is less arched than in *D. pteronyssinus* (Figure 4- 8, Figure 4- 9, and Figure 4- 10). Adult males of this species have a large first pair of legs relative to the rest of their legs (Figure 4- 11). Males also have a V or Y-shaped structure formed by the epimeres of coxae I, which is not seen in male *D. pteronyssinus* (Figure 4- 11).

Identifying *Blomia tropicalis*

Both sexes of this species have legs that become thinner as they reach their apex (taper off) (Figure 4- 13), a hairy body (Figure 4- 13), a round shape (Figure 4- 13), and have a rugose (wrinkled) cuticular surface (Figure 4- 4) (Mariana et al. 2007). Figure 4- 14 shows an adult female and adult male. Males of this species do not have anal suckers, unlike the other mites in this guide. The leg of males of this species is flexed at the apex of tarsi IV (Figure 4- 14) (Mariana et al. 2007).

Identifying *Suidasia* spp.

Suidasia spp. mites can be identified by the presence of a propodosomal shield (Figure 4- 15) on the anterior dorsal surface. This structure is not found in the other mite species treated in this guide. These species also have a cuticle that looks like it is covered in scales (Figure 4- 4). Figure 4- 16 shows an adult female and adult male, and Figure 4- 17 shows another image of an adult male.

Identifying *Tyrophagus* spp.

The genus *Tyrophagus* has a prodorsal shield with a groove present (Figure 4- 18). Seta *vi* is less than three times as long as seta *ve* (Figure 4- 18). Figure 4- 19 shows an adult female and adult male.

Conclusion

House dust mites and storage mites are found throughout the world. Their presence within the home can lead to allergen sensitization which induces allergic reactions from mild to severe as well as health problems like asthma. This identification guide uses microscope pictures and descriptions to introduce anyone interested in HDMs and related health to common mites within the home. This guide also contains an introduction to understanding the taxonomy and

biology of both selected common HDMs and storage mites. It is intended to be an easy-to-use guide for identifying mites most commonly encountered within a home.

Dichotomous Key for Common House Dust Mites and Storage Mites

1. Round without legs, egg-shaped (Figure 4- 2).....egg

   Has three pairs of legs (Figure 4- 2) ..... larva

   Has 4 pairs of legs (Figure 4- 2) 2

2. No genitalia present ..... nymph

   Genitalia present. Males will have an aedeagus (Figure 4- 3) and females will have a vulva (Figure 4- 3) (adult specimens)..... 3

3. Cuticle is striated (Figure 4- 4) and setae *sce* is 5x longer than *sci* 4

   Cuticle is not striated, but has a different pattern 5

4. **Genus *Dermatophagoides* spp.**: If male specimen, epimeres of coxae 1 are formed into a Y or V shape and first pair of legs are enlarged in comparison with the other legs (Figure 4- 11). If female specimen, receptaculum seminis is shaped like a cup (Figure 4- 6 and Figure 4- 7) and the epigynum is less arched (Figure 4- 8 and Figure 4- 10) .....*D. farinae*

   If male specimen, epimeres of coxae 1 are not joined to form a Y or V shape and the first pair of legs are not enlarged in comparison with the other legs (Figure 4- 11). If female specimen, receptaculum seminis is shaped like a hat (Figure 4- 6 and Figure 4- 7) and the epigynum is more arched (Figure 4- 8 and Figure 4- 10) .... *D. pteronyssinus*

5. Cuticle is scaly (Figure 4- 4), males do have anal suckers .....*Suidasia* sp.

   Mite cuticle not scaly, mites appear to have many setae (hairy) 6

6. Cuticle is rugose (Figure 4- 4), legs are tapered (Figure 4- 13), males contain a flexed tarsi IV but no anal suckers (Figure 4- 14) ..... *Blomia tropicalis*

Seta *ve* is similar in size to seta *vi* (Figure 4- 18) .....*Tyrophagus* sp.

Glossary

Aedeagus – (ae) also known as the penis, it is the male copulatory organ.

Anal suckers – (as) an external structure found on male mites that is used to attach to the female during copulation.

Anterior - towards the front end.

Apodeme - a groove in the cuticle that is often a site where muscles attach internally.

Bursa copulatrix – (bc) found in female mites on the ventral side and is where females receive sperm from males.

Coxa - the first part of the mite leg where it joins at the body.

Coxae - plural of coxa.

Cuticle - the outer covering of a mite

Dorsal - the upper side of the mite body; the side of the mite that is usually exposed.

Epigynum – (e) a hardened structure in females that is shaped like an inverted U found posterior to the vulva.

Epimere - an apodeme that is found on the anterior coxae.

Gnathosoma - the anterior portion, head region, of the mite that contains the palps and the chelicerae which are used for feeding.

Idiosoma - the posterior portion (tail-end), or main body of the mite that contains the legs, digestive system, and sex organs.

Larva - a juvenile stage in mite development that comes after emerging from an egg. Larval-stage mites have a total of 6 legs.

Nymph - a juvenile stage in mite development that comes after the larval stage. Mite nymphs have a total of 8 legs.

Posterior - towards the rear or tail end.

Prodorsal shield - a shield found on the anterior part of the dorsal side.

Prodorsum - dorsal part of the propodosoma.

Propodosoma - the anterior part of the idiosoma (the part of the mite following the gnathosoma) in acariform mites that contains the first two pairs of legs.

Propodosomal shield - a shield on the anterior dorsal side of the propodosoma.

Receptaculum seminis – (rs) internal structure in females that is used to store sperm.

Rugose - wrinkled.

Seta *ve* - also known as the external vertical seta, this is found on the prodorsum.

Seta *vi* - also known as the internal vertical seta, this is found prodorsum.

Seta - hair-like structures found on mites and other invertebrates.

Setae - plural of seta.

Seta *sce* - also known as the external scapular seta, it is a hair found on the dorsal side (the back) of the mite near the gnathosoma, outside of seta *sci*.

Seta *sci* - also known as the internal scapular seta, it is a hair found on the dorsal side (the back) of the mite near the gnathosoma, internal to seta *sce*.

Striations - a type of raised pattern of fine ridges found on the cuticle that is similar to a fingerprint

Tarsi - plural of tarsus.

Tarsus - the last part of the leg.

Ventral - the underside of the body, the underbelly

Vulva – (v) found on female on the ventral side. It is between legs III and IV, and is the site where eggs emerge from the mite.

References cited

Arlian, L. G. 1989. Biology and ecology of house dust mites, *Dermatophagoides* sp. and *Euroglyphus* sp. Immunol Allergy Clin North Am 9: 339-356.

Arlian, L. G., and J. S. Dippold. 1996. Development and fecundity of *Dermatophagoides farinae* (Acari: Pyroglyphidae). J Med Entomol 33: 257-260.

Arlian, L. G., and M. S. Morgan. 2003. Biology, ecology, and prevalence of dust mites. Immunol Allergy Clin North Am 23: 443-468.

Arlian, L. G., C. M. Rapp, and S. G. Ahmed. 1990. Development of *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). J Med Entomol 27: 1035-1040.

Arlian, L. G., M. S. Morgan, and J. S. Neal. 2002. Dust mite allergens: ecology and distribution. Curr Allergy Asthma Rep 2: 401-411.

Arlian, L. G., D. Bernstein, I. L. Bernstein, S. Friedman, A. Grant, P. Lieberman, M. Lopez, J. Metzger, T. Platts-Mills, and M. Schatz. 1992. Prevalence of dust mites in the homes of people with asthma living in eight different geographic areas of the United States*. J Allergy Clin Immunol 90: 292-300.

Bernini, F. 1991. Fossil acarida, pp. 253-262. *In* A. M. Simonetta and S. Conway Morris (eds.), The early evolution of Metazoa and the significance of problematic taxa. Cambridge University Press, Cambridge and New York.

Bonnefoy, X., H. Kampen, and K. Sweeney. 2008. House dust mites, pp. 85-129, Public health significance of urban pests. World Health Organization.

Caplin, J., A. Capriles-Hulett, V. Iraola, H. Pinto, M. Sanchez-Borges, G. de los Santos, and E. Fernandez-Caldas. 2009. Allergic sensitization to domestic mites in Corpus Christi, Texas. Allergy Asthma Proc 30: 166-170.

Chew, F. T., L. Zhang, T. M. Ho, and B. W. Lee. 1999. House dust mite fauna of tropical Singapore. Clin. Exp. Allergy 29: 201-206.

Chmielewski, W. 2009. Pollen pellets as a medium for culture of mites *Suidasia pontifica* (Oud.)(Acarina, Suidasiidae). J Apic Sci 53: 37-41.

Colloff, M. J. 1987. Effects of temperature and relative humidity on development times and mortality of eggs from laboratory and wild populations of the European house-dust mite *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). Exp Appl Acarol 3: 279-289.

Colloff, M. J. 1991. Practical and theoretical aspects of the ecology of house dust mites (Acari: Pyroglyphidae) in relation to the study of mite-mediated allergy. Rev Med Vet Entomol 79: 611-630.

Colloff, M. J. 2009. Dust mites, 1 ed, vol. CSIRO Publishing and Springer, Collingwood, Australia.

Fain, A., B. Guérin, and B. J. Hart. 1990. Mites and allergic disease, vol. Allerbio, Varennes en Argonne.

Fan, Q. H., and Z. Q. Zhang. 2007. *Tyrophagus* (Acari: Astigmata: Acaridae). Fauna N.Z. 56: 1-291.

Fernandez-Caldas, E., L. Puerta, D. Mercado, R. F. Lockey, and L. R. Caraballo. 1993. Mite fauna, Der p I, Der f I and *Blomia tropicalis* allergen levels in a tropical environment. Clin. Exp. Allergy 23: 292-297.

Fernandez-Caldas, E., R. W. Fox, G. A. Bucholtz, W. L. Trudeau, D. K. Ledford, and R. F. Lockey. 1990. House dust mite allergy in Florida. Mite survey in households of mite-sensitive individuals in Tampa, Florida. Allergy Proc 11: 263-267.

Glauert, A. M. 1987. Fixation, dehydration and embedding of biological specimens - Cytological staining, pp. 5-110, Practical methods in electron microscopy, vol. 3. Elsevier, Amsterdam.

Glauert, A. M., and P. R. Lewis. 1998. Biological specimen preparation for transmission electron microscopy - Embedding methods., pp. 147-69, Practical methods in electron microscopy, vol. 16. Portland Press, London.

Gorham, J. R. 1991. Insect and mite pests in food: An illustrated key, vol. 1. U.S. Dept. of Agriculture, Agricultural Research Service : U.S. Dept. of Health and Human Services, Public Health Service, Food and Drug Administration, Washington, D.C.

Hart, B. J., and A. Fain. 1987. A new technique for isolation of mites exploiting the difference in density between ethanol and saturated NaCl: qualitative and quantitative studies. Acarol. 28: 251-254.

Ho, T. M. 1996. First report of *Suidasia pontifica* (Acari: Acaridae) in milk powder. Southeast Asian J Trop Med Public Health 27: 853-854.

Hughes, A. M. 1976. The mites of stored food and houses, 2 ed, vol. H.M.S.O., London.

Klaus, A. V., and V. Schawaroch. 2006. Novel methodology utilizing confocal laser scanning microscopy for systematic analysis in arthropods (Insecta). Integr Comp Biol 46: 207-214.

Krantz, G. W., D. E. Walter, and V. Behan-Pelletier. 2009. A manual of acarology, 3 ed, vol. Texas Tech University Press.

Liao, E. C., C. M. Ho, M. Y. Lin, and J. J. Tsai. 2010. *Dermatophagoides pteronyssinus* and *Tyrophagus putrescentiae* allergy in allergic rhinitis caused by cross-reactivity not dual-sensitization. J Clin Immunol 30: 830-839.

Mariana, A. 2002. The biology & distribution of allergen producing mites with particular reference *to Blomia tropicalis* (Acarina: Astigmata: Echimyopodidae) in the Klang Valley, Malaysia. University of Malaya, Kuala Lumpur, Malaysia.

Mariana, A., and T. M. Ho. 1996. Distribution of *Blomia tropicalis* in Malaysia. Trop Biomed 13: 85-88.

Mariana, A., T. M. Ho, and S. K. Heah. 1996. Life-cycle, longevity and fecundity of *Blomia tropicalis* (Acari: Glycyphagidae) in a tropical laboratory. Southeast Asian J Trop Med Public Health 27: 392.

Mariana, A., A. S. Santana Raj, S. N. Tan, and T. M. Ho. 2007. Scanning electron micrographs of *Blomia tropicalis* (Acari: Astigmata: Echimyopodidae), a common house dust mite in Malaysia. Trop Biomed 24: 29-37.

Mariana, A., T. M. Ho, T. Y. Lau, S. K. Heah, and A. L. Wong. 2009. Distribution of arthropods in rice grains in Malaysia. Asian Pac J Trop Med 2: 1-7.

Mercado, D., L. Puerta, and L. Caraballo. 2001. Life-cycle of *Suidasia medanensis* (= *pontifica*)(Acari: Suidasiidae) under laboratory conditions in a tropical environment. Exp Appl Acarol 25: 751-755.

OConnor, B. M. 1982. Astigmata, pp. 146-169, Synopsis and classification of living organisms, vol. 2. McGraw Hill, New York.

OConnor, B. M. 1994. Life-history modifications in astigmatid mites. Mites, ecological and evolutionary analyses of life-history patterns: 136-159.

Samšiňàk, K. 1962. Beitrage zur eknntnis der gattung *Tyrophagus* Oudemans. Cas. Cs. Spol. Entomol 59: 266-280.

Schram-Bijkerk, D., G. Doekes, M. Boeve, J. Douwes, J. Riedler, E. Üblagger, E. Von Mutius, J. Budde, G. Pershagen, and M. Van Hage. 2006. Nonlinear relations between house dust mite allergen levels and mite sensitization in farm and nonfarm children. Allergy 61: 640-647.

Sporik, R., S. T. Holgate, T. A. E. Platts-Mills, and J. J. Cogswell. 1990. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. N Engl J Med 323: 502-507.

Stanaland, B. E., E. Fernandez-Caldas, C. M. Jacinto, W. L. Trudeau, and R. F. Lockey. 1994. Sensitization to *Blomia tropicalis*: skin test and cross-reactivity studies. J Allergy Clin Immunol 94: 452-457.

Stanaland, B. E., E. Fernández-Caldas, C. M. Jacinto, W. L. Trudeau, and R. F. Lockey. 1996. Positive nasal challenge responses to *Blomia tropicalis*. J Allergy Clin Immunol 97: 1045-1049.

van Bronswijk, J. 1981. House dust biology for allergists, acarologists and mycologists, vol. The Author, the University of Michigan.

van Hage-Hamsten, M., and S. G. O. Johansson. 1992. Storage mites. Exp Appl Acarol 16: 117-128.

Vargas, M. V., and H. A. Mairena. 1991. House dust mites from the metropolitan area of San José, Costa Rica. Int J Acarol 17: 141-144.

Walter, D. E., and H. C. Proctor. 1999. Mites: ecology, evolution and behaviour, vol. UNSW Press, the University of Michigan.

Wharton, G. W. 1976. House dust mites. J Med Entomol 12: 577-621.

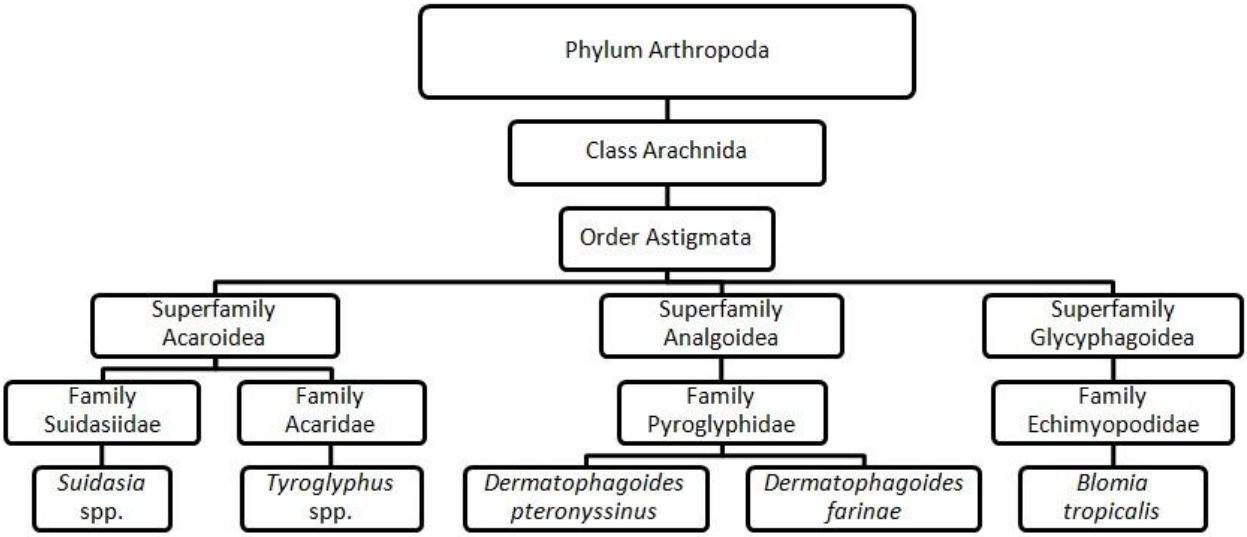


Figure 4- 1. Taxonomic overview of species of HDMs and storage mites.

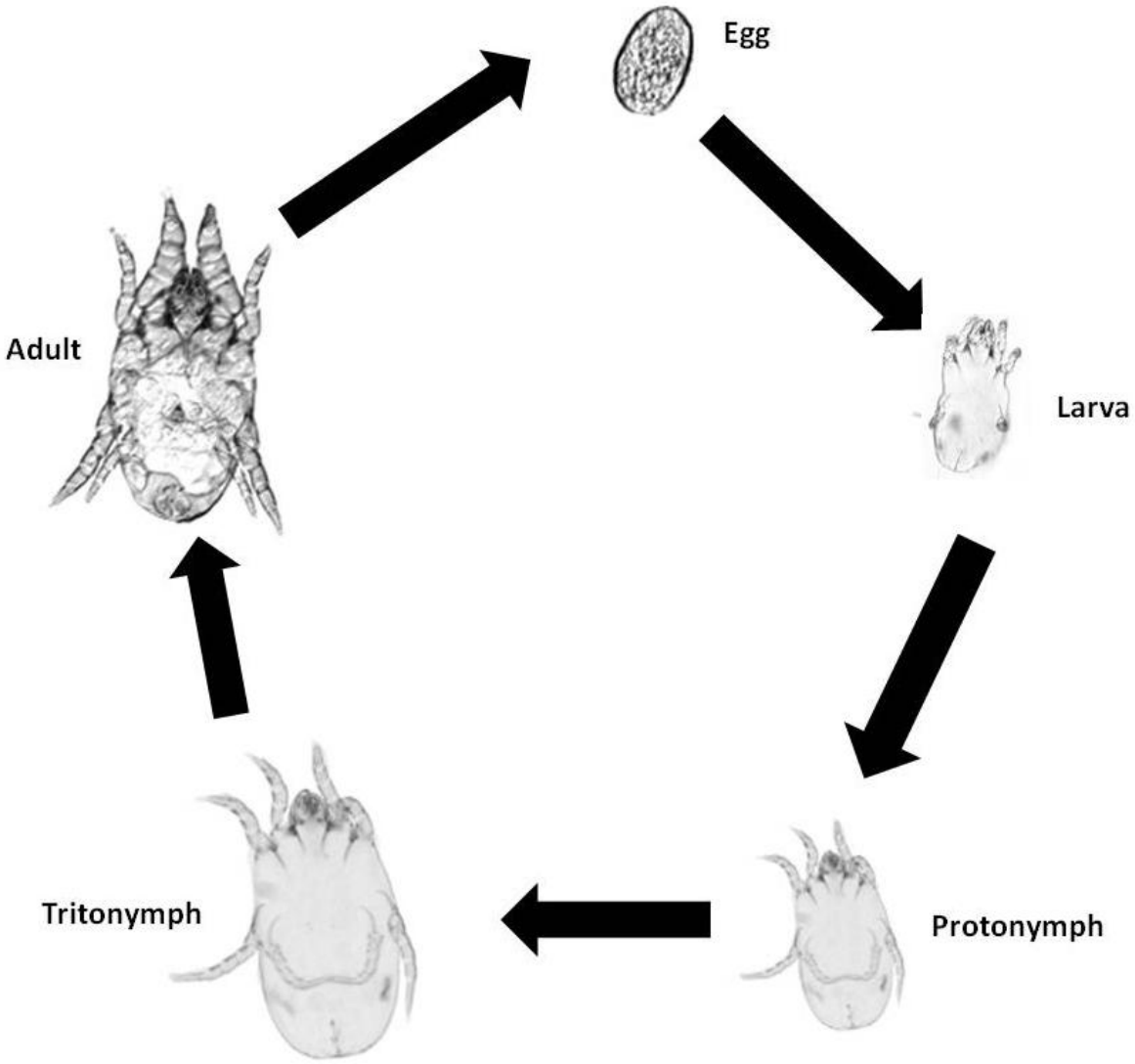


Figure 4- 2. Basic mite lifecycle from egg to mature adult.

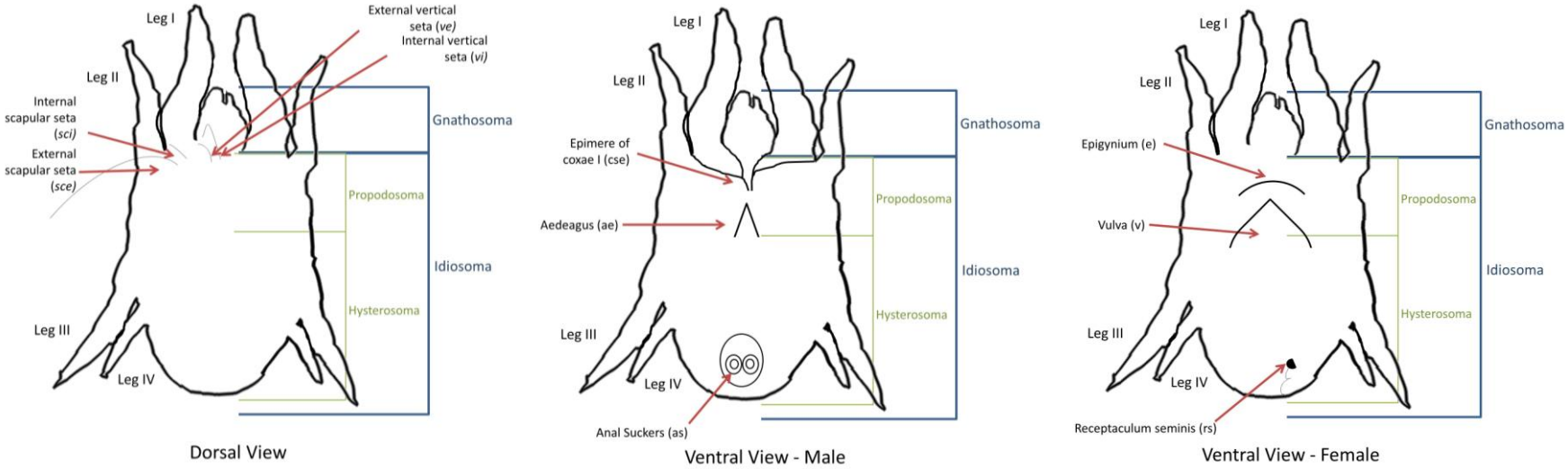


Figure 4- 3. Basic HDM and storage mite anatomy from the dorsal side, along with sex specific characters found on the ventral side. It should be noted that the receptaculum seminis is an internal structure.

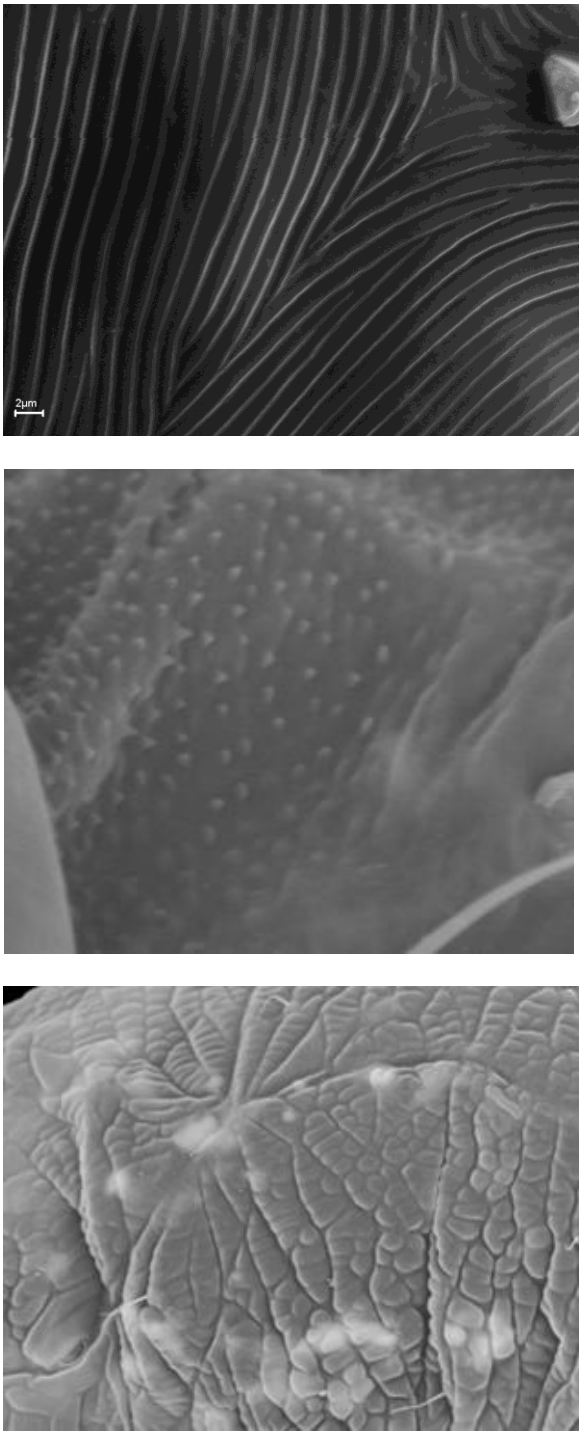


Figure 4- 4. *Dermatophagoides* spp., *Blomia tropicalis* and *Suidasia* sp. cuticular surfaces (arranged top to bottom). Pictures were taken with SEM. *Dermatophagoides* spp. cuticle has fine striations, *Blomia tropicalis* cuticle is rugose, and *Sudasia* sp. looks like it is covered in many scales.

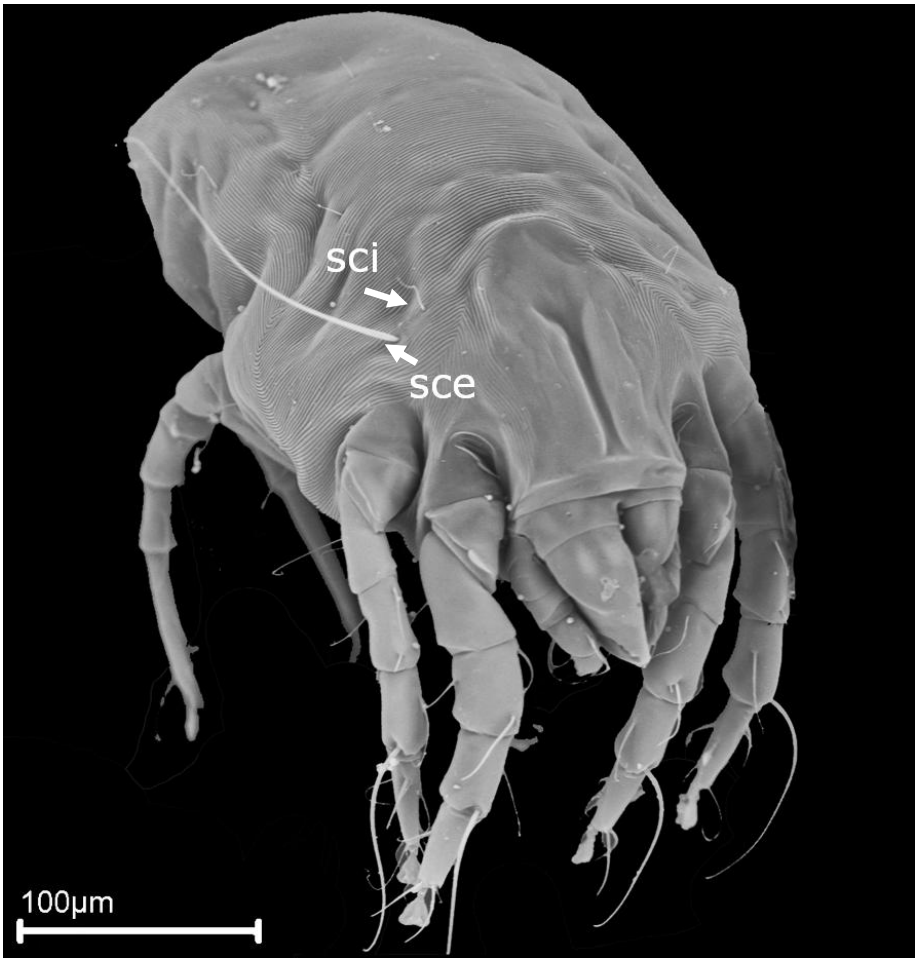


Figure 4- 5. *Dermatophagoides* spp., adult, dorsal side, SEM. The cuticle is covered in fine striations and the external scapular seta (*sce*) is at at least 5x longer than the internal scapular seta (*sci*).

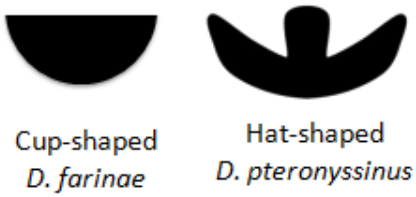


Figure 4- 6. *D. pteronyssinus* and *D. farinae*, female, adult, receptaculum seminis drawings. *D. farinae* has one that is cup-shaped, while *D. pteronyssinus* has a hat-shaped one.

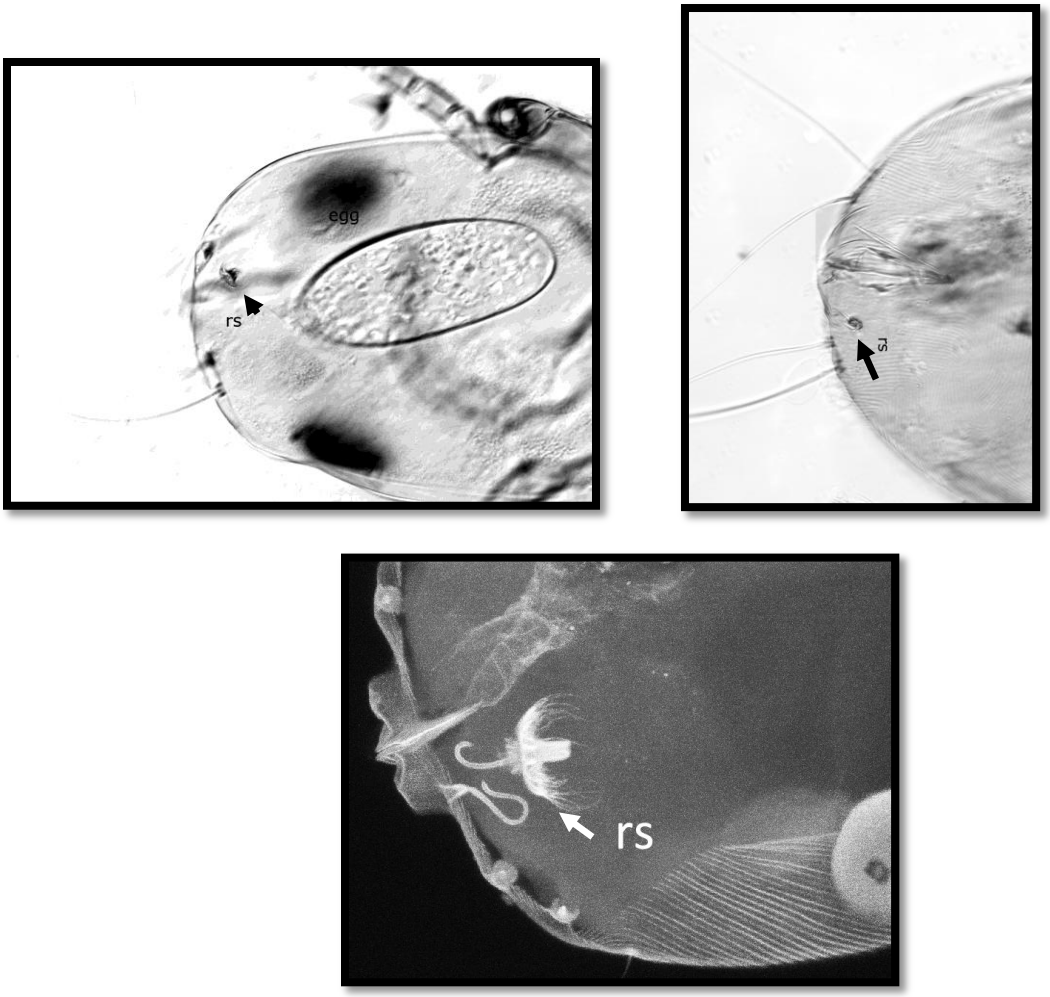


Figure 4- 7. *Dermatophagoides pteronyssinus* and *D. farinae* adult females, views of the receptaculum seminis (rs). Top left – *D. pteronyssinus,* light microscope, with a hat-shaped receptaculum seminis. An egg is also present in the picture. Top right – *D. farinae*, light microscope, with a cup-shaped receptaculum seminis. Bottom – *D. pteronyssinus,* confocal microscope, with another view of the hat-shaped receptaculum seminis.

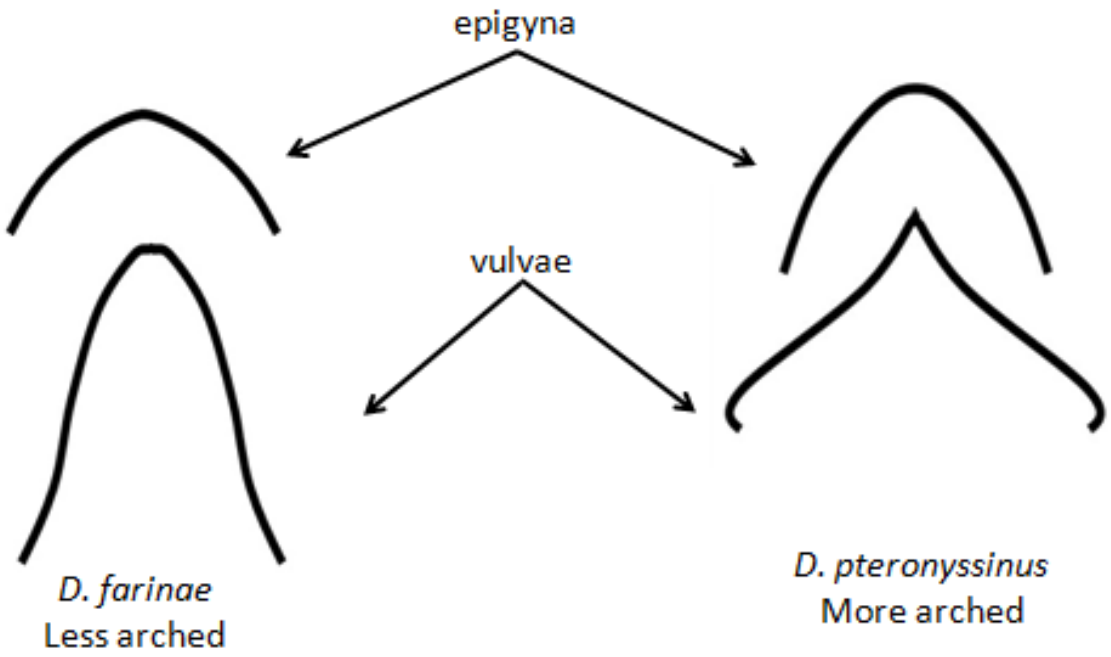


Figure 4- 8. *Dermatophagoides pteronyssinus* and *D. farinae* female adult vulvae and epigyna, line drawings. *D. farinae* has an epigynum that is less arched than in *D. pteronyssinus.*

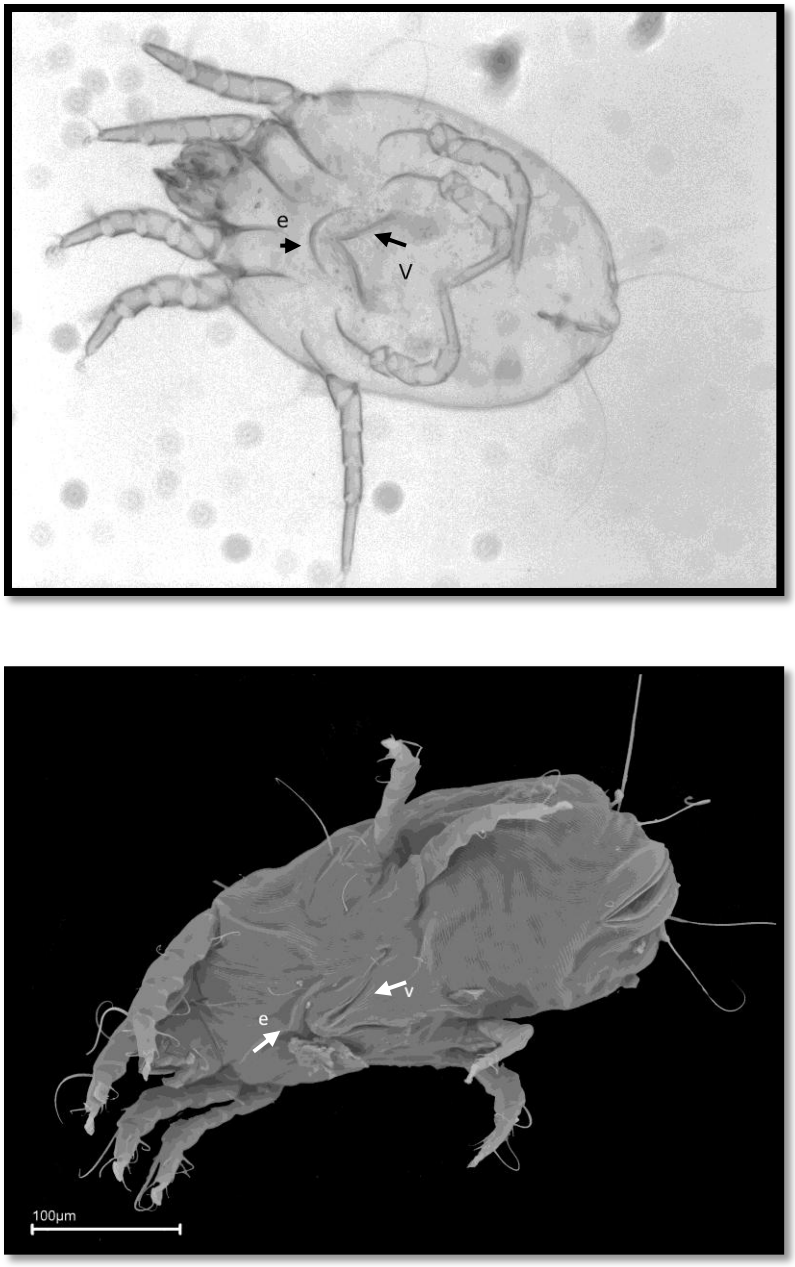


Figure 4- 9. *Dermatophagoides pteronyssinus* and *D. farinae*, females, adults. Top – *D. pteronyssinus*, light microscope, with the U-shaped epigynum (e) and vulva (v). Bottom – *D. farinae*, SEM, epigynium (e) and vulva (v) are shown. The epigynum in *D. farinae* is less arched than in *D. pteronyssinus.*

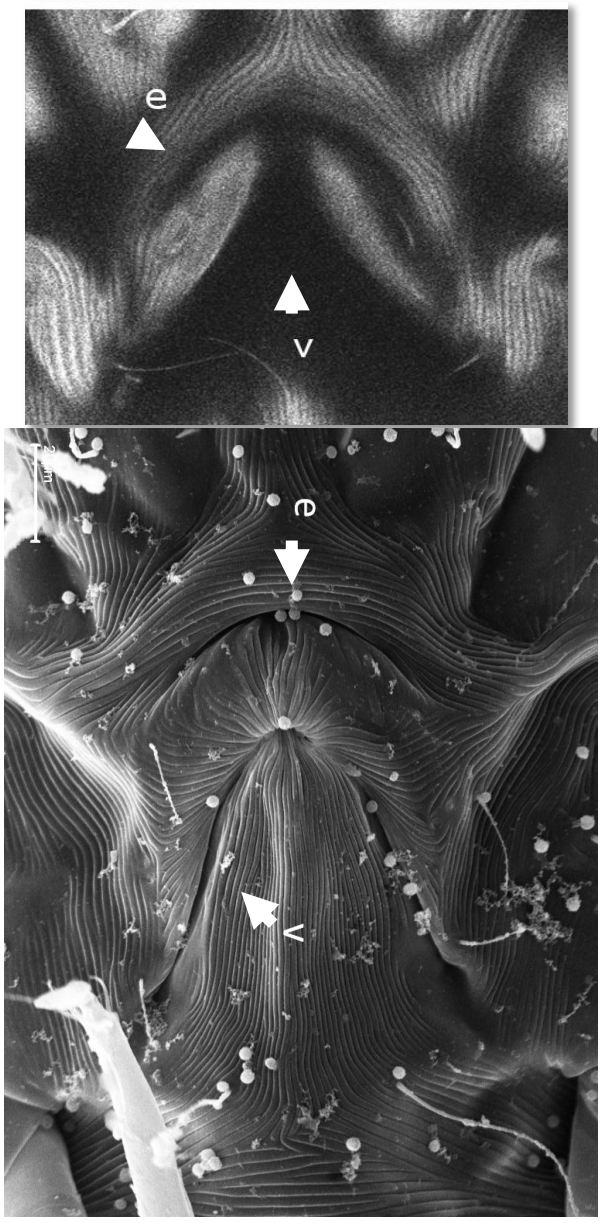


Figure 4- 10. *Dermatophagoides pteronyssinus* and *D. farinae,* females, adults, ventral. Top – *D. pteronyssinus*, confocal, epigynium (e) and vulva (v) can be seen. Bottom – *D. farinae*, SEM, epigynium and vulva are shown. The epigynium in *D. farinae* is less arched than in *D. pteronyssinus*.

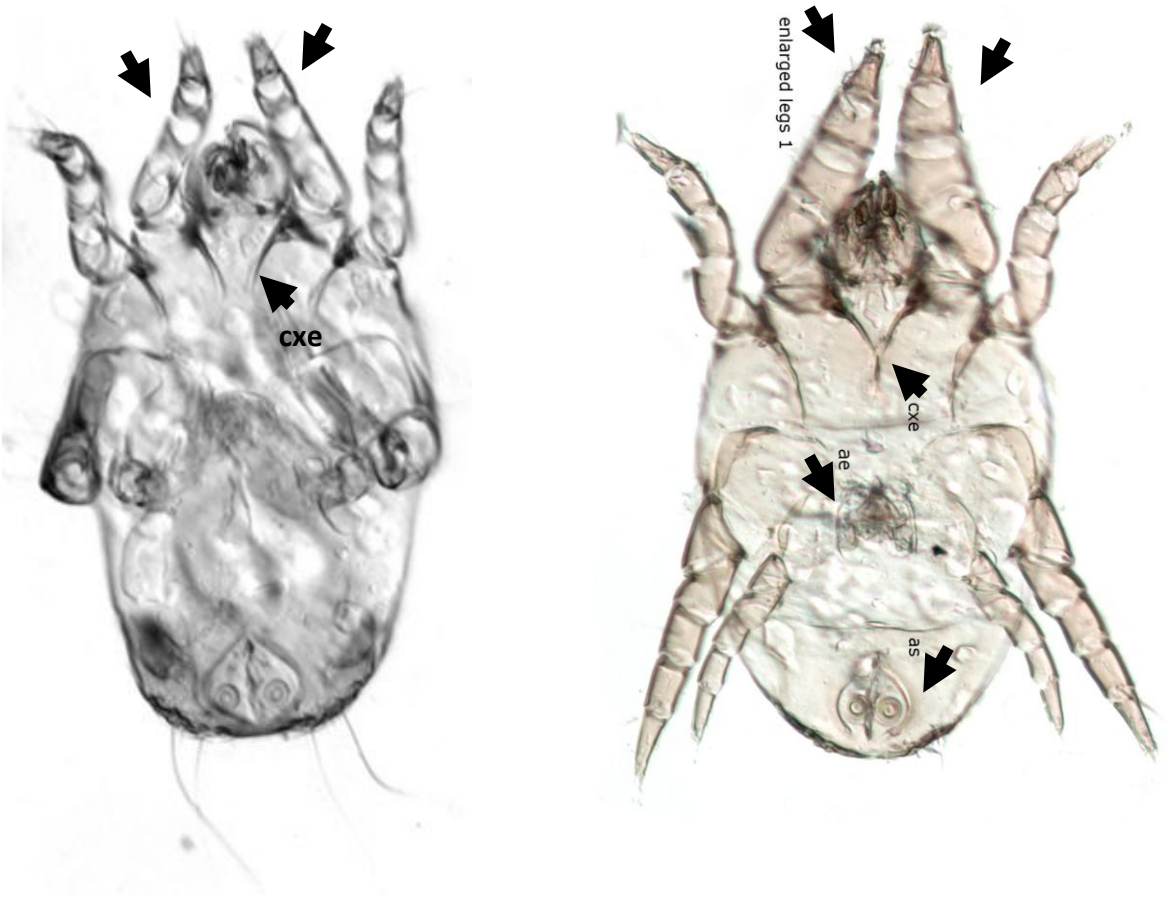


Figure 4- 11. *Dermatophagoides pteronyssinus* and *D. farinae,* males, adults, light microscope. Left – *D. pteronyssinus* has non-enlarged first pair of legs. The epimeres of coxae I (cxe) are not in a Y or V shape. Right – *D. farinae* enlarged first pair of legs shown, along with a Y or V shape formed by the epimers of coxae I. The aedeagus (ae) and anal suckers (as) are also labeled.

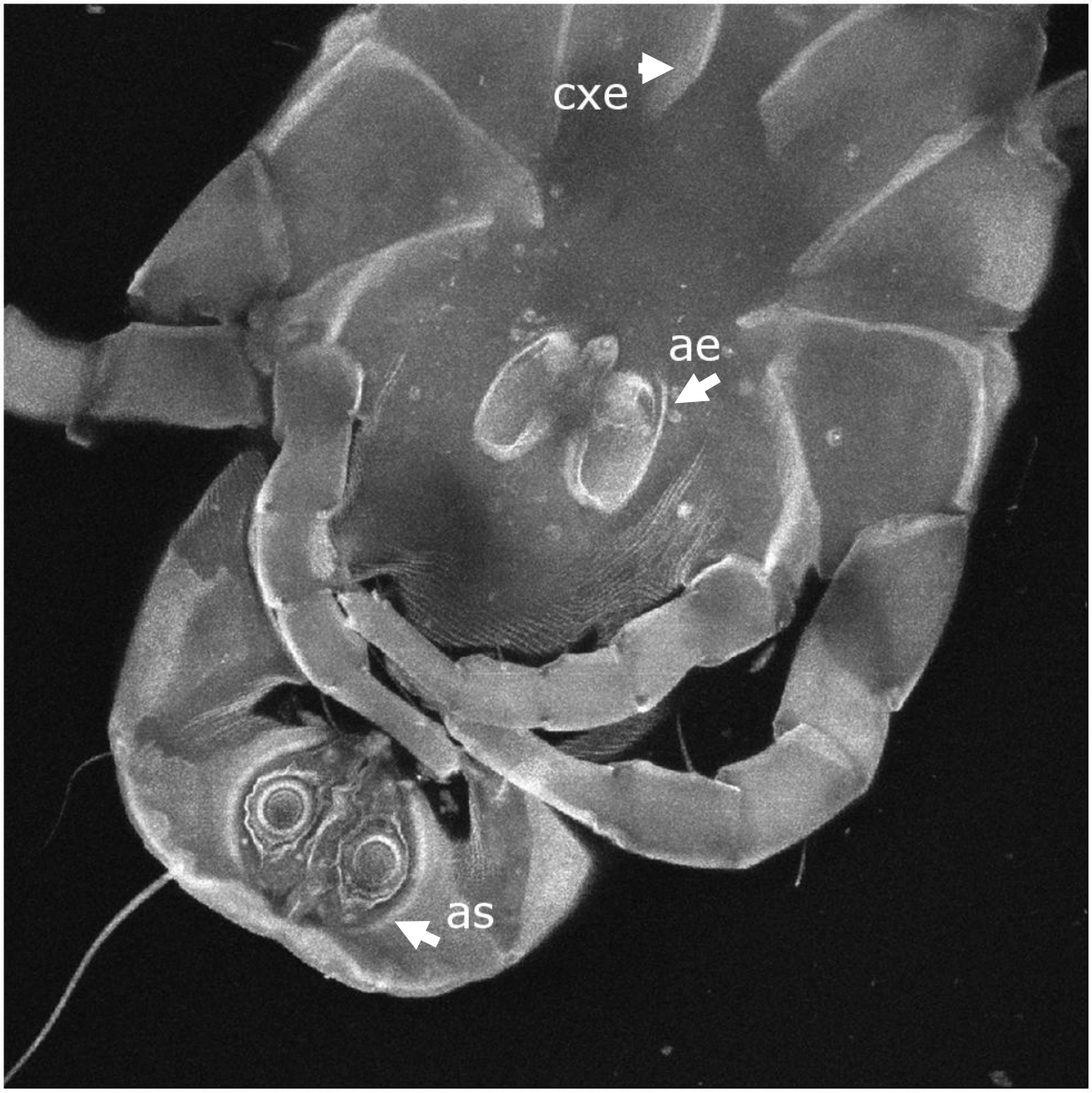


Figure 4- 12. *Dermatophagoides pteronyssinus*, male, adult, confocal. The aedeagus (ae) and anal suckers (as) are labeled. The epimeres of coxae I (cxe) do not form a Y or V shape.

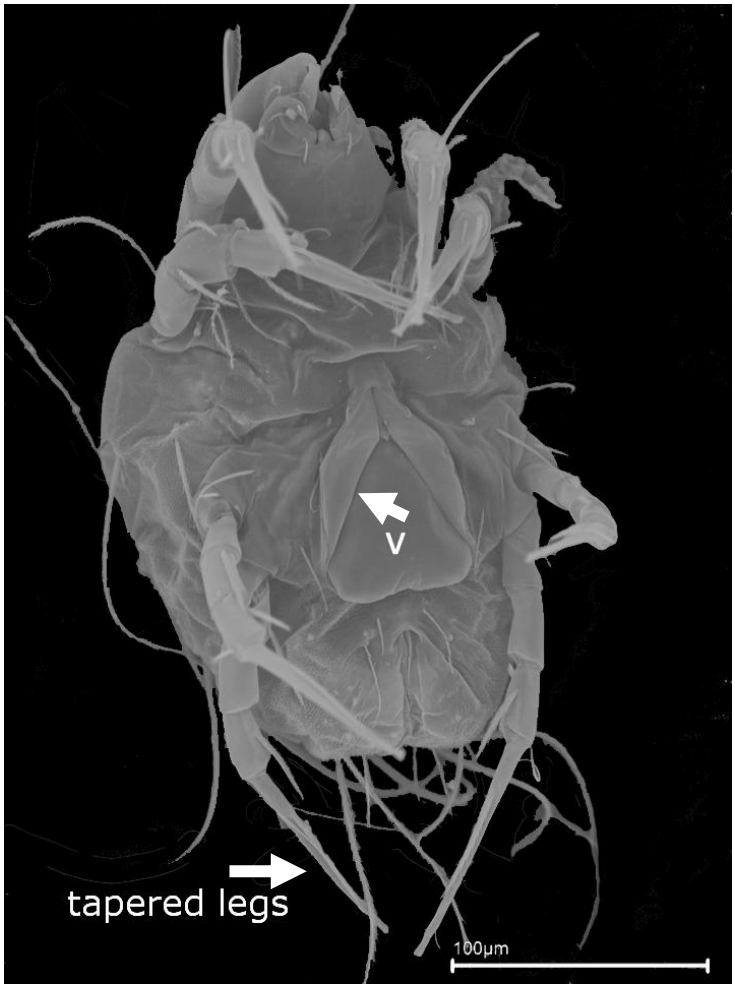


Figure 4- 13. *Blomia tropicalis*, female, adult, ventral, SEM. This species has many setae and tapered legs. The vulva (v) is shown.

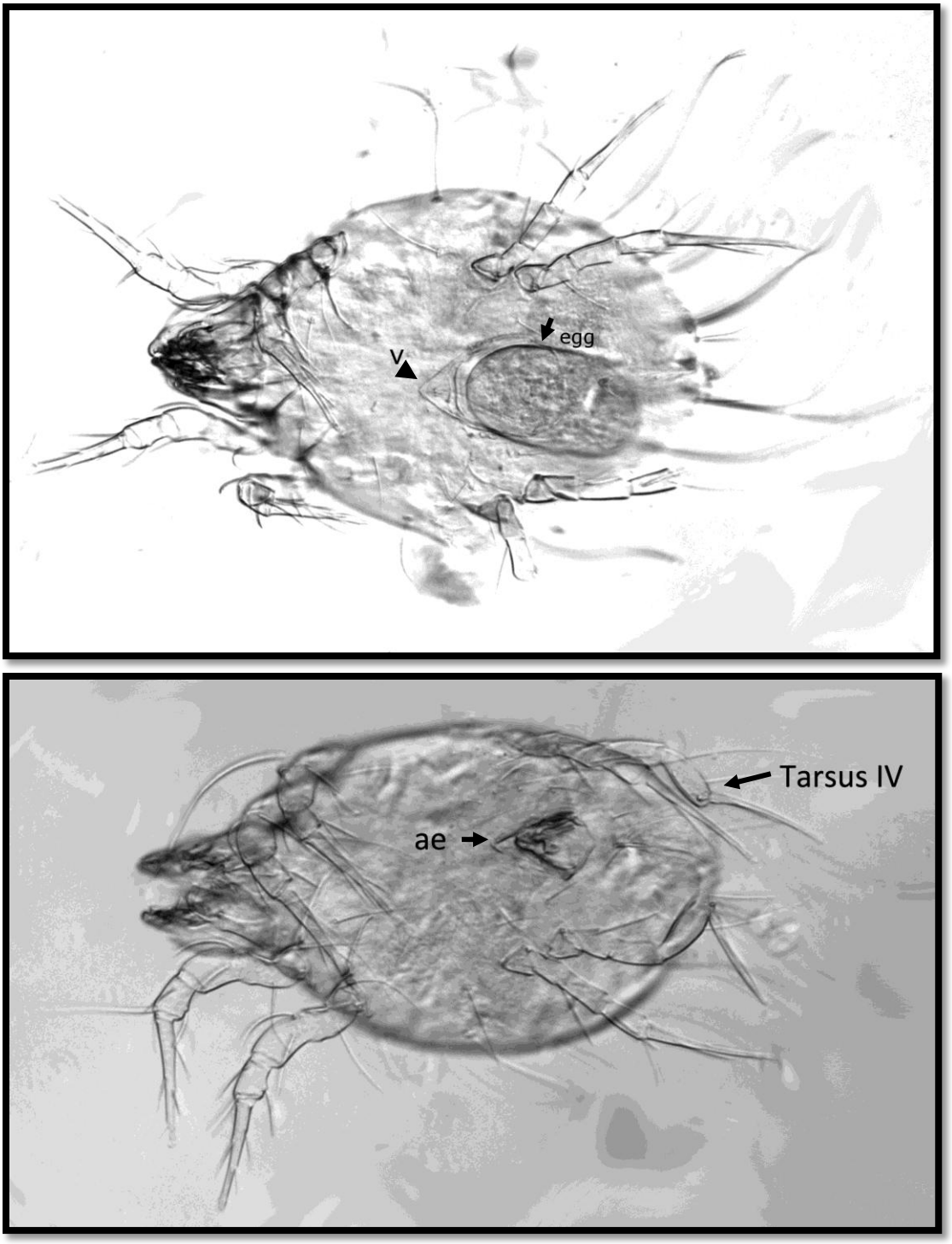


Figure 4- 14. *Blomia tropicalis*, female and male, adults, light microscope. Top – Female with vulva (v) and egg shown. Bottom – Male, with aedeagus (ae) and flexed tarsus IV.

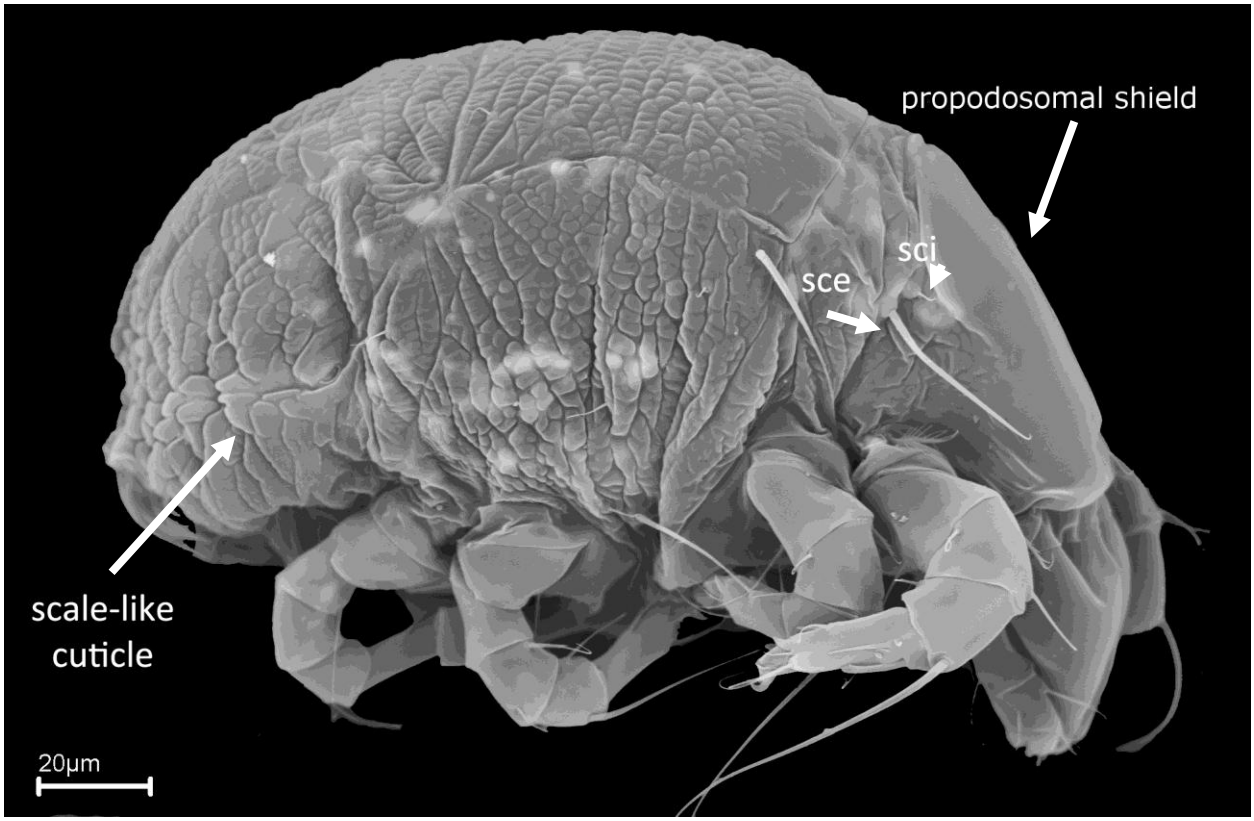


Figure 4- 15. *Suidasia* sp., adult, SEM. Has a scale-like cuticle and a propodosomal shield. The internal scapular seta (*sci*) is shorter than the external scapular seta (*sce*).

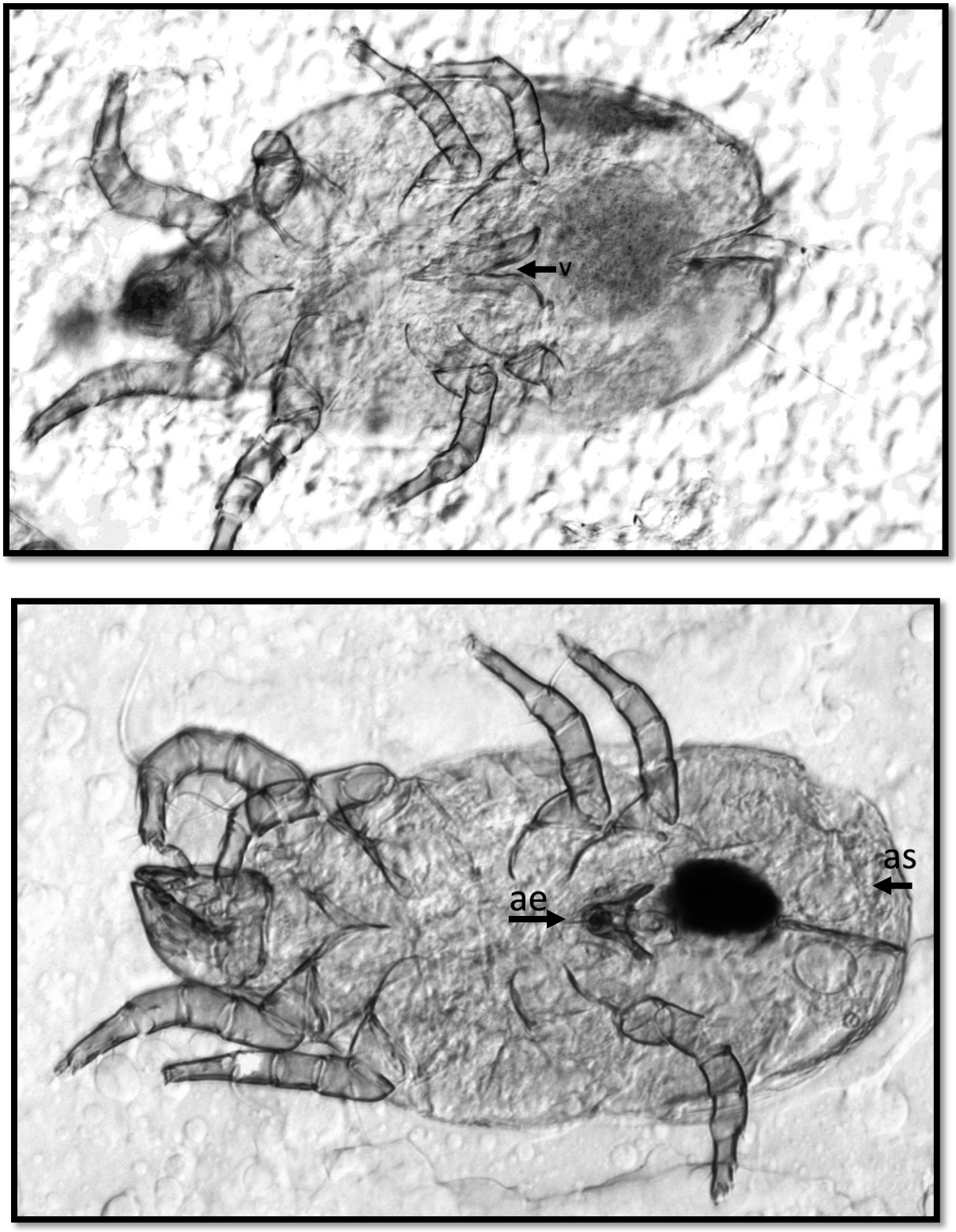


Figure 4- 16. *Suidasia* spp., female and male, adults, light microscope. Top- female has a vulva (v). Bottom – Male has anal suckers (as) and aedeagus (ae).

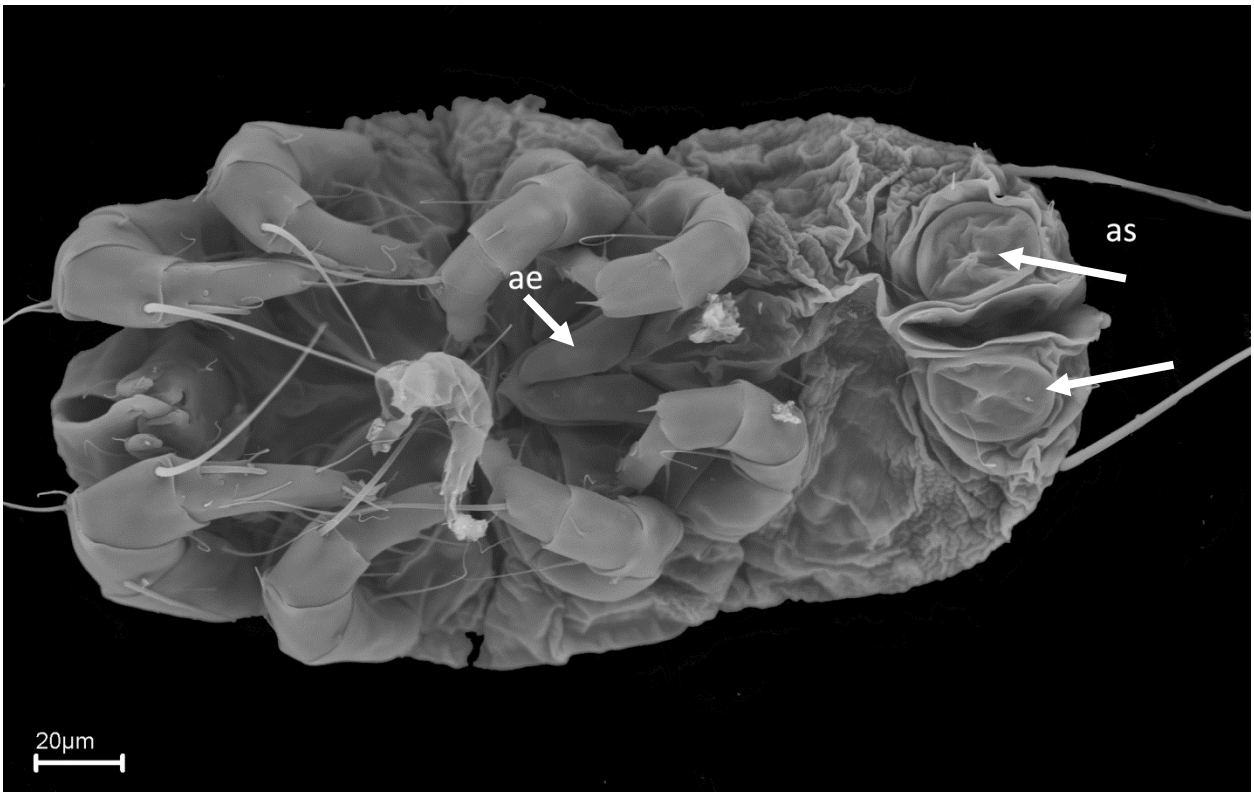


Figure 4- 17. *Suidasia* sp. male, adult, ventral, SEM. The anal suckers (as) can be seen, along with the aedeagus (ae), which is used for copulation.

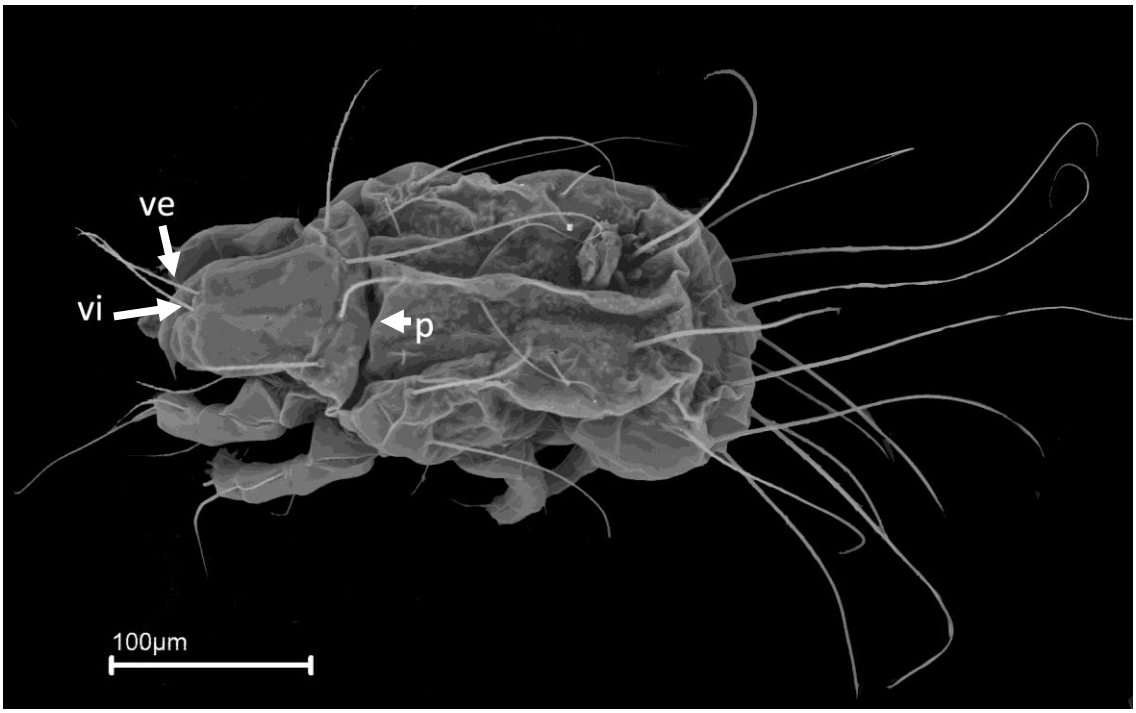


Figure 4- 18. *Tyrophagus* sp. adult, dorsal, SEM. Seta *vi is* seen to be less than three times as long as seta *ve,* which is the main characteristic for identifying this genus. The groove of the prodorsal shield is also shown (p).

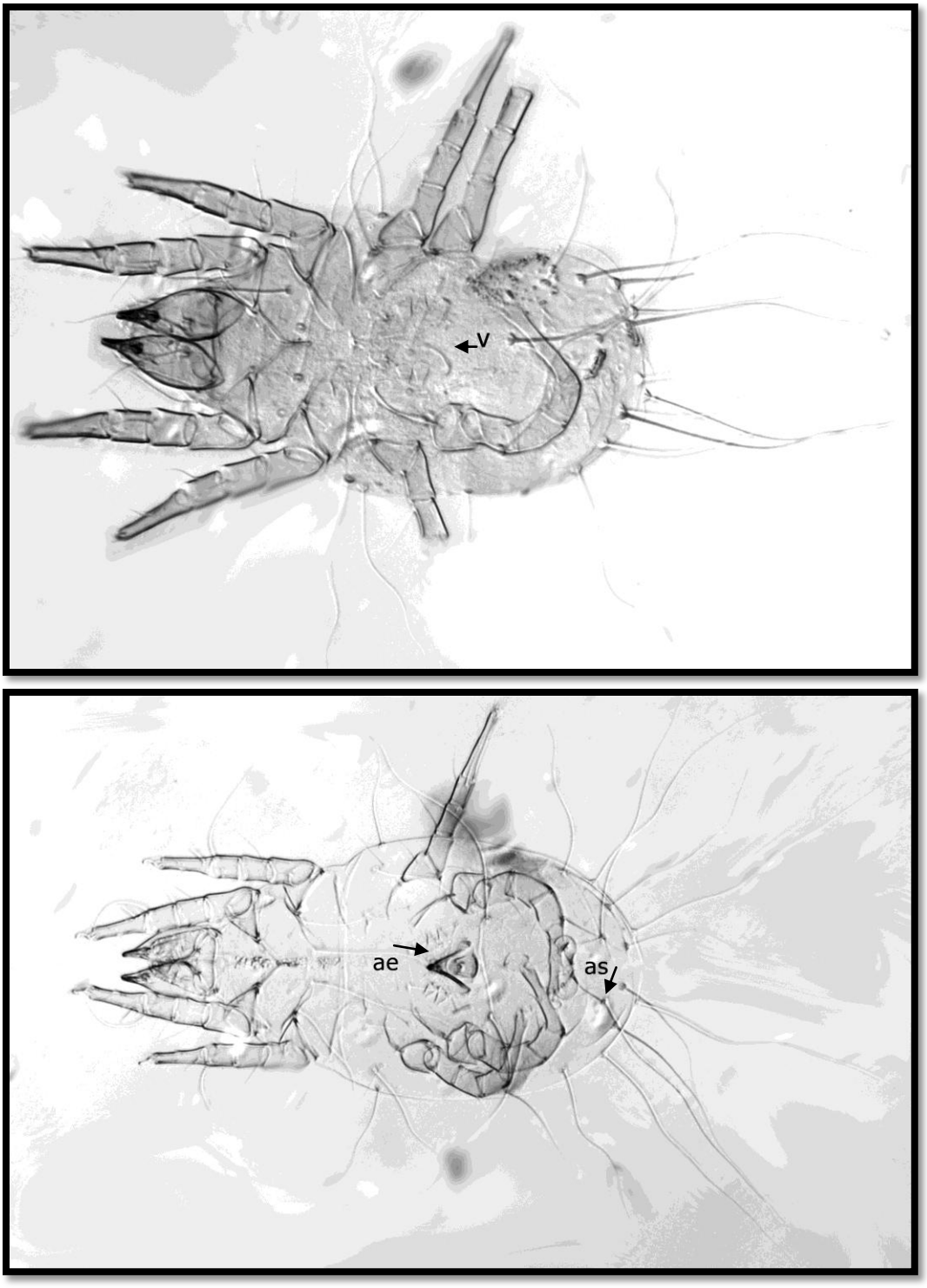


Figure 4- 19. *Tyrophagus* spp., female and male, adults, light microscope. Top - female with vulva (v). Bottom – male with aedeagus (ae) and anal suckers (as*).*

CHAPTER 5

CONCLUSION

House dust mites and storage mites are found worldwide. Human sensitization to the allergens that these animals produce and leave in the urban environment is a serious issue. Finding the efficiency of methods that are used to remove these animals from the home is extremely important in order to further study them. An easy to use identification handbook containing microscopy photographs of these animals will also facilitate the study of these animals.

A strong correlation was found between weights and the number of *Dermatophagoides pteronyssinus* and *D. farinae* mites. This research was able to be completed because of the extremely pure/clean samples of mites that are available at the Mahidol University, Department of Parasitology at Siriraj Hospital in Bangkok, Thailand. This would provide the basis for increased confidence for the extraction experiments, which is found in the second part of Chapter 3. The number of mites for those experiments could be weighed out before using the heat escape of flotation method instead of individually counting the mites.

The heat escape and flotation methods for extracting mites were tested and both provided low numbers of mites compared to those originally placed into the test apparatus or substrate, respectively (termed extraction efficiency). It is recommended that the flotation method should be used because it provided higher and more consistent extraction efficiencies than the heat escape method, and it is able to remove both live and dead mites from materials, while the heat

escape method can only remove live mites. Knowing the accuracy of both of these methods will aid researchers in estimating mite numbers within homes during survey studies.

An identification guide to common house dust and storage mites is presented in Chapter 4. This guide is intended to serve as an introductory text to those who wish to start studying these mites. The guide features pictures from different types of microscopes to highlight important features of the mites that are essential for identification.