SARCOPTIC MANGE IN BLACK BEARS (*URSUS AMERICANUS*) IN PENNSYLVANIA

by

SARAH KNOX PELTIER

(Under the Direction of Michael J. Yabsley)

ABSTRACT

There has been an increase in cases and geographic distribution of severe mange in black bears (*Ursus americanus*) in Pennsylvania. We evaluated several diagnostic assays for detection and identification of the mite(s) associated with bear cases. Samples from 72 black bears with mange were examined and *Sarcoptes scabiei* was the only mite species identified. Molecular testing of skin scrapes and full-thickness skin confirmed diagnosis. Fecal PCR testing was not useful for diagnostics. Antibodies against *S. scabiei* were detected in bears with mange suggesting serologic testing can be a useful epidemiologic tool. The genetic diversity of mites collected from black bears and wild canids in Pennsylvania and surrounding states indicated that the ITS-2 marker was highly conserved but two haplotypes, one bear specific was identified. The *cox*1 gene sequences were more variable but phylogenetic analysis failed to distinguish mites from bears from other mites from other hosts and geographic regions.

INDEX WORDS: mange, black bears, *Sarcoptes scabiei*, ITS-2, *cox*1

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DEDICATION

This work is dedicated to my dad, Matt Knox, who introduced me to the world of wildlife biology and management at a young age.

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CHAPTER 1

INTRODUCTION

The black bear (*Ursus americanus*) is widely distributed throughout much of Canada and the United States and extends into northern Mexico. Although black bear populations are steady across much of the country, in recent years, there have been increasing reports of diseases that are novel to bears or are being reported in increased numbers. For example, the first report of clinical disease caused by canine distemper virus in a bear occurred in 2011 in Pennsylvania (Cottrell et al., 2013). Another disease that has historically only been reported sporadically in black bears is mange, a skin disease caused by mites. Since 1991, there has been an increase in the number of cases and geographic distribution of severe sarcoptic mange in black bears in Pennsylvania (Sommerer, 2014). The reason for this increase in mange cases remains unknown but could be due to increased interactions with other *Sarcoptes* hosts [e.g., coyotes (*Canis latrans*), red fox (*Vulpes vulpes*), etc], increased bear density or interactions, emergence of a novel *S. scabiei* strain or other mite species, or some other factor making bears more susceptible to clinical disease.

The goal of this research is to investigate the epidemiology of mange in black bears in Pennsylvania and neighboring states. Ultimately, the data obtained in this thesis should help managers understand what is causing this epizootic, why it may be occurring, and provide tools for mitigation and management of the disease.

Specific objectives of this study include the following:

1) to determine the etiologic agent(s) of mange in Pennsylvania black bears;

2) to evaluate several diagnostic assays for detection and identification of mites in bears with and without evidence of overt disease and;

3) to characterize the genetic diversity of mange mites from black bears in Pennsylvania

LITERATURE CITED

Cottrell, W. O., M. K. Keel, J. W. Brooks, D. G. Mead, and J. E. Phillips. 2013. First report of clinical disease associated with canine distemper virus infection in a wild black bear (*Ursus americanus*). Journal of Wildlife Diseases **49:** 1024-1027.

Sommerer, A. 2014. A spatial analysis of the realtionship between the occurrence of mange in Pennyslvania's black bear population and impervious land cover. M.S. Thesis. Indiana University of Pennsylvania, Indiana, Pennsylvania, 1-131 p.

CHAPTER 2

LITERATURE REVIEW

MANGE AND MANGE MITES

Mange is a highly contagious skin disease in wild and domestic animals caused by multiple species of parasitic mites. These mites are microscopic arthropods that live on or burrow into the skin, hair follicles, or other tissues of their host. Mites within several orders, including Trombidiformes (includes *Demodex*) and Sarcoptiformes (includes *Sarcoptes, Ursicoptes, Psoroptes, Knemidokoptes*), can cause mange in birds and mammals, including people. For carnivores, mites within the Family Sarcoptidae are very important causes of disease and all 117 known species are parasitic in mammals. Two notable examples with the Sarcoptidae family are the *Sarcoptes* and *Notoedres*, both of which are burrowing mange mites of carnivores (Klompen, 1992). Mites are transmitted by direct or indirect (i.e., fomites) contact, as such, transmission tends to be density-dependent (Pence and Ueckermann, 2002). Burrowing mange mites will often leave their burrows and wander onto the surface of the skin, thereby facilitating transmission by direct contact. Mites may also dislodge from the host onto fomites or into the environment (Arlian and Vyszenski-Moher, 1988). For example, when dislodged, *S. scabiei* mites perceive host odor and body temperature as stimuli to search for a new host. Fomites can be important sources of mite transmission, especially under the right conditions. Mite survival off the host is dependent on relative humidity and ambient temperature. Longer survival is

favored by higher relative humidity and lower temperatures (Arlian, 1989; Arlian and Vyszenski-Moher, 1988).

Mange is characterized by varying degrees of alopecia and thickened, crusty skin, which can lead to poor body condition, secondary infections and possibly death (Bornstein et al., 2001; Pence and Ueckermann, 2002). In general, mange cases are sporadic and are not associated with specific seasons or geographic regions. Also, because cases tend to be sporadic, this disease is rarely associated with population-level impacts; however, there are notable examples of large epizootics such as sarcoptic mange in ibex/chamois, and red fox, and notoedric mange in bobcats in California (USA; Trainer and Hale, 1969; Pencefrank et al., 1982; Fernandez-Moran et al., 1997; Leon-Vizcaino et al., 1999).

SARCOPTES SCABIEI

Worldwide, *Sarcoptes scabiei*, a parasitic mite in the Family Sarcoptidae, is the most common cause of mange in mammals. There are over 100 species of domestic and wild mammals, including humans that are documented hosts for *S. scabiei*. Within the United States, most reports of sarcoptic mange have occurred in six families (Table 2.1). Worldwide, reports of sarcoptic mange have occurred in various hosts (Table 2.2). Sarcoptic mange is a contagious skin disease characterized by varying degrees of hair loss and thickened, crusty skin, which can lead to poor body condition, secondary infections and possibly death. While some infections lead to mild lesions, some hosts [e.g., red fox (*Vulpes vulpes*)] can develop extremely severe disease with marked skin thickening and crusting, pruritus, alopecia, and death (Bornstein et al., 2001; Pence and Ueckermann, 2002). Little et al. (1998) demonstrated that red foxes infected with *S. scabiei* develop an immediate hypersensitivity reaction within two weeks of exposure. In

addition, foxes failed to develop resistance to reinfection (Little et al., 1998). In comparison, domestic dogs have been shown to express protective immunity when reinfested with *S. scabiei*. Reinfested dogs that displayed resistance cleared *Sarcoptes* infection within three months (Arlian et al., 1996).

Table 2.1. Reports of sarcoptic mange in wild mammals from the United States.

Family	Species	Location	Reference(s)
Canidae			
	Coyote (*Canis latrans*)	Texas Wisconsin	Pence et al., 1983 Trainer and Hale, 1969
	Gray Fox (*Urocyon cinereoargenteus*)	New York	Stone et al., 1982
	Gray wolf (*Canis lupus*)	Montana Wyoming Canada	Jimenez et al., 2010 Todd et al., 1981
	Red fox (*Vulpes vulpes*)	Wisconsin New York Pennsylvania	Trainer and Hale, 1969 Stone et al., 1972 Pryor, 1956
	Red wolf (*Canis rufus*)	Texas	Pence et al., 1981
Ursidae			
	Black bear (*Ursus americanus*)	Michigan	Schmitt et al., 1987 Fitzgerald et al., 2008
Erethizonntidae			
	Porcupine (*Erethizon dorsatum*)	Maine	Payne and O'Meara, 1958
Mustelidae			
	Fisher (*Martes pennanti*)	Maine	O'Meara et al., 1960
Suidae			
	Wild Boar (*Sus scrofa*)	USA	Smith et al., 1982
Leporidae			
	Marsh Rabbit (*Sylvilagus palustris*)	North Carolina	Stringer et al., 1969

Table 2.2. Selected reports of sarcoptic mange in various hosts from around the world.

Species	Location	Reference(s)	Notes
Wombat (*Vombatus* spp.)	Australia	Skerratt et al., 1998	outbreaks
Ibex (*Capra* spp.)	Spain	Leon-Vizcaino et al., 1999 Alasaad et al., 2013 Perez et al., 2015	outbreaks
Giraffe (*Giraffa camelopardalis reticulata*)	Africa	Alasaad et al., 2012a	sporadic
European rabbit (*Oryctolagus cuniculus*)	Spain	Millan et al., 2010	sporadic
Cheetah (*Acinonyx jubatus*)	Africa	Mwanzia et al., 1995	sporadic
Iberian Wolf (*Canis lupus signatus*)	Spain	Dominguez et al., 2008	outbreaks
Capybara (*Hydrochaeris hydochaeris*)	Europe	Fain, 1968	sporadic
Koala (*Phascolarctos cinereus*)	Australia	Obendorf, 1983	sporadic
Chamois (*Rupicapra pyrenaica parva*)	Spain	Fernandez-Moran et al., 1997	outbreaks
Pampas Fox (*Pseudalopex gymnocercus*)	South America	Deem et al., 2002	sporadic

Life Cycle

Sarcoptes scabiei mites burrow into the lower stratum corneum of the skin and directly drain energy from the host by consuming tissue fluid (Figure 2.1; Arlian, 1989). As a member of Sarcoptidae, mite development occurs in five stages: egg, larva, protonymph, tritonymph, and adult (Klompen, 1992). Adult females create burrows where they lay 40-50 eggs and then die. The eggs hatch after approximately two days, and the larva crawl to the surface of the skin where they will create new burrows and molt into the next two nymphal stages. Finally, the nymphs molt into adults and mate. Complete development from egg to adult takes approximately 12 days (Arlian and Vyszenski-Moher, 1988; Arlian, 1989).

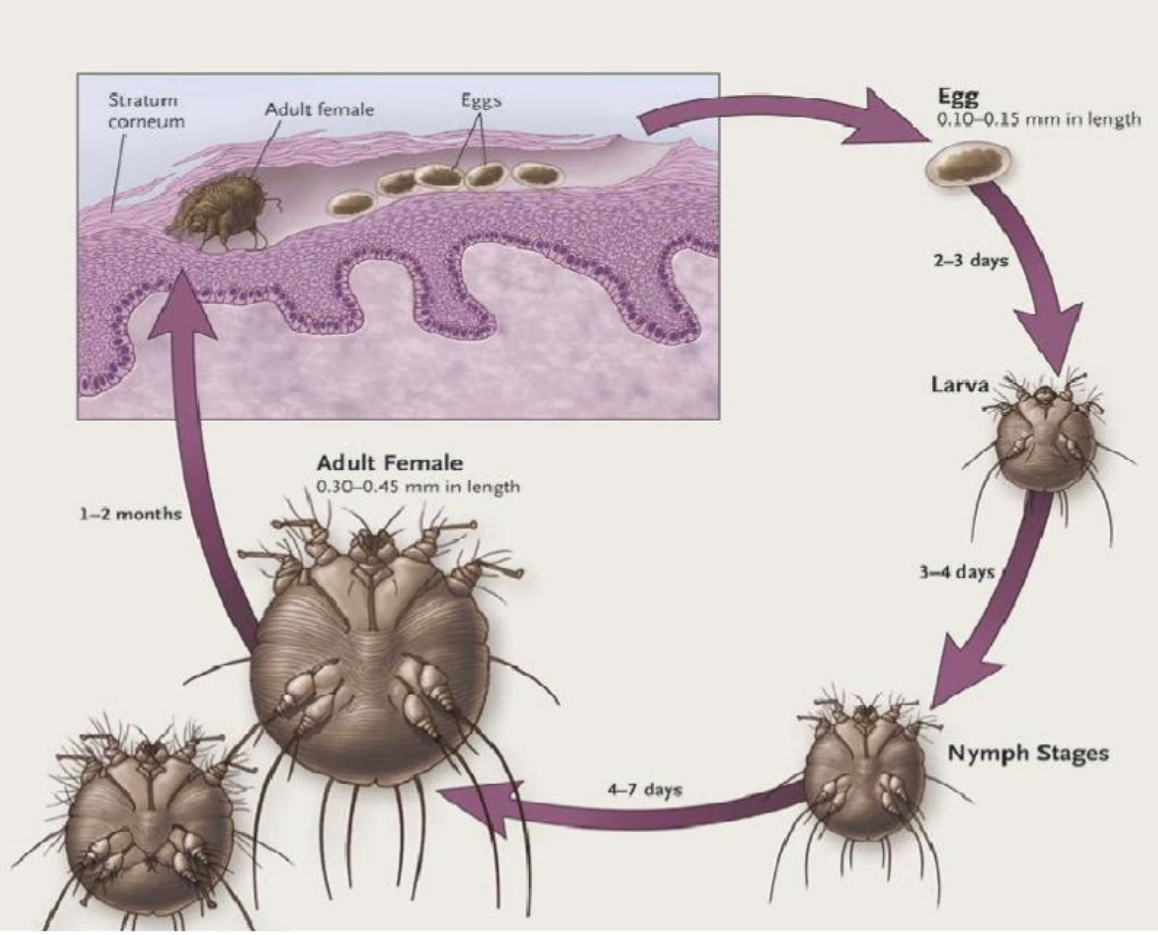

Figure 2.1. Generalized life cycle of *Sarcoptes scabiei* illustrating the burrows created by females where eggs and feces are deposited.

Host Specificity and Cross-Infestivity

Prior to the availability of molecular tools for the characterization of *S. scabiei*, mites from different hosts were classified as variants of *S. scabiei*, some of which were considered host-specific and others that were known to infest a wide range of hosts. However, based on morphology alone, it is difficult or impossible to distinguish between mites from different hosts, and cross-transmission trials were needed (Arlian, 1989). For example, *S. scabiei* mites from dogs were successfully transferred to white lab rabbits in New Zealand. In comparison, the same

rabbits could not be infested with *S. scabiei* mites from pigs and humans (Arlian et al., 1984; Arlian et al., 1988). In the natural environment, transmission of *Sarcoptes* between raccoon dogs (*Nyctereutes procyonoides*) and companion dogs (*Canis lupus familiaris*) has been documented genetically in Japan (Matsuyama et al., 2015). The factors associated with host specificity are not known but could include host seeking stimuli (i.e., body temperature and odor), the host skin environment, the host's immune response, and/or the mite's resistance to the host's immune response (Arlian, 1989).

Transmission of *S. scabiei* mites from domestic animals to humans is not uncommon, in fact dogs are the most frequent non-human reservoir of mites infecting humans (Thomsett 1968; Aydingoz and Mansur 2011). Pseudoscabies, or human scabies of animal origin, have been reported from direct contact with wildlife less frequently. The few episodes of pseudoscabies that have occurred due to exposure to wildlife were due to a lack of protective clothing (i.e., gloves) and/or animals infested with large numbers of *S. scabiei*. Outbreaks of pseudoscabies have been associated with red fox from Canada and Germany (Samuel, 1981 and Birk et al., 1999, respectively), chamois (*Rupicapra rupicapra*) from Italy (Menzano et al., 2004), wombats (*Vombatus ursinus*) from Australia (Skerratt and Beveridge, 1999), and black bears (*Ursus americanus*) from Pennsylvania, USA (personal communication, M. Ternent, 2015).

Although all of these data suggest that there are biological differences among various *S. scabiei* populations, numerous genetic characterization studies have determined that strains from different hosts are not taxonomically significant, and as such the genus *Sarcoptes* remains monotypic (Fain, 1968).

Molecular epidemiology

Sarcoptes scabiei is a genetically diverse species, and host specialists or generalists have been identified (Pence and Ueckermann, 2002). Historically, due to the difficult nature of obtaining mites, there have been few studies investigating the genetics of *S. scabiei*. However, given advances in molecular technology over the past few decades, numerous genetic targets have been used to investigate the genetic relationship among *Sarcoptes* samples from different hosts and geographic regions, and some of these techniques are more useful than others.

The most frequently used gene target for validation of a species status is the internal transcribed spacer (ITS)-2 region in nuclear rDNA (Dabert, 2006). Many studies have shown that ITS-2 sequences from different hosts from different countries exhibit limited diversity and therefore belong to the same species (Zahler et al., 1999; Berrilli et al., 2002; Gu and Yang, 2008; Alasaad et al., 2009; Makouloutou et al., 2015). Although successful at resolving phylogenetic problems, the high substitution rate of ITS-2 affects intraspecific diversity of sequences. As such, amplified ITS-2 fragments should be cloned in plasmids, however, this technique is more expensive and labor intensive (Dabert, 2006).

Researchers have also investigated the mitochondrial genes coding for cytochrome *c* oxidase subunit 1 (*cox*1), 12S rRNA, and 16S rRNA as a DNA barcode to distinguish *Sarcoptes* strains. Studies on other acarine species have shown mitochondrial DNA (mtDNA) to be a suitable population marker (Crosbie et al., 1998; Navajas et al., 1998). Distinct, geographically separate, and host-adapted *S. scabiei* populations have been identified using *cox*1 and 16S as gene targets (Berrilli et al., 2002; Walton et al., 2004; Amer et al., 2014; Makouloutou et al., 2015; Zhao et al., 2015). Conversely, phylogenetic analysis of *S. scabiei* 12S sequences from humans, a dog, and wombats in Australia revealed that all had similar sequences and did not

diverge phylogenetically (Skerratt et al., 2002). Mitochondrial DNA has a higher base substitution rate compared that that of nuclear genes and may successfully recover intraspecific relationships (Dabert, 2006).

Another genetic marker system for the characterization of *S. scabiei* is amplification of microsatellites, which are sequence repeats dispersed throughout a genome. Microsatellites can be useful for the study of genetic relationships between and within populations. Primers are chosen from the sequence flanking the microsatellite and then polymerase chain reaction (PCR) is used to amplify the repeat region. This procedure is simple and efficient, assuming a successful DNA extraction. Walton et al. (1997) isolated three single locus microsatellites that display fragment length polymorphisms from *S. scabiei*. Polymorphic markers would make it possible to distinguish between homozygous *S. scabiei* populations within and between host species (Walton et al., 1997). Comparing mitochondrial DNA (16S rRNA and cox1) and a microsatellite marker system for identification of *S. scabiei* from Australia, Walton et al. (2004) found that mtDNA genes failed to distinguish *S. scabiei* haplotypes from different host species; however, microsatellites identified genetically distinct *S. scabiei* var. *canis* and var. *hominis* populations (Walton et al., 2004).

Epizootics

While mange typically does not affect long-term population dynamics, there are examples in which mange epizootics have caused devastating short-term mortality and in extreme cases, population level impacts. Naïve hosts, immunocompromised individuals, isolated populations, or endangered species, in particular, may experience more serious population-level impacts when faced with a mange epizootic (Pence and Ueckermann, 2002). For example,

populations of Iberian Ibex (*Capra pyrenaica*) from the Sierra Nevada mountain range in southern Spain are heavily impacted by sarcoptic mange. Given the limited access and difficult terrain of the Sierra Nevada Mountains, researchers have implemented the use of radio collars to monitor ibex affected by sarcoptic mange (Alasaad et al., 2013). The harsh environmental conditions under which these ibex live have been shown to influence general health parameters such as body weight, serum chemistry, and hematology, all of which may increase the probability of severe mange (Perez et al., 2015). Similar to the use of radio collars to monitor wildlife diseases, disease-detector dogs have been shown to be successful in following the scent of *Sarcoptes*-infected animals and may therefore be a useful surveillance and control tool for sarcoptic mange in wild populations (Alasaad et al., 2012b).

Another example of the impact of sarcoptic mange on a population is in the wombat (*Vombatus ursinus*) in Australia (Borchard et al., 2012; Skerratt et al., 1998, 2004). Researchers have used dowels to administer drugs to those wombats they are able to approach successfully. For those they cannot, burrows are identified and "flaps" with product are installed in the opening of the burrow. "Flaps" are intended to administer product directly onto wombats during each entrance and exit from burrows. This undertaking to try and reduce the prevalence of sarcoptic mange in wombat populations has now become a citizen science project (Wombat Protection Society of Australia, 2011).

In general, reports of sarcoptic mange epizootics are more common in canid populations [i.e., red fox, coyote (*Canis latrans*), and gray wolves (*Canis lupus*)]. Mange epizootics in red fox have been reported in Pennsylvania (Pryor, 1956) and parts of Europe (Sreter et al., 2003; Soulsbury et al., 2007), epizootics in coyotes have been reported in Texas (Pence et al., 1983;

Pence and Windberg, 1994), and epizootics in gray wolves have been reported in Montana, Wyoming (Jimenez et al., 2010), and parts of Canada (Todd et al., 1981).

A recent sarcoptic mange outbreak has been documented in gray wolves in Montana and Wyoming. Over 70 gray wolves between the two states were either confirmed to have mange or were observed in the field with clinical signs of mange. As such, the severity of mange in gray wolf populations in Montana and Wyoming is of management concern (Jimenez et al., 2010). Using infrared thermography, researchers have been able to investigate the energetic costs of sarcoptic mange in wolves. Thermal cameras were used to estimate heat loss associated with mange-induced hair loss. The energetic demands associated with mange resulted in altered behavioral patterns in individual wolves. This shift in behavior may affect predator-prey interactions and prey consumption rates (Cross et al., 2016).

The reintroduction of gray wolves to the Northern Rockies has given researchers the unique opportunity to study the patterns of parasite invasion, such as that of *S. scabiei*. Areas supporting the largest density of wolves due to high resource quality appear to be the most susceptible to parasite invasion and species declines. Current fluctuations within wolf populations in Yellowstone National Park may signal that a point of natural regulation has been reached, possibly due in part by parasites (Almberg et al., 2012). The sociality of wolves is thought to increase infection risk. However, in respect to *S. scabiei*, group size does not predict infection risk. In fact an increase in group size, specifically an increase in healthy pack-mates, appears to offset an individual's costs of infection. Conversely, an increase in infected pack-mates may increase mortality for infected individuals (Almberg et al., 2015).

DIAGNOSTIC METHODS

Several different techniques can be used to diagnosis mange and the most appropriate test may vary based on individual, goal, or availability of funds or specialized equipment.

Cytology

The gold standard for diagnosis of mange and determination of the etiologic agent is to cytologically examine skin scrapes of effected skin. The success in this technique is dependent on the quantity of the mites in the skin and scraping the correct anatomic location. Detection of mites based on skin scrapes is generally low (Hill and Steinberg, 1993). This limits the value of this diagnostic approach for animals with mild or no overt disease. Some species, such as pigs, have high numbers of *Sarcoptes* mites while others may not have very many mites, even with severe lesions. For example, red fox and coyote can develop extremely severe disease (i.e., alopecia, encrusted lesions, etc.) but have a very low mite burden and in some cases mites cannot be found on skin scrapings (Samuel, 1981). As such, cytology can provide negative results even when mites are present.

Polymerase Chain Reaction (PCR)

Another option for mange diagnosis is the use of PCR to detect mite DNA in skin scrapings or biopsies. Similar to cytology, this diagnostic method is dependent on the quantity of mites in the skin scraping. In addition, extra steps during DNA extraction (e.g., grinding or collection of individual mites) may be necessary to facilitate mite digestion. For example, Silbermayr et al (2015) used conventional PCR to detect and discriminate between three feline *Demodex* (*D. cati*, *D. gatoi*, *D. felis*) mite species. Skin scrapings were collected from affected

cats and individual mites were placed by hand into lysis buffer for DNA extraction, which resulted in an average of 0.36 ng DNA per extracted mite. Conventional PCR was successful in producing a *Demodex*-specific band (Silbermayr et al., 2015). In comparison, Fukuyama et al (2010) used nested PCRs to detect *S. scabiei* mite DNA in skin scrapings from human scabies patients. Nested PCRs targeting the ITS-2 gene were positive for *S. scabiei* mite DNA in all skin scrapings from mite-positive scabies patients (Fukuyama et al., 2010). One limitation of these methods is the need to capture or directly sample individuals. Thus, in an effort to create a noninvasive diagnostic test, Stephenson et al (2013) used fecal PCR to identify notoedric mange in bobcats (*Lynx rufus*) feces, which can be collected without the need to capture animals. However, felids are active groomers, especially those with mange, so it may not be possible to detect mites in feces of other species.

Serology

Although cytology remains the gold standard for wildlife, there have been some efforts to develop and validate serologic-based diagnostic assays. Serology has the potential to detect previous exposures and/or mild infections. An enzyme-linked immunosorbent assay (ELISA) assay developed for the detection of *S. scabiei* in dogs with mange has been validated for use in red fox in Europe using experimentally and naturally-infected animals (Bornstein et al., 1995, 1996, 2006). Serologic testing of red fox during an epizootic of sarcoptic mange in Norway was used to show that ten years after the initial epidemic started, there had been substantial host-parasite adaptation. The ratio of seropositive-mange negative to seropositive-mange positive foxes increased significantly confirming that either the fox or parasite had adapted and fewer clinical cases were observed as a result (Davidson et al., 2008).

With the exception of the red fox studies in Europe, few studies have utilized serologic based assays for *S. scabiei* in other wildlife species. An ELISA developed for the detection of *S. scabiei* in domestic swine with mange has been validated for use in wild boar (*Sus scrofa*; Haas et al., 2015). A labelled avidin-biotin enzyme-linked immunosorbent assay (LAB-ELISA) was developed to detect antibodies to *S. scabiei* in chamois (*Rupicapra* spp.) from Spain and Italy. Twenty-six of 169 (15%) clinically healthy chamois had antibodies to *S. scabiei*, suggesting the ability of an ELISA to reveal asymptomatic infestations (Rambozzi et al., 2004). Sarasa et al. (2010) assessed the IgG response to sarcoptic mange infestation in Iberian ibex in Spain using a LAB-ELISA. In experimentally infested ibex, IgG response to *S. scabiei* infestation was affected by sex and previous exposure. Male ibex that had been previously exposed to *S. scabiei* had a lower IgG response than that of naïve males (Sarasa et al., 2010). The sensitivity of an indirect ELISA may be increased with the addition of an avidin-biotin detection system vs. direct detection of species-specific IgG. Some advantages of this system include, low background absorbance and a strong complex (since avidin has four binding sites for biotin) which remains stable during incubations and washes (Rambozzi et al., 2004).

Histology

Histological examination of skin is commonly done for wildlife cases of mange. Histology can be used to classify or score mange lesions and to detect mites. However, with this diagnostic method, sarcoptiform mites cannot be identified to species. Nimmervoll et al (2013) proposed a classification scheme to describe sarcoptic mange skin lesions in red foxes. A scoring system (0- absent, 1- mild, 2- moderate, and 3- severe) was used to analyze specific features (crusts, alopecia, mites, eosinophils, lymphocytes, and mast cells) of the lesions (Nimmervoll et al., 2013).

MANGE IN BLACK BEARS

In black bears, mange has been associated with one of three species of mites, *Ursicoptes americanus*, *Demodex ursi*, or *Sarcoptes scabiei* (Table 2.3).

Table 2.3. Reports of mites associated with black bears (*Ursus americanus*).

Causative Agent	Location	Reference(s)
Demodex ursi		
	Wisconsin	Manville, 1978
	Florida	Forrester et al., 1993; Desch, 1995; Foster et al., 1998
Ursicoptes americanus		
	Idaho Virginia Pennsylvania	Yunker et al., 1980 Joyner et al., 2004 Ternent and Cottrell, 2007
Sarcoptes scabiei		
	Michigan	Schmitt et al., 1987 Fitzgerald et al., 2008

Demodectic Mange

To date, clinical demodicosis, caused by *Demodex ursi*, has been restricted to a single population of black bears in central Florida and a single bear from Wisconsin (Manville, 1978; Forrester et al., 1993; Desch, 1995; Foster et al., 1998). These cases typically presented with varying degrees of alopecia and crusty, scaly skin with occasional yellowish exudate due to secondary pyoderma (Forrester et al., 1993; Foster et al., 1998). A study investigating the geographical distribution of *D. ursi* in black bears from Florida sampled 55 bears from 21 counties found *D. ursi* in only four bears that presented with clinical demodicosis. For the remaining 51 bears with no signs of mange, no *D. ursi* were found on skin scrapes (Foster et al., 1998).

Ursicoptic Mange

Compared with *S. scabiei*, little is known about *Ursicoptes americanus*-associated mange in bears. The mite *Ursicoptes americanus* was first described from healthy captive black bears in Kansas in 1970 but clinical ursicoptic disease has subsequently been reported in individual cases in black bears from Idaho (Yunker et al., 1980), Virginia (Joyner et al., 2004), and Pennsylvania (Ternent and Cottrell, 2007). Similar to sarcoptic mange, bears with ursicoptic mange present with variable alopecia, lichenification, and pruritus [Southeastern Cooperative Wildlife Disease Study (SCWDS), unpublished data].

Sarcoptic Mange

The first report of mange due to *S. scabiei* in black bears was in 1984 in Michigan. Three bears presented with alopecia and crusty skin consistent with sarcoptic mange. Further diagnosis confirmed that *S. scabiei* was in fact the etiologic agent (Schmitt et al., 1987). Since 1984, the only additional published report of sarcoptic mange in black bears was in Michigan (Fitzgerald et al., 2008) although cases from Maryland and Virginia have been diagnosed at SCWDS. A potential *Sarcoptes* suspect, but ultimately an undiagnosed dermatitis, in black bears from New Mexico was reported from 1992-2000 (Costello et al., 2006). *Sarcoptes scabiei* mites are easily recognized by their ventrally flattened, oval shaped body and cuticular spines. Larval mites have six legs, compared to eight legs in all other stages (protonymph, tritonymph, and adult). Adult males are approximately two thirds smaller than the adult females (Arlian, 1989).

In the United States, severe sarcoptic mange occurs sporadically or commonly in a number of carnivore species but severe disease in black bears have been relatively rare (Pence and Ueckermann, 2002). In Pennsylvania, however, mange began to be observed more regularly

in black bears beginning in the early 1990s, and has subsequently become a significant source of morbidity and mortality in an expanding area of the state (Sommerer, 2014; Figure 2.2). In 2014, Pennsylvania Game Commission (PGC) staff documented 56 bears that had either died or were euthanized because of mange (Figures 2.3 and 2.4). The reason for this increase in mange cases remains unknown but could be due to increased interactions with other *Sarcoptes* hosts (e.g., coyotes, fox), increased bear density or interactions, or emergence of a novel *S. scabiei* strain.

Currently, detection of mange in bears relies largely on syndromic surveillance, in which infection is identified by grossly visible lesions in the skin. Previous attempts to utilize more sensitive surveillance approaches, focused on the detection of mite infection or host antibody response, have been hindered by a lack of validated diagnostic assays and a poor understanding of the disease ecology and epidemiology among bears. A small pilot study conducted by the PGC tested the use of an ELISA to detect antibodies to *S. scabiei*. A total of 329 serum samples, collected during the 2007 and 2008 bear harvest, were tested. Ultimately, the commercially available canine ELISA was judged unreliable for diagnosis of mange in bears; however, importantly, the causative mites in the bears used in that study was not determined (Cottrell and Ternent, 2013). In addition, in 1996 the PGC attempted to determine the prevalence of sarcoptic mange in black bears across the state, but were unable to make an accurate estimate of prevalence due to the skin sampling method used. A total of 1,366 skin scrape samples were examined, and mites were detected on only 2.2%.

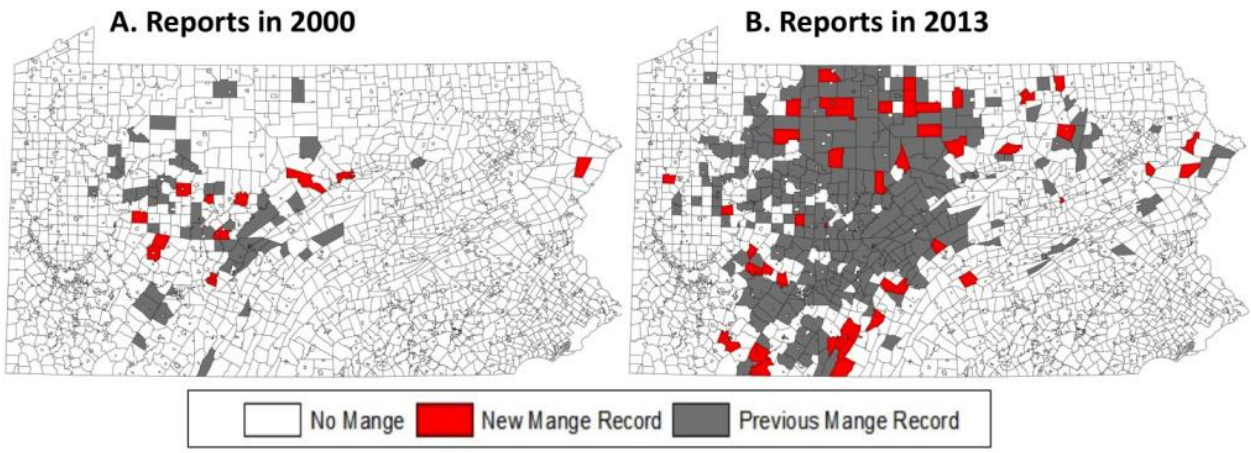

Figure 2.2. Reports of clinical mange in black bears (*Ursus americanus*) in Pennsylvania by township, 2000 and 2013.

Figure 2.3. Black bear (*Ursus americanus*) that died of sarcoptic mange from Pennsylvania.

Figure 2.4. Live captured black bear (*Ursus americanus*) with sarcoptic mange from Pennsylvania.

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CHAPTER 3

EVALUATION OF DIFFERENT DIAGNOSTIC ASSAYS FOR DETECTION AND IDENTIFICATION OF THE CAUSATIVE AGENT OF MANGE IN BLACK BEARS (*URSUS AMERICANUS*) FROM PENNSYLVANIA[1]

[1]Peltier S, J Brown, M Ternent, H Fenton, and MJ Yabsley. To be submitted to the *Journal of Wildlife Diseases*

ABSTRACT

At least three mite species can cause mange in black bears (*Ursus americanus*) including *Demodex ursi*, *Ursicoptes americanus*, and *Sarcoptes scabiei*. Recently, the number and geographic distribution of severe mange cases in black bears in Pennsylvania has increased. We evaluated several diagnostic assays for detection and identification of the mite(s) associated with these mange cases. Samples from 72 black bears with mange were examined and, based on morphology, *S. scabiei* was the only mite species present in 66 of the 72 bear samples; no mites were identified in the other six samples. Molecular testing targeting the ITS-2 region and *cox*1 gene were used to test skin scrapes (ITS-2), skin biopsies (ITS-2 and *cox*1) and feces (ITS-2 and *cox*1) from selected bears. For each full-thickness skin sample, DNA was extracted from three separate sections which were ground with a micropestal. For each feces sample, DNA was extracted twice and was also ground with a micropestal. Sixty of the 69 (87%) skin scrape samples were positive while only 40 of the 56 (71%) full-thickness samples tested by ITS-2 were positive. Of the two remaining full-thickness extractions, 30 of 56 (53%) and 18 of 56 (32%) tested by *cox*1 were positive. PCR testing of feces for mites was uniformly negative. Microscopic lesions from 40 bears with mange were consistent with those previously described in free-ranging carnivores with sarcoptic mange. Using a commercial indirect ELISA, the prevalence of antibodies against *S. scabiei* was significantly higher in the bears with mange (39/46, 85%) compared to those with no gross lesions suggestive of mange (1/26, 4%; $p<0.0001$). These data confirm that the most common cause of mange in black bears in Pennsylvania during this study period was *S. scabiei* and suggests that serologic testing may be useful for studying the epidemiology of this outbreak.

INTRODUCTION

The black bear (*Ursus americanus*) is widely distributed throughout much of Canada, the United States, and into northern Mexico. Although black bear populations are steady across much of the country, in recent years, there have been increasing reports of diseases that are novel to bears or are being reported in increased numbers. For example, the first report of clinical disease caused by canine distemper virus in a bear occurred in 2011 in Pennsylvania (Cottrell et al. 2013). Another disease that has historically only been reported sporadically in black bears is mange. Several different mite species can cause mange but all can cause varying degrees of hair loss and thickened, crusty skin, which can lead to poor body condition, secondary infections and possibly death (Pence and Ueckermann 2002; Bornstein et al., 2001). In black bears, mange has been associated with infection with *Sarcoptes scabiei*, *Ursicoptes americanus*, and *Demodex ursi* (Fitzgerald et al., 2008; Schmitt et al., 1987; Desch, 1995; Yunker et al., 1980).

Since 1991, the Pennsylvania Game Commission (PGC) has recorded mange cases and a notable increase in the number of records and the geographic distribution has been noted (Sommerer 2014). To date, these records have been based on syndromic surveillance (i.e., detection of overt disease based on gross lesions) and the mites associated with this epidemic have not been identified. The gold standard for diagnosis of mange and determination of the etiologic agent is to cytologically examine skin scrapes of effected skin. The success of this technique is dependent on the quantity of the mites in the skin and scraping the correct anatomic location. Detection of mites based on skin scrapes is generally low (Hill and Steinberg, 1993). This limits the value of this diagnostic approach for wildlife with mild or no overt disease.

Another option for mange diagnosis of mites is the use of polymerase chain reaction (PCR) to detect mite DNA in skin scrapings (Fukuyama et al., 2010, Silbermayr et al., 2015).

Similar to cytology, this diagnostic method is dependent on the quantity of mites in the skin scraping and sampling requires the capture of the bear to collect a sample. However, recently, in an effort to create a noninvasive diagnostic test, Stephenson et al (2013) used fecal PCR to identify *Notoedres cati* in feces of bobcats (*Lynx rufus*) in an effort to understand a notoedric mange outbreak.

Cytology and DNA based diagnostics only detected affected animals with mites present; however, to understand the impacts of mange on a population level, a serologic approach may be more useful. An enzyme-linked immunosorbent assay (ELISA) developed for the detection of *S. scabiei* in dogs with mange has been validated for use in red fox (*Vulpes vulpes*) in Europe using experimentally and naturally-infected fox (Bornstein et al. 1995, 1996, 2006). With the exception of the red fox studies in Europe, few studies have utilized serologic based assays for *S. scabiei* in wildlife.

Finally, histological examination of skin is commonly used to classify or score mange lesions and to detect mites (Pence et al., 1983; Schmitt et al., 1987; Deem et al., 2002; Dominguez et al., 2008; and Nimmervoll et al., 2013). However, with this diagnostic method, sarcoptiform mites cannot be determined down to species. Variation in lesion severity is common between species and even among individuals of the same species (Nimmervoll et al., 2013). For example, many canid species present with mild to severe encrustations with or without alopecia, whereas, severe forms characterized by large thick crusts have been observed in wombats (*Vombatus* spp.) and ibex (*Capra* spp.; Pence et al., 1983; Leon-Vizcaino et al., 1999; and Skerratt et al., 1998). Histology can also be used to identify secondary infections or dual infections with *Pelodera strongyloides* that may contribute to lesions.

Previous attempts to utilize more sensitive surveillance approaches, focused on the molecular detection of mites, however, identification may be limited by difficulty in obtaining mites, successful DNA extraction, or a low PCR success rate (Zhao et al., 2015). Additionally, detection of host antibody responses have been hindered by a lack of validated diagnostic assays and a poor understanding of the disease ecology and epidemiology among bears. The objective of this study was to identify the causative agent(s) of mange in black bears in Pennsylvania. In addition, we aimed to evaluate the utility of several diagnostic assays (cytology, PCR, serology, and histology) for detection and identification of mites in these black bears.

MATERIALS AND METHODS

Sample collection

Black bears with mange that were captured or found dead by the Pennsylvania Game Commission (PGC) or were legally harvested between 2011 and 2015 were opportunistically sampled. For humane reasons, the PGC euthanizes captured bears if severe mange lesions cover >50% of the body. Multiple samples were collected from each bear, although not all samples were available for each individual bear. Skin scrapes, full-thickness skin (1cm x 1cm), serum, and feces were collected from bears euthanized by PGC due to severe mange or from legally harvested bears. For bears captured and released by PGC personnel, skin scrapes and serum were collected. Feces, if present in traps, were collected. Scrapes were stored in 70% ethanol for cytologic examination and PCR analysis. Full-thickness skin samples were preserved in ethanol and 10% neutral-buffered formalin for PCR analysis and histologic examination, respectively. Serum was stored at -20°C until serologic testing. Feces were frozen at -20°C until PCR analysis. Permission to sample bears on the Sproul State Forest managed by the Pennsylvania Department

of Conservation and Natural Resources was granted under permit SFRA-0216. Capture and handling was done by employees of the Pennsylvania Game Commission following approved operating procedures 40.1, 40.4, and 40.9. In addition, all animal sampling protocols were reviewed by and approved by University of Georgia's IACUC committee (A2013- 10-016 and A2015-05-13).

Laboratory analysis

Morphologic identification

Ethanol fixed skin scrapes were examined under a microscope and mites were identified to species using published keys (Fain 1968; Yunker et al. 1980; Desch 1995).

Molecular detection

For skin scrapes, a small amount (≤ 800 µl) of material was divided into a tube, which was centrifuged to form a pellet. Ethanol was then pipetted off, the pellet washed with phosphate-buffered saline (PBS), and the remaining pellet dried. DNA was extracted using a commercial extraction kit (DNeasy blood and tissue kit, Qiagen, Valencia, California, USA). For full-thickness skin, DNA was extracted from three separate sections of skin (25mg). Sections of skin were divided into three tubes and ethanol was allowed to evaporate. Prior to DNA extraction, each skin sample was ground with a micropestle (Kimble Chase, Grainger). For fecal samples, a small amount (≤ 800 ul) was ground with a micropestle and DNA was extracted as described above.

Mite DNA was amplified by PCR using primers RIB-18 and RIB-3 to amplify the ITS-2 gene (Zahler et al. 1999) and primers Cyto F and Cyto R to amplify a partial region of the cytochrome oxidase subunit 1 (*cox*1) gene (Walton et al. 2004). Cycling conditions for the ITS-2

PCR was 94 C for 2 min followed by 40 cycles of 94 C for 45 sec, 60 C for 45 sec, 72 C for 1 min, and a final step at 72 C for 5 min. For the *cox*1 gene, cycling conditions were 94 C for 5 min followed by 40 cycles of 94 C for 30 sec, 48 C for 1 min, 72 C for 1 min, and a final extension at 72 C for 1 min. The DNA extraction conducted on skin scrape samples was used to target ITS-2 (n = 69). Of the three DNA extractions conducted on each full-thickness skin sample (all with grinding), DNA from one extraction was used to target ITS-2 (n = 56) and the remaining two were used to target *cox*1 (n = 56 for both). Of the two DNA extractions conducted on each feces sample (both with grinding), DNA from one extraction was used to target ITS-2 (n = 36), and the other to target *cox*1 (n = 36).

Precautions were taken to prevent and detect contamination including performance of DNA extraction, PCR reaction setup, and product analysis in distinct, designated areas. Negative controls were included in each DNA extraction and PCR reaction. A positive control (DNA sample from *S. scabiei* mite) was included in each PCR set.

To confirm identity, representative PCR amplicons were purified from a GelRed stained 1.5% agarose gel using a gel-purification kit (Qiagen) and bi-directionally sequenced at the University of Georgia Genomics Facility (Athens, GA).

Histology

Several sections of formalin-fixed skin were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE) stain. Microscopic lesions were described by a board certified wildlife pathologist in a blind fashion.

Serology

Sera were analyzed for IgG antibodies against *Sarcoptes scabiei* with a commercially available indirect ELISA (*Sarcoptes*-ELISA 2001® Dog, Afosa, Germany). A serum sample

from *Sarcoptes scabiei* var. canis-infested dogs was used as a positive control. Negative controls included a normal canine serum sample and sera samples from three brown bears (*Ursus arctos*) from Alaska where mange has not been reported. Testing followed the conditions indicated by the manufacturer. Optical densities (OD) (450nm) were read 15 minutes after substrate addition and were expressed as a percentage (OD sample – OD negative control)/(OD positive control – OD negative control). Converted OD values >15% were considered positive, those <10% were considered negative, and those between 10-15% were considered equivocal.

Statistical analyses

A Fisher's exact test was used to determine if the prevalence of antibodies to *S. scabiei*, was different between bears with mange compared to those with no gross lesions suggestive of mange. In addition, prevalence rates were compared among age classes.

RESULTS

A total of 72 bears with mange from Pennsylvania were sampled [65 adults (39 females, 26 males) and 7 yearlings (4 females, 3 males], although not all samples were collected from each individual (Table 3.1). Morphologic identification of ethanol fixed skin scrapes revealed that *S. scabiei* was the only mite species present in 66 of the 72 bear samples analyzed. Mites were not detected in skin scrapings from the remaining six bears. Of the 66 skin scrape samples with mites present, an average of 24 mites were observed on cytology, ranging from 1-113 mites present.

A total of 69 skin scrape samples were tested by PCR. Sixty of the 69 (87%) samples tested with the ITS-2 protocol were positive (Table 3.2). A total of 56 full-thickness skin samples were tested by PCR. Forty of 56 (71%) full-thickness skin samples tested with ITS-2 protocol

were positive while only 30 (53%) and 18 (32%) of the samples tested using the *cox*1 protocol were positive (Table 3.3). However, not all positives were detected using the ITS-2 protocol, as four samples were positive using the cox1 protocol in extractions 2 and 3. A total of 36 fecal samples were tested by PCR. All fecal samples were PCR negative, by both assays, for *S. scabiei*.

Histology

Histologically, mites were found within the epidermis in 38 of 40 (95%) bears. Lesions consisted of moderate to severe acanthosis and neutrophilic epidermitis with large serocellular crusts with marked parakeratotic hyperkeratosis. Evidence of secondary bacterial colonization within the serocellular crust was present in most bears (37/40, 93%) and superficial yeast (presumably *Malassezia*) was present in 23 of 40 (58%) bears (Figure 3.1). Yeast spores consistent with *Candida* spp. were observed within the serocellular crust of one bear. Additionally, *Pelodera strongyloides*, a free-living saprophytic nematode was present associated with the hair follicles in four of the 40 bears (10%; Figure 3.2).

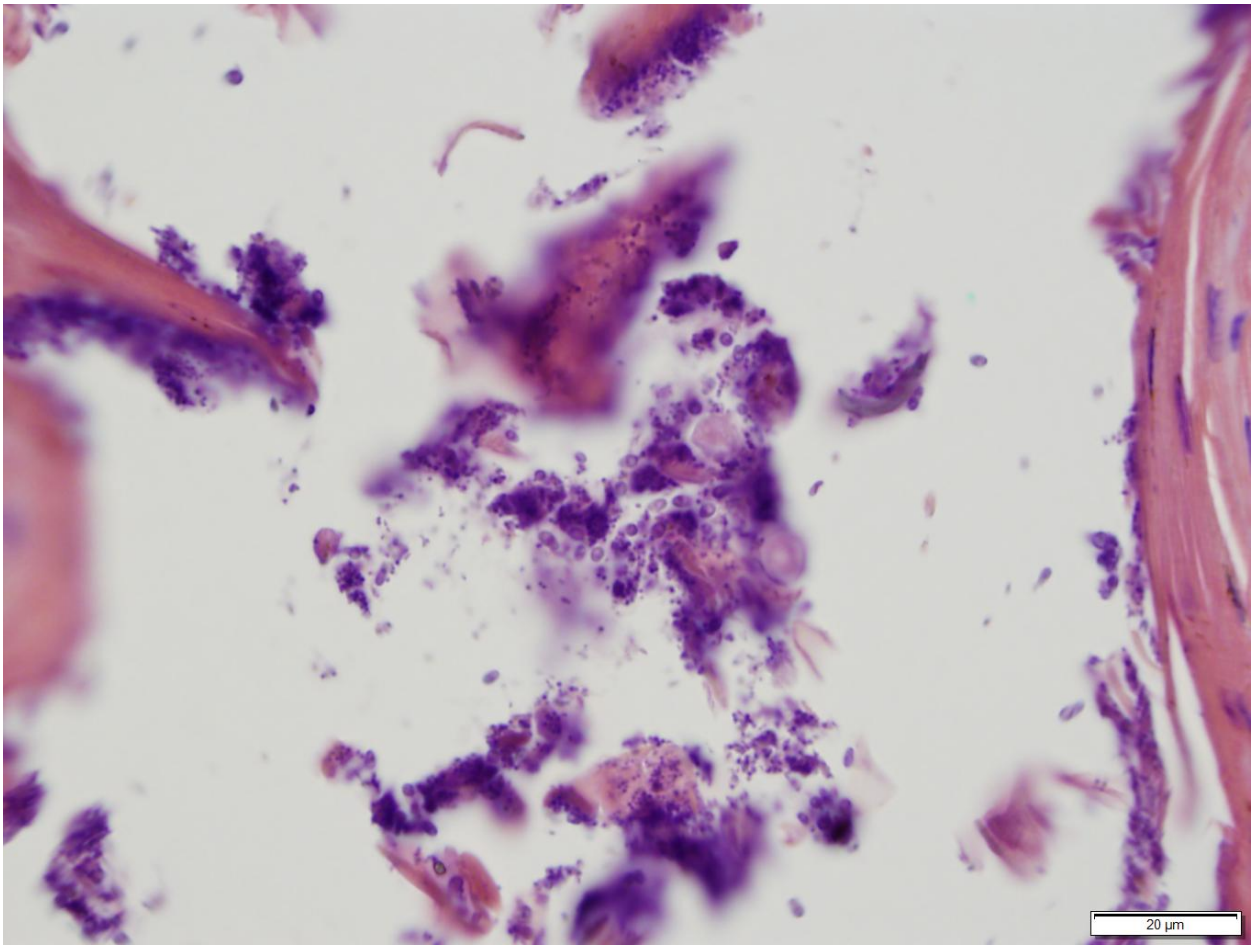

Figure 3.1. Histologic section of skin showing secondary bacteria and yeast colonization.

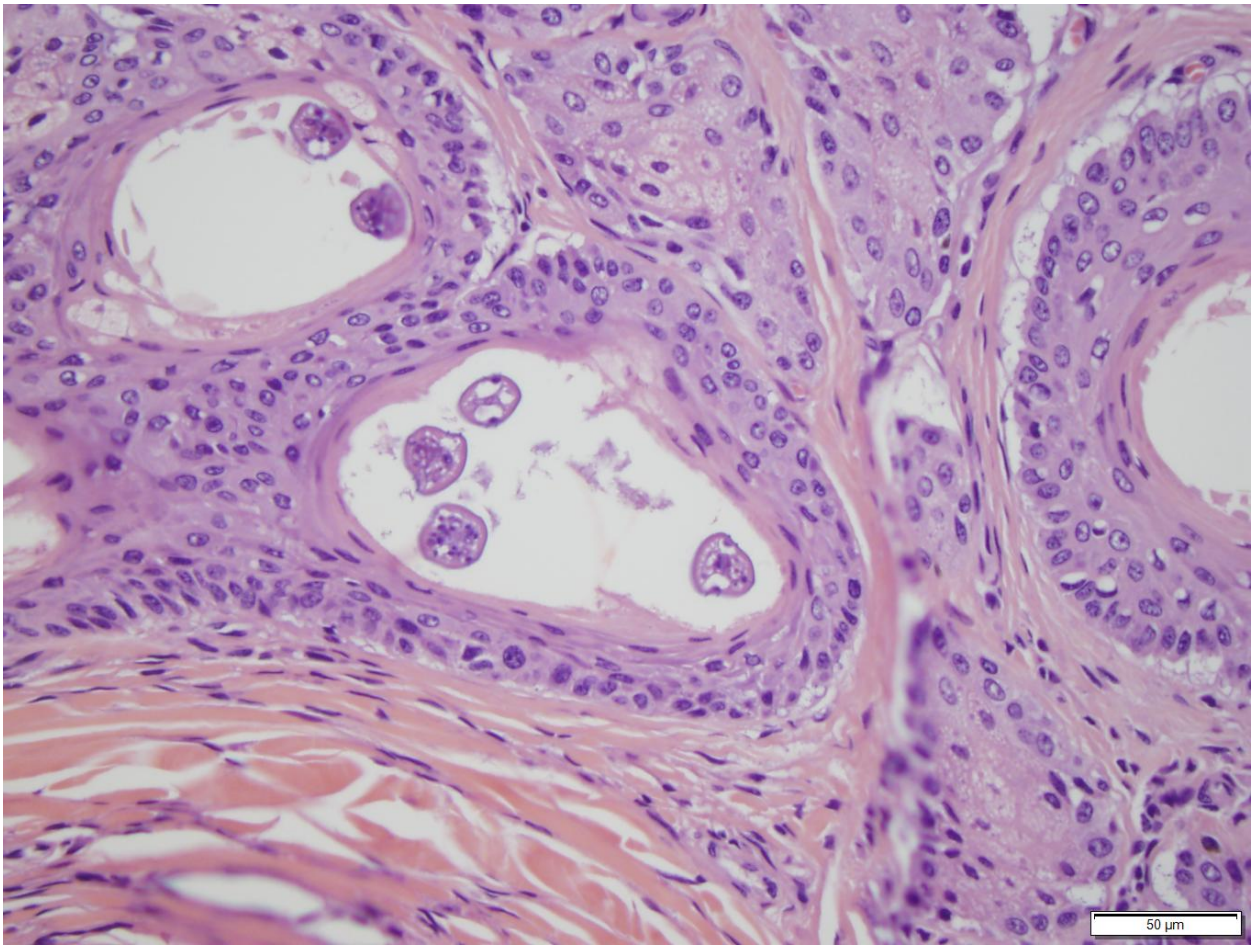

Figure 3.2. Histologic section of skin showing intra-follicular *Pelodera* nematodes.

Serology

Serum samples from a total of 72 bears were tested and 40 (56%) were positive for antibodies to *S. scabiei*. Of the 72 bears tested, 46 had confirmed *S. scabiei*- associated mange and 26 had no gross lesions suggestive of mange. The prevalence of antibodies to *S. scabiei* was significantly higher in adult and yearling bears with mange (39/46, 85%) compared to those with no gross lesions suggestive of mange (1/26, 4%; $p<0.0001$; Table 3.4). Only five bears (three adults and two yearlings) with mange lesions were negative for antibodies while an additional one adult and one yearling with mange lesions were classified as equivocal according to the

ELISA manufacturer's directions (Table 3.4). Of these five, three were positive for *S. scabiei*, while the other two only had mild alopecia with no confirmation of mites in skin scrapes. Among bears with no lesions of mange, one adult bear tested positive for *S. scabiei* antibodies and one yearling bear was classified as equivocal (Table 3.4). No difference in antibody prevalence was noted between males and females (Table 3.4).

DISCUSSION

In this study, we confirmed that *S. scabiei* currently appears to be the primary cause of mange in black bears in Pennsylvania. In general, reports of clinical mange in bears are rare and often, the causative agent is not determined. Previously, only two reports of sarcoptic mange (Schmitt et al., 1987; Fitzgerald et al., 2008), two reports of demodectic mange (Manville, 1978; Desch, 1995) and three reports of ursicoptic mange exist for black bears (Yunker et al., 1980; Joyner et al., 2004; and Ternent and Cottrell, 2007). Since so few studies have been published on mange in black bears, it is imperative to identify the mites to better understand the epidemiology of bear mange cases. The gold standard for diagnosis of mange and determination of the etiologic agent is to cytologically examine skin scrapes of effected skin. The success in this technique is dependent on the quantity of the mites in the skin and scraping the correct anatomic location. Therefore, in the present study we aimed to evaluate the utility of several diagnostic assays (cytology, PCR, serology, and histology) for detection and identification of mites in these black bears.

Molecular assays have been used in numerous studies to diagnose and characterize the species of mites present in mange cases; however, the utility of molecular testing may be limited by the mite loads and the successful extraction of DNA (Alasaad et al., 2009; Amer et al., 2014;

Berrilli et al., 2002; Makouloutou et al., 2015; Walton et al., 2004; Zahler et al., 1999; and Zhao et al., 2015). A high percentage of the black bear mange cases were PCR positive by one or both of our PCR methods but we did find the ITS-2 protocol was more sensitive for the detection of *S. scabiei* compared with the *cox*1 protocol. Fukuyama et al (2010) evaluated the diagnostic usefulness of nested PCR targeting *S. scabiei* myosin heavy chain (SMH) and ITS-2 in scabies patients and also found that the ITS-2 protocol was more sensitive compared to the SMH target (Fukuyama et al., 2010). Surprisingly, the addition of a grinding step during extraction did not increase the sensitivity of the PCR protocol. The bears with mange, confirmed by cytology, that were PCR negative all had low numbers of mites present which emphasizes the need to collect high quality scrapes to increase the sensitivity. Due to difficulties in capturing bears, a noninvasive diagnostic test on feces would be ideal, similar to that reported from bobcats with notoedric mange in California which could detect a few as 1.9 mites/200 µg feces (Stephenson et al., 2013); however, we did not detect mite DNA in any of the fecal samples from bears with severe mange. Although bears groom, these data suggest that insufficient numbers of mites are ingested during the grooming process for detection.

Microscopic lesions from the black bears with mange were consistent with those previously described in free-ranging carnivores with sarcoptic mange (Pence et al., 1983; Schmitt et al., 1987; Deem et al., 2002; Dominguez et al., 2008; and Nimmervoll et al., 2013). While histological examination of skin is commonly used to classify or score mange lesions and to detect mites, sarcoptiform mites cannot be determined down to species with this diagnostic method (Nimmervoll et al., 2013). Therefore, this diagnostic method is not ideal for mite species identification. However, our results did demonstrate the presence of a dual infection with *Pelodera stronglyoides* in four bears. *Pelodera* is a free-living saprophytic nematode that is

commonly associated with organic material and moist soils. Parasitism occurs when an animal's skin, especially those animals with a preexisting dermatitis such as mange, comes into contact with contaminated soils or bedding, (Dykstra et al., 2012). *Pelodera* has been reported in dogs (Schlotthauser and Zollman, 1955; Willers, 1970; Horton, 1980; Morisse et al., 1994; and Saari and Nikander, 2006), sheep (Bergeland et al., 1976; and Ramos et al., 1996), cattle (Levine et al., 1950; Rhode et al., 1953; Scott and Gourreau, 1993; and Yeruham and Perl, 1998), guinea pigs (Todd et al., 1982), and humans (Pasyk, 1978; and Jones et al., 1991). Recently, however, there have been two reports of *Pelodera* in black bears. Interestingly, the first report of *Pelodera* was from a black bear with sarcoptic mange in Michigan (Fitzgerald et al., 2008). The only other report was from a black bear in Minnesota that died from disseminated blastomycosis (Dykstra et al., 2012). Black bears suffering from mange may be more susceptible or predisposed to invasion by *Pelodera* due to skin damage. Additionally, black bears may especially be prone to invasion during hibernation when they are in contact with damp soils for a prolong period of time (Fitzgerald et al., 2008). Further research is needed to better understand the role this nematode may play in contributing to the overall condition of mange-impacted animals.

Serologic testing has many advantages such as the ability to detect animals that have had past episodes of sarcoptic mange. Previously, an ELISA that detects antibodies against *S. scabiei* in dogs has been validated for use in red fox (Bornstein et al., 2006). The commercial ELISA used in the current study, developed for the detection of *S. scabiei* in domestic dogs, was useful for detecting IgG antibodies against *S. scabiei* in black bears. Although a significantly higher proportion of bears with mange were seropositive (85%) compared to bears with no signs of mange (4%), five bears that had signs of mange but were negative for antibodies. Of these five bears, mites were only detected in three bears so the other two bears with alopecia may not have

had sarcoptic mange. For the small number of bears with confirmed mange that were negative for antibodies may have been in the early stages of infection and had not yet seroconverted. Alternatively, bears with mange that were seronegative may response similar to sheep and goats which may only develop a transient increase in IgG antibodies during secondary challenges with *S. scabiei* (Tarigan and Huntley, 2005; Rodriguez-Cadenas et al., 2010). Also, Davidson et al (2008) identified two red foxes that were positive for sarcoptic mange but remained seronegative suggesting that some individuals may take a long period of time to seroconvert or were immune suppressed resulting in no seroconversion. We did not note a difference in antibody prevalence between male and female bears which is similar to previous serologic studies on wild foxes that did not find differences in antibody prevalence between sex classes (Bornstein et al., 2006; Davidson et al., 2008). Serology has the potential to detect previous exposures and/or, potentially mild infections. Currently, surveillance for mange in black bears in Pennsylvania is syndromic which has limitations that may be overcome by serologic testing. Future work needs to investigate, the likelihood that a bear seroconverts following infestation and/or during different extents of clinical disease (i.e., mild vs. severe mange), antibody persistence following clinical mange, lower immune responses following subsequent periods of clinical mange, maternal antibody production, and possible serologic cross-reactivity with *U. americanus*. Although there are many unknowns, these serologic data suggest that a serologic approach may be useful in understanding the extent of *S. scabiei* infestation and mange in this population.

Overall, these data confirm that the severe mange cases in black bears from Pennsylvania that we examined were caused by *S. scabiei*. Sarcoptic mange is widespread in mammals, but only sporadically reported in bears. The current outbreak in Pennsylvania's black bear population is ongoing and sustained. The reason for this increase in mange cases remains

unknown but could be due to the emergence of a novel *S. scabiei* strain. Genetic characterization of *S. scabiei* associated with this outbreak is needed to determine if an unusual strain of *Sarcoptes* has emerged. In addition, our data suggests that serologic testing may be useful for studying the epidemiology of this outbreak. These are critical data as wildlife managers struggle to determine appropriate diagnostic approaches for detection and management of mange wildlife species, including free-ranging bears.

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Table 3.1. Laboratory tests performed on each black bear (*Ursus americanus*).

(X denotes that the sample was collected and tested).

		Tests				
Bear ID	**Mange?**	**Scrape**	**Full-thickness Skin**	**Feces**	**Serum**	**Histology**
35494	Y	X			X	
35791	Y	X		X	X	
22308	Y	X	X	X	X	X
21775	Y	X	X		X	X
19933	Y	X	X		X	X
20269	Y	X	X		X	X
20268	Y	X	X		X	X
22001	Y	X	X		X	X
21773	Y	X	X	X	X	X
21774	Y	X	X	X	X	X
22000	Y	X	X		X	X
35477	Y	X	X	X	X	X
20267	Y	X	X	X	X	X
21974	Y	X	X	X	X	X
21890	Y	X	X		X	X
21329	Y	X	X	X	X	X
18598	Y	X	X	X	X	X
22009	Y	X	X	X	X	X
22002	Y	X	X	X	X	X
34244	Y	X	X		X	
22305	Y	X	X		X	X
27228 A	Y	X			X	X
27228 B	Y	X	X		X	
35640	Y	X	X	X	X	X
35484	Y	X			X	
15BB0002	Y	X	X	X		X

22616	Y	X	X	X	X	
21487	Y	X	X	X	X	
21488	Y	X		X		
22370	Y	X	X	X	X	
22369	Y	X	X	X		
19168	Y	X	X	X	X	
21627	Y	X	X	X	X	
19404	Y	X	X		X	
22614	Y	X	X	X	X	
22008	Y	X	X	X	X	
22361	Y	X	X	X	X	
15SC011	Y	X	X		X	
15NC0096	Y				X	
2-65-3 (#2)	Y	X	X		X	X
2-65-3A	Y	X	X		X	X
2-65-2A	Y	X	X			X
2-11-2A	Y	X	X		X	X
2-65-3 (#3)	Y	X	X			X
402840	Y	X	X			X
403242	Y	X	X		X	X
403245	Y	X	X		X	X
403241	Y	X	X	X	X	X
402394	Y	X	X		X	X
402383	Y	X	X	X	X	X
402376	Y	X	X		X	X
402382	Y	X	X		X	X
402379	Y	X	X			X
402373	Y	X	X		X	X
400828	Y	X	X	X		X
405124	Y	X	X	X		X

405111	Y	X	X	X		X
406312	Y	X	X			X
401321	Y					
403944	Y	X		X		
403990	Y	X		X		
402886	Y	X				
404050	Y	X		X		
403612	Y	X		X		
403627	Y	X		X		
405167	Y	X		X		
403634	Y	X		X		
1503957	Y	X	X	X	X	
1503007	Y		X			
1503193	Y	X	X			
1510720	Y	X	X		X	
1503327	Y	X				
18607	Y				X	
35469	Y				X	
51450	Y				X	
51752	Y				X	
33144	Y				X	
51460	N				X	
30975	N				X	
30984	N				X	
33536	N				X	
35868 A	N				X	
33546	N				X	
25146	N				X	
27151	N				X	
33247	N				X	

35035	N	X
35032	N	X
35664	N	X
35977	N	X
36892	N	X
35224	N	X
35728	N	X
41114	N	X
31920	N	X
51626	N	X
35602	N	X
29749	N	X
41216	N	X
33764 A	N	X
40044	N	X
35979	N	X
35732	N	X

Table 3.2. PCR results for skin scrape samples tested with the ITS-2 protocol, without grinding.

		PCR result	
	n	**pos**	**neg**
ITS-2	69	60	9

Table 3.3. PCR results for three full-thickness skin samples tested with either the ITS-2 or *cox*1 protocols.

	PCR protocol		**PCR result**	
Sample		***n***	**pos**	**neg**
1	ITS-2	56	40	16
2	*cox*1	56	30	26
3	*cox*1	56	18	38

Table 3.4. Serologic results for black bears (*Ursus americanus*) with sarcoptic mange or with no lesions suggestive of mange.

	Bears with mange			Bears with no lesions of mange		
	No.	**No. Pos (%)**	**Equivocal (%)**	**No.**	**No. Pos (%)**	**Equivocal (%)**
Adults	39	35 (90)	1 (2.6)	17	1 (5.9)	nd
Females	33	30 (91)	nd	11	1 (9)	nd
Males	6	5 (83)	1 (17)	6	0	nd
Yearlings	7	4 (57)	1 (14)	4	0	1 (25)
Females	3	2 (67)	nd	3	0	1 (33)
Males	4	2 (50)	1 (25)	1	0	nd
Cubs	nd	nd	nd	5	0	nd
Females	nd	nd	nd	4	0	nd
Males	nd	nd	nd	1	0	nd

*nd = no data

CHAPTER 4

GENETIC CHARACTERIZATIONS OF *SARCOPTES SCABIEI* FROM BLACK BEARS (*URSUS AMERICANUS*) AND OTHER CARNIVORES IN NORTHEASTERN UNITED STATES[2]

[2]Peltier S, J Brown, M Ternent and MJ Yabsley. To be submitted to the *Journal of Parasitology*

ABSTRACT

In the past several years, an outbreak of severe mange has impacted the black bear (*Ursus americanus*) population in Pennsylvania. To better understand the possible causes of this increased number of cases, we genetically characterized *Sarcoptes scabiei* samples from black bears in the northeastern United States. These sequences were compared to *S. scabiei* sequences from wild canids [red fox (*Vulpes vulpes*) and coyote (*Canis latrans*)] from Pennsylvania and other sequences in GenBank. The internal transcribed spacer (ITS)-2 region and cytochrome *c* oxidase subunit 1 (*cox*1) gene were amplified and sequenced. Nineteen ITS-2 sequences were obtained from mites on bears (n = 14), red fox (n = 3), and coyote (n = 2). The bear sequences were identical to each other and four of the five *S. scabiei* from canids were identical and differed from all *S. scabiei* sequences from bears. The remaining fox sequence only differed from other canid sequences by a single polymorphic base. Eighteen *cox*1 sequences obtained from mites from bears represented six novel haplotypes. Phylogenetic analysis of *cox*1 sequences revealed four clades: two clades of mites of human origin from Panama or Australia, a clade of mites from rabbits from China, and a large unresolved clade that included the remaining *S. scabiei* sequences from various hosts and regions, including sequences from the bears from the current study. Numerous studies have suggested that the ITS-2 is not ideal for distinguishing spatial or host-associated strains of *S. scabiei*, but, interestingly, in our study, we did find that a single nucleotide difference segregated bear from wild canid *S. scabiei* samples. As expected, the *cox*1 gene was more variable; however, phylogenetic analyses failed to detect any clustering of *S. scabiei* from the northeastern US states. Instead, sequences from bears were present in the large clade which included *S. scabiei* from numerous hosts from Europe, Asia, Africa, and Australia. Our results suggest that there is not a single strain of *S. scabiei* causing this large

outbreak in bears in Pennsylvania and thus isn't due to emergence of a novel pathogenic strain. Finally, alternative molecular characterization methods (e.g., microsatellites) or novel gene targets may be necessary in future studies of the possible association of *S. scabiei* haplotypes with certain host or geographic areas.

INTRODUCTION

Sarcoptic mange, caused by the mite *Sarcoptes scabiei*, is a contagious skin disease occurring in over 100 species of domestic and wild animals worldwide. This disease is characterized by varying degrees of hair loss and thickened, crusty skin, which can lead to poor body condition, secondary infections and possibly death (Pence and Ueckermann, 2002). Since 1991, there has been an increase in the number and geographic distribution of severe sarcoptic mange in black bears (*Ursus americanus*) in Pennsylvania (Sommerer, 2014). The reason for this increase in mange cases remains unknown but could be due to increased interactions with other *Sarcoptes* hosts (e.g., coyotes, fox), increased bear density or interactions, or emergence of a novel *S. scabiei* strain.

Sarcoptes scabiei is a genetically diverse species and host specialists or generalists have been identified (Pence and Ueckermann, 2002). Numerous genetic targets have been used to investigate the genetic relationship among *Sarcoptes* samples from different hosts and geographic regions and some show of these techniques are more useful than others. For example, sequences of the internal transcribed spacer (ITS)-2 region from different hosts from different countries exhibit limited diversity (Zahler et al., 1999; Berrilli et al., 2002; Gu and Yang, 2008; and Alasaad et al., 2009; Makouloutou et al., 2015). Researchers have also investigated the use of cytochrome *c* oxidase subunit 1 (*cox*1) as a DNA barcode to distinguish *Sarcoptes* strains and

distinct geographically separate and host-adapted *S. scabiei* populations have been identified (Walton et al., 2004; Amer et al., 2014; Makouloutou et al., 2015; and Zhao et al., 2015).

A considerable number of genetic characterization studies have been conducted on *Sarcoptes* mites from Asia, Europe, Africa, and Australia, but relatively few studies have been conducted in North America and none in the region where the current sarcoptic mange outbreak is occurring in black bears. In this study, we examined the genetic diversity of mites collected from black bears in Pennsylvania and compared these sequences to those from mites from other hosts or geographic regions. In addition, we analyzed mites from black bears with mange from states surrounding Pennsylvania (i.e., Virginia, Maryland).

MATERIALS AND METHODS

Mite collection and DNA extraction

Samples were obtained from black bears with mange that were captured by the Pennsylvania Game Commission (PGC) or were legally harvested during hunting season between 2011 and 2015. For harvested bears, skin scrapes and full-thickness skin (1cm x 1cm) samples were collected and for bears captured and released by PGC personnel, only skin scrapes were collected. Scrapes and full-thickness skin were stored in 70% ethanol until analysis. All mites included in this study were confirmed to be *S. scabiei* based on morphologic criteria (Fain 1968; Yunker et al. 1980; Desch 1995).

To evaluate the utility of grinding mites prior to DNA extraction, skin scraping samples were divided into two microcentrifuge tubes. Samples were centrifuged to form a pellet, the ethanol was pipetted off, and the pellet was washed with phosphate-buffered saline (PBS). After washing, tubes were left open to allow any remaining ethanol to evaporate. One tube was

processed following the manufacture's (DNeasy blood and tissue kit, Qiagen, Valencia, California, USA) instructions for tissue while the other tube was first ground with a micropestle (Kimble Chase, Grainger) before being processed following the tissue protocol. For full-thickness skin, the ethanol was allowed to evaporate and then DNA was extracted from three separate sections of skin (≤ 25mg) which were each ground with a micropestle.

Molecular analysis and sequencing

Mite DNA was amplified using primers RIB-18 and RIB-3 which amplify the internal transcribed spacer (ITS)-2 region (Zahler et al., 1999) and primers Cyto F and Cyto R which amplify a partial region of the cytochrome *c* oxidase subunit 1 (*cox*1) gene (Walton et al., 2004). Cycling conditions for the ITS-2 region were 94 C for 2 min followed by 40 cycles of 94 C for 45 sec, 60 C for 45 sec, 72 C for 1 min, and a final step at 72 C for 5 min. For the *cox*1 gene, cycling conditions were 94 C for 5 min followed by 40 cycles of 94 C for 30 sec, 48 C for 1 min, 72 C for 1 min, and a final extension at 72 C for 1 min.

Precautions were taken to prevent and detect contamination including performance of DNA extraction, PCR reaction setup, and product analysis in distinct, designated areas. Negative water controls were included in each set of DNA extractions and PCR reactions. A positive control (DNA from *S. scabiei* mite) was included in each PCR set.

To determine the intraspecific variability of *S. scabiei* samples, PCR amplicons were purified from a GelRed (Biotium, Hayward, CA) stained 1.5% agarose gel using a gel-purification kit (Qiagen, Valencia, California, USA) and bi-directionally sequenced at the University of Georgia Genomics Facility (Athens, GA, USA).

Sequence and phylogenetic analysis

Sequences were aligned and edited using Geneious 8.1.8. A phylogenetic tree was constructed using Phylogeny.fr (Dereeper et al., 2008; Dereeper et al., 2010; http://www.phylogeny.fr/index.cgi)

RESULTS

ITS-2

In total, 19 *S. scabiei* ITS-2 sequences were obtained from bears (n = 14), fox (n = 3), and coyote (n = 2). All were confirmed to be *S. scabiei* and were 98-99% similar to *S. scabiei* sequences in Genbank. Most of these sequences (n = 8) were approximately 439 bp in length but 11 ranged from 311-427 bp in length. Among the fourteen bear sequences, 8 haplotypes were detected, most differed only due to the presence of polymorphic bases (Table 4.1). Four of the five *S. scabiei* from fox and coyotes were identical and differed from all *S. scabiei* sequences from bears, but one sequence from a fox sample had a single polymorphic base at this site, similar to sequences from bears (Table 4.1; GenBank reference sequence accession number EF514468).

*cox*1

A total of 18 cox1 sequences were successfully amplified and sequenced, all from black bears. All were confirmed to be *S. scabiei* and were 95-99% similar to *S. scabiei* sequences in Genbank. The majority of these sequences (n=16) were approximately 696 bp in length but two were only ~450 bp in length. Overall, a total of six unique haplotypes were identified and these were 99.5-99.8% similar to each other. Eight bears were infested mites with one haplotype (called haplotype A), six bears were infested with mites in haplotype B, and the remaining four bears had unique mite sequences (haplotypes C, D, E, and F). Nucleotide variation by haplotype

is reported in Table 4.2 (GenBank reference sequence accession number CM003133). Three of the eight haplotype A mites were collected from Mifflin County, two from Centre County, one from Elk County, and one from Bedford County Pennsylvania, and one from Maryland (Garrett County). Five of the six haplotype B sequences came from McKean County and the remaining sequence came from Potter County Pennsylvania. Of the four unique bear sequences, three are from Pennsylvania (Potter, Indiana, and Westmoreland Counties), and one from Virginia (Frederick County; Figure 4.1).

The nucleotide differences in the *cox*1 gene resulted in several amino acid (AA) substitutions which occurred at four positions on the AA alignment (10, 12, 35, and 213) within four of the six unique haplotypes (haplotype B, C, D, and E). Two of the substitutions were synonymous (I vs. F, M vs. I) and two were nonsynonymous (G vs. W, S vs. Y). The three unique mite sequences (haplotypes C, D, and E) each had two substitutions, and the haplotype B sequence had one substitution (Table 4.3; GenBank reference sequence accession number CM003133).

Phylogenetic analysis of nucleotide sequences revealed four clades of *Sarcoptes* mites, 1) a clade of mites of human origin from Panama, 2) a clade of human origin mites from Australia, 3) a clade of mites from rabbits in China, and 4) a large unresolved clade including the remaining *S. scabei* sequences from various hosts and regions, including sequences from the bears from the current study (Figure 4.2).

DISCUSSION

In this study, we genetically characterized *Sarcoptes scabiei* isolates from black bears, fox, and coyote in the Northeastern U.S. using ITS-2 and/or *cox*1 gene targets. This study is the

first to genetically characterize *S. scabiei* from free-ranging bears and contributes significantly to the number of sequences from wildlife species in North America. Overall, we found only a limited amount of variation in both gene targets; however, the *cox*1 gene target suggested multiple strains of *S. scabiei* are circulating among black bears.

Our results showed that ITS-2 sequences of *S. scabiei* from bears are highly conserved with only five polymorphic sites. Our findings support those of previous studies, which demonstrated that *S. scabiei* isolates from numerous hosts and geographic regions have almost identical ITS-2 nucleotide sequences (Alasaad et al., 2009; Zahler et al., 1999; and Gu and Yang 2008; Makouloutou et al., 2015). Despite the limited variability, a single base could be used to distinguish two genotypes that, for the most part, segregated by species with one being found in fox or coyotes and the other in bears. There was one fox sample that had a single polymorphic base at the site so could have been a co-infestation with the two haplotypes. However, in general, the ITS-2 is not ideal for genetic characterization within a mite species but is often used for molecular diagnostic testing because it is highly sensitivity (Zahler et al., 1998; Berrilli et al., 2002; Alasaad et al., 2009).

In contrast to the ITS-2 data, several haplotypes of cox1 sequences were identified. All of these haplotypes were novel and suggest that the current outbreak of severe mange in black bears in the northeastern US is not due to the emergence of a single highly-pathogenic strain. Similar to previous studies, our phylogenetic analysis of *cox1* gene sequences revealed two distinct clades of human origin *Sarcoptes* from Panama and Australia and another clade of mites that originated from rabbits in China (Makouloutou et al., 2015; Andriantsoanirina et al., 2015). The remaining *S. scabiei* sequences from various hosts and regions, including sequences from the bears from the current study, occurred within a large unresolved clade which has been reported

previously (Walton et al., 2004; Amer et al., 2014; Makouloutou et al., 2015; Zhao et al., 2015). Historically, *S. scabiei* in domestic and wild animals was thought to be a transfer from humans (Fain, 1968; Currier et al., 2011; Alasaad et al., 2013); however, recent analyses suggests that *S. scabiei* in dogs is not of human origin and could actually be the source of human infestations (Andriantsoanirina et al., 2016). The rate of nonsynonymous substitutions observed for our cox1 sequences was similar to that reported by Walton et al., (2004). Currently, the importance of these substitutions is unknown.

The small sample size of the current study is a limitation, especially the absence of *cox*1 sequences from fox or coyotes. Many of these canid samples were PCR negative using the cox1 protocol and those that were positive failed to sequence. Because red fox and coyote can develop severe disease (i.e., alopecia, encrusted lesions, etc.) with very low mite burdens, the mites can be missed unless large samples are collected (Samuel, 1981). Of the nine fox and coyote samples tested in this study, only two mites were observed on skin scrapings. Future work will evaluate more sensitive extraction methods to maximize the chances of obtaining *cox*1 sequences from fox or coyotes for comparison with those from bears.

Host adaptation and geographic segregation in *S. scabiei* has important implications in our understanding of the epidemiology of this parasite. Most studies have focused on phylogenetic analysis of different gene targets and this has often resulted in poor segregation of mites in contrast to use of microsatellites which did demonstrate host species and geographic location clustering of *S. scabiei* (Walton et al., 1997; Walton, 2004). Use of microsatellites allowed the genetic distinction of *S. scabiei* var. *canis* and var. *hominis* populations from Australia (Walton et al., 1997). Because of this success, microsatellites may be a useful technique to investigate the intraspecific variation of *S. scabiei* from black bears and sympatric

carnivores in the northeastern US. Fain (1994) suggests that *Sarcoptes* remains in a continuous process of adaptation due to the large number of hosts it parasitizes. Speciation may be prevented in part because of frequent interbreeding between *Sarcoptes* mites, which ultimately provides genetic characteristics that enhance adaptability, allowing mites to infest new hosts (Fain, 1968 and 1994). More research is necessary to better understand host adaptation and geographic segregation in *S. scabiei* (Makouloutou et al., 2015).

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Table 4.1. Nucleotide variation in *Sarcoptes scabiei* ITS-2 sequences from bears (n = 14), fox (n =3) and coyote (n = 2). Identity with the first sequence is indicated by dots. Polymorphic codes are designated with IUPAC codes.

		Nucleotide Alignment Position					
Host Species and ID	**State**	**163**	**226**	**393**	**448**	**458**	**475**
Bear 35494	PA	C	A	G	A	T	C
Bear NY	NY	.	.	.	.	.	.
Bear 19933	PA	.	.	R	.	.	.
Bear 35477	PA	.	.	R	.	.	.
Bear 21890	PA	.	.	R	.	.	.
Bear VA	VA	.	.	R	.	.	.
Bear 22000	PA	Y	.	R	.	.	.
Bear 35640	PA	Y	.	.	.	.	.
Bear 20267	PA	Y	.	.	.	.	.
Bear 21329	PA	Y	.	.	.	.	.
Bear MD	MD	Y	.	.	W	.	.
Bear 22001	PA	.	.	.	W	.	.
Bear 21974	PA	Y	.	.	.	.	M
Bear 22305	PA	.	.	R	W	Y	.
Red Fox 40	PA	.	R	.	.	.	.
Red Fox 23	PA	.	G	.	.	.	.
Red Fox 14	PA	.	G	.	.	.	.
Coyote B	PA	.	G	.	.	.	.
Coyote D	PA	.	G	.	.	.	.

Table 4.2. Nucleotide variation in the *Sarcoptes scabiei* haplotypes from mites on bears, the number of sequences comprising each haplotype, and the states they come from. Identity with the first sequence is indicated by dots.

			Nucleotide Alignment Position					
Haplotype	**No.**	**State(s)**	**750**	**756**	**763**	**834**	**1,213**	**1,358**
A	8	PA & MD	G	G	A	G	C	C
B	6	PA	.	.	.	A	T	.
C	1	PA	.	T	.	A	T	.
D	1	PA	.	.	.	A	T	A
E	1	PA	.	.	T	A	T	.
F	1	VA	A	.	.	.	.	.

Table 4.3. Amino acid (AA) variation detected in the *Sarcoptes scabiei* haplotypes from mites on bears. Identity with the first sequence is indicated by dots.

	AA Alignment Position			
Haplotype	10	12	35	213
A	G	I	M	S
B	.	.	I	.
C	W	.	I	.
D	.	.	I	Y
E	.	F	I	.
F	.	.	.	.

Figure 4.1. Locations of *S. scabiei cox*1 haplotypes in Pennsylvania, Maryland, and Virginia.

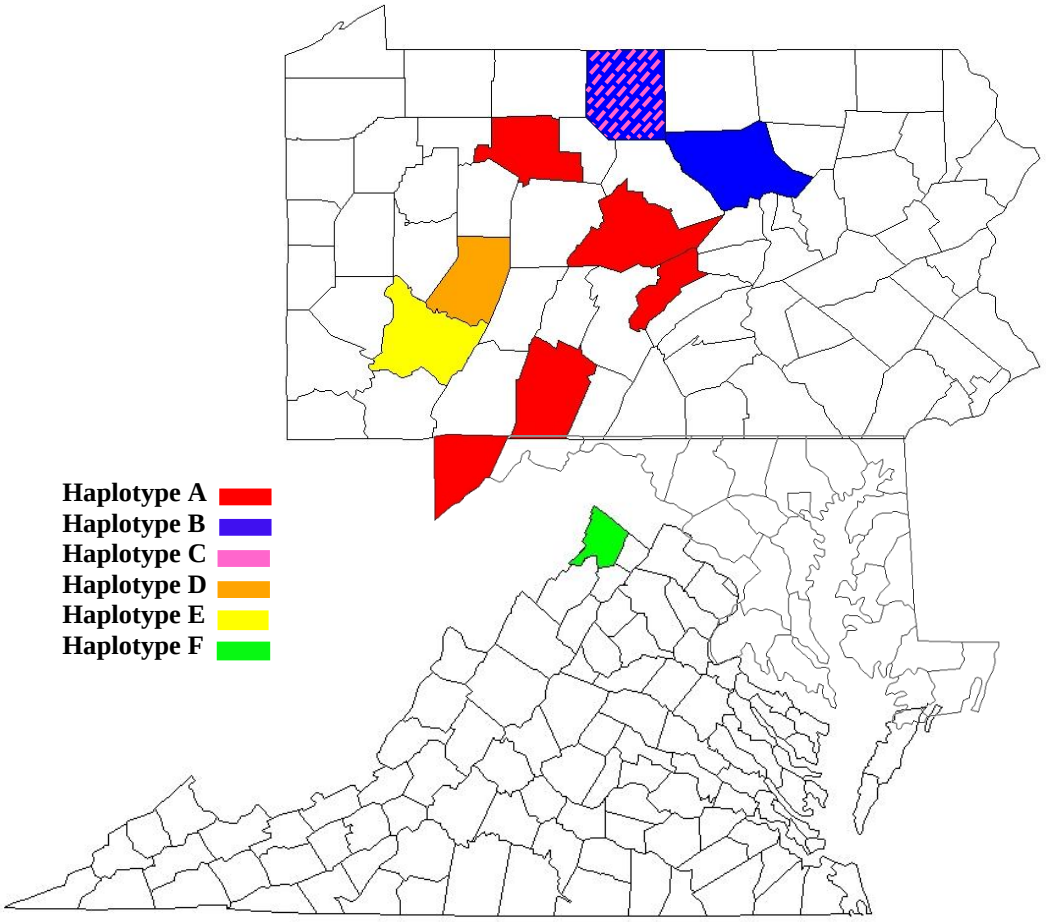
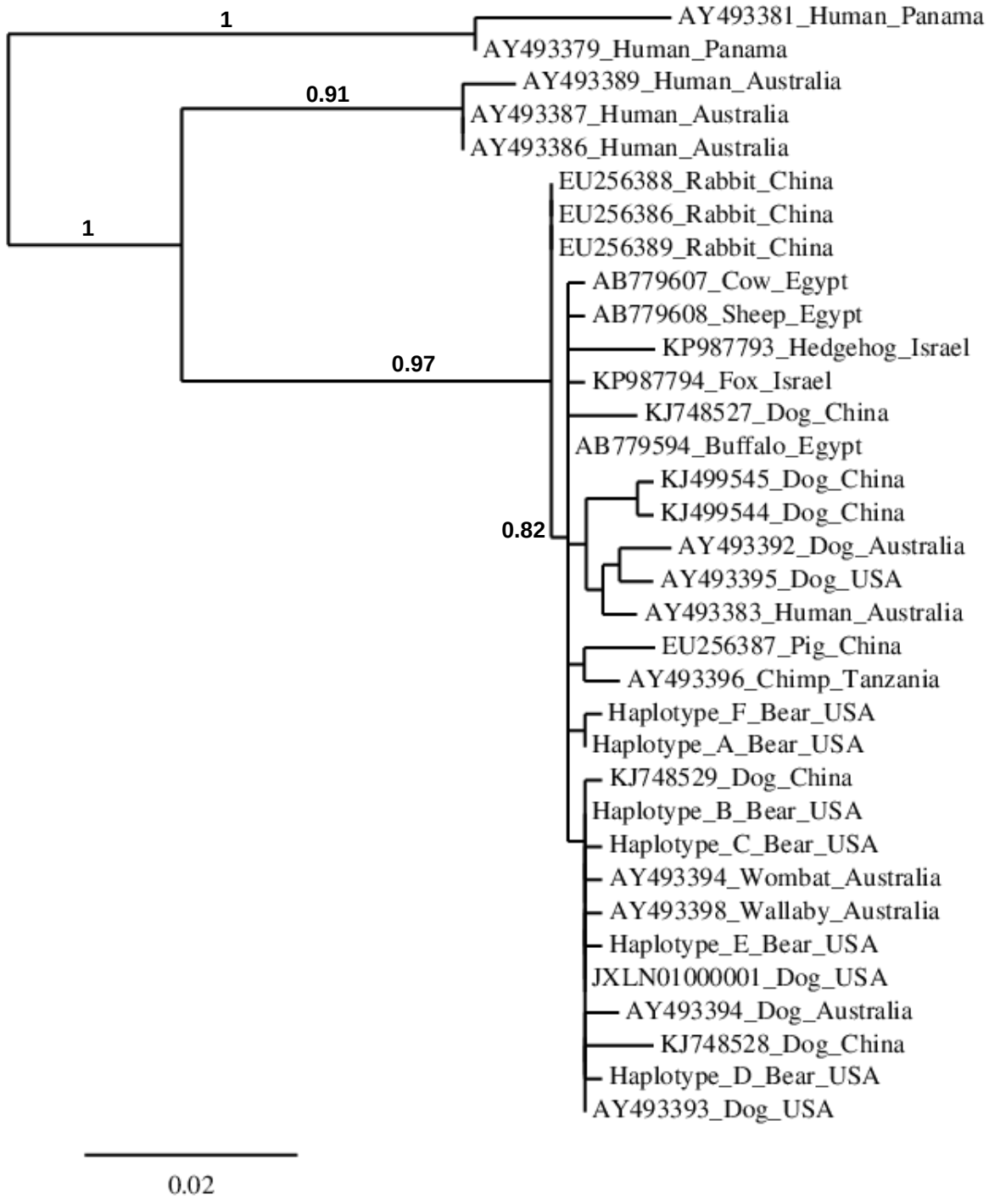


Figure 4.2. Phylogenetic analysis of *Sarcoptes scabiei cox*1 gene sequences from various hosts and geographic regions. Bootstrap values >95% shown at branches.



CHAPTER 5

SUMMARY AND CONCLUSIONS

At least three mite species can cause mange in black bears (*Ursus americanus*) including *Demodex ursi, Ursicoptes americanus*, and *Sarcoptes scabiei*. Recently, the number and geographic distribution of severe mange cases in black bears in Pennsylvania has increased. The reason for this increase in mange cases remains unknown but could be due to increased interactions with other hosts of *Sarcoptes* hosts [e.g., coyotes (*Canis latrans*), red fox (*Vulpes vulpes*), etc], increased bear density or interactions, emergence of a novel *S. scabiei* strain or other mite species, or some other factor making bears more susceptible to clinical disease.

We evaluated several diagnostic assays for detection and identification of the mite(s) associated with these mange cases. Samples from 72 black bears with mange were examined and, based on morphology, *S. scabiei* was the only mite species present in 66 of the 72 bear samples; no mites were identified in the other six samples. Molecular testing targeting the ITS-2 region and *cox*1 gene were used to test skin scrapes (ITS-2), skin biopsies (ITS-2 and *cox*1) and feces (ITS-2 and *cox*1) from selected bears. For each full-thickness skin sample, DNA was extracted from three separate sections which were ground with a micropestal. For each feces sample, DNA was extracted twice and was also ground with a micropestal. Sixty of the 69 (87%) skin scrape samples were positive while only 40 of the 56 (71%) full-thickness samples tested by ITS-2 were positive. Of the two remaining full-thickness extractions, 30 of 56 (53%) and 18 of 56 (32%) tested by *cox*1 were positive. PCR testing of feces for mites was uniformly negative.

Microscopic lesions from 40 bears with mange were consistent with those previously described in free-ranging carnivores with sarcoptic mange. Using a commercial indirect ELISA, the prevalence of antibodies against *S. scabiei* was significantly higher in the bears with mange (39/46, 85%) compared to those with no gross lesions suggestive of mange (1/26, 4%; $p<0.0001$). These data confirm that the most common cause of mange in black bears in Pennsylvania during this study period was *S. scabiei* and suggests that serologic testing may be useful for studying the epidemiology of this outbreak (Chapter 3).

In addition, we genetically characterized *Sarcoptes scabiei* samples from black bears in the northeastern United States. These sequences were compared to *S. scabiei* sequences from wild canids [red fox (*Vulpes vulpes*) and coyote (*Canis latrans*)] from Pennsylvania and other sequences in GenBank. The internal transcribed spacer (ITS)-2 region and cytochrome *c* oxidase subunit 1 (*cox*1) gene were amplified and sequenced. Nineteen ITS-2 sequences were obtained from mites on bears (n = 14), red fox (n = 3), and coyote (n = 2). The bear sequences were identical to each other and four of the five *S. scabiei* from canids were identical and differed from all *S. scabiei* sequences from bears. The remaining fox sequence only differed from other canid sequences by a single polymorphic base. Eighteen *cox*1 sequences obtained from mites from bears represented six novel haplotypes. Phylogenetic analysis of *cox*1 sequences revealed four clades: two a clade of mites of human origin from Panama or Australia, a clade of mites from rabbits from China, and a large unresolved clade that included the remaining *S. scabiei* sequences from various hosts and regions, including sequences from the bears from the current study. Numerous studies have suggested that the ITS-2 is not ideal for distinguishing spatial or host-associated strains of *S. scabiei*, but, interestingly, in our study, we did find that a single nucleotide difference segregated bear from wild canid *S. scabiei* samples. As expected, the *cox*1

gene was more variable; however, phylogenetic analyses failed to detect any clustering of *S. scabiei* from the northeastern US states. Instead, sequences from bears were present in the large clade which included *S. scabiei* from numerous hosts from Europe, Asia, Africa, and Australia. Our results suggest that there is not a single strain of *S. scabiei* causing this large outbreak in bears in Pennsylvania and thus isn't due to emergence of a novel pathogenic strain. Finally, alternative molecular characterization methods (e.g., microsatellites) or novel gene targets may be necessary in future studies of the possible association of *S. scabiei* haplotypes with certain host or geographic areas (Chapter 4).

Management Implications

Collectively, these data improve our knowledge of mange in black bears in Pennsylvania. The solitary nature and overall lack of contact between bears generally would restrict a widespread outbreak for a directly transmitted pathogen such as *S. scabiei*; however, sarcoptic mange in Pennsylvania's black bear population continues to spread. Despite this extensive outbreak, black bear populations in Pennsylvania are currently stable, although future expansion of severe mange may be problematic for maintaining a healthy population. Also, severely emaciated bears with mange are more likely to emerge from their dens and spend time in residential areas searching for food. These nuisance bears are often trapped and euthanized. Our data have laid the groundwork for wildlife managers to investigate large-scale questions related to mechanisms of *S. scabiei* spread (i.e., environmental transmission, asymptomatically infected bears, current research capture procedures), risk factors for disease (e.g., recreational feeding), and treatment or preventive management strategies.