IDENTIFICATION AND EXPRESSION OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN *SARCOPTES SCABIEI.*

By

NOEL MICHAEL COTE'

Bachelor of Arts/Science in Professional Aeronautics

Embry-Riddle Aeronautical University

Oklahoma City, OK

2005

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2010

IDENTIFICATION AND EXPRESSION OF

MACROPHAGE MIGRATION

INHIBITORY FACTOR IN

SARCOPTES SCABIEI

Thesis Approved:

Dr. Deborah C. Jaworski

Thesis Adviser

Dr. Justin L. Talley

Dr. Michael H. Reiskind

Dr. A. Gordon Emslie

Dean of the Graduate College

ACKNOWLEDGMENTS

I would first like to thank my wife, Heather. Her support, strength, love, and encouragement have made this all possible. I would not be where I am today if not for her by my side. I sincerely appreciate her willingness to relocate when it had been required and support all of my endeavors to further my career. Words cannot express my gratitude. I also appreciate the sacrifices my children, Cameron, Chandler, Christopher, and Cydney, have had to endure. Their untiring love and admiration are all a father could ask for, and I could not be any prouder of them. I would like to thank Dr. Deborah Jaworski for serving as my major professor, and for her guidance, patience, and editorial skills, not to mention taking a chance on someone with absolutely no molecular background. I am also grateful for the guidance and input of my other committee members, Dr. Justin Talley and Dr. Michael Reiskind. Completing my research would have been virtually impossible without the help of Nalinda Wasala and Dr. Jerry Bowen. I owe much to both for showing me the ropes and for all the advice. I would also like to thank the United States Navy for allowing me the opportunity to complete my master's degree and for funding my academic career. Finally I would like to thank my parents, Robert and Sandie Cote' for their love and encouragement.

TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION 1

Objectives 4

II. REVIEW OF LITERATURE 5

Introduction to Macrophage migration inhibitory factor (MIF) 5
MIF in skin 7
MIF in parasites 8
History of *Sarcoptes scabiei* 10
Appearance and life cycle 12
The epidemiology of scabies 14
Survival of mites 15
Symptoms and diagnosis of scabies 16
Treatment of scabies 19
Conclusion 20

III. MATERIALS AND METHODS 21

Sarcoptes scabiei samples 21
RNA isolation 21
RT-PCR and RT-qPCR 22
Primers 24
Sequencing of *Sarcoptes scabiei* cDNA 25
Cloning and plasmid transfer 25
Rapid Amplification of cDNA Ends 28
16S and 18S reactions 30
Cataloging of *Sarcoptes scabiei* expressed sequence tags (ESTs) and genes 31

IV. RESULTS 32

Real Time PCR (qPCR) of scabies MIF 36
Catalogue of *Sarcoptes scabiei* expressed sequence tags (ESTs) and genes 37

V. DISCUSSION AND CONCLUSIONS 39

LITERATURE CITED 43

APPENDICES 47

LIST OF TABLES

Table Page

1. Primers synthesized for RT-PCR and RT-qPCR assays.....24
2. Synopsis of existing genes and expressed sequence tags (ESTs) for *Sarcoptes scabiei* 38

LIST OF FIGURES

Figure Page

1. Classification of scabies mite, *Sarcoptes scabiei* 2
2. Structural representation of MIF in *Leishmania,* humans, and hookworms.....6
3. Alignment of MIF genes of various ticks and *Sarcoptes scabiei* 11
4. The life cycle of *Sarcoptes scabiei* 13
5. pGEM-T vector system.....26
6. Initial scabies MIF cDNA compared to *D. variabilis* and *A. americanum*.....27
7. Rapid Amplification of cDNA Ends (RACE) 29
8. *Sarcoptes scabiei* MIF cDNA sequence 32
9. Alignment of *Dermacentor variabilis, Amblyomma americanum, Haemaphysalis longicornis,* and *Sarcoptes scabiei* nucleotide sequences.....34
10. Amino acid sequence alignment between *Dermacentor variabilis, Amblyomma americanum, Haemaphysalis longicornis,* and *Sarcoptes scabiei*.....35
11. *Sarcoptes scabiei* 18S cDNA sequence comparison 36
12. MIF real time PCR assay with *Sarcoptes scabiei* and *Dermacentor variabilis* 37
13. *Sarcoptes scabiei* research model for further experiments in the role of anti-mite MIF antibodies to limit scabies infestations 42

CHAPTER I

INTRODUCTION

Macrophage migration inhibitory factor (MIF) is found in a number of organisms, including humans, mammals, arthropods, and parasites. MIF is found throughout the bodies of these organisms, and is used as a defense to wounds and diseases. Regulating inflammatory responses and immunological processes, as well as responding to tissue damage is another role MIF plays in organisms (Steinhoff et al, 1999). MIF was the first cytokine discovered more than 30 years ago (Review, Bucala et al, 2003). A cytokine is a signaling molecule used in cellular communications. Cytokines are secreted by specific cells in the immune system, which carry local signals between cells. When stimulated, usually by a wound, macrophages produce MIF which inhibits the random migration of macrophages (Shimizu et al, 2003). MIF does not always support a healing role in the body. MIF has been found in elevated levels in people with skin disorders such as atopic dermatitis and psoriasis vulgaris.

MIF has been found in a number of parasites. Some of these parasites include: ticks, hookworms, trypanosomes, coccidians, roundworms, and whipworms. MIF within parasites acts in many ways to evade or confuse the host immune responses.

For example, MIF in ticks is thought to increase inflammation at the feeding site, and thereby increasing blood for the tick at the feeding site. Hookworms are able to thrive in the host by evading the natural immune response during tissue migration (Cho et al, 2007). Some trypanosomes inhibit the macrophages in humans, keeping the trypanosome in the host system (Cho et al, 2007). New evidence identified a putative MIF in the scabies mite, *Sarcoptes scabiei* (Jaworski, unpublished). A scabies MIF might function in permitting mites to evade the immune response, as well as increase inflammation at the feeding site, to increase food intake. To date, the role of MIF in *S. scabiei* has not been studied.

The role of MIF in *S. scabiei* may be similar to its role in ticks. Scabies mites and ticks are closely related. This is shown genetically and taxonomically. Taxonomically scabies mites and ticks are separated at the super order level (Figure 1).

Figure 1. Classification of scabies mite, *Sarcoptes scabiei*.

Phylum: Arthropoda

Class: Arachnida

Sub Class: Acari

Super Order: Parasitiformes – ***Dermacentor, Ixodes, Amblyomma***

Acariformes – ***Sarcoptes, Psoroptes, Oribatida***

Scabies is a contagious skin condition caused by the parasitic mite, *Sarcoptes scabiei*, commonly known as the scabies mite. Scabies are known to infest humans, canines, sheep, pigs and other mammals. Scabies have been documented as early as 350

B.C. (Walton et al, 2007). Scabies infest over 300 million people worldwide each year (Kuhn et al, 2008). Human scabies, also called itch or the seven-year itch is a contagious skin disease which is caused by the burrowing of the scabies mite into the skin. The tunnels or burrows that contain the mite, eggs, and waste products cause the intense itching in the skin (Routh et al, 1994). Scabies mites can be found anywhere on the body, but commonly infest the hands and trunk area. The lesions from the scabies mite cause intense itching, especially at night. Scratching of the lesions can lead to secondary infections. The itching sensation of the affected areas is caused by the toxic secretions and excretions from the scabies mite.

Female scabies burrow into the skin to lay eggs. The female usually lays two to three eggs daily. The eggs hatch and the larvae make their way to the surface of the skin to create molting pouches, where they will molt into nymphs then adults (Routh et al, 1994). Scabies are most commonly transferred by direct contact with an infested host. Newly infested hosts may not show any symptoms for two to six weeks, but are still able to spread the infestation. A rash usually appears in the area that the mite is infesting, but can also occur on other areas of the body. There is only one species of scabies mite, but many different variants, depending on the host. During experimental situations it has been proven that other mammalian scabies can infest humans, but do not complete their life cycle when on a human host (Estes et al, 1983).

A more severe form of scabies is known as crusted or Norwegian scabies. This is a scabies infestation that is usually associated with the immunocompromised, elderly, or persons unable to identify the infestation such as the mentally ill or paralyzed persons.

Hosts with crusted scabies can harbor as many as two million mites and are extremely contagious. A rash may be present when a host is infected with crusted scabies.

Scabies can be hard to diagnose since the signs and symptoms look like many other conditions. Skin scrapings are the most common method of diagnosing scabies infestations. Treatment of scabies infestations is generally accomplished with pyrethroid creams although some success has been found using Ivermectin. Currently, studies are looking at other treatments and strategies for control, since there is a fear of the scabies mite becoming resistant to the current treatments. In addition, a catalog of *Sarcoptes scabiei* genes was constructed to begin to assess what is known about scabies at the gene expression level and to provide context for scabies MIF research.

My hypothesis is that *Sarcoptes scabiei* has a MIF gene whose likely function is to facilitate feeding and reproduction while evading the host immune response. The overall goal of this research was to identify Macrophage migration inhibitory factor from *Sarcoptes scabiei*.

Objectives

1. Cloning and sequencing of Macrophage Migration Inhibitory Factor in *Sarcoptes scabiei*.

2. Cataloging of the existing genes and expressed sequence tags (EST) for *Sarcoptes scabiei.*

CHAPTER II

REVIEW OF LITERATURE

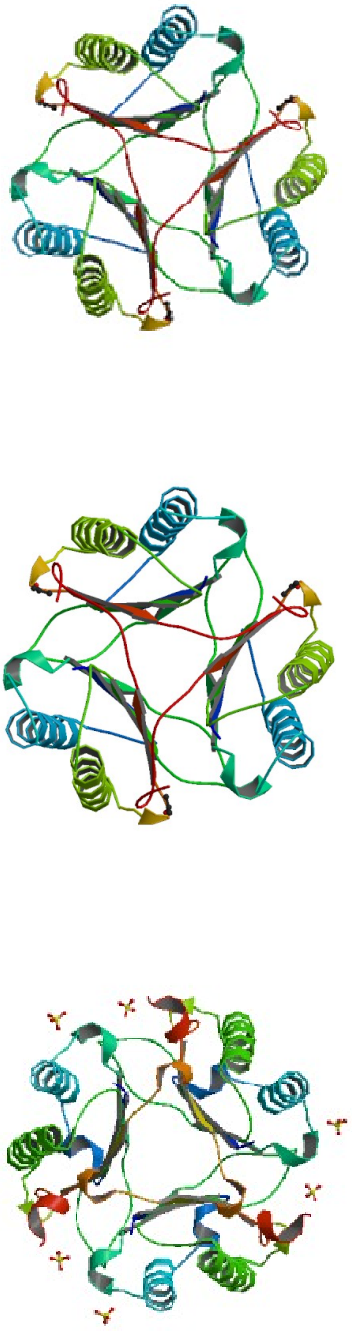
A Review of the Characteristics and Function of Macrophage Migration Inhibitory Factor in *Sarcoptes scabiei*

Macrophage migration inhibitory factor (MIF)

Macrophage migration inhibitory factor is a proinflammatory cytokine, (signaling molecule used in cellular communication), that is used in the human body for immune responses. It is also a critical mediator of diseases such as septic shock, rheumatoid arthritis, and cancer (Dewor et al, 2007; Gomez et al, 2007; Kitaichi et al, 2006). MIF has been found in various organs, such as the skin, brain, and kidneys (Zhao et al, 2005). Macrophage migration inhibitory factor responds to tissue damage and regulates inflammatory and immunological processes (Steinhoff et al, 1999). MIF was first identified more than 30 years ago as a T-cell-derived factor that inhibits the random migration of macrophages (Bucala et al, 2003). T-cells and macrophages produce MIF in response to stimulation such as wounds and infections (Shimizu et al, 2003). MIF has a three polypeptide, or trimer, structure with an open channel in the middle (Figure 2).

MIF looks similar in structure whether from a human or produced by parasites inside the human body.

Figure 2. Structural representation of MIF in Leishmania (top) compared to human MIF (middle) and hookworm MIF (bottom).



All three MIF structures are very similar in appearance. The differences in the structures are found in the arrangement of the beta sheets and alpha helices arranged around the open channel. All the MIF structures have a clear open channel through the middle. This clear channel in the middle of the structure facilitates the movement of solvents through the MIF.

MIF in the skin

MIF has many functions, from mediating and regulating inflammatory responses and wound healing inside the human body. MIF also plays a role in the wound healing process. Studies performed by Dewor et al (2007) looked at the effect of MIF on fibroblast migration in wounded monolayers *in vitro*. Fibroblasts are used in the body as synthesizers of the extracellular matrix that makes up connective tissue. Fibroblasts are also instrumental in the wound healing process.

Human foreskin dermal fibroblasts were used in *in vitro* experiments. The samples were scraped or "wounded" to measure the migration response of the cells into scrape wounds. Microscopic pictures were taken at 0 and 24 hour intervals. When the wounded cells were treated with macrophage migration inhibitory factor a marked response was observed. The result demonstrated that MIF was able to promote fibroblast migration in *in vitro* experiments mimicking wound healing situations (Dewor et al, 2007). A similar experiment was performed using rats, and showed that the wound healing process was significantly delayed by adding anti-MIF antibodies *in vivo* (Abe et al, 2000). These data strongly indicate that MIF could play a critical role in skin injury, cell growth, inflammation, and cutaneous immunity.

Although MIF can have beneficial effects on skin, MIF also have antagonist effects on skin. Atopic dermatitis is a chronic pruritic inflammatory skin disorder. Skin lesions from patients with atopic dermatitis show MIF protein through the entire epidermal layer, in contrast on normal skin MIF is limited to the basal layer (Shimizu, 2005). Another chronic skin disease is psoriasis vulgaris. A symptom of elevated MIF serum levels were found in psoriasis vulgaris patients (Shimizu, 2005). MIF has also been found in the lymph nodes in high numbers with patients with tumors. MIF was higher in patients with breast carcinoma cells compared with normal control tissues (Shimizu, 2005).

MIF in parasites

MIF has been found in a number of parasites including, ticks, hookworms, trypanosomes, coccidians, roundworms, and whipworms. MIF has been found recently in a well known trypanosome, *Leishmania*. It is hypothesized that *Leishmania* uses MIF to evade human immune systems. The MIF in *Leishmania* inhibits the activation-induced apoptosis of macrophages in humans (Kamir et al, 2008). This inhibition of apoptosis in the human host may actually help keep *Leishmania* inside the host's macrophages and actually contribute to the evasion from immune destruction (Kamir et al, 2008).

Hookworms also use MIF homologues to evade the human immune system and avoid destruction. It is hypothesized that adult and juvenile stages of the hookworm, *Ancylostoma ceylanicum* use MIF to modulate the host immune response, during tissue migration in juveniles, and while attached to the intestinal mucosa by adults (Cho et al, 2007). It is proposed that MIF homologues in helminth parasites alter macrophage

influx, immune cell activation, and host cytokine production (Cho et al, 2007). Humans show no evidence of a sterile immunity to hookworms after an infestation and hookworms can live outside a host for a number of years, suggesting that the worms are able to evade or reduce the host immune response that would kill other parasites or have them exuded from the host (Cho et al, 2007). It is possible that MIF may contribute to the success of the hookworm in avoiding the host immune system.

Work has been done on the expression of MIF in ticks. Jaworski et al (2001) performed experiments using the tick *Amblyomma americanum*. They found a tick specific MIF in the salivary glands and midgut tissue of the ticks. In an *in-vitro* functional assay the tick MIF inhibited the migration of human macrophages in the same manner as when in humans (Jaworski et al, 2001). This study demonstrates that a possible role of tick MIF is to increase inflammation at the feeding site.

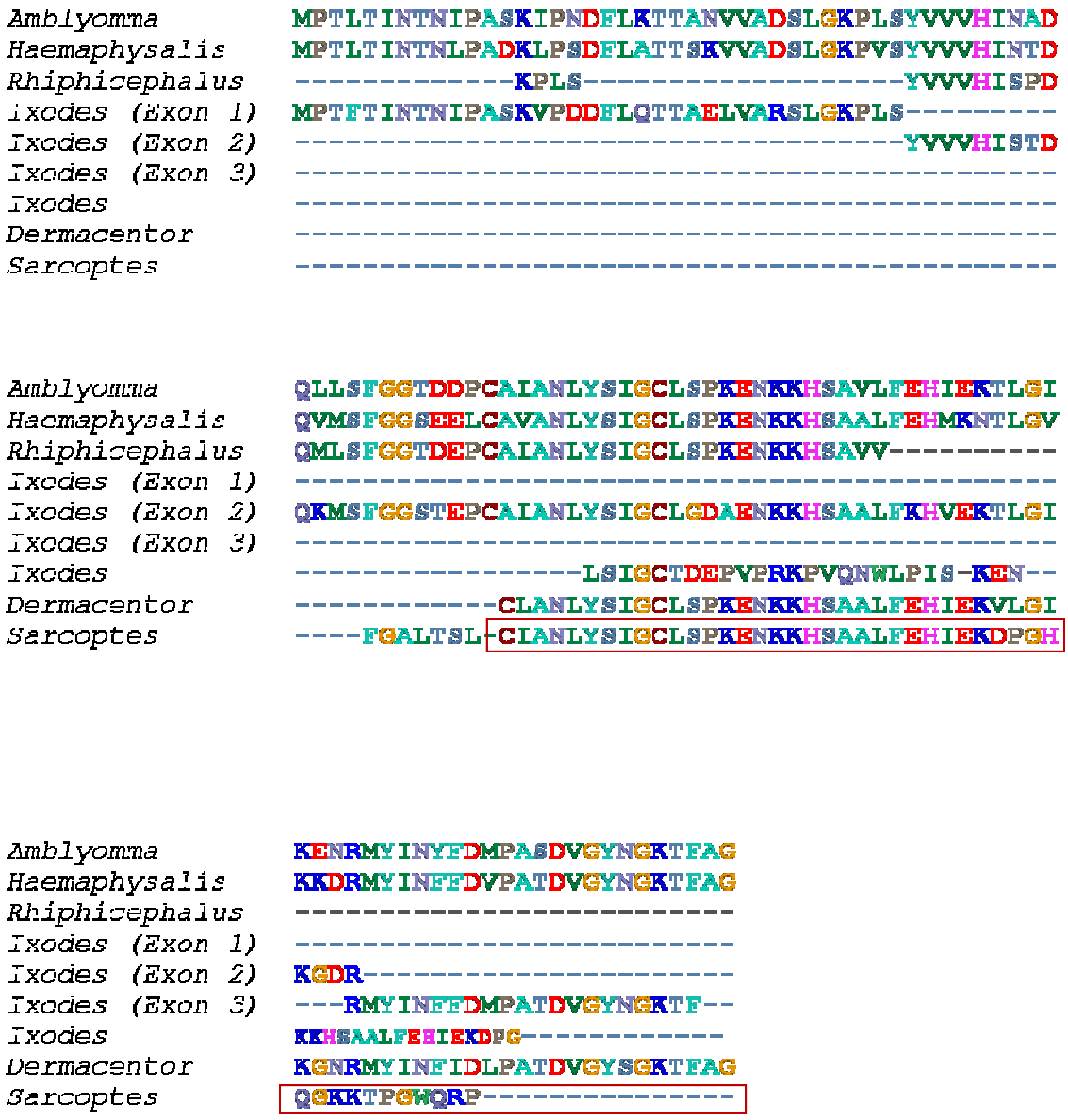
This increase in blood flow that comes with inflammation could benefit the tick. Increased inflammation could also increase host immune and cellular responses and cause changes in feeding and/or pathogen transmission (Jaworski et al, 2009). Results showed that tick MIF is rendered neutral in the tick feeding lesion or in the tick midgut by circulating anti-MIF antibodies (Jaworski et al, 2009). They showed that a specific peptide found in the MIF protein lengthens the feeding interval for ticks fed on peptide-immunized hosts (Jaworski et al, 2009). The localization of MIF using specific antibody confirmed an abundance of MIF protein in the tick midgut cells (Bowen et al, 2010). MIF protein was also localized in unfed adult salivary glands, which creates a MIF protein pool that could be secreted early during the tick feeding process.

To date, MIF in arthropods has been characterized in *Ixodid* ticks. Other putative MIFs are present in the gene database (NCBI) and are found in aphids, hookworms, ticks and trypanosomes. Interestingly, there are no MIF genes in Dipterans (Jaworski et al, 2001). In preliminary experiments, a small portion of *Sarcoptes scabiei* MIF gene has been amplified and sequenced (Jaworski, unpublished). The preliminary experiment amplifying the *Sarcoptes scabiei* gene was a novel finding. Since the scabies mite live in an environment of the host's skin it is not surprising that anti-MIF compounds might be useful in scabies mite infestations to reduce inflammation at the lesions and create an unproductive environment for mites. Since MIF and scabies mites have not been extensively studied, the field is wide open to the possibilities of finding a way to slow the spread of scabies or treat infestations. Figure 3 shows the amino acid alignment of known mite MIFs with the scabies MIF sequence in blocked letters. The highlighted portions in the amino acid sequence are 68% identical.

History of *Sarcoptes scabiei*

Scabies is a contagious skin condition that is caused by the parasite *Sarcoptes scabiei*, or the scabies mite. Scabies mites are in the class Arachnida, order Acari, family Sarcoptidae. Scabies mites can infest humans and other mammals, usually canines. Aristotle was the first person believed to have identified scabies mites, describing them as "akari" or "lice in the flesh" (Walton et al, 2007). Scabies continues to be a persistent problem, affecting as many as 300 million people across the world, even though effective treatments are available (Kuhn et al, 2008).

Figure 3. Alignment of various tick species and *Sarcoptes scabiei* amino acids.



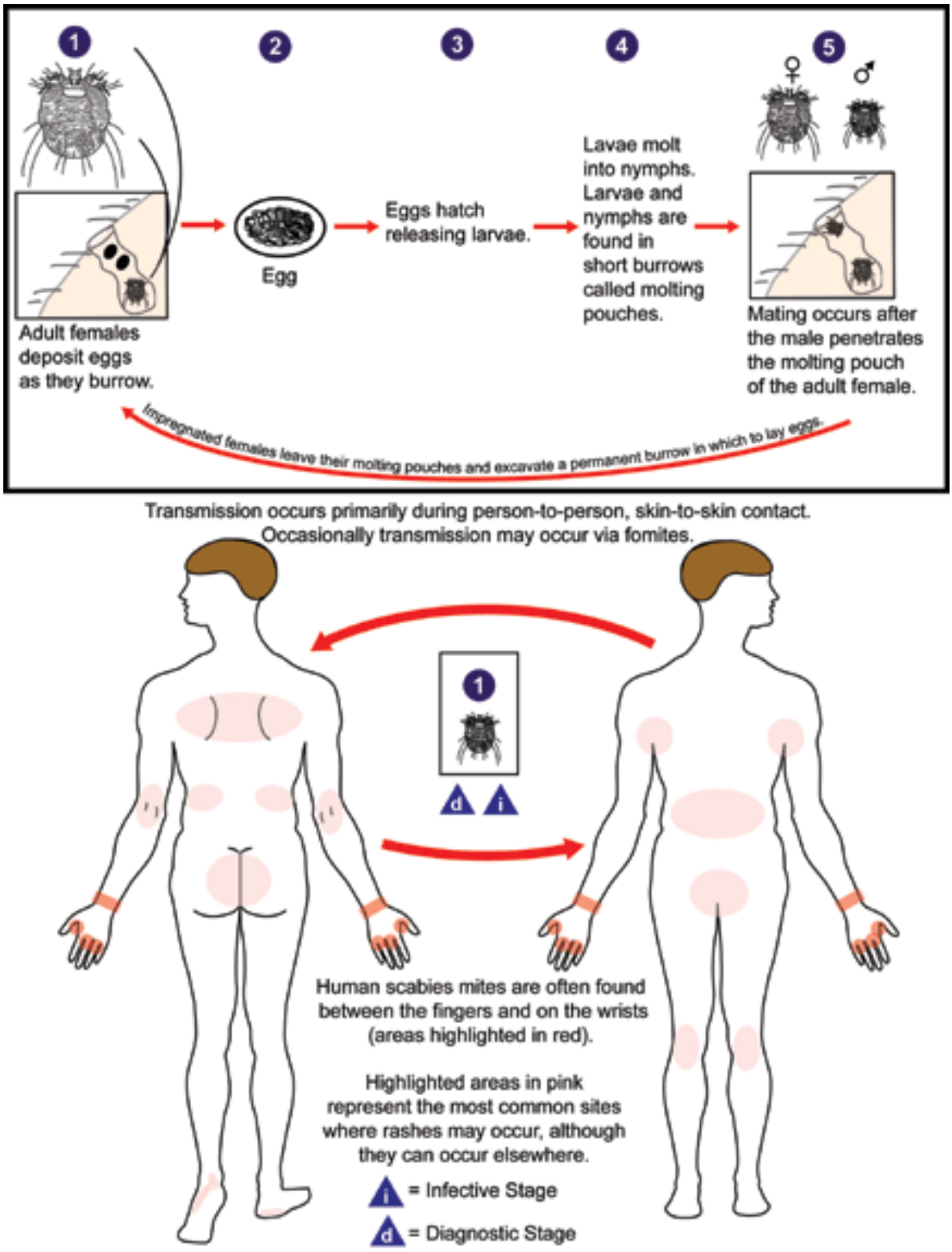
Appearance and life cycle

Sarcoptes scabiei is an eight legged mite that is tan in color and difficult to see without magnification, due to its small size (250-400 µm). The symptoms of scabies mites are usually utilized to diagnose an infestation. Female scabies mites cause the symptoms and skin irritations by burrowing down into the skin to lay eggs. Females lay 2-3 eggs daily and continue to burrow through the skin laying eggs until their death, usually in 1-2 months (Routh et al, 1994). The eggs hatch and larvae emerge, making their way to the skin surface to create molting pouches or small burrows. Once inside the molting pouch the larvae will molt into nymphs, and then adults. Male mites will pierce the molting pouch of the female to mate and the cycle starts over (Figure 4).

Scabies mites do not actually dig into the skin while infesting a host. After acquiring a host, the mite will flatten against the host skin. After a one to two minute period the mite begins to sink into the skin as an unknown clear liquid forms around the mite (Arlian et al, 1984). Digging is not necessary, since the liquid appears to lyse the host's skin. Once the mite has cleared a depression in the skin it propels itself forward as the tissue around it dissolves (Arlian et al, 1984).

Studies performed on penetration time were performed and showed that female mites took the longest to penetrate with a time of 31 minutes (+/- 15 minutes). Males followed with the next longest time of 17 minutes (+/- 7.2 minutes). Nymphs and larvae had the fastest penetration time with larvae penetrating in 9 minutes (+/- 2.5 minutes) (Arlian et al, 1984).

Figure 4. The life cycle of *Sarcoptes scabiei.* (Centers for Disease Control, 2008).



The epidemiology of scabies

Scabies was first thought to be spread by unclean, personally poor hygiene, sexual promiscuity, or overcrowded human populations. This hypothesis has been proven to be untrue since clean and affluent people, as well as isolated families, became infested with *Sarcoptes scabiei* (Arlian et al, 1988). Scabies are spread through direct contact with persons or, rarely, inanimate objects (fomites) infested with scabies mites. Scabies rarely survive off a host for long periods of a time. The normal survival rate of *Sarcoptes scabiei* off the host is from 30 minutes to a few hours, although in the laboratory scientists have gotten scabies mites to live off a host for up to 96 hours (Arlian et al, 1984). Infestation is more likely from direct human contact.

As scabies almost exclusively requires direct host contact, sexual transmission is the easiest way to spread scabies. Scabies seem to prefer this method as the friction and moisture associated with sexual contact provide an excellent environment for the mite to be spread (Routh et al, 1994). In addition, scabies has many routes of transmission besides sexual. Close contact of hosts without sex is another way that scabies are spread, and nursing homes and extended care facilities often suffer outbreaks of scabies. For example, the Northport Veterans Affairs Medical Center in Long Island, New York is a 742 bed facility that houses eligible veterans for short or long term care. This facility suffered an outbreak of scabies in 1991 with 112 patients and staff infested with scabies. Nurses who worked with scabies infested patients had the highest infestation rates (49%). Patients that shared a room were also at high risk with 78% of roommates becoming infested (Jimenez-Lucho et al, 1995).

Scabies mites are known to be spread through close contact or in facilities with highly populated group living conditions. Scabies infest nursing homes and extended living facilities frequently due to the close contact of patients, the immunocompromised, and the disabled. All life stages of scabies mites leave the burrows and wander onto the skin during the life cycle (Arlian et al, 1988). A study was performed using five nursing homes or extended care facilities that had patients that were identified positive for scabies through skin scrapes. Dust samples were taken from six sites. The dust sample sites included the patient's mattress (on top of the bottom sheet), the floor next to the bed, the bathroom floor, the living room floor, the floor space in the clothes changing area in the bedroom, and a frequently used chair or couch (Arlian et al, 1988).

A surface of 1 m^2 was vacuumed for 2 minutes with a Hoover™ vacuum cleaner, with a special dust trap in the hose. All samples were analyzed within 72 hours. Live scabies mites were recovered from the dust samples of 80% of the nursing homes studied. Numbers were low, with one mite found from a chair, four from mattresses, and five on the floor beside the bed (Arlian et al, 1988). This study shows that not only can mites survive off the host, but can infest others from fomites as well as direct contact between hosts. Nursing homes also show prevalence for scabies mites due to the close living conditions and relatively poor health of the patients.

Survival of mites

The traditional theory of scabies mites is that mites cannot survive away from a host for more than a few hours. Studies by Arlian et al (1984) rejected this theory. Mites survived for 24-36 hours at room temperature away from their host, and female mites

survived longer than males. Live mites taken from host bed linens would still penetrate a host after 96 hours with 12 hour alternating periods of refrigeration and room conditions (Arlian et al, 1984). Scabies mite survival is dependent on temperature and relative humidity while off the host. Scabies mites, unlike related mites, cannot take in water from water vapor in the air and always desiccate when away from the host. Survival time when away from the host is proportional to the ambient relative humidity, due to desiccation (Arlian et al, 1984).

Scabies mites infest many mammals and have species specific variants. Canine scabies mites do not usually infest humans or swine for instance. It has been proven that other mammal scabies mites can infest humans in experimental situations or through repeated close contact. Canine scabies mites were taken from a canine infested with *Sarcoptes scabiei var canis* and put onto human skin for 96 hours in an experimental chamber. The mites burrowed, defecated, and produced eggs that developed normally. Two eggs hatched out of nine, and the life cycle ended at this point (Estes et al, 1983).

Arlian et al (1984) performed similar experiments using canine and human scabies mites. The mites were removed from a canine or human host and held off a host for 96 hours with 12 hour alternating periods of refrigeration and normal room temperatures. After 96 hours, the mites would still infest and penetrate a rabbit host (Arlian et al, 1984). While this is not likely to happen naturally, it does show that scabies mites can and will cross hosts in experimental situations.

Symptoms and diagnosis of scabies

The most common symptom of scabies is intense itching (pruritus). The itching

sensation has been described as one of the worst itches ever felt by patients. The skin irritations are usually worse at night than in daylight hours. A person infested for the first time might not show symptoms for 2-6 weeks, but can still spread scabies without showing symptoms. A person who has had scabies in the past can show symptoms much quicker usually 1-4 days. A rash that has a pimple-like look and feel (papular) usually accompanies the pruritus (Centers for Disease Control, 2008) The rash and itching can be across the whole body, but is usually located in certain locations, such as the hands and wrists, groin regions, armpits, buttocks, bra lines on females, and the waist area. Most of these areas are where clothing rubs against the skin, or are moist, warm places on the body. The head and face region are not usually infested unless it is in infants and immunocompromised adults (Routh et al, 1994). Secondary infections are also common due to the intense scratching of infested areas (Pasay et al, 2006).

A more severe form of scabies is known as Crusted or Norwegian scabies. This is the same species of scabies mite in drastically higher numbers. This is a more severe infestation of scabies and usually associated with the immunocompromised, elderly, or persons that cannot itch themselves like the paralyzed or mentally ill. Crusted scabies is characterized by the scab-like crusts on the skin that can hold many mites. A host with crusted scabies can be infested with as many as 2 million mites and is considered highly contagious (Centers for Disease Control, 2008). The symptoms of itching may be absent in crusted scabies due to the ability of the patient to notice the itch or not have the ability to itch themselves. A rash may be present in crusted scabies.

Scabies can be hard to diagnose since the signs and symptoms often look like other conditions, such as bites from other insects, infections, eczema, dermatitis, and

allergic reactions (Walton et al, 2007). There are many ways to diagnose scabies, but the most common is scraping the skin lesions, adding potassium hydroxide solution to the skin sample on a slide, and looking at the sample through microscopy for mites (Katsumata, 2006). Yoshizumi et al (2008), found a way to diagnose scabies without the use of a microscope. The finding of "wake signs" can point to the mite in the skin and can be seen by the naked eye (Yoshizumi et al, 2008). The "wake" sign is a Y shaped lesion that is caused when the female mite burrows into the skin. This is useful for a number of reasons: 1) it is specific for scabies, 2) it is large enough to be seen by the naked eye, 3) it shows the location of the mite and products, 4) it is usually the first sign found during the incubation period (Yoshizumi et al, 2008).

There can be problems by trying to diagnose mites by skin scrapings. Handling of the sample by many people before diagnosis can increase the time it takes to make a positive diagnosis and start treatment for scabies. The technique of using a hand-held dermoscope is being implemented in Canada. The dermoscope is an illuminated magnifier (magnification of 20-60). The dermoscope is held perpendicular to the area of skin believed to be infested. With the use of a dermoscope, a diagnosis can be made in minutes with a success rate for identification of scabies of 91% (Neynaber et al, 2008).

Another method that was tested in Japan recently in disabled patients or in patients that are bed-ridden is the use of clear adhesive tape. Tape is applied to an affected skin area and then removed. The tape is then cut and put on a slide to look for scabies mites. A test of 30 patients was conducted using this method. Six patients were positive for scabies on the tape (Katsumata, 2006). The results from this method are not very encouraging for a couple of reasons. The first reason being that the percentage of

mites found (6) out of the total number of patients (30) is not high at all, approximately 15%. The other reason is that this method does not detect the *Sarcoptes scabiei* eggs, which are also an important diagnostic characteristic.

Treatment of scabies

Treatment of scabies mites involves the use of topical creams with pyrethroids for control. Pyrethroids alter the function of voltage-sensitive sodium channels in arthropod nervous systems, causing paralysis and death (Pasay et al, 2006). Permethrin, in the form of a topical cream, is becoming more commonly used, especially in community-based programs to control endemic scabies (Pasay et al, 2006). The standard treatment for a scabies infestation is application of a topical with permethrin concentration of 5%. The concern from over using a treatment is the development of mite resistance to the drug. This has not been seen as of yet, but other instances of arthropod resistance to drugs are quite common.

Treatment of scabies with ivermectin has also been successful. Ivermectin is an anti-helminth that has been safe in the treatment of other parasitic infestations when given in a single oral dose (Meinking et al, 1995). A study was performed using 22 patients with scabies, 11 of whom were healthy and 11 who had AIDS or HIV. A single oral dose of ivermectin (200-ug per kg) was administered to all patients in the study. The severity of scabies ranged from mild to severe in the study. Five patients in the healthy group were cured in two weeks (45%) and the rest were cured by four weeks. Six of the patients in the AIDS/HIV group were cured after two weeks (55%), while the rest were cured by the four week mark (Meinking et al, 1995).

Conclusion

Macrophage Migration Inhibitory Factor (MIF) is a vital component in humans and many other animals, including parasites. MIF in the human body regulates immune responses, mediates diseases, causes skin conditions, and may cause cancer. MIF in parasites acts as a buffer against the host immune response. *Leishmania* trypanosomes use MIF to stay inside the host and flourish while avoiding the immune response. Hookworms also use MIF to evade the immune response of the host, but also keep the host from developing immunity against it. Ticks use MIF to increase inflammation at feeding sites, increasing the blood flow to the tick and change the host immune response while the tick feeds. Scabies, theoretically, like other parasites use MIF to increase their chances of feeding, whether by evading the host immune system or simply increasing inflammation at the bite site to increase food intake. Finding the way that scabies use MIF will help us understand how it helps propagate the scabies life cycle and eventually give us a way to combat scabies themselves.

Sarcoptes scabiei is an important pest to humans even though it is not a vector for any diseases. Scabies infests over 300 million people yearly, causing skin irritations and responsible for many secondary infections. Scabies mites have been found to be closely related to ticks that have been found to have MIF present in their systems. Ticks with MIF have been given anti-MIF compounds that reduce inflammation at the feeding site. It is the intention of this study to identify and level of expression of MIF in *Sarcoptes scabiei* will eventually lead to the reduction in infestations by scabies mites through anti-MIF compounds.

CHAPTER III

MATERIALS AND METHODS

Samples

Samples of *Sarcoptes scabiei* were obtained from Dr. Larry Arlian at Wright University in Ohio. The samples were enclosed in a cryovial containing 25 mg of *Sarcoptes scabiei var. canis* in 1.0 ml TriReagent® solution. The mites were collected while alive, washed briefly with phosphate buffered saline with 0.05% Tween® 20, water, and 70% ethanol. The scabies mites were then placed in the cryovial with the TriReagent® solution and were frozen at -80°C.

RNA isolation

Scabies mite total RNA was extracted from the tissues using the TriReagent® (Molecular Research Center Inc., Ohio, USA) manufacturer's RNA protocol. The final RNA pellet was reconstituted with nuclease-free water (Ambion, Canada) and stored at -80°C. Template solutions were aliquoted into two concentrations of 50 ng/ml and 250 ng/ml. The total RNA in the samples was quantified using the ND-1000 nanodrop spectrophotometer located in the OSU Biochemistry department. Samples with an $A_{260/280}$ ratio below 1.7 were not used.

Purified samples of DNA have an $A_{260/280}$ ratio of 1.8. DNA samples with $A_{260/280} < 1.8$ are more than likely contaminated by proteins. DNA samples with $A_{260/280} > 1.8$ may be contaminated by RNA.

Reverse transcriptase-PCR and reverse transcriptase-relative quantitative PCR

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) involves a three step cycle: denaturation, annealing, and extension, which is repeated numerous times (Roche 2006). The denaturation step requires heat usually over 90°C, which separates double stranded DNA into two single strands. The annealing step takes place between 40-65°C and replicates a target sequence between 100 and 35,000 base pairs that is specific to the organism. Primers make up the ends of the target sequence. The extension step takes place around 72°C and is where synthesis of new double stranded DNA molecules identical to the original DNA are formed. The new synthesis extends from the primers creating a double stranded molecule from a single stranded template (Roche, 2006). A MJ Research Inc.® PTC-100 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) thermocycler was used to amplify scabies MIF. A modified Ambion AgPath-ID™ One-Step RT-PCR Kit (Ambion, Canada) protocol was used. MIF amplification conditions were 50°C for 4 min, 95°C for 15 min, 35 cycles of 94°C for 1 min (denature cycle), 58°C for 1 min (annealing cycle), 72°C for 1 min (extension cycle), followed by 72°C for 10 min then stored at 4°C. Three 25µl samples of scabies RNA and one negative control were used. Gel electrophoresis was performed at 50 V for 30 minutes using 14-µl product per well in a 0.1% ethidium bromide (EtBr) in 1.5% agarose gel to confirm presence of an amplicon.

Reverse transcriptase-relative quantitative-PCR (RT-qPCR) measures the accumulation of PCR products during amplification with fluorescent dyes. RT-PCR only provides a qualitative answer: whether or not the target sequence is present or absent. In real time (qPCR) data is collected from every cycle and can be displayed as an amplification curve or other graphical representations. This can be used to determine the amount of PCR product during the extension phase as well as the initial amount of template from each reaction (Roche 2006). Reverse transcriptase-relative quantitative-PCR (RT-qPCR) was completed using a modified Ambion AgPath- ID™ One-Step RT-PCR Kit (Ambion, Canada). FastStart Universal SYBR Green Master with Rox (Roche Diagnostics, Indiana, USA) replaced the AgPath 2x RT-PCR Buffer. Primers (as described in Table 1) were diluted to 10 µM concentrations. AgPath-ID protocols were used for appropriate reagent volumes for a 25 µl final volume. 10 ng of template was used for each reaction. Samples were mixed in 96-well plates and then placed into an Applied Biosystems 7500 Real Time PCR system. Amplification conditions were 50°C for 4 min, 95°C for 15 min, 40 cycles of 95°C for 15 sec and 55°C for 1 min. Ct (cycle threshold) values are the number of cycles required for the fluorescent signal to exceed background signals (cross the threshold). Ct values are inversely proportional to the amount of nucleic acid in the sample, or the lower the Ct value the higher the amount of nucleic acid in the sample (Wisconsin Veterinary Diagnostic Laboratory, 2009). Microsoft Excel was used for Ct values and MIF expression levels were normalized against human 18S ribosomal RNA control (Bowen et al, 2010). Three replicates were averaged and the averages were then used to calculate the Ct value for each sample (Bowen et al, 2010).

Primers

For reverse transcriptase polymerase chain reaction (RT-PCR) and reverse transcriptase –relative quantitative-PCR (RT-qPCR), MIF 1Q primers (Table 1) were generated from the scabies MIF sequence and yielded at 180 bp product (Jaworski et al, 2001). Primers were used successfully on *A. americanum* ticks in the lab and were used for this study. All primers were designed using the Integrated DNA Technologies website. Two primer sets were evaluated for use as internal controls for gene expression in the RT-PCR assays (Table 1). Human 18S ribosomal RNA primer set (Table 1) was used as an internal control to normalize gene expression for MIF quantification (Bowen et al, 2010). The product resulting from this primer set was sequenced to verify scabies 18S RNA. The product column in Table 1 shows whether a product was able to be sequenced using the primer listed. All primers with a "yes" in the product column were sequenced using that specific primer.

Table 1. Primers synthesized for RT-PCR assays.

Gene	Forward primer	Reverse primer	Product
16S rRNA	5'-GACAAGAAGACCCTA-3'	5'-ATCCAACATCGAGGT-3'	No
Human 18S rRNA	5'-TTCGAACGTCTGCCCTATCAA-3'	5'-GATGTGGTAGCCGTTTCTCAGG-3'	Yes
MIF	5'-AAGCCGCTTTCGTATGTTGTGG-3'	5'-TTCCTTGATGCCCAGGGTCTTT-3'	Yes
SsRACE	5'-GGCCATTGTGTATTTGGAGCCCTG-3'	5'-TTCCTTGATGCCCAGGGTCTTTGC-3'	No
D.v. MIF	5'-CTCCTTTGGAGAGAGGCAGCCAATGCTG-3'	5'-TGTTGTGGTGCACATCAGTCCTGGCCAAT-3'	Yes
Ixd MIF	5'-TGACGAGCCTGTGCCTCGCAAACCTGTA-3'	5'-TCCTTGATGCCCAGGGTCTTTCTCAATGTGCTC-3'	Yes
Dv MIF Exp1	5'-TGTGTGCTTCTTCTGTTGCGAGT-3'	5'-AATCCGAGATACGCAGACTTCTCTCC-3'	Yes
Dv MIF Exp2	5'-CATATGTGTGTGCTTCTTCTGTTGCGAGT-3'	5'-TTCGAAAATCCGAGATACGCAGACTTCTCTCC-3'	Yes

Sequencing

RNA bands were excised from 1.5% agarose/EtBr gels and purified through the Gene Clean II kit® (California, USA). Gel electrophoresis was conducted at 50 V for ~ 30 min using 17-µl product per well in a 0.1% EtBr in 1.5% agarose gel. Sequencing was carried out at the OSU Biochemical department Core Facility using the Applied Biosystems BigDye® terminator cycle sequencing kit version 1.1 and analyzed with an Applied Biosystems Model 3730 DNA Analyzer®. The resulting sequence was analyzed against possible other sequences using the Basic Local Alignment Search Tool nucleotide collection (BLASTn) on the National Center for Biotechnology Information (NCBI) website **(**Figure 6).

Cloning and Plasmid Transfer

Cloning of the scabies MIF DNA was performed by using the Promega pGEM-T® (Wisconsin, USA) protocol. A standard reaction from the scabies PCR was used along with a positive control. A ligation reaction was set up using 1 µl of pGEM-T®, 5 µl scabies PCR product, 5 µl 2X Rapid Ligation Buffer, and 1 µl T4 DNA Ligase. The reaction was incubated over-night at 4°C to allow the maximum number of transformations to occur. This step allows the scabies DNA to combine with the bacterial vector in a circular form (plasmid DNA). Two µl of the scabies ligation reaction were combined with 50-µl of the vector, a recombinant *E. coli* (JM109 High Efficiency competent cells). The reaction was then heat-shocked at exactly 42°C for 45-50 sec then put on ice for 2 min. The heat-shocking of the reaction opens the vector so that the scabies DNA can insert itself. Immediately cooling the reaction on ice closes the vector

so the scabies DNA does not pass through the vector completely. Briefly, the vector that contained a plasmid DNA clone of the scabies MIF sequence (~ 200 bp) (Jaworski et al, 2001) was plated onto 1.5% agar in Luria-Bertoli media with 0.2 µg/µl ampicillin (Figure 5). A single colony was transferred to 50 ml of Luria broth with 0.2 µg/µl ampicillin then incubated overnight. An Eppendorf Fast Plasmid Mini kit® was used to isolate the plasmid from the Luria broth. The scabies DNA was then sequenced and compared to tick sequences in the NCBI database to further verify cloning success (Figure 6). In addition, a restriction enzyme digest was performed using Apa I and Spe I restriction enzymes to excise a MIF insert and verify successful cloning.

Figure 5. pGEM-T® vector system (Promega, 2010).

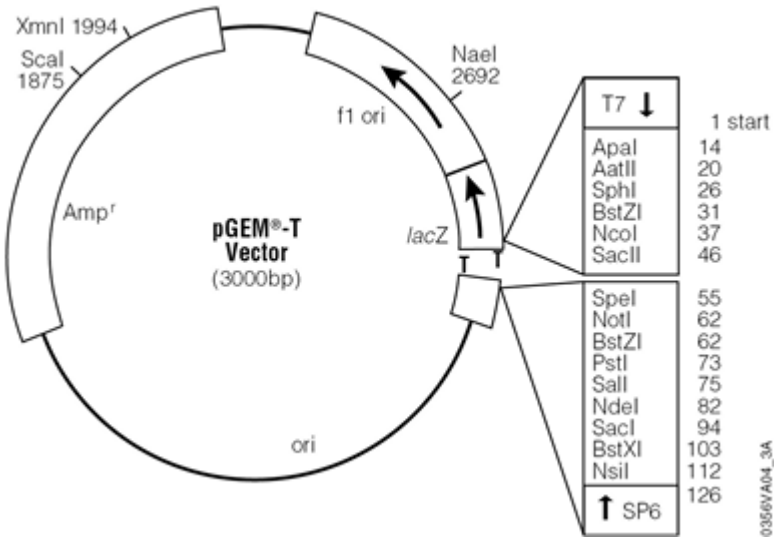
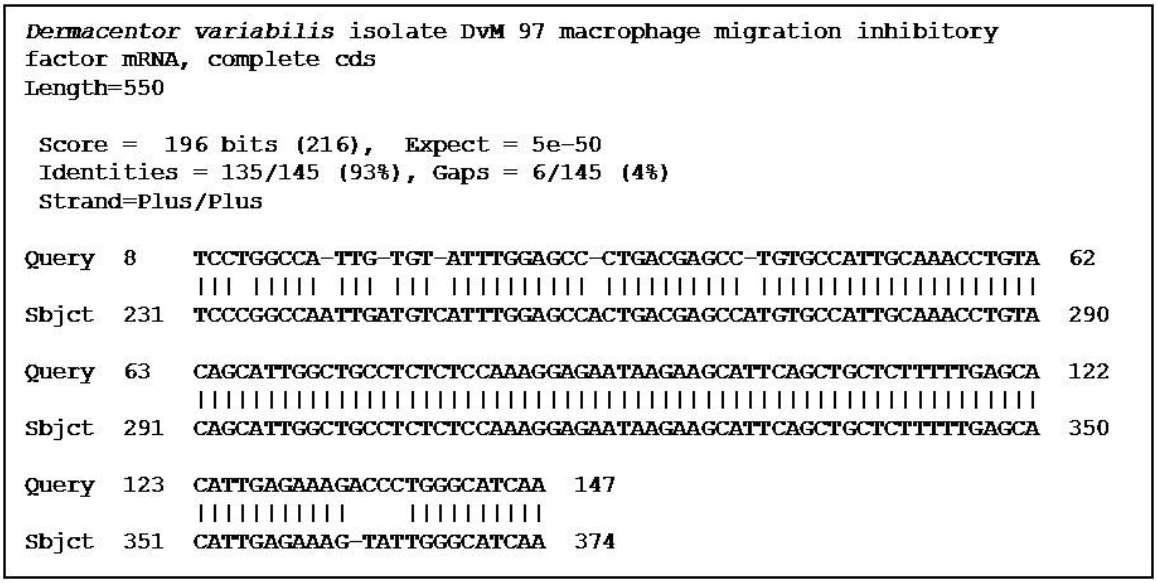


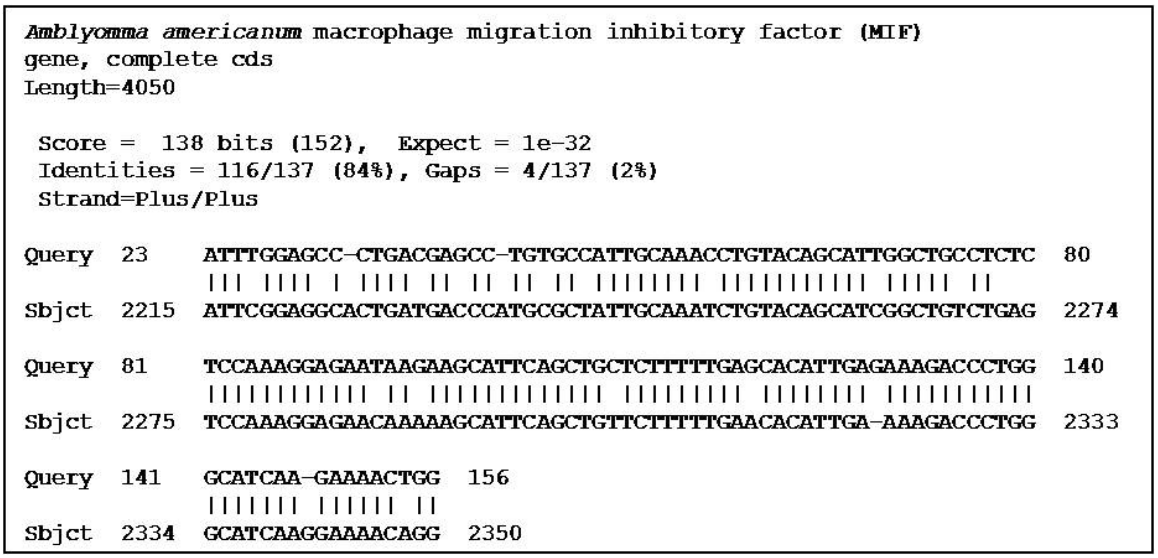
Figure 6. Initial scabies MIF cDNA compared to *Dermacentor variabilis* (top) and *Amblyomma americanum* (bottom)**.**



Dermacentor variabilis isolate DvM 97 macrophage migration inhibitory factor mRNA, complete cds
Length=550

Score = 196 bits (216), Expect = 5e-50
Identities = 135/145 (93%), Gaps = 6/145 (4%)
Strand=Plus/Plus

```
Query  8    TCCTGGCCA-TTG-TGT-ATTTGGAGCC-CTGACGAGCC-TGTGCCATTGCAAACCTGTA  62
            ||| ||||| ||| ||| |||||||||| |||||||||| ||||||||||||||||||||
Sbjct  231  TCCCGGCCAATTGATGTCATTTGGAGCCACTGACGAGCCATGTGCCATTGCAAACCTGTA  290

Query  63   CAGCATTGGCTGCCTCTCTCCAAAGGAGAATAAGAAGCATTCAGCTGCTCTTTTTGAGCA  122
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  291  CAGCATTGGCTGCCTCTCTCCAAAGGAGAATAAGAAGCATTCAGCTGCTCTTTTTGAGCA  350

Query  123  CATTGAGAAAGACCCTGGGCATCAA  147
            ||||||||||||    ||||||||||
Sbjct  351  CATTGAGAAAG-TATTGGGCATCAA  374
```



Amblyomma americanum macrophage migration inhibitory factor (MIF) gene, complete cds
Length=4050

Score = 138 bits (152), Expect = 1e-32
Identities = 116/137 (84%), Gaps = 4/137 (2%)
Strand=Plus/Plus

```
Query  23    ATTTGGAGCC-CTGACGAGCC-TGTGCCATTGCAAACCTGTACAGCATTGGCTGCCTCTC  80
             ||| |||| | |||| || || || || |||||||| |||||||||| ||||| ||
Sbjct  2215  ATTCGGAGGCACTGATGACCCATGCGCTATTGCAAATCTGTACAGCATCGGCTGTCTGAG  2274

Query  81    TCCAAAGGAGAATAAGAAGCATTCAGCTGCTCTTTTTGAGCACATTGAGAAAGACCCTGG  140
             |||||||||||| || |||||||||||| |||||||| |||||||| ||||||||||||
Sbjct  2275  TCCAAAGGAGAACAAAAAGCATTCAGCTGTTCTTTTTGAACACATTGA-AAAGACCCTGG  2333

Query  141   GCATCAA-GAAAACTGG  156
             ||||||| |||||| ||
Sbjct  2334  GCATCAAGGAAAACAGG  2350
```

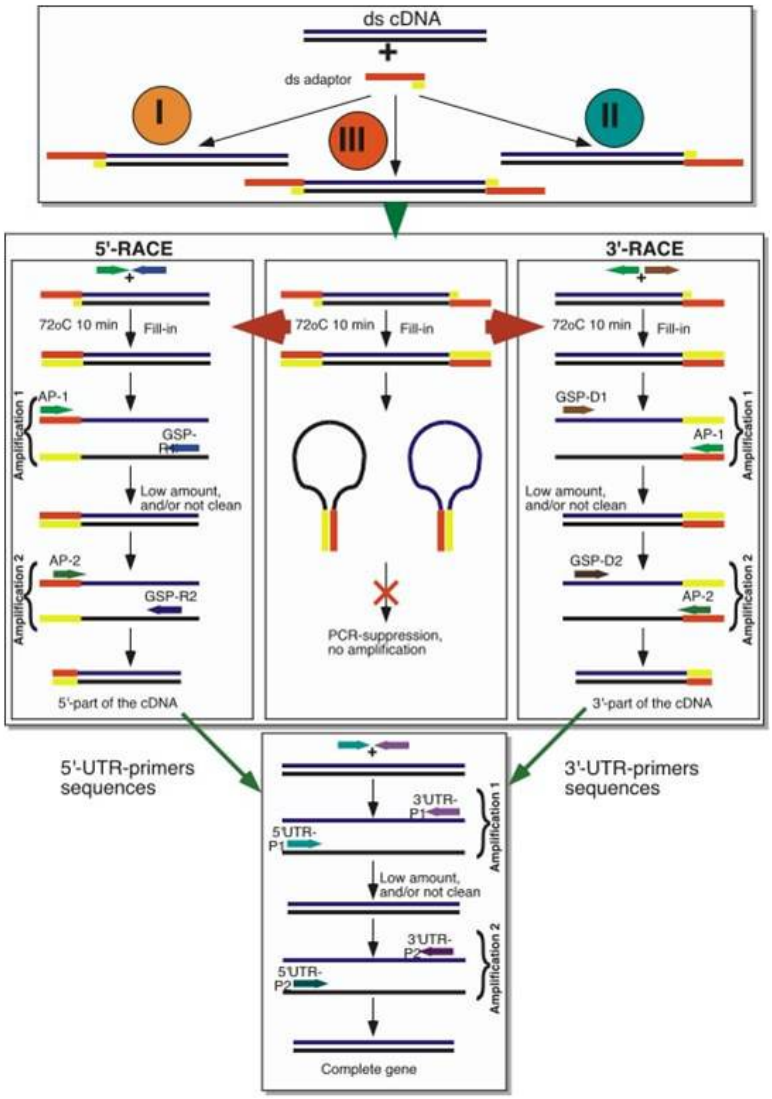
Rapid Amplification of cDNA Ends (RACE)

During first strand cDNA synthesis 50 ng of scabies RNA was used. First strand cDNA synthesis, or 5' RACE, uses the scabies mRNA as a template, along with a gene specific primer (GSP) that recognizes a part of the scabies MIF sequence. Following first strand synthesis a group of identical nucleotides is added to the 3' end of the cDNA. Control Human Placental Poly A+ RNA was used as a positive control. These control reactions amplify the ends of the cDNA. This ensures that the RACE protocol works with the thermal cycler, or if problems arise, it pinpoints whether it is with the thermal cycler or the cDNA. For the second strand cDNA synthesis 10 µl of the first strand reaction was used. Adaptor ligation was performed on the dsDNA from the second strand synthesis using the Marathon cDNA Amplification kit® (California, USA) protocol. The adaptor ligation reaction was incubated at 16°C over-night. After the incubation, the reaction was heated at 70°C for 5 min to inactivate the ligase. Undiluted scabies cDNA was stored at -20°C for future use. Two templates of scabies adaptor ligated cDNA were diluted to concentrations of 1/25 and 1/50 adaptor-ligated ds-cDNA in Tricine-EDTA Buffer® for use in RACE reactions. The diluted ds-cDNA was heated at 94°C for 2 min to denature the ds-cDNA. A cDNA library of adaptor ligated scabies DNA was completed.

RACE produces a cDNA copy of the RNA sequence, using reverse transcription followed by PCR amplification to make copies of the cDNA. RACE allows for the synthesis of unknown sequences at the end of the 5' mRNA transcript. The first strand cDNA is synthesized from total RNA using a gene specific primer (GSP, Table 1). After the first strand cDNA is purified, a poly A+ tail is added to the 3' end of the sequence.

The 5’ and 3’ reactions copy the ends of the unknown sequence using the known middle section of the sequence, and the copies are attached to either the 5’ or 3’ ends (Sambrook et al 2001). Gene specific primers used were SsRvse3’RACE (GSP2) and SsFwd5’RACE (GSP1, Table 1). These were designed using the Integrated DNA Technologies website and the scabies DNA sequence. Concentrations of 1/25 and 1/50 scabies cDNA in Tricine-EDTA Buffer® were both used during RACE reactions.

Figure 7. Rapid Amplification of cDNA Ends (RACE, Weizmann, 2008).



Differences between the 16S, 18S, and SsRACE primer melting temperatures (T_m) required different annealing temperatures for each primer set. SsRACE amplification conditions were 94°C for 30 sec, 35 cycles of 94°C for 5 sec, 68°C for 2 min, then stored at 4°C. 16S amplification conditions were 94°C for 30 sec, 35 cycles of 94°C for 5 sec, 57°C for 2 min, then stored at 4°C. 18S amplification conditions were 94°C for 30 sec, 35 cycles of 94°C for 5 sec, 60°C for 2 min, then stored at 4°C. Amplification conditions were 94°C for 30 sec, 35 cycles of 94°C for 5 sec, 65°C for 2 min, then stored at 4°C. Gel electrophoresis was conducted at 50 V for ~ 35 minutes using 17-µl product per well in a 0.1% EtBr in 1.5% agarose gel.

Analysis of the 5' and 3' cDNA fragments were run on a 0.1% EtBr in 1.5% agarose gel at 50 V for 40 minutes to verify product. Bands of the 5' and 3' fragments were then excised and purified using the Gene Clean II kit®. Sequencing was carried out at the OSU Biochemical department Core Facility using the Applied Biosystems BigDye® terminator cycle sequencing kit version 1.1 and analyzed with an Applied Biosystems Model 3730 DNA Analyzer®. DvMIF Exp1 and DvMIF Exp2 primers (Table 1) were used to amplify scabies MIF. Amplification conditions were 94°C for 30 sec, 35 cycles of 94°C for 5 sec, then 55°C for 2 min, then stored at 4°C. Gel electrophoresis was conducted at 100 V for ~ 30 minutes using 15-µl product per well in a 0.1% ethidium bromide in 1.5% agarose gel.

16S and 18S Reactions

The 16S and 18S ribosomal PCR reactions were used on the scabies cDNA as controls. The same procedures were used as the 5' and 3' cDNA templates. The primers

used were 16S and 18S human ribosomal cDNA primers designed from the NCBI website. Gel electrophoresis was conducted at 50 V for 40 minutes using 17-µl product per well in a 0.1% ethidium bromide in 1.5% agarose gel. The samples were purified using the Gene Clean II kit®. The 18S ribosomal cDNA was sequenced at the OSU Core Facility.

Cataloging of *Sarcoptes scabiei* expressed genes

A catalogue of *Sarcoptes scabiei* expressed genes (Appendix 1) and expressed sequence tags (ESTs, Appendix 2) were compiled using the NCBI website. The tables were compiled to assess what is known about *Sarcoptes scabiei* at the level of gene expression and to provide context for our scabies MIF research.

CHAPTER IV

RESULTS

To identify a MIF gene homolog for *Sarcoptes scabiei* a MIF gene product of 192 bp was found using RT-PCR. Using the Basic Local Alignment Search Tool nucleotide collection (BLASTn) the scabies sequence identified 93% with *Dermacentor variabilis*, 83% with *Amblyomma americanum,* and 96% with *Ixodes scapularis* MIF. These data established that there was a putative MIF gene in *Sarcoptes scabiei.*

Figure 8. *Sarcoptes scabiei* MIF cDNA sequence**.**

5’GAGTCGCTCCTGGCCATTGTGTATTTGGAGCCCTGACGAGCCTGTGCC

ATTGCAAACCTGTACAGCATTGGCTGCCTCTCTCCAAAGGAGAATAAGA

AGCATTCACTGCTCTTTTTTGAGCACATTGAGAAAGACCCTGGGCATCAA

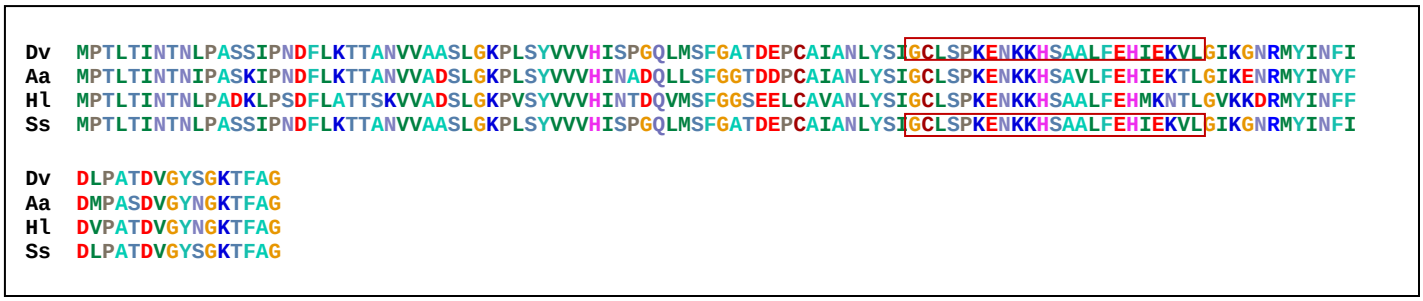
GAAAACTGGGGAACGGCTGGCAAAGACCCTGGGCATCAAGGAAAC3’

Using several RT-PCR and RACE strategies, we were able to clone and sequence the complete open reading frame for *S. scabiei* MIF. In addition, using RNA from *Sarcoptes scabiei* and adaptor primers, a cDNA library was produced. Sequence data obtained from different primer sets and methods is shown in Figure 9. Our nucleotide comparisons showed that scabies MIF bore a high percentage identity with tick MIFs (Figure 9).

Figure 9. Alignment of *Dermacentor variabilis*, *Amblyomma americanum*, *Haemaphysalis longicornis*, and *Sarcoptes scabiei* nucleotide sequences.

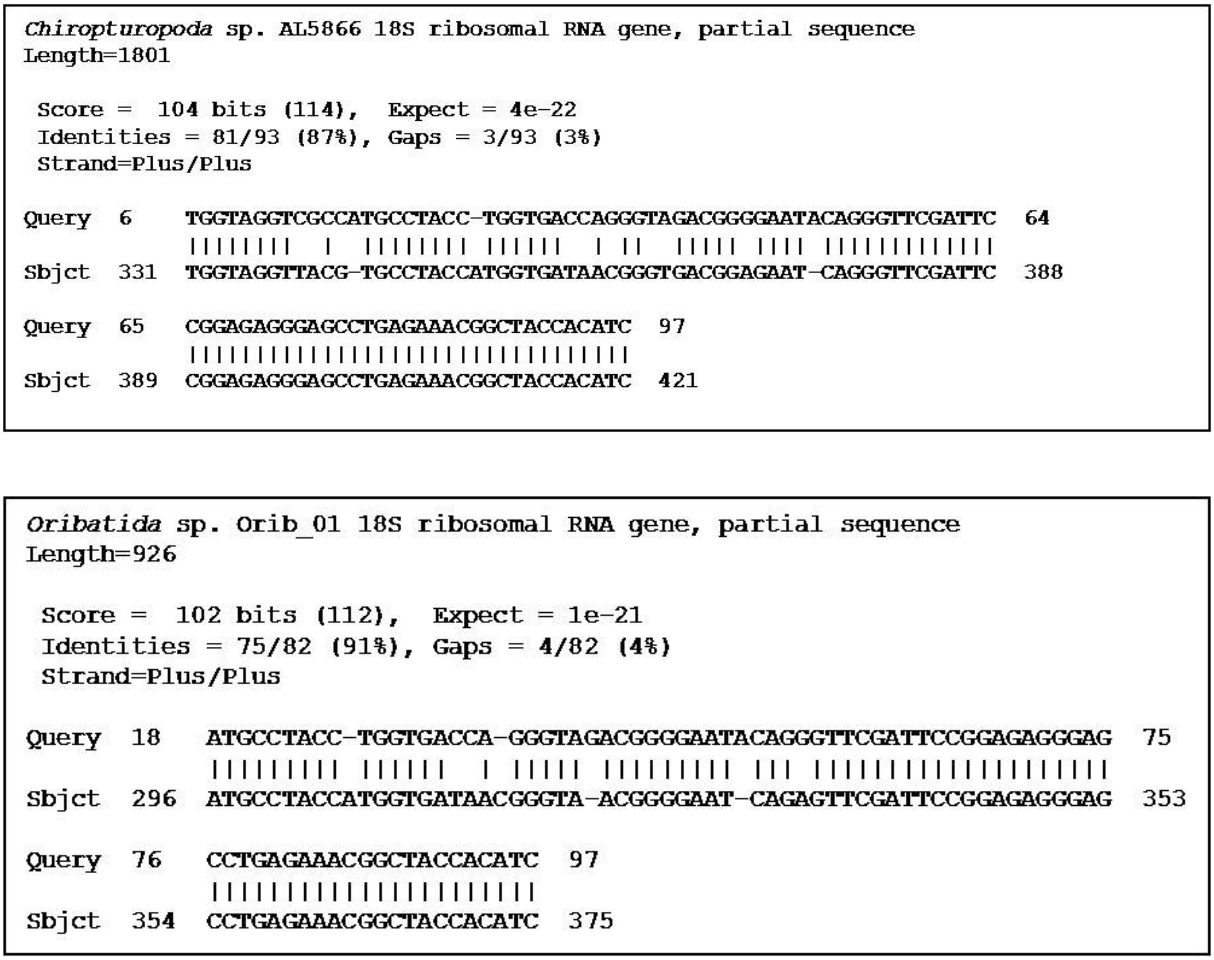
At the nucleotide level, *Sarcoptes scabiei* MIF showed 98% identity with *D. variabilis.* The putative amino acid sequence traversed the complete open reading frame (ORF) of 405 nucleotides. An alignment of the amino acid sequences showed a high identity between the three tick species and the scabies mite MIFs (Figure 10). In fact, the putative amino acid sequences for *S. scabiei* and *D. variabilis* were identical. The putative amino acid sequence from the scabies mite MIF (Figure 10) shows a peptide that is specific only to ticks and now, mites.

Figure 10. Amino acid sequence alignment between *Dermacentor variabilis, Amblyomma americanum, Haemaphysalis longicornis,* and *Sarcoptes scabiei*.



In addition to identifying the scabies MIF gene, we confirmed a novel sequence for the scabies 18S ribosomal RNA gene DNA (Figure 11). This is the first time an 18S ribosomal RNA gene has been identified from a scabies mite.

Figure 11. *Sarcoptes scabiei* 18S cDNA sequence comparison to *Chiropturopoda* (top) and *Oribatida* (bottom) spp.



Chiropturopoda sp. AL5866 18S ribosomal RNA gene, partial sequence
Length=1801

Score = 104 bits (114), Expect = 4e-22
Identities = 81/93 (87%), Gaps = 3/93 (3%)
Strand=Plus/Plus

```
Query  6    TGGTAGGTCGCCATGCCTACC-TGGTGACCAGGGTAGACGGGGAATACAGGGTTCGATTC  64
            ||||||||  |  |||||||| ||||||  | ||  ||||| |||| ||||||||||||
Sbjct  331  TGGTAGGTTACG-TGCCTACCATGGTGATAACGGGTGACGGAGAAT-CAGGGTTCGATTC  388

Query  65   CGGAGAGGGAGCCTGAGAAACGGCTACCACATC  97
            |||||||||||||||||||||||||||||||||
Sbjct  389  CGGAGAGGGAGCCTGAGAAACGGCTACCACATC  421
```


Oribatida sp. Orib_01 18S ribosomal RNA gene, partial sequence
Length=926

Score = 102 bits (112), Expect = 1e-21
Identities = 75/82 (91%), Gaps = 4/82 (4%)
Strand=Plus/Plus

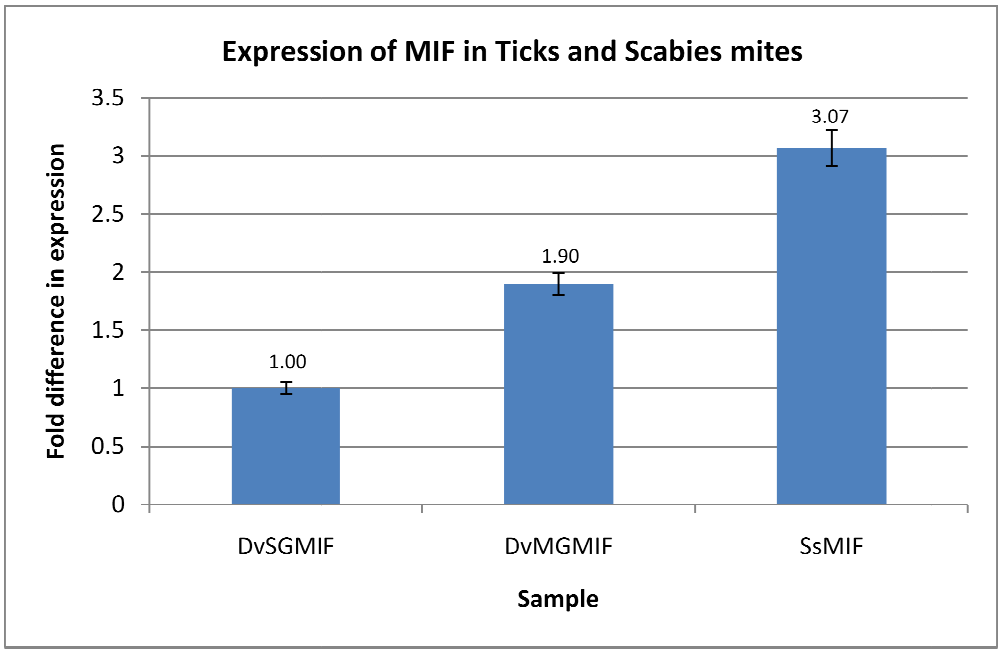
```
Query  18   ATGCCTACC-TGGTGACCA-GGGTAGACGGGGAATACAGGGTTCGATTCCGGAGAGGGAG  75
            ||||||||| ||||||  | ||||| ||||||||| ||| ||||||||||||||||||||
Sbjct  296  ATGCCTACCATGGTGATAACGGGTA-ACGGGGAAT-CAGAGTTCGATTCCGGAGAGGGAG  353

Query  76   CCTGAGAAACGGCTACCACATC  97
            ||||||||||||||||||||||
Sbjct  354  CCTGAGAAACGGCTACCACATC  375
```

Reverse transcriptase –relative quantitative-PCR (RT-qPCR)

To gain some information about the relative expression of scabies MIF compared to tick MIF, a real time (qPCR) assay was done. Scabies MIF expression was over three times that of *D. variabilis* salivary gland MIF (Figure 12). In Figure 12, *D. variabilis* salivary gland is set at 1.0 as the reference sample to show comparisons between it and *D. variabilis* midgut MIF samples and the samples from *S. scabiei*. The *D. variabilis*

midgut samples are 1.3 times that of *D. variabilis* salivary gland MIF, and the scabies sample is over three times the reference sample.

Figure 12. MIF real time PCR assay with *Sarcoptes scabiei* and *Dermacentor variabilis*.



Cataloging of *Sarcoptes scabiei* expressed genes

The cataloging of *Sarcoptes scabiei* expressed genes and expressed sequence tags (ESTs) was performed using the NCBI database to create a table that shows a compilation of *Sarcoptes scabiei* genes and ESTs. This catalog was constructed to assess what is known and show a larger grasp of *Sarcoptes scabiei* at the gene expression level. The table was compiled from 324 partial or full genes and over 1000 ESTs. Table 2 shows a synopsis of the compiled catalog. Overall many of these genes relate directly to allergy and acaricide resistance. After compiling these data, it shows that there is still much to learn about scabies at molecular level.

Table 2. Synopsis of sequence data for existing genes and expressed sequence tags (ESTs) in *Sarcoptes scabiei.*

Gene	Type	Frequency	Proposed function
glutathione S-transferase	**Allergen**	**16**	**Homologue of dust mites**
Major allergen 1	**Allergen**	**1**	**Homologue of dust mites**
Cytochrome oxidase subunit I	**Acaricide resistance**	**2**	**Characterization**
Sar s 1 allergen	**Allergen**	**1**	**Inactivated cysteine proteases**
Allergen 1 mRNA	**Allergen**	**2**	**Homologue of dust mites**
Vitellogenin-like protein	**Allergen**	**1**	**Homologue of dust mites**
Voltage-sensitive sodium channel	**Acaricide resistance**	**1**	**Knockdown resistance to acaricides**
E5 mRNA	**Allergen**	**1**	**Characterization of immunoreactive antigens**
pH gated chloride channel	**Acaricide resistance**	**1**	**Characterization**
Antigen 1 (ASA1)	**Allergen**	**1**	**Antigen containing MADF domain**
Paramyosin mRNA	**Allergen**	**1**	**Homologue of dust mites**
Actin mRNA	**Allergen**	**1**	**Elevated transcription of a Glutathione S-transferase**
Expressed sequence tags	**Unknown**	**73**	**Unknown**

CHAPTER V

DISCUSSION AND CONCLUSION

Using molecular methods, we identified a MIF gene from the scabies mite. Surprisingly, while the nucleotide sequence differed slightly, the putative amino acid sequence was exactly the same as the putative amino acid sequence from *D. variabilis* ticks. We ruled out the possibility of contamination of human MIF or MIFs from symbiotic organisms. The scabies amino acid sequence contained a specific peptide that previously had only been found in tick MIFs (Jaworski et al, 2001; Wasala et al, unpublished data). In a phylogenetic analysis of ticks, nematodes, and vertebrate MIFs, the tick MIFs were closely related and clustered with nematode MIFs, while the human and other vertebrate MIFs were distinctly separated (Jaworski et al, 2001; Wasala et al, unpublished). Previously the genomic data for tick MIF was characterized from *Amblyomma americanum* ticks (Jaworski et al, 2001). The genomic structure for the tick MIF gene was different from that of other organisms. The organization of the tick gene revealed three exons separated by wide intronic regions. Pastrana et al, 1998 compared the gene structure between *Brugia malayi* (a parasitic nematode), human, mouse, and *Caenorhabditis elegans* (a non parasitic nematode) MIFs. All of these organisms, including ticks had a similar length first exon. Subsequent spacing between the exons

and introns differed greatly between ticks and the other organisms. Also, the *B. malayi* and *C. elegans* MIFs consisted of a different number of exons than three. We suggest that scabies MIF is likely to have the same gene structure as *A. americanum.* While the genomic clones for other species of tick MIFs have not been explored, the *Ixodes scapularis* genome contains the same exons (exon for exon) as *A. americanum* (Jaworski et al, unpublished).

In addition to the discovery of scabies MIF, we report the novel finding of a partial sequence for the scabies 18S ribosomal RNA gene. The partial sequence for scabies 18S ribosomal RNA identified 91% with *Oribatida* spp mites and 87% with *Chiropturopoda* spp and not the tick 18S ribosomal RNA gene. These results together with our MIF results suggest that MIF arose by convergent evolution and positive selection due to parasitism.

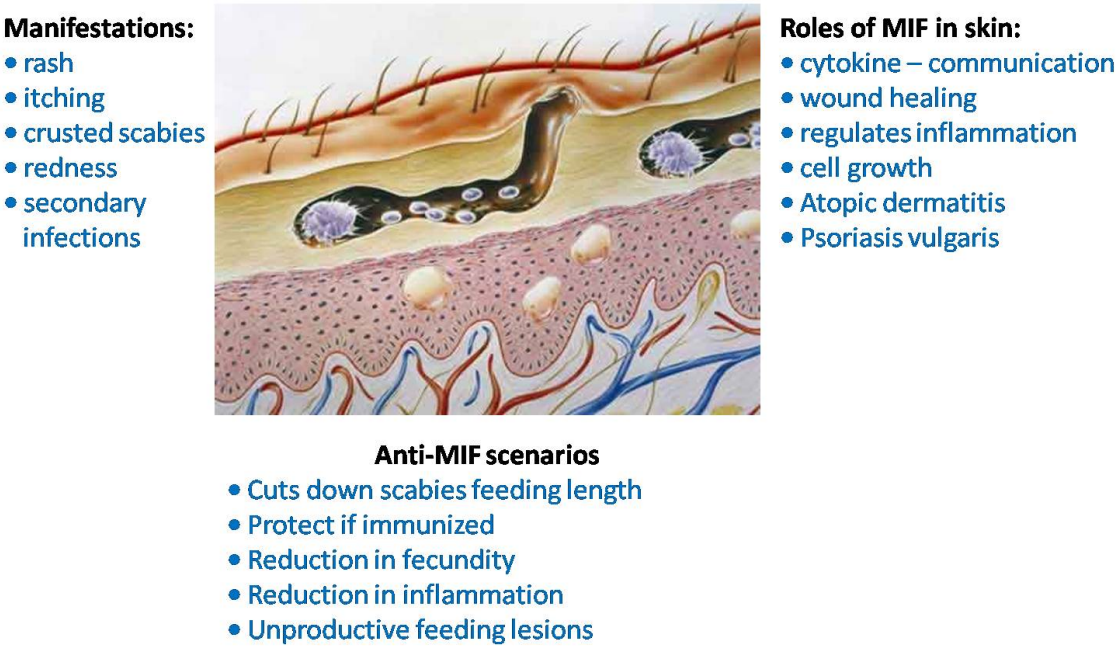
As a prelude to future studies on the relative expression of scabies mites MIF, we utilized our established MIF qPCR assay to compare scabies MIF to tick MIF. From these studies, we found that scabies MIF was expressed at a higher level than that of *D. variabilis* ticks. Previously, Bowen et al (2010) established that MIF expression in *A. americanum* was abundant in the midgut cells at five days of tick feeding, and that MIF protein pools are present in the midgut before attachment to a host. The ticks used in our experiment were unfed, while the scabies mites were feeding before being removed from the host. This intake of host nutrients could lead to a higher expression level of MIF in the scabies mite versus the MIF of unfed ticks. These expression studies also indicate that MIF is not a house-keeping gene in scabies.

A catalog of known scabies mite expressed genes and expressed sequence tags (ESTs) were compiled. This compilation was to assess what is known about *Sarcoptes scabiei* at the level of gene expression and to give context to our research. The expressed genes fell into two categories: allergens and acaricide resistance. Acaricide resistance continues to be an important issue for scabies mite infestation control. As acaricide resistance develops, the need for alternative control strategies becomes a priority in minimizing the mite's impact. This catalog aids in the efforts of characterizing the molecular biology of the scabies mite.

The research model we are using is summarized in Figure 13. We know the symptoms of scabies on a host and the role MIF plays in the skin. Thus, scabies MIF may be a suitable target for vaccine development to prevent the impact of scabies infestations. Jaworski et al (2009) showed that by using a peptide immunization on *A. americanum*, the ticks did not attach as readily to the immunized hosts and the feeding period was lengthened due to an unproductive feeding lesion (Jaworski et al, 2009). Some scenarios for using anti-mite MIF or peptide would be to decrease the feeding interval, protection from mite infestations if immunized, a reduction in mite fecundity, a reduction in inflammation to the host, and unproductive feeding lesions.

The full role MIF plays in *Sarcoptes scabiei* is crucial to begin to understand important interactions for scabies mite infestations. This research has provided the molecular basis for future projects to develop anti-mite MIF antibodies and use those to reduce infestations or immunize against future scabies infestations.

Figure 13. *Sarcoptes scabiei* research model for further experiments in the role of anti-mite MIF antibodies to limit scabies mite infestations (modified from Lindane, 2009).



LITERATURE CITED

Abe, R., Shimizu, T., Ohkawara, A., Nishihira, J. 2000. Enhancement of macrophage migration inhibitory factor (MIF) expression in injured epidermis and cultured fibroblasts. *Biochimica et Biophysica Acta*. **1500**: 1-9.

Arlian, L.G., Runyan, R.A., Achar, S., Estes, S.A. 1984. Survival and infestivity of *Sarcoptes scabiei var. canis* and *var. homnis*. *Journal of the American Academy of Dermatology*. **11(2)**: 210-215.

Arlian, L.G., Estes, S.A., Vyszenski-Moher, D.L. 1988. Prevalence of *Sarcoptes scabiei* in the homes and nursing homes of scabietic patients. *Journal of the American Academy of Dermatology*. **19(5)**: 806-811.

Bowen, C.J., Jaworski, D.C., Wasala, N.B., Coons, L.B. 2010. Macrophage migration inhibitory factor expression and protein localization in *Amblyomma americanum* (Ixodidae). *Experimental and Applied Acarology*. **50**: 343-352.

Bucala, Richard, Morand, E. F., Leech, Michelle. 2003. Macrophage migration inhibitory factor: An emerging therapeutic target in Rheumatoid arthritis. *Arthritis and Rheumatism*. **48(2)**: 291-299.

Centers for Disease Control. 2008. Centers for Disease Control and Prevention. (http://www.cdc.gov).

Cho, Y., Jones, B.F., Vermeire, J.J., Leng, L., DiFedele, L., Harrison, L.M., Xiong, H., Kwong, Y.A., Chen, Y., Bucala, R., Lolis, E., Cappello, M. 2007. Structural and functional characterization of a secreted hookworm Macrophage Migration Inhibitory Factor (MIF) that interacts with the human MIF receptor CD74. *Journal of Biological Chemistry*. **282(32):** 23447-23456.

Chosidow, O. 2006. Clinical practices. Scabies. *New England Journal of Medicine*. **354(16)**: 1718-1727.

Dewor, M., Stephens, G., Krohn, R., Weber, C., Baron, J., Berhnhagen, J. 2007. Macrophage migration inhibitory factor (MIF) promotes fibroblast migration in scratch-wounded monolayers in vitro. *Federation of European Biochemical Societies*. **581(24)**: 4734-4742.

Estes, S. A., Kummel, B., Arlian, L. 1983. Experimental canine scabies in humans. *Journal of the American Academy of Dermatology*. **9**: 397-401.

Fischer K., Holt D. C., Harumal P., Currie B. J., Walton S. F., and Kemp D. J. 2003. Generation and Characterization of cDNA clones from *Sarcoptes scabiei* var. hominis for an expressed sequence tag library; Identification of homologues of house dust mite allergens. *American Journal of Tropical Medicine and Hygiene*. **68**: 61-64.

Gomez, L.M., Sanchez, E., Ruiz-Narvaez, A., Lopez-Nevot, M.A., Anaya, J.M., Martin, J. 2007. Macrophage migration inhibitory factor gene influences the risk of developing tuberculosis in northwestern Columbian population. *Tissue Antigens.* **70:** 28-33.

Jaworski, D.C., Jasinskas, A., Metz, C.N., Buccala, R., Barbour, A.G. 2001. Identification and characterization of a homologue of the pro-inflammatory cytokine Macrophage Migration Inhibitory Factor in the tick, *Amblyomma americanum*. *Insect Molecular Biology*. **10**(4), 323-331.

Jaworski, D.C., Bowen, C.J., Wasala, N.B. 2009. *Amblyomma americanum* (L): Tick macrophage migration inhibitory factor peptide immunization lengthens lone star tick feeding intervals *in vivo*. *Experimental Parasitology*. **121**: 384-387.

Jimenez-Lucho, V.E., Fallon, F., Caputo, C., Ramsay, K. 1995. Role of prolonged surveillance in the eradication of nosocomial scabies in an extended care Veterans Affairs medical center. *American Journal of Infection Control*. **23(1)**: 44-49.

Kamir, D., Zierow, S., Leng, L., Cho, Y., Diaz, Y., Griffith, J., McDonald, C., Merk, M., Mitchell, R.A., Trent, J., Chen, Y., Kwong, Y.A., Xiong, H., Vermeire, J., Cappello, M., McMahon-Pratt, D., Walker, J., Bernhagen, J., Lolis, E., Bucala, R. 2008. A *Leishmania* ortholog of Macrophage Migration Inhibitory Factor modulates host macrophage responses. *Journal of Immunology*. **180:** 8250-8261.

Katsumata, K., Katsumata, K. 2006. Simple Method of Detecting *Sarcoptes Scabiei Var Hominis* Mites among bedridden elderly patients suffering from severe scabies infestation using an adhesive-tape. *Internal Medicine*. **45(14)**: 857-859.

Kitaichi, N., Shimizu, T., Honda, A., Abe, R., Ohgami, K., Shiratori, K., Shimizu, H., Ohno, S. 2006. Increase in macrophage migration inhibitory factor levels in lacrimal fluid of patients with severe atopic dermatitis. *Graefe's Archive of Clinical Experimental Ophthalmology*. **244**: 825-828.

Kuhn, C., Lucius, R., Matthes, H.F., Meusel, G., Reich, B., Kalinna, B.H. 2008. Characterisation of recombinant immunoreactive antigens of the scab mite *Sarcoptes scabiei*. *Veterinary Parasitology*. **153**: 329-337.

Lindane. 2009. (http://www.lindane.com/scabiesandlice/perspective/).

Meinking, T.L., Taplin, D., Hermida, J.L., Pardo, R., Kerdel, F.A. 1995. The treatment of scabies with ivermectin. *The New England Journal of Medicine.* **333(1)**: 26-31.

Neynaber, S., Wolff, H. 2008. Diagnosis of scabies with dermoscopy. *Canadian Medical Association Journal*. **178(12)**, 1540-1541.

Pasay, C., Walton, S., Fischer, K., Holt, D., McCarthy, J. 2006. PCR-based assay to survey for knockdown resistance to pyrethroid acaricides in human scabies mites (*Sarcoptes scabiei var hominis).* *American Journal of Tropical Medicine and Hygiene*. **74(4)**: 649-657.

Pastrana, Diana, V., Raghavan, Nithy, A., Fitzgerald, Peter, Eisinger, Stephen, W., Metz, Christine, Bucala, Richard, Schleimer, Robert, P., Bickel, Carol, Scott, Alan, L. 1998. Filarial nematode parasites secrete a homologue of the human cytokine Macrophage migration inhibitory factor. *Infection and Immunity*. **66(12)**: 5955-5963.

Promega. 2010. Promega pGEM-T® Vector System. (http://www.promega.com/).

Roche. 2006. *PCR Applications Manual 3rd edition.*

Routh, H.B., Mirensky, Y.M., Parish, L.C., Witkowski, J.A. 1994. Ectoparasites as sexually transmitted diseases. *Seminars in Dermatology.* **13(4)**: 243-247.

Sambrook, J. & Russell, D.W. 2001. Rapid amplification of 5' cDNA ends. *Molecular Cloning: A Laboratory Manual*. **8(9)**: 8.54−8.60.

Shimizu, T., Abe, R., Nishihira, J., Shibaki, A., Watanabe, H., Nakayama, T., Taniguchi, M., Ishibashi, T., Shimizu, H. 2003. Impaired contact hypersensitivity in macrophage migration inhibitory factor-deficient mice. *European Journal of Immunology*. **33**: 1478-1487.

Shimizu, T. 2005. Role of macrophage migration inhibitory factor (MIF) in the skin. *Journal of Dermatological Science*. **37**: 65-73.

Steinhoff, M., Meinhardt, A., Steinhoff, A., Gemsa, D., Bucala, R., Bacher, M. 1999. Evidence for a role of macrophage migration inhibitory factor in psoriatic skin disease. *British Journal of Dermatology*. **141**: 1061-1066.

Walton, S.F., Currie, B.J. 2007. Problems in diagnosing scabies, a global disease in human and animal populations. *Clinical Microbiology Reviews*. **20(2)**: 268-279.

Weizmann Institute of Science. 2008. (http://bioinfo2.weizmann.ac.il/~sasha/thesis/Materials_and_methods.html).

Wisconsin Veterinary Diagnostic Laboratory. 2009. (http://www.wvdl.wisc.edu/).

Yoshizumi, J., Harada, T. 2008. "Wake-sign": an important clue for the diagnosis of scabies. *Clinical and Experimental Dermatology*. **30(10)**: 1365-2230.

Zhao, Y., Shimizu, T., Nishihira, J., Koyama, Y., Kushibiki, T., Honda, A., Watanabe, H., Abe, R., Tabata, Y., Shimizu, H. 2005. Tissue regeneration using macrophage migration inhibitory factor-impregnated gelatin microbeads in cutaneous wounds. *American Journal of Pathology*. **167(6)**: 1519-1531.

APPPENDICES

Appendix 1: Catalogue of the partial or full length existing genes for *Sarcoptes scabiei*.

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Glutathione S-transferase delta class 3	*Sarcoptes scabiei v. suis*	654 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	tgggttctat tcgaccgata atctattgga tggcggaaag tccaccatgt cgaactcttt atgcggttac caaattgctt ggcatcgatt gcgaatggaa agttcttgat ctgtcgcaaa aagaacacat gaagccggat ttcctaacta ttaatccatt tcattgtgtc ccgacgatgg tggaaagcga tggattcaaa ctttgggagt ccagagtgat ttgcaagtat ttgattgaaa gtcgaaatat agaaacggca ttgtatccga aagacttgaa aaaacgagcg atcattgatc gctgtcttca tttcgatctt ggaacactgt atcgtgcgtt agccgatgtt gtgtacgatg ctttctatgt tggcaaaccg aatcttgcga aattacctcg tcttgaagaa gttctacaga tgatggaaga taatcttgct aagactaatt ctaattatct agctcaaacc gatgagccta ctctggcaga tatctcaact tatttctctt tgtcgattct tgagatcgtg agcgagtttg atttggcaaa atactttaaa ttattttctt ggaaacaacg aatgaatgaa ttcattaaat cgatcgatga tggaacattc gcgaccggac aagctaacat cattggcatt cgca
Glutathione S-transferase delta class 3	*Sarcoptes scabiei v. canis*	633 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	tcgaccgata atctattgga tggcggaaag tccaccatgt cgaactcttt atgcggtaac caaattgctt ggcatcgatt gcgaatggaa agttcttgat ctgtcgcaaa aagaacacat gaagtcggat ttcctaacta ttaatccatt tcattgtgtc ccgacgatgg tggaaagcga tggattcaaa ctttgggagt ctagagtgat ttgcaagtat ttgattgaaa gtcgaaatat agaaacggca ttgtatccga aagacttgaa aaaacgagcg atcattgatc gttgtcttca tttcgatctt ggaacactgt atcgtgcgtt agccgatgtt gtgtacgatg ctttctatgt tggcaaacca aatctagcga aattacctcg tcttgaagaa gttctacaga tgatggaaga taatcttgct aagactaatt ctaattacct agctcaaagc gatgagccta ctctggcaga tatctcaact tatttctctt tgtcgattct tgagatcgtg agcgagtttg atttggcaaa atactttaaa ttattttctt ggaaacaacg aatgaatgaa ttcatcaaat cgatcgatga tggaacattc gcgaccggac aagctaacat cat

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Glutathione S-transferase delta class 2	*Sarcoptes scabiei v. suis*	603 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	agtcgtagtg tatatttggt ggccaagatt ctcggcatcg attgtgaatg gaaagttttc aatctattca aaaaagaaca gatgaatccg gaatttctag ctattaatcc attgcattgt gtcccgacgc tggtggaaag cgatggattc actctttggg aatccagagt gatttgtagc tatctgatcg agagccgtga tcccgattcg gctttgtatc cgaaagatct aaagaaacga gcgatcatcg atcgttgtct tcatttcgat ctgggaacat tgtatcgtgc attggccgat gttgtgtacg atattttatt cttcggcaaa cctaatctga caaaattacc tcgtcttgaa gaggttctac aattgatgga agatggtttc gctaaaatcg attccgatta tctggctggc ggtgatggac caacattagc cgatatcgta tcgtatttca ccttacaaat gctcgatatt ttgcaagagc ttgatcttac gaaatattca aaactgtacg cttggagaga gcgaatggaa gaattcgtta aatcgaacga tgatggttcg cttgctaaag ggcttcagaa tttcgttgga ttc
Glutathione S-transferase delta class 2	*Sarcoptes scabiei v. canis*	430 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	gtagtgtata tttggtggcc aagattctcg gcatcgattg tgaatggaaa gttttcaatc tattcaaaaa agaacagatg aatccggaat ttctagctat taatccattg cattgtgtcc cgacgctggt ggaaagcgat ggattcactc tctgggaatc cagagtgatt tgtagctatc tgatcgagag ccgtgatccc gattctgctt tgtatccgaa agatctaaag aaacgagcga tcatcgatcg ttgtcttcat ttcgatctgg gaacattgta tcgtgcattg gccgatgttg tgtacgatat tttattcttc ggcaaaccta atctgacaaa attacctcgt cttgaagagg ttctacaatt gatggaagat ggtttcgcta aaatcgattc cgattatctg gctggcggtg atggaccaac
Glutathione S-transferase delta class 1	*Sarcoptes scabiei v. suis*	510 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	gtcgtacggt tatggctgtg gcccgaatga ttggtctcga tatggagatg aaaaaattga atcttcgtaa caaagaacat ctcacaccag aattcttgaa aattaaccca atgcataagg taccgacctt ggtggaacca gatggattcg ctttgggaga atcgagagcc atctctactt atatcattca aaaatacaag cctagttcgc ctctatatcc agtcgatgat cttcgtagaa gagctcatat tgatggatgg cttcaatatg attgttccac tcttggacca gctttacgag ctgtcataat ggatcgaatg tatggaggtg gtttgaacga gaatcgtctc aatcaaacca aagagacctt gaaaacattg aatgaagttc taaaagcatt ggaaggacga tatcttcttg atgatcaaat aacggtggca gatatttcga tgtatttcag ttgcaatatg attgaagttt taccggatct agaaatgtcc gattatgaat
Glutathione S-transferase delta class 1	*Sarcoptes scabiei v. canis*	555 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	gtcgtacggt tatggctgtg gcccgaatga ttggtctcga tatggagatg aaaaaattga atcttcgtaa caaagaacat ctcacaccag aattcttgaa aattaaccca atgcataagg taccgacctt ggtggaacca gatggattcg ctttgggaga atcgagagcc atctctactt atatcattca aaaatacaag cctagttcgc ctctatatcc agtcgatgat cttcgtagaa gagctcatat tgatggatgg cttcaatatg attgttccac tcttggacca gctttacgag ctgtcataat ggatcgaatg tatggaggtg gtttgaacga gaatcgtctc aatcaaacca aagagacctt gaaaacattg aatgaagttc taaaagcatt ggaaggacga tatcttcttg atgatcaaat aacggtggca gatatttcga tgtatttcag ttgcaatatg attgaagttt taccggatct agaaatgtcc gattatgaac atctttgtaa atggtataag aacatgaccg aagcgatgaa tgctg

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Glutathione S-transferase mu class 3	*Sarcoptes scabiei v. suis*	724 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	tcgaactact gatagcaatt cgaacgatag caaactaccg atattggcat attggaatat tcgaggaaac gctcaaccga taaggttgtt gttgcgttac accaaaacac cttacaaaga gaagagctac aatttcggta aatacgagca agataaagca atctggcgtg cagataaacc gcatctaggt ttagattttc ccaatcttcc ctactacatc gatggtgatc tacgattgac acaaagtctt acgattttgc gttatctggc aaaaaaacat catctagctg gaatcaatga aacggaacga atcagaatcg atctgatgga acagcagcta agagattttc gtaaccagtt catcgacgcc acaaacgatg ccaatttcga gaaggctcga gtgatctatt tggctcgatt gccagagaaa ctacaatcgc tatcgaattt cttaaaagat cggccatttt ttgctggcaa ctctataagc tatgttgatt ttatggcgta tgaattcatc gatcaacact attatctgaa tccagatctc tttgggcaaa atcaacaatg gagaaatttg attgattttc tacatcgaat cgaatcgttt ccaaccataa aagaatatca atattctgag gattatattc gtcatcctag tggcctattg atcgcctggt acgaggcgaa atttttctcc acattcaatc gatcgttagg tgat
Glutathione S-transferase mu class 3	*Sarcoptes scabiei v. canis*	729 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	actcgaacta ctgatagcaa ttcgaacgat agcaaactac cgatattggc atattggaat attcgaggaa acgctcaacc gataaggttg ttgttgcgtt acaccaaaac accttacaaa gagaagagct acaatttcgg taaatacgag caagataaag caatctggcg tgcagataaa ccgcatctag gtttagattt tcccaatctt ccctactaca tcgatggtga tctacgattg acacaaagtc ttacgatttt gcgttatctg gcaaaaaaac atcatctagc tggaatcaat gaaacggaac gaatcagaat cgatctgatg gaacagcagc taagagattt tcgtaaccag ttcatcgacg ccacaaacga tgccaatttc gagaaggctc gagtgatcta tttggctcga ttgccagaga aactacaatc gctatcgaat ttcttaaaag atcggccatt ttttgctggc aactctataa gctatgttga ttttatggcg tatgaattca tcgatcaaca ctattatctg aatccagatc tctttgggca aaatcaacaa tggagaaatt tgattgattt tctacatcga atcgaatcgt ttccaaccat aaaagaatat caatattctg aggattatat tcgtcatcct agtggcctat tgatcgcctg gtacgaggcg aaatttttct ccacattcaa tcgatcgtta ggtgatcaa
Glutathione S-transferase mu class 2	*Sarcoptes scabiei v. suis*	494 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	atgctttcat atgctggtgt ggatttcgtt gacaaacgct acaattatgg tccagctcca gatttcgatc gaagcgaatg gttgaacgag aaattcaatc ttggtctcga ttttccaaac ttaccttatt acatcgatgg cgatgtaaaa ttgactcaat cattggctat tcttcgttat ctagctagaa agcacaaatt ggatggccat aacgaacaag aatggttacg aattgcgctc tgcgagcaac aaatcgtcga tctttatatg gcgatgggtc gaatttctta tgatccgaat ttcgaaaaac taaagccaga ttatcttgag aagcttcccg ataatttgaa attgttttcg gaatttctcg gtgaccatcc attcgtcgct ggaactaatc taagctatgt ggatttttc gtctacgaat atcttattcg attgaaagcg atgacaccgg aagtttttgc aaaatttcaa aacctaggca acta

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Glutathione S-transferase mu class 1	*Sarcoptes scabiei v. suis*	603 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	cgtggattag gtcaatcaat ccgaatttta ttgacatatg ctggcgtgga tttcgtagac aaacgttata aaattggttc agctccagat ttcgatcgag gagaatggtt gaacgataaa ttcaatcttg ggctcgattt tccaaactta ccctattata ttgagggtga tgtgaaatta acccaatcga tagctattct ccggtatttg ggcagaaaac acaagctaga tggtcaaaat gaacaagaat ggcgacggat tacgctttgt gaacagcaga tcatggattt attgatggca ttggcccgaa tctgttacga tccaaatttt gaaaaactga aactcgattt ggttgctaag cttcctgatg atcttaaatt gttttctaaa tttcttggcg atcatcaatt tgtagctgga acaaatataa gctatatcga ttttctggtt tatgaatatc ttatccgtgt caaaattttt gcaccagaaa ttttcaccaa atttccaaac ctaaatagct acattactcg cattgaatcg atgccgaaaa tctctgccta catcaaacaa caagagcctc aattattcaa cggtccaatg gcg
Glutathione S-transferase mu class 1	*Sarcoptes scabiei v. canis*	605 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	ttcgtggatt aggtcaatca atccgaattt tattgacata tgctggcgtg gatttcgtag acaaacgtta taaaattggt tcagctccag atttcgatcg aggagaatgg ttgaacgata aattcaatct tgggctcgat tttccaaact taccctatta tattgagggt gatgtgaaat taacccaatc gatagctatt ctccggtatt tgggcagaaa acacaagcta gatggtcaaa atgaacaaga atggcgacgg attacgcttt gtgaacagca gatcatggat ttattgatgg cattggcccg aatctgttac gatccaaatt ttgaaaaact gaaactcgat ttggttgcta agcttcctga tgatcttaaa ttgttttcta aatttcttgg cgatcatcaa tttgtagctg gaacaaatat aagctatatc gatttttgg tttacgaata tcttatccgt gtcaaaattt ttgcaccaga aattttcacc aaatttccaa acctaaatag ctacattact cgcattgaat cgatgccgaa aatctctgcc tacatcaaac aacaagagcc tcaattattc aacggtccaa tggcg
Sarcoptes scabiei delta glutathione S-transferase	*Sarcoptes scabiei*	684 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	atggcttcag agaaaccaac aatctattgg atgccagaaa gtgcaccatg tcgtagtgta tatttggtgg ccaagattct cggcatcgat tgtgaatgga aagttttcaa tctattcaaa aaagaacaga tgaatccgga atttctagct attaatccat tgcattgtgt cccgacgctg gtggaaagcg atggattcac tctctgggaa tccagagtga tttgtagcta tctgatcgag agccgtgatc ccgattctgc tttgtatccg aaagatctaa agaaacgagc gatcatcgat cgttgtcttc atttcgatct gggaacattg tatcgtgcat tggccgatgt tgtgtacgat attttattct tcggcaaacc taatctgaca aaattacctc gtcttgaaga ggttctacaa ttgatggaag atggtttcgc taaaatcgat tccgattatc tggctggcgg tgatggacca acattagccg atatcgtatc gtatttcacc ttacaaatgc tcgatatttt gcaagagctt gatcttacga aatattcaaa actgtacgct tggagagagc gaatggaaga attcgttaaa tcgaacgatg atggttcgct tgctaaaggg cttcagaatt tcgttggatt cgctcaacaa atgcaacaac aacatagctc ttaa

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Sarcoptes scabiei major allergen 1	*Sarcoptes scabiei v. hominis*	991 bp mRNA linear	Identification of homologues of house dust mite allergens	ttttgatttt ttggacatcc agaagtttac attcttatta ttcctggatt tgggataatt tctcatatta ttacttattc aagtaataaa agagaaccat ttggatcttt aggtataatt tatgctataa tttctattgc aactttaggt tttattgtat gagctcatca tatatttact gttggattag atgttgatac tcgagcttat tttacttcag ctactataat tatcgctgtt cctacgggag taaaaatttt tagttgatta tctacaatat taggaggaaa attagatttt aacccttcta tgtattgagc aattggcttt gtgtttctat ttagaatggg aggtcttacg ggtattattt tatctaactc ttctttagat gttagattac acgatactta ctatgttgta gctcactttc attatgtttt atctataggt gctgtttttg ctcttatagg aggtttttct ttttgatata taatgtttac aggttatttt ttaaaacctt ctataataaa aagacaattt tgaacaatat ttataggagt taatataact ttttccctc aacatttttt aggtttaaga ggtatacctc gacggtattc tgattatcct gataatttct caacttgaaa tactatttca tctttaggaa ctataattac aatattctca atattatttt ttatgtatat tttatgagat tcattatcaa aatataaaat tatttca
Glutathione S-transferase delta class 2	*Sarcoptes scabiei v. suis*	654 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	tgggttctat tcgaccgata atctattgga tggcggaaag tccaccatgt cgaactcttt atgcggttac caaattgctt ggcatcgatt gcgaatggaa agttcttgat ctgtcgcaaa aagaacacat gaagccggat ttcctaacta ttaatccatt tcattgtgtc ccgacgatgg tggaaagcga tggattcaaa ctttgggagt ccagagtgat ttgcaagtat ttgattgaaa gtcgaaatat agaaacggca ttgtatccga aagacttgaa aaaacgagcg atcattgatc gctgtcttca tttcgatctt ggaacactgt atcgtgcgtt agccgatgtt gtgtacgatg ctttctatgt tggcaaaccg aatcttgcga aattacctcg tcttgaagaa gttctacaga tgatggaaga taatcttgct aagactaatt ctaattatct agctcaaacc gatgagccta ctctggcaga tatctcaact tatttctctt tgtcgattct tgagatcgtg agcgagtttg atttggcaaa atactttaaa ttattttctt ggaaacaacg aatgaatgaa ttcattaaat cgatcgatga tggaacattc gcgaccggac aagctaacat cattggcatt cgca
Glutathione S-transferase delta class 2	*Sarcoptes scabiei v. canis*	633 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	tcgaccgata atctattgga tggcggaaag tccaccatgt cgaactcttt atgcggtaac caaattgctt ggcatcgatt gcgaatggaa agttcttgat ctgtcgcaaa aagaacacat gaagtcggat ttcctaacta ttaatccatt tcattgtgtc ccgacgatgg tggaaagcga tggattcaaa ctttgggagt ctagagtgat ttgcaagtat ttgattgaaa gtcgaaatat agaaacggca ttgtatccga aagacttgaa aaaacgagcg atcattgatc gttgtcttca tttcgatctt ggaacactgt atcgtgcgtt agccgatgtt gtgtacgatg ctttctatgt tggcaaacca aatctagcga aattacctcg tcttgaagaa gttctacaga tgatggaaga taatcttgct aagactaatt ctaattacct agctcaaagc gatgagccta ctctggcaga tatctcaact tatttctctt tgtcgattct tgagatcgtg agcgagtttg atttggcaaa atactttaaa ttattttctt ggaaacaacg aatgaatgaa ttcatcaaat cgatcgatga tggaacattc gcgaccggac aagctaacat cat

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Isolate 14 cytochrome oxidase subunit I gene	*Sarcoptes scabiei v. hominis*	747 bp DNA linear	Genetic epidemiology of *Sarcoptes scabiei*	ttttgatttt ttggacaccc agaagtttat attcttatta ttcctggatt tgggataatt tctcatatta ttacttattc aagtaataaa agagaaccat ttggatcttt aggtataatt tatgctatga tttctattgc aactttaggt tttattgtat gagctcatca tatatttact gttggattag atgttgatac tcgagcttat tttacttcag ctactataat tatcgctgtc cctacgggag taaaaatttt tagttgatta tctacaatat taggaggaaa attagatttt aacctctcta tgtattgagc aattggcttt gtgtttctat ttagaatggg aggtcttacg ggtattattt tatctaactc ttctttagat gttagattac acgatactta ctatgttgta gcccactttc attatgtttt atctataggt gctgtttttg ctcttatagg gggtttttct ttttgatata taatgtttac aggttatttt ttaaaacctt ctataataaa aagacaattt tgaacaatat ttataggagt taatataact ttttccctc aacatttttt aggtttaagaggtatacctc gacggtattc tgattatcct gataatttct caacttgaaa tactatttca tctctaggaa ctataattac aatattctca atattatttt ttatgtatat tttatgagat tcattatcaa aatataaaat tatttca
Sar s 1 allergen	*Sarcoptes scabiei v. hominis*	1203 bp mRNA linear	Inactivated cysteine proteases in *Sarcoptes scabiei*	gtctgttctc atagaataag agattgttga taagaatcta gaagaagcca aataatcgaa aatgaattgg cttggaaaaa aaagctcaac gattatcgtt gcaatgttga tgataatcca ttttgatgta tcatttacgc aagaattgaa tgaatctcct ccgacagcaa cagaatctac tctgacaaca acagaatcgc ctccgacaac tacagaatct tctacgacta caacagaatc tccaacggaa aatactgatt tattccgaga acaacctttc tcatataatg aagaggatta ttacgaagtt ttatacaatg attatagaaa gtgtctgaaa gattttgcac aatttcggca tggcagacac tttcgattct gtaatccacc accacgtcca aaacttccga aagaattcga tttaagaaaa ttgaaagtaa taccaccggt tcgtaatcaa aaaagatgta acgcatcctg ggctttcggt ccacttggag ctgttgaatc ggcactcatc catagatttc atctgccaca tcgacatttt caactttcta ctcaagaatt ggttgattgc gctggtaatc aaggttgcag aggaggcgta gatgtcaccc aagctttctc gtatttgatg gagaaaggcg tcgtcactga atttgaatat ccttacacag caaagaaagg aatatgccac gcgagaagat atcggaaata ttatcatgtt aaaattaaag attattgtgc catttgtccg catacggtgc ccatattgaa atcgtttatt tatcactata agagaccatt gacaacgatc cttcacattc gaaatctcaa agcttataat agaatcggaa tttatgctgt tgaagatgat tttggtaaaa aagttcaaca tcaacaagtg gtcaacatcg ttggttgggg ttatcatcat agaggcaata ttagctattg gatcgtaaaa aatagtatgg gtctccattg gggccataaa ggttacgctt tcgtcgacat cgatagtgat gcattcgaga tcagaaagaa taacttctat gtcagattgg gtagaccaga attctaaaca aattcatttc tagactgaaa ctgtttgact gaggacttaa tcttggctac ggcatcattg tttttacaac aattcggata aggatttaca atttattcga atcaaaacaa ttc

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Isolate 208 cytochrome oxidase subunit I gene	*Sarcoptes scabiei v. hominis*	747 bp DNA linear	Genetic epidemiology of *Sarcoptes scabie*	ttttgatttt ttggacaccc agaagtttac attcttatta ttcctggatt tgggataatt tctcatatta ttacttattc aagtaataaa agagaaccat ttggatcttt aggtataatt tatgctatga tttctattgc aactttaggt tttattgtat gagctcatca tatatttact gttggattag atgttgatac tcgagcttat tttacttcag ctactataat tatcgctgtc cctacaggag taaaaatttt tagttgatta tctacaatat taggaggaaa attagatttt aacccctcta tgtattgagc aattggcttt gtgtttctat ttagaatggg aggtcttacg ggtattattt tatctaactc ttctttagat gttagattac acgatactta ctatgttgta gctcactttc attatgtttt atctataggt gctgtttttg ctcttatagg gggtttttct ttttgatata taatgtttac aggttatttt ttaaaacctt ctataataaa aagacaattt tgaacaatat ttataggagt taatataact ttttccctc aacatttttt aggtttaaga ggtatacctc gacggtattc tgattatcct gataatttct caacttgaaa tactatttca tctttaggaa ctataattac aatattctca atattatttt ttatgtatat tttatgagat tcattatcaa aatataaaat tatttca
Group 3 allergen precursor RNA	*Sarcoptes scabiei v. hominis*	1040 bp RNA linear	Novel immunevasion strategy in the scabies mite	cgttcataat gtcatcacaa cgatttcgat caattgctct cgtagtctct gttttaatat tgatttatca ttcaccatca ttcgcgatcc atggtggaac aaagattgac atcaaagatg caccatggac cgttgcaata ttcacaataa ctacattttg cggtgggagt attctttcca aagattacgt tctgactgca gctagttgtg tcgaagggtg agcagcgata gaaaaaatcc atgaaaaaat tggaattgat taattcaacc taaattaaag acaagcagtg agtgaaatct tgatccaata tgaatcaagt aatctctata cgggacgaac gaaaattgta tgggctgaga tggtttatat tttcgatcga tatcggaatg ataccttgca aaataatatc gccttaatca aaaccaacac atcaatgaca ttggaccaag agaaaagtaa agctattgat ttgccgaaag tcgaatatga gcctgagaaa gatagtaatg tctcagtttc tggctatgga gatgtagatg cgaagcctat taatgagaaa ttaacagaca cttcaaaata tgatttgaag agagctgatt tcactgtgca agataggtct gaatgtgctc aaaaatatac agataaatac actgattatg agacattctg tgctaaagga tgtggcgctt atatcgaaca aggtgatata ggcgatcctg ctgtgcaaaa aaatgaatct tcaatcgaag tcttagctgg tttcgtttct tatgccaaaa tacaaaaccc tttgacaata tttaccaagg taggatcata tgtcgaatgg atattggaaa tcatgaaaaa aaattcaaaa tcctaaaatt gctcaaaatc agtacactaa aatcatctca taaatgaaaa gacgaaactt aaatcatttt aattgcatct tctaatttaa aatttatttt tttaagaaaa accgaattct atcccaatgg ccttataatt gttcctgact atttttcttt ttcaatattc tattaatttt

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Glutathione S-transferase delta class 1	*Sarcoptes scabiei v. canis*	430 bp mRNA linear	Elevated transcription of glutathione S-transferases in pyrethroid resistant scabies mites	gtagtgtata tttggtggcc aagattctcg gcatcgattg tgaatggaaa gttttcaatc tattcaaaaa agaacagatg aatccggaat ttctagctat taatccattg cattgtgtcc cgacgctggt ggaaagcgat ggattcactc tctgggaatc cagagtgatt tgtagctatc tgatcgagag ccgtgatccc gattctgctt tgtatccgaa agatctaaag aaacgagcga tcatcgatcg ttgtcttcat ttcgatctgg gaacattgta tcgtgcattg gccgatgttg tgtacgatat tttattcttc ggcaaaccta atctgacaaa attacctcgt cttgaagagg ttctacaatt gatggaagat ggtttcgcta aaatcgattc cgattatctg gctggcggtg atggaccaac
Sar s 3 allergen	*Sarcoptes scabiei v. hominis*	934 bp mRNA linear	Novel immune evasion strategy in the scabies mite	ctcctaattt gcaacaatga aaactgtttt tttgatttta ttcttattca atctgctctg tttcggatgt ttatcagcgc acattggcga ctttcagaaa ttgattgtcg gcggtcgttt agctaagcca aatgaatttc cttatcaagt acagcttaga aaaaacgata cacactggtg tggtggatcg atcctaaatg acagatggat tcttacagct gcacattgta ctttcggtat tcttccagaa ttactcacaa tctattatgg atcgagtaat agaaaatgcg gcggtagatc agttaaagtg aaagatattt tcaaccatgg aatgtatcat tcaagaatat atttgttcga tatctctttg atcaaaaccg aaaaaccatt gattcttgat caaaacgcat ctgcgataac tttatctgcc gaacctgatg ttcgacctgg cttgaaagtg acagtctccg gatggggtct gctgagagag gatgccgatt tttttcccga agatcttaga gtagcagaca tacctgtggt aactagagat gagtgtaaag ttgcctacca tgatgagcca gaatataaga tcaccgggca aatgttttgt gccggcgatc tcgttagagg taatcttgat tcgtgcagag gcgattcagg tggcccagct gtcttgaatg gcgtacaagt aggaatcgtt tcttggggta acaaatgtgg cgaccgtaaa catcctggcg tttacacact tgtttcgttc tttctccaat ggatcaaaaa tatccttaga aataattaat tatcaatgta cctatatagt gcaatgattt cgagaaaata tttgttctaa gtaatcaaca tctgaatcga tatcgattga taatgattta attccttcct taatccttga ttgagaaaca aaaaatgaac ataa
Voltage-sensitive sodium channel (vssc)	*Sarcoptes scabiei v. suis*	648 bp mRNA linear	Knockdown resistance to pyrethroid acaricides	ttgtgcatcg ttagcaacac cctattcatg gccatggatc atcataatat ggacaaagat ttcgagaaca ttctacagaa aggaaactac ttttttactg cgatttttgc gatcgaagct atgatgaaat tgatggctct tagtccaaaa ttttattttc gtgaaggatg gaatatattc gatttcgtaa ttgttgtact ttcactactc gatgttagtc tttcatcggt ctctggactt tctgttctac gatcgtttcg tttgctaagg gttttcaaac ttgctaaatc ttggccaaca ttgaatctgc tcatatcgat catgggaaaa actattggcg atcttggaaa tctgaccttt gttttggtga taatcatttt catattcgct gtgatgggaa tgcaattgtt cggaaaaaac tacactgaag aaagtttcgg tggtaaagag attccgagat ggaatttcaa agattttatg cattcattta tgattgtgtt tcgtgtgctt tgcggcgaat ggatcgaatc gatgtgggat tgtatgcgtg tttcaggcgc agcttgcgta ccattcttct tagctactgt tgtgattgga aatctggttg ttttgaatct gttcttggct ttgttgctct cttcgttc

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Allergen 1 mRNA	*Sarcoptes scabiei v. hominis*	991 bp mRNA linear	Identification of homologues of house dust mite allergens	atttcgtcga acttaaaaaa gataaagatt tatattcgat gaaatcgaat gtgaaacgaa acaatgagat tttctatgag aacaatatgg atttggagaa gaacggtaaa atgaattggt attacaaacg aaacgatcga acatggaata tggatctcga taatgcattc aatccaagag atggtacaat gaaacttcaa gtgaaagatc gtatctatga tatcaaattg aaacgagaac cgttccgata cggtgatcta catatcgaag gaaatgagaa tgctttgatc aaaaagggtg atttacatat gtctctcgtc gatccgctta ctttgaatgt tttgaccaag aatgatggaa tcgtcgatat gacattggat ttggtctctc ccaacaccaa aaaagcagcg ctaaaaatca attcgaaaaa atacgatctt gatcatgatg gtgagattac cgtttcgatc tttaatcctc gaatgacttg gaaacatcac actagaaaag gtgatatgga attgaatatt gatgctgata tcactcgaaa aggttcattg atcacctatt cccgtaaaga gccagatgat tcgacaaaag ttcgatattc aagacaagga aatcaagttt cgatggaagt cgattctaaa ttgatcgaag gccatgcgaa cggaactttg accgatggca aaattcatgt caaaggtcga gagagtgatt tcgaaatcga aagcacctat aaagttgaag atggtaagct tatgattgag ccaaccaaaa ctcagaatgg aaaattagaa ggtcttcttt cgagaaaagt accatcacat cttgttcttg aaacaccaag agtgaaaatg aacatgaaat atgatagatt tgctccggtg aagatattga aattagatta cgatggtttg aattatgaga aacatatcga tgctgaatac gagccatcaa atcattacaa atactttacc gatggtaaat c
Vitellogenin-like protein	*Sarcoptes scabiei v. hominis*	591 bp mRNA linear	Identification of homologues of house dust mite allergens	aagggaacca aagtgtgtcc atcgaccact cgagaaatg gaaaatattc agtgccaatc tatgaaccat tctcaaggct aatggataaa tggagcgctg agactagaac caacaatcta agacagatcg ctcgacaagc cgcccaagag aagctgctc gtcaacagca gatgaatttg gaacgaatta tggctaggca acacaaaatt gttcagcaac aagaacaaga gcaagagcag cagaagcaag agcaaaaaat ggagcattat cgattgagaa cgatggctgt tcaacagact gacaaaattt gcttttcagt tcaaccagtg atgtcgtgta ttgaaggaat ttcgcgacct actcgagttc agcaacaaac tttaggattc cattgtctgc catctcaatc gatttccgcg aagaaattgg cggagaaatc tcaatatcaa gtgctggaaa tatttggcaa gaaacaagtt gatttcatgg cgccgttcca agtgccagtt tcgtgccaag cctaatttat tctgtaaacg aatcttctat aatcatgatt gattgaaaaa tttttataat aaattttaat g

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Glutathione S-transferase	*Sarcoptes scabiei v. hominis*	705 bp mRNA linear	Identification of homologues of house dust mite allergens	caagactcaa cgatcgagaa tctaaaatcg taaaacgtaa aaatgtcttc gaaaccaact ctaggctatt gggatcttcg tggattaggt caatcaatcc gaattttatt gacatatgct ggcgtggatt tcgtagacaa acgttataaa attggttcag ctccagattt cgatcgagga gaatggttga acgataaatt caatcttggg ctcgattttc caaacttacc ctattatatt gagggtgatg tgaaattaac ccaatcgata gctattctcc ggtatttggg cagaaaacac aagctagatg gtcaaaatga acaagaatgg cgacggatta cgctttgtga acagcagatc atggatttat tgatggcatt ggcccgaatc tgttacgatc caaattttga aaaactgaaa ctcgatttgg ttgctaagct tcctgatgat cttaaattgt tttctaaatt tcttggcgat catcaatttg tagctggaac aaatataagc tatatcgatt ttctggttta tgaatatctt atccgtgtca aaatttttgc accagaaatt ttcaccaaat ttccaaacct aaatagctac attactcgca ttgaatcgat gccgaaaatc tctgcctaca tcaaacaaca agagcctcaa ttattcaacg gtccaatggc gaaatggaat acaaaatatt aataa
E5 mRNA	*Sarcoptes scabiei v. suis*	644 bp mRNA linear	Characterisation of recombinant immunoreactive antigens	caactaactc ttgttagtat aatgattcgt ttctcagtgc tatttttgtt ggtcttttgt gctacgatct atttgatcga tgctaaaggt gtttcgataa atcctggggg aaaacctgga ggaaaacatg taggtaaatc tcaccgtggg acacctttg gcaaacgatt aggaggtgga cacgagccta aacctcatac gaagcctaaa tcaaaaccca aacccaaacc tcctaaacca catcctaaga agcatcatgg aaaaccatct aaaccttctc caaagaagcc aggacacaga cccggacata agcctgcaca taaacctcgt aaaccagttc ataagaagca tcataaaaaa tcccctaaac ctaagcgtaa gaagccagga cacagacccg gacataagcc tgcacataaa cctcgaaaac cagttcataa gaagcatcat aaaaaatccc ctaaacctaa gcgtaagaag ccagggcaca aaccacgtaa accgaatcct aagaagcatc atggaaaacc atctaaacct tctcatagaa agtctggaca ccgtaaacct ggtcacaaaa agccaggacg taaacatgga aaaaataaga aggaatcgga aaaaaaaaaa aaaaaaaaaa aaaa

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
pH gated chloride channel	*Sarcoptes scabiei v. hominis*	1470 bp mRNA linear	Molecular characterisation of a pH-gated chloride channel	atgtttttga agcaaaaatt atatcaaatt ttattgatta agatcgtaat aatcgcattt tatattcaaa tttcaagttc caacaatgtc atcattgatg agacattcat aaagacattt aataaaactg atagattgat tcggccatca ttcaatgaca aagcggatgt aatcgatgtt agcatgttga tcgatcgttt cgcttactat catgacatcg aatcgatttt agagattcag gctcaattcg aatatcattg gttcgatcag agagtgaaat ttgattgtga ccgatcatca agaatcgaag gcaatcacta ccatgaacag atttgggtac cagatttacg tgtctcacgt accgaagata tcgatgtttt tgaatcggag aatctaacca gattgatttc gatccaaatc gattgtgatg gacatgttcg aatgagattt cgatcgaatt tggatttgat ttgtgtgatg aactatcaaa attatccgtt tgatgagcaa acttgtgaga ttgaattgat tcctagctat atggaaataa atagattaca attgagatgg aaagatcaga acattatgat aagagatgat ttctatatgt cgggccattt gctgaaaggc tattcggtcc atcagaaaga tgtggaattg atgccgtaca acgaaatcta tagcgcctta tttgttcatc ttcatcttaa gcggcaattc atttatcata tcctagtttt atttcttccc tcgatcttca tagtactaac atcatggatc tctttttgga tagaaatcac ttgtatacca gcaagagtaa cactttgtgt gacaacattg ctagcaatgg tgactgtttc gaaggaatcc aaacaaaata ttccaaaagt gccatatgtc aaagctgttg atctttggtt cgctggttgt attgtatcaa ttttcatcac actaatcgaa tacatctttg tttgctatgt ttatcgagaa gaaagaaaca aactgaaaca aagaaaacgc atcaaacgta gtctaagcac catttcattc acatcattcg ataagcagat cagcaacaat gataattcat catcatcatt gtttttgaca ccatcgcaag atcgtatcgc tcccagacgt ttcagcagta actgtttgcc tagtagaaat acatttctaa aacaaattgg aagtaaatca caggtgaatg tctctgatct ctcatcaacc acaacaaacg ttttagctag cggattagct gctgaagctg gcataggcag ccatagcaat caattgcaat cagataccga tatcaatgca tcgaatagca atcaaatcaa caatgctaac atagtttttc cattcaaaga tacaccacaa gaggttgccg aatcgatcga tcgaaaatgt cgctatctaa ttcctttggc atttattctg tttaatctaa ttcattggtc atatttgtaa

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Antigen 1 (ASA1)	*Sarcoptes scabiei*	2847 bp mRNA linear	Characterization of an atypical antigen containing an MADF domain	catagattcc gattcgaatt ccaatgaatt aaataatttt gttcatattt gattttactt aatgatctca tagaaaaaat ctgaaacacc attttccctg aacctctaat aacaagttca aattatatgg ttccgttcta tgcatttccg ataacttgaa attcgcctta attcttttcc aatcaaaaca aaaaaagtat tcatttgtta ctcgatgctg aatatatccg accatgcatc gcttgtcttt cgagcaagaa aaaaaattga tcgactattt tcgtgatcat ccatcgttgt ggaacacaaa gaataaagat tacaataatc gtttgttgcg cacacaaaaa ttacaaatca tcggagacga acttggctta acacgcaaag atgtttatga aaaataccga aatctaagaa ccacctttt ccgtgaacac aaacgtgtta cccgtggatc ttgcaaagcc aatgcgttag gagcgaacgg acctcactta caaggatatg tatctcgatg gaggcattat aatcacatgc ttttttaac aattaaatca tccacatccg atgatgatga agttaatgtt gctagaagac aatgctcaac tagggaaaat tctgtatcga ttattggacg tccggcgtcc agtctcttta ctgtatcgaa cgatgattcc gcatctcttc caaatggtca tgaaattgag ttgatcagta cagctagttt gccaatttct catccccatc aacaagatca acgctatgaa tctcaaatat tgcctgtcaa tctgaattct gatgaactca ttttcattaa tacaaatgtt ccaaactgtg caaccctaca atctgctaat aatgttggca tcaataataa taaccagcaa caagcgattt tatcaactgg atcaaatttg atttcttcta catcatctcc aattgtctcc accgtaccag ttgctggtct ttctgtaatt catgcggcat cttcatgttc aaaccctaat cagcccatca atgtagccaa tcgtcgaatc aagatagaac caatcatcaa cgtcaatcca acaaatggct tgtcttctta tcaatccggg attaactcgc aacaacagca atctgctttt ctgattaaga gttcaatcgg acttatgcct tctattttga caaattgcaa tcctagtgtt gcgaatgctt caacgaatgt ttccaataat gtcgtcagca ataatttgac agtttccaat caatccatta actcttctct taatgactct ggagattcta tgatcaataa tagcaataca gtttcacagc aatcaaattc ctgtaagaac agagttaacg tttctacccc taaagcaaat caaattactg tggacacaat caatggaaat tcatcgtctt atcaatctca atcacaatcg aattcatctc aaactagcgc aaaaaaaagt tcatttttt tgatgaccac cactccggcg ttacaaagtt cacagatatc ggccaccaaa gatcgttctt catcagattc ggtgcagcaa aatcgaagct ctatacagcc tggatcggct gcatcatctt caatgagtta tcaaaccaaa aacaatccta atactacaac tttgttgcta acgaatggta ataacgttgt cggagggcag aacaatttat ctcaggtctc tggcagaaat actgtcatgg ctaatctgaa ttccaataca gattgcagtg aaatgactca aatcatcaag aaatttgttc aagaagccat tcttgaattc gaggatgaag acatggcatt ctgtcgtagc atagcaggag ctttgaaacg tatagagaaa tcaaagcgtg aaaccgccaa attagatcta caaaaaatca tcgttcgata cattgatcca gagaatagtggttcaccttc atctccatca tgctttaact ggtcttcgtc ctcttgctat agctcgacta ataacaatca aagtgaaaca ccaggagaaa atattttgaa taatgttagc ggcgaaactatggtgaacac ttctcaggtc atccagaatt tcaataatta tgtaagcaat acagaatcga ctgcaacaga gcaatcatct ccatcctcaa ataatataaa tcaaactcgt gccacttcgaacgctgttag cactatcata acatacgaga ctgcaaatct tagctcttcg tcaacaaataaatccgatta tgtaatgagc agaattgatt ctctaccgtc gaacaatata attgtttctaacaagaaaat aaagaggcaa gactcggaaa cagaaactaa aactgttgat aaatatgacatcgatcagaa tcaagaaaaa aactcatcga aaaatgactc atcaaaacag tgagaattaaatttagattt aatttaaatt tacattgaaa tttttagcgc aaatcaccgt tatcatttttaattgttttt gggatattct tactttgaga taataaaatt tttacattga tataaacgaattgaatccac aatcggatgg catttcaatt gctgattttc tattcaaaat ttttcgttctgttttttaaa ttcttcatca atctaaactt tcgatcgaga acttttgtac agatttcattgttcattaaa ttcaattgtt aattcaaatt gatctaaaat gacaatttat cttgtgtcatctctcgagaa acgatgctac aatgattatg attgttattg attattatta tttctttttttttctattga ctatgttatg attgttattg atgatttgtt aataataaat attcttctttttctataacaa aaaaaaaaaa aaaaaaa

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Paramyosin mRNA	*Sarcoptes scabiei v. hominis*	2362 bp mRNA linear	Identification of homologues of house dust mite allergens	aagataccaa acatcgtttg gaggaagagg aaagaaaacg tgccagtctc gagaatcatg cccatacttt ggaagtggaa ttggaatcat tgaaagtaca attggatgaa gaatctgagg ctcgacttga acttgaacga caattgacca aagcgaatgg tgatgctgca tcgtggaaat ctaaatacga agccgaactt caagcccatg ctgatgaagt tgaagaactt cgtcgtaaga tggcacagaa gatttcggaa tacgaggaac aattagaagc attattgaac aaatgtagct cattggagaa acaaaaatct agactacaaa gcgaagtgga agttttgatt atggatttgg agaaggccac cacacacgct caacaattag agaaacgtgt tgcacaattg gagaaactta atctggatct taagaacaaa ttggaagagg tcacaatgct tatggaacag gcacagaaag aggcccgagc gaaagcggct gagttgcaaa aattacagca cgagtatgag aaactacgag atcaacgaga tgctttggct agagagaaca aaaaacttac cgatgatttg gctgaatgca aatcacagct caacgatgct catcgtagaa tccacgaaca agagatcgaa atcaaacgat tggagaacga gagagaagag ttatctgctg cctacaaaga agcggaaacc ttacgcaaac aagaggaggc taagaatcaa cgattgactg ccgagctggc tcaagtacga cacgattacg agaaacgttt ggcacagaaa gaggaagaaa tcgaagcact tagaaaacaa tatcaaatcg agatcgaaca gcttaatatg cgattagctg aagctgaagc taaattgaaa accgagatcg cacgattgaa gaagaaatat caagctcaaa tcactgaatt ggaattgtca ttggatgctg ccaataaagc caatattgat ctacagaaaa caatcaaaaa acaagcccta caaattacgg agcttcaagc acactatgat gaggtccatc gtcaattgca acaggcagtt gatcaattgg gagtcaccca aagacgatgc caagcgttac aagcagagct ggaagagcaa agaatcgctt tggaacaagc gaatcgtgcc aaacgacaag ccgaacaatt gcatgaggaa gctgtcgcaa gagttaatga acttaccacc atcaatgtta atcttgcttc agctaaaagc aaattggaat cggaattcgc tgctctacaa aacgattacg atgaagtcca caaagaactc agaatttctg atgaacgagt acagaaactt accattgagc tcaaatctac caaggactta ttagtggagg aacaggaacg attagtgaaa atggagaccg taaagaaatc tttggagcaa gaagttcgta ccctacatgt ccgtatcgaa gaggtcgaag ctaatgcttt ggctggcggt aaacgtgtca ttgccaaact ggagagcaga attcgtgatg ttgagatcga agtggaagag gaacgaagac gacacgctga gacggaaaaa atgcttagaa aaaaagatca tcgactcaag gaattgttgg tgcaaaacga ggaggaccac aaacaaatcc aattgcttca agaaatggtc gacaaaatga acgaaaaggt caaagtttat aaacgacaaa tgcaagaaca ggagggaatg agccaacaaa atctgactcg tgttcgacgc ttccaacgtg aattggaagc tgctgaagat cgagcggatc aagctgaatc aaatctatcg ttcatccgtg ccaaacaccg ttcatgggtg accactagcc aagttccggg cggcactcga caagtcttcg taacgcaaga agagcaatcc aattattaaa gataaatctc aacaaacaaa caaacaaaca aaaaaaatga ataaataaat aaataaaaac aaaatactat ttctaataga attgatgaga tcttcgagaa gaaacaaatc aaacaatcgtcatcataatc gaatttgatt ctttttgttt tccgttntgt ttcgaaaatc ttttttccat ctaaaaatga ttgttggaaa aaacgaagct atacgagana atagagcaag atagagatctactatgacag actttatatt ataaaaaaat ntttaattta caatcgatta agcgaaaaatctaaaaaaaa aaacttcaag tctctaagcg gcgggccatc gtttcaccgg gtggtccaaggaggtcccat cgcttagggg cgatacaata tggcgcgtta aaagtcgatg ggaccctgggtaccattatg ctgagcatcc tt

Gene/Protein	Species	Size of Gene/Protein	Role	Sequence
Actin mRNA	*Sarcoptes scabiei v. hominis*	1131 bp mRN linear	Elevated transcription of glutathione S-transferases and P-glycoprotein in ivermectin exposed Sarcoptes scabiei v. hominis	atgtgtgacg acgaagtaac cgcattggtt gttgacaatg gttccggcat gtgcaaggcc ggtttcgctg gagatgatgc tccacgagct gtcttcccgt ccatcgtcgg tcgaccaaga catcaaggtg tcatggtcgg tatgggtaac aaagattcat acgtcggaga tgaagctcaa tcgaaaagag gtattctcac cttgaaatac cccattgagc acggtatcgt caccaattgg gatgacatgg agaaaatctg gcatcacacc ttctataacg aactccgtat tgctccagaa gaaagtccag ttcttttgac cgaggctcca ttgaatccaa aagctaacag agaaaagatg acccagatca tgttcgagac cttcaacagt cctgcagtct acatcgccat ccaggctgtc ttgtccttgt acgcttctgg tcgtaccacc ggtattgtgc tcgactccgg tgatggtgtc actcacaccg taccaatcta tgaaggttac gcccttcctc atgccattct ccgtttggat atggctggtc gagatttgac tgattatttg atgaaaatct tgaccgaacg aggttactct ttcgttacca ccgccgaaag agaaattgtc cgagatatca aagaaaaact ttgctatgtc gctttggact tcgacaatga aatggccacc gccgccacct catcggcttt ggaaaaatcc tatgaattgc ctgatggtca agtcatctcc atcggtagcg aacgtttccg tgccccagag gctctcttcc aaccatcctt cttgggtatg gaagctgtcg gtatccatga aacctcatac aactccatca tgaaatgcga tatcgatatc cgtaaagatt tgttcgccaa cactgtattg tctggaggta ccaccatgta tccaggtatt gctgacagaa tgcaaaaaga aatcactgcc ttggccccag ctaccatcaa gatcaagatc atcgctccac cagaacgaaa atactccgtc tggatcggag gttcaatctt ggcctcattg tccacctttc aacagatgtg gatctcgaaa caagaatacg acgaagctgg tccagctatc gtacacagaa aatgctttta a

Appendix 2: Catalogue of the existing expressed sequence tags (ESTs) for *Sarcoptes scabiei*

EST	Size of EST	Sequence
ESSU904	406 bp linear mRNA	CATTTCCAGGTGATCGATTCGATCGTTCAATTTTCGTTTGTTCACT TAAGTAAGCGGAAACAAAACCAACAAAAATGGAGGCCATCAAA AAAAAAATGCAAGCGATGAAGCTCGAGAAAGATAATGCTATCGA TCGAGCTGAAATCGCTGAACAAAAAGCCCGAGATGCTAATCTAC GAGCCGAAAAATCTGAAGAGGAGGTTTGTGGTCTACAGAAAAAG ATCCAACAAATCGAGAACGAATTAGATCAAGTCCAAGAACAATT ATCGGCTGCCAATACCAAACTAGAGGAGAAAGAGAAAGCCTTAC AAACTGCTGAAGGCGATGTTGCCGCATTGAATCGTCGTATTCAAT TGATCGAAGAAGATTTGGAACGTTCCGAAGAGCGTCTCAAGATC GCTACAGC

EST	Size of EST	Sequence
ESSU903	423 bp linear mRNA	ACCCAAGCCTTCTCGTATTTGATGGAGAAAGGCGTCGTCACTGAA TTTGAATATCCTTACACAGCAAAGAAAGGAATATGCCACGCGAG AAGATATCGGAAATATTATCATGTTAAAATTAAAGATTATTGTGC CATTTGTCCGCATACGGTGCCCATATTGAAATCGTTTATTTATCAC TATAAGAGGCCATTGACAACGATCCTTCACATTCGAAATCTCAAA GCTTATAATAGAATCGGAATTTATGCTGTTGAAGATGATTTTGGT AAAAAAGTTCAACATCAACAAGTGGTCAACATCGTTGGTTGGGG TTATCATCATAGAGGCAATATTAGCTATTGGATCGTAAAAAATAG TATGGGTATCCATTGGGGCCATAAAGGTTACGCTTTCGTCGACAT CGATAGTGATGCATTCGAG
ESSU902	494 bp linear mRNA	TCGTGATGAACATGGAAAATTGGGTGGCGAAATTCTATTCGGTGG ATCAGATGCCCGATTTATGAGGGTGAATTTCATTATGTTCCCTTA ACCAGAATGACATATTGGCAATTCGCAATGTCAGCTGTCTATGTT GAGAGTAAATCAAAAAAGAGACCTATAGGACATCTGTGTGAACA TGGTTGTCAAGCGATCGCTGATACTGGAACTTCTTTGATCGCTGG TCCAAGTGCTGAGGTGAATCATCTTAATAAAGCACTTGGAGCTAT CGATCCTGTGAAAGGAATTTACACTTTTGATTGTAAAATGATACC AACGCTTCCGGATGTAGTGTTCCGAATCCATGGAAAAGATTTTCC ATTAACCTCAGAGCAATATGTGATGAAAGTTACAGCGGTTGGAC ATACCACCTGTATTAGTTCGTTCATAGGCATCGATATTGGCAATC TCTGGATTCTTGGAGATACTTTTATTGGATACTATTACACCGAAT
ESSU901	493 bp linear mRNA	CAAAATTCTTGCTCCAAGCCAAAGCATATTCTGTAGATTCGAATC GATCTCGATATCTTGATTATCATTGTAGATGTAGAATAGATTCAA TGAGCTACCACAATCGATCTTCTCATCACGACCATTACCACAATT GTTTTCGCCATTGCATCTTAATGATTCTGGGATACAAAATTTTCCA TTCGATTCACAATCGAACGTTCCTTTCGGACAGGTCTTTTGTTGTA TATCCTATTGATGCTTTGTCGATTGTGTGATCGTCTTCGATCGAAT TCAGGACCATTGTATTTGATTCCGAAAGGAAATTATATCTCAATT TCGGGAGATAGTATCGAACAGAAACCACTGAAATCGATGCGTTC GAAAATCGATTCAAAAGATGCTCAAATTCGTGAATATATCTATGT TAATAATAATTATGGACAACCTCAAGGCTCTACAGTCGTCTCGAC CAATGTTGATCCGATGAATAAATCTAATTACAAACCTGCTG
ESSU900	609 bp linear mRNA	AATAAATGGACACTATCCGAACAACAACTTGTCGATTGTGCTTGA CCAAAAGGATGTGATGGCGAAAAACCGACCACCGGTTTCAAATA TCTTCTCGAAAAAGGTGTAACAACTGGCGATAGATATCCTTATGT TGGAAAGGTTCAACCTTGCAGGCCTCCGATCGGTTCATACTATAA GATTAGATCGTTTTGCTGGGTCTATCCTCCTGATCCTAAGAAGAT ACAAGTACTCCTCTCAAATCGGAAAGCCGCATTGACGACAGTGA TGAAAATCACCAATTATATGCAATTCCGTCACTACGATGGTAAAA GTGTAATTGAGACCGAGGTAAGGGAGGGAAAAACTTTATCGCAT GCTGTAAACATCGTAGGATATGGCAAATATTTTGGCAAGGATGCT TGGATCGTTCGCAACAGTTGGGGTACTAGCTGGGGTGATAAAGG ATATTGCTATGTCTCAATGAACAGTCAAGTATTCCGTTTACTGGA ATTAGTCTATTCTGCTTCTGTCGTCTAATTAGCTTTGAAGCAGGAA CTTTTTATGATGATTTATTCGTATTTCTCGAAATTTCCAATAAATT AAAATTTGGTAAAAAAAAAAAAAAA

EST	Size of EST	Sequence
ESSU899	539 bp linear mRNA	GTAGCAGATATTTTCGATTCTGTAATCTTCCACCCCACACTAAAC TTCCGAAAGAATTCGATTTAGGAAAATTGAAAGTGATACCACCTG TTCGTAATCAAAAAAGATGTAACGCATCCTGGGCTTTCGGTCCAC TTGGAGCTGTTGAATCGGCACTCATCCATAGATTTCATCTGCCAC ATCGACATTTTCAACTTTCTACTCAAGAATTGGTTGATTGCGCTG GTAATCAAGGTTGCAGAGGAGGCGTAGATGTCACCCAAGCCTTC TCGTATTTGATGGAGAAAGGCGTCGTCACTGAATTTGAATATCCT TACACAGCAAAGAAAGGAATATGCCACGCGAGAAGATATCGGAA ATATTATCATGTTAAAATTAAAGATTATTGTGCCATTTGTCCGCAT ACGGTGCCCATATTGAAATCGTTTATTATCACTATAAGAGGCCAT TGACAACGATCCTTCACATTCGAAATCTCAAAGCTTATAATAGAA TCGGAATTTATGCTGTTGAAGATGATTTTGGTAAAAAAGTTCAAC
ESSU898	221 bp linear mRNA	CGAGAAGAAGCTTACGAACAACAGATCCGGGTCAGTACCGCTAA ACTTAAGGAAGCGGAAGCTAGAGCTGAATTCGCTGAACGATCGG TTCAAAAACTACAGAAAGAAGTCGATCGATTGGAAGATGAGCTT GTACACGAGAAAGAGAAATACAAATCGATCTCGGATGAATTGGA TCAGACATTTGCCGAGCTTACTGGCTATTAATAAAAAAAAAAAA A
ESSU897	510 bp linear mRNA	CAACGCCAAGTGATGCTGAAGAAAATGCCAGGTTGTCTGGATGT TAGATATACCGGTGAACCAGATGATCCCGATTTTAAGCTTCCAGA TTCATTCATTCGAAAGTTCAAGAAAGATTTGATGAGAGAATTGAC CAGAAAATGCCGCAAAGGTTTCAGACACATGCGATTAGAACATC CTGAGTTACAGTTTTGTGAAATTCCCAAAGTTTTTCAAAAACTAC CAAAATGGTTCGATCTTAGGAATCTAGAATTGGTCACTCCAACTA GAGATAATTCAACCGAAAGTAAATGTAAAGCATCGTGGGCGTTC GGTCCGGTCGCTAGTATGGAATCCGCTTGGTTGGAATCGCATGAT CGAATCGCTTCCGATTCATTCTTTGTCTCTCCACAAAATCTAATCG ATTGTGCTGGTTACCAAGGTTGGGAATGGGTGGGAGTCGATGTTA TCGAAAGCTTTCAACTTTTTTGAAAACATAAGGGTATCTCAAAAG AGGAATTTCTATAAATT
ESSU896	719 bp linear mRNA	ATTTTGCACAATTTCGACGTAGCAGATATTTTGCGATTCTGTAATC TTCCACCCCACACTAAACTTCCAAAAGAATTCGATTTAAGAAAAT TGAAAGTGATACCACCTGTTCGTAATCAAAAAAGATGTAACGCA TCCTGGGCTTTCGGTCCACTTGGAGCTGTTGAATCGGCACTCATC CATAGATTTCATCTGCCACATCGACATTTTCAACTTTCTACTCAAG AATTGGTTGATTGCGCTGGTAATCAAGGTTGCAGAGGAGGCGTA GATGTCACCCAAGCCTTCTCGTATTTGATGGAGAAAGGCGTCGTC ACTGAATTTGAATATCCTTACACAGCAAAGAAAGGAATATGCCA CGCGAGAAGATATCGGAAATATTATCATGTTAAAATTAAAGATTA TTGTGCCATTTGTCCGCATACGGTGCCCATATTGAAATCGTTTATT TATCACTATAAGAGGCCATTGACAACGATCCTTCACATTCGAAAT CTCAAAGCTTATAATAGAATCGGAATTTATGCTGTTGAAGATGAT TTTGGTAAAAAAGTTCAACATCAACAAGTGGTCAACATCGTTGGT TGGGGTTATCATCATAGAGGCAATATTAGCTGTTGGATCGTAAAA AATAGTATGGGTATCCATTGGGGCCATAAAGGTTACGCTTTCGTC GACATCGATGGGGATGCATTCGAGATCAGAAAGAATACCTTTTT

EST	Size of EST	Sequence
ESSU895	670 bp linear mRNA	CTCGTGCCGCAGCCACCAAACAAAACAGCAAAAATGAAATTCGC CTTGTTGGTTCTTGCTAGCGTTTGCGCCGCAGTTAGCGGTGTTCCA ACCTATTCTCTCGGCTACTCAGGTTTAGGCAATTTGGGTAGTACC TATACTTTGGGTGGTTACCGAACATCTGGCCTCACTGGTCTCGCT GGTCTTGGTGGTTTGACTTATGGTACCGGATATGGTCTTGCAGGT CTCGGTGGTTTGACCTATGGTGCTGGTTATGGTTATGGCCGAACT CTTGCTTTGTCTGCTGCTCCAGCTGTCCAATTGGTTGCTGCTGCTC CAGCCGTTGCCGCTGCCCCAGCTGTCCAATATGTTGCTGCTGCTG CTCCAGCCGTTGCCGCTGCTCCAGCCATTGCCGCTGCTCCAGCTA TTGCTGCTGCTCCAGCTATCACCAGCTTCGCTGCTGCCCCAGCTGT CCAAACTACCCAAGTTACTGGTCCAATCCTGCCGCCATCGAAACT CGACAAACTGTCGAAGTTGTTGATGTCCCAACCCAAAGCGAAGG GCTGTTGCCCAAACTGTTGCATTGGACCAAATGTTAACCAATCAC TTGGAATTCCAAATTAAGCCAGCCATTGGCCGTTACTAAAAACAC TTGCCATTTGCCCAAGTGAAACTAACACAAGCTAATACG
ESSU895	670 bp linear mRNA	CTCGTGCCGCAGCCACCAAACAAAACAGCAAAAATGAAATTCGC CTTGTTGGTTCTTGCTAGCGTTTGCGCCGCAGTTAGCGGTGTTCCA ACCTATTCTCTCGGCTACTCAGGTTTAGGCAATTTGGGTAGTACC TATACTTTGGGTGGTTACCGAACATCTGGCCTCACTGGTCTCGCT GGTCTTGGTGGTTTGACTTATGGTACCGGATATGGTCTTGCAGGT CTCGGTGGTTTGACCTATGGTGCTGGTTATGGTTATGGCCGAACT CTTGCTTTGTCTGCTGCTCCAGCTGTCCAATTGGTTGCTGCTGCTC CAGCCGTTGCCGCTGCCCCAGCTGTCCAATATGTTGCTGCTGCTG CTCCAGCCGTTGCCGCTGCTCCAGCCATTGCCGCTGCTCCAGCTA TTGCTGCTGCTCCAGCTATCACCAGCTTCGCTGCTGCCCCAGCTGT CCAAACTACCCAAGTTACTGGTCCAATCCTGCCGCCATCGAAACT CGACAAACTGTCGAAGTTGTTGATGTCCCAACCCAAAGCGAAGG GCTGTTGCCCAAACTGTTGCATTGGACCAAATGTTAACCAATCAC TTGGAATTCCAAATTAAGCCAGCCATTGGCCGTTACTAAAAACAC TTGCCATTTGCCCAAGTGAAACTAACACAAGCTAATACG
ESSU893	262 bp linear mRNA	AATGAACCCGATTGTTCACCAATCGATCTCGATCATGGAGTCGCT GTTGTAGGATATGGAGTTTTGAACGGTGTACCATATTGGAAAGTT CGTAACTCTTGGGGAGTTTCGTGGGGTATGGATGGCTATATTTTG ATGTCTCGAAATCGACACAATCAATGTGGTATTGCTTCAAGAGCT AGCTATCCACTTATTTGAAGTTTTTTTTTTGTTCTTCACATCGAATT CTGACTGATTGAATAAAATAATTTAATAATTTAAA
ESSU892	673 bp linear mRNA	TTCGATTCTGTAATCTTCCACCCCACACTAAACTTCCAAAAGAAT TCGATTTAAGAAAATTGAAAGTGATACCACCTGTTCGTAATCAAA AAAGATGTAACGCATCCTGGGCTTTCGGTCCACTTGGAGCTGTTG AATCGGCACTCATCCATAGATTTCATCTGCCACATCGACATTTTC AACTTTCTACTCAAGAATTGGTTGATTGCGCTGGTAATCAAGGTT GCAGAGGAGGCGTAGATGTCACCCAAGCCTTCTCGTATTTGATGG AGAAAGGCGTCGTCACTGAATTTGAATATCCTTACACAGCAAAG AAAGGAATATGCCACGCGAGAAGATATCGGAAATATTATCATGT TAAAATTAAAGATTATTGTGCCATTTGTCCGCATACGGTGCCCAT ATTGAAATCGTTTATTTATCACTATAAGAGGCCATTGACAACGAT CCTTCACATTCGAAATCTCAAAGCTTATAATAGAATCGGAATTTA TGCTGTTGAAGATGATTTTGGTAAAAAAGTTCAACATCAACAAGT GGTCAACATCGTTGGTTGGGGTTATCATCATAGAGGCAATATTAG CTATTGGATCGTAAAAAATAGTATGGGTATCCATTGGGGCCATAA AGGGTACGCTTTCGTCGACATCGATAGGGATGCATTCGAAATCAG

EST	Size of EST	Sequence
ESSU891	523 bp linear mRNA	TTTTGTTTGGTTTGCTTAATTTTTATTTCTCAACAAATTGTTTCAAT TCAAATAATTTCTGTTTTTAGATTCGTGAGACTTAATCATGCAAAT TTTTTTAAGGCATCAAGGCATTCATTCGATCGAGTGCAACGAAAA TGATCGAATTTATGCTCTCAAATTGAAAATCGAACAATCTCATCA GATTGCAGTTGATGAGCAGATTCTCTACCATCAAGGCCAGGTGTT GGAAGATGATCAAATCATCGCGGAACATTTGACTCAAGATGCTA ATGTCGATTTGAATCTCCGTCTACTTGGTGGTAAAGTACACGGAT CTTTGGCTCGAGCTGGTAAAGTACGAGCTCAGACTCCTAAAGTCG AGAAACAAGAGAAAAAAAAGAAACCAACCGGTCGAGCGAAACG CAGAATTCAATACAATCGTCGGTTTGTGAATGCTGTCAAATTCCC AGGACGAAGACGAGGACCTAATGCTAATTCTTAAAACTTGAATG CCCGAATAAACTTGAGAATTTATCATTTC
ESSU890	350 bp linear mRNA	CTGATTTCGAAGAACCAGGTACTCCAGTCGCTCCATACAATTTCG CTTTCGACGAAACTGATGAATTTGGAATGAATTTGAAACGACAA GAAGCTAGCGAAAACGGTATTGTTACTGGATCCTATTCTTTCACC ACCCCAGAAGGATACACTCGAGTTGTCAACTACGTTTCTGATGAG AAAGGTTTCCGAGCTCAAGTGCAAACAAACGAACCAGGTACTGC CTCTTCCGCTCCTGCTGATGCCGAATATATCTCTTCAGCTCCAGCT CAAAAATAAAAATTGAACTCAAATATAAATTTTGAAAATAAACC CATCTCTTAGAAATAAAAACAATTTTTTTTGACGATC
ESSU0882	435 bp linear mRNA	TTTTTGTTTGCATCACCGATCGTCTCTAAGATTTTTGCATCAAATC AATCTCGCTCCAAGATTTTTGCGAAAAAAAAGGAAAAAAAATTT GCGAAGTTTTCTTCTTTGAAATCCAACTTTCTACAACAAACCTAA CATTCGTACGTTAATCGTTTATCTTTGGAGAGAGCGAATCGATTA AATCATTCATTCATTCATTCATTTATTCATTCAATTCTTAATC GAATTTCGATTATCATTCGATTTCACTAATCAAATGTTATTATTCG GTTTGGATTGATACTGTTGTGGGACTTTTTTTTTCGTTTTTTTTTGA ACTTGGGTTCAGCTCGATCTTCATTCGACTAGATACATTCGATCG AATCATAAACATCAAAATGTTTTCTCACTGATCTGATCGAATTGT GGTCAATACTCAAAAAAAAAAAAAA
ESSU0717	429 bp linear mRNA	AGAGAATATTGAATCGTTTCATTTGAATCGTTCTCATCTTGATCCA AGAAGCATCAGAAAATCGTTCATATCACCGACGTTTGGATTATTG TACCAGCATCGTTGTCGGAGTGATTTAATAAACCAATTTAGAATT CGATATATTCAGCCATCGATTTTACATTCAAGCAAATCATCAGTG TTCTCGTCTAAATCTTTCCAACGCCTAGACGATCCGATTCGGCTTA TTTCCAACAGAATTTTACACACATCTGCTTCTTCATTCATCATGAA AAATAAAGCCGTGTTGTTGGGTGTTTACGAGAATTCGGACAAAA AAGACGCGTTCATTTTGACACCGTCTGCGGAAAAAATCAATAATG AATGTGGTGGTGCCATCGAAAGACAAATCAATATTATTGGCCCTC TGAAAAAAGGTAAAGTTCGAAT
ESSU0889	507 bp linear mRNA	TGTGGAAGCAAAAAGAATCAAAATGTTACGACAATTATTGCTAC TCTGTTTAGTGGCTATCGTCATCGATCAAGCTTTAATAGTCTCGGC AAAAGCTCCGAAATCGAAACACTTGAAGCATCCCCACCATCCCC ATTGTCCTCCACATTCTCATTGGGATCCTTGTGGTCGTTCATGCCC GAAAACATGTGCACGTCCTTATGGTCCATTTAGATGTCCACTATC GTGTAAACCCGGATGCAAATGCGATAGAGGCTATGTACGAAAAG GCAGCGATGGCAATGGTCCGTGTGTCCGCAGAAGACATTGCAAA AGACCTCACCACAGATGCCCTAAACATTCTCATTGGGCTCCTTGT GGTCGTTCATGCCCGAGAACATGTGCACGTCCTCATGGCCCATTG AAATGTCCACTATCGTGTAAACCCGGATGCAAATGCGATAGAGG CTATGTACGAAAAGGCAGCGATGGCAATGGTCCGGGTGTCCGCA GAAGACATTGCAAAAG

EST	Size of EST	Sequence
ESSU0888	527 bp linear mRNA	TTTCAGTTAATTTCAAAGTATTTGGAAAAGTTCAAGGTTCGGTAA TCGAAAATGGCCAATGCCCATTTAATTTCAATATCTATTTATTCAT TGCGAAAAAAAAAGGTGTCTTCTTCCGTAAATATACTCAAGCCAAA GCCAGAGAGCTAGGACTTTTTGGATACGTCAAAAATGAATTCGAT GGTACGGTTGTAGGAATTATGCAAGGAATCGAATCAAGCGTTCG AGAAATGAAAGTATGGCTAGAGACAAAAGGTAGTCCACAATCTT CAATACAAAAGGTGGAGTTCACCAATGAGAAAATTATATCGAAA CCAGATTACGATTCTTTTACGATAAACAAATAAATGATCGATTTT GATCAAATTTCATATCGACTTGAAATTGATGTAGGGCTGCAATAC TTTAGGGATTCTAATAAGCTGATTCTCTGTTTGACCTTGTTCGATC AAAGCTATCAACATTCTTGGAATCGCACAAGCTGTACCGTTGACG GTATGGACAAAGTTTCGTTTCAAATCTATTTTC
ESSU0887	746 bp linear mRNA	CTCGTGCCGCTCGCGTTTCCATTTCGAAGTTATTTAAATTCCAATT TTTTAAATTTTTTTTCCCAATTTTTTTAACAATAATTTTTGTGGTGC AACATTTTTTTTCAAATTGGTAGAGCGATAATCTTCAATATTATCA TTAAAGCTCATCCACACCACAAAAATGCCACCTAAAATCGATCCG AATGAAATCAAAATCGTCTATCTTAGAGCGGTAGGTGGAGAAGT TGGTGCTACTTCATCGTTGGCTCCTAAAATCGGTCCATTAGGTTTG TCTCCGAAAAAAGTCGGTGATGATATTGCAAAAGCTACAGCAGA TTGGAAAGGTTTGAAGATTACTTGCAAACTAATCATACAGAATCG TCAAGCTCAAATAGAAGTTGTACCTTCGGCTGCTTCACTTCTGAT CAAAGCTTTAAAAGAGCCACCGAGAGATCGCAAAAAGGTCAAAA ACATCAAACATAATGGTAATCTATCGTTGGATGAAATAATCAAG ATTGCCCGAACCATGCGTCCTCGTAGTATGGCCAGGAAACTAGA AGGTACTGTCAAAAGAAATTCTTGGCACTGCAGTATCTGTTGGCT GTACTGTGGAGAATGAAAATCCTCAAGACATAATTACAAAAAAT CAAGGAGAATGAAATTCAAAATTCCGAATGAATGAAATCAATTG TTTGAAAGTTTTGAATAGAATTTTATTCTTTTCAAAAATAAATCAA ATTACTGGTTTATAAAAAAAAAAAAAA
ESSU0886	397 bp linear mRNA	AAAGCTAAGAGCACTTCAGCAAGTCCAGCATCGAAAAAAGTCAA ACCTACCAAAACAGCTTCGAAAAAATTGGCATCAAAAAAATCTG CTGTTATGAAGAAAACTGTATCGAAGAAATGAAATTAATATAATC TGTAAACAAATTCAGCACCAATTAATCTTGCCTATAATCGTTTTG GAATCTCATGTTGTAAACATCATTTGGAACTTTCTCTTTCATATAG TCATTGTAAACATACCAAAATATTATAATTATAATTACATTGAAA ATGATTGGTTATCATCACCTTTATTATTGGATTATAATTTACTTTA AACTTGAAAACTTACGATTGTTACTGATAATTAATAAAATTTTAA GTATATCCAAACTTTCAATTATAAAAAAAAAAAAAA
ESSU0885	319 bp linear mRNA	AAAAAATTTATAAAAGAACTTTTTATAAAGAAAAATTGACTAAAT ACTCTAGGGATAACAGCTTTATATTTTTTAAGAGAACTTATTAAA ATAAAAGTTTAAGACCTCGATGTTGGATAAAAGTTTTTATTAAGC GCAGAAGCTTAAAAAAATGGATTGTTCATCCATTAAATCTTTACT TGATCTGAGTTAAAGTCGGCGTGAGCCAGACTGGTTTTTATCTGG AATAAAATTATATAAATAAACAGTACGAAAGGACAATTATTTTTT CTTAAAGAAATTTATTTTCCCNAAAAAAAAAAAAAAAAAAAAA AAAAA
ESSU0884	287 bp linear mRNA	TTTTCGTTGGTGTTCAAAATTTTCAACAAAATCTCAATTAATAGAT GAGATTAGTATGAAAATGTTTAATCTTGCCTCCCAACAGTTGAAA ATAATCCAAAGAGAAGATAATTTTATGAATCCTTATGAGCGAAA AAATCAATTGCCTACTAATCGCTTGGAAAATGCTCAAATCAAATC CAAACCAAAAAAGACCACAAATGTTCGAAAAGGTCCACGAATGA GTAGAACAGAGTTTTGAATTGAGAAATAAACCATTTGATTGTTGC AACAAAAAAAAAAAAAAA

EST	Size of EST	Sequence
ESSU0883	441 bp linear mRNA	TCGAAATCTAAACAAGCTAGATCTAAACATCATCACCATCATCAT CATCATCATCGTGGCTACTGTAATGATGATTATATCTTTGCCATCA GAGTTAAAAAACAATATAGTTTTGCTTATGAATCTTATAGACACG AAACTGGTTATCGTAGTCAACATGATGTCTATTATGGGATGCAAG TGGATTATATCATTAAAGATCGTTATGGATTCTCGAATTACAATA AAAAACTTCGTGGTGTAGAGATCTATGGATTTTCGTGTGGACAAA CACTTCAAGTTGGTCAAAGTTATCTAGTGTCAGCTCGATATAATA AGAGACAAAAACATTTGGAAGTTAATCTATGCTCTGGTCTTCGAC GAAGATTACCTCAAAAGCATAAAATGACTAGAAAAGATCGATTT AGAGTTTTTTTACAATTGAAACACCAATGTCCTAAT
ESSU0881	446 bp linear mRNA	GTCGGATCAACATTGGAACAGTTTGTTAATGGCTAAAAAAATTGA AATCTACCTAGCTTTCGAGACAATTCTGAATTGATGTAATCCTTT AAGAGAAAAGTTTTCAACTAAAGTTAGCAATTAATCTATTGAGAA TTCTTGAATTTTTAAATTATGAGTCCCAGCAATCGGCAGATTTGA AACTAAAACTGGAATTTTTACTTTGAATTCAATTGAATTCAGTGG ATCGATTGTTCAAGCAAAATGATTAAGTCATTATTCAAATCTCAA ATGTTCATCAAGAAACAATCATCATCGTCATTATCATTATTATTAT ACCCATTACCAGCATCATCGACTTATAAAATGATCATCAAAAACA GACACCGAAAACGGTGGTCCAAATCGAAGCAATTACAGCCTGGA TGGTTTTCAATGTTCATCATAATGATAATAATTTATAATTT
ESSU0880	478 bp linear mRNA	CTTTCTTGTCTCTGCGAGATTTATTGATTCTTTGAATATCTTGCTA ACTTCGACCAACATATCCAGAAATTTTCGTTATTTCGAATTCAAA TCAAATTCTCGATTTTCAATCTCTTGGCCTTTAATCTCGTCATTTTT TACTGTTGAAAATGTTGGCTTTACCAAGACACCTTTCTCAAATGG TTTCCGATGTTCTCGATGATGACATCTTCTATTGGCCATTGAATTC GTTGCAAAATCGTTGTGCTGGATTGATGTCCACCATGAATCACGA CACCAGTGTTCCGACATTCAAAAACGATAAATACCAGATGTGTTT CGATGTTCGAAATTTCGATCCAAACACTGTCAAAGTTAAATTGGA TGGCGATCGATTACACGTCAGCGCTAAACAAGAGAAGAAAGGCG ATGGTCACTACGAATATCGTGAATTCATTCGTGAAGTTAATGTTC CGGATAATGTTCACGCGGAAAAGTT
ESSU0879	327 bp linear mRNA	CATGTATTATGTGCTGTCGGATACTACGCAGACTTCAAATTAAA AAAGTATTCTACGGCGCCAGCAATGAAAGATTCGGCGGAGCCGG ATCGGTTCTCTCAGTTCATTGTGATCAACGAATCCAAGATCCAGA GCTTCATTGTGTCTCCGGTAGTCTTGATCGAGAACGATCGATAAA ATTGCTACAAAAATTCTACGATCAAACTAATGAAAATGCTCCGA AAAATAAATCAACCAATTGAATCCAAGATAATTTTTTTGTTAATA AGATAATTGTTTTTTTGAATGCAATAAATTGTTAATTAAAATGAA AAAAAAAAAAAAAAA
ESSU0878	552 bp linear mRNA	CGGCACGAGGTAAAATCACCCAACAAATCTTGCCAGTCCAAGAA GAAGTCCACAGCATCGTGTCCAAGGGAGAGAAACGATCCAACA TTGAACAAGTCACTGATGATGCCGCATTGGCCAGCAGCATTCCA GAAGAAGAACAAATCGAACAAGCTGCAGTGCAAGCTCAACAAC GATCCCGAGAACCACAACAATTGGGTTTCCCACAAGGCCAATTC AAAACCATCGCTCAACAACCACAAGCCGCCTTCATTAGCGCTCA ACAACCATCAAACACTGTTATCCAAGCCGGTTTGGTCGGTGGAA CCAGAATTTTGCCAGCTAGCGAAGCCGTTTACGAGCAACAACGA GAAGTGGTCTCAGCTATCGCCGCTCCAGGCTTGAAAAAGAAACG ATCTGCTAACATTTTTCGATTCGATATCCCATAAACAGTGATAGA AATCAATGAAATATTATCATCATCAACACACCAGACCTAGAAAC ATTGATAACAATAATGAAATAAAGAGCAACAGCATTAGTTCAAT CGCTATGCCTTTCCTTTTGAAGTAA

EST	Size of EST	Sequence
ESSU0877	520 bp linear mRNA	CAAGATTCCTGTTCTTTTACTATAATAGAATTATCTTTATTTCATG ACTATATATTATCTATCCTAATAGGAATTGTAGTAATAATTACTT ATATTTTATTTTATATTATTTTTAATAAAAATTTTTACAAAAATTT ATCTGAAAGAACAAAAATTGAAATCATCTGATCAGTTGTACCAG TTTTTATTTTAATTATACTAGTAATACCTTCAATAAAAGTTTTATA CTTAATAGAAGATATTAAAACTCCTTCTTTAAATTTTAAAATTGT GGCTCATCAATGATATTGATCTTACATTGTCCCTTTTTTTAAAAG ATATTTTTATAAAACTAACAGAAAAGAATATTTTTTTCATGAATT TGATTCAATTATAGAAAATGAAAAAATACCTCGTTTATTAAATT GTGATAAAAGATTAATTATTCCTTATAAAACTAATTCTCGTCTTT TATTATTCTCTACAGATGTGATTCATTCATTTAGAATACCATCTA TAGGTTTAAAAGTTGATGCTCTCC
ESSU0876	170 bp linear mRNA	GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA GAGAGAGATCTCGCGCCTCTCGCGCCAAATTCCGCACGAAAAGA GGCACGCGCAGAAAAAAAAGAAAGCGCGTGCGCGAGAGAAAG AGAGAGCGCGAGCGCCTGTGTGGCGCAAGAAAAAAACGCACA
ESSU0875	467 bp linear mRNA	CGAGTTTAAATTTTTTTTTAAAATAAAATAAAATCAAAAATTTTT AAAGGGGTAATACGCTATCGTTTGAATATTTATCTAAAATAGAC AAAAATTAAAAATAGTAAATATTTTTATTATCATTGAATAAATTT TTAAATATTCTTTAAAAGTAAGTTTACGTTGTTATAACAAAACAT TTTATTATTATATTTAATTATTATATTATAAAAATATTAAATTAA AAAATTAGTATAAATATTATAAAATTTTTATATTTAATAAAATTA AATAATAAAAAATAAAAAAAATTTATTCATTAAATTATTAGACT GTTTAACAAAATCTTATTTATAATTTTTATAATCTATTCTGCTCAA TGAAATTATTTAAATAGCTGTTAATAACACTAAGGTAGCGAAAT CATTAGCTACTTAATTGGTAACTTGTATGAAGGGACTAACTAAA AATTTATTTTATAAAAAATA
ESSU0874	401 bp linear mRNA	CAAAGTGTTCGGTGCATATCTAGATCGATCTTCTGGTCGAAATCC TATGACTGGAAAAAAATGTCCTATTCTAATCAAAAGTGAAGATG CTAATATCACCAAAAATAAATTGAGAGAATCGTTGACTTGTACA GTGGACGGTCCAACTAAACCCGAGGTGATTTGGTCCACCGTTGC CGATCAAGAGCCCGATTTTGATGAGAGAACTTTGGTCGCTTATTT TGTGCCCATCAAACCTGGTAAACATAAATTGACGATTAGATGCA ATGGTAAAAAACTTCCTTCAACTCCTTTTGAGTATGAAGTGTCTG GAGAAGCAATAGATTTATCCAAACTTTTAGAAAAGGTCACTGTG CACGGACGTGGACATGAATTGGGAAAAGCATTCACCCATAATCA AT
ESSU0873	419 bp linear mRNA	CCGAAATTTAAAAAAAATTAGATTCAAAATGATGCTTAAGTTCA TTTTGTTGAGCGCCTTGGCTTTGACCTGCTCTGCTCAAACTTATA AACAAACCTATGGTGGTCTCCATCATGGTGGATTTACCGGTTTGG GTGGTGGTTTGATGTCCTATGGTACATATGGATCCGGTCTCGGCT ACGGAAGCGGTCTACACTTCGGTGGTGCACAGAAAAAAGTTGTC GGCTATGGTTTAGGCTATGGTGGCTATGGCGGTTACGGTCAAGG TTTGGGCTATTCTAGCTTCTTGCCTGGTGGTTACCGATTCGGTGG TCTCGGATACACTTTGGGTCATCAATCATATGCCCCAATCGCCAC TCATCAATATGTCAGCCAACCATACGTCTCTGGTTTGTCGCTCGG TCACGCTAACATGCAAA
ESSU0872	237 bp linear mRNA	TTTTTTAGACTAAAAAATACAGAAATTTTTATTCTCTAAACATAT TTTTTGTGTTTCGAGAATGGAAGAGAGACGATTTTTAATTTAATA ATTGAAAATTTTTTTTTCTTTTCAAAACAACCACACTTGGTGCGA GAGAAATATGTTGATAATCGAGTTGAGTTTTTCCGAAAAACAGA TATGAAATATCGCCTGAAAATTTAGCCAATTACTGCAACTCGTGC CGGCCATTCTTAA

EST	Size of EST	Sequence
ESSU0871	438 bp linear mRNA	ATCCCAAAAAAAAAAAAAAAAAAAATTTTTTCCCTCCTGGGGTT GGGCAGACCAATTGCGTTGGGTTTCCCTAAAAAAAAATTCTTGG TTAAAAAAAATGTTTTAAACCCGCCACCTCCAAATTCGTTCCACC TTTTTAAACCTTCCCTTTTTTTTTTTTGGCCCTTTGGGGGGAAATT CCATGGAAACCGTTAAAAAACCTTTTTTTGGGGGAAGGATTCCC CCCTGCAAAAAGAGAGAGAGAGAGAAAGAAAAAGAGTTTTTCC CCCCTCCAAAAAAAAAAATCAAATTTTGTTACACATCCAAAATC ATTCCTTTATTTTTTTCCCTTGGAAAATTTATTGGCCCCTTCCCCC CTGGAAGGGAGAGAGAAAAAATTTTTTTTTTGAAAAGAGAGACCC GAAAAAAAAAAATTTTTTTCCAATTTTTTTTTTTTTTA
ESSU0870	369 bp linear mRNA	GGCAAAGGCAAAGGAAAAGGCCAAGGCGAAGGAAAAGGTAAG GCCGAAGGCCAAGGTGGAGCAGGAGGAAAAGGCAAAGGTAAAG GAAAAGGCCAAGGCGAAGGAGAAGGTGAAGGCGAAGGCCAAG GTGGAGCAGGAGGAAAAGGCAAAGGTAAAGGAAAAGGTCACGG TGAAGGCGAAGGAGAAGGAGAAGGCGAAGGCCAAGGTGGAGC AGGAGGAAAAGGCAAAGGCAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAANTTTTTGGGGGGGGGGGG GGGGCCCCCCCCCCTTTTTTTTTTTGGGGG
ESSU0869	447 bp linear mRNA	GAAATCGTAGATTTGTCCCAAGTGAATCCAATCTTGGTTTTTGAA GCAATCAAGCGTTCGAGGAAGAGCAAAACCTAACCAAAATGTC GGTCGAAGAATTAACCAAAGAACAAGTACAAATGTTACGAAAA GCTTTCGATATGTTTGATCGAGAGAAGAAAGGCCACATACATAC CAATATGGTCTCGACCATCCTAAGAACTTTAGGTCAAACTTTTGA AGAGAAAGATCTTCAACAATTGATCACCGAGATCGATGCCGATG GTAGTGGTGAATTAGAATTCGATGAGTTTCTAACACTGACCGCA AGATTTCTGGTGGAAGAGGATGCTGAAACAATGCAAGAAGAGCT ACGTGAAGCATTCAGGATGTACGATAAGGAAGGAAATGGTTATA TCCCGACCTCAGCTTTGAGAGAGATTCTTCGAGCCTTGGATGATA AACTAA
ESSU0868	552 bp linear mRNA	GAACAAATCATAATTTTTAGCGAACAAGTCGGTCAAAAGACTCA ATTCTCCAATCCAAATTATTTGTATAAAAATCATCTCCAAGTGAA CAAAATGTCATTGATTGAGAAGCACAATTCAATCGATGGGAACA AATTCATATTGAAATCGAAAGATCCGCAACAGGATGGATTAGCG TTTGTTTGCGAAGGTCTCACAAAAGAAGATTCTCAAGAATGGAT CTCAAACATTCGTGCCATTCTCGATACGCAATTAGATTTTTTGCG TGCCCTACAATCTCCGATCGCATATCAAAAAGAGTTAACAAAAG AAATGTGAGCTATGACTTAGTCTTTAAACAAAAAATCCTTTCCGT TTCTCACTGTGTGATGATCGCCTGATGATCCTAATTTAAAGCAAT GAATTTGATTGATTTAAATGATCGAAGAATATCGAAAGAAAACA GCATCCGTTTGATGGAAGACCAATTTGTTTGCTCTCCTTAATCTT ATCCTGCACAAAAAACAATTTTCACATCCTTGTAACCAACGTCTG AAAATCTTGAATCATTTT
ESSU0867	366 bp linear mRNA	AACCGTTTCAATCAATGATATTATCTTCTCGATCAACGCCACCAC CCCACCACCACCACCATCATCATCATCATCATCATCATCATCATC ATTATAAATTTAATCAAACTATCTTCTCGATGAAGAGAAGAGAT GATAGCTTCGTTATAATTAGATTCACGAATCGTTTTTCTTTTGCCT GGTTCTTTTCCCTTCATTCTGGAACTATCAAAAGAAAGAAGCAA GCAAGCAGCCCCGCTCCTTCGCTTCTAGAGAAAAAAAAATTCAA GATGATGCCTTGAAGATATTACGGAGACTATTATCGTTGGCGAC ACTATTTTCACCATTCTTTTTTGCAATGAAAATTTCCAAAAAAAA AAAAAAAAA

EST	Size of EST	Sequence
ESSU0866	483 bp linear mRNA	TCGAGTTTTTTTTTTTTTTTTTTTTTGGGAAAAAAATTTTTTATTG CCAAAGGAGGTTTTTAAATTTTAAAGGTTTTCCCAATTTCCCCCC CCAAAATACCCTGGGGGGTTGAAAAACGAAAAAATTTTGGCGGG GGATTCCCTTTTTTCCCGGCCCCCCCCCAATTTCAACCCAAAAAA ATTTTCCCCTAAAAAAAATCCCCCAAAATTTTCCCCCAAAAAAA AAATTTCTTTGCAAAAAAATTGGGCCCTTTTTAACCAAAAAATG GCGGTTTCCTTTTTTACCAAATTTTTTTTCAAAACAACCCCCTTTT AAACCCCCCCCGGGAAAACCCTTGGGGGAACCCAACCCTTTTTT AAAAAGGTTTTTTTTAAATTCCAACAAAGGGGGAATTAAAGCTT TTCCCGGGAAATGGGGCCTTCCCCAAGGGGCCTTCTTTTTCGCCT TTAAAGGGGCCCCCCCCCAAAATTTTTTTTTTTTG
ESSU0865	338 bp linear mRNA	ATTATAATGATGAAAATCAGAATAATATCAACATTTCAAACATG TTCCGGCAAATAAGATCCCTCCAAATGAACATCTAGATAACACA GCTTTGTCTAACGATCAGAATGCCAATAATATAGCTCCCGCCGGT AATTCTGATCAAAGTGAAGTTGCCATAGATAATAAAATCGAACA AGCTTGGGTTTGGGCCAAGTACATTTGATGGTTGGAAGTCCAAA AAAAATCCATGGTTTGAAAGAACGAATTAAAAAACTTGGATCTG ACTTACAAATCTTAATTGGTCATGCGACACCTGAAACCCTAGCTT TGGTAAGTAATAACCGGTCAACTGGCAA
ESSU0864	419 bp linear mRNA	ATTTTTTATAGGAAAAATAAAATATTTTCTAATAATTAAAATTTT TTAAAAAATGTAAAAGTCCTAAAAGATAAATACTAGTGCCAGCA TTCGCGGTTATTCTATATTTTTTTAAGTCTTTAAATTCTTAAAAAT ATACAAAATAAAAAAATATAATTAAGTAAAATTATTTTATGAAA TTTAATAAATTTTAAGAAAGTAAATAAAAATAAAACTAGGATTA GATACCCTATTATTTTTTAGTTGTATTAAAGTAGTAAATTATAAT AACAAAACTTAATTTTTTTGGCGGTTTTCTATATATTCACAGGAA CTTGTATAATTAAAAAGATAACCCACTTTTAATTTTACCTTTTTTA TTTTTTTTTTGTACGGTTGTTAGATAAAAATTTATTTTTTTTTCTC AAAAAATAAAATAT
ESSU0863	504 bp linear mRNA	TTTTTGATCTGAATGATCTTTTGACTGAACGAGAATAGCTTCGCA AATGCATTATTAGATTGAACTTGGAACCGTCTAAAATCTTTGGCT TTACTTTTGAATAATTTTTCCTTAATTTCAAATATTATTTCAACGC GTATAATTGAGTAATTATCAAGAAAATAAGATGAGTTCCAAACG AGACTACGATTATTTCGATTCGAATTCCAATAGTAATGATTCTTT TGGCAAAAAAAGAAAACGAAAATCACGATTCAGTGATGAGCAA GAGAAAATTTTCATTCCTGGTCTACCGACAATAATCCCATCGAAT TTGAGCAAACAACAAGAAGAGATCTATATTTTGCAACTTCAAAT AGAATCGTTGACAAGACGTTTATTAACTGGTGATCTTGGAATTCC TGACAATCCTGAAGAAAGGTTTGATTGATTGAATTATTTAGAATT TGCTGATTAAATATTTTTTCTTTCAATCCAAATCAATGAGAATCA ATATCTTTAGTT
ESSU0862	519 bp linear mRNA	GCCGAAAGAGGTTTTTATTTGATCATCGAACACAAGCAAAACGA TTGAATTGTTGCGAAGCTTTCGATTAATTCTGCTCGAGTTTTTTTT CTGTTTTCTTGTTTCAATTTTTGGTGCCATCGGCAATATTTTATCG GATTTTTCAAGTTTTTCAAAGTAGCATTAGATCTCTCATTCTGAC ATCAGATTGAAATCAAATATTGTCTCACTCCATATTGAAAACTGT TCACGTTGAAGATTTAACAATCGAACAATTATCGAGCTAATTAT ATTGCAGATCATCTTCGTTAGATTGTTAATTAAATTTCGCCGATT GGAATTTGAATTTTTCTCTTTTTCATCACTGATAATATACAGTCTT TTACAATCCAATTTAAAATCGCAATCTAACGAGATGGAGAACAA CACTAATGAAACGAATAATATCGAACAGAAATCGGAACAAACA CCAGATGTTAGTGAATCGAACAAAATCGAAGAATTGAAAACTGA ACAAGTAGATCAAGCTACTAAAGATGA

EST	Size of EST	Sequence
ESSU0861	500 bp linear mRNA	AAAACAAGAAATTGAGCAGTGAAATGCAAAAGGCATTAGTTGG ATCACATCAAGATTGTGCTGAAGTGTCCAAAACTCAGAAGAAAC AATCGAAAACTCCAATGGATGTGGTTGAGCAAGTACAAATCTAT CAAGGATGCATGGATGCTCATATAACTCAACATTGTCAAATTAA AATTGGAGCCTAGAGATGATGATTTGCTTCGTAATGATTTGATTC ATTCTTTCTATCTATGCTAGTGTTTAAACTTTCATTTAAAAAACA AAAAAAAAATTCTCAAAGAATTAATAAACTTTCTGGTCGATCGA TTGTCCAAGAATAATTTTGAAAAAAAAAAAAAAAAAAAAAAAA CACGAGGGGGGGGCCCCAACCCAAAAAAAAAAAAAAAAAAATT TTAATNCCAAAANNNNNACCCCCCCAAGGGGGGGGGGGGGGTG GAAACATTTTTTATATACCCCCCCCCGGGGCGGGAAAAAAAAAA AAAATTTTTTTTTTTTT
ESSU0860	349 bp linear mRNA	TTTACTTCGCCATGTTCTGCATCGAATCTACTCAAAAAAAAAAAA AAAATTCTCAAAAATTTTCGAGGTGGCCATGGGTTCTGCCTAAAT CTTTTCTCATCACCAGAAACCATCATCTTTTAAATGGATAGCCTT TTGGCCGTTTTCCCCGGCAAAATTTTCCGGTTTCCATTATCCCCA CCTTCTCCCTATTTATCTTGGCATTACCCCACCTCATGGATCCGG GTCTTTAATAAACCCAAATTTATCTTGGGTTCCAAATTCGACCCT CACGAAATTTTTCTTTCGATTACCAGACCCCTTTGGACCACCTTT AAATCTTATTAAATTTAAGAAAAAAAAAAAAAAA
ESSU0859	455 bp linear mRNA	CCACAACGATAATAAAACATTTTTGATTTGGGTCAATGAAGAAG ATCATTTGCGAATCATTTCAATGCAAAAAGGGGGTGATCTTAAG CAAGTCTTCAGCCGATTGATCAACGGAGTCAGCCATATTGAAAA GAAATTGCCATTCTCTAGAGACAACCGTCTAGGTTTCTTGACTTT CTGCCCAACAAACCTTGGTACTACAATCCGTGCTTCCGTACACAT CAAATTGCCTAAATTGGCCGCAGATCGAAAGAAATTGGAAGAGA TTGCTGGAAAATACAACCTACAAGTCCGTGGTACTGCCGGTGAG CATACCGAAAGTGTCGGTGGCGTCTATGATATCAGCAACAAACG TCGAATGGGTTTGACTGAATACCAAGCCGTCAAAGAGATGCAAG ATGGTATTTTGGAATTGATTAAGATTGAGAAATCGATGTAAGAT AATAATCTCTGTC
ESSU0858	448 bp linear mRNA	CGATTTTCAAACTCTACTTTCAAGTTTTCTAATTTCAAAAGCAAA ATTTTGTAGCCAAATAAAAAAACTTTTAAAATTTTCACCGGAAG AAAATAAGTAAAAAAAGAAAAAACTATGAACCAATCGAATGAA TCAGTGAAGATTTTCTGCAAAGATTGCAACTGTGGTTGCAATTGT TCCAGTGATAAATGCAAATGCGATGGTTGTAAAGGATGCAAATG AATCCGTTTGGATCGTAATCGATCGTAATAAAGATATCAACGAT CTACTTCCTTCTTATGGAGCAAAAAAAAAAAAAACCCAAAGGG GGTGGGCAACCGGGTGAAAAAAGGGACCCAATCCCCCCGGTGG ACCCTGAACCGGGGGACAAAATTTGGAATGGATAAAATTGGTTC CCTTTTTTCCCAAAAAAAAAATTTTTGATTTTTTAACCAAAAAAA AAAAAAA
ESSU0857	507 bp linear mRNA	TTGCAATACCATTTTGAATGTAATAAAGACACCAATCTAATTTAT CTCTCTATTTGAATGTGAATATATGTGTATGTTTTCAGTTTTTAGT ATGTGTGCGTGTTAGCAAATAATCCATACTTGTTTGTCTGTCTCT GTCTAACTGTGTGTGTATCTCTTGTGTTGTATGTGTGTATGTTCTG TCTACAGCACAAAACAAAACAAAAAAATATCAACTGAAAAAAA GAAGTAAAGCTAAATGCTGATAGAAAGAAGTGAAAAAGAAAAG AAGAAAAAATCCTACAACCATAAAATAATATCTCATAAATAATA TCTCATCCTCATATTTAATCTCATCGATTGAGAATCAGCAAATCT TCGACCAACGACCTGTTCTTTTTTATATCTTGTTTTCAATAATATG TCTGAATACGCCGATTATCATCAAGCAATGGCGCATCATGCCGC TACATTGAATGCTAATCAATTGCACCCAGCCACGATGACACAGA TCCCAATGTCTCAAAT

EST	Size of EST	Sequence
ESSU0856	401 bp linear mRNA	TGGAAATAGAGATTGTCCTTTTGTTCTTCGTCCTGGTTGTGTTTGC AACGAAGGTTACGTTCGTACCCACAAAAATGGTAAAGGCAAATG TGTTCGTTTGAAACACTGCAAACATTGTCCTCAACATGAACGGA AAGTTCTTTGTAGAGGTCATTGTCAACCGACTTGTAAGAAACCTC ATCCTAAGTGCTCTTTGAAATGTAAGAAAGGTTGCAGATGTAAA TTGGGCTTTGTTCGACAAAGACATCATGGTCCTTGTATCAAATGG AAATATTGTAAAAACAAGAAAAGTTTTTTTAAATTTTTCTTCAAA AAATAATTCCACTAACTTCAAAATTCCATTTGATTCAATTCTCTA CTGAATAGAGATGTAATTAGATGAAATCAAAAAAAAAAAAAAAA
ESSU0855	541 bp linear mRNA	TCGAAACATTGAAGAATATCAAAGCTTGTCAGTGGCATTGGGAT GAGGATGCGTTCAAAAACATCTCGGATGAGGGAAAAGATTTTGT TCGAAGATTGTTGGTGAAAGAGAAAGAGAAACGAATGACTGCA CATGAATGTCTTGAACATCCTTGGCTAAAACAAACCGATGTACA ACGAACCGATAGTATTTCCAATCGCAAATATCAAGATATTCGAG ATAGAACACGTGCCAAATATCCGATGTGGGATAAAGCCATCGTA CCATTGGGACATTCCGCTAATTATAGCTCATTGAGAAAGCTTCAA GATGAAAAATATCGTTTGCATGATGTTTTCTTGGATCGACGTGAG ATGTTACCGAGATTCGTTTTGAAACCACAATCCACTATGGTTTAT GAAGGCCAATCAGCGAAATTCTATTGTCGAGTGATTGCTGAAGC ACCGCCAATGTTGACTTGGTATCGCGAGGGAGCCGAATTACGGC AATCGGTGAAATTTATGAAACGATACGCAGAGAGTGATTTCACT TTCATCATTAA
ESSU0854	519 bp linear mRNA	CTCGTGCCGTGGCAGCTGGCGATCAAAACTTTGGATCTCCAAGC ACCAAATTGAGCATGAAGAATTCAAACACTGAAAATTCTATGTA TCTATCAATGGAACAGTTGAAAAAGACTCTTATCCATCTGTTGAA AAATGATGCCGATTTTTTACATGCCATTCATACTGCTTATGTCGA AAATATCCGCAAATAATCTTTAATTTTTTCAATCATAAATTGGCA AAATTTTCATTCTTATATCCTCTTGTAATGGCACGTTGCGAACTT GATCAATTTTCACTCATCATTTGATTGATTCCTCTTGAAAATTCTT CCGAAATTCTAGAAGTTTTTTTTTGTTCTCAATTTAATCTAACTCT AAACTATCGACAAAGATTGGATAATTCTATTTTTCATTCCCCAAT CATTCATTTATCGATTTGTTTTCTATATGAGAAAATATTTTGTTTT ATTTGATTTTAAGATTAAGATGAGAATTAAATGATCTATTTTTCA AGTAAAAAAAAAAAAAAAAAAA
ESSU0853	468 bp linear mRNA	AAATCATTAGCTACTTAATTGGTAACTTGTATGAAGGGACTAACT AAAAATTTATTTTATAAAAAATATAAATTATAAAATTGTTGAAA ATAACAATATTTATTCCAAGACAAAAAGACCCTAGAATTTTAAT GAAAATTTTCATTTAGTTGGGGAAATAAAATTAATAAAATAAAA ATAAAAAAATTTATAAAAGAACTTTTTATAAAGAAAAATTGACT AAATACTCTAGGGATAACAGCTTTATATTTTTTAAGAGAACTTAT TAAAATAAAAGTTTAAGACCTCGATGTTGGATAAAAGTTTTTATT AAGCGCAGAAGCTTAAAAAAATGGATTGTTCATCCATTAAATCT TTACTTGATCTGAGTTAAAGTCGGCGTGAGCCAGACTGGTTTTTA TCTGGAATAAAATTATATAAATAAACAGTCCGAAAGGACAATTA TTTTTTCTTAAAGAAATTTATTTT

EST	Size of EST	Sequence
ESSU0852	572 bp linear mRNA	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTCCCGGAAAAAAAAAAAAAATTTTTTTTTTTTCTTAAAAAA AAACCCCCAAAAAAGTTCCCTTTTTTTTTTCCCCCCCCACACAAA AAAAAAGGGGGGGGTTTCCCCCCCCAAAAAAATTCCCCCCCCAAAT TTTCCCCCCCCCCCAAAAAAAAAAAAAAAGCCCCCCCATTTTTTTTT AAAAAAACCCCCCCCCCCAAAAACGGGGGGGGGGGGTTTTCCCCCC CCCCCCATAGGGGGGGTTTTTTTTCCCAAAAAAAAAAAAATTTTT TTTTTTCCCTTTGGGCCCCCCCCCCCCCGGGAAAATTTGGGGGGA AAATTCTTTTCCCCCCCCCCCCAAAAAAAAAAACCTTTTTTTTTTT TTTTTCCCCCCCCCCCCCCCCGGGGGGGGAAAAAGAAATTTTTTT TNNNCAAAAATTAACCCCCCCCCCCCTTTTTTTTAGGCCCCCCCC CCTTCAAAAAGGGGAAAATAAAAAAAAGGGTTTTTTTTTTTAAA AAACCCCGCCGCACAAAATTTTTTTTTTTT
ESSU0851	567 bp linear mRNA	CGGCTACACTCAAGGCGTTGTCGCTCAACCAGTCACCGCTAGCT ACGGATACCAACAAAAATTGATCGCTCAACCAGTCTCAACCATC AGCACTGGTCTTGTCGGTGGAACCGGCCTCGTTGGTGGCTCCGGT CTTGTCGGCGGTAGCGGATCCTCATTCGGATTCGGACCATCATCA GTCGGAACCAGCTTCCAAACCACTGGTTTGGTTAGCAAACCAGC TTCAGTCTCCACCTCGTTCACTACCACTGGTGCTAAGCCATTGAC CTCAACCAGCTTCTCGGGAACCACCGGAACAACCTCAACCACCT ATTAAATTCCTGAAACTGCCAATAGCTGATGTTTTAGTCTAAGAT TCTCTAATTTTTGAAAATAATTCTCATTGTTGCATTCTGCCAGAT ATTTTGTTCTCTCCTCTTTCCATCAACAAAAACATAAAAAATTTT GTTGAAAAACTTTCTCCTTTGAAAAAAAAACCAACAATATTCTG AATGAATTGAAAAAAAAATTCAATAAAAAGGTCAAATTGACTGG TTTTTGGAAAAAAAAAANNNNNAAAAAAAAAAA
ESSU0850	554 bp linear mRNA	ATCATCAAGTCCGATTGTCTTTTGATGCTTTTGGTGATGTTTTACA GCCTCAAAATGTTTCTTCGAACGTTAATAGCAACAGTAATGATA ATTTGTTGTTCAATTCTAACATGACCAGTTTGTTTTCTAATGAAA TCAATCATACCGTGACAACAAAAGATAAAATTACAACGACAGCA ACATCAACATCAACATCATCATCGACTGTGACGACCAGTTCTTCT GGCTTGATAAAAGGTGATCTTGATGCTGCACTTGCTTCATTGGCC CAAAACCTTGAAATAAATGGTTTCAATAAAACGCCAGGAAATTT TAAAAAAGGGTTCAATTCTAATCAAAAAATCGCATGGAAAACCT CTGGTGGAAATGCGGCAAGCGGGAACTCTTTGCCCACATCGACA TCGTTGAGCGGTAGCTCTATTTCTCCATCAATGAATATGATGGTT AGCAGTGGTAGTAATATTAGCTGGAACACAAATCAATCTCTCGG ACAATCTTCTAAAATTGATTGGGCTGTCAAGCAGTCACCAATGC CAATGTTGAATCAAAACACT
ESSU0849	606 bp linear mRNA	TTCGAATTCGATAAAGATTTTAGGCCAAGCATAGCATTCCAGCA TATCGGCTACGATCATTATGCCTCCAGCCGTAATATGGTCATCTT GATCGAACAAAAACTTCGAATACAATCAGATTTCGATGTCAATA ACCAGACAGTTTTTCATCGTCTCTTCACCAGACGTTGGCAAATCT ATTCGTTCGATAATCTCAATAGAATCACAAATCTAACCTATAGTG CCATAATTCCAGATTATCTCGAAACGATACCACCACAGCTTGAA TTGACTGGAGTTTTCGGTTGGACTAGAAGAAAAACGAACGAAGA TGAATTTCTAATTCTCACTTTCAATGGATCCAATCATCTCTACTGT TTGAATGAGAATTGTGCCAATATTGAAAACTATAGAGTTTATCTA ACCGAATGTAACGCTCCAAGTGAGCTGATTCGAATGAATCAAAA CGATTTCGAATCTTTTAATCGAGGTCTTATCGTCACTAAATGCTG TGGTCAATGGCGTCAGTTTGATGAGGACTACGACAATAACAACA ACAACGCATTCGATGTCAATGATGCTAGATGGAAATTAAAAGAT GGAAAGCTCTATCTAATCAAGTCAGCT

EST	Size of EST	Sequence
ESSU0848	580 bp linear mRNA	CCGAATGAAATTTGAAAATTTTTACTAAATAATTATAATTCTCTG TGTTTGAAATCAAAAAATAAACTTGCACAAATACAAAAATGGTC AAACCAATTTTCAAACCTACGATCGTTAAAAAACGAACCAAAAA ATTTCGTCGTCATCAATCAGATCGATATCGAAAATTGAAGATGA ATTGGAGGAAGCCAAAAGGTATCGATAATCGAGTAAGAAGACG ATTCAAGGGGCAGATTTTGATGCCAAACATCGGTTATGGGTCGG CTAGAAAAACTAAACACATGCTACCGAATGGTTTTCGTAAAGTT TTGGTCCATAATGTCAAGGAACTTGAAGTCTTGTTGATGATGAAT CGAAGATTTTGTGCCGAAGTAGCTCATTCAGTTTCATCTAAAAAA CGTAAAGACATTGTTGAGCGAGCCAGACAATTGTCCATCAAATT GACCAATGGTTCAGCTCGTCTTCGTACTGAAGAGAATGAATAAA CTTGATGACTTTTTCTCTATCATTCATCAAACTTCTTGTTATATTA AGATTAAATTAAAATTCCCTGTCTTTTTTGAAAAAAAAAAAAAA AAA
ESSU0847	367 bp linear mRNA	AAACAAATCTCTCTCTCTCTCTCAGTGAATCCCTGCCAAAGATCA TCTCCTCTTGTCAAACGGAATCAAGCAGAAAATAACGGAATAGA TTCTTGTTTTCTTTCTCTCATTTTTTCTCTCTTTCTCAATGAAACAA ACCATTCTTTGTTTGATTGAAAAAGAAGAAAATAATCCTTTGTAC AATTGTGTGAAACAGTCATAAATCAAAAAAAGAATGATGATGAT GATGATTTGATGATTTGATGAAGCCATCATCGATCGAATGAATA AATATATTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA
ESSU0846	563 bp linear mRNA	ATTTCTGCTTCAATCGAATAAGATTTAAATAAATTTTATTTTAAT TAAAAATGGCGGATGAAGTTAAAGAAAAGAAGAAGAAAAGTAA AAAATCATCGGTGAAAGCTGCAGCTGCGGAAGCTGCTCCTGCAG CCGACCCAGCACCAGCGGAAGAACCAGCAGCAGCACCGGCTCC AGCTCCAGCTGCTCCAAAACCAAGCTCTACCAAGAAACGTGCTC AAAGGACTGGATCCAATGTGTTTGCAATGTTCACTCAGCATCAA GTGCAAGAATTCAAAGAAGCTTTCCAGTTTATTGATCAAGATAA AGATGGTTTCATTTCGAAAAACGATATAAGAGCCACTTTCGACT CTCTCGGTCGTATTTGTACCGATGCCGAATTAGAATCGATGATCA AAGAAGCACCTGGTCCAATCAATTTCACAATGTTTTTGACTATTT TCGGTGATCGAACCCAAGGCACTGATGAAGAAGAAGTTATTCTC AACGCTTTCGCGCAATTCGATGAAGGTGAAGGATTGTGCAAAGA GGAAACACTTCGTCATTCACTAGTAACATGGGGA
ESSU0845	514 bp linear mRNA	GGTAGTACCTATACTTTGGGTGGTTACCGAACATCTGGCCTCACT GGTCTCGCTGGTCTTGGTGGTTTGACTTATGGTACCGGATATGGT CTTGCAGGTCTCGGTGGTTTGACCTATGGTGCTGGTTATGGTTAT GGCCGAACTCTTGCTTTGTCTGCTGCTCCAGCTGTCCAATTGGTT GCTGCTGCTCCAGCCGTTGCCGCTGCCCCAGCTGTCCAATATGTT GCTGCTGCTGCTCCAGCCGTTGCCGCTGCTCCAGCCATTGCCGCT GCTCCAGCTATTGCTGCTGCTCCAGCTATCACCAGCTTCGCTGCT GCCCCAGCTGTCCAAACTACCCAAGTTACTGGTCCAGTCGCTGCC GCCATCGAAACTCGACGAACTGTCGAAGTTGTTGATGTCCCAAC CCAAAGCGAAGGTGCTGCTGCCCAAACTGTTGTCATTGGACCAA ATGTTCAACCAATCAACTTGGAATTCCAAACTCAAGCCAGCCCA TTGGCCGCTACTCAAAACCACT

EST	Size of EST	Sequence
ESSU0844	564 bp linear mRNA	ATTTATTGATTGATGCTTTTCGAGAGAATCTATCAACAAGATAGA ATTAAGTCTATTATATTTCTCAAATTGAAAATAATCAAAATTGGT AGTCTTTAATTCAAGATGGTCAGCAATAAGCAGAGAACTGACAA GAAGAATAAATCTACATTGAATGATGTTATAACAAGAGAGTATA CCATTCATCTGCATAAACGGTTGCATGGAGTCGGTTTCAAGAAA CGTGCACCAAGAGCTATCAAAGAGATCCGAAAATTCGCTCAGAA ACAAATGTGCACCGAAGATGTCCGAATCGATACCAGATTGAATA AATACATTTGGTCACAAGGAATCCGAAACGTTCCATTCAGAGTA CGTGTTCGATTAGCTAGACGAAGGAATGAAGATGAAGATAGTGT ACATAAATTATACACTCTTGTAACATTGGTTGCTGTACGTTCATT CAAAGGGCTACAAACTGATAATGTTGATGAATCAACCTAAATAA TTTTGAAGAATGTTTTTTGAAATCTCTAAATTTCCAAATAAAATT GAATGTTTGGATAAAAAAAAAAAAAAAAAAA
ESSU0843	505 bp linear mRNA	TGCCTGTTGAGATTGAGAAAATTCAAACATGAATATCAAAGAGT GATTCTACAAGCAATGAAATGTCATTTCTCGACCTCAGATCAAG CGAGAATACCCGCTGAATTTAAGCATATAAATAAGCGGAGGAAA AGAAACCAAACGGGATTCCCTTAGTAACGGCGAGCGAAACGGG AACTGTCCAGCGCCAAGTCTTGACACTCAAGAGTGCTCAAGAGA TGCGGCGTTAATGTGTTGGTGCATCTTGTATTGTCCGATGATTCA AGTTCCCTTGAACGGGATTTCCGGAGCGGGTGCCAGACCCATAG AATCGATGGACTGATATGAGCAAACCAATACATTCTAGAGTCAG GTTGCTTGAGAGTGCAGCTTAAAGTCGGTGGTAAACTCCATCCA AGACTAAATATTGCAGCGAGACCGATAGCAAACAAGTACCGTGA GGGAAAGTTGAAAAGCACTTTGAAGAGAGAGTTCAATAGCACGT GAAACCACTGGGAGGCAAACA
M4B6.ab1.bin	726 bp linear mRNA	TTGAGCGAGGCACTTTAATCCCTGTCTGAATCCACGTTGCCTGCC GGAGCACCGAATATAACTGCGCTAAAGAAAGTTCACGTCTGCGA GCTCTTTAGAGTTTCGACGAACTTGAGTAAAGGAATTCGACCAC TCACAGCACGAATAACTAGGCTCCGAGCATGGCCACTCCTACCG TAGGCTACACAAGCGCCAGGGGTCTGGCCCAAAGCATCCGCAAC CTGCTCGTGTACAAAGGGGTGCATTTCCAGGACAAGCGCTACGA GTTTGGACCCGCACCGACCTACGAGAAGCAGGGCTGGGCCGCTG ACAAAGCATCGCTGGGACTGGCCTTCCCGAATTTGCCCTACTAC ATTGAGGGAGACGTCCGACTCACGCAGACCGTCGCCATTCTGCG CTACCTCGGAAAGAAGCACGGTCTTGACGCAAGGACTGAGCGGG ATGCCGTGGAGCTGTCGCTGCTGGAGCAGCAGGCACACGACCTG CAGTGGGCCCTCGTGCTGACCTCCATGAACCCCAACACGACCCA GGCCCGCGAGTCACACGAGCGGAACTTGGCCGACTCCCTGAGCC AGTGGCAGAAGCACCTGAAGACGCGAAAGTGGGCCCTCGGAGA CTCGCTCACCTACGTCGACTCCCTACTGTACGANGGCGTTTGACT GGAACCGCCATTTTGCTCCCCGGGTGTTTTGAGGGCCGTCCCGAG ATCCCTGACTACTTGAAGAA
Mg_AFT_03F05_M13F	479 bp linear mRNA	GGGGAACCTGAGCTTGTCCAACTTCTCCACCAGCGGGGGGAAAG GAAATAGCACGTGGGGACCGAATTTAAGGCATTAAGTCGAAACA AGCTTGTGGAGTGTGGCAGTGAATCGCTGTGTGAACATCCCGCA ACTTACGAATCCAAGATGTCTTCAGTCAAGACCACTAAATATGT CTACCGTTCTACTACTGGAACGTCCGGAGACGTTTCCGTCGGGTA CGGTACGGATCTCGGAGCCCTGACTCGTTTAGAGGATAAGATCA GGCTGCTGCAAGAAGATCTAGAATTCGAGAGGGAACTGCGACA AAAGATTGAAAGAGAAAAATCTGAGTTAACAGTTCAGCTCTTTT CCGTTAGCGACCGATTGGAAGAAGCAGAGGGAAGTTCCGAAAC CAACGTCGAGCTTAATAAACGAAGAGATGCCGAACTCGCCAATT TGCGCAAACTGTTGGAGGATGTTCACTTGGAGAGCGAGGA

EST	Size of EST	Sequence
Mg_AFB_01G03_M13F	455 bp linear mRNA	GGGGGTGGAGTGTGGCAGTGAATCGCTGTGTGAACATCCCGCAA CTTACGAATCCAAGATGTCTTCAGTCAAGACCACTAAATATGTCT ACCGTTCTACTACTGGAACGTCCGGAGACGTTTCCGTCGAGTAC GGTACGGATCTCGGAGCCCTGACTCGTTTAGAGGATAAGATCAG GCTGCTGCAAGAAGATCTAGAATTCGAGAGGGAACTGCGACAA AAGATTGAAAGAGAAAAATCTGAGTTAACAGTTCAGCTCTTTTC CGTTAGCGACCGATGGGAACAAGCAGAGGGAAGTTCCGAAACC AACGTCGAGCTTAATAAACGAAGAGATGCCGAACTCGCCAAATA GCGCAAACTGTTGGAGGATGTTCACTTGGAGAGCGAGGAAACTG CCCATCACCTCAGAAAGAAACATCAAGAGGCCATCGCCGAGATG CAAGACCAAATCGAAA
ESSU0202	474 bp linear mRNA	ATCGAAGGCCCCAAAATTGATGAAGAACAAATGGAAAAAATTG AATATTGGGCACATGTTCATGCGATCACAACCAGAAATATGACT GGCTTTCGTAAATACAATAAAATAGCTCTAAAATTGAAAGCTGA TCTCGATCGCTTTTACGGCAATGGTAATGTTTGGTCGGTGGTGAT CGTTAAGAACCCTTTTCTTGTCAAAGCAAAAATTTTGGAGGATCC AGAATATCATTTAGAGTTCTCTAGACTGAATGGTGGTCATTTCAT TGTTTGGCGTGGTGGACAGAACATCGAANATGGCCAACGCAACG CTAGAGATTTGATCATACCGGAAACTGAAGACAATCTTCTTGAA CCAGAAACCGAGCATGAAGACTATCGAAACAATATCTATCTGAT GACCTATCGGTGATAGTTTTTTTAGTATTTTCCATTTTGTAAATAA GAATCCATTCTGATTAAACGTCCATTTTTT
Mg_AFT_02F07_M13F	478 bp linear mRNA	GGGGAACCTGAGCTTGTCCAACTTCTCCACCAGCGGGGGGAAAG GAAATAGCACGTGGGGACCGAATTTAAGGCATTAAGTCGAAACA AGCTTGTGGAGTGTGGCAGTGAATCGCTGTGTGAACATCCCGCA ACTTACGAATCCAAGATGTCTTCAGTCAAGACCACTAAATATGT CTACCGTTCTACTACTGGAACGTCCGGAGACGTTTCCGTCGAGTA CGGTACGGATCTCGGAGCCCTGACTCGTTTAGAGGATAAGATCA GGCTGCTGCAAGAAGATCTAGAATTCGAGAGGGAACTGCGACA AAAGATTGAAAGAGAAAAATCTGAGTTAACAGTTCAGCTCTTTT CCGTTAGCGACCGATTGGAAGAAGCAGAGGGAAGTTCCGAAAC CAACGTCGAGCTTAATAAACGAAGAGATGCCGAACTCGCCAAAT TGCGCAAACTGTTGGAGGATGTTCACTTGGAGAGCGAGG
Mg_AFB_09G02_M13F	531 bp linear mRNA	GGGGAACCTGAGCTTGTCCAACTTCTCCACCAGCGGGGGGAAAG GAAATAGCACGTGGGGACCGAATTTAAGGCATTAAGTCGAAACA AGCTTGTGGAGTGTGGCAGTGAATCGCTGTGTGAACATCCCGCA ACTTACGAATCCAAGATGTCTTCAGTCAAGACCACTAAATATGT CTACCGTTCTACTACTGGAACGTCCGGAGACGTTTCCGTCGAGTA CGGTACGGATCTCGGAGCCCTGACTCGTTTAGAGGATAAGATCA GGCTGCTGCAAGAAGATCTAGAATTCGAGAGGGAACTGCGACA AAAGATTGAAAGAGAAAAATCTGAGTTAACAGTTCAGCTCTTTT CCGTTAGCGACCGATTGGAAGAAGCAGAGGGAAGTTCCGAAAC CAACGTCGAGCTTAATAAACGAAGAGATGCCGAACTCGCCAAAT CGCGCAAACTGTTGGAGGATGTTCACTTGGAGAGCGAGGAAACT GCCCATCACCTCAGAAAGAAACATCAAGAGGCCATCGCCGAGAT GCAA

EST	Size of EST	Sequence
Mg_AFB_08F10_M13F	446 bp linear mRNA	GGGGGGAGTGTGGCAGTGAATCGCTGTGTGAACATCCCGCAACT TACGAATCTAAGATGTCTTCAGTCAAGACCACTAAATATGTCTAC CGTTCTACTACTGGAACGTCCGGAGACGTTTCCGTCGAGTACGGT ACGGATCTCGGAGCCCTGACTCGTTTGGAGGATAAGATCAGGCT GCTGCAAGAAGATCTAGAATTCGAGAGGGAACTGCGACAAAAG ATTGAAAGAGAAAAATCTGAGTTAACAGTTCGGCTCTTTTCCGTT AGCGACCGATTGGAAGAAGCAGAGGGAAGTTCCGAAACCAACG TCGAGCTTAATAAACGAAGAGATGCCGAACTCGCCAAATTGCGC AAACTGTTGGAGGATGTTCACTTGGAGAGCGAGGAAACTGCCCA TCACCTCAGAAAGAAACATCAAGAGGCCATCGCCGAGATGCAA GACCAA
Mg_AFB_07C05_M13F	565 bp linear mRNA	GGGGAACCTGAGCTTGTCCAACTTCTCCACCAGCGGGGGGAAAG GAAATAGCACGTGGGGACCGAATTTAAGGCATTAAGTCGAAACA AGCTTGTGGAGTGTGGCAGTGAATCGCTGTGTGAACATCCCGCA ACTTACGAATCCAAGATGTCTTCAGTCAAGACCACTAAATATGT CTACCGTTCTACTACTGGAACGTCCGGAGACGTTTCCGTCGAGTA CGGTACGGATCTCGGAGCCCTGACTCGTTTAGAGGATAAGATCA GGCTGCTGCAAGAAGATCTAGAATTCGAGAGGGAACTGCGACA AAAGATTGAAAGAGAAAAATCTGAGTTAACAGTTCAGCTCTTTT CCGTTAGCGACCGATTGGAAGAAGCAGAGGGAAGTTCCGAAAC CAACGTCGAGCTTAATAAACGAAGAGATGCCGAACTCGCCAAAT TGCGCAAACTGTTGGAGGATGTTCACTTGGAGAGCGAGGAAACT GCCCATCACCTCAGAAAGAAACATCAAGAGGCCATCGCCGAGAT GCAAGACCAAATCGAAATGGCCAACAAGAGCAAGATCA
Mg_AFB_03B10_M13F	548 bp linear mRNA	GGGAACCTGAGCTTGTCCAACTTCTCCACCAGCGGGGGGAAAGG AAATAGCACGTGGGGACCGAATTTAAGGCATTATGTCGAAACAA GCTTGTGGAGTGTGGCAGTGAATCGCTGTGTGAACATCCCGCAA CTTACGAATCCAAGATGTCTTCAGTCAAGACCACTAAATATGTCT ACCGTTCTACTACTGGAACGTCCGGAGACGTTTCCGTCGAGTAC GGTACGGATCTCGGAGCCCTGACTCGTTTAGAGGATAAGATCAG GCTGCTGCAAGAAGGAACACCGAATTAAGGAACTGATTGTCCAA GCAGAAGAGGACCATCAAACCTTAGTTGTTTTGAATGATTCCGT GGACAAACTGAGCGAAAAATGCAAGATGTACAAGCGACAATTG GTAGAACAGGAGGGAATGTCTCAACAGAATCTGACCCGAGTGCG TAGATTCCAGAGAGAATTGGAAGCAGCCGAAGAACGTGCAGAT CAGGCCGAGAGCAACCTGAATTTGATTCGTGCCAAGCACAGGTC CTGGGTAACGACGAGCCAAAT
Mg_AFB_01G01_M13F	220 bp linear mRNA	GGGGGCAGTGAATCGCTGTGTGAACATCCCGCAACTTACGAATC CGAGATGTCTTCAGTCAAGACCACTAAATATGTCTACCGTTCTAC TACTGGAACGTCCGGAGACGTTTCCGTCGAGTACGGTACGGATC TCGGAGCCCTGACTCGTTTAGAGTATAAGATCAGGCTGCTGCAA GAAGATCTAGAATTCGAGAGGGAACTGCGACCAAAGATTGAAA

VITA

Noel Michael Cote'

Candidate for the Degree of

Master of Science

Thesis: IDENTIFICATION AND EXPRESSION OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) IN *SARCOPTES SCABIEI*

Major Field: Entomology and Plant Pathology

Biographical:

Education:

Completed the requirements for the Master of Science in Entomology at Oklahoma State University, Stillwater, Oklahoma in May, 2010.

Completed the requirements for the Bachelor of Science in Professional Aeronautics at Embry-Riddle Aeronautical University, Oklahoma City, Oklahoma in 2005.

Name: Noel Michael Cote' Date of Degree: May, 2010

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: Identification and expression of Macrophage Migration Inhibitory Factor (MIF) in *Sarcoptes scabiei*

Pages in Study: 76 Candidate for the Degree of Master of Science

Major Field: Entomology and Plant Pathology

Scope and Method of Study: The two objectives of this study were: 1) the cloning and sequencing of Macrophage migration inhibitory factor in *Sarcoptes scabiei*, 2) the cataloging of the existing genes and expressed sequence tags (EST) for *Sarcoptes scabiei*.

Findings and Conclusions: Macrophage migration inhibitory factor (MIF) was sequenced from *Sarcoptes scabiei,* the scabies mite, using RT-PCR and RACE molecular techniques. The resulting nucleotide sequence had a length of 405 base pairs. A partial portion of the 18S ribosomal RNA gene was also sequenced from the scabies mite. The partial 18S nucleotide sequence had a length of 98 nucleotide base pairs. The intial steps for the project resulted in the production of a scabies mite cDNA expression library. A real time (qPCR) assay was performed with MIF from scabies mites and various tick species. The results showed that the scabies MIF was expressed three times more than that of the control of *Dermacentor variabilis* salivary gland MIF and 1.3 times that of *D.variabilis* midgut MIF. Finally, a catalogue of *S. scabiei* partial and full length gene sequences and expressed sequence tags (ESTs) was compiled to assess what is known about *S. scabiei* at the level of gene expression and to provide context for research.

ADVISER'S APPROVAL: Dr. Deborah C. Jaworski