Host specificity and species boundaries of beetle-associated mites

by

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in

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Abstract

Understanding the evolution of host specificity and the determinants of host range are fundamental challenges to the study of the evolutionary ecology of symbionts. Host specificity is a continuous variable, and it is shaped by the biogeographical and evolutionary history of species. The study of host specificity is constrained by how species are defined, which requires a thorough understanding of species boundaries. The objectives of Chapters 2 and 3 were to explore the species boundaries, the extent of cryptic diversity, the evolution of specificity (only Chapter 3), and the degree of coevolution of mites (Mesostigmata: Uropodoidea) associated with carrion-feeding beetles (Silphidae: *Nicrophorus*), and bark beetles (Scolytinae) respectively, using molecular and morphological characters. In Chapter 2, I found that the widespread host generalist, *Uroobovella nova* (Uropodoidea) collected from 14 *Nicrophorus* species across six countries, actually represents at least five species with varying levels of specificity, as shown by morphological characters, as well as cytochrome oxidase I (COI) and internal transcribed spacer 2 (ITS2) markers. Additionally, it appears that associated uropodoids may be coevolving with their silphid hosts. In Chapter 3, morphometric analysis, as well as COI and large subunit ribosomal DNA (28S) markers, indicated that host generalist uropodoid species associated with scolytines, are truly generalists, with one exception. The majority of uropodoid species were specialists, and there was no evidence that the evolution of host specificity of uropodoids, collected from 51 scolytine species across 11 countries, is directional or irreversible. There was little evidence of coevolution or ecological tracking in associated uropodoids; however, it is possible that

the evolutionary history of this group reflects a blend of both factors. In Chapter 4, the acarofauna (Mesostigmata, Oribatida) of scolytines was surveyed across four sites in Eastern Ontario over two years. The findings indicate that Ontario scolytines are associated with a diverse and common acarofauna, and the majority of species are specialists. I used this dataset to explore some of the additional obstacles surrounding the study of host specificity. Overall, the findings of my thesis research further our understanding of species boundaries, cryptic diversity, and the evolution of host specificity in symbiotic taxa.

Preface

I have followed the integrated thesis format, and each chapter has been formatted as an independent research article, however none of the chapters have been submitted for publication at the time of thesis submission. I have attempted to reduce redundancy in the introduction, methods and discussion sections across thesis chapters. My contributions to the research performed in this thesis were as follows: I proposed and developed the specific concepts and research objectives in collaboration with Drs. M. R. Forbes and F. Beaulieu. I was responsible for the design of each research project used to explore the thesis concepts. I was primarily responsible for the development and deployment of the field sampling protocol and laboratory methods used in each thesis chapter. I performed all insect and mite identifications. In the summer of 2009, I co-supervised a summer laboratory assistant, T. Hartzenberg, who assisted me with field collections, processing samples, isolating mites from beetles, as well as assisting with slide mounting mites from *Monochamus scutellatus* beetles. I was trained in molecular techniques by S. Kelso at Agriculture and Agri-Food Canada, after which I was responsible for performing these techniques and acquiring molecular data independently. Dr. J. H. Skevington provided me with advice and direction throughout my molecular research, and provided editorial comments on Chapters 2 and 3. Dr. A. I. Cognato allowed me to examine and remove uropodoid mites from preserved bark beetles in the A. J. Cook Arthropod Research Collection, which were used in Chapter 3. A. I. Cognato also provided me with editorial comments on Chapter 3, and useful feedback on the bark beetle phylogeny used for the coevolution aspect of Chapter 3. I have performed all of the data analyses in each

chapter, I also wrote the first draft of each thesis chapter, and incorporated subsequent revisions. M. R. Forbes and F. Beaulieu have provided feedback and editorial comments on all of the thesis chapters. To reference my thesis as a whole, I recommend the following citation:

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I thank the countless colleagues, friends and family members that I subjected to my discussions of everything mite related, I thank them for enduring such talks whether they wanted to or not. I also thank my Forbes lab mates for their help along the way, the Forbes lab covers a broad range of research interests, and this diversity provides a fresh perspective. I am eternally grateful to my wife, Georgia Pettit, and my parents, Bessie and Hubert Knee, for their never-ending support and encouragement throughout my

studies. I thank Georgia for enduring through this process with me, for providing an optimistic point of view, and for always being there to listen.

Numerous researchers and individuals graciously sent specimens from around the world, and these specimens expanded the reach and impact of the findings in the molecular chapters. I particularly thank Dr. Anthony I. Cognato and the Michigan State University A. J. Cook Arthropod Research Collection, who allowed me to collect mites from their preserved bark beetle material from around the world. I also would like to thank Hume Douglas and CFIA Invasive Alien Species Monitoring program for allowing me to go through their collections of Canadian bark beetles. I am forever grateful to my father, Hubert Knee, and uncle, Ted Knee, for collecting bark beetle and cerambycid specimens for me in Northern Alberta. I would like to thank B. Jones, J. Dombroskie, G. Smith, and R.J. Buss for donating bark beetle specimens from various locales. I would also like to thank D. Johnson, D. Sikes, J. Müller, J. Sweeney, M. Nishikawa, M. Schilthuizen, M. Scott, R. Dawson, S. Peck, S. Suzuki, S. Trumbo, M. Locke, W. Hoback, and W. Hunting for providing me with silphid beetles from around the world. I also thank the private land owners who allowed me to trap bark beetles on their property.

This research was conducted with a permit to collect in Provincial Parks issued by Ontario Parks to W. K. This study was funded by an NSERC Discovery Grant to M. R. F., and an NSERC CGS D award to W. K. The bark beetle molecular study was also funded in part by the USDA-FS EDRR program (# 07-DG-11420004-182) and by US-NSF (DEB-0328920) to A. I. C.

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CHAPTER ONE

General Introduction

General Introduction

Host specificity and species boundaries

A fundamental question of the evolution and ecology of host-symbiont associations is why some species have narrow host ranges, while others are associated with a broad range of hosts (Van Tienderen 1991; Kassen 2002; Krasnov et al. 2004; Poulin et al. 2006). **Host specificity**¹, or host range, operationally defined as the number of host species used by a symbiont at a given life cycle stage (Poulin and Keeney 2007), has received extensive interest due to its linkage to testing ideas, such as local adaptation (Lajeunesse and Forbes 2002), **coevolution** (Paterson and Banks 2001), evolution of discrimination (Brown and Wilson 1992), and the expression of resistance (Poulin and Combes 1999). The evolution of host specificity is a continuous variable, and it is ultimately a function of the biogeographical and evolutionary history of a symbiont and its host species (Poulin 2007).

The study of host specificity is constrained by how species are defined and by our understanding of species boundaries and the extent of cryptic diversity (Bickford et al. 2007). The definition of a species is subjective, and there are many approaches to defining species, the best of which remains contentious (Coyne and Orr 2004). Thorough knowledge of species boundaries is fundamental to understanding the evolution and ecology of host-symbiont associations. Parasite and symbiont identifications have been historically based on morphological characteristics. However, in some taxa, morphological characteristics alone are ill-suited for delineating species boundaries, especially in groups that are poorly understood taxonomically, or those which include

¹ Refer to Appendix 1.1 for the operational definitions of terms in bold.

cryptic species, which are defined as species distinguishable by no or very subtle morphological differences, and using morphology in these groups may provide a poor assessment of host specificity (Donald et al. 2004; Locke et al. 2010). Elucidating species boundaries has an impact on many fields, such as biodiversity conservation, pest management, and biocontrol (Bickford et al. 2007). An understanding of the extent of cryptic diversity and host specificity of a given species is particularly important in order to accurately model biodiversity losses through coextinction events, since the fate of a **specialist** is linked to that of its host (Koh et al. 2004).

Modern molecular techniques are often more effective than morphology-based approaches, when exploring cryptic diversity and species boundaries (Navajas and Roderick 2008). However, the best approach is an integrative approach combining morphological and molecular techniques (Bickford et al. 2007). Over the last two decades there has been an exponential increase in molecular-based research on species boundaries and cryptic species, largely due to the increased access to nucleotide sequencing (Bickford et al. 2007). The use of molecular techniques has had a massive impact on our ability to classify, describe, and quantify biological diversity (Caterino et al. 2000). However, molecular data may provide misleading results when attempting to delineate species boundaries in cases where there has been hybridization and gene introgression, incomplete lineage sorting, recent time of divergence, or *Wolbachia* infections (Hurst and Jiggins 2005; Schmidt and Sperling 2008).

Molecular techniques are frequently used to assess species boundaries of parasitic and symbiotic taxa, and many studies have shown that species with broad host ranges are

actually groups of cryptic specialist species, but in some instances **generalists** are shown to be valid species (Donald et al. 2004; Stireman 2005; Smith et al. 2007). For example, the barcoding region of mitochondrial cytochrome oxidase I (sensu Hebert et al. 2004), indicated that 16 generalist morphospecies of parasitoid flies (Tachinidae) from Costa Rica, were actually a complex of 9 generalist species and 64 specialist species (Smith et al. 2007). Mitochondrial 16S and nuclear internal transcribed spacer 2 markers indicated that an apparent generalist digenean morphospecies, endoparasitic on trochid snails in New Zealand and Australia, is not a single species but it is actually two specialist and two generalist species (Donald et al. 2004). Generalists may also represent **host races**, which are defined as genetically distinct sympatric populations of symbionts, associated with different host species, in which appreciable gene flow occurs between populations (Drès and Mallet 2002). Across host-symbiont systems, there are often a greater proportion of specialists, with much fewer generalist species (Poulin and Keeney 2007; Loxdale et al. 2011; Schmid-Hempel 2011).

Historically, it has been assumed that specialists are adapted to exploit a narrow range of hosts, and that such adaptation to a few host species constrains the ability of specialists to establish on new host species, which results in specialists being susceptible to extinction (Poulin et al. 2006). It has also been assumed that adaptation to exploit relatively few host species may provide specialists with an adaptive advantage over generalists, which could result in greater local **abundance** of specialists (Poulin and Mouillot 2004). On the other hand generalists, capable of exploiting a wider range of host species, may have a greater evolutionary potential, allowing generalists to switch

between hosts and reduce the likelihood of extinction (Poulin et al. 2006). Historically, the evolution of host specificity was assumed to be directional, with specialists evolving from generalists, and that this increase in specificity was irreversible, progressing towards an “evolutionary dead end” (Poulin et al. 2006). However, over the past decade many studies have indicated that host specificity is not directional or irreversible (Poulin 2007). Poulin et al. (2006) reported a weak trend towards the evolution of reduced host specificity in ectoparasitic fleas of small mammals. Stireman (2005) reported that the evolution of generalists from specialists occurred more frequently than the reverse, in tachinid parasitoid flies.

The evolutionary steps that give rise to the fauna associated with a given host species are a function of historical events and ecological conditions (Poulin 2007). The evolution of associated symbionts may reflect a history of coevolution with their hosts, or a history of **ecological fitting** (Kethley and Johnston 1975; Brooks et al. 2006). The evolution of symbiont fauna is a function of two processes: the loss and the acquisition of species through time. These processes are driven by various types of events, such as host switching, cospeciation, duplication (intrahost speciation), sorting (extinction), and inertia (lack of parasite speciation) (Paterson and Banks 2001; Krasnov et al. 2010). Paterson and Banks (2001) have reported considerable congruence between the phylogenetic relationships of Procellariiformes seabirds and their associated chewing lice (*Halipeurus*), suggesting that *Halipeurus* lice are coevolving with their hosts. Strict cospeciation has been reported between rhinonyssid nasal mites of the *Ptilonyssus sairae* species complex and their passerine hosts (Morelli and Spicer 2007). The perfect

congruence between the phylogenies of hosts and their symbionts (Fahrenholz's rule), is however rarely observed in nature (Paterson and Banks 2001). In comparison, the evolution of symbiont fauna may instead represent a history of tracking ecologically-similar species, where phylogenetically unrelated but ecologically similar hosts, share symbiont species (Kethley and Johnston 1975; Brooks et al. 2006). Desdevise et al. (2002) observed that phylogenetically unrelated but ecologically related host species of Sparidae fish, living in sympatry, often shared the same species of Monogenean gill parasites.

Challenges surrounding the study of host specificity

Understanding species boundaries and the extent of cryptic diversity are fundamental challenges associated with the study of host specificity, but there are also other obstacles, such as variation in defining and measuring host specificity. The definition of what constitutes a specialist or generalist is often variable and sometimes idiosyncratic, such that specialists have been defined as symbionts which exploit eight or less host species (Stireman 2005), or those which use a single host species (Krasnov et al. 2004). Often, the definition of what constitutes a specialist or a generalist is 'in the eye of the beholder' (Futuyma and Moreno 1988). As a result of this ambiguity, a clear definition of what is considered a specialist or a generalist is required.

Another challenge to studying host specificity is that there are multiple approaches to measuring host specificity (Schmid-Hempel 2011). The number of host species used is the clearest approach to measuring host specificity. Another approach is the specificity index S_{TD} , which accounts for the taxonomic distance between pairs of

host species (Poulin and Mouillot 2003), or S_{TD}^* which combines the S_{TD} index with symbiont **prevalence** (Poulin and Mouillot 2005). Rohde (1980) proposed multiple host specificity indices, such as the diversity index (S_i) which accounts for the abundance of symbionts across host species, and the frequency index which accounts for the prevalence of a symbiont species across host taxa. For a review of these and other indices, refer to Schmid-Hempel (2011) or Poulin (2007).

The publication of incorrect identifications of host-associated symbionts can obfuscate estimates of host specificity. Species level identifications, performed by non-specialists and specialists, are occasionally incorrect (Moss 1978; Lindquist and Wu 1991). The likelihood of incorrect identifications is higher in groups with little or no identification material. Indeed, in many groups of symbiotic taxa there are no dichotomous keys or reliable identification materials, as seen in helminth endoparasites of vertebrates (Kijewska et al. 2002; Locke et al. 2010). To interpret host specificity patterns and compare records across studies, it is important that species are identified correctly.

Occasionally there are disagreements about status of particular species in many groups of arthropods, in which a given species may be synonymized with other species and then later separated (McGraw and Farrier 1969; Pence and Casto 1976). Instances where the status of symbiotic species has changed repeatedly can hamper any interpretations of host specificity across studies.

Another potential obstacle associated with the study of host specificity is that the level of host specificity can vary with the spatial scale of investigation (Krasnov et al.

2004, 2011). A given symbiont species may appear to be a host specialist at a local scale and a generalist at a global scale, resulting from variation in the availability of host species throughout the geographic range of a symbiont (Krasnov et al. 2011). The diversity of host species used by a symbiont is ultimately constrained by the evolutionary history of a symbiont, but it is proximally determined by the local abundance and availability of host species (Poulin 2007).

Research objectives:

Host specificity and species boundaries of beetle associated mites

My thesis research focuses on questions of host specificity and species boundaries of mites (Acari) associated with carrion-feeding beetles (Silphidae: *Nicrophorus*) and bark beetles (Curculionidae: Scolytinae). Insects and mites represent two of the most diverse groups of life, occurring on every continent worldwide, as well as freshwater and marine habitats. Mites are second in diversity to insects, with approximately 48,200 described species, and an estimated diversity of 0.5–1 million species (Walter and Proctor 1999; Halliday et al. 2000). In addition to many other habitats, mites also live in close association with many families of beetles, and they are often one of the most diverse and common associates of beetles (Lindquist 1970; Wilson and Knollenberg 1987; Hunter and Rosario 1988; Kinn and Linit 1989; Seeman 2001).

In my first data chapter (Chapter 2), I explored the morphological and molecular diversity of tortoise mites (Mesostigmata: Uropodoidea) associated with *Nicrophorus* (Silphidae) beetles. *Nicrophorus* beetles are cosmopolitan, with 60 described species worldwide, and these large-bodied beetles feed and mate on decaying organic matter,

primarily vertebrate carcasses (Anderson and Peck 1985; Sikes et al. 2008). *Nicrophorus* beetles are associated with a diverse acarofauna, with at least 14 species of mites from four families living in close association with *Nicrophorus* hosts (Wilson and Knollenberg 1987). *Nicrophorus* beetles are also hosts to the apparent widespread generalist species, *Uroobovella nova* (Oudemans, 1902) (Uropodoidea: Urodinychidae). Uropodoid deutonymphs (last juvenile instar) glue themselves to their insect host with an anally-secreted pedicel, and *Nicrophorus* hosts provide a means of dispersal to and from carcasses (Athias-Binche et al. 1993). The semi-permanent mode of attachment of uropodoids precludes host-switching during dispersal, which provides a useful association for planned studies.

In Chapter 2, there were two specific objectives; the first was to assess whether the apparent widespread generalist, *U. nova*, collected from *Nicrophorus* species worldwide, is truly a single generalist species, or is it instead a complex of cryptic specialist species. I hypothesized that this apparent generalist is multiple specialist species as seen in rhinonyssid nasal mites of birds (Morelli and Spicer 2007) or parasitoid tachinid flies from Costa Rica (Smith et al. 2007). The second objective was to explore whether the evolutionary history of associated uropodoids reflects a history of tracking ecologically-similar host species or coevolution with their *Nicrophorus* hosts. To explore these two objectives, I employed morphological characters, including morphometrics, as well as partial mitochondrial cytochrome oxidase I (COI) and the entire nuclear internal transcribed spacer 2 (ITS2) markers, from uropodoid specimens collected from 14 host species across six countries. To assess the extent of coevolution between uropodoids and

their hosts, the diversification of associated mites, based on molecular and morphological characters, was interpreted and contrasted with the known ecology and phylogenetic history of their *Nicrophorus* hosts.

In Chapter 3, I explored the same questions as those examined in Chapter 2, in a related but different natural system with many more host and symbiont species. I examined the species boundaries, extent of cryptic diversity and the evolution of host specificity, as well as coevolution in tortoise mites associated with bark beetles (Scolytinae). Scolytines are one of the most diverse and abundant groups of wood-boring arthropods, and they reside in the cambium and xylem tissue of many tree species worldwide (Wood 1982; Rabaglia 2002). Mites are one of the most diverse associates of bark beetles, with at least 97 species of mites from 40 families, residing in the galleries of scolytine infested pines (Moser and Roton 1971). The symbiotic relationship between mites and their scolytine hosts may vary from beneficial to detrimental across congeners and even within species (Hirschmann and Rühm 1955; Kinn 1967, 1980, 1984; Moser 1975). Tortoise mites of the genera *Trichouropoda*, *Nenteria* (Trematuridae), and *Uroobovella* (Urodinychidae) are among the most frequently collected associates of bark beetles (Moser and Roton 1971). The symbiotic relationship between uropodoids and their hosts is poorly understood, uropodoids are typically considered to be omnivorous, feeding on fungi, nematodes, and occasionally the eggs and larvae of bark beetles (Moser 1975; Kinn 1982, 1987; Athias-Binche 1989; Hofstetter et al. 2009). Uropodoids residing in bark beetle galleries glue themselves to dispersing scolytines, and hitch a ride to host trees which would otherwise be inaccessible.

In Chapter 3, I had four primary objectives; the first was to explore the species boundaries of bark beetle associated uropodoids and the extent to which morphologically defined species are supported by molecular data. To this end, I employed morphometrics and morphological characters, as well as partial COI and partial nuclear large subunit ribosomal DNA (28S, D2–D4 region) markers, from 35 species of bark beetle associated uropodoids, collected from 51 scolytine species across 11 countries. My second objective was to assess whether apparent widespread generalist species are truly generalists or are they instead complexes of cryptic specialists, using morphological and molecular markers. The findings from Chapter 2, and published studies, suggest that widespread generalists are often shown to be multiple specialist species (Morelli and Spicer 2007; Smith et al. 2007). The third objective was to explore whether associated uropodoids exhibit a pattern towards increasing host specificity. The directionality of host specificity was assessed based upon the observed distribution of specialist and generalist uropodoids in the phylogenetic trees. I hypothesized that these mites would add support to the notion that generalists can evolve from specialists, and specialists can evolve from generalists (Stireman 2005; Poulin et al. 2006). The fourth objective was to assess whether the evolution of associated uropodoids reflects a history of coevolution with their hosts or tracking ecologically-similar scolytine species. To this end, I compared and contrasted the uropodoid phylogeny with the phylogeny and known ecology of their scolytine hosts.

Despite their known diversity, the mite fauna associated with Canadian bark beetles is poorly known. The mites associated with several species of scolytines have been surveyed in other countries, but usually these surveys focus on economically

important species; including *Ips typographus* (Moser et al. 1989), *Dendroctonus frontalis* (Moser and Roton 1971), *D. ponderosae* (Mori et al. 2011), *Scolytus multistriatus* and *S. pygmaeus* (Moser et al. 2005). In Chapter 4, I addressed this lack of knowledge by describing the acarofauna (Mesostigmata, Oribatida) of bark beetles in Eastern Ontario. The main objective of Chapter 4 was to describe the diversity, prevalence, abundance, **intensity** and host specificity of mesostigmatic and oribatid mites associated with scolytines in Eastern Ontario. Scolytines were collected across four sites in Eastern Ontario in 2008 and 2009 using baited Lindgren funnel traps, and species level identifications were based on morphospecies concepts. A secondary objective of Chapter 4 was to compare the degree of host specificity observed in this study with that of published host records. Instances where the level of host specificity differed between my findings and published records were used to explore some of the challenges associated with the study of host specificity and species boundaries, such as unrecognized cryptic diversity (Smith et al. 2007), incorrect taxonomic identifications (Moss 1978; Lindquist and Wu 1991), instability in the status of species (McGraw and Farrier 1969; Pence and Casto 1976), and the spatial scale of investigation (Krasnov et al. 2004).

In the general discussion (Chapter 5), I compared and contrasted the major findings from each of my data chapters and explored how this research contributes to our understanding of species boundaries, the evolution of host specificity, and coevolution between hosts and symbionts. Additionally, I unveiled some of the possible future directions of this research, such as host-choice experimentation, the interaction between

the nature of **symbiosis** and host specificity, and potential determinants of host specificity.

Appendix 1.1. Operational definitions for terms bolded in text, and frequently used abbreviations.

Operational Definitions

- **Abundance** is the total number of individuals of a particular symbiont on or in all examined hosts. Mean abundance is the average number of symbionts on all examined hosts (Criscione et al. 2005).
- **Coevolution** or cophylogenetic descent, is where host and associated symbiont lineages evolve and adapt together over time (Paterson and Banks 2001).
- **Cryptic species** are defined as species distinguishable by no or very subtle morphological differences.
- **Ecological fitting** is where the evolution of symbiont fauna represents a history of tracking ecologically-similar species, where phylogenetically unrelated but ecologically similar hosts, share symbiont species (Brooks et al. 2006).
- **Generalist** species are those which exploit three or more host species.
- **Host races** are defined as genetically distinct sympatric populations of symbionts, associated with different host species, in which appreciable gene flow occurs between populations (Drès and Mallet 2002).
- **Host specificity** or host range, operationally defined as the number of host species used by a symbiont at a given life cycle stage (Poulin and Keeney 2007).
- **Intensity** is the number of individuals of a given symbiont in or on a single infested host, and mean intensity is the average number of symbionts per all infested hosts (Criscione et al. 2005).

- **Prevalence** is the proportion of all hosts examined which carry one or more individuals of a given symbiont species (Criscione et al. 2005).
- **Specialist** species are those which exploit one or two host species.
- **Symbiosis** is loosely defined as two or more distinct species “living together”, irrespective of whether the association is mutualistic, commensal or parasitic. Specifically, symbiosis is the acquisition and maintenance of one or more species by another species which results in novel structures or metabolism (Zook 1998).

List of Abbreviations

- 28S = Large subunit ribosomal DNA
- BI = Bayesian inference
- bp = Base pairs
- BS = Bremer support
- COI = Cytochrome oxidase subunit I
- ITS2 = Internal transcribed spacer 2
- JKS = Jackknife support
- NJ = Neighbour-joining
- PBS = Partitioned Bremer support
- PCR = Polymerase chain reaction

CHAPTER TWO

Cryptic species complex revealed in uropodoid mites associated with *Nicrophorus* (Silphidae) beetles based on phylogenetic inference and morphometric analysis.

Abstract

Uroobovella (Uropodoidea: Urodinychidae) species are among the most prevalent groups of mites associated with carrion-feeding *Nicrophorus* (Silphidae) beetles. Previous taxonomic understanding suggested that a single widespread generalist species, *U. nova*, was associated with *Nicrophorus* species worldwide. Using morphometrics and morphological characteristics, as well as partial cytochrome oxidase I (COI) and the entire internal transcribed spacer 2 (ITS2) markers, I examined whether this apparent widespread generalist was truly a generalist or is it instead a complex of cryptic specialist species. Uropodoids were collected from 14 host species across six countries and 17 provinces or states. The morphological, mitochondrial and nuclear markers indicated that *U. nova* is not a single species, but is instead at least five distinct species with differing levels of host specificity. Additionally, it appears that associated uropodoids may be coevolving with their silphid hosts, rather than tracking ecologically-similar species. Understanding extant biodiversity hinges largely on having a thorough knowledge of species boundaries, and the extent of cryptic diversity.

Introduction

Cryptic species, defined as species distinguishable by no or very subtle morphological differences, have been reported across numerous taxa, and more cryptic species are being uncovered as more genetic and phylogeographical studies are conducted, as seen in various invertebrate groups (Bensch et al. 2004; Miura et al. 2005; Williams et al. 2006). There has been an exponential increase in research on cryptic species over the last two decades, primarily as a result of the increased availability of nucleotide sequencing (Bickford et al. 2007). In order to accurately assess the biodiversity, or the ecological and evolutionary history of a given group, a thorough understanding of the extent of cryptic diversity is essential.

The use of molecular markers to elucidate species boundaries and uncover cryptic diversity, can significantly resolve our understanding of host specificity. The use of molecular markers has often revealed that a host generalist is actually multiple specialist species. The avian nasal mite, *Ptilonyssus sairae* (Mesostigmata: Rhinonyssidae), associated with numerous passerine species worldwide was shown to be a complex of cryptic species with monospecific host specificity (Morelli and Spicer 2007). In Costa Rica, the 5'-end of mitochondrial cytochrome oxidase I (barcoding region, sensu Hebert et al. 2004) revealed that 16 apparent generalist morphospecies of parasitoid tachinid flies are actually a complex of 64 specialist and 9 generalist species (Smith et al. 2007).

In addition to being used to uncover cryptic species, modern molecular techniques have also been used to assess the extent to which associated symbionts are tracking the ecology or the phylogenetic history of their hosts. Rhinonyssid nasal mites have been

shown to track the phylogeny of their host; Morelli and Spicer (2007) observed strict cospeciation between four lineages of the *P. sairae* species complex, and five host species. The phylogenetic relationships of pocket gophers and their associated lice have shown considerable congruence (Page 1996). On the other hand, the evolution of symbionts may reflect a history of ecological fitting, where a symbiont is associated with phylogenetically unrelated hosts that are ecologically similar (Kethley and Johnston 1975; Brooks et al. 2006). Multiple species of endoparasitic thelastomatoid nematodes have been collected from phylogenetically unrelated log-dwelling and wood-feeding arthropods, which share a common feature of dwelling in woody material (Jex et al. 2006).

Mites associated with *Nicrophorus* (Silphidae: Nicrophorinae) beetles provide a model system to explore species boundaries and cryptic diversity, as well as coevolution and ecological fitting. *Nicrophorus* species are large-bodied beetles, which breed and feed on decaying organic matter, most often vertebrate carcasses (Anderson and Peck 1985). *Nicrophorus* beetles are cosmopolitan, with at least 60 extant species worldwide, and 21 of which occur in the New World (Sikes et al. 2008). *Nicrophorus* beetles have been of interest to behavioural ecologists because most species provide biparental care. The life cycle of *Nicrophorus* species and other silphid genera is summarized by Anderson and Peck (1985). *Nicrophorus* beetles are associated with at least 14 species of mites from four families, and these mites can occur at very high prevalences of 95% (Wilson and Knollenberg 1987). *Nicrophorus* beetles provide a means of dispersal for mites to and from carcasses. The symbiotic relationship between mites and their silphid

hosts appears to be a blend of commensalism and mutualism (Wilson and Knollenberg 1987).

The uropodoid species *Uroobovella nova* (Oudemans, 1902) (Uropodoidea: Urodinychidae) is a common associate of *Nicrophorus* beetles, and has been reported from at least six *Nicrophorus* species (Athias-Binche et al. 1993). There are at least 2,000 described species of uropodoids worldwide, occurring in various habitats such as forest litter and soil, as well as patchy habitats such as carcasses, nests, coarse woody debris, and dung (Bloszyk et al. 2003). Known uropodoids are phoretic as deutonymphs, and the species associated with insects glue themselves to their host with an anally-secreted pedicel. Athias-Binche et al. (1993) measured body length and width, as well as sternal shield length of *U. nova* deutonymphs collected from *Nicrophorus vespillo*, *N. vespilloides*, and *N. humator* in Bielefeld Germany. It was reported that *U. nova* displayed two significantly different size morphs: a small morph associated with *N. vespillo* and *N. vespilloides*, and a large morph on *N. humator* (Athias-Binche et al. 1993). The consistent variation in body size across host species could indicate that *U. nova* is not a single species, but is instead at least two morphologically similar species.

Numerous acarological studies have used cytochrome oxidase I (COI) and internal transcribed spacer 2 (ITS2) either alone or combined with other markers to uncover cryptic diversity in mites, or to dissolve apparent generalist mite species into complexes of specialist species (Anderson and Trueman 2000; Webster et al. 2004; Morelli and Spicer 2007; Kawazoe et al. 2008; Mahani et al. 2009; Schäffer et al. 2010). In this study, I employed morphometrics and morphological characters, as well as

mitochondrial (COI) and nuclear (ITS2) markers, to investigate whether the widespread uropodoid species, *U. nova*, associated with *Nicrophorus* beetles worldwide, is actually a single species with a broad host preference, or is it instead a complex of cryptic specialist species. I also explored whether associated uropodoids are tracking the ecology or the phylogeny of their hosts, by comparing the uropodoid phylogeny with the known ecology and phylogenetic relationships of *Nicrophorus* host species.

Materials and Methods

Biological material

Silphids were collected by various researchers across eight countries and 21 provinces or states. In Canada, the majority of silphids were collected as bycatch from xylophagous beetle traps. Specimens from other countries were typically collected in pitfall traps, or were hand collected. Beetle specimens were shipped to Carleton University, and upon receipt specimens were placed in 95% ethanol and stored at -20°C. Silphids were identified to species using keys from Anderson and Peck (1985). Using a dissecting microscope, *Nicrophorus* beetles were examined for uropodoid mites, all mites were removed and placed in a 0.5 ml microfuge tube with 95% ethanol and stored at -20°C. Four species of uropodoids collected from bark beetles (Scolytinae), and one uropodoid species collected from *Nicrophorus* beetles were used as outgroup specimens. Following DNA extraction, mites were recovered from the extraction buffer and slide-mounted in a polyvinyl alcohol medium, and slides were cured on a slide warmer at about 40°C for 3–4 days. Slide-mounted specimens were examined using a compound microscope and identified to species using descriptions from the literature based on morphospecies concepts, focusing on taxonomically informative morphological characteristics (Hirschmann and Zirmgibl-Nicol 1962). Species were identified prior to examining the molecular reconstructions, and in any instances where a conflicting result emerged between the molecular data and identifications, the morphological characteristics and sequence data were reexamined. Voucher specimens are deposited in the Canadian

National Collection of Insects, Arachnids and Nematodes, in Ottawa, Canada (Appendix 2.1).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from whole specimens for 24 hours using a DNeasy Tissue kit (Qiagen Inc., Santa Clara, CA, USA). Following extraction, mites were removed from the extraction buffer, and genomic DNA was purified following the DNeasy Tissue kit protocol.

PCR amplifications were performed in a total volume of 25 µl, with 16 µl ddH₂O, 2.5 µl 10x PCR buffer, 2.5 µl 25 mM MgCl₂, 0.5 µl of each 10 µM primer, 0.5 µl 10 mM dNTPs, 0.5 µl Taq DNA polymerase (Promega Corp., Madison, WI, USA), and 2 µl genomic DNA template. PCR amplification cycles were performed on an Eppendorf ep Gradient S Mastercycler (Eppendorf AG, Hamburg, Germany). Primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994) were used to amplify a 709bp fragment of the mitochondrial COI gene. Specimens which did not produce detectable PCR products using LCO1490 and HCO2198 were reattempted using LCO1490 and LoDog (5'-GGRTCAAAAAAAGAWGTRTTTRAARTTTTCG-3') which amplified 643bp fragment of COI. The thermocycler protocol for COI amplification was as follows: initial denaturation cycle at 94°C for 3 min, followed by 40 cycles of 94°C for 45 s, 45°C for 45 s, 72°C for 1 min, and a final extension at 72°C for 5 min. The entire nuclear ribosomal ITS2 (333bp) marker, was amplified using ITS2-F1 and ITS2-R1

primers (5'-ATATGCTTAAATTCAGCGGG-3', 5'-GGGTCGATGAAGAACGCAGC-3') (Navajas et al. 1998), which are anchored in the highly conserved 5.8S and 28S regions. The PCR protocol for ITS2 amplification was as follows: initial denaturation cycle at 95°C for 3 min, followed by 30 cycles of 95°C for 1 min, 52°C for 1.5 min, 72°C for 2 min, and a final extension at 72°C for 5 min.

Amplified products and negative controls were visualized on 1% agarose electrophoresis gels, and purified using pre-cast E-Gel CloneWell 0.8% SYBR Safe agarose gels (Invitrogen, Carlsbad, CA, USA) following the protocol of Gibson et al. (2010). Sequencing reactions were performed in a total reaction volume of 10 µl, with 3 µl ddH₂O, 1.5 µl of 5x sequencing buffer, 0.5 µl of primer, 1 µl of BigDye Terminator (PE Applied Biosystems, Foster City, CA, USA), and 4 µl of purified PCR product. Sequencing was performed at the Agriculture & Agri-Food Canada, Eastern Cereal and Oilseed Research Centre Core Sequencing Facility (Ottawa, ON, Canada). Purification of sequencing reactions was performed using the ABI ethanol/EDTA/sodium acetate precipitation protocol and reactions were analysed on an ABI 3130xl Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA).

Sequence alignment and phylogenetic analysis

Sequence chromatograms were edited and contiguous sequences were assembled using Sequencher v4.7 (Gene Codes Corp., Ann Arbor, MI, USA). COI sequences were aligned manually in Mesquite v2.74 (Maddison and Maddison 2010) according to the translated amino acid sequence. ITS2 was initially aligned in ClustalX v2.0.12 (Larkin et al. 2007)

with the default settings, and subsequently adjusted manually in Mesquite. Sequences will be submitted to GenBank.

Phylogenetic reconstructions of COI, ITS2, and concatenated datasets were performed using neighbour-joining (NJ) analyses in PAUP* v4.0b10 (Swofford 2003), parsimony analyses in TNT v1.1 (Goloboff et al. 2008), and Bayesian inference (BI) in MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). NJ analysis was used to depict the relationships between species based on pair-wise distances, and to reveal the extent of genetic divergence between species. Bayesian inference and parsimony were used to assess the evolutionary relationships between species; these two approaches are analytically distinct and possess their own inherent strengths and weaknesses (Sanderson and Kim 2000). BI is a parametric statistical approach, in which trees are evaluated based on the probability of getting the observed data given the tree, which is calculated with reference to a specific model of evolution. Parsimony is a nonparametric statistical approach, in which trees are evaluated based on the minimum number of character state changes necessary to generate the observed data on a given tree (Sanderson and Kim 2000; Kolaczowski and Thornton 2004).

NJ trees were generated using the pairwise distances calculated with the Kimura-2-parameter (K2P) model, and branch support was assessed using jackknife resampling with 36% of characters removed and 1000 replicates. Parsimony analysis was performed using a heuristic search with tree bisection-reconnection (TBR) branch swapping and 1000 random addition sequence replicates, all characters were treated as unordered, equal weighting, and gaps were treated as missing. Gaps scored as a fifth state produced the

same topology as that observed for gaps as missing for each of the analytical approaches. Analyses of the COI dataset excluding the third codon positions produced poorly supported reconstructions with similar topology to the analyses including the third codon position. Multiple trees were obtained and these were presented in a semistrict consensus tree. Node support was assessed in TNT, using jackknife resampling with 36% of characters removed and 1000 replicates, Bremer supports and partitioned Bremer supports (PBS) were also determined using TNT.

MrModeltest v2.3 (Nylander 2004) was used to determine the best-fit model of molecular evolution for each molecular marker, which was determined to be GTR+I+G. Bayesian analysis was performed in MrBayes with a Markov Chain Monte Carlo (MCMC) method, two independent runs, with nucmodel = 4by4, $N_{st} = 6$, rates = invgamma, samplefreq = 1000, four chains = one cold and three heated. The ITS2 and concatenated datasets ran for 10 million generations, burn-in of 378 and 1000 respectively, and the COI dataset ran for 20 million generations with a burn-in of 1000. In Mesquite, the remaining trees, excluding the burn-in, were used to generate a majority-rule consensus tree displaying the posterior probability supports for each node. Bayesian analyses were performed using the on-line Computational Biology Service Unit at Cornell University, and at the Cyberinfrastructure for Phylogenetic Research (CIPRES) portal (Miller et al. 2010).

Morphological analysis

In order to assess the extent of morphological divergence across mites used in the molecular analyses, slide-mounted specimens were examined using a Leica DM 5500B compound microscope, and 15 characters were measured using Leica Application Suite, Live and Interactive Measurements Modules v3.5. Characters were selected based on prior knowledge for potential variation in mesostigmatic mites, and for their prominence across specimens. The 15 characters measured were: maximal length and width of the dorsal shield and ventrianal shield; sternal shield (SS) median length; SS width at five points (from anterior to posterior), the maximal width of the anterior margin anterior of coxa II, the maximum medial width near coxa III, the maximum width of the posterior margin, and the minimum width of the two medial constrictions level with coxa II and with coxa IV; the length of tarsus I; and the length of the following setae: dorsal seta *J5* (Hirschmann and Zirngiebl-Nicol 1962), opisthogastric seta *V3* (*JV3* Evans and Till 1965), anterodorsal setae of tarsus I, palp-trochanter seta *Pa1* (*v1* Evans and Till 1965). Morphological divergence was visualized using an ordination based on semistrong hybrid multidimensional scaling (SSH MDS), generated in PATN v2.27 (Belbin 2003). A distance matrix between mite specimens was created using morphometric data standardized for body size to eliminate possible bias linked to body size, then transformed $((\text{value} - \text{minimum})/\text{range})$ to balance the weight of all measured characters, and visualized in a three-dimensional SSH MDS ordination based on Bray-Curtis distance. The ordination was generated using 1000 replicates and 1000 random starts.

Significant differences among groups of mites were tested using ANOSIM (analysis of similarity), with 1000 iterations.

The effect of DNA extraction on mite morphology was examined in order to discern whether morphological characteristics of mites that underwent DNA extraction could be considered as unchanged and usable in analyses. Wilcoxon signed rank tests were performed in SPSS v17, to determine the effect of DNA extraction on mite morphology by comparing 15 morphological characters (standardized for body size) of specimens that underwent DNA extraction versus specimens that did not undergo DNA extraction.

Results

Uropodoids were collected from 14 species and three genera of silphids, across six countries and 17 provinces or states (Appendix 2.1). Amplification of COI was attempted with 89 mites across 39 sites, from which only 56 mites, from five countries and 36 sites yielded COI sequence data; COI was also amplified from six outgroup individuals (Appendix 2.1). COI was amplified from 62 individuals (56 ingroup and six outgroup specimens), with 673 characters in total, 410 constant, 38 parsimony-uninformative, and 225 parsimony-informative. Mean base pair frequencies (A: 0.298, C: 0.172, G: 0.158, T: 0.371) were found to be homogeneous across all taxa ($\chi^2 = 82$, $p = 1.0$). ITS2 was used to assess the branching patterns observed in the COI reconstructions, and to confirm species boundaries. ITS2 was amplified from 28 mites from 19 sites, as well as from four outgroup specimens (Appendix 2.1), with 377 characters in total, 180 constant, 9 parsimony-uninformative, and 188 parsimony-informative. Mean base pair frequencies (A: 0.33, C: 0.225, G: 0.183, T: 0.262) were also found to be homogeneous across taxa ($\chi^2 = 15.7$, $p = 1.0$). In each reconstruction, specimens are labeled with a unique number for each mite individual (Appendix 2.1), followed by the host species and abbreviated state, province or country.

Initial identifications based on morphology indicated that the uropodoids associated with *Nicrophorus* beetles were all the same species, *Uroobovella nova* (sensu Hirschmann and Zirngiebl-Nicol 1962). In contrast, subsequent molecular analyses strongly suggested that these mites are not a single species but instead are at least four morphologically similar species, herein referred to as, *Uroob. sp. 1*, *Uroob. sp. 2*, *Uroob.*

sp. 3, and *Uroob.* sp. 4. The dissolution of *U. nova* into multiple distinct species is strongly supported across all analytical approaches.

Neighbor-joining

The neighbor-joining analysis (K2P) of COI from 62 uropodoid specimens including 56 ingroup specimens (four *Uroobovella* spp.), and six outgroup specimens (five spp.), generated a well supported NJ tree (Fig. 2.1) (TL = 772, CI = 0.4948, RI = 0.8801). Most nodes had moderate to high jackknife support (JKS) values, and four had 100% JKS, three of which are basal nodes to three of the four putative ingroup species (Fig. 2.1). Average intraspecific pairwise distance was relatively low ($1.5\% \pm 0.6$), and the maximum intraspecific divergence was reported for *Uroobovella* sp. 2 (3.3%) (Table 2.1). Mean interspecific divergence was very high ($16.3\% \pm 0.6$), and the maximum interspecific divergence was observed between *Uroob.* sp. 1 and *U.* sp. 2 (18.7%) (Table 2.1). The range of intra- (0–3.3%) and interspecific (13.4–18.7%) pairwise distances did not overlap (Table 2.1).

The NJ analysis of ITS2 from 32 uropodoid specimens including 28 ingroup specimens (four *Uroobovella* spp.), and four outgroup specimens (two spp.), generated a well supported NJ tree (Fig. 2.2) (TL = 250, CI = 0.944, RI = 0.9754). Similar to the COI NJ tree, most nodes had moderate JKS values, and seven nodes had 100% JKS, two of which are basal nodes to two ingroup species, and one of which is the basal node to the ingroup (Fig. 2.2). Average intraspecific pairwise divergence was very low ($0.3\% \pm 0.4$), the maximum intraspecific divergence was reported from *Uroob.* sp. 3 (2.3%) (Table

2.1). *Uroobovella* sp. 1 showed 0% intraspecific divergence for the entire ITS2 marker. Average interspecific divergence ($6.4\% \pm 0.9$) was markedly greater than intraspecific divergence, and the range of intra- (0–2.3%) and interspecific (2.2–11.6%) pairwise distances overlapped slightly. As seen in COI, the maximum interspecific divergence was observed between *Uroob.* sp. 1 and *U.* sp. 2 (11.6%) (Table 2.1).

Table 2.1. Intra- and interspecific nucleotide divergence (%) $\pm$ standard deviation (range) of COI and ITS2 amplified from uropodoid mites associated with silphids.

	COI	ITS2
	mean (range)	mean (range)
Intraspecific		
<i>Uroob.</i> sp. 1	1.2 $\pm$ 0.4 (0–2.4)	0 $\pm$ 0 (0)
<i>Uroob.</i> sp. 2	1.6 $\pm$ 0.8 (0–3.3)	0.05 $\pm$ 0.1 (0–0.4)
<i>Uroob.</i> sp. 3	1.8 $\pm$ 0.5 (0.8–2.5)	0.9 $\pm$ 1.2 (0–2.3)
Total	1.5 $\pm$ 0.6 (0–3.3)	0.3 $\pm$ 0.4 (0–2.3)
Interspecific		
<i>Uroob.</i> sp. 1 – 2	16.7 $\pm$ 0.5 (15.5–18.7)	10.05 $\pm$ 0.9 (8.0–11.6)
<i>Uroob.</i> sp. 1 – 3	15.7 $\pm$ 0.4 (14.7–17.0)	6.7 $\pm$ 1.3 (5.7–9.9)
<i>Uroob.</i> sp. 1 – 4	17.1 $\pm$ 0.4 (16.7–18.0)	7.5 $\pm$ 0.5 (7.0–8.3)
<i>Uroob.</i> sp. 2 – 3	15.0 $\pm$ 0.9 (13.4–17.3)	6.4 $\pm$ 1.1 (4.2–8.8)
<i>Uroob.</i> sp. 2 – 4	17.4 $\pm$ 0.5 (16.7–18.5)	5.3 $\pm$ 0.5 (4.2–5.8)
<i>Uroob.</i> sp. 3 – 4	15.5 $\pm$ 1.0 (14.4–16.6)	2.7 $\pm$ 0.9 (2.2–4.4)
Total	16.3 $\pm$ 0.6 (13.4–18.7)	6.4 $\pm$ 0.9 (2.2–11.6)

The NJ analysis of the concatenated dataset from 29 specimens, including 26 ingroup specimens (four *Uroobovella* spp.), and three outgroup specimens (two spp.), produced a well supported tree (Fig. 2.3) (TL = 748, CI = 0.7393, RI = 0.8924). Most nodes with JKS had moderate to high JKS, and many nodes had no JKS: six nodes had 100% JKS, three of which are basal to three ingroup species, and one is basal to the ingroup (Fig. 2.3).

Figure 2.1. NJ tree (K2P) constructed from 673bp fragment of COI from 62 uropodoid specimens, 56 ingroup specimens representing four *Uroobovella* species, and six outgroup specimens representing five species, jackknife support (1000 replicates) >50% shown above branches (TL = 772, CI = 0.4948, RI = 0.8801).

Figure 2.2. NJ tree (K2P) constructed from the entire ITS2 (377bp) marker from 32 uropodoid specimens, 28 ingroup specimens representing four *Uroobovella* species, and four outgroup specimens representing two species, jackknife support (1000 replicates) >50% shown above branches (TL = 250, CI = 0.944, RI = 0.9754).

Figure 2.3. NJ tree (K2P) constructed from the concatenated dataset of 673bp fragment of COI and entire ITS2 (377bp) from 29 uropodoid specimens, 26 ingroup specimens representing four *Uroobovella* species, and three outgroup specimens representing two species, jackknife support (1000 replicates) >50% shown above (TL = 748, CI = 0.7393, RI = 0.8924).

Parsimony

The parsimony heuristic analysis of COI resulted in 54 most parsimonious trees (TL = 752, CI = 0.508, RI = 0.8863) presented in a semistrict consensus tree (Fig. 2.4). Multiple nodes had no JKS, a few nodes had moderate JKS, and only four nodes had 100% JKS. Most nodes had poor Bremer support, and only four nodes had relatively strong (≥ 19) support. Three of the four well supported nodes are basal nodes to three of the four putative ingroup species (Fig. 2.4). *Uroobovella* sp. 1 and *U.* sp. 2 had multiple unresolved nodes, collapsing into a few intraspecific polytomies. The heuristic analysis of ITS2 produced two most parsimonious trees (TL = 248, CI = 0.9516, RI = 0.9789) presented in a semistrict consensus tree (Fig. 2.5). All nodes had JKS, and five nodes had 100% support, two of which are the basal nodes to two ingroup species, and one is the basal node to the ingroup. Bremer support of each node was moderate, with only two nodes showing relatively strong Bremer support within the ingroup (Fig. 2.5). The low level of intraspecific divergence observed in ITS2 resulted in each species collapsing into an intraspecific polytomy. The parsimony analysis of the concatenated dataset resulted in 44 most parsimonious trees (TL = 737, CI = 0.7503, RI = 0.8985) presented in a semistrict consensus tree (Fig. 2.6). Most nodes had moderate to high JKS: five nodes had 100% JKS, three of which are basal to three ingroup species, and one is basal to the ingroup (Fig. 2.6). The PBS varied from poor to strong, with the strongest PBS reported for the basal nodes for three of the ingroup species, and the basal node to the ingroup (Fig. 2.6). The PBS revealed conflict between the COI and ITS2 datasets for four intraspecific nodes (Fig. 2.6). PBS also indicated that ITS2 showed no support for the

intraspecific nodes of *Uroob.* sp. 2. *Uroobovella* sp. 1 showed little intraspecific divergence, and collapsed into a polytomy (Fig. 2.6).

Figure 2.4. Semistrict consensus tree of 54 most parsimonious trees (TL = 752, CI = 0.508, RI = 0.8863) generated from parsimony heuristic analysis (1000 replicates) of 673bp fragment of COI from 62 uropodoid specimens, 56 ingroup specimens representing four *Uroobovella* species, and six outgroup specimens representing five species, jackknife support (1000 replicates) >50% shown above and Bremer support shown below branches.

Figure 2.5. Semistrict consensus tree of two most parsimonious trees (TL = 248, CI = 0.9516, RI = 0.9789) generated from parsimony heuristic analysis (1000 replicates) of the entire ITS2 (377bp) marker from 32 uropodoid specimens, 28 ingroup specimens representing four *Uroobovella* species, and four outgroup specimens representing two species, jackknife support (1000 replicates) >50% shown above and Bremer support shown below branches.

Figure 2.6. Semistrict consensus tree of 44 most parsimonious trees (TL = 737, CI = 0.7503, RI = 0.8985) generated from parsimony heuristic analysis (1000 replicates) of the concatenated dataset of 673bp fragment of COI and entire ITS2 (377bp) from 29 uropodoid specimens, 26 ingroup specimens representing four *Uroobovella* species, and three outgroup specimens representing two species, jackknife support (1000 replicates) >50% shown above and partitioned Bremer support (COI, ITS2) shown below branches.

Bayesian Inference

The Bayesian analysis of COI was performed for 20×10^6 generations, which produced 38002 trees (after burn-in) which were summarized in a majority rule consensus tree (TL = 796, CI = 0.4799, RI = 0.8727) (Fig. 2.7). The BI consensus tree was well supported, with most nodes having moderate to high posterior probabilities, and three nodes with 100% support, two of which are the basal nodes to two ingroup species (Fig. 2.7). Similar to the parsimony analysis of COI, *Uroob. sp. 1* and *U. sp. 2* had multiple unresolved nodes, collapsing into a few intraspecific polytomies. BI of ITS2 was performed for 10×10^6 generations, which produced 19246 trees (after burn-in) which were summarized in a majority rule consensus tree (TL = 249, CI = 0.9478, RI = 0.9771) (Fig. 2.8). The consensus tree was well supported: five nodes had 100% support, two of which are basal to two ingroup species, and one is basal to the ingroup (Fig. 2.8). As seen with the parsimony analysis of ITS2, each species collapsed into an intraspecific polytomy. BI of the concatenated dataset was performed for 10×10^6 generations, which produced 18002 trees (after burn-in) which were summarized in a majority rule consensus tree (TL = 766, CI = 0.7219, RI = 0.8825) (Fig. 2.9). The consensus tree was well supported: six nodes had 100% support, three of which are basal to three ingroup species and one is basal to the ingroup (Fig. 2.9). *Uroobovella sp. 1* collapsed into an intraspecific polytomy (Fig. 2.9).

Figure 2.8. Majority rule consensus tree of 19246 trees generated by Bayesian MCMC analysis (10×10^6 generations) of the entire ITS2 (377bp) marker from 32 uropodoid specimens, 28 ingroup specimens representing four *Uroobovella* species, and four outgroup specimens representing two species, posterior probabilities >50% shown above branches (TL = 249, CI = 0.9478, RI = 0.9771).

Figure 2.9. Majority rule consensus tree of 18002 trees generated by Bayesian MCMC analysis (10×10^6 generations) of the concatenated dataset of 673bp fragment of COI and entire ITS2 (377bp) from 29 uropodoid specimens, 26 ingroup specimens representing four *Uroobovella* species, and three outgroup specimens representing two species, posterior probabilities >50% shown above branches (TL = 766, CI = 0.7219, RI = 0.8825).

Summary of molecular reconstructions

The NJ, parsimony, and BI analyses of COI, ITS2 and concatenated datasets yielded very similar results, which strongly indicate that *U. nova* is not a single species but instead at least four morphologically similar species. In most reconstructions, except the parsimony analysis of COI, the ingroup was shown to be monophyletic with relatively strong support. Additionally, in most reconstructions, except the parsimony and Bayesian analyses of ITS2, *Uroob.* sp. 1, *U.* sp. 2 and *U.* sp. 3 were monophyletic with moderate to strong support. All analyses, except BI of COI and concatenated dataset, placed *Uroob.* sp. 2 as the sister taxa to the rest of the ingroup, suggesting that *U.* sp. 1, *U.* sp. 3 and *U.* sp. 4 are more closely related to each other than to *U.* sp. 2; however, the basal placement of *U.* sp. 2 was not strongly supported. With the exception of the parsimony and BI analyses of ITS2 and the concatenated dataset, all other analyses indicated, with weak support, that *U.* sp. 4 and *U.* sp. 3 formed a monophyletic clade sister to *U.* sp. 1.

Molecular analyses indicated that mites of the same species, from the same host individual were not more closely related to each other than to mites of the same species from different host individuals of the same host species. Additionally, collection site appears to have no impact on the degree of relatedness, mites from different host individuals from the same collection site, were not more closely related to each other than to mites from the same host species from different sites.

Morphological analysis

Initial morphological identifications indicated that the uropodoids collected from silphids were a single species, *Uroobovella nova*. Closer investigation revealed five relatively subtle morphological characteristics which consistently varied between each of the putative species (Table 2.2). These additional characters supported the existence of the four species (*Uroob.* sp. 1, *U.* sp. 2, *U.* sp. 3, and *U.* sp. 4) delineated in the molecular analyses (Table 2.2). A fifth species, *Uroob.* sp. 5, collected from *N. quadripunctatus* in Japan, did not yield COI and ITS2 data, although this species could be distinguished morphologically (Table 2.2).

Based on morphometric measurements, the SSH MDS ordination (stress = 0.1344) (Fig. 2.10) and ANOSIM revealed that *Uroob.* sp. 1 is morphologically distinct from *U.* sp. 2 and *U.* sp. 3 ($p < 0.0001$), it was also shown that *U.* sp. 2 and *U.* sp. 3 are not significantly different from each other ($p = 0.0521$).

The extraction of DNA did not significantly alter the morphology of a mite, and morphological characteristics of mites that undergo DNA extraction can be considered unaltered. Wilcoxon signed rank tests revealed that the extraction of DNA had no statistically significant effect on the morphology of a mite specimen when compared to mites that did not undergo DNA extraction, based on measurements of 15 characters from 23 mites with 11 pairwise comparisons ($p = 0.062\text{--}0.79$).

Table 2.2. Morphological characteristics delineating between *Uroobovella* species collected from silphids.

Mite species	ventrianal shield shape	seta J5 shape	sternal and ventrianal shield pattern	light-refractant pits on endopodal shield	anterodistal projection on femur II
<i>Uroob. sp. 1</i>	rounded corners, lateral margins rounded	filamentous	widely spaced pits absent or barely noticeable	no light-refractant pits	rounded tip, not curved
<i>Uroob. sp. 2</i>	sharp corners, lateral margins parallel	scalloped	fine widely spaced pits	widely spaced irregularly shaped light-refractant pits	pointed tip, not curved
<i>Uroob. sp. 3</i>	"	"	"	"	rounded tip, not curved
<i>Uroob. sp. 4</i>	"	"	"	"	pointed tip, curved
<i>Uroob. sp. 5</i>	very sharp corners, lateral margins parallel	"	"	widely spaced oval-shaped light-refractant pits	pointed tip, not curved

Figure 2.10. Three-dimensional SSH MDS ordination with Bray-Curtis distance (1000 replicates, 1000 random starts) performed on measurements $((\text{value} - \text{min})/\text{range})$ of 15 morphological characters from 58 uropodoids representing four species, *Uroob. sp. 1*, *Uroob. sp. 2*, *Uroob. sp. 3*, and *Uroob. sp. 4* (stress = 0.1344).

Discussion

This study is the first to use the barcoding region of COI and the entire ITS2 markers, to examine the relationships between uropodoid species. The level of variation, and the relative utility of any given marker, can vary between taxa, and it is difficult to predict the relative effectiveness of a particular marker when attempting to examine a previously unstudied group. COI and ITS2, either alone or combined with other markers, have been widely used to clarify species boundaries and to assess the degree of host specificity (Webster et al. 2004; Morelli and Spicer 2007; Kawazoe et al. 2008; Mahani et al. 2009; Schaffer et al. 2010). This study indicates that both COI and ITS2 are suitable markers for distinguishing between closely related uropodoid species; however, unlike COI, ITS2 was not particularly variable intraspecifically.

COI and ITS2, as well as previously overlooked morphological character states indicate that an otherwise widespread generalist, *Uroobovella nova*, associated with at least 14 species of silphids, is not a single species but rather multiple distinct species with differing degrees of host specificity. Overall, the molecular reconstructions strongly support the monophyly of each *Uroob.* sp. 1, *U.* sp. 2 and *U.* sp. 3, with the exception of the parsimony and Bayesian analyses of ITS2, which does not support the monophyly of *U.* sp. 3. The evolutionary relationships between *Uroobovella* species were poorly resolved in the molecular reconstructions, indicating that *U.* sp. 1, *U.* sp. 3 and *U.* sp. 4 are more closely related to each other than to *U.* sp. 2. Examination of additional deutonymphal specimens, as well as adults, from more host species, would likely improve the understanding of relationships between uropodoid species.

The interspecific divergence of COI between uropodoid species was relatively high (13.4–18.7%), considering that these species only showed slight morphological variation. Cryptic species showing high levels of COI divergence have been reported from other groups of mites. The cereal rust mite *Abacarus hystrix* showed an average COI divergence of 22.6% between strains (Skoracka and Dabert 2010). The water mite *Hygrobatas nigromaculatus* exhibited an average COI divergence of 17.8% between stream- and lake-inhabiting populations (Martin et al. 2010). *Stereotydeus mollis* (Penthalodidae), collected across the trans-Antarctic mountains, exhibited a maximum of 18% intraspecific divergence in COI (Stevens and Hogg 2006). The ITS region can also exhibit considerable divergence between cryptic species; the avian nasal mite, *Ptilonyssus sairae*, exhibited mean intraspecific divergence of 10.4% (maximum 17.6%) across five species of hosts (Morelli and Spicer 2007).

The cryptic species that were fettered from the widespread host generalist, *U. nova*, exhibited differing levels of host specificity. *Uroobovella* sp. 4 and *U. sp. 5* appear to be the most host specific, each occurring on a single host species. *Uroobovella* sp. 3 and *U. sp. 2* are host specialists, both living in association with two host species. The COI reconstructions indicate that *Uroob. sp. 2*, associated with *N. sayi* and *N. defodiens*, may be represented by two host races. The separation of these two host races is not perfectly consistent across all analyses; however, there is enough support to suggest that these host races are diverging intraspecifically. *Uroobovella* sp. 1 appears to be the least specialized, occurring primarily on *N. orbicollis* as well as eight other silphid species. However, the association with at least five host species, *N. pustulatus*, *N. carolinus*, *N.*

defodiens, *Heterosilpha ramosa* and *Necrodes surinamensis*, was very infrequent, indicating that these may be incidental host records.

It appears that silphid-associated uropodoids might be coevolving with their hosts, at least to some extent. The strongest evidence that these mites are not tracking the ecology of their hosts lies in the association of *Uroob.* sp. 2 with *N. sayi* and *N. defodiens*, and *U.* sp. 1 with *N. orbicollis*. *Nicrophorus sayi* and *N. orbicollis* are ecologically similar; they are large, nocturnal beetles that dwell in forests and open habitats, and they bury carcasses relatively deep in the ground, *N. sayi* emerges in mid-April, and *N. orbicollis* emerges in mid-May (Anderson 1982; Anderson and Peck 1985). In contrast, *N. defodiens* is a small, crepuscular, forest dwelling species, which buries carcasses shallowly beneath the leaf litter, and emerges in mid-May (Anderson 1982; Anderson and Peck 1985). Overall, *Nicrophorus sayi* and *N. defodiens* are quite dissimilar ecologically, though they share the same mite species, *Uroob.* sp. 2. If mites were tracking the ecology of their hosts, it would be expected that ecologically similar species, like *N. sayi* and *N. orbicollis*, would share the same species, or closely related species of mites. A plausible explanation why *N. sayi* and *N. defodiens* share the same mite species is because they are phylogenetically closely related (Peck and Anderson 1985; Sikes 2003). Additionally, *Uroob.* sp. 1 may be distantly related to *U.* sp. 2 because its primary host, *N. orbicollis*, is a distant relative of *N. sayi* and *N. defodiens* (Peck and Anderson 1985; Sikes 2003). Additional silphids and associated uropodoids need to be examined in order to draw any conclusions about the extent to which these mites are tracking their hosts ecologically or phylogenetically.

As shown for *Uroobovella* species, I also think that *Poecilochirus* (Mesostigmata: Parasitidae) species may be tracking the phylogenetic history of their hosts. *Poecilochirus carabi* G. & R. Canestrini, is a frequently encountered associate of *Nicrophorus* beetles, and historically it was considered to be a widespread generalist. Investigations into the host associations between *Nicrophorus* and *P. carabi*, revealed that *P. carabi* exhibited specific host races in North America and Europe (Müller and Schwarz 1990; Brown and Wilson 1992), and subsequent enzyme electrophoresis, and detailed morphological assessments, revealed that these specific host races were possibly distinct species (Schwarz et al. 1991; Baker and Schwarz 1997). In Michigan, two host races of *P. carabi* were reported from three ecologically dissimilar host species; one host race was associated with *N. orbicollis*, and the other with *N. tomentosus* and *N. defodiens* (Anderson 1982; Anderson and Peck 1985; Brown and Wilson 1992). It is possible that *N. tomentosus* and *N. defodiens* share the same putative mite species because they are phylogenetically more closely related to each other than to *N. orbicollis* (Sikes 2003). *Poecilochirus* mites are vagile and they have been shown to switch between *Nicrophorus* species at carcasses (Brown and Wilson 1992), but despite their ease of host switching, they have managed to diverge genetically and morphologically, and the observed divergence somewhat reflects the phylogenetic history of their hosts.

An understanding of the life cycle of the host and associated mites is necessary in order to explore the nature of divergence of *Uroobovella* species. In general, a mating pair of *Nicrophorus* beetles will search for a small dead animal, and upon discovery the carcass will be buried. The burial culminates in the creation of a crypt-like chamber, and

the carcass is molded into a sphere and stripped of its fur (Anderson and Peck 1985). The female beetle excavates a tunnel in which eggs are laid; larvae hatch and move to the carcass to feed, although larvae are initially fed by the adults; eventually the parent beetles leave the crypt, larvae pupate in the surrounding soil, and progeny adults eclose (Anderson and Peck 1985). Typically, a single pair of reproductive adults claim a carcass, but occasionally more than one couple of the same or different species claim a carcass (Anderson and Peck 1985). Interspecific competition occurs occasionally between *Nicrophorus* species, and often the smaller species, such as *N. defodiens*, have to contend with larger species, such as *N. orbicollis* and *N. sayi*, for ownership of a carcass (Trumbo 1990). Competition between hosts may provide opportunities for host switching, which may explain in part the instances where a mite was on a presumably incorrect host.

Uropodoids and their silphid hosts have a rather close relationship. *Uroobovella* deutonymphs are transported by adult *Nicrophorus* beetles into the crypt, and after the mites detach from the host, the rest of the life cycle takes place in the crypt, from molting into adults, mating, egg laying and development into deutonymphs (Athias-Binche et al. 1993). The new deutonymphs do not attach to parent beetles, but instead they disperse solely on progeny adults emerging from the crypt (Wilson and Knollenberg 1987). Uropodoids develop much more slowly than most silphid associated mites (*Poecilochirus* sp., *Macrocheles* sp., and *Histiostoma* sp.) (Wilson and Knollenberg 1987), and it is possible that their slow development constrains their dispersal to progeny adults. Phoretic deutonymphs glue themselves to their host, and this semi-permanent mode of attachment would preclude host switching at larger carcasses, which do not serve as brooding

material (Athias-Binche et al. 1993). The marked differences in phenology, development time, behaviour, and habitat preferences among *Nicrophorus* species may have conferred an adaptive advantage to the evolution of carrier-specific preferences in mites (Athias-Binche et al. 1993), which may have reinforced barriers to gene flow among uropodoids, and possibly resulted in the observed divergence between uropodoid species.

Overall, molecular and morphological characters strongly suggest that the widespread generalist, *U. nova*, is multiple distinct species. The degree of host specificity of these species is variable, in that the majority of putative cryptic species are specialists, and one species is a generalist. These findings also reveal that the mite fauna associated with *Nicrophorus* beetles is more diverse than previously understood; additional investigations may uncover more cryptic diversity. Lastly, the evolutionary history of associated uropodoids may reflect a history of coevolution with their *Nicrophorus* hosts, rather than a history of tracking ecologically-similar host species.

Appendix 2.1. Collection locale and host species records of uropodoid mites collected from silphid beetles (ingroup) and scolytines (outgroup), 1 indicates that either COI or ITS2 was amplified (**Uroob.*= *Uroobovella*, *Urop.*= *Uropoda*, *Trich.*= *Trichouropoda*).

Beetle no.	Beetle species	Collection location	Lat	Long	Coll date	Mite species*	COI	ITS2
1 - WKN078	<i>Nicro. vespilloides</i>	Netherlands, North Holland, Bloemendaal	52.406	4.623	17 iv 2009	<i>Uroob. sp. 3</i>	1	1
2 - WKN084	<i>Nicro. sayi</i>	Can, QE, Pont-Rouge	46.806	-71.679	05 vi 2009	<i>Uroob. sp. 2</i>	1	1
3 - WKN085	<i>Nicro. sayi</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	11 vi 2009	<i>Uroob. sp. 2</i>	1	1
4 - WKN114	<i>Nicro. orbicollis</i>	Can, PEI, Wellington, Route 2	46.452	-63.949	06 vii 2009	<i>Uroob. sp. 1</i>		
5 - WKN131	<i>Nicro. defodiens</i>	Can, BC, Prince George, nr. UNBC	53.904	-122.783	12 vi 2009	<i>Uroob. sp. 2</i>	1	1
6 - WKN157	<i>Nicro. orbicollis</i>	Can, MB, Vassar, Hwy 12	49.084	-95.875	12 vii 2009	<i>Uroob. sp. 1</i>	1	1
7 - WKN165	<i>Nicro. orbicollis</i>	Can, ON, Carbine Rd.	45.330	-76.371	23 vii 2009	<i>Uroob. sp. 1</i>	1	1
8 - WKN184	<i>Nicro. vespilloides</i>	Germany, Mooswald Forest, nr. Freiburg	48.0	7.85	vi 2009	<i>Uroob. sp. 3</i>	1	1
9 - WKN186	<i>Nicro. vespillo</i>	Germany, Mooswald Forest, nr. Freiburg	48.0	7.85	vi 2009	<i>Uroob. sp. 3</i>	1	1
10 - WKN193	<i>Nicro. sayi</i>	USA, CT, Bethany	41.462	-72.961	14 viii 2009	<i>Uroob. sp. 2</i>	1	1
11 - WKN211	<i>Nicro. sayi</i>	USA, NH, Durham	43.134	-70.926	10 vi 2009	<i>Uroob. sp. 2</i>		
12 - WKN224	<i>Nicro. defodiens</i>	USA, NH, Durham	43.134	-70.926	07 vi 2009	<i>Uroob. sp. 1</i>	1	1
13 - WKN228	<i>Nicro. orbicollis</i>	USA, NH, Durham	43.134	-70.926	07 vi 2009	<i>Uroob. sp. 1</i>	1	1
14 - WKN232	<i>Nicro. defodiens</i>	Can, AB, nr. Drayton Valley	53.358	-114.984	05 vii 2009	<i>Uroob. sp. 2</i>		1
15 - WKN305	<i>Nicro. carolinus</i>	USA, NE, Kearney Co.			vii 2009	<i>Uroob. sp. 1</i>	1	1
16 - WKN307	<i>Nicro. pustulatus</i>	USA, NE, Kearney Co.			vii 2009	<i>Uroob. sp. 1</i>	1	1
17 - WKN345	<i>Nicro. quadripunctatus</i>	Japan, Niigata, Matsunoyama Matsuguchi			25 x 2009	<i>Uroob. sp. 5</i>		
18 - WKN349	<i>Nicro. orbicollis</i>	Can, NS, Juniper Lake	44.7	-63.6	2009	<i>Uroob. sp. 1</i>	1	1
19 - WKN355	<i>Nicro. sayi</i>	Can, NS, McNabs Island	44.6	-63.517	2009	<i>Uroob. sp. 2</i>	1	
20 - WKN357	<i>Nicro. defodiens</i>	Can, NS, McNabs Island	44.6	-63.517	2009	<i>Uroob. sp. 2</i>		1
21 - WKN165	<i>Nicro. orbicollis</i>	Can, ON, Carbine Rd.	45.330	-76.371	23 vii 2009	<i>Uroob. sp. 1</i>	1	
22 - WKN184	<i>Nicro. vespilloides</i>	Germany, Mooswald Forest, nr. Freiburg	48.0	7.85	vi 2009	<i>Uroob. sp. 3</i>	1	1
23 - WKN079	<i>Nicro. vespilloides</i>	Netherlands, North Holland, Bloemendaal	52.406	4.623	18 iv 2009	<i>Uroob. sp. 3</i>	1	1
24 - WKN082	<i>Nicro. sayi</i>	Can, ON, Hwy 132, Dacre	45.369	-76.988	16 v 2009	<i>Uroob. sp. 2</i>		
25 - WKN083	<i>Nicro. sayi</i>	Can, PEI, Milburn, Route 176	46.677	-64.323	26 v 2009	<i>Uroob. sp. 2</i>	1	
26 - WKN084	<i>Nicro. sayi</i>	Can, QE, Pont-Rouge	46.806	-71.679	05 vi 2009	<i>Uroob. sp. 2</i>		
27 - WKN086	<i>Nicro. orbicollis</i>	Can, ON, Windsor, Elgin St.	42.261	-83.057	18 vi 2009	<i>Uroob. sp. 1</i>		
28 - WKN087	<i>Nicro. sayi</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	25 vi 2009	<i>Uroob. sp. 2</i>	1	
29 - WKN088	<i>Nicro. orbicollis</i>	Can, ON, Hwy 132, Dacre	45.369	-76.988	25 vi 2009	<i>Uroob. sp. 1</i>	1	
30 - WKN090	<i>Nicro. nepalensis</i>	Taiwan, nr. Meifeng, 5 km Sungkang	24.088	121.171	02 v 2007	<i>Uroob. sp. 4</i>	1	1
31 - WKN096	<i>Nicro. quadripunctatus</i>	Japan, Nagano, Honshu, Koshimizu-ga-hara			04 vii 2008	<i>Uroob. sp. 5</i>		
32 - WKN099	<i>Nicro. sayi</i>	Can, NS, Hantsport	45.055	-64.176	19 vi 2009	<i>Uroob. sp. 2</i>	1	

Appendix 2.1. continued.

Beetle no.	Beetle species	Collection location	Lat	Long	Coll date	Mite species*	COI	ITS2
33 - WKN100	<i>Nicro. orbicollis</i>	Can, NS, Hantsport	45.055	-64.176	19 vi 2009	<i>Uroob. sp. 1</i>	1	
34 - WKN116	<i>Nicro. orbicollis</i>	Can, PEI, Wellington, Route 2	46.452	-63.949	06 vii 2009	<i>Uroob. sp. 2</i>	1	
35 - WKN117	<i>Nicro. sayi</i>	Can, QE, Pont-Rouge	46.562	-71.545	08 vi 2009	<i>Uroob. sp. 2</i>	1	
36 - WKN118	<i>Nicro. defodiens</i>	Can, NS, Aylesford, Lake Rd.	44.981	-64.635	25 vi 2009	<i>Uroob. sp. 2</i>		
37 - WKN119	<i>Nicro. orbicollis</i>	Can, NS, Wolfville, Deep Hollow Rd.	45.047	-64.416	02 vii 2009	<i>Uroob. sp. 1</i>	1	
38 - WKN135	<i>Nicro. defodiens</i>	Can, BC, Prince George, nr. UNBC	53.904	-122.783	12 vi 2009	<i>Uroob. sp. 2</i>	1	1
39 - WKN147	<i>Nicro. orbicollis</i>	Can, ON, Hamilton, Site 4-7			07 vii 2009	<i>Uroob. sp. 1</i>		
40 - WKN155	<i>Nicro. defodiens</i>	Can, NS, Debert Industrial Park	45.428	-63.429	25 vi 2009	<i>Uroob. sp. 2</i>	1	
41 - WKN158	<i>Nicro. sayi</i>	Can, MB, Sundown, Hwy 201	49.102	-96.277	09 vii 2009	<i>Uroob. sp. 2</i>	1	1
42 - WKN164	<i>Nicro. orbicollis</i>	Can, ON, Waterloo	43.540	-80.211	07 vii 2009	<i>Uroob. sp. 1</i>	1	
43 - WKN167	<i>Nicro. orbicollis</i>	Can, NS, Glenmont, Black Hole Rd.	45.111	-64.296	17 vii 2009	<i>Uroob. sp. 1</i>	1	
44 - WKN168	<i>Nicro. orbicollis</i>	Can, NS, Cold Brook, Hwy 101	45.079	-64.592	13 vii 2009	<i>Uroob. sp. 1</i>	1	1
45 - WKN169	<i>Nicro. orbicollis</i>	Can, NB, Chipman	46.174	-65.899	08 vii 2009	<i>Uroob. sp. 1</i>	1	
46 - WKN170	<i>Nicro. defodiens</i>	Can, NB, Darktown, 200 South Rd.	46.553	-66.123	23 vii 2009	<i>Uroob. sp. 2</i>	1	1
47 - WKN171	<i>Nicro. defodiens</i>	Can, NS, Goodwood	44.603	-63.677	13 vii 2009	<i>Uroob. sp. 2</i>	1	
48 - WKN176	<i>Nicro. defodiens</i>	Can, NS, Sandy Cove, Liverpool Bay	44.053	-64.699	12 viii 2009	<i>Uroob. sp. 2</i>	1	
49 - WKN178	<i>Nicro. orbicollis</i>	Can, NS, East River off Hwy 329	44.583	-64.164	10 viii 2009	<i>Uroob. sp. 1</i>	1	
50 - WKN179	<i>Nicro. orbicollis</i>	Can, NS, Debert Industrial Park	45.428	-63.429	05 viii 2009	<i>Uroob. sp. 1</i>		
51 - WKN192	<i>Nicro. orbicollis</i>	USA, CT, Bethany	41.462	-72.961	14 viii 2009	<i>Uroob. sp. 1</i>	1	
52 - WKN193	<i>Nicro. sayi</i>	USA, CT, Bethany	41.462	-72.961	14 viii 2009	<i>Uroob. sp. 2</i>	1	
53 - WKN203	<i>Nicro. sayi</i>	USA, NH, Durham	43.134	-70.926	10 vi 2009	<i>Uroob. sp. 2</i>		
54 - WKN222	<i>Nicro. defodiens</i>	USA, NH, Durham	43.134	-70.926	07 vi 2009	<i>Uroob. sp. 1</i>		
55 - WKN230	<i>Nicro. orbicollis</i>	USA, NH, Durham	43.134	-70.926	07 vi 2009	<i>Uroob. sp. 1</i>		
56 - WKN233	<i>Nicro. defodiens</i>	Can, AB, nr. Drayton Valley	53.358	-114.984	05 vii 2009	<i>Uroob. sp. 2</i>		
57 - WKN296	<i>Nicro. carolinus</i>	USA, NE, Kearney Co.			03 vi 2009	<i>Urop. orbicularis</i>		1
58 - WKN297	<i>Nicro. pustulatus</i>	USA, NE, Kearney Co.			13 vii 2009	<i>Uroob. sp. 1</i>	1	1
59 - WKN299	<i>Necrodes surinamensis</i>	USA, NE, Kearney Co.			19 vii 2009	<i>Uroob. sp. 1</i>	1	1
60 - WKN300	<i>Heterosilpha ramosa</i>	USA, NE, Kearney Co.			11 vii 2009	<i>Uroob. sp. 1</i>	1	1
61 - WKN302	<i>Nicro. marginatus</i>	USA, NE, Kearney Co.			06 vii 2009	<i>Urop. orbicularis</i>	1	1
62 - WKN337	<i>Nicro. tomentosus</i>	USA, NE, Kearney Co.			vi 2009	<i>Urop. orbicularis</i>		
63 - WKN344	<i>Nicro. quadripunctatus</i>	Japan, Niigata, Matsunoyama Matsuguchi			25 x 2009	<i>Uroob. sp. 5</i>		
64 - WKN348	<i>Nicro. orbicollis</i>	Can, NS, Conrads	44.65	-63.6	2009	<i>Uroob. sp. 1</i>		
65 - WKN350	<i>Nicro. sayi</i>	Can, NS, Portobello	44.75	-63.6	2009	<i>Uroob. sp. 2</i>	1	

Appendix 2.1. continued.

Beetle no.	Beetle species	Collection location	Lat	Long	Coll date	Mite species*	COI	ITS2
66 - WKN351	<i>Nicro. orbicollis</i>	Can, NS, Portobello	44.75	-63.6	2009	<i>Uroob. sp. 1</i>	1	
67 - WKN361	<i>Nicro. defodiens</i>	Can, NS, McNabs Island	44.6	-63.517	2009	<i>Uroob. sp. 1</i>	1	
68 - WKN364	<i>Nicro. sayi</i>	Can, NS, Devon	44.9	-63.5	2009	<i>Uroob. sp. 2</i>	1	
69 - WKN368	<i>Nicro. sayi</i>	Can, NS, McNabs Island	44.6	-63.517	2009	<i>Uroob. sp. 2</i>		
70 - WKN082	<i>Nicro. sayi</i>	Can, ON, Hwy 132, Dacre	45.369	-76.988	16 v 2009	<i>Uroob. sp. 2</i>		
71 - WKN086	<i>Nicro. orbicollis</i>	Can, ON, Windsor, Elgin St.	42.261	-83.057	18 vi 2009	<i>Uroob. sp. 1</i>		
72 - WKN098	<i>Nicro. quadripunctatus</i>	Japan, Nagano, Honshu, Koshimizu-ga-hara			04 vii 2008	<i>Uroob. sp. 5</i>		
73 - WKN118	<i>Nicro. defodiens</i>	Can, NS, Aylesford, Lake Rd.	44.981	-64.635	25 vi 2009	<i>Uroob. sp. 2</i>		
74 - WKN145	<i>Nicro. orbicollis</i>	Can, ON, Hamilton, Site 4-7			07 vii 2009	<i>Uroob. sp. 1</i>		
75 - WKN159	<i>Nicro. orbicollis</i>	Can, PEI, Wellington, Route 2	46.452	-63.949	17 vii 2009	<i>Uroob. sp. 1</i>	1	
76 - WKN196	<i>Nicro. sayi</i>	USA, NH, Durham	43.134	-70.926	10 vi 2009	<i>Uroob. sp. 2</i>		
77 - WKN232	<i>Nicro. defodiens</i>	Can, AB, nr. Drayton Valley	53.358	-114.984	05 vii 2009	<i>Uroob. sp. 2</i>		
78 - WKN260	<i>Nicro. obscurus</i>	Can, AB, Onefour	49.121	-110.47	12 vi 2001	<i>Uroob. sp. 1</i>		
79 - WKN261	<i>Nicro. guttula</i>	Can, AB, Onefour	49.121	-110.47	12 vi 2001	<i>Uroob. sp. 1</i>		
80 - WKN292	<i>Nicro. marginatus</i>	Can, AB, Onefour	49.121	-110.47	17 vi 2004	<i>Uroob. sp. 1</i>		
81 - WKN348	<i>Nicro. orbicollis</i>	Can, NS, Conrads	44.65	-63.6	2009	<i>Uroob. sp. 1</i>		
82 - WKN098	<i>Nicro. quadripunctatus</i>	Japan, Nagano, Honshu, Koshimizu-ga-hara			04 vii 2008	<i>Uroob. sp. 5</i>		
83 - WKN118	<i>Nicro. defodiens</i>	Can, NS, Aylesford, Lake Rd.	44.981	-64.635	25 vi 2009	<i>Uroob. sp. 2</i>	1	
84 - WKN145	<i>Nicro. orbicollis</i>	Can, ON, Hamilton, Site 4-7			07 vii 2009	<i>Uroob. sp. 1</i>	1	
85 - WKN196	<i>Nicro. sayi</i>	USA, NH, Durham	43.134	-70.926	10 vi 2009	<i>Uroob. sp. 2</i>	1	
86 - WKN232	<i>Nicro. defodiens</i>	Can, AB, nr. Drayton Valley	53.358	-114.984	05 vii 2009	<i>Uroob. sp. 2</i>	1	
87 - WKN260	<i>Nicro. obscurus</i>	Can, AB, Onefour	49.121	-110.47	12 vi 2001	<i>Uroob. sp. 1</i>	1	
88 - WKN261	<i>Nicro. guttula</i>	Can, AB, Onefour	49.121	-110.47	12 vi 2001	<i>Uroob. sp. 1</i>		
89 - WKN282	<i>Nicro. marginatus</i>	Can, AB, Onefour	49.121	-110.47	17 vi 2003	<i>Uroob. sp. 1</i>		
90 - WKN348	<i>Nicro. orbicollis</i>	Can, NS, Conrads	44.65	-63.6	2009	<i>Uroob. sp. 1</i>	1	1
91 - WKN375	<i>Nicro. orbicollis</i>	USA, MD, Scientist's Cliffs	38.504	-76.518	26 vii 2009	<i>Uroob. sp. 1</i>	1	
92 - WKN376	<i>Nicro. orbicollis</i>	USA, MD, Scientist's Cliffs	38.504	-76.518	26 vii 2009	<i>Uroob. sp. 1</i>	1	1
50 - WKHD080	<i>Dendro. rufipennis</i>	Can, NS, Victoria Beach	44.703	-65.747	22 vi 2009	<i>Uroob. orri</i>	1	1
65 - WKHD142	<i>Dryoc. affaber</i>	Can, NS, Sheet Harbour	44.909	-62.503	19 vi 2009	<i>Uroob. orri</i>	1	1
77 - WKHD184	<i>Gnatho. materiarius</i>	Can, NS, Debert, Industrial Park	45.428	-63.429	25 vi 2009	<i>Trich. parisiana</i>	1	
78 - WKHD185	<i>Ips pini</i>	Can, NS, Debert, Industrial Park	45.428	-63.429	25 vi 2009	<i>Trich. australis</i>	1	
95 - WKB5929	<i>Dendro. valens</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	25 vi 2009	<i>Uroob. americana</i>	1	

CHAPTER THREE

Species boundaries and host specificity of uropodoid mites (Trematuridae, Urodinychidae) associated with bark beetles (Scolytinae) based on phylogenetic inference and morphometric analysis.

Abstract

Understanding species boundaries, cryptic diversity and host specificity is the foundation to exploring the evolutionary history of hosts and symbionts. Mites are one of the most diverse arthropod associates of bark beetles (Curculionidae: Scolytinae), with tortoise mites (Mesostigmata: Uropodoidea) being one of the most frequently encountered. There are numerous species of uropodoids associated with bark beetles; however, the species boundaries of these mites have never been assessed using modern systematic techniques. Hence, the diversity of these mites may be greater than what is currently known.

Morphometric data, as well as partial cytochrome oxidase I (COI) and nuclear large subunit ribosomal DNA (28S), were used to explore the species boundaries, the evolution of host specificity, and the extent of coevolution of bark beetle associated uropodoids. A total of 35 species of *Trichouropoda*, *Nenteria* and *Uroobovella* were collected from 51 host species, across 11 countries and 103 sites. Morphometric data as well as phylogenetic reconstructions indicate that the majority of uropodoid species are strict host specialists, while fewer species are generalists. In most cases where an apparent widespread generalist species occurs, it is often a generalist, and not a complex of specialists. Additionally, the evolution of host specificity in scolytine associated uropodoids appears to be neither directional nor irreversible. Lastly, the evolution of associated uropodoids does not reflect a history of tracking their scolytine hosts phylogenetically or ecologically.

Introduction

Research into species boundaries and cryptic species has increased exponentially over the last twenty years, largely due to the increased access to nucleotide sequencing (Bickford et al. 2007). Cryptic species, defined as species distinguishable by no or very subtle morphological differences, are being discovered increasingly as more molecular investigations are conducted, as seen in a wide range of invertebrate groups (Wilcox et al. 1997; Hebert et al. 2004; Fontaneto et al. 2009). Species boundaries of associated symbionts are frequently explored using molecular markers, and it is often revealed that an apparent widespread generalist is not a generalist, but instead a complex of cryptic specialist species. *Ixodes uriae* (Ixodidae) was previously considered to be a host generalist, but microsatellite analysis strongly suggested that *I. uriae* is much more specialized, exhibiting strong genetic divergence across host species (McCoy et al. 2001).

Phylogenetic inference, using molecular markers, has also been effective in exploring the evolution of host specificity. Historically, it has been assumed that the evolution of host specificity is directional and irreversible, in which specialists evolve from generalists, and specialists are an “evolutionary dead end” (Poulin et al. 2006). The understanding is that specialists are adapted to exploit a narrow range of hosts, and this adaptation constrains their ability to colonize new hosts, which makes specialists more susceptible to extinction (Poulin et al. 2006). However, it is currently accepted that host specificity is not directional nor is it irreversible (Poulin 2007); as seen in tachinid parasitoid flies, where generalists are frequently more derived than specialists (Stireman

2005), or in ectoparasitic fleas of small mammals, where the trend is towards the evolution of lower host specificity (Poulin et al. 2006).

Molecular techniques have also been used to explore the extent of coevolution between symbionts and their host species, where the evolution of symbionts may reflect a history of coevolution or ecological fitting, in which symbionts track ecologically-similar host species (Kethley and Johnston 1975; Brooks et al. 2006). The phylogenetic relationships of Procellariiformes seabirds and their associated chewing lice (*Halipeurus*) have shown considerable congruence (Paterson and Banks 2001). However, Fahrenholz's rule, defined as the perfect congruence between the phylogenies of hosts and symbionts, is rarely observed in natural systems (Paterson and Banks 2001). The phylogenetic relationships of Sparidae fish and associated specialist Monogenean gill parasites (*Lamellodiscus*) showed no evidence of coevolution, instead phylogenetically unrelated, but ecologically similar host species, living in sympatry, often shared the same parasite species (Desdevises et al. 2002).

Bark beetle (Scolytinae) associated mites (Acari) provide a model system to examine species boundaries and cryptic diversity, as well as the evolution of host specificity, and the extent of coevolution and ecological fitting. The subcortical niche of living, dead or dying trees provides the habitat for a diverse assemblage of burrowing beetles (Coleoptera: Scolytinae, Cerambycidae, Buprestidae) and their associates, primarily mites, nematodes, and fungi. Bark beetles feed and mate in the cambium or xylem tissue of numerous tree species worldwide (Wood 1982). Mites are one of the most diverse and abundant associates of scolytines, with at least 97 species, representing

65 genera and 40 families, have been collected from the galleries of bark beetle infested pine trees (Moser and Roton 1971). Mites residing in the galleries of bark beetles attach to dispersing scolytines, hitching a ride to host trees and coarse woody debris, which would otherwise be inaccessible to most free-living mites.

The Uropodoidea genera, *Trichouropoda*, *Nenteria* (Trematuridae) and *Uroobovella* (Urodinychidae), are some of the most frequently collected and abundant mite associates of bark beetles. Scolytine associated uropodoids are often collected at a relatively high prevalence, up to 36.3% of 8475 beetles (Kinn and Witcosky 1978). The cohort Uropodina represents more than 2,000 described species worldwide, occurring in various habitats such as forest litter and soil, bird and mammal nests, coarse woody debris, and dung (Bloszyk et al. 2003). Deutonymphal uropodoids glue themselves to their insect host with an anally-secreted pedicel.

Mitochondrial cytochrome oxidase I (COI) and nuclear large subunit ribosomal DNA (28S), either alone or combined with other markers, have been used to uncover cryptic diversity, assess phylogenetic relationships, and to explore host specificity patterns of mites (Anderson and Trueman 2000; Kawazoe et al. 2008; Dowling and OConnor 2010; Martin et al. 2010; Schäffer et al. 2010; Skoracka and Dabert 2010). The specific objectives of this study were to employ morphological and molecular markers, COI and 28S (D2–D4), to explore the species boundaries and evolutionary relationships of bark beetle associated uropodoid species, and to investigate whether widespread generalist species are truly single species with a broad host preference, or rather complexes of cryptic specialists. Additionally, I explored whether the evolution of host

specificity in scolytine associated uropodoids is directional. Lastly, I investigated whether associated mites are coevolving with their scolytine hosts, or are they tracking ecologically-similar host species. To this end, I compared and contrasted the uropodoid phylogeny with the phylogeny and known ecology of their bark beetle hosts.

Materials and methods

Biological material

Bark beetle specimens were collected across 11 countries and 103 sites, with the majority of sites in Canada and USA. Canadian specimens were collected in Ontario by W.K., and in various provinces by the Canadian Food Inspection Agency (CFIA) staff as part of the Invasive Alien Species Monitoring program. Specimens from USA and other countries were collected by A. I. Cognato.

In Ontario, bark beetles were collected across four study sites from mid-April to early-August 2009. The four sites were: Algonquin site 1 (45.902, -77.605), Algonquin site 2 (45.895, -78.071), one site near Pakenham (45.33, -76.371), and another on Hwy 132 near Dacre (45.369, -76.988). Four Lindgren traps with propylene glycol were placed in each study site. Traps were baited with 95% ethanol and/or α -pinene lures (Synergy Semiochemicals). Traps were emptied every two weeks, trap lures were replaced every eight weeks, and the propylene glycol was replaced at each visit. Bark beetles were placed individually into 1.5 ml microfuge tubes with 95% ethanol and stored at -20°C. Scolytines were identified to species using keys from Bright (1976) and Wood (1982), and tribes were based on Alonso-Zarazaga and Lyal (2009). Beetles were examined for uropodoid mites using a dissecting microscope, all mites were removed and placed into a 0.5ml microfuge tube with 95% ethanol and stored at -20°C.

A portion of the bark beetles collected by CFIA staff in 2009 from Canadian provinces, as well as scolytine specimens collected by A.I.C. from USA and several other countries were examined for associated uropodoid mites, all mites were removed, and

stored in 95% ethanol at -80°C. Four species of uropodoids collected from *Nicrophorus* beetles (Silphidae) in Ontario were used as outgroup specimens. Following DNA extraction, mites were recovered from the extraction buffer and slide-mounted in a polyvinyl alcohol medium, and slides were cured on a slide warmer at about 40°C for 3–4 days. Slide-mounted specimens were examined using a compound microscope and identified to species using descriptions from the literature based on morphospecies concepts, focusing on taxonomically informative morphological characteristics (Hirschmann and Zirngiebl-Nicol 1961; Hirschmann 1972; Hirschmann and Wisniewski 1985, 1986, 1987, 1989). Species were identified prior to examining the molecular reconstructions, and in any instances where a conflicting result emerged between the molecular data and identifications, the morphological characteristics and sequence data were reexamined. Voucher specimens are deposited in the Canadian National Collection of Insects, Arachnids and Nematodes, in Ottawa, Canada, and the Michigan State University A.J. Cook Arthropod Research Collection (Appendix 3.1).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from whole specimens for 24 hours using a DNeasy Tissue kit (Qiagen Inc., Santa Clara, CA, USA). Following extraction, mites were removed from the extraction buffer, and genomic DNA was purified following the DNeasy Tissue kit protocol.

PCR amplifications were performed in a total volume of 25 µl, with 13 µl ddH₂O, 2.5 µl 10x PCR buffer, 2.5 µl 25 mM MgCl₂, 0.5 µl of each 10 µM primer, 0.5 µl 10 mM

dNTPs, 0.5 μ l Taq DNA polymerase (Promega Corp., Madison, WI, USA), and 5 μ l genomic DNA template. In the instances where semi-nested or nested primers were employed, 1 μ l of primary PCR product was used as template and the ddH₂O was increased to 17 μ l. PCR amplification cycles were performed on an Eppendorf ep Gradient S Mastercycler (Eppendorf AG, Hamburg, Germany). Primer pairs LCO1490 + LoDog, and LCO1490 + BB R4 (Table 3.1), were used to amplify 643 and 603bp fragments, respectively, of the mitochondrial COI gene. Specimens which did not produce detectable PCR products using either of these primer pairs were reamplified using 1 μ l of the primary PCR product and semi-nested, LCO1490 + BB R3Lo, or nested, BB F + BB R3Lo, primer combinations (Table 3.1), which amplified 592 and 475bp fragments, respectively. Additional primers were designed to amplify COI from uropodoids, the position of all primers designed or employed is shown in the primer map (Table 3.1, Fig. 3.1). The thermocycler protocol for COI amplification was as follows: initial denaturation cycle at 94°C for 3 min, followed by 40 cycles of 94°C for 45 s, primer annealing at 45°C for 45 s, 72°C for 1 min, and a final extension at 72°C for 5 min. The primer annealing temperature was reduced to 43°C when primer BB R4 was employed.

Primer pairs D23F + 28S R2, and 28S Fb + 28S R2 (Table 3.1), were used to amplify a 990 and 980bp fragment, respectively, from the 5' end of the nuclear ribosomal 28S gene, spanning the D2–D4 region. In the instances where neither primer pair produced a detectable PCR product, the specimens were reamplified using 1 μ l of the primary PCR product and semi-nested primer pairs, D23F + 28Sb or 28S Fb + 28Sb,

which amplified an 800 and 790bp fragment of 28S rDNA, respectively (Table 3.1). Additional primers were designed to amplify 28S from uropodoids, all primers designed or employed are shown in the primer map (Table 3.1, Fig. 3.1). The PCR protocol for D23F + 28S R2, and D23F + 28Sb was as follows: initial denaturation cycle at 95°C for 2 min, followed by 30 cycles of 95°C for 1 min, primer annealing at 44°C for 1.5 min, 72°C for 2 min, and a final extension at 72°C for 10 min. The primer annealing temperature was changed to 56°C for 28S Fb + 28S R2, and it was changed to 50°C for 28S Fb + 28S R2.

Amplified products and negative controls were visualized on 1% agarose electrophoresis gels, and purified using pre-cast E-Gel CloneWell 0.8% SYBR Safe agarose gels (Invitrogen, Carlsbad, CA, USA) following the protocol of Gibson et al. (2010). Sequencing reactions were performed in a total reaction volume of 10 µl, with 3 µl ddH₂O, 1.5 µl of 5x sequencing buffer, 0.5 µl of primer, 1 µl of BigDye Terminator (PE Applied Biosystems, Foster City, CA, USA), and 4 µl of purified PCR product. Sequencing was performed at the Agriculture & Agri-Food Canada, Eastern Cereal and Oilseed Research Centre Core Sequencing Facility (Ottawa, ON, Canada). Purification of sequencing reactions was performed using the ABI ethanol/EDTA/sodium acetate precipitation protocol and reactions were analysed on an ABI 3130xl Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA).

Table 3.1. Primer sequences (5'–3') used to amplify partial COI and 28S D2–D4 sequences in uropodoid mites associated with bark beetles.

Gene	Primer	Sequence 5'–3'	Reference
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994
	BB F	TAATTGGWRATGAYCAAATTTTAA	
	BB R2	AATHGTDGTAATAAAATTAATTGA	
	BB R3Lo	CCTCCTGCTAADACHGG	
	BB R4	GTATAGTAATRGCTCCTGC	
	LoDog	GGRTCAAAAAAGAWGTRTTTRAARTTTTCG	
28S	D23F	GAGAGTTCAAGAGTACGTG	Park and Foighil 2000
	28S Fb	GAGTACGTGAAACCGCWTWGA	
	28Sa	GACCCGTCTTGAAACACGG	
	28S F1	GGCGHAATGAAATGTGAAGG	
	28S R3	GGCTTCRTCTTGCCCAGGC	Whiting et al. 1997 (modified)
	28S R4	GGCTTCGTCTTGCCCAGGC	
	28Sb	CGGAAGGAACCAGCTAC	
	28S R2	CCAGTTCTGCTTACCAAAAATGG

Figure 3.1. Primer map showing the relative location of primers used to amplify (A) partial COI, and (B) 28S D2–D4 sequences from uropodoid mites collected from bark beetles.

Sequence alignment and phylogenetic analysis

Sequence chromatograms were edited and contiguous sequences were assembled using Sequencher v4.7 (Gene Codes Corp., Ann Arbor, MI, USA). COI sequences were aligned manually in Mesquite v2.74 (Maddison and Maddison 2010) according to the translated amino acid sequence. 28S was initially aligned in ClustalX v2.0.12 (Larkin et al. 2007) with the default settings, and subsequently adjusted manually in Mesquite, no regions were excised, and due to the absence of any secondary structure for mites for this gene region, no secondary structure alignment was performed. Sequences will be submitted to GenBank.

Phylogenetic reconstructions of COI, 28S, and concatenated datasets were performed using neighbour-joining (NJ) analyses in PAUP* v4.0b10 (Swofford 2003), parsimony analyses in TNT v1.1 (Goloboff et al. 2008), and Bayesian inference (BI) in MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). NJ analysis was used to depict the relationships between species based on pair-wise distances, and to reveal the extent of genetic divergence between species. Bayesian inference and parsimony were used to assess the evolutionary relationships between species; these two approaches are analytically distinct and possess their own inherent strengths and weaknesses (Sanderson and Kim 2000). BI is a parametric statistical approach, in which trees are evaluated based on the probability of getting the observed data given the tree, which is calculated with reference to a specific model of evolution. Parsimony is a nonparametric statistical approach, in which trees are evaluated based on

the minimum number of character state changes necessary to generate the observed data on a given tree (Sanderson and Kim 2000; Kolaczkowski and Thornton 2004).

NJ trees were generated using the pairwise distances calculated with the Kimura-2-parameter (K2P) model, and branch support was assessed using jackknife resampling with 36% of characters removed and 1000 replicates. Parsimony analysis was performed using a heuristic search with tree bisection-reconnection (TBR) branch swapping and 1000 random addition sequence replicates, all characters were treated as unordered, equal weighting, and gaps were treated as missing. Gaps scored as a fifth state produced the same topology as that observed for gaps as missing for each of the analytical approaches. Analyses of the COI dataset excluding the third codon positions produced poorly supported reconstructions with similar topology to the analyses including the third codon position. Multiple trees were obtained and these were presented in a semistrict consensus tree. Node support was assessed in TNT, using jackknife resampling with 36% of characters removed and 1000 replicates, Bremer supports and partitioned Bremer supports (PBS) were also determined using TNT.

MrModeltest v2.3 (Nylander 2004) was used to determine the best-fit model of molecular evolution for each gene, which was determined to be GTR+I+G. Bayesian analysis was performed in MrBayes with a Markov Chain Monte Carlo (MCMC) method, two independent runs, with nucmodel = 4by4, $N_{st} = 6$, rates = invgamma, samplefreq = 1000, four chains = one cold and three heated. The COI dataset ran for 20 million generations, and the 28S and concatenated datasets ran for 10 million generations with a burn-in of 1000. In Mesquite, the remaining trees, excluding the burn-in, were

used to generate a majority-rule consensus tree displaying the posterior probability supports for each node. Bayesian analyses were performed using the on-line Computational Biology Service Unit at Cornell University, and at the Cyberinfrastructure for Phylogenetic Research (CIPRES) portal (Miller et al. 2010).

Coevolution of mites and scolytines

To assess the extent of coevolution between uropodoids and their bark beetle hosts, a tanglegram was created based on the topology of the uropodoid COI BI phylogeny and a phylogeny of 39 bark beetle species. The bark beetle phylogeny was based on Jordal et al. (2008, 2011) and Cognato and Sun (2007), as well as specific adjustments from A.I.C. The uropodoid and bark beetle phylogenies were created and adjusted in Mesquite v2.74 and Adobe Illustrator CS3.

Morphological analysis

To assess intraspecific morphological divergence of mites used in the molecular analyses, slide-mounted specimens were examined using a Leica DM5500B compound microscope, and 15 and 14 characters (for Trematuridae and Urodinychidae, respectively) were measured using Leica Application Suite, Live and Interactive Measurements Modules v3.5. Characters were selected based on prior knowledge for potential variation in mesostigmatic mites, and for their prominence across specimens. The 15 characters measured for trematurid species were: maximal length and width of the dorsal shield and ventrianal shield; sternal shield (SS) median length; SS width at five levels (from anterior

to posterior), the maximal width of the anterior margin anterior of coxa II, the maximum width of the two medial expansions between coxa II–III and coxa III–IV, the minimum width at the posterior constriction near coxa IV, and the width of the posterior margin; length of tarsus I; and the length of the following setae: opisthogastric setae *V8* and *V4* (Hirschmann 1972) (*JV4* and paranal of Evans and Till 1965), anterodorsal setae of tarsus I, and proximoventral setae of femur I. The same characters were measured for urodynychid species, minus the length of seta *V4* and proximoventral setae of femur I, and plus the length of dorsal seta *j1*. Morphological divergence was visualized by generating an ordination based on semistrong hybrid multidimensional scaling (SSH MDS), with PATN v2.27 (Belbin 2003). The ordination is based on a Bray-Curtis distance matrix between mite specimens created using morphometric data standardized for body size to eliminate bias linked to body size, and transformed $((\text{value} - \text{minimum})/\text{range})$ to balance the weight of all measured characters. The ordination was generated using 1000 replicates and 1000 random starts. Significant differences among groups of mites were tested using ANOSIM (analysis of similarity), with 1000 iterations.

The effect of DNA extraction on mite morphology was examined in order to discern whether morphological characteristics of mites that underwent DNA extraction could be considered as unchanged and usable in analyses. Wilcoxon signed rank tests were performed in SPSS v17, to determine the effect of DNA extraction on mite morphology by comparing 13 morphological characters (standardized for body size) of specimens that underwent DNA extraction versus specimens that did not undergo DNA extraction.

Results

A total of 35 species of uropodoids from three genera and two families, were collected from 51 scolytine species from 20 genera and 10 tribes, collected across 11 countries (Appendices 3.1, 3.2). Among the 35 species of uropodoids collected, 22 were found on a single host species and 13 were associated with two or more host species. Most scolytine species were only associated with one or two mite species; four host species had three mite species, and *Polygraphus rufipennis* was associated with four mite species (Appendix 3.1). Amplification of COI was attempted with 176 mites, from which only 116 mites, representing 28 species and three genera, from nine countries and 74 sites yielded COI sequence data (Appendix 3.1). COI was also amplified from 6 outgroup specimens. COI was amplified from 122 specimens, with 608 characters in total, 328 constant, 19 parsimony-uninformative, and 261 parsimony-informative. Mean base pair frequencies (A: 0.294, C: 0.187, G: 0.153, T: 0.366) were found to be heterogeneous across all taxa ($\chi^2 = 504.83$, $p < 0.0001$). The 28S D2–D4 region was used to assess and explore the branching patterns observed in the COI reconstructions, and to test species boundaries. Partial 28S was amplified from 31 mites from 24 species and three genera, collected across nine countries and 26 sites, as well as from two outgroup specimens (Appendix 3.1), with 1069 characters in total, 446 constant, 114 parsimony-uninformative, 509 parsimony-informative. Mean base pair frequencies (A: 0.239, C: 0.199, G: 0.283, T: 0.279) were found to be homogeneous across all taxa ($\chi^2 = 92.12$, $p = 0.59$). In each reconstruction, specimens are labeled with a unique number for each mite

individual (Appendix 3.1), followed by the host species and abbreviated state, province or country.

Neighbor-joining

The neighbor-joining analysis (K2P) of COI from 122 mite specimens including 116 ingroup specimens (28 spp.), and six outgroup specimens (four spp.), generated a well supported NJ tree (Fig. 3.2) (TL = 1978, CI = 0.2513, RI = 0.8326). Most nodes had moderate to high jackknife support (JKS) values, and 26 nodes had 100% JKS, 11 of which are basal nodes to 11 of the ingroup species (Fig. 3.2). The three genera were well supported, and the basal node to the Trematuridae had high JKS (Fig. 3.2). Average COI intraspecific pairwise distance was lowest between *Trichouropoda* species ($1.5\% \pm 1.8$), and highest between *Uroobovella* species ($3.5\% \pm 4.3$) (Table 3.2). The maximum divergence was rather high for both genera, with a maximum of 10.4% for *T. polytricha*, and 12.5% for *U. orri*, both of which were between new and old world specimens (Table 3.2). Mean interspecific divergence within each genus was rather high for all three genera (16.7–17.9%) (Table 3.2). The maximum divergence between *Trichouropoda* species was between *T. hirsuta* and *T. moseri* (23.4%), and the minimum was between *Trich. sp.* 11 and *T. idahoensis* (0.5 %). The maximum for *Uroobovella* was between *U. americana* and *U. orri* (20.8%), and the minimum was between *U. americana* and *U. vinicolora* (8.4%) (Table 3.2). Average intergeneric divergence was very high (18.6–21.5%), with the maximum divergence between *T. hirsuta* and *U. australiensis* (28.1%) (Table 3.2).

Mean intra- and interspecific pairwise distances were distinctly divergent, but the range of intra- and interspecific divergence did overlap partially (Table 3.2).

The NJ analysis of 28S from 33 mite specimens including 31 ingroup specimens (24 spp.), and two outgroup species, generated a well supported NJ tree (Fig. 3.3) (TL = 1465, CI = 0.6881, RI = 0.8204). As seen in the COI NJ tree, most nodes had moderate to high JKS, with 14 nodes with 100% JKS, one of which was the node to the ingroup (Fig. 3.3). The three genera were very well supported, as well as the node to the Trematuridae which had 100% JKS (Fig. 3.3). Average 28S intraspecific pairwise distance was lowest between *Trichouropoda* species ($0.3\% \pm 0.2$), and highest between *Uroobovella* species ($2.1\% \pm 2.4$) (Table 3.2). The maximum divergence was relatively low for *Trichouropoda* with a maximum of 0.5% for *T. californica*, while the maximum distance was higher for *Uroobovella* with a maximum of 4.2% for *U. orri* (Table 3.2). Mean interspecific divergence within each genus was rather high for all three genera (7.1–36.0%) (Table 3.2). The maximum between *Trichouropoda* species was between *T. hirsuta* and *Trich.* sp. 11 (16.6%), and the min was between *T. lamellosa* and *Trich.* n.sp. 10 (0%) (Table 3.2). The maximum for *Uroobovella* species was between *U. dryocoetes* and *U. orri* (42.3%), and the min was between *U. vinicolora* and *U. americana* (1.5%) (Table 3.2). Average intergeneric divergence was very high (16.0–34.9%), with the maximum pairwise distance between *Trichouropoda lamellosa* and *Uroobovella dryocoetes* (41.6%) (Table 3.2). Mean intra- and interspecific pairwise distances are highly divergent and do not overlap, whereas the ranges did overlap slightly (Table 3.2).

Table 3.2. Intra- and interspecific nucleotide divergence (%) $\pm$ standard deviation (range) of COI and 28S amplified from uropodoid mites associated with bark beetles.

	COI mean (range)	28S mean (range)
Intraspecific		
<i>Trichouropoda</i>	1.5 $\pm$ 1.8 (0–10.4)	0.3 $\pm$ 0.2 (0.1–0.5)
<i>Nenteria</i>	–	–
<i>Uroobovella</i>	3.5 $\pm$ 4.3 (0–12.5)	2.1 $\pm$ 2.4 (0–4.2)
Interspecific		
<i>Trichouropoda</i>	16.7 $\pm$ 2.9 (0.5–23.4)	7.1 $\pm$ 5.0 (0–16.6)
<i>Nenteria</i>	16.9 $\pm$ 0 (16.9)	10.0 $\pm$ 0 (10.0)
<i>Uroobovella</i>	17.9 $\pm$ 2.0 (8.4–20.8)	36.0 $\pm$ 13.0 (1.5–42.3)
Intrageneric		
<i>Trichouropoda</i>	15.1 $\pm$ 5.4 (0–23.4)	7.0 $\pm$ 5.1 (0–16.6)
<i>Nenteria</i>	16.9 $\pm$ 0 (16.9)	10.0 $\pm$ 0 (10.0)
<i>Uroobovella</i>	13.4 $\pm$ 7.3 (0–20.8)	29.6 $\pm$ 18.0 (0–42.5)
Intergeneric		
<i>Trich</i> – <i>Nent</i>	18.6 $\pm$ 1.2 (16.3–23.2)	16.0 $\pm$ 1.1 (13.8–20.0)
<i>Trich</i> – <i>Uroob</i>	21.3 $\pm$ 1.4 (17.7–28.1)	34.9 $\pm$ 3.9 (28.5–41.6)
<i>Nent</i> – <i>Uroob</i>	21.5 $\pm$ 1.3 (18.6–23.6)	34.5 $\pm$ 3.7 (29.0–41.1)

The NJ analysis of the concatenated dataset from 31 specimens including 29 ingroup specimens (24 spp.), and two outgroup species, produced a well supported NJ tree (Fig. 3.4) (TL = 2933, CI = 0.4988, RI = 0.6777). Most nodes had moderate to high JKS, with 19 nodes with 100% JKS, which includes the node to the ingroup (Fig. 3.4). The three genera were very well supported, as well as the Trematuridae, these nodes all had 100% JKS (Fig. 3.4).

Figure 3.3. NJ tree (K2P) constructed from 1069bp fragment of 28S from 33 uropodoid specimens, 31 ingroup specimens representing 24 species, and two outgroup species, jackknife support (1000 replicates) >50% shown above branches (TL = 1465, CI = 0.6881, RI = 0.8204).

Figure 3.4. NJ tree (K2P) constructed from concatenated dataset of 608bp fragment of COI and 1069bp fragment of 28S from 31 specimens, 29 ingroup specimens representing 24 species, and two outgroup species, jackknife support (1000 replicates) >50% shown above branches (TL = 2933, CI = 0.4988, RI = 0.6777).

Parsimony

The parsimony heuristic analysis of COI resulted in 34 most parsimonious trees (TL = 1928, CI = 0.2578, RI = 0.8383) presented in a semistrict consensus tree (Fig. 3.5). Many nodes had moderate to high JKS, 18 nodes had 100% JKS. Many nodes had poor Bremer support, with 24 nodes with moderate to strong support (≥ 10). Nine of the nodes with 100% JKS and strong Bremer support are basal nodes to ingroup species (Fig. 3.5). Some species, such as *T. australis*, *T. californica*, *U. orri*, *U. dryocoetes*, and *U. americana*, had multiple unresolved nodes, collapsing into multiple intraspecific polytomies. The heuristic analysis of 28S produced 14 most parsimonious trees (TL = 1462, CI = 0.6895, RI = 0.8216) presented in a semistrict consensus tree (Fig. 3.6). Most nodes had moderate to strong Bremer support, nearly every node had JKS, 12 nodes had 100% JKS, one of which was the basal node to the ingroup (Fig. 3.6). The three genera, as well as the Trematuridae (*Trichouropoda*, *Nenteria*), were strongly supported (Fig. 3.6). Numerous *Trichouropoda* species showed little interspecific divergence resulting in a large polytomy. The parsimony analysis of the concatenated dataset resulted in three most parsimonious trees (TL = 2924, CI = 0.5003, RI = 0.6797) presented in a semistrict consensus tree (Fig. 3.7). Most nodes had moderate to strong JKS, with 10 nodes with 100% JKS, including the basal node to the ingroup and to the Trematuridae. Many nodes had moderate to strong PBS, and the strongest PBS was seen between *Uroobovella* species (Fig. 3.7). The PBS revealed conflict between the COI and 28S datasets for 10 nodes; most notably COI showed negative support for the node to the ingroup, and 28S had strongly negative support for the node basal to *T. fallax* (Fig. 3.7).

Figure 3.5. Semistrict consensus tree of 34 most parsimonious trees (TL = 1928, CI = 0.2578, RI = 0.8383) generated from parsimony heuristic analysis (1000 replicates) of 608bp fragment of COI from 122 uropodoid specimens, 116 ingroup specimens representing 28 species, and six outgroup specimens representing four species, jackknife support (1000 replicates) >50% shown above and Bremer support shown below branches.

Figure 3.6. Semistrict consensus tree of 14 most parsimonious trees (TL = 1462, CI = 0.6895, RI = 0.8216) generated from parsimony heuristic analysis (1000 replicates) of 1069bp fragment of 28S from 33 uropodoid specimens, 31 ingroup specimens representing 24 species, and two outgroup species, jackknife support (1000 replicates) >50% shown above and Bremer support shown below branches.

Figure 3.7. Semistrict consensus tree of three most parsimonious trees (TL = 2924, CI = 0.5003, RI = 0.6797) generated from parsimony heuristic analysis (1000 replicates) of concatenated dataset of 608bp fragment of COI and 1069bp fragment of 28S from 31 specimens, 29 ingroup specimens representing 24 species, and two outgroup species, jackknife support (1000 replicates) >50% shown above and partitioned Bremer support (COI, 28S) shown below branches.

Bayesian Inference

The Bayesian analysis of COI was performed for 20×10^6 generations, which produced 38002 trees (after burn-in) which were summarized in a majority rule consensus tree (TL = 2021, CI = 0.2459, RI = 0.8277) (Fig. 3.8). The BI consensus tree was well supported, with most nodes having moderate to high posterior probabilities, 26 nodes had 100% support, eight of which are basal nodes to ingroup species (Fig. 3.8). The node to the ingroup, as well as the three genera had strong support. Similar to the parsimony reconstruction, *T. australis*, *T. californica*, *U. orri*, *U. dryocoetes*, and *U. americana*, had multiple unresolved nodes, collapsing into intraspecific polytomies. BI of 28S was performed for 10×10^6 generations, producing 18002 trees (after burn-in) that were summarized in a majority rule consensus tree (TL = 1465, CI = 0.6881, RI = 0.8204) (Fig. 3.9). The consensus tree was well supported: 12 nodes had 100% support, one of which was the node to the ingroup (Fig. 3.9). The nodes to the three genera as well as the Trematuridae were well supported. Branch lengths within *Trichouropoda* were very short, especially when compared to the relatively longer branch lengths within *Uroobovella* (Fig. 3.9). BI of the concatenated dataset was performed for 10×10^6 generations, which produced 18002 trees (after burn-in) which were summarized in a majority rule consensus tree (TL = 2947, CI = 0.4964, RI = 0.6746) (Fig. 3.10). The total evidence consensus tree was well supported: 13 nodes had 100% support, which includes the basal node to the ingroup, as well as to the three genera (Fig. 3.10).

Figure 3.8. Majority rule consensus tree of 38002 trees generated by Bayesian MCMC analysis (20×10^6 generations) of 608bp fragment of COI from 122 uropodoid specimens, 116 ingroup specimens representing 28 species, and six outgroup specimens representing four species, posterior probabilities >50% shown above branches (TL = 2021, CI = 0.2459, RI = 0.8277).

Figure 3.9. Majority rule consensus tree of 18002 trees generated by Bayesian MCMC analysis (10×10^6 generations) of 1069bp fragment of 28S from 33 uropodoid specimens, 31 ingroup specimens representing 24 species, and two outgroup species, posterior probabilities >50% shown above branches (TL = 1465, CI = 0.6881, RI = 0.8204).

Figure 3.10. Majority rule consensus tree of 18002 trees generated by Bayesian MCMC analysis (10×10^6 generations) of concatenated dataset of 608bp fragment of COI and 1069bp fragment of 28S from 31 specimens, 29 ingroup specimens representing 24 species, and two outgroup species, posterior probabilities >50% shown above branches (TL = 2947, CI = 0.4964, RI = 0.6746).

Summary of molecular reconstructions

The NJ, parsimony, and BI analyses of COI, 28S and concatenated datasets yielded similar results. In most reconstructions, the monophyly of the three genera, *Trichouropoda*, *Nenteria* and *Uroobovella*, was well supported, except for the COI parsimony analysis where the two *Nenteria* species were not sister to the *Trichouropoda* clade. Across all COI analyses, most trematurid species were monophyletic, with the exception of *T. moseri* and *T. polytricha*. Across all COI analyses, *T. moseri* was not monophyletic, and in particular, *T. moseri* collected from *Pityokteines sparsus* was consistently grouped separately from those collected from *Polygraphus rufipennis*. Across all COI analyses, *T. polytricha* was not monophyletic, in that the *T. polytricha* collected from *Ips cembrae* from Switzerland was consistently shown to be more closely related to *Trich. n.sp. 5* from Norway than to other North American *T. polytricha* specimens. Generalist and specialist species were evenly distributed throughout the molecular reconstructions, and there was seemingly no consistent direction in host specificity.

Molecular analyses of COI indicate that mites of the same species, from the same host individual were more closely related to each other than to mites of the same species from different host individuals of the same host species. Collection site did not appear to impact the degree of relatedness, since mites from different host individuals from the same site were not more closely related to each other than to mites from the same host species from different sites.

Overall, the relationships between trematurid species was poorly resolved using 28S, with slightly better resolution in the concatenated dataset, and better resolution only using COI. The D2–D4 region of 28S was not very effective for examining the relationships between many closely related *Trichouropoda* species. The 28S and COI analyses were not completely congruent. In all 28S reconstructions *T. hirsuta* was basal to all other species in the genus, while COI indicated that *Trich. n.sp. 2* was the basal species of the genus. Additionally, COI and 28S disagreed on the placement of *T. fallax* and *T. alascaae*. COI provided more insight into the relationships between trematurid species than 28S. The concatenated dataset produced well supported trees which were more resolved than 28S alone. The placement of a few *Trichouropoda* species differed between the 28S and concatenated reconstructions, reflecting the shifts in trematurid species relationships independently inferred from COI and 28S.

In all COI reconstructions, there appeared to be a major division within the trematurids generating two distinct groups of trematurid species, this separation was well supported and shown most clearly in the Bayesian analysis. Most trematurid species in one of the two major divisions of the family are associated with beetles of the Ipini tribe, while most species in the other major division are associated with beetles of tribes other than Ipini. With the exception of the placement of a few trematurid species, which were represented by only one or two specimens, the relationships between trematurid species were consistent across all COI reconstructions.

Across all reconstructions, the monophyly of all *Uroobovella* species was very well supported, and the relationships between *Uroobovella* species was consistent across

all analyses. In particular, *U. orri*, *U. dryocoetes* and *U. australiensis* were most closely related to each other, while *U. americana* and *U. vinicolora* were most closely related to each other. Across all COI analyses, there was a small clade of *U. orri* associated with *Orthotomicus caelatus* beetles, which was very well supported.

Coevolution of mites and scolytines

The degree of coevolution between uropodoids and their bark beetle hosts was explored using a tanglegram which compared the overlap between the uropodoid COI B1 phylogeny and a phylogeny of 39 scolytine species, which was based on Jordal et al. (2008, 2011) and Cognato and Sun (2007). The tanglegram clearly shows that there is no evidence of coevolution between bark beetles and uropodoids (Fig. 3.11).

Figure 3.11. Tanglegram showing the lack of cospeciation between uropodoids and their bark beetle hosts, lines show the observed associations: (A) majority rule consensus tree of 38002 trees generated by Bayesian MCMC analysis (20x10⁶ generations) of 608bp fragment of COI from 29 uropodoid species compared with the (B) phylogeny of 39 bark beetle species which had mites, based on Jordal et al. (2008, 2011) and Cognato and Sun (2007).

Morphological analysis

The level of intraspecific morphological divergence was assessed in five widespread generalist species (*T. australis*, *T. parisiana*, *T. polytricha*, *U. orri*, *U. dryocoetes*), and two relatively more specialized species, (*T. californica* and *U. americana*), in order to explore whether widespread generalist species displayed cryptic morphological diversity.

Uroobovella orri was the only species of the seven examined that showed prominent morphological variation, with two apparent groupings: mites from *Orthotomicus caelatus*, labelled as *U. 'caelatus'* (to indicate host association), and mites from hosts other than *O. caelatus* (Fig. 3.12). The SSH MDS ordination (stress = 0.1571) (Fig. 3.12) and ANOSIM based on 14 morphological characters from 22 *U. orri* specimens, indicate that *U. orri* and *U. 'caelatus'* are significantly divergent morphologically ($p = 0.01$).

Mean COI divergence between *U. 'caelatus'* specimens was very low ($0.5\% \pm 0.31$), where as the mean divergence between *U. 'caelatus'* and all other *U. orri* specimens from North America, was 20 times higher ($10.5\% \pm 0.4$).

Figure 3.12. Two-dimensional SSH MDS ordination with Bray-Curtis distance (1000 replicates, 1000 random starts) performed on measurements ((value – min)/range transformed) of 14 morphological characters from 22 uropodoids representing *Uroobovella orri* and *U. 'caelatus'* (stress = 0.1571).

The remaining generalist and specialist species displayed no significant intraspecific morphological variation; these species also showed low COI intraspecific divergence (<1%), with the exception of *T. polytricha* and *T. parisiana* with 4.6 and 2.8% divergence, respectively. The high divergence between *T. polytricha* specimens is largely due to a single specimen from Switzerland, since the intraspecific divergence among North America specimens was only 2%.

Overall, it appears that DNA extraction did not significantly alter the morphology of a mite, and morphological characteristics of mites that undergo DNA extraction can be considered unaltered, with a couple exceptions. Wilcoxon signed rank tests revealed that the extraction of DNA had no statistically significant effect on the morphology of *U. orri*

specimens when compared to mites that did not undergo DNA extraction, based on measurements of 13 characters and 15 pairwise comparisons ($p = 0.078\text{--}0.995$). The extraction of DNA had no statistically significant effect on the morphology of *T. californica* specimens, based on 13 characters and 10 pairwise comparisons ($p = 0.139\text{--}0.799$), with the exception of two characters: median length and width of the sternal shield ($p = 0.037$, $p = 0.009$). The variation of these two characters is most likely an artefact of slide mounting following DNA extraction, in that extraction weakens sclerotized tissue which may encourage shields to fracture. Slide-mounted *T. californica* specimens that underwent DNA extraction had small fractures on either side of the sternal shield just posterior to the midpoint, which may have increased sternal shield medial length and width relative to that of mites that did not undergo DNA extraction.

Discussion

This study is the first to use the barcoding region of COI and the D2–D4 region of 28S to examine the relationships between uropodoid species. The effectiveness and variability of any specific marker can vary between taxa, thus it is difficult to predict the relative effectiveness of a given marker when attempting to investigate relationships between previously unexplored taxa. This study indicates that both partial COI and 28S D2–D4 are suitable markers for distinguishing between closely and distantly related uropodoid species. COI was far more effective than 28S at distinguishing between closely related *Trichouropoda* species. In contrast, 28S does appear to be a good marker for distinguishing between closely related *Uroobovella* species.

This study also represents the first investigation into the evolutionary relationships of uropodoid species associated with scolytines. A total of 35 species were collected, 12 of which are undescribed. The scolytine tribe Ipini had the greatest diversity of uropodoids, with nearly half of all specimens and species collected. Overall, COI, 28S and concatenated datasets yielded similar results across different analytical approaches. Overall, the monophyly of all three uropodoid genera was well supported. Most morphologically defined species were well supported by the COI reconstructions, with the exception of *T. moseri* and *T. polytricha*.

The uropodoids examined in this study showed various levels of host specificity. Among the 35 uropodoid species collected, the majority were host-specialists, with much fewer generalists. Molecular reconstructions and morphometric analyses indicate that for the most part, widespread generalist species are not complexes of cryptic specialist

species, but instead they are truly generalists. Either gene flow between populations of generalist species has not been inhibited, or COI has not diverged significantly for these species. *Uroobovella orri* was the only generalist species that may instead represent at least two distinct species in North America, including a widespread generalist, associated with at least eight species and six genera of hosts, and a host-specialist (*U. 'caelatus'*) associated only with *Orthotomicus caelatus*. Interestingly, *O. caelatus* is not a host-tree specialist, but it is instead a broad generalist attacking many species of *Pinus*, *Picea* and *Larix* throughout its range (Wood 1982). In addition to *U. 'caelatus'*, it is possible that *U. orri* collected from *Pityokteines curvidens* in Croatia is also a distinct cryptic species, based upon the level of COI divergence from all other *U. orri* specimens (11.5%). Intraspecific variation of the cosmopolitan *U. orri* requires further investigation with additional markers and specimens.

In all COI reconstructions, *T. moseri* and *T. polytricha* were not monophyletic, which may suggest that these two generalist species are actually complexes of cryptic specialists. *Trichouropoda moseri* collected from Ipini hosts was consistently distinct from those collected from Polygraphini hosts. It is possible that *T. moseri*, from these two tribes, represents two cryptic specialist species rather than a single generalist species; however, this was not supported by morphometric analysis. *Trichouropoda polytricha* collected from *Ips cembrae* from Switzerland was more closely related to the morphologically dissimilar species, *Trich. n.sp. 5* from Norway, than to North American *T. polytricha*. *Trichouropoda polytricha* did not show significant morphological variation, but the non-monophyly of the species may indicate that it is instead a complex

of cryptic species. The species boundaries of *T. moseri* and *T. polytricha* need to be assessed with additional markers and specimens.

Specialist and generalist species were seemingly evenly distributed throughout the phylogenetic trees, which emphasizes that neither generalists nor specialists represent an evolutionarily more derived strategy for scolytine uropodoids. There were a greater proportion of specialist species than generalists, but there was no evidence to suggest that host specificity was directional, indicated by the somewhat random distribution of specialists and generalists. However, the relationships between species were poorly resolved, especially between *Trichouropoda* species, and taxon sampling was not exhaustive. As a result of these shortcomings this dataset only provides a limited view of the evolution of host specificity in this group, and conclusions should be drawn tentatively. To gain a more in-depth understanding of the evolution of host specificity a more complete and robust phylogeny is required, and the direction of host specificity should be assessed using statistical analyses, such as generalized least squares and likelihood-ratio tests (Poulin et al. 2006).

The evolutionary history of associated symbionts may reflect a history of host switching and ecological tracking, or it may reflect a long-term coevolutionary relationship (Kethley and Johnston 1975; Paterson and Banks 2001; Morelli and Spicer 2007). The evolution of scolytine-associated uropodoids does not reflect the phylogenetic history or the shared ecology of their bark beetle hosts. Alternatively, it is possible that the evolution of scolytine uropodoids represents a blend of coevolution and tracking ecologically-similar species, as seen with platyhelminth parasites of anuran amphibians

(Brooks et al. 2006). Overall, the tanglegram indicates that bark beetle and uropodoid phylogenies are not congruent, with little evidence that closely related hosts share closely related mites. Multiple bark beetle species often occupy the same tree concurrently, and occasionally their galleries cross, thus providing mites with an opportunity to transfer across host species (Moser et al. 1971). There are a few examples of closely related hosts sharing closely related mites, but the shared ecology of these hosts confounds any inferences about the extent of coevolution. For example, *T. californica* is associated with the sister-species, *Ips hoppingi* and *I. confusus*, and *T. californica* specimens associated with these host species, are also very closely related (Cognato and Sun 2007). Initially, it may appear that these two host species share the same mite species because they are sister-species, but *I. hoppingi* and *I. confusus* are also similar ecologically, with peripatric ranges and feeding on pinyon pine (*Pinus*) species (Wood 1982), and as a result there is no way to discern if they share the same mite species due to shared phylogeny or shared ecology.

On the other hand, there is little evidence of ecological tracking of associated uropodoids, in that the scolytine species that feed on similar host trees, have overlapping habitat ranges, or similar phenologies (Wood 1982), are not necessarily associated with the same mite species. Most mite species were collected from phloeophagous scolytines, and these mite species demonstrated no specific association with hosts from a particular tree species or habitat type. A notable exception is *T. parisiana* and *Trich. n.sp. 1* which are monophyletic species associated with “ambrosia beetles”, which are an ecological grade of scolytines and platypodines that carry symbiotic fungi in complex glandular

mycangial structures, which is inoculated into host trees and cultivated as a food source (Wood 1982). *Trichouropoda parisiana* was collected from three distantly related ambrosia beetle species, *Gnathotrichus materiarius* (Corthylini), *Xyleborinus saxesenii* (Xyleborini) and *Trypodendron retusum* (Xyloterini), which attack a range of coniferous and deciduous host trees. *Trichouropoda* n.sp. 1 is morphologically and genetically similar to *T. parisiana*, and it was only collected from the ambrosia beetle *Corthylus* sp. (Corthylini), which uses deciduous host trees. It is possible that the closely related *T. parisiana* and *Trich.* n.sp. 1 are tracking some aspect of the mycetophagous life history of their hosts.

Scolytines are associated with a diverse fauna of uropodoid mites, and for the most part COI and 28S were effective for exploring the species boundaries of these mites. The majority of bark beetle associated uropodoid species are strict monospecific specialists, and fewer species are generalists. The phylogenetic reconstructions and morphometric analyses indicate that widespread generalist species are truly generalists rather than complexes of cryptic specialist species, with one exception. The evolution of host specificity in scolytine associated uropodoids was not directional or irreversible, in that neither specialists nor generalists represent an evolutionarily more derived strategy. Lastly, the evolutionary history of associated uropodoids does not reflect a history of tracking the phylogenetic relationships or shared ecology of their scolytine hosts. Our understanding of the ecological and evolutionary relationships between interacting species hinges on having thorough knowledge of species boundaries and the extent of cryptic diversity.

Appendix 3.1. Collection and host species records of uropodoid mites collected from scolytines (ingroup) and *Nicrophorus* beetles (outgroup), 1 indicates that either COI or 28S was amplified (**Uroob.*= *Uroobovella*, *Trich.*= *Trichouropoda*, *Nent.*= *Nenteria*).

Beetle no.	Beetle species	Collection location	Lat	Long	Date	Mite species*	COI	28S
1 - WKB4051	<i>Pityokteines sparsus</i>	Can, ON, Hwy 132, Dacre	45.369	-76.988	16 v 2009	<i>Uroob. orri</i>	1	
2 - WKB4057	<i>Orthotomicus caelatus</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	16 v 2009	<i>Uroob. orri</i>	1	
3 - WKB4095	<i>Gnathotrichus materiarius</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	16 v 2009	<i>Trich. parisiana</i>	1	
4 - WKB4109	<i>Ips grandicollis</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	16 v 2009	<i>Trich. australis</i>		
5 - WKB4190	<i>Pityokteines sparsus</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	28 v 2009	<i>Trich. moseri</i>	1	
6 - WKB4232	<i>Polygraphus rufipennis</i>	Can, ON, Carbine Rd.	45.330	-76.371	16 v 2009	<i>Uroob. orri</i>		
7 - WKB4429	<i>Dendroctonus valens</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	16 v 2009	<i>Uroob. americana</i>	1	
8 - WKB4850	<i>Polygraphus rufipennis</i>	Can, AB, Fort McMurray	56.016	-110.88	23 vii 2009	<i>Trich. moseri</i>	1	
9 - WKB4869	<i>Dryocoetes affaber</i>	Can, AB, Fort McMurray	56.016	-110.88	29 vi 2009	<i>Uroob. orri</i>		
10 - WKB4943	<i>Hylesinus aculeatus</i>	Can, ON, Hwy 132, Dacre	45.369	-76.988	1 v 2009	<i>Trich. bipilis</i>	1	
11 - WKB4987	<i>Ips pini</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	1 v 2009	<i>Trich. australis</i>	1	
12 - WKB4995	<i>Trypodendron retusum</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	1 v 2009	<i>Trich. parisiana</i>		
13 - WKB5224	<i>Polygraphus rufipennis</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	28 v 2009	<i>Uroob. orri</i>	1	
14 - WKB5226	<i>Dryocoetes affaber</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	28 v 2009	<i>Uroob. orri</i>	1	
15 - WKB5261	<i>Hylastes porculus</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	28 v 2009	<i>Uroob. dryocoetes</i>	1	
16 - WKB5344	<i>Gnathotrichus materiarius</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	28 v 2009	<i>Trich. parisiana</i>	1	
17 - WKB5351	<i>Dendroctonus valens</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	28 v 2009	<i>Uroob. dryocoetes</i>		
18 - WKB5563	<i>Pityogenes hopkinsi</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	28 v 2009	<i>Trich. n.sp. 3</i>		
19 - WKB5564	<i>Polygraphus rufipennis</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	28 v 2009	<i>Trich. moseri</i>		
20 - WKB5568	<i>Ips pini</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	28 v 2009	<i>Trich. australis</i>		
21 - WKB5639	<i>Orthotomicus caelatus</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	28 v 2009	<i>Uroob. orri</i>	1	
22 - WKB5682	<i>Dryocoetes autographus</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	25 vi 2009	<i>Uroob. dryocoetes</i>	1	
23 - WKB5759	<i>Ips grandicollis</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	25 vi 2009	<i>Trich. lamellosa</i>		
24 - WKB5759	<i>Ips grandicollis</i>	Can, ON, Algonquin P.P. 1	45.902	-77.605	25 vi 2009	<i>Uroob. orri</i>	1	
25 - WKB5797	<i>Hylurgops pinifex</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	25 vi 2009	<i>Trich. hirsuta</i>		
26 - WKB5882	<i>Hylastes porculus</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	25 vi 2009	<i>Trich. hirsuta</i>	1	1
27 - WKB5970	<i>Dendroctonus ponderosae</i>	Can, AB, Grande Prairie			2007	<i>Trich. lamellosa</i>	1	1
28 - WKHD001	<i>Gnathotrichus materiarius</i>	Can, QE, La Patrie, Route 212	46.345	-72.576	22 v 2009	<i>Trich. parisiana</i>		
29 - WKHD004	<i>Pityokteines sparsus</i>	Can, QE, La Patrie, Route 212	46.345	-72.576	22 v 2009	<i>Uroob. orri</i>	1	
30 - WKHD008	<i>Dendroctonus valens</i>	Can, QE, La Patrie, Route 212	46.345	-72.576	22 v 2009	<i>Uroob. americana</i>		
31 - WKHD009	<i>Polygraphus rufipennis</i>	Can, QE, East Hereford	45.029	-71.505	22 v 2009	<i>Uroob. orri</i>		
32 - WKHD010	<i>Gnathotrichus materiarius</i>	Can, QE, East Hereford	45.029	-71.505	22 v 2009	<i>Trich. parisiana</i>		

Appendix 3.1. continued.

Beetle no.	Beetle species	Collection location	Lat	Long	Date	Mite species*	COI	28S
34 - WKHD012	<i>Gnathotrichus materiarius</i>	Can, QE, Pont Rouge	46.806	-71.679	05 vi 2009	<i>Trich. parisiana</i>	1	
35 - WKHD014	<i>Polygraphus rufipennis</i>	Can, QE, Pont Rouge	46.806	-71.679	05 vi 2009	<i>Uroob. dryocoetes</i>	1	
36 - WKHD018	<i>Dendroctonus rufipennis</i>	Can, NS, West Northfield			01 vi 2009	<i>Uroob. orri</i>		
37 - WKHD030	<i>Hylastes porculus</i>	Can, NS, Westfield	44.403	-64.975	28 v 2009	<i>Uroob. dryocoetes</i>	1	
38 - WKHD037	<i>Hylastes porculus</i>	Can, NB, Bayside, Route 127	45.205	-67.140	15 vi 2009	<i>Uroob. dryocoetes</i>		
39 - WKHD042	<i>Xyleborinus saxesenii</i>	Can, BC, Stanley Park, Pipeline Dr.			06 vi 2008	<i>Trich. parisiana</i>	1	
40 - WKHD057	<i>Gnathotrichus materiarius</i>	Can, QE, Parc des iles de Boucherville	45.601	-73.466	26 v 2009	<i>Trich. parisiana</i>		
41 - WKHD062	<i>Dendroctonus valens</i>	Can, QE, Sorel-Tracy	46.030	-73.083	09 vi 2009	<i>Uroob. americana</i>	1	
42 - WKHD065	<i>Gnathotrichus materiarius</i>	Can, QE, Sorel-Tracy	46.030	-73.083	09 vi 2009	<i>Uroob. orri</i>		
43 - WKHD066	<i>Hylastes porculus</i>	Can, QE, Sorel-Tracy	46.030	-73.083	09 vi 2009	<i>Uroob. dryocoetes</i>	1	1
44 - WKHD067	<i>Dryocoetes autographus</i>	Can, QE, Sorel-Tracy	46.030	-73.083	09 vi 2009	<i>Uroob. dryocoetes</i>		
45 - WKHD070	<i>Dryocoetes affaber</i>	Can, QE, Sorel-Tracy	46.030	-73.083	09 vi 2009	<i>Uroob. dryocoetes</i>		
46 - WKHD075	<i>Hylastes ruber</i>	Can, BC, McPhee Creek Rd.	49.323	-117.61	29 iv 2009	<i>Trich. fallax</i>	1	1
47 - WKHD078	<i>Hylurgops pinifex</i>	Can, NS, Greenfield	44.335	-64.915	11 vi 2009	<i>Trich. fallax</i>		
48 - WKHD079	<i>Dendroctonus rufipennis</i>	Can, NS, Annapolis, Granville ferry	44.810	-65.537	22 vi 2009	<i>Trich. alascae</i>	1	
49 - WKHD079	<i>Dendroctonus rufipennis</i>	Can, NS, Annapolis, Granville ferry	44.810	-65.537	22 vi 2009	<i>Uroob. orri</i>	1	
50 - WKHD080	<i>Dendroctonus rufipennis</i>	Can, NS, Victoria Beach	44.703	-65.747	22 vi 2009	<i>Uroob. orri</i>	1	
51 - WKHD085	<i>Dendroctonus rufipennis</i>	Can, NS, Blomidon, Stewart Mtn. Rd.	45.227	-64.397	19 vi 2009	<i>Trich. alascae</i>	1	1
52 - WKHD085	<i>Dendroctonus rufipennis</i>	Can, NS, Blomidon, Stewart Mtn. Rd.	45.227	-64.397	19 vi 2009	<i>Uroob. orri</i>	1	
53 - WKHD114	<i>Dendroctonus rufipennis</i>	Can, QE, Degelis	47.561	-68.644	16 vi 2009	<i>Uroob. orri</i>		
54 - WKHD116	<i>Hylastes porculus</i>	Can, QE, Saint Come De liniere	46.014	-70.483	23 vi 2009	<i>Uroob. dryocoetes</i>	1	
55 - WKHD117	<i>Gnathotrichus materiarius</i>	Can, QE, Degelis	47.551	-68.642	26 vi 2009	<i>Trich. parisiana</i>		
56 - WKHD118	<i>Hylastes porculus</i>	Can, QE, Degelis	47.551	-68.642	26 vi 2009	<i>Uroob. dryocoetes</i>	1	
57 - WKHD120	<i>Dendroctonus valens</i>	Can, QE, Pont Rouge	46.562	-71.545	08 vi 2009	<i>Uroob. americana</i>	1	
58 - WKHD121	<i>Dendroctonus valens</i>	Can, QE, Saint Pamphile	46.943	-69.764	17 vi 2009	<i>Uroob. dryocoetes</i>	1	
59 - WKHD129	<i>Dendroctonus rufipennis</i>	Can, QE, Saint Pamphile	46.947	-69.761	17 vi 2009	<i>Uroob. orri</i>		
60 - WKHD130	<i>Dryocoetes autographus</i>	Can, NB, Monument	45.954	-67.767	24 vi 2009	<i>Uroob. dryocoetes</i>	1	
61 - WKHD133	<i>Dendroctonus rufipennis</i>	Can, NS, Sheet Harbour	44.907	-62.491	19 vi 2009	<i>Uroob. orri</i>	1	
62 - WKHD136	<i>Polygraphus rufipennis</i>	Can, NS, Sheet Harbour	44.907	-62.491	19 vi 2009	<i>Uroob. orri</i>	1	
63 - WKHD140	<i>Dryocoetes autographus</i>	Can, NS, Sheet Harbour	44.909	-62.503	19 vi 2009	<i>Uroob. dryocoetes</i>	1	
64 - WKHD142	<i>Dryocoetes affaber</i>	Can, NS, Sheet Harbour	44.909	-62.503	19 vi 2009	<i>Trich. hirsuta</i>		
65 - WKHD142	<i>Dryocoetes affaber</i>	Can, NS, Sheet Harbour	44.909	-62.503	19 vi 2009	<i>Uroob. orri</i>	1	
66 - WKHD149	<i>Polygraphus rufipennis</i>	Can, QE, Cookshire	45.389	-71.513	02 vii 2009	<i>Trich. hirsuta</i>		

Appendix 3.1. continued.

Beetle no.	Beetle species	Collection location	Lat	Long	Date	Mite species*	COI	28S
67 - WKHD158	<i>Dryocoetes autographus</i>	Can, QE, Cookshire	45.389	-71.513	02 vii 2009	<i>Uroob. dryocoetes</i>	1	
68 - WKHD169	<i>Dryocoetes affaber</i>	Can, QE, Cookshire	45.389	-71.513	02 vii 2009	<i>Uroob. dryocoetes</i>	1	
69 - WKHD172	<i>Dryocoetes affaber</i>	Can, QE, Saint Malo	45.197	-71.527	02 vii 2009	<i>Uroob. dryocoetes</i>		
70 - WKHD175	<i>Hylastes porculus</i>	Can, QE, La Patrie, Route 212	46.345	-72.576	02 vii 2009	<i>Uroob. dryocoetes</i>	1	
71 - WKHD177	<i>Dryocoetes autographus</i>	Can, QE, La Patrie, Route 212	46.345	-72.576	02 vii 2009	<i>Uroob. dryocoetes</i>		
72 - WKHD178	<i>Orthotomicus caelatus</i>	Can, NS, Goodwood	44.603	-63.677	27 v 2009	<i>Uroob. orri</i>	1	1
73 - WKHD179	<i>Ips pini</i>	Can, NS, Goodwood	44.603	-63.677	27 v 2009	<i>Trich. australis</i>	1	1
74 - WKHD181	<i>Polygraphus rufipennis</i>	Can, NS, Purcell's Cove	44.624	-63.575	03 vi 2009	<i>Uroob. orri</i>	1	
75 - WKHD182	<i>Dryocoetes affaber</i>	Can, NS, Purcell's Cove	44.624	-63.575	03 vi 2009	<i>Uroob. orri</i>	1	
76 - WKHD183	<i>Dendroctonus rufipennis</i>	Can, NS, Purcell's Cove	44.624	-63.575	13 vii 2009	<i>Uroob. orri</i>		
77 - WKHD184	<i>Gnathotrichus materiarius</i>	Can, NS, Debert, Industrial Park	45.428	-63.429	25 vi 2009	<i>Trich. parisiana</i>	1	
78 - WKHD185	<i>Ips pini</i>	Can, NS, Debert, Industrial Park	45.428	-63.429	25 vi 2009	<i>Trich. australis</i>	1	
79 - WKHD189	<i>Ips borealis</i>	Can, NS, Debert, Industrial Park	45.428	-63.429	25 vi 2009	<i>Trich. polytricha</i>	1	
80 - WKHD193	<i>Dryocoetes autographus</i>	Can, NS, Debert, Industrial Park	45.428	-63.429	25 vi 2009	<i>Uroob. dryocoetes</i>	1	
81 - WKHD194	<i>Dryocoetes affaber</i>	Can, QE, Saint Roch de Mekinac	46.792	-72.748	23 vi 2009	<i>Uroob. orri</i>		
82 - WKHD199	<i>Hylastes porculus</i>	Can, QE, Saint Severin, Route 159	46.686	-72.525	23 vi 2009	<i>Uroob. dryocoetes</i>	1	
83 - WKHD204	<i>Ips grandicollis</i>	Can, ON, Brampton	43.708	-79.728	06 vii 2009	<i>Trich. australis</i>	1	
84 - WKHD208	<i>Ips grandicollis</i>	Can, ON, Argentia Rd. Century Ave	43.598	-79.744	07 vii 2009	<i>Trich. australis</i>	1	1
85 - WKHD228	<i>Ips pini</i>	Can, QE, Boucherville	45.601	-73.466	09 vii 2009	<i>Trich. australis</i>	1	
86 - WKHD230	<i>Ips pini</i>	Can, ON, Argentia Rd. Century Ave	43.598	-79.744	20 vii 2009	<i>Trich. australis</i>		
87 - WKHD232	<i>Ips grandicollis</i>	Can, ON, New Market, 500 Water St.	44.047	-79.456	23 vii 2009	<i>Uroob. orri</i>	1	
88 - WKHD234	<i>Ips pini</i>	Can, ON, New Market, 500 Water St.	44.047	-79.456	23 vii 2009	<i>Trich. australis</i>	1	
89 - WKHD235	<i>Polygraphus rufipennis</i>	Can, QE, Saint Zacharie	46.130	-70.262	21 vii 2009	<i>Trich. moseri</i>	1	1
90 - WKHD236	<i>Polygraphus rufipennis</i>	Can, QE, Woburn	45.342	-70.898	21 vii 2009	<i>Trich. moseri</i>	1	
91 - WKHD237	<i>Polygraphus rufipennis</i>	Can, QE, Saint Benjamin	46.268	-70.617	21 vii 2009	<i>Trich. moseri</i>		
92 - WKHD252	<i>Ips borealis</i>	Can, NS, Hantsport, Cobesquid Bay	45.099	-64.184	21 vii 2009	<i>Trich. polytricha</i>		
93 - WKHD254	<i>Ips pini</i>	Can, NS, Hantsport, Cobesquid Bay	45.099	-64.184	11 viii 2009	<i>Trich. australis</i>	1	
94 - WKHD261	<i>Hylastes subopacus</i>	USA, NM, Bernalillo			10 x 2008	<i>Nent. chiapasa</i>		
95 - WK85929	<i>Dendroctonus valens</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	25 vi 2009	<i>Uroob. americana</i>	1	
96 - WK85639	<i>Orthotomicus caelatus</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	28 v 2009	<i>Uroob. orri</i>	1	1
97 - WK85929	<i>Dendroctonus valens</i>	Can, ON, Algonquin P.P. 2	45.895	-78.071	25 vi 2009	<i>Uroob. americana</i>	1	1
98 - MSU001	<i>Pityophthorus</i> sp.	USA, CA, El Dorado N.F. Ice House Res.	38.5	-120.22	25 v 2007	<i>Trich. n.sp. 2</i>	1	1
99 - MSU004	<i>Dendroctonus valens</i>	USA, OH, Secrest Arboretum	40.782	-81.916	v 2007	<i>Uroob. americana</i>		

Appendix 3.1. continued.

Beetle no.	Beetle species	Collection location	Lat	Long	Date	Mite species*	COI	28S
100 - MSU006	<i>Ficicis</i> sp.	China, Yunnan, Xishuangbanna	22.163	100.871	30 v 2008	<i>Uroob. australiensis</i>	1	
101 - MSU010	<i>Dendroctonus valens</i>	USA, PA, Keystone Rd.	40.739	-76.308	30 iv 2009	<i>Uroob. americana</i>		
102 - MSU012	<i>Polygraphus</i> sp.	Thailand, Doi Pui			iv 2005	<i>Trich. polygraphi</i>		
103 - MSU014	<i>Scolytus ventralis</i>	USA, CA, El Dorado N.F. Ice House Res.	38.5	-120.22	17 vi 2003	<i>Trich. n.sp. 10</i>	1	1
104 - MSU016	<i>Hylurgops rugipennis pinifex</i>	USA, UT, Ashley N.F., Gray Head Peak	39.54	-110.45	11 vi 2003	<i>Trich. fallax</i>		
105 - MSU020	<i>Monarthrum dentigerum</i>	USA, TX, Davis Mt. S.P.			25 v 2001	<i>Trich. n.sp. 8</i>		
106 - MSU024	<i>Monarthrum dentigerum</i>	USA, TX, Big Bend N.P.			iv 2004	<i>Trich. n.sp. 8</i>		
107 - MSU025	<i>Hylurgops</i> sp.	Mex, South of Amecameca	19.016	-98.741	11 v 2004	<i>Uroob. vinicolora</i>	1	
108 - MSU028	<i>Hylastes</i> sp.	USA, WI, Cobma			11 iv 2004	<i>Trich. perissops</i>		
109 - MSU030	<i>Dendroctonus valens</i>	USA, WI, nr. Madison			v 2005	<i>Uroob. americana</i>	1	
110 - MSU032	<i>Pseudips mexicanus</i>	Mex, Jalisco			5 xi 2003	<i>Nent. moseri</i>	1	1
111 - MSU036	<i>Pityokteines curvidens</i>	Croatia			2003	<i>Uroob. orri</i>	1	1
112 - MSU038	<i>Pseudips mexicanus</i>	Mex, Jalisco, nr. Ciudad Guzman			9 ii 2006	<i>Trich. n.sp. 9</i>	1	
113 - MSU040	<i>Orthotomicus erosus</i>	Italy, Tuscany, nr. San Gusme	43.360	11.501	29 xii 2006	<i>Trich. n.sp. 4</i>	1	1
114 - MSU045	<i>Ips hunteri</i>	USA, UT, Ashley N.F., Hwy 191	40.43	-109.29	10 vi 2003	<i>Trich. polytricha</i>		
115 - MSU049	<i>Ips pilifrons utahensis</i>	USA, CO, San Isabel N.F. Monarch Pass	38.31	-106.19	9 vi 2003	<i>Trich. polytricha</i>		
116 - MSU050	<i>Ips cribricollis</i>	USA, NM, Big Burro Mts			20 viii 2003	<i>Trich. australis</i>	1	
117 - MSU051	<i>Ips perturbatus</i>	USA, MN, Cascade River Park			12 vi 2001	<i>Trich. polytricha</i>	1	
118 - MSU053	<i>Ips cribricollis</i>	Mex, South of Amecameca	19.016	-98.741	11 v 2004	<i>Trich. tegucigalpae</i>	1	1
119 - MSU055	<i>Ips cribricollis</i>	Mex, Landa de Matamoros	21.263	-99.177	14 v 2004	<i>Trich. australis</i>		
120 - MSU056	<i>Ips nitidus</i>	China, Sichuan			9 vii 2004	<i>Nent. eulaelaptis</i>	1	1
121 - MSU057	<i>Ips cribricollis</i>	Mex, Jalisco, nr. Ciudad Guzman			9 ii 2006	<i>Trich. sp. 13</i>	1	
122 - MSU060	<i>Ips pilifrons</i>	USA, CO, White River N.F. Lost Lake			30 vi 2005	<i>Trich. polytrichasimilis</i>		
123 - MSU066	<i>Ips calligraphus</i>	USA, FL, Naples, Collier	26.157	-81.660	iii - iv 2007	<i>Trich. australis</i>		
124 - MSU067	<i>Ips hoppingi</i>	USA, TX, McDonald Observatory			12 iv 2002	<i>Trich. californica</i>	1	
125 - MSU069	<i>Ips montanus</i>	USA, WA, Hwy 410, nr. Chinook Pass			11 v 2001	<i>Trich. polytrichasimilis</i>		
126 - MSU071	<i>Ips pini</i>	USA, AK, Douglas is. nr. Juneau			4 v 2001	<i>Trich. idahoensis</i>	1	
127 - MSU073	<i>Ips pini</i>	USA, CA, Lassen N.F. Polesprings Rd.			3 vii 2001	<i>Trich. idahoensis</i>	1	
128 - MSU079	<i>Ips plastographus</i>	USA, CA,			v 2001	<i>Trich. sp. 11</i>	1	1
129 - MSU084	<i>Ips paraconfusus</i>	USA, CA, Mt. Diablo S.P. Contra Costa			10 vi 2001	<i>Trich. sp. 7</i>		
130 - MSU085	<i>Ips lecontei</i>	USA, AZ, Coronado N.F. Ladybug Peak			18 vii 2001	<i>Trich. australis</i>	1	
131 - MSU086	<i>Ips cembrae</i>	Switzerland			v 2002	<i>Trich. polytricha</i>	1	1
132 - MSU090	<i>Ips montanus</i>	USA, CA, El Dorado, Hwy 50 nr. Meyer			13 vi 2001	<i>Trich. polytricha</i>	1	

Appendix 3.1. continued.

Beetle no.	Beetle species	Collection location	Lat	Long	Date	Mite species*	COI	28S
133 - MSU091	<i>Pityogenes chalcographus</i>	Norway			v 2002	<i>Trich. n.sp. 5</i>	1	1
134 - MSU094	<i>Ips confusus</i>	USA, NV, Mt. Charleston Recreation	36.16	-115.32	27 vi 2003	<i>Trich. californica</i>	1	
135 - MSU099	<i>Ips confusus</i>	USA, UT, nr. Baker Dam	37.23	-113.39	28 vi 2003	<i>Trich. californica</i>	1	
136 - MSU104	<i>Ips confusus</i>	USA, AZ, Kaibab N.F. Hwy 389	36.51	-112.16	30 vi 2003	<i>Trich. californica</i>	1	
137 - MSU108	<i>Ips confusus</i>	USA, AZ, Kaibab N.F. nr. Flagstaff	35.24	-111.35	2 vii 2003	<i>Trich. californica</i>	1	
138 - MSU111	<i>Ips confusus</i>	USA, NM, Carson N.F. nr. Los Pinons	36.25	-106.01	9 vi 2003	<i>Trich. californica</i>	1	1
139 - MSU114	<i>Ips confusus</i>	USA, NM, Santa Fe			17 vi 2003	<i>Trich. californica</i>	1	
140 - MSU119	<i>Ips confusus</i>	USA, NV, Risue Canyon			4 vi 2003	<i>Trich. californica</i>	1	
141 - MSU123	<i>Ips confusus</i>	USA, AZ, Coconino, nr. Red Mt.	35.31	-111.5	vi 2003	<i>Trich. californica</i>	1	
142 - MSU124	<i>Ips confusus</i>	USA, CO, F.R. 504	37.669	-108.70	9 viii 2004	<i>Trich. californica</i>	1	
143 - MSU125	<i>Ips perturbatus</i>	Can, ON, Marlborough Forest			19 v 1995	<i>Trich. australis</i>		
144 - MSU127	<i>Pseudips mexicanus</i>	USA, CA, San Francisco			20 viii 1995	<i>Trich. n.sp. 9</i>	1	
145 - MSU131	<i>Ips emarginatus</i>	USA, CA, Lassen, Black Mt.			7 vii 1995	<i>Trich. polytrichasimilis</i>		
146 - MSU132	<i>Ips calligraphus</i>	USA, NY, Smithtown			11 ix 1994	<i>Trich. australis</i>		
147 - MSU133	<i>Ips pini</i>	USA, NY			18 x 1995	<i>Trich. australis</i>		
148 - MSU137	<i>Ips paraconfusus</i>	USA, CA, Mt. Diablo			3 ix 1995	<i>Trich. sp. 7</i>	1	
149 - MSU139	<i>Ips woodi</i>	USA, AZ, Coronado N.F. Hospital Flat			4 ix 1996	<i>Trich. polytricha</i>	1	
150 - MSU143	<i>Dendroctonus valens</i>	USA, PA, 225 Yeager Rd. Woodland	41.049	-78.349	30 iv 2009	<i>Uroob. americana</i>	1	
151 - MSU144	<i>Ips woodi</i>	USA, AZ, Apache N.F. Hannagan Meadow			1 ix 1996	<i>Trich. polytricha</i>	1	
152 - MSU147	<i>Ips pilifrons</i>	USA, AZ, Apache N.F. Hannagan Meadow			31 viii 1996	<i>Trich. australis</i>	1	
153 - MSU148	<i>Ips cribricollis</i>	USA, NM, Otero			v 1994	<i>Trich. australis</i>	1	
154 - MSU150	<i>Ips hunteri</i>	USA, AZ, Apache N.F. Hannagan Meadow				<i>Trich. australis</i>	1	
155 - MSU152	<i>Pseudips mexicanus</i>	USA, CA, Albion River Rd. nr. Rt. 1			23 iii 1996	<i>Trich. n.sp. 9</i>	1	1
156 - MSU154	<i>Ips emarginatus</i>	USA, CA, El Dorado N.F. Ice House Res.			6 ix 1997	<i>Uroob. orri</i>	1	
157 - MSU155	<i>Dendroctonus valens</i>	USA, CA, University of California Berkeley			14 x 1996	<i>Uroob. vinicolora</i>	1	
158 - MSU157	<i>Ips cribricollis</i>	USA, NM, Cloudcroft			11 v 1994	<i>Trich. australis</i>	1	
159 - MSU162	<i>Ips bonanseai</i>	Mex, Nuevo Leon			xii 1993	<i>Trich. tegucigalpae</i>		
160 - MSU163	<i>Ips hoppingi</i>	Mex, Nuevo Leon	24.505	-99.985	25 x 1993	<i>Trich. californica</i>		
161 - MSU167	<i>Ips plastographus</i>	USA, CA, Santa Cruz			13 x 1993	<i>Uroob. orri</i>	1	
162 - MSU168	<i>Ips pini</i>	USA, RI, Lincoln S.P.			19 vii 1997	<i>Trich. australis</i>	1	
163 - MSU173	<i>Ips emarginatus</i>	USA, CA, Lassen, Bogard Bultes			6 xii 1996	<i>Uroob. orri</i>	1	
164 - MSU174	<i>Ips cembrae</i>	Germany, Dresden			28 v 1986	<i>Trich. polytricha</i>		
165 - MSU179	<i>Gnathotrichus materiarius</i>	USA, MI, Mt. Pleasant			28 v 1998	<i>Trich. parisiana</i>	1	

Appendix 3.1. continued.

Beetle no.	Beetle species	Collection location	Lat	Long	Date	Mite species*	COI	28S
166 - MSU180	<i>Camptocerus auricomis</i>	Panama			4 ix 2008	<i>Trich. n.sp. 6</i>		
167 - MSU185	<i>Corthylus</i> sp.	Panama	8.862	-82.743	26 viii 2008	<i>Trich. n.sp. 1</i>	1	
168 - MSU010	<i>Dendroctonus valens</i>	USA, PA, Keystone Rd.	40.739	-76.308	30 iv 2009	<i>Uroob. americana</i>		
169 - MSU084	<i>Ips paraconfusus</i>	USA, CA, Mt. Diablo S.P. Contra Costa			10 vi 2001	<i>Trich. sp. 7</i>	1	1
170 - MSU123	<i>Ips confusus</i>	USA, AZ, Coconino, nr. Red Mt.	35.31	-111.5	vi 2003	<i>Trich. californica</i>		1
171 - MSU143	<i>Dendroctonus valens</i>	USA, PA, 225 Yeager Rd. Woodland	41.049	-78.349	30 iv 2009	<i>Uroob. americana</i>	1	1
172 - MSU148	<i>Ips cribricollis</i>	USA, NM, Otero			v 1994	<i>Trich. australis</i>	1	1
173 - MSU154	<i>Ips emarginatus</i>	USA, CA, El Dorado N.F. Ice House Res.			6 ix 1997	<i>Uroob. orri</i>		
174 - MSU185	<i>Corthylus</i> sp.	Panama	8.862	-82.743	26 viii 2008	<i>Trich. n.sp. 1</i>	1	1
175 - MSU025	<i>Hylurgops</i> sp.	Mex, South of Amecameca	19.016	-98.741	11 v 2004	<i>Uroob. vinicolora</i>	1	1
176 - MSU049	<i>Ips pilifrons utahensis</i>	USA, CO, San Isabel N.F. Monarch Pass	38.31	-106.19	9 vi 2003	<i>Trich. polytricha</i>		1
177 - MSU179	<i>Gnathotrichus materiarius</i>	USA, MI, Mt. Pleasant			28 v 1998	<i>Trich. parisiana</i>	1	1
2 - WKN084	<i>Nicrophorus sayi</i>	Can, QE, Pont-Rouge	46.806	-71.679	05 vi 2009	<i>Uroob. sp. 2</i>	1	
7 - WKN165	<i>Nicrophorus orbicollis</i>	Can, ON, Carbine Rd.	45.330	-76.371	23 vii 2009	<i>Uroob. sp. 1</i>	1	1
8 - WKN184	<i>Nicrophorus vespilloides</i>	Germany, Mooswald Forest, nr. Freiburg	48.0	7.85	vi 2009	<i>Uroob. sp. 3</i>	1	1
21 - WKN165	<i>Nicrophorus orbicollis</i>	Can, ON, Carbine Rd.	45.330	-76.371	23 vii 2009	<i>Uroob. sp. 1</i>	1	
30 - WKN090	<i>Nicrophorus nepalensis</i>	Taiwan, nr. Meifeng, 5 km Sungkang	24.088	121.171	02 v 2007	<i>Uroob. sp. 4</i>	1	
65 - WKN350	<i>Nicrophorus sayi</i>	Can, NS, Portobello	44.75	-63.6	2009	<i>Uroob. sp. 2</i>	1	

Scolytine tribe		
species	Uropodoid species	Country
Corthylini		
<i>Corthylus</i> sp.	<i>Trich.</i> n.sp. 1	Panama
<i>Gnathotrichus materiarius</i>	<i>Trich. parisiana</i> Wisniewski & Hirschmann, 1987	Canada
	<i>Trich. parisiana</i>	USA
	<i>Uroob. orri</i> Hirschmann, 1972	Canada
<i>Monarthrum dentigerum</i>	<i>Trich.</i> n.sp. 8	USA
<i>Pityophthorus</i> sp.	<i>Trich.</i> n.sp. 2	USA
Dryocoetini		
<i>Dryocoetes affaber</i>	<i>Trich. hirsuta</i> Hirschmann, 1972	Canada
	<i>Uroob. dryocoetes</i> (Vitzthum 1923)	Canada
	<i>Uroob. orri</i>	Canada
<i>D. autographus</i>	<i>Uroob. dryocoetes</i>	Canada
Hylastini		
<i>Hylastes porculus</i>	<i>Trich. hirsuta</i>	Canada
	<i>Uroob. dryocoetes</i>	Canada
<i>H. ruber</i>	<i>Trich. fallax</i> (Vitzthum, 1926)	Canada
<i>Hylastes</i> sp.	<i>Trich. perissops</i> Wisniewski & Hirschmann, 1986	USA
<i>H. subopacus</i>	<i>Nent. chiapasa</i> Hirschmann, 1978	USA
<i>Hylurgops pinifex</i>	<i>Trich. fallax</i>	Canada
	<i>Trich. hirsuta</i>	Canada
<i>H. rugipennis pinifex</i>	<i>Trich. fallax</i>	USA
<i>Hylurgops</i> sp.	<i>Uroob. vinicolora</i> (Vitzthum, 1926)	Mexico
Hylesinini		
<i>Ficicis</i> sp.	<i>Uroob. australiensis</i> Wisniewski & Hirschmann, 1992	China
<i>Hylesinus aculeatus</i>	<i>Trich. bipilis</i> (Vitzthum, 1921)	Canada
Hylurgini		
<i>Dendroctonus ponderosae</i>	<i>Trich. lamellosa</i> Hirschmann, 1972	Canada
<i>D. rufipennis</i>	<i>Trich. alascas</i> Wisniewski & Hirschmann, 1987	Canada
	<i>Uroob. orri</i>	Canada
<i>D. valens</i>	<i>Uroob. americana</i> Hirschmann, 1972	Canada
	<i>Uroob. americana</i>	USA
	<i>Uroob. dryocoetes</i>	Canada
	<i>Uroob. vinicolora</i>	USA
Ipini		
<i>Ips bonansea</i>	<i>Trich. tegucigalpae</i> Hirschmann & Wisniewski, 1986	Mexico
<i>I. borealis</i>	<i>Trich. polytricha</i> (Vitzthum, 1923)	Canada
<i>I. calligraphus</i>	<i>Trich. australis</i> Hirschmann, 1972	USA
<i>I. cembrae</i>	<i>Trich. polytricha</i>	Germany
	<i>Trich. polytricha</i>	Switzerland
<i>I. confusus</i>	<i>Trich. californica</i> Wisniewski & Hirschmann, 1988	USA
<i>I. cribricollis</i>	<i>Trich. australis</i>	Mexico
	<i>Trich. australis</i>	USA
	<i>Trich.</i> sp. 13	Mexico
	<i>Trich. tegucigalpae</i>	Mexico
<i>I. emarginatus</i>	<i>Trich. polytrichasimilis</i> Hirschmann, 1972	USA
	<i>Uroob. orri</i>	USA

Appendix 3.2. continued.

Scolytine tribe		
species	Uropodoid species	Country
Ipini		
<i>Ips grandicollis</i>	<i>Trich. australis</i>	Canada
	<i>Trich. lamellosa</i>	Canada
	<i>Uroob. orri</i>	Canada
<i>I. hoppingi</i>	<i>Trich. californica</i>	Mexico
	<i>Trich. californica</i>	USA
<i>I. hunteri</i>	<i>Trich. australis</i>	USA
	<i>Trich. polytricha</i>	USA
<i>I. lecontei</i>	<i>Trich. australis</i>	USA
<i>I. montanus</i>	<i>Trich. polytricha</i>	USA
	<i>Trich. polytrichasimilis</i>	USA
<i>I. nitidus</i>	<i>Nent. eulaelaptis</i> (Vitzthum, 1930)	China
<i>I. paraconfusus</i>	<i>Trich. sp. 7</i>	USA
<i>I. perturbatus</i>	<i>Trich. australis</i>	Canada
	<i>Trich. polytricha</i>	USA
<i>I. pilifrons</i>	<i>Trich. australis</i>	USA
	<i>Trich. polytrichasimilis</i>	USA
<i>I. pilifrons utahensis</i>	<i>Trich. polytricha</i>	USA
<i>I. pini</i>	<i>Trich. australis</i>	Canada
	<i>Trich. australis</i>	USA
	<i>Trich. idahoensis</i> Hirschmann & Wisniewski, 1986	USA
<i>I. plastographus</i>	<i>Trich. sp. 11</i>	USA
	<i>Uroob. orri</i>	USA
<i>I. woodi</i>	<i>Trich. polytricha</i>	USA
<i>Orthotomicus caelatus</i>	<i>Uroob. orri</i>	Canada
<i>O. erosus</i>	<i>Trich. n.sp. 4</i>	Italy
<i>Pityogenes chalcographus</i>	<i>Trich. n.sp. 5</i>	Norway
<i>P. hopkinsi</i>	<i>Trich. n.sp. 3</i>	Canada
<i>Pityokteines curvidens</i>	<i>Uroob. orri</i>	Croatia
<i>P. sparsus</i>	<i>Trich. moseri</i> Hirschmann, 1972	Canada
	<i>Uroob. orri</i>	Canada
<i>Pseudips mexicanus</i>	<i>Nent. moseri</i> Hirschmann, 1972	Mexico
	<i>Trich. n.sp. 9</i>	Mexico
Polygraphini		
<i>Polygraphus rufipennis</i>	<i>Trich. hirsuta</i>	Canada
	<i>Trich. moseri</i>	Canada
	<i>Uroob. dryocoetes</i>	Canada
	<i>Uroob. orri</i>	Canada
<i>Polygraphus sp.</i>	<i>Trich. polygraphi</i> (Vitzthum, 1923)	Thailand
Scolytini		
<i>Camptocerus auricomis</i>	<i>Trich. n.sp. 6</i>	Panama
<i>Scolytus ventralis</i>	<i>Trich. n.sp. 10</i>	USA
Xyleborini		
<i>Xyleborinus saxesenii</i>	<i>Trich. parisiana</i>	Canada
Xyloterini		
<i>Trypodendron retusum</i>	<i>Trich. parisiana</i>	Canada

CHAPTER FOUR

**Diversity, prevalence and host specificity of mites (Acari: Mesostigmata, Oribatida)
associated with bark beetles (Curculionidae: Scolytinae) in Ontario.**

Abstract

Mites represent one of the most diverse groups of arthropods associated with bark beetles (Curculionidae: Scolytinae), and there is little known about the acarofauna of Canadian bark beetles. The objectives of this study were to describe the mesostigmatic and oribatid mite fauna associated with bark beetles, sampled across four sites in Eastern Ontario, in 2008 and 2009. In Lindgren funnel traps baited with α -pinene and/or ethanol lures, and traps baited with *Ips pini* pheromone lures, a total of 5635 bark beetles (30 species) were collected, and 16.4% of these beetles had at least one mite. A total of 2424 mites representing 33 species, were collected. The majority of mite species were specialists from one (33.3%) or two (36.4%) host species, with fewer generalist species on three or more host species (30.3%). This study represents the first broad investigation into the acarofauna of Ontario bark beetles. The level of host specificity of some of the mite species collected in this study differed from that of published records, and this variation in the apparent level of host specificity may reflect some of the obstacles associated with the study of host specificity.

Introduction

One of the biggest puzzles in the evolutionary ecology of symbiotic species is why some symbionts have broad host ranges, whereas other species tend to be specialists (Van Tienderen 1991; Kassen 2002; Krasnov et al. 2004; Poulin et al. 2006). Host specificity, or host range, operationally defined as the number of host species used by a symbiont, has received continued interest because of its linkage to testing ideas about local adaptation (Lajeunesse and Forbes 2002), expression of resistance (Poulin and Combes 1999), and the evolution of discrimination (Brown and Wilson 1992). Questions about what influences host specificity are themselves open to broad interpretation depending on how species are defined. Many recent studies have shown that symbiont species with broad host ranges are actually groups of cryptic species, but in some instances generalists are shown to be truly generalists (Smith et al. 2007). This potential problem remains as long as taxonomic species are the units of investigation. Additional obstacles associated with the study of host specificity include the publication of incorrect identifications (Moss 1978; Lindquist and Wu 1991), changes and instability in the status of species (McGraw and Farrier 1969; Pence and Casto 1976), and the geographic range of study (Krasnov et al. 2004).

Mites (Acari) associated with bark beetles, provide a diverse and abundant natural system to explore some of the challenges surrounding the study of host specificity. Dead, dying and living trees provide habitat for a diverse assemblage of wood-burrowing beetles (Cerambycidae, Buprestidae, Curculionidae: Scolytinae), mites, nematodes, and fungi. Scolytines feed and reproduce in the cambium or xylem tissue of many tree species

across the globe, many species feed on dead or dying coarse woody debris, and some species of the genera *Ips*, *Dendroctonus*, and *Scolytus* cause extensive economic losses in North America (Wood 1982). *Dendroctonus rufipennis* is reportedly the most destructive spruce feeding bark beetle, killing approximately up to 0.55 billion board feet of spruce timber on average per year (Wood 1982). *Dendroctonus ponderosae* is considered to be the most destructive of all *Dendroctonus* species, with an estimated timber loss of 1.5 billion board feet per year since 1895 (Wood 1982). Over 5,800 described species of scolytines are known worldwide, and there are approximately 525 species and subspecies in Canada and the United States (Rabaglia 2002).

The association between bark beetles and mites is an ancient and diverse ecological relationship. One of the oldest records of mites associated with insects are tortoise mites (Mesostigmata: Uropodina) attached to bark beetles in 20–40 million year old amber collected in the Dominican Republic (Poinar 1982). A total of 97 species of mites from 65 genera and 40 families were collected from the galleries of bark beetle infested pine trees (Moser and Roton 1971), although, some of the mites collected may not have a true association with bark beetles. Lindquist (1969) observed that many species of the scolytine genera *Ips*, *Dendroctonus*, *Dryocoetes*, *Hylastes*, *Hylesinus*, *Orthotomicus*, *Tomicus*, and *Scolytus* are typically associated with assemblages of 15–20 mite species. Mites dwelling in bark beetle galleries attach to dispersing beetles, hitching a ride to a new tree or coarse woody debris, which would otherwise be inaccessible to most mite individuals. Scolytine-associated mites include fungivores, predators of insects and nematodes, omnivores, and scolytine parasites and parasitoids (Lindquist 1970). The

relationship between mites and their hosts may vary from beneficial to detrimental across and even within mite species (Hirschmann and Rühm 1955; Kinn 1967, 1980, 1984; Moser 1975), although overall, the impact that each mite species has on their bark beetle hosts is largely unknown.

The acarofauna associated with bark beetles in Canada are virtually unstudied. Several bark beetle species have been surveyed for mites in other localities, and typically these surveys focus on individual species of economic importance; including *Ips typographus* in Germany and Sweden (Moser and Bogenschütz 1984; Moser et al. 1989), *Dendroctonus frontalis* in Louisiana, Central America and Mexico (Moser and Roton 1971; Moser et al. 1974), *D. ponderosae* in Alberta (Mori et al. 2011), *Scolytus multistriatus* and *S. pygmaeus* in Austria (Moser et al. 2005), *Pityokteines curvidens*, *P. spinidens* and *P. vorontzowi* in Croatia (Pernek et al. 2008), *Trypodendron lineatum* in Germany (Strube and Benner 1984). The specific objectives of this study were to describe the diversity, prevalence, abundance, and host specificity of mesostigmatic and oribatid mites associated with scolytines in Eastern Ontario over four sites sampled in 2008 and 2009, using baited Lindgren funnel traps. In addition, the observed variation in the level of host specificity between this study and published host records was used to explore some of the challenges associated with the study of host specificity.

Materials and Methods

Study sites and sampling design

Bark beetles were sampled in four study sites from mid-April to late-August in 2008. Two sites were in the mixed wood ecozone, one near Charleston Lake (S1) (44.500, -76.072) and the other near Frontenac Provincial Park (P.P.) (S2) (44.447, -76.577). The other two sites were in the boreal shield ecozone in Algonquin P.P., Algonquin site 1 (N1) (45.902, -77.605) and Algonquin site 2 (N2) (45.895, -78.071). Sites S1 and S2 were in isolated stands of white pine, along the Frontenac axis, surrounded by deciduous forests. Sites N1 and N2 were in large contiguous stands of white and red pines. Five Lindgren 12-unit funnel dry traps with four 1 cm³ pest strips (Ortho Home Defense Max), baited with lures (Synergy Semiochemicals Corp. Burnaby, BC), were placed in each site at least 16 m apart, with the collection cups ~80 cm off the ground and at least 2 m from any host trees (distance from trees, trap height, and distance between traps based upon Miller and Duerr (2008)). Three traps were baited with ipsdienol (100 mg released at ~400 ug/day) and lanerione (4 mg) to attract *I. pini* adults. The other two Lindgren traps were baited with α -pinene and 95% ethanol (released at 2 g/day and 400 mg/day, respectively) lures to attract beetles seeking dead or dying coarse woody debris. At mid-May, the trap lures were changed so that in each site there were four traps baited with α -pinene and 95% ethanol lures, and one trap baited with ipsdienol and lanerione lures. Traps were emptied every two weeks, trap lures were replaced every eight weeks, and the

insecticide strips were replaced during each visit to maintain effectiveness. Each beetle specimen captured was placed individually into a microfuge tube with 80% ethanol.

In 2009, sampling was repeated in Algonquin P.P. due to the high abundance and diversity of bark beetles in these sites, and sampling was also performed in deciduous stands in order to collect a broader diversity of scolytines and mites. From mid-April to early-August 2009, bark beetles were sampled in four sites: N1, N2, and two new sites, one near Carbine road (S3) outside of Pakenham (45.330, -76.371), and another on Hwy 132 (S4) near Dacre (45.369, -76.988). Sites S3 and S4 were in mixed wood forest dominated by deciduous trees. Four Lindgren traps with propylene glycol were placed in each study site following the same protocol. Traps were baited with α -pinene and 95% ethanol lures in sites N1 and N2, and baited with 95% ethanol lures in sites S3 and S4. Traps were emptied every two weeks, trap lures were replaced every eight weeks, and propylene glycol replaced at each visit. Each beetle captured was placed individually into a microfuge tube with 95% ethanol.

Identifications and mite associations

Scolytines were identified to species using a dissecting microscope and keys from Bright (1976) and Wood (1982), and tribes were based on Alonso-Zarazaga and Lyal (2009). The presence, abundance, and attachment location of mesostigmatic and oribatid mites was recorded; other mites (Prostigmata, Astigmata) were not studied. Prevalence was defined as the proportion of beetles with mites of a given species. Intensity was defined as the number of mites of a given species, per beetle carrying mites. Average abundance

was defined as the average number of mites of a given species, across all host individuals examined. All mesostigmatic and oribatid mites were removed from the host, cleared in 85% lactic acid for 1–24 hours depending on the degree of opacity, slide-mounted in a polyvinyl alcohol medium and cured on a slide warmer at about 40°C for 3–4 days. Slide-mounted specimens were examined using a compound microscope and identified to species using descriptions from the literature, in which species concepts were based on taxonomically informative morphological characteristics as indicated by species descriptions (Hirschmann 1960, 1972; Hirschmann and Zirngiebl-Nicol 1961; Hurlbutt 1967; Hirschmann and Wisniewski 1982, 1986, 1987, 1989; Wunderle et al. 1990). Voucher specimens are deposited in the Canadian National Collection of Insects, Arachnids and Nematodes, in Ottawa, Canada.

Results

General traps

A total of 4006 bark beetles from 29 species, 21 genera and 10 tribes, were collected in traps with general lures (α -pinene and/or ethanol), across four sites in 2008 and 2009 (Table 4.1). Beetle abundance was higher in 2008 than in 2009 (2421 and 1585 beetles, respectively), and species richness was higher in 2009 than in 2008 (26 and 23 species, respectively) (Table 4.1). Beetles were collected almost exclusively in the spring; in 2008, 96% of all beetles were collected from mid-April to mid-June, and in 2009, 98% from mid-April to late-June. *Hylastes porculus* (28%), *H. opacus* (20%), and *Dendroctonus valens* (10%) were the three most abundant species across all sites in 2008 and 2009 (Table 4.1). The majority of beetle individuals were collected from Algonquin P.P. in 2008 (89%) and 2009 (87%), and species diversity was accordingly highest in Algonquin in both years (Table 4.1).

Across all sites and both years, 12% of the 4006 beetles examined had at least one mesostigmatic or oribatid mite (hereafter mites), and of the 29 host species examined, only 17 species (13 genera and 9 tribes) had mites (Table 4.1). Overall prevalence was highest in 2009 than in 2008 (13.5% and 10%, respectively). Nine bark beetle species (242 individuals) that feed on deciduous hosts were collected (Table 4.1), three of which had mites, with a low overall prevalence of 1.7%. A total of 19 conifer feeding bark beetle species (3706 individuals) were collected, 14 of which had mites, with an overall prevalence of 12.3% (Table 4.1).

Table 4.1. Total abundance of scolytine species collected across four sites in Eastern Ontario in 2008 and 2009 (*mites present).

Tribe	Beetle species	2008 sites ¹				2009 sites ¹			
		N1	N2	S1	S2	N1	N2	S3	S4
Corthylini	<i>Corthylus punctatissimus</i> ^d	-	-	1	1	-	-	-	1
	<i>Gnathotrichus materiarius</i>	135*	26	8*	14*	19*	39*	-	-
	<i>G. materiarius</i> ⁺	2	2	-	-	-	-	-	-
	<i>Monarthrum mali</i> ^d	-	-	-	-	-	1	-	-
	<i>Pityophthorus</i> sp.	15	28	2	2	3	5	-	3
	<i>Pityophthorus</i> sp. ⁺	-	4	3	3	-	-	-	-
Crypturgini	<i>Crypturgus pusillus</i>	2	-	-	-	-	-	-	-
Dryocoetini	<i>Dryocoetes affaber</i>	9	-	-	7	5*	1	-	-
	<i>D. affaber</i> ⁺	1	-	-	-	-	-	-	-
	<i>D. autographus</i>	22*	-	-	-	11*	-	1	-
	<i>Lymantria decipiens</i> ^d	-	-	-	-	-	1	-	-
	<i>L. decipiens</i> ^{d+}	-	-	1	40	-	-	-	-
Hylastini	<i>Hylastes opacus</i>	154*	221*	10	11	17	388	-	-
	<i>H. opacus</i> ⁺	1	3	4	-	-	-	-	-
	<i>H. porculus</i>	603*	42*	14*	67	291*	104*	2	1
	<i>H. porculus</i> ⁺	-	1	-	-	-	-	-	-
	<i>Hylurgops pinifex</i>	26*	11*	1	1	3*	6*	-	-
	<i>H. pinifex</i> ⁺	1	-	-	-	-	-	-	-
Hylesinini	<i>Hylesinus aculeatus</i> ^d	-	-	1	-	-	-	3	15*
	<i>H. aculeatus</i> ^{d+}	-	-	1*	-	-	-	-	-
Hylurgini	<i>Dendroctonus rufipennis</i>	2	-	-	-	-	-	-	-
	<i>D. valens</i>	102*	93*	1	3	12*	194*	-	-
	<i>D. valens</i> ⁺	-	2	1	-	-	-	-	-
	<i>Hylurgopinus rufipes</i> ^d	-	-	-	-	-	-	-	4
	<i>Tomicus piniperda</i>	-	-	-	-	-	1	-	-
Ipini	<i>Ips grandicollis</i>	80*	93*	16*	23	30*	83*	-	-
	<i>I. grandicollis</i> ⁺	-	2	-	-	-	-	-	-
	<i>I. perroti</i> ⁺	-	1	-	-	-	-	-	-
	<i>I. pini</i>	9*	5*	-	6	2*	7*	-	-
	<i>I. pini</i> ⁺	170*	504*	511*	230*	-	-	-	-

Table 4.1. continued.

Tribe	Beetle species	2008 sites ¹				2009 sites ¹			
		N1	N2	S1	S2	N1	N2	S3	S4
Ipini	<i>Orthotomicus caelatus</i>	142*	36*	12*	10*	15*	83*	-	-
	<i>O. caelatus</i> ⁺	1	-	-	-	-	-	-	-
	<i>Pityogenes hopkinsi</i>	6	25	1	-	1	14*	-	3
	<i>P. hopkinsi</i> ⁺	4	14	2	1	-	-	-	-
	<i>P. plagiatus</i>	-	6	-	-	1	1	-	-
	<i>P. plagiatus</i> ⁺	1	6	-	-	-	-	-	-
	<i>Pityogenes</i> sp.	-	1	-	-	-	-	-	-
	<i>Pityokteines sparsus</i>	1	3	1	-	-	1*	-	2*
	<i>P. sparsus</i> ⁺	-	3	-	-	-	-	-	-
Polygraphini	<i>Polygraphus rufipennis</i>	18	8*	-	1	11*	1*	2*	1
	<i>P. rufipennis</i> ⁺	-	1	-	-	-	-	-	-
Xyleborini	<i>Xyleborus dispar</i> ^d	-	-	-	-	-	-	14	60
	<i>X. sayi</i> ^d	3*	2	19	24	-	-	39	31
Xyloterini	<i>Trypodendron lineatum</i>	232*	-	2	-	21	4	-	6
	<i>T. lineatum</i> ⁺	9	-	1	-	-	-	-	-
	<i>T. retusum</i> ^d	-	-	-	-	2*	2	-	-
	<i>Xyloterinus politus</i> ^d	1	-	-	-	2	3	6	6
	<i>X. politus</i> ^{d+}	7	-	90*	1	-	-	-	-
Total no. individuals		1562	600	89	170	446	939	67	133
no. individuals ⁺		197	543	614	275	-	-	-	-
Total no. species		19	15	14	13	17	20	7	12
no. species ⁺		10	12	9	5	-	-	-	-

¹N1 = Algonquin park 1, N2 = Algonquin park 2, S1 = Charleston lake, S2 = Frontenac, S3 = Packenham, S4 = Dacre.

⁺ traps baited with *Ips pini* pheromone lures.

^d scolytine species feeds on deciduous host species (Wood 1982)

Among bark beetles, *Dendroctonus valens* and *Hylastes porculus*, two of the three most abundant beetle species, exhibited the highest mite abundance (43.8 and 26.2% of all mites collected, respectively) and mite diversity (both with 17 species) (Table 4.2). *Ips grandicollis*, the fourth most abundant beetle, also showed a high mite diversity (11 species), despite a relatively lower mite abundance and prevalence (Table 4.2).

Dendroctonus valens, *Hylurgops pinifex* and *Ips pini* had the highest mite prevalence (35–38%), although *H. pinifex* and *I. pini* were less common than *D. valens* (Table 4.2).

A total of 1025 mites representing 33 species, eight genera and seven families, were collected across all sites and both years (Table 4.2). Site N1, in 2008, was the most species rich site, with 26 mite species from seven genera and seven families (Appendix 4.1). In 2008, sites S1 and S2 had relatively few mite species, with six and two species, respectively (Appendix 4.1). In 2009, sites S3 and S4 also had poor mite diversity, with one and two species, respectively (Appendix 4.1). The Digamasellidae was the most abundant and diverse family, with 11 species (2 genera) and 39% (395 mites) of all mite individuals collected (Table 4.2). Also abundant and diverse were the Urodynychidae (241 mites, 4 species), Trematuridae (88 mites, 9 species), and Melicharidae (94 mites, 5 species) (Table 4.2). Oribatulidae (194 mites) and Macrochelidae (5 mites) were less diverse, with two and one species, respectively (Table 4.2).

Table 4.2. Prevalence (%) (abundance) of mites associated with scolytines collected across four sites in Eastern Ontario in 2008 and 2009.

Mite family*	Mite species	<i>Gnathotrichus materianus</i>	<i>Dryocoetes affaber</i>	<i>D. autographus</i>	<i>Hyalestes opacus</i>	<i>H. porculus</i>	<i>Myiuraps pinifex</i>	<i>Hyalestinus aculeatus</i> d	<i>Hyalestinus aculeatus</i> d +	<i>Dendroctonus valens</i>	<i>Ips grandicollis</i>	<i>I. pini</i>	<i>I. pini</i> +	<i>Orthotomicus caelatus</i>	<i>Pityogenes hopkinsi</i>	<i>Pityokteines sparsus</i>	<i>Polygraphus rufipennis</i>	<i>Xyleborus sayi</i> d	<i>Trypodendron lineatum</i>	<i>T. retusum</i> d	<i>Xyloterinus politus</i> d +	Total mite abundance
AMR	<i>Epicriopsis</i> sp.	0.4(1)	-	-	-	0.1(1)	2.1(1)	-	-	1(4)	0.3(1)	-	-	-	-	-	-	-	-	-	-	8
DGM	<i>Dendrolaelaps hexaspinosus</i>	0.4(1)	-	-	0.4(3)	0.5(6)	8.3(7)	-	-	4.9(26)	3.1(11)	-	-	0.3(1)	-	-	-	-	-	-	-	55
	<i>D. louisianae</i>	-	-	-	-	-	-	-	-	-	0.9(3)	-	-	-	-	-	-	-	-	-	-	3
	<i>Dendra.</i> n.sp. 1	-	-	-	0.1(1)	0.1(1)	-	-	-	18.3(156)	-	-	-	-	-	-	-	-	-	-	-	158
	<i>Dendra.</i> n.sp. 2	-	-	-	-	0.4(8)	22.9(20)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28
	<i>Dendra.</i> n.sp. 3	-	-	-	-	-	-	-	-	-	-	0.1(1)	-	-	-	-	-	-	0.4(1)	-	-	2
	<i>Dendra.</i> n.sp. 4	-	-	-	-	-	-	-	-	0.2(1)	-	-	-	-	-	-	-	-	-	-	-	1
	<i>D. neodisetus</i>	-	-	-	-	-	-	-	-	0.2(1)	-	-	-	-	-	-	-	-	-	-	-	1
	<i>D. quadrisetosimilis</i>	-	-	-	-	-	-	-	-	-	-	-	-	18.1(112)	4(3)	-	-	-	-	-	-	115
	<i>D. quadrisetus</i>	-	-	-	-	-	-	-	-	-	1.2(4)	17.2(18)	30.2(1302)	-	-	-	-	-	-	-	-	1324
	<i>D. quadritorus</i>	-	-	-	0.1(1)	0.3(3)	-	-	-	1.2(5)	0.3(1)	-	-	-	-	-	-	-	-	-	-	10
	<i>Longoseius cuniculus</i>	-	-	-	-	0.1(1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
MAC	<i>Macrocheles</i> n.sp.	-	-	-	-	0.3(3)	-	-	-	0.5(2)	-	-	-	-	-	-	-	-	-	-	-	5
MLC	<i>Proctolaelaps fiseri</i>	-	-	-	-	1.4(20)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20
	<i>P. hystrix</i>	-	-	-	0.1(1)	-	-	-	-	1(5)	-	-	-	-	-	-	-	-	-	-	-	6
	<i>Procto.</i> n.sp. 1	-	-	-	-	0.2(6)	-	-	-	0.2(1)	-	-	-	-	-	-	-	-	-	-	-	7
	<i>Procto.</i> n.sp. 2	-	-	-	-	2.3(60)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60
	<i>Procto.</i> n.sp. 4	-	-	-	-	-	-	-	-	-	-	-	-	0.3(1)	-	-	-	-	-	-	-	1
TRM	<i>Trichouropoda australis</i>	-	-	-	-	-	-	-	-	0.2(1)	1.5(7)	17.2(14)	4(86)	-	-	-	-	-	-	-	1(1)	109
	<i>T. bipilis</i>	-	-	-	-	-	-	10.5(3)	100(1)	-	-	-	-	-	-	-	-	-	-	-	-	4
	<i>T. fallax</i>	-	-	-	-	-	6.3(4)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
	<i>T. hirsuta</i>	-	-	-	-	0.4(10)	2.1(3)	-	-	2(10)	0.9(4)	-	0.1(2)	-	-	-	-	-	-	-	-	29
	<i>T. lamellosa</i>	-	-	-	-	0.2(2)	-	-	-	0.7(3)	0.6(3)	-	-	-	-	-	-	0.8(1)	-	-	-	9
	<i>T. moseri</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.5(1)	2.4(1)	-	-	-	-	2
	<i>Tricho.</i> n.sp. 12	-	-	-	0.1(1)	0.1(1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	<i>Tricho.</i> n.sp. 3	-	-	-	-	-	-	-	-	-	-	-	-	-	6(3)	-	-	-	-	-	-	3
	<i>T. parisiana</i>	4.1(15)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25(1)	-	16
URO	<i>Uroobovella americana</i>	-	-	-	-	0.4(5)	-	-	-	8.9(151)	-	-	-	-	-	-	-	-	-	-	-	156
	<i>U. dryocoetes</i>	-	-	17.6(6)	-	1.6(35)	-	-	-	0.5(2)	0.3(1)	-	-	-	-	-	-	-	-	-	-	44
	<i>U. orri</i>	-	4.5(1)	-	-	-	-	-	-	-	0.6(4)	-	-	4.4(21)	-	12.5(2)	7.1(11)	-	-	-	-	39
	<i>U. vinicolora</i>	-	-	-	-	-	-	-	-	0.5(2)	-	-	-	-	-	-	-	-	-	-	-	2
ORB	<i>Parakeius leontomycha</i>	0.4(1)	-	-	-	0.5(7)	-	-	-	0.7(3)	0.9(3)	-	0.4(6)	1.3(4)	-	-	-	-	-	-	-	24
	<i>Paral.</i> n.sp.	-	-	-	-	5.2(100)	-	-	-	10.1(76)	-	-	-	-	-	-	-	-	-	-	-	176
Total	Beetle abundance	241	22	34	801	1124	48	19	1	405	325	29	1415	298	50	8	42	118	265	4	98	
	no. mite species (33)	4	1	1	5	17	5	1	1	17	11	2	5	5	2	2	2	1	1	1	1	
	Mite abundance (2424)	18	1	6	7	269	35	3	1	449	42	32	1397	139	6	3	12	1	1	1	1	
	Mite prevalence (%)	5.4	4.5	17.6	0.9	12.4	37.5	10.5	100	38.0	9.5	34.5	32.7	22.1	10	25	9.5	0.8	0.4	25	1	

*AMR = Ameroseiidae, DGM = Digamasellidae, MAC = Macrochelidae, MLC = Meichariidae, TRM = Trematuridae, URO = Urodymychidae, ORB = Oribatulidae.

d = scolytine species feeds on deciduous host species (Wood 1982).

+ traps baited with *Ips pini* pheromone lures

Typically, only one mite individual, occasionally two, were collected per beetle (58 and 19% of all beetles with mites, respectively); more rarely, three (7%), four (7%), or 5–11 mites (9%) were found on an individual beetle. Most bark beetle individuals with mites were associated with only one species (84%), fewer had two (12%), or three species (3%), and the maximum was four species collected from a single *Dendroctonus valens* specimen. *Dendroctonus valens* reached an exceptionally high number of mites, with 24, 27 and a maximum of 29 mites per host, and an instance with four species of mites from a single host.

In terms of prevalence on a given beetle species, from general traps, *Dendrolaelaps* n.sp. 2 (22.9% prevalence on *Hylurgops pinifex*), *Dendrolaelaps* n.sp. 1 (18.3% on *D. valens*), *D. quadrisetosimilis* (18.1% on *Orthotomicus caelatus*), *Uroobovella dryocoetes* (17.6% on *Dryocoetes autographus*) were the most prevalent mite species (excluding the instances with <5 beetles collected) (Table 4.2). In terms of overall abundance from general traps, *Paraleius* n.sp. (Oribatulidae) (17.2% of all mites collected), *Dendrolaelaps* n.sp. 1 (15.4%), *Uroobovella americana* (15.2%), and *D. quadrisetosimilis* (11.2%) were the most frequently collected mite species (Table 4.2).

Most mite species showed minimal variation in total abundance, prevalence or mean intensity across sites or years, with the exception of a few species. *Dendrolaelaps* n.sp. 1 showed a ~threefold increase (8.6–29.4%) in prevalence on *D. valens* from 2008 to 2009 in site N2, as well as an eightfold increase in total abundance from 2008 to 2009 (Appendix 4.1). *Paraleius* n.sp. associated with *D. valens* showed a ~fourfold increase (4.3–18.6%) in prevalence, and 14 times increase in abundance in site N2 from 2008 to

2009 (Appendix 4.1). *Dendrolaelaps quadrisetosimilis* prevalence on *Orthotomicus caelatus* dropped from 20.4 to 6.7%, and abundance dropped from 68 to 2, in site N1 from 2008 to 2009 (Appendix 4.1). In contrast, *D. quadrisetosimilis* prevalence increased from 8.3 to 20.5%, and abundance increased from 9 to 28, in site N2 from 2008 to 2009 (Appendix 4.1).

Ips pini pheromone traps

A total of 1629 bark beetles from 19 species, 15 genera and eight tribes, were collected in traps baited with *Ips pini* pheromone lures, across four sites in 2008 (Table 4.1). Only three of the 19 host species examined had mites (29% prevalence), with *I. pini* having the highest mite prevalence (32.7%), diversity (five species) and abundance (99.9% of all individuals) (Appendix 4.1). A total of 1399 mites representing six species, three genera and three families, were collected across four sites in 2008 (Appendix 4.1).

Dendrolaelaps quadrisetus (sensu Hirschmann 1960) and *Trichouropoda australis* were collected from *I. pini* in all four sites in 2008 (Appendix 4.1).

Dendrolaelaps quadrisetus had the highest prevalence (20–33.5%), abundance (93% of all individuals), and mean intensity (3.2), whereas *Trichouropoda australis* had the second highest prevalence (3–4.7%), abundance (6%), and mean intensity (1.9) (Appendix 4.1).

Combined across general traps and *I. pini* pheromone traps, a total of 5635 bark beetles, from 30 species, were collected, and 16.4% of these hosts had at least one mite. In total, 2424 mites representing 33 species were collected from both trapping methods.

Host specificity, phenology and attachment

Across general and pheromone specific traps, most mite species were specialists, occurring on one (33.3%) or two (36.4%) host species; much fewer species were generalists occurring on three (3%), four (12.1%), five (9.1%), or six (3%) host species, and one species, *D. hexaspinosus*, on seven host species (Fig. 4.1). If mite species with an overall abundance <5 are removed then slightly more mite species are specialists from one or two host species (54%), and slightly fewer are generalists from three or more host species (47%). There was no apparent specificity of mite taxa towards particular beetle taxa: each mite family and most genera were found on a range of scolytine tribes and genera (Table 4.2). *Proctolaelaps* species showed the narrowest host breadth occurring on three genera from three tribes, whereas *Trichouropoda* species exhibited the greatest host breadth, being collected from 12 genera and eight tribes (Table 4.2).

Figure 4.1. Distribution of the level of host specificity of 33 species of mites collected from bark beetles sampled across four sites in Eastern Ontario, showing the percentage of total mite species and the number of scolytine species used by each mite species (number of mite species shown above columns).

There was no emergent pattern in the abundance of mites throughout the spring and summer, such that mites were overall most frequently collected when beetles were most abundant. For example, *Hylastes porculus* and *D. valens* were collected primarily from May to late-June and associated mites were most abundant during that interval (Appendices 4.2, 4.3). There was also no apparent seasonality in the abundance of each mite family, such that digamasellids, trematurids and oribatulids were collected most frequently when host beetles were most abundant (Appendices 4.2, 4.3). *Ips pini* was collected from mid-April to late-August in 2008 (using *I. pini* pheromone lures), and the two most common mite species, *D. quadrisetus* and *T. australis*, were most frequently encountered when their host was most abundant (Appendix 4.4).

The attachment location of mites, summarized across all sites, indicates that different groups of mites attach to different locations on host beetles. Mites from the Order Mesostigmata, excluding Uropodoidea, were collected almost exclusively from under the elytra of their hosts, with 97.2% of mites under the elytra, 0.5% on the elytra, 1% on the ventral thorax or abdomen, 0.8% on the legs, and 0.5% on the head or pronotum of their hosts. Uropodoids (Trematuridae, Urodinychidae) were most often found glued with their anal pedicel to the venter of their hosts, 55% on the ventral thorax or abdomen, and 9% on the legs. Uropodoids were also found on the dorsum of their hosts, 20% on the elytral declivity, 7% on the elytra (excluding the declivity), and 9% on the head or pronotum. Oribatids were also typically located on the venter of their hosts, 86% on the ventral prothorax, 12% on the ventral meso- or metathorax, and 2% on the elytral declivity.

Discussion

In Ontario, bark beetles were associated with a rich and abundant assemblage of mesostigmatic and oribatid mites. The prevalence of mites varied between host species, in that some scolytine species, such as *Dendroctonus valens*, *Ips pini*, *Orthotomicus caelatus* and *Hylastes porculus*, were frequently associated with mites, while other host species were seemingly devoid of mites. The majority of bark beetle species collected in this study are host generalists feeding on a range of host trees, with fewer specialists (Wood 1982). Overall, host tree specialists were not associated with unique mite species, with the exception of *Hylesinus aculeatus* which was associated with a rare mite species, *Trichouropoda bipilis*. It is not understood why the diversity or abundance of mites varies widely between scolytine species, these differences could be in part a function of particular aspects of the evolutionary history, ecology, behaviour, or feeding habits of scolytines or associated mites.

Larger hosts have been reported as having a more diverse and abundant symbiont fauna in some animal taxa, such as endoparasitic helminths from freshwater fish, or gastrointestinal nematodes from terrestrial mammals (Bell and Burt 1991; Morand and Guégan 2000; Poulin 2004). *Dendroctonus valens* was the largest bark beetle species collected in this study, ranging from 5.3–8.3 mm; this species had the most abundant, prevalent and diverse mite fauna of all scolytine species examined. However, considering that many scolytine species with a substantially smaller body size than *D. valens*, such as *H. porculus*, *I. pini*, *I. grandicollis*, and *O. caelatus* (ranging 2.4–5.0 mm) (Wood 1982),

were hosts to an abundant and diverse mite fauna, it is likely that host body size is not a major determinant of the diversity or abundance of bark beetle associated mites.

The definition of what constitutes a specialist or a generalist is variable; herein, mites collected from one host species are monospecific specialists, mites from two host species are specialized but less so than monospecific species, and those from three or more hosts are generalists. The majority of scolytine associated mites collected in Ontario are specialized; of the 33 species of mites collected, 33.3% were monospecific, 36.4% on two host species, and 30.3% were host generalists associated with three to seven host species. However, some mite species were present at relatively low abundances, which may falsely increase the number of host specialists. Following the removal of mite species with an overall abundance of less than five mites, there are still slightly more host specialists from one or two host species (53%), with slightly fewer mite species collected from three or more host species (47%).

The level of host specificity or apparent host preferences may vary among studies or geographically, in that some generalists are reportedly specialists, or some specialists are reportedly generalists, when compared to published host records, and this variation may be in part a product of the challenges surrounding the study of host specificity. However, it should be noted that published host records often fail to provide an accurate assessment of host specificity of symbionts at the population level (Poulin 2007). The total diversity of host species exploited by a symbiont species worldwide represent the global host range of a symbiont species, but it does not necessarily reflect the host species

that can be exploited by individuals in a given population, due to local adaptation or variation in host availability (Poulin 2007).

The mutualistic species, *Dendrolaelaps neodisetus* (Kinn 1980), was rarely encountered in Ontario, and it was collected only from *Dendroctonus valens* in this study, indicating that this species may be a specialist. *Dendrolaelaps neodisetus* has being collected from at least seven additional host species in southern USA, Honduras and Alberta (Hurlbutt 1967; Moser and Roton 1971), and it is considered to be one of the most frequently encountered mites associated with *Dendroctonus frontalis* (Kinn 1984), indicating that this species is a broad generalist. *Dendrolaelaps quadritorus* was collected from four host species and three genera in Ontario, indicating that this species is a generalist; however, published records suggest that *D. quadritorus* is more specialized, being reported from only two congeneric host species, *Ips avulsus* and *I. grandicollis*, from Louisiana (Robillard 1971). The most abundant and prevalent species collected in this study was *D. quadrisetus*. In Ontario, *D. quadrisetus* was collected predominately from *I. pini*, and secondarily from *I. grandicollis*, indicating that this species may be a specialist. However, published host records suggest that this mite is a broad generalist, reported from at least 13 scolytine species from four genera (Hirschmann 1960; Kinn 1967; McGraw and Farrier 1969; Levieux et al. 1989; Cardoza et al. 2008; Hofstetter 2008; Pernek et al. 2008).

Across the study sites examined in Ontario, *Proctolaelaps fiseri* was only associated with *Hylastes porculus*. However, *P. fiseri* has been reported from at least nine additional scolytine species from seven genera, across Germany and southern USA,

suggesting that this species is a host generalist (Hirschmann and Rühm 1953; Westerboer 1963; Lindquist and Hunter 1965; Moser and Roton 1971). Similarly, *P. hystrix* was collected from two bark beetle species in this study, but this species has been reported from five additional host species (four genera), in southern USA, British Columbia, Austria and Poland (Lindquist and Hunter 1965; Moser and Roton 1971).

In Ontario, *Trichouropoda fallax* was collected from a single host species in the Hylastini tribe, indicating that this species is a specialist; however, *T. fallax* has been collected from three additional Hylastini species across Siberia, Belgium and North America, which suggests that this species is not a strict specialist (Hirschmann and Wisniewski 1989). In the study sites examined in Ontario, *Uroobovella americana* was collected from two hosts, *D. valens* and *H. porculus*, with *D. valens* being the primary hosts, which suggests that in Ontario this mite is not a broad generalist, but instead at least somewhat specialized. *Uroobovella americana* has also been reported from six additional scolytine species (four genera), as well as *D. valens*, from southern USA (Moser and Roton 1971; Hofstetter 2008). *Uroobovella orri* was collected from five host species and five genera in this study, which suggests that this species is a host generalist. *Uroobovella orri* has been collected from at least six additional scolytine species from three genera across southern USA (Moser and Roton 1971). However, the barcoding region of COI has indicated that *U. orri* may not be a generalist but instead multiple species with varying levels of host specificity (Chapter 3).

The association of *Paraleius* species with bark beetles is among the few established cases of phoresy in oribatid mites, and the association is typically considered

uncommon and infrequent (Norton 1980; Moser et al. 2005). *Paraleius* n.sp. was quite common, with 5 and 10% prevalence on *H. porculus* and *D. valens*, which contrasts with the assumption that the association between oribatids and scolytines is infrequent and transient.

Studies on scolytine associated mites often fail to consider host specificity in the context of mite associations with other families of wood boring beetles. Overall, mites associated with scolytines are infrequently collected from other families of wood boring beetles, with the exception of a few species (Kinn and Linit 1989). Three species of mites collected from bark beetles in Ontario, were also collected from the widespread cerambycid species, *Monochamus scutellatus*, across the sites examined in this study (Appendix 4.5). *Trichouropoda hirsuta* is a broad host generalist, occurring on many families of wood boring beetles, such as cerambycids (Kinn 1987; Kinn and Linit 1989), scolytines, weevils, and tenebrionids (unpubl. data), and *T. hirsuta* occasionally reaches high abundances (2,578 mites with 10 mites/beetle) on cerambycid species (Kinn 1987). *Trichouropoda lamellosa* is occasionally collected from scolytines, and it has been reported from four other scolytine species (Moser and Roton 1971), however, this species often exhibits a much higher abundance on cerambycids (9,308 mites with 37 mites/beetle) (Kinn 1987). *Longoseius cuniculus*, which was infrequently collected from bark beetles in Ontario, was originally described from the galleries of *Monochamus notatus* (Cerambycidae) (Chant 1961), and has been reported from four additional cerambycid species (Moser and Roton 1971).

The variation in the degree of host specificity between the findings of this study and published host records may be in part a reflection of some of the challenges surrounding the study of host specificity. Defining the boundaries between species represents one of the primary obstacles to assessing host specificity. Parasite and symbiont identifications have historically been exclusively based on morphological characteristics; however, morphological based taxonomy is ill-suited for recognizing cryptic species (Poulin and Keeney 2007). The advent of modern molecular techniques, and increased access to nucleotide sequencing, has resulted in an exponential increase in research into cryptic diversity and species boundaries (Bickford et al. 2007). It is possible that the differences in host specificity between published records and this study are in part a result of unrecognized cryptic diversity, in which a widespread generalist, such as *Dendrolaelaps quadrisetus*, may not be a single species, but instead a complex of cryptic species with relatively narrow host preferences, as seen in parasitoid tachinid flies from Costa Rica (Smith et al. 2007).

Incorrect identifications can obfuscate host specificity estimates, and they may explain some of the variability in the level of host specificity between this study and published records. Lindquist and Wu (1991) performed a revision of the genus *Mucroseius* following the discovery of several publications with incorrectly identified mite species associated with long-horned beetles (Cerambycidae), from North America and Japan. Prior to the revision of the genus *Dermanyssus* (Mesostigmata), many researchers misidentified multiple *Dermanyssus* species as *D. gallinae* (Moss 1978). Species are occasionally identified incorrectly, and this may occur more frequently when

non-specialists are performing identifications. However, there are instances where specialists, with decades of experience on a particular group, have difficulty correctly identifying specimens to species (Packer et al. 2009). Incorrect identifications are more likely in groups where there are no dichotomous keys or reliable identification materials, which is the case in many groups of symbiotic taxa, such as helminth endoparasites of vertebrates (Kijewska et al. 2002; Locke et al. 2010).

The variation in host specificity may also be a product of changes to the status of species, where species are synonymized and then later separated once again. Instability and disagreements in the status of species is present in most groups of arthropods, and published host records may be unstable as a result. For example, *Dendrolaelaps quadrisetus* and *D. quadrisetosimilis* are currently recognized as distinct species, but historically *D. quadrisetosimilis* was synonymized under *D. quadrisetus* (McGraw and Farrier 1969). Interpreting host specificity patterns across studies can be hampered in groups where the status of symbiotic species has changed repeatedly.

The level of host specificity can vary with the geographic scale of study (Krasnov et al. 2004, 2011), and the differences observed between this study and published records could be a result of the spatial scale of investigation. The degree of host specificity was found to vary across the geographic range of ectoparasitic fleas of small mammals, and the variation was a function of environmental conditions (Krasnov et al. 2004). At a local scale a given symbiont species may appear to be a specialist, while at a global scale the same species may be a generalist (Krasnov et al. 2011). Spatial scale also influences host

sampling, and when host sampling is insufficient there tends to be an increased incidence of specialists (Poulin and Keeney 2007).

Overall, these findings indicate that bark beetles in Ontario are frequently associated with a diverse and abundant acarofauna. Some species of mites are relatively common, while other species are rare. The majority of mite species are specialists, while fewer species are generalists; however, the level of host specificity for some species, differed between this study and published host records. The observed variation in host specificity may be a result of unrecognized cryptic diversity, incorrect identifications, changes in taxonomy, or the spatial scale of investigation. To gain a much clearer understanding of host specificity in mites associated with bark beetles, specimens need to be collected over a greater geographic range, and species boundaries need to be assessed using molecular techniques.

Appendix 4.1 Prevalence (%) (abundance) and mean intensity (maximum) of mites associated with scolytines collected across four sites in Eastern Ontario in 2008 and 2009

Scolytine tribe			Prevalence(abundance) / mean intensity(maximum) in each site ^a							
species	Family ^a	Mite species	R1 08	R2 08	S1 08	S2 08	R1 09	R2 09	S3 09	S4 09
Corthylini										
<i>Gnathotrichus materianus</i>	AMR	<i>Epicrotopis</i> sp.	0.7 / 1	-	-	-	-	-	-	-
	DGM	<i>Dendrolaelaps hexaspinosus</i>	-	-	-	7.1 / 1	-	-	-	-
		Hirschmann, 1960								
	TRM	<i>Trichouropoda pansane</i> Wosniowska & Hirschmann, 1987	2.2(3) / 1	-	12.5 / 1	-	15.8(5) / 1.7(2)	7.7(6) / 2(4)	-	-
Dryocoetini	ORB	<i>Paraleius leontonycha</i> (Berlese, 1910)	-	-	-	-	-	2.6 / 1	-	-
<i>Dryocoetes effaber</i>	URO	<i>Uroabovella orn</i> Hirschmann, 1972	-	-	-	-	20 / 1	-	-	-
<i>Dryocoetes eutographus</i>		<i>U. dryocoetes</i> (Vitzthum, 1923)	4.5 / 1	-	-	-	45.5(5) / 1	-	-	-
Hylastini										
<i>Hylastes opacus</i>	DGM	<i>Dendrolaelaps hexaspinosus</i>	-	1.4(3) / 1	-	-	-	-	-	-
		<i>Dendra.</i> n.sp. 1	-	0.5 / 1	-	-	-	-	-	-
		<i>D. quadratus</i> Robillard, 1971	-	0.5 / 1	-	-	-	-	-	-
	MLC	<i>Proctolaelaps hystris</i> (Vitzthum, 1923)	-	0.5 / 1	-	-	-	-	-	-
<i>Hylastes parvulus</i>	TRM	<i>Trichouropoda</i> n.sp. 12	0.6 / 1	-	-	-	-	-	-	-
	AMR	<i>Epicrotopis</i> sp.	0.2 / 1	-	-	-	-	-	-	-
	DGM	<i>Dendrolaelaps hexaspinosus</i>	0.8(5) / 1	2.4 / 1	-	-	-	-	-	-
		<i>Dendra.</i> n.sp. 1	0.2 / 1	-	-	-	-	-	-	-
		<i>Dendra.</i> n.sp. 2	0.8(8) / 1.8(4)	-	-	-	-	-	-	-
		<i>D. quadratus</i>	0.2 / 1	2.4 / 1	-	-	0.3 / 1	-	-	-
		<i>Longosius cuniculus</i> Chant, 1961	-	-	-	-	0.3 / 1	-	-	-
	MAC	<i>Macrocheles</i> n.sp.	-	-	-	-	-	2.9(3) / 1	-	-
	MLC	<i>Proctolaelaps fiseri</i> Samsinik, 1960	2(16) / 1.3(3)	4.8(2) / 1	-	-	0.7(2) / 1	-	-	-
		<i>Procto.</i> n.sp. 1	0.3(6) / 3(5)	-	-	-	-	-	-	-
		<i>Procto.</i> n.sp. 2	4(58) / 2.4(11)	-	-	-	0.3 / 1	1 / 1	-	-
	TRM	<i>Trichouropoda hirsuta</i> Hirschmann, 1972	-	-	-	-	0.7(6) / 3(5)	2.9(4) / 1.3(2)	-	-
		<i>T. lamellosa</i> Hirschmann, 1972	0.3(2) / 1	-	-	-	-	-	-	-
		<i>Tricho.</i> n.sp. 12	0.2 / 1	-	-	-	-	-	-	-
	URO	<i>Uroabovella americana</i> Hirschmann, 1972	0.7(4) / 1	-	7.1 / 1	-	-	-	-	-
<i>Hylurgops pinifex</i>		<i>U. dryocoetes</i>	1.3(14) / 1.8(5)	2.4 / 1	-	-	3.1(20) / 2.2(6)	-	-	-
	ORB	<i>Paraleius leontonycha</i>	0.3(2) / 1	-	-	-	1.4(5) / 1.3(2)	-	-	-
		<i>Paral.</i> n.sp.	4.3(37) / 1.4(4)	2.4 / 1	-	-	6.9(41) / 2.1(5)	11.5(21) / 1.8(3)	-	-
	AMR	<i>Epicrotopis</i> sp.	3.8 / 1	-	-	-	-	-	-	-
	DGM	<i>Dendrolaelaps hexaspinosus</i>	7.7(2) / 1	18.2(5) / 2.5(4)	-	-	-	-	-	-
		<i>Dendra.</i> n.sp. 2	27(13) / 1.9(5)	18.2(4) / 2(3)	-	-	33.3 / 1	16.7(2) / 2	-	-
	TRM	<i>T. fallax</i> (Vitzthum, 1926)	-	9.1 / 1	-	-	-	33.3(3) / 1.5(2)	-	-
		<i>T. hirsuta</i>	-	-	-	-	-	16.7(3) / 3	-	-
Hylesiniini										
<i>Hylesinus aculeatus</i>		<i>T. bipilis</i> (Vitzthum, 1921)	-	-	-	-	-	-	13.3(3) / 1.5(2)	-
<i>H. aculeatus</i> +		<i>T. bipilis</i>	-	-	100 / 1	-	-	-	-	-
Hylurgini										
<i>Dendroctonus valens</i>	AMR	<i>Epicrotopis</i> sp.	2(2) / 1	2.2(2) / 1	-	-	-	-	-	-
	DGM	<i>Dendrolaelaps hexaspinosus</i>	5.9(7) / 1.2(2)	15.1(19) / 1.4(3)	-	-	-	-	-	-
		<i>Dendra.</i> n.sp. 1	7.8(13) / 1.6(4)	8.4(15) / 1.9(5)	-	-	8.3(2) / 2	29.4(126) / 2.2(8)	-	-
		<i>Dendra.</i> n.sp. 4	-	1.1 / 1	-	-	-	-	-	-
		<i>D. neodactylus</i> Hurlbutt, 1967	1 / 1	-	-	-	-	-	-	-
		<i>D. quadratus</i>	1 / 1	1.1 / 1	-	-	-	1.5(3) / 1	-	-
	MAC	<i>Macrocheles</i> n.sp.	1 / 1	-	-	-	-	0.5 / 1	-	-
	MLC	<i>Proctolaelaps hystris</i>	2.9(3) / 1	-	-	-	-	0.5(2) / 2	-	-
		<i>Procto.</i> n.sp. 1	-	-	-	-	-	0.5 / 1	-	-

Scotching with

Scorpion's wife	Family*	Mite species	901 08	902 08	52 08	91 09	92 09	53 08	54 09
			Prevalence(absolute) / mean intensity(mean/mite) in each site*						
Dendroctonus valens	TMA	Trichouragopsis australis Herschmann, 1972		1.3/1	-	-	-	-	-
		<i>T. hirsuta</i>	2.8(3)/1	1.3/1	-	-	2.1(6)/(1.5)(2)	-	-
		<i>T. lamellatus</i>	-	1.3/1	-	-	1(2)/1	-	-
	URO	<i>Uroabovella americana</i>	4.9(22)/(4.4)(10)	10.8(66)/(6.4)(4)	-	8.3/1	10.3(62)/(3.1)(22)	-	-
		<i>U. dybowskii</i>	1/1	-	-	8.3/1	-	-	-
		<i>U. weicollae</i> (Vitzthum, 1936)	1/1	-	-	-	0.5/1	-	-
	OAB	<i>Pandinus leontorhyche</i>	1/1	-	-	-	1(2)/1	-	-
		<i>Pandus n. sp.</i>	-	4.3(5)/(1.3)(2)	-	8.3/1	18.6(70)/(3.9)(9)	-	-
Ipsini									
Ips grandicollis:	AAMR	<i>Ectophasia</i> sp.	1.3/1	-	-	-	-	-	-
	DGM	<i>Dendrolaelaps krasnovianus</i>	2.5(2)/1	7.5(8)/(1.1)(2)	-	-	1.2/1	-	-
		<i>D. luciae</i> Herschmann & Wainwiler, 1962	1.3/1	2.2(2)/1	-	-	-	-	-
		<i>D. quadrisetus</i> (Berlese, 1920)	-	1.1/1	12.5(2)/1	-	1.2/1	-	-
		<i>D. quadrisetus</i>	-	1.1/1	12.5(2)/1	3.3/1	2.4(4)/(3)(3)	-	-
	TMA	<i>Trichouragopsis australis</i>	-	1.1/1	-	-	2.4(3)/(1.5)(2)	-	-
		<i>T. hirsuta</i>	1.3/1	-	-	3.2(1)/2	-	-	-
		<i>T. lamellatus</i>	1.3/1	-	-	-	-	-	-
	URO	<i>Uroabovella dybowskii</i>	1.3/1	-	-	3.2(3)/3	1.2/1	-	-
		<i>U. orn</i> Herschmann, 1972	-	-	-	-	2.4(2)/1	-	-
	OAB	<i>Pandinus leontorhyche</i>	1.3/1	-	-	-	-	-	-
Ips pini	DGM	<i>Dendrolaelaps quadrisetus</i>	33.3(12)/(4)(10)	40(6)/(3)(4)	-	50(3)/3	57.1(11)/(2.8)(8)	-	-
I. pini +	TMA	<i>Trichouragopsis australis</i>	-	-	-	-	-	-	-
	DGM	<i>Dendro n. sp. 3</i>	-	-	0.2/1	-	-	-	-
		<i>D. quadrisetus</i>	20(107)/(3.1)(10)	32.7(477)/(2.9)(17)	33.5(539)/(3.2)(16)	-	-	-	-
	TMA	<i>Trichouragopsis australis</i>	4.7(8)/1	4.2(39)/(1.9)(7)	4.1(32)/(1.5)(4)	3(7)/1	-	-	-
		<i>T. hirsuta</i>	-	-	0.4(2)/1	-	-	-	-
	OAB	<i>Pandinus leontorhyche</i>	-	-	1.2(6)/1	-	-	-	-
Orthotomicus coelestis	DGM	<i>Dendrolaelaps krasnovianus</i>	-	2.8/1	-	-	-	-	-
		<i>D. quadrisetos</i> Herschmann, 1960	20.4(68)/(2.3)(8)	8.3(9)/(3)(6)	8.3/1	6.1(2)/2	20.5(28)/(1.4)(3)	-	-
	MLC	<i>Proctolaelaps n. sp. 4</i>	0.7/1	-	-	-	-	-	-
	URO	<i>Uroabovella orn</i>	4.2(6)/1	-	-	6.7(5)/5	7.2(10)/(1.7)(3)	-	-
Pygospes hapkinsi	OAB	<i>Pandinus leontorhyche</i>	0.7/1	-	-	-	2.4(2)/1	-	-
	DGM	<i>Dendrolaelaps quadrisetos</i>	-	-	8.3/1	-	14.3(37)/(1.5)(2)	-	-
	TMA	<i>Trichouragopsis n. sp. 3</i>	-	-	-	-	21.4(3)/1	-	-
Pygospes sparvus	URO	<i>T. mosseri</i> Herschmann, 1972	-	-	-	-	100/1	-	50(2)/2
		<i>Uroabovella orn</i>	-	-	-	-	-	-	-
Polygraphini	TMA	<i>Trichouragopsis mosseri</i>	-	-	-	-	100/1	-	-
Polygraphus rufipennis	URO	<i>U. orn</i>	-	12.5(3)/3	-	9.1(7)/7	50/1	-	-
Nyctoserini									
Nyctoserius sepi	TMA	<i>T. lamellatus</i>	33.3/1	-	-	-	-	-	-
Nyctoserius	DGM	<i>Dendrolaelaps n. sp. 3</i>	0.4/1	-	-	50/1	-	-	-
Trypodendron levatum	TMA	<i>Trichouragopsis parviana</i>	-	-	-	-	-	-	-
Zygodereon retusum									
Zygodereon pedica +	Total	nra. mite species	26	20	6	16	22	1	2
		nra. mite species +	2	2	6	2	-	-	-
		nra. abundance	330	167	8	118	393	1	5
		nra. abundance +	115	516	562	1306	-	-	-

	mean abundance + range	S.E.
TH1 = Algonquin park 1, MZ = Algonquin park 2, S1 = Charlestown lake, S2 = Frontenac, S3 = Packardham, S4 = Ocre	113	318
AAR = Amnionocissus, DGM = Diguanilic acid, MAC = Macrochelidae, MIC = Micetichidae, TRM = Trematodidae, URO = Urodelechidae, ORB = Orbiculidae

Appendix 4.2. The cumulative abundance of *Hylastes porculus* and five associated mite species (*Proctolaelaps fiseri*, *Procto. n.sp. 2*, *Trichouropoda hirsuta*, *Uroobovella dryocoetes*, and *Paraleius n.sp.*), across 14-day collection intervals in sites N1 and N2 (Algonquin P.P.) (A) from 17th April to 26th August 2008 and (B) 17th April to 6th August 2009.

Appendix 4.3. The cumulative abundance of *Dendroctonus valens* and five associated mite species (*Dendrolaelaps* n.sp. 1, *D. hexaspinosus*, *Uroobovella americana*, *Trichouropoda hirsuta*, and *Paraleius* n.sp.), across 14-day collection intervals in sites N1 and N2 (Algonquin P.P.) (A) from 17th April to 26th August 2008 and (B) 17th April to 6th August 2009.

Appendix 4.4. The cumulative abundance of *Ips pini* and two associated mite species, *Dendrolaelaps quadrisetus* and *Trichouropoda australis*, across 14-day collection intervals from 17th April to 26th August 2008 (A) across sites N1 and N2 (Algonquin P.P.), and (B) sites S1 (Charleston lake) and S2 (Frontenac P.P.).

Appendix 4.5

The natural history of mesostigmatic mites associated with the widespread white-spotted sawyer beetle, *Monochamus scutellatus* (Cerambycidae): diversity, phenology, host attachment, and sex bias.

Abstract

Little is known about the acarofauna associated with wood-boring beetles in Canada, including long-horned beetles (Cerambycidae). The objectives of this study were to describe the mesostigmatic mite fauna of *Monochamus scutellatus* (Say, 1824), the white-spotted sawyer beetle, to assess the prevalence, abundance, species richness, phenology and attachment location of associated mites, and to test whether the abundance or prevalence of mites differed between male and female beetles. A total of 176 beetles were collected in Eastern Ontario in 2008 and 2009, using Lindgren funnel traps baited with α -pinene and ethanol lures, and 71% of hosts had mesostigmatic mites. A total of 2486 mites were collected, representing eight species, four genera and three families. There was no significant pattern in mite phenology throughout the season. Overall, there was no significant sex bias in the abundance or prevalence of mites between male and female *M. scutellatus*. This study represents the first examination of mites associated with *M. scutellatus* in Ontario, and several of the species collected in this study have been reported from other cerambycid species at comparable and differing levels of intensity.

Introduction

Weakened, living and dead trees provide habitat for a diverse assemblage of wood-burrowing beetles (Cerambycidae, Buprestidae, Curculionidae: Scolytinae), mites (Acari), fungi, and nematodes. Cerambycidae is one of the largest insect families, with at least 20,000 described species worldwide, and an estimated 1,100 species in Canada and the United States (Yanega 1996). Most cerambycid species bore into and feed on plant tissue, attacking a wide range of woody and herbaceous plants either living or dead (Yanega 1996).

The white-spotted sawyer beetle, *Monochamus scutellatus* (Say, 1824) (Cerambycidae), feeds on dead or dying conifers, including fresh cut pulpwood, logging debris, and fire-killed trees (Wilson 1962). *Monochamus scutellatus* is found throughout most of North America (Yanega 1996), its primary host is Eastern white pine (*Pinus strobus*), but it will also attack other species of pine, spruce and fir throughout its range (Wilson 1962). The white-spotted sawyer is not a primary pest species, but it is capable of killing healthy trees, and significantly degrading the economic value of standing trees (Richmond and Lejeune 1945; Wilson 1962). *Monochamus scutellatus* has also been reported as vectoring the pinewood nematode, *Bursaphelenchus xylophilus*, which is the causative agent of pine wilt disease in North America and Asia (Nickle et al. 1980; Kinn 1987). However, *M. scutellatus* also serves a beneficial role as a nutrient recycler, feeding on deadwood and returning organic nutrients to the soil (Cobb et al. 2010).

There have been few investigations into the acarofauna of cerambycids, and there is little know about the mites associated with the widespread cerambycid species, *M.*

scutellatus. In Maine, six families of mites, including four mesostigmatic families, were collected from four cerambycid species, including *M. scutellatus* (Soper and Olson 1963). In Louisiana, 13 species of mites, including eight species and three families of mesostigmatic mites, were collected from five cerambycid species (Kinn and Linit 1989). The mite fauna of *M. scutellatus* in Canada has been virtually unstudied. The main objective of this study was to characterize the natural history (diversity, prevalence, abundance, phenology and attachment site specificity) of mesostigmatic mites associated with the white-spotted sawyer beetle in Eastern Ontario, sampled in two sites over two years. The abundance or prevalence of mites occasionally differs between male and female insect hosts (e.g. Lajeunesse et al. 2004); therefore, I assessed whether there was a sex bias in the abundance or prevalence of mites associated with *M. scutellatus*.

Materials and Methods

Study sites and sampling design

Wood-boring beetles were sampled in two study sites in Eastern Ontario from mid-April to late-August in 2008, and from mid-April to early-August in 2009. The two study sites were in the boreal shield ecozone in Algonquin Provincial Park (P.P.), Algonquin site 1 (N1) (45.902, -77.605) and Algonquin site 2 (N2) (45.895, -78.071). Four Lindgren 12-unit funnel dry traps with four 1 cm³ pest strips (Ortho Home Defense Max), baited with lures (Synergy Semiochemicals Corp. Burnaby, BC), were placed in each site at least 16 m apart, with the collection cups ~80 cm off the ground and at least 2 m from any host trees (distance from trees, trap height, and distance between traps based upon Miller and Duerr (2008)). In 2009, propylene glycol was used in place of pest strips. Traps were baited with α -pinene and 95% ethanol (released at 2 g/day and 400 mg/day, respectively) lures to attract beetles seeking dead or dying coarse woody debris. Traps were emptied every two weeks, trap lures were replaced every eight weeks, and the insecticide strips were replaced during each visit to maintain effectiveness. Each beetle specimen captured was placed individually into a 2.0ml microfuge tube with 80% ethanol.

Identifications and mite associations

Cerambycids were identified to species using a dissecting microscope and identification material from Yanega (1996). The presence, abundance, and attachment location of mesostigmatic mites (hereafter mites) was recorded; other mites (e.g. Prostigmata, Astigmata) were not studied. Prevalence was defined as the proportion of all examined

hosts with one or more mites of a given species. Intensity was defined as the number of mites of a given species, carried per beetle with mites. Average abundance was defined as the average number of mites of a given species, across all host individuals examined, including hosts with no mites. All mites were removed from the host, cleared in 85% lactic acid for 1–24 h depending on the degree of opacity, slide-mounted in a polyvinyl alcohol medium and cured on a slide warmer at about 40°C for 3–4 days. Slide-mounted specimens were examined using a compound microscope and identified to species using descriptions from the literature based on morphospecies concepts (Hirschmann 1960, 1972; Hurlbutt 1967; McGraw and Farrier 1969). Voucher specimens are deposited in the Canadian National Collection of Insects, Arachnids and Nematodes, in Ottawa, Canada. Statistical tests were performed using SPSS v.17.

Results

A total of 176 *Monochamus scutellatus* specimens were collected in Algonquin, across both sites and years (Appendix 4.5.1). Beetle abundance was greater in 2008 than in 2009, with 65.3% of total beetle abundance in 2008. Site N2 in 2008 had the highest abundance with 46% of all beetles collected (Appendix 4.5.1).

Across both study sites and years, 71% of hosts had at least one mite. Overall prevalence was highest in site N2 in 2008 (79%) and second highest in N1 in 2009 (72%) (Appendix 4.5.1). A total of 2486 mites representing eight species, four genera and three families (Digamasellidae, Melicharidae, and Trematuridae), were collected across both sites and years (Appendix 4.5.1). Mite species richness was greater in each site in 2008 than in 2009, despite a slightly greater abundance in 2009, with 54.3% of total mite abundance collected in 2009 (Appendix 4.5.1). Mite diversity and abundance was highest in site N2 in both years, with eight species and 73.1% of all mites collected (Appendix 4.5.1). Typically, when mites were present there were 1–10 (59.8%) or 11–20 (17.7%) mites per beetle; more rarely, 21–30 (8.1%) or 31–100 (11.2%) mites were found per beetle, few hosts had 100+ mites (3.2%), and one male specimen from site N2 in 2009 had 328 mites. Most beetles that had mites were associated with only one or two species of mites (35.5% and 28.2% respectively), fewer had three (22.6%), four (9.7%) or five (3.2%) species, and the maximum was six species collected from a female specimen from site N2 in 2008.

In 2008, beetles and mites were collected from early-June to late-August, and the majority of specimens were collected from mid-June to mid-July (Appendix 4.5.2a). In

2009, beetles and mites were collected from mid-June to early-August, and the majority were collected from mid-June to late-July (Appendix 4.5.2b). Peak beetle abundance was in the first two weeks of July in both 2008 and 2009 (Appendix 4.5.2). There were no patterns in the abundance of mites throughout the spring and summer, such that mites were most frequently encountered when beetles were most abundant (Appendix 4.5.2). It appeared that most mite species reached peak cumulative abundance before that of *M. scutellatus* in both years; although, this pattern was less obvious in 2009 due to the relatively narrower flight interval of beetles in 2009. There were also no apparent differences in seasonality in the abundance of each mite family throughout the season, such that digamasellids and trematurids were collected most frequently when host beetles were most abundant (Appendix 4.5.2).

The Digamasellidae (*Dendrolaelaps* spp.) was the most prevalent, abundant and diverse family of mites overall, with five species representing 52.9% of all mite individuals collected (Appendix 4.5.1). The Trematuridae (*Trichouropoda* spp.) was the second most prevalent, abundant and species rich family, with two species representing 47% of all mites collected (Appendix 4.5.1). The Melicharidae (*Proctolaelaps*, *Mucroseius*) was rarely encountered, with two species and 0.1% of all mites (Appendix 4.5.1). Across all sites, *Dendrolaelaps* nr. *punctatus* and *Trichouropoda* *hirsuta* Hirschmann, showed the highest prevalence (35.6% and 26.6%, respectively) and greatest abundance (802 and 1107 mites, respectively) (Appendix 4.5.1). Among sites, *D.* nr. *punctatus* and *T. hirsuta*, in site N1 in 2009, were the most prevalent species (55.6% and 50%, respectively) (Appendix 4.5.1). *Trichouropoda* *hirsuta* and *T. lamellosa*

Hirschmann, had the highest mean intensity, with 45 and 22 mites/beetle in site N2 in 2009 and N1 in 2008, respectively (Appendix 4.5.1).

There was no significant sex bias in the abundance or prevalence of mites between male and female *M. scutellatus*, ($z = -0.192$, $N = 176$, $p = 0.848$; $\chi^2 = 0.317$, $df = 1$, $p = 0.573$). Six of the eight mite species collected were sufficiently abundant to test for sex bias within a single mite species. All six species showed no significant sex bias in the prevalence of each mite species (Chi-square $p = 0.056$ – 0.823), or abundance (Mann-Whitney $p = 0.050$ – 0.876); although, one species, *Dendrolaelaps isodontatus* Hurlbutt, showed a marginally insignificant female sex bias in mite prevalence ($p = 0.056$) and abundance ($p = 0.050$).

Different families and species of mites appear to attach to different regions on host individuals. All mesostigmatic specimens, excluding uropodoids, were collected under the elytra of their hosts. Uropodoids were found most often glued to the prothorax with an anally-secreted pedicel, with 79% of mites on the pronotum and 11% on the ventral prothorax, and they were also found on the underside of the elytra (10%). Uropodoids showed site specific preferences, in that *T. lamellosa* was collected predominately (83%) under the elytra, and *T. hirsuta* was found mostly (98.4%) on the prothorax.

Appendix 4.5.1. Prevalence (%) (abundance) and mean intensity (maximum) of mites associated with *M. scutellatus* collected across two sites in Eastern Ontario in 2008 and 2009.

Mite family species	Prevalence(abundance) / mean intensity(maximum) in each site ¹				
	N1 08	N2 08	N1 09	N2 09	Overall
Digamasellidae					
<i>Dendrolaelaps isodentatus</i>	23.5(17) / 2.1(6)	27.2(79) / 3.6(14)	5.6 / 1	16.3(35) / 5(12)	17.1(132) / 3.5(14)
<i>D. neodisetus</i>	2.9 / 1	23.5(61) / 3.2(18)	-	7(3) / 1	10.4(65) / 2.8(18)
<i>D. nr. punctatus</i>	32.4(168) / 15.3(49)	50.6(431) / 10.5(122)	55.6(93) / 9.3(16)	39.5(110) / 6.5(18)	35.6(802) / 10.2(122)
<i>Dendro. n.sp. 5</i>	29.4(52) / 5.2(28)	37(128) / 4.3(44)	22.2(82) / 20.5(36)	25.6(54) / 4.9(30)	24.8(316) / 5.7(44)
Trematuridae					
<i>Trichouropoda hirsuta</i>	20.6(35) / 5(17)	32.1(120) / 4.6(17)	50(189) / 21(91)	39.5(763) / 44.9(304)	26.6(1107) / 18.8(304)
<i>T. lamellosa</i>	2.9(22) / 22(22)	7.4(19) / 3.2(5)	11.1(7) / 3.5(6)	14(13) / 2.2(7)	6.8(61) / 4.1(22)
Melicharidae					
<i>Proctolaelaps nr. hystricoides</i>	2.9 / 1	1.2 / 1	-	-	0.9(2) / 1
<i>Mucroseius monochami</i>	-	1.2 / 1	-	-	0.5 / 1
Beetle abundance	34	81	18	43	176
no. mite species	7	8	5	6	8
Mite abundance	296	840	372	978	2486
Prevalence (%)	59	79	72	63	71

¹N1 = Algonquin park site 1, N2 = Algonquin park site 2

Instances where abundance or maximum intensity = 1 were not reported.

Appendix 4.5.2. The cumulative abundance of *Monochamus scutellatus* and six associated mite species (*Dendrolaelaps nr. punctatus*, *D. isodontatus*, *Dendro. n.sp. 5*, *D. neodisetus*, *Trichouropoda hirsuta* and *T. lamellosa*), across 14-day collection intervals in sites N1 and N2 (Algonquin P.P.) (A) from 17th April to 26th August 2008 and (B) 17th April to 6th August 2009.

Discussion

Monochamus scutellatus was associated with a prevalent and moderately diverse assemblage of mites in Ontario. The diversity of mites observed in this study was similar to that of mites on other cerambycid species. In Louisiana, *M. titillator* was associated with eight mesostigmatic species from three genera and three families (Digamasellidae, Melicharidae, and Trematuridae), while *M. carolinensis* had four species and *Neacanthosinus obsoletus* had five species (*Dendrolaelaps* and *Trichouropoda* species) of mesostigmatic mites (Kinn 1987; Kinn and Linit 1989). The abundance of mites collected in this study somewhat differed from that of other cerambycid species. In this study, average mite abundance was 14 mites/beetle, while in Louisiana average mite abundance for *M. titillator* and *N. obsoletus* was 106 and 16 mites/beetle, respectively (Kinn 1987). This large divergence in mite abundance may be a result of differing collection techniques: in Louisiana, beetles were collected immediately after emergence, while in this study beetles were collected in Lindgren traps, and mites could have been lost during flight. The abundance, prevalence and diversity of mites from *M. scutellatus* in Algonquin P.P. were greater than that of *M. scutellatus* from northern Alberta (56.950, -111.417), which had 277 mites (*T. hirsuta* and *T. lamellosa*) from 38 beetles, with a prevalence of 58% (unpubl. data, available upon request). The mite load of *M. scutellatus* in Algonquin P.P. was also greater than that of *M. scutellatus* from two sites in southern Ontario (44.500, -76.072; 44.447, -76.577), which had 151 mites (*D. nr. punctatus*, *D. isodontatus*, *Dendro. n.sp. 5*, *T. hirsuta* and *T. lamellosa*) from 8 beetles, with a prevalence of 62.5% (unpubl. data, available upon request).

Several of the mite species collected in this study have been reported from other species of cerambycids, as well as other families of wood-boring beetles, but at different levels of abundance or prevalence. The Digamasellidae was the most dominant family across both sites and years. Except for a few species, the ecology and impact of digamasellids on their hosts is poorly understood. *Dendrolaelaps neodisetus* Hurlbutt, a putative mutualistic species of bark beetles that feeds on entopathogenic nematodes (Kinn 1980), was infrequently collected in this study. However, this species is primarily associated with bark beetles, and is considered to be one of the most frequently encountered mites associated with *Dendroctonus frontalis* and *Ips* species (Scolytinae) in southern pines (Kinn 1984). *Dendrolaelaps neodisetus* has also been collected from three other cerambycid species in Louisiana (Kinn and Linit 1989). The large-bodied *D. isodontatus* was frequently collected in Ontario, and this species has also been collected from *M. titillator* and *M. carolinensis* (Moser and Roton 1971; Kinn and Linit 1989), as well as five bark beetle species from southern USA and Ohio (Moser and Roton 1971).

Only two melicharid species were collected in this study, both of which were rarely encountered. *Monochamus scutellatus* and *M. notatus* are the primary hosts of *Mucroseius monochami* Lindquist (Lindquist and Wu 1991). *Mucroseius monochami* can reach high abundance levels, reportedly filling the spiracular atria of its host (Lindquist 1962). However, *M. monochami* was rarely collected in Ontario, and it was also rarely collected from *M. titillator* in Louisiana (Kinn 1987). *Mucroseius monochami* has been collected from five *Monochamus* species, including *M. scutellatus* and *M. notatus*, from

Quebec, British Columbia, Manitoba, and southern USA, and this widespread mite species may represent a species complex (Lindquist and Wu 1991; Kinn and Linit 1989).

The ecology and impact of uropodoids on cerambycids is unknown.

Trichouropoda hirsuta was one of the most prevalent and abundant species collected in this study. This species is a broad host generalist, occurring on multiple families of wood-boring beetles, including scolytines, weevils, tenebrionids (unpubl. data), and at least four additional cerambycid species from Louisiana (Kinn and Linit 1989). The high prevalence of *T. hirsuta* on Ontario *M. scutellatus* (26.6%) was similar to that of Albertan *M. scutellatus* (23.7%) (unpubl. data). *Trichouropoda lamellosa* is often found in association with cerambycids, but in this study it was sporadically associated with *M. scutellatus*. *Trichouropoda lamellosa* is a host generalist, occurring on at least four other species of cerambycids in Louisiana (Kinn and Linit 1989), as well as four bark beetle species (Moser and Roton 1971). The prevalence of *T. lamellosa* on *M. scutellatus* from Ontario (6.8%) was much lower than that of Albertan *M. scutellatus* (42.1%) (unpubl. data). *Trichouropoda hirsuta* and *T. lamellosa* occasionally reach high abundances on other cerambycid species, with an average abundance of 10 and 37 mites/beetle, respectively (Kinn 1987).

Phoresy evolved independently numerous times within mites, and phoretic species display a wide range of morphological and behavioural adaptations of varying complexity (Moser and Cross 1975; Binns 1982). Adaptations for a phoretic life history often reflect the location of attachment to a host. Phoretic mites attach to different body parts of a host, and this may reflect the relative level of adaptation by the mites;

mesostigmatans, excluding uropodoids, were collected solely under the elytra, whereas uropodoids were attached to the prothorax or the underside of the elytra. Uropodoid species attach to a host using an anally-secreted pedicel which allows for attachment to any location on the host. Although the two species collected in Ontario exhibited strong attachment location specificity: *T. hirsuta* was mostly found on the prothorax, and *T. lamellosa* under the elytra. These two species showed the same site preferences on four additional cerambycid species (Kinn 1987). Attachment location specificity is often considered to be proportional to the specificity of host associations, in that many species of highly host-specific mites exhibit strong site specificity; however, mites occurring on multiple host species also show strong site specificity (Lindquist 1975), as seen with the two generalist *Trichouropoda* species collected in this study. Given that *Dendrolaelaps*, *Proctolaelaps* and *Mucroseius* species lack specialized structures for phoretic dispersal, residing under the elytra may provide the greatest protection against dislodgement by the host. *Dendrolaelaps* species use their leg ambulacra and chelicerae to clamp onto hairs and attach to the body surface of their host (Hirschmann and Rühm 1953). *Mucroseius* species are often found in the metathoracic spiracular atria of their host (Lindquist and Wu 1991); although, in this study, and in Louisiana (Kinn 1987), *M. monochami* was only found under the elytra.

Mites are dispersing equally on males and females: there was no sex bias in the abundance or prevalence of mites between male and female *M. scutellatus*. The absence of sex bias in mite load has been reported for other cerambycid species: the mesostigmatic species, *Dendrolaelaps fukikoe*, *D. unispinatus* and *Proctolaelaps*

hystrix, showed no preference for male or female *Monochamus alternatus* (Tamura and Enda 1980). The mating and ovipositioning behaviour of *M. scutellatus* is as follows: mating takes place on host trees selected by *M. scutellatus* females for ovipositioning, and males often closely guard females before and after copulation (Hughes 1979).

Following copulation, females walk and palpate the bark surface, they select an ovipositioning site and chew a scar in the bark in which eggs are laid, and a light brown jelly is deposited over the scar (Peddle et al. 2002). Mites must detach from their host and enter the egg niche, in which they reside and complete development. Unfortunately, the behaviour of associated mites during mating or ovipositioning has never been studied.

Mites attached to females should have the best opportunity to access the egg niche, while those attached to males may have a lesser chance. Since males often guard females during ovipositioning, mites could detach from males and enter the egg niche at this time. However, some *M. scutellatus* males do not guard females (Peddle et al. 2002), and mites attached to those males may fail to enter the egg niche and perish. The absence of a sex bias in mite load observed in this study, suggests that the overall selective advantage for mites dispersing on female beetles is weak or absent. There may be other routes that male associated mites reach the egg niche, for example, by dispersing across the bark of a host tree, or via sexual transmission during copulation and mate guarding, as seen in the sexually transmitted mite *Coccipolipus hippodamiae* associated with coccinellids beetles (Webberley et al. 2004), or the mite, *Parobia husbandi*, from chrysomelid beetles (Seeman and Nahrung 2004).

Overall, these results suggest that the widespread cerambycid species, *M. scutellatus*, is frequently associated with a diverse and abundant mite fauna, and the acarofauna does not differ between male and female hosts. The species richness and abundance of mites associated with the white-spotted sawyer in Ontario is somewhat similar to that of other cerambycid species, taking sampling approach into consideration. There are clear differences in the host attachment location of different species of mites, which may reflect the degree of adaptive specialization for phoresy. Lastly, most species of mites appear to reach peak abundance prior to that of the host beetles, collected in 2008, which may result from a loss of mites from beetles collected later in the season, or it may reflect a preference to disperse on earlier emerging beetles. This pattern is less obvious in 2009 where beetles occurred in a much narrower time period.

CHAPTER FIVE

General Discussion

General Discussion

The range of host species used by a symbiont represents one of its most fundamental characteristics, and the underlying mechanisms determining why some species are specialists, while others have broad host ranges, are not fully understood (Poulin et al. 2006; Dick and Patterson 2007; Poulin 2007; Schmid-Hempel 2011). Host specificity is determined by ecological and physiological factors, and ultimately constrained by the evolutionary and biogeographical history of associated species (Poulin 2007). The study of host specificity is constrained by the definition of a species, by our understanding of the boundaries between species, and the extent of cryptic diversity (i.e. species distinguishable by no or very subtle morphological differences) (Bickford et al. 2007; Poulin 2007). Modern molecular techniques are one of the most effective and versatile approaches for assessing species boundaries and cryptic diversity (Donald et al. 2004; Bickford et al. 2007).

Throughout my thesis research I was interested in elucidating species boundaries and uncovering cryptic diversity, as well as exploring the evolution of host specificity, and the extent of coevolution of beetle associated mites, using molecular and morphological approaches. I examined mites (Uropodoidea) associated with carrion-feeding beetles (Silphidae: *Nicrophorus*), and those associated with bark beetles (Scolytinae), and by examining related mites in two distinct natural systems I was able to assess the generality of my findings for the following questions: is a widespread generalist truly a generalist, are mites evolving towards increased specificity, and to what extent are mites coevolving with their hosts. Another aspect of my thesis research

focused on characterizing the biodiversity and host specificity of mites (Mesostigmata, Oribatida) associated with bark beetles in Ontario. I then used this dataset to explore some of the obstacles associated with the study of host specificity. Herein, I compare and contrast the major findings of my research on the study of host specificity and species boundaries, as well as expand upon specific topics for future research, including live-choice experimentation, the relationship between specificity and the nature of symbiosis, and further study on the determinants of host specificity.

Widespread generalists and cryptic specialists

As mentioned, investigations into the boundaries between species, and the extent of cryptic diversity, have become increasingly common over the last two decades, primarily due to the increased accessibility of nucleotide sequencing (Bickford et al. 2007). As more molecular and phylogeographic studies are performed it often appears that cryptic species are becoming commonplace, and that most widespread generalist species are actually complexes of cryptic specialist species (McCoy et al. 2001; Morelli and Spicer 2007; Smith et al. 2007). Overall, in many host-symbiont systems there are typically a greater proportion of specialist species, and a smaller proportion of generalists (Poulin and Keeney 2007; Loxdale et al. 2011; Schmid-Hempel 2011).

In Chapter 2, I employed molecular and morphological markers to assess whether the apparent widespread generalist species, *Uroobovella nova*, associated with *Nicrophorus* beetles, was truly a generalist or instead a complex of cryptic specialists. The two markers employed (COI and ITS2), as well as previously unacknowledged

morphological characteristics clearly indicated that *U. nova* is not a single species, but it is instead at least five cryptic species with differing levels of host specificity. These putative species were distinguishable by only a few subtle morphological characteristics, but despite their apparent similarities, the level of COI divergence between these species was rather high (13.4–18.7%). The degree of host specificity was variable, with four specialist species (*Uroobovella* sp. 2–5), occurring on one or two host species, and one generalist species (*Uroob.* sp. 1), occurring on nine host species. *Uroobovella* sp. 1 is primarily associated with *N. orbicollis*, which is considered to be a large-bodied competitive species that occasionally invades the crypts of other *Nicrophorus* species (Trumbo 1990), and instances where crypt takeover is unsuccessful could result in novel host associations, which may explain in part the broad host range of *Uroob.* sp. 1. The findings from Chapter 2 add support to the published notion that typically, widespread generalists are actually complexes of cryptic specialists; however, in some instances, including the results of Chapter 2, generalists are shown to be valid species (Donald et al. 2004; Stireman 2005; Smith et al. 2007).

In Chapter 3, I explored the generality of the findings of Chapter 2, by assessing species boundaries in a closely related group of mites occurring in a distinct and more diverse natural system. I used molecular and morphological characters to explore the species boundaries, and the extent of cryptic diversity, in bark beetle associated uropodoids. Initial identifications were based on morphospecies concepts, and these identifications indicated that the majority of uropodoid species collected were specialists, with much fewer generalists. Molecular findings supported most of the morphologically

defined species, with the exception of *Trichouropoda polytricha* and *T. moseri* which were not monophyletic. The molecular markers employed (COI and 28S), as well as morphometric analyses indicated that for the most part, widespread generalist species were not complexes of cryptic specialists, but instead they were truly generalists, with one exception. Analyses indicated that the widespread generalist species, *Uroobovella orri*, is not a single species, but it is instead at least two cryptic species, including a generalist and a host specialist. Overall, the findings of Chapter 3 are somewhat contradictory to those of Chapter 2 and the published literature, which show that widespread generalists are typically cryptic specialist species (Kawazoe et al. 2008; Skoracka and Dabert 2010). However, it should be noted that even though widespread generalist uropodoid species were found to be truly generalists, the majority of bark beetle associated uropodoids were specialists.

The somewhat contradictory results of Chapters 2 and 3 pose an interesting question about the evolution of specificity. Why in one natural system is a generalist actually a complex of cryptic specialists, while in the other system, generalists are truly generalists? What is different about these two systems that host specificity is selected for in one, but not in the other? One of the most likely factors driving host specificity in these two systems is the opportunity for host switching (Poulin 2007). The entire life cycle of associated uropodoids takes place in the breeding habitat of their host species, which may initially appear as a barrier to host-switching, however, the degree of isolation varies between these two systems (Wood 1982; Anderson and Peck 1985; Athias-Binche et al. 1993). A breeding pair of *Nicrophorus* beetles will pair up and bury a carcass, and in the

absence of interspecific competition, host switching is unlikely (Anderson and Peck 1985). On the other hand, multiple scolytine species may occupy the same tree concurrently, and their galleries occasionally intersect, which may provide mites with opportunities for host switching (Moser et al. 1971); although, some scolytines actively avoid interspecific competition by avoiding or deterring other bark beetle species (Birch et al. 1980).

The findings of this thesis have only begun to scratch the surface of the genetic diversity of silphid and scolytine associated mites. The examination of additional uropodoid specimens from the same and different host species, as well as different locales, would further elucidate the host associations, and improve our understanding of the evolution of host specificity in these groups. The Uropodina is a diverse group, and many of them are associated with arthropods from many orders and families, including long-horned beetles (Cerambycidae) (Kinn and Linit 1989), Hymenoptera (Gotwald 1996), centipedes (Bloszyk et al. 2006), and coprophagous beetles (Aphodiidae, Geotrupidae and Scarabaeidae) (Bajerlein and Bloszyk 2004), as well as occasional vertebrate hosts (Domrow 1981). Further research on species boundaries and cryptic diversity of uropodine mites associated with other arthropod groups, would broaden the taxonomic scale of investigation, and likely provide a better understanding of the evolution of host specificity.

Directionality of host specificity

Historically, the evolution of host specificity was considered to be directional and irreversible, with specialists evolving from generalists, and specialists representing an evolutionary dead end (Poulin et al. 2006; Poulin 2007). However, it is now widely accepted that host specificity is not necessarily directional or irreversible (Poulin et al. 2006; Poulin 2007). Transitions from generalists to specialists are typically more common, but in some groups the reverse is observed more frequently (Nosil 2002; Stireman 2005).

Exploring the directionality of host specificity was not an objective of Chapter 2; however, the findings in this chapter can be used to some extent to discuss host specificity in silphid associated uropodoids. In Chapter 2, four of the five putative cryptic species were host specialists, and one species (*Uroobovella* sp. 1) was a generalist, collected from nine host species. In all molecular reconstructions, the single generalist species was the most derived species, indicating that there could be a trend towards lower host specificity; however, the phylogenetic relationships between putative species were only weakly supported. Considerably more data is required in order to make broader conclusions on the directionality of host specificity in silphid associated mites.

In Chapter 3, generalist and specialist uropodoid species were distributed approximately evenly throughout the phylogenetic trees, suggesting that there is no overall trend towards increased or decreased host specificity, and that neither generalists nor specialists represent an evolutionarily more derived strategy. Specialization appeared to be a more common strategy, with a majority of specialists, and many fewer generalists,

but there was no evidence to suggest that host specificity was directional. The findings from this chapter need to be interpreted with caution, primarily because the evolutionary relationships between many *Trichouropoda* species were poorly resolved, with multiple polytomies at deeper nodes within the genus. Additional uropodoid species need to be examined, and alternative complementary genes should be employed, in order to increase the resolution at deeper nodes, clarify phylogenetic reconstructions, and further our understanding of the evolution of host specificity.

Coevolution and ecological fitting

The evolution of host associated symbionts is a function of ecological and evolutionary constraints (Poulin 2007). The evolutionary history of associated symbionts may reflect a history of coevolution with their hosts, and/or a history of ecological fitting, in which symbionts track ecologically-similar host species (Kethley and Johnston 1975; Brooks et al. 2006). The findings of Chapter 2 indicate that the evolution of *Nicrophorus* associated uropodoids appears to represent more a history of coevolution, than tracking ecologically-similar species, although more data is necessary before any robust conclusion can be drawn. The available data does provide an example of coevolution, where ecologically dissimilar but phylogenetically closely related hosts, *N. sayi* and *N. defodiens* (Peck and Anderson 1985; Sikes 2003), shared the same mite species. While ecologically similar but distantly related hosts, *N. sayi* and *N. orbicollis* (Peck and Anderson 1985; Sikes 2003) were associated with distantly related mite species.

In contrast, in Chapter 3, there was little evidence of coevolution or ecological fitting between bark beetles and their uropodoid associates, and although this was not shown specifically, I suspect that the evolution of uropodoids represents a blend of tracking ecologically-similar host species and coevolution with their scolytine hosts. The tanglegram showed no evidence of coevolution, and there was no evidence of ecological fitting, in that ecologically related hosts that feed on similar trees, which have overlapping habitat ranges or similar phenologies (Wood 1982), were not particularly associated with the same mite species. Additionally, many phloeophagous bark beetle species feed on the same host tree concurrently and larval galleries occasionally cross, which may provide mites with a chance to transfer between host species (Moser et al. 1971). An obstacle in the study of coevolution between bark beetles and uropodoids is that phylogenetically related hosts are often ecologically similar, which makes it difficult to discern the determinant factors of host associations.

Overall, Chapters 2 and 3 provide a limited understanding of the extent to which mites are coevolving with their hosts or tracking ecologically-similar host species. In both chapters the relationships between mite species were poorly resolved and taxon sampling was not exhaustive, and as a result of these shortcomings any conclusions should be drawn tentatively. Additionally, bark beetles and associated uropodoids do not represent an ideal natural system for studying coevolution, specifically the presence of widespread generalist uropodoid species and the extent to which unrelated host species are ecologically similar. Host-symbiont systems in which the majority of symbionts are monospecific, such as fig wasps and their fig hosts (Cook and Rasplus 2003) or avian

nasal mites of the *Ptilonyssus sairae* species complex (Morelli and Spicer 2007), provide a more ideal natural system to explore the extent of coevolution. In order to gain a more complete understanding of the extent to which mites are coevolving with their hosts a more complete phylogeny of associated mites and their hosts is required, and the extent of coevolution needs to be assessed using a robust computational approach.

Computational methods for assessing host-symbiont phylogenies are divided into two well established *a posteriori* and *a priori* categories, which differ based on whether the symbiont phylogeny is assessed with or without a predefined model of host association (Stevens 2004). For an in-depth review of the various analytical approaches used to explore host-symbiont coevolution refer to Stevens (2004).

The perfect congruence of host and symbiont phylogenies is rarely observed in nature, and in some instances congruence is only observed under atypical circumstances, such as that seen in pocket gophers and their lice, where host species isolation is particularly high (Page 1996). It is probable that many host-symbiont associations are a product of both coevolution and tracking ecologically-similar host species, as seen in platyhelminth parasites of anuran amphibians, and the relative importance of either factor likely varies across systems (Brooks et al. 2006).

Challenges surrounding the study of host specificity

The research effort of Chapters 2 and 3 focused on elucidating species boundaries and detecting cryptic species, which are some of the most important obstacles associated with the study of host specificity (Poulin 2007). There are many other obstacles surrounding

the study of host specificity (Poulin 2007), and in Chapter 4, I explored some of those obstacles.

In Chapter 4, I conducted the first broad investigation into the mesostigmatic and oribatid mite fauna of bark beetles in Ontario, collected across four sites over two years, and this survey represents one of the largest in Canada. The scolytines included in this study were frequently associated with an abundant and diverse mite fauna. Some hosts were associated with numerous mite species and individuals, while other host species were seemingly devoid of mites. The relative proportion of generalist and specialist mite species was similar in this chapter to that observed for uropodoids in Chapter 3, in that the majority of mesostigmatic and oribatid mite species were specialists, with much fewer generalists.

The level of host specificity for some species of mites collected in this study differed from that of published records, and this variation could be due in part to some of the challenges surrounding the study of host specificity. First, unrecognized cryptic diversity could obscure true host associations (Smith et al. 2007). Alternatively, the publication of incorrect species level identifications (Lindquist and Wu 1991), or changes to the status of species (McGraw and Farrier 1969). In order to validate published host records, all of the identified material from each study would have to be reexamined. Another potential source of variation is the spatial scale of investigation, in that some species may appear to be specialists at a local scale, and generalists at a regional scale, due to variation in the local diversity and availability of hosts (Krasnov et al. 2004; Šimková et al. 2006).

Inferring host specificity solely based upon published host records is a potentially dangerous approach (Poulin 2007). In addition to the aforementioned challenges, published records also provide an inaccurate assessment of host range or specificity at the population level (Poulin 2007). The known hosts exploited by a species worldwide is applicable to a species as a whole, but it does not accurately reflect the hosts that can be exploited by individuals in a given population, which may be constrained by local adaptation (Poulin 2007). Host specificity is a continuous variable, and it is a function of the local diversity and availability of host species (Poulin 2007).

Future directions:

Live-choice experimentation

In this study, the evolution of host specificity and species boundaries of beetle associated mites were explored using molecular techniques, which appeared to be effective tools (Chapters 2, 3). However, in order to truly assess host specificity and species boundaries, an integrated approach incorporating morphological and molecular analyses, as well as live host-choice experimentation is necessary (Poulin and Keeney 2007). The conclusions drawn from an integrated approach are more robust than those drawn solely from individual analyses (Poulin and Keeney 2007). The primary criticism of experimental approaches is that the associations observed *in vitro* do not necessarily occur in nature; however, the benefit of an experimental approach is that it can demonstrate potential host associations by removing ecological constraints (Poulin and Keeney 2007; Schmid-Hempel 2011). Typically, the degree of host specificity of a given group decreases

resulting from the findings of host-choice experimentation, while the level of specificity often increases following molecular investigations due to the discovery of cryptic species (Poulin and Keeney 2007).

There have been numerous live-choice experimentations on *Poecilochirus* mites associated with *Nicrophorus* beetles, and these studies have revealed a lot about the ecology and evolution of these mites, as well as the nature of the relationship with their hosts (Brown and Wilson 1992; Schwarz and Müller 1992). There have been virtually no host-choice experimentations on bark beetle associated mites, but the few studies that have been performed have furthered our understanding of host-symbiont associations (Kinn 1971). I think that live-choice experiments could teach us a lot about the host preferences, behaviour, and evolutionary history of beetle associated mites. However, the interpretation of experimental results should be done cautiously, since associations observed in the laboratory may not occur in nature (Poulin and Keeney 2007). Additionally, experimental results should not be generalized across congenics or populations, because host preferences often vary between species, and preferences can be a function of local adaptation (Lajeunesse and Forbes 2002).

Nature of symbiosis and host specificity

Another possible avenue for future research is to examine the level of host specificity in context of the symbiotic relationship, whether the association between the host and symbiont is parasitic, mutualistic or commensal. It may be expected that the closer the symbiosis, the greater the dependency between the host and symbiont, then the higher the

trade-off between host specificity and average fitness on other host species (Poulin 2007). Bark beetle associated mites could provide a natural system to explore the potential relationship between the nature of symbiosis and the level of host specificity. Overall, the symbiosis between bark beetles and associated mites are poorly understood; however, it is known that the symbiotic relationship may vary from beneficial to detrimental across congeners and even within species (Russo 1938; Novák 1960; Kinn 1967; Kinn 1980). The variation in the nature of the symbiotic relationship, coupled with the observed variability in the degree of host specificity of bark beetle associated mites, may provide an opportunity to explore the potential relationship between symbiosis and host specificity.

Determinants of host specificity

Another possible direction to expand upon the findings of this thesis is to explore some of the determinants of host specificity in beetle associated mites. The determinants of host specificity have been the focus of many studies, but despite such interest the determinants of host specificity remain poorly understood overall (Poulin 2007). The evolution and maintenance of host-symbiont associations is in part, a function of compatibility and encounter filters (Combes 2001). To be compatible, a host species must provide sufficient resources to meet the spatial and metabolic needs of a symbiont, and the host species must not eliminate the symbiont via defense mechanisms. To encounter a host species, the symbiont and host species must occupy the same ecosystem, and the behaviour of either must not prevent contact between the two (Combes 2001). Factors influencing the

compatibility or encounter filters are possible determinants of host specificity (Poulin 2007).

The specific circumstances under which host specificity is expected to evolve are poorly understood, and our incomplete understanding is likely due to a lack of investigation (Poulin 2007). Overall, most potential determinants of host specificity can be grouped into two factors: the opportunity for a symbiont to transfer across host species, and the extent of fitness trade-offs (Poulin 2007). Fitness trade-offs are based upon the notion that adaptations required to exploit a given host species are costly, and that there is a trade-off between the number of host species that a symbiont species can exploit and the relative success of a symbiont on a given host species, as measured by abundance (Poulin 2007).

The extent of fitness trade-offs or the likelihood of host switching are a function of many symbiont- and host-related factors (Poulin 2007). Some studies have shown that the adaptations to exploit and overcome the immunological defenses of a given host species are costly, and these adaptations can constrain a symbiont's ability to exploit other host species (Møller et al. 2005). Fitness trade-offs may be expected in natural systems where the developmental timeline of a given symbiont species is adapted and constrained to track the life cycle of a given host species (Théron and Combes 1995; Kiss et al. 2011). The adaptation to track the developmental timeline of a given host species may also reduce the likelihood of host transfers. Fitness trade-offs have also been observed in natural systems where symbiont species require highly specialized structures for attaching to a specific host species, such as that seen in feather lice or in monogenean

parasites (Clayton et al. 2003; Šimková et al. 2006; Poulin 2007). It is important to acknowledge that host specificity and host specialization are not necessarily linked, in that a given symbiont species may be host specific but not specialized. The specialized adaptations associated with fitness trade-offs can be used as an indicator of the degree of host specialization.

There are a number of host-related factors that may influence the opportunity of host switching, such as host social behaviour and the local availability and diversity of hosts (Poulin 2007). In natural systems where hosts are highly isolated from other host species, such as that seen in pocket gophers (Hafner et al. 2003), there are few opportunities for transmission between host species (Poulin 2007). On the other hand, in natural systems where host species are gregarious, such as icterid passerines (Strandtmann 1958), there are many opportunities for host switching (Poulin 2007). However, in some instances host specificity does evolve even when there are many opportunities for host switching. For example, streblid and nycteribiid flies are highly host specific and vagile parasites of bats, and a high level of host specificity has evolved in these flies despite there often being several species of bats dwelling in the same cave. The selection for being host specific is reportedly a function of mate availability and adaptive immunocompatibility (Dick and Patterson 2007).

Overall, generalists are expected to evolve in the absence of fitness trade-offs, or in natural systems where there is a high likelihood of host switching. On the other hand, host specificity may be expected to evolve when there are high fitness trade-offs, or when there are no chances to transfer between host species. Unfortunately, many of the

potential determinants of host specificity do not provide easily testable predictions (Poulin 2007). Additionally, it is difficult to isolate the impact of individual factors, since the influence of a given host or symbiont factor may be masked by other factors, such as changing environmental conditions (Zander 1998; Poulin 2007).

The determinants of host specificity in beetle associated mites could be a function of any number of symbiont- and host-related factors, and the relative impact of each likely varies. I suspect that one of the strongest determinants of host specificity in beetle associated mites is the opportunity for host switching. Opportunities are likely a function of the local diversity and availability of host species, as well as the specific ecology of host species. The spatial and temporal overlap of host species is determined in part by host ecology, and the greater the overlap between host species, then the higher the likelihood of host switching. I also speculate that fitness trade-offs play a relatively minor role in host specificity of mite species which only have a phoretic association with their hosts, because there are no immunological barriers to overcome. Phoresy is the most common strategy amongst arthropod-associated mite species (Hunter and Rosario 1988). The most likely fitness trade-off, for phoretically associated mite species, may be the adaptation to track the developmental timing of a particular host species. Fitness trade-offs would be expected to play a larger role in mite species which a closer relationship with their host, such as parasitic species (Møller et al. 2005).

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