UNIVERSITY OF TURKU

HIDDEN DIVERSITY OF MOSS MITES (ACARI: ORIBATIDA) UNVEILED WITH ECOLOGICAL AND GENETIC APPROACH

Riikka A. Elo

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
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ACADEMIC DISSERTATION

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Biodiversity is a chest of jewels that so few open

Maddison *et al*.

Look closely at nature. Every species is a masterpiece, exquisitely adapted to the particular environment in which it has survived.

E. O. Wilson

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ABSTRACT

Moss mites (Acari: Oribatida) are microscopic (0.1–1 mm) soil-dwelling arachnids that function as soil decomposers. The oribatids are among the most numerous soil animals with abundances reaching up to 200,000 specimens and 50 species per one square meter of soil. Despite their ubiquitous and abundant existence, they are classified as a poorly known animal group, both in Finland and elsewhere.

In this thesis I study the diversity of oribatids in a previously undocumented microhabitat, red wood ant nest mounds (of the *Formica rufa* group), and the special characteristics of that habitat. In addition, I study whether forest clear felling detrimentally affects the physical properties of ant nests and the oribatid fauna inhabiting those nests. Using DNA-based methods, I also investigate species richness, phylogeny, and species boundaries within the genus *Phthiracarus* Perty 1841.

First, I compared the community composition of oribatids between ant (*Formica polyctena*) nest mounds and the surrounding soil. The study revealed that equally abundant fauna inhabited the two habitats, but the community composition differed; these two habitats were predominantly inhabited by different species. Second, I compared the community composition of oribatids between the parts of an ant mound. I found that, as was presumed, the oribatids predominantly inhabited the surface layer of mounds, which was also observed to host the highest moisture. This study revealed that the distribution of oribatids is moisture-related within ant mounds. These results revealed that ants and their nest mounds providing optimal conditions for decomposer fauna are important factors in maintaining oribatid diversity in the forest landscape.

The next study investigated how the physical properties of ant nests (those of *F. aquilonia*) change due to forest clear felling by comparing mounds located in mature spruce forest and its clear fells. The study showed that the surface layer of mounds was significantly drier in clear fells than in undisturbed forest, and due to the dryness, the mounds were also relatively cooler as they lose thermal capacity on clear fells. Next, I studied whether these carry-over effects have an impact on the oribatid communities inhabiting the ant nests. The study revealed that the species richness was lower in clear fell mounds, but there were no clear changes in the total abundance or community composition of oribatids.

Morphology-based identification of these minute animals is difficult due to the phenotypic variation of species. Therefore, using molecular systematic methods I investigated the species delineation of the genus *Phthiracarus* among nine species. Despite the challenges in obtaining DNA sequences, the DNA-based analysis (using markers COI, 28S D3, ITSS) showed that five species formed clear entities (clades), while the other four species were split into two haplotypes, indicating cryptic diversity. These results highlight that the actual species diversity may be higher than previously known. Hence, the results reveal a need to develop further the DNA-based taxonomic methods for oribatids.

This thesis provides novel information about the diversity, ecology, and habitat selection of oribatid mites in a distinct habitat: wood ant nest mounds. Using systematic sampling and a species-specific approach I showed that ant mounds are central factors in maintaining the oribatid diversity in forests. Moreover, the ant mounds are inhabited by a large variety of other invertebrates, and hence these microhabitats form diversity hotspots in the forest landscape. Thus, the red wood ant colonies should be taken into consideration when making conservation decisions. The red wood ant species are still viable (to use the IUCN category) in the boreal forest of Finland, but in many other European countries they

are classified as near-threatened species. Hence, conservation of these distinct habitats is of great value.

Appropriate identification of organisms (taxonomy, systematics) is a cornerstone in the studies focusing on biodiversity research. For this purpose, DNA taxonomy may provide a fast and precise tool in characterizing species, especially in the case of microscopic organisms that are otherwise challenging to identify. This thesis provided the first DNA reference library for the genus *Phthiracarus,* revealing possible cryptic diversity, but also highlighting the need for developing new laboratory protocols for the future studies of this poorly known animal group.

Figure 1. Photos of oribatid mites taken with microscope (by R. Elo). Species from left to right: **a)** *Eupelops torulosus* (C.L. Koch, 18401) **b)** *Quadroppia michaeli* Mahunka, 1977 **c)** *Oribotritia fennica* Forsslund & Märkel, 1963 **d)** *Hypochthonius rufulus* C. L. Koch, 1835 **e)** *Pergalumna nervosa* (Berlese, 1914) **f)** *Eniochtonius minutissimus* (Berlese, 1903) **g)** *Heminothrus targionii* (Berlese, 1885) **h)** *Conchogneta traegardhi* (Forsslund, 1947) **i)** *Hermaniella dolosa* Grandjean, 1931.

TIIVISTELMÄ

Sammalpunkit (Acari: Oribatida) ovat maaperässä hajottajina toimivia mikroskooppisia selkärangattomia eläimiä. Sammalpunkit ovat yksi runsaimmista maaperäeläinryhmistä, sillä niitä esiintyy neliömetrillä metsämaata yli 200 000 yksilöä ja 50 lajia. Tästä huolimatta ne luokitellaan puutteellisesti tunnetuksi eläinryhmäksi sekä Suomessa että muualla maailmalla.

Tässä väitöskirjassa kartoitin sammalpunkkien monimuotoisuutta ja syntymisen syitä aiemmin heikosti tunnetussa elinympäristössä, kekomuurahaispesissä (*Formica rufa* -ryhmä). Lisäksi tutkin metsähakkuiden haittavaikutuksia sekä muurahaiskekojen olosuhteisiin että sammalpunkkifaunaan. Tarkastelin myös sammalpunkkien evoluutiohistoriaa ja lajimäärää malliryhmän ja DNA-menetelmien avulla.

Ensiksi vertailin sammalpunkkilajistoa ja yhteisökoostumusta muurahaiskekojen (*Formica polyctena*, kaljukekomuurhainen) ja tätä ympäröivän maaperän välillä. Tutkimukseni mukaan, näitä kahta elinympäristöä asuttaa yhtä runsas sammalpunkkifauna, mutta pääosin eri lajit. Toiseksi vertailin sammalpunkkilajistoa keon eri osissa, sekä tähän vaikuttavia tekijöitä. Tulosteni mukaan, sammalpunkit esiintyivät pääosin keon pintakerroksessa, jonka havaittiin olettamusten mukaan olevan myös keon kostein osa. Tutkimus siis osoitti kosteuden olevan merkittävä tekijä määräämässä sammalpunkkien esiintymistä muurahaiskekojen sisällä.

Seuraavaksi tarkastelin, miten metsän avohakkuut häiritsivät muurahaiskekojen (*F. aquilonia*, tupsukekomuurahainen) olosuhteita, ja aiheuttivatko nämä muutokset elinympäristössä häiriöitä kekojen sammalpunkkilajistoon. Tutkimuksessa vertailtiin muurahaiskekoja luonnon metsässä ja tämän avohakkuuaukeilla. Tutkimuksessa kävi ilmi, että hakkuuaukeilla muurahaiskekojen tärkeä kostea pintakerros kuivui, ja lisäksi tämän vuoksi keot myös viilenivät suhteessa ympäröivän ilman lämpötilaan. Tutkin seuraavaksi samojen kekojen sammalpunkkilajistoa, ja tulosteni mukaan sammalpunkkien yhteisökoostumuksessa ei ollut eroa metsän ja hakkuualueiden kekojen välillä. Myöskään punkkien kokonaismäärän välillä ei ollut eroa, mutta sammalpunkkien lajimäärä oli alhaisempi hakkuuaukeiden keoissa.

Mikroskooppisten eläinten morfologiaan perustuva tutkimus ja lajinmääritys on hankalaa yksilöiden ulkoisen muuntelun takia. Tämän vuoksi tarkastelin lisäksi sammalpunkkien todellista lajimäärää käyttäen DNA-tuntomerkkejä malliryhmän *Phthiracarus*-suvun ja sen yhdeksän lajin tutkimuksessa. Vaikka DNA:n monistamisessa oli hankaluuksia, DNA-taksonominen analyysi osoitti (markkerit COI, 28S, ITSS), että viisi lajia muodosti selkeitä ryhmiä (kladi), kun taas neljä muuta lajia jakautui kahdeksi kladiksi. Tulos korostaa, että lajiryhmän todellinen lajimäärä on todennäköisesti suurempi kuin aiemmin on luultu. Tutkimus osoitti, että DNA-taksonomia on käyttökelpoinen työkalu sammalpunkien monimuotoisuuden tutkimuksessa.

Väitöskirjani tulokset tuottivat uutta tärkeää tietoa sammalpunkkien monimuotoisuudesta, ekologiasta ja elinympäristövaatimuksista erikoiselinympäristössä, muurahaiskeoissa. Osoitin, että muurahaiskeot ovat merkittäviä sammalpunkkilajiston ylläpitäjiä. Sammalpunkkien lisäksi muurahaiskeoissa elää hyvin runsas selkärangattomien eläinten fauna, minkä vuoksi ne ovat monimuotoisuuden polttopisteitä. Tällaiset elinympäristöt ovat erityistärkeitä suojelukohteita. Kekomuurahaiset ovat Suomen metsissä runsaita ja elinvoimaisia (IUCN kategoria), mutta monissa muissa Euroopan maissa ne ovat lähes

vaarantuneita. Väitöskirjani tulokset ovat näin ollen sovellettavissa myös laajempiin suojelusuunnitelmiin ja päätöksen teon pohjaksi.
Hyvä lajintuntemus (taksonomia, systematiikka) on luonnon monimuotoisuuden kartoituksen kulmakivi. Tähän tarkoitukseen DNA-taksonomia voi tarjota nopean ja tarkan työkalun, etenkin mikroskooppisille eläimille, joiden tutkimus on muutoin hankalaa. Väitöskirjani tuotti *Phthtiracarus*-suvulle määritystyön avuksi ensimmäisen DNA-kirjaston ja osoitti kryptistä lajimonimuotoisuutta, mutta korosti myös laboratoriomenetelmien kehitystarpeen tämän puutteellisesti tunnetun eläinryhmän todellisen monimuotoisuuden tutkimuksiin tulevaisuudessa.

LIST OF ORIGINAL PAPERS

This thesis is based on the following publications and manuscripts, referred to in the text by their Roman numerals:

I Elo RA, Penttinen R, Sorvari J (2016) A comparative study of oribatid mite communities in red wood ant *Formica polyctena* nests and surrounding soil in a Finnish oak forest. *Insect Conservation and Diversity*, 9, 210-223.

II Elo RA, Penttinen R, Sorvari J (2018) Distribution of oribatid mites is moisture-related within red wood ant *Formica polyctena* nest mounds. *Applied Soil Ecology*, 124, 203-210.

III Sorvari J, Elo RA, Härkönen SK (2016) Forest-built nest mounds of red wood ant *Formica aquilonia* are no good in clear fells. *Applied Soil Ecology*, 101, 101-106.

IV Elo RA, Sorvari J. Impacts of forest clear felling on oribatid mite fauna inhabiting red wood ant *Formica aquilonia* nest mounds. Resubmitted manuscript.

V Elo RA, Penttinen R, Vesterinen E, Vahtera V. Molecular-based investigation on phylogeny, taxonomy and species richness for the genus *Phthiracarus* Perty, 1841 (Acari: Oribatida) in Finland region. Manuscript.

	I	II	III	IV	V
Study question	JS, RP	RE	JS, RE, SH	JS, RE	RE, RP, VV
Literature review	RE	RE	JS, RE, SH	RE	RE
Fieldwork	JS, RP	RE, JS, RP	JS	JS	RP
Material sorting	RE	RE	-	RE	RP, RE
Identification	RE, RP	RE, RP	-	RE	RP
Laboratory work	-	-	-	-	RE, EV
Analyses	RE, JS	RE, JS	JS	RE, JS	RE, VV
Writing	RE	RE	JS	RE	RE
Commenting	JS, RP, VV	JS, RP	RE, SK	JS	VV, RP

RE = Riikka Elo, JS = Jouni Sorvari, VV = Varpu Vahtera, RP = Ritva Penttinen, SH = Salla Härkönen, EV= Eero Vesterinen

1. INTRODUCTION

1.1 Oribatid mites with many names

Soil ecosystems are described as a "poor man's rainforest" (Giller 1996), referring to the vast abundance and species richness of soil animals. A substantial portion of soil mesofauna consists of oribatid mites (Acari: Oribatida) (Fig. 1), which are microscopic (0.1–1 mm) soil-dwelling arthropods (Maraun et al. 2007; Huhta et al. 2011). Oribatids have an important role in soil ecosystems as detrivorous and fungivorous decomposers, since they contribute to soil formation by comminuting organic material and by altering soil structure and aeration. Consequently, they also play an important role in soil nutrient cycles (Reichle 1977; Behan-Pelletier 1999).

The common name for oribatids, "moss mites," refers to their main habitat in the moist soil surface where they inhabit moss, lichen, vegetation roots, and decaying litter, forming abundant and species-rich communities. In boreal and temperate forests their abundance may yield up to 200,000 specimens and 50 species per square meter of soil (Schatz and Behan-Pelletier 2008; Huhta et al. 2011). In addition to soil, oribatids occur abundantly in various other moist and stable microhabitats such as decaying wood (Siira-Pietikäinen et al. 2008), tree hollows (Taylor and Ranius 2014), tree canopies (Lindo and Winchester 2008), and ant nest mounds (Laakso and Setälä 1998). Low numbers of oribatids can be found from many parts of ecosystems: some species live inside spruce needles (Hågvar 1998), some species are aquatic (Schatz and Behan-Pelletier 2008), and some are obligatory ant associates (i.e., myrmecophiles) (Aoki et al. 1994; Ito and Takaku 1994). Occasionally they can also be found in rodent and bird nests (Bukva et al. 1976; Shakhab 2006) to which they are brought via wind, nest construction materials, or carried by the host animal. Oribatids also disperse via phoresy, that is, some species are able to attach onto flying insects and drop off when conditions are suitable (Norton 1980).

Due to their huge abundance in soil, the oribatids are also often called "soil mites." This name, however, is not strictly correct, as soil is also inhabited by other mite groups from the suborders Mesostigmata, Prostigmata, and Astigmata (Walter and Proctor 2013). The oribatids outnumber the other mite groups in soil, but these different mite groups together form the mite fauna of soils (Behan-Pelletier 1999). These different mite groups are systematically and morphologically separated by the location of breathing stigmata in the body. However, detecting the stigma can be difficult, even for experienced specialists (Walter and Proctor 2013). Yet another name for oribatids is "beetle mites" referring to their morphological appearance, having a polished and rounded body shape gained via a hard, shell-like cuticle. This morphological characteristic best separates oribatids from other mite groups. Other names for oribatids include "shell mites" or "armored mites," referring to their hard cuticle, and these shells may be preserved as fossils in soil

sediments. The oldest oribatid fossil is from the Devonian period (450 mya) (Norton et al. 1988) making it one of the oldest animal groups on Earth (Schaefer et al. 2010).

In general, with over 50,000 described species mites are one of the most speciose orders of arthropods. Yet there are many more species to discover as the estimation about the total mite diversity exceeds one million species (Walter and Proctor 2013). Mites in general are a diverse set of organisms that include decomposers, predators, and parasites (such as Ixodidae ticks) with habitat ranging from soil and water to vegetation and animals. Some groups are also pests in food and storages, and may cause allergies or diseases. Mites are classified as arachnids, together with spiders, harvestmen, pseudoscorpions, and scorpions (Walter and Proctor 2013).

1.2 Global and local diversity of oribatids

Conducting an oribatid survey is a difficult task due to the vast abundance and species richness of oribatids, accompanied by difficult species identification and the scarcity of taxonomic experts. Currently, approximately 11,000 oribatid species are described worldwide (Subías 2018).

In general, the species data is often published as location- or country-specific checklists based on a few identified specimens. To mention a few, 357 species are reported from Finland (Rassi et al. 2010; R. Penttinen unpublished data), 200 from Latvia (Baranovska 2007), 477 from Hungary (Mahunka and Mahunka-Papp 2000), 677 from Austria (Krisper et al. 2017), and 960 species from Spain (Subías et al. 2013). On the other hand, the species data is rarely mapped and only a few countries—like Finland (Niemi et al. 1997), Hungary (Mahunka and Mahunka-Papp 2000), and Spain (Subías et al. 2013)—provide distribution maps for species. Nonetheless, the distribution maps may often be biased since the data normally only originates from the locations where the researchers have collected samples.

Due to their ubiquitous presence in soil, the community compositions of oribatids in relation to various environmental elements have long been studied. Community composition analyses are based on systematic sampling of different microhabitats or treatments where mass identification of oribatid fauna may reflect the differences in habitats. Hence, checklists provide data about species presence or absence, while community surveys also take the species abundance into account. Community composition analyses have been used to study, for example, the species assemblages of canopies (Lindo and Winchester 2008), decaying wood (Siira-Pietikäinen et al. 2008), tree hollows (Taylor and Ranius 2014), and ant nests (Arroyo et al. 2015) in comparison with the species assemblages in soil. Additionally, considering the bioindication aspects, the effects of drought (Lindberg et al. 2002), heavy metal pollution (Skubała and Kafel 2004), and forest

clear felling (Lóšková et al. 2013) have been studied with oribatid communities. Hence, various studies contribute to the knowledge of the global and local diversity of oribatids.

Based on the spatial occurrence of species, it has been shown that many species have continental or even worldwide distribution, reflecting their ability to live in various microhabitats and conditions (Subías 2018). Oribatids are considered as omnivorous feeders on decaying plant material, pollen, moss, lichen, fungi, and bacteria, but some predators occur as well (Maraun and Scheu 2000; Schneider et al. 2004a). On the other hand, recent studies have demonstrated that there is also niche differentiation among oribatids, that is to say, specialist feeders exist (see Schneider et al. 2004a). The variation of the habitats of oribatids is probably the outcome of the ubiquitous presence of food resources that allows them to use different habitats within a forest landscape.

1.3 Oribatid identification

Traditional morphology-based identification of oribatids—these minute, abundant, and species-rich animals—is a difficult task. Species identification can, however, be done with training and the help of identification books, such as those of Pérez-Ìñigo (1993; 1997), Subías and Arillo (2001), and Weigmann (2006). The species identification in oribatids is usually focused on adult individuals, while the immature stages (egg, larva and three nymphs)—that form a substantial portion of the species material typically obtained during the surveys—remain unidentified (Weigmann 2006). However, some effort to describe the juvenile morphology has been made (Seniczak and Seniczak 2007; Seniczak 2018).

Species identification is also often difficult because of phenotypic variation, which is the intraspecific morphological variation of species caused by genetic and environmental factors (Kagainis 2014; Pfingstl and Baumann 2017). In oribatid species, phenotypic variation can be seen through different body sizes and shapes (ranging from oval to round), color shades (ranging from pale brown to blackish), and different forms of body setae (i.e., the characteristics that are usually used in species identification) (Weigmann 2006). While interspecific morphological variation traditionally discriminates between closely related species, the intraspecific variation might still overlap with characters among different species. The difficulty in species discrimination is also demonstrated in the history of taxonomy, for example the synonym list of the common species *Phthiracarus longulus* (C.L. Koch 1841) includes over 20 synonymic names that reflect the high variability in morphology (Niedbała and Liu 2018).

Unlike among many other arthropod and even mite groups, the sexual dimorphism in oribatids is generally low, and therefore determining the sex of the specimens has traditionally been seen as unnecessary in species identification (Weigmann 2006). Still, as there are known cases of morphological variation between females and males (Weigmann

2006; Behan-Pelletier 2015), the variability between the two sexes might be one of the factors causing disagreements in taxonomy. In other words, the two sexes may have been described as individual species in the course of history, but later synonymized into one species, or vice versa.

An interesting characteristic of oribatids is that circa 10% of the species reproduce parthenogenetically (Heethoff et al. 2009). Many of these species have wide, even cosmopolitan distribution, but specimens contain some morphological variation in different locations (Subías 2018; Weigmann 2006). New DNA-based analyses have, however, demonstrated that there is cryptic speciation within these nominal species, in other words, there are several species that resemble each other morphologically (Heethoff et al. 2007). Cryptic diversity has also been observed within many sexual species too (Schäffer et al. 2010b; Lienhard et al. 2014). Knowledge about genetic cryptic diversity might guide taxonomists in looking into characters previously unstudied, like occurrence, reproduction timing, food consumption, and niche differentiation, and hence find new traits for species discrimination (Heethoff et al. 2011).

1.4 Hidden diversity of oribatids

1.4.1 Oribatids as associates of red wood ants and their threats

A diverse set of invertebrates, known as ant associates or ant guests, inhabit ant nests (Hölldobler and Wilson 1990). However, the oribatids—despite being abundant and ubiquitous soil animals that have been studied in many aspects,— have been poorly documented in this particular microhabitat.

Out of the 15,000 described ant species of the world (AntWeb 2018), the studies concerning ant associates have been largely focused on red wood ants of the *Formica rufa* group (Hymeoptera: Formicidae) (Hölldobler and Wilson 1990). These ants build large and long-lived nest mounds using organic plant material (needles, sticks, hay) and soil particles (small rocks) in Eurasian forests (Seifert 2007; Kilpeläinen et al. 2008). The *Formica rufa* group can be considered to consist of six closely related red wood ant species (*F. rufa* Linnaeus 1761; *F. pratensis* Retzius 1783; *F. lugubris* Zetterstedt 1838; *F. polyctena* Förster 1850; *F. aquilonia* Yarrow 1951; *F. paralugubris* Seifert 1996). Red wood ants are abundant in various locations in Eurasia, for example, in Finland where on average three red wood ant nest mounds occur per hectare (Kilpeläinen et al. 2008); but, based on the IUCN Red List of Threatened Species, many *Formica* species are classified as near-threatened (NT) in several other European countries (Stockan and Robinson 2016).

Despite the aspect that red wood ants are polyphagous predators that hunt invertebrates both on the forest floor and in the tree canopy (Domisch et al. 2009), various invertebrates

are still able to live with ants in their nests. While some studies have focused on documenting only one specific taxonomic group among ant-associated organisms—such as beetles (Päivinen et al. 2002, 2004), spiders (Cushing 1997), myriapods (Stoev and Lapeva-Gjonova 2005), earthworms (Laakso and Setälä 1997), and mesostigmatid mites (Lehtinen 1987; Berghoff et al. 2009; Campbell et al. 2013)—several studies provide comprehensive lists of ant-associated invertebrates (Hölldobler and Wilson 1990; Laakso and Setälä 1998; Robinson and Robinson 2013; Parmentier et al. 2014; Härkönen and Sorvari 2014). With their high amount of invertebrate ant associates, ant mounds can be called biodiversity hotspots in the forest landscape (Parmentier et al. 2014).

The presence of oribatids in ant nests has long been noted; for example, A. Berlese already described some oribatid species found in ant nests 100 years ago (Weigmann 2006). On a larger scale, oribatids have been generally disregarded when studying ant associate fauna, mainly due to their minute size, high abundance, and difficult identification (Eickwort 1990; Berghoff et al. 2009; Uppstrom 2010; Rettenmeyer et al. 2011; Parmentier et al. 2014). However, a few studies provide checklists (Huhta et al. 2011; Constantinescu et al. 2011; Robinson and Robinson 2013) or even community composition data (Laakso and Setälä 1998) about oribatid species (but some groups are identified only to family or genus level) that inhabit the nest mounds of red wood ants.

Within ant nests the associates may occur continually, occasionally, or temporally as predators, commensals, mutualists, trophobionts, or ectoparasites (Päivinen et al. 2004). Some associate species are “myrmecophiles” that are directly dependent on ants or on their nests during either their entire life cycle or part of it (Hölldobler & Wilson 1990; Robinson & Robinson 2013). The term “myrmecophilous species” is, however, often misused as it does not indicate when the relationship is obligatory or facultative (Kistner 1982). According to Rettenmeyer et al. (2011) and Parmentier et al. (2014), most organisms within ant colonies can be considered as “non-integrated” or “facultative” myrmecophiles. These associate species maintain their populations in ant nests, but they are also found in other microhabitats in nature (Stoev & Lapeva-Gjonova 2005).

Three main reasons are suggested to cause the accumulation of invertebrate associates on ant nest mounds. First, invertebrates are ectothermic and hence dependent on temperature (Chown and Nicolson 2004). Ants, as social insects working together, are able to maintain higher temperatures inside the mounds compared to the temperature of ambient air (Frouz 2000; Frouz and Finer 2007). Second, moisture is also one key factor in the development of invertebrates, and by building a nest mound, ants are able to regulate the moisture content of their microhabitats (e.g., by active ventilation and closing the tunnels during rain) (Hölldobler and Wilson 1990). Third, the nest mounds consist of decaying organic plant material with high humidity and temperature, forming an ideal environment for the primary decomposers (fungi and bacteria) that are exploited by secondary consumers

(oribatids, springtails, nematodes, earthworms) and hence, create the basis for multi-level food webs (Laakso and Setälä 1997; Jílková and Frouz 2014).

Red wood ants are often described as a key species of forest ecosystems, particularly due to their nest-building activity that alters the soil structure, nutrient accumulation, and the distribution of various other organisms including, for example, plants, invertebrates, and vertebrates (Hölldobler and Wilson 1990; Sorvari and Hakkarainen 2004; Kilpeläinen et al. 2008). Various environmental threats, especially deforestation and clear felling, alter ant colonies detrimentally. It has been shown, for example, that forest clear felling lowers the body fat content of worker ants, weakens the physiological condition of ants by reducing food resources, decreases the production of sexual offspring, and splits polydomous colonies into smaller units (Sorvari 2016). The effects of forest management and clear felling on oribatids and other soil animals has also been the focus of much published literature (Huhta et al., 1969; Abbott et al. 1980; Bird and Chatarpaul 1986; Lindo and Visser 2004; Déchêne and Buddle 2009; Lóšková et al. 2013), but the effect on the oribatid fauna of this special microhabitat, red wood ant nests, has remained unstudied.

1.4.2 DNA methods in unveiling diversity

Nowadays, the morphology-based species identification is often accompanied with DNA characters (i.e., DNA taxonomy). The most commonly used method is DNA barcoding, that is, species identification based on the cytochrome c oxidase I (the 5'-3' region of the COI) gene sequences, which has proved to be an efficient, cost-effective, and convenient analytical tool since its establishment in 2003 (Hebert et al. 2003; Hebert and Gregory 2005). DNA barcoding relies on the assumption that the inter-specific variance of species' COI sequences is higher than the intra-specific variance, and it is commonly thought that the differences between species are >2% and within species <2%, however the percentage can vary considerably in different animal groups and genera (Hebert et al. 2003).

Among arthropods, several analyses have shown that the DNA barcodes were able to discriminate between species, for example, 1000 species of Finnish and Austrian butterflies (Huemer et al. 2014), almost 2000 North European beetles (Pentinsaari et al. 2014), and over 1300 Canadian spider species (Blagoev et al. 2016). On the other hand, DNA barcoding was not an appropriate method for discriminating between some fly species (Meier et al. 2006; Whitworth et al. 2007) and grasshoppers (Trewick 2008) due to the identical COI sequences between morphological species or due to high intraspecific variation. As a consequence, the DNA barcoding methodology has gathered not only praise but also criticism (DeSalle et al. 2005). A common way to untangle this problem is to use information from several additional molecular markers in phylogenetic inference, that is, to use multiloci methods instead of a one-locus method. This approach is used in

much of the recent studies concerning mites too (Dabert et al. 2010; Schäffer et al. 2010b; Kreipe et al. 2015; dos Santos and Tixier 2017).

Within oribatid mites, and mites in general, the molecular systematics are still in the beginning. The NCBI GenBank is still lacking oribatid DNA sequences to a great extent. Also, a substantial proportion of the existing sequences come from non-identified specimens, that is to say, from specimens that are identified only to suborder or genus level (e.g., Oribatida sp., *Galumna* sp.). Many of the existing publications using the molecular approach focus on resolving phylogenetic relationships at deep taxonomic levels, such as suborder, family, or genus level (Maraun et al. 2004; Domes et al. 2007; Dabert et al. 2010; Schaefer et al. 2010; dos Santos and Tixier 2017). In contrast, there are only a few studies focusing on species delimitation and those usually concentrate on one or a few species or genera (Schäffer et al. 2010a; 2010b; Lienhard et al. 2014; Kreipe et al. 2015). On the other hand, these studies often report cryptic diversity among the studied oribatid species, resulting in a higher species number than was previously known (Schäffer et al. 2010b; Lienhard et al. 2014).

Moreover, the DNA barcoding methodology using the COI gene sequences is not well-optimized for mites, resulting in a lack of knowledge of how this marker varies between and within species. Few studies report rather high identification success with COI among ticks (Lv et al. 2014; Zhang and Zhang 2014), spider mites (Ros and Breeuwer 2007), and even oribatids (Kreipe et al. 2015). In contrast, some studies report high intraspecific variance among species, usually correlating with the geographic origin of the population. For example, in oribatids the study of the sexual species *Steganacarus magnus* (Nicolet 1855) revealed up to 30% intraspecific variance in COI among European populations (Rosenberger et al. 2013). Similarly, the study of the parthenogenetic species *Platynothrus peltifer* (C.L. Koch 1839) revealed over 50% variation among the populations of separate continents (Heethoff et al. 2007).

It has been suggested that the amount of variation in COI might be too high for it to be a suitable marker to resolve species-level questions in oribatids, and Lehmiz and Decker (2017) proposed that the nuclear ribosomal 28S rDNA (D3) could be more appropriate for this purpose. In their work, the phylogenetic analysis of the 28S D3 region, comprising over 60 oribatid genera, showed high success in species discrimination, and similar results were also noted in the analysis of Maraun et al. (2003). However, Kreipe et al. (2015) showed that this marker was almost identical between the *Steganacarus* Ewing 1917 species and it could only discriminate taxa on the subgenera level.

In addition to these, several other molecular markers, alternative to COI, have been proposed as species identification tools for mites. For example, i) for *Ixodes* ticks the nuclear marker internal transcribed spacer 2 (ITS2) and mitochondrial 16S rDNA and 12S rDNA have been proposed as alternative delineation markers (Lv et al. 2014), ii) for

Phytoseiidae mites, plants, and animals ITS2 has been proposed (Ben-David et al. 2007; Yao et al. 2010), and iii) for mesostigmatid mites the mitochondrial 12S rDNA and cytochrome oxidase B (CytB), as well as the nuclear markers elongation factor 1-alpha (EF-1a), internal transcribed spacer 1 and 2 (ITSS), 28S (D1–D3), and HSP90, have been proposed. Moreover, it is worthwhile investigating various alternative markers for COI because sometimes studies face problems in laboratory protocols, that is, yielding DNA sequences. For example, in COI sequencing Young et al. (2012) reported 20% failure in yielding sequences for oribatids, Stålstedt et al. (2013), reported 50% failure for water mites (Prostigmata), and in the study of Bowman and Hoy (2012) all the investigated Phytoseiidae (Astigmata) failed to yield COI sequences. The failures of other markers have also been reported (Dos Santos and Tixier 2017).

Since the single-locus approach may not be able to discriminate between all species, the analysis is often improved with other markers, of which concatenated analysis yields better representation of the evolution of the organism group. For mites, the multiloci approach, while being still rather poorly developed protocol, has enabled the study of the phylogeny, taxonomy, and species status of several taxa, such as mesostigmatids (Dos Santos and Tixier 2017), water mites (Stålstedt et al. 2013), and various oribatid groups including *Steganacarus* (Kreipe et al. 2015), *Scutovertex* Michael 1879 (Schäffer et al. 2010b), and Eremaeidae Oudeman 1900 species (Lienhard et al. 2014).

Still, as in general the COI has been selected as species identifier among animals (Hebert et al. 2003), it is important to develop DNA libraries suitable for metabarcoding approaches, in others words, to develop a protocol where large amount of species can be identified simultaneously via COI sequences. Attempts towards this have already been accomplished, for example, the study of Young et al. (2012) showed a high identification success among soil mites using the DNA metabarcoding methodology (with identification made to genus or some higher level) in the Canadian subarctic. Several other large-scale meta-analysis with molecular investigation of soil animal communities have also been conducted recently, but all state that the oribatids and other soils mites are excluded from the analysis due the lack of reference sequences in DNA libraries (Hamilton et al. 2009; Wu et al. 2011; Orgiazzi et al. 2015; Arribas et al. 2016).

1.5 Aims of the thesis

The aims of this thesis can be summarized with three main points. First, my aim was to create an overall picture about the abundance and species richness of oribatid fauna inhabiting red wood ant nests and illustrate how the oribatid fauna differs from the fauna of forest soil and between different parts of ant mounds. Through this, I aimed to provide knowledge for conservation purposes. Second, my aim was to examine how forest clear felling impacts the physical properties, that is, the temperature and moisture content of ant nest mounds and the oribatid fauna inhabiting these nests. With this aim I intended to provide knowledge for use in forest management and utilization. Third, I aimed to create DNA taxonomic tools for species identification by comparing molecular markers for oribatid species delineation and revealing possible cryptic speciation.

In the following, the aims of this thesis are described in more detail, chapter by chapter:

I. In this ecological study I aimed to summarize the existing knowledge about oribatids in ant nests, and conduct the first systematic and taxonomically comprehensive comparative study of oribatid mite communities in red wood ant nests and their surrounding soil.
II. Since the variability of oribatid assemblages within ant mounds remained uninvestigated in the previous study, in this ecological study I aimed to compare the community composition of oribatids between different parts of the ant mounds. Based on earlier literature, I predicted that the distribution of oribatids might be moisture-related and thus I also investigated the relationship between nest moisture content and the distribution of oribatids.
III. In this environmental study, the goal was to investigate the impacts of forest clear felling on the moisture content and temperature of ant nest mounds and how the size and shape of the nest mounds related to these factors.
IV. By using the same experimental design as in the previous study, in this study I aimed to investigate the impacts of forest clear felling and its side effects on the oribatid fauna inhabiting these ant nest mounds.
V. In this genetic study, I aimed to compare different molecular markers for oribatid species discrimination and provide a phylogeny for *Phthiracarus* by using nine species occurring in Finland. Special interest (an extra dataset) was paid to the marker COI that is commonly used in the DNA barcoding of various animal groups.

2. MATERIALS AND METHODS

2.1 Ecological studies

2.1.1 Study areas and ant species

Studies **I** and **II** were conducted on the island of Ruissalo (60°26'N, 22°10'E), Southwest Finland, located close to the mainland. The island of Ruissalo belongs to a hemiboreal vegetation zone, indicated by the distribution of oak (*Quercus robur*), which reaches the coastal areas of Southwestern and Southern Finland. The oak forests of Ruissalo are protected under the European Union Natura 2000 network. The Ruissalo area is one of the most species rich areas in Finland, but the oribatid fauna of Ruissalo have remained poorly surveyed. Study **I** was performed in two oak forest patches in the eastern part of the island, while study **II** was performed in three patches of oak, mixed, and coniferous forest running along the island. Samples collected from protected areas were collected with permission obtained from the Centre for Economic Development, Transport and the Environment of Southwestern Finland (LOS-2006-L-291-259).

The island of Ruissalo is inhabited by abundant colonies of red wood ants, of which *Formica polyctena* is the most common and was therefore selected as the host ant species in studies **I** and **II** (Fig. 2). In general, *F. polyctena* is the dominant ant species in Southern Finland, and it forms polydomous colonies (a network of mounds connected with trails) with polygynous mounds (several queens in one mound) (Collingwood 1979; Seifert 2007). In Ruissalo, *F. lugubris, F. pratensis,* and *F. rufa* occur alongside *F. polyctena* (J. Sorvari, unpublished data).

Studies **III** and **IV** were conducted simultaneously in coniferous forest of central Finland, in three separate areas close to the town of Kuopio (62°52N, 27°29E). The area belongs to boreal forest zone, indicated by the distribution of Norway spruce (*Picea abies*) mixed with Scots pine (*Pinus sylvestris*). Each of the three separate sites contained a clear-felled forest stand and a bordering non-felled stand (side by side). The clear felling had occurred one to two summer periods before the study.

In Kuopio, *Formica aquilonia* was selected as a host ant species since it occurred abundantly in the area (also in clear-cut areas of forest). Similar to *F. polyctena*, F. *aquilonia* is also a polydomous and polygynous species (Collingwood 1979; Wuorenrinne 1994).

Figure 2. Red wood ant (*Formica rufa*; photo by J. sorvari) carrying nest material. The photo below is the nest mound of *Formica polyctena* at the island of Ruissalo (photo by R. Elo).

2.1.2 Experimental designs and oribatid sampling

In study **I,** in total ten *F. polyctena* mounds were selected in the oak forest on the island of Ruissalo on 29 July 2009. The locations of the mounds were pre-documented in other studies by J. Sorvari and S. Härkönen. The minimum distance between selected mounds was 15 meters. The locations of the selected nest mounds are hereafter referred to as *study plots*. Two samples from each study plot were collected: one from an ant mound and another one from adjacent soil 4 m from the mound (10 samples in total from mounds and 10 from soil). The size of the samples was 0.75 liters. From ant mounds, altogether three subsamples of 0.25 liter size were collected from separate mound locations (top, middle of outer surface, rim), but then combined into one sample of 0.75 liters volume. The subsamples were combined because the aim of the study was to create an overall picture about the oribatid fauna of nests via representative sampling. The soil sampling was conducted by collecting three subsamples from a 1 m x 1 m area by gathering soil litter by hand and then combining it into one sample of 0.75 liters volume.

In study **II**, in total nine *F. polyctena* mounds were selected in the island of Ruissalo on 18 July 2013. Out of the nine mounds, three were located in oak forest, three in mixed forest, and three in spruce forest. Within each forest patch the distance between sampled mounds varied from 8 to 65 m. From each of the nine nests three litter samples were collected: i) from the surface, ii) from the rim, and iii) from the core (in total, 27 samples). The size of the sample was 0.75 liters each, and they were studied individually.

Studies **III** and **IV** were conducted simultaneously with the same study design on three separate clear fell–forest pairs in Kuopio by investigating *F. aquilonia* nest mounds in September 2014 (on the 5th, 7th, and 8th). Seven nest mounds per clear fell and seven nest mounds per forest were sampled, except in one forest where only six suitable nest mounds of *F. aquilonia* were found (in total, there were 41 investigated mounds). The distance between mounds per area varied from 11 to 82 meters. All the selected mounds were viable, in other words, deserted or semi-deserted and young, small post-harvesting nests were excluded. In study **IV**, one oribatid sample of 0.5 liters was collected from each of the selected mounds. The samples were collected from the moist surface layer, directly down from the top point at the approximate depth of 10–15 cm (the uppermost, almost dry crust of 0–10 cm was moved away by hand).

2.1.3 Environmental data collection

In studies **I–III,** the above-ground dimensions of each mound were measured and volumes calculated using the formula for a half-ellipsoid: volume = $(4/3\ \pi abc)/2$, where a is the height and b and c are the horizontal radius of mound (the shape of the mound base was nearly a circle so $b = c$). Additionally, in study **III** the shape of a nest mound was

described using a nest shape index calculated by dividing the height of the mound with the basal diameter of the mound.

In studies **II** and **III,** the temperature of both mound interiors and ambient air in the shade was measured with a thermometer. In these studies, the moisture content of mounds (study II: the surface, core, and rim were measured; study III: only the surface was measured) was also measured simultaneously with the oribatid sampling by collecting separate mound litter units (ca. 0.5 liters) close to the oribatid units. The moisture content of each litter unit was measured gravimetrically as the difference between fresh weight and dry weight after drying the litter (study II: the litter was dried at room temperature for two weeks; study III: the litter was dried in an oven for 48 hours). The environmental variables of study **III** (moisture and temperature measurements) were used in the analysis of study **IV**.

2.1.4 Oribatid extraction and species identification

In studies **I, II,** and **IV,** in the field each collected litter sample was sieved (Fig. 3) into a plastic bag through a 2 mm mesh in order to exclude the ants and larger particles, which were carefully returned to the mounds. The oribatids were extracted from the sieved material using Berlese–Tullgren funnels (Weigmann, 2006) for one week. For studies **I–II,** the extraction of specimens was done at the Zoological Museum of the University of Turku (ZMUT), and for study **III** it was done at the University of Kuopio, from where the extracted samples were later moved to Turku. In studies **I–II** the specimens were extracted and preserved in 70% alcohol. In study **IV** the extraction was done into soap-water, from where the specimens were transferred to 70% alcohol after the extraction.

The oribatids were sorted from other soil animals under a light microscope (Fig. 3) and preserved in glass vials in the oribatid collections of the ZMUT. Only adult specimens were identified and included in the datasets. The identification of oribatids was done according to Weigmann (2006) and (for the Phthiracaroidea Perty 1841) Niedbala (1992). All specimens were identified to species level, except for the family Suctobelbidae Jacot 1938, which were only identified to family level. Of this family, three specimens representing three species were identified to species level in study **I**. Additionally, in study **I** some difficult *Ramusella* Hammer 1962 specimens were only identified to genus level and titled "*Ramusella spp.*" These two taxa (*Ramusella* spp. and Suctobelbidae) were treated as "species" in the statistical analyses.

Figure 3. The soil litter samples are typically sieved in order to exclude the large particles prior to funnel extraction. Second picture shows the extracted and sorted oribatid material viewed from microscope (photos by R. Elo).

2.1.5 Community composition and statistical analyses

In studies **I, II,** and **IV,** the oribatid community composition was examined with principal coordinate analysis (PCoA), an ordination method that examines the faunistic similarity (or distance) with a similarity index based on species data (a species matrix combining the species dataset). The commonly used Bray–Curtis index, which uses the species' absolute abundances, was selected as the distance measure (Legendre and Legendre 1998). Additionally, in study **I** the ordinations were made also using Bray–Curtis index based on relative abundance and with the Sørensen index that only takes into account the species' presence–absence data. In studies **I** and **II** the effect of geographical distance on the faunistic similarity of nest mounds was examined with the Mantel test, which calculates a linear correlation between two distance matrices. The geographical locations were transformed to Euclidean distances based on the mounds' coordinates (Legendre & Legendre, 1998). The PCoA and Mantel test were performed with the software Le Progiciel R v. 4.0 (Casgrain and Legendre 2001).

The statistical analyses were conducted using SAS 9.4 statistical software with Enterprise Guide 6.1 (SAS Institute Inc., Cary, NC, USA) (Littell et al. 2006). In study **I** the relationship between abundance and diversity of each sample was analyzed using Pearson's correlation, and the effect of mound size on abundance and diversity was analyzed with regression analysis. In study **II** the nest mound conditions (i.e., moisture content, temperatures, and differences among forest types) were analyzed using linear mixed models (LMM). Mound identity was added as a categorical random factor to control for the clustering of the observations and Kenward-Roger's approximation was used for

estimating the degrees of freedom. The effect of moisture content and nest mound part on the abundance and species richness of oribatids was analyzed using generalized linear models (GLM) with Poisson and negative binomial distribution parameters and a log link function. In study **III** the relationship of moisture and temperature was studied with LMMs using the study stand (a clear fell–forest pair) as a random factor in the models and using Kenward-Roger approximation for the degrees of freedom. In study **IV** the effects of felled/non-felled forest and mound moisture content on oribatid abundance and species richness were analyzed with GLMs.

2.2 Genetic study

In this study nine *Phthiracarus* species occurring in Finland—that is, *P. boresetosus, P. bryobius, P. clavatus, P. crinitus, P. ferrugineus, P. globosus* (Fig. 4), *P. laevigatus, P. longulus,* and *P. nitens* (Niemi *et al.*, 1997; R. Penttinen, unpublished data)— were analyzed in a molecular phylogenetic framework.

The specimens for DNA analysis were selected from the collections of the ZMUT. Four to eight specimens from all nine investigated species were selected depending on the presumptions about the uncertainty of species status and the availability of specimens. The final material consisted of 47 specimens that represented various localities across Finland including geographical, habitat-related, and morphological variation. The selected specimens had been collected during 1992–2016 by several persons, they had been identified by R. Penttinen and stored in the museum collections in 70% alcohol. For the purpose of genetic analysis, all the selected specimens were re-identified (with a few exceptions) according to species based on identification books.

DNA extraction was made from individual specimens using the NucleoSpin Tissue DNA extraction kit (Macherey-Nagel) and following the manufacturer's protocol. The purpose was to amplify altogether six molecular markers: mitochondrial COI and CytB, nuclear 28S D3, ITSS (i.e. ITS1-5.8S-ITS2), EF-1a, and HSP90. Polymerase chain reaction (PCR) amplifications were conducted with the following primer pairs (forward–reverse): Arch1-Arch2 (one other was also tested), CytR-CytB, D3A-D3B, ITSSF-ITSSR, 40.74-52.RC, and hsp1.2-hsp8.x. The PCR protocols were similar as in the work of Kreipe et al. (2015) and dos Santos and Tixier (2017); the used protocols are described in detail in paper IV. Amplification proved to be more challenging than surmised since only three (28S D3, COI, ITSS) of the six markers amplified successfully. This was despite the multiple set of primers and annealing temperatures tested. The possible reasons for the low success of PCR amplifications are discussed in detail in paper IV (e.g., primer unsuitability, PCR unsuitability, DNA preservation).

The quality of the obtained DNA sequences was checked with Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA), aligned with Mafft (Katoh et al. 2017), and trimmed with Gblocks (Castresana 2000). The used outgroups were obtained from GenBank and included oribatid *Hypochtonius rufulus* for COI and 28S D3 (JF264166, AY273495; not from the same specimen), and gamasid *Amblyseius andersoni* (Chant 1957) for COI and ITSS (KU318176, KU318230; from the same specimen; in dos Santos and Tixier 2017).

Phylogenetic analyses were done under a maximum likelihood criterion utilizing RAxML (Stamatakis et al. 2005) and the resulting trees were visualized with FigTree version 1.4.3 (Rambaut 2009). For the multiloci analysis the sequences were concatenated with Sequence-Matrix 1.7.8 (Vaidya et al. 2011). Genetic distances were measured with Mega 7 (Kumar et al. 2016).

Figure 4. *Phthiracarus globosus* walking on lichen (photo by R. Elo).

3. MAIN RESULTS AND DISCUSSION

3.1 Differences in community composition between oribatid fauna of ant nest mounds and surrounding soil (I)

Based on the distribution of the 10,529 observed specimens and 74 species (and two genera), the ant nest mounds of *F. polyctena* and the surrounding soil were inhabited by an equally abundant and species rich oribatid fauna. In nest habitats the abundance and species richness had a positive relationship indicating that mounds form rich habitat patches with a diverse set of niches. Mound size had no effect on the abundance or species richness. The estimated species richness was higher than that observed among all of the samples. This indicates that I did not detect all the possible species with the sampling effort used, (i.e., by taking more samples, the species list might have been even more extensive); however, the identification work would then have been exhausting.

Interestingly, the study showed that the nest and soil habitats were predominantly occupied by different species, resulting in dissimilarities in community composition. A total of 34% of the species were categorized as having a preference for a nest habitat and 50% were categorized as having a preference for soil habitats. Altogether 16% of the categorized species were indifferent to habitat, with equal abundances in both nest and soil habitats. The results would imply that the species with a preference for nest habitats would be myrmecophilous, but the literature review showed that these species commonly inhabit various microhabitats, both in Finland and in other parts of Europe (Subías 2018; Weigmann 2006). Hence, the oribatids within ant mounds may be classified as facultative myrmecophiles, possibly preferring nests for the abiotic conditions promoted in wood ant nests, that is, the nest preference is accounted for by habitat preferences which the ants have no direct role.

Since there is basically no information about the relationship between ants and oribatids, it was speculated—similarly to the earthworm–ant association (Laakso and Setälä 1997)—that oribatids, as bacterivores and fungivores, may eliminate the potentially pathogenic species and hence function as “cleaners” in ant nests, which is why they are tolerated in such abundance by ants.

The study is in line with the findings of Laakso and Setälä (1998) and showed that ant nests host abundant and diverse oribatid assemblages, which differ from the fauna in adjacent soil, and wood ants therefore form an important factor in maintaining biodiversity. Moreover, I showed that in the studies investigating the myrmecophily of oribatids, it is essential to sample the ambient habitat and not only the ant mounds.

3.2 Moisture content related distribution of oribatids within ant nest mounds (II)

In this study, in total 18,614 specimens, 93 species, and one higher taxon were identified. The oribatids occurred predominantly on the nest surface (77.9% of the total abundance), followed by the rim (16.6%), and the core (5.5%). The nest surface was the moistest (24.3% moisture content) and the nest core was the driest (6.4%), while the moisture of the rim was intermediate (14.1%). Thus, the study showed that the abundance and species richness were positively related to the moisture content of mounds.

The oribatid community composition differed both between the ant nest parts and between forest types. The difference between nest temperature and ambient temperature increased with increasing moisture content in the surface layer. The mound volume had no effect on moisture, temperature, or community composition.

Oribatids were generally most abundant in mounds located in mixed forest (43.6% of the total abundance), followed by spruce forest (36.4%), and oak forest (20.0%). It has been shown in many studies (Hansen and Coleman, 1998; Schneider et al., 2004b) that soil animal communities are more abundant and species rich in mixed litter (polycultures) compared to simple litter (monocultures); this is chiefly explained by the more diverse availability of niches. Our results are in accordance with this observation since it is likely that, as decomposers, many oribatid species find the most versatile food resources in mixed forest and therefore abundantly inhabit ant nests in those forests.

As was hypothesized based on earlier literature, I found that the nest mound surface layer was significantly moister than the other parts of mounds and I was therefore able to verify the earlier observations (Laakso and Setälä 1997, 1998; Rosengren et al. 1987). I also found that the moist nest surface had a positive correlation with the nest's core temperature, which is in accordance with the findings of Sorvari et al. (2016); hence, these studies suggest that the moist surface layer has a role in nest thermoregulation. The results indicate that the moisture harbored in the surface layer of ant nest mounds is a vital factor for maintaining the rich oribatid fauna within the nests. In general, moisture is one of the key factors affecting the distribution of soil animals (Taylor et al. 2004). Additionally, the study provided guidance for sampling procedures; I recommend sampling the surface layer of mounds to obtain a high number of oribatids, but when a diverse set of species and a representative sample per mound are desired, I recommend sampling the different parts of mounds.

3.3 Effects of forest clear felling on physical conditions of ant nest mounds (III)

In this study, we found that nest surfaces were significantly drier in clear fells (average: 21% moisture content) than in forests (average: 55% moisture content). Moreover, the nest temperatures relative to ambient temperature were higher in forests than in clear fells and the relative temperature increased with the increasing moisture content of the nest surface layer.

The loss of surface layer moisture and the loss of the nest's thermal capacity may have direct detrimental effects on ants, but also on their abundant associated fauna. Invertebrates are ectotherms (Chown and Nicolson 2004), thus, temperature variations may not harm individuals seriously, but may slow down developmental rates and consequently decrease population growth (Laakso and Setälä, 1997).

Low moisture is most likely to be a more serious threat to nest-dwelling invertebrates than temperature oscillations. In particular the oribatids, which were earlier observed as predominantly inhabiting the surface layer (chapter II), may suffer from the changes in habitats. Moreover, I speculated in study I that oribatids may prefer the food resources of nest mounds instead of directly preferring the ants' mounds. Therefore, the food (bacteria, fungi) availability for oribatids may decrease with decreasing nest surface moisture. This might potentially be reflected in community composition and the abundance of oribatids.

Nevertheless, studies II and III together suggest that the nest mound architecture, a moist surface layer covering a dry central core, is similar in Finland regardless of the habitat or *Formica* species. At a larger scale, this kind of nest architecture, a dry core with a moist surface layer preventing mounds from losing heat, might be an adaptation to the cold climate conditions in the boreal and temperate forests (Frouz and Finer, 2007).

3.4 Effects of forest clear felling on oribatid fauna inhabiting ant nest mounds (IV)

A total of 16,499 specimens, representing 67 oribatid species, were observed. The ten most abundant species represented 90.9% of the total abundance. The oribatid species richness was significantly lower in clear fell mounds and was positively related to the surface moisture content of the mounds.

Based on previous observations, I predicted that, since oribatids predominantly inhabit the moist surface layer of ant mounds (chapter II), the observed reduction in the moisture of the nest surface (chapter III) might have negative carry-over effects onto the oribatids. In

the current study, however, there was no correlation between the surface moisture content and oribatid abundance.

In my study, the oribatid abundance or community composition did not markedly differ between clear fell and forest mounds. Despite the attempt to standardize the microhabitat of oribatid fauna, the use of oribatid mites as biological indicators of harvesting disturbance was limited with this study design due to the lack of changes in community composition. The result is hence in accordance with previous observations indicating that forest management practices do not cause detectable changes in oribatid fauna (Bird and Chatarpaul 1986; Lindo and Winchester 2008; Déchêne and Buddle 2009). Then again, the oribatid species richness of this characteristic microhabitat may provide usable quality measures about the harms of forest clear felling.

The ant nests remained viable regardless of the clear felling and the surface moisture content of nest mounds stayed above 21% in clear fells, although this was significantly lower than in forest stands. The abundant presence of oribatids in these mounds may indicate that moisture is still above a critical level, and hence, this may partly explain the abundant existence of oribatids in clear fell mounds. For this reason it was speculated that the mounds might become refuges for soil animals during environmental stress.

3.5 Phylogeny and DNA barcoding of the genus *Phthiracarus* (V)

In this study, in total 50 specimens, comprising nine nominal *Phthiracarus* species, were analyzed with three molecular markers (COI, 28S D3, ITSS). Three others were also considered (CytB, EF-1a, HSP90), but their amplification did not succeed in this study. The marker 28S D3 was sequenced successfully from 47 specimens, COI from 20 specimens, and ITSS from 19 specimens (the markers sequenced per specimens varied). These results are in line with other studies reporting the low success of PCR amplification for various mite genera (Young et al. 2012; Stålstedt et al. 2013; Bowman and Hoy 2012; Dos Santos and Tixier 2017), and this might be due to, for example, primer incompatibility, differences in sample preservation conditions, DNA extraction methods, or PCR protocols.

In this work, with the extra datasets the study of COI enabled the phylogenetic analysis of a total of 72 *Phthiracarus* sequences. The COI sequences were 658 bp in length, of which 330 were variable sites. The phylogeny of COI revealed five clear clades, indicating rather high identification success for five species (*P. laevigatus, P. globosus, P. ferrugineus*, *P. boresetosus, and P. nitens*). Interestingly, the sequences of *P. boresetosus* from Finland matched the Canadian specimens almost perfectly (1.6% intraspecific variance), indicating high identification success for the species. Four nominal species (*P. longulus, P. clavatus*, *P. crinitus,* and *P. bryobius*) in the phylogenetic analysis were split into two haplotypes,

which may indicate the presence of cryptic species or misidentification. Interestingly, the Canadian specimens of *P. borealis* matched the Finnish specimens of *P. crinitus 1* almost perfectly (0.4% intraspecific variance), highlighting the need for re-identification of these specimens. Moreover, the COI phylogeny enabled the detection of misidentifications and also the identification of a few difficult specimens that were not identified prior to sequencing. Overall, the interspecific variance between clades in the COI data varied between 3.8–30.7% (mean: 24.2%), while the intraspecific variation was between 0–6.2% (mean: 1.7%).

For *Phthiracarus*, the ribosomal 28S showed a limited genetic variation (19 variable characters within a 317 bp sequence). The modest amount of variation is in line with the findings of Kreipe et al. (2015), showing that this marker was not able to discriminate between *Steganacarus* species. In contrast, the same marker was recently proposed as a suitable species identification marker for all oribatids (Lehmiz and Decker 2017), something our data does not support. Despite the great length variance, the ITSS showed moderate variation (64 variable character within 385 bp sequences) and was also usable in species discrimination. Overall, the multiloci phylogeny conducted showed higher support values for clades than COI used alone, hence both markers, 28S and ITSS, provided supportive information for the evolutionary analysis confirming species statuses and indicating cryptic species.

To conclude, with the high amount of variable characters, the COI, which has been selected as the species identification marker in DNA barcoding protocol (Hebert et al. 2003), was also relatively suitable for oribatid DNA discrimination, but the analyses were improved with the additional markers 28S and ITSS that provided higher support values for the species.

3.6 Oribatid identification (I, II, IV, V)

In the course of this thesis, altogether 117 oribatid species with the taxa Suctobelbidae were identified from red wood ant nests (I, II, IV). Accompanied with four species identified from the soil in study I and two species identified in study V, this thesis provided records accounting for a total of circa 125 oribatid taxa. The sum makes up a substantial proportion (35%) of the known 357 Finnish oribatid species (Rassi et al. 2010; R. Penttinen, unpublished data). Interestingly, as reported in study I, the inventory revealed four species that were previously undiscovered in Finland: *Mesoplophora pulchra* Sellnick 1928, *Pergalumna willmanni* Zachvatkin 1953, *Suctobelba regia* Moritz 1970, and *S. aliena* Moritz 1970.

However, in the course of this thesis several misidentifications were detected, demonstrating the difficulties in the inference of the intra- and interspecific morphological

variation of species. The errors are as follows: In study I, all specimens of the genus *Ceratozetes* Shaldybina 1966 are identified to *C. gracilis* (Michael 1884), but the group most likely contains altogether three species, because in study II *C. thienemanni* Willmann 1943 and *C. sellnicki* Rajski 1958 were also identified from the same locality. Also in study I, both *Galumna elimata* (C.L. Koch 1941) and *G. lanceata* Oudemans 1900 were identified, but in study II these were determined as *C. dimorpha* Krivoluckaja 1952, representing both sexes with wide sexual dimorphism. In studies I–II and IV, specimens of *Carabodes ornata* Storkan 1925 most likely also contains the morphologically very similar *C. marginata* (Michael 1884) since it was later found from the same localities in thorough analysis of that particular genus (R. Elo, unpublished data).

Additionally, in study I, *Phthiracarus* should have been marked as *Phthiracarus* spp., but all specimens were erroneously assigned as *P. longulus*. This group most likely contains altogether the same six species as in study II; also, *P. crinitus*, *P. boresetosus, P. bryobius, P. globosus,* and *P. nitens* were identified from the same locality. Some greater variation with possible cryptic species was moreover detected in the thorough analysis of the *Phthiracarus* with DNA methods in study V.

These misidentifications highlight the difficulties of oribatid studies and the great need for fast, precise, and convenient identification tools for which DNA taxonomy may provide an efficient tool in the future. Moreover, the time-consuming task of identifying all the circa 50,000 specimens (I, II, IV, V) led to a situation in which I needed to exclude the juveniles, but with DNA taxonomy these could have been matched to adults and thus could have provided accurate knowledge about the oribatid diversity in soil ecosystems.

4. CONCLUSIONS

This thesis demonstrates that along with soils and some special microhabitats of forest, red wood ant nest mounds in boreal forests are also inhabited by abundant and species rich oribatid fauna (I, II, IV). Moreover, the results of this thesis demonstrate that the oribatid assemblages vary even within a small geographic scale as the community composition of oribatids differed, for example, between ant mounds and soil (I), among the three parts of ant mounds (II), and between forest types even within an island (the island Ruissalo) (I).

This knowledge adds information about oribatids among other ant-associated fauna (Laakso and Setälä 1998; Robinson and Robinson 2013), highlighting the value of red wood ants in maintaining the arthropod diversity in forests. These special environments, biodiversity hotspots, should be taken into consideration in land use planning, conservation activities, and forest management and utilization.

In Finland the mound-building *Formica* ants still build large, dense, and long-lived nest mounds and are considered viable species. Therefore, unlike in many other countries where *Formica* ants are classified as near-threatened species, the ant studies in Finland do not require any special permits. This aspect makes studies conducted in Finland of high value and the results are usable for conservation and forest management decisions in other countries too.

The slow process of identifying the almost 50,000 oribatid specimens, accompanied with the difficult species identification demonstrated by the list of misidentifications and the cryptic speciation (V), highlight the need for the development of DNA-based taxonomy. The faster, more precise, and cost-effective tools that are already used in various other animal groups would enhance oribatid studies in the future.

For the development of a usable DNA database for oribatid species, we need knowledge of suitable molecular markers that would trustworthily discriminate between species. For oribatid discrimination the nuclear marker 28S has been proposed, but our results (V) showed that, similarly to *Steganacarus* species (Kreipe et al., 2015), this marker could not discriminate the *Phthiracarus* species due to the low sequence variation. In contrast, the COI that is commonly used in DNA barcoding showed great value in species discrimination, with 3.8–30.7% divergence between species. But the amplification success of that marker for oribatids, at least for some genera, seems to be low, hampering its use in oribatid identification. Also, other applicable markers , such as ITSS, would provide usable information for species delineation, but amplified weakly. Therefore, the development of PCR protocols, and specifically oribatid-specific primers and additional molecular markers, should be the focus of future studies.

With a comprehensive species sequence database, which is in slow development due the scarcity of oribatid taxonomists, the oribatids could be taken into account in a meta-analysis, such as in the metabarcoding of soil organisms: these investigations are already being accomplished without knowledge about oribatids (Arribas et al. 2016; Wu et al 2011).

These metabarcoding approaches could also be used in faster environmental inventories, such as in my study about the effect of forest clear felling on oribatid mites (IV). Although, the species richness was lower in clear fell mounds, I did not detect clear changes in oribatid community composition. However, this study was made only once and hence investigated only the short-term effects of clear felling. More research is needed to uncover the long-term effects of clear felling on oribatid communities, which could in the future be done with metabarcoding approaches in order to save time in studies. Moreover, I only investigated these effects on adult oribatids, but there might be underlying effects—for example, effects on the reproduction and development of oribatids—and with DNA taxonomy these juvenile stages of species could be taken into account.

Overall this thesis added highly valued species data and knowledge about Finnish oribatid species that are considered a poorly known animal group. This thesis provided records of circa 35% of the Finnish species with four new species that were discovered from ant nests. These results show that discovering the hidden diversity of oribatids by surveying poorly documented microhabitats and cryptic speciation, the oribatid species richness may be higher than previously known, both locally and globally.

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REFERENCES

Abbott DT, Seastedt TR, Crossley DA (1980) Abundance, Distribution, and Effects of Clearcutting on Cryptostigmata in the Southern Appalachians.Environmental Entomology 9:618–623.

AntWeb (2018). Available from https://www.antweb.org. Accessed 25 October 2018.

Aoki J, Takaku G, Ito F (1994) Aribatidae, a new myrmecophilous oribatid mite family from Java. International Journal of Acarology 20:3–10.

Arribas P, Andújar C, Hopkins K, Shepherd M, Vogler AP (2016) Metabarcoding and mitochondrial metagenomics of endogean arthropods to unveil the mesofauna of the soil. Methods in Ecology and Evolution 7:1071–1081.

Arroyo J, O'Grady A, Vance H, Bolger T (2015) The mite (Acari: Oribatida, Mesostigmata) assemblages associated with *Lasius flavus* (Hymenoptera: Formicidae) nests and surrounding soil in an Irish grassland. Biology and Environment: Proceedings of the Royal Irish Academy 115B:1.

Baranovska A (2007) A Checklist of Latvian Oribatida. Latvijas entomologs, 44:5–10.

Behan-Pelletier VM (1999) Oribatid mite biodiversity in agroecosystems: role for bioindication. Agriculture, Ecosystems & Environment 74:411–423.

Behan-Pelletier VM (2015) Sexual dimorphism in *Autogneta*, with description of three new species from North America and new diagnosis of the genus (Acari, Oribatida, Autognetidae). Zootaxa 3946:55–78.

Ben-David T, Melamed S, Gerson U, Morin S (2007) ITS2 sequences as barcodes for identifying and analyzing spider mites (Acari: Tetranychidae). Experimental and Applied Acarology 41:169–181.

Berghoff SM, Wurst E, Ebermann E, Sendova-Franks AB, Rettenmeyer CW, Franks NR (2009) Symbionts of societies that fission: mites as guests or parasites of army ants. Ecological Entomology 34:684–695.

Bird GA, Chatarpaul L (1986) Effect of whole-tree and conventional forest harvest on soil microarthropods. Canadian Journal of Zoology 64:1986–1993.

Blagoev GA, deWaard JR, Ratnasingham S, deWaard SL, Lu L, Robertson J, Telfer AC, Hebert PDN (2016) Untangling taxonomy: a DNA barcode reference library for Canadian spiders. Molecular Ecology Resources 16:325–341.

Bowman HM, Hoy MA (2012) Molecular discrimination of phytoseiids associated with the red palm mite *Raoiella indica* (Acari: Tenuipalpidae) from Mauritius and South Florida. Experimental and Applied Acarology 57:395–407.

Bukva V, Daniel M, Mrciak M (1976) Oribatid mites (Acari, Oribatei) in the nests of small mammals in the Western Carpathians. Vestn Cesk Spol Zool. 40:240–254.

Campbell KU, Klompen H, Crist TO (2013) The diversity and host specificity of mites associated with ants: the roles of ecological and life history traits of ant hosts. Insectes Sociaux 60:31–41.

Casgrain P, Legendre P (2001) The R Package for multivariate and spatial analysis, version 4.0 Département de sciences biologiques, Université de Montréal, Montreal

Castresana J (2000) Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis. Molecular Biology and Evolution 17:540–552.

Chown SL, Nicolson S (2004) Insect physiological ecology: mechanisms and patterns. Oxford University Press. New York City, New York.

Collingwood CA (1979) The Formicidae (Hymenoptera) of Fennoscandia and Denmark. Fauna Entomologica Scandinavica, 8:1–175.

Constantinescu I, Ivan O, Călugăr A, Markó B (2011) Mite Fauna of Ant Nests - Comparative Study of Mite Fauna in the Argeş River Basin (South Romania). Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa" 54: 327–342.

Cushing PE (1997) Myrmecomorphy and myrmecophily in spiders: a review. The Florida Entomologist 80:165–193.

Dabert M, Witalinski W, Kazmierski A, Olszanowski Z, Dabert J (2010) Molecular phylogeny of acariform mites (Acari, Arachnida): Strong conflict between phylogenetic signal and long-branch attraction artifacts. Molecular Phylogenetics and Evolution 56:222–241.

Déchêne AD, Buddle CM (2009) Effects of experimental forest harvesting on oribatid mite biodiversity. Forest Ecology and Management 258:1331–1341.

DeSalle R, Egan MG, Siddall M (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. Philosophical Transactions of the Royal Society B: Biological Sciences 360:1905–1916.

Domes K, Althammer M, Norton RA, Scheu S, Maraun M (2007) The phylogenetic relationship between Astigmata and Oribatida (Acari) as indicated by molecular markers. Experimental and Applied Acarology 42:159–171.

Domisch T, Finér L, Neuvonen S, Niemelä P, Risch AC, Kilpeläinen J, Ohashi M Jurgensen MF (2009) Foraging activity and dietary spectrum of wood ants (*Formica rufa* group) and their role in nutrient fluxes in boreal forests. Ecological Entomology 34:369–377.

Eickwort GC (1990) Associations of mites with social insects. Annual review of entomology 35:469–488.

Frouz J (2000) The effect of nest moisture on daily temperature regime in the nests of *Formica polyctena* wood ants. Insectes Sociaux 47:229–235.

Frouz J, Finer L (2007) Diurnal and seasonal fluctuations in wood ant (*Formica polyctena*) nest temperature in two geographically distant populations along a south – north gradient. Insectes Sociaux 54:251–259.

Giller PS (1996) The diversity of soil communities, the "poor man's tropical rainforest". Biodiversity and Conservation 5:135–168.

Hågvar (1998) Mites (Acari) developing inside decomposing spruce needles: biology and effect on decomposition rate. Pedobiologia, 42:358–377.

Hamilton HC, Strickland MS, Wickings K, Bradford MA, Fierer N (2009) Surveying soil faunal communities using a direct molecular approach. Soil Biology and Biochemistry 41:1311–1314.

Hansen RA, Coleman DC (1998). Litter complexity and composition are determinants of the diversity and species composition of oribatid mites (Acari: oribatida) in litterbags. Applied Soil Ecology 9:17–23.

Härkönen SK, Sorvari J (2014) Species richness of associates of ants in the nests of red wood ant *Formica polyctena* (Hymenoptera, Formicidae). Insect Conservation and Diversity 7:485–495.

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270:313–321.

Hebert PDN, Gregory TR (2005) The Promise of DNA Barcoding for Taxonomy. Systematic Biology 54:852–859.

Heethoff M, Domes K, Laumann M, Maraun M, Norton RA, Scheu S (2007) High genetic divergences indicate ancient separation of parthenogenetic lineages of the oribatid mite *Platynothrus peltifer* (Acari, Oribatida). Journal of Evolutionary Biology 20:392–402.

Heethoff M, Laumann M, Weigmann G, Raspotnig G (2011) Integrative taxonomy: Combining morphological, molecular and chemical data for species delineation in the parthenogenetic *Trhypochthonius tectorum* complex (Acari, Oribatida, Trhypochthoniidae). Frontiers in Zoology 8:2.

Heethoff M, Norton RA, Scheu S, Maraun M (2009) Parthenogenesis in Oribatid Mites (Acari, Oribatida): Evolution Without Sex. In: Schön I, Martens K, Dijk P (eds) Lost Sex. Springer Netherlands, Dordrecht, 241–257.

Hölldobler B, Wilson EO (1990) The ants. The Belknap Press of Harvard University Press Cambridge, Massachusetts.

Huemer P, Mutanen M, Sefc KM, Hebert PDN (2014) Testing DNA Barcode Performance in 1000 Species of European Lepidoptera: Large Geographic Distances Have Small Genetic Impacts. PLoS ONE 9:e115774. .

Huhta V, Nurminen M, Valpas A (1969) Further notes on the effect of silvicultural practices upon the fauna of coniferous forest soil. Annales Zoologici Fennici 6:327–334.

Huhta V, Siira-Pietikäinen A, Penttinen R, Räty M (2011) Soil fauna of Finland: acarina, collembola and enchytraeidae. Memoranda Societas pro Fauna et Flora Fennica 86:59–82.

Ito F, Takaku G (1994) Obligate myrmecophily in an oribatid mite: Novel symbiont of ants in the oriental tropics. Naturwissenschaften 81:180–182.

Jílková V, Frouz J (2014) Contribution of ant and microbial respiration to CO2 emission from wood ant (*Formica polyctena*) nests. European Journal of Soil Biology 60:44–48.

Kagainis U (2014) A morphometrical study of oribatid mites (Acari: Oribatida)

of the genus Carabodes C.L. Koch, 1835 (Carabodidae) using a confocal laser scanning microscope: an alternative approach to quantitative analysis of various features. Zoomorphology 133:227–236.

Katoh K, Rozewicki J, Yamada KD (2017) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics bbx108.

Kilpeläinen J, Punttila P, Finér L, Niemelä P, Domisch T, Jurgensen MF, Neuvonen S, Ohashi M, Risch AC, Sundström L (2008) Distribution of ant species and mounds (*Formica*) in different-aged managed spruce stands in eastern Finland. Journal of Applied Entomology 132:315–325.

Kistner DH (1982) The social insects' bestiary. Social insects 3:1–244.

Kreipe V, Corral-Hernández E, Scheu S, Schaefer I, Maraun M (2015) Phylogeny and species delineation in European species of the genus *Steganacarus* (Acari, Oribatida) using mitochondrial and nuclear markers. Experimental and Applied Acarology 66:173–186.

Krisper G, Schatz H, Schuster R (2017) Oribatida (Arachnida: Acari). Checklists of the Austrian Fauna, No. 9. Biosystematics and Ecology Series No. 33.

Kumar S, Stecher G, Tamura K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution, 33:1870–1874.

Laakso J, Setälä H (1998) Composition and trophic structure of detrital food web in ant nest mounds of *Formica aquilonia* and in the surrounding forest soil. Oikos 81:266–278.

Laakso J, Setälä H (1997) Nest mounds of red wood ants (*Formicaaquilonia*): hot spots for litter-dwelling earthworms. Oecologia 111:565–569.

Legendre P, Legendre L (1998) Numerical ecology. 2nd. Elsevier Science, Amsterdam, NL.

Lehmitz R, Decker P (2017) The nuclear 28S gene fragment D3 as species marker in oribatid mites (Acari, Oribatida) from German peatlands. Experimental and Applied Acarology 71:259–276.

Lehtinen PT (1987) Association of uropodid, prodinychid, polyaspidid, antennophorid, sejid, microgynid, and zerconid mites with ants. Entomologisk tidskrift 108:13–20.

Lienhard A, Schäffer S, Krisper G, Sturmbauer C (2014) Reverse evolution and cryptic diversity in putative sister families of the Oribatida (Acari). Journal of Zoological Systematics and Evolutionary Research 52:86–93.

Lindberg N, Engtsson JB, Persson T (2002) Effects of experimental irrigation and drought on the composition and diversity of soil fauna in a coniferous stand: Long-term drought affects soil animal diversity. Journal of Applied Ecology 39:924–936.

Lindo Z, Visser S (2004) Forest floor microarthropod abundance and oribatid mite (Acari: Oribatida) composition following partial and clear-cut harvesting in the mixedwood boreal forest. Canadian Journal of Forest Research 34:998–1006.

Lindo Z, Winchester N (2008) Scale dependent diversity patterns in arboreal and terrestrial oribatid mite (Acari:

Oribatida) communities. Ecography 31:53–60.

Littell RC, Milliken GA, Stroup WW., Wolfinger RD,Schabenberger, O. (2006) SAS® for Mixed Models (second ed.)SAS institute Inc., Cary, IL, USA (2006).

Lóšková J, Ľuptáčik P, Miklisová D, Kováč Ľ (2013) The effect of clear-cutting and wildfire on soil Oribatida (Acari) in windthrown stands of the High Tatra Mountains (Slovakia). European Journal of Soil Biology 55:131–138.

Lv J, Wu S, Zhang Y, Chen Y, Feng C, Yuan X, Jia G, Deng J, Wang C, Wang Q, Mei L, Lin X (2014) Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). Parasites & Vectors 7:93.

Mahunka S, Mahunka-Papp L (2000) Checklist of the oribatid mites of Hungary (Acari: Oribatida). Folia entomologica hungarica, 61:27–54.

Maraun M, Heethoff M, Schneider K, Scheu S, Weigmann G, Cianciolo J, Thomas RH, Norton RA (2004) Molecular phylogeny of oribatid mites (Oribatida, Acari): evidence for multiple radiations of parthenogenetic lineages. Experimental and Applied Acarology 33:183–201.

Maraun M, Heethoff M, Scheu S, Norton RA, Weigmann G, Thomas RH (2003) Radiation in sexual and parthenogenetic oribatid mites (Oribatida, Acari) as indicated by genetic divergence of closely related species. Experimental & applied acarology 29:265–277.

Maraun M, Schatz H, Scheu S (2007) Awesome or ordinary? Global diversity patterns of oribatid mites. Ecography 30:209–216.

Maraun M, Scheu S (2000) The structure of oribatid mite communities (Acari, Oribatida): patterns, mechanisms and implications for future research. Ecography 23:374–383.

Meier R, Shiyang K, Vaidya G, Ng PKL (2006) DNA Barcoding and Taxonomy in Diptera: A Tale of High Intraspecific Variability and Low Identification Success. Systematic Biology 55:715–728.

Niedbała W, Liu D (2018) Catalogue of ptyctimous mites (Acari, Oribatida) of the world. Zootaxa 4393:1–238.

Niemi R, Karppinen E, Uusitalo M (1997) Catalogue of the Oribatida (Acari) of Finland. Acta Zoologica Fennica 207:1–39.

Norton RA (1980) Observations on phoresy by oribatid mites (Acari: Oribatei). International Journal of Acarology 6:121–130.

Norton RA, Bonamo PM, Grierson JD, Shear WA (1988) Oribatid mite fossils from a terrestrial Devonian deposit near Gilboa, New York. Journal of Paleontology 62:259–269.

Orgiazzi A, Dunbar MB, Panagos P, de Groot GA, Lemanceau P (2015) Soil biodiversity and DNA barcodes: opportunities and challenges. Soil Biology and Biochemistry 80:244–250.

Päivinen J, Ahlroth P, Kaitala V (2002) Ant-associated beetles of Fennoscandia and Denmark. Entomologica Fennica 13:20–40.

Päivinen J, Ahlroth P, Kaitala V, Suhonen J (2004) Species richness, abundance and distribution of

myrmecophilous beetles in nests of *Formica aquilonia* ants. Annales Zoologici Fennici 41:447–454.

Parmentier T, Dekoninck W, Wenseleers T (2014) A highly diverse microcosm in a hostile world: a review on the associates of red wood ants (*Formica rufa* group). Insectes Sociaux 61:229–237.

Pentinsaari M, Hebert PDN, Mutanen M (2014) Barcoding Beetles: A Regional Survey of 1872 Species Reveals High Identification Success and Unusually Deep Interspecific Divergences. PLoS ONE 9:e108651.

Pérez-Ìñigo (1993) Acari, Oribatei, Pronota. En: Fauna Ibèrica vol 3. Ramos, M.A et al. (Eds.). Museo Nacional de Ciancias Naturales. CSIC. Madrid.

Pérez-Ìñigo (1997) Acari, Oribatei, Gymnota I. En: Fauna Ibèrica vol 9. Ramos, M.A et al. (Eds.). Museo Nacional de Ciancias Naturales. CSIC. Madrid.

Pfingstl T, Baumann J (2017) Morphological diversification among island populations of intertidal mites (Acari, Oribatida, Fortuyniidae) from the Galápagos archipelago. Experimental and Applied Acarology 72:115–131.

Rambaut A (2009) FigTree, ver. 1.3.1. [Online]. http://tree.bio.ed.ac.uk/software/figtree

Rassi P, Hyvärinen E, Juslén A, Mannerkoski I (2010) The 2010 Red List of Finnish Species, Ministry of the Envi ronment and Finnish Environment Institute, Helsinki, Finland. (In Finnish with English summary).

Reichle (1977) The role of soil invertebrates in nutrient cycling. Ecological Bulletins 25:145–156.

Rettenmeyer CW, Rettenmeyer ME, Joseph J, Berghoff SM (2011) The largest animal association centered on one species: the army ant Eciton burchellii and its more than 300 associates. Insectes Sociaux 58:281–292.

Robinson NA, Robinson EJH (2013) Myrmecophiles and other invertebrate nest associates of the red wood ant *Formica rufa* (Hymenoptera: Formicidae) in north-west England. British Journal of Entomology and Natural History 26:67–88.

Ros VID, Breeuwer JAJ (2007) Spider mite (Acari: Tetranychidae) mitochondrial COI phylogeny reviewed: host plant relationships, phylogeography, reproductive parasites and barcoding. Experimental and Applied Acarology 42:239–262.

Rosenberger M, Maraun M, Scheu S, Schaefer I (2013) Pre- and post-glacial diversifications shape genetic complexity of soil-living microarthropod species. Pedobiologia 56:79–87.

Schaefer I, Norton RA, Scheu S, Maraun M (2010) Arthropod colonization of land – Linking molecules and fossils in oribatid mites (Acari, Oribatida). Molecular Phylogenetics and Evolution 57:113–121.

Schäffer S, Koblmüller S, Pfingstl T, Sturmbauer C, Krisper G (2010a) Contrasting mitochondrial DNA diversity estimates in Austrian *Scutovertex minutus* and *S. sculptus* (Acari, Oribatida, Brachypylina, Scutoverticidae). Pedobiologia 53:203–211.

Schäffer S, Pfingstl T, Koblmüller S, Winkler KA, Sturmbauer C, Krisper G (2010b) Phylogenetic analysis of European *Scutovertex* mites (Acari,

Oribatida, Scutoverticidae) reveals paraphyly and cryptic diversity: A molecular genetic and morphological approach. Molecular Phylogenetics and Evolution 55:677–688.

Schatz H, Behan-Pelletier V (2008) Global diversity of oribatids (Oribatida: Acari: Arachnida). Hydrobiologia 595:323–328.

Schneider K, Renker C, Scheu S, Maraun M (2004a) Feeding biology of oribatid mites: a minireview. Phytophaga 14:247–256.

Schneider K, Migge S, Norton RA, Scheu S, Langel R, Reineking A, Maraun M (2004b) Trophic niche differentiation in soil microarthropods (Oribatida, Acari): evidence from stable isotope ratios (15N/14N). Soil Biology and Biochemistry 36:1769–1774.

Seifert B (2007) Die Ameisen Mittel-und Nordeuropas. Lutra Verlags-und Vertriebsgesellschaft Tauer Germany.

Shakhab SV (2006) Oribatid mites (Oribatei, Acariformes) in nests of passerine birds. Entomological Review 86:173–176.

Siira-Pietikäinen A, Penttinen R, Huhta V (2008) Oribatid mites (Acari: Oribatida) in boreal forest floor and decaying wood. Pedobiologia 52:111–118.

Skubała P, Kafel A (2004) Oribatid mite communities and metal bioaccumulation in oribatid species (Acari, Oribatida) along the heavy metal gradient in forest ecosystems. Environmental Pollution 132:51–60.

Sorvari (2016) Wood ants: threats, conservation and management. In: Stockan JA, Robinson EJH (Eds.), Wood Ant Ecology and Conservation, Cambridge University Press, Cambridge, UK.

Sorvari J, Hakkarainen H (2004) Habitat-related aggressive behaviour between neighbouring colonies of the polydomous wood ant *Formica aquilonia*. Animal Behaviour 67:151–153.

Stamatakis A, Ludwig T, Meier H (2005) RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics 21:456–463.

Stålstedt J, Bergsten J, Ronquist F (2013) "Forms" of water mites (Acari: Hydrachnidia): intraspecific variation or valid species? Ecology and Evolution 3:3415–3435.

Stockan and Robinson (2016) Wood ant ecology and conservation. Cambridge University Press.

Stoev P, Lapeva-Gjonova A (2005) Myriapods from ant nests in Bulgaria (Chilopoda, Diplopoda) Peckiana 4:131–142.

Subías LS (2004; updated 2018) Listado sistematico, sinonimico y biogeografico de los acaros oribatidos (Acariformes: Oribatida) del mundo (1758-2002). Graellsia 60:3–305 [Updated 2018] :<http://escalera.bio.ucm.es/usuarios/bba/cont/docs/RO_1.pdf> 25th October 2018.

Subías and Arillo (2001) Acari, Oribatei, Gymnota II. En: Fauna Ibèrica vol 15. Ramos, M.A et al. (Eds.). Museo Nacional de Ciancias Naturales. CSIC. Madrid.

Subías LS, Shtanchaeva UY, Arillo A (2013) Oribátidos (Acari, Oribatida) de España peninsular e islas Baleares. Distribution. Monografías electrónicas SEA, 5.

Taylor AR, Ranius T (2014) Tree hollows harbour a specialised oribatid mite fauna. Journal of Insect Conservation 18:39–55.

Taylor AR, Schröter D, Pflug A, Wolters V (2004) Response of different decomposer communities to the manipulation of moisture availability: potential effects of changing precipitation patterns. Global Change Biology 10:1313–1324.

Trewick SA (2008) DNA Barcoding is not enough: mismatch of taxonomy and genealogy in New Zealand grasshoppers (Orthoptera: Acrididae). Cladistics 24:240–254.

Uppstrom KA (2010) Mites (Acari) associated with the ants (Formicidae) of Ohio and the harvester ant, *Messor pergandei*, of Arizona. Master Thesis. The Ohio State University.

Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27:171–180.

Vicente dos Santos V, Tixier M-S (2017) Which molecular markers for assessing which taxonomic level? The case study of the mite family Phytoseiidae (Acari: Mesostigmata). Cladistics 33:251–267.

Walter DE Proctor HC (2013) Mites: Ecology, Evolution & Behaviour Springer, Dordrecht.

Weigmann (2006) Hornmilben (Oribatida). Die Tierwelt Deutschlands, 76. Teil. Goecke and Evers, Keltern, Germany.

Whitworth T., Dawson R., Magalon H, Baudry E (2007) DNA barcoding cannot reliably identify species of the blowfly genus Protocalliphora (Diptera: Calliphoridae). Proceedings of the Royal Society B: Biological Sciences 274:1731–1739.

Wu T, Ayres E, Bardgett RD, Wall DH, Garey JR (2011) Molecular study of worldwide distribution and diversity of soil animals. Proceedings of the National Academy of Sciences 108:17720–17725.

Wuorenrinne H (1994) Some interesting features of Finnish wood ant mounds. Memorabilia Zoologica 48:261–265.

Yao H, Song J, Liu C, Luo K, Han J, Li Y, Pang X, Xu H, Zhu Y, Xiao P, Chen S (2010) Use of ITS2 Region as the Universal DNA Barcode for Plants and Animals. PLoS ONE 5:e13102.

Young MR, Behan-Pelletier VM, Hebert PDN (2012) Revealing the Hyperdiverse Mite Fauna of Subarctic Canada through DNA Barcoding. PLoS ONE 7:e48755.

Zhang RL, Zhang B (2014) Prospects of using DNA barcoding for species identification and evaluation of the accuracy of sequence databases for ticks (Acari: Ixodida). Ticks and Tick-borne Diseases 5:352–358.