University of Reading

Parasitellus fucorum: A beneficial mite for bumblebees.

A thesis submitted by

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

It is known that bumblebees (*Bombus*) have a wide variety of different mite (Acari) species associated with them, however research into the nature and effects of these relationships is scarce. For many species our understanding of their biology and life cycles are incomplete, and our understanding of the effects that most bumblebee-associated mites have on their hosts is likewise inadequate. There have been many studies conducted on a small number of bumblebee associated mite species, such as the obligate endoparasite *Locustacarus buchneri*, but information on the majority of mite species is limited, and much of the information available is outdated due to changes in the ecological context of British bumblebees in the intervening time (the extinction of several species, introduction of a 'new' species (*Bombus hypnorum*), fragmentation of habitats, and potential importation of foreign mites).

One species of particular interest is *Parasitellus fucorum*. *P. fucorum* is frequently suggested in previous literature to be a potential mutualist with the bumblebees it associates with, based on the hypothesis that the predatory actions of *P. fucorum* in bumblebee colonies will result in a net benefit to colony fitness, outweighing any negative effects from the kleptoparasitic instars of *P. fucorum*'s life cycle. However, no empirical testing has previously taken place to evaluate this hypothesis. *P. fucorum* is a relatively large, highly mobile and predatory mite species. All species within the genus *Parasitellus* are obligate associates of bumblebees in the Holarctic region, with broadly similar ecology.

In order to address this issue, we aimed to determine what effects *Parasitellus fucorum* association had on bumblebees at the individual or the colony level. To achieve this we tested the ability of *P. fucorum* to predate various bumblebee pests and parasites including greater wax moth (*Galleria mellonella*) eggs and bumblebee wax moth (*Aphomia sociella*) eggs and 1st instar larvae, and found that *P. fucorum* deutonymphs were capable of predating all three. It was also shown that *P. fucorum* predates *Tyrophagus putrescentiae* mites, a fungivorous pest species commonly found within bumblebee colonies. This work represents the first evidence of these predatory relationships. A field trial was conducted to test the impact of *P. fucorum* inoculums on commercial bumblebee colony development, the results of which showed that the presence of a *P. fucorum* population within bumblebee colonies leads to a 165% greater colony mass increase, a 12% relative increase in syrup consumption and a 119% higher number of workers on average compared with controls. This is the first empirical evidence of benefits to bumblebee fitness resulting from association with *P. fucorum*. These experiments required large numbers of *P. fucorum* mites, so a suitable method for rearing *P. fucorum* in laboratory conditions was developed. It was also shown during this project that phoretic *P. fucorum* numbers on spring queens are inversely correlated with the likelihood of the queen being infested by the parasitic nematode *Sphaerularia bombi*. A survey of phoretic mite groups and trends in their associations with bumblebee queens in Reading was conducted in order to better inform the project with up-to-date information on local mites, which showed that all mites groups except *Parasitellus* varied significantly in abundance

between different bumblebee species, and that the most common local bumblebee associated mites were *Kuzinia* spp., *Scutacarus* spp., *Pneumolaelaps* spp., and *Parasitellus fucorum*.

From the results of this project it has been shown that the presence of *Parasitellus fucorum* within bumblebee colonies is beneficial to their development, likely due to the predatory activity of *P. fucorum* within the colony upon bumblebee pests and parasites. Our findings will be useful to pollinator researchers and acarologists, and may be of interest for future efforts in bumblebee conservation.

Acknowledgements

I express my thanks to my supervisors Dr. Alejandra Perotti and Prof. Dave Goulson for their help and guidance throughout my PhD project. Dr. Perotti's knowledge of acarology and Prof. Goulson's understanding of bumblebee ecology were invaluable throughout my research, and I would not be where I am today without their contributions to this work.

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Chapter 1 Introduction

1.1 Bumblebees and their associates

Bumblebees are insects of the genus *Bombus* (Order Hymenoptera), widely known for their pollination activity and their distinct large and furry appearance amongst flying insects (Alford, 1975). Bumblebees are abundant throughout much of the temperate Northern Hemisphere and South America. There are over 250 different species globally, of which 27 are currently found in the UK (Goulson, 2010). This figure includes the recently established species *Bombus hypnorum* Linnaeus, 1758, which was first recorded in Britain in 2001 and has since spread throughout the UK up to central Scotland (Goulson and Williams, 2000, Edwards, 2009). The number of British species may increase to 28 dependent upon *Bombus subterraneus*, which was declared extinct in the UK in the year 2000 but is subject to ongoing reintroduction efforts (Gammans, 2019).

Bumblebees are important pollinators, particularly for certain species of flowering plants such as tomatoes which benefit from 'buzz pollination'. Buzz pollination is essentially the vibration of the anthers by a pollinating insect to release pollen grains, a service only provided by bumblebees and some solitary bees (Buchmann, 1983, van den Eijnde et al., 1991, King, 1993, Vallejo-Marín, 2018). The pollination service provided by bumblebees and other insect pollinators is estimated to be worth £139 billion annually (Gallai et al., 2009), and the value of the ecosystems in which bumblebees are an essential component is certainly higher. Despite their obvious importance, bumblebee numbers are in decline globally as a result of pesticide usage, habitat loss and imported pests and parasites (Goulson et al., 2015).

Bumblebees are social insects which form communal colonies based around a single reproductive female (the Queen) who typically gives birth to all other bumblebees within the colony, which consist mostly of females (Workers) and some males (Drones) (Goulson, 2010). The numbers of bumblebees within colonies varies over time, but can reach upwards of 400 in *Bombus terrestris* (Linnaeus, 1758) colonies (Alford, 1975). In the earliest stages of the colony development foraging is performed solely by the queen, until the first group of workers emerge and assume this responsibility. Bumblebee workers will regularly leave the colony to forage for pollen and nectar, both of which are collected from flowering plants within a wide radius (up to 1-2 km in some species) of the colony and are brought back to the colony to place within wax pots as part of the colony's food store (Osborne et al., 2008). The bumblebees within the colony use wax secreted from their abdomens to build up the colony as its population increases, regulate the colony's internal temperature at around 30°C (or 24°C (Chapter 3)) using their wing muscles, and defend the colony against threats (Alford, 1975, Goulson, 2010). Despite these communal efforts wild bumblebee colonies are thought to have a very high failure rate, with Cumber (1953) noting that of 80 *Bombus pascuorum* (Scopoli, 1763) colonies he observed during development only 29% produced new queens. This facet of bumblebee ecology likely contributes to the

devastating effects that habitat fragmentation has wrought on certain species (Potts et al., 2010, Lepais et al., 2010).

Bumblebee colonies can provide a richly rewarding environment for many organisms aside from bumblebees themselves. As anyone who has dissected a bumblebee colony can attest, there are a surprisingly large variety of organisms which do so, and many take full advantage of the abundant food supplies and regulated conditions within. These include detrivorous beetles (Coleoptera) such as *Antherophagus nigricornis* (Fabricius, 1787), which has been observed clinging to bumblebees for phoretic travel (Frisch, 1952). Other common colony inhabitants include various flies (Diptera) e.g. *Fannia canicularis* (Linnaeus, 1761) (the lesser house fly) which can be detrivorous or, in the case of conopid flies such as *Physocephala rufipes* (Fabricius, 1781), directly parasitic (Alford, 1975). Cumber (1949) found that 12% of bumblebee workers near London were parasitized by conopid flies. Other flies such as the bumblebee mimic *Volucella bombylans* (Linnaeus, 1758) (a hoverfly) commonly lay their eggs within bumblebee colonies, where the larvae proceed to feed upon nest debris and other detritus towards the base of the colony (Hasselrot, 1960). Moths (Lepidoptera) including the parasitic species *Aphomia sociella* (Linnaeus, 1758) (the bumblebee wax moth) also commonly invade bumblebee colonies (Alford, 1975, Kindl et al., 2012). The greater wax moth *Galleria mellonella* (Linnaeus, 1758) is also sometimes found within bumblebee colonies, and is similar to *A. sociella* in its mode of parasitism (Miyamoto, 1957). These parasitic moths are discussed in detail in Chapter 4 and Appendix I. Other species like the house moth *Endrosis sarcitrella* (Linnaeus, 1758) are thought to be fairly harmless, merely laying eggs within the colony so its detrivorous larvae can feed upon nest debris. Bumblebee colonies are also subject to invasion by other members of the order Hymenoptera such as the braconid wasp *Syntretus splendidus* (Marshall 1887), an endoparasite of adult bumblebees. The adult female of this species attacks bumblebee queens or workers and lays eggs within the thorax, with developed larvae later emerging through the abdominal wall, resulting in the death of the parasitized bee (Alford, 1975). Ants such as *Lasius niger* (Linnaeus, 1758) and *Myrmica rubra* (Linnaeus, 1758) are also commonly found within bumblebee colonies, particularly in the case of commercial colonies (pers. obs.) or artificial nest-boxes, in order to feed on brood and stored foodstuffs (Sladen, 1912). They are not thought to pose a serious threat to mature colonies however, and ants will not attack healthy adult bumblebees (Alford, 1975). Predatory earwigs (*Forficula auricularia* Linnaeus, 1758, Order Dermaptera) are also a common sight within bumblebee colonies, where they feed on developing brood (Holm, 1960, Alford, 1975). Bumblebee colonies also attract a wide variety of mite species.

1.2 Bumblebee mites

Though research into bumblebee ecology has been increasing in recent years, little is known about the nature of relationships between bumblebees and the many mites that associate with them (Eickwort, 1994). This chapter examines prior research on bumblebee associated mites, particularly in European populations, identifying knowledge gaps and summarising the current knowledge within this field.

Mites (Acari) are small or microscopic arachnids belonging to the subphylum Chelicerata. Bumblebee associated mites can be broadly defined as any mite which lives part (or all) of its life cycle in close association with bumblebees and may have developed adaptations to this lifestyle (Eickwort, 1994). This covers a wide range of mites, from species which become phoretic upon bumblebees and inhabit their colonies (e.g. *Parasitellus fucorum* de Geer, 1778) through to obligate endoparasites of bumblebees which live inside bumblebee tracheae and air sacs for most of their life-cycle, e.g. *Locustacarus buchneri* (Stammer 1951) (Schousboe, 1986, Eickwort, 1994, Otterstatter et al., 2004). The mite species associated with bumblebees can be broadly divided into 3 taxonomic groupings. These comprise members of the Cohort Astigmata (Order Oribatida), the Suborder Heterostigmata and the Order Mesostigmata (Eickwort, 1994). The number of bumblebee associated mite species has been variously estimated between 54 and 110 depending on how strictly 'bumblebee association' is defined (Eickwort, 1994, O'Connor and Klimov, 2011).

One common feature used to determine bumblebee association in mite species is phoresis upon bumblebees (Eickwort, 1994). Phoresy is defined as one organism using another simply for transportation (Houck and OConnor, 1991), and many mite species utilise the far greater mobility of bumblebees by using them as transport into and between bumblebee colonies (Schwarz and Huck, 1997). The phoretic instar in astigmatid mites (phoretic deutonymph) is highly specialised for phoresy or dispersal, with a characteristic sucker plate or attachment organ in a posterior/ventral location on the body (Houck and OConnor, 1991, Eickwort, 1994, OConnor, 2009). In the Suborder Heterostigmata and the Order Mesostigmata the phoretic instar will typically grip bumblebee setae using its pretarsal claws or chelicerae in order to adhere to its host (Houck and OConnor, 1991, Eickwort, 1994). The phoretic instar in many mite species is the deutonymph, though this can vary e.g. in *Pneumolaelaps* it is typically adults that are found phoretic upon bumblebees (Hunter and Husband, 1973, Krantz and Walter, 2009, OConnor and Klimov, 2012c).

Bumblebee associated mites are not particularly host-specific beyond the genus level, and associate with most if not all bumblebees within their geographical range (Eickwort, 1994). There are many possible evolutionary reasons for this, one being that in rich yet ephemeral habitats like bumblebee colonies crowding is likely to occur rapidly, so dispersal between these habitats would clearly benefit phoretic mites (Houck and OConnor, 1991). Another tangible benefit is the avoidance of inbreeding depression by keeping genetic variation high through the horizontal transmission of mites between bumblebee colonies (whether intra- or inter-species). This horizontal transmission has been shown previously in a variety of pathogens associated with bumblebees (Durrer and Schmid-Hempel, 1994), and horizontal transmission of mites between bumblebees has been observed in some mite species (*Parasitellus fucorum*, *Parasitellus ignotus* (Vitzthum, 1930), *Hypoaspis hyatti* Evans & Till, 1966 and *Scutacarus acarorum* Goeze, 1780) in controlled laboratory experiments (Schwarz and Huck, 1997).

1.2.1 Cohort Astigmata

Bumblebee associated mites within Cohort Astigmata, characterised by their lack of stigmata or tracheal systems, largely belong to the diverse Acaridae family (Eickwort, 1994, Krantz and Walter, 2009). The principal genera found in association with bumblebees are *Kuzinia* and *Tyrophagus* (both family Acaridae). Both *Kuzinia* spp. and *Tyrophagus* spp. are often found within bumblebee colonies (Eickwort, 1994, Rożej et al., 2012).

Mites of the genus *Kuzinia* are ubiquitous bumblebee kleptoparasites, and can frequently become the most abundant mites in bumblebee colonies (Goldblatt and Fell, 1984, Eickwort, 1994). There are 6 species in this genus known to associate with bumblebees, the most commonly found being *Kuzinia laevis* (Dujardin, 1849) (in Europe) and *K. americana* Delfinado and Baker 1976 (in the USA) (Delfinado & Baker, 1976). There is well-founded dispute over the validity of species distinctions within the genus *Kuzinia*, as acarologists have examined *K. laevis*, *K. americana* and *K. affinis* Delfinado and Baker 1976 samples and found no morphological differences significant enough to indicate definite speciation (Klimov et al., 2016e).

K. laevis is known to have six stages in its life cycle; egg, larva, protonymph, deutonymph, tritonymph and adult (Figure 1.1) (Zamec, 2014). The deutonymphal instar is specialised for phoresy and preferentially becomes phoretic upon bumblebee queens (Zamec, 2014, Chmielewski and Baker, 2008). In *K. laevis* the appearance of deutonymphs is facultative; protonymphs will only moult into this instar if the host bumblebee colony is coming to the end of its lifespan (i.e. a dying bumblebee colony), in a healthy colony protonymphs will moult into tritonymphs instead. These biological characteristics are not well researched for the other mites of this genus.

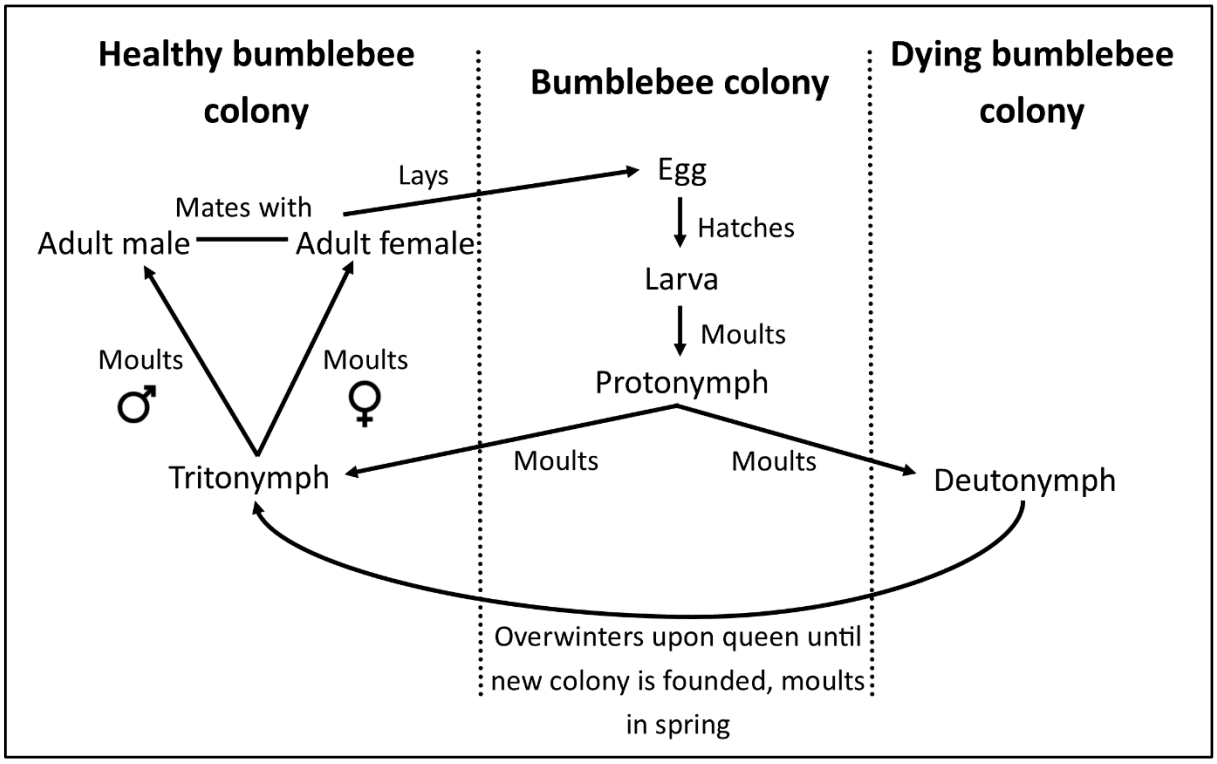

Figure 1.1 – Summary of *Kuzinia laevis* life-cycle (Zamec, 2014). A 'dying' bumblebee colony is a colony nearing the end of its lifespan, with gynes and drones produced by the queen (if still alive) rather than workers. A 'healthy' bumblebee colony is still developing, producing workers, and has a healthy queen. The general 'bumblebee colony' section in the centre refers to a colony in either state of health.

Tyrophagus spp. are well-known cosmopolitan fungivores (Fan and Zhang, 2007, Canfield and Wrenn, 2010). *Tyrophagus putrescentiae* (Schrank, 1781) is not a specific bumblebee associate and can be found in a wide variety of different environments (Kheradmand et al., 2007). However it is frequently found in bumblebee colonies where the mite is known to feed upon fungi growing on detritus and stored pollen (Rożej et al., 2012, Maggi et al., 2011). The life cycle of *T. putrescentiae* is well characterised and has only 4 stages; egg, larva, protonymph and tritonymph (Kheradmand et al., 2007). Unusually amongst the Acaridae, most mite species of the genus *Tyrophagus* do not have a phoretic deutonymphal instar (with the notable exception of *Tyrophagus formicetorum* Volgin, 1948) and instead disperse incidentally as feeding stages (OConnor, 1982, Houck and OConnor, 1991, Perotti, 2009, Revainera et al., 2019).

1.2.2 Suborder Heterostigmata

Mites of the Suborder Heterostigmata (within the Order Trombidiformes) are defined by the presence of paired stigmata without associated peritremes (Krantz and Walter, 2009). This group includes the endoparasite *Locustacarus buchneri* (Family Podapolipidae), and *Scutacarus acarorum* (Family Scutacaridae) (Husband and Sinha, 1970, Schousboe, 1986, Otterstatter et al., 2004).

Locustacarus buchneri is an obligate bumblebee-specific endoparasitic mite (Husband and Sinha, 1970, Eickwort, 1994). The species has 3 instars in its life cycle; egg, larviform females, and adult males and females (Figure 1.2) (Husband and Sinha, 1970). *Locustacarus buchneri's* life cycle has become highly specialised towards internally parasitizing bumblebees and the species has lost life stages seen in other bumblebee associated mites, for instance *L. buchneri* has no phoretic instar (Husband and Sinha, 1970).

Only adult males and larviform females are mobile. Males hatch in their adult form, and mate with larviform females that have hatched within the same bumblebee. The males die soon afterwards within their host bumblebee (Husband and Sinha, 1970, Yoneda et al., 2008a). Females may spend the rest of their lives developing in the same bee, or they may migrate to infest another bumblebee within the colony (Eickwort, 1994, Yoneda et al., 2008a). After this occurs the mites will infest the tracheae and begin to feed on their host's haemolymph by piercing the thin tracheal walls, triggering the female to moult into her sedentary adult form (Yoneda et al., 2008b).

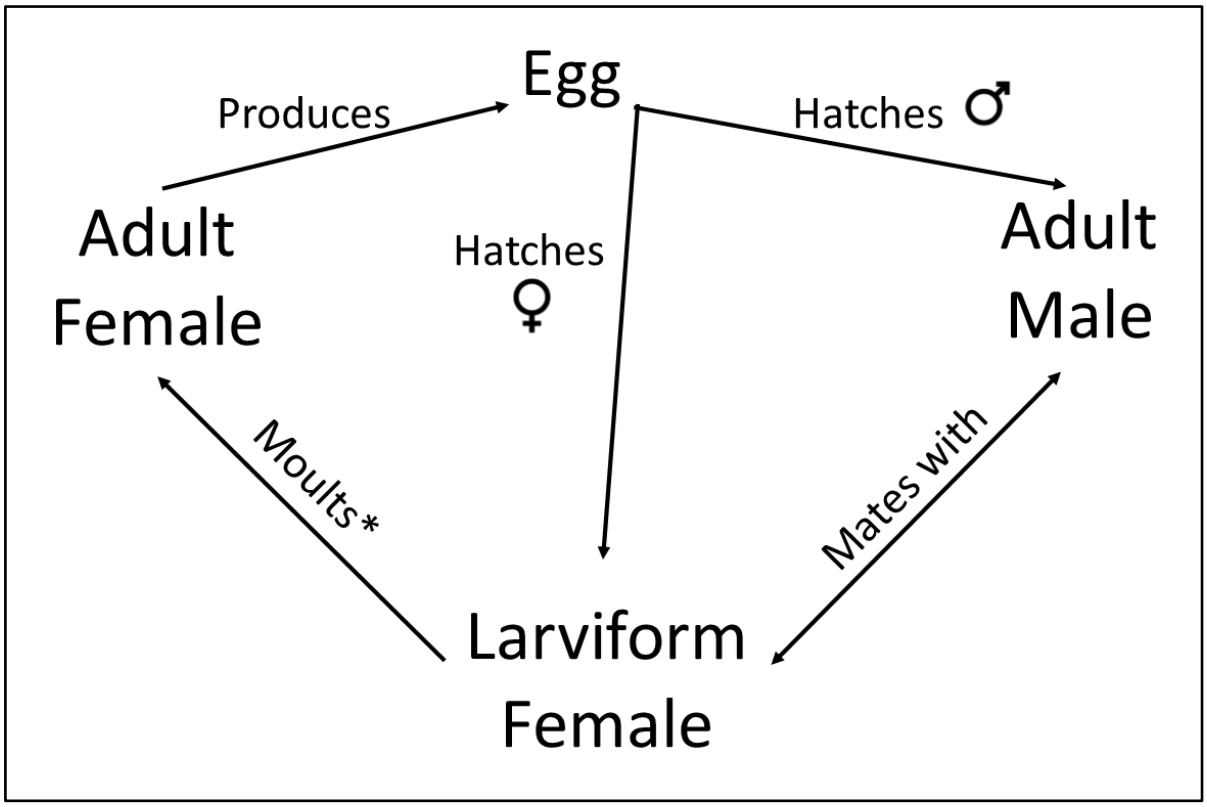

Figure 1.2 – Summary of *Locustacarus buchneri* life cycle. The moult marked by an asterisk (*) is triggered by the larviform female feeding upon the haemolymph of the bumblebee that becomes its host for the rest of the life cycle.

Scutacarus acarorum is a small fungivorous mite commonly found in bumblebee colonies (Chmielewski and Baker, 2008, Schousboe, 1986). *S. acarorum* has also been described as a facultative parasite of bumblebees, as it has been observed to feed directly on bumblebee haemolymph given the opportunity. However this claim by Maggi et al. (2011) is based on a single source (Chmielewski, 1971) and has not been reported since. Unfortunately the details of the *S. acarorum* life cycle are not well defined. *S. acarorum* become phoretic in larger numbers upon bumblebee queens towards the end of a colony cycle and overwinter phoretic upon these queens, suggesting adaptation towards association with bees (Chmielewski and Baker, 2008, Jagersbacher-Baumann, 2014). Due to its small size *S. acarorum* is often found phoretic upon larger mites e.g. *Parasitellus fucorum*, which are themselves phoretic upon bumblebees (Eickwort, 1994). This behaviour is called hyperphoresy, and is reminiscent of a phrase from the poet Jonathon Swift; "So, naturalists observe, a flea has smaller fleas that on him prey, and these have smaller still to bite 'em, and so proceed ad infinitum." (Swift, 1733, Perotti and Braig, 2009).

Previously *Scutacarus acarorum* was thought to be the only species within this genus to associate with bumblebees, however it has since been found that there are 4 similar *Scutacarus* species (*S. acarorum*, *S. deserticolus* Mahunka, 1969, *S. mendax* Karafiat, 1959 and *S. occultatus* Sevastianov, 1975) associated with bumblebees, collectively referred to as the *acarorum* species-complex (Eickwort, 1994, Jagersbacher-Baumann, 2014).

1.2.3 Order Mesostigmata

Mites of the Order Mesostigmata are characterised by a single pair of spiracles located laterally on either side of the body typically associated with long peritremes (i.e. grooves extending from stigmata) (Krantz and Walter, 2009, Koehler, 1999). The most common bumblebee associated genera within this order are *Parasitellus* (Family Parasitidae), *Pneumolaelaps* and *Hypoaspis* (both family Laelapidae), and *Macrocheles* (Family Macrochelidae) (Hunter and Husband, 1973, Richards and Richards, 1977, Royce and Krantz, 1989, Chmielewski and Baker, 2008). Mesostigmatid mites are typically the largest found in bumblebee colonies, and many are free-living predators and/or kleptoparasitic on bumblebee colony provisions (Richards, 1976, Eickwort, 1994).

The relationship between *Pneumolaelaps* and *Hypoaspis* has been the subject of some debate within the literature (Costa and Hunter, 1970, Hunter and Costa, 1971). *Pneumolaelaps* has been considered a subgenus of *Hypoaspis* (Costa, 1966), and as a genus in its own right (Hunter, 1966). For this work the classifications given by Hunter and Husband (1973) were followed, with *Pneumolaelaps* considered a genus of *Hypoaspis*-like mites with some distinctive characteristics (Hunter and Husband, 1973).

Pneumolaelaps and *Hypoaspis* mites are both known to feed both on pollen stores within the colony and to be predatory, although this may vary by species (Costa, 1966, Hunter and Husband, 1973). *H. hyatti* is said to be predatory upon *Tyrophagus* mites (Costa, 1966, Schwarz et al., 1996), and *H. marginepilosa* Sellnick, 1938 preys upon astigmatid mites in bumblebee colonies (Rożej et al., 2012). *Pneumolaelaps longanalis* Hunter and Husband, 1973 has been suggested as a kleptoparasite of stored pollen and honey (Hunter and Husband, 1973). Unfortunately the life cycles of these genera are not well known beyond their preference to become phoretic upon bumblebee queens rather than on other castes/sexes (Eickwort, 1994).

The genus *Macrocheles* (Family Macrochelidae) has two species known to associate with bumblebees; *Macrocheles praedafimetorum* Richards and Richards, 1977 and *M. rotundiscutis* Bregetova and Koroleva, 1960 (Richards and Richards, 1977, Klimov et al., 2016a). These mites are poorly known beyond being predatory associates of bumblebee colonies, though they are not specific to this environment (Richards and Richards, 1977). Both species depend upon beetles (such as the dung-inhabiting beetle *Onthophagus nuchicornis* (Linnaeus, 1758)) for phoretic transport into bumblebee colonies (Eickwort, 1994, Richards and Richards, 1977).

There are up to 18 described species in the genus *Parasitellus*, and all are obligate bumblebee associates with broadly similar biology (Eickwort, 1994, O'Connor and Klimov, 2011, Klimov et al., 2016d).

Parasitellus are large predatory/kleptoparasitic mites that live within bumblebee colonies (Hyatt, 1980). *Parasitellus fucorum* is the most common of these (Figure 1.3) (Schwarz et al., 1996, Chmielewski and Baker, 2008). *P. fucorum* has 5 stages in its life cycle; egg, larva, protonymph, deutonymph and adult (Figure 1.4). In the larval, protonymphal, and the adult male instar, this species is thought to feed upon astigmatid mites and other bumblebee pests (Koulianos and Schwarz, 1999, Schousboe, 1987). The adult female is thought to be kleptoparasitic, feeding primarily upon pollen stored by the bumblebees within the colony (Koulianos and Schwarz, 1999). Deutonymphs have been observed to feed via both kleptoparasitism and predation, and to engage in cannibalism (Koulianos and Schwarz, 1999). This variation of feeding strategies is not uncommon among bumblebee associated predatory mites (Evans and Till, 1966, Eickwort, 1994).

Figure 1.3 – Phoretic *Parasitellus fucorum* deutonymph on *Bombus terrestris* queen.

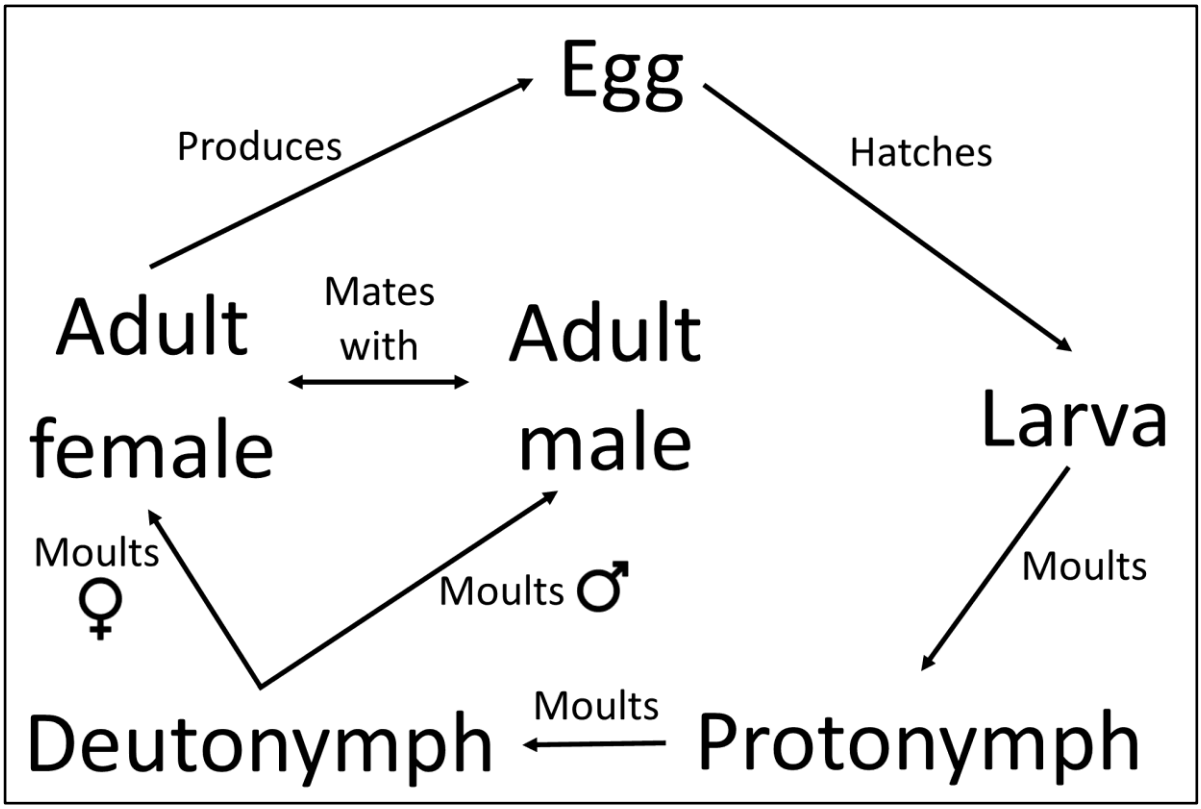

Figure 1.4 – Summary of *Parasitellus fucorum* life-cycle.

1.3 The 'known' effects of mites upon bumblebees

Much of the literature concerning bumblebee associated mites describes various observed associations without attempting to determine whether the presence of mites within the bumblebee colony effects the fitness of their hosts. Where possible effects on bumblebees are mentioned, they are typically assumed or implied from circumstantial evidence, with no empirical testing taking place to verify the claims (Allendes and Montalva, 2011, Maggi et al., 2011, Rożej et al., 2012). Even within the limited information that is available about these effects, there is significant debate.

Locustacarus buchneri is a bumblebee endoparasite. It is agreed that heavy infestations can cause lethargy, diarrhoea and cessation of foraging (Husband and Sinha, 1970, Goka et al., 2001, Yoneda et al., 2008b). The results of such infestations are thought to include shortened bumblebee lifespans, altered foraging behaviour, and (ultimately) a weakened colony (Otterstatter et al., 2004, Otterstatter et al., 2005, Allen et al., 2007, Plischuk et al., 2013). However others have stated that most infestations do not affect pollen collection or ovarian function in individual bees, and that the effects of such infestations at the colony level are minimal (Shykoff and Schmid-Hempel, 1991, Macfarlane et al., 1995, Otterstatter et al., 2005, Yoneda et al., 2008a). Rutrecht and Brown (2008) have even claimed that *L. buchneri* infestation may have a positive effect on bumblebee populations as a whole by the selective removal of weaker queens which would have "exhibited a negative effect of parasitism post-hibernation". It appears that the severity of effects from *L. buchneri* infestation are linked to the intensity of infestation at both the colony and individual bumblebee levels.

It has been suggested in the literature that infestations of predatory *Parasitellus fucorum* may benefit the fitness of bumblebee colonies due to the reduced abundance of kleptoparasitic astigmatid mites in the colony as a result of *Parasitellus*' predation (Eickwort, 1994, Schmid-Hempel, 1998, Koulianos and Schwarz, 1999, Chmielewski and Baker, 2008). An inverse correlation has been observed between the numbers of phoretic *Parasitellus* mites upon bumblebee queens and the prevalence of infestation by the bumblebee-parasitic nematode *Sphaerularia bombi* Dufour, 1837 (Schousboe, 1987). Some subsequent literature has interpreted this as evidence that *Parasitellus* mites are responsible for lowering *Sphaerularia* infestation levels, however there is no experimental evidence showing that this is actually the case (Eickwort, 1994, Schmid-Hempel, 1998).

Other predatory mites that are considered potentially beneficial towards their bumblebee hosts include *Macrocheles praedafimetorum*, which preys upon various pests and their eggs in bumblebee colonies (Richards and Richards, 1977). Other examples include various *Pneumolaelaps* and *Hypoaspis* species which are both kleptoparasitic and predatory (Eickwort, 1994, Schwarz et al., 1996). However (as with *Scutacarus acarorum*) some *Pneumolaelaps* species have been observed to feed on the haemolymph of injured bumblebees given the opportunity (Hunter and Husband, 1973).

These examples only represent a selection of known bumblebee associated mites. The effects of most bumblebee associated mites on their hosts are unknown, representing a significant knowledge gap.

1.4 Importance of understanding bumblebee-mite relationships

1.4.1 Pollinator decline

Global pollinator populations are in decline across the planet, with 11% of bumblebee species listed on the IUCN Red list (Williams and Osborne, 2009). This is due to a multitude of different factors including loss of habitat due to increasing arable field sizes and a reduction in wildflower meadows and hedgerows, increasing pesticide use (and misuse) and the effects of various introduced parasites and pathogens, including some mite species (Goka et al., 2006, Osborne et al., 2008, Goulson et al., 2015). Some bumblebee species, such as *Bombus franklini* (Frison, 1921) in North America and *B. dahlbomii* Guérin-Méneville, 1835 in South America, are already facing extinction (Thorp, 2005, Arbetman et al., 2012, Schmid-Hempel et al., 2014). Therefore gaining a detailed understanding of the effects resulting from bumblebee association with certain mite species to the detriment or benefit of bumblebee fitness is more important than ever.

1.4.2 Global spread of mites

Bumblebee associated mites have been introduced to countries around the world, largely by being phoretic upon or endoparasitic within bumblebees that were themselves introduced to different countries. The earliest recorded introductions were various European species being transported to New Zealand in 1885 and 1906. *Bombus terrestris*, *B. hortorum* (Linnaeus, 1761), *B. ruderatus* (Fabricius, 1775) and *B. subterraneus* (Linnaeus, 1758) became established along with the mites that were associated with them, including *Locustacarus buchneri* and *Kuzinia laevis* (Macfarlane and Gurr, 1995,

Macfarlane et al., 1995, Goulson, 2003, Allendes and Montalva, 2011). The effects of naturalised *Locustacarus buchneri* and *Kuzinia laevis* are not well known in this region however. *Bombus terrestris* (and phoretic *Kuzinia laevis*) from New Zealand subsequently invaded Tasmania by unknown means, and since 1992 both have spread rapidly across the island (Semmens et al., 1993, Buttermore, 1997). *K. laevis* has been found in far greater numbers in Tasmanian bumblebee colonies than anywhere else, but otherwise the effects of this introduction are unknown (Allen et al., 2007).

The European bumblebee species *Bombus ruderatus* and *B. terrestris* were introduced to Chile (in 1982 and 1998 respectively) for greenhouse tomato pollination (Arretz and Macfarlane, 1986, Ruz, 2002, Whittington et al., 2004, Kraus et al., 2010). The imported bumblebees quickly escaped and became naturalised in Chile, and have since spread to Argentina (Abrahamovich et al., 2001, Torretta et al., 2006, Maggi et al., 2011, Arbetman et al., 2012, Schmid-Hempel et al., 2014). In Chile the European mite *Kuzinia laevis* has been found upon naturalised populations of *B. terrestris* (Allendes and Montalva, 2011). Recently *Kuzinia* spp., *Pneumolaelaps longanalis*, *P. longipilus*, *Scutacarus acarorum* and *Tyrophagus putrescentiae* have all been found phoretic on native bumblebee species in Uruguay (Revainera et al., 2019). A study of museum specimens dated 1945-1986 from Argentina found 3 - 4 different species of *Kuzinia* (Including *K. laevis* and *K. americana*), *Pneumolaelaps longanalis*, *P. longipilus* Hunter, 1966, *Scutacarus acarorum* and *Tyrophagus putrescentiae* in association with native bumblebee species (Revainera et al., 2014). Given that *B. ruderatus* was only introduced into Chile in 1982, this suggests two possibilities: Either *K. laevis* and the other mite species have been misidentified in some cases, or our understanding of the bumblebee associated mite groups present in South America has been characterised incorrectly. Due to the lack of previous information regarding bumblebee associated mites in the region, further research will be needed to determine whether the native bumblebee species may be threatened by the spread of invasive mite species, or if these are actually native mite species that have long been present. To further confuse matters, *Locustacarus buchneri* has been found parasitizing native bumblebee species in Argentina, but has yet to be described in Chile (Plischuk et al., 2013). If it is European *L. buchneri* that has been discovered with these mites then it follows logically that *L. buchneri* must be present in Chile, however the mites described by Plischuk et al. (2013) may represent a new species or sub-species. As with *L. buchneri*'s introduction to New Zealand, the effects of this potential introduction have not yet been investigated.

European *Locustacarus buchneri* escaped from commercial colonies of *Bombus terrestris* that were imported from Europe into Japan during the 1990s. While it was predicted that this would occur as these mites has been discovered within commercial colonies, no action was taken until European *L. buchneri* had escaped alongside *Bombus terrestris* and had spread to native Japanese bumblebees (Goka et al., 2000, Goka et al., 2001, Goka et al., 2006). The impacts of feral *B. terrestris* became apparent, including competition for resources with native species and changes in seed sets of native flora (Nagamitsu et al., 2006, Kenta et al., 2007, Inoue et al., 2007). After this the Japanese government implemented strict conditions for commercially produced colonies of *B. terrestris* to limit damage to their ecosystem

(Tomoo, 2008). Commercial bumblebee producers also took preventative measures, and the results can be inferred by the absence of any mites in commercial bumblebee colonies imported into Japan in 2001, and by Rożej et al. (2012) who tested commercial colonies purchased from Israel, Denmark and the Netherlands in 2007-2009 and found no mites present in any of them (Goka et al., 2006). It has since been discovered that European *L. buchneri* infests 17% of commercial colonies of the native Japanese bumblebee *Bombus ignitus* (Smith, 1869) whereas the native Japanese genotype *L. buchneri* did not infest *B. ignitus* at all (Goka et al., 2001). European genotype *L. buchneri* was also found to infest natural populations of *Bombus hypocrita sapporoensis* Cockerell 1911 in the northern Japanese island of Hokkaido (Goka et al., 2006). It has been theorised that this invasion by foreign parasites may have severe consequences on native bumblebee species (Goka et al., 2006), as invasive foreign species including pests and parasites spread by human activities are thought to be one of the biggest threats to global biodiversity (Vitousek et al., 1997, Daszak et al., 2000, Meeus et al., 2011, Vanbergen and The Insect Pollinators Initiative, 2013).

While the invasion of *L. buchneri* in Japan is being well studied and monitored, this is an exceptional case. As has been shown, where European mite species have invaded different regions like South America or Australasia we know virtually nothing about how these mites may behave in these regions, or if they may have different effects on the bumblebees native to these regions compared with their natural host species. Part of this problem is that we also know very little about the effects of these mites on their natural hosts. It is therefore important that further research is conducted into the effects of bumblebee associated mites on bumblebee fitness, in order to better inform future bumblebee conservation efforts and future research into their effects in regions they have successfully invaded.

1.5 Research questions

The effects that most bumblebee associated mite species have on their hosts are unknown, or at best assumed. It is hypothesised that these mite species have a variety of effects on bumblebees, both beneficial and detrimental. Further knowledge of these effects, and in particular whether there are any mites that are beneficial towards bumblebees, will result in better informed conservation and control strategies in future. This thesis contains the first empirical studies of this research area, particularly focussing on the predatory mite *Parasitellus fucorum* and its impacts upon the bumblebees that play host to these mites. The reasons for this focus were that *P. fucorum* were locally abundant, easily identified, and the only mite species commonly theorised to benefit bumblebees within the literature. Within this thesis the following research questions were addressed:

- Which mite groups/species are associated with the local bumblebee species in England, and are there any trends within these associations based on bumblebee biology? (Chapter 2)
- Can *Parasitellus fucorum* be reared in laboratory conditions for use in experimentation? (Chapter 3)
- Can *Parasitellus fucorum* predate common bumblebee or honeybee pests? (Chapter 4)

- Does infestation with the parasitic nematode *Sphaerularia bombi* have a significant effect on mite association in bumblebee queens, and can *Parasitellus fucorum* protect queens against infestation? (Chapter 5)
- Do *Parasitellus fucorum* or *Tyrophagus putrescentiae* have a significant impact on the development of bumblebee colonies in field conditions? (Chapter 6)

Chapter 2 Associations between bumblebee queens and their phoretic mites

2.1 Abstract

One hundred and seventeen overwintered bumblebee (*Bombus*) queens of eight species were captured during April 2016 in Reading, England. These queens carried 16,363 phoretic mites in total. All mites were counted and identified to the genus or species level with the aim of both characterising the various mite taxa and examining trends in their associations with bumblebee queens. Mites were sorted into four groupings based on external morphology; *Parasitellus*, all 'other Mesostigmata', *Kuzinia* and *Scutacarus*. The vast majority of mites belonged to the *Kuzinia* group (95 %), while the *Parasitellus* (2 %), *Scutacarus* (2 %) and other Mesostigmata (2 %) groups had far lower abundancy. The most abundant mites found were (in order) *Kuzinia* spp. (15,576), *Scutacarus* spp. (273), *Pneumolaelaps* spp. (241) and *Parasitellus fucorum* (122).

All mite groupings (except for *Parasitellus*) were significantly more abundant upon some bumblebee species than others, suggesting discrimination by these mites. This was shown by every bumblebee species in a generalised linear model being assigned significant p-values when compared using total phoretic mite numbers as a predictor (all $p < 0.001$). *Scutacarus* numbers were significantly positively correlated with the numbers of both *Parasitellus* (0.615, $p < 0.001$) (correlation, p-value) and 'other Mesostigmata' (0.553, $p < 0.001$) phoretic on bumblebee queens. This is likely due to *Scutacarus acarorum* commonly becoming hyperphoretic on larger mites. *Parasitellus* and other Mesostigmata numbers were also strongly correlated (0.859, $p < 0.001$), suggesting that they are likely to inhabit the same bumblebee colonies.

This study provides an up to date snapshot of the varieties of mites found in association with different bumblebee species in England, and should be useful for informing current research on bumblebee-associated mites.

2.2 Introduction

Bumblebees and their colonies support a wide variety of different organisms. These include 110 different mite (Acari) species (according to Klimov et al. (2016g)), of which 54 are thought to be directly associated with bumblebees (Eickwort, 1994). These mite associates range from obligate endoparasites such as *Locustacarus buchneri* through to potentially beneficial predatory mites like *Parasitellus fucorum* and cosmopolitan detrivores and fungivores such as *Scutacarus acarorum* (Alford, 1975, Koulianos and Schwarz, 1999, Chmielewski and Baker, 2008). Bumblebee-mite associations are described frequently in older literature, however the last relevant studies of mites associated with bumblebees in the UK were conducted in the 1980s (Hyatt, 1980, Hyatt and Embersom, 1988). The ecological context of bumblebees in the UK has since changed due to extensive habitat loss, increased

pesticide usage, imported bumblebee colonies potentially bringing new mites into the UK and the arrival of a new bumblebee species (*Bombus hypnorum*) from the European mainland (Goulson and Williams, 2000, Potts et al., 2010, Graystock et al., 2013). An up-to-date study of wild bumblebee populations in the UK and their associated mites was therefore needed.

Bumblebee mites preferentially become phoretic upon bumblebee queens in order to overwinter with them in the hibernaculum and to be transported into the newly founded colony in the spring (Huck et al., 1998). This study examined the association of bumblebee species present in Reading, England with their phoretic mites. For the purposes of this study only overwintered queens were collected and examined. Bumblebee queens were caught during April 2016 in the Harris Garden, University of Reading. Queens were identified and their phoretic mites removed and identified to the genus or species level.

2.3 Methods

2.3.1 Bumblebee capture and mite removal

The capture of all 117 bumblebee queens took place between 1/04/16 - 28/04/16. Overwintered queens were identified by their characteristic flying pattern or their size (Alford, 1975). After capture all relevant information about each queen was recorded (date/time, location, preliminary species identification, etc.). The queen was then euthanized and stored in a 15ml tube (Corning, UK) filled with 70% ethanol so that the queen and any phoretic mites were fully submerged.

Each 15ml tube containing a bumblebee queen was agitated in a MultiBio RS-24 rotator-mixer for 30 minutes. After this the tube was vortexed for 30 seconds using a VWR lab dancer S42 and the bumblebee was removed and placed into a cavity dish. The bumblebee's thorax width was measured at the widest point using digital callipers (Tacklife, China) and any mites present were removed, separated into 4 morphological groupings (*Parasitellus*, other Mesostigmata, *Kuzinia* and *Scutacarus*) based on anatomical observations, and counted using a hand tally counter before being transferred to labelled Eppendorf tubes filled with 70% ethanol for later identification.

This process of agitating the bumblebee and removing, counting and storing the mites was repeated as necessary. The Eppendorfs containing all removed mites were stored for later identification work.

2.3.2 Mite clearing and mounting

Mites were prepared for mounting on microscope slides by first transferring the mites within each Eppendorf into others containing 50% lactic acid solution. Mites were left in this solution until they had 'cleared' i.e. had their internal structures dissolved by the lactic acid, allowing their external morphological feature to be clearly observed under a microscope (Dhooria, 2016). If necessary, Eppendorfs containing mites were placed in a heating block at 50°C to speed the clearing process.

Once cleared the mites were dehydrated in 70% then 96% ethanol for 15 minutes each. Mites were transferred to a microscope slide using a paintbrush. A drop of Hoyers liquid was then placed onto the mites and they were covered by a cover slip and sealed with Glyptal .

2.3.3 Identification of mite groups/species

Mites were separated into 4 morphological groups by examination of their external morphological characteristics using a Motic SMZ-171 microscope. Mites in the *Parasitellus* group were identified by their size, red-brown colouration and the distinctive triangular shape of their opisthonotal shield (Hyatt, 1980). All other mites with stigmata associated with peritremes were placed in the 'other Mesostigmata' group (Krantz and Walter, 2009). Mites in the *Kuzinia* group were distinguished primarily by their size and distinctive arrangement of body parts (4 anterior legs, 4 posterior, anal sucker, etc.) (Klimov et al., 2016e). Mites in the *Scutacarus* group were easily distinguished based on their unique size and shape among bumblebee associated mites (Khaustov, 2008). The < 0.1% of all mites collected which did not fit within these 4 groups were left uncategorised, and were not included in subsequent analyses.

Mounted mites were identified by close examination using a Nikon Optiphot microscope with 10x, 40x and 100x objective lenses. Morphological characteristics of the mites were used in combination with dichotomous identification keys contained in the many works of reference for this field (Evans and Till, 1966, Hyatt, 1980, Baker et al., 1999, Fan and Zhang, 2007, Krantz and Walter, 2009, OConnor and Klimov, 2012c, Jagersbacher-Baumann, 2014, Klimov et al., 2016e). After these keys had been followed mites were compared against species descriptions as a confirmatory step if needed. In some cases the mites being identified did not feature in any identification keys. To identify these mites group/species descriptions alone were used, for instance Klimov et al. (2016e) was used to identify *Kuzinia* mites. Mites were identified to the species level where possible (e.g. *Parasitellus fucorum*) or otherwise to their genus (e.g. *Pneumolaelaps* spp.). For each species/group of mite identified an ID report was prepared (Appendix E).

2.3.4 Statistical analysis

The data collected and analysed consisted of a database of 117 bumblebees captured including the following factors; bumblebee species, thorax width, date captured, and the numbers of phoretic mites within the *Parasitellus*, other Mesostigmata, *Kuzinia* and *Scutacarus* morphological groupings found upon each bee.

A Generalised Linear Model (GLM) was created in SAS v9.4 using the GENMOD procedure (SAS Institute Inc., 2013). This model used total phoretic mite numbers present upon captured bumblebees as the dependant variable. Thorax width (held at the overall average for the dataset (7.684 mm)), bumblebee species, date of capture, and the interaction of bumblebee species and date of capture were used as predictors. This model structure was found to be the most parsimonious based on the AIC. All bumblebee species represented by under 5 queens within the dataset were grouped for analysis and labelled as "other". Maximum likelihood estimates were obtained for each parameter and adjusted sum

of square tests were used to determine overall p-values for each predictor following the chi-square method.

All other statistical analyses were performed using R v3.4.2. and Rstudio (RStudio Team, 2015, R Core Team, 2017). Most figures were produced using the ggplot2 package, the correlation matrix was created using the Hmisc package and the correlogram was produced using the corrplot package (Wickham, 2016, Wei and Simko, 2017, Harrell Jr and Dupont, 2019). A multinomial logistic regression model was estimated using the nnet package, relative risk ratios were calculated for regression coefficients, and 2-tailed z-tests were conducted to produce p-values for each coefficient (Venables and Ripley, 2002). The model used bumblebee species as the response variable and *Parasitellus*, Mesostigmata, *Kuzinia*, *Scutacarus*, capture date and thorax width as the predictors. *Bombus terrestris* was used as the baseline to which all other species were compared. All bumblebee species where $n < 5$ were grouped for analysis and labelled as "other" or "other *Bombus*".

Kolmogorov-Smirnov tests were used to determine whether data were normally distributed. Where data were not normal non-parametric tests were used. Spearman's correlations were used to construct a correlation matrix. The GLM, correlation matrix and multinomial model outputs were used to determine which factors in the dataset were related (e.g. bumblebee species and phoretic *Kuzinia* numbers). All correlated factors were explored, with a particular focus on variation in phoretic mite numbers between the captured bumblebee species.

2.4 Results

Among the 117 bumblebees captured there were 70 *Bombus terrestris*, 6 *B. hortorum*, 5 *B. hypnorum*, 17 *B. lapidarius* (Linnaeus, 1758), 5 *B. lucorum* (Linnaeus, 1761), 3 *B. pascuorum*, 2 *B. pratorum* (Linnaeus, 1761), and 9 *B. vestalis* Geoffroy, 1785. To test the relationships between all factors in the dataset a Spearman's correlation matrix was produced (Appendix A). The significant correlations are listed in Table 2.1.

Row	Column	Correlation	p-value
Date captured	Thorax width	0.357	< 0.001
Species	Thorax width	0.434	< 0.001
Parasitellus	Mesostigmata	0.859	< 0.001
Date captured	*Kuzinia*	0.196	0.035
Species	*Kuzinia*	0.204	0.028
Thorax width	*Kuzinia*	0.274	0.003
Parasitellus	*Scutacarus*	0.615	< 0.001
Mesostigmata	*Scutacarus*	0.553	< 0.001

Table 2.1 – Table of Spearman's correlation values comparing factors within the results collected for each bumblebee queen captured, including p-values. Only significant ($p < 0.05$) correlations are shown in this table. Date captured, Thorax width and Species refer to characteristics of the captured bumblebee queens. Full table including non-significant correlations shown in Appendix A. The 'Species' factor was coerced into numeric format (in alphabetical order) for the purposes of these correlations.

The results of a multinomial logistic regression comparing all other bumblebee species with *Bombus terrestris* showed that 'other *Bombus'* (-12.037, $p < 0.001$) (coefficient, p-value) and *B. vestalis* (-2.432, $p = 0.031$) both differed significantly from *B. terrestris* in numbers of phoretic *Parasitellus* mites. 'Other *Bombus'* also differed significantly in phoretic *Scutacarus* numbers (0.531, $p = 0.022$). *B. vestalis* (-0.029, $p = 0.03$) and *B. lapidarius* (-0.014, $p = 0.018$) both had significantly different prevalences of phoretic *Kuzinia* mites compared to *B. terrestris*. *B. hortorum* (0.692, $p = 0.016$) and *B. vestalis* (0.389, $p = 0.023$) differed significantly from *B. terrestris* in dates of capture. Finally, every species tested within the model (including 'other *Bombus'*) differed significantly from *B. terrestris* in thorax width (varied coefficients, all $p < 0.005$). The model's residual deviance was 147.791, and the AIC was optimised at 231.791. Full model outputs are shown in Appendix B.

The results of a GLM which used total phoretic mite numbers as the dependant variable showed that thorax width, date of capture, bumblebee species, and the interaction between date of capture and bumblebee species were all significant predictors of total phoretic mite numbers on bumblebee queens (all $p < 0.001$). These results are shown in Table 2.2, and full model outputs are displayed in Appendix C. The deviance of this model was 10752.66 and the AIC was optimised at 11460.284.

Source	DF	Chi-Square	p-value
Thorax width	1	25.38	**< .0001**
Date	13	1678.12	**< .0001**
Species	6	2308.4	**< .0001**
Date*Species	23	3208.02	**< .0001**

Table 2.2 - Results of adjusted sum of squares test on GLM in which total phoretic mite numbers present upon bumblebee queens was the dependant variable. Thorax width, bumblebee species, date of capture, and the interaction of bumblebee species and date of capture were used as predictors. Significant p-values are shown in bold. p-values were calculated following the chi-square method (Pr > ChiSq).

Bumblebee species	Mite grouping	Prevalence (%)	Mean phoretic mites	Standard deviation	p - value
B. terrestris	*Parasitellus*	56	2.557	8.64	
B. hortorum	*Parasitellus*	33	3.833	5.947	
B. hypnorum	*Parasitellus*	20	0.2	0.447	
B. lapidarius	*Parasitellus*	59	1.941	2.925	
B. lucorum	*Parasitellus*	60	2	2.549	0.582
B. pascuorum	*Parasitellus*	67	0.667	0.577	
B. pratorum	*Parasitellus*	0*	0*	0*	
B. vestalis	*Parasitellus*	56	0.778	0.972	
B. terrestris	Other Mesostigmata	60	3.014	9.478	
B. hortorum	Other Mesostigmata	33	0.5	0.837	
B. hypnorum	Other Mesostigmata	60	1.6	2.074	
B. lapidarius	Other Mesostigmata	47	0.706	0.92	
B. lucorum	Other Mesostigmata	40	2.6	5.27	**0.041**
B. pascuorum	Other Mesostigmata	0*	0*	0*	
B. pratorum	Other Mesostigmata	0*	0*	0*	
B. vestalis	Other Mesostigmata	11	0.111	0.333	
B. terrestris	*Kuzinia*	93	192.6	203.746	
B. hortorum	*Kuzinia*	33	3.333	6.408	
B. hypnorum	*Kuzinia*	60	232	419.273	
B. lapidarius	*Kuzinia*	71	27.35	102.246	
B. lucorum	*Kuzinia*	80	77.966	23.722	**<0.001**
B. pascuorum	*Kuzinia*	100	12.33	4.163	
B. pratorum	*Kuzinia*	0*	0*	0*	
B. vestalis	*Kuzinia*	89	20	34.489	
B. terrestris	*Scutacarus*	30	2.614	9.577	
B. hortorum	*Scutacarus*	33	0.5	0.837	
B. hypnorum	*Scutacarus*	0*	0*	0*	
B. lapidarius	*Scutacarus*	65	3.588	6.032	
B. lucorum	*Scutacarus*	40	0.6	0.894	**0.005**
B. pascuorum	*Scutacarus*	100	7.333	10.116	
B. pratorum	*Scutacarus*	0*	0*	0*	
B. vestalis	*Scutacarus*	11	0.111	0.333	

Table 2.3 – Percentage prevalence, mean numbers and standard deviations of phoretic mites from 4 morphological groupings found on bumblebee queens of different species. Prevalence was defined as the percentage of bumblebees which carried at least 1 phoretic mite. Where no mites were found, results are marked by an asterisk (*). The p-values quoted in the final column show the results of Kruskal-Wallis tests used to determine whether differences in phoretic mite numbers between bumblebee species were statistically significant. Significant p-values (< 0.05) are shown in bold.

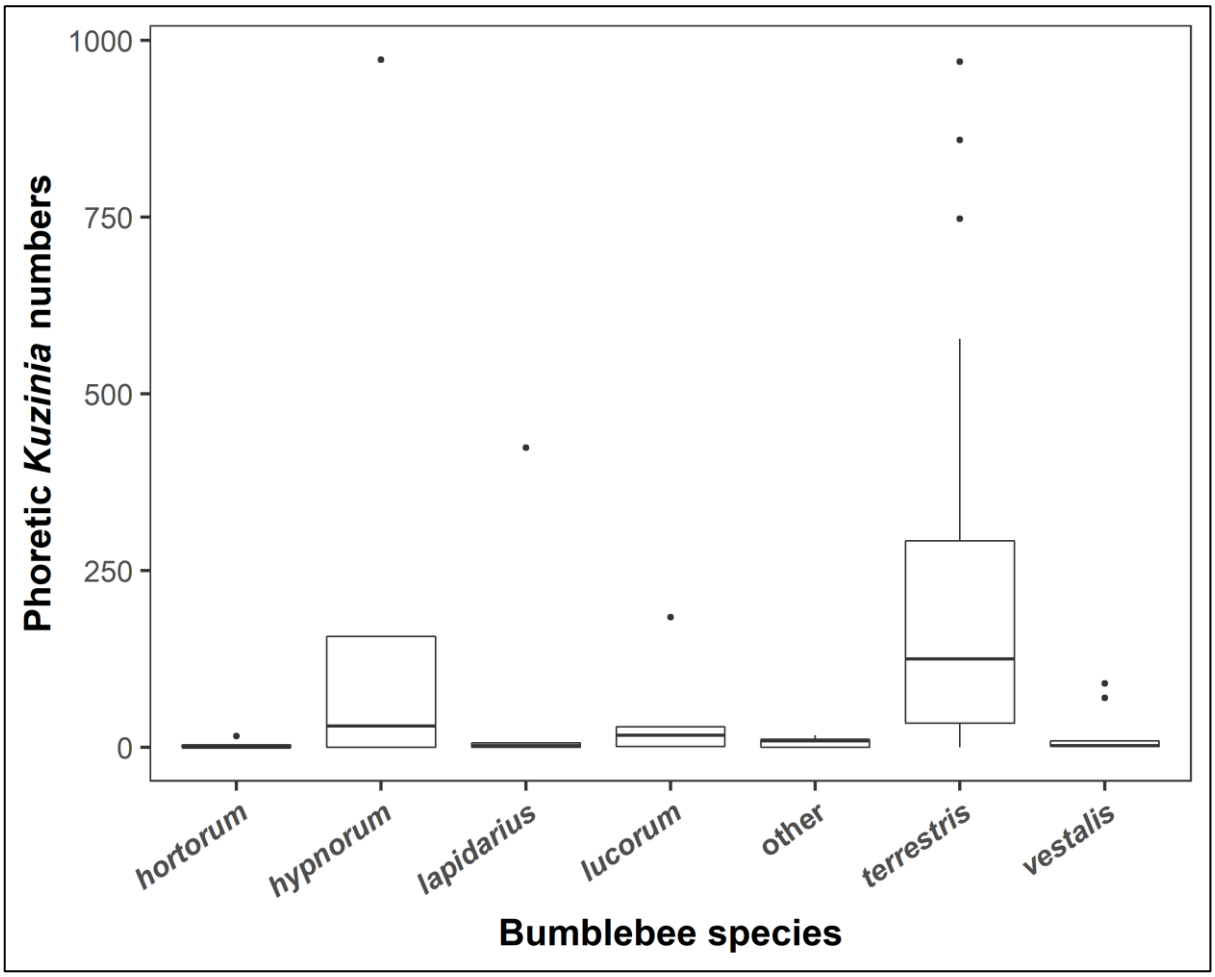

Figure 2.1 – Boxplots displaying numbers of phoretic mites from the genus *Kuzinia* found on bumblebee queens of all species collected. Boxplots are in the Tukey style, where the thick horizontal bar shows the median value, the 'box' extends to the 25th and 75th percentiles, and the 'whiskers' extend vertically to all data within 1.5x the Inter-Quartile Range (IQR) of the median. Any outlying results are denoted by black dots.

Among the 255 mites within the *Parasitellus* group, *P. fucorum* was the most common species (48 %), followed by *P. ignotus* (28 %), *P. talparum* (Oudemans, 1913) (22 %) and *P. crinitus* (Oudemans, 1903) (2 %). Within the 248 'Other Mesotigmata' collected there were two genera represented, the most common by far was the genus *Pneumolaelaps* which accounted for 97 % of all mites in this morphological grouping. The other genera found was *Proctolaelaps* (3 %). Within the 273 mites of the *Scutacarus* group, *S. acarorum* was the most common species (93 %), followed by *S. mendax* (4 %), *S. occulatus* (2 %), and *S. deserticolus* (1 %). A subsample (4 %) of the 15,576 mites within the *Kuzinia* morphological group were examined. This confirmed that all were indeed *Kuzinia* spp., and no further identification was conducted. The prevalence of phoretic mites from each morphological group and their average phoretic numbers for each bumblebee species are shown in Table 2.3 and Appendix D.

2.5 Discussion

All *Parasitellus* species are obligatory bumblebee associates with similar biology (Eickwort, 1994). The most commonly found mite species in the *Parasitellus* group was *Parasitellus fucorum* (Family Parasitidae, Order Mesostigmata). *P. fucorum* is a well-known associate of bumblebees, and the deutonymph is the phoretic instar which overwinters upon bumblebee queens (Huck et al., 1998).

Deutonymphs and adult females feed on stored pollen (specifically the pollenkitt surrounding pollen grains) within bumblebee colonies, while adult males, larvae, protonymphs and deutonymphs are predatory (Richards, 1976, Koulianos and Schwarz, 1999). *P. fucorum* has been suggested as a potentially beneficial mite associate for bumblebees due to their predatory activities within the colony (Schmid-Hempel, 1998, Koulianos and Schwarz, 1999, Rożej et al., 2012).

Within the 'other Mesostigmata' group the two most common mite genera found were *Pneumolaelaps* spp. (Family Laelapidae) and *Proctolaelaps* spp. (Family Ascidae). *Pneumolaelaps* mites are common kleptoparasites within bumblebee colonies, where they can often be found in abundance (OConnor and Klimov, 2012c). All *Pneumolaelaps* species are thought to be obligate bumblebee associates (Hunter and Husband, 1973). Adult females of this genera become phoretic upon bumblebee queens to overwinter (Royce and Krantz, 1989). *P. longanalis* has been observed to feed on the nectar/pollenkitt coating stored pollen in bumblebee colonies, and the mites congregate around brood cells where they can feed on the pollen brought to the developing brood by bumblebee workers (Royce and Krantz, 1989). *Proctolaelaps* is a less well-known genera than *Pneumolaelaps*. There are several species known to associate exclusively with bumblebee colonies but their life cycles and feeding behaviours are completely unknown, though some have suggested they may be kleptoparasites or predatory within bumblebee colonies (Alford, 1975, Eickwort, 1994). Adult females are known to travel phoretic upon bumblebee queens and to overwinter with them in the hibernaculum (Klimov et al., 2016b). Other generalist species within the *Proctolaelaps* genus will feed on fungi, microarthropods, and the eggs or larvae of larger insects, however the bumblebee associated species have notably different chelicera suggesting a possible difference in feeding habits (Klimov et al., 2016b). Whether the kleptoparasitism of these two mite groups has any negative effect on the bumblebee colonies they inhabit is not known.

Mites in the genus *Kuzinia* (Family Acaridae) are bumblebee associates, and frequently become the most abundant Acari within bumblebee colonies (Chmielewski, 1971, Eickwort, 1994). The genus is in need of revision, so for this study *Kuzinia* spp. were only identified to the genus level (Klimov et al., 2016e). *Kuzinia laevis* (the European species) has six developmental instars; egg, larva, protonymph, deutonymph, tritonymph and adult (Zamec, 2014). *Kuzinia laevis* is also known to feed on a variety of foodstuffs within bumblebee colonies; it is a kleptoparasite of stored pollen, honey and colony materials, a fungivore, and a predator of nematodes (Chmielewski, 1969, Klimov et al., 2016e). Despite these feeding habits, *Kuzinia* infestations are not thought to have any significant effects on bumblebee colonies (Chmielewski, 1969, Klimov et al., 2016e). This has never been tested empirically however.

The genus *Scutacarus* (Family Scutacaridae) contains at least 4 bumblebee associated species, though previously the only species thought to associate with bumblebees was *Scutacarus acarorum* (Eickwort, 1994, Jagersbacher-Baumann, 2014). It has been theorised that the other species associated with bumblebees (*S. mendax*, *S. occultatus* and *S. deserticolus*) only recently speciated from *S. acarorum*, though the means by which this speciation occurred are not known (Jagersbacher-Baumann, 2014). *Scutacarus* mites are fungivorous, and those that are not found in bumblebee colonies can be found

inhabiting soil or detritus (Schousboe, 1986, Eickwort, 1994, Jagersbacher-Baumann, 2014). Their life-cycle is poorly understood, however these mites are known to become phoretic upon larger mites which are themselves phoretic upon bumblebees, a behaviour known as hyperphoresy (Swift, 1733, Alford, 1975, Perotti and Braig, 2009). It seems unlikely that *Scutacarus* spp. have a major impact on the bumblebee colonies they inhabit, as they are extremely small and tend not to occur in great numbers (pers. obs.).

The results of this study showed that a degree of host preference is demonstrated by bumblebee associated mites from all mite groups tested, as significant differences were found in the numbers of phoretic mites from any group (e.g. *Kuzinia*) between at least two bumblebee species in the multinomial regression, and every bumblebee species predictor was assigned statistical significance by the GLM. The sample sizes for several bumblebee species were too low to be certain that this is the case for the population at large, so bumblebee species where $n < 5$ (*Bombus pascuorum* and *B. pratorum*) were combined into the 'other' species group for analysis. The current literature on the subject shows that mites in the *Parasitellus*, *Pneumolaelaps*, *Kuzinia* and *Scutacarus* genera are found in association with all bumblebee species within their geographical range (and occasionally in honeybee colonies) but has little to say on preferential associations between mites and different bumblebee species (Eickwort, 1994, Chmielewski and Baker, 2008, Klimov et al., 2016g). Schwarz et al. (1996) reported that *Parasitellus fucorum* was found phoretic on 46-49 % of all overwintered queens in Switzerland of the species *Bombus terrestris*, *B. lucorum* and *B. lapidarius*. The percentages reported here were roughly 10 % higher for the same 3 bumblebee species (Table 2.2). The results of this study partially match the conclusions drawn by Schwarz et al. (1996) after host-choice preference experiments using *Parasitellus fucorum* and bumblebee queens of different species. They found that *P. fucorum* had no significant preference between *Bombus lucorum* and *B. lapidarius*. However Schwarz et al. (1996) also found that *P. fucorum* preferred both *B. lucorum* and *B. lapidarius* to *B. pascuorum*, which we could not test due to low *B. pascuorum* numbers. Why all mite groups appear to have significantly different relative abundances upon bumblebee queens of different species is a subject worthy of investigation. Likely reasons include differences in the behaviour of bumblebee queens or in the environments within their colonies, which may have an impact on the fitness of different mite species and encourage preferential association with certain bumblebee species.

The number of phoretic *Scutacarus* mites collected in this study had a significant positive correlation with the numbers of both *Parasitellus* and all 'other Mesostigmata' collected from the same bumblebee queens. It has been noted that *Scutacarus* mites are frequently found engaged in hyperphoresy (Goulson, 2010). In this study many *Scutacarus* mites recorded were indeed found attached to the legs of larger mites belonging to the order Mesostigmata, and those which were not had likely been separated from a larger mite during the vortexing/mite removal process, which explains the strength of the observed correlations.

Bumblebee queens with phoretic mites from the 'other Mesostigmata' group were likely to have more phoretic *Parasitellus* mites. This suggests that bumblebee colonies containing *Parasitellus* mites are likely to contain other Mesostigmata as well, as this is the most likely way in which different mites within these groups could find themselves sharing space phoretic upon the same bumblebee queens. This is supported by Rożej et al. (2012), who left commercial bumblebee colonies in greenhouses and later extracted the mites within. They found various mites from the Laelapidae family and the genus *Parasitellus* (both in the order Mesostigmata) within their colonies after greenhouse exposure, with the only other mites found being large numbers of *Tyrophagus* mites (abundant due to a favourable greenhouse environment) (Rożej et al., 2012). However they did not conduct any tests of association, so this study is the first to show a correlation between the presences of these mite groups upon bumblebee queens.

It is interesting that only the *Kuzinia* group did not show any statistically significant correlation or differences with another mite group. A negative correlation was observed between *Kuzinia* and *Parasitellus* numbers, but not *Kuzinia* and other Mesostigmata or *Scutacarus* numbers (Appendix A). The reasons for this are hard to guess, but it is possible that the very high numbers of *Kuzinia* often found on bumblebee queens mean that they may effectively exclude other mites from sites for phoretic attachment on the queens, or that bumblebee colonies with abundant *Kuzinia* populations are less suited to support populations of other mite groups. However this cannot be determined from the results of this survey, and must remain a subject for future research.

This study characterised the associations between bumblebees and their phoretic mites in Reading, England, and provided the first up to date information for the UK in this field in around 40 years. The various trends of association shown here between different mites groups and bumblebee species should provide food for thought for future research in this field, as currently little is known about the reasons for bumblebees to tolerate phoresy by many different mite species, or why certain mite species may preferentially associate with particular bumblebee species over others.

Chapter 3 Development of methods for laboratory rearing of *Parasitellus fucorum*

3.1 Abstract

Parasitellus fucorum is a potentially mutualistic mite species and an obligate associate of bumblebees. There is limited information on rearing methods for *P. fucorum*, especially regarding food preferences. This work explored the mites' food preferences with the aim of finding optimal foodstuffs for maintaining *P. fucorum* laboratory colonies. Three different foodstuffs (honeybee-collected pollen, bumblebee-collected pollen and fresh sliced mealworm) were tested using captive *P. fucorum* kept in conditions similar to those found within bumblebee colonies (24°C/55%rh).

Four foodstuff combinations were trialled with test colonies containing *P. fucorum* deutonymphs. Five replicates were conducted using UK *P. fucorum*, while three more used Belgian *P. fucorum*. Each test colony started with eight *Parasitellus fucorum* deutonymphs. Number of mites per colony was counted weekly and final mite numbers were obtained by destructive sampling at the end of the experiment. The results showed that, while all treatments eventually resulted in overall population decline, pollen-fed foodstuff combinations resulted in similar rates of reproduction and observed population growth prior to the declines.

The bumblebee pollen (BB) group had the highest observed mite numbers throughout the experimental period (11 ± 5.25) (median ± inter quartile range). This was closely followed by the bumblebee pollen + mealworm (BB+MW) group (10 ± 7.25), with honeybee pollen (HB) (8.5 ± 1.75) and mealworm (MW) (2.5 ± 1.25) both showing considerably lower average mite numbers. At the end of the experiment the HB group (5 ± 3) contained the most live mites, followed by the BB+MW group (4 ± 3.5) and the BB group (2.5 ± 5.5). The mealworm (MW) group had no live mites at the end of the experiment (0 ± 0.25). Fresh mealworm was an inadequate foodstuff for *P. fucorum* reproduction and led to the most rapid population decline. These results showed that commercially available honeybee pollen may be suitable for rearing *P. fucorum* in laboratory conditions, contrary to received wisdom.

3.2 Introduction

Mites of the genus *Parasitellus* (Order Mesostigmata) are obligate bumblebee associates which develop through their lifecycle within bumblebee colonies (Koulianos and Schwarz, 1999, Rożej et al., 2012), and *Parasitellus fucorum* is the most commonly found species in this genus (Alford, 1975, Eickwort, 1994). Many bumblebee associated mites are thought to have little effect on their hosts (Eickwort, 1994, Chmielewski and Baker, 2008), but mites of the genus *Parasitellus* are frequently suggested as potential mutualists with bumblebees, despite the pollen feeding behaviour observed in some developmental instars (Schmid-Hempel, 1998, Rożej et al., 2012). This is due to the predatory behaviour of *Parasitellus*

mites, which are thought to feed on various kleptoparasites and pest species within bumblebee colonies (Eickwort, 1994, Schmid-Hempel, 1998).

Every stage of the life cycle of *P. fucorum*, except for the phoretic deutonymphal instar, typically occurs only within bumblebee colonies (Alford, 1975). The phoretic deutonymph specialises in leaving the colony by travelling upon a bumblebee, preferably a young queen (gyne), after overwintering within the hibernaculum (Richards, 1976). *Parasitellus* deutonymphs also become phoretic upon bumblebee workers or males to move between bumblebee colonies if no queens are available (Schwarz and Huck, 1997, Huck et al., 1998). *Parasitellus fucorum* deutonymphs have been observed to engage in interspecific predation, cannibalism, and kleptoparasitism (Koulianos and Schwarz, 1999).

To better understand the nature of the relationships between *Parasitellus fucorum* and its various bumblebee hosts, large numbers of this mite species are required for experimentation. While it is possible to collect these mites from bumblebees in the field or harvest them from commercial bumblebee colonies (Schousboe, 1987, Chmielewski and Baker, 2008, Rożej et al., 2012), it should be feasible to rear *P. fucorum* in laboratory conditions in order to ensure a reliable supply. There is a single example in the literature of *P. fucorum* being reared successfully in laboratory conditions which lists examples of acceptable foodstuffs including bumblebee-collected pollen and dead mealworm (Koulianos and Schwarz, 1999), however when tested it transpired that their results could not be replicated using British *P. fucorum* populations. Therefore experiments were designed in order to determine which foodstuffs were of key importance for encouraging reproduction and population growth in laboratory colonies of *P. fucorum*. Four different foodstuff regimens (honeybee pollen (HB), bumblebee pollen (BB), sliced mealworm (MW), and bumblebee pollen + sliced mealworm (BB+MW)) were tested in appropriate environmental conditions, and the resulting effects on captive populations of *Parasitellus fucorum* deutonymphs collected in the UK (Reading) and Belgium (Westerlo) were recorded and analysed. The foodstuff combinations used were chosen based on information from Koulianos and Schwarz (1999) and the results of a previous pilot experiment (Appendix F).

3.3 Methods

3.3.1 Bumblebee colony conditions measurement and pollen collection

The conditions within 5 commercial colonies of *Bombus terrestris audax* (Biobest, Belgium) were measured using remote temperature/%rh probes (Lascar, UK). The probes recorded surrounding conditions every 5 minutes, and were placed within the bumblebee colonies from which *Parasitellus* deutonymphs were later harvested. Probes were put in place by anaesthetising the colonies with CO_2, peeling back the wool/wax covering over the bumblebee colony, placing the probe underneath and replacing the covering afterwards. The bumblebee colony was then placed in the field for the duration of data recording.

The probe was recovered days later by following the same procedure, and the data recorded by the probe analysed in order to calculate the mean temperature and humidity within the bumblebee colony during

the recording period. From these five colonies overall average internal conditions of 24°C/55%rh were calculated and used for the experiment.

Bumblebee pollen was collected for experimental use by catching *Bombus terrestris* (*audax/terrestris*) workers returning from foraging trips as they attempted to enter their commercial colonies. The workers were caught in 50ml falcon tubes (with drilled air holes) then taken to the lab and rendered torpid using CO_2, after which their pollen balls were gently removed using probes. Afterwards the workers were released outdoors, and the pollen was frozen at -20°C. For the food preference experiment only pollen balls collected in Belgium (Westerlo) by *Bombus terrestris terrestris* were used, and all prior work used pollen collected in the UK (Reading) by *Bombus terrestris audax*.

3.3.2 Initial *Parasitellus fucorum* collection and rearing

Parasitellus fucorum deutonymphs for experimental use were collected from commercial colonies of *Bombus terrestris audax* (in the UK) or *Bombus terrestris terrestris* (in Belgium). In the UK these bumblebee colonies had previously been inoculated with *P. fucorum* deutonymphs and placed in the field for 2-8 weeks to allow a large population of mites to develop. In Belgium no mite inoculations were conducted prior to placement in the field, but they were otherwise treated in the same way.

To collect *P. fucorum* deutonymphs from matured *B. terrestris* colonies, the whole colonies were placed into large containers and anaesthetised using CO_2 gas. Colonies were opened and any bumblebees within that had visible phoretic *Parasitellus* deutonymphs were taken from the colonies and all phoretic *Parasitellus* mites were removed from the bees. Any freely-moving *Parasitellus* mites (including adults) observed within the colony were also collected.

The method for rearing and maintaining captive populations of *Parasitellus* mites prior to the food preference experiment was developed based on both the results of a previous pilot experiment (Appendix F) and the work of Koulianos and Schwarz (1999). A 650ml tissue culture flask with vented lid (Greiner, Austria) was filled halfway with autoclaved peat (Peltracom, Belgium), on top of which were placed two small ‘feeding trays’ made from cut plastic weighing boats (Heathrow, USA). One tray was filled with a mixture of organic honeybee-collected pollen (Aspermuehle, Hungary) and bumblebee-collected pollen. The other feeding tray was filled with slices of locally purchased fresh mealworms. All foodstuffs were frozen at -20°C prior to usage to ensure their freshness and that no pests were added to the culture flasks, hereafter referred to as *Parasitellus* colonies (Figure 3.1). After food addition, the field-caught *Parasitellus* deutonymphs were added and the colony was placed into an unlit Controlled Environment (CE) chamber. All *Parasitellus* colonies were kept at 24°C/55%rh based on measurements taken from within bumblebee colonies. All foodstuffs were replaced on a daily basis (except during weekends, when foodstuffs were replaced every other day), and any fungal growths observed within the colony were removed.

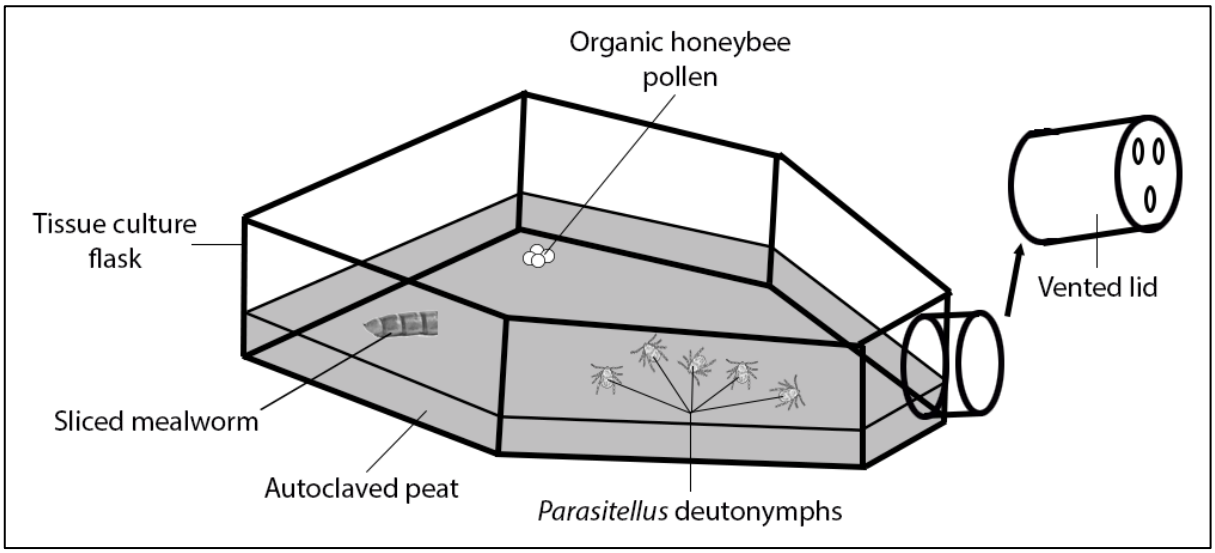

Figure 3.1 – *Parasitellus* laboratory colony design. Mites and foodstuffs not to scale. Feeding trays not shown.

3.3.3 Experimental procedure

Eight replicates were conducted in a fractional factorial design which tested 4 different foodstuff combinations. The combinations tested were; Organic honeybee pollen (Control/HB), fresh bumblebee pollen supplemented with organic honeybee pollen (BB), fresh slices of mealworm (MW), and a combination of the bumblebee/honeybee pollen mixture and fresh slices of mealworm (BB+MW). All foodstuffs were kept at -20°C and defrosted as needed for experimental use.

All experimental *Parasitellus* colonies were established by filling 800ml vented cap tissue culture flasks (Falcon, USA) with 85g (±2g) moist autoclaved peat (Peltracom). The moisture content of this peat was tested using a Sartorius Moisture Analyzer (MA35) and found to be 55% (±2%). Colonies had equal weights of their required foodstuffs added on small plastic feeding trays (Appendix H). After the addition of peat and food, eight *Parasitellus fucorum* deutonymphs were added. The first five replicates of this experiment were established using *Parasitellus fucorum* deutonymphs collected in Reading, England. The final three replicates (replicates 6 - 8) were established using *P. fucorum* deutonymphs collected in Westerlo, Belgium, in the grounds at Biobest Group NV. This was because the experiment was conducted in the Research and Development department of Biobest Group NV during a research placement, and in order to reach the desired number of replicates the previously collected UK mites had to be supplemented with mites collected locally. Mites from different geographical origins were kept separate at all stages of the experiment in case it later proved necessary to analyse them separately.

Replicates were established over 2 days in order to keep the work required to observe and process *Parasitellus* colonies manageable. The first 4 replicates of the experiment were set up on 23/10/18, and the final 4 set up on 24/10/18. After each test colony was established and the mites added, the colony was stored in an unlit CE chamber maintained at a constant 24°C/55%rh. Every colony had all foodstuffs replaced on a daily basis Monday-Friday, in order to ensure a supply of fresh foodstuffs for as much of the experimental period as possible.

Mite counts were taken on a weekly basis, after the removal of all feeding trays within each test colony. Counts were taken through uninterrupted observation of the test colony for 3 minutes with the aid of a dissecting microscope with 1-5x objective lenses where appropriate. Colonies were placed so that the entire surface of the colony was visible, every observation of *P. fucorum* instars was tallied. To keep the counting method consistent, if a mite burrowed beneath the surface of the peat within the colony and later re-emerged this was counted as a new observation. The population counts taken from weeks 2-6 of the experiment therefore reflect both the population size in each colony and (to a lesser degree) the level of activity within it, as more active mites are more likely to burrow and re-emerge from the peat frequently.

Mites were removed from the test colonies manually at the end of the experiment by first pouring a small sample of the colony materials into a large plastic tray. This sample was spread out on the base of the tray by gentle shaking, and the materials were then closely examined both by eye and with 1-5x objective lenses using a dissecting microscope. Any mites observed were counted and removed using a paintbrush into an Eppendorf containing 70% ethanol. This was repeated for all materials from every colony. After this the colony materials were frozen at -20°C and discarded. This method of mite extraction was used in lieu of the more common process of filtering colony material through progressively finer sieves until only mites and small substrate particles are left, after pilot testing showed that this method was unsuitable for use with peat-based mite colonies. Additional precautions were also taken to minimise the risk of any mites being missed during manual processing (Appendix H).

3.3.4 Mite species identification

All mites collected from test colonies were first cleared in 50% lactic acid, dehydrated using 70% and then 95% ethanol solutions, mounted on a microscope slide in Hoyer's liquid, and sealed using glyptal paint. This method was informed by the recommendations of Krantz and Walter (2009). Mounted mites were identified to the species level by close observation of their external morphology using a Nikon Optiphot microscope with 10 – 100x objective lenses following dichotomous keys and comparing observations with species descriptions in the standard works of reference for *Parasitellus* mites (Hyatt, 1980, Baker et al., 1999, Krantz and Walter, 2009).

3.3.5 Statistical analysis

The data collected from these experiments consisted of the observed counts of live *Parasitellus* mites of different developmental instars in every test colony from weeks 2 - 6, and practical counts of the same taken start and end of the experimental period (weeks 1 & 7).

All statistical analyses (except survival analysis) were carried out using R v3.4.2 (R Core Team, 2017). Kolmogorov-Smirnov tests were used to test whether data were normally distributed, and Bartlett's test was used to determine homogeneity of variances. ANOVA and Tukey multiple comparisons of means tests were used to determine whether there were significant differences between groups. R studio and the ggplot2 package were used to produce all scatterplots and boxplots (RStudio Team, 2015, Wickham,

2016). Survival analyses in the form of log-rank tests and Cox proportional hazards models, and the production of Kaplan-Meier graphs, were carried out using Stata SE 15 (StataCorp, 2017). For the purposes of the survival analysis, the 'failure' event used for time-to-event calculations was defined as the direct observation of evidence indicating that *Parasitellus fucorum* mites had reproduced within each test chamber (i.e. the presence of larvae or protonymphs). Each test chamber used in the experiment was counted as an individual unit for the purposes of these analyses, and chambers were grouped by treatment.

3.4 Results

The average mite counts taken from replicates established using mites collected in the UK (1 - 5) and replicates which used Belgian mites (6 - 8) were compared using a Kruskal-Wallis test. This showed that there were no significant differences between the two datasets when all treatment groups were pooled, therefore replicates conducted using *Parasitellus fucorum* deutonymphs collected in the UK or Belgium were analysed together.

There were significant differences in the average numbers of *Parasitellus* mites observed within colonies during the experimental period, depending on treatment group (Figure 3.2). The honeybee pollen (HB) group had (8.5 ± 1.75) (median ± Inter Quartile Range (IQR)), the bumblebee pollen (BB) group (11 ± 5.25), the mealworm (MW) group had (2.5 ± 1.25), and the BB+MW group (10 ± 7.25). An ANOVA showed that these differences between treatment groups were statistically significant ($F = 5.891$, $p = 0.003$). A Tukey multiple comparisons of means test showed there were significant differences between the MW group and both the BB group (-8.625, 0.002) (difference, p-value) and the BB+MW group (-6.750, 0.02). When the mean results were calculated for each treatment group, all pollen-fed groups contained at least 1 adult mite, while the MW treatment group contained none. Full mite counts for each developmental instar at each weekly observation are shown in Appendix G.

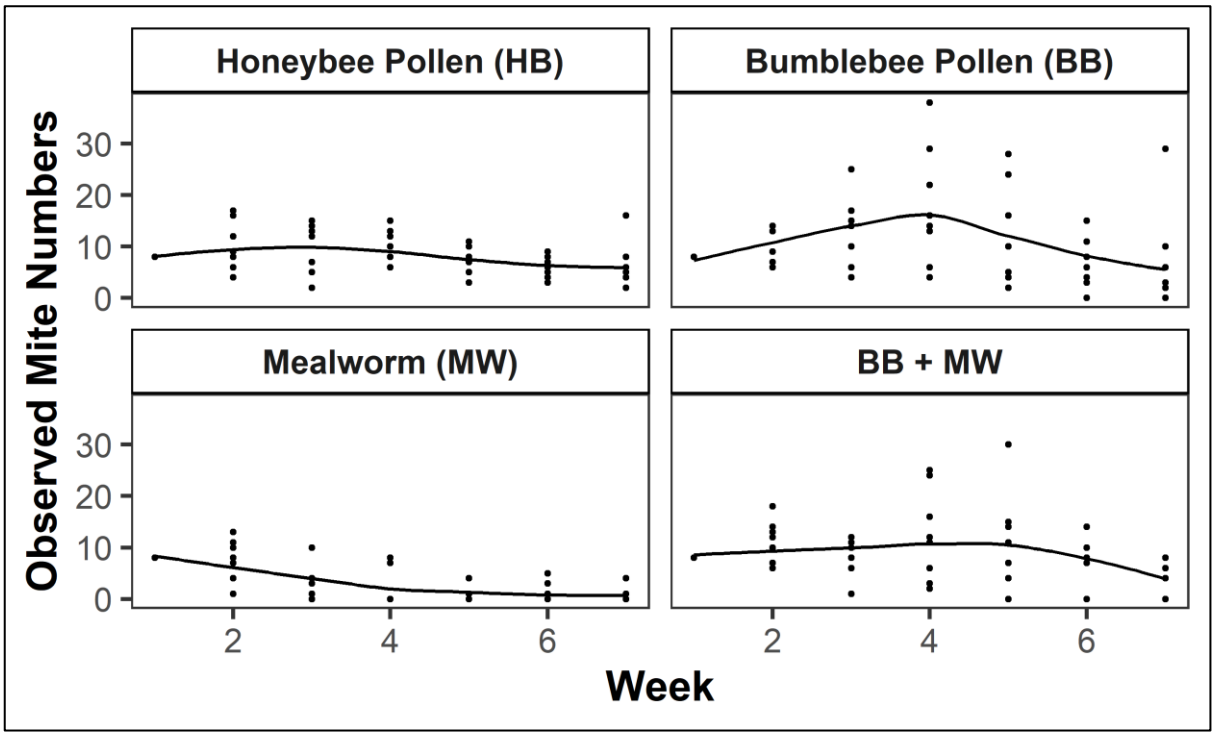

Figure 3.2 – Observed numbers of *Parasitellus* mites (of any developmental instar) recorded for each test colony from weeks 2-6 of the experimental period, separated by treatment group. Black trendline added using a smoothed rolling mean to reflect population trend over time.

While there were variations between treatment groups in the mite numbers collected at the end of the experiment (Figure 3.3), an ANOVA showed these were not statistically significant ($F = 1.803$, $p = 0.17$). There was an apparent trend however, with all treatment groups fed with pollen showing broadly similar median numbers of mites present at the end of the experiment (HB (5 ± 3) (median ± IQR), BB (2.5 ± 5.5), BB+MW (4 ± 3.5)), and the mealworm treatment group very clearly having no live mites (0 ± 0.25).

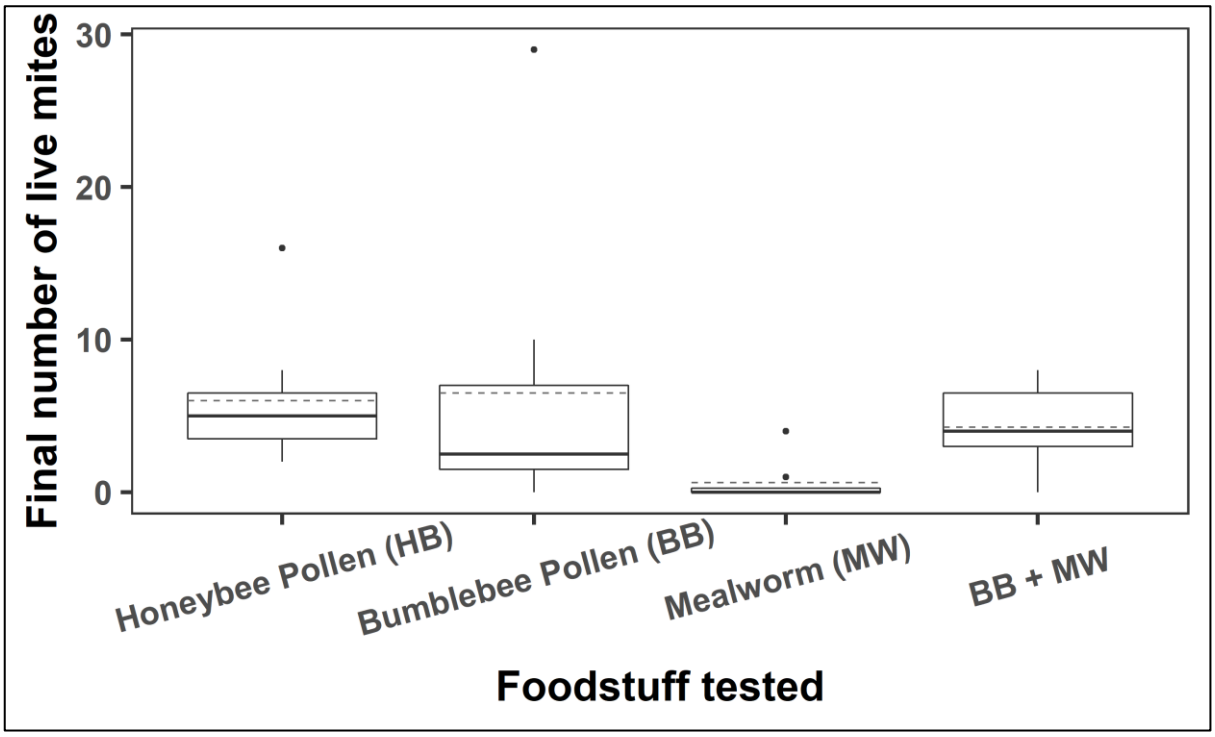

Figure 3.3 – Numbers of live *Parasitellus* mites (of any instar) collected from test colonies at the conclusion of the experiment (week 7), separated by treatment group. Thick horizontal line shows median value, thin horizontal lines represent 1st and 3rd quartiles, and vertical whiskers extend to maximum and minimum values within 1.5x inter-quartile range, with points showing outlying results beyond this range. Dashed grey horizontal lines show arithmetic mean values of each dataset.

A survival analysis was conducted to determine if significant differences existed between treatment groups in the time taken for the mites within each colony to reproduce and the resulting offspring to be recorded during weekly observations. A Kaplan-Meier failure graph was produced to visualise this data (Figure 3.4), and showed a similar trend to the final observed mites numbers (Figure 3.3). All pollen-fed treatment groups (HB, BB & BB+MW) had broadly similar results (i.e. 3/8 colonies tested had shown evidence of reproduction by the end of the experiment (Figure 3.4, Appendix G)), and the mealworm group (MW) markedly different (no reproduction at all). However, a Cox proportional hazards model showed that these differences were not statistically significant ($z = -0.43$, $p = 0.67$). The proportional hazards assumption was not met, so a log-rank test was also conducted and confirmed the Cox model result ($X^2 = 3.81$, $p = 0.283$).

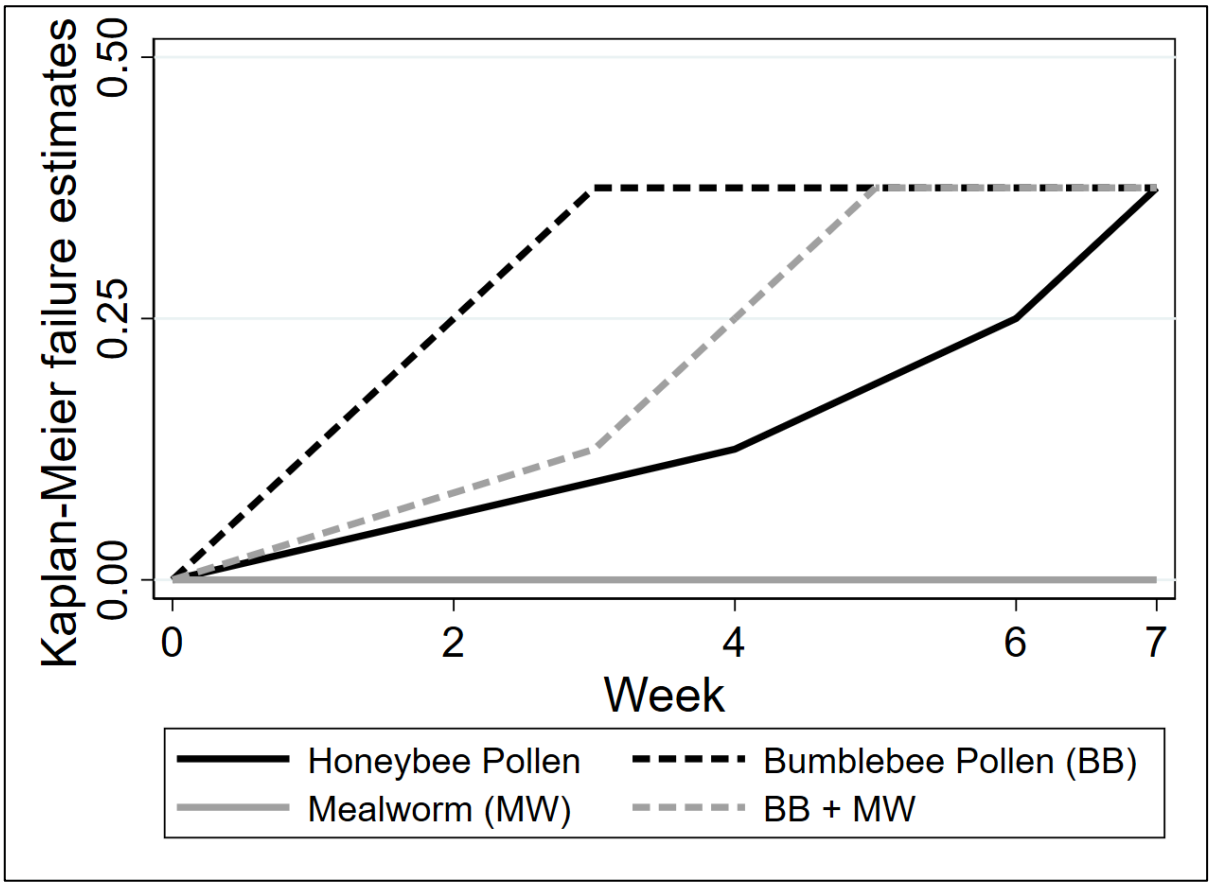

Figure 3.4 – Kaplan-Meier failure graph showing the proportion of colonies in each treatment group that demonstrated 'failure' events over time. A 'failure' event was defined as the first recorded visual evidence of reproduction within each colony (i.e. direct observation of larvae or protonymphs within a colony). Note that for clarity the y-axis has been capped at a failure estimate of 0.5.

3.5 Discussion

This experiment was designed to give insight into which foodstuffs are of key importance for the development of *Parasitellus fucorum* populations in the laboratory. Honeybee collected pollen (purchased commercially) was chosen as a control foodstuff and used to add bulk to bumblebee collected pollen (collected by hand) because it was thought to lack an essential component for *P. fucorum* development. This theory was based on both the results of a pilot experiment (Appendix F) and previous literature on this subject (Koulianos and Schwarz, 1999). Bumblebee collected pollen and sliced fresh mealworm were used as test foodstuffs because they resulted in the greatest observed population increases during the pilot experiment (Appendix F). While regional variation may exist within a mite species such as *Parasitellus fucorum*, no significant differences were found between the UK and Belgian mite groups used in this experiment, and so they were analysed together.

The results of this experiment showed that supplying *Parasitellus fucorum* deutonymphs with different foodstuffs will result in markedly different effects on survival. While the general trend in all treatment groups was one of population decline (after initial population increases in some cases) the clearest result was that supplying *P. fucorum* deutonymphs with mealworm as their sole food source will prevent mites from moulting into adults (the mealworm group alone contained no adult mites at any stage) and result in rapid population decline and the eventual death of all mites within the colony (Figure 3.2). The

average numbers of mites observed during the experiment varied significantly by treatment group, and the final mite numbers in the mealworm treatment group were markedly different from all other groups (honeybee pollen (HB), bumblebee pollen (BB), and bumblebee pollen with mealworm (BB+MW)) though these differences were not statistically significant. The 3 pollen-fed treatment groups did not differ significantly from each other, and had broadly similar median final mite numbers. This suggests that the use of both bumblebee collected and honeybee collected pollen allowed the development of *P. fucorum* through its life cycle. Mite reproduction was evidenced visually in all pollen-fed treatments (Figure 3.4), and 1st generation offspring (larvae/protonymphs) were collected from colonies in both the HB and BB groups at the end of the experiment (Appendix G). There were also interesting differences in reproductive results between the treatment groups. Colonies fed bumblebee pollen reached higher observed populations mid-experiment and showed evidence of reproduction earlier than those fed with honeybee pollen, while colonies fed with mealworm alone never exhibited reproduction at all (Figures 3.2 & 3.4). However, a Cox proportional hazards model found no significant differences in the rate at which reproduction occurred in colonies from different treatment groups. Taking this into account, the results suggest that pollen of either variety (bumblebee or honeybee collected) is required for the reproduction and development of *Parasitellus fucorum* in laboratory conditions. This was an unexpected result, as it has previously been asserted that *P. fucorum* would not feed on honeybee-collected pollen (Koulianos and Schwarz, 1999), and in the pilot experiment no reproduction or population increases were observed in colonies supplied with only honeybee collected pollen (Appendix F). In retrospect this seems to have been a result of the different conditions and availability of fresh foodstuffs in the pilot experiment, rather than the foodstuff itself.

When the numbers of mites present at the end of the experiment were compared by treatment group, no significant differences were found. This evident lack of variation in the final mite numbers was likely influenced by rapid population declines during the experimental period caused primarily by two factors. All experimental colonies were inaccessible for two days a week due to issues beyond our control, which meant that there was no fresh food available to the mites on these days. *Parasitellus fucorum* are also known to be cannibalistic, and therefore it is likely that during days without fresh food the populations within colonies were reduced by intra-specific predation (Koulianos and Schwarz, 1999). Cannibalistic behaviour has been observed in various predatory Mesostigmata, including *Parasitus bituberosus* and *Macrocheles glaber* (Marquardt et al., 2015, Rueda-Ramírez et al., 2019). These issues are likely to have stunted the rapid population growth that can be observed in some *P. fucorum* colonies around weeks 3-5 (Figure 3.2). The fresh foodstuffs available within colonies were doubled for the final 2 weeks of the experiment in an effort to mitigate against this effect, but to no avail (Appendix H).

The results of this experiment demonstrate that both honeybee collected pollen and bumblebee collected pollen are suitable foodstuffs to encourage *Parasitellus fucorum* deutonymphs to moult into adult instars and reproduce in appropriate laboratory conditions, though they do not show that the rearing methods employed here are suitable for maintaining or increasing the size of a captive mite population. While

bumblebee pollen appeared to encourage faster population growth and earlier reproduction than honeybee pollen, these differences were not significant. This result should be useful for future research requiring captive *P. fucorum*, as honeybee collected pollen can be purchased commercially in large quantities whereas bumblebee collected pollen must be painstakingly gathered by hand. The results also clearly show that sliced mealworm is an unsuitable foodstuff for *P. fucorum* and serves no purpose as an additional foodstuff when used to supplement pollen. While this experiment did not demonstrate continuous population growth in any condition tested, an obvious improvement that could be employed would be to ensure that foodstuffs were replaced on a daily basis with no weekly gaps, in order to remove the effects on the mites from lacking fresh food for extended periods of time. Other ways to take this work forward could involve testing a wider variety of foodstuffs e.g. bumblebee colony wax, or testing the impact of exposing the mites to a live bumblebee or bumblebee pheromones during captivity. Further research is needed to explore the viability of any *P. fucorum* rearing methods in order to facilitate future work studying these fascinating mites and their relationships with other organisms within bumblebee colonies.

Chapter 4 Bumblebee mite *Parasitellus fucorum* predates greater wax moth (*Galleria mellonella*) eggs and mould mites (*Tyrophagus putrescentiae*)

4.1 Abstract

Greater wax moth (*Galleria mellonella*) eggs and mould mites (*Tyrophagus putrescentiae*) were used to conduct a series of predation tests with deutonymphs of the bumblebee associated predatory mite *Parasitellus fucorum*. These were carried out to determine whether *P. fucorum* could predate either of these species, as it has been theorised previously that *P. fucorum* predates pests within bumblebee colonies, but there is little empirical evidence to demonstrate this.

Three different experimental 'conditions' were used, based on the inclusion of 15 *Galleria mellonella* eggs, 15 *Tyrophagus putrescentiae* mites, or both together within the experimental chamber. These 3 conditions were each divided into Test and Control groups, with Test groups having 4 *Parasitellus fucorum* deutonymphs added and Control groups being left unchanged. The survival of all organisms was closely monitored for 24 hours after the setup of each predation test.

There were significant differences in the survival of both varieties of prey when the Test and Control groups were compared in all conditions. *Tyrophagus putrescentiae* mites were predated at a higher rate than *Galleria mellonella* eggs by the *Parasitellus fucorum* deutonymphs, however both were clearly predated. At the end of the experiment the combined condition (i.e. *T. putrescentiae* + *G. mellonella*) Test group had far fewer live *T. putrescentiae* mites (7 ± 6.5) (median ± inter-quartile range) or *G. mellonella* eggs (12 ± 3.75) than the Control group (15 ± 1 and 14.5 ± 2, respectively). The Test vs Control group results for the *T. putrescentiae* (3 ± 2 vs 15 ± 0) and *G. mellonella* (12.5 ± 6.75 vs 15 ± 1) conditions were broadly similar. Generalised Estimating Equation analyses showed that for both *G. mellonella* eggs (4.42, 0.035) (X^2, p-value) and *T. putrescentiae* mites (30.66, < 0.001) the presence or absence of *P. fucorum* deutonymphs had a significant effect on prey survival.

These results show for the first time that *Parasitellus fucorum* deutonymphs can predate both *Tyrophagus putrescentiae* mites and *Galleria mellonella* eggs. This supports the hypothesis that the presence of predatory *P. fucorum* within bumblebee colonies may result in a net benefit towards the bumblebees through the predation of different colony pests.

4.2 Introduction

One area of bumblebee ecology that has not received a great deal of attention in recent years is their relationships with various other organisms within their colonies, including pests such as the bumblebee wax moth (*Aphomia sociella*) and a diverse range of mite (Acari) species (Eickwort, 1994, Goulson, 2010). The number of different mite species found within bumblebee colonies is estimated at around

110 (Klimov et al., 2016g). These include active predators such as *Parasitellus fucorum*, kleptoparasites (stored pollen feeders) such as *Pneumolaelaps longanalis*, and fungivores like *Tyrophagus putrescentiae* (Royce and Krantz, 1989, Chmielewski and Baker, 2008, Rożej et al., 2012). The effects on the bumblebees that result from these mites becoming established in colonies are largely unknown, as there has not been a great deal of empirical research into the subject.

Parasitellus fucorum is a large bumblebee associated mite of the Order Mesostigmata known to be predatory and oophagous upon other organisms within bumblebee colonies, as well as a kleptoparasite (Alford, 1975, Eickwort, 1994). *P. fucorum* has also been found within honeybee colonies (Chmielewski, 2003). The species has often been theorised to have a net beneficial impact on the fitness of its bumblebee hosts due to its predatory and oophagous activities within bumblebee colonies (Koulianos and Schwarz, 1999, Rożej et al., 2012).

Tyrophagus putrescentiae is a cosmopolitan mite species belonging to the Cohort Astigmata which feeds on mould that develops on stored food products, hence its common name – the mould mite (Fan and Zhang, 2007, Kheradmand et al., 2007). *T. putrescentiae* is found in a variety of environments including both honeybee and bumblebee colonies, in which it can become the most numerous mite species (Chmielewski, 2003, Rożej et al., 2012, Klimov et al., 2016g). The effects of *T. putrescentiae* infestation in bumblebee or honeybee colonies have not been established, though Chmielewski (2003) noted that bee-bread from honeybee colonies heavily infested by mites including *T. putrescentiae* were often damaged/contaminated.

Galleria mellonella (the greater wax moth) is a major pest of honeybees (*Apis mellifera* Linnaeus, 1758), known for the tenacity of its larval stages in devouring honeycomb, developing brood and detritus within infested honeybee colonies (Kwadha et al., 2017). Adult female moths enter honeybee colonies and lay their eggs which rapidly hatch, develop into larvae, and begin feeding. The larvae protect themselves from honeybees within the colony by spinning tunnels of silk that the honeybees cannot penetrate, leading to the eventual destruction of the colony if untreated (Kwadha et al., 2017). This species has also been shown to have an economic impact; beekeepers in the southern USA were estimated to have lost 5.1% of their profits ($4 million) due to colony losses attributed to *G. mellonella* infestations in 1976 (Kwadha et al., 2017). The reason for using *Galleria mellonella* eggs in these predation experiments (instead of a bumblebee associated wax moth species such as *Aphomia sociella*) was that *G. mellonella* larvae can be purchased commercially. This enabled the rearing of *G. mellonella* larvae en masse and the subsequent collection of a large number of eggs at one time in order to conduct the predation tests. Since *G. mellonella* occupies a similar ecological niche to *A. sociella* its use as a substitute seemed reasonable. It is also likely that *G. mellonella* and *P. fucorum* encounter each other in the field since *P. fucorum* is sometimes found within honeybee colonies, and *G. mellonella* has been found in bumblebee colonies in Japan (Alford, 1975, Chmielewski, 2003).

Biological pest control is a well-established technique in which live organisms are used to control pest species and prevent crop damage or increase yields in agriculture without the use of chemical means such as pesticides. One example of this is the use of the predatory mite *Hypoaspis miles* to control various pests on vegetable and mushroom crops (Enkegaard et al., 1997). Biological control of bumblebee or honeybee pests represents a potentially useful avenue of research for pollinator conservation. The aim of this experiment was to test whether the bumblebee-associated mite *Parasitellus fucorum* could predate the eggs of the greater wax moth (*Galleria mellonella*) or adult *Tyrophagus putrescentiae* mites.

4.3 Methods

4.3.1 Wax moth rearing

Greater wax moth (*Galleria mellonella*) larvae were commercially purchased (UK Waxworms Ltd., UK). Upon arrival the larvae were separated into small circular storage containers dubbed 'moth tubs' for this experiment (Vinida, UK) in groups of 15 (Figure 4.1). The bottom of each tub was lined with a ground bran cereal (Harvest Morn, UK) and honey (Aldi, UK) mixture, and crumpled wax paper (Reynolds, UK) was added. The lid of each tub was drilled with air holes, and women's tights (George, UK) were cut to size and put between the lid and base of each tub to prevent any larvae or moths escaping. The tubs were then stored in a Controlled Environment (CE) chamber kept in darkness at a constant 30°C/80% H_20, based on common protocols for rearing *G. mellonella* larvae (Karsten, 2002, wikiHow Staff, 2019).

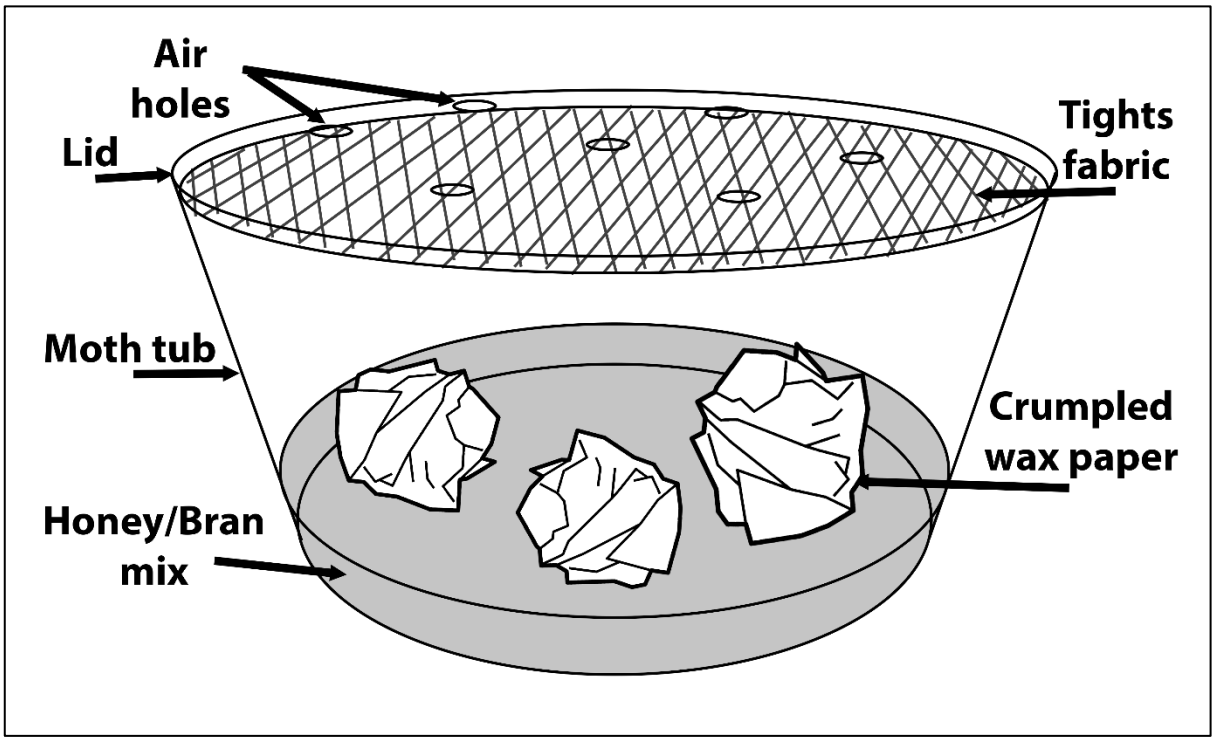

Figure 4.1 – Diagram of the moth tub design used for this experiment.

The moth tubs were inspected every 2 days to check for pupating and adult moths, and once adult moths and eggs were observed the colony box was opened and the eggs removed using forceps. These eggs

were then carefully separated and manipulated for experimental use using probes and forceps under 1-5x objective lenses using a Motic SMZ171 microscope.

4.3.2 Mite rearing

The *Tyrophagus putrescentiae* used in this predation experiment were reared in 50ml tissue culture flasks with vented caps (Greiner Bio-One Ltd., UK) with a base layer of food (a mixture of yeast, oats and flour) added. Mites were initially added from another colony (Acarology Lab, University of Reading) previously established in this way which had become overpopulated. The culture flask was then stored in a sandwich box with air holes containing a layer of damp tissue paper in order to maintain high humidity. This box was wrapped in foil to maintain internal darkness, and left at room temperature (21-24°C) until the mites were needed for experimental purposes.

When the mites were needed, a spatula was used to remove a large number of adult mites from the tissue culture flask, after which the required numbers were carefully transferred using probes or very fine forceps as needed.

The *Parasitellus fucorum* deutonymphs used in the predation experiment were taken from laboratory colonies of this mite species (started using phoretic mites taken from field-caught bumblebees) which were being used for other work (Acarology Lab, University of Reading). These laboratory colonies consisted of 650ml tissue culture flasks with vented caps (Greiner Bio-One Ltd., UK) half filled with moist autoclaved peat (Sycamore Trading, UK), kept at 21C in a controlled environment (CE) chamber, and fed on a daily basis with organic honeybee pollen (Aspermuehle, Germany), fresh bumblebee-collected pollen (collected by hand) and slices of fresh mealworm (livefoods4u, UK). All foodstuffs given to these colonies were frozen at -20C prior to usage to prevent contamination. Deutonymphs were removed as needed for experimental usage using a paintbrush.

4.3.3 Experimental protocol

For the predation experiments, each test chamber consisted of a sterile 50ml tissue culture flask with a vented cap (Greiner Bio-One Ltd., UK). The 3 conditions used in the predation experiment were as follows; 15 *Galleria mellonella* eggs (GM), 15 *Tyrophagus putrescentiae* mites (TP), or 15 *G. mellonella* eggs and 15 *T. putrescentiae* mites together (GMTP). Each condition was used for both a Test group and a Control group, where the Test group had 4 *Parasitellus fucorum* deutonymphs added and the Control group did not. For each of the 6 possible condition/grouping combinations, 10 replicates were conducted.

The 'prey' specimens (*G. mellonella* eggs and *T. putrescentiae* mites) were added to each test chamber first, followed by the addition of any required *P. fucorum* deutonymphs. The start time of each test chamber was recorded immediately after the addition of the *Parasitellus* deutonymphs (or the prey if in the Control group). Once each test chamber had all organisms added it was moved into a 30°C/80%rh controlled environment (CE) kept in constant darkness, and test chambers were only removed from the CE for survival counts.

Survival counts were performed every hour for 6 hours, and consisted of visually counting the numbers of surviving *Parasitellus* deutonymphs, *Tyrophagus* mites, and *Galleria mellonella* eggs using a Motic SMZ171 microscope using 1-5x objective lenses for 3 minutes. Dead *Tyrophagus* mite were obvious by their shrivelled remains or complete absence, and dead *G. mellonella* eggs were determined by either having been shrivelled or 'hollowed out' by *P. fucorum* feeding (Figure 4.2). After the 6th hour of observations the colonies were returned to the CE chamber overnight, and final observations were taken for each test chamber 24 hours after the start time.

After the final survival counts had been conducted all organisms in each test chamber were euthanised by the addition of 10ml of 70% ethanol, after which they were separated and mounted. This was done by storing the mites in 50% lactic acid until their internal organs dissolved, dehydrating them by submersion in 70% and then 95% ethanol, before placing the mites on a microscope slide then covering with Hoyer's liquid and a cover slip (Krantz and Walter, 2009). The mounted mites were later identified to the species level using identification keys and species descriptions (Hyatt, 1980, Fan and Zhang, 2007).

4.3.4 Statistical analysis

The data resulting from these predation tests consisted of the survival counts of all organisms present in each test chamber (*Galleria mellonella* eggs, *Tyrophagus putrescentiae* mites, and *Parasitellus fucorum* deutonymphs) from the beginning of the experimental period until the end (0 – 24 hours).

Generalised Linear Models (GLMs) using Generalized Estimating Equation (GEE) analysis were created in SAS v9.4 using the GENMOD procedure (SAS Institute Inc., 2013). These models used numbers of live *G. mellonella* eggs or *T. putrescentiae* mites within experimental chambers as the dependant variable. Experimental Group (i.e. Test or Control), Conditions (i.e. GM / TP / GMTP) within the Groups, Hour of measurement, and the interactions of Hour with both Group and Condition within the Groups were used as predictors. Experimental chambers were specified as the repeated subject within the model. The most parsimonious model structures were determined based on the QIC. Maximum likelihood estimates were obtained for each parameter and used to determine overall p-values following the chi-square method. Figures were produced using the PLM procedure showing predicted numbers of live prey (GM or TP) in both Test and Control group in both conditions containing those prey (i.e. GM and GMTP for *Galleria mellonella* eggs). All other figures were produced using the ggplot2 package within R 3.5.2 and Rstudio (RStudio Team, 2015, R Core Team, 2017, Wickham, 2016).

4.4 Results

The numbers of prey specimens (*Tyrophagus putrescentiae* mites or *Galleria mellonella* eggs) that were still alive at the 24 hour survival counts in each experimental Group/Condition were as follows. The final numbers of live *T. putrescentiae* mites in the *Tyrophagus* (TP) Test (3 ± 2) (median ± Inter-Quartile Range (IQR)) and Control (15 ± 0) groups differed more than in any other condition (Figure 4.3). The

final numbers of live *Galleria mellonella* eggs from the *G. mellonella* (GM) Test (12.5 ± 6.75) and Control (15 ± 1) groups did not differ as significantly (Figure 4.4). Live *T. putrescentiae* mites and *G. mellonella* eggs from the *G. mellonella* + *Tyrophagus* (GMTP) Test and Control groups mirrored the results of the other conditions; the final numbers of live *T. putrescentiae* mites in the Test (7 ± 6.5) and Control (15 ± 1) groups varied more than the final numbers of live *G. mellonella* eggs in the same Test (12 ± 3.75) and Control (14.5 ± 2) groups (Figure 4.5). No *Parasitellus fucorum* mites died in any conditions tested. Numbers of each prey species observed at every measurement in each test chamber are shown in Appendix J.

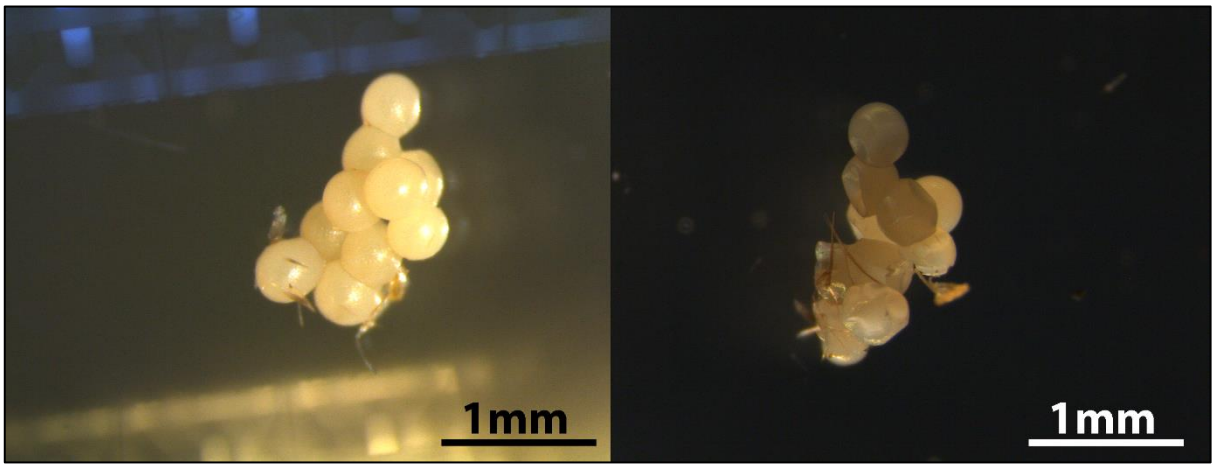

Figure 4.2 – A cluster of *Galleria mellonella* eggs pictured at the beginning (left) and end (right) of a 24 hour predation test. Predated eggs are hollowed or greatly shrunken, while healthy eggs remain full and transmit light more clearly.

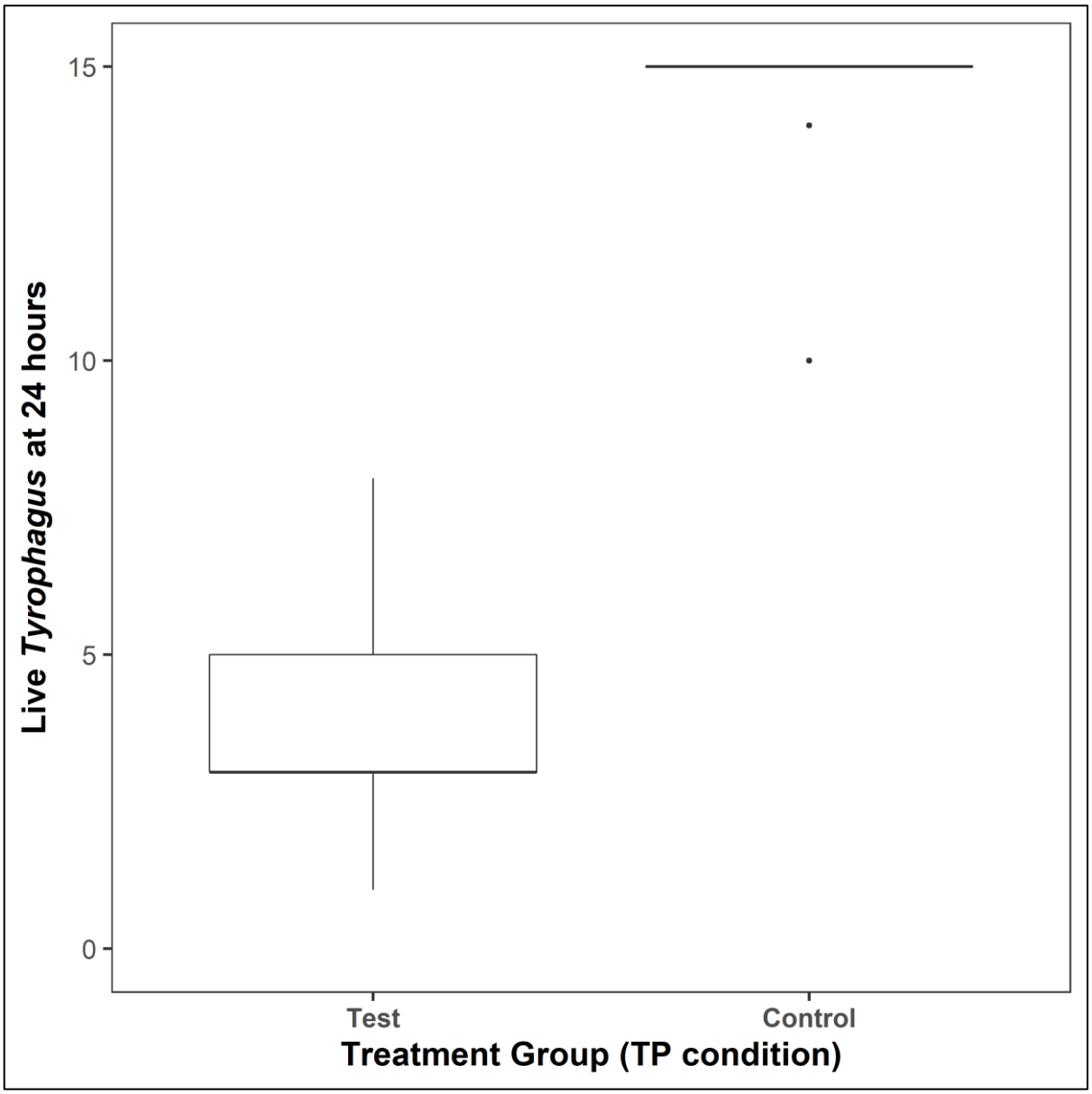

Figure 4.3 – Numbers of live *Tyrophagus putrescentiae* mites present in the *Tyrophagus* (TP) test and control colonies at the 24 hour survival count. Test colonies had *Parasitellus fucorum* added, controls did not. Thick horizontal line shows median value, thin horizontal lines represent 1st and 3rd quartiles, and vertical whiskers extend to maximum and minimum values within 1.5x inter-quartile range, with points showing outlying results beyond this range.

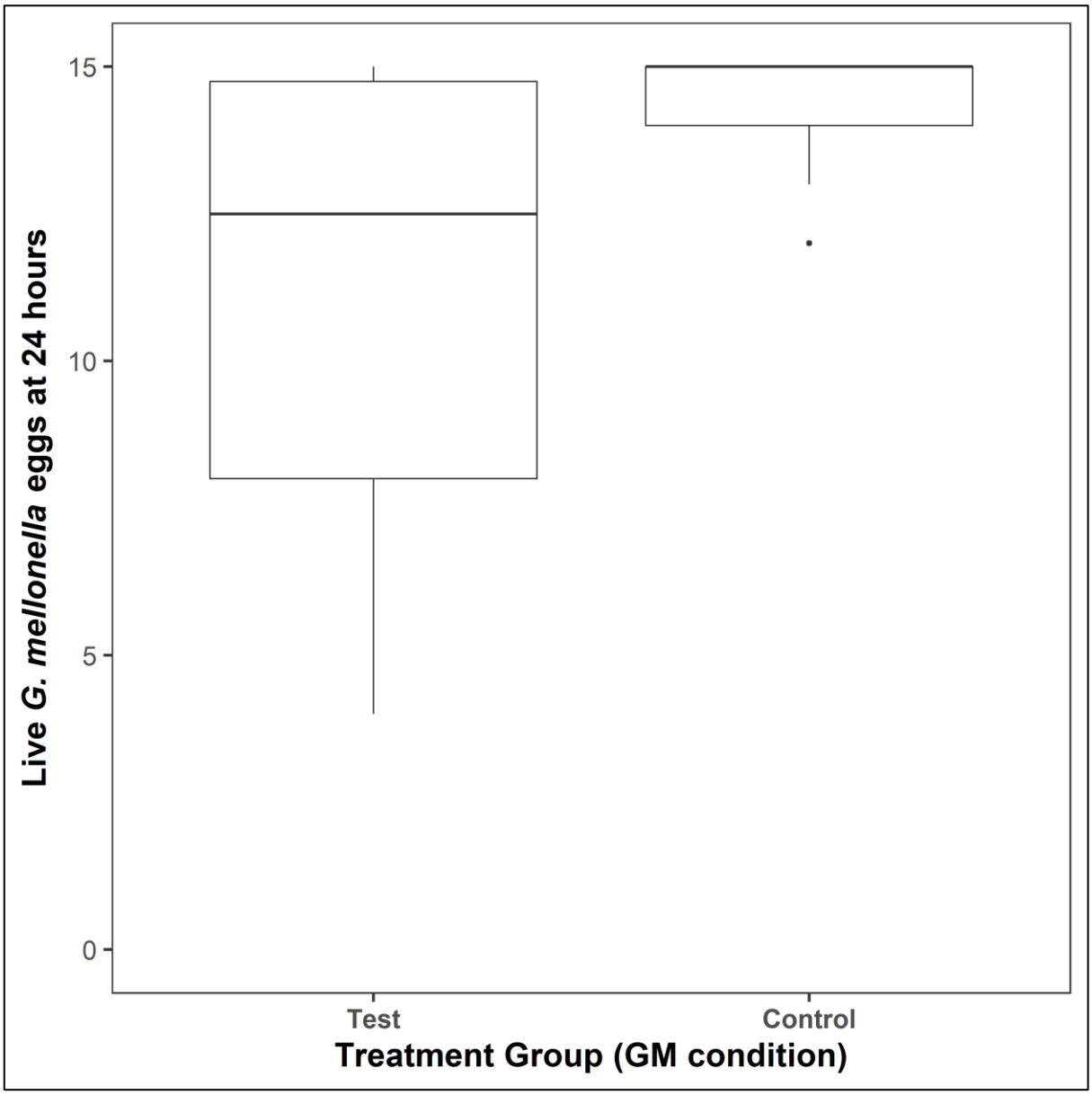

Figure 4.4 – Numbers of live wax moth eggs present in the *Galleria mellonella* (GM) test and control colonies at the 24 hour survival count. Test colonies had *Parasitellus fucorum* added, controls did not. Thick horizontal line shows median value, thin horizontal lines represent 1st and 3rd quartiles, and vertical whiskers extend to maximum and minimum values within 1.5x inter-quartile range, with points showing outlying results beyond this range.

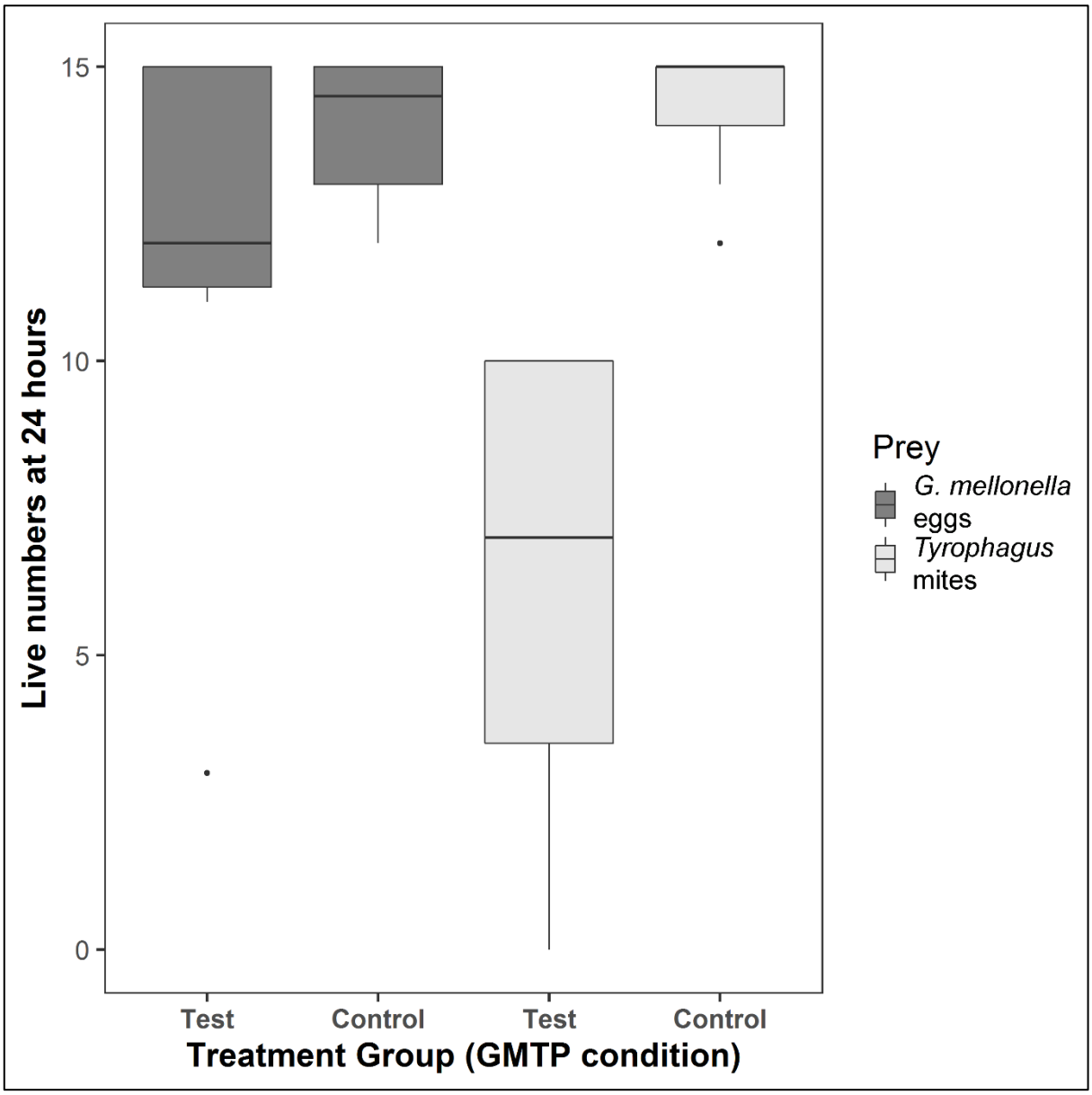

Figure 4.5 – Numbers of live *Galleria mellonella* eggs (light grey) and *Tyrophagus putrescentiae* mites (dark grey) present in the Wax Moth + *Tyrophagus* (GMTP) test and control groups at the final 24 hour survival counts. Test colonies had *Parasitellus fucorum* added, controls did not. Thick horizontal line shows median value, thin horizontal lines represent 1st and 3rd quartiles, and vertical whiskers extend to maximum and minimum values within 1.5x inter-quartile range, with points showing outlying results beyond this range.

The results of GEE analyses based on two GLMs testing the impact of Group, Condition, Hour, and various interactions of these parameters on live prey numbers (*G. mellonella* eggs or *Tyrophagus putrescentiae* mites, respectively) in all conditions containing either prey species were clear in both cases. In the model which analysed live *G. mellonella* egg numbers throughout the experiment, two factors were found to have a significant effect. These were Group (i.e. Test/Control) ($X^2 = 4.42$, $p = 0.035$) and Hour ($X^2 = 17.53$, $p = 0.014$) (Table 4.1). Full model outputs for each parameter and fitted *G. mellonella* egg numbers are shown in Appendix L.

Source	DF	Chi-Square	p - value
Group	1	4.42	**0.035**
Hour	7	17.53	**0.014**
Group*Hour	7	11.11	0.134
Condition(Group)	2	2.66	0.265
Condition*Hour(Group)	11	10.12	0.519

Table 4.1 – Summary statistics from analysis of a GLM with GEE examining the effects of Group, Condition, Hour, and various interactions between these on live *Galleria mellonella* eggs numbers, calculated using maximum likelihood ratios. DF = Degrees of Freedom. p – values were calculated based on chi-square distributions (Pr > ChiSq), and significant results are shown in bold.

The results of a GLM which included numbers of live *Tyrophagus putrescentiae* mites during the experiment as the response variable showed that three parameters had a significant effect; Group (Test/Control) ($X^2 = 30.66$, $p < 0.001$), Hour of measurement ($X^2 = 33.95$, $p < 0.001$), and the interaction between these two parameters ($X^2 = 33.6$, $p < 0.001$). Full model outputs for each parameter and a figure showing fitted values for *T. putrescentiae* numbers at each hour are shown in Appendix K.

Source	DF	Chi-Square	p - value
Group	1	30.66	**< .001**
Hour	7	33.95	**< .001**
Group*Hour	7	33.6	**< .001**
Condition(Group)	2	2.9	0.234
Condition*Hour(Group)	12	10.37	0.584

Table 4.2 – Summary statistics from analysis of a GLM with GEE examining the effects of Group, Condition, Hour, and various interactions between these on numbers of live *Tyrophagus putrescentiae* mites, calculated using maximum likelihood ratios. DF = Degrees of Freedom. p – values were calculated based on chi-square distributions (Pr > ChiSq), and significant results are shown in bold.

4.5 Discussion

The results of this experiment demonstrate for the first time that the bumblebee associated mite *Parasitellus fucorum* is capable of predating both *Tyrophagus putrescentiae* mites and the eggs of the greater wax moth, *Galleria mellonella*. Predation of both prey species in laboratory conditions is shown by the greater reduction in live *G. mellonella* eggs or *T. putrescentiae* mites in Test groups (containing *P. fucorum* deutonymphs) over time when compared with Control groups (with no *P. fucorum*), as well as the significant effect of Group (Test/Control) on both live *G. mellonella* egg numbers and *T. putrescentiae* mites in all experimental conditions shown by the GEE results. Hour of measurement had a significant impact on live numbers of both prey varieties during the experiment, and in the case of *T. putrescentiae* mite numbers the interaction between Group and Hour also had a significant effect.

Tyrophagus putrescentiae was chosen as a prey species for these experiments for three reasons; it is often found within bumblebee colonies, *T. putrescentiae* is a small soft bodied mite species and represent likely prey for a large predatory mite such as *Parasitellus fucorum*, and protocols for rearing large numbers of *Tyrophagus* mites are well established (Sanchez-Ramos and Castanera, 2005, Canfield and

Wrenn, 2010, Hubert et al., 2012, Rożej et al., 2012). All results were conclusive regarding *T. putrescentiae* predation by *P. fucorum*, with the GEE analysis confirming that the presence of *P. fucorum* significantly reduced *T. putrescentiae* survival. No previous literature could be found demonstrating the predatory relationship between *P. fucorum* and *T. putrescentiae*, so the results presented here represent the first empirical evidence for this.

Galleria mellonella (the greater wax moth) was unlikely to represent a frequent 'natural' prey species for *Parasitellus fucorum* as it is not often found in bumblebee colonies and *P. fucorum* is only occasionally found within honeybee colonies (Alford, 1975, Chmielewski, 2003). *Aphomia sociella* (the bumblebee wax moth) had been successfully used in a pilot study prior to these experiments, which showed that the eggs and larvae of this species can be predated by *P. fucorum* (Appendix I). However for reasons of practicality *G. mellonella* was chosen as the second prey species for this experiment (see Introduction). The GEE analysis showed clearly that *P. fucorum* significantly reduced the survival of *G. mellonella* eggs in both experimental conditions where it was present (GM and GMTP). *P. fucorum* deutonymphs were therefore shown to predate *G. mellonella* eggs, though not as readily as they will predate *Tyrophagus putrescentiae* mites. This is supported by the greater reduction in live *T. putrescentiae* numbers than in *G. mellonella* eggs in the combined condition where both species were present (Figure 4.5), as well as the greater reduction in *T. putrescentiae* numbers when compared with *G. mellonella* eggs in the conditions containing a single prey variety (Figures 4.3 & 4.4).

This study represents a novel contribution to the current knowledge of the relationships between *Parasitellus fucorum* and two common pests of bumblebees and/or honeybees, *Galleria mellonella* and *Tyrophagus putrescentiae*. The results here also go some way towards validating the hypothesis that *Parasitellus fucorum* infestation may be beneficial for bumblebee colonies due to their predatory action against various pests.

Chapter 5 *Sphaerularia bombi* associations with English bumblebee species and their phoretic mites

5.1 Abstract

Sphaerularia bombi is a common parasite of bumblebee queens. This endoparasitic nematode invades the queen's abdomen, develops, and produces thousands of offspring. This results in the eventual death of the queen. It has been reported previously in some literature that *Parasitellus fucorum* (a predatory mite) may provide a form of defence to queens against infestation. This work was performed in order to explore this hypothesis.

We captured 121 bumblebee queens in the Harris Garden of the University of Reading, England from March – July of 2017/18. These queens were tested for infestation by *Sphaerularia bombi* for two purposes. Firstly to examine any relationships between nematode infestation and other factors of bumblebee queen ecology e.g. species, thorax width, month of capture, and the numbers/presence of different phoretic mites groups (*Parasitellus*, all other Mesostigmata and *Kuzinia*). Secondly, to run predation tests to determine whether mobile (infective) phase *S. bombi* could be predated by phoretic *P. fucorum* deutonymphs. While the predation test was ultimately unsuccessful and should not be considered robust, the preliminary results indicated that *P. fucorum* was unable to predate *S. bombi*.

The data collected on bumblebee queens proved more informative. A binomial generalised linear model was constructed to examine trends within the data. Our model strongly associated nematode infestation in bumblebee queens with their month of capture (79.823, 0.006) (residual deviance, p-value), the bumblebee species (94.313, 0.01), and phoretic *Parasitellus* numbers (73.197, 0.014). The proportion of captured queens infested with *Sphaerularia bombi* peaked in May (43 %), and the species with the highest proportions of infested queens were *Bombus lapidarius* (50 %) and *B. terrestris* (38 %). Queens with more phoretic *Parasitellus* mites were less likely to be infested by *S. bombi*, and a contrast was revealed as this association did not exist with either the 'other Mesostigmata' or the *Kuzinia* mite groups. The results of this study show that *Parasitellus* may be unique among bumblebee phoretic mites in their negative correlation with *Sphaerularia bombi* infestation in bumblebee queens. Further investigation is required to determine the causes of this inverse correlation.

5.2 Introduction

One insidious parasite of bumblebee queens is the nematode *Sphaerularia bombi* (Alford, 1969). This obligate endoparasite infests the abdomen of a hibernating bumblebee queen and alters their behaviour so that after emerging from hibernation, instead of founding a colony the infested queen visits different potential hibernation sites and excretes nematodes into the soil, where they will later attempt to infest other queens (Alford, 1975). This behaviour continues until the host queen's death (Alford, 1975, Schmid-Hempel, 1998). The queens are initially parasitized by one or more adult female nematodes,

which develop by everting and enlarging their uterus into a sac up to 20,000 times larger than the rest of the nematode. After the queen emerges from hibernation the nematode releases many eggs from this sac into the queen's abdominal haemocoel (Poinar and Hess, 1972). These eggs subsequently hatch into 1st instar larvae, moult twice, and the resulting 3rd instar larvae are excreted by the host bumblebee queen at potential hibernation sites. In the following months the nematodes enter their 4th instar, mate in the soil, and develop into their adult infective form. The nematodes then await an opportunity to infest a suitable bumblebee queen (Poinar and Hess, 1972, Kelly et al., 2009).

Parasitellus are a genus of predatory mites within the order Mesostigmata, and all *Parasitellus* species are obligate bumblebee associates (Chmielewski and Baker, 2008). *Parasitellus* deutonymphs will preferentially become phoretic upon bumblebee queens over any other caste, likely because phoresy upon a queen while she overwinters is how *Parasitellus* mites survive between bumblebee colony cycles (Huck et al., 1998). Due to their predatory activities *Parasitellus* are theorised to benefit their bumblebee hosts, although many instars are kleptoparasites (Koulianos and Schwarz, 1999, Schmid-Hempel, 1998). It was reported that an inverse correlation exists between the numbers of phoretic *Parasitellus* deutonymphs present on a bumblebee queen and infestation by *S. bombi* within the queen (Schousboe, 1987). In more recent literature this correlation seems to have been interpreted as evidence that *Parasitellus* mites were responsible for reducing *S. bombi* infestations (Eickwort, 1994, Schmid-Hempel, 1998).

It is possible that previous claims of *Parasitellus* reducing *S. bombi* infestation prevalence are true, as *Parasitellus* deutonymphs phoretic upon an overwintering queen may predate mobile *S. bombi* nematodes within the hibernaculum. In this work, an experiment was designed in order to test the hypothesis that *Parasitellus* deutonymphs were capable of predating *Sphaerularia bombi* nematodes in controlled laboratory conditions. A number of overwintered bumblebee queens were caught while foraging in spring and then reared in captivity. Any mobile *S. bombi* nematodes excreted by infested queens were collected, counted and used for a predation trial with *Parasitellus fucorum* deutonymphs. While the predation trial ultimately proved unsuccessful, the data collected from the captured bumblebee queens proved very useful. These data were analysed and trends were explored in order to shed light on any relationships between *S. bombi* infestation and the other factors measured in spring bumblebee queens.

5.3 Methods

5.3.1 Bumblebee queen capture, processing and rearing

Queens were caught from March – July of 2017 and 2018 using an insect net. The date of capture and species of each queen was recorded, and queens were taken to the laboratory. Captured bees were anaesthetised using CO_2 and all 'large' mites (*Parasitellus* and other mesostigmatid mite genera) were removed using a paintbrush, separated into morphological groups (*Parasitellus* and all Other Mesostigmata), and counted. Mites from the Order Mesostigmata other than the *Parasitellus* genus were

stored in 70% ethanol, and *Parasitellus* deutonymphs were reared in the laboratory for use in the predation experiment (Appendix M). The presence of phoretic *Kuzinia* (Cohort Astigmata) was recorded, but these mites were not removed or counted due to the difficulty in doing so without damaging the bumblebee. Processed bumblebees were moved into modified sandwich boxes containing a 45ml base layer of autoclaved terrarium sand (Trixie, Germany) and two feeding trays. One feeding tray was filled with Invertbee syrup (36% Fructose/31% Sucrose/30% Dextrose/3% other sugars) (Wyefield Apiaries, Wales) and the other filled with organic honeybee pollen (Naturwaren Niederrhein GmbH, Hungary). These were refilled regularly to provide an *ad libitum* food supply (Figure 5.1). Once set up these boxes (henceforth referred to as 'queen boxes') were moved into a Controlled Environment (CE) chamber (Weiss Technik, Germany) kept dark at constant conditions of 21°C/80%rh, and only removed for food additions and nematode testing. High humidity was maintained to slow the desiccation of any nematodes excreted into the sand substrate.

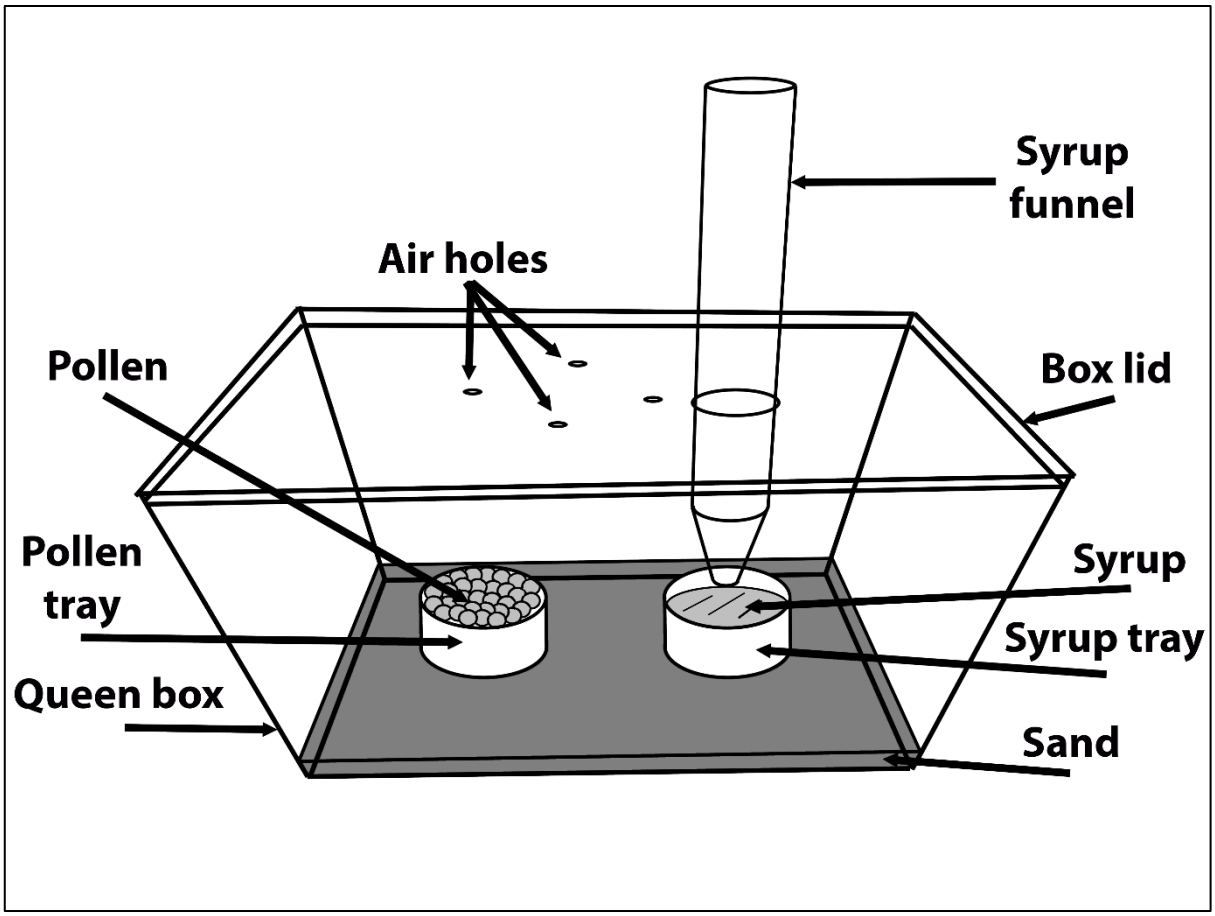

Figure 5.1 – Diagram of bumblebee queen storage box.

All queen boxes were maintained until either the queen died or two weeks passed, after which the queen was either stored in 70% ethanol for later dissection or transferred to a new box depending on *S. bombi* infestation (all infested queens were kept alive until their natural death, while all queens shown to lack *S. bombi* infestation after storage in two boxes were euthanised).

5.3.2 Nematode testing and extraction protocol

Every two weeks (or upon the death of the bumblebee queen) the sand substrate from used queen boxes was tested for the presence of mobile phase *Sphaerularia bombi* nematodes. To do this a modified 'decant and sieve' method was used, based upon that employed by Kelly (2009).

If alive, the queen within the box was transferred into a newly prepared box and returned to the CE chamber. Dead queens were stored as outlined above. The feeding trays were removed from the old box, along with any stray pollen balls or detritus that were present in the sand sample. The cleaned sand was poured into a 1L conical flask and covered with 400-600ml filtered water, then agitated strongly to suspend the sand sample. The sand was allowed to settle for 30 seconds, then some of the supernatant was poured through a funnel containing grade 1 filter paper (Whatman, UK) suspended over another 1L conical flask. The agitation and decanting of supernatant was repeated twice more, after which the filter paper was removed and the retentate rinsed into a petri dish. This was examined closely using a Motic SMZ171 microscope with 1-5x objective lenses, and if nematodes were observed then this was recorded (and the queen noted as *Sphaerularia bombi* infested), the nematode suspension transferred to an insect jar (Sterilin, UK), and the sand sample processed further (see below). If no nematodes were observed then the sand sample was discarded and a negative result recorded.

To extract nematodes from an *S. bombi* positive sand sample the decant and sieve method was repeated continuously until all supernatant had been put through a new filter paper. Afterwards another 400-600ml filtered water was added to the sand sample, and the decant and sieve method was repeated until the supernatant was completely clear after agitation. All retentate on this filter paper was then rinsed off into an insect jar as before, and the filter paper was replaced. To ensure that all nematodes had been removed from the sand sample the decant and sieve method was repeated once more, and if <10 nematodes were counted in the petri dish at the end then no more washes were performed and the sand was disposed of. If >10 nematodes were counted then the extraction protocol was repeated in full until this testing returned <10 nematodes. All nematode suspensions taken from a single sand sample were stored in an insect jar for later counting and nematode dosage preparation for the predation experiment (Appendix M).

5.3.3 Queen dissection protocol

All queens collected were dissected to search for reproductive stage female *Sphaerularia bombi* within their abdomens. This was done by first removing the queen from the 70% ethanol in which it was stored, after which the thorax width was measured (at the widest point) using digital callipers (Tacklife, China) and the queen was pinned to a dissecting board ventral side up. The sternites on the abdomen were carefully cut before pulling the ventral side of the abdomen away and pinning it open. The internal contents of the abdomen were carefully examined using a Motic SMZ171 microscope with 1-5x objective lenses, and the number of reproductive stage female *Sphaerularia bombi* observed was recorded. The bumblebee thorax and head were then returned to storage.

Ultimately the dissection results were not used due to some instances where nematodes were excreted by the queen but no mature nematodes were found internally due to decay in storage. As a result of this the data on queens which excreted live *Sphaerularia bombi* in their queen boxes were used instead, as mobile *S. bombi* excretion represents conclusive evidence of an internal infestation (Alford, 1975).

5.3.4 Statistical analysis

The data from this work consisted of a variety of results from each bumblebee captured. These included bumblebee species, month of capture, thorax width, nematode infestation, phoretic *Parasitellus* mite numbers, phoretic mite numbers from all 'other Mesostigmata' genera, and the presence of phoretic *Kuzinia* mites.

All statistical testing was performed using R v3.4.2 (R Core Team, 2017) and RStudio (RStudio Team, 2015). All figures were constructed using the ggplot2 package (Wickham, 2016). Kolmogorov-Smirnov tests were carried out in order to determine normality, and Bartlett's test was used to test for homogeneity of variances. A binomial Generalised Linear Model (GLM) was constructed using the glm function within R on a data subset containing only complete records (see below), with all bumblebee species where $n < 5$ were combined into the 'Other *Bombus'* group within the model. Nematode infestation was used as the response variable and month of capture, bumblebee species, thorax width, presence of phoretic *Kuzinia* mites, and the numbers of phoretic *Parasitellus* and 'other Mesostigmata' were included as fixed effects. Comparative tests using reduced models were conducted to optimise both AIC and residuals. The Hosmer-Lemeshow Goodness of Fit (GOF) test in the ResourceSelection R package was used to determine the GOF of the model (Lele et al., 2019). An ROC curve and the calculation of the model's Area Under Curve (AUC) were both produced using the ROCR package (Sing et al., 2005).

Queens of 9 different bumblebee species were captured, with 121 queens caught in total. In the early stages of the study some data were not recorded and so some records were incomplete. To resolve this only complete records were used for each test conducted, and a more conservative dataset of 109 records (complete for all factors except phoretic mites) was used in most instances. Only testing of nematode presence vs bumblebee species and bumblebee species vs month of capture used the full dataset of 121 queens, as using any data subset significantly changed the results of analyses. All tests involving phoretic mites used a smaller subset of 95 records with complete data for all factors, as did the binomial GLM. Where any other subsets of the data (e.g. *Bombus terrestris* data alone) were used, n-numbers are quoted in the text.

5.4 Results

From most to least common (in the full dataset of 121 queens), the bumblebee species captured were *Bombus terrestris* (61, 56 %) (n, percentage of total), *B. vestalis* (12, 11 %), *B. lucorum* (10, 9 %), *B. hypnorum* (8, 7 %), *B. ruderatus* (5, 5 %), *B. pascuorum* (4, 4 %), *B. hortorum* (4, 4 %), *B. lapidarius* (4, 4 %), and *B. pratorum* (1, 1 %).

The relative rates of *Sphaerularia bombi* infestation among the different bumblebee species collected were as follows; *Bombus lapidarius* (50 %), *B. terrestris* (38 %), *B. pascuorum* (20 %), *B. ruderatus (*17 %) and *B. lucorum* (15 %). No queens from other species collected (*B. hortorum, B. hypnorum, B. pratorum* and *B. vestalis*) were infested by nematodes (Figure 5.2). The proportion of *B. terrestris* infested queens captured per month was found to peak in May (Figure 5.3).

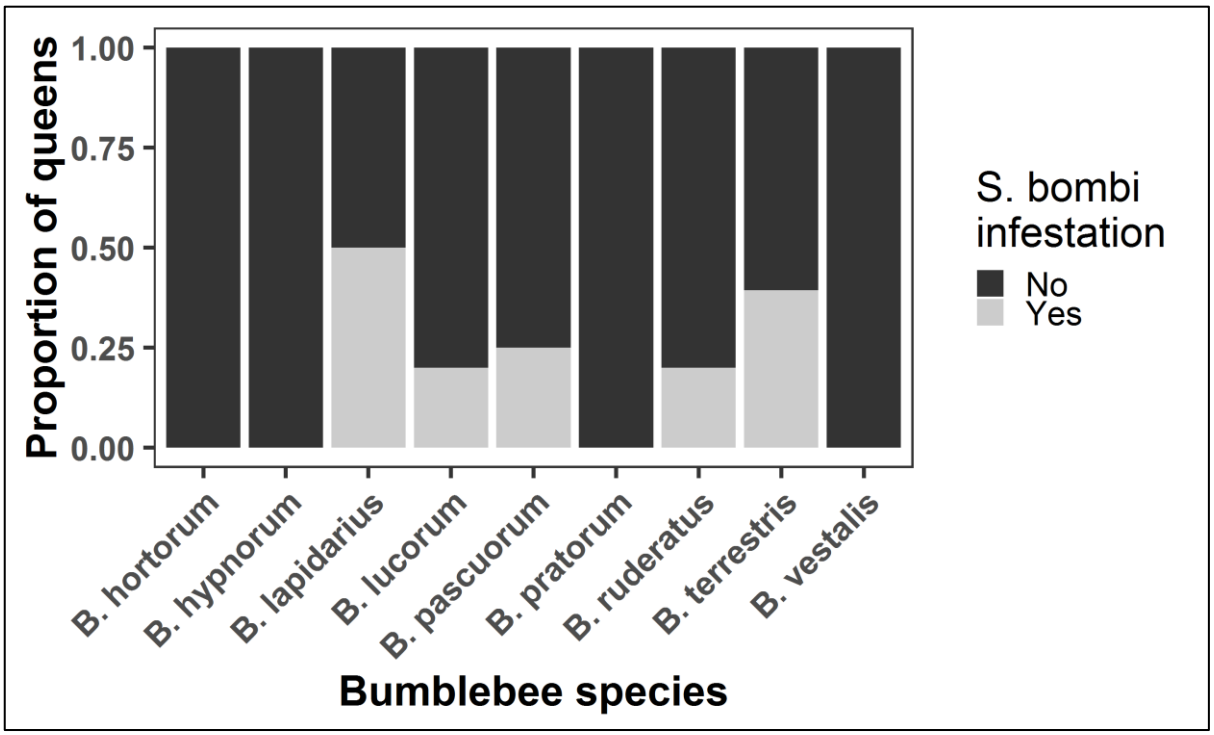

Figure 5.2 – Proportion of bumblebee queens infested or not infested by *Sphaerularia bombi* shown for all species collected.

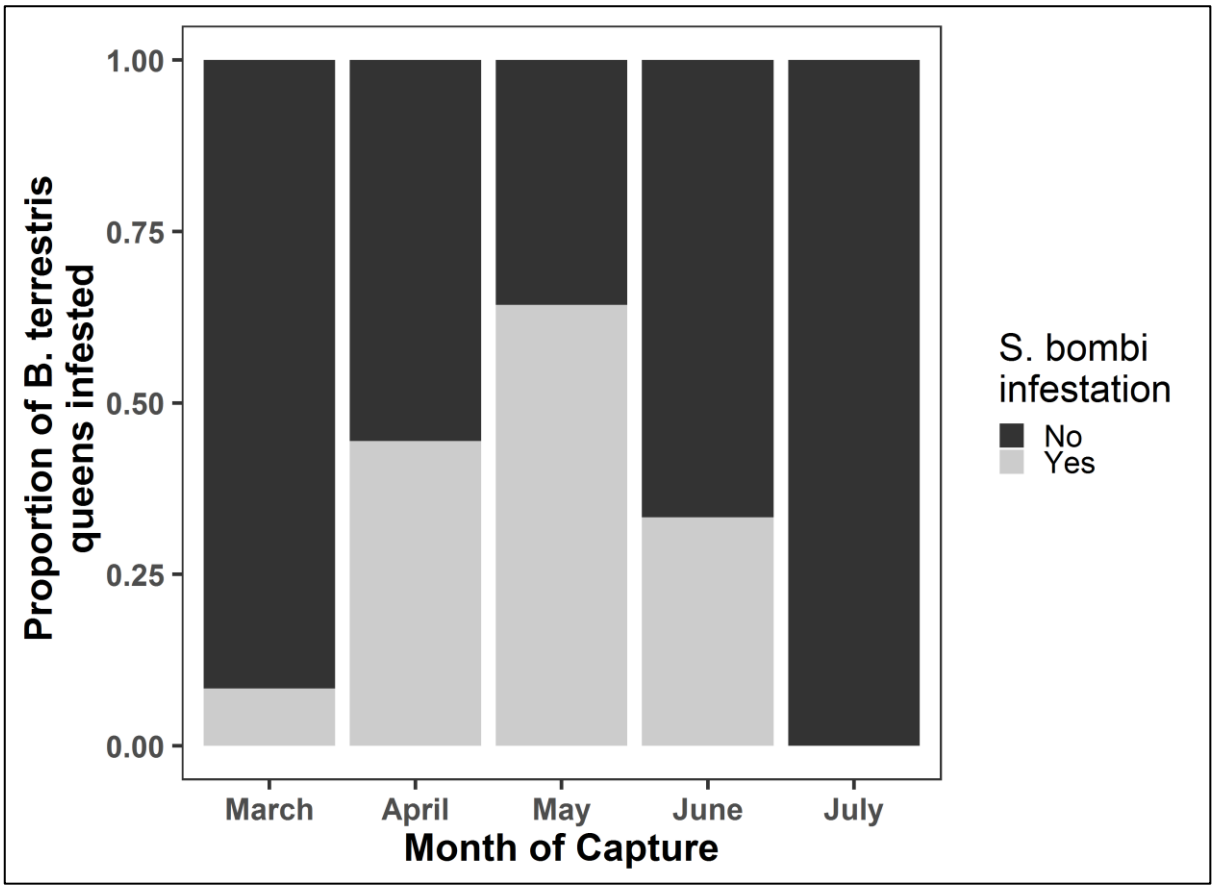

Figure 5.3 – Proportion of *Sphaerularia bombi* infested *Bombus terrestris* queens caught by month of capture.

The numbers of phoretic *Parasitellus* mites found on bumblebee queens infested (4.44, 8.417) (mean, standard deviation) or not infested (3.586, 8.659) with *Sphaerularia bombi* differed noticeably, and it was observed in the results that (with the exception of *B. lucorum*) nematode infested queens carried equal or lower average numbers of phoretic *Parasitellus* mites (Appendix N). The same was true of the numbers of all other Mesostigmata phoretic on queens infested (0.32, 0.9) or not (0.429, 1.314) with *S. bombi*. When the presence of phoretic *Kuzinia* mites was compared against nematode infestation, it was observed that a similar percentage of queens not infested by *S. bombi* (25.7 %) carried phoretic *Kuzinia* to queens which were nematode infested (28 %).

5.4.1 Binomial GLM for nematode infestation

A binomial generalised linear model constructed using only the data which were complete in all aspects (n = 95) showed that month of capture, bumblebee species and phoretic *Parasitellus* numbers were significantly associated with *Sphaerularia bombi* infestation in the bumblebee queens, as seen in the analysis of deviance table (Table 5.1). The model was also compared (by ANOVA) against a series of null models missing a single explanatory variable, and the missing variables which resulted in significant differences between the two models were month of capture (-15.666, 0.003) (deviance, p-value), bumblebee species (-13.603, 0.018) and phoretic *Parasitellus* numbers (-6.027, 0.014). A Hosmer-Lemeshow Goodness of Fit (GOF) test was conducted and showed that the model was well fitted to the data ($X^2 = 3.144$, $p = 0.925$). A ROC curve was also plotted and found satisfactory with the Area Under Curve (AUC) calculated at 0.810 (Appendix N).

Coefficients	Df	Residual Deviance	p value
(NULL)	94	109.503	-
Species	89	94.313	0.01
Month	85	79.823	0.006
Thorax Width	84	79.49	0.564
Other Mesostigmata	83	79.384	0.744
Kuzinia	82	79.224	0.689
Parasitellus	81	73.197	0.014

Table 5.1 – Analysis of deviance table for binomial generalised linear model of Nematode infestation response variable against the following explanatory variables; Month, Species, Thorax width, phoretic *Parasitellus* and other Mesostigmata numbers, and phoretic *Kuzinia* presence. Binomial model used with logit link function. p values shown are Pr(>Chi) output calculated using chi-square method. Significant p values (>0.05) are underlined.

Coefficients	Estimate	Standard Error	z value	p value
Species - *B. terrestris*	-9.203	4.584	-2.008	0.045
Species - *B. hypnorum*	-22.990	2754.759	-0.008	0.993
Species - *B. lucorum*	-0.1	1.286	-0.078	0.938
Species - *B. ruderatus*	3.254	1.925	1.690	0.091
Species - *B. vestalis*	-18.766	2579.964	-0.007	0.994
Species - Other *Bombus*	0.170	1.049	0.162	0.871
Month - April	2.145	1.059	2.026	0.043
Month - May	4.307	1.419	3.035	0.002
Month - June	2.715	1.327	2.047	0.041
Month - July	-15.730	2336.516	-0.007	0.995
Thorax width	0.774	0.511	1.515	0.13
M	-0.550	0.474	-1.160	0.246
K (Y)	-0.537	0.726	-0.739	0.46
P	0.142	0.064	2.240	0.025

Table 5.2 – Summary results for all parameters in binomial generalised linear model of Nematode infestation response variable against the following explanatory variables; Month, Species, Thorax width, phoretic *Parasitellus* numbers (P), phoretic 'other Mesostigmata' numbers (M), and phoretic *Kuzinia* presence (K (Y/N)). Binomial model used with logit link function. p value shown is summary output Pr(>|z|) and was calculated using chi-square method. Significant p values (>0.05) are underlined.

5.5 Discussion

The results of this study demonstrated that *Sphaerularia bombi* infestation in bumblebee queens is strongly associated with the time of year (month of capture), the species of bumblebee, and the numbers of phoretic *Parasitellus* mites.

The month of capture arguably had the strongest association of any factor with *Sphaerularia bombi* infestation. The evidence for this comes from the binomial model which showed significant variation in the numbers of infested queens captured during different months. Removal of this factor from the model

resulted in the most significant differences of any factor when compared with the full model, and its inclusion caused a large reduction in residual deviance (Table 5.1). Logically this makes sense, as the incidence of internal *Sphaerularia bombi* parasites is tied to the emergence of bumblebee queens in spring. It is probable that as the season progresses, the queens available for capture in the field (i.e. queens that have not yet founded colonies) from any particular species will be more likely to be infested with *S. bombi*, as parasitised queens make up an ever-larger proportion of the available population (Hattingen, 1956, Alford, 1975). This is supported by the fact that in our study captured *Bombus terrestris* numbers peaked in April while the proportion of infested *B. terrestris* queens was highest in May, although this decreased subsequently in June and July (perhaps as a result of British *B. terrestris* going through multiple colony cycles per year in recent times, meaning 'fresh' queens may have entered the field (Goulson, 2010)). The differences in nematode infestation between different months of capture for *B. terrestris* queens alone were also statistically significant.

Bumblebee species was also associated with *Sphaerularia bombi* infestation. This was demonstrated by the significant differences to the binomial model caused by the removal of this variable and by the large decrease in residual deviance observed in Table 5.1. Significant differences in nematode infestation between bumblebee species are not surprising when the results are examined (i.e. *Bombus terrestris* having a 38 % infestation rate and *B. vestalis* a 0 % infestation rate), particularly when previous studies on this subject have concluded along the same lines (Hattingen, 1956, Alford, 1975). The reasons for differing infestation rates between bumblebee species are commonly suggested to be their choice of hibernation site and the relative abundance of each species. This is because mobile phase nematodes will undoubtedly have environmental preferences and will likely transmit more easily between individuals of a locally abundant bumblebee species (Cumber, 1949, Hattingen, 1956, Alford, 1975). These hypotheses seem credible based on the results of this study, as the most numerous species collected (*B. terrestris*) during this study also had the 2nd highest infestation rate of all species tested, and the only species with a higher infestation rate (*B. lapidarius*) had a sample size of 4 so its results cannot be considered robust. Due to the lack of significant differences in *S. bombi* infestation between individual bumblebee species no predictions are made here regarding which species are statistically more likely to be infested with *S. bombi*, though the summary of the binomial model does suggest *B. terrestris* might be more likely to become infested than other species (Table 5.2). It can however be said with confidence that species is a strongly associated factor in the probability of nematode infestation in bumblebee queens.

Phoretic *Parasitellus* numbers did not vary significantly between *Sphaerularia bombi* infested and non-infested queens when univariate tests were used, however the binomial model assigned the *Parasitellus* explanatory variable significance in the residual deviance table (Table 5.1), and significant differences between models resulted if the *Parasitellus* variable was removed. This shows that lower phoretic *Parasitellus* numbers are associated with higher chances of a bumblebee queen having been infested by *S. bombi*. Previous research has shown a negative correlation between *Parasitellus* numbers and the

likelihood of a queen being infested with *S. bombi*, and the results here support this (Schousboe, 1987). It is possible that *Parasitellus* deutonymphs protect bumblebee queens against nematode infestation (as others seem to have concluded previously), but we propose that is more likely *Parasitellus* may simply recognise when queens are infested by *S. bombi* and abandon them. This is supported by the fact that no *Parasitellus fucorum* deutonymphs were observed predating mobile phase *S. bombi* nematodes during the predation experiment (Appendix M), and that more phoretic *Parasitellus* were generally found on non-infested queens within each species (Appendix N).

Neither the numbers of phoretic mites from all 'other Mesostigmata' genera, nor the presence of phoretic *Kuzinia* mites were significantly associated with nematode infestation in bumblebee queens. This was shown by the results of the binomial model. These are intriguing results, as it might have been expected that all phoretic bumblebee mites would behave similarly in response to *Sphaerularia bombi* infestation, however this is evidently not the case. This suggests that *Parasitellus* deutonymphs may be unique in their apparent ability to recognise and avoid/abandon *S. bombi* infested bumblebee queens.

The results of this study therefore provide support to Schousboe (1987)'s reported negative correlation between phoretic *Parasitellus* mite numbers and the chances of bumblebee queen infestation by the parasitic nematode *Sphaerularia bombi.* The link between phoretic *Parasitellus* numbers and *S. bombi* infestation in queens is shown in stark contrast to the lack of any such link when other phoretic mite groups are examined. Why only phoretic *Parasitellus* deutonymphs appear to have a negative correlation with *S. bombi* infestation when the 'other Mesostigmata' or *Kuzinia* mites do not is a question worthy of further research. It would also be useful to understand whether the differences in *S. bombi* infestation between bumblebee species are simply a result of local abundance and colony locations, or if there are other factors not yet identified. It is also of key importance to determine whether *Parasitellus* deutonymphs can predate mobile *S. bombi* nematodes and protect bumblebee queens. The answers to these questions would shed more light on the nature of relationships between bumblebees and their associated mites, as well as revealing more detail on the complex relationship between *Sphaerularia bombi* and its bumblebee hosts, and how phoretic mites may impact this.

Chapter 6 *Parasitellus fucorum* inoculation benefits bumblebee colonies

6.1 Abstract

We tested the effects of inoculations of two different mite species (*Parasitellus fucorum* and *Tyrophagus putrescentiae*) on the development of commercial colonies of *Bombus terrestris audax* in field conditions, in order to determine whether the presence of either mite species impacted the bumblebee host at the colony level. Four treatment groups were established based on bumblebee colonies' mite inoculations; Controls, *P. fucorum*, *T. putrescentiae* and both mite species in combination. Colonies were supplied *ad libitum* with 'Invertbee' syrup and left to develop naturally for 8 weeks after setup in the field. Colony weight changes and syrup consumption were monitored over this period, and finally all colonies were destructively sampled and the numbers/weights of bees within were counted. Samples of colony materials were processed for mites, and all mites phoretic upon the bumblebees were removed, counted and identified.

In overall colony weight changes, the group inoculated with *P. fucorum* (P) showed the greatest weight gain (165%) relative to the control group, followed by the *T. putrescentiae* (T) group (72%) and the combined (PT) group (15%). This trend was mirrored in the overall syrup consumption results. Colonies in the *T. putrescentiae* group contained the highest numbers of bumblebee workers at the end of the experiment (38 ± 9) (median ± Inter-Quartile Range (IQR)), closely followed by the *Parasitellus* group (35 ± 24), the PT group (26 ± 20) and the controls (16 ± 12). All mite-inoculated treatment groups on average gained more weight, consumed more syrup, and had higher bumblebee populations than the control group.

The results of this experiment provide the first empirical evidence that the presence of *Parasitellus fucorum* within bumblebee colonies provides a benefit to the overall fitness of these colonies. This result has major implications for our understanding of the relationships between bumblebees and their associated mite species, and suggests a mutualistic relationship at the bumblebee colony level.

6.2 Introduction

The effects that bumblebee associated mites have on their hosts, either at the individual or colony level, have been discussed often in the literature (Huck et al., 1998, Rożej et al., 2012). However there is little in the way of empirical evidence to support any claimed effects on bumblebees, except in the case of the internal parasitic mite *Locustacarus buchneri* (Otterstatter et al., 2004, Otterstatter et al., 2005, Yoneda et al., 2008a). While the lack of evidence places these claims in some doubt, a consensus has emerged in the literature that bumblebee colonies infested by *Parasitellus fucorum* are likely to receive some benefit from their presence (Schmid-Hempel, 1998, Chmielewski and Baker, 2008, Koulianos and Schwarz, 1999). It is commonly suggested that this is because *P. fucorum,* a large mite from the Order

Mesostigmata, predates pests and smaller kleptoparasitic mites within bumblebee colonies (Koulianos and Schwarz, 1999, Eickwort, 1994, Chmielewski and Baker, 2008, Schmid-Hempel, 1998). *Parasitellus fucorum* mites are predatory and/or kleptoparasitic (feed on bumblebee collected pollen within the colony) depending on sex and instar of development, and the net effect upon bumblebees from both the pollen feeding (presumed negative) and predatory (presumed positive) activities have been the subject of speculation in the literature (Richards, 1976, Koulianos and Schwarz, 1999, Chmielewski and Baker, 2008). *P. fucorum* is also an obligate bumblebee associate which engages in phoresy upon bumblebees for transport between colonies and to overwinter upon queens (Chmielewski and Baker, 2008, Schwarz and Huck, 1997). Phoresy is the act of one organism using another purely as a means of transportation (Houck and OConnor, 1991).

Tyrophagus putrescentiae (the 'mould mite') is a small mite belonging to the Cohort Astigmata which feeds on fungi that develops on decaying foodstuffs (Smrž and Čatská, 1987, Klimov et al., 2016f). *T. putrescentiae* is frequently found within bumblebee colonies despite not engaging in phoresy (Rożej et al., 2012, Revainera et al., 2014). Bumblebee colonies commonly become mouldy (Alford, 1975), and this might negatively impact the health of the colonies. *T. putrescentiae's* fungivorous diet might reduce any negative effects to colony health by feeding on fungal hyphae, and therefore benefit the bumblebee colony (Smrž and Čatská, 1987).

This experiment was designed to test the hypothesis that the presence of *Parasitellus fucorum* or *Tyrophagus putrescentiae* has a measurable impact on the fitness of bumblebee colonies which they inhabit. In order to explore this, commercially purchased bumblebee colonies were inoculated with one, both, or neither mite species before being placed outdoors and allowed to develop naturally. Differences in colony development and bumblebee numbers within the colonies were examined during the experimental period, and particularly at the end when all colonies were destructively sampled.

6.3 Methods

In this experiment 4 conditions were tested based on the mites inoculated into each bumblebee colony at the start of the experimental period:

1. Control group (C)
2. *Parasitellus fucorum* (P)
3. *Tyrophagus putrescentiae* (T)
4. *Parasitellus fucorum* and *Tyrophagus putrescentiae* (PT)

Nine replicates of the experiment were conducted, using 36 bumblebee colonies in total. The bumblebee colonies were commercially purchased *Bombus terrestris audax* colonies supplied through Agralan ltd (Swindon, United Kingdom) by Biobest Group NV (Westerlo, Belgium). All colonies used were taken from the same production run and were 4 weeks younger than standard commercial colonies at the time of shipping. Delivery of the colonies was split over two days, with 16 delivered and established on

24/05/18 and the remaining 20 on 25/05/18. All checks and final processing of colonies were likewise staggered across two days.

6.3.1 Mite preparation

Bumblebee colonies from 2 conditions (P & PT) were each inoculated with 10 *Parasitellus fucorum* deutonymphs obtained from commercial *Bombus terrestris audax* colonies which had been kept outdoors in natural conditions. The collections of *Parasitellus* deutonymphs from these bumblebee colonies were performed by anaesthetising the colonies using CO_2 before removing bees with visible phoretic *Parasitellus* deutonymphs. The deutonymphs were removed from these bees using probes and paintbrushes then transferred to laboratory colonies where they were kept until the start of the experiment, at which time deutonymphs were used for experimental inoculations.

Parasitellus laboratory colonies (Figure 6.1) were set up by first filling an 750ml vented cap tissue culture flask (Greiner Bio-One Ltd., UK) with moist, autoclaved peat (Sycamore Trading, UK) up to the halfway point, before adding commercially purchased fresh mealworm (livefoods4u, UK) (in small slices) and organic honeybee pollen (Naturwaren Niederrhein GmbH, Hungary) supplemented with fresh bumblebee-collected pollen. All foodstuffs were frozen at -20°C prior to usage to ensure freshness. After this, *Parasitellus* deutonymphs were added. Colonies were kept in a controlled environment at 24°C with ambient humidity and no lighting between feeding. The colonies were maintained by replacing their food daily and transferring any observed adult mites into new colonies, which were maintained in the same way. This protocol was developed based upon previous work in this area by Koulianos and Schwarz (1999).

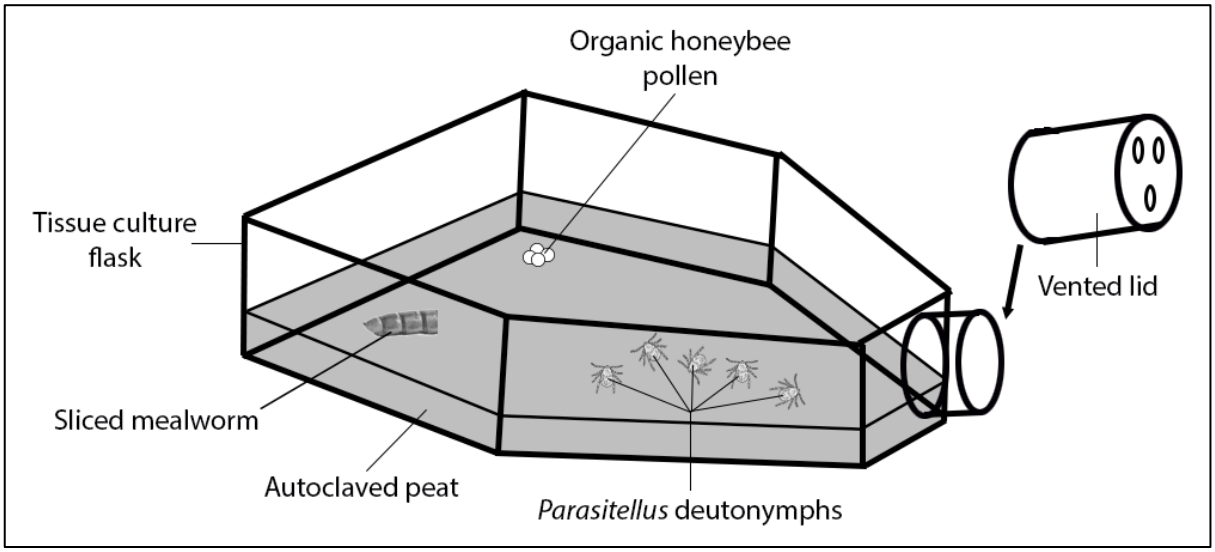

Figure 6.1 – *Parasitellus* laboratory colony design.

Bumblebee colonies inoculated with 10 *Tyrophagus putrescentiae* mites (T & PT conditions) had their mites taken directly from laboratory colonies (Acarology Lab, University of Reading) which had been maintained long-term following a standard protocol (Hubert et al., 2012). These colonies were established in 25ml vented cap tissue culture flasks, and given a mixture of yeast, oats and flour for food

as needed after the addition of the first mites. The colonies were kept within a larger box containing a base of moist tissue paper, wrapped in foil to maintain internal darkness and high humidity at room temperature (21-24°C) (Hubert et al., 2012). The colonies were checked weekly to determine when more food was needed, and when the population of any mite colony became saturated a new colony was established using mites taken from the older colony.

For both species (*P. fucorum* and *T. putrescentiae*), the mites were transferred into 50ml Falcon tubes using probes or paintbrushes in their required numbers (separate tubes used for *Tyrophagus* and *Parasitellus* with PT colonies) before the start of the experiment for later inoculation in the field.

6.3.2 Experimental protocol

Upon delivery the commercial bumblebee colony syrup feeders were standardised. Syrup feeders beneath the colony boxes were removed, emptied, and each was then filled with ~1.6kg Invertbee syrup (36% Fructose/31% Sucrose/30% Dextrose/3% other sugars) (Wyefield Apiaries, Wales). All colonies were also given 10g organic honeybee pollen (Naturwaren Niederrhein GmbH, Hungary) to ensure an adequate food supply during the experimental setup. Each syrup feeding tray was then weighed, as were the colony boxes. These measurements provided the principle metrics for comparing colony development over time by recording the change in weight of each colony box and syrup feeding tray.

After this, all colonies were placed in the field at Sonning Farm, University of Reading (51°28'58.2"N 0°53'55.7"W). The area in which colonies were placed was bordered by fallow fields containing many wildflowers and unmaintained forest/scrubland. Colonies were arranged by treatment, with 4 metres separating each colony within treatment groups and 8 metres separating treatment group rows from each other. This was to limit cross-infestation of phoretic mites between treatment groups caused by bumblebee workers visiting neighbouring colonies from other treatment groups (Schwarz and Huck, 1997, Huck et al., 1998).

Each colony was placed within an insulating Bee-Coat (Biobest Group NV, Belgium) and had unique ‘landmarks’ placed on the front and top including variously coloured foam stickers (Grafix, USA) and plastic egg skewers (Easter Wishes, USA) and roman numerals used as labels (Figure 6.2). This was done to further reduce any cross-contamination of mites between colonies due to bumblebees entering colonies other than their own (Free, 1958, Birmingham, 2003). Finally, mite inoculations were performed by agitating the 50ml tubes containing the required numbers of mites directly above the opened colony boxes, ensuring that all mites fell from the tubes into the bumblebee colonies.

Figure 6.2 – Two examples of unique 'landmarks' placed on the front of each bumblebee colony box. Roman numerals and letters indicating treatment group are visible on top of each colony.

Colonies were then left undisturbed except for weekly checks. Checks took place at the same time on the same days every week, with each colony syrup feeding tray and colony box removed and weighed. If any colony syrup feeding tray fell below 750g in weight (from the starting weight of ~1640g) then more Invertbee syrup was added to ensure a consistent *ad libitum* supply. Syrup feeders were weighed before and after syrup addition so that this could be accounted for in the data recording.

The only exception to this protocol was between weeks 4/5 of the experiment when all colonies had their Bee-Coats unfolded (except for the 'landmarked' front panel) and their container lids partly opened. This was done in order to allow greater ventilation of the colonies during an extended heatwave which saw daytime temperatures maintained well above 25°C (the temperature at which Agralan recommends treating colonies this way to avoid damage) and nightly temperatures remained above 15°C. All colonies were left this way until the conclusion of the experiment as conditions remained fairly constant during this period.

After 8 weeks the experiment was concluded, and all colony boxes and syrup feeding trays were weighed one final time. The colonies were first anaesthetised with CO_2 gas in an enclosed box then carefully dissected using forceps. Live bumblebees were removed from each colony and stored in zip-lock bags then frozen at -20°C for later processing. Fifty grams (50g) of colony materials were then transferred into sealed sandwich boxes so that any mites free-living within the sample could be removed, mounted and identified to species level. If 50g of colony materials could not be collected due to the whole colony weighing less than this, then all colony material was removed and the total weight recorded.

Each colony material sample was processed by hand to collect mites living within the colonies. This was done by taking a small quantity of the material, placing it within a petri dish, examining and manipulating it under a microscope with 1-5x objective lenses and removing all observed mites. Mites were removed using a paintbrush or probe and stored in 70% ethanol for later analysis. This was repeated until the whole sample had been analysed, after which the colony materials were discarded. Processing all samples took 4 days and was conducted solely by the lead researcher.

To process the bees removed from the experimental colonies, each bumblebee was weighed and examined under a dissecting microscope with 1-5x objective lenses to determine sex. After this the bee was manipulated using forceps and probes, and all phoretic mites observed were removed and stored in 70% ethanol for later mounting and identification. If many mites were observed on the bee, or there was any doubt that all mites had been removed, the bee was put through the 'ethanol wash' method of phoretic mite removal to ensure that all mites were counted. To do this, the bee was placed into a 50ml tube, covered with 5ml 70% ethanol, and vortexed for 30 seconds (VWR Lab Dancer S42). The ethanol was then removed and all mites present within the ethanol were counted, separated by morphospecies, and stored in Eppendorf tubes containing 70% ethanol for later analysis. This process was repeated until no more mites were found after two consecutive washes. The bumblebee was then left until the ethanol had evaporated off, and returned to the freezer bag with all other bees from its experimental colony. Once all mites had been removed from all bees from a colony, the freezer bag was placed back into storage at -20°C. This process was repeated for every colony.

6.3.3 Mite identification

All mites collected from colony materials and found phoretic upon bumblebees were cleared (i.e. internal organs dissolved to allow observation of external features) using 50% lactic acid and mounted on microscope slides following suggestions from Krantz and Walter (2009). After mounting, mites were identified morphologically by following the identification keys within the usual works of reference for this field of study, and by comparing observed morphologies with species descriptions within these same references (Hyatt, 1980, Baker et al., 1999, Fan and Zhang, 2007, Krantz and Walter, 2009, OConnor and Klimov, 2012b, OConnor and Klimov, 2012a, Jagersbacher-Baumann, 2014, Klimov et al., 2016g).

6.3.4 Statistical analysis

The data from this work consisted of weekly measurements of colony weight and syrup consumption taken for each bumblebee colony throughout the experimental period, and the data collected from every colony at the end of the experiment during destructive sampling. These included the overall colony weight changes and syrup consumption, and the numbers of *Parasitellus*, other Mesostigmata, *Kuzinia* and *Tyrophagus* mites present within the colony materials. These data also included the numbers and weights of all live bumblebees of different castes within each colony, and the numbers of phoretic mites present on each bee (split into the same 4 morphological categories as above).

All univariate analyses were conducted using R v3.4.2, as was a linear mixed effects analysis (R Core Team, 2017). Kolmogorov-Smirnov normality testing showed that almost all data collected were not normally distributed, so non-parametric testing was used unless otherwise stated. Kruskal-Wallis and Wilcoxon rank sum tests were used to analyse the variance in results. One-way ANOVA was used to test differences in the numbers of queens found within bumblebee colonies. The numbers of mites collected from colony samples were scaled based on the disparity between sample weight and total weight of colony materials, after which they were analysed.

R studio and the lme4/lmerTest packages were used to conduct linear mixed effects analyses examining the relationship between bumblebee colony weight and the treatment group (RStudio Team, 2015, Bates et al., 2015, Kuznetsova et al., 2017). To determine if treatment group affected bumblebee colony weight it was used as the main response factor, with syrup consumption included as an additional fixed effect. Week of measurement and individual colonies were included as random effects in order to account for variation from these factors. A random slope model was used, and p-values for fixed effects were obtained using lmerTest t-tests following Satterthwaite's method. A p-value for the model was obtained by performing an ANOVA of the full model against a reduced model lacking the treatment group fixed effect.

SAS 9.4 was used to produce two Generalised Linear Models (GLMs) which utilised Generalised Estimating Equation (GEE) analysis (SAS Institute Inc., 2013). The first model predicted the effects of treatment group, week of measurement, the interaction between treatment group and week of measurement, and colony syrup consumption on changes in bumblebee colony weight. Predicted colony weights over time were then fitted for each treatment group with syrup consumption set at the overall mean (197.2 g) and a figure was produced to display this. The second model examined the effects of treatment group, overall colony weight change, and the numbers of drones and queens collected on final numbers of worker bees. A table of exponentiated Least Square Means was produced in this case to predict final worker numbers in each treatment group, and a figure showing the standard estimates was produced. Maximum likelihood estimates were obtained for each parameter and used to determine overall p-values following the chi-square method for both models. The most parsimonious structure was chosen in both cases based on the QIC goodness of fit statistic. Individual colony numbers were specified as a repeated subject within the models.

6.4 Results

6.4.1 Bumblebee colonies

The overall changes in weight of bumblebee colonies during the experimental period were compared between treatment groups. Bumblebee colonies inoculated with *Parasitellus fucorum* (P) showed the greatest mean increase in weight (372.3 g, 207.1) (mean, Standard Deviation (SD)) during the experimental period, followed by those inoculated with *Tyrophagus putrescentiae* (T) (242.7 g, 111), and both mite species in combination (PT) (160.8 g, 226.8). All mite treatment groups had a greater mean weight increase than the Control group (C) (140.3 g, 76.2), however the PT group had a lower median weight increase (Figure 6.3, Figure 6.4). Relative to the Control group, the *Parasitellus* group increased in weight by an average of 165%, the *Tyrophagus* group by 72%, and the PT group by 15%.

Overall consumption of Invertbee syrup during the experimental period demonstrated the same trend as was observed in bumblebee colony weight changes. Colonies in the *Parasitellus* treatment group consumed the most syrup on average (1899 g, 323.1) (mean, SD), followed by those in the *Tyrophagus*

group (1810 g, 164), the PT group (1701 g, 526.8) and finally the Control group (1689 g, 367.7) (Figure 6.5). Syrup consumption was a significant predictor for colony weight change over time (Table 6.1).

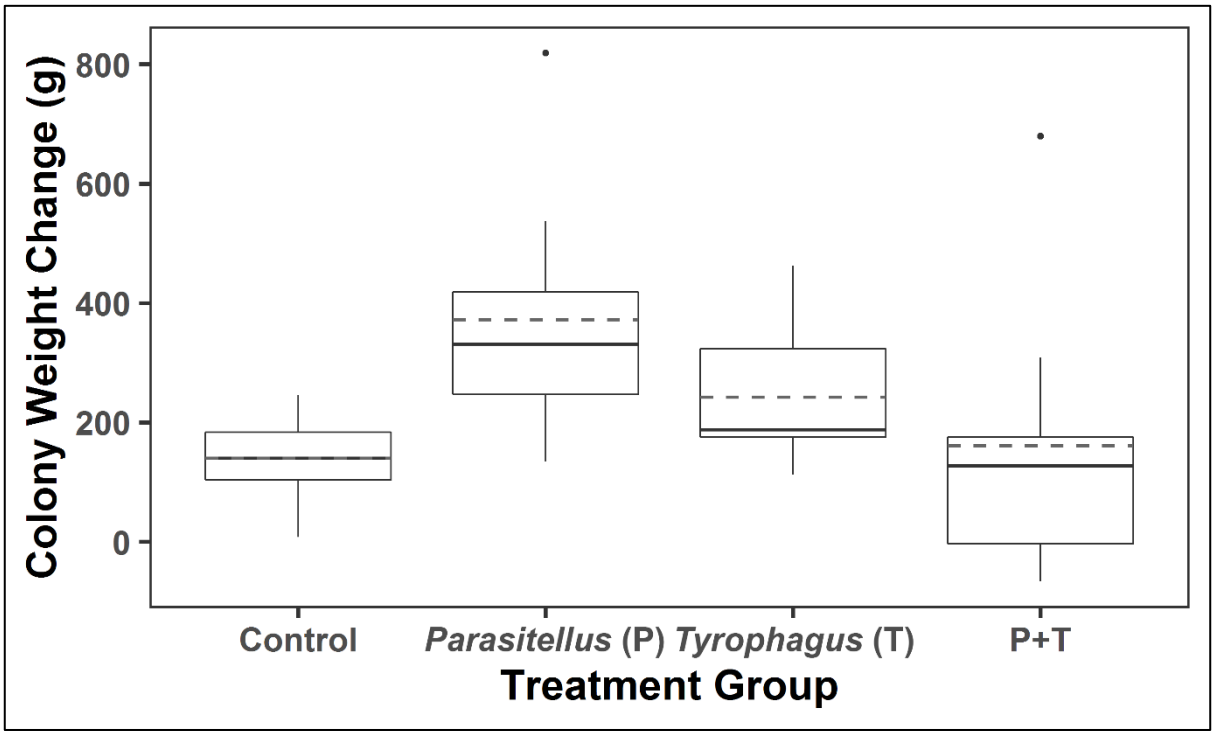

Figure 6.3 – Overall weight changes in commercial bumblebee colonies compared by treatment group. Plotted in the Tukey style. Medians are marked by thick horizontal lines, the 25th and 75th percentiles by thin horizontal lines, and vertical lines extend to all data within ≤1.5x the inter-quartile range. Any outlying results are marked by dots. Mean values are also displayed as grey dashed horizontal lines.

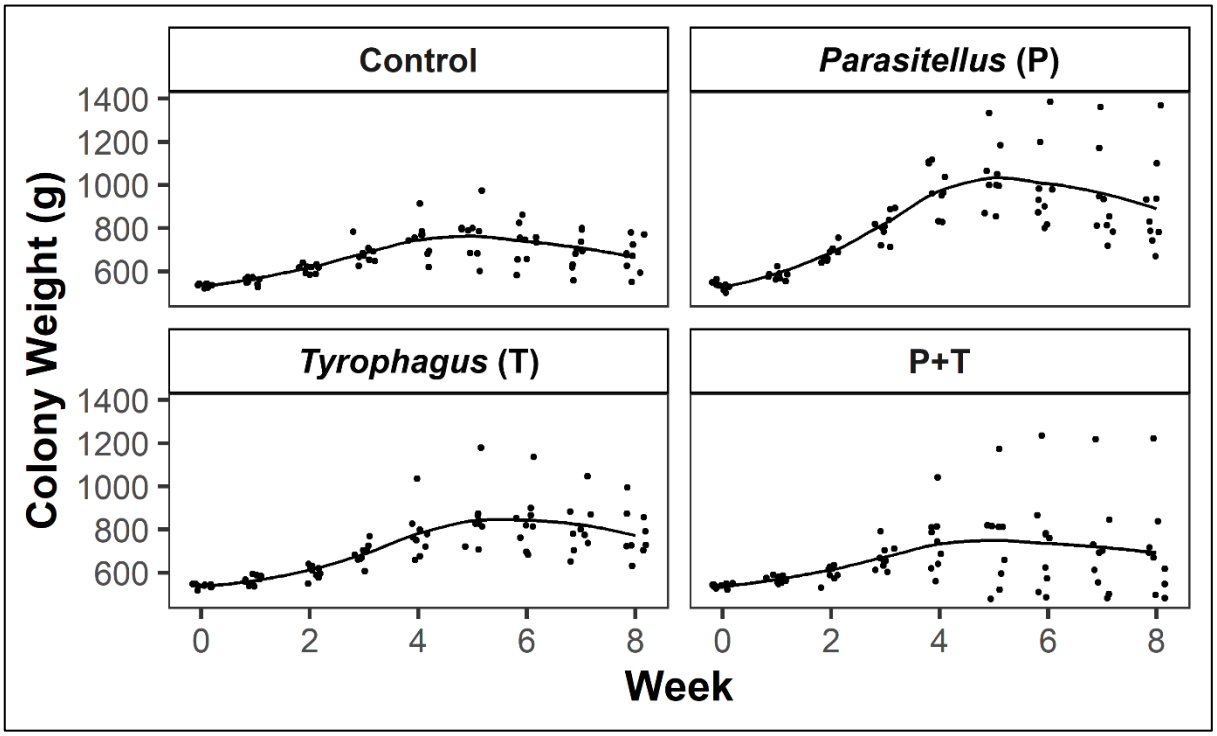

Figure 6.4 – Bumblebee colony weight measurements taken weekly from the beginning of the experiment until the end. A trendline has been added to each treatment group, calculated using a smoothed rolling mean fitted to the data. A combined figure with all treatments overlaid on a single plot can be seen in Appendix O.

The key results of a mixed linear effects analysis examining the effects of treatment group on colony weight are shown in Table 6.1. This model predicted that a bumblebee colony in the *Parasitellus* treatment group would gain 172.41 g ± 51.772 (estimate ± Standard Error (SE)) more weight than a colony in the Control group, which was a significant difference (p = 0.004). The model also predicted a comparative 50.35 g ± 32.653 weight increase for colonies in the *Tyrophagus* treatment group. Colonies in the PT group were predicted to lose 0.11 g ± 40.155 compared to Control colonies. Neither the *Tyrophagus* nor PT group results were significantly different from the Control group. The model also predicted that syrup consumption had a significant impact on colony weight gain (SE = 0.067, p < 0.001), with an estimated colony weight gain of 0.357 g per gram of syrup consumed. An ANOVA comparing the test model against a null model lacking the treatment group fixed effect returned a significant p-value (X^2 = 8.504, p < 0.001), and showed the test model to have a lower AIC (3466.6) compared with the null model (3469.1).

Fixed Effects	**Estimate (g)**	**Standard Error**	**p-value**
(Intercept)	607.467	31.252	< 0.001
Parasitellus (T. group)	172.412	51.772	0.004
Tyrophagus (T. group)	50.351	32.653	0.14
PT (T. group)	-0.11	40.155	0.998
Syrup consumption	0.357	0.067	<0.001

Table 6.1 – Summary results of mixed linear effects model examining effect of treatment group and syrup consumption on colony weight change. Intercept that all results are derived from was the Control treatment group. Significant p-values are underlined.

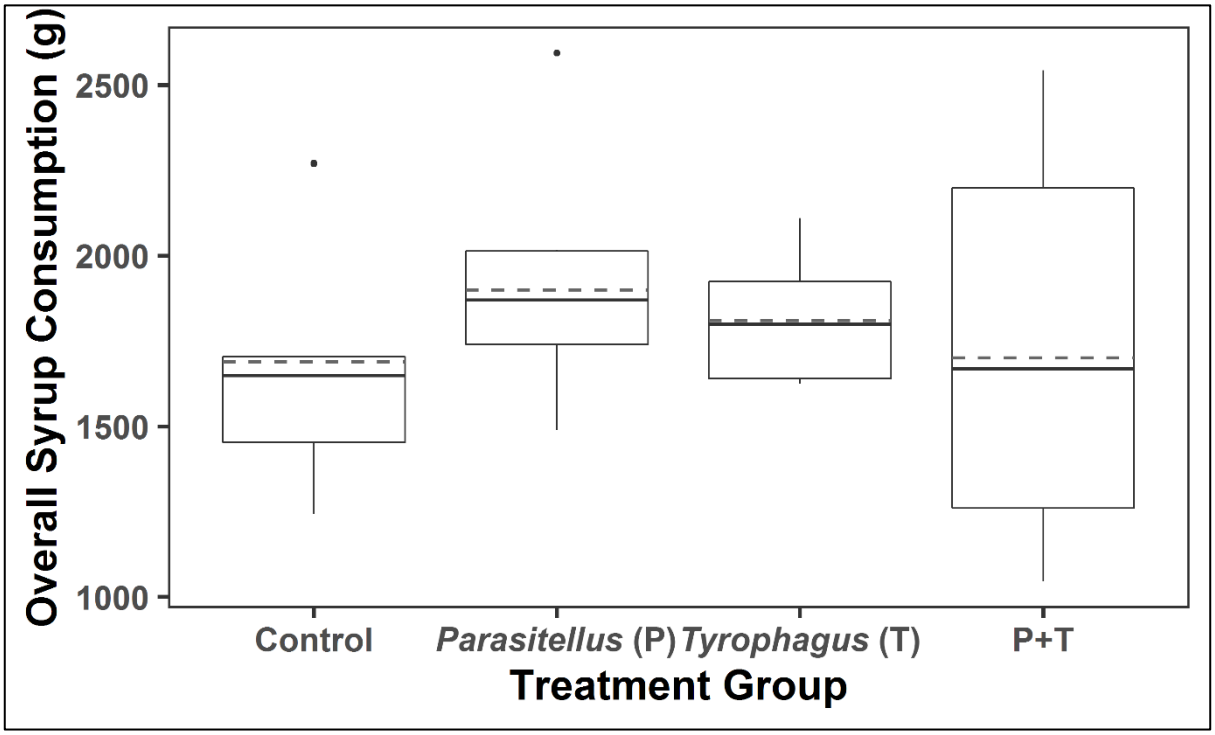

Figure 6.5 – Overall Invertbee syrup consumption in commercial bumblebee colonies compared by treatment group. Medians are marked by thick horizontal lines, the 25th and 75th percentiles by thin horizontal lines, and vertical lines extend to all data within ≤1.5x the inter-quartile range. Any outlying results are marked by dots. Mean values are also shown by dashed grey horizontal lines.

A Generalised Estimating Equation (GEE) analysis tested the effects of treatment group, week of measurement and syrup consumption on colony weight, and had broadly similar results to the mixed effects analysis. This model showed that treatment group ($X^2 = 11.13$, $p = 0.011$), week of measurement ($X^2 = 31.85$ $p < 0.001$), and syrup consumption ($X^2 = 6.55$, $p = 0.01$) each had significant effects on colony weight (Table 6.2). A figure was produced to display predicted colony weights over time based on treatment group (Figure 6.6). This showed that *Parasitellus* inoculated colonies were predicted to gain the most weight over time, followed by *Tyrophagus* inoculated colonies, with the PT and Control groups predicted to have very similar results. Full model parameters and related p-values are shown in Appendix P.

Source	**DF**	**Chi-Square**	**p-value**
Treatment Group	3	11.13	**0.011**
Week	8	31.85	**<.001**
Week*Treatment Group	24	33.05	0.103
Syrup Consumed	1	6.55	**0.01**

Table 6.2 – Score statistics produced by Type 3 analysis of likelihood ratios within GLM/GEE model examining effects of treatment group, week of measurement, the interaction of treatment group and week of measurement, and colony syrup consumption on colony weight changes. All p-values shown were calculated using the chi-square method (Pr > ChiSq). Significant results are highlighted in bold.

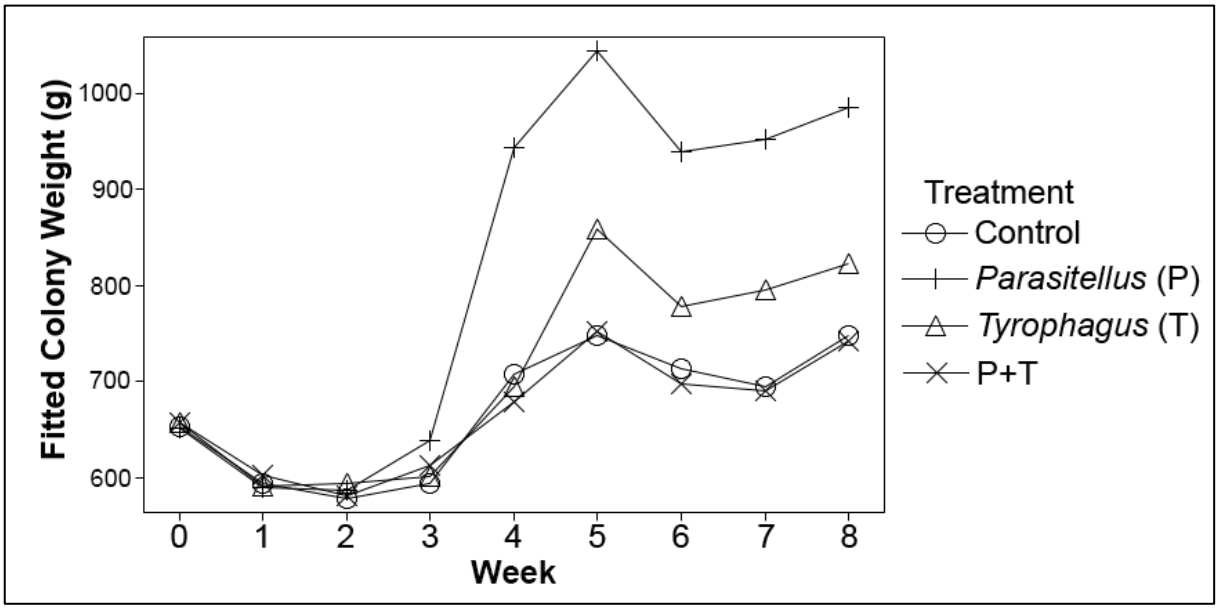

Figure 6.6 – Fitted colony weight (g) for each treatment group at each week of measurement, produced based on the GLM/GEE model results. For these estimates syrup consumption was held at the overall mean (197.2 g)

6.4.2 Bumblebee samples

All bumblebees were removed from their colonies at the end of the experimental period. The median number of bumblebees removed from colonies belonging to different treatment groups varied, with 24 (± 11) (median ± Inter-Quartile Range (IQR)) removed from the Control group, 44 (± 37) from the *Parasitellus* group, 59 (± 39) from the *Tyrophagus* group, and 29 (± 20) from the PT group. A Kruskal-Wallis test showed that these differences were statistically significant ($X^2 = 8.698$, $p = 0.034$), and

pairwise Wilcoxon rank sum tests showed that the significant differences lay mainly between the *Tyrophagus* and Control groups (p = 0.011) (Appendix O).

The number of specimens from each bumblebee caste collected from colonies in different treatment groups were analysed. The highest numbers of worker bees were removed from colonies in the *Tyrophagus* (38 ± 9) (median ± IQR) and *Parasitellus* (35 ± 24) treatment groups. The numbers of drones collected showed that the *Tyrophagus* group again had the highest median number (16 ± 35). The PT group had the highest median number of queens per colony (1 ± 1) and all other groups had a median of 0 queens per colony. A Kruskal-Wallis test showed that variation in worker numbers between treatment groups was statistically significant ($X^2 = 8.615$, p = 0.035), and a pairwise Wilcoxon rank sum test showed the groups which varied significantly from each other were *Tyrophagus* and the Control group (p = 0.014) (Appendix O). Neither drone or queen numbers varied significantly between treatment groups. Bumblebee caste results are shown in full in Table 6.2.

Treatment group	Bumblebee caste	Median number collected	Inter-quartile range	p-value
Control	Worker	16	12	0.035
Parasitellus	Worker	35	24	
Tyrophagus	Worker	38	9	
Parasitellus + *Tyrophagus*	Worker	26	20	
Control	Drone	8	6	0.076
Parasitellus	Drone	8	10	
Tyrophagus	Drone	16	35	
Parasitellus + *Tyrophagus*	Drone	3	6	
Control	Queen	0	1	0.93
Parasitellus	Queen	0	1	
Tyrophagus	Queen	0	1	
Parasitellus + *Tyrophagus*	Queen	1	1	

Table 6.3 – Median numbers (and Inter-Quartile Ranges (IQR)) of bumblebees from different castes removed from bumblebee colonies at the end of the experimental period, divided by treatment group. Kruskal-wallis test p-value results are also shown, and significant (p < 0.05) results are underlined.

The key results of a GEE analysis examining the effects of treatment group, overall colony weight change, and drone/queen numbers on bumblebee worker numbers are shown below. The results showed that treatment group ($X^2 = 10.95$, p = 0.012) and overall colony weight change ($X^2 = 4.42$, p = 0.035) had a significant effect on the numbers of bumblebee workers collected from colonies at the end of the experiment, while the numbers of drones ($X^2 = 0.18$, p = 0.669) and queens ($X^2 = 0.05$, p = 0.819) did not (Table 6.4). Least Square Means were calculated for each treatment group, and the predicted numbers of workers for each group are shown in Table 6.5. This model predicted that colonies in the *Tyrophagus* group (38.761, < 0.001) (exponentiated estimate, p-value) would contain the highest number of bumblebee workers after 8 weeks, followed by the PT group (32.95, < 0.001), the Control

group (23.619, < 0.001), and finally the *Parasitellus* group (20.265, p < 0.001). The estimates for all treatment group were found to be significant (Table 6.5). Full Least Square Means output and GLM parameters are shown in Appendix Q.

Source	**DF**	**Chi-Square**	**p-value**
Treatment group	3	10.95	**0.012**
Overall colony weight change (g)	1	4.42	**0.035**
Drone numbers	1	0.18	0.669
Queen numbers	1	0.05	0.819

Table 6.4 – Score statistics produced by Type 3 analysis of likelihood ratios within GLM/GEE model examining effects of treatment group, overall colony weight change, drone numbers and queen numbers on final bumblebee worker numbers. All p-values shown were calculated using the chi-square method (Pr > ChiSq). Significant results are highlighted in bold.

Treatment Group	**Estimate**	**Exp. Estimate**	**Exp. Lower**	**Exp. Upper**	**Std. Error**	**z value**	**p-value**
Control	3.162	23.619	17.756	31.417	0.146	21.72	**< .001**
Parasitellus (P)	3.009	20.265	15.981	25.698	0.121	24.83	**< .001**
P+T	3.495	32.95	26.31	41.266	0.115	30.44	**< .001**
Tyrophagus (T)	3.657	38.761	33.29	45.133	0.078	47.1	**< .001**

Table 6.5 – Least Square Mean estimates for bumblebee worker numbers separated by treatment group. Estimates, exponentiated estimates (Exp. Estimate) and exponentiated lower and upper confidence limits (Exp. Lower and Exp. Upper) are shown, along with standard errors (Std. Error), z-values and p-values calculated based on the test statistic (Pr > |z|). Significant results are shown in bold.

No significant differences were found in bumblebee weights between different treatment groups when all castes were counted together, or when each caste was analysed separately (Appendix O).

6.4.3 Mites

The numbers of mites collected both from bumblebee colony materials and phoretic mites found upon bumblebees taken from colonies during destructive sampling were sorted into 4 different morphological groupings for analysis. These were *Parasitellus*, all other Mesostigmata, *Kuzinia*, and *Tyrophagus*.

From the bumblebee colony material samples 297 mites were collected in total, and these numbers were scaled appropriately to estimate mite numbers in the entire colonies. No *Kuzinia* or *Tyrophagus* mites were collected from any samples. Samples taken from *Tyrophagus* inoculated colonies had the highest estimated mean (13.333, 14.151) (mean, Standard Deviation (SD)) number of *Parasitellus* mites present, followed by the PT group (9.333, 19.83), the Control group (8.444, 18.756) and finally the *Parasitellus* group (2.222, 2.862). When the estimated numbers of mites from all other Mesostigmatid genera were compared a different trend was observed. The *Parasitellus* group (16.889, 31.35) (mean, SD) had the highest estimated numbers of these mites, followed by the Control group (14.667, 23.812), the *Tyrophagus* group (9.444, 13.612), and finally the PT group (8.333, 13.426). None of these differences were found to be significant when compared between treatment groups using Kruskal-Wallis tests however.

From the bumblebees themselves 1648 phoretic mites were collected. For mites from both the *Parasitellus* and other Mesostigmata morphospecies the medians and IQRs for all treatment groups were 0 due to the majority of bees collected carrying no phoretic mites, therefore means and standard deviations were compared instead (Table 6.3). No *Tyrophagus* mites were found phoretic upon any bees collected.

Phoretic *Kuzinia* numbers varied between treatment groups, with bumblebees from the *Parasitellus* groups carrying the highest average number (1.47, 2.128) (mean, SD), and all other treatment groups having means of less than 1 mite per bumblebee (Table 6.3). These differences were shown to be significant ($X^2 = 8.693$, $p = 0.034$), however a subsequent pairwise Wilcoxon rank sum test showed that no two groups differed significantly (all $p > 0.05$).

Treatment group	**Mite morphospecies**	**Mean**	**Standard Deviation**	**p-value**
Control	*Parasitellus*	0.129	0.358	0.344
Parasitellus	*Parasitellus*	0.095	0.232	
Tyrophagus	*Parasitellus*	0.069	0.116	
Parasitellus + *Tyrophagus*	*Parasitellus*	0.058	0.141	
Control	Other Mesostigmata	0.07	0.134	0.342
Parasitellus	Other Mesostigmata	0.035	0.04	
Tyrophagus	Other Mesostigmata	0.012	0.01	
Parasitellus + *Tyrophagus*	Other Mesostigmata	0.011	0.019	
Control	*Kuzinia*	0.617	0.58	**0.034**
Parasitellus	*Kuzinia*	1.47	2.128	
Tyrophagus	*Kuzinia*	0.764	1.224	
Parasitellus + *Tyrophagus*	*Kuzinia*	0.241	0.461	

Table 6.6 – Means and standard deviations of different mite morphospecies found phoretic upon bumblebees taken from experimental colonies, separated by treatment group. The means quoted were calculated using the mean numbers of mites present upon bumblebees from each experimental colony to avoid pseudoreplication, as were the standard deviations. Kruskal-Wallis test results comparing phoretic mite numbers between treatment groups also shown. Significant p-values are shown in bold.

Phoretic mites were identified to the species or genus level where possible. It was shown that *Parasitellus fucorum* accounted for ≥ 95% of all mites within the *Parasitellus* group in all treatments, while the remaining mites consisted of some *P. ignotus* and *P. talparum*. 100% of other Mesostigmata which could be identified were *Pneumolaelaps* spp., however 6 mites within this morphospecies from the PT group could not be identified as they did not fit any available keys. All mites identified from the *Kuzinia* morphospecies were determined to be *Kuzinia* spp. (Appendix O).

6.5 Discussion

It has been suggested previously in acarological literature that *Parasitellus fucorum* may be beneficial towards bumblebees when present within their colonies (Eickwort, 1994, Koulianos and Schwarz, 1999, Rożej et al., 2012). The results of this experiment support this hypothesis in the case of whole commercial bumblebee colonies in field conditions. This experiment demonstrated that colonies of *Bombus terrestris audax* inoculated with *Parasitellus fucorum* deutonymphs became more populous, consumed more syrup and had a greater increase in colony mass than colonies which were not. This was also shown to be the case (to a lesser degree) for colonies which were inoculated with *Tyrophagus putrescentiae*, however colonies inoculated with a combination of both *T. putrescentiae and P. fucorum* benefitted very little or not at all.

The bumblebee colony weight changes during the experiment showed a clear trend; colonies inoculated with *Parasitellus fucorum* increased in mass more than any other treatment group. The results also showed that inoculation with *Tyrophagus putrescentiae* or a combination of both *P. fucorum* and *T. putrescentiae* resulted in smaller net increases in colony mass compared to the control group. These results (especially differences between the *Parasitellus* and Control groups) were confirmed as significant by both a linear mixed effects analysis of the weights of all colonies throughout the experiment, and the results of a Generalised Estimating Equation (GEE) analysis. The linear mixed effects analysis however predicted a statistically significant difference from the controls only for colonies in the *Parasitellus* group, and both models predicted an insignificant drop in weight for colonies within the PT group. The reasons for *Parasitellus fucorum* inoculations resulting in an increased bumblebee colony mass compared to the control group are likely related to the predatory activity of *P. fucorum* limiting the population growth of various pests within the colony, resulting in freer reproduction and growth for the bumblebees. Such pests may include smaller mites like the mobile female instar of the internal parasitic bumblebee mite *Locustacarus buchneri*, and a variety of other small invertebrates (Alford, 1975, Schmid-Hempel, 1998). The reasons why this was also shown to be case (to a lesser degree) with *Tyrophagus putrescentiae* inoculated colonies are less likely to be due to the initial inoculations of *Tyrophagus* mites, and more with the high numbers of *P. fucorum* and other Mesostigmata which were present in these colonies by the end of the experiment. This is suggested by the fact that colony samples taken from the *Tyrophagus* group had the highest number of *Parasitellus* mites, and that no *Tyrophagus* mites were recovered at all. Given the famous fecundity of *T. putrescentiae* and the favourable conditions for this species found within bumblebee colonies, it is clear that the *Tyrophagus* mites died out or fled during the experiment (Rożej et al., 2012). It is possible that the *T. putrescentiae* in these colonies acted as a pull factor for predatory mite species including *P. fucorum* and the *Tyrophagus* were subsequently predated, or that the inoculated *T. putrescentiae* died out quickly and left the colony free for other mites to colonise, though the differences in results between the *Tyrophagus* group and the Control group strongly suggests this was not the case. The fact that the beneficial effects observed were greatly neutered when both mite species were inoculated in

combination (i.e. the PT group) also seems incongruous at first. It is possible that introducing two mite species at once within bumblebee colonies creates a greater variability of outcome (as was observed in the PT group's colony weight and syrup consumption results (Figure 6.4, Figure 6.5)).

The consumption of Invertbee syrup was measured as a proxy for colony activity and population, as a more populous and/or active colony will likely consume more syrup. The results of these measurements by treatment group followed the same trend as the colony weight changes (i.e. *Parasitellus* group > *Tyrophagus* group > PT group > Control group). The differences between treatment groups in this metric were smaller however, and found to lack statistical significance. The reasons behind these differences were probably the same as for the colony weight changes, as the number of bees within a colony and their activity levels have been linked to the rate at which the colony gains mass (Vaidya et al., 2018).

Colonies from the *Tyrophagus* treatment group had the highest number of bumblebees within them at the end of the experiment, followed by those from the *Parasitellus* and PT groups. All mite-inoculated groups had a higher average number of bees per colony than the Control group. When this result was broken down by bumblebee caste it became clear that many of the bees present within the *Tyrophagus* group were drones; twice as many as were removed from colonies in the Control and *Parasitellus* groups, with the PT group having the lowest average number. All mite-inoculated treatment groups had significantly higher numbers of workers than the Control group, with the *Tyrophagus* group containing the most closely followed by *Parasitellus* and the PT group. These results were contested by a GEE analysis which predicted worker numbers based on treatment group, which suggested that colonies in the *Tyrophagus* group should contain the highest number of workers (matching the observed results) but also that colonies in the *Parasitellus* group would contain the lowest number (which did not reflect the results). This model may have been unduly influenced by the numbers of drones and queens collected from colonies however (see below), and should be interpreted with caution. Overall, the varied numbers of bumblebees collected at the end of this experiment suggests that a lack of associated mites present within bumblebee colonies at an early stage in development negatively impacts population growth, as colonies inoculated with mites were more populous on average during destructive sampling, even after late-experiment degradation.

The number of mites collected from hand-processing samples of bumblebee colony materials was relatively low compared to the numbers of mites found phoretic upon bumblebees, and did not show any statistically significant variation. The only result of importance from the examination of colony materials was that no *Tyrophagus* were found in any samples. The phoretic mite numbers collected from the bumblebees themselves were also fairly homogenous when mean numbers per colony were compared. There was no significant variation in the phoretic numbers of either *Parasitellus* or any other Mesostigmatid mites between treatment groups. The only significant differences were found in the numbers of phoretic *Kuzinia* mites, where bees from the *Parasitellus* group had the highest mean number. These results were surprising, and suggested that the numbers of phoretic mites within the bumblebee colonies decreased prior to destructive sampling. While relatively few *Parasitellus* mites

were collected at the end of the experiment, a far greater number (and certainly more than the initial inoculums) were observed freely moving within the colony boxes and their containers during the first 2-5 weeks of the experiment, particularly those belonging to the *Parasitellus* inoculated colonies (pers. obs.).

It should be considered when drawing conclusions from the results of the mite collections that many colonies, and the *Parasitellus* inoculated group in particular, are likely to have produced gynes and drones weeks before the end of the experiment and many would have subsequently left the colonies, taking most of the phoretic mites with them (Huck et al., 1998, Vesterlund and Sorvari, 2014). This is supported by the fact that most colonies lacked any queens at the end of the experiment, and many colonies were degrading (i.e. losing weight) significantly for up to 3 weeks before destructive sampling. While colonies in other treatment groups were degrading for the same length of time, those in the *Parasitellus* treatment group degraded faster than other groups (Figure 6.4). The causes of this degradation were likely related to the very hot and dry weather experienced during this part of the experiment, which resulted in the colonies being opened to prevent overheating. Prior to this, the weather conditions (and local abundance of flowering plants such as poppy and red/white clover) had been highly favourable, both of which are known to contribute to colony development (Vaidya et al., 2018). Opening the colonies may have also changed internal conditions in a way that disadvantaged some mite species within the colonies or resulted in their migration out of the colony via phoresy (Schwarz and Huck, 1997). This may go some way to explaining these aspects of the results, which necessitate further experimentation in this vein be conducted to confirm beyond all doubt that the beneficial effects observed in some treatment groups were caused by the presence of *Parasitellus fucorum* or other mite species. The late-experiment degradation of colonies, the flight of reproductive gynes and drones, and the likely resulting effects upon the mite fauna within experimental colonies also necessitate that the overall changes in colony weight, syrup consumption and the number of bumblebee workers present at the end of the experiment be considered of primary importance to understanding the results of this experiment. These factors are less likely to have been affected by the loss of drones, gynes or mites in the final weeks of the experimental period, and can therefore be considered more robust.

The results of the experiment were remarkably clear in some ways. In 2 out of the 3 factors outlined above (colony weight, syrup consumption, and numbers of bumblebee workers within the colonies), colonies treated with *Parasitellus fucorum* outperformed all other groups, especially the Controls. The only exception to this was the average number of workers per colony, where the *Tyrophagus* group outperformed the *Parasitellus* group. This shows that the presence of *Parasitellus fucorum* within commercial bumblebee colonies benefits their development. Colonies treated with *Tyrophagus putrescentiae* demonstrated similarly clear benefits, though whether this was a result of *Tyrophagus* activity within the colonies is doubtful. Colonies treated with both mite species in combination demonstrated insignificant benefits when compared to the Control group.

This represents the first empirical evidence of benefits to bumblebee colony development resulting from the presence of a bumblebee-associated mite species (*Parasitellus fucorum*). These results should be of great interest to the field of bumblebee ecology. However, more research into the relationships between bumblebees and their associated mites is needed to better understand the reasons behind such an evidently mutualistic relationship, and to investigate the effects that other bumblebee-associated mites have on bumblebee ecology.

Chapter 7 Conclusions

The aim of this research project was to determine whether any of the various species of mites (Acari) associated with bumblebees (*Bombus*) had a net beneficial effect on bumblebee fitness. To this end, a survey of local bumblebee queens and their phoretic mites was conducted in order to characterise the local mites available for study, and a literature review was carried out in order to determine the mites most likely to benefit their bumblebee hosts. The survey of bumblebee queens (Chapter 2) provided much scope, as there were a variety of mite groups found in the local environment. This survey also gave the first up-to-date information on bumblebee-mite associations in England in decades, and revealed that *Kuzinia* spp., *Scutacarus* spp., and some Mesostigmata genera (*Pneumolaelaps* and *Proctolaelaps*) demonstrate different phoretic abundancies based on bumblebee species, while the genus *Parasitellus* does not. The survey also showed that *Parasitellus fucorum* was by far the most common species within the *Parasitellus* genus present in the local area. Based on the results of the queen survey and literature review, *Parasitellus fucorum* was chosen as the most likely mutualistic mite associate of bumblebees and a series of experiments were designed to test the hypothesis that association with this mite species was beneficial for bumblebees.

For all planned experiments a stable/growing population of captive *Parasitellus fucorum* mites was required, so attempts were made to develop a protocol for rearing field-caught *P. fucorum* deutonymphs in laboratory conditions. This method (Chapter 3) went through numerous changes and developments over time. While the empirical testing of both the initial and more developed methods had highly stochastic results, this method for rearing *P. fucorum* was successful in maintaining and growing captive *P. fucorum* populations for use in all other experiments. It can therefore be said to represent a workable, if unreliable, method of keeping live *P. fucorum* in laboratory conditions. One observation from the *P. fucorum* rearing tests that was both novel and of value was the discovery that *P. fucorum* deutonymphs can feed, develop and reproduce successfully when given a diet consisting wholly of commercially purchased honeybee-collected pollen. This was shown in stark contrast to the wisdom in the literature, which stated that *P. fucorum* would not even feed on honeybee-collected pollen, let alone moult and reproduce afterwards (Koulianos and Schwarz, 1999).

Parasitellus fucorum's predatory activities were then tested against a variety of bumblebee pests and parasites (Chapter 4). A pilot experiment was conducted using the larvae and eggs of the bumblebee wax moth (*Aphomia sociella*) which clearly showed that *P. fucorum* deutonymphs could predate both without issue. A larger experiment was subsequently conducted using *Tyrophagus putrescentiae* mites and the eggs of the greater wax moth (*Galleria mellonella*) and again both were shown to be predated by *P. fucorum* deutonymphs, though *T. putrescentiae* was consumed at a significantly higher rate. This provided circumstantial evidence to support *P. fucorum*'s hypothetical beneficial effects, as logically the predation of pests within bumblebee colonies must be of some benefit to the bumblebees.

A theory within some areas of the literature that phoretic *Parasitellus* deutonymphs provide some form of active protection to bumblebee queens against infestation by the parasitic nematode *Sphaerularia bombi* was next to be tested (Chapter 5). To this end a large number of spring bumblebee queens were captured, their infestation with *S. bombi* determined, and a predation test conducted using mobile-phase *S. bombi* nematodes and *Parasitellus fucorum* deutonymphs. While the predation test was unsuccessful, the data collected on the bumblebee queens did show a significant negative correlation between *S. bombi* infestation and the phoretic *Parasitellus* numbers. Interestingly, *Parasitellus* was the only mite group in which phoretic abundance varied based on nematode infestation, this was not observed in other Mesostigmatid mite genera (*Pneumolaelaps*/*Proctolaelaps*) or with the presence of phoretic *Kuzinia*. The authors consider it more likely that *Parasitellus* deutonymphs can recognise and abandon nematode infested queens than these mites providing direct defence against infestation, but more research will be required to determine this conclusively.

Finally, a large-scale field trial was conducted using commercial bumblebee colonies inoculated with different mite species (Chapter 6). Some were inoculated with *Tyrophagus putrescentiae*, some with *Parasitellus fucorum*, some with both mite species in combination, and a control group were not inoculated with any mites. These colonies were placed into field conditions and their development monitored over time before destructive sampling. The results were clear; commercial bumblebee colonies inoculated with *Parasitellus fucorum* prior to field exposure developed more successfully than colonies which had any other inoculum. They gained the most weight (i.e. colony mass), consumed the most syrup on average, and came a close 2nd in the number of bumblebee workers present after 8 weeks (35 median workers per colony vs 38 for the *Tyrophagus* inoculated group). Broadly similar effects were observed in colonies initially inoculated with *T. putrescentiae*, however it is a distinct possibility that these effects resulted from large populations of *Parasitellus* mites which developed within these colonies during the experiment. This experiment demonstrated for the first time that the presence of particular mite species within bumblebee colonies has a direct beneficial impact upon colony development, and therefore upon the bumblebees themselves.

Other results of interest throughout the project related to broad trends of bumblebee and mite ecology. *Bombus terrestris* was found to be the bumblebee species (with a reasonable sample size) most commonly infested by *Sphaerularia bombi* nematodes, while it's socially parasitic counterpart *Bombus vestalis* was not infested at all, nor was *B. hypnorum*. This supports the hypothesis that the abundance and choice of hibernation site are key in determining the likelihood of infestation for a bumblebee species, as *B. terrestris* was the most abundant local species while both *B. hypnorum* and *B. vestalis* were much less abundant. It was also found that *S. bombi* infestation peaked in British bumblebee queens during May, April and June (in descending order of proportion of infested queens) (Chapter 5).

The phoretic abundance of different mite groups were found to be positively correlated. *Parasitellus* numbers were strongly correlated with those of all other Mesostigmata, and these were both correlated with phoretic *Scutacarus* numbers. This suggests that multiple varieties of Mesostigmatid mites are

likely to be found within the same bumblebee colonies, and shows that hyperphoresy by *Scutacarus* mites is of key importance for their associations with bumblebees, as these mites were typically found phoretic upon larger Mesostigmatid mites. Interestingly, phoretic *Kuzinia* numbers did not correlate with any other mite group, suggesting that the very high numbers of phoretic *Kuzinia* found on individual queens may exclude phoresy by other mite genera (Chapter 2).

Based on direct evidence from the field experiment (Chapter 6) and supporting evidence from the predation experiments (Chapter 4) it can be stated with confidence that *Parasitellus fucorum* is a beneficial mite for bumblebees, and that *Bombus terrestris* enjoys a mutualistic relationship with the phoretic mite *Parasitellus fucorum*. In this relationship the mites benefit from phoretic transportation upon bumblebees, housing (and stable environmental conditions) within bumblebee colonies or a queen's hibernaculum, a food supply in the form of stored pollen/wax, and ample prey among the various organisms found within bumblebee colonies. In turn, the bumblebees can be said to benefit from the predatory activities of *Parasitellus fucorum* within their colonies, the effects of which clearly outweigh any drawbacks from kleptoparasitism by *P. fucorum* and result in more effective bumblebee colony development.

7.1 Applications of this work

Up to date information on the bumblebee-mite associations found in England will be useful for any researchers planning work in this area, as the previously most up-to-date information in this niche area came from the 1980s (Hyatt, 1980, Hyatt and Embersom, 1988). This was not ideal considering the various changes in the environmental context of British bumblebees since then, which were thought likely to have impacted upon their mite fauna.

Keeping *Parasitellus fucorum* in laboratory conditions is a likely prerequisite for most large-scale or long-term experimentation involving this species, and therefore the development of a proven, replicable protocol for maintaining and growing a population of this mite species in such conditions should prove useful for researchers in this vein. Clearly this work fell short of that mark and more work is needed before the methods trialled herein can be considered a real success, including the demonstration of continuous population growth over time. Of particular interest for future work will be the fact that commercially available honeybee-collected pollen is a suitable foodstuff for *P. fucorum*, as previous research had suggested that only bumblebee-collected pollen (which must be painstakingly gathered by hand) would suffice.

Parasitellus fucorum's ability to predate *Tyrophagus putrescentiae* mites, *Aphomia sociella* eggs and larvae and *Galleria mellonella* eggs should be of great interest to anyone interested in potential mechanisms for the biological control of these common pest species. It is also likely that *Parasitellus fucorum* can predate a wide range of pollinator pests and parasites beyond those tested within this work. The fact that commercial bumblebee colonies inoculated with *Parasitellus fucorum* outgrow colonies which are not will also likely prove of interest to companies which produce such colonies, though history

shows that care must be taken whenever commercial bumblebee colonies and mites are concerned if further introductions of non-native species around the world are to be avoided (Chapter 1). Colonies inoculated with *P. fucorum* likely provide a greater pollination service than those which are not, as it is logical that a larger, more active colony with more workers would out-pollinate a smaller, less populous competitor colony. The knowledge that introducing a particular mite species into bumblebee colonies results in the improved development of that colony could have a variety of implications beyond those suggested here, particularly if this effect could be proven to exist using wild bumblebee colonies and with different bumblebee species.

7.2 Limitations and further research

While the survey on bumblebee queens (Chapter 2) provides useful and up-to-date information on bumblebee-mite associations, its collection area was limited to the Harris Garden within the University of Reading Whiteknights campus in April 2016, and so the survey cannot be considered representative of England as a whole or throughout the year, though it does provide a useful snapshot of current associations.

There were also limitations to the mite ID work, for instance all mites in the Order Mesostigmata (with the exception of the genus *Parasitellus*) were identified only to the genus level. The reason for this was that a large number of *Pneumolaelaps* and *Proctolaelaps* mites were collected, but since they were not of interest to the central aim of the project it was not deemed a priority to determine their species. Mites of the genus *Kuzinia* were only identified to the genus level for another reason; it has been noted by taxonomists that the genus *Kuzinia* is in dire need of revision, with the differences between the 'European' species *Kuzinia laevis* and the 'American' species *K. americana* being minimal, and both having been correctly identified phoretic upon bumblebees in South American museum collections (with some samples predating the first invasion of European *K. laevis* in that region) (Revainera et al., 2014, Klimov et al., 2016e). This shows that the current criteria for morphological speciation are not fit for purpose, and so the *Kuzinia* sampled during this project were not identified past the genus level (Klimov et al., 2016e). Were all mites collected identified to the species level, a more in-depth analysis of species-level associations between local mite species and their bumblebee hosts could be conducted.

The study of nematode infestation trends among local bumblebee queens (Chapter 5) experienced similar limitations to the bumblebee queen survey. Though the nematode study was somewhat broader in scope, it was still limited to bumblebee queens captured throughout the University of Reading Whiteknights campus during March – July of both 2017 and 2018. The nematode study therefore cannot be said to be representative of these associations throughout England as a whole. The predation experiment that had been initially planned was abandoned due to the unforeseen ability of mobile-phase *Sphaerularia bombi* nematodes to move into water droplets which formed on the inner surfaces of the tissue culture flasks in which predation tests were conducted. Once the nematodes had entered water droplets they were effectively invisible to the *Parasitellus fucorum* deutonymphs, and the condensation

within the tissue culture flasks also made it impossible to accurately count the numbers of surviving nematodes. As a solution to this problem could not be developed while maintaining the moisture levels required for *S. bombi* survival, the tests were discontinued. If these obstacles were overcome testing *P. fucorum* predation upon mobile-phase *S. bombi* would be a novel and useful experiment, and would help lay to rest the question of *P. fucorum*'s hypothetical 'protection' of bumblebee queens against *S. bombi* infestation.

In the *Parasitellus fucorum* rearing chapter (Chapter 3) the pilot test was limited in success due to the foodstuffs only being replaced every other day, while the main experiment was limited due to the lead researcher being unable to access the mite colonies during weekends, which meant that every colony went without fresh food for a minimum of 2 days out of every 7. This (coupled with *P. fucorum*'s cannibalistic tendencies) is likely to have seriously stunted potential population growth within the test colonies. It should be noted that the same method which was shown to fail in Chapter 3 was successful in rearing larger populations for other experiments, but this mite rearing method only succeeded when the foodstuffs could be replaced on a consistent daily basis. If this rearing method could be further refined and a successful trial conducted it would be a useful tool for further research in this vein.

The *Parasitellus fucorum* predation tests (Chapter 4) were both limited in different ways. The pilot experiment which used *Aphomia sociella* eggs and larvae was limited by low numbers of available *P. fucorum* deutonymphs and *A. sociella* eggs/larvae. Moreover, the experiment's usefulness was seriously limited by the use of inert pollen balls as a control group, and by the lack of any control groups containing only *A. sociella* eggs/larvae without *P. fucorum* present. The larger scale experiment using *Galleria mellonella* eggs was limited by the use of *G. mellonella* as a substitute for *A. sociella* (due to the commercial availability of *G. mellonella*, and the lack of such for *A. sociella*), since *G. mellonella* is a less likely prey species for *P. fucorum*. The experiment was also limited by the exclusive use of *G. mellonella* eggs when 1st instar larvae could also have been included in the larger predation test. An issue present in both experiments was the recorded time at which each test chamber 'started'. This was recorded at the time when the last live organisms were added, which meant that 'prey' organisms in chambers which subsequently had predatory *P. fucorum* added would have been within the chamber for some time prior to the recorded starting time. It may have been better to record the 'start' time from the final addition of 'prey' organisms, though this would present its own problems (i.e. if *P. fucorum* deutonymphs were added before prey, they would vigorously try to escape during the prey addition).

The field experiment testing the effect of mite inoculation on commercial colony development (Chapter 6) was limited in a few ways. Firstly the effects shown by this experiment are limited to commercial bumblebee colonies of *Bombus terrestris audax* produced by Biobest, and it cannot be stated conclusively that the outcomes would be the same were the experiment repeated using colonies produced by a different supplier, or if another bumblebee species was tested. It also cannot be stated conclusively that the results would be the same if wild colonies of *B. terrestris audax* were tested in the same way. Clearly the only way to determine whether the effects observed during the experiment would also apply

to the other situations outlined above is to actually test them, which is the next logical step in the research conducted during this project. The interpretation of results from this chapter was also severely stunted by an unfortunate combination of factors in that the experiment was run for 2-3 weeks too long (due to initial confusion between the producer, supplier and researcher about the age of the colonies at the beginning of the experiment, which was only clarified in retrospect) and exceptional environmental conditions which resulted in rapid bumblebee colony development. This meant that after 8 weeks when the colonies were destructively sampled very few queens and relatively few males were found, which strongly suggests that the colonies had already 'peaked' and the reproductive gynes and males had largely left their colonies, likely taking most of the colony mites with them. This meant that the numbers of males, queens, and mites found within the colonies were much less reliable as measures of colony development than they otherwise might have been.

7.3 Final remarks

In conclusion, this project has achieved its central aim of discovering a mite species commonly associated with bumblebees which has a beneficial effect upon bumblebee fitness. *Parasitellus fucorum* has been shown to benefit the development of commercial *Bombus terrestris* colonies by its presence within the colony, and *P. fucorum* deutonymphs have been shown to predate common (and uncommon) pests of bumblebee colonies. This project makes a strong case for *Parasitellus fucorum* as a beneficial mite for bumblebees.

References

ABRAHAMOVICH, A. H., TELLERÍA, M. C. & DÍAZ, N. B. 2001. *Bombus* species and their associated flora in Argentina. *Bee World,* 82**,** 76-87.

ALFORD, D. V. 1969. *Sphaerularia bombi* as a Parasite of Bumble Bees in England. *Journal of Apicultural Research,* 8**,** 49-54.

ALFORD, D. V. 1975. *Bumblebees*, Davis-Poynter.

ALLEN, G. R., SEEMAN, O. D., SCHMID-HEMPEL, P. & BUTTERMORE, R. E. 2007. Low parasite loads accompany the invading population of the bumblebee, *Bombus terrestris* in Tasmania. *Insectes Sociaux,* 54**,** 56-63.

ALLENDES, J. L. & MONTALVA, J. 2011. First record of the mite *Kuzinia laevis* (Dujardin, 1849)(Acarina: Acaridae) in Chile. *Boletín de Biodiversidad de Chile,* 5**,** 36-38.

ARBETMAN, M. P., MEEUS, I., MORALES, C. L., AIZEN, M. A. & SMAGGHE, G. 2012. Alien parasite hitchhikes to Patagonia on invasive bumblebee. *Biological Invasions,* 15**,** 489-494.

ARRETZ, P. V. & MACFARLANE, R. P. 1986. The Introduction of *Bombus ruderatus* to Chile for Red Clover Pollination. *Bee World,* 67**,** 15-22.

BAKER, A. S., YORK, P. V. & MUSEUM, N. H. 1999. *Mites and ticks of domestic animals: an identification guide and information source,* The Stationary Office.

BATES, D., MAECHLER, M., BOLKER, B. & WALKER, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software,* 67**,** 1-48.

BIRMINGHAM, A. 2003. *Bumble Bee Drift In A Greenhouse Environment.* Master of Science, Simon Fraser University.

BUCHMANN, S. L. 1983. Buzz pollination in angiosperms. *Handbook of experimental pollination biology; 1983. :73-113.* New York, NY: Van Nostrand Reinhold Company.

BUTTERMORE, R. E. 1997. Observations of Successful *Bombus terrestris* (L.) (Hymenoptera: Apidae) Colonies in Southern Tasmania. *Australian Journal of Entomology,* 36**,** 251-254.

CANFIELD, M. S. & WRENN, W. J. 2010. *Tyrophagus putrescentiae* mites grown in dog food cultures and the effect mould growth has on mite survival and reproduction. *Veterinary Dermatology* 21**,** 58-63.

CHMIELEWSKI, W. 1969. Obserwacje nad biologia nowego dla akarofauny polskiej gatunku *Kuzinia laevis* (Dujardin, 1849)(Acarina, Acaridae). *Polskie pismo entomologiczne. Bulletin entomologique de la Pologne,* 39**,** 603-617.

CHMIELEWSKI, W. 1971. *The Mites (Acarina) Found on bumble-bees (Bombus latr.) and in their nests*, Państwowe Wydawnictwo Naukowe.

CHMIELEWSKI, W. 2003. Insects and mites-pests of bee-bread stores in honey combs. *Journal of apicultural science,* 47**,** 87-92.

CHMIELEWSKI, W. & BAKER, R. A. 2008. Mites (Acarina) phoretic on some common bumblebee species (*Bombus* spp.) from the Pulawy area (south-eastern Poland). *Journal of Apicultural Science,* 52.

COSTA, M. 1966. The biology and development of *Hypoaspis (Pneumolaelaps) hyatti* (Acari: Mesostigmata). *Journal of Zoology,* 148**,** 191-200.

COSTA, M. & HUNTER, P. E. 1970. genus Coleolaelpas Berlese, 1914 (Acarina: Mesostigmata). *Redia*.

CUMBER, R. A. 1949. Humble bee parasites and commensals found within a thirty mile radius of London. *Proceedings of the Royal Entomological Society of London. Series A, General Entomology,* 24**,** 119-127.

CUMBER, R. A. 1953. Some Aspects of the Biology and Ecology of Humble-Bees Bearing Upon the Yields of Red-Clover Seed in New Zealand. *New Zealand Journal of Science and Technology,* 34**,** 227-240.

DASZAK, P., CUNNINGHAM, A. A. & HYATT, A. D. 2000. Emerging Infectious Diseases of Wildlife-- Threats to Biodiversity and Human Health. *Science,* 287**,** 443-449.

DHOORIA, M. S. 2016. *Fundamentals of Applied Acarology,* Springer Singapore.

DURRER, S. & SCHMID-HEMPEL, P. 1994. Shared Use of Flowers Leads to Horizontal Pathogen Transmission. *Proceedings of the Royal Society of London B: Biological Sciences,* 258**,** 299-302.

EDWARDS, M. 2009. *Bombus hypnorum (Linnaeus, 1758)* [Online]. Bees, Wasps & Ants Recoding Society. Available: http://www.bwars.com/index.php?q=bee/apidae/bombus-hypnorum [Accessed 29/08/19.

EICKWORT, G. C. 1994. Evolution and Life-History Patterns of Mites Associated with Bees. *In:* HOUCK, M. (ed.) *Mites.* Springer US.

ENKEGAARD, A., SARDAR, M. A. & BRØDSGAARD, H. F. 1997. The predatory mite *Hypoaspis miles*: biological and demographic characteristics on two prey species, the mushroom sciarid fly, *Lycoriella solani*, and the mould mite, *Tyrophagus putrescentiae*. *Entomologia Experimentalis et Applicata,* 82**,** 135-146.

EVANS, G. O. & TILL, W. M. 1966. *Studies on the British Dermanyssidae (Acari: Mesostigmata): Part II Classification*, Bulletin of the British Museum (Natural History).

FAN, Q.-H. & ZHANG, Z.-Q. 2007. *Tyrophagus* (Acari: Astigmata: Acaridae). *Fauna of New Zealand,* 56**,** 291.

FREE, J. B. 1958. The Defence of Bumblebee Colonies. *Behaviour,* 12**,** 233-242.

FRISCH, K. V. 1952. Hummeln als unfreiwillige Trasportflieger. *Natur und Volk,* 83**,** 171-174.

GALLAI, N., SALLES, J.-M., SETTELE, J. & VAISSIÈRE, B. E. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics,* 68**,** 810-821.

GAMMANS, N. 2019. *Short-haired bumblebee reintroduction project* [Online]. Bumblebee Conservation Trust. Available: https://www.bumblebeeconservation.org/short-haired-bumblebee-reintroduction-project/ [Accessed 29/08/19.

GOKA, K., OKABE, K., NIWA, S. & YONEDA, M. 2000. Parasitic Mite Infestation in Introduced Colonies of European Bumblebees, *Bombus terrestris*. *Japanese Journal of Applied Entomology and Zoology,* 44**,** 47-50.

GOKA, K., OKABE, K. & YONEDA, M. 2006. Worldwide migration of parasitic mites as a result of bumblebee commercialization. *Population Ecology,* 48**,** 285-291.

GOKA, K., OKABE, K., YONEDA, M. & NIWA, S. 2001. Bumblebee commercialization will cause worldwide migration of parasitic mites. *Molecular Ecology,* 10**,** 2095-2099.

GOLDBLATT, J. W. & FELL, R. D. 1984. Parasites and Parasitization Rates in Bumble Bee Queens, *Bombus* spp. (Hymenoptera: Apidae), in Southwestern Virginia. *Environmental Entomology,* 13**,** 1661-1665.

GOULSON, D. 2003. Effects of Introduced Bees on Native Ecosystems. *Annual Review of Ecology, Evolution, and Systematics,* 34**,** 1-26.

GOULSON, D. 2010. *Bumblebees: Behaviour, Ecology, and Conservation*, Oxford University Press.

GOULSON, D., NICHOLLS, E., BOTÍAS, C. & ROTHERAY, E. L. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science,* 347.

GOULSON, D. & WILLIAMS, P. 2000. *Bombus hypnorum (Hymenoptera: Apidae), a new British bumblebee?*

GRAYSTOCK, P., YATES, K., EVISON, S. E. F., DARVILL, B., GOULSON, D. & HUGHES, W. O. H. 2013. The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies. *Journal of Applied Ecology,* 50**,** 1207-1215.

HARRELL JR, F. E. & DUPONT, C. 2019. Hmisc: Harrell Miscellaneous.

HASSELROT, T. B. 1960. *Studies on Swedish bumblebees (genus Bombus Latr.) : their domestication and biology.* Entomogoliska Saellskapet.

HATTINGEN, R. 1956. Beitrage zur Biologie von *Sphaerularia bombi* Leon Dufour (1837). *Zentbl. Bakt. ParasitKde Abt. II,* 109**,** 236-249.

HOFFER, E. 1882-3. *Die Hummeln Steiermarks. Lebensgeschichte und Beschreibung derselben. Graz.*

HOLM, S. N. 1960. Experiments on the domestication of bumblebees (*Bombus* Latr.) in particular *B. lapidarius* L. and *B. terrestris* L.

HOUCK, M. A. & OCONNOR, B. M. 1991. Ecological and Evolutionary Significance of Phoresy in the Astigmata. *Annual Review of Entomology,* 36**,** 611-636.

HUBERT, J., KOPECKY, J., PEROTTI, M. A., NESVORNA, M., BRAIG, H. R., MARKETA, S.-M., MACOVEI, L. & ZUREK, L. 2012. Detection and Identification of Species-Specific Bacteria Associated with Synanthropic Mites. *Microbial Ecology,* 63**,** 919-928.

HUCK, K., SCHWARZ, H. H. & SCHMID-HEMPEL, P. 1998. Host choice in the phoretic mite *Parasitellus fucorum* (Mesostigmata: Parasitidae): which bumblebee caste is the best? *Oecologia,* 115**,** 385-390.

HUNTER, P. E. 1966. The Genus *Pneumolaelaps* with Descriptions of Three New Species (Acarina: Laelaptidae). *Journal of the Kansas Entomological Society,* 39**,** 357-369.

HUNTER, P. E. & COSTA, M. 1971. Description of Gymnolaelaps shealsi n. sp.(Acarina: Mesostigmata) associated with the imported fire ant. *Georgia Entomological Society Journal*.

HUNTER, P. E. & HUSBAND, R. W. 1973. *Pneumolaelaps* (Acarina: Laelapidae) Mites from North America and Greenland. *The Florida Entomologist,* 56**,** 77-91.

HUSBAND, R. W. & SINHA, R. N. 1970. A Revision of the Genus *Locustacarus* with a Key to Genera of the Family Podapolipidae (Acarina). *Annals of the Entomological Society of America,* 63**,** 1152-1162.

HYATT, K. H. 1980. Mites of the subfamily Parasitinae (Mesostigmata: Parasitidae) in the British Isles. *Bulletin of the British Museum (Natural History) Zoology,* 38.

HYATT, K. H. & EMBERSOM, R. M. 1988. *A review of the Macrochelidae (Acari: Mesostigmata) of the British Isles,* London, ROYAUME-UNI, British Museum (Natural History).

INOUE, M. N., YOKOYAMA, J. & WASHITANI, I. 2007. Displacement of Japanese native bumblebees by the recently introduced *Bombus terrestris* (L.) (Hymenoptera: Apidae). *Journal of Insect Conservation,* 12**,** 135-146.

JAGERSBACHER-BAUMANN, J. 2014. Species differentiation of scutacarid mites (Heterostigmatina) using multivariate morphometric methods. *Experimental and Applied Acarology,* 62**,** 279-292.

KARSTEN, P. 2002. Raising Waxworms as Food Insects for Birds and Reptiles. *Journal of the American Federation of Aviculture,* 29**,** 37-38.

KELLY, D. W., PATERSON, R. A., TOWNSEND, C. R., POULIN, R. & TOMPKINS, D. M. 2009. Parasite spillback: A neglected concept in invasion ecology? *Ecology,* 90**,** 2047-2056.

KELLY, M. D. 2009. *Investigation into the host-parasite relationship of Sphaerularia bombi and its host(s) Bombus spp.* PhD, Trinity College Dublin.

KENTA, T., INARI, N., NAGAMITSU, T., GOKA, K. & HIURA, T. 2007. Commercialized European bumblebee can cause pollination disturbance: An experiment on seven native plant species in Japan. *Biological Conservation,* 134**,** 298-309.

KHAUSTOV, A. A. 2008. Mites of the family Scutacaridae of Eastern Palaearctic. *Kiev: Akademperiodika***,** 290 pp.

KHERADMAND, K., KAMALI, K., FATHIPOUR, Y. & GOLTAPEH, E. M. 2007. Development, life table and thermal requirement of *Tyrophagus putrescentiae* (Astigmata: Acaridae) on mushrooms. *Journal of Stored Products Research,* 43**,** 276-281.

KINDL, J., JIROŠ, P., KALINOVÁ, B., ŽÁČEK, P. & VALTEROVÁ, I. 2012. Females of the Bumblebee Parasite, *Aphomia sociella*, Excite Males Using a Courtship Pheromone. *Journal of Chemical Ecology,* 38**,** 400-407.

KING, M. J. 1993. Buzz foraging mechanism of bumble bees. *Journal of Apicultural Research,* 32**,** 41-49.

KLIMOV, P., OCONNOR, B., OCHOA, R., BAUCHAN, G., REDFORD, A. & SCHER, J. 2016a. *Macrocheles* [Online]. Bee Mite ID: University of Michigan. Available: http://idtools.org/id/mites/beemites/factsheet.php?name=15279 [Accessed 26/07/19.

KLIMOV, P., OCONNOR, B., OCHOA, R., BAUCHAN, G., REDFORD, A. & SCHER, J. 2016b. *Proctolaelaps* [Online]. Bee Mite ID: University of Michigan. Available: http://idtools.org/id/mites/beemites/factsheet.php?name=15275 [Accessed 07/08/19.

KLIMOV, P., OCONNOR, B., OCHOA, R., BAUCHAN, G., REDFORD, A. & SCHER, J. 2016c. *Scutacarus* [Online]. Bee Mite ID: University of Michigan. Available: http://idtools.org/id/mites/beemites/factsheet.php?name=15289 [Accessed 22/09/2019.

KLIMOV, P., OCONNOR, B. M., OCHOA, R., BAUCHAN, G., REDFORD, A. & SCHER, J. 2016d. *Parasitellus* [Online]. Bee Mite ID: University of Michigan. Available: http://idtools.org/id/mites/beemites/factsheet.php?name=15277 [Accessed 18/09/2019.

KLIMOV, P. B., OCONNOR, B., OCHOA, R., BAUCHAN, G. R., REDFORD, A. J. & SCHER, J. 2016e. *Kuzinia* [Online]. Available: http://idtools.org/id/mites/beemites/factsheet.php?name=15316 [Accessed 12/09/2017.

KLIMOV, P. B., OCONNOR, B., OCHOA, R., BAUCHAN, G. R., REDFORD, A. J. & SCHER, J. 2016f. *Tyrophagus* [Online]. Bee Mite ID: Bee-Associated Mite Genera of the World.: USDA APHIS Identification Technology Program (ITP), Fort Collins, CO. . Available: http://idtools.org/id/mites/beemites/factsheet.php?name=15324 [Accessed 17/12/2018.

KLIMOV, P. B., OCONNOR, B. M., OCHOA, R., BAUCHAN, G. R., REDFORD, A. J. & SCHER, J. 2016g. *Bee Mite ID: Bee-Associated Mite Genera of the World.* [Online]. Available: http://insects.ummz.lsa.umich.edu/beemites/Species_Accounts/index.html [Accessed 07/09/2017.

KOEHLER, H. H. 1999. Predatory mites (Gamasina, Mesostigmata). *Agriculture, Ecosystems & Environment,* 74**,** 395-410.

KOULIANOS, S. & SCHWARZ, H. H. 1999. Reproduction, development and diet of *Parasitellus fucorum* (Mesostigmata: Parasitidae), a mite associated with bumblebees (Hymenoptera: Apidae). *Journal of Zoology,* 248**,** 267-269.

KRANTZ, G. W. & WALTER, D. E. 2009. *A Manual of Acarology*, Texas Tech University Press.

KRAUS, F. B., SZENTGYÖRGYI, H., ROŻEJ, E., RHODE, M., MOROŃ, D., WOYCIECHOWSKI, M. & MORITZ, R. F. A. 2010. Greenhouse bumblebees (*Bombus terrestris*) spread their genes into the wild. *Conservation Genetics,* 12**,** 187-192.

KUZNETSOVA, A., BROCKHOFF, P. B. & CHRISTENSEN, R. H. B. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software,* 82**,** 1-26.

KWADHA, C. A., ONG'AMO, G. O., NDEGWA, P. N., RAINA, S. K. & FOMBONG, A. T. 2017. The Biology and Control of the Greater Wax Moth, *Galleria mellonella*. *Insects,* 8**,** 61.

LELE, R., KEIM, J. L. & SOLYMOS, P. 2019. ResourceSelection: Resource Selection (Probability) Functions for Use-Availability Data.

LEPAIS, O., DARVILL, B., O'CONNOR, S., OSBORNE, J. L., SANDERSON, R. A., CUSSANS, J., GOFFE, L. & GOULSON, D. 2010. Estimation of bumblebee queen dispersal distances using sibship reconstruction method. *Mol Ecol,* 19**,** 819-31.

MACFARLANE, R. P. & GURR, L. 1995. Distribution of bumble bees in New Zealand. *New Zealand Entomologist,* 18**,** 29-36.

MACFARLANE, R. P., LIPA, J. J. & LIU, H. J. 1995. Bumble Bee Pathogens and Internal Enemies. *Bee World,* 76**,** 130-148.

MAGGI, M., LUCIA, M. & ABRAHAMOVICH, A. H. 2011. Study of the acarofauna of native bumblebee species (*Bombus*) from Argentina. *Apidologie,* 42**,** 280-292.

MARQUARDT, T., KACZMAREK, S. & HALLIDAY, B. 2015. Ovoviviparity in *Macrocheles glaber* (Müller) (Acari: Macrochelidae), with notes on parental care and egg cannibalism. *International Journal of Acarology,* 41**,** 71-76.

MEEUS, I., BROWN, M. J. F., DE GRAAF, D. C. & SMAGGHE, G. 2011. Effects of Invasive Parasites on Bumble Bee Declines - Efectos de Parásitos Invasores sobre Declinaciones de Abejorros. *Conservation Biology,* 25**,** 662-671.

MIYAMOTO, S. 1957. Biological studies on Japanese bees. VI. Observations on the nest of *Bombus ardens* Smith. *Science reports of the Hyogo University of Agriculture. Series, agriculture. Biology.,* 3**,** 12-14.

NAGAMITSU, T., KENTA, T., INARI, N., KATO, E. & HIURA, T. 2006. Abundance, body size, and morphology of bumblebees in an area where an exotic species, *Bombus terrestris*, has colonized in Japan. *Ecological Research,* 22**,** 331-341.

O'CONNOR, B. M. & KLIMOV, P. B. 2011. *Species and subspecies of mites associated with bees of the World* [Online]. University of Michigan. Available: http://insects.ummz.lsa.umich.edu/beemites/Species_Accounts/index.html [Accessed 24/08/17.

OCONNOR, B. & KLIMOV, P. 2012a. *Family Ascidae Voigts & Oudemans, 1905* [Online]. University of Michigan. Available: http://insects.ummz.lsa.umich.edu/beemites/Species_Accounts/Ascidae.htm [Accessed 14/08/19.

OCONNOR, B. & KLIMOV, P. 2012b. *Family Laelapidae Berlese, 1892* [Online]. University of Michigan. Available:

http://insects.ummz.lsa.umich.edu/beemites/Species_Accounts/Laelapidae.htm [Accessed 14/08/19.

OCONNOR, B. & KLIMOV, P. 2012c. *Genus Pneumolaelaps* [Online]. University of Michigan. Available: http://insects.ummz.lsa.umich.edu/beemites/Species_Accounts/Pneumolaelaps.htm [Accessed 17/07/2019.

OCONNOR, B. M. 1982. Evolutionary ecology of astigmatid mites. *Annual review of entomology,* 27**,** 385-409.

OCONNOR, B. M. 2009. Chapter 169 - Mites. *In:* RESH, V. H. & CARDÉ, R. T. (eds.) *Encyclopedia of Insects (Second Edition).* San Diego: Academic Press.

OSBORNE, J. L., MARTIN, A. P., SHORTALL, C. R., TODD, A. D., GOULSON, D., KNIGHT, M. E., HALE, R. J. & SANDERSON, R. A. 2008. Quantifying and comparing bumblebee nest densities in gardens and countryside habitats. *Journal of Applied Ecology,* 45**,** 784-792.

OTTERSTATTER, M., WHIDDEN, C. & LORNE, T. 2004. Patterns of parasitism by tracheal mites (*Locustacarus buchneri*) in natural bumble bee populations. *Apidologie,* 35**,** 351-357.

OTTERSTATTER, M. C., GEGEAR, R. J., COLLA, S. R. & THOMSON, J. D. 2005. Effects of parasitic mites and protozoa on the flower constancy and foraging rate of bumble bees. *Behavioral Ecology and Sociobiology,* 58**,** 383-389.

PEROTTI, M. A. 2009. Megnin re-analysed: the case of the newborn baby girl, Paris, 1878. *Experimental and Applied Acarology,* 49**,** 37-44.

PEROTTI, M. A. & BRAIG, H. R. 2009. Phoretic mites associated with animal and human decomposition. *Experimental and Applied Acarology,* 49**,** 85-124.

PLISCHUK, S., POCCO, M. E. & LANGE, C. E. 2013. The tracheal mite *Locustacarus buchneri* in South American native bumble bees (Hymenoptera: Apidae). *Parasitology International,* 62**,** 505-507.

POINAR, G. O. & HESS, R. 1972. Food Uptake by the Insect-Parasitic Nematode, *Sphaerularia bombi* (Tylenchida). *Journal of nematology,* 4**,** 270-277.

POTTS, S. G., BIESMEIJER, J. C., KREMEN, C., NEUMANN, P., SCHWEIGER, O. & KUNIN, W. E. 2010. Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution,* 25**,** 345-353.

R CORE TEAM 2017. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

REVAINERA, P., LUCIA, M., ABRAHAMOVICH, A. H. & MAGGI, M. 2014. Spatial aggregation of phoretic mites on *Bombus atratus* and *Bombus opifex* (Hymenoptera: Apidae) in Argentina. *Apidologie,* 45**,** 579-589.

REVAINERA, P. D., SALVARREY, S., SANTOS, E., ARBULO, N., INVERNIZZI, C., PLISCHUK, S., ABRAHAMOVICH, A. & MAGGI, M. D. 2019. Phoretic mites associated to *Bombus pauloensis* and *Bombus bellicosus* (Hymenoptera: Apidae) from Uruguay. *Journal of Apicultural Research,* 58**,** 455-462.

RICHARDS, K. W. & RICHARDS, L. A. 1977. A new species of *Macrocheles* (Acarina: Macrochelidae) found in bumble bee nests (Hymentoptera: Apidae). *The Canadian Entomologist,* 109**,** 711-719.

RICHARDS, L. A. 1976. Parasitid mites associated with bumblebees in Alberta, Canada (Acarina: Parasitidae; Hymenoptera: Apidae). I. Taxonomy. *University of Kansas science bulletin,* 51**,** 1-18.

ROYCE, L. A. & KRANTZ, G. W. 1989. Observations on pollen processing by *Pneumolaelaps longanalis* (Acari: Laelapidae), a mite associate of bumblebees. *Experimental & Applied Acarology,* 7**,** 161-165.

ROŻEJ, E., WITALIŃSKI, W., SZENTGYÖRGYI, H., WANTUCH, M., MOROŃ, D. & WOYCIECHOWSKI, M. 2012. Mite species inhabiting commercial bumblebee (*Bombus terrestris*) nests in Polish greenhouses. *Experimental & Applied Acarology,* 56**,** 271-282.

RSTUDIO TEAM 2015. RStudio: Integrated Development for R. Boston, MA RStudio, Inc.

RUEDA-RAMÍREZ, D., RIOS-MALAVER, D., VARELA-RAMÍREZ, A. & MORAES, G. J. 2019. Biology and predation capacity of *Parasitus bituberosus* (Acari: Mesostigmata: Parasitidae) on *Frankliniella occidentalis* (Thysanoptera: Thripidae), and free-living nematodes as its complementary prey. *Pest Management Science,* 75**,** 1819-1830.

RUTRECHT, S. T. & BROWN, M. J. F. 2008. The life-history impact and implications of multiple parasites for bumble bee queens. *International Journal for Parasitology,* 38**,** 799-808.

RUZ, L. 2002. Bee pollinators introduced to Chile: a review. *Pollinating bees***,** 155-167.

SANCHEZ-RAMOS, I. & CASTANERA, P. 2005. Effect of temperature on reproductive parameters and longevity of *Tyrophagus putrescentiae* (Acari: Acaridae). *Experimental and Applied Acarology* 36**,** 93-105.

SAS INSTITUTE INC. 2013. SAS/STAT®13.1 User's Guide. Cary, NC: SAS Institute Inc.

SCHMID-HEMPEL, P. 1998. *Parasites in Social Insects*, Princeton University Press.

SCHMID-HEMPEL, R., ECKHARDT, M., GOULSON, D., HEINZMANN, D., LANGE, C., PLISCHUK, S., ESCUDERO, L. R., SALATHÉ, R., SCRIVEN, J. J. & SCHMID-HEMPEL, P. 2014. The invasion of southern South America by imported bumblebees and associated parasites. *Journal of Animal Ecology,* 83**,** 823-837.

SCHOUSBOE, C. 1986. On the biology of *Scutacarus acarorum* Goeze (Acarina: Trombidiformes). *Acarologia,* 27**,** 151-158.

SCHOUSBOE, C. 1987. Deutonymphs of *Parasitellus* phoretic on Danish bumblebees (Parasitidae, Mesostigmata: Apidae, Hymenoptera. *Acarologia,* 28**,** 37-41.

SCHWARZ, H. H. & HUCK, K. 1997. Phoretic mites use flowers to transfer between foraging bumblebees. *Insectes Sociaux,* 44**,** 303-310.

SCHWARZ, H. H., HUCK, K. & SCHMID-HEMPEL, P. 1996. Prevalence and Host Preferences of Mesostigmatic Mites (Acari: Anactinochaeta) Phoretic on Swiss Bumble Bees (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society,* 69**,** 35-42.

SEMMENS, T. D., TURNER, E. & BUTTERMORE, R. 1993. *Bombus terrestris* (L.) (Hymenoptera: Apidae) now established in Tasmania. *Australian Journal of Entomology,* 32**,** 346-346.

SHYKOFF, J. A. & SCHMID-HEMPEL, P. 1991. Incidence and effects of four parasites in natural populations of bumble bees in Switzerland. *Apidologie,* 22**,** 117-125.

SING, T., SANDER, O., BEERENWINKEL, N. & LENGAUER, T. 2005. ROCR: visualizing classifier performance in R. *Bioinformatics,* 21**,** 7881.

SLADEN, F. W. L. 1912. *The Humble-Bee, its life history and how to domesticate it.*, Cambridge University Press.

SMRŽ, J. & ČATSKÁ, V. 1987. Food selection of the field population of *Tyrophagus putrescentiae* (Schrank) (Acari, Acarida). *Journal of Applied Entomology,* 104**,** 329-335.

STATACORP 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC.

SWIFT, J. 1733. On Poetry: a Rhapsody.

THORP, R. W. 2005. Species profile: *Bombus franklini*. *Red list of pollinator insects of North America*.

TOMOO, M. 2008. Japanese IAS law and its practical implementation for addressing intentional animal imports. *Current National Practices and Available Tools for Pre-import Screening of Animal Species in International Trade*.

TORRETTA, J. P., MEDAN, D. & ABRAHAMOVICH, A. H. 2006. First record of the invasive bumblebee *Bombus terrestris* (L.) (Hymenoptera, Apidae) in Argentina. *Transactions of the American Entomological Society,* 132**,** 285-289.

VAIDYA, C., FISHER, K. & VANDERMEER, J. 2018. Colony Development and Reproductive Success of Bumblebees in an Urban Gradient. *Sustainability,* 10**,** 1936.

VALLEJO-MARÍN, M. 2018. Buzz pollination: studying bee vibrations on flowers. *New Phytologist*.

VAN DEN EIJNDE, J., DE RUIJTER, A. & VAN DER STEEN, J. 1991. Method for rearing *Bombus terrestris* continuously and the production of bumblebee colonies for pollination purposes. *Acta Horticulturae,* 288**,** 154-158.

VANBERGEN, A. J. & THE INSECT POLLINATORS INITIATIVE 2013. Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment,* 11**,** 251-259.

VENABLES, W. N. & RIPLEY, B. D. 2002. Modern Applied Statistics with S. New York: Springer.

VESTERLUND, S.-R. & SORVARI, J. 2014. Longevity of starved bumblebee queens (Hymenoptera: Apidae) is shorter at high than low temperatures. *European Journal of Entomology,* 111**,** 217-220.

VITOUSEK, P. M., D'ANTONIO, C. M., LOOPE, L. L., REJMANEK, M. & WESTBROOKS, R. G. 1997. Introduced species: A significant component of human-caused global change. *New Zealand Journal of Ecology,* 21**,** 1-16.

WEI, T. & SIMKO, V. 2017. R package "corrplot": Visualization of a Correlation Matrix.

WHITTINGTON, R., WINSTON, M. L., TUCKER, C. & PARACHNOWITSCH, A. L. 2004. Plant-species identity of pollen collected by bumblebees placed in greenhouses for tomato pollination. *Canadian Journal of Plant Science,* 84**,** 599-602.

WICKHAM, H. 2016. ggplot2: Elegant Graphics for Data Analysis. *Springer-Verlag New York*.

WIKIHOW STAFF. 2019. *How to Breed Waxworms* [Online]. wikiHow. Available: https://www.wikihow.com/Breed-Waxworms [Accessed 16/04/19 2019].

WILLIAMS, P. H. & OSBORNE, J. L. 2009. Bumblebee vulnerability and conservation world-wide. *Apidologie*, 40**,** 367-387.

YONEDA, M., FURUTA, H., KANBE, Y., TSUCHIDA, K., OKABE, K. & GOKA, K. 2008a. Reproduction and transmission within a colony of bumblebee tracheal mite *Locustacarus buchneri* (Acari: Podapolipidae) in *Bombus terrestris*(Hymenoptera: Apidae). *Applied Entomology and Zoology*, 43**,** 391-395.

YONEDA, M., FURUTA, H., TSUCHIDA, K., OKABE, K. & GOKA, K. 2008b. Commercial colonies of *Bombus terrestris* (Hymenoptera: Apidae) are reservoirs of the tracheal mite *Locustacarus buchneri* (Acari: Podapolipidae). *Applied Entomology and Zoology*, 43**,** 73-76.

ZAMEC, R. 2014. New distributional records of mite *Kuzinia laevis*(Dujardin, 1849)(Astigmata, Acaridae) and notes on its life cycle. *Folia faunistica Slovaca*, 19**,** 5-7.

Chapter 8 Appendices

Appendix A Full correlation table of results

Row	**Column**	**Correlation**	**p-value**
Date captured	Species	0.159	0.086
Date captured	Thorax width	0.357	< **0.001**
Species	Thorax width	0.434	< **0.001**
Date captured	*Parasitellus*	-0.040	0.668
Species	*Parasitellus*	0.000	0.999
Thorax width	*Parasitellus*	0.092	0.326
Date captured	Mesostigmata	-0.046	0.626
Species	Mesostigmata	0.082	0.377
Thorax width	Mesostigmata	0.141	0.130
Parasitellus	Mesostigmata	0.859	< **0.001**
Date captured	*Kuzinia*	0.196	**0.035**
Species	*Kuzinia*	0.204	**0.028**
Thorax width	*Kuzinia*	0.274	**0.003**
Parasitellus	*Kuzinia*	-0.026	0.785
Mesostigmata	*Kuzinia*	0.029	0.755
Date captured	*Scutacarus*	0.019	0.837
Species	*Scutacarus*	0.016	0.863
Thorax width	*Scutacarus*	0.026	0.778
Parasitellus	*Scutacarus*	0.615	< **0.001**
Mesostigmata	*Scutacarus*	0.553	< **0.001**
Kuzinia	*Scutacarus*	0.016	0.862

Table A.1 – Spearman's correlation values comparing factors within the results collected for each bumblebee queen captured, including p-values. Significant p-values are shown in **bold**. Date captured, Thorax width and Species refer to characteristics of the captured bumblebee queens. Species was coerced into numeric format (in alphabetical order) for the purpose of these correlations.

Appendix B Multinomial logistic regression outputs

Coefficients	***Parasitellus***	**Mesostigmata**	***Kuzinia***	***Scutacarus***	**Date caught**	**Thorax width**
hortorum	0.462	-0.352	-0.068	-0.721	0.692	-4.478
hypnorum	-1.079	0.136	0.003	-1.040	-0.199	-3.437
lapidarius	0.122	-0.402	-0.014	0.061	0.219	-2.291
lucorum	0.074	-0.140	0.002	-0.608	-0.322	-4.526
other	-0.520	-12.037	-0.022	0.531	-0.712	-4.167
vestalis	0.047	-2.432	-0.029	-1.769	0.389	-2.418
Std. Errors	***Parasitellus***	**Mesostigmata**	***Kuzinia***	***Scutacarus***	**Date caught**	**Thorax width**
hortorum	0.261	0.548	0.055	0.720	0.289	1.343
hypnorum	0.945	0.171	0.002	2.082	0.182	0.794
lapidarius	0.163	0.286	0.006	0.062	0.129	0.657
lucorum	0.317	0.326	0.004	1.200	0.284	1.000
other	0.969	0.659	0.112	0.232	0.523	1.176
vestalis	0.348	1.125	0.013	1.141	0.172	0.800
Relative risk ratios	***Parasitellus***	**Mesostigmata**	***Kuzinia***	***Scutacarus***	**Date caught**	**Thorax width**
hortorum	1.587	0.703	0.934	0.486	1.998	0.011
hypnorum	0.340	1.146	1.003	0.353	0.819	0.032
lapidarius	1.130	0.669	0.986	1.063	1.245	0.101
lucorum	1.076	0.869	1.002	0.545	0.724	0.011
other	0.594	0.000	0.979	1.701	0.491	0.016
vestalis	1.048	0.088	0.972	0.170	1.476	0.089
z – values	***Parasitellus***	**Mesostigmata**	***Kuzinia***	***Scutacarus***	**Date caught**	**Thorax width**
hortorum	1.771	-0.643	-1.244	-1.002	2.399	-3.335
hypnorum	-1.142	0.796	1.273	-0.500	-1.097	-4.330
lapidarius	0.749	-1.403	-2.367	0.986	1.704	-3.488
lucorum	0.233	-0.430	0.518	-0.506	-1.137	-4.524
other	-0.537	-18.278	-0.193	2.293	-1.361	-3.542
vestalis	0.135	-2.163	-2.171	-1.551	2.269	-3.021
p – values	***Parasitellus***	**Mesostigmata**	***Kuzinia***	***Scutacarus***	**Date caught**	**Thorax width**
hortorum	0.077	0.520	0.213	0.316	**0.016**	**0.001**
hypnorum	0.254	0.426	0.203	0.617	0.273	< **0.001**
lapidarius	0.454	0.160	**0.018**	0.324	0.088	< **0.001**
lucorum	0.816	0.667	0.604	0.613	0.256	< **0.001**
other	0.591	< **0.001**	0.847	**0.022**	0.174	< **0.001**
vestalis	0.893	**0.031**	**0.030**	0.121	**0.023**	**0.003**
Residual Deviance = 147.791, AIC = 231.791						

Table B.1 – Outputs of multinomial logistic regression (Formula = Species ~ *Parasitellus* + Mesostigmata + *Kuzinia* + *Scutacarus* + Date caught + Thorax width) and subsequent tests. *Bombus terrestris* was used as the baseline outcome, and all bumblebee species where n < 5 were removed. Relative risk ratios were calculated through exponentiation of the regression coefficients, giving ratios for a 1 unit change in the predictor variables. z-values were calculated by dividing regression coefficients by standard errors, and p-values were calculated using 2-tailed z tests.

Appendix C GLM supplementary data

C.1 Parameter estimates

Parameter			**DF**	**Est.**	**Std. Err.**	**95% Confidence Limits**		**Chi Square**	**p-value**
Thorax width			1	2.587	0.199	2.197	2.978	168.510	**<.0001**
Date	5		1	0.112	0.022	0.069	0.156	25.390	**<.0001**
Date	6		1	-6.417	1.000	-8.378	-4.456	41.140	**<.0001**
Date	12		1	-3.611	0.355	-4.306	-2.915	103.590	**<.0001**
Date	13		1	-0.740	0.056	-0.850	-0.630	172.760	**<.0001**
Date	14		1	-0.533	0.052	-0.634	-0.431	106.260	**<.0001**
Date	18		1	-0.917	0.054	-1.023	-0.811	286.630	**<.0001**
Date	19		1	-0.321	0.045	-0.409	-0.234	51.690	**<.0001**
Date	20		1	-1.508	0.296	-2.089	-0.928	25.930	**<.0001**
Date	21		1	-2.780	0.715	-4.180	-1.379	15.130	**<.0001**
Date	22		1	0.955	0.161	0.640	1.270	35.290	**<.0001**
Date	25		1	-0.935	0.061	-1.054	-0.817	238.430	**<.0001**
Date	26		1	-2.858	0.715	-4.259	-1.457	15.980	**<.0001**
Date	27		1	-0.128	0.034	-0.195	-0.061	13.930	**<.0001**
Date	28		0	-1.393	0.368	-2.115	-0.671	14.310	**<.0001**
Species	*hortorum*		1	0.000	0.000	0.000	0.000	.	.
Species	*hypnorum*		1	-2.314	0.421	-3.139	-1.488	30.180	**<.0001**
Species	*lapidarius*		1	1.769	0.135	1.505	2.033	172.480	**<.0001**
Species	*lucorum*		1	-1.841	0.459	-2.740	-0.941	16.090	**<.0001**
Species	other		1	4.600	0.711	3.206	5.993	41.850	**<.0001**
Species	*terrestris*		1	3.009	0.736	1.567	4.451	16.730	**<.0001**
Species	*vestalis*		0	2.206	0.107	1.996	2.417	421.560	**<.0001**
Date*Species	5	*hypnorum*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	5	*terrestris*	0	2.187	1.099	0.034	4.340	3.960	**0.047**
Date*Species	6	*lucorum*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	6	*terrestris*	0	-1.974	0.822	-3.584	-0.363	5.770	**0.016**
Date*Species	12	*hortorum*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	12	other	1	2.325	0.498	1.350	3.301	21.820	**<.0001**
Date*Species	12	*terrestris*	0	-3.183	0.762	-4.678	-1.689	17.430	**<.0001**
Date*Species	13	*lucorum*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	13	*terrestris*	0	-3.881	0.744	-5.339	-2.423	27.220	**<.0001**
Date*Species	14	*hortorum*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	14	*hypnorum*	1	2.446	0.497	1.473	3.420	24.250	**<.0001**

Parameter			DF	Est.	Std. Err.	95% Confidence Limits		Chi Square	p-value
Date*Species	14	*lapidarius*	1	2.601	0.099	2.408	2.794	696.470	**<.0001**
Date*Species	14	*terrestris*	0	2.094	0.520	1.076	3.113	16.250	**<.0001**
Date*Species	18	*lapidarius*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	18	*terrestris*	0	0.909	0.560	-0.188	2.006	2.640	0.104
Date*Species	19	*hypnorum*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	19	*lapidarius*	1	-0.021	0.356	-0.719	0.677	0.000	0.954
Date*Species	19	*terrestris*	1	2.813	0.547	1.742	3.884	26.490	**<.0001**
Date*Species	19	*vestalis*	0	1.072	0.299	0.485	1.659	12.830	**<.0001**
Date*Species	20	*lapidarius*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	20	*lucorum*	0	7.249	0.844	5.594	8.904	73.700	**<.0001**
Date*Species	20	other	0	0.000	0.000	0.000	0.000	.	.
Date*Species	20	*terrestris*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	20	*vestalis*	0	2.511	0.716	1.107	3.914	12.290	**0.001**
Date*Species	21	*lapidarius*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	21	*terrestris*	1	-1.273	0.607	-2.462	-0.083	4.400	**0.036**
Date*Species	21	*vestalis*	0	-2.126	0.169	-2.457	-1.795	158.540	**<.0001**
Date*Species	22	*lapidarius*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	22	*terrestris*	0	1.184	0.536	0.134	2.234	4.880	**0.027**
Date*Species	25	*hortorum*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	25	*lapidarius*	1	3.912	0.858	2.230	5.594	20.770	**<.0001**
Date*Species	25	*terrestris*	1	1.931	1.100	-0.226	4.087	3.080	0.079
Date*Species	25	*vestalis*	0	2.804	0.716	1.401	4.207	15.350	**<.0001**
Date*Species	26	*hypnorum*	0	0.000	0.000	0.000	0.000	.	.
Date*Species	26	*lapidarius*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	26	*terrestris*	0	0.139	0.549	-0.937	1.214	0.060	0.801
Date*Species	27	*lapidarius*	1	0.000	0.000	0.000	0.000	.	.
Date*Species	27	*terrestris*	1	0.511	0.914	-1.281	2.302	0.310	0.577
Date*Species	27	*vestalis*	0	1.080	0.370	0.355	1.805	8.520	**0.004**
Date*Species	28	*hortorum*	0	0.000	0.000	0.000	0.000	.	.
Date*Species	28	*lapidarius*	0	0.000	0.000	0.000	0.000	.	.
Date*Species	28	*terrestris*	0	0.000	0.000	0.000	0.000	.	.
Date*Species	28	*vestalis*	0	0.000	0.000	0.000	0.000	.	.

Table C.1 – Analysis of maximum likelihood parameter estimates (Est.) for each parameter combination. Significant (< .05) p-values are shown in bold. p-values were calculated using the chi-square method.

C.2 Goodness of fit

Criterion	DF	Value	Value/DF
Deviance	72	10752.66	149.342
Scaled Deviance	72	10752.66	149.342
Pearson Chi-Square	72	11393.863	158.248
Scaled Pearson X2	72	11393.863	158.248
Log Likelihood		71775.572	
Full Log Likelihood		-5686.142	
AIC		11460.284	
AICC		11516.059	
BIC		11581.442	

Table C.2 – Goodness of fit test results for GLM. AIC was used as the criteria by which the most parsimonious model was determined.

C.3 Sequential sum of squares results

Source	Deviance	DF	Chi-Square	p-value
Intercept	25893.717			
Thorax width	22897.706	1	2996.01	**<.0001**
Date	19731.799	13	3165.91	**<.0001**
Species	13960.681	6	5771.12	**<.0001**
Date*Species	10752.66	23	3208.02	**<.0001**

Table C.3 – Results of sequential sum of squares test on GLM (i.e. SAS Type 1 Analysis), with significant p-values shown in bold. p-values we calculated using the chi-square method.

C.4 Date*Species least square means results

Species	Date	Estimate	Standard Error	z Value	Pr > |z|	Mean	SEM
hypnorum	5	0.987	0.447	2.21	**0.027**	2.684	1.201
terrestris	5	-0.762	1.000	-0.76	0.446	0.467	0.467
lucorum	6	2.464	0.185	13.3	**<.001**	11.756	2.179
terrestris	6	2.044	0.354	5.78	**<.001**	7.722	2.731
hortorum	12	2.720	0.279	9.75	**<.001**	15.182	4.237
other	12	2.534	0.176	14.39	**<.001**	12.608	2.221
terrestris	12	4.915	0.050	98.08	**<.001**	136.280	6.829
lucorum	13	3.635	0.193	18.82	**<.001**	37.885	7.316
terrestris	13	5.122	0.040	126.5	**<.001**	167.680	6.789
hortorum	14	2.664	0.278	9.58	**<.001**	14.355	3.991
hypnorum	14	6.901	0.032	213.52	**<.001**	993.330	32.105
lapidarius	14	2.785	0.259	10.77	**<.001**	16.196	4.189

Species	Date	Estimate	Standard Error	z Value	Pr > |z|	Mean	SEM
terrestris	14	4.738	0.047	101.1	**<.001**	114.140	5.349
lapidarius	18	2.196	0.333	6.59	**<.001**	8.984	2.995
terrestris	18	5.333	0.036	149.51	**<.001**	207.130	7.389
hypnorum	19	3.688	0.182	20.24	**<.001**	39.972	7.283
lapidarius	19	2.913	0.106	27.61	**<.001**	18.402	1.941
terrestris	19	5.219	0.033	156.73	**<.001**	184.650	6.148
vestalis	19	1.940	0.278	6.99	**<.001**	6.961	1.933
lapidarius	20	6.078	0.049	125.14	**<.001**	435.920	21.171
lucorum	20	5.269	0.074	71.37	**<.001**	194.190	14.335
other	20	3.678	0.201	18.29	**<.001**	39.564	7.954
terrestris	20	5.386	0.039	137.04	**<.001**	218.220	8.576
vestalis	20	0.669	0.707	0.95	0.344	1.952	1.381
lapidarius	21	1.290	0.378	3.41	**0.001**	3.634	1.374
terrestris	21	4.484	0.043	103.69	**<.001**	88.579	3.831
vestalis	21	4.404	0.123	35.68	**<.001**	81.751	10.090
lapidarius	22	1.856	0.289	6.42	**<.001**	6.400	1.849
terrestris	22	4.719	0.054	87.13	**<.001**	112.080	6.071
hortorum	25	2.189	0.243	9.02	**<.001**	8.924	2.166
lapidarius	25	0.680	0.707	0.96	0.336	1.974	1.396
terrestris	25	5.601	0.025	227.27	**<.001**	270.620	6.669
vestalis	25	0.591	0.707	0.83	0.404	1.805	1.277
hypnorum	26	5.090	0.079	64.09	**<.001**	162.320	12.890
lapidarius	26	1.619	0.316	5.12	**<.001**	5.047	1.596
terrestris	26	5.527	0.021	264.35	**<.001**	251.400	5.256
lapidarius	27	0.725	0.707	1.03	0.305	2.065	1.460
terrestris	27	5.341	0.021	250.24	**<.001**	208.770	4.456
vestalis	27	2.055	0.354	5.81	**<.001**	7.809	2.761
hortorum	28	1.135	0.408	2.78	**0.005**	3.111	1.270
lapidarius	28	1.608	0.447	3.59	**<.001**	4.991	2.232
terrestris	28	5.655	0.030	188.44	**<.001**	285.610	8.570
vestalis	28	3.449	0.103	33.43	**<.001**	31.454	3.244

Table C.4 – Least mean square results resulting from Date*Species interaction within the GLM. Significant p-values are shown in bold.

Appendix D Median phoretic mite numbers by group

Bumblebee species	**Mite grouping**	**n**	**Median # phoretic mites**	**IQR**
B. terrestris	*Parasitellus*	70	1	2.75
B. hortorum	*Parasitellus*	6	0	8.25
B. hypnorum	*Parasitellus*	5	0	0
B. lapidarius	*Parasitellus*	17	1	2
B. lucorum	*Parasitellus*	5	1	3
B. pascuorum	*Parasitellus*	3	1	0.5
B. pratorum	*Parasitellus*	2	0*	0*
B. vestalis	*Parasitellus*	9	1	1
B. terrestris	Other Mesostigmata	70	1	2.75
B. hortorum	Other Mesostigmata	6	0	0.75
B. hypnorum	Other Mesostigmata	5	1	2
B. lapidarius	Other Mesostigmata	17	0	1
B. lucorum	Other Mesostigmata	5	0	1
B. pascuorum	Other Mesostigmata	3	0*	0*
B. pratorum	Other Mesostigmata	2	0*	0*
B. vestalis	Other Mesostigmata	9	0	0
B. terrestris	*Kuzinia*	70	125	258
B. hortorum	*Kuzinia*	6	0	3
B. hypnorum	*Kuzinia*	5	30	157
B. lapidarius	*Kuzinia*	17	2	6
B. lucorum	*Kuzinia*	5	17	28
B. pascuorum	*Kuzinia*	3	11	4
B. pratorum	*Kuzinia*	2	0*	0*
B. vestalis	*Kuzinia*	9	2	8
B. terrestris	*Scutacarus*	70	0	1
B. hortorum	*Scutacarus*	6	0	0.75
B. hypnorum	*Scutacarus*	5	0*	0*
B. lapidarius	*Scutacarus*	17	1	4
B. lucorum	*Scutacarus*	5	0	1
B. pascuorum	*Scutacarus*	3	2	9
B. pratorum	*Scutacarus*	2	0*	0*
B. vestalis	*Scutacarus*	9	0	0

Table D.1 – Median numbers and Inter-Quartile Ranges (IQR) of phoretic mites from 4 morphological groupings found on bumblebee queens of different species. n-numbers state total number of bumblebees of this species captured. Where no mites were found, results are marked by an asterisk (*).

Appendix E Mite ID reports

E.1 Parasitellus

E.1.1 Parasitellus fucorum

Identifier – Robin McArthur

Mite location – Phoretic on a *Bombus terrestris* queen

Order – Mesostigmata

Family – Parasitidae

Genus – *Parasitellus*

Species – *P. fucorum* de Geer, 1778

Key used for ID – Hyatt, K. H. (1980). "Mites of the subfamily Parasitinae (Mesostigmata: Parasitidae) in the British Isles." Bulletin of the British Museum (Natural History) Zoology 38. Key to deutonymphs used.

ID steps taken	Notes/Reasoning
1 → 4	Began ID from Parasitinae deutonymph, as the mites' distinctive shield arrangement, brownish/reddish colouration, large size and phoretic behaviour upon *Bombus spp.* distinguish it as such (Fig. 1). • Leg II lacks ventral spurs in example (Fig. 2) • Sternal shields formed differently to example given (Fig. 4)
4 → 8	• Sternal shield without granular area shown in example (Fig. 3/5) • Chelicera cannot be seen clearly due to mounting
8 → 9	• Dorsal shields otherwise than example given in key (Fig. 6) • Corniculi shorter and triangular, only reaching around half the length of palp trochanter (Fig. 7/8)
9 → 13	• Dorsal setae overall without any extreme difference in length, though j1, z5 and r3 are longest (Fig. 9)
13 → 14	• >40 pairs of opisthogastric seta (Fig. 10/11) • Associated with *Bombus*
14 → ID	• Sternal shield broad with longitudinal striations within laterally arranged reticulations (Fig. 12) • Opisthonotal shield triangular and narrower than podonotal shield, with 15 pairs of seta (Fig.13) Additionally, the mite's palps were compared against various *Parasitellus* species and the seta were found to most closely match those of *P. fucorum* (Fig 14/15).
Result	**Mite identified as *Parasitellus fucorum* deutonymph**.

Table E.1 – *Parasitellus fucorum* ID steps taken with reasoning and pictorial references. Text prefixed by a bullet point refers to specific morphological features mentioned in the key. Bracketed references indicate figures within the key.

Pictorial references

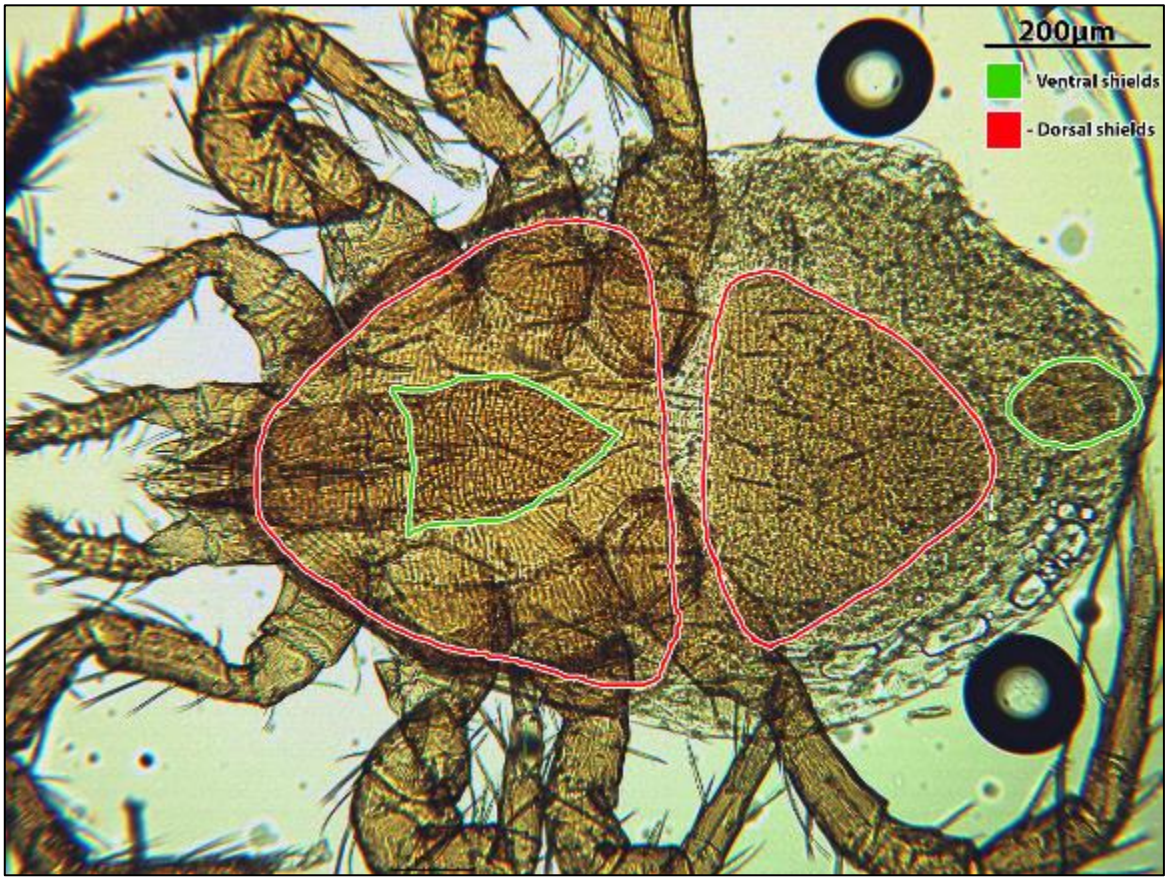

Figure E.1 – Distinctive shield arrangements and size (~1.1mm) show that this mite belongs to the Parasitidae family. *Parasitellus fucorum* ventral shields (Left - Sternal and Right - Anal) are outlined in green, while dorsal shields (Left - Podonotal and Right - Opisthonotal) are outlined in red.

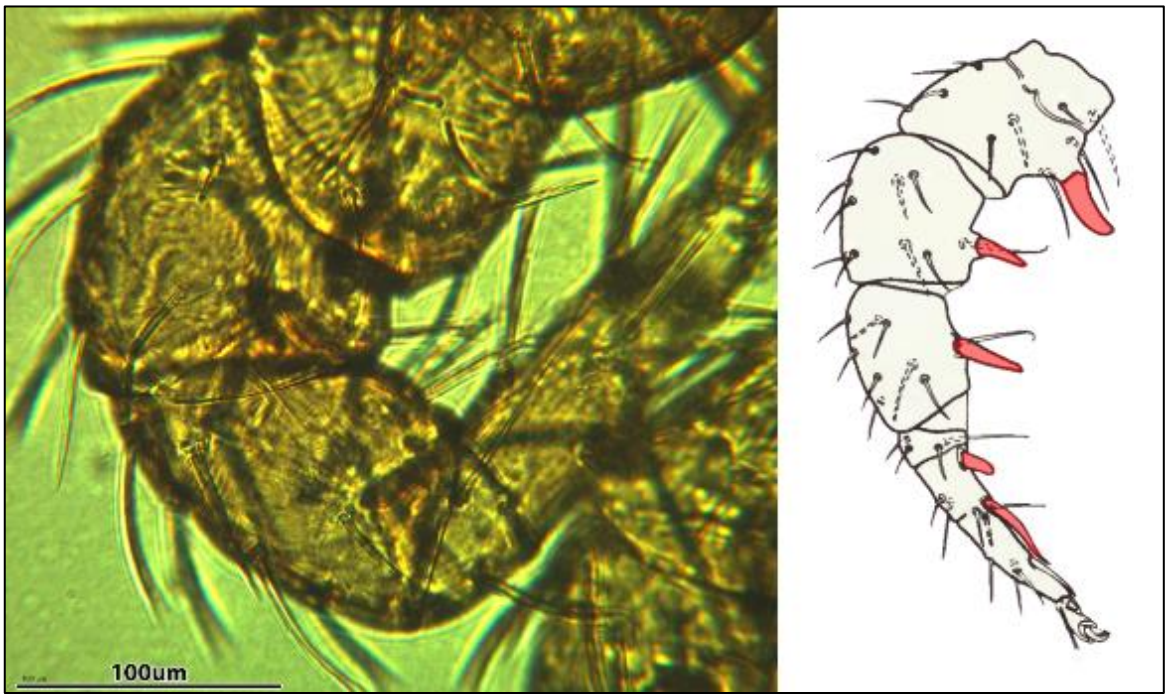

Figure E.2 – Leg II of *Parasitellus fucorum* compared with Fig. 50G from (Hyatt, 1980), showing leg II of a *Gamasodes spiniger* deutonymph with conspicuous ventral spurs highlighted in red.

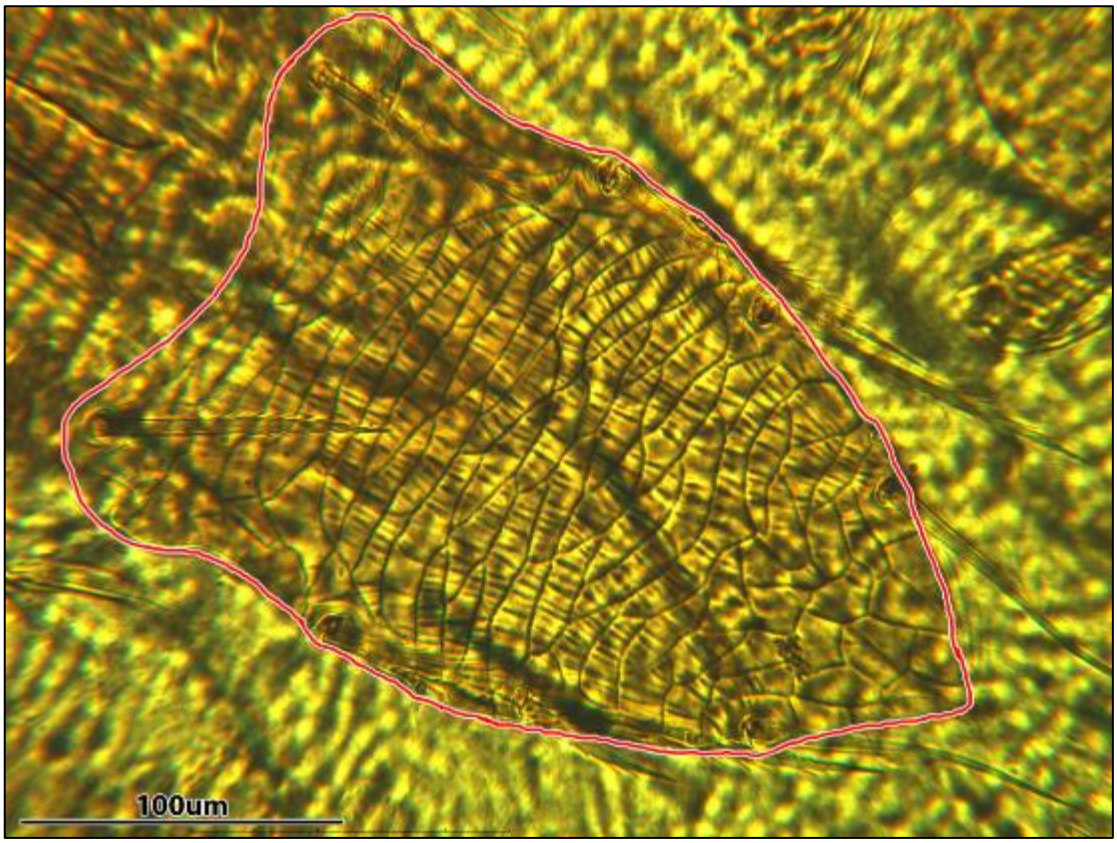

Figure E.3 – *Parasitellus fucorum* sternal shield outlined in red.

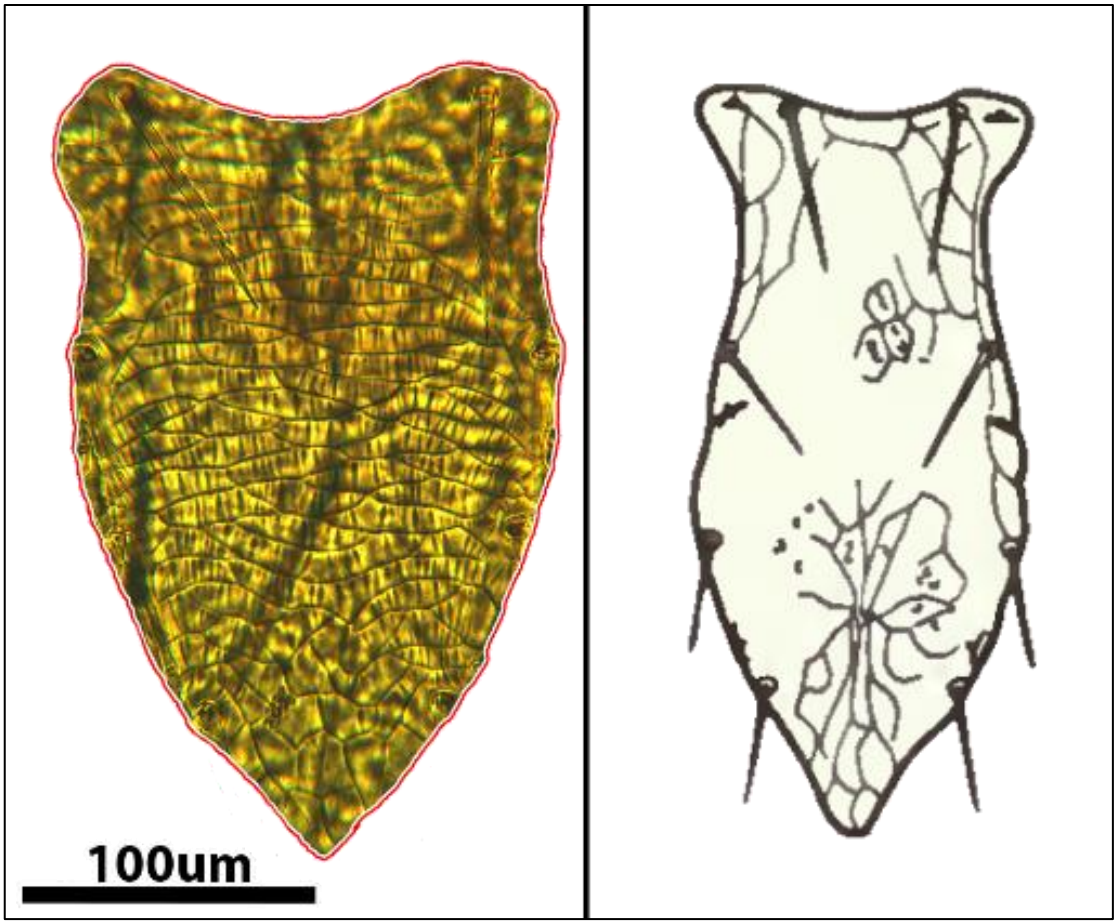

Figure E.4 – Sternal shield of *Parasitellus fucorum* vs sternal shield example given in Fig. 50G from (Hyatt, 1980), showing the sternal shield of a *Gamasodes spiniger* deutonymph.

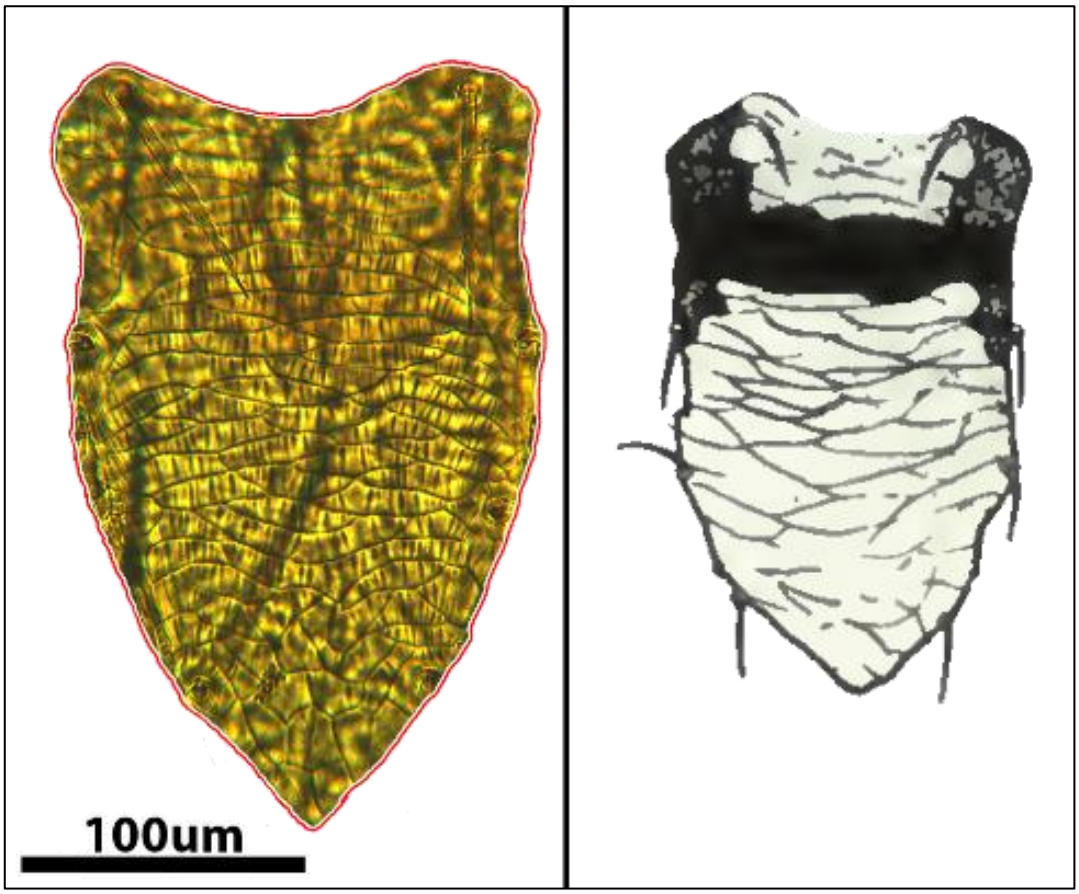

Figure E.5 – Sternal shield of *Parasitellus fucorum* vs sternal shield example given in Fig. 55I from (Hyatt, 1980), showing the ventral features of a *Poecilochirus carabi* deutonymph.

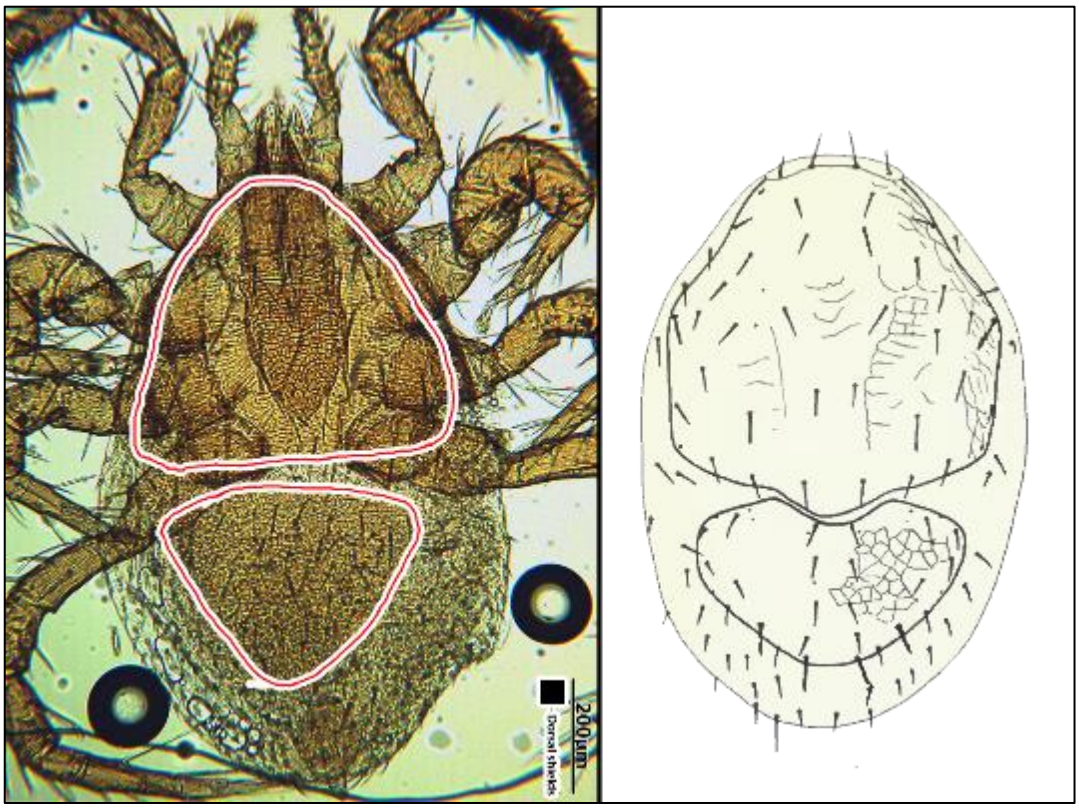

Figure E.6 – Comparison of s *Parasitellus fucorum*'s dorsal shield shape with example given in (Fig. 41A) from (Hyatt, 1980), showing the dorsal features of a *Cornigamasus lunaris* deutonymph.

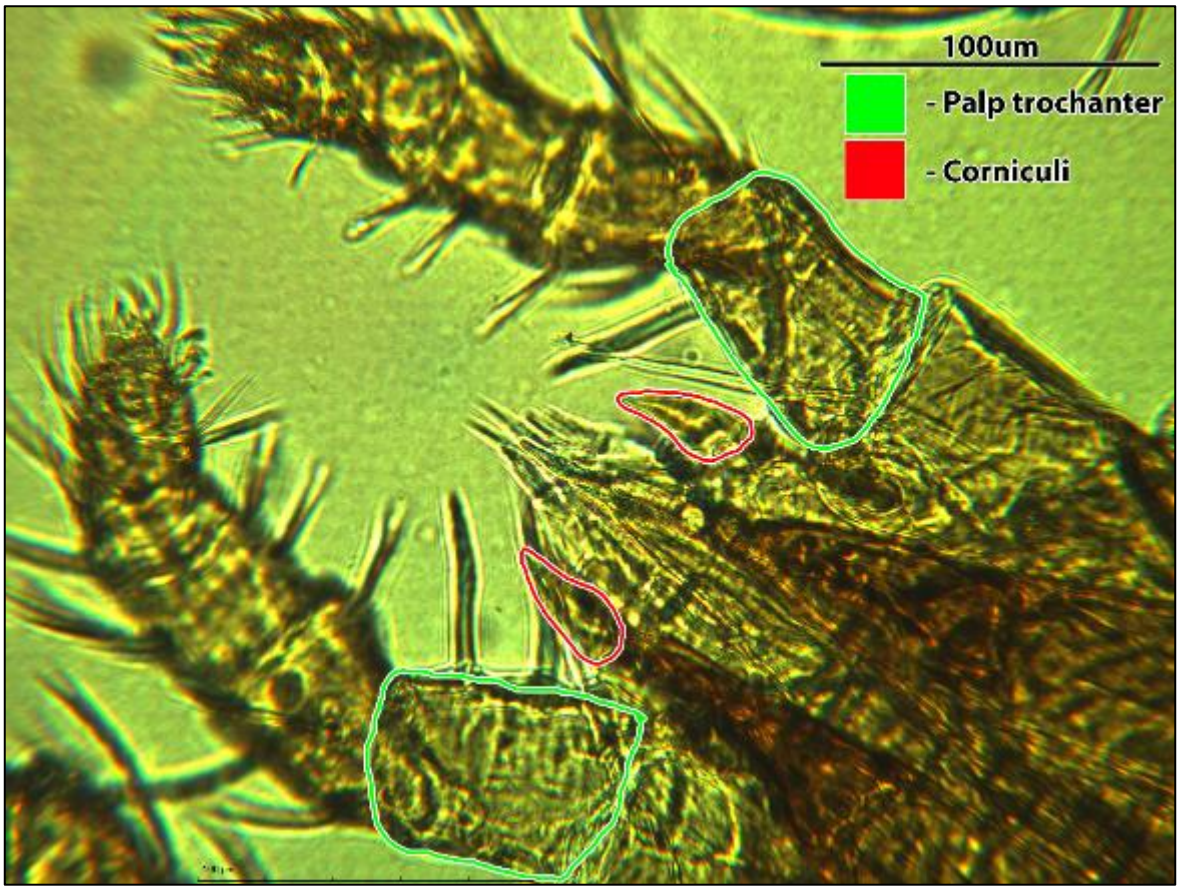

Figure E.7 – Mouthparts of *Parasitellus fucorum*, including palps. Corniculi outlined in red, palp trochanters outlined in green.

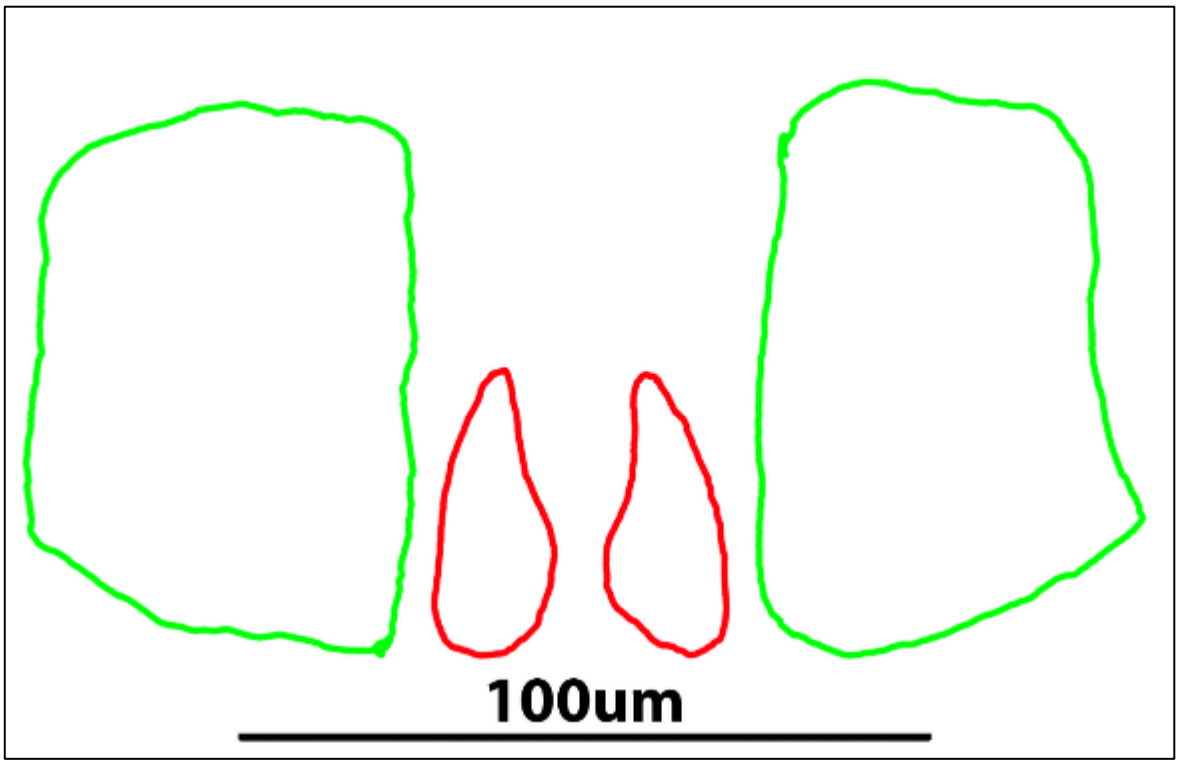

Figure E.8 – Direct comparison of corniculi (red) and palp trochanter (green) sizes, showing that the corniculi reach roughly half the length of the trochanters.

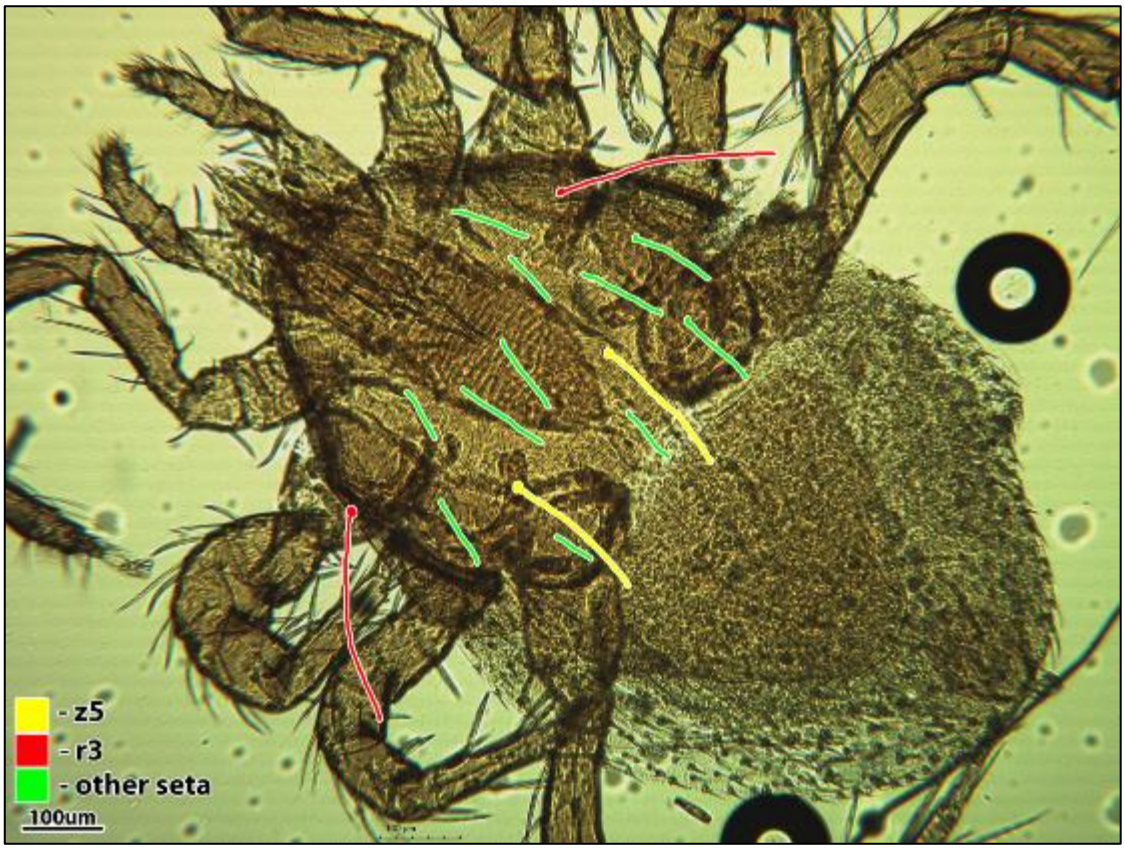

Figure E.9 – Some visible dorsal seta highlighted. z5 highlighted yellow, r3 highlighted red, all others highlighted green.

Figure E.10 – Opithogastric seta. While >40 pairs were observed, it proved impossible to get a photograph with them all in focus due to the mite size and lack of clearing.

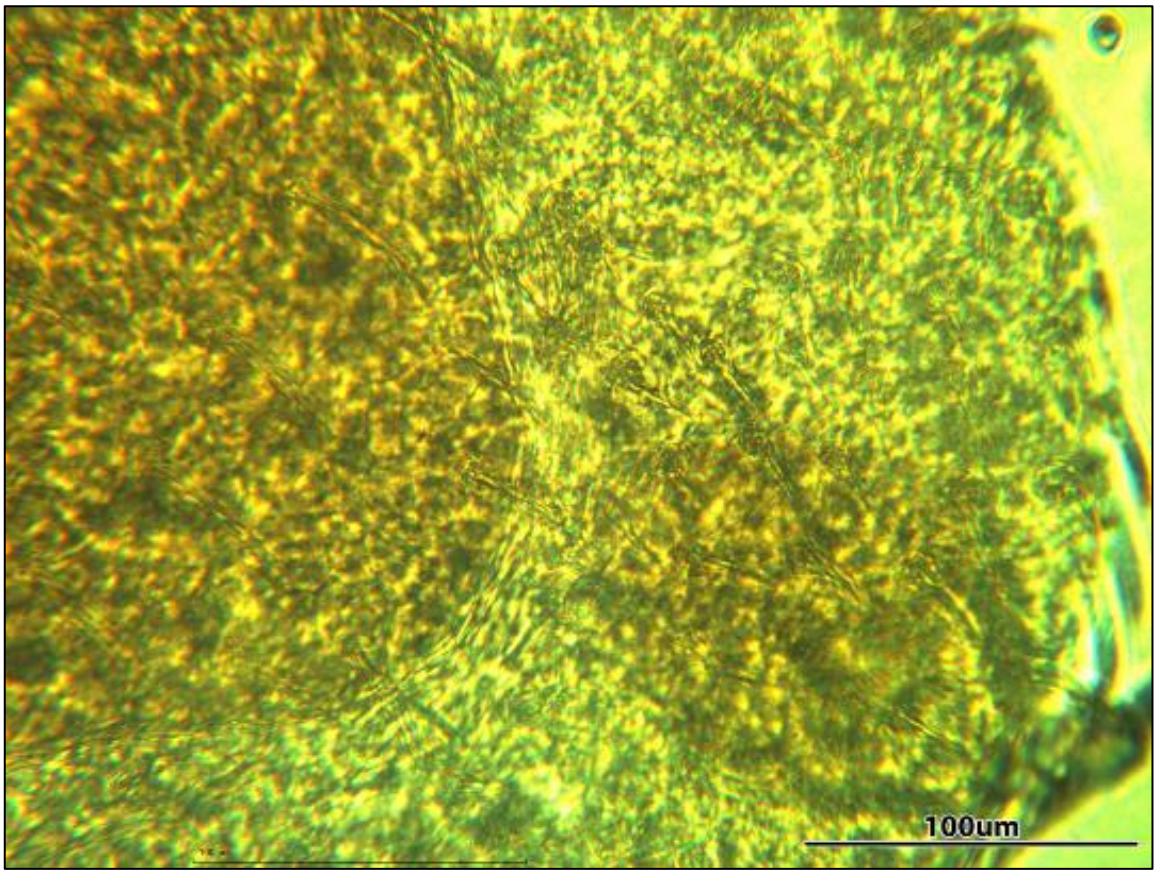

Figure E.11 – Closer photograph of opithogastric seta.

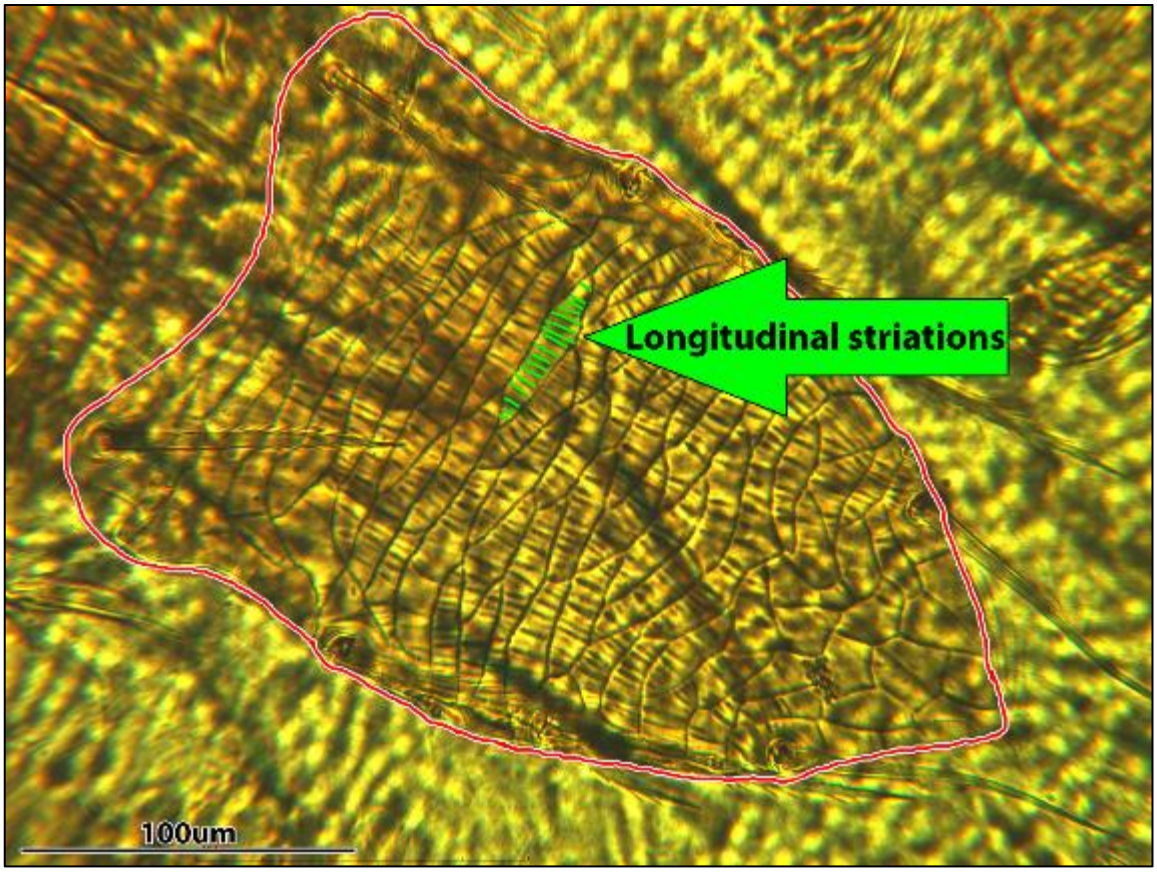

Figure E.12 – Sternal shield outlined in red. Clear longitudinal striations within the laterally arranged reticulations highlighted in green.

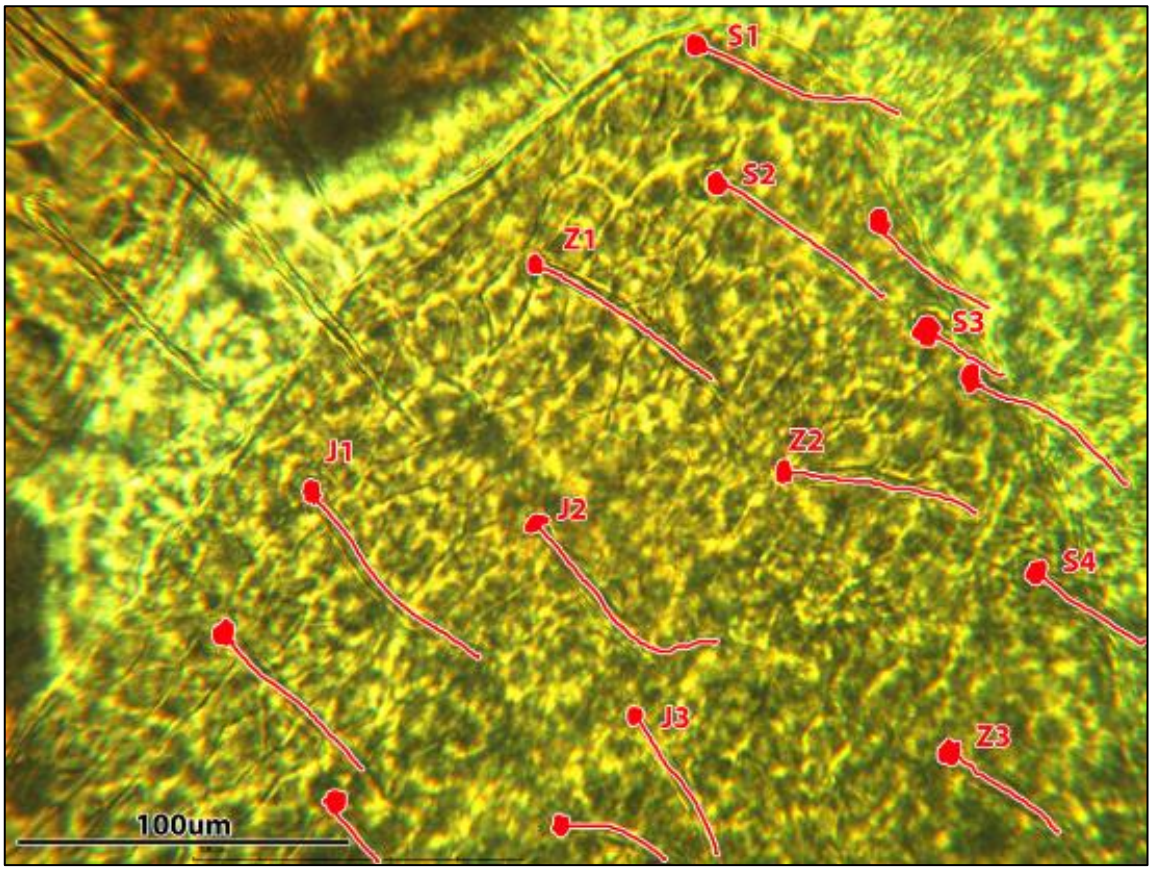

Figure E.13 – Opisthonotal seta, with diagnostic labelling for each seta applied where possible following the layout given in (Hyatt, 1980). 15 pairs of seta in total were counted on this shield.

Figure E.14 – Palp seta of *Parasitellus fucorum* in focus.

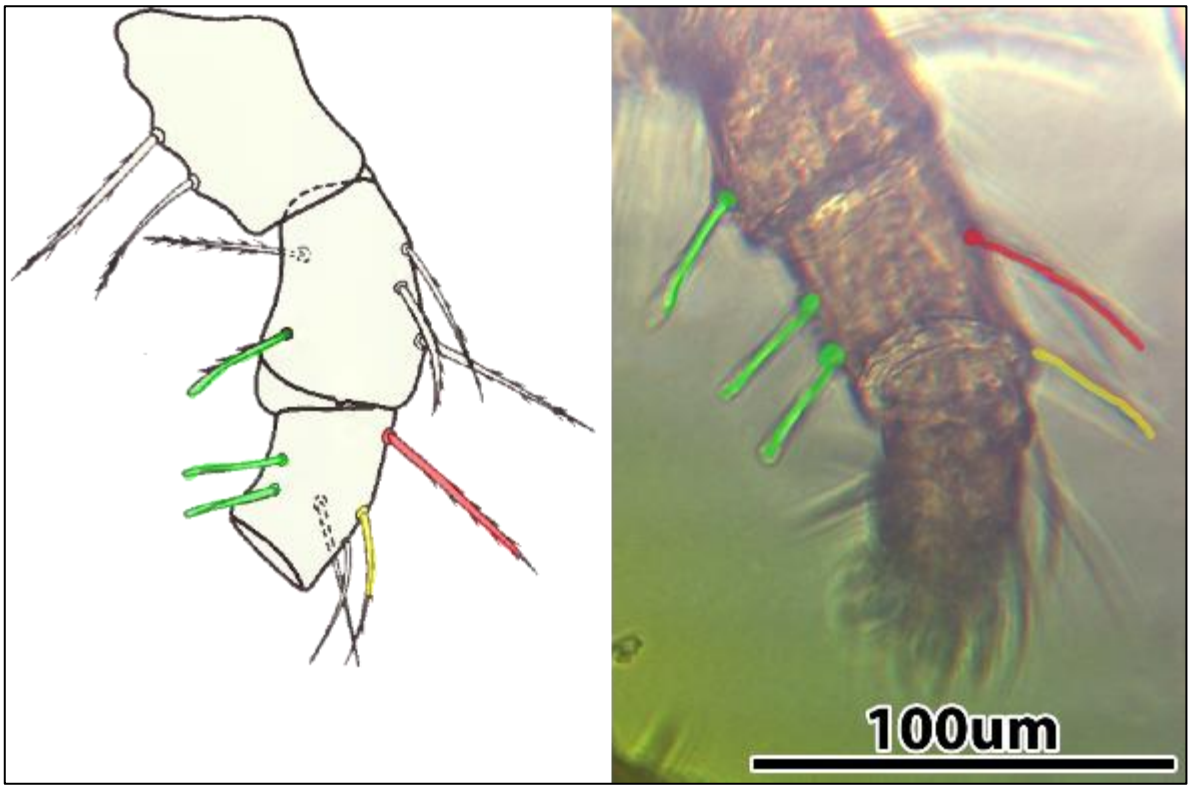

Figure E.15 – Left; (Figure 42E) from (Hyatt, 1980) showing the palp of *Parasitellus fucorum* deutonymph. Right; palp from the sample deutonymph. Certain seta in (Figure 42E) and their counterparts in the sample have been highlighted different colours for ease of comparison.

E.1.2 Parasitellus talparum

Identifier – Robin McArthur

Mite location – Phoretic on a *Bombus terrestris* queen

Order – Mesostigmata

Family – Parasitidae

Genus – *Parasitellus*

Species – *P. talparum* (Oudemans, 1913)

Keys used for ID – Hyatt, K. H. (1980). "Mites of the subfamily Parasitinae (Mesostigmata: Parasitidae) in the British Isles." Bulletin of the British Museum (Natural History) Zoology 38. Key to deutonymphs used.

ID steps taken	Notes/Reasoning
1 → 4	Began ID from Parasitinae deutonymph, as the mites' distinctive shield arrangement (Figs. 1/4), brownish/reddish colouration, large size and phoretic behaviour upon *a Bombus* worker distinguish it as such. • Leg II lacks ventral spurs in example (Fig. 2) • Dorsal setae long • Sternal shields formed differently to example given (Fig. 3)
4 → 8	• No granular area on sternal shield (Fig. 3) • Fixed digit of chelicera without membranous process
8 → 9	• Dorsal shields otherwise than example given in key (Fig. 4) • Corniculi shorter and triangular, only reaching around half the length of palp trochanter (Figs. 5/6)
9 → 13	• Dorsal setae overall without any extreme difference in length, though j1, z5 and r3 are longest (Fig. 7)
13 → 14	• Associated with *Bombus* • >40 pairs of opithogastic seta (Figs. 8/9)
14 → 15	• Sternal shield lacks striations (Fig. 3) • Opisthonotal shield >20 pairs seta (Fig. 10)
15 → ID	• Opisthogastic seta very short (Figs. 8/9) • Opisthonotal shield ~24 pairs seta (Fig. 10) • Presternal shields match species description (Figs. 11/12)
Result	**Mite identified as *Parasitellus talparum* deutonymph**.

Table E.2 – *Parasitellus talparum* ID steps taken with pictorial references. Text prefixed by a bullet point refers to specific morphological features mentioned in the key. Bracketed references indicate figures within the key.

Pictorial references

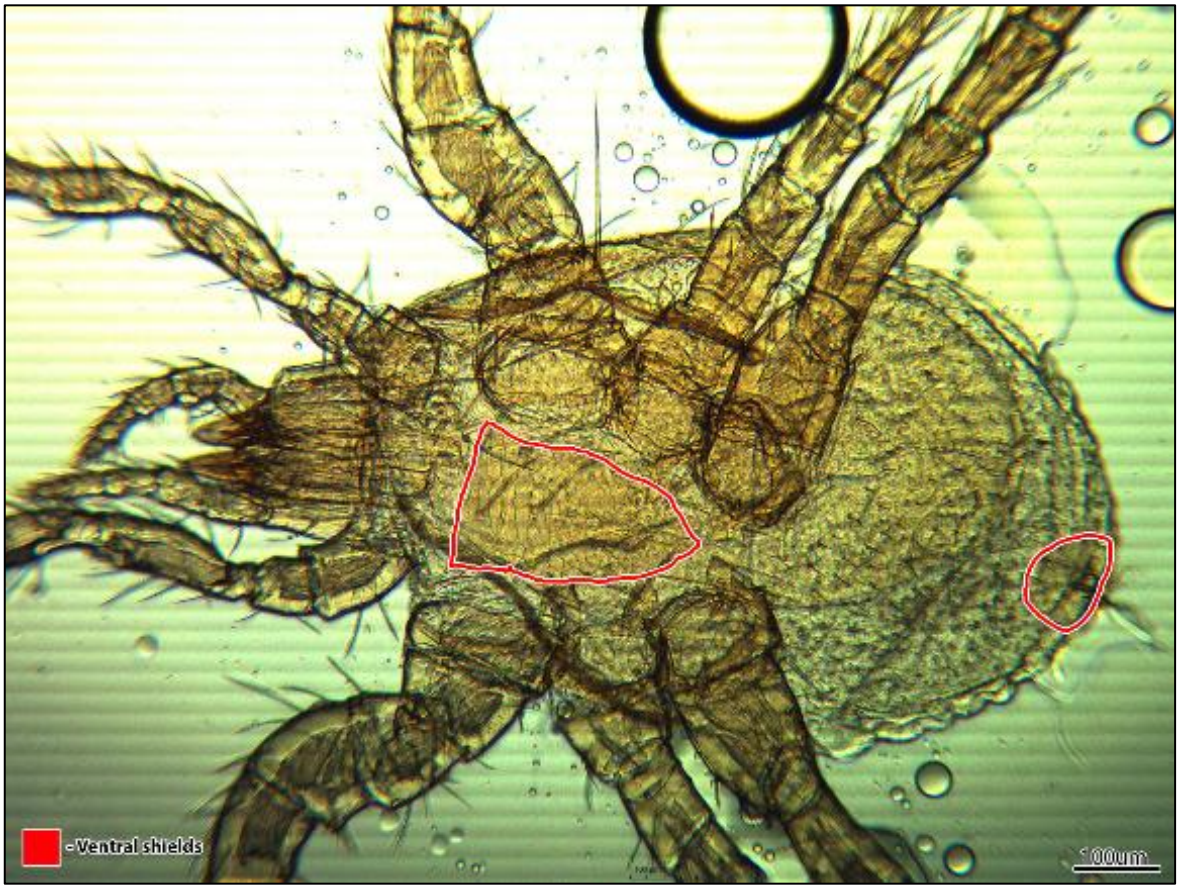

Figure E.16 – Distinctive shield arrangements and size (~1.1mm) show that this mite belongs to the Parasitidae family. *Parasitellus talparum* ventral shields (Left - Sternal and Right - Anal) are outlined in red.

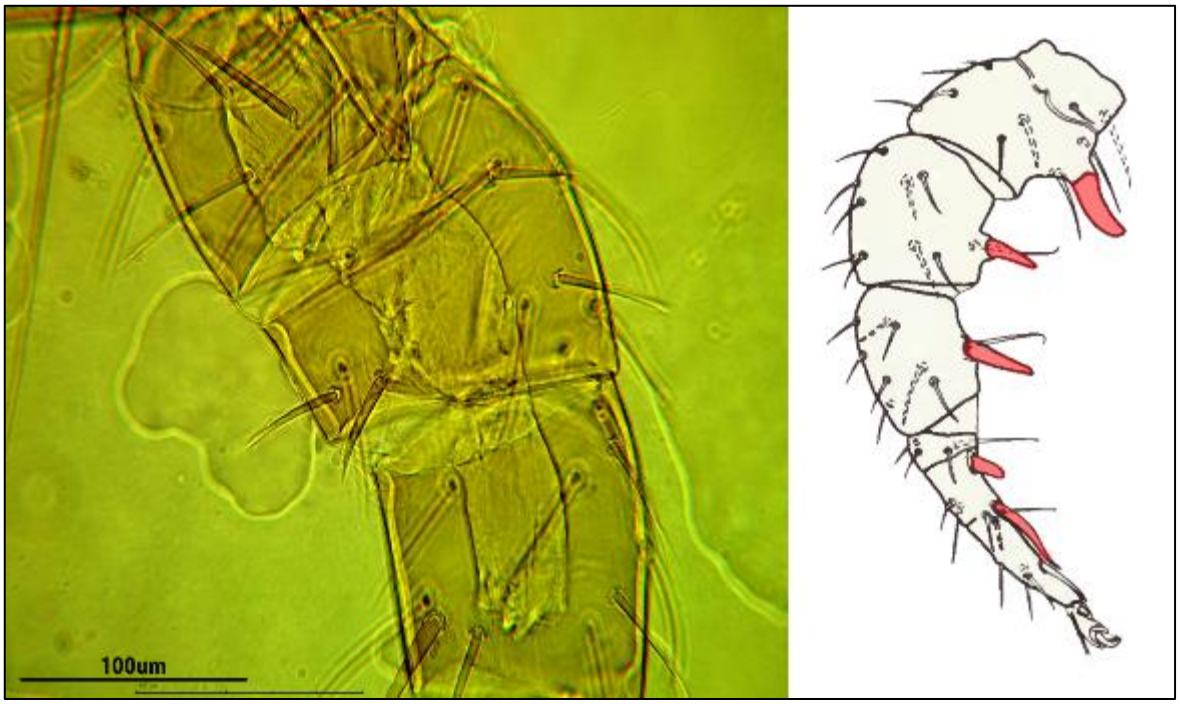

Figure E.17 – Leg II of *Parasitellus talparum* compared with Fig. 50G from (Hyatt, 1980), showing leg II of a *Gamasodes spiniger* deutonymph with conspicuous ventral spurs highlighted in red.

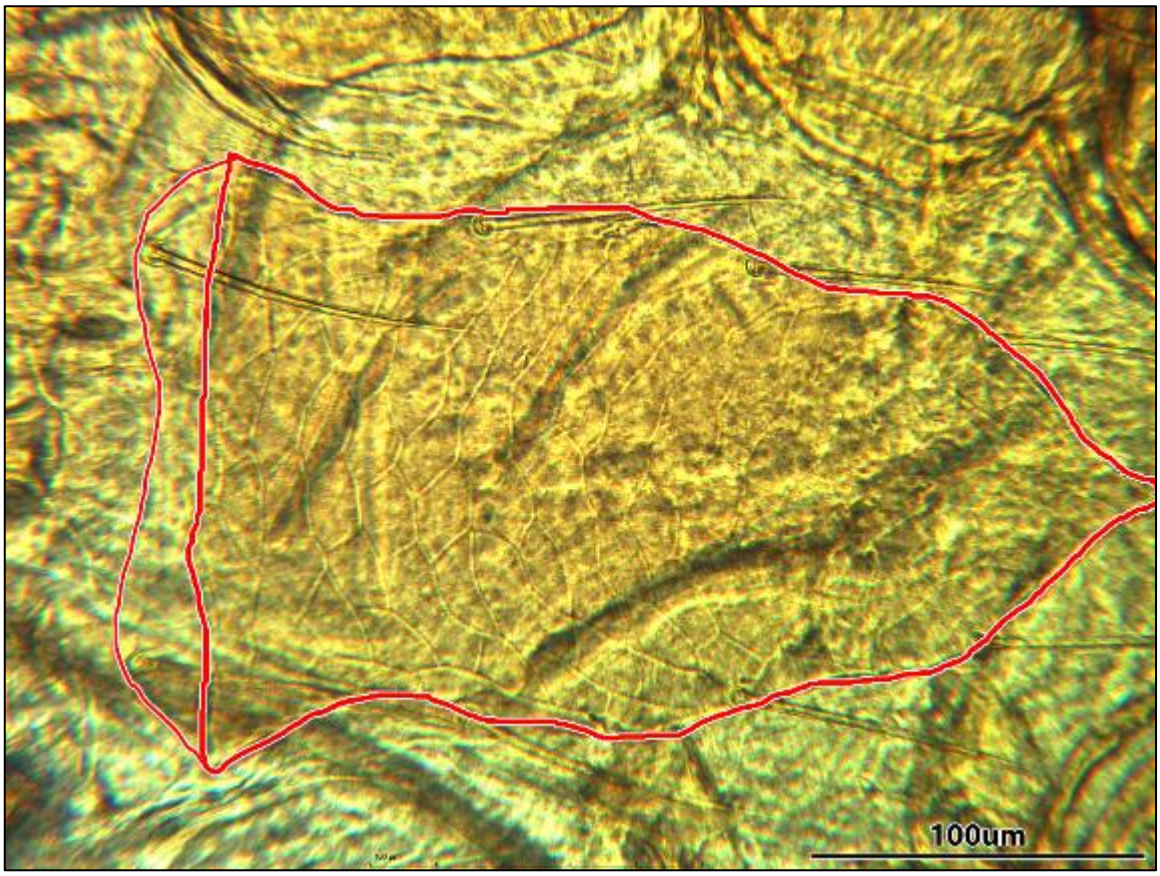

Figure E.18 – Sternal shield outlined in red.

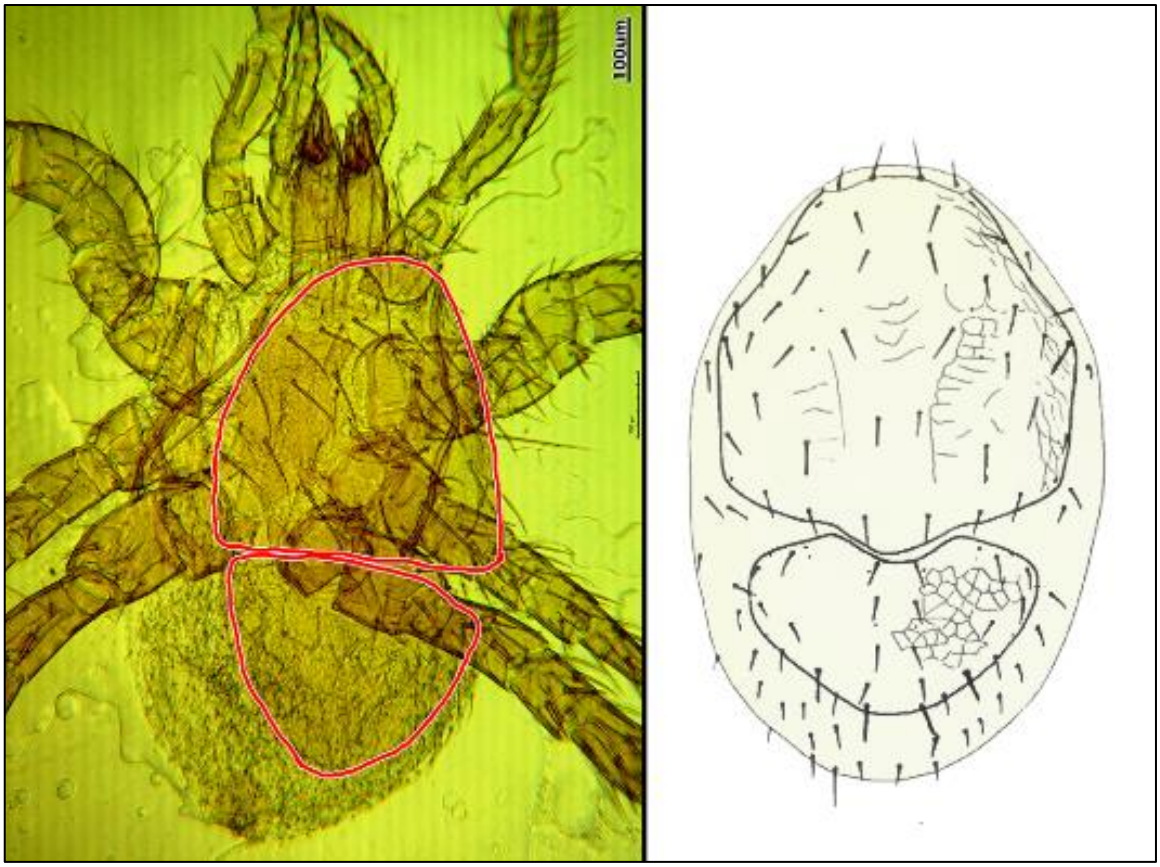

Figure E.19 – Comparison of *Parasitellus talparum*'s dorsal shield shape with example given in (Fig. 41A) from (Hyatt, 1980), showing the dorsal features of a *Cornigamasus lunaris* deutonymph.

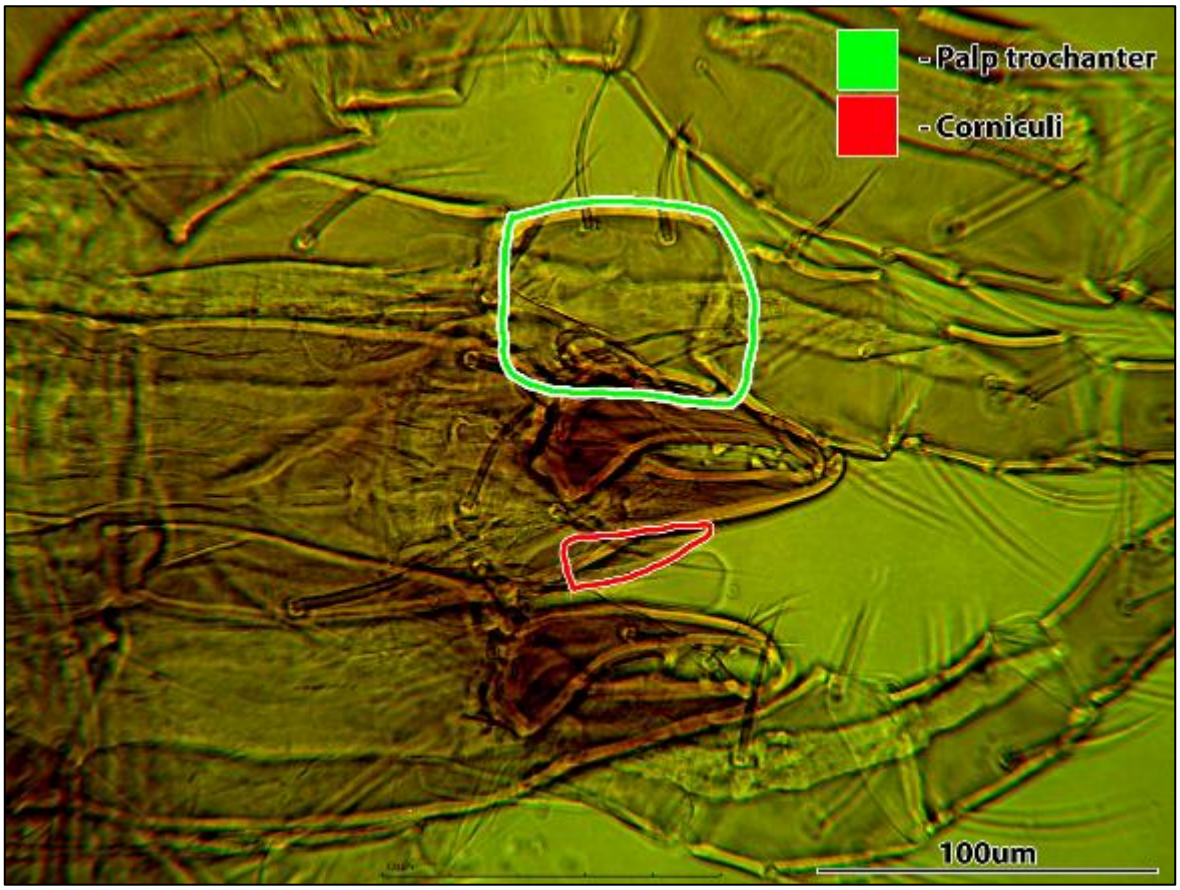

Figure E.20 – Mouthparts of *Parasitellus talparum*, including palps. Corniculi outlined in red, palp trochanters outlined in green.

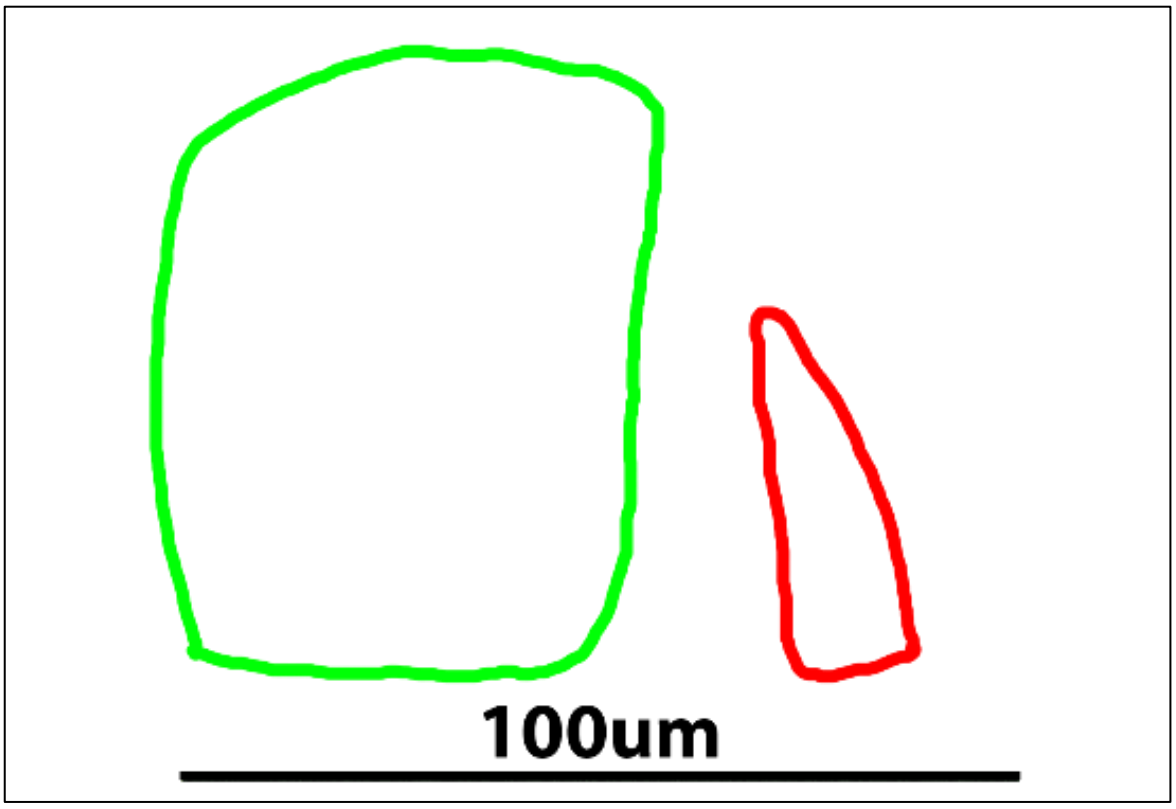

Figure E.21 – Direct comparison of corniculi (red) and palp trochanter (green) sizes, showing that the corniculi reach roughly half the length of the trochanters.

Figure E.22 – Some visible dorsal seta highlighted. z5 highlighted yellow, r3 highlighted green, all others highlighted yellow.

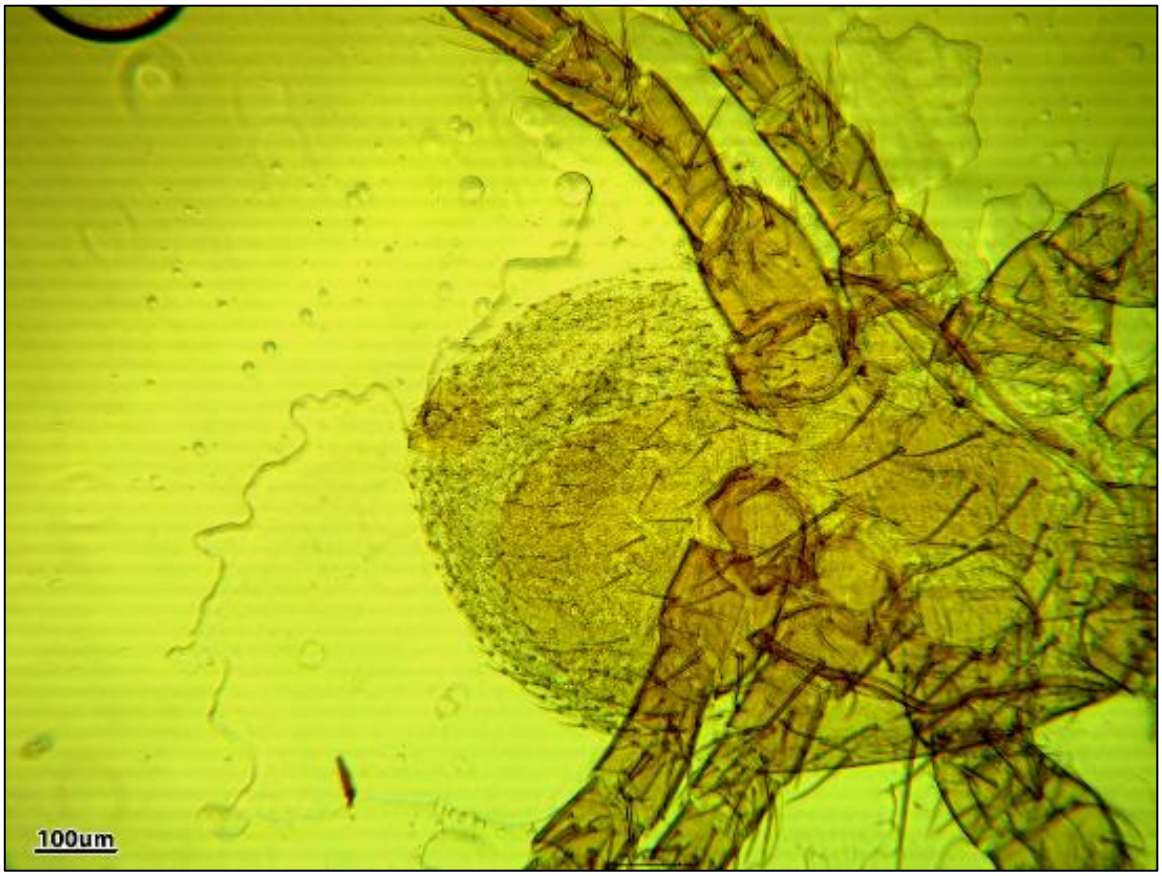

Figure E.23 – Opithogastric seta. While >40 pairs were observed, it proved impossible to get a photograph with them all in focus due to the mites size.

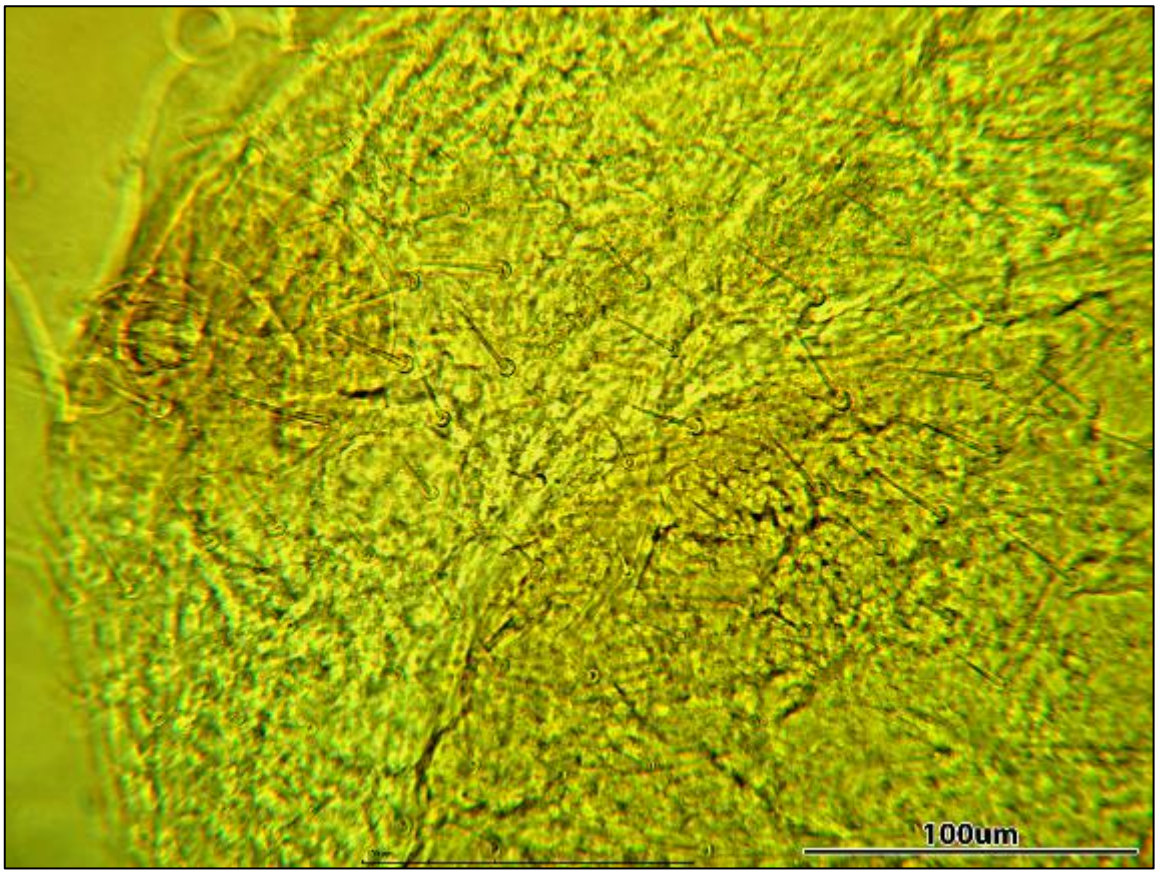

Figure E.24 – Closer photograph of opithogastric seta.

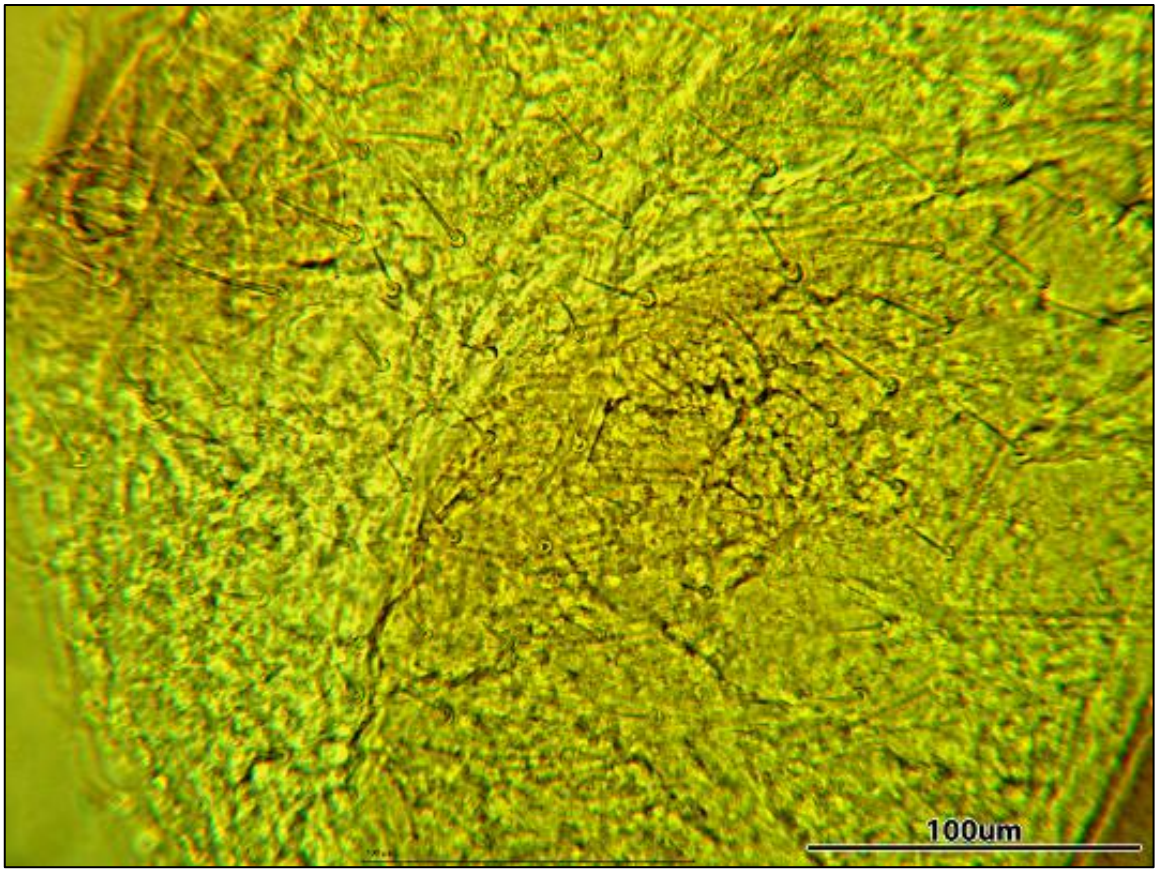

Figure E.25 – Opisthonotal seta.

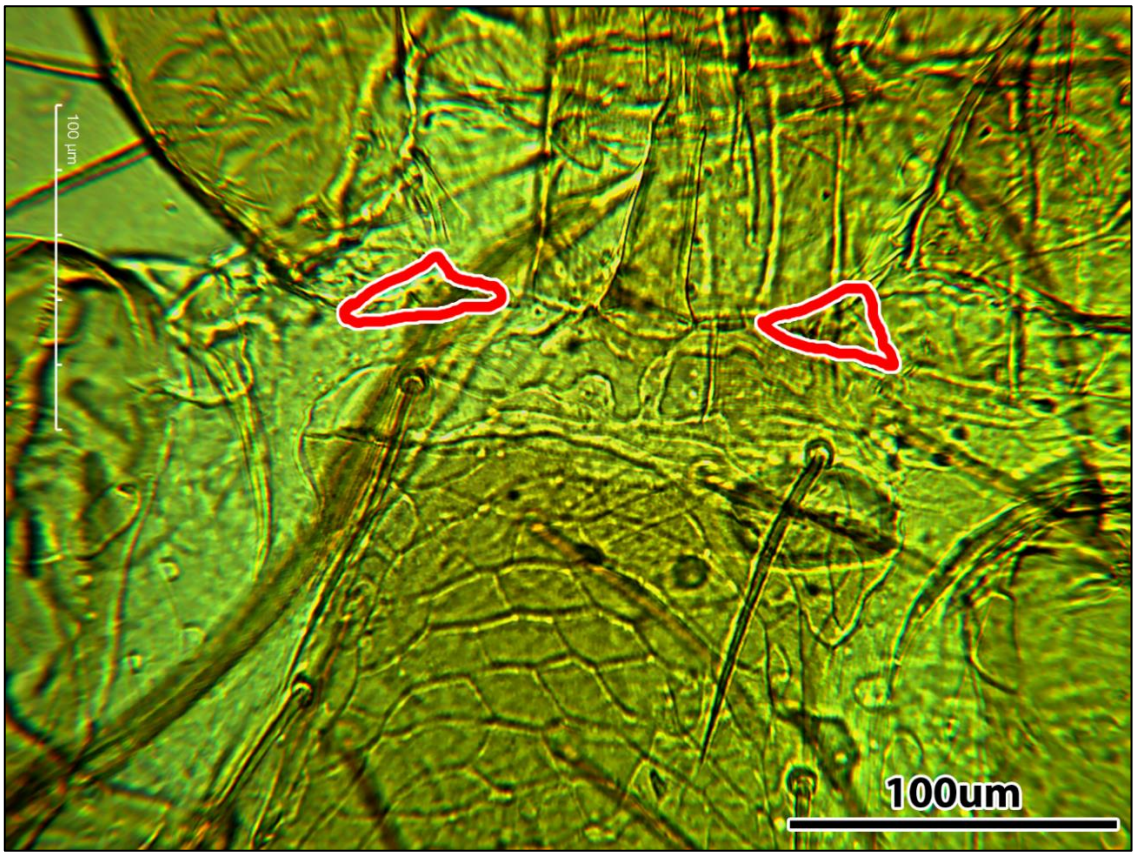

Figure E.26 – Presternal shields outlined in red.

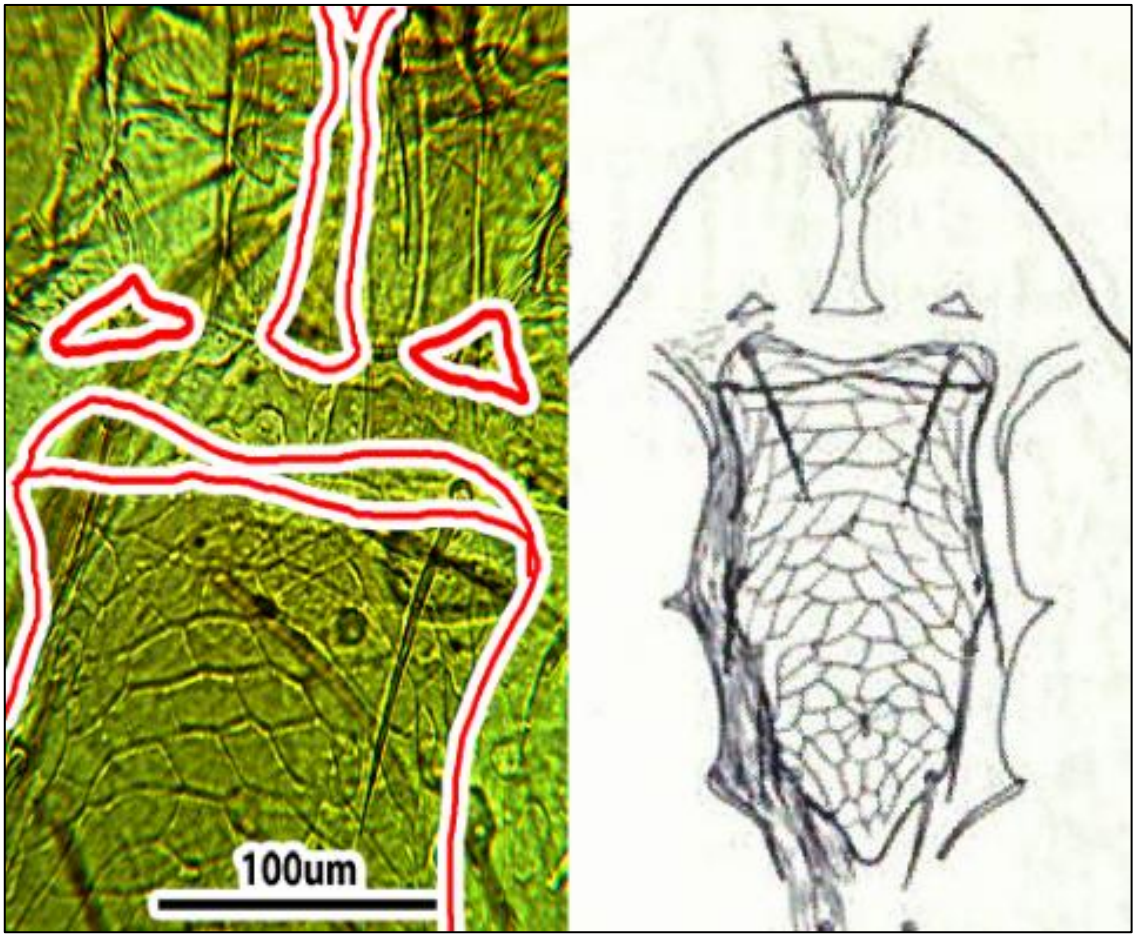

Figure E.27 – Sternal shield, presternal shields, and other characters from *Parasitellus talparum* compared to species description diagram.

E.1.3 Parasitellus ignotus

Identifier – Robin McArthur

Mite location – Phoretic on a *Bombus terrestris* queen

Order – Mesostigmata

Family – Parasitidae

Genus – *Parasitellus*

Species – *P. ignotus* (Vitzthum, 1930)

Keys used for ID – Hyatt, K. H. (1980). "Mites of the subfamily Parasitinae (Mesostigmata: Parasitidae) in the British Isles." Bulletin of the British Museum (Natural History) Zoology 38. Key to deutonymphs used.

ID steps taken	Notes/Reasoning
1 → 4	Began ID from Parasitinae deutonymph. The mites' distinctive shield arrangements, brownish/reddish colouration, large size and phoretic behaviour upon *Bombus* distinguish it as such. • Leg II lacks ventral spurs • Dorsal setae long • Sternal shields differ from example given
4 → 8	• No granular area on sternal shield • Fixed digit of chelicera lacks membranous process
8 → 9	• Dorsal shields differ from example given in key
9 → 13	• Dorsal setae mostly without extreme differences in length, though z5 and r3 are longest. j1 seta could not be seen.
13 → 14	• Associated with *Bombus* • >40 pairs of opithogastic seta
14 → 15	• Sternal shield lacks striations • Opisthonotal shield >20 pairs seta
15 → 16	• Opisthogastic seta mostly >35μm length • Opisthonotal shield ~35 pairs seta
16 → ID	• Additional dorsal seta between r5 and s6. Additional seta on J-series shorter than other J-series seta. Anal shield with usual 3 seta.
Result	**Mite identified as *Parasitellus ignotus* deutonymph**.

Table E.3 – Steps taken to identify *Parasitellus ignotus*.

Pictorial references

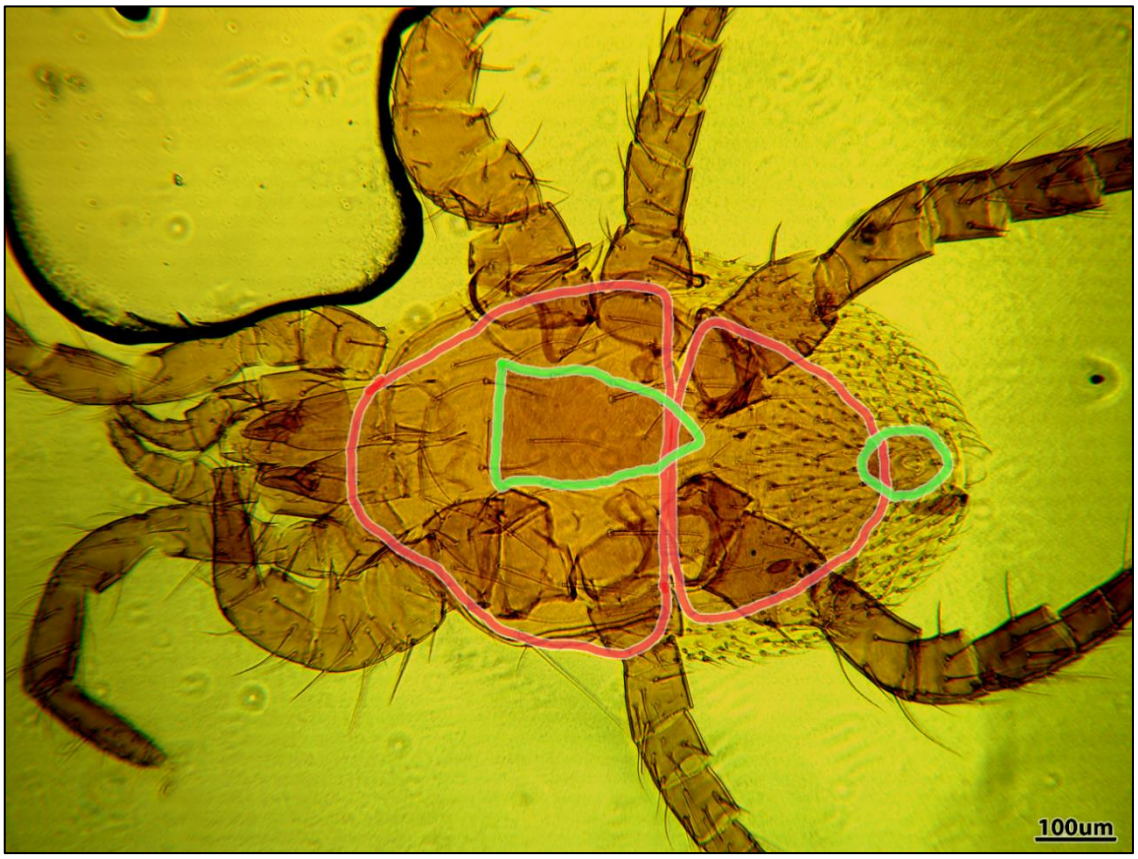

Figure E.28 – *Parasitellus ignotus* with shield outlines highlighted. Red outlines used for dorsal shields (podonotal and opishtonotal shields) and green outlines used for ventral shields (sternal and anal shields).

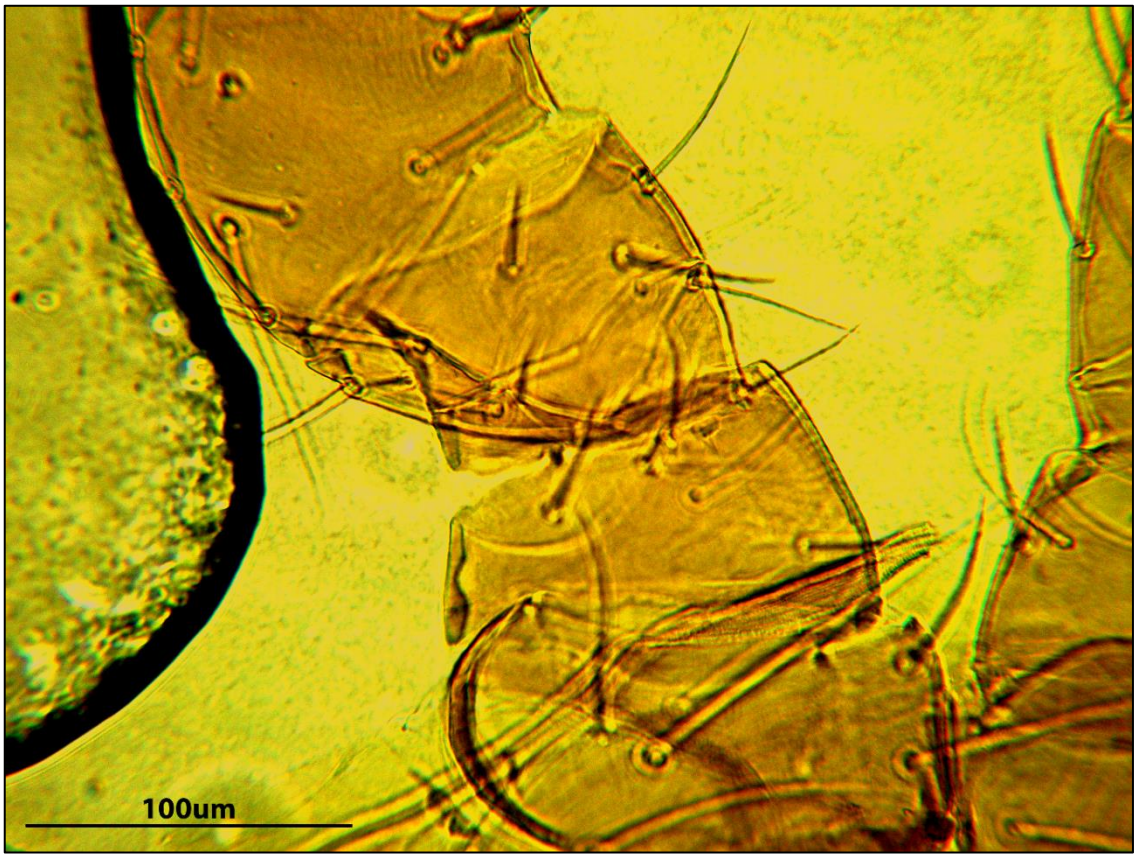

Figure E.29 – Leg II of *Parasitellus ignotus*, lacking conspicuous ventral spurs.

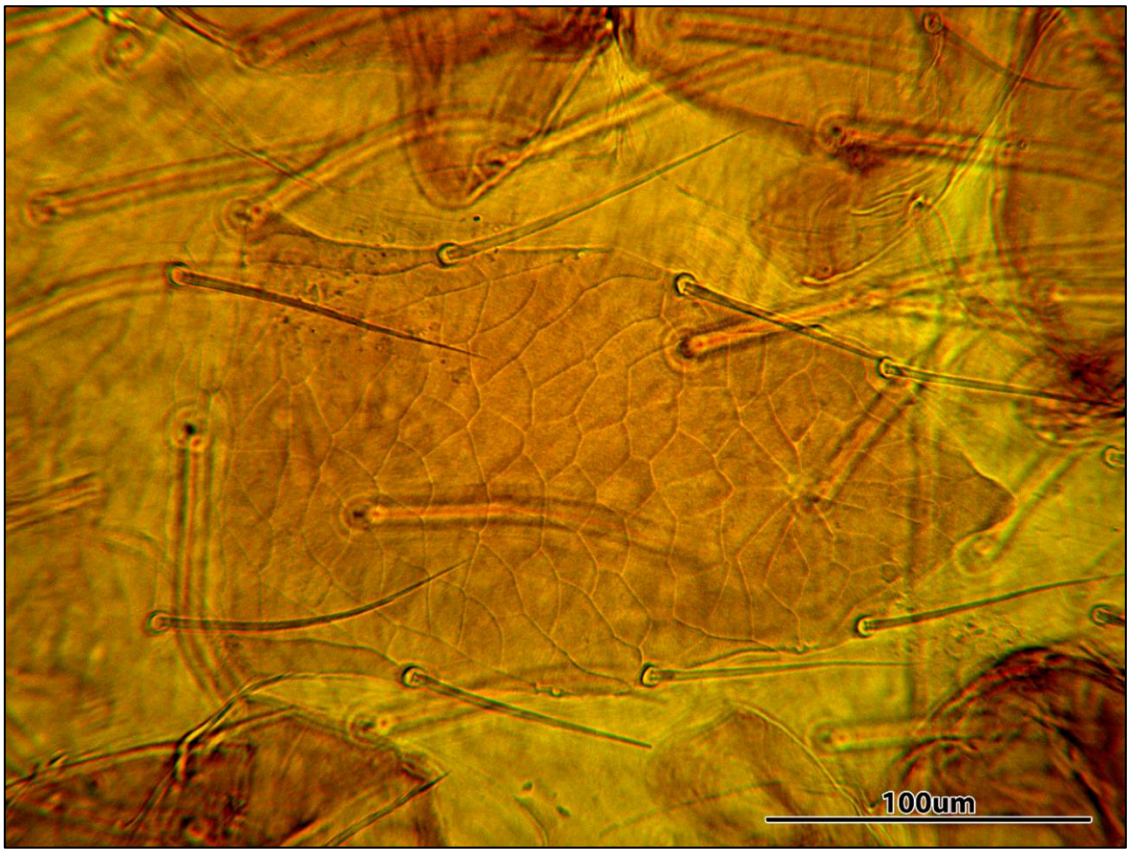

Figure E.30 – *Parasitellus ignotus* sternal shield.

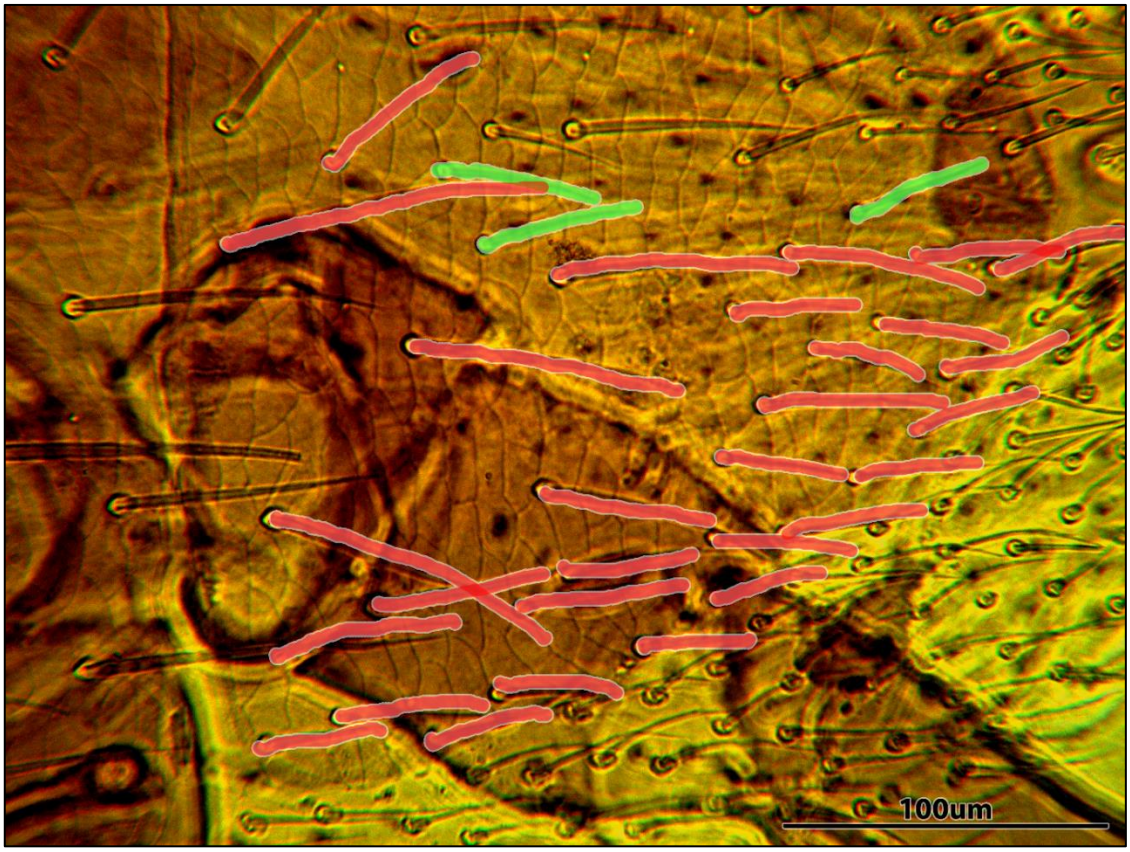

Figure E.31 – Opisthonotal shield seta, showing some clearly longer than 35µm. 35 pairs of seta were counted in total, though not all are shown clearly here, and some additional seta were situated on J-series. One seta from each pair visible highlighted in red, additional J-series seta highlighted green.

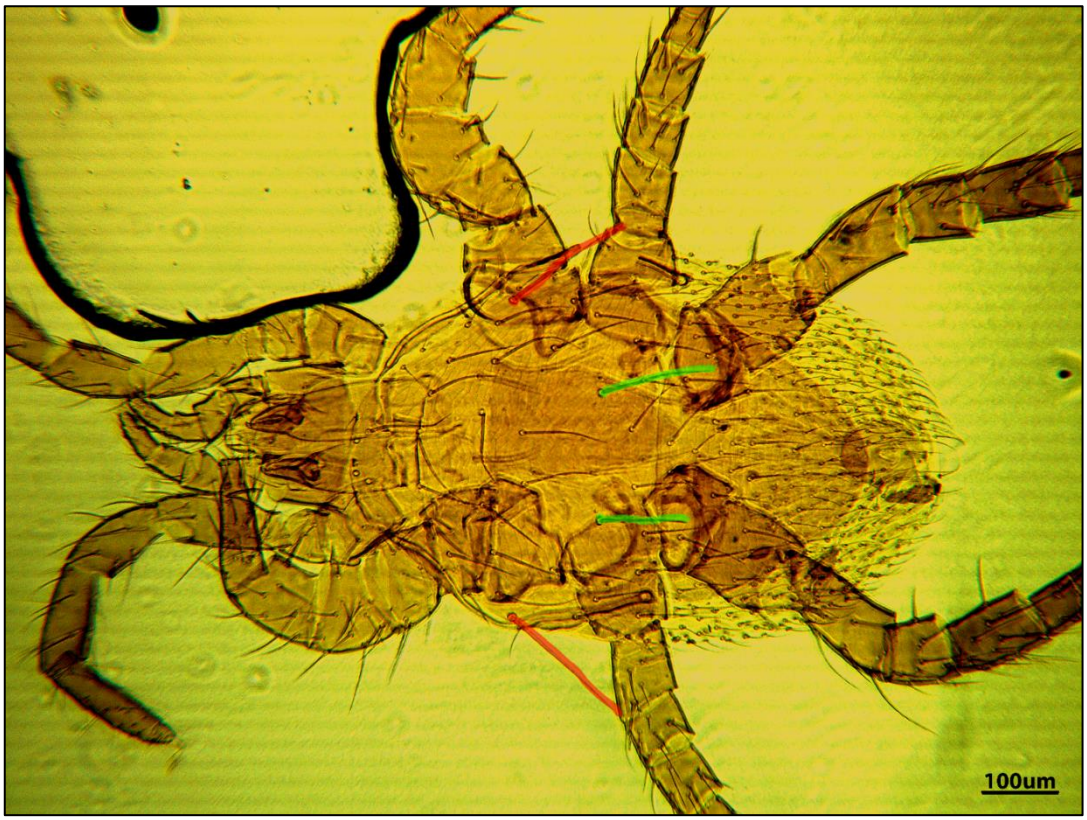

Figure E.32 – Dorsal seta of *Parasitellus ignotus* with r3 (red) and z5 (green) highlighted. While one of the z5 seta had broken near the end and no j1 seta were visible, it was clear that both r3 and z5 were longer than all other dorsal seta. No other dorsal seta differed greatly in size.

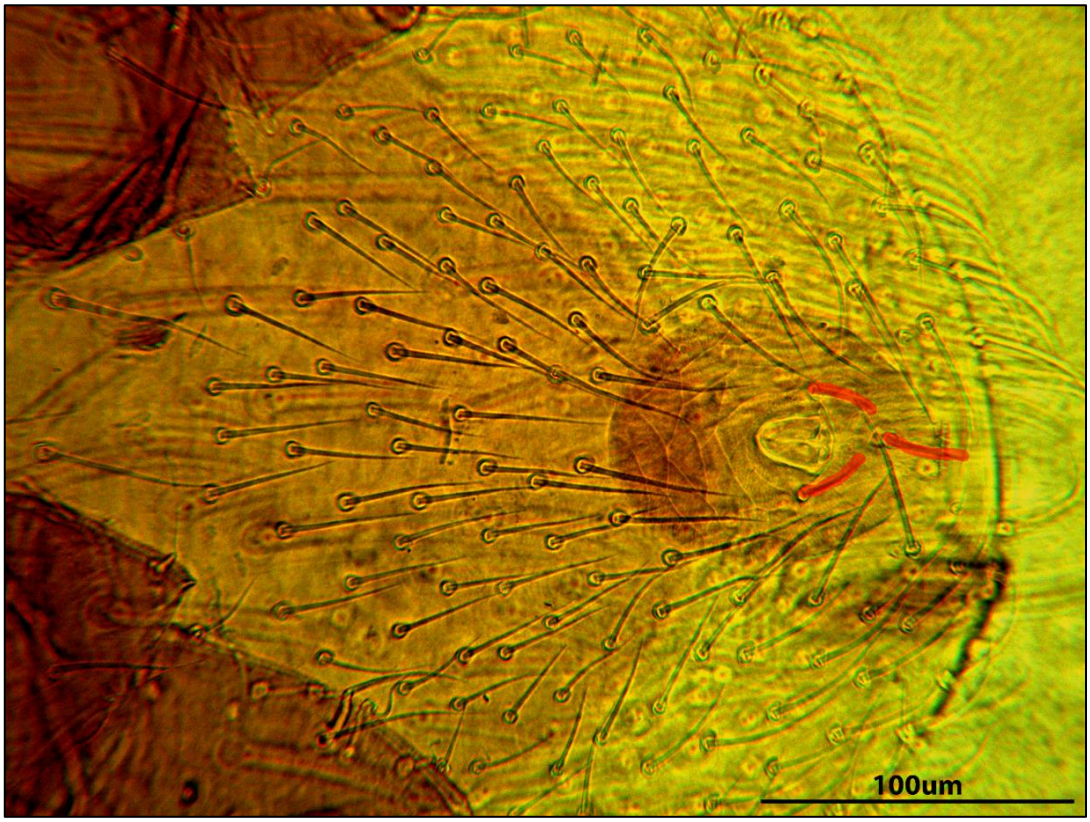

Figure E.33 – *Parasitellus ignotus* opisthogastric seta, with >40 pairs present. Anal shield seta highlighted red.

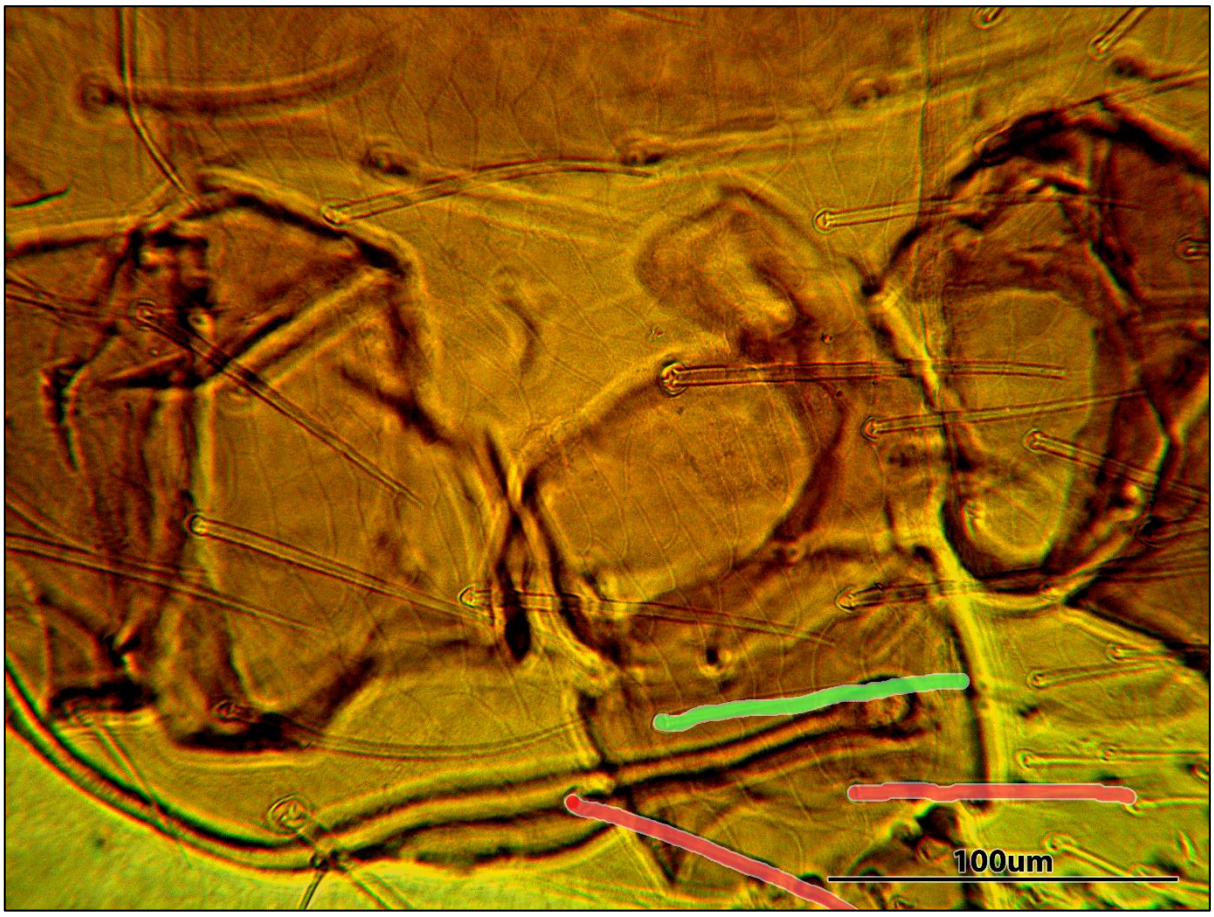

Figure E.34 – Podonotal shield seta with r5 and s6 highlighted red, and additional seta highlighted green.

E.1.4 Parasitellus crinitus

Identifier – Robin McArthur

Mite location – Phoretic on a *Bombus terrestris* queen

Order – Mesostigmata

Family – Parasitidae

Genus – *Parasitellus*

Species – *P. crinitus* (Oudemans, 1903)

Keys used for ID – Hyatt, K. H. (1980). "Mites of the subfamily Parasitinae (Mesostigmata: Parasitidae) in the British Isles." Bulletin of the British Museum (Natural History) Zoology 38. Key to deutonymphs used.

ID steps taken	Notes/Reasoning
1 → 4	Began ID from Parasitinae deutonymph. The mites' distinctive shield arrangements, brownish/reddish colouration, large size and phoretic behaviour upon *Bombus* distinguish it as such. • Leg II lacks ventral spurs • Dorsal setae long • Sternal shields differ from example given
4 → 8	• No granular area on sternal shield • Fixed digit of chelicera lacks membranous process
8 → 9	• Dorsal shields differ from example given in key
9 → 13	• Dorsal setae mostly without extreme differences in length, though j1, z5 and r3 are longest.
13 → 14	• Associated with *Bombus* • >40 pairs of opithogastic seta
14 → 15	• Sternal shield without striations • Opisthonotal shield >20 pairs seta
15 → 16	• Opisthogastic seta mostly >35μm length • Opisthonotal shield > 35 pairs seta, some on J-series.
16 → ID	• Podonotal shield without added setae between r5 and s6. Additional J-series setae not longer than surrounding setae. Anal shield has additional setae.
Result	**Mite identified as *Parasitellus crinitus* deutonymph**.

Table E.4 – Details of steps taken to identify *Parasitellus crinitus*.

Pictorial references

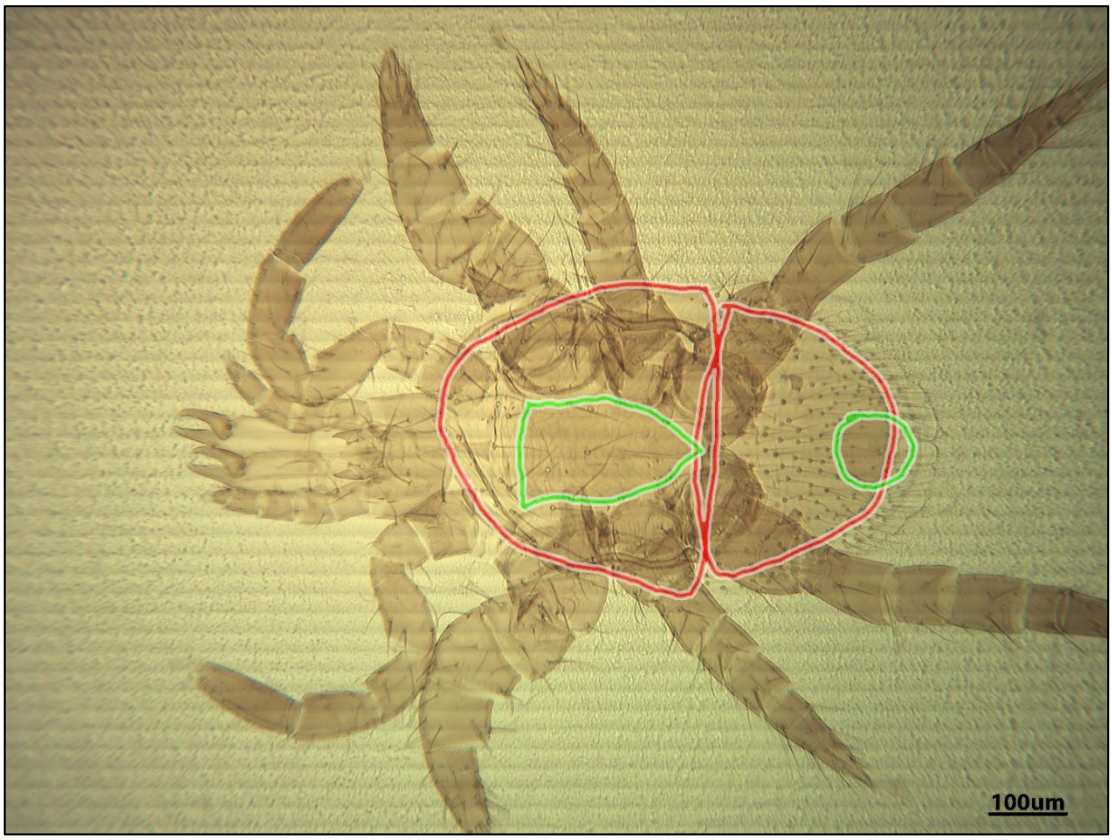

Figure E.35 – *Parasitellus crintus* with shield outlines highlighted. Red outlines used for dorsal shields (podonotal and opishtonotal shields) and green outlines used for ventral shields (sternal and anal shields).

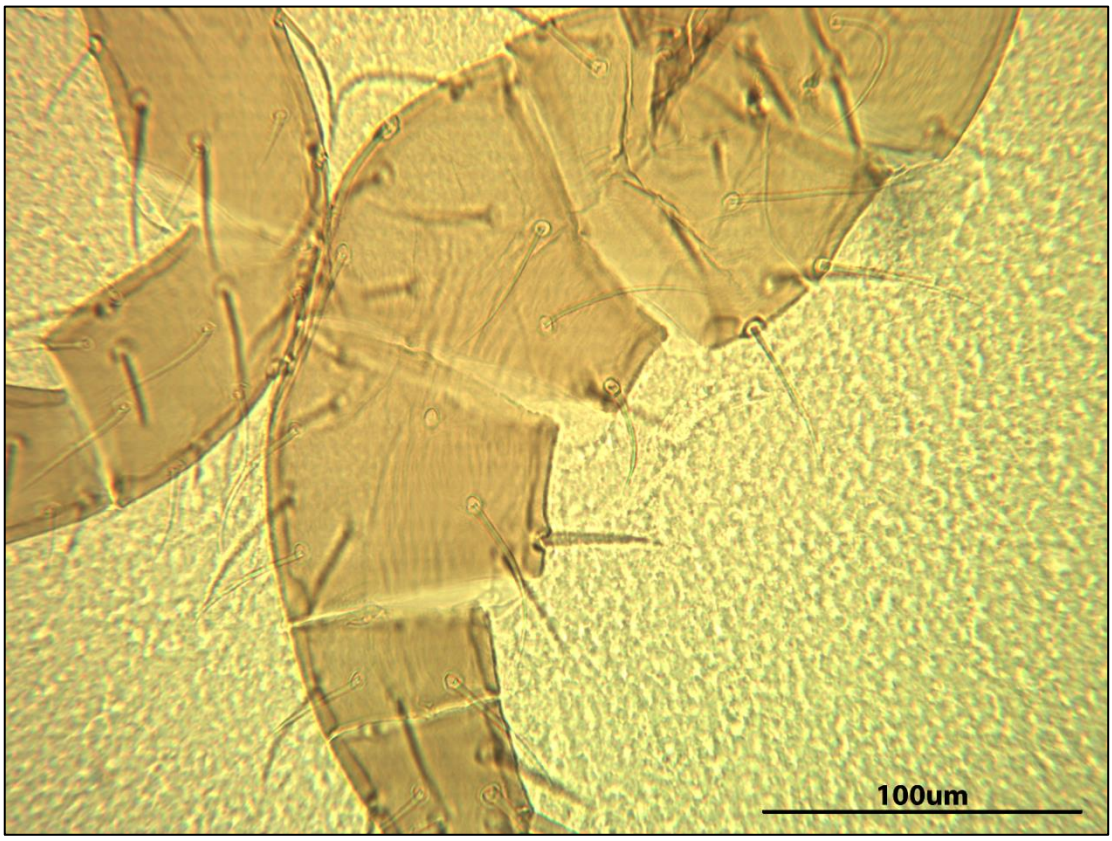

Figure E.36 – Leg II of *Parasitellus crintus*, lacking conspicuous ventral spurs.

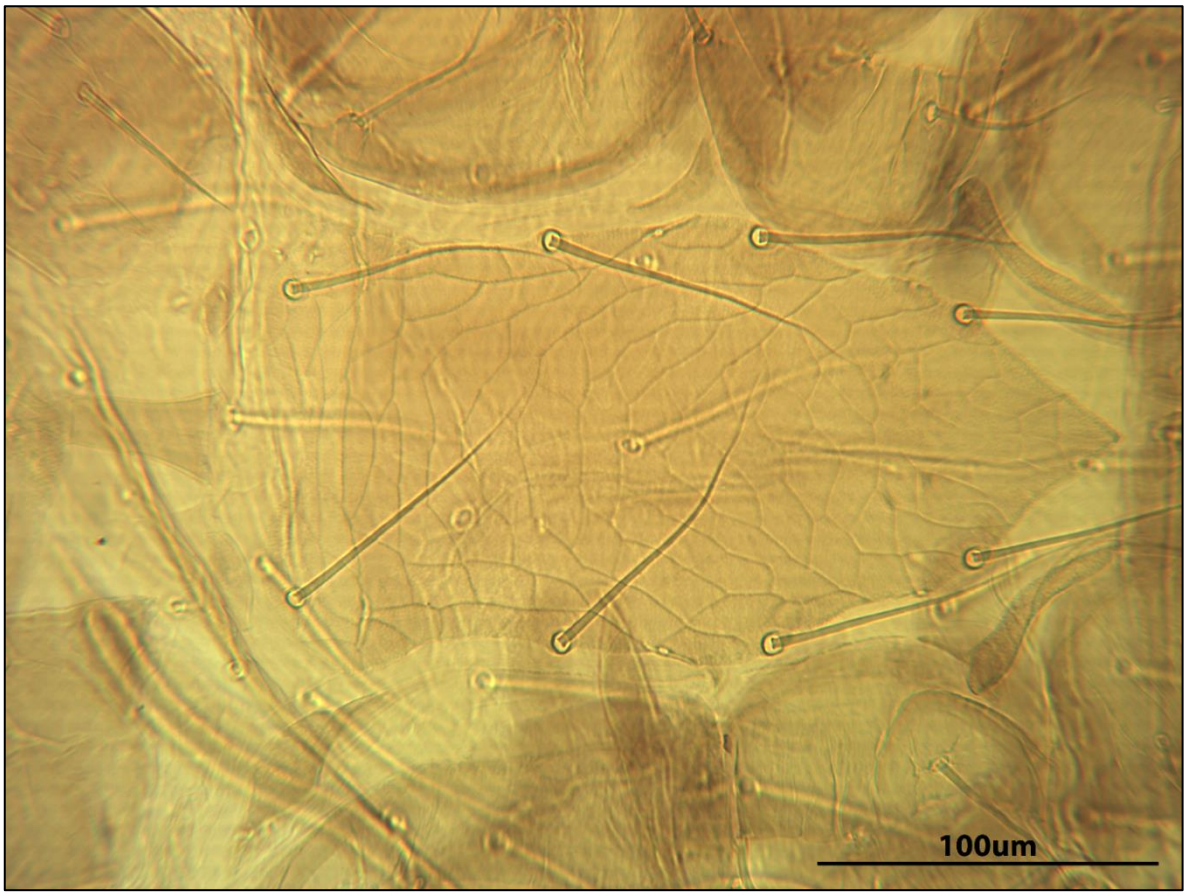

Figure E.37 – *Parasitellus crintus* sternal shield.

Figure E.38 – Dorsal seta of *Parasitellus crintus* with j1 (yellow), r3 (red) and z5 (green) highlighted. While one seta from each pair had broken near the base it was clear that both r3 and z5 were longer than all other dorsal seta.

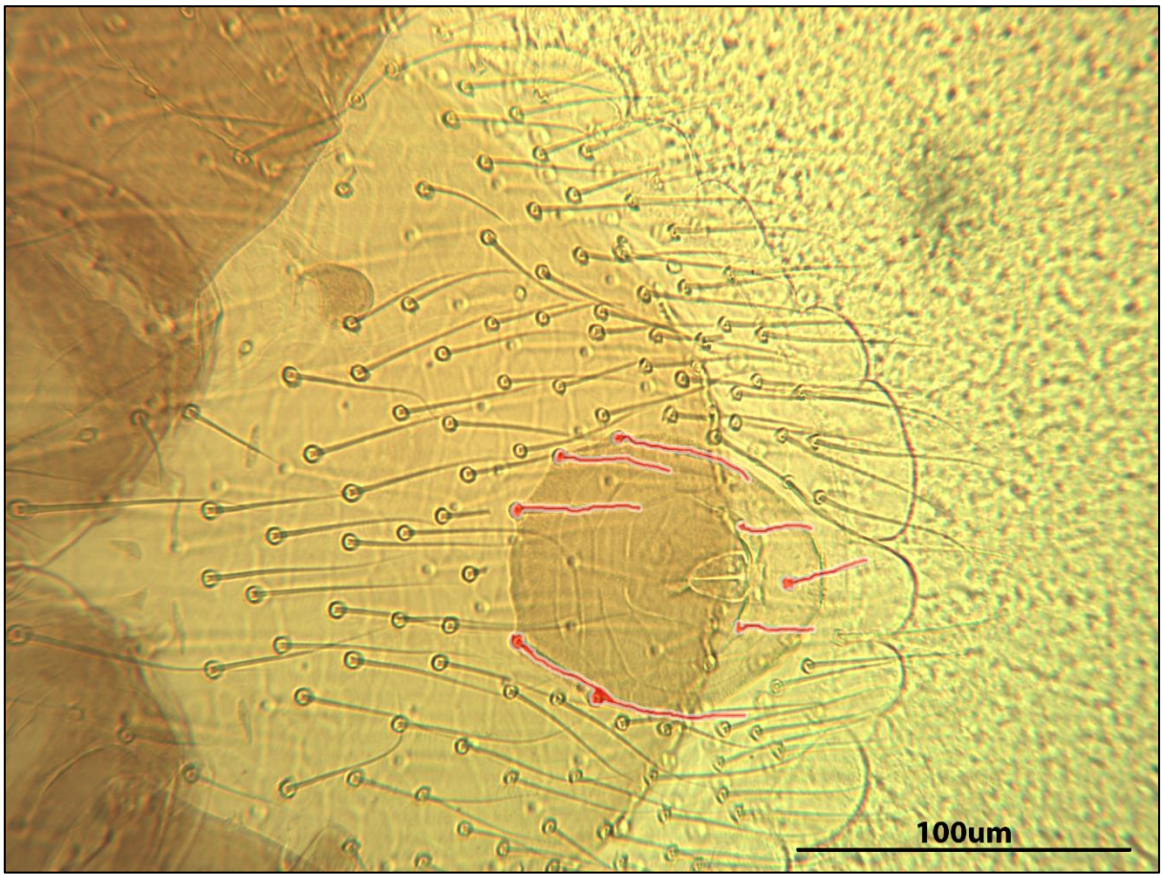

Figure E.39 – *Parasitellus crintus* opisthogastric seta, with >40 pairs present. Anal shield seta highlighted red. Presumably 3 additional pairs in most cases, however in this sample the upper side (as shown here) had 3 additional setae while the lower side only had 2.

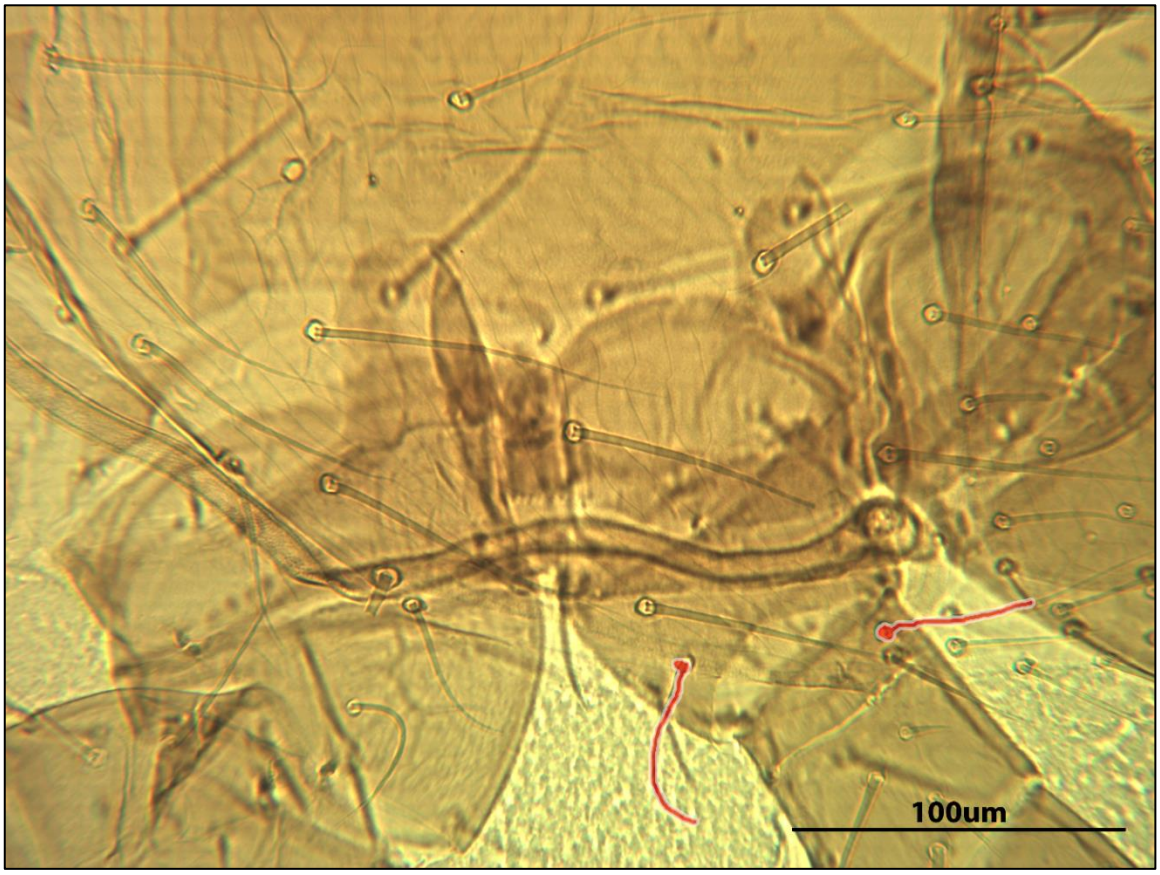

Figure E.40 – Podonotal shield seta with r5 and s6 highlighted red, with no additional seta between them.

E.2 Pneumolaelaps

Identifier – Robin McArthur

Mite location – Phoretic upon *Bombus terrestris* queen

Order – Mesostigmata

Family – Laelapidae

Genus – *Pneumolaelaps* Berlese, 1920

Keys/papers used for ID:

1. Baker AS, York PV, Museum NH. (1999). Mites and ticks of domestic animals: an identification guide and information source: The Stationary Office.
2. OConnor B, Klimov P. (2012). Family Laelapidae Berlese, 1892 University of Michigan [updated 07/11/201514/08/19]. Available from: http://insects.ummz.lsa.umich.edu/beemites/Species_Accounts/Laelapidae.htm.

ID steps taken	Keys used	Notes/Reasoning
1 → 2	1	Gnathosoma lacked recurved teeth.
2 → 3	1	Genital opening without associated shields, mite < 2mm long.
3 → 53	1	Palp tarsus with 2-tined apotele, legs with 6 discrete segments, 1 pair of stigma with ribbon-like peritreme.
53 → 54	1	Idiosoma longer than width of idiosoma.
54 → 55	1	Peritreme straight posteriorly, ambulacrum I has two claws.
55 → 56	1	Genital shield with rounded anterior margin.
56 → 60	1	Dorsal shield lacks conspicuous patterning.
60 → 62	1	Middle segment of chelicera shorter and thicker than anterior.
62 → ID	1	Female genital shield expands posterior to coxae IV. Sample mite is in the **family Laelapidae**.
1 → 2	2	Sternal shield lacks porose area at coxae III, Legs III – IV not wider than legs I – II, femora III – IV lack dorsal spur.
2 → 3	2	Presternal shields distinct, paired, situated near sternal shield.
3 → 4	2	Anal shield with 3 setae, and seta not distinctly stouter than other ventral seta.
4 → 5	2	Peritemes not looped, reach well past coxae II.
5 → 7	2	No idiosomal setae distinctly wider than others, spatulate setae absent.
7 → 8	2	Anal shield lacks cuticular spur. Presternal region lacking keel-like ridge. Presternal shields present.
8 → 10	2	Anal shield narrower than posterior margin of ventrogenital shield.

ID steps taken	**Keys used**	**Notes/Reasoning**
10 → 11	2	Sternal shield between coxae I – II widened, but with pointed margins. Seta st4 on metasternal shields.
11 → 12	2	Apotele 2-tined.
12 → 13	2	Holodorsal shield covers entire dorsum.
13 → 14	2	Genu IV with 10 setae. Anterior ends of peritremes reach coxae I.
14 → 15	2	Pilus dentilis weakly developed.
15 → 16	2	Dorsal seta all simple or pilose, none are flattened.
16 → ID	2	Genu IV has 10 setae, with 2 ventral. Peritremes broad. Associated with *Bombus*.
ID	2	**Mite is a *Pneumolaelaps* female.**

Table E.5 – Steps taken through multiple keys to identify *Pneumolaelaps* female.

Pictorial references

Figure E.41 – *Pneumolaelaps* adult female (ventral side shown).

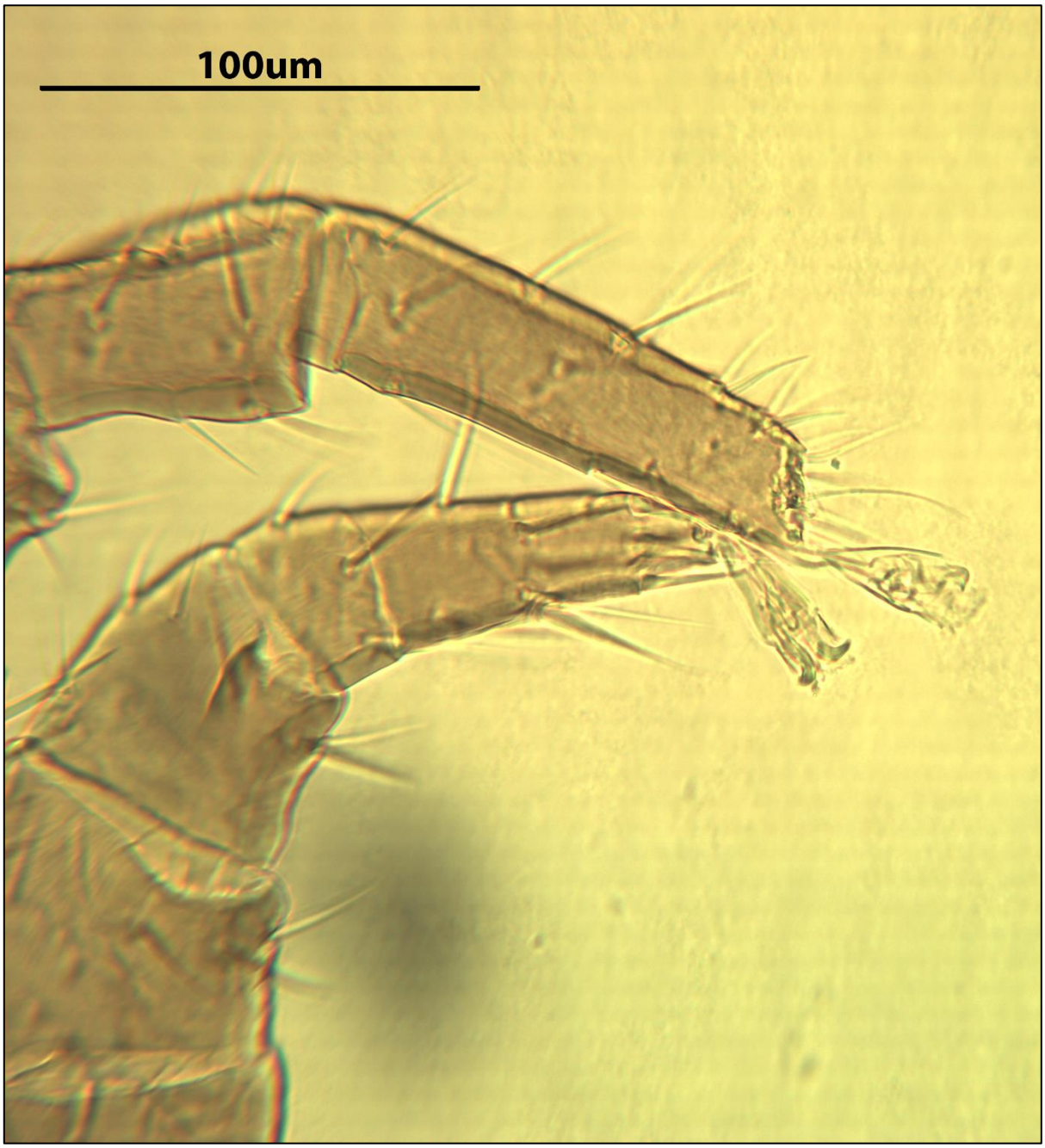

Figure E.42 – *Pneumolaelaps* apoteles (Legs I/II) with 2 claws each.

Figure E.43 – *Pneumolaelaps* with stigma and peritremes highlighted.

Figure E.44 – *Pneumolaelaps*, dorsal side and seta shown.

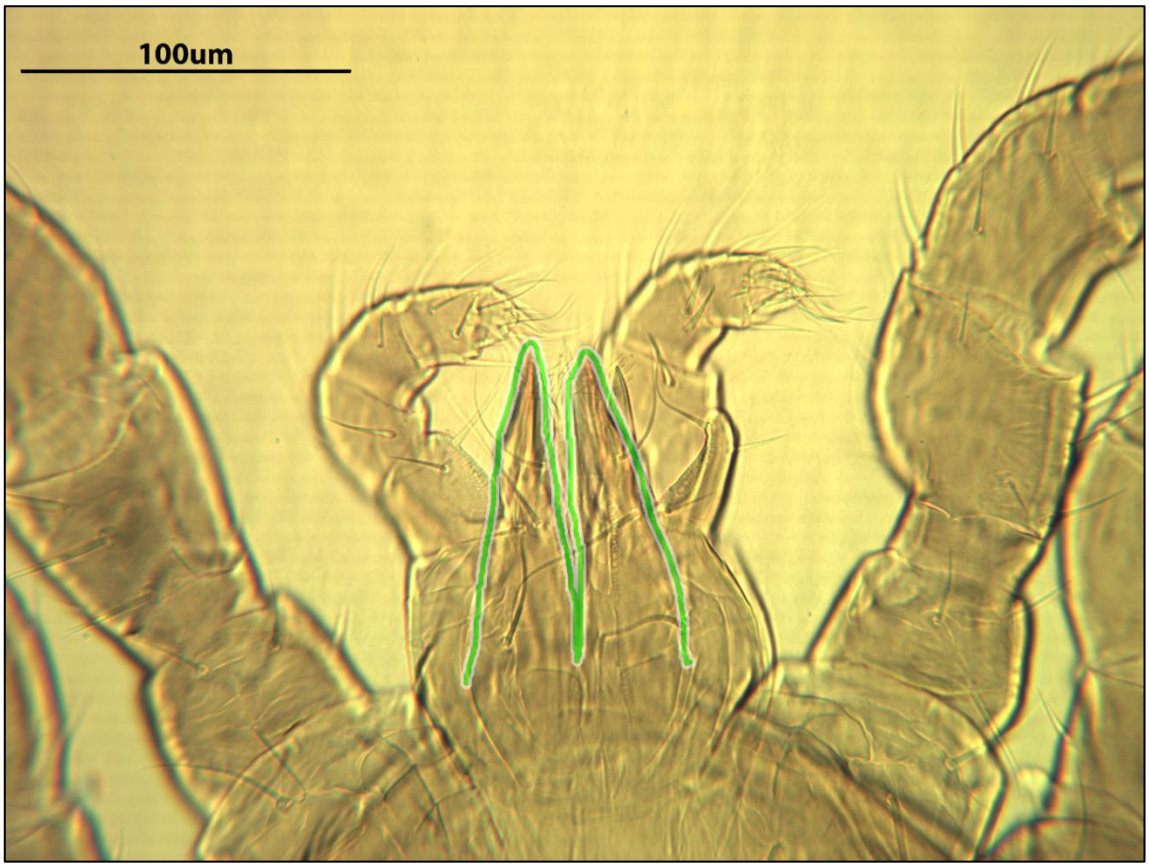

Figure E.45 – *Pneumolaelaps* chelicera, showing increasing thickness towards middle section (highlighted).

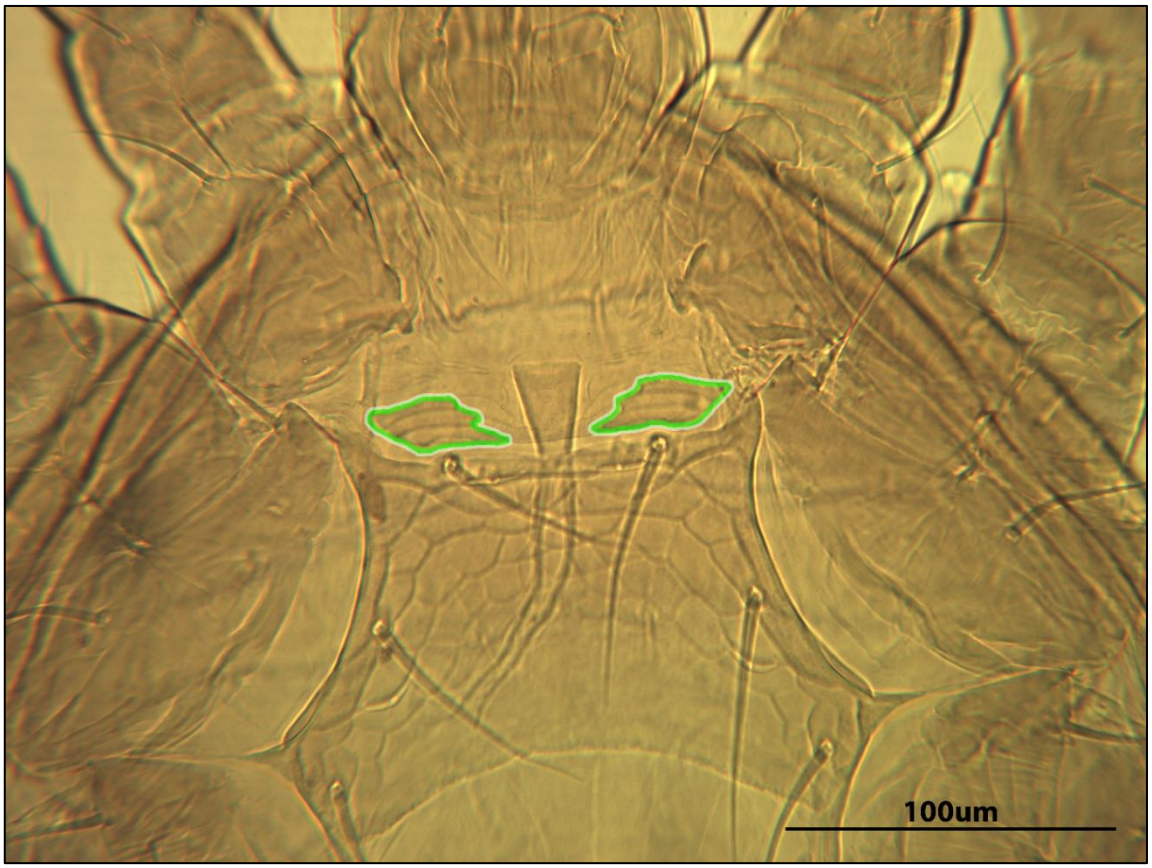

Figure E.46 – *Pneumolaelaps* presternal shields (highlighted).

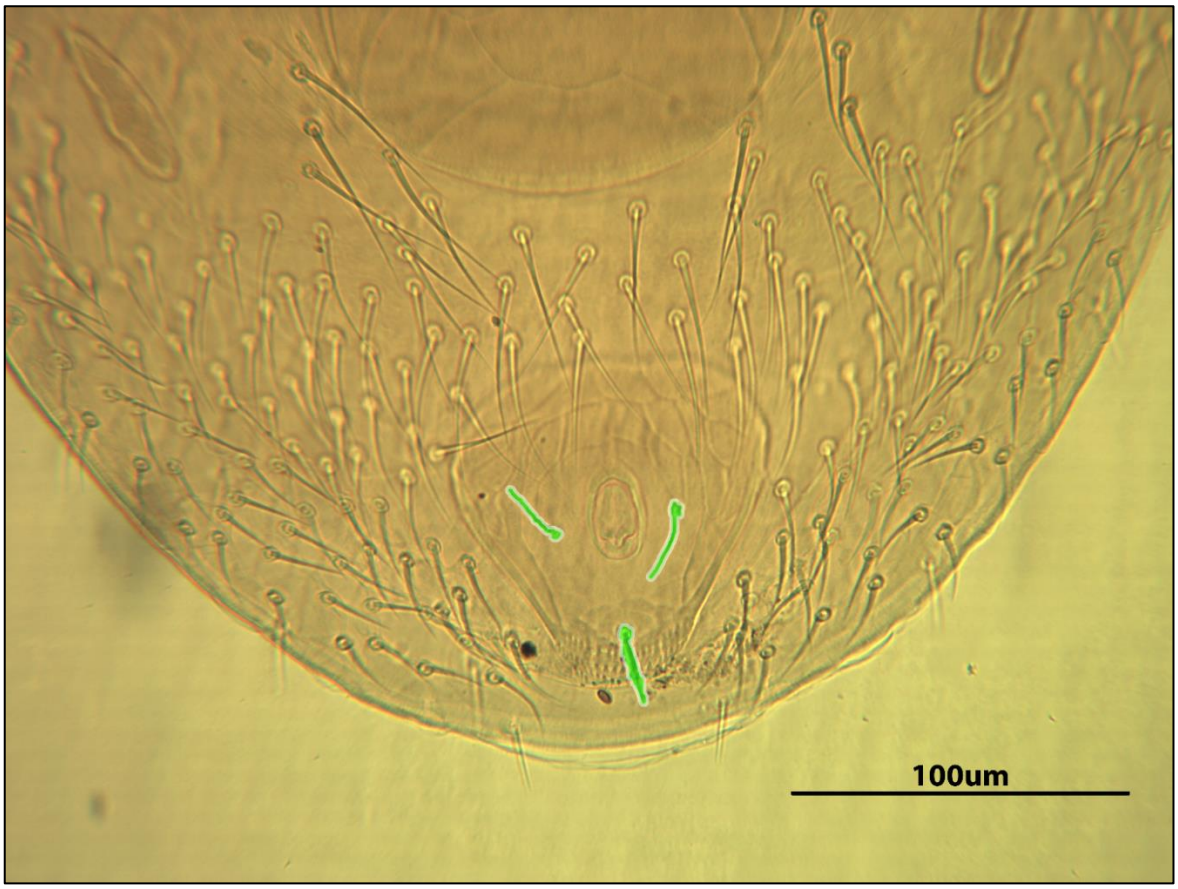

Figure E.47 – *Pneumolaelaps* anal shield with 3 seta highlighted.

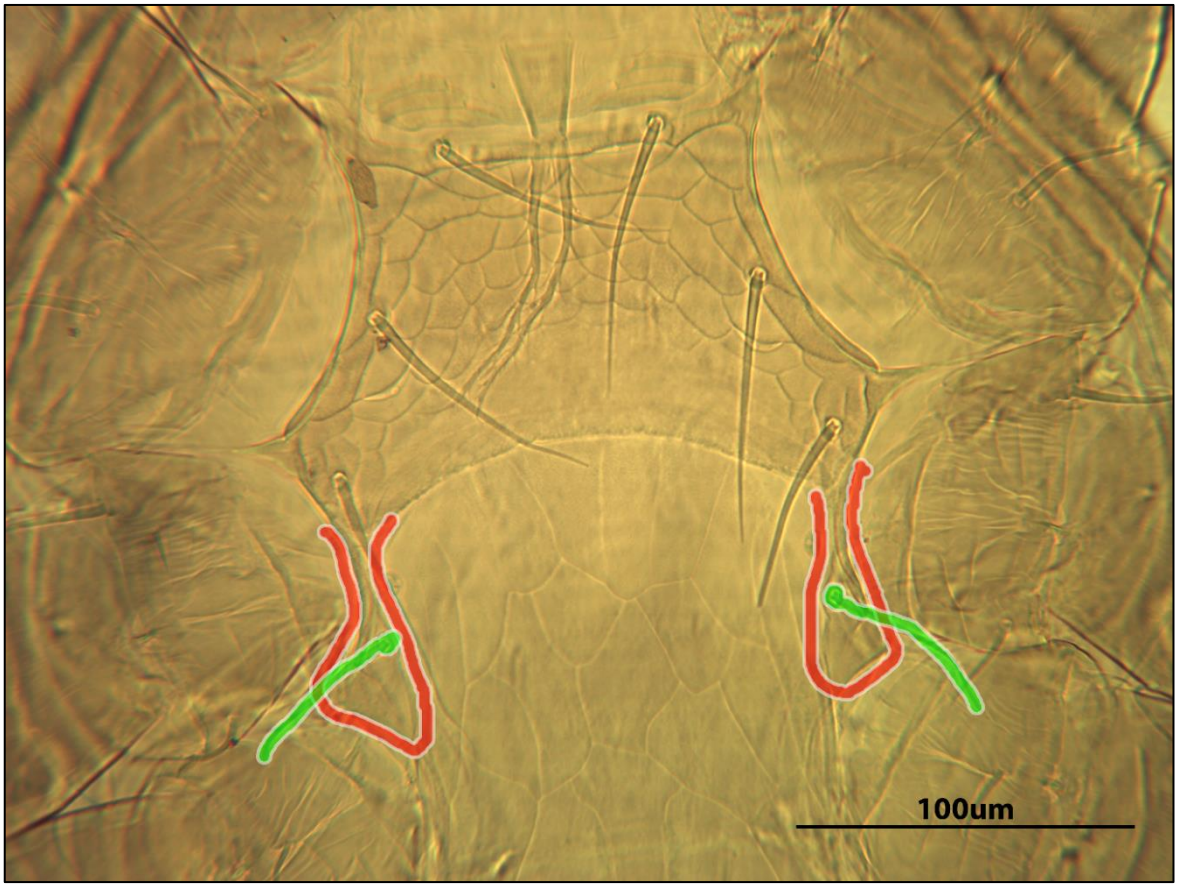

Figure E.48 – *Pneumolaelaps* metasternal shields (red) and seta st4 (green).

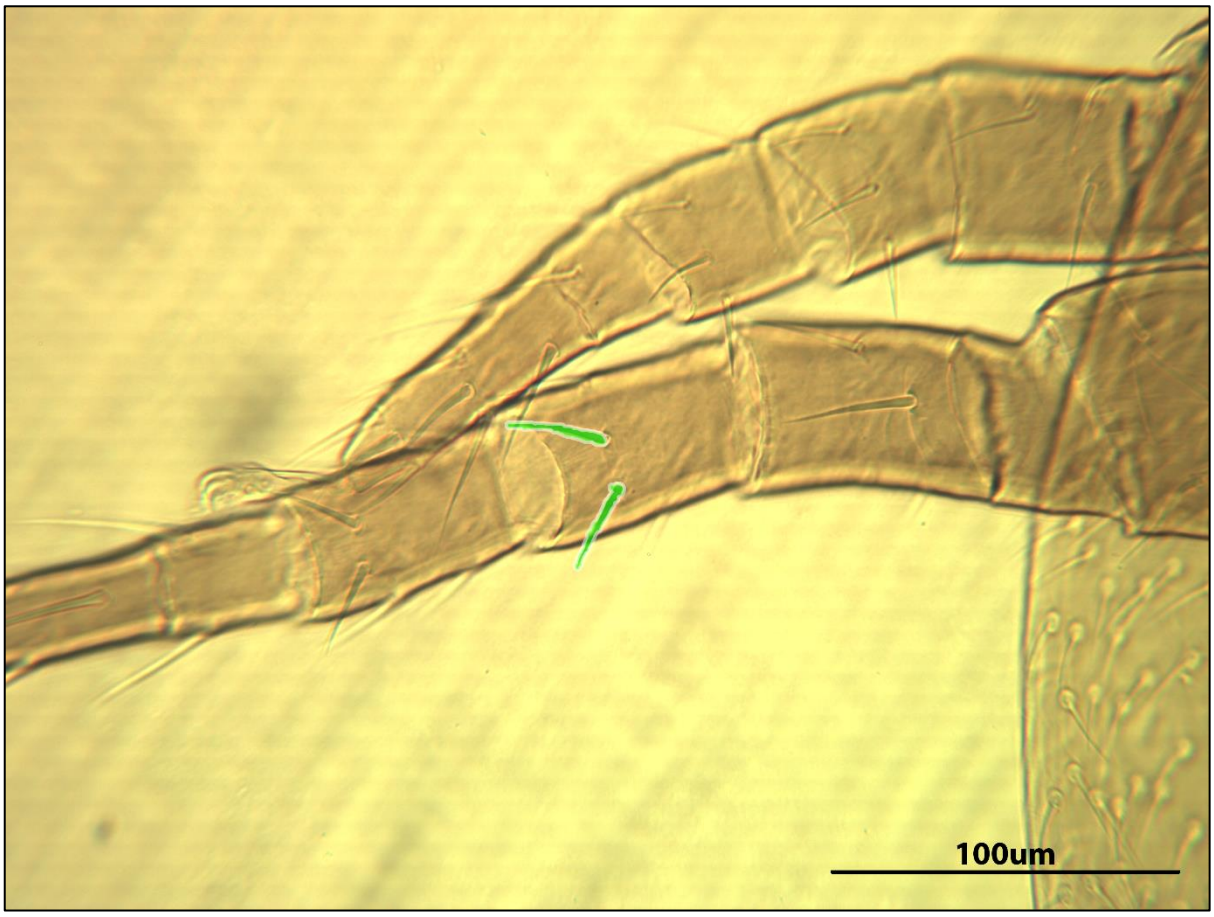

Figure E.49 – Genu IV of *Pneumolaelaps* with 2 ventral setae shown. Eight other setae were counted on the dorsal and lateral sides of this structure, totalling 10 setae.

E.3 Proctolaelaps

Identifier – Robin McArthur

Mite location – Phoretic upon *Bombus terrestris* queen

Order – Mesostigmata

Family – Ascidae

Genus – *Proctolaelaps* Berlese, 1923

Keys/papers used:

1. Baker AS, York PV, Museum NH. (1999). Mites and ticks of domestic animals: an identification guide and information source: The Stationary Office.
2. Krantz GW, Walter DE. (2009). A Manual of Acarology: Texas Tech University Press. Key to Families of the Order Mesostigmata used (pages 157 – 167).
3. OConnor B, Klimov P. Family Ascidae Voigts & Oudemans, 1905 University of Michigan2012 [updated 05/06/201214/08/19]. Available from: http://insects.ummz.lsa.umich.edu/beemites/Species_Accounts/Ascidae.htm.
4. Klimov P, OConnor B, Ochoa R, Bauchan G, Redford A, Scher J. (2016). *Proctolaelaps* Bee Mite ID: University of Michigan; [07/08/19]. Available from: http://idtools.org/id/mites/beemites/factsheet.php?name=15275.

ID steps taken	Keys used	Notes/Reasoning
1 > 2	1	Sample mite lacks recurved teeth.
2 > 3	1	Mite < 2mm long, genital opening without associated shields.
3 > 53	1	Legs with 6 discrete segments. One pair of stigma with ribbon-like associated peritremes. Sample mite belongs to **order Mesostigmata**.
1 > 32	2	Oviporus covered by a single shield. Tarsus of leg IV with ≤ 18 seta.
32 > 34	2	Opithogaster without adhesive organs. Sternal shield well-developed.
34 > 35	2	Sample mite not wormlike.
35 > 38	2	Sternal setae st1-3 all on same shield.
38 > 39	2	Epigynal shield flask shaped, expands into opithogaster, bears seta st5. Peritreme fairly linear.
39 > 43	2	Genua II and IV have ≤ 9 setae.
43 > 44	2	Sternal setae st4 on small metasternal plates. Mite belongs to **subcohort Dermanyssiae**.
44 > 45	2	Genu 4 has ≤ 9 setae.
45 > 50	2	Sternal shield not fused with metasternal plates, where setae st4 are located.
50 > 52	2	Both epigynal and anal shields are present.

ID steps taken	**Keys used**	**Notes/Reasoning**
52 > 53	2	Epigynal and anal shields are present and separate. Genu IV has 2 anterolateral setae.
53 > 54	2	3 pairs setae on sternal shield. Corniculi entire.
54 > 55	2	Epigynal shield weakly convex posteriorly, and widely separated from anal shield.
55 > 58	2	Over 20 pairs of dorsal setae.
58 > ID	2	3rd pair of sternal poroids on posterolateral corners of sternal shield, setae st4 off on cuticle or small metasternal plates. Sample mite belongs to **family Ascidae.**
1 > 2	3	Only 3 setae on anal shield. Epigynal shield rounded posteriorly.
2 > ID	3	Metasternal plates present. 18 pairs opisthonotal setae.
N/A	4	Fixed digit of chelicera with membranous pilus dentilis. Movable digit with pointed process (mucro) on external side. Metasternal shields present. Epigynal shield rounded posteriorly. Anal shield with 3 setae.
ID	3/4	**Mite is a *Proctolaelaps* female.**

Table E.6 – Steps taken to identify *Proctolaelaps* female.

Pictorial references

Figure E.50 – *Proctolaelaps* adult female (ventral side shown).

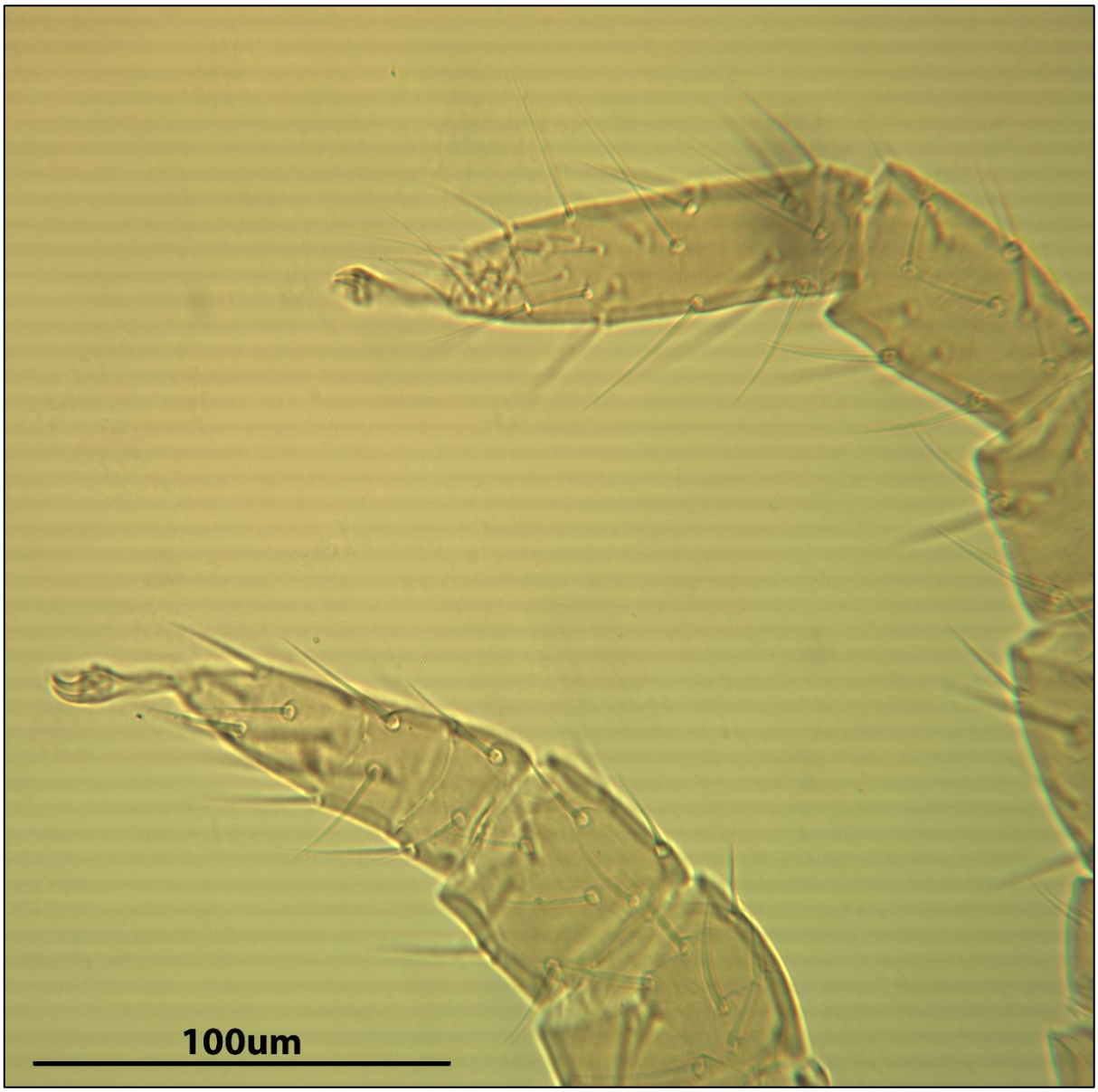

Figure E.51 – *Proctolaelaps* apoteles (Legs I/II) with 2 claws each.

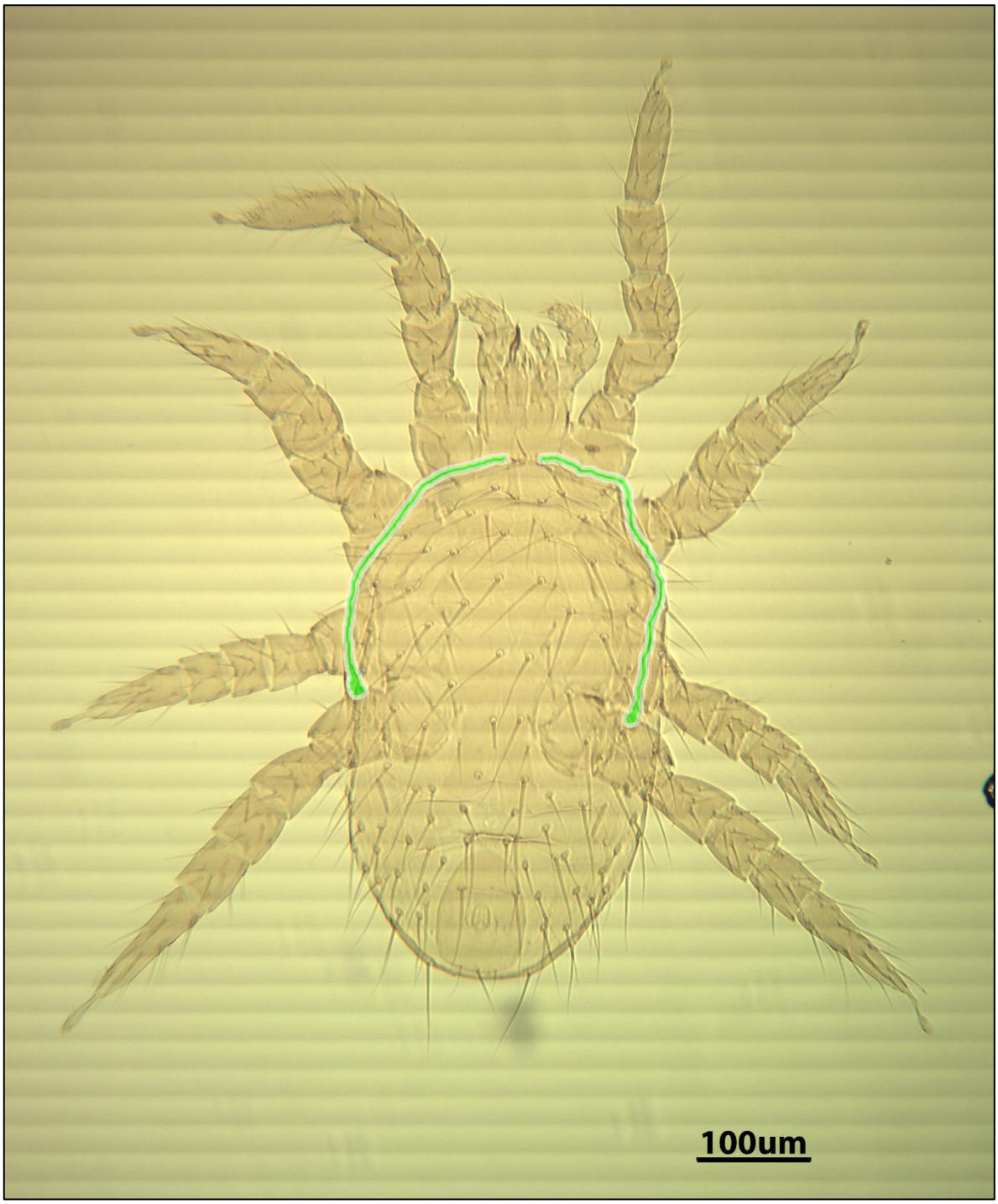

Figure E.52 – *Proctolaelaps* with stigma and peritremes highlighted.

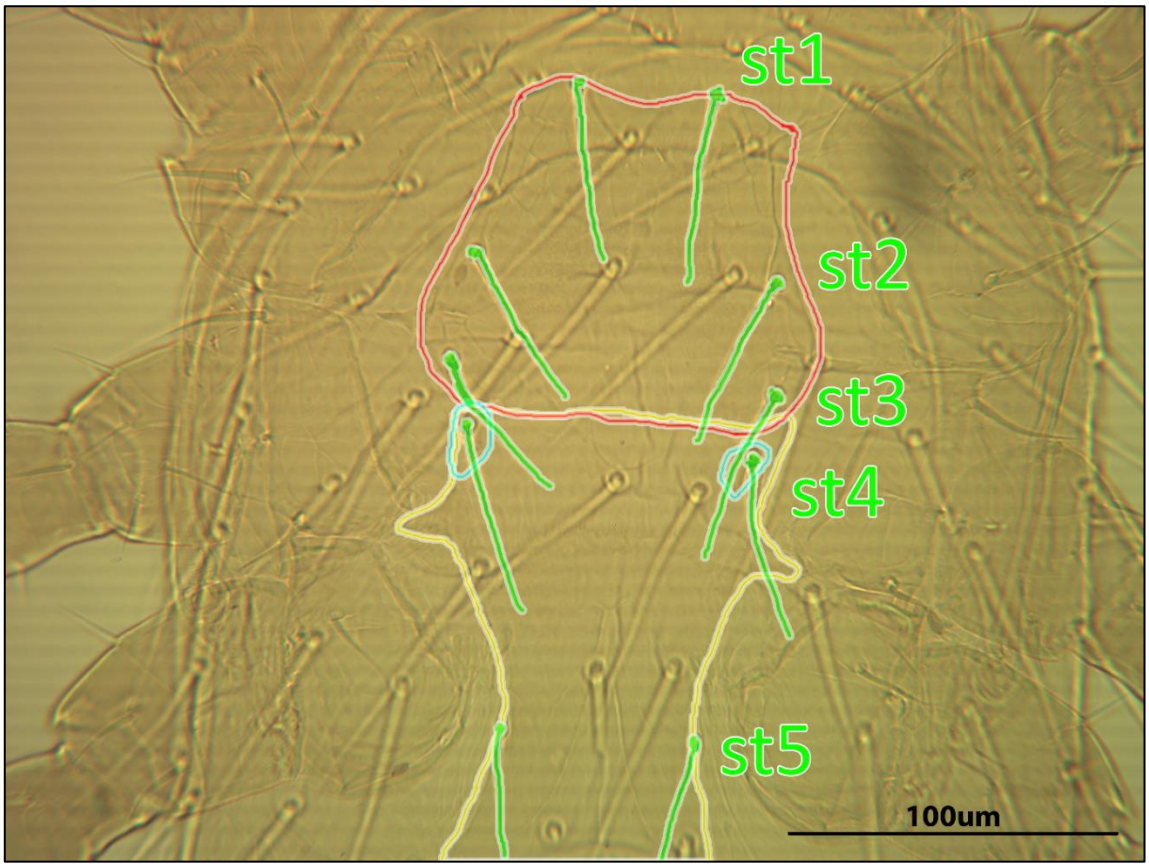

Figure E.53 – *Proctolaelaps* ventral shields and setae highlighted. Red – Sternal shield, Yellow – Epigynal shield, Blue – Metasternal plates, Green – Sternal setae st1 – st5.

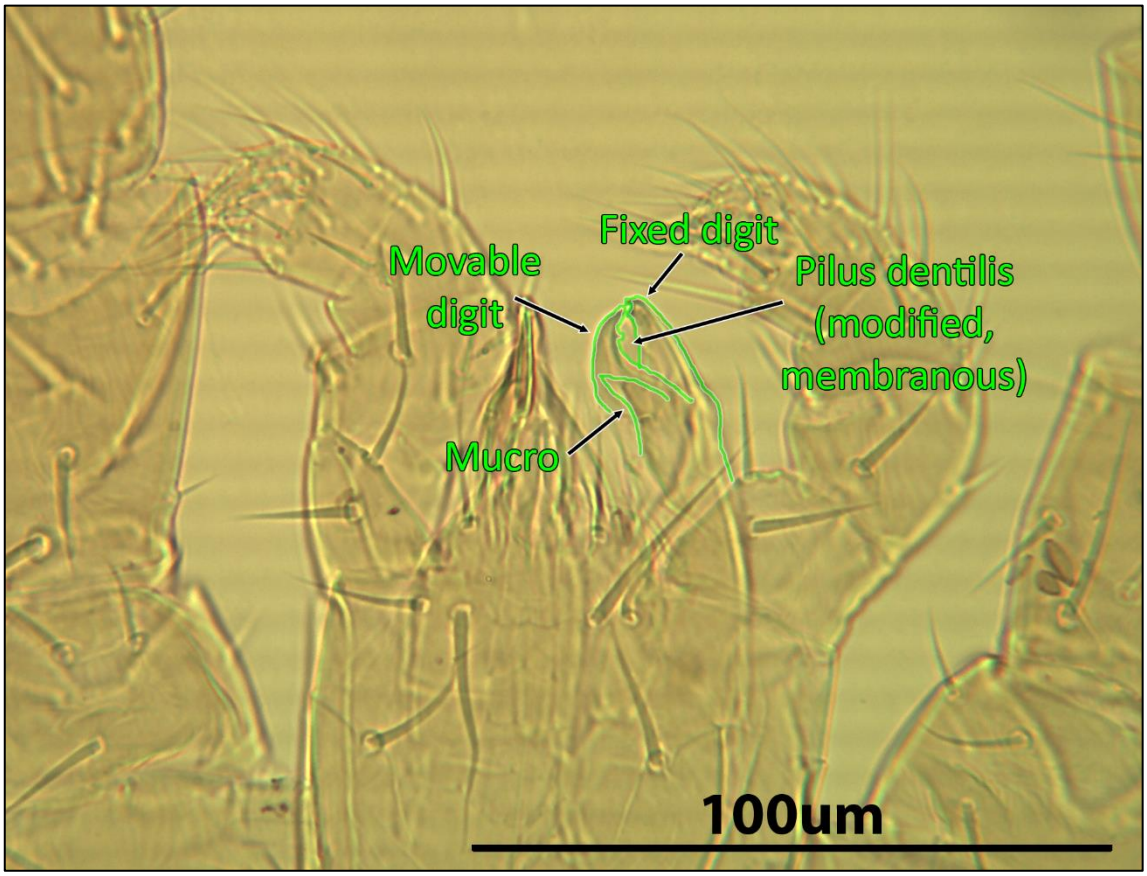

Figure E.54 – *Proctolaelaps* chelicera, showing fixed and moveable digits, membranous pilus dentilis and the pointed mucro structure.

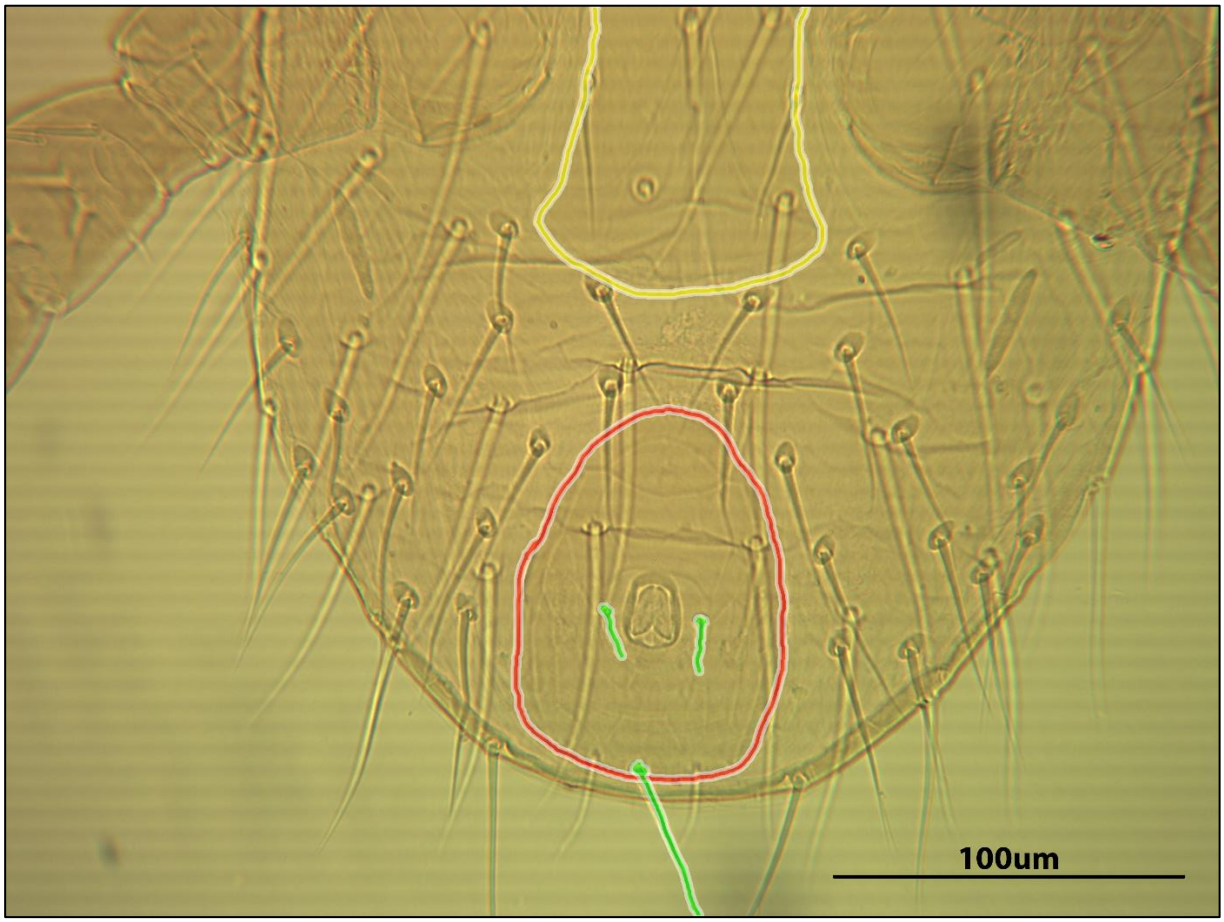

Figure E.55 – *Proctolaelaps* anal shield (red) with 3 cicrumanal setae (green). Rounded posterior of epigynal shield (yellow) also highlighted.

E.4 Kuzinia

Identifier – Robin McArthur

Mite location – Phoretic upon *Bombus terrestris* queen

Cohort – Astigmata

Family – Acaridae

Genus – *Kuzinia* Dujardin, 1849

Keys/papers used to guide ID:

1. Baker AS, York PV, Museum NH. (1999). Mites and ticks of domestic animals: an identification guide and information source: The Stationary Office.
2. Klimov P, OConnor B, Ochoa R, Bauchan G, Redford A, Scher J. (2016). Mites found on adult bumblebees (genus Bombus) Bee Mite ID: University of Michigan; [23/09/2019]. Available from: https://idtools.org/id/mites/beemites/Bumble_bees-Bombus.pdf.
3. Klimov PB, OConnor B, Ochoa R, Bauchan GR, Redford AJ, Scher J. Kuzinia 2016 [12/09/2017]. Available from: http://idtools.org/id/mites/beemites/factsheet.php?name=15316.

ID steps taken	**Guide used**	**Notes/Reasoning**
1 > 2	1	Gnathosoma without recurved teeth.
2 > 3	1	Mite < 2mm long.
3 > 4	1	Stigma and peritremes absent. Leg coxae fused to ventral idiosomal wall, outlined by cuticular apodemes.
4 > 5	1	Moderately sclerotized, gnathosoma lacking rutella.
5 > 6	1	Gnathosoma absent/modified, appears as in step 6(a). Ambulacrum of legs with 1 claw.
6 > 7	1	Gnathosoma modified as in diagram (a), bearing 2 pairs long setae.
7 > 8	1	Gnathosoma represented by structure bearing 2 pairs long setae.
8	1	Idiosoma with well-developed suckers posterior to legs IV. Gnathosoma as in step 6(a). Mite belongs to **family Acaridae**.
N/A	2/3	Decided to follow key #3 after reviewing visual keys (key #2) and checking visible sample mite characters against likely *Bombus*-associated mite groups.
N/A	3	Saucer on tarsal setae e I – II present. External conoidal setae (ps2) of attachment organ slightly anterior to median suckers (ad1 and ad2). ps2 without long endosclerites. Gnathosoma typical, with base present and 2 pairs of setae. Coxal setae 1a minute and filiform.
N/A	ID	Sample mite is a ***Kuzinia* phoretic deutonymph**.

Table E.7 – Details of steps taken to identify *Kuzinia* phoretic deutonymph.

Pictorial references

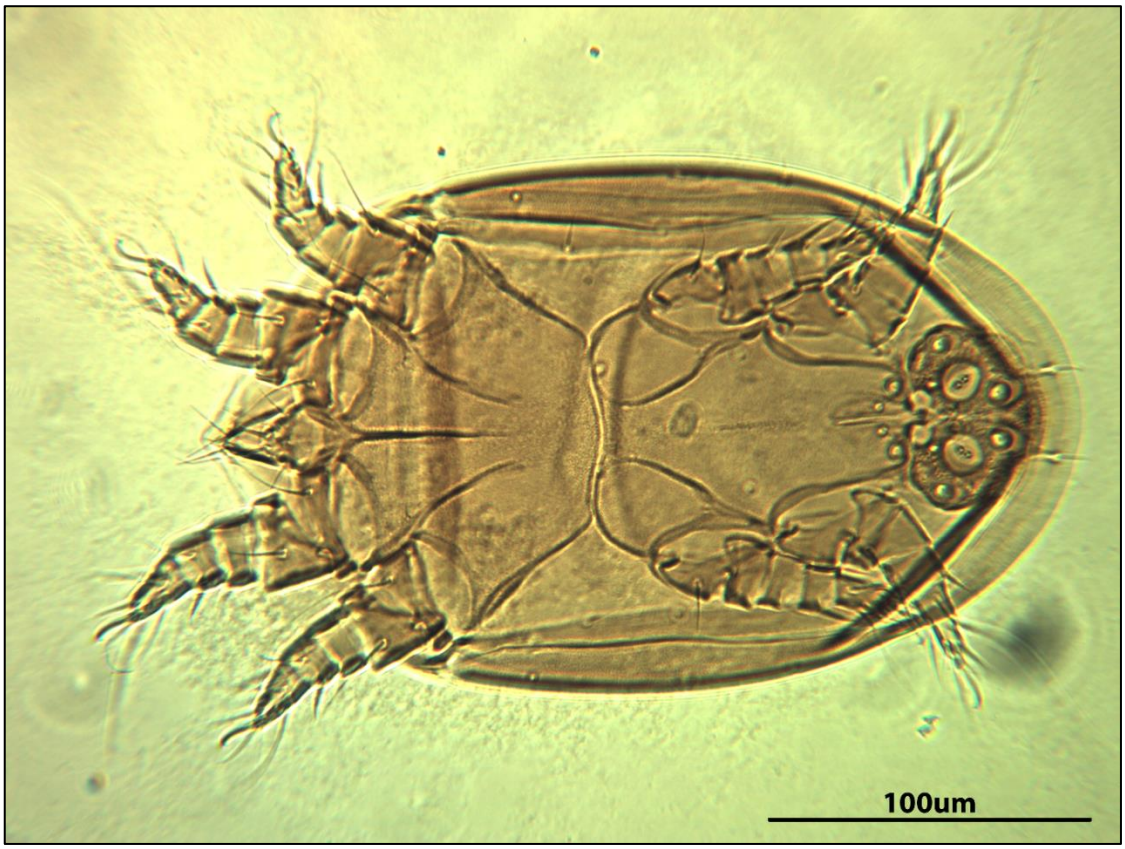

Figure E.56 – *Kuzinia* phoretic deutonymph ventral surface/seta.

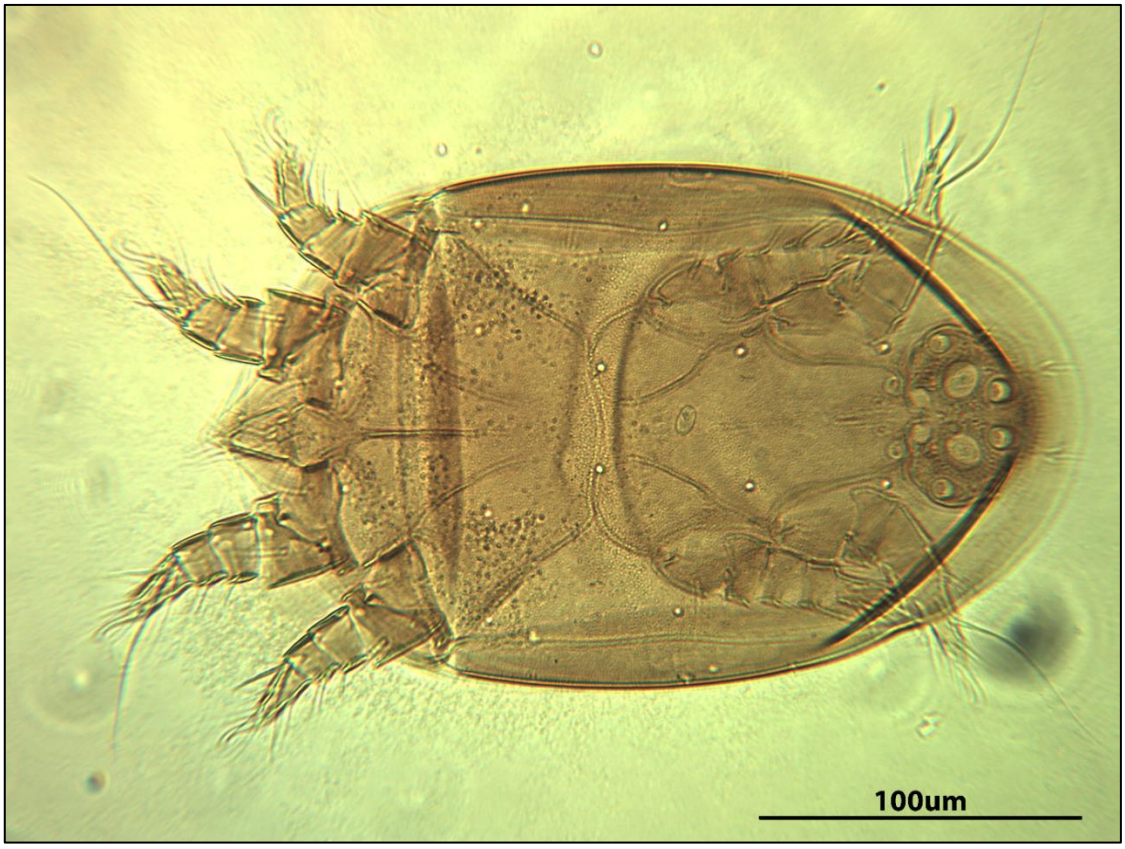

Figure E.57 – *Kuzinia* dorsal side with setae.

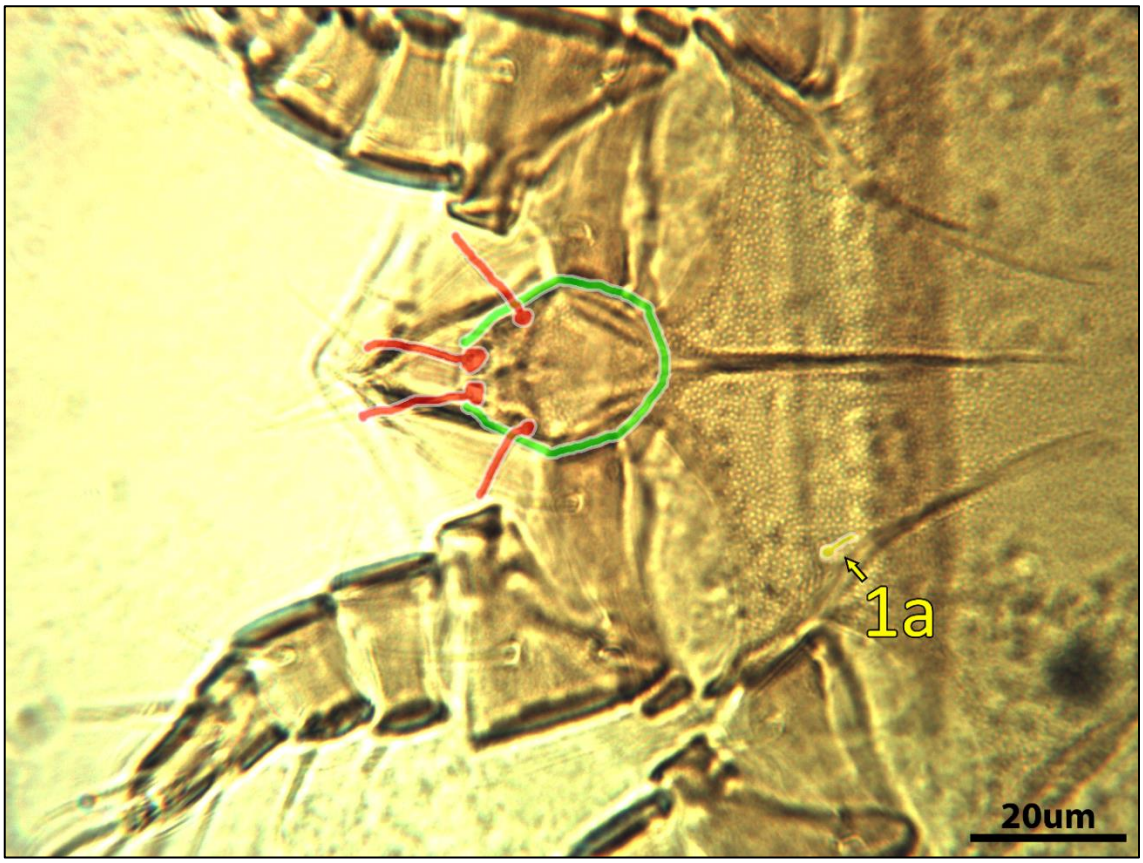

Figure E.58 – *Kuzinia* gnathosoma. Basal structure outlined in green, 2 setae highlighted in red. Coxal seta 1a highlighted yellow and labelled.

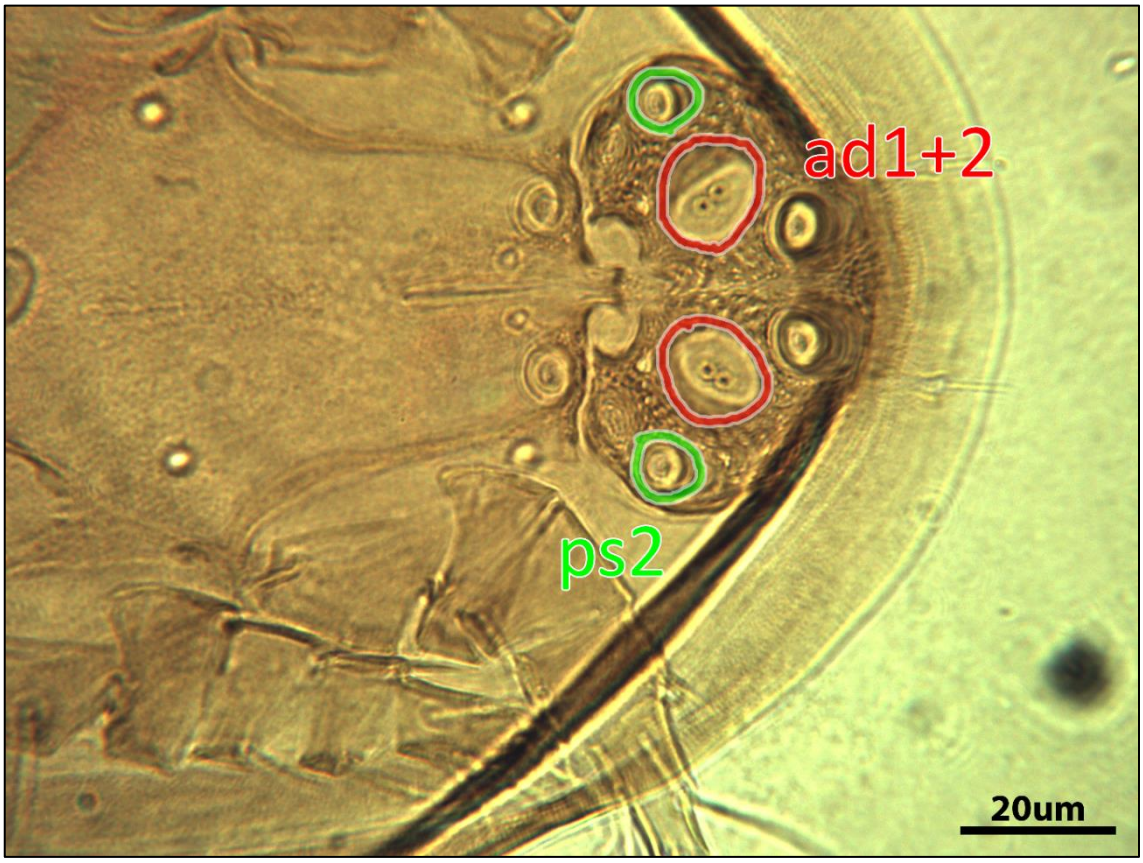

Figure E.59 – *Kuzinia* attachment organ with relevant structures highlighted and labelled.

E.5 Scutacarus

E.5.1 Scutacarus acarorum

Identifier – Robin McArthur

Mite location – Phoretic upon *Bombus hortorum* queen

Suborder – Heterostigmata

Family – Scutacaridae

Genus – *Scutacarus*

Species – *S. acarorum* (Goeze, 1780)

Keys/papers used to guide ID:

1. Baker AS, York PV, Museum NH. (1999). Mites and ticks of domestic animals: an identification guide and information source: The Stationary Office.
2. Klimov P, OConnor B, Ochoa R, Bauchan G, Redford A, Scher J. (2016). Mites found on adult bumblebees (genus Bombus) Bee Mite ID: University of Michigan; [23/09/2019]. Available from: https://idtools.org/id/mites/beemites/Bumble_bees-Bombus.pdf.
3. Klimov P, OConnor B, Ochoa R, Bauchan G, Redford A, Scher J. (2016). Scutacarus Bee Mite ID: University of Michigan. [22/09/2019]. Available from: http://idtools.org/id/mites/beemites/factsheet.php?name=15289.
4. Jagersbacher-Baumann J. (2014). Species differentiation of scutacarid mites (Heterostigmatina) using multivariate morphometric methods. Experimental and Applied Acarology. 62(3):279-92.

ID steps taken	**Guide used**	**Notes/Reasoning**
1 > 2	1	Gnathosoma without recurved teeth.
2 > 3	1	Sample mite small (<2mm), genital opening different to example in key.
3 > 4	1	Palp tarsus lacks apotele, cuticular apodemes present.
4 > 5	1	Weakly/moderately sclerotized sample mite, idiosoma varies from oribatida example, palps lack rutella.
5 > 40	1	Ambulacrums each with 2 claws, gnathosoma present. Sample mite belongs to **order Prostigmata**.
N/A	2/3	Visual keys and species description used. Decision based on seta c1 being in correct position, legs each having 4 segments, and tibiotarsus IV having >4 seta (7 by my count). Sample mite is ***Scutacarus* adult female**.
N/A	4	Seta tc" shorter than both pv" & tc', no spur.
ID	4	Mite is a ***Scutacarus acarorum* adult female**

Table E.8 – Steps taken through multiple keys and species decriptions to identify *Scutacarus acarorum* female.

Pictorial references

Figure E.60 – *Scutacarus acarorum* adult female full body, ventral side shown.

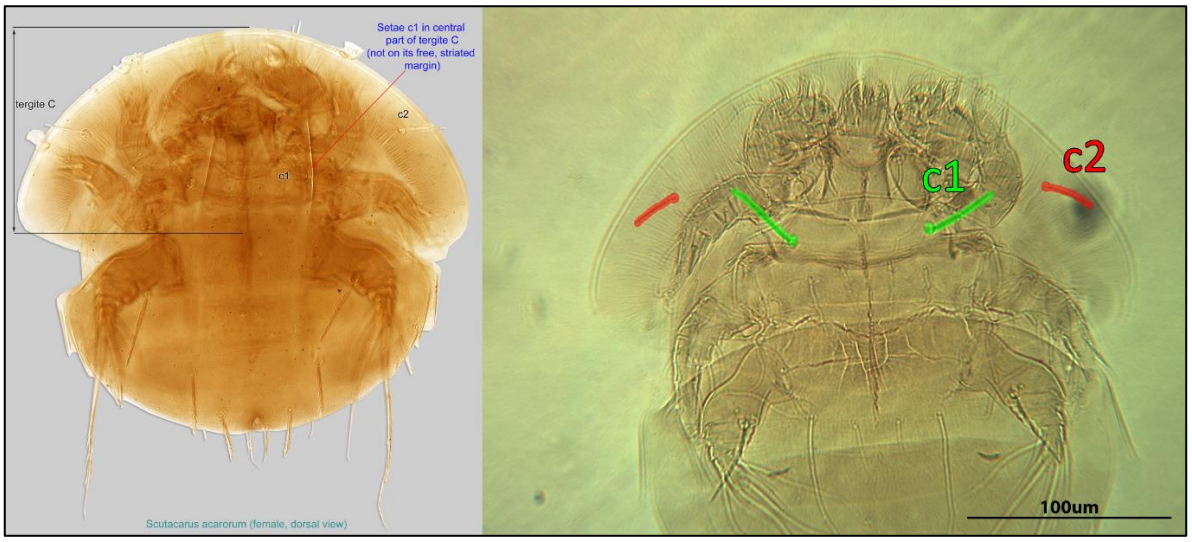

Figure E.61 – (Left) *Scutacarus* example given by Klimov et al. (2016c). (Right) *Scutacarus acarorum* with relevant seta highlighted.

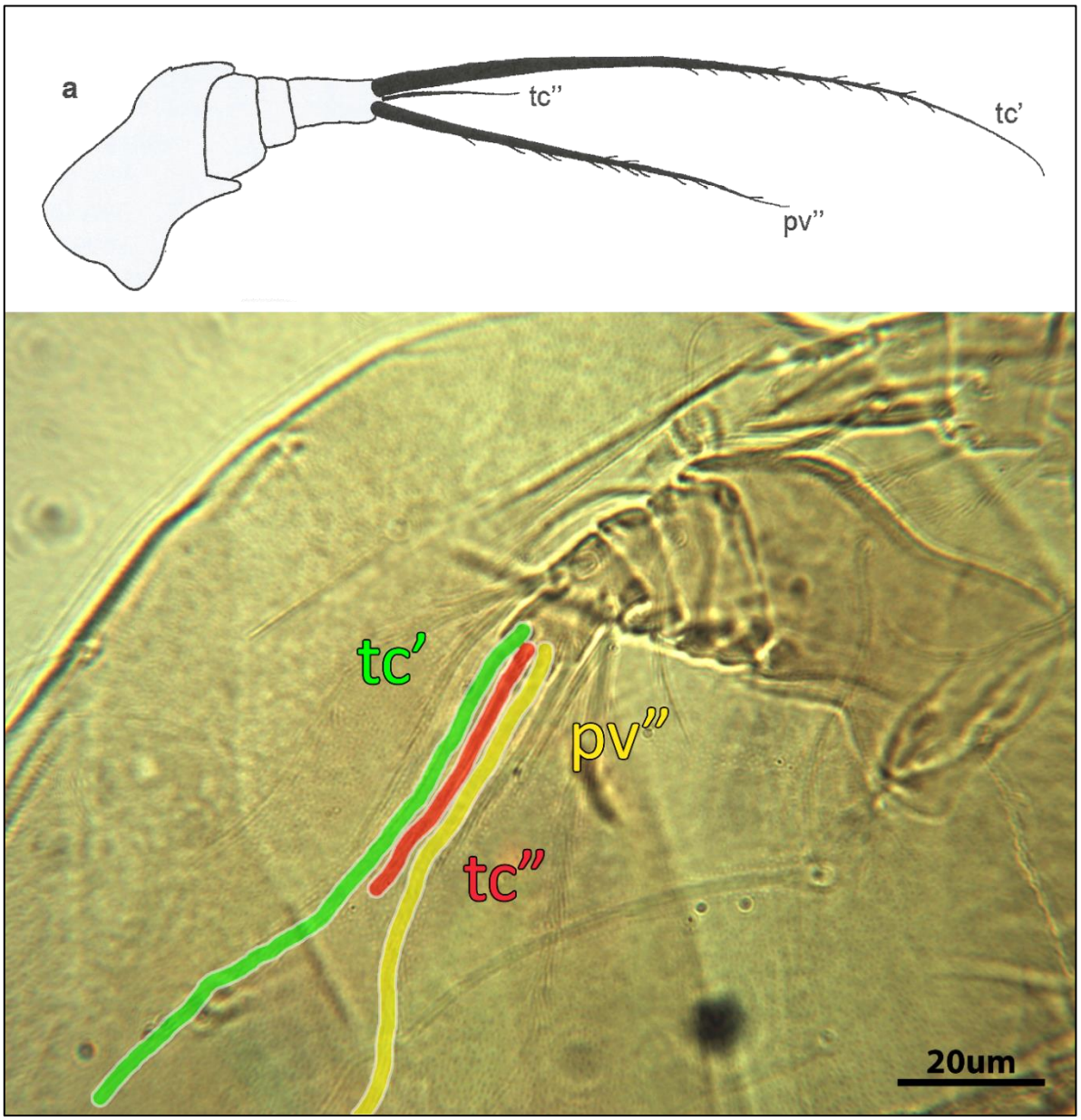

Figure E.62 – (Top) *Scutacarus acarorum* trochanter IV with discriminant characters from Jagersbacher-Baumann (2014). (Bottom) *Scutacarus acarorum* with relevant characters highlighted.

E.5.2 Scutacarus mendax

Identifier – Robin McArthur

Mite location – Phoretic upon *Bombus terrestris* queen

Suborder – Heterostigmata

Family – Scutacaridae

Genus – *Scutacarus*

Species – *S. mendax* Karafiat, 1959

Keys/papers used to guide ID:

1. Baker AS, York PV, Museum NH. (1999). Mites and ticks of domestic animals: an identification guide and information source: The Stationary Office.
2. Klimov P, OConnor B, Ochoa R, Bauchan G, Redford A, Scher J. (2016). Mites found on adult bumblebees (genus Bombus) Bee Mite ID: University of Michigan; [23/09/2019]. Available from: https://idtools.org/id/mites/beemites/Bumble_bees-Bombus.pdf.
3. Klimov P, OConnor B, Ochoa R, Bauchan G, Redford A, Scher J. (2016). Scutacarus Bee Mite ID: University of Michigan. [22/09/2019]. Available from: http://idtools.org/id/mites/beemites/factsheet.php?name=15289.
4. Jagersbacher-Baumann J. (2014). Species differentiation of scutacarid mites (Heterostigmatina) using multivariate morphometric methods. Experimental and Applied Acarology. 62(3):279-92.

ID steps taken	**Guide used**	**Notes/Reasoning**
1 > 2	1	Gnathosoma lack recurved teeth.
2 > 3	1	Sample mite small (<2mm), genital opening different to example in key.
3 > 4	1	Palp tarsus lacks apotele, cuticular apodemes present.
4 > 5	1	Weakly/moderately sclerotized sample mite, idiosoma varies from oribatida example, palps lack rutella.
5 > 40	1	Ambulacra each with 2 claws, gnathosoma present. Sample mite belongs to **order Prostigmata**.
N/A	2/3	Visual keys and species description used. Decision based on seta c1 being in correct position, legs each having 4 segments, and tibiotarsus IV having >4 seta. Sample mite is ***Scutacarus* adult female**.
N/A	4	Seta tc" similar in length to pv", tc' much longer, and no spur.
ID	4	Mite is a ***Scutacarus mendax* adult female**

Table E.9 – All steps taken to identify *Scutacarus mendax* adult female.

Pictorial references

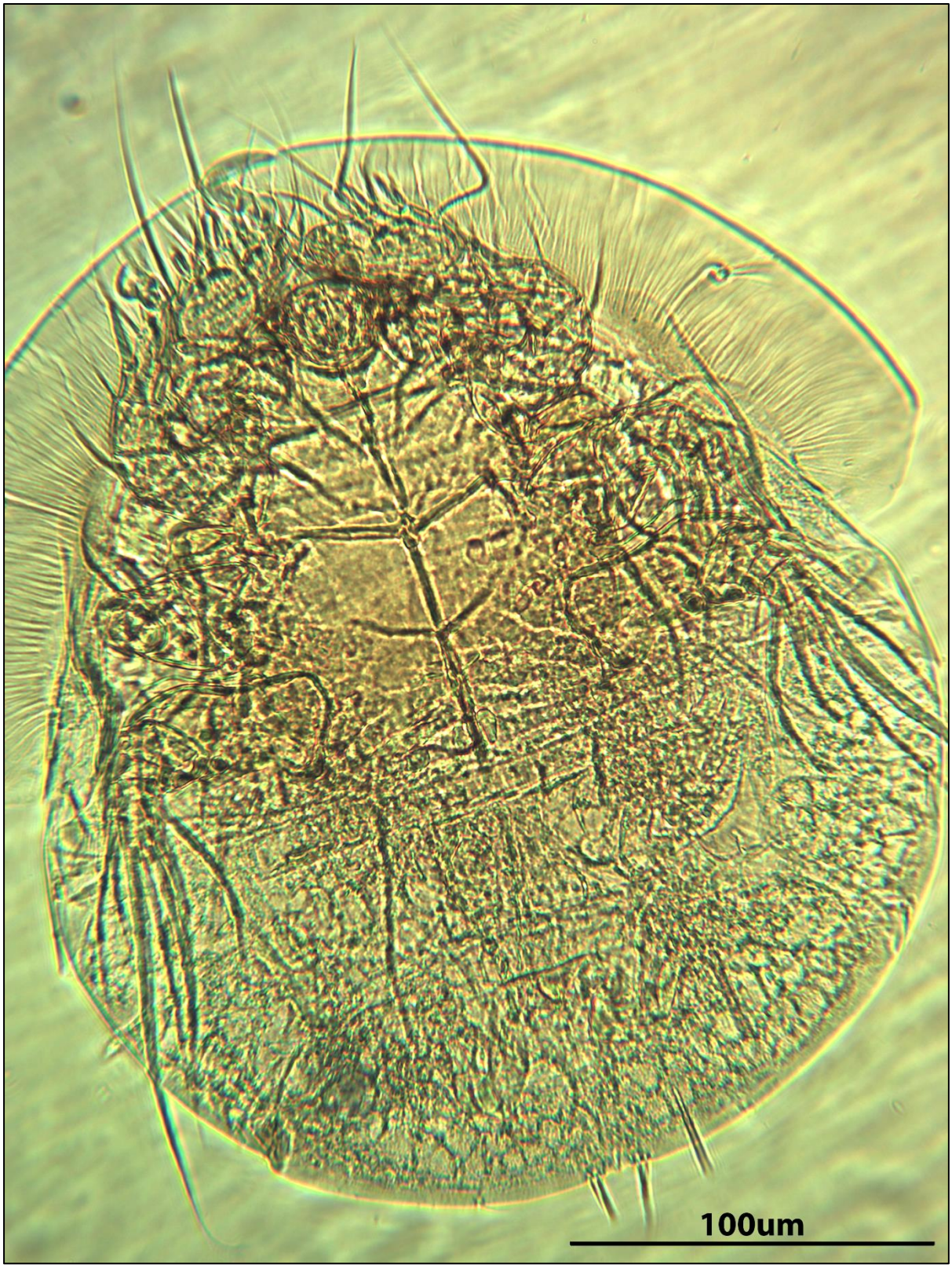

Figure E.63 – *Scutacarus mendax* adult female full body, ventral side shown.

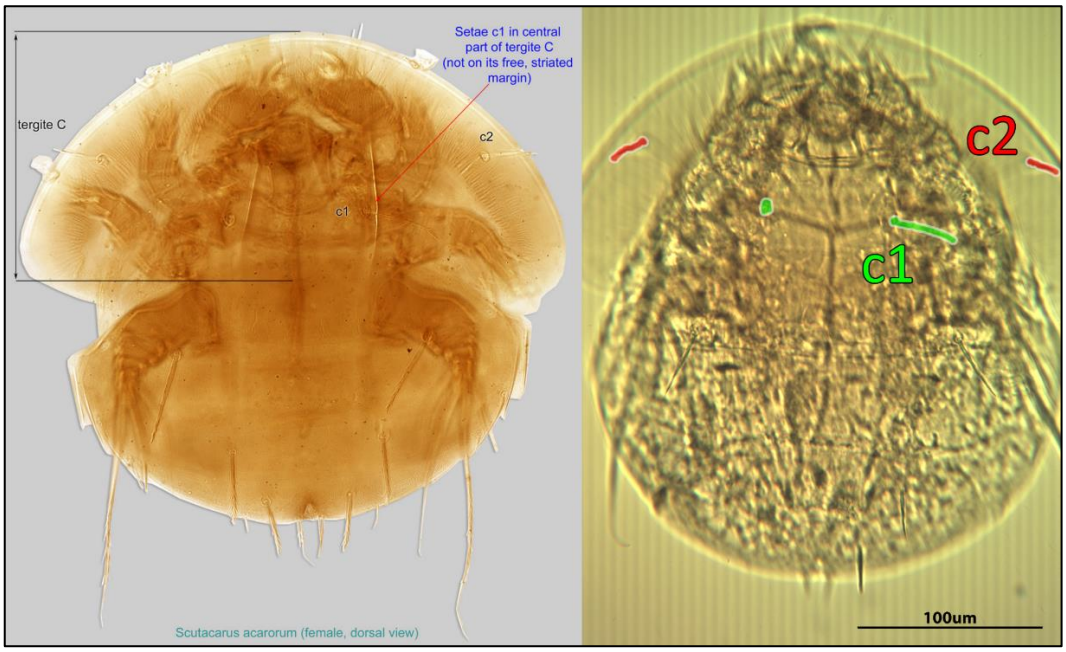

Figure E.64 – (Left) *Scutacarus* example given by Klimov et al. (2016c). (Right) *Scutacarus mendax* with relevant seta highlighted.

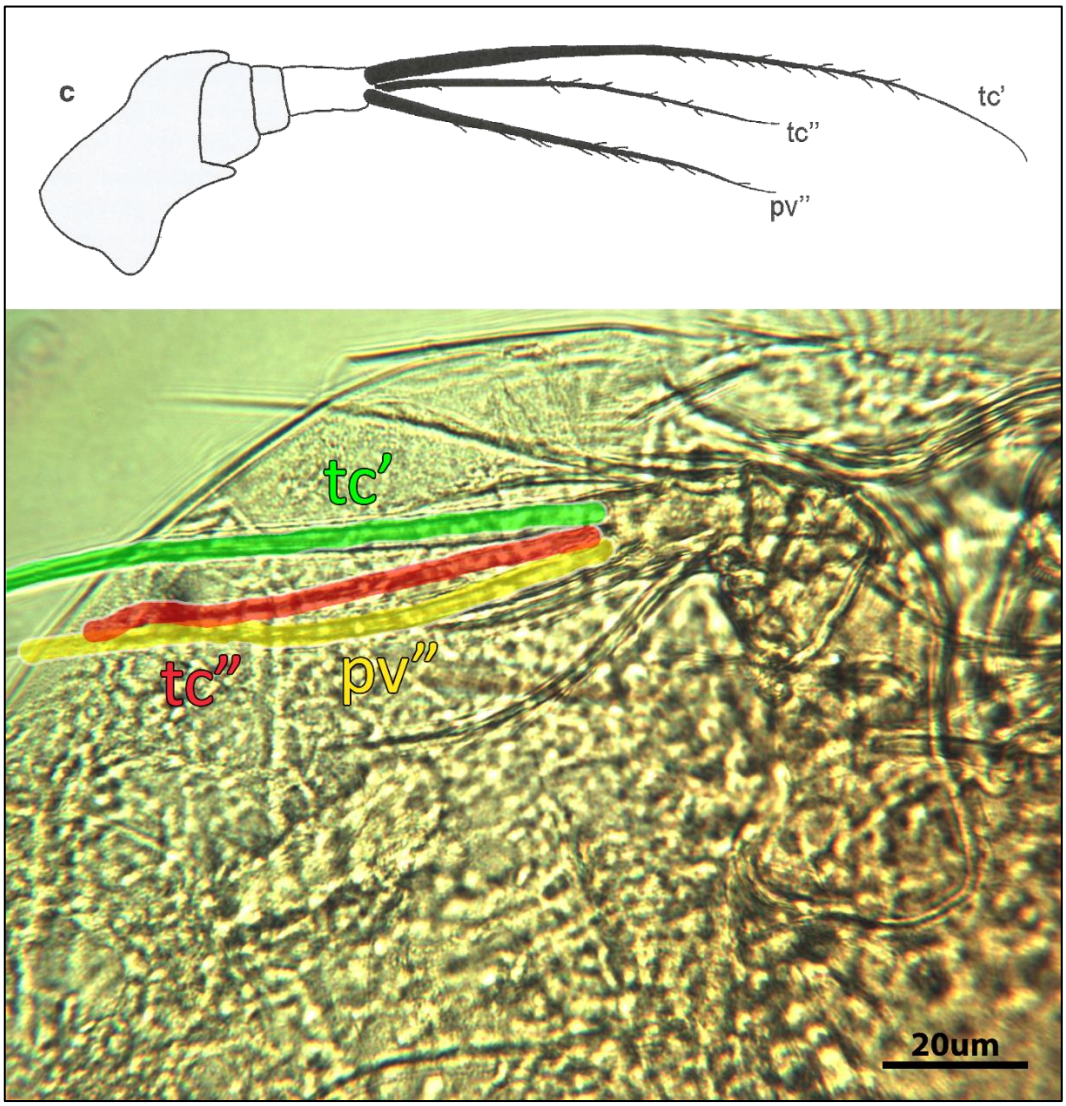

Figure E.65 – (Top) *Scutacarus mendax* trochanter IV with discriminant characters from Jagersbacher-Baumann (2014). (Bottom) *Scutacarus mendax* with relevant characters highlighted.

E.5.3 Scutacarus occultatus

Identifier – Robin McArthur

Mite location – Phoretic upon *Bombus terrestris* queen

Suborder – Heterostigmata

Family – Scutacaridae

Genus – *Scutacarus*

Species – *S. occultatus* Sevastianov, 1975

Keys/papers used to guide ID:

1. Baker AS, York PV, Museum NH. (1999). Mites and ticks of domestic animals: an identification guide and information source: The Stationary Office.
2. Klimov P, OConnor B, Ochoa R, Bauchan G, Redford A, Scher J. (2016). Mites found on adult bumblebees (genus Bombus) Bee Mite ID: University of Michigan; [23/09/2019]. Available from: https://idtools.org/id/mites/beemites/Bumble_bees-Bombus.pdf.
3. Klimov P, OConnor B, Ochoa R, Bauchan G, Redford A, Scher J. (2016). Scutacarus Bee Mite ID: University of Michigan. [22/09/2019]. Available from: http://idtools.org/id/mites/beemites/factsheet.php?name=15289.
4. Jagersbacher-Baumann J. (2014). Species differentiation of scutacarid mites (Heterostigmatina) using multivariate morphometric methods. Experimental and Applied Acarology. 62(3):279-92.

ID steps taken	**Guide used**	**Notes/Reasoning**
1 > 2	1	Gnathosoma lacked recurved teeth.
2 > 3	1	Sample mite small (<2mm), genital opening differed to example in key.
3 > 4	1	Palp tarsus lack apotele, cuticular apodemes present.
4 > 5	1	Weakly/moderately sclerotized sample mite, idiosoma varies from oribatida example, palps lack rutella.
5 > 40	1	Ambulacra each with 2 claws, gnathosoma present. Sample mite belongs to **order Prostigmata**.
N/A	2/3	Visual keys and species description used. Decision based on seta c1 being in correct position, legs each having 4 segments, and tibiotarsus IV having >4 seta. Sample mite is ***Scutacarus* adult female**.
N/A	4	Seta tc" similar in length to pv", tc' much longer, with conspicuous thin spur.
ID	4	Mite is a ***Scutacarus occultatus* adult female.**

Table E.10 – Details of steps taken to identify *Scutacarus occultatus* adult female.

Pictorial references

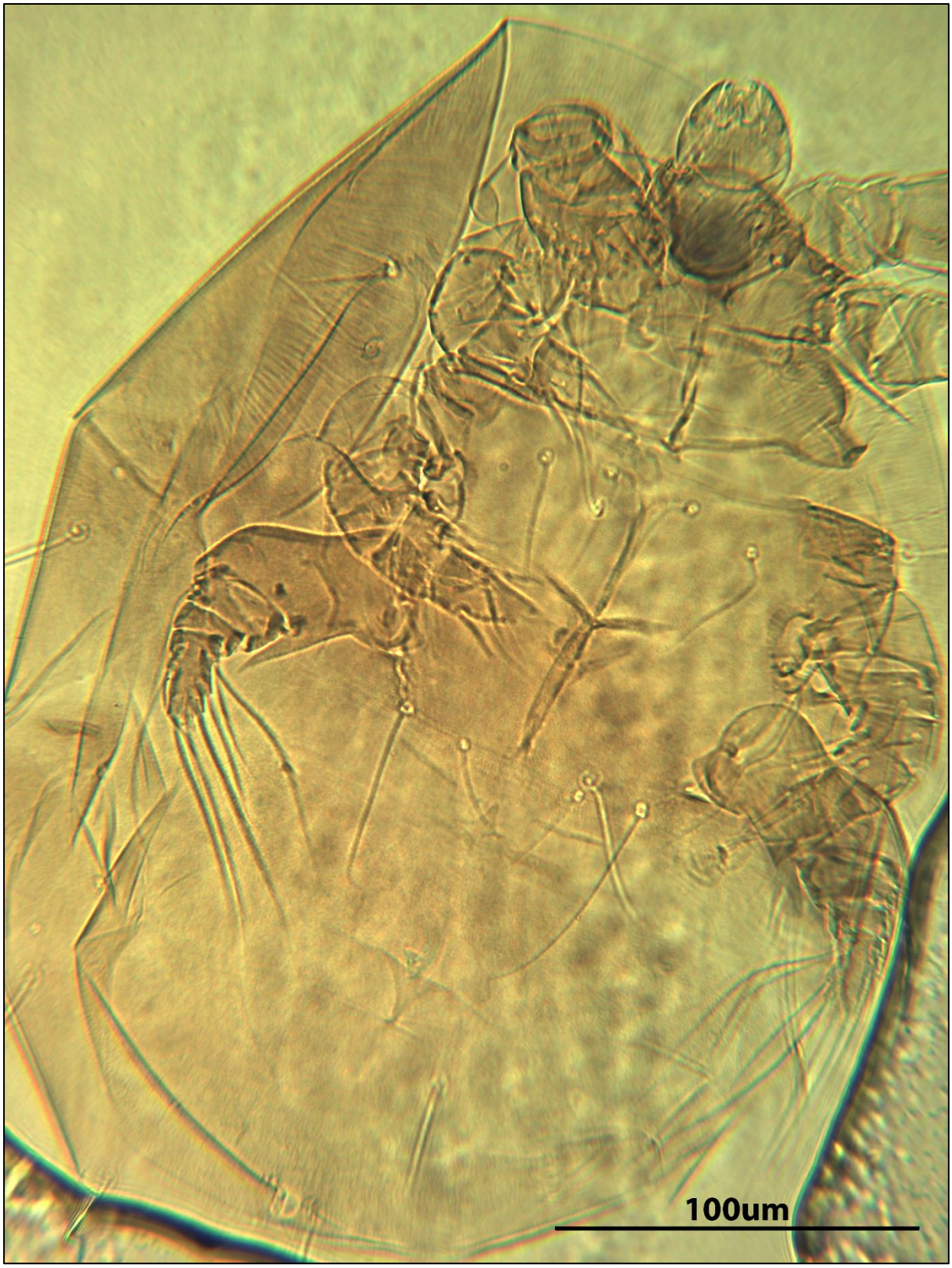

Figure E.66 – *Scutacarus occultatus* adult female full body, ventral side shown.

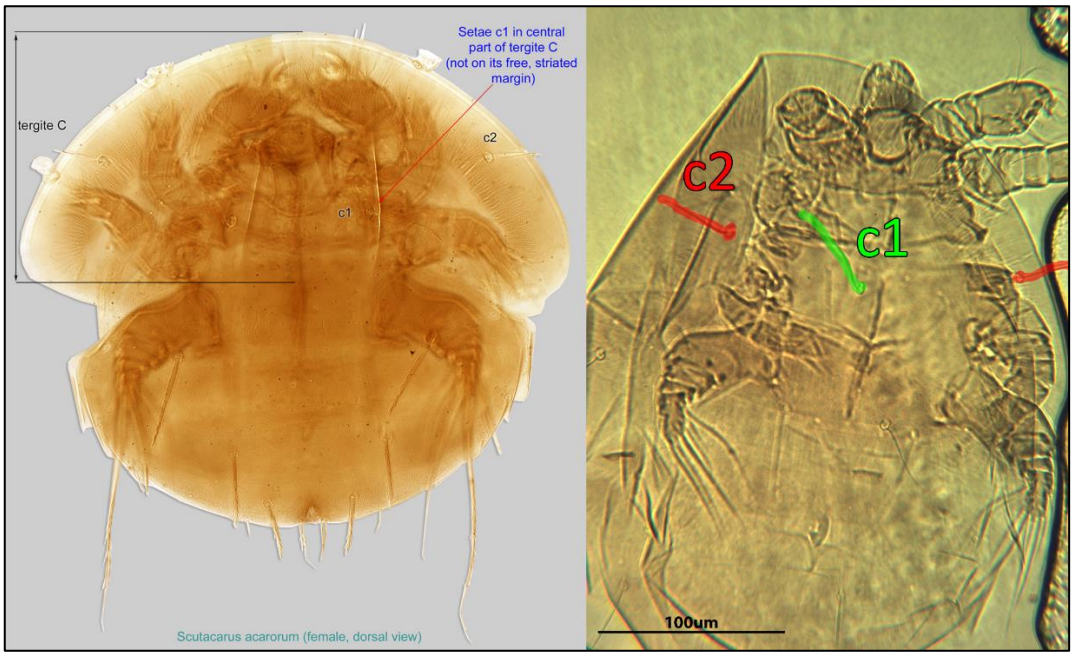

Figure E.67 – (Left) *Scutacarus* example given by Klimov et al. (2016c). (Right) *Scutacarus occultatus* with relevant seta highlighted.

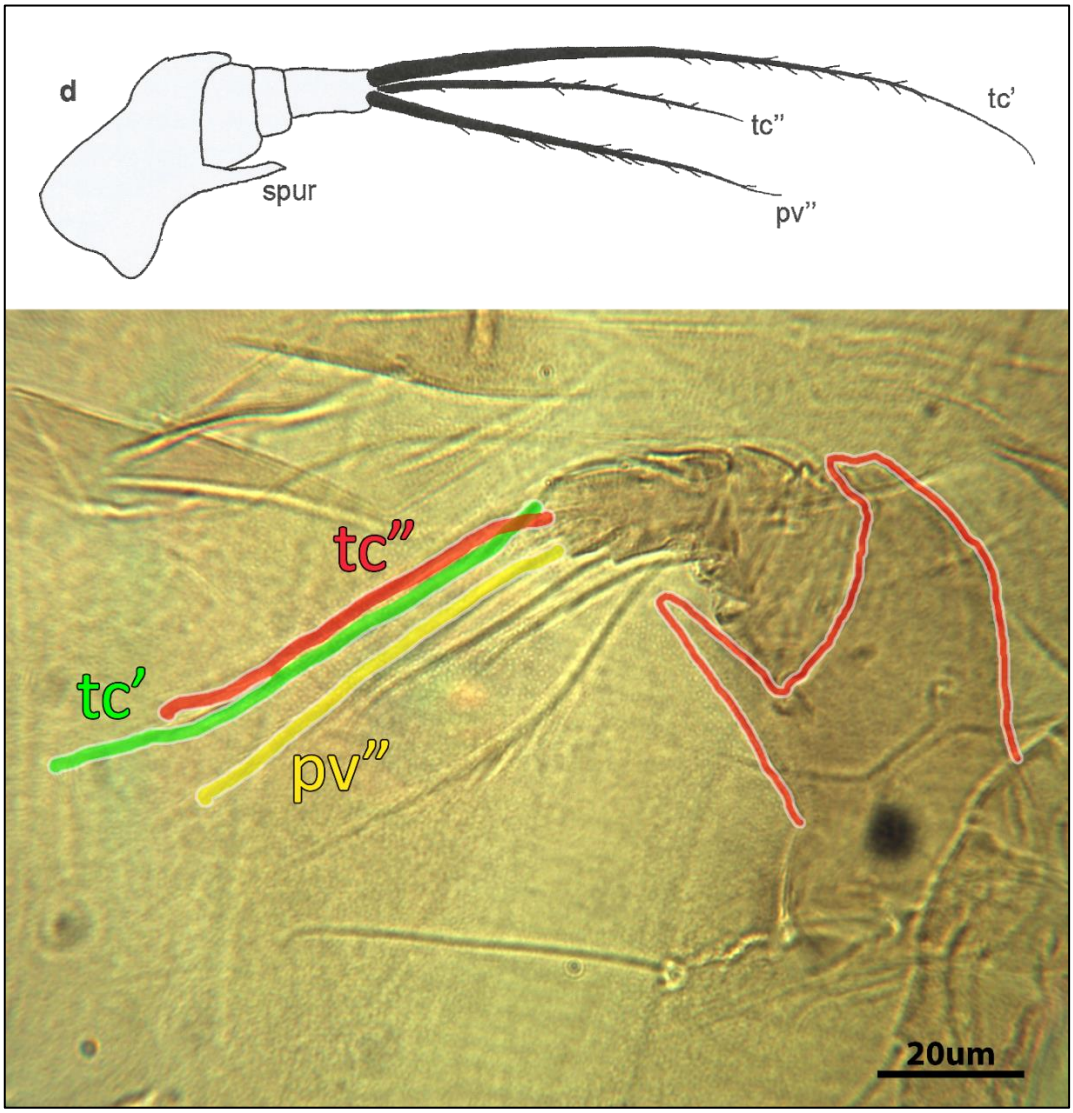

Figure E.68 – (Top) *Scutacarus mendax* trochanter IV with discriminant characters from Jagersbacher-Baumann (2014). (Bottom) *Scutacarus occultatus* with relevant characters highlighted.

E.5.4 Scutacarus deserticolus

Identifier – Robin McArthur

Mite location – Phoretic upon *Bombus terrestris* queen

Suborder – Heterostigmata

Family – Scutacaridae

Genus – *Scutacarus*

Species – *S. deserticolus* Mahunka, 1969

Keys/papers used to guide ID:

1. Baker AS, York PV, Museum NH. (1999). Mites and ticks of domestic animals: an identification guide and information source: The Stationary Office.
2. Klimov P, OConnor B, Ochoa R, Bauchan G, Redford A, Scher J. (2016). Mites found on adult bumblebees (genus Bombus) Bee Mite ID: University of Michigan; [23/09/2019]. Available from: https://idtools.org/id/mites/beemites/Bumble_bees-Bombus.pdf.
3. Klimov P, OConnor B, Ochoa R, Bauchan G, Redford A, Scher J. (2016). Scutacarus Bee Mite ID: University of Michigan. [22/09/2019]. Available from: http://idtools.org/id/mites/beemites/factsheet.php?name=15289.
4. Jagersbacher-Baumann J. (2014). Species differentiation of scutacarid mites (Heterostigmatina) using multivariate morphometric methods. Experimental and Applied Acarology. 62(3):279-92.

ID steps taken	**Guide used**	**Notes/Reasoning**
1 > 2	1	Gnathosoma lacked recurved teeth.
2 > 3	1	Sample mite small (<2mm), genital opening differed to example in key.
3 > 4	1	Palp tarsus lack apotele, cuticular apodemes present.
4 > 5	1	Weakly/moderately sclerotized sample mite, idiosoma varies from oribatida example, palps lack rutella.
5 > 40	1	Ambulacra each with 2 claws, gnathosoma present. Sample mite belongs to **order Prostigmata**.
N/A	2/3	Visual keys and species description used. Decision based on seta c1 being in correct position, legs each having 4 segments, and tibiotarsus IV having >4 seta. Sample mite is ***Scutacarus* adult female**.
N/A	4	Seta tc" obviously shorter than pv" and tc', with conspicuous thick spur.
ID	4	Mite is a ***Scutacarus deserticolus* adult female.**

Table E.11 – Steps taken to identify *Scutacarus deserticolus* adult female.

Pictorial references

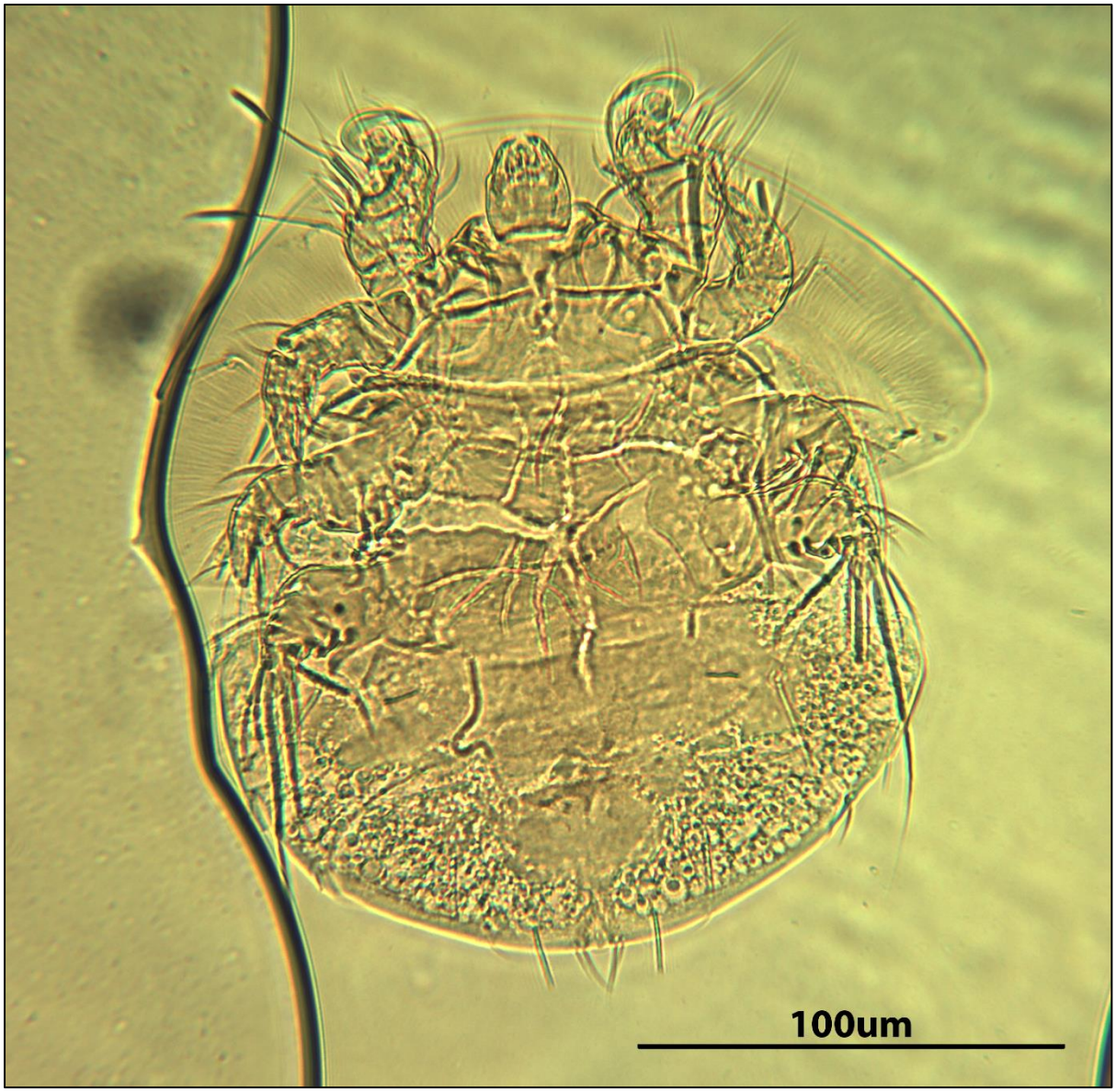

Figure E.69 – *Scutacarus deserticolus* adult female full body, ventral side shown.

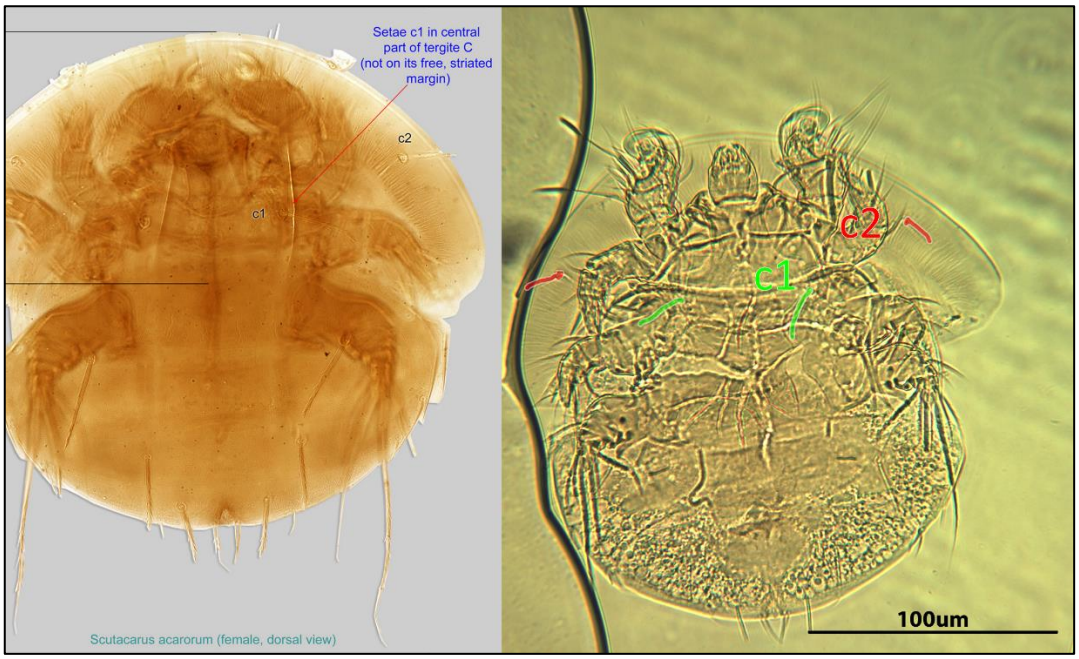

Figure E.70 – (Left) *Scutacarus* example given by Klimov et al. (2016c). (Right) *Scutacarus deserticolus* with relevant seta highlighted.

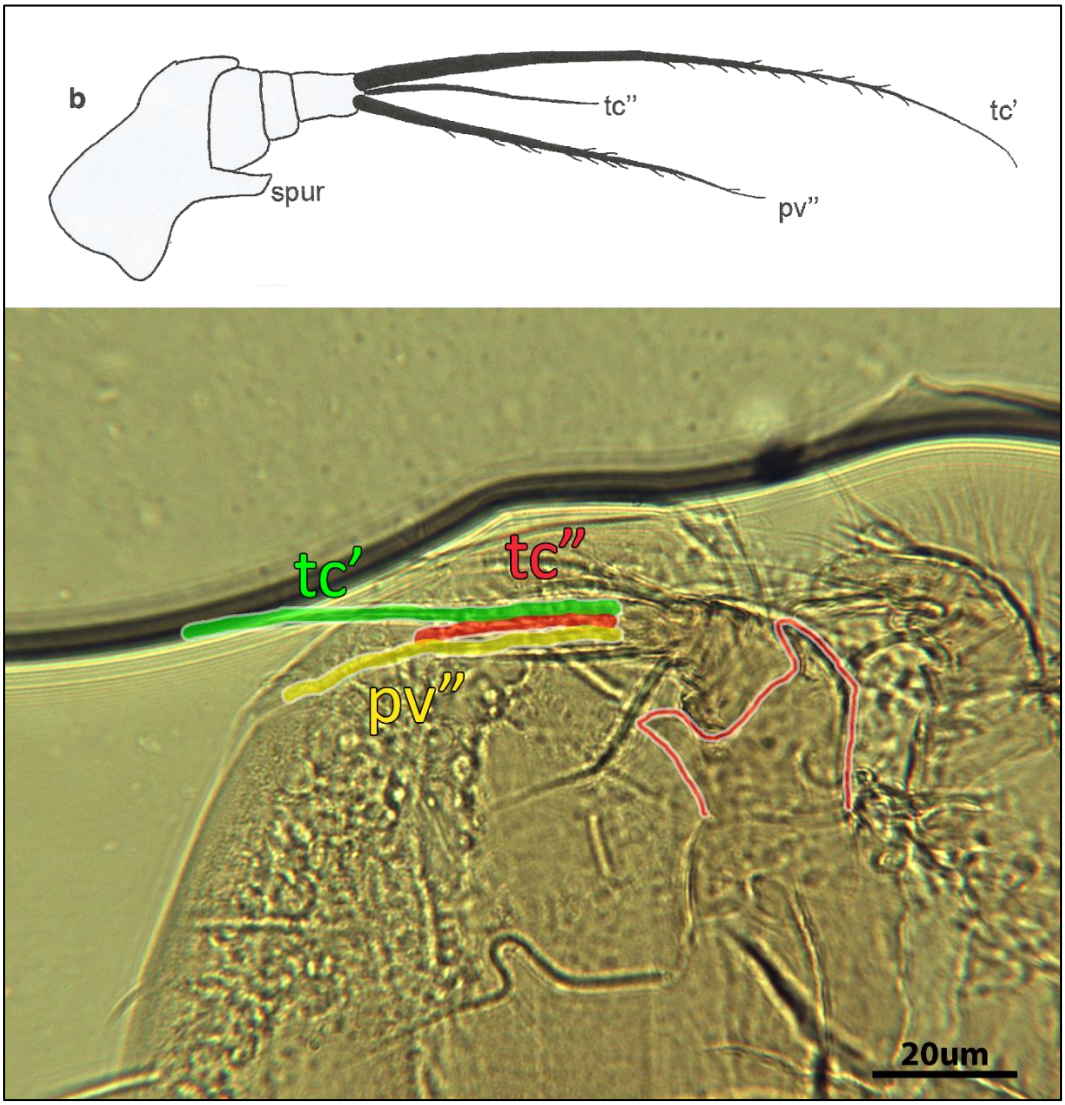

Figure E.71 – (Top) *Scutacarus deserticolus* trochanter IV with discriminant characters from Jagersbacher-Baumann (2014). (Bottom) *Scutacarus deserticolus* with relevant characters highlighted.

Appendix F Pilot *Parasitellus fucorum* rearing experiment

F.1 Introduction

This pilot experiment was designed to test different foodstuff combinations in trial colonies of *Parasitellus fucorum* in order to determine what effect the foodstuffs had on population growth over time. The design of this experiment was heavily based upon information in the only previous literature in which *P. fucorum* had been successfully kept captive and allowed to reproduce in laboratory conditions (Koulianos and Schwarz, 1999).

F.2 Methods

F.2.1 Test colony setup and maintenance

Seven different foodstuff combinations were tested using three base foodstuffs in a full factorial design, and ten replicates of the experiment were conducted in total. The base foodstuffs were fresh bumblebee collected pollen (gathered by hand in the field), organic honeybee collected pollen (Aspermuehle, Germany) and sliced fresh mealworm (livefoods4u, UK). All foodstuffs were kept frozen at -20°C until needed to ensure freshness and to prevent contamination. The seven combinations of foodstuffs (hereafter referred to as conditions) used were as follows; bumblebee pollen (BB), honeybee collected pollen (HB), sliced mealworm (MW), BB+HB, BB+MW, HB+MW and BB+HB+MW. All test colonies were set up by filling a 650ml tissue culture flask (Greiner, UK) halfway with moist autoclaved peat (Sycamore Trading) before adding whatever foodstuffs were required. Foodstuff quantities were not weighed for this experiment, but semi-standardised units were used for each foodstuff. All colonies containing honeybee pollen were given 3 balls of pollen, and all colonies containing bumblebee pollen were given ½ a ball each. All colonies containing mealworm were given roughly ⅓ of a mealworm. These foodstuff quantities were each thought to represent an *ad libitum* supply so no weight comparisons were conducted. Colonies which were given more than one foodstuff were given the same quantities in combination so that e.g. the BB+HB group were given 3 balls of honeybee pollen and ½ ball of bumblebee pollen. The *Parasitellus fucorum* deutonymphs required for this experiment were removed from commercial colonies of *Bombus terrestris audax* (Biobest Group NV) that had been left in the field for a number of weeks, and the mites were temporarily stored in colonies identical to the BB+HB+MW condition of this experiment until they were moved into their experimental colonies.

Four *Parasitellus fucorum* deutonymphs were added to each test colony (the tissue culture flasks described above) after setup, and all colonies were then moved into a Controlled Environment (CE) chamber maintained at 21°C with ambient humidity and no lighting (as used by Koulianos and Schwarz (1999)) (Figure F.1). Due to variation in the numbers of *P. fucorum* deutonymphs that could be collected at any one time, replicates were established in groups of 2-3 on different dates. Other than this there were no differences in how replicates were treated.

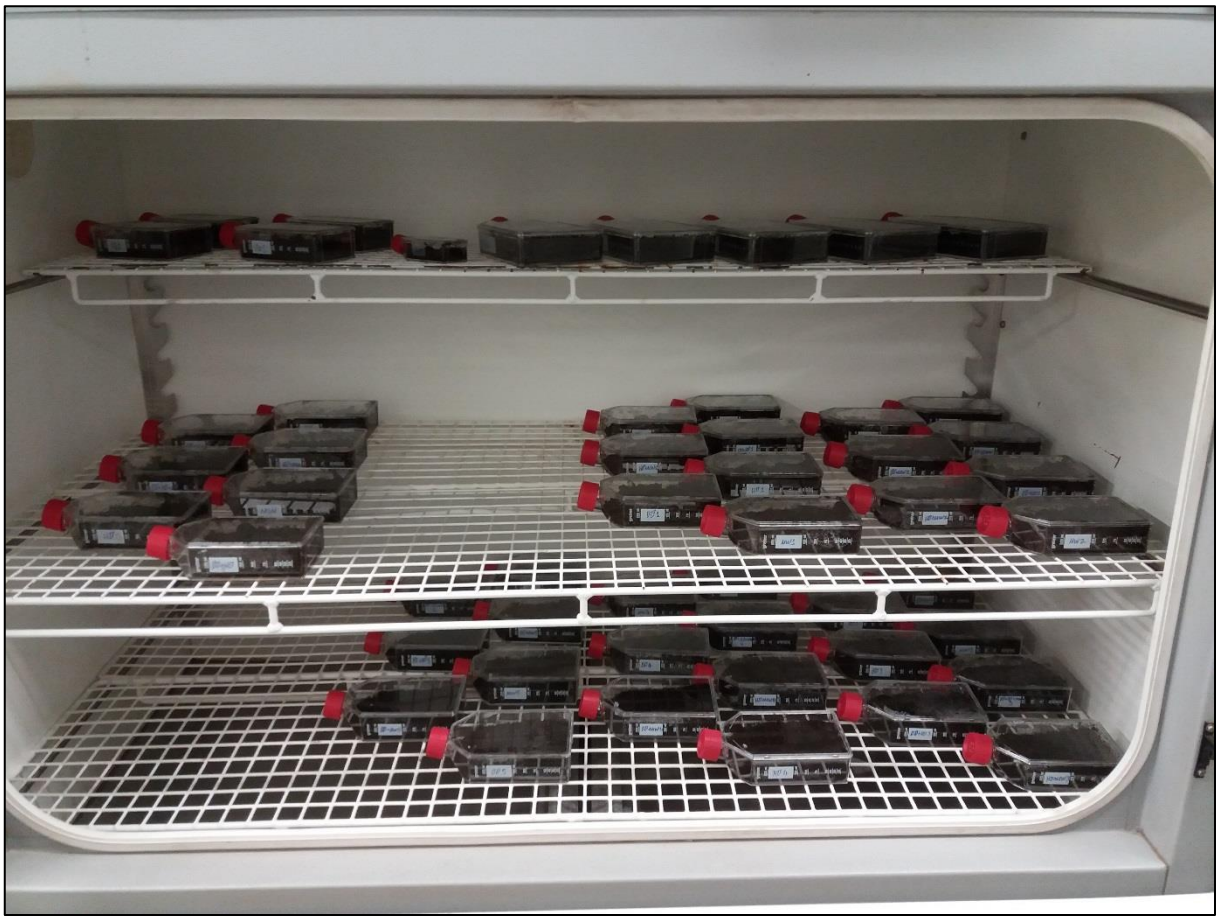

Figure F.1 – *Parasitellus* colonies within the CE chamber.

All colonies were removed from the CE chamber every 2 days and all foodstuffs were replaced to ensure an ongoing *ad libitum* supply of food. Colonies were also given a brief visual inspection daily, and if foodstuffs had spoiled early or developed fungal growths then they would be replaced. During the final colony maintenance of each week, 1ml of sterile H_2O was pipetted into each test colony and the peat substrate was gently shaken to distribute the water. This ensured that the substrate remained moist over the course of the experiment.

F.2.2 Population observations

After the foodstuffs were replaced every second day, mite observations were performed and recorded. Each test colony was observed continuously for 3 minutes by eye and using a dissecting microscope with 1-5x objective lenses where necessary. The numbers and developmental instars of all mites observed were recorded, and the colonies were returned to their CE chamber afterwards. If a mite moved out of observation range by moving deeper into the peat substrate and emerged shortly afterwards, this was not counted as an additional observation.

Each replicate of the experiment continued for 28 days after setup, and 14 observations of mite numbers were taken in total for each test colony. The final observations on day 28 were conducted immediately after foodstuffs had been removed, as no new foodstuffs were added on this occasion.

F.2.3 Berlese funnel extractions

After the final observations had been conducted, the contents of each test colony were transferred into a customised Berlese funnel made from 1.5L plastic water bottles (Nestle), placed under a light/heat source, and left undisturbed for 8 days (Figure F.2). The design of the Berlese funnel was altered to deal with *Parasitellus* mites, which are relatively large and mobile compared to other mite species and capable of crossing typical barriers used to prevent mite escapes (e.g. Vaseline) with ease. Autoclave tape was used to seal gaps between the two sections of each Berlese funnel. Sections of 60 denier tights (George) were also cut to size and held taught over the top of the funnel using clear tape in order to prevent the *Parasitellus* mites climbing out and escaping from the funnels. 100ml of 70% ethanol was poured into the bottom section of the funnels to trap, euthanise and store mites present in the test colonies for later examination. After 8 days the ethanol in the bottom section of each funnel was collected and transferred into a labelled sample pot for later examination.

Figure F.2 – Customised Berlese funnels.

F.2.4 Ethanol sample processing

The ethanol samples collected from Berlese funnels were analysed by transferring some of their contents into a petri dish for examination under a stereoscopic microscope with 1-5x objective lenses. Any mites observed in the ethanol were counted and transferred to Eppendorf tubes for later clearing and identification to the species level. This was repeated as many times as necessary until the entire ethanol sample had been examined.

F.2.5 Mite identification

Mites were identified to the species level following the standard protocol; first they were cleared in 50% lactic acid at 50°C, then dehydrated using 70% and 95% ethanol, and finally mounted on a microscope slide in Hoyers liquid, in a process similar to the recommendations in Krantz and Walter (2009). Mounted mites were sealed using glycol solution and identified under a compound microscope with 10-100x objective lenses by observing their morphological characteristics and following dichotomous keys in the standard work of reference for this genus (Hyatt, 1980, Baker et al., 1999).

F.2.6 Statistical analysis

All statistical analyses were carried and all graphs produced using R v3.4.2, R studio, and the ggplot2 package (R Core Team, 2017, RStudio Team, 2015, Wickham, 2016). Normality testing was performed using the Kolmogorov-Smirnov test, and as all data were not normally distributed the Kruskal-Wallis and Wilcoxon signed rank tests were used to test for differences in results between different treatments.

F.3 Results

All colonies in replicates 1 and 2 were infested with *Tyrophagus* mites from an unknown source during the course of the experiment and so were removed from all analyses.

Very little reproduction or increases in population were observed in any colonies during the experiment, with the only exception being a single colony in the mealworm treatment group (Figure F.3). Differences in the numbers of mites observed in colonies from different treatment groups were analysed using a Kruskal-Wallis test (after the data were shown to be of a non-normal distribution by a Kolmogorov-Smirnov test). The Kruskal-Wallis test showed that there were statistically significant differences between the treatment groups ($p < 0.001$), and subsequent pairwise comparisons using Wilcoxon signed rank tests showed that these differences were widespread, especially between the bumblebee pollen and honeybee pollen groups when compared to every other treatment (though the honeybee pollen treatment did not differ significantly from the mealworm treatment) (Table F.1).

The same tests were conducted in order to analyse any key differences in the numbers of mites observed on the final day of the experiment. Observed counts were used for this purpose due to the failure of the Berlese funnel method of extracting live mites from the colonies. Kolmogorov-Smirnov testing showed that these observed counts were not normally distributed, so a kruskal-wallis test was used to analyse differences in total mite numbers between treatment groups. This test showed that the differences in these final counts were not significant ($p = 0.13$).

Pairwise comparisons using Wilcoxon rank sum test						
	Bumblebee pollen (BB)	BB+HB	BB+HB+MW	BB+MW	Honeybee pollen (HB)	HB+MW
BB+HB	**<0.001**	-	-	-	-	-
BB+HB+MW	**<0.001**	0.861	-	-	-	-
BB+MW	**0.02**	0.736	0.428	-	-	-
Honeybee pollen (HB)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	-	-
HB+MW	**<0.001**	0.677	0.861	0.267	**0.006**	-
Sliced mealworm (MW)	**<0.001**	0.051	0.099	**0.002**	0.845	0.267

Table F.1 – p-values obtained from pairwise Wilcoxon rank sum test comparisons between all treatment groups. Data used were total numbers of mites counted during observations from day 2-28 of the experiment. p-values which indicated significant differences in observed mite numbers are shown in **bold**.

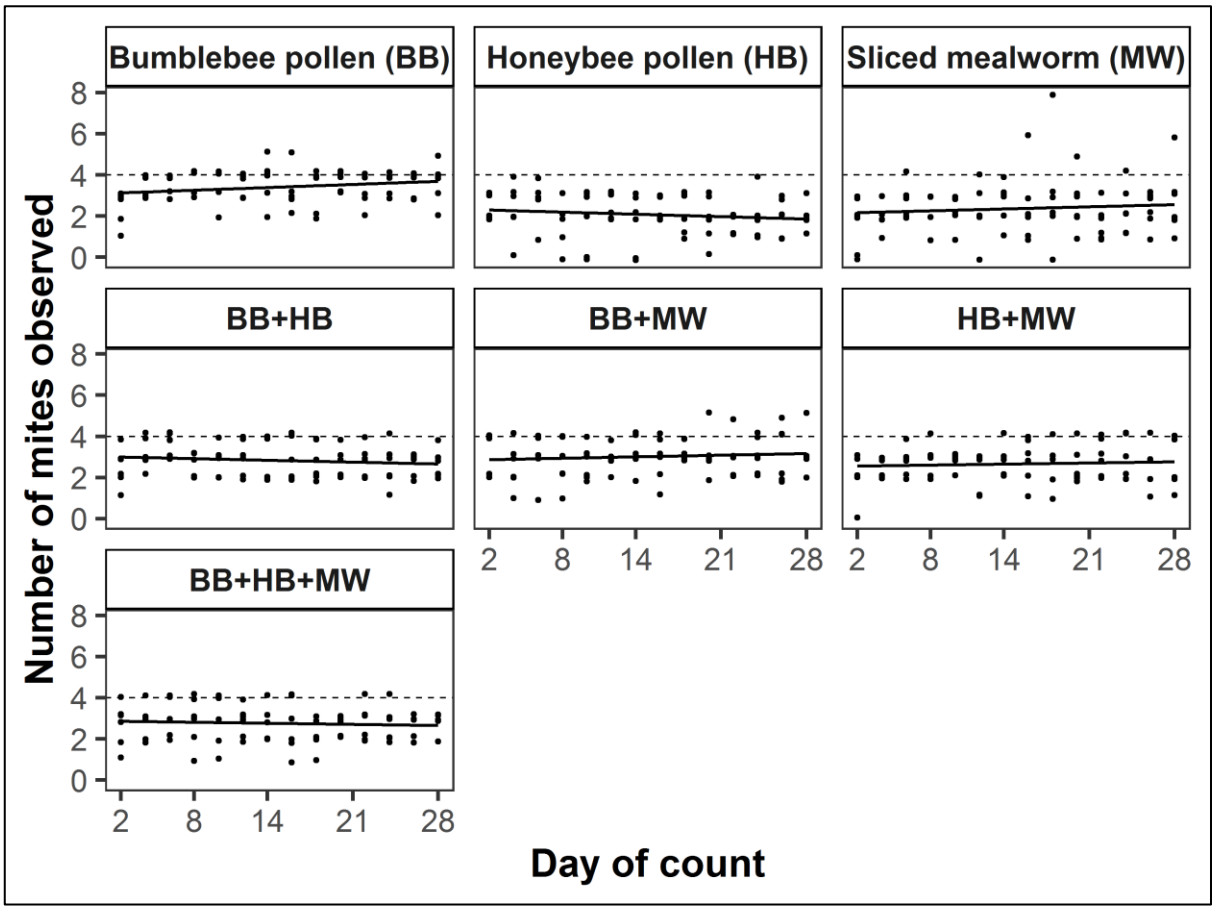

Figure F.3 – Total numbers of mites counted during each observation of replicates 3-8 during the experimental period, separated by treatment group. Dashed horizontal line shows original number of deutonymphs added (4) and a black trendline was added to each treatment group's data using a linear model.

F.4 Discussion

This experiment was designed to investigate in a fully factorial manner the effects of 3 different foodstuffs on the population growth of *Parasitellus fucorum* deutonymphs in laboratory conditions. However, as became apparent when the results were analysed, there were flaws in the experimental design. This contributed to an unclear result which did not achieve the central aim of the experiment.

The only results that could be analysed were population observation counts taken every two days when the foodstuffs were replaced. This was because the final results which were intended to provide objective mite counts for each colony at the end of the experimental period could not be collected, due to the failure of the Berlese funnel method of extracting mites from soil (or in this case peat) samples. Despite several changes to the standard funnel design in order to prevent *P. fucorum* from escaping, when the funnels were checked the day after setup, many *P. fucorum* mites were found wandering the area surrounding the funnels having escaped during the night. As a result of this the funnel results were irreversibly compromised and thus had to be discarded.

The observed population counts showed a confusing picture. Reproduction (i.e. actual *Parasitellus fucorum* offspring in the form of larvae or protonymphs) were only observed in a single colony within the mealworm (MW) group. However there were significant differences in observed mite numbers between many treatment groups. The bumblebee pollen (BB) group differed significantly from all other

groups, and the honeybee pollen (HB) group differed significantly from all groups except the mealworm group, which differed significantly from all treatments which did not contain mealworm as a foodstuff (and also honeybee pollen). The fact that reproduction (and therefore actual population growth) was restricted to a single colony renders useful interpretation of these statistical differences useless, as the aim of the experiment was to compare how these foodstuffs effected population growth over time, and in nearly all colonies none was observed. It is also clear that something was fundamentally wrong with the experimental design, as colonies used prior to this experiment when trialling different foodstuffs in an ad-hoc fashion (and treated in broadly similar ways) resulted in reproduction when fed with all 3 foodstuffs used in this experiment.

As a result of this, the experimental design at every stage was examined and redesigned for a subsequent experiment. The number of replicates would be kept the same if possible, however more mites would be used per colony. Therefore the number of foodstuffs tested would be restricted based on which foodstuffs seemed to result in the most population 'growth' (i.e. the least reduction over time as evidenced by the gradient of the trendline fitted by the linear model (Figure F.3)). The conditions in which the colonies were kept would also be adjusted to more accurately reflect those found within bumblebee colonies. To ensure that there was a constant supply of entirely fresh food, foodstuffs would be replaced daily instead of every other day. The weight of foodstuffs available at any one time would also be standardised for all treatment groups. Final mite extraction would also be performed in a different way so that accurate final counts of the mite population of each colony could be collected and analysed. In order to provide more time for population development, the length of the experimental period would also be extended. It was thought that with these adjustments, the subsequent experiment should provide useful information on how effective these foodstuffs are at maintaining or growing a captive population of *Parasitellus fucorum*.

Appendix G *Parasitellus* rearing experiment supplementary data

G.1 Mean mite numbers at start and end of experiment

Week	Treatment	Larvae	Protonymphs	Deutonymphs	Males	Females	Total
1	C	0	0	8	0	0	8
7	C	1.25	0.5	3.625	0.375	0.25	6
1	BB	0	0	8	0	0	8
7	BB	2.375	0.25	2.875	0.75	0.25	6.5
1	MW	0	0	8	0	0	8
7	MW	0	0	0.625	0	0	0.625
1	BB+MW	0	0	8	0	0	8
7	BB+MW	0	0	4	0.25	0	4.25

Table G.1 – Mean numbers of all *Parasitellus fucorum* instars counted in test chambers from different treatment groups. Only objective counts (Weeks 1 & 7) are included. Totals marked with an asterisk (*) indicate that when total mite numbers from all colonies in this treatment were compared between weeks 1 & 7 using a Wilcoxon rank sum test, significant differences were found ($p < 0.05$).

G.2 Total counts per week for each treatment

Week	Treatment	Larvae	Protonymphs	Deutonymphs	Males	Females	Total
1	BB	0	0	64	0	0	64
2	BB	0	0	66	4	3	73
3	BB	35	0	57	12	4	108
4	BB	31	7	90	10	4	142
5	BB	9	18	60	5	2	94
6	BB	0	3	32	10	2	47
7	BB	19	2	23	6	2	52
1	BB+MW	0	0	64	0	0	64
2	BB+MW	0	0	81	5	4	90
3	BB+MW	1	0	33	18	8	60
4	BB+MW	33	10	44	9	3	99
5	BB+MW	7	20	53	1	0	81
6	BB+MW	0	14	42	0	0	56
7	BB+MW	0	0	32	2	0	34
1	C	0	0	64	0	0	64
2	C	0	0	78	0	0	78
3	C	0	0	71	1	1	73
4	C	10	0	62	6	2	80
5	C	0	2	44	4	4	54
6	C	2	0	41	3	4	50
7	C	10	4	29	3	2	48
1	MW	0	0	64	0	0	64
2	MW	0	0	58	0	0	58
3	MW	0	0	23	0	0	23
4	MW	0	0	23	0	0	23
5	MW	0	0	5	0	0	5
6	MW	0	0	9	0	0	9
7	MW	0	0	5	0	0	5

Table G.2 – Total mite counts (physical counts for weeks 1 & 7, observed counts for weeks 2 – 6) for each *Parasitellus fucorum* instar per week for each treatment group.

Appendix H Additional method details for Chapter 3

H.1 Foodstuff weight calculations

The average weights of replicable units of each foodstuff e.g. individual pollen balls collected from bumblebees, honeybee pollen balls, ⅓ of a fresh mealworm (with the first and last segments of the body removed), were calculated from 10 measurements taken using a very precise scale (Sartorius Cubis Micro Balance). These were used to calculate quantities of each foodstuff combination that would be roughly equal in weight. For the first 5 weeks of the experiment the following average weights were used for each foodstuff combination with a ±5mg tolerance for variance; Honeybee pollen – 37mg, Bumblebee pollen (BB) – 34mg, Sliced mealworm (MW) – 32mg, BB+MW – 35mg. To reach these average weights, colonies in each treatment group were given foodstuffs in set quantities. Honeybee pollen colonies were given 2 ½ balls of honeybee collected pollen, bumblebee pollen colonies were given 2 balls of honeybee collected pollen and ½ ball of bumblebee collected pollen, mealworm colonies were given ⅓ of a sliced mealworm, and BB+MW colonies were given 1 honeybee pollen ball, ½ bumblebee pollen ball and ⅙ of a slice mealworm. For the final 2 weeks of the experiment these quantities of foodstuffs were doubled for all colonies, as was the tolerance for variance. Due to only limited quantities of bumblebee collected pollen being available for use in the experiment, in all conditions where bumblebee pollen was used it was supplemented with honeybee collected pollen to reach the required weight. This compromise was necessary to ensure that all colonies would be supplied with the correct weight of foodstuffs throughout the experimental period.

H.2 Final mite extraction additional precautions

As an additional precaution, once all materials from a colony had been examined and all visible mites removed, the materials were returned to the colony and the colony stood so that the neck of the flask was vertically above the base. Four hours later these test colonies were examined a final time, and any mites which had climbed towards the flask neck were collected, counted and added to the 70% ethanol sample for that colony. This was performed as it was thought that a few mites might be missed during hand processing, and it has been consistently observed that *Parasitellus* mites in a tall vertical container will move towards the top (pers. obs.).

Appendix I *Aphomia sociella* pilot predation test

I.1 Introduction

The larval stages of the bee moth (*Aphomia sociella*) are a common and damaging pest of bumblebee colonies (Figure H.1). Adult female moths enter bumblebee colonies and lay their eggs within, and these rapidly hatch and moult into large larvae which proceed to consume much of the colony including wax, stored honey and pollen, and even developing brood (Alford, 1975). Hoffer (1882-3) considered *A. sociella* to be the greatest natural enemy of bumblebees.

Parasitellus fucorum is a predatory bumblebee associated mite from the Order Mesostigmata which is often found within bumblebee colonies (Rożej et al., 2012). This mite species has been suggested as a potential mutualist with its bumblebee hosts (in spite of often kleptoparasitic feeding habits) due to its likely predation and oophagy upon pests and parasites within bumblebee colonies (Alford, 1975, Eickwort, 1994).

The aim of this pilot experiment was to determine whether *Parasitellus fucorum* were capable of predating the eggs or early instar larvae of *Aphomia sociella*. To test this a series of chambers were established containing either *Aphomia sociella* eggs or a single 1st instar larva, after which *P. fucorum* deutonymphs were added. The survival of all organisms within these test chambers were then monitored at intervals over the next 29 hours, and the results were examined in order to determine whether *P. fucorum* had predated *A. sociella* eggs/larvae during the experimental period.

I.2 Methods

I.2.1 Pilot protocol

A small number of 1st instar *Aphomia sociella* larvae and several eggs were carefully removed from a section of the cardboard box surrounding a commercial colony of *Bombus terrestris* (Biobest Group NV, Belgium) which had been left in the field for many weeks (Figure H.1). The species of these larvae and eggs were determined based on 1) their location beneath a thick layer of silk tunnels constructed by other *A. sociella* larvae in which no other species were found and 2) a visual comparison with descriptions of the species found in (Alford, 1975).

Figure I.1 – Commercial *Bombus terrestris audax* colony destroyed by *Aphomia sociella* infestation.

The larvae and eggs were stored overnight in at 5°C in a dark fridge in a 250ml tissue culture flask with a vented cap (Greiner Bio-One Ltd., UK) placed within a sandwich box containing a base layer of damp tissue paper and air holes, and removed the following morning for experimental use.

The *Parasitellus fucorum* deutonymphs used in this pilot experiment were reared and collected in the same way as outlined in the main Methods section.

The pilot experiment was run using 3 replicates of 3 different conditions. All tests involved the exposure of 2 *Parasitellus fucorum* deutonymphs to different potential prey species/foodstuffs; a single 1st instar *Aphomia sociella* larva, two *A. sociella* eggs, or a single ball of organic honeybee pollen (control). The aim of this experiment was simply to demonstrate if *P. fucorum* deutonymphs were capable of predating

eggs or early larvae of *A. sociella* in order to inform a future large scale predation experiment using these species.

For each replicate of the experiment, all 'test chambers' (25ml tissue culture flaks with vented caps (Greiner Bio-One Ltd., UK)) were set up by first adding the 'prey' specimens (*Aphomia sociella* eggs/larva or pollen ball) followed by the *Parasitellus fucorum* deutonymphs. All specimens were transferred carefully using forceps or a paintbrush as appropriate. Once all specimens had been added to a test chamber the lid was closed and the test chamber moved into a large box containing moistened paper towels (referred to as the 'humid box') in order to maintain a high level of humidity to prevent the experimental organisms from dehydrating. The humid box itself was stored in a dark, room temperature (21-24°C) cupboard.

The start time for each test chamber was recorded when the lid was closed after all specimens were added, and survival counts were taken every hour for 5 hours from the recorded start time. Survival counts were performed by removing the test chamber from the humid box and observing the chamber as needed using a Motic SMZ171 microscope with 1-4x objective lenses in order to determine how many live organisms remained in each test chamber (in the case of the control group fed honeybee pollen, only the live *Parasitellus fucorum* deutonymphs were counted). Pictures of the organisms within each colony were taken where possible to provide visual evidence of predation having taken place. After this the test chamber was returned to the humid box until the next survival count. After the 5 hourly observations had been conducted the humid box was left in the cupboard overnight, and a final set of survival counts were conducted exactly 24 hours after the last counts (i.e. 29 hours from the experiment start time).

After the final survival counts both *Parasitellus fucorum* deutonymphs were removed from each colony using a paintbrush and euthanised/stored in 70% ethanol for later species confirmation. This was done by dissolving the mites internal organs using 50% lactic acid, dehydrating them by 15 minutes submersion in first 70% and then 95% ethanol, before finally mounting the mites on a microscope slide before covering with Hoyer's liquid and a cover slip (Krantz and Walter, 2009). The mounted mites were later identified to the species level using an identification key and species descriptions (Hyatt, 1980).

I.2.2 Statistical analysis

All statistical tests were conducted using R version 3.5.2 and RStudio (RStudio Team, 2015, R Core Team, 2017). All figured were produced using the ggplot2 package (Wickham, 2016)., except for the Kaplan-Meier survivor graphs showing larvae/egg survival over time which were produced using Stata 15 (StataCorp, 2017). Due to the use of a single pollen ball as the control 'prey' specimen the statistical comparisons which could be conducted within the results were limited. After Kolmogorov-Smirnov normality testing showed that prey survival counts were not normally distributed, non-parametric Kruskal-Wallis or Wilcoxon rank sum tests were used to compare differences between treatment groups.

I.3 Results

In all colonies from all conditions, no *Parasitellus fucorum* deutonymphs died during the experiment. Since a non-animate source of food was used for the Control group (i.e. a pollen ball) no summary statistics could be calculated for this group's prey survival. The average number of surviving prey specimens in the Larva group at the final survival counts was 0 ± 0 (median ± Inter-Quartile Range (IQR)), and for the Eggs treatment group the average was 1 ± 1 (median ± IQR) (Figure H.2). In colonies from both the Larva and Eggs treatment groups, there were direct observations of *Aphomia sociella* larvae (Figure H.4) or eggs (Figure H.3) being predated by *Parasitellus fucorum* deutonymphs. Since the results for prey survival in the Control group were unusable, a Wilcoxon rank sum test was conducted to compare the prey survival counts from the Eggs and Larva treatment groups, returning a non-significant result ($p = 0.079$). Kaplan-Meier curves were also plotted for the *A. sociella* egg and larvae treatment groups, showing survival over time (Figure H.5, Figure H.6)

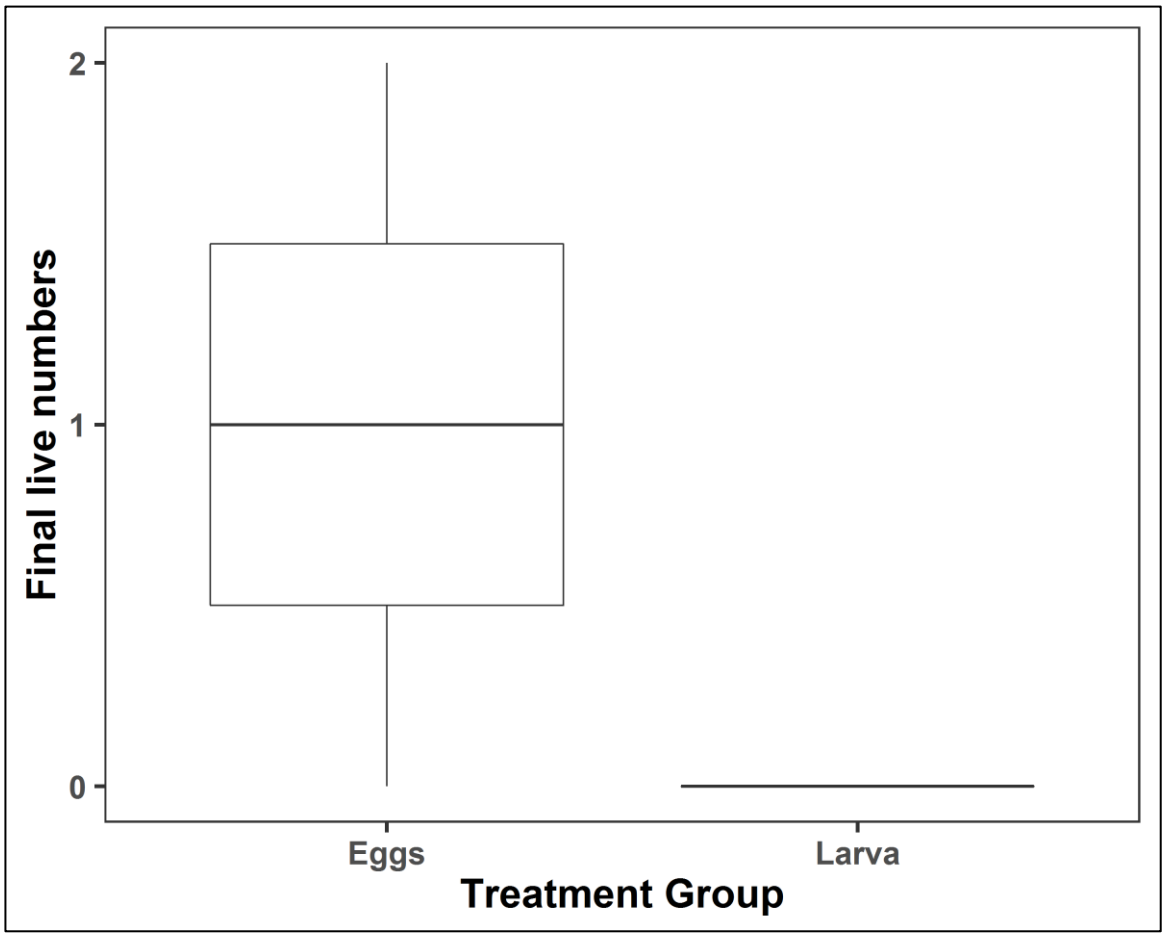

Figure I.2 – Final numbers of surviving prey specimens shown by treatment group. Control group left out as no live prey were used in the Control group.

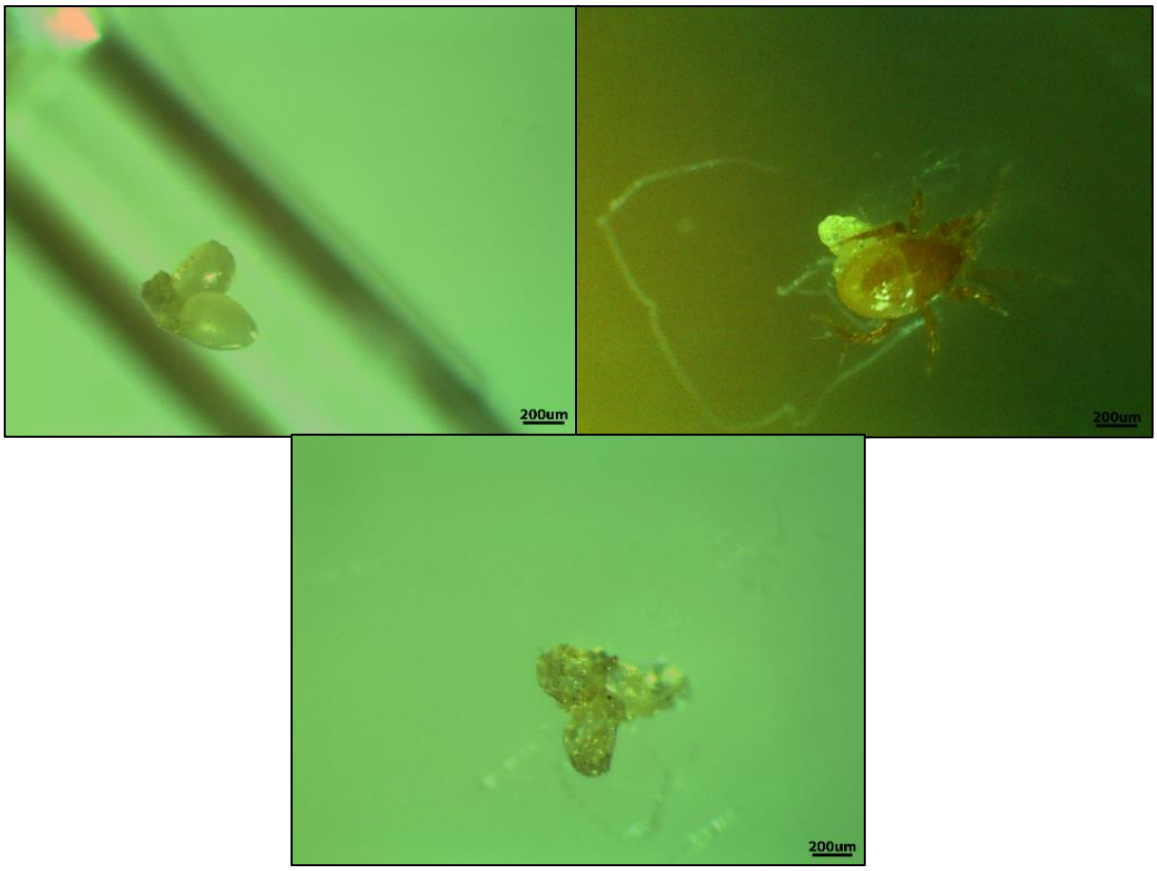

Figure I.3 – *Aphomia sociella* eggs predated by *Parasitellus fucorum* deutonymph. Bottom-right image shows remains at 29 hour inspection.

Figure I.4 – *Aphomia sociella* larva attacked and consumed by *Parasitellus fucorum* deutonymph. Bottom-right image shows remains at 29 hour inspection.

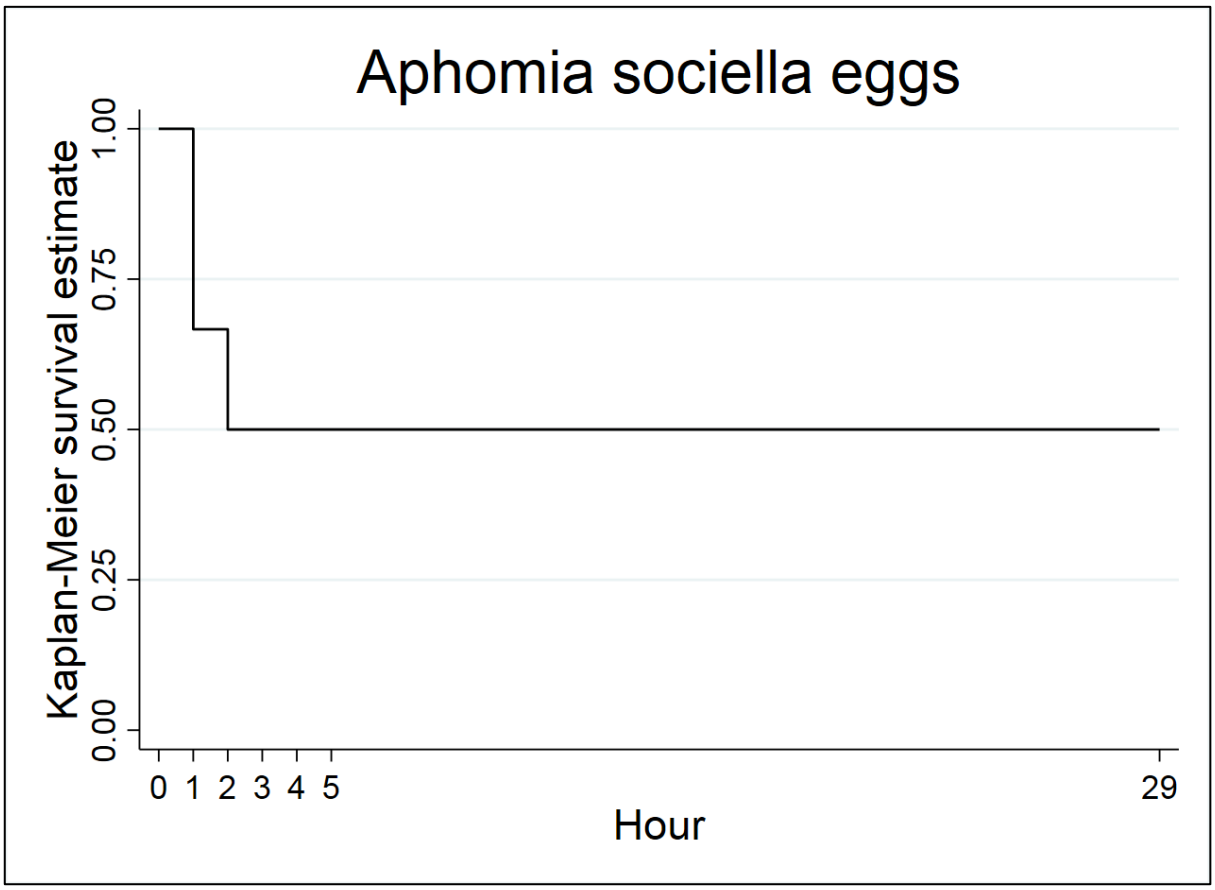

Figure I.5 – Survival estimates for *Aphomia sociella* eggs plotted across all survival count times.

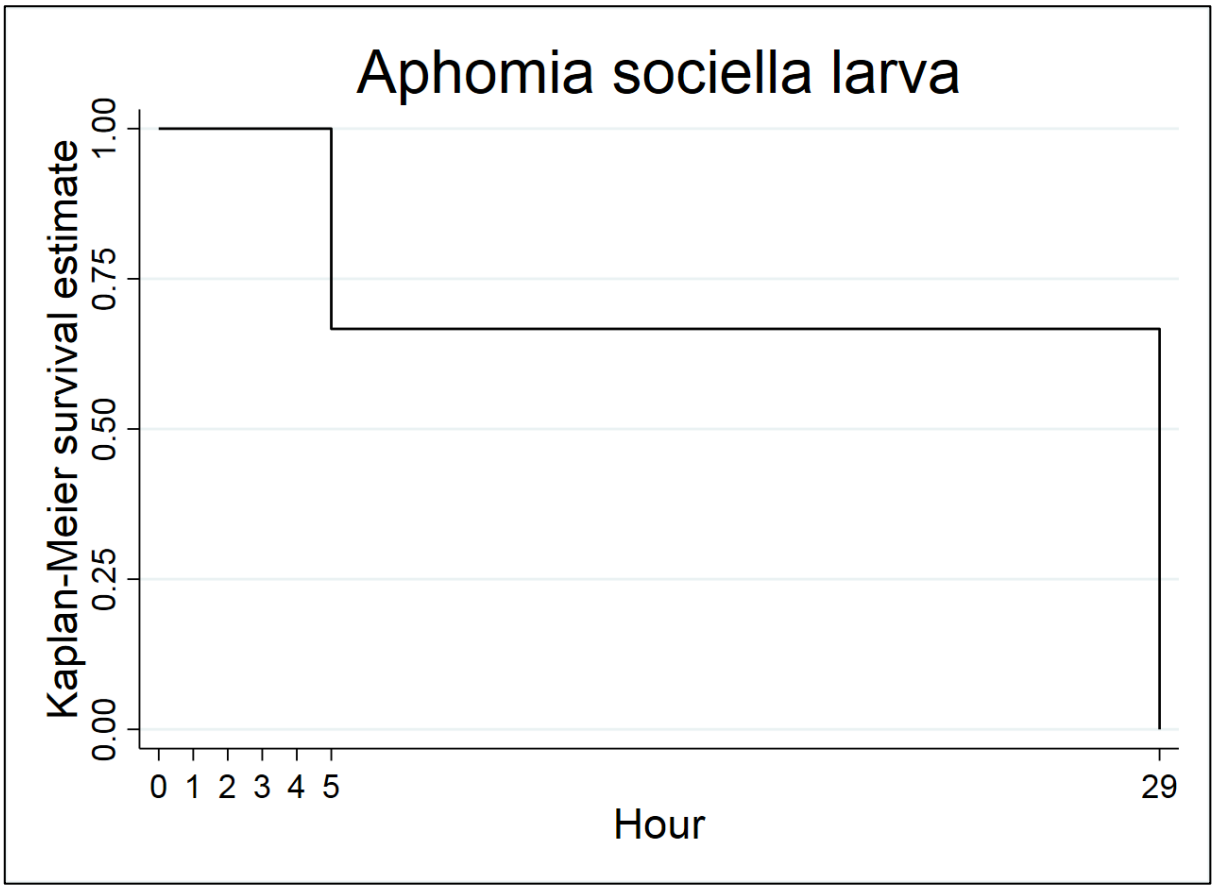

Figure I.6 – Survival estimates for *Aphomia sociella* larvae plotted across all survival count times.

I.4 Discussion

While the design of this initial pilot experiment was clearly flawed by the use of an inert food source in the 'Control' group of colonies (i.e. a pollen ball) and the lack of objective control groups without added *Parasitellus fucorum* deutonymphs, there were nonetheless some clear results. In every colony from the 'Larva' group the *Aphomia sociella* larvae had been predated by the *Parasitellus fucorum* deutonymphs before the final survival counts were performed. In the 'Eggs' group, on average half of all *A. sociella* eggs were consumed by the *P. fucorum* deutonymphs by the end of the experiment. No significant differences were found in the relative numbers of prey predated between these two conditions.

This experiment was only a small-scale pilot, however it nonetheless represents the first reported evidence that *Parasitellus fucorum* deutonymphs can predate the larvae and eggs of the bumblebee wax moth *Aphomia sociella*. It is impossible to know from the results of this experiment whether this predation occurs in the field, has any impact on the health of bumblebee colonies, or slows the damage caused by an *A. sociella* infestation, however the fact that *P. fucorum* is able to predate this common bumblebee pest should encourage further research in this area in order to answer these questions.

Appendix J Prey survival counts plotted over time

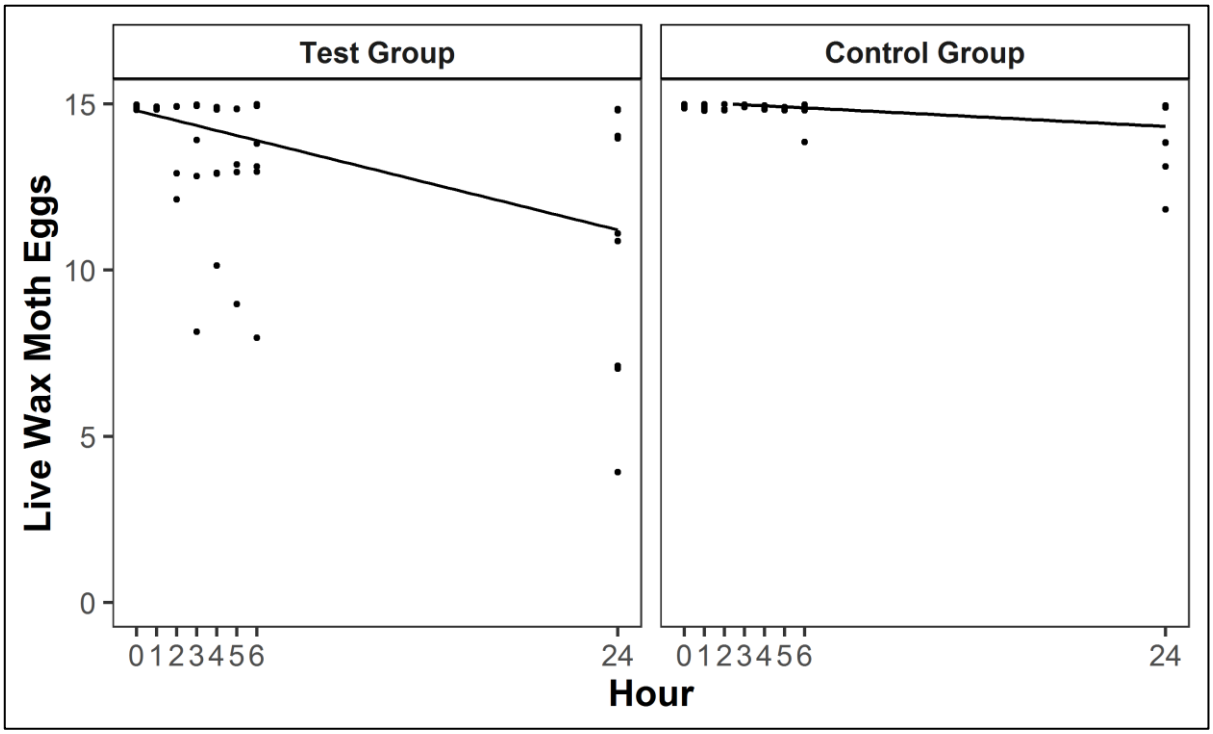

Figure J.1 – Numbers of live wax moth (*Galleria mellonella*) eggs at each survival count for all colonies in the Wax Moth Egg Test and Control groups.

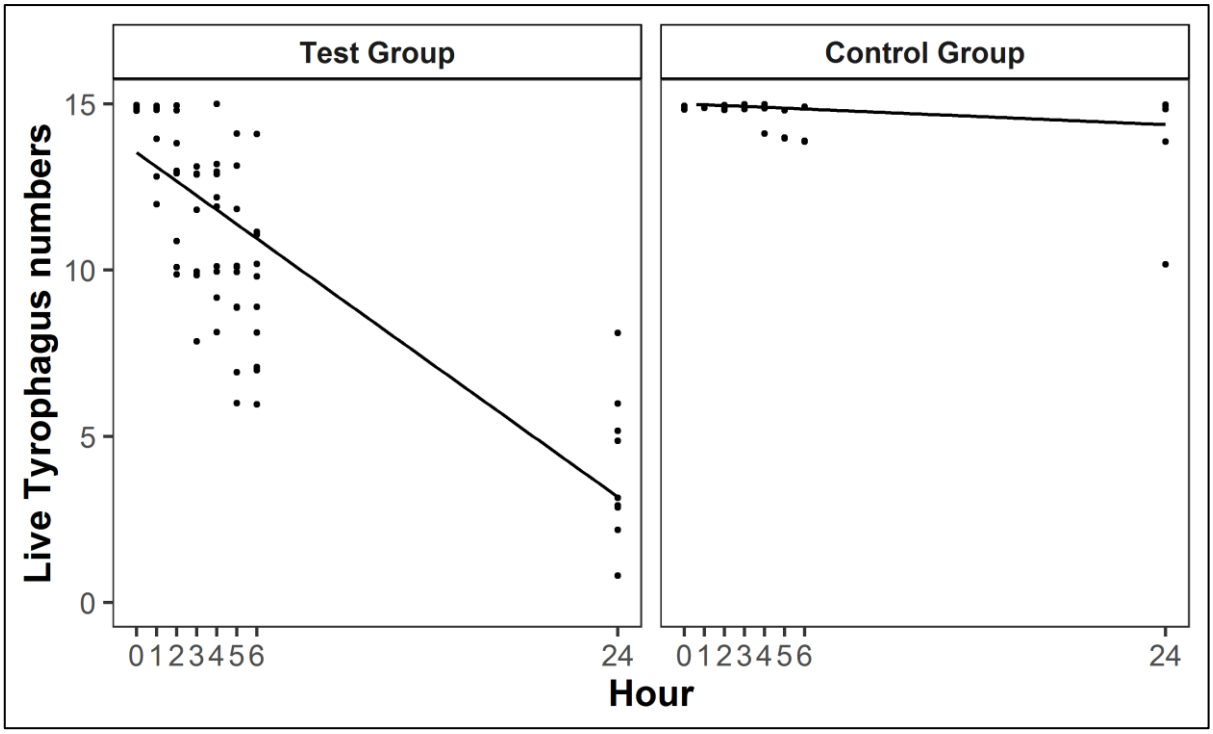

Figure J.2 – Numbers of live *Tyrophagus putrescentiae* mites at each survival count for all colonies in the *Tyrophagus* Test and Control groups.

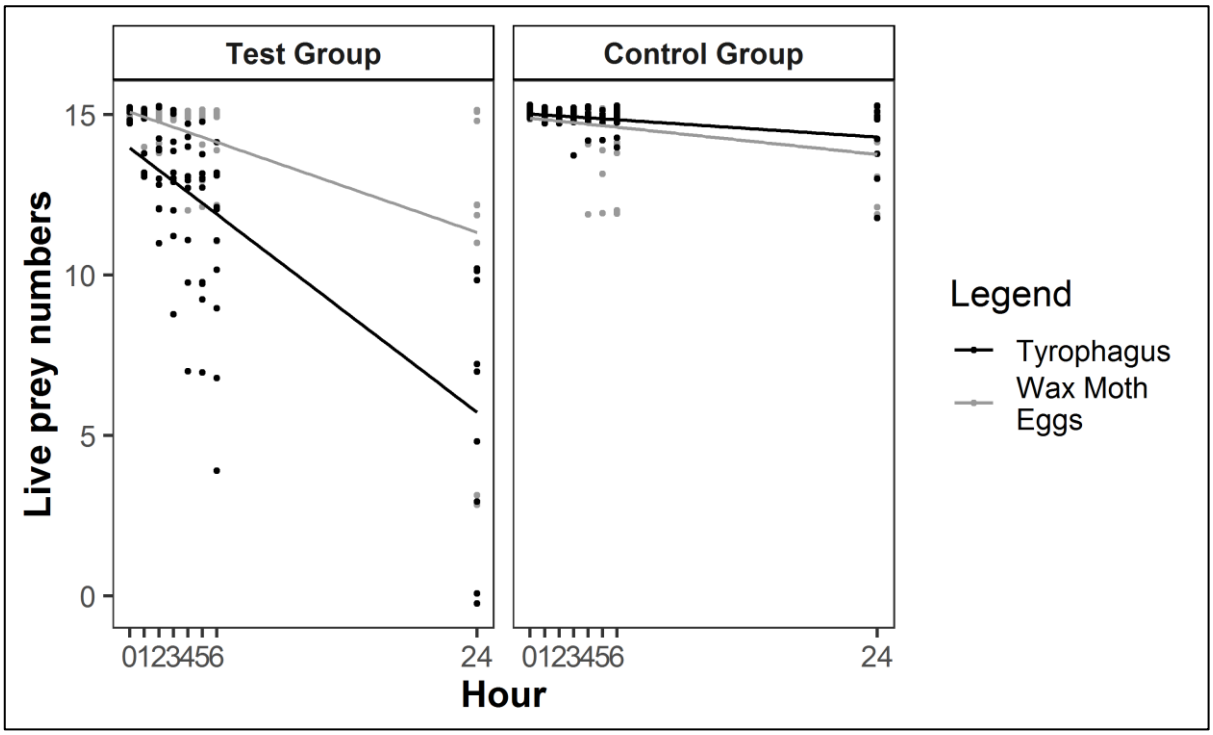

Figure J.3 – Live numbers of *Tyrophagus putrescentiae* mites (black) and *Galleria mellonella* eggs (grey) at each survival count from the combined (*T. putrescentiae* + *G. mellonella*) Test and Control groups.

Appendix K *Tyrophagus putrescentiae* GLM parameter estimates and fitted values

Parameter				**Estimate**	**Standard Error**	**95% Confidence Limits**		**Z**	**Pr > |Z|**
Intercept				1.8245	0.196	1.4405	2.2086	9.31	<.0001
Group	C			0.8357	0.1972	0.4492	1.2222	4.24	<.0001
Group	T			0	0	0	0	.	.
Hour	0			0.8835	0.196	0.4994	1.2676	4.51	<.0001
Hour	1			0.8357	0.1867	0.4697	1.2017	4.48	<.0001
Hour	2			0.7632	0.1949	0.3812	1.1453	3.92	<.0001
Hour	3			0.7327	0.1942	0.3521	1.1133	3.77	0.0002
Hour	4			0.685	0.202	0.2891	1.081	3.39	0.0007
Hour	5			0.635	0.1915	0.2596	1.0104	3.32	0.0009
Hour	6			0.5268	0.1792	0.1756	0.878	2.94	0.0033
Hour	24			0	0	0	0	.	.
Group*Hour	C	0		-0.8357	0.1972	-1.2222	-0.4492	-4.24	<.0001
Group*Hour	C	1		-0.7879	0.188	-1.1565	-0.4194	-4.19	<.0001
Group*Hour	C	2		-0.7154	0.1962	-1.1	-0.3309	-3.65	0.0003
Group*Hour	C	3		-0.6916	0.195	-1.0738	-0.3094	-3.55	0.0004
Group*Hour	C	4		-0.6439	0.2028	-1.0415	-0.2464	-3.17	0.0015
Group*Hour	C	5		-0.5939	0.1924	-0.971	-0.2169	-3.09	0.002
Group*Hour	C	6		-0.4925	0.1801	-0.8454	-0.1395	-2.73	0.0062
Group*Hour	C	24		0	0	0	0	.	.
Group*Hour	T	0		0	0	0	0	.	.
Group*Hour	T	1		0	0	0	0	.	.
Group*Hour	T	2		0	0	0	0	.	.
Group*Hour	T	3		0	0	0	0	.	.
Group*Hour	T	4		0	0	0	0	.	.
Group*Hour	T	5		0	0	0	0	.	.
Group*Hour	T	6		0	0	0	0	.	.
Group*Hour	T	24		0	0	0	0	.	.
Condition(Group)	CTR	C		0.007	0.0397	-0.0708	0.0847	0.18	0.8606
Condition(Group)	CWM+TR	C		0	0	0	0	.	.
Condition(Group)	TR	T		-0.4636	0.2529	-0.9593	0.0322	-1.83	0.0668
Condition(Group)	WM+TR	T		0	0	0	0	.	.
Condition*Hour(Group)	CTR	0	C	-0.007	0.0397	-0.0847	0.0708	-0.18	0.8606
Condition*Hour(Group)	CTR	1	C	-0.007	0.0397	-0.0847	0.0708	-0.18	0.8606
Condition*Hour(Group)	CTR	2	C	-0.007	0.0397	-0.0847	0.0708	-0.18	0.8606
Condition*Hour(Group)	CTR	3	C	-0.0003	0.0374	-0.0736	0.073	-0.01	0.994
Condition*Hour(Group)	CTR	4	C	-0.007	0.0374	-0.0804	0.0664	-0.19	0.8524
Condition*Hour(Group)	CTR	5	C	-0.0137	0.0386	-0.0894	0.062	-0.35	0.7227
Condition*Hour(Group)	CTR	6	C	-0.007	0.0386	-0.0827	0.0688	-0.18	0.8569
Condition*Hour(Group)	CTR	24	C	0	0	0	0	.	.

Parameter				**Estimate**	**Standard Error**	**95% Confidence Limits**		**Z**	**Pr > |Z|**
Condition*Hour(Group)	CWM+TR	0	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	1	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	2	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	3	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	4	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	5	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	6	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	24	C	0	0	0	0	.	.
Condition*Hour(Group)	TR	0	T	0.4636	0.2529	-0.0322	0.9593	1.83	0.0668
Condition*Hour(Group)	TR	1	T	0.4705	0.2444	-0.0085	0.9496	1.93	0.0542
Condition*Hour(Group)	TR	2	T	0.433	0.2625	-0.0814	0.9475	1.65	0.099
Condition*Hour(Group)	TR	3	T	0.3829	0.2634	-0.1334	0.8991	1.45	0.146
Condition*Hour(Group)	TR	4	T	0.3963	0.2798	-0.1521	0.9448	1.42	0.1567
Condition*Hour(Group)	TR	5	T	0.3066	0.2711	-0.2248	0.8379	1.13	0.2581
Condition*Hour(Group)	TR	6	T	0.3422	0.2596	-0.1666	0.8511	1.32	0.1875
Condition*Hour(Group)	TR	24	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	0	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	1	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	2	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	3	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	4	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	5	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	6	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	24	T	0	0	0	0	.	.

Table K.1 – Analysis of GEE parameter estimates for GLM examining effects of Group, Condition, Hour and various interactions of these factors on numbers of live *Tyrophagus putrescentiae* mites during the Chapter 4 predation experiment. Significant results ($p < 0.05$) are underlined.

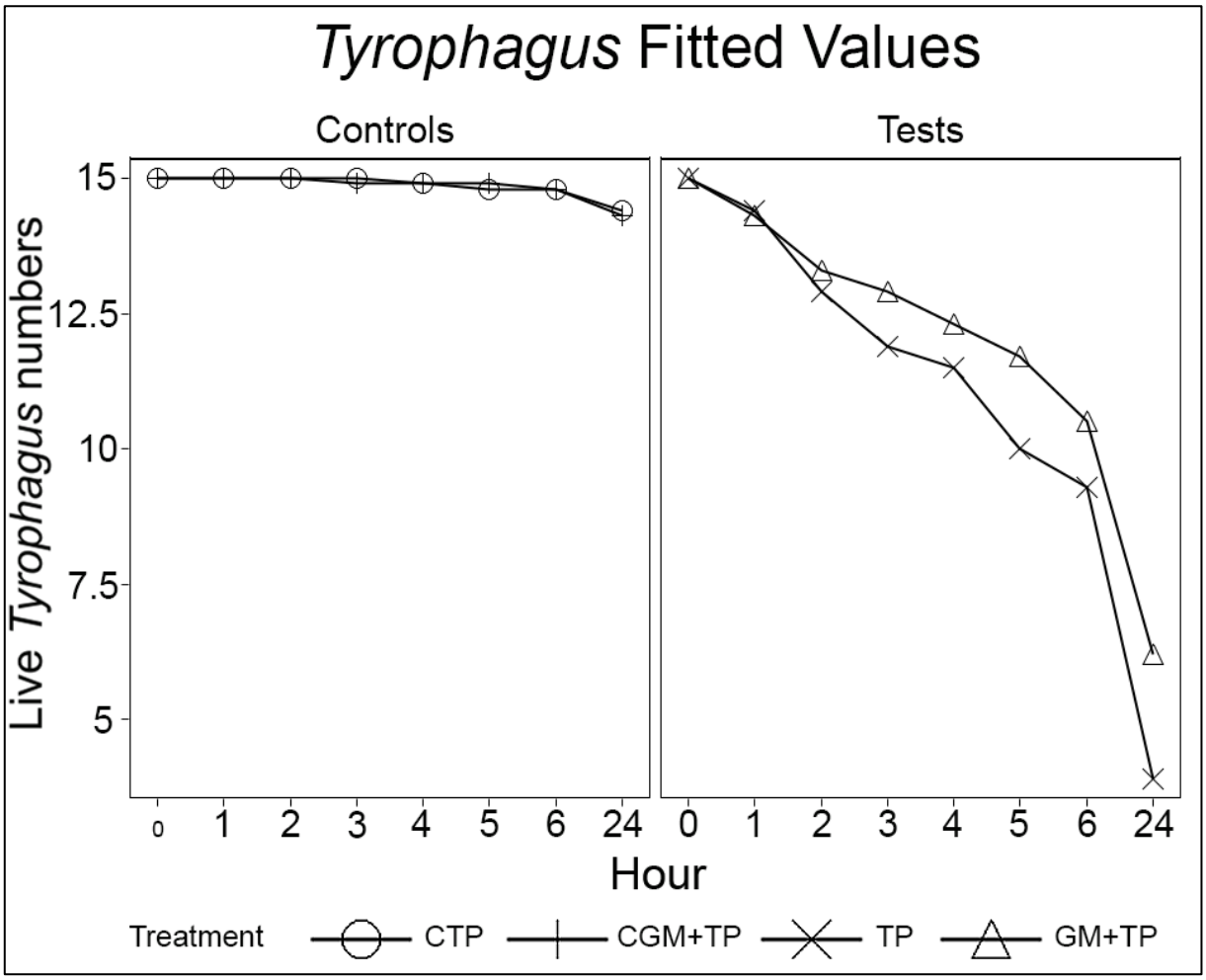

Figure K.1 – Fitted value figure showing predicted numbers of live *Tyrophagus putrescentiae* mites at each observation in all experimental Groups/Conditions which contained this prey variety, based on results of a GLM (Chapter 4).

Appendix L *Galleria mellonella* egg GLM parameter estimates and fitted values

Parameter				**Estimate**	**Standard Error**	**95% Confidence Limits**		**Z**	**Pr > |Z|**
Intercept				2.4248	0.1233	2.1832	2.6665	19.67	<.0001
Group	C			0.2071	0.1264	-0.0406	0.4548	1.64	0.1013
Group	T			0	0	0	0	.	.
Hour	0			0.2832	0.1233	0.0416	0.5249	2.3	0.0216
Hour	1			0.2766	0.1194	0.0425	0.5106	2.32	0.0205
Hour	2			0.2698	0.1153	0.0439	0.4958	2.34	0.0192
Hour	3			0.2562	0.1071	0.0463	0.4662	2.39	0.0168
Hour	4			0.2424	0.1034	0.0398	0.445	2.34	0.019
Hour	5			0.2424	0.1034	0.0398	0.445	2.34	0.019
Hour	6			0.2284	0.095	0.0423	0.4146	2.41	0.0162
Hour	24			0	0	0	0	.	.
Group*Hour	C	0		-0.2071	0.1264	-0.4548	0.0406	-1.64	0.1013
Group*Hour	C	1		-0.2004	0.1226	-0.4407	0.0399	-1.63	0.1021
Group*Hour	C	2		-0.1937	0.1186	-0.4261	0.0387	-1.63	0.1024
Group*Hour	C	3		-0.1801	0.1107	-0.397	0.0368	-1.63	0.1037
Group*Hour	C	4		-0.1933	0.1058	-0.4007	0.0142	-1.83	0.0678
Group*Hour	C	5		-0.2071	0.1055	-0.4138	-0.0004	-1.96	0.0496
Group*Hour	C	6		-0.2142	0.0959	-0.4022	-0.0261	-2.23	0.0256
Group*Hour	C	24		0	0	0	0	.	.
Group*Hour	T	0		0	0	0	0	.	.
Group*Hour	T	1		0	0	0	0	.	.
Group*Hour	T	2		0	0	0	0	.	.
Group*Hour	T	3		0	0	0	0	.	.
Group*Hour	T	4		0	0	0	0	.	.
Group*Hour	T	5		0	0	0	0	.	.
Group*Hour	T	6		0	0	0	0	.	.
Group*Hour	T	24		0	0	0	0	.	.
Condition(Group)	CWM	C		0.0284	0.0356	-0.0413	0.0981	0.8	0.4251
Condition(Group)	CWM+TR	C		0	0	0	0	.	.
Condition(Group)	WM	T		0	0.1632	-0.3199	0.3199	0	1
Condition(Group)	WM+TR	T		0	0	0	0	.	.
Condition*Hour(Group)	CWM	0	C	-0.0284	0.0356	-0.0981	0.0413	-0.8	0.4251
Condition*Hour(Group)	CWM	1	C	-0.0284	0.0356	-0.0981	0.0413	-0.8	0.4251
Condition*Hour(Group)	CWM	2	C	-0.0284	0.0356	-0.0981	0.0413	-0.8	0.4251
Condition*Hour(Group)	CWM	3	C	-0.0284	0.0356	-0.0981	0.0413	-0.8	0.4251
Condition*Hour(Group)	CWM	4	C	-0.0013	0.0318	-0.0636	0.0609	-0.04	0.9663
Condition*Hour(Group)	CWM	5	C	0.0125	0.0305	-0.0472	0.0721	0.41	0.6826
Condition*Hour(Group)	CWM	6	C	0.0268	0.0224	-0.0172	0.0708	1.19	0.2322

Parameter				**Estimate**	**Standard Error**	**95% Confidence Limits**		**Z**	**Pr > |Z|**
Condition*Hour(Group)	CWM	24	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	0	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	1	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	2	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	3	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	4	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	5	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	6	C	0	0	0	0	.	.
Condition*Hour(Group)	CWM+TR	24	C	0	0	0	0	.	.
Condition*Hour(Group)	WM	0	T	0	0.1632	-0.3199	0.3199	0	1
Condition*Hour(Group)	WM	1	T	0.0067	0.1603	-0.3075	0.3209	0.04	0.9667
Condition*Hour(Group)	WM	2	T	-0.0205	0.1467	-0.3079	0.267	-0.14	0.8889
Condition*Hour(Group)	WM	3	T	-0.042	0.1326	-0.3018	0.2179	-0.32	0.7516
Condition*Hour(Group)	WM	4	T	-0.0211	0.1328	-0.2814	0.2393	-0.16	0.8741
Condition*Hour(Group)	WM	5	T	-0.0282	0.131	-0.2849	0.2286	-0.22	0.8297
Condition*Hour(Group)	WM	6	T	-0.0286	0.1239	-0.2715	0.2144	-0.23	0.8177
Condition*Hour(Group)	WM	24	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	0	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	1	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	2	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	3	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	4	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	5	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	6	T	0	0	0	0	.	.
Condition*Hour(Group)	WM+TR	24	T	0	0	0	0	.	.

Table L.1 – Analysis of GEE parameter estimates for GLM examining effects of Group, Condition, Hour and various interactions of these factors on numbers of live *Galleria mellonella* eggs during Chapter 4 predation experiment. Significant results ($p < 0.05$) are underlined.

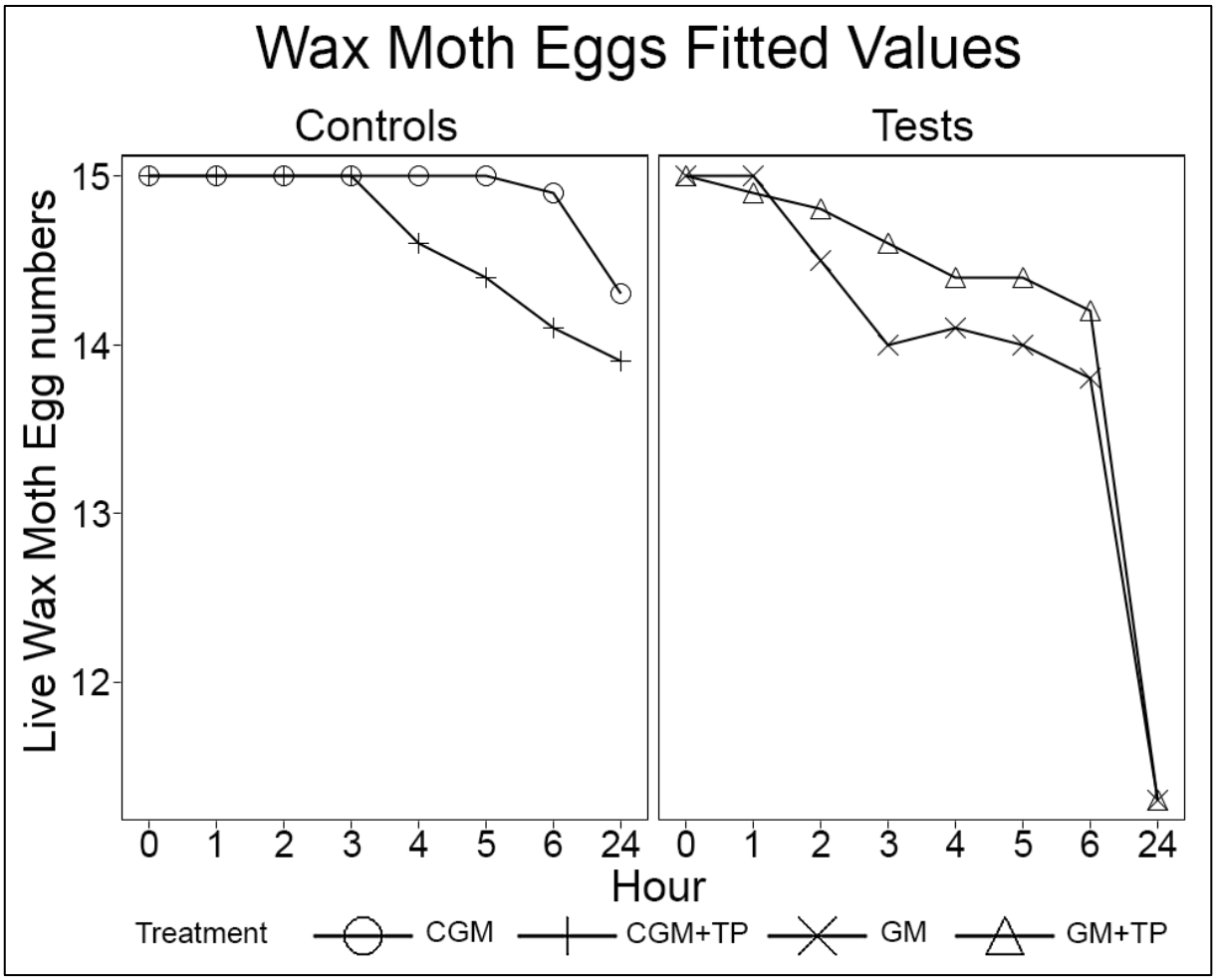

Figure L.1 – Fitted value figure showing predicted numbers of live *Galleria mellonella* eggs at each observation in all experimental Groups/Conditions which contained this prey variety, based on results of a GLM (Chapter 4).

Appendix M *Sphaerularia bombi* predation test

M.1 Introduction

The predation test outlined herein was conceived in order to determine whether it was possible for *Parasitellus fucorum* deutonymphs too predate mobile phase *Sphaerularia bombi* nematodes in controlled conditions, in order to shed light on the theory proposed by some researchers that phoretic *P. fucorum* provide some form of protection against the infestation of bumblebee queens during hibernation by *S. bombi* (Eickwort, 1994, Schmid-Hempel, 1998). This idea is based on a negative correlation between phoretic *Parasitellus* numbers and *S. bombi* infestation first shown by (Schousboe, 1987). Logically the only way phoretic mites could protect queens from being infested would be to predate these nematodes in their mobile instar in the soil of the hibernaculum before they invade the queen's abdomen (Alford, 1975). Therefore a test was designed to expose mobile *S. bombi* to *Parasitellus fucorum* deutonymphs, as shown below.

M.2 Methods

M.2.1 Nematode counting and dosage preparation

Once a sample of mobile *Sphaerularia bombi* suspended in water had been acquired (Main paper methods) the density and number of nematodes in the sample had to be estimated. To do this, the sample was first agitated thoroughly to evenly suspend the nematodes. Immediately after this 2ml of the sample was pipetted into a Nematode Counting Slide (Chalex LLC, USA). All nematodes present in the counting area of this slide were tallied by examining the slide using a Motic SMZ171 microscope with 1-5x objective lenses, and the number of nematodes counted was recorded as the average number of nematodes per ml of sample.

To prepare a dosage of ~20 nematodes in 200µl water for experimental use, the counting process was conducted 5 times and an overall average count taken, in order to lessen the effect of human variance in the counting process. This overall average was used to calculate the necessary dilution required to reach a concentration of 100 nematodes/ml, and a 5ml sample was then prepared to this specification for immediate experimental use.

M.2.2 *Parasitellus* predation trial

To test whether *Parasitellus fucorum* deutonymphs could predate 3rd instar *Sphaerularia bombi* nematodes, doses of ~20 nematodes in 200µl water were pipetted into test chambers consisting of a 50ml tissue culture flask with vented caps (Greiner Bio-One Ltd., UK)containing a 6cm x 3cm rectangle of black grade 918 filter paper (Camlab, UK). Black filter paper was used to absorb and retain the water used to transfer the mites, and to aid in counting the white nematodes by making them more visible. The nematode 'doses' were pipetted directly onto the centre of the filter papers. The control group were left unchanged after nematode addition, whereas the test group had 4 *P. fucorum* deutonymphs transferred into the chamber immediately afterwards. After this all test chambers were closed and left undisturbed in a controlled environment maintained at 21°C/80%rh with no lighting, except during survival counts.

Survival counts were conducted at the following times after colony closure; 2 hours, 4 hours, 6 hours, 24 hours and 48 hours. Survival counts were conducted by closely examining each test chamber using a Motic SMZ171 microscope with 1-5x objective lenses, moving the area of observation slowly along the entire filter paper area and its surroundings to ensure that no nematodes or mites were left uncounted. Once the count had been completed the test chamber was returned to the controlled environment. After the final survival count had been conducted, the test chamber was filled with 20mls 70% ethanol and agitated to suspend all organisms inside in the ethanol. Nematodes and mites were pipetted into separate Eppendorf tubes for (respectively) storage or species-level identification.

M.3 Results

Test chambers which were inoculated with *P. fucorum* had a higher median final number of dead nematodes (7.5 ± 6.25) (median ± Inter-Quartile Range (IQR)) than test chambers from the control group (5.5 ± 1.25). This was reflected in the numbers of dead nematodes present in both treatments groups during the final survival counts at 48 hours (Figure L.1). Dead nematode counts were used instead of live counts due to variation in the starting numbers of live nematodes between colonies. After Kolmogorov-Smirnov testing and a Bartlett's test showed that the data were normal and of equal variation, a t-test was used to compare the numbers of dead nematodes found in both treatment groups throughout the experimental period. This returned a p-value of 0.24, showing that significant differences did not exist between in the numbers of dead nematodes found in either treatment group. When the results over time were plotted against each other it was apparent that while the results varied greatly, the overall trend of increasing numbers of dead nematodes over time (shown by the trendlines plotted over each dataset) occurred at very similar rates in both treatment groups (Figure L.2).

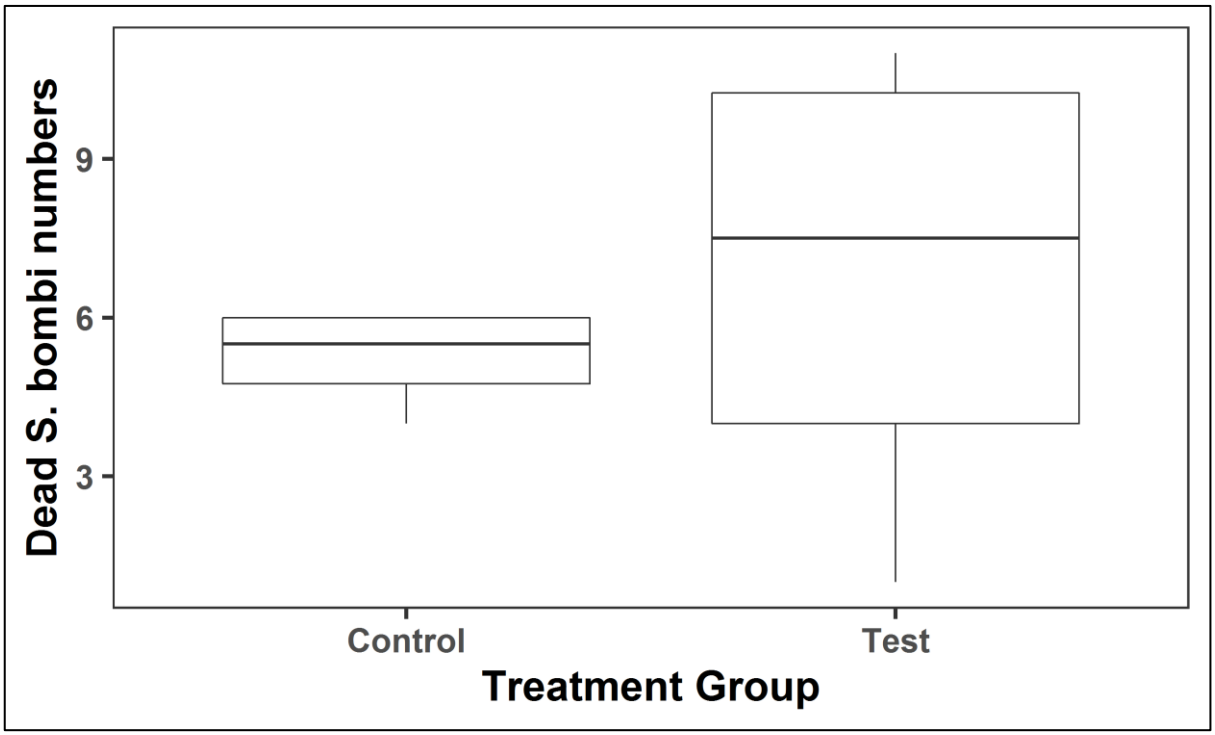

Figure M.1 – Final numbers of dead *Sphaerularia bombi* nematodes observed in colonies from the treatment group inoculated with 4 *Parasitellus fucorum* deutonymphs (Test) and the group with no added mites (Control). Thickened horizontal line shows the median value, two narrower horizontal line show the 1st and 3rd quartiles, while vertical 'whiskers' extend to the 5th and 95th percentiles.

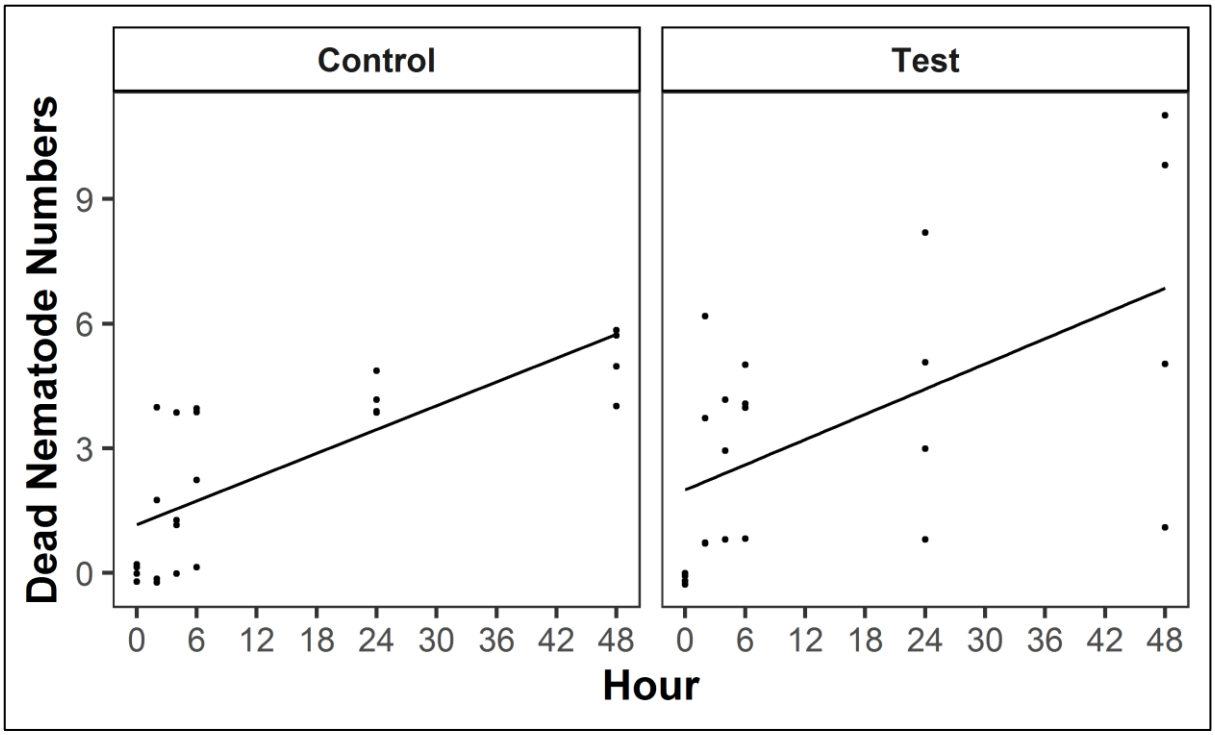

Figure M.2 – Observed numbers of dead *Sphaerularia bombi* nematodes observed during each survival count of each colony, comparing Test (*Parasitellus fucorum* deutonymphs added) and Control (no added mites) groups. Trendlines were added using a linear model to show the overall trend in each dataset. Results are slightly jittered vertically to reduce overplotting.

M.4 Discussion

The predation trial proved unsuccessful due a combination of several unforeseen factors. The water used to transfer the *Sphaerularia bombi* nematodes onto the black filter paper was essential to prevent the

nematodes drying out and dying, and the filter paper was required so that the nematodes were not simply contained in water droplets (and therefore completely inaccessible for the *Parasitellus fucorum* deutonymphs). However this resulted in condensation forming on all interior surfaces of the test chambers, which made observing the nematodes impossible and formed small water droplets into which some nematodes escaped, making nematode survival counts from then onwards effectively meaningless. A similar issue occurred when it was observed that nematodes from the 24 hour mark onwards often manoeuvred themselves beneath the filter paper, again making them inaccessible to the mites and invalidating survival counts. Other substrates besides black filter paper were trialled to try and resolve these issues (sand, vermiculite, etc.), but none solved the issue of internal condensation, and all increased the likelihood of miscounting the number of live nematodes as many would hide beneath the substrate in these trials. Therefore the predation trial was ended after 4 replicates, and the results (which appeared to lean towards no significant differences between the test and control groups in any case, supported by the fact that no interactions between the mites and the nematodes were observed) were not included in the main paper.

Appendix N Supplementary data for Chapter 5

N.1 ROC curve

The ROC curve produced using the binomial regression model in the main paper and the Rstudio package ROCR are shown below. The greater the area 'under the curve', the more appropriately fitted the model is likely to be.

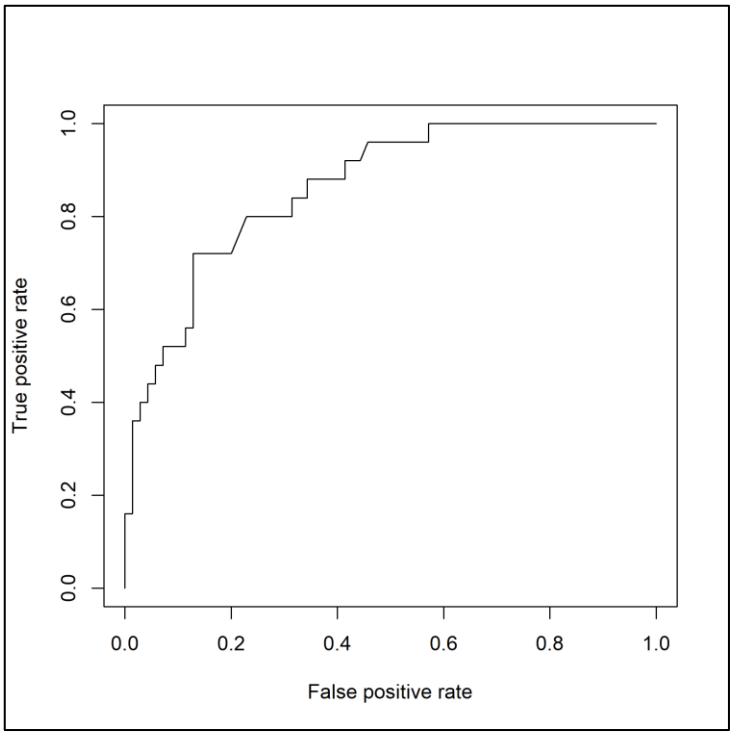

Figure N.1 – ROC curve for binomial logistic regression model.

N.2 Mean phoretic mites stratified by bumblebee species and nematode infestation

Bumblebee species	***S. bombi***	**n**	**Mean *Parasitellus***	**St. Dev.**	**p-value**	**Mean other Mesostigmata**	**St. Dev.**	**p-value**
B. lapidarius	Yes	1	0	NA		0	0	
B. lapidarius	No	2	9	8.485		0	0	
B. lucorum	Yes	2	15.5	3.535		0	0	
B. lucorum	No	8	1.125	2.475		0.25	0.463	
B. pascuorum	Yes	1	0	NA	0.55	0	NA	0.71
B. pascuorum	No	1	0	NA		0	NA	
B. ruderatus	Yes	1	0	NA		0	NA	
B. ruderatus	No	4	1	2		1.25	1.893	
B. terrestris	Yes	20	4	8.522		0.4	0.995	
B. terrestris	No	32	4.438	6.677		0.25	0.622	

Table N.1 – Mean phoretic mites (of the genus *Parasitellus* or all other mites of the order Mesostigmata) on bumblebee queens stratified by species and nematode infestation. Number of queens captured within each category shown under n. Standard deviation (St. Dev.) of all mean results is also given, and p-values shown are results of Wilcoxon rank sum tests.

Appendix O Chapter 6 supplementary data

O.1 Pairwise Wilcoxon test results

	Control	***Parasitellus***	***Tyrophagus***	***Parasitellus* + *Tyrophagus***
Control	-	0.025	0.201	0.666
Parasitellus	0.025	-	0.227	0.094
Tyrophagus	0.201	0.227	-	0.21
Parasitellus* + *Tyrophagus	0.666	0.094	0.21	-

Table O.1 – Results of pairwise Wilcoxon rank sum tests comparing the overall weight changes in bumblebee colonies belonging to each treatment group tested. Significant (< 0.05) p-values are underlined.

	Control	***Parasitellus***	***Tyrophagus***	***Parasitellus* + *Tyrophagus***
Control	-	0.863	0.011	1
Parasitellus	0.863	-	0.579	1
Tyrophagus	0.011	0.579	-	0.2
Parasitellus* + *Tyrophagus	1	1	0.2	-

Table O.2 – Results of pairwise Wilcoxon rank sum tests comparing total numbers of bumblebees removed from commercial colonies at the end of the experimental period, compared by treatment group. Significant (< 0.05) p-values are underlined.

	Control	***Parasitellus***	***Tyrophagus***	***Parasitellus* + *Tyrophagus***
Control	-	0.619	0.014	0.802
Parasitellus	0.619	-	0.802	0.802
Tyrophagus	0.014	0.802	-	0.510
Parasitellus* + *Tyrophagus	0.802	0.802	0.510	-

Table O.3 – Results of pairwise Wilcoxon rank sum tests comparing number of bumblebee workers removed from colonies belonging to each treatment group. Significant (< 0.05) p-values are underlined.

O.2 Additional summary statistics

Treatment group	Bumblebee caste	Mean weight (g)	Standard deviation	p-value
Control	Worker	0.224	0.058	0.55
Parasitellus	Worker	0.193	0.03	
Tyrophagus	Worker	0.198	0.02	
Parasitellus + *Tyrophagus*	Worker	0.206	0.032	
Control	Drone	0.281	0.026	0.13
Parasitellus	Drone	0.285	0.056	
Tyrophagus	Drone	0.306	0.036	
Parasitellus + *Tyrophagus*	Drone	0.26	0.046	
Control	Queen	0.811	0.11	0.596
Parasitellus	Queen	0.881	0.299	
Tyrophagus	Queen	0.735	0.192	
Parasitellus + *Tyrophagus*	Queen	0.714	0.152	

Table O.4 – Mean weights (and standard deviation) of bumblebees collected from colonies at the end of the experimental period, split by bumblebee caste and treatment group. Results of Kruskal-Wallis tests comparing caste results between treatment groups also shown. Significant (< 0.05) p-values are underlined.

Treatment group	Caste	n	Mean P	SD (P)	Mean M	SD (M)	Mean K	SD (K)
C	Worker	176	0.074	0.355	0.051	0.245	0.591	1.524
C	Queen	6	6	9.317	1.833	2.041	5.000	4.195
C	Drone	62	0.081	0.275	0.081	0.329	0.629	2.966
P	Worker	290	0.041	0.232	0.031	0.193	0.293	1.194
P	Queen	5	5.4	5.177	0.200	0.447	90	174.093
P	Drone	94	0.021	0.145	0.011	0.103	0.723	3.056
PT	Worker	291	0.134	0.545	0.010	0.131	0.186	2.005
PT	Queen	112	2	4.472	0.000	0.000	7.4	15.453
PT	Drone	5	0.196	0.499	0.009	0.094	0.143	1.512
T	Worker	364	0.066	0.269	0.011	0.104	0.252	0.765
T	Queen	8	2.250	2.866	0	0	3.875	6.266
T	Drone	207	0.058	0.234	0.014	0.120	1.797	14.551

Table O.5 – Mean numbers (and standard deviations) of phoretic mites from three morphological groupings (P – *Parasitellus*, M – other Mesostigmata, K – *Kuzinia*) found present upon bumblebees removed from experimental colonies within different treatment groups, separated by bumblebee caste. n-number refers to number of bumblebees of each caste caught in total from all 9 experimental colonies within each treatment group.

Treatment Group (TG)	Morphological Group (MG)	Mite species/group	n	% MG	% TG
C	K	*Kuzinia*	33	100	29
C	M	*Pneumolaelaps*	23	100	21
C	P	*Parasitellus fucorum*	53	95	47
C	P	*Parasitellus ignotus*	2	4	2
C	P	*Parasitellus talparum*	1	2	1
P	K	*Kuzinia*	67	100	55
P	M	*Pneumolaelaps*	12	100	10
P	P	*Parasitellus fucorum*	41	98	34
P	P	*Parasitellus talparum*	1	2	1
PT	K	*Kuzinia*	15	100	15
PT	M	*Pneumolaelaps*	9	60	9
PT	M	Unknown	6	40	6
PT	P	*Parasitellus fucorum*	71	100	70
T	K	*Kuzinia*	42	100	41
T	M	*Pneumolaelaps*	7	100	7
T	P	*Parasitellus fucorum*	54	100	52

Table O.6 – Aggregated mite identification results for phoretic mites removed from all bumblebees removed from experimental colonies. Results are split by different species/groups of mites identified, as well as mite morphological groupings and colony treatment groups. n-number shown refers to number of mites directly identified, not including some *Kuzinia* mites which were identified visually. % MG column shows the percentage each mite species represented within own morphological grouping and treatment group (e.g. % of 'P' group within treatment group 'C' that were *Parasitellus fucorum*). % TG column shows percentage each mite species represented of all identified mites from that treatment group (e.g. % of identified mites from treatment group 'C' that were *Parasitellus fucorum*).

O.3 Combined colony weight graph

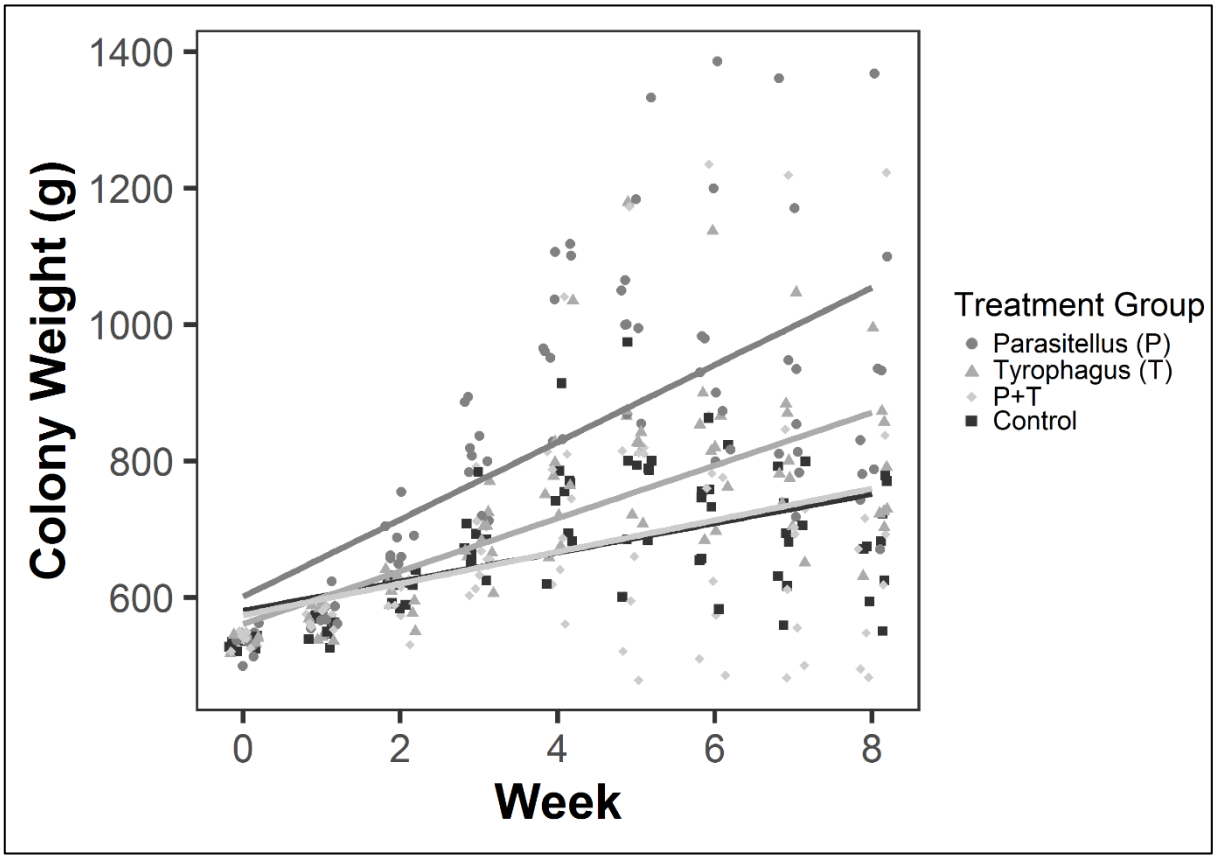

Figure O.1 – Bumblebee colony weight measurements taken weekly from the beginning of the experiment until the end. Each data point represents a measurement from a single colony. Data points are slightly jittered to reduce overplotting. A trendline has been added to each treatment group, calculated using a linear model fitted to the data. Factors in the legend are listed in the same order as the trendlines at week 8 (i.e. *Parasitellus* – top, Control – bottom)

Appendix P Colony weight GLM supplements

P.1 Parameter estimates

Parameter			**Estimate**	**Standard Error**	**95% Confidence Limits**		**Z**	**Pr > |Z|**
Intercept			6.513	0.0581	6.399	6.6269	112.01	**<.0001**
Treatment Group	C		-0.0952	0.0558	-0.2047	0.0142	-1.71	0.0882
Treatment Group	P		0.1799	0.0735	0.036	0.3239	2.45	**0.0143**
Treatment Group	PT		-0.1039	0.0893	-0.2789	0.0711	-1.16	0.2447
Treatment Group	T		0	0	0	0	.	.
Week	0		-0.2247	0.0582	-0.3388	-0.1106	-3.86	**0.0001**
Week	1		-0.3323	0.0446	-0.4196	-0.2449	-7.46	**<.0001**
Week	2		-0.3258	0.0401	-0.4043	-0.2472	-8.13	**<.0001**
Week	3		-0.315	0.0502	-0.4134	-0.2166	-6.27	**<.0001**
Week	4		-0.1688	0.0526	-0.2718	-0.0658	-3.21	**0.0013**
Week	5		0.0419	0.0374	-0.0314	0.1151	1.12	0.2624
Week	6		-0.0556	0.0417	-0.1374	0.0261	-1.33	0.1821
Week	7		-0.0341	0.0245	-0.0822	0.014	-1.39	0.1645
Week	8		0	0	0	0	.	.
Week*Treatment Group	0	C	0.088	0.0565	-0.0228	0.1987	1.56	0.1194
Week*Treatment Group	0	P	-0.1891	0.0695	-0.3253	-0.0528	-2.72	**0.0065**
Week*Treatment Group	0	PT	0.1031	0.0895	-0.0725	0.2786	1.15	0.2498
Week*Treatment Group	0	T	0	0	0	0	.	.
Week*Treatment Group	1	C	0.1004	0.0571	-0.0115	0.2123	1.76	0.0786
Week*Treatment Group	1	P	-0.1812	0.0721	-0.3224	-0.0399	-2.51	**0.0119**
Week*Treatment Group	1	PT	0.1245	0.094	-0.0596	0.3087	1.33	0.185
Week*Treatment Group	1	T	0	0	0	0	.	.
Week*Treatment Group	2	C	0.0671	0.0577	-0.046	0.1802	1.16	0.2451
Week*Treatment Group	2	P	-0.1922	0.0814	-0.3518	-0.0327	-2.36	**0.0182**
Week*Treatment Group	2	PT	0.0815	0.0878	-0.0906	0.2537	0.93	0.3531
Week*Treatment Group	2	T	0	0	0	0	.	.
Week*Treatment Group	3	C	0.0837	0.0486	-0.0115	0.179	1.72	0.085
Week*Treatment Group	3	P	-0.1178	0.0764	-0.2676	0.0321	-1.54	0.1234
Week*Treatment Group	3	PT	0.123	0.0882	-0.0499	0.2958	1.39	0.1632
Week*Treatment Group	3	T	0	0	0	0	.	.

Week*Treatment Group	4	C	0.1124	0.0485	0.0173	0.2076	2.32	**0.0206**
Week*Treatment Group	4	P	0.1255	0.0822	-0.0357	0.2867	1.53	0.127
Week*Treatment Group	4	PT	0.0805	0.0627	-0.0424	0.2034	1.28	0.1994
Week*Treatment Group	4	T	0	0	0	0	.	.
Week*Treatment Group	5	C	-0.042	0.0469	-0.134	0.05	-0.9	0.3704
Week*Treatment Group	5	P	0.0167	0.0533	-0.0877	0.1212	0.31	0.7536
Week*Treatment Group	5	PT	-0.0273	0.0435	-0.1127	0.058	-0.63	0.53
Week*Treatment Group	5	T	0	0	0	0	.	.
Week*Treatment Group	6	C	0.0088	0.0309	-0.0517	0.0694	0.29	0.7755
Week*Treatment Group	6	P	0.0081	0.0394	-0.0691	0.0854	0.21	0.8364
Week*Treatment Group	6	PT	-0.0059	0.0364	-0.0771	0.0654	-0.16	0.8721
Week*Treatment Group	6	T	0	0	0	0	.	.
Week*Treatment Group	7	C	-0.0393	0.025	-0.0884	0.0098	-1.57	0.1165
Week*Treatment Group	7	P	0	0.0204	-0.04	0.0399	0	0.9987
Week*Treatment Group	7	PT	-0.0381	0.0265	-0.09	0.0137	-1.44	0.1495
Week*Treatment Group	7	T	0	0	0	0	.	.
Week*Treatment Group	8	C	0	0	0	0	.	.
Week*Treatment Group	8	P	0	0	0	0	.	.
Week*Treatment Group	8	PT	0	0	0	0	.	.
Week*Treatment Group	8	T	0	0	0	0	.	.
Syrup Consumed			0.001	0.0002	0.0006	0.0015	4.49	**<.0001**

Table P.1 – Analysis of parameter estimates for each parameter combination. Significant (< 0.05) p-values are shown in bold. p-values were calculated using the chi-square method.

Appendix Q Bumblebee worker numbers GLM supplements

Q.1.1 Parameter estimates

Parameter		**Estimate**	**Standard Error**	**95% Confidence Limits**		**Z**	**Pr > |Z|**
Intercept		3.1407	0.0977	2.9493	3.3321	32.16	**<.0001**
Treatment Group	C	-0.4954	0.1638	-0.8163	-0.1744	-3.02	**0.0025**
Treatment Group	P	-0.6485	0.1393	-0.9216	-0.3755	-4.66	**<.0001**
Treatment Group	PT	-0.1624	0.131	-0.4191	0.0943	-1.24	0.2149
Treatment Group	T	0	0	0	0	.	.
Overall Weight Change		0.0024	0.0003	0.0018	0.0029	7.94	**<.0001**
Drones		-0.0012	0.0029	-0.0068	0.0044	-0.42	0.6712
Queens		-0.0118	0.049	-0.1078	0.0842	-0.24	0.8095

Table Q.1 – Analysis of parameter estimates for each parameter combination. Significant (< 0.05) p-values are shown in bold. p-values were calculated using the chi-square method.

Q.1.2 Least Square Means results

Treatment Group	**Estimate**	**Standard Error**	**z Value**	**Pr > |z|**	**Lower**	**Upper**	**Exponentiated**	**Exponentiated Lower**	**Exponentiated Upper**
Control	3.162	0.1456	21.72	**<.0001**	2.8767	3.4474	23.619	17.7563	31.4174
Parasitellus (P)	3.0089	0.1212	24.83	**<.0001**	2.7714	3.2464	20.2653	15.9813	25.6978
P+T	3.495	0.1148	30.44	**<.0001**	3.2699	3.72	32.9497	26.3097	41.2656
Tyrophagus (T)	3.6574	0.07765	47.1	**<.0001**	3.5052	3.8096	38.7612	33.289	45.1329

Table Q.2 – Full Least Square Means outputs produced using GLM/GEE predicting number of bumblebee workers based on treatment group, overall colony weight change (g), and the numbers of drones and queens collected from colonies. p-values calculated based on the test statistic using the chi-square method. Significant results shown in bold.