The influence of co-infections on the reservoir competence of peridomestic rodents for tick-borne pathogens

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Declaration on plagiarism:

I hereby confirm that I have independently composed this thesis and that no other than the indicated aid and sources have been used. This work has not been presented to any other examination board.

Berlin, 15th of May 2018

Denny Maaz

List of publications

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Manuscript	90	90	60

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List of abbreviations

Ag	Antigen
AIC	Akaike information criterion
ANOVA	Analysis of variance
B.a.	*Borrelia afzelii*
BALB/c	Albino laboratory-bred strain of *Mus musculus*
bp	Base pair
BSA	Bovine serum albumin
C3H	laboratory-bred strain of *Mus musculus*
C57BL/6	“C57 black 6“, laboratory-bred strain of *Mus musculus*
CD3	Cluster of differentiation 3
CD4	Cluster of differentiation 4
CD28	Cluster of differentiation 28
CI	Confidence interval
CO2	Carbon dioxide
DEBONEL	*Dermacentor*-borne necrosis erythema and lymphadenopathy
DNA	Desoxyribonucleic acid
dsDNA	Double-stranded DNA
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
et al.	*Et alii* (and others)
FACS	Fluorescence-activated cell sorting
GATA-3	Transcription factor encoded by the GATA3 gene
hbb	Gene encoding for the histone-like protein HBb
HGA	Human granulocytic anaplasmosis
H.p.	*Heligmosomoides polygyrus*
HRM	High resolution melting curve analysis
IFN-γ	Gamma interferon
IgG	Immunoglobulin G
IgE	Immunoglobulin E
IL-2	Interleukin 2
IL-10	Interleukin 10
IL-13	Interleukin 13
IL-17	Interleukin 17
i.e.	*Id est* (that means)

I.r.	*Ixodes ricinus*
L1	First-stage larvae
L2	Second-stage larvae
L3	Third-stage larvae
L4	Fourth-stage larvae
LD	Lyme disease
MgCl2	Magnesium chloride
MLN	Mesenteric lymph nodes
Mya	Million years
n	Sample size
NMDS	Non-metric multidimensional scaling
ns	Not significant
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PE	Phycoerythrin
p.i.	*Post infectionem* (after infection)
PMA	Phorbol myristate acetate
qPCR	Quantitative (real-time) polymerase chain reaction
RPMI	Roswell Park Memorial Institute medium
rDNA	Ribosomal Desoxyribonucleic acid
RNA	Ribonucleic acid
rRNA	Ribosomal Ribonucleic acid
s.l.	*Sensu lato* (in the broad sense)
SD	Standard deviation
SEM	Standard error of the mean
SENLAT	Scalp eschar and neck lymph adenopathy after tick bite
SLN	Skin-draining lymph nodes
sp.	*Species*
SPF	Specific pathogen-free
spp.	*Species pluralis*
TBE	Tick-borne encephalitis
Th1	Type 1 T helper cells or cell responses
Th2	Type 2 T helper cells or cell responses
TIBOLA	Tick-borne lymphadenopathy
Tregs	Regulatory T cells

Chapter 1

General introduction

1 General introduction

1.1 *Ixodes ricinus* ticks and tick-borne pathogens

Ticks are of great medical and veterinary importance as vectors of pathogens. The most common tick species in Europe is the hard tick *Ixodes ricinus*, which is by far the most important arthropod vector for vector-borne pathogens in Central Europe. Humans and companion animals are mainly infested by the nymphal and adult life stages, while the larvae predominantly feed on small mammals. Rodents and insectivores are not only important for the life cycle of *I. ricinus*, they are also reservoir hosts for the causative agents of a number of zoonotic tick-borne pathogens transmitted by *I. ricinus* in Europe, including the Tick-borne encephalitis virus, Louping ill virus, *Borrelia burgdorferi* sensu lato (s.l.), relapsing fever *Borrelia*, *Francisella tularensis*, zoonotic *Babesia* spp. and probably *Candidatus* Neoehrlichia mikurensis and *Anaplasma phagocytophilum* (Burri *et al.*, 2014; Durden, 2006). Reservoir competence of the host is thereby characterised by (1) the ability to become infected with a pathogen by the bite of a vector tick, (2) stay infected at least for a considerable time span and (3) the ability to infect another vector tick during feeding (Richter *et al.*, 2000). Accordingly, recent studies showed that rodents are probably no reservoirs for *Rickettsia helvetica* and *Rickettsia monacensis* as suggested (Burri *et al.*, 2014).

Tick-borne encephalitis (TBE) is caused by a same-named single-stranded RNA virus from the family Flaviviridae. It occurs in Europe and Asia and the principal vectors are *I. ricinus* and the Asian tick *Ixodes persulcatus* (Mansfield *et al.*, 2009). In 2017, 505 cases were reported from patients in Germany (SurvStat@RKI_2.0, 2018). Tick-borne encephalitis is mostly biphasic with a short febrile period including symptoms such as fatigue, pain in neck and shoulders, headache as well as particularly vomiting and high fever. This is often (45-56%) followed by the second phase including the central nervous system (meningitis, encephalitis, myelitis, radiculitis) with a considerable mortality rate (Mansfield *et al.*, 2009).

The Louping ill virus is another flavivirid virus which causes louping ill in sheep and red grouse in the UK and occasionally encephalo-myelitis in humans, although most cases have been acquired in the laboratory (Mansfield *et al.*, 2009). It is transmitted by *I. ricinus*.

Lyme disease is the most common human tick-borne disease in temperate parts of the northern hemisphere and can be transmitted by a number of *Ixodes* spp., including *I. ricinus* (Stanek *et al.*, 2012) (Figure 1-1). In Germany, 7799 human cases were reported from nine federal states in 2017

(SurvStat@RKI_2.0, 2018). But also many other mammals can become infected. It is caused by different spirochete species of the *Borrelia burgdorferi* s.l. complex. The clinical symptoms of many human patients include erythema migrans at the site of attachment, but further, more severe manifestations can occur in skin (acrodermatitis chronica atrophicans, Borrelial lymphocytoma), nervous system (neuroborreliosis), joints (arthritis) or heart, which are often specific to particular spirochete genospecies (Stanek *et al.*, 2012).

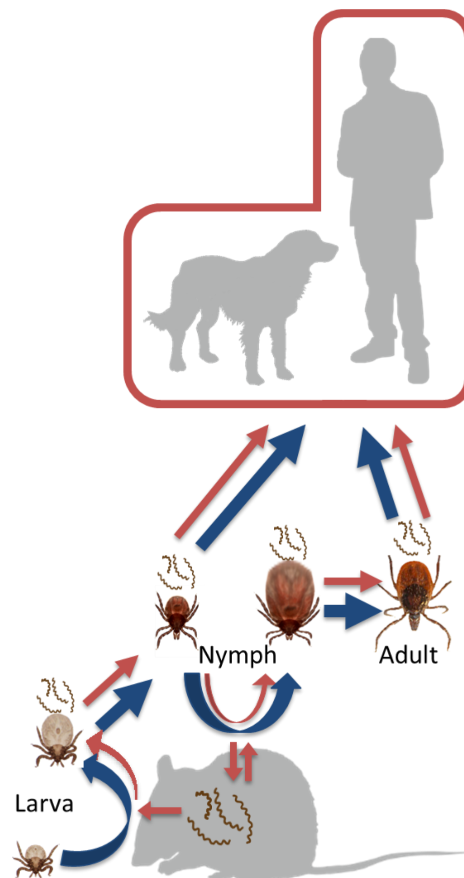

Figure 1-1. Transmission of Lyme disease spirochetes (red) from wild rodents to humans and companion animals during blood-meal of ticks (blue)

Tick-borne relapsing fever spirochetes are mainly transmitted by soft ticks. However, three species are transmitted by hard ticks and there are already case reports of *Borrelia myiamotoi* infections in humans from Russia, the USA, Japan and the Netherlands (Siński *et al.*, 2016). *Ixodes ricinus* is the main vector in Europe although it also occurs in other *Ixodes* spp. in Asia and North America. Transmission is horizontally from the rodent reservoirs to humans, but there is also transovarial (vertical) transmission within the tick population. It causes a nonspecific febrile illness with fever that may exceed 40 °C, as well as headache, fatigue, myalgia, chills, nausea and joint pain (Siński *et al.*, 2016).

Within the bacterial family Anaplasmataceae, *Ehrlichia*, *Anaplasma* and *Neoehrlichia* spp. are tick-borne pathogens. The pathogens *A. phagocytophilum*, *Ehrlichia muris* and *Candidatus* Neoehrlichia mikurensis are transmitted by *Ixodes* ticks. Numerous species of mammals including humans can be infected by *A. phagocytophilum* in the USA, Europe and Asia. Human infection with *A. phagocytophilum* causing human granulocytic anaplasmosis (HGA) is quite common in North America, but rare in Europe with about 60 reported cases (Rar & Golovljova, 2011). However, seroprevalence can be considerably high in Europe with 0-28%, particularly in tick-exposed risk groups (Strle, 2004). Infections in humans range from asymptomatic to mild febrile illness up to severe fever and often include myalgia and headache. In Europe, *A. phagocytophilum* is mainly of veterinary importance, since there are numereous cases of granulocytic anaplasmosis in sheep, goats, cattle ("tick-borne fever"), horses, dogs and cats. Apart from rodents, cervids and even sheep are considered reservoir hosts, at least for a considerable life span (Rar & Golovljova, 2011) but this is questioned by others for rodents (Obiegala *et al.*, 2014). *Ehrlichia muris* is a pathogen mainly of mice and voles and causes murine splenomegaly. *Ixodes ricinus* ticks are also the principal vector for the emerging *Candidatus* N. mikurensis (Rar & Golovljova, 2011). Currently, this pathogen was reported from at least 16 human cases in Europe and one from China. All but one patient were immune-compromised due to immuno-suppressive treatment or asplenic and quite old (Silaghi *et al.*, 2016a). The patients had severe symptoms and some of them had recurrent fever, arthralgia and edema. One patient died, while the others recovered after antibiotic treatment (Rar & Golovljova, 2011). However, also immune-competent people can be infected, but infections are asymptomatic or mild with fever, headache and malaise. This pathogen also caused illness in a dog after intense surgery and probably a puppy which died from haemolytic anaemia (Silaghi *et al.*, 2016a). The reservoir competence of rodents is currently not fully proven, since only one of three requirements of a reservoir host was confirmed using xenodiagnosis by Burri *et al.* (2014). However, potential transplacental transmission was observed in rodent hosts (Obiegala *et al.*, 2014).

The bacteria within the genus *Rickettsia* currently comprise 32 valid species (LPSN, 2018) and a number of further subspecies and uncultured strains (Parola *et al.*, 2013). At least five valid, human pathogenic species from the spotted-fever group, *Rickettsia helvetica* and three uncultured ("Candidatus") species of unknown pathogenity were detected in *I. ricinus* in Europe. *Rickettsia massiliae* and *Rickettsia aeschlimanni* causing Mediterranean spotted fever (MSF)-like symptoms, and *Rickettsia raoultii*, causing scalp eschar and neck lymph adenopathy (SENLAT, former TIBOLA or DEBONEL) in humans, have been detected in *I. ricinus*. However, the principal vector ticks are most likely *Rhipicephalus*, *Hyalomma* and *Dermacentor* spp., respectively. In addition, *Rickettsia sibirica sibirica*, causing Siberian tick typhus in Asia, was once detected in *I. ricinus* larvae collected from

migratory birds, but the main vectors are *Dermacentor* spp.. *Rickettsia monacensis* causes fever and flu-like symptoms in patients and occurs in *I. ricinus*, but is probably associated with lizards (Parola *et al.*, 2013). Only *Rickettsia helvetica* is mainly transmitted by *I. ricinus* ticks and was sometimes detected in rodents (Schex *et al.*, 2010; Sprong *et al.*, 2009). There are only few cases of *R. helvetica* in patients, which had mild illness with headache, myalgias and occasionally eschar and rash at the attachment site. However, wild mice and voles with attached tick larvae positive for *R. helvetica* did not reveal infection in the blood and were unable to infect xenodiagnotic ticks (Burri *et al.*, 2014). Since many *Rickettsia* spp., including *R. helvetica*, are vertically transmitted within invertebrate populations, ticks themselves may act as natural reservoirs (Sprong *et al.*, 2009).

The bacterium *Francisella tularensis* is the causative agent of tularemia in North America, Eurasia and Australia (Gunnell *et al.*, 2016). It is classified into four subspecies and only *F. tularensis holarctica* (or "Type B") causes the disease in Europe (Maurin & Gyuranecz, 2016). Two transmission cycles were observed. The predominant terrestrial cycle includes rodents and ticks, whereby *Dermacentor reticulatus* and *I. ricinus* are the most commonly infected. The aquatic cycle occurs mainly in South-East Europe and Scandinavia and includes mosquitos and their larvae as well as lagomorphs and semiaquatic rodents. Wild lagomorphs (*Lepus* spp.) and rodents are considered as main reservoirs although infections in these animals are often fatal. Disease in humans is most often associated with rats, mice, voles, lemmings and beavers. Transmission occurs via direct contact with these animals, bites of ticks and mosquitoes or by drinking of contaminated water (Maurin & Gyuranecz, 2016). In Germany, 56 cases were reported in 2017, which is the highest number ever since it is reported (2001) (SurvStat@RKI_2.0, 2018). Here, consumption or contact with hares is the most frequent cause but also tick-borne tularemia was reported (Boone *et al.*, 2015). Human patients in Europe often reveal influenza-like symptoms, but six different forms are common. The form of the disease that develops depends on the route of infection. Arthropod-transmitted tularemia causes the ulceroglandular and glandular forms, which are the most common in Europe. These are characterised by lymphadenopathy near the attachment site (glandular) and/or a skin inoculation ulcer (ulceroglandular). In animals, tularemia can be severe in highly susceptible species such as house mice, including sepsis and hepato- and splenomegaly, and subacute in hares, which develop granulomatous lesions in the pericardium, lungs and kidneys (Maurin & Gyuranecz, 2016).

Hard ticks are vectors for many protozoa, namely Piroplasmida (*Babesia* and *Theileria* spp.) of medical and veterinary importance. In Europe, however, human infections are rare and mostly caused by the zoonotic *Babesia divergens*, *Babesia* EU1 (also "Babesia venatorum") and the *Babesia microti* complex. The latter has a natural reservoir in wild rodents and insectivores, where zoonotic as well as presumably non-zoonotic strains occur (Yabsley & Shock, 2013). While *B. microti* infections

are common in asplenic and immunocompromised patients in North America, only a few autochtonous cases were reported in Europe, such as in a patient with leukemia from Germany (Hildebrandt *et al.*, 2007) and asymptomatic or mild infections in immunocompetent people in Poland (Moniuszko-Malinowska *et al.*, 2016; Welc-Faleciak *et al.*, 2015). However, there are probably many undiagnosed cases, since seroprevalence in tick-exposed persons in Germany was 5.4% (Hunfeld *et al.*, 2002). It can cause a febrile syndrome which is similar to malaria, including fever, myalgia and headache in the European cases (Hildebrandt *et al.*, 2007; Moniuszko-Malinowska *et al.*, 2016).

1.2 Important rodent hosts for *I. ricinus*

A number of rodent species live in urban areas and partly in close proximity to humans. In Berlin, 18 rodent species were recorded of which 13 species are regularly observed (Klawitter *et al.*, 2005). Apart from the semiaquatic Eurasian beaver and coypu (= nutria) or the arboreal European red squirrel, the majority of these species belongs to ground-dwelling species of two families of the superfamily Muroidea: Cricetidae (five species) and Muridae (five species) (Klawitter *et al.*, 2005). Two of them, the commensal murids Western house mouse (*Mus domesticus*) and Norway rat (*Rattus norvegicus*) are closely adapted to humans and primarily live inside buildings and feed on human food or waste. Only the Norway rat secondarily populates other habitats, such as banks of water bodies with dense vegetation. Due to the high affinity to humans, both species can cause economic damage at food storages, but more importantly, are vectors for zoonotic rodent-borne parasites, bacteria and viruses (Ulrich *et al.*, 2009). For *I. ricinus*, house mice are less suitable hosts. They rarely get in contact with typical tick habitats, since this species regularly lives inside buildings (Hauer *et al.*, 2009). In contrast, the Norway rats can be abundant in urban parks and represents there a competent host for ticks and a reservoir for Lyme disease spirochetes (Matuschka *et al.*, 1996).

The remaining, non-commensal, or “peridomestic” rodents that live in Berlin are not dependent on human housings, but can be abundant in proximity to humans and partly benefit from organic food of anthropogenic origin and urbanised habitats (Luniak, 2004). Due to the large area of potential habitats in urban regions, these species are a consistent part of cities (Hauer *et al.*, 2009; Matuschka *et al.*, 1990; Schmitt, 2007) and it can be assumed that rodent control measures would be, even if efficient, not long-lasting due to regular migration from rural areas. The peridomestic rodents in Berlin comprise three species of mice of the genus *Apodemus* and five species of voles from the genera *Microtus*, *Myodes*, *Arvicola* and *Ondatra*. The muskrat *Ondatra zibethica* and the European water vole *Arvicola amphibius* primarily occur at banks of standing and flowing waters or other moist

habitats with high ground water although at least *A. amphibius* secondarily populates farmland, gardens, orchards or ditches (Hauer *et al.*, 2009). The remaining six mouse-like species presumably represent the most abundant non-commensal rodent species in Europe, since they have, together with *Sciurus vulgaris*, *Micromys minutus*, *Arvicola amphibius* and *Ondatra zibethicus*, the largest geographic range in Europe (IUCN, 2017), but normally with higher population densities than the latter four rodent species. At the same time they are likely the most abundant rodents of green biotopes in Berlin and therefore should constitute the most important rodent hosts for ticks. These six species will be introduced in more detail:

1.2.1 The bank vole *Myodes glareolus*

The bank vole (Cricetidae, subfamily Arvicolinae) has a Eurasian range from the Atlantic coast to the Baikal Lake in the east and from the Arctic Circle to northern Spain in the south (Hutterer *et al.*, 2016). It repopulated Central Europe in the Holocene together with the establishing woodland (Hauer *et al.*, 2009). The bank vole is the most widespread rodent in Central European forests (Schröpfer, 1989) and has a broad tolerance towards the structure and humidity of the biotope. It is abundant in old-wood forests as well as reclamation fields, but a marked herb layer seems to be important (Hauer *et al.*, 2009). In urban areas, the bank vole also populates parks, although a suitable migration corridor connecting to rural habitats is important (Hauer *et al.*, 2009). Especially in winter, it enters buildings, although mainly in rural areas (Schaefer, 1962). In cycles of three to five years, mass reproduction occurs in optimal habitats (Hauer *et al.*, 2009). The lifespan is density-dependent, but rarely exceeds one year (Viro & Niethammer, 1982). The bank vole feeds mainly on green parts of plants and seeds but especially during the reproductive period in summer, a considerable part of the nutrition includes insects and their larvae. During autumn, also fruits, berries and fungi are part of their diet (Viro & Niethammer, 1982). Underground passages are mainly close below the ground surface. The nest is situated either in a blind ending tunnel of the burrows up to 45 cm under the surface or aboveground under trunks or in the vegetation (Viro & Niethammer, 1982). It consists of moss and frayed wood (Viro & Niethammer, 1982). Natural predators of the bank vole are forest-dwelling birds of prey, such as owls and sparrow hawks, as well as stone and pine martens, stoats and wild cats (Viro & Niethammer, 1982).

1.2.2 The common vole *Microtus arvalis*

Common voles (Cricetidae, subfamily Arvicolinae) are distributed from the Atlantic ocean to the Baikal region in the east (Yigit *et al.*, 2016). The most northern part of its range includes Denmark and southernmost Finland whereas in the South it ranges towards northern Spain and the Caucasus mountains (Yigit *et al.*, 2016). This species prefers open habitats such as grassland, fields and is

synanthropic, since it reaches high abundances in reclamation fields and agricultural areas (Hauer *et al.*, 2009). Common voles are only absent in contiguous forests and at waterlogged grounds (Dolch, 1995; Niethammer & Krapp, 1982b). In urban areas they populate gardens, lawns and cemeteries (Hauer *et al.*, 2009). Only 1% of a common vole population have a lifespan longer than nine months (Niethammer & Krapp, 1982b). The diet comprises green parts of grasses and herbs but also subterranean plant parts, seeds, bark, moss and arthropods (Niethammer & Krapp, 1982b). While seeds are important during late summer, subterranean plant parts and grasses dominate during winter and spring. Animal food could be found in the stomach of every fifth vole during summer (Niethammer & Krapp, 1982b). The nest is a chamber situated 8-75 cm below the ground surface and contains dry grass (Niethammer & Krapp, 1982b). Occasional mass reproduction occurs as a result of high abundance in the previous year and appropriate weather conditions in spring (Hauer *et al.*, 2009). In general, the common vole is a very abundant small mammal species and contributes to 57% and 75% of the preyed animals in casts of owls in Brandenburg and Saxony, respectively (Dolch, 1995; Hauer *et al.*, 2009). Other predators are the common buzzard, common kestrel, red fox, mouse weasel, stoat and many more (Niethammer & Krapp, 1982b).

1.2.3 The field vole *Microtus agrestis*

The range of the field vole has a similar east-west extension as the common vole, but also occurs more northern throughout Scandinavia and Great Britain, where common voles are absent (Kryštufek *et al.*, 2016). It prefers moist to wet areas with dense ground vegetation, whereby it mainly occurs at clearings of forests or at non-wooded places (Dolch, 1995; Hauer *et al.*, 2009). After closure of the canopy, field voles disappear from forest patches due to the reduction in ground vegetation (Hauer *et al.*, 2009). The maximum life span under natural conditions are about 14-15 months (Myllymäki, 1977). The field vole mainly feeds on grasses and to a lesser extent on mosses and dicotyledonous herbs. The proportion of animal food is very small (Krapp & Niethammer, 1982). The nest is filled with grass, but its position is depending on the wetness of the ground. In dry grounds, the burrows are, similar to those of the common vole, subterranean. Since field voles prefer wet patches, nests are mostly aboveground under roots or in tufts of grass (Krapp & Niethammer, 1982). The cyclic mass reproductions every three to five years reveal a lower intensity compared to the common voles, but also during phases of low population density, field voles can cause economic damage in winter months by feeding on the bark at the base of trees (Hauer *et al.*, 2009). The predators are nearly the same as for common voles (Krapp & Niethammer, 1982).

1.2.4 The striped field mouse *Apodemus agrarius*

The striped field mouse has two isolated distribution ranges, one in Taiwan, northern and eastern China and the other one from Central Europe to the Baikal Lake. The European area ranges from Denmark and southern Finland to northern Greece and the Caucasus mountains (Kaneko *et al.*, 2016). Today, the western margin of its range runs through Germany and south of the Alps in the Po Valley towards the 8th to 10th degree of eastern longitude (Spitzenberger & Engelberger, 2014). The striped field mouse prefers moist habitats, such as pond areas and floodplains and was the sole rodent species at a moist sewage farm in Gatow, Berlin (Dolch, 1995). However, it has a broad ecological tolerance and also populates agricultural land and is a typical immigrant of urban areas, such as parks, gardens and cemeteries (Hauer *et al.*, 2009). Mass reproduction occurs in some years and particularly in urban areas the striped field mouse can reach high abundances, such as in a mass occurrence in the park of Sanssouci in Potsdam (Schmidt, 1965). In the winter, striped field mice sometimes enter buildings (Ansorge, 1986; Schaefer, 1962). The maximal lifespan in the field comprises about one and a half years (Böhme, 1978). The diet of the striped field mouse includes seeds and fruits but is characterised by a high proportion of animal food, such as insects and their larvae, but also arachnids, chilopods, molluscs and annelids. Especially in the reproductive period, the proportion of animal food increases (Böhme, 1978). The striped field mouse digs tunnels, but often also uses burrows of other small mammals (Böhme, 1978). Predators are carnivores and birds of prey although this rodent is underrepresented in casts of owls due to its marked day activity (Böhme, 1978).

1.2.5 The yellow-necked mouse *Apodemus flavicollis*

The yellow-necked mouse has a mainly European distribution from northern Spain, southern Great Britain and eastern France in the west to the southern Ural Mountains. In the north it is restricted to southern Scandinavia and ranges towards the northern and eastern Mediterranean area with an isolated population in Iran (Amori *et al.*, 2016). The yellow-necked mouse is, together with the bank vole, a typical representative of forest habitats (Dolch, 1995; Hauer *et al.*, 2009). It prefers old-growth deciduous- and mixed forests with rich undergrowth, but also populates parks in urban areas (Hauer *et al.*, 2009). Particularly in winter, it enters buildings, where it also moves to the upper floors (Hauer *et al.*, 2009). If occurring at the same location, the bigger yellow-necked mouse displaces the wood mouse (Dolch, 1995). The maximum natural lifespan is about 18 months (Niethammer, 1978a). The yellow-necked mouse feeds on seeds, mainly from trees, but a high proportion of animal food of 13-37% by volume was observed, depending on the season with a maximum in early summer. Animal feed includes insects, arachnids, myriapods and less often annelids, molluscs and vertebrate musculature (Obrtel, 1973). The burrows are preferably under tree stumps or roots with a nest filled

with leaves and moss. Due to their climbing ability of up to 23 m in the trees, also nest boxes and tree holes are used for nests or storage (Hauer *et al.*, 2009; Niethammer, 1978a).

1.2.6 The wood mouse *Apodemus sylvaticus*

The wood mouse is a sister species to the yellow-necked mouse and both are included in the same subgenus *Sylvaemus*. The geographic distribution of the wood mouse includes the complete western part of Europe to the Ukraine in the east, including the British Isles, Iceland and South Scandinavia as well as north Africa (Schlitter *et al.*, 2016). In contrast to what its name suggests and to the yellow-necked mouse, it is a characteristic species of the open land and a pioneer species at reclamation sites (Hauer *et al.*, 2009; Maaz, 2010). It populates edges of agricultural land and non-wooded urban areas, such as railway systems and gardens (Hauer *et al.*, 2009; Schmitt, 2007). Occasionally, it enters houses in the winter and invades also upper floors (Niethammer, 1978b). The maximal lifespan in the field is estimated to be about 12 months, whereby only about 10% of the animals become considerably older than 6-8 months (Niethammer, 1978b). The diet of the wood mouse is mainly comprised of seeds, supplemented with animal feed, in particular snails and slugs, adult and larval beetles, lepidopteran pupae and spiders (Niethammer, 1978b; Obrtel, 1975). The tunnels of the burrows are situated about 8-18 cm below the ground with a maximum of 50 cm. The nest is subterranean or near tree stumps and filled with leaves, moss and dry grass (Niethammer, 1978b). Natural enemies of wood mice are owls and carnivores, such as red foxes and wild cats (Niethammer, 1978b).

1.3 Compendium of rodent-associated Protozoa, macroparasites and other associated invertebrates

About 87 years ago, Elton *et al.* (1931) were the first, and until now only scientist, who ever examined the (nearly) complete range of parasites occurring in and on wild non-commensal small mammals. Their study on the health of wild mice and voles was initiated to clarify the observed density fluctuations in small rodent populations. Since then, only subsets of parasites were investigated by researchers without a general view on the wide species diversity associated with the rodent hosts. Since the macroparasite species diversity was never reviewed before for the most abundant non-commensal rodents in Europe, the following chapters will introduce all parasites and other associated animals that have been described in and on the mentioned six muroid rodent species in Europe (exluding the Russian part and Turkey) and give brief information about their taxonomic status and biology (if known). Parasite groups are condensed by a similar parasitic life style with simultaneous taxonomic relationship, but without respect to the hierarchical level. After

an extensive literature review, invertebrates from four phyla (Platyhelminthes, Acanthocephala, Nematoda, Arthropoda) and at least 92 families and 460 species were recorded (Figure 1-2, Appendix 8-1) as well as about 69 species of Protozoa (here including Microsporidia, Appendix 8-1):

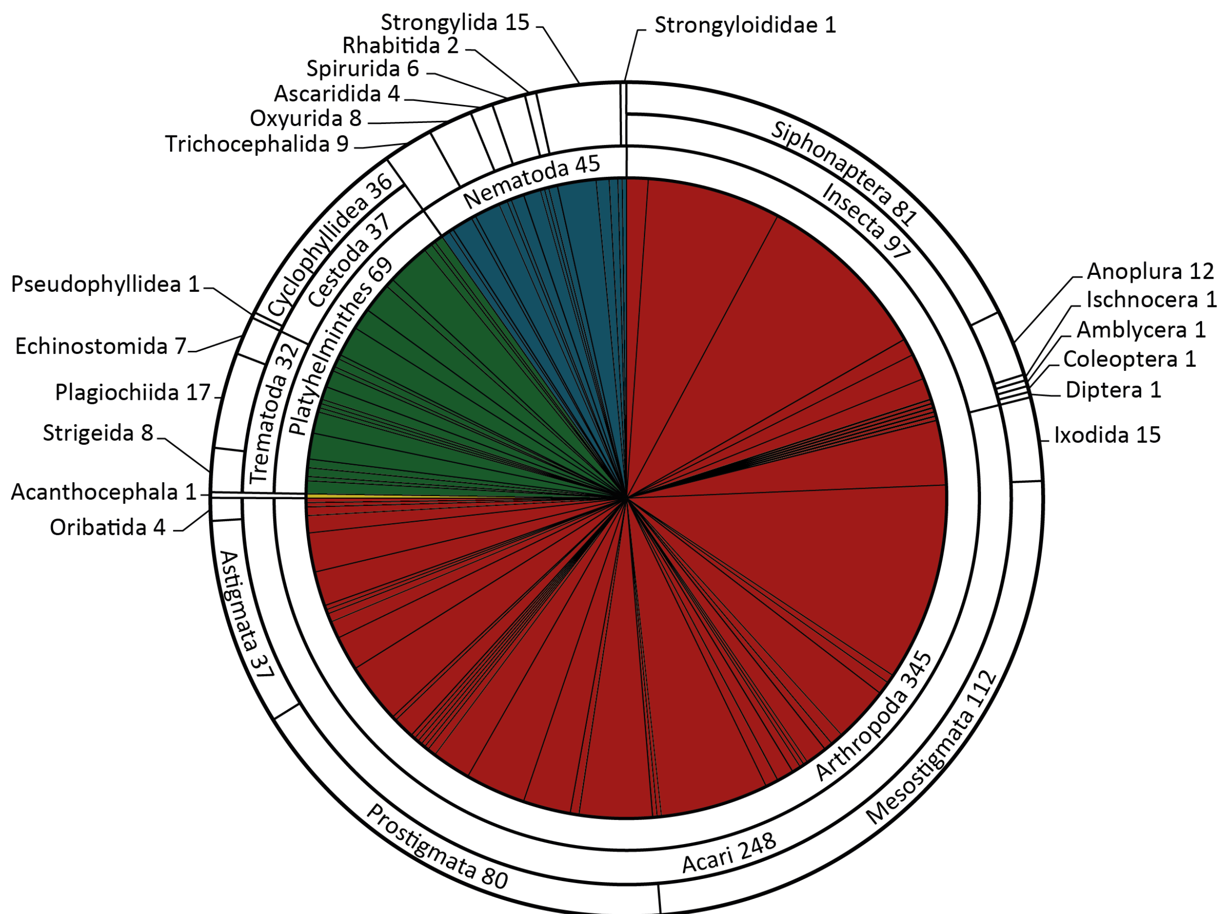

Figure 1-2. Taxonomic classification of metazoan invertebrates associated with the six rodent species in Europe. Pie chart including all 460 invertebrate species from the four phyla Arthropoda (red), Acanthocephala (yellow), Platyhelminthes (green) and Nematoda (blue). Slices indicate the 92 families. Higher taxa together with the number of reported species are depicted outside the chart with decreasing taxonomic level from inside out. Protozoan parasite species are not included, since the species concept for Protozoa (or Protista) and Metazoa is not comparable.

1.3.1 Protozoa

Various protozoan parasites infect the six mouse and vole species in Europe. These include blood-parasites (*Trypanosoma* spp., *Hepatozoon* spp., *Babesia microti*), parasites of carnivores and birds of prey using rodents as intermediate hosts in diverse body sites (*Sarcocystis* spp. (including former *Frenkelia* spp. (Dubey *et al.*, 2016)), *Toxoplasma gondii*, *Neospora caninum*) and intestinal Protozoa, such as amoebae (*Entamoeba muris*), flagellates (*Giardia* spp., *Spironucleus muris* (syn. *Hexamita muris*), *Chilomastix* spp., *Tetratrichomonas microti*, *Tritrichomonas muris*, *Trichomonas sylvatici*),

ciliates (*Blepharomonas mollis*), *Cryptosporidium* spp. and Coccidia (*Eimeria* spp., *Isospora* spp.). Microsporidians in brain, skeletal muscle and other body sites (*Encephalitozoon cuniculi*, *Thelohania apodemi*) were once included in Protozoa but are actually fungi (Hibbett *et al.*, 2007). Since only the intestinal Coccidia were studied in the present project, this is the only group, which is further introduced here.

1.3.1.1 Intestinal Coccidia (Alveolata: Myzozoa: Apicomplexa: Coccidia = Coccidiasina)

Intestinal Coccidia in wild rodents are poorly studied and the majority of species were exclusively described from the sporulated oocyst life stage. Hence, information about the life history of most species is sparse (Levine & Ivens, 1990; Lewis & Ball, 1983). The two genera *Eimeria* and *Isospora* have a direct life cycle. Morphologically, they are classically discriminated by the features of the sporulated oocysts: *Eimeria* oocysts harbour four sporocysts with two sporozoites, each, while *Isospora* oocysts have two sporocysts each containing four sporozoites. *Eimeria* spp. are very common and diverse, and at least 32 species have been reported from the six rodent species (Grikienienė, 2005; Levine & Ivens, 1990). In contrast, *Isospora* spp. are rare and based on single reports of *Isospora golemanskii* in wood mice and yellow-necked mice, *Isospora buxea* in striped field mice and *Isospora clethrionomydis* in bank voles (Grikienienė, 2005; Levine & Ivens, 1990). According to phylogenetic studies, the tetrasporozoic, diplosporocystic oocysts without Stieda bodies of *Isospora* spp. infecting mammals were transferred to the genus *Cystoisospora* (Sarcocystidae), while the remaining species (parasites of birds) possessing Stieda bodies remain in the genus *Isospora* (Eimeriidae) (Barta *et al.*, 2005). Since at least *I. clethrionomydis* has a Stieda body (Levine & Ivens, 1990) and birds and rodents also share parasitic families from various orders of invertebrates, it remains to be investigated to which genus and family the three *Isospora*-like species of these rodents belong. However, the six rodents have never been observed to be intermediate or paratenic hosts for *Cystoisospora* spp. with facultative heteroxenous life cycles.

In the life history strategy of *Eimeria* spp., the hosts become infected by ingestion of the sporulated oocysts, which release their sporozoites in the intestine (Ball *et al.*, 1989). These enter epithelial cells, become meronts and divide to form numerous merozoites (merogony or schizogony). These merozoites leave the host cell, reinfect other cells and become meronts again. The number of these asexual cycles is genetically determined. Some merozoites follow a sexual phase (gamogony), where they develop either to macrogametes or microgamonts. The latter develop into microgametes, which fertilise macrogametes and form the zygote with a thick wall, called oocyst (Ball *et al.*, 1989). The oocysts are shed in the faeces and sporulate in the environment (sporogony) to become infective within 2-5 days (Levine & Ivens, 1990). As an example, prepatent and patent periods are 6 and 4-6 days in *Eimeria cernae* in bank voles and 3-5 days and 8 days in *Eimeria hungaryensis* in

Apodemus mice, respectively (Levine & Ivens, 1990). Repeated schizogonic cycles can cause considerable damage in the intestine of the host (Cox, 1970).

1.3.2 Helminths

1.3.2.1 Digenean Trematodes (Trematoda: Digenea)

The Digenea within the class Trematoda include about 7200 species, all of them parasites. The complex life cycle starts when the free-swimming miracidium larvae either enter a first intermediate host or when they, or eggs including these larve, are ingested by this host. Here, the larvae develop to (mother-) sporocysts, which produce numerous progenies by vegetative reproduction (metagenesis), which are termed daughter sporocysts or rediae, depending on absence or presence of an intestine, respectively. Within daughter sporocysts or rediae either a second generation of these life stages or the mobile cercariae develop. The latter actively or passively leave the host. Subsequently, they either actively infect the definite host through skin penetration (diheteroxenous, e.g. *Schistosoma* spp.) or form a resting stage, called metacercaria, either after attachment to substrates, such as plants, or after infection of a second intermediate host (triheteroxenous). The definite host becomes infested by ingestion of these free-living metacercariae or by predation on infected second intermediate hosts (Xylander, 2006). In some species (e.g. *Alaria* spp.), cercariae only develop to an interjectional stage, called mesocercaria, in the second intermediate host and become metacercariae not until ingestion by the definite host (Möhl *et al.*, 2009). In the definite host, the infective stages develop to the adult flukes. The adults feed on blood, mucus, intestinal content or host tissues and may either be hermaphroditic or dioecious. After fertilisation, partly or completely embryonated eggs are shed with faeces, urine, saliva or sputum, depending on the parasite species. (Xylander, 2006).

The taxonomy within Digenea in general, and in several families such as Plagiorchiidae and Echinostomatidae in particular, is not finally solved due to the high degree of morphological variability within fluke species and their often low host specifity. A wide variety of 29 digenean families infect muriod rodents (Feliu *et al.*, 2006). Based on the taxonomy in Gibson *et al.* (2002), Jones *et al.* (2005) and Bray *et al.* (2008), species from 13 families in 7 superfamilies from all three digenean orders were reported to regularly or accidently infect the six species of rodents from the Muroidea in Europe.

1.3.2.1.1 Brachylaimid digeneans (Digenea: Strigeida: Brachylaimoidea: Brachylaimidae)

Within the family Brachylaimidae, the three species *Brachylaima recurvum* (syn. *Brachylaema recurva*, *Brachylaemus recurvatus, Brachylaemus aequans*), *Brachylaima spinulosum* (syn. *Brachylaemus*

spinulosus) and *Scaphiostomum palaearcticum* were detected in the six above listed host species. *Brachylaima recurvum* infects *Apodemus* spp. mice, while *B. spinulosus* was reported from common voles (Behnke *et al.*, 1999; Genov *et al.*, 1998; Tenora *et al.*, 1973). In the cosmopolitan genus *Brachylaima*, land snails or slugs (Gastropoda) are first and second intermediate hosts and birds and mammals, as definite hosts, become infected by feeding on them (Klimpel *et al.*, 2007b). *Scaphiostomum palaearcticum* occurs in the hepatic ducts, and secondarly, the pancreatic ducts of *Mus spretus* and *A. sylvaticus* on the Balearic islands (Mas-Coma *et al.*, 1986). The closely-related species *Scaphiostomum pancreaticum* has terrestrial snails as first and second host (Mas-Coma *et al.*, 1986).

1.3.2.1.1 Panopistid digeneans (Digenea: Strigeida: Brachylaimoidea: Panopistidae)

Only once, the shrew fluke *Pseudoleucochloridium soricis* was observed in the small intestine of a bank vole in Hungary (Gubányi *et al.*, 2002). First and second intermediate hosts of this species are several terrestrial snails (Jourdane, 1976; Pojmańska, 1961).

1.3.2.1.2 Diplostomatid digeneans (Digenea: Strigeida: Diplostomatoidea: Diplostomatidae)

Occasionally, rodents are paratenic hosts for the diplostomatid fluke *Alaria alata* (e.g. Grikienienė (2005); Shimalov (2013)). Aquatic snails (*Planorbis* spp.) are first intermediate hosts and the resulting cercariae infect tadpoles or adult frogs and develop to mesocercariae, which are able to survive several host switches in paratenic hosts, such as reptiles, birds and mammals. Definite hosts are canids including dogs but occasionally also cats, which become infected after predation of intermediate or paratenic hosts. Here, the mesocercariae develop during a somatic migration via the lungs to adult flukes in the small intestine. The life cycle can be completed in about 92-114 days (Lucius *et al.*, 1988). Also larvae of *Neodiplostomum major* were once reported from striped field mice in Bulgaria, which are paratenic hosts for this species (Genov *et al.*, 1998).

1.3.2.1.3 Strigeid digeneans (Digenea: Strigeida: Diplostomatoidea: Strigeidae)

Reports of digeneans from the genus *Strigea* are very rare in intermediate hosts of the six rodent species in Europe. *Strigea falconis* was once detected in striped field mice (Shimalov, 2002) and *Strigea sphaerula* once in bank voles and striped field mice (Shimalov, 2013) in Belarus. For both species, the four-host-cycle includes aquatic pulmonate gastropods as first intermediate hosts, while mesocercariae develop in anurans and metacercariae in amphibians, snakes, lizards and mammals. Definite hosts are birds from the family Corvidae (*S. sphaerula*) or birds of prey (*S. falconis*) (Gibson *et al.*, 2002; Pojmańska *et al.*, 2007).

1.3.2.1.4 Plagiorchiid digeneans (Digenea: Plagiorchiida: Plagiorchioidea: Plagiorchiidae)

The large family Plagiorchiidae includes several endoparasites of rodents. Natural and experimental infections with *Plagiorchis* reveal aquatic snails (e.g. *Lymnaea* spp.) as first, and aquatic amphipods, insect larvae and fish as second intermediate hosts (Gorman, 1980; Rogan *et al.*, 2007). *Plagiorchis elegans* and *Plagiorchis muris* (e.g. Hildebrand *et al.* (2004); Rogan *et al.* (2007); Tenora *et al.* (1983)) are the most often reported species within this genus in the small intestine of muroid rodents, but also *Plagiorchis arvicolae* and *Plagiorchis talassensis* can be found in Europe (Feliu *et al.*, 1997; Ivanov & Semenova, 2000; Wahl, 1967). *Plagiorchis elegans* infects many vertebrates but the main hosts are probably passerine birds. Due to its morphological variability, it was considered to be synonymous with *P. muris* (Tenora *et al.*, 1983), which was found to be zoonotic (Hong *et al.*, 1996). *Rubenstrema exasperatum* is a parasite of shrews from the family Soricidae, but the synonymised (Gorman, 1980) *Plagiorchis microti* was once described to parasitise the oesophagus and stomach of *M. arvalis* in Poland (Soltys, 1949). Finally, *Skrjabinoplagiorchis polonicus* was detected in a yellow-necked mouse in Belarus (Shimalov, 2013) and *Skrjabinoplagiorchis vigisi* in the liver of the wood mouse in western Russia and probably also occurs in Europe (Bugmyrin *et al.*, 2015; Petrov & Merkuscheva, 1963). The life cycle of both species is unknown.

1.3.2.1.5 Lecithodendriid digeneans (Digenea: Plagiorchiida: Microcephalloidea: Lecithodendriidae)

Within the Lecithodendriidae, *Macyella apodemi* was found in the duodenum of wood mice, *Paraleyogonimus baeri* in the bile ducts of bank voles and *Posterocirrus clethrionomi* in the liver and mesenteries of bank voles (Grikienienė, 2005; Jourdane & Triquell, 1973; Vaucher, 1968). The life cycles are not known. The species *Encystodendrium rotundum* infects the aquatic snail *Bythinella compressa* as first intermediate host, which is endemic to the low mountain ranges Rhön and Vogelsberg in Germany (Brendow, 1970) and indeed, it was only found to infect yellow-necked mice in the Vogelsberg region as definite hosts. However, under experimental conditions it infects house sparrows (Memaran, 1970). Second intermediate hosts are insects of the orders Plecoptera and Trichoptera and their larvae (Memaran, 1970). *Cephalotrema elasticum* is more probably a shrew parasite, but was once detected in a bank vole in Hungary (Matskasi, 1971).

1.3.2.1.6 Prosthogonimid digeneans (Digenea: Plagiorchiida: Microcephalloidea: Prosthogonimidae)

Mediogonimus jourdanei is a species in the Prosthogonimidae which was detected in the liver of bank voles in the Pyrenees and northwestern Spain (Mas-Coma & Rocamora, 1978; Ribas *et al.*, 2009). The life cycle was not further studied.

1.3.2.1.7 Microcephallid digeneans (Digenea: Plagiorchiida: Microcephalloidea: Microcephallidae)

One member of the family Microcephallidae, *Maritrema apodemicum*, infects the small intestine of wood mice on Skomer Island, UK (Lewis, 1966). The intermediate hosts are not known.

1.3.2.1.8 Pleurogenid digeneans (Digenea: Plagiorchiida: Microcephalloidea: Pleurogenidae)

According to a ribosomal marker, Kanarek *et al.* (2015) placed *Collyricloides massanae*, formerly belonging to the Collyriclidae in the superfamily Gorgoderoidea, now in the family Pleurogenidae in the superfamily Microphalloidea. The species was described from spherical cysts in the wall of the intestine of wood mice, yellow-necked mice and bank voles (Jourdane & Triquell, 1973; Kanarek *et al.*, 2015; Vaucher, 1969). The Xiphidocercariae develop in the snail *Bythinella dunkeri* as first intermediate host and in stream-associated insects of the orders Trichoptera, Ephemeroptera and Plecoptera as second intermediate hosts (Schwarz, 1981).

1.3.2.1.9 Dicrocoelid digeneans (Digenea: Plagiorchiida: Gorgoderoidea: Dicrocoelidae)

The elongate species *Corrigia vitta* (syn. *Lyperosomum vitta*) from the family Dicrocoelidae is one of the most often detected digenean species in *Apodemus* spp. mice (e.g. Behnke *et al.* (1999); Debenedetti *et al.* (2016)) and infects, like all dicrocoelids, the interlobary ducts of pancreas and the duodenum of their definite hosts (Lewis, 1968). Unfortunately, the life cycle has not been revealed yet but the first intermediate host appears to be the terrestrial gastropod *Clausilia bidentata* and Schmidt (1967) assumed isopods as second intermediate hosts. Comparatively, *Brachylecithum glareoli* infects the bilary ducts of bank voles in Poland (Hildebrand *et al.*, 2007) and these authors also mentioned an undescribed *Brachylecithum* sp. from striped-field mice (Hildebrand & Popiolek, 2001). *Dictyonograptus muris* (earlier placed in the genera *Platynosomum*, *Platynosomoides* and *Skrjabinus* (Nguyen & Pham, 2004)) was detected in the intestine of a bank vole in Switzerland and in liver and *ductus choledochus* of yellow-necked mice in Hungary (Gubányi *et al.*, 2002; Matskási, 1967; Vaucher & Hunkerer, 1967). For *B. glareoli* and *D. muris*, the intermediate hosts were not investigated, but can be assumed to be similar as for *C. vitta*.

1.3.2.1.10 Notocotylid digeneans (Digenea: Echinostomida: Pronocephaloidea: Notocotylidae)

In the cosmopolitan genus *Notocotylus*, the ventral suckers are absent (Tenora *et al.*, 1983). Four species were found in the caecum of voles in Europe: *Notocotylus neyrai*, *Notocotylus gonzalezi*, *Notocotylus noyeri* and *Notocotylus malhamensis* (Boyce *et al.*, 2012; Feliu *et al.*, 1997; Memaran, 1970; Simón-Vicente *et al.*, 1985). After a revision by Simón-Vicente *et al.* (1985), *Notocotylus wetlugensis* (known from the Russian part of Europe) is a *species inquerenda* (= doubtful identity) and probably not valid. In addition, many, if not all reports of *N. noyeri* in rodents in the past were

probably one of the other species in this morphologically variable genus. Aquatic pulmonate gastropods are probably the only intermediate hosts and definite hosts may become infected by ingestion of metacercariae, which are attached to the vegetation (Haukisalmi, 1986).

1.3.2.1.11 Psilostomid digeneans (Digenea: Echinostomida: Echinostomatoidea: Psilostomidae)

The parasite of the muskrat, *Psilotrema pharyngeatum* was once detected in a field vole in Hungary (Edelényi, 1966).

1.3.2.1.12 Echinostomatid digeneans (Digenea: Echinostomida: Echinostomatoidea: Echinostomatidae)

There were occasional reports of infections with Echinostomatidae in muroid rodents. *Isthmiophora melis* (syn. *Euparyphium melis*) is a typical endoparasite of badgers, polecats and hedgehogs (Dönges, 1967) but occurs in a range of 30 species of definite hosts (Radev *et al.*, 2009) including humans and *Apodemus* spp. mice (Goüy de Bellocq *et al.*, 2003; Hildebrand *et al.*, 2015). The water snail *Lymnaea stagnalis* and amphibians as well as fishes are first and second intermediate hosts, respectively (Radev *et al.*, 2009). Also *Echinostoma miyagawai* and *Echinostoma* sp. were found in the small intestine of striped field mice in Hungary, Poland and Bulgaria (Edelényi, 1966; Genov *et al.*, 1998; Hildebrand *et al.*, 2004). The cosmopolitan *Echinostoma* spp. preferably infect semi-aquatic and aquatic birds, reptiles and mammals, as well as humans. The triheteroxenous life cycle includes aquatic snails as first, and clams, snails, frogs and fishes as second intermediate hosts (Huffman & Fried, 1990).

1.3.2.2 Tapeworms (Cestoda)

Tapeworms are highly specialised, obligate endoparasites with almost exclusive heteroxenous life cycles. Most of them live in the intestinal tract of their vertebrate definite hosts and at different sites in the body of their intermediate or paratenic hosts, which are represented by invertebrates and vertebrates (Xylander, 2006). The majority of species, including all cestodes in the muroid rodents from the six mentioned species in Non-Russian Europe, belong to the order Cyclophyllidea, which currently comprise about 3100 species in 380 genera and 18 families (Georgiev *et al.*, 2006). Only one member of the Pseudophyllidea, *Spirometra erinacei*, was rarely found to use rodents as intermediate or paratenic host. Adult cyclophyllidean cestodes are tape-like, elongated worms and mainly protandric hermaphrodites, where the body is segmented into three to numerous proglottids, all harbouring a set of male and female sexual organs. The anterior body is formed by a scolex with musculous suckers and often a rostellum with hooks as attachment organs (Xylander, 2006). The intermediate hosts become infected by ingestion of eggs where the larvae (oncosphaera) hatch and develop to metacestodes. The final tetrapod host normally preys on the intermediate hosts and

hence ingests the metacestodes, which develop to adult tapeworms. Eggs are released either via the oviporus in the proglottids or when one or several posterior gravid proglottids fall off the tapeworms and desintegrate outside the host (Xylander, 2006). The six mouse and vole species of interest here are reported to be definite hosts for four families (Hymenolepididae, Catenotaeniidae, Anoplocephalidae, Dilepididae), 14 genera and 21 species as well as intermediate or paratenic hosts for another four families (Taeniidae, Paruterinidae, Dipylidiidae, Mesocestoididae), seven genera and 15 species of Cyclophyllidea in Europe.

1.3.2.2.1 Diphyllobothriid tapeworms (Cestoda: Pseudophyllidea: Diphyllobothridae)

Spirometra erinacei (syn. *Spirometra erinaceieuropaei*, *Diphyllobothrium mansoni*) was rarely detected in the six rodent species in Europe, such as in yellow-necked and striped field mice in Bulgaria (Genov & Georgiev, 1998a) and in bank voles in eastern Poland (Soltys, 1954). The species has a triheteroxenous life cycle including copepods as first intermediate host, harbouring procercoids, and amphibians, reptiles and mammals as second intermediate or paratenic host, harbouring the plerocercoid larvae, also called spargana. Feline and canine carnivors are definite hosts for adult intestinal tapeworms (Georgiev *et al.*, 2006; Liu *et al.*, 2015). Also humans can become infected with *Spirometra* spp. plerocercoid larvae as second intermediate host through drinking of water contaminated with infected copepods or as paratenic or, very rarely, definite host through consumption of spargana in raw flesh of snakes, frogs and probably wild boar (Kołodziej-Sobocińska *et al.*, 2016; Liu *et al.*, 2015). The spargana can invade the brain, breast, scrotum, eyes, spinal cord and subcutaneous tissues of human patients which cause considerable morbidity and even death (Liu *et al.*, 2015). Human sparganosis is a worldwide, although mainly southeast Asian disease with about 15 European cases in Italy, France, UK, Czech Republic and Germany, although some of them were travel-related (Bracaglia *et al.*, 2015; Liu *et al.*, 2015; Lo Presti *et al.*, 2015).

1.3.2.2.2 Hymenolepidid tapeworms (Cestoda: Cyclophillidea: Hymenolepididae)

The cosmopolitan and speciose Hymenolepididae are mainly parasites of birds. At least the six species *Hymenolepis hibernia*, *Arostrilepis horrida*, *Rodentolepis fraterna, Rodentolepis straminea, Rodentolepis asymmetrica* (syn. *Hymenolepis ampla*) und *Microsomacanthus crenata* (syn. *Variolepis crenata, Passerilepis crenata*, *Microsomacanthus murissylvatici*) infect the six mice and vole species in Europe (e.g. Behnke *et al.* (1999); Haukisalmi (2015)). Reports of the human pathogenic species *Hymenolepis diminuta*, *Rodentolepis microstoma* and *Rodentolepis nana* in these rodent hosts are most likely misidentifications (see Casanova *et al.* (2001); Macnish *et al.* (2002a); Tenora (2004)). Also the detection of the shrew tapeworm *Staphylocystoides stefanskii* in field voles (Bluszcz *et al.*, 1987) was questioned by Pojmańska *et al.* (2007). A single report of the fat dormouse parasite *Hymenolepis myoxi* (syn. *Armadolepis myoxi, Hymenolepis sulcata*) in bank voles (Pojmańska *et al.*, 2007) requires

reexamination after the recent redescription of this problematic species by Makarikov (2017). Hymenolepidid intermediate hosts are arthropods and annelids (Georgiev *et al.*, 2006), such as oribatid mites in *R. asymmetrica* and *R. straminea*, beetles in *M. crenata* or collembolans in *A. horrida* (Gubanyi *et al.*, 1992; Prokopic, 1962; Rausch, 1994; Tenora *et al.*, 1973).

1.3.2.2.3 Catenotaeniid tapeworms (Cestoda: Cyclophillidea: Catenotaeniidae)

The Catenotaeniidae are a small family of exclusively rodent parasites in Africa, Eurasia and America (Georgiev *et al.*, 2006). The species *Catenotaenia pusilla*, *Catenotaenia henttoneni*, *Catenotaenia asiatica, Skrjabinotaenia lobata* and *Pseudocatenotaenia matovi* were detected in the six rodent species in Europe (e.g. Haukisalmi and Henttonen (2000); Klimpel *et al.* (2007b); Mas-Coma *et al.* (1978); Tenora *et al.* (1973)). Only the life cycle of *C. pusilla* is known with *Glycyphagus domesticus* (mite), *Tenebrio molitor* (beetle) and *Leptopsylla segnis* (flea) as intermediate hosts (Kisielewska, 1970a).

1.3.2.2.4 Anoplocephalid tapeworms (Cestoda: Cyclophillidea: Anoplocephalidae)

In the family Anoplocephalidae, species are often parasites of herbivorous mammals, which become infected by accidental ingestion of intermediate hosts (Georgiev *et al.*, 2006). In Europe, the six species *Paranoplocephala janickii*, *Paranoplocephala kalelai*, *Paranoplocephala omphalodes*, *Eurotaenia gracilis*, *Microticola blanchardi* and *Anoplocephaloides dentata* (syn. *Paranoplocephala brevis*) infect voles, while *Gallegoides arfaai* infects mice (e.g. Haukisalmi (2015); Haukisalmi *et al.* (2014)). The intermediate host of *P. omphalodes* are probably collembolans (Georgiev *et al.*, 2006).

1.3.2.2.5 Dilepidid tapeworms (Cestoda: Cyclophillidea: Dilepididae)

The bird parasite *Dilepis undula* is one of only two species within the large cosmopolitan family Dilepididae, which was observed as adult tapeworm in the small intestine of the six mice species (e.g. Vaucher and Hunkerer (1967)). Intermediate hosts of *D. undula* are earthworms of the family Lubricidae (Stammer, 1956). In a single common vole, adults of the blackbird parasite *Choanotaenia unicoronata* were observed (Tenora *et al.*, 1973).

1.3.2.2.6 Taeniid tapeworms (Cestoda: Cyclophillidea: Taeniidae)

The Taeniidae comprise the four genera *Taenia, Hydatigera, Versteria* and *Echinococcus* (Nakao *et al.*, 2013). The former three genera were earlier often united in the genus *Taenia*, since they are morphologically similar as adults in the definite hosts, although having very different types of metacestodes in the intermediate hosts. Metacestodes of some taeniid species have the ability of vegetative reproduction. The ten taeniid species and subspecies occurring in the six mouse and vole species in Europe are the following: *Hydatigera taeniaeformis* (syn. *Cysticercus fasciolaris* for the larval form), *Hydatigera kamiyai* and *Hydatigera parva* form a Strobilocercus. *Taenia crassiceps*,

Taenia pisiformis, *Taenia martis martis* (syn. *Taenia intermedia*), *T. martis americana* (syn. *Taenia sibirica*), *Taenia polyacantha polyacantha* and *Versteria mustelae* (syn. *Taenia tenuicollis*) form a Cysticercus, *Echinococcus multilocularis* forms alveolar cysts (e.g. Debenedetti *et al.* (2016); Führer *et al.* (2010b); Lavikainen *et al.* (2016); Memaran (1970); Pfaller and Tenora (1972); Schmidt (2001)). In addition, also *Taenia hydatigena* metacestodes were rarely observed (Jančev & Stoykova-Hadjinikolova, 1980; Memaran, 1970) but their proper identification remains questionable, since their normal intermediate hosts are ruminants. Taeniid metacestodes were detected in the thoracic and abdominal cavity, subcutaneous tissue and in different organs such as liver, brain or lung. The typical definite hosts of the Taeniidae are, depending on the species, carnivorous canid, felid, mustelid, viverrid or herpestid mammals. While *H. taeniaeformis*, *T. crassiceps* and *T. martis* metacestodes very rarely also infect humans, *E. multilocularis* is a major public health thread and causes alveolar echinococcosis in the northern hemisphere (Nakao *et al.*, 2010).

1.3.2.2.7 Paruterinid tapeworms (Cestoda: Cyclophillidea: Paruterinidae)

The members of the family Paruterinidae infect mainly insectivorous birds as definite hosts (Georgiev *et al.*, 2006), but the two species *Cladotaenia circi* and *Cladotaenia globifera* (syn. *Cladotaenia cylindracea*) infect the mice and voles in Europe (e.g. Murai (1982)), with birds of prey as definite hosts. Both species form “cladothyridia” metacestodes in the liver of the rodents (Georgiev *et al.*, 2006). *Paruterina candelabraria* is the most common cestode in holarctic owls (Rausch, 1949) and infects North American rodents including voles (Freeman, 1957). However, it was never observed in its rodent intermediate hosts in Europe.

1.3.2.2.8 Dipylidiid tapeworms (Cestoda: Cyclophillidea: Dipylidiidae)

Joyeuxiella pasqualei is the only species in the Dipylidiidae, which was detected as metacestodes in the six rodent species in Europe, or more precisely, only in the wood mouse on the Balearic Islands (Mas-Coma *et al.*, 2000). There, it was found in the abdominal cavity. The normal life cycle includes an unidentified first intermediate host, a reptile as second intermediate or paratenic host, as well as domestic and wild felid, canid and mustelid mammals (Bowman *et al.*, 2008).

1.3.2.2.9 Mesocestoidid tapeworms (Cestoda: Cyclophillidea: Mesocestoididae)

The last family to be addressed herein are the Mesocestoididae. Two species, *Mesocestoides litteratus* (syn. *Mesocestoides leptothylacus* (Literák *et al.*, 2006)) and *Mesocestoides lineatus*, were detected as tetrathyridia mesocestodes in the body cavity of mice and voles in Europe (e.g. Behnke *et al.* (2008); Loos-Frank (1980)). In contrast to *Mesocestoides vogae* (syn. *Mesocestoides corti*), they do not multiply asexually (Loos-Frank, 1991). The life cycle is not fully known. Since eggs and oncospheres are not infectious to rodent intermediate hosts, another, probably arthropod (first)

intermediate host is assumed (Loos-Frank, 1991), which means that the rodents are either second intermediate or paratenic hosts for the tetrathyrid larvae. Definite hosts are mainly foxes, but also other carnivorous mammals (Literák *et al.*, 2006; Pojmańska *et al.*, 2007). Also humans can become infected with *M. lineatus* as definite hosts through consumption of raw or undercooked intermediate hosts, which can be also numerous other mammals, birds, reptiles and amphibians. However, cases are very rare and only known from southeast Asia (Fuentes *et al.*, 2003b).

1.3.2.3 Acanthocephalans (Acanthocephala)

Occasionally, the spiny-headed worm *Moniliformis moniliformis* (Moniliformidae) was reported to infect the small intestine of yellow-necked mice and bank voles in Hungary (Edelényi, 1966; Gubányi *et al.*, 2002), common voles in former Czechoslovakia (Tenora *et al.*, 1973) and common voles without detailed reference in the “Lehrbuch der Helminthologie” (Sprehn, 1932). Since this is a very rare infection, this parasitic group is not further introduced.

1.3.2.4 Nematodes (Nematoda)

Nematoda is a monophyletic phylum including more than 27,000 described species and with an approximation of 500,000 to 1,000,000 species worldwide considered to be not only the second largest phylum after Arthropoda but also the group with the most highest number of individuals within the Metazoa (Lorenzen, 2006; Morand *et al.*, 2006; Schmidt-Rhaesa *et al.*, 2014). About 12,000 described species are parasites of vertebrates which phylogenetically evolved at least seven times from free-living ancestors (Morand *et al.*, 2006). Their morphology and development is relatively consistent with a wormlike, cylindrical body with a hydrosceleton and five postembryonal life stages including first- to fourth-stage larvae (L1, L2, L3, L4) developing through four moults to adults. Most species reproduce bisexually, but there are also hermaphroditic (e.g. *Muspicea borreli* in house mice) and parthenogenetic (e.g. *Strongyloides ratti*) parasitic nematodes (Anderson, 2000; Lorenzen, 2006). In contrast, their life cycles are highly diverse ranging from simple, monoxenous life-styles to indirect cycles including one or more intermediate or paratenic hosts and sometimes tissue migration (Morand *et al.*, 2006). Nematodes reported to infect the six rodent species include 45 species and 17 families from a wide phylogenetic spectrum.

1.3.2.4.1 Trichurid nematodes (Nematoda: Trichocephalida: Trichinelloidea = Trichuroidea: Trichuridae)

The family Trichuridae includes two valid species infecting the voles (*Trichuris arvicolae*) and mice (*Trichuris muris*, syn. *Trichocephalus muris*) in Europe (e.g. Feliu *et al.* (2000)). The identification of *Trichuris madisonensis* in voles in Germany (Schmidt, 1961; Stammer, 1956) is unsatisfactory according to Feliu *et al.* (2000) and these specimens might have been also *T. arvicolae*. The adult

worms have a narrow, whip-like anterior body part attached to the mucosa of the caecum or colon of the host and a handle-like posterior part. They induce a epithelial syncytium at the attachment site and feed on it (Anderson, 2000). As in the vast majority of trichinelloid nematodes (except *Trichinella* spp.), the life cycle involves only one host, which becomes infected by ingestion of embryonated eggs (Anderson, 2000). The infective L1 hatch from the eggs in the small intestine, migrate to the colon and devolop after four moults to adults. The full development inside the host lasts 35-43 days until females start shedding eggs which are expelled with the host faeces. Eggs embryonate and become infective outside the host in, depending on the temperature, 16-42 days (Anderson, 2000; Kulke *et al.*, 2014).

1.3.2.4.2 Trichosomoidid nematodes (Nematoda: Trichocephalida: Trichinelloidea = Trichuroidea: Trichosomoididae)

Trichosomoides crassicauda is a parasite of rats infecting the urinary tract, but there is a single report of this nematode in striped field mice in Bulgaria (Genov & Georgiev, 1998b).

1.3.2.4.3 Capillariid nematodes (Nematoda: Trichocephalida: Trichinelloidea = Trichuroidea: Capillariidae)

The related Capillariidae are a speciose family of hair-like endoparasites of vertebrates. Due to the dispute regarding the generic classification of this family, I here follow Gibbons (2010) and consider all the following species as part of the genus *Capillaria*. The five species *Capillaria* (*Aonchotheca*) *annulosa* (syn. *Pterothominx sardovskoi* according to Moravec and Barus (1991)), *Capillaria* (*Aonchotheca*) *murissylvatici* (syn. *Capillaria halli*), *Capillaria* (*Eucoleus*) *bacillatus*, *Capillaria* (*Eucoleus*) *gastrica* and *Capillaria* (*Calodium*) *hepatica* were reported to infect mice and voles in Europe (e.g. Feliu *et al.* (1997); Memaran (1970); Tenora (2004)). They are localised in the small intestine (*C. murissylvatici*, *C. annulosa*), stomach and esophagus (*C. bacillatus*, *C. gastrica*) or liver parenchyma (*C. hepatica*) of their hosts (Moravec, 2000). However, recently, also *C. gastrica* and *C. annulosa* were detected in the liver of wood mice (Debenedetti *et al.*, 2014). For the majority of them, the life cycle is unknown and monoxeny is assumed, although some non-rodent *Capillaria* spp. use earthworms as intermediate hosts, where non-infective L1 hatch from the egg after ingestion and grow to infective L1 (Anderson, 2000). Only biology of the cosmopolitan and zoonotic *C. hepatica* was studied in detail (Anderson, 2000): The host ingests embryonated eggs, the L1 hatch and migrate to the liver parenchyma, probably via the hepatic portal system, where they develop to adults. Females start to deposit eggs 21 days *post infectionem* in small groups in the liver. In contrast to other (gut) *Capillaria* spp., release of eggs only occurs, if the liver of the infected host is eaten during cannibalism, scavenging or predation by another animal and after a passage through its gut. The

viability of eggs from decaying livers after the host's death is very low. In the environment, eggs embryonate and are infective after 35-45 days.

1.3.2.4.4 Trichinellid nematodes (Nematoda: Trichocephalida: Trichinelloidea = Trichuroidea: Trichinellidae)

The zoonotic trichinellid nematodes *Trichinella* spp. were only once reported as larvae from a common vole in Belarus by Tenora *et al.* (1973) and another two times in yellow-necked mice and striped-field mice in Ukraine and Poland, but without references, in the reviews of Dick and Pozio (2001) and Pozio (2007). Since this is mainly a parasite of wild boars and carnivores and very rare in the six non-commensal rodent species, it is not further introduced.

1.3.2.4.5 Oxyuroid nematodes (Nematoda: Oxyurida: Oxyuroidea: Oxyuridae and Heteroxynematidae)

In the order Oxyurida, two families infect the six rodent species: Oxyuridae with six *Syphacia* spp. and Heteroxynematidae with two *Aspiculuris* spp. These endoparasites are very host specific and mostly infect only hosts from the same host species or genus: *Syphacia stroma* and *Syphacia frederici* in *Apodemus* sp., *Syphacia agraria* in the striped field mouse, *Syphacia petrusewiczi* and *Aspiculuris tianjinensis* in bank voles, *Syphacia nigeriana* in *Microtus* sp. (Behnke *et al.*, 2015; Tenora & Mészéros, 1975). Only *Syphacia montana* infects a broader range of voles includung bank voles (Tenora & Mészéros, 1975), and *Aspiculuris tetraptera*, a parasite of the house mouse, has been reported to incidentially infect wood mice (e.g. Eira *et al.* (2006)). However, Behnke *et al.* (2015) showed that *A. tetraptera* from *Apodemus* spp. are genetically distinct from those found in house mice and probably represent a separate, undescribed species. Reports of the strict house mouse specific oxyurid *Syphacia obvelata* (Tenora & Mészéros, 1975) from other rodents are probably erroneous (Stammer, 1956; Stewart *et al.*, 2017; Tenora, 2004) and the detection of the snow vole oxyurid *Aspiculuris dinniki* in bank voles (Mažeika *et al.*, 2003; Pojmańska *et al.*, 2007) may refer to *A. tianjinensis*, which was described only after that time. The worms have a direct life cycle and live in the lumen of the small intestine (*S. stroma*), caecum (other *Syphacia* spp.) and colon (*Aspiculuris* spp.) (Lewis, 1987; Tenora & Mészéros, 1975). Gravid *Syphacia* sp. females migrate to the perianal region, primarily around noon and deposit their eggs (Lewis, 1987). Infection of the host occurs by ingestion of embryonated eggs from the environment or by grooming. Migration of larvae hatching in the perianal region back to the rectum (retrofection) was observed but questioned by some authors (Anderson, 2000). The life cycle of *S. obvelata* is very short, since eggs become infective after 6-42 h at 30 °C and the prepatent period in laboratory mice is only 11-15 days (Grice & Prociv, 1993).

1.3.2.4.6 Heterakid nematodes (Nematoda: Ascaridida: Heterakoidea: Heterakidae)

The family Heterakidae are gut parasites of tetrapods (Anderson, 2000) and the typical rat nematode *Heterakis spumosa* (syn. *Ganguleterakis spumosa*) regularly infects *Apodemus* spp. mice (e.g. Ondríková *et al.* (2010); Schmidt (1961); Zaleśny *et al.* (2010)). The endoparasite infects the lumen of the upper colon and occasionally the caecum (Anderson, 2000). Females of this monoxenous endoparasite shed unembryonated eggs via the faeces, which embryonate outside (12-14 days at 30 °C). The host becomes infected by ingestion of embryonated eggs containing the infective larvae which moult to the L3 before hatching. The prepatent period is 26-47 days in rats (Anderson, 2000). Earthworms can be infected as paratenic hosts of *H. spumosa* (Saitoh *et al.*, 1993).

1.3.2.4.7 Ascaridid nematodes (Nematoda: Ascaridida: Ascaridoidea: Ascarididae)

For members of the Ascarididae, the rodent species are intermediate or paratenic hosts. In a number of reports, *Porrocaecum* sp. was observed to use mice and voles as paratenic hosts (Portoles *et al.*, 2000; Shimalov, 2013; Tenora *et al.*, 1973). Definite hosts are birds of prey, where the adults are endoparasites in the alimentary tract (Lewis, 1987). Eggs passed in the faeces are ingested by earthworm intermediate hosts, where the infective L3 develops in the blood vessels. Paratenic hosts feeding on the earthworms are normally shrews and moles, but also rodents, which transfer the worms to the final host (Anderson, 2000). Except for the field vole, all other rodent species were already observed to be seropositive for *Toxocara* spp., but also undetermined larvae were detected in different organs, which reacted with rabbit anti-*Toxocara* hyperimmune serum (Dubinský *et al.*, 1995; Reiterová *et al.*, 2013). However, the possibility of cross-reaction with *Porrocaecum* sp. larvae was not considered. In a publication which also emerged from this rodent trapping campaign, Krücken *et al.* (2017) recorded the presence of *Toxocara cati* and *Toxocara canis* in tissues of European wild rodents after sequencing of PCR products. *Toxocara cati* infects felids and *T. canis* canids as definite hosts. Eggs produced by female worms in the intestine are shed with the faeces, embryonate and develop to third stage larvae after nine days at 26-30 °C in the environment and then these are infective to the definite host (Anderson, 2000). However, also small mammals can serve as paratentic hosts and accumulate infective L3 which directly hatch from the eggs after they become ingested or maybe scavenger insects as mechanical vectors of eggs (Thyssen *et al.*, 2004). Hence, the definite host also becomes infected while preying on infected small mammals. In both, paratenic or definite hosts, the hatched L3 performs a somatic migration to different body parts. In mice it becomes encapsulated after two or three weeks *post infectionem* in the subcutaneous tissues of the skin, the liver, brain, kidneys, lungs, heart and muscles (Anderson, 2000). Infection of the offspring through transmammary transmission (*T. cati*) or, in addition, prenatal transmission (*T. canis*) occurs in definite and paratenic hosts (Anderson, 2000). Also infections of humans occur,

causing visceral or ocular larva migrans (Antolová *et al.*, 2004). Although the ascarid *Toxascaris leonina* is endemic in canids and felids in Europe (Antolová *et al.*, 2004), and was detected in the intermediate host *Microtus oeconomus* in North America (Rausch & Fay, 2011), the author is not aware of any detection of this parasite in the six rodent species in Europe.

1.3.2.4.8 Rictulariid nematodes (Nematoda: Spirurida: Rictulariidae)

The spirurid family Rictulariidae includes two genera with the species *Rictularia proni*, *Rictularia cristata* and *Pterygodermatites hispanica* infecting *Apodemus* mice (e.g. Eira *et al.* (2006); Quentin (1973); Stammer (1956)) and rarely bank voles (Bjelic-Cabrilo *et al.*, 2011) in Europe. In addition, *Pterygodermatites kolimensis*, a typical parasite of the snow vole, was once described from bank voles in the Alps (Barus *et al.*, 1972). Like all members of the Spirurida, the life cylce includes an intermediate host, which is a scavenger insect (grasshopper, cricket, cockroach, earwig, beetle) or a diplopod in rictulariid nematodes (Anderson, 2000; Kabilov & Siddikov, 1990; Luong & Hudson, 2012). They become infected by ingestion of eggs which were shed in the faeces by the adult worms in the small intestine of the definite host. Rodents become infected by feeding on these intermediate hosts harbouring the encysted, infective L3. The life cycle may be completed in 7-8 weeks (Luong & Hudson, 2012).

1.3.2.4.9 Spirocercid nematodes (Nematoda: Spirurida: Spirocercidae)

The Spirocercidae include the cosmopolitan species *Mastophorus muris* as a parasite of rodents, marsupials, carnivores and lemurians (Rojas & Digiani, 2003), which also infects all the six mentioned rodent species in Europe (e.g. Bernard (1961); Genov (1984); Schmidt (1961); Stammer (1956)). This large nematode with a body size of up to 56 mm in males and 87 mm in females lives in the stomach of its definite hosts (Wertheim, 1962). Intermediate hosts, which may harbour infective L3 can be grasshoppers, crickets, earwigs, cockroaches, ground beetles and even fleas such as *Ctenophthalmus agyrtes* (Grzybek *et al.*, 2015b). The life cycle needs about six weeks to be completed (Quentin, 1970).

1.3.2.4.10 Gongylonematid nematodes (Nematoda: Spirurida: Gongylonematidae)

Gongylonema neoplasticum (syn. *Gongylonema problematicum*) is the only member in the monogeneric (= only one genus in the family) family Gongylonematidae, which was detected in yellow-necked mice, wood mice and bank voles (Eira *et al.*, 2006; Genov & Georgiev, 1998b; Stammer, 1956). The species is cosmopolitan, since it is mainly associated with rats and infects the mucosa of stomach and esophagus of its definite hosts (Kruidenier & Peebles, 1958). Intermediate hosts are coprophagous insects, such as cockroaches, beetles and also crickets (Anderson, 2000; Kabilov & Siddikov, 1990).

1.3.2.4.11 Peloderid nematodes (Nematode: Rhabditida: Peloderidae)

The nematodes *Pelodera cutanea* and *Pelodera orbitalis* are assigned to the Peloderidae within the paraphyletic order Rhabditida. They infect the hair follicles of the skin of *A. flavicollis* and *A. sylvaticus* and the conjunctival sacs of the eyes of all six rodent species, respectively (Schulte, 1989). Both species are facultative parasites and at least *P. orbitalis* can maintain a complete, non-parasitic life for many generations (Schulte, 1989). The *Pelodera* spp. generate three behaviourally and morphologically different types of L3: the “normal”, “dauer” and “infective” third larva (larval triphenism). The infective L3 of *P. orbitalis* actively invades the conjunctival sacs and although they do not further develop in the host and do not have a feeding apparatus, they obtain nutrients from the lachrymal fluid by osmotical absorption and grow considerably in size (Schulte, 1989). Hence, the infective L3 can be considered as truly and obligatory parasitic. The rodent serves the nematode for protection and dispersal, if conditions for the free-living life cycle are adverse (Anderson, 2000). After 3-19 days, they leave the host and continue development (Schulte, 1989). Despite the fact that hundreds of worms may infect a single eye, no pathological effects were observed in the host. At least the reports of *Pelodera strongyloides* from the mice and voles in Stammer (1956) belong to the two mentioned *Pelodera* species (Sudhaus *et al.*, 1987), just as probably other observations of *P. strongyloides* in Germany and the UK (Behnke *et al.*, 2009; Behnke *et al.*, 1999; Klimpel *et al.*, 2007a; Klimpel *et al.*, 2007b; Lewis, 1987; Montgomery & Montgomery, 1988). The dauer larvae of the species *Pelodera nidicolis*, detected in a nest of the field vole show winking behaviour and may be phoretic on the voles, but this was not observed, yet (Sudhaus *et al.*, 1987).

1.3.2.4.12 Heligmosomoid nematodes (Nematoda: Strongylida: Heligmosomoidea: Heligmosomidae and Heligmonellidae)

Two heligmosomoid families, Heligmosomidae and Heligmonellidae represent common endoparasites of the stomach and small intestine of rodents, lagomorphs and insectivores (Anderson, 2000). The nine heligmosomid species *Heligmosomoides polygyrus* (syn. *Nematospiroides dubius*, *Heligmosomoides skrjabini*), *Heligmosomoides neopolygyrus*, *Heligmosomoides laevis*, *Heligmosomoides glareoli, Heligmosomum costellatum*, *Heligmosomum pseudocostellatum*, *Heligmosomum yamagutii*, *Heligmosomum mixtum* and *Heligmosomum borealis* and the heligmonellid species *Carolinensis minutus* (syn. *Longistriata wolgaensis*, *Boreostrongylus minutus*) infect the six mice and vole species in Europe (e.g. Feliu *et al.* (1997); Haukisalmi and Henttonen (2000); Tenora (2004); Tenora *et al.* (2002); Tenora *et al.* (1983); Tenora *et al.* (1973); Zaleśny *et al.* (2014)). The heligmonellid dormouse nematode *Paraheligmonina gracilis* (syn. *Longistriata schulzi*) was only once reported in a common vole in former Czechoslovakia (Tenora *et al.*, 1973) and the shrew parasite *Longistriata depressa* once in striped field mice in Poland (Pojmańska *et al.*, 2007).

Reports of *Heligmosomum halli* from Germany (Memaran, 1970; Stammer, 1956) actually represented *H. costellatum* (Tenora *et al.*, 2002) or *H. mixtum* (Haukisalmi & Henttonen, 2000). Heligmosomoid nematodes are brick-red-coloured worms and coiled into spirals. They have a direct life cycle, which was studied for *H. polygyrus* in house mice (probably in this host the species *Heligmosomoides bakeri*) by Ehrenford (1954): Larvae hatch in the environment after 26 hours at 23-28 °C and develop to infective, non-feeding L3 after 4-6 days. The L3s are still sheated by the cuticle of the L2. When larvae are ingested by the host, they exsheet, move to the duodenum within 24 hours, where they penetrate the intestinal mucosa and develop there to adults. Afterwards, they move back to the lumen and feed on intestinal tissue (Anderson, 2000). Eggs are first observed in faeces nine days *post infectionem* and hence the life cycle needs about 15 days to be completed. Grooming seems to be important for the transmission to other hosts (Hernandez & Sukhdeo, 1995). Tissue damage and inflammation was observed due to the larval penetration of the gut mucosa during high infestations (Memaran, 1970). In temperate regions, where transmission cannot occur in winter, L4 of heligmosomoid nematodes remain as hypobiotic stages in the intestinal wall without further development until spring (“arrested development”) (Anderson, 2000).

1.3.2.4.13 Trichostrongylid nematodes (Nematoda: Strongylida: Trichostrongyloidea: Trichostrongylidae)

A quite rare nematode in the colon and rectum of the three vole species is *Trichostrongylus retortaeformis*, since it is predominantly an endoparasite of rabbits and hares (e.g. Bernard (1961); Thomas (1953)). In addition, the zoonotic sheep nematode *Trichostrongylus colubriformis* was once accidentally reported from bank voles (Grikienienė, 2005; Sato *et al.*, 2011).

1.3.2.4.14 Metastrongyloid nematodes (Nematoda: Strongylida: Metastrongyloidea)

Most members of the superfamily Metastrongyloidea occupy the lungs of their mammalian hosts and have an indirect life cycle, including mainly gastropods as intermediate host (Anderson, 2000). A single species of Angiostrongylidae, namely *Angylostrongylus dujardini*, infects wood mice and bank voles (Eira *et al.*, 2006; Mészáros, 1978; Ribas *et al.*, 2009). The hosts become infected most likely by ingestion of mainly aquatic gastropods (*Biomphalaria*, *Lymnaea*, *Planorbius*, *Retinella* spp.) harbouring infective L3 (Anderson, 2000). The larvae penetrate the intestine and move via the liver to the lungs within 28 hours. There, as well as in arteries and right heart, they develop to the L4 within 72 hours and mature to fertile adults twelve days *post infectionem*. After another four days, eggs are deposited and embryonate in the lungs (Anderson, 2000). The L1 move up the airways to the larynx, where they are swallowed, and can be detected in the faeces starting 24-26 days after infection (Spratt, 2015). The L1 are infective to the intermediate hosts and, apparently after ingestion, develop to infective L3 within about 18 days (Anderson, 2000). A small number of L3 is well

tolerated by the rodents, while a large dose of more than 50 larvae may cause clinical signs and occasionally death (Spratt, 2015). Clarke *et al.* (2004) found nematode larvae in the epididymides of 19.6% of male wood mice from UK, which were assumed to be transmitted during copulation by ejaculation of the male host. The worms were not determined morphologically, but belong to bursate nematodes (Strongylida) according to 18S rDNA sequence and grouped together with members of the Metastrongyloidea. They were genetically distinct from *A. dujardini* and probably belong to an undescribed species.

1.3.2.4.15 Strongyloidid nematodes (Nematoda: Strongyloididae)

Strongyloides ratti was observed several times in the small intestine of all six rodent species (e.g. Bernard (1961); Frank (1977); Schmidt (1961)) although it is a common parasite of rats (Schmidt, 1961; Viney, 1999). Phylogenetic studies showed that *Strongyloides* spp. (probably all Strongyloididae), which were originally included in the order Rhabditida, are distantly related to other orders including parasites of vertebrates (van Megen *et al.*, 2009). The cosmopolitan genus *Strongyloides* includes 40 species infecting the gut mucosa of tetrapod parasites. The life history of *S. ratti* includes two pathways, a single free-living generation with males and females (heterogonic) and one or several generations of parasitic females (homogonic) in the intestinal mucosa that produce eggs via mitotic parthenogenesis. The free-living adults produce eggs, which develop to infective L3. These larvae penetrate the skin of the rat host and migrate via the lymph, blood and the heart to the lungs, then up the trachea and are swallowed to reach the intestine approximately 48 hours *post infectionem* (Abadie, 1963). This route was questioned by others, who noted that larvae move directly through the subcutis of the skin to the nasofrontal region (Masataka *et al.*, 1999). Some larvae may remain as hypobiotic stages in the superficial tissues and are transmitted to sucklings during lactation (Anderson, 2000). In the intestine, larvae develop exclusively to hair-like female adults to the fourth day after skin penetration (Abadie, 1963). These females produce parthenogenetic, already embryonated eggs and L1 rapidly hatch in the faeces (Anderson, 2000). First stage larvae of *S. ratti* either develop again to infective, female L3 after two moults in the environment and repeat a homogonic, parasitic life cycle or develop to heterogonic, free-living adults of both sexes through four moults (Viney, 1994). The proportion of these progenies is determined by the genetic backround of the nematode and by several environmental factors which obviously favour one of both life cycles in or off the host (Streit, 2008).

1.3.3 Arthropoda

1.3.3.1 Fleas (Insecta: Siphonaptera)

The fleas or Siphonaptera are an order of holometabolic insects which includes about 2,000 exclusively parasitic species and 600 subspecies. Four out of five families associated with Palaearctic rodents (treating the not generally accepted Ctenophthalmidae as family and Leptopsyllidae as a subfamily of Ceratophyllidae) occur in Germany, i.e. Hystrichopsyllidae, Ctenophthalmidae, Ceratophyllidae and Pulicidae (Kutzscher & Striese, 2003; Marshall, 1981). At least 81 flea species, all belonging to these four families, have been collected from the six mouse and vole species in focus here in Europe, although some fleas of carnivores, squirrels, dormice, insectivores and birds only accidentally infested them (e.g. Beaucournu and Launay (1990); Beaucournu and Alcover (1984); Biocca *et al.* (1975); Brelih and Trilar (2000); Brinck-Lindroth and Smit (2007); Dudich and Szabó (1984); Gheoca *et al.* (2013); Gómez and Blasco-Zumeta (2002); Mahnert (1969); Peus (1959); Peus (1964); Peus (1968); Peus (1970); Rosicky *et al.* (1959); Skuratowicz (1967); Sosnina (1973); Suciu (1969); Szabó and Dely (1965); Whitaker (2007)). These species include 42 out of 72 species endemic in Germany (Kutzscher & Striese, 2003).

Fleas are wingless, laterally flatted and mostly 2-3 mm large. Despite their considerable large size, their anatomy and the sclerotinised bristles on the body surface allow rapid movement through the host fur and resistance to host grooming (Medvedev & Krasnov, 2006). The six legs of most species are specialised for jumping and crawling. The life cycle includes the egg, three larval instars, the non-feeding pupa surrounded by a silken cocoon and the adult. With their piercing-sucking mouthparts, adult fleas puncture the host skin and suck blood. Simultaneously they inject saliva, which prevents blood coagulations (Brinck-Lindroth & Smit, 2007). Flea larvae are non-parasitic and almost exclusively live in the host's nest. With the help of their chewing mouthparts, they feed on detritus and dried blood which is provided by the adult fleas with their faeces. Rodents are important hosts, since 74% of extant species are rodent fleas. The remaining species are associated with other mammals and birds. The association with the host ranges from temporary parasitic nest-dwellers to some species with females exhibiting stationary (some Pulicidae and Tungidae) or even subcutaneous parasitism (*Tunga* spp.) (Kim, 1985). The former are often categorised into "nest fleas" and "fur fleas" depending on the proportion of time they spend on the host, although intermediate life styles exist (Medvedev & Krasnov, 2006). The parasite-host-association varies from strictly host-specific species to highly opportunistic species, which are usually habitat-dependent (Medvedev & Krasnov, 2006). In the latter case, the fleas are considered to have a principal host species but are able to survive on other hosts (Brinck-Lindroth & Smit, 2007). Under optimal conditions, the life cycle

lasts 3-4 weeks. However, in univoltine species (= a single generation per year, e.g. *Peromyscopsylla*), transformation from the third larva to the pupa can take several months. Also the hatching of the adult fleas from the silk cocoon can take up to a year or even more since egress is triggered by host-derived signals such as odour or CO_2, a rapid increase in temperature, as well as air movements or vibrations (Brinck-Lindroth & Smit, 2007; Marshall, 1981).

1.3.3.2 Sucking lice (Insecta: Phthiraptera: Anoplura)

Sucking lice are like all members of the hemimetabolic order Phthiraptera obligate permanent ectoparasites of eutherian mammals (Kim, 1985). More than 532 valid species from 15 families were described and the whole diversity was estimated to be around 1,500 species worldwide (Kim, 2006). About 70% of these species are ectoparasites of rodents (Kim, 2006). Representatives of other suborders of Phthiraptera are absent on palearctic rodents, although there are two accidental reports of the crow louse *Myrsidea cornicis* (syn. *Myrsidea consimilis*, Amblycera) (Haitlinger, 1989b) and of a typical louse of the least weasel (*Mustela nivalis*), *Trichodectes mustelae* (syn. *Stachiella mustelae*, Ischnocera) (Haitlinger, 1981), on two common voles in Poland (Haitlinger, 1989b). The three families Enderleiniidae (only on squirrels), Polyplacidae and Hoplopleuridae occur on rodents in the Palaearctic, and six species were reported to regularly infest the six mice and voles in Europe discussed here: *Hoplopleura edentula*, *Polyplax hannswrangeli* and *Polyplax borealis* mainly prefer bank voles, *Hoplopleura acanthopus* field and common voles, *Hoplopleura affinis* striped field mice and *Polyplax serrata Apodemus* mice, (e.g. Beaucournu (1968); Durden and Musser (1994)). Apart from the latter species, all of them were already observed in Germany (Mey, 2003). However, there are accidental reports from these six rodents of lice normally specific to other mammals, such as *Hoplopleura longula* and *Polyplax gracilis* (Eurasian harvest mouse), *Hoplopleura captiosa* (house mouse), *Polyplax spinulosa* (rats), *Polyplax spinigera* (water voles) and *Enderleinellus nitzschi* (red squirrel) (Wegner, 1966).

Sucking lice are secondarily wingless and dorsoventrally flattened insects with piercing-sucking mouthparts. The life cycle comprises the egg, which is glued to the hair near the skin, three nymphal stages and the adult stage. Depending on the species, this cycle can be completed in up to 18 days, as for the human louse *Pediculus humanus capitis* (Kim, 2006). Since all the postembryonal stages are feeding on blood, the coevolved association of these stationary ectoparasites with the host is very close. Hence, over 63% of the anopluran species are specific to a single host species, more specifically 62% and 58% within the two families Hoplopleuridae and Polyplacidae, respectively (Kim, 2006). Transmission of sucking lice between different hosts occurs by direct contact (e.g. copulation), by sharing the same burrow or nest or by phoresy. However, the latter was only reported from sucking lice of Artiodactyla and man by different dipteran species (Marshall, 1981). The lice are able to locate

the host by its smell or the odour of lice excrements, as well as by the local increase of temperature (Marshall, 1981). On small mammals, sucking lice are more concentrated at the foreparts and head and spread lateral and dorsal with increasing infestation intensities. The most important factors determining the sites and density of sucking lice on small mammals are host grooming, followed by the hair size and configuration, the regional body temperature and moulting frequency of the host (Marshall, 1981).

1.3.3.3 Platypsylline beetles (Insecta: Coleoptera: Leiodidae)

Less than 0.03% of the enormous species diversity of holometabolic Coleoptera (beetles) is associated with vertebrates, all of them with mammals (Kim, 1985). Marshall (1981) and Kim (1985) listed 72 species from different families most of which are commensals or use the mammal for phoresy. True ectoparasites are only some members of the Staphylinidae (tribe Amblyopinini) in the Neotropics and four holarctic genera (*Leptinus*, *Leptinillus*, *Platypsyllus*, *Silphopsyllus*) (Kim, 1985; Marshall, 1981) nowadays condensed in the subfamily Platypsyllinae within the family Leiodidae (Newton, 2016). Two of the latter are feeding on Holarctic beavers and one on rodents in North America. Only six *Leptinus* spp. are associated with Palaearctic small rodents and insectivores and *Leptinus testaceus* is the only species reported to be parasitic on the mentioned six rodent species (Besuchet, 1980; Marshall, 1981; Peck, 1982). Beetles of the Platypsyllinae are small, 2-3 mm long insects with broad, dorsoventrally flattened head and body and covered with dense setae. The wings are reduced or absent (Kim, 1985). The life cycle of all beetles includes the egg, three larval instars, the pupa and the imagines. *Leptinus testaceus* adults feed on dead skin, hair, excrement and probably other secretions, whereas the larvae are non-parasitic feeding on detritus in the nest (Marshall, 1981). All species pupate off the host at the surface of the nest or below. On the host, adult *L. testaceus* can be usually found at the posterior end of the body, but they also frequently occur in the nest together with the larvae (Marshall, 1981). At least two related platypsylline beaver beetles find their host by positive CO_2 gradients and geotropism (Marshall, 1981). Although most platipsyllin beetles are relatively host-specific, *L. testaceus* was found in nests of mice, shrews and moles, but clearly prefers *Apodemus* species (Marshall, 1981).

1.3.3.4 Oestrid flies (Insecta: Diptera: Oestridae)

Parasitic diptera on birds and mammals are included in six families: Carnidae, Mystacinobiidae, Hippoboscidae (nowadays including Nycteribiidae and Strebilidae), and the myasis-producing families Calliphoridae, Sarcophagidae and Oestridae (nowadays including Cuterebridae, Gasterophilidae and Hypodermatidae) (Kim, 1985; Marshall, 1981; Systema Dipterorum, 2018). Only a few members of the Oestridae, which otherwise mainly infest large herbivorous mammals, are associated with rodents (Kim, 1985). In the Palaearctic, only the hypodermatin genera *Oestromyia* (five species) and

Portschinskia (seven species) are rodent parasites (Colwell *et al.*, 2006). *Oestromyia leporina* (syn. *Oestromyia satyrus*) is the only species occurring on rodents in Europe, since nothing is known about the biology of the parasitic stages of *Portschiskia neugebaueri,* which was never observed again since its description exclusively from its non-parasitic adult stage from the Swiss Alps in 1881 (Colwell *et al.*, 2006; Volf *et al.*, 1989). Oestrid flies are obligate parasites causing myasis in mammals, or accidentally on birds and reptils (Colwell *et al.*, 2006). Only the larvae are parasitic and have to achieve all the necessary energy to complete the life cycle, since the non-parasitic adults have reduced mouthparts, do not feed and only serve for reproduction and vectoring (Colwell *et al.*, 2006). Larvae of the subfamily Hypodermatinae, to which the two mentioned genera belong, cause dermal or subdermal myasis by penetrating the skin and developing in swellings and furuncles (Colwell *et al.*, 2006). After copulation, the adult fly is assumed to find its host mainly by olfactory stimuli at long-distances, including CO_2, but visual stimuli are most important for host finding at short ranges (Colwell *et al.*, 2006). Fertilised females glue eggs on the host. Hence, *O. leporina* glues about 30-40 eggs per host on individual hairs, mainly at the posterior part of the body and from mid-September to mid-October (Rietschel & Baumann, 1975). After about 48 hours, the 1-2 mm first-stage larvae hatch, quickly penetrate the skin and migrate for 3-4 days subcutaneously to the region near the tail and legs (Rietschel, 1975). There, the larvae create a respiratory opening in the skin, moult to the second larvae and form a warble, encapsulated by a granulomatous immune reaction (Colwell *et al.*, 2006). The *O. leporina* larvae feed on serous and cellular exudates provided by the immune reaction of the host, moult to the third larvae and grow within 26-40 days p.i. to 20-30 mm size (Colwell *et al.*, 2006; Rietschel, 1975). Mostly during movement of the rodent, the larvae leave the warble, drop to the ground, burrow about 3-5 cm into the ground and pupate (Rietschel & Baumann, 1975). In this stage, the species goes through a diapause of about 10 months, overwinters and emerges as imagines around mid-August (Rietschel & Baumann, 1975). This univoltine life cycle leads to a very short period of host infestation in late summer and autumn (Rietschel & Baumann, 1975). Even during high infestations, mortality is low and wounds often heal completely even if larvae die during infestation. However, sometimes restriction of motion was observed, which likely increases the probability of predation of the rodent host (Rietschel & Baumann, 1975). Oestrid flies are mostly very host-specific, but *O. leporina*, although quite specific to common voles in Europe, was found on other voles and muskrats, and experimentally infects wood mice, house mice and rats (Rietschel & Baumann, 1975).

1.3.3.5 Hard ticks (Acari: Ixodida: Ixodidae)

Ticks are cosmopolitan obligate blood feeders on terrestrial and semi-aquatic vertebrates and of great medical and veterinary importance as vectors of pathogens. However, 90% of the species

normally do not feed on humans and their livestock (Oliver Jr, 1989). The order includes three families: the monospecific (= only one species in the family) Nuttalliellidae, the Argasidae, called soft ticks and the Ixodidae, or hard ticks (Guglielmone *et al.*, 2010). Only members of the latter infect rodents from Germany (Petney *et al.*, 2012) and Europe. Hard ticks include 12 extant and two fossil genera with 702 species (Guglielmone *et al.*, 2010). Seven genera infest rodents from the Palaearctic: *Ixodes*, *Dermacentor, Haemaphysalis*, *Rhipicephalus*, *Hyalomma*, *Amblyomma* and *Anomalohimalaya* (Guglielmone *et al.*, 2013). At least 15 valid species (according to Guglielmone *et al.* (2013)) from the former five genera were described from the six mouse and vole species in Europe (e.g. Martyn (1988); Mihalca *et al.* (2012); Nosek *et al.* (1967); Santos-Silva *et al.* (2011)). *Ixodes ricinus* is by far the most common tick in Europe and also abundant on rodents.

The life cycle of ixodid ticks includes the egg, as well as three ectoparasitic stages: the larva, a single nymphal stage and the adult with a relatively large size, compared to other Acari, of 2-30 mm (Krantz & Walter, 2009). In the temperate region, rodents are only infested by immature stages of most ixodid ticks including *I. ricinus* (Durden, 2006) while the adults feed on larger mammals. However, there are also some species where the complete host range may include rodents (e.g. *Ixodes trianguliceps*, *Ixodes apronophorus*). Most rodent ixodids feed on three different and progressively larger individual hosts during their life, while one- and two-host ticks, remaining on the same host individual after moults, are rare (Durden, 2006). Every life stage feeds only once, but blood feeding lasts 3-7 days in larvae, 4-8 days in nymphs and 7-12 days in adult females (Oliver Jr, 1989). Only engorged hard ticks drop to the ground (in three-host-ticks) and moult to the next life stage (larva, nymph) or produce a large egg mass of 1,000-10,000 eggs before dying (female). Males in some *Ixodes* spp. feed only short-time on blood if they feed at all. *Ixodes* spp. males mate with the females on host or off the host in the nest, while members of other genera solely mate on the host (Durden, 2006). Unfed ticks actively search for a host and are able to perceive vibrations, sudden shadows, warmth and carbon dioxide from the host (Krantz & Walter, 2009). At appropriate levels in the vegetation, often at tops of grass and other herbs, ticks wait for the host in a questing posture. Attached to the host, ticks pierce the skin with their chelicerae and anchor with the toothed hypostome (Krantz & Walter, 2009). The injected saliva, a cocktail of numerous immune-modulatory and anticoagulant molecules, also includes cementitious substances for fixation (Francischetti *et al.*, 2009). The typical life span of hard ticks is very variable. In tropical one-host-ticks three to four generations can develop per year whereas life spans of usually one year occur in warmer temperate and of one to three years in colder temperate regions (Oliver Jr, 1989). The latter is the case for *I. ricinus* in Germany. Hence, the life stages are able to starve for a long time. The host spectrum of many species is relatively wide, most notably for *I. ricinus* which feeds on more than 300 mammalian,

avian and reptile species (Stanek, 2009). Infestation with ticks causes injury to the host, and during high infestations and especially in small animals, hosts may suffer from blood-loss and die through exsanguination (Krantz & Walter, 2009). More importantly, ticks transmit a wider variety of pathogens, than any other arthropod group, as already addressed (see chapter 1.1).

1.3.3.6 Laelapid mites (Acari: Mesostigmata: Gamasina: Laelapidae)

The majority of mites from the order Mesostigmata (Parasitiformes) are free-living predators feeding on arthropods and nematodes (Dowling, 2006). Traditionally, all parasites of mammals within the mite order Mesostigmata are members of the superfamily Dermanyssoidea, although modern phylogenetics tends to exclude two families of bat parasites. Within this superfamily, members of four families are associated with Palaearctic rodents, namely the Laelapidae, Dermanyssidae Macronyssidae and Halarachnidae. The author here considers Haemogamasidae and Hirstionyssidae as subfamilies of Laelapidae according to Mašán and Fenďa (2010). Halarachnidae are, within rodents, only parasites of the respiratory tracts of squirrels and porcupines (Dowling, 2006). Dermanyssidae and Macronyssidae are not introduced in this work about parasites of non-commensal rodents, since the former family only includes rodent ectoparasites in the genus *Liponyssoides* infesting house mice and the latter only includes neotropical rodent parasites, of which only *Ornithonyssus bacoti* was introduced in Europe on rats (Dowling, 2006). However, the macronyssid bat parasite *Steatonyssus spinosus* and *O. bacoti* were once accidentally reported on a single field vole and striped field mice, respectively (Haitlinger, 1983b; Haitlinger, 2007).

The Laelapidae are important and diverse nest inhabitants of wild rodents and insectivores and at least 46 species have been collected from the six rodent species in Europe (e.g. Edler and Mehl (1972); Haitlinger (1986a); Haitlinger (1989b); Haitlinger (1997); Haitlinger (2008); Haitlinger (2011); Mašán and Fenďa (2010)). As most other dermanyssoids, these mites have five life stages with eggs, larvae, proto- and deutonymphs and adults. Only the larvae have six legs, the later stages are octopod. They are fluid feeders and if not feeding on body fluids, the chelicerae macerate the food prior to feeding (Colwell *et al.*, 2006). Life styles of members of this family represent all forms from free-living over obligate nidicolous predation to facultative and finally obligate ectoparasitism, which has evolved multiple times within Laelapidae (Kim, 1985). Hence, classification as a parasite is challenging, since members closely related to the basal, non-parasitic subfamily Hypoaspidinae are at least able to feed on dried or fluid blood and occasionally have been observed feeding on pre-existing wounds, such as *Androlaelaps fahrenholzi* (Laelapinae) (Kim, 1985). This species, but also *Haemogamasus nidi* (Haemogamasinae) is able to live and reproduce with blood diet, but has highest reproduction rates during mixed diet with arthropods (Kim, 1985). For many species it is not clear if they are able to penetrate host skin or only feed on blood and lymph if a host is already injured. The

highest adaptation to parasitism occurs in the subfamilies Hirstionyssinae and Myonyssinae where members have more slender, elongate chelicerae specialised for piercing skin compared to the mainly chelate dentate mouthparts in the other subfamilies used to abrade skin to induce flow of body fluids (Kim, 1985; Krantz & Walter, 2009). Hirstionyssinae, or particularly *Echinonyssus* sp. also reveal other adaptations such as decrease in size, spurs for better attachment to host hair and skin, non-feeding, ephemeral larvae and protonymphs and obligate blood feeding in deutonymphal and adult life stages (Kim, 1985). In a number of species, response to CO_2 was observed. Particularly noticeable is the pronounced female bias in the adult stage of many species. This was partly explained by a shorter lifespan of males. With increasing adaptation to the host in Hirstionyssinae, their mouthparts are increasingly adapted for sperm transfer, which hinders feeding and leads to starvation (Kim, 1985). In addition, parthenogenesis is assumed for species with rare or absent males (Mitchell, 1968).

1.3.3.7 Trombiculid mites (Acari: Parasitiformes: Prostigmata: Trombiculidae)

While only about 100 species were described in this family until 1929, the recognition, that some species transmit the causative agent of Tsutsugamushi disease during the Second World War led to strong interest in these mites and the number of described species today exceeds 3,000 (Brennan & Goff, 1977). Most of them are only known from the larval life stage, called chiggers. Hence, the taxonomy, only based on larval morphology, is not finally solved and chiggers of Leeuwenhoekiinae are either included in this family or treated as a separate family (Shatrov & Kudryashova, 2006). At least 17 species were collected from the six *Microtus*, *Myodes* and *Apodemus* spp. in Europe (e.g. Haitlinger (1980); Kepka (1964); Kovacik (1984); Moniuszko and Mąkol (2014)).

The parasitic life of Trombiculidae as obligate periodic ectoparasites of diverse vertebrates is short, since only the larva feeds on the host for several hours or days (Shatrov & Kudryashova, 2006). The subsequent life stages are either quiescent (proto- and tritonymph) or soil-dwelling predators of arthropods, preferably of their eggs (deutonymph and adults) (Shatrov & Kudryashova, 2006). While the duration of the calyptostatic (= neither legs nor mouthparts are functional) proto- and tritonymphs typically does not exceed 25-30 days, the active larvae can survive 200 days without food, the deutonymphs can live more than 500 days and the adults even 1,000 days in *Hirsutiella zachvatkini* (Shatrov & Kudryashova, 2006). However, the natural life cycle, at least in boreal regions, lasts about 150-400 days. Females lay several tens to hundreds eggs in 1-3 oviposition cycles (Shatrov & Kudryashova, 2006). The ectoparasitic larvae are small mites (about 200 µm) with a wrinkled cuticula allowing rapid increase of the body volume during feeding. Larvae of some *Neotrombicula* spp. hatch from the egg in summer and autumn. Comparable to hard ticks, they are positively phototactic as well as negatively geotactic. Particularly on warm and dry days, they climb towards

the tips of grass and other elevated sites waiting for a host (Kampen, 2002; Stekolnikov *et al.*, 2014). However, the mites normally do not climb higher than 20-30 cm in the vegetation since they are strongly dependent on a high relative humidity of at least 78% (Kampen, 2002). On the host, trombiculid larvae form a typical stylostome, a feeding tube in the epidermis of their host, to feed on lysed host tissue (Krantz & Walter, 2009). With their chelicerae, they cut the *stratum corneum* and inject saliva. This contains substances to form the walls of the feeding chamber and to lyse the tissue. Extra-oral digestion and feeding on fluid, dissolved lymphoid and epithelial cells is necessary, since the larvae have a discontinuous alimentary tract and cannot defecate solid faeces (Krantz & Walter, 2009; Shatrov & Kudryashova, 2006). Some species are invaginated in capsules during feeding, because the site of the stylosome is depressed and concave and the surrounding epidermis undergoes strong hyperplasia and hyperkeratosis (Shatrov & Kudryashova, 2006). The preferred attachment sites on the host are the head and ears, abdomen, armpits, genitalia, anus and tail base (Shatrov & Kudryashova, 2006). There, chiggers can cause skin irritations, scrub itch and pruritic dermatitis, called trombidiosis or trombiculosis (Kim, 1985). The host specifity of trombiculid species is very low. For example, the harvest mite *Neotrombicula autumnalis* was found on more than 39 host species of mammals, birds and reptiles (Fuller, 1952; Kepka, 1964). Chiggers are obviously more dependent on a specific habitat, where they attack all or most of the occurring vertebrate species, but often prefer rodents (Shatrov & Kudryashova, 2006).

1.3.3.8 Speleognathine mites (Acari: Parasitiformes: Prostigmata: Ereynetidae: Speleognathinae)

The Ereynetidae are a family with about 180 described species from three subfamilies (Krantz & Walter, 2009). The members of the subfamily Ereynetinae are mostly free-living or associated with insects and snails, while Lawrencarinae live in the nasal cavities of amphibians and Speleognathinae in those of mammals and birds (André & Fain, 2000). Both, Ereynetidae, as well as Speleognathinae are considered monophyletic (André & Fain, 2000). Within the latter subfamily, three out of five tribes are associated with diverse mammals (Dasyuromorph marsupials, bats, rodents, primates, even-toed ungulates), but only within Paraspeleognathopsini, ten species are endoparasites on the nasal mucosa of rodents (Fain, 1985). *Paraspeleognathopsis bakeri* infects wood mice and *Speleorodens michigensis* (syn. *Paraspeleognathopsis clethrionomys*) bank and field voles (Fain & Lukoschus, 1968).

Ereynetid mites appear to be restricted to wet or humid habitats, hence they evolved from using habitats such as soils in temperate and tropical regions to the elytra of aquatic insects and to the respiratory system of vertebrates such as members of the cosmopolitan Speleognathinae (André &

Fain, 2000; Krantz & Walter, 2009). Mites from this subfamily have no nymphal life stages or only calyptostatic nymphs and develop from the hexapod larvae directly to the adults (Krantz & Walter, 2009). The females are ovoviviparous or viviparous (Fain, 1972). If the host dies, Speleognathinae are able to leave the nasal cavities to find a new host (Fain, 1956a).

1.3.3.9 Myobiid mites (Acari: Parasitiformes: Prodstigmata: Myobiidae)

The cosmopolitan mite family Myobiidae comprises over 580 species from 53 genera and five subfamilies, all of them obligate, stationary ectoparasites of small mammals (Bochkov, 2009b; Bochkov, 2011). Only the subfamily Myobiinae with nine genera and 137 species includes several ectoparasites of rodents but only the two genera *Radfordia* and *Myobia* were detected on muroid rodents in Europe (Bochkov, 2009b). Findings of the shrew parasites *Amorphacarus elongatus* (Haitlinger, 1977a; Haitlinger, 2009a), *Amorphacarus phillipsae* (syn. *Amorphacarus parvisetosus*) (Haitlinger, 1980) and *Protomyobia onoi* (Haitlinger, 1989b; Haitlinger, 2010a) were likely contaminations according to Bochkov (2009b) and not considered here.

The highly specialised mites with a size of about 0.3-0.6 mm are characterised by modified first legs used for clasping two host hairs (Haitlinger, 1988; Krantz & Walter, 2009). With their stylet-like chelicerae, they pierce the skin and feed on lymph, blood and other extracellular tissue fluids. On laboratory rodents, myobiid mites were reported to cause dermatitis, alopecia and trauma. The life cycle includes five postembryonal stages: the hexapod larvae and the octopod proto-, deuto- and tritonymphs and adults (Krantz & Walter, 2009). Eggs are glued at the bases of the hairs by the female (Baker, 2007). In *Myobia murismusculi*, the life cycle is completed in about 23 days. Transmission occurs by direct contact between hosts and also from mother to sucklings (Baker, 2007). Probably due to the high specialisation of the clasping organs, host specificity is high and every species only infests hosts from the same species or genus (Bochkov & Labrzycka, 2003). Therefore, phylogeny of the Myobiidae is in strong coherence to the phylogeny of the therian mammalian hosts (Bochkov, 2011).

1.3.3.10 Psorergatid and Demodecid mites (Acari: Parasitiformes: Prostigmata: Psorergatidae and Demodecidae)

Both of these groups are included in the superfamily Cheyletoidea, and these two closely related families comprise highly specialised, obligate and exclusive skin parasites of mammals. As other Cheyletoidea, the life cycle includes eggs, larvae, proto- and deutonymphs and adults.

At least 70 species from three genera were described from the cosmopolitan Psorergatidae (Izdebska & Fryderyk, 2012). While *Psorergatoides* spp. infect bats and *Psorobia* spp., among other mammals,

only the Canadian beaver and South African porcupines and mole-rats, some species of *Psorergates* also occur on Palaearctic rodents (Giesen, 1990). Nine *Psorergates* species have been observed on the six rodent species in Europe (Fain *et al.*, 1966; Giesen, 1990; Lukoschus *et al.*, 1967). Psorergatid mites are small (90-220 µm), round and dorso-ventrally flattened, and live in pits and hair follicles of the superficial skin layers of the hosts (Giesen, 1990; Krantz & Walter, 2009). They can cause dermatitis, mange and follicular infection. This is often aggravated by biting and rubbing by the host (Krantz & Walter, 2009). Three types of infections were described: (1) in pits of the inner ear concho, (2) in pits at the tibia region (or occasionally the genital region) with scarce hair implantation or (3) in hair follicles at the femur with thicker hair implantation. In all of them, hypertrophy and hyperkeratosis of the epidermis occurs, though to different extents (Giesen, 1990).

The Demodicidae included seven genera and 86 species in 2008 (Bochkov, 2008) but recently several *Demodex* species have been newly described alone from muroid rodents from Poland (Izdebska & Rolbiecki, 2013a; Izdebska, 2012; Izdebska & Rolbiecki, 2013c; Izdebska & Rolbiecki, 2014; Izdebska *et al.*, 2014). In total, 38 demodicid species were recorded from rodents (Izdebska & Rolbiecki, 2014; Izdebska *et al.*, 2014) of which 14 were reported from the six rodents of interest here in Europe (Bochkov, 2009b; Izdebska & Rolbiecki, 2013a; Izdebska, 2012; Izdebska & Rolbiecki, 2013c; Izdebska *et al.*, 2014). Several species can be detected on individual hosts, since they inhabit different microhabitats (Izdebska & Rolbiecki, 2014). These obligate, stationary parasites are small, usually wormlike and live in the hair follicles and Meibomian glands of their hosts. Occasionally, they were reported from auricular tissue, and even digestive tract and circulatory system (Krantz & Walter, 2009). Demodicid mites feed on cells of sebaceous glands and epithelium. They can induce hyperplasia, epidermal destruction and granuloma, but pathology is low in most wild animals. Nevertheless, hyperkeratosis and alopecia can occur in cases of high infestation intensities (Krantz & Walter, 2009). As in Psorergatidae, transmission occurs by direct contact of hosts, particularly from mother to sucklings (Baker, 2007).

1.3.3.11 Epimyodicid mites (Acari: Parasitiformes: Prostigmata: Epimyodicidae)

Four species have been descibed from this monogeneric family infesting moles (*Epimyodex talpae*), greater white-toothed shrews (*Epimyodex crocidurae*), Trowbridge's shrews (*Epimyodex soricis*), or the rodents common vole, Savi's pine vole and wood mouse (*Epimyodex microti*) from Europe and North America (Bochkov, 2002; Fain & Bochkov, 2001a; Fain *et al.*, 1982). Little is known about these parasites detected in the connective subcutaneous or perimusculature tissues of their hosts (Fain *et al.*, 1982). The mites are 165-250 µm in size, slightly dorso-ventrally flattened and the postembryonal development includes a larval, one nymphal and the adult stage (Bochkov, 2002; Fain & Bochkov, 2001a; Fain *et al.*, 1982). Fain *et al.* (1982) suggested that mites migrate to the reproductive system

of the host and that transmission occurs during pregnancy via transplacentar infection or during copulation by infected sperm, which was suggested for the closely related *Cloacarus* spp. (Cloacaridae). However, they were not detected in the uteri or foetuses of infested, pregnant moles, but present in the preputial gland of males. Lesions were absent at the sites of infestation.

1.3.3.12 Myocoptid mites (Acari: Acariformes: Astigmata: Myocoptidae)

This relatively small, cosmopolitan family comprises 64 species from six genera infecting rodents and a single species of mustelid parasites (Bochkov, 2016). The three genera *Myocoptes*, *Trichoecius* and *Criniscansor* occur on muroid rodents in Europe and of those, eight species parasitise the six rodent species of interest here (Bochkov, 2010). The obligate, permanent stationary ectoparasites are dorso-ventrally flattened with the exception of female *Trichoecius* sp. which are subcylindrical (Bochkov, 2010). The Myocoptidae are characterised by morphological adaptations of legs III and IV in females and immatures, or legs III in males specialised for clasping one (*Trichoecius* spp. females) or two hairs (other myocoptids) (Fain *et al.*, 1970; Krantz & Walter, 2009). The life cycle of the oviparous mites comprises eggs, larvae, proto- and tritonymphs and adults (Bochkov, 2010; Fain *et al.*, 1970). Myocoptidae are mainly feeding on tissue fluids from skin, which is locally abraded with the mouthparts. Only *Trichoecius* spp. feed on substances in the fur (Krantz & Walter, 2009). While *Trichoecius* spp. is rather located particularly at the posterior part of the body, other myocoptids prefer the inside of the legs of the host, spreading towards head and other body parts in cases of high infestation intensity (Fain *et al.*, 1970). *Myocoptes musculinus* is known as a pest in laboratory mouse colonies, where high infestation intensities can lead to skin irritation and hair loss (Fain *et al.*, 1970; Krantz & Walter, 2009).

1.3.3.13 Listrophorid mites (Acari: Acariformes: Astigmata: Listrophoridae)

Listrophoridae are mites infesting the fur of diverse therian mammals and comprise two subfamilies with 20 genera and 167 species (Bochkov, 2010). While the monospecific Aplodontochirinae were described from North American rodents, the Listrophorinae infest mammals in the Holarctic, Africa and South America (Bochkov, 2010). Only four genera were reported from rodents in Europe, with *Listrophorus brevipes*, *Listrophorus leuckarti* and *Listrophorus mediterraneus* on cricetid and *Afrolistrophorus apodemi* on murid rodents (Bochkov, 2010). Listrophorid mites are considered obligate, permanent and stationary ectoparasites feeding on sebaceous material in the fur of mammals, although they generally cause no damage of the hair (Krantz & Walter, 2009). Pathogenicity has not been described although thousands of mites can infest a single host, which looks like powdered if it dies, since mites move to the hair-ends (Willmann, 1952). The body of Listrophorinae is subcylindrical and elongate and characterised by a specialised clasping-apparatus at

the gnathosoma to contact a single host hair (Krantz & Walter, 2009). Males are usually smaller and more sclerotised than the oviparous females and have paranal suckers to attach to the females (Bochkov, 2010; Krantz & Walter, 2009). The life cycles include eggs, proto- and tritonymphs as well as the adults (Wurst, 1993).

1.3.3.14 Yunkeracarine mites (Acari: Acariformes: Astigmata: Gastronyssidae: Yunkeracarinae)

The family Gastronyssidae with nine genera and 42 species is divided into the two subfamilies Gastronyssinae with 29 species parasitising on bats, and the Yunkeracarinae with 13 rodent parasite species (Bochkov, 2010; Fain, 1994; Kim, 1985). The ovoviviparous yunkeracarine mites live in the nasal cavity of squirrels (*Sciuracarus*), cricetid and murid hosts (*Yunkeracarus*) in Africa, America and Eurasia (Bochkov, 2010; Smith *et al.*, 1985). Only three species of *Yunkeracarus*, were described from European mice and voles, in particular *Yunkeracarus apodemi* from wood mice and *Yunkeracarus microti* from common voles (Bochkov, 2010). However, nearly nothing is known about their biology or life cycle.

1.3.3.15 Sarcoptid mites (Acari: Acariformes: Astigmata: Sarcoptidae)

The family Sarcoptidae comprises three subfamilies with 15 genera and 117 species (Bochkov, 2010). Only members of the two genera *Trixacarus* (Sarcoptinae) and *Notoedres* (Teinocoptinae) infest rodents (Bochkov, 2010). Three species of *Trixacarus* were described from guinea pigs and muroid rodents, however, in Europe, only rats have been described to be infested. The 45 species of the genus *Notoedres* are primarily bat parasites, but eight species were secondarily associated with rodents and three species with other mammals. Thereof, two species are ectoparasites on rodents in Europe, i.e. *Notoedres muris* on Eurasian hamster, European water vole, black and Norway rat, and *Notoedres musculi* on house mouse and yellow-necked mouse (Bochkov, 2010). The Sarcoptidae are small, obligate permanent and stationary parasites in the upper epidermal layers of mammals (Krantz & Walter, 2009). The life cycle, including eggs, larvae, proto-, tritonymphs and adults, can be completed in two weeks (Foley *et al.*, 2016; Kim, 1985). The sexually dimorph mites have globose or slightly dorsoventrally flattened and oviparous females and smaller, more sclerotised males (Krantz & Walter, 2009). With the help of short spines at the tarsi of the short legs, the mites form tunnels in the corneous layers of the hosts' skin (Kim, 1985). *Notoedres* mites can thereby cause mange with potentially fatal contagious dermatitis. This is initiated by the burrowing and the secretions of the mites in the epidermis and is mediated by the immune response of the host (Foley *et al.*, 2016). An intensive pruritus leads to scratching and self-mutilation, accompanied with secondary bacterial infections as well as behavioural changes (Foley *et al.*, 2016). Fatal notoedric mange caused by

Notoedres centrifera and *Notoedres cati* was reported from tree squirrels and felids, respectively. Such infestations have been shown to kill half of the population, but such reports from small rodents are absent (Foley *et al.*, 2016). *Notoedres muris* was detected in the stratum corneum of the extremities, ear pinnae, nose, eyelids and tail of the muroid hosts leading to scab formation and epidermal cell proliferation (Foley *et al.*, 2016).

1.3.3.16 Pneumocoptid mites (Acari: Acariformes: Astigmata: Pneumocoptidae)

Members of this monogeneric family are, together with the genus *Pneumonyssus* (Mesostigmata: Halarachnidae), the only mites living as endoparasites in the lungs of mammals (Loos-Frank & Abel, 1983). *Pneumocoptes* with four known species are restricted to Holarctic rodents, but only *Pneumocoptes tiollaisi* was found in Europe, more precisely in three reports in bank voles in France, Germany and Switzerland (Kouchakji & Loos-Frank, 1984). The ovoviviparous mites live in the bronchi, bronchioli and alveoli and are 140-200 µm in size in the adult stages (Bochkov, 2010; Loos-Frank & Abel, 1983). In cases of high infestation intensities, mites were also detected in the trachea. For transmission to other hosts, the authors suggested, that mites migrate along the trachea or are expectorated and that mainly sucklings are infected during licking and cleaning by the mother. Although sucklings of other vole species can be infected experimentally, *P. tiollaisi* was only detected in wild bank voles (Loos-Frank & Abel, 1983).

1.3.3.17 Glycyphagid mites (Acari: Acariformes: Astigmata: Glycyphagidae)

Within the family Glycyphagidae with 41 genera and at least 192 species, most species are associated with the nests of insectivores, rodents, and New World marsupials (Krantz & Walter, 2009). None of them are typical parasites, since they do not feed on mammals, but the initially phoretic association with the hair of the hosts evolved to an endofollicular localisation of the non-feeding hypopi (= heteromorphic deutonymph without functional mouthparts) in the subfamilies Ctenoglyphinae, Lophuromyopidinae and some members of the Metalabidophorinae (Kim, 1985; Krantz & Walter, 2009; OConnor, 1982). In these species, the organs for attachment to the hair are reduced or completely regressed (OConnor, 1982). Some species were described to cause skin lesions in the rodent host (Kim, 1985). The life cycle of *Lophioglyphus liciosus* (= *Apodemopus apodemi*) infesting *Apodemus* mice was studied by Lukoschus *et al.* (1972): Tissue hypopi, infesting the hair follicles under the scales of the tail of wood mice, developed outside the host to tritonymphs after three days and to adults after seven days. First eggs were observed at the 9th day, larvae at the 12th day, protonymphs at the 14th day and free (probably infective) hypopi at the 17th day. However, infection of adult wood mice with these hypopi was not succusful. The authors suggested that the hypopi leave the host and maintain their short free-living lifespan during pregnancy of the mice.

Subsequently, they infest the resulting nestlings, where they complete their long endofollicular period. This was supported by high prevalences of 79% (n=154) and intensities of 1-182 mites per host in wood mice in September in Belgium, decreasing infestation rates in winter and spring and absence in lactating mice. Despite absence of mouthparts, tissue hypopi drastically increase in size during infestation, since they absorb nutrients through the cuticle (Krantz & Walter, 2009). Hence, this species can be considered a true parasite, comparable to the above mentioned nematode *Pelodera orbitalis* (Peloderidae). Hypertrophy and paraceratosis of the *stratum corneum* around the endofollicular hypopi and degeneration of connective tissue was observed during high infestations and hence, the mite considered low grade pathogenic.

1.3.4 Phoretic mites (different taxonomic groups)

Several groups of mites attach to rodents only for the purpose of dispersal to other habitats, mainly nests of other mammals. These phoretic mites do not feed on the host and normally do not cause any harm to the host (except for some Glycyphagidae as mentioned above).

Most of the about 50 *Pygmephorus* mite species (Acari: Parasitiformes: Prostigmata: Pygmephoridae) occur in the nests of mammals. Many Pygmephoridae (e.g. *Pediculaster*, *Pediculitopsis* spp.) have two morphologically different types of females: phoretic and non-phoretic females (phoretomorphy). Whether this is the case in *Pygmephorus* sp. is not known, yet (Krantz & Walter, 2009). The phoretic females have specialised fist-like clasping organs at first legs to attach to host fur.

In Astigmata, the deutonymphs are generally highly specialised for dispersal and able to withstand adverse environmental conditions (Krantz & Walter, 2009). Apart from the mentioned endofollicular species, the non-feeding hypopi of some glycyphagid mites (Acari: Acariformes: Astigmata: Glycyphagidae) have evolved a clasping organ at the ventral, posterior part of the body used for clasping a hair of the host. A similar organ has evolved in the deutonymphs of the nidicolous genus *Prowichmannia* (Acari: Acariformes: Astigmata: Histiostomatodae = Anoetidae) to attach to rodent hair (Krantz & Walter, 2009). Deutonymphs of some Acaridae (Acari: Acariformes: Astigmata), such as *Acarus nidicolous*, are indirectly phoretic on rodents, since they are actually phoretic on their fleas (Fain & Beaucournu, 1993).

1.3.5 Other arthropods in the fur of rodents

Many other arthropod species were detected occasionally in the fur of wild rodents, which are neither specialised for parasitism nor for phoresy. Almost all are nidicolous mites using the special habitat of the small mammal nest. One could argue they have no benefit from the (temporary) association, since they probably moved accidentally onto the rodent in the nest or were stripped off

by the host. Nethertheless, the population might benefit at least from dispersal by the host in this "accidental phoresy". About three fourth of the mite species of the family Cheyletidae (Acari: Parasitiformes: Prostigmata: Cheyletidae) are free-living predators. However, specimens of *Hemicheyletus* sp. and *Chelacaropsis moorei* were observed preying on ectoparasitic arthropods in the fur of mammals (Krantz & Walter, 2009).

Apart from some not mentioned, free-living (non-phoretic) members of the introduced families Glycyphagidae, Acaridae and Histiostomatidae, the following additional mite families were observed in the fur of muroid rodents from the six mentioned species in Europe: Mesostigmata: Eviphididae, Macrochelidae, Pachylaelapidae, Phytoseiidae, Blattisociidae, Ameroseiidae, Melacharidae, Ascidae, Ologamasidae, Veigaiidae, Parasitidae, Zerconidae, Uropodina (not further determined), Prostigmata: Anystidae, Tetranychidae, Tarsonemidae, Trombidiidae, Erythraeidae, Siteroptidae, Oribatida: Damaeidae, Nothridae, Camisiidae (e.g. Haitlinger (1983a); Haitlinger (1986a); Haitlinger (1989b); Haitlinger (1997); Haitlinger (2015); Stammer (1956)). Apart from Tetranychidae and Tarsonemidae, which were only once detected by Haitlinger (1997), and Oribatida, all other families are predators or parasites of invertebrates (Krantz & Walter, 2009) and hence considerably mobile, which may be the reason for their occasional random occurrence in the rodent fur.

1.4 Human-infesting and zoonotic rodent parasites

The tick *I. ricinus* and the tick-borne pathogens, for which it is a vector, were already introduced in chapter 1.1. However, a number of other rodent-associated parasites infect humans and some of them are vectors of zoonotic pathogens (Appendix 8-1).

The digenean trematodes *I. melis* and *P. muris* are zoonotic pathogens using also humans as definite hosts (Hong *et al.*, 1996; Radev *et al.*, 2009). The same apparently applies for *P. elegans*, since it was considered to be a synonym of *P. muris* (Tenora *et al.*, 1983). Infection probably occurs through ingestion of insufficiently heated freshwater fishes, which are second intermediate hosts (Hong *et al.*, 1996; Radev *et al.*, 2009). Similarly, seven *Echinostoma* spp. are zoonotic parasites with cases in Far East and Southeast Asia (Chai, 2009), but the specimens detected in the rodents by Edelényi (1966) and Hildebrand *et al.* (2004) were not identified to species level. Humans as definite hosts were infected by ingestion of undercooked molluscs, snails, amphibians and fishes as second intermediate hosts (Graczyk & Fried, 1998; Huffman & Fried, 1990). Also *Alaria alata* is considered potentially zoonotic, since mesocercariae of other *Alaria* spp. caused disease in patients in North America, mostly after ingestion of frog legs (Möhl *et al.*, 2009). Reports of *I. melis*, *Echinostoma* sp. and

A. alata are very rare in the rodents, but *P. muris* pose a zoonotic risk and infected rodents contribute to the enzootic cycle.

As mentioned above, the zoonotic tapeworms *H. diminuta*, *R. microstoma* and *R. nana* were probably misidentified in the rodents of focus here. Plerocercoids of *S. erinacei and* metacestodes of *M. lineatus*, *H. taeniaeformis*, *T. crassiceps* and *T. martis* were rarely detected in patients, but a major threat are infections with *E. multilocularis* (Nakao *et al.*, 2010). Humans become infected by ingestion of taeniid eggs after contact with faeces of infected carnivorous definite hosts. The hatching oncosphere larvae invade various tissues and develop to hyatid metacestodes in *E. multilocularis*, which multiply and enlarge. In humans, the invasive larval development, called alveolar echinococcosis is lethal without treatment (Nakao *et al.*, 2010).

The acanthocephalan *M. moniliformis* is zoonotic and humans become infected after ingestion of invertebrate intermediate hosts (Salehabadi *et al.*, 2008) but the six rodent species virtually do not play a role in the epidemiology of *M. moniliformis* in Europe, since infections are very rare.

Rodents are reservoir hosts for the zoonotic nematode *C. hepatica*. The route of transmission for mammals, including humans, is through ingestion of infective eggs in faeces, after passage through a predator, that fed infected rodent liver. Prevalences in mice and voles can be quite high (Schmidt *et al.*, 1998). In contrast, *Trichinella* sp. and *T. colubriformis* are very rare and the six rodent species do not play a role in the transmission cycle of these zoonotic parasites in Europe. *Toxocara* sp. infections are quite common in the paratenic wild rodents according to seroprevalence studies (Dubinský *et al.*, 1995; Reiterová *et al.*, 2013) and there are many cases of human larval toxocariasis manifesting as *larva migrans visceralis* or *larva migrans ocularis* (Kinčeková *et al.*, 2009). Patients were infected by *T. canis* and *T. cati* through contact with infective eggs shed with faeces by definite canine and felid hosts. The disease is caused by the larvae which hatch from the ingested eggs and migrate through different organs (Kinčeková *et al.*, 2009).

At least eleven species of fleas have been reported to infest humans (Beaucournu & Launay, 1990; Brinck-Lindroth & Smit, 2007; Peus, 1968; Peus, 1972; Whitaker, 2007). However, most species are either (1) commonly reported from humans but only accidentally on the mice and voles (*Pulex irritans*, *Ctenocephalides canis*, *Ctenocephalides felis*, *Ceratophyllus gallinae*, *Monopsyllus sciurorum*) or (2) common on rodents but with rare reports of human infestation (*Leptopsylla segnis*, *Amalareus penicilliger*, *Dasypsyllus gallinulae*) or (3) rare on both (*Ceratophyllus rusticus*, *Ceratophyllus garei*). Only the species *Nosopsyllus fasciatus*, which is also common on rats, is a frequent flea on the mice and voles and also one of the common fleas infesting humans (Brinck-Lindroth & Smit, 2007). Hence, people who get in contact to the rodents or their nests may become infested. This species is a

competent vector for the tapeworms *R. nana* and *H. diminuta* (Marshall, 1981) and the pathogens *Yersinia pestis*, *Rickettsia typhi* (Eisen & Gage, 2012) and probably *Bartonella* spp. (Silaghi *et al.*, 2016b).

Within the hard ticks, which were detected on the six rodent species in Europe, all other 14 species than *I. ricinus* have also, although less commonly, been reported to infest humans (Guglielmone *et al.*, 2013). In addition to the mentioned *I. ricinus*-borne pathogens, these species transmit the zoonotic disease agents Crimean Congo haemoragic fever virus, Bhanja virus, Tribeč virus, *Rickettsia conorii*, *R. raoulti*, *Rickettsia slovaca*, *R sibirica*, *R. aeschlimanni*, *R. massiliae* and probably Erve virus, Dhori virus, Thogoto virus and *Bartonella* spp. in Europe (Hubálek, 2009; Hubálek & Rudolf, 2012; Maltezou *et al.*, 2010; Petney *et al.*, 2015; Petney *et al.*, 2012; Treib *et al.*, 1998).

Human infestations with the gamasid mite species occurring on the six mouse and vole species in Europe are rare and often doubtful. *Haemogamasus pontiger* was reported to parasitise soldiers in England during Second World War but that was more likely caused by *Pyemotis* mites. Dermatitis caused by *Androlaelaps casalis* in England and Israel resulted from contacts with pigeon or rat nests (Baker *et al.*, 1956; Rosen *et al.*, 2002) but the species is probably not able to penetrate intact skin (Lesna *et al.*, 2009; McKinley, 1963).

Chiggers of the trombiculid harvest mite *N. autumnalis* and probably *Neotrombicula inopinata* infest rodents as well as humans, causing pruritic dermatitis and scrub itch (Stekolnikov *et al.*, 2014). Although *Rickettsia* DNA was detected in high prevalence in *N. autumnalis* (Miťková *et al.*, 2015), vector function has not been confirmed so far.

The astigmate storage mite *G. domesticus* is a causal agent of allergic dermatitis called “grocer’s itch” (Krantz & Walter, 2009) but rodents are at most rare mechanical vectors for this mite species.

1.5 Co-occurrence and interaction of parasites

Polyparasitism is a common pattern in wild vertebrates including rodents. Due to the large diversity of rodent-associated parasitic species (chapter 1.3), co-infections are very common and not at all an exception. If co-infections of hosts by different species occur more/less commonly than expected, the reason for this may be simply passive, due to similar/different transmission routes or seasonality. But they can also result from active heterologous synergistic/antagonistic interaction between the species (Behnke *et al.*, 2001). These interactions can range from increase/reduction in growth, fecundity and lifespan to enhancement/inhibition of establishment or death of one or both parasites (Christensen *et al.*, 1987). In natural systems, the verification of patterns of co-existence is

challenging, since extrinsic factors and their interaction is complex. Season, year, site, host abundance, as well as the intrinsic factors age and sex are important factors determining prevalence and intensity of parasite infections (Abu-Madi *et al.*, 1998; Abu-Madi *et al.*, 2000; Behnke *et al.*, 1999; Haukisalmi *et al.*, 1988; Kiffner *et al.*, 2011; Kisielewska, 1970a) and have to be thourougly controlled as confounding factors (Behnke *et al.*, 2001; Haukisalmi & Henttonen, 1993a). Consequently, co-infections were mainly studied in laboratory rodents under controlled conditions.

The majority of experimental studies on co-infections in rodents were conducted on endoparasites, either between different helminth species or between helminths and protozoa. Most of these investigations were conducted to study interactions of parasites of human and lifestock (e.g. *Schistosoma*, *Fasciola*, *Taenia*, *Trichuris*, *Trichinella*, *Ascaris*, *Ancylostoma, Plasmodium, Babesia, Trypanosoma* spp.) (See Christensen *et al.* (1987)), which often do not occur/co-occur in wild rodents. Nematodes were most often included in such experiments to analyse the influence of co-infections with other endoparasites in laboratory mice (Behnke *et al.*, 2001). *Heligmosomoides polygyrus* (in *Mus musculus* probably in fact *H. bakeri*) enhances survival and/or establishment of the nematodes *Trichuris muris* (Jenkins & Behnke, 1977), *Trichinella spiralis* (Behnke *et al.*, 1978), the tapeworm *Hymenolepis citelli* (Alghali *et al.*, 1985), as well as proliferation of the protozoan *Eimeria falciformis* (Rausch *et al.*, 2010) in pairwise co-infection experiments. This nematode is immune-modulatory and modifies the intestinal environment to ensure its own survival, which also downregulates the response towards other intestinal parasites (Behnke *et al.*, 2001). In contrast to these synergistic effects, expulsion of the nematodes *T. muris* and *Nippostrongylus brasiliensis* (Burce & Wakelin, 1977; Kennedy, 1980) and the tapeworm *H. diminuta* (Behnke *et al.*, 1977) was observed as a consequence of acute *T. spiralis* infection, but also intestinal protozoan infections are impaired by *T. spiralis* infection in mice and rats (Roberts-Thomson *et al.*, 1976; Stewart *et al.*, 1980). These effects on other endoparasites were neither caused by direct interaction of the parasites (competition) nor by specific immune responses (cross-immunity), but rather by non-specific effector mechanisms of the host which are elicited by acute infection with nematodes. However, cross-immunity was observed between the phylogenetically relatively closely related parasites *T. muris* and *T. spiralis.* Mice became resistant to one species after primary infection with the other species, as well as by immunisation with worm antigens or adoptive transfer of mesenteric lymph node cells from infected mice (Lee *et al.*, 1982).

Patterns of co-occurrence in wild rodents were studied in wood mice (Behnke *et al.*, 2005; Montgomery & Montgomery, 1990) and bank voles (Haukisalmi & Henttonen, 1993a; Haukisalmi & Henttonen, 1993b; Kisielewska, 1970b). Apart from Kisielewska (1970b), who did not consider confounding factors, pair-wise associations, which differed significantly from the predicted

occurrence, were mostly positive. Haukisalmi and Henttonen (1993a) found consistent positive associations between the nematodes *H. mixtum* and *H. glareoli* (presence-absence) and *M. muris* and *Capillaria* sp. (presence-absence and abundance) in bank voles. Behnke *et al.* (2005) detected positive associations between *H. polygyrus* and *T. muris* (presence-absence) and between *H. polygyrus* and the tapeworm *C. pusilla* (presence-absence and abundance) in wood mice. However, these and other (Montgomery & Montgomery, 1990) co-occurrence patterns were weak, often not consistent across several data sets and strongly dependent on extrinsic and intrinsic factors. Only Behnke *et al.* (2009) found consistent interaction of helminths in wood mice from three distinct habitats and geographical regions in Europe: Prevalence of other helminths increased if the hosts were co-infected with *H. polygyrus* and the helminth diversity increased with *H. polygyrus* worm burden. Frequent co-occurrence of parasites in rich helminth communities favours the development of competitive behaviour (Holmes & Price, 1986). The absence of negative interaction in wild rodents was therefore attributed to the comparatively low prevalence and diversity of helminths in rodents compared to other vertebrates (e.g. water fowl) (Haukisalmi & Henttonen, 1993a). Common helminths, which occur throughout the year and are the first that colonise young hosts are less likely to be competitive than helminths, which are rare (e.g. due to seasonal occurrence of intermediate hosts), since the latter more often have to co-occur with the common species than the other way around (Haukisalmi & Henttonen, 1993b). Indeed, the intestinal distribution of the common *H. mixtum* was shifted away from the preferred habitats of other species during heavy infections with the rare *Catenotaenia* and *Capillaria* spp. in bank voles. But during normal infection intensities, different intestinal parasites co-occur in wild rodents without signs of competition due to radial (mucosa - lumen) and linear (localisation in different compartments) spatial segregation in the intestinal tract and different feeding strategies. Hence, in natural systems, interspecific interactions are less important than intraspecific interactions (Haukisalmi & Henttonen, 1993b).

Co-infections between ectoparasites or between ecto- and endoparasites were rarely studied experimentally or in the field. The main reasons for this are probably two issues: (I) the surface of the host is much larger than the narrow intestinal tract, therefore competition for space and food should rarely occur between two ectoparasite species, probably only during heavy infestations. (II) In contrast to the majority of helminths, many ectoparasites, such as fleas, chiggers and ticks, have a low host-specifity. Hence, co-occurrence on the same host is much rarer than between host-specific endoparasites. Both issues hinder coevolution between two parasites and the necessity for the development of competitive behaviour. But as was revealed in experimental helminth co-infections, interaction of parasites occured most often indirectly when the protective immune responses against

one parasite impairs the survival of another or when immunomodulatory behaviour of one parasite facilitates the survival of another endoparasite species. Moreover, ectoparasites such as ticks, which have to survive and feed on the host for several days, have evolved several bioactive molecules, which are released via the saliva during blood feeding. Besides antiplatelet, anticoagulant, vasodilatory and anti-inflammatory functions, tick saliva molecules also display immunomodulatory activities (reviewed in Brossard and Wikel (2004); Kazimírová and Štibrániová (2013); Schoeler and Wikel (2001)). This could also generate indirect interactions between ectoparasites (locally and systemically) or between endo- and ectoparasites (systemically).

Until now, only Lundqvist and Brinck-Lindroth (1990) studied pairwise co-occurrence of ectoparasites on wild shrews and voles including field and bank vole with simple two-by-two-tables. They found significant pairs on every host species but the the associations were not consistent between host species or trapping locations. Noteworthy is also the cross-sectional field study of Ferrari *et al.* (2009) in Italy, who found a significant negative correlation between the abundances of *H. polygyrus* nematodes and *I. ricinus* tick larvae in yellow-necked mice in a regression model considering several confounding factors. After treatment of mice with a non-acaricidal anthelmintic, they hosted significantly more *I. ricinus* larvae in comparison to untreated mice from comparable habitats. The authors attributed this to a negative influence of the nematode *H. polygyrus* on tick larvae, which was most likely mediated by the immune response of the host.

1.6 Central aims of the present studies

Summing up one can say that wild rodents are important hosts of parasites also infecting humans, lifestock and companion animals, and reservoirs for numerous zoonotic rodent- and vector-borne pathogens. In Central Europe, the most important zoonotic dieseases are those transmitted by the tick *I. ricinus*, whose larval life stage most commonly aquires infection during the first blood meal on rodents. The influence of co-infections with members of the diverse parasite fauna on the enzootic cycles of ticks and tick-borne pathogens is largely unknown. Hence, the present studies were conducted to (I) analyse important factors determining the diversity and quantity of virtually the whole array of macroparasites as well as intestinal Coccidia in the most abundant, non-commensal rodent host species of Europe, (II) determine the influence of urbanisation on these rodent-associated parasites at four study sites with different grades of anthropologic influence, (III) assess the risk of parasitic and vector-borne zoonotic infections in an urban area like Berlin, (IV) examine potential correlations between the abundance of *I. ricinus* ticks and other parasite groups, and (V) examine a potential interaction of parasites of wild rodents via the immune system in laboratory

rodents to assess its effect on tick feeding success and the reservoir competence of rodents for tick-borne pathogens.

The first manuscript (chapter 2) addressed the first four of these five aims for the ectoparasites and other rodent-associated arthropods, the second manuscript (chapter 3) the same for helminths and intestinal Coccidia and the third manuscript (chapter 4) addressed the experimental co-infection in laboratory rodents.

Chapter 2

Factors associated with diversity, quantity and zoonotic potential of ectoparasites on urban mice and voles

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2 Factors associated with diversity, quantity and zoonotic potential of ectoparasites on urban mice and voles

2.1 Abstract

Wild rodents are important hosts for tick larvae but co-infestations with other mites and insects are largely neglected. Small rodents were trapped at four study sites in Berlin, Germany, to quantify their ectoparasite diversity. Host-specific, spatial and temporal occurrence of ectoparasites was determined to assess their influence on direct and indirect zoonotic risk due to mice and voles in an urban agglomeration. Rodent-associated arthropods were diverse, including 63 species observed on six host species with an overall prevalence of 99%. Eight mite species represent new records for the German fauna. The tick *Ixodes ricinus* was the most prevalent species found on 56% of the rodents. The trapping location clearly affected the presence of different rodent species and, therefore, the occurrence of particular host-specific parasites. In Berlin, fewer temporary and periodic parasite species as well as non-parasitic species (fleas, chiggers and nidicolous Gamasina) were detected than reported from rural areas. In addition, abundance of parasites with low host-specificity (ticks, fleas and chiggers) apparently decreased with increasing landscape fragmentation and level of urbanisation from periurban, wooded sites in the periphery of Berlin to an urban park to a backyard in the city centre. In contrast, stationary ectoparasites, closely adapted to the rodent host, such as the fur mites Myobiidae and Listrophoridae, were most abundant at the two urban sites. A direct zoonotic risk of infection for people is only posed by *Nosopsyllus fasciatus* fleas, which were prevalent even in the city centre. More importantly, peridomestic rodents clearly supported the life cycle of ticks in the city as hosts for their subadult stages. In addition to trapping location, season, host species, rodent abundance and host sex, infestation with gamasid Laelapidae mites was associated with significantly reduced abundance of *I. ricinus* larvae on mice and voles. Whether this is caused by predation, grooming behaviour or interaction with the host immune system is unclear. The present study constitutes a basis to identify interactions and vector function of rodent-associated arthropods and their potential impact on zoonotic diseases.

2.2 Introduction

Small mammals are essential hosts for the immature life stages of the most important arthropod vector of pathogens in Central Europe, the hard tick *Ixodes ricinus*. Furthermore, a large number of other arthropods is associated with these mammals, such as fleas, lice and numerous mite species. In

most studies about ectoparasites of wild rodents, only single arthropod species/groups were investigated. Moreover, the majority of publications focuses on qualitative data, such as species lists and descriptions of new species. Although hard ticks and fleas infesting rodents are thoroughly studied, data on mites are scarce.

Commensal rodents, such as the house mice or the Norway or black rats, living inside buildings, are often considered to be the principal risks of zoonotic infections for humans. However, bank voles and *Apodemus* mice are also known to enter cellars and storage areas during winter (Hauer *et al.*, 2009; Niethammer, 1978b; Viro & Niethammer, 1982). These and other mice and voles live in close proximity to humans and are abundant in parks and other greenspaces of urban agglomerations. The population density of these “peridomestic” rodents, such as the striped field mouse, *Apodemus agrarius*, appears to be even higher in urban than rural regions, due to a prolonged breeding season and better winter survival (Luniak, 2004). Changes in the seasonality of the rodent hosts in terms of reproduction, abundance, behaviour and motility may affect the seasonal abundance of rodent-associated arthropods. Also, behavioural differences of the host, such as changes in circadian rhythm and home range as well as an increased longevity, were observed in urban areas (Luniak, 2004). This may also affect the species diversity and quantity of rodent-associated arthropods. In the last decades, the geographical distribution of arthropod vectors changed (Dautel *et al.*, 2006; Medlock *et al.*, 2013). Monitoring of ectoparasite communities of wild animals may provide important information on such processes. Since the proportion of people living in urban areas is constantly increasing worldwide, this is especially important in human agglomerations.

Peridomestic rodents spread and maintain the enzootic cycles of tick-borne pathogens in cities (Matuschka *et al.*, 1996; Matuschka *et al.*, 1990; Rizzoli *et al.*, 2014). Although, Lyme-*Borrelia*, spotted-fever *Rickettsia* spp., tick-borne encephalitis virus and other pathogens have been detected in rodent ectoparasites other than ticks (Hornok *et al.*, 2015; Miťková *et al.*, 2015; Špitalská *et al.*, 2015; Valiente Moro *et al.*, 2005), their vector competence has not been verified. Assessing the diversity and quantity of other mite and insect species on rodent hosts might provide a basis for studies on vector competence of the most abundant arthropod species to elucidate their role in enzootic cycles. Furthermore, the examination of different ectoparasite groups co-occurring with ticks allows to evaluate their effects on tick infestation.

The aim of the present study was (I) to determine and quantify the total species diversity of arthropods located in the fur and on the skin of peridomestic rodents in Berlin, Germany, (II) to examine their distribution in respect to host species, trapping location/urbanisation and seasonality,

(III) to assess the zoonotic risk due to these arthropods for people in urban agglomerations and (IV) to determine parameters affecting the abundance of *I. ricinus* larvae on peridomestic mice and voles.

2.3 Materials and methods

2.3.1 Ethics statement

Rodent trapping and euthanasia were performed in accordance with the German laws on animal protection (*Tierschutzgesetz*) and nature conservation (*Bundesnaturschutzgesetz*) and were approved by the *Landesamt für Gesundheit und Soziales* (*LAGeSo*) Berlin under the registration number G 0256/10 and the *Obere Naturschutzbehörde* Berlin under the reference number I E 210(V)–OA-SG/LSG2a/602;OA-AS/G/825. The three mouse species from the genus *Apodemus* included "besonders geschützte Arten" (= specially protected species) according to the German *Bundesnaturschutzgesetz*.

2.3.2 Rodent trapping

Wild rodents were trapped at four study sites in Berlin in November 2010 and between April and November 2011. Two forest sites at the periphery were chosen as periurban sites with limited human influence (Gatow and Tegel), while two urban study sites were situated in the densely populated city (Steglitz and Moabit). The trapping location Gatow (N52° 28.167 E013° 08.460) is a wooded area at the General-Steinhoff-Barracks characterised by pinewood with a few oaks and sparse ground vegetation. Traps were placed along paths and in an adjacent meadow situated at the margin of the former runways. Another study site was situated within a large forested area in the district Tegel (N52° 36.351 E013° 16.288). Trapping was performed in the surroundings of the forestry office and along a wide path through the forest. The forest comprised mainly pines, beeches, oaks and little ground vegetation. The Botanic Garden Berlin in the district Steglitz comprises about 43 ha and is surrounded by widely spaced single-family houses with garden properties (N52° 27.233 E013° 18.151). The park is tended by gardeners and watered during dry periods. The vegetation of this study site is characterised by widely-spaced old trees, many shrubs and a dense ground layer of ivy and ground elder (Vollack *et al.*, 2017). The most urban study site in the district Moabit was a backyard of an apartment building in the densely populated city centre (N52° 31.286 E013° 21.571). Trapping was performed in six-week blocks, with one site visited per week (termed "occasions" henceforth), followed by two weeks without trapping (Figure 2-1). In total, every site was sampled during seven occasions. Rodents were trapped for three consecutive nights at each occasion. Traps were emptied and cleaned in the morning and deactivated during the daytime. Before trapping, they were filled with cotton, rodent pellets and a piece of apple.

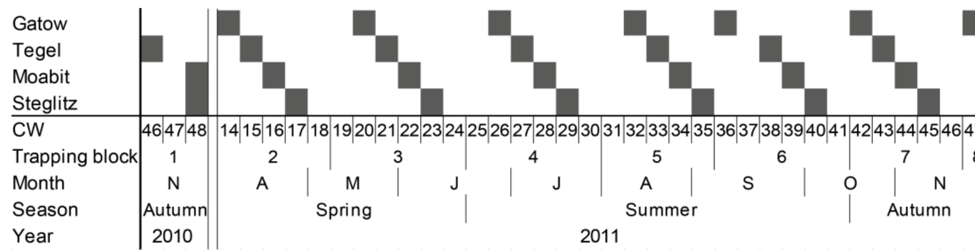


Figure 2-1. Trapping schedule. Trapping blocks and time as calendar week (CW), month, season and year (columns) for every trapping location (rows). The season was categorised by means of trapping blocks. Trapping occasions with three consecutive trap nights are shown in grey.

Initially, 30 Longworth live traps and 10 Tomahawk rat live traps were placed at every location. According to the size of the location, the number of Longworth traps was increased to 40 traps in Steglitz and to 50 traps in Gatow and Tegel in July 2011. Because only two persons were available for the necropsies, no more than eight rodents were examined after each trap night. Therefore, the traps were checked according to random matrices and only the first eight animals were examined and the remaining rodents were released.

Trapped rodents were anaesthetised intraperitoneally with 0.1 mg/g ketamine and 0.012 mg/g xylazine and subsequently euthanised by cervical dislocation followed by cardiac bleeding into serum tubes. The rodents were wrapped in individual plastic bags and traps as well as the cotton wool were thoroughly screened for fleas, gamasid mites and other detached arthropods. Animals were placed on heat packs (approximately 38 °C) to improve the survival of the parasites and transferred to the Institute for Parasitology and Tropical Veterinary Medicine for necropsies. After each week of trapping, the traps were cleaned, cotton wool and food were removed.

2.3.3 Necropsies

In the laboratory, animals were placed on electric heating blocks at 38 °C and species, sex, body weight and size were determined. For some animals (especially *Microtus*), sculls were prepared and teeth morphology was used for species determination (Niethammer & Krapp, 1982a).

The fur and skin of the animals were thoroughly screened for arthropods under a stereo microscope with clean forceps from head to tail and approximately 15 minutes were spent per animal. In addition, the plastic bags used for transportation were checked. Whereas ticks attached to the skin remained there, detached ticks were transferred into glass vials with screened caps and placed in a desiccator filled with oversaturated magnesium sulphate solution to allow moulting to the next life stage. All other arthropods were preserved in tubes with 70% ethanol, except for rodents heavily infested with Myocoptidae or Listrophoridae, of which not all specimens were sampled. During a

necropsy, additional organ samples were obtained for other studies and the reproduction status of female rodents was determined by checking for embryos. After removal of the eyes for age determination (described in Krücken *et al.* (2017)), the remaining carcasses were placed in glass beakers over water to allow detachment of still attached ticks and to improve the recovery of small arthropods. The water and the rodent bodies were examined microscopically during the following week and remaining ticks were placed in the desiccator, whereas other arthropods were preserved in 70% ethanol. After six weeks, any ticks from the desiccator were transferred to 70% ethanol.

2.3.4 Arthropod species determination

The preserved arthropods were determined to species level wherever possible with the help of a stereo microscope and a microscope with up to 1000× magnification. Several specimens of fleas and gamasid mites were cleared in 10% potassium hydroxide before examination. Different literature was used for species determination of fleas (Brinck-Lindroth & Smit, 2007; Kutzscher & Striese, 2003; Skuratowicz, 1967; Whitaker, 2007), lice (Beaucournu, 1968), ticks (Márquez *et al.*, 1992; Morel & Perez, 1972; Morel & Perez, 1973; Morel & Perez, 1978a; Morel & Perez, 1978b), Gamasina (Mesostigmata) (Karg, 1993; Mašán & Fenďa, 2010; Mrciak, 1964; Strandtmann & Garrett, 1970), Myobiidae (Bochkov, 2009a; Bochkov, 2011; Bochkov & Labrzycka, 2003), Trombiculidae (Kepka, 1964; Kudryashova, 1998; Vercammen-Grandjean, 1960), Cheyletidae (Fain & Bochkov, 2001b; Volgin, 1989), Pygmephoridae (Krczal, 1959; Smiley & Whitaker, 1984), Ereynetidae (Fain, 1956b; Fain & Lukoschus, 1968), Myocoptidae (Fain *et al.*, 1969; Fain *et al.*, 1970; Haitlinger, 1986b; Haitlinger, 1987; Labrzycka & Dabert, 2008), Listrophoridae (Dubinina, 1968; Dubinina, 1967; Fain, 1970; Fain, 1981), Gastronyssidae (Fain *et al.*, 1967; Smith *et al.*, 1985), Glycyphagidae and Acaridae (Fain, 1969; Fain & Beaucournu, 1993; Fain & Lukoschus, 1974; Lukoschus *et al.*, 1972; Turk & Turk, 1957). For rodents, on which nits of sucking lice containing nymphs were observed but no postembryonal life stages, the number of lice for measures of prevalence and mean intensity was set to one for the respective species. Similarly, for closed eggs of Myobiidae and Myocoptidae despite absence of postembryonal stages, specimen number was set to one for the respective family.

2.3.5 Rodent abundance

Rodent population densities for every site and every trapping occasion of three trap nights were calculated with the Minimum Number known Alive (MNA) method (Boye, 1996) where all euthanised animals were added to the minimal number of remaining animals which were released and their recapture assumed.

2.3.6 Seasonality

To compare the seasonal occurrence between the different rodent-associated arthropods, abundance was normalised to host species and location. This avoided that strictly host-specific parasites appear to be more abundant in seasons when their preferred host was trapped more frequently. In order to analyse representative data, only the following four rodent subsets (species-location-combinations) were included for which two or more animals were caught in at least five out of six trapping occasions: *Myodes glareolus* in Gatow, *Apodemus flavicollis* in Gatow, *A. flavicollis* in Steglitz und *Apodemus agrarius* in Steglitz. Abundance of the four subsets were averaged, 152 trapped rodents from six trapping blocks (at least eight rodents each) from April to November 2011 were used for the analysis. The normalised mean abundance was calculated as the mean of the mean abundance of every rodent subset to prevent overweighing one factor. The standard error of the mean was calculated, whereby the respective mean abundance of every subset was subtracted from the parasite counts to generate the sums of deviation squares. Only those parasite species were included in the analysis, which reached an overall prevalence of at least 10%, i.e. they were detected on at least 15 animals. Hence, the normalised mean abundance for every arthropod was adjusted for better visualisation of the time course of occurrence and the y axes are not shown because the interpretation of the normalised values would be unreasonable.

2.3.7 Statistics

As the mean abundance (mean number of parasites on all screened rodents) provides only limited information about the parasite distribution across host species and other parameters, mainly prevalence (number of infested rodents divided by total number of screened rodents) and mean intensity (mean number of parasites on infested rodents) were used in combination, as:

$$\text{prevalence} \times \text{mean intensity} = \text{mean abundance.}$$

Local differences in prevalence and intensity of rodent-associated arthropods often depended on the presence of certain rodent host species, particularly for host-specific parasites. Therefore, regression analyses served to identify associations with either host species (n=6) or host family (voles/mice) and trapping location (n=4) or location category (urban/periurban). In order to model prevalence data, logistic regression analyses (LRA) were calculated with the parasite occurrence (infested/non-infested) as dependent variable and trapping location (or location category: urban/periurban) and host species (or rodent family: voles/mice) as independent variables. For modelling mean infestation intensities, a negative binomial regression analysis (NRA) was used with the ectoparasite counts of infested hosts as dependent variable and trapping location and host species as independent variables. Non-infested animals were excluded from the NRA. The negative binomial distribution

appears to be the most adequate approach for the analysis of distribution of most ectoparasitic groups (Lundqvist & Edler, 1987; Shaw *et al.*, 1998). The combination of both regression methods in a zero-inflated negative binomial regression analysis of the abundance was not successful, presumably because of missing data for several host species-location-combinations, collinearity between both factors and small sample size. For both regression analysis methods, the odds ratio (OR) and rate ratio (RR) of the level of interest with the corresponding p-value of the F statistics are presented in the text and the full model with all odds ratios and rate ratios with 95% confidence intervals (95% CI) and p-values in Supplemental Table 2-4.

For statistical testing of differences between groups of rodents within one trapping location or host species, Mann-Whitney-U-test was used for the nonparametric mean intensities (two groups) and mid-p-exact-test for prevalence, with Holm-corrected post-tests if more than 2 groups were analysed.

For the analysis of parameters affecting the number of *I*. *ricinus* larvae on wild rodents, a NRA was performed including all screened rodents with the exception of *Microtus agrestis* voles (n=2). The infestation parameters prevalence and intensity were combined to abundance and not separately analysed to increase statistical power. Modelling was limited to the larval stage because nymphs were only found on every 6th rodent. After exclusion of rodents with missing values (including all from 2010), 219 rodents trapped in 2011 were used for the analysis. The counts of *I. ricinus* larvae on the rodents were used as dependent variable. As independent variables, spatial factors (trapping location, 4 levels), temporal factors (season, 3 levels), host species (5 levels), host abundance, host characteristics (sex, body condition, age) and co-infestations with other frequent ectoparasites (counts of fleas, lice, parasitic laelapid mites, myobiid mites, trombiculid mites, myocoptid mites and listrophorid mites) were included. The season was categorised (see Figure 2-1) into “spring” (calendar week (CW) 14-24 including 2 trapping blocks, n=33), “summer” (CW 25-41 including three trapping blocks, n=125) and “autumn” (CW 42-47 with 2 trapping occasions in Gatow and one at the other locations, n=61). The host abundance was the number of all trapped rodents per 100 trap nights at a certain trapping occasion (3 consecutive nights at one location). Since explanatory variables must be statistically independent and many host characteristics are correlated with each other, three condensed parameters as used in Rossin et al. (Rossin *et al.*, 2010) were included: (I) The sex, including reproductive condition, was used in the three levels male (n=115), non-pregnant female (n=64) and pregnant female (n=40). (II) The different methods for age estimation (e.g. head-body-length, weight) most strongly depend on the season of birth and sexual dimorphism and are thus inappropriate in mature animals. The strongest, nearly linear relationship was described between the age and the weight of the formol-fixed and dried eye lenses at least for *Apodemus*

sylvaticus (Vandorpe & Verhagen, 1979), *A. agrarius* (Adamczewska-Andrzejewska, 1973) and *Microtus arvalis* (Martinet, 1966). Since calibration curves were not available for all trapped rodent species, z-transformations of the mean weight of both lenses for every rodent species were used as a proxy for age to ensure independence of the variable from host species. To calculate z-values, the weights of the dried eye lenses subtracted by the mean weight of the dried eye lenses of the same species was divided by the standard deviation of the latter. (III) In order to estimate the nutritional or body condition, an adaptation of the method in Rossin *et al.* (2010) was used, where the differences (residuals) from an average weight to body-length ratio were calculated using linear regression. A regression of the log of the body weight against the head-body-length was calculated. However, as voles and mice differ morphologically and residuals of voles and mice of a combined linear regression differ significantly (unpaired t test: $p<0.001$), but not within species of each group (mice: one-way ANOVA of three *Apodemus* spp.: $p=0.09$, voles: unpaired t test of *M. glareolus* and *M. arvalis*: $p=0.25$), separate linear regressions were used for either (mice: log(weight in g)= 0.3964 + 0.0285 × head-body-length in mm, adjusted $R^2=0.83$, $p<0.001$; voles: log(weight in g)= 0.3696 + 0.0277 × head-body-length in mm, adjusted $R^2= 0.66$, $p<0.001$). Positive residuals from the calculated curves indicate a good body condition, because rodents are comparatively heavier than expected for their body length; negative residuals indicate a low nutritional status.

The NRA regression analysis started with the full model including all variables followed by backward variable selection using the “step” function in R statistics. In the process, variables were successively excluded which least reduced the Akaike Information Criterion (AIC) until AIC did not improve by further model reduction.

For the calculation of the 95% CI of mean intensities, a bootstrapping method was performed with 2,000 bootstrap replications calculating bias-corrected and accelerated confidence intervals in the online software Quantitative Parasitology 3.0 (Reiczigel & Rózsa, 2005). Wilson-Score confidence intervals from proportions were used for prevalence. Wilson-Score confidence intervals (package PropCIs), mid-P-exact-test (package epitools), χ^2-test, one-way and two-way ANOVA, generalised linear models for logistic regression analysis and negative binomial regression analysis (package MASS) were performed in R Statistics. D’Agostini-Pearson omnibus test for normality was recommended and performed in GraphPad Prism 7.

2.4 Results

Other data derived from this rodent trapping campaign have been published previously (Krücken *et al.*, 2017; Krücken *et al.*, 2013; Maaz *et al.*, 2016 = chapter 4).

2.4.1 Rodent sampling

During November 2010 and from April to November 2011, 276 mice and voles belonging to six species were trapped at four study sites in Berlin. Of those, 256 were thoroughly screened for ectoparasites, whereas nineteen animals were released (18 in Steglitz, one in Gatow) and one fell victim to carnivores (in Steglitz). During the seven trapping occasions at each of the sites, the highest rodent abundance with 17 rodents per 100 trap nights was found at the urban Steglitz site, followed by the periurban forest habitat in Gatow (Table 2-1). The forest site Tegel and the most urbanised site, Moabit in Central Berlin, exhibited the lowest rodent abundance with only four rodents per 100 trap nights. The highest activity of rodents was observed in June and July 2011 with a second peak for some species in October/November (Supplemental Figure 2-7).

Table 2-1. Rodent abundance at the study sites. The mean abundance of mice and voles per 100 trap nights for every trapped species (columns) was calculated using the “minimum number known alive (MNA) method” for the four study sites (rows). Total mean abundance of each rodent species and of all rodent species at each trapping location are shown in the last row or column, respectively. The hyphen indicates absence of the respective rodent.

Trapping location	Rodent species						Total
	Myodes	*Microtus*		*Apodemus*			
	glareolus	*agrestis*	*arvalis*	*agrarius*	*flavicollis*	*sylvaticus*	
Moabit	0.2	-	-	-	-	4.0	4.1
Steglitz	-	-	0.6	12.2	4.2	-	16.9
Tegel	1.6	-	-	-	2.5	-	4.1
Gatow	5.1	0.2	0.8	0.6	3.9	-	10.6
Total	1.7	0.1	0.3	2.7	2.5	0.7	8.0

The wood mouse *A. sylvaticus* was exclusively found in Moabit and constituted the dominant rodent species at this site. The two sylvatic species, yellow-necked mouse (*A. flavicollis*) and bank vole (*M. glareolus*), were the most frequent species in Gatow and Tegel, although *A. flavicollis* was also abundant in Steglitz. Nevertheless, the striped field mouse (*A. agrarius*) was the dominant species at this study site with 12.2 rodents per 100 trap nights. At the only other site where *A. agrarius* was trapped, in Gatow, it occurred in low numbers. The field vole (*M. agrestis*) and the common vole (*M. arvalis*) were rarely caught in Gatow and Steglitz with a maximum of 0.8 animals per 100 trap nights. The occurrence of a single *M. glareolus* in Moabit seems to be an exception.

2.4.2 The diversity of rodent-associated arthropods

From 256 examined mice and voles, a total of 5,429 arthropods belonging to 63 different species was collected from fur and skin and identified (Figure 2-2 and Supplemental Table 2-5). Taxonomic

arthropod groups of different levels (order/suborder/family) were compared and grouped according to phylogenetic position and feeding habits. The arthropods represented two orders of Insecta, fleas (Siphonaptera, ten species) and lice (Phthiraptera, four species, all suborder Anoplura), but most importantly four orders of mites (Acari, 49 species). The latter were comprised of three species of hard ticks (Ixodida), 19 species of Mesostigmata (all cohort Gamasina), 13 species of Trombidiformes (all suborder Prostigmata) and 14 species of Sarcoptiformes (all cohort Astigmata). These species ranged in body sizes from 0.2 (astigmate mite: *Trichoecius tenax*) to 8 mm (flea: *Hystrichopsylla orientalis*).

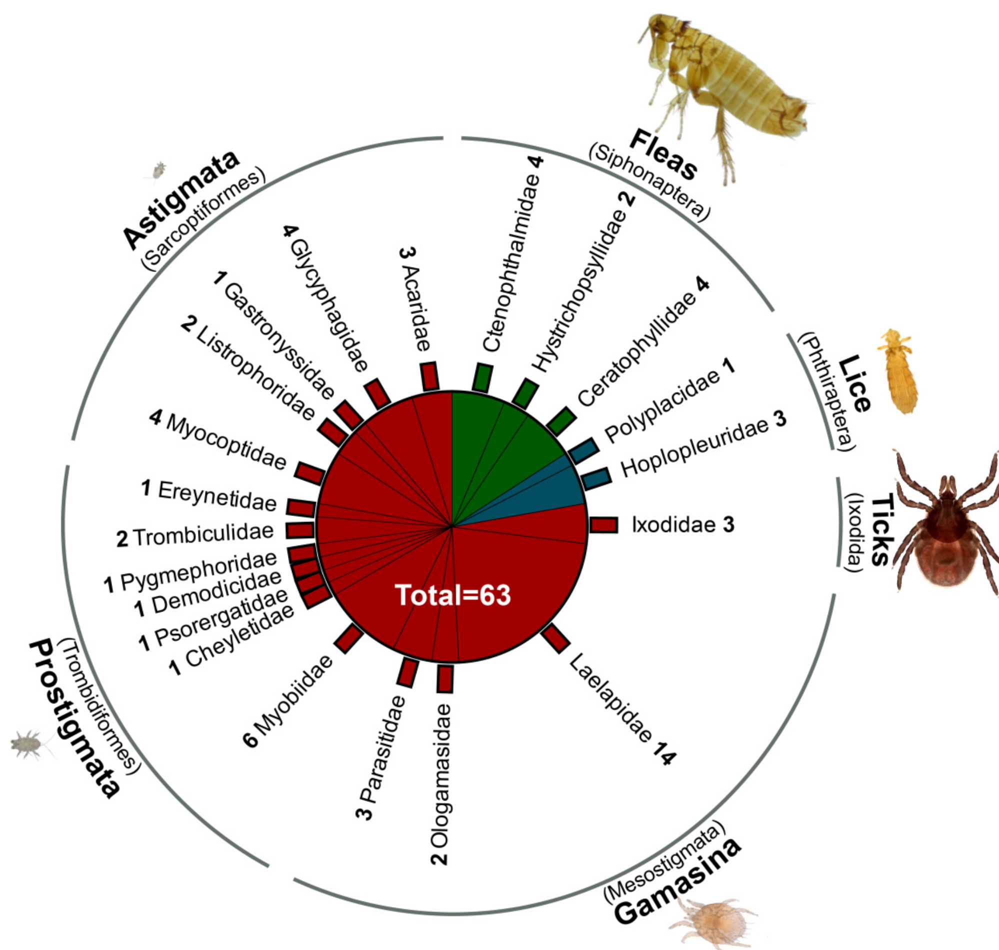


Figure 2-2. Taxonomic distribution of 63 detected species across families and higher taxa of arthropods. Numbers of species are accompanied by family names. Families shown in red belong to mites (Acari), those in blue to lice (Phthiraptera) and those in green to fleas (Siphonaptera). Parasite micrographs show representative specimens from the different groups depicted in the same size ratio.

The diversity of rodent-associated arthropods consisted of facultative and obligatory ectoparasites (fleas, lice, 35 mite species) and also included phoretic life stages of non-parasitic arthropods (four mite species) and nidicolous mites, rarely occurring in the fur of rodents (10 mite species). The

phoretic mites comprised life stages attached to the hair of rodents, such as female *Pygmephorus forcipatus* and deutonymphal hypopi of *Xenoryctes krameri* and *Glycyphagus hypudaei* (Supplemental Figure 2-8), as well as the hypopial stages of *Acarus nidicolous* which were found phoretic on fleas (Supplemental Figure 2-8). Arthropods, in general, and ectoparasites, in particular, infested virtually every mouse and vole and amounted to an overall prevalence of 99% with an average of 16 specimens per host. The most frequent ectoparasite groups were Laelapidae (67%, Gamasina), fleas (63%), hard ticks (57%, Ixodida), Myobiidae (47%, Prostigmata), sucking lice (41%, Anoplura), Listrophoridae (32%, Astigmata), Myocoptidae (20%, Astigmata) and Trombiculidae (7%, Prostigmata) (Table 2-2 and Figure 2-3). Although the skin-inhabiting mites *Demodex* sp. (Demodicidae, Prostigmata), *Psorergates* sp. (Psorergatidae, Prostigmata), *Lophioglyphus liciosus* (Glycyphagidae, Astigmata) and mites living in the nasal cavities (Gastronyssidae, Astigmata and Ereynetidae, Prostigmata) were detected as incidental findings, the study was not designed to quantify these species. To the best knowledge of the authors, the finding of the eight mite species *Laelaps jettmari*, *Hirstionyssus (Echinonyssus) sunci*, *Radfordia clethrionomys*, *Paraspeleognathopsis bakeri*, *Trichoecius widawaensis*, *Listrophorus brevipes*, *Yunkeracarus apodemi* and *Acarus nidicolous* represent first records for the German fauna.

Table 2-2. Distribution of ectoparasitic arthropod groups on wild rodents. Total number and sex ratio (male : female) of parasites as well as number of infested rodents. For each parasite group, prevalence and mean intensity for six rodent species are shown. The number of examined rodents and the number of male/female are given below the species name. The last column shows values for the sum of all rodents. Hyphens represent absence of parasites. n: Number of parasites. No: Number of infested rodents. P [%]: Prevalence in %. 95% CI: 95% confidence interval. mI [n]: mean intensity = mean number of parasites on infested rodents. max: maximum of parasite intensities. n.d.: no adults observed or not determined.

Ectoparasite			Rodent host species																												
			M. glareolus 29/29 = 59[a]				*M. arvalis* 4/7 = 11				*M. agrestis* 0/2 = 2				*A. agrarius* 44/33 = 77				*A. flavicollis* 41/41 = 82				*A. sylvaticus* 14/11 = 25				All Species 132/123 = 256[a]				
Taxon	n	Sex ratio	No	P [%]	95% CI	mI [n]	No	P [%]	95% CI	mI [n]	No	P [%]	95% CI	mI [n]	No	P [%]	95% CI	mI [n]	No	P [%]	95% CI	mI [n]	No	P [%]	95% CI	mI [n]	No	P [%]	95% CI	mI [n]	max
Siphonaptera (Fleas)	504	1 : 1.2	41	70	57-80	2.0	8	73	43-90	3.1	1	50	9-90	2.0	46	60	49-70	3.0	56	68	58-77	4.1	9	36	20-55	2.7	161	62.9	56.8-68.6	3.1	15
Anoplura (Sucking lice)	463	1 : 2.2	6	10	5-20	2.7	3	27	8-57	5.0	-		0-66		66	86	76-92	5.4	20	24	16-35	2.9	10	40	23-59	1.9	105	41.0	35.2-47.1	4.4	44
Ixodidae (Hard ticks)	1370	n.d.	44	75	62-84	8.1	8	73	43-90	27.0	2	100	34-100	10.5	31	40	30-51	5.4	54	66	55-75	11.0	6	24	11-43	2.5	145	56.6	50.5-62.6	9.5	108
Parasitic Laelapidae	987	1 : 12.3	18	31	20-43	2.1	9	82	52-95	13.4	-		0-66		59	77	66-85	2.8	63	77	67-85	7.9	22	88	70-96	7.3	171	66.8	60.8-72.3	5.8	91
Myobiidae	444	1 : 4.1	22	37	26-50	4.0	4	36	15-65	2.2	2	100	34-100	11.0	38	49	38-60	2.6	38	46	36-57	3.8	17	68	48-83	4.8	121	47.3	41.2-53.4	3.7	20
Trombiculidae	88	n.d.	13	22	13-34	4.8	2	18	5-48	3.0	1	50	9-90	1.0	-		0-5		3	4	1-10	6.0	-		0-13		19	7.4	4.8-11.3	4.6	38
Myocoptidae[b]	302	1 : 3.5	35	59	47-71	≥6.8	7	64	35-85	≥7.7	1	50	9-90	≥2.0	6	8	4-16	≥1.0	1	1	0.2-7	≥1.0	-		0-13		50	19.5	15.1-24.8	≥6	≥29
Listrophoridae[b]	1057	n.d.	9	15	8-26	≥16.0	-		0-26		2	100	34-100	≥13.0	32	42	31-53	≥11.0	26	32	29-50	≥14.0	13	52	33-70	≥14.0	82	32.0	26.6-38.0	≥12.9	≥146

[a] Sex of one bank vole was not determined

[b] Because of small body size of Myocoptidae and Listrophoridae, not all specimens were sampled when high intensities occurred. Values should be treated as minimum numbers. Sex ratio of Listrophoridae was not determined

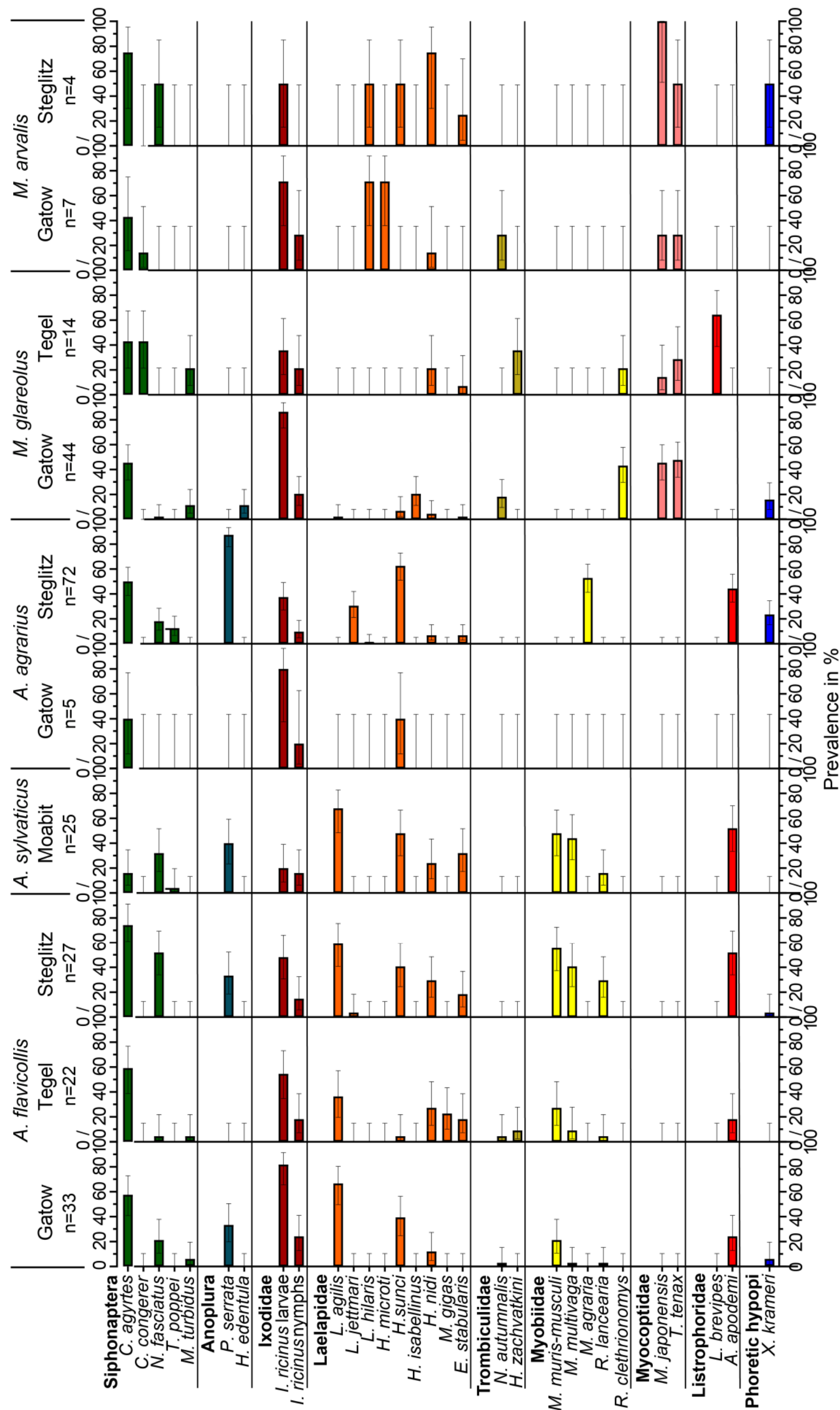


Figure 2-3. Prevalence of the most frequently observed rodent-associated arthropods. Bar plots with 95% CI showing percentage of infested rodent for every arthropod species on five rodent host species trapped at four trapping locations. One *M. glareolus* (Moabit) and two *M. agrestis* voles (Gatow) are not shown because of the small sample size. Only arthropod species are depicted which occurred at least five times on one of the illustrated host-location-combinations. Numbers below rodent species names depict the sample size (n) of examined mice or voles for every study site. Horizontal solid lines border species of the same parasite group.

2.4.3 Infestation differences between host species and between trapping location

Hematophagous fleas, of which only the parasitic adults were observed, occurred on four of the five regularly trapped rodent species at a comparable prevalence of 60-73% (Table 2-2). Only the wood mouse seemed to be less frequently infested (36%), although this may also result from an overall reduced flea density in Moabit. Mice carried slightly more fleas than voles (NRA including parameters host family (reference mouse) and location, vole-RR=0.57, p<0.001, Supplemental Table 2-4, model J). Especially the largest species, the yellow-necked mouse, hosted an average of 4.1 fleas per infested animal (Table 2-2). Most flea species revealed little host-specificity and the dominant species *Ctenophthalmus agyrtes* (Supplemental Figure 2-8), which represented nearly one third of all observed fleas, was found on all rodent species and also at every trapping location (Figure 2-3 and Supplemental Table 2-5). Another frequently observed flea *Nosopsyllus fasciatus* appeared to infest *Apodemus* species more frequently than voles (LRA: host family (reference mice) and location (reference Gatow), voles-OR=0.27, p=0.055, Supplemental Table 2-4, model A). Whereas *Typhloceras poppei* solely occurred on *Apodemus* mice (Figure 2-3 and Supplemental Table 2-5), *Ctenophthalmus assimilis*, *Ctenophthalmus congerer* and *Peromyscopsylla sylvatica* only infested voles (Supplemental Table 2-5). Occurrence differed also at particular study sites. For instance, *C. assimilis*, *P. sylvatica* and *H. orientalis* were only detected in Gatow although their main host species were also trapped at other locations.

The stationary parasitic, hematophagous lice parasitised every rodent species, with the exception of the two field voles. They were found in a markedly host-specific manner, i.e. each parasite species was identified on only a single host genus (Supplemental Table 2-5). Prevalence was higher in mice than in voles (LRA: host family (reference mouse) and location, vole-OR=0.25, p=0.003, Supplemental Table 2-4, model B), which was most obvious in comparison to bank voles (Table 2-2). The louse species *Polyplax serrata* (Supplemental Figure 2-8), accounting for 80% of all louse specimens, infested exclusively *Apodemus* mice with prevalence rates of up to 82% (*A. agrarius*). The spatial distribution of the sucking lice was surprisingly inconsistent. In fact, *Hoplopleura affinis* was the dominant louse species on the striped field mouse in Gatow, but absent on such hosts in Steglitz (mid-P-exact-test: p<0.001, Supplemental Table 2-5). In contrast, 88% of this host species were infested by *P. serrata* in Steglitz, but none of the five animals in Gatow (mid-P-exact-test: p=0.047, Figure 2-3 and Supplemental Table 2-5). Moreover, lice were abundant on wood mice in Moabit and on bank voles and yellow-necked mice in Gatow and Steglitz, but completely absent from the latter hosts in Tegel (two-way-ANOVA: species p=0.18, location p=0.003, interaction p=0.15).

Hard ticks exhibited a low diversity with only three species, whereby *I. ricinus* was by far the most frequent, representing 99.2% of all rodent-attached ticks, followed by *Ixodes trianguliceps* (0.7%) and *Dermacentor reticulatus* (0.1%). *Ixodes ricinus* (Supplemental Figure 2-8) was the most prevalent arthropod species on rodents in Berlin, infesting 56% of the animals with an average of 9.4 ticks per infested rodent. The majority of individuals from this species represented the larval life stage (94%), the remaining were nymphs, while no adult ticks were observed. Prevalence and intensity of infestation with larval ticks did not differ between mice and voles (Figure 2-4A, LRA: host family (reference mice) and location, voles-p=0.78, Supplemental Table 2-4, model C, NRA: host family (reference mouse) and location, vole-p=0.32, Supplemental Table 2-4, model K). On the species level, infested yellow-necked mice and common voles hosted significantly more *I. ricinus* larvae than did bank voles (NRA: host species (reference *M. glareolus*) and location, *A. flavicollis*-RR=1.84, p=0.006, *M.* arvalis-RR=3.58, p=0.001, Supplemental Table 2-4, model L). In contrast, intensity of infestation with larval ticks was lower in wood mice (same model, *A. sylvaticus*-RR=0.23, p=0.007). The location appears to be more important for the abundance of larval ticks. Host-associated *I. ricinus* larvae were most prevalent and numerous in Gatow followed by Steglitz and Tegel and were relatively rare at the most urban site in Moabit (Figure 2-3 and Figure 2-4A). In Gatow, they were not only more prevalent with 84% of all rodents infested (LRA: host species and location (reference Gatow), Tegel-OR=0.17, p<0.001, Steglitz-OR=0.17, p<0.001, Supplemental Table 2-4, model F), but also more numerous. On average, 13 *I. ricinus* larvae were observed on infested rodents, which was 6.5 fold higher than in Moabit (Supplemental Figure 2-8) and, after correction for host species, 4.1 and 2.3 fold higher than in Tegel and Steglitz, respectively (NRA: host species and location (reference Gatow), Tegel-RR=0.24, p<0.001, Steglitz-RR=0.43, p=0.002, Supplemental Table 2-4, model L). In contrast, only 20% of the dominant wood mice at the Moabit site were infested and hosted only about two *I. ricinus* larvae. Nymphal ticks were generally less frequent and were observed on every 6th rodent host. Infested *A. flavicollis* mice hosted with on average 2.7 nymphs which is somewhat more than most other inspected rodent species (Supplemental Table 2-5), although this difference was only significant when compared to *M. glareolus* which were infested with 1.3 nymphs (NRA: host species (reference *A. flavicollis*) and location (reference Gatow), *M. glareolus*-RR=0.43, p=0.02, Supplemental Table 2-4, model M). In the same model, a trend of an increased intensity of infestation was recognised in Gatow with 2.8 nymphs compared to the other sites, where typically only one nymph was observed per rodent (Tegel-RR=0.42, p=0.052, Steglitz-RR=0.43, p=0.063; Figure 2-4B). With 18-28% prevalence, nymphs seemed to be slightly more frequent on rodents in Gatow and Tegel (periurban) than in Steglitz and Moabit (urban), where they reached 10-17% (Figure 2-4B and Figure 2-3). However, this difference was not significant (LRA: host species and location category (reference periurban), urban-OR=0.47 p=0.174, Supplemental Table 2-4, model G).

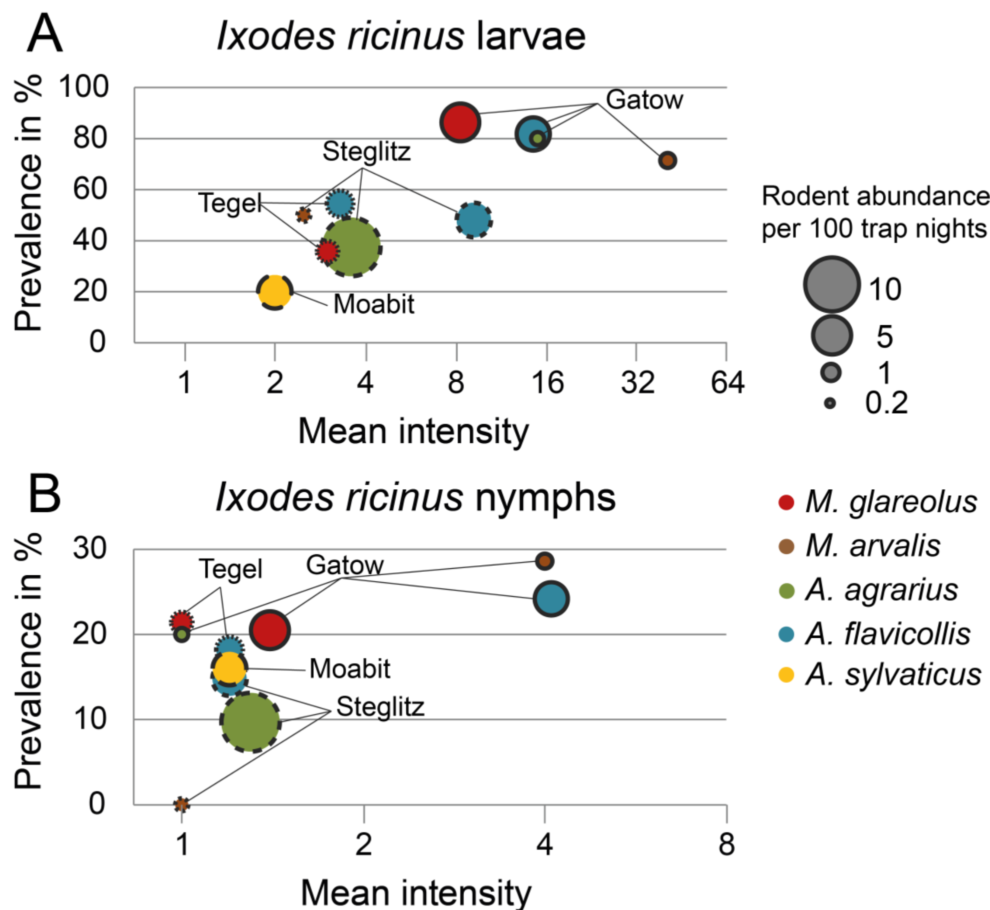


Figure 2-4. Prevalence and mean intensity of *I. ricinus* ticks. Bubble diagram showing prevalence and mean intensity of *I. ricinus* larvae (A) and nymphs (B) combined with rodent abundance for five rodent species and the four trapping locations. Bubble colour indicates rodent species and the shape of margins the trapping location (also labelled). Bubble size indicates mean rodent abundance during the study period for each species and location. The two *M. agrestis* and the one *M. glareolus* captured in Moabit are not shown.

The encountered specimens of gamasid mites (Mesostigmata) belonged to the family Laelapidae and two families of non-parasitic, nest-associated mites, represented by three species of Parasitidae and two species of Ologamasidae. Most Laelapidae, represented by 13 species, are at least facultatively parasitic feeding on skin, lymph and blood. The only non-parasitic member of this family was *Hypoaspis sardoa* of the nidicolous subfamily Hypoaspidinae. The parasitic laelapid species were very frequent with 67% prevalence and were absent only from the two field voles. These mites parasitised bank voles with a 31% prevalence considerably less frequently than other rodent species which revealed a prevalence of 77-88% (Table 2-2 and Figure 2-3, LRA: host species (reference *M. glareolus*) and location, *M. arvalis*-OR=7.57, p=0.018, *A. flavicollis*-OR=5.77, p<0.001, *A. agrarius*-OR=3.15, p=0.059, Supplemental Table 2-4, model D). The mean intensity of infestation was the highest in common voles with 13.4 (95% CI 8.4-25) gamasid mites per infested rodent compared to

M. glareolus, *A. agrarius* and *A. sylvaticus*/location Moabit (undistinguishable due to collinearity), while *A. flavicollis* just missed significance (Table 2-2, NRA: host species (reference *M. arvalis*) and location, *M. glareolus*-RR=0.13, p<0.001; *A. agrarius*-RR=0.37, p=0.001; *A. sylvaticus*-RR=0.38, p=0.002; *A. flavicollis*-RR=0.63, p=0.09, respectively, Supplemental Table 2-4, model N). The gamasid mite species exhibited marked host preferences. Within the genera *Laelaps* and *Hyperlaelaps*, which are strongly adapted to parasitism, *L. jettmari* occurred nearly exclusively on the striped field mouse, whereas *Laelaps agilis* infested animals of the rodent subgenus *Sylvaemus* comprising the yellow-necked mouse and the wood mouse. *Laelaps hilaris* (Supplemental Figure 2-8) and *Hyperlaelaps microti* virtually only parasitised the common vole (Figure 2-3 and Supplemental Table 2-5). With 35% overall prevalence, the most frequent gamasid species was the obligate parasite *H. sunci*, which clearly preferred *Apodemus* mice to voles (Figure 2-3). The related *Hirstionyssus (Echinonyssus) isabellinus* was strictly host-specific and represented the most abundant gamasid mite of bank voles. In contrast, the common, but facultative parasitic mite *Haemogamasus nidi* had a broad host range (Figure 2-3). The occurrence of the parasitic Gamasina was much more host-species-specific than location-specific. Indeed, the prevalence of infested yellow-necked mice was nearly the same in Steglitz, Tegel and Gatow or between bank voles in Tegel and Gatow. However, *Myonyssus gigas* infested yellow-necked mice solely in Tegel (Figure 2-3, mid-P-exact-test p<0.001, post-tests with Holm-correction: Gatow p=0.015, Steglitz p=0.015) and *H. isabellinus* (Figure 2-3) and *Hirstionyssus soricis* occurred only on bank voles in Gatow, although case numbers were too low for significance compared to Tegel (mid-P-exact-Test p=0.067 and p=0.76, respectively).

The prostigmatic mites of the Trombiculidae (chiggers) are only hematophagous as larvae, whereas the deutonymphal and adult life stages live predatorily in the soil. In agreement with this life style, only larvae were found on the rodents. With only two species, *Hirsutiella zachvatkini* and the harvest mite *Neotrombicula autumnalis* (Supplemental Figure 2-8), the diversity was low. They occurred with only about 5% prevalence, however, with a high mean intensity of nearly five mites per infested rodent. They showed a marked preference for voles as compared to *Apodemus* mice (Figure 2-3 and Table 2-2, LRA: host family (reference vole) and location, mouse-OR=0.25, p=0.004, Supplemental Table 2-4, model E). Trombiculidae exclusively infested sylvatic rodents in Gatow and Tegel. Since *H. zachvatkini* was exclusively present in Tegel (Figure 2-3), about eight times more trombiculid mite larvae fed on rodents in Tegel than in Gatow (NRA: host species and location (reference Tegel), Gatow-RR=0.12, p<0.001, Supplemental Table 2-4, model O).

Myobiidae are hematophagous stationary ectoparasites. Every species was specific to a particular host species (*Myobia agraria* on *A. agrarius* and *R. clethrionomys* on *M. glareolus*) or host genus (*Myobia muris-musculi* (Supplemental Figure 2-8), *Myobia multivaga* and *Radfordia lancearia* on

Apodemus (*Sylvaemus*), *Radfordia lemnia* on *Microtus*). Prevalence rates of Myobiidae did not differ between rodent species or between mice and voles. The mean intensity of infestation was independent of the host species and trapping site and remained mostly within a small range between two and six mites per rodent (Table 2-2). In contrast, the Myobiidae infested mice in the periurban sites with 33-34% prevalence markedly less frequently than in the urban sites in Steglitz (59%) and Moabit (65%) (LRA: host species and location category (reference periurban), urban-OR=6.53, p<0.001, Supplemental Table 2-4, model H).

The astigmatic family Myocoptidae predominantly infested voles (Figure 2-3), except for seven mite specimens of *T. widawaensis* and *Criniscansor* sp. which were a rare finding on *Apodemus* mice and exclusively detected in Steglitz. The latter appeared to be an undescribed species from the genus *Criniscansor*. The most prevalent myocoptid species were *Myocoptes japonensis* (Supplemental Figure 2-8) and *T. tenax*, occurring on all three vole species. Together, they infested about 60% of all voles with a mean intensity of seven mites per infested animal (Table 2-2). The Myocoptidae were about two-times more prevalent in bank voles in Gatow than in Tegel (Mid-P-Exact-Test p=0.008), although the infestation intensity was approximately the same at both periurban study sites (Mann-Whitney-U-Test: p=0.65).

Listrophoridae are parasites feeding on organic material in the fur of rodents. Occasionally, they were found in high numbers with more than 100 mites per rodent host. In contrast to the Myocoptidae, the Listrophoridae preferred mice. The species *Afrolistrophorus apodemi* (Supplemental Figure 2-8) occurred on 39% of *Apodemus* mice, whereas the vole parasite *L. brevipes* only infested 15% of the voles, where it was only absent from *M. arvalis*. *Afrolistrophorus apodemi* was less abundant on *Apodemus* mice at the periurban sites Gatow and Tegel with 21% and 18% prevalence and a mean intensity of seven mites per mouse than on those in Moabit and Steglitz with 52% and 46% prevalence and an average of 14 mites per rodent (Figure 2-3 , LRA and NRA within *Apodemus*: host species and location category (reference periurban), LRA: urban-OR=4.64, p=0.001, Supplemental Table 2-4**,** model I, NRA: urban-RR=2.96, p=0.022, Supplemental Table 2-4, model P). Surprisingly, the vole parasite *L. brevipes*, which infested 9 of 14 bank voles in Tegel was absent on 44 bank voles in Gatow (Figure 2-3 , Mid-P-Exact-Test: p<0.001), although it was found on both field voles at this location.

Overall, rodents were infested by an average of 4.5 arthropod or 4.2 ectoparasite species. To test whether the diversity of arthropod species differed between hosts (both *Microtus* species combined), a D'Agostini-Pearson omnibus normality test was performed revealing that normality was only rejected for *M. glareolus* (p=0.005). As species numbers per host also resembled normality in

Quantile-Quantile-plots of the four other hosts, normal distribution was similarly assumed for the bank vole. The number of arthropod species differed significantly between rodents (one-way-ANOVA with Holm-corrected post-tests: p=0.011), as *Microtus* voles hosted significantly more arthropod species (mean=6.5) than did *A. agrarius* (4.4, p=0.006), *A. flavicollis* (4.4, p=0.010) and *M. glareolus* (4.0, p=0.005). The difference to *A. sylvaticus* was not quite significant (4.68, p=0.057). The highest arthropod diversity with 11 species was observed on two *A. flavicollis* and one *M. arvalis* in Steglitz and on one *M. glareolus* in Tegel. As an example, an individual *A. flavicollis* mouse was infested by the fleas *N. fasciatus* and *C. agyrtes*, the louse *P. serrata*, the tick *I. ricinus*, the gamasid mites *H. nidi*, *Eulaelaps stabularis* and *H. sunci* and the fur mites *A. apodemi*, *M. muris-musculi*, *M. multivaga* and *R. lancearia*.

2.4.4 Seasonality

Most groups of ectoparasites occurred with specific patterns of seasonality. In the study period from April to October 2011, when the four study sites were trapped six times, fleas were most abundantly found in the fur of the rodents in late spring and early summer and became increasingly rare until the end of October (Figure 2-5). In contrast, *P. serrata* lice were quite rare in spring and reached their highest normalised mean abundance not before June/July, although the number of lice strongly varied between host individuals. Their peak of abundance occurred simultaneously with the peak abundance of their hosts, *Apodemus* spp. mice (Supplemental Figure 2-7). Different patterns of seasonality were detected for larval and nymphal ticks. Whereas larval *I. ricinus* were detected continuously and frequently throughout the study period with only a slight peak in August, nymphs infested rodents at higher abundance until June/July and were nearly absent from rodents in August to October. No common pattern of seasonality was evident for the most prevalent species of gamasid mites. However, species strongly adapted to parasitism, such as *L. agilis*, *L. jettmari* and *H. sunci,* were most abundant in June/July with a second peak in September for the latter two species. In contrast, the facultative parasite *H. nidi* seemed to most abundantly infest rodents early in May. Some strongly adapted stationary parasite groups, such as the prostigmatic Myobiidae or the astigmatic Myocoptidae and Listrophoridae, occurred relatively late in the year starting only in June/July with a peak abundance in August. Also the nonparasitic, phoretic hypopial stages (deutonymphs) of *Xenoryctes krameri* increased in occurrence on the rodents in the early summer with an apparent peak abundance at the end of the study period.

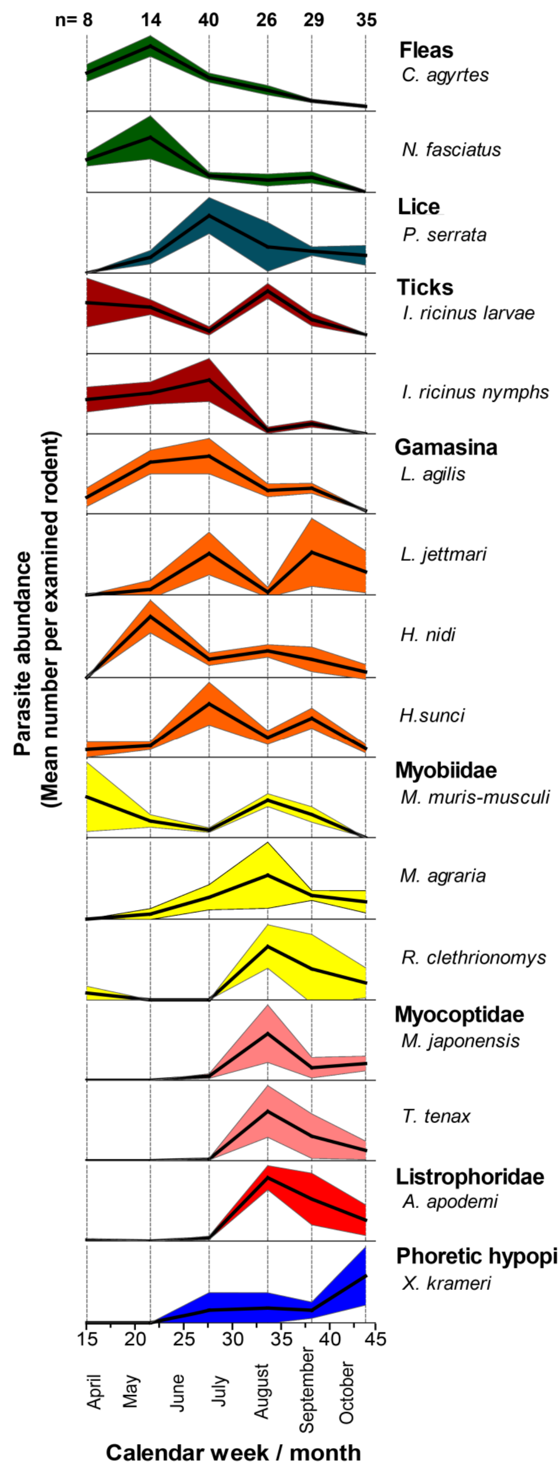


Figure 2-5. Seasonal abundance of common rodent-associated arthropods. The normalised mean abundance (thick solid line) per rodent host and standard errors of the mean (thin solid lines), normalised for trapping location and host species are shown for the most prevalent arthropod species on 152 rodents (three species from two study sites) from April to November 2011. The y axes were adjusted between species for better comparability of the time course, whereas abundance values were omitted, because of the lack of comparability between species due to normalisation. Dashed vertical lines indicate the mid time-point of trapping from every trapping block. Numbers of examined rodents are shown in the first row for every trapping block.

2.4.5 Regression model for the abundance of host-associated *I. ricinus* larvae

After stepwise backward variable selection starting with the full model, model 9 appeared to be the best model (Figure 2-6A). Six parameters significantly affected the number of larval ticks on trapped rodents: (I) The trapping location, (II) the season of trapping, (III) the rodent host species, (IV) the rodent abundance at the time of trapping at the respective location, (V) the host sex including reproductive condition and (VI) the number of parasitic Laelapidae mites co-infesting the rodents (Figure 2-6B). Because of total collinearity between the rodent species *A. sylvaticus* and the trapping location Moabit (all wood mice were trapped exclusively in Moabit), the regression was not able to calculate estimates for both levels in these two factors. With all other variables remaining constant, markedly more larval ticks were found on rodents in Gatow than in any other location which was consistent with the regression analyses of prevalence and intensity including only location and host species (see above and Supplemental Table 2-4, models F and I). Compared to Gatow, only 11% (95% CI 5-24%, p<0.001) and 15% (95% CI 8-28%, p<0.001) of the number of ticks were found on rodents in Tegel and Steglitz, respectively. In addition, the season of trapping as external factor affected the number of ticks. According to the model about 5.7 times (95% CI 2.8-11.4, p<0.001) and 2.9 times (95% CI 1.8-4.7, p<0.001) more larval ticks were found on the rodents in spring (April to early June) and summer (end of June to early October), respectively, compared to autumn (mid of October to end of November). Another important determinant of tick infestation was the host species. *Myodes glareolus* voles were significantly less densely infested by *I. ricinus* larvae than were *M. arvalis* voles (4.3 times more, 95% CI 1.7-10.6, p=0.002) and *A. agrarius* (2.3 times more, 95% CI 1.1-4.8, p=0.023) and *A. flavicollis* mice (2.2 times more, 95% CI 1.3-3.8, p=0.003). The abundance of rodents at the respective trapping location at the time of trapping was a variable which significantly affected tick infestation. In this model, each additional rodent per 100 trap nights reduced the number of larval ticks by 10.6% (95% CI 6.3-14.7%, p<0.001). The biological host characteristics, such as age (estimated from the z values of eye lens weight) and body condition (the nutritional status as the relation of the log of body weight and body length), were poorly correlated with the tick count and were hence excluded from the final model. Only host sex combined with reproductive condition significantly affected the larval *I. ricinus* count. Although tick abundance did not differ between pregnant and non-pregnant females, male rodents hosted two times (95% CI 1.3-3.2, p=0.003) more larval ticks than did non-pregnant females. The abundance of most of the ectoparasite groups did not affect the number of ticks on mice and voles and were excluded from the best model selected. However, the counts of parasitic Laelapidae mites influenced the level of tick infestation. Theoretically, each additional mite on a rodent reduced the number of larval ticks by 3.3% (95% CI 0.23-6.2%, p=0.035).

A Model selection

Model variables		Model number 1	2	3	4	5	6	7	8	9
Spacial factors	Trapping location	■	■	■	■	■	■	■	■	■
Temporal factors	Season	■	■	■	■	■	■	■	■	■
Host species	Rodent species	■	■	■	■	■	■	■	■	■
Host abundance	Rodent abundance	■	■	■	■	■	■	■	■	■
Host characteristics	Sex	■	■	■	■	■	■	■	■	■
	Age	■	■	■	■	■				
	Body Condition	■	■	■	■					
Co-infestations with other parasites (count)	Fleas	■	■	■	■	■	■			
	Lice	■	■	■	■	■	■	■	■	
	Parasitic Laelapidae	■	■	■	■	■	■	■	■	■
	Myobiidae	■								
	Trombiculidae	■	■							
	Myocoptidae	■	■	■	■	■	■	■		
	Listrophoridae	■	■	■						
	n	21	20	19	18	17	16	15	14	13
	AIC	959.9	955.9	953.9	952.0	951.0	949.8	949.6	949.4	949.1
	Δ	10.8	6.81	4.87	2.92	1.94	0.74	0.51	0.32	0.00

B Best Model (Model 9)

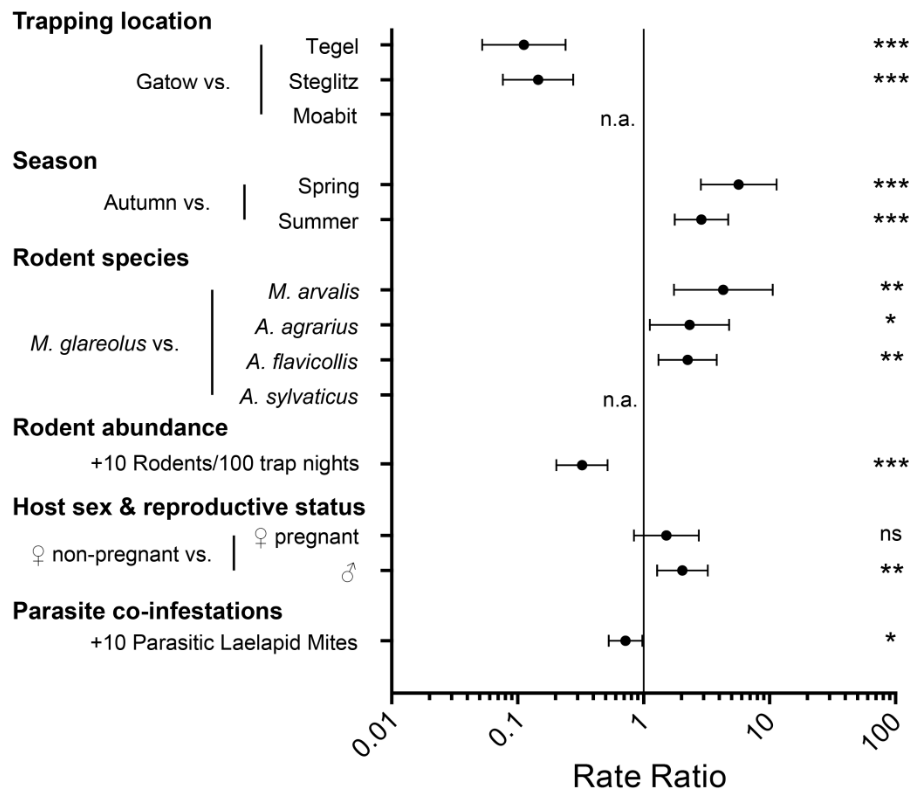


Figure 2-6. Parameters affecting number of host-associated *I. ricinus* larvae on wild rodents. (A) Model selection and (B) Forest Plot of negative binomial regression analysis of the count of *I. ricinus* larvae. (A) Analysis started with full model 1 including all the listed variables and was reduced by stepwise backwards variable selection to the best model 9. Number of variables (n), AIC values and difference of AIC to best model (Δ) are shown below. (B) Rate ratios with 95% CI for variables of model 9. The Y axis depicts additional counts (+) for metric parameters and reference levels in front of the other levels for categorical factors. Vertical line depicts rate ratio of 1 (no influence). * p<0.05; ** p<0.01; *** p<0.001; n.a.: not applicable because of total collinearity between *A. sylvaticus* and the trapping location Moabit.

2.5 Discussion

This study represents the first comprehensive investigation on the diversity as well as prevalence and intensity of rodent-associated arthropod infestations in wild mice and voles comparing these aspects in an urban/periurban context. The variety of rodent species and ectoparasite species differed significantly among the four trapping sites in Berlin. The trapping location affected not only the rodent abundance, but also the rodent species. The highest number of rodents was captured in the urban Botanic Garden Berlin in Steglitz followed by the periurban forest site Gatow. The site in Steglitz with its old trees and a marked shrub and ground vegetation provided ideal conditions for the striped field mouse *A. agrarius*. The sylvatic species yellow-necked mouse (*A. flavicollis*) and bank vole (*M. glareolus*) prefer old forests (Niethammer, 1978a; Viro & Niethammer, 1982) and were thus the most abundant species in the forest sites Tegel and Gatow. In Tegel, fewer rodents were observed than expected, possibly because at least in parts of that study site concurrent rodent control measures were undertaken. The trapping site in Moabit, an inner-city backyard, was the most urbanised location, with the wood mouse *A. sylvaticus* constituting the dominant rodent. In contrast to its name, it is a rather euryoecious species (Niethammer, 1978b; Zejda, 1965) and able to colonise anthropologically disturbed areas (Halle, 1987) in close proximity to humans.

The diversity of rodent-associated arthropods from fur and skin consisted of 63 species, of which 49 species were at least facultative parasites. The five mite species inhabiting nasal cavity and skin were only detected accidentally during microscopical examination of scabbed skin and after placing the carcass of the examined rodents over water for one week, which allowed the mites to leave the body. Parasitic beetles from the family Leptinidae (*Leptinus testaceus*) and myasis-causing flies from the family Oestridae (*Oestromyia* spp.) found in other areas of Germany (Artz, 1975; Schober, 1958; Stammer, 1956) were absent on rodents trapped in the present study. Most studies on the host species investigated here only examined certain rodent-associated arthropod groups (Table 2-3). Only three reports from non-Russian Europe investigated the whole array of rodent-associated arthropods: One of them is so old that comparisons may not be drawn due to new and re-descriptions, as for ectoparasites of British mice and voles (Elton *et al.*, 1931). In a study on larger arthropods, small fur mites and other Astigmata infesting the yellow-necked mouse and the bank vole in Poland, the number of examined hosts was not reported, small mite groups were not determined to species level and rare species were likely overlooked (Harris *et al.*, 2009). The sole directly comparable studies are those by Ryszard Haitlinger, who examined numerous small mammals, including 3,307 specimens of the rodent species examined here (Haitlinger, 1997; Haitlinger, 2006; Haitlinger, 2007; Haitlinger, 2008; Haitlinger, 2009a; Haitlinger, 2010a; Haitlinger,

2010b; Haitlinger, 2011; Haitlinger, 2015) at numerous sites in Poland over the last 20 years. Overall, he differentiated approximately 150 arthropod species. The 20 trapping sites in the northern Lubuskie province, Poland (Haitlinger, 2009a), were the closest to Berlin with 90-170 km distance. Here, he found a similar species richness of 69 arthropod species, 43 of which are parasitic, on a comparable number of 277 mice and voles. Haitlinger found fewer small arthropod species (Myobiidae, Myocoptidae). Because of their small body size, these species are easily overlooked, and the examination method presented herein seems to be more sensitive. Nevertheless, all myobiid and myocoptid species (except the undescribed *Criniscansor* sp.) were found by Haitlinger in other regions in Poland, probably only when they densely infested their hosts. However, true differences in fur mite diversity between the rural area in Poland and Berlin cannot be excluded. In accordance with most other studies from Europe (Ambros, 1984; Artz, 1975; Dudich, 1984; Kovacik, 1984; Mahnert, 1971c; Mahnert, 1972; Stammer, 1956; Willmann, 1952), Haitlinger determined more flea, trombiculid mite and non-parasitic gamasid mite species than recorded herein (Table 2-3). These arthropods exhibit a poor association with the mammal host: First, the majority of flea species detected on the rodents were nest-associated fleas, which live most of their life in the nest and only the adults temporarily parasitise the host. In addition, these species are little host-specific. Second, Trombiculidae are predatory mites, which are parasitic only in their larval stage, so-called chiggers. They have a wide host array feeding on numerous terrestrial vertebrates (Kepka, 1964). Third, the nidicolous, non-parasitic Gamasina feed on other arthropods, nematodes and dead organic material from mammals. The occurrence of these three groups, therefore, depends rather on habitat preferences and structure than on the rodent host. As rodents were trapped at rural, sparsely populated areas by Haitlinger (2009a), these natural habitats appear to facilitate higher species richness in arthropods with lower association to rodent hosts compared to highly fragmented urban wooded and non-wooded areas examined herein. In other studies, fragmentation led to a decreased species richness and abundance of pollinators (Aizen & Feinsinger, 1994), dung and carrion beetles (Klein, 1989) and butterflies (Rodrigues *et al.*, 1993). In contrast, the diversity and composition of highly specialised stationary ectoparasite species was very similar between the present and the mentioned studies from Europe and distinctly determined by the presence of the respective host species. Urbanisation may influence the presence of highly adapted rodent ectoparasites only if it affects the presence and abundance of the specific rodent host species.

Table 2-3. Number of species of different rodent-associated arthropod groups infesting the rodent species of the present study from similar studies from non-Russian Europe.

	Reference	Elton et al., 1931	Willmann, 1952	Stammer, 1956	Mahnert, 1971abc, 1972	Artz, 1975	Ambros, 1984, Kovacik, 1984, Dudich, 1984	Harris et al., 2009	Haitlinger, 2009a	present study
	Locality	England	Germany/ Poland	Germany	Austria	Germany	Slovakia	Poland	Poland	Germany
Number of hosts	*M. glareolus*	281a[a]	150	144	203	98	209-219[e]	?[f]	97	59
	M. arvalis	-	59	107	2	219	7-9[e]	-	35	11
	M. agrestis	368[a]	2	51	46	19	-	-	7	2
	A. agrarius	-	36	3	-	-	9	-	92	77
	A. flavicollis	-	245	25[b]	79	311	25-40[e]	?[f]	36	82
	A. sylvaticus	988[a]	178	198	16	44	-	-	10	25
	Total	1637[a]	670	528	349[c]	691	9	?[f]	277	256
Number of arthropod species	Diptera	0	-	1	-	0	-	0	0	0
	Coleoptera	1	-	0	-	1	-	0	0	0
	Siphonaptera	11	-	11	20[d]	14	12	9	12	10
	Anoplura	2	-	-	4	3	3	1	4	4
	Ixodida	1	-	-	3	2	1	2	2	3
	Gamasina parasitic	7	14	-	13	-	10	6	14	13
	Gamasina non-parasitic	7	7	-	15	-	7	1	18	6
	Myobiidae	0	3	-	-	-	-	1	2	6
	Trombiculidae	1	4	3		-	6	1	6	2
	Pygmephoridae	0	4	7	2	-	-	0	1	1
	Myocoptidae	0	2	-	-	-	-	1[g]	1	4
	Listrophoridae	1	1	-	-	-	-	1[g]	2	2
	other parasitic	1	0	-	0	-	-	0	0	5[h]
	other non-parasitic	3	0	9	1	-	-	1[g]	7	7
	Total	35	35	31	58	20	39	24	69	63

Only the study closest to Germany from Ryszard Haitlinger is illustrated. Some studies investigated further host species which are not displayed here. Hyphens indicate (presumably) not investigated arthropod groups

[a] Number of examined hosts not clear and different between arthropod group

[b] 25 *A. flavicollis* according to the lists but 35 according to the text

[c] Three *Apodemus* sp. specimens not determined to species level

[d] 21 if two subspecies of *Doratopsylla dasycnema* are considered

[e] Number of examined hosts differed between arthropod groups

[f] Number of examined hosts are not reported. Only *M. glareolus* screened for fur mites and other Astigmata

[g] Arthropod group not determined to species level and probably include more species

[h] all parasites in nasal cavity or skin

Concerning the quantity of infestations, there are no comprehensive data on the whole array of arthropods for comparison of the apparently high prevalence (99%) and mean intensity

(16 specimens per host) in the present study. However, this may be common in nature or even low, as in a longitudinal study from south-central Sweden (Tälleklint & Jaenson, 1997), tick larvae alone infested 100% of rodents with mean intensities of 34 (*M. glareolus*, n=106) to 68 larvae (*A. flavicollis*, n=31). Our live trapping method allowed us to detect the majority of arthropods living in the fur of rodents. Nevertheless, fleas, in particular, are known to leave the host rapidly during disturbance (Stark & Kinney, 1962), although in an experiment only about 5% of the fleas left the live trap when a rodent was trapped for 10 hours (Artz, 1975). Since also trap contents (cotton, apple, faeces) were screened, it can be expected that only a small number of fleas and other arthropods has been lost. In contrast, it cannot be excluded, that individual, non-rodent-associated (probably ground-dwelling) arthropods were examined, which entered the traps independently from the trapped host.

The quantity and species diversity of rodent-associated arthropods strongly depended on the rodent species and/or trapping location. Similarly, Timm (1975) recognised three primary categories of ectoparasites of mammals: (1) the host-specific, (2) the habitat-specific and (3) the cosmopolitan parasites. It was, therefore, decided to analyse both factors in combination in multivariate regression analyses. Voles (family Cricetidae), represented by three species, were much more frequently infested by trombiculid larvae and Myocoptidae than mice of the genus *Apodemus* (family Muridae). In contrast, mice more often hosted lice (most of all *A. agrarius*), Listrophoridae and the infestation intensity with fleas was higher than on voles. Hard ticks, gamasid and myobiid mites, on the other hand, revealed comparable prevalence and infestation intensities among the different rodent families. The reason, although not the cause, for differences in the quantitative occurrence of stationary parasitic groups, is that mice and voles are infested by different host-specific species, such as Anoplura, Myocoptidae and Listrophoridae. Differences may be caused by the width of species-specific niches of the arthropods concerning texture, density and diameter of hairs (Fain, 1994) as well as grooming behaviour of mice and voles.

Fleas are not markedly host-specific parasites. The reason for the higher number of fleas per mouse may be their larger body surface with their relatively long extremities compared to the rather compact vole body. Hence, fleas were most numerous on yellow-necked mice, the largest rodent in our study. Chiggers have a broad host range and the more subterranean life of voles may be the reason for the higher prevalence of the periodically parasitic trombiculid larvae. Hard ticks, most notably *I. ricinus* larvae and to a smaller extent nymphs, use a wide host array and accordingly occurred with an overall similar prevalence on all host species trapped at the same location. However, on the yellow-necked mouse, both life stages were more numerous than on the bank vole, often occurring syntopically, confirming earlier observations in Berlin (Matuschka *et al.*, 1990) and in south-central Sweden (Tälleklint & Jaenson, 1997). In addition, engorged larvae and nymphs feeding

on *A. flavicollis* and the resulting nymphs moulted from fed larvae appear to have a higher weight compared to those ticks feeding on *M. glareolus* (Tälleklint & Jaenson, 1997). The yellow-necked mouse, therefore, might be the more suitable host for this tick species. The lower prevalence of subadult ticks on *A. sylvaticus* may be explained by the trapping location Moabit (see below). Differences in prevalence and infestation intensity of the mainly host-specific gamasid and myobiid mites were not pronounced among rodents, because almost every host species was infested by a specific mite species. Only the bank vole was less frequently infested by Gamasina presumably because its specific *Laelaps* species, *L. clethrionomydis*, was absent, as it primarily occurs in submontane and montane regions (Ambros, 1995).

The trapping location was an equally important factor for the occurrence of different parasitic groups on the rodents. The backyard in Moabit in the city centre of Berlin was the most urbanised location and the surrounding habitat was highly fragmented. Periodic and temporary parasites, such as fleas, ticks and Trombiculidae live most of their life span off the host. For this reason, these parasites strongly depend on environmental parameters. Particularly, those parasites that require undisturbed habitats were infrequently found on rodents at this urbanised location. For nest-associated fleas and especially their juvenile stages, the properties of the host's nest, such as temperature and relative humidity of 70-80% (Brinck-Lindroth & Smit, 2007) are more important than host characteristics (Mahnert, 1972). Similarly, *I. ricinus* larvae require high levels of relative humidity (Randolph & Storey, 1999). As the different stages of this tick prefer various hosts, their further development depends on access and habitat quality for these vertebrates. The trombiculid mites require high humidity (Kampen, 2002) and undisturbed ground vegetation and fauna where they live as predators in their deutonymphal and adult stages. On the other hand, stationary parasites, such as Myobiidae and Listrophoridae, closely adapted to the host fur, were very prevalent and numerous on mice and voles in Moabit. As these observations mainly derive from *A. sylvaticus* being the dominant rodent species at the Moabit site and being absent from the other sites, this collinearity makes it statistically impossible to differentiate them from the host species as factor. But because of the close phylogenetic relation to *A. flavicollis*, these differences are unlikely to be particular features of the wood mouse.

The area around the forestry office in Tegel with the adjacent state forest and the wooded area at the General-Steinhoff-Barracks in Gatow were comparable habitats situated at the periphery of Berlin. Little fragmentation and anthropogenic impact characterised these periurban sites in contrast to Moabit. At these trapping locations, temporary or periodic parasites, especially chiggers, were more prevalent and numerous than at any other site. Also, subadult *I. ricinus* infested more rodents with higher intensities than in the more urbanised locations. The differences between the two forest

sites were mainly on the species level for some frequent parasites (e.g. *H. zachvatkini* (Trombiculidae), *L. brevipes* (Listrophoridae), *H. isabellinus* (Gamasina)), whereas sucking lice were completely absent in Tegel. In general, louse species showed a very peculiar and focal occurrence. Although stationary parasites, they were not substantially more abundant on urban sites and they were occasionally absent from rodents in particular locations. Whether this observation results from differing vegetation, microclimate, predators, or simply from founder effects in these particular habitats remains to be examined.

The Botanic garden in Steglitz revealed an intermediate composition of rodent ectoparasites. Although in the centre of an urban area, it is characterised by diverse and structured vegetation with old trees and constituted a habitat even for the sylvatic yellow-necked mouse. A quantity of ticks and fleas comparable to that at the forest sites was found, but no chiggers and higher prevalence of stationary fur mites, such as Myobiidae and Listrophoridae, similar to the urban backyard Moabit.

Seasonal differences in the abundances of rodent-associated arthropods are to be expected in temperate regions. The seasonal abundance of ectoparasites on rodents must be interpreted in respect to the population dynamics of their hosts. The bank vole and the yellow-necked mouse are both mainly bivoltine, with the first litter from overwintered females in spring and the second from these females together with their progenies in early summer (Harris *et al.*, 2009; Niethammer, 1978a; Stenseth & Gustafsson, 1985). Therefore, the greatest recruitment of “new” rodent hosts occurs in July/August resulting in a dilution of parasite numbers on individual hosts (Harris *et al.*, 2009), which may be most noticeable for parasites with long generation times. In the present study, two flea species, including *C. agyrtes* as the second most prevalent parasite, both occurred throughout the study period and showed a unimodal occurrence with peak abundance on the rodents in early spring. This was similarly observed for *C. agyrtes* in Poland (Haitlinger, 1983a), but in other studies a second peak in late summer/autumn was observed (Brinck-Lindroth & Smit, 2007; Harris *et al.*, 2009). The sucking louse *P. serrata* was most abundant in summer which is consistent with the occurrence of *Hoplopleura edentula* and *Hoplopleura acanthopus* in Tyrol, Austria (too few data for *P. serrata*) (Mahnert, 1971b), *H. edentula* in Poland (Haitlinger, 1983a) and other studies (Sosnina *et al.*, 1981; Stanko *et al.*, 2015). Lice produce only one generation of offspring during the summer (Mahnert, 1971b; Smetana, 1962) presumably because of the higher fecundity during moderate temperatures (Marshall, 1981) and the higher density and activity of the rodent hosts (Sosnina *et al.*, 1981). Similar to the present observations, the seasonality of rodent-associated *I. ricinus* larvae is often described as bimodal with peaks in early summer and autumn (Nilsson, 1988; Paziewska *et al.*, 2009) and near absence in winter. In some other studies, the depression in midsummer was missing (Matuschka *et al.*, 1990; Pérez *et al.*, 2012). Theoretically, the reduced

infestation of rodents with larval ticks in July may have three reasons: First, the increase in rodent abundance at this time may result in fewer ticks feeding on individual rodents (Matuschka *et al.*, 1990). Second, a probable bimodal recruitment of "new" *I. ricinus* larvae may derive from overwintering eggs or overwintering engorged females in spring and from engorged females of the same year in late summer. Third, Randolph and Storey (1999) demonstrated experimentally that *I. ricinus* larvae quest for hosts in the ground vegetation during periods of low saturation deficits as an index of humidity, whereas nymphs move to vantage points high above the ground. In contrast, during periods of high saturation deficits, larvae remain inactive, whereas nymphs continue to quest for hosts low in the vegetation, where they are more likely to encounter small mammals (Randolph & Storey, 1999). Since saturation deficits were highest in the summer in Berlin (Supplemental Figure 2-9), this may explain why larvae were rarely detected on rodents in summer while nymphs parasitised rodents most abundantly. Parasitic gamasid mites showed differences in seasonality depending on the species. The closely host-associated mites were most abundant in summer and autumn which is also described for *L. agilis* in southern Sweden (Edler, 1973). In Poland, only one peak in July was observed (Harris *et al.*, 2009). The facultative parasite *H. nidi* most abundantly infested rodents in early summer, but seasonal occurrence differed between trapping sites in the Swedish study. All gamasid mites reproduce throughout the year (Edler, 1973). Only little is known about seasonality of the very small fur mites and astigmat hypopi which use rodent fur for phoresy. Surprisingly, all of them revealed a similar pattern being most abundant in August (fur mites) or October (phoretic hypopi), but were nearly absent until July. This may be a methodological bias because higher awareness presumably increased the sensitivity of detection of these small mites towards the end of the study. In contrast, these mites were abundant throughout the year on bank voles in Poland (Harris *et al.*, 2009), whereas *Listrophorus* was slightly less abundant in summer. Further long-term studies are needed to verify the present results.

Because of their mobility, broad host array and the ability to penetrate human skin, only fleas, hard ticks, chiggers and laelapid mites may potentially constitute a direct or indirect zoonotic and public health relevance. Specimens of these groups occurred on 95% of the rodents in Berlin with an average of 12 arthropod specimens per rodent. However, only a small number of these species have actually been recorded to infest humans. Rodent fleas are mainly nest-associated and humans generally do not come in close contact with rodent nests (Brinck-Lindroth & Smit, 2007).

Of the flea species found in the present study, only the squirrel or dormouse flea *Monopsyllus sciurorum* and *N. fasciatus*, which primarily infests rats and house mice, have been described to attack humans (Brinck-Lindroth & Smit, 2007). Both species have a wide host array and whereas *M. sciurorum* was rare, *N. fasciatus* infested 18% of the rodents in our study, mainly *Apodemus* mice.

The vector role of *M. sciurorum* is unknown, but *N. fasciatus* is able to transmit the tapeworms *Hymenolepis diminuta* and *Rodentolepis nana* (syn. *Hymenolepis nana*) (Marshall, 1981) and is a competent vector for *Yersinia pestis* and *R. typhi* (Eisen & Gage, 2012). Nevertheless, it is unlikely that people ingest fleas infected with these tapeworms (Marshall, 1981) and infections in humans are exceedingly rare in Central Europe (Kołodziej *et al.*, 2014; Tomaso *et al.*, 2001). The plague is no longer endemic in Europe and the role of these fleas in the transmission of *R. typhi* is presumably poor (Eisen & Gage, 2012). DNA of two zoonotic *Bartonella* species was detected in *N. fasciatus*, *C. agyrtes* and *Megabothris turbidus* fleas from rodents in Saxony (Silaghi *et al.*, 2016b), but the vector competence has not been examined. Likewise, the sole detection of *Rickettsia* DNA in *C. agyrtes* (Špitalská *et al.*, 2015) fails to prove the role as vector.

Of the chiggers species, at least the harvest mite *N. autumnalis*, which was abundant at periurban sites, infests humans causing scrub itch and pruritic dermatitis. The hard tick *I. ricinus* abundantly parasitised rodents in all study sites in Berlin. As long as they are attached to rodents, both ectoparasites do not pose a direct risk of infestation for humans, but the hosts promote the development of the next generation (chiggers) or life stage (ticks) of ectoparasites in proximity to humans. More importantly, rodents are reservoirs for tick-borne pathogens and may maintain the transmission cycles of these pathogens, constituting a risk of contact with infected nymphal or adult ticks (Matuschka *et al.*, 1996). *Rickettsia* spp. DNA may be detected in mites, such as Trombiculidae, and an 18% DNA prevalence was found for *H. zachvatkini* (*Rickettsia helvetica, Rickettsia monacensis*) and *N. autumnalis* in Slovakia (Miťková *et al.*, 2015). Whether these findings only reflect the gut content of the mite containing blood from infected rodents or whether the mites are vector-competent remains to be examined in appropriate transmission experiments.

Reports regarding the infestation of humans with gamasid mites from the family Laelapidae are very rare and mostly doubtful. An often cited case of mite dermatitis caused by *Haemogamasus pontiger* in soldiers in England during the Second World War was challenged by Halliday (2011), as it was more likely caused by *Pyemotis* mites. Likewise, reddish papulous dermatitis caused by *Androlaelaps casalis* on humans in England (personal communication from Evans in Baker *et al.* (1956)Baker et al.) and in Israel derived from contacts with rat and pigeon nests (Rosen *et al.*, 2002) were reported and the mite was shown to feed on droplets of human blood (McKinley, 1963). In contrast, the latter author suggested that the mite was unable to penetrate vertebrate (including human) skin which was confirmed by Lesna *et al.* (2009). The species *Laelaps nutalli*, *Laelaps echidninus* and *Androlaelaps fahrenholzi* are unable to feed on intact skin, but feed on abraded human skin (Wharton & Cross, 1957). Nevertheless, infestations of humans are mainly conceivable after rodent

control measures, when the natural hosts have been eliminated. However, the peridomestic rodent species trapped in the present study usually do not live inside houses.

The direct zoonotic risk of arthropods associated with peridomestic rodents is low in Berlin because these rodent species rarely come in close contact with humans and the majority of ectoparasite species has never been reported to infest people. Apart from *N. fasciatus*, typical rodent-borne zoonotic arthropods, such as the tropical rat mite *Ornithonyssus bacoti* and *Liponyssoides sanguineus*, a vector for *Rickettsia akari* (Renvoisé *et al.*, 2012), were absent. These ectoparasites are primarily associated with rats and house mice that live in close proximity to humans, but are rarely found on other mice and voles.

However, if the mentioned transmission of pathogens by C. *agyrtes* would be possible, this abundant flea species, infesting every second rodent in the present study, may be important for the circulation of *Bartonella* spp. and *Rickettsia* spp. in the rodent population. Likewise, laelapid mites may transmit pathogens within the rodent population and enhance their reservoir competence for pathogens. For example, DNA from *Rickettsia helvetica* was found in as much as 36.4% of *L. agilis* in Slovakia and also in *H. nidi* (Miťková *et al.*, 2015). Further pathogens were isolated and identified by culture, such as *Francisella tularensis* from *H. nidi*, *L. hilaris* and *H. isabellinus*, *Coxiella burnetii* from *H. nidi*, *Haemogamasus hirsutus*, *E. stabularis* and *A. fahrenholzi* and the TBE virus from *H. nidi*, *H. hirsutus*, *H. isabellinus, E. stabularis* and *A. fahrenholzi* (Valiente Moro *et al.*, 2005). At least *E. stabularis* is able to become and remain experimentally infected with the TBE virus and to transmit it to rodents (Naumov & Gutova, 1984).

Peridomestic rodents as reservoir hosts for tick-borne pathogens constitute a relevant health risk for people. The multivariate regression analysis identified the following parameters affecting the abundance of larval ticks: Study location, season, rodent species, rodent abundance, host sex and the abundance of co-infesting laelapid mites. Rodents in Gatow were more heavily infested by ticks than those at any other study site. The study was not designed to examine the co-factors for spatial variation. The host species clearly affected the abundance of *I. ricinus* larvae on the rodents. The broad host array of this tick suggests that differences in the quantity of ticks on rodent species may depend on the frequency of tick-host encounters. The infestation intensity of rodents with ticks is positively correlated with the distance rodents migrate between successive captures (Sonenshine & Stout, 1968). *Apodemus* mice have larger home ranges than bank voles (Macdonald & Barrett, 1993) and, therefore, should encounter more ticks than bank voles. Also, different levels of tick mortality during the blood meal may result from different grooming behaviour, immune reactions and development of immunity (Dizij & Kurtenbach, 1995). Partial co-linearity between rodent species and

location may have further confounded the model estimates. The model revealed that larval ticks infest rodents less abundantly in the late autumn than in spring and summer. The abundance of rodent hosts had an impact on levels of tick infestation, as density of infestation decreased when the number of rodents trapped increased. Besides the dilution effect, home ranges of rodents seem to become smaller, when rodents become more abundant (reviewed by Tälleklint and Jaenson (1997)) which reduces the probability of encountering ticks from the vegetation or ground. Male rodents hosted more *I. ricinus* larvae than non-pregnant females. Male-biased parasitism is a commonly observed phenomenon for many mammals and it is often attributed to differences in body size, behaviour, such as grooming or home range, and physiology, such as the immunosuppressive effect of testosterone (see Harrison *et al.* (2010) and Marshall (1981) for review). Although body size increases with age, this variable had only a minor effect on the tick infestation in the present study. Behavioural reasons for a male-bias are more likely, since the home ranges of male *Myodes*, *Microtus* and *Apodemus* rodents are larger than those of conspecific females especially during the breeding season (reviewed by Tälleklint and Jaenson (1997)) and consequently they should encounter more ticks. The pregnancy of females did not affect the tick count, although an energy-consuming active reproductive status may reduce the immune status and affect the successful blood meal of ticks. Malnutrition is known to impair immunity and resistance to parasites (Hughes & Kelly, 2006), but the body condition did not sufficiently affect the abundance of *I. ricinus* larvae. Limitation of food presumably did not really occur during the study period since rodent densities were moderate. In a study in Hesse, Germany, a regression analysis with a forward variable selection was performed on a comparable number of mice and voles (Kiffner *et al.*, 2011). They similarly identified location, season, host species and rodent density as important factors for tick infestation on rodents. In contrast, they found that age and/or body mass had an impact as well as to some extent relative humidity and vegetation cover, whereas host sex failed to improve their model.

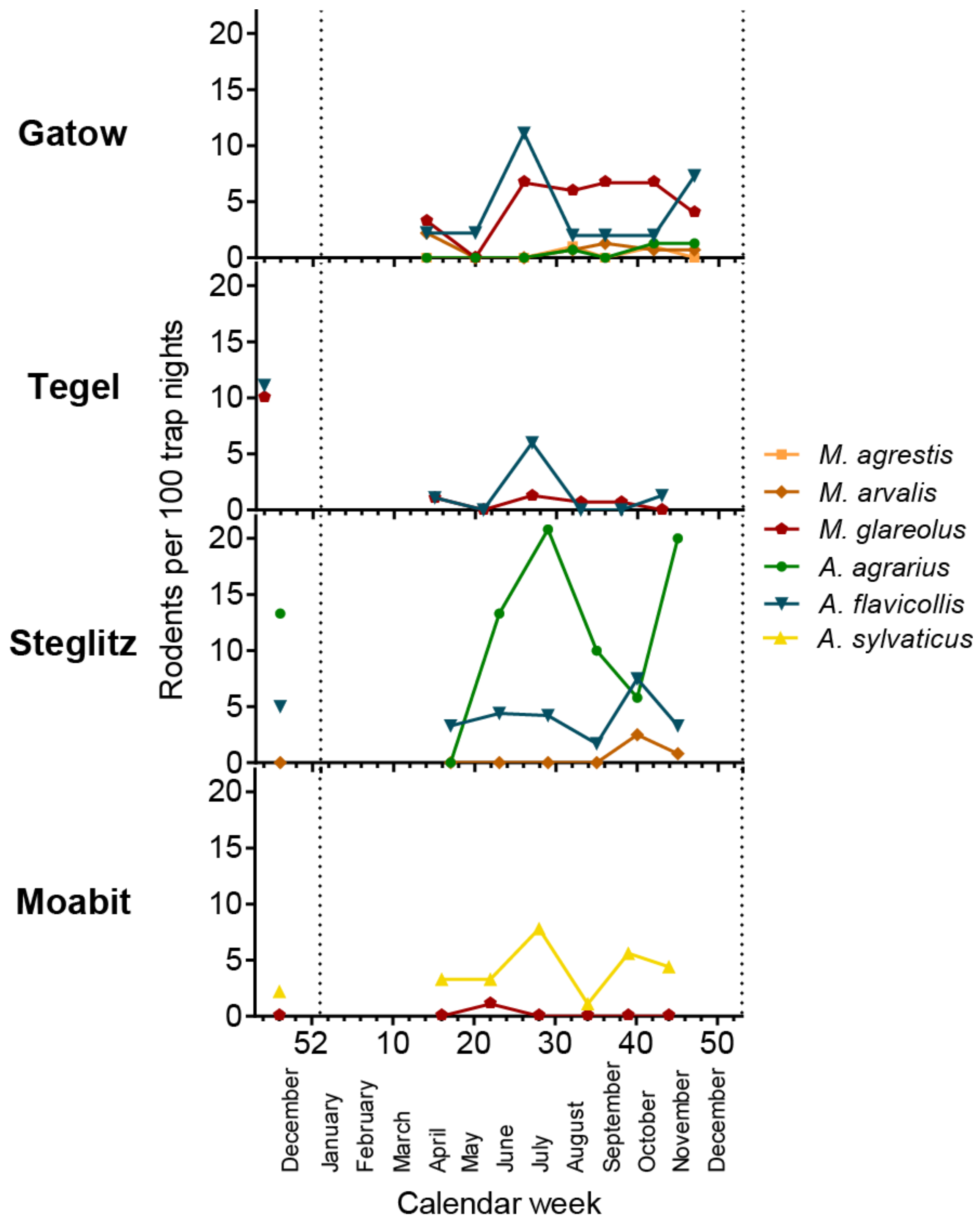
It was analysed whether ectoparasitic co-infestations affect tick abundance on rodent hosts considering all the co-factors in the regression model. An increasing abundance of co-infesting ectoparasitic gamasid mites of the family Laelapidae was significantly correlated with a reduced abundance of *I. ricinus* larvae on mice and voles. Some mites are known to predate on ectoparasites in the fur of mammals, such as the Cheyletidae *Cheyletiella parasitivorax*, *Chelacaropsis moorei* and *Hemicheyletus* spp. on Listrophoridae and other parasitic arthropods (Kim, 1985; Krantz & Walter, 2009). But according to Samish and Alekseev (2001), mites were reported only once to feed on host-attached ticks, namely the chigger *Parasecia gurneyi* on larval *Ixodes scapularis* feeding on a lizard in the USA (Oliver *et al.*, 1986). Parasitism in laelapid mites derives from predation on arthropods (subfamily Hypoaspidinae) and species of the family represent all stages from facultative to obligate

blood-sucking parasitism (Kim, 1985). The normal diet of at least *A. fahrenholzi*, *E. stabularis* and *H. nidi* contains arthropods. In laboratory feeding tests *H. ambulans*, *L. echidninus* and *A. fahrenholzi* fed on blood-filled sucking lice (Kim, 1985). Ticks, which feed on their hosts for several days, can hardly defend themselves and may be an easily accessible food resource for these mites. Increased grooming due to mite infestation may result in simultaneous removal of feeding ticks. And competition for space, release of toxic products by feeding mites, or an interaction of both ectoparasites via the immune system are further potential explanations. Although the immune responses towards feeding gamasid mites are poorly studied, they presumably initiate type 2 T helper cell responses, as ticks do in the skin of rodents. Immune reactions against mites may, therefore, impair tick feeding as well. Although infections with intestinal endoparasites in laboratory mice failed to affect the success of tick infestation (Maaz *et al.*, 2016 = chapter 4), interspecific local immune reactions in the same compartment, the skin, may be conceivable. Whether the negative relationship of the abundance of laelapid mites and *I. ricinus* larvae is caused by direct interactions between the parasites or via the rodent host remains to be examined experimentally. However, the abundant co-infestations of peridomestic rodents with parasitic Laelapidae may influence the suitability of rodents as host for ticks and their reservoir competence for tick-borne pathogens.

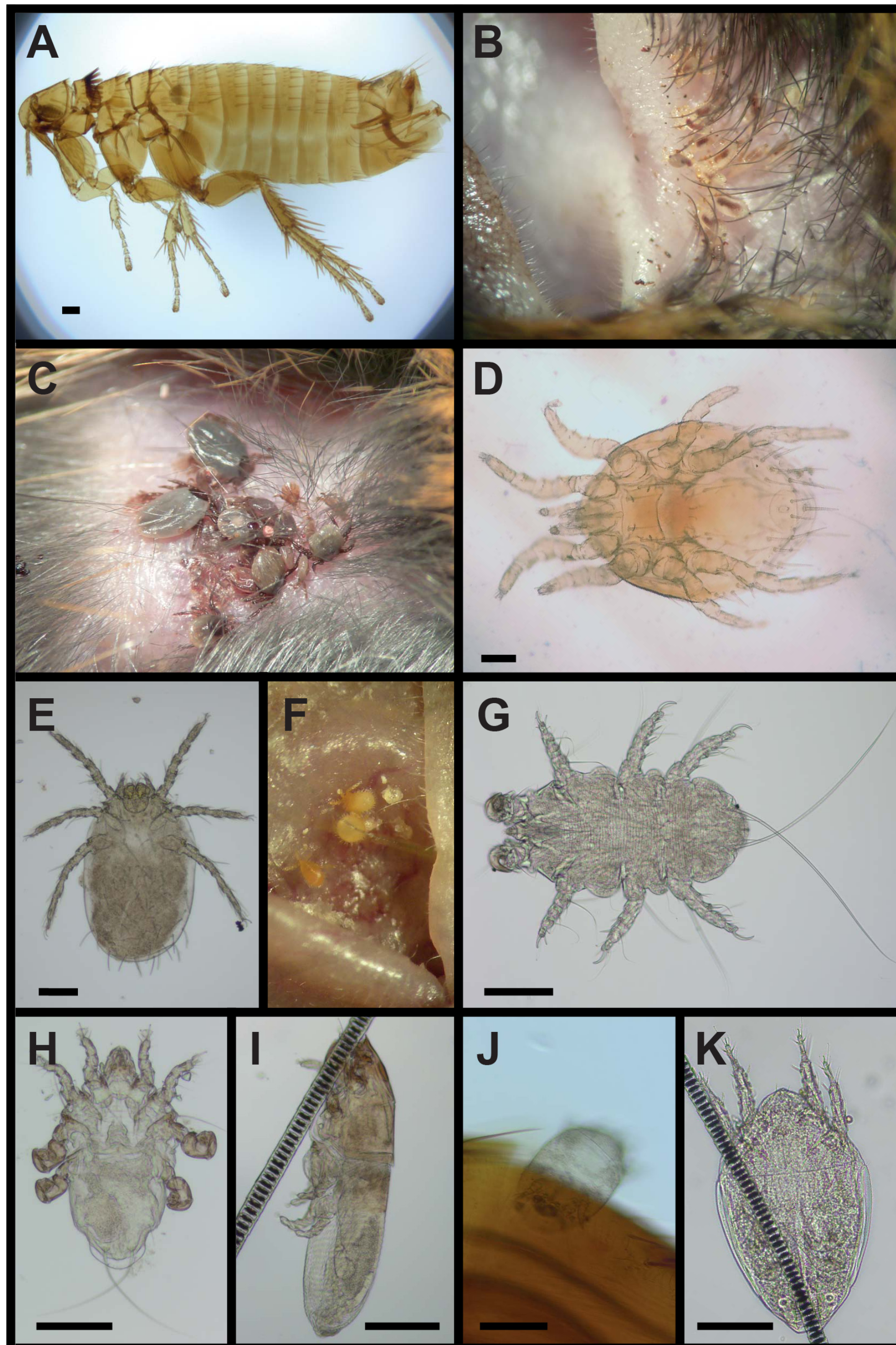
2.6 Acknowledgements

We thank Eberhard Wurst (Senckenberg Museum of Natural History, Görlitz) and Andre V. Bochkov (Zoological Institute of the Russian Academy of Sciences, Saint Petersburg, Russia) for their help determining some of the rare species. Claudia Baldermann and Marcus Groß (Statistical Consulting fu:stat, Freie Universität Berlin) are acknowledged for statistical support. We also thank the Botanic Garden Berlin, the proprietors of the apartment building, the General-Steinhoff-Barracks and the forestry office in Tegel for permitting us to conduct the study on their premises.

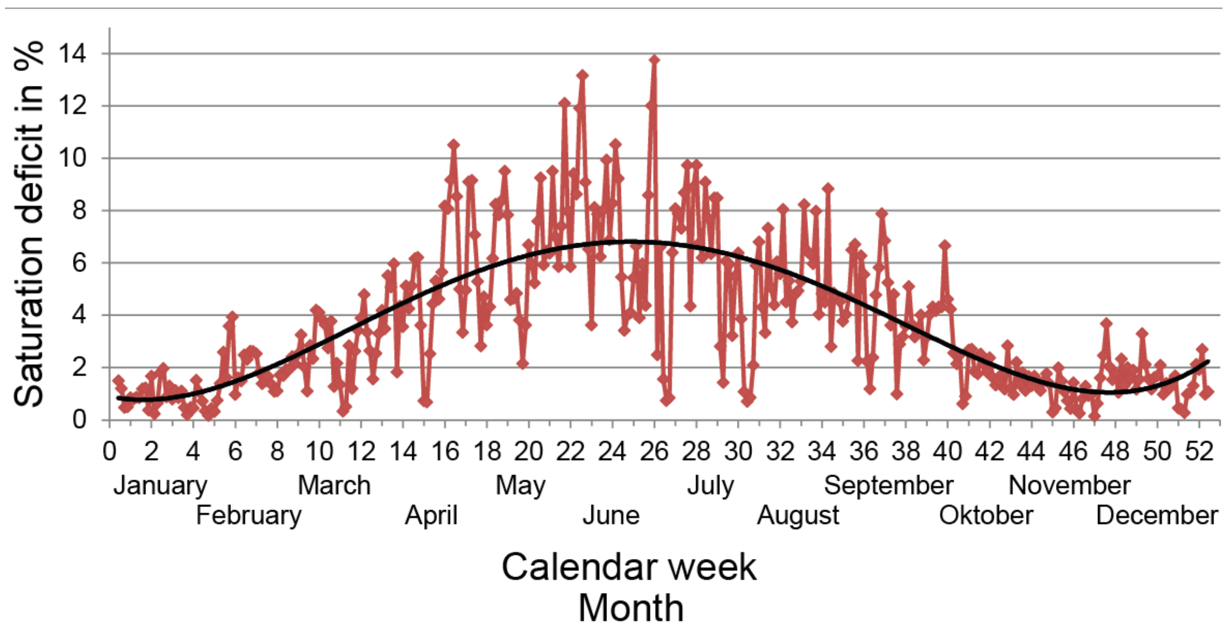
2.7 Supporting information



Supplemental Figure 2-7. Seasonal rodent activity at trapping sites. For every rodent species, the numbers of animals per 100 trap nights are shown for every trapping week (three consecutive nights) and for four different study sites. Dashed line indicates the turn of the year.



Supplemental Figure 2-8. Light micrographs (A, D, E, G-K) and photographs (B, C, F) of diverse rodent-associated arthropod species collected from mice and voles from Berlin. A *Ctenophthalmus agyrtes* (Siphonaptera) male, B *Polyplax serrata* (Anoplura) infesting the ear margin of *A. agrarius*, C *Ixodes ricinus* (Ixodidae) larvae and nymphs infesting neck of *A. flavicollis*, D *Laelaps hilaris* (Laelapidae) female, E *Neotrombicula autumnalis* (Trombiculidae) larva, F *Hirsutiella zachvatkini* larvae infesting ear of *M. glareolus*, G *Myobia muris-musculi* (Myobiidae) female, H *Myocoptes japonensis* (Myocoptidae) female, I *Afrolistrophorus apodemi* (Listrophoridae) female, J phoretic hypopus (deutonymph) of *Acarus nidicolous* attached to sternal plates of *Megabothris turbidus* (Siphonaptera), K phoretic hypopus of *Glycyphagus hypudaei* attached to a hair of *M. glareolus*, scale bars 0.1 mm, specimens in A and D were cleared in potassium hydroxide.



Supplemental Figure 2-9. Course of saturation deficit in an urban park in Berlin Steglitz in 2011. Daily means of minute values of saturation deficit calculated from relative humidity and temperature (table 10 in (Deutscher-Wetterdienst, 1998)). Data were measured by a climate station (THIES Clima) at a height of 2 m every day of the year 2011 and were provided by the Institute of Meteorology, Freie Universität Berlin. Line chart (red) is depicted together with an order 4 polynomial trend line (black).

Supplemental File 2-1. Original table with raw data of rodent trapping and arthropod identification (on attached CD). Rows in the sheet “Data” represent trapped rodent individuals with data on rodent taxonomy, trapping, rodent body measures and arthropod counts. Shaded rodents were released or escaped and were not further investigated. The sheet “Codebook” lists the description and scale levels of every column.

Supplemental Table 2-4. Regression analyses for modelling presence/absence (=prevalence) and count (=intensity of infestation) of ectoparasites on peridomestic rodents from Berlin as dependent variable. Rodent host species (6 levels) or family (2 levels) and trapping location (4 levels) or location category (2 levels) were used as independent variables. Odds Ratios (OR) for logistic regression (left panel) or Rate Ratios for negative binomial regression (right panel) are shown together with 95% CI and p-value. * p<0.05, ** p<0.01, *** p<0.001 n.a.: not applicable because of total collinearity between *A. sylvaticus* and the trapping location Moabit.

Logistic Regression Analyses

	Odds Ratio	95%CI	p-Value	
A Prevalence of *N. fasciatus*				
Intercept	0.17	0.07-0.41	<0.001	***
Mouse vs. Vole	0.27	0.07-1.03	0.055	
Gatow vs. Tegel	0.23	0.03-1.96	0.179	
Gatow vs. Steglitz	2.33	0.90-6.00	0.080	
Gatow vs. Moabit	2.64	0.81-8.66	0.109	
B Prevalence Lice				
Intercept	2.47	1.61-3.78	<0.001	***
Mouse vs. Vole	0.25	0.10-0.62	0.003	**
Steglitz vs. Gatow	0.26	0.12-0.53	<0.001	***
Steglitz vs. Tegel	0	0-Inf	0.985	
Steglitz vs. Moabit	0.31	0.13-0.76	0.010	**
C Prevalence of *I. ricinus* larvae				
Intercept	5.41	2.61-11.22	<0.001	***
Mouse vs. Vole	0.89	0.41-1.97	0.78	
Gatow vs. Tegel	0.17	0.07-0.41	<0.001	***
Gatow vs. Steglitz	0.13	0.06-0.29	<0.001	***
Gatow vs. Moabit	0.04	0.01-0.15	<0.001	***
D Prevalence of parasitic Laelapidae				
Intercept	0.45	0.25-0.82	0.009	**
M. glareolus vs. *M. arvalis*	7.57	1.42-40.42	0.018	*
M. glareolus vs. *M. agrestis*	0	0-Inf	0.989	
M. glareolus vs. *A. agrarius*	3.15	0.96-10.39	0.059	
M. glareolus vs. *A. flavicollis*	5.77	2.59-12.85	<0.001	***
M. glareolus vs. *A. sylvaticus*	>999	0-Inf	0.990	
Gatow vs. Tegel	0.96	0.40-2.29	0.928	
Gatow vs. Steglitz	2.45	0.88-6.84	0.086	
Gatow vs. Moabit	0	0-Inf	0.992	
E Prevalence of Trombiculidae				
Intercept	0.25	0.13-0.48	<0.001	***
Vole vs. Mouse	0.13	0.04-0.53	0.004	**
Gatow vs. Tegel	2.40	0.78-7.34	0.125	
Gatow vs. Steglitz	0	0-Inf	0.992	
Gatow vs. Moabit	0	0-Inf	0.996	

Negative Binomial Regression Analyses

	Rate Ratio	95%CI	p-Value	
J Intensity of fleas				
Intercept	3.7	2.88-4.74	<0.001	***
Mouse vs. Vole	0.57	0.42-0.77	<0.001	***
Gatow vs. Tegel	1.25	0.92-1.73	0.184	
Gatow vs. Steglitz	0.92	0.68-1.23	0.560	
Gatow vs. Moabit	0.7	0.41-1.20	0.195	
K Intensity of *I. ricinus* larvae				
Intercept	14.74	10.48-20.72	<0.001	***
Mouse vs. Vole	0.81	0.53-1.23	0.320	
Gatow vs. Tegel	0.23	0.13-0.43	<0.001	***
Gatow vs. Steglitz	0.36	0.23-0.57	<0.001	***
Gatow vs. Moabit	0.14	0.04-0.42	<0.001	***
L Intensity of *I. ricinus* larvae				
Intercept	8.66	6.38-11.77	<0.001	***
M. glareolus vs. *M. arvalis*	3.58	1.63-7.84	0.001	**
M. glareolus vs. *M. agrestis*	1.21	0.30-4.91	0.788	
M. glareolus vs. *A. agrarius*	1.07	0.56-2.05	0.831	
M. glareolus vs. *A. flavicollis*	1.84	1.19-2.84	0.006	**
M. glareolus vs. *A. sylvaticus*	0.23	0.08-0.67	0.007	**
Gatow vs. Tegel	0.24	0.14-0.44	<0.001	***
Gatow vs. Steglitz	0.43	0.26-0.73	0.002	**
Gatow vs. Moabit	n.a.	n.a.	n.a.	
M Intensity of *I. ricinus* nymphs				
Intercept	3.69	2.31-5.88	<0.001	***
A. flavicollis vs. *M. glareolus*	0.43	0.21-0.87	0.020	*
A. flavicollis vs. *M. arvalis*	1.08	0.37-3.19	0.883	
A. flavicollis vs. *A. agrarius*	0.70	0.26-1.85	0.471	
A. flavicollis vs. *A. sylvaticus*	0.34	0.11-1.02	0.055	
Gatow vs. Tegel	0.42	0.17-1.01	0.052	
Gatow vs. Steglitz	0.43	0.18-1.05	0.063	
Gatow vs. Moabit	n.a.	n.a.	n.a.	
N Intensity of parasitic Laelapidae				
Intercept	19.47	11.66-32.52	<0.001	***
M. arvalis vs. *M. glareolus*	0.13	0.06-0.25	<0.001	***

Supplemental Table 2-4. Continued

Logistic Regression Analyses

	Odds Ratio	95%CI	p-Value	
F Prevalence of *I. ricinus* larvae				
Intercept	4.98	2.45-10.12	<0.001	***
M. glareolus vs. *M. arvalis*	0.72	0.15-3.32	0.670	
M. glareolus vs. *M. agrestis*	>999	0-Inf	0.989	
M. glareolus vs. *A. agrarius*	0.70	0.23-2.15	0.529	
M. glareolus vs. *A. flavicollis*	1.13	0.47-2.71	0.790	
M. glareolus vs. *A. sylvaticus*	>999	0-Inf	0.992	
Gatow vs. Tegel	0.17	0.07-0.40	<0.001	***
Gatow vs. Steglitz	0.17	0.07-0.44	<0.001	***
Gatow vs. Moabit	0	0-Inf	0.991	
G Prevalence of *I. ricinus* nymphs				
Intercept	0.26	0.14-0.40	<0.001	***
M. glareolus vs. *M. arvalis*	1.09	0.20-5.89	0.924	
M. glareolus vs. *M. agrestis*	0	0-Inf	0.989	
M. glareolus vs. *A. agrarius*	0.89	0.23-3.45	0.864	
M. glareolus vs. *A. flavicollis*	1.16	0.48-2.77	0.742	
M. glareolus vs. *A. sylvaticus*	1.55	0.30-7.98	0.597	
Periurban vs. Urban	0.47	0.16-1.39	0.174	
H Prevalence of Myobiidae				
Intercept	0.58	0.34-0.98	0.042	*
M. glareolus vs. *M. arvalis*	0.46	0.10-2.07	0.311	
M. glareolus vs. *M. agrestis*	>999	0-Inf	0.988	
M. glareolus vs. *A. agrarius*	0.29	0.09-0.86	0.026	*
M. glareolus vs. *A. flavicollis*	0.82	0.38-1.74	0.602	
M. glareolus vs. *A. sylvaticus*	0.56	0.15-2.13	0.399	
Periurban vs. Urban	6.53	2.67-16.00	<0.001	***
I Prevalence of Listrophoridae within *Apodemus* mice				
Intercept	0.17	0.06-0.46	<0.001	***
A. agrarius vs. *A. flavicollis*	1.56	0.67-3.61	0.302	
A. agrarius vs. *A. sylvaticus*	1.41	0.57-3.50	0.459	
Periurban vs. Urban	4.64	1.83-11.77	0.001	**

Negative Binomial Regression Analyses

	Rate Ratio	95%CI	p-Value	
M. arvalis vs. *A. agrarius*	0.37	0.20-0.67	0.001	**
M. arvalis vs. *A. flavicollis*	0.63	0.36-1.08	0.094	
M. arvalis vs. *A. sylvaticus*	0.38	0.20-0.69	0.002	**
Gatow vs. Tegel	0.41	0.26-0.66	<0.001	***
Gatow vs. Steglitz	0.39	0.26-0.57	<0.001	***
Gatow vs. Moabit	n.a.	n.a.	n.a.	
O Intensity of Trombiculidae				
Intercept	10.12	5.06-20.25	<0.001	***
M. glareolus vs. *M. arvalis*	2.44	0.53-11.26	0.254	
M. glareolus vs. *A. agrestis*	0.81	0.06-10.69	0.874	
M. glareolus vs. *A. flavicollis*	0.95	0.29-3.10	0.932	
Tegel vs. Gatow	0.12	0.04-0.33	<0.001	***
P Intensity of Listrophoridae within *Apodemus* mice				
Intercept	3.67	1.33-10.14	0.012	*
A. agrarius vs. *A. flavicollis*	1.82	0.86-3.83	0.116	
A. agrarius vs. *A. sylvaticus*	1.31	0.61-2.83	0.488	
Periurban vs. Urban	2.96	1.17-7.48	0.022	*

Supplemental Table 2-5. Distribution of arthropods on wild rodent species. Total number and sex ratio (male : female) of parasites as well as number of infected rodents, prevalence and mean intensity for six rodent species are shown for every arthropod species. The number of examined rodents and the number of male/female are given below the species name. The last column shows values for the sum of all rodent species. Capital letters next to the families indicate higher arthropod taxa: Si Siphonaptera (fleas), Ph Phthiraptera (lice), Ix Ixodida (ticks), Ga Gamasina (Mesostigmata), Pr Prostigmata, As Astigmata. n: Number of arthropods. Hyphens indicate absence of arthropods. No: Number of infested rodents. P [%]: Prevalence in %. mI [n]: mean intensity = mean number of parasites on infected rodents. max: highest arthropod intensity. n.d.: no adults observed or not determined.

Arthropod				Rodent host																					
				M. glareolus 29/29 = 59[a]			*M. arvalis* 4/7 = 11			*M. agrestis* 0/2 = 2			*A. agrarius* 44/33 = 77			*A. flavicollis* 41/41 = 82			*A. sylvaticus* 14/11 = 25			All Species 132/123 = 256[a]			
	Species	n	Sex ratio	No	P [%]	mI [n]	No	P [%]	mI [n]	No	P [%]	mI [n]	No	P [%]	mI [n]	No	P [%]	mI [n]	No	P [%]	mI [n]	No	P [%]	mI [n]	max
Parasitic arthropods																									
Si Ctenophthalmidae	*Ctenophthalmus agyrtes*	369	1 : 1.31	27	46	1.8	6	55	2.3	1	50	1.0	38	49	2.8	52	63	3.7	4	16	2.2	128	50.0	2.9	14
	Ctenopthalmus assimilis	7	1 : 1.3	3	5	1.0	1	9	3.0	1	50	1.0		-			-			-		5	2.0	1.4	3
	Ctenophthalmus congerer	9	1 : 0.3	6	10	1.3	1	9	1.0		-			-			-			-		7	2.7	1.3	2
	Rhadinopsylla pentacantha	11	1 : 1.2	4	7	1.8	1	9	1.0		-		1	1	2.0	1	1	1.0		-		7	2.7	1.6	3
Si Hystrichopsyllidae	*Hystrichopsylla orientalis*	5	1 : 4.0	2	3	1.0	2	18	1.0		-		1	1	1.0		-			-		5	2.0	1.0	1
	Typhloceras poppei	15	1 : 0.7		-			-			-		9	12	1.3		-		1	4	3.0	10	3.9	1.5	3
Si Ceratophyllidae	*Peromyscopsylla sylvatica*	3	1 : 2.0	2	3	1.0	1	9	1.0		-			-			-			-		3	1.2	1.0	1
	Nosopsyllus fasciatus	68	1 : 0.9	1	2	1.0	2	18	1.0		-		13	17	1.3	22	27	1.6	8	32	1.5	46	18.0	1.5	8
	Megabothris turbidus	15	1 : 4.0	8	14	1.5		-			-			-		3	4	1.0		-		11	4.3	1.4	2
	Monopsyllus sciurorum	2	1 : 1.0		-		1	9	1.0		-		1	1	1.0		-			-		2	0.8	1.0	1
Ph Polyplacidae	*Polyplax serrata*	373	1 : 2.2		-			-			-		63	82	4.7	20	24	2.9	10	40	1.9	93	36.3	4.0	34
Ph Hoplopleuridae	*Hoplopleura affinis*	59	1 : 2.9		-			-			-		3	4	20.0		-			-		3	1.2	19.7	44
	Hoplopleura acanthopus	15	1 : 0.8		-		3	27	5.0		-			-			-			-		3	1.2	5.0	3
	Hoplopleura edentula	16	1 : 1.6	6	10	2.7		-			-			-			-			-		6	2.3	2.7	6
Ix Ixodidae	*Ixodes ricinus* total	1359	n.d.	44	75	7.9	8	73	27.0	2	100	10.5	31	40	5.4	53	65	11.2	6	24	2.5	144	56.3	9.4	108
	larvae	1277		43	73	7.7	7	64	29.7	2	100	10.5	31	40	5.1	52	63	10.6	5	20	2.0	140	54.7	9.1	101
	nymphs	82		12	20	1.3	2	18	4.0		-		8	10	1.2	16	20	2.7	4	16	1.2	42	16.4	2.0	17
	Ixodes trianguliceps total	9	n.d.	4	7	1.8		-			-			-		2	2	1.0		-		6	2.3	1.5	3
	larvae	6		3	5	1.7		-			-			-		1	1	1.0		-		4	1.6	1.5	3
	nymphs	3		1	2	2.0		-			-			-		1	1	1.0		-		2	0.8	1.5	2
	Dermacentor reticulatus (nymphs)	2	n.d.	1	2	2.0		-			-			-			-			-		1	0.4	2.0	2

Supplemental Table 2-5. Continued

Arthropod				Rodent host																					
				M. glareolus 29/29 = 59[a]			*M. arvalis* 4/7 = 11			*M. agrestis* 0/2 = 2			*A. agrarius* 44/33 = 77			*A. flavicollis* 41/41 = 82			*A. sylvaticus* 14/11 = 25			All Species 132/123 = 256[a]			
	Species	n	Sex ratio	No	P [%]	mI [n]	No	P [%]	mI [n]	No	P [%]	mI [n]	No	P [%]	mI [n]	No	P [%]	mI [n]	No	P [%]	mI [n]	No	P [%]	mI [n]	max
Ga Laelapidae	*Laelaps agilis*	405	1 : 7.7	1	2	1.0		-			-			-		46	56	6.7	17	68	5.6	64	25.0	6.3	56
	Laelaps jettmari (L. pavlovskyi)	48	1 : 11.0		-			-			-		22	29	2.1	1	1	1.0		-		23	9.0	2.1	8
	Laelaps hilaris	87	1 : 9.7		-		7	64	12.3		-		1	1	1.0		-			-		8	3.1	10.9	35
	Hyperlaelaps microti	21	1 : 3.0		-		5	46	4.2		-			-			-			-		5	2.0	4.2	7
	Androlaelaps fahrenholzi	3	1 : >3.0	1	2	1.0	1	9	1.0		-			-		1	1	1.0		-		3	1.2	1.0	1
	Haemogamasus nidi	89	1 : 12.3	5	9	1.4	4	36	1.5		-		5	7	1.2	18	22	2.7	6	24	3.7	38	14.8	2.3	16
	Haemogamasus hirsutus	1	n.d.		-			-			-			-		1	1	1.0		-		1	0.4	1.0	1
	Haemogamasus hirsutosimilis	3	n.d.		-			-			-			-		1	1	3.0		-		1	0.4	3.0	3
	Eulaelaps stabularis	49	1 : 48.0	2	3	1.0	1	9	1.0		-		5	7	2.2	9	11	1.9	8	32	2.2	25	9.8	2.0	7
	Hirstionyssus (*Echinonyssus*) *sunci*	241	1 : 116.5	3	5	1.3	2	18	3.0		-		47	61	2.2	25	31	4.1	12	48	2.2	89	34.8	2.7	27
	Hirstionyssus (*Echinonyssus*) *isabellinus*	22	1 : 20.0	9	15	2.4		-			-			-			-			-		9	3.5	2.4	4
	Hirstionyssus (*Echinonyssus*) *soricis*	1		1	2	1.0		-			-			-			-			-		1	0.4	1.0	1
	Myonyssus gigas	17	1 : 14.0		-			-			-			-		5	6	3.4		-		5	2.0	3.4	9
Pr Myobiidae	*Myobia muris-musculi*	102	1 : 4.8		-			-			-			-		28	34	2.5	12	48	2.7	40	15.6	2.6	10
	Myobia multivaga	95	1 : 7.6		-			-			-			-		14	17	3.7	11	44	3.9	25	9.8	3.8	15
	Myobia agraria	100	1 : 7.4		-			-			-		38	49	2.6		-			-		38	14.8	2.6	12
	Radfordia lemnia	31	1 : 0.5		-		4	36	2.2	2	100	11.0		-			-			-		6	2.3	5.2	14
	Radfordia clethrionomys	88	1 : 1.9	22	37	4.0		-			-			-			-			-		22	8.6	4.0	17
	Radfordia lancearia	26	1 : 5.0		-			-			-			-		10	12	2.0	4	16	1.5	14	5.5	1.9	5
Pr Trombiculidae	*Neotrombicula autumnalis*	22	n.d.	8	14	1.1	2	18	3.0	1	2	1.0		-		2	2	3.0		-		13	5.1	1.7	5
	Hirsutiella zachvatkini	66	n.d.	5	9	11.0		-			-			-		2	2	6.0		-		7	2.7	9.4	28
Pr Psorergatidae[d]	Psorergates spec.	≥ 10	n.d.		-			-			-			-		1				-		1			
Pr Ereynetidae[d]	*Paraspeleognathopsis bakeri*	≥ 2	n.d.	1			1				-			-			-			-		2			
Pr Demodicidae[d]	*Demodex* spec.	≥ 1	n.d.		-			-			-			-		1				-		1			
As Myocoptidae[b]	*Myocoptes japonensis*	160	1 : 2.8	22	37	≥5.7	6	55	≥5.8		-			-			-			-		28	10.9	≥5.7	≥24
	Trichoecius tenax	132	1 : 4.8	25	42	≥4.4	5	36	≥4.8	1	50	≥2.0		-			-			-		30	11.7	≥4.4	≥20
	Trichoecius widawaensis	3	1 : 2.0		-			-			-		3	4	≥1.0		-			-		3	1.2	≥1	1
	Criniscansor spec.	4	n.d.		-			-			-		3	4	≥1.0	1	1	≥1.0		-		4	1.6	≥1	1
As Listrophoridae[b]	*Listrophorus brevipes*	169	n.d.	9	15	≥16.0		-		2	100	≥13.0		-			-			-		11	4.3	≥15.4	≥100

Family	Species																								
	Afrolistrophorus apodemi	888	n.d.		-			-			-		32	42	≥11.0	26	32	≥14.0	13	52	≥14.0	71	27.7	≥12.5	≥146
As Gastronyssidae[d]	*Yunkeracarus apodemi*	≥ 2	n.d.		-			-			-		1				-			-		1			
As Glycyphagidae[d]	*Lophioglyphus liciosus* (*Apodemopus apodemi*)	≥ 1	n.d.		-			-			-			-			-		1			1			
Phoretic arthropods					-			-																	
P Pygmephoridae	*Pygmephorus forcipatus*	2	1 : > 2		-		1	9	2.0		-			-			-			-		1	0.4	2.0	2
As Glycyphagidae	*Xenoryctes krameri* (hypopi)	82	n.d.	7	12	2.7	2	18	4.0	1	50	1.0	17	22	2.7	3	4	2.7		-		30	11.7	2.7	10
	Glycyphagus hypudaei (hypopi)	78	n.d.	3	5	1.0		-		1	50	1.0	2	3	2.5	3	4	23.0		-		9	3.5	8.7	56
As Acaridae	*Acarus nidicolous* (hypopi)[c]	16	n.d.	4	7	2.0		-						-		5	6	1.6		-		9	3.5	1.8	4
Nidicolous arthropods					-			-																	
Ga Laelapidae	*Hypoaspis sardoa*	3	1 : > 3		-			-			-		1	1	1.0	2	2	1.0		-		3	1.2	1.0	1
Ga Ologamasidae	*Euryparasitus emarginatus*	2	n.d.		-			-			-			-		2	2	1.0		-		2	0.8	1.0	1
	Cyrtolaelaps mucronatus	3	n.d.		-		1	9	1.0	1	50	1.0	1	1	1.0		-			-		3	1.2	1.0	1
Ga Parasitidae	Parasitinae spec.	2	n.d.		-			-			-			-			-		2	8	1.0	2	0.8	1.0	1
	Pergamasus spec.	1	n.d.		-			-			-			-		1	1	1.0		-		1	0.4	1.0	1
	Eugamasus spec.	1	n.d.		-			-			-			-		1	1	1.0		-		1	0.4	1.0	1
Pr Cheyletidae	*Eucheyletia flabellifera*	6	1 :>4		-		2	9	3.0		-			-			-			-		2	0.8	3.0	5
As Glycyphagidae	*Glycyphagus domesticus*	4	1 :<0.5		-		1	9	1.0		-		1	1	1.0	1	1	1.0	1	4	1.0	4	1.6	1.0	1
As Acaridae	*Tyrophagus dimidiatus*	2	1 :>2		-			-			-		1	1	1.0		-		1	4	1.0	2	0.8	1.0	1
	Acaridae spec.	1	n.d.		-		1	9	1.0		-			-			-			-		1	0.4	1.0	1

[a] Sex of one bank vole was not determined

[b] Because of small body size of Myocoptidae and Listrophoridae, not all specimens were sampled when high intensities occurred. Values should be treated as minimum numbers. Sex ratio of Listrophoridae was not determined

[c] The mite was not directly phoretic on rodents but on fleas

[d] Incidental findings of skin-inhabiting or nasal mites without quantification

Chapter 3

Effects of urbanisation on endoparasite diversity and quantity in rodents in Berlin

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3 Effects of urbanisation on endoparasite diversity and quantity in rodents in Berlin

3.1 Abstract

Wild rodents are an omnipresent part of the fauna of urban parks and gardens. Rodents themselves may carry numerous invertebrate and protozoan species, most of them parasitic. Due to the close proximity to humans, it is important to know, how urbanisation affects the distribution and quantity of these species and consequently the zoonotic risk in a city like Berlin. The aim of the current study was to examine the endoparasite diversity of mice and voles and to assess the influence of urbanisation on the zoonotic infection risk. After examination of 257 live-trapped rodents (*Apodemus*, *Myodes*, *Microtus* spp.) from four study sites in Berlin for helminths and intestinal Coccidia, 21 different taxa were detected infecting 90% of the animals. Including the arthropods described elsewhere (chapter 2), wild mice and voles were associated with at least 84 species of parasites and commensals. Zoonotic risk emerging from endoparasites was considerably low, since *Trichinella* spp., *Capillaria hepatica* or *Echinococcus* spp. were not detected and also the observed adult cestodes presumably did not include zoonotic species. Taxon richness clearly decreased along a periurban-urban gradient and especially species with complex, heteroxenous life cycles were less diverse, prevalent and/or numerous in urban areas. This was comparable to the effects on the rodent-associated arthropod fauna and was attributed to different determinants of urbanisation. To determine co-infection patterns, effects of endoparasites on the number of zoonotic *Ixodes ricinus* tick larvae infesting the rodents were analysed. Multi-variate regression analysis revealed that abundance of three helminth species had significant effects on larval tick counts on the rodent hosts with hymenolepidid tapeworms increasing and heligmosomoid nematodes and *Syphacia* spp. decreasing the number of ticks. Interaction of helminths and ticks may affect the enzootic cycle of ticks and consequently tick-borne pathogens in urban agglomerations, but laboratory or field experiments with natural hosts are necessary to verify this interaction.

3.2 Introduction

The spectrum of eukaryotic endoparasites associated with wild rodents is very wide including numerous species of protozoa, helminths and even mites. The rodent is the single host for homoxenous species or the intermediate, paratenic or final host of heteroxenous parasites. Results from a comprehensive parasitological investigation about ecto- and partly endoparasitic arthropods

were recently published (Maaz et al., submitted = chapter 2) as well as about the diversity of extraintestinal or tissue-dwelling protozoan and helminth parasites using rodents as paratenic or intermediate host (Krücken *et al.*, 2017). In the present report, particular emphasis was placed on protozoa and helminths from the gastro-intestinal and urinary tract to extend the results about the diversity of micro- and to complete the diversity of macroparasites (metazoan parasites) infecting the same wild mouse and vole specimens in Berlin, Germany.

Intestinal Protozoa include a relatively small number of species of flagellates, amoebae and *Cryptosporidium* spp. with low host specifity and a large diversity of the host-specific Coccidia (Cox, 1987). Most of the intestinal Coccidia were only described according to the morphology of the sporulated oocysts and the detailed life cycle of most species is still unknown (Levine & Ivens, 1990; Lewis & Ball, 1983). The occurrence in natural hosts of the superfamily Muroidea was mainly studied in Eastern Europe and the British Isles (Lewis & Ball, 1983) but were poorly examined in Western and Central Europe. In Germany, apart from a study about *Cryptosporidium* spp. in muskrats (Petri *et al.*, 1997) and the apparently sporadic examination of small mammals by Stammer (1956), the present authors are not aware of any study about intestinal Protozoa in wild rodents.

The helminth communities of the non-commensal murine and arvicoline rodent species have been researched in many places in Europe in the last decades, most intensively in the United Kingdom (UK) (e.g. Behnke *et al.* (2005)), Finnland (e.g. Haukisalmi and Henttonen (1993a)), Spain (e.g. Feliu *et al.* (1997)), Poland (e.g. Grzybek *et al.* (2015a)), Czech Republic (e.g. Tenora (2004)), Slovakia (e.g. Murai and Mészáros (1984)) and Hungary (e.g. Murai *et al.* (1992)), but also in Ireland (Loxton *et al.*, 2016), Portugal (Eira *et al.*, 2006), Italy (Milazzo *et al.*, 2003), Norway (Tenora *et al.*, 1979), France (Mishra & Bercovier, 1975), Switzerland (Wahl, 1967), Austria (Pfaller, 1974), Denmark (Tenora *et al.*, 1991), Belgium (Bernard, 1969), Serbia (Bjelic-Cabrilo *et al.*, 2011), Bulgaria (Genov, 1984), Romania (Mészáros & Murai, 1979), Belarus (Shimalov, 2013), Maldova (Andrejko, 1973) and Lithuania (Grikienienė, 2005). In Germany, studies about wild rodent helminth fauna are scarce and, in addition, often overlooked by international researchers since reports were written in German language (Gässlein, 1954; Schmidt, 1961; Schmidt *et al.*, 1998; Stammer, 1956) or furthermore only published as a thesis (Heddergott, 2008; Memaran, 1970; Schmidt, 2001; Schmitt, 2007). Only once a study about the helminth fauna from three species of rodents from Dormagen in the West of Germany was published in an international journal (Klimpel *et al.*, 2007a; Klimpel *et al.*, 2007b). In particular, studies on the striped field mouse *Apodemus agrarius* are interesting in Germany since here it has the most Western occurrence of its geographic range and is still spreading.

Research on helminths of wild animals is not only important from a faunistic point of view. There are several other reasons to obtain more information about the endoparasite community associated with wild rodents.

First, many rodents have an economic importance since bank voles and other voles are a major forest pest. High intensities of natural infections with several helminth species were shown to induce pathological symptoms in the host (Memaran, 1970; Stammer, 1956; Tenora *et al.*, 1979). Although it is unlikely that the helminth parasites shape the population dynamics of the rodent hosts by induction of high mortality under natural conditions (Haukisalmi & Henttonen, 2000), it remains to be investigated if this is the same under rather unnatural conditions in urban areas. This is particularly import if very high rodent population densities occur in such environments.

Second, rodents are important reservoirs for zoonotic pathogens. A number of cestode and nematode species infecting rodents e.g. *Echinococcus multilocularis*, *Hymenolepis diminuta*, *Trichinella spiralis* are endemic in Europe (Burlet *et al.*, 2011; Franssen *et al.*, 2016; Pozio, 2007) and the surveillance of potential pathogens of medical and veterinary importance is even more important in urban areas. Using the same rodent samples, it has recently been shown that mice and voles from Berlin are obligate intermediate hosts for parasites of carnivorous pet animals as well as birds of prey (Krücken *et al.*, 2017).

Third, interactions among the members of the helminth infra-community are intensively studied and partly revealed strong antagonistic or synergistic effects in laboratory studies (Christensen *et al.*, 1987), part of which were also confirmed in natural infections (Behnke *et al.*, 2009). Interactions among endo- and ectoparasites were rarely considered but may markedly affect the relevance for humans especially with respect to ticks. In a study from Italy, yellow-necked mice, which were dewormed with the non-acaricidic anthelmintic pyrantel pamoate and recaptured some weeks later, hosted significantly more tick larvae compared to non-treated mice from comparable habitats. This was attributed to a negative influence of the nematode *Heligmosomoides polygyrus* on *Ixodes ricinus* larvae most likely mediated by the host immune system (Ferrari *et al.*, 2009). Also in urban areas such as Berlin, rodents were shown to maintain the enzootic cycles of ticks and tick-borne pathogens in areas with high human population density (Matuschka *et al.*, 1996; Matuschka *et al.*, 1990; Rizzoli *et al.*, 2014). Altered prevalence and infection intensities of helminths compared to rural areas may therefore affect public health concerning the transmission of tick-borne pathogens to humans.

In the present study, the quantitative occurrence of helminth and intestinal coccidian taxa infecting peridomestic rodents from Berlin will be presented and their zoonotic potential will be discussed. In

addition, interrelationships between the abundance of those parasites with that of ticks will be analysed under the consideration of confounding factors.

To the knowledge of the authors there is only one study from Europe where the (nearly) complete fauna of metazoan invertebrates associated with non-commensal mice or voles was investigated: A 86 year old study on parasites of three species of mice and voles from England (Elton *et al.*, 1931) which is considerably outdated since many species were synonymised or newly described meanwhile. There are only four more investigations where in addition to helminths at least some groups of arthropods were thoroughly studied on the same animals from Germany (Stammer, 1956), Austria (Mahnert, 1971a; Mahnert, 1971b; Mahnert, 1971c; Mahnert, 1972; Prokopic & Mahnert, 1970), Slovakia (Ambros, 1984; Dudich, 1984; Kovacik, 1984; Murai & Mészáros, 1984) and Ireland (Langley & Fairley, 1982). Comprehensive studies like these are essential for the detection of co-occurrence patterns within the diverse parasite assemblages of wild vertebrates. According to Bush *et al.* (1997), all three studies only observed the component community (species richness within host population) of the rodent-associated invertebrate fauna, but never before those species were analysed at the infra-community level (within individual hosts). Data about 63 species of rodent-associated arthropods from fur and skin of the same rodents from Berlin have been published recently (Maaz et al., submitted = chapter 2). Complementing these data, the nearly complete macroparasite fauna (and some Protozoa) will be surveyed in the present paper and visualised in ordination plots.

3.3 Material and Methods

3.3.1 Rodent trapping

Wild small mammals were trapped at four study locations in Berlin in November 2010 and between April and November 2011. Two study sites situated in the densely populated city (Steglitz and Moabit) were categorised as urban and two forest sites at the periphery of Berlin with limited human influence (Gatow and Tegel) as periurban sites. The habitat characteristics of the study locations were already described in Krücken *et al.* (2017) and Maaz et al., (submitted). Trapping time setup was described in Figure 2-1 in Maaz et al., (submitted, chapter 2). Depending on the size of the trapping site, 30-50 Longworth live traps and 10 rat live traps were placed. Every site was sampled seven times during the study period and rodents were always trapped overnight for three consecutive days. Traps were filled with cotton, rodent pellets and a piece of apple. Every morning, traps were emptied, cleaned and deactivated during the daytime. At the end of the third night the traps were cleaned and cotton and food was removed.

Trapped rodents were anaesthetised by injecting a combination of 0.1 mg/g ketamine and 0.012 mg/g xylazine intraperitoneally. Subsequently, they were euthanised by cervical dislocation followed by cardiac bleeding into serum tubes. After urine was massaged out of the rodents and collected together with occasionally passed fresh faeces in collection tubes for flotation, the rodents were put in individual plastic bags and placed on heat packs (approximately 38 °C) to improve the survival of the parasites. Finally, they were transferred to the Institute for Parasitology and Tropical Veterinary Medicine for necropsies. Trapping and euthanasia of all animals was in accordance with the German laws on nature conservation (Bundesnaturschutzgesetz) and animal protection (Tierschutzgesetz) and were approved by the Obere Naturschutzbehörde Berlin under the reference number I E 210(V)–OA-SG/LSG2a/602;OA-AS/G/825 and the Landesamt für Gesundheit und Soziales (LAGeSo) Berlin under the registration number G 0256/10, respectively.

3.3.2 Necropsies

In the laboratory, rodent species, sex, body weight and head-body length were determined. For *Microtus* species, teeth morphology was used for species determination (Niethammer & Krapp, 1982a). Animals were placed on heat packs and fur and skin were thoroughly screened for arthropods under a stereo microscope (published in Maaz et al., submitted = chapter 2). Afterwards, rodents were necropsied on a 38 °C electric heating block. The reproduction status of female rodents was determined by the detection of embryos. The abdominal and thoracic cavity and organs (liver, lung, heart, spleen, kidneys) were examined for metacestodes and other morphological alterations and parasite specimens or organ samples were stored in tubes with 80% ethanol. The gastro-intestinal system was removed, transferred to a petri dish with physiological saline solution on a heat pack. The bladder was washed with 1 ml of physiological saline solution in a clean syringe and the solution was added to the flotation tube. The diaphragm together with a pea-sized piece of the femoral muscle were sampled for later *Trichinella* screening and different further organ samples were frozen for other studies. The eyes were removed and the lenses were dissected out and stored in tubes with water and 10% formol for later rodent age determination. The remaining carcass was placed in glass screw-top jars over water to allow detachment of arthropods and other parasites. The water and the rodent body were examined microscopically a few times during the following week. The warmed gastro-intestinal tract was sliced open from the stomach to the rectum and the content of the large intestine was screened for helminths under a stereo microscope and then transferred to the faeces/urine tube for flotation. The gastro-intestinal tract was thoroughly screened for helminths. Parasite specimens were stored in tubes with 80% ethanol for later species determination. Afterwards a migration technique was used to allow overlooked helminths or those living inside the intestinal tissue to leave the organ. Therefore, Baermann funnels with sieves with a

mesh size of 0.3 mm were filled with physiological saline solution and placed in an incubator set at 38 °C and 80% humidity and the opened gastro-intestinal tracts were transferred onto the sieves. Small migrated helminths sank to the ground of the funnels and every hour the sediment was screened for parasites. After three hours, the sediment, the sieves and the gastrointestinal tract were screened a last time.

3.3.3 Flotation

For the detection of helminth eggs and coccidian oocysts/sporocysts in faeces, urine and bladder washing solution, the FLOTAC method was performed using the FLOTAC-400 apparatus. Faeces, urine and content of the large intestine were pooled and stored at 4 °C for up to 48 h before flotation was performed. Samples were transferred into a petri dish and homogenised with wooden scoopulas. They were flushed into 10 ml tubes, filled with tap water and centrifuged for 3 min at 1500 rpm. The supernatant was discarded and saturated zinc sulphate solution (density 1.35 g/ml measured with a hydrometer) was added to the pellet to a final volume of 10 ml. This flotation solution was chosen since it supports the detection of the broadest spectrum of parasite stages (eggs of flukes, tapeworms and nematodes, nematode larvae, coccidian oocysts) (Cringoli *et al.*, 2010) and it is less toxic and environmentally hazardous than solutions containing mercury. After homogenisation, 5 ml were pipetted in each of the two flotation chambers. The FLOTAC apparatus was closed and centrifuged at 1000 rpm for 5 min. Afterwards the samples were immediately screened using meandering patterns under a microscope with 100 × magnification. Parasite life stages were identified at 400 × magnification. Parasite stages were counted as faecal eggs/oocysts per rodent as nearly the whole large intestine/bladder content was used in the flotation and FLOTAC method has an analytical sensitivity of nearly 100% (Cringoli *et al.*, 2010). If more than 500 parasite life stages were counted, only a fraction of the 24 reading grid areas in each chamber was counted and the result multiplied accordingly.

Only the gut/bladder contents of the first 29 rodents captured in 2010 were analysed with a non-quantitative method. Faeces were homogenised with some tap water, transferred to a 10 ml tube, filled and homogenised with saturated salt solution and centrifuged for 5 min at 2500 rpm. The surface of the sample solution was transferred to a microscope slide with an eyelet and screened with 100 × magnification under a microscope. Quantity was only categorised in the four levels "absent", "+", "++" and "+++".

3.3.4 Helminth identification

The preserved helminths were identified to species level wherever possible with the help of a microscope with up to 1000 × magnification and a stereo microscope. Different publications were

used for identification of flukes (Bray *et al.*, 2008; Gibson *et al.*, 2002; Jones *et al.*, 2005; Ryzhikov *et al.*, 1978), tapeworms (Khalil *et al.*, 1994; Ryzhikov *et al.*, 1978; Schmidt, 1986; Schmidt, 2001) and nematodes (Anderson *et al.*, 2009; Behnke *et al.*, 2015; Sudhaus *et al.*, 1987). Flukes, fixed in 80% ethanol, were hydrated in an ethanol series, stained with haematoxylin, dehydrated in an ethanol series and xylene and mounted in Canada balsam prior to determination.

To increase the sensitivity of helminth detection, the worm counts observed in necropsies were combined with flotation results, i.e. when at least 3 helminth eggs were detected in flotation but no worm was observed microscopically, the helminth number of the respective taxon was set to one (female) worm as it was obviously overlooked. This happened 21 times (5% of all cases when a helminth species was detected in an individual host) in 19 rodents (7% of all examined hosts). Ten of these cases involved *Capillaria* spp. which are difficult to find in the intestine. Adult or larval specimens of *Syphacia* or *Heterakis* counted in the flotation were added to the helminth counts in the necropsies, respectively.

3.3.5 Screening for *Trichinella* spp.

Muscle samples were examined for tissue nematodes within 72 h after necropsies and stored at 4 °C in the meantime. The diaphragm, which is a predilection site for *Trichinella* spp. even at low infection intensities, was fixed in a compressorium and screened under a microscope with 100 × magnification for encapsuled nematode larvae. Furthermore, from the 228 animals trapped in 2011, an enzymatic digestion of muscle fibres of diaphragm and femoral muscle was performed using acidified pepsin to release muscle larvae. Digestion solution (30 ml of an enzyme solution containing 17.5 U/ml pepsin) was individually prepared for each rodent in 50 ml tubes on a thermo shaker set at 37 °C and 200 rpm. Muscle samples were sliced into small pieces and added to the solution. Subsequently, 0.45 ml of fuming hydrochloric acid were added and the digestion solution was incubated in the thermo shaker for 1.5 h. The digestion solution was rinsed through a 0.3 mm sieve into another falcon tube and transferred into a fridge at 4 °C for sedimentation for 20 min. Finally, two third of the supernatant were discarded, the pellet was resuspended and screened under a microscope with 100 × magnification for free nematode larvae. This procedure was tested with diaphragms from experimentally infected laboratory mice (kindly provided by Dr. Daniel Kulke, Bayer Animal Health) prior to the study and *Trichinella* larvae were successfully detected.

3.3.6 Statistics

For the quantification of helminth/oocyst infections in rodent hosts, not only the prevalence of infection but also the infection intensity (number of parasites per infected host) was determined. Since these measures markedly differed not only among host species but simultaneously among

trapping locations, both factors always had to be considered in combination for statistical testing of differences. For this purpose, trapping location (n=4) or location category (urban/periurban) and host species (n=6) or host family (mouse/vole) were always included as independent variables in regression analyses to explain presence of parasite and infection intensities and for statistical analysis of differences among the levels. For presence/absence data (prevalence) logistic regression analyses (LRA) were calculated with the parasite occurrence (infected/not infected) as dependent variable and for modelling infection intensities, a negative binomial regression analysis (NRA) was used with the parasite counts of infected hosts as dependent variable. Non-infected hosts were excluded from NRA. The odds ratio (OR) or rate ratio (RR) with 95% confidence intervals (CI) of the level of interest against the reference level with the corresponding p-value of the F statistics for both regression analysis methods are presented in the text and the full model with all OR or RR with 95% CI and p-values in Supplemental Table 3-3. OR and RR were obtained by calculating the exponential function of the model estimates.

For the investigation of differences in diversity of parasites among host species and location category (urban/periurban), both factors were included in a two-way ANOVA with subsequent Tukey multiple comparisons of means since factors were significant. Although count data (number of parasites) were used, normal distribution of residuals was assumed since no boundary value (zero parasites) was present in the data, the range was large enough (1-14 parasites) to avoid problems with discrete structure of data and since the residuals-versus-fitted plots and QQ-plots illustrated normality of residuals.

For univariate comparison of prevalence, mid-P exact tests were performed, whereas differences in infestation intensity for non-parametric parasite counts were analysed with Mann-Whitney-U tests (two groups). Diversity of parasites per rodent host was compared among host species using Kruskal-Wallis-Test (>two groups) with Dunn's multiple comparison post-tests, since parasite counts were not normally distributed for *M. glareolus* in D'Agostini-Pearson omnibus test. The Wilson Score interval was used as 95% CI of prevalence and for calculation of bias-corrected and accelerated confidence intervals for mean intensities, a bootstrapping was performed with 2,000 replications in the online software Quantitative Parasitology 3.0 (Reiczigel & Rózsa, 2005).

The estimation of taxon richness for five host species based on abundance (count) data was calculated using bootstrap estimation and Chao's estimation with the functions bootstrap and chao1 in the package "fossil" in R Statistics.

LRA (glm function), NRA (glm.nb function package "MASS"), two-way ANOVA (aov function) with Tukey (TukeyHSD function), mid-P-exact test (ormidp function, package "epitools"), Wilson Score (scoreci function, package "PropCIs") and residual plots (ggplot function, package "ggplot2") were performed in R Statistics. The D'Agostini-Pearson omnibus test for normality was recommended and

performed in GraphPad Prism 5. In addition, Kruskal-Wallis test and Dunn's multiple comparison post-test were calculated in the same software.

Kruskal's non-metric multidimensional scaling (NMDS) was performed using square root and Wisconsin double transformation of abundance (counts) of rodent-associated helminths and arthropods. The five accidentally found endoparasitic or endodermal arthropods living in the skin, lungs and nasal cavity, as well as the few non-determined nematode larvae in faecal flotation were excluded from the analysis. In addition, intestinal Coccidia were not included, since they were only counted in rodents trapped in 2011 but not in 29 rodents from 2010. The larval and nymphal life stages of *I. ricinus* ticks were included separately. For NMDS calculations, Bray-Curtis dissimilarity index was used in the wrapper function metaMDS in the package "vegan" in R Statistics. This function called the monoMDS engine and performed minimum 200 random starts (iterations) until two convergent solutions with lowest stress values (global maximum) or a maximum of 10,000 iterations was reached. Every analysis was repeated with two and three dimensions. Step-across dissimilarities was enabled since a large proportion of rodents had no shared species and this markedly improved the ordination. Finally, centring, principal components rotation and halfchange scaling was conducted for better visualisation. Species scores were calculated as weighted averages. The 2D plots were drawn using ordiplot function and ordihull for polygons. The 3D plots were generated using functions in the package "rgl". The polyhedron indicating the convex hull of the three-dimensional data was calculated using the QuickHull algorithm in the function convhulln in the package "geometry".

Since ticks appeared to be the most important parasites with regard to public health, a negative binomial regression analysis was performed to identify potential parameters including parasite co-infections affecting the abundance of *I. ricinus* larvae on wild rodents. With the exception of two *Microtus agrestis* voles, data from all screened rodents was included, including those without ticks. After exclusion of rodents with missing values, 218 rodents trapped in 2011 were used for the analysis, since rodents from 2010 were lacking quantitative data for intestinal Coccidia and for dried eye lens weights (parameter age, see below). The counts of *I. ricinus* larvae per examined rodent were used as dependent variable. As independent variables, spatial factors (trapping location, 4 levels), temporal factors (season, 3 levels), host species (5 levels), host abundance, host characteristics (sex, body condition, age) were included. Furthermore, the count of co-infestations with other frequent ectoparasites (fleas, sucking lice, parasitic laelapid mites, myobiid mites, trombiculid mites, myocoptid mites and listrophorid mites) as well as co-infections with parasitic helminths detected in at least ten hosts (*Capillaria* spp, Heligmosomidae/Heligmonellidae, *Heterakis spumosa*, *Syphacia* spp., *Aspiculuris tianjinensis*, *Pelodera orbitalis*, Hymenolepididae., *Brachylaima* sp., *Corrigia vitta*) and intestinal *Eimeriidae* oocysts served as independent variables. A very similar

regression analysis, but without the helminth and coccidian parasites presented here, was already described previously (Maaz et al., submitted = chapter 2) and independent variables are explained there in more detail. The season was categorised into “spring” (calendar weak (CW) 14-24, n=33), “summer” (CW 25-41, n=125) and “autumn” (CW 42-47, n=60). The number of trapped rodents per 100 trap nights in 3 consecutive nights (one trapping week) at one location was used as host abundance at time of trapping. Further host characteristics were included as a combination of the condensed parameters age (z-transformed weight of dried eye lenses for every rodent species), nutritional or body condition (differences (residuals) from the average weight-to-body-length-ratio derived from two linear regressions of the trapped mice and voles) and sex including reproductive condition (three categories male n=114, female pregnant, n=40, female not pregnant, n=64). This combination (adapted from Rossin *et al.* (2010)) was used to minimise correlations among the host-intern parameters, since explanatory variables must be statistically independent and this is not completely possible here. The regression was performed in R Statistics using the “MASS” package. First, all parameters were included in the full model followed by a backward variable selection using the “step” function, where successively variables were excluded to optimise (reduce) the AIC (Akaike Information Criterion). Data fitting of full (p=0.170) and best model (p=0.386) was verified using goodness-of-fit-test and improvement of both negative binomial models compared to null models and Poisson models (all p<0.001) were validated using likelihood-ratio-tests. The forest plot was prepared in GraphPad Prism 5 and RR were obtained by calculating the exponential function of the model estimates.

3.4 Results

Some of the results presented here were already submitted for publication or published, i.e. trapped rodents (Krücken *et al.*, 2017; Krücken *et al.*, 2013), infestation of rodents with arthropods (Maaz *et al*, submitted = chapter 2) as well as prevalence and mean intensity of nematodes of the superfamily Heligmosomoidea in *Apodemus sylvaticus* and *Apodemus flavicollis* (Maaz *et al.*, 2016 = chapter 4).

3.4.1 Rodent sampling

At four study sites in Berlin, 257 mice and voles belonging to six species were trapped during November 2010 and from April to November 2011 and analysed parasitologically. The number of examined rodents per rodent species and trapping location is illustrated in Table 3-1. The most frequent rodent species were the yellow-necked mouse *A. flavicollis* (n=82) followed by the striped field mouse *A. agrarius* (n=78), the bank vole *Myodes glareolus* and the wood mouse *A. sylvaticus*. Voles from the genus *Microtus* rarely entered the traps (common vole *Microtus arvalis* n=11, field vole *M. agrestis* n=2). The species composition clearly differed among the study sites. The backyard

in Moabit (n=26) was almost only populated by the wood mouse and was at the same time the only location where this species was trapped. In Steglitz (n=104) the striped field mouse was most numerous followed by the yellow-necked mouse. In Gatow (n=91) and Tegel (n=36) bank voles and yellow-necked mice were most frequent, although in Gatow, in addition, the striped field mouse and the two *Microtus* voles could be trapped.

Table 3-1. Rodents trapped at the study sites. The number of trapped and examined mice and voles for every species (columns) is presented for every study site (rows). Total numbers for the rodent species and trapping locations are shown in the last row or column, respectively. The hyphen indicates absence of the respective rodent.

Trapping location	Rodent species						Total
	Myodes	*Microtus*		*Apodemus*			
	glareolus	*agrestis*	*arvalis*	*agrarius*	*flavicollis*	*sylvaticus*	
Moabit	1	-	-	-	-	25	26
Steglitz	-	-	4	73	27	-	104
Tegel	14	-	-	-	22	-	36
Gatow	44	2	7	5	33	-	91
Total	59	2	11	78	82	25	257

3.4.2 Detection of parasites

A total of 9,226 helminths and approximately 411,110 intestinal Coccidia oocysts were detected infecting the gastro-intestinal tract, the body cavity and organs as well as the lachrymal fluid of 257 wild rodents from Berlin. Parasites living in the bladder and *Trichinella* spp. in muscle tissue of the diaphragm and the femoral muscle were not detected. The digestion method did also not detect any ascarid nematode larvae that had previously been described to be present at low prevalence in the sample set using PCR from muscle and brain samples (Krücken *et al.*, 2017). The helminths were differentiated morphologically to at least 20 taxa belonging to 3 families of flukes (Trematoda), 6 families of tapeworms (Cestoda) and at least 9 families of nematodes (Nematoda), while the intestinal Coccidia were not further differentiated (Table 3-2). The majority of taxa were detected in the gastro-intestinal tract (15 helminth and one coccidian taxa). Three taxa of juvenile tapeworms (metacestodes) were detected in the body cavity and in the liver. Data about these metacestodes and about the molecular biological detection of further tissue-infecting nematodes and Coccidia using rodents as intermediate hosts were already published by Krücken *et al.* (2017). Another two nematode species, *P. orbitalis* and *Pelodera cutanea*, were observed in the larval life stage on the eyes of the rodents and in the water under the rodent carcass in the week after necropsies, respectively. Finally, undetermined nematode larvae were detected during flotation but it was not possible to identify the larvae in the hyperosmotic flotation solution.

Table 3-2. Distribution of endoparasites in wild rodent species. Absolute numbers of infected rodents, prevalence and mean intensity for six rodent species are shown for every parasite taxon (rows) for every rodent species (columns). The number of examined rodents and the number of male/female are given below the species name. The last column shows values for the sum of all rodent species. Hyphens represent absence of parasites. *Trichinella* and *Klossiella* were not detected and omitted from the table. n: Number of parasites. No: Number of infected rodents. P [%]: Prevalence in %. 95% CI: 95% confidence interval. mI [n]: mean intensity = mean number of parasites per infected rodent. max: maximum of parasite intensities (l): only larval life stage detected.

Endoparasite				Rodent host																												
				M. glareolus 29/29 = 59[a]				*M. arvalis* 4/7 = 11				*M. agrestis* 0/2 = 2				*A. agrarius* 44/34 = 78				*A. flavicollis* 41/41 = 82				*A. sylvaticus* 14/11 = 25				All Species 132/124 = 257[a]				
	Family	Genus/Species	n	No	P [%]	95% CI	mI [n]	No	P [%]	95% CI	mI [n]	No	P [%]	95% CI	mI [n]	No	P [%]	95% CI	mI [n]	No	P [%]	95% CI	mI [n]	No	P [%]	95% CI	mI [n]	No	P [%]	95% CI	mI [n]	max
	Intestinal Protozoa		411110	22	37	26-50	923	6	55	28-79	954	1	50	9-91	888	50	64	53-74	4776	32	39	29-50	2993	18	72	52-86	4915	129	50.2	44.1-56.3	3455	42960
Trematoda	Dicrocoeliidae	*Corrigia vitta*	79		-	0-6			-	0-26			-	0-66		7	9	4-17	2.7	16	20	12-29	3.8		-	0-13		23	9.0	6.0-13.1	3.43	16
	Brachylaimidae	*Brachylaima* spp.	35		-	0-6			-	0-26			-	0-66		18	23	15-34	1.9		-	0-4			-	0-13		18	7.0	4.5-10.8	1.94	7
	Plagiorchiidae	*Plagiorchis* spp.	1	1	2	0.3-9	1		-	0-26			-	0-66			-	0-5			-	0-4			-	0-13		1	0.4	0.07-2.2	1	1
Cestoda	Hymenolepididae	spp.	520		-	0-6			-	0-26			-	0-66		10	13	7-22	1.1	22	27	18-37	23.1		-	0-13		32	12.5	9.0-17.1	16.25	215
	Catenotaenidae	spp.	32	1	2	0.3-9	11		-	0-26			-	0-66			-	0-5		3	4	1-10	7		-	0-13		4	1.6	0.6-3.9	8	18
	Anoplocephalidae	spp.	35		-	0-6		7	64	35-85	4.4	1	50	9-91	4		-	0-5			-	0-4			-	0-13		8	3.1	1.6-6.0	4.38	7
	Mesocestoididae	*Mesocestoides litteratus* (l)	890	3	5	2-14	218	1	9	2-38	6		-	0-66		1	1	0.2-7	30	1	1	0.2-7	200		-	0-13		6	2.3	1.1-5.0	148	512
	Taeniidae	*Taenia* spp. (l)	8	2	3	1-12	1.5		-	0-26			-	0-66		1	1	0.2-7	1	4	5	2-12	1		-	0-13		7	2.7	1.3-5.5	1.14	2
	Paruterinidae	*Cladotaenia globifera* (l)	12	1	2	0.3-9	12		-	0-26			-	0-66			-	0-5			-	0-4			-	0-13		1	0.4	0.07-2.2	12	12
Nematoda	Trichuridae	*Trichuris* spp.	9	1	2	0.3-9	1	1	9	2-38	4		-	0-66			-	0-5		3	4	1-10	1.3		-	0-13		5	2.0	0.8-4.5	1.8	4
	Capillariidae	*Capillaria* spp.	499	23	39	26-50	16.4	2	18	5-48	1.5		-	0-66		3	4	1-11	1	13	16	10-25	5.5	11	44	27-63	4.1	52	20.2	15.8-25.6	9.6	167
	Oxyuridae	*Syphacia* spp.	1684		-	0-6		3	27	10-57	4		-	0-66		1	1	0.2-7	59	18	22	14-32	77.2	7	28	14-48	32	29	11.3	7.9-15.7	58.07	459
	Heteroxynematidae	*Aspiculuris tianjinensis*	181	12	20	12-32	14.9		-	0-26		1	50	9-91	2		-	0-5			-	0-4			-	0-13		13	5.1	3.0-8.5	13.92	100
	Heterakidae	*Heterakis spumosa*	3246		-	0-6			-	0-26			-	0-66		68	87	78-93	46.1	21	26	17-36	4.9	5	20	9-39	1.8	94	36.6	30.9-42.6	34.53	295

Spirocercidae	*Mastophorus muris*	15	5	9	4-18	1.6		-	0-26			-	0-66			-	0-5		2	2	1-8	3.5		-	0-13		7	2.7	1.3-5.5	2.14	6
Peloderidae	*Pelodera orbitalis* (l)	844	8	14	7-25	23	5	46	21-72	79.6		-	0-66		9	12	6-21	29.1		-	0-4			-	0-13		22	8.6	5.7-12.6	38.4	215
	Pelodera cutanea (l)	63		-	0-6			-	0-26			-	0-66			-	0-5		4	5	2-12	15.8		-	0-13		4	1.6	0.6-3.9	15.8	45
Heligmosomidae/ Heligmonellidae	spp.	1059	6	10	5-20	12.5	5	46	21-72	2		-	0-66			-	0-5		30	37	27-47	12.03	21	84	65-94	29.2	62	24.1	19.3-29.7	17.08	246
Strongyloididae	*Strongyloides ratti*	1	1	2	0.3-9	1		-	0-26			-	0-66			-	0-5			-	0-4			-	0-13		1	0.4	0.1-2.2	1	1
undetermined nematode larvae (l)		13	1	2	0.3-9	5	1	9	2-38	7		-	0-66			-	0-5		1	1	0.2-7	1		-	0-13		3	1.2	0.4-3.4	4.3	7

[a] Sex of one bank vole not determined

The combination of microscopical helminth detection, the migration technique, placing the carcass over water for one week and faecal/urinal flotation clearly increased the sensitivity of detection and quantification of helminths. Hence, for some nematode species (i.e. *Syphacia* spp. and *Mastophorus muris*), eggs were only accidentally observed in faeces whereas small juvenile specimens of e.g. *Syphacia* spp. were sometimes overlooked during necropsies but detected during flotation. Occasionally, mites and their eggs, which were presumably swallowed by the rodent during grooming, were detected during faecal flotation. However, they were neither included in the present study nor in the related paper concerning rodent-associated arthropods (Maaz *et al*., submitted = chapter 2).

The parasites investigated in the present study infected 90.3% (95% CI 86.0-93.5) of the rodents in Berlin. Intestinal Coccidia oocysts were detected in 50.2% (95% CI 44.1-56.3, Table 3-2) and helminths infected 80.9% (95% CI 75.7-85.3), of which the majority were gastro-intestinal helminths which were detected in 77.8% (95% CI 72.4-82.5) of the wild rodents. The gastro-intestinal helminths comprised nematodes, adult tapeworms and flukes accounting for an overall prevalence of 73.2% (95% CI 67.4-78.2), 17.1 (95% CI 13.0-22.2) and 16.0% (95% CI 12.0-20.9) with a mean intensity of 35.7 (95% CI 28.1-48.2), 13.3 (95% CI 6.18-31.0) and 2.8 (95% CI 2.1-4.0) specimens per infected rodent, respectively. An average of 75.8 (95% CI 22.0-235.0) metacestodes were detected in the 4.7% (95% CI 2.7-8.0) of rodents infected with these larval cestodes.

3.4.3 Host species and spatial distribution of the parasites

The analysis of the distribution of parasites revealed marked preferences in terms of host species and trapping location. The prevalence for the parasite taxa are shown in Table 3-2 and Figure 3-1. Whether differences were elicited by location or by host was sometimes difficult or even impossible to determine, because of partial collinearity between both factors. For instance Moabit was virtually only populated by wood mice and simultaneously the species was only trapped at this site. For this reason in the following chapter, the independent variables host species (n=6) or host family (voles/mice) were always used in combination with trapping location (n=4) or location category (periurban/urban) for statistical testing in regression analyses.

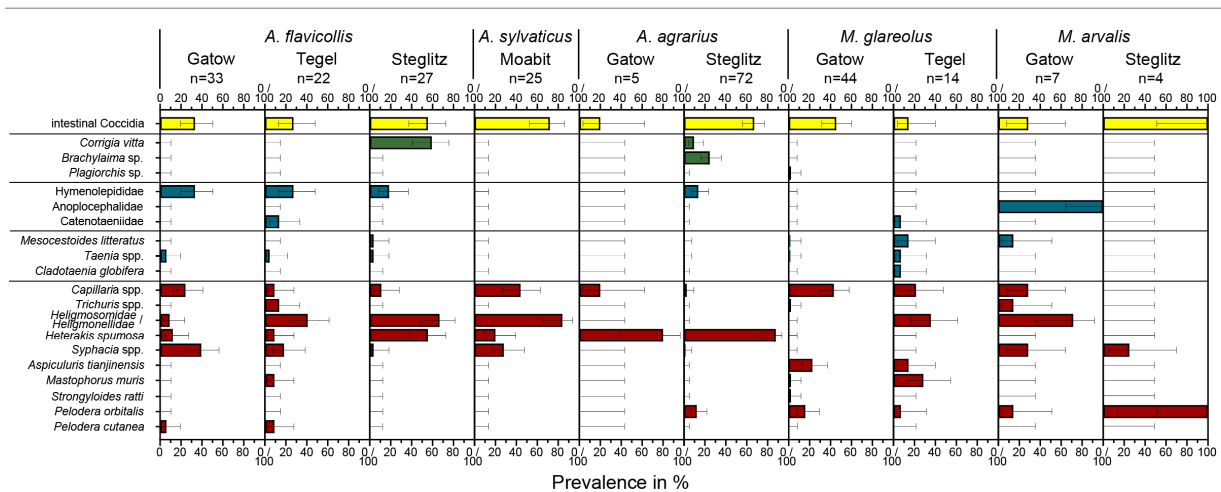

Figure 3-1. Prevalence of coccidian and helminth parasites of rodents from Berlin. Prevalence with 95% confidence intervals are shown for parasites for different host species/trapping location combinations. Prevalence for *M. agrestis* (n=2) and for one *M. glareolus* in Moabit are not shown because of the small number of trapped specimens.

3.4.3.1 Intestinal Coccidia

The intestinal Coccidia were frequently detected as oocysts during flotation in all species and at all locations, however the oocysts were not allowed to sporulate for further species determination. Staining of faeces for the detection of Cryptosporidium spp. (no longer placed within Coccidia) was not conducted. Giardia spp. cysts, which were occasionally observed in faecal flotation were not quantified in the present study. No sporocysts of the coccidian genus Klossiella (Klossiellidae) were detected in urine and the bladder of the examined animals. Coccidia oocysts were detected in the faeces of all host species and at all trapping locations. In the four host species which were trapped at both location categories, oocysts were more prevalent in urban compared to periurban sites (Figure 3-1), which was also significant in the regression model including both factors (LRA: host species (reference *M. glareolus*) and location category (reference periurban), Urban-OR: 3.6 p=0.002, Supplemental Table 3-3, model A), whereas prevalence were not significantly different among host species, at least when compared to bank voles. In contrast, infection intensity, which was measured in the 226 rodents trapped in 2011, was more dependent on the host since *Apodemus* mice contained 6.0 times more intestinal coccidian oocysts (95% CI 2.6-14.0) than did voles (NRA: host family (reference mouse) and trapping location, vole-RR: 0.17 p<0.001, Supplemental Table 3-3, model B).

3.4.3.2 Trematoda

Three digenean genera were detected in the intestine (including pancreatic ducts) of rodents. They were rare in voles, since only once *Plagiorchis* sp. was detected in a bank vole in Gatow. But also in

mice they exhibited a very focal occurrence as they only infected striped field mice and yellow-necked mice in Steglitz (Figure 3-1). At this location, *Corrigia vitta* was about 6-times more prevalent in yellow-necked mice (59%, 95% CI 41-75) compared to striped field mice (10%, 95% CI 5-18, mid-P-exact-Test: p<0.001) whereas *Brachylaima* sp. was only detected in the latter host species.

3.4.3.3 Cestoda

Tapeworms were detected in all host species except for the wood mouse and thus also at every trapping location except for Moabit (Figure 3-1). Adult tapeworms solely infected the gastrointestinal tract, whereas metacestodes were detected in the body cavity (*Mesocestoides litteratus*, *Taenia* spp.) and attached to or inside the liver (*Taenia* spp., *Cladotaenia globifera*). Tetrathyridia larvae of the genus *Mesocestoides* spp. revealed DNA sequence identity to *M. litteratus* (Krücken *et al.*, 2017), however, the ITS-2 sequences of the related species *Mesocestoides lineatus*, known to occur in Germany, were not available. *Mesocestoides litteratus* was only found in six animals and, those were from four species and from three study sites indicating an opportunistic occurrence with little specificity regarding host species or location. Similarly, *Taenia* spp. larvae were only detected seven times in rodents from three host species and three study locations. They were either freely moving in the abdominal or thoracic cavity or fixed at the diaphragm or at the liver. Infected rodents only hosted one or two taeniid metacestodes, but the mean intensity of *M. litteratus* (Mesocestoididae) was very high with 148 (95% CI 49.3-358) tetrathyridia per infected host with a maximum of 512 metacestodes in a bank vole from Tegel. This vole was the only host where metacestodes from *C. globifera* were detected with 12 larvae located at the edge of the liver.

Three families of adult cestodes infected rodents from Berlin. Hymenolepididae were only detected in yellow-necked and striped field mice (Figure 3-1 and Table 3-2). Within these two host species there were no significant differences in prevalence and intensity of infection with Hymenolepididae (LRA and NRA: host species (reference *A. flavicollis*) and location (reference Gatow), *A. agrarius*-p=0.198 and p=0.150, Supplemental Table 3-3, model C and D). In the same regression analyses, mice in Gatow were not more often (LRA: Tegel-p=0.790, Steglitz-p=0.400) but 4.9 fold (95% CI: 1.4-17.6, p=0.014) and 11.8 fold (95% CI: 2.9-48.2, p<0.001) more heavily infected with these tapeworms than in Tegel and Steglitz, respectively. The other host species where the tapeworms were absent could not be included in the models, because regression analyses cannot handle levels without positive values. The adult cestodes of the Anoplocephalidae infected exclusively *Microtus* voles with a very high prevalence of 62% (95% CI 36-82). More precisely, they occurred only in *Microtus* spp. at the periurban site Gatow where they infected eight out of nine animals (Figure 3-1). Also members of the Catenotaenidae were only detected in a periurban area in Tegel, where they infected three yellow-necked mice and one bank vole.

Taken together, not only the family richness of tapeworms was more diverse in rodents at the periurban study locations Gatow and Tegel, but they were also significantly more prevalent and 7.5 times (95% CI 2.1-27.4) more numerous than at the urban sites (LRA and NRA: host species (reference *M. arvalis*) and location category (reference urban), periurban-OR: 3.0 p=0.023, periurban-RR: 7.5 p=0.002, Supplemental Table 3-3, model E and F) and were even completely absent in Moabit. On host species level, odds of infection with tapeworms was significantly higher for common voles compared to yellow-necked mice (4.5 fold, 95% CI 1.15-17.8, p=0.031), striped field mice (6.8 fold, 95% CI 1.5-30.4, p=0.012) and especially bank voles (153.8 fold, 95% CI 14.2-1664, p<0.001) which only once were infected with tapeworms of the family Catenotaenidae (model E). In contrast, mean infection intensity was 5.8 fold higher (95% CI 1.9-17.6, p=0.002) in yellow-necked mice compared to common voles (model F), because the frequent mouse-specific Hymenolepididae revealed high mean infection intensities of 16.3 compared to Anoplocephalidae with only 4.4 tapeworms in voles (Table 3-2).

3.4.3.4 Nematoda

Eleven different nematode taxa were differentiated in rodents from Berlin which undoubtedly include more than eleven species, since many specimens were identified at best to genus level. They comprised a very wide phylogenetic spectrum within the phylum Nematoda.

The whipworms *Trichuris* sp. (Trichuridae) were relatively rare, infecting the caecum of only 2.0% (95% CI 0.8-4.5) of the hosts. They were only detected in yellow-necked mice, bank voles and common voles, but solely at the periurban sites in Gatow and Tegel (Figure 3-1).

In contrast, specimens of *Capillaria* spp. (Capillariidae) were frequently found in the gastro-intestinal tract of all host species except for the two field voles and at every trapping location. *Capillaria* worms were most prevalent in wood mice (44%, 95% CI 27-63) and bank voles (39%, 95% CI 26-50, Figure 3-1), which means a significantly higher odds of infection than for striped field mice (LRA: host species (reference *M. glareolus*) and trapping location (reference Gatow), *A. agrarius*-OR: 0.17 p=0.036 Supplemental Table 3-3, model G) and yellow-necked mice, although difference to the latter was just not significant (OR: 0.45 p=0.067). Also mean intensity was, after correction for trapping location, in bank voles significantly 5.7 fold (95% CI 2.2-14.8, p<0.001), 43.3 fold (95% CI 4.9-381, p<0.001) and 11.6 fold (95% CI 1.5-90, p=0.020) higher than in yellow-necked mice, striped field mice and common voles, respectively (NRA: host species (reference *M. glareolus*) and trapping location (reference Gatow), Supplemental Table 3-3, model H). Under consideration of spatial differences, *Capillaria* spp. were most prevalent in Moabit with 46% (95% CI 29-65), but this is probably linked to frequent occurrence in wood mice. Prevalence in Gatow appeared to be slightly lower with 33%

(95% CI 24-43) but still constituted a 3.1 fold (95% CI 1.1-9.0, p=0.036) and 3.8 fold (1.03-14.0, p=0.045) higher odds of infection compared to Tegel and Steglitz (model G). Although morphological abnormalities in the liver were screened for worms and egg packages, life stages of *Capillaria* (*Calodium*) *hepatica* were not detected.

Two taxa of the superfamily Oxyuroidea were differentiated in the rodents from Berlin, i.e. *A. tianjinensis* (Heteroxynematidae) and *Syphacia* sp. (Oxyuridae) living in the lumen of the small intestine, caecum or colon. This is the first record of *A. tianjinensis* from Germany, which solely infected one of two field voles and 20% (95% CI 12-32) of the bank voles (Table 3-2) and was consequently only detected at the periurban sites (Figure 3-1). In contrast, representatives of the genus *Syphacia* were absent in the bank vole and the two field voles. They were only once (1.2%) detected in striped field mice but in the other three host species they were frequently detected in 23.7% (95% CI 17.0-32.2) with a mean intensity of 58.0 worms per infected rodent (95% CI 28.1-115). There were no obvious differences in the quantitative occurrence of this parasite among the three host species or the trapping locations (Figure 3-1). Only within yellow-necked mice, *Syphacia* spp. were significantly more prevalent in Gatow compared to Steglitz (mid-P exact test: p=0.010). Eggs of *Syphacia* sp. were very rarely detected in faecal flotation since these nematodes cement their eggs to the perianal skin, but comparable to *H. spumosa*, in four out of 28 infected rodents, additional small worms were detected during flotation.

The heterakoid species *H. spumosa* only infected the colon of mice from the genus *Apodemus*. In nearly half of the infected rodents, additional, mostly larval specimens (most of them <1 mm length) were detected in faecal flotation, which were overlooked during necropsies. This illustrates the higher sensitivity due to the combination of microscopical examination and flotation as well as a better quantification of infestation intensity of up to 295 *H. spumosa* worms. Notably, not only the odds of being infected were 7.3 fold (95% CI 2.9-18.1, p<0.001) and 29.8 fold higher in *A. agrarius* (95% CI 9.1-97.7, p<0.001) than for *A. flavicollis* and *A. sylvaticus*, respectively, but also the infestation intensity was 6.1 fold (95% CI 3.4-10.9, p<0.001) and 20.1 fold (95% CI 6.3-64.2, p<0.001) higher compared to the mentioned hosts (LRA and NRA within *Apodemus*: host species (reference *A. agrarius*) and location category (reference Periurban), Supplemental Table 3-3, model J and K, Figure 3-1). In addition, mice from urban sites had a 6.6 fold higher infection risk (95% CI 2.6-17.0, p<0.001, model I) and 3.7 times more *H. spumosa* worms per infected rodent (95% CI 1.7-8.4, p=0.002, model J) in comparison to periurban sites. Consequently, the infection rate was extremely high in the striped field mice in Steglitz with a prevalence of 88% (95% CI 78.2-93.4, Figure 3-1) and a mean intensity of 48.2 worms (95% CI 36.5-66.4).

In the stomach of seven rodents (2.7%, 95% CI 1.3-5.5) the spiruroid nematode *M. muris* (Spirocercidae) was detected. Eggs were never observed in faecal flotation but worms were found in yellow-necked mice and bank voles but only at the periurban sites (Figure 3-1). Considering only these two host species in Gatow and Tegel, odds of infection was 21.2 fold higher in Tegel (95% CI 2.3-194, p=0.007) compared to Gatow (LRA: host species and trapping location (reference Gatow), Supplemental Table 3-3, model L).

Two species of the paraphyletic suborder Rhabditina infected the rodents in the larval life stage. *P. cutanea* was detected on the hair-ends of and in the water under four rodent carcasses of *A. flavicollis* from Gatow and Tegel in the week after necropsies. Since skin, where these nematodes most likely emigrated from, was not additionally screened for these parasites, the prevalence could be actually higher. The same applies to *P. orbitalis* with a prevalence of 8.6% (95% CI 5.7-12.6, Table 3-2), which was first recognised in the lachrymal fluid of the eyes of the 162nd examined rodent (of 257). From then on also the eyes of every rodent were thoroughly screened for this nematode. *P. orbitalis* was detected in striped field mice, bank voles and common voles from three study locations except for Moabit. In agreement with the relatively rare cases there were no obvious differences among these hosts or the trapping locations.

Heligmosomoid nematodes belonging to the families Heligmosomidae and/or Heligmonellidae (Strongylida) were frequently detected in mouse and vole species from all study sites, although they were absent in striped field-mice and the two field voles. Within the four host species, differences in quantitative occurrence were more pronounced among trapping locations than among hosts. The odds of infection with heligmosomoid nematodes was 4.2 fold higher (95% CI 1.7-10.4, p=0.002) for rodents from urban sites than from the periurban forest sites (LRA: host species (reference *M. glareolus*) and location category (reference periurban), Supplemental Table 3-3, model I). Indeed, the observed prevalence within the four rodent species which appeared to be competent hosts was highest in Moabit with 85% (95% CI 67-94, n=26) followed by Steglitz with 58% (95% CI 41-74, n=31), Tegel with 39% (95% CI 25-55, n=36) and Gatow with 10% (95% CI 5-18, n=84). Simultaneously, bank voles, which were virtually only trapped at periurban sites, revealed a significantly reduced odds of infection compared to yellow-necked mice (OR: 3.1, 95% CI 1.1-8.7, p=0.028), wood mice (OR: 11.4, 95% CI 2.3-57.1, p=0.003) and, although significance was not reached, in trend common voles (OR: 4.5, 95% CI 0.96-21.1, p=0.056, model I). However, it was not possible do determine if bank voles carried Heligmosomidae/Heligmonellidae generally less often or because these rodents occurred predominantly at the periurban study sites.

A single female of *Strongyloides ratti* (Stongyloididae) was detected in the intestine of a bank vole in Gatow.

Further larval nematode specimens were detected during flotation in the absence of *H. spumosa* and *Syphacia* sp. These larvae could not be determined more accurately because of morphological alteration due to the hyperosmotic flotation solution. They were detected in one *M. glareolus* (n=5) and one *M. arvalis* (n=7) from Gatow and one *A. agrarius* (n=1) in Steglitz.

Taken together, nematodes were more prevalent at the urban sites Moabit 92% (95% CI 76-98) and Steglitz 88% (95% CI 80-93) compared to the periurban sites in Gatow (63%, 95% CI 52-72) and Tegel (64%, 95% CI 48-78) (LRA: host species and location category (reference periurban), urban-OR: 4.2 p=0.017, Supplemental Table 3-3, model M). This results from the observation that nematode species, which only occurred at periurban sites such as *Trichuris* spp, *A. tianjinensis*, *M. muris*, *P. cutanea* and *S. ratti* were relatively rare whereas the frequent Heligmosomidae/ Heligmonellidae spp. and *H. spumosa* were more prevalent at urban sites.

3.4.4 Parasite taxon diversity and taxon richness

Every examined rodent from Berlin hosted an average of 2.03 (median 2) of the 21 parasite taxa presented here. This diversity of the endoparasite infra-community was significantly lower in bank voles (mean 1.48, Kruskal-Wallis test without *M. agrestis*: p<0.001, Dunn's multiple comparison post-tests) compared to field and common voles (2.82, p<0.01), yellow-necked mice (2.07, p<0.01), wood mice (2.48, p<0.001) and striped field mice (2.15, p<0.01). Nevertheless, the highest diversity with six parasite taxa was observed in one of the bank voles from Tegel as well as in one yellow-necked mouse from Steglitz. For example, the bank vole was infected with all three metacestode genera and the three nematode taxa Heligmosomoidea, *Capillaria* spp. and *A. tianjinensis*.

The ectoparasites and other rodent-associated arthropods from the rodents of the present study were already described in Maaz et al., (submitted, chapter 2). When all the morphologically differentiated 84 coccidian, helminth and arthropod taxa associated with the rodents from Berlin are considered, at least one species was detected in every rodent with a mean diversity of 6.48 taxa (median 6) and a maximum of 14 taxa per host which was observed in one *M. glareolus* in Tegel and two *A. flavicollis* in Steglitz. The bank vole for instance hosted two tick, three flea, one Gamasina, one Myobiidae, two Myocoptidae, one Listrophoridae, one phoretic glycyphagid species and furthermore intestinal Coccidia, Heligmosomoidea and *M. litteratus* metacestodes. In a two-way ANOVA including host species and trapping location, species diversity significantly differed within both parameters (host p<0.001, location p<0.001, interaction p=0.799, normality of residuals was verified in residual

plots and QQ-plots). After Tukey's multiple comparisons of means, the highest diversity was associated with common voles with an average of 9.27 taxa per examined rodent compared to 5.47 with bank voles (p<0.001), 6.50 with yellow-necked mice (p=0.006) and 6.58 with striped field mice (p=0.009), but did not differ significantly from wood mice with 7.16 (p=0.160) due to the small sample sizes. The diversity per rodent was higher in Moabit (7.04) and Steglitz (7.39) compared to Tegel (5.44, Moabit-p=0.773, Steglitz-p=0.020) and Gatow (5.71, Moabit-p=0.808 Steglitz-p=0.002), although the differences to Moabit could not be calculated adequately because of collinearity of host species and location. The total taxon richness was not easy to compare among the host species since sample size was far from being equal. Therefore the richness was extrapolated by bootstrapping estimation (BO) and Chao's species richness estimator (CH) described in Poulin (1998), whereby the author recommended the BO. The taxon richness seemed to be highest in yellow-necked mice (BO 73.4, CH 63.7, 47 observed in 82 specimens) followed by bank voles (BO 67.3, CH 51.0, 42 observed in 59), common voles (BO 58.2, CH 52.7, 36 observed in 11), striped field mice (BO 56.3, CH 51.0, 35 observed in 77) and wood mice (BO 37.4, CH 26.5, 22 observed in 25), which is consistent with the order according to the actually observed numbers of taxa. The differences between the results of both estimators occurred because taxon richness estimation becomes difficult if a large number of rare species are detected and e.g. of the 47 species found to be associated with the yellow-necked-mouse, 26 (55%) had prevalence less than 5%.

3.4.5 Visualisation of the fauna associated with rodents from Berlin

It is difficult to maintain an overview of the host-species and location-specific distribution of all 84 observed rodent-associated arthropod, helminth and intestinal coccidian taxa. Therefore, the ordination method NMDS was used to analyse parasite infra-community similarities/dissimilarities among host animals. NMDS scales down the complex dissimilarities among the non-vertebrate taxa found in/on every rodent to two or three dimensions. This makes it possible to visualise and interpret these dissimilarities in 2D or 3D plots. The Bray-Curtis dissimilarity index was used to quantify differences in the quantitative occurrence (counts) of parasite taxa between rodent specimens on a non-metric basis. Since this index is dependent on the order by which host specimens are compared, a number of repeated measures (iterations) was conducted to find the best (global) solution with the lowest stress value as a measure of disagreement between the data visualised in the graphic plot and the original rank order. The stress value in a two-dimensional solution was quite high (0.269), therefore an additional NMDS was calculated with three dimensions, which appeared to have a good stress of 0.191 indicating a good representation of the true dissimilarities in a 3D plot. The results are shown in Figure 3-2. According to the species carried by the hosts, rodents clearly clustered better on the basis of host species (Figure 3-2A, D) than on the

trapping location of the hosts (Figure 3-2C, E). Especially most *A. agrarius* revealed a distinct parasite spectrum compared to the other host species, which was most obvious in the more representative three-dimensional solution (Figure 3-2D). The two rodent species from the subgenus *Sylvaemus*, *A. flavicollis* and *A. sylvaticus*, shared a very common parasite composition which largely differed from that of *A. agrarius* and voles. The parasite occurrence of the voles did not clearly differentiate among the three trapped species. The separate clustering of rodents within rodent species but also within trapping locations becomes increasingly better, if rodents carrying less than three (Supplemental Figure 3-4A-E) or even less than five parasite taxa (Supplemental Figure 3-4F-J) were excluded from the procedure. This illustrates, that some scattered data points in the plots are represented by rodent specimens carrying only a small number of, in addition, often non-host species- or location-specific helminth and arthropod taxa (not shown). Figure 3-2B shows the distribution of the weighted averages of parasite taxa which can be compared to the dissimilarities of the host specimens. Taxa in the centre revealed no strong influence on dissimilarities. For example the phoretic hypopi *Glycyphagus hypudaei* (G.hy), the non-parasitic Gamasina *Cyrtolaelaps mucronatus* (C.mu) and nematode larvae of *P. orbitalis* (P.or) living on the eyes were largely independent from the occurrence of other parasites with a more restricted infection/infestation pattern. In contrast, for instance the parasite fauna typical for striped field mice was mainly represented by the shared occurrence of the flea *Tyrophagus poppei* (T.po), the louse *Polyplax serrata* (P.se), the laelapid mite *Laelaps jettmari* (L.je), the myobiid *Myobia agraria* (M.ag), the myocoptid *Trichoecius widawaensis* (T.wi) and *Criniscansor* sp. (Crin), the nematode *H. spumosa* (H.sp) and the fluke *Brachylaemus* sp. (Bra), since these parasites are associated with the majority of *A. agrarius* specimens in Figure 3-2A and B. The nymphs of the hard tick *I. ricinus* are very central and indeed there were no marked quantitative differences in host-species or location-specific occurrence apart from significantly higher infestation intensity in yellow-necked-mice compared to bank voles (Maaz et al., submitted = chapter 2). *Ixodes ricinus* larvae were more associated with parasites which were more frequent in rodents in Gatow and Tegel (Figure 3-2C) or in *A. flavicollis* and *M. glareolus* (Figure 3-2A) which were most frequently trapped at these study sites.

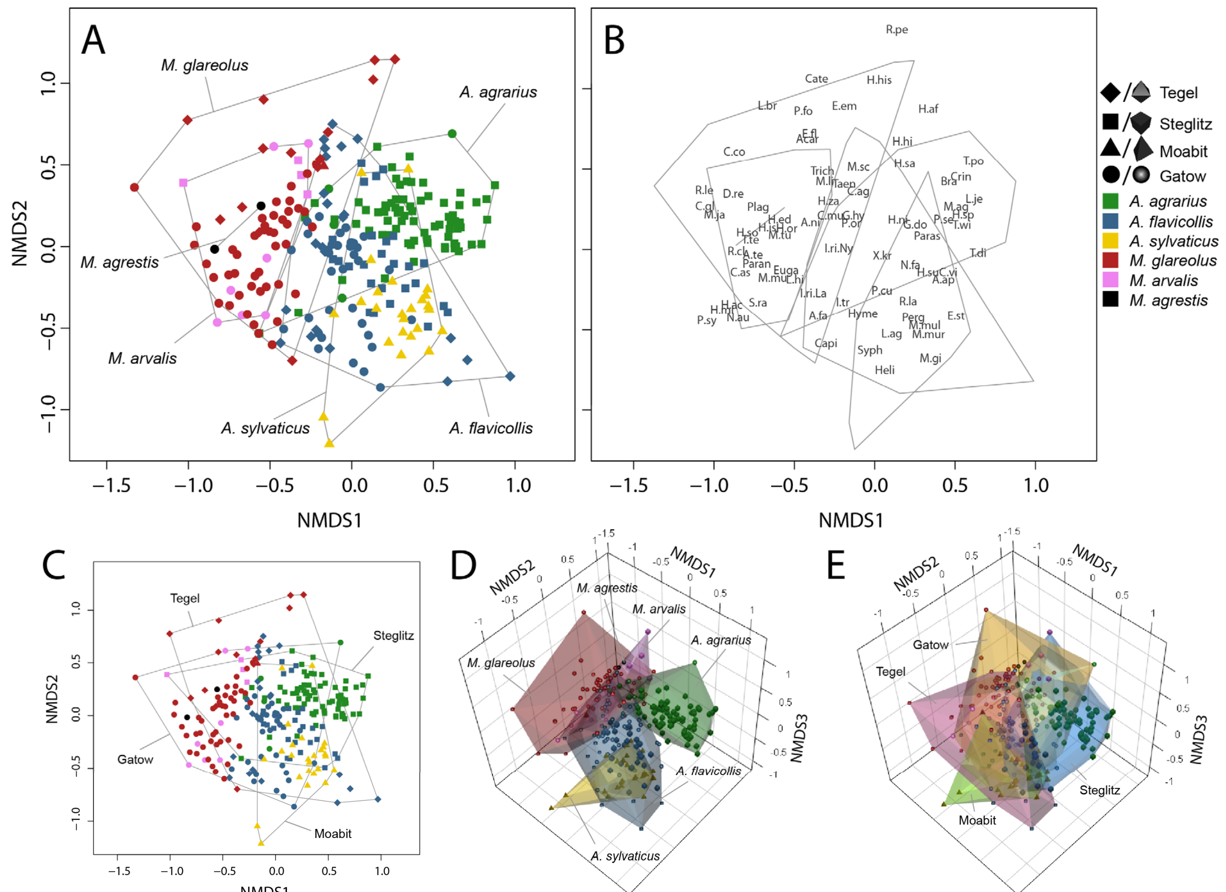

Figure 3-2. Non-parametric multidimensional scaling of 77 helminth and arthropod taxa associated with 256 mice and voles from Berlin. NMDS was calculated for two (A-C) and three dimensions (D, E). Grey polygons border all rodents from one host species (A, B) or trapping location (C). Shaded coloured polyhedrons depict the convex hull including all rodents within one species (D) or from one trapping location (E). Point colours indicate the rodent species and point symbols the trapping locations (see legend). Stress of best (presented) 2D solution was 0.269 after 375 iterations, those of the best 3D solution 0.191 after 200 iterations. The names of helminth and arthropod taxa in B were abbreviated with the first letter of the genus and the first two to three letters of the species. If only genus or family were determined, the first three to five letters are shown.

3.4.6 Determination of parameters affecting tick abundance on rodents

Based on the regression analysis conducted in Maaz et al., (submitted, chapter 2) including several confounding factors and ectoparasites, the present analysis now further included the (mainly) endoparasitic helminths and Coccidia, to focus on the statistical influence of the count of these parasites on the tick count as dependent variable. First, all parameters were included in the full Model A which obtained an AIC of 950.9 (Figure 3-3A). During stepwise backward variable selection, parameters which reduced the AIC most clearly, were excluded from the regression until the best model (Model N, AIC=923.3) was reached. At the end, eleven parameters (18 including dummy variables for categorical parameters with more than two levels) were left in Model N as predictors of

tick abundance on rodents, of which nine were significant: The trapping location, the season of trapping, the rodent host species, the rodent abundance in the week of trapping at the respective location, the host sex including reproductive condition and finally five parasite taxa including the count of parasitic laelapid mites. Therefore, the same parameters again appeared to be important predictors for tick larvae and, in addition, significantly affected the tick number (Figure 3-3B) compared to the regression without the helminths and Coccidia (Maaz et al., submitted = chapter 2). Because of total collinearity between the trapping location Moabit and the host species *A. sylvaticus* (all exclusively trapped in Moabit), it was not possible to calculate estimates for these two levels within the two parameters. In contrast to the regression only including ectoparasites, the abundance of fleas on rodents was still included in the present best model, although it was not significant after statistical testing (p=0.133). Furthermore, four helminth taxa now appeared to be important predictors of larval tick abundance, of which three were significant: Keeping all the other parameters constant, the heligmosomoid nematodes and *Syphacia* spp. significantly reduced the tick count on rodents by 2.2% (95% CI 0.2-4.2, p=0.030) or 1.9% (95% CI 0.4-3.2, p=0.012) per additional nematode, respectively, whereas the adult tapeworms of the Hymenolepididae spp. increased tick infestation by 1.1% (95% CI 0.2-2.0, p=0.020) per tapeworm. The fluke *C. vitta* revealed a trend to a negative influence, but it was not significant.

Risk factors affecting *I. ricinus* larvae count

A Model selection

Model variables		A	B	C	D	E	F	G	H	I	J	K	L	M	N
Spacial factors	Trapping location	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Temporal factors	Season	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Host species	Rodent species	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Host abundance	Rodent abundance	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Host characteris-tics	Sex	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Age	x	x	x	x	x	x	x	x	x	x				
	Body Condition	x	x	x	x	x	x	x	x	x	x	x			
Co-infestations / -infections with other parasites	Fleas Count	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Lice Count	x	x	x	x	x	x	x	x	x	x	x	x		
	Parasitic Laelapid Count	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Myobiid Count	x	x	x	x	x	x								
	Trombiculid Count	x	x	x	x										
	Myocoptid Count	x	x	x	x	x	x	x	x	x	x	x	x	x	
	Listrophorid Count	x	x												
	Capillaria spp. Count	x	x	x	x	x	x	x	x	x					
	Heligmosomid/-nellid Count	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Heterakis spumosa Count	x	x	x	x	x									
	Syphacia spp. Count	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Aspiculuris tianjinensis Count	x	x	x	x	x	x	x							
	Pelodera orbitalis Count	x	x	x											
	Hymenolepidid Count	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Corrigia vitta Count	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Brachylaima spp. Count	x	x	x	x	x	x	x	x						
	Eimeria spp. Count	x													
	n	31	30	29	28	27	26	25	24	23	22	21	20	19	18
	AIC	950.9	948.9	947.0	945.0	943.1	941.4	939.7	938.2	936.8	935.4	934.0	932.8	932.5	932.3
	Δ	18.7	16.7	14.7	12.7	10.8	9.1	7.4	5.9	4.5	3.1	1.7	0.6	0.2	0.0

B Best Model (Model N)

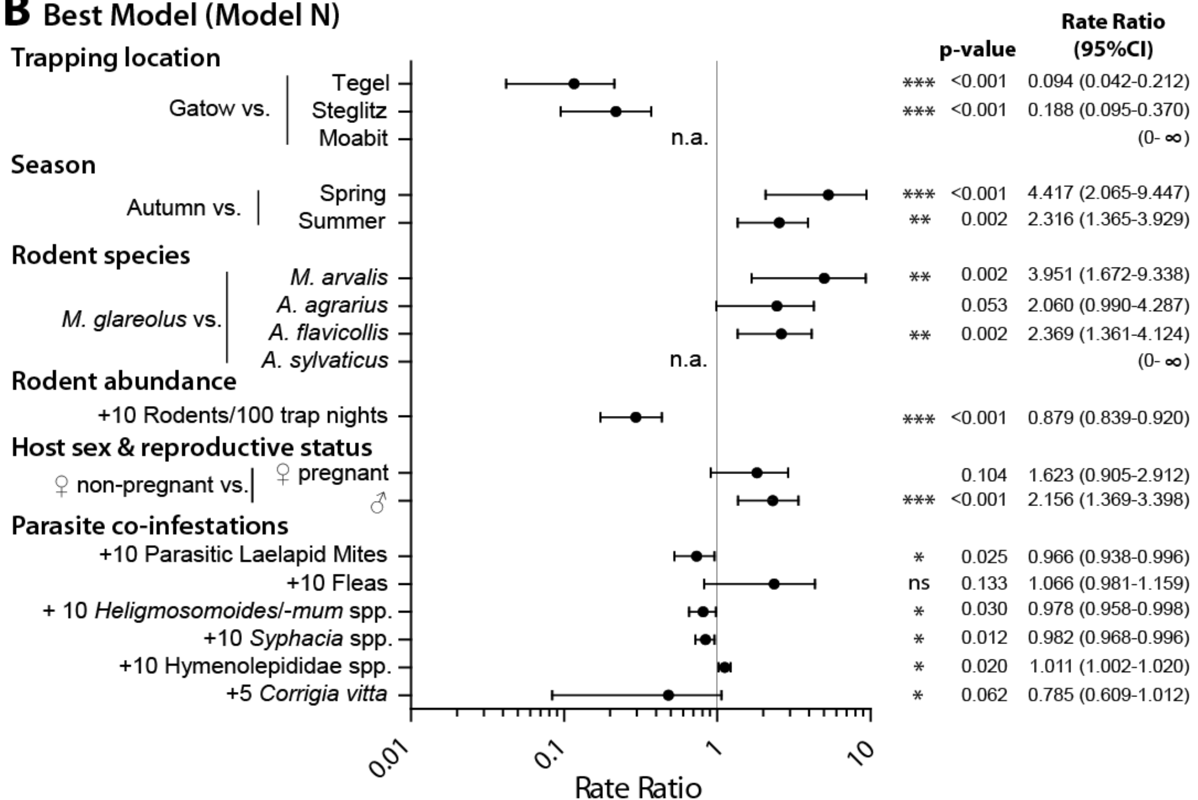

Figure 3-3. Negative binomial regression analysis for the estimation of *I. ricinus* larvae count on rodents from Berlin. (A) Model selection and (B) Forest Plot of best regression model. (A) Full model A including all the listed variables was stepwise reduced by exclusion of parameters until best model N was reached with lowest AIC. Number of variables including dummies (n), Akaike Information Criterion (AIC) and difference in AIC to the best model (Δ) are depicted below. (B) Rate Ratios (RR) with 95% confidence intervals (95% CI) for all variables included in the best model N. The y axis depicts reference levels in front of the compared level within categorical parameters and additional counts (+) for metric variables. P-values and RR with 95% CIs are indicated next to the variables. Estimates of metric variables (rodent abundance and parasites) are depicted in the Forest Plot for 5 or 10 counts increase for better visualisation, whereas written values on the right represent one count increases. Vertical line indicates rate ratio of 1 (no influence). * p<0.05; ** p<0.01; *** p<0.001; n.a.: not applicable because of total collinearity between the trapping location Moabit and *A. sylvaticus.*

3.5 Discussion

3.5.1 Taxonomic discussion

With at least 21 different taxa detected, the helminth and coccidian fauna of rodents in the urban area of Berlin is not only diverse but also very prevalent since only every tenth animal was uninfected. The true species diversity is undoubtedly higher in the examined rodents because many parasite taxa were not determined to species level and in particular the intestinal Coccidia surely comprise a number of species. Although the detected intestinal oocysts were not allowed to sporulate for further species determination, only *Eimeria* spp. and *Isospora* spp. were reported from the intestinal tract of rodents in Europe (Levine & Ivens, 1990). Only twice in Europe, *Isospora* species were reported to infect the host species investigated in the present study in Bulgaria and Eastern Lithuania (Grikienienė, 2005; Levine & Ivens, 1990). Since in contrast, at least 32 *Eimeria* spp. and several undescribed species are reported from these six rodent species in Europe (Grikienienė, 2005; Levine & Ivens, 1990), the detected oocysts most likely belong to this genus. The free sporocysts of the coccidian genus *Klossiella* (Klossiellidae), of which only *Klossiella muris* is known to infect the urinary system of house mice in Europe, were not detected in urine and the bladder of the examined animals.

The taxonomy within the digenean Trematode genera *Plagiorchis* and *Brachylaima* is not finally solved and probably includes synonyms. The specimens were therefore not further determined.

Three families of cestode parasites were detected using rodents as their final host. The tapeworm specimens were not stained and therefore not further differentiated. According to published records from Europe, the Hymenolepididae infecting *A. flavicollis* and *A. agrarius* in the present study were

most likely *Rodentolepis straminea*, *Rodentolepis fraterna*, *Hymenolepis hibernia* and/or *Variolepis crenata* (syn. *Hymenolepis muris-sylvatici*) but also the vole tapeworms *Rodentolepis asymmetrica* and *Arostrilepis horrida* were reported from these hosts (e.g. Haukisalmi (2015); Tenora (2004)). The human-pathogenic *Rodentolepis nana* is often synonymised with *R. fraterna*, but there is increasing evidence, that both are cryptic species which are morphologically identical but genetically distinct and that *R. fraterna* infecting rodents are not zoonotic (Macnish *et al.*, 2002a). In Germany (Schmidt, 1961; Stammer, 1956) and elsewhere (Lewis, 1987; Wahl, 1967), the potentially zoonotic *Rodentolepis microstoma* (Macnish *et al.*, 2003) was detected in the same rodent species of the present study. However, these reports were most likely *R. straminea*, since this species was often synonymised with the morphologically similar *R. microstoma* at that time and *R. microstoma* is very specific to the house mouse (Casanova *et al.*, 2001). Reports on the zoonotic tapeworm *H. diminuta* in *Apodemus* spp. from Germany (Memaran, 1970; Schmidt, 1961) are presumably erroneous since this is a host-specific parasite of rats and morphologically similar to *H. hibernia* infecting *Apodemus* spp. (Montgomery *et al.*, 1987). The observed catenotaeniid specimens from *A. flavicollis* are supposed to be *Catenotaenia pusilla*, *Catenotaenia lobata* or *Pseudocatenotaenia matovi* (Klimpel *et al.*, 2007b; Tenora, 2004), those from *M. glareolus Catenotaenia henttoni* (Haukisalmi & Henttonen, 2000). The Anoplocephalidae detected in *M. arvalis* and *M. agrestis* should be *Anoplocephaloides dentata*, *Paranoplocephala omphalodes*, *Paranoplocephala janickii*, *Eurotaenia gracilis* and/or *Microticola blanchardi* (Haukisalmi, 2015; Tenora *et al.*, 1973; Tenora *et al.*, 1985).

Specimens of metacestodes from the family Taeniidae for which the rodent is the intermediate host probably comprised the species *H. taeniaeformis*, *T. martis* (synonym *T. intermedia*), *T. polyacantha*, *T. pisiformis* and *T. crassiceps* according to morphological descriptions of Schmidt (2001), Schmidt (1961) and Memaran (1970) and partly sequencing in Krücken *et al.* (2017), but this remains hypothetical, since larval taeniids were not differentiated by the number and shape of the rostellar hooks.

According to Gibbons (2010), all capillariid genera (*Aonchotheca*, *Calodium*, *Capillaria*, *Eucoleus*) were collectively considered to be subgenera of *Capillaria*. The specimens from the present study were not determined to species level, but those nematodes from *M. glareolus*, *M. arvalis*, *A. flavicollis* and *A. sylvaticus* may belong to the species *Capillaria* (*Aonchotheca*) *annulosa*, *Capillaria* (*Aonchotheca*) *muris-sylvatici* or *Capillaria* (*Eucoleus*) *gastrica* (e.g. (Feliu *et al.*, 1997; Memaran, 1970; Tenora, 2004)). In *A. sylvaticus* and *M. arvalis*, *Capillaria* (*Eucoleus*) *bacillatus* was further detected in Spain (Feliu *et al.*, 1997; Fuentes *et al.*, 2000; Fuentes *et al.*, 2004; Fuentes *et al.*, 2010) and Germany (Stammer, 1956). *Capillaria* spp. in *A. agrarius* were not reported in at least ten publications from Europe where more than ten animals were screened (Bjelić-Čabrilo *et al.*, 2013;

Grikienienė, 2005; Hildebrand *et al.*, 2004; Kucia *et al.*, 2006; Murai *et al.*, 1992; Ondríková *et al.*, 2010; Ondríková & Stanko, 2009; Schmidt, 1961; Shimalov, 2002; Shimalov, 2013). In the present study only three animals appeared to be infected. In one *A. agrarius* from Steglitz, only one female nematode and 11 eggs in faeces were observed, whereas infection in two more mice from Steglitz and Gatow was only determined by the detection of 4 and 16 *Capillaria* eggs during faecal flotation. Despite their considerable length of about 10-20 mm, these hairlike nematodes are only about 60 µm thick, transparent and can easily be overlooked. In addition, they infected striped field mice in habitats where also yellow-necked mice hosted *Capillaria* spp. with prevalence of 11% in Steglitz and 24% in Gatow. The low intensity of infection suggests that *A. agrarius* might be an incidental host for intestinal *Capillaria* spp. Worms or egg packages of *Capillaria* (*Calodium*) *hepatica* in the liver were not detected in any host according to examinations of morphologically altered liver tissues but these examinations are probably insensitive and specimens may have been easily overlooked.

Members of the genus *Trichuris* detected in the caecum of *M. glareolus*, *M. arvalis* and *A. flavicollis* most likely belong to the sibling species *Trichuris muris* occurring in mice and *Trichuris arvicolae* infecting voles according to Feliu *et al.* (2000). The authors further confirm that the description of *Trichuris madisonensis* detected in *M. glareolus*, *M. arvalis* and *M. agrestis* from Germany (Schmidt, 1961; Stammer, 1956) is unsatisfactory and the specimens might refer to *T. arvicolae*.

Within the heligmosomoid families Heligmosomidae and Heligmonellidae, several species may have infected the four species of rodents: the heligmonellid *Paraheligmonina gracilis* and *Carolinensis minutus* and the heligmosomid *Heligmosomoides polygyrus*, *Heligmosomoides neopolygyrus*, *Heligmosomoides laevis*, *Heligmosomoides glareoli*, *Heligmosomum costellatum*, *Heligmosomum pseudocostellatum*, *Heligmosomum yamagutii*, *Heligmosomum mixtum* and *Heligmosomum borealis* (Feliu *et al.*, 1997; Haukisalmi & Henttonen, 2000; Tenora, 2004; Tenora *et al.*, 1983; Tenora *et al.*, 1973; Zaleśny *et al.*, 2014).

Seven species from the oxyurid genus *Syphacia* were reported in Europe to infect the common vole and the three *Apodemus* spp. which appeared to be infected in the present study: *Syphacia stroma* and *Syphacia frederici* mainly infect *A sylvaticus* and *A. flavicollis*, but there are also reports from *A. agrarius* (Hildebrand *et al.*, 2004; Murai *et al.*, 1992). *Syphacia agraria* is a specific parasite of *A. agrarius* (Tenora & Mészéros, 1975). *Syphacia petrusewiczi* and *Syphacia montana* are usually considered to be specific to voles, but the latter was also once reported from *A. flavicollis* (Grikienienė, 2005). The main host for *Syphacia nigeriana* are *Microtus* spp. voles but the species was also detected in the wood mouse in Spain (Fuentes *et al.*, 2003a). The species *Syphacia obvelata* was reported from numerous mouse and vole species but this is a parasite specific for *Mus musculus*

(Tenora & Mészéros, 1975) and many reports from other hosts should be doubted (Stammer, 1956; Tenora, 2004). The heteroxynematid *A. tianjinensis* from field and bank voles, which was first described from *Myodes rufocanus* from China in 2012, closely resembled the descriptions of *Aspiculuris tetraptera*. But Behnke *et al.* (2015) showed that the latter species is morphologically similar but only infects mice.

Two species of *Pelodera* infected rodents from Berlin; *Pelodera orbitalis* were detected in the lachrymal fluid of striped field mice, bank voles and common voles and *Pelodera cutanea* on the hair ends and in the water under the carcasses of yellow-necked mice, which presumably emigrated from the skin of the rodents. The life cycle of these *Pelodera* spp. can generate three morphologically and behaviourally distinct types of third larvae (“normal-”, “dauer-”, “infective-”). Only one is obligatory parasitic but at least *P. orbitalis* is also able to maintain a complete free-living nidicolous life for many generations (Schulte, 1989). Stammer (1956) also reported nematodes in these compartments from different rodents from Germany and attributed them to *Pelodera strongyloides*, but later Sudhaus *et al.* (1987) revealed that two other sibling species regularly infect rodents, *P. orbitalis* in conjunctival sacs of the eyes and *P. cutanea* in the skin and that the nematodes from Stammer (1956) also belong to these two species. The nematode larvae from the present study were already reported from the host species and closely resemble the descriptions from Sudhaus *et al.* (1987) although larvae were not allowed to develop to the adult stage to further confirm this. Similar observations of nematode larvae from these compartments in rodents from Germany and the UK determined as *P. strongyloides* (Behnke *et al.*, 2009; Behnke *et al.*, 1999; Klimpel *et al.*, 2007a; Klimpel *et al.*, 2007b; Lewis, 1987; Montgomery & Montgomery, 1988) probably also refer to the mentioned species.

Remarkable was the detection of a single female of *S. ratti* in a bank vole in Gatow. This parasite normally is an exclusive and common parasite of rats (Schmidt, 1961; Viney, 1999) and was in Europe, to the knowledge of the authors, only detected in other wild rodents from Austria (Frank, 1977), Belgium (Bernard, 1961) and Germany (Memaran, 1970; Schmidt, 1961; Stammer, 1956), where it was detected in all host species from the present study with considerable prevalence. This small, hairlike parasite is easily overlooked (Schmidt, 1961) and may be confused with *Capillaria* spp. at first glance, but high prevalence of up to 18% in 34 *A. agrarius* from the area of Halle (Schmidt, 1961) indicate that this finding is a particularity of Central Europe. Tenora *et al.* (1973) suggested *S. ratti* only infects rodents at sites where also Norway rats occur and this could be true in Gatow which was situated in the vicinity of barrack buildings even though no rats were captured in the study period.

3.5.2 The component community of the host species

The helminth community of bank voles in the present study was mainly comparable to that from other reports on this intensively studied species. Helminths infected 64% of this host species which is in a range of most other longitudinal studies in Germany (Schmidt, 1961) and Europe (Grzybek *et al.*, 2015a; Ribas *et al.*, 2009; Shimalov, 2013; Wahl, 1967) with 51-80% prevalence but obviously higher than observed in Italy, Norway and Austria with 19-44% (Milazzo *et al.*, 2003; Pfaller, 1974; Tenora *et al.*, 1979). The most prevalent helminth taxon was *Capillaria* which is more typical for bank voles from the UK than for continental Europe where Heligmosomidae normally dominate (Haukisalmi & Henttonen, 2000). *Aspiculuris* spp. were only reported from bank voles in Northern Scotland (Thomas, 1953), Southern England and Wales (Lewis, 1987), Western Ireland (Loxton *et al.*, 2016), in a single vole from Serbia (Bjelic-Cabrilo *et al.*, 2011) and very frequently in Eastern Poland (Grzybek *et al.*, 2015a) with a high prevalence of 42% (n=922). In contrast, this genus was absent in bank voles from other European sites including Germany (Klimpel *et al.*, 2007a; Memaran, 1970; Schmidt, 1961; Stammer, 1956). Interestingly, this perfectly matches the presumed geographic range of the Carpathian lineage of the bank vole according to the map in Filipi *et al.* (2015). Among the populations positive for *Aspiculuris* spp., only the introduced bank voles from Western Ireland were described as part of the Western lineage. The other oxyurid genus *Syphacia* with *S. petrusewiczi* as the species most specific for *M. glareolus* is generally rare or absent in most studies from Europe and also did not infect bank voles from the present study. But real absence at the study sites cannot be claimed, since this oxyurid parasite was shown to vary drastically in long-term studies and can be even absent in some years (Grzybek *et al.*, 2015a). It was striking that a low prevalence and diversity of adult cestodes was detected with only 2% prevalence and solely from the Cataenotaeniidae. In a review of Haukisalmi and Henttonen (2000) combining 28 studies on bank voles from Europe, Anoplocephalidae and Hymenolepididae were detected in 22 and 16 studies, respectively and *Catenotaenia* spp. revealed an averaged prevalence of 14%. Reasons for this may be the absence or low infection rate of arthropod intermediate hosts at the trapping sites in Berlin, which should be oribatid mites for Anoplocephalidae (Haukisalmi, 1986) and R. *asymmetrica* (Gubanyi *et al.*, 1992; Tenora *et al.*, 1973) and collembolans for *A. horrida* (Rausch, 1994).

Only eleven common voles and two field voles were trapped during the study period, therefore quantitative statements about single parasite taxa are not possible but the spectrum of observed helminth taxa is consistent with other studies on *Microtus* spp. in Europe (e.g. (Tenora *et al.*, 1973)). The absence of uninfected animals, which means a prevalence of at least 77% according to the lower 95% confidence limit was high compared to other studies with 52-62% in *M. arvalis* (Schmidt, 1961; Shimalov, 2013; Soltys, 1949) and 67% in *M. agrestis* (Mažeika *et al.*, 2003). On the other side,

Stammer (1956), who did not report a total helminth prevalence, found at least *H. polygyrus* in 75% of the common voles in the area of Erlangen, Germany.

Apart from the above mentioned rare *Capillaria* spp. infections, the diversity of the helminth community of the striped field-mouse at the Western margin of its range largely reflects those from other studies from Europe. Animals appeared to be highly infected with 90% prevalence but this was in the range with other populations with 29% (n=45) to 96% (n=96) in European studies including at least 30 animals (Hildebrand *et al.*, 2004; Murai *et al.*, 1992; Ondríková *et al.*, 2010; Ondríková & Stanko, 2009; Schmidt, 1961; Shimalov, 2002; Shimalov, 2013). However, overall prevalence of helminth was largely depending on the obviously focal occurrence of the two nematodes *H. polygyrus* and *H. spumosa*. *H. polygyrus* (or other Heligmosomoidea) were absent in *A. agrarius* in the present study which was the same in a study from Halle, Germany (Schmidt, 1961) and Central-Slovakia (Ondríková & Stanko, 2009) but were very prevalent in the same study in Eastern Slovakia (and Ondríková *et al.* (2010)) as well as in Poland (Kucia *et al.*, 2006) and Belarus (Shimalov, 2002; Shimalov, 2013) with up to 93% (n=96). Similarly *H. spumosa* was absent or rare in the mentioned studies from Germany, Slovakia and Belarus but infected 79% (n=14) and 46% (n=35) in Poland (Hildebrand *et al.*, 2004; Kucia *et al.*, 2006). Interestingly, *H. spumosa* also infected a considerable number of yellow-necked and wood mice which has rarely been reported from Europe and apart from Klimpel *et al.* (2007b) only in habitats where the preferred host *A. agrarius* was also abundant (Bjelić-Čabrilo *et al.*, 2013; Schmidt, 1961). Due to the presumably high infection pressure in Steglitz because of highly abundant striped-field mice with a prevalence of 88%, the sympatric yellow-necked mice showed a prevalence of 56% which is extremely high for this incidental host, albeit low intensity of infection. Similarly, the digenean *C. vitta*, particularly a parasite of *A. sylvaticus* and *A. flavicollis*, also infected *A. agrarius* in Steglitz. This was to the knowledge of the authors not reported before in Europe. Obviously this parasite, which was only reported from Western and Middle Europe in the last decades, finds at its Eastern range margin a competent new host with the striped field mouse, which here reveals its most Western occurrence. An apparently very high infection pressure elicited from infected intermediate hosts, leading to 59% infected yellow-necked mice at this trapping location as well. But also the prevalence of 25% *Brachylaima* sp. in the striped field-mouse in Steglitz is extremely high for this triheteroxenous parasite compared to other studies.

The helminth community of the closely related *A. sylvaticus* and *A. flavicollis* is described as similar (Stammer, 1956) and indeed the most prevalent helminth taxa for both hosts were Heligmosomidae/Heligmonellidae, *Capillaria* spp. and *Syphacia* spp., which is consistent with most reports from Europe, as well as the above mentioned *H. spumosa*. General helminth prevalence is with 78% in yellow-necked mice in a range similar to what has been reported from elsewhere in

Europe (44-93% (Debenedetti *et al.*, 2016; Murai *et al.*, 1992; Schmidt, 1961; Shimalov, 2013)) but the high prevalence of 92% in wood mice is according to an intensive literature review on longitudinal studies at the highest margin of reported infection rates of 45-91% (e.g. Abu-Madi *et al.* (2000); Debenedetti *et al.* (2015); Milazzo *et al.* (2005); Schmidt (1961)). As a consequence of the reported similar endoparasite communities of both hosts, differences in diversity and quantity of helminths and Coccidia should be mainly assigned to the trapping location. Hymenolepidid tapeworms were prevalent in yellow-necked mice from three trapping locations infecting every fourth mouse but were absent in wood mice in Moabit (or according to the upper 95% confidence limit at least below a prevalence of 13%). In contrast, similar infection rates of both hosts were observed if investigated at the same trapping sites (Debenedetti *et al.*, 2016; Debenedetti *et al.*, 2015; Klimpel *et al.*, 2007b; Memaran, 1970; Schmidt, 1961; Shimalov, 2013). On the other hand, prevalence of *Capillaria* spp. was very high in wood mice in Moabit with 44% prevalence, which is more than in other studies with 0-38% (e.g. (Abu-Madi *et al.*, 2000; Debenedetti *et al.*, 2015; Milazzo *et al.*, 2005; Schmidt, 1961; Shimalov, 2013)) but similar only to a dry mountainous region in Spain with about 52% (Fuentes *et al.*, 2000) and an urban area in Dormagen, Germany with about 45% (Klimpel *et al.*, 2007b). Dry conditions with a regularly mowed lawn and a sewage system in the backyard in Moabit may positively affect the transmission of the infective stages, which are comparably drought-resistant embryonated eggs in the case of *Capillaria* spp. Hence, such conditions should negatively affect the infective L3 of Heligmosomoidea which were still very prevalent infecting 84% of the animals which is comparable to other studies e.g. from UK where prevalence could be even higher (Lewis, 1987). In contrast, cestodes and digenean parasites are obligatory heteroxenous. In particular, the life-cycle of the latter includes at least one terrestrial or freshwater mollusc as intermediate host (Feliu *et al.*, 1997). Dry conditions in Moabit should be unfavourable for these animals leading to an absence of infection in the final host. In contrast the regularly watered area in Steglitz surely improved the environmental conditions for gastropods which probably lead to a very high prevalence of Digenea in *A. agrarius* and *A. flavicollis*.

3.5.3 Urbanity as a factor of rodent endoparasite species richness, diversity and abundance

Most endoparasites with a direct life cycle such as intestinal Coccidia (most likely *Eimeria* spp.), Heligmosomoidea and *H. spumosa* were more prevalent and/or more numerous in rodents from urban areas than from periurban sites and only the rare homoxenous *Trichuris* spp., *S. ratti* and *P. cutanea* were exclusively detected in Gatow and Tegel. In contrast, apart from the mentioned Trematoda infecting *Apodemus* spp., heteroxenous parasites such as the digenean *Plagiorchis* spp., adult and larval cestodes and *M. muris* were more diverse, prevalent and/or numerous at periurban

sites. The present authors already reported from the same animals that species richness and abundance of some rodent-associated arthropod groups were reduced at the urban trapping locations, especially for those with low host-specifity and with higher demands on habitat quality such as fleas, chiggers and ticks (Maaz et al., submitted = chapter 2). Some of these arthropods (e.g. fleas) may also participate in the transmission cycles of the observed helminths. The same can be concluded in the present study where the parasites with a more complex life cycle including obligate invertebrate intermediate hosts mainly occurred at the periphery of Berlin. Most studies investigating urban-rural gradients exhibited a reduction of invertebrate and vertebrate species richness already towards medium levels of urbanisation (McKinney, 2008). But "Urbanisation" is a term accompanying many different factors. The key determinants of species richness in an urban area are according to a metaanalysis about urban studies on different taxonomic groups the size of the patch area, the presence of corridors important for migration of species and the vegetation structure (Beninde *et al.*, 2015). Parasites are largely neglected in terms of biodiversity, but the same can be concluded for the rodent endoparasites in the present study. Helminth taxa infecting rodents were most numerous at the large, open forest habitats in Gatow and Tegel, were lower in the urban park in Steglitz with a considerable area of 43 ha and a well-structured vegetation although absence of real corridors, up to the lowest number in Moabit with less than 1 ha area, no corridors and a poor vegetation structure. In contrast to species richness, the overall prevalence of helminths was the highest in the backyard in Moabit with 92%, although only nematodes of four taxa were detected. The reasons for this could be a higher host density, altered food spectrum or migration behaviour which is reported from striped field mice in urban areas compared to rural sites (Luniak, 2004). High host densities should enhance the transmission of parasites with a direct life cycle since the infective eggs or larvae are more likely taken up by another competent host. The probably higher food availability in the proximity of human houses should reduce the necessity of feeding on invertebrates in poor seasons and therefore the ingestion of intermediate hosts. Finally a reduced migratory behaviour and home range, the reduced vegetation coverage and the increased density of predators reported from urban areas (Luniak, 2004) could reduce the space where rodents are active solely to some covered paths and consequently increase the density of infective stages of endoparasites with direct life cycle on these particular areas.

Despite the low helminth species richness in Moabit, the number of endoparasite taxa simultaneously infecting every wood mouse was high which was caused by the high prevalence of every taxon. Also if all the 84 detected taxa of helminths, arthropods and intestinal Coccidia are considered, every rodent in Moabit and also in Steglitz hosted on average more invertebrate/protozoan taxa at the same time compared to the periurban sites. Again the reason for

this was the higher prevalence of some parasites despite lower taxon richness for both, arthropods and helminths, in the urban city centre compared to the periphery of Berlin. The yellow-necked mouse revealed the highest species richness in the present study but also was the only host species which was abundant at three trapping locations and therefore was more likely to acquire more location-specific parasites compared to the other rodents. More studies with larger numbers of animals are required for the comparison of the macroparasite species richness between these rodent species from the same location.

3.5.4 NMDS for visualisation of the macroparasite assemblages

To create an overview over the incredibly large number of helminth and arthropod species which are hosted by wild rodents, a multidimensional scaling was performed, which tried to reduce and to visualise the complex dissimilarities of the quantitative parasite occurrence patterns among all examined hosts. Hence, the distribution of hosts according to its associated invertebrate fauna in 2D and 3D plots revealed that the host species explains much more of the clustering than the trapping location. Already Stammer (1956) noticed that the species of a host can be well predicted from its parasite fauna, except for some closely related species such as *A. flavicollis* and *A. sylvaticus* as well as *M. arvalis* and *M. agrestis*. In the present study, too few specimens were investigated of the latter species but indeed, the invertebrate fauna of the yellow-necked mouse and the wood mouse was very similar if not undistinguishable even though they were not trapped at the same locations. But also Klimpel *et al.* (2007b) revealed this similarity in an ordination of both species of the same trapping location. Most specimens of the striped field mouse clearly showed a distinct community which is characterised by a number of host-specific arthropods as well as helminths such as *Brachylaima* sp. and largely *H. spumosa*.

3.5.5 Zoonotic risk and influence of rodent endoparasites on larval tick abundance

The data allow concluding that the risk of infection for people arising from endoparasites of non-commensal muroid rodents in Berlin is low. *Eimeria* spp. are mainly parasites of herbivorous animals, very host-specific and have not been reported from humans. Although cestodes were not determined to species level, the three hymenolepidid species reported from man are very unlikely to occur in the present rodent specimens. Metacestodes of the zoonotic *Echinococcus multilocularis* were not detected although morphologically altered liver parts were further analysed by PCR (Krücken *et al.*, 2017) and *Taenia* spp., which probably included *T. crassiceps* and *H. taeniaeformis*, are very rare human parasites (Nakao *et al.*, 2010). Furthermore, *Trichinella* spp. as well as *C. hepatica* were absent or infecting rodents at least with only low intensities. The same was

concluded for the rodent-associated arthropods since only the flea *Nosopsyllus fasciatus* represented a direct zoonotic risk (Maaz et al., submitted = chapter 2). More importantly, the rodents represented competent host for the larval and nymphal life stage of the hard tick *Ixodes ricinus* and therefore maintain the life cycle of ticks and tick-borne pathogens also in urban areas. A number of external and intrinsic factors significantly affected the number of *I. ricinus* larvae infesting mice and voles in a regression analysis and these factors were already discussed in Maaz et al., (submitted = chapter 2). Moreover, the number of ectoparasitic mites of the family Laelapidae (Gamasina) revealed a significant negative influence on the abundance of host-associated *I. ricinus* larvae which might be attributed to predation, competition for space and food as well as an interaction via the immune system of the host. In the present study also endoparasitic helminth taxa were included in the same regression analysis. Parameters which showed a significant influence in the previous publication remained present and significant also in the best model of the present analysis. But furthermore, three helminth taxa appeared to have a significant influence on the number of tick larvae infesting rodents from Berlin. The heligmosomoid nematodes as well as *Syphacia* spp. revealed a significant negative correlation to tick infestation of the wild rodents, whereas the adult tapeworms Hymenolepididae exhibited a positive effect on larval tick count. However, it has to be considered that this only shows that, keeping other co-factors constant, mice and voles infected with these helminths hosted more/fewer ticks compared to non-infected rodents and does not prove a mechanism of cause and effect. In a cross-sectional study on yellow necked mice in Italy, Ferrari *et al.* (2009) found the same negative association of *H. polygyrus* and larval *I. ricinus* ticks in a similar regression analysis taking in consideration several confounding factors. To verify that this is a mechanism of cause and effect they exhibited experimentally, that if mice were dewormed and recaptured, the tick infestation was increased compared to non-treated mice from a comparable habitat. The authors concluded that due to their different nutrition (tissue feeder versus blood sucker) and microhabitat on the host, competition for space and food can be ruled out. Also behavioural reasons related to age, sex and reproductive status as well as the intensity of grooming, which would remove ticks and enhance infection with *H. polygyrus* larvae, were excluded due to the cause-effect experiment. But in the present study the abundance of *Syphacia* sp. also showed a negative relationship to tick larvae. These nematodes migrate to the perianal region where they deposit their eggs (Lewis, 1987) which should lead to intense pruritus and agitation. The resulting scratching of the host or probably grooming due to the agitation should remove feeding ticks. The anthelminthic pyrantel pamoate used to deworm mice in Ferrari *et al.* (2009) most likely also expelled *Syphacia* spp. from the mice which may have contributed to the observed increase of tick larvae.

The most likely reason for the observed correlations of helminths and ticks is an interaction via the host immune system or the release of toxic products by one parasite. A number of studies detected antagonistic as well as synergistic interactions of parasites in experimental infections of laboratory animals, although mostly from the same compartment (Christensen *et al.*, 1987). Recently the influence of prepatent, acute and chronic *H. polygyrus* (probably a synonym for *Heligmosomoides bakeri*) infection on nymphal and repeated larval *I. ricinus* infestation in C57BL/6 laboratory mice was studied and no influence of co-infection on the success of tick feeding or nematode survival was found (Maaz *et al.*, 2016). Worms of the intensively studied model organism *H. bakeri* are able to supress the host immune system to permit their own survival but also facilitate the infection by other helminth species (Christensen *et al.*, 1987; Ferrari *et al.*, 2009). While laboratory studies often demonstrated strong interactions of helminths mediated by the immune system, these interaction seem to play a minor role in naturally infected rodents (Behnke *et al.*, 2001; Behnke *et al.*, 2005). Differences to field observations in laboratory experiments especially occur, if non-natural hosts are used (Pedersen & Babayan, 2011). Therefore, the absence of effects of *H. polygyrus* co-infections on tick feeding in laboratory mice may be related to this artificial setting. However, experiments with the natural hosts are challenging for immunological studies since the immunological tools such as antibodies for cell types and cytokines are mostly not available yet.

Although the extent of influence of helminths on tick infestation in the present regression appeared to be relatively low, the present dataset again found a negative relationship of nematodes and ticks and an interaction would have an important impact on the life cycle of ticks and tick-borne pathogens. However, the pattern of polyparasitism in wild rodents demonstrated in the present study is clearly not feasible in experimental infections. Capture-mark-recapture experiments in the field, adequately controlled for the numerous confounding factors presumably have the best chances to verify these findings and to prove mechanisms of cause and effect.

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3.7 Supplemental information

◆/ Tegel
■/ Steglitz
▲/ Moabit
●/ Gatow
A. agrarius
A. flavicollis
A. sylvaticus
M. glareolus
M. arvalis
M. agrestis

Supplemental Figure 3-4. Non-parametric multidimensional scaling of 77 helminth and arthropod taxa associated with mice and voles from Berlin hosting at least three (A-E) or at least five (F-J) arthropod and helminth taxa at the same time. NMDS was calculated for two (A-C, F-H) and three dimensions (D, E, I, J). Grey polygons border all rodents from one host species (A, B, F, G) or trapping location (C, H). Shaded coloured polyhedrons depict the convex hull including all rodents within one species (D, I) or from one trapping location (E, J). Point colours indicate the rodent species and point symbols the trapping locations (see legend). Stress of best (presented) 2D solutions were 0.273 after 760 iterations (A-C) and 0.252 after 231 iterations (F-G), those of the best 3D solution 0.193 after 200 iterations (D, E) and 0.186 after 200 iterations (I, J). The names of helminth and arthropod taxa in B and G were abbreviated with the first letter of the genus and the first two to three letters of the species. If only genus or family were determined, the first three to five letters are shown.

Supplemental Table 3-3. Regression analyses for modelling presence/absence (=prevalence) and count (=intensity of infestation) of parasites in rodents from Berlin as dependent variables. Rodent host species (6 levels) or family (2 levels) and trapping location (4 levels) or location category (2 levels) were used as independent variables. Odds Ratios for logistic regression (left panel) or Rate Ratios for negative binomial regression (right panel) are presented together with 95%CI and p-value. * p<0.05, ** p<0.01, *** p<0.001

Logistic Regression Analyses

	Odds Ratio	95% CI	p-value	
A Prevalence of intestinal Coccidia oocysts in faecal flotation (within 2011)				
Intercept	0.58	0.34-0.99	0.045	*
M. glareolus vs. *M. arvalis*	1.33	0.34-5.22	0.685	
M. glareolus vs. *M. agrestis*	1.72	0.10-28.92	0.706	
M. glareolus vs. *A. agrarius*	0.92	0.33-2.56	0.874	
M. glareolus vs. *A. flavicollis*	0.70	0.33-1.49	0.354	
M. glareolus vs. *A. sylvaticus*	1.21	0.33-4.42	0.769	
Periurban vs. Urban	3.64	1.63-8.15	0.002	**
C Prevalence of Hymenolepididae				
Intercept	0.44	0.22-0.90	0.024	*
A. flavicollis vs. *A. agrarius*	0.51	0.18-1.43	0.198	
Gatow vs. Tegel	0.85	0.26-2.76	0.789	
Gatow vs. Steglitz	0.64	0.22-1.83	0.401	
E Prevalence of adult tapeworms				
Intercept	0.91	0.22-3.60	0.888	
M. arvalis vs. *M. glareolus*	0.0065	0.0006-0.07	<0.001	***
M. arvalis vs. *M. agrestis*	0.37	0.017-8.06	0.529	
M. arvalis vs. *A. agrarius*	0.15	0.033-0.66	0.012	*
M. arvalis vs. *A. flavicollis*	0.22	0.056-0.87	0.031	*
M. arvalis vs. *A. sylvaticus*	0	0-Inf	0.989	
Urban vs. Periurban	2.96	1.16-7.55	0.023	*
G Prevalence of *Capillaria* spp.				
Intercept	0.77	0.44-1.37	0.383	
M. glareolus vs. *M. arvalis*	0.41	0.07-2.20	0.299	
M. glareolus vs. *M. agrestis*	0	0-Inf	0.988	
M. glareolus vs. *A. agrarius*	0.17	0.032-0.89	0.036	*
M. glareolus vs. *A. flavicollis*	0.45	0.19-1.06	0.067	
M. glareolus vs. *A. sylvaticus*	0	0-Inf	0.991	
Gatow vs. Tegel	0.32	0.11-0.93	0.036	*
Gatow vs. Steglitz	0.26	0.072-0.97	0.045	*
Gatow vs. Moabit	>999	0-Inf	0.991	

Negative Binomial Regression Analyses

	Rate Ratio	95% CI	p-value	
B Intensity of intestinal Coccidia oocysts in faecal flotation (within 2011)				
Intercept	5878	2785-12405	<0.001	**
Mouse vs. Vole	0.17	0.072-0.38	<0.001	**
Gatow vs. Tegel	0.36	0.094-1.41	0.143	
Gatow vs. Steglitz	0.68	0.30-1.51	0.343	
Gatow vs. Moabit	0.84	0.29-2.41	0.740	
D Intensity of Hymenolepididae				
Intercept	40.27	19.17-84.62	<0.001	**
A. flavicollis vs. *A. agrarius*	0.32	0.07-1.51	0.150	
Gatow vs. Tegel	0.20	0.06-0.73	0.014	*
Gatow vs. Steglitz	0.08	0.02-0.34	<0.001	**
F Intensity adult tapeworms				
Intercept	0.58	0.11-2.95	0.518	
M. arvalis vs. *M. glareolus*	2.48	0.18-35.11	0.501	
M. arvalis vs. *M. agrestis*	0.90	0.06-14.29	0.942	
M. arvalis vs. *A. agrarius*	1.87	0.29-12.26	0.512	
M. arvalis vs. *A. flavicollis*	5.79	1.91-17.60	0.001	**
Urban vs. Periurban	7.54	2.08-27.41	0.002	**
H Intensity of *Capillaria* spp.				
Intercept	17.33	10.22-29.38	<0.001	**
M. glareolus vs. *M. arvalis*	0.087	0.011-0.68	0.020	*
M. glareolus vs. *A. agrarius*	0.023	0.0026-0.20	<0.001	**
M. glareolus vs. *A. flavicollis*	0.17	0.068-0.45	<0.001	**
M. glareolus vs. *A.*	4.09	0.18-92.8	0.376	
Gatow vs. Tegel	0.91	0.28-2.99	0.877	
Gatow vs. Steglitz	4.07	0.90-18.46	0.068	
Gatow vs. Moabit	0.058	0.0027-1.25	0.069	
K Intensity of *Heterakis spumosa* (within *Apodemus* spp.)				
Intercept	9.75	4.34-21.93	<0.001	**
A. agrarius vs. *A. flavicollis*	0.16	0.092-0.29	<0.001	**
A. agrarius vs. *A. sylvaticus*	0.050	0.016-0.16	<0.001	**
Periurban vs. Urban	3.72	1.65-8.39	0.002	**

Logistic Regression Analyses

	Odds Ratio	95% CI	p-value	
I Prevalence of *Heligmosomoides/Heligmosomum* spp.				
Intercept	0.11	0.047-0.25	<0.001	***
M. glareolus vs. *M. arvalis*	4.50	0.96-21.12	0.056	
M. glareolus vs. *M. agrestis*	0	0-Inf	0.998	
M. glareolus vs. *A. agrarius*	0	0-Inf	0.988	
M. glareolus vs. *A. flavicollis*	3.15	1.13-8.73	0.028	*
M. glareolus vs. *A. sylvaticus*	11.39	2.27-57.14	0.003	**
Periurban vs. Urban	4.24	1.73-10.40	0.002	**
J Prevalence of *Heterakis spumosa* (within *Apodemus* spp.)				
Intercept	1.12	0.40-3.19	0.826	
A. agrarius vs. *A. flavicollis*	0.14	0.055-0.34	<0.001	***
A. agrarius vs. *A. sylvaticus*	0.034	0.010-0.11	<0.001	***
Periurban vs. Urban	6.62	2.59-16.95	<0.001	***
L Prevalence of *Mastophorus muris* (within *A. flavicollis* and *M. glareolus* from Gatow and Tegel)				
Intercept	0.020	0.0027-0.146	<0.001	***
M. glareolus vs. *A. flavicollis*	0.22	0.037-1.32	0.097	
Gatow vs. Tegel	21.16	2.31-193.67	0.007	**
M Prevalence nematodes				
Intercept	1.54	0.91-2.60	0.107	
M. glareolus vs. *M. arvalis*	4.75	0.56-40.63	0.155	
M. glareolus vs. *M. agrestis*	0.65	0.039-10.91	0.764	
M. glareolus vs. *A. agrarius*	1.49	0.45-4.98	0.515	
M. glareolus vs. *A. flavicollis*	1.07	0.51-2.24	0.863	
M. glareolus vs. *A. sylvaticus*	2.26	1.24-8.85	0.381	
Periurban vs. Urban	4.24	1.73-10.40	0.017	*

Chapter 4

Susceptibility to ticks and Lyme disease spirochetes is not affected in mice coinfected with nematodes

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Susceptibility to Ticks and Lyme Disease Spirochetes Is Not Affected in Mice Coinfected with Nematodes

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Small rodents serve as reservoir hosts for tick-borne pathogens, such as the spirochetes causing Lyme disease. Whether natural coinfections with other macroparasites alter the success of tick feeding, antitick immunity, and the host's reservoir competence for tick-borne pathogens remains to be determined. In a parasitological survey of wild mice in Berlin, Germany, approximately 40% of *Ixodes ricinus*-infested animals simultaneously harbored a nematode of the genus *Heligmosomoides*. We therefore aimed to analyze the immunological impact of the nematode/tick coinfection as well as its effect on the tick-borne pathogen *Borrelia afzelii*. Hosts experimentally coinfected with *Heligmosomoides polygyrus* and larval/nymphal *I. ricinus* ticks developed substantially stronger systemic type 2 T helper cell (Th2) responses, on the basis of the levels of GATA-3 and interleukin-13 expression, than mice infected with a single pathogen. During repeated larval infestations, however, anti-tick Th2 reactivity and an observed partial immunity to tick feeding were unaffected by concurrent nematode infections. Importantly, the strong systemic Th2 immune response in coinfected mice did not affect susceptibility to tick-borne *B. afzelii*. An observed trend for decreased local and systemic Th1 reactivity against *B. afzelii* in coinfected mice did not result in a higher spirochete burden, nor did it facilitate bacterial dissemination or induce signs of immunopathology. Hence, this study indicates that strong systemic Th2 responses in nematode/tick-coinfected house mice do not affect the success of tick feeding and the control of the causative agent of Lyme disease.

Ticks are the most important arthropod vectors of pathogens in temperate regions of the Northern Hemisphere. In particular, ticks of the genus *Ixodes* transmit several pathogens affecting the health of humans and livestock, such as *Borrelia* spp., which cause Lyme disease (LD) or relapsing fever, and *Rickettsia* spp. of the spotted fever group, which cause diseases such as aneruptive fever and tick-borne lymphadenopathy (1). Small rodents serve as reservoir hosts for many tick-borne pathogens, and the larval stage of *Ixodes ricinus*, the main vector species in Western and Central Europe, frequently feeds on mice and voles (2). The larval tick stage efficiently acquires the pathogen when feeding on infected rodents (3). After they molt, nymphal ticks constitute the major source for infections in people (4). Two genospecies of LD spirochetes account for the majority of infections in Eurasia, *Borrelia garinii* and *Borrelia afzelii*, the latter of which mainly and efficiently perpetuates in rodents (5–8). Mice and voles in urban and rural areas are frequently infected by intestinal helminths, such as the trichostrongyloid *Heligmosomoides polygyrus* (9–11), raising the question of whether such natural coinfections may affect the feeding success of ticks and the transmission of tick-borne pathogens.

Ticks initiate type 2 T helper cell (Th2) responses during their blood meal (12). Although feeding of *I. ricinus* ticks results in protective immunity in guinea pigs and rabbits, animals nonnative to this tick's distribution, the immune responses of most autochthonous wild mouse species and laboratory mouse strains are insufficient to protect them from repeated infestations with ticks (12–16). On the other hand, feeding *Ixodes* ticks suppress Th1 responses against tick-borne LD spirochetes in mice (17), and the suppression of Th2 cytokines or the reconstitution of tumor necrosis factor alpha, interleukin-2 (IL-2), and gamma interferon (IFN-γ) after tick attachment reduces infection rates in disease-susceptible laboratory mouse strains (18, 19). Infections with the enteric nematode *H. polygyrus* elicit strong local and systemic Th2 and regulatory responses (20, 21) and thereby affect the Th1-dependent control of concomitant bacterial and protozoan infections (22–28). It may be that the concurrent presence of enteric nematodes synergistically enhances Th2 and associated innate effector cell responses toward repeated tick infestations and as a result affects tick feeding success and the transmission of tick-borne pathogens. The expected strong general skewing of immune responses toward a Th2 response in mice simultaneously harboring the two macroparasites *H. polygyrus* and *I. ricinus* may restrain

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the immune control of tick-borne pathogens and subsequently increase the reservoir competence of rodents.

To investigate how frequently mice simultaneously harbor enteric nematodes and ticks in nature, we captured wild rodents in Berlin, Germany, and determined the frequency of infection with *Heligmosomoides* spp. and infestation with *I. ricinus*. Using a defined experimental laboratory system, we then assessed parameters of antitick immunity and the competence of the mice as a reservoir for LD spirochetes using inbred house mice. To examine whether the expected strong Th2 immune responses associated with intestinal nematode infections interfere with, first, the experimental host's development of antitick immunity (measured by means of determination of tick-specific cytokine responses and the success of repeated tick feeding) and, second, the control of tick-borne pathogens, we established an experimental model of coinfection in inbred laboratory mice, compared susceptibility to tick feeding and to tick-borne infection with LD spirochetes, and evaluated local and systemic immune responses.

Here we show that (i) *H. polygyrus* and *I. ricinus* frequently parasitize wild *Apodemus* mice in Berlin; (ii) local anti-*I. ricinus* immune responses in laboratory house mice are not altered by a concurrent nematode infection, despite the induction of extraordinarily high systemic Th2 responses by nematode/tick coinfections; (iii) the nematode infection does not alter the feeding success of pathogen-free tick larvae and spirochete-infected nymphs or the development of partial immunity toward repeated tick infestations; and (iv) the nematode coinfection does not affect the tick-borne transmission, replication, and dissemination of *B. afzelii* spirochetes in house mice.

MATERIALS AND METHODS

Wild rodent trapping. Wild rodents were trapped at two urban study sites (Moabit, Steglitz) and two periurban study sites (Gatow, Tegel) in Berlin, Germany, in November 2010 and from April to November 2011 (license number G0210/10, Landesamt für Gesundheit und Soziales, Berlin, Germany). The sites, trapping method, and type of euthanasia used were described by J. Krücken, J. Blümke, D. Maaz, J. Demeler, S. Ramünke, D. Antolová, R. Schaper, and G. von Samson-Himmelstjerna (submitted for publication). After the animals were transferred to the laboratory, the fur was screened for ticks under a stereomicroscope. Subsequently, a complete necropsy was performed, the gastrointestinal tract was thoroughly screened for *Heligmosomoides* spp., and any *Heligmosomoides* organisms detected were stored in 80% ethanol. The remaining carcass was placed on metal grids over water in screw-top jars to allow detachment of the remaining ticks. The water and the rodent body were examined during the following week. Ticks were stored in 70% ethanol. The parasite species were determined microscopically according to the morphological literature for nematodes (29) and ticks (30–32).

Laboratory mice, parasites, and *Borrelia*. Six- to 8-week-old specific-pathogen-free (SPF) female C57BL/6JRj, BALB/cJRj, and C3H/HeNRj mice (Janvier, France) were used for the experiments. The trichostrongyloid nematode *H. polygyrus* was maintained by serial passage in C57BL/6 mice. Specific-pathogen-free larval *I. ricinus* ticks were obtained from batches of eggs from two adult females after they had fed on laboratory beagle dogs (license number H0078/10, Landesamt für Gesundheit und Soziales, Berlin, Germany). The absence of tick-borne pathogens in the tick larvae used for experimentation was surveyed by published PCRs (33, 34) for the detection of a 153-bp fragment of the *hbb* gene of *Borrelia burgdorferi sensu lato*, a 203-bp fragment of the *gltA* gene of spotted fever *Rickettsia* spp., a 602- to 639-bp fragment of the 18S rRNA gene of piroplasmida (33), and a 257-bp fragment of the 16S rRNA gene of the *Anaplasmataceae* (34) in DNA isolated (33) from subsets of 20 larvae randomly chosen from each tick batch. *Borrelia afzelii*-infected *I. ricinus* nymphs were derived from larvae that had engorged on experimentally infected mice (license number 23-2347-A-24-1-2010, Landesamt für Umwelt, Gesundheit, und Verbraucherschutz, Potsdam, Germany).

Animal experimentation. Mice were infected with 250 *H. polygyrus* L3 larvae via oral gavage. In an initial experiment, mice of three strains (C57BL/6, BALB/c, and C3H) were infested with *I. ricinus* larvae on day 15 after *H. polygyrus* infection and dissected 20 days later. To survey the feeding success of larval ticks and the antilarval tick immune responses, C57BL/6 mice were infected with *H. polygyrus* and subsequently repeatedly infested with 50 specific-pathogen-free *I. ricinus* larvae by biweekly tick infestations on days 4, 18, and 34 postinfection (p.i.) in order to cover the early phase of local Th2 reactivity (at approximately day 6 p.i.), the phase of maximal Th2 reactivity (at about day 21 p.i.), and the chronic phase of infection with a declining magnitude of the nematode-induced Th2 reaction (at approximately day 35 p.i.). Mice exposed either to nematodes or to ticks and naive mice served as controls. To investigate the effect of *H. polygyrus* on the control of a tick-borne pathogen, nematode-infected and -free groups were infested with 4 to 6 *B. afzelii*-infected *I. ricinus* nymphs on day 16 after *H. polygyrus* infection in order to challenge the mice with *B. afzelii* at the peak of nematode-induced Th2 reactivity. Age-matched naive mice served as controls. During the period of tick infestation, all mice, including the naive controls, wore a collar to prevent removal of the ticks placed in the head region. Mice were housed individually at 80% humidity in filter-topped cages in ventilated cabinets. To optimize the recovery of detached ticks, the mice were kept on metal grids over water, and food was restricted to 3 g per day during tick feeding. Mice received water *ad libitum*. In several experiments, mice were anesthetized with isoflurane once a week for retroorbital blood collection with hematocrit capillary tubes. To assess the feeding success of the ticks, *I. ricinus* larvae or nymphs detaching from hosts were collected from the water and counted twice a day, pooled for every mouse in a screen-capped glass vial, and transferred to a desiccator with supersaturated $MgSO_4$ solution. The feeding duration was calculated as the mean recovery time of engorged ticks. Seven days after all ticks had detached, the engorgement weight of the pooled ticks was determined using a microscale, and the mean weight was calculated. The molting rates of fed larvae from the 1st and 2nd infestations or of fed nymphs were determined 3 months after infestation. All animal experiments were approved by and conducted in accordance with the guidelines of the appropriate committee (Landesamt für Gesundheit und Soziales, Berlin, Germany) under license number G0282/12.

Preparation of nematode, tick, and spirochete Ags. *H. polygyrus* antigen (Ag) was prepared as described previously (28). Antigen was acquired from specific-pathogen-free feeding *I. ricinus* larvae 48 h after attachment to C57BL/6 mice at a time of intensive saliva production. The ticks were removed from the host, washed in sterile phosphate-buffered saline (PBS), homogenized, and sonicated (3 times for 10 s each time, 60 W) in PBS on ice, and the sonicate was centrifuged at $20,000 \times g$ for 10 min at 4°C. The supernatant was sterile filtered through 0.22-μm-pore-size filters (Millipore, USA). To acquire *B. afzelii* antigen, spirochetes cultured in Barbour-Stoenner-Kelly H medium (Sigma-Aldrich, Germany) supplemented with 6% rabbit serum were washed three times by centrifugation at $10,000 \times g$ and 4°C for 30 min each time in PBS containing 5 mM $MgCl_2$ and sonicated on ice (8 times for 15 s each time), and the sonicate was centrifuged at $10,000 \times g$ at 4°C for 30 min. The protein contents of the supernatants were determined by use of a bicinchoninic acid protein assay kit (Pierce, USA). The antigens were stored at −80°C until use.

Cell culture. Splenocytes and cells were isolated from the gut-draining and head-skin-draining lymph nodes of euthanized mice by passing the organs through a 70-μm-mesh-size cell strainer (BD, USA). The cells were cultured as triplicates of 3.5×10^5 to 5×10^5 cells in 200 μl RPMI 1640 medium (PanBiotech, Germany) containing 10% fetal calf serum, 20 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cultures were stimulated with 40 μg/ml worm or tick Ag, 30 μg/ml *B. afzelii* Ag (all for 72 h), or 1 μg/ml anti-CD3 and anti-CD28 (BD, USA) for 48 h

at 37°C in a 5% CO_2 atmosphere. The supernatants were stored at −20°C for cytokine detection.

Immunohistology. Formalin-fixed tissue samples were dehydrated and embedded in paraffin. Tibiotarsal joints were decalcified before they were embedded in paraffin. The tissues in the paraffin sections (1 to 2 μm) were dewaxed, hydrated, and histochemically stained with hematoxylin and eosin (Merck) to obtain an overview, toluidine blue to observe mast cells, and a modified Sirius red staining protocol to observe eosinophil granulocytes (35). Sections were stained with toluidine blue (Sigma) for 10 min, washed with distilled water, and dehydrated by dipping them in 1% acetic acid and 100% ethanol. Sections were cleared in xylol, and coverslips were mounted with Corbit balsam (Hecht). For staining of eosinophils, sections were stained for 1 min with hematoxylin (Merck), washed with tap water, and dehydrated with 100% ethanol before staining with direct red 80 dye (Sigma) for 2 h. Sections were washed with tap water, dehydrated with 100% ethanol, and cleared in xylol before coverslips were mounted with Corbit balsam. Images were acquired using a fluorescence microscope (AxioImager Z1) equipped with a charge-coupled-device camera (AxioCam MRm) and processed with Axiovision software (Carl Zeiss AG, Germany).

ELISA. Culture supernatants were screened by enzyme-linked immunosorbent assay (ELISA) for IFN-γ, IL-13 (Ready-Set-Go! ELISA; eBioscience, USA), and IL-10 (BD, USA) according to the manufacturer's instructions.

Flow cytometry. Blood samples were placed in PBS with 0.2% bovine serum albumin (BSA) and 5 mM EDTA, and cells were stained with anti-CD4–fluorescein isothiocyanate (clone RM4-5; eBioscience, USA) and fixable viability dye eFluor 780 (eBioscience, USA) for dead cell exclusion. Red blood cells were lysed in fluorescence-activated cell sorting (FACS) lysing solution (BD, USA), before leukocytes were stained intracellularly with anti-Foxp3–eFluor 450 (clone FJK-16S; eBioscience, USA), anti-GATA-3–eFluor 660 (clone TWAJ; eBioscience, USA), and anti-T-bet–phycoerythrin (PE) (clone eBio4B10; eBioscience, USA) using a fixation/permeabilization buffer kit (eBioscience, USA). Cells from the spleen and lymph nodes were labeled with anti-CD4–peridinin chlorophyll protein–eFluor 710 (clone GK1.5; eBioscience, USA) and fixable viability dye eFluor 780 (eBioscience, USA) in PBS with 0.2% BSA. Transcription factors were stained as described above for the white blood cells. For the intracellular detection of cytokines, cells were incubated with 1 μg/ml phorbol myristate acetate (PMA) and ionomycin for 30 min and 5 μg/ml brefeldin A (Sigma, Germany) for another 2.5 h. Subsequently, they were stained with anti-IFN-γ–eFluor 450 (clone XMG1.2; eBioscience, USA), anti-IL-13–Alexa Fluor 647 (clone eBio13A; eBioscience, USA), anti-IL-10–PE (clone JES5-16E3; eBioscience, USA), and anti-IL-17–Alexa Fluor 488 (clone TC11-18H10; BD, USA). Cells were acquired using LSRII and Canto II flow cytometers (BD, USA) and analyzed using FlowJo (version 8.8.7) software (TreeStar, USA).

Spirochete quantification. For quantification of spirochetes in mouse organs in relation to tissue weight, a touchdown quantitative PCR (qPCR) assay for absolute quantification was developed, and λ phage genome copies were used as an external standard. For DNA isolation, 80 ± 1 mg of head skin and 70 ± 1 mg of heart were sampled with sterile scalpel blades, and the skinned left tibiotarsal joint (weight range, 32.6 to 39.1 mg) was obtained using clean scissors. Prior to DNA isolation with a FastDNA Spin kit with Lysing Matrix A (MP Biomedicals, France), 10^4 copies of the 48,502-bp λ phage genome (Thermo Fisher Scientific, USA) per mg head skin or heart and 10^5 copies per tibiotarsal joint were added as an external standard for tissue weight. To calculate copy numbers, the DNA concentration (0.3 μg/μl, measured spectrophotometrically) and the molecular weight of the genome were used. Samples were homogenized with a FastPrep 24 kit (MP Biomedicals, France) 4 times for 30 s each time at 6.0 m/s and cooled on ice between homogenization steps. Variations in the amount of DNA in the samples used in the qPCR have a major impact on the accuracy of absolute quantification, since qPCR efficiency is decreased with higher DNA concentrations. As common spectrophotometric DNA quantification is strongly impacted by contamination with salts and proteins, a more precise fluoroscopic DNA quantification assay for fluorescence measurement was developed using LCGreen Plus 10× dye (BioChem, USA), exclusive binding on double-stranded DNA (dsDNA), and a LightCycler 480 II system (Roche Molecular Diagnostics, USA). Accurate DNA quantification over the range of 1 to 100 ng was obtained. DNA samples, standards (20, 40, 60, 80, and 100 ng λ phage DNA), and water controls were prepared in duplicate in a 10-μl assay volume for each well in 96-well plates. After vigorous vortexing of the sealed plates, samples were centrifuged and fluorescence was measured by the LightCycler 480 II system after 2 min of incubation at 25°C during a short heating from 25 to 26°C with 100 acquisitions per degree Celsius. For extrapolation of the dsDNA quantity in the samples, a calibration curve was calculated from the fluorescence values of the standards using a 4-parameter logistic regression model. Two real-time PCR assays targeting a 153-bp fragment of the *hbb* gene of LD spirochetes and a 158-bp fragment of the λ phage genome were conducted with the LightCycler 480 II system using LightCycler 480 SYBR green I Master mix (Roche Molecular Diagnostics, USA), 0.5 μM each primer, and exactly 100 ng sample DNA in a 20-μl volume. The primers used for the *hbb* fragment are published elsewhere (36), and the forward and reverse primers used for the λ phage genome were 5′-TTTTGATTTTGCTGTTTCAA-3′ and 5′-ACCTTTTCCATGA ATTGGTA-3′, respectively. All PCRs were performed in duplicate and included a negative control and standards, each with 100 ng target-free mouse DNA from the respective organ. The standards consisted of a dilution series of 10^4 to 10^1 and 3 copies of a plasmid carrying the *hbb* gene standard or λ phage DNA, respectively. The thermocycling conditions consisted of an initial denaturation step at 95°C for 5 min, followed by 50 cycles of 95°C for 5 s, annealing for 30 s (at an initial temperature of 62°C and then a decrease in the temperature by 0.25°C/cycle to 57°C), and 72°C for 15 s. Touchdown amplification was performed to increase the binding specificity of the primers during the first cycles. After amplification, a high-resolution melting step consisting of 95°C for 5 s, 40°C for 1 min, and progressive heating from 65 to 95°C with 20 acquisitions per degree Celsius was performed to identify target fragments by melting temperature in combination with their fragment size in subsequent agarose gel electrophoresis. The numbers of *Borrelia hbb* copies extrapolated from the standard curve were multiplied by the ratio of the extrapolated λ phage DNA copy numbers in the sample and the number of copies previously added per milligram of skin/heart or per tibiotarsal joint. The detection limit was less than 3 copies, and the quantification limit was 5 to 10 copies per 100 ng DNA for both touchdown qPCR assays.

Statistics. The confidence intervals (CIs) of the prevalences were calculated as Wilson score intervals using R Statistics software and the PlotCIs package. Negative binomial regression of tick numbers was performed using R Statistics software and the MASS package to estimate the influence of the variable *Heligmosomoides* sp. count and the categorical variables mouse species (*Apodemus flavicollis* and *Apodemus sylvaticus*) and trapping location (Gatow, Moabit, Steglitz, and Tegel) on the number of *I. ricinus* ticks on wild mice.

GraphPad Prism (version 5.01) software (San Diego, CA, USA) was used for the plotting and statistical analysis of the experimental infection data. The data were tested for statistically significant differences using an unpaired *t* test, one-way analysis of variance (ANOVA) with a Bonferroni-corrected posttest (see Fig. 3 and 5), and two-way-ANOVA (see Fig. 2) under the assumption of a normal distribution of the FACS fluorescence data, ELISA optical density data, and the majority of the experimental parasite infection data. The posttests following the two-way ANOVA for individual pairs using the Bonferroni correction were performed with the posttest calculator (GraphPad Software). The number of engorged tick nymphs and the molting rate in Fig. 7A and D, respectively, and the cell counts in Fig. S1B in the supplemental material were not normally distributed, and differences were tested using the Mann-Whitney U test. Differences with *P* values below 0.05 were considered to be significant.

TABLE 1 Numbers of wild *Apodemus* (*Sylvaemus*) mouse species trapped at the different study sites and *I. ricinus* and *H. polygyrus* infection parameters[a]

	No. of mice trapped		*Ixodes ricinus* (larvae and nymphs) infection parameters				*H. polygyrus* infection parameters			
			Prevalence		Intensity		Prevalence		Intensity	
Trapping location	*Apodemus flavicollis*	*Apodemus sylvaticus*	% of specimens	95% CI	Mean	Range	% of specimens	95% CI	Mean	Range
Gatow	33		81.8	65.6–91.4	15.7	1–52	9.1	3.1–23.6	2.7	1–6
Tegel	22		54.5	34.7–73.1	3.8	1–9	40.9	23.3–61.3	24.1	1–200
Steglitz	27		51.9	34.0–69.3	8.9	1–59	66.7	47.8–81.4	7.6	1–52
Moabit		25	24.0	11.5–43.4	2.5	1–6	84.0	65.4–93.6	29.2	1–246
Total[b]	82	25	55.1	45.7–64.2	10.3	1–59	47.7	38.5–57.0	19.1	1–246

[a] Prevalences (in percent with 95% CIs) and intensities (mean number of parasites per infected/infested mouse and range) are shown for *H. polygyrus* and subadult live stages of *I. ricinus*.
[b] Total, the sums of the number of trapped mice per species as well as the infection parameters for all trapped mice ($n = 107$).

RESULTS

Nematode/tick coinfection is frequent in urban and periurban wild mice. A total of 107 potential murine hosts of a *Heligmosomoides* sp. representing 82 yellow-necked mice (*Apodemus flavicollis*) and 25 long-tailed wood mice (*A. sylvaticus*) were trapped alive at 2 urban sites (Moabit, Steglitz) and 2 periurban sites (Gatow, Tegel) in Berlin and examined for their parasite burden. No house mice (*Mus musculus*) were caught, as the traps were placed exclusively outside buildings. Both species frequently hosted *H. polygyrus* (47.7%; 95% CI, 38.5 to 57.0%; $n = 107$) with intensities of from 1 to 246 specimens (Table 1). In addition, 55.1% (95% CI, 45.7 to 64.2%) were infested by up to 59 subadult *I. ricinus* ticks (92.1% larvae, 7.9% nymphs). A high percentage (41.3%; 95% CI, 29.1 to 53.4%) of tick-infested mice ($n = 59$) were simultaneously infected by *H. polygyrus*. A negative binomial regression model including all 107 mice showed an important influence of the trapping location and/or the rodent species on the number of *I. ricinus* ticks on the rodents but could not distinguish between the two variables because of total colinearity (Fig. 1). *A. sylvaticus* mice were exclusively trapped in Moabit, whereas *A. flavicollis* occurred only at the three other locations. *A. flavicollis* mice from Gatow were infested with significantly more ticks than those from Steglitz ($P < 0.05$) and Tegel ($P < 0.001$) and *A. sylvaticus* mice from Moabit ($P < 0.001$). Although the difference was not significant ($P = 0.15$), the number of *Heligmosomoides* organisms showed a negative trend versus the number of ticks, with every nematode reducing the number of ticks by 2.4% (95% CI, −6.6% [decrease] to +0.17% [increase]). Likelihood ratio tests confirmed that the negative binomial model was more appropriate than a Poisson model ($P < 0.001$) and a significant improvement over the null model without coefficients ($P < 0.001$). In order to assess a possible influence of a nematode infection on the parameters of tick infestation, host development of antitick immunity, and dissemination of tick-borne pathogens, we established an experimental nematode/tick coinfection model under defined laboratory conditions with house mice.

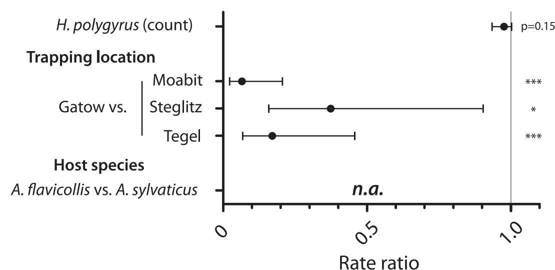

FIG 1 Incident rate ratios with 95% CIs for risk factors affecting the number of host-associated *Ixodes ricinus* ticks on wild mice. A negative binomial regression for the *I. ricinus* count (larvae and nymphs) as the dependent variable was performed using the *H. polygyrus* worm count and the categorical factors trapping location with three dummy variables and host species as independent variables. Reference categories for the trapping location and host species were Gatow and *A. flavicollis*, respectively. *, $P < 0.05$; ***, $P < 0.001$; n.a., not applicable because of total colinearity with the trapping location. Data are for 107 wild mice.

Nematode/tick coinfection leads to highly polarized Th2 responses in inbred mouse strains. To compare the spectrum of Th2 reactivity against ticks and nematodes depending on the host's genetic background, mice of three inbred strains with reported differences in immune reactivity (13, 37, 38) were either infested with *I. ricinus* larvae alone or infested with *I. ricinus* larvae and coinfected with the nematode *H. polygyrus* (Fig. 2A). Systemic Th2 responses in spleens were characterized by flow cytometry 20 days after *I. ricinus* infestation on the basis of cytokine (IL-13) production and expression of the lineage transcription factor of Th2 cells, GATA-3 (Fig. 2B). Coinfected mice of all strains exhibited elevated Th2 responses compared to the responses of the controls infested only with ticks. C57BL/6 mice produced more splenic IL-13-producing ($IL\text{-}13^+$) $CD4^+$ cells and exhibited higher frequencies of GATA-3-expressing ($GATA\text{-}3^+$) $CD4^+$ cells in response to coinfection or infestation with ticks only than BALB/c and C3H mice. Following the hypothesis that a strong Th2 response during nematode/tick coinfection might affect the feeding success of ticks and the control of tick-borne pathogens, C57BL/6 mice were chosen for subsequent experiments.

The frequencies of Th2 cells peak during acute nematode and tick coinfection. To evaluate the effects of a nematode infection on antitick immune responses, we next determined the kinetics of the systemic Th2 and regulatory T cell responses provoked by the nematode infection, repeated tick infestations, and a coinfection regimen with repeated tick feeding. We chose an infective dose of 250 *H. polygyrus* L3-stage larvae and 50 *I. ricinus* larvae to reflect the parasite burdens also detectable in wild mice (Table 1).

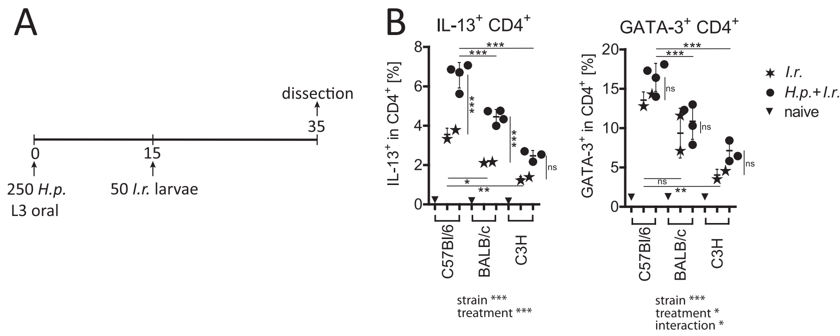

FIG 2 Comparison of systemic Th2 responses to tick and nematode/tick coinfections in three inbred mouse strains. (A) Experimental setup showing the time line (in days) of exposure to *H. polygyrus* (*H.p.*) and to *I. ricinus* (*I.r.*) larvae. (B) Frequencies (mean ± SD) of splenic IL-13$^+$ CD4$^+$ T cells in response to PMA-ionomycin stimulation (left) and GATA-3$^+$ CD4$^+$ cells (right) detected by flow cytometry. Values of significance determined by two-way ANOVA are shown below the graph. Values of significance determined by individual Bonferroni-corrected pairwise posttests are depicted with horizontal lines for comparisons between mouse strains (lines at the top are for mice coinfected with *H. polygyrus* and *I. ricinus*; lines at the bottom are for mice infested with *I. ricinus*) and vertical lines for comparison between treatments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. Data are for 1 to 4 mice per group.

C57BL/6 mice were either infested with specific-pathogen-free *I. ricinus* larvae three times at biweekly intervals or first infected with *H. polygyrus* and subsequently infested three times with *I. ricinus* starting on day 4 after nematode infection (Fig. 3A), a regimen chosen to cover the early, acute, and chronic phases of nematode infection reflecting expanding, maximal, and declining nematode-driven Th2 reactivity, respectively. Mice infected only with *H. polygyrus* and naive littermates served as controls. To survey the development of systemic Th2 and regulatory responses, blood was sampled once a week and GATA-3 and Foxp3 protein expression by CD4$^+$ T cells was monitored (Fig. 3B). Infection with *H. polygyrus* alone led to the typical pattern of a strong systemic Th2 response during the acute phase of infection and the subsequent decline of Th2 cell levels to near the background levels in the chronic phase of infection (Fig. 3C), despite the presence of high worm burdens at the end of the experiment on day 41 p.i. (Fig. 3E). Tick infestations resulted in strong systemic Th2 responses of similar magnitudes after each infestation and a subsequent decline in Th2 cell frequencies (Fig. 3C). In mice coinfected with both parasites, systemic Th2 cells were substantially more numerous, roughly reflecting the sum of the Th2 cell populations detected in controls infected/infested with a single pathogen. Interestingly, the suppression of nematode-elicited systemic immune responses in the chronic phase of *H. polygyrus* infection at day 41 did not spill over to tick-induced responses, as GATA-3 cells expanded systemically in response to the third tick infestation with a magnitude similar to that in the controls infested with ticks only (Fig. 3C). Infections with *H. polygyrus* activate Foxp3-expressing regulatory T cells (Tregs), which control the developing Th2 response (39–41). The frequencies of Foxp3$^+$ Tregs in the peripheral blood of mice exposed only to *H. polygyrus* or only to *I. ricinus* were not significantly altered compared to those in the peripheral blood of the naive controls (Fig. 3D). In coinfected mice, however, the dominant increase in GATA-3-expressing Th2 cells led to a reciprocal transient decline in the frequencies of Tregs detected in peripheral blood. Hence, we found that systemic Th2 responses were greatly increased in mice that were acutely infected by intestinal nematodes and concomitantly infested by ticks.

Th2 cytokine responses to ticks are not affected by concurrent nematode infection. We next analyzed whether systemic and local cytokine responses to repeated tick infestations were affected by a concurrent nematode infection. For this purpose, mice were dissected 1 day after the last *I. ricinus* larva of the third infestation had detached, and cells from spleen and local skin-draining lymph nodes (SLNs) were harvested and stimulated for cytokine production. As expected, the frequencies of splenic CD4$^+$ T cells producing IL-13 in response to unspecific PMA-ionomycin-induced stimulation were the highest in coinfected mice (Fig. 4A). In response to larval tick antigen, however, similar levels of IL-13 were detected in cultures of splenocytes from coinfected mice and control mice infested only with ticks, a pattern also reflected by anti-CD3/anti-CD28 antibody-induced stimulation (Fig. 4A and not shown). The tick-specific local IL-13 responses of cells from cervical and axillary lymph nodes draining the head and neck skin region were also equivalent (Fig. 4A). A similar pattern was detected for IL-10: the highest frequencies of IL-10$^+$ CD4$^+$ cells were detected after unspecific activation of spleen cells from coinfected/infested mice, and systemic and local *I. ricinus*-specific IL-10 responses were equal in both tick-infested groups (Fig. 4B). Splenocytes from coinfected mice contained the highest proportion of CD4$^+$ cells responding to PMA-ionomycin with IFN-γ production; however, IFN-γ-positive (IFN-γ$^+$) responses to tick antigen in spleen cells were close to the detection limit of the assay and similar in spleen and SLNs for both tick-infested groups (Fig. 4C). Because Th2-associated innate effector cells contribute to immunity against ticks (42, 43), we compared the mast cell and eosinophil numbers of tick-infested and *H. polygyrus*-infected mice by histological analyses of the skin region affected by repeated tick feeding. They did not vary between the tick-infested experimental groups (see Fig. S1 in the supplemental material). Taken together, mice coinfected with nematodes and ticks develop very strong systemic Th2 responses, while local antitick immune responses are unaltered by an infection with enteric nematodes.

Tick feeding success is not affected by concurrent *H. polygyrus* infection. In line with the unaltered tick-specific local cytokine responses in coinfected mice, the parameters of the success of tick feeding and subsequent molting were unaffected by the presence of *H. polygyrus* (Fig. 5). During successive infestations, the number of successfully engorged larval ticks and their feeding duration did not differ among the single infection and coinfection groups. Although ticks feeding on nematode-infected hosts dur-

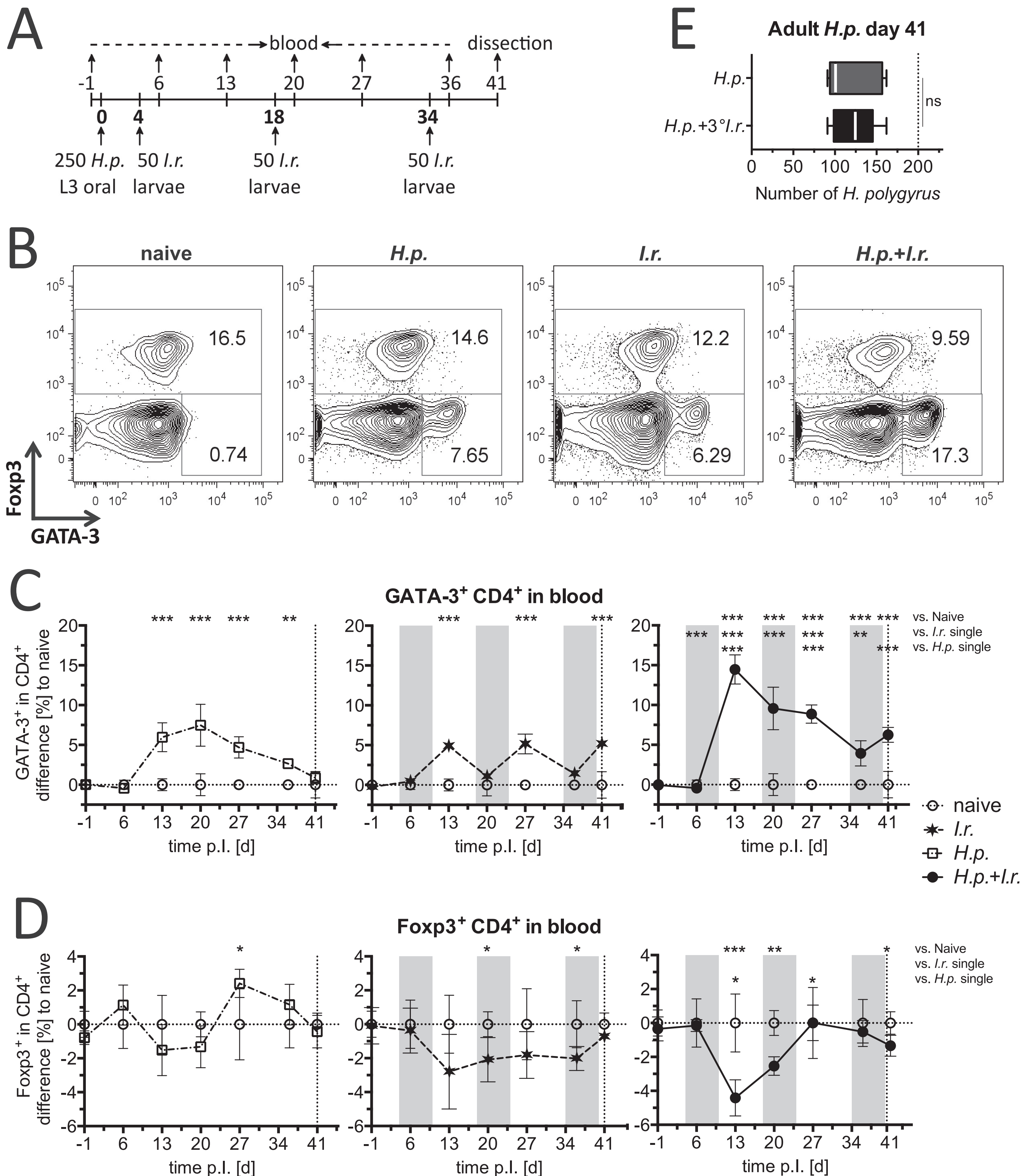

FIG 3 Kinetics of GATA-3$^+$ CD4$^+$ Th2 cells from C57BL/6 mice during infestation with *I. ricinus* ticks alone and during infestation with *I. ricinus* ticks and coinfection with *H. polygyrus*. (A) Experimental setup showing the time line (in days) of exposure to *H. polygyrus* (*H.p.*) and to *I. ricinus* (*I.r.*) larvae and blood collection. (B) Representative flow cytometry density plots of peripheral blood CD4$^+$ cells stained for Foxp3 and GATA-3 on day 13. (C, D) Frequencies of GATA-3$^+$ (C) and Foxp3$^+$ (D) CD4$^+$ cells (mean ± SD) in the peripheral blood of *H. polygyrus*-infected mice (left), *I. ricinus*-infested mice (middle), and *H. polygyrus*- and *I. ricinus*-coinfected mice (right) normalized to those in the peripheral blood of naive controls. Gray shading and vertical lines, period of larval tick feeding and the day of dissection, respectively. One-way ANOVAs with a Bonferroni-corrected pairwise *t* test were performed for every time point in the kinetic analysis. d, day. (E) Number of adult *H. polygyrus* worms isolated from small intestines on day 41. The box plot shows the medians and quartiles, with whiskers indicating 95% CIs. Differences were tested using an unpaired *t* test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. Data are for 5 or 6 mice per group.

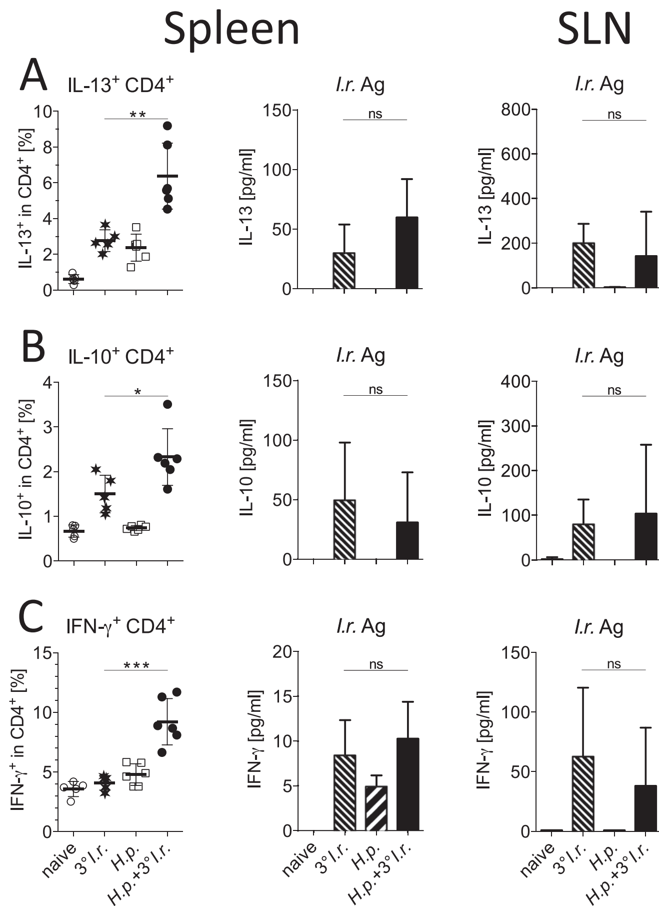

FIG 4 Systemic and local cytokine responses of mice infected with *H. polygyrus* and *I. ricinus*. The production of IL-13 (A), IL-10 (B), and IFN-γ (C) was measured in spleen CD4$^+$ T cells by flow cytometry after intracellular cytokine staining following PMA-ionomycin stimulation (left) and in bulk culture supernatants of spleen and SLN cells by ELISA after specific stimulation with larval *I. ricinus* antigen (middle and right, respectively). Means and SDs are shown. Symbols represent the intracellular cytokine responses detected in cells from individual mice. Differences between groups were tested using an unpaired *t* test. *, $P < 0.05$; ** $P < 0.01$; ***, $P < 0.001$; ns, not significant. Data are for 5 or 6 mice per group.

ing the 1st infestation were significantly lighter than ticks feeding on nematode-free mice, no such difference was observed during subsequent infestations. The effect of partial immunity against larval ticks after repeated feeding was also similar in both groups when the detachment rates of engorged ticks of the primary and tertiary infestations, their feeding durations, and their weights were compared (Fig. 5). The rates of molting to the nymphal stage did not differ for ticks feeding on worm-free and worm-infected hosts after the primary and secondary infestations. Ticks from the tertiary infestations, unfortunately, succumbed to fungal growth. Hence, we detected a partial protection of laboratory house mice against repeated tick feeding which was unaffected by a concurrent nematode infection.

Concurrent *H. polygyrus* infection does not significantly affect Th1 responses to tick-borne *B. afzelii* infection. Next, we examined whether increased systemic Th2 responses in mice coinfected with ticks and intestinal nematodes might inhibit Th1 responses against tick-borne spirochetes. *Borrelia afzelii*-infected *I. ricinus* nymphs were permitted to feed on mice infected with *H. polygyrus* and naive controls. As the maximum systemic Th2 response, based on peripheral blood Th2 cell frequencies, occurred in the second week of *H. polygyrus* infections (Fig. 3C), we chose day 16 after nematode infection for tick attachment (Fig. 6A). High frequencies of GATA-3$^+$ Th2 cells in the peripheral blood briefly before and during tick feeding were confirmed (Fig. 6B). Mice were dissected 19 days after *I. ricinus* infestation, when *B. afzelii* dissemination in the natural rodent hosts results in maximum infectivity for feeding tick larvae (3). Upon PMA-ionomycin-induced stimulation, spleen cells from mice coinfected with nematodes and ticks again responded with the highest frequencies of CD4$^+$ cells producing IL-13, IL-10, and IFN-γ (Fig. 6C to E). A similar response to PMA-ionomycin-induced stimulation was observed for local CD4$^+$ T cells derived from SLNs (Fig. 6C to E). To survey antispirochete responses, bulk cultures were stimulated with *B. afzelii* antigen. Surprisingly, low levels of spirochete-specific IL-13 production by splenocytes but not by SLN cells were detectable in both groups of mice infested with *B. afzelii*-infected ticks (Fig. 6C). Splenocytes from mice infested only with *Borrelia*-infected ticks produced high levels of IL-10 in response to spirochete antigen, but this response was significantly suppressed in spleen cells derived from mice coinfected with nematodes and

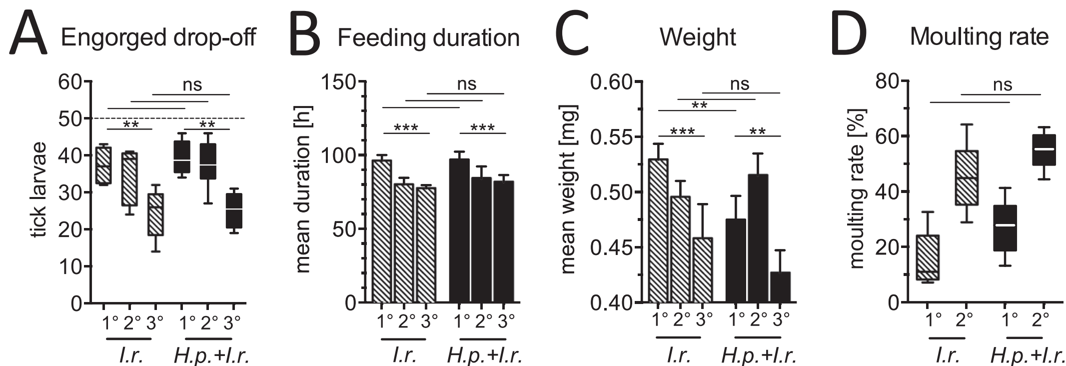

FIG 5 Feeding success of *I. ricinus* larvae on mice with and without *H. polygyrus* coinfection. (A and D) The number of engorged larvae recovered after they dropped off the mice (A) and the rates of molting to the nymph stage by engorged larvae (D) are depicted as box plots (medians and quartiles, with whiskers representing 95% CIs). (B and C) The feeding duration (B) and tick weight after detachment (C) are shown as means ± SDs. The dotted line in panel A depicts the total number of ticks applied to individual mice. Asterisks indicate the significance values obtained by Bonferroni-corrected pairwise posttests following one-way ANOVA. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.001$; ns, not significant. Data are for 5 or 6 mice per group.

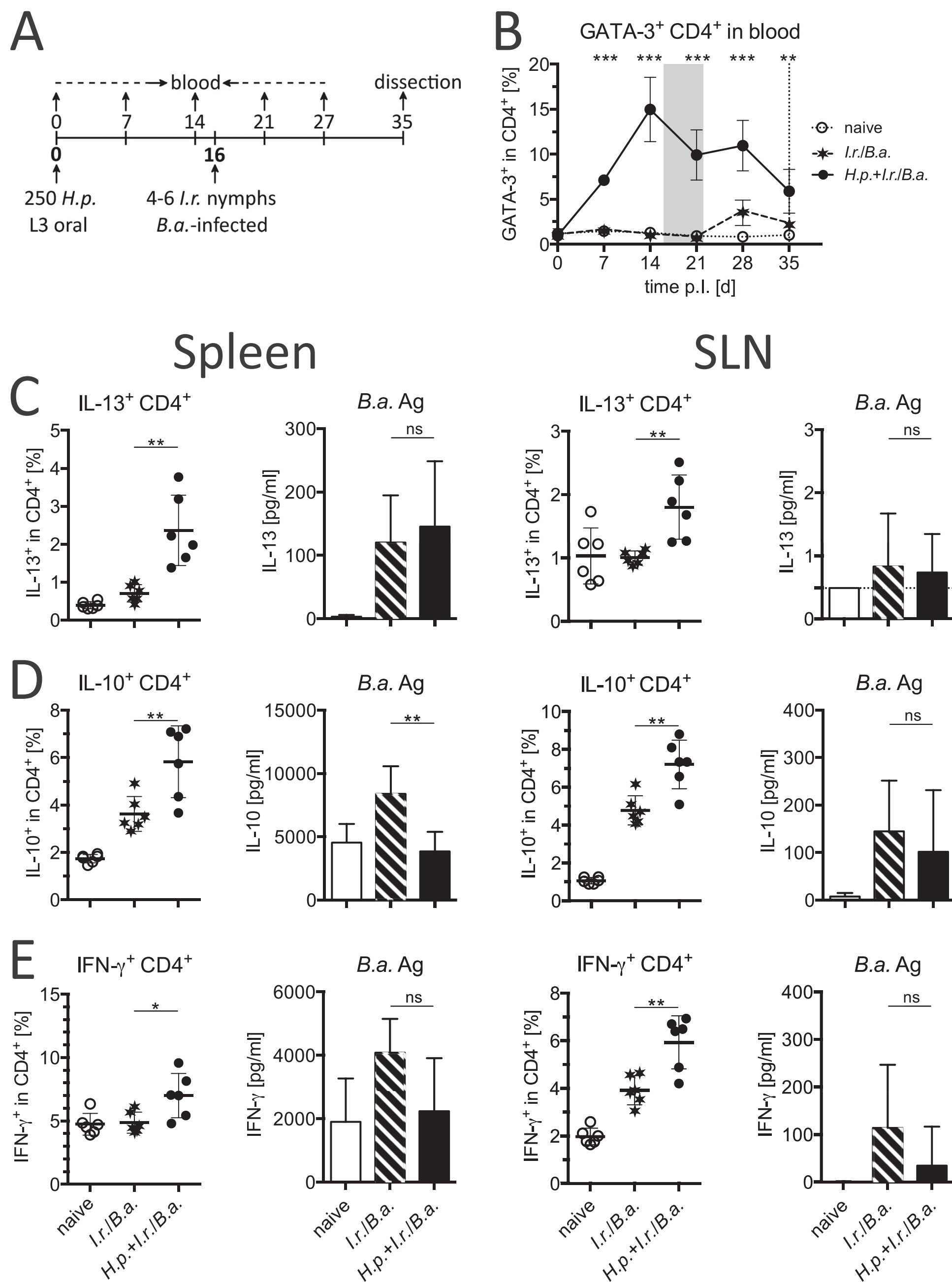

FIG 6 Systemic and local immune responses in mice coinfected with *H. polygyrus* and *I. ricinus*-transmitted *B. afzelii* (*B. a.*). (A) Experimental setup showing the time line (in days) of exposure to parasites and blood sampling. (B) Kinetics of GATA-3$^+$ CD4$^+$ cell frequencies (mean ± SD) in peripheral blood. Gray shading and the vertical line, period of nymphal tick feeding and the day of dissection, respectively. Asterisks indicate significant differences between the coinfected group and the group infested with *I. ricinus* only for every time point. (C to E) Production of IL-13 (C), IL-10 (D), and IFN-γ (E) by spleen and SLN CD4$^+$ T cells stimulated with PMA-ionomycin and bulk cultures stimulated with *B. afzelii* antigen. Means and SDs are shown. Symbols represent the intracellular cytokine responses detected in cells from individual mice. Differences between groups were analyzed using unpaired *t* tests. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. Data are for 6 mice per group.

ticks. In contrast, spirochete-specific IL-10 production by local SLN cells was unaffected by the presence of intestinal nematodes (Fig. 6D). Although the highest frequencies of CD4$^+$ IFN-γ$^+$ cells were detected in the spleens and SLNs of coinfected mice, the *B. afzelii* antigen-specific responses of splenocytes and SLN cells appeared to be slightly but not significantly lower in mice coinfected with nematodes and ticks, which was also reflected by anti-CD3/anti-CD28 antibody stimulation (Fig. 6D and not shown). Thus, systemic and local *Borrelia*-specific Th1 reactivity is only mildly affected, if it is affected at all, by a concurrent intestinal nematode infection.

***H. polygyrus* infection does not affect the transmission or control of tick-borne *B. afzelii* infection.** Because coinfections with intestinal nematodes reduce the control of concomitant bac-

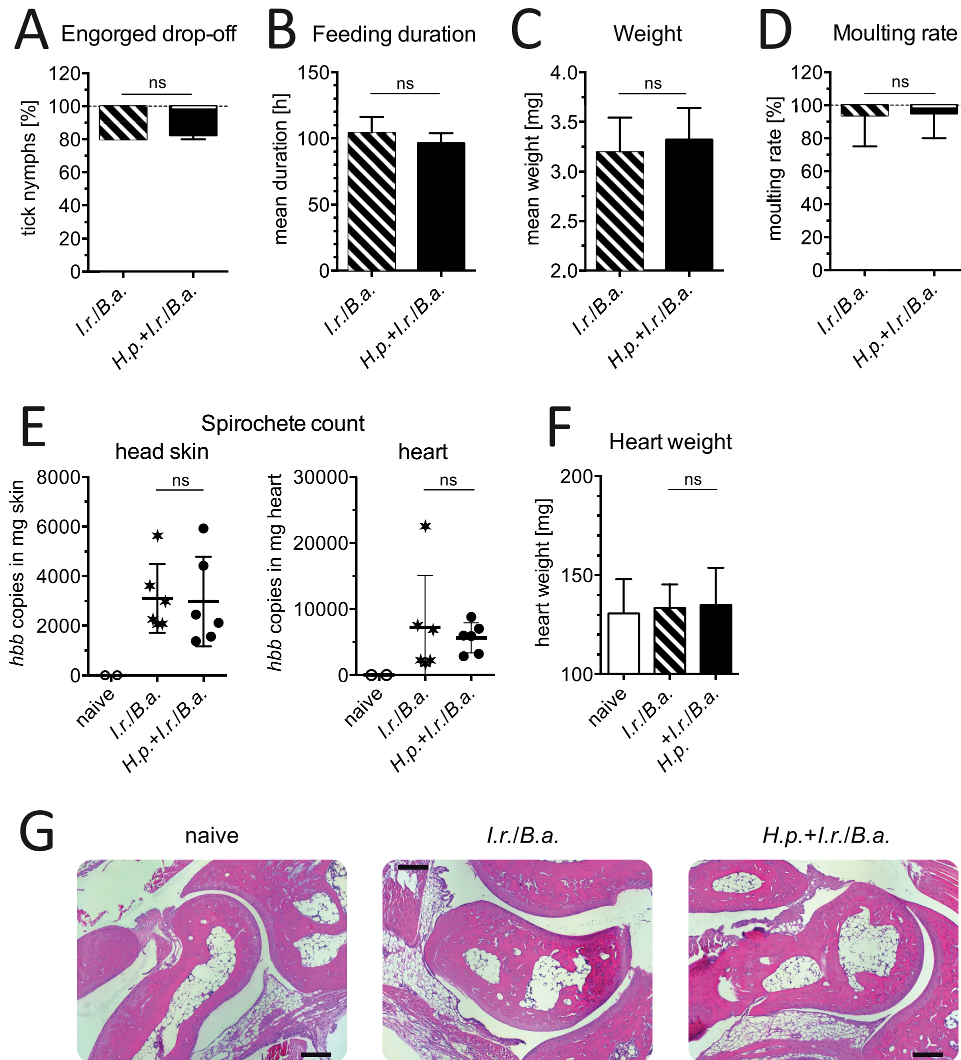

FIG 7 Feeding parameters, spirochete load in mouse tissues, and tibiotarsal joint histology after infection with *H. polygyrus* and tick-*B. afzelii* infection. (A and D) The number of engorged nymphs recovered after detachment (A) and rates of molting to adult ticks (D) are depicted as box plots (medians and quartiles, with whiskers representing 95% CIs). The dotted line in panel A depicts the total number of ticks applied to individual mice, and that in panel D depicts the total number of engorged ticks. (B and C) Feeding duration (B) and tick weight after detachment (C) are shown as means ± SDs. (E) Number (mean ± SD) of *B. afzelii* spirochetes per milligram of head skin (left) and heart (right), measured by absolute quantification of the number of copies of the *hbb* gene via touchdown qPCR. (F) Heart weight of mice (mean ± SD). (G) Representative cross sections from tibiotarsal joints stained with hematoxylin and eosin for histopathological scoring. Bar, 100 μm. Differences between groups were tested using unpaired *t* tests (B, C, E, F) or the Mann-Whitney U test (A, D). ns, not significant. Data are for 6 mice per group.

terial infections (22–24), we examined whether the transmission and dissemination of tick-borne LD spirochetes were affected in nematode-infected hosts. As was observed during primary infestation with larval *I. ricinus*, *H. polygyrus* did not affect the feeding success of *B. afzelii*-infected *I. ricinus* nymphs (Fig. 7A to D). Nearly all ticks engorged and subsequently molted successfully. We quantified the number of *B. afzelii* bacteria in the skin of the head, the heart, and tibiotarsal joint by applying a newly established touchdown real-time PCR assay quantifying spirochetes in relation to organ weight. All mice had acquired *B. afzelii* from the infected nymphal *I. ricinus* ticks. They harbored about 3,000 spirochetes per mg head skin and about 6,400 spirochetes per mg heart, while the spirochete numbers in the tibiotarsal joints were under the detection limit (Fig. 7E). Spirochete numbers were independent of the presence of *H. polygyrus* infections. All mice were free of clinical signs of Lyme disease, such as carditis or arthritis. The weight of the hearts of infected mice was comparable to that of the hearts of naive mice (Fig. 7F), and the low numbers of spirochetes that had disseminated to the joints did not lead to signs of pathological inflammation (Fig. 7G). Furthermore, food and water intake, mobility, and body weight were unaltered in *B. afzelii*-infected mice (data not shown). Taken together, we demonstrate that coinfection with an intestinal nematode fails to facilitate the proliferation and systemic dissemination of tick-borne Lyme disease spirochetes in house mice under experimentally controlled conditions.

DISCUSSION

Small rodents serve as reservoir hosts for several tick-borne pathogens in Europe, and the *I. ricinus* tick is the vector for the agents of

Lyme disease. Numerous mice and voles inhabit urban areas and thus constitute a considerable source for tick-borne pathogens in close proximity to humans. Hosts serving as competent reservoirs for tick-borne pathogens must permit repeated tick feeding, must be susceptible to the pathogen, and must become and remain infectious for feeding ticks for prolonged periods. Acquired immunity to repeated tick infestations, observed in some artificial hosts, concomitantly reduces the susceptibility to tick-borne pathogens and thus curtails the pathogens' transmission to a new vector (44–46). Currently, it is not known whether and to what extent coinfections affect the susceptibility of rodents to tick feeding and the competence of rodents as a reservoir for tick-borne pathogens. As nematode coinfections are highly prevalent in wild mice and the worm burdens can reach high levels (9–11), they might influence the success of pathogen transmission by repeated tick feeding and the immune control of tick-borne pathogens, such as *B. afzelii*. Thus, coinfections of rodents with macroparasites may increase the efficiency of transmission of tick-borne pathogens and thereby affect public health. In this study, we present epidemiological data derived from wild mice showing that coinfections with ticks and nematodes are very frequent and combine them with immunological data and parameters related to the competence of the rodent as a reservoir for LD spirochetes obtained from a defined experimental laboratory system. This system was chosen by consideration of the fact that factors likely to affect the surveyed parameters, such as preexposure to tick infestations and LD spirochetes, the time since spirochete infection, and the number of bites by infected ticks, were unknown for rodents trapped in the wild.

We analyzed wild mice captured in Berlin for the occurrence of a *Heligmosomoides* sp. and *I. ricinus* and found that both are frequent parasites in wild mice from urban and periurban sites in Berlin; i.e., 41.3% of tick-infested *Apodemus* mice were coinfected by the nematode. This is in line with the findings of a previous Italian field study reporting that 68% of tick-infested yellow-necked mice were coinfected with *H. polygyrus* (47). A regression analysis revealed that rodent species and the trapping location mainly affect the number of ticks on the rodents. Only a trend for a negative influence of the number of *Heligmosomoides* organisms on the tick count was observed, although it did not reach statistical significance. Ferrari et al. (47) reported that besides the trapping location and different host factors, *H. polygyrus* had a negative influence on the number of *I. ricinus* ticks infesting wild *A. flavicollis* mice. These statistical results suggest that the nematode infection may influence the tick infestation, but validation by use of an experimental infection under defined laboratory conditions was lacking. Hence, we examined experimentally whether concurrent nematode infections influence the development of (i) antitick immunity and (ii) the control of the tick-borne pathogen *B. afzelii*.

Antitick immunity is associated with the development of Th2 immune responses and is mainly accomplished by basophils, mast cells, and eosinophils, which are Th2-associated innate effector cells (13, 42, 48). In our experimental model, C57BL/6 mice acquired partial immunity against repeated tick infestations, detected as a significantly reduced number of larval ticks and a significantly reduced engorgement weight of the larval ticks after the third infestation. A reduction in feeding time during a third tick infestation, as previously documented in BALB/c mice (16), was also evident in our study but may not reliably reflect the immune status of the infested host (49). Our observations during repeated larval infestations are in contrast to those in a study in which several laboratory inbred mouse strains, including C57BL/6 mice, did not develop protective immunity in response to repeated feeding of nymphal *I. ricinus* ticks (13). It is known that the pattern of tick antigenic molecules recognized by the tick hosts is dependent on the tick life stage (50); therefore, it is possible that the larval *Ixodes* stage is more immunogenic or more susceptible to discrete immune defense mechanisms than the nymphal stage.

In our experimental coinfections of laboratory mice with nematodes and ticks, the systemic Th2 immune responses against the distinct macroparasites resulted in the generation of extraordinarily high levels of Th2 cells in the peripheral blood and spleens, and these peaked during the second tick infestation and simultaneous acute nematode infection. This, however, did not affect the anti-tick Th2 responses measured locally in skin-draining lymph nodes after repeated infestations with larval ticks. Consequently, the success of larval tick feeding was unaffected by prepatent, acute, and chronic nematode coinfection, and partial immunity to tick feeding developed independently of the concurrent nematode infection. In addition, infestation with infected nymphal ticks was unaffected. Taken together, we demonstrate that a concurrent *H. polygyrus* infection does not reduce the susceptibility of mice to tick feeding.

A previous study revealed a negative correlation between *H. polygyrus* infection (the number of eggs per gram in feces) and tick numbers on wild yellow-necked mice and showed that tick infestation was increased when mice were dewormed, released, and recaptured compared to the level of infestation for the nontreated controls (47). Competition for space and food could not explain that correlation between these two macroparasites, and the influence of factors such as sex and host sex-related behavior, age, breeding condition, and habitat was at least partly ruled out, as the sites where the treated and control mice were trapped were in close proximity and similar parasite abundances were observed on the mice *a priori* (47). The intensity of grooming behavior leading to reduced tick infestation and increased ingestion of nematode larvae from the fur could also explain the negative correlation but not the increased level of tick infestation after deworming in the study of Ferrari et al. (47). Our findings suggest that the possibility of an immunologic basis for this negative correlation or the release of toxic products by one parasite affecting the other, which was suggested by the authors of the previous study (47), can also be excluded.

Several reasons may account for the differences observed between the mice obtained from the wild and those raised in the laboratory. First, the antitick immune responses and susceptibility to coinfection-induced immune changes observed in the *Mus musculus* mice used in our experimental study may differ from those observed in other mouse species, such as *A. flavicollis* and *A. sylvaticus*. Second, recent comparative immunological analyses show that SPF laboratory mice and those obtained from the wild differ not only in their expression of immune functions but also in specific and generalized immune reactivity. C57BL/6 mice raised under SPF conditions activate natural killer cells less readily than wild *M. musculus* mice (51), which also display a stronger general status of immune cell activation and higher antibody titers and avidities (52). Thus, coinfections with nematodes may differentially affect the success of tick feeding and antitick immunity in wild mice with a highly activated immune system. Third, sex-related differences in the immune reactivity of the host to many

parasite species have been described (47, 53, 54). We restricted our experimental coinfection studies to female mice, but factors such as host sex, breeding condition, nutritional status, and population density may clearly alter immune reactivity to single infections and coinfections with parasites.

Infections with intestinal nematodes affect immune responses to unrelated pathogens, such as bacteria and protozoa (22–28). This results from efficient immune modulation by parasitic worms and the counterregulation of Th1 and associated cytotoxic T cell responses (25, 27), as well as modulated innate effector cell functions during strongly biased Th2 responses (22, 55). Immune control of LD spirochetes depends on the development of Th1 responses in mice and humans (56–59). In the present study, mice coinfected with *H. polygyrus* and *I. ricinus* were only slightly inhibited in their mounting of a Th1 response to *B. afzelii*, as seen by a trend of reduced IFN-γ production by local and systemic T cells in response to the spirochete antigen. Surprisingly, the general capacity for IFN-γ production, as assessed after unspecific $CD4^+$ T cell activation, was significantly elevated even in mice that had been coinfected/infested with nematodes and ticks, further arguing against a counterregulation of Th1 reactivity in the face of two macroparasites coinducing strong Th2 responses. We also assessed whether a concurrent nematode infection increased local and systemic spirochete-specific IL-10 levels; however, local responses were not altered and the *Borrelia*-specific IL-10 production was significantly suppressed in the splenocytes of mice coinfected with nematodes. In line with these data, the presence of an acute worm infection at the peak of systemic Th2 cell reactions did not affect either the susceptibility of mice to *B. afzelii* or spirochetal proliferation or dissemination to different organs. Because two of the three essential components of reservoir competence, susceptibility to repeated tick feeding and susceptibility to the vector-borne pathogen, were not influenced, nor were spirochetal numbers modified by coinfection with *H. polygyrus*, we speculate that the competence of mice as a reservoir for LD spirochetes is not enhanced by the presence of enteric nematodes. Hence, this study indicates that the strong systemic Th2 responses in house mice coinfected with nematodes and ticks fail to affect the success of tick feeding and the control of the causative agent of Lyme disease.

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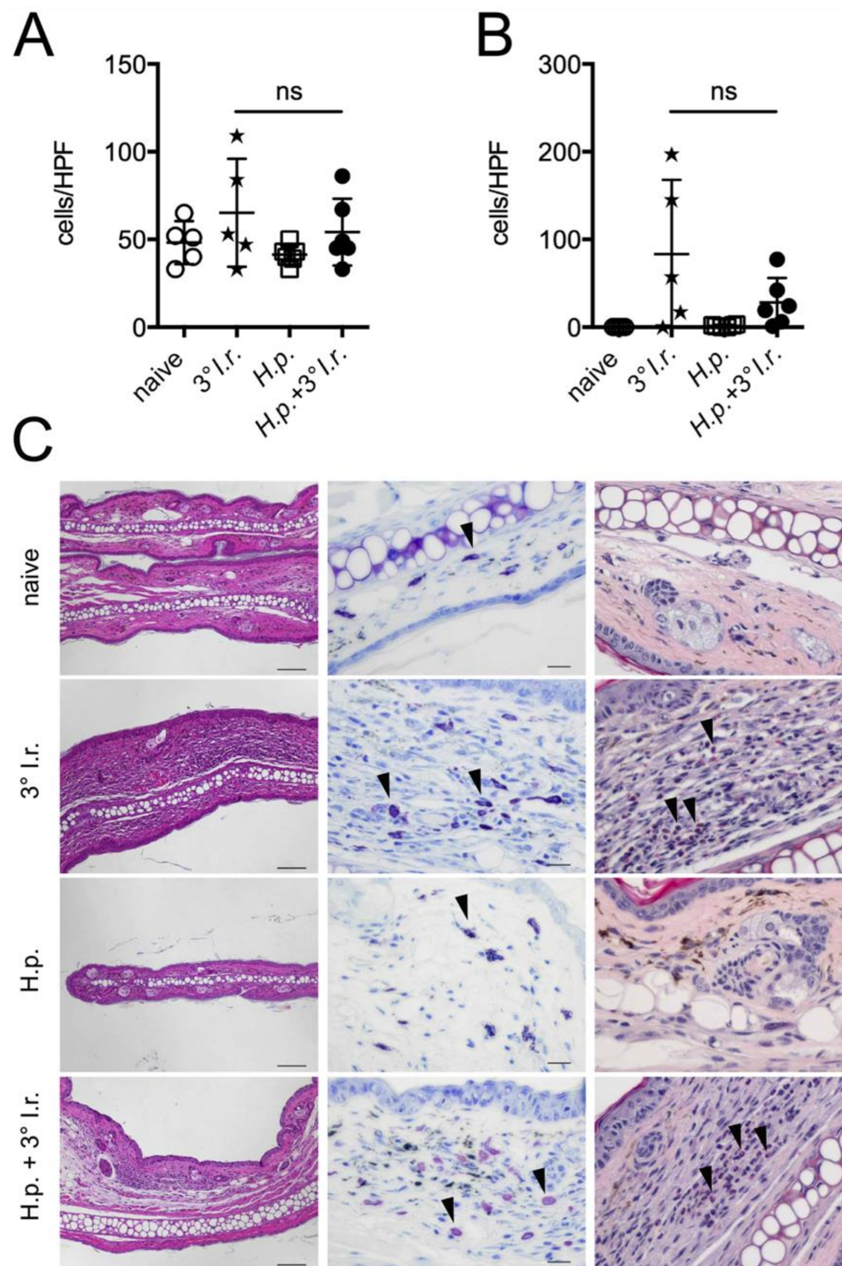

Figure S1. Histological analysis of local Th2-associated innate effector cells in skin. Number of (A) mast cells and (B) eosinophils as determined in histological ear skin sections (mean ±SD). ns: not significant. N=5-6 mice/group. Differences between groups were analysed using unpaired t-test (A) or Mann-Whitney-U-test (B). (C) Representative examples of skin sections stained with H&E (left column, scale bar 100µm), toluidine blue for mast cell detection (mid column, scale bar 20µm) and Sirius red for detection of eosinophils (right column, scale bar 20µm). Arrowheads depict mast cells and eosinophils, respectively.

Chapter 5

Comprehensive discussion

5 Comprehensive discussion

The current investigations are the first in 87 years since Elton *et al.* (1931) that analysed the overall macroparasite species richness of wild non-commensal rodent species. These field investigations are also the first that examined the influence of important extrinsic factors affecting the occurrence and partly co-occurrence of all major macroparasite groups and intestinal Coccidia at the component-community and infra-community level. As many as 84 different taxa of parasites and other rodent-associated invertebrates were differentiated on the six most abundant European mouse and vole species in Berlin. Since not all taxa were differentiated to species level and some small endoparasitic/intradermal nematode and mite groups were not in focus (*Angiostrongylus*, *Toxocara*, *Porrocaecum*, *Pneumocoptes*, *Demodex*, *Psorergates* spp.), the 257 investigated rodents likely hosted in fact more than 100 species. The findings of eight mite and one nematode species represent first records for Germany and one mite species from the genus *Criniscansor* (Myocoptidae) is currently undescribed. Between 22 and 47 taxa were identified per host species on the five regularly trapped rodent species. No wild rodent was free of parasites and a maximum of 14 different taxa on individual hosts was observed. Compared to commensal house mice, which mainly live in the human environment with lower infection pressure, invertebrate species richness is very high in wild rodents. Izdebska and Rolbiecki (2013b) identified only 14 metazoan ecto- and endoparasite species and no phoretic or other non-parasitic arthropods on 74 house mice in two different regions in Poland. The impressive natural polyparasitism in peridomestic rodents shows the complexity of potential interactions between parasite species and the extent of different, more or less invasive agents, the rodent immune system is exposed to. The observed average parasite diversity of every host individual of about six taxa depicts, that by far not every observed taxon occurred on every rodent individual. In fact, more than half of the taxa associated with yellow-necked mice, for example, had prevalences of less than five percent. Presence, as well as infection/infestation intensity of parasites of rodents are strongly dependent on extrinsic factors such as trapping location, season, year, rodent species, rodent abundance and intrinsic factors, e.g. age, sex and reproductive status (Abu-Madi *et al.*, 1998; Abu-Madi *et al.*, 2000; Behnke *et al.*, 1999; Grzybek *et al.*, 2015b; Haukisalmi *et al.*, 1988; Kiffner *et al.*, 2011; Kisielewska, 1970a). Due to the broad array of parasites in the present study, it was focused on the factors location, rodent species and partly season (ectoparasites) and on consideration of taxonomic groups of parasites together instead of single species.

5.1 Seasonality of rodent ectoparasites

Several ectoparasites revealed seasonal changes in abundance on the rodents. Much of these were likely caused by a non-continuous reproduction throughout the year. One the one hand, many

rodent louse species, for example, reproduce only in the warm season (Mahnert, 1971b; Sosnina *et al.*, 1981). On the other hand, most gamasid mites are reproductive throughout the year (Edler, 1973). Especially for species with long generation times, abundance is markedly influenced by the seasonal population dynamics of the hosts. The strong recruitment of new rodent offspring in July/August born by both, overwintered females and their meanwhile matured female progenies born in spring, leads to a transient dilution of ectoparasites with a reduced abundance (Harris *et al.*, 2009). This may be a reason for the reduction of selected flea, louse and tick species in autumn. In contrast, the abundance of stationary myobiid, myocoptid and listrophorid mites was the highest in autumn, but unfortunately, it could not be ruled out, that an increased awareness and/or competence regarding the detection of these small mites during the study period was responsible for this. For tick species, which live, apart from the period of the blood meal, off the host and outside the protective rodent nest with slight microclimate fluctuations, weather conditions are also important for the activity and hence for their abundance on rodents. In particular, saturation deficit as a measure of relative humidity appeared to affect the extant and vertical questing height of active *I. ricinus* larvae and nymphs (Randolph & Storey, 1999): During high saturation deficit, larvae are inactive and nymphs are host-seeking low in the vegetation, which should affect the subsequent infestation of rodents. Indeed, rodents hosted fewer larvae and more nymphs during July, when saturation deficit was the highest in Berlin. However, for *I. ricinus*, a combination of tick population dynamics, host population dynamics and weather should have likely elicited the observed seasonality of rodent-associated *I. ricinus* abundance.

5.2 Host-specificity of rodent parasites

The present studies revealed strong dependence of presence and intensity of most parasite groups on the host species or host family. Voles hosted more often trombiculid and myocoptid mites and anoplocephalid tapeworms than mice. In contrast, prevalence of lice, listrophorid mites, digenean flukes, hymenolepidid tapeworms, *H. spumosa* and mean intensity of fleas and intestinal Coccidia was higher in mice compared to voles. As expected, on parasite species level, host species appeared to be most important for the abundance of most parasites compared to trapping location in Non-metric multidimensional scaling (NMDS) analysis. Most rodent specimens could be assigned to one of three host groups, only based on the quantitative occurrence of the associated helminth and arthropod taxa: (a) voles, (b) *Apodemus* subgenus *Sylvaemus* spp. (*A. flavicollis* and *A. sylvaticus*) or (c) *A. agrarius* (subgenus *Apodemus*). This grouping was particularly unambiguous, if only rodents were included in the analysis that hosted at least three or five invertebrate taxa. The degree of specialisation of parasites to distinct host species corresponded largely with the evolutionary time

since when the rodent taxa diverged. Mice (Muridae) and voles (Cricetidae) split into two lineages about 24 million years ago (Mya) (Steppan *et al.*, 2004) and the mentioned two *Apodemus* subgenera about 7.9 Mya (Michaux *et al.*, 2002). In contrast, the divergence of the rodent taxa, where parasite communities were nearly undistinguishable was much later: Between *Myodes* and *Microtus* about 6 Mya (Steppan *et al.*, 2004) and between *A. flavicollis* and *A. sylvaticus* only about 2.6 Mya (Michaux *et al.*, 2002). Habitat preferences of macroparasites and coevolution with their hosts obviously led to specialisation, isolation and speciation. However, further differentiation of the helminth taxa to species level probably would have refined the ordination plot.

5.3 Influence of urbanisation on rodent parasites

A major focus of the present studies was the question, to which extant spatial differences affect the species richness, prevalence and mean intensity of rodent parasite groups, particularly those differences, which can be attributed to the degree of urbanisation. The reduction of free-living vertebrate and invertebrate species richness along a rural-urban gradient is an often observed issue (McKinney, 2008), but parasites are largely neglected as a considerable part of biodiversity (Gómez & Nichols, 2013). A reduction in biodiversity of free-living species leads to a decrease in parasite diversity and Lafferty (2012) hypothesised that this is most pronounced for parasites with complex life cycles, such as the dependence on more than one host species. The effect of urbanisation on parasite fauna was studied for bird helminths (Calegaro-Marques & Amato, 2014; Sitko & Zaleśny, 2012) and for some zoonotic species (Reperant *et al.*, 2009; Rizzoli *et al.*, 2014). Only recently, some data for rodent parasites are available (Cevidanes *et al.*, 2016; Dwużnik *et al.*, 2017). Cevidanes *et al.* (2016) studied differences in species richness, prevalence and intensity of ectoparasites (fleas, ticks, Gamasina) of wood mice between natural and residential (periurban) areas near Barcelona, Spain. The authors found only little support for differences resulting from urbanisation on ectoparasite species and group level and attributed this to a lack of considerable differences between those areas concerning environmental conditions. In contrast, house mice from rural areas in Poland hosted more parasite species with broader host range (*C. agyrtes*, *I. ricinus*) in comparison to those from urban houses, which hosted more house-mouse-specific parasites (*L. segnis*, lice) and the mite *O. bacoti* (Izdebska & Rolbiecki, 2013b). Dwużnik *et al.* (2017) studied the influence of urbanisation on the intestinal helminth fauna in yellow-necked and striped field mice in Warzaw, Poland and slightly differing patterns. While the mean helminth diversity, prevalence and abundance of yellow-necked mice was slightly, although not significantly decreased in urban areas, these measures of striped field mice were higher in urban areas. This finding was mainly elicited by the higher

parasitisation with nematodes, but also digeneans were more diverse and prevalent in striped field mice in urban parks.

In the present studies, urbanisation affected in the first place the presence and abundance of the rodent host species themselves. While at the periurban forest sites in Gatow and Tegel the yellow-necked mouse and bank vole were most abundant, the urban park in Steglitz was dominated by the striped field mouse, but also permitted the abundant occurrence of the sylvatic species yellow-necked mouse. At the most urban site in Moabit, the wood mouse was virtually the only trapped species. Due to the mentioned very similar parasite communities of yellow-necked and wood mice (both subgenus *Sylvaemus*), they can be used for comparisons between trapping sites. Considering arthropods, where comparisons of the present study to other studies (e.g. Ambros (1984); Dudich (1984); Haitlinger (2009a); Kovacik (1984)) were possible, since they were identified to species level, rodents from Berlin hosted a comparable species richness of most ectoparasitic groups. In contrast, considerably less species of groups with low host specifity or non-parasitic arthropods, including the opportunistic fleas, trombiculid mites and the nidicolous gamasid mites, were found compared to rural regions. Similarly, for both, ectoparasites and endoparasites, there was one consistent finding which confirms the mentioned hypothesis of Lafferty (2012): Parasite species richness was higher at the periurban sites in Gatow and Tegel than at the urban sites in Steglitz and Moabit, since species with low adaptation to the host and high dependence on other factors, such as other (intermediate) hosts, composition of the rodent nest and microclimate, were more prevalent and/or numerous at these less urbanised sites. These parasites included fleas, trombiculid mites, ticks, larval and adult cestodes, the trematode *Plagiorchis* sp. and the nematode *M. muris*. On the other hand, stationary ectoparasites and monoxenous endoparasites with a close adaptation to and dependence on the rodent hosts and comparable low demands on other factors, such as members of the Myobiidae, Listrophoridae, Heligmosomoidea, *H*. *spumosa* and intestinal Coccidia were more prevalent and/or numerous at the urban sites. However, the botanical park in Steglitz with its well-structured and diverse vegetation and regular watering permitted high prevalence and mean intensities of digenean flukes, fleas and ticks also in a densely populated urban area. Based on free-living invertebrates, the most important determinants of species richness related to urbanisation are the vegetation structure, the size of the patch area and the availability of corridors important for migration of species (Beninde *et al.*, 2015). The present studies confirmed these conclusions also for rodent parasite species: A gradient of urbanisation from rural areas 90-170 km away in western Poland (Haitlinger, 2009a) via the periurban sites in Tegel and Gatow to Steglitz and finally to Moabit corresponds with a reduction of species richness, which was most pronounced for parasites with strong demands on the off-host environment.

5.4 Zoonotic potential of parasites of peridomestic rodents in Berlin

In Europe, 74% of the people live in urban areas (Population-Reference-Bureau, 2016). Wild rodents can be abundant in cities and provide a connection between wildlife and humans. Mice and voles can live in areas of high human population density, exposing people to zoonotic pathogens. Moreover, the three *Apodemus* species and bank voles occasionally enter buildings in the winter (Ansorge, 1986; Hauer *et al.*, 2009; Niethammer, 1978b; Schaefer, 1962), bringing pathogens in close proximity to humans. Therefore, surveillance of rodent zoonotic parasites and disease vectors should be important in urban areas to assess the risk of infections for people. In Berlin, the rodents hosted several parasites, which can cause zoonosis or infest humans. The single specimen of the trematode *Plagiorchis* sp. likely represented *P. muris*, which caused disease in Southeast Asia (Hong *et al.*, 1996), or *P. elegans*, which was considered a synonym of *P. muris* (Tenora *et al.*, 1983). The detected taeniid metacestodes included the zoonotic species *T. martis* and presumably *H. taeniaeformis* and *T. crassiceps*. Infections with these four helminths in humans are very rare (Brunet *et al.*, 2015; Eberwein *et al.*, 2013; Nakao *et al.*, 2010). People become infected by ingestion of insufficiently heated freshwater fishes (*Plagiorchis* sp.) or through contact with faeces of infected definitive hosts, which are cats, foxes and mustelids (Taeniidae). Krücken *et al.* (2017) detected *T. canis* and *T. cati* in muscles of some of the rodents from the present study by PCR. Consequently, the peridomestic rodents support the life cycles of these nematode parasites of humans as paratenic hosts. The zoonotic helminths *C. hepatica*, *T. spiralis* and *E. multilocularis*, causing severe disease in humans, were not detected in the rodents from Berlin. Considering the ectoparasites, the fleas *M. sciurorum* and *N. fasciatus* have been reported to infest humans (Brinck-Lindroth & Smit, 2007) and while *M. sciurorum* was rare, N. *fasciatus* was the second most abundant flea on the rodent hosts. The latter is a cosmopolitan vector for several pathogens (Eisen & Gage, 2012; Marshall, 1981; Silaghi *et al.*, 2016b). However, infections with the tapeworms *H. diminuta* and *R. nana* through ingestion of the infected fleas are very rare in Europe (Kołodziej *et al.*, 2014; Tomaso *et al.*, 2001). Its epidemiological importance as a vector for *Rickettsia typhi* is presumably low (Eisen & Gage, 2012) and the vector competence for *Bartonella* sp. has not been demonstrated yet. Nevertheless, people may suffer considerable discomfort, if they become infested by *N. fasciatus* through contact with the rodent nest or if rodents enter buildings. At the periurban sites, mice and voles supported the life cycles of the harvest mite *N. autumnalis*, which infests humans mainly from late summer to autumn and causes pruritic dermatitis and scrub itch (Stekolnikov *et al.*, 2014). However, the mite is currently not known to transmit diseases to humans.

The most important macroparasites of mice and voles in Berlin affecting risk of infection for people were ticks. All three detected species (*I. ricinus*, *I. trianguliceps*, *D. reticulatus*) are able to infest humans (Guglielmone *et al.*, 2013) and are competent vectors for tick-borne pathogens (Petney *et al.*, 2012). However, *I. ricinus* is undoubtedly the most important vector tick for zoonotic pathogens in Europe and also represented 99.2% of the rodent-associated tick specimens in the present study. It was in general the most frequent macroparasite species with an overall prevalence of 56% and a mean intensity of 9.4 ticks per infested rodent. Accordingly, it can be concluded that rodents maintain an important part of the enzootic cycle of *I. ricinus* at all study sites in Berlin, even in the backyard in the city centre. We already reported the presence of the zoonotic tick-transmitted pathogens *A. phagocytophilum* and Candidatus *N. mikurensis* in the mice and voles from the present study (Krücken *et al.*, 2013), and they are likely reservoir hosts at least for the latter bacteria (Burri *et al.*, 2014; Obiegala *et al.*, 2014). Since rodents are also important reservoirs for numerous other zoonotic pathogens, the mice and voles supported particularly the transmission from the larval to the potentially infected nymphal life stage. Nymphs represent the most important source for human infection with Lyme disease spirochetes (Matuschka *et al.*, 1992) and other pathogens, since attached nymphs are less often or later recognised than adults, due to its small size. However, after another blood meal of these tick nymphs on rodent species of the present study or other hosts, they may also infect humans as adults. In addition, the fact that several nymphs fed on the rodents is the prerequisite for the transmission cycle of pathogens without transovarial transmission. Thereby, new competent reservoir hosts can become infected, which perpetuates the transmission cycle for other larval ticks (Radolf *et al.*, 2012).

Compared to the house mouse as a commensal rodent species, peridomestic rodents are much more important hosts for ticks. Izdebska and Rolbiecki (2013b) found none of the 40 house mouse specimens trapped inside houses infested with ticks and only two out of 34 specimens trapped on fields in a rural area of Poland hosted *I. ricinus* with an average of three ticks per infested mouse. House mice are not in general poor hosts for *I. ricinus*, since numerous experimental studies, including the present one (chapter 4) were successfully conducted on laboratory mice, which descend from wild congeners. More likely, they seldom come in contact with typical *Ixodes* habitats, such as forests, shrubs and meadows. Similarly, commensal black rats (*Rattus rattus*) nearly exclusively live inside buildings (Hauer *et al.*, 2009) and may not be important hosts of ticks. In contrast to its little importance for the transmission cycle of tick-borne pathogens, and apart of several non-arthropod borne bacterial and viral pathogens, which are not considered here, house mice may have particular importance as hosts of mites infesting also humans. *Liponyssoides sanguineus*, the main vector for rickettsialpox, and the so-called tropical rat mite *O. bacoti* are

associated with house mice. While the former mite was only detected in Ukraine and Sicily in Europe (Raoult & Parola, 2007), *O. bacoti* was also detected on house mice inside houses in Poland (Izdebska & Rolbiecki, 2013b) and there are several human cases in Europe, including Germany (Beck, 2008; Beck & Fölster-Holst, 2009). These infestations of humans mainly occurred after rodent control measures, when the rodent hosts were eliminated. *Ornithonyssus bacoti* is a potential vector for Lyme disease, Tularaemia, Q-fever and even Hantavirus haemorrhagic fever (Valiente Moro *et al.*, 2005). The other commensal rodents, the Norway rat (*R. norvegicus*) and the black rat, are probably even more important hosts of *O. bacoti*, although the black rats are becoming increasingly rare in regions such as Saxony (Hauer *et al.*, 2009) and Berlin-Brandenburg (Klawitter *et al.*, 2005), likely due to a reduction of private animal husbandry and renovation of suitable buildings (Hauer *et al.*, 2009). But comparable to the peridomestic rodents, Norway rats can be abundant in urban parks, where they are susceptible hosts of ticks and particularly contribute to the transmission cycle of Lyme disease (Matuschka *et al.*, 1996). Unfortunately, complex parasitological investigations on wild rats in Central Europe are scarce, which impedes comparison of the parasite diversity and the resulting zoonotic importance in comparison with the peridomestic rodent species from the present study. However, at least the helminth fauna of Norway and black rats in the Netherlands revealed a comparable low zoonotic risk provoked by the tapeworms *H. diminuta*, *H. nana* and the fluke *P. muris* (Franssen *et al.*, 2016) with very rare or no human cases in Europe.

The present study investigated the zoonotic risk of arthropod-borne pathogens based on the presence and quantity of rodent-associated ectoparasites (chapter 2), but especially for fleas, and some tick and gamasid mite species, a considerable part of the specimens lives most of the time in the nest and attach to the host only for the blood meal. The infestation of culled wild animals reveals only a snapshot information on the current ectoparasite burden, which was already emphasised by Behnke *et al.* (2001) for helminths. Hence, further studies should also observe the parasite diversity in the rodent burrows and nests, since humans may come in contact with these shelters in urban backyards and gardens.

Studies on the vector potential for pathogens mainly focused on the major arthropod species infesting people and the direct transmission pathways from wild reservoirs to humans. The participation of the great diversity of other, non-human-infesting, rodent-associated arthropods on the transmission-cycles of pathogens within the wild animal reservoirs is largely unknown. However, recent investigations exhibited high prevalences (PCR) of different tick- and flea-borne pathogens in fleas, gamasid and trombiculid mites in Europe, which do not infest humans (Miťková *et al.*, 2015; Silaghi *et al.*, 2016b; Špitalská *et al.*, 2015). Especially the non-stationary fleas and gamasid mites may be important participants in the urban enzootic transmission cycles, if vector competence becomes

verified, since they were very prevalent on the rodents in Berlin and may regularly switch hosts due to their high mobility. Future investigations should concentrate especially on the fleas *C. agyrtes, N. fasciatus* and the mite *H. nidi*, which occurred with low host-specifity on every second (50% prevalence), fifth (18%) and seventh (15%) rodent individual, respectively. Furthermore, focus should be on the gamasid mites *L. agilis* on yellow-necked and wood mice (here 59% prevalence), *H. sunci* on *Apodemus* mice (46%), *L. jettmari* on striped-field mice (29%), *E. stabularis* on wood mice (32%), *L. hilaris* and *H. microti* on common voles (64% and 46%) and *H. isabellinus* on bank voles (15%). In addition, trombiculid mites may be important in periurban and rural areas.

5.5 Co-occurrence of ticks and other parasites in wild rodents

Ixodes ricinus ticks were, in terms of zoonotic risk, the most important macroparasites of peridomestic rodents in Berlin as vectors for tick-borne pathogens. The present studies investigated the influence of co-infections/infestations on tick abundance on wild rodents as a measure for successful tick attachment and the susceptibility of rodents to tick feeding. Polyparasitism generates large possibilities of interaction between ticks and the numerous other parasites. Hence, we used parasite groups for arthropods instead of single species in multidimensional regression analyses in addition to the most important confounding factors (chapter 2 and 3). Although the latter factors appeared to be most important determinants of larval tick count on the rodents, the abundance of ectoparasitic laelapid mites, as well as of the endoparasitic nematode taxa Heligmosomoidea, *Syphacia* spp. and the tapeworms Hymenolepididae revealed significant association with the larval *I. ricinus* abundance. The negative relationship between the counts of laelapid mites and that of tick larvae may result from a predation on ticks by the mites either in the nest or on the host, which would explain the reduction of host-associated tick larvae. Although this was never observed in this family, many rodent-associated gamasid mite species are only facultative ectoparasites and an important part of their diet includes arthropods (Kim, 1985). Some species were observed to feed on blood-filled lice (Kim, 1985). The mouthparts of some obligate ectoparasite species in this family are adapted for piercing host skin and these mites may also pierce and feed on host-attached ticks. However, other possible interactions between laelapid mites and ticks may include competition for space, release of toxins by one parasite or indirect affects through host grooming or the immune responses of the host. Whether the observed correlation will be consistent in data sets from other geographical regions and if this interaction is one of cause and effect remains to be investigated in further field and experimental laboratory studies. Similarly, the positive and negative correlation of tick larvae with hymenolepidid tapeworms and *Syphacia* spp., respectively, can only provide

indications of true interaction and passive reasons, such as differences/similarities in transmission of infective stages, may also determine co-occurrence in parasite infra-communities.

The observed negative correlation between heligmosomoid nematodes and larval *I. ricinus*, however, was particularly notable, since it was in accordance to another field study from yellow-necked mice in Italy and these authors could demonstrate a causal relationship (Ferrari *et al.*, 2009): The experimental reduction of the nematode *H. polygyrus* by deworming resulted in an increase of host-associated *I. ricinus* larvae in subsequently recaptured mice compared to non-treated mice from comparable habitats. It was attributed to a negative influence of the nematode *H. polygyrus* on larval tick infestation. Since competition for food and space can be excluded between these parasites living in completely different microhabitats, the interaction was most likely mediated via the host immune response. It has been known for decades that the heligmosomoid nematode (earlier under the synonym *Nematospiroides dubius*) is immunomodulatory and enhances the establishment and survival of other helminths in laboratory mice (Alghali *et al.*, 1985; Behnke *et al.*, 1978; Jenkins & Behnke, 1977) and wild wood mice (Behnke *et al.*, 2009; Behnke *et al.*, 2005). Hence, Ferrari *et al.* (2009) initially hypothesised a positive association between the nematode and ticks, counter to their results.

5.6 Experimental co-infection with rodent parasites

Following the present field results and that of Ferrari *et al.* (2009), the present laboratory study was conducted to investigate the influence of *H. polygyrus* on the susceptibility of mice for *I. ricinus* tick feeding and their reservoir competence for Lyme disease spirochetes (chapter 4). We analysed the systemic and local immune responses towards the parasites and the reason for a hypothesised indirect interaction of both species via the host immune system. Co-infection/infestation with *H. polygyrus* and *I. ricinus* larvae and nymphs resulted in substantially high levels of systemic T helper cell type 2 (Th2) responses, independent of the mouse strain. Co-infected mice reflected nearly the sum of $CD4^+$ cells expressing the Th2 lineage transcription factor GATA-3 or the cytokine interleukin-13 (IL-13) in peripheral blood and spleen detected in both single-parasite control groups. However, antigen-specific systemic and local cytokine response toward larval ticks was not altered by a concurrent nematode infection and no cross-reactivity between larval *I. ricinus*- and *H. polygyrus* antigen was observed (see Figure 4-4 for cytokine response toward tick antigen and Appendix 8-1 for cytokine response toward *H. polygyrus* antigen). Also the number of Th2-associated innate effector cells (mast cells, eosinophils) in skin did not differ between single- and co-infected groups. According to these observations, larval and nymphal tick feeding success, measured by engorged drop-off of fed ticks, feeding duration, partly weight of fed ticks and moulting rate were not affected by early,

acute and chronic nematode infection. Also, a developing anti-tick immunity after repeated infestations was similar in single- and co-infected laboratory mice. *Ixodes ricinus* nymphs, infected with the Lyme disease spirochete *Borrelia afzelii*, successfully infected laboratory mice with the spirochete during their blood meal, but increased proportions of IL-13-producing $CD4^+$ cells in spleen and skin-draining lymph nodes did not or only marginally counterregulate the spirochete-specific type 1 T helper cell response (Th1) measured by gamma interferon (IFN-γ) production. Therefore, transmission, proliferation, dissemination and induction of pathology by *B. afzelii* were not increased in mice co-infected with *H. polygyrus*, since spirochete numbers were similar in head skin and heart and no signs of carditis or arthritis were observed in groups with or without acute intestinal nematode infection. These results on spirochete counts in infected rodents were generated by an innovative, newly developed touchdown qPCR with subsequent High Resolution Melt, which enabled for the first time the absolute quantification of pathogens per gram organ or per organ. In conclusion, the study revealed no signs of interactions between concurrent intestinal infections with heligmosomoid nematodes and larval *I. ricinus* infestation or development of anti-tick immunity in laboratory house mice. This was cotradictory to the prediction based on the present statistical results in wild mice and voles (chapter 2.4.5 and 3.4.6) and those of a field study on wild mice in Italy (Ferrari *et al.*, 2009). The difference between the laboratory and the field observations may either result from the deficiency of the laboratory study to replicate the processes in wild rodent species or from other particularities in the life history of both parasites in/on wild hosts that provoked the significant co-occurrence pattern. Therefore, (I) the present experimental settings were either inadequate for the detection of natural interspecific interaction or (II) both parasites are truly non-interactive:

(I) The immune responses of laboratory inbred mouse strains toward parasites may differ from that of wild hosts, especially if they are from another genus (*Apodemus* mice) or family (voles). Already within the same species (*M. musculus*), wild animals reveal a more activated immune system due to the permanent encounter with pathogens compared to mice reared under specific pathogen-free (SPF) conditions. They elicit higher antigen-specific IgE and IgG concentrations and avidities and a higher activation level of immune cells (Abolins *et al.*, 2011). Natural killer cells are more readily activated than in laboratory mouse strains, since microbial priming is largely restricted under SPF conditions (Boysen *et al.*, 2011). Laboratory experiments to verify natural observations should be most meaningful, if they are conducted in the respective host species, but important molecular and immunological tools for quantification of specific signal molecules and for characterisation of immune cell populations are currently not developed for most mice and vole species. Thus, many studies on the immune status of wild animal species only use simple measures of

immunocompetence, such as spleen size and white blood cell number (Babayan *et al.*, 2011). But also the nematode parasite *H. polygyrus*, which was passaged through mice in the laboratory for many generations with particularly different selection pressures compared to that in the natural life cycle, should behave different to their wild counterparts in terms of infectivity and immunogenicity (Behnke *et al.*, 2001; Dobson & Owen, 1977). Moreover, there is a considerable debate about the identity of the *Heligmosomoides* species used in laboratory experiments with house mice. Mitochondrial and ribosomal markers suggest that it might not be identical to *H. polygyrus* found in *Apodemus* mice but instead represent a separate, closely related species *H. bakeri* (Behnke & Harris, 2010; Cable *et al.*, 2006). The infection doses used in the present study leading to about 100-150 adult *H. polygyrus* worms and about 40 feeding *I. ricinus* larvae per mouse were used in relation to naturally observed infection/infestation intensities, but were at the highest margins of that range. They may have been inappropriate to reproduce natural co-infections, since the average wild rodent in Berlin hosted only about 17 heligmosomoid nematodes and nine tick larvae per infected individual. Finally, the large variability of age, sex, reproduction status, life history and genetic background of wild host individuals and the natural polyparasitism is impossible to simulate in laboratory experiments. Therefore, field experiments like that of Ferrari *et al.* (2009), carefully controlled for the most important confounding factors may require large numbers of investigated host individuals to permit statistical power but are likely the most promising settings to verify pathogen interaction in nature.

(II) On the other hand, direct interaction between parasites of different compartments such as intestinal helminths and ectoparasitic ticks may be generally unlikely since the evolutionary development of competitive or symbiotic behaviour between two species, which do not come in contact, cannot be expected. Hence, at most indirect interaction, e.g. via the immune system are conceivable. In mice with induced allergic airway disease in the lungs, indirect influence of chronic intestinal *H. polygyrus* infection was observed (Hartmann *et al.*, 2009). Nematode infection was associated with increased numbers of regulatory T cells (Tregs) in bronchial lymph nodes, which obviously diminished the infiltration of innate effector cells (eosinophils) into lungs, the allergen-specific IgE production and the hyper-reactivity of airways in this mucosal compartment. However, in the same study, *H. polygyrus* infection had no noteworthy effect on the development of induced allergic atopic dermatitis, although it influenced the number of $CD4^+$ and mast cells in the skin. In contrast to the allergic airway disease, Tregs were not increased in skin and skin-draining lymph nodes. While the nematode is able to alter the immune reaction of the host in the same compartment, the intestine, and in other mucosal-associated tissues of the host, its immunomodulatory properties have only minor effect on the immune responses in the skin compartment and consequently *H. polygyrus* did also not affect immunity to ticks.

5.7 Résumé and Outlook

The present longitudinal field study demonstrates and describes the enormous species richness of parasites and other invertebrates associated with six wild mouse and vole species, which likely represent the most abundant rodent species in Europe. Since parasites of wild animals were largely neglected in the past as part of biodiversity, the study represents an acquisition and quantification of rodent-associated species in an urban/periurban area in Germany. It may serve as a basis for further studies on biology and life cycles of many poorly studied parasitic arthropods, helminths and intestinal protozoa. The study verified a negative influence of urbanisation on parasite species richness, which was previously mainly attributed to free-living invertebrates. Apart from ticks, the zoonotic risk resulting from parasites of peridomestic rodents appears to be comparatively low in Berlin, since the species causing the most severe diseases were absent and rodent-borne infections caused by the zoonotic species detected in the present study are rare in human patients in Europe. Although they are not considered to be zoonotic, many ectoparasite species may act as vectors of arthropod-borne and other pathogens between wild animals, and their contribution in the enzootic transmission cycles should be investigated in future studies. Unambiguously, the hard tick *I. ricinus* was the most important health threat for humans within rodent-associated parasites in Berlin. In addition, the rodents certainly contributed to the infection of ticks in urban and periurban areas, since *A. phagocytophilum*, *Candidatus* N. mikurensis (Krücken *et al.*, 2013) and several other tick-borne pathogens were detected in organ samples by PCR by the present author. Unfortunately, data on these results could not be considered here, since this would go beyond the scope of the present thesis. Co-infections with ticks and other parasites were in focus and within several significant co-occurrence patterns in regression analyses, the most conspicuous co-infection was examined in an experimental laboratory infection. However, this approach revealed no measurable influence of heligmosomoid nematodes on the susceptibility of mice for ticks and their reservoir competence for Lyme disease spirochetes. Even if this originated from an inappropriate laboratory model, the statistic influence of the abundance of the heligmosomoid nematodes on that of tick larvae on wild rodents was low compared to other extrinsic and intrinsic factors, especially during low infection intensities. Similarly, effects of intestinal *Syphacia* spp. and hymenolepidid tapeworm infections on the anti-tick immunity in the skin compartment are unlikely in wild rodent hosts. However, interactions between ticks and other ectoparasites, especially mites from the family Laelapidae, from the same (skin) compartment via predation or mediated by host immune responses should be further investigated in future studies, since they may have important impacts on the reservoir competence of rodents for tick-borne pathogens.

Chapter 6

Summary / Zusammenfassung

6 Summary / Zusammenfassung

6.1 Summary

Small rodents and their nests are fascinating and existential biotopes for numerous arthropod, helminth and protozoan animals. On the other side, some of the parasite species can cause disease in humans and companion animals and wild mice and voles are reservoir hosts for many tick-borne pathogens. Despite that, the biodiversity and quantitative occurrence of many wild rodent parasites and their zoonotic potential is largely unknown.

Six rodent species from the genera *Apodemus*, *Myodes* and *Microtus* are presumably the most abundant non-commensal rodent species in Europe and also among the most important hosts for immature life stages of ticks in urban areas like Berlin. In the first instance, the present thesis therefore reviewed for the first time all the 460 invertebrate and 69 protozoan species which were reported from these six rodent species in Europe and gave a brief overview about the taxonomy, major morphological characteristics, life cycles and zoonotic potential of the macroparasites and intestinal Coccidia associated with these rodents.

In a longitudinal field study, 257 rodents of these six species were trapped at four study sites in Berlin and examined for intestinal Coccidia, macroparasites and other invertebrates. The ectoparasites, as well as phoretic and non-parasitic arthropods detected in the fur and on the skin of the rodents were covered in **manuscript 1**, while helminths and intestinal Coccidia were addressed in **manuscript 2**. A high taxon richness of at least 84 species, comprising 63 arthropod, 20 helminth and one higher coccidian taxon was observed on/in the rodents in Berlin and no rodent individual was free of parasite infections. The tick *Ixodes ricinus* was the most frequent species with 56% prevalence and a mean intensity of 9.4 ticks per rodent host. Eight mite and one nematode species represent new records for the fauna of Germany. Rodent species, trapping location and season were clearly associated with the quantitative occurrence of most parasite groups. Mice were more often parasitised by digenean flukes, hymenlepidid tapeworms, the nematode *Heterakis spumosa*, lice and listrophorid mites but less often by anoplocephalid tapeworms, trombiculid and myocoptid mites than voles. In non-metric multidimensional scaling, host species even appeared to be more important than trapping location, since rodent individuals could mostly be allocated to one of three host taxon groups, only according to the parasite taxa they harboured. However, the different degrees of urbanisation between the trapping locations affected not only the presence and abundance of the rodent species, but also the species richness of rodent parasites. Compared to rural areas, mice and voles in Berlin carried less arthropod species with low host dependence and

host specifity, such as fleas, trombiculid and non-parasitic gamasid mites. Furthermore, ecto- and endoparasites with lower host adaptation and high dependence on intermediate hosts or other external factors were decreasingly diverse, prevalent and/or numerous with increasing degree of urbanisation from periurban to urban sites. Endoparasites without host change (monoxenous) and stationary ectoparasites, in contrast, were more prevalent and/or numerous at urban sites. Apart from ticks, the parasites detected on the six peridomestic rodent species in Berlin only pose a minor zoonotic risk of infection for people, since species causing severe diseases, such as *Echinococcus multilocularis* were absent. In contrast, the role of these hosts in the urban life cycle of ticks is important and they most certainly participate in the maintenance of well-known and emerging tick-borne pathogens in Berlin. Hence, regression analyses were performed to assess the influence of co-infections on tick abundance on the rodent host. Considering several confounding factors, the abundance of *I. ricinus* larvae was negatively associated with the abundance of nematodes from the superfamily Heligmosomoidea and *Syphacia* sp., as well as of gamasid mite parasites from the family Laelapidae, while that of hymenolepidid tapeworms was positively associated.

To verify a relationship of cause and effect, laboratory co-infection experiments were conducted and published in **manuscript 3**. Mice simultaneously infected with *Heligmosomoides polygyrus* nematodes as well as with larval or nymphal *I. ricinus* ticks exhibited substantially higher systemic type 2 T helper cell (Th2) responses, based on interleukin-13 and GATA-3 expression compared to single-infected mice. However, the development of partial immunity and the Th2 reactivity towards ticks were unaffected by the nematode infection during repeated larval tick infestations. Co-infections with *H. polygyrus* were also unable to affect the susceptibility for tick-transmitted *Borrelia afzelii* Lyme disease spirochetes and their replication, dissemination and induction of signs of pathology in the rodent host.

The negative association between heligmosomoid nematodes and ticks in wild rodents could not be confirmed in laboratory mice. However, the observed negative effect of laelapid mites on tick abundance, which may be caused by predation, should be further investigated. These mites have the potential to affect feeding success of ticks and therefore the transmission of many tick-borne pathogens. The present field study constitutes a current basis for studies on parasite diversity, arthropod vector competence and natural co-infections.

6.2 Zusammenfassung

Kleine Nagetiere und ihre Nester sind faszinierende und existentielle Biotope für zahlreiche Arthropoden-, Helminthen-, und intestinale Protozoenarten. Auf der anderen Seite können einige der parasitischen Arten Krankheiten bei Menschen und Haustieren verursachen. Zudem sind wilde Wühlmäuse und Mäuse Reservoirwirte für viele Zecken-übertragene Pathogene. Trotz allem sind die Biodiversität und das quantitative Vorkommen vieler Wildnagerparasiten, sowie ihr zoonotisches Potential noch immer weitgehend unbekannt.

Sechs Nagetierarten aus den Gattungen *Apodemus*, *Myodes* und *Microtus* umfassen wahrscheinlich die individuenreichsten, nicht-kommensalen Nagetierarten in Europa und gehören zugleich zu den wichtigsten Wirten für juvenile Zeckenstadien in urbanen Gebieten wie Berlin. Daher fasst diese Thesis zu Beginn erstmalig alle 460 Invertebraten- und 69 Protozoenarten zusammen, welche diese sechs Nagetierarten in Europa parasitieren oder anderweitig mit ihnen assoziiert sind und gibt zudem einen kurzen Überblick über die Taxonomie, die wichtigsten morphologischen Charakteristiken, die Lebenszyklen und das zoonotische Potential der Makroparasiten und intestinalen Kokzidien.

In einer longitudinalen Freilandstudie wurden 257 Nagetiere dieser sechs Arten an vier Fangorten in Berlin gefangen und auf intestinale Kokzidien, Makroparasiten und andere Invertebraten untersucht. Die Ektoparasiten, sowie phoretische und nicht-parasitische Arthropoden, welche im Fell und auf der Haut der Nagetiere gefunden wurden, werden im **Manuskript 1** behandelt, während Helminthen und intestinale Kokzidien in **Manuskript 2** präsentiert werden. Eine hohe Taxonvielfalt von mindestens 84 Arten, welche sich aus 63 Arthropoden-, 20 Helminthenarten und einem höheren Kokzidientaxon zusammensetzt, wurde auf/in den Nagetieren in Berlin festgestellt und kein einziges Tier war frei von parasitischen Infektionen. Die Zecke *Ixodes ricinus* war die häufigste Art mit einer Prävalenz von 56% und einer mittleren Intensität von 9,4 Zecken pro Nagetierwirt. Acht Milbenarten und eine Nematodenart stellten Erstnachweise für die deutsche Fauna dar. Nagetierart, Fangort und Jahreszeit hatten deutlichen Einfluss auf das quantitative Vorkommen der meisten Parasitengruppen. Mäuse waren häufiger mit Trematoden, hymenolepididen Bandwürmern, dem Nematoden *Heterakis spumosa*, Tierläusen und listrophoriden Milben, aber seltener mit anoplocephaliden Bandwürmern, trombiculiden und myocoptiden Milben befallen als Wühlmäuse. Unter Verwendung einer Nicht-metrischen multidimensionalen Skalierung erschien die Wirtsart von größerer Bedeutung zu sein als der Fangort, da die Nagetierindividuen allein aufgrund ihrer Parasitentaxa einer von drei Wirtstaxongruppen zugeordnet werden konnten. Der unterschiedliche Grad an Urbanisierung zwischen den Fangorten hingegen beeinflusste nicht nur die Präsenz und Abundanz der Nagetierarten, sondern auch den Artenreichtum der Nagetierparasiten. Im Vergleich zu ländlichen Gebieten waren die Mäuse und Wühlmäuse in Berlin mit weniger Arthropodenarten mit geringer Wirtsabhängigkeit und -spezifität, wie Flöhen, trombiculiden und nicht-parasitischen gamasiden

Milben, infestiert. Zudem waren Ekto- und Endoparasiten mit geringerer Wirtsadaptation und starker Abhängigkeit von Zwischenwirten oder anderen externen Faktoren weniger divers, prävalent und/oder individuenreich mit steigendem Grad der Urbanisierung von periurbanen zu urbanen Fangorten. Endoparasiten ohne Wirtswechsel (monoxen) und stationäre Ektoparasiten waren dagegen prävalenter und/oder zahlreicher an den urbanen Standorten. Mit Ausnahme der Zecken stellen die in dieser Studie gefundenen Parasiten der sechs peridomestischen Nagetierarten in Berlin nur ein geringes zoonotisches Infektionsrisiko für den Menschen dar. Arten wie der Fuchsbandwurm *Echinococcus multilocularis*, welche schwere Krankheiten verursachen, fehlten. Im Gegensatz dazu ist die Rolle dieser Wirte bedeutend für die urbanen Lebenszyklen von Zecken und sie nehmen unzweifelhaft auch an der Zirkulation von bekannten und neuartigen Zecken-übertragenen Krankheitserregern in Berlin teil. Aus diesem Grund wurden Regressionsanalysen zum Einfluss von Koinfektionen auf die Abundanz von Zecken auf ihren Nagetierwirten durchgeführt. Unter Berücksichtigung von wichtigen Störfaktoren war die Abundanz von *I. ricinus* Larven sowohl mit der Abundanz von Nematoden der Überfamilie Heligmosomoidea und von *Syphacia* sp., als auch mit der Abundanz von parasitischen, gamasiden Milben der Familie Laelapidae negativ assoziiert, während jene von hymenolepididen Bandwürmern positiv assoziiert war.

Für den Nachweis einer kausalen Beziehung zwischen den Parasiten wurden experimentelle Koinfektionen in Labornagern durchgeführt, welche in **Manuskript 3** publiziert wurden. Mäuse, welche gleichzeitig sowohl mit *Heligmosomoides polygyrus* Nematoden, als auch mit Zeckenlarven und -nymphen von *I. ricinus* infiziert waren, zeigten beträchtlich erhöhte systemische Typ 2 T-Helfer-Zellantworten (Th2), auf der Basis der Interleukin-13- und GATA-3-Expression im Vergleich zu Mäusen mit Einzelinfektionen. Die Entwicklung einer teilweisen Immunität und die Th2 Reaktivität gegenüber Zecken waren hingegen unbeeinflusst von der Nematodeninfektion während wiederholter Infestationen mit Zeckenlarven. Koinfektionen mit *H. polygyrus* waren zudem nicht in der Lage, die Suszeptibilität gegenüber Zecken-übertragenen *Borrelia afzelii* Lyme-Spirochäten, sowie deren Replikation, Dissemination und Induktion von pathologischen Veränderungen im Nagetierwirt zu beeinflussen.

Die in Wildnagern beobachtete negative Assoziation zwischen heligmosomoiden Nematoden und Zecken konnte somit in Labormäusen nicht bestätigt werden. Allerdings sollte ein womöglich negativer Effekt von laelapiden Milben auf die Zeckenabundanz, welcher eventuell auf Prädation beruht, in Zukunft näher untersucht werden. Diese Milben haben das Potential, den Erfolg der Blutmahlzeit von Zecken und somit die Transmission von Zecken-übertragenen Pathogenen zu beeinflussen. Die hier präsentierte Feldstudie bietet eine aktuelle Basis für weitere Studien zur Biodiversität von Parasiten, zur Vektorkompetenz von Arthropoden und zu natürlichen Koinfektionen.

Chapter 7

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7 References

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Chapter 8

Appendices

Appendix 8-1. Species of invertebrate and protozoan animals reported from the six muroid rodent species in Europe. At least 69 species of Protozoa (here including Microsporidia) and 460 species of invertebrates from 92 families and four phyla (Platyhelminthes, Acanthocephala, Nematoda, Arthropoda) were reported from *Apodemus flavicollis* (syn. *A. tauricus*), *Apodemus sylvaticus* (syn. *A. callipidis*), *Apodemus agrarius*, *Myodes glareolus* (syn. *Clethrionomys glareolus*), *Microtus arvalis* (syn. *Arvicola arvalis*) and *Microtus agrestis* (syn. *Arvicola agrestis*) and its synonyms in Europe excluding Turkey and the Russian part but including Crimea. Relevant synonyms are listed in brackets. Bold marked species are at least facultatively parasitic. From underlined species, only their DNA was detected by PCR in the rodents and not the whole organisms. Grey shaded species have reported ability to infect (zoonosis) or infest humans. For every species, at least one reference was attached confirming their detection. Asterisks (*) indicate rare accidental infections (only where adult parasites were observed), since the parasites are specific to other host species.

Phylum	Family	Species	References
Microsporidia	Unikaryonidae	***Encephalitozoon cuniculi***	Führer *et al.* (2010a)
Microsporidia	Thelohaniidae	***Thelohania apodemi***	Doby *et al.* (1963)
Amoebozoa	Entamoebidae	***Entamoeba muris***	Cox (1970)
Metamonada	Giardiidae[a]	***Giardia muris***, ***Giardia microti***	Cox (1987); Koudela (1994); Perec-Matysiak *et al.* (2015)
Metamonada	Hexamitidae	***Spironucleus muris*** **(*Hexamita muris*)**	Cox (1970); Frank (1978)
Metamonada	Tetramitidae	***Chilomastix bettencourti***	Cox (1970)
Metamonada	Trichomonadidae	***Tetratrichomonas microti*** **(*Trichomonas microti*)**, ***Trichomonas sylvatici***, ***Tritrichomonas muris***	Cox (1970); Ring (1959)
Euglenozoa	Trypanosomatidae	***Trypanosoma grosi***, ***Trypanosoma evotomys***, ***Trypanosoma microti***	Cox (1970); Frank (1978)
Ciliophora	Buetschliidae	***Blepharomonas mollis***	Cox (1970)
Myzozoa	Cryptosporidiidae	***Cryptosporidium muris***, ***Cryptosporidium parvum***, ***Cryptosporidium scrofarum***, ***Cryptosporidium hominis***, ***Cryptosporidium suis***, ***Cryptosporidium ubiquitum***	Chalmers *et al.* (1997); Danišová *et al.* (2017); Perec-Matysiak *et al.* (2015)

Appendix 8-1. Continued

Phylum	**Family**	**Species**	**References**
Myzozoa	Eimeriidae	***Eimeria agrarii***, ***Eimeria arnastauskieni***, ***Eimeria apionodes***, ***Eimeria apodemi***, ***Eimeria arkutinae***, ***Eimeria arvalis***, ***Eimeria arvicolae***, ***Eimeria clethrionomydis***, ***Eimeria cernae***, ***Eimeria derenica***, ***Eimeria divichinica***, ***Eimeria gandobica***, ***Eimeria golemanskii***, ***Eimeria gomurchaica***, ***Eimeria hungaryensis***, ***Eimeria iradiensis***, ***Eimeria iwanoffi***, ***Eimeria kaunensis***, ***Eimeria monocrustae***, ***Eimeria montgomeryae***, ***Eimeria muris***, ***Eimeria naye***, ***Eimeria prasadi***, ***Eimeria primbelica***, ***Eimeria rugosa***, ***Eimeria russiensis***, ***Eimeria rysavyi***, ***Eimeria rysavyensis***, ***Eimeria svanbaevi***, ***Eimeria sylvatica***, ***Eimeria uptoni,*** ***Eimeria zuvandica***, ***Isospora golemanskii***, ***Isospora clethrionomydis***, ***Isospora buxea***	Grikienienė (2005); Levine and Ivens (1990)
Myzozoa	Sarcocystidae	***Sarcocystis microti*** **(*Frenkelia microti*)**, ***Sarcocystis glareoli*** **(*Frenkelia glareoli*, *Frenkelia clethrionomyobuteonis*)**, ***Sarcocystis cernae***, ***Sarcocystis clethrionomyelaphus***[b], ***Sarcocystis muris***[c], ***Sarcocystis putorii***, ***Sarcocystis cymruensis*** **(*Sarcocystis rodentifelis*)**, ***Sarcocystis sebeki***, ***Toxoplasma gondii***, ***Neospora caninum***	Cox (1987); Führer *et al.* (2010a); Levine and Ivens (1990); Matuschka (1986); Odening (1998)
Myzozoa	Hepatozoidae[d]	***Hepatozoon erhardovae***, ***Hepatozoon microti*** **(*H. lavieri*)**, ***Hepatozoon sylvatici***	Cox (1987); Smith (1996)
Myzozoa	Babesiidae	***Babesia microti***	Karbowiak (2004)
Platyhelminthes	Brachylaimidae	***Brachylaima recurvum*** **(*Brachylaema recurva*, *Brachylaemus recurvatus*, *Brachylaemus aequans*)**, ***Brachylaima spinulosum*** **(*Brachylaemus spinulosus*)**, ***Scaphiostomum palaearcticum***	Behnke *et al.* (1999); Mas-Coma *et al.* (1986); Tenora *et al.* (1973)
Platyhelminthes	Panopistidae	***Pseudoleucochloridium soricis******	Gubányi *et al.* (2002)
Platyhelminthes	Diplostomatidae	***Alaria alata***[e], ***Neodiplostomum major***	Genov *et al.* (1998); Grikienienė (2005); Shimalov (2013)

Platyhelminthes	Strigeidae	***Strigea falconis***, ***Strigea sphaerula***	Shimalov (2002); Shimalov (2013)
Platyhelminthes	Plagiorchiidae[f]	***Plagiorchis muris***, ***Plagiorchis elegans*** **(*P. stefanskii*)**, ***Plagiorchis arvicolae***, ***Plagiorchis talassensis***, ***Rubenstrema exasperatum*** **(*Plagiorchis exasperatus, Plagiorchis microti*)***, ***Skrjabinoplagiorchis polonicus*** **(*Plagiorchis polonicus*)**	Feliu *et al.* (1997); Hildebrand *et al.* (2004); Ivanov and Semenova (2000); Rogan *et al.* (2007); Shimalov (2013); Soltys (1949); Tenora *et al.* (1983); Wahl (1967)
Platyhelminthes	Lecithodendriidae	***Paraleyogonimus baeri***, ***Posterocirrus clethrionomi***, ***Macyella apodemi***, ***Cephalotrema elasticum***, ***Encystodendrium rotundum***	Grikienienė (2005); Jourdane and Triquell (1973); Matskasi (1971); Memaran (1970); Vaucher (1968)
Platyhelminthes	Prosthogonimidae	***Mediogonimus jourdanei***	Mas-Coma and Rocamora (1978); Ribas *et al.* (2009)
Platyhelminthes	Microcephallidae	***Maritrema apodemicum***	Lewis (1966)
Platyhelminthes	Pleurogenidae	***Collyricloides massanae***	Jourdane and Triquell (1973); Kanarek *et al.* (2015); Vaucher (1969)
Platyhelminthes	Dicrocoelidae	***Corrigia vitta (Lyperosomum vitta***), ***Dictyonograptus muris*** **(*Platynosomum muris, Platynosomoides muris, Skrjabinus muris*)**, ***Brachylecithum glareoli***	Behnke *et al.* (1999); Debenedetti *et al.* (2016); Gubányi *et al.* (2002); Hildebrand *et al.* (2007); Matskási (1967); Vaucher and Hunkerer (1967)
Platyhelminthes	Notocotylidae[g]	***Notocotylus noyeri*** **(*N. marinus*)**[h], ***Notocotylus neyrai***, ***Notocotylus gonzalezi***, ***Notocotylus malhamensis***	Boyce *et al.* (2012); Feliu *et al.* (1997); Memaran (1970); Simón-Vicente *et al.* (1985)
Platyhelminthes	Psilostomidae	***Psilotrema pharyngeatum****	Edelényi (1966)

Appendix 8-1. Continued

Phylum	**Family**	**Species**	**References**
Platyhelminthes	Echinostomatidae	***Isthmiophora melis* (*Euparyphium melis*)**, ***Echinostoma miyagawai***	Edelényi (1966); Genov *et al.* (1998); Goüy de Bellocq *et al.* (2003); Hildebrand *et al.* (2004)
Platyhelminthes	Diphyllobothriidae	***Spirometra erinacei* (*S. erinaceieuropaei, Diphyllobothrium mansoni*)**	Genov and Georgiev (1998a); Soltys (1954)
Platyhelminthes	Hymenolepididae[ijkl]	***Hymenolepis hibernia***, ***Hymenolepis myoxi* (*Armadolepis myoxi, H. sulcata*)***[m], ***Arostrilepis horrida* (*Hymenolepis horrida*)**, ***Rodentolepis fraterna* (*Hymenolepis fraterna*)**, ***Rodentolepis straminea***, ***Rodentolepis asymmetrica* (*Hymenolepis ampla*)**, ***Microsomacanthus crenata* (*Variolepis crenata, Passerilepis crenata, M. murissylvatici, Hymenolepis muris-sylvatici, Staphylocystis muris-sylvatici*)**	Behnke *et al.* (1999); Haukisalmi (2015); Pojmańska *et al.* (2007)
Platyhelminthes	Catenotaeniidae	***Pseudocatenotaenia matovi***, ***Cataenotaenia pusilla***, ***Catenotaenia henttoneni* (*C. cricetorum*)**, ***Catenotaenia asiatica***, ***Skrjabinotaenia lobata* (*Catenotaenia lobata, Catenotaenia capensis*)**	Haukisalmi and Henttonen (2000); Klimpel *et al.* (2007b); Mas-Coma *et al.* (1978); Tenora *et al.* (1973)
Platyhelminthes	Anoplocephalidae	***Paranoplocephala janickii***, ***Paranoplocephala kalelai***, ***Paranoplocephala omphalodes***, ***Eurotaenia gracilis***, ***Microticola blanchardi***, ***Anoplocephaloides dentata* (*Paranoplocephala dentata*, *Paranoplocephala brevis*)**, ***Gallegoides arfaai***	Haukisalmi (2015); Haukisalmi *et al.* (2014)
Platyhelminthes	Dilepididae	***Dilepis undula****, ***Choanotaenia unicoronata****	Tenora *et al.* (1973); Vaucher and Hunkerer (1967)
Platyhelminthes	Taeniidae	***Hydatigera taeniaeformis* (*Taenia taeniaeformis, Cysticercus fasciolaris*)**, ***Hydatigera kamiyai***, ***Hydatigera parva* (*Taenia parva*)**, ***Taenia crassiceps***, ***Taenia pisiformis***, ***Taenia martis* (*T. m. martis* (*T. intermedia*) and *T. m. americana***	Debenedetti *et al.* (2016); Führer *et al.* (2010b); Jančev and Stoykova-Hadjinikolova (1980); Lavikainen *et al.*

		(*T. sibirica*)), ***Taenia polyacantha*** **(*Tetratirotaenia polyacantha*)**, ***Taenia hydatigena***, ***Versteria mustelae*** **(*Taenia mustelae, Taenia tenuicollis*)**, ***Echinococcus multilocularis***	(2016); Memaran (1970); Pfaller and Tenora (1972); Schmidt (2001)
Platyhelminthes	Paruterinidae[n]	***Cladotaenia circi***, ***Cladotaenia globifera*** **(*Cladotaenia cylindracea*)**	Murai (1982)
Platyhelminthes	Dipylidiidae	***Joyeuxiella pasqualei***	Mas-Coma *et al.* (2000)
Platyhelminthes	Mesocestoididae	***Mesocestoides lineatus***, ***Mesocestoides litteratus*** **(*M. leptothylacus*)**	Behnke *et al.* (2008); Loos-Frank (1980)
Acanthocephala	Moniliformidae	***Moniliformis moniliformis***	Edelényi (1966); Gubányi *et al.* (2002); Tenora *et al.* (1973)
Nematoda	Trichuridae	***Trichuris arvicolae*** **(*Trichuris madisonensis*)**, ***Trichuris muris***	Feliu *et al.* (2000)
Nematoda	Trichosomoididae	***Trichosomoides crassicauda******	Genov and Georgiev (1998b)
Nematoda	Capillariidae	***Capillaria annulosa*** **(*Aonchotheca annulosa*, *Pterothominx sardovskoi*)**, ***Capillaria muris-sylvatici*** **(*Aonchotheca murissylvatici, C. halli*)**, ***Capillaria bacillatus*** **(*Eucoleus bacillatus*)**, ***Capillaria gastrica*** **(*Eucoleus gastricus, Thominx gastrica*)**, ***Capillaria hepatica*** **(*Calodium hepaticum, Hepaticola hepatica*)**	Feliu *et al.* (1997); Memaran (1970); Tenora (2004)
Nematoda	Trichinellidae	***Trichinella* spp.**	Dick and Pozio (2001); Pozio (2007); Tenora *et al.* (1973)
Nematoda	Oxyuridae[o]	***Syphacia stroma***, ***Syphacia frederici***, ***Syphacia agraria***, ***Syphacia petrusewiczi***, ***Syphacia nigeriana***, ***Syphacia montana***	Tenora and Mészéros (1975)
Nematoda	Heteroxynematidae[p]	***Aspiculuris tianjinensis***, ***Aspiculuris tetraptera***	Behnke *et al.* (2015); Eira *et al.* (2006)
Nematoda	Heterakidae	***Heterakis spumosa*** **(*Ganguleterakis spumosa*)**	Ondríková *et al.* (2010); Schmidt (1961)

Appendix 8-1. Continued

Phylum	Family	Species	References
Nematoda	Ascarididae[q]	***Toxocara cati***, ***Toxocara canis***, ***Porrocaecum* sp.**	Portoles *et al.* (2000); Shimalov (2013); Tenora *et al.* (1973)
Nematoda	Rictulariidae	***Rictularia proni***, ***Rictularia cristata***, ***Pterygodermatites hispanica***, ***Pterygodermatites kolimensis***	Barus *et al.* (1972); Bjelic-Cabrilo *et al.* (2011); Eira *et al.* (2006); Quentin (1973); Stammer (1956)
Nematoda	Spirocercidae	***Mastophorus muris***	Bernard (1961); Genov (1984); Schmidt (1961); Stammer (1956)
Nematoda	Gongylonematidae	***Gongylonema neoplasticum* (*G. problematicum*)**	Eira *et al.* (2006); Stammer (1956)
Nematoda	Peloderidae[rs]	***Pelodera cutanea***, ***Pelodera orbitalis***	Schulte (1989)
Nematoda	Heligmosomidae[t]	***Heligmosomoides polygyrus* (*Nematospiroides dubius, Heligmosomoides skrjabini, Heligmosomum azerbaidjani*)**, ***Heligmosomoides neopolygyrus***, ***Heligmosomoides laevis***, ***Heligmosomoides glareoli***, ***Heligmosomum costellatum***, ***Heligmosomum pseudocostellatum***, ***Heligmosomum yamagutii***, ***Heligmosomum mixtum***, ***Heligmosomum borealis***	Feliu *et al.* (1997); Haukisalmi and Henttonen (2000); Tenora (2004); Tenora *et al.* (2002); Tenora *et al.* (1983); Tenora *et al.* (1973); Zaleśny *et al.* (2014)
Nematoda	Heligmonellidae	***Carolinensis minutus* (*Longistriata wolgaensis, Boreostrongylus minutus*)**, ***Paraheligmonina gracilis* (*Longistriata schulzi*)***, ***Longistriata depresssa*****	Feliu *et al.* (1997); Haukisalmi and Henttonen (2000); Pojmańska *et al.* (2007); Tenora (2004); Tenora *et al.* (1973)
Nematoda	Trichostrongylidae	***Trichostrongylus retortaeformis*****, ***Trichostrongylus colubriformis*****	Bernard (1961); Grikienienė (2005); Thomas (1953)
Nematoda	Angiostrongylidae[u]	***Angiostrongylus dujardini***	Eira *et al.* (2006); Mészáros (1978);

			Ribas *et al.* (2009)
Nematoda	Strongyloididae	***Strongyloides ratti***	Bernard (1961); Frank (1977); Schmidt (1961)
Arthropoda	Pulicidae	***Pulex irritans****, ***Echidnophaga murina, Ctenocephalides felis****, ***Ctenocephalides canis****, ***Spilopsyllus cuniculi****	Peus (1959); Skuratowicz (1967); Whitaker (2007)
Arthropoda	Ceratophyllidae	***Leptopsylla segnis***, ***Leptopsylla taschenbergi***, ***Leptopsylla sciurobia****, ***Peromyscopsylla bidentata***, ***Peromyscopsylla silvatica***, ***Peromyscopsylla spectabilis***, ***Peromyscopsylla fallax***, ***Amphipsylla sibirica***, ***Amphipsylla rossica***, ***Nosopsyllus fasciatus*** (***N. paganus***), ***Nosopsyllus consimilis***, ***Nosopsyllus londiniensis***, ***Nosopsyllus mokrzeckyi***, ***Orchopeas howardi****, ***Megabothris walkeri***, ***Megabothris turbidus***, ***Megabothris rectangulatus***, ***Megabothris calcarifer***, ***Callopsyllus saxatilis***, ***Amalareus penicilliger*** (***Malareus penicilliger***), ***Amalareus arvicolae*** (***Malareus arvicolae***), ***Myoxopsyllus laverani****, ***Citellophilus martinoi****, ***Tarsopsylla octodecimdentata****, ***Monopsyllus sciurorum***, ***Ceratophyllus gallinae****, ***Ceratophyllus styx****, ***Ceratophyllus vagabundus****, ***Ceratophyllus garei****, ***Ceratophyllus rusticus****, ***Dasypsyllus gallinulae****	Beaucournu and Launay (1990); Beaucournu and Alcover (1984); Biocca *et al.* (1975); Brelih and Trilar (2000); Brinck-Lindroth and Smit (2007); Dudich and Szabó (1984); Gheoca *et al.* (2013); Mahnert (1969); Peus (1968); Skuratowicz (1967); Whitaker (2007)
Arthropoda	Ctenophthalmidae	***Ctenophthalmus assimilis***, ***Ctenophthalmus agyrtes***, ***Ctenophthalmus congener***, ***Ctenophthalmus uncinatus***, ***Ctenophthalmus obtusus***, ***Ctenophthalmus solutus***, ***Ctenophthalmus bisoctodentatus****, ***Ctenophthalmus nobilis***, ***Ctenophthalmus russulae****, ***Ctenophthalmus andorrensis***, ***Ctenophthalmus baeticus***, ***Ctenophthalmus ubayensis***, ***Ctenophthalmus apertus***, ***Ctenophthalmus nivalis***, ***Ctenophthalmus orphilus***, ***Ctenophthalmus egregius***, ***Ctenophthalmus niethammeri***, ***Ctenophthalmus capriciosus***, ***Ctenophthalmus fransmiti***, ***Ctenophthalmus bureschi*** (***C. congerer bureši***), ***Ctenophthalmus savii***,	Beaucournu and Launay (1990); Beaucournu and Lorvelec (2014); Beaucournu and Alcover (1984); Brinck-Lindroth and Smit (2007); Dudich and Szabó (1984); Gheoca *et al.* (2013); Gómez and Blasco-Zumeta (2002); Haitlinger (1983a); Lundqvist and Brinck-Lindroth (1990);

Appendix 8-1. Continued

Phylum	**Family**	**Species**	**References**
Arthropoda	Ctenophthalmidae (continued)	***Ctenophthalmus wagneri***, ***Ctenophthalmus proximus***, ***Ctenophthalmus secundus*** **(*C. congerer germinus*)**, ***Doratopsyllus dasycnema***, ***Corrodopsylla birulai****, ***Catallagia dacenkoi***, ***Rhadinopsylla pentacantha***, ***Rhadinopsylla integella***, ***Rhadinopsylla isacantha***, ***Rhadinopsylla pitymydis***, ***Rhadinopsylla eivissensis*** **(*R. masculana eivissensis*)**, ***Rhadinopsylla beillardae***, ***Rhadinopsylla mesa****, ***Rhadinopsylla mesoides***, ***Palaeopsylla soricis***, ***Palaeopsylla similis****, ***Palaeopsylla steini****, ***Palaeopsylla kohauti****, ***Palaeopsylla minor****, ***Stenoponia tripectinata***	Mahnert (1969); Peus (1959); Peus (1964); Rosicky *et al.* (1959); Skuratowicz (1967); Sosnina (1973); Suciu (1969); Whitaker (2007)
Arthropoda	Hystrichopsyllidae	***Typhloceras poppei***, ***Hystrichopsylla talpae***, ***Hystrichopsylla orientalis***, ***Atyphloceras nuperus*** **(*A. nuperum*)**	Brinck-Lindroth and Smit (2007); Skuratowicz (1967)
Arthropoda	Oestridae	***Oestromyia leporina***	Rietschel and Baumann (1975); Schober (1958); Stammer (1956)
Arthropoda	Leiodidae	***Leptinus testaceus***	Artz (1975); Haitlinger (2008)
Arthropoda	Hoplopleuridae	***Hoplopleura acanthopus***, ***Hoplopleura affinis***, ***Hoploplera edentula***, ***Hoplopleura longula****, ***Hoplopleura captiosa****	Beaucournu (1968); Durden and Musser (1994); Wegner (1966)
Arthropoda	Polyplacidae	***Polyplax serrata***, ***Polyplax hannswrangeli*** **(*P. glareoli*)**, ***Polyplax borealis***, ***Polyplax gracilis****, ***Polyplax spinulosa****, ***Polyplax spinigera****	Beaucournu (1968); Durden and Musser (1994); Haitlinger (1983a); Wegner (1966)
Arthropoda	Enderleinellidae	***Enderleinellus nitzschi****	Wegner (1966)
Arthropoda	Trichodectidae	***Trichodectes mustelae*** **(*Stachiella mustelae*)***	Haitlinger (1981)
Arthropoda	Menoponidae	***Myrsidea cornicis*** **(*Myrsidea consimilis*)***	Haitlinger (1989b)
Arthropoda	Ixodidae	***Ixodes ricinus***, ***Ixodes trianguliceps***, ***Ixodes acuminatus***, ***Ixodes apronophorus***,	Martyn (1988); Mihalca *et al.* (2012);

		Ixodes hexagonus, ***Ixodes redikorzevi***, ***Haemaphysalis concinna***, ***Haemaphysalis punctata***, ***Haemaphysalis inermis***, ***Dermacentor marginatus***, ***Dermacentor reticulatus***, ***Hyalomma marginatum***, ***Rhipicephalus bursa***, ***Rhipicephalus pusillus***, ***Rhipicephalus sanguineus*** **s.l.**	Nosek *et al.* (1967); Santos-Silva *et al.* (2011)
Arthropoda	Laelapidae[v]	***Laelaps agilis***, ***Laelaps hilaris***, ***Laelaps jettmari*** **(*L. pavlovskyi*)**, ***Laelaps clethrionomydis***, ***Laelaps micromydis***, ***Laelaps muris****, ***Laelaps algericus****, ***Hyperlaelaps microti*** **(*H. arvalis*)**, ***Hyperlaelaps amphibius****, ***Androlaelaps fahrenholzi***, ***Androlaelaps casalis*** **(*Haemolaelaps casalis*)**, ***Hirstionyssus isabellinus*** **(*Echinonyssus isabellinus*)**, ***Hirstionyssus sunci*** **(*Echinonyssus sunci, Hirstionyssus apodemi*)**, ***Hirstionyssus butantanensis*** **(*Echinonyssus butantanensis*, *Echinonyssus latiscutatus*)***, ***Hirstionyssus soricis*** **(*Echinonyssus soricis*)**, ***Hirstionyssus carnifex*** **(*Echinonyssus carnifex*)***, ***Hirstionyssus talpae*** **(*Echinonyssus talpae*)**, ***Hirstionyssus gudauricus*** **(*Echinonyssus gudauricus*)**, ***Hirstionyssus sciurinus*** **(*Echinonyssus sciurinus*)***, ***Hirstionyssus criceti*** **(*Echinonyssus criceti*)***, *Hypoaspis sardoa* (*Androlaelaps sardous*), *Hypoaspis aculeifer*, *Hypoaspis vacua*, *Hypoaspis claviger*, *Hypoaspis lubrica*, *Hypoaspis karawaiewi*, *Hypoaspis austriacus*, *Hypoaspis astronomica*, *Hypoaspis forcipata*, *Hypoaspis heselhausi*, *Hypoaspis helianthi*, *Hypoaspis oblongus* (*Alloparasitus oblongus*), *Ololaelaps placentula*, ***Myonyssus gigas*** **(*Myonyssus rossicus*)**, ***Myonyssus ingricus****, ***Myonyssus decumani***, ***Haemogamasus nidi***, ***Haemogamasus nidiformes***, ***Haemogamasus horridus***, ***Haemogamasus hirsutus***, ***Haemogamasus hirsutosimilis***, ***Haemogamasus liponyssoides***, ***Haemogamasus ambulans***, ***Haemogamasus mandschuricus*** **(*H. bregetovae*)**, ***Haemogamasus pontiger***, ***Eulaelaps stabularis*** **(*E. oudemansi*)**	Edler and Mehl (1972); Haitlinger (1980); Haitlinger (1983a); Haitlinger (1986a); Haitlinger (1989b); Haitlinger (1997); Haitlinger (2008); Haitlinger (2011); Mašán and Fenďa (2010)

Appendix 8-1. Continued

Phylum	Family	Species	References
Arthropoda	Macronyssidae	***Steatonyssus spinosus****, ***Ornithonyssus bacoti****	Haitlinger (1983b); Haitlinger (2007)
Arthropoda	Eviphididae	*Alliphis halleri*, *Eviphis ostrinus*, *Iphidosoma fimetarium*	Haitlinger (1997); Haitlinger (2008)
Arthropoda	Macrochelidae	*Geholaspis longispinosus*, *Geholaspis alpinus*, *Glyptholaspis americana*, *Macrocheles glaber*, *Macrocheles tardus*, *Macrocheles matrius*, *Macrocheles tridentinus*, *Macrocheles decoloratus*, *Macrocheles montanus*, *Macrocheles rotundiscutis*, *Macrocheles muscaedomesticae*, *Macrocheles punctoscutatus*, *Neopodocinum mrciaki*	Ambros (1987); Ambros (1995); Haitlinger (1977b); Haitlinger (1979); Haitlinger (1983a); Haitlinger (1986a); Haitlinger (1997); Haitlinger (2008); Haitlinger (2011)
Arthropoda	Pachylaelapidae	*Olopachys suecicus*, *Pachylaelaps furcifer*, *Pachylaelaps dubius*	Haitlinger (1997); Haitlinger (2010a); Haitlinger (2010b)
Arthropoda	Veigaiidae	*Veigaia kochi*, *Veigaia cervus*, *Veigaia nemorensis*	Ambros (1995); Haitlinger (1983a); Haitlinger (2006)
Arthropoda	Ologamasidae	*Cyrtolaelaps minor*, *Cyrtolaelaps mucronatus*, *Ologamasus* sp., *Euryparasitus emarginatus*	Mahnert (1971c)
Arthropoda	Melacharidae	*Proctolaelaps pygmaeus*	Haitlinger (2008); Haitlinger (2009a)
Arthropoda	Ascidae	*Asca nova*, *Neojordensia levis*	Haitlinger (1986a)
Arthropoda	Ameroseiidae	*Ameroseius corbiculus*	Haitlinger (2009a)
Arthropoda	Blattisociidae	*Lasioseius berlesei*, *Lasioseius confusus*, *Platyseius italicus*, *Platyseius major*, *Cheiroseius neocorniger*	Haitlinger (2008); Haitlinger (2009b); Haitlinger (2011)
Arthropoda	Phytoseiidae	*Amblyseius* sp., *Typhlodromus meridionalis*	Haitlinger (1983a); Haitlinger (1997)
Arthropoda	Parasitidae	*Pergamasus septentrionalis* (*Amblygamasus septentrionalis*), *Eugamasus magnus*, *Eugamasus berlesei*, *Gamasodes spiniger*, *Holoparasitus excipuliger*, *Holoparasitus*	Ambros (1995); Haitlinger (1983a); Haitlinger (1983b); Haitlinger (1989a);

		pseudoperforatus, *Holoparasitus intermedius*, *Parasitus consanguineus*, *Parasitus loricatus*, *Pergamasus quisquiliarum* (*Parasitus quisquiliarum*), *Pergamasus alpestris*, *Pergamasus brevicornis*, *Pergamasus crassipes*, *Pergamasus longicornis*, *Pergamasus runcatellus*, *Pergamasus runciger*, *Pergamasus mediocris*, *Poecilochirus carabi*, *Poecilochirus subterraneus*, *Poecilochirus necrophori*, *Porrhostaspis lunulata* (*Eugamasus lunulatus*), *Cornigamasus lunaris*, *Vulgarogamasus kraepelini* (*Parasitus kraepelini, Eugamasus kraepelini*), *Vulgarogamasus oudemansi*, *Vulgarogamasus remberti*	Haitlinger (1997); Haitlinger (2006); Haitlinger (2008); Haitlinger (2009a); Haitlinger (2009b); Haitlinger (2010b); Haitlinger (2011); Haitlinger (2015)
Arthropoda	Zerconidae	*Zercon peltatus*	Haitlinger (1989b)
Arthropoda		Uropodina sp.	Haitlinger (1986a); Haitlinger (1989b)
Arthropoda	Trombiculidae	***Neotrombicula autumnalis* (*Trombicula autumnalis*)**, ***Neotrombicula earis***, ***Neotrombicula nagayoi***, ***Neotrombicula talmiensis***, ***Neotrombicula japonica***, ***Neotrombicula inopinata***, ***Neotrombicula vulgaris***, ***Neotrombicula elegans***, ***Leptotrombidium russicum* (*Trombicula russica*)***, ***Leptotrombidium silvaticum***, ***Leptotrombidium europaeum* (*Leptotrombidium intermedia*)**, ***Hirsutiella zachvatkini* (*Trombicula zachvatkini, T. multisetosa, H. multisetosa*)**, ***Miyatrombicula muris***, ***Ascoschoengastia latyshevi***, ***Schoutedenichia anatolicum***, ***Cheladonta ikaoensis***, ***Cheladonta constulata* (*Euschoengastia costulata*)**	Haitlinger (1980); Kepka (1964); Kovacik (1984); Moniuszko and Mąkol (2014)
Arthropoda	Erythraeidae	*Erythraeus regalis*, *Erythraeus phalangoides* (*E. dubiosus*), *Hauptmannia kazimierae*, *Leptus molochinus*, *Leptus clethrionomydis*	Haitlinger (1989b); Haitlinger (1997); Haitlinger (2015)
Arthropoda	Trombidiidae	Trombidiidae sp.	Haitlinger (1997)
Arthropoda	Anystidae	*Anystis baccarum*	Haitlinger (1989b)
Arthropoda	Ereynetidae	***Paraspeleognathopsis bakeri***, ***Speleorodens michigensis* (*Paraspeleognathopsis clethrionomys*)**	Fain and Lukoschus (1968)

Appendix 8-1. Continued

Phylum	Family	Species	References
Arthropoda	Myobiidae[w]	***Myobia murismusculi*** **(*M. musculi*)**, ***Myobia agraria***, ***Myobia multivaga***, ***Radforia lemnia***, ***Radfordia clethrionomys***, ***Radforia lancearia***, ***Radfordia affinis***, ***Radfordia mironovi***, ***Amorphacarus elongatus****, ***Amorphacarus phillipsae*** **(*A. parvisetosus*)***, ***Protomyobia onoi****	Bochkov (1997); Bochkov (2009b); Haitlinger (1980); Haitlinger (1989b); Haitlinger (2009a)
Arthropoda	Cheyletidae	*Eucheyletia flabellifera* (*E. taurica*), *Cheyletus eruditus*	Haitlinger (1983b); Haitlinger (1997)
Arthropoda	Psorergatidae	***Psorergates musculinus***, ***Psorergates muricola***, ***Psorergates apodemi***, ***Psoregates callipidis***, ***Psorergates meati***, ***Psorergates dissimilis***, ***Psorergates arvalis***, ***Psorergates microti***, ***Psoregates agrestis***	Fain *et al.* (1966); Giesen (1990); Lukoschus *et al.* (1967)
Arthropoda	Demodecidae	***Demodex auricularis***, ***Demodex lacrimalis***, ***Demodex longior***, ***Demodex rosus***, ***Demodex corniculatus***, ***Demodex hutteri***, ***Demodex agrarii***, ***Demodex apodemi*** **(*Demodex arvicolae apodemi*)**, ***Demodex gracilentus***, ***Demodex arvicolae***, ***Demodex glareoli***, ***Demodex buccalis***, ***Demodex microti***, ***Ophthalmodex apodemi***	Bochkov (2009b); Izdebska and Rolbiecki (2013a); Izdebska (2012); Izdebska and Rolbiecki (2013c); Izdebska *et al.* (2014)
Arthropoda	Epimyodicidae	***Epimyodex microti***	Fain *et al.* (1982)
Arthropoda	Tetranychidae	Tetranychidae sp.	Haitlinger (1997)
Arthropoda	Pygmephoridae	*Pygmephorus stammeri* (*P. krczali*), *Pygmephorus spinosus*, *Pygmephorus forcipatus*, *Pygmephorus microti*, *Pygmephorus brevispinus*, *Pygmephorus elegans*, *Pygmephorus incognitus*, *Pygmephorus sensillosus*, *Pygmephorus erlangensis*, *Pygmephorus soricis*, *Bakerdania bavarica* (*Pygmephorus bavaricus*), *Bakerdania cultrata* (*Pygmephorus cultratus*), *Bakerdania sellnicki*, *Bakerdania amplus*	Haitlinger (1983a); Haitlinger (1997); Smiley and Whitaker (1984); Stammer (1956)
Arthropoda	Siteroptidae	*Siteroptes liliarum* (*Pygmephorus liliarum*)	Stammer (1956)
Arthropoda	Tarsonemidae	*Tarsonemus* sp.	Haitlinger (1997)

Arthropoda	Myocoptidae	***Myocoptes japonensis***, ***Myocoptes musculinus***, ***Trichoecius clethrionomydis***, ***Trichoecius tenax***, ***Trichoecius apodemi***, ***Trichoecius blaszaki***, ***Trichoecius widawaensis, Criniscansor apodemi***	Bochkov (2016)
Arthropoda	Listrophoridae	***Listrophorus brevipes***, ***Listrophorus leuckarti***, ***Listrophorus mediterraneus***, ***Afrolistrophorus apodemi***	Bochkov (2010)
Arthropoda	Gastronyssidae	***Yunkeracarus apodemi***, ***Yunkeracarus microti***	Bochkov (2010)
Arthropoda	Sarcoptidae	***Notoedres musculi***	Bochkov (2010)
Arthropoda	Pneumocoptidae	***Pneumocoptes tiollaisi***	Bochkov (2010)
Arthropoda	Glycyphagidae	***Lophioglyphus liciosus*** **(*Apodemopus apodemi*)**, *Glycyphagus domesticus*[x], *Glycyphagus ornatus*, *Glycyphagus hypudaei* (*Labidophorus hypudaei, Myacarus arvicolae*), *Labidophorus talpae*, *Orycteroxenus soricis*, *Xenoryctes punctatus*, *Xenoryctes krameri* (*Labidophorus oudemansi*)	Haitlinger (1977b); Haitlinger (1986a); Haitlinger (2007); Haitlinger (2015); Stammer (1956)
Arthropoda	Acaridae	*Tyrophagus dimidiatus*, *Tyrophagus infestans*, *Tyrophagus humerosus*, *Mycetoglyphus fungivorus*, *Acotyledon pedispinifer*, *Acarus nidicolous*, *Acarus farris*, *Acarus siro* (*Tyroglyphus farinae*), *Rhizoglyphus echinopus*	Haitlinger (1983a); Haitlinger (1989b); Haitlinger (2011); Stammer (1956)
Arthropoda	Histiostomatidae	*Prowichmannia spinifera* (*Wichmannia spinifera*), *Pelzneria crenulata*, *Copronomoia sphaerocerae* (*Bonomoia sphaerocerae*), *Histiostoma sapromyzarum* (*Anoetus sapromyzarum*)	Haitlinger (1983a); Stammer (1956)
Arthropoda	Damaeidae	*Belba corynopus*, *Belba verticillipes*	Haitlinger (1983a)
Arthropoda	Camisiidae	*Platynothrus pelifer*	Haitlinger (1983a)
Arthropoda	Nothridae	*Nothrus silvestris*	Haitlinger (1983a)

[a] Sequences of the zoonotic *Giardia intestinalis* were mentioned in Bajer (2008) but remained unpublished

[b] *Myodes* and *Microtus* spp. were only infected experimentally by Matuschka (1986), but are very likely intermediate hosts

[c] Reports from other intermediate hosts than *Mus musculus* probably erroneous according to Levine and Tadros (1980)

[d] The rat parasite *Hepatozoon muris* was mentioned from *A. sylvaticus* in Great Britain in the review of Cox (1970) but not in that of Cox (1987). Hence, the record in wood mice is likely erroneous

[e] *Alaria alata* is potentially zoonotic, since other *Alaria* spp. cause disease in North America, but there are currently no reported cases in humans

[f] *Skrjabinoplagiorchis vigisi* likely also occurs in these rodents in Europa

[g] *Notocotylus wetlugensis* is known from bank voles from Western Russia, but it is a *species inquerenda* according to Simón-Vicente *et al.* (1985)

[h] Most or all reports of *N. noyeri* in rodents are likely another *Notocotylus* sp. according to Simón-Vicente *et al.* (1985)

[i] Reports of *Hymenolepis diminuta* are likely misidentifications in these rodents, since this is a rat parasite according to Tenora (2004) and Haukisalmi (2015)

[j] The zoonotic dwarf tapeworm *Rodentolepis nana* is closely related and morphologically indistinguishable from *R. fraterna*, but human isolates are non-infective for mice and rats and only barely infective for typical intermediate hosts (Macnish *et al.*, 2002a; Macnish *et al.*, 2002b). Hence, reports of *R. nana* in these rodents are likely misidentifications

[k] The zonotic tapeworm *Rodentolepis microstoma* was synonymised with *R. straminea* by Baer and Tenora (1970), but these are two distinct, although hardly distinguishable species (Casanova *et al.*, 2001). Since *R. microstoma* is a specific parasite of *Mus musculus*, reports in other rodents are likely erroneous

[l] The report of *Staphylocystoides stefanskii* in field voles in Poland (Bluszcz *et al.*, 1987) was questioned by Pojmańska *et al.* (2007), since this is a parasite of insectivores

[m] The report of the fat dormouse parasite *Hymenolepis myoxi* in bank voles (Pojmańska *et al.*, 2007) requires reexamination after the redescription by Makarikov (2017)

[n] *Paruterina candelabraria* probably also occurs in these rodents in Europe

[o] Reports of *Syphacia obvelata* in other hosts than house mice are likely misidentifications according to Stammer (1956), Tenora (2004) and Stewart *et al.* (2017)

[p] Report of the snow vole oxyurid *Aspiculuris dinniki* in bank voles (Mažeika *et al.*, 2003) are most likely *A. tianjinensis*, which was described after that time

[q] *Toxascaris leonina* probably also occurs in these rodents in Europe

[r] Most or all reports of *Pelodera strongyloides* (*Rhabditis strongyloides*) from these rodents are one of the other two species of *Pelodera* (Sudhaus *et al.*, 1987)

[s] *Pelodera nidicolis* occurs in the nest of field voles and may be phoretic as well according to Sudhaus *et al.* (1987)

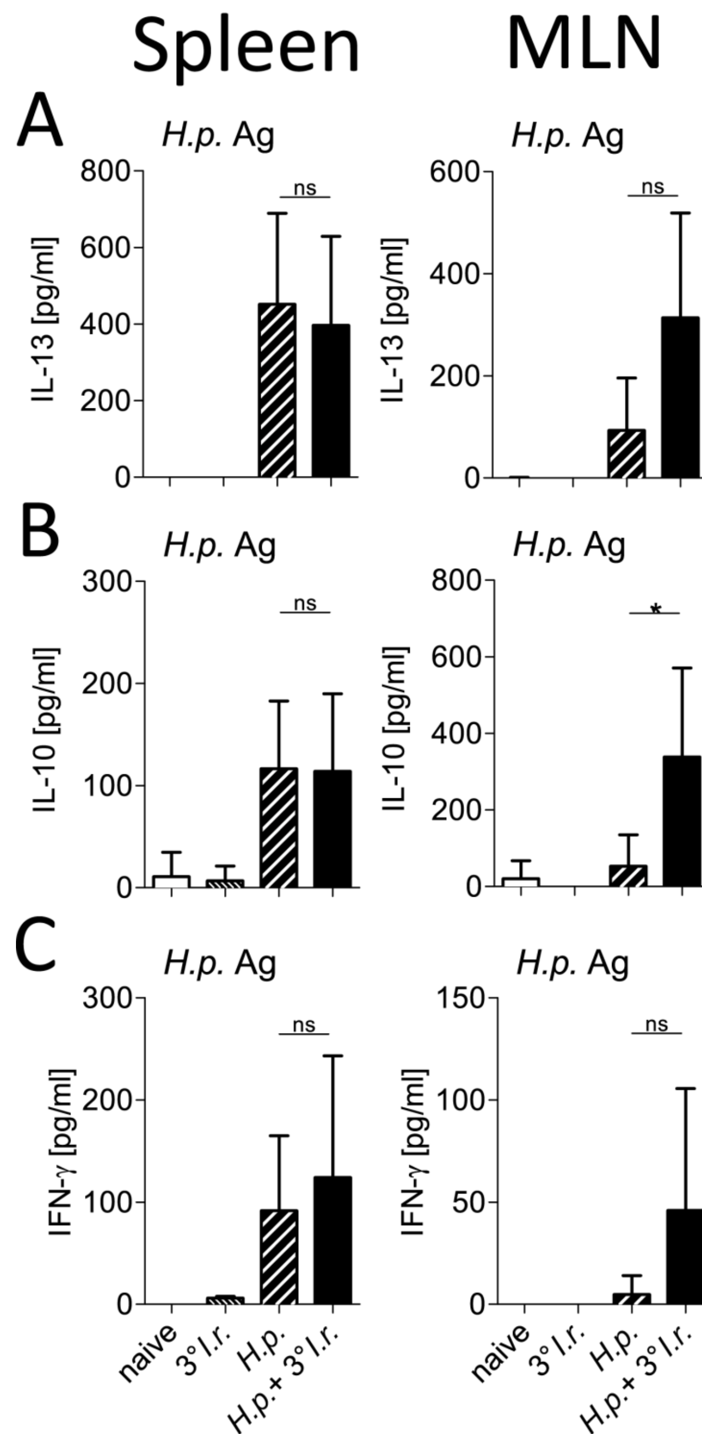
[t] *Heligmosomum halli* is either a synonym of *H. costellatum* according to Tenora *et al.* (2002) or of *H. mixtum* according to Haukisalmi and Henttonen (2000)

[u] A further undetermined nematode of the Metastrongyloidea was detected in the epididymides of wood mice (Clarke *et al.*, 2004)

[v] Report of *Hirstionyssus musculi* are most likely *Hirstionyssus sunci* or *Hirstionyssus butantanensis* according to Mašán and Fenďa (2010)

[w] Detections of *Amorphacarus elongatus*, *A. philipsae* and *Protomyobia onoi* by Ryszard Haitlinger were considered inaccurate by Bochkov (2009b)

[x] *Glycyphagus domesticus* can cause allergic dermatitis called “grocer’s itch” in high densities (Krantz & Walter, 2009)



Appendix 8-1. Systemic and local cytokine responses of mice infected with *H. polygyrus* and *I. ricinus*. The production of IL-13 (A), IL-10 (B), and IFN-γ (C) was measured in bulk culture supernatants of spleen and mesenteric lymph node (MLN) cells (left and right, respectively) by ELISA after specific stimulation with *H. polygyrus* antigen. Means and SDs are shown. Symbols represent the intracellular cytokine responses detected in cells from individual mice. Differences between groups were tested using an unpaired t test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. Data are for 5 or 6 mice per group.