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eingereicht über
den Fachbereich Veterinärmedizin
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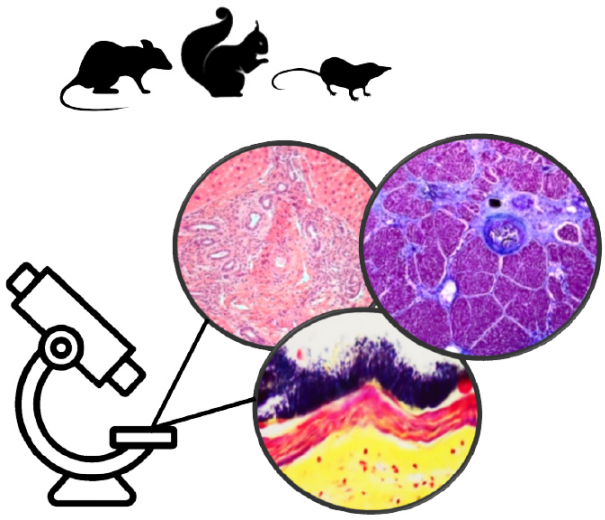
Disease occurrence in small mammals from Northern Borneo: Histopathology and selected pathogens

Inaugural-Dissertation
zur Erlangung des Grades eines Doktors der Veterinärmedizin
an der Freien Universität Berlin

vorgelegt von
Paula Ortega Pérez
Tierärztin aus Madrid, Spanien



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To my parents, brother and husband, for always supporting me.

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LIST OF ABBREVIATIONS

bp	base pair
cPCR	conventional polymerase chain reaction
DNA	deoxyribonucleic acid
Ev:	Evaluated
Fig.	figure
h	hour
kbp	kilo-base pair
min	minute
mRNA	messenger RNA
nm	nanometre
ND	not determined
PCR	polymerase chain reaction
qPCR	real-time polymerase chain reaction
RNA	ribonucleic acid
sec	second
sp.	single species
spp.	multiple species
Tab.	table
µm	micrometre

1 INTRODUCTION

Small mammals are key for the maintenance of the ecosystem balance. Their influence in the flora by pollinizing and dispersing seeds through contact and faeces is crucial for the preservation of the vegetation species diversity (Wells and Bagchi, 2005). Moreover, the bottom position small mammals occupy in the trophic pyramid, define them as essential prey for the survival for a wide variety of predator species such as reptiles, mammals, and birds (Burgin et al., 2017; Denys et al., 2017; Hawkins, 2017; Koprowski et al., 2016). These interactions occurring between prey and predator are also needed for parasite species which require different hosts to complete their life cycle (Mehlhorn, 2016). Beyond the prey-predator relationship, small mammals also interact with other small mammal species inhabiting the same or neighbouring areas as well as interactions with pathogens and vectors, influencing the transmission of diseases from different species as well as taxa groups (Suzán et al., 2015). From the public health perspective, within small mammals, rats are responsible for the transmission of numerous zoonotic pathogens as a consequence of their role as reservoir (Battersby, 2015; Han et al., 2015; Herbreteau et al., 2012). However, they are not an exclusive reservoir and to the date, the range of small mammal species which potentially can become reservoir hosts remains unknown (Han et al., 2015). Besides the implication of the reservoir species in zoonotic diseases, modifications in the ecosystem based on deforestation and fragmentation, can also significantly influence the transmission of infectious pathogens (Jones et al., 2013; Patz et al., 2000). For example, in anthropogenic disturbed habitats some small mammals species such as the commensal rats *Rattus* spp. and the shrew *Suncus murinus*, have successfully adapted to these environments, increasing their population (Khanam et al., 2017; Morand et al., 2015; Rubio et al., 2014). The close proximity between human and reservoir species inhabiting in urban areas, together with the high prevalence of zoonotic pathogens these species carry, become a risk for human health (Firth et al., 2014; Tung et al., 2013). In anthropogenically disturbed environments it is crucial to study not only the presence of pathogens in reservoir species from urban areas, but also from small mammal species inhabiting adjacent territories in order to understand the general occurrence of pathogens and potential interspecific exchanges, because species inhabiting urban areas also establish contact with other small mammal species (Blasdell et al., 2019; Bordes et al., 2015).

Considering that habitat fragmentation can have a significant effect on pathogen occurrence, the study of subclinical diseases in small mammal species inhabiting an anthropogenic modified environment becomes essential to understand not only the pathogens that these species are exposed to, but also the effect they might have on the health status of these animals. Small mammals are exposed to multiple pathogens which can cause diseases in the

animals, negatively influencing their health (Mühldorfer et al., 2011; Seguel et al., 2017). The evaluation of subclinical diseases provides the crucial information to understand the morbidity range among the different species (Rothenburger et al., 2015a, 2015b).

In this study, we investigated pathological changes affecting 15 small mammal species including rodents, treeshrews and shrews, captured along a land-use gradient from urban to forest areas in Northern Borneo. This tropical territory has faced a severe environmental degradation caused by anthropogenic pressure based on the expansion of urban territories and the transformation of forest into agricultural land (Dirzo et al., 2014). Although Northern Borneo has been the scenario of multiple studies focussing on the habitat of a diverse range of small mammal species (Meiri et al., 2008; Wells et al., 2007, 2006), the occurrence of subclinical changes or clinical diseases and their impact on the general health of the different species remains unknown. The ecological background of the species included in this study, provides information about the species distribution, classifying them in five ecological groups: forest rodents and forest treeshrews, generalist *Sundamys muelleri* found in all areas along the land-use gradient, the urban *Rattus* genus and the urban shrew *Suncus murinus* (Wells et al., 2014).

Considering the importance of small mammals as areservoir for zoonotic pathogens, a parasite host and prey species, it is vital to understand their natural diseases occurrence. In contrast to molecular studies based only on pathogen detection, the superiority of a histopathological analysis is not only the evaluation of lesions observed in different organ tissues but also the assessment of possible aetiologies. The histopathological evaluation gives precise information on the condition of the organs and allows scoring the overall impact of the detected pathological changes as an estimate of the individual health status. The integrative study of the subclinical pathologic lesions of the species in combination with their ecological information allows determining the health status of the species inhabiting the different territories from the forest to the urban area. The accurate evaluation of the health status combined with the ecology of the animals can also indicate a possible relationship between the habitat in their pathological lesions.

Since wildlife species are exposed to a wide variety of pathogens, it is important to investigate multiple pathogens in the host. The detection of multiple pathogens from animal species living along disturbed environment gradients can also improve understanding pathogen epidemiology (Bordes et al., 2015). In this context selected pathogens belonging to major pathogen groups (bacterial, protozoa and viruses) were chosen for this study to exemplarily investigate pathogen burdens: *Leptospira* spp., *Trypanosoma* spp, *Sarcocystis* spp and herpesviruses.

This study is a continuation of Dr. K. Wells' project ""Host-parasite interactions and rodent-borne diseases: native and invasive small mammal occurrence and parasitism along land use gradients from natural to anthropogenic habitats in Borneo" (Wells et al., 2014). All histopathologic and electron microscopic investigations as well as nucleic acid detection of *Leptospira* spp., trypanosomes and *Sarcocystis* spp. were performed at the IZW. For *Sarcocystis* spp., phylogenetic evaluations were carried out by Dr. T. Jäkel, (University of Hohenheim, Germany) and the description of a new *Sarcocystis* species was elaborated under his supervision. Nucleic acid investigations on herpesviruses were basis to a bachelor thesis at the Robert Koch Institute (Berlin, Germany) by Heiko Pietsch, supervised by Dr. Andreas Kurth.

2 HYPOTHESIS AND AIMS

Small mammals are naturally exposed to pathogens which can lead to the development of diseases however, little is known about their influence in small mammals' morbidity. As both pathogen and host ecological dynamics are modified by environmental degradation (Bordes and Morand, 2011; Jones et al., 2013; Patz et al., 2000), we postulate that the pathogens impact on the animals' well-being differs depending on the grade of anthropogenic modification of the habitat they live in, with forest animals being healthier than urban animals. Thus, we aimed to elucidate the health status of small mammals living along a land-use gradient in Northern Borneo by evaluating histopathological changes in their organs. The study of the natural disease occurrence together with the ecological background aids to understand whether the ecosystem can have an effect on the morbidity of small mammals.

Every habitat contains specific challenges for the animals living in the given environment like food availability, population density, level of pollution etc. Alongside, pathogen distribution will be realted to the environmental background. Therefore, we hypothesize that the occurrence of pathogens differs between forest and urban species.

Pathogen transmission among wild small mammals is not only influenced by the environment, but also by the interactions with other individuals. The distribution of small mammals can facilitate interaction not only among animals from the same habitat, but also between species living in different areas due to the capacity of generalist species to bridge interaction between individuals inhabiting distant territories. Hence, we predict that pathogens investigated in this study (*Leptospira* spp., *Trypanosoma* spp., *Sarcocystis* spp. and herpesvirus) will follow the land-use gradient, with the highest presence in urban areas. In order to elucidate the presence of these pathogens in different species we have combined different methods including histopathology, electron transmission microscopy and molecular techniques.

3 LITERATURE REVIEW

3.1 Tropical rainforest

Tropical forests are located in the latitudes of the Tropic of Cancer (latitude 23,5 °N) and the Tropic of Capricorn (latitude 23,5 °S) (Perry et al., 2008). This geographic location provides exceptional climate conditions based on unvaried and high moisture and warmth, needed to create an extraordinary environmental richness. In contrast to the temperate zones where seasonality is divided in four periods, the seasons in the tropical forest are divided according to precipitations into dry and wet (Newman, 2002). For example: within the dry season, the driest month in Borneo is August with 52 mm of rainfall, in contrast with an average of 474 mm rainfall register in March, the month with the highest precipitation of the wet season. Both seasons are characterized by high temperature around 23 ºC and 32 ºC in dry and wet season (Climate-Data.org., 2019).The high temperature together with the elevated humidity (71.7%) permit vegetation to remain evergreen during the entire year (Newman, 2000).

Aside climate conditions, there are two other crucial environmental factors involved in the structure of tropical forest: soil and light (Perry et al., 2008). The soil represents an essential element in the development and preservation of the terrestrial ecosystem (Perry et al., 2008). The singularities of the soil from tropical forest are based on the low mineral concentration and high heterogeneity of the microbiota (fungi and bacteria) (Camenzind et al., 2018). Soil microorganism activities, as well as their interactions (i.e. mutualism) play a fundamental role in the composition and structure of the soil (Cleveland et al., 2013; Savage, 1987; Wall et al., 2008). Although light intensity in the equator area is very high, the tropical forest has developed a system to distribute the light concentration among the different layers of the forest. The top of the canopy works as an umbrella absorbing most of the light and only letting pass a small percentage to layers beneath (Thery, 2001). Specially the ground layer, which receives around 0.1 to 1.9% of light in comparison to the top layer (Bazzaz and Pickett, 2003). This modification of light intensity and spectrum found from the top canopy to the ground influences significantly the life of animals and plants (Ribeiro et al., 2005).

The combination of water, soil and light provides the perfect scenario for the development of immense vegetation species richness, with approximately 250 plant species per hectare, and hosting almost 50% of the total fauna diversity of the world (Chazdon et al., 1996; Zakaria et al., 2016). This vegetation variety is the basic energy supply in the food web from primary consumers (herbivores) to secondary consumers (carnivores), interconnecting the different components of the ecosystem. However, all the components of the tropical rainforest ecosystems are threatened by accelerated habitat destruction. To better understand the unique environmental status of tropical forests in the world, Myers defined them as “hot-spot”,

based on the species richness (animals and plants) in combination with the high rate of degradation (Myers, 1988).

In Southeast Asia four hot-spots are described: Indo-Burma, Sundaland, Wallacea, and the Philippines (Myers et al., 2000; Woodruff, 2010), which are also suffering from high rates of deforestation (Sodhi et al., 2010; Wilcove et al., 2013). But, not all territories are equally affected. The island of Borneo is the most threatened area in the Sundaland region, with an almost two times faster deforestation rate in comparison to other tropical rainforests (Achard, 2002; Gaveau et al., 2014). The development of intense forest degradation simultaneously with a rapid expansion of the urban and suburban areas into rural and forest landscapes has a significantly negative impact on the forest ecosystem (Gaveau et al., 2014).

3.2 Urban areas in the tropics

In the last decades, urban territories in tropical region have experienced a rapid growth through the expansion of cities into rural and forest areas, and the increment of their population growth. Considering tropical countries, Malaysia stands out in the dramatic raise of its urbanization level from 34% in 1980 to 71% in 2010 (Siwar et al., 2016). The change of the urban economical model from agriculture to industrialization has been responsible for massive urbanization in Southeast Asian territories (Drakakis-Smith, 1997; Elhadary et al., 2013; Ghani, 2002). This rapid urban development in tropical regions has often been associated with inadequate sanitation infrastructures, that led to multiple problems including environmental pollution and deficient hygienic conditions (Al-Mekhlafi et al., 2007). This situation is aggravated in suburban slum areas, which has favoured the transmission of infectious agents, some of them zoonotic (Haines et al., 2006; Maciel et al., 2008).

Disease outbreaks in Southeast Asian urban and suburban areas are commonly related to floods, occurring after adverse environmental conditions such as heavy rains during the wet season (Ahern et al., 2005; Torti, 2012). The accumulation of stagnant water during these natural disasters is the perfect scenario for a rapid development of pathogen vectors such as mosquitos, increasing the prevalence of vector-borne diseases (Acuin et al., 2011). At the same time, flooding also augments the occurrence of rodent-borne infectious diseases such as leptospirosis (Boey et al., 2019; Koay et al., 2004; Sarkar et al., 2012).

3.3 Borneo

From the different regions in Southeast Asia, the island of Borneo exemplifies the land transformation in all the territories: in the cities due to rapid urbanization, rural areas with cropland expansion, and the tropical rainforest as a consequence of deforestation (Gin, 2011).

Since the 1960s the rainforest of Borneo has suffered a severe land-use transformation as a result of the intensive logging extraction for valuable timber (Brookfield and Byron, 1990). This environmental degradation is aggravated by forest fires, which increase considerably in deforested forests (Wooster et al., 2012). After logging, the land is rapidly used for oil-palm (*Elaeis guineensis*) plantations (Gaveau et al., 2016), which create one of the most important national incomes (Wahid et al., 2006).

However, the ecological impact does not affect all parts of the island equally. Comparative studies investigating the impact of degradation form the different areas of the island showed Northern Borneo (Fig. 1) as the most affected (Bryan et al., 2013; Labrière et al., 2015). This area has suffered from a substantial environmental transformation mainly resource depletion and habitat fragmentation (Gaveau et al., 2014; Labrière et al., 2015). As a result, only 8% of the Northern area is covered by intact forest (Bryan et al., 2013).

Fig. 1: Global map with the island of Borneo magnified in the frame. The Malaysian territories of the island are the state of Sarawak and Sabah. Kota Kinabalu is the capital of the state of Sabah. Modified images from https://d-maps.com/carte.php?num_car=241753 and https://www.freepik.com/free-photos-vectors/travel.

These environmental modifications have a catastrophic effect on wildlife habitats (Gibson et al., 2013; Mckinney, 2008). Overall environmental threats, urbanization is strongly correlated with the decrease of species richness and their population (Czch et al., 2006; Kitzes and Shirley, 2016; McKinney, 2008). Moreover, an ecological study conducted by Wells et al. (2014) along a broad territory of Northern Borneo, which included different types of habitats,

showed how modification on the landscape of Northern Borneo also affected the small mammal species distribution (Wells et al., 2014). In their study the different habitats were classified according to the land-use types in different gradients from urban areas to the forest.

The results from this study showed that urban and suburban areas were the preferred habitat for the invasive *Rattus* spp. (including *Rattus rattus*, *R. norvegicus* and *R. tiomanicus*) and the shrew *Suncus murinus*, while forest environment is the chosen habitat of native small mammal species (treeshrews, squirrels and murids). One exception is the native generalist rat *Sundamys muelleri,* which is found in all land-use gradients from forest to suburban and urban areas.

3.4 Small mammal species: main characteristics

The island of Borneo is the habitat of the highest concentration of mammalian species (n=230) in Southeast Asia (De Bruyn et al., 2014). However, this biodiversity is threatened due to environmental degradation as well as a dramatic raise of hunting pressure (Harrison et al., 2016; Mckinney, 2008).

From the habitat perspective, small mammals can be found in all three dimensions of a tropical forest: terrestrial, low above-ground and arboreal space (Wells et al., 2014). Because for most of the species the habitat is not restricted to a single forest layer, there is an existing interaction among small mammals living in the same area (Kelt et al., 1995). At the same time, generalist species, which live in a wide range of habitats, interact with species located in different habitats becoming the link between them (Bordes et al., 2015). These interactions can favour a pathogen transmission among the different animals leading to a cross-species transmission (Bordes et al., 2015).

The ecological information of the small mammal species can elucidate possible interactions between the species in their habitat. Because these interactions can influence the occurrence of pathogen, it is important to consider multiple species in a study in order to understand the pathogens and resulting diseases existing among small mammals. For this reason, we included 15 different small mammal species from the families Sciuridae, Muridae, Tupaiidae and Soricidae with differing ecological background. A summary of the animal species can be found in Tab. 1.

3.4.1 Family Tupaiidae

Systematics

The Tupaiidae family is composed of 22 species originating from Southeast Asia, ten of these species can be found on the island of Borneo (Payn et al., 2016). From the evolutionary point of view, this family has been called “living fossil” due to similarities of the fossil ancestors to the current treeshrew species (Hawkins, 2017). However, in contrast to the other small mammal families from this study (Family Sciuridae, Muridae and Soricidae), the phylogeny of the Scandentia Order is ambiguous (Kriegs et al., 2007; Song et al., 2012). The unclear phylogenetic position of treeshrews with regard to the other mammalian orders makes it not only difficult to evaluate their evolution, but their genetic relationship with other mammal species. An initial morphologic classification included them in the Order Primates. This was later modified, classifying them in their own order Scandentia (Dagosto, 1991). Current discussions concern the unique phylogenetic position of *Tupaia* spp. and the difficulty to determine whether they are closest relatives to primates or to rodents remains unresolved (Kriegs et al., 2007; Madsen et al., 2001; Song et al., 2012). The Euarchonta theory proposes to include the Scandentia order in the primate group (Song et al., 2012), meanwhile other studies even suggest that treeshrews are phylogenetically closer to lagomorphs (Madsen et al., 2001; Schmitz et al., 2000).

Habitat

Treeshrews inhabit different type of forests in Southeast Asia from mountain to low-elevated forests (Hawkins, 2017). In our investigation we focused on three treeshrew species (*Tupaia gracilis Tupaia minor* and *Tupaia tana*) inhabiting the lower altitudes (<1000 m) from the Northern tropical forest of Borneo. Their ability to climb provides a broad habitat utilization (Wells et al., 2004), being able to set their nests from the arboreal heights (*Tupaia minor)* to above-ground (*Tupaia tana* and *Tupaia gracilis*) (Emmons, 2000; Wells et al., 2006). The three species of treeshrews included in this study are classified as least concern according to the IUCN list (IUCN 2020).

Generalities: diet, reproduction and predation

Tupaia spp. include in their diet a wide variety of food: fruits, invertebrates and also small vertebrates such as small lizards or birds (Payn et al., 2016). The reproduction period of treeshrews is markedly seasonal, occurring mostly in the wet season due to the increase of food availability, with a small litter of one to three offspring (Emmons, 2000).

Their diurnal lifestyle and their small size make *Tupaia* spp. prey for many different predators including mammalian carnivores, snakes and lizards (Emmons, 2000).

3.4.2 Family Sciuridae

Systematic

The family Sciuridae is one of the largest mammalian families consisting of 273 species (Koprowski et al., 2016; Moore, 1959). The current phylogeny based on molecular markers classified this family into five subfamilies: Ratufinae (Oriental giant squirrels), Sciurinae (tree squirrels and true flying squirrels), Sciurillinae (neotropical pygmy squirrel), Callosciurinae (Asian ornate squirrels) and Xerinae (terrestrial squirrel) (Hawkins et al., 2016; Steppan et al., 2004).

The island of Borneo hosts 34 squirrel species from the subfamilies Ratufinae, Callosciurinae, and Sciurinae (Payn et al., 2016). Although the molecular biology has helped to elucidate the phylogeny of this family, the high diversity of Bornean squirrels needs further investigation to better understand their evolution (Hawkins et al., 2016).

Habitat

The three squirrel species included in this thesis (*Callosciurus notatus*, *Callosciurus prevostii* and *Sundasciurus lowii*) live in Northern Borneo. All present diurnal behaviour and can be found in lowlands and hills with an altitude lower than 1400 m. The majority of their activities occurs in the arboreal habitat and they only descend to the ground when there is a gap between trees (Payn et al., 2016). The three squirrel species are classified as least concern according to the IUCN list (IUCN 2020).

Generalities: diet, reproduction and predation

Their diet includes fruits with high concentration of sugar and oil, as well as a variety of insects (ants, termites, and beetles). The species *Callosciurus notatus* has adapted to anthropogenic modified Bornean ecosystems, being able to live and feed from monoculture plantations (Payn et al., 2016). Similar to treeshrews, the food availability is the main limiting factor for the reproduction in squirrels. When food is easily accessible, they are able to breed up to three times per year worth up to seven offspring (Koprowski et al., 2016).

From the different types of animals that prey on squirrels, including terrestrial carnivores and raptors, snakes are defined as the main predator (Becker et al., 1985).

3.4.3 Family Muridae

Systematic

The family Muridae is exceptionally heterogeneous, composed by 155 genera, 816 species and at least 916 extant taxa (Denys et al., 2017).

For the last decades, the definition of new species and genera by molecular techniques has helped to elucidate the taxonomy of this family (Monadjem et al., 2015). However, the genetic classification has faced multiple obstacles, for example, the initial misclassification of the reference species which are essential for the construction of the phylogenetic tree, or the lack of consensus to determine the phylogenetic relationship based on the rate of genetic distance (Pagès et al., 2010). Although the evaluation of the morphological characteristics allows to differentiate multiple rodent species (Musser and Carleton, 2005) they are not sufficient to discriminate the *Rattus* spp. (Aplin et al. 2003; Pagès et al., 2010). Within the Muridae family, the species belonging to the *Rattus* group represent the most diverse group, composed of 65 *Rattus* species and 120 other species (Denys et al., 2017). The species from the *Rattus* group originate from South and Southeast Asia and the majority of the species can be found in this region (Myers et al., 2000). The main phylogenetic feature of the species of the *Rattus* group is the high divergence of numerous phylogenetic lines in a relatively short geological period (some tens of millions of years) (Aplin et al., 2011). The possible explanation for this phylogenetic event could be the lack of complete reproductive barriers between these species, which tolerates species hybridization (Lack et al., 2012). Although multiple studies have tried to elucidate the phylogeny of the Muridae family the genetic complexity within the members of this family will require further studies to establish the full extent of their phylogenetic relationships (Kergoat et al., 2018).

Habitat

a) Forest species have a nocturnal behaviour and are able to occupy the three-dimensional space from terrestrial habitat (*Maxomys* spp.), above the ground (*Niviventer* spp.), to the arboreal space (*Leopoldamys sabanus*). Although they usually occur in primary and secondary forests, *Maxomys suriferi*, and *Maxomys whiteheadi* can also easily be found in rice plantations. Meanwhile, *Maxomys rajah* is associated to areas near the coast (Payn et al., 2016; Wilson et al., 2006). The environmental degradation occurring in Northern Borneo also affects the population of the forest murid species *Maxomys rajah, Maxomys whiteheadi* and *Niviventer cremoriventer*, which are now classified as vulnerable according to the IUCN red list (IUCN 2020). In contrast to the species *Leopoldamys sabanus* and *Maxomys suriferi*, which are registered as least concern (IUCN 2020).

b) *Sundamys muelleri* is a nocturnal terrestrial species with a broad habitat distribution. In contrast to the forest rodents, *Sundamys muelleri* is a generalist species and can be found in a high variety of habitats: from forests to anthropogenic disturbed environments including housing areas with nearby vegetation (Wells et al., 2014). This species is classified as least concern (IUCN 2020).

c) Members of the *Rattus* spp. are predominantly nocturnal, mostly terrestrial and are found in the proximity of human settlements (Payn et al., 2016). The species *Rattus rattus* and *Rattus norvegicus* are highly adapted to human environments and therefore classified as commensal species (Aplin et al. 2003). Another *Rattus* sp., *Rattus tiomanicus*, is more related to agricultural fields, where it is considered as an opportunistic pest (Singleton et al., 1999). Species from the *Rattus* group are classified as least concern (IUCN 2020).

Generalities: diet, reproduction and predation

The forest species and *Sundamys muelleri* have an omnivorous diet based on different insects, crabs and land snails as well as a variety of fruits, seeds and vegetable matter (Kitamura et al., 2006; Medway, 1969; Nakagawa et al., 2007). Members of the *Rattus* group feed on a wide range of food resources available in anthropogenic environments, being considered as highly opportunistic (Aplin et al. 2003).

Sundamys muelleri and the forest rodents have a seasonal reproduction and litter have fewer offspring, for example *Sundamys muelleri* give birth to 3 to 4 young (Denys et al., 2017; Tanner, 1990). In contrast, *Rattus* spp. can breed the year round, having approximately five litters per year of four to eight young (Feng and Himsworth, 2014; Tamarin and Malecha, 1972).

All members of the family Muridae are considered prey for a broad spectrum of predators, from mammalian carnivores to birds of prey and reptiles such as snakes and lizards (Denys et al., 2017).

3.4.4 Family Soricidae

Systematic

The family Soricidae is formed by 448 species grouped in three subfamilies: Soricinae, Crocidurinae and Myosoricinae (Solari and Baker, 2007). The shrew species included in this project, *Suncus murinus* belongs to the subfamily Crocidurinae and the genus *Suncus,* which contains 19 species (Burgin et al., 2017).

In contrast to the family Muridae, the phylogeny of the family Soricidae is well defined. The use of molecular techniques has considerably improved the knowledge of the phylogenetic relationship between the different species (Dubey et al., 2007; Ohdachi et al., 2006).

Habitat

The shrew *Suncus murinus* (common name Asian House Shrew) originates from India, however, their commensal lifestyle has facilitated their distribution throughout entire Asia (Heim de Balsac and Lamotte, 1956). In Southeast Asia they are commonly seen in anthropogenic habitats like agricultural fields or urban areas and housing (Ruedi et al., 1996). This species is registered as least concern (IUCN 2020).

Generalities: diet, reproduction and predation

Physiologically, shrews have a very high metabolic rate in comparison to other small mammals like rodents. Their body demands continuous intake of food to maintain their basal metabolism (Buckner, 1964). For this reason, they have developed a continuous diurnal and nocturnal lifestyle to maximize the feeding hours. They feed on a broad spectrum of prey including vertebrates like frogs or lizards, as well as invertebrates, mainly insects and snails, providing an easily accessible food source (Burgin et al., 2017).

Suncus murinus can breed year-round, however, there is a reduction of the breeding activity during the wet season due to a decrease of light hours and lower food availability (Rissman et al., 1987; Temple and Rissman, 2000). The litter size ranges between two to three (Hasler et al., 1977).

Shrews are prey for different predators such as birds, snakes and mammalian carnivores like weasels and minks (Burgin et al., 2017).

3.5 Small mammals and human health

From the animals included in this study, the family Muridae has been considered the most relevant in terms of Global Health due to the high number of species which serve as vector or reservoir for numerous pathogens that can affect humans, domestic animals and wildlife. To the date, 66 zoonotic pathogens including viruses, bacteria, fungi and parasites (helminths and protozoa) have been detected in murid species (Han et al., 2015).

Some of these potential reservoir species (i.e. *Rattus* spp.) are usually found in urban areas, which increases the probability of pathogen transmission to humans (Morand et al., 2019). The rats' ability to maximize resources available in disturbed anthropogenic environments (Aplin et al., 2003; Himsworth et al., 2013b) facilitated their proliferation, making them one of the most relevant pest species in Southeast Asia (Singleton et al., 1994). Beside rats, other species such as the commensal shrew species *Suncus murinus* are also host for relevant pathogens like the parasites *Angiostrongylus cantonensis* and *Taenia taeniaeformis* or the

bacteria *Yersinia pestis* and *Leptospira* spp. (Mayer-Scholl et al., 2014; Rahelinirina et al., 2017; Tung et al., 2013).

The interaction between reservoir species and other small mammal species increases the risk of pathogens transmission. Certain species, such as generalist species with the capacity to live in different habitats, can potentially become a vector for pathogen transmission for species inhabiting distant environments, which naturally would not interact (Bordes et al., 2015). Among the species of this study, *Sundamys muelleri* is the species which connects small mammal species from urban areas, such as *Rattus* spp. and shrews, with species inhabiting the forest. The lack of information of the range of species serving as reservoir hosts and the possible risk of pathogen transmission between well-known reservoirs (i.e. *Rattus* spp.) and potential hosts (forest rodents), encouraged to also investigate the presence of pathogens with zoonotic potential in these multiple species.

3.6 Health status

Small mammals are naturally exposed to wide variety of pathogens including parasites, bacteria and viruses (VanderWaal and Ezenwa, 2016; Vaumourin et al., 2015). Studies based solely on pathogen detection are useful to understand the prevalence of pathogens in certain population and species, but they are insufficient to investigate the impact of these pathogens on their host. Histopathological investigations on small mammals can provide such crucial information by elucidating subclinical diseases affecting different organs of an individual (Mühldorfer et al., 2011; Seguel et al., 2017). To evaluate the health status of animal species inhabiting modified environments is also important, because environmental degradation has shown to negatively affect wildlife health (Acevedo-Whitehouse and Duffus, 2009).

The health of small mammals influences significantly their behaviour by becoming less active, reducing social interest, grooming and also mating (Hart, 1991; Wobeser, 2006). These behavioural changes have an impact not only in the interactions with individuals of the same species but also with predators. The reduction of the activity in sick individuals, makes them the preferred prey for predator because they are easier to catch (Hudson et al., 1992). These interactions between infected prey and predators can also have a significant effect on pathogen transmission (Su and Hui, 2011), meanwhile increasing the risk of infection for predators via trophic transmission (Hall et al., 2007; Orlofske et al., 2014).

The possible implication of certain small mammal species in the transmission of zoonotic pathogens, encourage to improve the knowledge on their habitat, diet and behaviour (Bordes et al., 2015). And because the health status can influence crucial aspects such as behaviour, motility and reproduction, its investigation is important. Up to date there is no information on

the possible occurrence, the degree and the quality of pathological changes in small mammals from Northern Borneo. In this study the evaluation of the small mammals' health status was based on the individuals' histopathological examination as this allows the assessment of all tissue changes regardless the aetiology: infectious (e.g. viral, bacterial or fungal), non-infectious or metabolic. In connection with information on the animals' ecological background the assessment of a baseline level of pathological changes can also help to comprehend possible implications of the environment on the health of the species.

3.7 Selected infectious pathogens of small mammals

Animal species formerly separated by environmental barriers can experience an increase of pathogen transmission due to novel encounters with invasive species (Clark et al., 2018; Faust et al., 2018; Ostfeld and Holt, 2004). These interspecific interactions can influence infection in native species, especially by parasites, which incorporate these species as intermediate hosts in their lifecycles based on trophic infection (Britton, 2013). Besides parasites with complex lifecycles, interspecific encounters can support infections by pathogens transmitted through direct or indirect contact. The behaviour of the host species together with their population density can propitiate the contact among them, thus, increasing the risk of infection (VanderWaal and Ezenwa, 2016). The transmission of pathogens can be expanded by abundant mammal species with a broad habitat spectrum (i.e. generalist species), facilitating the propagation of pathogens by interacting with species from different habitats (Vaumourin et al., 2015).

Wildlife fauna is naturally infected by multiple pathogens and the transmission of these pathogens is influenced by the environment (Bordes and Morand, 2011). However, investigations on small mammal pathogens are mostly focussed on a single agent (Bordes and Morand, 2011), while the host ecology is often neglected (Bordes et al., 2015). Although publications based on pathological changes and natural diseases in free-ranging small mammal wildlife tackle different diseases affecting the individual animals as well as the aetiologic agent in many cases, these studies are restricted to a single animal species or species group (e.g. *Rattus* spp.) (LaRose et al., 2010; Rothenburger et al., 2015a; Seguel et al., 2017). Ideally, to better understand pathogen dynamics, multiple pathogens as well as their hosts ecology should be considered (Bordes et al., 2015).

In this study we have selected relevant pathogens of each major pathogen group to exemplarily investigate pathogen burdens: *Leptospira* spp. (bacteria), *Trypanosoma* spp. (protozoa), *Sarcocystis* spp. (protozoa), and herpesvirus (viruses). The detection of these pathogens in a variety of small mammal species can help to elucidate their role as host species.

3.7.1 *Leptospira* spp.

Generalities

Leptospira spp. are Gram negative, obligate aerobic bacteria belonging to the phylum Spirochaetes. The genus is composed by 22 species classified in three groups according to the phylogeny and pathogenicity: saprophytic, intermediate, and pathogenic. Within the species more than 300 different serovars are found (Xu et al., 2016). All species have a singular rotational movement created by the endoflagellum and their helicoidally (corkscrew) shape. These allow the spirochetes to penetrate cellular membranes and to migrate through the tissue (Charon et al., 1992).

Leptospira spp. are responsible for one of the most common world-wide bacterial zoonosis (Picardeau, 2017). Almost all mammals are susceptible for infection, including wildlife and domestic animals (Monahan et al., 2009). The capacity of *Leptospira* spp. to survive in the environment, especially in water and high humidity areas, facilitates the transmission of the bacteria (Andre-Fontaine et al., 2015). Animals get infected by direct contact with *Leptospira* spp. in contaminated environments through the skin or mucosa due to the capability of *Leptospira* spp. to penetrate tissue. In susceptible animals infected with pathogenic *Leptospira* spp. the bacteria initially replicate in the adipose tissue (Ozuru et al., 2017) followed by a haematogenous systemic dissemination until they finally reach multiple organs such as lung, liver and brain, but the kidney is the primary target organ (Monahan et al., 2009; Picardeau, 2017). The pathological lesions are tubulointerstitial nephritis, pulmonary haemorrhage and necrosis in the lung, necrotizing hepatitis and meningitis. The clinical manifestation of the infection varies from subclinical, sub-acute, acute to chronic, depending on the leptospiral serovar and the age and the immune status of the host (Adugna, 2016; Kohn et al., 2010; Monahan et al., 2009; Picardeau, 2017; Sykes et al., 2011).

The presence and spread of *Leptospira* spp. is often associated with rodents especially mice and rats, which are considered the principal reservoir for the *Leptospira* spp. (Bharti et al., 2003; Costa et al., 2015; Picardeau, 2017). They are chronic asymptomatic carriers, characterized by an active replication of the bacterium in the renal tubules without inflammatory reaction. The *Leptospira* spp. are excreted via urine and contaminate the environment (Vihol et al., 2017; Levett, 2001; Picardeau, 2017). Although the mechanism of colonization in the renal tubules of the reservoir is not fully known, it was proven that *Leptospira* spp. develop biofilms on the surface of the tubular epithelial cells (Yamaguchi et al., 2018).

Detection

In contrast to other bacteria easily isolated by culture, *Leptospira* spp. have a very slow growth in solid and liquid media (Adler et al., 2010). The detection methods described by Picardeau (Picardeau, 2017) are based on molecular methods (DNA, RNA sequencing and next-generation sequencing) and cross-agglutinin absorption test (CAAT) for serotype identification. Additionally, dark field microscopy and histologic argyrophilic staining allows the direct detection of the spirochetes in a host's blood or urine or in infected tissue, respectively (Bhatia et al., 2015). In tissues actively colonized by spirochetes, for example the lumen of the proximal renal tubules, silver staining can be used to their detection. However, tissues containing lower numbers of bacterial aggregations such as liver or lung argyrophilic staining has a much lower sensibility (Athanazio et al., 2008; Blendedn and Goldberg, 1965).

Importance

The majority of human leptospirosis cases are highly associated with urban areas of tropical developing countries (Andersen-Ranberg et al., 2016). There, the presence of the sufficient numbers of reservoir hosts and deficiencies of the sanitation infrastructures increase the spread of *Leptospira* spp. and thus the risk of infection (Santos et al., 2017). Although *Leptospira* spp. are associated with urban environments, they can also be found in habitats other than cities including agricultural fields, forests and floodable areas (Cosson et al., 2014). The occurrence and distribution of *Leptospira* spp. is highly influenced by climate conditions, especially heavy rains, which facilitate the occurrence of the disease (Andersen-Ranberg et al., 2016).

Another relevant aspect of this pathogen is an economic loss due to infection of farm animals. These infections are most likely to occur in peridomestic cattle from developing areas, due to the closer contact with murid-like rodents (Gamage et al., 2011). Infected dairy cattle suffer subclinical manifestations of the disease characterized by a reduction of milk production, infertility and abortions (Adugna, 2016).

Due to the lack of effective vaccines for humans and animals and the increment of cases in tropical regions, leptospirosis has become one of the most relevant neglected diseases (Picardeau, 2017; Tan et al., 2016).

Given the importance of *Leptospira* spp. in tropical regions and the limited information on the range of reservoir species responsible for their transmission, it is crucial to evaluate the role as reservoir of different species (Harstskeerl et al., 2010; Picardeau, 2017; Thayaparan et al., 2014). In this study we have investigated the presence of this *Leptospira* spp. in classic reservoir species such as rats, as well as other reservoir species (e.g. treeshrews and shrews) to elucidate the occurrence of these bacteria in different ecological territories in Northern Borneo and the role of the species living there.

3.7.2 *Trypanosoma* spp.

Generalities

The genus *Trypanosoma* is composed of numerous protozoal parasites with an obligatory two host life cycle and dependent on a hematophagous vector for their transmission (Hoare, 1972). The morphological and phylogenetic classification defined by Wheeler included the *Trypanosoma* spp. in the juxtaform superclass. They are characterized by the presence of a distinct lateral flagellum in the extracellular stages epimastigote and trypomastigote (Wheeler et al., 2013). The trypanosomatid genera are classified based on the number of intermediate hosts, in monoxenus (single intermediate host) or dixenous which require more than one intermediate hosts (Kaufer et al., 2017). The parasites have a broad host range including amphibians, fish, birds, reptiles and mammals (Noyes, 1998; Stevens et al., 1999). The host specificity of these protozoa is still unclear, although some species are highly specific for their host (e.g. *T. minasense* infecting non-human primates), others like *T. cruzi* are considered generalists because of the capacity to infect a diverse range of host species (Rodrigues et al., 2019). The vectors involved in the infection of mammals are insects: bugs such as *Triatoma infestans, Rhodnius prolixus* or tsetse flies (*Glossina* spp.) or flees (*Siphonaptera*). Aquatic leeches (e.g. *Zeylanicobdella arugamensis*) were discovered to be responsible for the transmission among fishes (Hayes et al., 2014).

The transmission pathway depends on the anatomic location where the parasite develops inside the vector. In Salivaria species this occurs in the buccal area with transmission through bites, in contrast to Stercoraria species, which develop in the posterior region of the invertebrate vector and the host is infected through small skin lesions contaminated by faeces containing the parasites (Stevens and Gibson, 1999). Once the parasite entered the host, it adopts the extracellular trypomastigote form, characterized by a flagellum which facilitates the mobility through the body. After infecting the host cells, it changes into the non-flagellated amastigote form, which replicates inside the cells causing cellular rupture and tissue damage in the host (Barrett et al., 2003). Any mammalian cell can be susceptible for infections by *Trypanosoma*, however they have a tropism for reticuloendothelial cells of the muscular, cardiovascular and nervous system (Tanowitz et al., 2009). The disease can be manifested in an acute or chronic form depending on the *Trypanosoma* spp. and host's immunity (Barrett et al., 2003)

Some *Trypanosoma* spp. are responsible for zoonotic diseases like African sleeping sickness caused by *T. brucei* or the American Chagas diseases caused by *T. cruzi* (Hoare, 1972). In the last decade, many studies have indicated the implication of rats in the transmission of atypical human trypanosomiasis in Southeast Asia, caused by *T. lewisi* (Lun et al., 2015; Pereira et al., 2017; Pumhom et al., 2014). This species has been isolated only from *Rattus* spp. and although initially it was considered non-pathogenic, these case reports have shown

that *T. lewisi* is potentially infective for humans, while rats are the reservoir hosts (Lun et al., 2015; Pumhom et al., 2014).

Detection

Morphological identification using light and electron microscopy is the classical method for *Trypanosoma* detection (Wheeler et al., 2013). Their singular phenotypic structure based on a nucleus with a circular DNA, lanceolated body and the single lateral flagellum makes them easily distinguished (Wheeler et al., 2013). Additionally to morphologic examination, other methods are used for the identification of *Trypanosoma* spp. like agglutination tests and antigen cross-reactions (Desquesnes et al., 2007; Milleliri et al., 1989). However, the advances in molecular biology have elevated molecular markers as the preferred method for *Trypanosoma* detection (Kaufer et al., 2017; Mafie et al., 2019; Pereira et al., 2017).

Importance

Beside the interest for *Trypanosoma* spp. concerning human health, wildlife populations have also been affected by this pathogen (Thompson et al., 2014). For example, the event of inter-species infection between invasive *Rattus* spp. and a native rodent (*Rattus macleari*) on Christmas Island, resulted in the extinction of this native species (Wyatt et al., 2008). Moreover, *Trypanosoma* infection has been reported as the most likely cause of population decline in the Australian marsupial brush tailed bettong (*Bettongia penicillata*) (Botero et al., 2013).

Wildlife is assumed to be more resistant to the disease in contrast to domestic animals, which are more susceptible. Pathogenic species of these parasites are responsible for severe clinical manifestations in domestic bovines, horses and camels (Barrett et al., 2003). One of the most pathogenic species is *T. evansi*, causing Surra disease present in the Middle East, Asia and in Central and South America, and responsible for serious economic losses due to the high mortality and fertility problems in horses and camels (Dobson et al., 2009; El Wathig et al., 2016). Cattle is likewise affected by *T. evansi*, usually developing a chronic manifestation characterized by edema, weight loss and reproduction problems (Barrett et al., 2003; Narnaware et al., 2016). In contrast, other host species like donkeys, goats and sheep are less vulnerable for trypanosomiasis with only mild clinical signs (Barrett et al., 2003).

Investigating the presence of *Trypanosoma* spp. in different species of free-ranging small mammals elucidates not only the diversity of *Trypanosoma* spp. among the different host species, but also the occurrence of this parasite in anthropogenic disturb areas in Northern Borneo.

3.7.3 *Sarcocystis* spp.

Generalities

Sarcocystis spp. are protozoal organisms belonging to phylum Apicomplexa with an obligatory two-host prey-predator cycle (Dubey et al., 2016). This parasite has a global distribution and includes more than 200 species, being able to infect a wide host variety including mammals, birds, and reptiles (Dubey et al., 2016; Odening, 1998). In the life cycle of *Sarcocystis* (Fig. 2) the prey species are usually herbivores and omnivores and become intermediate hosts after the ingestion of water or feed contaminated with *Sarcocystis* sporocysts. In the intermediate host occurs the asexual maturation of the parasite, starting with the release of sporozoites in the gastrointestinal tract and followed by migration through the lymphatic or circulatory system. Subsequently, schizogony occurs in a specific type of host cells depending on *Sarcocystis* spp., for example *S. singaporensis* develops in pneumocytes of rats (*Rattus norvegicus*), *S. neurona* in neurons of the dog, *S. markusi* in the myocytes of macaques (*Macaca mulatta*) (Dubey et al., 2016; Lane et al., 1998). Also, the number of schizogony generations differs between the different species. Merozoites released from the last schizonts enter striated muscle fibres resulting in the sarcocystis phase, which remains infective from a few months to a host's lifetime. After a predator feeds on an infected intermediate host the parasite enters the sexual phase characterized by the fertilization of female macrogamonts by male microgamonts to produce the oocytes. The cycle ends with the excretion of infective oocytes containing two sporocysts via faeces of the definitive host (Dubey et al., 2016; Mehlhorn and Heydorn, 1978).

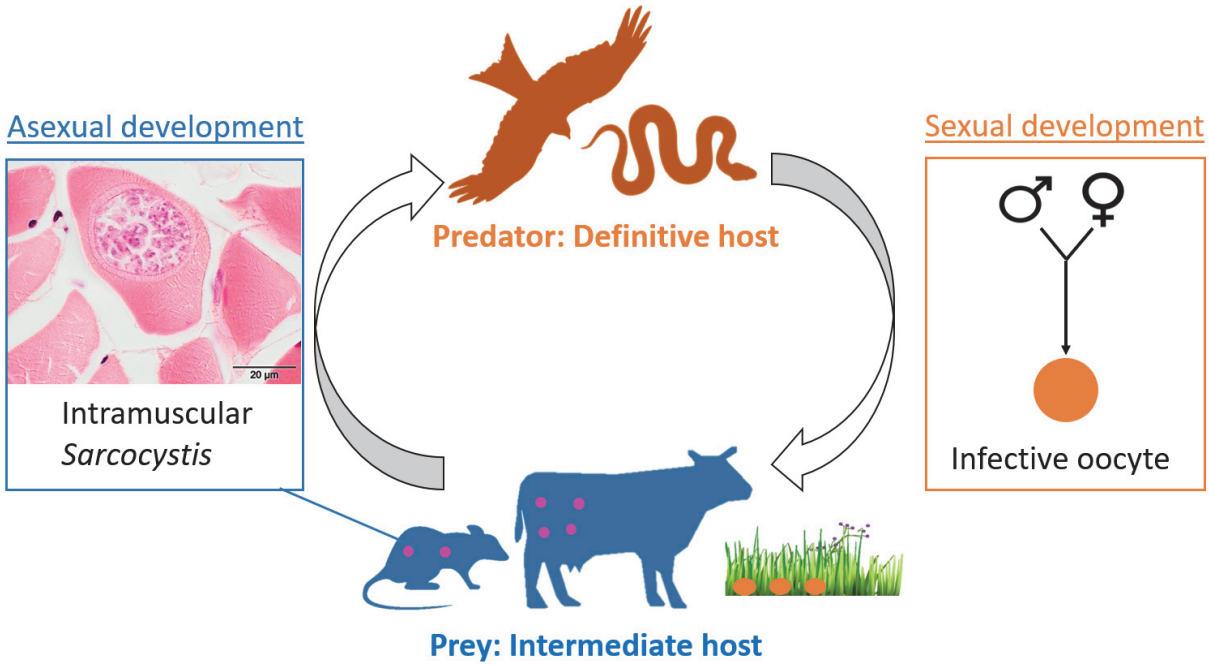

Fig. 2: Life cycle of *Sarcocystis* spp. modified after Dubey et al. (2006) and Mehlhorn and Heydorn, (1978). The cycle is divided in two parts according to the type of maturation development: sexual and asexual. The sexual stage occurs in the definitive host producing the infective oocysts. In the

intermediate host the asexual transformation concludes with a latent intramuscular infectious *Sarcocystis*. Modified images from: https://www.freepik.com/vectors/animals. Animals' vector created by rawpixel.com – www.freepik.com

Sarcocystis infection in the definitive host is non-pathogenic, however the development of this parasite in the intermediate host can be pathogenic, depending on the *Sarcocystis* spp., the concentration of sporocysts ingested and the immunological status of the host (Dubey et al., 2016). For example, cattle infected with *S. cruzi* can suffer a wide spectrum of clinical signs from mild anaemia and weight loss to severe neurological signs, abortion and death, depending on the ingested dose of the sporocysts, the reproductive status of a cow (e.g. pregnant or lactating) and the animal's health status in general (Dubey et al., 1982, 2016; Nakamura et al., 1982). A study on experimentally infected *Rattus* spp. with high concentration of sporocysts from *S. singaporensis*, caused the death of the individuals by severe damage on the lung tissue due to the schizogonic development (Jäkel et al., 1996).

Detection

The presence of intramuscular stages of *Sarcocystis* in the intermediate host can be detected by gross inspection, histological evaluation, microscopic examination of unstained fresh squashed muscle or tissue digestion (Dubey et al., 2016). In contrast to other parasites were morphology investigations are not sufficient for a precise species identification (e.g. *Trypanosoma* spp.), the unique morphological features of *Sarcocystis,* permit a species recognition (Dubey et al., 2016). Histological examination of *Sarcocystis* spp. can determine the different structures of the cyst, allowing an initial species classification (Dahlgren et al., 2007; Verma et al., 2015) as well as the host's cellular reaction against the parasite. Since the cyst wall is characterized by unique species specific features, the morphological evaluation by transmission electron microscopy of the *Sarcocystis* wall allows an accurate species identification. Moreover, *Sarcocystis* spp. infecting closely related intermediate host species often have a similar wall pattern, being indicative also of the phylogenetic relationship among these intermediate host species (Dubey et al., 2016). However, this morphological species identification can only be conducted on the intramuscular cystic form, in contrast to the oocyst and sporocysts released by the definitive host that does not allow any species morphological differentiation (Xiang et al., 2009).

In contrast to other pathogens, where molecular investigations have replaced the morphological evaluation for the species identification, the phylogenetic analysis of *Sarcocystis* spp. has been defined as too ambiguous, due to difficulties to discern closely related species (Morrison, 2008). Therefore, morphological evaluation combined with

molecular characterization, is the most reliable approach to determine *Sarcocystis* species (Dubey et al., 2016).

Importance

There are two main *Sarcocystis* spp. that infect humans (*S. hominis* and *S. suihominis*), which lead to a clinical manifestation with mild gastroenteritis including vomiting, diarrhoea or abdominal pain (Dubey et al., 2016; Poulsen and Stensvold, 2014). Recent investigations found an additional *Sarcocystis* sp. (*S. nesbitti*) also responsible for human sarcocystosis outbreaks in Malaysia (AbuBakar et al., 2013; Italiano et al., 2014). Because humans can get infected through the consumption of meat from domestic or wildlife animals, which could be contaminated with different species of *Sarcocystis*, the range of *Sarcocystis* spp. that can infect humans remains undetermined (Fayer et al., 2015).

Among domestic animals, the high prevalence of this parasite in livestock leads to important economic losses due to the downgrading of meat (Dubey and Fayer, 1986) and a decrease in milk production (Fayer et al., 1983). Although in cattle and pigs a few investigations have attempted to develop immunization against *Sarcocystis* spp., they have not succeeded in producing protective immunity (Fayer and Dubey, 1984; O'Donoghue et al., 1985).

The wide spectrum of wildlife animal species involved in the life cycle of this parasite is responsible for a global distribution of *Sarcocystis* spp. (Dubey et al., 2016). Among different taxa, free-ranging small mammals such as shrews (*Crocidura russula* host for *S. russuli*), squirrels (*Spermophilus richardsonii* host for *S. bozemanensis*), rats (*Rattus norvegicus* host for *S. singaporensis*) and treeshrews (*Tupaia belangeri chinensis* host for *S. Tupaia*) are a common intermediate host for multiple *Sarcocystis* spp. (Beaver and Maleckar, 1981; Pak et al., 1989; Dubey 1983., Xiang et al., 2010). The low position small mammals occupy in the trophic pyramid as prey, makes them a suitable host for the development of this parasite. Amongst small mammals, the relationship between prey animals and *Sarcocystis* spp. has been well studied in various rodent species from Southeast Asia, the majority belonging to *Rattus* spp., as murids show a high prevalence of sarcosporidia infections as well as a large diversity of *Sarcocystis* spp. (Ambu et al., 2011; Jäkel et al., 1997; Tung et al., 2009). However, the range of small mammal species acting as intermediate hosts is not fully determined (Dubey et al., 2016; Jäkel et al., 1997). Thus, the evaluation of *Sarcocystis* spp. from additional small mammal host species would reveal not only the possible occurrence of this parasite in these animal species but also further elucidate the parasite's diversity.

3.7.4 Herpesvirus

Generalities

The family Herpesviridae is composed of the subfamilies *Alphaherpesvirinae, Betaherpesvirinae* and *Gammaherpesvirinae* (Davison et al., 2009).

Herpesviruses are defined by a linear, double-stranded DNA genome of 125–290 kbp surrounded by a icosahedron capsid formed by 16 proteins, covered by an poorly defined matrix or tegument and the external envelope with viral glycoproteins (Pellett and Roizman, 2007). These specific receptors of the external capsid interact with the host cells, facilitating the entry of the virus into the cell. For viral replication, the herpes-DNA migrates towards the nucleus, where it is transcribed into mRNA by using the host's own transcription mechanism. The viral mRNA is then translated by ribosomes into viral proteins, which migrate to the nucleus for the assembling of viral particles. This cycle is finalized by the rupture of the cell (lytic cycle) releasing the new virions into the extracellular matrix, where they will infect neighbouring cells and restarting the cycle (Payne, 2017). One of the main characteristics of the herpesvirus family is their capacity to remain latent in the host cell without causing damage until certain conditions such as immune depression, UV light or stress again activates the lytic cycle (Boehmer and Nimonkar, 2003).

Within the *Herpesviridae* subfamilies the different viruses are classified according to their biological properties into alpha-, beta- and gammaherpesviruses (Pellett and Roizman, 2007). The alphaherpesviruses have a variable host range, replicate rapidly in the host cells and establish latent infections primarily in sensory ganglia, in contrast to betaherpesviruses which have a limited host range, a slow replication cycle and infected cells show cellular enlargement. The gammaherpesvirus group is characterized by a tropism for T- and B-cells and in latent infections gammaherpesviruses can be detected in lymphoid tissue (Roizman and Baines, 1991).

In general, herpesviruses are commonly found in a large number of different animal species (Davison, 2002; Ehlers et al., 2008, 2007; Wibbelt et al., 2007), often causing asymptomatic but persistent infections in the host (Ehlers et al., 2008). Due to the diverse type of target cells of the different herpesviruses and their capacity to remain latent in the tissue, there spectrum of clinical signs can be rather large (Boehmer and Nimonkar, 2003). In addition, some herpesviruses have the capacity to induce neoplasia (Kheimar et al., 2019), for example, the *Gallid alphaherpesvirus* 2 causes Marek's diseases in poultry, which is characterized by lymphoproliferative disease (Woźniakowski and Samorek-Salamonowicz, 2015).

Co-evolution between host species and the viruses resulted in distinct host specificity for most herpesviruses (Davison et al., 2009). Still, some herpesviruses have shown cross-species

transmission, representing also a zoonotic risk such as the Asian alphaherpesvirus *Cercopithecine herpesvirus* 1 (B virus) transmitted from non-human primates to humans (Huff and Barry, 2003).

Detection

Direct identification techniques, such as transmission electron microscopy, allow the examination of virus structures as well as the interaction with the host cell. The detection of virus antigen by fluorescent antibody test (FAT), is a common method to evaluate if an individual was infected by a herpesvirus, however there are limitations in this technique as the specific herpesvirus type cannot always be identified (Rissi and Barros, 2013). The most precise way to identify the specific herpesvirus types are molecular methods detecting viral nucleic acids (Pellett and Roizman, 2007).

Importance

Zoonotic transmission of herpesvirus has been reported as a rare event, and only occurring after a close contact between macaques infected with CeHV-1 and animal keepers (Tischer and Osterrieder, 2010a). But humans can also transmit herpesviruses, to primates and other animal species leading to high mortality rates, for example *Human herpesvirus* 1 (HHV-1) transmitted to New World primates (Mätz-Rensing et al., 2003) or pet rabbits (de Matos et al. 2014).

Herpesviruses are responsible for important economic losses in the livestock industry (mostly porcine, cattle and poultry) due to mortality and decrease in production. One example is poultry farms suffering from Marek's disease caused by *Gallid alphaherpesvirus* 2. Although a highly protective vaccine against this virus is available, the sporadic presence of novel virus strains decreases the efficacy of the vaccine resulting in disease outbreaks (Payne and Venugopal, 2000).

Studies on wildlife rodent populations from Africa, Europe and Asia have shown a high diversity of herpesviruses, including the identification of many novel herpesviruses (Ehlers et al., 2007; Ntumvi et al., 2018). Considering the high diversity of rodent species in Borneo one can assume to discover even more novel herpesviruses in these animals. Interactions within this high species richness could potentially also contribute to an inter-species transmission of herpesvirus (Azab et al., 2018; Ntumvi et al., 2018).

The investigation of the presence of herpesviruses in different small mammal species living in Northern Borneo will indicate an even broader diversity of herpesvirus among different host species as well as extent the knowledge on the distribution of these viruses in the different habitats.

4 MATERIAL AND METHODS

4.1 Material

4.1.1 Animals and tissue samples

Tissue samples from a number of different small mammal species for histopathological investigations as well as nucleic acid-based methods for pathogen detection were provided by Dr. Konstans Wells (Dept. of Biosciences, Swansea University, UK) as a collaboration from the long term project: “Host-parasite interactions and rodent-borne diseases: native and invasive small mammal occurrence and parasitism along land-use gradients from natural to anthropogenic habitats in Borneo”. The animals were caught in Northern Borneo, Sabah, Malaysia, in the greater surroundings of Kota Kinabalu, the capital of Sabah. They originate from a land-use gradient from forests to urban habitats (see Tab. 1). All necessary permits and documents were obtained by Dr. Konstans Wells and issued by the Sabah Biodiversity Centre (JKM-MBS.1000-2/2(35), JKM-MBS.1000-2/2(63)), access permits to forest field sites were approved by Sabah Parks and individual land owners (Wells et al., 2014).

Between 2012 and 2013, the small mammals were live-trapped by locally made drop-door, wire-mesh traps (ca. 280 x 140 x 140 mm) baited with mixtures of raw banana, fried banana and dried fish. Traps were set in different habitat types from mature forest to urban habitats, and frequently checked for captures. For each capture location, geographical coordinates were recorded using a GPS device (Garmin GPSmap62st, Olathe, USA). All animals were transferred to nearby mobile field laboratories within their traps, which were covered with cloth to reduce stress. Animals were moved from the traps into small cotton bags and further into a plastic container for subsequent anaesthesia by diethyl ether inhalation (anaesthetic grade) and killed by cervical dislocation (according to guidelines by the American Veterinary Medical Association, https://www.avma.org). Animal species identification was based on the phenotypic characteristics according to relevant publications (Aplin et al. 2003; Musser and Carleton 2005).

In total, 346 individuals were caught, belonging to 15 different small mammal species from the following families: Sciuridae (*Callosciurus notatus, Callosciurus prevostii, Sundasciurus lowii),* Muridae *(Leopoldamys sabanus, Maxomys rajah, Maxomys surifer, Maxomys whiteheadi, Niviventer cremoriventer, Sundamys muelleri, and Rattus* spp.*)* and Tupaiidae *(Tupaia gracilis, Tupaia minor, Tupaia tana)* and Soricidae (*Suncus murinus*). Because of the difficulty to discriminate some individuals from the *Rattus* species group with full confidence to the species level based on morphometric data alone (Pagès et al., 2013, 2010), all individuals were grouped as a complex of single species for the purpose of this study, hereafter noted as *Rattus* complex (*Rattus* compl.). However, to estimate trends of pathological lesions for certain findings, the phenotypically assumed species was indicated, i.e. *Rattus norvegicus*.

Animal data recorded in the field included gender and age group (classified as ‘juvenile’, ‘immature’, ‘sub adult’, ‘adult’ according to size, body weight, pelage, sexual maturity, tooth wear and ossification (Aplin et al. 2003; Herbreteau et al. 2011; Musser and Carleton 2005)).

After euthanasia, all animals were dissected and organ samples were fixed in 70% ethanol and stored in 35 ml plastic containers until shipment to the Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany. Each container included tissue samples of the organs, i.e. lung, heart, liver, kidneys, and gastrointestinal tract as well as samples of striated muscles (diaphragm, lateroventral abdominal wall (*Musculus latissimus dorsi*, *M. spinotrapezius* or *M. obliquus externus* or *M. quadriceps femoris* and occasionally laryngeal muscle attached to the trachea).

Parts of the ethanol-fixed tissue samples of liver and kidney were initially employed for the molecular detection of *Leptospira*-DNA and *Trypanosoma*-DNA but proved unsuitable for these investigations. Long-term storage of the organs in 70% ethanol at ambient tropical temperature (30-40°C) had rendered the tissues into rubber-like blocks impeding standard DNA extraction protocols. The high value and low availability of sample material from wildlife animals from Northern Borneo encouraged establishing an improved protocol for DNA extraction with an initial tissue rehydration step, supported by advice of Macherey-Nagel (Macherey-Nagel, Düren, Germany).

As DNA extraction control, a partial sequence of the cytochrome b gene was amplified in a subset of samples to confirm the presence of host-DNA in the extracted product. Moreover, the amount and quality of the DNA extracted from ethanol-fixed tissue was assessed by NanoDrop and its ratio of absorbance at 260 and 280 nm. However, although DNA extraction with this improved protocol was successful, the DNA quality was not satisfying enough and would clearly decrease the sensitivity of PCR analyses. Fortunately, small aliquots of liver and kidney samples were available from most individuals of this study, which have been stored in 2 ml tubes containing RNAlater® at the same ambient temperature and shipped to the Robert Koch Institute, Berlin, Germany for virological analyses. Homogenized tissue of these samples had a sufficient quality for DNA extractions and subsequent molecular analyses in comparison to the ethanol-fixed tissue.

Tissues fixed in RNAlater® were used to investigate the occurrence and diversity of herpesviruses in the captured small mammals as part of a bachelor thesis project of Heiko Pietsch (supervised by Dr. Andreas Kurth, Centre for Biological Threats and Special Pathogens (Pietsch, 2013). The results of the bachelor thesis were included in this doctoral thesis to compare the different pathogen groups of the study (bacteria, protozoa and viruses) in their occurrence in the different animal species and along a land-use gradient.

Tab. 1: Summary of sampled animals sorted by habitat (forest to urban) and assignment to ecological groups as referred to in this study

Habitat	Ecological group	Order	Family	Genus	Species	Sex			Age					Total number of individuals
						Female	Male	NA	Inmature	Juvenile	Subadult	Adult	NA	
Forest	Forest rodents (n= 42)	Rodentia	Sciuridae	Callosciurus	*Callosciurus notatus*	3	2	/	/	/	/	5	/	5
					Callosciurus prevostii	1	2	/	/	/	/	3	/	3
				Sundasciurus	*Sundasciurus lowii*	5	1	/	1	1	/	4	/	6
			Muridae	Leopoldamys	*Leopoldamys sabanus*	/	1	/	/	/	/	1	/	1
				Maxomys	*Maxomys rajah*	/	2	/	/	/	/	2	/	2
					Maxomys surifer	2	3	/	/	/	3	2	/	5
					*Maxomys surifer/rajah**	5	5	/	3	2	2	3	/	10
					Maxomys whiteheadi	2	4	/	/	/	4	2	/	6
				Niviventer	*Niviventer cremoriventer*	2	2	/	/	/	/	4	/	4
	Treeshrews (n=15)	Scadentia	Tupaiidae	Tupaia	*Tupaia gracilis*	1		/	/	/	1	/	/	1
					Tupaia minor	4	4	/	/	/	8	/	/	8
					Tupaia tana	4	2		1		1	4	/	6
Forest to urban	Generalist (n=76)	Rodentia	Muridae	Sundamys	*Sundamys muelleri*	42	33	1	/	4	18	52	2	76
Urban	Rattus (n=155)	Rodentia	Muridae	Rattus	*Rattus spp.*	46	61	/	1	8	8	88	2	155
					Rattus norvegicus	24	24	/	4	8	7	29	/	
	Suncus (n= 58)	Insectivora	Soricidae	Suncus	*Suncus murinus*	16	40	2	/	/	/	55	3	58
Total number						157	186	3	10	23	52	254	7	**346**

* *Maxomys suriferi/rajah*: Some individuals from the species *Maxomys rajah* and *M. surifer* were phenologically indistinguishable and were therefore grouped together. ** *Rattus* compl. comprises phenologically indistinguishable *Rattus rattus* and *R. tiomanicus*

4.1.2 Buffer and reagents

4.1.2.1 Histopathology main stainings

Hematoxylin-Eosin staining

Solutions:

- 1 g Hematoxylin in 1000 ml distilled water
- 0.2 g of sodium iodate 50 g of aluminium potassium sulphate, 50 g of chloral hydrate and 1 g of citric acid
- Incubate for 24 h in a dark place. Filtrate afterwards

Reagents:

- Hematoxylin (Carl Roth, Karlsruhe, Germany)
- Sodium iodate (Merck Millipore, Darmstadt, Germany)
- Potassium sulphate (Merck Millipore, Darmstadt, Germany)
- Chloral hydrate (Carl Roth, Karlsruhe, Germany)
- Citric acid (Carl Roth, Karlsruhe, Germany)
- Eosin (Merck Millipore, Darmstadt, Germany)

Warthin-Starry (WS) staining

Solutions:

- Solution A: Dilution of 4.1 g sodium acetate in 250 ml distilled water
- Solution B: Dilution of 11.8 ml of glacial acetic acid in 988.2 ml of distilled water
- Base solution for preparing 1% silver nitrate solution: Mixing 1.5 ml of solution A and 18.5 ml of solution B with 480 ml of distilled water
- Developer solution: Preheat at 60° C, 15 ml of 2% silver nitrate solution, 90 ml of 7% gelatine solution and 8 ml of 4% hydroquinone solution in separate tubes. Mix shortly before use

Reagents:

- Silver Nitrate (Carl Roth, Karlsruhe, Germany)
- Sodium acetate (Carl Roth, Karlsruhe, Germany)
- Acetic acid, 100% (Merck Millipore, Darmstadt, Germany)
- Gelatine (Carl Roth, Karlsruhe, Germany)
- Hydroquinone (Merck Millipore, Darmstadt, Germany)
- Sodium thiosulfate (Merck Millipore, Darmstadt, Germany)

4.1.2.2 Transmission electron microscopy

Solutions:

- Glyceryl ether 100 mixture: prepared from two mixtures; glyceryl ether 100 A and B. Solutions A and B are prepared in a ratio of 2 parts A + 3 parts B + 2% Tris-(dimethyl amino methyl) phenol (DMP 30). The polymerization DMP 30 is used as an accelerator.
    - Glyceryl ether 100 mixture A: 62 ml of glycidyl ether 100 and 100 ml of Dodecenylsuccinic anhydride (DDSA)
    - Glyceryl ether 100 mixture B: 100 ml glycidyl ether 100 and 89 ml Methylnadic anhydride (MNA)
- Phosphate buffer according to Sörensen (0,1M) (to pH 7.2)
    - Solution A: 0.1 M Monopotassium phosphate (KH_2PO_4): 13.76 g/l
    - Solution B: 0.1 M Sodium phosphate dibasic dihydrate ($Na_2PO_4.2H_2O$): 18 g/l
- Lead citrate (Reynolds'stain) (34.42 mg/ml)
    - 1.33 gr of Lead nitrate ($Pb(NO_3)_2$)
    - 1.76 gr of Sodium Citrate ($Na_3C_6H_5O_7$)
    - 30 ml of distilled water
    - 8 ml 1N Sodium hydroxide (NaOH)
    - Fill with distilled water to 50 ml
    - Adjust to pH 12
- Uranyl acetate (35 mg/ml)
    - 100 µl of saturated uranyl solution + 100 µl of acetone

Reagents:

- Xylene (Medite, Burgsdorf, Germany)
- Monopotassium phosphate (KH_2PO_4) (Merck Millipore, Darmstadt, Germany)
- Sodium phosphate dibasic dihydrate ($Na_2PO_4.2H_2O$) (Carl Roth, Karlsruhe, Germany)
- Osmium tetroxide (Plano, Wetzlar, Germany)
- Epoxy resin = glycidyl ether 100 (Carl Roth, Karlsruhe, Germany)
- Dodecenylsuccinic anhydride (DDSA) (Carl Roth, Karlsruhe, Germany)
- Methylnadic anhydride (MNA) (Carl Roth, Karlsruhe, Germany)
- Propylene oxide (Carl Roth, Karlsruhe, Germany)
- Uranyl acetate (Merck Millipore, Darmstadt, Germany)
- Lead citrate (SERVA Electrophoresis, Heidelberg, Germany)
- Tris-(dimethyl amino methyl) phenol (DMP 30) (Carl Roth, Karlsruhe, Germany)

4.1.2.3 PCR

PBS buffer

Dissolution in initial 800 ml of distilled water of 8 g of NaCl (Carl Roth, Karlsruhe, Germany), 0.2 g of KCl (Carl Roth, Karlsruhe, Germany), 1.44 g of Na2HPO4 (Carl Roth, Karlsruhe, Germany) and 0.24 g of KH2PO4 (Carl Roth, Karlsruhe, Germany). Addition of distilled water to a volume of 1 l. Adjusting the pH to 7.4 with HCl.

TEA 50X buffer

Initial dissolution of 242 g Tris base (Carl Roth, Karlsruhe, Germany) in distilled water. Followed by the addition of 57.1 ml glacial acetic acid (Carl Roth, Karlsruhe, Germany), and 100 ml of 500 mM EDTA (VWR, Darmstadt, Germany). Adjustment of the pH to 8.0 in a final volume of 1 l.

DNA extraction kit NuleoSpin® Tissue (Macherey-Nagel, Düren, Germany)

1. Lysis Buffer RLY (lyophilized)
2. Binding Buffer RLB
3. Wash Buffer RLE
4. Liquid Proteinase K (lyophilized) 1-3%
5. NuleoSpin® DNA RapidLyse Columns

DNA extraction kit NuleoSpin® DNA RapidLyse (Macherey-Nagel, Düren, Germany)

- Lysis Buffer T1
- Lysis Buffer B3
- Wash Buffer B5
- Elution Buffer BE
- Proteinase K
- Proteinase Buffer PB
- NuleoSpin® Tissue Columns

DNA extraction kit PureLink™ Viral RNA / DNA Mini Kit (Invitrogen™, Darmstadt, Germany)

- Viral Lysis Buffer (L22)
- Wash Buffer (WII)
- Proteinase K (20 mg/ml)
- Carrier RNA (lyophilized)
- Viral Spin Columns with Collection Tubes

Supermix SsoAdvanced™ Supermix (Bio-Rad, Munich, Germany)

- Antibody mediated hot-start Sso7d-fusion polymerase
- dNTPs-Mix
- MgCl2
- Enhancers
- Stabilizers
- Blend of passive reference dye

My Taq HS Mix (Bioline, Luckenwalde, Germany)

- Antibody mediated hot-start polymerase
- dNTPs-Mix
- MgCl2
- Stabilizers

PCR master mix final volume of 25 µl:

- 1U Taq DNA polymerase from Thermus aquaticus (Merck, Darmstadt, Germany)
- 3 mM dNTPs (Thermo Fisher Scientific, Schwerte, Germany)
- 2.5 mM MgCl2

Cloning kit (Invitrogen™, Glasgow, UK)

- TOPO TA Cloning® Kit for Sequencing
- One Shot® TOP10 Chemically Competent *E. coli*

DNA-ladder 100 bp (Thermo Fisher Scientific, Schwerte, Germany)

- 100 bp DNA-Ladder 50µl (0.5 µg/µl)

Midori Green (Biozym, Hessisch Oldendorf, Germany)

Amplicon purification kit:

a) QIAquick® Gel Extraction (Qiagen, Hilden, Germany)
- Silica membrane assembly for binding of DNA
- Binding buffers
- High-salt buffer
- Elution buffer

b) Agencourt® AMPure® (Beckman Coulter, Krefeld, Germany)

Primers

Tab. 2: Primer sequence for partial cytochrome b gene amplification

Analysis	Target	Primer name	Sequence 5'- 3'	Product length	Reference
Conventional PCR	cytochrome b gene	Kocher 1	CATCCAACATCTCAGCATGATGAAA	307 bp	(Kocher et al., 1989)
		Kocher 2	ATGTTTCATGTTTCGGTGAATATAT		

Tab. 3: Primer sequences for the detection of DNA of pathogenic *Leptospira* spp.

Analysis	Target	Primer name	Sequence 5'- 3'	Product length	Reference
Real-time PCR	LipL32 gene	LipL32-45F	AAG CAT TAC CGC TTG TGG TG	242 bp	(Stoddard et al., 2009)
		LipL32-286R	GAA CTC CCA TTT CAG CGA TT		
		LipL32-189P	FAM- AAA GCC AGG ACA AGC GCC G -BHQ1	Probe	
Conventional PCR	pLIPs60 recombinant plasmid	G1	CTG AAT CGC TGT ATA AAA GT	266-285 bp	(Gravekamp et al., 1993)
		G2	GGA AAA CAA ATG GTC GGA AG		
Conventional PCR	LipL21 gene	LipL21 F	CGC GGT CGA CAT GAT CAA TAG ACT TAT AGC T	561 bp	(Cheema et al., 2007)
		LipL21 R	CGC GCT GCA GTT ATT GTT TGG AAA CCT CTT G		

Tab. 4: Primer sequences for detection of *Trypanosoma*-DNA

Analysis	Target	Primer name	Sequence 5'- 3'	Product length	Reference
Nested PCR	18S rRNA gene	TRY927F*	GAA ACA AGA AAC ACG GGA G	927 bp	(Noyes et al., 2000)
		TRY972R*	CAT CTG GGC AGC TTG GA		
		SSU561F#	TGG GAT AAC AAA GGA GCA	561 bp	
		SSU561R#	CTG AGA CTG TAA CCT CAA AG C		

*outer and #inner primer pairs

Tab. 5: Primer sequences for detection of *Sarcosporidia*-DNA and Sanger sequencing

Analysis	Target	Primers name	Sequence 5'- 3'	Product length	Reference
Conventional PCR	18S rRNA gene	SarAF	CTGGTTGATCCTGCCAGTAG	1440 bp	(Prakas et al., 2014)
		SarDR	GCAGGTTCACCTACGGAAA		
Sanger sequencing internal primers	18S rRNA gene	SarCF	TTAACTGTCAGAGGTGAAATTCTT		
		SarBR	GGCAAATGCTTTCGCAGTAG		
Conventional PCR	COI gene	SF1	ATGGCGTACAACAATCATAAAGAA	1000–1070 bp	(Gjerde., 2013)
		SR8D	CATTGCCCATDACTACGCC		

Tab. 6: Primer sequences for detection of Herpesvirus-DNA

Analysis	Target	Primer name	Sequence 5'- 3'	Product length	Reference
Generic nested PCR	Herpesvirus-DNA polymerase	285s DFA	GAY TTY GC(N/I) AGY YT(N/I) TAY CC	150 - 200 bp	(Ehlers et al., 1999)
		285s ILK	TCC TGG ACA AGC AGC AR(N/I) YSG C(N/I)M T(N/I)A A		
		285as KG1	GTC TTG CTC ACC AG(N/I) TC(N/I) AC(N/I) CCY TT		
		286s TGV	TGT AAC TCG GTG TAY GG(N/I) TTY AC(N/I) GG(N/I) GT		

PCR positive controls

For *Trypanosoma*-DNA detection:

- *Trypanosoma dionisii* isolated from the spleen of a bat (*Eptesicus serotinus*). Concentration 1:10 Source: Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany.

For *Leptospira*-DNA detection:

- DNA from a laboratory strain of Leptospira interrogans serovar Icterohaemorrhagiae Source: the German Federal Institute for Risk Assessment, Consultant laboratory for *Leptospira* (Prof. Dr. Karsten Nöckler), Berlin, Germany.

4.1.3 Equipment

4.1.3.1 Histopathology

Dehydrator Excelsior AS (Thermo Fisher Scientific, Dreieich, Germany)

Paraffin-station EG 1150H (Leica, Wetzlar, Germany)

Microtome HM 360 (Microm, Dreieich, Germany)

4.1.3.2 Transmission electron microscopy

Trimmer Reichert ultratrim (Leica, Wetzlar, Germany)

Ultramicrotome (Boeckeler Instruments, Tuscon, USA)

Transmission electron microscope (Tecnai BioSpirit, FEI, Netherlands)

Nickel grid 200 mesh (Plano, Wetzlar, Germany)

4.1.3.3 PCR

Autoclave (Varioklav, Oberschleißheim, Germany)

Micropipette 1-1000 µm (Eppendorf, Hamburg, Germany)

Thermal cycler Biometra TRIO (Analytik Jena, Jena, Germany)

Thermo Shaker Incubator Eppendorf ThermoMixer® C (Eppendorf, Berzdorf, Germany)

Homogenizer (Bertin Technologies, Frankfurt, Germany)

Electrophoresis (Bio-Rad, Munich, Germany)

Centrifuge Heraeus™ (Thermo Fisher Scientific, Hennigsdorf, Germany)

NanoDrop 2000cqPCR (Thermo Fisher Scientific, Hennigsdorf, Germany)

UV-light transilluminator type UVT2053, 302 nm, medium wave (Herolab, Wiesloch, Germany)

MxPro software for qPCR analysis (Thermo Fisher Scientific, Hennigsdorf, Germany)

StratageneRMx 3005P (Thermo Fisher Scientific, Hennigsdorf, Germany)

MinION (Oxford Nanopore Technologies, Oxford, UK)

4.1.4 Consumables

- Reaction tubes 0.5 ml; 1.5 ml and 2 ml (Sarstedt, Nümbrecht, Germany)
- Screw cap micro tubes (Sarstedt, Nümbrecht, Germany)
- Sterile micropipette tips 10 µm, 100 µm, 1000 µm (Biozym, Hessisch Oldendorf, Germany)
- Precellys ceramic beads (Peqlab/VWR, Darmstadt, Germany)
- Nuclease free Water (Thermo Fisher Scientific, Schwerte, Germany)
- Ethanol 99% (Carl Roth, Karlsruhe, Germany)
- Blades for microtomy, TissueCut® (Medite, Burgdorf, Germany)
- Paraffin for tissue embedding, Paraplast Plus® (Leica, Wetzlar, Germany)
- Histology cassette (Leica, Wetzlar, Germany)

4.2 Methods

4.2.1 Histopathology

Ethanol-fixed tissue specimens were trimmed, processed routinely, embedded in paraffin and sectioned at 3 µm. Tissue sections included: multiple liver and lung lobes, longitudinal sections of heart, striated muscle (lateroventral abdominal wall (*Musculus latissimus dorsi*, *M. spinotrapezius* or *M. obliquus externus*), *M. quadriceps femoris* and occasionally laryngeal muscle), kidneys, spleen, stomach, small intestine and large intestine. The tissue stains were conducted according to protocols published by Denk et al. (1989). All tissue sections were stained with hematoxylin and eosin (H&E). Kidney sections were also stained after Warthin Starry (WS) for spirochete detection. Additional specific staining was used depending on detected histological lesions: Azan, Prussian blue, Giemsa, PAS, Gram, Best's carmine and Richardson-staining. The specific staining protocols are described in detail in the supplementary material (page 186).

Hematoxylin and eosin staining (H&E)

1. Dewaxing of paraffin sections in a slide holder in xylene for 2 x 10 min
2. Rehydration of the sections through decreasing grades of alcohol (1 x 2 min 99% ethanol, 1 x 1 min 96% ethanol, 1 x 1min 80% ethanol and 1 x 1 min 70% ethanol) and distilled water
3. Hematoxylin staining: Incubation in hematoxylin solution for 4 min
4. Washing step in distilled water for 5-10 min
5. Incubation for 3 min in 0.2% eosin solution
6. Washing step in distilled water for 5-10 min

7. Dehydration through increasing grades of alcohol: 1 x 1 min 70% ethanol, 1 x 1 min 80% ethanol, 1 x 1min 96% ethanol and 2 x 1 min 99% ethanol)
8. Washing step in xylene 3 x 15 min
9. Mounting of coverslips to glass slides by Canada balsam (Carl Roth, Karlsruhe, Germany)
10. Dry overnight

Warthin-Starry staining (WS)

1. Dewaxing of paraffin sections in a slide holder in xylene for 2 x 10 min
2. Rehydration of the section through decreasing grades of alcohol (1 x 2 min 99% ethanol, 1 x 1 min 96% ethanol, 1 x 1 min 80% ethanol and 1 x 1 min 70% ethanol) and distilled water
3. Incubation for 1 h at 60 °C in 1% silver nitrate solution
4. Incubation in warm developer solution until sections turn into a golden/yellow colour
5. Washing step in tap water
6. Washing step 3 x in Buffer solution
7. Fixation for 2 min in 1% sodium thiosulfate
8. Washing step in distilled water
9. Dehydration through graded alcohols: 1 x 1 min 96% ethanol and 2 x 1 min 99% ethanol
10. Washing in xylene 3 x 15 min
11. Mounting of coverslips to glass slides by medium Canada balsam (Carl Roth, Karlsruhe, Germany)
12. Dry overnight

4.2.2 Health status classification

In this study, the interpretation of the histopathological lesions was used as an estimate of the overall health status of the individual (Crissman et al., 2004). Specifically, the potential impact of the sum of all detected histopathological changes on the clinical well-being of the animal was estimated and expressed as a) clinically healthy, b) mildly clinically affected and c) overt clinical disease likely. It was based on an ordinal numeric scoring with an ascending scale (Stevensen, 1946), using an optimal range of five levels (Shackelford et al., 2002; Thoolen et al., 2010) from 0 (no lesions) to 5 (more than three organs with moderate lesions or one vital organ (lung, liver, heart, kidney) with severe lesions) (see Tab. 7). To reduce a possible performance error, two pathologists scored each animal independently, compared their results and determined the final score.

The histopathological assessment was conducted by examining the different patterns of cellular organ tissue response to an insult regardless of the aetiology, allowing the identification of the individuals' natural diseases (Maxie et al., 2015) and providing the essential information of an animal's lesions' background needed for an accurate scoring (Gibson-Corley et al., 2013). The histopathological information of all organs was considered for the evaluation, except spleen tissue due to limited availability and findings of this organ were excluded from the health status assessment.

The histopathological findings were grouped into four broad categories, according to previous publications (Gibson-Corley et al., 2013; Kleiner et al., 2005; Snider et al., 2010; Thoolen et al., 2010): 1) Inflammation: infiltration by inflammatory cells (neutrophilic, mixed inflammatory cells with neutrophilic predominance, mixed inflammatory cells with lymphoplasmacytic predominance and lymphoplasmacytic) or inflammatory reactions e.g. like granuloma formation. As mild inflammation of the gastrointestinal was a regular background finding in all animals of this study, it was only included in the evaluation when two or more sections of the gastrointestinal tract revealed at least a moderate degree of inflammatory reaction. 2) Parasitic infection: although the presence of parasites was a common finding in the animals of this study, they were mostly not associated with reactive changes however in certain tissue sections dense accumulations of parasites was detected. If these aggregations affected more than 20% of the organ or single or multiple parasites resided in critical locations like the heart vessels, they were included in this category. 3) Non-neoplastic proliferative changes: hyperplasia and metaplasia. 4) Cellular damage: Necrosis, hemorrhage and reparative changes (i.e. deposition of collagenous connective tissue (fibrosis)). For each of the four categories organ location and distribution of the changes (focal, focally extensive, multifocal to coalescing, generalized) were noted. Further the quality of the changes was graded into mild, moderate and severe according to criteria established by Shackelford et al. (2002): Mild – a noticeable, but not prominent histopathological change, affecting up to 20% of the tissue section. In hyperplasic lesions, the affected tissue shows an estimated volume increase about 10% up to 20%. Moderate – distinct tissular modification affecting 20 to 40% of the tissue section. In hyperplasic lesions, the affected tissue shows an increased volume from 20% up to 40%. Severe – extensive tissular modification affecting 40% to 100% of the tissue section. In hyperplastic lesions, the affected tissue shows an increased volume from 40% up to 100%.

Tab. 7: Scoring of pathological lesions and estimated health status

Score and definition	Health status
0: No lesions observed	Clinically healthy
1: Mild lesions only	
2: One organ with moderate lesions	Mildly clinically affected
3: Two organs with moderate lesions	
4: Three organs with moderate or severe lesions	Overt clinical disease likely
5: More than three organs with moderate lesions or at least one vital organ (lung, liver, heart, kidney) with severe lesions	

4.2.3 Transmission electron microscopy

The processing of sample material for the ultrastructural evaluation was conducted following the procedure described by Bergmann and Kinder (1987) and Robinson et al. (1987).

Re-embedding of paraffin material in epoxy resin

a) Removal of organ tissue from paraffin block
b) Trimming tissue pieces down to 2-4 mm
c) Deparaffinization of the tissue
   - Incubation of tissue 2 x 1 h in xylene
   - Overnight incubation in xylene
   - Rehydration with decreasing grades of alcohol: 1 x 10 min 90% ethanol, 1 x 10 min 70% ethanol, 1 x 10 min 50% ethanol
   - Overnight incubation in Phosphate Sörensen buffer
   - Incubation at 1% osmium tetroxide (4°C) for 1h 30 min
   - Washing step 3 x 15 min with PBS pH 7.2
   - Dehydration with increasing grades of alcohol: 1 x 15 min 30% ethanol, 1 x 15 min 50% ethanol, 2 x 15 min 70% ethanol, 2 x 15 min 96% ethanol, 3 x 20 min absolute ethanol and 2 x 7.5 min in propylene oxide
d) Embedding in epoxy resin
   - Incubation with propylene oxide / epoxide mixture (1:1) for 45 min
   - Incubation in EPON for 1 h
   - Place sample into mold until completely covered
   - Polymerization: incubation overnight at 60 °C

Semi-thin sections

- Trim epoxy blocks
- Sectioning of resin embedded tissue with an ultra-microtome at 1 µm and mounting sections on glass slides
- Dyeing of sections with Richardson solution
- Mounting of coverslip slides

Ultrathin sections

Equipping the ultra-microtome with a diamond knife for ultrathin sections of 70 nm thickness. Transfer sections to 200 mesh nickel grid.

Section contrasting

- Incubating sections in a 1:1 mixture of uranyl acetate / acetone for 20 sec
- Rinse in distilled water
- Incubation in lead citrate for 10 min
- Rinse with double-distilled water

4.2.4 Molecular investigations

4.2.4.1 Preparation of ethanol-fixed tissue for DNA extraction

Initially, kidney (n=119) and liver (n=120) tissue from all forest rodents, *Tupaia* spp. and *Sundamys muelleri* and striated muscle from *Tupaia* spp. (n= 14) fixed in 70% ethanol were attempted to homogenize. (Note: Material of three kidneys from one *Maxomys whiteheadi* and two *Maxomys surifer/rajah* and two livers from two *Sundamys muelleri* were entirely used for the histopathological evaluation and therefore unavailable for molecular analysis.)

From each sample six grams of ethanol-fixed tissue were placed in a screw cap micro tube filled with 25 precellys ceramic beads and PBS buffer and homogenized at 6300 rpm for two cycles of 30 seconds. But as tissue samples proved resistant to this treatment, they were manually cut into fine pieces by using a sterile scalp. The first DNA extraction attempt using these tiny tissue fragments in the proteinase K digestion step was unsuccessful for several samples. In a second attempt double or threefold volume of proteinase K and extending the incubation time up to 48 hours in total. A third attempt of DNA extraction was performed by washing and rehydrating the tissue samples, which finally enabled proteinase K digestion overnight. The sample materials were placed in a sterile 1.5 ml reaction tube, washed three times with 100 µl of sterile distilled water and incubated in 100 µl of sterile distilled water at room temperature for two hours.

4.2.4.2 DNA extraction from ethanol-fixed samples

For DNA extraction of the rehydrated ethanol-fixed kidney and liver tissues the NuleoSpin® Tissue kit (Macherey-Nagel, Düren, Germany) was used. The manufacturer's standard protocol for human or animal tissue and cell culture was followed:

1. Tissue preparation: 25 µl of homogenized tissue or tissue cut into fine pieces was placed in 1.5 ml tube
2. Pre-lysing sample: Addition of 180 µl Buffer T1 + 25 µl Proteinase K; vortexed to mix
3. Incubation at 56 °C overnight. (Incubation time was increased when tissue material was not completely digested)
4. Lysing sample: Addition of 200 µl of Buffer B3 and incubation at 70°C for 10 min
5. Adjust DNA binding conditions: Adding 210 µl of 96-100% ethanol
6. Transfer the mixture into NuleoSpin® Tissue columns
7. For DNA binding: Centrifugation for 1 min at 11,000 rpm. Discharge collection tube.
8. Washing of silica membrane (twice):
   - Addition of 500 µl of buffer BW for the first washing step and 500 µl buffer B5 for the second washing step
   - Centrifugation for 1 min at 11,000 rpm and discharge of the collection tube. Placing silica membrane into a new tube
9. Drying of the silica membrane by centrifugation for 1 min at 11,000 rpm. Discharge collection tube
10. Elution of the DNA: Addition of 100µl of buffer BE into a final collection tube. Incubation at room temperature for 3 min. Final centrifugation for 1 min at 11,000 rpm

For DNA extraction of the rehydrated ethanol-fixed striated muscle tissue NuleoSpin® DNA RapidLyse kit (Macherey-Nagel, Düren, Germany) was used:

1. Tissue preparation: 30 µl of homogenized tissue into 2 ml tube
2. Pre-lysing sample: Addition of 150 µl of Lysis Buffer RLY + 10 µl liquid Proteinase K
3. Incubation at 56 °C for 1 h
4. Adjusting DNA binding conditions: Addition of 440 µl Binding Buffer RLB
5. Application of the mixture into the NuleoSpin® DNA RapidLyse columns
6. Binding of DNA: Centrifugation for 1 min at 11,000 rpm. Discharge collection tube
7. Washing steps (twice)
8. Addition of 500 µl of Buffer RLW
9. Centrifugation for 1 min at 11,000 rpm and discharge collection tube. Place silica membrane into new tube
10. Dried of the silica membrane (Centrifuge for 1 min at 11,000 rpm). Discharge collection tube

11. Elution of the DNA: Addition of 100 µl of Buffer RLE into a final collection tube. Incubation at room temperature for 3 min and a final centrifugation for 1 min at 11,000 rpm

4.2.4.3 DNA extraction from homogenized tissue stored in RNAlater®

Tissue concentration

A volume of 100 µl of homogenized kidney (n= 108) and liver (n= 277) tissues each were placed in a 1.5 ml reaction tube and centrifuged at 10,000 rpm for 10 min. The supernatant was discharged, and the pellets were used for DNA extraction.

For all forest rodents, *Sundamys muelleri* and all *Tupaia* spp. the DNA was extracted using the NuleoSpin® Tissue kit (Macherey-Nagel, Düren, Germany). The DNA from all *Rattus* spp. and *Suncus murinus* was extracted using NuleoSpin® DNA RapidLyse kit (Macherey-Nagel, Düren, Germany).

For the herpesvirus detection, the DNA extraction was based on the phenol-chloroform method by using the Purelink™ Viral RNA / DNA Mini Kit (Invitrogen™, Darmstadt, Germany). For this purpose, 140 µl of suspensions from centrifuged liver (n=106) and spleen (n=50) homogenates were used according to the manufacturer's instructions. In addition, 11 spleen and 14 liver samples were extracted using the RTP© DNA / RNA Virus Mini Kit (Invitek GmbH, Berlin, Germany). (All herpesvirus analyses were conducted by Heiko Pietsch, Robert Koch Institute, Berlin, Germany (Pietsch, 2013)*)*

DNA quantification

Quantity and quality of all the extracted DNA samples were measured with a spectrophotometer (NanoDrop 2000cqPCR, Thermo Fisher Scientific, Hennigsdorf, Germany). The quality assessment was based on the absorbance parameter (A260/A280), which indicates pure DNA at the ratio of 1.8.

4.2.4.4 PCR analyses

The detection of pathogen-DNA was conducted using the methods polymerase chain reaction (PCR) described by Mullis et al. (1986) and Saiki et al. (1988), and real-time polymerase chain reaction (qPCR) stablished by Higuchi et al. (1992, 1993).

Partial cytochrome b amplification from ethanol-fixed samples

A subset of DNA extracted from ethanol-fixed tissue of 61 livers and 44 kidneys were analysed by PCR for the detection mitochondrial DNA, targeting the cytochrome b gene.

Table 8: PCR- protocol cytochrome b

Reagent	Volume
SsoAdvanced™ Universal Probes Supermix	22.5 µl
Primer Kocher 1 (10 µM)	1 µl
Primer Kocher 2 (10 µM)	1 µl
Template	1 µl
Total volume	25 µl

Table 9: Thermocycler protocol cytochrome b

PCR-step	**Temperature**	**Time**
Pre-denaturation	94 °C	4 min
30 cycles		
Denaturation	94 °C	30 sec
Annealing	50 °C	30 sec
Amplification	72 °C	2 min
Final extension	72 °C	5 min

Molecular detection of pathogenic *Leptospira* spp.

The molecular detection of *Leptospira*-DNA was conducted on DNA (n=106) from homogenized kidney tissue stored in RNAlater®, using a real-time PCR analysis, followed by conventional PCRs for sequencing and confirmation of qPCR positive or questionable samples.

1. Real-time PCR (qPCR)

The analysis followed a protocol published by Stoddard et al. (2009) using the SsoAdvanced™ Supermix (Bio-Rad, Munich, Germany). The DNA concentration was adjusted to 500 ng in all samples. PCR reactions included positive (see page 35) and negative controls. Samples with a Ct value of ≤40 were considered positive or questionable. The analysis was carried out in the StratageneRMx 3005P thermocycler (Stratagene, Amsterdam, The Netherlands).

Tab. 10: Real-time PCR- protocol

Reagent	Volume
SsoAdvanced™ Universal Probes Supermix	10 µl
Primer LipL32-45F (10 µM)	1 µl
Primer LipL32-286R (10 µM)	1 µl
Probe LipL32-189P (10µM)	1 µl
Distilled water	1-6 µl
Template	1-5 µl
Total volume	20 µl

Tab. 11: Thermocycler protocol for qPCR

PCR-step	**Temperature**	**Time**
Denaturation	95 °C	5 min
Amplification: 45 cycles	95 °C	10 sec
	58 °C	30 sec

2. Conventional PCR (cPCR)

All qPCR positive or questionable samples (n= 25/106) were further investigated by cPCR sequencing and for confirmation. The cPCR protocol was based on a protocol from Cheema et al. (2007) with minor modifications: the use of MyTaq™ HS master Mix (Bioline, Germany) and adjusted cycling conditions (Tab. 11). DNA from *Leptospira interrogans* serovar Icterohaemorrhagiae served as positive control, as described above. Three different sets of primers were used for the cPCR analyses to amplify *Leptospira*-DNA from all samples.

The first set of LipL21 primers was used for all qPCR positive or questionable samples to amplify a partial LipL21 gene sequence of 561 bp length conserved in all pathogenic *Leptospira* serovars (Cullen et al., 2003).

The second set of primers G1 was used for all samples in comparison including those with negative results for LipL21. The G1 and G2 primers amplify a short but highly conserved DNA region of 266-285 bp length enabling the detection of pathogenic *Leptospira* spp. except *Leptospira kirschneri* (Gravekamp et al., 1993).

The third set of LipL32 primers correspond to the qPCR primers and amplifies a short LipL32 gene sequence of 240 bp length conserved in all pathogenic *Leptospira* serovars (Stoddard et

al., 2009). The LipL32 primers were used for sample E346/13 (*Sundasciurus lowii*) only, because *Leptospira*-DNA could not be amplified with LipL21 and G1/G2 primers.

Tab. 12: cPCR- protocols for amplification of *Leptospira*-DNA

Reagent	Volume
Taq HS Mix	12.5 µl
Nuclease-free water	3.5 µl
Primer LipL21 F (10 µM)	1 µl
Primer LipL21 R (10 µM)	1 µl
Template	5 µl
Total volume	25 µl

Reagent	Volume
Taq HS Mix	12.5 µl
Nuclease-free water	3.5 µl
Primer G1 (10 µM)	1 µl
Primer G2 (10 µM)	1 µl
Template	5 µ
Total volume	25 µ

Tab. 13: Cycling conditions for LipL21F/ LipL21R and G1/G2

PCR-step	**Temperature**	**Time**
Pre-denaturation	94 °C	2 min
35 cycles		
Denaturation	94 °C	30 sec
Annealing	55 °C	30 sec
Amplification	72 °C	1 min
Final extension	72 °C	7 min

3. Evaluation of qPCR and cPCR sensitivity used for *Leptospira*-DNA detection

To determine the sensitivity of both, qPCR and cPCR, we used a dilution series of up to 10^{-5} from the positive control DNA of *Leptospira interrogans* serovar *Icterohaemorrhagiae*. The qPCR (LipL32 primers) showed in 40 cycles the highest sensitivity detecting dilutions of up to 10^{-3}, meanwhile the cPCR detected dilutions of up to 10^{-2} for LipL21 and G1/G2 primers.

Molecular analysis for *Trypanosoma* spp. detection

The detection of *Trypanosoma*-DNA was conducted using homogenized liver tissue (n=277) stored in RNAlater®.

A nested-PCR targeting the 18S rRNA gene was used with a modified protocol from Noyes (Noyes et al., 2000, 1999). The external primers TRY927F and TRY927R were used in the first PCR, producing a sequence length of 927 bp. This product was amplified in a second PCR with internal primers SSU561F and SSU561R which resulted in a sequence length of 561 bp.

Tab. 14: Nested PCR- protocol for detection of *Trypanosoma*-DNA

Reagent	Volume
2.5 units Taq polymerase (GoTaq® Promega)	0.25 µl
MgCl2 (0.3 mM)	5 µl
100 mM-dNTPs	1 µl
Primer TRY927F (0.4 µM)	1 µl
Primer TRY927R (0.4 µM)	1 µl
Template	5 µl
Total volume	25 µl

Reagent	Volume
3 units Taq polymerase (GoTaq® Promega)	0.25 µl
MgCl2 (0.3 mM)	10 µl
100mM-dNTPs	2 µl
Primer SSU561F (0.4 µM)	2 µl
Primer SSU561R (0.4 µM)	2 µl
Template (PCR product)	2 µl
Total volume	50 µl

Tab. 15: Thermocycler protocol for the first and second round of nested PCR

PCR-step	**Temperature**	**Time**
30 cycles		
Denaturation	94 °C	30 sec
Annealing	58 °C **	1 min
Amplification	72 °C	1 min
Final extension	72 °C	7 min

** Second PCR round 56 °C for 1 min.

Molecular analysis for *Sarcocystis* spp. detection

The molecular detection of *Sarcocystis*-DNA was conducted from ethanol-fixed striated muscle (n=58) from forest rodents (10/38), treeshrews (11/14) and *Sundamys muelleri* (37/70) that presented sarcosporidiasis by light microscopy evaluation.

Partial 18S RNA gene sequences were amplified using the primers SarAF and SarDR following the protocol of Prakas (Prakas et al., 2014). The amplicons were cloned using the TOPO TA Cloning® Kit for sequencing with One Shot® TOP10 Chemically Competent *E. coli* (Invitrogen™, Glasgow, UK) according to the manufacturer's instructions. Ten clones per PCR product were selected and transferred to 20µl water. The clones were used as template in a PCR as published by Andree et al. (2010).

The mitochondrial gene cytochrome oxidase subunit 1 (COI) was targeted by using the primers SF1 and SR8D according to the protocol by Gjerde (Gjerde, 2013) with minor modifications like the use of DNA polymerase (Thermo Fisher Scientific, Vilnius, Lithuania) and an annealing temperature of 56 ºC.

Tab.16: PCR protocol for partial 18S rRNA gene amplification

Reagent	Volume
Nuclease-free water	12 µl
5 x PCR-Puffer + MgCl (0.3 mM)	5 µl
dNTPs 100mM	1 µl
SarAF (10 µM)	1 µl
SarDR (10 µM)	1 µl
Taq-Polymerase (GoTaq® Promega)	0.2 µl
Template	5 µl
Total volume	25 µl

Tab. 17: Thermocycler protocol for partial 18S rRNA gene amplification

PCR-step	**Temperature**	**Time**
5 cycles		
Denaturation	94 °C	45 sec
30 cycles		
Annealing	64 °C	60 sec
Amplification	72 °C	70 sec
Final extension	72 °C	10 min

Tab. 18: PCR for COI gene amplification

Reagent	Volume
Taq HS Mix	6 µl
Nuclease free water	15 µl
Primer SF1 (10 µM)	1 µl
Primer SR8D (10 µM)	1 µl
Template	2 µl
Total volume	25 µl

Tab. 19: Thermocycler protocol COI gene amplification

PCR-step	**Temperature**	**Time**
35 cycles		
Denaturation	94°C	45 sec
Annealing	56°C	45 sec
Amplification	72°C	2 min
Final extension	72°C	10 min

Molecular analysis for herpesvirus detection (Bachelor thesis Heiko Pietsch, Robert Koch Institute, Berlin, Germany)

The molecular analysis of herpesvirus was conducted by Heiko Pietsch (Pietsch 2013) as part of his bachelor thesis on the surveillance of emerging infectious diseases in small mammals from Northern Borneo. The aim of his thesis was the identification of relevant viral pathogens and their diversity among small mammal species inhabiting disturbed anthropogenic environment. Due to sample preservation in RNAlater® under tropical conditions (30-40 ºC), the RNA content of the samples was degraded. For this reason, the presence of RNA viruses such as arenaviruses and hantaviruses could not be confirmed. Nevertheless, herpesviruses being DNA viruses could be successfully detected in liver and spleen tissue.

The tissue samples used for this analysis belonged to 156 animals included in this study. The herpesvirus analysis was based on a generic nested-PCR targeting phylogenetically conserved regions in the herpesvirus genome (Ehlers et al., 1999).

Sequence analyses

- Identification of pathogenic *Leptospira* spp.

Amplicons from the LipL21 and LipL32 primers were purified with the MSB® Spin PCRapace kit (Stratec, Birkenfeld, Germany). The PCR products from the G1/G2 primers showed unspecific bands, therefore the amplicons were extracted directly from the gel and purified

using QIAquick® Gel Extraction Kit (Qiagen, Hilden, Germany). Sanger sequencing was performed at LGC Genomics (Berlin, Germany) by using the corresponding cPCR primers.

Leptospira sequences were checked for quality, trimmed and aligned according to the primer sets used for cPCR and sequencing with the ClustalW algorithm as implemented in BioEdit (Thompson et al. 1994; Hall 1999). Identification results were obtained by BLAST analysis against the NCBI GenBank database (supplementary material Tab. 40).

- Identification of *Trypanosoma* spp.

PCR products from the nested-PCR analyses were purified with the MSB® Spin PCRapace kit (Stratec, Birkenfeld, Germany) and sequenced at LGC Genomics (Berlin, Germany). *Trypanosoma* sequences were checked for quality, trimmed, and aligned as described above. Identification results were obtained by BLAST analysis against the NCBI GenBank database (supplementary material Tab. 42).

Subsequent sequence alignment and identification were carried out through BLAST analysis to find the most similar sequences from the NCBI GenBank database (supplementary material Tab. 42).

- Identification of *Sarcocystis* spp.

PCR products from 18S rRNA and COI gene amplification were purified with the ExoSAP (Thermo Fisher Scientific, Waltham, USA) and MSB® Spin PCRapace kit (Stratec, Birkenfeld, Germany) respectively.

The 18S rRNA sequences were directly sequenced using the BigDye Terminator v1.1 Cycle Sequencing Kit for Sanger sequencing (Thermo Fisher Scientific, Waltham, USA) and the primers SarAF, SarCF, SarBR and SarDR (Prakas et al., 2014). Sequences were analysed using a 3130xl Genetic Analyzer (Thermo Fisher Scientific, Waltham, USA). Raw sequences were manually checked and refined using MEGA6 (Tamura et al., 2013).

The sequencing analysis of the amplicons from COI was conducted by Dr. Annika Brinkmann and Prof. Dr. Andreas Nitsche (Robert Koch Institute, Berlin, Germany) by using a MinION (Oxford Nanopore Technologies, Oxford, UK).

- Identification of herpesviruses

Purification of the amplicons was conducted using the MSB® Spin PCRapac kit (Invitek, Berlin, Germany). Sequencing of the PCR herpesvirus products was carried out by the dideoxy chain termination method according to Sanger. For sequencing, the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) was used. Capillary electrophoresis was performed by the in-house sequencing laboratory of the Robert Koch Institute (Berlin, Germany).

4.2.5 Morphologic and phylogenetic evaluation of *Sarcocystis* spp.

4.2.5.1 Morphologic and morphometric evaluation

Tissue sections of striated muscles from all forest species (rodent species and treeshrews) and the generalist species, *Sundamys muelleri*, were stained with H&E and examined by light microscopy at 100- to 400-times magnification to record the number of cysts of *Sarcocystis* spp. per tissue slide. *Sarcocystis*-positive samples were removed from the paraffin block and re-embedded in epoxy resin. Semi-thin sections of these tissues were stained with Richardson staining. Morphometric measures were conducted on all Sarcocystis cysts stained either with H&E or Richardson. The measurements of the cyst structures were conducted as follows:

- Cyst: Length and width
- Wall: Width, including ground substance and protrusions
- Cystozoites: Length and width as well as description of their shape

Autolytic cysts could not sufficiently be evaluated. For this reason, only well-preserved cysts were included in the morphological analysis. In the measurement results the median and the standard deviation (±SD) were included when more than 15 measurements could be taken from different cysts with the same morphology.

4.2.5.2 Transmission electron microscopy

Ultrathin sections of *Sarcocystis*-positive samples were examined by transmission electron microscopy (Tecnai BioSpirit, FEI, Hillsboro, Netherlands). The morphological examination of the cysts comprised the description of their ultrastructural features and the measurements of the cysts' structures (Fig. 3):

- Protrusions: Length and width
- Wall: Width, including protrusions
- Ground substance: Width
- Cystozoites: Length and width as well as description of their shape

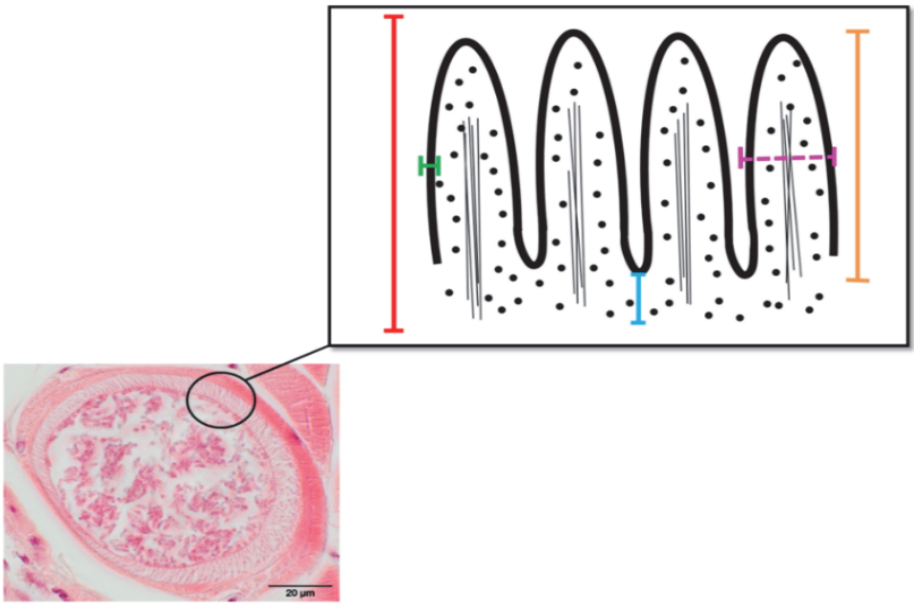

Fig. 3: Scheme of the basic structures of the *Sarcocystis* wall highlighting the most importantfeatures for the morphometric evaluation. The total width of the cyst wall is represented by the red line. Considering the villous protrusions, the orange line indicated the length, the pink the width and the green the width of the protrusion wall. The blue line indicated the ground substance width.

4.2.5.3 Phylogenetic analysis of *Sarcocystis* spp.

The phylogenetic analysis of the SSU (small subunit) 18S rRNA gene and cytochrome C oxidase subunit I (COI) gene for a novel *Sarcocystis* sp. discovered in *Tupaia* spp. from this study, were conducted by Dr. Thomas Jäkel (University of Hohenheim, Stuttgart, Germany) (Ortega Pérez et al., 2020).

In summary, the SSU 18S rRNA gene sequences of the *Sarcocystis* isolates from *Tupaia* spp. were aligned with published Apicomplexan species available in GenBank (Clark et al., 2016) using the multiple sequence alignment algorithm 'R-Coffee' of the T-Coffee web server (version 11.00.d625267/2016-01-11/Revision-d625267-Build 507).
For Maximum Likelihood analysis (ML), the appropriate model for nucleotide substitution rates was determined employing the MEGA 7 software package (Kumar et al., 2016).
Bayesian Inference (BI) was executed with MrBayes Version 3.2 (Ronquist et al., 2012) (supplementary material Fig. 21).
Analyses of sequences of the COI gene were all executed within MEGA 7 software. For phylogenetic reconstruction, the Tamura-Nei model was selected (supplementary material Fig. 22).

5 RESULTS

5.1 Histopathology

In total 346 small mammals were caught in the study area in Northern Borneo. Out of these, tissues from 331 individuals were suitable for histopathological investigations (Tab. 1). Due to autolysis, some organs were removed from the sample pool, resulting in varying organ numbers compared to the total number of individuals per species. In the following paragraphs the species with such discrepancy in organ numbers are marked (*), the total number of evaluated organs is always mentioned at the beginning of the description of each organ system. The different organ systems are listed in a decreasing order of affection by pathological changes. A summary of all the histopathological changes described below can be found in Tab. 20A, 20B and 20C.

Respiratory system (Lung n=310)

Callosciurus notatus (n=5), **Callosciurus prevosti* (n=2), *Sundasciurus lowii* (n=6), **Leopoldamys sabanus* (n=0), *Maxomys rajah* (n=3), *Maxomys suriferi* (n=7), **Maxomys suriferi/rajah* (n=6), *Maxomys whiteheadi* (n=6), *Niviventer cremoriventer* (n=4), *Tupaia gracillis* (n=1), *Tupaia minor* (n=8), **Tupaia tana* (n=5), **Sundamys muelleri* (n=71), *Rattus* compl. (n=101), *Rattus norvegicus* (n=41) and **Suncus murinus* (n=44).

(Due to autolysis "*" indicates a different number of the respective organ compared to the total number of individuals per species.)

Inflammation of the lung (pneumonia and bronchopneumonia) was the most common pathological finding occurring in 89% (277/310) of all individuals from all species groups: forest species (forest rodents 37/39 and *Tupaia* spp.14/14), *Sundamys muelleri* (63/71), *Rattus* spp. (*Rattus* compl. 79/101 and *Rattus norvegicus* 41/41) and the shrew *Suncus murinus* (43/44).

There were distinct differences in the prevailing type of inflammation and extension of the pneumonia or bronchopneumonia between the examined animal species. Mild to moderate lymphoplasmacytic pneumonia and bronchopneumonia were predominant in the forest species like *Sundasciurus lowii* (6/6), *Niviventer cremoriventer* (4/4) and in *Tupaia* spp. (14/14) as well as in the generalist *Sundamys muelleri* (48/71). Meanwhile the forest species *Maxomys* spp. (20/22), *Callosciurus notatus* (5/5) and the urban shrew *Suncus murinus* (43/44) were more often affected by a mild to moderate neutrophilic pneumonia and bronchopneumonia (Fig. 4A). Individuals from the *Rattus* spp. were affected by both kinds of inflammation: mild to moderate lymphoplasmacytic (*Rattus* compl. (44/101), *Rattus norvegicus* (19/41)) and neutrophilic infiltration (*Rattus* compl. (35/101), *Rattus norvegicus*

(22/41)). Presence of mild eosinophilic homogeneous material in the alveolar lumen, comparable with alveolar edema, was found in forest rodents (19/39), treeshrews (3/14), *Sundamys muelleri* (20/71) and the urban rat *Rattus norvegicus* (2/41). In addition to the inflammatory processes a mild to severe hyperplasia of the bronchus-associated lymphoid tissue (BALT) was observed in forest rodents (4/39), *Sundamys muelleri* (24/71), the shrew *Suncus murinus* (3/44) and *Rattus* spp. (*Rattus* compl. (9/101), *Rattus norvegicus* (22/41)).

Moderate to severe parasitic pneumonia with evidence of nematode cross sections in alveolar tissue was a common finding in rats (*Rattus* compl. (37/101) and *Rattus norvegicus* (8/41)) and *Tupaia* spp. (5/14). In *Rattus* spp. lung sections revealed up to 50% to 80% of the tissue affected by multiple variably sized parasite nodules containing nematode eggs and larvae demarcated by lymphocytes, plasma cells and macrophages (Fig. 4B). Furthermore, in *Rattus* spp. adult nematodes, characterized by hypodermal bands and coelomyarian-polymyarian musculature, were often located in the lumen of the pulmonary blood vessels with moderate to severe reactive hypertrophy of the tunica media, sometimes almost entirely obstructing the lumen of the vessels. The location and morphology of these parasites is consistent with *Angiostrongylus* sp.

The same distinct hyperplasia of the vascular tunica media of pulmonary arteries, but without evidence of intravascular nematodes in the section plane (Fig. 4C), was also found in *Rattus* spp. (*Rattus* compl. (54/101) and *Rattus norvegicus* (25/41)) as well as in a high number of the generalist *Sundamys muelleri* (44/71). This contrasts with the forest animals, where such lesion was only found in a single squirrel (*Sundasciurus lowii*).

As an additional finding, seven individuals from the *Rattus* compl. had multifocal mild to moderate squamous metaplasia of bronchiolar epithelium (Fig. 4D).

Hepatic system (Liver n=321)

Callosciurus notatus (n=5), **Callosciurus prevosti* (n=2), *Sundasciurus lowii* (n=6), **Leopoldamys sabanus* (n=0), *Maxomys rajah* (n=3), **Maxomys suriferi* (n=7), **Maxomys suriferi/rajah* (n=6), *Maxomys whiteheadi* (n=6), *Niviventer cremoriventer* (n=4), *Tupaia gracillis* (n=1), *Tupaia minor* (n=8), **Tupaia tana* (n=5), **Sundamys muelleri* (n=70), **Rattus* compl. (n= 98), **Rattus norvegicus* (n=42) and *Suncus murinus* (n=58).

Overall, hepatic lesions were detected in 60% (192/321) of all individuals. Histopathological changes of the liver in all species groups consisted mostly of mild to moderate predominantly lymphoplasmacytic infiltration of the portal spaces. Frequently this lesion was associated with mild to moderate hyperplasia of the bile ducts (Fig. 5A), which was a common finding in the majority of the study species with higher presence in *Sundamys muelleri* (36/70). Hepatitis

suggestive for bacterial origin was detected in three *Suncus murinus* and two individuals from *Rattus* compl., which presented multiple foci of neutrophilic infiltration in the parenchyma.

Lesions associated with parasite infection, most likely nematode migration tracts, were found in all taxa groups with an overall occurrence of 30% (96/321), with *Rattus* spp. and *Sundamys muelleri* being the most commonly affected with 41% (58/140 (30/98 *Rattus* compl., 28/42 *Rattus norvegicus*) and 29% (20/70) respectively.

The development from acute, subacute to chronic stages of parasitic lesions as a result of parasite migration through the parenchyma and final granulomatous encapsulation of parasite remnants could be retraced. The acute stage was the most commonly observed lesion (42/96), characterized by focal haemorrhage and necrosis composed by a central area with detritus, amorphous eosinophilic material (Splendore-Hoeppli material) (Fig. 5B) and severe mixed inflammatory infiltration with predominance of neutrophils, and mild lymphoplasmacytic infiltration. In some individuals with acute lesions, the detection of adult nematodes with a thin cuticula and hypodermal bacillary bands on cross sections and barrel shaped nematode eggs with bipolar lugs and thick cuticle (Fig. 5C) allowed a morphological classification: Morphology and location are comparable to *Calodium hepaticum* (previous nomenclature*: Capillaria hepatica)*. The extension of egg aggregations in the parenchyma ranged from single eggs to 80% of the liver tissue containing helminthal eggs. Subacute lesions (8/96) had increasing numbers of macrophages and fibroblasts, and chronic lesions (29/96) were characterized by tissue repair with deposition of collagen-rich fibrous connective tissue, readily observed with Azan staining (Fig. 5D). A mix of coexisting acute and chronic lesions was found in 5 out of 96 infected individuals. Moreover, in twelve individuals eggs or adult nematodes were found without inflammatory response.

Formation of large parasitic cysts was found in three individuals from the *Rattus* compl*.* The cysts had a thick fibrous capsule infiltrated by moderate mixed inflammatory cells and were compressing the adjacent parenchyma with local atrophic hepatocyte degeneration. These lesions are consistent with hepatic cysticercosis most likely caused by a *Taenia* sp.

In two individuals from *Rattus norvegicus* a small number of hepatocytes contained multiple protozoal organisms suggestive of merogony stages of *Sarcosporidia* spp. with the characteristic rosette appearance of the basophilic internal structures. There was no inflammatory response to these infected cells.

The livers of eight *Tupaia* spp. and one *Niviventer cremoriven*ter had generalised extensive swelling of the hepatocytes, which appeared almost empty except for some finely stippled cytoplasm and small densely basophilic central nuclei. Specific staining by Periodic acid–

Schiff (PAS) for glycogen detection was negative. There was no inflammatory reaction and sinusoidal spaces were completely obscured by the swollen hepatocytes.

Digestive system:

Stomach n=299; *_Callosciurus notatus_ (n=2), *_Callosciurus prevosti_ (n=1), *_Sundasciurus lowii_ (n=5), *_Leopoldamys sabanus_ (n=0), _Maxomys rajah_ (n=3), _Maxomys suriferi_ (n=7), *_Maxomys suriferi/rajah_ (n=6), _Maxomys whiteheadi_ (n=6), _Niviventer cremoriventer_ (n=4), _Tupaia gracillis_ (n=1), _Tupaia minor_ (n=5), *_Tupaia tana_ (n=6), *_Sundamys muelleri_ (n=69), *_Rattus_ compl. (n=93), *_Rattus norvegicus_ (n=36) and *_Suncus murinus_ (n=55).

Small intestine n=305; *_Callosciurus notatus_ (n=4), *_Callosciurus prevosti_ (n=2), _Sundasciurus lowii_ (n=6), *_Leopoldamys sabanus_ (n=0), _Maxomys rajah_ (n=3), *Maxomys suriferi (n=5), *_Maxomys suriferi/rajah_ (n=5), *_Maxomys whiteheadi_ (n=5), *_Niviventer cremoriventer_ (n=3), _Tupaia gracillis_ (n=1), _Tupaia minor_ (n=8), *_Tupaia tana_ (n=4), *_Sundamys muelleri_ (n=67), *_Rattus_ compl. (n=100), _Rattus norvegicus_ (n=36) and *_Suncus murinus_ (n=56).

Large intestine n=274; *_Callosciurus notatus_ (n=4), *_Callosciurus prevosti_ (n=1), *_Sundasciurus lowii_ (n=5), *_Leopoldamys sabanus_ (n=0), _Maxomys rajah_ (n=3), *_Maxomys suriferi_ (n=6), *_Maxomys suriferi/rajah_ (n=6), _Maxomys whiteheadi_ (n=6), _Niviventer cremoriventer_ (n=4), _Tupaia gracillis_ (n=1), _Tupaia minor_ (n=8), *_Tupaia tana_ (n=4), *_Sundamys muelleri_ (n=66), *_Rattus_ compl. (n=95), *_Rattus norvegicus_ (n=38) and *_Suncus murinus_ (n=27).

A surprising finding in some murid species was the presence of a highly dense layer of long rod-shaped bacteria orientated perpendicular to the mucosal lining of the forestomach resembling a bacterial “lawn”. This was commonly seen in the forest rodent species Maxomys (_Maxomys rajah_ (3/3), _M. surifer_ (5/7), _M. whiteheadi_ (6/6)) and one _Niviventer cremoriventer_ (1/4). Gram staining showed Gram-positive rod-shaped bacterial aggregation on the surface of the keratinized epithelium, suggestive of _Lactobacilli_ sp. (Fig. 6A).

Gastro-enteric nematodiasis was a common finding with varying prevalence between animal groups as well as digestive tract locations. The stomach was highly parasitized in _Sundamys muelleri_ (34/69), _Maxomys_ spp. (14/22) where barrel-shaped bipolar eggs were deposited in the non-keratinized squamous epithelium without causing an inflammatory reaction (Fig. 6B). The morphology and location of the eggs were suggestive of _Trichomoides crassicauda_ (Family Trichomoididae) or _Eucoleus_ sp. (Family Trichuridae) according to Gardiner and Percy (Gardiner and Fayer, 1999; Percy and Barthold, 2007). In contrast, gastric nematodiasis in species with only a glandular stomach such as _Tupaia_ spp. (6/12) and the shrew _Suncus murinus_ (8/56) showed more often submucousal granulomatous lesions. The

glandular part of the stomach of almost half of the study animals was affected by mild to moderate neutrophilic infiltration of the lamina propria.

Considering the individuals where the small and large intestines were present for histological evaluation inflammatory reaction was found in 38% (102/266). Generally, in the small and large intestine the lamina propria contained mild to moderate degrees of lymphoplasmacytic infiltration with some intermixed neutrophilic granulocytes.

With the exception of an extensive suppurative peritonitis detected in an individual from *Sundamys muelleri*, the only significant lesions found in the intestine were caused by nematodal infection. More than half of *Suncus murinus* (28/56) had high numbers of nematodes in the duodenum. These were located within the Brunner's glands in the submucosal layer leading to moderate to severe hyperplasia of the glands and mild to moderate neutrophilic infiltration of the submucosa (Fig. 6C). In all taxa groups jejunum and ileum were also commonly infected by nematodes: forest species (forest rodent species (14/33) and *Tupaia* spp. (6/12)), *Sundamys muelleri* (26/67), the urban shrew *Suncus murinus* (28/56), *Rattus* compl. (39/100) and *Rattus norvegicus* (6/36) (Fig. 6D). The infection was not associated with an increase of inflammatory cells in the lamina propria as compared to individuals without evidence of parasitic infection.

Intraepithelial coccidian protozoal organisms with different developmental stages were observed in the enterocytic epithelium of villi and crypts of the small and large intestine in forest rodents and treeshrews (*Niviventer cremoriventer* (1/3) and *Maxomys whiteheadi* (1/6), *Tupaia minor* (2/8), *Tupaia tana* (1/4)), the generalist *Sundamys muelleri* (6/ 67) and one shrew *Suncus murinus* (1/56). The urban *Rattus* spp. showed coccidiosis of the small intestine only (*Rattus* compl. (1/100) and *Rattus norvegicus* (10/36)). A mild lymphoplasmacytic inflammation of the lamina propria was found alongside with the infection. The morphology and location of the parasite is consistent with *Eimeria* sp.

Musculature (Striated muscle n=309)

Callosciurus notatus (n=5), **Callosciurus prevosti* (n=2), *Sundasciurus lowii* (n=6), **Leopoldamys sabanus* (n=0), *Maxomys rajah* (n=3), *Maxomys suriferi* (n=7), **Maxomys whiteheadi* (n=5), **Maxomys suriferi/rajah* (n=6), *Niviventer cremoriventer* (n=4), *Tupaia gracillis* (n=1), *Tupaia minor* (n=8), **Tupaia tana* (n=5), **Sundamys muelleri* (n=70), **Rattus* compl. (n=90), *Rattus norvegicus* (n=41) and **Suncus murinus* (n=56).

About one third (108/309) of all individuals presented numerous cysts of apicomplexan protozoa from the genus *Sarcocystis* in their striated muscles. The species *Tupaia* spp. (11/14), *Sundamys muelleri* (37/70) and *Rattus* compl. (38/90) showed a rather high prevalence in contrast to, for example, only two *Suncus murinus*. From the forest species five

squirrels (*Sundasciurus lowii* (4/6), *Callosciurus notatus* (1/4) and five murids (*Maxomys whiteheadi* (2/5), *Niviventer cremoriventer* (3/4) were infected. All *Sarcocystis* spp. were localized in muscle fibers without any inflammatory reaction.

Urinary system (Kidney n=314)

Callosciurus notatus (n=5), **Callosciurus prevosti* (n=2), **Sundasciurus lowii* (n=5), **Leopoldamys sabanus* (n=0), *Maxomys rajah* (n=3), *Maxomys suriferi* (n=7), **Maxomys suriferi/rajah* (n=6), **Maxomys whiteheadi* (n=5), *Niviventer cremoriventer* (n=4), *Tupaia gracillis* (n=1), *Tupaia minor* (n=8), **Tupaia tana* (n=5), **Sundamys muelleri* (n=71), and **Rattus* compl. (n= 100), **Rattus norvegicus* (n=43) **Suncus murinus* (n=49).

Kidney lesions affected 28% (89/314) of the individuals and were mostly characterized by a mild to moderate lymphoplasmacytic interstitial nephritis, often associated with perivascular cuffs of mononuclear inflammatory cells (Fig. 7A). Granulomatous nephritis was observed in one individual from *Rattus norvegicus*.

In contrast to lung and liver, renal nematodiasis was very low (5/314). In five individuals (*Rattus norvegicus* (2/143), *Tupaias* spp. (2/14) and *Sundamys muelleri* (1/71)) epithelial hyperplasia of the renal pelvis was observed in association with the presence of adult nematodes in this location, compatible with *Triichomoides crassicauda* (Fig. 7B). Two squirrels (*Sundasciurus lowii* (2/5)) and one rat from *Rattus* compl. presented renal coccidiosis revealing different developmental stages of the parasite in the epithelial cells of renal tubules predominately located at the corticomedullary junction (Fig. 7C).

Circulatory system (Heart n= 324)

Callosciurus notatus (n=5), **Callosciurus prevosti* (n=2), *Sundasciurus lowii* (n=6), **Leopoldamys sabanus* (n=0), *Maxomys rajah* (n=3), *Maxomys suriferi* (n=7), **Maxomys suriferi/rajah* (n=6), **Maxomys whiteheadi* (n=5), *Niviventer cremoriventer* (n=4), *Tupaia gracillis* (n=1), *Tupaia minor* (n=8), **Tupaia tana* (n=5), **Sundamys muelleri* (n=71), **Rattus* compl. (n=102), **Rattus norvegicus* (n=43) and **Suncus murinus* (n=56).

Generalized lymphoplasmacytic myocarditis was detected in 19% (60/324) of all individuals: forest rodents (6/38), *Sundamys muelleri* (19/71), *Rattus* compl. (14/102), *Rattus norvegicus* (18/43) and *Suncus murinus* (3/56). Further lesions of the heart consisted mostly of focal mild to moderate lymphoplasmacytic inflammation of the myocardial walls extending from epicardium, myocardium to endocardium or the atrioventricular valves (Fig. 8A). Such lesions affected 18% (61/324) of all animals: forest rodents (9/38), *Tupaia* spp. (3/14), *Sundamys muelleri* (22/71), *Rattus* compl. (15/102), *Rattus norvegicus* (11/43) and *Suncus murinus* (1/56).

In two individuals of *Sundamys muelleri* Giemsa staining revealed bacterial myocarditis characterized by multifocal dense foci of bacterial colonies (Fig. 8B).

In eight individuals from *Rattus* compl. adult nematodes were observed intraluminal in the arteries of the heart similar to the arterial vessels in the lung (Fig. 8C). The location and morphology of these nematodes was again suggestive of *Angiostrongylus* spp.

In one *Tupaia tana* multiple very small, long and slender nematode-like helminths consistent with parasitic microfilaria were found inside blood vessels of heart, liver and lung (Fig. 8D).

Spleen n=111

**Callosciurus notatus* (n=2), **Callosciurus prevosti* (n=1), **Sundasciurus lowii* (n=0), **Leopoldamys sabanus* (n=0), **Maxomys suriferi* (n=0), **Maxomys rajah* (n=1), **Maxomys whiteheadi* (n=0), **Maxomys suriferi/rajah* (n=0), **Niviventer cremoriventer* (n=0), **Tupaia gracillis* (n=0), **Tupaia minor* (n=0), **Tupaia tana* (n=0), **Sundamys muelleri* (n=42), **Rattus* compl. (n=41), **Rattus norvegicus* (n=21) and **Suncus murinus* (n=3).

The most common change observed was mild to moderate follicular hyperplasia in *Sundamys muelleri* (40/42) and *Rattus* spp. (*Rattus* compl. (29/41), R*attus norvegicus* (13/21)). Additionally, two *Suncus murinus* showed moderate neutrophilic splenitis. Lacking spleen material from most of the animals impeded further comparison among the ecological groups.

Tab. 20A: Summary of histopathological lesions in the lung and liver from forest rodents

Group		**Forest rodents**							
Species		*C. notatus*	*C. prevostii*	*S. lowii*	*M. rajah*	*M. surifer*	*M. rajah/surifer*	*M. whiteheadi*	*N. cremoriventer*
no.		5	2	6	3	7	6	6	4
Main histo-pathological lesions	**Lung**	(5/5) Mild/moderate mix-neut. interstitial pneumonia (1/5) Mild hyperplasia of the BALT (4/5) Mild alveolar edema	(2/2) Mild/moderate lypl. Interstitial bronchopneumonia (1/2) Mild/moderate hyperplasia of the BALT (1/2) Mild alveolar edema	(6/6) Mild/moderate lypl. interstitial pneumonia (1/6) Mild hyperplasia of the BALT (4/6) Mild alveolar edema	(3/3) Mild/moderate lypl. interstitial pneumonia (1/3) Mild alveolar edema	(5/7) Mild/moderate neut. interstitial pneumonia (3/7) Alveolar edema	(6/6) Mild/moderate lypl. (4/6) and neut. (2/6) interstitial pneumonia (4/6) Alveolar edema	(1/6) Mild parasitic pneumonia (6/6) Mild/moderate interstitial neut. Bronchopneumonia (2/6) Alveolar edema	(4/4) Mild/moderate interstitial mix-lypl. pneumonia (1/4) Mild hyperplasia of the BALT
	Liver	(3/5) Mild lypl. periportal inflammation (1/5) Granuloma (1/5) Mild BDH	(1/2) Mild lypl. periportal inflammation (1/2) Mild BDH	(1/6) Focal haemorrhage (parasitic migration) (2/6) Mild/moderate BDH	(2/3) Mild lypl. periportal inflammation (1/3) Mild BDH	(4/7) Mild lypl. periportal inflammation (1/7) Mild BDH	(3/6) Focal necrosis (parasitic migration) (2/6) Mild BDH	(1/6) Focal necrosis (parasitic migration) (2/6) Mild lypl. periportal inflammation (2/6) Mild BDH	(1/4) Focal fibrosis (parasitic migration) (2/4) Mild lypl. periportal inflammation (1/4) Mild/moderate BDH

Type of inflammation: lypl., lymphoplasmacytic. mix-neut., mixed inflammatory infiltration predominantly neutrophilic. neut., neutrophilic.
Lung: BALT, bronchus associated lymphoid tissue
Liver: BDH, bile duct hyperplasia

Continuation Tab. 20A: Summary of histopathological lesions in the gastrointestinal tract from forest rodents

Group		Forest rodents							
Species		*C. notatus*	*C. prevostii*	*S. lowii*	*M. rajah*	*M. surifer*	*M. rajah/surifer*	*M. whiteheadi*	*N. cremoriventer*
no.		5	2	6	3	7	6	6	4
Main histo-pathological lesions	Stomach	(1/2) Mild nematodiasis (2/2) Mild/moderate neut. inflammation of the lamina propria	NAD	(1/5) Mild/moderate neut. inflammation of the lamina propria	(2/3) Moderate nematodiasis (3/3) Mild/moderate bacterial accumulation (1/3) Mild/moderate neut. inflammation of the lamina propria	(5/7) Moderate/severe nematodiasis (5/7) Moderate bacterial accumulation (2/6) Mild/moderate neut. inflammation of the lamina propria	(3/6) Moderate/severe nematodiasis (6/6) Mild/moderate bacterial accumulation (2/6) Mild/moderate neut. inflammation of the lamina propria	(2/6) Mild nematodiasis (6/6) Moderate/severe bacterial accumulation (3/6) Mild/moderate neut. inflammation of the lamina propria	(1/4) Moderate nematodiasis (1/4) Mild bacterial accumulation (3/4) Mild/moderate neut. inflammation of the lamina propria
	Small intestine	(4/4) Mild/moderate lypl. inflammation of the lamina propria	NAD	(5/6) Mild/severe nematodiasis (6/6) Mild/moderate lypl. inflammation of the lamina propria	(1/3) Mild nematodiasis (3/3) Mild/moderate lypl. inflammation of the lamina propria	(1/5) Moderate nematodiasis	(1/5) Mild nematodiasis (4/5) Mild/moderate lypl. and neut. inflammation of the lamina propria	(3/5) Mild/moderate nematodiasis (5/5) Mild/moderate lypl. inflammation of the lamina propria	(3/3) Mild nematodiasis (3/3) Mild/moderate lypl. inflammation of the lamina propria
	Large intestine	(4/4) Mild/moderate lypl. inflammation of the lamina propria	NAD	(2/5) Mild nematodiasis (5/5) Mild/moderate lypl. inflammation of the lamina propria	(2/3) Mild/moderate lypl. inflammation of the lamina propria	(2/5) Mild nematodiasis (5/5) Mild/moderate lypl. inflammation of the lamina propria	(2/6) Mild nematodiasis (5/6) Mild/moderate lypl. inflammation of the lamina propria	(4/6) Moderate nematodiasis (1/6) Mild/moderate coccidiosis (6/6) Mild/moderate lypl. inflammation of the lamina propria	(1/3) Mild/moderate coccidiosis (2/3) Mild/moderate lypl. inflammation of the lamina propria

Type of inflammation: lypl., lymphoplasmacytic; mix-neut., mixed inflammatory infiltration predominantly neutrophilic; neut., neutrophilic;
NAD: Nothing abnormal discovered

Continuation Tab. 20A: Summary of histopathological lesions in the striated muscle, kidney, heart and spleen from forest rodents

Group		**Forest rodents**							
Species		*C. notatus*	*C. prevostii*	*S. lowii*	*M. rajah*	*M. surifer*	*M. rajah/surifer*	*M. whiteheadi*	*N. cremoriventer*
no.		5	2	6	3	7	6	6	4
Main histo-pathological lesions	**Striated muscle**	(1/4) *Sarcocystis*	NAD	(4/6) *Sarcocystis*	NAD	NAD	NAD	(2/5) *Sarcocystis*	(3/4) *Sarcocystis*
	Kidney	(1/4) Mild lypl. proximal interstitial nephritis	NAD	(2/6) Mild coccidiosis (Klossiella-like)	NAD	(1/4) Mild lypl. proximal interstitial nephritis	NAD	(2/5) Mild focal lypl. interstitial nephritis	(2/4) Mild focal lypl. interstitial nephritis
	Heart	(2/5) Mild lypl. generalized myocarditis	NAD	NAD	(3/3) Focal mild lypl. inflammation	(3/7) Focal mild lypl. inflammation	(2/6) Mild mild/moderate lypl. generalized myocarditis	(1/5) Mild lypl. generalized myocarditis (1/5) Focal lypl. mild inflammation	(3/4) Focal lypl. mild/moderate inflammation
	Spleen	(1/2) Moderate follicular hyperlpasia	NAD	NO	(1/1) Moderate follicular hyperlpasia	NO	NO	NO	NO

Type of inflammation: lypl., lymphoplasmacytic; mix-neut., mixed inflammatory infiltration predominantly neutrophilic; neut., neutrophilic.
NAD: nothing abnormal discovered
NO: no organ

Tab. 20B: Summary of histopathological lesions in the lung and liver from treeshrews

Group		**Treeshrews**		
Species		*T. gracilis*	*T. minor*	*T. tana*
no.		1	8	5
Main histo-pathological lesions	**Lung**	(1/1) Mild/moderate lypl. interstitial pneumonia	(4/8) Mild/moderate parasitic pneumonia (8/8) Mild to moderate lypl. interstitial pneumonia (2/8) Mild alveolar edema	(1/5) Severe microfilariosis (5/5) Mild to moderate lypl. interstitial pneumonia (1/5) Mild alveolar edema
	Liver	(1/1) Focal necrosis (parasitic migration)	(2/8) Focal necrosis (parasitic migration) (7/8) Generalized swelling of the hepatocytes (3/8) Mild/moderate BDH	(2/5) Parasite within bile duct (1/5) Generalized swelling of the hepatocytes (2/5) Mild/moderate BDH

Type of inflammation: lypl., lymphoplasmacytic; mix-neut., mixed inflammatory infiltration predominantly neutrophilic; neut., neutrophilic;
Lung: BALT, bronchus associated lymphoid tissue
Liver: BDH, bile duct hyperplasia

Continuation Tab. 20B: Summary of histopathological lesions in the gastrointestinal tract from treeshrews

Group		**Treeeshrews**		
Species		*T. gracilis*	*T. minor*	*T. tana*
no.		1	8	5
Main histo-pathological lesions	**Stomach**	(1/1) Mild nematodiasis (1/1) Mild/moderate neut. inflammation of the lamina propria	(1/5) Mild nematodiasis (3/5) Mild/moderate lypl. inflammation of the lamina propria	(4/6) Mild/moderate nematodiasis (3/6) Mild to moderate neut. inflammation of the lamina propria
	Small intestine	(1/1) Moderate nematodiasis	(3/8) Moderate/severe nematodiasis (1/8) Moderate coccidiosis (8/8) Mild/moderate lypl. inflammation of the lamina propria	(2/4) Moderate/severe nematodiasis (1/4) Moderate coccidiosis (4/4) Mild/moderate lypl. inflammation of the lamina propria
	Large intestine	NAD	(1/8) Mild nematodiasis (2/8) Moderate/severe coccidiosis (8/8) Mild/moderate lypl. inflammation of the lamina propria	(1/4) Moderate coccidiosis (4/4) Mild/moderate lymphoplasmacytic lypl. inflammation of the lamina propria

Type of inflammation: lypl., lymphoplasmacytic; mix-neut., mixed inflammatory infiltration predominantly neutrophilic; neut., neutrophilic;
NAD: Nothing abnormal discovered

Continuation Tab. 20B: Summary of histopathological lesions in the striated muscle, kidney, heart and spleen from treeshrews

Group		**Treeshrews**		
Species		*T. gracilis*	*T. minor*	*T. tana*
no.		1	8	5
Main histo-pathological lesions	**Striated muscle**	NAD	(7/8) *Sarcocystis*	(4/5) *Sarcocystis*
	Kidney	(1/1) Mild focal lypl. interstitial nephritis	(3/8) Mild focal lypl. interstitial nephritis (1/8) Mild focal fibrosis	(2/5) Mild parasitic infection (Trichomoides-like)
	Heart	NAD	(1/8) Mild/moderate focal lypl. inflammation	(2/5) Mild focal inflammation
	Spleen	NO	NO	NO

Type of inflammation: lypl., lymphoplasmacytic; mix-neut., mixed inflammatory infiltration predominantly neutrophilic; neut., neutrophilic;
NAD: Nothing abnormal discovered
NO: no organ

Tab. 20C: Summary of histopathological lesions in the lung and liver from generalist species, urban rats and shrew

		Generalist	Urban rats		Shrew
Species		*S. muelleri*	*R. rattus compl.*	*R. norvegicus*	*S. murinus*
no.		72	105	43	58
Main histo-pathological lesions	**Lung**	(3/71) Moderate parasitic pneumonia (63/71) Mild/moderate predominant lypl. interstitial pneumonia bronchopneumonia (44/71) Mild/moderate vascular hyperplasia (24/71) Mild/severe BALT hyperplasia (20/71) Mild edema	(37/101) Mild/severe parasitic pneumonia (79/101)* Mild/moderate interstitial pneumonia/bronchopneumonia (54/101) Moderate vascular hyperplasia (9/101) Mild/moderate BALT hyperplasia	(8/41) Mild/severe parasitic pneumonia (41/41)**Mild/moderate interstitial pneumonia/bronchopneumonia (25/41) Moderate vascular hyperplasia (22/41) Mild/severe BALT hyperplasia (2/41) Mild edema	(1/44) Mild parasitic pneumonia (43/44) Mild/moderate neut. predominance interstitial pneumonia (3/44) Moderate hyperplasia of the BALT
	Liver	(12/70) Focal necrosis (parasitic migration) (3/70) Fibrosis-granuloma (parasitic migration) (5/70) Eggs-adults nematodes no inflammation (56/70) Mild to moderate lypl. periportal inflammation (36/70) Mild/moderate BDH	(10/98) Focal necrosis (parasitic migration) (12/98) Fibrosis-granuloma (parasitic migration) (7/98) Eggs-adults nematodes no inflammation (1/98) Mix-acute and chronic parasitic migration (15/98) Mild/moderate lypl. sinusoidal and periportal inflammation (2/98) Moderate bacterial hepatitis (5/98) Mild/moderate BDH	(1/42) Focal necrosis (parasitic migration) (19/42) Fibrosis-granuloma (parasitic migration) (4/42) Eggs-adults nematodes no inflammation (4/42) Mix-acute and chronic parasitic migration (12/42) Mild/moderate lypl. sinusoidal and periportal inflammation (3/42) Mild/moderate BDH	(12/58) Focal necrosis (parasitic migration) (2/58) Fibrosis-granuloma (parasitic migration) (1/58) Eggs-adults no inflammation (26/58) Mild to severe lypl. and neut. sinusoidal and periportal inflammation (3/58) Moderate bacterial hepatitis (8/58) Mild/moderate BDH

Type of inflammation: lypl., lymphoplasmacytic; mix-neut., mixed inflammatory infiltration predominantly neutrophilic; neut., neutrophilic;
Lung: BALT, bronchus associated lymphoid tissue
Liver: BDH, bile duct hyperplasia
BALT: bronchus associated lymphoid tissue
*Lymphoplasmacytic predominance (44/101) and neutrophilic predominance (35/101)
**Lymphoplasmacytic predominance (19/41) and neutrophilic predominance (22/41)

Continuation Tab. 20C: Summary of histopathological lesions in the gastrointestinal tract from generalist species, urban rats and shrew

Group		**Generalist**	**Urban rats**		**Shrew**
Species		*S. muelleri*	*R. rattus compl.*	*R. norvegicus*	*S. murinus*
no.		72	105	43	58
Main histo-pathological lesions	**Stomach**	(34/69) Moderate/severe nematodiasis (8/69) Mild bacterial accumulation (54/69) Mild/moderate neut. inflammation of the lamina propria	(15/93) Moderate/severe nematodiasis (8/93) Mild to moderate neut. inflammation of the lamina propria	(11/36) Moderate/severe nematodiasis (2/36) Mild/moderate neut. inflammation of the lamina propria	(8/56) Moderate nematodiasis (36/56) Mild/moderate neut. inflammation of the lamina propria
	Small intestine	(19/67) Mild/moderate nematodiasis (6/67) Mild/severe coccidiosis (65/67) Mild/moderate lypl. inflammation of the lamina propria	(39/100) Mild/moderate nematodiasis (1/100) Mild/moderate coccidiosis (56/100) Mild/moderate lypl. inflammation of the lamina propria	(6/36) Mild/moderate nematodiasis (10/36) Moderate coccidiosis	(28/56) Moderate/severe nematodiasis (1/56) Mild coccidiosis (48/56) Mild/moderate lypl. inflammation of the lamina propria
	Large intestine	(20/66) Moderate/severe nematodiasis (3/66) Mild/moderate coccidiosis (56/66) Mild/moderate lypl. inflammation of the lamina propria (1/66) Severe suppurative peritonitis	(5/95) Mild nematodiasis (15/95) Mild/moderate lypl. inflammation of the lamina propria	(7/38) Mild/severe lypl. inflammation of the lamina propria	(3/27) Mild nematodiasis (1/27) Moderate coccidiosis (14/27) Mild/moderate neut. inflammation of the lamina propria

Type of inflammation: lypl., lymphoplasmacytic; mix-neut., mixed inflammatory infiltration predominantly neutrophilic; neut., neutrophilic.

Continuation Tab. 20C: Summary of histopathological lesions in the striated muscle, kidney, heart and spleen from generalist species, urban rats and shrew

Group		Generalist	Urban rats		Shrew
Species		*S. muelleri*	*R. rattus compl.*	*R. norvegicus*	*S. murinus*
no.		72	105	43	58
Main histo-pathological lesions	Striated muscle	(37/70) *Sarcocystis*	(38/90) *Sarcocystis*	(10/41) *Sarcocystis*	(2/56) *Sarcocystis*
	Kidney	(1/71) Mild nematodiasis (Trichomoides-like) (35/71) Mild focal/multifocal lypl. inflammation (3/71) Mild/ moderate generalized lypl. inflammation	(1/100) Moderate coccidiosis (14/100) Mild/moderate focal/multifocal lypl. inflammation (2/100) Mild/moderate lypl. diffuse inflammation	(2/43) Mild nematodiasis (Trichomoides-like) (9/43) Moderate focal lypl. inflammation (1/43) Mild generalized lypl. inflammation	(3/49) Mild focal lypl. inflammation
	Heart	(19/71) Mild generalized lypl. myocarditis (22/71) Focal mild/moderate inflammation	(8/102) Moderate nematodiasis (14/102) Mild/moderate generalized lypl. myocarditis (15/102) Focal mild inflammation	(18/43) Mild generalized lypl. myocarditis (11/43) Focal mild lypl. inflammation	(3/56) Mild generalized lypl. myocarditis (1/56) Focal mild lypl. inflammation
	Spleen	(40/42) Mild/moderate follicular hyperplasia	(29/41) Mild/moderate follicular hyperplasia	(13/21) Mild/moderate follicular hyperplasia	(2/3) Moderate generalized neut. splenitis

Type of inflammation: lypl., lymphoplasmacytic; mix-neut., mixed inflammatory infiltration predominantly neutrophilic; neut., neutrophilic;

Histopathological findings related to gender and age

The animals of the entire study comprised 178/331 males and 149/331 females in total, for 4/331 gender was not determined. To investigate a possible effect of gender and age amongst histopathological findings (Tab. 21 and 22) the most common lesions detected in the parenchymal organs lung, liver, heart and kidney the gastrointestinal tract and spleen as well as striated muscle were analysed per number of available organs and the respective gender and age distribution.

The comparison between gender and lesions revealed that inflammatory changes in the lung, liver, small intestine and heart occurred in similar rates in both genders with a variation from 1% to 4% depending on the organ. Similarly, no association between gender and lesions was found for follicular hyperplasia of the spleen. In contrast, female individuals showed a higher inflammatory rate in stomach (47% (62/133)) and in large intestine (47% (60/128), in comparison with males' stomach (36% (59/162) and 36% (35/167) large intestine). This difference was also notable for the kidney where the occurrence of inflammatory changes was again higher in females with 37% (53/144)) than in males with only 21% (35/167).

250/331 of all animals were adult, 42/331 subadult, 23/331 juvenile and 9/331 immature, for 7/331 the age group was not available. Inflammatory processes were detected in individuals from all age groups. In general, adults and subadults showed higher occurrence of inflammatory changes in the in parenchymal organs such as liver (61% (146/240) in adults and 64% (27/42) in subadults), kidney (31% (74/236) in adult and 26% (11/42) in subadults), as well as in the gastrointestinal tract (e.g., stomach with 40% (89/221) in adults and 50% (22/40) in subadults) and striated muscle (41% (93/233), subadults 22% (9/41). Whereas juvenile and immature animals presented lower rate of inflammatory lesions in these organs (liver (48% (11/23) in juveniles and 44% (4/9), in immature), kidney (10% (2/21) juvenile and absence of inflammation in immature (9) animals), gastrointestinal (e.g., stomach, 27% (6/22) juvenile and 33% (3/9) immature animals) and striated muscle 5% (1/20)).

In contrast inflammatory changes in the lung and heart presented similar rates in adults, subadults and juveniles, showing immature animals the highest occurrence (lung: 89% (206/230) adults, 88% (37/42) subadults and 82% (19/23) juveniles, 100% (9/9) immature, heart: 36% (88/245) adults, 35% (14/40) subadults and 35% (8/23) juveniles and 44% (4/9) immature). In regard to splenic lesions, from the mature groups 74% (67/90) adults and 94% (15/16) subadults showed follicular hyperplasia. Only for two juvenile individuals spleen samples were available, showing absence of pathological changes.

Tab. 21: Main histopathological lesions of the organs classified according to the gender

Organ	**Total number**	**Total number with inflammation**	**Sex**					
			Female		Male		NA	
			Analysed	Inflammation	Analysed	Inflammation	Analysed	Inflammation
Lung	310	277	144	130 (90%)	163	144 (88%)	3	3
Liver	321	192	145	88 (61%)	172	101 (60%)	4	3
Stomach	299	124	133	62 (47%)	162	59 (36%)	4	3
Small intestine	305	213	137	98 (72%)	164	111 68%	4	4
Large intestine	274	113	128	60 (47%)	145	52 (36%)	2	1
Kidney	314	89	144	53 (37%)	167	35 (21%)	3	1
Heart	324	121	146	56 (38%)	174	64 (37%)	4	1
Organ	**Total number**	**Total number with follicular hyperplasia**	**Sex**					
			Female		Male		NA	
			Analysed	Hyperplasia	Analysed	Hyperplasia	Analysed	Hyperplasia
Spleen	111	84	57	44 (77%)	53 (74%)	39	1	1
Organ	**Total number**	**Total number with *Sarcocystis***	**Sex**					
			Female		Male		NA	
			Analysed	*Sarcocystis*	Analysed	*Sarcocystis*	Analysed	*Sarcocystis*
Striated muscle	309	108	142	51 (36%)	164	56 (34%)	4	1

Tab. 22: Main histopathological lesions of the organs classified according to the age

Organ	**Total number**	**Total number with inflammation**	**Age**									
			Immature		Juvenile		Subadult		Adult		NA	
			Analysed	Inflamm.	Analysed	Inflamm.	Analyzed	Inflamm.	Analysed	Inflamm.	Analysed	Inflamm.
Lung	310	277	9	9 (100%)	23	19 (82%)	42	37 (88%)	230	206 (89%)	6	6
Liver	321	192	9	4 (44%)	23	11 (48%)	42	27 (64%)	240	146 (61%)	7	4
Stomach	299	124	9	3 (33%)	22	6 (27%)	40	22 (50%)	221	89 (40%)	7	4
Small intestine	305	213	9	3 (33%)	23	13 (57%)	37	29 (78%)	229	161 (70%)	7	7
Large intestine	274	113	9	3 (33%)	21	5 (24%)	40	22 (55%)	200	81 (40%)	5	2
Kidney	314	89	9	/	21	2 (10%)	42	11 (26%)	236	74 (31%)	6	2
Heart	324	121	9	4 (44%)	24	10 (4%)	40	14 (35%)	251	91 (36%)	7	2
Organ	**Total number**	**Total number with follicular hyperplasia**	**Age**									
			Immature		Juvenile		Subadult		Adult		NA	
			Analysed	Hyperplasia	Analysed	Hyperplasia	Analysed	Hyperplasia	Analysed	Hyperplasia	Analysed	Hyperplasia
Spleen	111	84	/	/	2	/	16	15 (94%)	90	67 (74%)	3	2
Organ	**Total number**	**Total number with *Sarcocystis***	**Age**									
			Immature		Juvenile		Subadult		Adult		NA	
			Analysed	*Sarcocystis*	Analysed	*Sarcocystis*	Analysed	*Sarcocystis*	Analysed	*Sarcocystis*	Analysed	*Sarcocystis*
Striated muscle	309	108	9	/	20	1 (5%)	41	9 (22%)	233	96 (41%)	7	2

Inflamm.: Inflammation

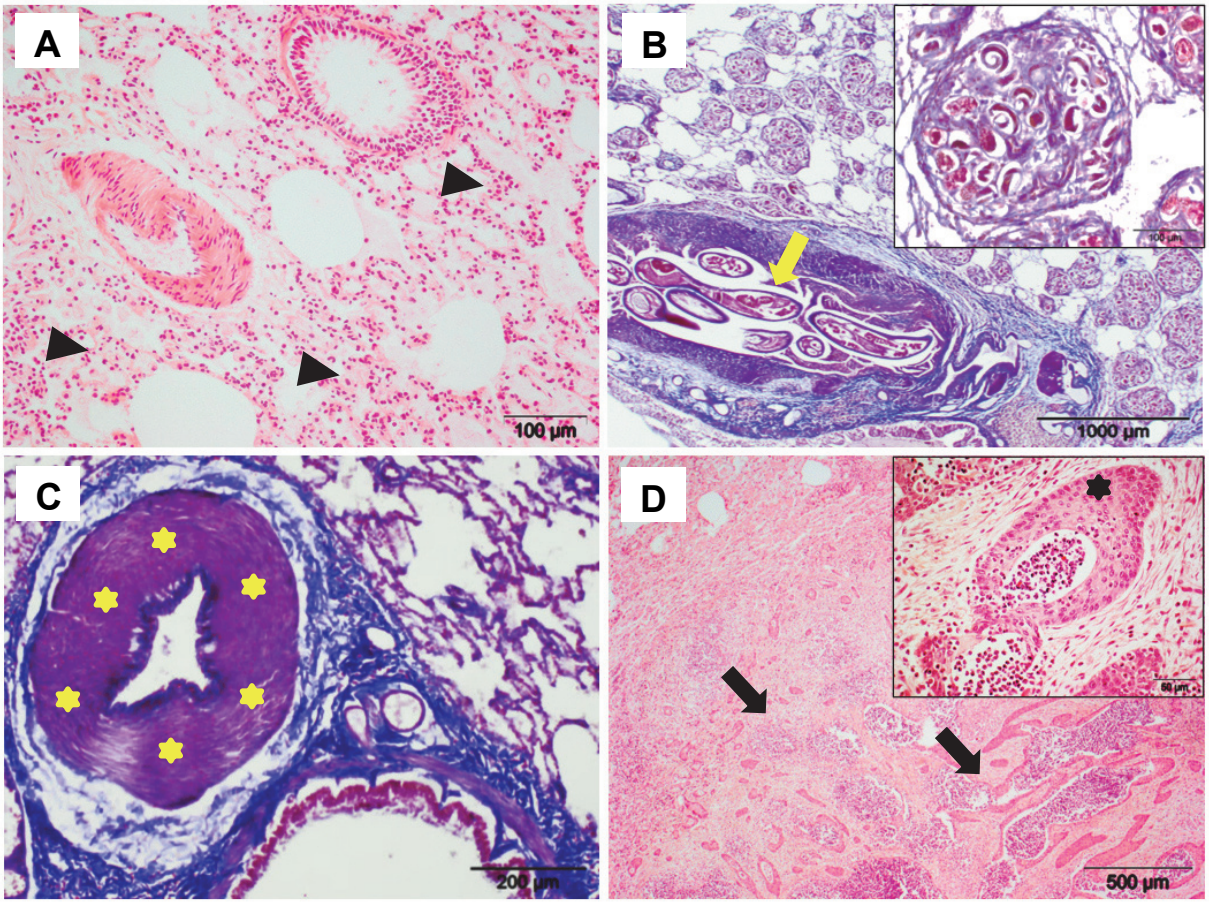

Fig. 4: Microscopic images of pulmonary lesions: A) *Suncus murinus,* lung (H&E): Moderate generalized diffuse neutrophilic pneumonia (black arrow heads). B) *Rattus* compl., lung (Azan): Severe generalized parasitic pneumonia (*Angiostrongylus* spp.) with numerous alveolar parasitic nodules containing aggregations of nematode larvae and a large artery with intravascular adult nematodes (black arrow). Inset: Magnification of a parasite nodule. C) *Rattus* spp., lung (Azan): Severe vascular hyperplasia of a pulmonary artery (yellow stars). D) *Rattus* spp., lung (H&E): Extensive area of bronchiolar metaplasia (black arrows). Inset: island of bronchiolar metaplasia (black star) with accumulation of neutrophils in the centre, surrounded by fibrous tissue.

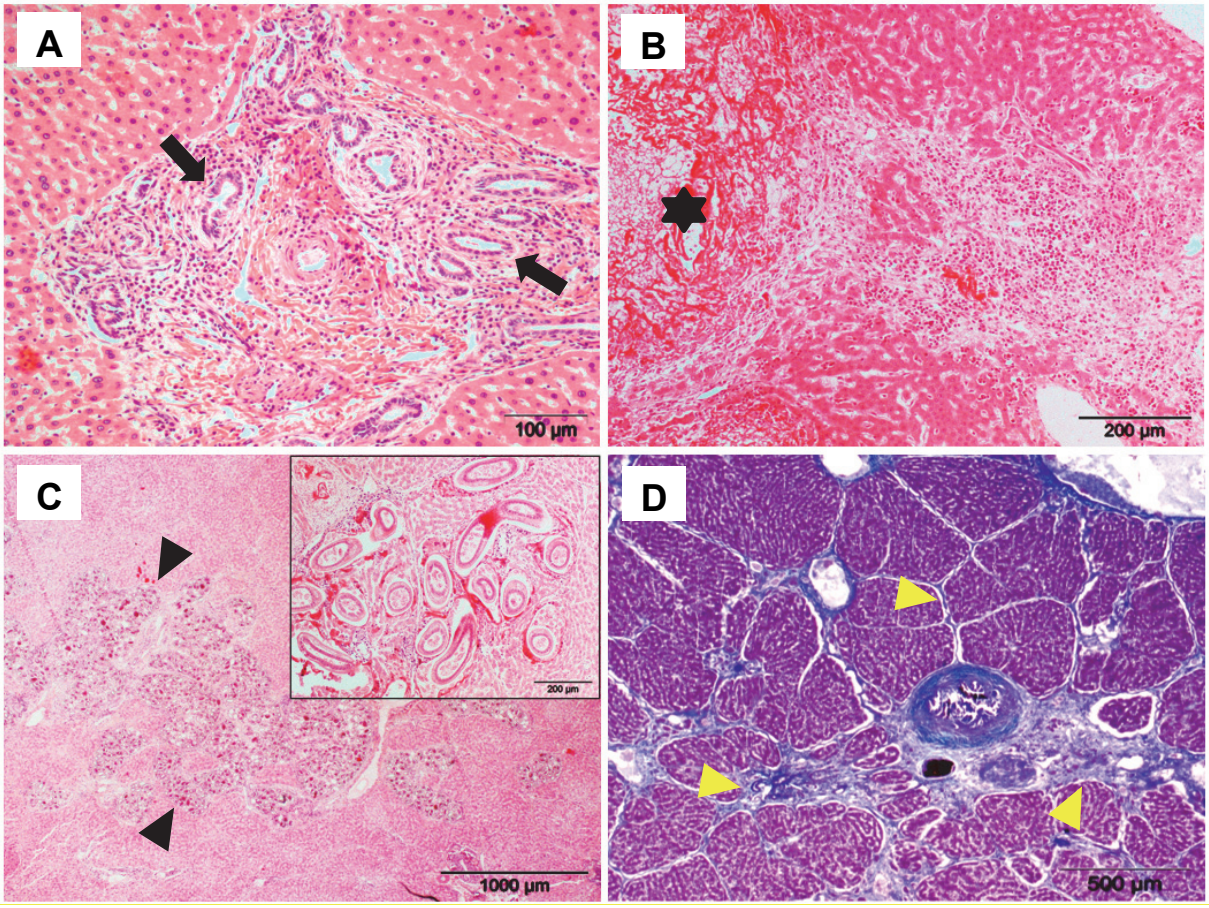

Fig. 5: Microscopic images of hepatic lesions: A) *Rattus* compl., liver (H&E): Severe bile duct hyperplasia (black arrows) with moderate lymphoplasmacytic infiltration B) *Tupaia minor*, liver (H&E): Acute coalescing necrosis and haemorrhages (black star) due to larval migration. C) *Rattus* compl., liver (H&E): Parasitic hepatitis (black arrow heads) caused by *Calodium hepaticum.* Inset: Magnification of cross sections of *Calodium hepaticum*. D) *Rattus norvegicus*, liver (Azan): Hepatic fibrosis (yellow arrows) due to chronic parasitic migration.

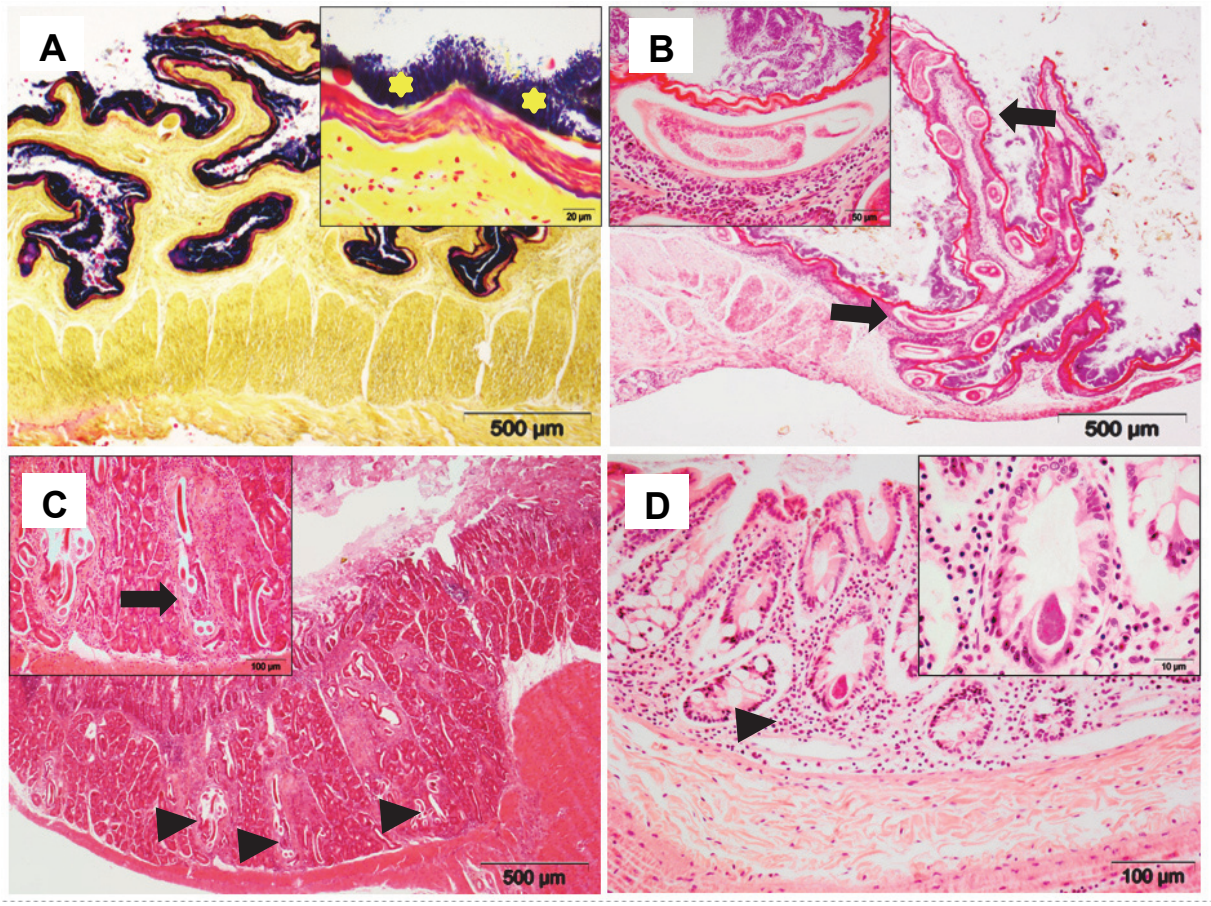

Fig 6: Microscopic images of intestinal lesions: A) *Maxomys rajah,* stomach, non-keratinized squamous epithelium (Gram): Gram positive (dark blue) aggregates of long slender bacteria perpendicular to the surface of the epithelium. Inset: Magnification of bacterial layer (yellow stars). B) *Maxomys whiteheadi*, stomach, non-keratinized squamous epithelium (H&E): Severe subepithelial nematodiasis (black arrows) and moderate bacterial aggregation on the epithelial surface (black star). The black arrows point to nematodes larva located underneath the keratinized squamous epithelium. Inset: Magnification of a subepithelial nematode and superficially located bacterial colonies (black star). C) *Suncus murinus,* duodenum (H&E): Intestinal nematodiasis. Nematode aggregation in the lumen of Brunner's glands (black arrow heads). Inset: Magnification of the area indicated by the arrows with multiple cross and longitudinal sections of nematode larvae and moderate neutrophilic infiltration. D) *Rattus* compl., large intestine (H&E): nematode larvae between enterocytes of a crypt (black star), with a moderate diffused lymphoplasmacytic infiltration of the lamina propria (black arrow head). Inset: Magnification of the nematode.

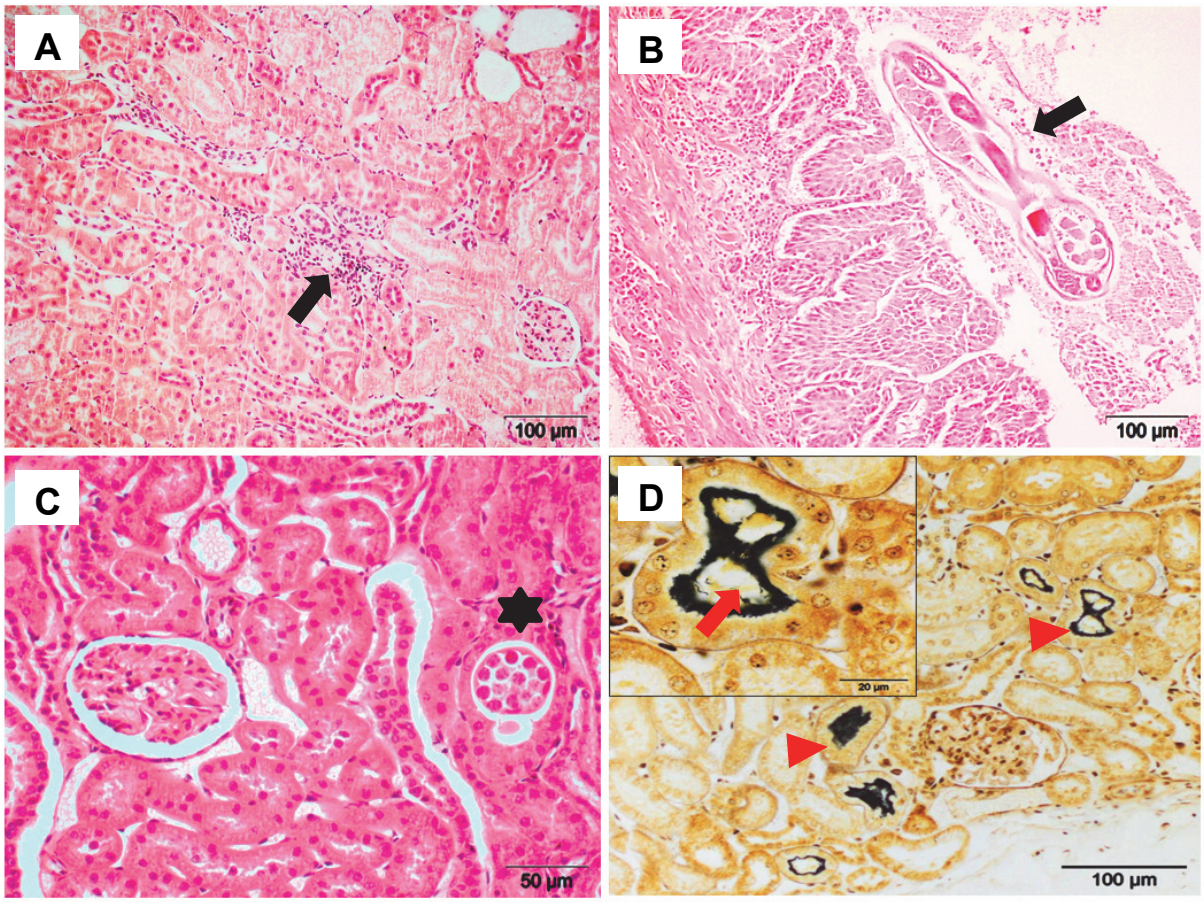

Fig. 7: Microscopic images of renal lesions: A) *Sundamys muelleri*, kidney (H&E): Proximal tubules with a focus of lymphoplasmacytic interstitial infiltration (black arrow). B) *Rattus norvegicus*, kidney (H&E): Renal pelvis, adult nematode (*Trichomoides crassicauda*) (black arrow). C) *Sundasciurus lowii*, kidney (H&E): Renal coccidiosis caused by *Klosiella*-like species (black star). D) *Rattus norvegicus*, kidney (WS): Proximal tubules with intratubular aggregations of spirochetes (red arrow heads). Inset: Magnification of spirochetes (red arrow) within the lumen of a proximal tubule.

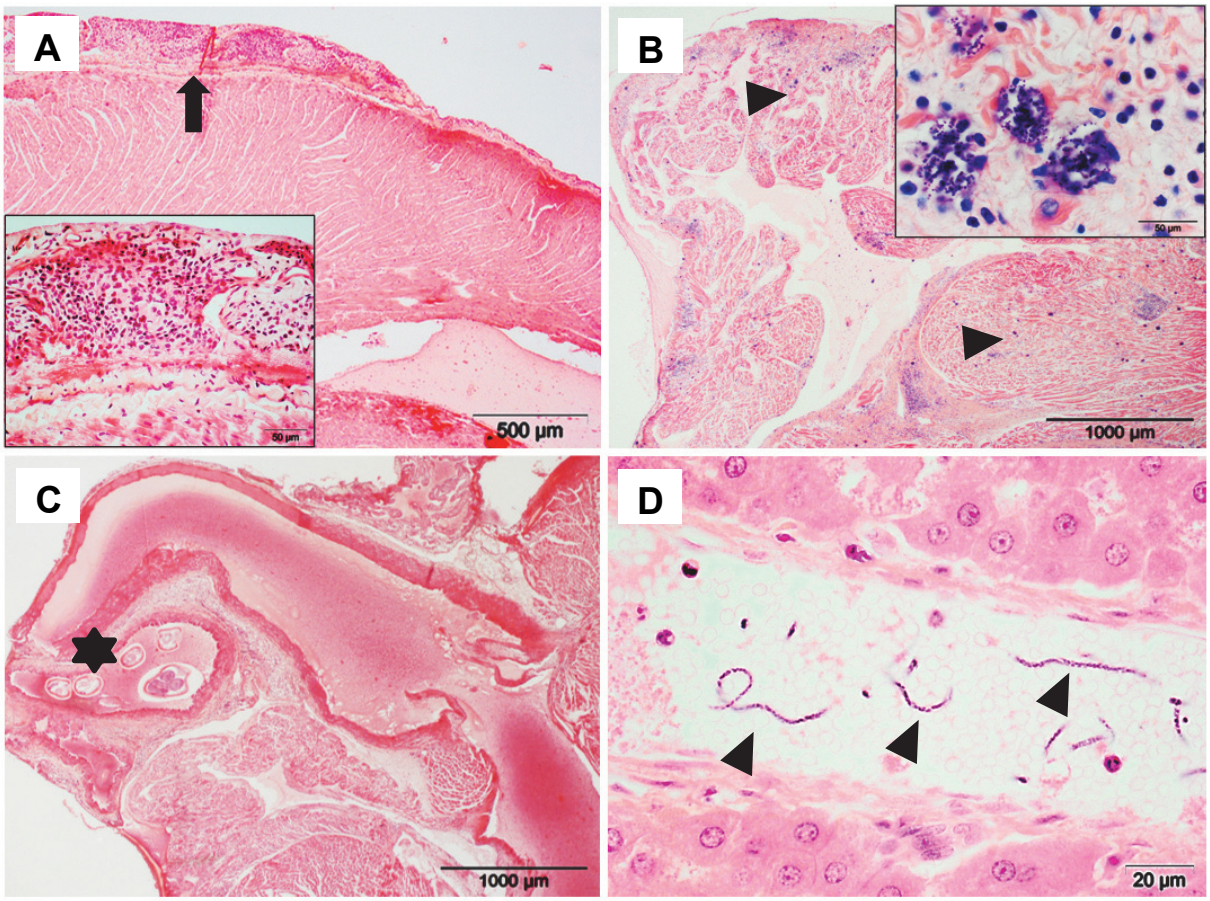

Fig. 8: Microscopic images of vascular lesions: A) *Tupaia tana*, left ventricle (H&E): Diffuse lymphoplasmacytic pericarditis (black arrow). Inset: Magnification of the pericardial inflammation. B) *Sundamys muelleri*, right atrium of the heart (Giemsa): Multifocal bacterial myocarditis (black arrow heads). Inset: Magnification of bacterial colonies. C) *Sundamys muelleri*, heart (H&E): Intravascular adult nematode (*Angiostrongylus* spp.) (black star). D) *Tupaia minor,* blood vessel (H&E): Multiple microfilaria in the lumen of a hepatic blood vessel (black arrow heads).

5.2 Health status

The histopathological evaluation of the 331 individual small mammals revealed various organ lesions of differing severity and quality. These results were employed to evaluate the clinical health of the individuals and to estimate their overall impact on the animals' health status (Fig. 9). A summary table of health status finding of the different species based on the gender and sex in included in the supplementary material Tab. 36 and Tab. 37.

Score 0 to 1: Clinically healthy

The health status of 22% (74/331) of all animals was estimated to be healthy but commonly with a mild degree of histopathological changes in varyring organs, 17% (25/149) were females, 26% (47/178) males and two animals' gender was undetermined. Regarding age groups 1/9 immatures, 26% (6/23) juveniles, 19% (8/42) subadult, 23% (57/250) adults and two unclassified belonged to this category.

Attending to the habitat, all ecologic groups included animals that were evaluated as clinically healthy: a) Forest species 25% (13/53): 15% (6/39) forest rodents (1/5 *Callosciurus notatus*, 1/2 *Callosciurus prevosti*, 2/6 *Sundasciurus lowii*, 2/7 *Maxomys suriferi*), treeshrews 50% (7/14) (6/8 *Tupaia minor,* 1/5 *Tupaia tana*), b) the generalist species *Sundamys muelleri* 10% (7/72), and c) urban species 27% (54/206): *Rattus* spp. 21% (32/148; *Rattus* compl. (28/105)*, Rattus norvegicus* (4/43) and *Suncus murinus* 38% (22/58) (Fig. 10).

The most frequent lesion detected in all groups of this category was mild lymphoplasmacytic predominance pneumonia (47/331), followed by inflammatory processes in the liver with focal mild inflammation in the parenchyma or portal spaces (26/331). To a lesser extent mild lymphoplasmacytic multifocal perivascular inflammation of the renal cortex and focal inflammation of the heart endocardium and atrioventricular valve, as well as generalized inflammation of the myocardium were found.

Score 2 to 3: Mildly clinically affected

Approximately 66% (219/331) of individuals were included in the category "mildly clinically affected" due to the presence of moderate lesions in one to two organs, with variations in the pattern of organ affection and/or the type of lesions. From these animals 72% (107/149) were females, 62% (110/178) males and two had unidentified gender. Attending to the age: eight out of nine immatures, 15/23 juveniles, 28/42 subadults, 164/250 adult unclassified.

From the forest rodent species, 17/22 rodents belonging to *Maxomys* spp. (3/3 *Maxomys rajah*, 5/7 *Maxomys suriferi*, 5/6 *Maxomys whiteheadi*, 4/6 *Maxomys surifer/rajah)* and 4/5 *Callosciurus notatus* squirrels showed a predominance of moderate suppurative pneumonia/bronchopneumonia and their hepatic lesions consisted of lymphoplasmacytic

periportal inflammation as well as mild focal or generalized inflammatory infiltration in the heart. Three out of four *Niviventer cremoriventer* and five squirrels (1/2 *Callosciurus prevosti* and 4/6 *Sundasciurus lowii*) showed hepatic lesions similar to *Maxomys spp.* as well as predominantly lymphoplasmacytic pneumonia. Two animals belonging to *Maxomys surifer/rajah* (1/6) and *Sundasciurus lowii* (1/6) had hepatic lesions associated with necrosis and haemorrhage respectively.

Half of the treeshrews 7/14 (2/8 *Tupaia minor*, 4/5 *Tupaia tana* and 1/1 *Tupaia gracilis*) (Fig. 10) had mild to moderate predominantly lymphoplasmacytic pneumonia, while lesions in their livers consisted of mild of lymphoplasmacytic periportal inflammation. Two out of seven *Tupaia* spp. showed hepatic lesions caused by parasitic migration and one presented moderate lymphoplasmacytic inflammation of the lamina propria of the gastrointestinal tract.

80% (58/72) of *Sundamys muelleri* included in this group had moderate inflammatory processes both in lung and liver alongside with focal mild inflammation of heart and kidney. In contrast to lesions of parasitic origin detected in *Rattus* spp. and *Suncus murinus,* the liver lesions observed in *Sundamys muelleri* were mostly limited to mild to moderate predominantly lymphoplasmacytic periportal inflammation and bile duct hyperplasia.

A high number of individuals in this category belonged to *Rattus* spp. (57/105 *Rattus* compl., 33/43 *Rattus norvegicus*) and *Suncus murinus* (35/58) revealing moderate pneumonia/bronchopneumonia alongside with hepatic lesions caused by parasitic migration based of necrosis, haemorrhage, granulomas or fibrosis: 20/57 *Rattus* compl., 24/33 *Rattus norvegicus* and 13/35 *Suncus murinus.* Amongst the individuals from *Suncus murinus* included in this category 19/35 also presented moderate duodenal inflammation with hyperplasia of Brunner´s gland.

Score 4 and 5: overt clinical disease likely

From the total number of individuals 12% (38/331) were estimated to have a compromised health status (Fig. 9) with both genders of similar rate (females 11% (17/149) and males 12% (21/178)). Based on the age group, 2/23 juveniles, 6/42 subadults, 29/250 adults and one age undetermined individual were classified with likely overt clinical disease.

The animals included in this group had at least three organs with moderate lesions as well as a single vital organ severely affected (e.g. extensive necrotic areas in one organ or parasitic pneumonia affecting more than 70% of the pulmonary parenchyma).

Only four forest rodents from the genus Maxomys and Niviventer were included in this category (1/6 Maxomys whiteheadi, 2/6 Maxomys surifer/rajah and 1/4 Niviventer cremoriventer). These showed numerous foci of severe acute necrosis and fibrosis in the liver

caused by parasitic migration with additional inflammatory processes in lung, kidney and gastrointestinal tract.

From the generalist species, *Sundamys muelleri*, 7/67 individuals were included in this category of likely overt clinical disease due to moderate mixed bronchopneumonia together with moderate lesions in liver, kidney and gastrointestinal tract. Furthermore, there was an individual with moderate acute suppurative peritonitis.

Most of the urban *Rattus* spp. included in this group (19/105 *Rattus* compl. and 3/43 *Rattus norvegicus)* presented severe extensive parasitic pneumonia with severe vascular hyperplasia. In addition, in six *Rattus* compl. of these affected individuals intravascular nematodes were detected in their arteries. Seven of these affected individuals also showed parasitic lesions in their livers comprising acute necroses and haemorrhage (1/105 *Rattus* compl. and 1/43 *Rattus norvegicus),* cestode cyst formation (2/105 *Rattus* compl.) or nematode egg aggregation in more than 25% of the parenchyma (3/105 *Rattus* compl.)

One individual from *Rattus* compl. showed multiple hepatic granulomas caused by parasitic migration which affected 80% of the parenchyma. Moreover, 3/43 *Rattus norvegicus* revealed different lesions in up to three vital organs consisting of moderate lymphoplasmacytic pneumonia, moderate myocarditis, parasitic hepatic lesions (one individual with acute haemorrhagic lesion and two with chronic parasitic granulomas) and a single animal with renal granulomas.

Only 1/58 shrews (*Suncus murinus*) was included in this category due to moderate to severe lesions in lung and liver. The animal had suppurative pneumonia together with parasitic granuloma formation and suppurative inflammation and necroses in the liver caused by parasitic migration.

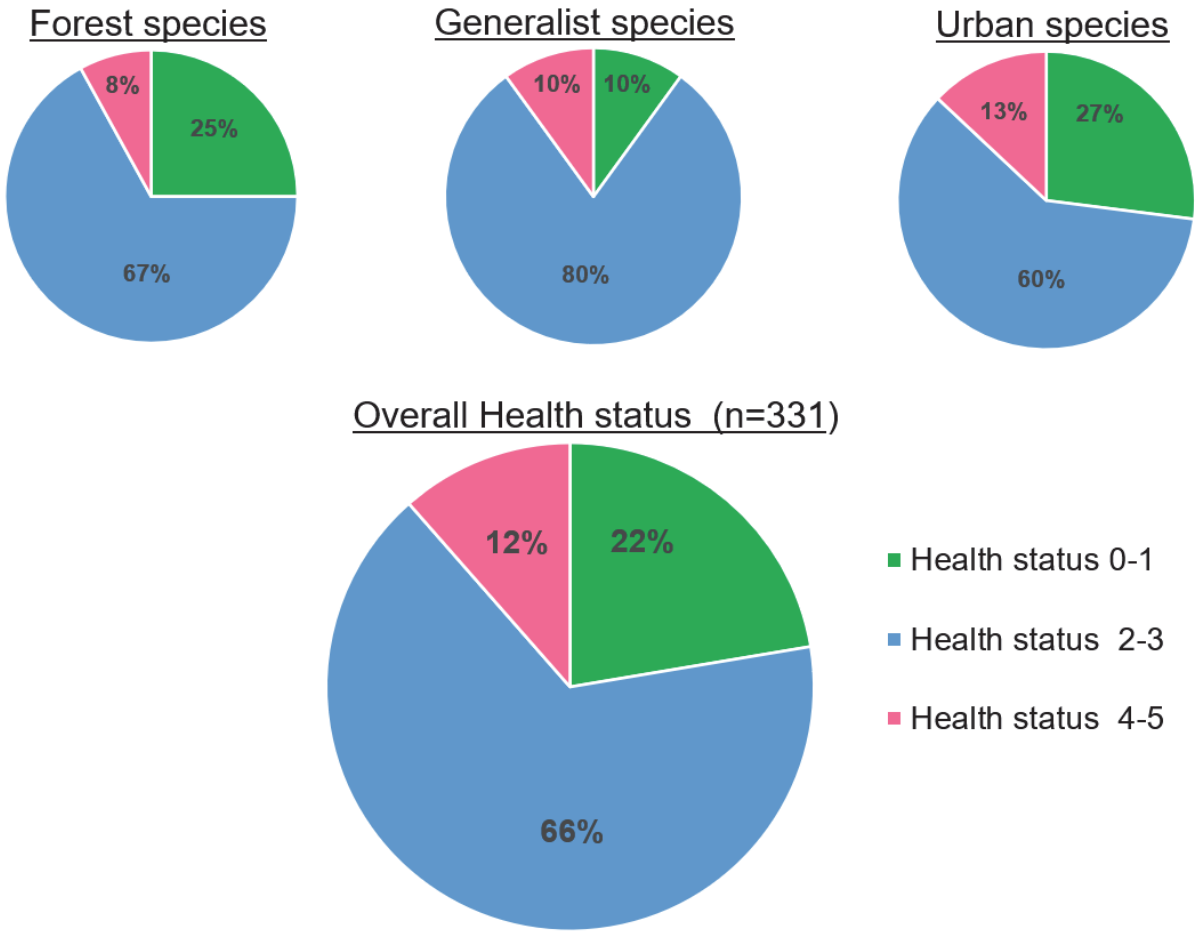

Fig. 9: Health status according to the habitat: Overall health status: Health status 0-1: 22% (74/331), Health status 2-3: 66% (219/331), Health status 4-5: 12% (38/331). Forest species: Health status 0-1: 25% (13/53), Health status 2-3: 67% (36/53), Health status 4-5: 8% (4/53). *Sundamys muelleri:* Health status 0-1: 10% (7/72), Health status 2-3: 80% (58/72), Health status 4-5: 10% (7/72). Urban species: Health status 0-1: 27% (54/206), Health status 2-3: 60% (125/206), Health status 4-5: 13% (27/206).

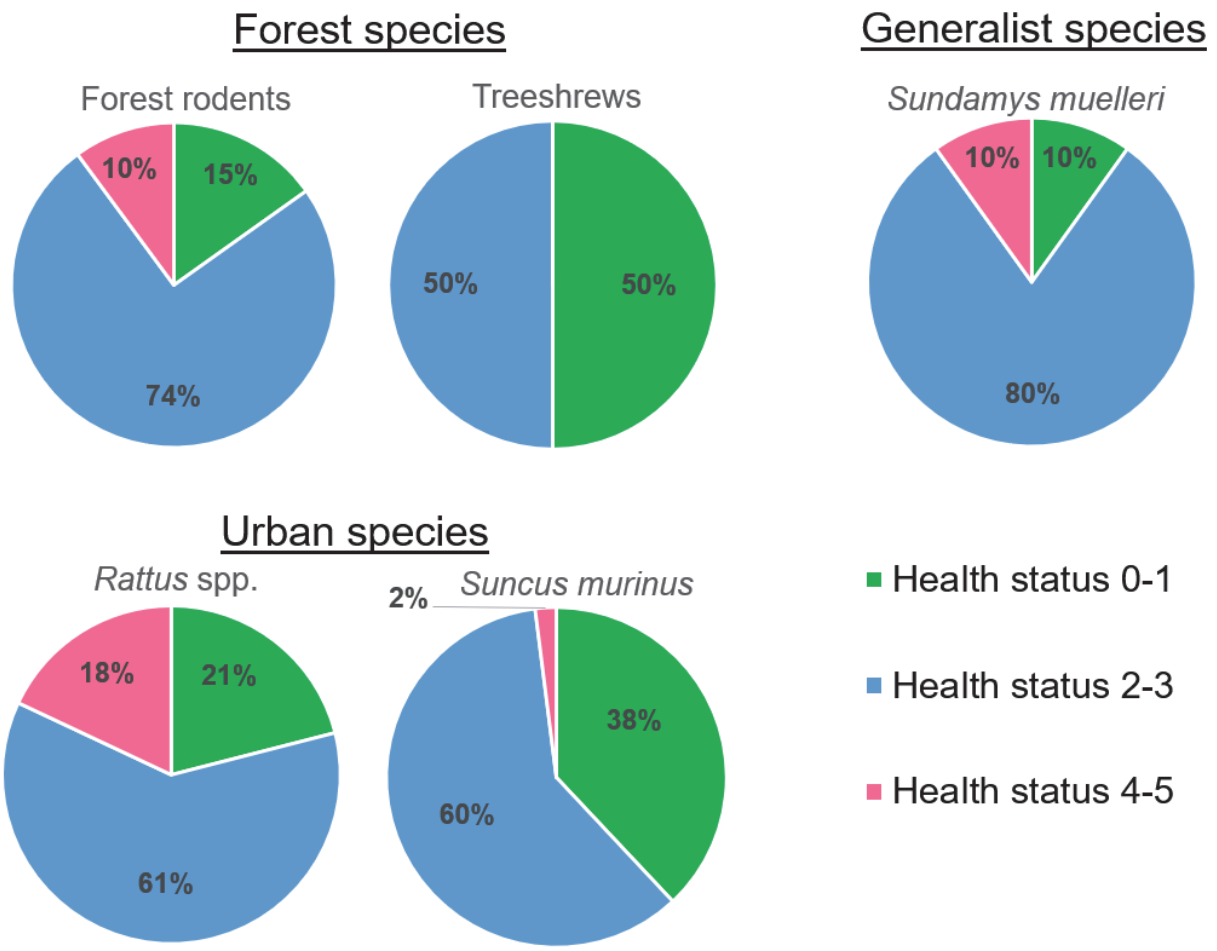

Fig. 10: Health status of the animal species groups: Forest rodents: Health status 0-1: 15% (6/39), Health status 2-3: 74% (29/39), Health status 4-5: 10% (4/39). Treeshrews: Health status 0-1: 50% (7/14), Health status 2-3: 50% (7/14). *Sundamys muelleri:* Health status 0-1: 10% (7/72), Health status 2-3: 80% (58/72), Health status 4-5: 10% (7/72). *Rattus* spp.: Health status 0-1: 21% (32/148), Health status 2-3: 61% (90/148), Health status 4-5: 18% (26/148). *Suncus murinus*: Health status 0-1: 38% (22/58), Health status 2-3: 60% (35/58), Health status 4-5: 2% (1/58).

5.3 Pathogen detection

5.3.1 Molecular investigation of ethanol-fixed samples

Evaluation of the DNA content extracted from ethanol-fixed kidney (n=119) and liver (n=120) tissue using the NanoDrop spectrophotometer, showed poor quality and quantity of the DNA. The concentration of the DNA extracted was lower than 50 ng/µl in 75% of the kidney tissues (67/119) and 75% (90/120) of liver tissues, with significant variations from no detection to a DNA maximum of 539.65 ng/µl (i.e. sample E27/14 liver). The DNA purity was based on the absorbance parameter (260/280) and did not reach a ratio above 1.8 in 79/119 kidneys and 93/120 livers (Tab. 23).

After several modifications to the standard protocol there was a significant improvement in the quality and quantity of the DNA extracted in the majority of the samples. Only one kidney samples (E374/13) had no increase of DNA concentration after pre-treatment, but the quality increased from 1.39 to 1.54 (Tab. 24).

Tab. 23: Results of DNA extracted from problematic ethanol-fixed samples after modifying the digestion conditions

Sample ID and organ	Species	DNA concentration ng/µl	Absorbance ratio (260/280)	Time of digestion	Proteinase K	Complete digestion
E352/13 liver	*T. minor*	0.41	-0.96	2 days	2 times	YES
E352/13 liver	*T. minor*	35.71	1.73	2 days	4times	NO
E357/13 liver	*T. minor*	0.51	0.33	2 days	2 times	NO
E357/13 liver	*T. minor*	0.92	-0.74	2 days	2 times	NO
E364/13 liver	*T. minor*	29.79	1.94	2 days	2 times	YES
E364/13 liver	*T. minor*	57.03	1.73	overnight + 4 h	2 times	YES
E370/13 liver	*T. minor*	13.27	1.95	overnight + 3 h	2 times	YES
E371/13 liver	*T. minor*	7.15	2.05	overnight + 3 h	2 times	NO
E371/13 liver	*T. minor*	35.42	1.79	2 days	4times	NO
E392/13 liver	*T. minor*	1.67	1.8	2 days	2 times	YES
E392/13 liver	*T. minor*	53.22	1.84	2 days	4 times	NO
E352/13 kidney	*T. minor*	0.58	1.32	overnight + 3 h	2 times	YES
E357/13 kidney	*T. minor*	4.08	1.4	2 days	4 times	NO
E357/13 Kidney	*T. minor*	10.13	1.4	2 overnight	2 times	YES
E364/13 kidney	*T. minor*	10.81	1.82	overnight + 4 h	2 times	YES
E370/13 kidney	*T. minor*	2.28	-4.7	overnight + 3 h	2 times	YES
E365/13 kidney	*T. minor*	7.56	1.84	overnight	2 times	YES
E371/13 kidney	*T. minor*	32.16	1.68	2 days	4 times	NO
E392/13 kidney	*T. minor*	25.43	1.46	2 days	4 times	NO

Tab. 24: Results from DNA extraction of tissue without treatment and re-hydrated tissue before Proteinase K digestion

Sample ID/ organ	Species	DNA concentration		Quality	
		Standard extraction ng/µl	Re-hydrated tissue ng/µl	Standard extraction 260/280	Re-hydrated tissue 260/280
E334/13 liver	*Sundamys muelleri*	/	21.06	/	1.36
E334/13 kidney	*Sundamys muelleri*	0.64	47.73	- 4.42	1.44
E374/13 liver	*Sundamys muelleri*	3.15	3.16	1.26	1.5
E374/13 kidney	*Sundamys muelleri*	16.33	4.13	1.39	1.54
E330/13 liver	*Sundamys muelleri*	0.52	0.93	0.43	-7.55
E330/13 kidney	*Sundamys muelleri*	1.2	2.95	1.31	1.27
E333/13 liver	*Sundamys muelleri*	2.84	7.37	1.65	1.46
E333/13 kidney	*Sundamys muelleri*	0.95	71.84	1.14	1.42
E373/13 liver	*Sundamys muelleri*	1.6	2.37	1.09	1.77
E373/13 kidney	*Sundamys muelleri*	1.6	29.2	1.09	1.47
E328/13 liver	*Sundamys muelleri*	0.83	42.23	1.69	1.42
E328/13 kidney	*Sundamys muelleri*	0.83	20.19	2.36	1.43
E372/13 liver	*Sundamys muelleri*	4.48	59.41	2.56	0.74
E372/13 kidney	*Sundamys muelleri*	2.64	10.06	1.69	1.44

In a subset of extracted DNA (44 kidneys, 61 livers) from ethanol-fixed tissues, cytochrome B gene was used as an internal control to confirm sufficient host DNA contents. The PCR results showed a positive band for cytochrome b in 33/44 kidneys and 51/61 livers without initial treatment. For eight problematic samples, however, the cytochrome b detection failed from DNA extractions of rehydrated kidney samples and seven liver samples detection failed due to insufficient sample preservation (Fig. 11).

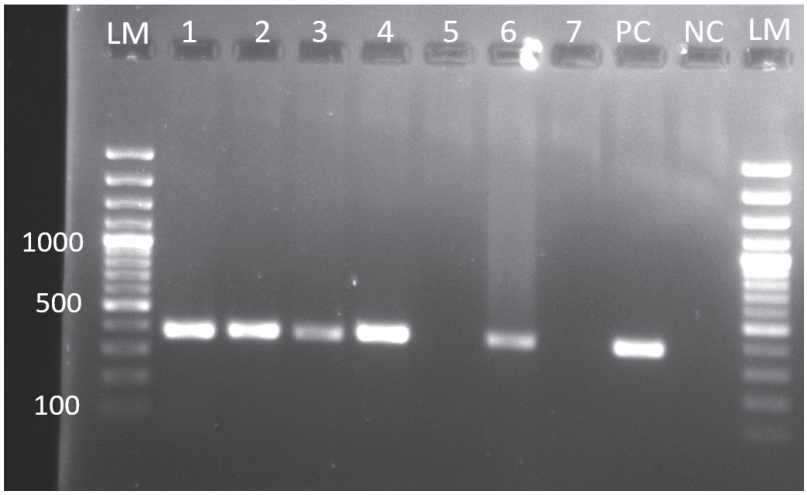

Fig. 11: PCR targeting cytochrome B gene with primers Kocher1/ Kocher2. Agarose gel 2% electrophoresis: Line 1 (re-hydrated liver from E27/14 *Niviventer cremoriventer*), line 2 (E72/14 re-hydrated liver from *Sundasciurus lowii*), line 3 (E390/13 re-hydrated liver from *Maxomys whiteheadi*), line 4 (E355/13 re-hydrated liver from *Maxomys rajah*), line 6 (E119/13 re-hydrated kidney from *Tupaia tana*) show bands of the same product (307 bp) as the positive control (PC). Line 5 (E334/13 re-hydrated liver from *Sundamys muelleri*) and line 7 (E365/13 re-hydrated kidney from *Tupaia minor* lack cytochrome b*,* similar to the negative control (NC). LM DNA-ladder marker (DNA-ladder 100 bp, Thermo Fisher Scientific).

Despite the efforts, due to the uncertain or poor quality of the DNA obtained from ethanol-fixed samples, all further investigations on pathogen detection were conducted on homogenized kidney and liver tissue stored in RNAlater®, received from collaboration partners (Heiko Pietsch, Dr. Andreas Kurth, Robert Koch Institute, Berlin, Germany). However, as only ethanol-fixed straited muscle tissue was available, molecular investigations on *Sarcocystis* spp. had to be performed with this tissue.

5.3.2 *Leptospira* results

5.3.2.1 Histopathology, Warthin-Starry staining (WS)

Silver staining after WS revealed the presence of abundant filiform, slender spiral-shaped bacteria, aggregated in the lumen and at the apical epithelial surface of multiple proximal tubules in 20% (64/314) of the histological kidney sections (Tab. 35). Occasionally these bacteria were seen intracellular inside the tubules' epithelial cells. The distribution pattern of the spirochetes varied from slightly affected single tubules to dense spirochete colonization along the inner surface of multiple cortical tubules (Fig. 7D, Fig. 12A and Fig. 12B). Comparing the gender of the positive animals, there was a similar proportion of approximately 20% positive females (30/144) and males (34/167), in three individuals the identification of the sex was not possible. Although the number of analysed immature (n=9) and juvenile (n=21)

individuals were much lower than subadult (n=100) and adults (n=178), in one immature and three juvenile animals spirochetes were detected. Moreove from the animal where the age was not determined (n=6), in a single individual was observed the presence of spirochetes. Positive WS staining was found in 2/100 subadults and 58/178 adult individuals (supplementary material Tab. 38). In addition, 3/4 animals without age information were also positive. Amongst the different species, the highest occurrence of WS positive staining was found in *Rattus norvegicus* 30% (13/43), *Rattus* compl. 27% (27/100) and *Sundamys muelleri* 21% (15/71) and to a lesser degree in forest rodents (19%, 7/37). Only a single positive individual was found among the urban *Suncus murinus* (1/49) and the forest treeshrews (1/14). Positive WS samples were compared with the corresponding H&E sections for possible inflammatory reactions associated with the spirochete aggregation. But no evidence of histopathological changes related to spirochete infection was found.

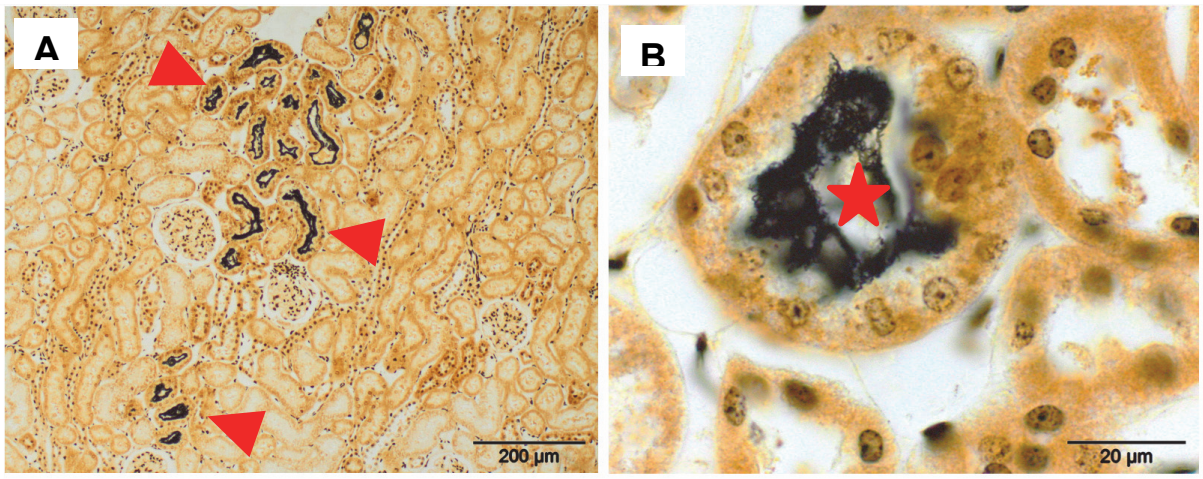

Fig. 12: Microscopic images of a kidney from *Rattus norvegicus*, kidney (WS): A) Proximal tubules with multiple foci of intratubular aggregations of spirochetes (red arrow heads). B) Cross section of a proximal tubule with spirochetes bacterial aggregation in the lumen (red star).

5.3.2.2 PCR

Only 106 of 314 kidney samples were available for DNA extraction and molecular analyses (give numbers of analised animals per species groups (forest rodents (n=15), treeshrews (n= 8), generalist (n=31), urban rats (n=38) and urban *Suncus murinus* (n=14)). The LipL32 gene-based qPCR analysis revealed pathogenic *Leptospira*-DNA in 25/106 samples (supplementary material Tab. 40). Subsequent cPCR successfully amplified *Leptospira*-DNA with LipL21 primers in 19 samples (Fig. 13), with G1/G2 primers in 29 samples (Fig. 14) and one further sample with LipL32 primers only in a single sample, respectively. Sequence analyses of these results revealed the presence of pathogenic *Leptospira* spp. in 26% (28/106) of investigated samples. From these positive samples, males showed a slightly

higher occurrence with 29% (16/56) in comparison to females with 24% (12/49) infected (supplementary material Tab. 39). Regarding the age groups, only two juvenile animals were available for analysis with negative results, but 2/9 subadults and 26/90 adults were qPCR positive for *Letospira*-DNA (supplementary material Tab. 39).

Pathogenic *Leptospira* spp. were identified in all habitats, but with distinct differences in their occurrence among the species. Only *Maxomys whiteheadi* (2/3) and *Sundasciurus lowii* (1/3) were *Leptospira*-positive amongst all tested forest individuals (13%, 3/23). While one third of the generalist species *Sundamys muelleri* (32%, 10/31) and 40% (15/38) of the urban *Rattus* species (*Rattus* compl. (12/26) and *Rattus norvegicus (*3/12)) were positive for *Leptospira*-DNA.

Leptospira interrogans was the most commonly identified bacterial species (23/28). *Leptospira borgpetersenii* was found in two *Sundamys muelleri*, one *Rattus* compl. and one *Rattus novegicus.* Further, a single *Sundasciurus lowii* carried a *Leptospira* sp. that could not be classified based on existing GenBank entries (supplementary material Tab. 40).

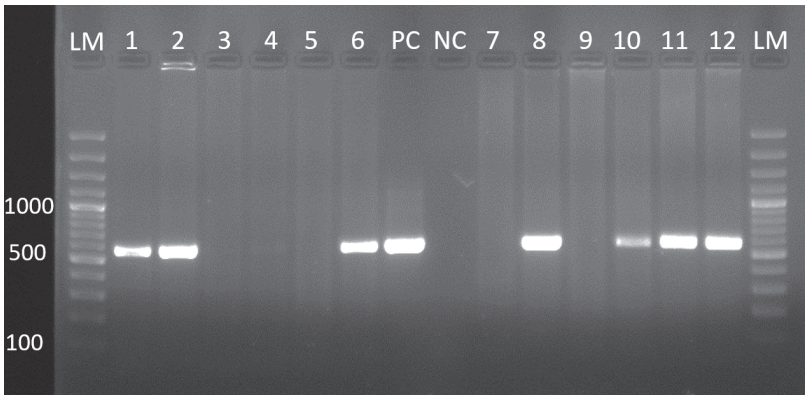

Fig. 13: PCR targeting LipL32gene from *Leptospira* with primers LipL21. Agarose gel 2% electrophoresis: Line 1 (E139/13 *Rattus* compl.), line 2 (E272/13 *Rattus* compl.), line 6 (E283/13 *Rattus norvegicus*), line 8 (E289/13 *Rattus* compl.), line 10 (E291/13 *Rattus* compl.), line 11 (E295/13 *Rattus* compl.), line 12 (E317/13 *Rattus* compl.) show bands of the same product (560 bp) like the positive control (PC). Line 3 (E8/14 *Rattus* compl.), line 4 (E9/14 *Rattus* compl.), line 5 (E281/13 *Rattus norvegicus*), line 7 (E285/13 *Suncus murinus*), line 9 (E290/13 *Rattus* compl.) are lacking *Leptospira*-DNA similar to the negative control (NC). LM DNA-ladder marker (DNA-ladder 100 bp, Thermo Fisher Scientific).

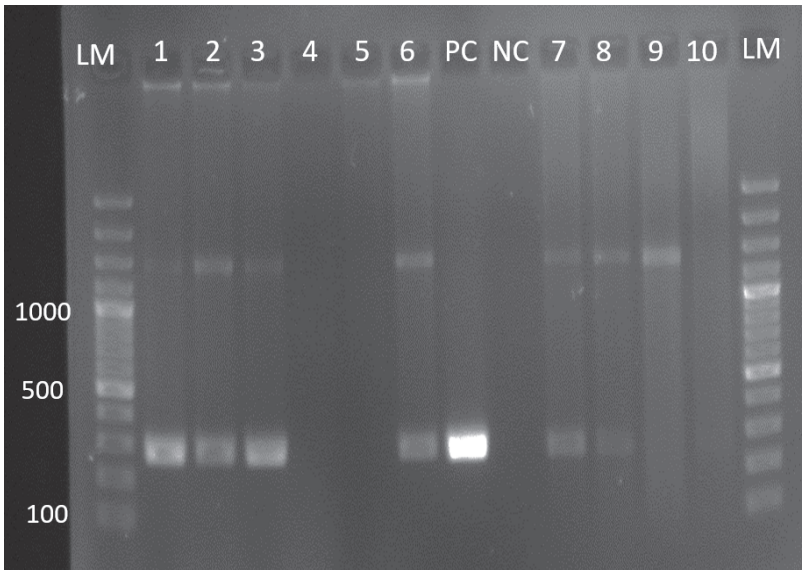

Fig. 14: PCR targeting pLIPs60 recombinant plasmid from *Leptospira* with primers G1/G2. Agarose gel 2% electrophoresis: Line 1 (E275/13 *Sundamys muelleri*), line 2 (E298/13 *Sundamys muelleri*), line 3 (E334/13 *Sundamys muelleri*), line 6 (E393/13 *Sundamys muelleri*), line 7 (E8/14 *Rattus* compl.), line 8 (E9/14 *Rattus* compl.) show bands of the same product (266-285 bp) like the positive control (PC). Line 4 (E346/13 *Sundasciurus lowii*), line 5 (E356/13 *Maxomys whiteheadi*), line 9 (E288/13 *Suncus murinus*), line 10 (E285/13 *Suncus murinus*) are lacking *Leptospira*-DNA similar to the negative control (NC). LM DNA-ladder marker (DNA-ladder 100 bp, Thermo Fisher Scientific).

5.3.2.3 WS and PCR comparison

A comparison between WS stained histology sections and PCR results showed that out of 28 PCR-positive kidney samples 17 were also positive by WS (supplementary material Tab. 40). From the remaining 208 kidney samples that could only be investigated by WS, but due to lack of suitable material not by PCR, spirochetes were detected in another 39 individuals (*Sundsciurus lowii* (1/5), *Tupaia tana* (1/5), *Sundamys muelleri* (6/71), *Rattus* compl. (18/100) and *Rattus norvegicus* (13/43).

5.3.3 *Trypanosoma* results

The nested 18S rRNA PCR analyses detected *Trypanosoma*-DNA in 24/277 liver samples (supplementary material Tab. 42) with a higher occurrence in female individuals (10% (13/126)) than in males (7% (11/149)). Comparing the age groups, subadults and adults had a higher rate of *Trypanosoma* infection with 21% (8/39) and 8% (15/192) respectively (supplementary material Tab. 41) in contrast to only 2/16 juvenile and 2/9 immature positive animals. From the forest individuals two murids (1/6 *Maxomys whiteheadi* and 1/4 *Maxomys surifer*), two squirrels (2/6 *Sundasciurus lowii*) and five treeshrews *(*1/8 *Tupaia minor*, 3/4 *Tupaia tana*, 1/1 *Tupaia gracillis)* were infected by *Trypanosoma* spp. (in total 18% (9/49)

forest rodents and treeshrews). *Trypanosoma* spp. were also detected in 11% (14/117) of the *Rattus* spp. (9/76 *Rattus compl.,* 5/41*Rattus norvegicus*) and one generalist *Sundamys muelleri* (1/66).

The 24 positive PCR products were sequenced in both directions and clean fragments of up to 560 bp length (Fig. 15), were used for sequence analyses. In two samples (E64/14 *Sundasciurus lowii* and E66/14 *Tupaia tana*), the sequences showed a partial overlap with differing nucleotides in their chromatogram files sequences showed a partial overlap of differing nucleotides in their chromatogram files, which might indicate co-infection of two *Trypanosoma* spp.

Thirteen sequences of 540 bp length retrieved from individuals of the urban *Rattus* spp. (8/76 *Rattus* compl, 5/41 *Rattus norvegicus*) and from *Sundamys muelleri* (1/66) were either 100% homologue to or differed by one nucleotide compared to the 18S rRNA gene sequence from the reference strain *Trypanosoma lewisi*. The remaining ten sequences (529 to 560 bp lengths) from forest rodents, treeshrew species and from one individual of *Rattus* compl. clearly differed from the *Trypanosoma lewisi* sequences. Their sequences could not be classified based on the existing GenBank entries (supplementary material Tab. 42).

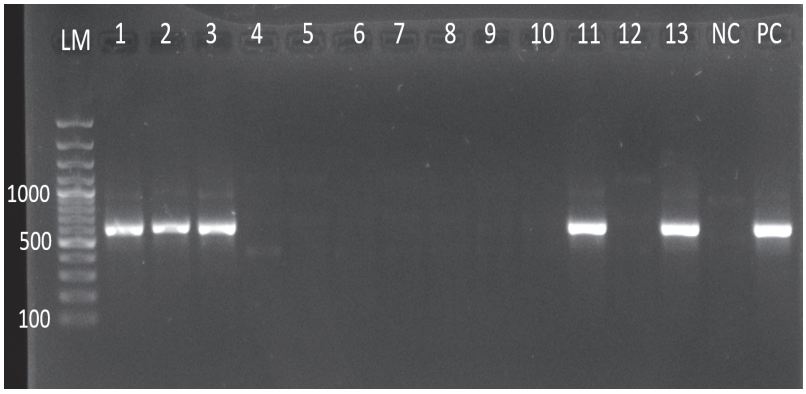

Fig. 15: PCR targeting 18SrRNA gene form *Trypanoma* with primers SSU561F/ SSU561R. Agarose gel 2% electrophoresis; Line 1 (E66/14 *Tupaia tana*), line 2 (E67/14 *Tupaia tana*), line 3 (E70/14 *Tupaia tana*), line 11 (E371/13 *Tupaia minor*), line 13 (E349/13 *Tupaia gracilis*) show bands of the same product (561 bp) like the positive control (PC). Line 4 (E88/14 *Tupaia tana*), line 5 (E351/13 *Tupaia minor*), line 6 (E352/13 *Tupaia minor*), line 7 (E357/13 *Tupaia minor*), line 8 (E364/13 *Tupaia minor*), line 9 (E365/13 *Tupaia minor*), line 10 (E370/13 *Tupaia minor*), line 12 (E392/13 *Tupaia minor*) are lacking *Trypanosoma*-DNA similar to the negative control (NC). LM DNA-ladder marker (DNA-ladder 100 bp, Thermo Fisher Scientific).

5.3.4 *Sarcocystis* spp. results

The histopathological investigations revealed that approximately 30% (108/309) of the individuals from this study were positive for intramuscular sarcosporidiasis. The *Sarcocystis* results related to gender and age are included in page 70, Tab. 21 and 22 as well as in supplementary material Tab. 43. All ecological groups were infected by these parasites; however, the number of infections differed among the groups. Treeshrews showed the highest rate of infection (11/14) (Tab. 29), followed by the generalist *Sundamys muelleri* (37/70) (Tab. 31), the urban rats (48/131), forest rodents (10/38) (Tab. 25) and finally the urban shrew, *Suncus murinus*, with only two individuals infected (2/56). As described above, all cysts were located inside skeletal myofibers (Fig. 16) without inducing any inflammatory response.

The range of cyst numbers detected per tissue slide varied from a single cyst to up to 50 cysts in one individual from *Sundamys muelleri.* Moreover, in forest species (forest rodents and treeshrews) and the generalist *Sundamys muelleri* the cysts observed by light microscopy revealed high morphological variability. Considering that each *Sarcocystis* species has a unique morphology, this variability is indicative for multiple *Sarcocystis* species amongst the different species as well as within individuals.

For *Sarcocystis* species identification, the detected muscular cysts were morphologically and genetically evaluated. The morphological identification was based on an initial analysis by light microscopy of the cysts' structures followed by a more detailed examination by transmission electron microscopy with special consideration of the wall characteristics. For the molecular investigations, sequences of the nuclear 18S rRNA and mitochondrial COI were analysed for a phylogenetic comparison.

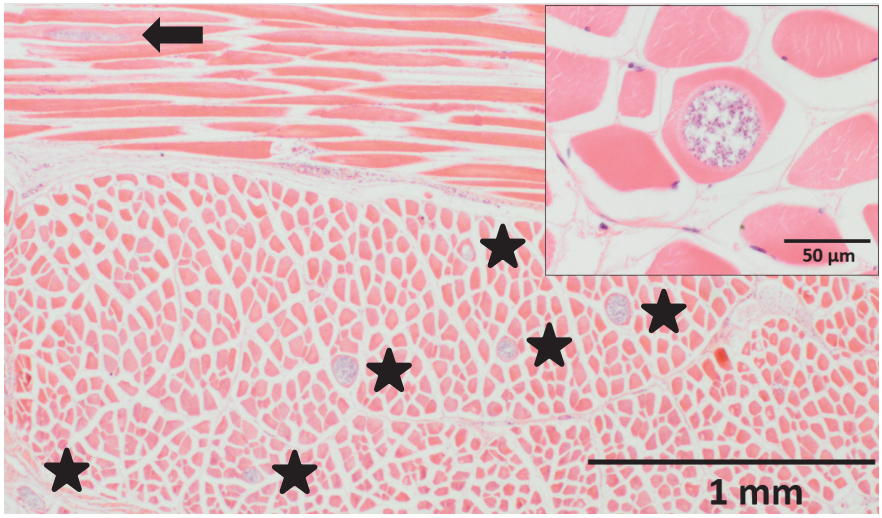

Fig. 16: Microscopic image of the diaphragm from *Sundamys muelleri* (H&E) infected with six *Sarcocystis* spp. cysts in cross sections (black stars) and one in longitudinal section (black arrow). Inset: Magnification of cross section of *Sarcocystis* spp. inside a myocyte with absence of inflammatory response.

5.3.4.1 Morphological evaluation

Based on the pattern of their wall structure the cysts detected by light microscopy in forest species and *Sundamys muelleri* were initially separated into five different morphology categories.

Forest species

Forest rodents

Tab. 25: Forest rodents infected with sarcosporidia

ID	Animal species	Sex	Age	(Number of cysts per tissue section) Origin of the striated muscle
E360/13	*Callosciurus notatus*	Female	Adult	(3) Diaphragm
E346/13	*Sundasciurus lowii*	Female	Adult	(9) Diaphragm
E367/13	*Sundasciurus lowii*	Female	Adult	(4) Thigh
E64/14	*Sundasciurus lowii*	Female	Adult	(2) Diaphragm
E71/14	*Sundasciurus lowii*	Female	Juvenile	(45) Diaphragm (3) Diaphragm
E369/13	*Maxomys whiteheadi*	Female	Adult	(4) Diaphragm
E389/13	*Maxomys whiteheadi*	Male	Adult	(13) Thigh
E27/14	*Niviventer cremoriventer*	Female	Adult	(3) Diaphragm (1) Thigh
E388/13	*Niviventer cremoriventer*	Male	Adult	(1) Diaphragm (3) Diaphragm
E102/14	*Niviventer cremoriventer*	Male	Adult	(2) Diaphragm

Category 1:

- Host species: *Maxomys whiteheadi.*
- Morphology of the cyst by light microscopy (Fig. 17A): Cross section plane revealed a round cyst with a thin wall with thick protrusions. The protrusions were widely separated from each other, characterized by a finger-like shape and bended in a 60º angle from the cyst wall surface. The ground substance was easily identifiable with a thin laminar shape. The interior of the cyst contained densely packed cystozoites characterized by a lanceolate shape.
- Morphology of the wall by transmission electron microscopy (Fig. 17B): The wall was composed of bended finger-like long protrusions with an electron-dense external membrane (parasitophorous vacuole membrane). Close to the cyst wall the protrusions had ramifications. In the middle and distal part of the protrusions the parasitophorous vacuole membrane showed multiple undulations giving an irregular appearance of the surface. The protrusions were characterized by a homogeneous content without microtubules or other structures. The cyst wall was clearly separated from the interior of the cyst by a very thin electron-dense linear ground substance.
- Measurements by light microscopy and transmission electron microscopy in Tab. 26.

Tab. 26: Measurements by light and electron microscopy of sarcosporidia cysts category 1

Light microscopy							
ID number_host species	**Number of cysts measured**	**Cyst orientation**	**Cyst (µm)**			**Cystozoites (µm) (n=6)**	
			Length	**Width**	**Wall**	**Length**	**Width**
E389/13_*M. whiteheadi*	1	cross section	88.32	70.19	1.95	4.72 to 5.84	1.3 to 2
Transmission electron microscopy							
ID number_host species	**Number of cysts measured**	**Protrusions (µm) (n=3)**			**Cystozoites (µm) (n=5)**		
		Length	**Width**	**Ground substance**	**Length**	**Width**	
E389/13_*M. whiteheadi*	1	6.94	1.24	0.04	4.2 to 5.3	1 to 1.7	

- Wall type classification according to Dubey (Dubey et al., 2016): **Type 17**
- Species classification: **Category 1 is comparable with *Sarcocystis zuoi.***

Category 2

- Host species: *Sundasciurus lowii.*
- Morphology of the cyst by light microscopy (as in Fig. 17C): The cross-section plane revealed a round big cyst with a compressed wall. Where the protrusions extended from the cyst wall, they appeared straight with a finger-like morphology and a well-defined, thin linear ground substance. The interior of the cysts presented loosely packed cystozoites with a lanceolate shape.
- Morphology of the wall by transmission electron microscopy (Fig.17D): The protrusions showed a finger-like shape with a narrowness at the junction with the ground substance. The parasitophorous vacuole membrane was thin along the contour of the protrusion and at the narrow area presented multiple small round electron-dense protuberances. The ground substance was linear, surrounding the interior of the wall without penetrating in inner cyst. The cystozoites presented a lanceolate shape and were loosely packed in the cyst matrix.
- Measurements by light microscopy and transmission electron microscopy in Tab. 27.

Tab. 27: Measurements by light and electron microscopy of cysts category 2

Light microscopy							
ID number_host species	**Number of cysts measured**	**Cyst orientation**	**Cyst (µm)**			**Cystozoites (µm) (n=3)**	
			Length	**Width**	**Wall**	**Length**	**Width**
E346/13_*S. lowii*	1	Cross	131.73	114.42	1.03	4.4 to 5.1	1.1 to 1.3
Transmission electron microscopy							
ID number_host species	**Number of cysts measured**	**Protrusions (µm)**			**Cystozoites (µm) (n=15)**		
		Length	**Width**	**Ground substance**	**Length**	**Width**	
E346/13_*S. lowii*	2	4.4 to 4.5	0.3 to 0.4	0.2 to 0.2	3.5 ± 0.6	1.1 ± 0.2	
E71/14_*S. lowii*							

- Wall type classification according to Dubey (Dubey et al., 2016): **Type 19.**
- Species classification: **Category 2 is comparable with *Sarcocystis singaporensis.***

Category 3

- Host species: *Callosciurus notatus, Sundasciurus lowii* and *Niviventer cremoriventer.*
- Morphology by light microscopy: In the cross section plane the cysts had a rounded appearance, and longitudinal sections showed a tube-like shape. The wall was characterized by compact short protrusions, limited towards the inner cyst by a well-defined linear ground substance. The cystozoites were loosely packed, presenting a lanceolate shape.
- Morphology of the wall by transmission electron microscopy: Due to autolysis, the ultrastructural analysis of category 3 was not possible.
- Measurements by light microscopy in Tab. 28.

Tab. 28: Measurements by light microscopy of cyst category 3

ID number_host species	Number of cysts measured	Cyst orientation	Cyst (µm)			Bradizoites (µm)	
			Length	Width	Wall	Length	Width
E27/14_*N. cremoriventer*	1	Longitudinal	197.6	77.6	3.8	4.9 ±0.10 (n=20)	1.40 ±0.04 (n=20)
E360/13_*C. notatus*	2	Cross	38-69.6	37.6-55.6	1.2-3.1		
E367/13_*S. lowii*	1	Longitudinal	207.86	72	1.1		

- Species classification: Not possible.
- Wall type classification according to Dubey (Dubey et al., 2016): Not possible.

Treeshrews

Tab. 29: Treeshrews infected by sarcosporidia

ID	Animal species	Sex	Age	(Number of cysts in a tissue section) Origin of the striated muscle
E351/13	*T. minor*	Male	Adult	(13) Diaphragm
E352/13	*T. minor*	Female	Adult	(6) Diaphragm, (28) Diaphragm
E357/13	*T. minor*	Male	Adult	(2) Diaphragm
E364/13	*T. minor*	Female	Adult	(3) Diaphragm, (2) Diaphragm, (11) Thigh, (7) Thigh
E370/13	*T. minor*	Male	Adult	(9) Laryngeal muscle {next to salivary gland}, (12) Laryngeal muscle {next to salivary gland}, (6) Abdominal muscle, (1) Abdominal muscle, (2) Abdominal muscle.
E371/13	*T. minor*	Male	Adult	(9) Abdominal muscle
E392/13	*T. minor*	Female	Adult	(3) Diaphragm
E70/14	*T. tana*	Male	Adult	(7) Laryngeal muscle {next to salivary gland}
E119/13	*T. tana*	Female	Adult	(8) Thigh
E120/13	*T. tana*	Male	Adult	(6) Diaphragm
E66/14	*T. tana*	Female	Adult	(5) Thigh

Category 4

- Host species: *Tupaia minor* and *Tupaia tana.*
- Morphology by light microscopy (Fig.18A): In the cross-sectional plane, *Sarcocysts* were spherical to slightly oval. Longitudinal sections showed cigar-shaped cysts with slightly pointed ends. Thick walls with striated pattern due to tightly packed fine villous protrusions. The protrusions were long and slender with a characteristic hair-like appearance. Depending on the section plane the protrusions varied from fully extended to mildly or completely bended at the interface between cyst and host tissue. The ground substance was characterized by a very thin layer underneath the villous protrusions. The interior of the cyst was occasionally septated, with chambers containing mostly loosely packed cystozoites with a lanceolate shape.
- Morphology of the wall by transmission electron microscopy (Fig. 18B): The wall presented a linear electron-dense parasitophorous vacuole membrane with minor undulation, folded into elongated villous protrusions with a hair-like appearance, anchored in the ground substance by microtubules that extended into each villous protrusion. The parasitophorous vacuole membrane delineating the villous protrusions appeared to be slightly undulating in the apical part, however, ultrastructural preservation was not sufficient to study this in detail. The villous protrusions were characterized by a distinct electron-dense, inverted U-shape apical structure in relation to the plane parallel to ground substance. Ground substance: remained in the proximity of the wall without infiltrating the interior of the cyst. Inside the cysts the lanceolate shaped cystozoites were loosely packed in the cyst matrix.
- Measurement by light and transmission electron microscopy in Tab. 30 A and Tab. 30 B.

Tab. 30A: Measurements by light and transmission electron transmission microscopy of cysts category 4

Light microscopy							
ID number_host species	**Number of cysts measured**	**Cyst orientation**	**Cyst (µm)**			**Cystozoites (µm) (n=85)**	
			Length	**Width**	**Wall**	**Length**	**Width**
E351/13_*T. minor*	32	Cross section	22 to 142 µm in diameter (mean = 53.2 ± 28.9 µm [± S.D.])		3.9 to 10.7 µm or bent down (1.8 to 3.6 µm)	5.3 (± 0.7)	1.3 (± 0.2)
E357/13_*T. minor*							
E364/13_*T. minor*							
E370/13_*T. minor*							
E120/13_*T.tana*							
E371/13_*T. minor*	4	Longitudinal section	545	176			
E392/13_*T. minor*			102	34.2			

Transmission electron microscopy						
ID number_host species	**Number of cysts measured**	**Protrusions**			**Cystozoites (µm) (n=17)**	
		Length	**Width**	**Ground substance**	**Length**	**Width**
E364.13_*T. minor*	6	average 5.02 µm	480 to 640 nm (300 – 400 nm near the tip)	150 - 210 nm	4.250 (± 650)	1.410 (± 120)
E357.13_*T. minor*						
E371.13_*T. minor*						
E351/13_*T. minor*						

Tab. 30B: Measurements of the ultrastructural features of the protrusion tip from cyst category 4

ID number_host species	**U-shape structure**		
	Length	**Width**	**Branch diameter**
E364.13_*T. minor*	150 nm	250 nm	74 to 99 nm
E357.13_*T. minor*			
E371.13_*T. minor*			
E351/13_*T. minor*			

- Wall type classification according to Dubey (Dubey et al., 2016): Unclassified.
- Species classification: **Novel cyst pattern**. The unique wall features suggest a novel *Sarcocystis* species.

Sundamys muelleri

Tab. 31: *Sundamys muelleri* infected by sarcosporidia

ID	Animal species	Sex	Age	(Number of cysts in a tissue section) Origin of the striated muscle
E115/13	*Sundamys muelleri*	Female	Adult	(25) Diaphragm
E123/13		/	/	(3) Diaphragm
E125/13		Female	Subadult	(5) Diaphragm (1) Diaphragm
E141/13		Female	Adult	(7) Diaphragm
E243/13		Male	Adult	(3) Diaphragm
E267/13		Male	Adult	(1) Diaphragm, (6) Diaphragm
E273/13		Female	Subadult	(2) Thigh
E274/13		Female	Adult	(14) Diaphragm, (7) Diaphragm
E275/13		Male	Adult	(1) Diaphragm
E276/13		Male	Adult	(10) Diaphragm, (1) Diaphragm, (2) Thigh
E277/13		Female	Adult	(1) Diaphragm
E28/14		Female	/	(9) Diaphragm
E318/13		Female	Adult	(3) Thigh
E319/13		Male	Adult	(1) Diaphragm
E328/13		Male	Adult	(5) Diaphragm
E330/13		Female	Adult	(3) Abdominal muscle
E331/13		Female	Subadult	(41) Diaphragm
E333/13		Male	Adult	(15) Diaphragm
E334/13		Female	Adult	(2) Thigh
E342/13		Male	Adult	(7) Diaphragm
E354/13		Female	Adult	(19) Diaphragm, (5) Thigh, (6) Thigh
E372/13		Male	Adult	(5) Diaphragm
E373/13		Male	Adult	(3) Diaphragm, (1) Diaphragm
E374/13		Female	Adult	(5) Diaphragm, (3) Diaphragm
E387/13		Male	Adult	(3) Diaphragm, (1) Diaphragm
E393/13		Female	Adult	(24) Diaphragm, (1) Diaphragm
E53/14		Male	Adult	(1) Diaphragm
E55/14		Male	Adult	(4) Diaphragm
E57/14		Male	Adult	(4) Diaphragm
E59/14		Male	Adult	(56) Diaphragm
E60/14		Male	Adult	(4) Diaphragm
E61/14		Female	Adult	(11) Diaphragm
E62/14		Female	Adult	(2) Diaphragm, (6) Diaphragm
E87/14		Female	Adult	(6) Diaphragm
E124/13		Female	Adult	(8) Thigh
E122/13		Male	Subadult	(3) Diaphragm, (2) Diaphragm
E37/13		Male	Adult	(15) Diaphragm (6) Thigh

Category 2

- Host species: *Sundamys muelleri.*
- Morphology by cyst light microscopy (Fig. 17C): Cross section plane revealed round cyst and the longitudinal section long elliptical shape morphology. The protrusions showed similar features to category 2 from *Sundasciurus lowii*, however because the wall was not compressed to the host myofiber, the protrusions were evaluated in more detail. The wall was composed by long finger-like villous protrusions, densely packed with a straight arrangement. The wall showed a distinct pattern and consisted of the main part of the protrusions and a small dense portion that bind to the cyst. The ground substance appeared well-defined, linear, and very thin. The interior of the cysts presented loosely packed cystozoites with a fusiform shape.
- Morphology of the wall by transmission electron microscopy (Fig. 17D):
  The villous protrusions showed a body consisting of a long cylindrical stalk with a finger-like shape and a narrowing part (neck of the protrusion) in the segment close to the cyst. The electron-dense parasitophorous vacuole membrane was thin and linear in the stalk with few mild invaginations. In contrast to this, the neck showed multiple invaginations giving a rugged appearance. The interior of the cysts was composed by a homogeneous matrix without the presence of microtubules. The ground substance was linear, surrounding the cyst wall without penetrating the interior of the cyst. Inside the cysts the cystozoites had a fusiform shape and were loosely packed in the cyst matrix.
  The ultrastructural evaluation confirmed the similarities to cyst category 2 described for the forest squirrel *Sundasciurus lowii*.
- Measurement by light and transmission electron microscopy in Tab. 32.

Tab. 32: Measurements by light and transmission electron microscopy of cysts category 2

Light microscopy							
ID number_host species	**Number of cysts measured**	**Cyst orientation**	**Cyst (µm)**			**Cystozoites (µm) (n= 58)**	
			Length	**Width**	**Wall**	**Length**	**Width**
E61/14_*S.muelleri*	43	Cross section	91.35 ± 105,69	66.75 ± 21.88	4.7 ± 0.24	5.11 ± 0.62	1.23 ± 0.16
E87/14_*S.muelleri*							
E115/13_*S.muelleri*							
E122/13_*S.muelleri*							
E123/13_*S.muelleri*							
E125/13_*S.muelleri*							
E141/13_*S.muelleri*							
E243/13_*S.muelleri*							
E276/13_*S.muelleri*							
E330/13_*S.muelleri*							
E331/13_*S.muelleri*							
E342/13_*S.muelleri*							
E354/13_*S.muelleri*							
E53/13_*S.muelleri*	4	Longitudinal section	431 to 780	41 to 85	2.7 to 5.4		
E141/13_*S.muelleri*							
E354/13_*S.muelleri*							
E276/13_*S.muelleri*							
Transmission electron microscopy							
ID number_host species	**Number of cysts measured**	**Protrusions (n=6) (µm)**			**Cystozoites (µm) (n=24)**		
		Length	**Width**	**Ground substance**	**Length**	**Width**	
E141/13_*S.muelleri*	2	6.21 to 5.66	0.48 to 0.51	0.13 to 1.17	4.2 ± 0.4	1.2 ± 0.2	
E273/13_*S.muelleri*							

- Wall type classification according to Dubey (Dubey et al., 2016): Type 19.
- Species classification: **Category 2 is comparable with *Sarcocysis singaporensis.***

Category 5

- Host species: *Sundamys muelleri.*
- Morphology by light microscopy (Fig. 18C): Cross sections revealed a round cyst morphology and a longitudinal plane section showed long elliptical shape. The cyst revealed a thin wall composed of small dense conical protrusions with a narrow part proximal to the cyst. The protrusions were widely separated and anchored in a well-defined, linear ground substance. The interior of the cysts presented loosely packed cystozoites with a fusiform shape.
- Morphology of the wall by transmission electron microscopy (Fig. 18D): The villous protrusions were characterized in the proximity to the cyst wall by a pointed conical base and a distal segment presented a radial pattern conferring a cocklebur-like shape. The parasitophorous vacuole membrane was thick and electron-dense, radiating short projections. A cross section of the distal segment of the protrusions revealed a cogwheel-like appearance. The interior of the protrusions contained

multiple microtubules. The ground substance was thin and linear without penetrating the interior of the cyst.

- Measurements by light microscopy and transmission electron microscopy in Tab. 33.

Tab. 33: Measurements by light and transmission electron microscopy of cysts category 5

Light microscopy							
ID number_host species	**Number of cysts measured**	**Cyst orientation**	**Cyst (µm)**			**Cystozoites (µm) (n= 30)**	
			Length	**Width**	**Wall**	**Length**	**Width**
E37/13_*S. muelleri*	6	Cross section	35 to 103.92	29 to 95	1.56 to 5.56	4.22 ± 0.27	1.71 ± 0.15
E333/13_*S. muelleri*							
E37/13_*S. muelleri*	1	Longitudinal section	589.95	52	5.79		
Transmission electron microscopy							
ID number_host species	**Number of cysts measured**	**Protrusions (n=6) (µm)**			**Cystozoites (µm) (n=4)**		
		Length	**Width**	**Ground substance**	**Length**	**Width**	
E37/13_*S. muelleri*	2	1.14 to1.86	0.69 to 0.73	0.06 to 0.1	2.6 to 3.4	1 to 1.1	
E333/13_*S. muelleri*							

- Wall type classification according to Dubey (Dubey et al., 2016): Type 22.
- Species classification: **Category 5 is comparable with *Sarcocysis villivillosi.***

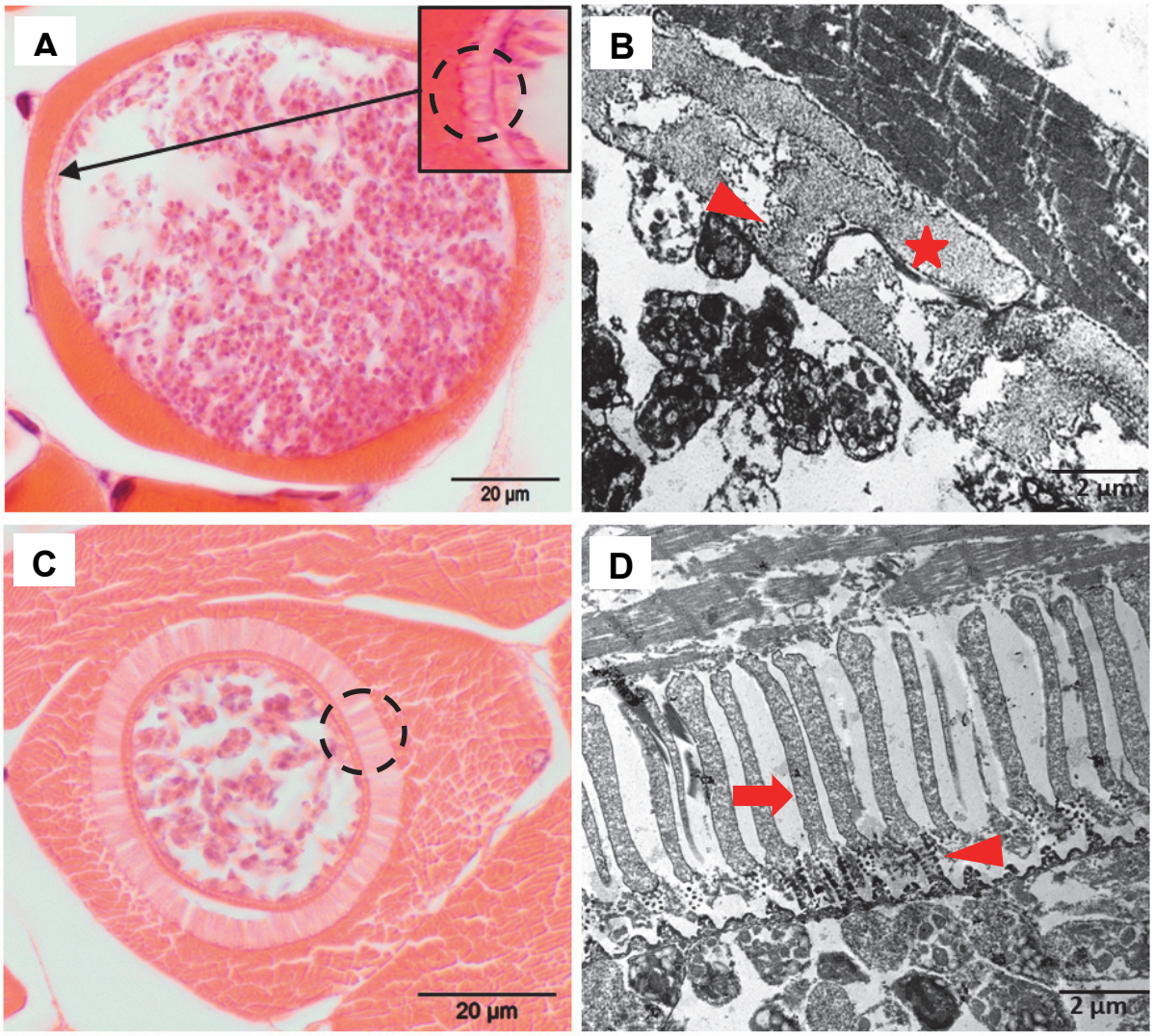

Fig 17: Microscopic images of the different *Sarcocystis* species detected in forest rodents and *Sundamys muelleri*. A) Cross section of *Sarcocystis zuoi* in an infected thigh muscle of *Maxomys Whiteheadi,* H&E. Inset: High magnification of the bended protrusions with finger-like shapes. B) Transmission electron micrograph of *Sarcocystis zuoi*. The wall is composed by multiple bended protrusions (red star) with rugged surface and branched in the base (red arrow head). C) Cross section of *Sarcocystis singaporensis* inside a diaphragm myofiber from *Sundamys muelleri,* H&E. The cyst wall is clearly defined by tight elongated straight protrusions with a denser portion at the junction with the ground substance (dash circle); note the absence of inflammatory reaction. D) Transmission electron micrograph of *Sarcocystis singaporensis*: wall protrusions characterized by cylindrical stalks (red arrow) and electron-dense necks (red arowhead).

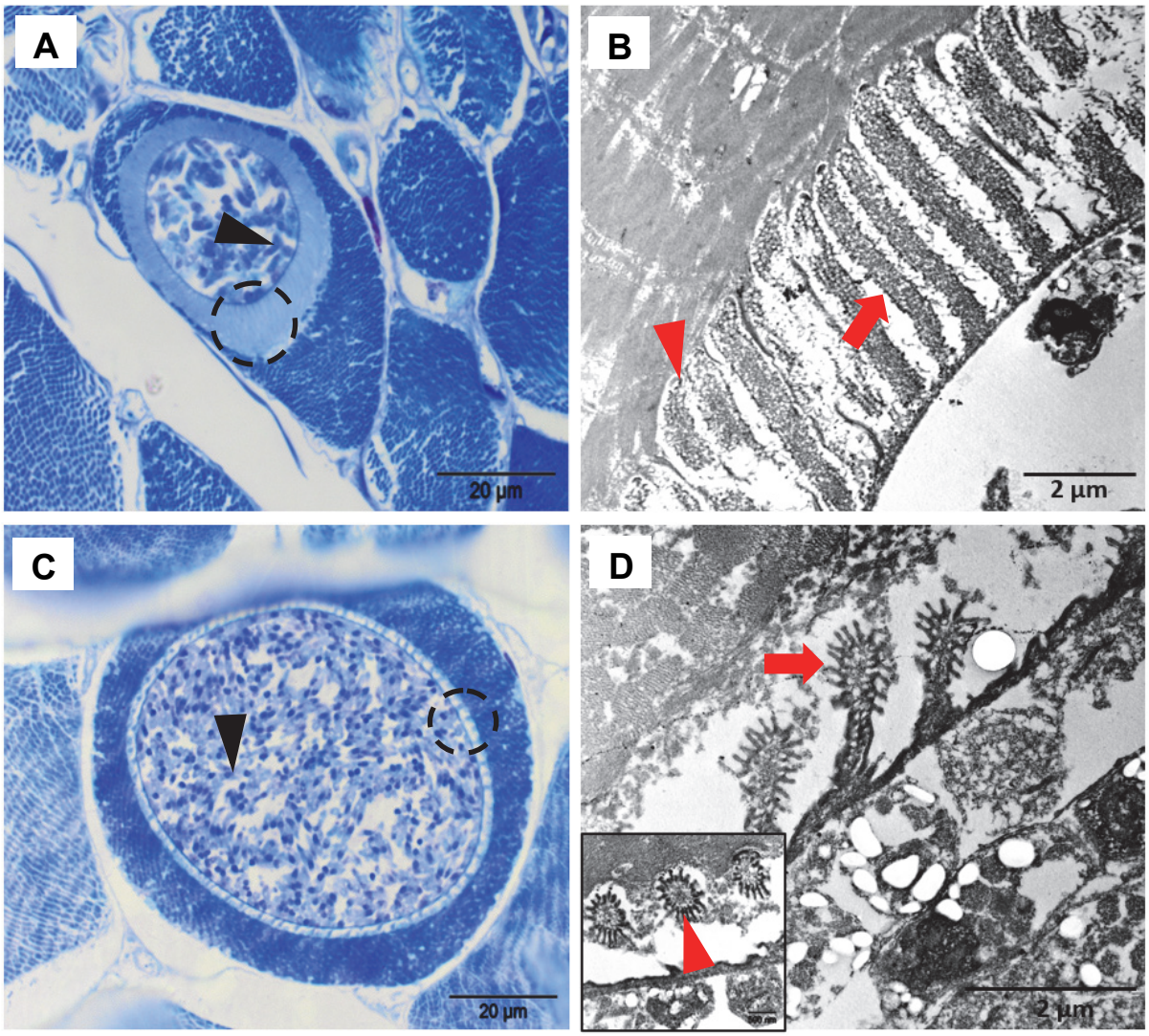

Fig. 18: Microscopic images of the different *Sarcocystis species* detected in treeshrews and *Sundamys muelleri.* A) Semithin section of a novel *Sarcocystis* species from *Tupaia minor* characterized by a thick wall with numerous hair-like villous protrusions (dash circle) and containing lanceolate cystozoites (black arrow head), Richardson staining Richardson staining. B) Transmission electron micrograph of the novel *Sarcocystis* sp. isolated from treeshrews defined by long and tight villous protrusions (red arrow) with a characteristic electron-dense tip with an inverted U-shape structure (red arrowhead). C) Semithin section of *Sarcocystis villivillosi* from *Sundamys muelleri* showing a thin wall (dash circle) and an inner cyst with tightly packed lanceolate cystozoites, Richardson staining. D) Transmission electron micrograph of the wall from *Sarcocystis villivillosi,* showing the radial pattern of the protrusions (red arrow). Inset: cross section of the protrusions with a characteristic cogwheel appearance (red arrow head).

The morphological investigations of the *Sarcocystis* species detected in forest rodents, treshrews and *Sundamys muelleri*, revealed the presence of five different cyst categories. A comparison between the identified cyst categories and the well-known wall pattern types of *Sarcocystis* species described by Dubey (2016), allowed the identification of three species: *S. zuoi* identified in forest rodents, *S. singaporensis* detected in forest rodents and *Sundamys muelleri*, and *S. villivillosi* observed in *Sundamys muelleri.* In addition, a novel cyst wall pattern was identified in the treeshrew species *Tupaia minor* and *T. tana*. Although category 3 was only evaluated by light microscopy and therefore the species identification was not possible, the distinct morphology of the wall is suggestive for a different species than *S. zuoi* or *S. singaporensis.*

Tab. 34: Summary *Sarcocystis* species identification by morphological analysis

Animal group	**Host species**	**Cyst category**	***Sarcocystis* species**	**Wall type classification according to Dubey (2016)**
Forest rodents	*Callosciurus notatus*	Category 3	/	/
	Sundasciurus lowii	Category 2	*Sarcocystis singaporensis*	Type 19
		Category 3	/	/
	Maxomys whiteheadi	Category 3	/	/
		Category 1	*Sarcocystis zuoi*	Type 17
	Niviventer cremoriventer	Category 3	/	Unknown
Treeshrew	*Tupaia minor*	Category 4	Unknown	Novel pattern
	Tupaia tana			
Generalist	*Sundamys muelleri*	Category 2	*Sarcocystis singaporensis*	Type 19
		Category 5	*Sarcocystis villivillosi*	Type 22

5.3.4.2 Molecular investigations

Forest species

Forest rodents

Initially, ten forest rodents were included in the molecular analysis, and a single sequence from the *Sarcocystis* spp. 18S rRNA gene was obtained from the forest squirrel species *Sundasciurus lowii* (E367/13: 1824bp) Moreover, for the *Sarcocystis* spp. cytochrome c-oxidase subunit 1 gene (COI) two sequences were obtained from forest rodent species *Niviventer cremeoriventer* (E102/14: 152bp) and *Sundasciurus lowii* (E346/13: 463bp).

Treeshrews

Molecular investigations (sequencing performed by Dr. Annika Brinkmann and Prof. Andreas Nitsche, Robert Koch Institute, Berlin, Germany) and phylogenetic analysis (performed by Dr. Thomas Jäkel, University of Hohenheim, Germany).

Out of the eleven treeshrews included in the molecular analysis two full-length sequences of the nuclear 18S rRNA gene were obtained from the novel *Sarcocystis* spp. of two *Tupaia minor* (E357/13: 1840 bp; E364/13: 1838 bp). Further, five partial sequences of cytochrome c-oxidase subunit 1 gene (COI) were retrieved from the novel *Sarcocystis* spp. of three *T. minor* (E364/13, 938 bp; E357/13, 944 bp, E351C/13, 908 bp) and from two *T. tana* (E120/13, 976 bp; E120B/13, 941 bp). All sequences obtained from the 18S rRNA and COI gene were used for the phylogenetic analysis.

Analysing the 18S rRNA sequences from *T. minor* by BLAST a high similarity with sequences of *Sarcocystis* spp. from colubrid snakes was found. Including these sequences in the phylogenetic analysis by Maximum Likelihood (ML) and Bayesian Inference (BI) the resulting phylogenetic trees located the 18S rRNA sequences from the novel *Sarcocystis* spp. of *T. minor* within a monophyletic clade with *Sarcocystis* spp. from colubrid snakes (supplementary material Fig. 21).

Due to the lack of known COI sequences within the Apicomplexa for the phylogenetic analysis of COI in this study further COI sequences with a high degree of similarity needed to be included in the BLAST search. The phylogenetic analysis conducted by Maximum Likelihood (ML) showed that sequences from the the novel *Sarcocystis* spp. of treeshrews formed a sister group with the only other *Sarcocystis* species isolated from snakes (supplementary material Fig. 22).

Sundamys muelleri

From the 37 *Sundamys muelleri* included in the molecular analysisseven *Sarcocystis* spp. sequences from the 18S rRNA gene were obtained (Fig. 19) (E333/13A: 1767bp, E333/13B: 1717bp, E37/13B: 1817bp, E37/13C: 1815 bp, E141/13A: 463bp, E141/13B: 1423bp, E274/13B: 668bp, E274/13A: 1387bp). In addition, for cytochrome c-oxidase subunit 1 gene (COI) also seven sequences were obtained (Fig. 20) (E125/13: 834bp, E330/13: 738bp, E333/13A: 385bp, E333/13B: 711bp, E393/13A: 511bp, E393/13B: 582 bp).

In contrast to other pathogens, the high intraspecific variability of the nuclear 18S rRNA due to paralogous sequences, complicates the molecular identification of coccidian parasites, preventing a direct molecular detection (El-Sherry et al., 2013). For this reason, the genetic species identification requires a phylogenetic evaluation. Further phylogenetic analyses (not included in this thesis) based on the structural and functional aspects of the COI and 18S rRNA would need to be conducted on the sequences obtained from *Sarcocystis* spp. infecting forest rodents and *Sundamys muelleri.*

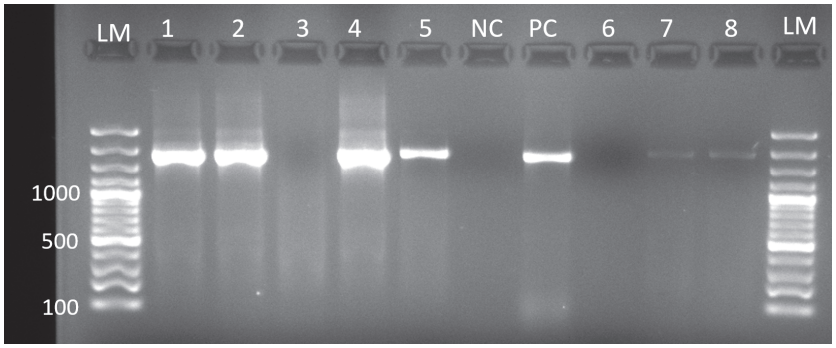

Fig. 19: PCR targeting 18S rRNA gene from *Sarcocystis* with primers SarAF/SarDR. Agarose gel 2% electrophoresis: Line 1 (E37/13B *Sundamys muelleri*), line 2 (E37/13C *Sundamys muelleri),* line 4 (E367/13 *Sudasciurus lowii*), line 7 (E333/13A *Sundamys muelleri*), line 8 (E333/13B *Sundamys muelleri*) show bands of the same product (1440 bp) like the positive control (PC). Line 3 (E120/13A *Tupaia tana*), line 6 (E351/13 *Tupaia minor*) are lacking *Sarcocystis*-DNA similar to the negative control (NC). LM DNA-ladder marker (DNA-ladder 100 bp, Thermo Fisher Scientific).

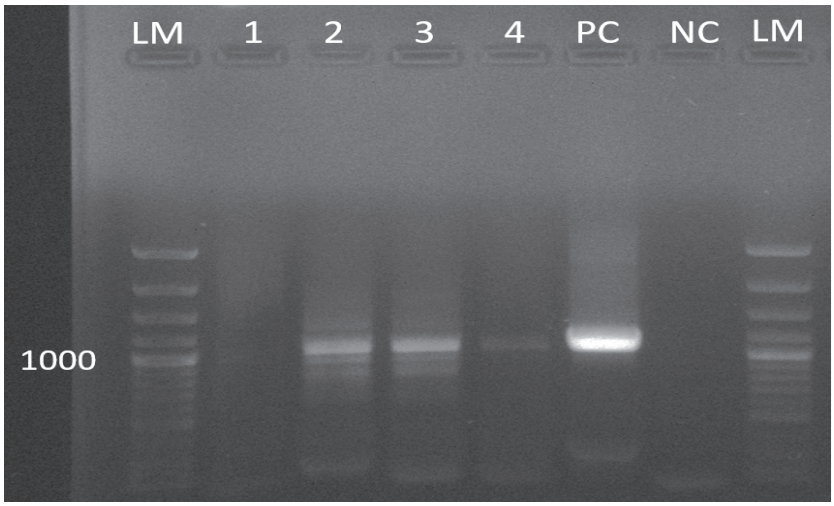

Fig. 20: PCR targeting the COI gene from *Sarcocystis* with SF1/SR8D. Agarose gel 2% electrophoresis: Line 2 (E364/13 *Tupaia minor*), line 3 (E351/13C *Tupaia minor),* line 4 (E357/13 *Tupaia minor*) show bands of the same product (1000-1070 bp) like the positive control (PC). Line 1 (E119/13C *Tupaia tana*) are lacking *Sarcocystis*-DNA similar to the negative control (NC). LM DNA-ladder marker (DNA-ladder 100 bp, Thermo Fisher Scientific).

5.3.4.1 Novel *Sarcocystis* sp. identified from *Tupaia minor* and *T. tana*

Considering the morphological, molecular, and phylogenetic results from the *Sarcocystis* isolated from treeshrees in this study, a new *Sarcocystis* sp. was detected in *Tupaia minor* and *T. tana* inhabiting Northern Borneo. This novel species was named after its intermediate host and the geographical origin as ***Sarcocystis scandentiborneensis* sp. nov**. The phylogenetic trees are included in the supplementary material Fig. 21 and Fig. 22.

5.3.5 Herpesvirus results (Bachelor thesis Heiko Pietsch, RKI, Berlin, Germany) (Pietsch, 2013)

From the 156 individuals screened for herpesviruses by nested PCR, a clear amplicon was retrieved from 49 samples, while herpesvirus sequencing was successful in 46/156 animals (29%) (supplementary material Tab. 45 and 46). From the evaluated animals, females had a slightly higher rate with 33% (23/68) in comparison to males with 27% (23/86). Two animals, where gender classification was not possible, had negative results. Considering the age of the individuals, 34% (38/112) were adults, 20% (6/30) were subadults and only one (1/7) juvenile were infected by herpesvirus. From the five individuals with unclassified age one was positive for herpesvirus-DNA (supplementary material Tab. 44).

The ratio of herpesvirus-positive individuals varied between the different host species. The highest herpesvirus infection rate was found in the urban *Rattus* spp. (35%, 20/57 (16/40 *Rattus* compl., 4/17 *Rattus norvegicus*)), *Sundamys muelleri* (36%, 15/41) and *Tupaia minor* (50%, 4/8). For all other species only one or two individuals per species were infected by herpesviruses (1/3 *Callosciurus notatus*, 1/2 *Callosciurus prevosti*, 2/5 *Maxomys whiteheadi*, 1/3 *Maxomys rajah*, 1/3 *Niviventer cremoriventer*, 1/26 *Suncus murinus*).

Overall, sequencing results indicated a broad range of different herpesvirus strains occurring in small mammals from Northern Borneo as at least 17 different strains were detected in ten different host species. Among these, the urban rats stood out as 30% of these individuals carried several herpesviruses.

The retrieved herpesvirus polymerase gene fragments had a maximum length of 166-178 bp due to insufficient DNA preservation. Sequence alignment based on these rather short PCR products showed a maximum homology of 72% to 99% to known herpesvirus sequences. Virus classification was therefore only indicative of a possible virus species. Thirteen different gammaherpesviruses and four betaherpesviruses were identified. Rattus tiomanicus rhadinovirus 1 (Rtiom_RHV1) was the most commonly detected gammaherpesvirus, found in 29% (13/46) of the individuals (5/41 *Sundamys muelleri*, 7/40 *Rattus* compl. and 1/17 *Rattus norvegicus*). The most frequently detected betaherpesvirus was Rattus tiomanicus cytomegalovirus 1 (Rtiom_CMV1) identified in three individuals from *Rattus* compl. (supplementary material Tab. 46).

The majority of homologous virus sequences occurred in host species phylogenetically closely related within the taxa Muridae and Tupaiidae. However, one herpesvirus sequence from Rattus rattus rhadinovirus 1 (Rrat_RHV1) was found in two individuals from different taxa: one forest squirrel *Calloscirius prevostii* and one individual from the urban *Rattus* compl. All gammaherpesviruses found in *Tupaia minor* showed a particular low identity match (<80%) with their closest related herpesvirus taxa.

The host species diversity carrying herpesviruses varied along the land-use gradient from urban to forest. The gammaherpesviruses Rtiom_RHV1, detected in the urban rats and *Sundamys muelleri*, was the dominant herpesvirus within the urban habitats. Gammaherpesvirus Rattus tiomanicus rhadinovirus 2 (Rtiom_RHV2) was also carried by urban rats and *Sundamys muelleri*. Moreover, the generalist species *Sundamys muelleri* living in suburban areas had infections with four different gammaherpesviruses indicative for Rtiom_RHV1, Rtiom_RHV2, Rattus rattus rhadinovirus 3 (Rrat_RHV3), and Bandicota indica rhadinovirus 4 (Bindi_RHV4). Amongst the forest rodents, beside Rrat_RHV1 found in *Calloscirius prevostii*, three further types of gammaherpesvirues were found: Bandicota savilei rhadinovirus 1 (Bsavi_RHV1) in *Maxomys whiteadi*, Otariid herpesvirus 4 (OtH4) from *Niviventer cremoriventer* and Oriental small-clawed otter gammaherpesvirus (OSOHV) in *Callosciurus notatus.* Only two betaherpesviruses sequence fragments were found in forest species, the Cytomegalovirus Guenon/muscle/BM-002 (CMV_Guenon) in *Maxomy rajah* and Rat cytomegalovirus Maastricht (RatCMV) from *Maxomys whiteadi*, however, the latter one had a sequence homology lower than 80%.

Tab. 35: Overview of results from molecular investigations on selected pathogens detected in the different small mammal species

Ecological group	Species	Pathogens									
		Leptospira				*Trypanosoma* (n= 277)		*Sarcocystis* (n=58)		Herpesvirus (n= 156)	
		WS (n= 314)		qPCR/PCR analysis (n= 106)							
		Analyzed	Positive	Analyzed	Sequenced	Analyzed	Sequenced	Analyzed	Sequenced	Analyzed	Sequenced
Forest rodents (n= 42)	*Callosciurus notatus*	5	0	3	/	2	/	1	/	3	1
	Callosciurus prevostii	2	1	1	/	3	/	/	/	2	/
	Sundasciurus lowii	5	2	3	1	6	2	4	2	3	/
	Leopoldamys sabanus	/	/	/	/	1	/	/	/	/	/
	Maxomys rajah	3	1	2	/	3	/	/	/	3	1
	Maxomys surifer	7	1	/	/	4	1	/	/	1	/
	*Maxomys surifer/rajah**	6	0	2	/	7	/	/	/	1	/
	Maxomys whiteheadi	5	1	3	2	6	1	2	/	5	2
	Niviventer cremoriventer	4	0	1	/	4	/	3	1	3	1
Forest rodents total		**37**	**7**	**15**	**3**	**36**	**4**	**10**	**3**	**21**	**6**
Treeshrews (n=15)	*Tupaia gracilis*	1	0	1	/	1	1	/		1	/
	Tupaia minor	8	0	7	/	8	1	7	5	8	4
	Tupaia tana	5	1	/	/	4	3	4	2	/	/
Treeshrews total		**14**	**1**	**8**	**/**	**13**	**5**	**11**	**7**	**9**	**4**
Generalist (n=76)	*Sundamys muelleri*	**71**	**15**	**31**	**10**	**66**	**1**	**37**	**14**	**41**	**15**
Rattus (n=155)	*Rattus* comp.	100	27	26	12	76	9	/	/	40	16
	Rattus norvegicus	43	13	12	3	41	5	/	/	17	4
Rattus total		**143**	**40**	**36**	**15**	**117**	**14**	**/**	**/**	**57**	**20**
Shrews (n=58)	***Suncus murinus***	**49**	**1**	**14**	**/**	**45**	**/**	**/**	**/**	**28**	**1**

6 DISCUSSION

Section 1: Histopathological findings

The histopathological investigation conducted in this project included 331 small mammals belonging to 15 species from three orders: Rodentia, Scandentia and Insectivora. The distribution of these species covered a land-use gradient from forest areas to urban territory of the region in and around the city Kota Kinabalu in Sabah, Northern Borneo, Malaysia. The study included forest dwelling species like forest-rats, squirrels and treeshrews, urban Muridae (*Rattus* spp.) and the shrew *Suncus murinus*, and lastly a generalist species, *Sundamys muelleri*, which habitat is bridging the forest and the urban environment. While all forest animals and *Sundamys muelleri* are endemic to Borneo, the urban species are considered invasive with *Rattus rattus* and *Rattus norvegicus* comprising the vast majority of the *Rattus* compl. in this study.

Regardless of the respective habitat an individual originated from, the majority of the organ lesions found in all species were infections by helminths and/or protozoal parasites. The parasitic lesions were either caused directly by the parasite, like acute migration tracts associated with haemorrhages and necrosis, or indirectly as a consequence of the inflammatory response, e.g. granuloma formation.

One of the most relevant findings was the high prevalence of inflammatory infiltration in the lungs in 89% (277/310) of all examined animals. Other studies in wildlife *Rattus* sp. also reported respiratory inflammatory diseases as a common finding with rates ranging from 47% to 54%, as well as in captive shrews *Macroscelides proboscideus* (Clancy et al., 2013; Rothenburger et al., 2015a; Seguel et al., 2017). Various respiratory pathogens were identified in wildlife and laboratory rats, amongst others the cilia-associated respiratory bacillus (CARB) and *Mycoplasma pulmonis* (Easterbrook et al., 2008; Rothenburger et al., 2015a) or *Pneumocystis* spp. (Chabé et al., 2010). In our study, a predominance of lymphoplasmacytic interstitial infiltration was found in the lungs (44%, 135/310), in the forest dwelling species *Sundasciurus lowii*, *Niviventer cremoriventer* and in *Tupaia* spp. as well as in the generalist *Sundamys muelleri*. However, the sample preservation prevented to elucidate possible causative agents. Although in laboratory rats *Pneumocystis* sp. is often responsible for interstitial pneumonia (Easton et al., 2004; Livingston et al., 2011), in wildlife rats histopathological evidence for *Pneumocystis* sp. in the lung has neither been observed by us nor by others (Rothenburger et al., 2015a). However, molecular analyses revealed infection with *Pneumocystis* spp. in 47% to 75% free-ranging *Rattus* spp. in multiple studies (Chabé et al., 2010; Palmer et al., 2000; Rothenburger et al., 2015a).

A high number of pulmonary inflammations with neutrophilic predominance was observed in forest *Maxomys* spp. (20/22) and *Callosciurus notatus* (5/5) and in urban *Suncus murinus*

(43/44). This type of inflammation is suggestive for a response against bacterial infections. Common bacterial pathogens associated with purulent pneumonia in laboratory rats are *Bordetella bronchiseptica*, *Mycoplasma spp., Streptococcus spp.,* and *Pasteurellaceae* (Clancy et al., 2013; Percy and Barthold, 2007).

The lack of suitable samples prevented the aetiological identification of pneumonia with a suspected bacterial or viral origin. In contrast, the morphological evaluation of the parasites found in the pulmonary parenchyma allowed the identification of the nematode *Angiostrongylus* spp. as responsible for parasitic pneumonias. In our study 38% (54/142) of all animals showed pulmonary angiostrongylosis, characterized by the presence of larval nodules in the parenchyma and juvenile forms in the pulmonary blood vessels migrating to the heart (Ubelaker, 1986). Moreover, six individuals from *Rattus* compl. infected by pulmonary nematodes presented multiple islands of squamous bronchiolar metaplasia in addition to the inflammatory reaction. Such lesions are a known consequence of chronic inflammation (Mohr and Dungworth, 1988; Nolte et al., 1993). Another common lesion induced by the migration of the parasite is a marked hypertrophy of the tunica media of pulmonary arterioles, which sometimes even obliterates the vascular lumen (Alicata, 1968). We found a high occurrence of pulmonary arterioles affected by vascular hyperplasia in individuals from the *Rattus* spp. with parasitic pneumonia (32/37 *Rattus* compl., 5/8 affected *Rattus norvegicus*). Although only in one half of the individuals with vascular hypertrophy nematode larvae were detected in the lumen of the pulmonary vessels, it seems feasible to assume the same causative agent for the other half, as this discrepancy is most likely attributed to the limitation of histopathology, when the causative agent is not revealed in the plane of a tissue section.

Lymphoplasmacytic myocarditis was observed amongst others in several *Rattus norvegicus* (18/43). This is in agreement with a previous publication from Canada, where in free-ranging *Rattus norvegicus* mononuclear myocarditis had a prevalence of 33.5% (Rothenburger et al., 2015b). In laboratory rats, myocardial mononuclear inflammation is a common finding and more related to myocardial cellular damage than pathogen infection (Berridge et al., 2016). Although in our study ethanol fixation of the samples did not allow viral or bacterial detection by culture, bacterial suppurative myocarditis was confirmed by special histological staining in two *Sundamys muelleri* individuals.

Focal changes in the heart were characterized by mild lymphoplasmacytic infiltration affecting 18% (61/325) of the individuals. The cause of this inflammatory pattern has been related to degenerative myocardial changes such as cardiomyocyte necrosis, being classified as a common lesion in laboratory and free-ranging *Rattus* spp. (Berridge et al., 2016; Rothenburger et al., 2015b). Although the atrioventricular valves are a preferred target for bacterial colonization, in this study evidence of bacterial infection of the valves such as neutrophilic infiltration or vegetative lesions were not found. However, multiple studies based

on the detection of the endotheliotropic bacterial *Bartonella* spp. in *Rattus* spp. from Southeast Asia, have revealed a high occurrence of up to 67.6% of these infectious agents (Hsieh et al., 2010; Neves et al., 2018; Soon Kim et al., 2016). Despite the infection dynamic of *Bartonella* spp. in rodents (including wild *R. rattus* and *R. norvegicus*) is well studied (Gutiérrez et al., 2015), the lack of histopathological evaluation from the infected individuals prevented to associate possible cardiac lesions caused by this pathogen in the studied species.

A single *Tupaia tana* had evidence of a haemoparasitic microfilaria infection. While the detection of this parasite is usually conducted through a blood smear in order to identify individual parasites in the blood, in this individual the microfilariae were observed in the lumen of the blood vessel from different organs (liver, heart and lung), indicating a high parasitaemia. The absence of inflammatory reaction surrounding the larva in the blood vessel, was also described in an histopathological study of cotton rats (*Sigmodon hispidus*) naturally infected with microfilaria (Wharton, 1947). *Tupaia tana* is a known host for the genus *Mansonella* (*Tetrapetalonema*), which is transmitted through hematophagous arthropods of the genus *Culicoides* (Eberhard and Orihel, 1984; Mullin and Orihel, 1972). A recent publication of a novel microfilaria species from *Tupaia glis* also occurring in Malaysia, has pointed out the importance of microfilaria species infecting treeshrews to understand the phylogeny of this parasite (Uni et al., 2017).

Follicular hyperplasia was found in the spleens of almost all *Sundamys muelleri* (40 out of 42) and more than half of the *Rattus* spp. (*Rattus* compl (29/40), *Rattus norvegicus* (13/23)). The increased cellularity in the follicles occurring during immune responses (acute or chronic) is highly related with the structure of the follicles. The central follicular arteriole transports antigens recognized by the periarteriolar T-cells, which activate the T-dependent B-cells of the outer follicular layer, that are responsible for the production of antibodies (Lewis et al., 2019; Willard-Mack et al., 2019). Given the high rate of inflammatory processes occurring in other organs of these species (i.e. lung or liver), the cellular proliferation can be interpreted as a normal physiological response to an infectious agent occurring somewhere in the body. In contrast, a true inflammation of the spleen characterized by a suppurative splenitis was observed in two *Suncus murinus.* These two animals also suffered a suppurative pneumonia, which could both have a similar origin. For example, haematogenous dissemination of *Streptococcus* spp. bacteria from infected lungs was described as a cause of splenitis in rodents (Schoeb, 2000) as well as in domestic animals (Boes and Durham, 2017).

Hepatic lesions associated with helminth migration were frequently observed in *Rattus* spp. (30/98 *Rattus* compl., 27/42 *Rattus norvegicus*) and *Sundamys muelleri* (20/70). Previous surveillance studies also showed a prevalence up to 88% of the hepatic nematode *Calodium hepaticum* in wild *Rattus norvegicus* inhabiting urban regions (Easterbrook et al., 2007;

Rothenburger et al., 2019). The elevated density of rats in these areas (Farhang-Azad, 1977) alongside with deficiencies in the sewer systems (Easterbrook et al., 2007; Walker et al., 2017) may facilitate the transmission of *Calodium hepaticum*. The majority of the infected animals in our study were adult, which is not surprising as infected individuals carry the parasite for their lifetime (Farhang-Azad, 1977). Depending on the section plane, false negative results could occur when a migrating parasite is located elsewhere in the tissue. If a thorough surveillance for these parasites was intended, an enzymatic tissue digestion is considered the best method for parasite detection (McGarry et al., 2014). Although this parasite is considered zoonotic, the cases reported of human capillariosis are rather low (Fuehrer et al., 2011). Another parasite commonly found in the liver of wild *Rattus norvegicus* is the larval stage of *Taenia taeniformis* (syn. *Cysticercus fasciolaris*) (Percy and Barthold, 2007). Frequently these larvae occur together with *Calodium hepaticum* (Seguel et al., 2017; Yi et al., 2010). Although in this study such co-infection was not observed, three out of 42 *Rattus* compl. had liver cyst formation suggestive of *Taenia* sp. infection.

One alteration commonly seen in all investigated species was bile duct hyperplasia. This lesion has been associated with different processes such as aging or an increase of the secretory activity (Alpini et al., 1988; Percy and Barthold, 2007). But in accordance to the major cause of hepatic lesions in this study, parasite infections are the most plausible aetiology for bile duct hyperplasia as the parasites' movement inside the bile ducts results in this reactive change (Choi et al., 2004; Foster, 1981; Modavi and Isseroff, 1984).

In contrast to this general change, a rather species-specific lesion was the generalised extensive swelling of the hepatocytes observed in 8/14 *Tupaia* spp. and in a single forest rodent *Niviventer cremoriven*ter. This pathological feature was also a common finding in a histopathological study of captive treeshrews (Clancy et al., 2013). This lesion type has been investigated in medical research, where *Tupaia* spp. are used as an animal model for hepatic storage diseases. It was shown that treeshrews are genetically predisposed to develop hepatic lipidosis (Zhang et al., 2016, 2015). Given that the *Niviventer cremoriven*ter was a female, this lesion could be related to fasting during pregnancy, commonly occurring in rodents. The decrease of the caloric intake leads to a mobilization of storage fat that further accumulates in the liver (Delaney et al., 2018).

Lymphoplasmacytic interstitial nephritis, detected in 28% (89/314) of all individuals, has been reported in other studies as a common pathological change in wild *Rattus* spp. (Ceruti et al., 2002; Rothenburger et al., 2019; Seguel et al., 2017; Tucunduva de Faria et al., 2007). This type of inflammation, also frequently reported in domestic species has been associated with antigen persistence (Cianciolo et al., 2015). Although interstitial nephritis has been found alongside with the presence of *Leptospira* spp. in wild *Rattus norvegicus* (Agudelo-Flórez et

al., 2012), the absence of lesions in kidneys positive for *Leptospira* spp., as noted in the present study, supports the concept that wild rats are asymptomatic carrier and reservoir hosts for *Leptospira* spp. (Adler and de la Peña Moctezuma, 2010; Picardeau, 2017; Yamaguchi et al., 2018).

A protozoal parasite infecting the kidney of two squirrels (*Sundasciurus lowii*) revealed different developmental stages of a coccidian parasite comparable with *Klosiella* spp. (Gardiner et al., 1998). The coccidian forms, consistent with sporonts and sporoblast, were located in the tubular epithelium without any significant inflammatory reaction. Renal coccidiosis caused by *Klosiella* spp. was identified in multiple small mammal species including mice (*Mus* spp.), water rat (*Hydromys chrysogaster*), guinea pig (*Cavia* spp.) and glider (*Petaurus* spp.) (Ardiaca et al., 2016; Percy and Barthold, 2007; Stevenson, 1915; Taylor et al., 1979; Winter and Watt, 1971). In the literature, no evidence for *Klossiella* infection or any other renal coccidia species in squirrels was found, making these results the first indication of a *Klossiella*-like infection in this species.

The presence of a dense bacterial layer, comparable with lactobacilli, in the non-glandular part of the stomach is well-described in laboratory mice (Almirón et al., 2013). To our surprise we found similar dense layers of rod-shaped bacteria in the stomachs of the forest rodent species *Maxomys whiteheadi*, *Maxomys surifer* and *Niviventer cremoriventer*. One study assumes the bacterial aggregation has a protective effect against the overgrowth with pathogenic bacteria (Tannock and Savage, 1974). Although the microbiome of the gut differs markedly between wild and laboratory rodents (Rosshart et al., 2017), a protective mechanism related to host fitness is suggested for both groups.

Studies on helminth parasites in the gastrointestinal tract of wild rodents from different geographical locations of the world reported a high prevalence of helminths in a wide range of host species (Azzam et al., 2016; Galán-Puchades et al., 2018; Julius et al., 2017; Mohd Zain et al., 2012; Smales, 2018). In contrast to endoparasites found in mesenchymal organs such as liver (i.e. *Calodium hepaticum*) or lung (i.e. *Angiostrongylus* spp.) the presence of intestinal helminths is usually not associated with significant pathological lesions (Rothenburger et al., 2019; Seguel et al., 2017). In addition to the inflammatory changes in the lamina propria of the duodenum the presence of the nematodes resulted in the hyperplasia of the Brunner's gland. As duodenal tissue from *Suncus murinus* was readily available it revealed a high occurrence of 50% (28/56) of parasites in this anatomic location of this animal species. The duodenum is known as a preferred place for nematode infection in different host species including cattle and poultry (Ackert and Herrick, 1928; Fuertes et al., 2015; Mavenyengwa et al., 2005). The high occurrence of intestinal nematodiasis among all ecological groups is highly related to the ingestion of invertebrates mostly arthropods and gastropods, which are intermediate hosts for multiple nematode species (Gibson et al., 2014). A previous study from Southeast Asia

reported a high presence of helminths (above 90%) in the rodents *R. rattus* and *R. norvegicus,* as well as in the shrew *Suncus murinus* (Tung et al., 2013).

Intestinal coccidiosis caused by *Eimeria* spp. was detected in all ecological groups but with a low occurrence. This finding is in accordance to the rare presence of coccidia in *R. norvegicus* from the Peninsular Malaysia (Lumpur, 2018). In rodents, *Eimeria* spp. are considered non-pathogenic (Morrisey, 1996; Schmidt, 1995). However, studies based on the detection of *Eimeria* spp. in other host species such as *Suncus murinus* or treeshrews, did not evaluate the host response to the parasite (Duszynski and Upton, 2000; Mullin et al., 1972). In this study here, the absence of inflammation in treeshrews and shrew *Suncus murinus* naturally infected by *Eimeria* sp. suggests also a low pathogenicity in these species.

The distribution rate of inflammatory lesions in the lung, liver, heart or large intestine as well as follicular hyperplasia in the spleen was similar between male and female individuals. Except, inflammatory changes in stomach, large intestine and kidney had a slightly higher occurrence in females. Inflammatory changes due to parasitic exposure were a common histopathological finding in all individuals, regardless the gender. Multiple studies have investigated the relationship between gender and parasitic infection in small mammals (Krasnov et al., 2012; Morales-Montor et al., 2004; Neupane et al., 2020), however the outcome of these studies reveals a major influence of multiple factors such as seasonality (weather conditions), reproductive stage (hormones) beside parasite taxonomy and host species (Krasnov et al., 2012). Of the different parasitic taxa responsible for the lesions detected in this study, nematodes were dominant. This type of parasite has shown absence of gender bias in rodents such as the field mice (*Apodemus flavicollis*) (Milazzo et al., 2010) and the white-footed mouse (*Peromyscus leucopus*) (Luong et al., 2009). But, cases of gender bias also exist where male wood mice (*Apodemus sylvaticus*) (Behnke et al., 1999) had higher parasitic occurrence than females, while the opposite occurrs in female Cape ground squirrels (*Xerus inauris*) (Hillegass et al., 2008). These examples point out how female and male's helminth burden can vary among host species and subsequently the lesions caused by these parasites in both sexes. The relationship between infection and gender has also been studied for other aetiological agents such as bacteria and viruses in laboratory mice as well as humans, where cases of sexual dimorphism have been related to the characteristics of the infectious agents as well as the host's gender specific immune response (McClelland and Smith, 2011; Vázquez-Martínez et al., 2018). Although the histological study allowed an approximation of the identification of some parasite species (i.e., *Calodium hepaticum, Trichosomoides crassicauda*) overall, the identification of aetiological background of the histopathological lesions mostly remained unclear, preventing a proper evaluation of a possible gender bias for infectious agents (bacteria, viruses or parasites).

On the other hand, the higher rate of females 37% (53/144) showing renal perivascular inflammation in comparison to males 21% (35/167) could for example be due to a gender specific immune response. Assuming that this type of inflammation is caused by antigen persistence (Cianciolo et al., 2015), the higher susceptibility of females to inflammatory processes due to a stronger immune response could increase the inflammatory response in comparison to males (Klein and Flanagan, 2016). However, further parameters such as host reproductive state would be needed in order to evaluate the influence of sexual hormones in inflammatory processes.

The classification of the lesions in regard to the age revealed the presence of inflammation in animals from all age groups and splenic follicular hyperplasia only in the evaluated groups (subadults and adults). Although the insufficient number of young animals, prevented an accurate comparison between the different age groups, the fact that inflammation is affecting animals with different maturity grade indicates that in general pathogen exposure is independent of age. One of the main differences between young, i.e. immature and juvenile, animals and mature individuals (subadults and adults) is the sexual development. In contrast to young animals, sexually mature individuals are affected by sex hormones which can have physiological repercussions in both genders. In males, testosterone is known to possess immunosuppressive properties, meanwhile oestrogen in female has a pro-inflammatory effect (Vázquez-Martínez et al., 2018). However, changes related to sexual maturation are not the only aging factors involved in the differences of diseases between young and adult individuals, multiple age related aspects such as species specific characteristics, behaviour or immunological components can modify the occurrence of diseases throughout the animals' life (Lemaître et al., 2020; Tidière et al., 2020).

The histopathological study showed that all individuals, regardless of the species or habitat, are naturally exposed to pathogens that can lead to organ lesions. Because the habitat preference can have a significant effect on the presence of pathogens and associated diseases (Himsworth et al., 2013b), it is important to consider the distribution of the investigated species. In this study, the small mammals species dwelling in the forest are in principal distributed in a three-dimensional complex: terrestrial (*Maxomys* spp*., Sundamys muelleri),* above the ground (*Niviventer* spp*., Tupaia tana, Tupaia gracilis)* and arboreal (*Sundasciurus* sp*., Callosciurus* spp*., Leopoldamys sabanus* and *Tupaia minor*), but characterized by an overlap between the microhabitat layers (Emmons, 2000; Payn et al., 2016; Wells et al., 2006). Moreover, the majority of these species do not remain exclusively in their microhabitats, but are also found in other layers (Wells et al., 2004). This allows the presence of different species in the same microhabitat, increasing the possibility of a close contact between individuals. This proximity between individuals is a determinant for pathogen transmission by direct contact (i.e. respiratory pathogens) (Kling, 2011) or transmission

through fomites of contaminated surfaces with faeces containing parasites or urine contaminated for example with *Leptospira* spp. (Easterbrook et al., 2008).

In contrast to the small mammal species richness in the forest, the suburban and urban areas are principally inhabited by the terrestrial species: the generalist *Sundamys muelleri* and the commensal *Rattus* spp. and the shrew *Suncus murinus* (Syed-Arnez and Mohd Zain, 2006; Wells et al., 2014). Within the urban distribution, *Sundamys muelleri* and the shrew *Suncus murinus* are most likely to be found in vegetation areas close to houses such as gardens. Although *Rattus* spp. can also be found in vegetation areas, they predominantly remain in highly urbanized areas, where they have successfully adapted (Wells et al., 2014). The high density of *Rattus* spp. in the urban environment favours the transmission of endoparasites between the individuals of a colony (Abad et al., 2017; Kataranovski et al., 2011), which is higher than in the other animal groups.

Section 2: Health status

All histopathological findings from 331 individual small mammals were evaluated to estimate their possible impact on the general health status of each animal. This approach allowed assessing the influence of all degrees of subclinical diseases on the well-being of the different individuals and species.

Considering all evaluated animals, the majority (66%, 219/331) showed a moderate health status (scored 2-3), followed by 22% classified as clinically healthy (scored 0-1) and 12% (38/331) estimated as severely affected (scored 4-5). The presence of subclinical moderately affected animals from all ecological groups (forest species, the generalist *Sundamys muelleri* and urban rats (*Rattus* spp.) and shrews) indicated that regardless the habitat, all animals developed diseases, which are likely to have an impact on their health status. Most of the different species with mild disease had inflammatory pulmonary lesions. Among laboratory rodents, respiratory diseases have been described as the most common cause of health problems, usually detected only during necropsies (National Research Council, 1991). Generally, the aetiology of these respiratory diseases in the laboratory rats as well as in the wild rat *Rattus norvegicus*, are caused by a combination of pathogens including bacteria and viruses (i.e. *Mycoplasma pulmonis,* cilia-associated respiratory bacillus (CARB) or Sendai virus) (National Research Council, 1991; Rothenburger et al., 2015a). The results of the health status evaluation indicated that respiratory diseases, commonly occurring in laboratory rats and *R. norvegicus*, are also the most common health problem in free ranging Bornean small mammal species from this study.

From the forest dwelling species, treeshrews had a better health status with 50% (7/14) clinically healthy and 50% (7/14) mildly affected, in comparison to all other forest rodents,

where 15% (6/39) were healthy, 74% (29/39) mildly affected and 10% (4/39) were estimated clinically diseased. Considering the three-dimensional distribution of forest dwelling species, differences among the health status of the species inhabiting the different layers were observed. The terrestrial *Maxomys* spp. and the above the ground species (*Niviventer cremoriventer, Tupaia tana* and *Tupaia gracilis)* presented a more compromised health status in comparison to the arboreal species. From all forest species, mild clinical disease was found in *Maxomys* spp. 17/22, *Niviventer cremoriventer* 3/4*, Tupaia tana* 4/5 and a single *Tupaia gracilis.* In one *Niviventer cremeriventer* and three *Maxomys* spp overt clinical disease was assumed. In contrast, none of the arboreal species (squirrels and *T. minor*)*,* was classified as clinically diseased, and these included also more truly healthy individuals (*Callosciurus spp.* 2/14, *Sundascirus* spp. 2/11 and *Tupaia minor* 6/10). A possible explanation for this variation of the health status between the species inhabiting different forest strata could be the higher occurrence of pathogens that terrestrial species are exposed to in contrast to arboreal individuals. Animal species living in terrestrial habitats are in much closer contact to soil-borne pathogens including helminths, protozoa or bacteria, many of them being transmitted by fecal contamination and affecting the animals' health (Santamaría and Toranzos, 2003; Wall et al., 2015). The arboreal strata could therefore be considered as a healthier environment due to the lower chance of pathogen exposure and accumulation in comparison to the ground.

The generalist species *Sundamys muelleri*, characterized by a broad spectrum of habitat utilization (Wells et al., 2014), showed a similar health status to the forest rodents with 10% (7/72) healthy animals, 80% (58/72) mildly clinically affected and 10% (7/72) with assumed overt clinical disease. The ability of generalist species to live in quite different territories was focus of many studies, which considered generalist species as potential pathogen transmitter to other species occupying the same areas (Bordes et al., 2015; McFarlane et al., 2012). Although the lack of suitable samples prevented us to detect potential pathogens by culture or PCR, at least in the histopathological evaluation a lesion patterns indicating a possible pathogen transmission from *Sundamys muelleri* to other ecological animal groups was not recognized. However, to clarify whether *Sundamys muelleri* was responsible for the transmission of certain pathogens, this would need to be investigated with a more appropriate sample set.

Among the investigated species living in urban and peri-urban areas, there was a difference between the health status of the rodent *Rattus* spp. and the shrew *Suncus murinus*. While 38% (22/58) of the shrews had no lesions and only 2% (1/58) were estimated clinically diseased, shrews were healthier than urban rats, with 18% (26/148) of *Rattus* spp. estimated to be clinically diseased and lesser healthy individuals (21% (23/148)). This difference of health status can be an expression of characteristic behaviour and social structure of each species group. While urban rats (*Rattus norvegicus* and *Rattus rattus)* live in colonies, where

social interactions are very frequent (Inglis et al., 1996; Whishaw and Whishaw, 1996), *Suncus murinus* lives solitary and avoids encounters with conspecifics, which also prevents pathogen transmission (Burgin et al., 2017; Loehle, 1995). Among *Rattus* spp. wounds caused by aggressive behaviour are common (Whishaw and Whishaw, 1996), often heavily contaminated with secondary bacterial infection (Himsworth et al., 2014). Additionally, close intraspecific contact is a route of transmission for *Calodium hepaticum* (Rothenburger et al., 2014). Although in this study no urban rat had lesions suggestive for resulting in septicaemia, wounds could still increase the disease burden of the individuals with a significant negative effect on their health status (Rothenburger et al., 2019; Snyder et al., 2016). Another type of interactions commonly occurring among *Rattus* spp. individuals is non-aggressive social behaviour such as grooming and sniffing, which also lead to a close contact between individuals of the colony (Whishaw and Whishaw, 1996) contributing to pathogen transmission within members of the colony (Loehle, 1995). Moreover, the transmission of pathogens can also be influenced by interspecies characteristics and a close phylogenetic relationship between different species from the *Rattus* genus might increase the probability of sharing the same parasite species (Wells et al., 2015).

Although less social interactions can decrease the transmission of pathogens among shrews, the high proportion of individuals mildly clinically affected 60% (35/58) shows that they do contract subclinical diseases. Shrews are characterized by a very high metabolic rate and a lack of significant adipose tissue storage, which demands continuous feeding (Burgin et al., 2017); Suzuki et al., 2007). Energy deficiency in shrews can lead to immunodeficiency, increasing the risk of infection (Laakkonen et al., 1993). In this regard, the high number of mild neutrophilic pneumonia, found in 43/44 lungs available, could be a result of bacterial infection due to such immunosuppression.

Attending to the gender of all small mammals investigated, the results indicate that approximately 10% more males (26% (47/178)) than females (17% (25/149)) were scored as clinically healthy. In addition, there fewer males were categorised as mildly clinically affected (62% (110/178)) in comparison to females (72% (107/149)). However, amongst the animals assumed to suffer overt clinical disease both genders showed similar percentages with 11% (17/149) females and 12% (21/178) males. This last category can be considered as most indicative to understand whether and to what degree the health status of both genders is compromised. Although, both sexes showed similar rates of severely affected animals, the number of healthy males was higher than in females, indicating that females from this study were more susceptible to develop mild subclinical diseases. Each gender has intrinsic characteristics based on behavioural, immunological and physiological differences which can influence the development of diseases (Klein, 2000; Klein and Flanagan, 2016; Van Lieshout et al., 2020) and subsequently affect the health of the individuals. For example, sexually

mature males from the studied families (Sciuridae, Muridae, Tupaiidae and Soricidae) are in general more prone and exposed to aggressive interactions commonly related to mating competition in contrast to females, which are usually restricted to the protection of the litter (Emmons, 2000, Kawano, 1992; Steiner, 1972; Whishaw and Whishaw, 1996; Wolff, 1993). However, the development of diseases is not only limited to interactions than can increase the infection of the animals, but also to the immunological mechanisms responsible to fight these agents. It was shown that females have a stronger adaptative and innate immune response which can increase their susceptibility for inflammatory processes (Klein and Flanagan, 2016) Considering that the lesions observed in mildly affected animals were predominantly inflammatory, the difference between both sexes included in this category can be as a result of a higher immunological response in females.

Though animals evaluated as severely affected are most likely to have higher mordidity might earlier perish or become an easier prey for predators (Genovart et al., 2010) a mortality rate of the studied animals cannot be determined by the information obtained from the health status evaluation. From the different factors that can influence the lifespan of wild mammals, gender together with specific physiological characteristics, such as rate of aging or environmental conditions, have been described as the most relevant (Lemaître et al., 2020). In terms of mortality, aging is understood as a deterioration process related to the decline of the immune system efficiency against infectious agents during the animal's life due to an overactivation of the immune system by a high pathogen exposure (Lemaître et al., 2020; Tidière et al., 2020). Although the limited number of animals from each age group prevented an accurate comparison between age and health status, the presence of only two young individuals (2/23 juveniles) severely affected in contrast to higher number of severely affected mature animals (6/42 subadults and 29/250 adults) can be related to the aging process.

In general terms, pathogen exposure in mammals represents a risk to their health, and thus animals have developed behavioural strategies to reduce the pathogen burden (Hart and Hart, 2018). However, pathogen-host interactions are rather complex, and the outcome of infections is not exclusively linked with detrimental effects on the host´s health status (Sachs et al., 2011). A theory postulated by Strachan (1989), named 'The hygiene hypothesis', affirms that infections occurring during early life stages could decrease the negative consequences of diseases in adulthood by reinforcing the immunological protection. Moreover, this exposure can contribute to mitigate the effect of the aging process in mature animals (Van Lieshout et al., 2020). Based on these premises, it cannot be excluded that the mild subclinical changes detected in immature and juvenile individuals might have positive influence in the lifespan of these animals. Although, long-term monitoring of the animals as well as immunological analysis would be needed to prove the long-term consequences of

infection in the host, the information obtained from the evaluation of the subclinical lesions can help to predict the lifespan and mortality rate of these species.

Considering the predilection of predators for sick animals (Genovart et al., 2010) the 12% (38/331) of all individuals from this study with a severely compromised health status, would be a preferred prey for predators. Although many studies have attempted to elucidate an effect between pathogen transmission through trophic routes and disease occurrence, these investigations usually focus only in a single pathogen or mathematical model, but lack information on the health status (Agnihotri, 2012; Hall et al., 2007). Here we show that subclinical diseases of prey species can be substantial in some cases. A finding which should be considered in studies concerning disease dynamics, particularly as some affected prey species might also transmit pathogens to their predator and subsequently influence the predator's health. In contrast to larger animals where surveillance of their health status can be possible with classical monitoring techniques, to investigate diseases of small mammals is often complicated by the fact, that they fall prey to predator species and easily vanish, and therefore information on small mammals' health status is very limited (Delaney et al., 2018). The scoring of pathological lesions based on a numeric gradient was conducted in multiple histopathological studies (Eaton et al., 2007; Ikeno et al., 2009; Kleiner et al., 2005). In contrast to studies with absolute results, as for example PCR investigations with clear positive or negative results, histopathological assessments are qualitative and could vary between different pathologists' perspectives. For this reason, it can be beneficial to include more than one pathologist for certain assessments. Whereas the health status evaluation using histopathology allows a precise evaluation of the different organs, this method also has limitations. One of the main disadvantages is that it is rather time consuming, including the training of personnel to evaluate the tissues. But because so much additional information can be retrieved by histopathology, invasive studies requiring euthanasia of animals should be encouraged to attempt a multi-disciplinary approach, including histopathology, to maximize the information obtained from the investigated individual animals.

Section 3: Pathogen detection

Leptospira

Histologic Warthin-Starry staining allows direct detection of bacterial spirochetes in infected tissues with high specificity (Chappel et al., 1992). Reservoir hosts usually carry these bacteria in the extracellular matrix of the proximal renal tubules (Agudelo-Flórez et al., 2012; Athanazio et al., 2008; Hookey, 1991). In some histological kidney sections from this study, *Leptospira* spp. were also observed within the tubules' epithelial cells but likewise without associated pathological changes. In general, positive Warthin-Starry samples contained numerous bacteria but these were limited to a small number of tubules.

Warthin-Starry staining and molecular methods showed an overall occurrence of *Leptospira* spp. in 23% (71/314) of all investigated individuals and the presence of pathogenic *Leptospira* spp. was confirmed in all ecological animal groups: the forest individuals, the generalist *Sundamys muelleri* and the urban *Rattus* genus and the shrew *Suncus murinus.* In 39/208 individuals, with only ethanol-fixed kidney samples available for histopathological examination but not moleculr methods, *Leptospira* spp. were detected by Warthin-Starry in histological sections. Although it is not possible to differentiate pathogenic from non-pathogenic bacteria, Warthin-Starry staining is an efficient method for the detection of spirochetes (i.e. *Leptospira* spp.), but it has a lower sensitivity compared to molecular analyses (Rossetti et al., 2004). This might explain, why Warthin-Starry staining allowed the detection of *Leptospira* spp. only in 17 from 28/106 *Leptospira*-positive PCR samples. One can assume that the actual prevalence of *Leptospira* spp. could be higher than the actual 23% identified in the 314 animals in total, if tissue suitable for molecular analyses would have been available from all individuals.

The marginally higher occurrence of pathogenic *Leptospira* detected in males (29% (16/56)) in comparison to females. (24% (12/49)), could be considered in agreement with studies conducted in *Rattus* spp. from different locations such as Malaysia and Germany (Benacer et al., 2013; Mohamed-Ha et al., 2012; Pui et al., 2017). In these studies, the higher predominance in males' is hypothesized to be caused by male-to-male fights during mating season. However, other studies showed a different outcome, where *Leptospira* infection was predominant in females (Benacer et al., 2016) or both genders were equally infected (Benacer et al., 2013; Mayer-Scholl et al., 2014, Moreno et al., 2009). Considering the age group, age appears to be positively related with *Leptospira* infection in the host (Benacer et al., 2013), due to a broader mobility range together with social interactions occurring among adult individuals (Benacer et al., 2013, 2016; Mohamed-Hassan et al., 2010). Unfortunately, due to an insufficient number of young individuals for *Leptospira* detection, the comparison of pathogen infection between age groups from this study was not possible.

Overall, *Leptospira* spp. were mainly detected in urban rodents, such as *Rattus* spp. (33%, 47/143) followed by the generalist *Sundamys muelleri* (23%, 16/71). In contrast, only 1/49 *shrews, Suncus murinus,* and 7/51 individuals from the forest group (1/2 *Callosciurus prevostii*, 1/5 *Sundasciurus lowii*, 1/7 *Maxomys suriferi,* 3/5 *Maxomys whiteheadi,* 1/5 *Tupaia tana*) were positive for *Leptospira* spp. Interestingly, a recent publication with similar investigatory approach as this study, based on trapping rodents along a land-use gradient in Western Borneo, but investigations were performed a few years later between 2015 and 2016 (Blasdell et al., 2019). The authors also found a higher prevalence of *Leptospira* spp. in anthropogenic disturbed areas. Despite the challenging sample material available for our study, our results are supported by their findings: the continuous occurrence of *Leptospira*

spp. along land-use gradients with a higher predominance in urban environments. As the animals used in our study were caught between 2012 and 2013, this indicates that the occurrence of *Leptospira* spp. in urban environments has remained at a similar level over time. Although in the study conducted by Blasdell et al., forest rodents species such as the terrestrial *Maxomys* sp. were included, further small mammal species inhabiting higher layers of the forest are missing. We investigated forest small mammals inhabiting the three-dimensional space of the forest and detected *Leptospira* spp. in the arboreal squirrel *Sundasciurus lowii* and the treeshrew *Tupaia tana* dwelling above the ground, indicating that this bacteria is distributed amongst all the forest's microhabitat layers.

Sequencing results from PCR analyses confirmed the presence of *Leptospira interrogans* (23/28) and *Leptospira borgpetersenii* (2/28) as well as of a novel, so far unclassified *Leptospira* sp. in a single squirrel. In Southeast Asia the pathogenic *Leptospira* species most commonly found are *Leptospira interrogans* and *Leptospira borgpetersenii* (Benacer et al., 2016; Cosson et al., 2014). *Leptospira interrogans* is also a dominant *Leptospira* species in urban areas in various parts of the world (Dietrich et al., 2014; Himsworth et al., 2013a; Koizumi et al., 2019). As urban areas are usually occupied by species of the *Rattus* genus and occasionally by *Sundamys muelleri*, both can considerably contribute to the maintenance of leptospirosis in areas with inadequate sanitation (Azhari et al., 2018; Santos et al., 2017). Moreover, the generalist species *Sundamys muelleri* is considered a "bridge host" being capable of carrying and disseminating pathogens while moving along different land-use gradients (Bordes et al., 2015). This animal species might also aid in the dispersal of pathogens between invasive urban small mammals and native forest species. A surprising finding was the detection of a potentially novel *Leptospira* sp. in a single squirrel (*Sundasciurus lowii*). The short LipL32 gene sequence of 240 bp had 100% homology with unclassified *Leptospira* sequences from farmed pigs (*Sus scrofa*) in Tahiti (Guernier et al., 2017) and closely related to sequences from *Leptospira weilii* (99.58% sequence homology) and *Leptospira mayottensis* (≤ 98.74% sequence homology) taken from GenBank database entries.

Trypanosoma

Trypanosoma-DNA was detected in 8.6% (24/277) of liver samples of animals from all three ecological groups. *Trypanosoma lewisi* was most consistently identified in *Rattus norvegicus* (5/32), *Rattus* compl. (8/85) and *Sundamys muelleri* (1/66) with 99.81% to 100% sequence homology to the corresponding type strain (ATCC 30085). This *Trypanosoma* species seems to dominate in the *Rattus* genus from Asia (Milocco et al., 2013; Pumhom et al., 2015), where its occurrence is highly related to human settlements (Pumhom et al., 2015) due to the presence of domestic animals in the urban and peri-urban environment, which positively

influences the flea vector population (Rafinejad et al., 2013). Moreover, it was found that the flea burden carried by rodents inhabiting anthropogenic disturbed areas is higher than in forest rodents (Laakkonen et al., 2003).

In contrast to the urban and peri-urban small mammals, the forest group showed a very heterogeneous distribution of several potentially novel and so far, unclassified *Trypanosoma* spp. Five *Trypanosoma* sequences from different treeshrews and one sequence from *Rattus* compl. were closely related and differed only by a short hypervariable region of up to 65 bp length. The sequence from a *Maxomys surifer* matched to 99.81% with that of a *Trypanosoma* sp. from a sand fly from Thailand (acc. no. KJ467217, Phumee et al. unpublished). Whereas the *Trypanosoma* sequence from a *Maxomys whiteheadi* was 100% homologue to that from a squirrel (*Sundasciurus lowii*) and at the time showed only ≤ 95.50% sequence homology to any other *Trypanosoma* sequence from the GenBank database. The results suggest that native forest species in Borneo carry a high diversity of *Trypanosoma* spp., but their importance for animal or human health is uncertain. Molecular identification is still challenging and requires more suitable target genes for reliable taxonomic classifications (Mafie et al., 2019).

Considering the influence of gender in *Trypanosoma* infection in the host, previous studies showed discrepancies in their results. While male *Rattus norvegicus* from Brazil were predominantly infected (Linardi and Botelho, 2002), there was no gender bias in different *Rattus* spp. analysed from Southeast Asia and Egypt (Dahesh and Mikhail, 2016; Pumhom et al., 2014). The infection of vector borne pathogens is linked to the presence of ectoparasites responsible for their transmission, which is highly influenced by the behaviour of the host (Cozzarolo et al., 2019; Krasnov et al., 2012). For example exposure in females, in certain cases such as isolation during nesting incubation described in the bird great tit (*Parus major*) decreases the ectoparasite infection (Cozzarolo et al., 2019), whereas nursery colonies in multiple bat species increases ectoparasite burden (Christe et al., 2007). Although in this study the presence of *Trypanosoma* was slightly higher in females (10% (13/126)) than in males (7% (11/149)), the low number of animals from certain species prevented the identification of gender biased at the species level.

Another factor that can influence the infection of *Trypanosoma* is the age of the host, while young individuals of *Rattus* spp. are more susceptible for the infection, adults acquire certain immunological resistance for re-infection (Alias et al., 2014; Linardi and Botelho, 2002). Whereas the comparison of infection between the different age groups was not possible in this study, the presence of positive individuals from all age groups indicated the predisposition of the host for infection regardless the age.

Recent investigations based on a survey of *Trypanosoma* spp. among an extensive range of mammalian wildlife species in Zambia, Tanzania and Australia have shown a wide spectrum of species that can act as hosts for these protozoa (Anderson et al., 2011; Auty et al., 2012; Thompson et al., 2014). Regarding wild rodents, the majority of studies are conducted in a domestic environment focusing in the commensal *Rattus* spp. as the responsible vector for the transmission of zoonotic *Trypanosoma* spp. (Jansen et al., 2018; Kocher et al., 2015; Ortiz et al., 2018; Schwan et al., 2016). An investigation based on the detection of *Trypanosoma* spp. in native rodents and marsupials from different territories of Australia, revealed a high diversity of *Trypanosoma* species, highlighting the importance to investigate multiple host species from different geographic areas to understand the occurrence of *Trypanosoma* among wildlife populations (Averis et al., 2009).

Sarcocystis

The *Sarcocystis* spp. detected by histology in the striated muscle of forest dwelling individuals (forest rodents 10/38, treeshrews 11/14), as well as generalist species (*Sundamys muelleri* 37/70)) were morphologically evaluated for species identification. Overall, four different species were identified: 1.) *Sarcocystis singaporensis* in the forest rodent *Sundascuirus lowii* (2/4) and the generalist *Sundamys muelleri* (16/37), 2.) *Sarcocystis zuoi* in the forest rodent *Maxomys whiteheadi*. (1/2), 3.) the novel species *Sarcocystis scandentiborneensis* sp. nov. in the treeshrews *Tupaia minor* (6/7) and *T. tana* (1/4), and 4.) *Sarcocystis villivillosi* in (2/37) *Sundamys muelleri*.

Among all positive individuals (108/309), females and males showed similar rates of infection, 36% (51/142) and 34% (56/164) respectively, indicating that the presence of *Sarcocystis* was independent of sex. This finding is in agreement with previous studies, where the detection of *Sarcocystis* in different intermediate host species such as sheep, *Rattus* spp. and humans was not associated with the gender (Italiano et al., 2014; Mirzaei and Rezaei, 2016; Tung et al., 2009). Moreover, in *Rattus* spp. as well as in humans the infection has also been proved to be independent from age (Italiano et al., 2014; Paperna et al., 2004). Although in this study the majority of the infected animals were adult and subadult, the single presence of a positive juvenile individual and absence of immatures carrying *Sarcocystis* could be attributed to the low number of young animals of the samples set.

The species identification of *Sarcocystis* spp. can be based on the wall ultrastructure. Focussing on the wall structures, Dubey and co-authors created a classification, where the different *Sarcocystis* spp. are grouped into 42 types (Dubey et al., 2016). The distinct ultrastructural characteristics among the *Sarcocystis* spp. detected in this study here, allowed identifying and discerning the different species. While *S. singaporensis* showed long stalk-like

protrusions of with a narrowness at the base (Beaver and Maleckar, 1981), *S. zuoi* was characterized by longer and thicker finger-like protrusions, (Hu et al., 2012). In treeshrew species the intricate wall structure of the novel species contrasts with the basic wall structure of the only known *Sarcocystis* from treeshrews: *S. tupaia*, which is classified as Type 1a due to the primitive wall development consisting of minor undulations of the parasitophorous vacuole membrane. The novel *Sarcocystis scandentiborneensis* sp. nov. has a more complex wall, including the presence of microtubules in the villous protrusions and a characteristic electron-dense inverted U-shape structure on the tip.

These morphological differences were also notable between the sarcosporidia cysts detected in the host *Sundamys muelleri.* The short protrusions of *S. villivillosi* in addition to the characteristic radiating projections made it clearly distinguishable from *S. singaporensis* (Beaver and Maleckar, 1981). Although a species identification from the type 3 detected in the forest rodents (*Callosciurus notatus, Sundasciurus lowii* and *Niviventer cremoriventer*) was not possible due to autolysis, the presence of a different cyst type than *S. singaporensis* and *S.zuoi* indicated a high species variability among forest rodents.

Generally *Sarcocystis* spp. are considered specific for their intermediate host (Dubey et al., 2016), however, the *Sarcocystis* spp. involved in a snake–rodent life cycle such as *S. singaporensis*, *S. villivillosi* and *S. zoui*, are known to have a broader spectrum of intermediate hosts (Hu et al., 2012). A study conducted by Häfner about host specificity of *S. singaporensis* and *S. villivillosi* revealed that species closely related to *Rattus* spp. and *Bandicota* spp. can become a suitable intermediate host for their *Sarcocystis* spp. (Häfner and Frank, 1984). Nevertheless, in our study the detection of *S. singaporensis* in *Sundamys muelleri* and in the forest squirrel *Sundasciurus lowii*, as well as *S. villivillosi* in *Sundamys muelleri*, indicates that the range of species that can act as intermediate hosts is even wider and not restricted to closely related species.

In regard to the parasites' life-cycle, the reticulated python (*Malayopython reticulatus*) has been defined as definitive host for *S. singaporensis*, *S. villivillosi* as well as the king rat snake *(Elaphe carinata)* for *S. zuoi.* (Häfner and Frank, 1984; Hu et al., 2012; Jäkel et al., 1997; Odening, 1998). The reticulated python is widely distributed in South-East Asia, including the island of Borneo, occupying tropical and degraded habitats (Das, 2001; Kasterine et al., 2012). The presence of *S. singaporensis* and *S. villivillosi* in the generalist species *Sundamys muelleri* as well as in the forest squirrel *Sundasciurus lowii* infected by *S. singaporensis* suggests that the reticulated python can be found in very different areas in Borneo: from habitats being highly transformed by humans like urban areas (Kota Kinabalu) to periurban areas to the tropical forest. Although the king rat snake, the known final host of *S. zuoi*, does not live on Borneo, *Sarcocsytis* spp. can develop in closely related species to the definitive

host, enabling the sexual developmental stages of *S. zuoi* (Häfner and Frank, 1984), which could explain the occurrence of *S. zuoi* in *Maxomys whiteheadi*

The high numbers of occurrence of *S. singaporensis* was not surprising since other studies indicated that *S. singaporensis* is a dominant species among rodents in Southeast Asia (Jäkel et al., 1997; O'Donoghue et al., 1987). This species has also been described as highly pathogenic for small rodents when heavily parasitized (Zaman and Colley, 1975) thus, a few studies have focused on the use of *S. singaporensis* of as an effective biological control (Jäkel et al., 1996; Jäkel et al., 1999). Despite the fact, that the spectrum of species affected by *S. singaporensis* is more extensive in this study, we have no information about the possible negative consequences of the infection in the forest squirrel *Sundasciurus lowii* or the generalist *Sundamys muelleri*.

Further extended molecular analysis on the sequences from *Sarcocystis* isolated from infected forest rodents and *Sundamys muelleri* should verify the species identification and allow broader phylogenetic investigations. The phylogeny of these species would provide a better understanding of the relationship among *Sarcocystis* spp. as well as between the parasites and their host species and thus helping to understand their interactions (Morrison et al., 2004).

Herpesvirus

Herpesvirus positive animals were identified in all ecological groups: 10/30 forest dwelling individuals (2/5 *Callosciurus* spp., 3/8 *Maxomys* spp. and 1/3 *Niviventer* sp. and 4/8 *Tupaia minor*), 15/41 generalist species (*Sundamys muelleri*), the urban *Rattus* genus (16/40 *Rattus* compl., 4/17 *Rattus norvegicus*) and one shrew (1/26, *Suncus murinus*).

The broad presence of 17 different herpesviruses detected in 10 different animal species was not unexpected, as previous studies from other geographical regions on various host species including livestock, bats, rodents and invertebrates discovered a high occurrence of, partly novel, herpesviruses (Ehlers et al., 2008, 2007; Smits et al., 2013; Sotomayor-Bonilla et al., 2019; Wibbelt et al., 2007). In regard to the ecological background, of the eight different herpesviruses detected in six forest-dwelling animal species (n=10) one herpesvirus (Rrat_RHV1) was shared with urban rats. While of the four different herpesviruses contained in the generalist *Sundamys muelleri* (n=15) three (Rtiom_RHV1+2 and Rrat_RHV3) were shared with herpesviruses from urban species. But 5/9 different herpesviruses carried by three urban small mammal species (*Rattus complex*, *R. norvegicus*, *Suncus murinus*; n= 21) were exclusively found in urban areas. This outcome supports the hypothesis that tropical rainforests harbouring a species-rich fauna can also be the source of a high number of novel

viruses (Murray and Daszak, 2014; Nakagawa et al., 2006).

Since the majority of the detected herpesviruses were gammaherpesviruses, which are most likely transmitted by close contact (Wald and Corey, 2019), the host's intra- and interspecific behaviour should be taken into account as it can influence virus transmission. A recent study on wild wood mice (*Apodemus sylvaticus*) found that the males' pre-mating scent marking is a dominant driver for the transmission of murine gammaherpesviruses within their population (Erazo et al., 2021). However, as multiple species with different behaviour were investigated in this study, the occurrence of herpesvirus infection based on the gender varies among the different species.

The gender-associated difference in viral susceptibility has been strongly linked with sex hormones and their positive influence in females' immune adaptative response in contrast to the detrimental effect of testosterone in males' immune system (Klein and Flanagan, 2016). However, the results of this study contrast with this hypothesis, being the number of females positive to herpesvirus (33% (23/68)) higher than in males (27% (23/86). Although the immune response can play a fundamental role in gender biased infection, there are other factors such as host behaviour or the route of infection that can influence the gender difference of infection (Glass et al., 1998, Mora et al., 2015, Yaremych et al., 2004). Considering that these results included multiple host species, in order to understand the possible gender biased in herpesvirus further analysis should be conducted in a sufficient number of individuals per species.

Animals infected by gamma- or betaherpesviruses show a diverse clinical manifestation from asymptomatic (e.g. betaherpesvirus simiae in capuchin monkeys (*Cebus apella*)) (Coulibaly et al., 2004), subclinical (e.g. bovine gammaherpesvirus 4 responsible for subclinical mastitis in bovine) (Ongradi, 2016) to highly pathogenic (e.g. ovine gammaherpesvirus 2 causing malignant catarrhal fever in bovids) (Russell et al., 2009). Among rodents the betaherpesvirus cytomegalovirus causes an inflammation of the salivary glands and the development of intranuclear inclusion bodies in laboratory and wild rats (Percy and Barthold, 2007; Priscott and Tyrrell, 1982). Unfortunately, salivary glands were not sampled for this study therefore such lesions could not be confirmed for animals indicating to carry Rat cytomegalovirus, Rattus tiomanicus cytomegalovirus 1 or Cytomegalovirus Guenon. The higher presence of gammaherpesviruses in comparison to betaherpesviruses could either be a consequence of the lower prevalence of betaherpesviruses in general or could be due to the detection method as the sensitivity of the generic PCR might vary between different herpesviruses subfamilies. Also, the tissue tropism of the different viruses could influence the results as, for example, betaherpesviruses are considered lympho-tropic. But as the number of available spleen samples in this study was distinctly lower than liver samples this could have influenced the detection rate of betaherpesviruses. This might have also been the reason why we found no

evidence for herpesvirus co-infections. The absence of alphaherpesviruses in our study seems to be in accordance to the literature as the *Rodentia* seem to lack this herpesvirus subfamily (Ehlers et al., 2007, ICTV Master species list, 2012).

Overall pathogen detection and health implications

The results from the different pathogens detected in this study (*Leptospira* spp., *Trypanosoma* spp., *Sarcocystis* spp. and herpesvirus) revealed a higher pathogen variability in forest areas based on the high diversity of small mammal species. In contrast, commensal species living in urban and peri-urban areas were usually infected by one or few dominant pathogen species. These findings are in agreement with previous studies which have investigated the relationship between host and pathogen diversity (vector-borne and non-vector-borne pathogens) in different anthropogenic disturbed environments (Civitello et al., 2015; Keesing et al., 2010, 2006). In general terms, pathogen occurrence and diversity are linked to characteristics of the host, the pathogen and additional hosts or vectors involved in the transmission (Keesing et al., 2006; Rohr et al., 2015). Environmental modifications can significantly influence the presence of a successfully adapted dominant host (i.e. commensal species in urban areas) by decreasing the presence of other host species (Wells et al., 2014), which subsequently can result in a modification of pathogen diversity (Keesing et al., 2010). One of the main theories related to pathogen-host diversity, the dilution effect, is based on the premisses that the presence of a dominant pathogen species, transmitted by a dominant efficient host, can be reduced by communities composed by a high diversity of less or non-efficient hosts (Johnson and Thieltges, 2010; Keesing et al., 2010; Ostfeld and Keesing, 2012). Although, to fully understand this effect in pathogen diversity, environmental factors such as temperature and humidity should be considered (Johnson and Thieltges, 2010). Further, the reduced presence of the dominant host (*Rattus* spp.) in forest areas together with the variety of small mammal species, could decreased the transmission of pathogen species commonly found in urban areas. This event becomes especially relevant in the transmission of zoonotic pathogens, where the reduction of host biodiversity can increase the risk of infection from directly transmitted pathogens such as *Leptospira* sp. and Hantavirus, as well as vector-dependant pathogens like *Borrelia burgdorferi*, responsible for Lyme disease (Allan et al., 2003; Mills, 2006; Ostfeld and Keesing, 2000).

From the pathogens investigated in this study, pathogenic *Leptospira* spp. are the most relevant in terms of zoonotic potential. The route of transmission is based on direct contact with contaminated water, food or surfaces and together with the capacity to infect any mammalian species this makes them one of the most important pathogens regarding global health (Karpagam and Ganesh, 2020). Although *Leptospira spp.* have been traditionally linked to areas close to water, they have also been detected in low humid environments, indicating

an even broader range of distribution (Cosson et al., 2014). Their environmental survival characteristics together with the detection of a seemingly unknown pathogenic *Leptospira spp.* from the arboreal dwelling squirrel *Sundasciurus lowii* could indicate an increasing risk of infection of mammals inhabiting different forest layers. However, considering the different habitats, in terms of zoonosis urban areas represent a higher risk than forest territories, due to the elevated concentration of reservoir species (i.e. commensal *Rattus* spp.) living in proximity to humans (Costa et al., 2015; Pépin, 2016). The urine marking behaviour described in *Rattus norvegicus* as a communication tool, characterized by excreting multiple small drops of urine while walking (Grant, 1963), can significantly contribute to the contamination of the environment and the dispersal of *Leptospira* spp.. This fact together with the high floatability of *Leptospira* spp. (Thibeaux et al., 2017) can increase their concentration on water surfaces during flood events as well as in peri-urban rivers and lakes (Bierque et al., 2020). The role of water as vehicle for *Leptospira* spp. transmission has been linked to outbreaks occurring in tropical regions during floods or water recreational activities such as the one reported in Sabah in 1999 affecting 46 persons after swimming in a creek (Karpagam and Ganesh, 2020; Koay et al., 2004).

The results obtained from investigatingthe protozoa *Trypanosoma* spp. and *Sarcocystis* spp. did not reveal the presence of a zoonotic species. However, although *Trypanosoma lewisi* was formerly considered non-pathogenic, multiple cases of human trypanosomiasis pointed to this species as a potential pathogen for humans and there seems to be a distinct relevance of rodents in the transmission (Lun et al., 2015; Pumhom et al., 2014). The high specificity between *Trypanosoma lewisi* and their host (*Rattus* spp.), usually prevents the natural infection of other animals including humans, however under certain circumstances involving an environment with high numbers of flees infected by *Trypanosoma lewisi* and an immunosuppression in the prospective host, the susceptibility for infection can be increased (Sarataphan et al., 2007; Truc et al., 2013; Wyatt et al., 2008). In contrast, the cases of human sarcocystiosis do not involve small mammals in the transmission but domestic species of cattle (*Sarcocystis hominis*) and swine (*Sarcocystis suihominis*) as well as wild reptiles (*Sarcocystis nesbitti*) (Fayer et al., 2015). Despite there is a reduced number of *Sarcocystis* species with zoonotic potential, the capacity to infect humans and the pathogenicity of multiple species is still unknown and would require further investigation (Dubey et al., 2016).

In terms of human health, multiple small mammal species inhabiting Southeast Asia carry relevant RNA viruses from the families *Arenaviridae, Hantaviridae, Flaviviridae, Picornaviridae* and *Reoviridae* which can lead to serious diseases in humans (Wu et al., 2020). The high number of potential host species included in this study in addition to the importance of synanthropic species in the transmission of these pathogens, would encourage the detection of these viruses from the studied individuals. Unfortunately, the poor quality of the samples

prevented the evaluation of RNA viruses, restricting investigations to DNA viruses like herpesviruses. The high specificity of herpesviruses to their host decreases their capacity to infect other taxa including humans, where cases of zoonosis have been reported as an unusual spill-over event (Tischer and Osterrieder, 2010b). Moreover, the absence of alphaherpesviruses from the detected sequences also reduces the zoonotic potential of herpesvirus since, and only under laboratory conditions certain species of this subfamily such as equid herpesvirus 1 (EHV-1) could infect human cells (Trapp et al., 2005).

Section 4: General discussion

In this study 331 small mammals of 15 different species were investigated, aiming to elucidate whether the health status of these animals or the occurrence of selected pathogens would differ along a land-use gradient. However, to understand possible interactions between small mammal species that could lead to pathogen transmission between them, it is important to determine their distribution pattern in the different habitats (Keesing et al., 2010). The information of the habitat utilization of these species from the forest environment to anthropogenic modified urban and suburban territory, allowed classifying these species into three larger groups (Wells et al., 2014): 1. Forest dwelling species: forest rodents (rats and squirrels) and treeshrews occupying all three layers of the forest, i.e. terrestrial, above the ground and arboreal. 2. Generalist terrestrial *Sundamys muelleri*: inhabiting areas from forest to urban environment. 3. Urban terrestrial species: commensal *Rattus* genus and the shrew *Suncus murinus*. Because pathogen transmission can be influenced by the habitat as well as the species' interactions (Bordes et al., 2015; Himsworth et al., 2013b) it is important to consider ecological background information in order to understand the occurrence of diseases among species inhabiting the same as well as neighbouring environments. Moreover, intraspecies features such as behaviour and social structure can also have an effect on disease occurrence and therefore on the animals' health status (Loehle, 1995). Thus, the evaluation of the health status and the detection of selected pathogens were considered at the species level and as well as at the habitat preference.

The evaluation of subclinical histopathological changes showed that the most common lesions among all species were of endoparasitic origin, followed by inflammatory processes in the lungs. Besides the organ lesions, the histopathological examination also allowed to investigate species specific histological features (e.g. dense layer of bacterial colonies in the stomach of *Maxomys* spp.). Since the knowledge of physiology and disease occurrence in small mammals is mostly limited to laboratory animals (Delaney et al., 2018; Hong et al., 2017; Smith et al., 2001), the pathological examination of free ranging small mammal species conducted in this study contributes to elucidate their physiologic features as well as some pathogens these species are naturally exposed to and the lesions they cause.

All histopathological information was used to estimate the health status of the individuals and to detect possible differences between animal species as well as between forest, peri-urban and urban habitats. In general, the health status evaluation revealed that truly healthy animals without pathological lesions were a rare finding in all groups. Interestingly, previously published histopathological surveys from Canada and Chile, although limited to wild *Rattus* spp. only, had similar results (Rothenburger et al., 2019; Seguel et al., 2017). It is known that the occurrence of multiple pathogens among animal populations is a common event (Cox, 2001). Yet, little is known about the repercussions of these pathogens in the animal's health (Vaumourin et al., 2015). When comparing the detected pathological changes between the three health status categories, many of the parasitic lesions, although differing in degree and extend, are indicative of the same or at least similar aetiology. This highlights that pathogen loads, and their impact in the host vary among different individuals.

The assessment of the health status in multiple species from different ecological groups, allowed evaluating the possible effect of the habitat on the health of the species. The lack of significant variations of the health status between the ecological groups, suggests that the habitat did not markedly influences the health status. On the other hand, the difference of the health status between species living in the same habitat indicates that intraspecies characteristics are most likely to be responsible for the health status. Amongst the species inhabiting the forest, the arboreal species (squirrels *Callosciurus* spp., and *Sundasciurus* spp., and the treeshrew *T. minor*) had a better health status with no clinically affected animals than species living terrestrial and above the ground (*Maxomys* spp, *Niviventer* sp, *T. tana* and *T. gracilis*). From the generalist species, the health status of *Sundamys muelleri* showed a similar number of clinically affected individuals 10% (7/72) as forest rodents and overall the highest rate of mildly clinically affected individuals with 80% (58/72). The ability of *Sundamys muelleri* to live in a broad spectrum of territories from forest to urban habitat (Wells et al., 2014) raises at the same time the possibility of pathogen transmission due to increased encounters with other species (Bordes et al., 2015). Unfortunately, the effect of such interactions on this species could not be revealed by histopathological and health status results.

For the urban species, behaviour and social interactions could be the main reason for the health status level of *Rattus* spp. and *Suncus murinus*. The aggressive behaviour and high populated colony structure of *Rattus* spp. (Whishaw and Whishaw, 1996) can favour a pathogen transmission (Abad A. et al., 2017; Himsworth et al., 2014), which could lead to the increment of clinically affected individuals to 18%. In contrast, the solitary behaviour of *Suncus murinus* (Burgin et al., 2017) might help to avoid pathogen transmission and results in a low number of individuals estimated to be clinically affected.

Although most of the observed inflammatory organ lesions were caused by pathogens, some cases of infections without inflammatory pathogen-host interactions were detected. Evidence of this concept was found at the histological examination of kidneys of individuals infected by *Leptospira* spp., which showed multiple large bacterial spirochete clusters in the tubular epithelium, but without causing pathological lesions. Another example of innocuous infection by a pathogen was observed during the histopathological examination of intramuscular sarcocystiosis. The parasitic cysts detected in the infected tissue contained cystozoites, confirming the mature stage of the asexual phase of *Sarcocystis*. The infection of mature cysts together with the absence of inflammation (host-response) confirmed that the infected small mammal species serve as intermediate reservoir hosts in *Sarcocysts* life cycles. Because the detection of a pathogen alone without a histological reference to its impact on the host cannot reflect the pathogen-host interaction or the health status of an individual, it is vital to include the histopathological evaluation in order to elucidate the severity of the impact on the host by determining the intensity and chronicity of the host's response at cellular level (Carnegie et al., 2016).

On the other hand, the development of molecular techniques, especially the polymerase chain reaction, has significantly optimized the detection of pathogens (Yang and Rothman, 2004), providing crucial information on the prevalence of pathogens occurring in a wide variety of host species around the world (Mühldorfer, 2013; Tokarz et al., 2016). In this study this technique was used to investigate the occurrence of *Leptospira* spp., *Trypanosoma* spp. and herpesviruses, as well as the phylogenetic comparison of *Sarcocystis*, in small mammal species living along a land-use gradient. The molecular results showed that *Leptospira* spp., *Trypanosoma* spp. and herpesviruses were detected in all habitats, with a higher occurrence of *Leptospira* spp. and *Trypanosoma* spp. in the urban environment, especially among *Rattus* spp. but with a lower diversity of these pathogens compared to the forest. The higher occurrence can be explain by the *Rattus* spp. exceptional adaptation skills to anthropogenic disturbed environments which can significantly increase their population numbers in limited areas (Feng and Himsworth, 2014). Highly dense *Rattus* spp. colonies lead to aggressive behaviour between conspecifics resulting in wounds, which can also facilitate the infection with *Leptospira* spp. (Andersen-Ranberg et al., 2016; Parker and Nilon, 2012). Moreover, close contact between individuals increases the risk of flee-bites thus enhancing in the transmission of vector-borne *Trypanosoma* spp. (Pereira et al., 2017). Although information obtained on herpesviruses was only indicative of the respective species, the identification of a high diversity of *Leptospira* spp. and *Trypanosoma* spp. as well as novel sequences in the forest habitat suggests high pathogen diversity in the tropical forest of Northern Borneo.

The ultrastructural evaluation of the *Sarcocystis* spp. detected in forest species and the generalist *Sundamys muelleri* allowed a precise species identification and revealed a high

diversity of *Sarcocystis* spp. This method also provided information on the developmental stages of the cysts in the host and to define whether the infected animal species is a true intermediate host or a paratenic host. In this regard, the morphological evaluation of *S. scandentiborneensis* sp. nov. at the ultrastructural level allowed not only the taxonomic classification, but also revealed the role of treeshrews as intermediate hosts for this pathogen. The morphological information combined with a phylogenetic evaluation elucidated the relationship between these host species and their possible predators as potential final hosts in the *S. scandentiborneensis* life cycle.

A study like this on a large number of different wildlife small mammal species captured along a land-use gradient from forests to anthropogenic disturbed environments implements a multidisciplinary approach, which ranges from ecological information of the host species to histopathological investigations and the assessment of the health status of the animals and to the search for selected relevant pathogens has rarely been conducted. But it demonstrates the power the different disciplines can achieve together if they are combined in such holistic manner.

7 SUMMARY

Disease occurrence in small mammals from Northern Borneo: Histopathology and selected pathogens

Small mammal species have been the study object for various scientific disciplines because their adaptation skills into different habitats, the basic position they occupy in the trophic pyramid and the role of multiple of these species as host and reservoir for parasites and zoonotic pathogens. From a veterinary point of view, there is a lack of information on the diseases of wild small mammal species and the pathogens they might carry, especially in degraded and anthropogenically modified environments, which could have a negative effect on the animal's health. For this reason, this project investigated 15 different small mammal species and a total number of 346 individuals captured between 2012 and 2013 along a land-use gradient in Northern Borneo from the tropical rainforest to the urban area of Kota Kinabalu. The animals belonged to the families Muridae, Sciuridae, Soricidae and Tupaiidae and were classified according to their habitat preference into forest species, i.e. forest rodents and treeshrews, the generalist *Sundamys muelleri* found along all habitat gradient and urban species as *Rattus* spp. and the shrew *Suncus murinus*. After capture animals were immediately euthanized, dissected and tissue samples were collected. Subsequently all animals were examined for subclinical histopathological changes followed by a scoring of their lesions to estimate the individuals' health status. Additionally, molecular analyses and transmission electron microscopy were conducted to identify selected pathogens.

The histopathological assessment showed that endoparasitic lesions were the most prevailing finding. Comparing the occurrence of pathological changes of the different organs the lung was predominantly affected with 89% (277/310), follow by lesions in liver (60% (192/321)), gastrointestinal tract (38% (102/266)) and striated muscle (sarcosporidiasis) (35% (108/309)). In all animals, the organs affected to a lesser degree were kidney and heart in 28% (89/314) and 19% (60/324), respectively. One the more notable findings were dense bacterial layers on the mucosal surface of the stomachs in the forest rodents *Maxomys* spp. and *Niviventer* sp., which so far was only described in laboratory rodents. Further, the first time a *Klossiella*-like infection in the kidneys of two *Sundascirurus lowii* squirrels was described. As the majority of pathological changes were observed in individuals from all species, the exposure to pathogens seems to occur regardless their chosen habitat. However, although lesions with parasitic origin were detected in forest species as well as in the generalist *Sundamys muelleri*, they were more predominant in urban individuals from the *Rattus* genus. Possible reasons could be the high density and the colony social structure, which favour parasite occurrence and transmission.

The evaluation and scoring of the health status, based on the histopathological findings showed that the majority of the individuals were estimated to be moderately affected 66% (219/331) by their lesions, while 22% (74/331) had no or only mild lesions and were classified as clinically healthy. In contrast to this, only 12% (38/331) of all individuals were assumed to have a severely affected health status. Comparing the ecological groups, the health status was similar, however, there were distinct differences of the health status between certain species living in the same habitat. Among forest species, treeshrews were healthier than forest rodents, which health status was similar to the generalist species *Sundamys muelleri.* Amongst urban species, the shrew *Suncus murinus* presented a better health status than individuals from the *Rattus* genus. These findings suggest that in general the habitat has not a significant influence of the health status. But intraspecific characteristics based on the behaviour, predation pressure and/or social structure may have an impact in the health of the species.

Species living in urban areas, mainly *Rattus* species, as well as the generalist *Sundamys muelleri*, were predominantly infected with *Leptospira interrogans*. While in contrast to this, infections of forest species showed a higher diversity of the detected *Leptospira* spp. DNA of the blood parasite *Trypanosoma* spp. was found in approximately 9% (24/277) of all investigated individuals. Amongst them, the potentially zoonotic *Trypanosoma lewisi* was predominantly found in *Sundamys muelleri* and urban *Rattus* species, meanwhile forest individuals were infected with either novel or unclassified *Trypanosoma* species. Histopathological investigations revealed that 30% (108/309) of all animals were infected by *Sarcocystis*. Further examination of the ultrastructural features of the *Sarcocystis* walls revealed the presence of three different *Sarcocystis* species (*S. singaporensis*, *S. zuoi* and *S. villivillosi*) in the forest species *Sundascuirus lowii* and *Maxomys whiteheadi,* and the generalist *Sundamys muelleri*. Additionally, a novel species, *Sarcocystis scandentiborneensis* sp. nov., was detected in treeshrews, corroborated by unique ultrastructural wall characteristics and phylogenetic evaluations. The morphological evaluation of *Sarcocystis* spp. showed a higher diversity among forest species and *Sundamys muelleri*, supporting that a broad range of small mammal species are intermediate hosts for the *Sarcocystis* spp. The occurrence of herpesvirus DNA was similar to *Sarocystis* spp. (29% (46/156) of all individuals), and although due to rather short fragments of herpesvirus DNA strain identification was not possible, sequence results indicated a wide diversity due to marked genetic heterogeneity of herpesviruses among animals from all habitats.

For the first time this study investigated multiple small mammal species trapped along a land-use gradient not only by molecular techniques, but by histopathology. This allowed to not only detect the presence of a given pathogen, but to get an estimate of the individuals' health status. Moreover, it lead to the identification of a novel parasite species (*S.*

scandentiborneensis sp. nov.). Such investigations are only possible with an interdisciplinary approach, but to be able to better understand the interconnectivity of multiple animal species and pathogens in an ecosystem such approaches are indispensable.

8 ZUSAMMENFASSUNG

Vorkommen von Krankheiten kleiner Säugetiere in Nord-Borneo: Histopathologie und ausgewählte Pathogene

Kleinsäugerarten sind aufgrund ihrer Anpassungsfähigkeit an verschiedene Lebensräume, der Position, die sie in der trophischen Pyramide einnehmen und der Rolle als Wirt und Reservoir für Parasiten und zoonotische Krankheitserreger das Untersuchungsobjekt für verschiedene wissenschaftliche Disziplinen. Aus veterinärmedizinischer Sicht mangelt es aber an Informationen über die Krankheiten wildlebender Kleinsäugerarten und die Krankheitserreger, die sie vor allem in degradierten und anthropogen veränderten Milieus ausgesetzt sind und die sich negativ auf den Gesundheitszustand der Tiere auswirken könnte. Das hier beschriebene Projekt untersuchte 15 verschiedene Kleinsäugerarten mit einer Gesamtzahl von 346 Individuen, die zwischen 2012 und 2013 im Norden Borneos entlang eines Landnutzungsgradienten vom tropischen Regenwald bis zum Stadtgebiet von Kota Kinabalu gefangen, euthanasiert und seziert wurden. Die Tiere gehörten zu den Familien Muridae, Sciuridae, Soricidae und Tupaiidae und wurden entsprechend ihrer Habitatpräferenz klassifiziert in Waldtierarten, darunter Waldnagetiere und Spitzhörnchen (*Tupaia*), den Generalisten *Sundamys muelleri,* der entlang des gesamten Habitatgradienten gefunden wurde, sowie urbane Tierarten wie Rattenarten (*Rattus* spp.) und Spitzmäuse (*Suncus murinus*). Nach dem Fang wurden die Tiere sofort euthanasiert, seziert und Gewebeproben entnommen. Alle Tiere wurden histopathologisch auf subklinische Veränderungen untersucht, gefolgt von einem Scoring der in einem Tier gefundenen Läsionen, um den Gesundheitszustandes der einzelnen Individuen abschätzen zu können. Zusätzlich wurden molekulare Analysen und Transmissionselektronenmikroskopie durchgeführt, um ausgewählte Krankheitserreger identifizieren zu können.

Die histopathologische Beurteilung zeigte, dass endoparasitäre Läsionen der häufigste Befund waren. Vergleicht man das Auftreten pathologischer Veränderungen der verschiedenen Organe, so war die Lunge mit 89% (277/310) überwiegend betroffen, gefolgt von Läsionen in der Leber (60% (192/321)), im Magen-Darm-Trakt (38% (102/266)) und der muskulären Sarcosporidiasis (35% (108/309)). Bei allen Tieren waren Niere und Herz in 28% (89/314) bzw. 19% (60/324) der Organe in geringerem Maße betroffen. Die bemerkenswertesten Befunde waren die dichten Bakterienrasen auf der Schleimhautoberfläche der Mägen der Waldnager *Maxomys* spp. und *Niviventer* sp., die bisher nur bei Labornagern beschrieben wurden, und ferner die erstmalige Beschreibung von *Klossiella*-ähnlichen Infektionen in den Nieren von zwei *Sundascirurus lowii*-Eichhörnchen. Da generell pathologische Veränderungen bei Individuen aller Arten beobachtet wurden, scheint

eine Erregerexposition unabhängig vom gewählten Habitat stattzufinden. Läsionen mit parasitärem Ursprung wurden zwar sowohl bei Waldtierarten als auch beim Generalisten *Sundamys muelleri* festgestellt, doch waren sie bei urbanen Individuen der Gattung *Rattus* deutlich stärker ausgeprägt. Mögliche Gründe könnten die hohe Dichte und die Sozialstruktur der Rattenkolonien sein, die das Vorkommen und die Übertragung von Parasiten begünstigen.

Die Bewertung und Einstufung des Gesundheitszustands auf Grundlage der histopathologischen Befunde ergab, dass die Mehrheit der Individuen mit 66% (219/331) als mäßig von ihren Läsionen betroffen eingeschätztz wurde, während 22% (74/331) keine oder nur leichte Läsionen aufwiesen und als klinisch gesund eingestuft wurden. Im Gegensatz dazu wurde bei 12% (38/331) aller Individuen von einem stark beeinträchtigten Gesundheitszustand ausgegangen. Beim Vergleich der ökologischen Gruppen miteinander war der Gesundheitszustand ähnlich, jedoch gab es deutliche Unterschiede im Gesundheitszustand zwischen bestimmten Arten, die im gleichen Lebensraum leben. Unter den Waldtierarten waren *Tupaia* gesünder als Nagetiere, letztere wiesen einen ähnlichen Gesundheitszustand auf wie die generalistische Art *Sundamys muelleri*. Bei den uranen Arten zeigte die Spitzmaus *Suncus murinus* einen besseren Gesundheitszustand auf als Individuen der Gattung *Rattus*. Diese Ergebnisse deuten darauf hin, dass der Lebensraum im Allgemeinen keinen signifikanten Einfluss auf den Gesundheitszustand hat. Aber intraspezifische Merkmale, die auf dem Verhalten, dem Prädationsdruck und/oder der Sozialstruktur beruhen, können einen Einfluss auf die Gesundheit der Art haben.

Kleinsäugerarten, die in städtischen Gebieten leben, hauptsächlich *Rattus* spp., sowie der Generalist *Sundamys muelleri*, waren überwiegend mit *Leptospira interrogans* infiziert. Im Gegensatz dazu zeigten Infektionen von Waldtierarten eine höhere Diversität der nachgewiesenen *Leptospira* spp. Die DNA des Blutparasiten *Trypanosoma* spp. wurde bei etwa 9% (23/277) aller untersuchten Individuen gefunden. Darunter wurde auch die potentiell zoonotischen *Trypanosoma lewisi* gefunden, vorwiegend in *Sundamys muelleri* und urbanen *Rattus*-Arten, während waldbewohnende Kleinsäugerarten entweder mit neuartigen oder nicht klassifizierten *Trypanosoma*-Arten infiziert waren. Herpesvirus-DNA wurde bei 29% (46/156) aller Individuen nachgewiesen, und obwohl aufgrund der relativ kurzen Fragmente der Herpesvirus-DNA keine Identifizierung des Stammes möglich war, zeigten die Sequenzergebnisse aufgrund der ausgeprägten genetischen Heterogenität der Herpesviren bei Tieren aus allen Lebensräumen eine große Diversität.

Histopathologische Untersuchungen ergaben, dass 30 % (108/309) aller Tiere mit *Sarcocystis* spp. infiziert waren. Die Untersuchung ultrastruktureller Merkmale der Sarcocystis-Wände ergab das Vorhandensein von drei verschiedenen Sarcocystis-Arten (*S. singaporensis*, *S. zuoi* und *S. villivillosi*) bei den Waldarten *Sundascuirus lowii* und *Maxomys whiteheadi* sowie

dem Generalisten *Sundamys muelleri*. Zusätzlich wurde eine neue Spezies, *Sarcocystis scandentiborneensis* sp. nov., in *Tupaia* nachgewiesen, was durch einzigartige ultrastrukturelle Wandmerkmale und phylogenetische Auswertungen bestätigt wurde. Die morphologische Auswertung von *Sarcocystis* spp. zeigte eine höhere Diversität unter Waldtierarten und *Sundamys muelleri*, was dafür spricht, dass eine breite Palette kleiner Säugetierarten als Zwischenwirte für die *Sarcocystis* spp. fungiert. Das Auftreten von Herpesvirus-DNA war ähnlich wie bei *Sarocystis* spp. (29 % (46/156) aller Individuen), und obwohl aufgrund der eher kurzen Fragmente der Herpesvirus-DNA eine Stammidentifizierung nicht möglich war, deuteten die Sequenzergebnisse auf eine große Diversität aufgrund einer ausgeprägten genetischen Heterogenität der Herpesviren bei Tieren aus allen Habitaten hin.

Zum ersten Mal wurden in dieser Studie nicht nur durch molekulare Techniken, sondern auch durch Histopathologie zahlreiche Kleinsäugerarten untersucht, die entlang eines Landnutzungsgradienten gefangen wurden. Dies ermöglichte nicht nur den Nachweis des Vorhandenseins eines bestimmten Erregers, sondern auch eine Einschätzung des Gesundheitszustandes der Individuen. Darüber hinaus führte es zur Identifizierung einer neuen Parasitenart (*S. scandentiborneensis* sp. nov.). Solche Untersuchungen sind nur mit einem interdisziplinären Ansatz möglich, aber um die Verflechtung mehrerer Tierarten und Erreger in einem Ökosystem besser verstehen zu können, sind solche Ansätze unverzichtbar.

9 REFERENCES

Abad A., D., Chávez V., A., Pinedo V., R., Tantaleán V., M., Gonzáles-Viera, O., 2017. Helmintofauna gastrointestinal de importancia zoonótica y sus aspectos patológicos en roedores (*Rattus* spp.) en tres medioambientes. Rev. Investig. Vet. del Perú 27, 736. https://doi.org/10.15381/rivep.v27i4.12568

AbuBakar, S., Teoh, B.T., Sam, S.S., Chang, L.Y., Johari, J., Hooi, P.S., Lakhbeer-Singh, H.K., Italiano, C.M., Omar, S.F.S., Wong, K.T., Ramli, N., Tan, C.T., 2013. Outbreak of human infection with *Sarcocystis nesbitti*, Malaysia, 2012. Emerg. Infect. Dis. 19, 1989–1991. https://doi.org/10.3201/eid1912.120530

Acevedo-Whitehouse, K., Duffus, A.L.J., 2009. Effects of environmental change on wildlife health. Philos. Trans. R. Soc. B Biol. Sci. 364, 3429–3438. https://doi.org/10.1098/rstb.2009.0128

Achard, F., 2002. Determination of deforestation rates of the world's humid tropical forests. Science. 297, 999–1002. https://doi.org/10.1126/science.1070656

Ackert, J., Herrick, C., 1928. Effects of the nematode *Ascaridia Lineata* (Schneider) on growing chickens. J. Parasitol. 15, 1–72.

Acuin, J., Firestone, R., Htay, T.T., Khor, G.L., Thabrany, H., Saphonn, V., Wibulpolprasert, S., 2011. Southeast Asia: an emerging focus for global health. Lancet 377, 534–535. https://doi.org/10.1016/S0140-6736(10)61426-2

Adler, B., de la Peña Moctezuma, A., 2010. *Leptospira* and leptospirosis. Vet. Microbiol. 140, 287–296. https://doi.org/10.1016/j.vetmic.2009.03.012

Adugna, S., 2016. A Review of bovine leptospirosis. Eur. J. Appl. Sci. 8, 347–355. https://doi.org/10.5829/idosi.ejas.2016.347.355

Agnihotri, K.B., 2012. The dynamics of disease transmission in a prey predator system with harvesting of prey. Int. J. Adv. Res. Comput. Eng. Technol. 1, 1-17.

Agudelo-Flórez, P., Murillo, V.E., Londoño, A., Rodas, J.D., 2012. Histopathological kidney alterations in rats naturally infected with *Leptospira*. Biomedica 33, 82–8. https://doi.org/10.7705/biomedica.v33i0.686

Ahern, M., Kovats, R.S., Wilkinson, P., Few, R., Matthies, F., 2005. Global health impacts of floods: epidemiologic evidence. Epidemiol. Rev. 27, 36–46. https://doi.org/10.1093/epirev/mxi004

Al-Mekhlafi, M.S.H., Atiya, A.S., Lim, Y.A.L., Mahdy, A.K.M., Ariffin, W.A.W., Abdullah, H.C., Surin, J., 2007. An unceasing problem: soil-transmitted helminthiases in rural Malaysian communities. Southeast Asian J. Trop. Med. Public Health. 38, 998–1007.

Alias, S.N., Sahimin, N., Edah, M.A., Mohd-Zain, S.N., 2014. Epidemiology of blood parasitic infections in the urban rat population in peninsular Malaysia. Trop. Biomed. 31, 230–240.

Alicata, J.E., 1968. *Angiostrongylus sandarsae* sp. n. (Nematoda: Metastrongyloidea), a lungworm of rodents in Mozambique. East Africa. J. Parasitol. 54, 896–899.

Allan, B.F., Keesing, F., Ostfeld, R.S., 2003. Effect of forest fragmentation on lyme disease risk. Conserv. Biol. 17, 267–272. https://doi.org/10.1046/j.1523-1739.2003.01260.x

Almirón, M., Traglia, G., Rubio, A., Sanjuan, N., 2013. Colonization of the mouse upper gastrointestinal tract by *Lactobacillus murinus*: a histological, immunocytochemical, and ultrastructural study. Curr. Microbiol. 67, 395–398. https://doi.org/10.1007/s00284-013-0367-9

Alpini, G., Lenzi, R., Sarkozi, L., Tavoloni, N., 1988. Biliary physiology in rats with bile ductular cell hyperplasia. Evidence for a secretory function of proliferated bile ductules. J. Clin. Invest. 81, 569–578. https://doi.org/10.1172/JCI113355

Ambu, S., Yeoh, E.Y.S., Mak, J.W., Chakravarthi, S., 2011. Prevalence of *Sarcocystis* spp. in rodents in Peninsular Malaysia. Int. E-Journal Sci. Med. Educ. 5, 29–38.

Andersen-Ranberg, E.U., Pipper, C., Jensen, P.M., 2016. Global patterns of *Leptospira* prevalence in vertebrate reservoir hosts. J. Wildl. Dis. 52, 468. https://doi.org/10.7589/2014-10-245

Andre-Fontaine, G., Aviat, F., Thorin, C., 2015. Waterborne leptospirosis: survival and preservation of the virulence of pathogenic *Leptospira* spp. in fresh water. Curr. Microbiol. 71, 136–142. https://doi.org/10.1007/s00284-015-0836-4

Aplin, K., Brown, P., Jacob, J., Krebs, C., Singleton, G., 2003. Field methods for rodent studies in Asia and the Indo-Pacific. Canberra: Australian Centre for International Agricultural Research.

Aplin, K.P., Suzuki, H., Chinen, A.A., Chesser, R.T., ten Have, J., Donnellan, S.C., Austin, J., Frost, A., Gonzalez, J.P., Herbreteau, V., Catzeflis, F., Soubrier, J., Fang, Y.P., Robins, J., Matisoo-Smith, E., Bastos, A.D.S., Maryanto, I., Sinaga, M.H., Denys, C., van Den Bussche, R.A., Conroy, C., Rowe, K., Cooper, A., 2011. Multiple geographic origins of commensalism and complex dispersal history of black rats. PLoS One 6. https://doi.org/10.1371/journal.pone.0026357

Ardiaca, M., Bennett, M.D., Montesinos, A., Juan-Sallés, C., Soriano-Navarro, M., 2016. *Klossiella dulcis* N. Sp. (Apicomplexa: Klossiellidae) in the kidneys of *Petaurus breviceps* (Marsupialia: Petauridae). J. Zoo Wildl. Med. 47, 622–627. https://doi.org/10.1638/2015-0258.1

Athanazio, D.A., Silva, E.F., Santos, C.S., Rocha, G.M., Vannier-santos, M.A., Mcbride, A.J.A., Ko, A.I., Reis, M.G., 2008. *Rattus norvegicus* as a model for persistent renal colonization by pathogenic *Leptospira interrogans*. Acta Trop. 105, 176–180. https://doi.org/10.1016/j.actatropica.2007.10.012

Averis, S., Thompson, R.C.A., Lymbery, A.J., Wayne, A.F., Morris, K.D., Smith, A., 2009. The diversity, distribution and host-parasite associations of trypanosomes in Western Australian wildlife. Parasitology 136, 1269–1279. Htt***ps://doi.org/10.1017/S0031182009990801

Azab, W., Dayaram, A., Greenwood,A.D., Osterrieder, N., 2018. How Host Specific Are Herpesviruses? Lessons from herpesviruses infecting wild and endangered mammals. Annu. Rev. Virol. 5, annurev-virology-092917-043227. https://doi.org/10.1146/annurev-virology-092917-043227

Azhari, N.N., Alia, S.N., Joseph, N., Philip, N., Mustapha, N.F., Ishak, S.N., Mohd-Taib, F.S., Nor, S.M., Yusof, M.A., Mohd Sah, S.A., Mohd Desa, M.N. Bin, Bashiru, G., Zeppelini, C.G., Costa, F., Sekawi, Z., Neela, V.K., 2018. Molecular characterization of pthogenic *Leptospira* sp. in small mammals captured from the human leptospirosis suspectd areas of Selangor state, Malaysia. Acta Trop. 188, 68–77. https://doi.org/10.1016/j.actatropica.2018.08.020

Azzam, K.M., El-Abd, N.M., Abd El-Hady, E.A., 2016. Survey of endoparasites of different rodent species in Egypt. Egypt. J. Biol. Pest Control 26, 815–820.

Barrett, M.P., Burchmore, R.J.S., Stich, A., Lazzari, J.O., Frasch, A.C., Cazzulo, J.J., Krishna, S., 2003. The trypanosomiases. Lancet 362, 1469–1480. https://doi.org/10.1016/S0140-6736(03)14694-6

Battersby, S.A., 2015. Rodents as carriers of disease. Rodent pests and their control. CAB International London; pp. 81-100. https://doi.org/10.1079/9781845938178.0000

Bazzaz, F.A., Pickett, S.T.A., 2003. Physiological ecology of tropical succession: a comparative review. Annu. Rev. Ecol. Syst. 11, 287–310. https://doi.org/10.1146/annurev.es.11.110180.001443

Beaver, P., Maleckar, J.R., 1981. Sarcocystis singaporensis Zaman and Colley, (1975) 1976,

Sarcocystis villivillosi sp. n., and *Sarcocystis zamani* sp. n.: development, morphology, and persistence in the laboratory rat, *Rattus norvegicus*. J. Parasitol. 67, 241–256.

Becker, P., Leighton, M., Payne, J.B., 1985. Why tropical squirrels carry seeds out of source crowns. J. Trop. Ecol. 1, 183–186. https://doi.org/10.1017/S0266467400000237

Behnke, J.M., Lewis, J.W., Zain, S.N.M., Gilbert, F.S., 1999. Helminth infections in *Apodemus sylvaticus* in southern England: Interactive effects of host age, sex and year on the prevalence and abundance of infections. J. Helminthol. 73, 31–44. https://doi.org/10.1017/s0022149x99000049

Benacer, D., Zain, S.N.M., Amran, F., Galloway, R.L., Thong, K.L., 2013. Isolation and molecular characterization of *Leptospira interrogans* and *Leptospira borgpetersenii* Isolates from the urban rat populations of Kuala Lumpur, Malaysia. Am. J. Trop. Med. Hyg. 88, 704–709. https://doi.org/10.4269/ajtmh.12-0662

Benacer, D., Mohd Zain, S.N., Sim, S.Z., Mohd Khalid, M.K.N., Galloway, R.L., Souris, M., Thong, K.L., 2016. Determination of *Leptospira borgpetersenii* serovar Javanica and *Leptospira interrogans* serovar Bataviae as the persistent *Leptospira* serovars circulating in the urban rat populations in Peninsular Malaysia. Parasit Vectors. 9, 117. https://doi.org/10.1186/s13071-016-1400-1

Bergmann, V., Kinder, E., 1987. Einsatz der Ultrahistologie in der veterinärmedizinschen Diagnostik – Methoden und Erfahrungen. Arch. exper. Vet. med. 41, 598-612.

Berridge, B.R., Mowat, V., Nagai, H., Nyska, A., Okazaki, Y., Clements, P.J., Rinke, M., Snyder, P.W., Boyle, M.C., Wells, M.Y., 2016. Non-proliferative and proliferative lesions of the cardiovascular system of the rat and mouse The INHAND Project (International harmonization of nomenclature and diagnostic criteria for lesions in rats and mice. J. Toxicol. Pathol. 29, 1–47. https://doi.org/10.1293/tox.2016-I001

Bharti, A.R., Nally, J.E., Ricaldi, J.N., Matthias, M.A., Diaz, M.M., Lovett, M.A., Levett, P.N., Gilman, R.H., Willig, M.R., Gotuzzo, E., Vinetz, J.M., 2003. Leptospirosis: a zoonotic disease of global importance. Lancet Infect. Dis. 3, 757–771. https://doi.org/10.1016/S1473-3099(03)00830-2

Bhatia, M., Umapathy, B., Navaneeth, B., 2015. An evaluation of dark field microscopy, culture and commercial serological kits in the diagnosis of leptospirosis. Indian J. Med. Microbiol. 33, 416. https://doi.org/10.4103/0255-0857.158570

Bierque, E., Thibeaux, R., Girault, D., Soupé-Gilbert, M.E., Goarant, C., 2020. A systematic review of *Leptospira* in water and soil environments. PLoS One 15, 1–22.

https://doi.org/10.1371/journal.pone.0227055

Blasdell, K.R., Morand, S., Perera, D., Firth, C., 2019. Association of rodent-borne *Leptospira* spp. with urban environments in Malaysian Borneo. PLoS Negl. Trop. Dis. 13, e0007141. https://doi.org/10.1371/journal.pntd.0007141

Blendedn, D.C., Goldberg, H.S., 1965. Silver impregnation stain for *Leptospira* and flagella. J. Bacteriol. 89, 899–900.

Boehmer, P.E., Nimonkar, A. V., 2003. Herpesvirus replication. IUBMB Life. 55, 13–22. https://doi.org/10.1080/1521654031000070645

Boes, K.M., & Durham, A.C., 2017. *Chapter*: Bone marrow, blood cells, and the lymphoid/lymphatic system. *In*: Pathologic basis of veterinary disease. Zachary J. (ed.): P.Elsevier Inc., St. Louis, MO, pp. 724–804. https://doi.org/10.1016/B978-0-323-35775-3.00013-8

Boey, K., Shiokawa, K., Rajeev, S., 2019. *Leptospira* infection in rats: a literature review of global prevalence and distribution. PLoS Negl. Trop. Dis. 13, e0007499. https://doi.org/10.1371/journal.pntd.0007499

Bordes, F., Blasdell, K., Morand, S., 2015. Transmission ecology of rodent-borne diseases: New frontiers. Integr. Zool. 10, 424–435. https://doi.org/10.1111/1749-4877.12149

Bordes, F., Morand, S., 2011. The impact of multiple infections on wild animal hosts: a review. Infect. Ecol. Epidemiol. 1, 7346. https://doi.org/10.3402/iee.v1i0.7346

Botero, A., Thompson, C.K., Peacock, C.S., Clode, P.L., Nicholls, P.K., Wayne, A.F., Lymbery, A.J., Thompson, R.C.A., 2013. Trypanosomes genetic diversity, polyparasitism and the population decline of the critically endangered Australian marsupial, the brush tailed bettong or woylie (*Bettongia penicillata*). Int. J. Parasitol. Parasites Wildl. 2, 77–89. https://doi.org/10.1016/j.ijppaw.2013.03.001

Britton, J.R., 2013. Introduced parasites in food webs: new species, shifting structures? Trends Ecol. Evol. 28, 93–99. https://doi.org/10.1016/j.tree.2012.08.020

Brookfield, H., Byron, Y., 1990. Deforestation and timber extraction in Borneo and the Malay Peninsula. Glob. Environ. Chang. 1, 42–56. https://doi.org/10.1016/0959-3780(90)90006-u

Bryan, J.E., Shearman, P.L., Asner, G.P., Knapp, D.E., Aoro, G., Lokes, B., 2013. Extreme differences in forest degradation in Borneo: comparing practices in Sarawak, Sabah, and Brunei. PLoS One 8. https://doi.org/10.1371/journal.pone.0069679

Buckner, C.H., 1964. Metabolism, food capacity, and feeding behavior in four species of shrews. Can. J. Zool. 42, 259–279. https://doi.org/10.1139/z64-026

Burgin, C., He, K., Haslauer, R., Sheftel, B., Jenkins, P., Ruedi, M., Hintsche, S., Motokawa, M., Hinckley, A., Hutterer, R., 2017. *Chapter*: Family Soricidae (Shrews). *In*: Handbook of the Mammals of the World. Insectivores, sloths and colugos. Mittermeier, R.A., Wilson, D.E., Rylands, A.B. (eds.): Lynx Edicions, Barcelona, pp. 288- 359.

Camenzind, T., Hättenschwiler, S., Treseder, K.K., Lehmann, A., Rillig, M.C., 2018. Nutrient limitation of soil microbial processes in tropical forests. Ecol. Monogr. 88, 4–21. https://doi.org/10.1002/ecm.1279

Carnegie, R.B., Arzul, I., Bushek, D., 2016. Managing marine mollusc diseases in the context of regional and international commerce: policy issues and emerging concerns. Philos. Trans. R. Soc. B Biol. Sci. 371. https://doi.org/10.1098/rstb.2015.0215

Ceruti, R., Ghisleni, G., Ferretti, E., Cammarata, S., Sonzogni, O., Scanziani, E., 2002. Wild rats as monitors of environmental lead contamination in the urban area of Milan, Italy. Environ. Pollut. 117, 255–9. https://doi.org/10.1016/S0269-7491(01)00273-1

Chappel, R.J., Prime, R.W., Millar, B.D., Mead, L.J., Jones, R.T., Adler, B., 1992. Comparison of diagnostic procedures for porcine leptospirosis. Vet. Microbiol. 30, 151–163. https://doi.org/10.1016/0378-1135(92)90110-F

Charon, N.W., Greenberg, E.P., Koopman, M.B.H., Limberger, R.J., 1992. Spirochete chemotaxis, motility, and the structure of the spirochetal periplasmic flagella. Res. Microbiol. 143, 597–603. https://doi.org/10.1016/0923-2508(92)90117-7

Chazdon, R.L., Percy, R.W., Lee, D.W., Fetcher, N., 1996. *Chapter:* Photosynthetic responses of tropical forest plants to contrasting light environments. *In:* Tropical forest plant ecophysiology. Mulkey, S.S., Chazdon, R.L., Smith, A.P., (eds.): Chapman and Hall, New York, pp. 5–55.

Cheema, P.S., Srivastava, S.K., Amutha, R., Singh, S., Singh, H., Sandey, M., 2007. Detection of pathogenic leptospires in animals by PCR based on lipL21 and lipL32 genes. Indian J. Exp. Biol. 45, 568–573.

Christe, P., Glaizot, O., Evanno, G., Bruyndonckx, N., Devevey, G.,Yannic, G., Patthey, P., Maeder, A., Vogel, P., Arlettaz, R., 2007. Host sex and ectoparasites choice: preference for, and higher survival on female hosts. J. Anim. Ecol. 76, 703–710. https://doi.org/10.1111/j.1365-2656.2007.01255.x

Choi, D., Hong, S.-T., Li, S., Chung, B.-S., Lim, J.H., Lee, S.-H., 2004. Bile duct changes in

rats reinfected with *Clonorchis sinensis*. Korean J. Parasitol. 42, 7. https://doi.org/10.3347/kjp.2004.42.1.7

Cianciolo, R.E., Mohr, F.C., 2015. *Chapter:* Urinary system. *In*: Jubb, Kennedy, and Palmer's pathology of domestic animals. Maxie, M.G., (ed.): Elsevier Saunders, Philadelphia, pp. 376–464

Civitello, D.J., Cohen, J., Fatima, H., Halstead, N.T., Liriano, J., McMahon, T.A., Ortega, C.N., Sauer, E.L., Sehgal, T., Young, S., Rohr, J.R., 2015. Biodiversity inhibits parasites: broad evidence for the dilution effect. Proc. Natl. Acad. Sci. U. S. A. 112, 8667–8671. https://doi.org/10.1073/pnas.1506279112

Clancy, M.M., Woc-Colburn, M., Viner, T., Sanchez, C., Murray, S., 2013. Retrospective analysis of mortalities in elephant shrew (Macroscelididae) and Tree shrew (Tupaiidae) at the Smithsonian National Zoological Park, USA. J. Zoo Wildl. Med. 44, 302–309. https://doi.org/10.1638/2012-0115R1.1

Clark, N.J., Seddon, J.M., Jan, Š., Wells, K., 2018. Parasite spread at the domestic animal - wildlife interface: anthropogenic habitat use, phylogeny and body mass drive risk of cat and dog flea (*Ctenocephalides* spp.) infestation in wild mammals. Parasit Vectors. 1–11. https://doi.org/10.1186/s13071-017-2564-z

Cleveland, C.C., Houlton, B.Z., Smith, W.K., Marklein, A.R., Reed, S.C., Parton, W., Del Grosso, S.J., Running, S.W., 2013. Patterns of new versus recycled primary production in the terrestrial biosphere. Proc. Natl. Acad. Sci. U. S. A. 110, 12733–7. https://doi.org/10.1073/pnas.1302768110

Cosson, J.F., Picardeau, M., Mielcarek, M., Tatard, C., Chaval, Y., Suputtamongkol, Y., Buchy, P., Jittapalapong, S., Herbreteau, V., Morand, S., 2014. Epidemiology of *Leptospira* transmitted by rodents in Southeast Asia. PLoS Negl. Trop. Dis. 8, e2902. https://doi.org/10.1371/journal.pntd.0002902

Costa, F., Hagan, J.E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M.S., Stein, C., Abela-Ridder, B., Ko, A.I., 2015. Global morbidity and mortality of leptospirosis: a systematic review. PLoS Negl. Trop. Dis. 9, e0003898. https://doi.org/10.1371/journal.pntd.0003898

Coulibaly, C., Hack, R., Seidl, J., Chudy, M., Itter, G., Plesker, R., 2004. A natural asymptomatic herpes B virus infection in a colony of laboratory brown capuchin monkeys (*Cebus apella*). Lab. Anim. 38, 432–438. https://doi.org/10.1258/0023677041958891

Cox, F., 2001. Concomitant infections, parasites and immune responses. Parasitology 122,

S23–S38. https://doi.org/10.1017/S003118200001698X

Cozzarolo, C.S., Sironi, N., Glaizot, O., Pigeault, R., Christe, P., 2019. Sex-biased parasitism in vector-borne disease: vector preference? PLoS One 14, 1–11. https://doi.org/10.1371/journal.pone.0216360

Crissman, J.W., Goodman, D.G., Hildebrandt, P.K., Maronpot, R.R., Prater, D.A., Riley, J.H., Seaman, W.J., Thake, D.C., 2004. Best practices guideline: toxicologic histopathology. Toxicol. Pathol. 32, 126–131. https://doi.org/10.1080/01926230490268756

Cullen, P.A., Haake, D.A., Bulach, D.M., Zuerner, R.L., Adler, B., 2003. LipL21 Is a novel surface-exposed lipoprotein of pathogenic *Leptospira* species. Infect. Immun. 71, 2414–2421. https://doi.org/10.1128/IAI.71.5.2414-2421.2003

Czch, B., Krausman, P.R., Devers, P.K., 2006. Economic associations among causes of species endangerment in the United States. Bioscience 50, 593.

Dagosto, M., 1991. Primate origins and evolution: a phylogenetic reconstruction. Int. J. Primatol. 12, 77–79 . https://doi.org/10.1007/BF02547560

Dahesh, S.M.A., Mikhail, M.W., 2016. Surveillance of *Trypanosoma* spp. of rodents and studies in their transmission probability by fleas in some rural Egyptian areas. J. Egypt. Soc. Parasitol. 46, 157–166. https://doi.org/10.12816/0026161

Dahlgren, S.S., Gjerde, B., Skirnisson, K., Gudmundsdottir, B., 2007. Morphological and molecular identification of three species of *Sarcocystis* in reindeer (*Rangifer tarandus tarandus*) in Iceland. Vet. Parasitol. 149, 191–198. https://doi.org/10.1016/j.vetpar.2007.08.015

Davison, A.J., 2002. Evolution of the herpesviruses. Vet. Microbiol. 86, 69–88. https://doi.org/10.1016/S0378-1135(01)00492-8

Davison, A.J., Eberle, R., Ehlers, B., Hayward, G.S., McGeoch, D.J., Minson, A.C., Pellett, P.E., Roizman, B., Studdert, M.J., Thiry, E., 2009. The order Herpesvirales. Arch. Virol. 154, 171–177. https://doi.org/10.1007/s00705-008-0278-4

De Bruyn, M., Stelbrink, B., Morley, R.J., Hall, R., Carvalho, G.R., Cannon, C.H., Van Den Bergh, G., Meijaard, E., Metcalfe, I., Boitani, L., Maiorano, L., Shoup, R., Von Rintelen, T., 2014. Borneo and Indochina are major evolutionary hotspots for Southeast Asian biodiversity. Syst. Biol. 63, 879–901. https://doi.org/10.1093/sysbio/syu047

De Matos, R., Russell, D., Van Alstine, W., Miller, A., 2014. Spontaneous fatal Human herpesvirus 1 encephalitis in two domestic rabbits (*Oryctolagus cuniculus*). J. Vet.

Diagnostic Investig. 26, 689–694. https://doi.org/10.1177/1040638714545111

Delaney, M.A., Treuting, P.M., Rothenburger, J.L., 2018. *Chapter*: Rodentia. *In*: Pathology of wildlife and Zoo animals. Terio. K., McAloose.D., (eds.): pp. 499-515. Academic Press, London;.. http://dx.doi.org/10.1016/B978-0-12- 805306-5.00020-1

Denk, H., Künzle, H., Plenk, H., 1989. Romeis–Mikroskopische Technik. Mulisch, M., Welsch, U. (eds.): Urban & Schwarzenberg, München, Wien, Baltimore, Germany.

Denys, C., Taylor, P., Aplin, K., 2017. *Chapter*: Family Muridae (True mice and rats, gerbils and relatives). *In*: Handbook of the Mammals of the World. Rodents II. Mittermeier, R.A., Wilson, D.E., Rylands, A.B. (eds.): pp. 536- 690. Lynx Edicions, Barcelona.

Desquesnes, M., Bosseno, M.F., Brenière, S.F., 2007. Detection of Chagas infections using *Trypanosoma evansi* crude antigen demonstrates high cross-reactions with *Trypanosoma cruzi.* Infect. Genet. Evol. 7, 457–462. https://doi.org/10.1016/j.meegid.2007.01.007

Dietrich, M., Wilkinson, D.A., Soarimalala, V., Steven, M., 2014. Diversification of an emerging pathogen in a biodiversity hotspot: *Leptospira* in endemic small mammals of Madagascar. Mol. Ecol. 2783–2796. https://doi.org/10.1111/mec.12777

Dirzo, R., Young, H.S., Galetti, M., Ceballos, G., Isaac, N.J.B., Collen, B., 2014. Defaunation in the Anthropocene. Science. 345, 401–406. https://doi.org/10.1126/science.1251817

Dobson, R.J., Dargantes, A.P., Mercado, R.T., Reid, S.A., 2009. Models for *Trypanosoma evansi* (surra), its control and economic impact on small-hold livestock owners in the Philippines. Int. J. Parasitol. 39, 1115–1123. https://doi.org/10.1016/j.ijpara.2009.02.013

Drakakis-Smith, D., 1997. Third world cities: sustainable urban development III-basic needs and human rights. Urban Stud. 34, 797–823. https://doi.org/10.1080/0042098975835

Drazen, D.L., Trasy, A., Nelson, R.J., 2003. Photoperiod differentially affects energetics of immunity in pregnant and lactating Siberian hamsters (*Phodopus sungorus*). Can. J. Zool. 81, 1406–1413. https://doi.org/10.1139/z03-120

Dubey, J., Speer, C., Epling, G., 1982. Sarcocystosis in newborn calves fed *Sarcocystis cruzi* sporocysts from coyotes. Am. J. Vet.Res. 43, 2147–2164.

Dubey, J.P., Calero-Bernal, R., Rosenthal, B., Speer, C.A., Fayer, R., 2016. Sarcocystosis of animals and humans. CRC Press, Boca Raton.

Dubey, J.P., Fayer, R., 1986. Sarcocystosis, Toxoplasmosis, and cryptosporidiosis in cattle. Vet. Clin. North Am. Food Anim. Pract. 2, 293–298. https://doi.org/10.1016/S0749-

0720(15)31239-1

Dubey, S., Salamin, N., Ohdachi, S.D., Barrière, P., Vogel, P., 2007. Molecular phylogenetics of shrews (Mammalia: Soricidae) reveal timing of transcontinental colonizations. Mol. Phylogenet. Evol. 44, 126–137. https://doi.org/10.1016/j.ympev.2006.12.002

Duszynski, Donald W. and Upton, Steve J.,2000. Coccidia (Apicomplexa: Eimeriidae) of the mammalian order Insectivora. Faculty Publications from the Harold W. Manter Laboratory of Parasitology 196. https://digitalcommons.unl.edu/parasitologyfacpubs/196

Easterbrook, J.D., Kaplan, J.B., Glass, G.E., Watson, J., Klein, S.L., 2008. A survey of rodent-borne pathogens carried by wild-caught Norway rats: a potential threat to laboratory rodent colonies. Lab. Anim. 42, 92–98. https://doi.org/10.1258/la.2007.06015e

Easterbrook, J.D., Kaplan, J.B., Vanasco, N.B., Reeves, W.K., Purcell, R.H., Kosoy, M.Y., Glass, G.E., Watson, J., Klein, S.L., 2007. A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA. Epidemiol. Infect. 135, 1192–1199. https://doi.org/10.1017/S0950268806007746

Easton, A.J., Domachowske, J.B., Rosenberg, H.F., 2004. Animal pneumoviruses: molecular genetics and pathogenesis. Clin. Microbiol. Rev. 17, 390–412. https://doi.org/10.1128/CMR.17.2.390-412.2004

Eaton, K.A., Danon, S.J., Krakowka, S., Weisbrode, S.E., 2007. A reproducible scoring system for quantification of histologic lesions of inflammatory disease in mouse gastric epithelium. Comp. Med. 57, 57–65.

Eberhard, M.L., Orihel, T.C., 1984. The genus *Mansonella* (syn. Tetrapetalonema): a new classification. Ann. Parasitol. Hum. comparée 59, 483–496. https://doi.org/10.1051/parasite/1984595483

Ehlers, B., Borchers, K., Grund, C., Frölich, K., Ludwig, H., Buhk, H.J., 1999. Detection of new DNA polymerase genes of known and potentially novel herpesviruses by PCR with degenerate and deoxyinosine-substituted primers. Virus Genes. 18, 211–20.

Ehlers, B., Dural, G., Yasmum, N., Lembo, T., de Thoisy, B., Ryser-Degiorgis, M.-P., Ulrich, R.G., McGeoch, D.J., 2008. Novel mammalian herpesviruses and lineages within the gammaherpesvirinae: cospeciation and interspecies Transfer. J. Virol. 82, 3509–3516. https://doi.org/10.1128/JVI.02646-07

Ehlers, B., Küchler, J., Yasmum, N., Dural, G., Voigt, S., Schmidt-Chanasit, J., Jäkel, T., Matuschka, F., Richter, D., Essbauer, S., Hughes, D.J., Summers, C., Bennett, M., Stewart, J.P., Ulrich, R.G., 2007. Identification of novel rodent herpesviruses, including

the first gammaherpesvirus of *Mus musculus*. J. Virol. 81, 8091–8100. https://doi.org/10.1128/JVI.00255-07

El-Sherry, S., Ogedengbe, M.E., Hafeez, M.A., Barta, J.R., 2013. Divergent nuclear 18S rDNA paralogs in a turkey coccidium, *Eimeria meleagrimitis*, complicate molecular systematics and identification. Int. J. Parasitol. 43, 679–685. https://doi.org/10.1016/j.ijpara.2013.03.005

El Wathig, M., Faye, B., Thevenon, S., Ravel, S., Bossard, G., 2016. Epidemiological surveys of camel trypanosomosis in Al-jouf, Saudi Arabia based on PCR and ELISA. Emirates J. Food Agric. 28, 212–216. https://doi.org/10.9755/ejfa.2015-09-759

Elhadary, Y.A.E., Samat, N., Obeng-Odoom, F., 2013. Development at the peri-urban area and its impact on agricultural activities: an example from the Seberang Perai region, Penang State, Malaysia. Agroecol. Sustain. Food Syst. 37, 834–856. https://doi.org/10.1080/21683565.2013.797950

Emmons, L., 2000. Tupai: a field study of Bornean treeshrews. University of California Press, Berkeley

Erazo, D., Pedersen, A.B., Gallagher, K., Fenton, A., 2021. Who acquires infection from whom? Estimating herpesvirus transmission rates between wild rodent host groups. Epidemics 35, 100451. https://doi.org/10.1016/j.epidem.2021.100451

Farhang-Azad, A., 1977. Ecology of *Capillaria hepatica* (Bancroft 1893) (Nematoda). 1. Dynamics of infection among Norway rat populations of the Baltimore Zoo, Baltimore, Maryland. J. Parasitol. 63, 117. https://doi.org/10.2307/3280114

Faust, C.L., McCallum, H.I., Bloomfield, L.S.P., Gottdenker, N.L., Gillespie, T.R., Torney, C.J., Dobson, A.P., Plowright, R.K., 2018. Pathogen spillover during land conversion. Ecol. Lett. 21, 471–483. https://doi.org/10.1111/ele.12904

Fayer, R., Dubey, J.P., 1984. Protective immunity against clinical sarcocystosis in cattle. Vet. Parasitol. 15, 187–201. https://doi.org/10.1016/0304-4017(84)90071-2

Fayer, R., Esposito, D.H., Dubey, J.P., 2015. Human infections with *Sarcocystis* species. Clin. Microbiol. Rev. 28, 295–311. https://doi.org/10.1128/CMR.00113-14

Fayer, R., Lynch, G.P., Leek, R.G., Gasbarre, L.C., 1983. Effects of sarcocystosis on milk production of dairy cows. J. Dairy Sci. 66, 904–908. https://doi.org/10.3168/jds.S0022-0302(83)81874-8

Feng, A.Y.T., Himsworth, C.G., 2014. The secret life of the city rat: a review of the ecology of

urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). Urban Ecosyst. 17, 149–162. https://doi.org/10.1007/s11252-013-0305-4

Firth, C., Bhat, M., Firth, M.A., Williams, S.H., Frye, M.J., Simmonds, P., Conte, J.M., Ng, J., Garcia, J., Bhuva, N.P., Lee, B., Che, X., Quan, P.-L., Lipkin, W.I., 2014. Detection of zoonotic pathogens and characterization of novel viruses carried by commensal *Rattus norvegicus* in New York City. MBio 5, e01933-14. https://doi.org/10.1128/mBio.01933-14

Foster, J.R., 1981. A study of the initiation of biliary hyperplasia in rats infected with *Fasciola hepatica.* Parasitology 83, 253–258. https://doi.org/10.1017/S0031182000085267

Fuehrer, H.-P., Igel, P., Auer, H., 2011. *Capillaria hepatica* in man—an overview of hepatic capillariosis and spurious infections. Parasitol. Res. 109, 969–979. https://doi.org/10.1007/s00436-011-2494-1

Fuertes, M., Pérez, V., Benavides, J., González-Lanza, M.C., Mezo, M., González-Warleta, M., Giráldez, F.J., Fernández, M., Manga-González, M.Y., Ferreras, M.C., 2015. Pathological changes in cattle naturally infected by *Calicophoron daubneyi* adult flukes. Vet. Parasitol. 209, 188–196. https://doi.org/10.1016/j.vetpar.2015.02.034

Galán-Puchades, M.T., Sanxis-Furió, J., Pascual, J., Bueno-Marí, R., Franco, S., Peracho, V., Montalvo, T., Fuentes, M. V., 2018. First survey on zoonotic helminthosis in urban brown rats (*Rattus norvegicus*) in Spain and associated public health considerations. Vet. Parasitol. 259, 49–52. https://doi.org/10.1016/j.vetpar.2018.06.023

Gamage, C.D., Koizumi, N., Muto, M., Nwafor-Okoli, C., Kurukurusuriya, S., Rajapakse, J.R.P.V., Kularatne, S.A.M., Kanda, K., Lee, R.B., Obayashi, Y., Watanabe, H., Tamashiro, H., 2011. Prevalence and carrier status of leptospirosis in smallholder dairy cattle and peridomestic rodents in Kandy, Sri Lanka. Vector-Borne Zoonotic Dis. 11, 1041–1047. https://doi.org/10.1089/vbz.2010.0153

Gardiner, C.H., Poynton, S.L., 1998. An atlas of protozoan parasites in animal tissues. U.S. Department of Agriculture handbook no. 651. U.S. Department of Agriculture, Washington D.C

Gaveau, D.L.A., Sheil, D., Husnayaen, Salim, M.A., Arjasakusuma, S., Ancrenaz, M., Pacheco, P., Meijaard, E., 2016. Rapid conversions and avoided deforestation: examining four decades of industrial plantation expansion in Borneo. Sci. Rep. 6, 32017. https://doi.org/10.1038/srep32017

Gaveau, D.L.A., Sloan, S., Molidena, E., Yaen, H., Sheil, D., Abram, N.K., Ancrenaz, M., Nasi, R., Quinones, M., Wielaard, N., Meijaard, E., 2014. Four decades of forest persistence,

clearance and logging on Borneo. PLoS One 9, 1–11. https://doi.org/10.1371/journal.pone.0101654

Genovart, M., Negre, N., Tavecchia, G., Bistuer, A., Parpal, L., Oro, D., 2010. The young, the weak and the sick: evidence of natural selection by predation. PLoS One 5, e9774. https://doi.org/10.1371/journal.pone.0009774

Ghani, S., 2002. Urbanisation and regional development in Malaysia. Utusan Publications and Distributors, Kuala Lumpur

Gibson, D.I., Bray, R.A., Hunt, D., Georgiev, B.B., Scholz, T., Harris, P.D., Bakke, T.A., Pojmanska, T., Niewiadomska, K., Kostadinova, A., Tkach, V., Bain, O., Durette-Desset, M.C., Gibbons, L., Moravec, F., Petter, A., Dimitrova, Z.M., Buchmann, K., Valtonen, E.T., de Jong, Y., 2014. Fauna Europaea: helminths (animal parasitic). Biodivers. Data J. 2. https://doi.org/10.3897/BDJ.2.e1060

Gibson, L., Lynam, A.J., Bradshaw, C.J. a., He, F., Bickford, D.P., Woodruff, D.S., Bumrungsri, S., Laurance, W.F., 2013. Near-complete extinction of native small mammal fauna 25 years after forest fragmentation. Science .341, 1508–1510. https://doi.org/10.1126/science.1240495

Gibson-Corley, K.N., Olivier, A.K., Meyerholz, D.K., 2013. Principles for valid histopathologic scoring in research. Vet. Pathol. 50, 1007–1015. https://doi.org/10.1177/0300985813485099

Gin, O.K., 2011. Borneo transformed: agricultural expansion on the Southeast Asian frontier (review). J. Malays. Branch R. Asiat. Soc. 84, 126–128. https://doi.org/10.1353/ras.2011.0017

Gjerde, B., 2013. Phylogenetic relationships among *Sarcocystis* species in cervids, cattle and sheep inferred from the mitochondrial cytochrome c oxidase subunit I gene. Int. J. Parasitol. 43, 579–591. https://doi.org/10.1016/j.ijpara.2013.02.004

Glass, G.E., Livingstone, W., Mills, J.N., Hlady, W.G., Fine, J.B., Biggler, W., Coke, T., Frazier, D., Atherley, S., Rollin, P.E., Ksiazek, T.G., Peters, C.J., Childs, J.E., 1998. Black Creek Canal virus infection in *Sigmodon hispidus* in southern Florida. Am. J. Trop. Med. Hyg. 59, 699–703. https://doi.org/10.4269/ajtmh.1998.59.699

Grant, E.C., 1963. An analysis of the social behaviour of the male laboratory rat. Behaviour 21, 260–281. https://doi.org/10.1163/156853963X00194

Gravekamp, C., Van de Kemp, H., Franzen, M., Carrington, D., Schoone, G.J., Van Eys, G.J.J.M., Everard, C.O.R., Hartskeerl, R.A., Terpstra, W.J., 1993. Detection of seven

species of pathogenic leptospires by PCR using two sets of primers. J. Gen. Microbiol. 139, 1691–1700. https://doi.org/10.1099/00221287-139-8-1691

Guernier, V., Richard, V., Nhan, T., Rouault, E., Tessier, A., Musso, D., 2017. *Leptospira* diversity in animals and humans in Tahiti, French Polynesia. PLoS Negl. Trop. Dis. 11, e0005676. https://doi.org/10.1371/journal.pntd.0005676

Gutiérrez, R., Krasnov, B., Morick, D., Gottlieb, Y., Khokhlova, I.S., Harrus, S., 2015. *Bartonella* infection in rodents and their flea ectoparasites: an overview. Vector-Borne Zoonotic Dis. 15, 27–39. https://doi.org/10.1089/vbz.2014.1606

Häfner, U., Frank, W., 1984. Host specificity and host range of the genus *Sarcocystis* in three snake-rodent life cycles. Zentralblatt für Bakteriol. Mikrobiol. und Hyg. 1. Abt. Orig. A, Medizinische Mikrobiol. Infekt. und Parasitol. 256, 296–299. https://doi.org/10.1016/S0174-3031(84)80005-0

Haines, A., Kovats, R.S., Campbell-Lendrum, D., Corvalan, C., 2006. Climate change and human health: impacts, vulnerability and public health. Public Health 120, 585–596. https://doi.org/10.1016/j.puhe.2006.01.002

Hall, S.R., Sivars-Becker, L., Becker, C., Duffy, M.A., Tessier, A.J., Cáceres, C.E., 2007. Eating yourself sick: transmission of disease as a function of foraging ecology. Ecol. Lett. 10, 207–218. https://doi.org/10.1111/j.1461-0248.2007.01011.x

Hamilton, P.B., Stevens, J.R., Gidley, J., Holz, P., Gibson, W.C., 2005. A new lineage of trypanosomes from Australian vertebrates and terrestrial bloodsucking leeches (Haemadipsidae). Int. J. Parasitol. 35, 431–443. https://doi.org/10.1016/j.ijpara.2004.12.005

Han, B.A., Schmidt, J.P., Bowden, S.E., Drake, J.M., 2015. Rodent reservoirs of future zoonotic diseases. Proc. Natl. Acad. Sci. 112, 7039–7044. https://doi.org/10.1073/pnas.1501598112

Hart, B.L., Hart, L.A., 2018. How mammals stay healthy in nature: the evolution of behaviours to avoid parasites and pathogens. Philos. Trans. R. Soc. B Biol. Sci. 373, 20170205. https://doi.org/10.1098/rstb.2017.0205

Harrison, R.D., Sreekar, R., Brodie, J.F., Brook, S., Luskin, M., O'Kelly, H., Rao, M., Scheffers, B., Velho, N., 2016. Impacts of hunting on tropical forests in Southeast Asia. Conserv. Biol. 30, 972–981. https://doi.org/10.1111/cobi.12785

Harstskeerl, R.A., Sertour, N., Ahmed, A., Rahelinirina, S., Le, A., Rahalison, L., Cornet, M., 2010. First isolation and direct evidence for the existence of large small-mammal

reservoirs of *Leptospira* sp. in Madagascar. PLoS One 5, e14111. https://doi.org/10.1371/journal.pone.0014111

Hart, B.L., 1991. The behavior of sick animals. Vet. Clin. North Am. Small Anim. Pract. 21, 225–237. https://doi.org/10.1016/S0195-5616(91)50028-0

Hasler, M.J., Hasler, J.F., Nalbandov, A. V., 1977. Comparative breeding biology of musk shrews (*Suncus murinus*) from Guam and Madagascar. J. Mammal. 58, 285–290. https://doi.org/10.2307/1379327

Hawkins, M., 2017. *Chapter*: Family Tupaiidae (Treeshrews). *In*: Handbook of the Mammals of the World. Insectivores, Sloths and Colugos. Mittermeier, R.A., Wilson, D.E., Rylands, A.B. (eds.): Lynx Edicions, Barcelona, pp. 242- 287.

Hawkins, M.T.R., Helgen, K.M., Maldonado, J.E., Rockwood, L.L., Tsuchiya, M.T.N., Leonard, J.A., 2016. Phylogeny, biogeography and systematic revision of plain long-nosed squirrels (genus *Dremomys*, Nannosciurinae). Mol. Phylogenet. Evol. 94, 752–764. https://doi.org/10.1016/j.ympev.2015.10.023

Hayes, P.M., Lawton, S.P., Smit, N.J., Gibson, W.C., Davies, A.J., 2014. Morphological and molecular characterization of a marine fish trypanosome from South Africa, including its development in a leech vector. ParasitVectors 7, 1–11. https://doi.org/10.1186/1756-3305-7-50

Heim de Balsac, H., Lamotte, M., 1956. Evolution et phylogénie des soricidés africains. Mammalia 20. https://doi.org/10.1515/mamm.1956.20.2.140

Herbreteau, V., Bordes, F., Jittapalapong, S., Supputamongkol, Y., Morand, S., 2012. Rodent-borne diseases in Thailand: targeting rodent carriers and risky habitats. Infect. Ecol. Epidemiol. 2, 18637. https://doi.org/10.3402/iee.v2i0.18637

Herbreteau, V., Jittapalapong, S., Rerkamnuaychoke, W., Chaval, Y., Cosson, J.F., Morand, S., 2011. Protocols for field and laboratory rodent studies. Retrieved from CERoPath project: http://www.ceropath.org/FichiersComplementaires/Herbreteau_Rodents_protocols_2011.pdf.

Hillegass, M.A., Waterman, J.M., Roth, J.D., 2008. The influence of sex and sociality on parasite loads in an African ground squirrel. Behav. Ecol. 19, 1006–1011. https://doi.org/10.1093/beheco/arn070

Himsworth, C.G., Bidulka, J., Parsons, K.L., Feng, A.Y.T., Tang, P., Jardine, C.M., Kerr, T., Mak, S., Robinson, J., Patrick, D.M., 2013a. Ecology of *Leptospira interrogans* in Norway

rats (*Rattus norvegicus*) in an inner-city neighborhood of Vancouver, Canada. PLoS Negl. Trop. Dis. 7, e2270. https://doi.org/10.1371/journal.pntd.0002270

Himsworth, C.G., Parsons, K.L., Jardine, C., Patrick, D.M., 2013b. Rats, Cities, People, and Pathogens: a systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. Vector-Borne Zoonotic Dis. 13, 349–359. https://doi.org/10.1089/vbz.2012.1195

Himsworth, C.G., Zabek, E., Tang, P., Parsons, K.L., Koehn, M., Jardine, C.M., Patrick, D.M., 2014. Bacteria isolated from conspecific bite wounds in Norway and black rats: Implications for rat bite-associated infections in people. Vector-Borne Zoonotic Dis. 14, 94–100. https://doi.org/10.1089/vbz.2013.1417

Higuchi, R., Dollinger, G., Walsh, P.S., Griffith, R., 1992. Simultaneous amplification and detection of specific DNA sequences. J. Biotechnol. 10, 413–417. https://doi.org/.10.1038/nbt0492-413

Higuchi, R., Fockler, C., Dollinger, G., Watson, R., 1993. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. J. Biotechnol. 11, 1026–1030. https://doi.org/.10.1038/nbt0993-1026

Hoare, C.A., 1972. The trypanosomes of mammals. A zoological monographc. Blackwell Scientific Publications, Edinburgh.

Hong, X.K., Zhang, X., Fusco, O.A., Lan, Y.G., Lun, Z.R., Lai, D.H., 2017. PCR-based identification of *Trypanosoma lewisi* and *Trypanosoma musculi* using maxicircle kinetoplast DNA. Acta Trop. 171, 207–212. https://doi.org/10.1016/j.actatropica.2017.04.007

Hookey, J. V., 1991. *Leptospira* and leptospirosis. J. Biol. Educ. 25, 169–172. https://doi.org/10.1080/00219266.1991.9655201

Hsieh, J.W., Tung, K.C., Chen, W.C., Lin, J.W., Chien, L.J., Hsu, Y.M., Wang, H.C., Chomel, B.B., Chang, C.C., 2010. Epidemiology of *Bartonella* infection in rodents and shrews in Taiwan. Zoonoses Public Health 57, 439–446. https://doi.org/10.1111/j.1863-2378.2009.01234.x

Hu, J.J., Meng, Y., Guo, Y.-M., Liao, J.Y., Song, J.L., 2012. Completion of the life cycle of *Sarcocystis zuoi*, a parasite from the Norway rat, *Rattus norvegicus*. J. Parasitol. 98, 550–553. https://doi.org/10.1645/GE-2831.1

Hudson, J.B.,1994. *Chapter*: Mouse cytomegalovirus (murid herpesvirus 1). *In*: Virus infections of rodents and lagomorphs. A.D.M. E. Osterhaus (ed.): Elsevier Science:

Amsterdam pp. 85–117.

Hudson, P.J., Dobson, A.P., Newborn, D., 1992. Do parasites make prey vulnerable to predation? Red grouse and parasites. J. Anim. Ecol. 61, 681–692.

Huff, J.L., Barry, P.A., 2003. B-virus (*Cercopithecine herpesvirus 1*) infection in humans and macaques: potential for zoonotic disease. Emerg. Infect. Dis. 9, 246–250. https://doi.org/10.3201/eid0902.020272

International Committee on Taxonomy of Viruses (ICTV): master species list, 2012. https://ictv.global/taxonomy/

Ikeno, Y., Hubbard, G.B., Lee, S., Cortez, L.A., Lew, C.M., Webb, C.R., Berryman, D.E., List, E.O., Kopchick, J.J., Bartke, A., 2009. Reduced incidence and delayed occurrence of fatal neoplastic diseases in growth hormone receptor/binding protein knockout mice. J. Gerontol. A Biol. Sci. Med. Sci. 64, 522–529. https://doi.org/10.1093/gerona/glp017

Inglis, I.R., Shepherd, D.S., Smith, P., Haynes, P.J., Bull, D.S., Cowan, D.P., Whitehead, D., 1996. Foraging behaviour of wild rats (*Rattus norvegicus*) towards new foods and bait containers. Appl. Anim. Behav. Sci. 47, 175–190. https://doi.org/10.1016/0168-1591(95)00674-5

Italiano, C.M., Wong, K.T., AbuBakar, S., Lau, Y.L., Ramli, N., Syed Omar, S.F., Kahar Bador, M., Tan, C.T., 2014. *Sarcocystis nesbitti* causes acute, relapsing febrile myositis with a high attack rate: description of a large outbreak of muscular sarcocystosis in Pangkor Island, Malaysia, 2012. PLoS Negl. Trop. Dis. 8. https://doi.org/10.1371/journal.pntd.0002876

IUCN 2020. The IUCN Red List of Threatened Species. Version 2020-2. https://www.iucnredlist.org. Downloaded on 09 July 2020.

Jacob, J., Sutherland, D.R., 2004. Murine cytomegalovirus (MCMV) infections in house mice: a matter of age or sex? Wildl. Res. 31, 369–373. https://doi.org/10.1071/WR03130

Jäkel, T., Burgstaller, H., Frank, W., 1996. *Sarcocystis singaporensis*: studies on host specificity, pathogenicity, and potential use as a biocontrol agent of wild rats. J. Parasitol. 82, 280. https://doi.org/10.2307/3284161

Jäkel, T., Khoprasert, Y., Endepols, S., Archer-Baumann, C., Suasa-ard, K., Promkerd, P., Kliemt, D., Boonsong, P., Hongnark, S., 1999. Biological control of rodents using *Sarcocystis singaporensis*. Int. J. Parasitol. 29, 1321–1330. https://doi.org/10.1016/S0020-7519(99)00081-8

Jäkel, T., Khoprasert, Y., Sorger, I., Kliemt, D., Seehabutr, V., Suasa-ard, K., Hongnark, S., 1997. Sarcosporidiasis in rodents from Thailand. J. Wildl. Dis. 33, 860–867.

Johnson, P.T.J., Thieltges, D.W., 2010. Diversity, decoys and the dilution effect: how ecological communities affect disease risk. J. Exp. Biol. 213, 961–970. https://doi.org/10.1242/jeb.037721

Jones, B.A., Grace, D., Kock, R., Alonso, S., Rushton, J., Said, M.Y., McKeever, D., Mutua, F., Young, J., McDermott, J., Pfeiffer, D.U., 2013. Zoonosis emergence linked to agricultural intensification and environmental change. Proc. Natl. Acad. Sci. U. S. A. 110, 8399–8404. https://doi.org/10.1073/pnas.1208059110

Julius, R.S., Schwan, E. V, Chimimba, C.T., 2017. Helminth composition and prevalence of indigenous and invasive synanthropic murid rodents in urban areas of Gauteng Province, South Africa. J. Helminthol. 4, 1–10. https://doi.org/10.1017/S0022149X17000761

Karpagam, K.B., Ganesh, B., 2020. Leptospirosis: a neglected tropical zoonotic infection of public health importance—an updated review. Eur. J. Clin. Microbiol. Infect. Dis. 39, 835–846. https://doi.org/10.1007/s10096-019-03797-4

Kasterine, A., Arbeid, R., Caillabet, O., Natusch, D., 2012. The trade in South-East Asian python skins. International Trade Centre: Geneva, Switzerland. 1-55 pp.

Kataranovski, M., Mirkov, I., Belij, S., Popov, A., Petrović, Z., Gačić, Z., Kataranovski, D., 2011. Intestinal helminths infection of rats (*Rattus norvegicus*) in the Belgrade area (Serbia): the effect of sex, age and habitat. Parasite 18, 189–196. https://doi.org/10.1051/parasite/2011182189

Kaufer, A., Ellis, J., Stark, D., Barratt, J., 2017. The evolution of trypanosomatid taxonomy. Parasit Vectors. 10, 287. https://doi.org/10.1186/s13071-017-2204-7

Kawano, K., 1992. Aggressive behavior of the domesticated house musk shrew (*Suncus murinus*) in inter-male, inter-female and heterosexed interactions. J. Ethol. 10, 119–131. https://doi.org/10.1007/BF02350116

Keesing, F., Holt, R.D., Ostfeld, R.S., 2006. Effects of species diversity on disease risk. Ecol. Lett. 9, 485–498. https://doi.org/10.1111/j.1461-0248.2006.00885.x

Keesing, F., Belden, L.K., Daszak, P., Dobson, A., Harvell, C.D., Holt, R.D., Hudson, P., Jolles, A., Jones, K.E., Mitchell, C.E., Myers, S.S., Bogich, T., Ostfeld, R.S., 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature 468, 647–652. https://doi.org/10.1038/nature09575

Kelt, D.A., Taper, M.L., Meserve, P.L., 1995. Assessing the impact of competition on community assembly: a case study using small mammals. Ecology 76, 1283–1296. https://doi.org/10.2307/1940935

Kergoat, G.J., Granjon, L., Dobigny, G., Bryja, J., Aghová, T., Kimura, Y., 2018. Fossils know it best: Using a new set of fossil calibrations to improve the temporal phylogenetic framework of murid rodents (Rodentia: Muridae). Mol. Phylogenet. Evol. 128, 98–111. https://doi.org/10.1016/j.ympev.2018.07.017

Khanam, S., Mushtaq, M., Nadeem, M.S., Kayani, A.R., 2017. Population ecology of the house mouse (*Mus musculus*) in rural human habitations of Pothwar, Pakistan. Zool. Ecol. 27, 106–113. https://doi.org/10.1080/21658005.2017.1307536

Kheimar, A., Trimpert, J., Groenke, N., Kaufer, B.B., 2019. Overexpression of cellular telomerase RNA enhances virus-induced cancer formation. Oncogene 38, 1778–1786. https://doi.org/10.1038/s41388-018-0544-1

Kitamura, S., Suzuki, S., Yumoto, T., Poonswad, P., Chuailua, P., Plongmai, K., Maruhashi, T., Noma, N., Suckasam, C., 2006. Dispersal of *Canarium euphyllum* (Burseraceae), a large-seeded tree species, in a moist evergreen forest in Thailand. J. Trop. Ecol. 22, 137–146. https://doi.org/10.1017/S0266467405002889

Kitzes, J., Shirley, R., 2016. Estimating biodiversity impacts without field surveys: a case study in northern Borneo. Ambio 45, 110–119. https://doi.org/10.1007/s13280-015-0683-3

Klein, S.L., 2000. The effects of hormones on sex differences in infection: from genes to behavior. Neurosci. Biobehav. Rev. 24, 627–638. https://doi.org/10.1016/S0149-7634(00)00027-0

Klein, S.L., Flanagan, K.L., 2016. Sex differences in immune responses. Nat. Rev. Immunol. 16, 626–638. https://doi.org/10.1038/nri.2016.90

Kleiner, D.E., Brunt, E.M., Van Natta, M., Behling, C., Contos, M.J., Cummings, O.W., Ferrell, L.D., Liu, Y.C., Torbenson, M.S., Unalp-Arida, A., Yeh, M., McCullough, A.J., Sanyal, A.J., 2005. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 41, 1313–1321. https://doi.org/10.1002/hep.20701

Kling, M.A., 2011. A review of respiratory system snatomy, physiology, and disease in the mouse, rat, hamster, and gerbil. Vet. Clin. North Am. Exot. Anim. Pract. 14, 287–337. https://doi.org/10.1016/j.cvex.2011.03.007

Koay, T.K., Nirmal, S., Noitie, L., Tan, E., 2004. An epidemiological investigation of an

outbreak of leptospirosis associated with swimming, Beaufort, Sabah. Med. J. Malaysia 59, 455–9.

Kohn, B., Steinicke, K., Arndt, G., Gruber, A.D., Guerra, B., Jansen, A., Klopfleisch, R., Lotz, F., Luge, E., No, K., 2010. Pulmonary abnormalities in dogs with leptospirosis. J. Vet. Intern. Med 24, 1277–1282. https://doi.org/10.1111/j.1939-1676.2010.0585.x

Koizumi, N., Muto, M., Tanikawa, T., Mizutani, H., Sohmura, Y., Hayashi, E., Akao, N., Hoshino, M., 2019. Human leptospirosis cases and the prevalence of rats harbouring *Leptospira interrogans* in urban areas of Tokyo, Japan. J. Med. Microbiol. 2 1227–1230. https://doi.org/10.1099/jmm.0.011528-0

Koprowski, J., Goldstein, E., Bennett, K. and Pereira, C. 2016. *Chapter*: Family Sciuridae (Tree, flying and ground squirrels, chipmunks, marmots and prairie dogs). *In*: Handbook of the Mammals of the World. Lagomorphs and Rodents. Mittermeier, R.A., Wilson, D.E., Rylands, A.B. (eds.): Lynx Editions, Barcelona, Spain, pp. 648–837.

Krasnov, B.R., Bordes, F., Khokhlova, I.S., Morand, S., 2012. Gender-biased parasitism in small mammals: patterns, mechanisms, consequences. Mammalia 76, 1–13. https://doi.org/10.1515/mammalia-2011-0108

Kriegs, J.O., Churakov, G., Jurka, J., Brosius, J., Schmitz, J., 2007. Evolutionary history of 7SL RNA-derived SINEs in Supraprimates. Trends Genet. 23, 158–161. https://doi.org/10.1016/j.tig.2007.02.002

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054

Laakkonen, J., Goodman, S.M., Duchemin, J.B., Duplantier, J.M., 2003. Trypomastigotes and potential flea vectors of the endemic rodents and the introduced *Rattus rattus* in the rainforests of Madagascar. Biodivers. Conserv. 12, 1775–1783. https://doi.org/10.1023/A:1023611727357

Laakkonen, J., Sukura, A., Haukisalmi, V., Henttonen, H., 1993. *Pneumocystis carinii* and helminth parasitism in shrew *Sorex areaneus araneus* and *Sorex caecutiens*. J. Wildl. Dis. 29, 273–277. https://doi.org/10.7589/0090-3558-29.2.273

Labrière, N., Laumonier, Y., Locatelli, B., Vieilledent, G., Comptour, M., 2015. Ecosystem services and biodiversity in a rapidly transforming landscape in northern Borneo. PLoS One 10, 1–18. https://doi.org/10.1371/journal.pone.0140423

Lack, J.B., Greene, D.U., Conroy, C.J., Hamilton, M.J., Braun, J.K., Mares, M.A., Van Den

Bussche, R.A., 2012. Invasion facilitates hybridization with introgression in the *Rattus rattus* species complex. Mol. Ecol. 21, 3545–3561. https://doi.org/10.1111/j.1365-294X.2012.05620.x

Lane, J.H., Mansfield, K.G., Jackson, L.R., Diters, R.W., Lin, K.C., MacKey, J.J., Sasseville, V.G., 1998. Acute fulminant sarcocystosis in a captive-born *Rhesus Macaque*. Vet. Pathol. 35, 499–505. https://doi.org/10.1177/030098589803500604

LaRose, J.P., Meredith, A.L., Everest, D.J., Fiegna, C., McInnes, C.J., Shaw, D.J., Milne, E.M., 2010. Epidemiological and postmortem findings in 262 red squirrels (*Sciurus vulgaris*) in Scotland, 2005 to 2009. Vet. Rec. 167, 297–302. https://doi.org/10.1136/vr.c4196

Lemaître, J.F., Ronget, V., Tidière, M., Allainé, D., Berger, V., Cohas, A., Colchero, F., Conde, D.A., Garratt, M., Liker, A., Marais, G.A.B., Scheuerlein, A., Székely, T., Gaillard, J.M., 2020. Sex differences in adult lifespan and aging rates of mortality across wild mammals. Proc. Natl. Acad. Sci. U. S. A. 117, 8546–8553. https://doi.org/10.1073/pnas.1911999117

Levett, P.N., 2001. Leptospirosis. Clin. Microbiol. Rev. 14, 296–326. https://doi.org/10.1128/CMR.14.2.296-326.2001

Lewis, S.M., Williams, A., Eisenbarth, S.C., 2019. Structure and function of the immune system in the spleen. Sci. Immunol. 4, eaau6085. https://doi.org/10.1126/sciimmunol.aau6085

Linardi, P.M., Botelho, J.R., 2002. Prevalence of T*rypanosoma lewisi* in *Rattus norvegicus* from Belo Horizonte, State of Minas Gerais, Brazil. Mem. Inst. Oswaldo Cruz 97, 411–414. https://doi.org/10.1590/S0074-02762002000300024

Livingston, R.S., Besch-Williford, C.L., Myles, M.H., Franklin, C.L., Crim, M.J., Riley, L.K., 2011. *Pneumocystis carinii* infection causes lung lesions historically attributed to rat respiratory virus. Comp. Med. 61, 45–59.

Loehle, C., 1995. Social barriers to pathogen transmission in wild animal populations. Ecology 76, 326–335. https://doi.org/10.2307/1941192

Lumpur, K., 2018. A survey of parasitic infections in wild rats from urban areas in Kuala Lumpur, Malaysia. Malaysian J. Vet. Res. 9, 31–38.

Lun, Z.R., Wen, Y.Z., Uzureau, P., Lecordier, L., Lai, D.H., Lan, Y.G., Desquesnes, M., Geng, G.Q., Yang, T.B., Zhou, W.L., Jannin, J.G., Simarro, P.P., Truc, P., Vincendeau, P., Pays, E., 2015. Resistance to normal human serum reveals *Trypanosoma lewisi* as an

underestimated human pathogen. Mol. Biochem. Parasitol. 199, 58–61. https://doi.org/10.1016/j.molbiopara.2015.03.007

Luong, L.T., Grear, D.A., Hudson, P.J., 2009. Male hosts are responsible for the transmission of a trophically transmitted parasite, *Pterygodermatites peromysci*, to the intermediate host in the absence of sex-biased infection. Int. J. Parasitol. 39, 1263–1268. https://doi.org/10.1016/j.ijpara.2009.03.007

Maciel, E.A.P., de Carvalho, A.L.F., Nascimento, S.F., de Matos, R.B., Gouveia, E.L., Reis, M.G., Ko, A.I., 2008. Household transmission of *Leptospira* infection in urban slum communities. PLoS Negl. Trop. Dis. 2, e154. https://doi.org/10.1371/journal.pntd.0000154

Madsen, O., Amrine, H.M., Springer, M.S., Stanhope, M.J., Scally, M., Douady, C.J., Kao, D.J., DeBry, R.W., Adkins, R., de Jong, W.W., 2001. Parallel adaptive radiations in two major clades of placental mammals. Nature 409, 610–614.

Mafie, E., Saito-Ito, A., Kasai, M., Hatta, M., Rivera, P.T., Ma, X., Chen, E.-R., Sato, H., Takada, N., 2019. Integrative taxonomic approach of trypanosomes in the blood of rodents and soricids in Asian countries, with the description of three new species. Parasitol. Res. 118, 97–109. https://doi.org/10.1007/s00436-018-6120-3

Mätz-Rensing, K., Jentsch, K.D., Rensing, S., Langenhuyzen, S., Verschoor, E., Niphuis, H., Kaup, F.-J., 2003. Fatal Herpes simplex Infection in a group of common marmosets (*Callithrix jacchus*). Vet. Pathol. 40, 405–411. https://doi.org/10.1354/vp.40-4-405

Mavenyengwa, M., Mukaratirwa, S., Obwolo, M., Monrad, J., 2005. A macro- and light microscopical study of the pathology of *Calicophoron microbothrium* infection in experimentally infected cattle. J. Vet. Res. 72, 321–332.

Maxie, M.G., Miller, M.A., 2015. *Chapter*: Introduction to the diagnostic process. *In*: Jubb, Kennedy and Palmer's pathology of domestic animals. Maxie, M.G. (ed.): Elsevier Saunders, Philadelphia, pp. 1-15

Mayer-Scholl, A., Hammerl, J.A., Schmidt, S., Ulrich, R.G., Pfeffer, M., Woll, D., Scholz, H.C., Thomas, A., Nöckler, K., 2014. *Leptospira* spp. in rodents and shrews in Germany. Int. J. Environ. Res. Public Health 11, 7562–7574. https://doi.org/10.3390/ijerph110807562

McClelland, E.E., Smith, J.M., 2011. Gender specific differences in the immune response to infection. Arch. Immunol. Ther. Exp.59, 203–213. https://doi.org/10.1007/s00005-011-0124-3

McFarlane, R., Sleigh, A., McMichael, T., 2012. Synanthropy of wild mammals as a

determinant of emerging infectious diseases in the Asian-Australasian region. Ecohealth 9, 24–35. https://doi.org/10.1007/s10393-012-0763-9

McGarry, J.W., Higgins, A., White, N.G., Pounder, K.C., Hetzel, U., 2014. Zoonotic helminths of urban brown rats (*Rattus norvegicus*) in the UK: neglected public health considerations? Zoonoses Public Health. 62, 44–52. https://doi.org/10.1111/zph.12116

McKinney, M.L., 2008. Effects of urbanization on species richness: a review of plants and animals. Urban Ecosyst. 11, 161–176. https://doi.org/10.1007/s11252-007-0045-4

Medway, L., 1969. The wild mammals of Malaya and offshore islands including Singapore., Oxford University Press, Kuala Lumpur

Mehlhorn, H., 2016. Animal Parasites. Springer Spektrum, New York https://doi.org/10.1007/978-3-319-46403-9

Mehlhorn, H., Heydorn, A.O., 1978. The Sarcosporidia (Protozoa, Sporozoa): life cycle and fine structure, in: Adv Parasitol. pp. 43–91. https://doi.org/10.1016/S0065-308X(08)60572-2

Meiri, S., Meijaard, E., Wich, S.A., Groves, C.P., Helgen, K.M., 2008. Mammals of Borneo - small size on a large island. J. Biogeogr. 35, 1087–1094. https://doi.org/10.1111/j.1365-2699.2008.01897.x

Milazzo, C., di Bella, C., Casanova, J.C., Ribas, A., Cagnin, M., 2010. Comunità elmintica del topo selvatico (*Apodemus sylvaticus*) lungo il fiume Avena (Calabria, Italia meridionale). Hystrix 21, 171–176. https://doi.org/10.4404/Hystrix-21.2-4477

Milleliri, J.M., Tirandibaye, H.N., Nan-Madjoum, B., 1989. The focus of human African trypanosomiasis in Moissala (Chad): prospective study of 16 villages using the direct card agglutination test (Testryp CATT) and the ion exchange mini-column (mAECT). Med. Trop. (Mars). 49, 253–8.

Mills, J.N., 2006. Biodiversity loss and emerging infectious disease: an example from the rodent-borne hemorrhagic fevers. Biodiversity 7, 9–17. https://doi.org/10.1080/14888386.2006.9712789

Milocco, C., Kamyingkird, K., Desquesnes, M., Jittapalapong, S., Herbreteau, V., Chaval, Y., Douangboupha, B., Morand, S., 2013. Molecular demonstration of *Trypanosoma evansi* and *Trypanosoma lewisi* DNA in wild rodents from Cambodia, Lao PDR and Thailand. Transbound. Emerg. Dis. 60, 17–26. https://doi.org/10.1111/j.1865-1682.2012.01314.x

Mirzaei, M., Rezaei, H., 2016. The role of sheep in the epidemiology of *Sarcocystis* spp. in

Tabriz area northwest of Iran. J. Parasit. Dis. 40, 285–288. https://doi.org/10.1007/s12639-014-0495-6

Modavi, S., Isseroff, H., 1984. *Fasciola hepatica*: Collagen deposition and other histopathology in the rat host's bile duct caused by the parasite and by proline infusion. Exp. Parasitol. 58, 239–244. https://doi.org/10.1016/0014-4894(84)90040-7

Mohamed-Ha, S.N., Bahaman, A.R., Mutalib, A.R., Khairani-B, S., 2012. Prevalence of pathogenic leptospires in rats from selected locations in peninsular Malaysia. Res. J. Anim. Sci. 6, 12–25. https://doi.org/10.3923/rjnasci.2012.12.25

Mohamed-Hassan, S.N., Bahaman, A.R., Mutalib, A.R., Khairani-Bejo, S., 2010. Serological prevalence of leptospiral infection in wild rats at the national service training centres in Kelantan and Terengganu. Trop. Biomed. 27, 30–32. https://doi.org/10.5540/tema.2008.09.01.0155

Mohd Zain, S.N., Behnke, J.M., Lewis, J.W., 2012. Helminth communities from two urban rat populations in Kuala Lumpur, Malaysia. Parasit Vectors. 5, 1–23. https://doi.org/10.1186/1756-3305-5-47

Mohr, U., Dungworth, D.L., 1988. Relevance to humans of experimentally induced pulmonary tumors in rats and hamsters. Inhal. Toxicol. 209–232. https://doi.org/10.1007/978-3-642-61355-5_13

Monadjem, A., Taylor, P.J., Denys, C., Cotterill, F.P., 2015. Rodents of sub-saharan Africa: a biogeographic and taxonomic synthesis. Walter de Gruyter GmbH & Co. KG, Berlin

Monahan, A.M., Callanan, J.J., Nally, J.E., 2009. Review paper: host-pathogen interactions in the kidney during chronic leptospirosis. Vet. Pathol. 46, 792–799. https://doi.org/10.1354/vp.08-VP-0265-N-REV

Moore, J.C., 1959. Relationships among the living squirrels of the Sciurinae. Bull. Am. Mus. Nat. Hist. 118, 157–206.

Mora, M., Napolitano, C., Ortega, R., Poulin, E., Pizarro-Lucero, J., 2015. Feline immunodeficiency virus and feline leukemia virus infection in free-ranging guignas (*Leopardus guigna*) and sympatric domestic cats in human perturbed landscapes on ChiloÉ Island, Chile. J. Wildl. Dis. 51, 199–208. https://doi.org/10.7589/2014-04-114

Morales-Montor, J., Chavarria, A., De León, M.A., Del Castillo, L.I., Escobedo, E.G., Sánchez, E.N., Vargas, J.A., Hernández-Flores, M., Romo-González, T., Larralde, C., 2004. Host gender in parasitic infections of mammals: an evaluation of the female host supremacy paradigm. J. Parasitol. 90, 531–546. https://doi.org/10.1645/GE-113R3

Morand, S., Blasdell, K., Bordes, F., Buchy, P., Carcy, B., Chaisiri, K., Chaval, Y., Claude, J., Cosson, J.F., Desquesnes, M., Jittapalapong, S., Jiyipong, T., Karnchanabanthoen, A., Pornpan, P., Rolain, J.M., Tran, A., 2019. Changing landscapes of Southeast Asia and rodent-borne diseases: decreased diversity but increased transmission risks. Ecol. Appl. 29, 1–15. https://doi.org/10.1002/eap.1886

Morand, S., Chaisiri, K., Chaval, Y., Claude, J., Tran, A., Herbreteau, V., 2015. Assessing the distribution of disease-bearing rodents in human-modified tropical landscapes. J. Appl. Ecol. 2, 784–794. https://doi.org/10.1111/1365-2664.12414

Moreno, N., Muñoz, L.F., Londoño, A.F., Ángel, J.C., Agudelo-Flórez, P., Loaiza, E.T., Rodas, J.D., Quiroz, V.H., 2009. Prevalence of *Leptospira* spp. in urban rodents from a groceries trade center of Medellín, Colombia. Am. J. Trop. Med. Hyg. 81, 906–910. https://doi.org/10.4269/ajtmh.2009.09-0195

Morrisey, J.K., 1996. Parasites of ferrets, rabbits, and rodents. Semin. Avian Exot. Pet Med. 5, 106–114. https://doi.org/10.1016/S1055-937X(96)80023-5

Morrison, D.A., 2008. Prospects for elucidating the phylogeny of the Apicomplexa. Parasite 15, 191–196. https://doi.org/10.1051/parasite/2008153191

Morrison, D.A., Bornstein, S., Thebo, P., Wernery, U., Kinne, J., Mattsson, J.G., 2004. The current status of the small subunit rRNA phylogeny of the coccidia (Sporozoa). Int. J. Parasitol. 34, 501–514. https://doi.org/10.1016/j.ijpara.2003.11.006

Mühldorfer, K., 2013. Bats and Bacterial Pathogens: A review. Zoonoses Public Health 60, 93–103. https://doi.org/10.1111/j.1863-2378.2012.01536.x

Mühldorfer, K., Speck, S., Wibbelt, G., 2011. Diseases in free-ranging bats from Germany. BMC Vet. Res. 7, 61. https://doi.org/10.1186/1746-6148-7-61

Mullis, K., Faloona, R., Scharf, S., Saiki, R., Horn, G. and Erlich, H. 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb. Symp. Quant. Biol. 51: 263–273. https://doi.org/10.1101/sqb.1986.051.01.032

Mullin, S.W., Colley, F.C., Stevens, G.S., 1972. Coccidia of Malaysian mammals: new host records and descriptions of three new species of *Eimeria*. J. Protozool. 19, 260–263. https://doi.org/10.1111/j.1550-7408.1972.tb03453.x

Mullin, S.W., Orihel, T.C., 1972. *Tetrapetalonema dunni* sp. n. (Nematoda: Filarioidea) from Malaysian Tree Shrews. J. Parasitol. 58, 1047. https://doi.org/10.2307/3278131

Murray, K.A., Daszak, P., 2014. Human ecology in pathogenic landscapes: two hypotheses

on how land use change drives viral emergence. Curr. Opin. Virol. 3, 79–83. https://doi.org/10.1016/j.coviro.2013.01.006

Musser, G., Carleton, M., 2005. *Chapter*: Superfamily Muroidea. *In*: Mammal species of the world: a taxonomic and geographic reference. Wilson, D.E., Reeder, D.M., (eds.): Johns Hopkins University, Baltimore, pp. 894-1531.

Myers, N., 1988. Threatened Biotas : "Hot Spots" in tropical forests. Environmentalist. 8, 187–208. https://doi.org/10.1007/BF02240252

Myers, N., Mittermeier, R., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature. 403, 853–858. https://doi.org/10.1038/35002501

Nakagawa, M., Miguchi, H., Nakashizuka, T., 2006. The effects of various forest uses on small mammal communities in Sarawak, Malaysia. For. Ecol. Manage. 231, 55–62. https://doi.org/10.1016/j.foreco.2006.05.006

Nakagawa, M., Miguchi, H., Sato, K., Nakashizuka, T., 2007. A preliminary study of two sympatric *Maxomys* rats in Sarawak, Malaysia: spacing patterns and population dynamics. Raffles Bull. Zool. 55, 381–387.

Nakamura, K., Shoya, S., Nakajima, Y., Shimura, K., Ito, S., 1982. Pathology of experimental acute sarcocystosis in a cow. Japanese J. Vet. Sci. 44, 675–679. https://doi.org/10.1292/jvms1939.44.675

Narnaware, S.D., Ghorui, S.K., Kumar, S., Patil, N. V., 2016. Vertical transmission of *Trypanosoma evansi* in dromedary camels and studies on fetal pathology, diagnosis and treatment. Acta Parasitol. 61, 329–336. https://doi.org/10.1515/ap-2016-0043

National Research Council, 1991. Infectious diseases of mice and rats. National Academies Press, Washington, D.C. https://doi.org/10.17226/1429

Neupane, B., Miller, A.L., Evans, A.L., Olsson, G.E., Höglund, J., 2020. Seasonal variation of *Mastophorus muris* (Nematoda: Spirurida) in the water vole *Arvicola amphibius* from southern Sweden. J. Helminthol. 94. https://doi.org/10.1017/S0022149X18000937

Neves, E.S., Mendenhall, I.H., Borthwick, S.A., Su, Y.C.F., Smith, G.J.D., 2018. Detection and genetic characterization of diverse *Bartonella* genotypes in the small mammals of Singapore. Zoonoses Public Health 65, e207–e215. https://doi.org/10.1111/zph.12430

Newman, A., 2002. Tropical Rainforest: our most valuable and endangered habitat with a blueprint for Its survival into the third millennium. Checkmark Books, New York.

Nolte, T., Thiedemann, K.-U., Dungworth, D.L., Ernst, H., Paulini, I., Heinrich, U., Dasenbrock, C., Peters, L., Ueberschär, S., Mohr, U., 1993. Morphology and histogenesis of squamous cell metaplasia of the rat lung after chronic exposure to a pyrolized pitch condensate and/or carbon black, or to combinations of pyrolized pitch condensate, carbon black and irritant gases. Exp. Toxicol. Pathol. 45, 135–144. https://doi.org/10.1016/S0940-2993(11)80489-6

Noyes, H., Stevens, J., Teixeira, M., Phelan, J., Holz, P., 2000. Corrigendum to “A nested PCR for the ssrRNA gene detects *Trypanosoma binneyi* in the platypus and *Trypanosoma* sp. in wombats and kangaroos in Australia”. Int. J. Parasitol. 29, 331–339. https://doi.org/10.1016/S0020-7519(00)00026-6

Noyes, H., 1998. Can *Trypanosoma* trees be trusted? Parasitol. Today 14, 49–50. https://doi.org/10.1016/S0169-4758(97)01159-9

Noyes, H.A., Perez Camps, A., Chance, M.L., 1996. *Leishmania herreri* (kinetoplastida; trypanosomatidae) is more closely related to *Endotrypanum* (kinetoplastida; trypanosomatidae) than to *Leishmania*. Mol. Biochem. Parasitol. 80, 119–123. https://doi.org/10.1016/0166-6851(96)02679-5

Noyes, H.A., Stevens, J.R., Teixeira, M., Phelan, J., Holz, P., 1999. A nested PCR for the ssrRNA gene detects *Trypanosoma binneyi* in the platypus and *Trypanosoma* sp. in wombats and kangaroos in Australia. Int. J. Parasitol. 29, 331–339. https://doi.org/10.1016/S0020-7519(98)00167-2

Ntumvi, N.F., Mbala Kingebeni, P., Tamoufe, U., Kumakamba, C., Ndze, V., Ngay Lukusa, I., LeBreton, M., Atibu Losoma, J., Le Doux Diffo, J., N’Kawa, F., Takuo, J.-M., Mulembakani, P., Nwobegahay, J., Makuwa, M., Muyembe Tamfum, J.J., Gillis, A., Harris, S., Rimoin, A.W., Hoff, N.A., Fair, J.M., Monagin, C., Ayukekbong, J., Rubin, E.M., Wolfe, N.D., Lange, C.E., 2018. High Herpesvirus diversity in wild rodent and shrew species in Central Africa. Intervirology 61, 155–165. https://doi.org/10.1159/000493796

O’Donoghue, P.J., Rommel, M., Weber, M., Weyreter, H., 1985. Attempted immunization of swine against acute sarcocystosis using cystozoite-derived vaccines. Vet. Immunol. Immunopathol. 8, 83–92. https://doi.org/10.1016/0165-2427(85)90112-6

O’Donoghue, P.J., Watts, C.H.S., Dixon, B.R., 1987. Ultrastructure of *Sarcocystis* spp. (Protozoa: Apicomplexa) in rodents from North Sulawesi and West Java, Indonesia. J. Wildl. Dis. 23, 225–232. https://doi.org/10.7589/0090-3558-23.2.225

Odening, K., 1998. The present state of species-systematics in *Sarcocystis Lankester*, 1882

(Protista, Sporozoa, Coccidia). Syst. Parasitol. 41, 209–233. https://doi.org/10.1023/A:1006090232343

Ohdachi, S.D., Hasegawa, M., Iwasa, M.A., Vogel, P., Oshida, T., Lin, L.K., Abe, H., 2006. Molecular phylogenetics of soricid shrews (Mammalia) based on mitochondrial cytochrome b gene sequences: with special reference to the Soricinae. J. Zool. 270, 177–191. https://doi.org/10.1111/j.1469-7998.2006.00125.x

Ongradi, J., 2016. Herpesviridae. IntechOpen, London. https://doi.org/10.5772/61923

Orlofske, S.A., Jadin, R.C., Hoverman, J.T., Johnson, P.T.J., 2014. Predation and disease: understanding the effects of predators at several trophic levels on pathogen transmission. Freshw. Biol. 59, 1064–1075. https://doi.org/10.1111/fwb.12329

Ortega Pérez, P., Wibbelt, G., Brinkmann, A., Galindo Puentes J.A., Tuh, F.Y.Y., Maklarin, L.B., Nitsche, A., Wells, K., Jäkel, T., 2020. Description of *Sarcocystis scandentiborneensis* sp. nov. from treeshrews (*Tupaia minor*, *T. tana*) in northern Borneo with annotations on the utility of COI and 18S rDNA sequences for species delineation. Int. J. Parasitol. Parasites Wildl. 12, 220–231. https://doi.org/10.1016/j.ijppaw.2020.07.003

Ostfeld, R.S., Keesing, F., 2000. The function of biodiversity in the ecology of vector-borne zoonotic diseases. Can. J. Zool. 78, 2061–2078. https://doi.org/10.1139/cjz-78-12-2061

Ostfeld, R.S., Keesing, F., 2012. Effects of host diversity on infectious disease. Annu. Rev. Ecol. Evol. Syst. 43, 157–182. https://doi.org/10.1146/annurev-ecolsys-102710-145022

Ostfeld, R.S., Holt, R.D., 2004. Are predators good for your health? Evaluating evidence for top-down regulation of zoonotic disease reservoirs. Front. Ecol. Environ. 2, 13-19. doi: 10.1890/1540-9295(2004)002[0013:APGFYH]2.0.CO;2

Ozuru, R., Saito, M., Kanemaru, T., Miyahara, S., Villanueva, S.Y.A.M., Murray, G.L., Adler, B., Fujii, J., Yoshida, S.I., 2017. Adipose tissue is the first colonization site of *Leptospira interrogans* in subcutaneously infected hamsters. PLoS One 12, 1–13. https://doi.org/10.1371/journal.pone.0172973

Pagès, M., Bazin, E., Galan, M., Chaval, Y., Claude, J., Herbreteau, V., Michaux, J., Piry, S., Morand, S., Cosson, J., 2013. Cytonuclear discordance among Southeast Asian black rats (*Rattus rattus* complex). Mol. Ecol. 22, 1019–1034. https://doi.org/10.1111/mec.12149

Pagès, M., Chaval, Y., Herbreteau, V., Waengsothorn, S., Cosson, J.-F., Hugot, J.-P., Morand, S., Michaux, J., 2010. Revisiting the taxonomy of the Rattini tribe: a phylogeny-

based delimitation of species boundaries. BMC Evol. Biol. 10, 184. https://doi.org/10.1186/1471-2148-10-184

Palmer, R.J., Settnes, O.P., Lodal, J., Wakefield, A.E., 2000. Population structure of rat-derived *Pneumocystis carinii* in Danish wild rats. Appl. Environ. Microbiol. 66, 4954–61.

Paperna, I., Peh, K.S.H., Martelli, P., Koh, L.P., Sodhi, N.S., 2004. Factors affecting *Sarcocystis* infection of rats on small tropical islands. Ecol. Res. 19, 475–483. https://doi.org/10.1111/j.1440-1703.2004.00661.x

Parker, T.S., Nilon, C.H., 2012. Urban landscape characteristics correlated with the synurbization of wildlife. Landsc. Urban Plan. 106, 316–325. https://doi.org/10.1016/j.landurbplan.2012.04.003

Patz, J.A., Graczyk, T.K., Geller, N., Vittor, A.Y., 2000. Effects of environmental change on emerging parasitic diseases. Int. J. Parasitol. 30, 1395–1405. https://doi.org/10.1016/S0020-7519(00)00141-7

Payn, J., Francis, C., Phillipps, K., 2016. A field guide to the mammals of Borneo. The Sabah Society and World Wildlife Fund, Kota Kinabalu.

Payne, L.N., Venugopal, K., 2000. Neoplastic diseases: Marek's disease, avian leukosis and reticuloendotheliosis. OIE Rev. Sci. Tech. 19, 544–564. https://doi.org/10.20506/rst.19.2.1226

Payne, S., 2017. Viruses: from understanding to investigation. Academic Press, London.

Pellett, P., Roizman, B., 2007. *Chapter*: The family Herpesviridae: a brief introduction. *In*: Fields Virology. Knipe, D.M., Howley, P.M., (eds.): Lippincott, Williams and Wilkins, Philadelphia, pp. 2479–2499.

Pépin, M., 2016. Of rats and pathogens: pathogens transmitted by urban rats with an emphasis on hantaviruses. CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 11. https://doi.org/10.1079/PAVSNNR201611019

Percy, D.H., Barthold, S.W., 2007. *Chapter*: Rat pathology. *In*: Pathology of laboratory rodents and rodents and rabbits. Percy, D.H., Barthold, S.W. (eds.): Blackwell Publishing Professional, Oxford, pp 125–177. https://doi.org/10.1002/9780470344613

Pereira, C.L., Teixeira, M.M.G., da Silva, F.M., Desquesnes, M., Ortiz, P.A., Camargo, E.P., Jittapalapong, S., Neves, L., Lima, L., Garcia, H.A., Campaner, M., 2017. Diagnosis and genetic analysis of the worldwide distributed Rattus-borne *Trypanosoma* (Herpetosoma) *lewisi* and its allied species in blood and fleas of rodents. Infect. Genet. Evol. 63, 380–

390. https://doi.org/10.1016/j.meegid.2017.09.001

Perry, D.A., Oren, R., Hart, S.C., 2008. Forest ecosystems. The Johns Hopkins University Press, Baltimore.

Picardeau, M., 2017. Virulence of the zoonotic agent of leptospirosis: still terra incognita? Nat. Rev. Microbiol. 15, 297–307. https://doi.org/10.1038/nrmicro.2017.5

Pietsch, H., 2013. Detektion viraler Erreger in Nagern. Robert Koch Institute, Bachelor thesis.

Poulsen, C.S., Stensvold, C.R., 2014. Current status of epidemiology and diagnosis of human sarcocystosis. J. Clin. Microbiol. 52, 3524–3530. https://doi.org/10.1128/JCM.00955-14

Prakas, P., Oksanen, A., Butkauskas, D., Sruoga, A., Kutkienė, L., Švažas, S., Isomursu, M., Liaugaudaitė, S., 2014. Identification and intraspecific genetic diversity of *Sarcocystis rileyi* from ducks, *Anas* spp., in Lithuania and Finland. J. Parasitol. 100, 657–661. https://doi.org/10.1645/13-395.1

Priscott, P.K., Tyrrell, D.A.J., 1982. The isolation and partial characterisation of a cytomegalovirus from the brown rat, *Rattus norvegicus*. Arch. Virol. 73, 145–160. https://doi.org/10.1007/BF01314723

Pui, C.F., Bilung, L.M., Apun, K., Su'ut, L., 2017. Diversity of *Leptospira* spp. in rats and environment from urban areas of Sarawak, Malaysia. J. Trop. Med. 2017, 1–8. https://doi.org/10.1155/2017/3760674

Pumhom, P., Morand, S., Tran, A., Jittapalapong, S., Desquesnes, M., 2015. *Trypanosoma* from rodents as potential source of infection in human-shaped landscapes of South-East Asia. Vet. Parasitol. 208, 174–180. https://doi.org/10.1016/j.vetpar.2014.12.027

Pumhom, P., Pognon, D., Yangtara, S., Thaprathorn, N., Milocco, C., Douangboupha, B., Herder, S., Chaval, Y., Morand, S., Jittapalapong, S., Desquesnes, M., 2014. Molecular prevalence of *Trypanosoma* spp. in wild rodents of Southeast Asia: influence of human settlement habitat. Epidemiol. Infect. 142, 1221–1230. https://doi.org/10.1017/S0950268813002161

Rafinejad, J., Piazak, N., Dehghan, A., 2013. Affect of some environmental parameters on fleas density in human and animal shelters. Am. J. Res. Commun. 1:2325–4076

Rahelinirina, S., Rajerison, M., Telfer, S., Savin, C., Carniel, E., Duplantier, J.M., 2017. The Asian house shrew *Suncus murinus* as a reservoir and source of human outbreaks of plague in Madagascar. PLoS Negl. Trop. Dis. 11, 1–14. https://doi.org/10.1371/journal.pntd.0006072

Ribeiro, R. V, Souza, G.M., Oliveira, R.F., Machado, E.C., 2005. Photosynthetic responses of tropical tree species from different successional groups under contrasting irradiance conditions. Rev. Bras. Botânica 28, 149–161. https://doi.org/10.1590/s0100-84042005000100012

Rissi, D.R., Barros, C.S.L., 2013. Necrotizing meningoencephalitis in a cow. Vet. Pathol. 50, 926–929. https://doi.org/10.1177/0300985813478209

Rissman, E.F., Nelson, R.J., Blank, J.L., Bronson, F.H., 1987. Reproductive response of a tropical mammal, the musk shrew (*Suncus murinus*), to photoperiod. Reproduction 81, 563–566. https://doi.org/10.1530/jrf.0.0810563

Robinson, D.G., Ehlers, U., Herken, R., Herrman, B., Mayer, F., Schurmann, F.W., 1987. Methods of preparation for electron microscopy: An Introduction for the biomedical sciences. Springer-Verlag, Tokyo.

Rodrigues, M.S., Lima, L., Xavier, S.C. das C., Herrera, H.M., Rocha, F.L., Roque, A.L.R., Teixeira, M.M.G., Jansen, A.M., 2019. Uncovering *Trypanosoma* spp. diversity of wild mammals by the use of DNA from blood clots. Int. J. Parasitol. Parasites Wildl. 8, 171–181. https://doi.org/10.1016/j.ijppaw.2019.02.004

Rohr, J.R., Civitello, D.J., Crumrine, P.W., Halstead, N.T., Miller, A.D., Schotthoefer, A.M., Stenoien, C., Johnson, L.B., Beasley, V.R., 2015. Predator diversity, intraguild predation, and indirect effects drive parasite transmission. Proc. Natl. Acad. Sci. U. S. A. 112, 3008–3013. https://doi.org/10.1073/pnas.1415971112

Roizman, B., Baines, J., 1991. The diversity and unity of herpesviridae. Comp. Immunol. Microbiol. Infect. Dis. 14, 63–79. https://doi.org/10.1016/0147-9571(91)90122-T

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542. https://doi.org/10.1093/sysbio/sys029

Rossetti, C.A., Vanasco, B.N., Pini, N., Carfagnini, J.C., 2004. Comparison of three diagnostic techniques for the detection of leptospires in the kidneys of wild house mice (*Mus musculus*). Pesqui. Vet. Bras. 24, 6–10.

Rosshart, S.P., Vassallo, B.G., Angeletti, D., Villena, F.P. De, Yewdell, J.W., Takeda, K., Hickman, H.D., Mcculloch, J.A., Badger, J.H., Ajami, N.J., Trinchieri, G., 2017. Wild mouse gut microbiota promotes host fitness and improves disease resistance article wild mouse gut microbiota promotes host fitness and improves disease resistance. Cell 171,

1015-1021.e13. https://doi.org/10.1016/j.cell.2017.09.016

Rothenburger, J.L., Himsworth, C.G., Chang, V., LeJeune, M., Leighton, F.A., 2014. *Capillaria hepatica* in Wild Norway Rats (*Rattus norvegicus*) from Vancouver, Canada. J. Wildl. Dis. 50, 628–633. https://doi.org/10.7589/2013-09-256

Rothenburger, Jamie L, Himsworth, C.G., La Perle, K.M.D., Leighton, F.A., Nemeth, N.M., Treuting, P.M., Jardine, C.M., 2019. Pathology of wild Norway rats in Vancouver, Canada. J. Vet. Diagnostic Investig. 31, 184–199. https://doi.org/10.1177/1040638719833436

Rothenburger, Jamie L., Himsworth, C.G., Nemeth, N.M., Pearl, D.L., Treuting, P.M., Jardine, C.M., 2019. The devil is in the details—Host disease and co-infections are associated with zoonotic pathogen carriage in Norway rats (*Rattus norvegicus*). Zoonoses Public Health 66, 622–635. https://doi.org/10.1111/zph.12615

Rothenburger, J.L., Himsworth, C.G., Treuting, P.M., Leighton, F. a, 2015a. Respiratory pathology and pathogens in wild urban rats (*Rattus norvegicus* and *Rattus rattus*). Vet. Pathol. 52, 201–208. https://doi.org/10.1177/0300985814528220

Rothenburger, J.L., Himsworth, C.G., Treuting, P.M., Leighton, F. a, 2015b. Survey of cardiovascular pathology in wild urban *Rattus norvegicus* and *Rattus rattus*. Vet. Pathol. 52, 201–208. https://doi.org/10.1177/0300985814528220

Rubio, A. V., Ávila-Flores, R., Suzán, G., 2014. Responses of small mammals to habitat fragmentation: epidemiological considerations for rodent-borne hantaviruses in the Americas. Ecohealth 11, 526–533. https://doi.org/10.1007/s10393-014-0944-9

Ruedi, M., Courvoisier, C., Vogel, P., Catzeflis, F.M., 1996. Genetic differentiation and zoogeography of Asian *Suncus murinus* (Mammalia: Soricidae). Biol. J. Linn. Soc. 57, 307–316. https://doi.org/10.1111/j.1095-8312.1996.tb01852.x

Russell, G.C., Stewart, J.P., Haig, D.M., 2009. Malignant catarrhal fever: a review. Vet. J. 179, 324–335. https://doi.org/10.1016/j.tvjl.2007.11.007

Sabra, L., Klein, S.L., 2010. Chapter: Sex differences in susceptibility to viral infection. In: Sex, hormones and immunity to infection. Keifer, G., Effenberger, F. (eds.): Springer, Berlin, pp. 93-122.

Sachs, J.L., Essenberg, C.J., Turcotte, M.M., 2011. New paradigms for the evolution of beneficial infections. Trends Ecol. Evol. 26, 202–209. https://doi.org/10.1016/j.tree.2011.01.010

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., Erlich, H.A., 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239: 487–491. https://doi.org/10.1126/science.239.4839.487

Santamaría, J., Toranzos, G.A., 2003. Enteric pathogens and soil: a short review. Int. Microbiol. 6, 5–9. https://doi.org/10.1007/s10123-003-0096-1

Santos, N. de J., Sousa, E., Reis, M.G., Ko, A.I., Costa, F., 2017. Rat infestation associated with environmental deficiencies in an urban slum community with high risk of leptospirosis transmission. Cad. Saude Publica 33, 1–13. https://doi.org/10.1590/0102-311x00132115

Sarataphan, N., Vongpakorn, M., Nuansrichay, B., Autarkool, N., Keowkarnkah, T., Rodtian, P., Stich, R.W., Jittapalapong, S., 2007. Diagnosis of a *Trypanosoma lewisi*-like (Herpetosoma) infection in a sick infant from Thailand. J. Med. Microbiol. 56, 1118–1121. https://doi.org/10.1099/jmm.0.47222-0

Sarkar, J., Chopra, A., Katageri, B., Raj, H., Goel, A., 2012. Leptospirosis: a re-emerging infection. Asian Pac. J. Trop. Med. 5, 500–502. https://doi.org/10.1016/S1995-7645(12)60086-8

Savage, D.C., 1987. Microorganisms associated with epithelial surfaces and stability of the indigenous gastrointestinal microflora. Nahrung 31, 383–395. https://doi.org/10.1002/food.19870310511

Schmidt, R.E., 1995. Protozoal diseases of rabbits and rodents. Semin. Avian Exot. Pet Med. 4, 126–130. https://doi.org/10.1016/S1055-937X(05)80036-2

Schmitz, J., Ohme, M., Zischler, H., 2000. The complete mitochondrial genome of *Tupaia belangeri* and the phylogenetic affiliation of Scandentia to other Eutherian orders. Mol. Biol. Evol. 17, 1334–1343. https://doi.org/10.1093/oxfordjournals.molbev.a026417

Schoeb, T.R., 2000. Respiratory diseases of rodents. Vet. Clin. North Am. Exot. Anim. Pract. 3, 481–496. https://doi.org/10.1016/S1094-9194(17)30083-X

Seguel, M., Muñoz, F., Paredes, E., Navarrete, M.J., Gottdenker, N.L., 2017. Pathological findings in wild rats (*Rattus rattus*) captured at Guafo Island, Northern Chilean Patagonia. J. Comp. Pathol. 157, 163–173. https://doi.org/10.1016/j.jcpa.2017.07.006

Shackelford, C., Long, G., Wolf, J., Okerberg, C., Herbert, R., 2002. Qualitative and quantitative analysis of non neoplastic lesions in toxicology studies. Toxicol. Pathol. 30, 93–96. https://doi.org/10.1080/01926230252824761

Singleton, G.R., Leirs, H., Hinds, L.A., Zhang, Z.B., 1999. Reevaluating our approach to an old problem. Ecologically-based management of rodent pests. Australian Centre for International Agriculture Research, Canberra. pp. 17-29

Singleton, G.R., Petch, D.E., 1994. A review of the biology and management of rodent pests in southeast Asia. Australian Centre for International Agricultural Research Technical Reports, 30, 65

Siwar, C., Ahmed, F., Bashawir, A., Mia, M.S., 2016. Urbanization and urban poverty in Malaysia: consequences and vulnerability. J. Appl. Sci. 16, 154–160. https://doi.org/10.3923/jas.2016.154.160

Smales, L.R., 2018. Gastrointestinal helminths of *Rattus steini* (Rodentia: Muridae) from Papua New Guinea. Comp. Parasitol. 85, 193–196. https://doi.org/10.1654/1525-2647-85.2.193

Smith, J.E., Friedman, M.I., Andrews, P.L., 2001. Conditioned food aversion in *Suncus murinus* (house musk shrew) — a new model for the study of nausea in a species with an emetic reflex. Physiol. Behav. 73, 593–598. https://doi.org/10.1016/S0031-9384(01)00538-8

Smits, S.L., Schapendonk, C.M.E., Leeuwen, M. Van, Kuiken, T., Bodewes, R., Raj, V.S., Haagmans, B.L., Neves, C.G., Tryland, M., Osterhaus, A.D.M.E., 2013. Identification and characterization of two novel viruses in ocular Infections in reindeer. PLoS One 8, 3–9. https://doi.org/10.1371/journal.pone.0069711

Snider, T.A., Confer, A.W., Payton, M.E., 2010. Pulmonary histopathology of cytauxzoon felis infections in the cat. Vet. Pathol. 47, 698–702. https://doi.org/10.1177/0300985810364527

Snyder, J.M., Ward, J.M., Treuting, P.M., 2016. Cause-of-death analysis in rodent aging studies. Vet. Pathol. 53, 233–243. https://doi.org/10.1177/0300985815610391

Sodhi, N.S., Koh, L.P., Clements, R., Wanger, T.C., Hill, J.K., Hamer, K.C., Clough, Y., Tscharntke, T., Posa, M.R.C., Lee, T.M., 2010. Conserving Southeast Asian forest biodiversity in human-modified landscapes. Biol. Conserv. 143, 2375–2384. https://doi.org/10.1016/j.biocon.2009.12.029

Solari, S., Baker, R.J., 2007. Mammal species of the world: a taxonomic and geographic reference. J. Mammal. 88, 824–830. https://doi.org/10.1644/06-MAMM-R-422.1

Song, S., Liu, L., Edwards, S. V., Wu, S., 2012. Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. Proc. Natl.

Acad. Sci. 109, 14942–14947. https://doi.org/10.1073/pnas.1211733109

Soon Kim, K., Inoue, K., Kabeya, H., Sato, S., Takada, T., Chiu, D., Chiu, S.H., Fujita, H., Kawabata, H., Takada, N., Kariwa, H., Maruyama, S., 2016. Prevalence and diversity of *Bartonella* species in wild small mammals in Asia. J. Wildl. Dis. 52, 10–21. https://doi.org/10.7589/2015-01-015

Sotomayor-Bonilla, J., Moreira-Soto, A., Mendizabal, D., Soley-Guardia, M., Ramírez-Fernández, J.D., Villalobos-Chaves, D., Niehaus, C., Gutiérrez-Espeleta, G., Rico-Chávez, O., Foley, J., Suzán, G., Chaves-Friedlander, A., 2019. Diverse beta- and gammaherpesviruses in neotropical rodents from Costa Rica. J. Wildl. Dis. 55, 663–667. https://doi.org/10.7589/2018-05-117

Steiner, A.L., 1972. Mortality resulting from intraspecific fighting in some ground squirrel populations. J. Mammal. 53, 601–603. https://doi.org/10.2307/1379051

Steppan, S.J., Storz, B.L., Hoffmann, R.S., 2004. Nuclear DNA phylogeny of the squirrels (Mammalia: Rodentia) and the evolution of arboreality from c-myc and RAG1. Mol. Phylogenet. Evol. 30, 703–719. https://doi.org/10.1016/S1055-7903(03)00204-5

Stevensn, S.S., 1946. On the theory of scales of measurement. Science 103, 677–80.

Stevens, J., Gibson, W., 1999. The evolution of salivarian trypanosomes. Mem. Inst. Oswaldo Cruz 94, 225–228. https://doi.org/10.1590/S0074-02761999000200019

Stevens, J.R., Noyes, H.A., Dover, G.A., Gibson, W.C., 1999. The ancient and divergent origins of the human pathogenic trypanosomes, *Trypanosoma brucei* and *T. cruzi*. Parasitology 118, 107–116. https://doi.org/10.1017/S0031182098003473

Stevenson, A.C., 1915. *Klossiella muris*. Q. J. Microsc. Sci. 61, 127–135.

Stoddard, R.A., Gee, J.E., Wilkins, P.P., McCaustland, K., Hoffmaster, A.R., 2009. Detection of pathogenic *Leptospira* spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. Diagn. Microbiol. Infect. Dis. 64, 247–255. https://doi.org/10.1016/j.diagmicrobio.2009.03.014

Strachan, D.P., 1989. Hay fever, hygiene, and household size. BMJ 299, 1259–1260. https://doi.org/10.1136/bmj.299.6710.1259

Su, M., Hui, C., 2011. The effect of predation on the prevalence and aggregation of pathogens in prey. BioSystems 105, 300–306. https://doi.org/10.1016/j.biosystems.2011.05.012

Suzán, G., García-Peña, G.E., Castro-Arellano, I., Rico, O., Rubio, A. V., Tolsá, M.J., Roche, B., Hosseini, P.R., Rizzoli, A., Murray, K.A., Zambrana-Torrelio, C., Vittecoq, M., Bailly,

X., Aguirre, A.A., Daszak, P., Prieur-Richard, A.-H., Mills, J.N., Guégan, J.-F., 2015. Metacommunity and phylogenetic structure determine wildlife and zoonotic infectious disease patterns in time and space. Ecol. Evol. 5, 865–873. https://doi.org/10.1002/ece3.1404

Suzuki, D., Murata, Y., Oda, S.I., 2007. Changes of Ucp1, D2 (Dio2) and Glut4 (Slc2a4) mRNA expression in response to short-term cold exposure in the house musk shrew (*Suncus murinus*). Exp. Anim. 56, 279–288. https://doi.org/10.1538/expanim.56.279

Syed-Arnez, A.S.K., Mohd Zain, S.N., 2006. A study on wild rats and their endoparasite fauna from the Endau Rompin National Park, Johor. Malaysian J. Sci. 25, 19–39.

Sykes, J.E., Hartmann, K., Lunn, K.F., Moore, G.E., Stoddard, R.A., Goldstein, R.E., 2011. 2010 ACVIM Small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. J. Vet. Intern. Med. 25, 1–13. https://doi.org/10.1111/j.1939-1676.2010.0654.x

Tamarin, R.H., Malecha, S.R., 1972. Reproductive parameters in *Rattus rattus* and *Rattus exulans* of Hawaii, 1968 to 1970. J. Mammal. 53, 513–528. https://doi.org/10.2307/1379041

Tan, W.L., Soelar, S.A., Suan, M.A.M., Hussin, N., Cheah, W.K., Verasahib, K., Goh, P.P., 2016. Leptospirosis incidence and mortality in Malaysia. Southeast Asian J. Trop. Med. Public Health 47, 434–440.

Tanner, E., 1990. Tropical rain forest ecosystems: biogeographical and ecological studies. Trends Ecol. Evol. 5, 377. https://doi.org/10.1016/0169-5347(90)90107-O

Tannock, G.W., Savage, D.C., 1974. Influences of dietary and environmental stress on microbial populations in the murine gastrointestinal tract. Infect. Immun.9, 591–598.

Tanowitz, H.B., Machado, F.S., Jelicks, L.A., Shirani, J., Campos de Carvalho, A.C., Spray, D.C., Factor, S.M., Kirchhoff, L. V., Weiss, L.M., 2009. Perspectives on *Trypanosoma cruzi*–Induced heart disease (Chagas Disease). Prog. Cardiovasc. Dis. 51, 524–539. https://doi.org/10.1016/j.pcad.2009.02.001

Taylor, J.L., Wagner, J.E., Kusewitt, D.F., Mann, P.C., 1979. *Klossiella* parasites of animals: a literature review. Vet. Parasitol. 5, 137–144. https://doi.org/10.1016/0304-4017(79)90005-0

Temple, J.L., Rissman, E.F., 2000. Brief refeeding restores reproductive readiness in food-restricted female musk shrews (*Suncus murinus*). Horm. Behav. 38, 21–28. https://doi.org/10.1006/hbeh.2000.1596

Thayaparan, S., Robertson, I.D., Abdullah, M.T., 2014. Leptospiral agglutinins in captive and free ranging non-human primates in Sarawak, Malaysia. Vet. World. 7, 428–431. https://doi.org/10.14202/vetworld.2014. 428-431

Thery, M., 2001. Forest light and its influence on habitat selection. Plant Ecol. 153, 251–261. https://doi.org/10.1023/A:1017592631542

Tidière, M., Badruna, A., Fouchet, D., Gaillard, J.M., Lemaître, J.F., Pontier, D., 2020. Pathogens shape sex differences in mammalian aging. Trends Parasitol. 36, 668–676. https://doi.org/10.1016/j.pt.2020.05.004

Thibeaux, R., Geroult, S., Benezech, C., Chabaud, S., Soupé-Gilbert, M.E., Girault, D., Bierque, E., Goarant, C., 2017. Seeking the environmental source of leptospirosis reveals durable bacterial viability in river soils. PLoS Negl. Trop. Dis. 11, 1–14. https://doi.org/10.1371/journal.pntd.0005414

Thompson, C.K., Godfrey, S.S., Thompson, R.C.A., 2014. Trypanosomes of Australian mammals: a review. Int. J. Parasitol. Parasites Wildl. 3, 57–66. https://doi.org/10.1016/j.ijppaw.2014.02.002

Thoolen, B., Maronpot, R.R., Harada, T., Nyska, A., Rousseaux, C., Nolte, T., Malarkey, D.E., Kaufmann, W., Küttler, K., Deschl, U., Nakae, D., Gregson, R., Vinlove, M.P., Brix, A.E., Singh, B., Belpoggi, F., Ward, J.M., 2010. Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. Toxicol. Pathol. 38, 5–81. https://doi.org/10.1177/0192623310386499

Tidière, M., Badruna, A., Fouchet, D., Gaillard, J.M., Lemaître, J.F., Pontier, D., 2020. Pathogens shape sex differences in mammalian aging. Trends Parasitol. 36, 668–676. https://doi.org/10.1016/j.pt.2020.05.004

Tischer, B.K., Osterrieder, N., 2010. Herpesviruses-A zoonotic threat? Vet. Microbiol. 140, 266–270. https://doi.org/10.1016/j.vetmic.2009.06.020

Tokarz, R., Markotić, A., Margaletić, J., Lipkin, W.I., Habuš, J., Jain, K., Turk, N., Svoboda, P., Vucelja, M., Desai, A., Tadin, A., 2016. Molecular survey of zoonotic agents in rodents and other small mammals in Croatia. Am. J. Trop. Med. Hyg. 94, 466–473. https://doi.org/10.4269/ajtmh.15-0517

Torti, J.M.I., 2012. Floods in Southeast Asia: a health priority. J. Glob. Health 2, 020304. https://doi.org/10.7189/jogh.02.020304

Trapp, S., von Einem, J., Hofmann, H., Köstler, J., Wild, J., Wagner, R., Beer, M., Osterrieder, N., 2005. Potential of Equine herpesvirus 1 as a vector for imunization. J. Virol. 79,

5445–5454. https://doi.org/10.1128/jvi.79.9.5445-5454.2005

Truc, P., Büscher, P., Cuny, G., Gonzatti, M.I., Jannin, J., Joshi, P., Juyal, P., Lun, Z.R., Mattioli, R., Pays, E., Simarro, P.P., Teixeira, M.M.G., Touratier, L., Vincendeau, P., Desquesnes, M., 2013. Atypical human infections by animal trypanosomes. PLoS Negl. Trop. Dis. 7. https://doi.org/10.1371/journal.pntd.0002256

Tucunduva de Faria, M., Athanazio, D.A., Gonçalves Ramos, E.A., Silva, E.F., Reis, M.G., Ko, A.I., 2007. Morphological alterations in the kidney of rats with natural and experimental *Leptospira* Infection. J. Comp. Pathol. 137, 231–238. https://doi.org/10.1016/j.jcpa.2007.08.001

Tung, K.C., Hsiao, F.C., Wang, K.S., Yang, C.H., Lai, C.H., 2013. Study of the endoparasitic fauna of commensal rats and shrews caught in traditional wet markets in Taichung City, Taiwan. J. Microbiol. Immunol. Infect. 46, 85–88. https://doi.org/10.1016/j.jmii.2012.01.012

Tung, K.C., Hsiao, F.C., Yang, C.H., Chou, C.C., Lee, W.M., Wang, K.S., Lai, C.H., 2009. Surveillance of endoparasitic infections and the first report of *Physaloptera* sp. and *Sarcocystis* spp. in farm rodents and shrews in central Taiwan. J. Vet. Med. Sci. 71, 43–47. https://doi.org/10.1292/jvms.71.43

Ubelaker, J., 1986. Systematics of species referred to the genus Angiostrongylus. J. Parasitol. 72, 237–244.

Uni, S., Mat Udin, A.S., Agatsuma, T., Saijuntha, W., Junker, K., Ramli, R., Omar, H., Lim, Y.A.-L., Sivanandam, S., Lefoulon, E., Martin, C., Belabut, D.M., Kasim, S., Abdullah Halim, M.R., Zainuri, N.A., Bhassu, S., Fukuda, M., Matsubayashi, M., Harada, M., Low, V.L., Chen, C.D., Suganuma, N., Hashim, R., Takaoka, H., Azirun, M.S., 2017. Morphological and molecular characteristics of *Malayfilaria sofiani* Uni, Mat Udin & Takaoka n. g., n. sp. (Nematoda: Filarioidea) from the common treeshrew *Tupaia glis* Diard & Duvaucel (Mammalia: Scandentia) in Peninsular Malaysia. Parasit Vectors. 10, 194. https://doi.org/10.1186/s13071-017-2105-9

VanderWaal, K.L., Ezenwa, V.O., 2016. Heterogeneity in pathogen transmission: mechanisms and methodology. Funct. Ecol. 30, 1606–1622. https://doi.org/10.1111/1365-2435.12645

Van Lieshout, S.H.J., Badás, E.P., Mason, M.W.T., Newman, C., Buesching, C.D., Macdonald, D.W., Dugdale, H.L., 2020. Social effects on age-related and sex-specific immune cell profiles in a wild mammal: immune cell profiles in a wild mammal. Biol. Lett. 16, 0–6. https://doi.org/10.1098/rsbl.2020.0234rsbl20200234

Vaumourin, E., Vourc'h, G., Gasqui, P., Vayssier-Taussat, M., 2015. The importance of multiparasitism: examining the consequences of co-infections for human and animal health. Parasit Vectors. 8, 545. https://doi.org/10.1186/s13071-015-1167-9

Vázquez-Martínez, E.R., García-Gómez, E., Camacho-Arroyo, I., González-Pedrajo, B., 2018. Sexual dimorphism in bacterial infections. Biol. Sex Differ. 9, 1–20. https://doi.org/10.1186/s13293-018-0187-5

Verma, S.K., Calero-Bernal, R., Lovallo, M.J., Sweeny, A.R., Grigg, M.E., Dubey, J.P., 2015. Detection of *Sarcocystis* spp. infection in bobcats (*Lynx rufus*). Vet. Parasitol. 212, 422–426. https://doi.org/10.1016/j.vetpar.2015.06.007

Vihol, D.P., Patel, H.J., Patel, J.M., Dabas, S.V., Kalyani, I.H., Chaudhari, C.F.,Patel, A.C., 2017. Identification of pathogenic *Leptospira* spp. carrier goats using polymerase chain reaction (PCR). Int. J. Curr. Microbiol. Appl. Sci. 6, 2174–2183. https://doi.org/10.20546/ijcmas.2017.612.249

Wahid, M.B., Weng, C.K., May, C.Y., Chin, C.M.E.E., 2006. The need to reduce national greenhouse gas emissions: oil palm industry's role. J. Oil Palm Res. Special Issue, 1–23.

Wald, A., Corey, L., 2019. HSV: persistence in the population: epidemiology, transmissio. In: Human herpesviruses. Arvin, A., Campadelli-Fiume, G., Mocarski, E., Moore, P.S., Roizman, B., Whitley, R., Yamanishi, K. (eds.): Cambridge University Press, Cambridge, pp. 656–672. https://doi.org/10.1017/CBO9780511545313.037

Walker, R., Carvalho-Pereira, T., Serrano, S., Pedra, G., Hacker, K., Taylor, J., Minter, A., Pertile, A., Panti-May, A., Carvalho, M., Souza, F.N., Nery, N., Rodrigues, G., Bahiense, T., Reis, M.G., Ko, A.I., Childs, J.E., Begon, M., Costa, F., 2017. Factors affecting carriage and intensity of infection of *Calodium hepaticum* within Norway rats (*Rattus norvegicus*) from an urban slum environment in Salvador, Brazil. Epidemiol. Infect. 145, 334–338. https://doi.org/10.1017/S0950268816002259

Wall, D.H., Bradford, M.A., St. John, M.G., Trofymow, J.A., Behan-Pelletier, V., Bignell, D.E., Dangerfield, J.M., Parton, W.J., Rusek, J., Voigt, W., Wolters, V., Gardel, H.Z., Ayuke, F.O., Bashford, R., Beljakova, O.I., Bohlen, P.J., Brauman, A., Flemming, S., Henschel, J.R., Johnson, D.L., Jones, T.H., Kovarova, M., Kranabetter, J.M., Kutny, L., Lin, K.C., Maryati, M., Masse, D., Pokarzhevskii, A., Rahman, H., Sabará, M.G., Salamon, J.A., Swift, M.J., Varela, A., Vasconcelos, H.L., White, D., Zou, X., 2008. Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. Glob. Chang. Biol. 14, 2661–2677. https://doi.org/10.1111/j.1365-2486.2008.01672.x

Wall, D.H., Nielsen, U.N., Six, J., 2015. Soil biodiversity and human health. Nature 528, 69–76. https://doi.org/10.1038/nature15744

Wells, K., Bagchi, R., 2005. Eat in or take away - Seed predation and removal by rats (Muridae) during a fruiting event in a dipterocarp rainforest. Raffles Bull. Zool. 53, 281–286.

Wells, K., Kalko, E.K. V, Lakim, M.B., Pfeiffer, M., 2007. Effects of rain forest logging on species richness and assemblage composition of small mammals in Southeast Asia. J. Biogeogr. 34, 1087–1099. https://doi.org/10.1111/j.1365-2699.2006.01677.x

Wells, K., Lakim, M.B., O'Hara, R.B., 2014. Shifts from native to invasive small mammals across gradients from tropical forest to urban habitat in Borneo. Biodivers. Conserv. 23, 2289–2303. https://doi.org/10.1007/s10531-014-0723-5

Wells, K., Lakim, M.B., Pfeiffer, M., 2006. Nest sites of rodents and treeshrews in Borneo. Ecotropica 12, 141–149.

Wells, K., O'Hara, R.B., Morand, S., Lessard, J.P., Ribas, A., 2015. The importance of parasite geography and spillover effects for global patterns of host-parasite associations in two invasive species. Divers. Distrib. 21, 477–486. https://doi.org/10.1111/ddi.12297

Wells, K., Pfeiffer, M., Lakim, M.B., Linsenmair, K.E., 2004. Use of arboreal and terrestrial space by a small mammal community in a tropical rain forest in Borneo, Malaysia. J. Biogeogr. 31, 641–652. https://doi.org/10.1046/j.1365-2699.2003.01032.x

Wharton, D.R.A., 1947. Pathological changes in natural and experimental filariasis in the cotton rat. J. Infect. Dis. 80, 307–318. https://doi.org/10.1093/infdis/80.3.307

Wheeler, R.J., Gluenz, E., Gull, K., 2013. The limits on trypanosomatid morphological diversity. PLoS One 8, e79581. https://doi.org/10.1371/journal.pone.0079581

Whishaw, I.Q., Whishaw, G.E., 1996. Conspecific aggression influences food carrying: Studies on a wild population of *Rattus norvegicus.* Aggress. Behav. 22, 47–66. https://doi.org/10.1002/(SICI)1098-2337(1996)22:1<47::AID-AB5>3.0.CO;2-R

Wibbelt, G., Kurth, A., Yasmum, N., Bannert, M., Nagel, S., Nitsche, A., Ehlers, B., 2007. Discovery of herpesviruses in bats. J. Gen. Virol. 88, 2651–2655. https://doi.org/10.1099/vir.0.83045-0

Wilcove, D.S., Giam, X., Edwards, D.P., Fisher, B., Koh, L.P., 2013. Navjot's nightmare revisited: logging, agriculture, and biodiversity in Southeast Asia. Trends Ecol. Evol. 28, 531–540. https://doi.org/10.1016/j.tree.2013.04.005

Willard-Mack, C.L., Elmore, S.A., Hall, W.C., Harleman, J., Kuper, C.F., Losco, P., Rehg, J.E., Rühl-Fehlert, C., Ward, J.M., Weinstock, D., Bradley, A., Hosokawa, S., Pearse, G., Mahler, B.W., Herbert, R.A., Keenan, C.M., 2019. Nonproliferative and proliferative lesions of the rat and mouse hematolymphoid system. Toxicol. Pathol. 47, 665–783. https://doi.org/10.1177/0192623319867053

Wilson, D.E., M.Helgen, K., Yun, C.Si., Giman, B., 2006. Small mammal survey at two sites in planted forest zone, Bintulu, Sarawak. Malayan Nat. J. 59, 165–187.

Winter, H., Watt, D., 1971. *Klossiella hydromyos* n.sp. from the kidneys of an Australian Water Rat (*Hydromys chrysogaster*). Vet. Pathol. 8, 222–231. https://doi.org/10.1177/030098587100800304

Wobeser, G.A., 2006.. Essentials of disease in wild animals. Blackwell Publishing Professional, Iowa.

Wolff, J.O., 1993. Why are female small mammals territorial? Oikos 68, 364-370. https://doi.org/10.2307/3544853

Woodruff, D.S., 2010. Biogeography and conservation in Southeast Asia: How 2.7 million years of repeated environmental fluctuations affect today's patterns and the future of the remaining refugial-phase biodiversity. Biodivers. Conserv. 19, 919–941. https://doi.org/10.1007/s10531-010-9783-3

Wooster, M.J., Perry, G.L.W., Zoumas, A., 2012. Fire, drought and El Niño relationships on Borneo (Southeast Asia) in the pre-MODIS era (1980-2000). Biogeosciences 9, 317–340. https://doi.org/10.5194/bg-9-317-2012

Woźniakowski, G., Samorek-Salamonowicz, E., 2015. Animal herpesviruses and their zoonotic potential for cross-species infection. Ann. Agric. Environ. Med. 22, 191–194. https://doi.org/10.5604/12321966.1152063

Wu, Z., Han, Y., Liu, B., Li, H., Zhu, G., Latinne, A., Dong, J., Sun, L., Du, J., Zhou, S., Chen, M., Kritiyakan, A., Jittapalapong, S., Chaisiri, K., Buchy, P., Duong, V., Yang, J., Jiang, J., Xu, X., Zhou, H., Yang, F., Morand, S., Daszak, P., Jin, Q., 2020. Decoding the RNA viromes of rodent lungs provides new visions into the origin and evolution pattern of rodent-borne diseases in Mainland Southeast Asia 1–19. https://doi.org/10.21203/rs.3.rs-17323/v1

Wyatt, K.B., Campos, P.F., Gilbert, M.T.P., Kolokotronis, S.-O., Hynes, W.H., DeSalle, R., Daszak, P., MacPhee, R.D.E., Greenwood, A.D., 2008. Historical mammal extinction on Christmas Island (Indian Ocean) correlates with introduced infectious disease. PLoS One

3, e3602. https://doi.org/10.1371/journal.pone.0003602

Xiang, Z., Chen, X., Yang, L., He, Y., Jiang, R., Rosenthal, B.M., Luan, P., Attwood, S.W., Zuo, Y., Zhang, Y., Yang, Z., 2009. Non-invasive methods for identifying oocysts of *Sarcocystis* spp. from definitive hosts. Parasitol. Int. 58, 293–296. https://doi.org/10.1016/j.parint.2009.03.004

Xiang, Z., Rosenthal, B.M., He, Y., Wang, W., Wang, H., Song, J., Shen, P., Li, M., Yang, Z., 2010. *Sarcocystis tupaia*, sp. nov., a new parasite species employing treeshrews (Tupaiidae, *Tupaia belangeri chinensis*) as natural intermediate hosts. Parasitol. Int. 59, 128–132. https://doi.org/10.1016/j.parint.2009.12.003

Xu, Y., Zhu, Yongzhang, Wang, Y., Chang, Y.-F., Zhang, Y., Jiang, X., Zhuang, X., Zhu, Yongqiang, Zhang, J., Zeng, L., Yang, M., Li, S., Wang, S., Ye, Q., Xin, X., Zhao, G., Zheng, H., Guo, X., Wang, J., 2016. Whole genome sequencing revealed host adaptation-focused genomic plasticity of pathogenic *Leptospira*. Sci. Rep. 6, 20020. https://doi.org/10.1038/srep20020

Yamaguchi, T., Higa, N., Okura, N., Matsumoto, A., Hermawan, I., Yamashiro, T., Suzuki, T., Toma, C., 2018. Characterizing interactions of *Leptospira interrogans* with proximal renal tubule epithelial cells. BMC Microbiol. 18, 64. https://doi.org/10.1186/s12866-018-1206-8

Yang, S., Rothman, R.E., 2004. PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. Lancet Infect. Dis. 4, 337–348. https://doi.org/10.1016/S1473-3099(04)01044-8

Yaremych, S.A., Levengood, J.M., Novak, R.J., Mankin, P.C., Warner, R.E., 2004. Gender determination and lack of sex specifc West Nile virus mortality in American crows. Wildl. Soc. Bull. 32, 893–899.

Yi, J.Y., Kim, Y.H., Kim, H.C., Hahn, T.W., Jeong, H., Choi, C.U., Woo, G.H., Kim, Y.B., Han, J.H., Yoon, B.I., 2010. Prevalence of hepatic parasites in korean wild rats (*Rattus norvegicus*) and their association with pulmonary arteriolar medial hypertrophy. Vet. Pathol. 47, 292–297. https://doi.org/10.1177/0300985809359306

Zakaria, M., Rajpar, M.N., Ozdemir, I., Rosli, Z., 2016. Fauna diversity in tropical rainforest: threats from land-use change, in tropical forests: the challenges of maintaining ecosystem services while managing the landscape. InTech, pp. 530–533. https://doi.org/10.5772/64963

Zaman, V., Colley, F.C., 1975. Light and electron microscopic observations of the life cycle of Sarcocystis orientalis sp. n. in the rat (*Rattus norvegicus*) and the Malaysian reticulated

python (*Python reticulatus*). Z. Parasitenkd. 47, 169–185. https://doi.org/10.1007/BF00418200

Zhang, L., Wu, X., Liao, S., Li, Y., Zhang, Z., Chang, Q., Xiao, R., Liang, B., 2016. Tree shrew (*Tupaia belangeri chinensis*), a novel non-obese animal model of non-alcoholic fatty liver disease . Biol. Open 5, 1545–1552. https://doi.org/10.1242/bio.020875

Zhang, L., Zhang, Z., Li, Y., Liao, S., Wu, X., Chang, Q., Liang, B., 2015. Cholesterol induces lipoprotein lipase expression in a tree shrew (*Tupaia belangeri chinensis*) model of non-alcoholic fatty liver disease. Sci. Rep. 5, 1–12. https://doi.org/10.1038/srep15970

10 SUPPLEMENTARY MATERIAL

10.1 Histological staining

1) Azan staining

1. Incubation for 10 min in Azocarmine solution at 56°C
2. Differentiate in 0.1% Aniline alcohol
3. Wash in 1% acetic acid-alcohol
4. Wash in distilled water
5. Incubation in phosphotungstic acid for 1 h
6. Wash in distilled water
7. Incubation for 1 h in Aniline blue-orange G solution
8. Wash in tap water
9. Dehydration through graded alcohols: 1 x 1 min 96% ethanol and 2 x 1 min 99% ethanol
10. Washing in xylene 3 x 15 min
11. Mounting of coverslips to glass slides by Canada balsam (Carl Roth, Karlsruhe, Germany)
12. Dry overnight

Solutions:

- Azocarmine dilution: dilution of 0.1 g Azocarmine in 100 ml of distilled water. Heat until boiling. After cooling, filtrate and add 1 ml of acetic acid
- Aniline blue-orange G solution: Dilution of 0.5 g of aniline blue and 2 g of orange G in 100 ml of distilled water. After the dilution add 8 ml of acetic acid, heat until boiling. After cooling, filtrate and dilute in solution 1:2 for staining

Reagents:

- Azocarmine (Chemapol (inactive company), Czech Republic)
- Aniline (Merck Millipore, Darmstadt, Germany)
- Phosphotungstic acid (Carl Roth, Karlsruhe, Germany)
- Aniline blue (Carl Roth, Karlsruhe, Germany)
- Orange G (Carl Roth, Karlsruhe, Germany)
- Acetic acid 100% (Merck Millipore, Darmstadt, Germany)

2) Prussian blue staining

1. Incubation in 2% potassium hexacyanoferrate II for 2 min
2. Wash in distilled water
3. Incubation for 5 min in nuclear fast red solution
4. Wash in distilled water
5. Dehydration through graded alcohols: 1 x 1 min 96% ethanol and 2 x 1 min 99% ethanol
6. Washing in xylene 3 x 15 min
7. Mounting of coverslips to glass slides by Canada balsam (Carl Roth, Karlsruhe, Germany)
8. Dry overnight

Solutions:

- Iron reaction solution: Dilution of 2 g of potassium hexacyanoferrate in 100 ml of distilled water. Addition of 5 ml of 25% hydrochloric acid immediately before use
- Nuclear fast red solution: Dilution of 1 g Nuclear fast red in 100 ml 5% aluminium sulphate solution. After cooling, filtrate

Reagents:

- Potassium hexacyanoferrate II (Carl Roth, Karlsruhe, Germany)
- Hydrochloric acid 25% (Carl Roth, Karlsruhe, Germany)
- Nuclear fast red (Fluka (inactive company), Switzerland)
- Aluminium sulphate (Carl Roth, Karlsruhe, Germany)

3) Giemsa's staining

1. 2 x 10 min xylene
2. Incubation in Giemsa solution 1:10 for 1 h
3. Wash in 1% acetic acid
3. Dehydration through graded alcohols: 1 x 1 min 96% ethanol and 2 x 1 min 99% ethanol
4. Washing in xylene 3 x 15 min
5. Mounting of coverslips to glass slides by Canada balsam (Carl Roth, Karlsruhe, Germany)
6. Dry overnight

Reagents:

- Giemsa solution (Carl Roth, Karlsruhe, Germany)
- Acetic acid 100% (Merck Millipore, Darmstadt, Germany)

4) PAS

1. Incubation in 1% periodic acid for 7 min
2. Wash in distilled water
3. Incubation in Schiff's reagent for 20 min
4. Wash 3 times for 2 min in sulphurous water
5. Wash for 15 min in tap water
6. Incubation of 1 min in Mayer's hemalum solution
7. Wash in tap water
8. Dehydration through graded alcohols: 1 x 1 min 96% ethanol and 2 x 1 min 99% ethanol
   Washing in xylene 3 x 15 min
9. Mounting of coverslips to glass slides by Canada balsam (Carl Roth, Karlsruhe, Germany)
10. Dry overnight

Solutions:

- Sulphurous water: Dilution of 5 ml of hydrochloric acid and 5 ml of 10% sodium disulfite in 100 ml of distilled water
- Mayer's hemalum solution: Dilution of 1 g of hematoxylin in 1000 ml of distilled water. After mixing the solution add to it 0.2 g of sodium iodate, 50 g of aluminium potassium sulphate, 50 g of chloral hydrate and 1 g of citric acid. Incubate at room temperature for 24 h and then filtrate

Reagents

- Periodic acid (Carl Roth, Karlsruhe, Germany)
- Schiff's reagent (Merck Millipore, Darmstadt, Germany)
- Hydrochloric acid 25% (Carl Roth, Karlsruhe, Germany)
- Sodium disulfite (Carl Roth, Karlsruhe, Germany)
- Hematoxylin (Carl Roth, Karlsruhe, Germany)
- Aluminium potassium sulphate (Merck Millipore, Darmstadt, Germany)
- Chloral hydrate (Merck Millipore, Darmstadt, Germany)
- Citric acid (Carl Roth, Karlsruhe, Germany)

5) Best carmine

1. Incubation for 1 min in in Mayer's hemalum solution
2. Wash in tap water
3. Stain in Best's Carmine solution for 20 min
4. Wash in Best's Carmine differentiator solution for few sec
5. Dehydration through graded alcohols: 1 x 1 min 96% ethanol and 2 x 1 min 99% ethanol
6. Washing in xylene 3 x 15 min
7. Mounting of coverslips to glass slides by Canada balsam (Carl Roth, Karlsruhe, Germany)
8. Dry overnight

Solutions

- Mayer's hemalum solution: Mix of 1 g of hematoxylin in 1000 ml of distilled water. After mixing the solution add 0.2 g of sodium iodate, 50 g of aluminium potassium sulphate, 50 g of chloral hydrate and 1 g of citric acid. Incubate at room temperature for 24 h and filtrate
- Best's Carmine stock solution: Mix of 2 g of carmine, 1 g of potassium carbonate and 5 g of potassium chloride in 60 ml of distilled water. Heat the solution at low temperature until it foams. After cooling, add 20 ml of ammonia
- Best's Carmine use solution: Filter 20 ml of Best's Carmine stock solution, add 30 ml of methanol and 30 ml of ammonia
- Best's Carmine differentiator solution: Mix 40 ml methanol, 80 ml 99% alcohol and 100 ml distilled water

Reagents

- Carmine (Serva, Heidelberg, Deutschland)
- Potassium carbonate (Carl Roth, Karlsruhe, Germany)
- Potassium chloride (Merck Millipore, Darmstadt, Germany)
- Ammonia (Merck Millipore, Darmstadt, Germany)
- Methanol (Carl Roth, Karlsruhe, Germany)
- Hematoxylin (Carl Roth, Karlsruhe, Germany)
- Sodium iodate (Merck Millipore, Darmstadt, Germany)
- Aluminium potassium sulphate (Merck Millipore, Darmstadt, Germany)
- Chloral hydrate (Carl Roth, Karlsruhe, Germany)
- Citric acid (Carl Roth, Karlsruhe, Germany)

6) Richardson staining

1. Mix equal volumes of stock solutions A and B
2. Rinse the slide in the mixed solution
3. Heat on a warm plate for 30 to 60 sec
4. Wash in distilled water
5. Dehydration through graded alcohols: 1 x 1min 96% ethanol and 2 x 1 min 99% ethanol
6. Washing in xylene 3 x 15 min
7. Mounting of coverslips to glass slides by Canada balsam (Carl Roth, Karlsruhe, Germany)
5. Dry overnight

Solutions:

- 1.0% Methylene blue in 1% borax: Dilution of 1 g of Methylene blue and 1 g of Borax in 100 ml of distilled water
- 1.0% Azure II: Dilution of 1 g of Azure II in 100 ml of distilled water

Reagents:

- Borax (disodium tetraborate decahydrate) (Merck Millipore, Darmstadt, Germany)
- Methylene blue (Serva, Heidelberg, Germany)
- Azure II (Serva, Heidelberg, Germany)

7) Gram staining (Method after Brown and Brenn)

1. Incubation for 1min in 1% Crystal violet
2. Incubation for 1min in Lugol's solution
3. Wash in distilled water
4. Dry using blotting paper
5. Rinse in acetone
6. Wash in tap water
7. Rinse for 3 min in 0.25% Carbon fuchsin
8. Dry using blotting paper
9. Wash twice in acetone
10. Decolourization in picric acid-acetone until the preparation turns into salmon colour
11. Wash twice in acetone
12. Washing in xylene 3 x 15 min

13. Mounting of coverslips to glass slides by Canada balsam (Carl Roth, Karlsruhe, Germany)
14. Dry overnight

Solutions:

- Picric acid-acetone: Mix of 100 ml saturated aqueous picric acid solution with 900 ml acetone

Reagents:

- Crystal violet (Merck Millipore, Darmstadt, Germany)
- Lugol's solution (Carl Roth, Karlsruhe, Germany)
- Acetone (Carl Roth, Karlsruhe, Germany)
- Fuchsin (Serva, Heidelberg, Germany)
- Picric acid (Merck Millipore, Darmstadt, Germany)

10.2 Health status results

Tab. 36: Health status results according to the ecological group, species and sex

Ecol. group	Species	No.	Sex											
			Female				Male				ND			
			Ev.	Health status			Ev.	Health status			Ev.	Health status		
				0-1	2-3	4-5		0-1	2-3	4-5		0-1	2-3	4-5
Forest rodents	*Callosciurus notatus*	5	3	1	2	/	2	/	2	/	/	/	/	/
	Callosciurus prevostii	2	1	/	1	/	1	1	/	/	/	/	/	/
	Sundasciurus lowii	6	5	2	3	/	1	/	1	/	/	/	/	/
	Leopoldamys sabanus	/	/	/	/	/	/	/	/	/	/	/	/	/
	Maxomys rajah	3	/	/	/		3	/	3	/	/	/	/	
	Maxomys surifer	7	3	2	1	/	4	/	4	/	/	/	/	/
	*Maxomys surifer/rajah**	6	3	/	1	2	3	/	3	/	/	/	/	
	Maxomys whiteheadi	7	2	/	2	/	4	/	3	1	/	/	/	/
	Niviventer cremoriventer	4	2	/	2	/	/	2	1	1	/	/	/	/
Treeshrews	*Tupaia gracilis*	1	1	/	1	/	/	/	/	/	/	/	/	/
	Tupaia minor	8	4	2	2	/	/	4	/	/	/	/	/	/
	Tupaia tana	5	3	/	3	/	2	1	1	/	/	/	/	/
Generalist	*Sundamys muelleri*	72	42	4	33	5	29	3	24	2	1	/	1	/
Rattus	*Rattus* comp.	105	44	8	27	9	61	20	30	11	/	/	/	/
	Rattus norvegicus	43	20	2	17	1	23	2	16	5	/	/	/	/
Shrews	*Suncus murinus*	58	16	4	12	/	39	16	22	1	3	2	1	/

Tab. 37: Health status results according to the ecological group, species and age

Ecol. group	Species	No.	Age																			
			Immature				Juvenile				Subadult				Adult				ND			
			Ev.	Health status			Ev.	Health status			Ev.	Health status			Ev.	Health status			Ev.	Health status		
				0-1	2-3	4-5		0-1	2-3	4-5		0-1	2-3	4-5		0-1	2-3	4-5		0-1	2-3	4-5
Forest rodents	*Callosciurus notatus*	5	/	/	/	/	/	/	/	/	/	/	/	/	5	1	4	/	/	/	/	/
	Callosciurus prevostii	2	/	/	/	/	/	/	/	/	/	/	/	/	2	1	1	/	/	/	/	/
	Sundasciurus lowii	6	1	/	1	/	1	/	1	/	/	/	/	/	4	2	2	/	/	/	/	/
	Leopoldamys sabanus	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Maxomys rajah	3		/	/			/	/	/	/	/	/	/	/	/	3	/	/	/	/	
	Maxomys surifer	7	/	/	/	/	/	/	/	/	/	2	2	/	/	/	3	/	/	/	/	/
	*Maxomys surifer/rajah**	6	2	/	2	/	2	/	2	/	/	1	/	1	/	1	/	1	/	/	/	/
	Maxomys whiteheadi	6	/	/	/	/	/	/	/	/	2	/	2	/	4	/	3	1	/	/	/	/
	Niviventer cremoriventer	4	/	/	/	/	/	/	/	/	/	/	/	/	4	/	3	1	/	/	/	/

Continuation Tab. 37

Ecol. group	Species	No.	Age																			
			Immature				Juvenile				Subadult				Adult				ND			
			Ev.	Health status			Ev.	Health status			Ev.	Health status			Ev.		Health status		Ev.	Health status		
				0-1	2-3	4-5		0-1	2-3	4-5		0-1	2-3	4-5		0-1	2-3	4-5		0-1	2-3	4-5
Treeshrews	*Tupaia gracilis*	1	/	/	/	/	/	/	/	/	1	/	1	/	/	/	/	/	/	/	/	/
	Tupaia minor	8	/	/	/	/	/	/	/	/	/	/	/	/	8	6	2	/	/	/	/	/
	Tupaia tana	5	1	/	1	/	/	/	/	/	1	/	1	/	3	1	2	/	/	/	/	/
Generalist	*Sundamys muelleri*	72	/	/	/	/	4	/	4	/	18	3	12	3	48	4	40	4	2	/	2	/
Rattus	*Rattus* comp.	105	1	/	1	/	8	6	1	1	8	3	5	/	86	19	49	18	2	/	1	1
	Rattus norvegicus	43	4	1	3	/	8	/	7	1	7	/	5	2	24	3	18	3	/		/	/
Shrews	*Suncus murinus*	58	/	/	/	/	/	/	/	/	/	/	/	/	55	20	34	1	3	2	1	/

10.3 Leptospira results

Tab. 38: *Leptospira* Warthin-Starry (WS) staining results according to the ecological group, species, sex and age

Ecological group	Species	*Leptospira* Warthin-Starry staining (WS)																	
		TOTAL WS (n= 314)		Sex					Age										
				Female		Male			Immature		Juvenile		Subadult		Adult		ND		
		Analy.	Pos	Analy.	Pos	Analy.	Pos	NA	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos
Forest rodents	*Callosciurus notatus*	5	/	3	/	2	/	/	/	/	/	/	/	/	5	/	/	/
	Callosciurus prevostii	2	1	1	/	1	1	/	/	/	/	/	/	/	2	1	/	/
	Sundasciurus lowii	5	2	4	2	1	/	/	1	/	1	1	/	/	3	2	/	/
	Leopoldamys sabanus	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Maxomys rajah	3	1	/	/	3	1	/	/	/	/	/	3	1	/	/	/	/
	Maxomys surifer	7	1	3	/	4	1	/	/	/	/	/	4	/	3	1	/	/
	*Maxomys surifer/rajah**	6	/	3	/	3	/	/	2	/	2	/	1	/	1	/	/	/
	Maxomys whiteheadi	5	2	2	1	3	1	/	/	/	/	/	2	/	3	2	/	/
	Niviventer cremoriventer	4	/	2	/	2	/	/	/	/	/	/	/	/	4	/	/	/
Forest rodents total		**37**	**7**															

Analy.: analysed. Pos: positive

Continuation Tab. 38

Ecological group	Species	*Leptospira* Warthin-Starry staining (WS)																	
		TOTAL WS (n= 314)		Sex					Age										
				Female		Male			Immature		Juvenile		Subadult		Adult		ND		
		Analy.	**Pos**	Analy.	Pos	Analy.	Pos	NA	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	
Treeshrews	*Tupaia gracilis*	1	0	1	/	/	/	/	/	/	/	/	1	/	/	/	/	/	
	Tupaia minor	8	0	4	/	4	/	/	/	/	/	/	8	/	/	/	/	/	
	Tupaia tana	5	1	3	1	2	/	/	1	/	/	/	1	/	3	1	/	/	
Treeshrews total		**14**	**1**																
Generalist	***Sundamys muelleri***	**71**	**15**	41	8	29	7	1	/	/	3	/	18	/	48	14	2	1	
Rattus	*Rattus* comp.	100	27	44	10	56	17	/	1	/	7	/	8	/	82	27	2	/	
	Rattus norvegicus	43	13	20	8	23	5	/	4	1	8	2	7	/	24	10	/	/	
Rattus total		**143**	**40**																
Shrews	***Suncus murinus***	**49**	**1**	13	/	34	1	2	/	/	/	/	47	1	/	/	2	/	

Analy.: analysed. Pos: positive

Tab. 39: *Leptospira* molecular detection results according to the ecological group, species, sex and age

Ecological group	Species	***Leptospira* molecular detection**																	
		qPCR/PCR análisis (n= 106)		**Sex**					**Age**										
				Female		Male			Immature		Juvenile		Subadult		Adult		ND		
		Analy.	**Seq.**	Analy.	Seq.	Analy.	Seq.	NA	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.
Forest rodents	*Callosciurus notatus*	3	/	2	/	1	/	/	/	/	/	/	/	/	3	/	/	/
	Callosciurus prevostii	1	/	1	/	/	/	/	/	/	/	/	/	/	1	/	/	/
	Sundasciurus lowii	3	1	3	1	/	/	/	/	/	/	/	/	/	3	1	/	/
	Leopoldamys sabanus	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Maxomys rajah	2	/	/	/	2	/	/	/	/	/	/	/	/	2	/	/	/
	Maxomys surifer	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	*Maxomys surifer/rajah**	2	/	/	/	2	/	/	/	/	/	/	/	/	2	/	/	/
	Maxomys whiteheadi	3	2	2	1	1	1	/	/	/	/	/	1	/	2	2	/	/
	Niviventer cremoriventer	1	/	1	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Forest rodents total		**15**	**3**															

Analy.: analysed. Seq.: sequenced

Continuation Tab. 39

Ecological group	Species	*Leptospira* molecular detection																
		qPCR/PCR analysis (n= 106)		Sex					Age									
				Female		Male			Immature		Juvenile		Subadult		Adult		ND	
		Analy.	**Seq.**	Analy.	Seq.	Analy.	Seq.	NA	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.
Treeshrews	*Tupaia gracilis*	1	/	1	/	/	/	/	/	/	/	/	/	/	1	/	/	/
	Tupaia minor	7	/	3	/	4	/	/	/	/	/	/	/	/	7	/	/	/
	Tupaia tana	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Treeshrews total		**8**	***/***															
Generalist	***Sundamys muelleri***	**31**	**10**	18	4	15	6	/	/	/	/	/	/	/	10	10	***/***	***/***
Rattus	*Rattus* comp.	26	12	13	6	13	6	/	/	/	/	/	4	/	20	12	2	/
	Rattus norvegicus	12	3	3	/	9	3	/	/	/	2	/	5	2	5	1	/	/
Rattus total		**38**	**15**															
Shrews	***Suncus murinus***	**14**	***/***	2	/	11	/	1	***/***	***/***	***/***	***/***	***/***	***/***	13	/	1	/

Analy.: analysed. Seq.: sequenced

Tab. 40: *Leptospira* sequencing results and comparison to Warthin-Starry staining (WS) results

Ecological group	Sample ID	Host	Sequence (bp)	BLAST results	% Identity	Positive to WS
Forest rodents (n=3)	E346/13	*Sundasciurus lowii*	LipL32 only	*Leptospira* sp.	≤ 100 [a]	/
				L.weilii	99.58 [b]	
				L. mayottensis	≤ 98.74	
	E347/13	*Maxomys whiteheadi*	G2 (216)	*L. interrogans*	≤ 100	/
			LipL 21 (500)		≤ 100	
	E369/13	*Maxomys whiteheadi*	G2 (216)	*L. interrogans*	≤ 100	/
			LipL21 (400)		≤ 100	
Generalist (n=10)	E141/13	*Sundamys muelleri*	G2 (216)	*L. interrogans*	≤ 100	Pos
			LipL21 (500)		≤ 99.80	
	E267/13	*Sundamys muelleri*	G1/G2 (270)	*L. interrogans*	≤ 99.26	Pos
			LipL21 (555)		≤ 99.10	
	E268/13	*Sundamys muelleri*	G2 (216)	*L. interrogans*	≤ 100	Pos
			LipL21 (500)		≤ 100	
	E275/13	*Sundamys muelleri*	G2 (216)	*L. interrogans*	≤ 98.61	Pos
	E298/13	*Sundamys muelleri*	G1/G2 (270)	*L. borgpetersenii*	≤ 100	Pos
	E319/13	*Sundamys muelleri*	G1/G2 (270)	*L. interrogans*	≤ 100	/
			LipL21 (555)		≤ 99.64	
	E328/13	*Sundamys muelleri*	G2 (216)	*L. interrogans*	≤ 100	Pos
			LipL21 (500)		≤ 100	
	E333/13	*Sundamys muelleri*	G1 (165)	*L. interrogans*	≤ 98.61	Pos
			LipL21 (555)		≤ 99.10	
	E334/13	*Sundamys muelleri*	G2 (216)	*L. interrogans*	≤ 99.39	Pos
	E393/13	*Sundamys muelleri*	G1/G2 (270)	*L. borgpetersenii*	≤ 100	Pos

[#]) The chromatogram file indicates co-infection with *L. interrogans* and *L. borgpetersenii.*
[a]) Accession nos. KY356930- KY356932, KY356944, KY356946, KY356949 and KY356950, host: farm pigs (*Sus scrofa*), Tahiti, French Polynesia (Guernier et al., 2017).
b) Accession nos. CP040840 and CP040843 (Kurilung et al., 2019 unpublished).
Pos: Positive to *Leptospira* Warthin-Starry staining

Continuation Tab. 40

Ecological group	Sample ID	Host	Sequence (bp)	BLAST results	% Identity	Positive to WS
Urban *Rattus* spp. (n=15)	E137/13	*Rattus* compl.	G1/G2 (270)	*L. interrogans*	≤ 98.89	/
			LipL21 (500)		≤ 99.00	
	E139/13	*Rattus* compl.	G2 [#]	-	-	/
			LipL21 (500)	*L. interrogans*	≤ 99.00	
	E272/13	*Rattus* compl.	G2 (216)	*L. interrogans*	≤ 100	Pos
			LipL21 (500)		≤ 99.60	
	E8/14	*Rattus* compl.	G2 (211)	*L. interrogans*	≤ 98.58	/
	E9/14	*Rattus* compl.	G1/G2 (216)	*L. interrogans*	≤ 99.07	/
	E281/13	*Rattus norvegicus*	G2 (158)	*L. interrogans*	≤ 100	/
	E283/13	*Rattus norvegicus*	G2 (216)	*L. interrogans*	≤ 100	/
			LipL21 (500)		≤ 100	
	E289/13	*Rattus* compl.	G2 (216)	*L. interrogans*	≤ 100	Pos
			LipL21 (500)		≤ 100	
	E290/13	*Rattus* compl.	G2 (211)	*L. borgpetersenii*	≤ 100	Pos
	E291/13	*Rattus norvegicus*	G2 (216)	*L. borgpetersenii*	≤ 100	/
			LipL21 (555)	*L. interrogans*	≤ 99.64	
	E295/13	*Rattus* compl.	G2 (216)	*L. interrogans*	≤ 99.54	Pos
			LipL21 (500)		≤ 99.00	
	E317/13	*Rattus* compl.	G2 (216)	*L. interrogans*	≤ 100	Pos
			LipL21 (500)		≤ 99.60	
	E323/13	*Rattus* compl.	G2 (216)	*L. interrogans*	≤ 100	Pos
			LipL21 (500)		≤ 99.60	
	E336/13	*Rattus* compl.	G2 (216)	*L. interrogans*	≤ 99.54	Pos
			LipL21 (500)		≤ 99.00	
	E343/13	*Rattus* compl.	G2 (216)	*L. interrogans*	≤ 99.07	Pos
			LipL21 (500)		≤ 99.00	

10.4 Trypanosoma results

Tab. 41: *Trypanosoma* molecular detection results according to the ecological group, species, sex and age

Ecological group	Species	***Trypanosoma* molecular detection**																	
		***Trypanosoma* (n= 277)**		Sex					Age										
				Female		Male			Immature		Juvenile		Subadult		Adult		ND		
		Analyzed	Sequenced	Analy.	Seq.	Analy.	Seq.	NA	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.
Forest rodents	*Callosciurus notatus*	2	/	1	/	1	/	/	/	/	/	/	/	/	2	/	/	/
	Callosciurus prevostii	3	/	1	/	2	/	/	/	/	/	/	/	/	3	/	/	/
	Sundasciurus lowii	6	2	5	2	1	/	/	1	/	1	/	/	/	4	2	/	/
	Leopoldamys sabanus	1	/	/	/	1	/	/	/	/	/	/	/	/	1	/	/	/
	Maxomys rajah	3	/	/	/	3	/	/	/	/	/	/	3	/	/	/	/	/
	Maxomys surifer	4	1	2	/	2	1	/	/	/	/	/	/	3	1	1	/	/
	*Maxomys surifer/rajah**	7	/	4	/	3	/	/	3	/	2	/	1	/	1	/	/	/
	Maxomys whiteheadi	6	1	2	1	4	/	/	/	/	/	/	2	1	4	/	/	/
	Niviventer cremoriventer	4	/	2	/	2	/	/	/	/	/	/	/	/	4	/	/	/
Forest rodents total		**36**	4															

Analy.: analysed. Seq.: sequenced

Continuation Tab. 41

Ecological group	Species	*Trypanosoma* molecular detection																	
		Trypanosoma (n= 277)		Sex					Age										
				Female		Male			Immature		Juvenile		Subadult		Adult		ND		
		Analyzed	Sequenced	Analy.	Seq.	Analy.	Seq.	NA	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.
Treeshrews	*Tupaia gracilis*	1	1	1	1	/	/	/	/	/	/	/	1	1	/	/	/	/
	Tupaia minor	8	1	4	/	4	1	/	/	/	/	/	/	/	8	1	/	/
	Tupaia tana	4	3	3	2	1	1	/	1	1	/	/	1	/	2	2	/	/
Treeshrews total		**13**	**5**																
Generalist	***Sundamys muelleri***	**66**	**1**	35	1	31	/	/	/	/	3	/	16	1	46	/	1	/
Rattus	*Rattus* comp.	76	9	33	4	43	5	/	1	/	5	1	8	1	60	7	2	/
	Rattus norvegicus	41	5	20	2	21	3	/	4	1	5	/	7	1	25	3	/	/
Rattus total		**117**	**14**																
Shrews	***Suncus murinus***	**45**	*/*	13	/	30		2	/	/	/	/	/	/	43	/	2	/

Analy.: analysed. Seq.: sequenced

Tab. 42: *Trypanosoma* sequencing results

Ecological group	Sample ID	Host	Sequence (bp)	BLAST results	% Identity
Forest rodents	E64/14	*Sundasciurus lowii*	380 bp	*Trypanosoma* sp.	≤ 97.24
	E367/13	*Sundasciurus lowii*	554 bp	*Trypanosoma* sp.	≤ 95.50
	E356/13	*Maxomys whiteheadi*			
	E368/13	*Maxomys surifer*	538 bp	*Trypanosoma* sp.	= 99.81[a]
Treeshrews	E349/13	*Tupaia gracilis*	567 bp	*Trypanosoma* sp.	≤ 97.71 [b]
				T. cyclops	= 97.53 [c]
	E371/13	*Tupaia minor*	572 bp	*T. cyclops*	= 97.38 [c]
				Trypanosoma sp.	≤ 96.68 [b]
	E66/14	*Tupaia tana*	2 fragments, 460 bp [#]	*T. cyclops*	(= 100.00) [c]
				Trypanosoma sp.	(≤ 99.06) [b]
	E67/14	*Tupaia tana*	580 bp	*T. cyclops*	= 95.52 [c]
				Trypanosoma sp.	≤ 95.34 [b]
	E70/14	*Tupaia tana*	529 bp	*Trypanosoma* sp.	≤ 97.54 [b]
				T. cyclops	=97.16 [c]
Generalist	E140/13	*Sundamys muelleri*	540 bp	*T. lewesi ATCC 30085*	99.81 to 100.00
Rattus	E200/15	*Rattus* compl.	560 bp	*Trypanosoma* sp.	≤ 98.58 [b]
				T. cyclops	= 97.51 [c]
	E306/13	*Rattus norvegicus*	540 bp	T. lewesi ATCC 30085	99.81 to 100.00
	E335/13	*Rattus* compl.			
	E336/13	*Rattus* compl.			
	E337/13	*Rattus norvegicus*			
	E12/15	*Rattus* compl.			
	E194/15	*Rattus* compl.			
	E197/15	*Rattus* compl.			
	E141/14	*Rattus* compl.			
	E149/14	*Rattus* compl.			
	E145/14	*Rattus norvegicus*			
	E162/14	*Rattus norvegicus*			
	E153/14	*Rattus norvegicus*			
	E217/15	*Rattus* compl.			

[#]) A complete clean fragment could not be obtained from forward and reverse sequences in repetitions. The two sequence fragments correspond to R408, R583, R585 and R588 but the hypervariable region is missing.
[a]) Accession no. KJ467217, host: sand fly, Thailand (Phumee et al., 2014 unpublished).
[b]) Accession no. AJ620574, host: terrestrial leech, Australia (Hamilton et al., 2005).
[c]) Accession no. AJ131958 (Stevens J.R., 1999 unpublished)

10.5 Sarcocystis results

Tab. 43: *Sarcocystis* histological results according to the ecological group, species, sex and age

Ecological group	Species	***Sarcocytis* histological detection**																	
		***Sarcocystis* (n=309)**		**Sex**						**Age**									
				Female		Male		NA		Immature		Juvenile		Subadult		Adult		ND	
		Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos
Forest rodents (n= 42)	*Callosciurus notatus*	5	1	3	1	2	/	/	/	/	/	/	/	/	/	5	1	/	/
	Callosciurus prevostii	2	/	1		1		/	/	/	/	/	/	/	/	3	/	/	/
	Sundasciurus lowii	6	4	5	4	1	/	/	/	1	/	1	1	/	/	4	3	/	/
	Leopoldamys sabanus	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Maxomys rajah	3	/	/	/	3	/	/	/	/	/	/	/	/	/	3	/	/	/
	Maxomys surifer	7	/	3	/	4	/	/	/	/	/	/	/	4	/	3	/	/	/
	*Maxomys surifer/rajah**	6	/	3	/	3	/	/	/	2	/	2	/	1	/	1	/	/	/
	Maxomys whiteheadi	5	2	2	1	3	1	/	/	/	/	/	/	2	/	3	2	/	/
	Niviventer cremoriventer	4	3	2	1	2	2	/	/	/	/	/	/	/	/	4	3	/	/
Forest rodents total		**38**	**10**																

Analy.: analysed. Pos: positive

Continuation Tab. 43

Ecological group	Species	***Sarcocystis* histological detection**																	
		***Sarcocystis* (n=309)**		**Sex**						**Age**									
				Female		Male		NA		Immature		Juvenile		Subadult		Adult		ND	
		Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos	Analy.	Pos
Treeshrews (n=15)	*Tupaia gracilis*	1	/	1	/	/	/	/	/	/	/	/	/	1	/	/	/	/	/
	Tupaia minor	8	7	4	3	4	4	/	/	/	/	/	/	/	/	8	7	/	/
	Tupaia tana	5	4	4	2	2	2	/	/	1	/	/	/	1	/	4	4	/	/
Treeshrews total		**14**	**11**																
Generalist (n=76)	*Sundamys muelleri*	**70**	**37**	41	17	28	19	1	1		/	3	/	17	5	48	30	2	2
Rattus (n=155)	*Rattus* comp.	90	38	39	17	51	21	/	/	1	/	6	/	8	4	73	34	2	/
	Rattus norvegicus	41	10	20	5	21	5	/	/	4	/	8	/	7	/	22	10	/	/
Rattus total		**131**	**48**																
Shrews (n=58)	***Suncus murinus***	**56**	**2**	15	**1**	38	**1**	3	/	/	/	/	/	/	/	53	2	3	/

Analy.: analysed. Pos: positive

Phylogenetic results from the novel *Sarcocytis* sp. (*Sarcocytis* scandentiborneensis sp. nov) (made by Dr. Thomas Jäkel, University of Hohenheim, Germany)

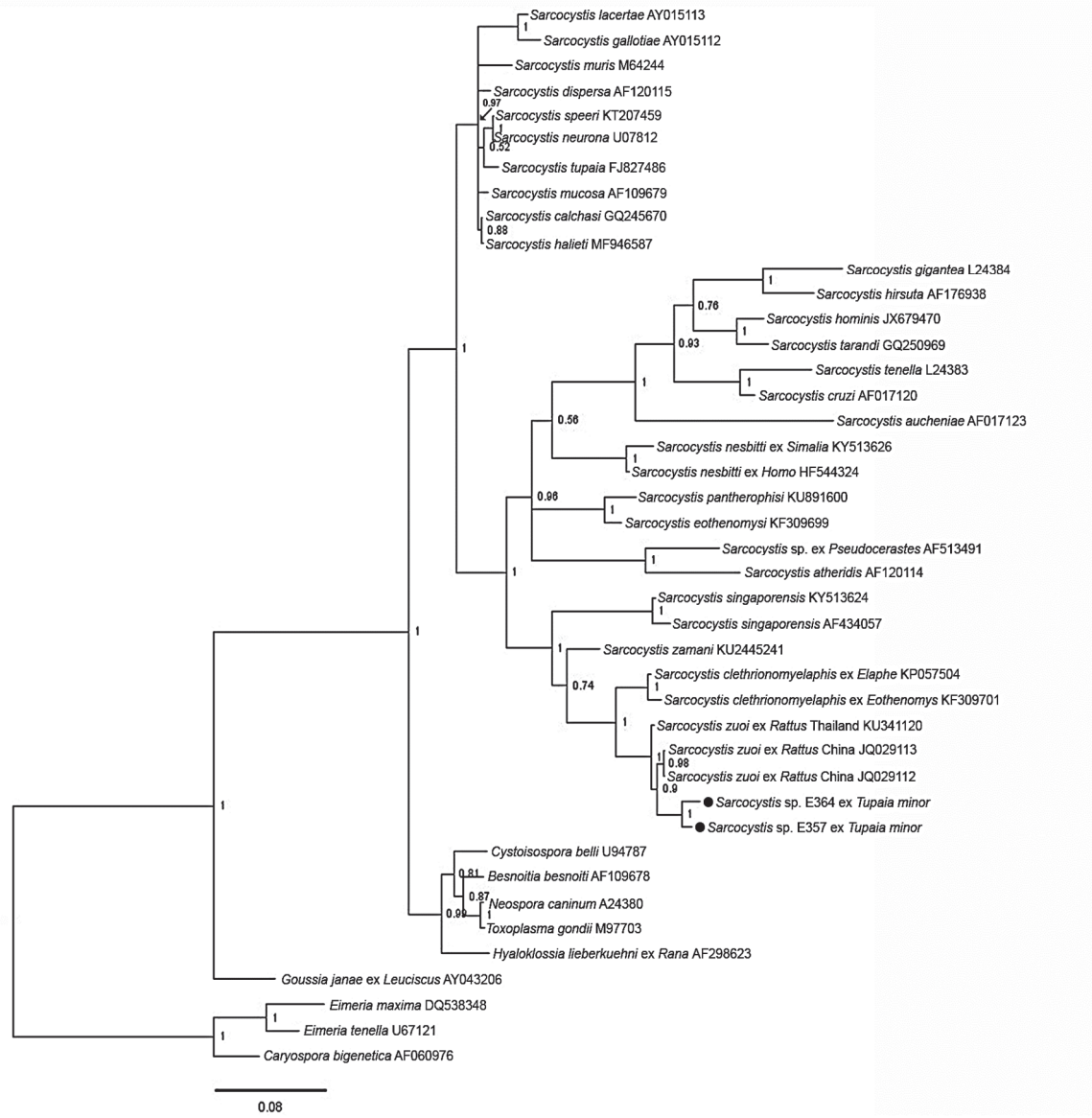

Fig. 21: Phylogenetic tree of nuclear 18S rDNA sequences of the Sarcocystidae, including the new *Sarcocystis* sp. from treeshrews (black dots) (Courtesy of Dr. T. Jäkel). Taxa of the Eimeriidae served as outgroup. Bayesian Inference was used for phylogeny reconstruction, whereby the general time-reversible substitution model (GTR+G+I) combined with an assumed among-site variation ('covariotide' model) was applied. Values for posterior probability are indicated behind nodes. Note that the new species is part of a monophyletic subclade previously tagged S1 (Wassermann et al., 2017), which includes taxa known to prefer snakes as definitive and rodents as intermediate hosts. Sequences E357-13 and E364-13 are available at GenBank under MN733816 and MN733817, respectively.

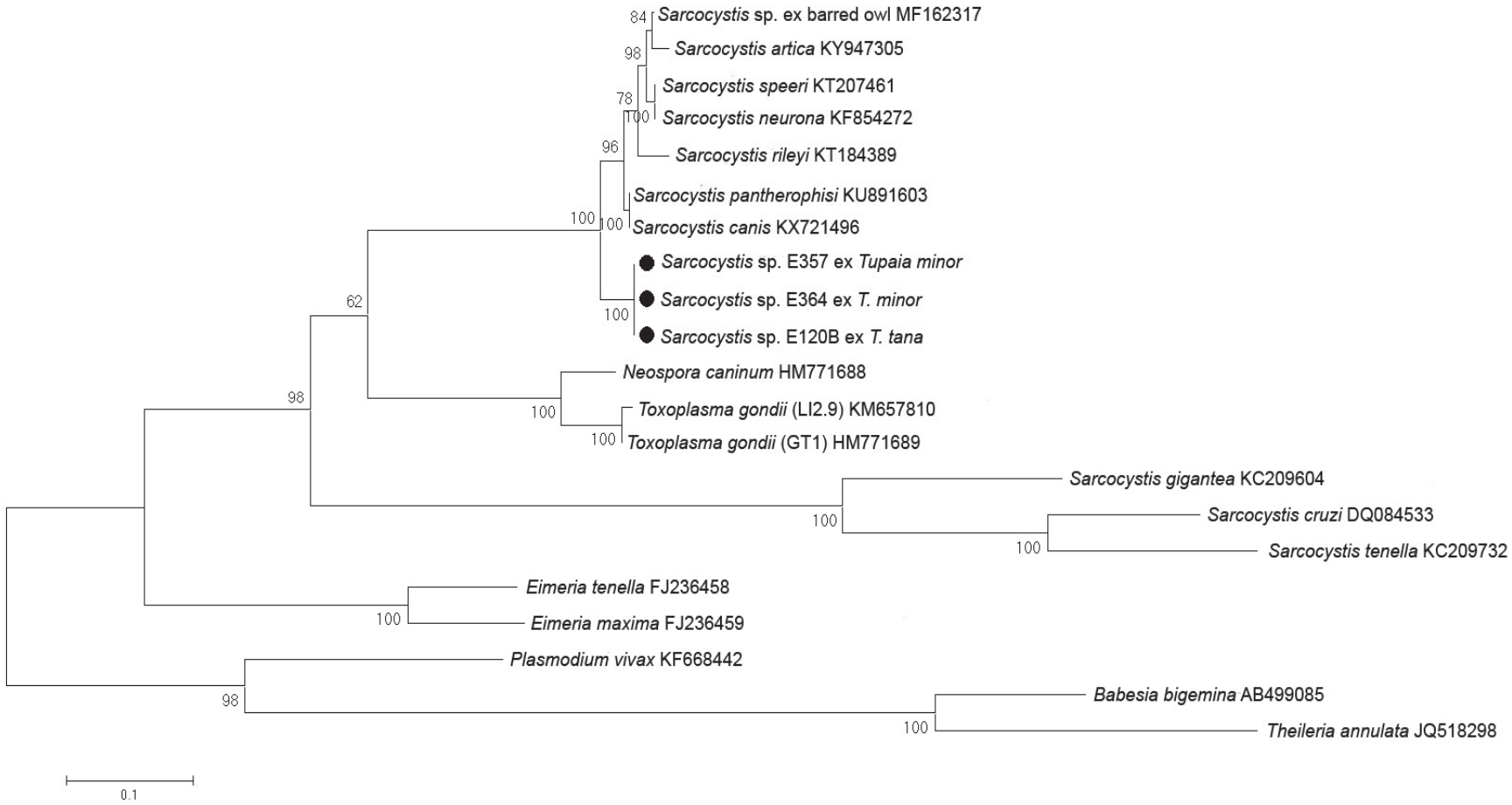

Fig. 22: Phylogenetic tree based on analysis of mitochondrial COI sequences of the Sarcocystidae including the new *Sarcocystis* sp. examined in this study (black symbols) (Courtesy of Dr. T. Jäkel). Other taxa of the Apicomplexa served as root. Evolutionary history was inferred by the Maximum Likelihood (ML) method based on the Tamura-Nei model, whereby 619 positions were included in the final data set. All positions with less than 95% site coverage were eliminated; that is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. Bootstrap percentages (1000 iterations) are shown next to branch.

10.6 Herpesvirus results

Tab. 44: Herpesvirus molecular detection results according to the ecological group, species, sex and age

Ecological group	Species	Herpesvirus molecular detection																
		Herpesvirus (n= 156)		Sex					Age									
				Female		Male			Immature		Juvenile		Subadult		Adult		ND	
		Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	NA	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.
Forest rodents	*Callosciurus notatus*	3	1	2	1	1	/	/	/	/	/	/	/	/	3	1	/	/
	Callosciurus prevostii	2	1	1	/	1	1	/	/	/	/	/	/	/	2	1	/	/
	Sundasciurus lowii	3	/	3	/	/	/	/	/	/	/	/	/	/	3	/	/	/
	Leopoldamys sabanus	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Maxomys rajah	3	1	/	/	3	1	/	/	/	/	/	3	1	/	/	/	/
	Maxomys surifer	1	/	/	/	1	/	/	/	/	/	/	/	/	1	/	/	/
	*Maxomys surifer/rajah**	1	/	/	/	1	/	/	1	/	/	/	/	/	/	/	/	/
	Maxomys whiteheadi	5	2	2	1	3	1	/	/	/	/	/	2	2	3	/	/	/
	Niviventer cremoriventer	3	1	2	/	1	1	/	/	/	/	/	/	/	3	1	/	/
Forest rodents total		**21**	**6**															

Analy.: analysed. Seq.: sequenced

Continuation Tab. 44

Ecological group	Species	Herpesvirus molecular detection																	
		Herpesvirus (n= 156)		Sex					Age										
				Female		Male			Immature		Juvenile		Subadult		Adult		ND		
		Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	NA	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.	Analy.	Seq.
Treeshrews	*Tupaia gracilis*	1	/	1	/	/	/	/	/	/	/	/	1	/	/	/	/	/
	Tupaia minor	8	4	4	3	4	1	/	/	/	/	/	/	/	8	4	/	/
	Tupaia tana	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Treeshrews total		**9**	**4**															
Generalist	*Sundamys muelleri*	**41**	**15**	23	9	18	6	/	1	/	/	/	12	1	27	13	1	1
Rattus	*Rattus* comp.	40	16	17	7	23	9	/	/	/	3	1	6	1	29	14	2	/
	Rattus norvegicus	17	4	5	2	12	2	/	/	/	4	/	6	1	7	3	/	/
Rattus total		**57**	**20**															
Shrews	***Suncus murinus***	**28**	**1**	8	/	18	1	2	/	/	/	/	/	/	26	1	2	/

Analy.: analysed. Seq.: sequenced

Tab. 45: List of herpesvirus abbreviations used in Tab. 46

Virus abbreviation	Virus name
BBWGHW	Blainville's beaked whale gammaherpesvirus
Bindi_RHV4	Bandicota indica Rhadinoherpesvirus 4
Bsavi_RHV1	Bandicota savilei rhadinovirus 1
CMV_Guenon	Cytomegalovirus Guenon/muscle/BM-002
EHV5	Equid herpesvirus 5
Mcerv_RHV1	Mus cervicolor rhadinovirus 1
MHV8	Murid herpesvirus 8
OSOHV	Oriental small-clawed otter gammaherpesvirus
OtH4	Otariid herpesvirus 4
RatCMV	Rat cytomegalovirus
Rnorv_RHV1	Rattus norvegicus rhadinovirus 1
Rrat_RHV1	Rattus rattus rhadinovirus 1
Rrat_RHV3	Rattus rattus rhadinovirus 3
Rtiom_CMV1	Rattus tiomanicus cytomegalovirus 1
Rtiom_RHV1	Rattus tiomanicus Rhadinoherpesvirus 1
Rtiom_RHV2	Rattus tiomanicus rhadinovirus 2
Tbel_GHV1	Tupaia belangeri gammaherpesvirus 1

Tab. 46: Herpesvirus sequencing results (*Bachelor thesis Heiko Pietsch, RKI, Berlin, Germany*) (Pietsch, 2013)

Ecological group	ID	Species	BLAST (abbreviation)	Type of herpesvirus	% Identity
Forest rodents	E366/13	*Callosciurus prevostii*	Rrat_RHV1	GAMMA	99%
	E360/13	*Callosciurus notatus*	OSOHV	GAMMA	80%
	E355/13	*Maxomys rajah*	CMV_Guenon	BETA	97%
	E388/13	*Niviventer cremoriventer*	OtH4	GAMMA	99%
	E369/13	*Maxomys whiteheadi*	RatCMV	BETA	72%
	E389/13	*Maxomys whiteheadi*	Bsavi_RHV1	GAMMA	84%
Treeshrews	E364/13	*Tupaia minor*	Mcerv_RHV1	GAMMA	79%
	E351/13	*Tupaia minor*	Tbel_GHV1	GAMMA	72%
	E392/13	*Tupaia minor*	Tbel_GHV1	GAMMA	74%
	E352/13	*Tupaia minor*	Mcerv_RHV1	GAMMA	79%
Generalist	E387/13	*Sundamys muelleri*	Bindi_RHV4	GAMMA	96%
	E280/13	*Sundamys muelleri*	Rrat_RHV3	GAMMA	98%
	E16/14	*Sundamys muelleri*	Rtiom_RHV1	GAMMA	99%
	E298/13	*Sundamys muelleri*	Rtiom_RHV1	GAMMA	99%
	E318/13	*Sundamys muelleri*	Rtiom_RHV1	GAMMA	99%
	E319/13	*Sundamys muelleri*	Rtiom_RHV1	GAMMA	99%
	E136/13	*Sundamys muelleri*	Bindi_RHV4	GAMMA	89%
	E275/13	*Sundamys muelleri*	Rtiom_RHV1	GAMMA	99%
	E145/13	*Sundamys muelleri*	Rtiom_RHV2	GAMMA	88%
	E330/13	*Sundamys muelleri*	Rtiom_RHV2	GAMMA	87%
	E334/13	*Sundamys muelleri*	Rtiom_RHV2	GAMMA	88%
	E372/13	*Sundamys muelleri*	Rtiom_RHV2	GAMMA	87%
	E374/13	*Sundamys muelleri*	Rtiom_RHV2	GAMMA	87%
	E28/14	*Sundamys muelleri*	Rtiom_RHV2	GAMMA	88%
	E40/14	*Sundamys muelleri*	Rtiom_RHV2	GAMMA	88%

Continuation Tab. 46

Ecological group	ID	Species	BLAST (abbreviation)	Type of herpesvirus	% Identity
Urban *Rattus* spp	E272/13	*Rattus* compl.	*Rtiom_RHV2*	*GAMMA*	*0,88*
	E41/14	*Rattus* compl.	*Rtiom_RHV1*	*GAMMA*	*0,98*
	E54/14	*Rattus* compl.	*Rtiom_RHV1*	*GAMMA*	*0,99*
	E336/13	*Rattus* compl.	*Rtiom_RHV1*	*GAMMA*	*0,99*
	E50/14	*Rattus* compl.	*Rtiom_RHV1*	*GAMMA*	*0,98*
	E310/13	*Rattus* compl.	*Rtiom_RHV1*	*GAMMA*	*0,97*
	E8/14	*Rattus* compl.	*Rtiom_RHV1*	*GAMMA*	*0,98*
	E282/13	*Rattus norvegicus*	*Rtiom_RHV1*	*GAMMA*	*0,98*
	E289/13	*Rattus* compl.	*Rtiom_RHV1*	*GAMMA*	*0,99*
	E34/14	*Rattus* compl.	*Rrat_RHV1*	*GAMMA*	*0,99*
	E9/14	*Rattus* compl.	*Rrat_RHV3*	*GAMMA*	*0,98*
	E48/14	*Rattus* compl.	*Rrat_RHV3*	*GAMMA*	*0,98*
	E394/13	*Rattus* compl.	*Rtiom_CMV1*	*BETA*	*0,97*
	E17/14	*Rattus* compl.	*Rtiom_CMV1*	*BETA*	*0,97*
	E47/14	*Rattus* compl.	*Rtiom_CMV1*	*BETA*	*0,98*
	E307/13	*Rattus norvegicus*	*Rnorv_RHV1*	*GAMMA*	*0,99*
	E308/13	*Rattus norvegicus*	*Rnorv_RHV1*	*GAMMA*	*0,99*
	E297/13	*Rattus* compl.	*Rrat_RHV1*	*GAMMA*	*0,98*
	E281/13	*Rattus norvegicus*	*MHV8*	*BETA*	*0,83*
	E24/14	*Rattus* compl.	*BBWGHW*	*GAMMA*	*0,97*
Urban *Suncus murinus*	E18/14	*Suncus murinus*	*EHV5*	*GAMMA*	*0,8*

11 LIST OF PRESENTATIONS AND PUBLICATION

1. **Ortega Pérez Paula, Wells Konstans, Mühldorfer Kristin, Lüschow Dörte, Lakim B. Maklarin, Krone Oliver, Jäkel Thomas, Wibbelt Gudrun:** Is nature a better place than urban life? Health status of native Bornean rodents and treeshrews compared to invasive rats. European Society of Veterinary Pathologists (ESVP) and European College of Veterinary Pathologists (ECVP) Annual Meeting 2018 (Cluj-Napoca, Romania) poster flash.
2. **Ortega Pérez Paula, Wells Konstans, Lakim B. Maklarin, Krone Oliver, Auls Susanne, Jäkel Thomas, Wibbelt Gudrun:** Investigations in Sarcosporidia in native rodents and treeshrews from Borneo. Annual Meeting of the German Society for Parasitology 2018 (Berlin, Germany) poster presentation.
3. **Ortega Pérez Paula, Wells Konstans, Lakim B. Maklarin, Krone Oliver, Auls Susanne, Wibbelt Gudrun:** Identification of *Sarcocystis* in native rodents and treeshrews from Borneo. Conference of the European Wildlife Disease Association (EWDA) 2016 (Berlin, Germany) poster presentation.
4. **Ortega Pérez Paula, Wibbelt Gudrun, Brinkmann Annika, Galindo Puentes John A., Tuh Fred Y. Y., Maklarin Lakim B., Nitsche Andreas, Wells Konstans, Jäkel, Thomas**, 2020. Description of *Sarcocystis scandentiborneensis* sp. nov. from treeshrews (*Tupaia minor*, *T. tana*) in northern Borneo with annotations on the utility of COI and 18S rDNA sequences for species delineation. Int. J. Parasitol. Parasites Wildl. 12, 220–231. https://doi.org/10.1016/j.ijppaw.2020.07.003.

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13 DECLARATION OF AUTHORSHIP

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen Anspruch genommen habe.

I hereby confirm that I have made this work autonomously. I assure that I have read and used only the specified sources claimed in this work.

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